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Pollen morphology of *Allium ampeloprasum* L. var. atroviolaceum and *Allium iranicum wendelbo* (wendelbo)

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Key words: LM, SEM, pollen grains, *Allium*.

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Abstract

Pollen morphology of two species of *Allium* belonging to *Allium* part, in Iran using LM and SEM has been studied. These species, includes *Allium ampeloprasum* L. var, *atroviolaceum* and *Allium iranicum* wendelbo (wendelbo), which in some refreces such as Flora Iranica (Rechinger) has been introduced as two subspecies of *Allium ampeloprasum* L. var, atroviolaceum and given the size of pollen grains is almost the same but have different surface decorations, also we focus on that they are separate species. Studies show that pollen in both single sulcus species which sulcus continues to the end, single sulcus, oval pollen with surface decoration is from the type Leaky-Lumpy and Lumpy-Leaky. Wall thickness of *Allium ampeloprasum* L. var, *atroviolaceum*'s lacy reticulum is more than *Allium iranicum* wendelbo (wendelbo). But the hole diameter between reticulum in *Allium iranicum* wendelbo (wendelbo)'s pollen is higher. The walls of pollen in *Allium iranicum* wendelbo (wendelbo)'s pollen (all are continuous, but in *Allium ampeloprasum* L var, *atroviolaceum* 's pollen are discrete). The overall picture of pollens in terms of pole is round oval and in terms of equator is oval., Pollen morphology of these species were similar to some species of *Tulipa,* and *Fritillaria* (from subfamily of *liliaceae*), In comparison with other genus of *Liiaceae*.

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Introduction

Prior to the commencement of the 19th century, a lot of herbs were regarded as traditional medicines and were comprised in medicinal prospective and formularies simultaneously (Gantait *et al.*, 2010). Hundreds of genera are used in herbal remedies and in traditional medicines all over the globe (Gantait *et al.*, 2009, 2009a).

As per one estimate, 35000 to 70,000 species have been used in some countries for medicinal purposes (Sebastian *et al.*, 2007). Most of aromatic plants posse's antimicrobial and antifungal ability which due to its essential oil constituents (Taran *et al.*, 2010, 2011). In many developing countries, some plant materials play an important role in PHC (Primary Health Care) (Zakaria, 1991; Sokmen *et al.*,1999). The genus *Allium* consists of hundreds of medicinal plant species and is one of the most imperative sources of life supporting drugs. (Gantait *et al.*, 2010). *Allium* genus is comprising of more than 600 different species, other common members are leek, chive, ampeloprasum, shallot and scallion.(Saleh Abu-Lafi *et al.*, 2004).

Allium ampeloprasum var. *porrum* l. (liliaceae), is a bulbous perennial plant, which is consumed daily. Also, is used as medicine. For years, the bulbs have been used as traditional medicine for treating inflammatory symptoms. The crushed bulbs are used to treat initial stages of cough, mucous secretion and sore throat. The fresh juice is taken orally as a stomachic and antispasmodic and is also reputed to possess digestive properties (Camila Rodrigues Adão *et al.*, 2011). In modern systems of Flowering Plants it is the largest genus of *Alliaceae* (order Asparagales), and in the APG III classification system, *Allium* is belonging to *Amaryllidaceae* family, Allioideae subfamily(Chase, 2009, Angiosperm Phylogeny Group, 2003). In the *Alliaceae* in classification systems in which that family is recognized as separate also (Knud, 1998, Bogdanović *et al.*, 2008).

Allium is taxonomically difficult and species boundaries are unclear. Most authorities accept 750 species (Hirschegger *et al.*, 2010). An phylogeny of *Allium* was published based on the nuclear ribosomal gene ITS in 2006. They divided.

