

Pollen Morphology of the Genera *Onobrychis* (*Hedysareae*, *Fabaceae*) in Iran

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Abstract

The *Onobrychis* is the genus which can be mostly found in the west Asia and the eastern Mediterranean and it belongs to *Hedysareae* tribe. This study investigated the pollen morphology of 17 taxa of *Onobrychis* Adans in Iran, using scanning electron microscopy. The size of pollen is medium or large; ornamentation is reticulate with tricolpate zonoapreturate shape. Some differences in dimensions, exine stratification, and aperture shape are observed. In polar and equatorial views, one pollen type and 3 subtypes are identified in terms of pollen size, shape and ornamentation. There is a close relationship, as it is supported by pollen morphology, among the species of genera.

Key Words: *Fabaceae*, Iran, *Hedysareae*, *Onobrychis*, Pollen Morphology.

INTRODUCTION

Leguminosae is one flowering plant and more than 50% of species of this family belong to subfamily *Papilionoideae*. Hutchinson, Benth and Hooker (1880) and Polhill and Raven (1981) divide *Papilionoideae* into 33, 11.6 and 24 tribes respectively. According to Polhill and Raven *Onobrychis* forms the major part of the tribe *Hedysareae* DC. The center of its variety is found in the west Asia and the eastern Mediterranean area and about 130 species of this type mostly covers northern mild regions. Some taxa are grown as fodder or ornamentals [1-2]. Rechinger (1984) classified 77 species in Flora Iranica under 9 sections including 7 species of *Dendrobrychis*, 5 species of *Lophobrychis*, 14 species *Onobrychis*, 3 species of *laxiflorae*, 7 species of *Anthyllium*, 3 species *Afghanicae*, 3 species *Insignes*, 21 species *Heliobrychis* and 12 species of *Hymenobrychis*, with two unnamed species. Although several studies of the family *Fabaceae* [3-5] have been done, there is little investigation regarding the pollen morphology of *Onobrychis*. Faegri (1956), Faegri and Iversen (1989) and Moore et al. (1991) have attributed the pollen grains of *Onobrychis* to a particular type (*Onobrychis* type). Such a conclusion is based on 3 apertures and suprareticulate ornamentation of

the exine. Later figures like Ferguson and Skvarla (1981), Guinet (1981), Polhill and Raven (1981), Guinet and Ferguson (1989), Moore et al (1991) and Reille (1992) completed the original description of the pollen morphology [6-8].

MATERIALS AND METHODS

The pollen material needed for this study was gained from populations of field and from collections in the Herbaria of Islamic Azad university, Science and Research Branch, Tehran (IZ) and Research Institute of Forests (IF) and Ranger lands (Table 1). In standard way the pollen was acetolysed (Erdtman, 1960). Fifty measurements of six characters at a magnification of x 1280 were done. The six measured characters were P (polar diameter), E (equatorial diameter), L (colpus length), S (colpus width), M (mesocolpium), A (apocolpium), and the P/E ratio. Pollen grains were coated with gold with a Hummel VII sputter coater and observations were made using a Philips model XL30 Scanning Electron Microscopy (SEM) at the Department of Biology, Modares University. The pollen terminology of Faegri and Iversen (1989) and Punt et al. (1994) was also used the botanical nomenclature of Kozhukharov (1976, 1992) was adopted.

