# Pollen Grains Characters and Their Evolutionary Significance in the family Papaveraceae in Iraq and some Iranian species

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# Abstract:

Pollen morphology of twenty seven species of Papaveraceae *sensu lato s.l.* werestudied under light and scanning electron microscopes. Seven pollen types are described of which *Eschscholzia californica*, *Roemeria hybrida* and *Corydalis rupestic* (Iranian sample) types are newly described here. Among the genera studies, pollen morphology has a very important taxonomic application. Almost all genera have its own distinct pollen type mainly distinguished by the shape and number of apertures, except *Glaucium* and *Corydalis* which have pollen grains nearly similar to those in *Papaver*.

On the Basis of Phenotypic characters of plants such as root, stem and leaf morphology, most of the taxonomists identify Plant species, but now palynological studies can provide accurate basis for the classification of plant species which may be considered as one of the most significant tools used by modern taxonomists to identify and differentiate closely related taxa (Mazari *et al.*, 2012). Pollen characters are valuable in solving confused problems of interrelationships between various taxa and estimate of their status in the classification, especially to the families, subfamilies, tribes, genera, species, and subspecies (Fazal *et al.*, 2013).

The oldest study of the family Papaveraceae was in (1832) by Fritzsch and followed by Mohl (1834). Erdtman (1971) gave excellent review of the systematic an applications of palynology in the plant kingdom; he studied pollen belonging to 60 species from 32 genera in Papaveraceae, and divided the Papaveraceae into 3 groups, these groups are:

1. Hypecoideae

#### 11. Papaveroideae

# III. Fumarioideae

He also indicated the general characteristics of the family and certain species. Many other authors who have dealt with this family using pollen as a character with systematic interest are: Ownbay (1958, 1961), Ernst (1962), Rutter (1968), Huynh (1970), as mentioned by Candau & Fernandez-paniagua (1985). Rachele (1974) studied eleven Papaveraceae genera endemic to the northeastern of the United State and Canada which belong to three subfamilies Papaveroideae, Fumarioideae, and Hypecoideae. Layka (1976) dealt with the morphology of 22 species of Argemone and on pollen structure in the Papaveraceae. Moore and Webb (1978) mentioned several species from Papaveraceae in their survey like, Papver argemone, Papver *Glaucium flavum* rhoeas. and Fumaria officinalis. Abou El-Naga (1982) studied Egyptian Papaveraceae and proved how far the detailed investigation of palynological studies combined with leaf anatomy, cytology, stomatal structure and morphology can be helpful in that context by using L.M. and S.E.M. She described a key to the genera based on pollen morphology: 1-Pollen colpate.

A. Dicolpate: Hypecoum.

B. Tricolpate:

- *I* Colpus neither operculate not covered by verrucae: *Papaver*.
- II- Colpus operculate: Argemone.
- III- Colpus covered by verrucae: Glaucium.
- 2-Pollen porate
- A. Pore annulate: Fumaria.
- B. Pore non-annulate: Roemeria.

Candau & Fernandeiz – paniagua (1985) in their study divided the family into four pollen types depending on the numbers and shape of aperture:

1- Hypecoum Imberbe type: 2-colpate.

2- *Glaucium flavum* type: 3-zonocolpate, apocolpaia acuminate.

3- *Papaver rhoeas* type: 3-zonocolpate, apocolpaia not acuminate.

4- Paver argemone type: pantoporate.

El-Ghazaly (1992) in pollen flora of Qatar, studied one species of the family which was *Papver syriacum*. While in Chaturvedi *et al.*, (1999) work on pollen morphology of *Argemone mexicana* and *Argemone ochroleuca*, the pollen grains of the two species are distinct on the basis of pollen size only. Romero *et al.*, (2003) studied the ontogeny of the pollen wall of *Hypecoum imberbe* by using transmission electron microscopy (T.E.M.), and mentioned the number of aperture in this species. While Perveen and Qaiser (2004) examined Pollen morphology of 9 species of the family Fumariaceae from Pakistan by light and scanning electron microscopes. They recognized two distinct pollen types on the basis of aperture type; these types are:

• *Corydalis diphylla* type: 3-colpate, 4-6 colpate.

• Fumaria indica type: 3-porate.

