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Variability of eight Algerian oleaster ecotypes (*Olea europaea* subsp. *europaea* var. *sylvestris* [Mill.] Lehr): pollen and exine morphology in relation to geo-climatic effect

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Abstract

Pollen characters of the Algerian oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris* [Mill.] Lehr) from eight ecotypes were analysed to evaluate the geo-climatic effect of their environment. Samples were collected along an altitudinal gradient from western to central Algeria. Eighteen morphological characters of the pollen grains were studied using scanning electron microscopy (SEM). The results show significant inter-populations variability. The geo-climatic conditions, duration and timing of dry periods vary from one area to another. Depending on the area, the length of the dry period ($P_p \leq 2T$) coincides more or less with anthesis periods. No significant geo-climatic effects on exine parameters were detected, except for lumina density (LD) and mean lumina diameter (Lm). However, polar axis (P), pollen size index (PSI) and pollen colpis length (PCL) were found to be related to latitude, longitude and temperature. LD and Lm were strongly linked to the pollen parameters, resulting in variation in pollen shape and size. Depending on the geo-climatic conditions, three morphotypes appear. The morphotype from a site with high temperature, low humidity associated with a long dry period is characterised by small pollen size, low lumina surface area and high lumina density/100 μm^2 and vice versa. The results indicate that the exine has played an important role in the environmental adaption in these wild populations.

Keywords: *wild olive, crop wild relatives, SEM, image analysis, multivariate analysis, geoclimatic adaptation*

The Mediterranean olive tree or oleaster (*Olea europaea* subsp. *europaea* var. *sylvestris* [Mill.] Lehr) is distributed all around the Mediterranean Sea (Breton et al. 2012; Kaniewski et al. 2012). The different Oleaster forms are considered by several researchers as the wild precursors of the cultivated olive tree because of their morphological and genetic affinities (Breton et al. 2012).

Olea europaea L. is classified as a complex group, either as a subspecies (subspecies *oleaster* [Hoffm. et Link] Hegi) or as a variety (var. *sylvestris* [Mill.] Lehr = var. *oleaster* [Hoffm. et Link] DC) (Zohary & Hopf 2000). Some authors consider oleaster populations as forms that have returned to the wild (feral form). However, there is so-called (true) oleaster that seems to be wild natural forms. The morphological distinction between the two forms is not clear (Besnard & Bervillé 2000). The confusion between the two forms of the wild olive tree (feral and true) as well as between the cultivars of the cultivated olive tree may be due to the fact that, the two wild forms as well as the cultivated olive tree are interfertile (Langgut et al. 2019).

Several studies has been carried out on pollen from *Olea europaea* var. *sativa* ([Weston] Lehr) to unravel

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its evolution (Liphschitz et al. 1991) as well as pollen development, morphology and exine structure as morphological markers for distinguishing different cultivars (Pacini & Juniper 1979a, 1979b; Fernández & Rodríguez-García 1989; Bartolini et al. 1992; Lanza et al. 1996; Najmaddin 2016; Messora et al. 2017; Laaribi et al. 2018), its viability and germination (Alba et al. 2011; Silva et al. 2016), and its conservation and various forms of sterility (Ateyyeh 2009). However, it was Roselli (1979) who pioneered the use of the pollen exine ultra-structure in his investigations for olive cultivars characterisations and identification (Ganino et al. 2006).

The real challenge for olive-growing countries is to achieve self-sufficiency and to develop better quality products. Some countries have breeding programmes, which initially focused on conventional cultivars and later found it necessary to draw on wild crop relatives as the wild crop relatives are more diverse genetically than cultivated types. Indeed, agro-morphological, biochemical and molecular studies have confirmed the high diversity of the wild species; these interesting characteristics hold agronomic and biotechnological potential and may allow cultivated species to acquire back some useful genetic material (Hannachi & Marzouk 2012; Klepo et al. 2013, 2014). Other countries have addressed the production challenge by increasing the area planted (extensive farming). For instance, Algeria set under the national plan for agriculture and rural development a target of one million hectares of olive trees for 2010–2014 (Bouarissa 2010).

The oleaster brings to the-cross-bred trees (oleaster × cultivars) good vigour, short juvenile period and good oil quality (Hannachi & Marzouk 2012; Klepo et al. 2013).

In North Africa, the oleaster most often grows in association with the mastic pistachio tree (*Pistacia lentiscus*), the *Oleo-Lentiscetum* group whose natural habitat covers the plains and low hills (up to 700 m in altitude), from the North African shores to the High Plains. Nevertheless, its current area is continuously shrinking due to land clearing for the expansion of olive growing (Harfouche et al. 2005).

The study of wild olive tree populations of different origins as a source of new genetic variation is one of the important objectives of olive breeding programmes (Abood et al. 2017). However, little has been done to assess the genetic resources of the oleaster in Algeria, despite being one of the most important forest and fruit crops in terms of socio-economic, environmental and agronomic impacts. To the best of our knowledge, no morpho-metric study of the *Olea europaea* subsp. *europaea* var. *sylvestris*) pollen has been carried out in Algeria, so far.

This study assesses inter-populations (intra-specific) variability among eight oleaster populations, collected according to an altitudinal gradient, from eight different sites in Algeria, and assesses the effect of the geoclimatic environment on this variability. The variability covers the micro-morphological parameters of pollen grains and exine structure, using 18 descriptors in total (seven pollen parameters as well as 11 exine features) through a combination of three tools, namely scanning electron microscopy (SEM), imaging and multivariate analysis.

Material and methods

Study sites and plant material

Flowers at the white-bud stage of *Olea europaea* subsp. *europaea* var. *sylvestris*) were collected from eight sites distributed over five regions from western to central Algeria. One site is sampled in each of the following localities: Ghelizane (GH), Chlef (Chl), Blida (B) and Tipaza (T), while four sites were sampled in the locality of Ain-Defla (ADL1, ADL2, ADL3 and ADL4) (Figure 1).

From each site flowers were collected randomly from three trees, making a total of 24 samples. The sampling was carried out according to an altitudinal gradient (Table I) with additional information on population, origin of samples and climatic characteristics of the sites. Climatic data were gathered over 35 years. Monthly temperature and precipitation data were plotted on the ombro-thermal diagrams of Bagnouls and Gaussen (1957) and used for establishing the P_p/T ratio (with, P_p for precipitation and T for temperature), the evaluation of the monthly arido-humidity index is calculated according to Rivas-Martinez (2005) method.

SEM observation of pollen grains

SEM observations of pollen grains followed the method of Bartolini et al. (1992) and Ćalić et al. (2013). The flowers were dried in open air at room temperature in the shade for about 10 days, and then stored in Kraft paper bags until SEM observation. About ten flowers per tree were attached to aluminium stubs using double-adhesive tape and coated with a CRESSINGTON SPUTTER COATER 108 auto for 160 s at a pressure of 0.1 bar and studied using a Philips ESSEMXL 30 SEM. The specimens were observed at 20 kV and at 1500 × magnification for general observation of pollen morphology and at 2500 × for details of exine ornamentation.

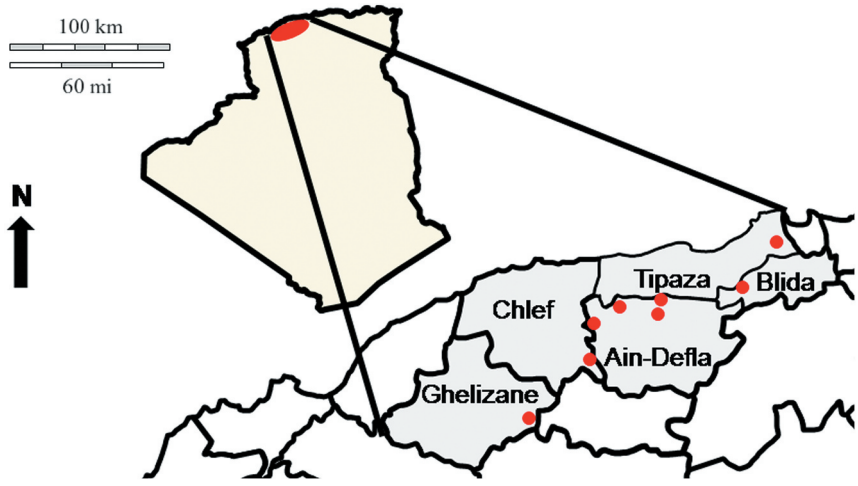


Figure 1. Location of the different sampling areas.

