

## Research Article

# Structure of Needle Highlights Ecological Adaptability and Microevolution of Natural Populations of *Cedrus atlantica* in Morocco

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The study of morphological and anatomical characteristics of leaves is important for assessing the geographical variation of species. The ecological adaptability of forty individuals from four populations of *Cedrus atlantica* were studied, based on analysis of morphological and anatomical traits. The results of the Spearman nonparametric coefficient of correlation showed that the number of stomatal lines (NLS) and the length of the needle (NL) are negatively correlated to altitude and positively to latitude and precipitation sums, while the width of the needle (NW), the thickness of the cuticle (CT), and the number of needles per rosette (NN/R) were negatively related to temperature. In addition, the sum of precipitation is negatively correlated with NW. The first two principal components account for 58.18% of the variation. According to Tukey's test and Kruskal–Wallis test, all populations had at least three characters separating them at a statistically significant variation. Moreover, the hierarchical classification led us to the individualization of three main groups. All these results show an adaptation of the structure of the needles of *C. atlantica* from Morocco to the geographical position and the climatic conditions of the populations.

## 1. Introduction

Atlas cedar (*Cedrus atlantica* Manetti) is a relict and endemic endangered species in the mountains of North Africa, whose distribution range has undergone a dramatic reduction over recent decades, that is attracting increasing international interest in its use in reforestation of degraded lands due to its large ecological range [1–4]. It occupies surfaces of unequal importance and spontaneously forms seven geographical blocks in North Africa, three of which are located in the Moroccan mountains: Rif, Middle Atlas, and High Atlas [5, 6]. *C. atlantica* is the most important timber resource in Morocco, occupying a surface area of 132,000 ha and representing 2.3% of the national forest [7]. In the central Middle Atlas, two groups of the geological formations are distinguished by their morphological and phytoecological structure: the group of the cause of the tabular

Middle Atlas in the north mainly made up of dolomitic limestones from the Lower Jurassic, which surmount the Triassic series formed by red argillites and basalts [8–10]; and the group of the Middle Atlas pleated to the south and differs from the subtabular cause by the presence of wrinkles which form reliefs oriented in the general direction of the chain. These anticlinal wrinkles frame synclinal depressions, which continued to function during the Middle Jurassic as fairly thick marly and marlcalcareous depocenters [11–13].

Needles are the most vigorous assimilation organs, especially in pine, because they have important effects on physiological and ecological adaptabilities [14, 15]. Although most of the morphological and anatomical traits of needles are more or less specific for the species, the genetic investigations have demonstrated intraspecies genetic variations [16, 17]. Adaptation of needle characteristics to the environment was also described [19–23], as well as

microevolution within species and needle evolution by comparing morphology and anatomy [24–27]. Nagy et al. [28] proposed the theory of the altitude-related biological phenomenon, which has had negative effects on plant communities reducing the number of plant species [29], the plant productivity [30], the organ size trends [31], the physiology and morphology of plants [32], the gene ecology [33] and the characteristics of the history of life [34]. In addition, Friend and Woodward [35] and Nagy [28] showed that the physical factors such as tree growth, altitude, decrease of air temperature with altitude, influence of exposition, solar radiation, access to the light, atmospheric pressure, increased precipitation, and wind speed affect plant development. The only studies published over the past decade on the taxonomic and geographical differentiation of conifers show that morphological and anatomical characteristics of needles are important in the recognition of phylogenetic relationships and geographic pattern of variation in Pinaceae [14, 35–39]. In Morocco and in Algeria, some morphological traits have been used as criteria for differentiating species and trees in nurseries and natural populations [40, 41]. These findings revealed that the populations of the High Atlas and the Rif are easier to characterize, while those of the Middle Atlas are morphologically more complex and require more detailed investigations on the morphology of needles. Despite the importance of the Moroccan forest genetic resources, few studies have focused on the variation of needle traits of *Cedrus* in nature [14], and the little information exists on the morphological traits and comes mainly from the nurseries [40–43].

The main objectives of the present study were to reveal the variations in needle morphological and anatomical traits among populations, illustrate the variations in needle traits among individuals within populations, and be interested in finding the link between the variation of traits with geography and environmental conditions that will be useful to understand the ecological adaptability and microevolution of *C. atlantica*.

## 2. Materials and Methods

**2.1. Sampling and Measurements.** The material used in this study was collected in 2015 in four geographical populations of *C. atlantica*: Moudemame (*M*), Aït Oufella (*O*), Aït Ayach (*A*) [23], and Tazekka (*T*), respectively, located in the central Middle Atlas and in the High Atlas and in eastern parts of the Middle Atlas (Table 1). The pure and dense population of *M* is formed on a basalt-calcareous substratum and characterizes with a fresh and stable wet bioclimate. The population of *T* represents one of the largest reserves of Atlas cedar and is a protected area for more than fifty years. This cedar forest covers area more than 850 hectares on a noncalcareous, primary shales substratum in a cold, perhumid bioclimate. The Aït Oufella (*O*) forest covers an area of 5 650 ha located on the southern edge of the Middle Atlas about 40 km northwest of Midelt on a calcareous substratum [44]. The general climate of *O* is characterized by a low rainfall and a marked drought for a long part of the year [45]. The population of *A* is less than 7 km from Cirque de Jaaffar

and is characterized by the presence of *C. atlantica* and *Quercus ilex* L. on a calcareous substratum with a semiarid continental climate. Ten adult and carrying cones trees were randomly sampled from each population, at distance of at least 30 m each other. Ten mature brachyblasts undamaged, fully developed with needles with no visible insect and/or fungi damage, from well-illuminated, north-facing parts of the tree crown about 2–3 m above ground level were collected from each tree. After collection, the lengths of the two-year-old needles were measured, and the plant material was then preserved in 70% ethanol and stored at 20°C until analysis [46, 47]. Semidurable preparations from the central part of ten needles representing the ten brachyblasts from each individual in each population were made by a cross-section. The anatomical preparations were performed freehand on the central part of each needle, and the cross-sections were treated with 5% NaOH for 4 h at 70°C, according to Arnott [48] and Brady et al. [49] cited by Ruzin [50]. In this study, six morphological and anatomical characters of needles were studied. The length of the needle (NL) was manually determined on fresh material with an accuracy of 0.25 mm. The number of the needle/brachyblast (NN/R) was counted for each sample (Figure 1). The number of facets (NF) was determined from the cross-sections in the central part of the needle. The number of stomatal lines (NLS) was determined for ten cross-sections of the same needle and keeping the highest number among the ten values obtained (Figure 1). Measurements of NF and NLS were taken under the optical microscope (Optika DM-15), and the preparations were then photographed with the integrated camera. Measurement of needle width (NW) and cuticle thickness (CT) (Figure 1) was determined with an accuracy of 1  $\mu$ m using the best image of a section for each needle, using the software Opmias ver.1.0. The climatic data in each geographic location of the population (Table 1) were obtained from <https://www.climate-data.org>.