Allium into 15 subgenera and 72 sections (Friesen *et al.*, 2006). Furthermore, pollen size is known to be correlated with ploidy level. This relationship was described in *Allium* by Bothmer (1974). 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; Karaca *et al.*, 2007; Ozler *et al.*, 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 Özhatay (2009). Hosseinzade *et al.* (2009) examined the Pollen morphology of 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 (Hosseinzade *et al.*,2009). 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 . Two species of the onion genus are (*Allium ampeloprasum* L. Var, *atroviolaceum* Regel) and (*Allium iranicum* Wendelbo (wendelbo). The *Allium* genus is one of the most diverse and taxonomically difficult groups of the monocots that formerly regarded as member of the Liliaceae s.l. (Block, 2010; James, 2008; Dilys, 1992; Haim, 2002; Woodward, 1996). *Allium* is taxonomically difficult and species boundaries are unclear. So the purpose of this study is comparing the pollination biology of these plants by LM and SEM which can help to study their systematic.

Material and methods

Plant material Pollen samples were collected from

different parts of Kermanshah and kurdestan provinces and compared with herbarium of Razi university of Iran. *Allium* taxa consist of section *Allium* (*A. ampeloprasum* L. var *atroviolaceum* and *A. iranicum* Wendelbo(wendelbo).The number of pollen samples of the genera were collected from 10 different location in the west of Iran.

For LM observations

Pollen was acetolysed following the technique of Erdtman(1960) and mounted in glycerine jelly. Slides were prepared for LM by mounting pollen in glycerin jelly. Dimensions of at least 25 pollen grains of per plant samples to help calibrate Eyepiece Leitz optical microscope with a magnification of 400 times with HM_LUX3 model was studied. Measurements, were taken based on 25 pollen grains, including the equatorial axis (E) or the major axis of pollen and polar axis (P) when the groove is on the sidewas (fig. 1, fig. 2).and the P/E ratio was calculated.

For SEM studies

For samples with a scanning electron microscope (SEM) Not acetolysed pollen into the metal block (sample stub) and then transferred to the Layhnshany gold (Sputter coater BAL-TEC model SCDOOS manufacturing companies of Switzerland), the blocks covered with deposits of gold or platinum and transferred electron microscope to see them. The microscope scanning electron (SEM) model LEO 440i build England and model Hitachi S-405 A made in Japan micrograph electron enlarge 10000-1000 for procurement Grdydkh to help detail level Agzyn such as the type of surface ornament, wall thickness Network The net-like wall distance networks from each other, and the pore structure were evaluated in the groovez (Fig. 3, Fig. 4, Fig. 5, Fig. 6).

Statistical analysis

The results were achieved using analysis of variance and mean comparison of experimental treatments by ward way.

Results

Study of Allium ampeloprasum L var, atroviolaceum using LM shows that Allium ampeloprasum of Kermanshah region had the smallest mean polar axis and Paveh region had the largest mean polar axis. Allium ampeloprasum L var, atroviolaceum of Sonqor has the smallest mean of Equatorial axis and Paveh hast the largest (Tab. 4, Tab. 8) and Kermanshah region had the smallest P/E ratio and Allium ampeloprasum L var, atroviolaceum of Songhor region was the largest. Which in comparing with the other species the size of polar axis dose not differ, but the size of Equatorial axis is more and P/E ratio is less than the other species (Tab. 4, Tab. 9). Results of studying Allium ampeloprasum L var, atroviolaceum using SEM shows 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 are almost the same and there were minimum differences. Furthermore, the surface decorations around the sulcus in Songhor and Kamyaran were different from the other habitations and from the leaky-lumpy type. The surface decorations and the wall continuity in this species were different from the other species (Tab. 9). LM study of iranic Allium shows that Sanandaj has the smallest mean of polar axis and Kermanshah has the largest mean. Kermanshah has the smallest mean equatorial axis and Sanandaj has the largest mean which is exactly vice versa to mean of polar axis (Tab. 4) And Sanandaj has the smallest P/E ratio and Kermanshah has the largest, which in compare to the other species, it has a much more polar axis and P/E ratio. Results of studying *Allium iranicum* by SEM shows that this species in the studied habitat has leaky-lumpy decorations and the walls were continuous and the wall thickness was different but the holes sizes are the same (Tab. 9, fig. 5, fig. 6). The surface decoration around the sulcus in *Allium iranic* pollen of Kermanshah was different from other pollens and different from lumpy-leaky type. The surface decoration around the sulcus in *Allium iranicum* pollen of Sanandaj was just from the leaky type. Results show that there were four pollens as Allium ampeloprasum L var, atroviolaceum from different habitats that all of them were in one group. Allium iranicum number seven and eight, due to the

