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POLLEN MICROMORPHOLOGY OF FOUR Onobrychis SPECIES (Fabaceae) FROM THE KURDISTAN REGION OF IRAQ

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ABSTRACT:

Polleniferous materials of four *Onobrychis* species were used in this study. Two different microscopes and one-way ANOVA analysis were applied. The pollen grains were tricolpate, isopolar, and consistently radially symmetrical. The value of the polar axis/equatorial diameter ranged from prolate to perprolate, which were categorized as tiny or medium in size. Exine sculpture patterns varied significantly when scanning electron microscopy was used: *Onobrychis megataphora, O. crista-galli, and O. caput-galli* have reticulate patterns; *O. galegifolia* has micro-reticulate perforates. The pollen morphology of *O. caput-galli* was noticeably perprolate due to its maximum polar axis/equatorial diameter value (2.02 µm). The *O. crista-galli* had the lowest polar axis/equatorial diameter value (1.37 µm), showing its prolate pollen morphology. A one-way ANOVA analysis revealed significant differences ($P < 0.05$) in the morphological features of polar length and equatorial diameter. The *O. megataphros* was primarily circular to ovoid-triangular in form, and *O. caput-galli* had a triangular outline. The two remaining species had circular shapes in polar view. In equatorial view, two species had elliptic-elongated shapes, while others had rectangular-obtuse outlines. Thus, these micromorphological dissimilarities indicate variations among taxa, and they can be used as taxonomic characters to identify and delimit *Onobrychis* species.
KEYWORDS: Fabaceae, Genus, Onobrychis, Pollen Morphology, SEM.

1. INTRODUCTIONS

Onobrychis Mill., belonging to the Fabaceae family, subfamily Faboideae, tribe Hedysareae DC. (Chase & Reveal, 2009), consists of approximately 342 perennial and annual species. This genus is found in southern Asia, the Eastern Mediterranean, and northern temperate zones (Hesamzadeh Hejazi & Ziaei Nasab, 2010). The Anatolian-Iran-Caucasia region shows a high concentration and diversity of these taxa (Shomurodov *et al*., 2021). One of its most prominent and significant families of flowering plants is known as Fabaceae (Dizkirici & Kaya, 2013). It comes in third place, behind the Orchidaceae and the Asteraceae family (Ghosh & Keshri, 2007). Pollen morphology is a key tool in plant taxonomy, offering insights into species-level classification due to the conserved yet diverse traits of pollen grains (Stuessy, 2009; Song *et al*., 2017). In Fabaceae, key palynological traits include aperture type, symmetry, and exine sculpturing (Perveen & Qaiser, 1998). The three most crucial characteristics of Fabaceae pollen grains are their symmetrical form as well as aperture (Perveen & Qaiser, 1998). Different taxa of the genus *Onobrychis* (Fabaceae) exhibit varied pollen morphologies. Typically, *Onobrychis* pollen grains are medium to large, tricolpate, and prolate or perprolate in structure (Khan *et al*., 2024). Variations in exine ornamentation, such as reticulate, suprareticulate, and microreticulate patterns, are beneficial for classification and identification within the genus. Despite the taxonomic importance of *Onobrychis* pollen, no previous detailed palynological studies have been conducted on this genus in Iraq, particularly within the Kurdistan Region (Mohamad, 2010). Therefore, this study aims to investigate the pollen morphology of *Onobrychis* species from the Kurdistan region of Iraq, using light microscopy (LM) and scanning electron microscopy (SEM), to assess the taxonomic significance of these traits.

2. MATERIALS AND METHODS

Plant Samples:

Plant materials from different parts of the Kurdistan Region of Iraq were used to collect the polleniferous materials (Table 1). Plant flowers were separated, placed in petri dishes, and left for a full day at room temperature. They were then sprinkled on a sanitized area.

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Table 1: Specimen details used in this study.

