

MORPHOLOGICAL STUDY OF POLLEN GRAINS FOR SOME GENERA OF SUBFAMILY PAPILIONOIDEAE

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Abstract

Pollen grain morphology of 18 species belonging to nine genera of the subfamily Papilionoideae was studied with light and scaning electron microscope. Pollen grains were monad and zonocolporate in all genera except *Onobrychis*, which was zonocolpate, the pollen is small or medium in size, the exine showed agreat variety in the patterns of sculpture (ornamentation), reticulate, microreticulate, Regulate, Psilate, Perforate. Morphology of pollen grain in polar and equatorial outline were found to be valuable, light microscope (LM.) and Scaning electron microscope (SEM) micrographs to each pollen grains were done.

Key words : Pollen grain, Colporate, Papilionoideae, Subprolate.

Introduction

Fabaceae is one of the most important and largest family of the flowering plants (Mabberley, 1997), it is the third family below the rank of Orchidaceae and Asteraceae (Ghosh and Kreshri, 2007). Fabaceae is a well represented family with (650-750) genera and more than 18.000 species (Tekin and Yilmeaz, 2015; Mirzaei *et al.*, 2015).

In Iraq, the family Fabaceae has economic importance after Gramineae, includes about 550 genera with 13,000 species (AL-Musawi, 1987; AL-Khatib, 2000).

The family are herbs, shrubs, woody vines or trees. Leaves are usually pinnately or palmately compound or unifoliate, alternate or opposite on the stem. Flower Zygomorphic, corolla has a standard, two wings and a keel formed by the fusion of the two lower petals. There are ten stamens and all are free or nine are fused into a tube and one is free (diadelphous) or all stamens are fused (Walters and Keil, 1988; Harlow *et al.*, 1991).

The most important characters of Fabaceae pollen grains are symmetry, shape of pollen grain and characters of aperture (Perveen and Qaiser, 1998). All Fabaceae pollen grains are isopolar, oblong-semioblong or oblate –oblate sphaerical, rarly prolate- spheroidal, tricolporate, rarly prolate or colpate only. Erdtman (1969), Melhem (1971), Primer (1974), Perveen and Qaiser (1998) pointed at the taxonomic importance of the study of the Legume pollen grains. They are usually monad, but sometimes Dyads, tetrads and polyads may also exist (Silvester and Capelato, 1993; Watson and Dallwitz, 1992; Guinet, 1981).

In Iraq, taxonomic studies based on the palynology of the family were developed by (AL-Aubaidy, 2006) for the genus *Trigonilla* L.), (AL-Dobassi, 2008) for different genera in Al-jadriaha district (AL-Saadi, 2013) for the genus *Trifolium* L. and (AL-Shammary, 2015) for the genera *Scorpiurus* L. and *Melilotus* Mill.

Aim of this study is to use different characteristics of pollen grains in delimitation of different genera within different tribes of subfamily Papilionoideae and clairly the evolutionary relationships among them.

Materials and Methods

The materials used for ihe present study were collected from the wild . plant specimens were deposited at Babylon University Herbarium (BUH).

Collectors and localities are shown in the table.

Pollen slides were preparded using the technique of AL-Mayah (1983). The pollen grains were mounted in safranin-glycerine jelly. Light microscope observation were made using SN 281166 microscope to measure polar axis length, equatorial width, thickness of the exine, colpus

Table 1 : Numbers and locations o	f the samples in which	the pollen was stu	udied with the date of	f collection and the herbicides
deposited therein.				