Table I. Population designation, origin and geo-climatic characteristics of the study sites.

Population designation	Population origin	Alt. (m)	Lat.	Long.	MaxT (°C)	MinT (° C)	MeanT (°C)	P_p (mm)	Q3	Bioclimatic stage
GH	Ghelizane	201	35.82	1.13	26.94	12.50	19.72	503.80	49.79	Arid very hot
Chl	Chelif	492	36.06	1.60	23.83	11.05	17.44	671.75	71.26	Semi-arid very hot
ADL1	Ain-Defla	667	36.34	1.78	22.36	13.44	17.90	676.54	84.78	Sub-humid very hot
ADL2		231	36.25	1.88	25.66	6.05	15.85	669.46	69.34	Semi-arid mild
ADL3		394	36.27	2.22	23.16	12.22	17.69	637.60	78.07	Semi-arid very hot
ADL4		529	36.29	2.23	22.22	11.68	16.95	662.84	81.41	Sub-humid hot
B	Blida	92	36.47	2.65	25.23	12.89	19.06	659.31	75.93	Semi-arid very hot
T	Tipaza	110	36.65	2.79	22.52	14.68	18.60	510.81	78.41	Semi-arid very hot

Note: Alt., Altitude; Lat., Latitude; Long., Longitude; MaxT (°C), maximum temperature; MinT (°C), minimum temperature; MeanT (° C), mean temperature; P_p, precipitation; Q3; Emberger pluviothermic quotient.

Image analysis with ImageJ/fiji software

SEM-micrographs were analysed using ImageJ/fiji1.46 software for the metric measurements of pollen morphology and exine structure according to Lanza et al. (1996), Messora et al. (2017) and Laaribi et al. (2018) protocols commonly used for the study of olive pollen. Characters used in the analysis follow the definition of Punt et al. (2007) and Hesse et al. (2009), and include polar axis P (in µm), equatorial axis E (in µm), pollen ratio P/E, pollen size index (PSI) = $(P \times E)/100$, providing information on both size and shape of the pollen, pollen colpus length PCL (in µm) and pollen shape factor (PSF) = $(R \times 100)$, where R, is the pollen ratio P/E. Exine characters include width of the muri Mw (in µm), lumina area LA (in µm^2), lumina perimeter LPm (in µm), maximum lumina diameter L (in µm), minimum lumina diameter W (in µm), lumina ratio L/W, mean lumina diameter Lm (in µm), lumina equivalent circle diameter (ECD) = $2\sqrt{\left(\frac{area}{\pi}\right)}$, lumina size index (LSI) = $(L \times W)/100$, lumina shape factor (LSF) = 4π(area/perimeter^2) and lumina density (LD) = number of lumina per 100 µm^2.

Twenty-five pollen grains from each tree, equivalent to 75 pollen per site and 600 for all sites, were measured and observed.

Statistical analysis

Using xlsat software, we carried out a variance analysis followed by a multiple comparison by Duncan's test, a correlation analysis supplemented by regression curves, principal component analysis (PCA) and hierarchical ascending classification (HAC) analysis.

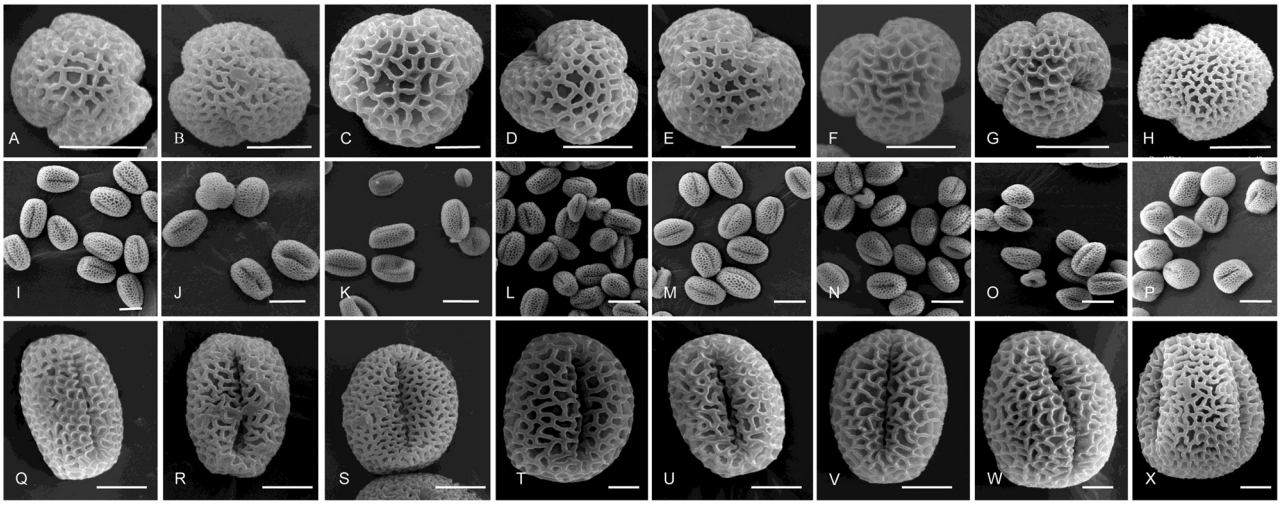


Figure 2. Scanning electron microscope observation of pollen grains from eight different localities under 6500× magnification. **A–H.** Polar pollen view. **A.** (Chl). **B.** (ADL2). **C.** (ADL4). **D.** (ADL1). **E.** (ADL3). **F.** (B). **G.** (T). **H.** (GH). **I–P.** Pollen overview. **I.** (Chl). **J.** (ADL2). **K.** (ADL4). **L.** (ADL1). **M.** (ADL3). **N.** (B). **O.** (T). **P.** (GH). **Q–X.** General pollen view. **Q.** (Chl). **R.** (ADL2). **S.** (ADL4). **T.** (ADL1). **U.** (ADL3). **V.** (B). **W.** (T). **X.** (GH). Scale bars – 20 µm (I–P), 10 µm (A, B, D, E–H, Q–S, U, V), 5 µm (C, T, W, X).

Results

Pollen grains from the eight sites are all tricolpate and trizonocolpate (Figure 2A–H).

Micromorphometric variability of pollen and exine

With the exception for *E* ($p = 0.037$), *P*/*E* ($p = 0.007$) and LD ($p = 0.019$) in the ADL2 population and the *P*/*E* ratio in the ADL3 ($p = 0.02$) and T ($p = 0.035$) populations; no intra-population variability in pollen and exine biometry was observed in any of the study populations; for the other parameters tested at the 5%. Nevertheless, the results of pollen and exine biometric parameters indicate a high phenotypic variability between populations (Tables II, III). Indeed, the inter-populations variability is significant at 1% ($p = 0.0001$), for all parameters tested on pollen morphology. The extreme values for *P* are obtained by the GH and T populations, for *E* by the ADL3 and T populations and for *P*/*E* ratio by ADL4 and GH populations. In addition, the *P*/*E* ratio or (*P*/*E*) × 100 and *P* can yield information on two qualitative characteristics (pollen size and shape) (Table II).