Name of taxa	Place of collection	Altitude	Latitude	Longitude
***O. crista-galli* (L.) Lam.**	Zakho/ Jawsaq village near Ibrahim Khalil	602 m	4454439.9N	4739782.8E
***O. caput-galli* (L.) Lam.**	Erbil/ Hujran	832 m	4356910.5N	4927331.0E
***O. megataphros* Bioss.**	Suliamaniya/ Qaradagh mountain	1300 m	4196730.1N	5050867.6E
***O. galegifolia* Bioss.**	Duhok/Rawinah	1200 m	4468553.9N	4826951.5E

Light Microscopy (LM)

Pollen grains were prepared for LM analysis utilizing fresh samples that had been kept. Following Radford *et al.* (1974), the pollen grains were mounted in methyl green–glycerine jelly (1 g of methyl green dissolved in one hundred ml of pure alcohol (99%). An examination of the slides was conducted using a compound trinocular microscope. The polar length (P), equatorial diameter (E), P/E ratio, aperture count, mesocolpium (in equatorial view), apocolpium, colpus length, exine thickness, as well as apertural form were among the morphological observations. For every measured morphological character, the mean, minimum (min), maximum (max), and standard deviation were calculated from twenty individual pollen grains per character. Erdtman (1945) was used to evaluate size classes, while Erdtman (1952) was used to define shape classes. In general, Erdtman (1971) and Punt *et al*. (1999) serve as the foundation for pollen terminology.

Scanning Electron Microscopy (SEM)

For later use in SEM analysis, the pollen was further dried at ambient temperature for five to eight hours before being placed in vials containing silica gel at 4 degrees Celsius. Utilizing a Quanta 450 model, scanning electron microscopy was carried out at the SEM center, Soran University, Erbil, Kurdistan Region, Iraq.

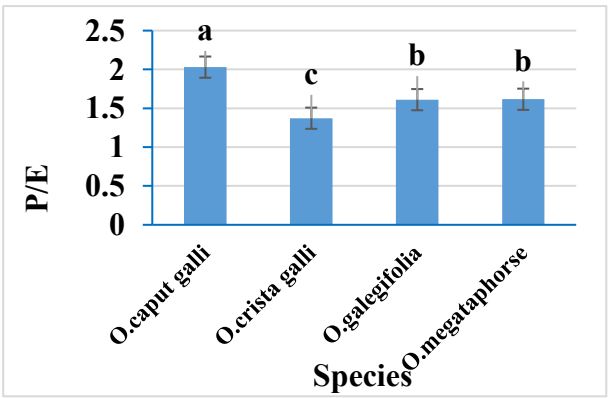
Statistical Analysis:

One-way Analysis of Variance (ANOVA) was performed to test the significant differences among the four *Onobrychis* species. All statistical analyses were conducted using SPSS Statistics software, and a p-value of less than 0.05 was considered statistically significant.

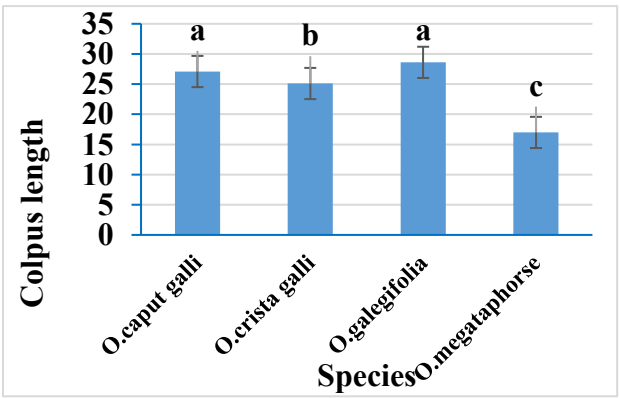
3. RESULTS

The morphological characteristics of four *Onobrychis* taxa were statistically analyzed using one-way ANOVA. Based on the P-values less than 0.05, the results showed significant differences throughout all investigated features, including equatorial diameter, polar length, apocolpium, and mesocolpium. There are noticeable variations in the group averages as well as variability between Charts 1-7 (Figure 1).