**2.2. Data Analysis.** The average values and coefficients of variation (CV) for each trait within populations were determined. Before starting the multivariate analysis, we used the Kolmogorov–Smirnov test to check the normality of the data and the Levene test to assess the homogeneity of the variances of the data. In addition, mean values were compared using the Tukey test at a level of significance of  $P \leq 0.01$  and  $P \leq 0.05$  for characters with a normal and Kruskal–Wallis test at  $P \leq 0.05$  for those of biased distribution (NW and NF). The Pearson correlation at the 0.01 and 0.05 thresholds between the pairs of characters studied was performed on the raw values of all the samples. The correlation between populations for each morphological trait and environmental factors such as altitude, latitude, longitude, temperature, and precipitation was studied using Spearman’s nonparametric correlation coefficient. This correlation coefficient is adequate for samples of small size and nonnormal distributions. Principal component analysis (PCA) was performed on the average of each tree. Finally, the agglomeration of the populations on the closest Euclidean distances according to Ward’s method was carried

TABLE 1: Location and geographic characteristics of studied populations of *Cedrus atlantica* in Morocco.

Populations	Region	Latitude	Longitude	Altitude (m)	$T$ (°C)	$T_{\max}$ (°C)	$T_{\min}$ (°C)	$P$ (mm)
Moudemame ( <i>M</i> )	Middle Atlas central	33°25'N	5°11'W	1780	14.2	24.6	6.5	779
Tazekka ( <i>T</i> )	Middle Atlas eastern	34°08'N	4°10'W	1750	12.1	22	3.9	456
Ait Oufella ( <i>O</i> )	Middle Atlas central	32°58'N	5°03'W	1982	14.7	25.2	6	263
Ait Ayach ( <i>A</i> )	High Atlas	32°31'N	4°59'W	1972	12	23.1	3.1	459

$T$ , annual mean temperature;  $T_{\max}$  and  $T_{\min}$  stand for mean maximum and mean minimum temperature;  $P$ , annual mean precipitation.

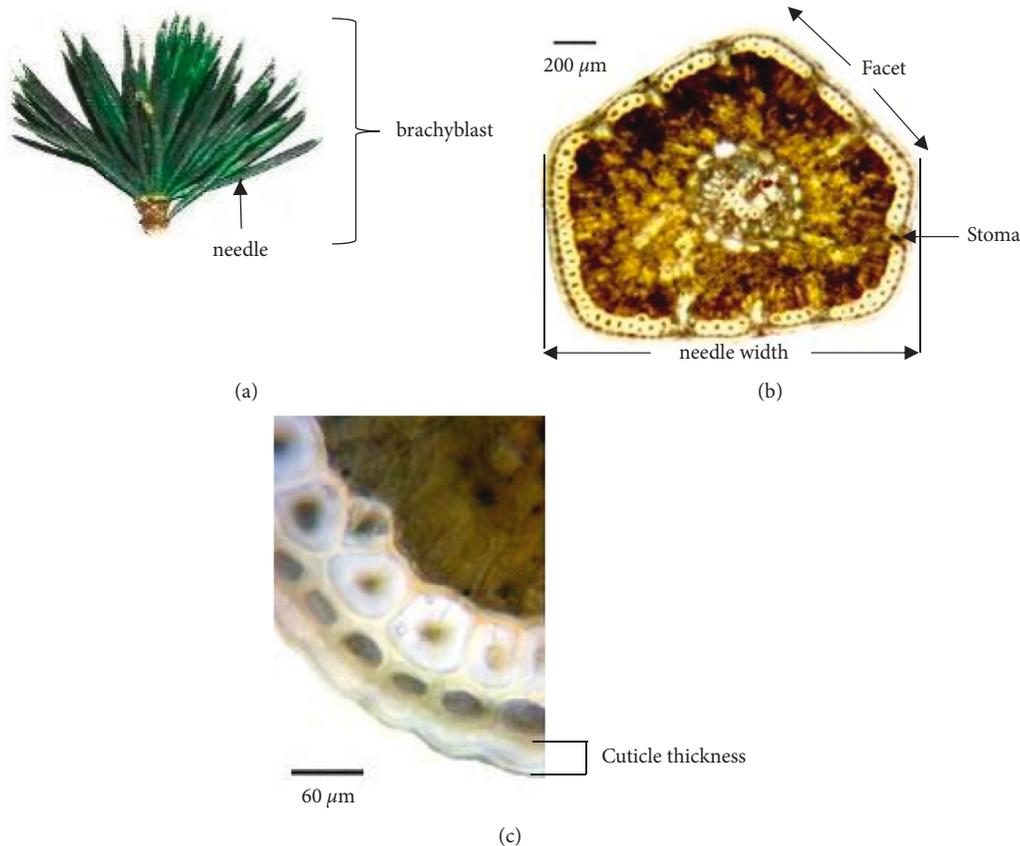


FIGURE 1: Measured characters of *C. atlantica* needle. (a) The image indicating needles of brachyblast, (b) The image indicating stoma, facet, and needle width. (c) The image indicating cuticle thickness.

out using all the characters to check out the affinities between *C. atlantica* populations. All statistical analyzes were performed using IBM SPSS software version 20.0.