same climate situation as to their habitats were in one group. Because of having similar characteristics of pollen with Allium ampeloprasum L var, atroviolaceum, they are located in the vicinity. Allium iranic number 10 due to specific forest cover and climate was in separated group (Fig. 7). The studies showed that two studied species in different habitats tend to have leaky surface decorations, however, about the wall continuity, Allium iranicum wendelbo (wendelbo) species, in different habitats was continuous. But the wall of Allium ampeloprasum L var, atroviolaceum had various kinds of continuous and discrete (Fig. 3, Fig. 4, Fig. 5, Fig. 6).

Table 1. Analysis of variance for the trait polar axis *A. ampeloprasum* L. Var *atroviolaceum* collected at five location.

Mean-square	Degrees of freedom	Sources changes
11.79 **	4	collected location
2.24	120	error

Level significant at the 5%.

Table 2. Analysis of variance for the trait Equatorial axis *A. ampeloprasum* L. Var *atroviolaceum* collected at five location.

Mean-square	Degrees of freedom	Sources changes
19.78 **	4	collected location
4.54	120	error

level significant at the 1%.

Table 3. Analysis of variance for the trait P/E *A. ampeloprasum* L. *Var atroviolaceum* collected at five location.

Mean-square	Degrees of freedom	Sources changes
0.016*	4	collected location
0.005	120	error

level significant at the 5%.

Table 4. Comparison of polar and Equatorial characteristics *A. ampeloprasum* L. Var *atroviolaceum* level significant at the 5%.

P/E	E	P	location
0.53(0.63±0.09)0.86 a	22.5(29.1±3.09)33.5 b	17(18.2±0.7)19.4 bc	Kamyaran
0.47(0.59±0.04)0.66 b	27.4(29.8±1.3)31.7 b	15(17.52±1.08)18.8 c	kermanshah
0.51(0.6±20.07)0.80 ab	25(30.1±1.74)32.5 ab	15(18.6±1.81)22.5 ab	Sarab Nelofar
0.53(0.66±0.07)0.81 a	25(29±1.73)31.8 b	15(19±1.74)22.5 ab	Songhor
0.50(0.62±0.07)0.81 ab	26.4(31.1±1.48)33.1 a	15(19.26±1.79)22.7 a	Paveh

Table 5. Analysis of variance for the trait polar axis *A. iranicum* Wendlbo(Wendlbo collected at five location.

Mean-square	Degrees of freedom	Sources changes
8.79 ns	4	collected location
10.24	120	error

Discussion

Recent studies have been suggested that all *Allium*'s pollens are single sulcus and single sulcus pollens in

seed plants are considered as primary, and widely are in Monocots (Ozler *et al*., 2007, Furness *et al*., 2001, Donmez *et al*., 2008).

Table 6. Analysis of variance for the trait Equatorial axis *A. iranicum* Wendelbo (Wendelbo) collected at five location.

Mean-square	Degrees of freedom	Sources changes
262.55 **	4	collected location
11.26	120	error

** level significant at the 1%.

Table 7. Analysis of variance for the trait P/E *Allium iranicum* Wendlbo(Wendlbo) collected at five location.

Mean-square	Degrees of freedom	Sources changes
0.349**	4	collected location
0.017	120	error

** level significant at the 1%.

Table 8. Comparison of polar and Equatorial characteristics *Allium iranicum* Wendelbo (Wendelbo).

