

POLLEN MORPHOLOGY OF SOME ADONIS L. SPECIES (RANUNCULACEAE) FROM IRAN

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The pollen grains of four species of *Adonis* consist of *A. wolgensis* Stev., *A. aestivalis* L., *A. dentata* Del. and *A. flammea* Jacq. were studied using scanning electron microscopy (SEM). In this work 10 different pollen characters were described, illustrated and compared. All pollen grains were tricolpate and exin ornamentation in two types, as echinate in *A. wolgensis* with small grains (mean = 19.40 μ) and in 3 other species with scabrate sculpturing and large grains. The result of SEM investigation of pollen characters revealed segregation of perennial *Adonis wolgensis* from other annual species. The pollen type of *A. wolgensis* is the first report in the world.

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مطالعه دانه گرده برخی گونه های جنس *Adonis* L. (تیره آلاله) در ایران

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دانه گرده چهار گونه از آدونیس های ایران شامل: *Adonis dentata* Del.، *Adonis aestivalis* L.، *Adonis wolgensis* Stev. و *Adonis flammea* Jacq. توسط دستگاه میکروسکوپ الکترونی نگاره (SEM)، مورد مطالعه و بررسی قرار گرفت. در طی این مطالعه ۱۰ خصوصیت مختلف گرده شناسی سنجیده و اندازه گیری شدند. تمامی دانه های گرده بررسی شده سه شیاره (Tricolpate) می باشند، تزئینات دیواره خارجی دانه گرده (Exin) در دو تیپ متفاوت دیده می شوند. در گونه *A. wolgensis* که از نظر اندازه با میانگین قطر ۱۹.۴ میکرومتر کوچکترین دانه گرده را دارا می باشد، سطح دانه گرده به صورت خار دار (Echinate) دیده می شود، در حالی که در سایر گونه ها، دانه های گرده بزرگتر بوده و سطح دانه همگی به صورت ریز خاردار (Scabrate) است. نتایج این تحقیق نشان دهنده کاربرد قابل توجه صفات گرده شناسی در تفکیک و جداسازی گونه های مختلف مورد مطالعه به ویژه گونه چند ساله *Adonis wolgensis* از یکدیگر می باشد. دانه گرده *Adonis wolgensis* برای اولین بار در دنیا گزارش می گردد.

Introduction

The genus *Adonis* L. comprises approximately 40 species, distributed in temperate region of north hemisphere. The genus has been divided into two distinct sections. Sect. *Adonis* comprises annual herbs and the second sect. *Consiligo* DC. consist of perennial plants. These two sections differ in many characters of

morphology, palynology and karyology (Tamura 1968, Hoot 1991, Hoffmann 1998 and Pushkurlat 2000).

Rechinger (1992) reported 7 species in 6 subspecies of *Adonis* for the flora of Iran. All of them belong to sect. *Adonis*, but he didn't mention *Adonis wolgensis* Stev., while Assadi (1988) had been recorded *Adonis wolgensis* Stev. (sect. *Consiligo*) from NW Iran in Arasbaran Protected Area.

Table 1. Materials used for pollen morphology of four species of the genus *Adonis* in Iran.

Population	Species	Habitat Location	Herbarium and Voucher no.	Collector
A ₁₀	<i>Adonis aestivalis</i>	Golestan: Golestan National Park, Tangerah, Savar baghi, 10/05/2005	SBUH,8400287	Ghorbani, Heidary
A ₁₁	<i>Adonis aestivalis</i>	Guilan: Damash, Loushan, Amarlou Region, Pakdeh, 04/04/2006	SBUH,8500118	Heidary, Habibi
A ₁₂	<i>Adonis aestivalis</i>	Mazandaran: Marzan abad, Dashte Nazir, 12/05/2005	SBUH,8400295	Habibi, Heidary
D ₇	<i>Adonis dentata</i>	Khozestan: Behbahan, Emamzadeh Shirali, Margin of Maroon river, 25/02/2006	SBUH,8500116	Ghorbani, Heidary
D ₆	<i>Adonis dentata</i>	Golestan: Azad shahr to Sharoud road, Khosh Yeilagh, 15/04/2005	SBUH,8400292	Habibi, Ghorbani
F ₁	<i>Adonis flammea</i>	Lorestan: Noor abad e delfan, Margin of Bad Avar River, 05/06/2006	SBUH,8500122	Rostami, Ghorbani
W ₀	<i>Adonis wolgensis</i>	Azerbaijan: Arasbaran Protected Area, Hasano Forests, 2600 m, 17/07/1967	IRAN, 49834	Damanabi

Pollen morphology of *Ranunculaceae* has been investigated by various authors (Wodehouse 1936, Kumazawa 1936, Erdtman 1971, Hamilton 1976, Al-Eisawi 1986). They recognized three pollen types as tricolpate, pantocolpate and pantoporate, and the exin sculpturing with scabrate to echinate ornamentation in the family. Savittski (1982) studied various genera of *Ranunculaceae* including *Adonis* species and proved the taxonomic value of pollen characters in *Ranunculaceae*. Recently Clarke et al. (1991) described two different pollen types of *Adonis* as *A. annua* L. type and *A. aestivalis* L. type.

