POLLEN MORPHOLOGY OF SAPONARIA L. (CARYOPHYLLACEAE) SPECIES AND ITS TAXONOMIC VALUES

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In this research pollen morphology of ten taxa (5 of them endemics) that belong to the genus *Saponaria* L. in Iran were investigated using scanning electron microscope (SEM). In order to study the pollen morphology of *Saponaria* and find its significance in taxonomy of the group, qualitative and quantitative variables of pollen characters related to the shape, size, ornamentation and pores were studied. Cluster and Principal Component Analysis (PCA) of qualitative and quantitative data were used to demonstrate the pollen grains similarities among the species. According to the results, pollen type of *Saponaria* species is polyporate and displays either peroblate, polyhederal and spheroidal pollen shapes. Pollen size also varies among different species. The longest polar axis length belongs to *S. officinalis* L. (51.03 µm) and the smallest one to *S. floribnuda* (Kir. & Kar.) Boiss. (22.82 µm). Pore ornamentation differs from prominent granular to slightly protuberant or distinctly sunken granular (deeply excavated). The pollen ornamentation is microechinate-punctate in all species except in *S. esfandiarii* which has microechinate ornamentation and lacks puncta. Based on the results, the morphological characters of the studied pollen grains have taxonomic value in distinguishing taxa at the species level.

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Key words: Caryophyllaceae; Saponaria; pollen; palynology; SEM; Iran

مورفولوژی دانه گرده در گونههای جنس .Saponaria L از تیره میخک و اهمیت تاکزونومیکی آن

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در این مطالعه مورفولوژی دانه گرده ده گونه از جنس .Saponaria L (پنج گونه انحصاری) در ایران با استفاده از میکروسکوپ الکترونی Saponaria مورد بررسی قرار گرفت. به منظور مطالعه مورفولوژی گرده گونههای جنس Saponaria و ارزیابی اهمیت آنها در طبقهبندی تاکسونومیکی، متغیرهای کمی و کیفی مربوط به شکل، اندازه، تزئینات و منافذ مورد بررسی قرار گرفتند. تجزیه و تحلیل خوشهای و تجزیه به مؤلفههای اصلی (PCA) دادههای کمی و کیفی برای نشان دادن شباهتهای دانه گرده در میان گونههای این جنس انجام شد. با توجه به نتایج، دانه گرده گونههای جنس انجام شد و کروی (spheroidal) هستند. اندازه دانه گرده از نوع polyporate و دارای سه شکل شبهکروی (peroblate)، چندوجهی گرد (polyhederal) و کروی (spheroidal) هستند. اندازه دانه گرده نیز در گونههای مختلف متفاوت است. بلندترین طول محور قطبی به گونه (polyhederal) S. officinalis L.

microechinate بهم و باارزش بودند. تزئینات منافذ دانه گرده نیز از صفات مهم و باارزش بودند. تزئینات منافذ دانه گرده نیز از صفات مهم و باارزش بودند. تزئینات هسطح دانه گرده در این جنس در همه گونهها به شکل microechinate-punctate و فقط در گونه S. esfandiarii به شکل به دو شکل بر آمده (protuberant) یا فرورفته (deeply excavated) به مشاهده شدند. نتایج این پژوهش نشان داد که صفات مورفولوژیکی دانه گرده به عنوان صفات با ارزش در جداکردن گونههای این جنس قابل توجه

هستند.

INTRODUCTION

The Caryophyllaceae is a large, cosmopolitan family of 86 genera, and about 2200 species of herbs or small shrubs (Bittrich 1993; Heywood 1998). Caryophyllaceae family includes 3 subfamilies, including Alsinoideae Burnett, Caryophylloideae Arn. and Paronychioideae A. St. Hil. ex Fenzl (Bittrich 1993).