### 3. Results

**3.1. Variation within Population.** The average values and variation coefficients (CV) of six needle traits in each population are given in Table 2. To estimate the proportion of within-population variation, we calculated the CV in the population according to means and standard deviations (SD) for each character (Table 2). Our results showed that the highest values of the coefficient of variation (>20%) in all populations were detected for cuticle thickness (CT) and the number of stomatal lines (NLS). The values of the coefficient of variation below 20% were recorded for needle length (NL) and needle width (NW) in most populations (Table 2). The remaining characters were as follows: the number of facets (NF) and the number of needles per brachyblast (NN/R)

show a considerable variation from 15.26 to 32.16% (Table 2). The description parameter of character distribution (curtosis) shows a similarity between population *T* and population *A* (Table 2). These two populations showed negative flattening values, so their characters are less clustered around the mean, with the exception of NL (needle length) of the population of *T* and NL and NN/R (length and number the needles per rosette) of the population of *A* (Table 2). While for the other two populations (*M* and *O*), the distribution character (curtosis) showed negative values for three characters at *M* and four characters at *O* and positive values for the rest of characters. The values of these characters are therefore less grouped around the mean.

**3.2. Variation among Populations.** Tukey's test for characters with a normal and Kruskal-Wallis test for those of biased distribution (NW and NF) revealed that among all compared populations, at least three characters separate them at

TABLE 2: Descriptive statistics of six quantitative traits of the studied populations of *C. atlantica* in Morocco.

Populations	Traits	Mean $\pm$ SD	Min	Max	CV	Skewness	Curtosis
M	Needle length (mm): NL	16.04 $\pm$ 1.02	14.89	17.56	10.35	0.60	-1.258
	Needle width ( $\mu$ m): NW	907.21 $\pm$ 35.19	849.58	961.69	10.87	-0.03	-0.14
	Cuticle thickness ( $\mu$ m): CT	8.93 $\pm$ 1.08	7.26	10.82	23.89	0.22	0.67
	Number of stomatal lines: NLS	6.46 $\pm$ 1.53	3.56	8.60	20.67	-0.85	1.15
	Number of facets: NF	4.12 $\pm$ 0.35	3.56	4.60	30.15	-0.44	-0.84
	Number of needles per brachyblast: NN/R	62.23 $\pm$ 8.75	46.56	73.20	18.79	-0.64	0.04
A	Needle length (mm): NL	11.35 $\pm$ 1.94	8.81	15.50	19.42	1.47	4.02
	Needle width ( $\mu$ m): NW	1408.91 $\pm$ 101.42	1280.65	1545.85	12.82	0.231	-1.58
	Cuticle thickness ( $\mu$ m): CT	13.62 $\pm$ 2.69	9.57	17.87	28.83	0.070	-0.35
	Number of stomatal lines: NLS	5.71 $\pm$ 1.31	4.43	8.20	18.24	0.88	-0.26
	Number of facets: NF	4.19 $\pm$ 0.32	3.71	4.60	33.73	-0.26	-1.75
	Number of needles per brachyblast: NN/R	71.03 $\pm$ 13.13	39.25	84.71	21.52	-2.01	5.04
T	Needle length (mm): NL	13.90 $\pm$ 1.35	11.90	16.60	13.16	0.51	0.51
	Needle width ( $\mu$ m): NW	1411.10 $\pm$ 146.90	1227.47	1571.79	14.45	-0.16	-2.07
	Cuticle thickness ( $\mu$ m): CT	15.44 $\pm$ 2.03	12.16	18.74	20.51	0.22	-0.44
	Number of stomatal lines: NLS	8.23 $\pm$ 1.37	6.50	10.33	19.31	0.36	-1.45
	Number of facets: NF	4.22 $\pm$ 0.33	3.78	4.83	23.49	0.56	-0.01
	Number of needles per brachyblast: NN/R	68.90 $\pm$ 7.14	59.10	81.20	15.26	0.38	-0.63
O	Needle length (mm): NL	10.45 $\pm$ 1.94	8.00	12.13	17.43	-0.77	-1.90
	Needle width ( $\mu$ m): NW	1133.47 $\pm$ 243.91	812.00	1462.94	23.51	-0.16	-1.08
	Cuticle thickness ( $\mu$ m): CT	9.59 $\pm$ 2.33	7.32	14.00	32.48	1.71	3.45
	Number of stomatal lines: NLS	5.90 $\pm$ 1.13	5.00	7.63	32.16	0.99	-1.12
	Number of facets: NF	3.98 $\pm$ 0.109	3.78	4.11	20.62	-1.44	3.60
	Number of needles per brachyblast: NN/R	60.98 $\pm$ 16.07	42.00	85.78	28.62	0.68	-0.51

M, Moudemame; T, Tazekka; O, Ait Oufella; A, Ait Ayach; T, annual mean temperature;  $T_{\max}$  and  $T_{\min}$  stand for mean maximum and mean minimum temperature; P, annual mean precipitation; mean, average values; SD, standard deviation; min, minimum; max, maximum; CV, variation coefficient.

a statistically significant level ( $P \leq 0.05$ ). All the studied traits differentiate *T* from *M* and from *O* (Table 3). The population *A* is differentiated from *M* by the trait NF and from *O* by NL, whereas it has distinguished from *T* by the traits NW, NF, and NN/R (Table 3).

**3.3. Geographical Structure of Morphological and Anatomical Traits Variation.** Interactions between traits were analyzed using the Pearson correlation coefficient matrix, which shows that NW is positively correlated with CT (Table 4). That is to say that the cuticle thickness increases with the needle width. The correlation between morphological and anatomical characters, with altitude, latitude, longitude, temperature, and precipitation, is given in Table 5. The NL and NLS are negatively related to altitude and positively related to latitude and precipitation at a 99% threshold, i.e., the longest needles with a high number of stomatal lines were found at low altitudes. The thickest needles with large cuticles were found in the low-longitude populations. The NW, CT, and NN/R correlate negatively with temperature, that is, populations with thick needles with high numbers per rosette are at low temperatures. While the precipitation is positively correlated with NL and negatively with NW, that is, populations located in the areas with significant rainfall have the needles long and thin (Table 5).

Principal component analysis (PCA) was performed on the average values of each tree and environmental factors. This analysis revealed four principal components with eigenvalues  $>1$  (Table 6).

TABLE 3: Differences between analyzed characters of needles of populations in Tukey's test and Kruskal-Wallis test.

Traits	M/A	M/T	M/O	T/A	T/O	A/O
NL	***	***	***	***	***	—
NW	***	***	***	—	***	***
CT	***	***	—	**	***	***
NF	—	**	***	—	***	***
NLS	***	***	***	***	***	***
NN/R	***	**	—	—	***	***

M, Moudemame; T, Tazekka; O, Ait Oufella; A, Ait Ayach; NL, needle length; NW, needle width; CT, cuticle thickness; NF, number of facets; NLS, number of stomatal lines; NN/R, number of needles per brachyblast. \* $P \leq 0.01$ ; \*\* $P \leq 0.05$ .

