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Original Research Article

Morpho-physiological parameters of *Cedrus atlantica* Manetti pollen discriminate genetically distinct populations of climatically different regions in the Moroccan Atlas

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ABSTRACT

In North Africa's forests of *Cedrus atlantica* many tree stands perished in the last 130 years due to environmental changes. Variations in some morpho-physiological characters of cedar pollens were studied in three populations localized in Azrou and Midelt regions of Moroccan Atlas having different local environmental conditions and levels of genetic diversity. Results of Spearman nonparametric coefficient of correlation showed significant correlations ($-0.726 \leq r \leq 0.812$) ($p < 0.01$). Corpus length (Lc) and volume (Vc), balloon volume (Vb) and the volume total of pollen (Vtp) were negatively related to annual mean precipitation (P) and positively to longitude (Long) and altitude (Alt). Lc and Vc were also negatively correlated with latitude (Lat) while positively with annual mean temperature (T) for Vc. Balloon height (Hb), number of germinated pollen (NGP) and germination frequency of pollen (GFP) were positively linked to Alt. Pollen starch content (Starch) was negatively correlated to T and Long and positively to P and Lat. Production of pollen (NTP) was positively correlated with P and negatively with Long and Alt. The PCA analysis showed that Lc, Vc, Lg, Hb, Vb, Vtp, NGP, GFP, NTP were influenced by climatic conditions and geographic location. The ascending hierarchical classification showed that populations of Ait Oufella and Ait Ayach are separated from the Seheb population. Tukey's test showed that at least five morpho-physiological characters separated significantly the populations. The structuration of morphological traits of Atlas cedar pollen of the populations was discussed in relation to physiological traits and local environmental conditions.

Keywords: Corpus, Balloon, Pollen, Population, Environment, *Cedrus*.

Introduction

Leaves and branches are key assimilative and reproductive organs in the plant, changing their shape in response to environmental change.[1] Generally, in photoperiodic plants, young leaves and roots inhibit flowering, while intense light and high carbon dioxide (CO_2) levels promote flowering.[2] Leaves affect male and female sexual functions in conifers.[3] Likewise, Piola et al.[4] found inhibition of bud growth by abscisic acid, temperature and leaves of *Cedrus libani*. These observations show the role of leaves in *Cedrus* reproduction.
Variability within woody species depends on several factors including changes in effective population size and mode of reproduction.[5] The research methods implemented for the study of pollen have made it possible to strengthen knowledge on the morphology and physiology of conifer pollen in general and cedar in particular.[11,22] Physiological studies on pollen grain in conifers have well established that germination and pollen tube length elongation are two parameters intrinsically linked to reproductive performances of the species.[6-8]

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Moreover, germination capacity and vigor of pollen tube growth were found to be correlated with pollen size.[9] In the same way pollen morphology and ultrastructure with pollination biology and growth habitat. [10,11] Schoch-Bodmer [12] and Kurtz and Liverman[13] demonstrated the influence of the degree of humidity in the ambient air during pollen development on its size and variability. Atlas cedar (*Cedrus atlantica* Manetti) is a relict and an endemic endangered species in the mountains of North Africa, whose distribution range has undergone a dramatic reduction
over the latest decades, attracting increasing international interest in its use in degraded lands reforestation due to its large ecological range.[14] The species covers the highest summits of Morocco (130000 ha) and Algeria (27000 ha) [15,16] and it is one of the most economically and ecologically important conifers in the Mediterranean region.[17,18] The variability of *Cedrus atlantica* has been addressed by several authors. Karam et al.[19] based on allelic frequencies and the presence of null alleles, revealed a clear hierarchical genetic structure among *C. atlantica* populations with a first level of differentiation between Algerian and Moroccan origins, and a sub-differentiation between coastal and internal mountains with the Algerian populations. Terrab et al. [16] and Cheddadi et al.[20] demonstrated a highest level of genetic diversity between the populations of the Rif and the High Atlas compared to those of the Middle Atlas. Likewise, El Bakkali and Bendriss Amraoui [14,21] showed that leaf characters are high discriminating characters among populations of *Cedrus atlantica*. Similarly, the work of Derridj et al.[22] and Bell et al.[23] revealed that pollen grain morphological characteristics of *Cedrus atlantica* vary widely with geographic area.
Recently, the Atlas cedar has suffered the effects of global warming which result in a massive loss of habitats linked to a significant decline

in the number of individuals [24,25] which can lead to a disproportionate loss of phenotypic diversity.[26] According to Pither[27] and Svenning[28] climate tolerance; particulary that of cold and aridity are crucial factors for trees survival. Several genetic studies have been made recently on *C. atlantica*, but no one has studied its morphological reproduction.[16,19,20, 29,30] Due to the fact that the reproductive stage is the most sensitive to abiotic stresses especially to climate variations, [31,32] studying pollen morphology and physiology in relation to environmental global warming is essential to understand the cedar loss of phenotypic diversity because pollen is the only part of male gametophytes in plants having direct role in heredity as well as in seed development and natural regeneration.[33] Thus, eco-physiological knowledge of mechanisms that control adaptation and resilience [34] of *Cedrus atlantica* pollen grain to drought is mandatory to maintain the diversity of genetic resources for this species since recurrent drought is one of the main climatic factors threatening forests, especially in the Mediterranean region.[35,36]

The only studies published during the last decade on the taxonomic and geographic differentiation of conifers show that morphological and anatomical characteristics of leaf are important in the recognition of the phylogenetic relationships and geographic pattern of variation in Pinaceae.[37-38] Again, to date, no research has been carried out on the variation in male gametophyte between genetically distinct populations of *C. atlantica*. The aim of this study is to highlight variations in morpho-physiological parameters of the male gametophyte with climate in genetically distinct populations.

Material and Methods

Study regions

In the High Atlas Mountains of Morocco, the cedar forests exist in an area of 10000 ha in a continental semi-arid bioclimate at an altitude of between 1900 and 3250 m between the latitudes of 32° 30' and 32° 15' and longitudes of 4° 24' to 5° 33'. [24] In the Middle Atlas, they cover an area of 70000 ha at latitudes between 32° 08' and 33° 44', for altitudes between 1600 and 2500 m [24,39] in a variable bioclimate from very cold to continental semi-arid.

