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Original Research Article

Micromorphological Study of Some Species of the Genus Papaver of Papaveraceae Family Growing Wildly in Middle and Northern Iraq

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Abstract: This research included a comprehensive (micro-phenotypic) pollen study that included 8 wild plant species are (*P.bornmuelleri, P.curviscapum, P.polychaetum, P.tauricola, P.pavoninum, P.rhoeas var. himerense, P.rhoeas var. strigosum and P.setigerum*) belonging to the genus *Papaver* of the Papaveraceae family growing in middle and northern Iraq. The samples were collected during 2023-2024 growing season. Approximately 17 field tours were carried out, including the central and northern governorates, in addition to a comprehensive survey of preserved herbarium samples in the Iraqi National Herbarium and other universities Herbarium for detection. Pollen grains were examined for the arrangement of species using a Light Microscope (LM) and Scanning Electron Microscope (SEM). It was found that the all of pollen grains were of the tricolpate type, and the species were classified based on the number of apertures and colpi and pollen grain shape, and its size varied between small and medium size (*P.curviscapum, P.rhoeas var. himerense, P.rhoeas var. strigosum* were medium size) and (*P.bornmuelleri, P.polychaetum, P.tauricola, P.pavoninum* and *P.setigerum were small size*), in addition to studying the surface ornamentation of the external grain wall (exine ornamentation).

Keywords: Papaveraceae ،SEM ،*Papaver* ،Micromorphological Studies.

INTRODUCTION

Palynology an important tool for the Ecologists, Palaeologist, medical scientists, and agriculturalists for discovering historic ecosystems. Thus, this science is considered one of the important and applied sciences (Abdulmajeed *et al.,* 2023). (Hyde and Williams, 1945) were the first to use the term Palynology, which means pollen grains and spores. The taxonomic and evolutionary importance of the external appearance of pollen grains may be at the species or genus or higher taxonomic rank than that (Payne, 1972). (Erdtman, 1943) stated that studying the cross-section of pollen grains is no less important than the study of micromorphological characteristics such as ornamentation, presence of spinulose, colpi, porate, the size of the pollen grain, and the structure of its wall.

Because it provides more evidences and information, especially after the invention of the (SEM) and the Transmission Electron Microscope (TEM) Using this information to study the micromorphological characteristics of pollen grains, which enables the detection and isolation of species and genera (Abdulmajeed, *et al.,* 2022). (Zhang *et al.,* 2020) show that morphological principles can be used to conduct quantitative analyses and estimate system parameters of anatomical structures. Thus, this science has contributed to solving complex taxonomic problems. The taxonomic order Taxon is called Stenopalynous if its pollen grains have fixed characteristics, which may be limited to that plant group only (Divis and Heywood, 1973). (Van Aenst and Van Went, 1974) reported that the pollen of the poppy family in the polar view is Tricolpate. In contrast. (Candao and Fernandez, 1985) divided the poppy family into four sections according to the shape and number of pollen grain porates.

(Abou El-Naga, 1982) studied the Papaveraceae in Egypt and gave a detailed report in his study of pollen grains, also the anatomical aspect of the leaf parts, biological cells, and stomatal complexes. He used the LM & SEM and he

described a classification key for the genera based on the pollen grains morphology. (Romero *et al.,* 2003) studied the development of the pollen grains wall of *Hypecoum imberbe* employ (TEM) and mentioned the number of porate in this species. (Fazal *et al*., 2013) reported that the properties of pollen grains have important taxonomic value in solving the confusing problems of mutual relationships between different taxonomic orders and estimating their taxonomic status, especially families, genera, species, and subspecies. (Alquran, 2010) he studied the pollen grains of 3 species of Papaver sp. (*P. polytricum*, *P. rhoeas*, *P. hybridum*) in Jordan, he noted that the shapes of the pollen grains were elliptic in the tropical view, and he made a comparison between these species according to the type of porate and apertures.

