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Research Article

Intraspecific polymorphism of pollen grains in *Artemisia herba Alba* Asso in the Tiaret region (West of Algeria)

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Abstract

This study allowed us to determine the appearance and pollen apertures of 200 individuals of *Atrémesia herba alba* from three areas in Algeria. The pollen morphology determined at the three stations studied is Tricolorpé, of which two grain shapes were encountered, spherical and elongated with three apertures and distinct diameters. Results obtained from pollen diameter measurements revealed a significant effect, which confirms a very marked intra- and inter-population variability of this character. Indeed, the spherical pollen diameters vary between 13.20 and 20 µm (Ain Dheb area) and between 16.56 and 29.90 µm in individuals from the two Chellala areas. The elongated pollen diameters from the Chellala region have diameters fluctuating between 34.31/25.46 to 29.90/16.56 µm and have diameters between 19/14.81 and 28.55 /18.54 µm for the Ain Dheb area.

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1. Introduction

Pollen is of great interest because It is a characteristic of the species that spawned it (Diot, 2000), but also because it presents for the plant one of the elements that are very fossilizable and resistant .It is also considered as an identity card,because " we can identify a plant simply by observing its pollen " (Roland & Claude, 1989). The pollen databases that have been set up to facilitate these reconstructions. Palynology applied to archaeology (paleopalynology) studies fossilized pollens and spores trapped in successive layers of sediments, in order to trace variations in the plant environment over very long periods of time Palynology concerns many different applications: Geology, Medicine, Ecology, Agronomy. Study of pollen morphology plays an important role as taxonomic tool in the accurate identification of plants species (Ullah et al., 2018). In the field of environment and health, anautomatic pollen analysis system is used, based on the use of digital images. Identifying an appropriate proxy for the reconstruction of paleoclimate and paleoenvironments is a major scientific challenge (Tierney et al., 2020; McClelland et al., 2021). The study of pollen morphology is very important in taxonomic studies, as it shows the phylogenetic relationships between genera, subfamilies and tribes (de Abreu et al., 2015). The interest of the study of pollen lies according to Doit (2000) in the sense that the morphological characters of pollen grains are specific to each taxon. Wodehouse (1928) also agrees by pointing out that in some families, such as Poaceae and Brassicales, the pollen grains of all species are very similar. To identify, differentiate and classify complex taxa, it is necessary to conduct studies of various characteristics of pollen grains as a preliminary step.

The diverse and severe selection forces that the diploid plant is exposed to typically do not have as much of an impact on pollen. Pollen traits can be conservative and have taxonomic significance since they may not change over millions of years due to selective pressures (such as temperature and precipitation) on pollen attributes being mostly absent or negligible in comparison to those on the diploid plant (Grímsson et al., 2014, 2016, 2017a, b).

Among taxonomic societies, it is generally acknowledged that the identification and classification of plants based on palynological characteristics is more accurate. Therefore, in order to generate proper plant names, taxonomists and researchers take into account various plant properties (Stephen, 2014). In terms of exine sculpting, size, shape, and other attributes, pollen grains exhibit significant morphological variety. (Umber et al., 2022).

In vegetation regeneration, Artemisia pollen is a crucial component and a valuable biological indicator due to its high yield, geographical and temporal extent, ease of identification, and homogeneity under light microscopy (LM). Environmental evaluations based on pollen have been effectively completed by the animal. While some researchers believe that there is little correlation between relative Artemisia pollen and humidity, others view it as an indicator of aridity (Cai et al., 2019; Cui et al., 2019; Wu et al., 2020; Cao et al., 2021) (Koutsodendris et al., 2019; Lu et al., 2020; Zhao et al., 2021). Determining if distinct Artemisia pollen species reflect various settings is crucial.

Since Wodehouse (1928), researchers have been examining the morphology of Artemisia pollen. Aspects of Artemisia pollen morphology, including as pollen structure, size and form, exine sculpture types, and aperture dimensions, were disclosed by later researchers like Jiang et al. (2005). A hierarchical classification analysis on pollen morphological traits was performed to subdivide Artemisia pollen into three types. By plotting these on global terrestrial biomes, it was found that the different types of Artemisia pollen grains had diverse habitat distributions. (Lu et al., 2022).

