Ann. For. Res. 67(1): 19-30, 2024
https://doi.org/10.15287/afr.2024.2573

ANNALS OF FOREST RESEARCH
www.afrjournal.org

Genetic diversity and population structuring of *Pistacia lentiscus* L. across Mediterranean region

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Mezni F., Roig A., Scotti-Saintagne C., Hamrouni L., Fady B., Khaldi A., 2024. Genetic diversity and population structuring of *Pistacia lentiscus* L. across Mediterranean region Ann. For. Res. 67(1): 19-30.

Abstract *Pistacia lentiscus* L., the mastic tree, is an evergreen shrub distributed across the entire Mediterranean Basin. This species, known for millennia for its medicinal, food and cosmetic value is currently threatened by overexploitation and climate change. To help prioritize conservation actions, we studied its genetic diversity using seventy four Mediterranean accessions and seven microsatellite loci. Genomic DNA was extracted from mastic tree leaves. Allelic frequency and population structure estimates were calculated as well as the relationship between studied populations. The observed heterozygosity (*Ho*) and expected heterozygosity (*He*) ranged from 0.493 to 0.788 and 0.518 to 0.781 respectively. Shannon's Information Index (*I*) ranged from 0.858 to 1.819 with a mean value of 1.420. The mean fixation index (*Fst*) value was estimated to 0.124. AMOVA analysis showed that only 7% of the variance existed among populations. In addition, the STRUCTURE analysis showed a clear distinction between the eastern and the western Mediterranean populations when the number of clusters (K) was set at 2. The study of genetic diversity of the Mediterranean *P. lentiscus* is of interest for conservation of genetic resources and it contributed to the understanding of the evolutionary history of this species.

Keywords: mastic tree, genetic resources, microsatellites, Mediterranean populations.

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Manuscript: received September 13, 2022; revised March 27, 2024; accepted April 09, 2024.

Introduction

The genus *Pistacia* of the family *Anacardiaceae* includes eleven species. The most well-known species are *Pistacia vera* L., the pistachio, recognized by its edible seeds; *Pistacia terebinthus* L., source of terebinth resin; *Pistacia chinensis* L., an ornamental tree from China and *Pistacia lentiscus* L., source of essential oil, mastic and edible oil.

Pistacia lentiscus L., mastic tree, is an evergreen shrub distributed across the

Mediterranean Basin. This species is known for its resin (the mastic) used in medicine, cooking and as a cosmetic. In some countries, the mastic tree is also known for its essential oil extracted from leaves and for its fixed oil extracted from fruits. The mastic tree is currently threatened by human exploitation for medicinal and ornamental purposes and by habitat destruction (Mezghani 1992). Exploitation of fruits and twigs is continuously increasing which requires appropriate conservation measures and sustainable management programs yet to be developed.

To maintain and improve this species and its genetic resources, there is a need to increase knowledge on its genetic diversity and understand how it is structured across its geographical range. There are only a few molecular studies that address genetic and taxonomic relationships of *Pistacia* species (Parfitt & Badenes 1997, Kafkas & Perl-Treves 2001, Kafkas et al. 2006a, b; Yi et al. 2008) and on the screening of sex markers (Hormaza et al. 1994, Verdu & Fayos 1998, Kafkas et al. 2001).

Previous studies, which have reported information on the diversity of *P. lentiscus*, were based on biochemical and morphological variations and on dominant random molecular markers (Barazani et al. 2003, Golan-Goldhirsh et al. 2004, Al Saghir & Porter 2006).

These studies revealed the phylogenetic and taxonomic relationships among *Pistacia* species and among Mediterranean populations of *P. lentiscus.* According to these findings, *P. lentiscus* from Tunisia showed a high similarity values with Spanish one indicating close relations between these two accessions.

To our knowledge, the genetic variation and differentiation of *P. lentiscus* has not yet been investigated range-wide using Mendelian, bi-parentally inherited markers. In the present study, using nuclear microsatellites, we investigated genetic diversity and differentiation in a large and representative portion of the geographic range of *P. lentiscus*, in order to shed light on the evolutionary history of this species understand whether current distribution has affected genetic variability and genetic structure of Mediterranean populations. Such a study could provide new and relevant information about the evolutionary history of this species.

Materials and Methods

Plant material

Individual samples were randomly collected from 74 accessions *P. lentiscus* representing the genetic material from 10 geographic regions (Table 1). The highest number of accessions in the collection was from Tunisia (44), followed by those from Algeria (11), Italy (5), Greece (4), Portugal (3), Spain and Lebanon (2) and France, Malta and Croatia (1) (Table 1).

Table 1 Geographic characterization of the 74 Mediterranean sites of *P. lentiscus* investigated in this study.

