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ORIGINAL RESEARCH PAPER

The length, number, and endodermis area of needles discriminate two genetically distinct populations of *Cedrus atlantica* Manetti in the Moroccan Middle Atlas

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Abstract

The variation in some adaptive characters of cedar needles was studied in two different regions of the Moroccan Middle Atlas that have different local environmental conditions and levels of genetic diversity. The two populations are localized in the Azrou and Ifrane regions. Tukey's tests showed that the needle/brachyblast number (Nn/R), length (Nl), and needle width (Nw) showed the greatest variation. In addition, all anatomical characters studied showed a significant correlation with Nw, whereas only the area of the vascular bundles (AVb) was related to Nl. Discriminant analysis revealed that Nn/R, Nl, and the area of the central cylinder (ACc) are high-discriminating characters among populations of Azrou and Ifrane and confirms their isolation. These adaptations of the morphological and anatomical traits of the Atlas cedar needles of the Azrou and Ifrane regions are discussed in relation to the local environmental conditions and have been found to be in harmony with their genetic distinctiveness revealed previously.

Keywords

anatomy; biometry; leaf; morphology; population

Introduction

Predicting how species will respond to future events by identifying structural differentiation is crucial in both preserving the species' potential and understanding their evolutionary forces [1]. The findings of several studies indicate the North-East Asiatic origin of the *Cedrus* tree and show that all *Cedrus* species, belonging to both the Mediterranean and Himalayan regions, were far more widely distributed across Europe, Asia, and Africa before the Quaternary [2–4]. Ivanov et al. [5] found that the retraction of the geographic range of the *Cedrus* occurred during the late Tertiary as a result of the increase in climate cooling.

In North Africa, *C. atlantica* is now distributed into five unequal, fragmented and distinct geographical regions. In Morocco, the largest area is localized in the Middle and High Atlas (116,000 ha) and the second in the Rif (15,000 ha). In Algeria, its natural area occupies only approximately 33,000 ha in the Tell Atlas and 17,000 ha in the Aurès Mountains [6]. Molecular analyses and fossil record have suggested that *C. atlantica* populations of the Rif and the central and the eastern Tell Atlas, in addition to those that occurred before the Holocene in Tunisia and eastern Algeria, represent the most ancient persistent cores in North Africa [7]. Such interpretations imply that *C. atlantica* would have persisted through the last glacial period in these distinct refugia located along the coast of the Western Mediterranean Sea, and then expanded south to west and colonized the Middle Atlas, which now harbors more recent stands that are genetically isolated and have contrasting levels of within-population diversity [7–9].

The genetic potential of *C. atlantica* acquired during its evolution confers this species a position in projects that revalorize Mediterranean populations, especially on calcareous land [10,11]. Its introduction and acclimatization in the Mediterranean mountains has proven to be successful [12,13]. Its exceptional power of adaptation to varied and often difficult ecological, climatic, and edaphic conditions has generated great interest in many foresters, ecologists, biologists, and botanists in this species for its potential outside its natural range for the reconstruction of unproductive or degraded forests and the restoration of denuded mountains [13–15]. In the past decade, three polymorphic cpSSR loci were analyzed in 162 individuals across six *C. atlantica* populations and five unique alleles were detected in the Middle and High Atlas populations, one unique allele was detected in the Rif, and seven haplotypes were not found in any individual of the Rif populations [8]. This high genetic diversity, due to heterogeneous environmental conditions, confirms the high adaptation potential of *C. atlantica* in the Middle Atlas of Morocco that are certainly linked to variation in the photosynthetic apparatus and needle structure of *C. atlantica* [16,17].

Thus, in Morocco and elsewhere, needle size, number of needles per brachyblast, number of stomata lines, etc. may be used as the criteria to differentiate the species and the trees in both nurseries and natural populations [18–20].These authors have also revealed that the populations in the High Atlas and the Rif are easier to characterize, while those in the Middle Atlas remain morphologically more complex, and require more detailed investigations on both structure of needles and genetics, which are limited. The only studies published during the last decade on the taxonomic and geographic differentiation of conifers show that morphological and anatomical characteristics are important in the recognition of the phylogenetic relationships and geographical pattern of variation in Pinaceae [21–31]. Moreover, to date, no research has been carried out on the variation in needle morphology between genetically distinct populations of *C. atlantica*.

The aim of the present study was to record and assess the distinguishing characteristics of *C. atlantica*, based on materials from nearby natural populations with different levels of genetic diversity [7] and local environmental conditions, by using biometric analysis of some adaptive anatomical and morphological traits of needles.