Some species of flowering plants produce several types of pollen. If these pollens are produced by different individuals, we speak of polymorphism. This is particularly the case in species that practice heterostyly (Mignot et al., 1994). A study of 186 species of Eudicotyledons showed that a third of the species studied were heteromorphic concerning pollen (Mignot et al., 1994).

Heteromorphism has also been documented in certain species of Monocotyledons, for example in the Haemodoraceae family (Pierce & Simpson, 2009).

Palynomorphological investigation using diverse microscopic techniques is conducted as an aid to the morphological studies and providing; significant tool for modern plant taxonomist for species delimitation (Ragho, 2020). Scanning electron microscopy (SEM) has been used for the dissimilarity of species on the basis of exine ornamentation (Khan, et al., 2021a; Khan, et al., 2021b). In this modern era recently use of SEM has developed essential research techniques in the micro morphological studies of pollen grains (Bahadur et al., 2018).

Pollen apertural polymorphism is used as a model for the study of evolutionary mechanisms that have led to the great diversity of pollen morphology. Variation in the number of apertures was mainly presented as the coexistence of different pollen types in all plants (heteromorphism), while genetic polymorphism is related to heterostyly. Sporophytic ploidy increases the average number of high apertures. However, by shifting the mode towards high aperture numbers. However, the different types of pollen found in the same individual do not correspond to reduced or unreduced gametophytes (Mignot et al., 1994). The present work consists of the study of intraspecific polymorphism of Artemisia herba alba pollen grains from three areas belonging west of Algeria.

2. Materials and methods

2.1. Areas study location

The two study sites 1 and 2 are located in the Chellala region which is located in the eastern part of the wilaya of TIARET. It is limited to the north and east by the commune of Rechaiga, to the south by Serguine and to the west by the wilaya of Djelfa. It is located 116 km from the head of the wilaya of Tiaret (Figure 1). The third study site is located at the level of the Daïra of Ain Dheb. The latter is located to the south of the Wilaya of Tiaret on 64 km.

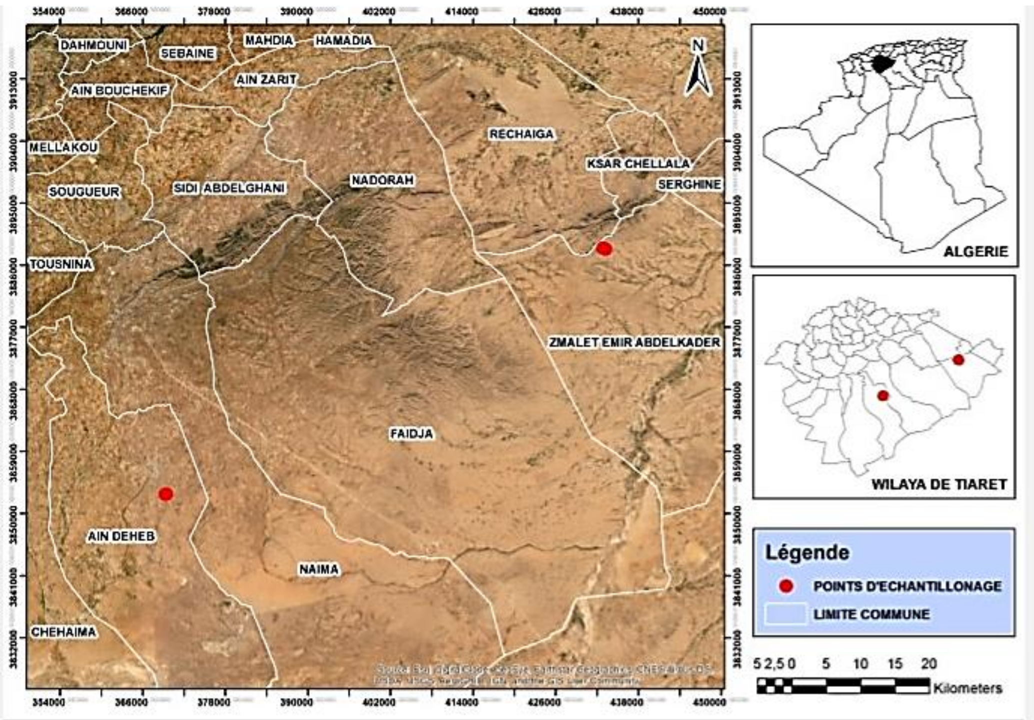

Figure 1. Geographic location map of the study sites (Tiaret Forestry Department)

The Altitudinal, Latitudinal and Longitudinal coordinates are shown in Table 1.

