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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Phylogenetic Analysis of *Pistacia* Species with *rbcL* Chloroplast Gene Region

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Abstract
The phylogeny of five species in the genus *Pistacia* was assessed using the plastid *rbcL* gene region. Total genomic DNA was isolated from plant leaf and polymerase chain reaction (PCR) with primer of *rbcL* gene region and DNA sequencing were performed. Using obtained sequence data, phylogenetic and PCoA (Principle Component Analysis) analyses were performed for reveal the phylogenetic relationships among *Pistacia* species. According to the result of phylogenetic analysis, closer grouping in clades was observed between *P. vera*; *P. khinjuk* and *P. atlantica*; between *P. palaestina* and *P. terebinthus*. Result of PCoA analysis supported the result of phylogenetic analysis. As a result, *rbcL* gene region was found powerful at species-based grouping. Revealed sequence information of chloroplast gene region is reliable to elaborate a molecular database to conduct breeding programs on local pistachio gene pool.
Keywords: *Pistacia*, chloroplast gene, *rbcL*, phylogeny.

Öz
Pistacia cinsindeki beş türün filogenisi, plastid *rbcL* gen bölgesi kullanılarak değerlendirilmiştir. Bitki yaprağından tüm genomik DNA izole edilmiş ve *rbcL* gen bölgesi primeriyle polimeraz zincir reaksiyonu (PZR) ve DNA dizilemesi yapılmıştır. Elde edilen dizi verileri kullanılarak *Pistacia* türleri arasındaki filogenetik ilişkileri ortaya çıkarmak için filogenetik ve PCoA (Principle Component Analysis) analizleri gerçekleştirilmiştir. Filogenetik analiz sonucuna göre, *P. vera, P. khinjuk* ve *P. atlantica* arasında; *P. palaestina* ve *P. terebinthus* arasında dallarda daha yakın gruplaşma gözlenmiştir. PCoA sonuçları filogenetik analiz sonucunu desteklemiştir. Sonuç olarak, *rbcL* gen bölgesi tür bazlı gruplandırmada güçlü bulunmuştur. Kloroplast gen bölgesi dizi bilgisi, yerel fıstık gen havuzu üzerinde yürütülecek ıslah programları kapsamında hazırlanacak moleküler bir veri tabanı için güvenilirdir.
Anahtar Kelimeler: *Pistacia*, kloroplast geni, *rbcL*, filogeni.

I. INTRODUCTION

Pistacia is a genus of aromatic trees of the cashew family that belongs to the Anacardiaceae family, which includes approximately 70 genera and 600 species. It contains pistachio (*Pistacia vera*) and several therapeutic plants. Pistachio's economic value stems from its status as one of the world's most popular nuts. Furthermore, pistachio has significant oil content, accounting for around 50-62 % of its weight. Other species and sub-species that produce smaller nuts and are mostly utilized as rootstocks or in oil, agroforestry, lumber production, and carpentry include; *P. integerrima, P. kinjuk, P. atlantica, P. lentiscus, P. cabulica, P. chinensis, P. terebinthus, P. mutica P. falcata, P. kurdica, P. palaestina* [1].

Regardless of their manner of conservation, cultivars and accessions require accurate identification. Conventional techniques of identification and characterisation might be problematic at times. Current cultivar identification and breeding programs use molecular approaches since morphological markers are time-and environment-consuming. Molecular markers provide significant benefits in breeding and reliable selection under various environmental conditions, and they also speed up the process of breeding by allowing elite varieties to be selected by identifying specific genes/genotypes throughout populations that have crossed [2, 3]. DNA markers are powerful tools for estimating and evaluating genetic diversity, DNA fingerprinting, evolutionary relations within and across cultivars, and better selection performance in plant breeding by marker-assisted selection for complex characteristics [4].

