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Genetic diversity in *Quercus petraea* Liebl. of North West of Iran based on SCoT markers

Yousef Mohammadi[1*], Shiva Gheytaranpour-Sehrigh[2], and Sajad Ghanbari[3]

[1]Assistant Prof., Research institute of Forests and Rangelands, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
[2]Ph.D. Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[3]Prof., Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Tabriz, Iran.

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Abstract

The Arasbaran forest is one of the genetic hotspots of the biosphere due to its high species diversity, and the white oak species plays a special role in the composition and diversity of the biological elements of Arasbaran forests. In this regard, it is necessary and important to evaluate the genetic diversity of the identified seed production area of white oak. In this research, the genetic diversity within and between seven different white oak populations, including 49 different trees, was investigated using the SCoT molecular marker. A total of 129 alleles were observed for these markers, and 100% of the produced alleles were polymorphic. The number of alleles varied from 16 to 29, with marker SCoT41 having the highest number at 29 alleles and marker SCoT7 having the lowest number at 16 alleles. Analysis of molecular variance (AMOVA) showed that 90% of the observed genetic diversity was distributed within populations, while 10% occurred among populations, suggesting that genetic variability within populations was more significant than that among populations. Based on the UPGMA dendrogram, individuals were partitioned into six distinct clades, and according to the UPGMA and PCA patterns, geographical proximity has not played an important role in the formation of the genetic structure of these populations. This research has provided initial insight into the genetic diversity within and between different populations of the Arasbaran white oak species. As a result, it is suggested that, by studying other markers, useful information for conservation and management measures for this valuable species can be obtained.

Keywords: Arasbaran, GenALEx, Molecular markers, *Quercus petraea*.

1. Introduction

The Arasbaran forest is one of the five vegetation areas in Iran and plays an important ecological and biological role in Iran due to its rich plant diversity. Situated in the northwest of Iran, it is one of the nine designated Biosphere Reserves in the country. According to the literature, 1067 plant species have been identified in this region, with 18 of these species being endemic to Iran (Hamzeh et al., 2010). The sessile oak (*Quercus petraea* Liebl.) is a deciduous tree species native to Europe and widely distributed in Europe, the Ural Mountains, and the Caucasus Mountains. It is important for forestry, providing valuable timber and supporting a diverse range of wildlife (Eaton et al., 2016; Gömöry et al., 2001). *Q. petraea* populations are threatened by habitat loss, fragmentation, and climate change. Understanding the genetic diversity and structure of *Q. petraea* populations is essential for the conservation and management of this species.

Genetic diversity is essential for species adaptation to changing environments and long-term survival (Hughes et al., 2008). Studying the genetic diversity within and among populations of a species can enhance comprehension of the historical mechanisms that have shaped genetic variation. Additionally, such analysis can yield crucial data for breeding initiatives and the development of strategies to conserve genetic resources (Alikhani et al., 2014). Molecular markers play a crucial role in evaluating the genetic resources of plants, enhancing our comprehension of the distribution and diversity

*Corresponding author Tel: 02144787282 Email: mohammadi.yousef63@gmail.com

of genetic variation within and among species (Banaei-Asl & Mohammadi, 2025; Mohammadi et al., 2025). Typically, for outbred forest tree species, the genetic diversity within populations exceeds that among populations of the same species, indicating minimal local spatial structure (Porth & El-Kassaby, 2014).

Despite the economic, biological, and ecological importance of *Q. petraea*, there is a lack of genetic research on this plant. Genetic studies on *Q. petraea* have mainly focused on intra-species genetic variability (Gömöry et al., 2001; Jensen et al., 2003; Muir et al., 2004; Sandurska et al., 2019; Jurkšienė et al., 2020; Dvořák et al., 2022; Rebrean et al., 2023; Tóth et al., 2023). These studies have shown significant levels of genetic variation in *Q. petraea.*

Different DNA marker systems used to evaluate genetic diversity have their advantages and disadvantages. The choice of marker system in research labs depends on factors such as technical expertise, available equipment, and funding (Aydın et al., 2022). The Start Codon Targeted (SCoT) marker (Collard & Mackill, 2009) is based on conserved regions flanking the ATG codon and uses a single primer for the amplification of PCR fragments. SCoT is considered to be more reproducible than RAPD and ISSR markers according to recent studies (Amom et al., 2020; Gogoi et al., 2020).