Table 1. Geographic coordinates of three study sites

Population	Latitude	Longitude	Altitude
Area1	433231 UTM	3888574 UTM	790 m
Area 2	432813 UTM	3888770 UTM	797 m
Area 3. Population1	34 ,81154 UTM	1,57258 UTM	1010 m
Area 3. Population2	34,81145 UTM	1,57077 UTM	1017 m

2.2. Climatic, edaphic and topographical characteristics

The climate of Ksar-chellala region is of the continental type with rains concentrated in the winter period, with a drought period of nine months, extending from March to November.

The average rainfall recorded for this region is 269 mm/year. In this area, there are several types of soils, the dominance of which is that of limestone soils characteristic of steppe regions, we also note the presence of hard dolomite soils and friable limestone and clay.The region of Ain dheb belongs to the lower semi-arid bioclimatic stage with cool winters with an average precipitation of 330.21mm / year. , The dry period is five months from May to October.

The Ain Deheb region is characterized by a mountainous relief with an average altitude of 1100 meters above sea level. The region is bordered to the east by the Djebel Amour mountains and to the west by the Djebel Babor mountains. The soils of the studied region are mainly clayey and loamy soils, with average fertility. According to a recent study, the soils of the region are characterized by a low organic matter content and high acidity.

Figure 2. Population of A. herba alba (ksar chellala area)

Sample collection this operation consists of harvesting the stems from each individual (Clump of *A. herba alba*) bearing inflorescences. it was mid-december accompanying the flowering period. thirty individuals were collected from each area.

Figure 3. Population of A. herba alba (Ain Dheb area)

2.3. Preparation of reference slides

Our experimental work was carried out at the plant protection laboratory of the Faculty of Natural and Life Sciences (University of Ibn Khaldoun of Tiaret). The plants were picked before their flower buds opened in order to conduct the reference pollen collection. To prevent anemophilous allopollens from floating in the atmosphere or pollen being carried back by insects, the hatching was carried out in a lab. The ripe anthers are either put in a watch glass or straight onto a slide. The pollen is released from the anthers that contain it when drops of chloroform are introduced. The preparation is encircled by Canada balsam shortly after the solvent has evaporated and the remnants of anthers have been removed. To preserve the true color of the pollen and its natural appearance, this preparation was made without coloring. Then, a label was stuck on the edges of the slide. The label mentions the number of the individuals, the population and the study area (Figure 4).

