Genetic diversity of ten Moroccan populations of *Tetraclinis articulata* as revealed by Inter Simple Sequence Repeat (ISSR) markers

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Photo 1.
Tetraclinis articulata a thuja in the region of Oulmès at an altitude of 640 m. Photo M. Makkaoui.

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FOCUS / GENETIC DIVERSITY OF MOROCCAN *TETRACLINIS ARTICULATA*

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RÉSUMÉ

Diversité génétique de dix peuplements de *Tetraclinis articulata* au Maroc révélée par marqueurs microsatellites (ISSR)

Tetraclinis articulata (Vahl) Masters est une des essences forestières les plus importantes pour le Maroc. Elle se trouve ponctuellement à Malte et en Espagne, ce qui témoigne d'une capacité significative d'adaptation à différentes conditions bioclimatiques. Cependant, cette essence est menacée par la fragmentation anthropogène, l'exploitation forestière et sa marginalisation par les pouvoirs publics, ce qui risque de conduire à la perte irrémédiable de cette ressource. La présente étude évalue la diversité génétique et la structure génétique de dix peuplements marocains de *T. articulata* à l'aide de quinze marqueurs microsatellites (ISSR). Ceux-ci ont généré 271 fragments polymorphes avec une moyenne de 18,06 par amorce et révèlent un polymorphisme à 79,59 %. Les 129 individus montrent un niveau de diversité génétique élevé (Hs = 0,221 ; Ht = 0,254) et 85 % de variation génétique au sein des peuplements. Cependant, le niveau de différenciation génétique est faible (Gst = 0,13), ce qui correspond à l'absence de corrélation entre les distances génétiques et géographiques révélées par le test de Mantel, qui se traduit par un flux génique élevé (Nm = 3,294). Les méthodes PCoA et *neighbour-joining* produisent un classement des dix peuplements sous l'effet d'un climat continental ou marin. Comparé à d'autres conifères, la diversité génétique actuelle et la structure populationnelle de *T. articulata* indiquent un patrimoine génétique important qui nécessite des stratégies de conservation efficaces.

Mots-clés : *Tetraclinis articulata,* variation génétique, fragmentation, ISSR, conservation, Maroc.

ABSTRACT

Genetic diversity of ten Moroccan populations of *Tetraclinis articulata* as revealed by Inter Simple Sequence Repeat (ISSR) markers

Tetraclinis articulata (Vahl) Masters is one of Morocco's most important forest species. It is also found occasionally in Malta and Spain, showing significant adaptability to different bio-climatic conditions. However, the species is being affected by anthropogenic fragmentation, logging and neglect from authorities, which could lead to the irretrievable loss of this resource. In this study, the genetic diversity and genetic structure of ten Moroccan populations of *T. articulata* were assessed. Fifteen Inter-Simple Sequence Repeat (ISSR) markers were used. These generated 271 polymorphic fragments with an average of 18.06 per primer and showed 79.59% of polymorphism. The 129 individuals revealed a high level of genetic diversity (Hs = 0.221; Ht = 0.254) and 85% of genetic variation within populations. However, the genetic differentiation level was low (Gst = 0.13), which is consistent with the lack of correlation between genetic and geographic distances revealed by the Mantel test, resulting in a high level of gene flow (Nm = 3.294). Based on PCoA and neighbour-joining methods, the ten populations clustered under the effect of continental and marine climates. Compared with other conifers, the current genetic diversity and the pattern of *T. articulata* population structure indicate an important gene pool requiring efficient conservation strategies.

Keywords: *Tetraclinis articulata,* genetic variation, fragmentation, ISSR, conservation, Morocco.

RESUMEN

Diversidad genética de diez poblaciones marroquíes de *Tetraclinis articulata* revelada por los marcadores microsatélites (ISSR)

Tetraclinis articulata (Vahl) Masters es una de las especies forestales más importantes de Marruecos. También se encuentra ocasionalmente en Malta y España, lo que muestra su significativa adaptabilidad a las diferentes condiciones bioclimáticas. Sin embargo, la especie está resultando afectada por la fragmentación antropogénica, la explotación forestal y la negligencia de las autoridades. Esto podría conducir a la irremediable pérdida de este recurso. En este estudio se evalúa la diversidad genética y la estructura génica de diez poblaciones marroquíes de *T. articulata*. Se utilizaron quince marcadores microsatélites (ISSR), que generaron 271 fragmentos polimórficos con una media de 18,06 por iniciador y se evidenció un 79,59 % de polimorfismo. Los 129 individuos revelaron un elevado nivel de diversidad genética (Hs = 0,221; Ht = 0,254) y un 85 % de variación génica en las poblaciones. Sin embargo, el nivel de diferenciación genética era bajo (Gst = 0,13), lo que es consistente con la falta de correlación entre las distancias genéticas y geográficas reveladas por el test de Mantel, que dio como resultado un elevado flujo genético (Nm = 3,294). Se clasificaron, según los métodos PCoA y neighbour-joining, las diez poblaciones bajo el efecto de los climas continental y marino. En comparación con otras coníferas, la actual diversidad genética y la estructura de la población de *T. articulata* reflejan un importante patrimonio genético que requiere estrategias de conservación eficientes.

