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Pollen morphology of the genus *Allium* in comparison with genus *Calochortus* of order Liliales

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Abstract

The pollen grains of two genera (*Allium* and *Calochortus*) of Liliaceae (*sensulato*) family were investigated. Pollens were obtained from the Herbarium at Komarov Botanical Institute of the Russian Academy of Science (RAN) and different parts of Kermanshah region and compared with herbarium of Razi University of Kermanshah, Iran. Both genera were investigated using LM (Light Microscopy) and SEM (Scanning Electron Microscopy). According to LM and SEM, the pollen grains of genera were monad, monosulcate percolate, heteropolar with bilateral symmetry, which the sulcus extends from distal to proximal. Also sulcus membrane ornamentations in both genera were regulate-perforate or perforate-regulate but wall thickness and diameter of the perforations in the mesh networks in *Calochortus* were upper than *Allium* genus. According to this study the species of *Calochortus* can be divided into five subsections (elegant, nitidus, puchelli, gunnisoniani andvenusti). Although are *Allium* and *Calochortus* genera were belonging to *Alliaceae* and *Liliaceae* family respectively, but in morphology of the pollen grains were very similar and their differences were minimum. In comparison with the other genera of *Liliaceae* (*sensulato*) family, these genera (*Allium* and *Calochortus)* in term of morphology of pollen grains were similar to some species of *Tulipa* and *Fritillaria* (*Liliaceae* family)

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Introduction

There are various genera in Liliaceae order, which have a lot of ornamental and edible and medicinal importance in monocots. Because of this importance,some genera belonging to this order have been chosen to study and comparison. For the first time, *Calochortus* genus was described by Pursh (1814) in the first volume of Book category and description of the plants of North America. Furthermore, 73 species of this genus have mentioned in check list of Botanical GardensKew and 27 species have been studied by Kosenco (1987) using LM and SEM. These studies showed that the most *Calochortus* species are single sulcus, but *C.kennedyi* Porter species was different in vent and has two holes and single sulcus. *Calochortus* has four different kinds of exine according to surface decorations (Reticular, leaky, leaky button) The *Allium* genus is one of the most diverse and taxonomically difficult groups of the monocots that formerly regarded as member of the Liliaceaes L. (James, 2008; Block, 2010;). In modern systems of flowering plants,it is the largest genus Of *Alliaceae* (*Asparagales* order) (Angiosperm Phylogeny Group, 2003) and in the APG III classification system, *Allium* is placed in Amaryllidaceae family, Allioideae sub family (Chase *et al*., 2009), and also in the Alliaceae in classification systems in which that family is recognized as separate. *Allium* is taxonomically difficult and species boundaries are unclear. Most authorities accept 750 species (Pablo Hirschegger, 2010). An phylogeny of *Allium* was published based on the nuclear ribosomal gene IT'S in 2006. They divided *Allium* into 15 subgenera and 72 sections (Nikolai Friesen *et al.,* 2006). Furthermore, pollen size is known to be correlated with ploidy level.The Liliaceae pollen grains are usually monocolpate. The colpus generally extends fully to the end of the grain, it may be very wide or may have an operculum, which may be ornamented (e.g. reticulate), or thin and scab rate (Dane, 1999;; Ozler and Pehlivan, 2007). Pollen morphology of 23 *Allium* species in European, Turkey and around Istanbul, belonging to the sections *Molium*, *Scorodon*, *Brevispatha*, *Codonoprasum*, *Allium* and *Melanocrommyum*, were investigated under LM (Light Microscopy) and by SEM (Scanning Electron Microscopy) by Neriman and Mine (2009). Hosseinzade *et al*., (2009) examined the Pollen morphology of 19 species belonging to wild onion using LM and SEM and showed that the exine's surface decorations in various species are leaky to lumpy. Neshati *et al.,* (2009) studied the pollen morphology of 30 species belonging to wild onion using LM and SEM and showed that onion's pollen is from the single sulcus type. The purpose of this study is comparing the pollination biology of these plants using LM and SEM. Results of this study can help us for systematic investigation of these genera.

Materials and methods

Pollen samples were obtained from the Herbarium in Komarov Botanical Institute of the Russian Academy of Science (RAN) and different region of Kermanshah province and compared with herbarium of Razi university of Iran. *Allium* taxa consist of section *Allium* (*A.ampeloprasum* L. varatroviolaceum and *A. iranicum* Wendlbo (wendlbo).