Material and methods

Materials for the study were collected from the Azrou and Ifrane regions of the Moroccan Middle Atlas during April and May 2014. These two regions were characterized by the highest percentage of polymorphic fragments (83.01 and 90.34), Shannon diversity (26.38 and 43.02), differences in the number of private fragments (0 and 1), and average gene diversity (0.113 and 0.203). The number of shared fragments between the two regions is only 66 and the pairwise F_{ST} distance is high (0.356) [7]. Two populations of the localities of Kha and Mou belonging to the Azrou region and a population of the locality of Tam in the Ifrane region were selected (Fig. 1, Tab. 1). The populations of Kha and Tam have been shown to be molecularly distinct [7], whereas Mou has never been studied genetically or structurally. The forest of Tam is characterized by a gradient of decreasing rainfall from the southwest to the northeast, following a descending altitudinal gradient [32]. This cedar forest differs from the Kha population in terms of climate and soil and the presence of a steep slope, and from Mou in terms of climate and soil only (Fig. 2, Tab. 1). The forest of Kha differs from that of Mou by the presence of a steep slope only (Tab. 1). These mountains are

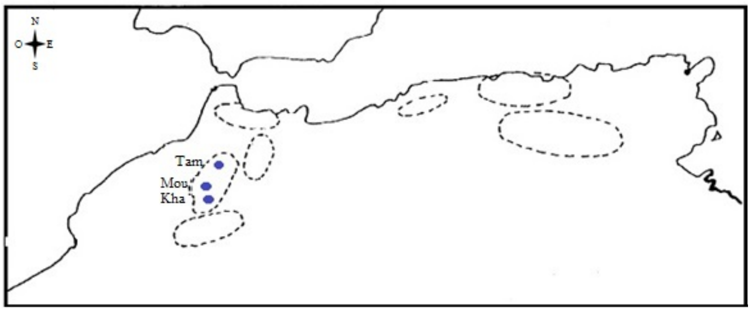

Fig. 1 Distribution and localization of sampled populations: Mou (Moudemame), Kha (Kharzouza), and Tam (Tamrabta). The dashed lines delineate the natural distribution areas of *C. atlantica* in North Africa.

Tab. 1 Environmental conditions of sampled populations of *C. atlantica* Manetti in Azrou and Ifran regions.

Regions	Localities	Coordinates	Elevation (m)	Distances between populations (km)	Parent rock	Substrate	Bioclimate
Azrou	Kha: clear population with green oak on a plateau at the top of a mountain which has a slope of 37%	33°24′ N 5°12′ W	1,823	Kha–Mou: 3	Basalt-calcareous	Volcanic substrate of post-villafranchian plio-quaternary age (basalt and ancaratrite lavas)	Fresh humid
	Mou: a pure and dense population on a plateau that has a slope of 0%	33°25′ N 5°11′ W	1,780	Mou–Tam: 27	Basalt-calcareous		Fresh humid
Ifran	Tam: a sparse and clear population with maritime pine or green oak on a plateau that has 10% slope	33°37′ N 5°03′ W	1,605	Tam–Kha: 30	Calcareous/dolomite/sander	Substrate rich in carbonate of calcium and magnesium of Liasic age rich in fossils	Cold subhumid to very cold

The forests of Moudemame (Mou) and Kharzouza (Kha) of Azrou occurred, respectively, in cedar forest soil with humus or in cedar and holm oak forest soil with humus, whereas the forest of Tamrabta (Tam) of Ifran took place in bare soil.