This study, was carried out in October 2019 on morphological and physiological traits of the pollen of the Seheb (Seh) population of *C. atlantica* in the Azrou region and Ait Oufella (A. Ouf) and Ait Ayach (A. Ay) in the Midelt region. The first region belongs to the central Middle Atlas, and the second belongs to the Middle and High Atlas as described in the study (Figure 1).[8]

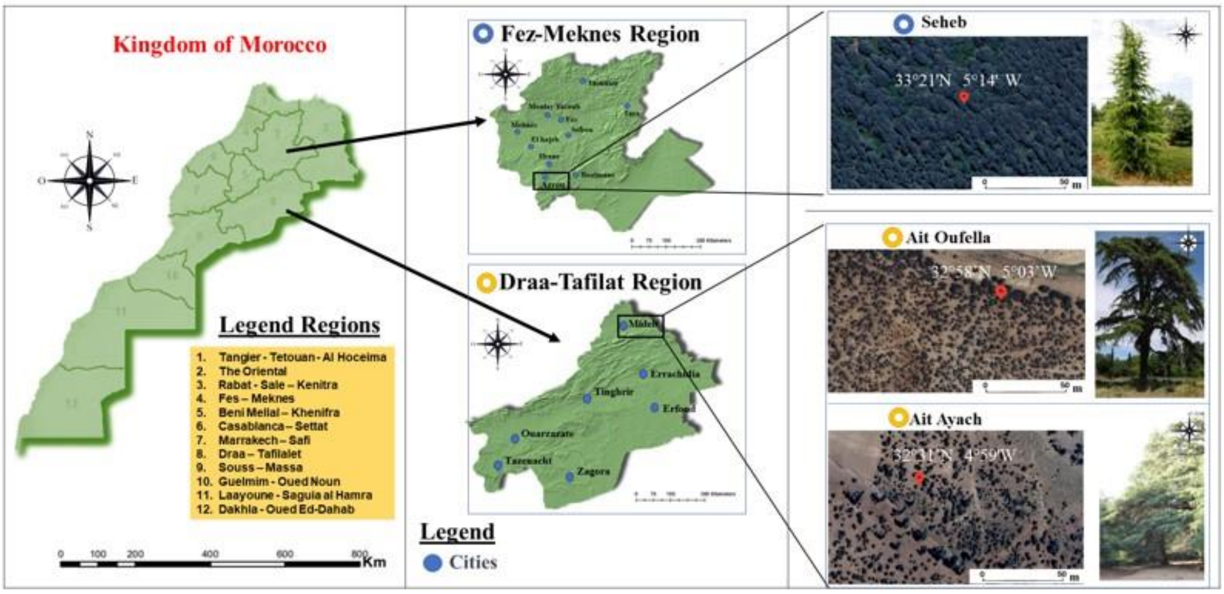

Figure 1: Localization of the natural areas of the sampled populations of *C. atlantica*

In the Seheb population, the nuclear DNA content (2C) is high (32.2 pg), the percentage of AT was 59.2%, whereas that of GC was 40.6%. The within population differences in DNA content were small and the total chromosome length was estimated to 139 µm. [40] The Ait Oufella population was shown to be molecularly distinct from Algerian populations by the null alleles and specifically marked by the mean allelic richness per locus = 3.691; the mean number of population-specific alleles per locus = 0.188; the expected diversity (He) not accounting for null alleles = 0.434; the expected diversity (He) accounting for null alleles = 0.519; the observed heterozygosity (Ho) = 0.352; the deficit of heterozygosity (F_{IS}) = 0.200. The coefficient of relatedness between individuals varies between -3.75 and 0.3 and corresponds to the expected distribution for unrelated individuals.[19] By contrast, the Midelt region, particulary the Ait Ayach population, has never been studied genetically or structurally but the Cirque de Jaffar population (32°22' N / 4°53' W) geographically very close to Ait Ayach was studied by Terrab et al.[16] who based on six cpSSR loci, found that the population was specifically characterized by the following values: the total number of haplotypes (No) = 13; the effective number of haplotypes (N_e) = 12.97; the haplotype diversity (H_e) = 1 ; the mean pairwise haplotype distance (D^2_{SH}) = 18.46; the proportion distinguishable (% PD) = 100, and it was also characterized by three chloroplast Simple Sequence Repeat (cpSSR) alleles that are specific to it: Pt63718: 95 base pairs (pb), Pt15169: 104 base pairs (pb) and Pt71936: 146 base pairs (bp).

The climatic data in each geographic location of the population (Table 1) had been obtained from the Regional Directorate of Meteorology North-East Region.

Sampling and extraction of catkins

For each population a total of 1000 catkins were collected at the time of pollination on ten healthy trees at the rate of one hundred catkins per tree. The pollen extraction technique consisted in a preliminary mixing of the catkins then we kneaded them and passed them through a series of sieves arranged in descending order (0.2 mm; 0.125 mm; 0.1 mm; 0.04 mm). The extracted pollen was stored at 4°C in airtight jars.

Table 1: Geographic characteristics and location of studied populations of *Cedrus atlantica* in Morocco

Populations	Regions	T	P	Lat	Long	Alt
Seh	Middle Atlas central	12.39	408	33.35	-5.23	1800
A. Ouf	Middle Atlas central	14.08	351.9	32.97	-5.05	1982
A. Ay	High Atlas	15.1	324.2	32.52	-4.98	1972

T (°C) : annual mean temperature ; P (mm) : annual mean precipitation, Lat : Latitude, Long : Longitude, Alt (m asl) : Altitude, Seh : Seheb, A. Ouf : Ait Oufella, A. Ay : Ait Ayach.