Palabras clave: *Tetraclinis articulata,* variación genética, fragmentación, ISSR, conservación, Marruecos.

Introduction

Tetraclinis articulata (Vahl) Masters consists of a monotypic Mediterranean genus of the Cupressaceae family, located in northern Africa and southern Spain (Rourke, 1991). Because of its rareness in Malta, the species is listed in the red list and protected by low (Sánchez-Gómez *et al.*, 2013). In Morocco, the coniferous occupy the most substantial extent estimated to 566,000 ha and thrive in various climates but highly represented in the semiarid and hot climates (Benabid, 1984) with an elevation of 1,000-1,100 m in shady areas and 1,500-1,700 m in sunny areas (Morte and Honrubia, 1996). Thus, *T. articulata* is a multifunctional tree offering a very desirable root burl wood regarding its quality in the handicraft industry (Fidah *et al.*, 2015). The chemical composition of essential oil extracted from *T. articulata*, showed antimicrobial activity against *Staphylococcus aureus* and *Micrococcus luteus* (Bourkhiss *et al.*, 2007). Ecologically, Thuya forests grant soil erosion control, biodiversity conservation, and CO_2 fixation, as well as suitability for afforestation programs in arid or semiarid environments and areas with severely eroded soils (Esteve-Selma *et al.*, 2010).

A combination of natural and human actions, including logging for industrial purposes, agriculture, and urbanization, increases *Tetraclinis* forest's loss and affects its genetic diversity, which usually has deleterious effects on species fitness and may threaten the survival of populations.

The study of genetic diversity is vital for conservation purposes. Genetic conservation aims to protect and preserve genetic variation. It is crucial for the maintenance of adaptive potential within populations and species (Aravanopoulos, 2016). Besides, genetic conservation is reliant on understanding the extent and distribution of the genetic diversity that exists in the current germplasm (Jubrael *et al.*, 2005). Markers have proven to be important tools for assessing the extent of the plant's genetics and understanding their distribution (Chung *et al.*, 2004; Porth and El-Kassaby, 2014).

To date, previous studies of *T. articulata* have mainly focused upon its wood characterization (Fidah *et al.*, 2015) and the antibacterial activity of its essential oil (Buhagiar *et al.*, 2000). The genetic diversity of *T. articulata* was assessed for the first time in its whole geographic range using ISSR (inter simple sequence repeat) markers, revealing a moderate genetic diversity at the intra-population level and high genetic differentiation (Sánchez-Gómez *et al.*, 2013). However, the investigations on genetic diversity remain insufficient to understand the intra-specific variation of the species.

The ISSR marker belongs to a class of multilocus, mostly dominant genetic markers that also include the amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) markers, and their derivatives (Ng and Tan, 2015). ISSR markers have been particularly useful in genetic fingerprinting and diversity analysis (Godwin *et al.*, 1997; Zietkiewicz *et al.*, 1994).

An essential step before the analysis of the genetic diversity of a population is the development of appropriate markers to avoid those that either fail to amplify or generate only a few fragments and low polymorphism levels (Rocha *et al.*, 2014). The selected primers have proved to be a reliable molecular tools for inter-populations genetic diversity studies of *T. articulata* and other conifers (Liu *et al.*, 2013; Sánchez-Gómez *et al.*, 2013; Tam and Hoa, 2006).

Our studies aimed to: (i) assess the levels of genetic differentiation and genetic structure of *T. articulata*; (ii) reveal the genetic variation within and among populations; (iii) establish conservation and management strategies.

Material and methods

Sample collection

The natural populations of *T. articulata* in Morocco is distributed in eight extents, according to High Commission for Water and Forests and Combating Desertification (HCWFCD). Leaf samples were collected from 10 populations (figure 1; table I) with a total of 129 individuals. Fresh leaves were wrapped and stored in ice then transferred into –80 °C until subsequent DNA extraction.

DNA extraction

Using a commercial kit (DNA mini kit Bioline, USA), genomic DNA was extracted from 129 lyophilized and ground leaves (50 mg powder), according to the manufacturer's instructions. Purified DNA quality was verified by spectrophotometry (ND-2000, Nanodrop, USA) at 260 and 280 nm and by electrophoresis on 1% Agarose gel. The DNA concentration was adjusted to a 50 ng/µL and stored at –20 °C for ISSR amplification.