Table 1. List of studied populations of *Onobrychis*

Taxon	Locality	Voucher Number
<i>O.altissima</i> (Wild)	Khoramabad, Lorestan, Iran.	IAUH:9999
<i>O.altissima</i> (Genotype)	Sanandaj, Kordestan, Iran.	IAUH:9970
<i>O.viciifolia</i> (Genotype 1)	Feridonshahr, Esfahan, Iran.	IAUH:9969
<i>O.viciifolia</i> (Wild)	Zanjan, Iran.	IAUH:9998
<i>Onobrychisbungei</i> Boiss .	Khas-Tayabad road, Khorassan, Iran.	TRAI:42213
<i>Onobrychis caput-galli</i> (L.) Lam .	Oshterankohe, Lorestan, Iran.	IAUH:9996
<i>Onobrychiscornuta</i> (L.) Desv .	Zanjan-Tarom road, Zanjan, Iran.	IAUH:9991
<i>Onobrychiscyri</i> Grossh .	Bandarabase-sirjan road, Hormozgan, Iran.	TRAI:24673
<i>Onobrychismejor</i> (Boiss.) Hand.	55Km Tabriz-Marand road, east Azarbayjan, Iran	IAUH:9989
<i>Onobrychismichauxii</i> DC .	Kohin, Qazvin, Iran.	IAUH:9990
<i>Onobrychismicrantha</i> Schrenk .	Khas-Tayabad road, Khorassan, Iran.	TRAI:84619
<i>Onobrychisnummularia</i> Boiss .	Bandarabase-sirjan road, Hormozgan, Iran.	TRAI:42206
<i>Onobrychispersica</i> Sirj.&Rech. F.	Kohin, Qazvin, Iran.	IAUH:9988
<i>Onobrychissintenissii</i> Bornm .	Gavazang, Zanjan, Iran.	IAUH:9995
<i>Onobrychistranscaspica</i> V. V. Nikitin .	Arasbaranprotected area NW of veinagh, Ardebil, Iran.	TRAI:20519
<i>Onobrychistavernieraefolia</i> Stocks ex Boiss.	Khash, Baluchestan, Iran.	TRAI:42756
<i>Onobrychisviciifolia</i> (Gentype2) .	Miyandoab, west Azarbayjan, Iran.	IAUH:9987

RESULT

SEM results showed thatexine sculpture to be scabrate and microechinate, and the tectum is perforate (Tab. 2, Figs. 1-7).The polar axis (P) ranges from19.1±1.9 to 36.2±2.6 μm the equatorial axis ranges from 9.4±2.4 to 18.7±1.9 μm .The polar axis is longest in *Onobrychisviciifolia* Genotype1(36.2 μm) and shortest in *Onobrychismicrantha* (18.7 μm); the equatorial axis is longest in *Onobrychisviciifolia* Genotype1(51.22 μm) and shortest in *Onobrychispersica* (9.4 μm). (Table 2). *Onobrychis* pollen grains are classified into 3- colpate, prolate and perprolate. The ectocolpi pollen subtype narrowing at the polesare made longer superficially or deeply and large granules cover colpus memberance. *Onobrychis* has reticulate ornamentation with different shapes. The pollen grains in equatorial view are longer and have oval to rectangular shape, while they have circular and triangular shape in polar view. Based on pollen size,shape in polar and equatorial view, and ornamentation, one pollen type and three tentative subtypes could be recognized. One pollen type and three subtypes can be identified in terms of characteristics like size, shape and ornatination

Subtype I

The diameter of polar in subtype I is less than 25 μm with reticulate ornamentation. The shape of grains in pollen view is triangular where asvaries from rectangular to oval. Ectocolpi is direct and sculptural elements with different sizes cover the membrane of colpus. Pollen grains prolate (*O.micrantha*) and prelate (*O.michauxii*). P/E=1.5-1.7, dimensions P×E=19.1-20.1×11.7-12.5 μm . (Tab:2, Fig.1A-G).

Subtype II

In polar view, the grains and colpi are circular and shallow respectively, the diameter is 25 μm with reticulate ornamentation. The size of lumina of the reticulum at the end margin of colpi decreases. In equatorial view the pollen grains have long oval shape.Pollen grains prolate (*O.cyri*,*O.mejor*) and prelate (*O.persica*). P/E=1.6-2.7,

dimensions P×E=23.1-36.2×18.7-9.4 μm . Large and small sculptural elements cover the membrane of colpus and ectocolpi are long and superficial. Although the pollen grains of some species like *Onobrychisviciifolia* Genotype1, *O. bungei*, *O. cyri*, *O. viciifolia* (Wild), *Onobrychisviciifolia* Genotype2, *O.cornuta*, *O. sintenissii*, *O. nummularia*, *O. mejor* and *O. persica* belong to the second subtype; there are some differences among them. The grains of *Onobrychisviciifolia* Genotype1are the largest and the smallest *O. persica*. The *O. nummularia*ornamentation due to existence of perforations around the openings is different from the other subspecies, eventhough it is similar closely to *Onobrychisviciifolia* Genotype2.(Tab2, Fig.2,3,4,5).