Saponaria L. is a member of Caryophylloideae subfamily with about forty species, found in temperate Eurasia, mainly in the Mediterranean region. This genus was formerly represented in Iran by 8 species, distributed in North (Gilan, Mazandaran and Golestan Provinces), NE (Khorasan Province), NW (Azerbaijan and Zanjan Provinces), West (Hamedan and Kurdistan Provinces), South (Kerman, Hormozgan and Sistan va Baluchestan Provinces) and in Center (Tehran, Markazi, Yazd and Qazvin Provinces).

More than half of the species occurring in Iran are reported from the N and NE of the country (Rechinger 1988). Most of the Iranian *Saponaria* species are morphologically similar, therefore, their taxonomy has been controversial for long time (Dashti & al. 2014).

Pollen morphology of Caryophyllaceae has been studied by different authors (Erdtman 1952; Barkoudah 1962; McNeill & Bassett 1974; Moore & Weeb 1978; Bittrich 1993; Perveen & Qsiser 2006; Atslar & al. 2009; Eröz & Ataslar 2010; Mostafavi & Mehregan 2014; Ulukus 2018). These studies revealed various pollen shapes for plants belonging to this family. Depending on species, pollen grain could be tricolpate, suboblate to subprolate, porate, pantacolpate, spherical or rounded polyhedral (McNeill & Bassett 1974). Tectum is mostly punctate-gillate, sometimes anulopunctate, rarely reticulate (Silene L. spp., Cerastium indicum Wight & Arnott), spinulate or microechinate. Pollen shape in the genus Saponaria is peroblate, polyhederal and spheroidal. The number of pores is variable in Caryophylloideae family from 15-38 (Erdtman 1968; Bittrich 1993; McNeill & Bassett 1974). Therefore, this character could be more useful for determining taxa. In the Caryophyllaceae family

most of the genera like *Arenaria* L., *Minuartia* L., *Dianthus* L., *Stellaria* L., *Gypsophila* L. and *Saponaria* L. are fairly uniform by having polyporate pollen grains with many pores and spinulose-scabrate to punctate tectum (Bittrich 1993; Perween & Qsiser 2006). According to the recent study (Ataslar & al. 2009), *Gypsophila* species from Caryophyllaceae family have spheroidal and pantoporate pollen grains. The exine is tectate and displays granulate-microechinate ornamentations in most species.

Simmler (1910), divided *Saponaria* into two subgenera, subgenus I: *Saponariella* Simmler and subgenus II: *Saporhizaea* Simmler. According to the latest taxonomic study, the genus *Saponaria* in Iran comprises ten species (Dashti & al. 2014). In this study, pollen morphology of 10 *Saponaria* species belonging to two subgenera is investigated to evaluate its taxonomic value.

MATERIAL AND METHODS

Pollen grains were taken from the herbarium materials deposited in TARI, IRAN, FUMH, HKS, Gilan and Nowshahr herbaria. The specimens were identified using Flora Iranica (Rechinger 1988), Flora de l'Iran (Parsa 1951), Flora of Turkey (Hedge 1967), Flora of the USSR (Gorshkova 1936), Flora Europaea (Chater 1964) and Monographie der Gattung *Saponaria* (Simmler 1910).

The list of the specimens and their localities are shown in table 1. Pollen grains from each species were mounted on stubs using double-sided adhesive tape. Each sample was gold coated, then the specimens were studied using scanning electron microscope (SEM), model EM 3200. Micro-morphological measurements were performed using Carnoy, digital measurements software (Scholes & al. 2002). Clustering analysis (Ward's Hierarchical method) and Principal Component Analysis (PCA) were used to identifying homogenous groups using SPSS 19 software. Thirteen quantitative and qualitative characters of pollen grain were considered in the analyses. Prior to the cluster analysis, our data were standardized (range of 0 to 1).

Table 1. List of species and their collection data.