The two first canonical variables components account for 58.18% of the total variation and demonstrated three dispersed clouds of single trees, representing four compared populations (Figure 2, Table 6). The first component  $C_1$  was determined primarily by the traits: NW, CT, and factors: longitude and temperature, while component  $C_2$  was determined by altitude, latitude, and precipitation (Figure 2). This dispersion shows that populations: Moudemame and Tazekka are dispersed without overlap with the Ait Oufella and the Ait Ayach populations (Figure 2). The individuals of the latter two populations are mostly intermingled with each other.

Finally, all morphological and anatomical traits were used in a hierarchical group analysis (Figure 3). Three groups of populations could be distinguished in the dendrograms. The first group is composed of the populations of

TABLE 4: Pearson coefficient of correlation between pairs of morphological and anatomical traits studied.

Traits	NL	NW	CT	NF	NLS
NW	-0.341 **				
CT	-0.106	0.541 **			
NF	-0.137 *	-0.085	-0.120 *		
NLS	0.159 **	0.284 **	0.250 **	-0.165 **	
NN/R	0.030	0.246 **	0.088	-0.159 **	0.025

NL, needle length; NW, needle width; CT, cuticle thickness; NF, number of facets; NLS, number of stomatal lines; NN/R, number of needles per brachyblast. \*\*Correlation is significant at 0.01 level. \*Correlation is significant at 0.05 level.

TABLE 5: Spearman nonparametric coefficient of correlation between single morphological traits and geographic parameters.

Traits	Altitude	Longitude	Latitude	T	P
NL	-0.561 * *	0.181 * *	0.514 * *	-0.143 * *	0.528 * *
NW	-0.078	-0.577 * *	-0.090	-0.401 * *	-0.275 * *
CT	-0.180 * *	-0.543 * *	0.083	-0.363 * *	-0.250 * *
NF	0.251 * *	0.009	-0.105	0.268 * *	-0.247 * *
NLS	-0.495 * *	-0.279 * *	0.446 * *	-0.295 * *	0.120 *
NN/R	-0.002	-0.160 * *	-0.076	-0.151 * *	-0.068

T, temperature; P, precipitation;; NL, needle length; NW, needle width; CT, cuticle thickness; NF, number of facets; NLS, number of stomatal lines; NN/R, number of needles per brachyblast. \*  $P < 0.05$  and \* \*  $P < 0.01$ .

TABLE 6: Total variance explained.

Components	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
	Total	% of variance	Cumulative%	Total	% of variance	Cumulative%	Total	% of variance	Cumulative%
1	3.648	33.166	33.166	3.648	33.166	33.166	3.604	32.762	32.762
2	2.752	25.016	58.182	2.752	25.016	58.182	2.796	25.421	58.182
3	1.633	14.847	73.029						
4	1.144	10.400	83.429						

T and A which show very close relationship between them. The second is composed of the populations T, A and O and shows rather close relationship. The third group is formed by populations of M, which is the most distinct of all the others (Figure 3).

### 4. Discussion

4.1. Environmental Effects on the Traits of the Needle of *Cedrus atlantica* Populations. Studies of leaf variation based on geographic locations and environmental changes are common in trees, especially those with a long life span [52]. In this study, we found a high level of morphological and anatomical variabilities of needles among and within the Moroccan populations of *Cedrus atlantica*. The results of the correlation of the Spearman analysis show that the majority of cedar needle traits correlated with environmental factors. The NL character was positively correlated with latitude and longitude and negatively with altitude. This relation of NL with the altitude is in agreement with those obtained at *P. roxburghii* [27] and *P. pinaster* [52]. While in these last two species, NL does not depend on latitude as was found in *Pinus yunnanensis* [17]. On the other hand, it was found that NL in *Pinus yunnanensis* [17] and *Pinus uncinata* [53] is negatively related to rainfall; whereas in our study, this trait is positively related to this factor, suggesting that this change of needle is species-related. Moreover, it well known that cell

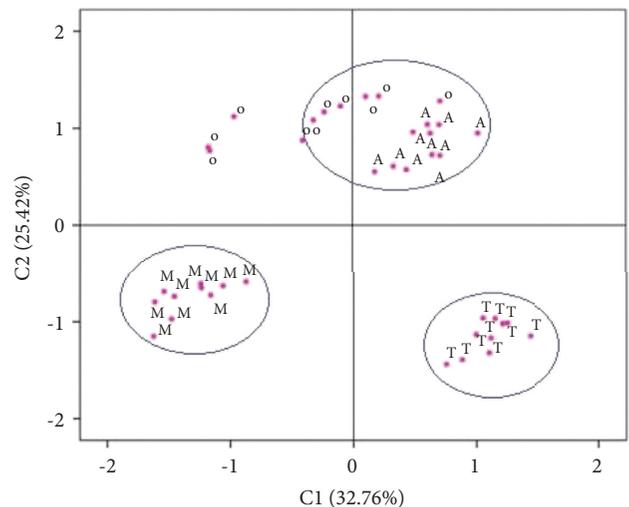


FIGURE 2: Dispersion diagram of the first two principal coordinates ( $C_1$  and  $C_2$ ) of the PCA for the traits studied in the four populations of *Cedrus atlantica*. A, Ait Ayach; T, Tazekka; O, Ait Oufella; M, Moudemame.

elongation induced by auxin needs more water and carbohydrate; this process is more water consuming in cedar, which was reported more sensitive to hydric deficit, adapting its shape to water availability [40, 54, 55]. In *Pinus mugo* and *Pinus yunnanensis*, NL was found to depend on the

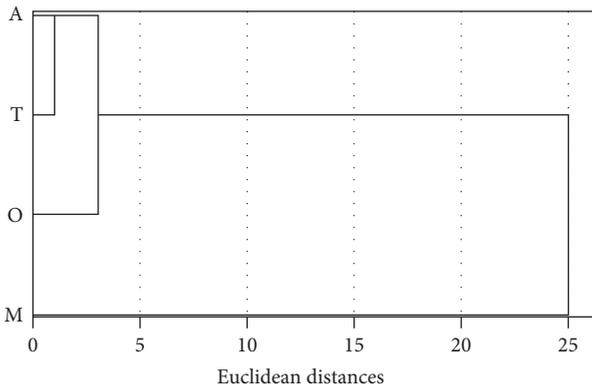


FIGURE 3: Relationships between populations of *Cedrus atlantica* (A, Ait Ayach; T, Tazekka; O, Ait Oufella; M, Moudemame) on the shortest Euclidean distances in relation to the morphological and anatomical characteristics of needles.

temperature [17, 56]. This ascertainment is in agreement with our results where NL was found to be significantly correlated with the temperature. This different response of NL to environmental conditions, compared to other morphological conifer studies, was supported by intraspecific variation of NL between genetically different *C. atlantica* populations in the Middle Atlas of Morocco [22].