The sample site	Date of collection	The herbicide deposited	Sample number	Species
-Assalman& from Shbecha	1977	BUH	004241	Astragalus kahiricus
-50 Km, from naiaf toward Ruhba	1993-3-7	BLN	-	
Ukhaidhir, 45Km.W.of Kerbala	1970-4-8		1607	
-Assalman & from Shbecha	1973-6-25	BLN	-	A. rhodosemius
Arbil		BLN	SN	Colutea cilicica
Arbil	2016-4-21	BLN	SN	Coronilla scorpioides
-Saran village, Karoukh region	1959-6-8	BAG	27275	1
-20 KM. NW of Sulaimaniya	14-6-1957	BAG	2172	
Arbil	2016-4-5	BLN	SN	Glycyrrhiza glabra var. glandulifera
Arbil	1978-4-6	BLN	SN	Hippocrepisunisiliquosa var. bisiliqua
-3 K m N. of Saadiya	1978-3-7	BAG	48671	
-5Km from Salman to Samawah	1978-2-15	BAG	48222	
Arbil	2016-4-7	BLN	SN	Hymenocarpos circinnatus
- Sharma dary	1979-4-28	SUH	00675	
- Jabal al-Muwaila near	1957-3-28	BAG	17538	
Kawait, E of JabalHamrine, 70 Km				
-20 Km. to Khanaqin	1973-3-7	BAG	39596	Onobrychis acaulis
- Badra, on Persian barder east of	1047 0 10	DAG	((22)	
Zerbatiya	1947-3-18	BAG	6633	
14 Km S. of Saadiya	19/8-3-6	BAG	48812	
Arbil	2016-4-6	BLN	SN	O.caput-galli
- Shana dary	1979-4-28	SUH	00673	
- Mawet alt. 900m.	19/9-5-9	SUH	00/41	
Arbil	2016-4-5	BLN	SN	O.carduchorum
- On the top of mountain with	1070 5 28	BAG	50287	
-W HagiOmran (200-300)m west of	1979-3-28	DAG	50287	
the spring	1959-6-14	BAG	27872	
Arbil	2016-4-5	BLN	SN	O. crista-galli
- Shana dary	1979-4-28	SUH	00677	
- Shewasoor near chemchemal	1980-5-14	SUH	1883	
Arbil	2016-4-5	BLN	SN	O. galegifplia
- Serseer alt. 1480 m.	1980-6-6	SUH	3960	
-Tirwanish 10 Km E KaniMasi	1976-7-7	BAG	45714	
- Shewasoor near chamchemal	1980-5-14	SUH	1913	O. haussknechtii
- WadiHauran near H1	1981-3-10	BAG	52990	
- Between Faida & AL Baqaq	1976-5-12	BAG	44925	O.ptolemaica subsp. ptolemaica
-170-190 Km. Wof Ramadi	1959-4-21	BAG	27032	
- 10 Km E. of Zmbatia	1976-3-31	BUH	44279	O.ptolemaica subsp. macroptera
- JabalMakhul near Baiji	1954-5-4	BAG	13197	
-10 Km. west of Razaza lake	1995-3-27	BLN	-	
- Near Bazian	1973-5-18	SUH	1376	O.schahuensis
- Qaradagn mountain Timara Singar, south face	19/3-3-18	BUH	40628	
- Jahal Singar	1981-4-22	BAG	-53344	Pisum sativum subsp platius
Pabilan University	2016 4 5		CNI	S municatury
Dablion University	2010-4-3	DLN	SIN	S.muricalusvar



Fig. 1 : Variations in pollen characteristics (polar view) of some species studied. A 100X B 40x.



Fig. 2 : Variations in pollen characteristics (polar view) of some species of genus Onobrychis studied (40x).

length (diameter). Measurements were based on 25 or more pollen grains for each species. For scaning electron microscope (SEM) dry pollen grains were mounted on stubs, morphological observation were made with a electron microscope.

The terminology used is mainly that of Punted *et al*. (2007).

Results

1. Genus : *Astragalus* (1: *A.kahiricus*). figs 1 and3, fig. SEM 5, table 2.

Pollen grains are 3-zonocolporate, subprolate (P/E: 1.18), medium in size, polar axis length32.5 (27.5-35) μ m., Equatorial axis 27.5 (17.5-35) μ m. The shape of pollen grain is triangular or trilobulate in polar outline and elliptic in equatorial outline, with ornamentation

microreticulate (medium reticulate), the exine is (1) μ m. thick, germinating pores 3, colporate.

(2: A. rhodosemius) (Figs. 1 and 3). Fig. SEM 5, table 2.