The present study aims to survey the pollen morphology of 4 Iranian species of *Adonis* as *A. wolgensis* Stev., *A. aestivalis* L., *A. flammea* Jacq. and *A. dentata* Del., using scanning electron microscopy and evaluating its significance in taxonomy of the genus.

Materials and Methods

The fully grown flowers of *Adonis aestivalis*, *A. dentata* and *A. flammea* were collected at full flowering stage from their natural habitats. Voucher specimens are deposited in Shahid Beheshti University Herbarium (SBUH). Pollen grains of *Adonis wolgensis* were obtained from herbarium samples of IRAN herbarium. The localities and numbers of voucher specimens are presented in Table 1. For SEM observations, unacetolyzed pollen grains were dusted onto stubs and coated with gold and then photographed using PHILIPS XL 30 scanning electron microscope at 15 KV voltages. For measurements "Image Tools" software with high accuracy and confidence degree were used. The mean of at least 10 pollen grain characters (consist of 8 quantitative and 2 qualitative characters from 7 populations as mentioned in table 1)

were considered and measured for each sample. The mean of quantitative characters were used, while qualitative characters coded in binary or multi state characters. Measured characters were presented in table 2.

Terminology

In general, the terms used for terminology are those proposed by Punt et al. (2007) and the site 'http://www.bio.uu.nl/~paleo/glossary/glos-int.htm'.

Results

The pollen grain characters of all species examined from different populations are presented in table 2. The pollen grains are isopolar and tricolpate but differ in size, shape and exin sculpturing. They are circular in polar view and elliptical in equatorial view (Fig. 1). The shape is apiculate in *A. aestivalis* (Fig. 2. A, C and E), while other species have prolate-suboblate pollen grains (Fig. 2.G and Fig. 3. I, K and M).

1. *Adonis wolgensis* Stev. (Fig. 3 M & N and table 2).

Pollen grains are isopolar and tricolpate with prolate-suboblate shape, it is only species that has echinate sculpturing. This species has the smallest pollen size with average = 19.40 μm in polar diameter and 13.43 μm in equatorial diameter. It has smallest colpi and mesocolpium length and Apocolpium index (0.22).

2. *A. dentata* Del. (Fig. 2 G & H, Fig. 3 I & J and table 2).

Pollen grains are isopolar, tricolpate and prolate-suboblate shape with scabrate sculpturing. This species

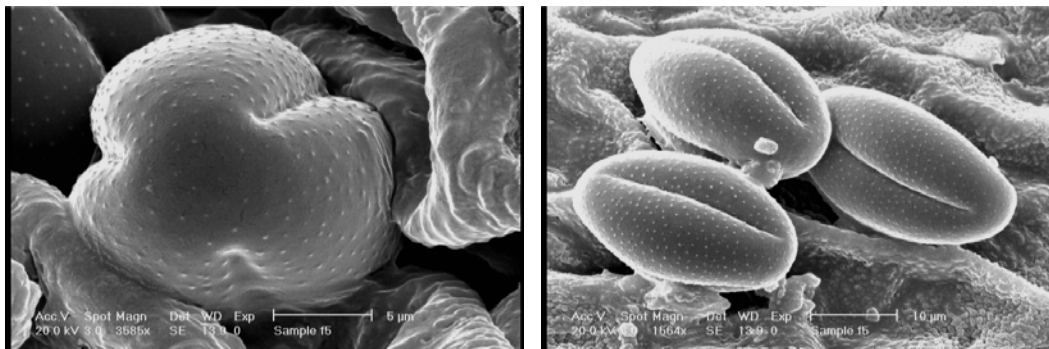


Fig. 1. Polar view of *Adonis* pollen grains in left and equatorial view of them in right.

with two populations have bigger pollen grains related to *A. wolgensis*, polar diameter 20.77-20.81 μm and equatorial diameter 13.69-14.97 μm . It has bigger colpi length, mesocolpium length and Apoculpium index (0.83).

3. *A. flammea* Jacq. (Fig. 3 K & L and table 2).

