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Genetic diversity evaluation of *Tetraclinis articulata L.* in Algeria

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Abstract

Tetraclinis articulata is an endemic, medicinal and aromatic species of the cupressaceae family with an economic importance. The objective of our study is based on the morpho-metric characterization of a 210 individual sample from seven populations distributed in five regions in western Algeria: Tlemcen (Ain fezza (Yfri forest), Ain Ghraba (Ahfire forest), Beni Snous (Zarifate forest)), Ain Temouchent (Madagh 2 forest) , Oran (the Virgin forest) , Relizane (Zemmora forest) and finely Sidi Bel Abbes (Tenira forest) using eight morphological traits :Circumference of the tree (CRF),The tree height (TH),The tree crown (TCR), The length of primary branch (LgB1),The length of secondary branch (Lg B2),The length of the needles(LgNd) which were statistically studied using the R software. Phenotypic diversity was determined by the Shannon-Weaver diversity index (H') at different levels. The estimated H' showed a large phenotypic variability for different traits with a mean of 0.90. The results of the multiple correspondence analysis (MCA) and hierarchical clustering (HCA) showed a clear distinction between the populations. The extraction of essential oils was done for all populations; the yield of essential oils from the trees of the Beni Snouss population was higher than the other populations which is very important for future economic perspectives and we all so found that there is a negative correlation between the yield of essential oils and The length of the needles (LgNd) which can be used to identify the subjects with the best performance.

Keywords: *Tetraclinis articulate L*, endemic species, morphometric characterization, genetic diversity, phenotypic variability, essential oil

الملخص

شجرة سرو الاطلس (*Tetraclinis articulata* L) هي من أنواع الاصناف مستوطنة ,طبية وعطرية ذات أهمية اقتصادية معتبرة. الهدف من دراستنا هو التوصيف المورفو المتري لمجموعة عينات متكونة من 210 فردا تنتمي الى سبع مجموعات موزعة في خمس مناطق على مستوى غرب الجزائر: تلمسان (عين فزا (غابة يفري) ، عين غرابة (غابة أحفير) ، بني سنوس (غابة زريفات)) ، عين تموشنت (غابة مداغ 2) ، وهران (غابة العذراء) ، غليزان (غابة زمورة) ، سيدي بلعباس (غابة تينيرا) باستخدام ثماني سمات مورفولوجية محيط الشجرة (CRF) ، ارتفاع الشجرة (TH) ، تاج الشجرة (TCR) ، طول الفرع الأولي (LgB1) ، طول الفرع الثانوي (Lg B2) ، طول الإبر (LgNd) التي تم دراستها إحصائيًا باستخدام برنامج R. تم تحديد التنوع المظهري بواسطة مؤشر تنوع (Shannon-Weaver H ') على مستويات مختلفة. أظهر تقدير H ' تنوعا ظاهريًا كبيرًا لصفات مختلفة بمتوسط 0.90. أظهرت نتائج تحليل المراسلات المتعددة (MCA) والتصنيف الهرمي (HCA) تمييزًا واضحًا بين المجموعات السبعة. تم استخراج الزيوت الأساسية لجميع العينات؛ كان محصول الزيوت الأساسية من أشجار عينات بني سنوس أعلى من العينات الأخرى وهو أمر مهم للغاية بالنسبة للمنظورات الاقتصادية المستقبلية، ووجدنا كذلك أن هناك علاقة سلبية بين محصول الزيوت الأساسية وطول الإبر (LgNd) .

الكلمات المفتاحية: سرو الاطلس، أنواع مستوطنة، التوصيف المتري، التنوع الوراثي، التباين الظاهري، زيت اساسي.

Introduction

Tetraclinis articulata (Vahl Masters) is a tree distributed throughout the western Mediterranean basin. It is included in the IUCN (International Union for Conservation of Nature) red list, From the Cupressaceae family, *Tetraclinis articulata* (Vahl) is geographically linked to the three Maghreb countries (Morocco, Algeria and Tunisia). There is also a minor population of *T. articulata* in some specific sectors, in the South-East of Spain (region Almeria) and on the island of Malte (Achhal et *al.*,1985).

According to Acherar (1981), Barbero *et al* (1990), Hadjadj-Aouel (1995), Benabdelli (1996) and Benabdellah(2011), in times of global warming and desertification, Thuja shows itself to be a resistant tree that can spread in the thermo-Mediterranean and even the lower meso-Mediterranean zone at the expense of other forest formations more demanding in terms of: biogeography, ecology, threats and conservation of freshness and humidity, which makes it an ideal candidate to combat desertification and forest fires.

Thuja forests ensure an important role in economic life, indeed, this species is used in carpentry, in the preparation of cosmetic products and in traditional medicine because of its therapeutic effects, especially its essential oil extracted from leaves (Bourkhiss *et al,* 2015), also from its branches which have an anti-infectious, anti-tumor property), and diuretic; used externally to treat benign tumors, water retention, inflammation of the genitals and painful periods (Bellamine K, 2017).

This study is based firstly on the morphometric characterization of different populations of Thuja found in the West of Algeria in seven study areas (Tlemcen (Ain fezza (Yfri forest), Ain ghraba (Ahfire forest), Beni snous (Zarifate forest)), Ain temouchent (Madagh 2 forest) , Oran (the Virgin forest), Relizane (Zemmora forest) and finely Sidi Bel Abbes (Tenira forest) in order to estimate the genetic diversity and secondary to begin a preliminary phytochemical screening by the extraction of essential oils from the leaves of trees.