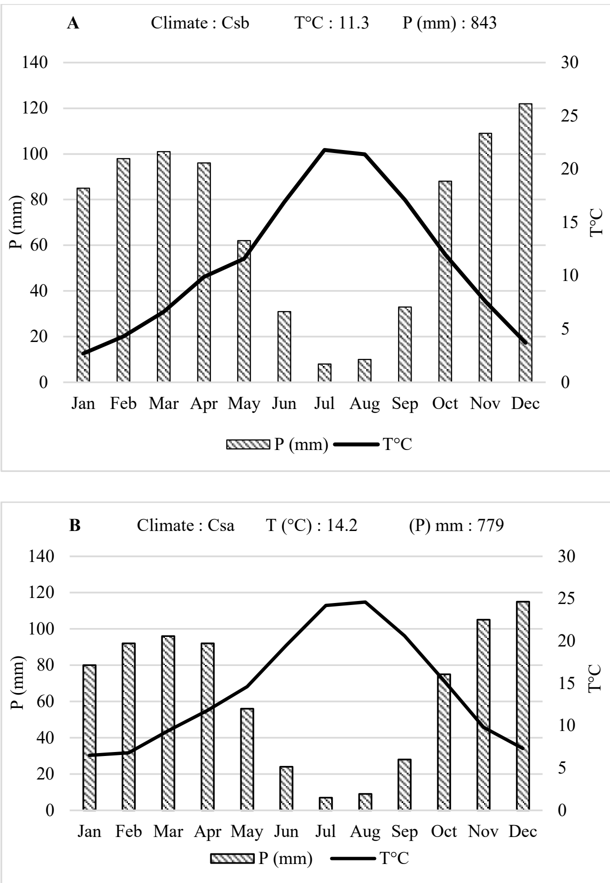

Fig. 2 Climate graph of the Ifrane region (**A**) [37] and the Azrou region (**B**) [38] during the year 2012.

characterized by the presence of *C. atlantica* and *Quercus ilex*. Kha is specifically marked by the dominance of *Q. ilex* compared to Mou. In each population, eight to ten trees aged approximately 150 years were selected (eight trees of Kha, 10 trees of Mou and Tam) spaced at a distance of no less than 30 m from each other. For tree selection of each population, similar light conditions were taken into account, with sampling of ten 2-year-old undamaged brachyblasts, and fully developed needles for each tree. The samples were preserved in 70% alcohol and stored at −20°C. A needle was selected from each brachyblast of each tree included in this study. Among these 10 mature needles, we selected two (the longest and the shortest) to prepare cross sections. Values were obtained from 16 to 20 replicates of needles. The anatomic preparations were performed freehand at the central portion of each needle, and the transversal sections were treated with 5% NaOH for 4 h at 70°C, according to the methods of Arnott and Brady cited by Ruzin [33].

The majority of the adaptive characters found in *C. atlantica* [34] and in *Pinus canariensis* [35] were measured. The length of the needle (Nl) was determined manually with an accuracy of 0.25 mm. The needle/brachyblast number (Nn/R) was counted for every sample. The number of the sclerenchymatic cells (NSc) adjacent to the phloem and the xylem was counted for each transversal section under a light microscope (Optika DM-15). The preparations were then photographed using the integrated camera of the same microscope. Measurement of traits (Fig. 3): needle width (Nw) and areas of: cross section (AS), mesophyll (AMp), central cylinder (ACc) (all tissues including and within

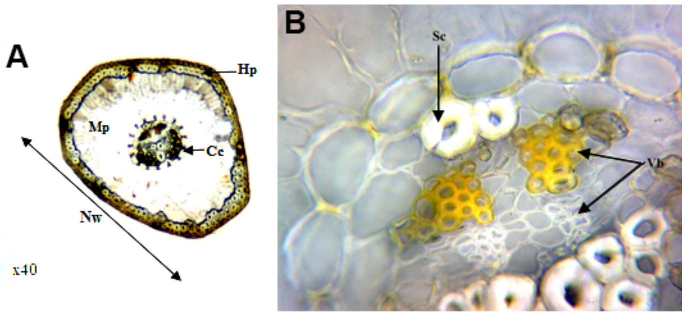

Fig. 3 Measured characters of the needle cross section. Hd – hypodermis; Mp – mesophyll; Cc – central cylinder; Sc – sclerenchymatic cells; Vb – vascular bundles; Nw – needle width.

the endodermis are referred to as central cylinder), hypodermis (AHd), and vascular bundles (xylem and phloem) (AVb), with an accuracy of 1 µm, were made using the best image of a section for every tree, using Opmias software ver. 1.0. All data were analyzed statistically using IBM SPSS Statistics 20.0 software. Descriptive statistics (means, minima, and maxima), and coefficient of variation (*CV*) were calculated for each trait. The Pearson correlation between characters was verified to avoid the most redundant ones, with $p = 0.01$ and 0.05. To determine the possibility of using multivariate statistical analyses, the distribution of each character was verified using the Shapiro–Wilk test. Each estimated character was standardized. Differences between means were calculated using the Tukey's test with a significance level of $p < 0.05$. The relationships among the populations were estimated on the scatter plot of function on the space between the first discrimination variables, after performing a stepwise discrimination analysis on the whole set of traits [36].