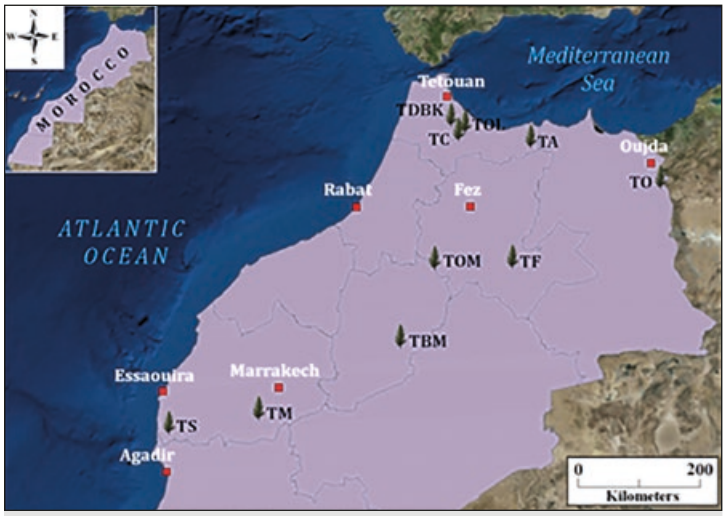

Figure 1.
Samples localities of *Tetraclinis articulata* (Vahl) Masters.
Population code and the numbers of plants collected and labeled correspond to that appeared in table I.

Table I.
The geographical locations of sampled *Tetraclinis articulata* populations.

Population code	Latitude (°N)	Longitude (°W)	Altitude (m)	Sample size
OL	35.280924	5.092205	193	13
BKT	35.324779	5.233056	267	11
A	35.095597	4.074480	362	21
C	35.181965	5.103973	635	20
O	34.54181	1.82779	946	10
OM	33.33236	5.53247	501	10
F	33.39778	4.26026	1,075	10
M	31.354677	8.235019	397	9
BM	32.330779	6.011252	1,049	15
S	31.15935	9.69343	534	10

Table II.
Primers used for ISSR amplification.

ISSR primer	Sequence	Tm (annealing temperature)
BTH1	$(AG)_8C$	50.4
BTH2	$(AG)_8T$	48.1
BTH3	$(AG)_8TC$	50.1
BTH4	$(AG)_8CA$	52.9
BTH5	$(AG)_8TA$	48.9
BTH6	$(AG)_8CC$	53.3
BTH9	$(CT)_8T$	47.1
BTH10	$(GA)_8C$	47.5
BTH11	$(GA)_8CT$	50.1
BTH12	$(GA)_8TT$	48.1
BTH13	$(GT)_8C$	46.5
B1	$(GT)_6CC$	39.8
B2	$(GT)_6GG$	40.5
TH1	$(GT)_6CG$	41.9
TH2	$(GT)_6TG$	38.3

DNA amplification for ISSR

A total of 51 primers were initially screened. Fifteen of them, which generated discernable and bright bands, were employed for the analysis of 129 samples (table II).

The ISSR amplifications were carried out in a total volume of 25 µl containing 2.5 mM $MgCl_2$, 2 mM dNTP, 4 µM of primer, 1 Unit Taq DNA Polymerase and 100 ng of total DNA. The reaction mixture was subjected to amplification in GenAmp® thermal cycler (Applied Biosystem, CA, USA) following the program: initial denaturation for 7 min at 94 °C; followed by 39 cycles of 30 s at 94 °C, 45 s annealing temperature (table II) 2 min at 72 °C and 7 min final extension at 72 °C (table II). A control was run by replacing genomic DNA with ddH_2O.

Amplified products were electrophoresed in 2.8% agarose gel in 1X TAE buffer at 120 V for two hours, and then stained with ethidium bromide for 20 min. Gels were visualized under ultraviolet and photographed with EnduroTM GDS (Labnet, USA). Size of amplified fragments was estimated using 1 kb ladder molecular size standards (Bioline, USA).

Data collection and analysis

For each ISSR reaction, only reproducible, well-resolved fragments were scored as present (1) or absent (0) and a binary matrix was generated using GEL COMPARE (v2.5). The data was firstly analyzed by POPGEN (v1.32) (ref) to estimate genetic diversity parameters for each population, mainly: percentage of polymorphic loci (%P), observed number of alleles per locus (Na), effective number of alleles per locus (Ne), Shannon's index (I) (Shannon and Weaver, 1964), Nei's gene diversity (He) (Nei, 1973) and genetic distance (D). Using Nei's gene diversity, the genetic structure parameters were calculated; Ht: total genetic diversity; Hs: genetic diversity within populations; G_{ST}: relative magnitude of genetic differentiation among populations by the equation (G_{ST} = (HT – HS)/HT). An estimate of gene flow among populations (Nm) was computed using the formula ($Nm = (1 - G_{ST})/2G_{ST}$) (Nei, 1973). These parameters were calculated, assuming Hardy-Weinberg equilibrium.