Morphological analysis

Pollen slides for light microscopy were prepared according to Wodehouse method.[41] The pollen was transferred onto a clean slide; absolute ethanol (95°) was added dropwise and the mixture was maintained at 70 °C until the ethanol was completely evaporated. Glycerin-gelatin with iodine green at a concentration of 0.2 mg/ml was dropped onto pollen, covered with cover-glass. To avoid excessive grain size due to the temporary turgor state in the glycerinated gelatin, we only started the measurements like Aytug [42] from the second month after mounting. Measurements were made with a software (Opmias Ver1.0, 2001-2008) at x400 magnification (Figures 2 and 3) on pollen grains photographed in distal view with an optical microscope (Optika DM-15, Italy) equipped with a camera. Pollen terminology was adopted following Erdtman [43] and Derridj et al.[22] Each of the variables in distal view for each provenance was measured on one hundred and thirty pollen grains, namely: corpus length (Lc), corpus height (Hc), thickness of the cap (Ec), balloon length (Lb), balloon height (Hb) and pollen grain entire length (Lg) (Figure 3). We calculated the volumes of the corpus (Vc) and balloon (Vb) according to Silveira [44] by using the formula 1 that corresponded to their shape such as an ellipsoid: [42]

$$(1)\ V = \frac{4(\pi e^2 p)}{3}$$

with V = volume, e = equatorial radius which corresponds to Lc for the corpus and to Hb for the balloon, p = polar radius which corresponds to Hc for the corpus and to Lb for the balloon. The total volume of pollen (Vtp) being the sum of the volume of the corpus (Vc) and that of the balloon (Vb).

Physiological analysis

On the extracted pollen we determined the germination in vitro, the number of total pollen (NTP), the total number of germinated pollen (NGP), the germination frequency of pollen (GFP), the mean pollen tube length (PTL) of all germinated grains, the starch reserve content (mg/g pollen) of 10 trees per population in the same way as reported in our study.[8]

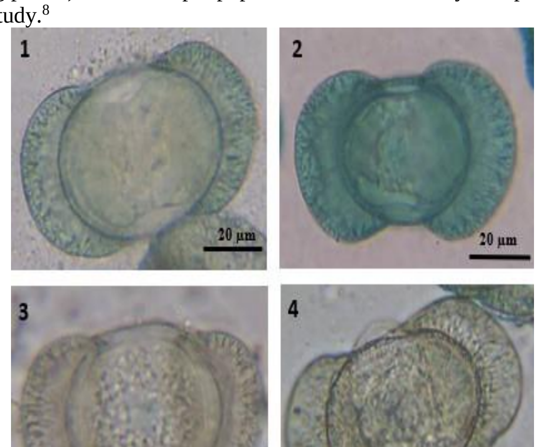

Figure 2: Pollen grain of *C. atlantica* Manetti (x400). 1-2: stained with iodine green; 3-4: without coloration

Statistical analysis

We determined descriptive statistics (mean, max, min, standard error, coefficient of variation, Skewness and Kurtosis) for all morphological and physiological parameters. Differences between the different geographical populations were calculated using the Tukey test at a significance level of 5% on pollen characteristics. Pearson correlation was performed at 0.01 and 0.05 significance level on morphological and physiological characters. The correlation between populations for each morphological and physiological trait and environmental factors such as temperature, precipitation, latitude, longitude and altitude were studied using Spearman's nonparametric correlation coefficient. This correlation coefficient is adequate for samples of nonnormal distribution. Since the number of correlation tests performed is very high for both Pearson and Spearman, we adjusted the obtained p-value values according to Bonferroni correction to avoid getting a significant p-value by chance. All these statistical analyses were done using IBM SPSS Statistics 20.0 software. Using R-STUDIO version 1.3.1093, we performed principal component analysis (PCA) to determine the nature and degree of diversity among the populations and determined the agglomeration of the populations on the closest Euclidean distances according to Ward's method using all the characters to check out the affinities between *C. atlantica* populations.

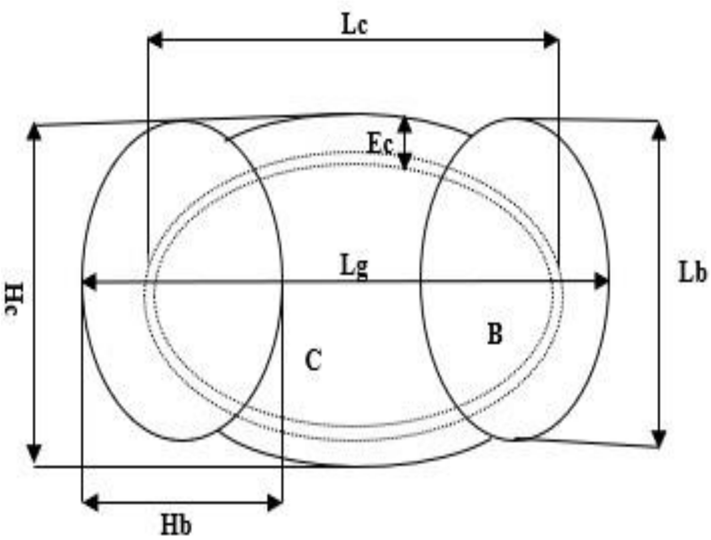

Figure 3: Measured parameters of *Cedrus atlantica* pollen grain in distal view. Lc: corpus length, Hc: corpus height, Hb: balloon height, Lb: balloon length, Ec: cap thickness, Lg: pollen grain total length, B: balloon, C: Corpus

Results and Discussion

Variation within-populations

The average values and variation coefficients (CV) of nine pollen morphological and five pollen physiological traits in each population were given respectively by tables 2 and 3. The within-population variation was studied according to the values of the coefficient of variation (CV). The variables Ec, Vc, Vb, Vtp, PTL, NGP, NTP and GFP showed high variation, whereas Hc, Lc and Lb showed low variation in the studied populations. The Balloon height (Hb) showed low variation, whereas the total length of the pollen grain (Lg) presented a strong variation only in the Seheb population. The variation of Starch variable was found to be low only in the Ait Oufella population. The description parameter of character distribution (Kurtosis) showed a similarity between populations of Ait Oufella and Ait Ayach. These two populations showed positive flattening values, so their characters were more clustered around the mean, with the exception of Ec, Lc and Starch for both two populations, Hb, NTP and GFP for the Ait Oufella population. In the Seheb population, the distribution character (Kurtosis) showed negative values for four characters meaning that the values of these characters were therefore less grouped around the mean; the rest of characters show positive values (Tables 2 and 3).