Population	Code	Country	Latitude	Longitude	Altitude (m)
Ain-Ben-Brahim	Ain Ben	Algeria	36°12'57"	0°26'52"	48
Ait-Essad	Ait Es	Algeria	36°39'25.9"	4°27'14.48"	523
Beni_Talla	Beni Tall	Algeria	35°11'5.11"	0°36'55.46"	552
Bouhanifa	Bouha	Algeria	35°18′ 58"	0°02′54"	237
Foret-Bainem 1	Foret B1	Algeria	36°48'05.36"	2°58'30.20"	247
Foret-Bainem 2	Foret B2	Algeria	36°48'05.36"	2°58'30.20"	247
Foret-Bainem 3	Foret B3	Algeria	36°48'05.36"	2°58'30.20"	247
Gouraya	Gour	Algeria	36°46'10.49"	5°5'3.75"	526
Hendou	Hendou	Algeria	36°45'35.42"	4°22'20.4"	342
Maascar	Maascar	Algeria	35°24'0.6"	0°8'23.46"	616
Zfisef	Zfisef	Algeria	35°33'16"	5°59'21"	1756
Murter-Island	Murter I	Croatia	43°49'5.39"	15°34'40.07"	25
Bormes_Le Trapan	Trap	France	43°08'02.89"	6°17'41.82"	77
Amaliada Fragapidima	Amal	Greece	38°15'39.85"	21°40'9.74"	60
Leonidion	Leonid	Greece	37°11'26.93"	23°29'8.96"	41
Previli_Crete	Previli	Greece	35°15'0"	24°47'0"	100

Population	Code	Country	Latitude	Longitude	Altitude (m)
Tatoi	Tatoi	Greece	38°16'26.36"	24°19'0.58"	243
Duna-Feniglia	Fenig	Italy	42°25'0"	11°17'0"	3
Punta-Rossa	Punta Ro	Italy	41°13'45.24"	13°2'41.19"	43
Sardaigne_Monte-Nieddu	Sardaigne Mon	Italy	39°3'34.2"	8°57'56.05"	146
Sardaigne_Torre-dei-corsari	Sardaigne Torre	Italy	39°19'22.5"	9°10'50.54"	231
Sardaigne_Porto-Columbu	Sardaigne Porto	Italy	39°1'37.81"	9°1'33.20"	16
Hamet	Hamet	Lebanon	34°17'9.39"	35°41'37.48"	202
Seydet_el_Nourieh	Seydet	Lebanon	34°18'18,09'	35°41'42,22'	160
Buskett	Busket	Malta	35°51'39.7"	14°23'57.21"	217
Amora	Amora	Portugal	38°37'42.03"	9°6'52.91"	26
PN_Arabida	PN Arab	Portugal	41°8'59.88"	8°36'36.87"	135
Sesimbra	Sesimbra	Portugal	38°26'39.17"	9°6'5.36"	21
Aznalcazar	Azn	Spain	37°18'16.96"	6°14'58.35"	66
Utrera	Utrera	Spain	37°11'5.33"	5°46'46.28"	51
Ain-Cristal	AC	Tunisia	35°55'21.47"	9°28'25.55"	489
Ain-Drahem	A Drah	Tunisia	36°42'36.8"	8°40'6.77"	597,64
Ain-El-Baya	A Baya	Tunisia	36°41'35.96"	8°40'33.25"	376
Ain-Snoussi	A Sn	Tunisia	36°49'22.52"	8°54'56.45"	496
Azib	Azib	Tunisia	37°12'35.7"	9°58'36.44"	39
Bellif	Bellif	Tunisia	37°2'8.4"	9°5'23.35"	55
Beni-Darraj	B Dar	Tunisia	36°23'49.2"	10°16'20.9"	109
Bouchoucha	Bouch	Tunisia	36°30'58.63"	10°26'27.52"	220
Boujelida	Boujel	Tunisia	36°24'53.81"	9°30'17.22"	212
Chaambi	Chaambi	Tunisia	35°11' 2.05"	8°40'3.12"	1517
Dmayen	Dmayen	Tunisia	37°3'28.65"	9°0'38.63"	277
Errtiba	Errtiba	Tunisia	36°52'15.87"	10°44'35.94"	50
Feija	Feija	Tunisia	36°28'48.19"	8°19'45.24"	545
Gamboura	Gamb	Tunisia	35°53'43"	9°32'33"	522
Henchir-Enaam	Hen Naa	Tunisia	36°8'23.12"	9°4'2.61"	511
Jbel-abderrahmen	J Abder	Tunisia	36°46'60"	10°43'60"	218
Jbel-Bou-Kehil	J Bouk	Tunisia	36°18'30.43"	9°7'10.83"	551
Jbel-Chedid	J Chehid	Tunisia	36°22'37.54"	9°19'23.97"	707
Jbel-Mansour	J Man	Tunisia	36°14'56.25"	9°47'27.76"	378
Jbel-Mansour2	J Man 2	Tunisia	36°14'56.25"	9°42'33.5"	550
Jbel-Mghila	J Mghila	Tunisia	35°28'8.16"	9°13'18.10"	849
Jbel-Orbata	J Orbata	Tunisia	34°26'2.09"	9°14'48.6"	1107
Jbel-Troza	J Troza	Tunisia	35°31'36.14"	9°34'46.27"	422
Kbouch	Kbouch	Tunisia	36°12'38.21"	8°53'17.19"	699
Kef-Erraai	Kef Err	Tunisia	37°10'48.03"	9°18'26.55"	112
Khorgalia	Khorgalia	Tunisia	37°3'46.12"	9°2'53.57"	74
Ksar-Lamsa	Ksar Lam	Tunisia	36°2'9.54"	9°41'33.06"	445
Majen-Essef	Majen Es	Tunisia	36°46'47.35"	8°45'50.48"	564
Marouhia	Marouh	Tunisia	36°16'26.65"	8°47'42.27"	606
Morneg	Morneg	Tunisia	36°30'50.09"	10°18'46.04"	174
Nahli	Nahli	Tunisia	36°53'3.64"	10°9'29.14"	55
Neber	Neber	Tunisia	36°19'43.08"	8°46'53.9"	424
Oued-El-Maaden	O Maaden	Tunisia	36°5'34.79"	9°6'43.63"	102
Oued-Ezzena	O Ezzena	Tunisia	37°11'23.52"	9°24'48.42"	143
Oueslatia	Oueslatia	Tunisia	35°51'11.72"	9°35'31.84"	475
Sidi-Amor	Sidi Amor	Tunisia	36°55'50.18"	10°9'52.39"	67
Sidi-Awidet	Sidi Awidet	Tunisia	36°13'34.56"	9°44'52.06"	514
Sidi-Zid	Sidi Zid	Tunisia	36°44'48.41"	10°19'9"	676
Sidi-Zid-BV	Sidi Zid BV	Tunisia	36°29'39.31"	10°18'13.65"	259
Tabarka	Tabarka	Tunisia	36°57'0"	8°46'0"	200
Takelsa	Takelsa	Tunisia	36°50'12.62"	10°41'15.14"	144
Teskreya	Teskreya	Tunisia	37°12'19.99"	9°32'47.28"	3
Zouainia	Zouainia	Tunisia	36°28'7.9"	8°20'52.86"	534
Zougueg	Zougueg	Tunisia	36°52'44.69"	10°48'21.99"	142