The phylogeny of the genus *Pistacia* has rarely been studied, with most studies relying on morphological analysis. The first molecular characterization study of pistachio (*P. vera*) was done by Hormaza et al. [5]. Researchers determined the relationship between 15 pistachio varieties using the RAPD technique. Using cpDNA sequence

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data, Parfitt and Badenes [6] categorized *Pistacia* species at the molecular level and divided the genus into Lentiscus and Terebinthus. Then, Kafkas and Perl-Treves [7] morphologically and molecularly defined *Pistacia* species in Türkiye, and the researchers revealed the misidentification of *P. khinjuk* Stocks as *P. eurycarpa*, which had already been reported by Yaltirik [8]. In another research, Kafkas and Perl-Treves [9] used RAPD markers to classify nine *Pistacia* species, determining that *P. palaestina* Boiss. is a subspecies of *P. terebinthus* L. and dividing the genus into two sections. At the molecular level, Werner et al. [10] characterized *P. saportae* Burnat. as a hybrid between *P. terebinthus* and *P. lentiscus*. *Pistacia* species were identified in Israel and Greece using AFLP and RAPD markers, respectively, by Katsiotis et al. [11] and Golan-Goldhirsh et al. [12].

Molecular systematics can benefit from the identification of polymorphic loci in chloroplast genomes [13]. The chloroplast genes *matK, ndhF, atpB, atpB-rbcL, rbcL, rps4-trnS, rpl16, rps16, trnS-G, trnL-F, trnH-psbA,* etc are now used for phylogenetic studies at several taxonomic levels within organelle-based markers [14]. These chloroplast genes can be used as potential plant DNA barcoding markers [15]. The key DNA barcodes for reliable determination of plants are *matK* and *rbcL*, according to the Consortium for the Barcode of Life (CBOL) [16]. The *rbcL* gene has the advantages of being easy to amplification, sequencing and alignment in many land plants, and being a useful DNA barcoding region for plants at the family and genus level [17, 18, 19, 20]. The aim of this study is to identify *Pistacia* species using *rbcL* chloroplast gene region and to reveal their relationships.

II. MATERIAL AND METHOD

2.1. Plant material

Five *Pistacia* species of which each species contains two specimens (**Table 1**) from Pistachio Research Institute (Gaziantep, Turkey) were used as plant material. *Anacardium excelsum* (Accession no: JQ590132.1) from Anacardiaceae family was used as an outgroup.

Table 1. *Pistacia* species used in this study.

No	Species
1	*Pistacia terebinthus* 1
2	*Pistacia terebinthus* 2
3	*Pistacia vera* 1
4	*Pistacia vera* 2
5	*Pistacia palaestina* 1
6	*Pistacia palaestina* 2
7	*Pistacia khinjuk* 1
8	*Pistacia khinjuk* 2
9	*Pistacia atlantica* 1
10	*Pistacia atlantica* 2

2.2. Genomic DNA Isolation and PCR Amplification

The Doyle and Doyle technique [21] which is modified by Kafkas et al. [22] was used to isolate total genomic DNA using *Pistacia* young leaf tissues. The Qubit 2.0 fluorometer was used to determine the concentration of isolated DNA using the Qubit dsDNA BR Assay Kit.

rbcL gene region of chloroplast genome was amplified using universal primer with accession number and sequence information given in **Table 2**.

Table 2. Accession number, primer sequence and reference of the *rbcL* gene used in the study.

Gene	Accession Number	Sequence (5'-'3)	Reference
rbcL	AF275990	**F:** gTggACTgATggACTTACC **R:** CgATgAATgTgAAgAAgTAgg	[23]

PCR amplification reactions were performed using 1×PCR buffer, 2.5 mM $MgCl_2$, 0.025 U Taq DNA polymerase, 0.2 mM dNTPs, 0.4 µM forward and reverse primers, and 4 ng of genomic DNA in final volume of 25 µl reactions. Amplification was performed as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 1 min., 60 °C for 1 min. and 72°C for 1 min, followed by an elongation step for 72°C for 10 min.

2.3. Sequence Analysis

For DNA sequencing of *rbcL* gene region, PCR products were purified with the ChargeSwitch-Pro PCR Clean-up Purification Kit. After the quantitative measurement of PCR products by Qubit 2.0 fluorometer, cycle sequencing reactions were performed with ABI BigDye Terminator v3.1 Cycle Sequencing Kit. The cycle sequenced products were cleaned with Applied Biosystems BigDye XTerminator Purification Kit and purified PCR products were sequenced with ABI 310 Genetic Analyzer. The results were carefully analysed by comparing the electropherograms and the sequence data.