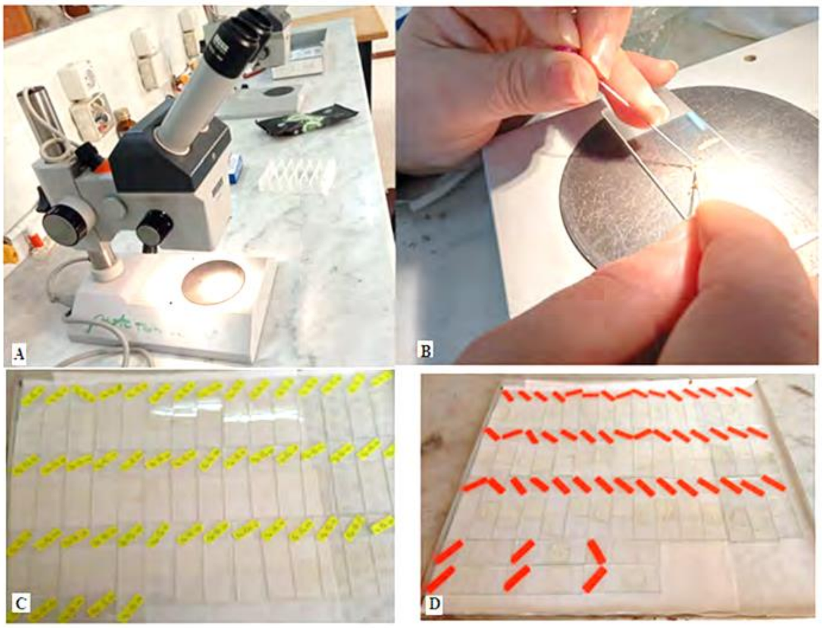

Figure 4. A: Binocular loupe; B: Deposit of pollen grains on the slide; C and D: Prepared slides with labels

2.4. Photography and measurements

The taking of photographs of pollen grains as well as the measurements of their diameters were made using a microscope with a camera model OPTICA (Figure 5) at the ecology laboratory of the SNV Faculty (Ibn Khaldou University of Tiaret). The measurements of the diameters of the pollen grains were taken in pixels and then converted into micrometers (um). The statistical analysis of the results obtained from different measurements carried out is the analysis of variance with Statistica 2008 software.

Figure 5. A: Optical microscope with camera model OPTICA; B: Portable microcomputer

3. Results and discussion

3.1. Identification of pollen aspects

Finding the pollen's characteristics (apertures and shapes) We were able to identify a form of Tricolporate pollen in the two areas under investigation by observing pollen grains under optical microscope. This aspect of pollen has either a spherical shape or elongated with different diameters. The apertures observed at the level of the pollen grains are either three colps or three pores. (Figure 7). Thus, Hayat et al. (2023) used optical and scanning electron microscopy technique to study the palynological characteristics of 19 species belonging to 15 genera of the family Asteraceae in Pakistan. The studied species produced pollen in various forms including spheroidal, prolate and subprolate. Trizoncolporate, Tricolporate and Tetracolporate are the three types of pollen apertures observed in the examined species. Using a scanning electron microscope (SEM), Hussain et al. (2019) investigated the pollen morphology of 15 species from the Gilgit-Baltistan area of Pakistan that belong to the genus Artemisia of the family Asteraceae. The findings showed that, with rare exceptions, the pollen grains of Artemisia species were tricolporate in shape and exhibited globular symmetry, which is defined by an ellipsoidal ball-shaped equatorial side and a three-lobed round-shaped polar face.

Pollen grains, from all observed taxa, are tricolporate, with globular symmetry (round with 3 lobes in polar view and ellipsoidal ball-shaped in equatorial view) and bear small spinules on their surface. Thus, the work of Maghni et al. (2020) found that in dicotyledons, three-apertured pollen is the primary morphology displayed by Rosidae and Asteridae, and triporate pollen is more frequently seen than tricolporate pollen. According to measurements made by Akinnubi et al. (2014), the pollen grains of the species they studied are spheroidal in shape, in the group media and minuta, eitherechinate or spinate, yellow to yellowish brown, polypentaporate, tricolporate, acolpate, and polycolporate. The spines are short and have thin walls, and the pores are densely arranged. While the interspecific differences in structures support their existence as separate species, the similarities in structure demonstrated interspecies interactions and reasons for belonging to the same family.