DNA extraction and PCR amplification

Genomic DNA was extracted from *P. lentiscus* leaves using the QIAGEN plant DNeasy Mini Kit (Quiagen, Courtabeuf, France) following the manufacturer's instructions.

Seven primer pairs were employed to amplify plastid regions containing short stretches of

simple mononucleotide repeats as described by Albaladejo et al. (Table 2) (2008, Stavridou et al. 2024). PCRs were performed in a final volume of 10 µl using a QIAGEN Multiplex PCR Kit (Qiagen, Courtabeuf, France) as follows: 2 mL diluted DNA, 1 mL Q solution (5x), 1.8 mL RNAse-free water, 5 mL QIAGEN Multiplex PCR Master mix 2x (6 mM $MgCl_2$, HotStarTaq DNA polymerase, dNTP mix), and 0.02 mL of each primer forward and reverse of 2 mM. The PCR program used was: 95°C 15 min, 94°C for 30 s, 57°C for 90 s, 72°C 90 s for 30 cycles, final elongation at 72°C for 10 min and 4°C for 1 min to stop Taq polymerase in an Eppendorf thermocycler. Electrophoresis of PCR products was performed on an ABI 3730XL sequencer as follows: 2 mL of PCR product for each individual was diluted (2 mL PCR product + 100 mL H_2O), combined with 8 mL of 600L12 Size Standard (20 mL GS600 + 900 mL Formamide), denatured at 95°C for 3 minutes and then injected into the sequencer. The microsatellite profiles were analysed in GeneMapper software version 4.1 (Thermo Fisher Scientific).

Data analysis

Allelic frequency and population structure were estimated using the GenAlEx 6.501 (Peakall & Smouse 2006).The number of different alleles (*Na*), the number of effective alleles (*Ne*), the fixation index (F_{ST}), the observed heterozygosity (*Ho*), the expected heterozygosity (*He*) and Shannon's Information Index (*I*) were calculated. Significance of differentiation estimates was tested by performing 1000 randomizations (genotypes among samples) as implemented also in GenAlEx 6.501 (Peakall & Smouse 2006).

GenAlex program was also used to determine molecular variance analysis (AMOVA) within and among groups and the existence of a correlation between genetic diversity and geographic distance using a Mantel test were also performed by the same program mentioned before.

The genetic structure of the sampled populations was detected using the software STRUCTURE 2.3.4. (Pritchard et al. 2000) with a burn-in of 10,000, run length of 100,000 and a model allowing for admixture and correlated allele frequencies. Five independent runs yielded consistent results. For each value of K, the log likelihood values were averaged and standard deviation calculated. We tried to infer the appropriate number of clusters by calculating the ΔK statistic (Evanno et al. 2005).

STRUCTURE HARVESTER was used to compute and plot the mean and variance of Ln P(D) again the range of K values (Campana et al. 2011).

