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Research Article

Morphological and Anatomical Characterization of Ecotype Needles of *Cedrus atlantica* in Morocco

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Atlas cedar (*Cedrus atlantica* (Endl.) G. Manetti ex Carrière) is an endemic species in the mountains of North Africa that is attracting international interest in its use in the reforestation of degraded ecosystems. This study aims to investigate and evaluate the morphoanatomical characteristics of needles of four cedar populations localized in the Middle and High Atlas Mountains. Descriptive statistics, analysis of variance (ANOVA), descriptive power, scatter-plot of the discrimination function, scatter-plot of discrimination, and dendrogram of the closest Euclidean distances were made on traits. The results of the linear model of ANOVA nested as population and tree within population suggest the differences statistically significant for the traits measured at a different level. Among these traits, the length of the needle, the width of a vascular bundle including endodermis, and thickness of the wall of hypodermis cell revealed the highest discriminating characters among populations of *C. atlantica* from the Middle and High Atlas and between the populations of the Middle Atlas. The agglomeration of populations over short Euclidean distances also showed a higher level of differentiation between two ecotypes of *C. atlantica* not very geographically distant in the Atlas Mountains of Morocco. The ecotype belonging to Aït Oufella and Aït Ayach confers this species a place of choice in the projects of revalorization of the Mediterranean populations, especially in semiarid areas.

1. Introduction

Cedrus is one of eleven commonly accepted genera in Pinaceae [1] and comprises four species with a highly disjunct distribution in the Mediterranean region and western Himalaya [2]. The results of many studies indicate that the Mediterranean cedars differ in some morphological traits [3–6] and a great debate in species taxonomic rank and phylogeny of *Cedrus* occurring in the Mediterranean Basin remains still unresolved [2, 3, 6–17].

Several studies have reported morphological traits of *C. atlantica* [5, 18–23]. The specimens with silver needles from the Atlas Mountains were described as a variety *C. atlantica* var. *glauca* [24, 25]. Depending on the different shapes of the crown and the needle color, several horticultural selections do exist [26]. In the Atlas Mountains, two morphologically well-differentiated ecotypes were reported in many studies: the *Meridionalis* type of the Moroccan High Atlas, and the Algerian Saharan Atlas, and the more recent *Tellica* type, located in the Tellian Atlas of Morocco (the Middle Atlas and the Rif) and Algeria [3, 27, 28]. Similarly, two groups distinguished by their morphological and phytoecological structure were also found in the central Middle Atlas: the group of the tabular Middle Atlas in the North and the Middle Atlas group folded in the South [29–31]. In a similar way, Terrab et al. [32, 33] have found contrasting genetic differentiation patterns at the Middle Atlas and among the Middle and the High Atlas populations of *C. atlantica*.

The current distribution of Atlas cedar does not reflect its ecological range [34, 35] and the vulnerability to temperature-induced drought stress in old cedar trees lead to an impending growth decline in the Moroccan Middle Atlas forests [36]. A new putative glacial refugia of *Cedrus atlantica* was found and revealed a fast contraction with shifting in altitude of the species range, showing more fragmented areas and even species disappearance in a number of nations of North Africa [37]. Moreover, these regions are expected to experience drastic climate change [38, 39], with an increase in temperature of 4-5°C and a reduction in precipitation of >30% in the next few decades [40] suggesting the disappearance of more *C. atlantica* populations. These data state that being an age-related drought-sensitive which may migrate to a new glacial refuge, the Atlas cedar is prone to disappearance by climate change in other regions. This leads to the reason why this investigation which is the first in North Africa was conducted on the characterization of potential ecotypes for effective cedar conservation of Morocco.

The aim of this paper was to provide a detailed description of the morphoanatomical characteristics of natural ecotypes of *C. atlantica* from the Middle and High Atlas Mountains of Morocco.

2. Materials and Methods

2.1. Sampling and Measurements. On transect from the Middle to High Moroccan Atlas, four populations of *Cedrus atlantica* were selected during 2015. The material for the study was collected from three populations on the localities Tamrabta, Moudemame [41], and Aït Oufella in the Middle Atlas and from one population on the locality Aït Ayach in the High Atlas region (Figure 1, Table 1). The annual mean temperatures and precipitations in each geographic location of populations (Table 1) were obtained from https://www.climate-data.org. The population of Tamrabta is located to the East with a subhumid bioclimate to very cold winter, unlike the population of Moudemame with a stable fresh humid bioclimate (Table 1). The Aït Oufella population with an area of 5 650 ha is located on the southern edge of the Middle Atlas about 40 km northwest of Midelt [42]. The lithology associates the calcareodolomitic Lias with the marl-limestone Cretaceous, to which are added some pockets of red clay and Triassic basalts [43]. The general climate of Aït Oufella is characterized by a low rainfall and a marked drought during a long part of the year [44]. The fourth cedar population of Aït Ayach developed before 7 km from Cirque de Jaaffar is characterized by the presence of *C. atlantica* and *Quercus ilex* (Table 1). The climate of this forest is semiarid continental. Healthy old trees in number of ten spaced at a distance of no less than 30 m from each other of each population were sampled (Table 1). Ten mature whorls undamaged, fully developed were collected from each tree formed in similar light conditions and conserved in 70% alcohol at −20°C during a month. One mature needle (representing the oldest and longest whorl needles) was analyzed from each whorl, with a total of $n = 10$ trees in each population and 10 needles within each tree. The anatomic preparations were performed freehand at the central portion of each needle, and the cross-sections were treated with 5% NaOH for 4 h at 70°C for clearing tissues, according to the methods of Arnott and Brady cited by Ruzin [45]; then, they are placed for a few minutes in a 0.05% solution of Toluidine blue (No. 52040, Mumbai 400005, India).