2.4. Data Analysis

The UPGMA (Unweighted pair-group method of arithmetic average) approach was used to do phylogenetic analysis based on DNA sequence data. Following the bootstrap test (100 replicates), the percentages of replicate trees in which the related taxa grouped together can be seen next to the branches). The Kimura 2-parameter method was used to calculate the evolutionary distances with MEGA11 software. Genetic diversity assessment was carried out by Principal Coordinates Analysis (PCoA). Using MEGA11 software, the specimens were pairwise aligned and pairwise distance was computed based Kimura 2-parameter model. Finally, GenAlEx 6.4 program was run to evaluate genetic distance.

III. RESULT AND DISCUSSION

rbcL chloroplast gene region was used to discover polymorphisms for five *Pistacia* species. DNA isolation, PCR amplification and sequencing of *rbcL* gene region with three replicates were performed successfully for each ten specimens. PCR products was found about 448-604 bp for all the studied specimens. The sequences of ten *Pistacia* specimens were given in **Table 3**. The genetic relationships among *Pistacia* species were revealed after data analysis with phylogenetic tree and PCoA.

Table 3. DNA sequences of 10 *Pistacia* specimens obtained from the rbcL barcode gene region.

>*P.khinjuk*_1
AGGAACTCCCAACTCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCATGTACCTGCAGTAG CATTCAAGTAATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAA ATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAG TCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTT AGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGA TCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGT AGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAG AACCCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATAACATATATATTGACTTTTCTTCTCAGC AACGGGCTCA
> *P.khinjuk*_2
AGGAACTCCCAACTCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCATGTACCTGCAGTAG CATTCAAGTAATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAA ATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAG TCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTT AGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGA TCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGT AGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAG AACCCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATAACATATATATTGACTTTTCTTCTCAGC AACGGGCTCA

>*P.terebinthus*_1
TCATGCATTACGATAGGAACTCCCAACTCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCAT GTACCTGCAGTAGCATTCAAGTATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTG CTTCCGCACAAAATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCA TCTTTGGTAAAGTCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGA TAAACCTAATTTAGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTC TCTCAACTTGGATCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGA TCCTCTAGACGTAGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACA TGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATACACATATATTGATTTT CTTCTCCAGCAACGGGCTCA
>*P.terebinthus*_2
TCATGCATTACGATAGGAACTCCCAACTCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCAT GTACCTGCAGTAGCATTCAAGTATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTG CTTCCGCACAAAATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCA TCTTTGGTAAAGTCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGA TAAACCTAATTTAGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTC TCTCAACTTGGATCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGA TCCTCTAGACGTAGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACA TGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATACACATATATTGATTTT CTTCTCCAGCAACGGGCTCA
>*P.atlantica*_1
CCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAAATAGGAAACGGTC TCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAGTCAAGTCCACCAC GTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTTAGGTTTAATAGTA CATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGATCCCATGTGGTGG TCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGTAGAGCGCGCAGG GCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAA AAAGGTCTAAAGGGTAAGCTACATAACATATATATTGATTTTTCTTCTCAGCAACGGGCT
> *P.atlantica*_2
CCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAAATAGGAAACGGTC TCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAGTCAAGTCCACCAC GTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTTAGGTTTAATAGTA CATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGATCCCATGTGGTGG TCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGTAGAGCGCGCAGG GCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAA AAAGGTCTAAAGGGTAAGCTACATAACATATATATTGATTTTTCTTCTCAGCAACGGGCT
>*P.vera*_1
GCCAGCTAGTATTTGCGGTAAATCCCCCTGTTAAGTAGTCATGCATTACGCTAGGAACTCCCAAC TCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCATGTACCTGCAGTAGCATTCAAGTAATGA CCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAAATAGGAAACGGTC TCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAGTCAAGTCCACCAC GTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTTAGGTTTAATAGTA CATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGATCCCATGTGGTGG TCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGTAGAGCGCGCAGG GCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCCTTCTTCA AAAAGGTCTAAAGGGTAAGCTACATAACATATATATTGACTTTTCTTCTCAGCAACGGGCTCAAT GTTGTAGCATCGTCCTTTGTA
>*P.vera*_2
GCCAGCTAGTATTTGCGGTAAATCCCCCTGTTAAGTAGTCATGCATTACGCTAGGAACTCCCAAC TCTCTTGCAAATACAGCCCTTTTTAGCATTTCTTCGCATGTACCTGCAGTAGCATTCAAGTAATGA CCTTTAATTTCACCTGTTTCAGCCTGCGCTTTATAAATTGCTTCCGCACAAAATAGGAAACGGTC TCTCCAACGCATAAATGGTTGGGAGTTCACGTTCTCATCATCTTTGGTAAAGTCAAGTCCACCAC GTAGACATTCATAAACAGCTCTACCGTAGTTCTTAGCGGATAAACCTAATTTAGGTTTAATAGTA CATCCCAATAGGGGACGTCCATACTTGTTCAATTTATCTCTCTCAACTTGGATCCCATGTGGTGG TCCTTGGAAAGTTTTTGTATACGCGGTAGGGATTCGTAGATCCTCTAGACGTAGAGCGCGCAGG GCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCCTTCTTCA AAAAGGTCTAAAGGGTAAGCTACATAACATATATATTGACTTTTCTTCTCAGCAACGGGCTCAAT GTTGTAGCATCGTCCTTTGTA
> *P.palaestina*_1