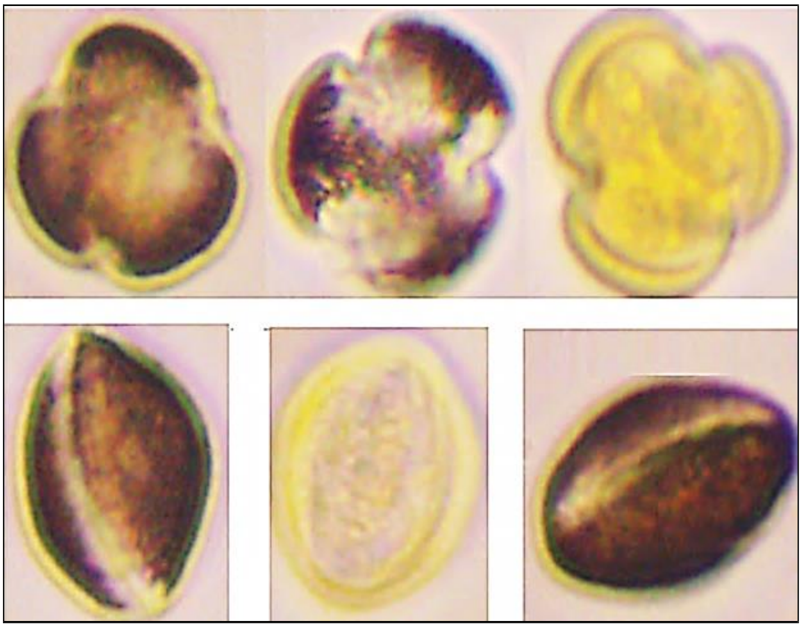

Figure 6. Pollen grains of Artémisia herba alba Asso from Area 1 (Ksar-Chellala)

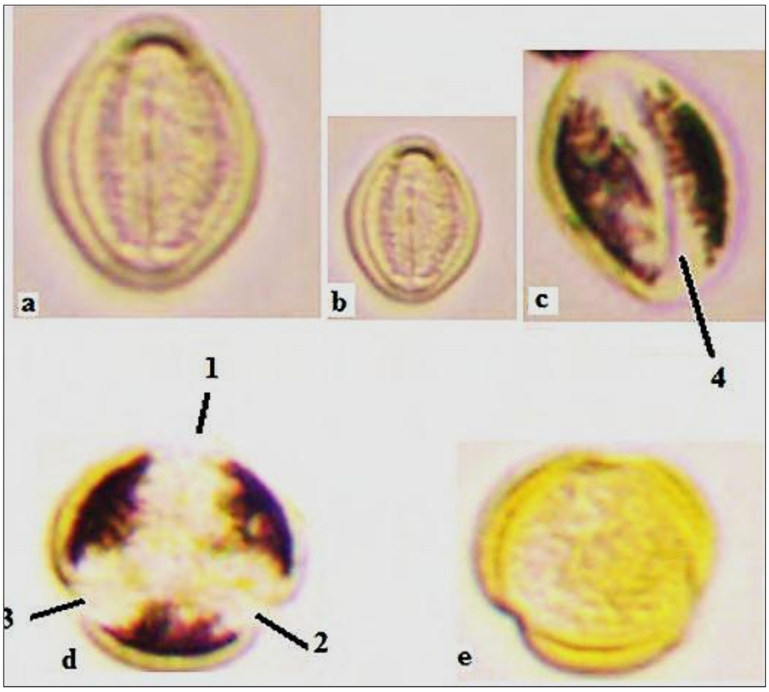

Figure 7. Pollen grains of Artemisia herba alba Asso from Area 2(Ksar-Chellala). a,b,c: Elongated shape; d,e: Spherical shape;1,2,3,4: Colpes

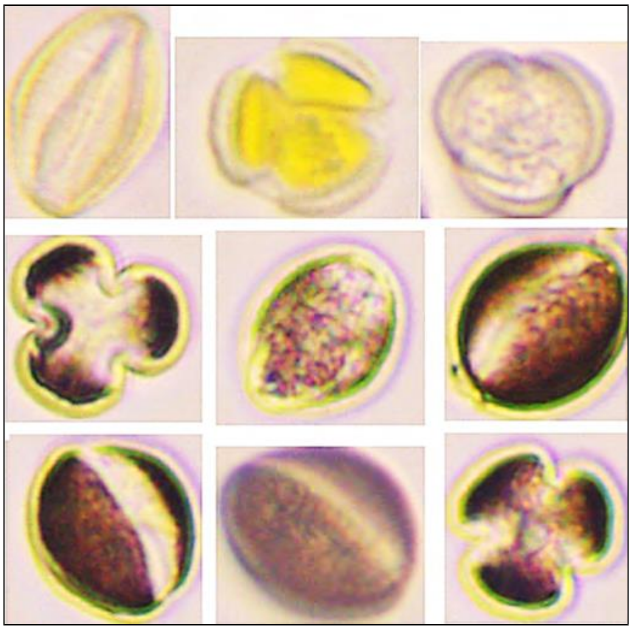

Figure 8. Pollen grains of Artemisia herba alba Asso from population 1 of Area (Ain Dheb)