Materials and methods

Study stations

Presentation of the Study Areas

The study stations visited (Fig 1) are regrouped in the west of Algeria (Table 1), Thuja is exclusively confined to the semi-arid bioclimatic stage with warm, mild and even cool variants that can develop at a maximum altitude of 1400 m. The difficult ecological conditions of the region allow this species to maintain itself where other species are present only in the state of scrub (benabdeli, 1996).

Table 1: Geographical locations of populations of *Tetraclinis articulata*

	Population	Latitude(N)	longitude (W)	Bioclimatic stage	Altitude (m)
Tlemcen	Forest of Yefri	34°52′10″N	1°14′32″W	Semi-arid superior	613m
	Ahfire Forest	34°47′31 ″N	1°25′54″W	Dry semi-arid	846m
	Zarifate Forest	34°37′31 ″N	1°34′41 ″W	Cold semi-arid	829m
Sidi Bel Abbes	Forest of Tenira	34°59′35 ″N	0°37′22 ″W	Semi-arid dry and cold	835m
AinTemouchen t	Forest of Madegh2	35°36′40 ″N	1°05′07 ″W	Semi-arid	100m
Oran	Forest of the virgin	35°38′34 ″N	0°43′18 ″W	Semi-arid	125m
Relizane	Forest of Zemmora	35°42′21 ″N	0°46′34 ″W	Semi-arid	310m

The experimental stations were selected according to the importance of *T. articulate* stands in the region, the accessibility to the field and possibility of assistance from foresters.

Field trip program

In total, we conducted 07 field trips,

- February 2019***:*** *wilaya of Tlemcen::* Forest of Yfri (Ainfezza),Forest of Ahfir (AinGhoraba)**,** Forest of Zarifatt (Beni Snous)
- February 2020: *wilaya of Relizane*:Forest of Zemmora
- January 2021:
  - Wilaya of Sidi Bel Abbes: Forest of Tenira,
  - Wilaya of AinTemouchent:Forest of Madegh 2
  - Wilaya of Oran:Forest of the virgin Misserghin

These prospections have concerned:

- Taking samples of the leaves of each station.
- Realization of the morpho-metric measurements on the various parameters

Figure 1. Geographical representation of study areas (1cm=20km)

Extraction of essential oils

Plant Material

A amount of 12 Kg of leaves of *T. articulata* were collected from trees which have approximately the same age except for the forest of Madagh 2 (trees were young) The samples were then dried in air at room temperature (25°C) and were protected from light for ten days.

Methods of preparation of essential oil "Steam distillation"

The extraction of the essential oil from the leaves of the tree was carried out by steaming with a Clevenger type apparatus. The extractions lasted three hours. The essential oil obtained was stored in small opaque bottles and placed in the refrigerator at 4 ° C.

The yield of essential oil

The yield of EO is the weight ratio between the weight of oil extracted and the weight of the leaves taken for the extraction.

The oil yield is expressed as a percentage and calculated by the following formula:

$$R = [PA/PB] \times 100$$

R = Yield of the oil in %.
PA = Weight of the oil in g.
PB = Weight of the leafin g.

Data Collection

Two hundred and ten samples (trees), 30 from each of the forest were used and served as a tool for this study.

Morphological data

Populations of thuja were evaluated using six quantitative and two qualitative traits. These characters are:

Quantitative traits:

Circumference of the tree (CRF)
The tree height (TH)
The tree crown (TCR)
The length of primary branch (LgB1)
The length of secondary branch (Lg B2)
The length of the needles (LgNd)

Qualitative parameters:

The color of the needles (ClNd)
The color of the badet of tree (ClBt)

Statistical analysis

Morpho metric measurements were analyzed using R software. The data collected were subjected to the following statistical analyses:

Shannon and Weaver Index

Before proceeding to this test, the quantitative traits were transformed into classes. This transformation was done using (summary) of the R software, which divided these data into the desired number of classes (in our case four classes). In parallel, we determined the limit of each class. The frequencies of these different phenotypic classes were calculated for each line.

The index of Shannon-Weaver (Shannon and Weaver, 1948) described by Jain *et al* (1975) was calculated for each line in order to estimate the genetic diversity.

The Shannon-Weaver index formula is:

$$H=-\sum[(pi)\times\ln(pi)]$$

H = Shannon and Weaver diversity index
Pi = Frequency of each phenotypic class i of a given character
n = Number of phenotypic classes of each character.

The index (H) is converted to the relative phenotypic diversity index (H') by dividing it by its maximum value: H max (Ln (n)) to obtain values from 0 to 1.

$$H=-\sum[(pi)\times\ln(pi)]/Ln(n)$$

The relative diversity index (H') reaches its minimum value, which is zero for monomorphic characters. In addition, the value of this index increases with the degree of polymorphism and reaches a maximum value 1 when all the phenotypic classes are present at equal frequencies.

Principal Components Analysis (PCA)

Population using the FactoMineR software (version R- 4.0.2).

