International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 7, p. 76-81, 2014

RESEARCH PAPER OPEN ACCESS

Pollen grain ultrastructure comparative of two species belonging to *Calochortus* Pursh (Liliaceae)

Samira Kiani[1], Seyed Mohammad Maassoumi[2], Ahmad Majd[3], Fatemeh Bareemizadeh[4*]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Department of Biology, Razi University, Kermanshah, Iran*
[3]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[4]*Department of Biology, Razi University, Kermanshah, Iran*

Key words: Caput, Columella, Intine, Tectum, TEM.

http://dx.doi.org/10.12692/ijb/4.7.76-81 Article published on April 01, 2014

Abstract

Pollen morphology of two genera of *Calochortus* was studied under transmission electron microscopy (TEM). The shape of caput in *Calochortus* is oblate spheroidal, flattened-spheroidal, rectangular that similar to caput of columella in other genera in Liliales. Absent endexine of *Calochortus* is advanced features in Liliales order, as well as monocotyledon plants. Also *Calochortus* has three layers intine, which consists of exintine, mesintine and endexine. The microreliefs on the surface of the muri in studied species *Calochortus* are smooth. The tectum to foot layer ratio (T/F) in studied species *Calochortus* is 1.6–2.6. This species was different from each other by shape and thickness of caput, length and width of columella and T/F ratio. The minimal thickness foot layer was in the pollen grain *C. albus* Douglas ex Benth The elliptical (oblate-spheroidal) caput form is in *C. eurycarpus* Wats, but from the caput in *C. albus is* spheroidal to indeterminate. In additional to sporoderm feature in *Calochortus* was similar to some species of genera *Tulipa* and *Fritillaria*.

* **Corresponding Author:** Fatemeh Bareemizadeh ✉ fbareemizadeh@yahoo.com

Introduction

The works are previously done including

Fritsche (1837) introduced exine and intine as two major layers of pollen grain. Also exine was divided into inner and outer layers (Fritzsche,1837). Praglowski and Raj (1979) described sculpture and structure of pollen grain (Punt *et al.*, 2001) For the first time, these two words were separated by Potonia (1934) (Potonib, 1934). Despite the differences between the word "sculpture" and "structure", they have used similarly in many researches. Also Faegri and Iversen (1975) expressed their investigation of two terms separately (Faegri *et al.*, 1975). Nilsson and Praglowski (1992) and Walker (1974) found types of tectum structure of pollen grain in angiosperms (Walker, 1974, Nilsson *et al.*, 2002).

In Liliales order, pollen grains are different. These differences include type of aperture, shape and exine ornamentation of pollen grain (Erdtman, 1952, Kosenko, 1992, Kuprianova, 1983, Maassoumi, 2005). Several studies have been made on different genera of this order.

Pollen morphology of two species in *Smilacina* were studied by Sohma and Takahashe (1982) and five species of *Clintonia* studied by Takahashe and Sohma (1982) using light and electron microscope (SEM and TEM) (Sohma *et al.*, 1982, Takahashi *et al.*, 1982) Harly (2003) examined the pollen of different species *Tulipa* and showed types of tectum in studied species using transmission electron microscope (TEM) (Harly, 2003). Tectum structure in pollen morphological studied in *Gagea* were examined by Zarei *et al* (2005) Maassoumi (2005) examined 48 pollen grains from 13 genera of Liliaceae using light microscopy, scanning electron microscopy and transmission electron microscopy (Maassoumi, 2005). Furthermore, he considered transmission electron micrograph of ultratin sections of *Fritillaria* and *Erythronium* (Maassoumi, 2012b, 2012a). Amiri (2010) investigated pollen morphology of some genera of Hyacinthaceae Batsch ex Borkh using light microscopy and electron microscopy SEM and TEM, and showed that studied species are easily separable from each other by exine and intine characteristics (Amiri, 2010). Bagheri (2012) studied ultrastructure of two pollen grain walls of *Colchicum* species using LM, SEM, and TEM (Bagheri, 2012). Kosenko (1987) studied surface ornamentation of pollen grains and 27 species in the genus *Calochortus* using LM and SEM [9] and obtained SEM micrograph for sporoderm of pollen grain in the *C. venustus* Douglas ex Benth species. Pollen morphology of 8 species of the genus *Calochortus* were examined under LM and SEM (Kiani, 2012). Pollen grains that used in this investigated were monosulcate, sulcus large, reaching end of grains, almost equal to equatorial diameter, striate-perforate ornamentation.

Thus, evaluation of light microscopy and SEM of two species in *Calochortus* did not much differ from each other. What still needs to be investigated using of TEM that will indicate differentiations. The aim of current study was compared of pollen wall ultrastructure using TEM carefully.

