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Study of Palynological traits for some species of the genus *Onobrychis* Mill

Chnar N. Fathulla[1], Bnar kh. Bakr[2]

[1,2]*Department of Biology, Science College, Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq*

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ABSTRACT

Onobrychis is a legume, and it contains blooms. Their distribution spans the entire world. A total of eleven *O.* were investigated for their pollen size and characteristics as part of this research project. A light microscope and a scanning electron microscope were used to analyze specimens originating from Northern Iraq. After conducting research, all types of *O.* possessed the type of germination aperture, prolate, sub-prolate and prolate shapes, and reticulate perforate orientation. Characterization is performed on the *O. acaulis* and *O. galegiofolia*, which are prolate in shape. At the same time, *O. susiana* is subprolate. In contrast, other species, such as *O. schhuensis, O. ptolemaica, O. hausskni, O. megataphrois, O. caputigalli, O. aequidentata, O. haespindicala*, and *O. galegioides,* are prolate. Additionally, tricolpate pollen was observed in each plant species. The larger pollen grain in size is *O. Susiana* (1.16µm), and the smaller is *O. hausskni* (0.34 µm).

Keywords: Fabaceae pollen grains, palynology of *Onobrychis*, leguminosae species.

Name: Chnar N. Fathulla **E-mail:** chnar.hussain@su.edu.krd

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دراسة خصائص حبوب اللقاح لأنواع مختارة من *Onobrychis*

جنار نجم الدين فتح الله، بنار خالد بكر

قسم علوم الحياة، كلية العلوم، جامعة صلاح الدين، أربيل، اقليم كردستان، العراق

الملخص

يعد نبات *Onobrychis* من النباتات البقولية، ويحتوي على أزهار. وينتشر في جميع أنحاء العالم. تم فحص مجموعة من النباتات المتكونة مناحد عشر نباتًا من جنس *O.* لتشخيص حجم حبوب اللقاح وخصائصها كجزء من هذا البحث. تم استخدام المجهر الضوئي والمجهر الإلكتروني الماسح لتشخيص العينات التي جمعت في شمال العراق. بعد إجراء البحث؛ تبين ان جميع أنواع *O.* تمتلك فتحة الإنبات germination aperture, per-prolate, sub-prolate and prolate shapes؛ والنحت الخارجي هو شبكي مثقوب. تم وصف الانواع على أن *O. acaulis* و *O. galegiofolia* لهما شكل per-prolate، في حين أن *O. susiana* له شكل sub-prolate، وأنواع أخرى مثل *O. schhuensis* و *O. ptolemaica* و *O. hausskni* و *O. megataphrois* و *O. caputigalli* و *O. aequidentata* و *O. haespindicala* و *O. galegi* لها شكل prolate بالإضافة إلى ذلك، لوحظت انواع حبوب اللقاح يكون tricolpate. حبوب اللقاح الأكبر حجمًا هي *O. Susiana* (1.16 ميكرومتر)، والأصغر حجمًا هي *O. hausskni* (0.34 ميكرومتر).

INTRODUCTION

The legume family comprises approximately 727 genera and 19,325 species, has a worldwide distribution, and is of significant economic importance. The family exhibits high diversity across neotropical flora, particularly in dry tropical forests. The species exhibits significant morphological diversity across macro- and micromorphological traits, including pollen-derived traits. [1-3].