Pollen grains are 3-zonocolporate or 4- zonocolporate, subprolate (P/E: 1.33), medium in size, polar axis length30 (27.5-32.5) μ m, E.axis 22.5 (15-25) μ m. The The shape of pollen grain is Triangular in polar outline and elliptic in equatorial outline, with ornamentation reticulate, the exine is (1-1.3) μ m. thick, germinating pores 3-4, colporate.

2. Genus : *Colutea* (*Col.cilicica*)., Figs. 1 and 3. Fig. SEM 5, table 2.

Pollen grains are zonocolporate , prolate (P/E: 1.37), medium in size, polar axis length27.5 (25-35) μ m , Ew





Fig. 3 : Variations in pollen characteristics (Equatorial view) of species studied. A: 100 x B : 40x



Fig. 4 : Variations in pollen characteristics (Equatorial view) of some species of genus Onobrychis studied. (40x)

20 $(15-22.5)\mu$ m. The The shape of pollen grain is triangular or trilobed in polar outline and semiprolate-spheroidal in equatorial outline, with ornamentation reticulate-fossulate, the exine is 1 or less than 1 μ m thick, germinating pores 3, colporate.

3. Genus: *Coronilla (Cor. scorpioides)*. Figs. 1 and. Fig. SEM 6, table 2.

Pollen grains are 3-zonocolporate, subprolate (P/E: 1.16), small in size, polar axis length 17.5 (17.5-22.5) μ m, Ew 15 (12.5-17.5) μ m. The The shape of pollen grain is triangular-circular in polar outline and rectangular-elliptic in equatorial outline, with ornamentation striate-

rugulate, the exine is 1 or less than 1 μ m thick, germinating pores 3, colporate.

4. Genus : *Glycyrrhiza* (*G glabra* var. *glandulifera*). Figs. 1 and 3, fig. SEM 6, table 2.

Pollen grains are 3-zonocolporate, prolate (P/E: 1.4), in size, polar axis length 25 (27.5-37.5) μ m, E. axis 25 (22.5-27.5) μ m. The The shape of pollen grain is trilobulate, oblate-spheroidal in polar outline and wide elliptical in equatorial outline with ornamentation Rugulate-reticulate or regulate-fossulate, the exine is 1 or less than 1 μ m thick, germinating pore 3, colporate.



Fig. 5 : Variations in the shape and ornementation of the surface of pollen grain of some studied species. A : Equatorial view. B : Polar view.

5. Genus : *Hippocrepis* (*H.unisiliquosa* subsp.), figs. 1 and 3. Fig. SEM 6, table 2.

Pollen grains are 3-zonocolporate, subprolate, (P/E: 1.33), small in size, polar axis length 20 (17.5-25) μ m, E. axis 15 (15-17.5) μ m. The The shape of pollen grain is triangular – circular in polar putline and elliptical or rectangular – elliptical in equatorial outline, with ornamentation perforate, the exine is 1 or less than 1, germinating pores 3, colporate.

6. Genus : *Hymenocarpos (Hy. circinnatus)*. Figs. 1 and 3. Fig. SEM 7, table 2.

Pollen grains are zonocolporate, subprolate (P/E: 1.22), medium in size, polar axis length 27.5 (17.5-32.5) μ m., E axis 22.5 (17.5-27.5) μ m. The shape of pollen grain



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Fig. 6 : Variations in the shape and ornementation of the surface of pollen grain wall of some studied species. A : Equatorial view. B : Polar view.

is hexagonal angular aperture in polar outline and rectangular-elliptical in equatorial outline, with ornamentation Psilate, finely perforate. The exine is 1 or less than 1 μ m thick, germinating pore 6-7, colporate.

7. Genus : *Onobrychis* (1: *O. acaulis*). Figs. 2 and 4. Fig. SEM 7, table 2.

Pollen grains are zonocolporate, prolate (P/E: 1.71), medium-small in size, polar axis length 30 (25-32.5) μ m, Ew 17.5 (15-20) μ m. The The shape of pollen grain is oblate-spheroidal in polar outline and rectangular-obtuse in equatorial outline with ornamentation Simple reticulate, the exine is less than 1 μ m thick, germinating pore 3, colpate.