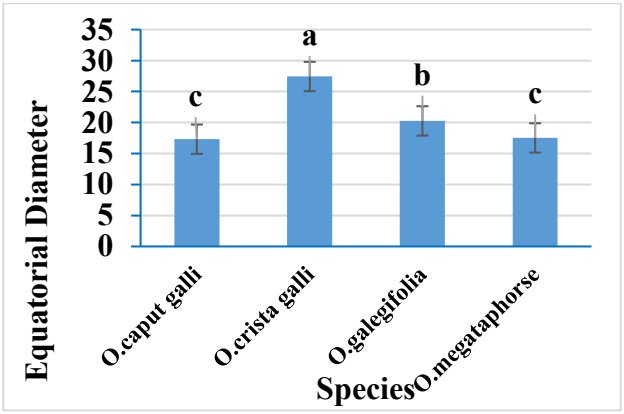
In Chart (A) (P/E), the mean value of the *O. caput galli* was high compared to the lowest value of the *O. crista galli*. The *O. galegifolia* and *O. megataphorse*, as represented in Chart (B) (colpus length), showed the highest and lowest mean values, respectively. The first and fourth groups (*O. caput galli* and *O. megataphorse*) have the lowest values for the Equatorial Diameter (Chart (C)). In contrast, the second group (*O. crista galli*) has the highest value. Similarly, the fourth group (*O. megataphorse*), with the lowest value, and the second group (O. crista galli), with the highest value, are displayed in Chart (D) (Mesocolpium). The second group (*O. crista galli*) in Chart (E) (Polar Length) has the most outstanding value, and the fourth group (*O. megataphorse*) has a much lower value. The third group (*O. galegifolia*) has the lowest mean, whereas the second group (*O. crista galli*) has the highest, as shown in Chart (F) (Apocolpium). Finally, the first and fourth groups (*O. caput galli* and *O. megataphorse*) have the highest and lowest mean values, respectively, as shown in Chart (G) (Exine thickness). Statistical analysis using one-way ANOVA confirmed that each of the chart's group differences was statistically significant ($P < 0.05$), indicating that the variation in the data was not the result of chance.



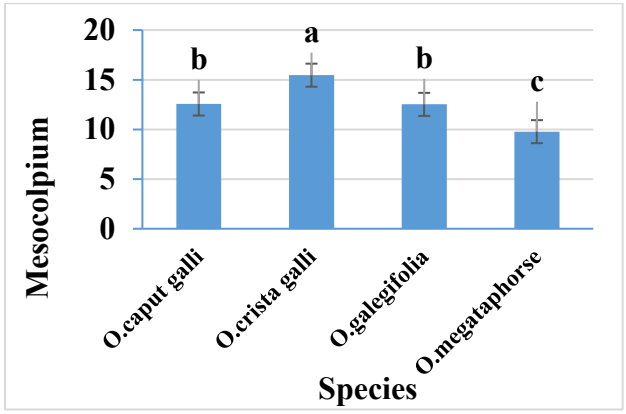
(A)P/E



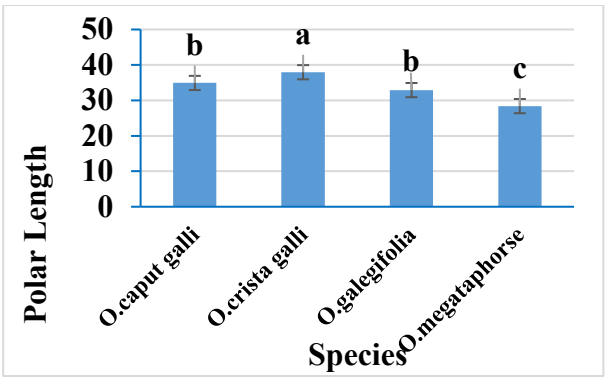
(B)Colpus Length



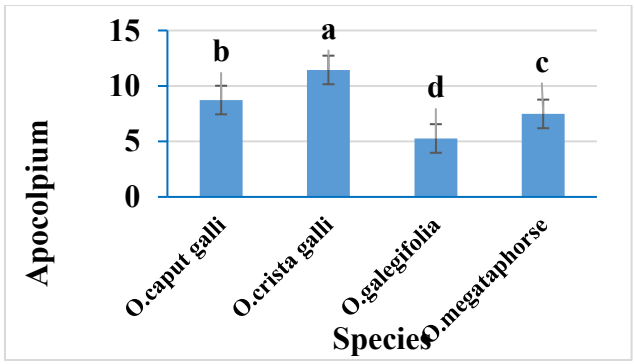
(C)Equatorial Diameter



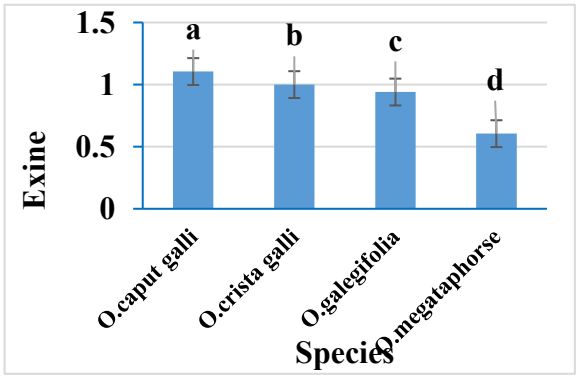
(D)Mesocolpium



(E)Polar Length



(F)Apocolpium



(G)Exine

Figure 1: Comparative pollen traits of four *Onobrychis* species based on one-way ANOVA analysis.