In Iran, (Keshavarzi *et al.,* 2011) studied five species of *Papaver* L. phenotypically by using a (SEM) and showed that pollen grain has tricolpate and the surface ornamentation is verrucate-psilate. (Hanif *et al.,* 2013) indicated in their study of pollen grains of *Papaver somniferum* employ LM, the type of pollen grain as being tricoplate and the shape ranged between spheroidal, prolate, or subprolate. In another study (Al-Mousawi, 2015) described seven types of pollen grain of Papaveraceae using LM and SEM and emphasized the importance of pollen grains in separating genera and species based on the shapes of their porate. This study aimed to a conduct a comprehensive (micro-phenotypic) pollen study that included 8 wild plant species are (*P.bornmuelleri, P.curviscapum, P.polychaetum, P.tauricola, P.pavoninum, P.rhoeas var. himerense, P.rhoeas var. strigosum and P.setigerum*) belonging to the genus *Papaver* of the Papaveraceae family growing in middle and northern Iraq which is not previously studied in the Iraqi plant encyclopaedia.

MATERIAL AND METHODS

Field Visits

To accomplish the various aspects of this study, fresh plant samples were collected directly from the field and within the study governorates, which were accessible to most orders of the poppy family. Surveys were conducted for genera and plant species for more than 17 field tours in various locations in central and northern Iraq governorates from mid-February 2022 until the end of May 2023.

Table 1: Natural regions and districts targeted for this study in central and northern Iraq (Guest, 1966).

(M) Mountain Range	
MAM	Amedia district
MRO	Rawandiz district
MSU	Sulaymaniyah district
MJS	Sinjar Mountains district
(F) Alluvial and Upland Plain	
FUJ	Upland Plain district
FNI	Ninawa district
FAR	Erbil district
FKI	Kirkuk district
FPF	Eastern Border highlands district

Mature flower buds collected directly from the field during field visits were fixed with Carnoy solution (Ethyl alcohol: Glacial acetic acid 3:1 v /v) for 24 hours, then it was washed with 70% ethyl alcohol and stored with the same concentration of alcohol in the refrigerator until further use.

Preparing Samples for Study in Light Microscope (LM)

The phenotypic study of pollen grains was based on fresh samples. In this study, the acetolysis method referred to by (Wilson and Goodman, 1964) was used with some modifications. Glycerine gel was ready according to the method of (Sass, 1958), 5 g of Gelatine dissolved in 30 ml of distilled water and 0.5 g of phenol dissolved in 35 ml of Glycerine.

Several anthers of small flowers and large buds preserved in 70% ethyl alcohol, were placed inside a watch glass bottle containing drops of water. The anthers were crushed using fine dissecting needles to release the pollen. The watch bottle was placed on a hot plate at 60°C until the water dries. 3-4 drops of acetolysis solution prepared from HCL: H2SO4 (9:1 v/v). The pollen grains were covered with the solution and gently heated on a hot plate at 70°C until the colour of the solution became brown. Add a few drops of 100% absolute ethyl alcohol to the hot mixture. Note that the solution is displaced towards the edge of the watch bottle and dried by wiping it with towel paper. Take a few drops of the solution containing the pollen with a clean dropper (use one for each sample). A drop of glycerine jelly (previously prepared) was added to the slide, covered with a slide cover, and the pollen grains were photographed using an NSZ-606 camera, Light Microscope (LM) mounted under the lens, with (40 x) magnification. Measurements were taken of the Polar view and the Equatorial view of the pollen grains, the P/E value was calculated, and the wall thickness of the grain was also measured using the Ocullar micrometre. It also described the shapes of pollen grains for the polar and tropical views and arranged the data in a table. In addition, the superficies ornamentation of pollen grains to explain the variations in the Polar and

Equatorial axis of species ranks studied for each species, at a rate of 10-16 pollen grains per sample, and the number of samples studied for each type of genus ranged between 5-8 samples depending on distribution place. The terms mentioned in have been adopted (Erdtman, 1969; Radford *et al.,* 1974).

Preparing Samples for Study in Scanning Electron Microscope (SEM)

In this method, pollen grains stored in 2 ml Eppendorf tubes containing 70% alcohol are used and prepared in the same way as they were prepared for light microscopy. Then, the pollen was withdrawn using Micropipettes and placed on a special disc Stup investigation after drying at room temperature. The disc was covered with double-sided adhesive tape. The samples were plated with gold using an ion sputter coating device. Then, the discs were transferred to an Inspect S50 scanning electron microscope, and the samples were photographed. This part of the research was carried out in the central service laboratory, university of Kufa/College of Science. In addition, most of the samples were sent to neighbouring countries, Iran and Jordan for investigation.