Subtype III

In polar view, the shape of grains is triangular, the diameter is a bove 25 μm with reticulate ornamentation and deep colpi. The size of the reticulum lumina at the end margin of the colpi is the same. The pollen grains of the following species and subspecies like *Onobrychis caput-galli*, *O. altissima* (wild), *O. altissima* (Genotype), *O. transcaspica*and *O. tavernieraefolia* are included in this subtype. The size of pollen grains is large or medium, P/E=2.1-2.6, dimensions P×E=32.7-35.9×13.5-16.2 μm . In polar view, the shape of pollen grains (*O.transcaspica*) is triangular-obtuse or triangular while in equatorial view whose shape is long elliptical to rectangular- obtuse. Large and small sculptural elements coat the membrane of colpus with long and deepectocolpi. L×W=28.1-33.1×0.4-0.8. The size and shape of lumina in the intercolpium and at the end margin of the colpi are the same, with reticulate ornamentation. The largest lumina has about 1.5-2 μm diameter. The developed new species of part *Lophobrychis* (*O.caput-galli*) and *Afghanicae* (*O. tavernieraefolia*) some elementary species of part *Onobrychis* (*O.transcaspica*) are studied in this paper. The colpi of the grains are the longest, narrow and nearly closed. The apocolpiumin *O.altissima* (Genotype), and mesocolpium have the lowest values in *O. tavernieraefolia* (Tab2, Fig.6,7).

Table 2. Taxa examined for pollen subtypes, with measurements(μm) of the mean and ranges for the polar(P) and equatorial(E) axes, length(L) and width(W) of the colpusmesocolpium(M) and apocolpium(A) and the shape index(P/E)

Subtypes & taxa	P	E	L	w	M	A	P/E
Subtype I							
<i>O. michauxii</i>	20.1 \pm 2.1	11.7 \pm 1.6	16.8 \pm 3.1	1.2 \pm 0.3	8.3 \pm 1.8	4.6 \pm 0.3	1.7 \pm 0.2
<i>O. micrantha</i>	19.1 \pm 1.9	12.5 \pm 1.4	15.1 \pm 2.6	1.1 \pm 0.2	12.1 \pm 1.1	3.5 \pm 0.9	1.5 \pm 0.1
Subtype II							
<i>O. cyri</i>	24.7 \pm 2.7	15.2 \pm 1.4	24.6 \pm 1.2	2.1 \pm 0.1	10.5 \pm 0.9	2.4 \pm 0.2	1.6 \pm 0.3
<i>O. bungei</i>	26.9 \pm 2.6	14.4 \pm 1.4	24.2 \pm 1.3	1.1 \pm 0.2	11.3 \pm 1.1	1.3 \pm 0.1	1.9 \pm 0.2
<i>O. persica</i>	25.7 \pm 1.4	9.4 \pm 2.4	20.7 \pm 1.5	1.1 \pm 0.1	10.6 \pm 0.9	2.1 \pm 0.6	2.7 \pm 0.4
<i>O. mejor</i>	24.9 \pm 2.5	14.7 \pm 1.8	19.8 \pm 2.4	1.7 \pm 0.2	10.7 \pm 0.8	1.8 \pm 0.4	1.7 \pm 0.1
<i>O. nummularia</i>	26.5 \pm 2.3	12.5 \pm 1.6	23.3 \pm 1.6	0.4 \pm 0.08	8.8 \pm 0.6	3.4 \pm 0.9	2.1 \pm 0.7
<i>O. sintenisii</i>	27.8 \pm 2.1	13.3 \pm 1.4	23.9 \pm 2.6	1.6 \pm 0.3	7.6 \pm 0.7	3.1 \pm 0.8	2.1 \pm 0.2
<i>O. cornuta</i>	23.1 \pm 1.8	13.9 \pm 1.5	21.1 \pm 2.1	1.8 \pm 0.2	9.8 \pm 0.9	3.1 \pm 0.8	1.7 \pm 0.1
<i>O. viciifolia</i> (Genotype 2)	31.7 \pm 1.4	14.5 \pm 2.1	27.9 \pm 1.8	1.7 \pm 0.2	10.2 \pm 1.2	2.8 \pm 0.8	2.2 \pm 0.2
<i>O. viciifolia</i> (wild)	30.9 \pm 2.3	15.2 \pm 1.9	27.7 \pm 2.4	2.3 \pm 0.1	8.1 \pm 1.3	2.1 \pm 0.7	2.1 \pm 0.1
<i>O. viciifolia</i> (Genotype 1)	36.2 \pm 2.6	18.7 \pm 1.9	33.0 \pm 1.5	1.6 \pm 0.04	12.1 \pm 0.4	4.1 \pm 0.6	1.9 \pm 0.5
Subtype III							
<i>O. altissima</i> (wild)	35.9 \pm 2.2	15.1 \pm 0.9	31.3 \pm 2.6	0.8 \pm 0.05	11.3 \pm 1.1	4.2 \pm 1.1	2.3 \pm 0.8
<i>O. altissima</i> (Genotype 1)	35.2 \pm 2.6	13.5 \pm 0.8	33.1 \pm 2.1	0.4 \pm 0.07	9.2 \pm 1.1	3.3 \pm 0.2	2.6 \pm 0.6
<i>O. caput-galli</i>	33.5 \pm 1.8	16.2 \pm 1.4	28.1 \pm 2.4	0.5 \pm 0.05	11.1 \pm 1.1	2.9 \pm 0.3	2.1 \pm 0.2
<i>O. transcaspica</i>	32.7 \pm 2.3	13.8 \pm 1	29.3 \pm 1.6	0.6 \pm 0.04	10.7 \pm 0.7	3.3 \pm 0.3	2.4 \pm 0.2
<i>O. tavernieraefolia</i>	34.5 \pm 2.1	14.2 \pm 1.3	31.2 \pm 1.7	0.7 \pm 0.07	10.2 \pm 0.6	2.8 \pm 0.3	2.4 \pm 0.1

