

Exploring morphological and anatomical trait diversity in Moroccan populations of *Juniperus oxycedrus* subsp. *oxycedrus*

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Abstract: *Juniperus oxycedrus* subsp. *oxycedrus* is a significant element of the forest ecosystems in High and Middle Atlas of Morocco. It is very well known for its resistant hard wood and for its essential oils used in traditional medicine. However, little data is available on its intra-specific variability and its adaptability. The objective of our study is to characterize and evaluate the phenotypic variation of this natural species. Using 22 morphological traits related to needles, cones, and stomata, we investigated the phenotypic variability of eight Moroccan wild populations to identify biogeographical trends and population relationships. Statistically significant differences were found between the studied populations for the most examined traits indicating a high level of phenotypic variability within this species. Principal component analysis (PCA) and hierarchical cluster revealed three groups of populations not related to geographical and climatic factors. Our study provides the first phenotypic and anatomical information on *Juniperus oxycedrus* subsp. *oxycedrus*, a previously under-studied plant in Morocco, and reveals a high level of phenotypic diversity. Our observations indicate that populations from distinctly different altitudes display clear morphological differences (Ouaouizeght and Chefchaouen), This diversity appears to be associated with environmental factors such as altitude and temperature, both of which demonstrate significant correlations with certain morphological traits. This approach supports informed decision-making in conservation efforts. The data gathered serves as a critical resource for future research and conservation planning.

keywords: *Juniperus oxycedrus* subsp. *oxycedrus*, wild populations, phenotypic diversity, Morocco.

Introduction

The cade juniper (*Juniperus oxycedrus* L.) is a species of the *Oxycedrus* section and *Juniperus* genus with four subspecies (Klimko et al., 2007) that differ in habitat, cone dimension and width of needles (Bayet et al., 1991; Klimko et al., 2007; Roma-Marzio et al., 2017): subsp. *oxycedrus*. subsp. *macrocarpa*. subsp. *transtagana* and subsp. *badia* (H.Gay) Debeaux. While the last research of Mao et al. (2010) and Boratyński et al. (2014) are considered the subsp. *macrocarpa* as species. *Juniperus oxycedrus* subsp. *oxycedrus* is considered a typical subspecies of Prickly Juniper, is found in the Mediterranean region with its natural range covering Southwest Europe, Northwest Africa and areas of the Middle East (Vasic and Dubak .2012; Ortiz et al., 2021). It can be found growing in several kinds of Mediterranean forests and occasionally even developing its own communities, as in Morocco, Greece and Anatolia (Klimko et al., 2007). It is an evergreen, dioecious upright shrub or small thermophilic tree that can reach heights of up to 14 meters (Yaltirik et al., 2007). The foliage is composed of needles that are green in color, sometimes with a bluish or greish tint. Female plants produce axillary seed cones called galbulus (Vilar et al., 2016).

In Morocco, *Juniperus oxycedrus* subsp. *oxycedrus* is a significant element of the country's forest ecosystem which has a wide range from the plain to around 3200 m.a.s.l., on all types of substrates (Benabid 2000). It participates in the organization of forested, pre-forested and various pre-steppic structures without forming a remarkable pure stand (Benabid and Fennane .1994). It is observed mainly in the High and Middle Atlas where they cover an area of about 41.000 km² with a situation between 31 and 32° N and 4 and 7° W ¹³. Its wood is used as fuel, charcoal or in the construction. Indeed, this species is actively involved in traditional medicine for the treatment of various diseases in several regions in Morocco.

Although its many benefits, *Juniperus oxycedrus* subsp. *oxycedrus* is subject to several constraints and threats such as deforestation, overexploitation, overgrazing and climate change. Furthermore, it belongs to the group of species that do not

reject strains. This poses a significant threat to the species' ability to persist and, consequently, to the diversity of Moroccan forest ecosystems. The distribution of genetic variability between populations plays a crucial role in conserving, managing, and restoring this natural genetic resource.