Hierarchical Ascendant Classification (HAC)

Hierarchical Ascendant Classification or cluster analysis was used to calculate the mean data of populations using the FactoMineR software (version R- 4.0.2).

Multiple Correspondence Analysis (MCA)

Multiple Correspondence Analysis was carried out on the correlation matrix. It calculated the mean data of trees' population using the FactoMineR software (version R- 4.0.2).

Significance analysis of these parameters was performed by $\chi 2$-test ($P < 0.05$), and Turkey's test ($P < 0.05$) with R statistical software.

Chi-Square test (contingency test)

The Chi-Square test of independence is used to determine if there is a significant relationship between two nominal (categorical) variables. The frequency of each category for one nominal variable is compared across the categories of the second nominal variable. The Chi square test formula is:

$$\chi^2 = n\left(\sum_{i,j} \frac{n_{ij}^2}{n_{i.}n_{.j}} - 1\right)$$

Results and discussions

Yield of essential oil extraction

The yield of essential oil extract from the leaves of *T. articulata* in each region expressed as percentage weight to dry plant is in Table 2

Table 2: Comparative oil yield from the leaf of the different populations of *T articulata* in the study regions

Populations	Plant weight (g)	Oil yield (ml)	Oil weight (g)	Oil yield (%) of plant weight
Yfri forest	12 600 g	11 ml	8 g	0.06 %
Ahfire forest	12 600 g	19 ml	17 g	0.13 %
Zarifate forest	12 600 g	22.3 ml	19 g	0.15 %
Tenira forest	12 600 g	4 ml	2 g	0.01 %
Virgin forest	12 600 g	6 ml	5 g	0.04%
Madagh 2 forest	12 600 g	12 ml	10 g	0.10%
Zemmora forest	12 600 g	7 ml	6 g	0.05%
Mean	12 600 g	11.61 ml	9.57 g	0.07 %

We notice that the yield of EO obtained after three hours in Zarifate forest is the highest (0.15%) followed by Ahfire forest (0.13%) than Madagh 2 forest (0.10%), Yafri forest (0.06%), Zemmora forest (0.05%), virgin forest (0.04%) and finelly Tenira forest (0.01%).

Our yield in EO of the leaves of *T. articulata* acquired is lower than that revealed by Barrero *et al*. (2005) in Morocco during six hours of distillation, also Boukhriss *et al.* (2007, 2009) in Morocco (0.22%) and to those revealed by Tékaya-Karaoui *et al.* (2007); and Ben Jemia *et al*. (2012) in Tunisia. The yields acquired are respectively 0.11% and 0.20%.

The low yield of Thuja present in Tenira, Virgin, Zemmora and Yfri forests, may be explained by the state of advanced degradation of trees in those forest (illicit cutting, overgrazing and fires), also by the ecological factors. Moreover, to the various mutilations, the tree reacts by emitting rejections of stumps, which induces an increase of the density and the output, so the length of the stems and volumes are reduced.

This difference in EO could be explained according to Kelen and Tepp (2008) by the choice of the harvest period because it is crucial in terms of yield and oil quality. The duration of drying, the geographical area, the climate and the part of the plant studied, are factors among others that can have a direct impact on the yield of EO (Vekiari *et al*, 2002).

The Table 3 presents the results of the measurements made on the trees of the seven populations studied (Ain Fezza, Ain Ghoraba, and Beni snouss, Oran, Temouchent, Relizane and Sidi Bel Abbes) after having performed the descriptive analysis.

Quantitative Parameters

Descriptive analysis

All genotypes showed highly significant differences for all the studied traits ($p<<0.01$) (Table 3).

The TH of the genotypes ranged from 161 for Ahfire forest to 1200 cm for Tenira forest , the tree crown TCR of all genotypes ranged from 85 for Madagh 2 forest to 650 cm for Zemmora forest, the circumference CRF of all genotypes ranged from 5 to 125 cm for Virgin forest, the length of the primary branch (Lg B1) of all genotypes ranged from 26 for Yfri forest to 380 cm for Zemmora forest, the length of the secondary branch (Lg B2) of all genotypes ranged from 15 to 190 cm for Tenira forest, the length of needles (LgNd) of all genotypes ranged from 1 for Zemmora forest and Tenira forest to 6.5 cm for Virgin forest.

Variance analysis ANOVA 1

All genotypes showed highly significant differences for all the studied traits ($p<<0.01$) (Table 3).

The Tukey HSD test was also carried out in order to find means of each trait that are significantly from each other., the results are represented by the table 05

As can be seen in the table above (ANOVA test), there is at least one significant difference between the samples, so we performed a HSD Tukey test, which gave results showing through the studied traits:

- Great similarity between genotypes of Ain GHoraba (Ahfire forest), Ain Fezza (Zarifate forest) and Ain Temouchent (Madagh 2 forest).
- An important difference between tree genotype of Sidi Bel Abbes (Tenira forest) and the other six-tree genotype (six forest).
- Low similarity between trees of the forest of Relizane (Zemmora forest) and Oran (Virgin forest).