Table 2: Statistical description of morphological parameters in the different populations studied

Statistics	Populations	Hc	Hb	Lb	Ec	Lc	Lg	Vc	Vb	Vtp
Mean ± ES	Seh	52.81±0.46	30.93±0.35	48.50±0.50	5.08±0.13	53.63±0.64	63.77±1.2	$6.36.10^{-4}$±$1.02.10^{-5}$	$1.94.10^{-4}$±$5.23.10^{-6}$	$8.30.10^{-4}$±$1.21.10^{-5}$
	A. Ouf	50.50±0.47	41.51±0.72	47.50±0.54	4.76±0.10	61.45±0.69	79.96±0.87	$8.01.10^{-4}$±$1.46.10^{-5}$	$3.42.10^{-4}$±$8.08.10^{-6}$	$11.41.10^{-4}$±$1.95.10^{-5}$
	A. Ay	53.86±0.46	39.25±0.82	49.94±0.50	4.71±0.10	60.20±0.67	78.99±0.78	$8.17.10^{-4}$±$7.61.10^{-6}$	$3.22.10^{-4}$±$6.69.10^{-6}$	$11.39.10^{-4}$±$1.12.10^{-5}$
Minimum	Seh	39.27	21.46	32.65	2.05	34.25	34.25	$7.17.10^{-5}$	$0.75.10^{-4}$	$3.34.10^{-4}$
	A. Ouf	36.00	16.97	28.44	2.29	36.11	60.78	$6.28.10^{-5}$	$2.53.10^{-4}$	$4.5.10^{-4}$
	A. Ay	40.29	11.24	26.83	2.05	42.72	61.69	$5.11.10^{-5}$	$2.12.10^{-4}$	$5.27.10^{-4}$
Maximum	Seh	66.44	43.27	68.26	9.63	62.32	88.95	$8.34.10^{-4}$	$9.76.10^{-4}$	$11.7.10^{-4}$
	A. Ouf	68.35	64.91	68.35	8.03	72.02	103.44	$14.84.10^{-4}$	15.10^{-4}	$17.7.10^{-4}$
	A. Ay	69.72	48.16	62.38	7.57	61.47	94.72	$10.51.10^{-4}$	$9.01.10^{-4}$	$12.4.10^{-4}$
CV	Seh	10.01	13.08	11.68	29.65	9.66	21.68	30.11	21.78	18.95
	A. Ouf	10.69	18.25	13.14	24.35	12.05	10.71	38.93	29.01	27.56
	A. Ay	9.86	16.36	11.55	23.86	7.42	7.93	33.83	15.01	15.84
Skewness	Seh	0.201	0.102	0.206	0.672	1.20	-0.082	0.060	0.397	0.408
	A. Ouf	-0.116	0.503	-0.424	0.152	0.355	-1.39	1.848	-0.803	1.035
	A. Ay	0.117	1.043	-0.768	0.136	0.664	-0.179	1.296	-1.396	0.823
Kurtosis	Seh	-0.150	-0.141	0.953	0.475	0.140	-1.095	-0.911	0.432	0.756
	A. Ouf	0.857	-0.495	0.931	-0.439	-0.518	0.198	0.672	2.120	2.580
	A. Ay	0.021	0.419	1.245	-0.253	-0.562	0.247	1.794	0.897	1.361

Hc (µm): corpus height, Hb (µm): balloon height, Lb (µm): balloon length, Ec (µm): cap thickness, Lc (µm): corpus length, Lg (µm): pollen grain total length, Vc (mm^3): volume of the corpus, Vb (mm^3): volume of the balloon, Vtp (mm^3): volume total of pollen, Seh: Seheb, A. Ouf: Ait Oufella, A. Ay: Ait Ayach.

Studies of leaf variation based on geographic locations and environmental changes are common in trees, especially those with a long-life span.[5] Many studies have reported significant variations in leaf characteristics within natural populations of several species.[45-47] These variations in leaf characteristics of the sporophyte in many species, [48-50] particulary in the atlas cedar [14,21] due to environmental and genetic factors [16,20,29] are communicated to the gametophytes because the pollen development occurring inside the anther and flowers of the mother plant is influenced by leaf development through water availability and sugars and hormone synthesis.[51,52]

Moreover, it's well known that the within-population variation in fertility parameters affects genetic diversity through reduction in effective population size which increases co-ancestry and in-breeding and through loss of genetic diversity.[53] In *Cedrus* genus and other species this variation is attributed to the genotype of individuals and year,[54,55] to the sunlight condition, gender and to the extent of masculinity on the crown [56,57] and to silvicultural practices.[58,59]

Even though many studies relating to the morphology of *C. atlantica* have already been carried out, they are yet very limited, since they are mainly oriented towards the search for morphological and genetic variability at the leaf level. The high within-population variability of the characters Ec, Vc, Vb, Vtp, PTL, NGP, NTP and GFP noted here in the pollen, in all the populations of *Cedrus atlantica* corroborates the results reported by El Bakkali and Bendriss Amraoui [21] who showed that adaptive variations in the length and number of leaves per whorl and the surface area of the endodermis are highly distinctive of cedar populations from the Ifrane and Azrou regions which have been previously shown as genetically distinct populations.[29] These pollen variations are also in agreement with those obtained on *C. atlantica* leaves by Karam et al.[19] which based on the allelic richness, the number of population-specific alleles, diversity, the heterozygosity and allele frequencies showed the existence of the within-population variation in the Ait Oufella population, showing here a strong variation in the balloon volume (Vb) and height (Hb), cap thickness (Ec), volume of the pollen corpus (Vc) as well as the volume total of pollen (Vtp); while the Ait Ayach population showed high variation only in the first three variables indicating high level of intra-population variation in the Ait Oufella population. This observation recommended the existence of a more significant gene flow in Ait Oufella.