In Morocco, no data related to its variability and adaptability is available. The research on *Juniperus oxycedrus* subsp. *oxycedrus* in Morocco primarily concerned its ecology (Benabid and Fennane. 1994; Benabid 2000) and the evaluation of its chemical composition (Rajouani et al., 2015; El Hajjouji et al., 2019). Therefore, it has become imperative to evaluate the diversity of the populations of this species. For a thorough evaluation of genetic diversity in *Juniperus oxycedrus* subsp. *oxycedrus*, we first assess genetic variation using ISSR markers (Neg et al., 2025). This is complemented by analyses of phenotypic and biochemical traits, with particular attention to their relationships with geographic and climatic factors. This paper provides an investigation of needles, cones and stomata morphological characters variability throughout a wide distribution of *Juniperus oxycedrus* L. subsp. *oxycedrus* in Morocco. Given the importance of this species for the development of marginal areas, study would provide interest in knowledge of its variability, adaptability and populations characterization.

Results

Needle morphometric and anatomical traits

The average values of quantitative traits measured on needles, cones and stomata are summarized in Table 3. Analysis of variance showed highly significant differences between populations for all parameters except needle ratio, cone weight and stomata surface index. The longest needles were found in Ouaouizeght and Tagleft populations (21.59 and 20.34 mm, respectively), while the shortest needles (17.75 mm) were recorded in population Azilal. The widest needles were observed in populations Tagleft and Ouaouizeght (1.56 mm and 1.55 mm, respectively), while the narrowest needles were recorded in population Chefchaouen (1.15 mm).

Moreover, stomatal characteristics also revealed significant differences between the studied populations. Indeed, stomata length ranged from 32.47 μm in population Azilal to 55.23 μm in population Ouaouizeght. Similarly, stomata width ranged from 20.49 μm in the Azilal population to 29.37 μm in population Demnate. The stomatal density was also variable and the highest value was observed in population Tagleft (242.51 stomata/ mm^2) and the lowest value in population Aghbala (174.69 stomata/ mm^2).

Morphometric cone traits

Regarding cones, the populations Tagleft, Chefchaouen and Ourika had the longest cones (12.33, 12.10 and 11.17 mm, respectively), while the widest cones were observed in populations Lekbab, Ourika and Aghbala (11.86, 11.58 and 11.57 mm, respectively). However, the shortest and the narrowest cones were observed in population Azilal (9.63 and 9.62 mm, respectively). Weight of cones was also variable among populations. The heaviest cones were found in populations Aghbala and Lekbab (0.99 and 0.91 g, respectively), while the lightest ones were found in the populations of Ouaouizeght, Demnate and Azilal (0.43 g).

In terms of qualitative parameters, analysis of variance revealed significant differences between populations for all morphological traits except for the presence of wax (Table 3). The observation data showed that 91% of plants showed needles with a symmetrical shape. Regarding the shape of the needle base, the result shows that 69.7% had a rounded base followed by a rounded base on one side (19.1%) and a flattened base (11.2%). On the other hand, 84.7% of needles had an acute apex, with the remaining having the narrowed apex (14.8%). In terms of color, most of trees showed light green needles (66.2%) followed by very light green (30%). The shape of the cone was mainly rounded (91.5%). As far as the form of the cone scar is concerned, the cone is most distinguished by a triangular scar at the base (98.7%). Moreover, 88.7% of the cones were dark red-brown followed by bright red brown (5.5%), dark brown (4.5%) and dark red (1.3%).

Correlation analysis

To highlight the strength and relationship between morphological traits and ecological parameters, Pearson correlation analysis was performed (Table 5). We observed significant correlations between some morphological parameters. Length of needles had positive and significant correlation with ratio of needles, length of median, length of stomata and width of stomata ($r = 0.782, 0.973, 0.904$ and 0.717 , respectively). Also, the ratio of needles showed a positive and significant correlation with both stomatal length and stomatal width ($r = 0.785$ and 0.772 , respectively). In the same trend, cone length was positively correlated with the cone ratio and stomatal density ($r = 0.842$ and 0.846 stomata/ mm^2 , respectively). Stomatal length was also positively correlated with stomatal width ($r = 0.887$). In contrast, needle width was negatively and significantly correlated with both stomatal surface area and stomatal surface index ($r = -0.863$ and -0.862 , respectively). In addition, a significant correlation was observed between geographic parameters and some morphological traits. Altitude exhibited a significant positive correlation with needle width ($r = 0.945$) and needle shape ($r = 0.827$), while it showed a significant negative correlation with stomatal surface area ($r = -0.850$) and stomatal surface index ($r = -0.860$). Furthermore, the average temperature was negatively correlated with median length ($r = -0.753$), stomatal length ($r = -0.743$) and stomatal weight ($r = -0.810$).