Table 3: Descriptive analysis of morphometric measurements of the different populations of *T. articulata* in the study regions

		Ahfire Forest	Zarifate Forest	Yfri Forest	Virgin Forest	Madagh 2 Forest	Zemmora Forest	Tenira Forest
TH (cm)	Mean	337.3	463.53	370.96	388.2	306.9	460.9	550.5
	Standard-deviation	87.81	169.70	95.10	120.11	55.44	200.99	207.89
	Variance	7712	28799	9368	14428	3074	40400	43221
	Stderror	16033	30.984	17.671	21.931	10.122	36.698	37.958
	Min	161	242	280	238	200	159	200
	Max	520	750	600	780	450	1100	1200
TCR (cm)	Mean	159.56	237.3	137.56	298.1	149.06	257.03	282.7
	Standard-deviation	48.94	98.75	65.34	112.25	39.19	141.03	132.63
	Variance	2395.21	9751.73	4394.83	9393.48	1536.06	19891.20	17592.56
	Stderror	8.935	18.030	12.103	17.695	7.155	25.750	24.217
	Min	96	113	50	140	85	96	34
	Max	300	570	310	500	260	640	650
CRF (cm)	Mean	15.86	32.76	18.3	37.63	16.63	32.56	41.7
	Standard-deviation	7.04	19.83	8.17	22.17	6.56	17.82	23.78
	Variance	49.567	393.28	69.233	491.89	43.13	317.77	565.94
	Stderror	1.285	3.620	1.519	4.049	1.199	3.254	4.343
	Min	6	9	7	5	8	7	9
	Max	36	76	37	125	35	100	117
Lg B1 (cm)	Mean	91.56	119.73	85.6	130.03	86.03	111.23	147.06
	Standard-deviation	23.46	71.04	37.63	49.38	21.60	66.46	56.07
	Variance	550.52	5047.30	1442.68	2438.65	466.92	4418.18	3144.34
	Stderror	4.283	12.971	6.934	9.016	3.945	12.136	10.238
	Min	55	53	26	90	40	45	65
	Max	135	350	220	350	120	380	280
Lg B2 (cm)	Mean	31.86	45.83	34.93	59.9	37.76	64.2	61.96
	Standard-deviation	5.95	23.35	17.97	25.127	10.90	35.00	33.22
	Variance	35.429	545.52	331.61	631.40	119.01	1225.26	1104.17
	Stderror	1.086	4.264	3.324	4.587	1.991	6.391	6.067
	Min	23	21	17	30	20	19	15
	Max	53	110	91	160	65	150	190
LgNd (cm)	Mean	2.09	1.99	2.48	3.37	2.75	2.49	2.64
	Standard-deviation	0.60	0.46	0.48	1.42	0.73	0.95	0.93
	Variance	0.366	0.216	0.221	2.025	0.537	0.917	0.881
	Stderror	0.110	0.084	0.085	0.259	0.133	0.174	0.171
	Min	1.1	1.4	1.6	1.4	1.3	1	1
	Max	3.7	3.1	3.6	6.5	4.2	4	5.4

Table 4: coefficient of variation by ANOVA

Parameters	**F value**	**P value**
TH (cm)	10.29	6.13e-10 ***
TCR (cm)	15.33	1.81e-14 ***
CRF (cm)	**12.61**	4.52e-12 ***
Lg B1 (cm)	6.769	1.49e-06 ***
Lg B1 (cm)	10.01	1.11e-09 ***
Lg Nd (cm)	8.543	2.77e-08 ***

*TH (tree height cm), CRF (Circumference of the tree cm), TCR (The crown cm)/ Lg B1 (The length of primary branch cm), Lg B2 (The length of secondary branch cm), Lg Nd (The length of the needles cm)***: ANOVA, very highly significant differences χ2-test (P < 0.001)*

Table 5: Tukey test (HSD)

	TH	TCR	CRF	Lg B1	Lg B2	Lg Nd
AH_YF	0.97	0.97	0.99	0.99	0.99	0.56
ZR_YF	0.17	0.001	0.015	0.11	0.57	0.27
TN_YF	6.15 e -5	4 e -7	2.9 e -6	7.3 e -5	3.6 e -4	0.99
VR_YF	0.99	0	0.0002	0.012	0.0013	0.001
MD_YF	0.60	0.99	0.99	1	0.99	0.89
ZM_YF	0.20	6.03 e-5	0.017	0.42	7.7 e-5	1
ZR_AH	0.01	0.03	0.002	0.30	0.26	0.99
TN_AH	0.9 e -6	3.11 e -5	2 e -7	5.1 e -4	4.2 e -5	0.17
VR_AH	0.82	4 e-7	1.7 e-5	0.04	0.0001	6 e-7
MD_AH	0.98	0.99	0.99	0.99	0.96	0.04
ZM_AH	0.01	0.002	0.002	0.73	7.9 e-6	0.55
TN_ZR	0.23	0.53	0.36	0.34	0.12	0.057
VR_ZR	0.40	0.091	0.91	0.98	0.25	1 e-7
MD_ZR	0.0008	0.008	0.004	0.12	0.84	0.011
ZM_ZR	1	0.98	1	0.99	0.049	0.26
TN_AH	0.0004	0.96	0.96	0.84	0.99	0.018
MD_AH	0.31	0	3.9 e-5	0.013	0.007	0.082
ZM_AH	0.45	0.44	0.90	0.76	0.99	0.001
TN_ZM	0.20	0.94	0.33	0.085	0.99	0.99
MD_ZM	0.001	0.0004	0.004	0.44	0.0005	0.89
TN_MD	0	4.3 e-6	4 e-7	8.5 e-5	0.002	0.99

AH : Ahfir – YF : Yfri – ZR : Zarifate – TN : Tenira – VR : Virgin – MD : Madagh 2 – ZM : Zemmora

Principal component analysis PCA (individuals)

This data set contains 210 individuals and 7 variables, one qualitative variable is illustrative.