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Table 3: Statistical description of physiological parameters in the different studied populations

Statistics	Populations	Starch	PTL	NGP	NTP	GFP
Mean ± ES	Seh	6.00±1.4	258.14±10.79	133	13416	0.99
	A. Ouf	4.03±0.09	166.34±2.63	1003	10182	9.85
	A. Ay	3.4±1.75	291.05±3.94	487	10230	4.76
Minimum	Seh	3.45	29.82	0.00	1.00	0.00
	A. Ouf	3.9	32.14	0.00	1.00	0.00
	A. Ay	0.83	67.91	0.00	1.00	0.00
Maximum	Seh	9.02	611.76	2.00	120.00	100
	A. Ouf	4.22	435	8.00	57.00	100
	A. Ay	7.04	607.07	3.00	60.00	100
CV	Seh	37.16	48.23	27.30	45.87	35.88
	A. Ouf	4.21	50.21	65.05	58.81	50.6
	A. Ay	77.78	29.91	17.12	63.88	74.22
Skewness	Seh	0.486	0.864	1.057	1.037	0.631
	A. Ouf	0.385	1.185	1.469	0.473	0.001
	A. Ay	-0.012	0.874	0.587	1.181	1.139
Kurtosis	Seh	0.156	1.087	1.753	0.494	0.566
	A. Ouf	-0.185	0.962	0.603	-0.413	-1.293
	A. Ay	-1.358	1.505	0.268	1.508	0.806

Starch reserves (mg/g pollen), PTL (µm): pollen tube length, NGP: number of germinated pollen, NTP: number of total pollen, GFP (%): germination frequency of pollen, Seh: Seheb, A. Ouf: Ait Oufella, A. Ay: Ait Ayach.

Many authors, [16,20,29] most recently Karam et al.[19] by using different methodologies on leaf showed that most of the variation in *Cedrus atlantica* occurs within populations in the North of Africa. A within-population variation in pollen morphological parameters in *Cedrus atlantica*, was previously reported by Bell et al.[23] and was attributed to irregular pollen development as found by Smith et al.[60] On the other hand, Alves Rodrigues et al.[61] reported that pollen development duration is linked to environmental conditions, suggesting that the differences between the data and those of Bell et al. [23] were due to pollen development duration as well as environmental conditions. Moreover, Cruzan [62] in *Erythronium grandiflorum* established a within-population variation in pollen production, its volume, its size and its quantity of reserves in opposite manner as found by Lopez et al.[63] although the relation between pollen size, production and its reserves content can be species dependent. [64,65] These observations suggest that variations of pollen morphology in *C. atlantica* as found in its leaf [14,21,66] may also be due more to species, ecotypes, sexual morphs, gene flow and methodology than to irregular pollen development.

Variation among populations

Tukey's test for morphological and physiological characters showed that the populations of Ait Oufella and Ait Ayach were similar in the variables Hb, Ec, Lc, Lg, Vc, Vb, Vtp, Starch and NTP. The Seheb population was significantly separated from the other populations by all the studied characters except Hc, Lb and Starch for the Ait Ayach population and Ec, Lb and Starch for the Ait Oufella population (Table 4).

The low genetic differentiation among leaf populations in the North of Africa was linked to a relatively infrequent gene flow although pollen dispersal by wind can span great distances.[16,20,29] This means that although cedar shows great variability between individuals in North Africa, this variability is not transmitted between populations due to poor pollen aerodynamics or another factor. Moreover, El Bakkali and Bendriss Amraoui [14,66] showed that needles per brachyblast traits induce variations between and within *Cedrus atlantica* populations of many forests of the Middle and High Atlas Mountains and are important longer-term adaptations to semi-arid climate. These authors also found that these variations are related to the latitude, altitude, longitude, rainfall and temperatures of sites and support the existence of two ecotypes of *C. atlantica* geographically distant in the Atlas Mountains of Morocco. The stronger variation among-population in pollen traits showed here are consistent with these biogeographic studies and suggest frequent pollen gene flow or different adaptation state to habitat and gender identity of father and mother plant; but genetic study on pollen, seed and seedling remains to be done for approving these assumptions.

The Seheb population is distinguished in almost all pollen traits from the populations of the Midelt region, which are very similar to each other. These data supported by principal component analysis show variations within the region of Azrou and Midelt studied here and suggest a local (intra) and distant (inter) regional adaptation of the *C. atlantica* pollen. These pollen morphological variations corroborate the genetic differences found between these two regions by Terrab et al.[29] and Karam et al.[19] and match with the existence of two ecotypes of *C. atlantica* geographically distant in the Atlas Mountains of Morocco as found in the previous study of El Bakkali and Bendriss Amraoui.[66] Similar results were established in Algeria by Derridj et al.[22] who showed that pollen grain size discriminates *C. atlantica* populations into different genetic and ecological groups. In Spain, afforested populations outcoming from natural stands of northern Morocco were found genomically different and showed a rapid local adaptation to drought in provenances of *C. atlantica.* [67] In the same way, Knight et al.[68] found a significant positive trend between pollen width and genome size across 464 species and Frenguelli et al.[69] reported a 12% reduction in pollen volume due to desiccation. These observations are consistent with the results which show that the pollen corpus and balloons of *C. atlantica* adapt to the climatic conditions of the geographical location of the mother plant.

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Table 4: P value of multiple comparison Tukey test on the morphological and physiological pollen grain's parameters of the three *Cedrus atlantica*'s populations studied

Localities	Hc	Hb	Lb	Ec	Lc	Lg	Vc	Vb	Vtp	Starch	PTL	NGP	NTP	GFP
Seh - A. Ouf	0.001*	0.000*	0.345	0.107	0.000*	0.000*	0.029*	0.000*	0.001*	0.802	0.000*	0.000*	0.000*	0.000*
Seh - A. Ay	0.250	0.030*	0.119	0.049*	0.010*	0.001*	0.010*	0.012*	0.001*	0.151	0.035*	0.000*	0.000*	0.000*
A. Ouf - A. Ay	0.000*	0.070	0.002*	0.942	0.337	0.714	0.925	0.467	0.965	0.999	0.000*	0.000*	1.000	0.000*

Hc (µm): corpus height, Hb (µm): balloon height, Lb (µm): balloon length, Ec (µm): cap thickness, Lc (µm): corpus length, Lg (µm): pollen grain total length, Vc (mm^3): volume of the corpus, Vb (mm^3): volume of the balloon, Vtp (mm^3): volume total of pollen, Starch reserves (mg/g pollen), PTL (µm): pollen tube length, NGP: number of germinated pollen, NTP: number of total pollen, GFP (%): germination frequency of pollen, Seh: Seheb, A. Ouf: Ait Oufella, A. Ay: Ait Ayach,* Statistically significant at the level of 5%.