Materials and methods

Plant material

Pollen grains of two species were taken from Komarov Botanical Institute of the Russian Academy of Science, St. Petersburg (LE), and Russia. Pollen grains for TEM were fixed in 1 % osmium tetroxide and stained with a solution of Uranylacetatein 70 % alcohol and lead citrate (Reynalds, 1963), then dehydrated in an ethanol series and embedded in Epon mixture (Epon 812, Epon Härter DDSA, Epon Härter MNA) according to the standard method (Weakly, 1977). Ultrathin sections of the pollen grains were obtained by a glass knife (LKB 8800 Ultratome III) and lead citrate (Reynalds, 1963). Observations were made using a JEOL JEM_100B transmission electron microscope.

For TEM studies

All measurements on TEM micrographs have been made in standard vision in several pollen grains. Height and width of caput thickness and its form, length of columella (from under foot layer until down of caput) and width of columella, foot layer thickness in ultrastructural exine of all pollen grains in

Calochortus were measured (Tab 1, Fig. 1-2) and also diagrams of caput of columella were drawn for every investigated species as Fig. 3. Descriptive terminology follows Kremp (1967) and Punt *et al.* (2007) (Kremp, *1967*, Punt *et al.*, 2007).

Results

Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. Albus* and *C. eurycarpus* S indicates parameters.

The parameters are studied including: (surface of the muri, exine thickness, tectum thickness, form of caput, caput thickness, Length of columella, width of columella, foot layer thickness, none present of endexine, T/F ratio, intine thickness in region of sulcus-less, exintine thickness, mesintine thickness, endintine thickness).

Ectexine in *C. albus* Douglas ex Benth (With 0.42-0.59 µm thickness) and *C. eurycarpus* S. Watson (with 0.46-0.59 µm thickness) include Tectum (with 0.7-0.9 µm thickness), columellae and foot layer (Fig. 1 and 2). The shape of caput in *C. albus* is oblate spheroidal, rectangular flattened-spheroidal with 0.33-0.46µm width and in *C. eurycarpus* is Spheroidal, Oblate spheroidal and rectangular with 0.26-0.4µm width. In studied species, the microreliefs on the surface of the muri are smooth (Tab. 1; Fig. 1C; 2C) and the thickness in the sulcus zone is equal to 4.4 µm (Fig. 2B and 2C). Intine is composed of three layers, including: (i) Exintine, which is a layer (in *C. albus* 0.10–0.20 µm and in C. *eurycarpus* 0.10–0.16 µm) thickness with high electron density and a lot of channels inside; (ii) Mesintine, a layer with 0.33–0.53µm and 0.20–0.26 µm thickness (in *C. albus* and *C. eurycarpus* respectively) with low electron density and (iii) Endintine, which is a layer with 0.05–0.10 µm and 0.04–0.10 µm thickness (in *C. albus* and *C. eurycarpus* respectively) with a more electron density (Tab. 1; Fig. 1C, 1D and 2D). The thickness of Entine below Exine, in an area with no sulcus was 0.8–1.4 µm and 0.5– 0.7 µm (in *C. albus* and *C. eurycarpus* (Fig. 2D, 1C and 1D).

Table 1. Measurements of sporoderm in Calochortus species investigated using TEM.Abbreviations: (M) more electron danse; (L) less electron dense.

Taxon	*C.albus* Douglas ex Benth	*C.eurycarpus Wats.*
surface of the muri (microrelief)	smooth	smooth
Exine thickness (µm)	0.42– 0.59	0.46- 0.59
Tectum thickness (µm)	0.33 – 0.53	0.22-0.40
form of caput	Oblate spheroidal, rectangular	Spheroidal, Oblate spheroidal, rectangular
Caput thickness (height; width) (µm)	0.16 – 0.26; 0.33 – 0.46	0.20 – 0.26; 0.26 – 0.40
Length of columella (µm)	0.26 – 0.33	0.26 - 0.33
Width of columella (µm)	0.10 – 0.26	0.13- 0.20
Foot layer thickness (µm)	0.13– 0.17	0.16 – 0.24
Endexine	absent	absent
T/F ratio	2.6	1.6
intine thickness in region of sulcus-less(µm)	0.8-1.4	0.5-.0.7
exintine thickness (µm)	0.10–0.20 L	0.10– 0.16 L
mesintine thickness (µm)	0.33 – 0.53 M	0.20-0.26 M
endintine thickness (µm)	0.05– 0.10 L	0.04-0.1 M

Discussion

Baranova (1985) showed three type of caput of collumela in *Lilium* that the form caputs are similar to *Calochortus* (Baranova, 1985). One of the tectum structures in Angiosperms is semitectate (Walker, 1974, Nilsson *et al.*, 2002).