Due to the high costs of conducting molecular studies and the difficult access to the white oak distribution areas in Arasbaran, there have been no prior studies conducted to assess the genetic variability of *Q. petraea* populations throughout Arasbaran. We used SCoT markers to estimate the genetic variability and differentiation within and among various *Q. petraea* populations from the Arasbaran forest. This information is essential for understanding the historical processes that have impacted genetic diversity and for creating effective conservation strategies and reforestation programs.

2. Materials and methods

2.1. Plant material

In the summer of 2022, young leaves from 49 individuals of *Q. petraea* were selected primarily based on their superior phenotypic traits (healthy trees, having enough healthy seeds) from seven populations of the Arasbaran forest located in the northwest of Iran, with each population represented by 7 individuals (Figure 1 and Table 1). A minimum distance of 50 m between sampled individuals was maintained to prevent the sampling of individuals with close genetic relationships (Ghamari Zare et al., 2023). The collected samples were labeled with individual IDs and were frozen at -80°C for subsequent processing.

2.2. DNA extraction and PCR amplification

Total genomic DNA was obtained from 50 mg of young leaves using a modified CTAB protocol following the method described by Saghai-Maroof et al. (1984). The extracted DNA was evaluated using 1% TAE agarose gel and spectrophotometry (Thermo Fisher Nanodrop 2000).

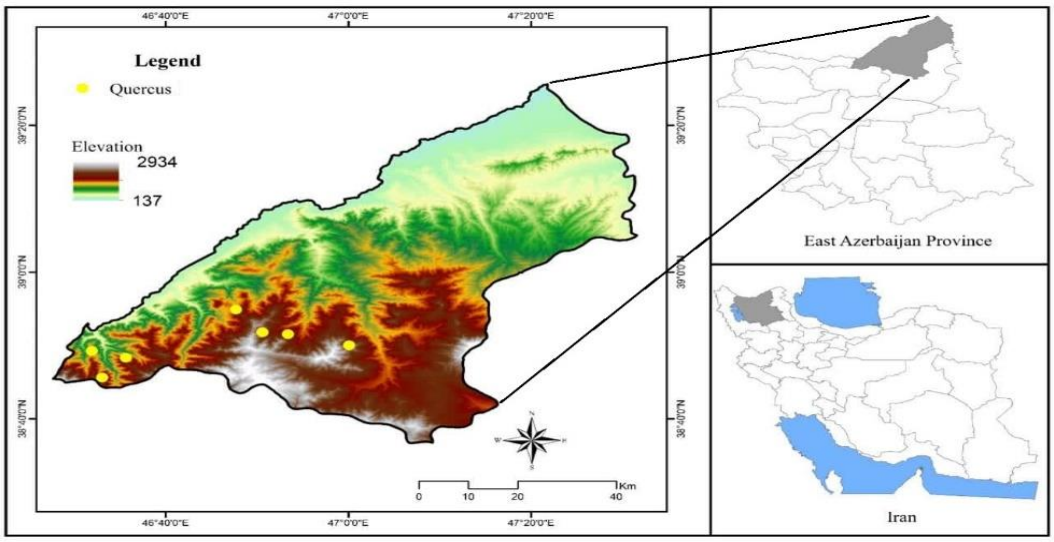

Figure 1. Geographic locations of *Q. petraea* populations

Table 1. Geographical features of the 7 populations of *Q. petraea*

Population name	Longitude	Latitude	Elevation (m)
A	N38° 50' 01.9"	E47° 00' 03.7"	1719
B	N38° 51' 32.5"	E46° 53' 23"	1684
C	N38° 51' 47.54"	E46° 50' 35.26"	1920
D	N38° 54' 53.8"	E46° 47' 41"	1462
E	N38° 56' 37.4"	E47° 26' 46.1"	1448
F	N38° 40' 54"	E46° 31' 55.1"	2218
G	N38° 49' 15.3"	E46° 31' 54.3"	1285