The first two axes of the analysis express 77.42% of the total inertia of the data set; this means that 77.42% of the total variability of the cloud of individuals is represented in this design. This is a high percentage, and the first plane therefore represents well the variability contained in a very large part of the active dataset, so the variability explained by this design is highly significant.

The projection of individuals on the factorial plane (Figure 2) shows that:

The tree populations of Tenira, Zemmora and Virgin regions are positively correlated with axis 1 (characterized by high values for the regions character); while the tree populations of Ahfir and fri regions were negatively correlated with axis 1.

On the other hand, the tree population of Madagh 2 is positively correlated with axis 2 while the population of Zarifate region correlated with the same axis but negatively.

The critical probability of the Wilks test indicates the variable whose modalities separates the individuals on the plane (i.e. which best explains the distances between individuals), in our case this variable is the regions (1.62499e-15).

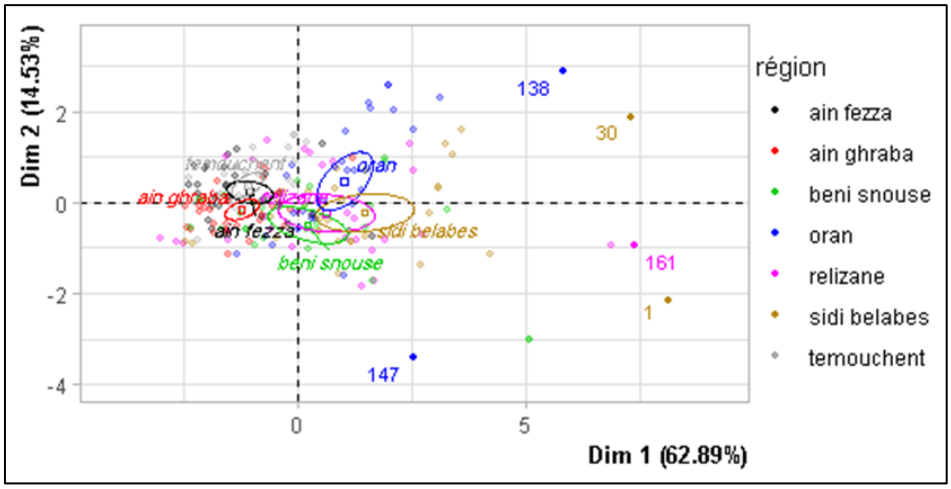

Figure 2: Graph of distribution of individuals of the seven different regions (PCA)

The labeled individuals are those with the greatest contribution to the construction of the design. The individuals are colored according to their belonging to the modalities of the region variable.

Principal component analysis PCA (variables)

The projection of the characters on the factorial plan (Figure 4) shows their division into three groups:

The first group composed of a single character, which is the length of the needle (LgNd), this character, is negatively correlated with axis 2,

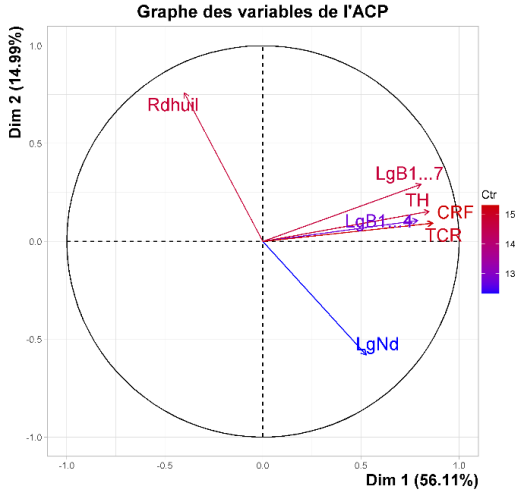

Figure 3: Graph of variables (PCA).The variables labeled are those best represented on the plane.

(TH (tree height) and TCR (tree crown), Lg B2 (secondary branch length), LgNd (needle length), CRF (girth) and Lg B1 (primary branch length), yield oil (Rdhuil)

The second group includes the following quantitative characters: the height of the tree (TH), the circumference of the tree (CRF), the crown (TCR), the length of the primary branch (Lg B1) and the length of secondary branch. (Lg B2). These have a positive correlation with axis 1,

The third group composed of a single character, which is the yield oil (Rdhuil), this character, is positively correlated with axis 2 and correlated negatively with the length of the needle (LgNd), the same result was confirmed by the test of correlation between those two parameters

Shannon and Weaver index

The relative diversity index (H' mean) of all populations is about 0.90 (Table 6).This index ranged from 0.82 to 0.975 for the characters Circumference of the tree (CRF) and the height (TH). Over all, all characters revealed diversity ranging from 0.888 for the length of secondary branch (LgNd) to 0.927 for the crown (TCR) (Table 06).

The table below presents the results of the Shannon-Weaver variability indices for the studied characters and for each population. The highest means were0.97 for the girth character (CRF) and 0.967 for the tree height (TH).