THE RESULTS AND DISCUSSION

Palynological Study

The study of variation in the phenotypic characteristics of pollen grains is considered one of the micromorphological characteristics and has a very high taxonomic value. The size, shape, and presence of spinulose, colpi, porate, and the type of surface ornamentation have provided a huge amount of important taxonomic information and evidences.

In the study of the pollen grains for the *Papaver* genus, it became clear that they were monads, regular, and Isopolar, in which the polar and tropical views could be distinguished, containing apertures and furrows for germination. This study included the following aspects:

Type

Pollen grains study of the species of the genus *Papaver*, the results of quantitative and qualitative characteristics and the use of LM and the S.E.M showed that all tricolpate species contain three germination pores.

Shape Pollen Grains

Most of the shapes of the pollen grain under study are classified into several shapes depending on the value of the ratio between the polar view and equatorial view (P/E), as illustrated in image (1) and Table (3). Shape of pollen grains have been determined as follows:

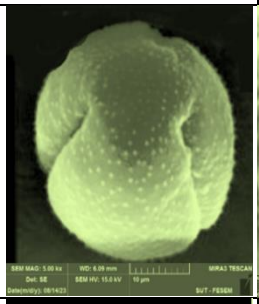
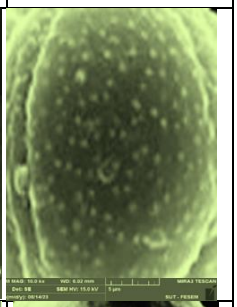
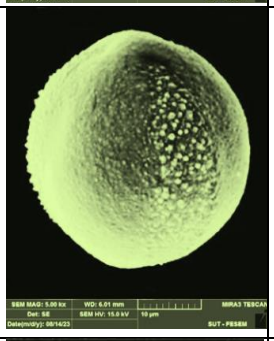
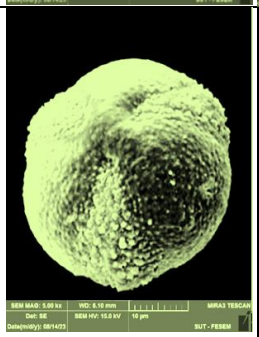
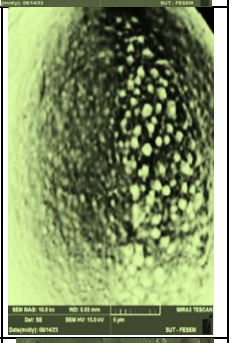
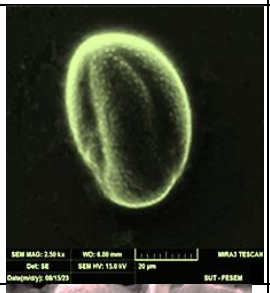
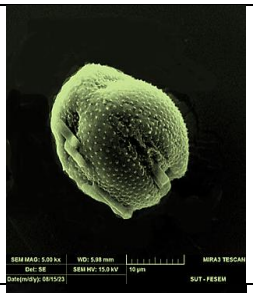
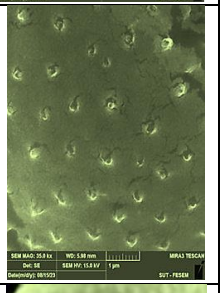
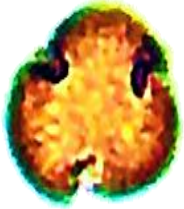
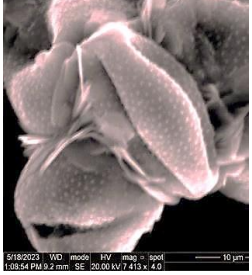
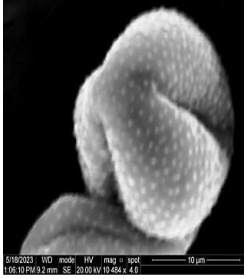
- Spheroidal: *P. bornmuelleri, P. curviscapum*
- Spheroidal – Subprolate: *P. tauricola*
- Prolate Spheroidal: *P. rhoeas* var. *himerense, P. setigerum*
- Oblate spheroidal: *P. rhoeas* var. *strigosum.*
- Subspheroidal: *P. pavoninum*
- Subspheroidal-Subprolate: *P. polychaetum*

Table 2: Quantitative characteristics of pollen grains of genera in the *Papaver* (measured in micrometres)