Based on previous research papers (Luo et al., 2010; Vivodik et al., 2019), a total of 29 SCoT primers were chosen and synthesized by Metabion (Germany). Initially, four individuals from each population were used to screen 29 SCoT markers to exclude markers that did not produce suitable scoring bands, as well as markers that had a low polymorphism percentage. Polymerase chain reaction (PCR) amplification was performed in a 10 μL reaction mixture consisting of 5 μL of red mastermix (Ampliqon, Denmark), 1 μL of a SCoT primer at a concentration of 10 pmol, 1 μL of genomic DNA (20 ng), and 3 μL of nuclease-free water. The amplification of SCoT fragments was carried out using a Mastercycler Gradient Thermal Cycler (Eppendorf, Germany) following a well-defined sequence, with an initial denaturation step at 95 °C for 5 min, followed by 40 cycles involving denaturation at 95 °C for 1 min, annealing at a specific temperature for 1 min, elongation at 72 °C for 90 sec, and a final elongation step at 72 °C for 5 min. To visualize the PCR products, 2% agarose gel electrophoresis was performed, with detection facilitated through a safe stain. Furthermore, to confirm the absence of contamination, a control PCR reaction without genomic DNA was included in every experimental run.

2.3. Data Interpretation and Statistical Analysis

For each SCoT, we calculated the polymorphism information content (PIC) using Excel. The PIC serves as an indicator of the discriminatory ability of each SCoT and relies on the number of polymorphic markers in each primer, as well as the frequency of each marker. GenALEx version 6.5 (Peakall & Smouse, 2012) was used to analyze genetic variability parameters such as the number of alleles (Na), the effective number of alleles (Ne), Shannon's information index (I), expected heterozygosity (He), and unbiased expected heterozygosity. Nei's genetic distances among populations were determined using NTSYS-2.10 software (Rohlf, 2000), and the UPGMA method was used for cluster analysis of the 7 populations. GenALEx was also used to analyze genetic differentiation among and within populations by analysis of molecular variance (AMOVA) and to visualize relationships among populations and individuals by principal coordinate analysis (PCA). This analysis takes all genetic data and simplifies it into a few significant coordinates, which can then be graphed in two or three dimensions to demonstrate the grouping of populations or individuals.

3. Results

3.1. SCoT analysis

A total of six highly polymorphic and reproducible SCoT primers were carefully selected for the genetic analysis of 49 individuals (Figure 2). The six SCoT primers yielded a total of 129 bands that could be scored. All bands (100%) were polymorphic, and the number of polymorphic bands varied from 16 (for the SCoT7 primer) to 29 (for SCoT41), with an average of 21.5 bands per primer (Table 2). The PIC parameter values varied from 0.248 (for SCoT21) to 0.347 (for SCoT30), with a mean of 0.306. In SCoT markers, the PIC parameter ranges from 0 to 0.5. A high PIC value for a marker indicates a high ability of the marker to determine the genetic distance of individuals.

Analysis of genetic diversity parameters in *Q. petraea* populations showed that the number of alleles ranged from 1.116 (for the G population) to 1.550 (for the C population), with an average of 1.303. The effective number of alleles was almost similar to the number of alleles (1.265, 1.311 and 1.287, respectively). Continuing our genetic variability evaluation, the C population showed the highest Shannon's information index (0.329) among the studied populations. Moreover, the C population displayed the highest expected heterozygosity

(0.206) and unbiased expected heterozygosity value (0.221). In contrast, the G population exhibited the lowest Shannon's information index (0.260), and D showed the minimum expected heterozygosity (0.165) and unbiased expected heterozygosity (0.178) (Table 3).

3.2. Genetic differentiation among populations

A high range of genetic identity values was observed among the studied populations. A and B were found to be the closest, with a genetic identity of 0.978, and B and G were more different, with a genetic identity of 0.929 (Table 4).