Results

Important variation in adaptive morphological characters of the needles among the three populations of cedar were observed (Tab. 2). The average number of needles per brachyblast was significantly low (32.80) in the population of Kha, intermediate in Mou (66), and high in Tam (Tab. 2). Similarly, the population of Kha is characterized by low values of average needle length (13.60 mm) compared to the other two populations that have a mean equal to 16 mm (Tab. 2). In contrast, the trees of Kha have very wide needles with a mean value of 1,099.54 µm and a high variation coefficient (18.60) (Fig. 4A, Tab. 2).

Tab. 2 Statistic description of analyzed morphological characters of needles sampled from the three populations.

Statistics	Localities	Nl (mm)	Nw (µm)	Nn/R
Mean	Kha	13.60	1,099.54	32.80
	Mou	16.00	930.50	66.00
	Tam	16.36	935.65	71.57
Minimum	Kha	10.00	846.66	18.00
	Mou	13.00	706.79	52.00
	Tam	12.00	763.33	34.00
Maximum	Kha	18.00	1,417.00	60.00
	Mou	20.00	1,130.06	94.00
	Tam	22.00	1,091.46	113.00
Standard error	Kha	2.01	49.59	6.05
	Mou	2.19	123.43	13.02
	Tam	2.84	116.79	22.38
Variation coefficient	Kha	15.72	18.60	32.71
	Mou	13.69	13.27	19.73
	Tam	17.39	12.48	31.27

Nl – length of the needle; Nw – needle width; Nn/R – needle/brachyblasts number.

Tab. 3 Statistic description of analyzed anatomical traits of needles sampled from the three populations. The values of surfaces must be multiplied by 103 μm².

Statistics	Localities	AS	AMp	ACc	AHp	NSc	AVb	AVb/AMp
Mean	Kha	563.65	356.07	66.62	77.72	11.00	10.62	0.03
	Mou	520.73	315.97	69.33	78.54	10.81	11.77	0.04
	Tam	534.57	336.49	70.86	76.01	11.00	11.80	0.04
Minimum	Kha	458.10	280.10	47.21	47.20	3.00	6.23	0.02
	Mou	366.30	204.70	48.00	60.60	5.00	7.94	0.03
	Tam	324.27	189.23	43.45	44.34	6.00	8.14	0.02
Maximum	Kha	659.82	420.30	85.60	94.50	24.00	15.07	0.04
	Mou	703.80	420.50	110.10	106.60	18.00	17.78	0.05
	Tam	739.68	528.76	113.06	106.90	17.00	18.89	0.05
Standard error	Kha	65.07	46.38	11.39	14.82	5.10	2.46	0.01
	Mou	87.74	61.08	17.20	13.10	3.71	3.12	0.01
	Tam	112.10	87.15	20.18	15.87	2.99	2.90	0.01
Variation coefficient	Kha	11.55	13.03	17.09	19.07	46.35	23.13	22.37
	Mou	16.85	19.33	24.80	16.68	34.31	26.49	22.36
	Tam	20.97	25.90	28.48	20.87	27.16	24.60	25.08

AS – area of the cross section; AMp – area of the mesophyll; ACc – area of the central cylinder; AHd – area of the hypodermis; NCc – number of the sclerenchymatic cells; AVb – area of the vascular bundles; AVb/AMp – proportion of area of the vascular bundle/area of the mesophyll.

The adaptive anatomical characters of needles varied considerably in the three populations (Tab. 3). The average area of the cross section in the Kha population was 563.65 × 103 μm², whereas in the Mou and Tam populations, this value was lower, 520.73 × 103 μm² and 534.57 × 103 μm², respectively. (Fig. 4A,C,E, Tab. 3). The lowest *CV* was observed in Kha (11.55%), followed by Mou (16.85%), and finally by Tam (20.97%) (Tab. 3). Furthermore, the average area value of the mesophyll (AMp, i.e., the main living tissue that has to be supplied with water) varied in the same direction as in the three populations. The highest value was in the Kha population (356.07 × 103 μm²) and lowest value was in the Mou population (315.97 × 103 μm²), with a low *CV* observed in Kha (13.03%) and higher *CV* in Mou (19.33%) and Tam (25.90%) (Fig. 4A,C, Tab. 3).

The average values of ACc varied slightly in the three populations, with a coefficient of variation of 17.09% detected at Kha, followed by 24.80% at Mou, and 28.48% at Tam (Fig. 4B,D,F, Tab. 3). The trees of Kha were distinguished by values of *CV* that indicate these three characters are more consistent compared with the other two populations. In contrast, the average and *CV* values of AHd varied slightly (from 76.01 × 103 μm² to 78.54 × 103 μm² and from 16.68% to 20.87%) in the three populations (Tab. 3). The average value of NSc adjacent to the vascular bundles varied between 10 and 11 in the three populations studied. This adaptive trait varied at 46.35% in Kha, 34.31% in Mou, and 34.31% in Tam (Tab. 3). The mean and *CV* values of AVb and the reported AVb/AMp in the populations were slightly higher for Tam and Mou than those of Kha (Tab. 3).

