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Taxonomic study of pollen grains for five species of Fabaceae Family*

Naznaz Nihmat Bapir [1]
Naznni3matB@gmail.com

Ali Mala Khedir Galalaey [2]
ali.galalaey@su.edu.krd

[1] Department of Forestry, College of Agricultural Engineering Science, Salahaddin University, Erbil, Iraq.
[2] Department of Field Crops and Medicinal Plants, College of Agricultural Engineering Science, Salahaddin University, Erbil, Iraq.

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Abstract

The objective of the study was to provide valuable palynological characteristics for the identification of Fabaceae taxa, which could potentially serve as distinguishing features for closely related species differentiation. Pollen samples were collected from fully developed flowers of 25 samples, encompassing five species within the Fabaceae Family Colutea *cilicica* Boiss. & Balansa, *Glycyrrhiza glabra* L., *Spartium junceum* L., *Robinia pseudoacacia* L., and *Anagyris foetida* L. The investigation involved the utilization of both light microscopy and electron scanning microscopy. Consequently, the observed pollen grains exhibited monad symmetry and were of medium to small dimensions. These grains displayed diverse ornamentation patterns including perforations, fossulate, reticulations, regular structures, and granulations. Moreover, a wide array of outline shapes was noted for pollen grains in both equatorial and polar views. Detailed examinations were performed using both light microscopy (LM) and electron scanning microscopy (SEM) techniques, revealing distinct differentiating characteristics among the examined taxa.

Key words: Pollen grain, Colporate, Perforate, Taxa, Fabaceae.

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Corresponding author: Naznaz Nihmat Bapir _ Naznni3matB@gmail.com

Introduction

Mabberley [1] asserted that the Fabaceae Family holds the distinction of being the most expansive and significant family among flowering plants. Historically, botanists have diligently endeavored to classify plants based on the size and shape of their pollen grains. Ghildiyal *et al*. [2], in their works on Plant Anatomy, noted that distinct species exhibit varying sizes and forms of pollen grains, while closely related species tend to have similar sizes and forms. Over time, this idea evolved, suggesting that the pollen grain's morphology remains consistent for each species. The study of pollen morphology furnishes compelling evidence for distinguishing between taxa at different hierarchical levels. For instance, in the Lamiaceae family, pollen plays a crucial role in identifying subfamilies, with only the Ajugeae tribe displaying distinctive pollen characteristics. Furthermore, specific genera—such as *Collinsonia*, *Salvia*, *Teucrium*, and *Trichostema*—possess markedly distinct pollen compared to others. At the infra-generic level, pollen grains offer valuable taxonomic insights for various genera, including *Hyptis, Monardella, Salvia, Stachys, Teucrium,* and *Trichostema* [3,4, and 5].

Researchers, [6 and 7] have employed characteristics like exine sculpturing, aperture structure, grain size, and shape to address taxonomic questions. These features prove instrumental in distinguishing plants not only at the genetic level but also at the specific level, particularly in the context of rosaceous pollen [8]. An electron and light microscope was used to test the polleniferous material of *Prunus* subg. *amygdalus* taxa in Iraq. A variety of taxa are classified as symmetric, isopolar, monads, colporates, and trizonocolpate. *P. argentea* var. *argentea* has the largest pollen grains. There is a large amount of elaeagnifolia. A discriminant analysis and principal component analysis can help predict taxa [9]. Furthermore, [10] emphasized the efficacy of pollen morphology in differentiating between Rosacea taxa. However, they cautioned against relying too heavily on pollen size due to the frequent occurrence of hybridization and polyploidy species within this family.

The morphology of pollen grains from 15 species within five genera of the Fabaceae family, including *Colutea cilicica* Boiss. & Balansa and *Glycyrrhiza glabra* L. were examined using a light microscope by [11]. Their observations revealed that the pollen grains were predominantly monad and colporate across all genera, except for *Spartium junceum, Anagyris foetida,* which displayed a colpate pattern. The size of the pollen was generally small to medium, with significant variability in exine patterns, encompassing ornamentation types like reticulate, micro reticulate, regulate, psilate, and perforate. The researchers highlighted the value of both polar and equatorial outline morphology in identifying species. This palynological investigation enhanced the understanding of pollen grain diversity and contributed to plant classification.

A collection of *Centaurea* pollen from Kurdistan-Iraq was examined using SEM and LM, which revealed triangular or triangular-circular grain boundaries, scabrate exine sculptures, and perforated tectums. Small to medium-sized pollen grains are common, and they have subprolate shapes. Using principal component analysis and discriminant analysis, 65.478% and 87.54% of variability are recognized, respectively, and 125 or 78.125% of observations are classified [12].

Despite modern laboratories vastly enriching knowledge of pollen morphology compared to the past, their impact on plant systematics remains somewhat limited. This is due to pollen grain studies not consistently integrating with results from other scientific disciplines. The intricate terminology in palynology and the challenges of describing comparative features based on pollen morphology, as noted by [13], contribute to this limitation. Pollen morphological characteristics offer valuable evidence for distinguishing taxa at various hierarchical levels. Multiple studies demonstrate that pollen morphology serves as an indicator in systematic botany, criminology, aeropalynology, allergies, paleobotany, stratigraphic correlation of oil-bearing rocks, and other fields. In quaternary studies, Palynology, as cited by [14, 15, 16, 17, and 18], is widely employed in plant taxonomy. Additionally, insights derived from pollen studies can reveal historical responses of natural vegetation to human impacts and climatic/environmental changes. Huntley [19] anticipates using pollen grain data to predict vegetation responses to future climatic conditions.