Analysis of molecular variance exhibited that 90% of the genetic diversity was distributed within populations, and 10% was among populations, indicating high genetic diversity within populations (Table 5).

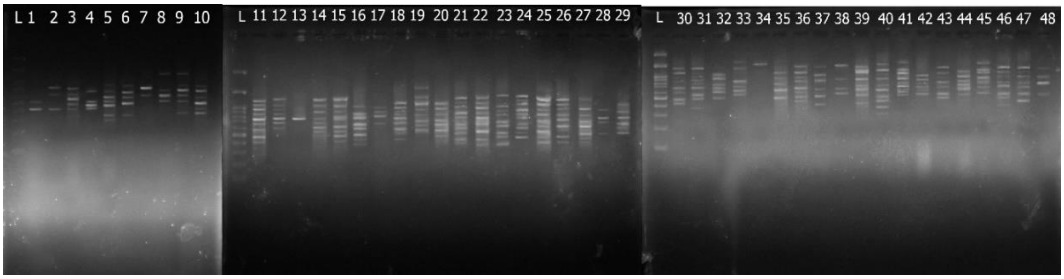

Figure 2. A sample of an Agarose gel for amplified fragments of *Q. petraea* using SCoT marker (SCoT 30 marker)

Table 2. The six SCoT primers used for *Q. petraea* genotyping

Primer name	Primer sequence	AT(°C)	PIC Value	PB
SCoT2	5′CAACAATGGCTACCACCC 3′	53	0.334	24
SCoT3	5′ CAACAATGGCTACCACCG 3′	53	0.263	17
SCoT7	5′ CAACAATGGCTACCACGG 3′	53	0.342	16
SCoT21	5′CACCATGGCTACCACCAT 3′	53	0.248	22
SCoT30	5′ CCATGGCTACCACCGGCG 3′	60	0.347	21
SCoT41	5′ CAATGGCTACCACTGACA 3′	51	0.306	29

AT Annealing Temperature, *PB* polymorphic bands

Table 3. Genetic diversity parameters of 6 SCoT primers in 49 individuals of *Q. petraea*

Population label	N	Na	Ne	I	He	uHe
A	7	1.271	1.293	0.293	0.187	0.202
B	7	1.310	1.302	0.297	0.190	0.205
C	7	1.550	1.311	0.329	0.206	0.221
D	7	1.271	1.253	0.265	0.165	0.178
E	7	1.310	1.287	0.289	0.183	0.197
F	7	1.295	1.304	0.302	0.193	0.208
G	7	1.116	1.265	0.260	0.167	0.182
Mean	7	1.303	1.287	0.290	0.184	0.199

Sample size (N); the number of alleles (Na); the effective number of alleles (Ne); Shannon's information index (I); expected heterozygosity (He); unbiased expected heterozygosity (uHe)

Table 4. Pairwise Population Matrix of Nei Genetic Identity

	A	B	C	D	E	F	G
A	1.000						
B	0.978	1.000					
C	0.977	0.968	1.000				
D	0.956	0.950	0.959	1.000			
E	0.959	0.945	0.958	0.958	1.000		
F	0.952	0.944	0.961	0.952	0.957	1.000	
G	0.941	0.929	0.949	0.936	0.945	0.955	1.000

Table 5. AMOVA analysis for studied populations of *Q. petraea* based SCoT markers

Source of variation	df	SS	MS	Est. Var.	%	*P*
Among Population	6	194.580	32.430	2.036	10%	<0.001
Within Population	41	757.357	18.472	18.472	90%	<0.001

Df, degree of freedom; SS, sum of squares; MS, mean squares; Est. var., estimate of variance; %, percentage of total variation; *P-value* is based on 1000 permutations.

3.3. Genetic relationships within populations

The genetic relatedness among the 49 individuals was represented using the UPGMA dendrogram, which was constructed based on Nei's genetic distance matrix. The individuals were partitioned into six distinct clades (Figure 3). Clade I comprised four populations including A1, A7, C3, and B6. Meanwhile, individuals A3–A6, B1–B5, B7, C1–C2, C4–C5, E1, and D6 belonged to clade II. Clade III consisted of C6, D1–D5, E2–E5, E7, and F1–F6. Clade IV comprised six populations including C7, E6, F7, and G4–G6. Finally, individuals A2 and D7 belonged to clade V and clade VI, respectively.