This index varies between 0.975 for the circumference character (CRF) of the population of the region of Zarifate and 0.833 for the same quantitative parameter of the population of the region of Ahfire.

The index also varies between 0.86 for the character tree crown (TCR) of the population of the region of Zemmora and 0.959 for the two populations of the two regions of Zarifate and Yfri.

The index also varies between 0.82 for the character height of the tree (TH) of the population of the region of Madaghe 2 and 0.959 for the population of the region of Zarifate.

The index also varies between 0.85 for the character length of the primary branch (Lg B1) of the tree of the populations of the regions of Tenira and Zemmora and 0.959 for the populations of the region of AinYfri and Zarifate.

The index varies from 0.83 for the character length of the secondary branch (Lg B2) of the tree of the population of the region of Tenira and 0.96 for the population of the region of Virgin.

The index oscillates between 0.84 for the character length of the needle (LgNd) of the tree of the population of the region of Ahfir and 0.95 for the population of the region of Virgin.

According to the high mean value of Shannon and weaver index that is 0.952 for Zarifate forest, we can deduce that this population is the most genetically diversified however, Madagh forest which have the low mean value (0, 87) of Shannon and weaver index is the lower diversified.

It should be noted that no similar work has been done, which has deprived us for a discussion and a comparison with other results and works.

Table 6: Relative diversity index of Shannon-Weaver (H') of characters for the seven different regions

	Yfri Forest	Ahfire Forest	Zarifate Forest	Tenira Forest	Virgin Forest	Madagh 2 Forest	Zemmora Forest	Mean
TH	0,946	0,913	0,959	0,946	0,94	0.82	0,94	0,925
TCR	0,959	0,953	0,959	0,933	0,91	0,93	0,86	0,927
CRF	0,913	0.833	0.975	0,932	0,88	0,91	0,85	0,899
Lg B1	0,959	0,942	0,953	0,854	0,86	0,87	0,85	0,896
Lg B2	0,888	0,856	0,934	0,834	0,96	0,84	0,95	0,895
Lg Nd	0,857	0,843	0,932	0,941	0,95	0,85	0,86	0,888
Mean	0,920	0,890	0,952	0,907	0,916	0,87	0,885	0,90

Hierarchical ascending classification (HAC)

The classification carried out on the individuals reveals three classes**.** (Figure 4, 5)

Cluster 1 is composed of individuals such as 18 from Virgin forest, 33, 45, and 47 from Zemmora forest, 65, 73, 76 from Madaghe 2 forest and 209 from Zarifate forest. This group is characterized by:

- Low values for the variables tree crown (TCR), tree height (TH), girth (CRF), primary branch length (Lg B1), secondary branch length (Lg B2) and needle length (LgNd) (from extreme to least extreme).

Cluster 2 is composed of individuals such as 16 from Virgin forest, 136, 141,149 from Yfri forest and 180 of Ahfire forest. This group is characterized by:

- High values for the variables tree crown (TCR), secondary branch length (Lg B2), primary branch length (Lg B1), tree height (TH), girth (CRF) and needle length (LgNd) (from extreme to least extreme).

Cluster 3 is composed of individuals such as 3, 5, and 30 from the Virgin forest and 94 from Tenira forestThis group is characterized by:

- High values for the variables tree height (TH), girth (CRF), tree crown (TCR), primary branch length (Lg B1), secondary branch length (Lg B2)and needle length (LgNd) (from extreme to least extreme).

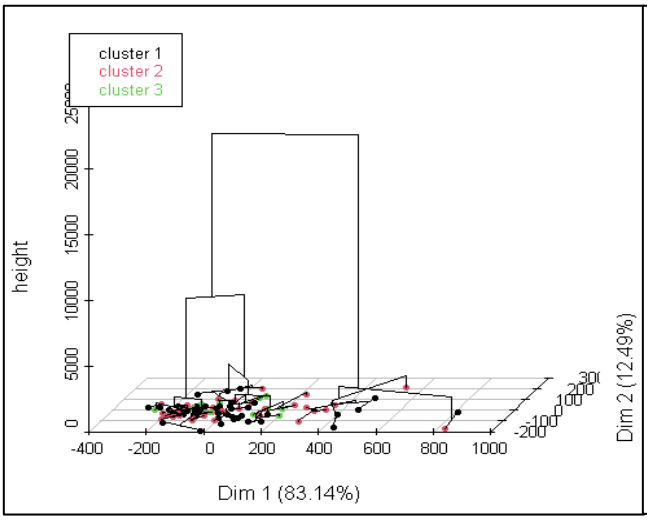

Figure 4. Ascending hierarchical classification (AHC)

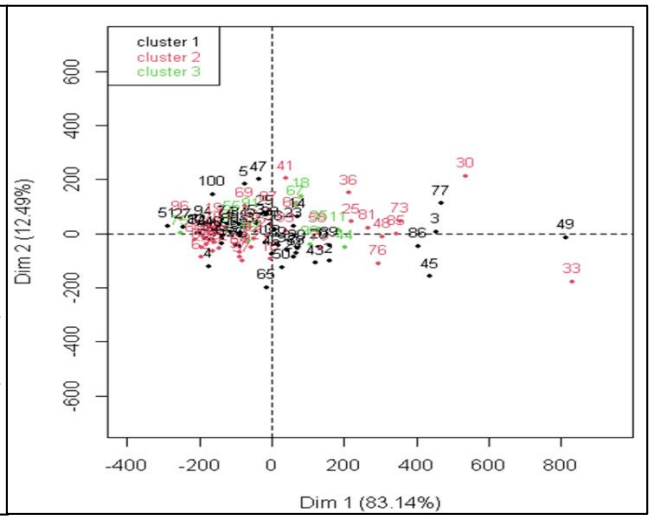

Figure 5. Hierarchical Ascending Classification of Individuals (HAC)

Qualitative Parameters:

Distribution of phenotypic classes.