Onobrychis Miller is a highly challenging genius within the Fabaceae family. Currently, 162 species have been identified. The genus *Onobrychis* is limited to the Palearctic region, spanning the Mediterranean, the Caucasus, the Zagros Mountains, and Central Asia. Onobrychis is primarily found in Anatolia (52 sp.), Iran (53 sp.), and the Caucasus (39 sp.). Only eight species of this genus are found in the western Iberian Peninsula. According to [4] Iran and Anatolia are the primary regions with the highest levels of genetic diversity. The studies by (5, 6) demonstrate the significant taxonomic value of pollen morphology. So far, the majority of the assessed *Onobrychis* species are classified within the category of section *Onobrychis*. *Onobrychis* sp. were classified under the *Onobrychis stewartii* Baker type. The palynological properties of *Onobrychis* species remain incompletely assessed. The provided material pertains to the local analysis of Fabaceae pollen morphology. Some researchers were provided with the original description of pollen morphology in *Onobrychis*. In addition, a separate researcher studies many morphological characteristics of pollen grains, including the forms of their polar and equatorial outlines, the sorts of apertures they possess, and the ornamentation of their exine. This study specifically focused on five different species of *Onobrychis* found in Pakistan. A group of experts conducted a study on the morphological characteristics of pollen grains in 12 species of Bulgarian *Onobrychis*, focusing on the *Laphobrychis* and *Onobrychis* sections. Subsequently, the palynological characteristics of 20 Turkish *Onobrychis* species were assessed using light and scanning electron microscopy. A study conducted in Iran examined the morphology of

pollen grains from 20 Onobrychis species. The study focused on evaluating the palynological inconstancy of nine species, particularly, and characterized the properties of the pollen grains of *Onobrychis iranensis Amirab* and *Ghanavati*, as well as *Onobrychis tavernieraefolia* Stocks ex Boiss.

The study addresses the lack of detailed pollen morphology data for Iraqi Onobrychis species. It examines the size, exine structure, and sculpture of pollen from 11 taxa in Kurdistan, assessing their taxonomic importance and filling a regional knowledge gap that enhances species differentiation.

MATERIALS AND METHODS

The samples of plants were prepared from herbarium specimens. The collected specimens were stored in the herbarium of the Department of Biology/College of Science /University of Salahadin/Erbil/Iraq. The pollens collected from open flowers or mature buds were prepared for examination under a light microscope (LM) using the methods described by [7]. The SEM method was washed three times with phosphate-buffered saline (PBS), then dehydrated using a series of aceton concentrations ranging from 50% to 95%, followed by three additional washes in 100% aceton for 30 minutes. Afterward, the pollens were dried at a critical point and coated with gold using a sputter coater.[8], and observed under a Quanta 450 Scanning Electron Microscope (SEM) at Soran University in Duhok, Iraq.

RESULTS AND DISCUSSION

The pollen grains of the studied taxa exhibited monads, tricolpate, isopolar, and radially coordinate characteristics. The size of the pollen grains varied, with *O.. hausskni* having small grains (0.34 µm) and *O. Sausain* having large grains (1.16 µm), all the samples that prepare for light microscope examined and imaged by Light Microscopes (Olympus AC100 with a camera, Japanese made), measure the polar view (P), then measure the equatorial view (E), finally divide (P) on (E) to get the size of pollen grains The polar axis (P) ranged in length 5.1-44.9 µm, while the equatorial diameter (E) ranged 11.2 - 16.2 µm. All species exhibited reticulate-perforate exine ornamentation, as shown in Table 1. Figures 1, 2, 3, 4, 5, and 6 show the polar view and equatorial view of pollen grains, the shape of pollen grains and the orientation of the pollen grains; this shows the different variations of pollen grains that are utilized to differentiate between the species that are used to classify and identify them. The present study shows variation in pollen grain characters, such as the dimensions of the polar and equatorial views. The shape of pollen was prolate, perorate, sub-prolate and tricolpate, such as the shape of O. acaulis (0.74µm) and O. galegiofolia (0.64µm) are prolate, O. susiana is sub-prolate (1.16µm). At the same time, another species are prolate, as in *O.schhuensis* (0.58µm), *O. ptolemaica* (0.85µm), *O. hausskni* (0.34µm), *O. megataphrois* (0.73µm), *O. caputigalli* (0.48µm), *O. aequidentata* (0.59µm), *O. haespindicala* (0.71µm) and *O. galegi* (0.64µm), and also shows shallow and deep colpi, the exaine ornamentation was reticulate in studied species; these traits help to the steady the taxonomic position of the species with in the family.