The 2D Principal Coordinate Analysis (PCA) of studied trees was performed, and a scatter plot was generated to illustrate the distribution of individuals according to the differentiation parameters. The PCA revealed that the first three principal coordinate components explained 60.30% of the overall variation. However, the two-dimensional representation of all individuals using the first two main coordinates (PCA 1 = 27.09 and PCA 2 = 18.35) did not show distinct differentiation based on geographic patterns (refer to Figure 4).

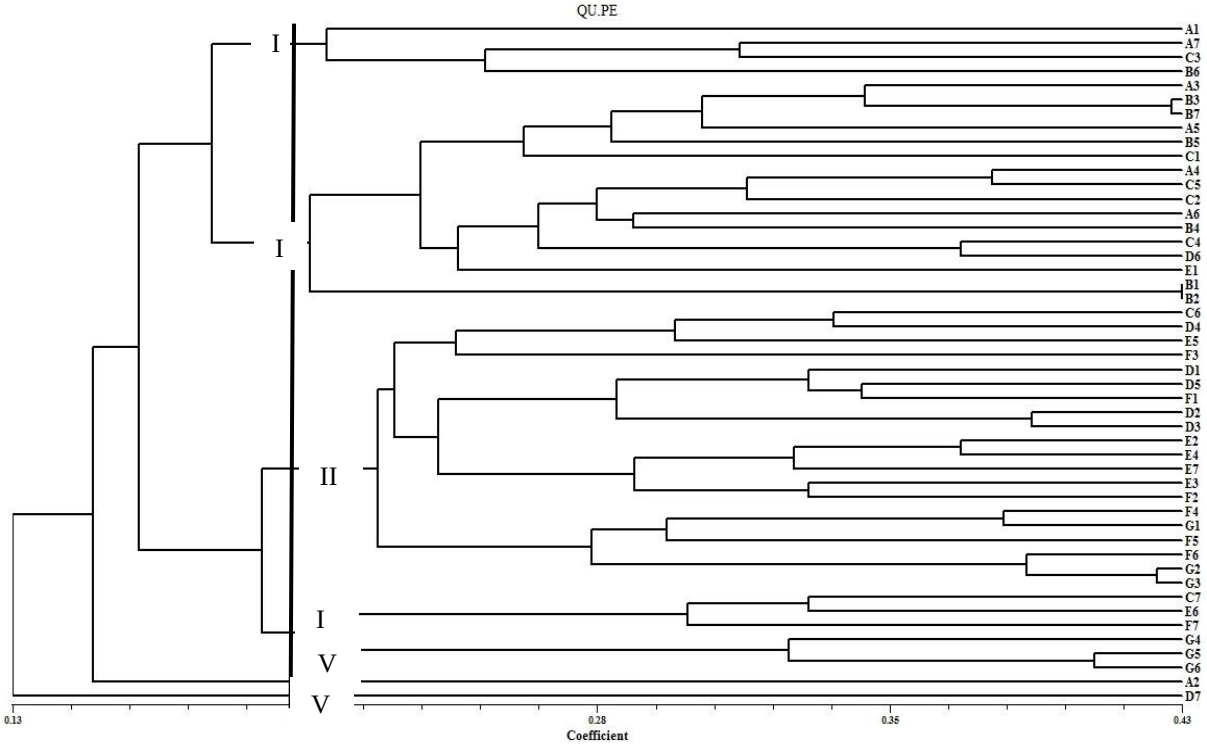

Figure 3. UPGMA of studied trees based on Nei's genetic distance matrix

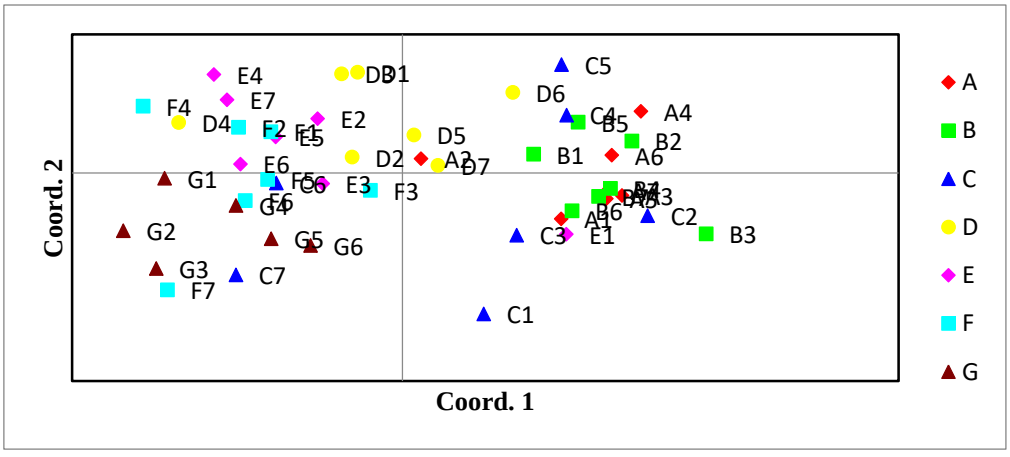

Figure 4. Principal coordinate analysis of the individuals

4. Discussion

The importance of genetic diversity in a species' evolutionary potential, adaptability, and long-term survival is well established. This is particularly relevant for the sessile oak, given its ecological significance and economic value in forestry (Rebrean et al., 2023). The present study aimed to evaluate the genetic variability within and among seven *Q. petraea* populations from the Arasbaran forest in Iran, using SCoT molecular markers. The results of this study provide scientific insight into the genetic structure and composition of these populations.

Genetic diversity plays a crucial role in facilitating the adaptation of forest tree populations to climate change conditions, and it is also essential for the development of future conservation programs (Kesić et al., 2021). A set of SCoT primers was effectively utilized to assess the genetic diversity of Arasbaran *Q. petraea* populations. All primers generated polymorphic bands, indicating the efficacy of these SCoT primers in evaluating genetic variability within and among *Q. petraea* populations. The successful application of SCoT markers to investigate genetic diversity has been validated in woody plants such as *Quercus brantii* Lindl. (Alikhani et al., 2014), *Taxus* x *media* (Hao et al., 2018), *Acer monspessulanum* L. (Motahari et al., 2021), and *Prunus sibirica* (Buer et al., 2022).