Principal component analysis was conducted based on the mean values of quantitative variables. The first two components provide a good summary of the data. They explained 65.48% of the total variability. The first axis accounts for 40.58% of the total variation and shows a strong positive correlation with needle length, median length, stomal length, stomal ratio, and stomal surface index. In contrast, it exhibits a negative correlation with needle width, cone length, and stomatal density.



Figure 1. Map depicting the locations of the eight *Juniperus oxycedrus* subsp. *oxycedrus* populations studied. The second axis explains 24.90% of the variation and is primarily positively correlated with needle weight, needle ratio, cone length, cone ratio, and both stomatal width and density. Conversely, it has a negative correlation with cone weight and the stomatal surface index.

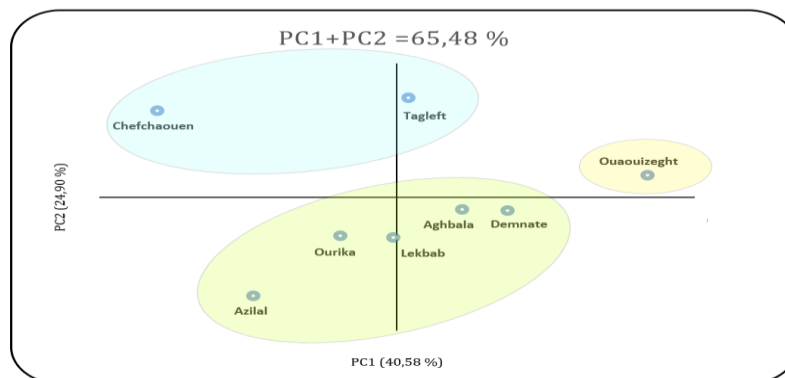


Figure 2. Principal component analysis of Moroccan populations of *Juniperus oxycedrus* subsp. *oxycedrus* on the space formed by the first two axes performed based on fourteen quantitative morphological characters.

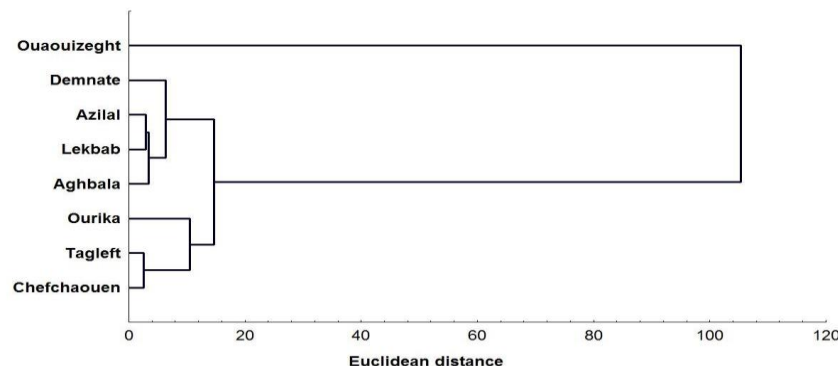


Figure 3. Dendrogram of eight populations of *Juniperus oxycedrus* subsp. *oxycedrus* based on twenty-two morphological traits.

The PCA plots show that Moroccan *Juniperus oxycedrus* subsp. *oxycedrus* populations constitute three distinct groups (Fig. 2). The first one is composed of five populations: three from Hight Atlas Mountain (Azilal, Demnate and Ourika) and two from Middle Atlas Mountain (Lekbab and Aghabala). These populations are characterized by relatively low values for needle length (17.45 – 19.35 mm) and length of cone (9.63-11.17 mm). The second group is made of two populations from Rif

Table 1. Geographical and environmental characteristics of studied *Juniperus oxycedrus* subsp. *oxycedrus* populations (According to MMD 2023).

Population	Code	Geographic origin	Mountain range	Precipitation (mm/year)	Latitude (Lat)	Longitude (Long)	Altitude (m)
Chefchaouen	CH	24 km South of Chefchaouen	Rif	753	34°8'N	5°1'E	389
Tagleft	TA	20 km Ouest of Tagleft	Middle Atlas	355	32°22'N	6°24'E	1287
Ouaouizeght	OA	3 km Ouest of Ououizeght	Middle Atlas	355	32°18'N	6°37'E	1450
Aghbala	AG	115 km East of Beni Mellal	Middle Atlas	450	32°30'N	6°1'E	1304
Lekbab	LE	34 km South of Khenifra	Middle Atlas	357	32°79'N	5°32'E	1120
Ourika	OU	35.9 km South of Marrakech	High Atlas	218	31°29'N	7°71'E	1106
Demnate	DE	20.2 km East of Demnate	High Atlas	246	31°72'N	6°91'E	1059
Azilal	AZ	16 km East of Azilal	High Atlas	333	31°9'N	6°7'E	1242

Table 2. Morphological traits and their acronym used for *Juniperus oxycedrus* subsp. *oxycedrus* studied populations.