Eight taxa of the *Onobrychis*, revealing that the pollen grains were 3-colpate, prolate, and perprolate. Palynological results showed oblong-elliptical pollen grains in equatorial view, as well as tricolpate and reticulate pollen grains with shallow or deep colpi. Two separate groups were identified based on pollen shape in polar view: 1) spherical-obtuse triangular pollen with shallow colpi, in which lumina diameter narrowed at the poles as in *O. arnacantha*, *O. amoena* subsp. meshedensis, *O. amoena* subsp. amoena, *O. chorassanica,* which were prolate, while *O. cornuta* and *O. ptycophylla* were perprolate; 2) obtuse triangular or triangular pollen with deep colpi, isodiametric lumina and perprolate in both varieties of *O. verae* [9]. A study was conducted on 31 species of the family Fabaceae. The study focused on the characteristics

of the pollen grains, which were found to be single and elongated, occasionally slightly prolate. The pollen grains had three colpi or occasionally three opercula. The exine exhibits several types of ornamentation, including microperforate, densely microperforate, psilate, perforate, polygonal perforate, macroperforate with granules in the holes, polygonal macroperforate, verrucate with pitted perforate striae, verrucate perforate, granulate, regulate, reticulate, or refined reticulate [10].

The characteristics of some taxa that belong to the tribe *Trifolieae*, which is part of the subfamily Papilionoideae, in Egypt [11]. Additionally, a study was conducted on 157 pollen morphotypes, designating 37 genera from the subfamily Papilionoideae in Pakistan. The shape is often elongated, either prolate or subprolate, and occasionally oblate spheroidal or suboblate. It is frequently problematic. The pollen grains exhibit radial symmetry, are isopolar trizonocolporate, and occasionally have colpate or porate structures with a perforate tectum. They also vary in shape and sculpting. The ora typically has an elongated or circular shape, with a rare occurrence of being elongated in a lopsided manner. The Colpal membrane has a psilate or somewhat ornamented surface. The sexine is equal to or thicker than the nexine. The reticulate tectum type is the most prevalent. [12].

The pollen grains of 11 species from Pakistan, belonging to 5 genera of the Caesalpinioideae subfamily (Fabaceae), are typically radially symmetrical, isopolar, tricolporate, and triangular trilobed. The technique typically exhibits a reticulate-rugulate or fossulate-foveolate texture and is frequently striated. The subfamily's pollen morphology is quite valuable for distinguishing among genera and species. Based on the characteristics of apocolpium, mesocolpium, and tectum, three varieties of pollen were identified as Bauhinia variegata, Caesalpinia pulcherrima, and Senna holosericea [1, 13-15].

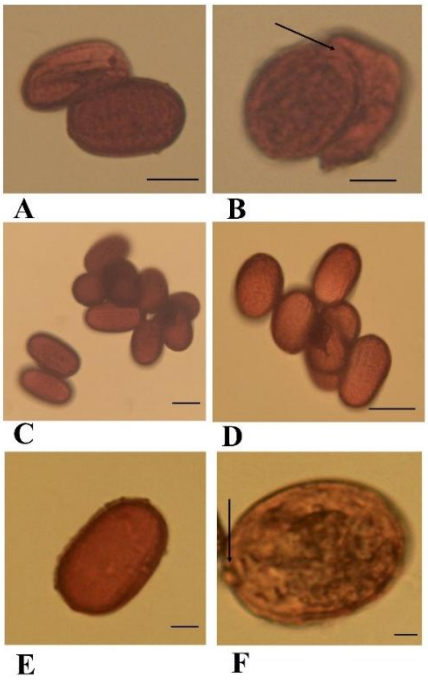

Fig.1: Pollen grains (Light microscope). A. equatorial view of *Ononis acaulis*, B. polar view of *Ononis acaulis*, C. equatorial and polar view of *Onobrychis schhuensis*, D. equatorial and polar view of *Onobrychis ptolemaica,* E. equatorial view of *Onobrychis hausskni*, F. polar view of *Onobrychis hausskni*. Pore: (Small black arrow) A,B,C,D,E,F=40X.