Table 2 Characteristics of the *Pistacia lentiscus* microsatellites markers used in this study

Locus name	Primer sequence 5'-3'	Repeated motif	Allele size range (bp)	Total number of alleles
Pislen 21	F: GGGAAGTGGGTTAGGAATTA R: GGGTGGTTACAATTAGGTCA	$(CT)_{23}$	245-281	12
Pislen 114	F: GTGACTTTGGTTGGTGTTTT R: CTGCTTTGACTGGATTTGAT	$(GTT)_4GCTGTTGCT$ $(GTT)_5GCTGTTGCT(GTT)_5$	183-207	6
Pislen 333	F: TTTGATAAGAACTCGCTTCC R: TTTCTGCCTTTGCTTTACTC	$(TA)_3(CA)_{21}(TA)_4$	181-217	13
Pislen 501	F: TTCAACTCAACAAATATGCAA R: ATTGTATTGGCGAAACCTAA	$(GA)_{28}$	185-211	9
Pislen 510	F: TGGTGGAGTCTTACTTTGCT R: TGACAATCAATATGCCTTCA	$(AC)_{20}$	216-234	6
Pislen 526	F: CAGTGAGGGTAAAAATGGAA R: ATTACCATTTTGAGGGAACC	$(GGT)_2GCT(GGC)_6$	142-148	3
Pislen R05	F : GGATTTTCCTCTACCATCCT R: GAAAACGAGGTTATTGGTCA	$(CCG)5CTG(CCG)_2$	188–212	3

Results

Mediterranean populations

This investigation assessed the genetic diversity between 74 Mediterranean accessions of *P. lentiscus* L.

In total, 186 alleles were observed at the 7 loci. The total number of alleles per locus varied from 9.311 (Pislen_R05) to 9.878 (Pislen_501) with a global mean of 9.701 alleles per locus.

The mean number of observed alleles (*Na*) and the mean number of effective alleles (*Ne*) were respectively 5.801 and 4.048, the observed heterozygosity (*Ho*) and expected heterozygosity (*He*) ranged from 0.493 to 0.788 and from 0.518 to 0.781 respectively (Table 3).

Shannon's Information Index (I) ranged from 0.858 to 1.819 with a mean value of 1.420.

The Mantel test indicated no correlation was found between the geographical and genetic distance (P=0.530).

Table 3 Genetic diversity of 74 Mediterranean populations of *Pistacia lentiscus* L.

Population	*Na*	*Ne*	*I*	*He*	*Ho*	Population	*Na*	*Ne*	*I*	*He*	*Ho*
Ain Ben Brahim	5.714	3.789	1.383	0.660	0.720	Kef Erraai	6.571	4.845	1.567	0.715	0.683
Ain Cristal	7.000	4.902	1.610	0.733	0.750	Khorgalia	6.286	4.771	1.526	0.715	0.667
Ain Draham	4.571	3.353	1.263	0.659	0.676	Ksar Lamsa	3.000	2.648	0.968	0.571	0.619
Ain Elbeya	5.857	4.031	1.464	0.707	0.788	Leonidion	7.000	4.448	1.626	0.740	0.627
Ain Snoussi	5.857	4.240	1.439	0.674	0.593	Seydet El Nourieh	6.429	4.343	1.520	0.706	0.614
Aït Issad	2.714	2.562	0.873	0.518	0.786	Maascar	5.000	3.254	1.192	0.583	0.535
Amaliada Fragapidima	7.429	4.483	1.605	0.730	0.654	Majen Essef	5.143	3.510	1.299	0.644	0.607
Amora	4.857	3.366	1.277	0.644	0.730	Marouhia	5.143	3.598	1.326	0.655	0.722
Azib	5.714	4.012	1.471	0.713	0.663	Morneg	5.714	3.703	1.407	0.684	0.661
Aznalcazar	7.286	4.459	1.444	0.654	0.693	Murter Island	5.857	3.726	1.389	0.661	0.579
Bellif	5.714	4.480	1.510	0.733	0.681	Nahli	5.000	3.608	1.285	0.633	0.653
Beni Talla	5.000	3.462	1.242	0.613	0.683	Neber	6.143	4.424	1.560	0.744	0.687
Beni Darraj	4.286	3.211	1.191	0.612	0.646	Oued El Maaden	6.429	4.553	1.559	0.728	0.736
Bouchoucha	6.714	5.306	1.642	0.754	0.695	Oued Ezzena	5.714	3.798	1.383	0.670	0.595
Bouhanifa	5.571	3.913	1.333	0.635	0.657	Oueslatia	6.857	4.596	1.564	0.722	0.643
Boujelida	5.429	4.221	1.444	0.711	0.694	Bormes_Le Trapan	7.429	4.891	1.680	0.749	0.688
Buskett	6.857	4.315	1.566	0.723	0.690	PN Arabida	7.143	4.625	1.596	0.731	0.713
Chaambi	2.571	2.290	0.858	0.546	0.671	Previli	5.857	3.372	1.326	0.648	0.554
Dmayen	5.714	4.257	1.515	0.732	0.599	Punta Rossa	8.571	5.743	1.819	0.781	0.671
Errtiba	5.714	4.121	1.445	0.695	0.650	Sardaigne_ Monte-Nieddu	6.571	4.046	1.510	0.702	0.638
Feija	6.571	4.361	1.599	0.752	0.654	Sardaigne_Torre-dei-corsari	6.429	3.368	1.369	0.664	0.615
Duna Feniglia	6.286	3.975	1.455	0.693	0.620	Sardaigne_Porto-Columbu	6.429	3.924	1.430	0.671	0.630
Foret Bainem 1	5.429	3.888	1.325	0.628	0.746	Sesimbra	5.429	4.077	1.425	0.695	0.698
Foret Bainem 2	4.000	3.082	1.123	0.594	0.600	Sidi Zid	5.000	3.457	1.317	0.661	0.625
Foret Bainem 3	4.429	3.417	1.165	0.583	0.493	Sidi Amor	5.429	4.032	1.392	0.687	0.639
Gamboura	6.000	4.065	1.477	0.704	0.644	Sidi Awidet	6.857	5.051	1.611	0.732	0.698
Hamet	6.714	3.829	1.369	0.624	0.544	Sidi Zid BV	5.571	3.871	1.430	0.700	0.677
Henchir Enaam	6.286	4.395	1.512	0.719	0.714	Tatoi	6.143	4.316	1.521	0.720	0.691
Hendou	5.143	3.570	1.314	0.643	0.684	Tabarka	7.000	4.769	1.643	0.749	0.636
Jbel Abderrahmen	4.857	4.245	1.346	0.669	0.657	Takelsa	6.143	5.111	1.611	0.761	0.728
Jbel Boukehil	6.571	3.613	1.443	0.664	0.746	Teskreya	6.571	4.937	1.563	0.724	0.732
Jbel Chedid	6.286	4.151	1.496	0.692	0.719	Utrera	6.857	4.321	1.532	0.705	0.721
Jbel Mansour2	5.714	4.075	1.425	0.682	0.675	Zfizef	4.714	3.420	1.224	0.614	0.516
Jbel Mansour	6.429	4.687	1.560	0.734	0.730	Zouainia	6.571	4.713	1.460	0.656	0.640
Jbel Mghila	4.571	3.265	1.278	0.673	0.700	Zougueg	6.143	5.014	1.557	0.729	0.721
Jbel Orbata	5.286	3.565	1.363	0.676	0.777						
Jbel Troza	4.429	3.672	1.313	0.685	0.700						
Kbouch	6.429	4.764	1.504	0.693	0.684						