TTCCCATGTACCTGCAGTAGCATTCAAGTATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTA TAAATTGCTTCCGCACAAAATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGT TCTCATCATCTTTGGTAAAGTCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTC TTAGCGGATAAACCTAATTTAGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAA TTTATCTCTCTCAACTTGGATCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGAT TCGTAGATCCTCTAGACGTAGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAA GTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATACATATATA TTGATTTTCTTCTCCAGCAACGGGCTCA
>*P.palaestina*_2
TTCCCATGTACCTGCAGTAGCATTCAAGTATGACCTTTAATTTCACCTGTTTCAGCCTGCGCTTTA TAAATTGCTTCCGCACAAAATAGGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACGT TCTCATCATCTTTGGTAAAGTCAAGTCCACCACGTAGACATTCATAAACAGCTCTACCGTAGTTC TTAGCGGATAAACCTAATTTAGGTTTAATAGTACATCCCAATAGGGGACGTCCATACTTGTTCAA TTTATCTCTCTCAACTTGGATCCCATGTGGTGGTCCTTGGAAAGTTTTTGTATACGCGGTAGGGAT TCGTAGATCCTCTAGACGTAGAGCGCGCAGGGCTTTGAACCCAAATACATTACCCACAATGGAA GTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAAGGGTAAGCTACATACATATATA TTGATTTTCTTCTCCAGCAACGGGCTCA

UPGMA algorithm was used to analyse *rbcL* region sequences, which included 10 in-groups and 1 outgroup taxa. With a difference in bootstrapping value, the UPGMA tree topology revealed that the species are closely connected to each other. In UPGMA tree, it was observed that the *Anacardium excelsum* used as an outgroup was the first to be separated. The ingroup consists of two main clades. The first main clade is divided into two subclades, the first subclade included two species namely *P. vera* and *P. khinjuk*; the second subclade included *P. atlantica*. The second main clade is also divided into two subclades, consisting of the first subclade *P. terebinthus*; the second subclade *P. palaestina* **(Figure 1)**. According to the UPGMA tree, *P. khinjuk*, *P. vera* and *P. atlantica; P. palaestina* and *P. terebinthus* were closely related to each other**.**