Except for the GH population, where the pollen form is prolate-spheroidal or subprolate, the pollen form of the other populations is prolate. The GH population is the most diverse. Pollen from all eight populations belongs to the 'small size' category; according to the terminology of Halbritter et al. (2018), where the pollen size is between 3.14 and 4.28 and the size of the largest diameter is between 10 µm and 25 µm (Figure 2I–X, Table II).

For the length of the colpus, the maximum and minimum values were observed from the T and GH populations, respectively (Table II).

For the 11 exine parameters, the inter-populations variability is significant at the threshold of 1% ($p = 0.0001$). Most of the extreme values of the exine parameters were produced by ADL4 and ADL1 populations, respectively, for the minimum and maximum values: LA, LPm, *L*, *W*, ECD, LSI and Mw. The ADL1 population also generated the highest mean for Lm, but the lowest value was observed for the GH population (Table III). The highest LD parameter was recorded from the GH population, while the T population gave the lowest lumina density (Figure 3, Table III). The highest and lowest LSF values were observed in the B and Chl populations, respectively. The three populations (ADL1, ADL3 and T) are at the top of Duncan's ranking and fall into Group A for almost all of the exine parameters (Table III).

Results of multivariate analysis of pollen and exine parameters

To avoid redundancy and confusion in the interpretation of results, we used a combination of two multivariate analysis models namely, PCA and HAC.

Results of the principal component analysis (PCA). — A reduced, centred and standardised PCA was carried out. The elbow method was used to select factors and the optimal number of groups. According to PCA results, 92.86% of the total variability can be explained

Table II. Biometric results of pollen grains from the eight studied localities.

Population Designation	Pollen Parameters						
	P (µm)	*E* (µm)	*P*/*E* ratio	PSI	PCL (µm)		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Shape pollen classes	size pollen classes
GH	18.49 ± 1.93^{e}	17.05 ± 1.83^{a}	1.1 ± 0.19^{d}	3.14 ± 0.39^{d}	9.94 ± 0.54^{d}	Prolate-sphéroidal; subprolate	Small size
Chl	23.34 ± 2.45^{b}	16.09 ± 2.03^{c}	1.47 ± 0.19^{b}	3.77 ± 0.69^{bc}	14.5 ± 2.001^{b}	Prolate	Small size
ADL1	23.001 ± 1.88^{b}	16.38 ± 1.87^{bc}	1.42 ± 0.17^{bc}	3.78 ± 0.61^{bc}	15.84 ± 1.07^{ab}	Prolate	Small size
ADL2	21.29 ± 2.08^{d}	15.002 ± 2.52^{de}	1.46 ± 0.28^{b}	3.20 ± 0.62^{d}	12.85 ± 0.9^{c}	Prolate	Small size
ADL3	22.33 ± 2.12^{c}	14.53 ± 1.68^{e}	1.55 ± 0.20^{a}	3.25 ± 0.56^{d}	14.65 ± 0.8^{b}	Prolate	Small size
ADL4	23.82 ± 2.96^{b}	15.36 ± 1.92^{d}	1.55 ± 0.28^{a}	3.65 ± 0.56^{c}	15.17 ± 1.81^{ab}	Prolate	Small size
B	23.03 ± 2.3^{b}	16.93 ± 1.62^{ab}	1.37 ± 0.19^{c}	3.9 ± 0.55^{b}	15.85 ± 1.93^{ab}	Prolate	Small size
T	24.96 ± 2.07^{a}	17.18 ± 1.80^{a}	1.47 ± 0.22^{b}	4.28 ± 0.54^{a}	16.12 ± 1.54^{a}	Prolate	Small size
Mean	22.62 ± 2.80	15.98 ± 2.13	1.44 ± 0.25	3.62 ± 0.69	14.13 ± 2.42	—	—

Note: *P*, polar axis; *E*, equatorial axis; *P*/*E*, pollen ratio; PSI, pollen size index; PCL, pollen colpus length.
The results are expressed as mean ± standard deviation (SD).
According to Duncan's test 1% the means with the same letter are not significantly different, and the significantly different means are followed by different letters.

Table III. Parameter results measured on pollen exine.

Population Designation	Pollen exine parameters										
	LA (µm^2)	LPm (µm)	(*L*) (µm)	(*W*) (µm)	LR: *L*/*W*	LSI	Lm (µm)	ECD (µm)	LSF	Mw (µm)	LD (nL/100 µm^2)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
GH	0.46 ± 0.09^{d}	2.5 ± 0.27^{c}	1.004 ± 0.11^{bc}	0.61 ± 0.06^{cd}	1.63 ± 0.04^{ab}	0.006 ± 0.001^{bc}	0.77 ± 0.09^{b}	0.77 ± 0.02^{cd}	0.96 ± 0.03^{ab}	0.58 ± 0.05^{bc}	24.12 ± 2.8^{a}
Chl	0.78 ± 0.17^{ab}	3.21 ± 0.35^{a}	1.27 ± 0.12^{a}	0.79 ± 0.09^{a}	1.61 ± 0.03^{ab}	0.012 ± 0.002^{a}	1.01 ± 0.12^{a}	0.99 ± 0.03^{a}	0.94 ± 0.04^{ab}	0.63 ± 0.09^{bc}	17.88 ± 3.02^{c}
ADL1	0.91 ± 0.07^{a}	3.44 ± 0.12^{a}	1.36 ± 0.05^{a}	0.86 ± 0.03^{a}	1.58 ± 0.02^{b}	0.012 ± 0.001^{a}	1.07 ± 0.05^{a}	1.08 ± 0.01^{a}	0.96 ± 0.01^{ab}	0.72 ± 0.04^{a}	19.52 ± 1.28^{bc}
ADL2	0.51 ± 0.1^{cd}	2.58 ± 0.22^{bc}	1.03 ± 0.09^{bc}	0.65 ± 0.06^{bc}	1.58 ± 0.07^{b}	0.007 ± 0.001^{bc}	0.85 ± 0.05^{b}	0.81 ± 0.02^{bc}	0.96 ± 0.062^{ab}	0.61 ± 0.04^{bc}	18.34 ± 2.48^{c}
ADL3	0.58 ± 0.09^{c}	2.74 ± 0.22^{b}	1.10 ± 0.08^{b}	0.68 ± 0.05^{b}	1.62 ± 0.06^{ab}	0.008 ± 0.001^{b}	0.87 ± 0.09^{b}	0.86 ± 0.02^{b}	0.97 ± 0.02^{a}	0.63 ± 0.08^{bc}	18.52 ± 5.25^{c}
ADL4	0.41 ± 0.13^{b}	2.35 ± 0.37^{c}	0.97 ± 0.14^{c}	0.58 ± 0.08^{d}	1.66 ± 0.07^{a}	0.002 ± 0.002^{c}	0.8 ± 0.13^{b}	0.72 ± 0.03^{d}	0.93 ± 0.07^{ab}	0.54 ± 0.07^{c}	21.92 ± 3.50^{ab}
B	0.82 ± 0.03^{ab}	3.40 ± 0.09^{a}	1.34 ± 0.03^{a}	0.82 ± 0.02^{a}	1.64 ± 0.06^{ab}	0.011 ± 0.001^{a}	1.06 ± 0.07^{a}	1.02 ± 0.007^{a}	0.89 ± 0.07^{b}	0.66 ± 0.01^{abc}	18.26 ± 1.61^{c}
T	0.76 ± 0.06^{b}	3.16 ± 0.18^{a}	1.25 ± 0.08^{a}	0.796 ± 0.04^{a}	1.57 ± 0.11^{b}	0.012 ± 0.001^{a}	1.016 ± 0.06^{a}	0.99 ± 0.001^{a}	0.96 ± 0.053^{ab}	0.59 ± 0.08^{bc}	15.72 ± 1.16^{d}
Mean	0.63 ± 0.19	2.85 ± 0.46	1.14 ± 0.17	0.71 ± 0.711	1.61 ± 0.07	0.008 ± 0.002	0.91 ± 0.14	0.71 ± 0.04	0.95 ± 0.05	0.60 ± 0.08	19.30 ± 3.82

Note: LA, lumina area; LPm, lumina perimeter; *L*, maximum lumina diameter; *W*, minimum lumina diameter; LR, *L*/*W* lumina ratio; LSI, lumina size index;
Lm, mean lumina diameter; ECD, equivalent circle diameter; LSF, lumina shape factor; Mw, lumina muri width; LD, lumina density (number of lumina per 100 µm^2).
The results are expressed as a mean ± standard deviation (SD).
According to Duncan's test 1% the means with the same letter are not significantly different, and the significantly different means are followed by different letter.