No.	Species	Polar axis	Equatorial axis	E/P	Pollen grain size	Wall thickness		Aperture dimensions	
						Entine	Extine	length	width
1	P. *bornmuelleri*	(20-16) 18	(19-14) 16	S	(1-0.6) 0.75	(1.3-1) 1.24	(2.4-1.3) 1.8	(8-5) 6	(16.6-13)13.5
2	P. *curviscapum*	(40-35) 37	(34-27) 30	M	(1.77-1.1) 1.41	(1.56-1) 1.30	(1.92-1.4) 1.71	(17-12) 14	(18-13) 15
3	P. *polychaetum*	(26-21) 22	(25-17) 21	S	(1.1-0.60) 0.80	(1.19-0.80) 1.0	(1.30-1) 1.11	(16-10) 13.6	(9-5) 7
4	P. *tauricola*	(19.2-14) 17	(22.5-19) 20.5	S	(1.37-1.2) 1.29	(1.25-1) 1.11	(0.95-0.7) 0.83	(17-13) 15	(11-7) 9
5	P. *pavoninum*	(27.5-21) 23.16	(28-19) 23	S	(1.8-1.36) 1.52	(1.38-1) 1.21	(1.40-1.3) 1.36	(19-12) 16	(11-6) 8
6	P. *rhoeas* var. *himerense*	(34-27) 30	(30-23) 28	M	(1.37-1.26) 1.3	(1.79-1.66) 1.72	(1.69-1.5) 1.58	(22-15) 19	(23-17) 20.33
7	P. *rhoeas* var. *strigosum*	(27.5-24) 25.5	(28.5-24) 25.5	M	(1.10-0.75) 0.95	(1-0.5) 0.76	(1.34-1.25) 1.29	(25-19.5) 22.5	(21-15) 18
8	P. *setigerum*	(26-20) 23	(30-19) 25	S	(1.4-1.1) 1.25	(1.44-1) 1.26	(1.60-1.44) 1.51	(20-18) 19	(18-14) 16

Table 3: Quantitative and qualitative characteristics of pollen of genera orders of the *Papaver* (measured in micrometres)

No.	Species	Colpate Length	Colpate Width	Mesocolpium average	Porate & Colpate count	Pollen Shape	Ornamentation
1	P. *bornmuelleri*	(18-12) 14	(9.6-8) 8.74	15.6	3 Colpate	Spheroidal	Verrucate
2	P. *curviscapum*	(19.4-14.1) 16.18	(10.22-7.3) 8.82	11.2	3 Colpate	Spheroidal	Verrucate
3	P. *polychaetum*	(17.8-12.2) 15	(9.3-6.8) 8.03	9.3	3 Colpate	Subspheroidal-Subprolate	Verrucate
4	P. *tauricola*	(22-15) 18.66	(12.7-8) 10.06	14	3 Colpate	Spheroidal - SubProlate	Verrucate
5	P. *pavoninum*	(13.5-11.58) 12.36	(7.2-6.1) 6.7	8.5	3 Colpate	Subspheroidal	Verrucate-Echinate
6	P. *rhoeas* var. *himerense*	(20.66-17.1) 18.58	(10.35-8.7) 9.38	14.8	3 Colpate	Prolate spheriodal	Verrucate
7	P. *rhoeas* var. *strigosum*	(20.90-17.33) 18.74	(9.18-7) 8.06	14.3	3 Colpate	Oblate spheroidal	Verrucate-Echinate
8	P. *setigerum*	(21.73-17) 19.07	(11.25-8) 9.5	13.21	3 Colpate	Prolate spheriodal	Verrucate

Taxa	Equatorial view LM	Polar view LM	Equatorial view SEM	Polar view SEM	Ornamentation SEM
P. bornmuelleri					
P. curviscapum					
P. polychaetum					
P. tauricola					

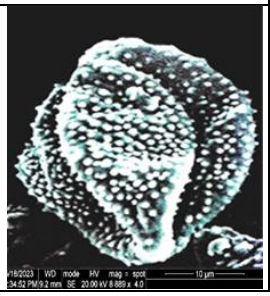
Taxa	Equatorial view LM	Polar view LM	Equatorial view SEM	Polar view SEM	Ornamentation SEM
P. pavoninum					