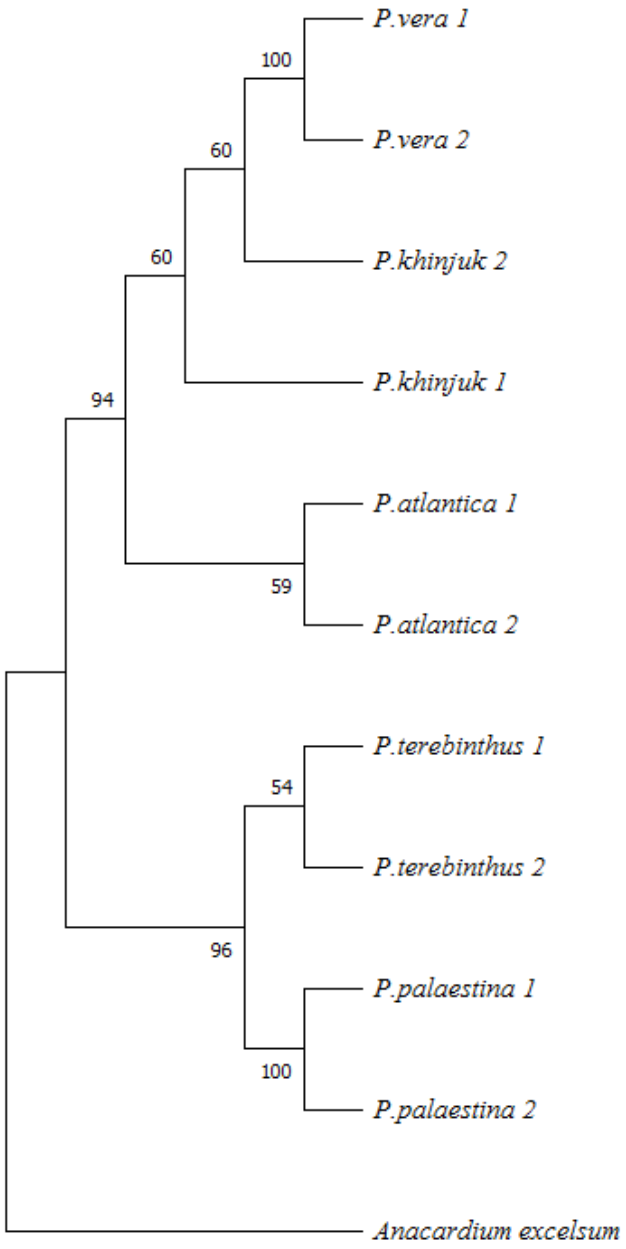

Figure 1. Phylogenetic tree of 10 *Pistacia* species with *rbcL* gene region based on UPGMA method (*Anacardium excelsum* an outgroup).

The PCoA plot derived from the the *rbcL* chloroplast gene region data showing the distribution of the *Pistacia* species belonging to their distance from themselves at the spatial level was given in **Figure 2**. PCoA results showed that percentage of variation explained by the first 3 axes as 96.98, 2.62 and 0.22%, respectively. When PCoA results were evaluated, the 10 specimens were divided into two clusters in the PCoA analysis. The cluster I consisted of *P. palaestina* and *P. terebinthus* species. The cluster II consisted of *P. vera*, P. *khinjuk* and *P. atlantica*. The phylogenetic tree and PCoA data show that, while intraspecific relationships were validated, interspecies genetic distances were also revealed.