The correlation analysis indicated a statistically significant relationship between needle length and Nn/R, AVb, and AVb/AMp characters. Nn/R was positively correlated with AVb/AMp ratio, while Nw was positively correlated with AS, AMp, ACc, AHd, and AVb (Tab. 4). This correlation indicated that the needle width determined by the cross section was significantly correlated with more anatomical characters than Nl and Nn/R.

Analysis of the differences between means by the Tukey's test revealed that only the morphological characters Nl, Nn/R, and Nw were significantly distinct at $p < 0.05$ between the population of Kha, Mou, and Tam (Tab. 5). In contrast, the dispersion of trees within the space between the two first canonical values U_1 and U_2 (responsible for

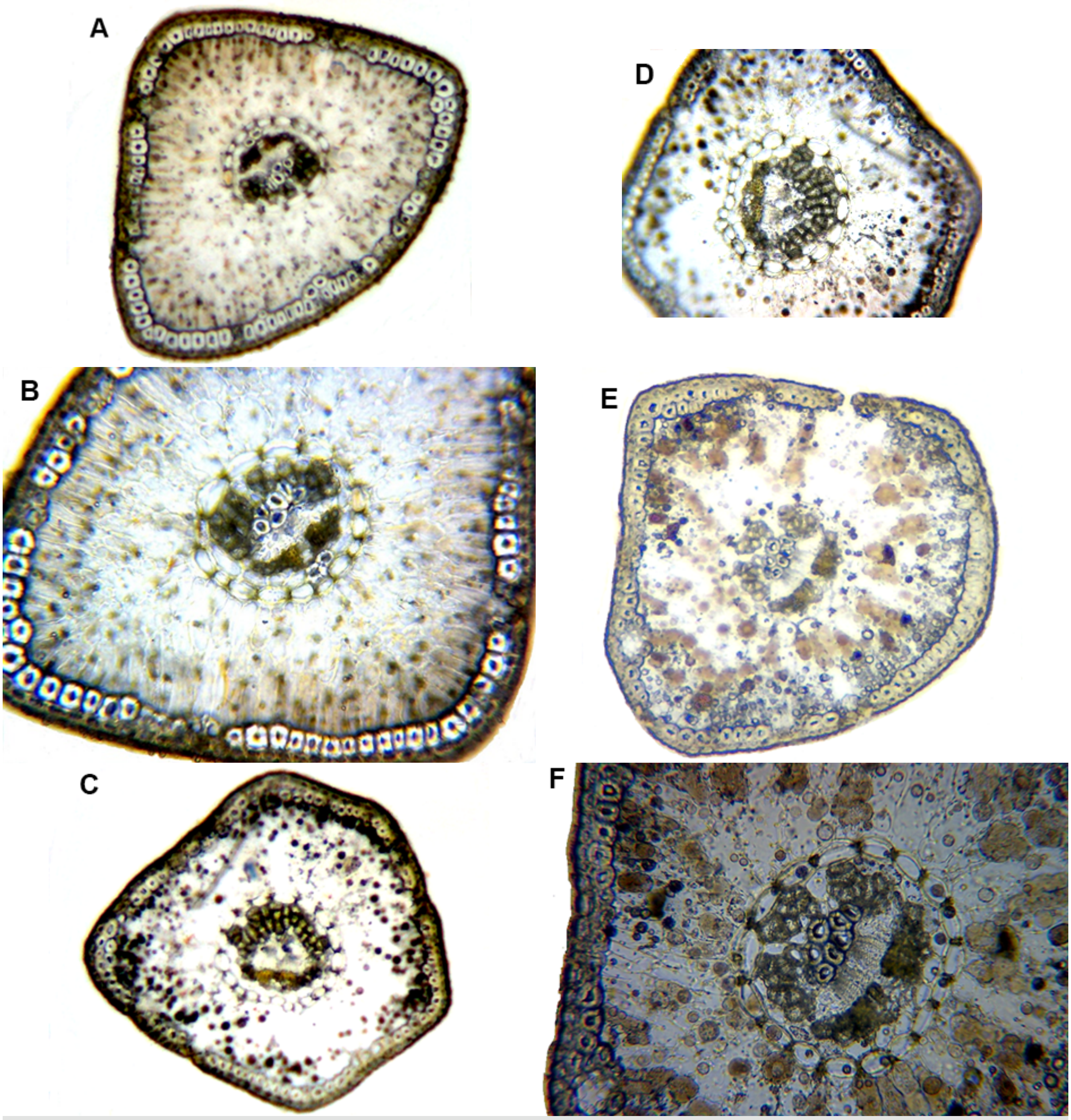

Fig. 4 Cross sections of needles – (**A,B**): Kha; (**C,D**): Mou; (**E,F**): Tam, with a magnification of ×40 (**A,C,E**) and ×100 (**B,D,F**).

100% variation among the populations), showed three dispersed clouds of individuals (Fig. 5). The variable U_1 was determined essentially by Nn/R, Nl, and ACc, while U_2 by AS, AMp, AHd, NSc, AVb, AVb/AMp, and Nw. This dispersion shows that all Kha trees are within the 95% confidence interval without any overlap with the populations of Mou and Tam. A partial separation was also observed between the Mou and Tam populations along the second axis but it was not very clear, because of a partial overlap among their variation ranges. Moreover, these two populations are spread out along the first axis, showing their larger within-population variation regarding characters correlated with this axis than the variation within the Kha population (Fig. 5).

Discussion

The traits needle/brachyblast number (Nn/R), needle length (Nl), and needle width (Nw) contributed most significantly to the morphological differences between the three studied populations of *Cedrus atlantica* (Tab. 2). The trait Nn/R is often listed