Table 3. Morphological parameters of *onobrychis* species pollen grains: section(S) pollen type(P.T) sculpture type (S.T) space between sculpture (S.S) diameter of the largest lumina(D.L) pollen grain in polar view (P.P) depth of lumina(D.L) sculpture inside of lumina (S.L) sculpture inside of colpi (S.C) depth of colpi (D.C).

Subtypes & taxa	S	P.T	S.T	S.S	D.L	P.P	L.M	C.M
Subtype I								
<i>O. michauxii</i>	Hymenobrychis	Porate	Reticulate clavate	0.1-0.3	0.6-0.7	Circular	Perforate	Sinuate Tubercle
<i>O. micrantha</i>	Lophbrychis	Porate	Reticulate	0.2-1	0.7-2	Circular	Tubercle	Tubercle striat
Subtype II								
<i>O. cyri</i>	Onobrychis	Porate	Reticulate perforate	0.2-0.5	0.4-0.7	Circular	Perforate	Tubercle striat & granulate
<i>O. bungei</i>	Onobrychis	Porate	Reticulate granulate	0.05-0.2	0.7-1	Triangular	microgranulate	Sinuate Tubercle
<i>O. persica</i>	Onobrychis	Perporate	Reticulate perforate	0.5-1	0.7-1	Circular	Perforate	Tubercle striat
<i>O. mejor</i>	Onobrychis	Porate	Reticulate perforate	0.2-0.6	0.7-1	Circular	Shallow Perforate	Sinuate Tubercle
<i>O. nummularia</i>	Afghanicae	Perporate	Reticulate micro granulate	0.2-0.5	0.7-1.2	Triangular	Tubercle	Sinuate Tubercle
<i>O. sintenisii</i>	Hymenobrychis	Perporate	Reticulate	0.1-0.2	0.7-1.2	Circular	Perforate Shallowly	Sinuate Tubercle
<i>O. cornuta</i>	Dendrobrychis	Porate	Reticulate	0.05-0.1	0.7-1	Circular	Perforate granulate	Densely Tubercle
<i>O. viciifolia</i> (Genotype 2)	Onobrychis	Perporate	Reticulate micro granulate	0.2-0.7	0.7-1.2	Triangular	Tubercle	Sinuate Tubercle
<i>O. viciifolia</i> (Wild)	Onobrychis	Perporate	Reticulate perforate	0.1-0.3	0.7-1	Circular	Perforate	Sinuate Tubercle
<i>O. viciifolia</i> (Genotype 1)	Onobrychis	Porate	Reticulate	0.2-0.4	0.7-1	Circular	Punctuate	Sinuate Tubercle
Subtype III								
<i>O. altissima</i> (Wild)	Onobrychis	Perporate	Reticulate	0.05-0.1	0.7-1.2	Triangula	Punctuate	Smooth deep
<i>O. altissima</i> (Genotype)	Onobrychis	Perporate	Reticulate micro granulate	0.3-1	0.7-1.2	Triangula	Tubercle	Smooth deep
<i>O. caput-galli</i>	Lophbrychis	Perporate	Reticulate	0.1-0.2	1.2-2	Triangula	geranulat	Smooth deep
<i>O. transcaspica</i>	Onobrychis	Perporate	Reticulate	0.1-0.4	1.2-2	Triangula	Tubercle	Smooth deep
<i>O. tavernieraefolia</i>	Afghanicae	Perporate 3	Reticulate micro granulate	0.1-0.2	0.7-1	Triangula	Tubercle	Smooth deep