P/E	E	P	location
0.49(0.61±0.06)0.74 c	25(29.4±2.11)33.6 a	15(17.9±1.45)20.5 a	Sanndag
0.55(0.68±0.1)0.86 bc	22.1(28±2.68)34 a	14(19±3.2)27 a	Kamyaran
0.67(0.88±0.14)1.24 a	17(22.2±4.31)34 c	14.5(19.4±3.81)28 a	kermanshah
0.53(0.73±1.01)0.89 b	20(26±3.18)34 b	14.2(18.8±3.38)26.8 a	Paveh
0.65(0.85±0.18)1.26 a	17.8 (22.4±3.99)33.8 c	15(19.2±3.65)28 a	Ravansar

Table 9. Pollen morphological parameters of the investigated *Allium* species using SEM *Allium iranicum* Wendlbo(Ai) and *A. ampeloprasum* L. Var *atroviolaceum*(Aa).

Number of Lumina size (around) in region of sulcus/ region of sulcus-less	Surface ornamentation around the track	Lumina size (density per square)	Continuity Width of muri	Lumina size (µm)	Width of muri (µm)	exine ornamention	location
high	Punching - Corrugated	Average	Discrete	0.18-0.37	0.35-0.6	Corrugated-punching	Aa Kamyaran
low	Corrugated -punching	Average	Discrete	0.2-0.4	0.16-0.43	Corrugated-punching	Aa kermanshah
low	Corrugated -punching	Average	Discrete	0.2-0.66	0.26-0.53	Corrugated-punching	Aa Paveh
homologous	Corrugated -punching	Average	Discrete	0.2-0.4	0.26-0.4	Corrugated-punching	Aa Sarab Nelofar
high	Punching - Corrugated	low	Discrete	0.18-0.37	0.23-0.4	Corrugated-punching	Aa songhor
high	Punching - Corrugated	high	Joined	0.26-0.6	0.2-0.32	Punching -Corrugated	Ai Kamyaran
homologous	Corrugated -punching	low	Joined	0.23-0.41	0.41-0.75	Punching -Corrugated	Ai kermanshah
homologous	Punching - Corrugated	Average	Joined	0.15-0.31	0.45-1.59	Punching -Corrugated	Ai Paveh
high	Punching - Corrugated	low	Joined	0.2-0.26	0.16-0.5	Punching -Corrugated	Ai Ravansar
high	punching	Average	Joined	0.2-0.46	0.3-0.75	Punching -Corrugated	Ai Sanndag

Previous studies have shown that in Allium genus, shape of pollens based on LA/SA ratio, oval and subprolate and in view distal is in form of ellipsoid and in polar view is in form of annular. Sulcus in Scorodon, Bervispatha, Codonoprasum, Allium, Melanocrommyum, Mollium parts, is expanding from distal to proximal, but in section Allium, sulcus clearly expands in both distal and proximal sides.

Studies done by Bogdanović *et al*., (2008), Neshati *et al*., (2009), Koc, (2001), Ozler, (2001),Guler, (2006) and Studies done by Department of Pharmaceutical Botany, College of Pharmacy, Istanbul University 2009 shows that section Molium of Allium genus has thin and round sulcus. Scorodon, Bogdanovice parts sulcus expands in round and flat form. In section melanocrommyum, sulcus expands roundly. Exine in these parts is in form of leaky-lumpy,lumpy-shady,shady. In results all these mentioned studies show that there are several significant features and characteristics in Allium genus. Results showed that defines that all species which studied in Allium genus from the section Allium have leaky-lumpy or lumpy-leaky pollen**.** This caused that these two species have differences in surface decoration of pollen. As regards in some sources (Flora Iranica) these two plants are known as subspecies, but we believe, because these two plants have different surface decoration in their pollen, so they are two Separate species as in also focused on being two species (Woodward, 1996, Gregory *et al*., 1998).