Tab. 4 Values of Pearson's correlation coefficients between characters of needles of the three populations.

	Nl	Nn/R	NSc	Nw	AS	AMp	ACc	AHd	AVb
Nn/R	0.540**								
NSc	0.064	−0.042							
Nw	0.084	−0.132	0.153						
AS	0.232	−0.175	0.083	0.725**					
AMp	0.042	−0.293	−0.026	0.543**	0.831**				
ACc	0.260	0.045	0.207	0.551**	0.769**	0.560**			
AHd	0.269	−0.029	0.006	0.564**	0.834**	0.607**	0.613**		
AVb	0.345*	0.089	0.073	0.301*	0.590**	0.424**	0.811**	0.461**	
AVb/AMp	0.330*	0.304*	0.060	−0.265	−0.194	−0.435**	0.259	−0.134	0.596**

Nl – length of the needle; Nw – needle width; Nn/R – needle/brachyblasts number; AS – area of the cross section; AMp – area of the mesophyll; ACc – area of the central cylinder; AHd – area of the hypodermis; NSc – number of the sclerenchymatic cells; AVb – area of the vascular bundles; AVb/AMp – proportion of area of the vascular bundle/area of the mesophyll. * The correlation is significant at the 0.05 level (bilateral). ** The correlation is significant at the 0.01 level (bilateral).

Tab. 5 *P* value of multiple comparison Tukey test for 10 characters of needles of the three populations of *Cedrus atlantica*.

	Nl	Nn/R	Nw	NSc	AS	AMp	ACc	AHd	AVb	AVb/ AMp
Kha-Mou	0.012*	0.000*	0.006*	0.993	0.442	0.275	0.909	0.988	0.566	0.070
Mou-Tam	0.990	0.422	0.971	0.866	0.678	0.429	1.000	0.911	0.980	0.610
Tam-Kha	0.013*	0.000*	0.009*	0.934	0.880	0.899	0.904	0.973	0.661	0.323

Nl – length of the needle; Nw – needle width; Nn/R – needle/brachyblasts number; AS – area of the cross section; AMp – area of the mesophyll; ACc – area of the central cylinder; AHd – area of the hypodermis; NSc – number of the sclerenchymatic cells; AVb – area of the vascular bundles; AVb/AMp – proportion of area of the vascular bundle/area of the mesophyll. * Statistically significant at the 0.05 level.