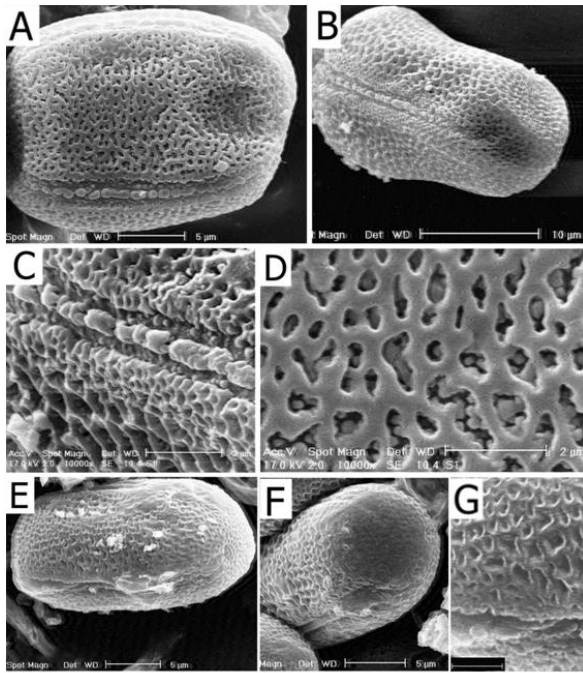


Figure 1. Scanning electron micrographs of pollen grains. A-D: *Onobrychismicrontha*. A-B: Equatorial view and colpus. C-D: Colpus and ornamentation. E-G: *O. michauxi*. E: Equatorial view and colpus. F: Polar view and apocolpium. G: Colpus and ornamentation. Scale bar= 2μm.

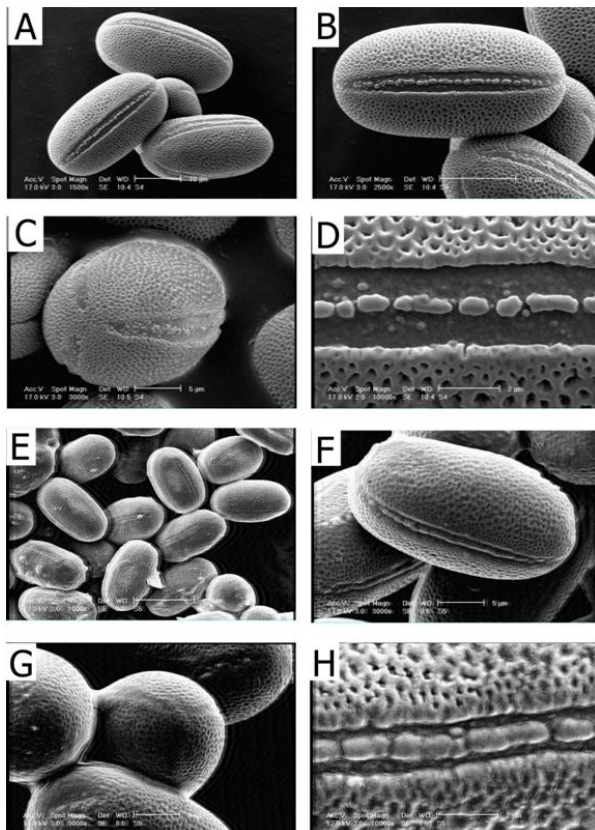


Figure 2. Scanning Electron Micrographs of pollen grains. A-D: *Onobrychiscyri*. A: Colpus view and equatorial view. B: Equatorial view and colpus. C: Polar view and ornamentation in apocolpium. D: Colpus and ornamentation. E-H: *O. mejor*. E: Polar view and equatorial view. F: Colpus view and equatorial view. G: Polar view. H: Colpus and ornamentation.