Taxa	Locality				
Saponaria officinalis L.	Tehran: Azadshahr, National botanical Garden, 1320 m, Dashti 98960 (TARI).				
S. bodeana Boiss.	Tehran: Firuzkuh to Pole Veresk, North of Pole Gaduk, 2000 m, Wendelbo & Frougi 13051 (TARI); Gorgan: Golestan National Park, Almeh, 1750 m Wendelbo & Froughi 12730 (TARI).				
S. cerastoides Fisch. ex C. A. Mey.	Mazandaran: Between Damghan and Sari, near Kiasar, 1600 m, Wendelbo & Assadi 29568 (TARI).				
S. esfandiarii Assadi	Hormozgan: 100 km S. E. Lar, Sartang village, Kuh-e Shab, 500-900 km, Assadi & Sardabi 42042 (TARI).				
S. viscosa C. A. Mey.	Between Tehran and Karaj, above Kalka village, 1500-1900 m, Assadi & Mozaffarian 27587 (TARI).				
S. orientalis L.	Khorasan: N. Shirvan, N. E. Serani Mt. 1864 m, Jouharchi 44946 (FUM); Tehran: Arak to Mahalat, near Bezijan Mt. 2300 m, Mozaffarian & Massoumi 48011 (TARI); Khorasan: W. Mashhad, Kang Mt. 1500-1600 m, Jouharchi & Zanguei 36500 (FUM).				
S. iranica Dashti, Assadi & Sharifnia	Kerman: Baft, Hararan, 2900m, Yazdani 4956 (IRAN).				
S. floribunda (Kir. & Kar.) Boiss.	Khorasan: 89 km Mashahd from Torbate Heidarieh, 1750 m, Assadi & Amirabadi 84850 (TARI).				
S. makranica Rech. f.	Khorasan: Nehabandan, Shoosef region, between Zahab and Mazare Bibi Maryam, spring, 1877 m, Joharchi and Zanguei 79240 (FUM).				
S. kermanensis Bornm.	Kerman: Kuhe Hezar, Dareh Hezargol, 2800-3200 m, Froughi & Assadi 17893 (TARI).				

RESULTS

Pollen morphological quantitative and qualitative characters used in this study are represented in tables 2 & 3 (terminology according to Punt & al. 2007). Selected SEM micrographs of the pollen grains are shown in figs. 1-6. Some characters of the pollen grains were variable among different species of *Saponaria*, for example, shape and color and margin of the testa cells. A set of characters were found as distinctive for the species (tables 2-4), as described in detail below.

Quantitative Characters

Pollen size

Polar axis length (P), equatorial diameter (E) and P/E ratio were measured. As shown in the table 2, the smallest and longest P were both devoted to S. floribunda (Kir. & Kar.) Boiss. as an annual species (22.82) and S. officinalis L. as a perennial species (51.03), respectively. Pollen grains of the annual S. floribunda (20.85 μ m) had the shortest E and the perennial S. officinalis (49.58 μ m) had the longest one.

The range of P/E ratio was between 1.015 µm in *S. bodeana* to 1.094 µm in *S. floribunda*.

Pore size and number

The largest and smallest pore diameter was observed in *S. officinalis* and *S. orientalis* with the value of 12.91 μ m and 4.37 μ m, respectively. The smallest distance between pores was seen in *S. orientalis* (1.40 μ m), while *S. officinalis* had the largest one (11.31 μ m), (table 2).

The number of pores was variable from 4 to 12 in one hemisphere, therefore 8-24 pores are present in each pollen (counting based on Monoszon 1952). Saponaria kermanensis had maximum pore numbers and S. officinalis showed minimum pore numbers. Saponaria bodeana and S. orientalis had 5 pore numbers. Also, S. esfandiarii, S. floribunda and S. makranica had equal pore numbers (N=6). The relation between pore diameter and pore distance obtained through the SEM analysis is shown in fig. 1.

Table 2. Morphological parameters of pollen grains in *Saponaria* species (values in μ m). Abbreviations, P: polar axis, E: equatorial axis, P/E: polar to equatorial diameter ratio, N: number of pores in equatorial surface, D: exine thickness (pore diameter with annulus), R: intine thickness (pore diameter without annulus), d: shortest distance between the

pores, n: number of echini in 10 microns area.