Using GenAlEx v6.5 software, the AMOVA (analysis of molecular variance) generated the genetic differentiation among and within populations using 999 permutations. A Mantel test was further carried out using the matrix of mean genetic distances and the matrix of the mean geographic distances to determine the correlation between genetic distance and geographic distance (Mantel, 1967). In addition, the obtained genetic distance matrix was then employed. At the species level, a dendrogram was generated using the Neighbor-joining method. PCoA (Principal co-ordinate analysis) was conducted to partition the populations studied on clusters. The genetic distance and the genetic identity were calculated for all pairwise combinations of populations using Nei's unbiased measures (Nei, 1973).

The Bayesian methods through STRUCTURE software version 2.1 (Pritchard *et al.*, 2000) was used to examine the number of differentiated and homogenous populations (K). K was run from 1 to 10, and for each K, 20 runs carrying out based on the burn-in and MCMC (Markov Chain Monte Carlo) length of 50,000 followed by 100,000 iterations. ΔK representing the number of clusters was estimated (Evanno *et al.*, 2005).

In order to verify the effectiveness of each primer, the following parameters were calculated; the resolving power

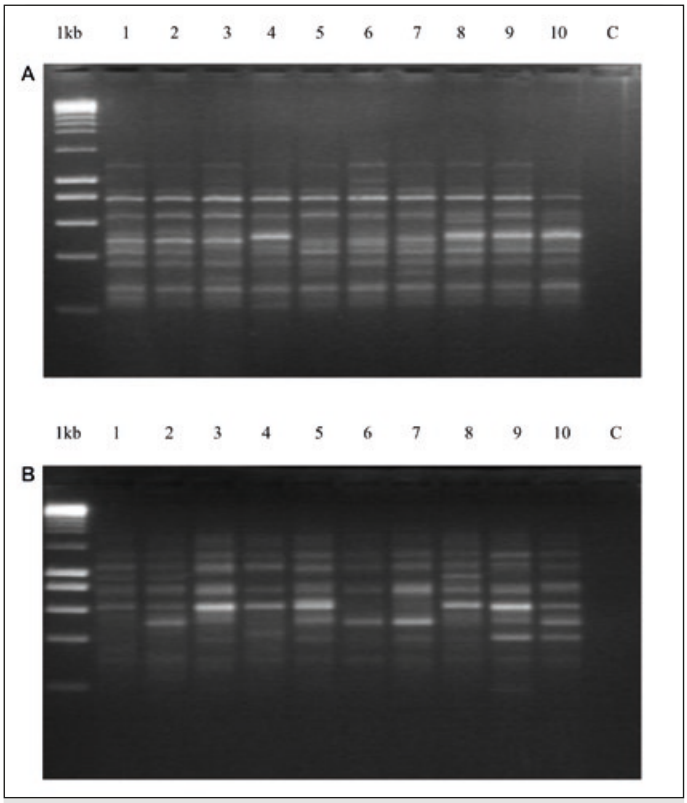

Figure 2.
ISSR amplification profiles using primer BTH11 (A) and BTH4 (B) for two regions (lanes 1kb, 1-10 individuals, C: control).

(RP) which is the ability of each primer to detect variation level between individuals where RP=$\sum I_b$ where I_b (band informativeness) takes the values of 1 – [2 |0.5 – p|], where p is the proportion of individuals containing the band (Prevost and Wilkinson, 1999); the polymorphic information content (PIC) is the probability in detecting polymorphism by a primer or primer combination between two randomly drawn genotypes, according to the formula PIC = 2fi (1-fi), fi is the frequency of the amplified allele (Roldán-Ruiz *et al.*, 2000); the effective multiplex ratio (EMR) is the number of polymorphic fragments detected per assay (Tonk *et al.*, 2014) where EMR = β x n (β is the product of the fraction of polymorphic loci, and n is the number of polymorphic loci); the marker index (MI) which is defined by the formula MI = PIC x EMR (Powell *et al.*, 1996).

Results

Marker system of ISSR

A total of 271 polymorphic fragments were generated using fifteen ISSR primers (table II). The number of fragments varied from 14 (BTH12) and 24 (BTH2) with an average of 18.06 per primer (figure 2). BTH2 primer generated a high number of markers (24), whereas BTH12 generated a low number of markers (14). The fragments with low intensity were optimized by the decrease or increase in the primers melting temperature (the case of BTH1, BTH6, BTH11, TH2). The main values concerning the polymorphism information content (PIC), the resolving power (Rp), the marker index (MI), and the effective multiplex ratio (EMR) are represented in table III.

ISSRs detected 225 of common bands ranging from 12 (TH2) to 18 (BTH6). The distribution of the unique, rare, common, and shared fragments are represented in table III.

Genetic diversity

Assuming Hardy-Weinberg equilibrium, fifteen ISSR primers were used to amplify 129 DNA samples (table II), and have generated a total of 271 fragments. The mean percentage of polymorphic fragments was 79.59% ranging from 63.47% (A) to 89.30% (C) at the population level (table IV). The number of observed alleles per locus ranged from 1,269 to 1,786, with an average of 1,597, while the number of

Table III.
Parameters evaluating the effectiveness of ISSR markers.