The levels of SCoT polymorphism percentage found in our study roughly correspond to those reported by previous studies, which range from 95% to 99% (Alikhani et al., 2014 & 2016). The findings demonstrate that SCoT markers exhibit a high capacity for assessing genetic diversity within plant populations. The genetic diversity of the seven *Q. petraea* populations (h = 0.184) was lower than that measured in the same genus: *Q. petraea* (h = 0.22, Rebrean et al., 2023) and *Quercus brantii* (h = 0.22, Alikhani et al., 2014).

The Polymorphic Information Content is influenced by the frequency and number of alleles within a population. Higher numbers of alleles, particularly when they are present at varying frequencies, tend to result in a higher PIC value, indicating greater genetic diversity (Nagy et al., 2012). In the present investigation, the mean PIC value was determined to be 0.306 across all loci. This substantial PIC value suggests a high degree of polymorphism among the individuals under study. The PIC value for different populations of the oak genus with different markers has been calculated in the range of 0.756 (SSR marker; Dvořák et al., 2022) and 0.38 (SCoT marker; Shabanian et al., 2015).

The results of the AMOVA showed a significant difference in intra-populations (90%) compared to inter-populations (9%). These results are in line with previous studies on oak species, such as *Q. brantii* (Alikhani et al., 2014), *Q. robur* (Burczyk et al., 2018), and *Q. petraea* (Rebrean et al., 2023). The significant genetic variability observed in intra-populations (90%) can be due to various reasons, including high levels of natural hybridization and long-life spans of oak species (Petit et al., 2004; Khadivi-Khub et al., 2015). Based on the UPGMA dendrogram, individuals were partitioned into six distinct clades, and according to the UPGMA and PCA patterns, geographical proximity has not played an important role in the formation of the genetic structure of these populations.