Fifteen morphological and anatomical characters of the needle were studied. The length of the needle (Nl) was determined manually with an accuracy of 0.2 mm. The number of the resin canals (Nc) is counted for each cross-sections under a light numeric microscope (DM-15 Optika, Ponteranica, Italy), and the measurement of the radial dimension of the hypodermal cell (Hh) and the radial dimension of the endodermal cell layer (Ce) is made on the largest cell of the hypodermis and the endoderm of each needle (Figure 2). The preparations were then photographed with the integrated camera of the same microscope. The measurements of the characters (Figure 2) with an accuracy of 1 μm were selected on the ten technically best images selected for every individual, using software for image analyses (Opmias Software ver. 1.3.0.0.). The majority of the selected characters studied here (Figure 2) has been detected, in previous studies, as discriminating among taxa in the anatomical and morphological examinations of the needle characteristics of *Pinus* and *Cedrus* species [10, 46, 47].

2.2. Analyses. Descriptive statistics (arithmetic mean, minima, maxima, standard error, and variation coefficient) for each trait among the populations were calculated. The average values of every feature for each tree were used to conduct discrimination analysis. The Shapiro–Wilks test was used to verify the data distribution, and the Levene test assessed the homoscedasticity of the data variance prior to multivariate comparisons. Then, analysis of variances (-ANOVAs) was carried out by using a nested-linear model for determining and assessing the significance of the variance of each morphological and anatomical trait of needles, which differs between populations and between trees within the population. The post hoc analysis was conducted using Tukey's test for multiple comparisons at $P < 0.05$. The discrimination power of a particular character was determined in discrimination analysis. The relationships among the populations were estimated on the scatter-plot of the discrimination function on the space between the two first discrimination variables, after stepwise discrimination analysis on the whole set of traits [48]. The relationships among taxa and populations were illustrated on the graph of discrimination. A dendrogram of the closest Euclidean distances using all characters except for ratios, according to Ward's method, was applied in order to check the affinities revealed in the discrimination analysis. Statistical analysis was performed using the IBM SPSS Statistics 20.0 software.

3. Results

3.1. Within Population Variation. The hierarchical analysis of variance showed the tree within population differentiated at the highest significance ($P \leq 0.01$) by Bw, Bh, Ct, Dy, Eh, Ew, Hh, and Hw and at a lower level ($P \leq 0.05$) for Ch, Cw, Ht, and Nl (Table 2).

The highest values of the variation coefficients (CV) were detected for the Nc character whereas the slightly lower values were detected for the following variables: Ch, Ct, Cw, Ht, and Dy. The value of CV below 10% was typical for Nl in

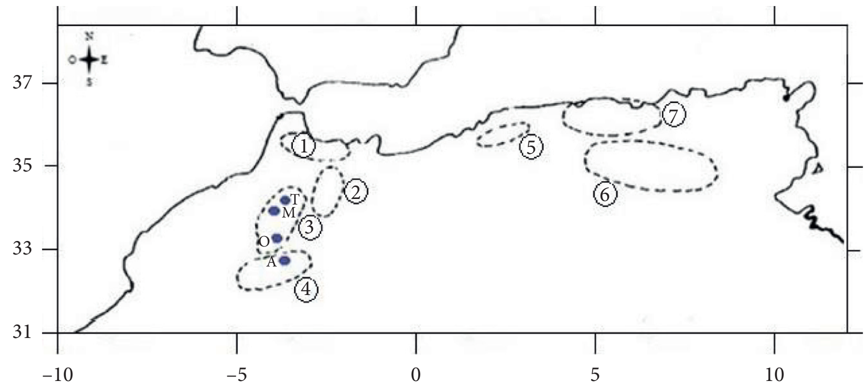

Figure 1: Distribution and localization of studied populations: Moudemame (M), Tamrabta (T), Aït Oufella (O), and Aït Ayach (A). The dashed lines delineate the natural distribution areas of *C. atlantica* in North African Rif (1), Eastern Middle Atlas (2), Central Middle Atlas (3), Eastern High Atlas (4), Ouarsenis (5); Aurès (6), and Djurdjure (7).

Table 1: Characteristics of four sampled populations of *C. atlantica* on transect from Middle to High Moroccan Atlas.