For LM studies

The number of pollen samples from 18 different species of the genera herbarium and also the nature and method of preparing gelatin, glycerin, Erdtman (1952) were prepared for light microscopic observations. Dimensions of at least 25 pollen grains in each plant samples to help calibrate Eyepiece Leitz optical microscope with a magnification of 400 times with HM_LUX3 model was studied. The equatorial axis (E) or the major axis of pollen and polar axis (P) when the groove was on the side was (Fig 1,2).

For SEM studies

Unacetolysed pollen grains were attached to aluminum stubs and sputter coaled (Eiko IB-3 ION COATER) with Au/Pb and examined under Hitachi S-405 A scanning electronic microscope (Fig 3,4,5,6,7 and 8).The purpose of this study was to compare the pollen morphology of the two genera of the order liliales.

Results

For LM observation

Measuring of equatorial axis, polar axis, P/E ratio, holes diameter, and wall thicknesses has been shown that pollens size of *Calochortus* samples were (26.1-42.2) µm and the P/E ratio was (0.53-0.69) µm. The shape of pollen is flattened oval and decorations tend to being leaky.LM study of *Allium ampeloprasum*L.var, at roviolaceum by showed that Kermanshah and Paveh were the smallest and largest mean polar axis respectively. Results showed that for *Allium ampeloprasum* L.var.atroviolaceum Sonqor and Paveh were the smallest and largest mean of Equatorial axis respectively (Figs 3 and 7). Kermanshah and Sonqor had the smallest and largest P/E ratio and *Allium ampeloprasum* L. var.atroviolaceum respectively. Size of the polar axis did notdiffer from other species, but size of Equatorial axiswas more and P/E ratio was less than other species (Fig 3 and 8).

Table 1. Comparison of polar and Equatorial with LM.

	Taxon	(1E)	(p)	P.E
1	*Allium*iranicumWendlboKamyaran	22.1-28.0-34.0	14.0-19.0-27.5	0.68
2	*Ai*.W.Kermanshah	17.0-22.2-34.0	14.5-19.4-28.0	0.88
3	*Ai*.W.Paveh	20.0-26.0-34.0	14.2-18.8-26.8	0.73
4	*Ai*.W.Ravansar	17.8-22.4-33.8	15.0-19.2-28.0	0.85
5	*Ai*.W.Sanandag	25.0-29.4-33.6	15.0-17.9-20.5	0.61
6	*A.ampeloprasum*L. VaratroviolaceumKamyaran	22.5-29.1-33.5	17.0-18.2-19.4	0.63
7	*Aa*.L.Kermanshah	27.4-29.8-31.7	15.0-17.52-18.8	0.59
8	*Aa*.L.Paveh	26.4-31.1-33.1	15.0-19.26-22.7	0.62
9	*Aa*.L.SarabNelofar	25.0-30.1-32.5	15.0-18.6-22.5	0.60
10	*Aa*.L.Songhor	25.0-29.0-31.8	15.0-19.0-22.5	0.66
11	*C. apiculatus* Baker	42.5-38.5-32.5	32.5-29.7-27.5	0.63
12	*C. coeruleusS*.Watson	32.6-27.9-22.0	20.2-17.8-15.3	0.63
13	*C. elegans*Pursh	42.5-38.0- 33.8	25.0-21.5-20.0	0.57
14	*C. eurycarpusS*.Watson	39.5-37.7-34.4	24.5-22.9-18.3	0.61
15	*C. greeneiS*.Watson	42.5-34.9-31.5	27.5-22.4-25.0	0.64
16	*C. albus*Douglas ex Benth.	32.6-27.9-22.0	20.2-17.8-15.3	0.66
17	*C. gunnisoniS*.Watson	47.1-42.2-39.5	32.0-27.4-22.5	0.64
18	*C. venustus*Douglas ex	42.5-39.89-37.5	35.0-27.6-25.0	0.69

Table 2. Pollen morphological parameters of the investigated Allium and Calochortus species using SEM.