Primer	*TNB*	PIC	Rp	EMR	MI	F_u	F_r	F_{sh}	F_s
BTH1	21	0.3	9.19	21	6.3	0	4	17	0
BTH3	15	0.35	7.89	15	5.25	0	1	13	1
BTH4	20	0.25	6.26	20	5.07	0	6	14	0
BTH11	17	0.37	9.58	17	6.25	0	1	15	1
BTH10	18	0.39	10.95	18	7.09	0	1	17	0
BTH2	24	0.28	9.58	24	6.74	0	7	16	1
BTH5	17	0.34	8.78	17	5.82	1	2	14	0
BTH6	21	0.36	11.12	21	7.47	0	3	18	0
BTH12	14	0.42	9.07	14	5.82	0	0	13	1
BTH13	18	0.31	8.08	18	5.64	0	4	14	0
TH2	17	0.33	8.47	17	5.65	0	4	12	1
TH1	19	0.34	9.61	19	6.41	1	2	16	0
BTH9	18	0.36	9.61	18	6.5	0	1	17	0
B1	17	0.38	10.47	17	6.5	0	2	15	0
B2	15	0.35	7.92	15	5.26	0	1	14	0
Total	271					2	39	225	5

TNB: total number of bands; PIC: polymorphism information content; Rp: resolving power; EMR: effective multiplex ratio; MI: marker index; F_u: number of unique fragments; F_r: number of rare fragments; F_{sh}: number of shared fragments; F_s: number of similar fragments.

Table IV.
Genetic diversity parameters of *Tetraclinis articulata* populations.

Pop	N_a	N_e	I	H_e	%P
Alhoceima	1.269	1.281	0.270	0.173	63.47%
Beni Mellal	1.605	1.324	0.328	0.206	80.07%
Chefchaouen	1.786	1.388	0.383	0.244	89.30%
DB Kerrich	1.701	1.353	0.349	0.221	84.87%
Fes-Boulemane	1.661	1.388	0.372	0.239	83.03%
Marrakech	1.594	1.412	0.378	0.248	78.23%
Oujda	1.458	1.304	0.303	0.191	72.69%
Oued Laou	1.528	1.327	0.325	0.206	76.01%
Oulmes	1.616	1.407	0.377	0.245	80.44%
Essaouira	1.756	1.364	0.380	0.239	87.82%
Average	1.597	1.355	0.346	0.221	79.59%

Na = observed number of alleles; Ne = effective number of alleles; I = Shannon's Information Index;
He = expected heterozygosity or Nei's genetic diversity;
%P = percentage of polymorphic loci.

Photo 2.
Tetraclinis articulata a thuja in the region of Oulmès at an altitude of 744 m.
Photo M. Makkaoui.

effective alleles per locus ranged from 1,281 to 1,412 with an average of 1,355. The expected heterozygosity varied from 0,173 to 0,248, and Shannon's index ranged from 0,270 to 0,383.

At the population level, the AMOVA analysis (Excoffier *et al.*, 1992) showed 14% of the genetic diversity among populations and 86% within populations (table V).

Genetic differentiation and genetic structure

The analysis of genetic differentiation parameters between populations showed that the total genetic diversity (Ht) was estimated to 0.2548, and the genetic diversity within populations (Hs) was estimated to 0.2212. And the coefficient of differentiation (G_{ST}) is 0.1318, while the gene flow (Nm) was rated to 3.2945.

Principal coordinate analysis (PCoA) was performed to provide spatial representation of the relative genetic distance among *T. articulata* populations. The first and second principal coordinates PCoA (a) and (b) account for 14.09% and 7.87% of total variation, respectively and portioned the 10 populations into 4 main clusters (figure 3).

Based on Nei's genetic distances (Nei, 1973) among the ten populations of *T. articulata*, genetic distances and genetic identities were calculated with an average of 0.952 and 0.049, respectively (table VI). The Neighbor-joining analysis grouped the populations into two groups. Each group contained two subgroups. The first subgroup included individuals from Chefchaouen, Oulmes, Fes-Boulemane, individuals from Marrakech, Chefchaouen, and Oulmes formed the second one. The Second subgroup contained individuals from Alhoceima and Oujda, Oued Laou and DB kerrich, Beni Mellal and Fes-Boulemane (figure 4). Whereas the Mantel test revealed no correlation between genetic and geographic distances (r = 0.069, *999* permutations). STRUCTURE analysis showed one genetic pool indicating high gene flow between the studied populations, which is consistent with the gene flow value (figure 5).

Table V.
AMOVA analysis of *Tetraclinis articulata* populations (P < 0.001).