The application of Start Codon Targeted (SCoT) markers in this study demonstrated robustness in evaluating the genetic diversity of *Q. petraea* populations within the Arasbaran forest. The substantial level of polymorphism detected across these populations indicates that SCoT markers are efficacious for quantifying genetic variability just for intra-population. These results corroborate previous findings in woody plant research, reinforcing the utility of SCoT markers for genetic diversity assessments.

5. Conclusions

Elucidating patterns of genetic variation is essential for guiding biodiversity conservation initiatives. Future investigations incorporating long-term monitoring and interdisciplinary approaches could provide deeper insights into the genetic dynamics of this species, thereby facilitating sustainable conservation practices. This research has provided initial insight into the genetic diversity within and between different populations of the Arasbaran white oak species. As a result, it is suggested that by studying other markers, useful information for conservation and management measures for this valuable species can be collected.

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تنوع ژنتیکی گونه بلوط سفید (*Quercus petraea* Liebl.) در شمال غرب ایران بر اساس نشانگرهای SCoT

یوسف محمدی[1*]، شیوا قیطران‌پور سهریق[2] و سجاد قنبری[3]

[1]استادیار، موسسه تحقیقات جنگلها و مراتع کشور، سازمات تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران
[2]دکترا، دانشکده کشاورزی، دانشگاه تبریز، ایران
[3]استاد، دانشکده کشاورزی و منابع طبیعی اهر، دانشگاه تبریز، ایران

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چکیده

جنگل ارسباران به دلیل تنوع گونه‌ای بالا، یکی از نقاط داغ ژنتیکی زیست‌کره است و گونه بلوط سفید نقش ویژه‌ای در ترکیب و تنوع عناصر زیستی جنگل‌های ارسباران ایفا می‌کند. در این راستا، ارزیابی تنوع ژنتیکی محاوط بذرگیری شناسایی شده بلوط سفید ضروری و مهم است. در این تحقیق، تنوع ژنتیکی درون و بین هفت جمعیت مختلف بلوط سفید شامل ۴۹ درخت مختلف، با استفاده از نشانگر مولکولی SCoT بررسی شد. در مجموع ۱۲۹ آلل برای این نشانگرها مشاهده شد و ۱۰۰ درصد آلل‌های تولید شده، چندشکل بودند. تعداد آلل‌ها از ۱۶ تا ۲۹ متغیر بود که نشانگر SCoT41 با ۲۹ آلل بیشترین تعداد و نشانگر SCoT7 با ۱۶ آلل کمترین تعداد را داشتند. تجزیه واریانس مولکولی (AMOVA) نشان داد که ۹۰ درصد از تنوع ژنتیکی مشاهده شده در درون جمعیت‌ها و ۱۰ درصد در بین جمعیت‌ها توزیع شده است، که نشان می‌دهد تنوع ژنتیکی در درون جمعیت‌ها قابل توجه‌تر از بین جمعیت‌ها است. بر اساس دندروگرام UPGMA، افراد به شش کلاد مجزا تقسیم شدند و طبق الگوهای UPGMA و PCA، نزدیکی جغرافیایی نقش مهمی در شکل‌گیری ساختار ژنتیکی این جمعیت‌ها نداشته است. این تحقیق بینش اولیه‌ای در مورد تنوع ژنتیکی درون و بین جمعیت‌های مختلف گونه بلوط سفید ارسباران ارائه داده است. در نتیجه، پیشنهاد می‌شود با مطالعه سایر نشانگرها، اطلاعات مفیدی برای اقدامات حفاظتی و مدیریتی این گونه ارزشمند جمع‌آوری شود.

واژه‌های کلیدی: ارسباران، بلوط سفید، نشانگرهای مولکولی، GenALEx.

[*] نویسنده مسئول
شماره تماس: ۰۲۱۴۴۷۸۷۲۸۲
ایمیل: mohammadi.yousef63@gmail.com