Continuity at Lumina size	Lumina size(µm)	Width of muri (µm)	Exineornamention	Taxon
Discrete	0.3-0.4	0.3-0.7	Corrugated-punching	*C. apiculatus* Baker
Discrete	0.1-0.3	0.2-0.6	Corrugated-punching	*C. coeruleus*S.Watson
Discrete	0.1-0.2	0.2-0.4	Corrugated-punching	*C. elegans*Pursh
Discrete	0.3-0.6	0.2-0.8	Corrugated-punching	*C. eurycarpus*S.Watson
Joined	0.1-0.4	0.1-0.3	Punching -Corrugated	*C. albusDouglas ex Benth.*
Joined	0.2-0.8	0.2-0.5	Corrugated-punching	*C. gunnisoni*S.Watson
Joined	0.1-0.6	0.3-0.8	Corrugated-punching	*C. venustus* Douglas ex
Discrete	0.3-0.8	0.2-0.6	Corrugated-punching	*C. greenei*S.Watson
Discrete	0.18-0.37	0.35-0.6	Corrugated-punching	AaKamyaran
Discrete	0.2-0.4	0.16-0.43	Corrugated-punching	Aa kermanshah
Discrete	0.2-0.66	0.26-0.53	Corrugated-punching	AaPaveh
Discrete	0.2-0.4	0.26-0.4	Corrugated-punching	AaSarabNelofar
Discrete	0.18-0.37	0.23-0.4	Corrugated-punching	Aasonghor
Joined	0.26-0.6	0.2-0.32	Punching -Corrugated	Ai Kamyaran
Joined	0.23-0.41	0.41-0.75	Punching -Corrugated	Ai kermanshah
Joined	0.15-0.31	0.45-1.59	Punching -Corrugated	Ai Paveh
Joined	0.2-0.26	0.16-0.5	Punching -Corrugated	Ai Ravansar
Joined	0.2-0.46	0.3-0.75	Punching -Corrugated	Ai Sanndag

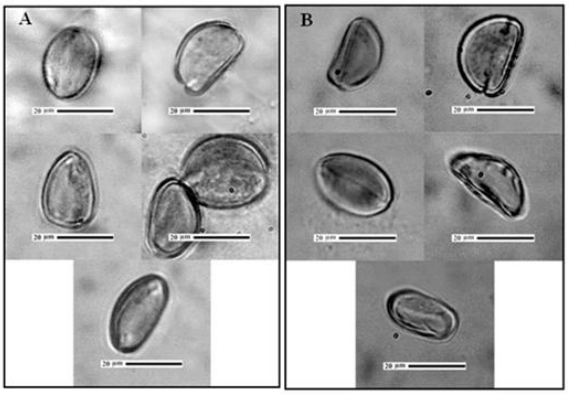

Fig. 1. Pollen shapes with light microscopic in *A. ampeloprasum* L.Var *atroviolaceu* (A) and *A. iranicum* Wendlbo (wendlbo) (B).

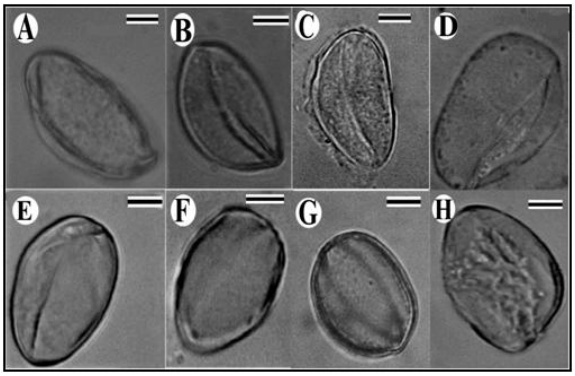

Fig. 2. Illustration of a light microscope (LM) of the overall shape of the pollen species:
(A) *C. apiculatus* Baker, (B) *C. coeruleus*S.Watson, (C) *C. elegans*Pursh, (D) *C. eurycarpus*S.Watson, (E) *C. greenei*S.Watson, (F) *C. albus*Douglas ex Benth.,(G) *C. gunnisoni*S.Watson, (H) *C. venustus* Douglas ex Benth.

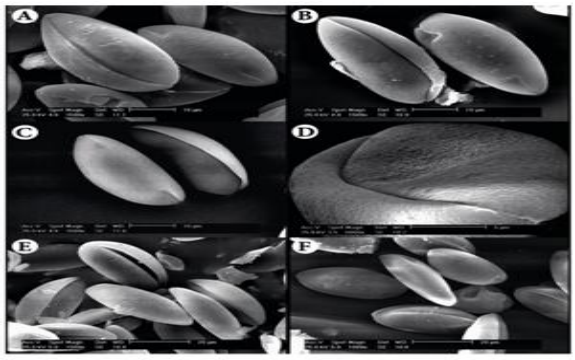

Fig. 3. Figures of pollen grain in equatoral view in *A. ampeloprasum* L. Var. a*troviolaceum* Regel in different regins of kermanshah Prevance with scanning electron microscope (SEM):
A)Kamyaran ,B) kermanshah , C,D)Paveh , E)Sarab nilofar , F)Songo.