In some genera of Liliales (e.g. genus of *Fritillaria*) has semitectate pollen grain (Pehlivan *et al.*, Kosenko, 1996). Also, tetcun of investigated species of *Calochortus* is semitectate.

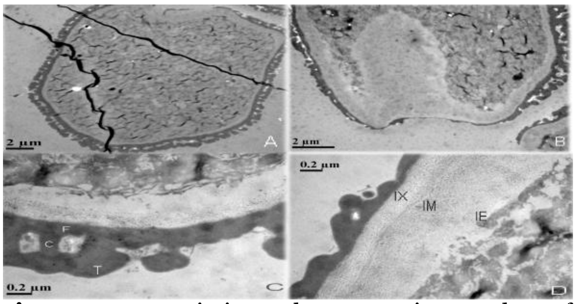

Fig. 1. Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. albus*. (C) = columellae, (F) = foot layer; (I) = intine, (T) = tectum , (Xi) = exintine, (Mi) = Mesintine, (Ei) = Endintine

We have revealed the minimal thickness foot layer in the pollen grain *C. albus* and the elliptical (oblate-spheroidal) caput form in columella in *C. eurycarpus* differs from the caputs (spheroidal to indeterminate). The surface of the muri (microrelief) in Subgenus of *Eriostemones* and *Leistemones* was microtuberculate tuberculate respectively (Maassumi, 2008) but microrelief in studied species was smooth. In addition, lack of Endexine in *Calochortus* indicates its similarity to the genus *Tulipa, Amana* and *Gagea* (Maassoumi, 2005). The obtained data indicate the similarity of two species *C. albus* and *C. eurycarpus* in terms of ectexine thickness, the tectum to foot layer ratio (T/F) (Tab. 1) and different layers of intine (Fig. 1D and 2D).

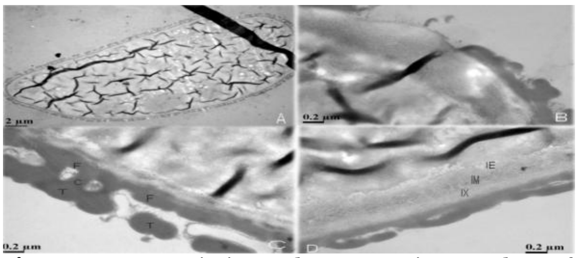

Fig. 2. Transmission electron micrographs of Sporoderm in pollen of investigated species of *C. eurycarpus*. (C) = columellae, (F) = foot layer; (I) = intine, (T) = tectum , (Xi) = exintine, (Mi) = Mesintine, (Ei) = Endintine

In addition, these two species were different from other species, by microreliefs on the surface of the muri, shape and thickness of caput, and the height and width of columellae (Tab. 1; Fig 1, 2 and 3).

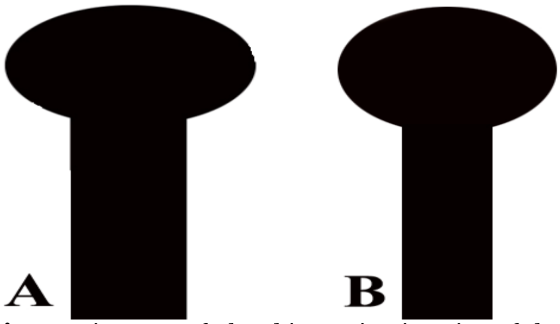

Fig. 3. Diagrams of ultrathin section in exine of the pollen grains in *Calochortus:* (A) *C.albus;* (B) C. *eurycarpus*.

Palynolomorphological data using TEM revealed more detail in comparison with SEM.

The shape of caput in *Calochortus* was oblate spheroidal, flattened-spheroidal, rectangular. The shape of caput of columella in *Calochortus* was similar to other genera in Liliales. Absent endexine in *Calochortus* is advanced features in Liliales order, as well as monocotyledon plants. *Calochortus* has three layers intine, which consists of exintine, Mesintine and endexine. The microreliefs on the surface of the muri in studied species of *Calochortus* were smooth. The tectum to foot layer ratio (T/F) in studied species of *Calochortus* was 1.6–2.6. Thus, palynolomorphological data showed that *Calochortus* was similar to some of species in *Tulipa* and *Fritillaria*.

Appendix

Specimen examined

The following specimens were included in the study:

C. albus Douglas ex Benth: Mariposa, CA, H. B. Ertter, 23/April/1989. *C. eurycarpus* S. Watson: Oregon, A. Cronquist, 6/jun/1953.

Acknowledgments

We would like to thank Dr. Svetlana Polevova for prepare ultrathin sections and micrograph of transmission electron microscope from Interfaculty Laboratory for Electron Microscopy at the Lomonosov State University, Moscow, Russia.