It has been reported that the stomatal parameters are specific to a particular species but are affected by multiple ecological factors [57, 58]. In our study, the NLS trait varies positively with latitude but negatively with altitude and longitude in cedar populations. On the convex side of the needles of *Pinus yunnanensis*, NLS is positively correlated with the longitude [17], and at *Pinus roxburghii*, Tiwari et al. [27] found that stomatal density, stomatal index, and length of guard cells are positively correlated with altitude. In *Pinus sylvestris*, the number of stomata on needles depends on geographical latitude [59, 60]. Otherwise, the NLS character found in this study varies negatively with temperature and positively with precipitation at a significant threshold, whereas it increases with increasing temperature at *Pinus sylvestris* [61] and varies negatively with rainfall in *Pinus pinaster* [52]. Huang et al. [17] found that the average density of stomata per needle in *Pinus yunnanensis* is positively related to precipitation and that the number of stomata on the needles also depends on rainfall in *Pinus sylvestris* and *Pinus uncinata* [53, 62].

In this study, the NW trait was negatively correlated with longitude, temperature, and precipitation, but no correlation was observed with altitude and latitude. In *Pinus pinaster*, NW is negatively related to altitude and positively to latitude, but no significant correlation was reported between NW, longitude, and precipitation [52]. In Asteraceae, Yuliani et al. [63] showed that the leaves of species belonging to this family and located at a low altitude are the largest. While in *Pinus yunnanensis*, this trait does not correlate with any environmental factors [17]. Our study showed that NF of cedar needles is independent of all environmental factors, whereas NN/R is negatively related to temperature and CT to temperature and longitude. In provenances of the Moroccan

High Atlas, NN/R was found maximum but reached the lowest values in the eastern part of the area in *Cedrus libani* and *Cedrus deodora* in Algeria [40]. In several studies, NN/R is not taken into account [14, 64–68] due to the leaf life span intraspecific variation which is largely explained as an environmentally determined phenotypic acclimation [69, 70]. These data are in accordance with the negative correlation found here between NN/R and temperature. However, even if the intraspecific variation in leaf life span is much less than the interspecific variation [71], it may be taken into account for such studies. Moreover, we recently found NN/R discriminates between genetically different populations in the Middle Atlas Morocco [22]. The study of the needles of the cedar populations by Jasińska et al. [14] shows that CT does not discriminate between cedar species, while distinguishing *C. atlantica* populations from the Moroccan Middle Atlas, with a CV from 12.29 to 21.64% in the Rif and the Middle Atlas and a CV of 14.65 to 28.66% for the *Cedrus libani*. These data are in agreement with our results from which we suggest a classification of the cedar habitats according to CT values: it is low in the Rif region (12.29%) and much more elevated in the High Atlas region (32.48%). Similarly, it has been reported that due to the heterogeneity and independence of habitats, directional selection may play a major role during the differentiation process in individual habitats, as was detected in the case of *Abies pinsapo*–*A. maroccana* [72].

Several studies have shown differences between the traits of cedar needles in Morocco [14, 22, 23, 41, 42, 66], but for the first time, our results showed highly significant intraspecific variations in needle traits between and within populations that covered a wide range of latitude and longitude from 32° 31'N 4°10'W to 34°08'N 5°11'W, displaying remarkable differences, which depend on the climatic conditions. The largest differences among populations were observed in the studied needles of the T and A populations. These phenotypic variations presented by the cedar tree in comparison with those found in the bibliography in various coniferous species are in agreement with previous studies [14, 27, 52, 17, 73–75] and highlight the most determinant needle traits for acclimation to environmental factors in *C. atlantica* natural populations.

**4.2. Environmental Effects on the Evolution of *Cedrus atlantica* Populations.** Environmental factors in areas of origin had significant impacts on plant growth [28, 77]. Schlichting [78]. found that phenotype changes were consistent with changes in environmental gradients, particularly in high altitude habitats where the climate was more complex and dynamic [28]. In this study, the analysis of the Euclidean distance shows that the populations A of the High Atlas and T of the Middle Eastern Atlas have been gathered in the same group, although they are very distant geographically. Whereas for the other two populations, the grouping is in line with their geographical positions.

## 5. Conclusion

The results of this study showed the plastic adaptation of *C. atlantica* needle shape to diverse environments.

They show for the first time that the needle length with number of stomatal lines and needle width with cuticle thickness are negatively sensitive to altitude and longitude, respectively. The populations at low temperatures are characterized by thick needles with a high number of rosettes. While, populations placed in areas with high rainfall have long, thin needles. All these results require that the geographical position and the climatic conditions of the populations change the structure of the needles of *C. atlantica* from Morocco. However, more in-depth studies (including several species and leading to different regions) are needed to determine the other anatomical, biochemical, and genetic parameters controlling tree structure for efficient cedar forest conservation.