The aim of this study was to offer valuable palynological features for defining Fabaceae taxa, potentially yielding diagnostic characteristics to differentiate closely related taxa.

Material and Method

A. Analysis Using a Light Microscope

Examination of pollen grains under a light microscope involved adhering to the standardized procedure outlined by [20] and [21], with certain adjustments incorporated into the preparation of pollen samples:

- Initially, desiccated pollen cones were positioned in 1.5 mm Eppendorf tubes and then pulverized using a glass rod.
- A copious amount of 70% ethanol was introduced, and the mixture was thoroughly agitated.
- The concoction was sifted through a sieve or gauze, facilitating the segregation of pollen from residual microsporophyll matter.
- Subsequently, the pollen-ethanol mixture was subjected to centrifugation until the pollen settled at the bottom of the Eppendorf tubes.
- About 2-3 drops of 5% safranin were introduced, and a waiting period of 5-10 minutes was observed.
- Dyed pollen was rinsed with 70% ethanol, promoting the breakup of pollen aggregates through agitation, followed by another round of centrifugation. This rinsing process was repeated 2-3 times, continuing until the mixture became colorless.
- Following the last centrifugation, the ethanol was meticulously decanted. The stained pollen was then placed onto slides, and a droplet of glycerin was added before affixing a cover slip.
- For every individual pollen species, 25 granules were subjected to observation using an Olympus AC100 light microscope fitted with a camera from Japan. Measurements were captured under both 40X and 100X magnification. The thickness of the wall (exine) was gauged in line with the approach delineated by [22].

B. Electron microscopy analysis

Pollens were prepared according to the procedure proposed by [23), at the Plant Taxonomy Lab/College of Agricultural Engineering Science/ University of Duhok. After sterilizing the laminar with alcohol (70%), a commercial cleaner was used to clean it. It was necessary to place within the laminar a flask filled with chloroform whose orifice was closed by cotton before closing the laminar. Using acetone in a series of concentrations, pollen cones are dehydrated using a glass vial.

1-Phosphate buffer, 3 times, each for 15 minutes.
2-Remove the phosphate buffer with a pipet, then add the 30% concentration acetone, for 30 min.
3-50% acetone, for 30 min.
4-70% acetone, for 30 min.
5-80% acetone, for 30 min.
6-90% acetone, for 30 min.
7-100% acetone, 2 times, each for 1 hour.

When the paraffin in the Petri dish has cooled, place a filter paper over it. Each pollen cone should be placed on a different Petri dish after the last acetone concentration. In the final stage of dehydration, the table lump (12W/ 3000K light bulb) was placed over all Petri dishes inside the sterilized laminar for two days. Because pollen samples are fragile, cover each Petri dish with the cover, seal them with ordinary tape, and place them inside a box tightly. Under a dissecting microscope, each sample was placed on a separate sample stage and cut into pieces with a surgical scalpel, if necessary, to avoid interfering with the other samples. A gold covering is then applied for

20-30 minutes to the sample. Finally, pollens were scanned using the (FEI Quanta 450) electron microscope. Studied characters included pollen shape, pollen surface texture, the type and characters of surface ornaments, and finally the kind and specification of aperture.

Results

Genus: *Colutea (C. cilicica*), as depicted in Figure 1 and outlined in Table 1, exhibits pollen grains with colpore aperture type, existing as monads in pollen units. These grains are of medium size, prolate in shape with a P/E ratio of 1.37. The polar axis measures 28 µm (ranging from 35 to 27.5 µm), while the equatorial length spans 21 µm (ranging from 21.5 to 15 µm). In the polar outline, the pollen grains take on a semi-circular or trilobate triangular form, while in the equatorial outline, they appear prolate-spheroidal. The surface of the grains displays reticulate-fossulate ornamentation. The exine of the cell wall is either 1 µm or less in thickness, and the germination pores exhibit tricolporate characteristics.

Genus: *Glycyrrhiza (G. glabra),* as illustrated in Figure 2 and detailed in Table 1, showcases pollen grains with colpore aperture type, existing as monads in pollen units. These grains are of medium size and prolate in shape with a P/E ratio of 1.4. The polar axis measures 34 µm (ranging from 37 to 27.5 µm), and the equatorial length is 24 µm (ranging from 27 to 21.5 µm). In polar outline, the pollen grains present as semi-triangular or circular, while in the equatorial outline, they adopt a prolate-spheroidal or elliptical shape. The surface ornamentation features regulate-reticulate or regulate-fossulate patterns. The exine of the cell wall has a thickness of 1 µm or less, and the germination pores display tricolporate or 3-colporate attributes.