Regions	Localities (code population)	Number of selected trees	Coordinates	Elevation (m, amsl)	Parent rock	Bioclimate (Koppen–Geiger scale)	T (°C)	P (mm)
Middle Atlas Ifrane	Tamrabta (T): less dense population with maritime pine or green oak	10	33°37′N 5°03′W	1605	Calcareous dolomite/ sander	Csb: cold subhumid to very cold	11.3	8438
Middle Atlas Azrou	Moudemame (M): a pure and dense population	10	33°25′N 5°11′W	1780	Basalt-calcareous	Csa: fresh humid	14.2	779
	Aït Oufella (O): less dense population with green oak and *Juniperus thurifea*	10	32°58′N 5°03′W	1982	Calcareous	Bsk: semiarid continental	14.7	263
High Atlas	Aït Ayach (A): less dense population with green oak	10	32°31′N 4°59′W	1972	Calcareous	Csa: semiarid continental	12	459

Csa: hot-summer mediterranean climate; Csb: warm-summer mediterranean climate; Bsk: cold semiarid climate; T: annual mean temperature; P: annual precipitation.

the population of Moudemame (Table 3). The individuals of Aït Oufella population are characterized by a high degree of average variation 22.17% compared to other populations, determined mostly by such characters, as Bh, Bw, Dy, Ew, Hh, Hw, Nh, Ht, and Nl and for the individuals of the Tamrabta population by average variation coefficient of 36% showed by Ch and Cw (Table 3).

This study has shown the average length of the needles differs greatly between the studied populations. The highest values are in the needles of Moudemame (16.09 mm) and Tamrabta (15.68 mm), while the lowest values are detected at Aït Oufella (10.77 mm) and Aït Ayach (11.06 mm) (Table 3). For characters, Bh, Bw, Ce, Ch, Cw, Dy, Ew, and Nh the highest mean values were found at Aït Ayach, and the lowest values at Moudemame and Tamrabta (Table 3). Nevertheless, the minimum of most of these characters was recorded in Aït Oufella and Moudemame while the maxima at Aït Ayach and Aït Oufella. Similarly, the highest mean values of Ct were found at Aït Ayach but the lowest at Moudemame and Tamrabta (Table 3). The population of Aït Ayach has the highest mean values of resin canal size Cw (96.32 μm) and Ch (83.74 μm), and a smaller number of resin canal (1.16) (Table 3). Similarly, the highest average values detected in this population were in radial dimension of the epidermal cell (Eh) with 22.55 μm and hypodermic cells size (Hh and Hw) with 46.55 and 38.77 μm, respectively (Table 3).

3.2. Differentiation of Populations. The analysis of variance showed that populations were differentiated for all traits measured at the highest significance ($P \leq 0.01$) except for Nc, who is statistically significant at a lower level ($P \leq 0.05$) (Table 2). The results of the Tukey's test showed that the value of Nc did not differ at a statistically significant level among populations studied, while the Ht character only differs the Aït Oufella population from the Moudemame and Aït Ayach populations. While all other characters could be used in separation at least between four to five combinations of populations at a significant level of $P \geq 0.05$ (Table 3). The Nl and Hw traits significantly distinguish the populations Tamrabta, Moudmame, and Aït Oufella from the Aït Ayach population. While, the characters Bh, Bw, Ce, Ct, Dy, Eh, Ew, and Nh differ significantly between the Middle Atlas and the High Atlas populations and even between the populations of the Aït Oufella and Aït Ayach. Conversely, the Hh trait does not distinguish between the populations of Aït Oufella and Aït Ayach, whereas the character Ch establishes a significant discrimination between the populations