2. O.caput-galli. Figs. 2 and 4. Fig. SEM 7, table 2.

	S		-	7	e	4	Ś	9	7	8	6	10	11	12	13	ed
	Species		Astragalus kahiricus	Astragalus rhodosemius	Colutea cilicica	Coronilla scorpioides	Glycyrrhiza* glabra	Hippocrepis unisiliquosa	Hymenocarpos circinnatus	Onobrychisacaulis	O. caput-galli	O.cardechorum	O.crista-galli	O. galegifolia	O. haussknechtii	Table 2 continiu
	Polar axis	length (P)	(35-27.5) 32.5	(32.5-27.5) 30	(35-27) 27.5	(22.5-17.5) 17.5	(37.5-27.5) 35	(25-17.5)20	(32.5-17.5) 27.5	(32.5-25)30	(42.5-27.7)40	(37.5-25)32.5	(45-25)37.5	(42.5-22.5) 37.5	(30-22.5)25	
	Equatorial	axıs length (E)	(35-17.5) 27.5	(25-15) 22.5	(22.5-15) 20	(17.5-12.5) 15	(27.5-22.5) 25	(17.5-15)15	(27.5-17.5) 22.5	(20-15)17.5	(22.5-12.5) 17.5	(20-15) 20	(30-20) 27.5	(22.5-17.5) 20	(17.5-12.5) 15	
	Ratio	r/F	1.18	1.33	1.37	1.16	1.4	1.33	1.22	1.71	2.28	1.62	1.87	1.67	1.67	
	Polle	n appe rture no.	ς	3-4	ς	3	3	m	6-7	3	3	3	ω	3	3	
by micrometer.	len grains	Polar outline	Triangular,	Triangular	Triangular or trilobed	Triangular or circular	Trilobulate obla- tespheroidal	Triangular-cir cular	Hexagonal angular perturate	Circular	Triangular- obtuse	Circular	Circular	Circular	Circular	
tudied are measured	Shapes of pol	Equatorial outline	Elliptic	Elliptic	Semiprolate- spheroidal	Rectangular- Elliptical	Wide elliptical	Elliptical or rectangular- elliptical	Rectangular- elliptical	rectangular-obtuse	Elliptical elongated	Elliptical	Elliptical elongated	rectangular-obtuse	rectangular-obtuse	
he species s	Aperture	type .	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate	Colpate	Colpate	Colpate	Colpate	Colpate	Colpate	
cteristics of tl	Pollen	graın size	Medium	Medium	Medium	Small	Medium	Small	Medium	Small- medium	Medium	Small- medium	medium	Small- medium	Small-medium	
ions in pollen chara	Ornamentation		Microreticulate (medium	Reticulate	Regulate-fossulate	Striate-regulate	Regulate-reticulate or regulate- fossulate	Perforate	Psilate, finely perforate	Reticulate	Reticulate	Suprareticulate	ReticulateSupra	Microreticulate	Suprareticulate	
: Variat	Wall	thick	1	1-1.3	1-less 1	1-less 1	1-less 1	1-less 1	1-less 1	Less 1	1-less 1		-	Less 1	Less 1	
Table 2	فطر فتحة الإثنيات	•	5-7	6-7	12-17	3-4	7-9		6-7				ı	1		