At the inter-population level, the mean fixation index (F_{ST}) value was estimated to 0.124. The level of gene flow (*Nm*) was estimated at 3.554.

In addition, AMOVA analysis showed that only 7% of the variance existed among populations and the STRUCTURE analysis showed a clear distinction between the eastern Mediterranean populations and the western Mediterranean populations when the number of clusters (K) was set at 2. This value of K = 2 was identified as the optimal number of clusters by the ΔK method (Fig. 2). Two groups were identified; the first group (green) regrouped the two Lebanese populations (Hamet & Sayedet), the four Greece populations (Amal, Leonid, Previli, Tatoi) and Malta population (Busket) while the second group (red) regrouped the other studied populations. Furthermore, at K = 4, the analysis produced three different groups: the first group (blue) included the Lebanese, the Greece and Malta populations; the second group (green) gathered populations from Portugal, Spain and Croatia and the individuals from Tunisia, Algeria, France and Italia were assigned to an admixed group (Fig. 1)

Tunisian populations

The total number of alleles detected over all Tunisian populations at each locus ranged from 2.857 to 7.0, with an average value of 5.718.

The observed (*Ho*) and expected (*He*) heterozygosities under the HWE ranged from 0.304 to 0.856 and from 0.468 to 0.825, respectively (Table 4). The mean gene diversity (*Ht*) was estimated to 0.765 and the mean expected heterozygocity (*Hs*) was 0.7. Of the 44 populations, only 14 had a negative fixation index while 30 populations had positive *Fis* values.

At the inter-population level, the mean diversity among populations, F_{ST}, was low (0.021) but statistically significant ($p<0.001$). This result was further confirmed by AMOVA analysis among populations, which showed that only 2% of the variance existed among populations. Hence, genetic diversity found

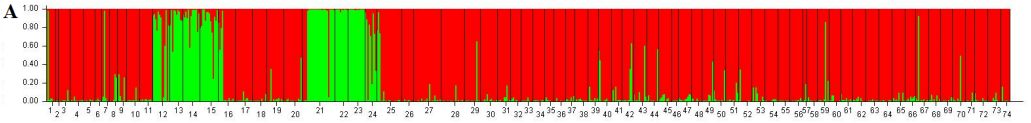

Figure 1 Inference of genetic clusters within the populations of study based on the population assignment analysis implemented using the software Structure. Bar plots in panels A–B show the results of the simulation for given value of K; (A) K = 2. Here, each vertical bar represents an individual, the different colors represent the genetic groups identified by the analysis, and the coloration of each bar indicates the probability with which an individual can be assigned to a particular ancestry. The populations sampled are separated by black vertical lines; Algeria (1-11), Greece (12-15), Italy (16-20), Lebanon (21-22), Malta (23), Portugal (24-26), Spain (27-28), Croatia (29), France (30) and Tunisia (31-74).