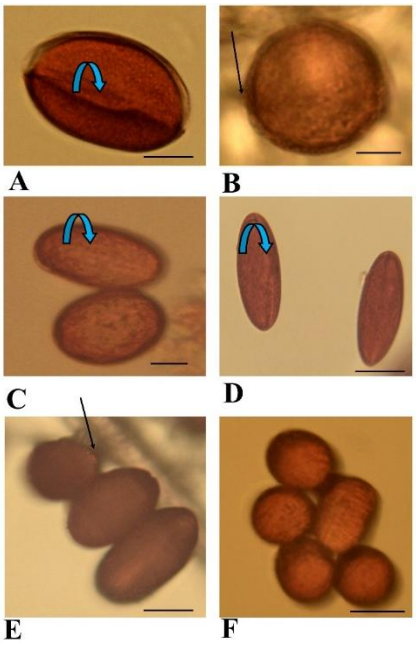

Fig.2: Pollen grains (Light microscope). A. equatorial view of *Onobrychis megataphrois*, B. polar view of *Ononis megataphrois* , C. equatorial and polar view of *Onobrychis caputigalli*, D. equatorial view of *Onobrychis aequidentata,* E. polar view of *Onobrychis aequidentata* , F. equatorial and polar view of *Onobrychis galegiofolia*. Pore: (Small black arrow) A,B,C,D,E,F=40X.

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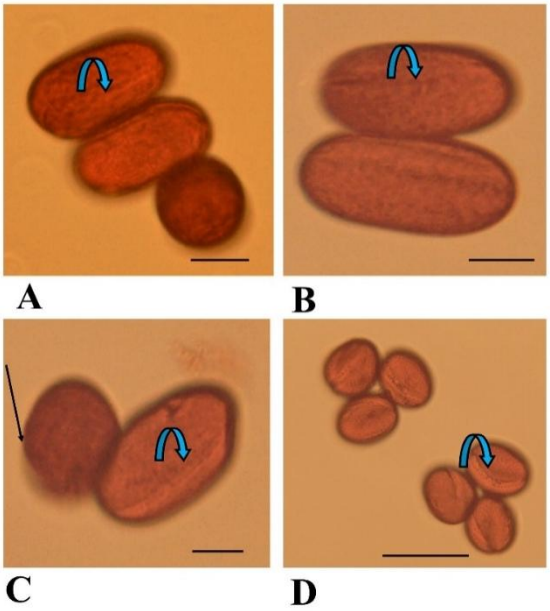

Figure 3: Pollen grains (Light microscope). A. equatorial and polar view of *Onobrychis haespindicala*, B. equatorial viewof *Onobrychis galegi,* C. equatorial and polar view of *Onobrychis galegi,* D. equatorial and polar view of *Onobrychis susiana*. colpus: Pore: (small black arrow), (large arrow). A,B,C,D=40X.