Needles traits	Trait acronym	Cone traits	Trait acronym	Stomata traits	Trait acronym
Length of the needle (mm)	LN	Length of the cone (mm)	LC	Length of stomata (μm)	LS
Width of the needle (mm)	WN	Width of the cone (mm)	WC	Width of stomata (μm)	WS
Ratio (length/width) of the needle	RN	Ratio (length/width) of the cone	RC	Ratio (Length/width) of stomata	RS
Length of the median of the needle (mm)	LM	Weight of the cone (g)	WeC	Stomatal density (stomata/mm ²)	SD
Shape of the needle	SN	Shape of the cone	SC	Stomatal surface (μm ²) (SS = π *L*.l.)	SS
Shape of needle base	SNB	Cone color	CC	Stomatal surface index (ISS = SD*LS*WS.)	SSI
Shape of needle apex	SNA	Form of scar of cone	FS		
Needle color	NC				
Wax	W				

Stomatal surface (SS). The stomatal surface is determined from the length of the stomata (LS) and their width (WS) according to the following formula: $SS = \pi *LS*WS$. Stomatal surface index (SSI). The stomatal surface index (SSI) corresponds to the total stomatal surface per 1 *mm² of leaf area. It is determined from the length of the stomata (LS), their width (WS) and their density (DS), according to the following formula: $ISS = DS*LS*WS$.

Mountain (Chefchaouen) and Middle Atlas (Tagleft) distinguished by higher values of the cone length (12.33mm-12.10mm) and stomatal density (242.8-235.91 stomata/mm²). The third group includes only one population from Middle Atlas (Ouaouizeght) characterized by the biggest needles (LN=21.59mm; RN=14.38; LM=18.45mm) and the highest values of stomatal length (55.23 μm) and stomatal surface area (4952.31 μm²).

Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) based on morphological distance, revealed that the eight populations could be divided into three main groups (Fig. 3). The first group contained three populations belonging to High Atlas (Ourika), Middle Atlas (Tagleft) and Rif Mountain (Chefchaouen). The second group is composed by four populations: two populations originated from the High Atlas (Demnate and Azilal) and two populations originated from the Middle Atlas (Lekbab and Aghbala). The population from the Middle Atlas and the highest altitude (Ouaouizeght) formed group three. It is highly divergent from the remaining population.

Epidermal surface and epicuticular waxes

To study the microstructure of epicuticular waxes and the epidermal surface of the leaf, we analyzed the stomatal distribution, the position of the stomatal band in relation to the median band, epidermal features and epicuticular waxes. A scanning electron microscope (SEM) analysis of stomata reveals that the upper (adaxial) leaf surface is devoid of stomata (Figure 4A), while the lower (abaxial) surface displays double stomatal bands. In these bands, the stomata are arranged in straight lines, with six to ten stomata per band separated by a medial band of epidermal cells (Figures 4B and 4C). The structure of the stomatal bands varies among populations. In the population Tagleft, the bands are aligned at the same level

Table 3. Mean value and significance levels of differences between *Juniperus oxycedrus* subsp. *oxycedrus* populations for quantitative morphological traits.