Genus: *Spartium (S. junceum),* as indicated in Figure 3 and outlined in Table 1, showcases pollen grains with colpus aperture type, existing as monads in pollen units. These grains are small in size and oblate, with a P/E ratio of 1.17. The polar axis measures 40 µm (ranging from 40.5 to 38.5 µm), and the equatorial length spans 34 µm (ranging from 35 to 28.5 µm). In the polar outline, the pollen grains appear semi-triangular or circular (round), while in the equatorial outline, they take on an oblate-spheroidal form. The ornamentation on the surface is faveolate. The exine of the cell wall measures 1 µm or less in thickness, and the germination pores display tricolpate or 3-colpate (colpus) characteristics.

Genus: *Robinia (R. pseudoacacia)*, as depicted in Figure 4 and detailed in Table 1, presents pollen grains with colpate aperture type, existing as dyads in pollen units. These grains are of medium size and oblate, with a P/E ratio of 1.2. The polar axis measures 25.5 µm (ranging from 28 to 24 µm), and the equatorial length is 23 µm (ranging from 25.8 to 20.2 µm). In the polar outline, the pollen grains take on an ovate or circular shape, while in the equatorial outline, they adopt an oblate-spheroidal structure. The surface is ornamented with a perforate pattern. The exine of the cell wall is either 1 µm or less in thickness, and the germination pores display tricolpate or 3-colpate (colpus) attributes.

Genus: *Anagyrus (A. foetida)*, as shown in Figure 5 and outlined in Table 1, features pollen grains with colpate aperture type, existing as monads in pollen units. These grains are of medium size and oblate, with a P/E ratio of 1.2. The polar axis measures 20.5 µm (ranging from 20.2 to 14.8 µm), and the equatorial length spans 16.5 µm (ranging from 19 to 15 µm). In the polar outline, the pollen grains appear semi-triangular or circular, while in the equatorial outline, they take on an oblate-spheroidal form. The surface ornamentation is characterized by a perforate-fossulate pattern. The exine of the cell wall measures 1 µm or less in thickness and the germination pores exhibit tricolporate or 3-colporate (colpus) features.

Table 1: Variations in Pollen Grain Qualitative and Quantitative Characteristics of the Fabaceae Family.

No.	Species	Shapes of Pollen Grains		Ornamentation	Aperture type	Wall thick	Pollen grain size	P/E ratio	Equatorial axis length	Polar axis length	Pollen units
		P/E Equatorial outline	Polar Outline								
1	*Colutea Cilicica*	Prolate spheroidal	Semi Circular or trilobate triangular	Reticulate fossulate	Colporate	1-less than 1 µm	Medium	1.3	(21.5-15)21	(35-27.5)28	Monad
2	*Glycyrrhiza glabra*	Prolate spheroidal	Semi-triangular or circular	Regulate reticulate or regulate fossulate	Colporate	1-less than 1 µm	Medium	1.4	(27-21.5)24	(37-27.5)34	Monad
3	*Spartium junceum*	Oblate spheroidal	Semi-traingular or circular	Reticulate-regulate	Colpus	Less than 1 µm	Small	1.17	(28.5-35)34	(38.5-40.70)40	Monad
4	*Robinia pseudoacacia*	Oblate spheroidal	Ovate or circular	Granulate-perforate	Colporate	Less than 1 µm	Small	1.1	(20.2-25.8) 23	(24.28)25.5	Monad
5	*Anagyris foetida*	Oblate spheroidal	Circular	Fossulate-perforate	Colpus	Less than 1 µm	Small	1.2	(15-19)16.5	(14.8-20.2)20.5	Monad

Table 2: Pollen grain outline shapes in both Polar and Equatorial views.

Pollen outline shape	Pollen outline shape	*Robinia pseudoacacia*	*Spartium junceum*	*Colutea cilicica*	*Glycyrrhiza glabra*	*Anagyris foetida*
Polar view	Circular	***	**	**	**	***
	Ovate	**	*	-	-	-
	Elliptic	-	-	***	*	-
	Triangular	*	***	*	***	**
	Triangular	-	-	-	-	-
Equatorial view	Circular	**	**	**	**	**
	Ovate	***	*	-	-	-
	Elliptic	-	-	*	*	-
	Triangular	*	**	***	**	**
	Triangular	-	-	-	-	-

Note: The symbol (*) represents the number of taxa.