Pollen grains are isopolar, tricolpate and prolate with scabrate sculpturing. But this species differs from the others in polar diameter as 26.77 μm to 15.62 μm in equatorial diameter and colpi length about 19.64 μm . Apoculpium index about (0.81).

4. *A. aestivalis* L. (Fig. 2 A-F and table 2).

This widespread species with different morphological populations, presents an apiculate shape of isopolar and tricolpate pollen. Therefore, 3 different populations (A_{10} , A_{11} , A_{12}) of the species have been studied. In addition to distinct pollen shape all populations have scabrate sculpturing. *A. aestivalis* has the largest pollen grains size between examined species with polar diameter 29.7-32.86 μm and equatorial diameter equal to 18.86-19.06 μm . The results demonstrate considerable diversity in populations of *A. aestivalis*, such as polar and equatorial diameter in A_{10} is about 32.86 μm and 14.49 μm , while in two other populations (A_{11} & A_{12}) are about 19.75 μm , 19.8 μm and 18.27 μm , 19.06 μm respectively. Apoculpium index also differs in different populations (0.78 in A_{10} , 0.77 in A_{11} and 0.86 in A_{12}).

Discussion

The results clearly demonstrate that palynological characters are useful in recognition of taxa in specific level and agreed with all the previous researches which offering similar results concerning shape, apertures and sculpturing of pollen grains. (Al-Eisawi 1986, Clarck et al. 1991). Considering these characters, separation of perennial *Adonis wolgensis* from the other annual species is possible. The pollen type of *A. wolgensis* is

the first report in the world. Moreover, separation of *A. dentata*, *A. flammea* and *A. aestivalis* from each other perceived. Palynological characters point to some infraspecies variation in *Adonis aestivalis* complex as it is seen in A_{10} population which differs from A_{11} and A_{12} populations in pollen characters.

As the species of *Adonis* exhibit variable morphological characters due to high adaptation potential (Riedl 1963, Steinberg 1971), so it seems that application of pollen morphology can help us to resolve the taxonomical problems within the species.

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Table 2. Summary of pollen morphological data of *Adonis* species (measurements in μm ; a=apiculate, ps=prolate-suboblate, s=scabrate, e=echinate).

Species & populations	character	Polar diameter	Equatorial diameter (D)	Polar/Equatorial diameter	Colpi length	Distance between apices of two ectocolpi (d)	Mesocolpium length	Apocolpium length	Apocolpium Index (d/D)	Pollen grain shape	Exin sculpturing
<i>Adonis aestivalis</i> A ₁₀	Min	31.32	17.26	1.62	24.18	5.81	10.91	8.59	.68	a	s
	Average	32.86	18.49	1.78	25.1	6.98	11.97	8.95	.78		
	Max	33.83	19.31	1.96	26.82	7.59	13.1	9.29	.85		
<i>Adonis aestivalis</i> A ₁₁	Min	30.43	17.82	1.56	21.47	5.73	13.06	7.56	.71	a	s
	Average	29.75	18.27	1.63	22.53	6.22	13.56	8.13	.77		
	Max	29.22	18.88	1.66	22.98	7.17	13.93	8.86	.81		
<i>Adonis aestivalis</i> A ₁₂	Min	29.66	18.95	1.56	21.7	6.11	12.94	7.51	.83	a	s
	Average	29.8	19.06	1.56	22.6	6.94	12.05	8.07	.86		
	Max	29.99	19.26	1.57	23.02	7.77	13.15	8.98	.90		
<i>Adonis dentata</i> D ₇	Min	19.11	13	1.36	13.93	4.97	8.17	7.3	.65	ps	s
	Average	20.77	13.69	1.52	15.36	6.43	8.7	7.7	.83		
	Max	22.86	14.04	1.76	16.15	7.39	9.34	8.11	.95		
<i>Adonis dentata</i> D ₉	Min	20.69	14.66	1.37	15.01	7.68	8.95	8.31	.82	ps	s
	Average	20.81	14.97	1.39	15.73	7.92	9.03	9.42	.83		
	Max	20.85	15.26	1.41	16.07	8.89	9.47	10.71	.92		
<i>Adonis flammea</i>	Min	25.01	14.93	1.49	18.32	6.13	8.95	7.15	.75	ps	s
	Average	26.27	15.62	1.68	19.64	6.66	9.95	8.27	.81		
	Max	27.13	16.74	1.79	21.87	7.18	11.12	9.58	.86		
<i>Adonis wolgensis</i>	Min	19.30	12.77	1.49	14.26	3.49	7.07	7.90	.25	ps	e
	Average	19.40	13.43	1.44	16.85	3.64	8.28	8.47	.27		
	Max	19.52	14.47	1.37	18.83	3.77	9.96	8.66	.29		