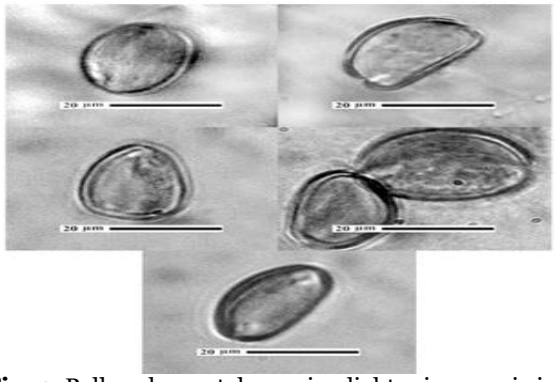

Fig. 1. Pollen shapes taken using light microscopic in *A. ampeloprasum* L. var *atroviolaceum*.

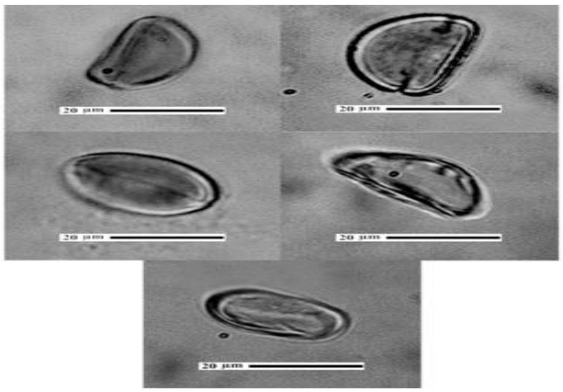

Fig. 2. Pollen shapes taken using light microscopic in *A. iranicum* Wendlbo (wendlbo).

Studies show that pollen in both single sulcus species which sulcus continues to the end, single sulcus, oval pollen with surface decoration is from the type Leaky-Lumpy and Lumpy-Leaky. Wall thickness of Allium ampeloprasum L. var, atroviolaceum's lacy reticulum is more than Allium iranicum wendelbo (wendelbo). But the hole diameter between reticulum in Allium iranicum wendelbo (wendelbo)'s pollen is higher. The walls of pollen in Allium iranicum wendelbo (wendelbo)'s pollen (all are continuous, but in Allium ampeloprasum L var, atroviolaceum 's pollen are discrete). The overall picture of pollens in terms of pole is round oval and in terms of equator is oval., Pollen morphology of these species were similar to some species of Tulipa,and Fritillaria (from subfamily of liliaceae), In comparison with other genus of Liiaceae (Kosenko, 1996; Maassoumi, 2005).

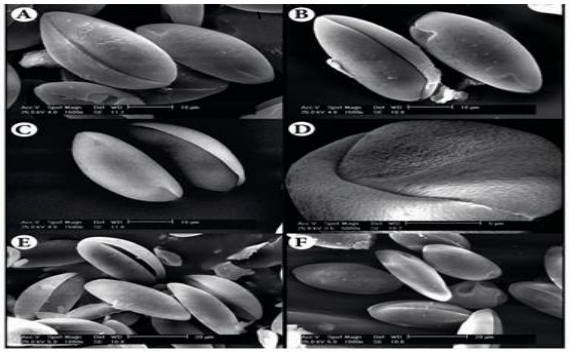

Fig. 3. Pollen grains in equatorial view in *A. ampeloprasum* L. Var. a*troviolaceum* Regel in different regions of kermanshah Province with scanning electron microscope (SEM): A) Kamyaran, B) Kermanshah C, D) Paveh E) Sarab nilofar F)Songhor.