	OA	DE	AZ	OU	TA	AG	LE	CH	Mean	F	CV %
LN	21.59	19.00	17.45	18.29	20.34	19.35	18.98	18.19	19.15	4.771***	6.23
WN	1.55	1.46	1.49	1.53	1.56	1.49	1.49	1.15	1.46	3.964***	38.34
RN	14.38	13.44	12.31	12.43	13.21	13.26	12.93	13.57	13.19	1.304ns	4.99
LM	18.45	16.42	14.34	15.50	17.36	17.02	16.17	14.94	16.28	3.940***	8.24
LC	10.06	10.14	9.63	11.17	12.33	10.25	10.05	12.10	10.72	3.939***	9.54
WC	10.57	11.48	9.62	11.58	10.52	11.57	11.86	11.35	11.07	2.068*	6.88
RC	0.95	0.98	1.00	0.98	1.17	0.98	0.97	1.06	1.01	2.309*	7.23
WeC	0.43	0.43	0.43	0.54	0.44	0.99	0.91	0.63	0.60	1.532ns	37.94
LS	55.23	50.72	32.47	41.59	50.63	47.97	43.01	40.49	45.26	18.341***	16.06
WS	28.58	29.37	20.49	21.65	28.24	28.90	23.07	25.10	25.68	8.468**	13.89
RS	1.95	1.73	1.60	2.00	1.79	1.66	1.89	1.63	1.78	3.485**	8.41
SD	178.96	199.65	200.51	212.32	242.51	174.69	205.17	235.91	206.21	3.396**	11.66
SS	4952.31	4699.16	2117.65	2857.27	4580.06	4436.82	3195.13	3205.82	3755.53	20.75***	27.69
SSI	283715.	295874.7	137941.1	192305.7	356890.3	238814.1	207114.0	240058.7	244089.3	1.84ns	27.84
	52	07	78	88	28	17	76	21	0		

***: significant at the 0.1% probability level; **: significant at the 1% probability level; *: significant at the 5% probability level; ns: non-significant. The minimum and maximum are written in Bold; CV: Coefficient of variation

Table 4. Analysis of variance and dominant frequencies of qualitative traits in *Juniperus oxycedrus* subsp. *Oxycedrus*.

Traits	Evaluation scale	Dominant character	Frequency	CV %
Shape of the needle	Symmetry-asymmetry	Symmetry	91***	26.27
Shape of needle base	Flattened -rounded -rounded on one side	Rounded	69.7***	26.23
Shape of needle apex	Acute -Expanded -shrunk	Acute	84.7***	57.16
Needle color	Vey light green -light green - green	Light green	66.2***	22.70
Wax	Presence – absence	Presence	100ns	0
Shape of the cone	Rounded-oval-elongated-cordiform –falciform	Rounded	91.5***	54.48
Shape of scar	Triangle -incomplete polygon -complete polygon - single line - two secant line	Triangle	98.7***	39.17
Cone color	dark brown- dark red- dark red brown- bright red brown	Dark red brown	88.7***	16.78

***: significant at the 0.1% probability level; ns: non-significant.

as the epidermis (Figure 5C), while in the population Chefchaouen, they are positioned below the epidermal level (Figure 5E). However, in population Aghbala, the bands are elevated above the epidermal level (Figure 5D). Additionally, stomatal bands contain epidermal cells instead of stomata, increasing the interstomatal surface. These cells are grouped in population Tagleft (Figure 5B) and dispersed in population Lekbab (Figure 5A). This study revealed that the different forms of epicuticular wax have distinct patterns of distribution on the surface of leaves.

Their form is:

- Small crystalloid particles around the stomata for population Ouaouizeght (Figure 6A).
- Large particles around epidermal cells such as in population Ourika (Figure 6B).
- Thin layer in the case of the population Chefchaouen (Figure 6C).
- Small crystalloid particles around epidermal cells and thin layer on stomatic bands as in population Lekbab (Figure 6D).
- Platelets in the case of population Demnate (Figure 6E).

Discussion

Morphological variability and stomatal traits of Moroccan populations of *Juniperus oxycedrus* subsp. *oxycedrus* were evaluated in this study in order to obtain information on the phenotypic diversity and ecological adaptations of this species. The results obtained demonstrate the high variability between the studied populations for the majority of examined morphological traits suggesting the existence of a high phenotypic variability within this species.

Needles length (17.45-19.15 mm) is similar to the results reported by Klimko et al. (2007) who found that the highest value for needle length were detected in Moroccan *J. oxycedrus* subsp. *oxycedrus* populations in comparison to Ukraine, Greece, Spain, France, Croatia and Bosnia populations. The needle width values (1.15–1.56 mm) obtained in this study are consistent with data reported for Turkish and Algerian populations (Yaltirik et al., 2007; Hafsi et al., 2017). While the width and length of needles are higher than reported by Brus et al., 2016 (LN=14.10 and WN=1.50).