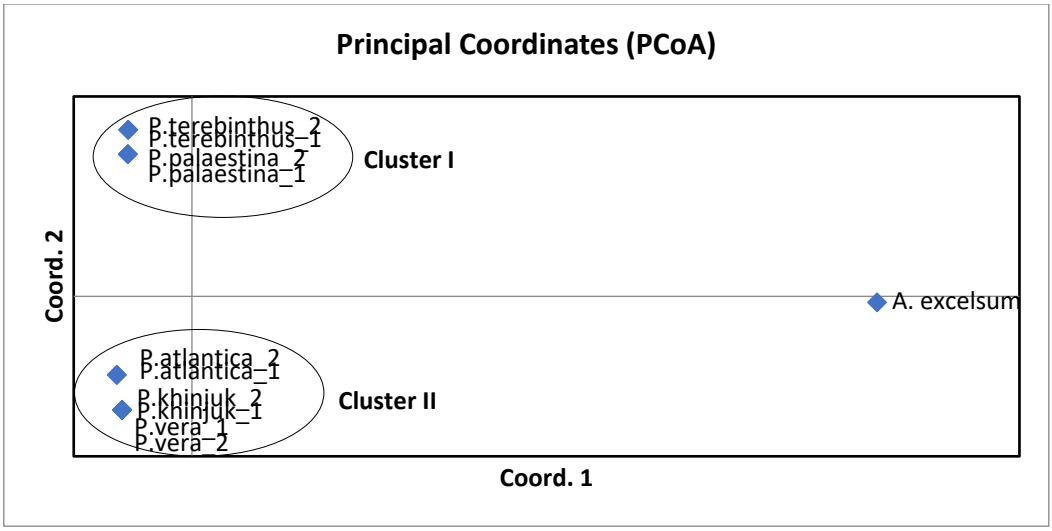

Figure 2. PCoA results of 10 *Pistacia* specimens with GenAlEx 6.4 program (*Anacardium excelsum* an outgroup).

Arabnezhad et al. [24] created two DNA libraries by enriching the dinucleotide (AG) with trinucleotide (ATG) from the *P. khinjuk* genome in order to evaluate the genetic relationships of wild and cultivated *Pistacia* species grown in Iran. A total of 27 SSR primer pairs were designed from the repetitive regions. Then, interspecies phylogenetic analysis was performed with these primers. According to the results obtained, *P. khinjuk* was found to be the closest species to *P. vera*. Talebi et al. [25] used the SRAP marker technique to characterize 36 pistachio genotypes and varieties originating from Türkiye, Iran, Syria and the USA. 30 SRAP primer pairs were tested. 11 primer pairs produced a total of 202 bands, of which 168 (83%) were found to be polymorphic. The closest species to *P. vera* were determined as *P. atlantica* and *P. khinjuk*, respectively. Yi et al. [26] used the nuclear ribosomal *ITS*, the nuclear nitrate reductase gene's third intron (*NIA-i3*), the plastid *ndhF*, *trnL-F*, and *trnC-trnD* sequences to analyse the phylogeny of *Pistacia*. They also reported that *P. terebinthus* and *P. palaestina*, as well as *P. khinjuk*, *P. atlantica* and *P. vera* share a close genetic relationship. Talebi et al. [27] conducted molecular characterization studies on 17 cultivated and wild pistachio genotypes using 4 different gene regions of cpDNA (*atpB-rbcl*, *trnCpetN*, *psbM-trnD* and *petN-psbM*) in their research. According to the results of their phylogenetic analysis with the UPGMA method, cultivated pistachio species and *P. vera* var. sarakhs have a common ancestor and *P. vera* was collected in a separate group. Also they showed that *P. palaestina* and *P. terebinthus* species were grouped together and reported that genomic chloroplast could accurately determine pistachio interspecies relations.

The results of this study's phylogenetic tree and PCoA analysis supported each other and were consistent with the molecular literatures researching into the relationships between various pistachio species. In sight of all of this data, the relationship between *P. khinjuk*, *P. vera*, and *P. atlantica;* between *P. terebinthus* and *P. palaestina* were shown in this study using *rbcl* chloroplast gene region sequence data. As a result of this study, it can be said that the *rbcl* chloroplast gene region is successful in describing the *Pistacia* species and showing the relationship of the species with each other.

IV. CONCLUSION

In this study, 5 *Pistacia* species (*P. vera*, *P. terebinthus*, *P. khinjuk*, *P. palaestina*, *P. atlantica*) were classified *rbcL* chloroplast gene region. The findings suggest that the species share a similar genetic sequence and, as a result, may have a tendency to follow the same evolutionary route. As a result of this research, it appears that the *rbcL* gene might be a useful marker for distinguishing *Pistacia* species and determining their evolutionary relationships. The identification and classification of *Pistacia* species are critical for the establishment of a systematic database. Also, molecular data and phylogenetic relationships seem to be very useful in facilitating classical breeding methods. Information provided on the cultivar relationships profile is significant since it may be utilized as a source of knowledge for effective hybridisation and the development of new cultivars in the future. The investigation of genetic diversity and relationships among the *Pistacia* species used in this study is required to highlight the priority for plant genetic resource conservation programs. Future studies can contribute to the studies of *Pistacia* species by obtaining more comprehensive findings with more and different combinations of barcode gene regions and more samples.

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