Geographical structure of morpho-physiological pollen traits Variation

The interactions between all traits were analyzed using the Pearson and Spearman correlation coefficient matrix (Tables 5 and 6). Almost all recorded correlations were significantly positive or negative. We found very high correlations ($0.7 \leq r < 1$) between Lc and the two characters Vc and Vb; Vc and Vtp; T and Starch; Alt and the three traits Lc, NGP and GFP. Similary high correlations ($0.5 \leq r < 0.7$) were detected between both Lc, Vc and Vb and the four parameters Starch, NGP, NTP and GFP; Vtp and the four variables Lc, Lg, Vb and Starch; Lg and the two traits Vc and Vb; Vc and Vb; NGP and GFP; T and Vc; both Long and P and the six parameters Lc, Vc, Vb, Vtp, Starch and NTP; Lat and the three traits Lc, Vc and Starch; Alt and the five characters Vc, Hb, Vb, Vtp and NTP. Finally, medium correlations ($0.3 \leq r < 0.5$) were recorded between both Lg, Hb, Vtp, Starch and the three parameters NGP, NTP and GFP; Hb and the five variables Lc, Vc, Vb, Vtp and Starch; Lg and the two traits Starch and Lc; Hc and Lb; NTP and the two parameters NGP and GFP; both T and Lat and the five characters Vb, Vtp, NGP, NTP and GFP; both P and Long and the four variables Hb, Lg, NGP and GFP; T and the three traits Lc, Lg, Hb; Lat and Hb; Alt and the three characters Lg, Starch and PTL.

Table 5: Pearson coefficient of correlation between pairs of morphological and physiological traits studied

	Hc	Hb	Lb	Ec	Lc	Lg	Vc	Vb	Vtp	Starch	PTL	NGP	NTP
Hb	-0.159												
Lb	0.355**	-0.070											
Ec	0.063	-0.087	0.070										
Lc	-0.035	0.454**	0.012	-0.125									
Lg	0.028	0.285**	0.181**	-0.047	0.460**								
Vc	0.049	0.444**	0.132	-0.105	0.733**	0.501**							
Vb	-0.004	0.439**	0.102	-0.095	0.762**	0.512**	0.639**						
Vtp	0.150	0.340**	0.220**	-0.003	0.631**	0.560**	0.759**	0.607**					
Starch	-0.067	-0.345**	-0.077	0.060	-0.689**	-0.384**	-0.615**	-0.617**	-0.526**				
PTL	0.128	0.105	0.139	-0.007	0.068	-0.064	0.104	0.026	0.056	-0.201**			
NGP	-0.094	0.435**	-0.031	-0.103	0.642**	0.355**	0.512**	0.590**	0.461**	-0.444**	0.051		
NTP	0.055	-0.405**	-0.006	0.197*	-0.662**	-0.430**	-0.629**	-0.580**	-0.489**	0.499**	-0.039	-0.488**	
GFP	-0.106	0.438**	-0.032	-0.027	0.610**	0.360**	0.510**	0.514**	0.485**	-0.399**	-0.113	0.589**	-0.459**

Hc (µm): corpus height, Hb (µm): balloon height, Lb (µm): balloon length, Ec (µm): cap thickness, Lc (µm): corpus length, Lg (µm): pollen grain total length, Vc (mm^3): volume of the corpus, Vb (mm^3): volume of the balloon, Vtp (mm^3): volume total of pollen, Starch reserves (mg/g pollen), PTL (µm): Pollen tube length, NGP: number of germinated pollen, NTP: number of total pollen, GFP (%): Germination frequency of pollen, ** $p < 0.01$.

Principal component analysis (PCA) was performed on morphological, physiological characters and geographic factors. This analysis revealed four principal components with eigenvalues > 1 (Table 7). The first two components explained 53.3 % of the total variation. The component 1 explained 44.2 % of the total variance and the second 9.1%. The component 1 is well correlated in a positive way with Starch, NTP, P and Lat and in a negative direction with Hb, Vb, Lc, Lg, Vc, Vtp, NGP, GFP, T, Long and Alt; however, the second component is negatively well correlated with Hc, Lb and PTL. The variable Ec is not well represented on the factorial plan.

The PCA allowed us to represent two population groups that are clearly discriminated. The dispersion shows the populations of Ait Oufella et Ait Ayach in overlap with each other at the left of the factorial plan, while the Seheb population at the right. The sites of Ait Oufella and Ait Ayach characterized by important values of T, Long and Alt are distinguished by high levels of pollen parameters Lc, Vc, Lg, Hb, Vb, Vtp, NGP and GFP, the population of Seheb characterized by important values of P and Lat presenting important values of Starch and NTP (Figure 4).

The hierarchical group analysis by use of all morphological and physiological traits showed that two groups of populations could be distinguished in the dendrogram. The first group composed by the Seheb population was very distinct from the second group of Ait Ayach and Ait Oufella populations which showed very close relationships between them (Figure 5).

Principal component analysis showed that pollen corpus length (Lc) and volume (Vc), pollen total length (Lg), balloon height (Hb) and volume (Vb) and pollen total volume (Vtp) of *C. atlantica* populations are influenced by climatic conditions and geographical location. These effects on pollen are in the same direction as those reported by Derridj et al.[22] in Algeria and are consistent with that of Ejsmond et al.[64,70] who demonstrated that when temperatures become high, pollen increases in

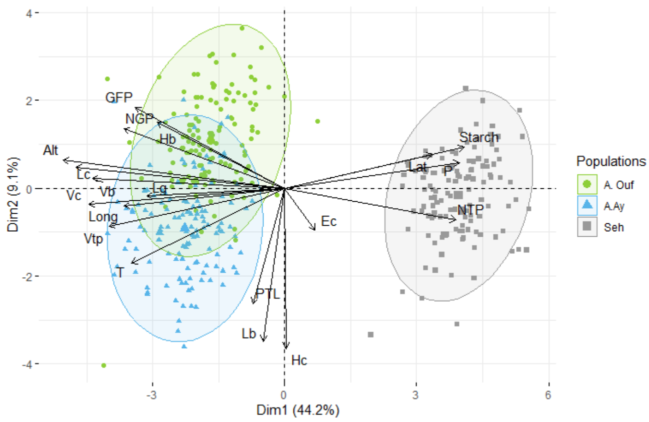

Figure 4: Biplot of the populations and parameters studied in the axes 1 and 2

Hc (µm): corpus height, Hb (µm): balloon height, Lb (µm): balloon length, Ec (µm): cap thickness, Lc (µm): corpus length, Lg (µm): pollen grain total length, Vc (mm^3): volume of the corpus, Vb (mm^3): volume of the balloon, Vtp (mm^3): volume total of pollen, Starch reserves (mg/g pollen), PTL (µm): pollen tube length, NGP: number of germinated pollen, NTP: number of total pollen, GFP (%): germination frequency of pollen, T (°C) : annual mean temperature ; P (mm) : annual mean precipitation, Lat : Latitude, Long : Longitude, Alt (m asl) : Altitude, Seh: Seheb, A. Ouf: Ait Oufella, A. Ay: Ait Ayach.