Source	Degree of freedom	Sum squares	Mean squares	Estimate variance	% Total variation
Among regions	4	579.948	144.987	1.896	4%
Among pops	5	556.344	111.269	5.299	11%
Within pops	119	4,781.320	40.179	40.179	85%
Total	128	5,917.612		47.375	100%

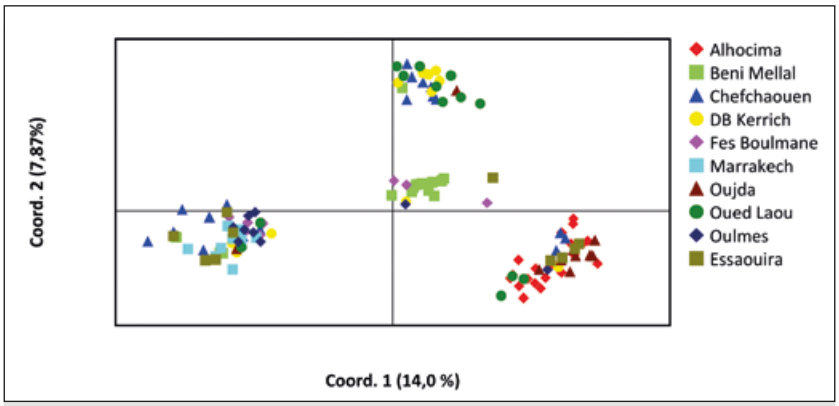

Figure 3.
Principal coordinates analysis of *Tetraclinis articulata* populations of ISSR based on pair-wise Nei's (1973) genetic distances.

Discussion and conclusion

Genetic diversity is essential to the long-term survival of tree species to avoid the risk of extinction (Li and Xia, 2005). However, the choice of the appropriate molecular tool for genetic analysis depends on the ability of the markers to estimate the genetic diversity in a reliable way (Roldán-Ruiz *et al.*, 2000). ISSR markers are one of the cheapest and easiest marker systems with high efficiency in generating polymorphism (Vijayan, 2005). They were used to assess the genetic diversity of the vast majority of conifers (Wang *et al.*, 2004; Meloni *et al.*, 2006; Xia *et al.*, 2008; Liu *et al.*, 2017). Recently, the genetic diversity of *T. articulata* was assessed; the study covered the species range (Sánchez-Gómez *et al.*, 2013). The mean of within-population gene diversity (Hs), derived from estimates of dominant markers, was 0.20 for endemic species (Nybom, 2004).

As an endemic species, the mean Nei's genetic diversity (Hs) of *T. articulata* is 0.221. The results are slightly high comparing to those found with *T. articulata* (Sánchez-Gómez *et al.*, 2013) but are comparative to those observed with conifers *Juniperus chinensis* (Kim *et al.*, 2018); *J. coreana* and *J. rigida* (Huh and Hong, 2000). Commonly, coniferous species hold a low average level of genetic diversity (h = 0.207) (Loveless and Hamrick, 1984). However, the genetic diversity observed in coniferous species from the Mediterranean regions estimated with a different set of genetic markers is considered to be high (Terrab *et al.*, 2008; Douaihy *et al.*, 2011). Long-lived, woody species showed the highest genetic diversity (including a significantly higher percentage of polymorphic loci and more alleles per locus) among all plant species (Porth and El-Kassaby, 2014): e.g., *Thuja occidentalis* (Hs = 0.64) (Pandey and Rajora, 2012); *Pilgerodendron uviferum* (Hs = 0.712) (Allnutt *et al.*, 2003) and low genetic differentiation (Hamrick *et al.*, 1992). However, some woody and long-lived species hold low within-population gene diversity case of *Cunninghamia lanceolata* var. *konishii* (Tam and Hoa, 2006) and *Glyptostrobus pensilis* (Li and Xia, 2005). Besides, genetic diversity within populations influenced by historical factors (e.g., founder effects, bottlenecks, extended periods with low numbers of individuals and gene flow rate), thus population sizes may not be a reliable indication of genetic diversity (Liu *et al.*, 2013). Another parameter showing the richness of genetic diversity of *T. articulata* in Morocco is the high percentage of polymorphic loci (average of 79.59%) which is similar to those found with (Sánchez-Gómez *et al.*, 2013), and all regions retained their genetic diversity and indicated that *T. articulata* didn't

Table VI.
Unbiased measures (Nei, 1973) of genetic distance (below diagonal) and geographic distance (above diagonal) between regions of *Tetraclinis articulata*.

Population	Alhoceima	Beni Mellal	Chefchaouen	DB Kerrich	Fes-Boulemane	Marrakech	Oujda	Oued Laou	Oulmes	Essaouira
Alhoceima	\	0.937	0.948	0.949	0.939	0.884	0.973	0.956	0.935	0.961
Beni Mellal	0.065	\	0.967	0.963	0.977	0.915	0.944	0.959	0.967	0.959
Chefchaouen	0.053	0.034	\	0.984	0.979	0.937	0.954	0.973	0.971	0.978
DB Kerrich	0.052	0.038	0.016	\	0.967	0.918	0.955	0.977	0.958	0.968
Fes-Boulemane	0.063	0.023	0.022	0.033	\	0.942	0.946	0.961	0.981	0.972
Marrakech	0.123	0.089	0.065	0.085	0.060	\	0.884	0.905	0.940	0.941
Oujda	0.027	0.057	0.047	0.046	0.056	0.123	\	0.956	0.931	0.972
Oued Laou	0.045	0.042	0.027	0.023	0.040	0.100	0.045	\	0.954	0.961
Oulmes	0.067	0.033	0.030	0.043	0.019	0.061	0.071	0.047	\	0.960
Essaouira	0.039	0.042	0.022	0.032	0.029	0.060	0.028	0.040	0.041	\

Unbiased measures (Nei, 1973) of genetic distance (below diagonal) and geographic distance (above diagonal) between regions of *Tetraclinis articulata*.