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ica.	1aica subsp.ptolema	. 2 = = O.ptolen	sp.macroptera	uica sub	tolema	naximum, $1=O.p$	de the minimum and r	e either insid	its the average	the brackets represen	than 11 outside	Values
							elliptical				Less	
			15				rectangular-			finely reticulate	1	
19	Scorpiurusmuricatus	(20-15)20	(17.5-12.5)	1.33		Circular	Elliptical or	Colporate	small	Microreticulate		2-3
18	Pisumsativum	(50-42.5)47.5			3	Circular	Elliptica	Colporate	medium	Reticulate	Less 1	
17	O.schahuensis	(32.5-25)27.5	(17.5-15) 15	1.83	ς	Spheroidal	rectangular-obtuse	Colpate	Small- medium	Supraerticulate		ć
							rectangular-obtuse				1-less 1	
							elongated to		medium		·	
16	0. ptolemaica 2	(32.5-20)25	(22.5-15) 15	1.67	3	Triangular	Ellipyical	Colpate	Small-	Reticulate		•
15	O.ptolemaica1	-	I	,	ı			I	ı	I		
									medium		Less 1	
14	O.megatophros	(32.5-25)30	(20-15) 20	1.5	3	Circular	rectangular-obtuse	Colpate	Small-	Reticulate		•

Table 2 continiued...



Fig. 7 : Variations in the shape and ornementation of the surface of pollen grain wall of some studied species.A : Equatorial view. B : Polar view.

Pollen grains are zonocolpate, perprolate (P/E: 2.28), medium in size, polar axis length 40 (27.5-42.5) μ m, E. axis 17.5 (12.5-22.5) μ m. The The shape of pollen grain is triangular-obtuse in polar outline and elliptic in equatorial outline, with ornamentation Supra reticulate, the exine is 1 0r less than 1 μ m. thick, germinating pores 3, colpate.

3. O.carduchorum. Figs. 2 and 4, fig. SEM 8, table 2.

Pollen grains are zonocolpate, subprolate (P/E: 1.62), medium in size, polar axis length 32.5 (25-37.5) μ m, E.axis 20 (15-20) μ m. The shape of pollen grain is Spheroidal in polar outline and elliptical in equatorial outline, with ornamentation supra reticulate, the exine is 1 μ m. thick, germinating pores 3, colpate .

4. O.crista-galli. Figs. 2 and 4. Fig. SEM 8, table 2.



Fig. 8 : Variations in the shape and ornementation of the surface of pollen grain wall of some studied species. A : Equatorial view. B : Polar view.

Pollen grains are zonocolpate, prolate (P/E: 1.87), medium in size, polar axis length 37 (25-42) μ m, E axis 27.5 (20-30) μ m. The The shape of pollen grain is circular in polar outline and elliptical elongated in equatorial outline, with ornamentation Supra reticulate, the exine is 1 μ m. thick, germinating pores 3, colpate.

5. O.galegifolia. Figs. 2 and 4. Fig. SEM 8, table 2.

Pollen grains are zonocolporate, subprolate (P/E: 1.67), medium in size, polar axis length 37.5 (22.5-42.5) μ m, Ew :20 (17.5-22.5) μ m. The The shape of pollen grain is Spheroidal in polar outline and rectangular-obtuse in equatorial outline, with ornamentation microreticulate-perforate, the exine is 1 μ m. thick, germinating pores 3, colpate.

6. O.haussknechtii. Figs. 2 and 4, fig. SEM 9, table 2.



Fig. 9: Variations in the shape and ornementation of the surface of pollen grain wall of some species of *Onobrychis* studied. A : Equatorial view. B : Polar view.

Pollen grains are zonocolpate, subprolate (P/E: 1.67), small-medium in size, polar length 25 (22.5-30) μ m, Ew 15 (12.5-17.5) μ m. The The shape of pollen grain is Spheroidal in polar outline and rectangular-obtuse in equatorial outline, with ornamentation Supra reticulate, the exine is less than 1 μ m. thick, germinating pores 3, colpate.

7. O.megatophros. Figs. 2 and 4. Fig. SEM 9, table 2.

Pollen grains are zonocolporate, prolate (P/E: 1.5), small-medium in size, polar axis length 30 (25-32.5) μ m, Ew 20 (15-20) μ m. The shape of pollen grain is Spheroidal



Fig. 10 : Variations in the shape and ornementation of the surface of pollen grain wall of some studied species. A : Equatorial view. B : Polar view.

in polar outline and rectangular-obtuse in equatorial outline, with ornamentation Simple reticulate, the exine is less than 1 μ m. thick, germinating pores 3, colpate.