Fig. 1: LM & SEM micrographs of pollen grains in Papveraceae

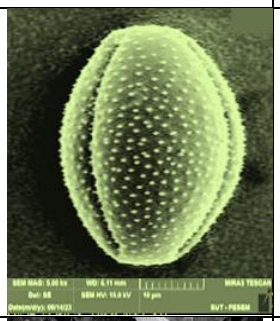
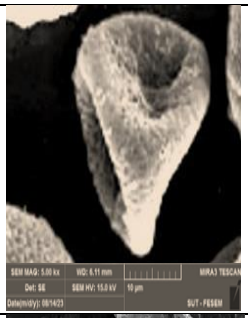
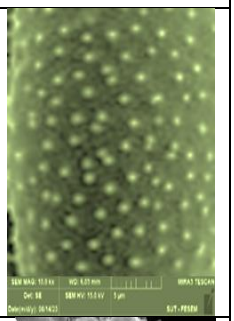
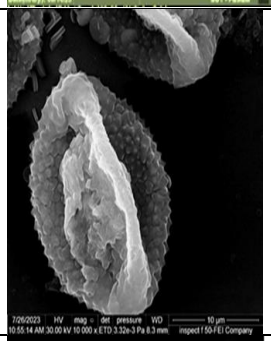
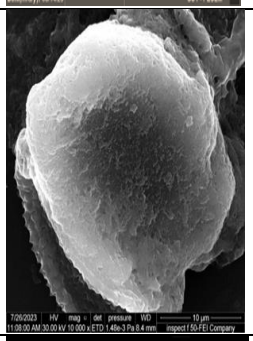
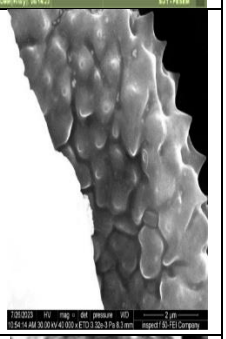
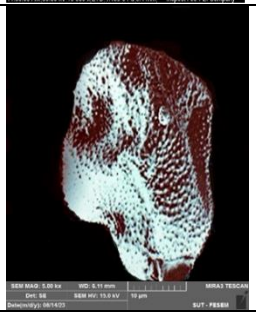
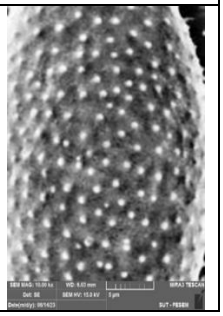
Taxa	Equatorial view LM	Polar view LM	Equatorial view SEM	Polar view SEM	Ornamentation SEM
P. rhoeas var. *himerense*					
P. rhoeas var. *strigosum*					
P. setigerum					

Fig. 2: LM&SEM micrographs of pollen grains in Papveraceae

The Size

The current study was based on measuring the lengths of the Polar and Equatorial axes the results showed that the medium length of the Polar axis ranged between a minimum of 17 µm in *P. tauricola* and a maximum recorded *P. curviscapum* at a rate of 37 µm. On the equatorial axis, the highest rate was recorded in *P. curviscapum* at 30 micrometres, and the lowest rate *in P. bornmuelleri* at 16 µm. Through it, the locations of the species were determined into two categories according to what (Erdtman, 1971) mentioned in dividing pollen grains based on size in general, depending on the length of its axes.

First category is small in size, rang (10-25) µm, and includes species *P. bornmuelleri, P. polychaetum, P. tauricola , P. Pavoninum.*

Second Category: Medium size, 25-50µm, except for the types mentioned in Table 2.

Apertures

the germination aperture dimensions varied in the pollen grains of the species of the studied genera, as the average length of the germination aperture reached 6-22.5 µm in the two species *P. bornmuelleri* and *P. rhoeas* var. *strigosum*, while the average width of the germination aperture ranged between 7-20.33µm in *P. polychaetum* and *P. rhoeas* var. *himerense* respectively, the other dimensions ranged between these two limits (Table 2 and Image 1).

Colpi

The current study showed a clear difference between the types of genera studied in terms of the dimensions of colpi. The average length of the colpi reached 12.36-19.07 µm in *P. pavoninum* and *P. setigerum* respectively, while the average width of colpi ranged between 6.7-10.06 µm in *P. pavoninum* and the *P. tauricola* respectively, and the other dimensions ranged between these two limits, Table 3.