### Data Availability

The data used to support this study are included within the article.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### References

- [1] L. Lanier, "Les champignons des cédraies en Algérie (Etude comparative)," *Le cedre de l'Atlas Cedrus atlantica (Manetti)*. Casablanca: Imprimerie Najah ElJadida, vol. 27, pp. 554–561, 1994.
- [2] C. Ripert and B. Boisseau, "Écologie et croissance du cèdre de l'Atlas en Provence CMAGREF," in *Le cedre de l'Atlas Cedrus atlantica (Manetti)*, O. Hirit, A. Samih, M. Malagnoux, Eds Imprimerie Najah ElJadida, Casablanca, Morocco, 1994.
- [3] M. Labhar, *Forest and Pre-Forest Environments of the Central and Western North Central Atlas: Geographical Approach, Phytoecology and Dynamics*, University Sidi Mohammed Ben Abdellah, Fez, Morocco, 1998.
- [4] D. A. Schaad, E. Iriarte, J. A. L. Sáez et al., "Are *Cedrus atlantica* forests in the Rif Mountains of Morocco heading towards local extinction?" *The Holocene*, vol. 28, no. 6, pp. 1023–1037, 2018.
- [5] M. Bariteau, K. P. Panetsos, O. M'hirit, and A. Scaltsoyiannes, "Genetic variability of Atlas cedar in comparison with other Mediterranean cedars," *Forêt Méditerranéenne*, vol. 20, pp. 175–190, 1999.
- [6] A. Moussouni and Z. Boubaker, *Diversity of Birds in the Forest Cedar Djurdjuran (Eastern Algeria)*. Revue Forestiere Française, Vol. 5, AgroParisTech, Paris, France, 2015.
- [7] B. R. Morata, S. G. Nebauer, E. Sales, J. Allainguillaume, P. Caligari, and J. Segura, "Genetic diversity and structure of natural and managed populations of *Cedrus atlantica* (Pinaceae) assessed using random amplified polymorphic DNA," *American Journal of Botany*, vol. 92, no. 5, pp. 875–884, 2005.
- [8] A. Charrière, *Héritage Hercynien et Évolution Géodynamique Alpine d'une Chaîne Intracontinentale: le Moyen-Atlas au Sud-Est de Fès (Maroc)*, Semantic Scholar, Seattle, WA, USA, 1990.
- [9] G. Lachkar, D. Ouarrache, and A. Charrière, "Nouvelles données palynologiques sur les formations sédimentaires associées aux basaltes triasiques du Moyen Atlas et de la haute Moulouya (Maroc)," *Revue de Micropaleontologie*, vol. 43, pp. 281–299, 1994.
- [10] D. Ouarhache, *Sédimentation et Volcanismes (effusif et explosif) Associés au Rifting Triasique et Infra-Liasique Dans le Moyen Atlas Sud-Occidental et la Haute Moulouya (Maroc)*, Université Mohammed-V, Rabat, Morocco, 2002.
- [11] C. Peyre, *Research on the Staging of Vegetation in the Bou Iblane Massif (Middle Eastern Atlas-Morocco)*, University of Aix-Marseille, Aix-en-Provence, France, 1979.
- [12] A. Achhal, O. Akabli, M. Barbero et al., "About the bioclimatic and dynamic value of some forest tree species in Morocco," *Ecologia Mediterranea*, vol. 5, pp. 211–249, 1980.
- [13] B. Fedan, "Evolution géodynamique d'un bassin intra-plaque sur décrochements: le Moyen Atlas (Maroc) durant le Méso-Cénozoïque, Travaux de l'Institut scientifique de Rabat," *Série Géologie et Géographie Physique*, vol. 18, p. 142, 1989.
- [14] A. K. Jasińska, K. Boratyńska, K. Sobierajska et al., "Relationships among *Cedrus libani*, *C. brevifolia* and *C. atlantica* as revealed by the morphological and anatomical needle characters," *Plant Systematics and Evolution*, vol. 299, pp. 35–48, 2013.
- [15] B. Ghimire, C. Lee, and K. Heo, "Leaf anatomy and its implications for phylogenetic relationships in Taxaceae s. l.," *Journal of Plant Research*, vol. 127, no. 3, pp. 373–388, 2014.
- [16] A. Terrab, A. Hampe, O. Lepais, S. Talavera, E. Vela, and T. F. Stuessy, "Phylogeography of North African Atlas cedar (*Cedrus atlantica*, Pinaceae): combined molecular and fossil data reveal a complex quaternary history," *American Journal of Botany*, vol. 95, no. 10, pp. 1262–1269, 2008.
- [17] Y. Huang, J. Mao, Z. Chen et al., "Genetic structure of needle morphological and anatomical traits of *Pinus yunnanensis* and its relation to environmental and phylogenetic signals," *Journal of Forest Research*, vol. 27, pp. 13–25, 2015.
- [18] M. P. Nobis, C. Traiser, and A. R. Nebelsick, "Latitudinal variation in morphological traits of the genus *Pinus* and its relation to environmental and phylogenetic signals," *Plant Ecology & Diversity*, vol. 5, no. 1, pp. 1–11, 2012.
- [19] O. M. Legoshchina, O. A. Neverova, and A. A. Bykov, "Variability of the anatomical structure of *Picea obovata* Ledeb. needles under the influence of emissions from the industrial zone of Kemerovo," *Contemporary Problems of Ecology*, vol. 6, no. 5, pp. 555–560, 2013.
- [20] F. Xing, J. F. Mao, J. Meng et al., "Needle morphological evidence of the homoploid hybrid origin of *Pinus densata* based on analysis of artificial hybrids and the putative parents, *Pinus tabulaeformis* and *Pinus yunnanensis*," *Ecology and Evolution*, vol. 4, no. 10, pp. 1890–1902, 2014.
- [21] N. El Bakkali and M. B. Amraoui, "The length, number, and endodermis area of needles discriminate two genetically distinct populations of *Cedrus atlantica* Manetti in the Moroccan Middle Atlas," *Acta Societatis Botanicorum Poloniae*, vol. 87, no. 3, p. 3591, 2018.
- [22] N. El Bakkali and M. B. Amraoui, "Morphological and anatomical characterization of ecotype needles of *Cedrus*