The investigated pollen grains of the four *Onobrychis* species were found to be tricolpate, radially symmetrical, isopolar, and monads. Comprehensive palynological features are presented in Table 2 and Figures 2–5. The majority of *Onobrychis* pollen grains were prolate and perprolate in form, with prolate being the most common shape among the taxa; in particular, the *O. caput-galli* clearly showed a perprolate pattern (Figure 5).

Table 2: Summary Quantitative for pollen morphological characters (µm)

Taxon	Statistics	Polar View	Equatorial View	Exine thickness	Apocolpium	Mesocolpium	Colpus length	P/E	Pollen shape	pollen Size
O. crista galli	**Mean**	**37.94**	**27.43**	**1**	**11.44**	**15.45**	**25.10**	**1.37**	**prolate**	

	STD	**±5.47**	**±2.19**	**±0.00**	**±2.56**	**±0.91**	**±2.83**	**±0.10**		**Medium**
	Min	**25**	**20**	**1**	**8**	**14**	**20**	**1.1**		
	Max	**45**	**30**	**1**	**16.5**	**17.1**	**28.5**	**1.50**		
O. caput galii	**Mean**	**34.91**	**17.31**	**1.10**	**8.73**	**12.55**	**27.08**	**2.02**	**perpolate**	**Medium**
	STD	**±4.44**	**±2.97**	**±0.05**	**±1.73**	**±0.88**	**±3.38**	**±0.97**		
	Min	**27.7**	**12.5**	**1.01**	**6**	**11.12**	**19.5**	**1.88**		
	Max	**42.5**	**22.5**	**1.20**	**11.6**	**13.99**	**31.9**	**2**		
O. megataphros	**Mean**	**28.38**	**17.51**	**0.60**	**7.48**	**9.77**	**16.98**	**1.61**	**prolate**	**Small-medium**
	STD	**±2.16**	**±1.54**	**±0.05**	**±0.29**	**±0.43**	**±0.33**	**±0.02**		
	Min	**25**	**15**	**0.51**	**7**	**9.06**	**16.5**	**1.59**		
	Max	**32.5**	**20**	**0.70**	**8**	**10.49**	**18**	**0.66**		
O. galegifolia	**Mean**	**32.89**	**20.26**	**0.94**	**5.26**	**12.52**	**28.60**	**1.61**	**prolate**	**Small-medium**
	STD	**±5.88**	**±2.48**	**±0.05**	**±0.65**	**±0.29**	**±5.27**	**±0.18**		
	Min	**23.3**	**17.5**	**0.9**	**4.2**	**12.04**	**20.1**	**1.23**		
	Max	**42.4**	**22.3**	**1**	**6.5**	**12.99**	**36.9**	**1.88**		

Note: P = Polar Length; E = Equatorial Diameter; P/E = Polar Length/Equatorial Diameter ratio; STD = Standard Deviation.

The pollen grain outlines of *O. megataphros* and *O. galegifolia* were rectangular-obtuse when viewed from an equatorial angle. At the same time, those of *O. crista-galli* and *O. caput-galli* were elliptic-elongated. In polar view, two of the species (*O. galegifolia* and *O. crista-galli*) exhibited a circular shape with more than 40% repeated patterns (Table 3). *O. megataphros* pollen grains were primarily round, with ovoid to triangular (more than 40 %) shapes also observed, while *O. caput-galli* displayed a triangular-obtuse morphology (Table 3, Figures 2 &5).

Table 3: Percentage of repeat morphological shape of pollen grains

Taxon	Polar view					Equatorial view				
	Triangular	Ovate	Circular	Elliptic	Rectangular-obtuse	Triangular	Ovate	Circular	Elliptic	Rectangular - obtuse
O. crista galli	-	-	****	-	-	-	-	-	****	-
O. caput galli	****	-	-	-	-	-		-	****	-
O. megataphros	**	-	****	-	-	-	-	-	*	****
O. galegifolia	-	-	****	-	-	-	-	-	*	****

Note**: *1-20 %, ** 20-30%, *** 30- 40 % and **** more than 40%, - not present.**A wide range of exine sculpture patterns was observed, with two different types identified: reticulate in *O. megataphros*, *O. crista-galli*, and *O. caput-galli* (Figures 2,4, and 5); and micro-reticulate perforate in *O. galegifolia* (Figure 3).