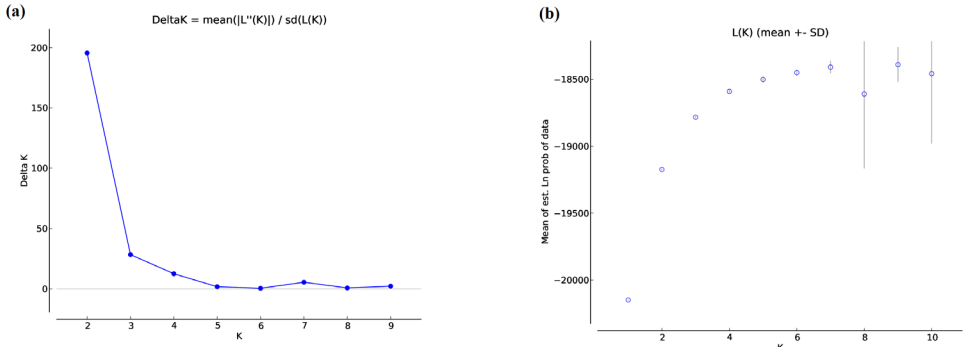

Figure 2 (a) Estimation of Mediterranean populations structure using mean of estimated log probability of data (LnP(K)) with cluster value (K) ranging from 1–10. (b) Estimation of Mediterranean populations using delta K with cluster value (K) ranging from 1–10.

among populations explains a maximum of 2% of the total genetic variance of *P. lentiscus* in Tunisia. Most of the diversity is found within population.

The Mantel test indicated that the geographical distance was not correlated with the genetic distance (r_{cp}=0.139, P=0.07) when using the complete data set.

The results from STRUCTURE revealed an average maximum log likelihood of posterior probability at K = 3 (population clusters Figure 3 and Figure 4). Among the 44 populations studied, only individuals of Chambi (CHA) accession have been shown to be homogeneous and belonged to the same population. These individuals represented a unique genetic group in our study. Individuals from the other accessions showed heterogeneous probabilities of belonging to the three populations determined by the structure software.

Discussion

Among Mediterranean populations, the majority of genetic variation (93%) occurred within populations, in agreement with patterns found among other *Pistacia* species (Khadivi et al. 2018, El Zerey-Belaskri et al. 2018). Similar results were obtained when we considered only the Tunisian populations were 2% of the variance existed among accessions.

Several factors, such as mating system, seed dispersal and geographic distribution, have been reported to be responsible of genetic variability in a plant species (Hamrick & Godt 1989, Hamrick & Godt 1996).

It is well known that *P. lentiscus* is a dioeciously species with an out-crossing mating system and a large distribution in the Mediterranean region. Based on previous studies focused on factors affecting genetic variability in natural populations, a high level of genetic diversity is expected in *P. lentiscus* (Hamrick & Godt 1989, Hamrick & Godt 1996, Amos & Harwood 1998, Petit et al. 2005, Bouta et al. 2024).

Hence, the high genetic variation portioned within *P. lentiscus* populations could be related to its mating system and/or to its wide geographic distribution.

Table 4 Number of alleles (*Na*), average observed (*Ho*), expected (*He*) heterozygosities, and fixation indices (*Fis*) in Tunisian populations of *P. lentiscus*.

Populations	*Na*	*Ho*	*He*	*Fis*
Ain Cristal	7.000	0.75	0.73	-0.009
Ain Drahem	4.571	0.676	0.659	0.029
Ain El Baya	5.857	0.788	0.707	-0.122
Ain Snoussi	5.857	0.593	0.674	0.114
Azib	5.714	0.663	0.713	0.076
Bellif	5.714	0.681	0.733	0.077
Beni Darraj	4.286	0.646	0.612	-0.065
Bouchoucha	6.714	0.695	0.754	0.104
Bou Jelida	5.429	0.694	0.711	0.031
Chaambi	4.143	0.643	0.623	-0.020
Dmayen	5.286	0.653	0.708	0.128
Errtiba	5.714	0.629	0.714	0.137
Feija	6.571	0.621	0.741	0.160
Gamboura	5.857	0.698	0.717	0.022
Henchir Enaam	6.000	0.668	0.703	0.058
Jbel Abderrahmen	4.714	0.643	0.664	0.047
Jbel Bou Kehil	6.571	0.770	0.702	-0.101
Jbel Chehid	6.286	0.737	0.691	-0.049
Jbel Mansour 2	6.000	0.649	0.693	0.034
Jbel Mansour	6.000	0.722	0.743	0.032
Jbel Mghila	5.000	0.700	0.659	-0.071
Jbel Orbata	6.143	0.749	0.689	-0.109
Jbel Troza	4.857	0.729	0.706	-0.035
Kebouche	6.571	0.668	0.707	0.085
Kef Erraai	6.857	0.683	0.737	0.098
Khorgalia	6.286	0.697	0.723	0.043
Ksar Lamsa	2.857	0.762	0.597	-0.262
Majen Essef	5.429	0.594	0.730	0.201
Marouhia	4.857	0.524	0.653	0.218
Morneg	5.571	0.665	0.667	0.001
Nahli	5.857	0.668	0.725	0.084
Neber	6.286	0.721	0.720	0.018
Oued El Maaden	5.286	0.611	0.676	0.088
Oued Ezzena	6.714	0.657	0.721	0.092
Oueslatia	6.286	0.665	0.729	0.083
Sidi Zid	6.000	0.649	0.689	0.084
Sidi Amor	6.429	0.725	0.701	-0.015
Sidi Awidet	5.143	0.629	0.715	0.113
Sidi Zid BV	5.571	0.714	0.736	0.040
Tabarka	6.857	0.686	0.715	0.057
Takelsa	5.286	0.750	0.713	-0.059
Teskreya	5.571	0.743	0.683	-0.099
Zouainia	6.286	0.651	0.744	0.104
Zougueg	5.286	0.697	0.665	-0.025