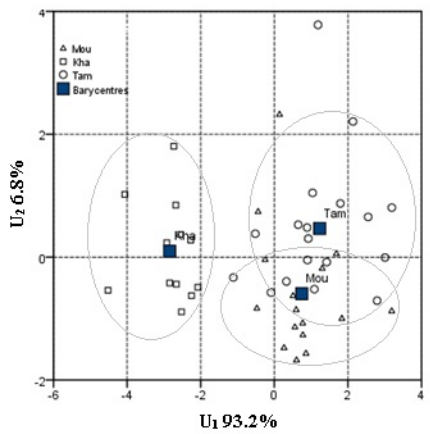

Fig. 5 Dispersion of individuals of the studied populations of *C. atlantica* in the first two discriminant axes (U_1, U_2).

as a discriminant character between populations of the Moroccan cedar [34]. It distinguishes the populations, despite the fact that they are not geographically distant from each other (Fig. 5, Tab. 1), and even confirms the study by de Lillo and Fusaro [39] in Italy for the provenance of populations in the Rif and the High Atlas of Morocco. The values of the average length of needles of the three populations (Tab. 2) were within the range of length of 10–25 mm reported by Farjon [40]. Arbez et al. [34] found a mean length of needles between 18.3 and 20.5 mm in the Middle Atlas of Morocco. Recently, Jasińska et al. [21] found mean values of Nl of 11.83 (7–17) mm, for brachyblasts of *C. atlantica* harvested at Jbel Anasan in the Rif and 14.78 (10–19) mm for brachyblasts harvested from the mountains of Azrou. The average length of needles at 13.6 (10–18) mm found in Kha was lower than those reported for Mou and Tam despite the fact that Mou is located close to Kha (Tab. 1, Tab. 2). In contrast, the value of Nl for Kha is similar to that found by Jasińska et al. [21] in Azrou. This result confirms that needle length is a discriminant trait within the Azrou region of the Middle Atlas as shown in Fig. 5. Various studies cited by Farjon [40] on the characteristics of needles have shown that only Nl, Nw, and Nh have been found to discriminate *C. atlantica*, *C. libani*, *C. brevifolia*, and *C. deodora*.

On the other hand, the variation in Nw in the present study indicate considerable efficiency of this character in distinguishing Mou and Tam trees from Kha trees, which have very wide needles (Fig. 5, Tab. 2, Tab. 5). This character of Kha is similar to that identified in a few species of pines subjected to drought [34,35,41].

In the present study, the decreases in Nn/R and Nl and the increase in Nw in trees in the Kha region (Tab. 2) indicate that there is some consistency between our results and those reported by Arbez et al. [34], Ducrey et al. [42], and Ladjal et al. [43]. These authors underline a positive relationship between stomata lines and needle width; however, to date, no information is available on the relationships between these traits and the diameter, height of stem, and root growth in *C. atlantica*. Moreover, Ladjal et al. [43] found a reduction in stomatal conductance, mesophyll photosynthesis, and hydraulic conductivity of the stem and the needles of *C. atlantica* following water deficit. Regardless of this, this drought-tolerant species is characterized by low values of critical predawn water potential (approximately −3.0 MPa), and 10% of the maximum value of mesophyll photosynthesis remains significantly higher for predawn water potential reaching −5.0 MPa [42]. However, its growth in height stopped below −2.1 MPa because the stomata remain open until −3.6 MPa at zero turgor, and its drought adaptation is mainly due to efficient root growth and good soil prospection ability. This author also reported that the growth in height and diameter is correlated to rainfall and temperature.

Based on the area measured, the *CV* of AS, AMp, and ACc detected in the present study (Tab. 3) are low and more stable in the Kha population compared to those in Mou and Tam populations. This low variation is in agreement with the low cellular structure changes of needle [21] and the low genetic diversity [7] present in the locality of Azrou (Kha) of the Middle Atlas of Morocco. In contrast, the largest variation in AS, AMp, and ACc revealed in Tam is in accordance with the high level of genetic diversity of *C. atlantica* [7], and the high polymorphism of maritime pine [44,45] present in this locality of Ifrane (Tam). Similarly, the fact that the population of Tam has the lowest and highest values of AS, AMp, and ACc indicates a high phenotypic variation of the needles in this population. Furthermore, the discriminant analysis revealed that area of the central cylinder (ACc) differentiates Kha, Mou, and Tam populations (Fig. 5), whereas Jasińska et al. [21] found that only height and not width of the central cylinder discriminate *C. atlantica*, *C. libani*, and *C. brevifolia*. The description suggests that different features should be used to distinguish between the *Cedrus* species (height of the central cylinder), and between other *Cedrus* populations (width of the central cylinder).