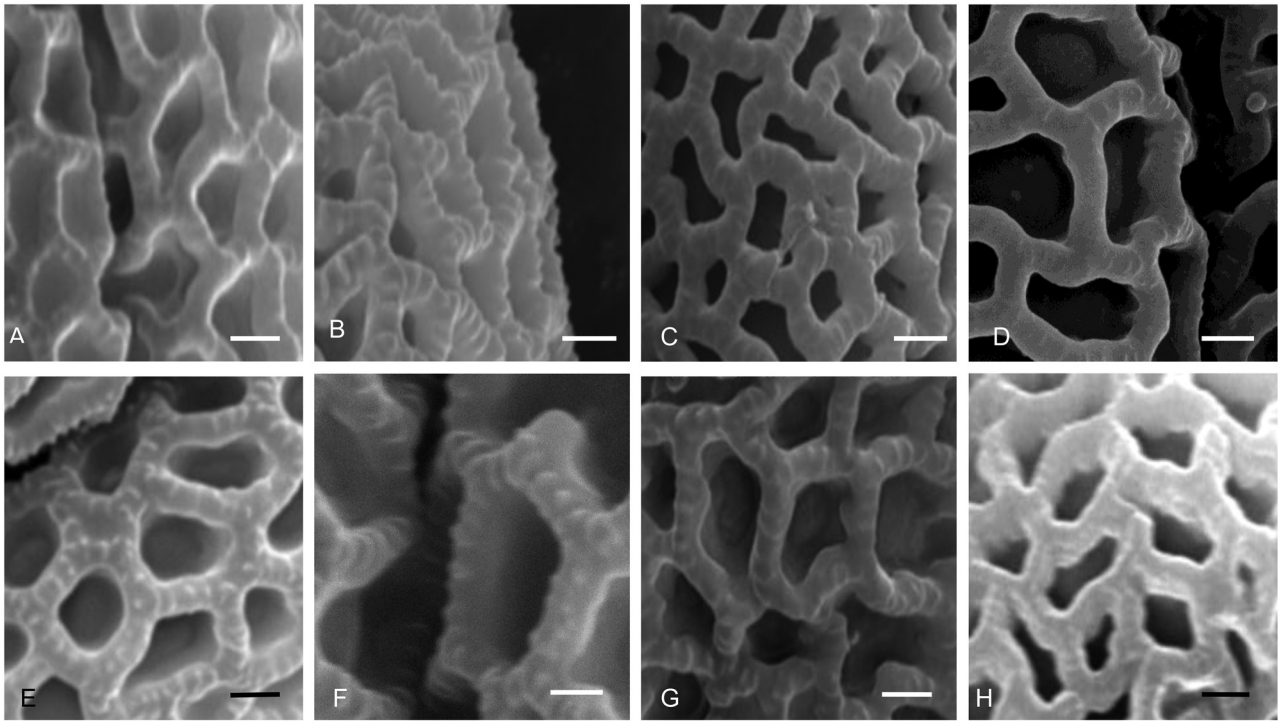


Figure 3. Scanning electron microscope observation of the exine pollen viewed under 2500× magnification. **A.** (Chl). **B.** (ADL2). **C.** (ADL4). **D.** (ADL1). **E.** (ADL3). **F.** (B). **G.** (T). **H.** (GH). Scale bars – 2 µm.

by three factors: F1, F2 and F3. Most of the information was obtained by F1 (63.72%) whereas those revealed by F2 are only 15.93% and 13.21% by F3. Factor F1 represents exine while factor F2 represents pollen (Figure 4A).

The pollen parameters *E* and *P*/*E* ratio are represented by F2; those of exine: *L*/*W* and LSF ratio are represented by F3. With the exception of LD which is positioned on the left side of F1, the other pollen and exine parameters are on the right side of F1 (Figure 4A).

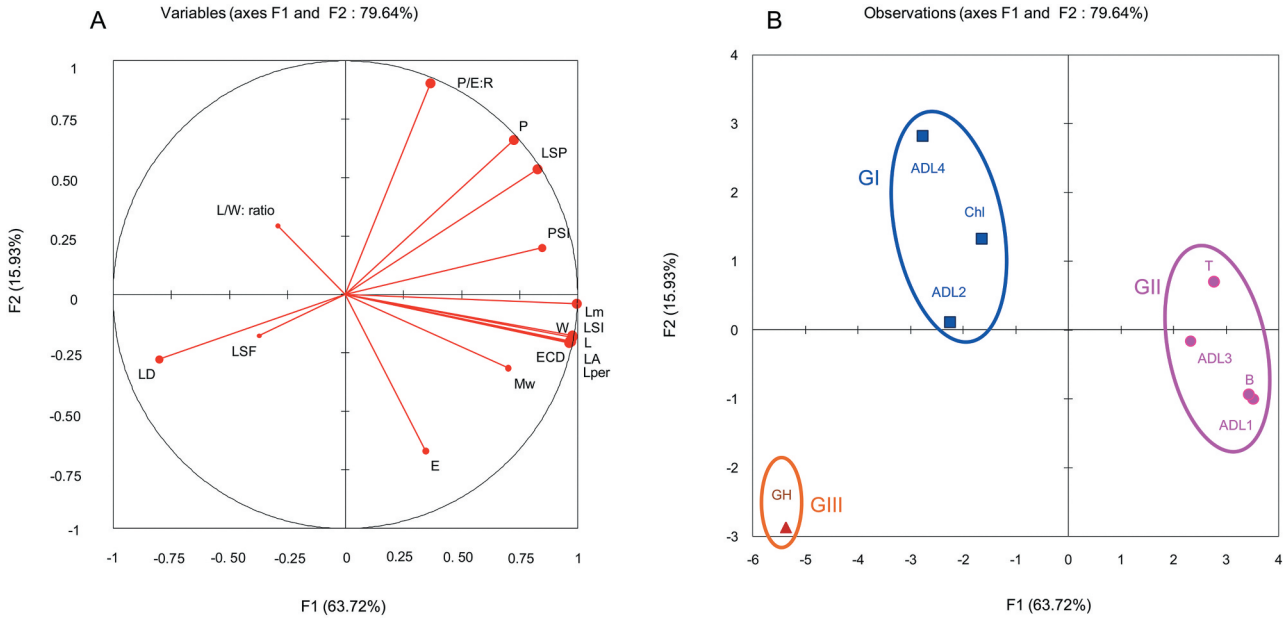


Figure 4. Principal component analysis. **A.** Projection of the variables measured on pollen and exine in the F1 and F2 axes. **B.** Projection of the populations in the F1 and F2 axes. Populations are GH, Chl, ADL1, ADL2, ADL3, ADL4, B and T. Abbreviations: *P*, polar axis; *E*, equatorial axis; *P*/*E*, pollen ratio; PSI, pollen size index; PCL, pollen colpus length; LA, lumina area; LPm, lumina perimeter; *L*, maximum lumina diameter; *W*, minimum lumina diameter; LR, *L*/*W* lumina ratio; LSI, lumina size index; Lm, mean lumina diameter; ECD, equivalent circle diameter; LSF, lumina shape factor; Mw, lumina muri width; LD, lumina density (number of lumina per 100 µm^2).