Gran and Sharifnia (2008) exammind the micro-macro morphological features of pollens and seeds for the genus Glaucium in Iran by using electron microscopy. Al-Qura'n (2010) studied the pollen morphology (by light and electron microscopy) of three Papaver species; Papaver hybridum, Papaver polytrichum and Papaver rhoeas in Jordan, he compares between them depending on type of apertures. Keshavarzi et al., (2011) studied the pollen morphology of seven Fumaria species in Iran scanning using light and electron by microscopy, and determined the number and types of apertures.

Fazal *et al.*, (2013) identified taxonomical markers for some medicinal plants by investigation and characterization of palynological types for these plants; one of these plants was *Fumaria indica*. Hanif *et al.*, (2013) studied the pollen grains of *Papaver*  somniferum in their palynological study on some ornamental plants by using (L.M.). Tavakkoli and Assadi (2013)studied morphological and micromorphological of some species of the genus *Papaver* that belong to the Sect. Oxytona in Iran. These studies were at the level of the world and they were limited, but in Iraq there are no studies that examined the pollen grains of Papaveraceae, only Kalaf (2012) give a brief illustration for one unidentified Fumaria sp. mentioning only one example without specifying the species in his study for pollen morphology of the wild plants in Basra. Therefore the aims of this study are:

1- To describe the pollen grains morphology of the genera of Papaveraceae and Fumariaceae.

2- To determine the taxonomical importance of pollen morphology in the classification of the genera under studied.

3- To describe the pollen types in the genera of Papaveraceae and Fumariaceae in Iraq and to assess their evolutionary importance.

# **Materials and Methods**

#### **Samples collections**

The study was based on fresh samples collected during field trips between the years (2013-2014). We also depended on the herbarium specimens from National Herbarium of Iraq (BAG), Herbarium of Bagdad University (BUH), Herbarium of Basra University/ Collage of Science (BSRA), National Herbarium of Iran (TARI), Herbarium of Azad University/Tehran and Herbarium of the Collage of Agriculture / University of Basra. The characters of fresh and herbarium specimens are studied by using dissection microscopy to identification of the plants. For species identification Cullen in Townsend and Guest (1980), Davis (1965), Zohary (1966) and Rechinger (1964) were consulted.

# Result

Quantitative and qualitative characters are shown in tables 1 (terminology according to Erdtman (1952). figures (20-26) and plates (1-13)

In this current study we noted that the ornamentation on the external surface of pollen was:

1- Echinate - Perforate

This includes most species of family Papaveraceae (in this study).

- 2- Granulate Tuberculate
  - This includes F. bracteosa and F. parviflora.
- 3- Punctate

This includes *C. rupestic*, as shown in plates (1-13).

# Pollen morphology of the genera studied Pollen morphology of *Papaver*

Pollen grains size varied between small to medium between (17.5- 41)  $\mu$ m and Shape varied between oblate spheriodal, suboblate, prolate spheriodal and spherical. Pollen grains have two types of apertures; 3-colpate in most of species and 6-pores which was found in *P.argemone*. Ornamentation was echinateperforate. Exine thicker than entine, thickness for entine was ranged between (0.375 - 1.125)  $\mu$ m while the thickness in exine was (0.875 -1.75)  $\mu$ m. Width of apertures was (5 -10)  $\mu$ m whereas the length of aperture was (12.6 -26.25)  $\mu$ m. Ratio of P/E was ranged between (0.75 -1.14).

# Pollen morphology of Glaucium

Pollen grains with medium size were between (0.97- 1.16)  $\mu$ m, shape oblate spheriodal, prolate spheriodal and subprolate; 3zonocolpate and ornamentation were echinateperforate. Exine is thicker than entine, thickness for entine was ranged between (0.875-1.875)  $\mu$ m, while in exine the thickness was ranged between (1.375-2.75)  $\mu$ m. Width of apertures was (6-15.5)  $\mu$ m, whereas length of aperture was (20-25.95)  $\mu$ m. Ratio of P/E was changed from (0.97-1.16).

#### Pollen morphology of Roemeria

Pollen grains with medium size were (29)  $\mu$ m, spherical. 8-poers and entine thickness was (0.875)  $\mu$ m and exine thickness was (1.375)  $\mu$ m, with echinate-perforate ornamentation. Aperture dimensions between (8.5-9)  $\mu$ m.