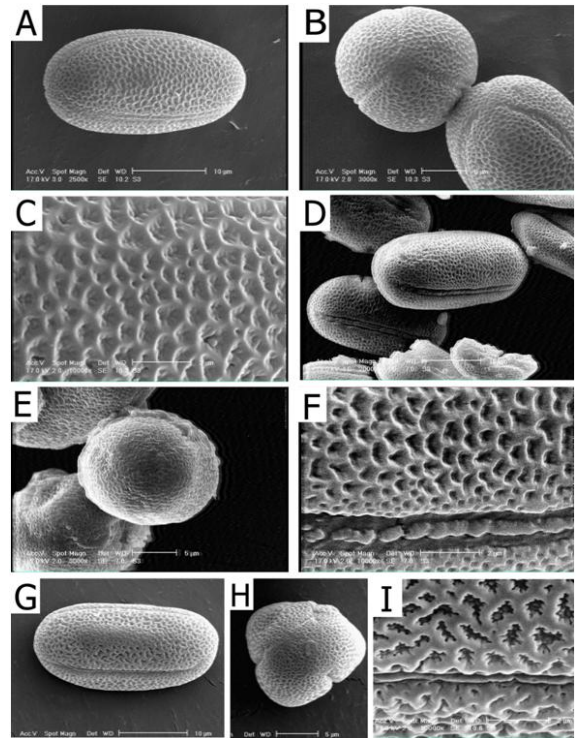


Figure 3. Scanning Electron Micrographs of pollen grains. A-C: *Onobrychisbongei*. A: Equatorial view and colpus. B: Polar view and apocolpium. C: Ornamentation. D-F: *O. persica*. D: Equatorial view and colpus view and apocolpium. E: Polar view. F: Colpus and ornamentation. G-I: *O. nummularia*. G: Equatorial view and ornamentation. H: Apocolpium and ornamentation. I: Colpus and ornamentation.

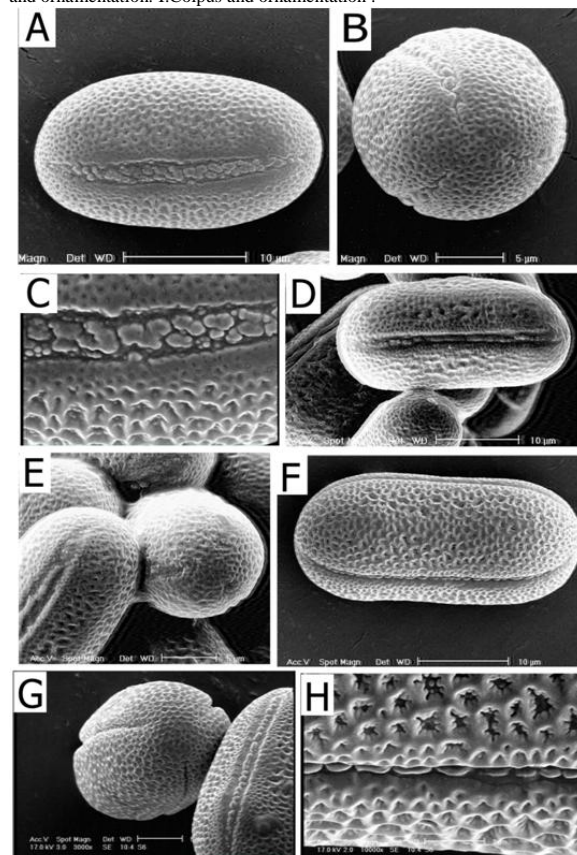


Figure 4. Scanning Electron Micrographs of pollen grains. A-C: *Onobrychiscornuta*. A: Equatorial view and ornamentation. B: Apocolpium, colpus and ornamentation. C: Colpus and ornamentation. D-E: *O. sintenissi*. D: Equatorial view and colpus. E: Polar view. F-H: *O. vicifolia*. F: Equatorial view and colpus ornamentation. G: Polar view and colpus. H: Colpus and ornamentation.