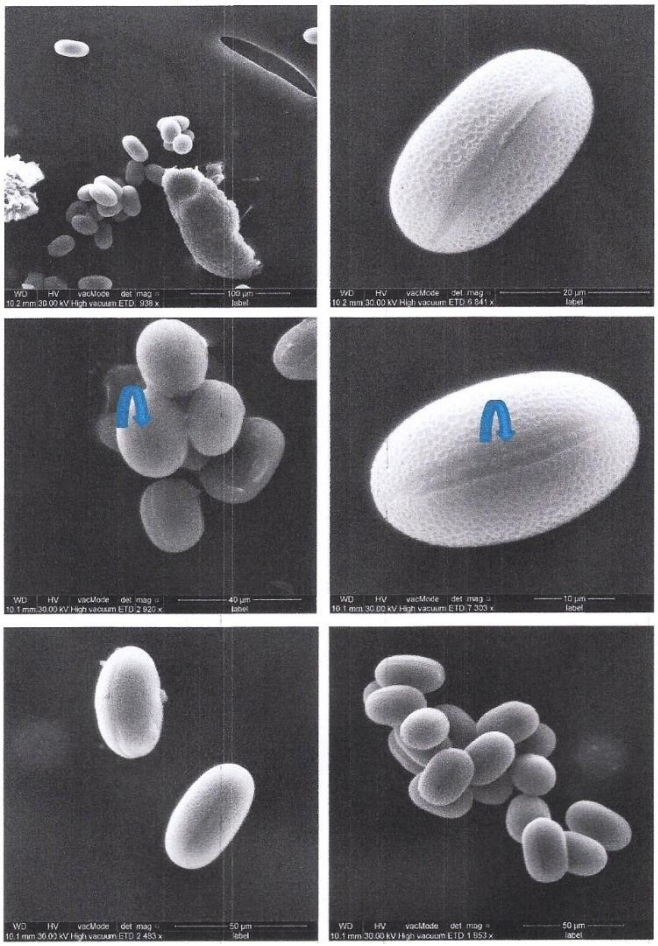

Figure 4: Pollen grains (Scan Electron Microscope). A. *Onobrychis acaulis, B. Onobrychis acaulis, C. Onobrychis schhuensis, D. Onobrychis ptolemaica, E. Onobrychis hausskni,* F. orientation of pollen grains. colpus: (large arrow).

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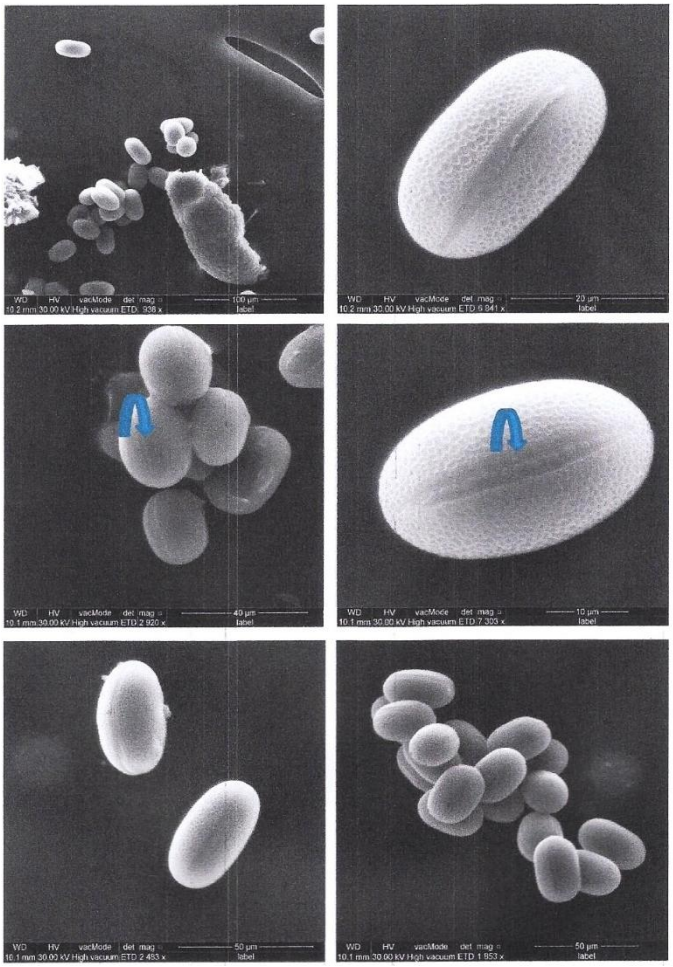

Fig. 5: Pollen grains (Scan Electron Microscope). A,B. *Onobrychis megataphrois*, C,D. *Onobrychis caputigalli*, E. *Onobrychis aequidentata*, F. *Onobrychis galegiofolia*. colpus: (large arrow).