Needle color (ClNd):

The distribution of the classes relating to the color of needles (Figure 6) shows the dominance of the class " green " for the trees of the population of Tenira and Virgin" light green " for the trees of the of Zarifate and Ahfir, "dark green" for the population of trees in Yfri, and Zemmora, and both "dark green and green" for trees in the forest of Madaghe 2

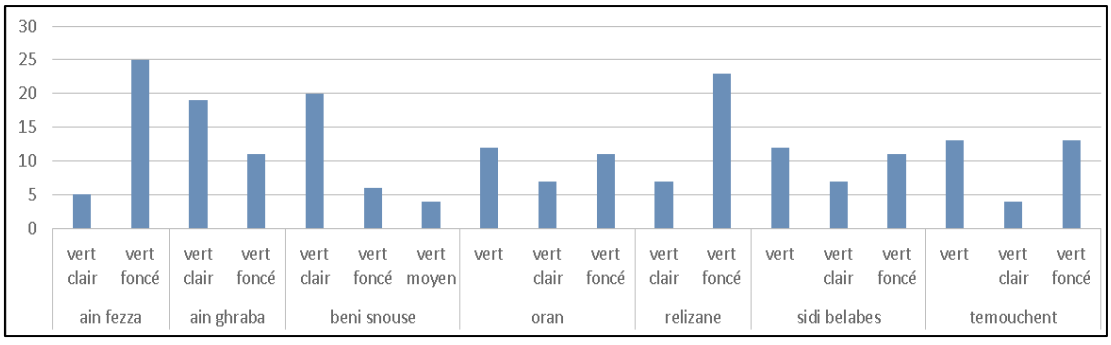

Figure 6. Representation of the number of repletion for the different colors of needles in the seven regions

Color of (ClBt):

The distribution of the classes relating to the color of balls (Figure 07) shows the dominance of the class "gray blue" for the trees of zarifate and Yfri, «gray" for Ahfire, "No balls" for Virgin, Madaghe 2, Tenira and Zemmora.

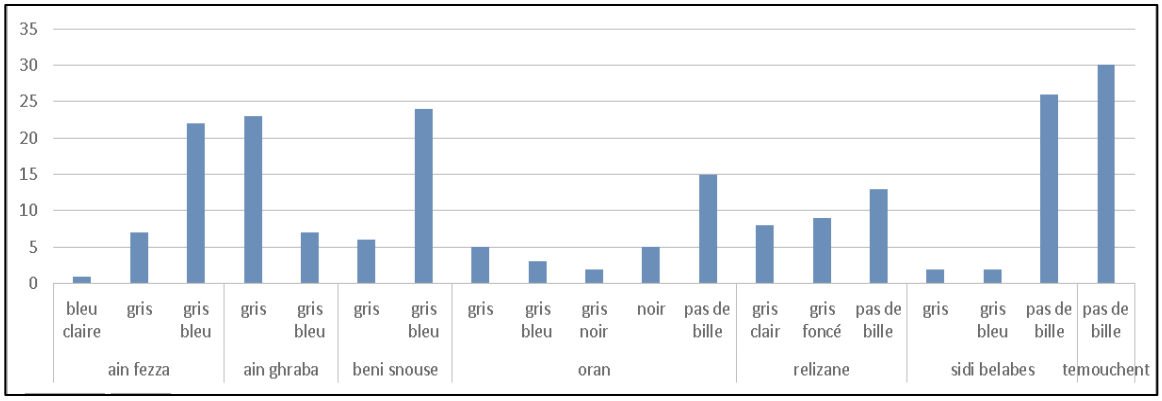

Figure 7. representation of the different colors of Thuja logs in the seven regions

Chi-square

In this chi-square test, we studied the relationship between the region and the color of the needles and that with the color of the log.

Color of the Babet (ClBt) / region:

According to the results of the chi-square test**)**; The two chi-square values (at 5% and at 1%) were respectively 58.12 and 66.20 which is lower than the chi-square threshold (282.96), so the null hypothesis (H0: no relationship between the variable and the region) is rejected), so H1 is accepted which means that there is a relationship between the character which is the color of the Babet and the region with a risk of error of 0.01.

Needle color (ClNd)/region:

According to the results of the chi-square test the two chi-square values (at 5% and at 1%) were respectively 28.86 and 34.80 which is lower than the chi-square threshold (95.32), so the null hypothesis (H0: no relationship between the variable and the region) is rejected, so H1 is accepted which means that there is a relationship between the character that is the color of the needles and the region with a risk of error of 1%.

Note: the color of the needles average green exists only in the region of Beni snous.