The mean polar axis length of the *Onobrychis* taxa varied numerically, ranging from 28.384 µm for *O. megataphros* to 37.94 µm for *O. crista-galli*. The *O. crista-galli* pollen grain recorded the highest mean equatorial diameter (27.43 µm), whereas *O. caput-galli* had the smallest value (17.31 µm) (Table 2).

In terms of the apocolpium area, *O. galegifolia* had the shortest mean value (5.26 µm), while *O. crista-galli* had the longest mean (11.44 µm). For mesocolpium, *O. megataphros* had the smallest mean area between two colpi (9.77 µm), whereas *O. crista-galli* had the most significant distance (15.45 µm). Similarly, the colpus length significantly differed among the taxa, with *O. megataphros* having the least mean colpus length (16.98 µm), and *O. galegifolia* having the longest (28.60 µm). The thickest exine thickness was 1.10 µm for *O. caput-galli*, and the thinnest was 0.60 µm for *O. megataphros*. The pollen grains were observed to possess a multi-cell wall layer composed of an exterior exine, an interior intine, and diverse surface sculpturing (Table 2, Figures 2-5).

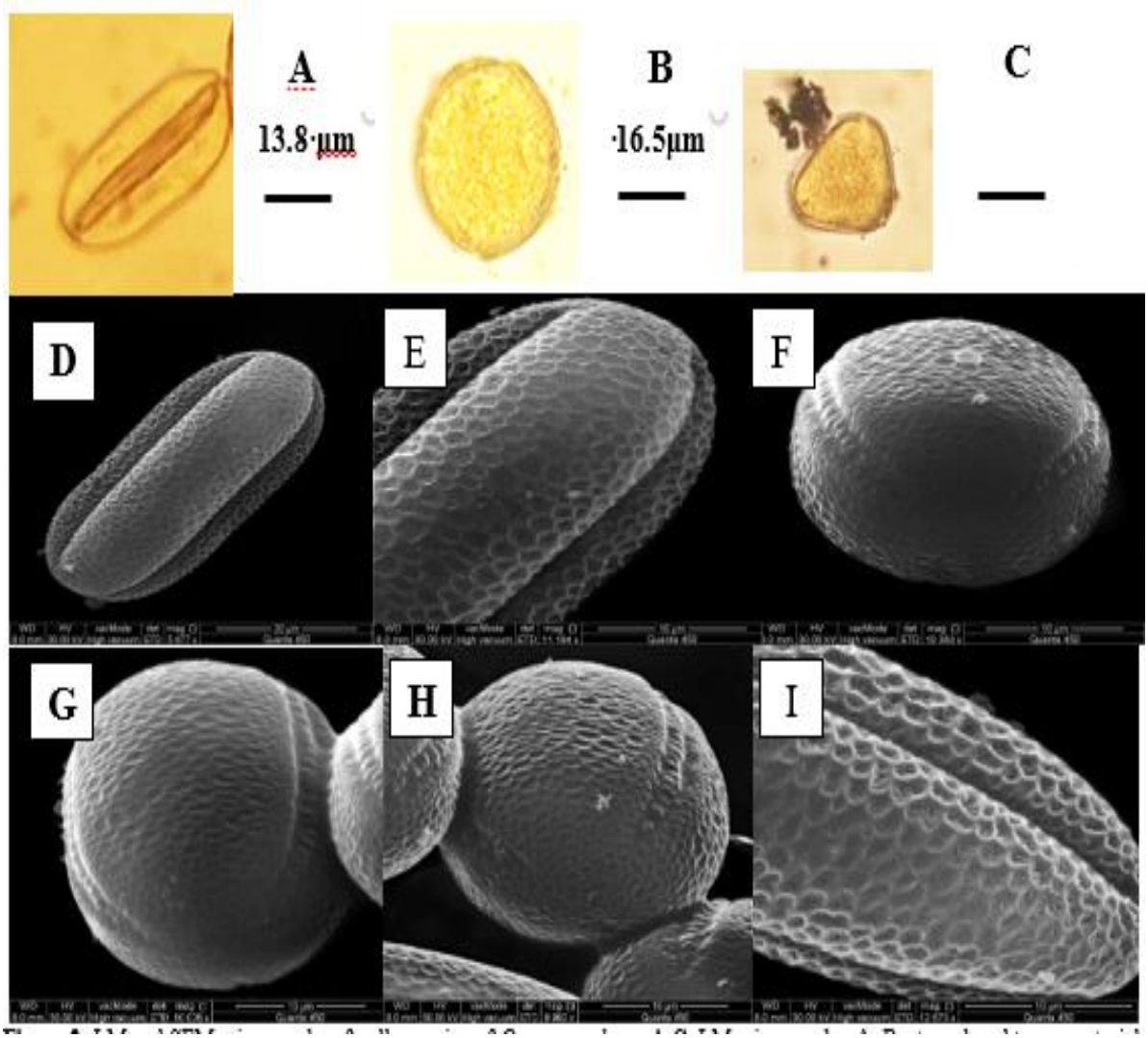


Figure 2: LM and SEM micrographs of pollen grains of *O. megataphros*: A-C. LM micrographs: A. Rectangular-obtuse equatorial view, B. Circular polar view; C.Ovoid-triangular polar view. D-I SEM micrographs: D.and E, Rectangular-obtuse equatorial view, F, G., and H., Circular polar view, I. Exine sculpture.

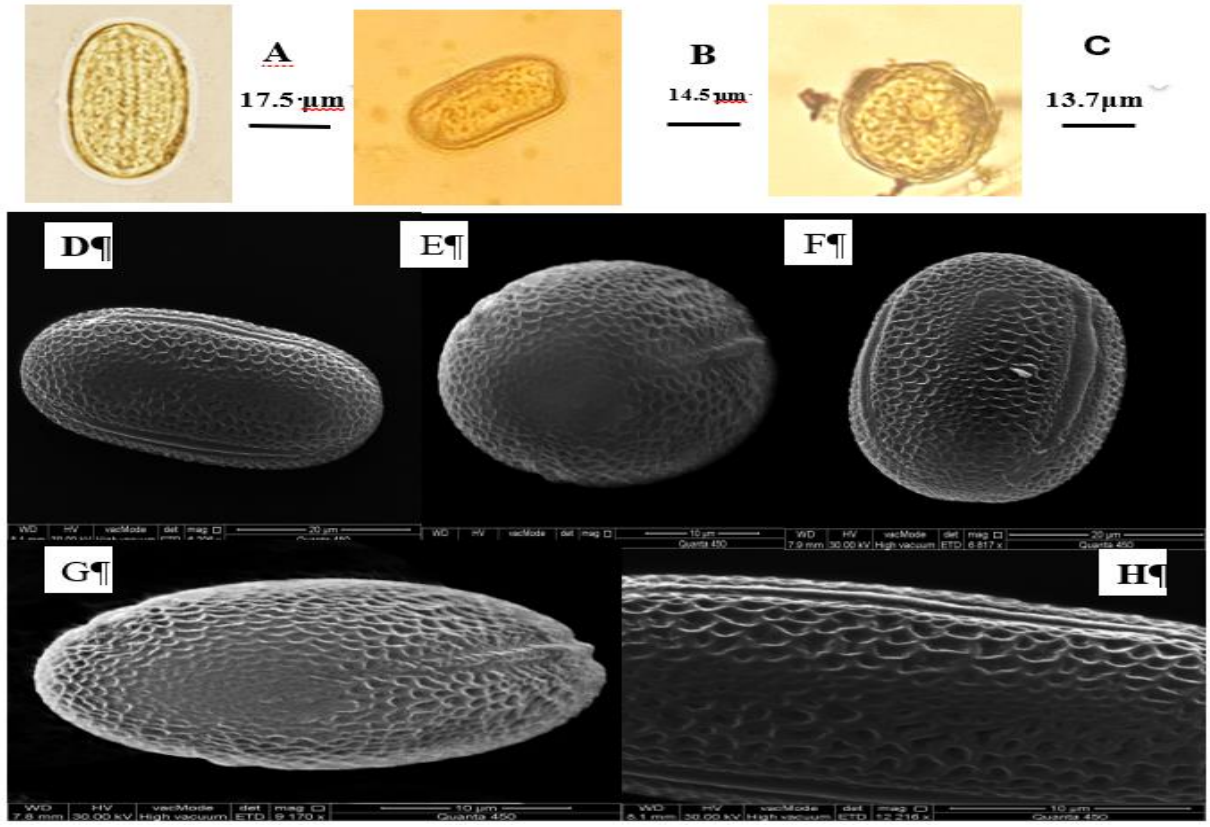


Figure 3: LM and SEM micrographs of pollen grains of *O. galegifolia*: A-C. LM micrographs: A and B. Rectangular-obtuse equatorial view, C. Circular polar view; D-H. SEM micrographs: D. Rectangular-obtuse equatorial view, E, F, and G. Circular polar view, H. Exine sculpture.