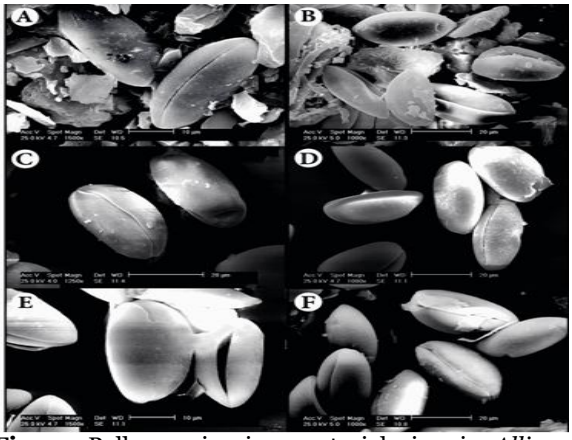

Fig. 4. Pollen grains in equatorial view in *Allium iranicum* Wendlbo (wendlbo) in different regions of

kermanshah province with scanning using electron microscope (SEM): A) Kamyaran, B) kermanshah, C)Paveh, D, E) Ravansar, F) Sanandag.

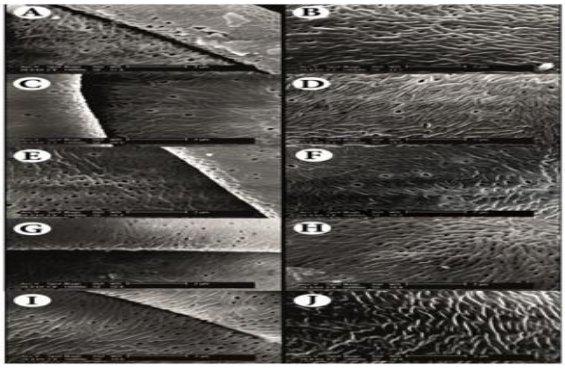

Fig. 5. Scanning electron micrographs of investigated species pollen of *Allium* ampeloprasum L.var atroviolaceum in in region of sulcus (A,C,E,G,I) and in region of sulcus-less(B,D,F,H,J): Kamyaran; C, D. kermanshah; E,F. Paveh; G,H. Sarab nelofar; I,J.Songhor ; A,B.

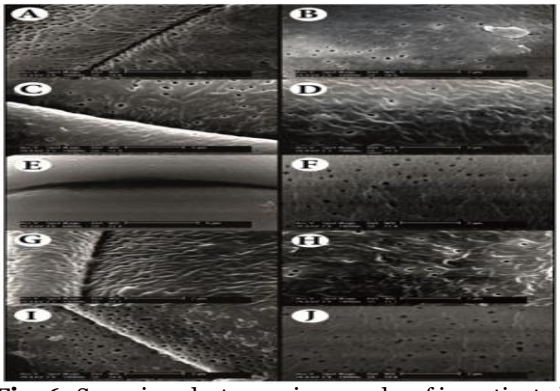

Fig. 6. Scanning electron micrographs of investigated species pollen of *Allium* iranicum Wendlbo (wendlbo) in region of sulcus (A,C,E,G,I) and in region of sulcus-less(B,D,F,H,J): Kamyaran; C,D. kermanshah; E.F. Paveh; G,H. Ravansar; I,J.Sanandag A,B.

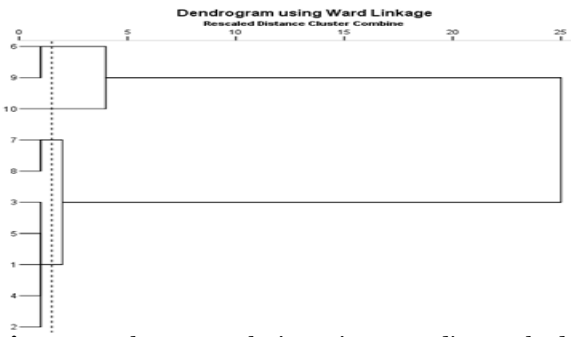

Fig. 7. cluster analysis using Ward's method (*A.ampeloprasum* L. var atroviolaceum and *A. iranicum* Wendlbo (wendlbo).1,2 Kamyaran ;3,4= kermanshah; 5,6= Paveh; 7,8= Ravansar; 9,10=Sanandag.

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