Shannon and Weaver index

The relative diversity index (H' mean) of all individuals is about 0.692 (Table 7).This index ranged from 0.441to 0.948, respectively, for the characters Color of balls and Color of needles. Over all, both characters revealed diversity ranging from 0.648 for the Color of needles to 0.737for the Color of Babet (Table 8).

The Table below presents the results of the Shannon-Weaver variability indices for the two qualitative characters studied. The highest character means are 0.97 for the needle color character and 0.88 for the Babet color character.

The index also varies between 0.57 for the character color of the needles of the tree of the population of the forest of the virgin and 0.88 for the population of the region of Madagh 2 forest.

The index also varies between 0.44 for the character color of Babet of the tree of the population of the

forest of Tenira and 0.78 for the population of the region of Ahfire forest.

According to the high mean value of Shannon and weaver index, which is 0.867 for Ahfire, forest we can deduce that this population is the most genetically diversified however, madagh 2 forest which have the low mean value (0.44) of Shannon and weaver index is the lower diversified.

We notice that compared to the results obtained of the relative index of Shannon and Weaver (H') for the quantitative characters (0.95) the H' of the two qualitative characters is low (0.69) which means that there is moderate genetic diversity of these two characters the bead color and the color of the needles.

Table 7. Relative Shannon and Weaver index of the different studied qualitative traits of Thuja genotypes.

Traits	Yfri Forest	Ahfire Forest	Zarifate Forest	Tenira Forest	Virgin Forest	Madagh2 Forest	Zemmora Forest	Mean
Clr balls	0.693	0.783	0.721	0.441	0.80	0.88	0.85	0.737
Clr needles	0.650	0.948	0.783	0.977	0.57	0 (no balls)	0.63	0.648
Mean	0.671	0.867	0.752	0.709	0.685	0.44	0.74	0.692

Multiple Correspondence Analyses (MCA)

Based on the qualitative characters (color of the ball and color of the needles), the MCA classifies the individuals in four groups (Figure 8), each with a set of characters that differentiates in:

The population of Tenira region negatively correlated with axis 2; characterized by

Needle color: green

- ✓ Color of babet: absence of ball

The population of the region of Ahfire correlated negatively with axis 2; characterized by:

- ✓ Needle color: light green
- ✓ Color of babet: gray

The population of the region of Zarifate correlated positively with axis 1; characterized by:

- ✓ Needle color: medium green
- ✓ Color of babet: blue gray

The population of the region of Yfri correlated positively with axis 2; characterized by:

- ✓ Needle color: dark green
- ✓ Color of babet: light blue and blue gray

The population of the region of Madagh 2 correlated negatively with axis 1; characterized by:

- ✓ Needle color: dark green
- ✓ Color of babet: no bead

The population of the region of Virgin correlated negatively with axis 1; characterized by:

- ✓ Color of needles: dark green
- ✓ Color of babet: black and gray black

The population of the region of Zemmora correlated positively with axis 2; characterized by:

✓ Color of babet: dark gray and gray black

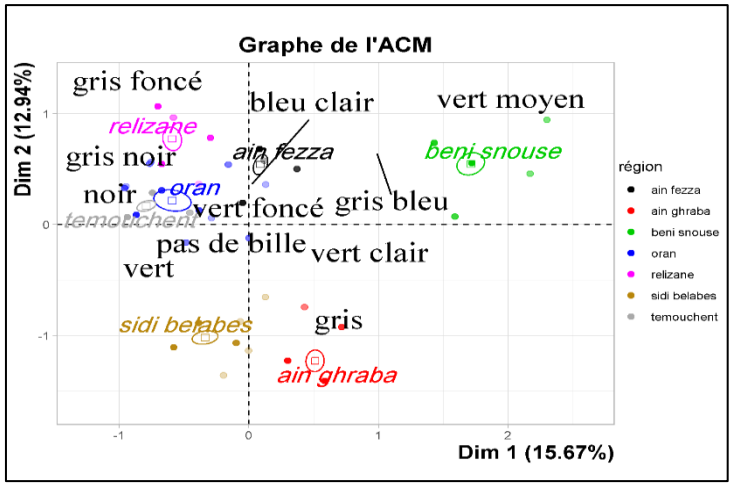

Figure 8: Multiple Correspondence Analysis (MCA)

Conclusion

Algeria, within the Mediterranean basin, is a country with high biological diversity given its contrasting landscape (Yahi *et al* .2012). The Tellian region, which covers more than 4% of the national territory, contains 2/3 of the Algerian fauna and flora. (Quezel, 1964; Véla & Benhouhou, 2007). A diversity that includes 289 species fairly rare, 647 rare species, 640 species very rare, 168 species endemic and 1779 interesting plants to be developed, according to data from FAO dated 2012 (FAO.2012).

The data collected during our field trips were statistically analyzed using the R software that demonstrated that the different samples of our seven populations were well differentiated and genetically diversified.

The results of the multiple correspondence analysis (MCA) and the hierarchical classification (HCA) showed a clear and very good diversity of the samples and have confirmed the negative correlation between oil yield and the length of needle (LgNd).The extraction of essential oils showed that the Beni snous region gives a good oil yield compared to other regions, which is very important for future economic perspectives and that the yield performance is related to the length of the needles(LgNd) wich can be used to identify the subjects with the best performance.

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