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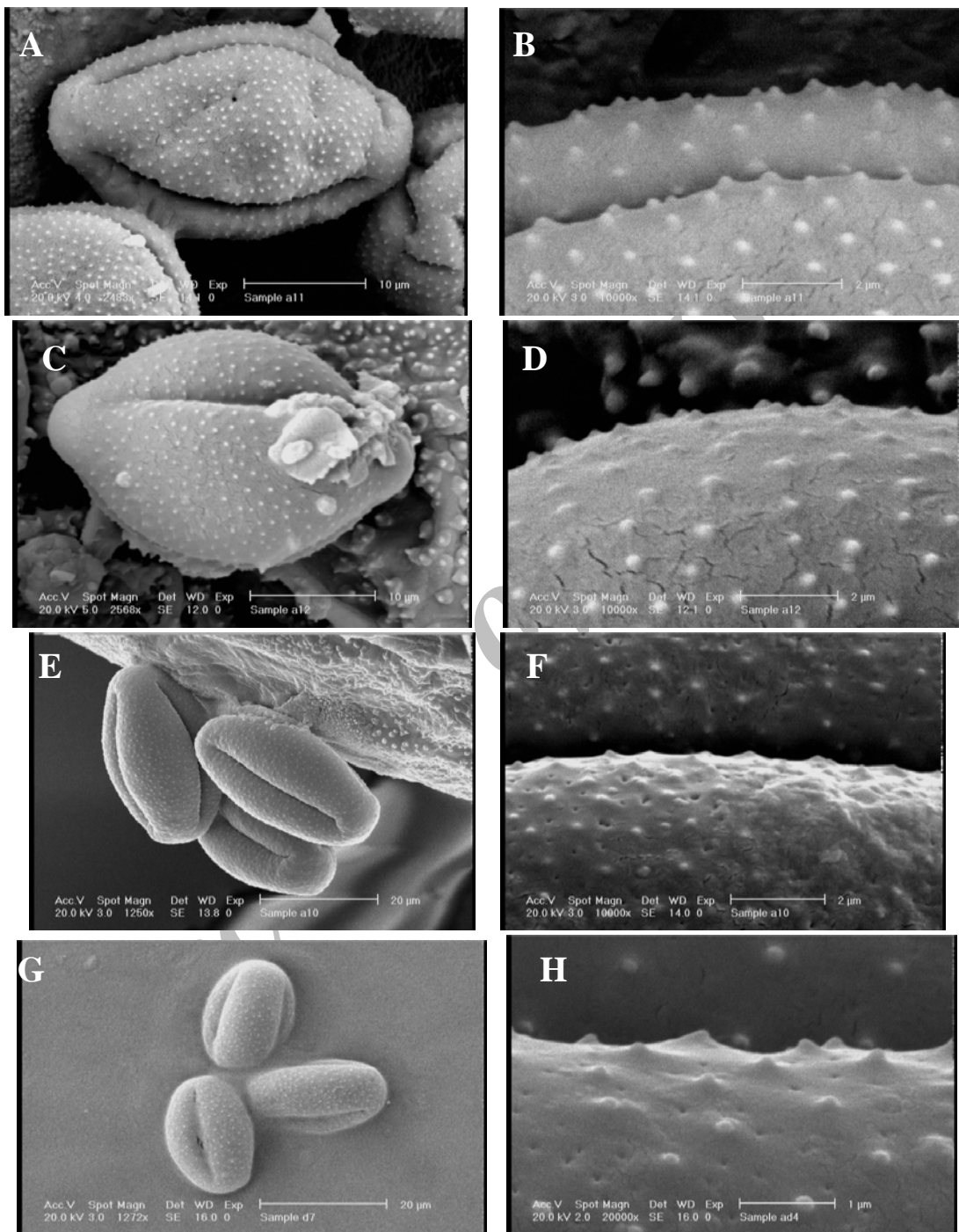


Fig. 2. Pollen grains (SEM Photomicrographs) of *Adonis* species. (A-B) *A. aestivalis* A₁₁ population (A: equatorial view, Scale bar = 10 μm.; B: ornamentation, scale bar = 2 μm.); (C-D) *A. aestivalis* A₁₂ population (C: equatorial view, Scale bar = 10 μm.; D: ornamentation, scale bar = 2 μm.); (E-F) *A. aestivalis* A₁₀ population (E equatorial view, Scale bar = 20 μm.; F: ornamentation, scale bar = 2 μm.); (G-H) *A. dentata* D₇ population (G: equatorial view, Scale bar = 20 μm.; H: ornamentation, scale bar = 1 μm.).