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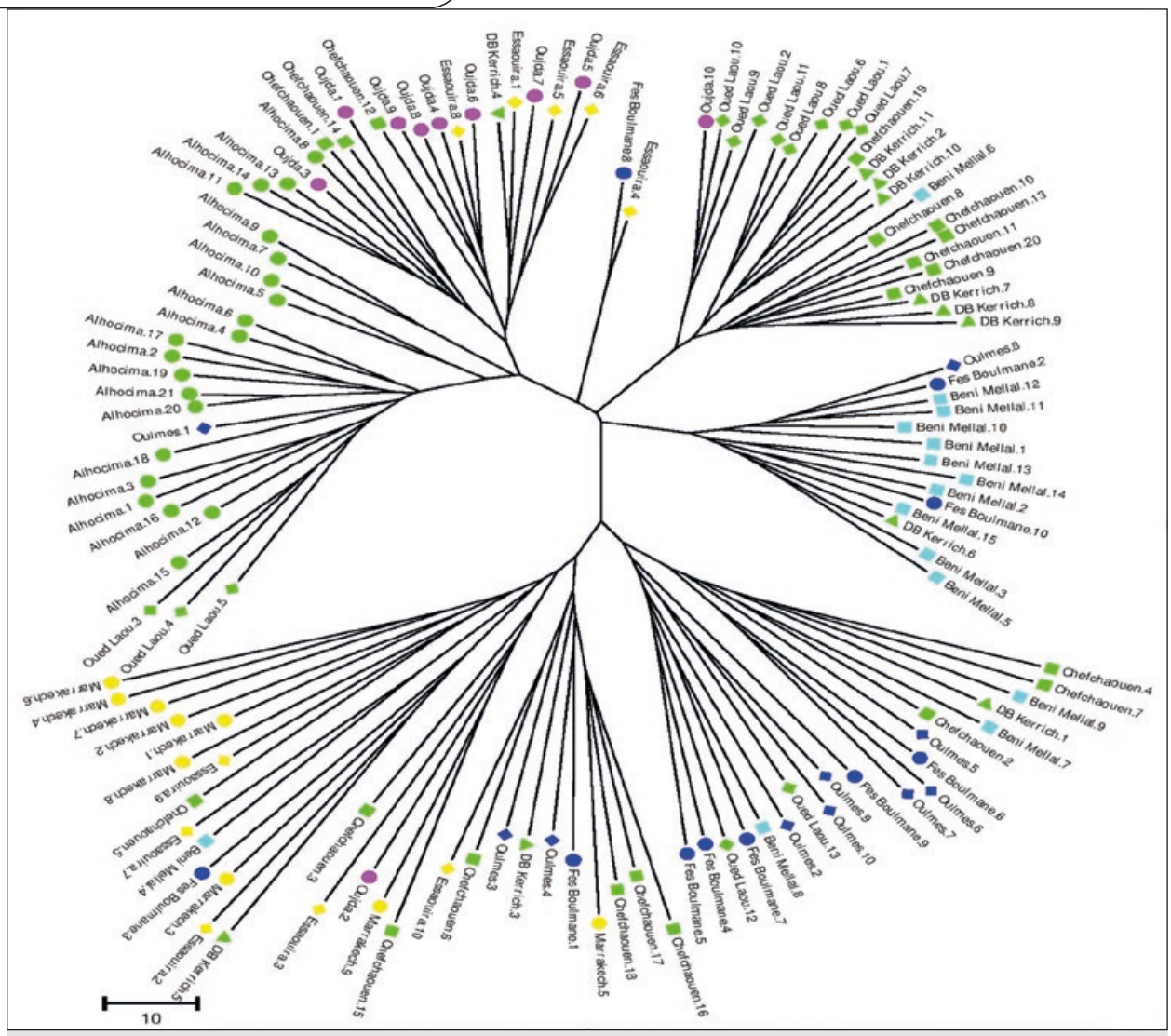

Figure 4.
The Neighbor-Joining tree based on the Nei's (1972) genetic distance of the 129 individuals *Tetraclinis articulata*.