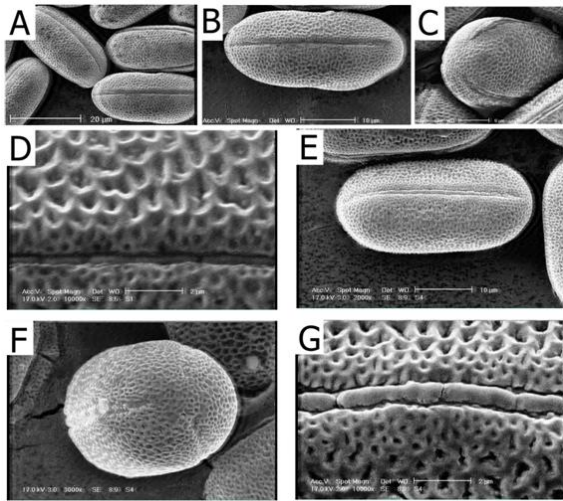


Figure 5. Scanning Electron Micrographs of pollen grains. A-D: *Onobrychisalbassplaconica*. A: Equatorial view and colpus. B: Equatorial view and apocolpium. C: Colpus and ornamentation. D: Colpus and ornamentation. E-G: *O.altissima*. E: Equatorial and colpus ornamentation. F: Polar view and ornamentation. G: Colpus and ornamentation.

Key to the species groups

- 1+ Colpus length less than 25µm-----2
- 1- Colpus length a bove 25µm-----3
- 2+ Diameter of the largest lumina about 0.1-0.7µm-----*O.michauxii*
- 2- Diameter of the largest lumina about 0.7-2µm-----*O.micrantha*
- 3+ Pollen grain circular, with shallow colpi in polar view-----4
- 3- Pollen grain triangular-obtuse or triangular in polar view with deep colpi-----13
- 4+ Pollen grain prolate-----15
- 4- pollen grain perporalate-----9
- 5+ Pollen grain triangular-obtuse in polar view-----6
- 5- Pollen grain triangular in polar view-----*O. bungi*
- 6+ Diameter of the largest lumina about 0.1-0.7µm-----*O.mejor*
- 6- Diameter of the largest lumina about 0.7-2µm-----7
- 7+ Width of the colpus 1.3-1.8µm-----8
- 7- Width of the colpus 1.8-2.3µm-----*O. cyri*
- 8+ Colpi without distinct colpal margins-----*O.albassplaconica*
- 8- Colpi with distinct colpal margins-----*O. cornuta*
- 9+ Pollen grain triangular-obtuse in polar view-----10
- 9- Pollen grain triangular in polar view-----*O. nummularia*
- 10+ Diameter of the largest lumina about 0.5-0.75µm-----11
- 10- Diameter of the largest lumina about 0.75-1.5µm-----12
- 11+ Colpi without distinct colpal margins-----*O.persica*
- 11- Colpi with distinct colpal margins-----*O.altissima*
- 12+ Colpi without distinct colpal margins-----*O. simenissii*
- 12- Colpi with distinct colpal margins-----*O.viciifolia*
- 13+ Pollen grain triangular-obtuse in polar view-----14
- 13- Pollen grain triangular in polar view-----16
- 14+ Diameter of the largest lumina about 0.1-0.3µm-----*O. transcaspica*
- 14- Diameter of the largest lumina above 0.3µm-----15
- 15+ Diameter of the largest lumina about 0.3-1µm-----*O.tavernieraefolia*
- 15- Diameter of the largest lumina about 1-2µm-----*O. caput-galli*
- 16+ Apocolpium 3-3.5µm-----*O. albasscalcarea*
- 16- Apocolpium 3.5-4.5µm-----*O. albassalba*

DISCUSSION

The results given by the present study reveal that the pollen morphology of Iranian Samples of *Onobrychis* is homogenous and is supported by what the authors like Ohashi (1971), Ferguson and Skvarla (1981), Faegri and Iversen (1989), and Moore and et. al. (1992) describe [3, 9-11]. Three pollen subtypes can be identified in terms of characteristics like pollen size, shape and ornamentation. No changes can be observed in the taxonomy of genera even though there are differences in the morphology of these three subtypes. According to Kozhukharou[12]. Ornamentation around the colpi, pollen and out line in pollen view can be used to identify species such as *O. viciifolia* which is macro morphologically similar. *Onobrychisalba* is the species which can be found largely in Bulgaria and Iran have many varieties with differences