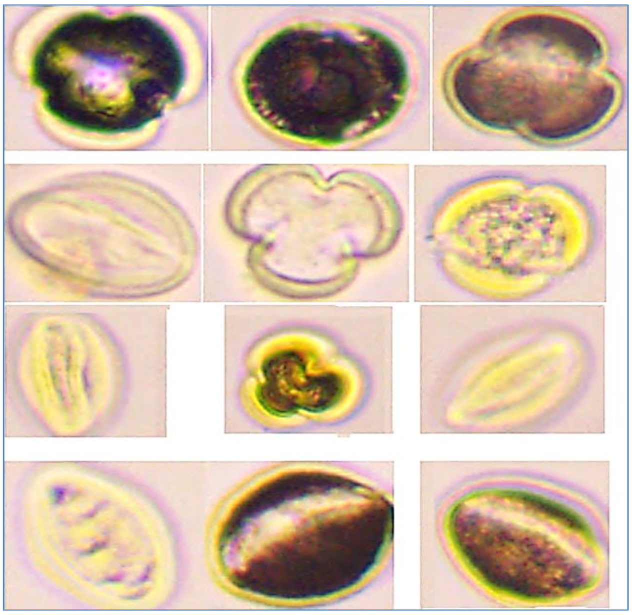

Figure 9. Pollen grains of Artemisia herba alba Asso from population 2 of Area (Ain Dheb)

According to the palynological study of Muhammad et al., (2010) carried out using optical and scanning electron microscopy on 22 taxa of the genus Artemisia. All of the taxa that have been observed have tricolored pollen grains with globular symmetry, which is rounded with tree lobes in polar view and ellipsoidal in equatorial view. Pollen form, spinule arrangement, exine sculpture, spinule base, polar length, equatorial breadth, exine thickness, and colpus width are the micro-morphological characteristics of Artemisia pollen grains that were recognized and categorized during the investigation. Five groups of Artemisia were identified in the resultant cluster. According to this finding, pollen morphology is a useful taxonomic identifier for the genus Artemisia's infrageneric classification.

According to LM and SEM measurements, the pollen grains' morphology is uniform across the species, confirming its monophyly as determined by Torrell et al. (1999) and Watson et al. (2002) molecular research. The overall characteristics of Artemisia pollen exhibit a very noticeable concordance, with globular or approximate symmetry, three-lobed spheres in equatorial view, and ellipsoidal in polar view with a tricolporate structure similar to the montioner Jiang (2005). Plesiomorphic (primitive) characteristics of Artemisia pollen include globular pollen form, dense spinule arrangement, granular exine sculpting, wide spinule base, large pollen size, wide colpus breadth, and thick exine. These characteristics have changed in apomorphic (derived) conditions, including flattened pollen shape, reduced exine thickness, thin colpus, tiny pollen volume, loss of spinule organization, and sinuate exine sculpture (without granules). Pollination patterns, such as the shift from entomophily to anemophily, are one factor contributing to this evolution. The shift of climatic regimes during the glacial age, The other primary cause of this evolution is the transition of the north temperate zone from high latitude to low latitude humid regions and low altitude evaluation (Jiang et al., 2005). There therefore seems to be a trade-off between survival and reproduction, and producing several types of pollen grains would allow several reproductive strategies to be combined. The case of another species of violet, Viola calcarata, is enlightening on this subject: this species produces pollen with four or five apertures, the pollen with four apertures surviving longer than the pollen with five apertures (which however germinates more quickly (Till-Bottraud et al., 1999).

The proportions of these two morphs vary with altitude, with pollen with four apertures being more frequent at high altitude than at low altitude (and vice versa for pollen with five apertures). This observation may be related to the fact that pollinators are rarer at high altitude, where it may be advantageous to produce pollen that survives for a long time to compensate for the vagaries of pollination; on the other hand, in an environment where pollinators are numerous, and pollination is less uncertain, it may be advantageous to produce pollen that germinates quickly, and which will have a competitive advantage over its competitors. These observations are consistent with game-theoretic models, which show that heteromorphism can be an evolutionarily stable strategy under a number of conditions (Till-Bottraud et al., 2001). Producing multiple types of pollen, each type adapted to particular environmental conditions, could be advantageous in the long term, and explain the high rate of heteromorphism in flowering plants (Mignot et al., 1994).