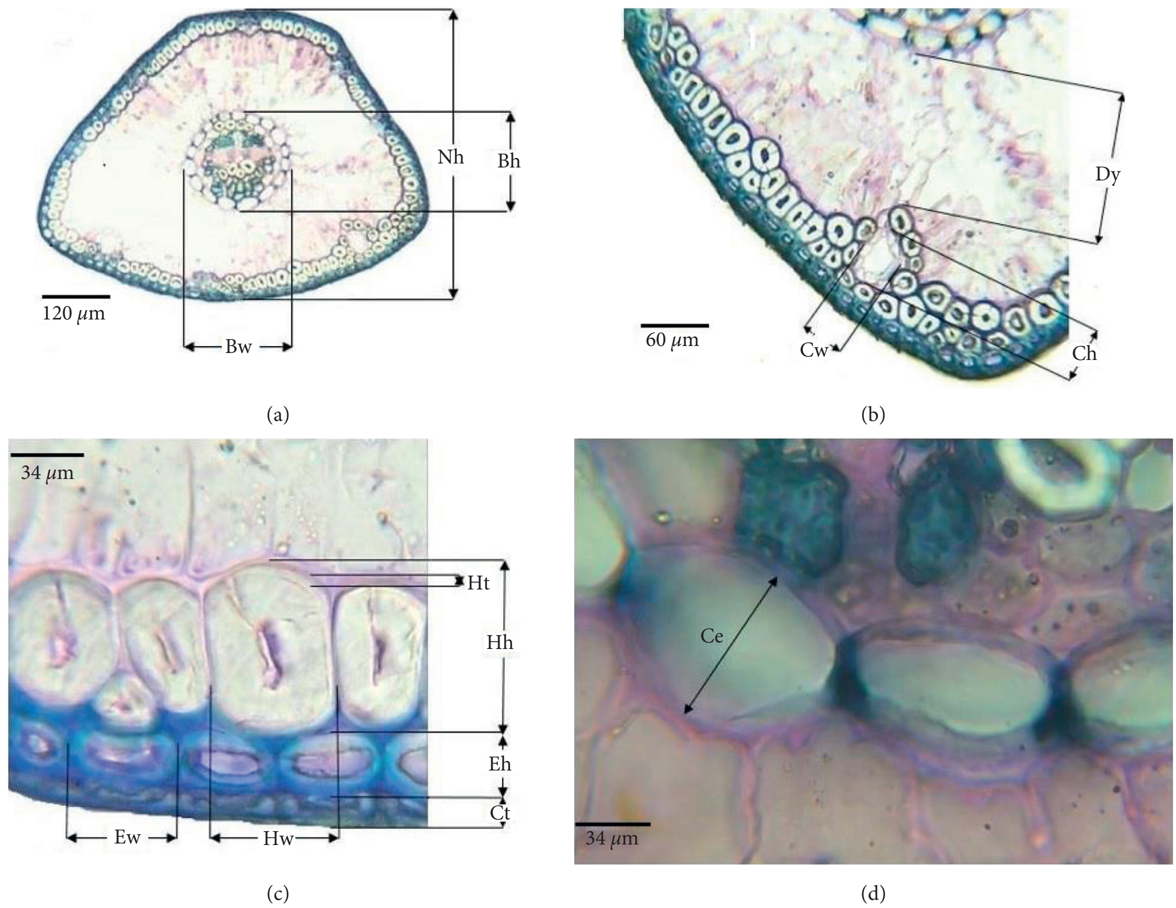

Figure 2: Measured characters of the cross-section of *C. atlantica* needle. (a) The image indicating height of needle cross-section (Nh), width of vascular bundle including endodermis (Bw), and height of vascular bundle including endodermis (Bh). (b) The image indicating width of resin canal (Cw), height of resin canal (Ch), and distance between resin canal and vascular bundle (Dy). (c) The image indicating cuticle thickness (Ct), tangential dimension of the epidermal cell layer (Ew), radial dimension of the epidermal cell layer (Eh), radial dimension of the hypodermal cell (Hh), tangential dimension of the hypodermal cell (Hw), and thickness of wall of hypodermis cell (Ht). (d) The image indicating radial dimension of the endodermal cell layer (Ce).

Tamrabta, Moudemame, and Aït Ayach and not between Tamrabta and Aït Oufella (Table 3).

The analysis of discrimination function revealed that 14 of 15 tested characters of the cedar needles had high discriminating power among populations ($P \leq 0.01$) and one character discriminate with $P \leq 0.05$ (Table 4).

The dispersion of individuals on the space between the two first discrimination values U_1 and U_2 (responsible for 98% of the total variation among populations) demonstrated three dispersed clouds of single trees, representing four compared populations (Figure 3). The discrimination variable U_1 was determined primarily by Nl, while component U_2 by Bw and Ht (Figure 3). This dispersion shows that the Middle Atlas populations (Moudemame and Tamrabta) are dispersed without overlap with the Aït Oufella populations of the Middle Atlas and the Aït Ayach one of the High Atlas (Figure 3). The individuals of the latter two populations are mostly intermingled with each other.

The agglomeration of populations over the shortest Euclidean distances indicated a higher level of differentiation between the Aït Ayach-Aït Oufella class and the Tamrabta-Moudemame class (Figure 4). This differentiation of populations confirms the results detected in the discrimination analysis (Figure 3).

4. Discussion

In the past, various studies were conducted on many characteristics of needles but only three analyzed set of characters of needle (needle length and width and height of needle cross-section) have been found to discriminate between *C. atlantica*, *C. libani*, *C. brevifolia*, and *C. deodara* [3, 5, 6, 49, 50]. Recently, Jasińska et al. [10] have reported an additional another set of needle characters (distance between resin canal and vascular bundle, number of the resin canals, tangential dimension of the hypodermal cell, etc.). Among these authors, only Farjon [6] and Jasińska et al. [10] indicated the origin of needles described by them (long shoots and short shoots). In addition, very few of these traits were used to distinguish between individuals of the same *Cedrus* species. In the case of *C. atlantica*, the present study shows the first results of needle morphology and anatomy on cedar

Table 2: Hierarchical analysis of variance based on the needle traits. *F* statistic value.

	Variance component	*F*	*P* value
Nl (mm)	Tree within population	3.80	0.01
	Between populations	370.08	0.001
Nh (μm)	Tree within population	0.45	0.72
	Between populations	439.31	0.001
Ct (μm)	Tree within population	13.97	0.001
	Between populations	175.48	0.001
Ew (μm)	Tree within population	219.55	0.001
	Between populations	219.55	0.001
Eh (μm)	Tree within population	7.19	0.001
	Between populations	89.93	0.001
Hh (μm)	Tree within population	4.58	0.001
	Between populations	169.87	0.001
Hw (μm)	Tree within population	4.23	0.001
	Between populations	107.92	0.001
Ht (μm)	Tree within population	2.81	0.03
	Between populations	109.81	0.001
Bw (μm)	Tree within population	247.78	0.001
	Between populations	707.84	0.001
Bh (μm)	Tree within population	219.75	0.001
	Between populations	608.28	0.001
Ce (μm)	Tree within population	2.16	0.09
	Between populations	143.61	0.001
Nc (no)	Tree within population	0.10	0.95
	Between populations	5.03	0.02
Cw (μm)	Tree within population	3.15	0.02
	Between populations	86.41	0.001
Ch (μm)	Tree within population	2.98	0.001
	Between populations	2.98	0.001
Dy (μm)	Tree within population	5.29	0.001
	Between populations	197	0.001