Except for ADL4 population, which corresponds to F2, F1 represents the other populations (Figure 4B). In terms of factors, F1 and F2, the populations are divided into three groups: the first group (G1) contains three populations (Chl, ADL2 and ADL4), the second group (G2) represents four populations (ADL1, ADL3, B and T) and the third (G3) includes of a single population (GH) (Figure 4B).

Results of the hierarchical ascending classification (HAC). — The HAC method used allowed classifying the study populations into three groups GI, GII and GIII (Figures 5, 6).

Environmental factors

The 35-year averages of climate data (maximum temperature, average temperature, minimum temperature and sum of annual precipitation), collected at the weather stations closest to the study sites, were corrected for the elevation of the sites (Table I).

According to the ombrothermal diagrams of Bagnouls and Gaussen (1953) and the P_p/T ratio (monthly arid-humidity Rivas-Martinez 1987), the duration of drought during the year varies from one location to another; it may start very early in the year or late.

8According to the Bagnouls and Gaussen method, the results show that drought may last from 3 months up to 5 months. The drought period may start from the second week of May to mid-October (Figure 7). However, according to Rivas-Martinez (1987), the dry period may last from 6 to 8 months depending on the study area.

Relationship between pollen parameters

At a threshold of 5%, the polar axis parameter (*P*) is positively and significantly related to PSI and PCL. At the same threshold and in the same direction as the first case, PCL, is significantly correlated with PSI (Table IV).

Relationship between exine parameters

The correlations between some exine parameters are very significant and positive at the threshold of 5%. The '*r*' values are > 0.99 (Table IV).

Relationship between pollen and exine parameters

Between the parameters (PCL and Lm; PSI and Lm) positive and highly significant relationships are observed. Negatively significant relationships

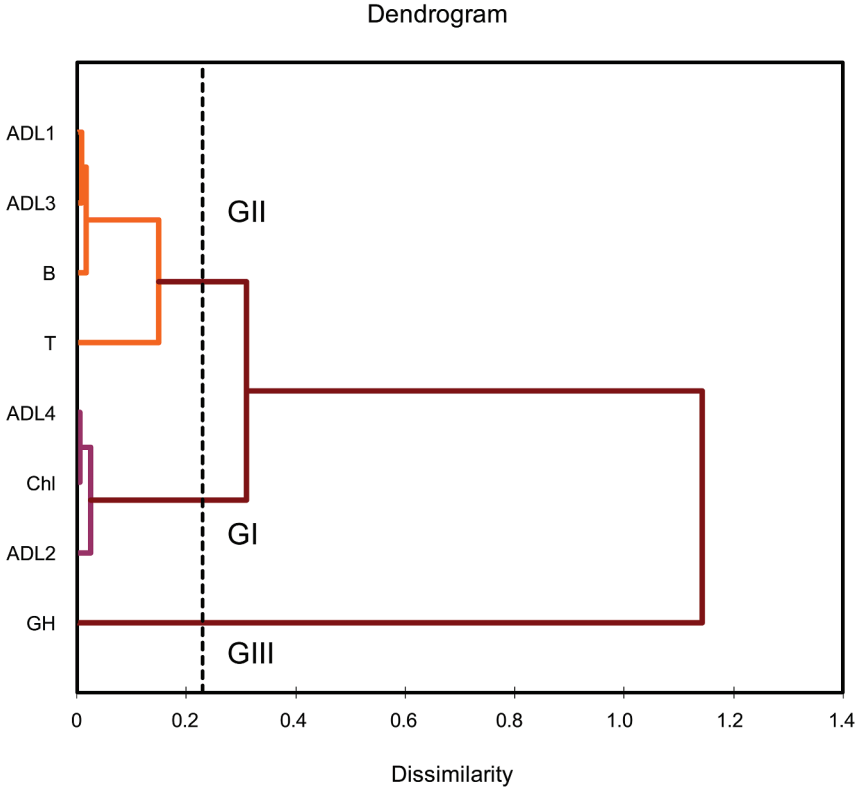


Figure 5. Cluster obtained by the hierarchical ascending classification analysis on pollen and exine micro-morphometric characters. Populations are GH, Chl, ADL1, ADL2, ADL3, ADL4, B and T.

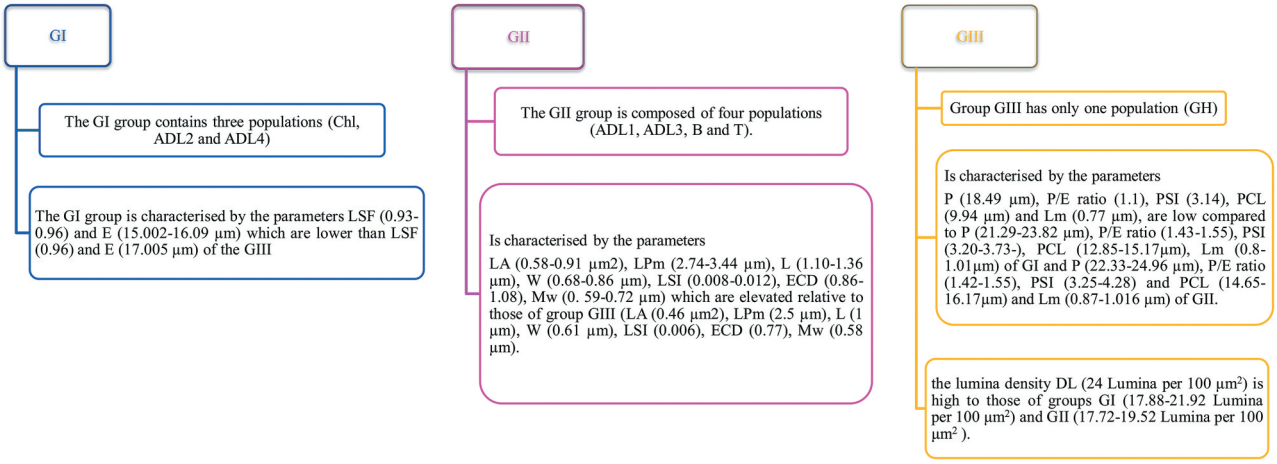


Figure 6. Characteristics of groups obtained by multivariate analysis groups are GI, GII and GIII. Abbreviations: *P*, polar axis; *P*/*E*, pollen ratio; PSI, pollen size index; PCL, pollen colpus length; LA, lumina area; LPm, lumina perimeter; *L*, maximum lumina diameter; *W*, minimum lumina diameter; LSI, lumina size index; Lm, mean lumina diameter; LD, lumina density (number of lumina per 100 µm^2).

are observed between *P* and LD. PSI and PCL are correlated with almost every parameter of the exine. This correlation is negative with LD and positive with the other parameters (Table IV).

Geo-climatic impact on pollen and exine parameters

The geo-climatic interaction between pollen and its exine parameters was tested by the slope of the regression model $y = ax + b$ and supplemented by the regression curves and the coefficient of determination (Figure 8).

Latitude and longitude are strongly and positively related to (*P*, PSI, and PCL). However, with the exception of LD and Lm, which are related to latitude, the remaining exine parameters are not related to the geo-climatic parameters (Figure 8, Table V).

Negatively significant to very highly significant relationships were recorded among pollen parameters (*P*/*E* ratio [r = −0.755], PCL [*r* = −0.787], *P* [r = −0.855]) and maximum temperature on the one hand, and between the warmest month's maxima temperature and PCL (*r* = −0.97), *P* (*r* = −0.88) and PSI (*r* = −0.887), on the other hand. Besides that, positively significant correlations were found between the minima temperature of the coldest month and PSI (*r* = 0.740), and between the average temperature and *E* (*r* = 0.742) (Figure 8, Table V). No significant relationship is recorded between the exine characteristics and the climate of the study sites.