# Pollen morphology of Hypecoum

Pollen grains with variation in size between small to medium, ranged between  $(18.5-30.5) \mu m$ . Suboblate to oblate, ornamentation was echinate-perforate. 2-colpate thickness for entine was  $(0.625-0.875) \mu m$ , while thickness in exine was  $(1.25-1.75) \mu m$ . Length of aperture was  $(13-26.2) \mu m$ . Ratio of P/E was ranged between (0.622-0.86).

#### Pollen morphology of Eschscholzia

Pollen grains with medium size were (31)  $\mu$ m, prolate spheriodal, 5-6 Colpate, entine thickness was (1.25)  $\mu$ m to and exine was (1.875)  $\mu$ m. Ornamentation was echinateperforate and the length of aperture was (16.75)  $\mu$ m. Ratio of P/E was (1.01).

#### Pollen morphology of Fumaria

Pollen grains medium size, ranged between (32-33)  $\mu$ m, spherical shape, 6-pores entine thickness was (0.875)  $\mu$ m and exine thickness was (1.375)  $\mu$ m. Ornamentation was granulate – tuberculate. Aperture dimensions between (11-11.5)  $\mu$ m.

# Pollen morphology of Corydalis

Pollen with medium size from  $(20-25) \mu m$ , was subprolate grains, 3-zonocolpate, entine thickness was  $(0.875) \mu m$  and exine thickness was  $(1.375) \mu m$ . Ornamentation was Punctate and the length of aperture was between (17-20) $\mu m$ . Ratio of P/E was (1.24).

#### **Pollen Types**

The results showed that pollen grains of species under studied of Papaveraceae and Fumariaceae have 7 types, four of these types were mentioned before, and the other types are: *Eschscholzia californica*, *Roemeria hybrida* type and *Corydalis rupestic* type, are described for the first time in this present study, and these types are:

#### 1- *Hypecoum imberbe* type (plate 12)

Pollen grain is characterized by 2-colpate with echinate - perforate ornamentation and this type includes *H.imberbe*, *H.geslinii* and *H. pendulum*.

# 2- Papaver rhoeas type (plates 1-8)

Pollen grain is characterized by 3-colpate with echinate - perforate ornamentation and this type includes *P.acrochaetum*, *P. armeniacum*, *P. bornmuelleri*, *P. curviscapum*, *P.cylindricum*, *P.decaisnei*, *P. dubium*, *P.fugax*, *P. glaucum*, *P.hybridum*, *P.*  macrostomum, P.persicum, P.rhoeas, P. somniferum.

3- Glaucium flavum type (plate 9)

Pollen grain is characterized by 3zonocolpate with echinate - perforate ornamentation and this type includes *G.cornculatum*, *G.grandiflorum*, *G. haussknechtii*.

4- *Papaver argemone* type (plates 1 and 13) Pollen grains are characterized by 6-pores

with echinate - perforate or granulatetuberculate ornamentation and this type include *P. argemone, F. bracteosa* and *F.parviflora*.

- 5- Eschscholzia californica type (plate 11)
   Pollen grain is characterized by 5-6colpate
   with echinate perforate ornamentation and
   this type includes *E. californica*.
- 6- Roemeria hybrida type (plate 10)

Pollen grain is characterized by 8-pores with echinate - perforate ornamentation and this type includes *R. hybrida and R. refracta*.

#### 7- Corydalis rupestic type (plate 13)

Pollen grain is characterized by 3-zonocolpate with Punctate ornamentation and this type includes *C. rupestic*. As for the summary results of the current study, we put a key to separate species under research

# Key to the genera and species of Papaveraceae in Iraq based on pollen types 1-Pollen grains 3- Pollen grains 5-6 colpate ..... Eschscholzia 4- Pollen grains 3-colpate subterminal ..... .....Papaver 5- Ornamentation punctate ..... Corydalis 5- Ornamentation echinate ..... Glaucium 6- Pollen grains 6- porate......7 6- Pollen grains 8- porate...... Roemeria 7- Ornamentation tuberculate ...... Fumaria 7- Ornamentation echinate ...... P.argemone

It is obvious that the pollen morphology characters provided significant taxonomical characteristic on generic level and can easily be used to separate the genera studied.