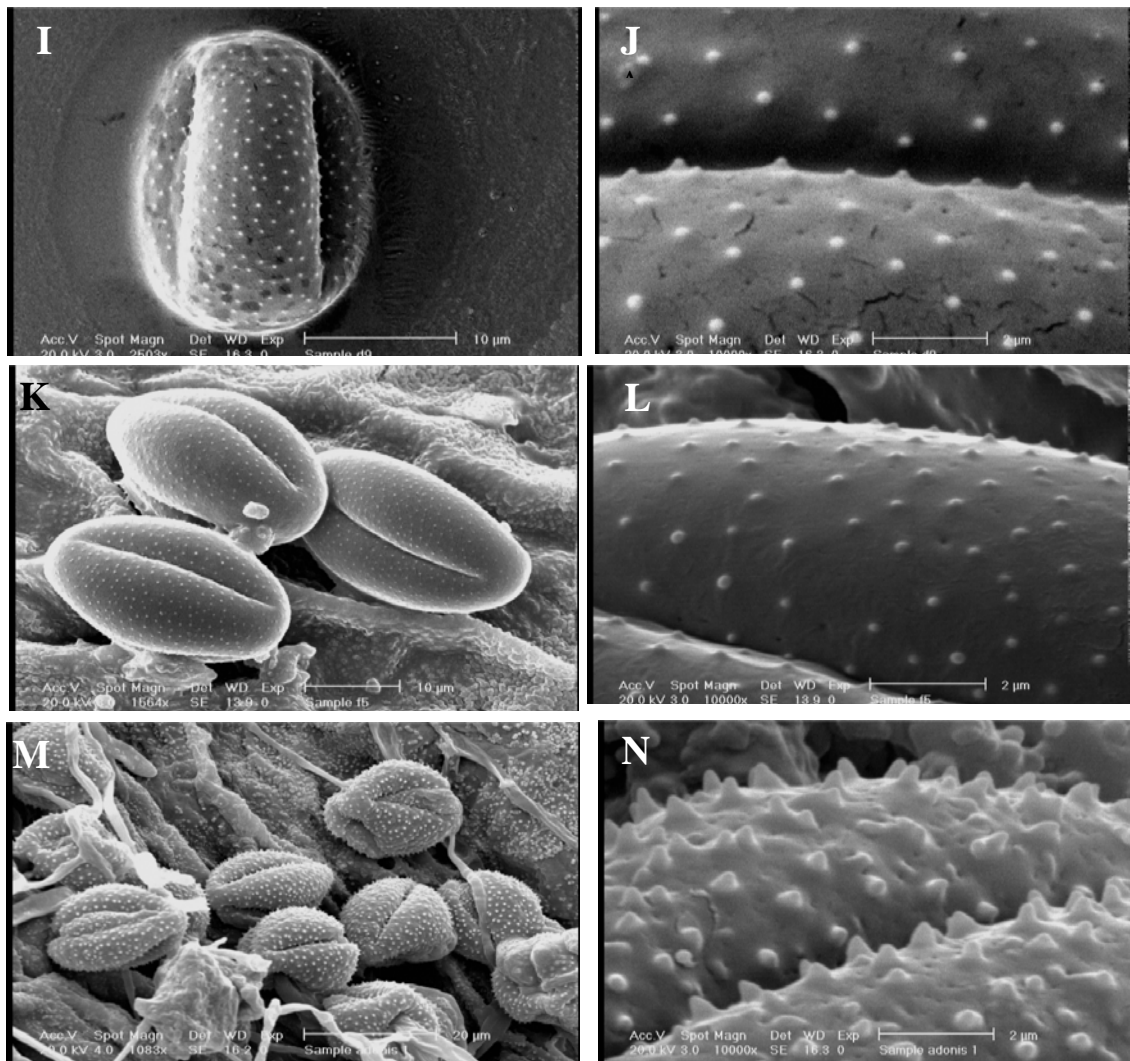


Fig. 3. Pollen grains (SEM Photomicrographs) of *Adonis* species. (I-J) *A. dentata* D₉ population (I: equatorial view, Scale bar = 10 μm. J: ornamentation, scale bar = 2 μm.); (K-L) *A. flammea* (K: equatorial view, Scale bar = 10 μm. L: ornamentation, scale bar = 2 μm.); (M-N) *A. wolgensis* (M: equatorial view, Scale bar = 20 μm. N: ornamentation, scale bar = 2 μm.).