References

Amiri S. 2010. Pollen morphology in Hyacinthaceae (Master's diss), Payam Nour University, Tehran, Iran.

Bagheri SH. 2012. Pollen morphology in *Colchicum* (Master's diss), Razi University, Kermanshah, Iran.

Baranova MV. 1985. Palynoderm Ultrastructure and morphological types of pollen grains in the genus *Lilium* (liliaceae). Botanicheskiy Zhurnal (Leningrad) **70,** 297-304. (in Russian).

Erdtman G. 1952. Pollen morphology and plant taxonomy. Angiosperms. Almqvist & Wiksell. Stockholm.

Faegri K, Iversen J. 1975. Textbook of Pollen Analysis. Munksgaard, Copenhagen, 3rd ed., 295 p.

Fritzsche J, Pollen ST, Petersburg, Kais. 1837. Academie der Wissenschaften. (in German).

Harly MM. 2003. A fresh look at *Tulipa* (Liliaceae) pollen // Polen. **13,** 175-183 p.

Kiani S. 2012. Pollen morphology in Liliaceae (Master's diss**),** Science and Research Branch, Islamic Azad University, Tehran, Iran.

Kosenko VN. 1987. Morphology of the Pollen of *Calochortus* spp. Calochortaceae. Botanicheskii Zhurnal (St. Petersburg) **72,** 758-64. (in Russian).

Kosenko VN. 1996. Palynomorpholoy of the order Liliales Lindley in connection with problems of systematization. Ph. D Thesis diss, Komarov botanical Institute, Petersburg. (in Russian).

Kosenko VN. 1999. Contributions to the pollen morphology and taxonomy of the Liliaceae. *Grana* **38,** 20-30.

Kremp GOW. 1967. Morphologic encyclopedia of palynology. Mir, Moscow (in Russian).

Kuprianova LA. 1983. Liliales. In: Spores of pteridophytes and pollen of gymnosperms and monocotyledons of the Flora of the European Russia (ed. L. A. Kuprianova), p. 134. Nauka, Leningrad (In Russian).

Maassoumi SM. 2012b. Exine Ultrastructure of *Fritillaria* (Liliaceae Juss).Journal of Basic and Applied Scientific Research **2(2),** 1101-1109.

Maassoumi SM. 2005. Sporoderm ultrasculpture and ultrastructure in representative of *Liliaceae*Juss. and in relationship between their systematic problems, Ph. D. Thesis diss, Lomonosov State University, Moscow, Russia(in Russian).

Maassumi SM. 2008. The exine ultrastructure and ultrasculpture of the pollen grains in *Tulipa* L. (*Liliaceae* Juss.). Materials of the All-Russian palynological conference XII "palynology: Stratigraphy and Geoecology" **1,** 87-92 p. (In Russian).

Massoumi SM. 2012a. Pollon Morphologe of Erythronium L.(Liliaceae) and its Systematic Relationships .Journal of Basic and Applied Scientific Research **2(2),** 1833-1838.

Nilsson S, Praglowski J. 2002: Erdtman's handbook of palynology. Copenhagen: Munksgaard.

Pehlivan S, Özler H. 1992. Pollen Morphology of Turkish endemic Fritillaria michailovskyi Fomin and Fritillaria armena Boiss. (Liliaceae) The Fourth Plant Life of Southwest Asia Symposium, Yüzüncü Yil Üniversitesi . 114 p.

Potonib R, Zur hlikrobotanik der Kohlen, ihrer Verwandten. 1934. Zur hlorphologie der fossilen Pollen und Sporen. - Arb. Inst. Palaeontol. Petrogr. Brenn- steine **4(5).**

Praglowski J, Raj B. 1979. On some pollen morphological concepts. Grana **18,** 109-113.

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. 2007: Glossary of pollen and spore terminology, Rev. Palaeobot. Palynol. **143,** 1–81.

Reynalds ES. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17,** 208–212.

Sohma K, Takahashi M. 1982. Exine formation of *Smilacina yesoensis* Fr.et Sav. and *S. japonica* A. Gray (*Liliaceae*) // Sci. Rep. Tohoku University. Ser.IV. (Biol.) **38(2),** 165-172 p.

Takahashi M, Sohma K. 1982. Pollen morphology of the genus Clintonia (Liliaceae**).** Sci. Rep. Tohoku University., IV **38(2),** 157-164.

Walker JW. 1974. Evolution of exine structure in the pollen of primitive angiosperms. American Journal of Botany **61,** 891-902.

Weakly BS. 1977. A Beginner's Handbook in biological Electron Microscopy. Edinburg and London (in Russian).

Zarrei M, Zarre S. 2005. Pollen morphology of the genus *Gagea* (Liliaceae) in Iran. Flora; **200 (1),** 96 p.