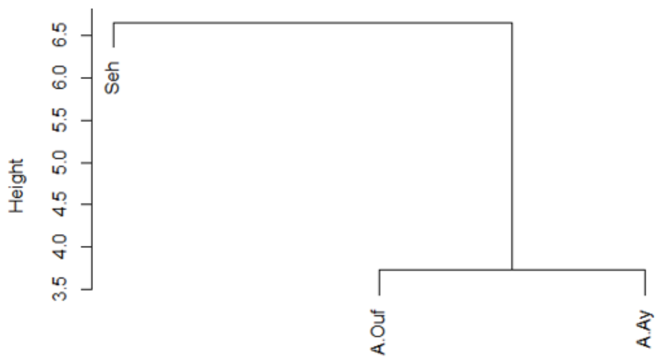

Figure 5: Relationships between populations of *Cedrus atlantica* (Seh = Seheb, A. Ay = Ait Ayach; A. Ouf = Ait Oufella) on the shortest Euclidean distances in relation to the morphological and physiological characteristics of pollen.

size to (i) reduce its surface area/volume ratio and thus resist desiccation and (ii) be more competitive on the stigma. They are important in the sites of Ait Oufella and Ait Ayach where T, Long and Alt are high and low in Seheb where P and Lat are important. However, these pollen characters are at least mediumly positively correlated with each other. Moreover, the dendrogram is congruent with the results of PCA analysis; the Euclidean distances showed that the populations of Seheb was separated from the others. On the other hand, the fact that morphologically Ait Oufella is significantly different from Ait Ayach only for the characters Lb and Hc and not for Ec, Lc, Lg, Hb, Vc, Vb, Vtp, showed too that this cedar population, which is geographically at the limit between the Middle and High Atlas, tend to sand out from Ait Ayach population. This observation is explained by the results of Karam et al. [19] who noted among-population variability in comparison with Algerian Cedar stands as well as a large within-population variability in this forest due to the strong presence of recessive null alleles which increases the dissimilarities between individuals and their level of homozygosity. These pollen morphologic differentiations reveled here among the Seheb population and the two others may be due to a relatively frequent gene flow and pollen dispersal as found by Karam et al. between the Ait Oufella population and the Algerian populations. This observation is in accordance with the differentiations of the natural populations of *C. atlantica* in Morocco reported by Terrab et al.,[16,29] Cheddadi et al.,[20] and El Bakkali and Bendriss Amraoui [66] who agree on the existence of a significant flow of genes between the Moroccan populations of the Middle and High Atlas and between those of Morocco and Algeria.

Statistical analyzes and the dendrogram show a trade-off between morphology (Lc, Lg, Vc, Hb, Vb and Vtp) and production (NTP) which is related to climatic factors and geographical position. For example, in the Seheb population these characters are low, but production is high and vice versa in the others. This is consistent with the results of many studies which concluded a negative relationship between pollen production and size because available resources for male function are limited.[64,71,72]

Many studies have reported that pollen grain corpus contains soluble sugars, starch, lipids and proteins that are involved in pollen germination and the first stages of pollen tube elongation.[7,73-76] However, the populations of Ait Oufella and Ait Ayach, although having high values of the morphological parameters of corpus, balloon and entire pollen, have with the Seheb population similar values of Starch. This observation is not consistent with a relationship between starch reserves and climate and geographical location. According to Kaufmann [77] and Pacini and Dolfeus [78] pollen grain remains metabolically active and stored starch is used for respiratory processes in conditions of high humidity, whereas in dry areas pollen enters a phase of metabolic arrest as reported in the studies of Wilson et al.[79] and Nelson et al.[80]. These data suggest that the starch reserves of *C. atlantica* pollen are insensitive to climate. Moreover, Franchi et al.[81] and Buchmann et al.[82] reported that in dry areas pollen does not contain starch because it is converted into sugars to allow the pollen to resist desiccation,[83,84] protect the cellular machinery [83,85] and maintain pollen viability.[86,87] In this sense, the populations of Ait Oufella and Ait Ayach, although leaving in semi-arid climate and significantly characterized by high levels of Lc, Lg, Vc, Hb, Vb and Vtp and the presence of starch, constitute an exception. Results partially similar to these were reported by Carrizo-Garcia [65] who found that in *Solanum pennellii* living in a dry environment, the pollen contained starch but had a low pollen corpus length. On the other hand, several other studies have reported a positive correlation between corpus length and its starch content in several species,[74,75] suggesting that cedar can't modulate its starch to resist desiccation as it does for its pollen size.

Correlations and principal component analysis showed that the number of germinated pollen (NGP) and germination frequency (GFP), high in Ait Oufella and Ait Ayach populations compared to the Seheb population, are positively correlated with Hb, Lc, Lg, Vb, Vc and Vtp showing a good state of viability and hydration. This showed that pollen germination of cedar is influenced by climatic conditions and geographical location. This data is confirmed by the shortest Euclidean distance of Ait Oufella and Ait Ayach compared to Seheb. The increase of pollen germination in Ait Oufella and Ait Ayach could be due to an adjustment in the conversion of pollen corpus starch reserves into sugars to maintain its viability in dry areas as reported by Franchi et al.[81] and Buchmann et al.[82]. This explanation, reinforced by the high Vc, Vb and Vtp, of these two populations, agree with the study of Dimou et al.[88] who found that pollen grains of big volume are rich in sugars. On the other hand, viability and the presence of sugars at similar levels in the pollen of two *Helleborus* species cohabiting together were reported by Vesprini et al.[89] while only one species had starchy pollen. Similarly, Pacini et Dolfeus [78] established that ripe pollen grain can be starchy or starchless, but there is always a variable proportion of soluble and insoluble cytoplasmic carbohydrates as a drought survival response.