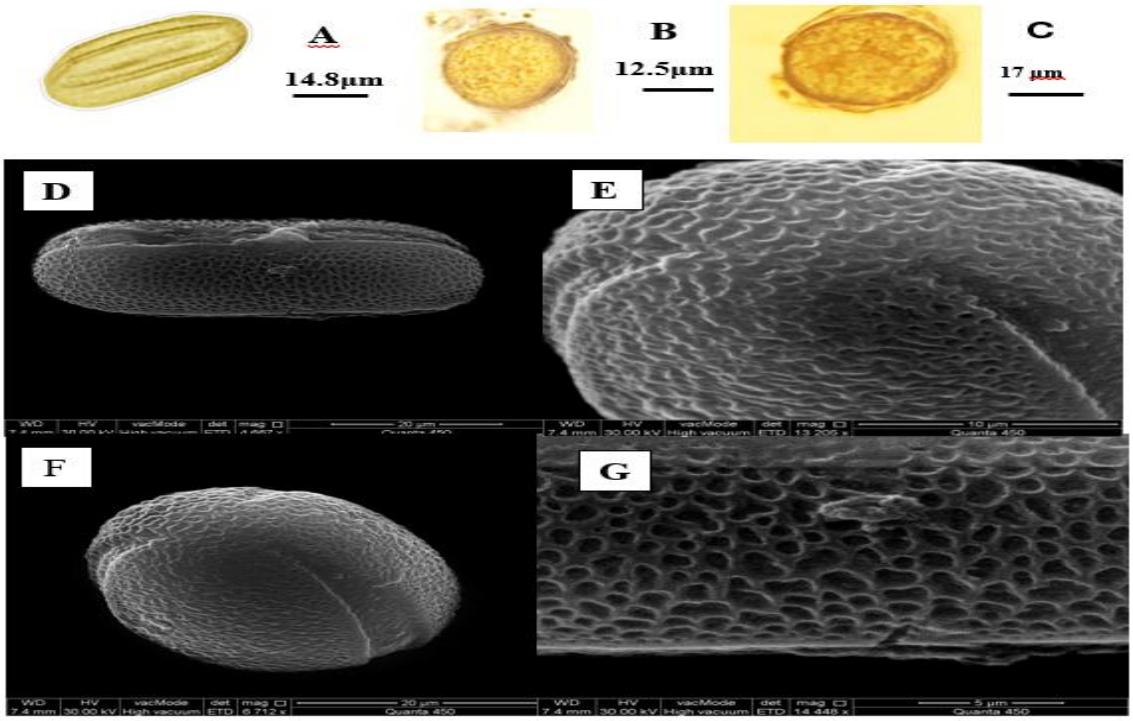


Figure 4: LM and SEM micrographs of pollen grains of *O. crista galli:* A-C. LM micrographs: A. Elliptical elongated equatorial view, B and C Circular polar view; D-G SEM micrographs: D. Elliptical elongated equatorial view, E. F Circular polar view, G. Exine sculpture.