8. O.ptolemaica. Figs. 2 and 4, fig. SEM 9, table 2.

Pollen grains are zonocolporate, prolate (P/E: 1.67), small-medium in size, polar axis length 25 (20-32.5) μ m, E.axis 15 (15-22.5) μ m. The shape of pollen grain is Spheroidal in polar outline and elliptical elongated to rectangular-obtuse in equatorial outline, with ornamentation Simple reticulate, the exine is 1 or less than 1 μ m. thick, germinating pores 3, colpate.

9. O.schahuensis. Figs. 2 and 4, Fig. SEM 10, table 2.

Pollen grains are zonocolpate, prolate (P/E: 1.83), small-medium in size, polar length $27.5(25.5-32.5) \mu m$,

E.axis 15 (15-17.5) μ m. The The shape of pollen grain is Spheroidal in polar outline and rectangular-obtuse elliptic in equatorial outline, with ornamentation suprareticulate, the exine is less than 1 μ m. thick, germinating pores 3, colpate.

8. Genus : *Pisum*. (*P. sativum* subsp. *elatius*). Figs. 1 and 3. Fig. SEM 10, table 2.

Pollen grains are 3-zonocolporate, oblate (P/E: 1.8), medium in size, polar axis length 40 (42.5-50) μ m, Ex. 22.5 (20-30) μ m. The The shape of pollen grain is Spheroidal in polar outline and in equatorial outline, with ornamentation reticulate, the exine is 1 μ m. thick, germinating pores 3, colporate.

9. Genus: *Scorpiurus (S. muricatus* var. *subvillosus)*. Figs. 1 and 3. Fig. SEM 10, table 2.

Pollen grains are 3-zonocolporate, subprolate (P/E: 1.33), small in size, polar axis length 20 (15-20) μ m, E axis 15 (12.5-17.5) μ m. The The shape of pollen grain is Spheroidal in polar outline and elliptic in equatorial outline, with ornamentation Microreticulate finely reticulate, the exine is 1 or less than 1 μ m. thick, germinating pores 3 colporate.

Discussion

In this investigation, pollen grains of eighteen species belonging to nine genera of the subfamily : Papilionoideae have been studied. The result show that the pollen grains are 3-zonocolpate in the genus *Onobrychis* and 3zonocolporate in other genera, sometimes 4-onocolporate in *As.rhodosemius*, 6-7 zonocolporate also occure in *Hy.circinnatus*, evolutionary tricolporate pollen grains may be considered as in advanced status (Bhattacharya *et al.*, 2015), pollen morphological study of Bhattacharya *et al.* (2015) supported this observation.

Tricolpate is the main and basic type found in eudicot, while other aperture types are regarded as derived among eudicot, such as 5-colpate, 6-colpate, porate, colporate (Walker and Doyle, 1975).

Size of pollen grains are valuable at generic level, the smallest pollen grains are those of *Hi. unisiliquosa* and *S.muricatus* and the largest one are those of *P.sativum* L. Varation in pollen size is caused by either anomalies in meiosis or hybridization (Matsuda, 1928; Aytug *et al.*, 1971). Chanda and Ghosh (1976) considered the size of pollen grains have teritiary importance, after the characters of germinating pores and ornamentation of exine.

In current study, all examined species showed a great varation in the shape of pollen grain at polar and equatorial outline.

Nair (1964) pointed out that characters of pollen morphology such as the ahape is a useful tool in solving problems at various taxonomic levels. Patterns of the sculpture of exine have a great taxonomic importance at generic and species level, in the terms of morphological evolution of pollen grain, exine sculturing evolution have secondary importance (Chanda and Ghase, 1976), also Ferguson (1985) mention to the Papilionoideae especially in the exine stratification and sculpturing.

The number of germinating pore was signification at generic level and help in differentiation of *Hy.circinnatus* and *A. rhodosemius*, which have more than 3-pores. Chanda and Ghosh (1976) considered the characters of germinating pore to be as a primary importance, because they are more conservative.

Conclusion

Characters of palynological study of some genera of Papilionoideae valuable at generic and species level especially shape of pollen and the exine sculpture.

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