- atlantica* in Morocco,” *International Journal of Forestry Research*, vol. 2022, Article ID 5836589, 11 pages, 2022.
- [23] K. Boratyńska and D. Lewandowska, “Differences among three populations of *Pinus uliginosa* and their relation to *P. sylvestris* as expressed by the needle characters,” *Dendrobiology*, vol. 61, pp. 37–46, 2009.
- [24] P. Androsiuk, Z. Kaczmarek, and L. Urbaniak, “The morphological traits of needles as markers of geographical differentiation in European *Pinus sylvestris* populations,” *Dendrobiology*, vol. 65, pp. 3–16, 2011.
- [25] B. Nikolić, S. Bojović, and P. Marin, “Variability of morphoanatomical characteristics of the needles of *Picea omorika* from natural populations in Serbia,” *Plant Biosystems*, vol. 10, pp. 1080–1126, 2013.
- [26] S. P. Tiwari, P. Kumar, D. Yadav, and D. K. Chauhan, “Comparative morphological, epidermal, and anatomical studies of *Pinus roxburghii* needles at different altitudes in the North-West Indian Himalayas,” *Turkish Journal of Botany*, vol. 37, pp. 65–73, 2013.
- [27] C. Körner, “The use of “altitude” in ecological research,” *Trends in Ecology & Evolution*, vol. 22, pp. 569–574, 2007.
- [28] L. Nagy, G. Grabherr, C. Körner, and D. B. A. Thompson, “Alpine biodiversity in space and time: a synthesis,” *Ecological Studies*, vol. 167, pp. 453–464, 2003.
- [29] T. Luo, Y. Pan, H. Ouyang et al., “Leaf area index and net primary productivity along subtropical to alpine gradients in the Tibetan Plateau,” *Global Ecology and Biogeography*, vol. 13, no. 4, pp. 345–358, 2004.
- [30] T. Fabbro and C. Körner, “Altitudinal differences in flower traits and reproductive allocation,” *Flora—Morphology, Distribution, Functional Ecology of Plants*, vol. 199, no. 1, pp. 70–81, 2004.
- [31] G. Hoch and C. Körner, “The carbon charging of pines at the climatic treeline: a global comparison,” *Oecologia*, vol. 135, no. 1, pp. 10–21, 2003.
- [32] C. Reisch, A. Anke, and M. Röhl, “Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps,” *Basic and Applied Ecology*, vol. 6, no. 1, pp. 35–45, 2005.
- [33] L. Klimes, “Life-forms and clonality of vascular plants along an altitudinal gradient in E Ladakh (NW Himalayas),” *Basic and Applied Ecology*, vol. 4, no. 4, pp. 317–328, 2003.
- [34] A. D. Friend and F. I. Woodward, “Evolutionary and eco-physiological responses of mountain plants to the growing season environment,” *Advances in Ecological Research*, vol. 20, pp. 59–124, 1990.
- [35] A. Boratyński, K. Boratyńska, and A. Lewandowski, “Evidence of the possibility of natural reciprocal crosses between *Pinus sylvestris* and *P. uliginosa* based on the phenology of reproductive organs,” *Flora*, vol. 198, pp. 377–388, 2003.
- [36] R. A. Jenner, “Accepting partnership by submission? morphological phylogenetics in a molecular millennium,” *Systematic Biology*, vol. 53, no. 2, pp. 333–359, 2004.
- [37] Q.-P. Xiang, R. Wei, Y.-Z. Shao, Z.-Y. Yang, X.-Q. Wang, and X.-C. Zhang, “Phylogenetic relationships, possible ancient hybridization, and biogeographic history of *Abies* (Pinaceae) based on data from nuclear, plastid, and mitochondrial genomes,” *Molecular Phylogenetics and Evolution*, vol. 82, pp. 1–14, 2015.
- [38] A. Benabid, “Biogeography phytosociology and phytodynamic of the cedars of the Atlas *Cedrus atlantica* (Manetti),” *Annales de la Recherche Forestière au Maroc*, vol. 7, pp. 61–76, 1994.
- [39] S. Sabatier, P. Baradat, and D. Barthelemy, “Intra-and interspecific variations of polycyclism in young trees of *Cedrus atlantica* (endl.) manetti ex. Carriere and *Cedrus libani* A. Rich (Pinaceae),” *Annals of Forest Science*, vol. 60, no. 1, pp. 19–29, 2003.
- [40] M. Arbez, P. Ferrandes, and N. Uyar, “Contribution à l’étude de la variabilité géographique des Cèdres,” *Annales des Sciences Forestières*, vol. 35, no. 4, pp. 265–284, 1978.
- [41] M. Illoul and O. Moualek, *Exploration of Intraspecific Variability in Atlas Cedar (Cedrus atlantica Manetti) in Algeria by Studying Needles and Seeds. Engineer Agronomist Memory*, University Tizi-Ouzou, Tizi Ouzou, Algeria, 1992.
- [42] H. Khris and M. Lounaci, *Exploration of the Intraspecific Variability in Cedrus Atlantica Manetti by the Comparative Study of Seedlings of Various Algerian Geographical Regions: Experimental Device of Tagma (Yakouren, Tizi-Ouzou). Engineer Agronomist Memory*, University Tizi-Ouzou, Tizi Ouzou, Algeria, 1994.
- [43] H. Bouzar and T. Loumi, *Exploration of Intervariability at Cedrus Atlantica Manetti in Tagma (Yakouren, W. de Tizi-ouzou). Biometrics and Juvenile Growth. Engineer Agronomist Memory*, University Tizi-Ouzou, Tizi Ouzou, Algeria, 1995.
- [44] M. Rhanem, “Aridification of the regional climate and rise of the lower limit of Atlas cedar (*Cedrus atlantica* Manetti) to the borders of the plain of Midelt (Morocco),” *Physio-Géo, Géographie Physique et Environnement*, vol. 5, 2011.
- [45] M. Rhanem, “L’alfa (*Stipa tenacissima* L.) dans la plaine de Midelt (haut bassin versant de la Moulouya, Maroc)—éléments de climatologie,” *Physio-Géo*, vol. 3, pp. 1–20, 2009.
- [46] K. Boratyńska, K. Marcysiak, and A. Boratyński, “*Pinus mugo* (Pinaceae) in the Abruzzi mountains: high morphological variation in isolated populations,” *Botanical Journal of the Linnean Society*, vol. 147, pp. 309–316, 2005.
- [47] K. Boratyńska and A. Boratyński, “Taxonomic differences among closely related pines *Pinus sylvestris*. *P. mugo*. *P. uncinata*. *P. rotundata* and *P. uliginosa* as revealed in needle sclerenchyma cells,” *Flora*, vol. 202, pp. 555–569, 2007.
- [48] H. J. Arnott, “Leaf clearings,” *Turtlox News*, vol. 37, pp. 192–194, 1959.
- [49] E. R. Brady, D. K. Wemple, and N. R. Lersten, “Floral vasculature as a potential taxonomic character in *Dalea* (Leguminosae),” *Iowa Academy, Science, Proceeding*, vol. 71, pp. 46–51, 1965.
- [50] S. E. Ruzin, *Plant Microtechnique and Microscopy*, Oxford University Press, Oxford, UK, 1999.
- [51] R. López, J. Climent, and L. Gil, “Intraspecific variation and plasticity in growth and foliar morphology along a climate gradient in the Canary Island pine,” *Trees*, vol. 24, pp. 343–350, 2010.
- [52] N. Wahid, S. C. G. Martínez, I. El Hadrami, and A. Boulli, “Variation of morphological traits in natural populations of maritime pine (*Pinus pinaster* Ait.) in Morocco,” *Annals of Forest Science*, vol. 63, no. 1, pp. 83–92, 2006.
- [53] K. Boratyńska and E. Muchewicz, “Do needle characteristics of *Pinus uncinata* depend on climatic factors?” *Biodiversity: Research and Conservation*, vol. 3, no. 4, pp. 220–226, 2006.
- [54] M. Ladjal, N. Deloche, R. Huc, and M. Ducrey, “Effects of soil and air drought on growth, plant water status and leaf gas exchange in three Mediterranean cedar species: *Cedrus atlantica*, *C. brevifolia* and *C. libani*: Trees *brevifolia* and *C. Libani*,” *Trees*, vol. 21, no. 2, pp. 201–213, 2007.
- [55] A. Güney, M. Küppers, C. Rathgeber, M. Şahin, and R. Zimmermann, “Intra-annual stem growth dynamics of