Discussion

The populations from the eight study areas have trizonocolpate pollen grains that show considerable similarity in terms of size and shape; most of the pollen grains, from each population, are small and prolate as also previously observed in pollen of olive cultivars by Lanza et al. (1996), Javadi and Arzani (2001) and Koubouris et al. (2012). However, Ribeiro et al. (2008) reported three types of shapes; elliptic, subprolate and spheroidal-prolate in 20 olive cultivars grown in Portugal. Furthermore, no intra-population variability was detected in the populations from ADL1, ADL4, GH, Chl, B and T areas. However, low intra-population variability in *E*, *P*/*E* ratio, and PSI parameter was noted for the ADL2 site and in the *P*/*E* ratio parameter for the population of ADL3.

High intra-specific variability in *Olea europaea* subsp. *europaea* var. *sylvestris*) was, however, observed for pollen and exine parameters according to the geo-climatic conditions of the study areas. The use of multivariate analysis HAC and PCA was very essential for a clearer vision of inter-populations variability. The results of the HAC and PCA analyses corroborate (Figures 4, 5). Thus, the eight populations tested were divided into three groups or morphotypes.

It should be noted that earlier studies have indicated that intra-specific polymorphism may be induced by two factors, which may be internal or external, or a combination of both (Derridj et al. 1991; El Ghazali et al. 1997; Belhadj et al. 2007). Internal factors are varied and are related to the species itself. External factors are related to the environment in which the different populations of the species have evolved. Environment factors may be geo-climatic (altitude, latitude, longitude, temperature, precipitations) or soil composition. The soil

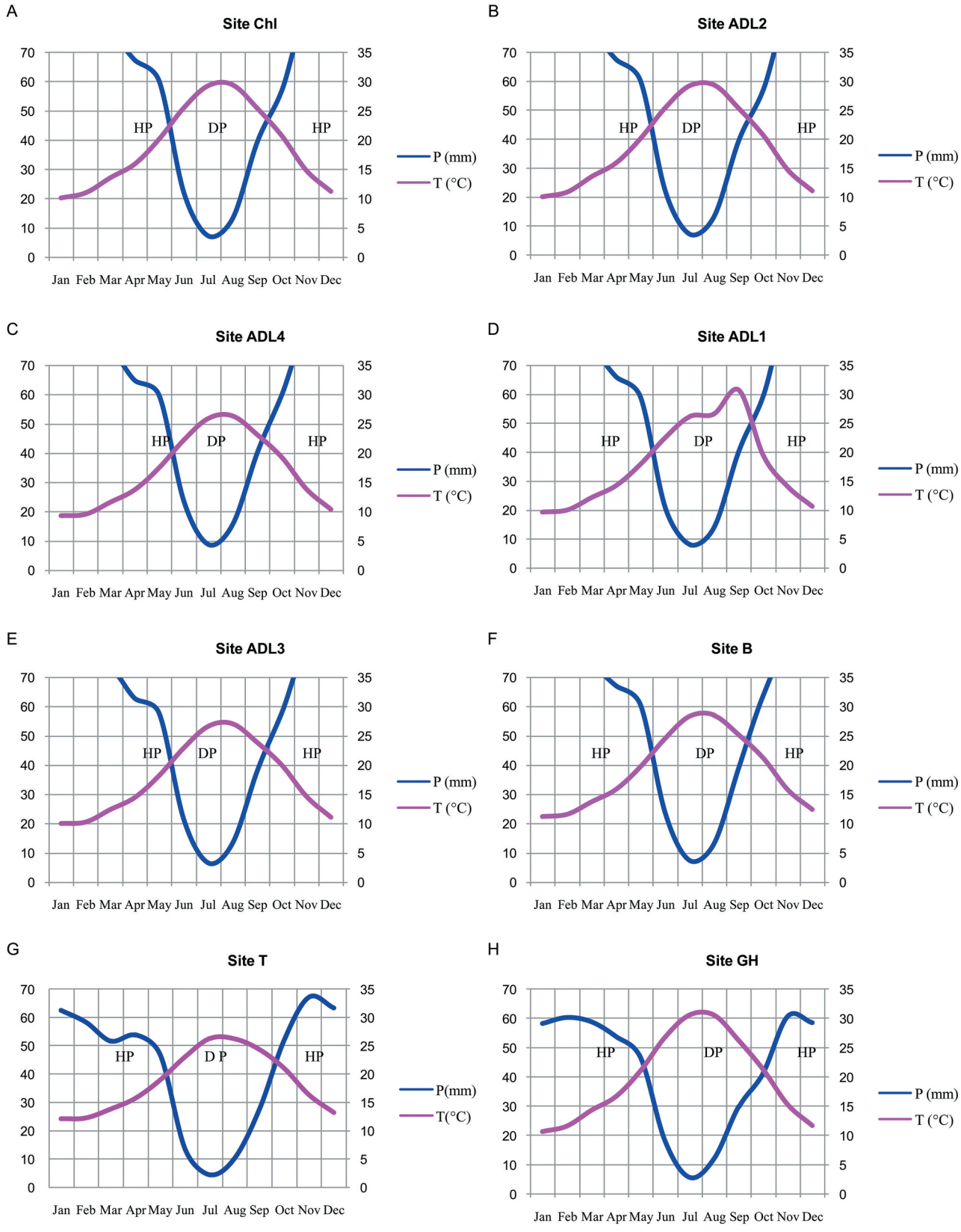


Figure 7. Ombrothermic diagram of Bagnouls and Gaussen method for a period of 35 years. Abbreviations: DP, dry period; HP, humid period; *P*, precipitation; *T*, temperature.

Table IV. Correlation matrix between the pollen and those of its exine parameters.

Variables		*P*	*E*	PR:*P*/*E*	PSI	PCL	Mw	LD	LA	LPm	*L*	*W*	LR: *L*/*W*	LSI	Lm	ECD	LSF
PR: *P*/*E*	*r*	**0.812****	-0.640^{NS}	**1.000**													
	p-Value	**0.014**	0.087	**0.000**													
PSI	*r*	**0.840*****	0.478^{NS}	0.366^{NS}	**1.000**												
	p-Value	**0.009**	0.231	0.373	**0.000**												
PCL	*r*	**0.957*****	-0.031^{NS}	**0.746***	**0.831*****	**1.000**	**1.000**										
	p-Value	**0.000**	0.942	**0.034**	**0.011**	**0.000**	**0.000**										
LD	*r*	**−0.776****	-0.022^{NS}	-0.577^{NS}	**−0.717***	**−0.767***	-0.362^{NS}	**1.000**									
	p-Value	**0.024**	0.958	0.134	**0.045**	**0.026**	0.378	**0.000**									
LA	*r*	0.545^{NS}	0.399^{NS}	0.162^{NS}	**0.721***	0.68^{NS}	0.718*	**−0.715***	**1.000**								
	p-Value	0.162	0.328	0.701	**0.044**	0.063	0.045	**0.050**	**0.000**								
LPm	*r*	0.573^{NS}	0.415^{NS}	0.175^{NS}	**0.753***	**0.710***	**0.763***	-0.705^{NS}	**0.994*****	**1.000**							
	p-Value	0.137	0.307	0.378	**0.031**	**0.048**	**0.028**	0.051	**< 0.0001**	**0.000**							
L	*r*	0.582^{NS}	0.429^{NS}	0.174^{NS}	**0.767***	**0.718***	**0.751***	-0.696^{NS}	**0.993*****	**0.999*****	**1.000**						
	p-Value	0.130	0.289	0.681	**0.026**	**0.045**	**0.032**	0.060	**< 0.0001**	**< 0.0001**	**0.000**						
W	*r*	0.552^{NS}	0.400^{NS}	0.168^{NS}	**0.729***	0.683^{NS}	**0.719***	**−0.743***	**0.998*****	**0.993*****	**0.990*****	**1.000**	**1.000**				
	p-Value	0.156	0.326	0.691	**0.040**	0.062	**0.044**	**0.030**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.000**	**0.000**				
LSI	*r*	0.560^{NS}	0.431^{NS}	0.156^{NS}	**0.750***	0.694^{NS}	**0.730***	-0.702^{NS}	**0.998*****	**0.998*****	**0.997*****	**0.996*****	-0.327^{NS}	**1.000**			
	p-Value	0.149	0.287	0.712	**0.032**	0.056	**0.040**	0.050	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.430	**0.000**			
Lm	*r*	0.695^{NS}	0.359^{NS}	0.306^{NS}	**0.827*****	**0.803****	**0.723***	**−0.772****	**0.973*****	**0.984*****	**0.984*****	**0.975*****	-0.268^{NS}	**0.980*****	**1.000**		
	p-Value	0.056	0.382	0.461	**0.011**	**0.016**	**0.040**	**0.020**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.520	**< 0.0001**	**0.000**		
ECD	*r*	0.551^{NS}	0.384^{NS}	0.175^{NS}	**0.718***	0.687^{NS}	**0.728***	**−0.734***	**0.999*****	**0.994*****	**0.992*****	**0.999*****	-0.386^{NS}	**0.996*****	**0.974*****	**1.000**	
	p-Value	0.157	0.348	0.678	**0.045**	0.060	**0.040**	**0.040**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.340	< 0.0001	**< 0.0001**	**0.000**	
LSF	*r*	-0.432^{NS}	-0.352^{NS}	-0.130^{NS}	-0.555^{NS}	-0.459^{NS}	-0.448^{NS}	-0.033^{NS}	-0.236^{NS}	-0.325^{NS}	-0.343^{NS}	-0.219^{NS}	**−0.713***	-0.293^{NS}	-0.379^{NS}	-0.223^{NS}	**1.000**
	p-Value	0.285	0.393	0.759	0.153	0.253	0.270	0.940	0.570	0.430	0.406	0.600	**0.050**	0.480	0.354	0.596	**0.000**