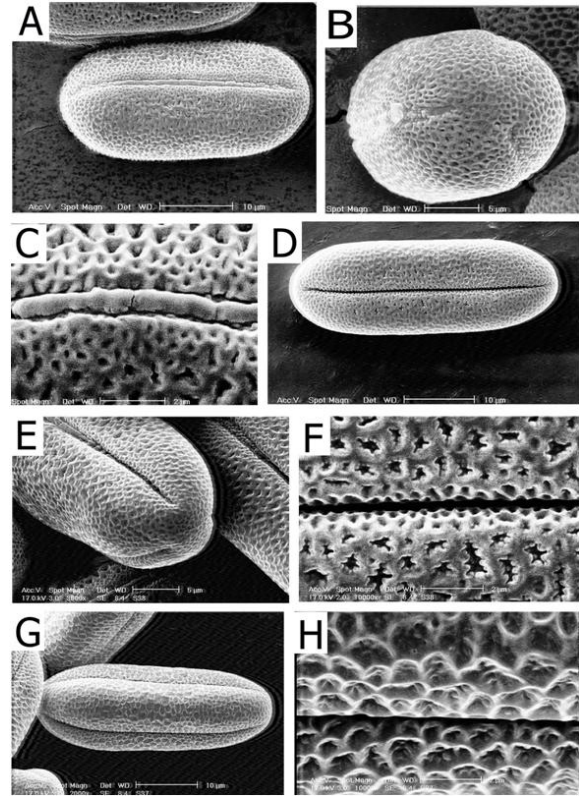


Figure 6. Scanning Electron Micrographs of pollen grains. A-C: *Onobrychistranscaspica*. A: Equatorial view and colpus. B: Polar view and apocolpium. C: Colpus and ornamentation. D-F: *O.altissima*(Genotype). Calcaria. D: Equatorial view and colpus. E: Apocolpium and ornamentation. F: Colpus and ornamentation. G-H: *O.altissima*(wild). G: Equatorial view and colpus. H: Colpus and ornamentation.

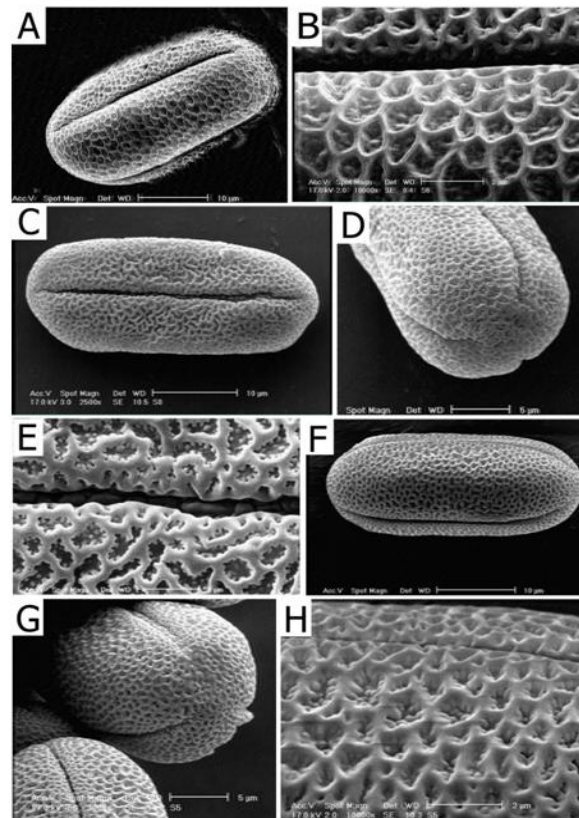


Figure 7. Scanning Electron Micrographs of pollen grains. A-B: *Onobrychiscaput-galli*. A: Equatorial view and colpus ornamentation. B: Colpus and ornamentation. C-E: *O.transcaspica* C: Equatorial view and colpus ornamentation. D: Polar view and ornamentation in apocolpium. E: Colpus and ornamentation. F-H: *O.taverniera*. F: Equatorial view and colpus ornamentation. G: Polar view and ornamentation in apocolpium. H: Colpus and ornamentation.

in pollen morphology [13-15]. In equatorial and polar view there are significant differences in the grains of studied *viciifolia* (Genotype1) in terms of ornamentation, dimensions and shape. This subspecies can be considered as a particular subtype. The results of this study did not demonstrate any significant differences between the annual and perennial taxa of *Onobrychis* with regard to pollen morphology. The development of pollen characters has passed through different stages, from a well-shape reticulum to a reticulum with decreasing size of lumina at the end margin of colpi like *Onobrychisviciifolia* (Genotype2), *O. cyri*, *O. transcaspica* *O.tavernieraefolia*, *O. altissima* (Genotype), *O. micrantha*, *O. nummularia*, *O. viciifolia* (Genotype1) and reticulum with lumina which has the same size at the margin of the colpi like *O. michauxii* and *altissima* (Genotype) [16-18].

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