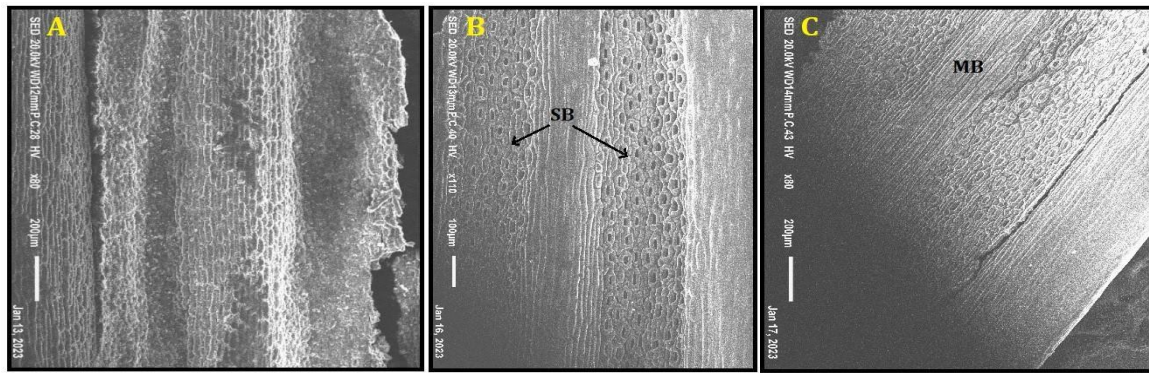


Figure 4. Micrographs of the adaxial face of untreated (A) and of treated (B-C) *Juniperus oxycedrus* subsp. *oxycedrus* leaf from different populations: Aghbala (A), Demnate (B), and Ouaouizeght (C), SB: stomatal band, MB: median band.

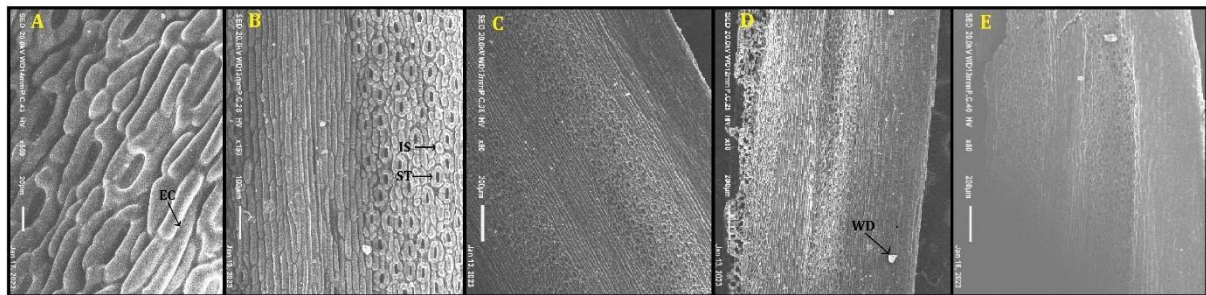


Figure 5. Micrographs of the adaxial face of a treated leaf of *Juniperus oxycedrus* subsp. *oxycedrus* from different populations: Lekbab (A), Tagleft (B, C), Aghbala (D), and Chefchaouen (E). EC: Epidermal cells, ST: stomates, WD: wax depot, IS: interstomatal.

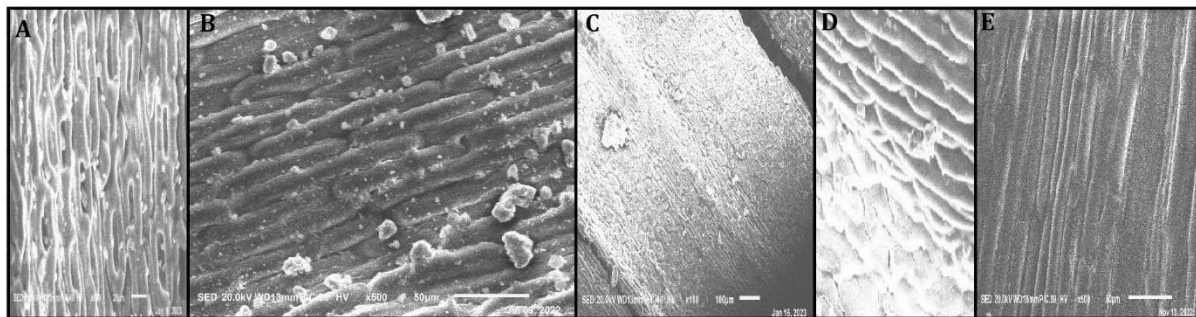


Figure 6. Surface Micrographs of a non-treated leaves of *Juniperus oxycedrus* subsp. *oxycedrus* from different populations: Ouaouizeght (A), Ourika (B), Chefchaouen (C), Lekbab p (D), and Demnate (E).