On the other hand, the population of Kha, compared with Tam, displayed a decrease in AS, AMp, and ACc. It is important to clarify the factors that cause variation in these characters. Grill et al. [35] showed that AS, AMp, and ACc of *P. canariensis* in seedling needles decreased under controlled conditions of drought, while Maurice and Crang [46] showed that AS of *P. strobus* needles increased in response to acid misting. In further studies, Bleweiss et al. [47] found that high levels of sulfur in the needles of *C. libani* caused needles to develop xeromorphic characteristics, with very thick cuticle, thick cutinized walls of the epidermis and hypodermal cells, sunken stomata, and plicate mesophyll cells. Similarly, Marin et al. [48] discovered that the surface area and volume density of mesophyll cells and intercellular spaces increased in the needles of *C. atlantica*, while the volume density of the epidermis, central cylinder, and resin ducts decreased in the polluted environment. These findings show that the anatomical traits of *Cedrus* taxon needles, like many conifers, increase in response to pollution but decrease in response to drought, indicating that the *Cedrus* trees adjusts its needle structure (AS, AMp, and ACc) depending on the type of stress. These data demonstrate that the decrease in AS, AMp, and ACc of the Kha *Cedrus* is caused by a tendency of the population to moderate their needle structure in response to the elevation in temperature and to the drought during summer caused by the steep slope of 37% inclination (Fig. 2, Tab. 1).

The *CV* of NSc varied more than AS, AMp, and ACc in the Kha population, compared to Mou and Tam populations (Tab. 3). Jasińska et al. [21] reported *CV* values of 47.91% for NSc in the Middle Atlas and only 18.11% in the Rif of Morocco. This could be attributed to fewer individuals (10 individuals) analyzed from the Middle Atlas in contrast to 30 from the Rif [21]. The similarity of NSc between the three populations is not in agreement with the results of Böcher [49] and Grill et al. [35], who found an increase in NSc in response to drought and/or high temperature in the year of growth of needles, to avoid the collapse of the vascular package under conditions of turgor loss. In contrast, the high *CV* of Kha trees, compared to Tam, may be confirmed by the trend of *C. atlantica* to adapt its photosynthetic apparatus to water deficits and by its low thermotolerance [16,17] in response to the elevation in temperature and the subsequent drought during the summer season caused by the steep slope of 37% inclination (Fig. 2, Tab. 1).

The results presented in Tab. 3 show neither an increase in the mean value of AHd nor in the *CV* in the Mou and Tam populations, compared to the Kha population. Moreover, the number of hypodermis cells, even if they were counted, does not necessarily indicate an increase in sclerification. Therefore, the number of cells will be dependent not only on the sclerification level but also on the needle circumference. The most important parameter which may explain an increase in the observed values was the hypodermis being composed of a single layer. Similarly, the results in Tab. 3 do not show an increase in the number of sclerenchymatic cells (NSc), while their number (mean value) is similar for all populations. These constancies of AHd and NSc suggest that *C. atlantica* does not adopt the mechanism of reinforcement of vascular bundles by increasing the number of the sclerenchymatic cells or increasing the number of hypodermis layers or the hypodermis surface size.

In the present study, the mean values of AVb and the report AVb/AMp were similar between the studied populations (Tab. 3). According to Grill et al. [35], under conditions of artificial droughts, the areas of the transverse section and the mesophyll (AS and AMp) decrease, while that of the vascular bundles (AVb) increase, leading to increased AVb/AMp in *P. canariensis*. On the basis of these findings, it is not clear whether AVb and AVb/AMp of *Cedrus* are under the influence of environmental conditions; thus, further extensive studies are required to clarify this aspect.

In the present study, the populations of Azrou (Kha) and Ifrane (Tam) regions (Tab. 1), which had only 66 fragments shared between them, high pairwise F_{ST} distances (0.356), and were found to be genetically distinct by Terrab et al. [7], displayed structural differentiation in Nl, Nw, Nn/R, AS, AMp, and ACc values of needles (Fig. 5, Tab. 2, Tab. 3). These needle trait distinctions resulted from the variations in environmental conditions, and are in accordance with the genetic characterization of these two regions by Terrab et al. [7].

All the populations studied therefore deserve some attention in genetic improvement programs of Atlas cedar, particularly the populations of Kha and Tam. The isolation and the distinctiveness between these populations confirmed in the present study can be useful for the Moroccan foresters for preserving their genetic characteristics. Useful markers for identification of beneficial genes controlling Nl, Nn/R, Nw, AS, AMp, and ACc may facilitate management and conservation of the genetic pool of this cedar species, which is threatened by climate change. Other DNA fragments that are used to investigate phylogenetic relationships of the genus *Cedrus*, such as the *nad5* intron [50,51] and the intergenic spacer trnH-psbA [52], may also bring more precision to determining the variation in needle traits and their specific genes.

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