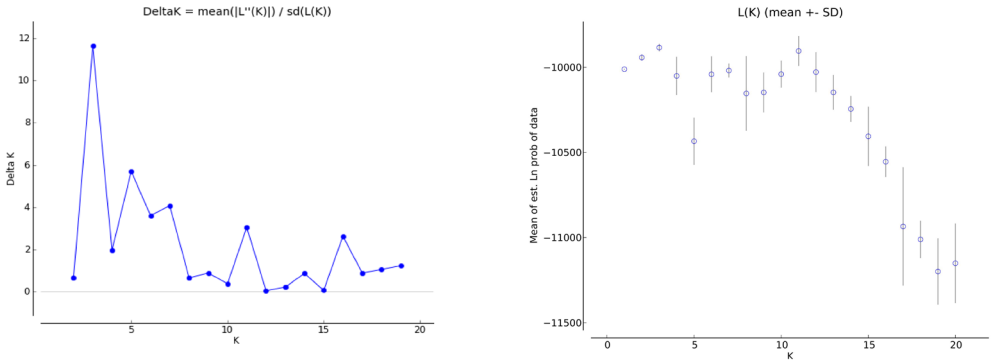

Figure 3 (a) Estimation of Tunisian populations structure using mean of estimated log probability of data (LnP(K)) with cluster value (K) ranging from 1–10. (b) Estimation of Tunisian populations using delta K with cluster value (K) ranging from 1–10.

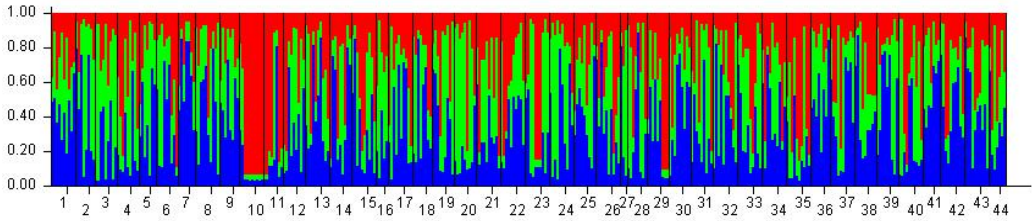

Figure 4 Evolutionary relationships among different *P. lentiscus* provenances as inferred from microsatellite loci according to the STRUCTURE clustering analysis best K = 3 (Each colour represents a genetic component and the vertical lines represent individuals) (1: AC, 2 : AD, 3 : AB, 4 : AS, 5 : AZ, 6: BEL, 7: BD, 8: BOUCH, 9: BOUJ, 10: CHA, 11: DM, 12: ER, 13: FEI, 14: GAM, 15: HEN, 16: JABD, 17: JBK, 18: JCH, 19: JM, 20: JM2, 21: JMGH, 22: JORB, 23: JTR, 24: KB, 25: KER, 26: KHOR, 27: KLAM, 28: MAJES, 29: MAR, 30: MOR, 31: NAH, 32: NEB, 33: OM, 34: OZ, 35: OUES, 36: SAM, 37: SAW, 38: SZ, 39: SZBV, 40: TAB, 41: TAK, 42: TESK, 43: ZOU, 44: ZOUG).

The number of migrants indicates that there is an elevated migration rate between populations. *P. lentiscus* is known to be a wind-pollinated species which seeds are dispersed particularly by birds, thus, there may be extensive gene flow even among distant populations.

Some Mediterranean populations show similar genetic diversity (He) values (Amora & Majen Es (0.644), Sidi Zid and Murter I (0.661), Sardaigne Mon and J Bouk (0.664), Kbouch and Fenig (0.693), Errtiba and Sesimbra (0.695) and Tabarka and Trap (0.749), demonstrating that they are probably sharing an analogue ecological history.

While no specific level of F_{ST} divergence is commonly accepted as designating different species, an F_{ST} value of 0.05–0.15 indicates a moderate genetic differentiation (Wright 1978). The average microsatellite genetic differentiation (F_{ST}) value between *P. lentiscus* Mediterranean populations was 0.124. By using this measure of divergence it can be concluded that there is a moderate genetic diversity between the Mediterranean studied populations. However, the F_{ST} value between the Tunisian populations was estimated to 0.021 (< 0.05) indicating a low genetic differentiation.