On the other hand, most of studied populations have larger cones in comparison with the populations from western Mediterranean, the Balkan Peninsula (Adams et al., 2005), from the Provence-Alpes-Côte d'Azur region in southern France and from three natural populations of *J. deltoides* from the north-eastern coast of the Adriatic Sea (Vidaković et al., 2024). Moreover, the measurements of *Juniperus turbinata* cones in Algeria reported by Elmir et al. (2024) are lower than those found in our study: we observed cone lengths exceeding 9.85 mm in maritime populations and 9.28 mm in Atlas Mountain populations, with cone widths exceeding 8.90 mm and 9.26 mm, respectively. However, these results are similar to those obtained by Hafsi et al. (2017) for Algerian populations and Avci and Zieliński (2008) for a new variety of this species in Turkey (var. *spilinanus* Yalt). Regarding cone shape, most of the populations studied showed rounded cones (91.5%) with a color ranging from brown to dark reddish brown (88.7%). Similar data were provided by Yaltirik et al. (2007) and Avci and Zieliński (2008) who revealed that the cone of *Juniperus oxycedrus* subsp. *oxycedrus* has a globular shape and a less glaucous orange, brown or reddish-brown color. Also, these results seem to be similar to those obtained by Hafsi et al. (2017) who found that the cone shape of the Algerian populations is rounded with a dark reddish-brown color. In addition, our results showed that Moroccan populations are characterized by smaller stomata dimensions and higher stomata density than those reported by Hafsi et al. (2017) in Algerian populations for the same species. Our findings reveal a highly significant morphological differentiation among populations of *Juniperus oxycedrus* subsp. *oxycedrus*. This pattern is consistent with results reported by Vidaković et al. (2024), who found greater variability among *J. oxycedrus* populations compared to *J. deltoides*.

The PCA plot and the hierarchical cluster analysis show that the structuration of Moroccan *Juniperus oxycedrus* subsp. *oxycedrus* populations based on their morphological traits but independently of geographic origin. This result suggests that there is no significant barrier to gene flow among populations. Our results are generally consistent with the trends of genetic variation observed in many outcrossing species. Similar results were obtained by Brus et al. (2011) who found the absence

of geographical structure of morphological variation in *J. deltoides* in the Balkan Peninsula and by Boratynski et al. (2014) who found a lack of significant genetic differences between European and African populations of *J. oxycedrus* subsp. *oxycedrus*. While, Douaihy et al. (2024) observed that the most distinct populations of *Juniperus excelsa* subsp. *excelsa*—based on both molecular and morphological markers—were those located in old, high-altitude, and isolated regions of Lebanon. While cluster analyses separated the populations into three primary groups, the degree of differentiation identified using molecular markers was moderate, and a less clear geographical pattern. Furthermore, the extent of within-population morphological diversity did not exhibit any obvious geographical gradient. In agreement with these trends, Elmir et al. (2024) reported that geographic variation in cone, seed, and needle traits corresponds closely with patterns observed using chemical taxonomy. They also documented distinct morphological differences between maritime and Atlas Mountain populations, supporting migratory routes for the species as hypothesized by previous researchers. While principal component analysis (PCA) and cluster analysis did not reveal a clear geographical pattern, the data indicate that some morphological traits remain relatively consistent across different environments, whereas others vary significantly in response to environmental gradients. For instance, altitude showed a strong positive relationship with needle width and shape. This finding is supported by Pan et al. (2013), who noted that higher elevations are associated with increased leaf size and greater mass allocation to larger leaves; similar trends were observed in studies by Guo et al. (2018) and Yang et al. (2022) across other plant species. On the other hand, altitude was found to correlate negatively with both stomatal surface area and the stomatal surface index. This pattern aligns with the work of Li et al. (2014), Wang et al. (2021), who documented negative relationships between altitude and stomatal traits, especially stomatal density, in various plant taxa. Additionally, our results showed that mean temperature was inversely related to both stomatal length and stomatal weight, a trend that was also documented by Wu et al. (2018), Jordan et al. (2020), and Holland et al. (2009). The last part in our study offers a comprehensive description of the microstructure of epicuticular waxes and the epidermal surface of *Juniperus oxycedrus* subsp. *oxycedrus*, as a crucial element in plant identification and taxonomy (Barthlott et al., 1998; Fernández et al., 2016). Our results are consistent with those reported by Kim (2012) and Hafsi et al. (2017) for Chinese and Algerian *Juniperus* species. However, Radoukova et al. (2024), found in *Juniperus pygmaea* and *Juniperus communis*, that the epidermal surface features numerous crust-like plates and the epicuticular waxes at the ends of the anticlinal walls of elongated epidermal cells are oval, while in *Juniperus sibirica*, they form comb-like crystals. Indeed, these characteristics can be influenced by various factors, including environmental conditions, the specific plant organs involved, and the ontogenetic development of the plants (Fernández et al., 2016). In addition, the evolutionary history of each species is a key factor shaping the distribution of its genetic diversity and the variability in its morphological and anatomical traits (Hamrick et al., 1992).