Table 2. Analysis of variance of the frequency of appearance of various pollen types for all the individuals studied from three Areas.

Analysis of Variance (P123FSTAT) Marked effects are significant at p < ,05000							
SS - Effect	df - Effect	MS – Effect	SS - Error	df - Error	MS - Error	F	p
2,625000	2	1,312500	27,07500	117	0,231410	5,671745	0,04457

3.2. Frequency of expression of pollen forms in the species studied

The statistical study by analysis of variance gave a significant effect at a $p < 0.05$ ($p=0.04457$) (Table 2). This indicates that the two forms of pollen are distributed in a heterogeneous manner and with different proportions at the scale of each site studied (Figure 9). We found that the spherical form is expressed in the majority of individuals of site 3 (79%) and it is dominant at site 1 (65%), while 62% of individuals of *A. herba alba* of Area 2 carry the elongated form of pollen grains (Figure 9). The similarity of the frequencies of appearance of the spherical form at the two sites 1 and 3 despite the difference in the ecological characteristics of the two geographical areas reduces the influence of the environment on this character's expression of pollen grains.

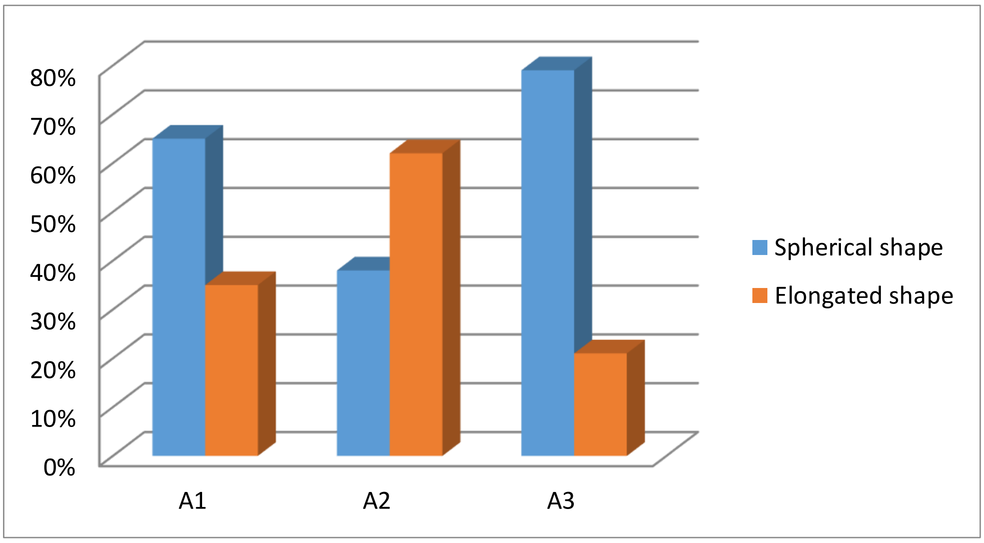

Figure 10. Frequency of appearance of different forms of pollen of A. herba alba at each area; A1: Area 1 (Ksar Chellala); A2; Area 2 (Ksar Chellala); A3: Area 3 (Ain Dheb)

3.3. Pollen diameters

The polar axis, denoted by P, connects the two poles, whereas the equatorial axis, denoted by E, is perpendicular to the polar axis and divides the pollen into two hemispheres. The configuration of the apertures on the single grains marks these two axes. The ANOVA of frequencies of appearance of different pollen diameters for every *individuals* analyzed from three locations gave a significant effect (p = 0.01294) (Table 3), which explains a variety of different pollen sizes at the three sites studied. The values obtained in pixels are multiplied by the microscopic coefficient 0.13.