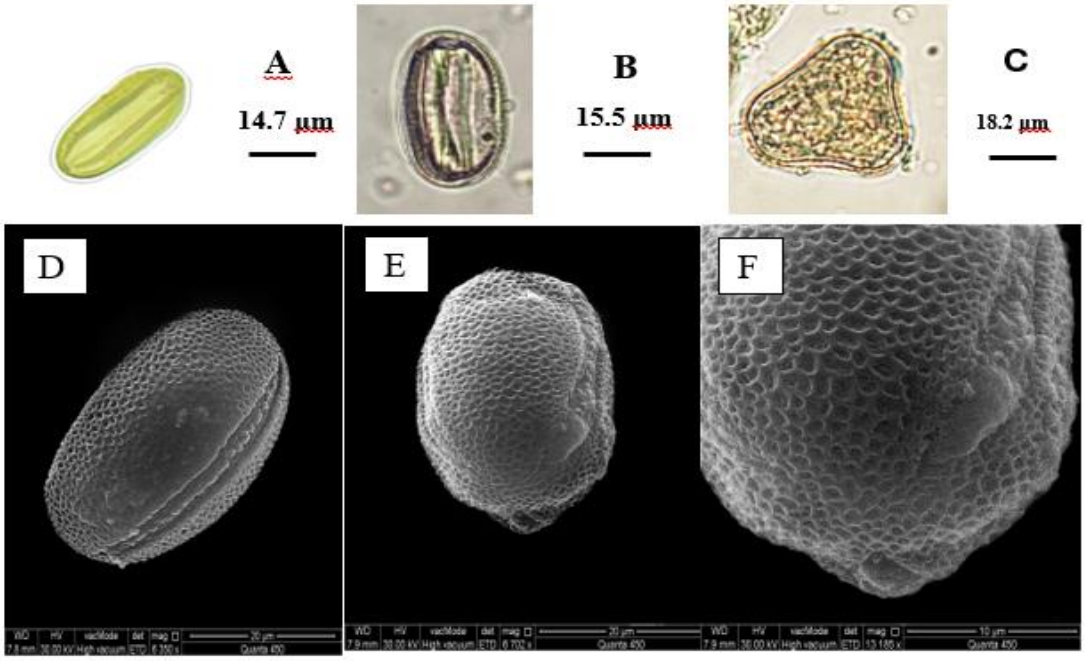


Figure 5: LM and SEM micrographs of pollen grains of *O. caput galii*: A-C. LM micrographs: A and B. Elliptical elongated equatorial view, C. Triangular-obtuse polar view; D-F SEM micrographs: D. Elliptical elongated equatorial view, E. Triangular-obtuse polar view, F. Exine sculptur

4. DISCUSSION

The results of this study consistently demonstrate that *Onobrychis* pollen is tricolpate. The current finding aligns with earlier studies by Ghanavati (2012) and Talebi *et al.* (2020). Our observations further corroborate recent research by Atasagun, Aksoy, and Martin (2024) on endemic Turkish *Onobrychis*, confirming the general pollen characteristics as radially symmetric, isopolar, tricolpate, prolate, and ornamented with reticulate-microreticulate patterns. Similarly, the prevalence of 3-colpate, prolate, and perprolate pollen grains in our study is consistent with descriptions by Pavlova and Manova (2000), who also noted the potential for diverse polar and equatorial shapes and ornamentation within the genus. Our findings on exine ornamentation agree with those of Avci *et al.* (2013), who observed comparable trends among *Onobrychis* species.

While these general morphological features are consistent across *Onobrychis*, the detailed palynological characteristics observed in the species from Kurdistan, Iraq, provide crucial insights for species delimitation. Specifically, variations in quantitative traits such as polar axis length, equatorial diameter, apocolpium area, mesocolpium distance, colpus length, and exine thickness, alongside qualitative differences in exine sculpture patterns, serve as robust taxonomic markers. Among these, the P/E ratio emerged as the most distinguishing character for the *Onobrychis* species examined, demonstrating significant interspecific variation that facilitates their clear differentiation. This highlights the importance of precise measurements of these features, rather than just their presence, for robust taxonomic discrimination. Furthermore, while exine thickness has historically been used for differentiation, the broader array of exine sculpture patterns revealed through scanning electron microscopy in this study underscores its increasing importance as a key trait for plant differentiation at the species level.

Limitations and Future Research:

This study provides valuable palynological data for four *Onobrychis* species from the Kurdistan Region of Iraq. However, due to the time constraints inherent to a master's program, the scope of species investigated was limited. Future research should aim to expand this investigation to a broader range of *Onobrychis* species across different geographical areas to further validate and refine the taxonomic significance of these palynological features, particularly the P/E ratio and exine ornamentation, for a more comprehensive understanding of species delimitation within the genus.

CONCLUSION

This palynological study on the Fabaceae taxa was conducted to figure out the micromorphology of pollen grains of four *Onobrychis* species growing naturally in the Kurdistan Region of Iraq. The results verify that the pollen grains are radially symmetric, isopolar, solitary, and tricolpate. Significant differences were found in exine sculpture patterns and important diagnostic traits, especially the polar and equatorial diameters. The pollen was classified as either prolate or perprolate based on the determined polar axis/equatorial diameter values. The study also revealed that the species had different outlines in polar and equatorial perspectives, as well as a variety of exine ornamentation patterns, such as reticulate and micro-reticulate perforate varieties. The use of these micromorphological differences as trustworthy taxonomic features for recognizing and defining *Onobrychis* species is strongly supported by the statistical significance of these morphological differences ($P < 0.05$), as confirmed by one-way ANOVA. This study makes a significant palynological contribution to the genus *Onobrychis*, particularly in an understudied region such as Iraq.

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Author Contributions:

The authors conducted all parts of this article.

Ethical Approval:

This research did not require ethical approval because it did not involve humans or animals.

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