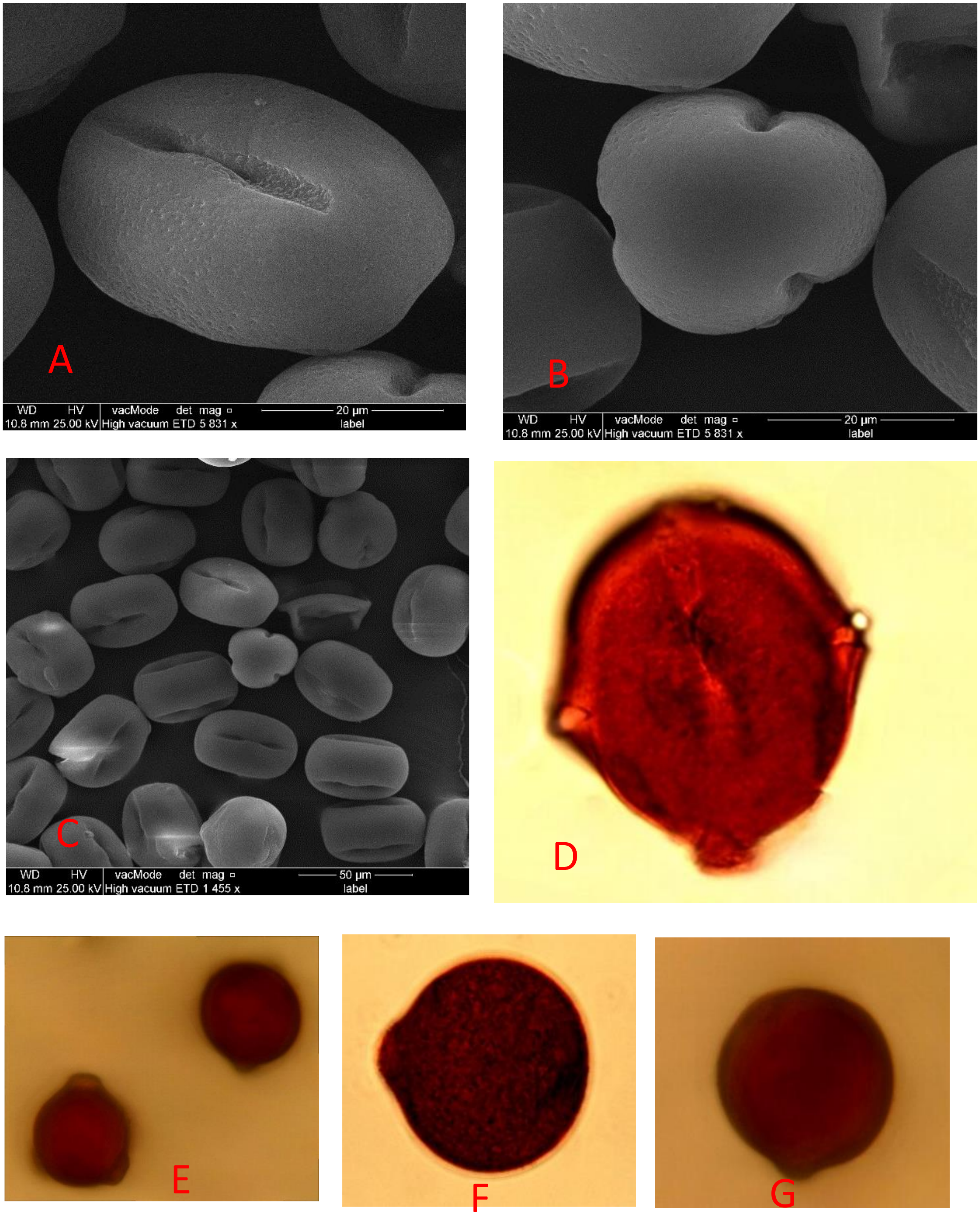

Figure 1: ***Colutea cilicica*** **pollen grains in different view LM & SEM. A-Equatorial, B-Polar, C-Equatorial and polar, D-Equarorial, E-Polar, F-Equatorial, G-Polar.**