However, because the values of GFP obtained did not follow pollen starch content in the populations suggests that other types of substances must be connected with the starch metabolism for pollen germination of *C. atlantica*. According to Baker and Baker,[90] lipids may be an important reserve in pollen grains and they are closely linked with the metabolism of carbohydrates same as proteins resources.[91] Carrizo-Garcia et al.[73] showed that in tomato cultivars containing little or no starch, the high germination rates obtained were due to molecules other than starch. These data suggest that other molecules are involved in cedar pollen germination.

Table 6: Spearman nonparametric coefficient of correlation between cedar traits and geographic factors.

	T	P	Lat	Long	Alt
Hc	0.108*	-0.042	-0.035	-0.002	-0.155*
Hb	0.304**	-0.363**	-0.319**	0.335**	0.548**
Lb	0.154*	-0.093	-0.108*	0.049	-0.041
Ec	-0.130**	0.298**	0.241**	-0.166**	-0.171**
Lc	0.484**	-0.562**	-0.502**	0.580**	0.726**
Lg	0.328**	-0.374**	-0.283**	0.302**	0.464**
Vc	0.546**	-0.606**	-0.545**	0.570**	0.665**
Vb	0.476**	-0.543**	-0.425**	0.522**	0.680**
Vtp	0.497**	-0.564**	-0.490**	0.530**	0.649**
Starch	-0.726**	0.655**	0.546**	-0.628**	-0.398**
PTL	0.258**	-0.218**	-0.187**	0.220**	-0.309**
NGP	0.351**	-0.408**	-0.426**	0.422**	0.713**
NTP	-0.381**	0.573**	0.475**	-0.548**	-0.651**
GFP	0.376**	-0.479**	-0.438**	0.459**	0.812**

Hc (µm): corpus height, Hb (µm): balloon height, Lb (µm): balloon length, Ec (µm): cap thickness, Lc (µm): corpus length, Lg (µm): pollen grain total length, Vc (mm^3): volume of the corpus, Vb (mm^3): volume of the balloon, Vtp (mm^3): volume total of pollen, Starch reserves (mg/g pollen), PTL (µm): pollen tube length, NGP: number of germinated pollen, NTP: number of total pollen, GFP (%): germination frequency of pollen, T (°C) : annual mean temperature ; P (mm) : annual mean precipitation, Lat : Latitude, Long : Longitude, Alt (m asl) : Altitude,** $p < 0.01$; * $p < 0.05$.

Table 7: 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	8.401	44.214	44.214	8.401	44.214	44.214	8.401	44.214	44.214
2	1.738	9.147	53.361	1.738	9.147	53.361	1.738	9.147	53.361
3	1.295	6.815	60.176						
4	1.007	5.302	65.479						

The length of the pollen tube (PTL) is not correlated with Lc, Lg, Hb, Vc, Vb and Vtp, explaining why in the Seheb population which has low dispersal parameters, the pollen tends to develop a long tube to increase the chances of fertilization. This indicates that pollen tube length (PTL) of *C. atlantica* reflects an equilibrium selection that balances pollen dispersion and fertility as was shown by Cruzan,[62] in *Erythronium grandiflorum*. In the same direction, the low correlation between PTL and Starch parameter shows that pollen tube growth and elongation in *C. atlantica* are heterotrophic phases in which pollen utilizes exogenous sucrose as reported by Rounds et al.[92] in *L. formosanum*. According to Nygaard,[93] pollen grain of conifers synthesizes intensively starch in the presence of optimal carbohydrates, sucrose or fructose concentrations. These observations suggest that the significant development of the pollen tube observed in the Ait Ayach and the Seheb populations unlike the Ait Oufella is due to factors other than the starch reserves of the pollen. These factors may be the biochemical mechanisms that drive pollen tube elongation like the steep calcium gradient within the pollen tube tip, the contribution of actin microfilaments,[94] or the proteins that are only synthesized at the onset of pollen tube elongation.[95]
According to Pither [27] and Svenning [28] climate tolerance; particularly that of cold and aridity are crucial factors for trees survival. The significant development of the dispersion characters Hb and Vb in the populations of Ait Oufella and Ait Ayach compared to Seheb shows that the pollen balloons react to extreme climatic conditions. This result is consistent with studies showing that the water status of the site affects the shape of the pollen grain and that of the balloons; [96,97] however, it disagrees with the study by Bell et al.[23]. These characteristics of the balloon (Vb and Hb), corpus (Lc, Vc) and entire pollen grain (Lg, Vtp) by increasing the surface area of pollen grains under the effect of extreme heat, while ideally adding a minimum of mass allow the pollen of the populations of Ait Oufella and Ait Ayach to move faster and further and to increase the distance of their dispersal while facilitating the orientation of these grains in pollination droplets as reported by several authors in other species.[41,98,99] These findings are also supported by the results of other studies which reported that the length of the entire grain, the volume of the pollen, its height, as well as the mass of the pollen grain by modulating the velocity of the pollen favors reproduction.[97,100] This conclusion confirms the existence of a significant potential pollen flow and gene between these two populations which have pollen characteristics very favorable to dispersal and shows consistence with the results of the ascending hierarchical classification.

Conclusion

This study shows that in *Cedrus atlantica* pollen grain physiology is intrinsically linked to phenotype when environmental conditions are taken into account. This morpho-physiological response of pollen grain to contrasting environments is fundamental in understanding the ability of natural populations of *Cedrus atlantica* from the High and Middle

Atlas to maintain their genetic potential in a context of global warming for efficient species conservation and degraded forest restoration. However, an analysis of both populations using other metabolic data and with focus on other molecules like carbohydrates, proteins and lipids would be advisable to further investigate the molecular basis of these observed responses. The metabolic pathways elucidated through this analysis will be guidelines for further search on other potential physiological differences between the studied populations and to better understand the mechanisms involved in drought resilience and gene flux from different sexual morphs of *Cedrus atlantica* populations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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