P. argemone

Plate (1): Pollen grains of some Papaver species (10 Micometer)



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Ornamentation

Plate (2): Pollen grains of P. bornmuelleri (10 Micometer)





Plate (3): Pollen grains of P. cylindricum (10 Micometer)



**Polar** view







Polar view

Equatorial view



Plate (5): Pollen grains of. P. dubium (10 Micometer)



Polar view

Omamentation

Polar view

P.hybridum





P.rhoeas

Plate (7): Pollen grains of some Papaver species (10 Micometer)

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Polar view

Equatorial view

Plate (9): Pollen grains of some Glaucium species (10 Micometer)



Plate (10): Pollen grains of Roemeria species (10 Micometer)

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Polar view

Equatorial view



Ornamentation





Plate (11): Pollen grains of E. californicain (10 Micometer)



Polar view

Equatorial view

Plate (12): Pollen grains of some Hypecoum species (10 Micometer)



#### Ornamentation.

#### Plate (13): Pollen grains of some Fumaria and Corydalis species (10 Micometer)

# Discussion

# Size

In respect to the size of pollen grains, the species were varied between small and medium-sized, and that most species of Papaver was small. The rest of the species that belongs to the related genera were mostly medium-sized except F.parviflora which was in large size and H.imberbe was in small size. Our pollen size classification was in

accordance with that in Erdetman (1971). The evolutionary trends in pollen size maybe

towards the large size of pollen grains and hence the small sizes are more primitive, these trends were found in pollen grains of many other families of flowering plants as reported by Al-Mayah (1983). This indicates that the pollen grains of *Papaver* were less developed from other species, and *F.parviflora* was the most developed.

#### Shape

The shape of pollen grains was various between oblate spheroidal and suboblate in most species of genus Papaver, whereas the is present in spherical shape species P.argemone, R. hybrida, Rrefracta,  $F_{\cdot}$ bracteosa and F. parviflora. While in Glaucium the shape of pollen grains become variable between oblate spheroidal, subprolate and prolate spheroidal. But in Hypecoum the shape was oblate - suboblate. From the above it is clear to us that the evolutionary trends towards the oblate and prolate, means that the spheroidal shape is considered as the primitive shape while the oblate and prolate shapes are considered as derived. This is in agreement with ideas of Al- Mayah (1983) in his study for evolutionary trend for the shape of pollen grains in the species of *Terminalia* and related genera.

#### Apertures

The results of the current study agreed with Erdtman (1971) who recorded three types of pollen grains included 2-colpate, 3-colpate, and 3-colpate or 6-rugate. The pollen grains apertures of Papaveraceae and Fumariaceae have a considerable taxonomical value, for both Papaver and Glaucium, in general, are 3colpate, but the genus Glaucium and Corydalis rupestic can be distinguished from other species by their 3-zonocolpate grains. This disagreed with Rachele (1974) who mentioned that P. dubium, P.rhoeas, P. somniferum, and G.flavum were 3-colpate, whereas agreed with Candau & Fernandeiz – paniagua (1985) they consider the species; P. dubium, P.rhoeas, P. somniferum, P.hybridum, G.corniculatum, G.flavum, and E.californica were 3zonocolpate.

The current study differed with the latter study regarding the type of *E.californica*, where it was a polycolpate (5-6 colpte) and this agreed with Kaderet (1993) who said that most of Papaveraceae are 3-colpate except *Roemeria* and *Papaver* Sect. *Argemonidium* with pollen grains with 4-5 rounded apertures and Eschscholzioideae polycolpate with 4-11

colpi. Al-Qura'n (2010) also mentioned that the species *P. hybridum*, *P. polytrichum* and *p.* rhoeas were 3-zonocolpate. Concerning the genus Hypecoum, our results have shown that all the species examined were 2-colpate and this is consistent with the results of each of Romero etal. (2003) and Candau & Fernandeiz - paniagua (1985). In Fumaria there were two types of apertures; porate (forate) found in F.parviflora and F.bracteosa while in genus Corydalis it was 3-zonocolpate in C.rupestic. This agreed with the suggestions of Erdtman (1971), Romero et al., (2003) and Perveen & Qaiser (2004) described pollen grains of Fumaria species usually between 6-12 forate and in *Corydalis* was 3-colpate or 6-12 rugate.