- Lebanon Cedar along climatic gradients,” *Trees*, vol. 31, no. 2, pp. 587–606, 2017.
- [56] J. Staszkiwicz, *Morphological Variability of Needles, Cones and Seeds*, PAN Instytut Dendrologii, Kórnik, Poland, 1993.
- [57] D. J. Beerling and C. K. Kelly, “Evolutionary comparative analyses of the relationship between leaf structure and function,” *New Phytologist*, vol. 134, no. 1, pp. 35–51, 1996.
- [58] M. J. Lockheart, I. Poole, P. F. Van Bergen, and R. P. Evershed, “Leaf carbon isotope compositions and stomatal characters: important considerations for palaeoclimate reconstructions,” *Organic Geochemistry*, vol. 29, no. 4, pp. 1003–1008, 1998.
- [59] E. Marcet, “On the detection of spontaneous hybrids of *Pinus mugo* turra and *Pinus sylvestris* L. due to needle characteristics,” *Berichte der Schweizerischen Botanischen Gesellschaft Bulletin de la Société Botanique de Suisse*, vol. 77, pp. 314–360, 1967.
- [60] A. L. Fedorkov, “Variation in the anatomical characters of needles and their resistance to technogenic and climatic stress in scots pine,” *Russian Journal of Ecology*, vol. 33, no. 1, pp. 65–67, 2002.
- [61] M. Kivimäenpää, H. Valolahti, E. Häikiö et al., “Warming and elevated ozone differently modify needle anatomy of Norway spruce (*Picea abies*) and scots pine (*Pinus sylvestris*),” *Canadian Journal of Forest Research*, vol. 47, pp. 488–499, 2017.
- [62] S. A. Mamaev, *Forms of Intraspecific Variability of Woody Plants*, Nauka, Moscow, Russia, 1972.
- [63] Y. Yuliani, B. Soemarno, B. Yanuwadi, and A. S. Leksono, “The relationship between habitat altitude, environmental factors and morphological characteristics of *Pluchea indica*, *Ageratum conyzoides* and *Elephantopus scaber*,” *Online Journal of Biological Sciences*, vol. 15, no. 3, pp. 143–151, 2015.
- [64] H. Gaussen, “Genus *Cedrus*,” *The Current Gymnosperms and Fossils*, vol. 7, pp. 295–320, 1964.
- [65] P. Maheshwari and C. H. Biswas, *Monographic*, Vol. 54, Council of Scientific and Industrial Research, , New Delhi, India, 1970.
- [66] A. Farjon, *A Handbook of the World's Conifers*, Vol. 1, Brill, , Leiden, Netherlands, 2010.
- [67] M. Vidaković, *Conifers*, Graficki Zavod Hrvatske, Zagreb, Croatia, 1991.
- [68] M. Brunetti, E. L. De Capua, N. Macchioni, and S. Monachello, “Natural durability, physical and mechanical properties of Atlas cedar (*Cedrus atlantica* Manetti) wood from Southern Italy,” *Annals of Forest Science*, vol. 58, no. 6, pp. 607–613, 2001.
- [69] P. B. Reich, J. Oleksyn, J. Modrzyński, and M. G. Tjoelker, “Evidence that longer needle retention of spruce and pine populations at high elevations and high latitudes is largely a phenotypic response,” *Tree Physiology*, vol. 16, no. 7, pp. 643–647, 1996.
- [70] Y. Xiao, “Variation in needle longevity of *Pinus tabulaeformis* forests at different geographic scales,” *Tree Physiology*, vol. 23, no. 7, pp. 463–471, 2003.
- [71] R. L. Eckstein, P. S. Karlsson, and M. Weih, “Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions,” *New Phytologist*, vol. 143, no. 1, pp. 177–189, 1999.
- [72] K. Sękiwicz, M. Sękiwicz, A. K. Jasińska et al., “Morphological diversity and structure of west mediterranean *Abies* species,” *Plant Biosystems*, vol. 147, no. 1, pp. 125–134, 2013.
- [73] G. E. Rehfeldt, “A model of genetic variation for *Pinus ponderosa* in the Inland Northwest (U.S.A.): applications in gene resource management,” *Canadian Journal of Forest Research*, vol. 21, no. 10, pp. 1491–1500, 1991.
- [74] K.-S. Woo, L. Fins, G. I. McDonald, D. L. Wenny, and A. Eramian, “Effects of nursery environment on needle morphology of *Pinus monticola* Dougl. and implications for tree improvement programs,” *New Forests*, vol. 24, no. 2, pp. 113–129, 2002.
- [75] L. G. Esteban, J. A. Martín, P. De Palacios, F. G. Fernández, and R. López, “Adaptive anatomy of *Pinus halepensis* trees from different Mediterranean environments in Spain,” *Trees*, vol. 24, no. 1, pp. 19–30, 2010.
- [76] V. Lavadinović, Z. Miletić, V. Lavadinović, and V. Isajev, “Variability in magnesium concentration in needles of different Douglas-fir provenances,” *Forestry*, vol. 17, pp. 74–79, 2011.
- [77] C. D. Schlichting, “The evolution of phenotypic plasticity in plants,” *Annual Review of Ecology and Systematics*, vol. 17, no. 1, pp. 667–693, 1986.