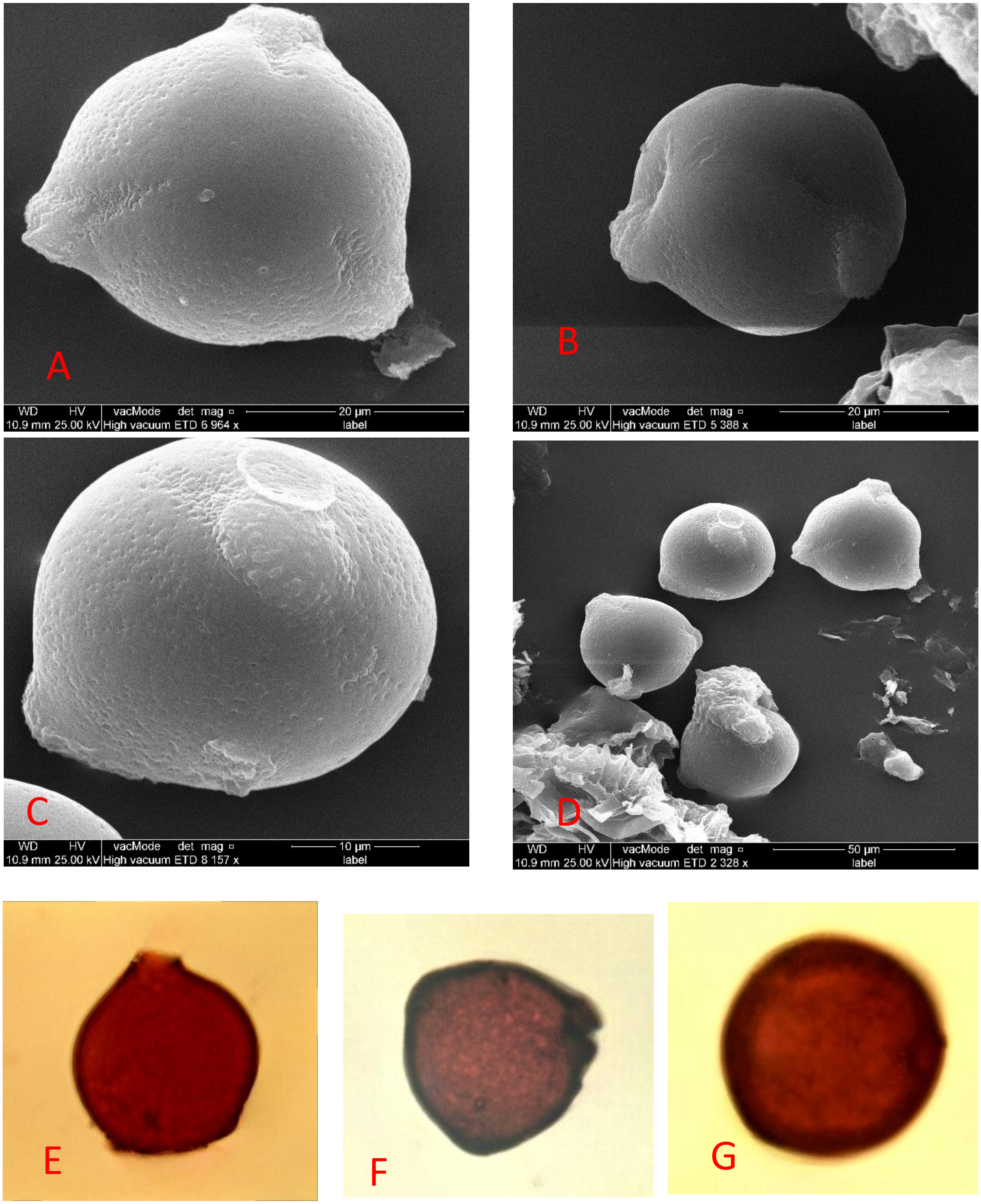

Figure 2: *Glycyrrhiza glabra* pollen grains in different view LM & SEM. A-Polar, B-Equatorial, C-

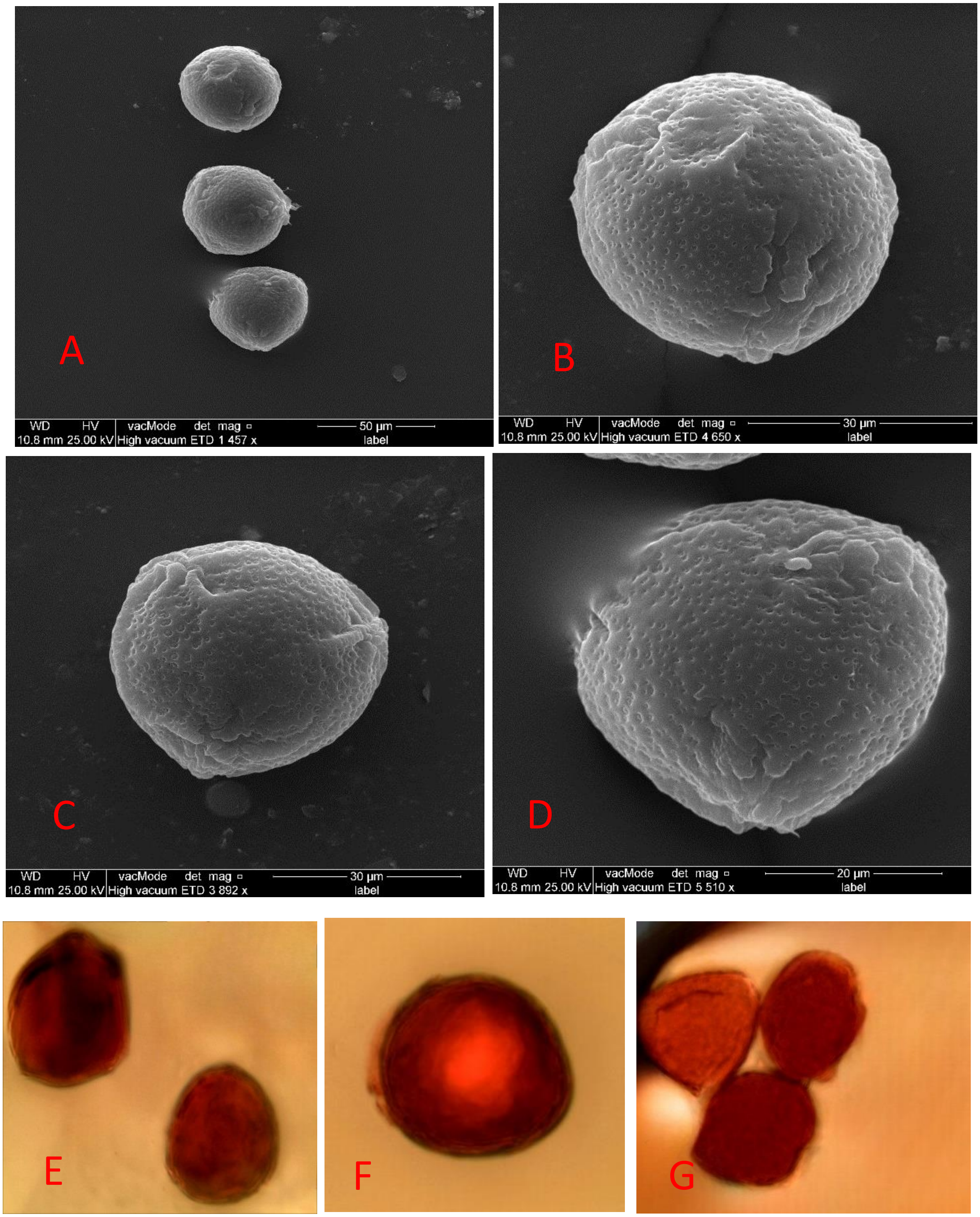

Figure 3: ***Spartium junceum*** **pollen grains in different view LM & SEM. A-Equatorial and Polar, B-Polar, C-Polar, D-Equatorial, E-Equatorial, F- Polar, G- Equatorial and Polar.**