Nl, length of the needle; Nh, height of needle cross-section; Bw, width of vascular bundle including endodermis; Bh, height of vascular bundle including endodermis; Nc, number of resin canals; Cw, width of resin canal; Ch, height of resin canal; Dy, distance between resin canal and vascular bundle; Ct, cuticle thickness; Ew, tangential dimension of the epidermal cell layer; Eh, radial dimension of the epidermal cell layer; Hh, radial dimension of the hypodermal cell; Hw, tangential dimension of the hypodermal cell; Ht, thickness of wall of hypodermis cell; Ce, radial dimension of the endodermal cell layer.

ecotypes from Morocco. The values of the average length of needles found here (Table 2) are slightly close to that (17–19.32 mm) found by Krouchi [51] on individual adults of the cedar population of Algeria (Tala-guilef) but are contained into the interval value 10–20 mm of the maximum average length of *C. atlantica* needles reported by Boudy [52]. Similarly, Jasińska et al. [10] found recently mean values of needle length of 14.78 mm between 10 and 19 mm of the Middle Atlas Mountain of Morocco. These values are included in the margin of the average length of needles found in two localities of the Middle Atlas of this study (Table 2). In contrast, our values of the average length of needles from the whorls are found to be more inferior to the value of the max average length of *C. atlantica* needles (25 mm) announced by Farjon [6]. This difference may be due to the use of the needles from the long shoots, which are generally longer than those of whorls. On the other hand, values obtained in the present study are still low even when compared to those of *C. atlantica* whorl needles reported by Saouab and Bendriss Amraoui [53] or to those of the needles of young seedlings of 2–5 years in the Middle Atlas of Morocco and higher than those of 16 months and 2 years of Algerian provenances [54]. The values of the average length of needles of the populations of Aït Ayach and Aït Oufella that were found to be included in the margin of length 10.77–11.06 mm are lower than those found in this study Tamrabta and Moudemame ones (Table 2). On seedlings of provenances from the High Atlas (Tounfite and Assaka) grown in Maures, Arbez et al. [54] found values of the average length of needles of 23.8 and 19.5 mm, respectively. These comparisons show that the average length of cedar needles is highly dependent on shoot growth and environmental conditions [53], making it one of the most important varietal selection criteria for cedar if it was taken at a fully developed stage. Thus, the ontogenetic variations of the needle traits may not mislead when we use mature needles to distinguish the ecotype in this study.

The dimensions of the needle cross-section (Nw and Nh) have been reported as 772.27–910.89 μm for *C. atlantica*, *C. libani*, and *C. brevifolia* [10]. Moreover, Vidaković [5] and Farjon [6] have found that the average values of these traits were much bigger and discriminating between these *Cedrus* species. Several studies reported that these two traits allow the distinction between *Pinus mugo* sensu stricto, *Pinus uliginosa*, and *Pinus uncinata*, respectively, with a very high probability [50, 55, 56]. In other studies, Huang et al. [57] and Nikolić et al. [58] found a significant percentage variation in the width and the height of the needle between natural populations of *P. yunnanensis* in Southwest China and *P. peuce* in Montenegro and Serbia, respectively. On the contrary, Boratyński et al. [59] and Boratyńska et al. [54] have found that the width of needle does not always distinguish between different pairs of populations of *P. mugo* and *Pinus sylvestris* at a significant level, or between *P. uliginosa* and *P. sylvestris.* In the same sense, Urbaniak et al. [60] reported that neither width of needle (Nw) nor height of needle (Nh) or Nh/Nw distinguish between the *P. sylvestris* populations in the Tatra Mountains. In contrast, the average value of the height of needle obtained in this study is discriminating among populations of the Middle and High Atlas at a statistically significant level suggesting long-lasting isolation between them and adaptation to the local environment of *C. atlantica* (Table 4). Values of the width and the height of the needle reported by Jasińska et al. [10] for *C. atlantica* from the Rif and the Middle Atlas populations are different and smaller than our Middle and High Atlas values, which stand out among themselves. No one has so far described this difference between the *C. atlantica* populations of North Africa and France.

Cheddadi et al. [61] reported that environmental changes in Northern Africa since the last glacial period have had an impact on the geographical distribution of *C. atlantica* and its modern genetic diversity. Moreover, several authors have also detected consistent growth declines and increased drought sensitivity in Atlas cedar by climate variability and age across

Table 3: Statistic description of analyzed characters for populations of *C. atlantica*: Moudemame (M), Tamrabta (T), Aït Oufella (O), and Aït Ayach (A).