Note: The values in bold are different from zero at an alpha significance level = 0.05; *, significant; **, highly significant; ***, very highly significant; NS, not significant.
P, polar axis; *E*, equatorial axis; PR, *P*/*E* pollen ratio; PSI, pollen size index; PCL, pollen colpus length; Mw, lumina muri width; LD, lumina density (number of lumina per 100 μm^2); LA, lumina area; LPm, lumina perimeter; *L*, maximum lumina diameter; *W*, minimum lumina diameter; LR, *L*/*W* lumina ratio; LSI, lumina size index; Lm, mean lumina diameter; ECD, equivalent circle diameter; LSF, lumina shape factor.

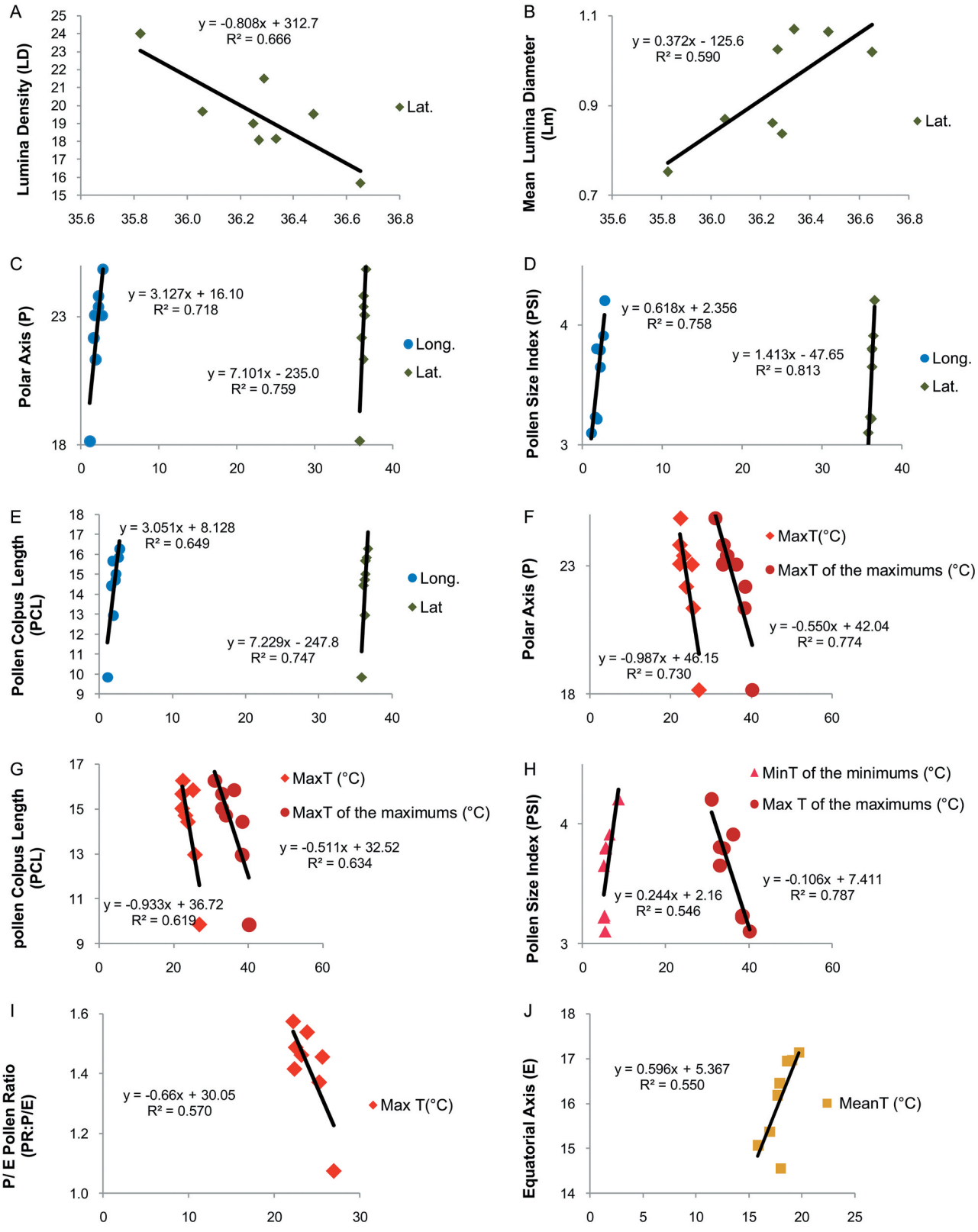


Figure 8. Regression curves obtained between the different geo-climatic parameters and the pollen and its exine parameters. Abbreviations: Lat., latitude; Long., longitude; MaxT (°C), maximum temperature; MaxT of the maximums (°C), the average temperature of the maxima of the warmest month; MinT of the minimums (°C), the average temperature of the minima of the coldest month; MeanT (°C), mean temperature; LD, lumina density (number of lumina per 100 μm^2); Lm, mean lumina diameter; *P*, polar axis; PSI, pollen size index; PCL, pollen colpus length; PR, *P*/*E* pollen ratio; *E*, equatorial axis.

Table V. Correlations between pollen and exine biometrics parameters with geo-climatic parameters.