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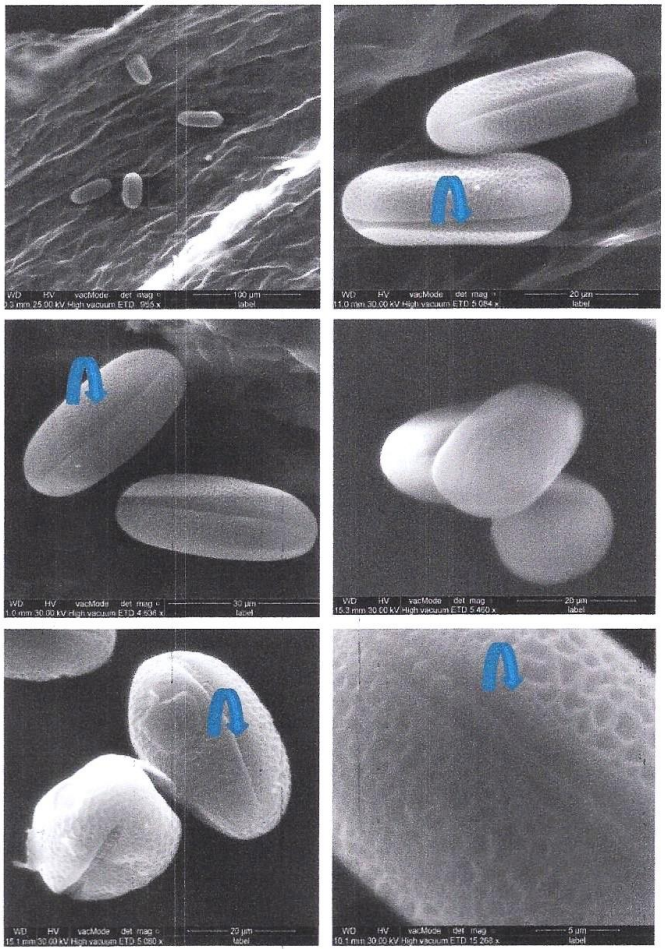

Fig. 6: Pollen grains (Scan Electron Microscope). A,B, *Onobrychis haespindicala*, C,D. *Onobrychis galegi*, E. *Onobrychis susiana*. F. orientation of pollen grains. colpus: (large arrow).

Table 1: Morphological characteristics of pollen grains

Taxon / traits	Polar view µm	Equatorial view µm	P/E	Shape	Orientation
Onobrychis acaulis	8.66±1.4	11.76±2.4	0.74	Per-prolate	Reticulate-perforate
O. schhuensis	7.64±1.9	13.08±2.6	0.58	prolate	Reticulate-perforate
O. ptolemaica	9.33±1.8	11±2.2	0.85	prolate	Reticulate-perforate
O. hausskni	5±1.0	14.9±2.9	0.34	prolate	Reticulate-perforate
O. megataphrois	10.25±2.1	14±2.8	0.73	Prolate	Reticulate-perforate
O. caputigalli	6.75±1.35	14±2.8	0.48	Prolate	Reticulate-perforate
O. aequidentata	10.25±2.1	17.25±3.5	0.59	Prolate	Reticulate-perforate
O. galegiofolia	9.25±1.85	14.5±2.9	0.64	Per-prolate	Reticulate-perforate
O. haespindicala	9.75±1.95	13.75±2.75	0.71	Prolate	Reticulate-perforate
O. galegi	10.75±2.15	16.67±3.3	0.64	prolate	Reticulate-perforate
O. susiana	44.93±1.7	16.67±2.1	1.16	Sub-prolate	Reticulate-perforate

CONCLUSION

The *Onobrychis* Species possess pollen grains that are elongated and prolate, and tricolpate with reticulate perforate orientation.

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Conflict of interest: There is no known conflict of interest for the found work.

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Author contribution: Authors contributed equally to the study.

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