Mesocolpium

The dimensions of the furrows diverse for the advised species depending on the pollen grain size. As the size of the pollen grains increased, the distance between them increased. Conversely, the average distance between two furrows increased to reach 8.5-15.6µm in the two species, *P. pavoninum*, *P. bornmuelleri* respectively, as for shortest and longest distance. Then the other species came in succession between them. Although they were close in some species ranks, they differed in other species to the extent that it was possible to benefit from successfully isolating the species.

Wall Thickness

The results in Table No. 2 showed that Exine is thicker than Entine. The reason for this is attributed to the nature of the chemical composition and its superior ability to resist environmental conditions and decomposition in addition to the nature of its covers. The thickness of Exine was varied. It reached 0.83-1.8µm in *P. tauricola* and *P. bornmuelleri*, respectively. The Entine of the pollen grain reached 0.76-1.72 µm in the two species *P. rhoeas* var. *strigosum* and *P. rhoeas* var. *himerense* respectively, and the other species ranged between these two rates.

Surface Ornamentation

Scanning electron microscope images showed clear differentiation in the outer surface of the pollen grain wall. Based on this, they were divided into two groups.

Group One:

Its outer surface was characterized by the presence of semi-circular granules resembling verrucate. It included the species *P.bornmuelleri*, *P.curviscapum*, *P. polychaetum, P.tauricola , P. rhoeas* var. *himerense, P. rhoeas* var. *strigosum.*

Group Two:

The pollen grain surface is described as Echinate type, and the spinulose are characterized as conical shape, that is wide at the bottom and then narrow and taper towards the top -Verrucate. characterized by the two species *P. pavoninum* , *P. rhoeas* var. *strigosum*.

DISCUSSION

The study showed that the pollen grains were of the tricolporate type. and that the pollen grain measurements were close to those of other previous studies (Erdtman.1943). Study agreed with (Rachele, 1974) who aforesaid that *p. bornmuelleri, p. tauricola*, *p. curviscapum*, were three- colpate, while concurrent with (Candau & Fernandeiz – Paniagua, 1985) they demonstrate the variety; *P. rhoeas* var. *himerense*, *p. rhoeas var. strigosum* were 3-zonocolpate. This is in convention for concept of (Al-Mayah, 1983) in his study for evolutional bearing for the style of pollen grains in the species of *terminalia* and regarding genera.

The present study differs with the latter study concerning the type of *P. polychaetum,* wherever it was a polycolpate 4-5colpte and this agreeing with (Kadereit, 1993b) who speak that most of Papaver are three-colpate except for *Papaver Sect. Argemonidium* with pollen grains with 5-4 circled apertures.

(Sharifnia *et al*., 2010) indicated that the Surface ornamentation of the pollen grain for the most order of the studied plant species of *Papaver* was characterized by its semicircular granular surface resembling verrucate with rough walls, which appeared evident in 8 subspecies of the current study.

This gives evidence of the stability and consistency of the order. Species are classified taxonomically. Therefore, the superficies ornamentation of pollen grains seemed more important and clearer through the study was conducted out by (Al-Moussawi, 2015), who studied 15 species belonging to the genus the poppy family in Iraq, based on the scanning electron microscope, including 8 subspecies current study. Furthermore, (Anderson, 2017), during his study of 9 species belonging to the *Papaver* using (SEM), described the surface trimmings of most species of the family with a granular surface resembling verrucate, and the results of the present study were consistent with what the researcher described. the study also agreed with what (Lee *et al*., 2021) said that pollen grain of Papaveraceae it has a considerable taxonomical account for both *Papaver*, in general, are three- colpate.

CONCLUSION

The study used SEM and LM to detect the micro-morphological characteristics of Papavereacea pollen grains. All studied species were found to be tricolpate based on the number of apertures and pollen grain shape, and its size varied between small and medium size, in addition to studying the surface ornamentation of the external grain wall (exine ornamentation). It was found that the all of pollen grains were of the tricolpate type, and the species were classified based on the number of apertures and colpi and pollen grain shape, and its size varied between small and medium size (P.*curviscapum,* P.*rhoeas var. himerense,* P.*rhoeas var. strigosum* were medium size) and (P.*bornmuelleri,* P.*polychaetum,* P.*tauricola,* P.*pavoninum* and P.*setigerum were small size*), in addition to studying the surface ornamentation of the external grain wall (exine ornamentation).

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