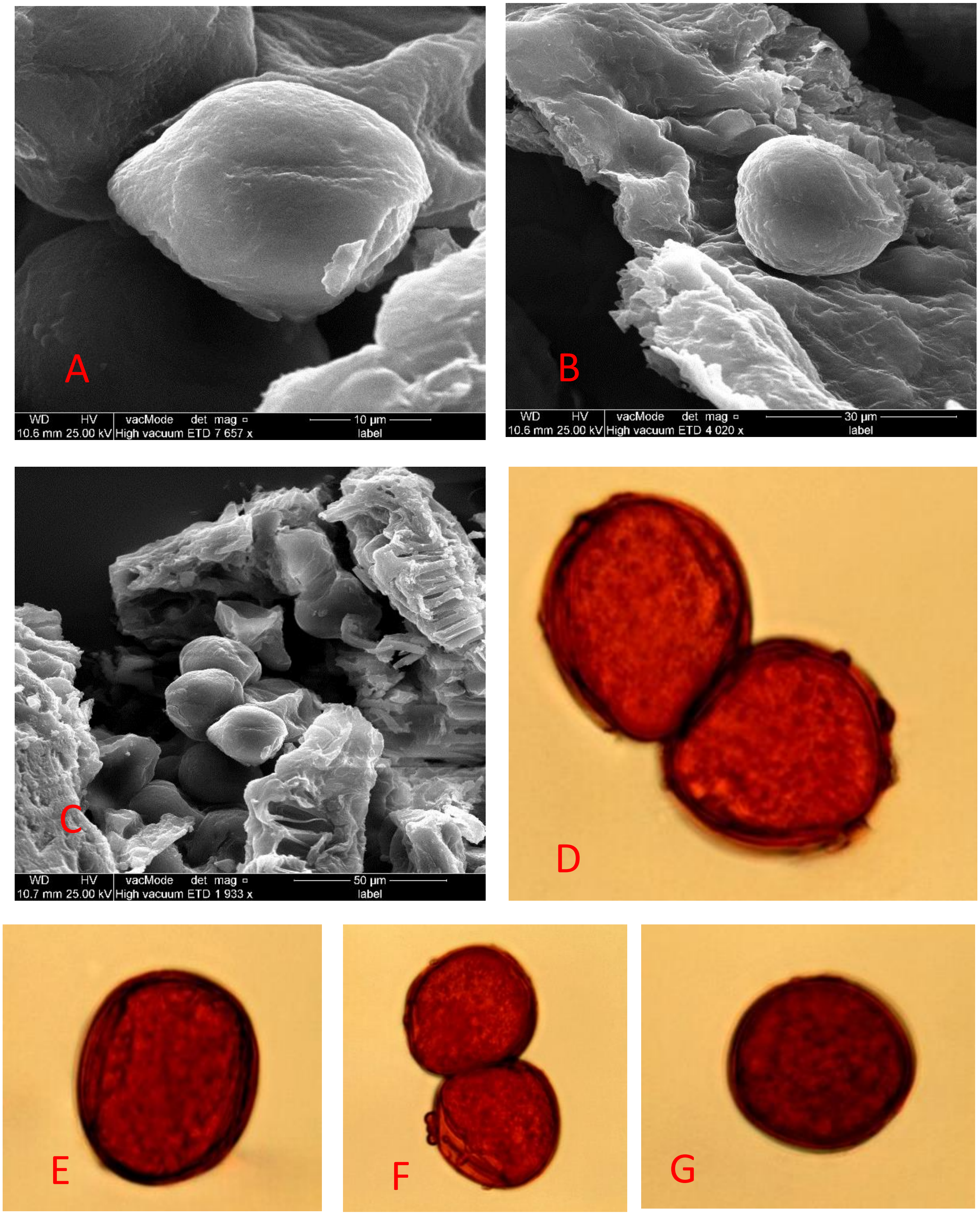

Figure 4: *Robinia pseudoacacia* pollen grains in different view LM & SEM. A-Equatorial, B-Equatorial, C-