When considering all the Mediterranean populations, two major genetic clusters were clearly identified, corresponding to the Oriental and Occidental Mediterranean regions, despite the fact that the genetic differentiation among 74 natural populations was moderate (F_{ST} = 0.124). This may be related to the different environmental conditions of the two Mediterranean sides, characterized by different chemical, physical and biological properties (Sara 1985).

The work developed by Al Saghir (2006) showed that *Pistacia* genus appeared, for the first time, in North America during the Paleocene (80 million years ago). The migration of this genus held to Europe, Africa and Asia during the lower Eocene (Hsu 1983, Muller 1984, Pell 2004). Several studies reported that, during this period, the Mediterranean region was characterized by marked events such as species extinction. It was also reported that the Eocene Epoch contained a wide variety of different climate conditions that includes the warmest climate and ends in an icehouse climate (Furon 1950, Sara 1985). It is well known that climate conditions are important factors that determine changes in species ranges and the evolution of locally adapted ecotypes (Araújo & Pearson 2005). In particular, the most recent climatic oscillations in the Pleistoscene, particularly, Glacial and interglacial events, have significantly shaped the present distribution of species around the Mediterranean basin (Petit et al. 2005, Médail & Diadema 2009). All these factors may be considered to explain the structure observed for *P. lentiscus* populations.

Furthermore, it is possible that historical human activities may have affected the genetic structure of *P. lentiscus* populations. This species is known to be more exploited in countries of occidental Mediterranean (Italy, Tunisia and Algeria) than oriental part.

Within *P. lentiscus* populations in Tunisia, Chambi (CHA) accession appears unusual, with low genetic diversity and a significant weight in the population structure of the whole dataset with homogeneous individuals.

CHA population is located at the highest altitude. This area is a protected and isolated zone where the human activity is weak. It is well known that low level of gene flow increases the genetic isolation among populations.

These suggest that the gene flow between CHA population and the other accessions is low. Considering relations between the genetic diversity and altitudes, several studies confirmed that the genetic diversity is lower for populations in high altitude in high altitude (Ohsawa & Ide 2008, Ohsawa et al. 2008). In this paper, the population CHA has the lowest genetic diversity at the higher altitude (1048 m). With the rise of altitude, the level of genetic diversity of *P. lentiscus* decreased. Those results supported the viewpoint that the genetic diversity of high elevation is lower than low and medium altitude populations.

CHA accession appeared to be genetically different from the rest of accessions and to be the most isolated when compared with other populations.

The Chambi locality in Tunisia, characterized as the only emergent area during the Eocene, is historically significant for its rich faunal and floral assemblages. Studies have shown that Chambi is one of Africa's oldest Eocene sites, with notable species such as *Chambius kasserinensis* and *Djebelemur martinezi*. This region's unique evolutionary history supports the hypothesis that Chambi could be the ancestral origin of *Pistacia lentiscus* in Tunisia. This finding is crucial for understanding the phylogenetic relationships and effective conservation of *P. lentiscus* genetic resources (Flandrin 1948, Burollet 1956, Hartenberger et al. 1985, Hartenberger et al. 2001, Tabuce 2018).

Migration of this species has probably occurred from CHA to lower altitude areas. CHA accession was than isolated from others and gene flow with other populations at lower altitudes has increased genetic diversity and changed genetic structure.

Besides the paleontological data about Chambi locality, the appearance and the migration of the genus *Pistacia* were previously studied.

The work developed by Al Saghir (2006) and by Zohary (1952) noted that, when considering fossil evidence, *P. lentiscus* appears to be originated 40 million years ago, and the genus *Pistacia*, as a whole, probably originated more than 80 million years ago.

By gathering the paleontological data of Chambi and the evolutionary history of the

genus *Pistacia*, we can conclude that, during Eocene, when *P. lentiscus* migrated to Africa, Tunisia was totally immerged by water and only the area of Chambi emerged. This strongly supports the hypotheses that the first appearance of *P. lentiscus* in Tunisia was in Chambi locality during Eocene.

Conclusions

The study of genetic diversity of Mediterranean *P. lentiscus* allowed the determination of the relationship between the different populations. An elevated migration rate was recorded between the Mediterranean populations of *P. lentiscus*.

When considering all the Mediterranean populations, two major genetic clusters were clearly identified, corresponding to the Oriental and Occidental Mediterranean regions, despite the fact that the genetic differentiation among 74 natural populations was moderate.

When focusing in *P. lentiscus* populations in Tunisia, Chambi (CHA) acession appears unusual, with low genetic diversity and a significant weight in the population structure of the whole dataset with homogeneous individuals. This might support the hypotheses that the first appearance of *P. lentiscus* in Tunisia was in Chambi locality during Eocene. Revealing the population structure and diversity of a species is crucial for the efficient management and conservation of plant genetic resources, as well as for understanding the phylogenetic relationships among accessions. In this context, our results are of interest for conservation of genetic resources and contribute to the understanding of the evolutionary history of this species.

Compliance with ethical standards

Conflict of interest

Authors declare that there is no conflict of interest.

Acknowledgments

This work was financially supported by FPS COST Action FP1202: Strengthening conservation: a key issue for adaptation of marginal/peripheral populations of forest trees to climate change in Europe (MaP-FGR).

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