Traits	Mean ± SD				Minimum				Maximum				Variation coefficient			
	M	T	O	A	M	T	O	A	M	T	O	A	M	T	O	A
Nl (mm)	$^{a}16.09 \pm 1.60$	$^{a}15.68 \pm 2.05$	$^{b}10.77 \pm 1.89$	$^{b}11.06 \pm 1.37$	13.00	11.00	7.00	8.00	20.00	22	15.00	15.00	9.96	13.10	17.56	12.40
Nh (μm)	$^{a}771.26 \pm 85.23$	$^{a}794.66 \pm 123.82$	$^{b}998.45 \pm 194.74$	$^{c}1180.11 \pm 168.33$	600.85	582.22	541.11	96.07	970.32	1268.82	1306.81	1610.75	11.05	15.58	19.50	14.26
Ct (μm)	$^{a}9.36 \pm 2.36$	$^{a}8.84 \pm 2.85$	$^{b}12.27 \pm 4.02$	$^{c}14.25 \pm 3.56$	2.25	1.00	3.52	4.85	14.14	15.81	21.84	22.09	25.27	32.27	32.74	24.98
Ew (μm)	$^{a}24.71 \pm 3.33$	$^{a}26.08 \pm 4.67$	$^{b}31.17 \pm 6.88$	$^{c}35.56 \pm 5.92$	17.28	18.60	16.75	23.37	33.49	43.35	48.10	55.84	13.47	17.90	22.06	16.65
Eh (μm)	$^{a}17.73 \pm 2.57$	$^{a}18.14 \pm 4.09$	$^{b}20.85 \pm 4.21$	$^{c}22.55 \pm 4.62$	11.18	10.85	11.31	13.25	26.41	47.53	35.93	39.00	14.47	22.55	20.21	20.50
Hh (μm)	$^{a}33.30 \pm 4.79$	$^{b}36.42 \pm 4.79$	$^{c}44.88 \pm 9.85$	$^{c}46.55 \pm 6.82$	23.83	26.11	24.77	29.79	42.93	51.04	70.90	63.45	14.37	13.16	21.96	14.64
Hw (μm)	$^{a}29.44 \pm 5.19$	$^{a}31.14 \pm 5.69$	$^{b}37.06 \pm 8.20$	$^{b}38.77 \pm 6.66$	13.33	18.56	21.81	24.78	44.28	51.04	56.93	54.93	17.63	18.26	22.11	17.19
Ht (μm)	$^{a}3.19 \pm 0.87$	$^{a,b}2.93 \pm 0.72$	$^{b}2.69 \pm 0.87$	$^{a}3.14 \pm 0.85$	1.94	1.13	0.97	1.00	6.43	4.93	5.08	5.37	27.40	24.44	32.39	27.18
Bw (μm)	$^{a}324.16 \pm 39.74$	$^{a}320.94 \pm 61.36$	$^{b}486.01 \pm 125.45$	$^{c}587.41 \pm 62.67$	241.74	241.07	222.31	398.33	422.15	629.13	783.98	732.20	12.26	19.12	25.81	10.67
Bh (μm)	$^{a}293.80 \pm 59.15$	$^{a}293.65 \pm 55.50$	$^{b}437.33 \pm 111.45$	$^{c}525.81 \pm 70.46$	227.12	221.05	202.83	365.00	377.64	569.29	778.26	672.27	12.11	18.90	25.48	11.25
Ce (μm)	$^{a}38.25 \pm 6.41$	$^{a}37.72 \pm 8.40$	$^{b}44.92 \pm 11.06$	$^{c}51.17 \pm 9.66$	25.08	21.27	22.20	28.88	61.12	72.92	73.32	77.69	16.75	22.28	24.63	18.87
Nc (no)	$^{a}1.27 \pm 0.80$	$^{a}1.23 \pm 0.72$	$^{a}1.24 \pm 0.79$	$^{a}1.16 \pm 0.71$	0.00	0.00	0.00	0.00	2.00	2.00	2.00	2.00	63.17	58.76	63.24	61.13
Cw (μm)	$^{a}55.15 \pm 12.13$	$^{b}70.69 \pm 25.03$	$^{c}87.15 \pm 23.64$	$^{d}96.32 \pm 27.27$	27.70	40.00	35.48	31.41	88.33	208.98	141.88	155.56	22.00	35.40	27.13	28.31
Ch (μm)	$^{a}46.88 \pm 11.41$	$^{b}61.40 \pm 22.47$	$^{a}74.07 \pm 20.94$	$^{c}83.74 \pm 24.49$	21.84	32.93	32.21	23.56	77.72	184.69	130.94	142.71	24.34	36.60	28.27	29.25
Dy (μm)	$^{a}196.38 \pm 45.09$	$^{a}183.30 \pm 43.44$	$^{b}275.48 \pm 93.20$	$^{c}316.79 \pm 79.40$	73.90	88.88	80.03	145.72	285.62	319.71	490.47	488.44	22.96	23.70	33.83	25.07

Different letters indicate significant differences between populations at $P \leq 0.05$ as revealed by Tukey's multiple comparison post hoc test; $n = 100$ needles for each population. Nl, length of the needle; Nh, height of needle cross-section; Bw, width of vascular bundle including endodermis; Bh, height of vascular bundle including endodermis; Nc, number of resin canals; Cw, width of resin canal; Ch, height of resin canal; Dy, distance between resin canal and vascular bundle; Ct, cuticle thickness; Ew, tangential dimension of the epidermal cell layer; Eh, radial dimension of the epidermal cell layer; Hh, radial dimension of the hypodermal cell; Hw, tangential dimension of the hypodermal cell; Ht, thickness of wall of hypodermis cell; Ce, radial dimension of the endodermal cell layer.

Table 4: Discrimination among sampled populations revealed by partial Wilks' lambda (λ) and P value for analyzed characters.