Also Keshavarzi *et al.*, (2011) studied the pollen morphology of seven *Fumaria* species in Iran which were panto-forate ranged between 6-12 forate. While our study disagreed with Khalf (2012) who mentioned that a species of *Fumaria* (unidentified) was with 3-colpate apertures. The length of colp varies from species to species in genus *Papaver* that is ranged between (12.6)  $\mu$ m to (16.25)  $\mu$ m, while the longest length of colp was (26.25)  $\mu$ m which was found in *P.hybridum*.

In other species with porate apertures, the biggest diameter of porate was (13) um in *P. argemone*, while the diameter in *Roemeria* species was close together between (8.5) µm to (9) µm and also in Fumaria species between (11)  $\mu$ m to (11.5)  $\mu$ m. The evolutionary trends of the number of apertures, which are often focused by other studies, become appeared that most of the pollen grains in genera Papaver, Glaucium and C. rupestic (18 species) were 3-colpate, and in Hypecoum species 2- colpate (3 species) while the species E. californicai is distinguished in polycolpate. So the evolutionary trends may start from the 2-colpate pollen then 3-colpate or vise versus and then rise and developed to polycolpate and polyporate. This shows that the types of genus Fumaria, Roemeria and P. argemone gives more developed pattern of pollen grains. So depending on the apertures, the pollen grains of Papaveraceae are divided into:

Hypecum type: included pollen grains with
 2-colpate.

2. Papaver type: included pollen grains with 3-colpate.

3. Eschscholzia type: included pollen grains with polycolpate

4. *P. argemone* type: included pollen grains with polyforate or polyporate.

# **Ornamentation and Wall thickness**

The ornamentation was echinate in genera Papaver, Glaucium, Roemeria. Eschscholzia and Hypecoum. But there is a variation in the ornamentation inside the colpe, which is characterized by the species G. haussknechtii where it was foveolate. In genus Fumaria the ornamentation was distinct from the other species it was granulate - tuberculate, pollen grains which are characterized by having bubbles protruding from pores with thick collars around pores. While in genus Corydalis differs from Fumaria by having different ornamentation which was punctuate. It also has unique ornamentation inside the colpe from the type pilate. As for the wall thickness of pollen grains the family is distinct in general by the exine thicker than entine or as thick as and the difference was very clear in genus Papaver.

# Pollen morphology and taxonomy

It is clear from above that the pollen grains morphology of Papaveraceae has considerable taxonomic value. The relation between species in based on type and numbers of apertures. For example, the pollen grains of genus *Papaver* have the same type of apertures 3-colpate except *P. argemone* which has polyforate, and this is similar to genus Roemeria, F. bracteosa and F. parviflora. Genus Hypecoum has pollen grain differ from others by having 2-colpate, the while Eschscholzia Pollen has 5-6 colpate. morphology evidence in current study suggests the affinity of *P.argemone* with genera Roemeria and Fumaria on the one hand, and with other species of Papaver on macromorphology on the other hand. Also with C. rupestic in this species, there is agreement with the species of Papaver in type of apertures, and with species of Fumaria on macro-morphology.

The palynological information available now support the proposal that Papaveraceae and Fumariaceae should be united under the same family.

#### **Evolutionary trends:**

As shown in Figure 87. the results indicate the evolutionary trends in the genera that were studied of this family in Iraq, it seems that the 3- colpate type represented by the species *P. rhoeas* is the primitive type and that may have been derived from it the rest of the types. This is consistent with this primitive character in all eudicot as confirmed by Walker and Doyle (1975). Accordingly, the 5-6 colpate type that representative by *E.californica* was derived from it evolutionary,

and maybe this type was evolution by reducing the dimensions of colps to the type 6-porate which representative by *R. hybrid* type. The last type led to polyporate by continues reducing the size of apertures. Or maybe the 5-6 colpate directly evolutionary by continue reducing the size and number of apertures to polyporate. There is another suggestion that the type polyporate was derived directly from the primitive type 3- colpate or from the transitional patterns representative by *P.argemone* type.



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