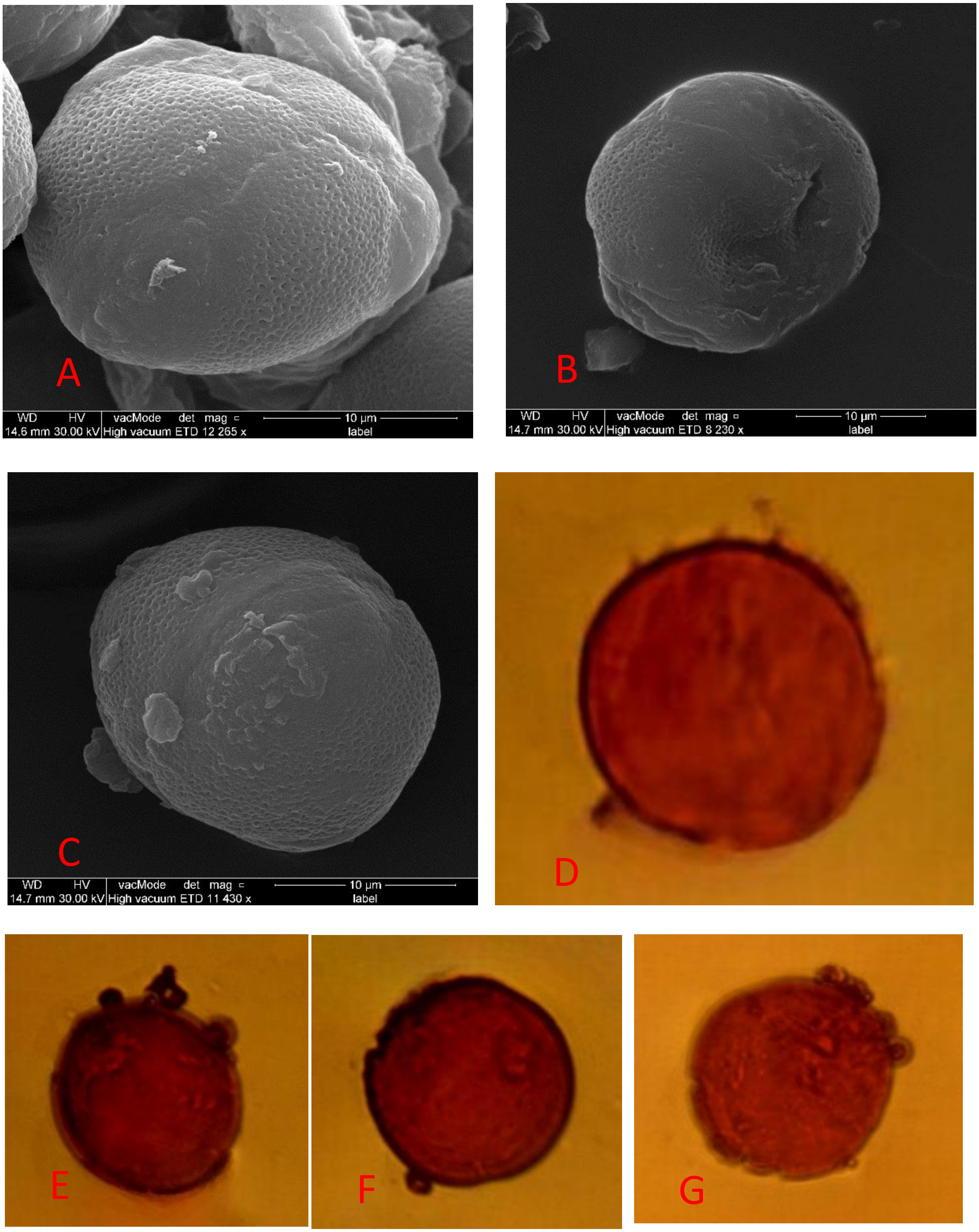

Figure 5: *Anagyris foetida* pollen grains in different view LM & SEM. A-Polar, B-Equatorial, C-Equatorial, D-Polar, E-equatorial. F-Polar G-Polar

Discussion

Table (1) in the research highlights the notable distinctions in pollen grains among various species, along with their consistent forms within each species. This recognition of diverse and visually appealing pollen forms captured the attention of eminent biological pioneers, as noted by [24]. However, the findings were met with mixed reactions. Some researchers encountered uncertainty in their observations, discovering instances where the pollen grains of seemingly unrelated plants exhibited identical appearances while those of closely related plants showed significant differences [25 and 26].

A comprehensive study revealed that although pollen grains from different plant species exhibited a range of shapes, these variations couldn't reliably be relied upon for indicating relationships or distinguishing species. This perspective was notably evident in their examination of the Gentianaceae family. Additionally, crucial divisions within the family and, at times, distinctions between various species were effectively illustrated through the characteristics of their pollen grains, as documented by [27].

Overall, the fundamental characteristics appear to exhibit a high degree of similarity across all taxa under study. The primary dispersal unit across the investigated taxa consistently presented as a single pollen grain (Monad). Among the twenty-five samples studied, encompassing five genera within the Fabaceae family, the pollen grains exhibited consistent traits. These traits included symmetry, monad structure, a 3-colporate configuration, and a sub-triangular or triangular-circular outline in the polar view. In the equatorial view, the pollen grains showed slightly less elliptical shapes.

Notably, the examination under a Light Microscope consistently revealed the presence of two layers within the Pollen grains, The intine and exine layers that demonstrated a relatively similar thickness. An interesting finding of this study was the identification of tricolpate pollen grains in some species and tricolporate pollen grains in others. The development of tricolporate pollen grains could potentially serve as an advanced indicator, as suggested by [28]. Consequently, this discovery reinforces the notion that morphological revisions of pollen can be supported by this particular observation.