	Nl (mm)	Nh (μm)	Ct (μm)	Ew (μm)	Eh (μm)	Hh (μm)	Hw (μm)	Ht (μm)	Bw (μm)	Bh (μm)	Ce (μm)	Nc (No)	Cw (μm)	Ch (μm)	Dy (μm)
λ	0.33	0.44	0.71	0.60	0.80	0.57	0.73	0.95	0.31	0.36	0.72	0.97	0.69	0.70	0.60
P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.020	0.001	0.001	0.001

Nl, length of the needle; Nh, height of needle cross-section; Bw, width of vascular bundle including endodermis; Bh, height of vascular bundle including endodermis; Nc, number of resin canals; Cw, width of resin canal; Ch, height of resin canal; Dy, distance between resin canal and vascular bundle; Ct, cuticle thickness; Ew, tangential dimension of the epidermal cell layer; Eh, radial dimension of the epidermal cell layer; Hh, radial dimension of the hypodermal cell; Hw, tangential dimension of the hypodermal cell; Ht, thickness of wall of hypodermis cell; Ce, radial dimension of the endodermal cell layer.

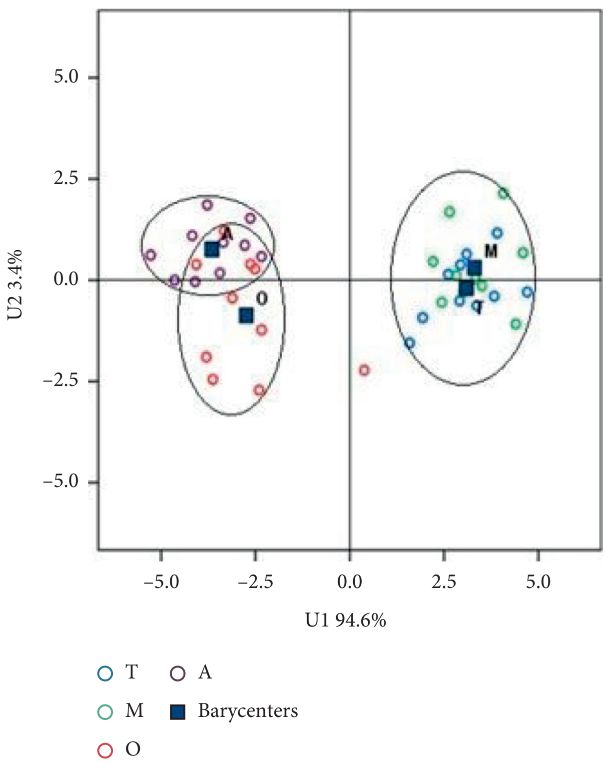

Figure 3: Dispersion of individuals of the studied populations of *C. atlantica* in the first two discriminant axes (U_1 and U_2). Moudemame (M), Tamrabta (T), Aït Oufella (O), and Aït Ayach (A). Barycenter: it is the point of average coordinates of all the explanatory variables of a group of observations.

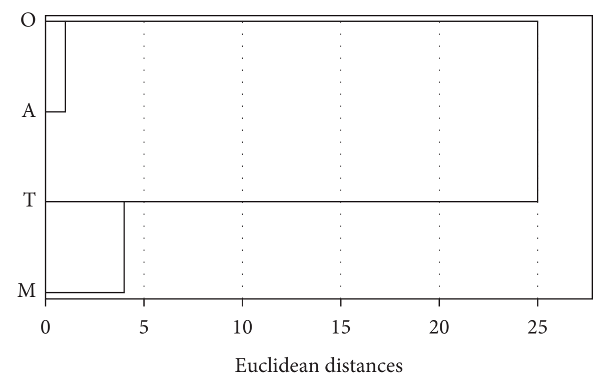

Figure 4: Relationship among populations of *C. atlantica* growing in Moudemame (M), Tamrabta (T), Aït Oufella (O), and Aït Ayach (A) on the shortest Euclidean distances from morphological and anatomical characteristics of the needles.

many sites since the early 1970s [36, 37, 42, 62–66]. For example, Rafael et al. [66] found that the decline of Algerian *Cedrus atlantica* forests is driven by a climate shift towards drier conditions. These observations are in agreement with our study, which showed that all needle characters were found separating distant populations (Figure 3) at a statistically significant level (Table 4), suggesting that they are under the control of environmental conditions more than the others are. Unlike this, on the genus, *Cedrus* Jasińska et al. [10] have found that only the traits: height of vascular bundle (Bh), size of epidermis cell layer (Eh, Ew), size of hypodermis cell (Hh, Hw), and the proportion of needle/vascular bundle width (Nw/Bw) distinguished between *C. atlantica*, *C. libani*, and *C. brevifolia*. Whereas they found that, the cuticle thickness (Ct), the width of vascular bundle (Bw), and the shape of needle (Nl/Nw) were not discriminant.