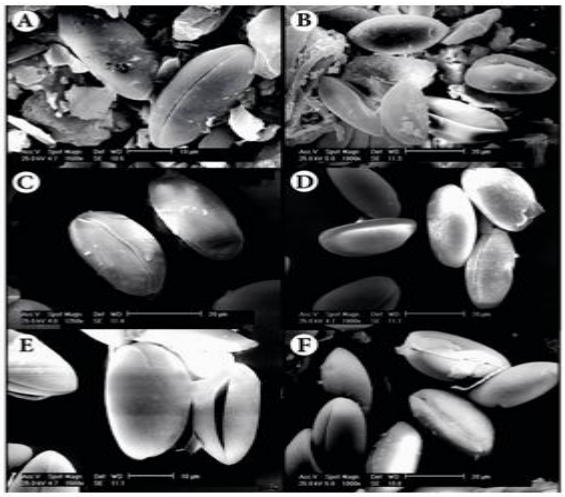

Fig. 4. Figures of pollen grain in equatoral view in *Alliumiranicum*Wendlbo (wendlbo)in different regins of kermanshah Prevance with scanning electron microscope (SEM):
A)Kamyaran, B) kermanshah , C)Paveh, D, E)Ravansar, F)Sanandagh.

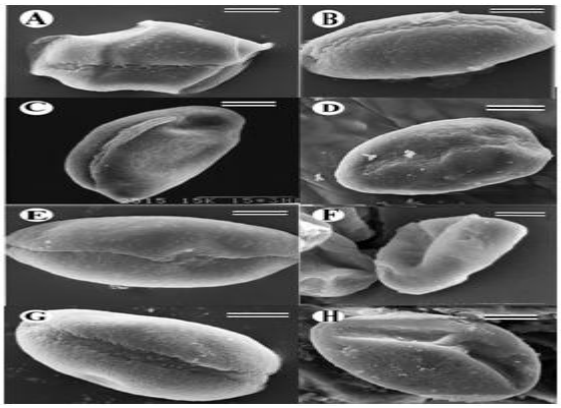

Fig. 5. Scanning electron microscope images (SEM) of the total pollen:6(µm):
A)*C. apiculatus* Baker, B) *C. coeruleus*S.Watson, C) *C. elegans*Pursh, D) *C. eurycarpus*S.Watson, E) *C. greenei*S.Watson, F) *C. albus*Douglas ex Benth., G) *C. gunnisoni*S.Watson, H) *C. venustus* Douglas ex Benth

For SEM observation

Results of studying *Allium ampeloprasum* L.var.atroviolaceum using SEM showed that this species has lumpy-leaky decorations in the studied habitats and the walls are discrete and the walls thickness and the holes sizes in the habitations were almost the same and there were minimum differences. The surface decorations around the sulcus in Sonqor and Kamyaran were different from

the other regions and were from the leaky-lumpy type. The surface decorations and wall continuity in this species was different from the other species (Fig 8).LM study of Iranic *Allium* shows that Sanandaj had the smallest mean of polar axis and Kermanshah had the largest mean. Kermanshah had the smallest mean equatorial axis and Sanandaj had the largest mean which is exactly vice versa to mean of polar axis (Fig-4).Sanandaj and Kermanshah were the smallest and largest P/E ratio respectively, which it had much more polar axis and P/E ration compare with other species. Results of studying *Allium* and *Calochortus* by SEM showed that this species in the studied habitat has leaky-lumpy decorations and the walls are continuous and the wall thickness is different but the holes sizes are the same.

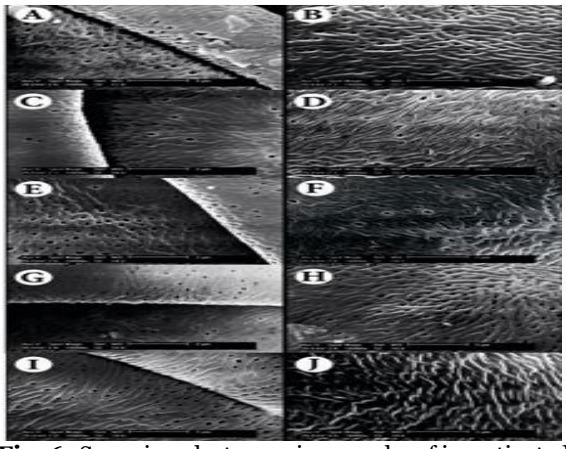

Fig. 6. Scanning electron micrographs of investigated species pollen of *Allium ampeloprasum* L.var atroviolaceum in region of sulcus (A,C,E,G,I) and in region of sulcus-less(B,D,F,H,J) Kamyaran ;C,D= kermanshah; E.F= Paveh; G,H= Sarab nelofar; I,J=Songhor=A,B.