The tricolpate aperture type is particularly prevalent among eudicots, whereas other aperture types like colpate, porate, and colporate are observed in dicots, as studied by [29]. In a broader context, the size of pollen grains holds significant importance, encompassing both small and medium-sized species under investigation [30]. Disparities in pollen sizes can be attributed to anomalies during meiosis, Beyond the characters of germination pores and exine cell wall ornamentation, pollen grain sizes hold tertiary significance, a perspective underscored by [30]. Additionally, in order to advance Quaternary palaeoecological, palaeoclimatological, and paleoenvironmental research, it is imperative to improve the taxonomic resolution of fossil pollen identification [31].

In this present study, the shape of pollen grains at both polar and equatorial outlines exhibited substantial variations across all observed species. The morphological characteristic of pollen shape has proven to be a potent tool in addressing taxonomic challenges, particularly at various levels, as confirmed by [32]. Furthermore, [33] painted the notable taxonomic significance of exine cell wall samples at the generic level, while the evolution of exine sculpturing holds a comparatively lesser role in the morphological development of pollen grains.

The study encompassed a total of 25 collected samples, with observations focused on key morphological aspects such as polar length (P), equatorial diameter (E), P/E ratio, and exine thickness. These parameters were assessed based on [34]'s classification of size classes, while shape classes were categorized following [20]'s. The standardized pollen terminology, as documented by [35], was followed throughout the study.

The findings revealed that some of the studied species, in accordance with [34]'s classification, exhibited medium-sized pollen grains, falling within the range of 25-50 µm, while others were characterized by small-sized pollen grains, falling within the range of 15-25 µm.

According to pollen shapes of [36] given in Table (1) may be divided into:

1. Oblate- spheroidal
   Anagyrus foitedia: P/E= 1.2
   Robinia pseudoacia: P/E= 1.1
   Spartium junecuim: P/E= 1.17

2. Prolate-spheroidal
   Cloutia cilicica: P/E =1.37
   Glychyrriza glabra: P/E= 1.4

Conclusion

Several species within the Fabaceae family were studied palynologically in order to determine their distinctive characteristics, both at the species and generic levels. The focus of this study was on the shapes and intricate patterns on the surfaces of pollen grains. This study aimed to determine how effectively morphological variations in pollen could identify unique attributes of Fabaceae species. Both light microscopy (LM) and scanning electron microscopy (SEM) were used in the examination.

The overarching objective was to evaluate the taxonomic relationships among different Fabaceae taxa based on pollen morphological data. With this approach, the study aimed to demonstrate how pollen grains can be used as taxonomic indicators. By discerning the variations in pollen characteristics, the research contributes to the ability to differentiate between closely related taxa.

This investigation has furnished valuable palynological insights that aid in the classification of Fabaceae taxa. The observed differences in pollen attributes can potentially serve as diagnostic traits, enabling the differentiation of closely related taxa. Ultimately, this study not only sheds light on the pollen morphology of Fabaceae species but also provides a foundation for understanding their taxonomic relationships.

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دراسة تصنيفية لحبات اللقاح لخمسة أجناس من العائلة البقولية

نازناز نعمة بابير [1]
Naznni3matB@gmail.com

علي ملا خضر گەلالەيي [2]
ali.galalaey@su.edu.krd

[1] قسم الغابات، كلية علوم الهندسة الزراعية، جامعة صلاح الدين، أربيل، العراق.
[2] قسم المحاصيل الحقلية والنباتات الطبية، كلية علوم الهندسة الزراعية، جامعة صلاح الدين، أربيل، العراق.

- تاريخ استلام البحث2023/09/19 وتاريخ قبوله 2023/09/29.
- البحث مستل من رسالة ماجستير للباحث الاول.

الملخص

استهدفت هذه الدراسة توفير خصائص قيمة لحبوب الطلع لتشخيص بعض أصناف العائلة البقولية، والتي يمكن أن تكون بمثابة سمات مميزة للتمايز بين الأنواع القريبة ذات الصلة الوثيقة. تم جمع عينات حبوب الطلع من 25 عينة لكل صنف من الأزهار الناضجة بالكامل لخمسة أنواع قيد الدراسة ضمن العائلة بقولية و شملت :
Colutea cilicica Boiss. & Balansa, *Glycyrrhiza glabra* L., *Spartium junceum* L., *Robinia pseudoacacia* L., and *Anagyris foetida* L.

من أجل تحقيق ذلك تم استخدام كل من المجهر الضوئي Light Microscopy (LM) والمجهر الالكتروني الماسح Scanning Electron Microscopy (SEM) في هذا البحث. وبالنتائج، أظهرت حبوب اللقاح المدروسة تماثلًا أحاديًا وكانت ذات أبعاد متوسطة إلى صغيرة. و أظهرت زخارف الجدران الخارجية للحبوب أنماطًا زخرفية متنوعة بما في ذلك الثقوب، و الاخاديد ، والشبكات، والهياكل المنتظمة، والتحبيبات. إضافة الى ذلك، تمت ملاحظة مجموعة واسعة من الأشكال التفصيلية لحبوب اللقاح في كل من المناظر الاستوائية والقطبية. تم إجراء فحوصات تفصيلية باستخدام كل من تقنيات المجهر الضوئي (LM) والمجهر الماسح الإلكتروني(SEM) ، حيث تم الكشف عن خصائص مميزة بين الأصناف التي تم فحصها.

الكلمات المفتاحية : حبوب لقاح، فتحات اخدودية، فتحات مثقبة، الانواع، بقولية