On the other hand, in a taxonomic study of the Central-European pines, Boratyńska et al. [56] have found only the tangential dimension of the epidermal cell layer (Ew) character discriminate between taxa whereas radial dimension of this trait (Eh) remains similar. Between the populations of Mediterranean firs, Sękiewicz et al. [67] have found that populations of Morocco differ more of Spanish populations in terms of radial dimension of the hypodermal cell (Hh) and not for radial dimension of the epidermal cell layer (Eh), which instead shows an intrapopulation variation. In contrast, Huang et al. [57] have shown that the width of vascular bundle including endodermis (Bw) trait contributes genetically in a similar way between and within populations of *P. yunnanensis*. In addition to these traits already known, a new group of characters (Ct, Bw, Ce, Ht, Cw, and Ch) found them for the first time here discriminating between populations of *C. atlantica*. Some of these traits like Ce, Ht, Cw, and Ch may be also discriminating between *Cedrus* species, so they should be tested in addition to those discovered by Jasińska et al. [10].

The number of the resin canals (Nc) does not discriminate between populations of *C. atlantica* (Tables 3 and 4) as found between *P. sylvestris* populations by Urbaniak et al. [60], but it was found to discriminate between species and populations in other studies [10, 57, 67, 68]. The stability of the Nc trait in our study is probably due to the fact that our samples were harvested at the same stage of maturity and position on the individuals because according to Lin et al. [69], the density of the number of the resin canals can be influenced by crown position and age of trees.

Analysis of discrimination on the individuals revealed the highest level of distance between the Middle Atlas populations (Tamrabta and Moudemame) and the two other compared populations of Middle and High Atlas (Aït Oufella and Aït Ayach) (Figure 3). No individual of Tamrabta and Moudemame populations enters the 95% confidential area of individual dispersion neither for Aït Oufella nor for Aït Ayach (Figure 3). The clustering analysis confirmed this result (Figure 3) and displayed a strong relation of Tamrabta to Moudemame and Aït Oufella to Aït Ayach populations (Figure 4). These multivariate analyses support the existence of two ecotypes of *C. atlantica* geographically distant in the Atlas Mountains of Morocco. As found for calcareous land [70, 71], the ecotype belonging to the population from Aït Oufella and Aït Ayach could be chosen in the projects of revalorization of the Mediterranean, especially on the semiarid and arid near- Sahara mountains [72] to stop a steppization process which is increasingly affecting *C. atlantica* forests in Morocco and Algeria [73]. Finally, further research may be made to characterize more of these two ecotypes at a genetic level. The genetic diversity and the population differentiation reported by many studies between distant populations of the Middle and High Atlas of *Cedrus atlantica* in Morocco are in agreement with this idea [32, 33, 61, 74, 75].

The capacity of trees to survive aridity depends on a group of physiological and morphological changes including stomatal closure [76], inhibition of shoot growth [77], and reduction of root longevity [78]. Similarly, EL Amrani and Bendriss Amraoui [79] have recently found that the continuous and partial mechanical barriers combined with the low availability of water reduce the aerial part growth and the main root length of *C. atlantica* seedlings. Studies on anatomical adaptation to drought were made by Kivimäenpää et al. [80] on Norway spruce needles vascular cylinder area. Abe and Nakai [81] found reduction by drought in tracheid lumen area of *Cryptomeria japonica* D. Don. Recently, Gebauer et al. [82] found that drought reduced the values of fifteen anatomical and hydraulic traits of sun needles of Norway spruce compared with shade needles, only tracheid maximum diameter was reduced by drought. In Mediterranean ecosystems, water availability plays an important role in determining phenological development [83]. In the present study, the length of needle (Nl), the width of vascular bundle including endodermis (Bw), and the thickness of wall of hypodermis cell (Ht) which were the highest discriminating characters between populations of the Middle and High Atlas and between populations of the Middle Atlas are important longer-term adaptations to semiarid climate. This acclimatization prevents evapotranspiration by increasing Ht and reducing Nl and favors needle water transfer by increasing Bw (Table 2). This observation is in agreement with the study of EL Amrani and Bendriss Amraoui [79] who found an increase of root phloem and xylem areas in the existence of mechanical impedance combined with the low availability of water.

These traits may be powerful tools to select individuals genotypically adapted to drought conditions, particularly in the arid area-Sahara mountains of Algeria in which Allen et al. [72] reported that the recent *C. atlantica* mortality began as small patches on drier aspects, eventually coalescing into large patches affecting all ages on all exposures.

5. Conclusion

The results obtained in this study support the existence of two ecotypes of *C. atlantica* geographically distant in the Atlas Mountains of Morocco. The ecotype belonging to population Aït Oufella and Aït Ayach could be chosen in the projects of revalorization of the Mediterranean semiarid zones because of their needles adaptations (BW, Ht, and Nl). This ecotype may be used outside its natural range (i.e., Moudemame), the basic essence for the restoration of denuded mountains areas. On the other hand, the morphoanatomical differences between populations from the Atlas Mountains of Morocco, although not very distant, seems here to be the most interesting finding and suggest possible future refugia and/or isolation within the Middle Atlas Mountains and between the Middle and High Atlas Mountains in the past. In the future, to more elucidate and support this conclusion for these cedar ecotypes, we should plan both (1) growing these ecotypes *ex situ* to see whether these morphological differences persist in a nursery over multiple years and (2) sequencing the genomes or doing other works (e.g., AFLP) on these ecotypes to look for genetic differences. Despite this, the four populations of *C. atlantica* characterized here deserve particular attention in the Atlas cedar genetic improvement programs. The isolation and distinction between these populations reported in this study may be useful by Moroccan foresters to preserve their genetic characteristics.

Data Availability

The data of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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