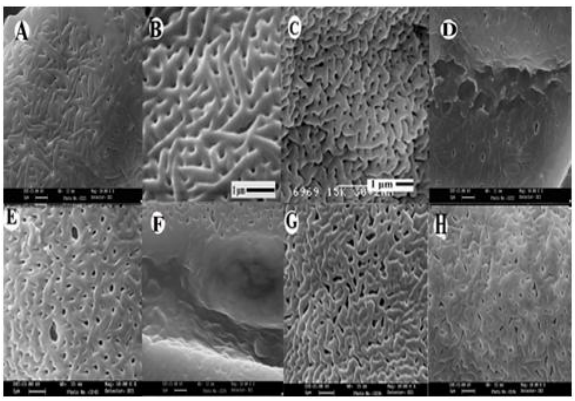

Fig. 7. Detail of pollen wall surface decoration using(SEM). 10(µm).

A) *C. coeruleus*S.Watson, B) *C. albus*Douglas ex Benth., C) *C. elegans*Pursh, D)*C. apiculatus* Baker, E) *C. greenei*S.Watson, F) *C. venustus* Douglas ex Benth., G) *C. gunnisoni*S.Watson, H) *C. eurycarpus*

Discussion

One of the advantages of studying pollen morphology is that it can help Taxonomy very well and Pollen diversity in different habitats will affect pollen morphology.In evolution of *Liliales* order by using the data of Pollination biology (Kosenco, 1996). Maassoumi *et al.*, (2005) has been considered that reticular surface decorations are earlier than smooth surface decorations, as in *Lilium*, surface decoration is from the coarse reticular type, while the in *Fritillria* is coarse reticular to leaky and in *Tulipa* genus, surface decorations are tend to leaky or smooth and also pollens of some *Tulipa* are totally round. On the other hand, there are not many differences between data of LM and data obtained by Kosenco, (1987) but in SEM studies the differences are much more. For example, lacy reticulum wall of the sample of *C. albus* is continuous, while the reticulum wall in Kosenco's sample is Separate. Also wall thickness of lacy reticulum in *C. elegans* Pursh is more than the thickness reported by Kosenco (1987) for this species. However the onion is in Alliaceae family and *Calochortus* in Liliaceae family, but the pollen morphology is very similar and the differences were minimum. According to LM and SEM investigations, the pollen grains of both genera are monad, monosulcate operculate, heteropolar with bilateral symmetry that the sulcus extends from distal to proximal. Also sulcus membrane ornamentations in both genera are regulate-perforate or perforate-regulate but wall thickness and diameter of the perforations in the mesh networks in *Calochortus* was higher than other genus. Furthermore present investigation aided the species *Calochortus* that divided into five subsections as: elegant, nitidus, puchelli, gunnisoniani and venusti. Although *Allium* genus belonging to Alliaceae family and *Calochartus* genus belonging to the Liliaceae family, but in terms of the morphology of the pollen grains werevery similar and their differences were minimum.

Furthermore both genera in comparison with the other genera of Liliaceae (sensulato) family, in term of morphology of pollen grains are similar to some of *Tulipa* and *Fritillaria* species of Liliaceae.

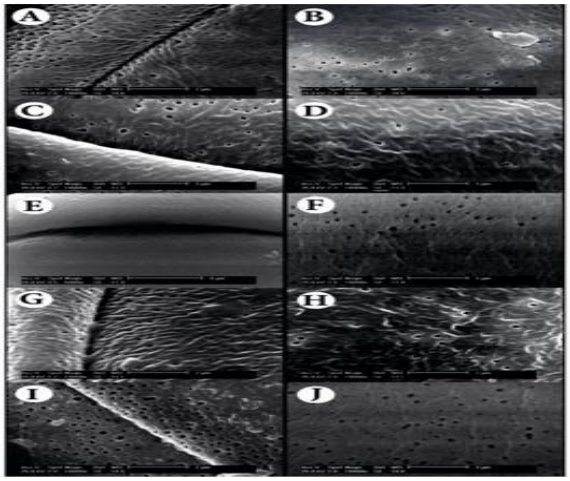

Fig. 8. Scanning electron micrographs of investigated species pollen of *Allium iranicum* Wendlbo (wendlbo) in region of sulcus (A,C,E,G,I) and in region of sulcus-less(B,D,F,H,J Kamyaran ;C,D= kermanshah; E.F= Paveh; G,H= Ravansar; I,J=Sanandag.

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