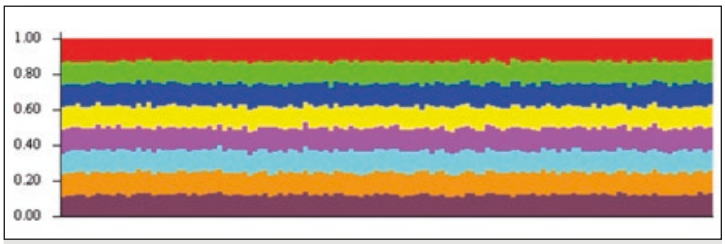

Figure 5.
Genetic structure of 129 genotypes assessed by STRUCTURE software (20 independent runs of each K value (from K = 1 to 10) were performed with 50,000 as the burn-in and 100,000 iterations.

endure drastic genetic bottlenecks in spite of anthropogenic fragmentation (intensive grazing, urbanization, cutting and pruning). The high within-population diversity might also be explained by a recent origin of the fragmentation coupled with a high initial level of diversity (Dagher-Kharrat *et al.*, 2007).

Genetic structure is the nonrandom distribution of alleles or genotypes in space or time (Li and Xia, 2005). Besides, it reflects the interactions of various evolutionary processes, including the long-term evolutionary history, such as shifts in distribution, habitat fragmentation, population isolation, mutation, genetic drift, mating system, gene flow, and selection (Schaal *et al*,. 1998). The structure of Moroccan populations, calculated by AMOVA, had revealed a high level of variation within populations (85%) and a low level of variation among populations (11%). Our results are in line with the previous study on genetic diversity of *T. articulata* (Sánchez-Gómez *et al.*, 2013) and other Cupressaceae species (Meloni *et al.*, 2006; Liu *et al.*, 2013; Hou *et al.*, 2018) that maintain more variation within species and within populations species than species with other life forms but have less variation among populations (Vashishtha *et al.*, 2013). The genetic differentiation values detected for gymnosperms (Gst = 0.18) (Nybom and Bartish, 2000) are similar to our

findings (Gst=0.13) and slightly lower than in the previous study (G_{ST} = 0.31) (Sánchez-Gómez *et al.*, 2013). On one hand, low level of genetic differentiation in gymnosperms usually attributed to the frequent wind-pollination and breeding systems that promote outcrossing (Bennett *et al.*, 2000). Still, on the other hand, the genetic differentiation into distinct populations is strongly influenced by genetic drift, gene flow, long-term evolution, mating systems, selection, and mutations (Hamrick *et al.*, 1992).

In our study, the gene flow (Nm = 3.29; Nm is the number of migrants per generation) showed a sort of continuity between the different populations of *T. articulata* insured by seed dispersal and pollen. In this case and being a wind-pollinated conifer, pollen can spread over long distances under proper environmental conditions (Lanner, 1966), which may explain the high level of genetic diversity (Hou *et al.*, 2018). Our results are similar to *Calocedrus macrolepis* (Wang *et al.*, 2004), *Thuja sutchuenensis* (Liu *et al.*, 2013) and *T. koraiensis* (Liu *et al.*, 2017) and higher than *G. pensilis* (Li and Xia, 2005), *J. phoenicea* (Meloni *et al.*, 2006) and *T. occidentalis* (Pandey and Rajora, 2012).

According to PCoA and the Neighbor-Joining methods, populations of Alhoceima, Oued Laou, Dar Ben Kerrich, and Essaouira influenced by the maritime climate formed an independent group. In contrast, the effect of continental climate clustered Marrakech, Beni Mellal, Fes-Boulemane, and Oulmes on other groups. Although, individuals of Chefchaouen were divided into two groups. Our findings supported the lack of correlation between genetic and geographic distances revealed by the Mantel test.

Implications for conservation. The level and distribution of genetic variation is a prerequisite for the establishment of effective and efficient conservation practices (Ge *et al.*, 1998). *T. articulata* has suffered severe fragmentation and reduction of its populations throughout history (Sánchez-Gómez *et al.*, 2013). Also, the most factor affecting *Tetraclinis* forests since Phoenician times is the demand of wood that steels today impacting the Moroccan populations, especially in the Essaouira region where the root burl wood produced by *T. articulata* is in intensive use. In spite of the overexploitation, populations of *Tetraclinis* showed an excellent mean of polymorphic loci, revealing an essential genetic resource (85% of the total genetic variation maintained within populations); i.e. a reliable indicator that the species are not endangered yet. A primary goal of conservation is the maintenance of genetic diversity and evolutionary processes in viable populations to prevent potential extinction (Falk and Holsinger, 1991). *In situ* and *ex situ* are the most recommended conservation measures in the light of climate changes in the Mediterranean area (Esteve-Selma *et al.*, 2010; Morte and Honrubia, 1996; Esteve-Selma *et al.*, 2012). Although the knowledge of the available genetic architecture is an appropriate strategy for sampling, and propagation could be formulated when *ex situ* conservation is carried out (Zhang *et al.*, 2005). Towards more profound research studying the gene flow, pollination mechanisms, the preservation of *T. articulata* is going to be more focused and optimized.

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