Geo-climatics parameters		Pollen parameters					Exine parameters	
		P	*E*	PR: *P*/*E*	PSI	PCL	LD	Lm
Latitude	*r*	**0.872*****	0.233^{NS}	0.540^{NS}	**0.902*****	**0.865*****	**−0.816****	**0.768****
	p-Value	**0.005**	0.578	0.167	**0.002**	**0.006**	**0.013**	**0.026**
Longitude	*r*	**0.849*****	0.229^{NS}	0.526^{NS}	**0.871*****	**0.806****	-0.678^{NS}	0.668^{NS}
	p-Value	**0.008**	0.586	0.180	0.005	**0.016**	0.065	0.070
MaxT (°C)	*r*	**−0.855*****	0.172^{NS}	**−0.755***	-0.650^{NS}	**−0.787****	0.585^{NS}	-0.493^{NS}
	p-Value	**0.007**	0.684	**0.030**	0.081	**0.020**	0.128	0.214
MeanT (°C)	*r*	-0.272^{NS}	**0.742***	-0.655^{NS}	0.159^{NS}	-0.167^{NS}	0.238^{NS}	0.090^{NS}
	p-Value	0.514	**0.035**	0.078	0.706	0.692	0.571	0.833
Minimum of the minima *T* (°C)	*r*	0.484^{NS}	0.565^{NS}	0.049^{NS}	**0.740***	0.458^{NS}	-0.635^{NS}	0.489^{NS}
	p-Value	0.224	0.144	0.909	**0.036**	0.254	0.091	0.219
Maximum of the maxima *T* (°C)	*r*	**−0.880*****	-0.221^{NS}	-0.553^{NS}	**−0.887*****	**−0.797****	0.673^{NS}	-0.643^{NS}
	p-Value	**0.004**	0.599	0.155	**0.003**	**0.018**	0.067	0.086

Note: Values in bold are different from zero at an alpha significance level = 0.05.
*, significant; **, highly significant; ***, very highly significant; NS, not significant; *r*, correlation coefficient.
P, polar axis; *E*, equatorial axis; PR, *P*/*E* pollen ratio; PSI, pollen size index; PCL, pollen colpus length; Lm, mean lumina diameter; LD, lumina density (number of lumina per 100 μm^2).
MaxT, maximum temperature; MeanT, mean temperature.

may have an effect on plant's nutrition (El Ghazali et al. 1997).

Furthermore, according to our results, each morphotype includes populations from localities with geo-climatic characteristics that seem to have an effect on phenotypic expression on the one hand, and on the adaptability of the population on the other hand. Indeed, the study areas are geo-climatically different from one another (Table I) and as a result, there is no single pattern for drought, periods of drought appear and disappear at different periods of the year and persist for varying lengths of time (Figure 7).

The first morphotype was collected from Chl, ADL2 and ADL4 site. The ADL4 site has the lowest minimum temperature and the highest annual precipitation compared to other sites. The second morphotype were collected from ADL1, ADL3, B and T. These sites are characterised by a more or less low maximum temperature, a somehow high minimum temperature, an intermediate average mean temperature and an average annual precipitation. Finally, the GH site, which is home to the third morphotype, stands out from the other sites by having the lowest latitude and longitude, the highest maximum and average temperatures and low annual precipitation (Table I).

With respect to the geographical impact on variability in our studied species (*Olea europaea* subsp. *europaea* var. *sylvestris*), three pollen characteristics and two exine parameters seem to be affected by latitude and longitude (Figure 8, Table V).

Our results show that for an increase in latitude by one decimal degree, LD decreases by 0.808 μm and Lm, *P*, PSI, PCL, increase by 0.372, 7.107, 1.413 and 7.229 μm, respectively. For one decimal degree increase in longitude, *P*, PSI, PCL increase by 3.127, 0.618 and 3.051 μm, respectively, (Figure 8A–E). Altitude has no effect on the inter-populations variability of our species (*Olea europaea* subsp. *europaea* var. *sylvestris*). Similar results were obtained by John and Birks (1978) on the geographical effect on pollen size and shape of *Picea abies* (L.) Karsten, and by Lobreau-Callen (1975) on the dimensional variations of pollen of the genus *Ilex* (Aquifoliaceae). This author was able to demonstrate that within each species or variety, measurements differ according to geographical distribution and more precisely according to the gradient of latitude. Derridj et al. (1991) and Belhadj et al. (2007) reported the same results for the Algerian Atlas cedar (*Cedrus atlantica* Manetti) and *Pistacia atlantica* pollen, respectively.

Climate can also affect pollen parameters (Porch & Jahn 2001) and in particular temperatures (Kurtz & Liverman 1958). In our case, the effect was observed for the maximum temperature, the average temperature of the maxima of the warmest month, and to a lesser degree for the average temperature of the minima of the coldest month and mean temperature (Figure 8, Table V).

The correlations noted between some of the parameters and the climatic data show that the variation of five pollen parameters is related to at least one of the temperatures considered in this work. In fact, any increase in the maximum temperature by 1°C results in a decrease in *P* by 0.987 μm (Figure 8F), PCL by 0.933 μm (Figure 8G) and by 0.66in *P*/*E* ratio (Figure 8I). Similarly, an increase in the average tem-

perature of the warmest month's maxima by 1°C, results in a decrease of P by 0.55 µm (Figure 8F), by 0.511 µm for PCL (Figure 8G) and by 0.106 µm for PSI (Figure 8H). The same conclusions can be made for the average minima temperature of the coldest month and the mean temperature where increases of 1°C can cause PSI and E to increases by 0.244 (Figure 8H) and 0.596 µm (Figure 8J), respectively.

Ejsmond et al. (2015) found that high temperatures associated with high humidity generate large pollen, whereas in our case, high temperatures accompanied by low precipitation generate small sized pollen. Therefore, humidity is the main parameter that can have an effect on pollen size. However, precipitation does not have a direct effect on the variability of pollen and exine characteristics, but the monthly aridity-moisture ($P_p \leq 2T$ on a monthly scale, Rivas-Martinez [1987]) of the study sites has a significant effect. It is also important to note that the duration of the dry period during the year together with the time and intensity of its occurrence has a determinant effect on the morphology and size of the pollen. Indeed, according to the ombrothermal diagrams of Bagnouls and Gaussen (1953) based on a 35-year period, the duration of the drought during the year varies from one area to another; it may appear very early in the year or even very late. The drought period can persist for 15 to 20 weeks. It can start as early as the second week of May and last until mid-October (Figure 7).

The morphotype from a site with high temperature, low humidity associated with a long dry period (GH site) is characterised by a small size, low LA and high LD and vice versa (Figures 2H, 3H).

One can see from the strong correlations between the pollen parameters and those of the ultra-structure of exine (Table IV), without a direct link between the latter and the characteristics of the environment; that the pollen of the oleaster exhibits an adaptation to the latitude, longitude and temperature gradient of its environment by adopting various phenotypes by modulation of the exine. Indeed, it seems that the ultra-structure of the exine fulfils, through the geometrical structure of its lumina, the function of extending and shrinking the latter according to the gradient of some environmental elements. This function allows for certain flexibility with regard to geo-climatic changes leading to the general phenotypic change of the pollen, which can be controlled by the parameters of the exine. These parameters may be: LA, LPm, L, W, LSI, ECD and Lm. We can infer from these findings that through exine, these wild populations have exhibited an adaptation to the conditions of their native environment.

This leads us to think that the variability of LD is only the result of variation in the LA of the lumina, and therefore both parameters reflect the same adaptive means to a particular abiotic parameter such as a lack of water or indirectly as a temperature increase provoking a demand for water.

Conclusions

The results showed high inter-populations variability. Our observations suggest that, the correlations connecting the exine parameters to each other on one side and to those of pollen on the other; may lead one to think without causation that changes in the size and shape of pollen could be a mechanism for adapting pollen to different environments. It seems that exine structure is of importance for resilience to aridity, by decreasing the LA of the exine and thereby decreasing the size and shape of the whole pollen. From these results it can be deduced that pollen micro-morphological parameters and exine are good markers and their variation may have a genetic origin, suggesting a genetic adaptation that must be evaluated and highlighted. The exploitation of the olive grove as a crop wild relative can supplement the olive tree, by enhancing its characteristics and enabling it to conquer other areas, in particular for varieties that are less adapted to certain geo-climatic conditions and whose economic benefits are not negligible.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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