Table 3. Analysis of variance of frequencies of appearance of different pollen diameters for all the individuals studied from three Areas

Analysis of Variance (P123FSTAT) Marked effects are significant at p < ,05000							
SS - Effect	df - Effect	MS - Effect	SS – Error	df – Error	MS - Error	F	p
283,8250	49	5,792347	227,1667	70	3,245238	1,784876	0,012941

The diameters of the spherical pollen fluctuate between 16.56 µm and 29.90 µm in *A. herba alba* individuals from the Area of Ksar Chellala and between 13.20µm and 20µm in Ain Dheb Area. The diameters of elongated pollen from region 1 have diameters fluctuating between 34.31/25.46 to 29.90/16.56 µm and have diameters between 19/14.81 and 28.55 /18.54 µm for region 2 We found that the largest pollen grain is found at Area 1 of Ksar Chellala with a diameter of 34.31 µm (figure 6). On the other hand, the smallest is the one manifested in individuals from area 3 (population 2) with a diameter of 13.20 µm (Figure 9). The equatorial diameter of Artemisia pollen grains in Turkey ranges from 16.48 to 27.47 µm, while the polar axis is between 15.23 and 27.20 µm. The grains are radially symmetrical, isopolar, oblate-spheroidal, and prolate-spheroidal (Kursat et al., 2023). The pollen grains range in size from tiny to medium when compared to the study conducted by Ghahreman et al. (2007) on other species of the genus Artemisia that were obtained from specimens at the Central Herbarium of Tehran University. The polar and equatorial axes of the smallest grains (*A. scoparia* and *A. turanica*) are 22.26 and 22.57 µm, respectively, and 12.8 and 13.16 µm. The largest grains (*A. vulgaris, A. absinthium*) have equatorial axes of 19.58 and 17.25 µm and polar axes of 34.68 and 32.84 µm.

According to Halbritte et al. (2018). The size of pollen ranges from less than 10 µm to over 100 µm. The biggest diameter is used to show the size of the pollen. (Hesse et al. 2009). The degree of hydration and the method of preparation determine the size. A variety of pollen size classifications is advised due to this and natural variation: very small (<10 µm), small (10–25 µm), medium (26–50 µm), big (51–100 µm), and very large (>100 µm) (Halbritte et al., 2018).

These variations reveal a very significant inter-population and inter-site polymorphism for the diameter of *A. herba alba* pollen grains. The intra-specific variability of pollen observed in this study consolidates the other types of variability confirmed in other studies of which they gave interpretations to explain the possible origins of this variability. Maghni et al. (2017) stated that genetic diversity is primarily responsible for the emergence of phenotypic variability in individuals of the same population (intra-population variability) exposed to the same edaphic conditions, and that the expression of morphological characters is still subject to the simultaneous action of the genotype and the environmental environment. Therefore, the spontaneous mutations affecting this species would largely account for the morphological diversity shown within our research locations and thus validated by our genetic analysis on the same species from the same site (Maghni et al., 2016). The anatomical polymorphism of A. herba alba seen at various bioclimatic levels in Algeria was explained by the same observations (Maghni et al., 2018). The stasis in pollen development in Eudicotyledons was due to stabilizing selection, indicating that the dominance of tricolpate pollen is likely due to selective processes rather than developmental constraints. The study of the development of the other major angiosperm clade, the Monocotyledons, may indicate that the dominance of monosulcate pollen in this group is also due to selection (Charlotte, 2015).

4. Conclusion

Examining the pollen grain morphology of each species of A. herba alba under study showed that there was a significant intraspecific morphological diversity, as evidenced by the emergence of two distinct pollen external appearances, spherical or elongated and apertures in the form of colps (furrows) and pores whose number is three for the two apertures. So, the pollen encountered is of the Tricolporate type. This study deserves to be carried out on other ecotypes of *A. herba alba* from different bioclimatic levels, including the possibility of having an interspecific polymorphism of the morphology of its pollen, because this species is characterized by a very pronounced intraspecific genetic variability. It will be more practical to determine the three-dimensional shape of the pollen using the electron microscope.

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Author’s declaration and contribution

The authors show no conflict of interest. All authors contributed to the study's conception and design. Material preparation, data collection and analysis.

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