Material and methods

Sampling areas

This work was conducted in eight sites of Morocco that were characterized by the presence of *Juniperus oxycedrus* subsp. *oxycedrus*. Geographical and ecological parameters of all sites studied are summarized in Table 1. For each population 30 female trees (healthy with a height of more than 3 m) were randomly chosen and sampled. From each sampled tree, thirty mature needles and thirty mature cones were collected and transported in a cool box (Population (8), Trees (30×8), Needles and cones (30 ×30× 8). Stomata were measured on 10 trees with ten needles per tree.

Morphological traits

The morphological variation analysis was based on nine needle traits, seven cone traits, and six stomatal traits (Table 2). The stomata were evaluated using the epidermal imprinting technique under an optical microscope with a 400× magnification attached to a computer and an image analysis program (ImageJ). The measured parameters were selected based on previous similar studies to ensure consistency and comparability (Bayet et al., 1991; Juan et al., 2003; Klimko et al., 2004, 2007; Marcysiak et al., 2007; Brus et al., 2011; Adam et al., 2014; Hafsi et al., 2017).

Epidermal surface and waxes

To examine the epidermal surfaces of the needles, an electronic scanning microscope was employed. Three needles from each population were treated with alcohol for five hours at 90°C under ultrasonography, followed by drying in open air at ambient humidity and temperature. Subsequently, the needles were analyzed using the electronic scanning microscope. Additionally, three untreated needles were also observed for comparison.

Statistical analysis

Descriptive statistics were computed for all traits, including mean, minimum, maximum and coefficient of variation ($CV\% = [SD/mean] \times 100$). Frequency distributions were analyzed for qualitative traits using the formula:

Relative frequency (fr) = $(f/N) \times 100$, where *f* is the count of observations in a category and *N* is the total sample size.

Normality of data distribution was verified using the Shapiro-Wilk test, while homogeneity of variances was assessed via Levene's test ($\alpha = 0.05$). For traits meeting these assumptions, one-way ANOVA identified population-level differences. Post-hoc Tukey tests ($p < 0.05$) clarified pairwise contrasts between populations. Furthermore, associations among traits were assessed by calculating bivariate Pearson correlation coefficients across population means of all morphological traits. The entirety of the statistical analyses was performed utilizing IBM SPSS Statistics, version 25.0.0

PCA was performed using a triangular distance matrix as input, in line with the procedure described by Legendre and Legendre (1998), and implemented with XLSTAT® version 2014.1. For cluster analysis, a separate rectangular matrix containing the mean values of all measured traits across populations was analyzed using the Euclidean distance metric and the UPGMA clustering algorithm (Sneath and Sokal, 1973), as implemented in STATISTICA® version 5.

Conclusion

The qualitative and quantitative character analyses of eight populations of *Juniperus oxycedrus* subsp. *oxycedrus* indicated the existence of a high level of phenotypic diversity among populations with regard to the leaves, cones and stomata. These results can be used in genetic resource conservation, management and selection programs. However, to obtain more precise information, a genetic study is essential, with the aim of identifying and developing molecular markers such as SSRs and SNPs.

Authors' contributions

These authors contributed to the article for publication. IN conceived the ideas, conducted the data collection and analysis, and wrote the manuscript. HZ and YA conducted data analysis. SB and YA conceived the ideas and reviewed the manuscript. AH supervised the research, reviewed and examined the manuscript. All authors read and approved the final manuscript.

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