Taxa	Character									
	P	Е	P/E	N	D	R	d	D/d	n	
S. officinalis	51.03 ± 0.18	49.58 ± 0.54	1.029	4	12.91 ± 0.48	6.24 ± 0.051	11.31 ± 0.11	1.14	5	
S. bodeana	31.01 ± 0.10	30.53 ± 0.51	1.015	5	10.42 ± 0.16	5.81 ± 0.18	4.8 ± 0.36	2.17	8	
S. cerastioides	34.12 ± 0.29	33.52 ± 0.51	1.017	7	9.02 ± 0.068	4.47 ± 0.14	4.3 ± 0.14	2.23	6	
S. esfandiarii	25.67 ± 0.41	24.3 ± 0.56	1.028	6	11.3 ± 0.42	7.29 ± 0.10	2.02 ± 0.03	5.46	12	
S. viscosa	32.05 ± 0.015	29.78 ± 1.99	1.025	9	8.96 ±0.66	8.61 ± 0.15	1.65 ± 0.24	1.08	9	
S. orientalis	30.1 ± 0.259	26.87 ± 0.020	1.093	5	4.37 ± 0.11	3.26 ± 0.06	1.40 ± 0.15	1.66	9	
S. iranica	32.05 ± 0.015	29.78 ± 1.99	1.044	11	8.96 ± 0.66	8.61 ± 0.15	2.60 ± 0.21	1.83	16	
S. floribunda	22.82 ± 0.10	20.85 ± 0.36	1.094	6	5.31 ± 0.051	3.52 ± 0.05	5.27 ± 0.15	1.1	12	
S. makranica	23.97 ± 0.082	23.23 ± 0.46	1.031	6	5.05 ± 0.64	13.66 ± 0.64	5.47 ± 0.18	0.93	11	
S. kermanensis	33.69 ± 0.17	31.69 ± 0.58	1.063	12	5.94 ± 0.63	4012 ± 0.63	2.3 ± 0.13	2.58	8	

Table 3. Comparison of qualitative pollen morphological data in *Saponaria* spp.

	Characters							
Taxa	Pollen type	Pollen shape	Pollen ornamentation	Pore ornamentation	Echini arrangement	Echini density	Puncta status	
S. officinalis	polyporate	peroblate	microechinate- punctate	deeply excavated	regular	dense	distinct	
S. bodeana	polyporate	peroblate	microechinate- punctate	deeply excavated	regular	very dense	almost indistinct	
S. cerastoides	polyporate	peroblate	microechinate- punctate	deeply excavated	irregular	medium	distinct	
S. esfandiarii	polyporate	polyhederal	microechinate	deeply excavated	rather regular	medium	no puncta	
S. viscosa	polyporate	spheroidal	microechinate- punctate	protuberant	rather regular	dense	distinct	
S. orientalis	polyporate	polyhederal	microechinate- punctate	deeply excavated	irregular	very dense	distinct	
S. iranica	polyporate	spheroidal	microechinate- punctate	protuberant	regular	dense	indistinct	
S. floribunda	polyporate	spheroidal	microechinate- punctate	protuberant	regular	very dense	almost indistinct	
S. makranica	polyporate	spheroidal	microechinate- punctate	protuberant	rather regular	very dense	distinct	
S. kermanensis	polyporate	spheroidal	microechinate- punctate	deeply excavated	irregular	medium	almost indistinct	

Qualitative characters Shape

Three different shapes of pollen grains are observed in *Saponaria* species, including peroblate, polyhederal and spheroidal. Peroblate pollen grains were observed

in *S. officinalis*, *S. bodeana* and *S. cerastioides* (fig. 1), Polyhederal pollen grains were observed in *S. esfandiarii* and *S. orientalis* (fig. 2 g, k), while the other species had spheroidal pollen grains (figs. 2 i, 3, 4).

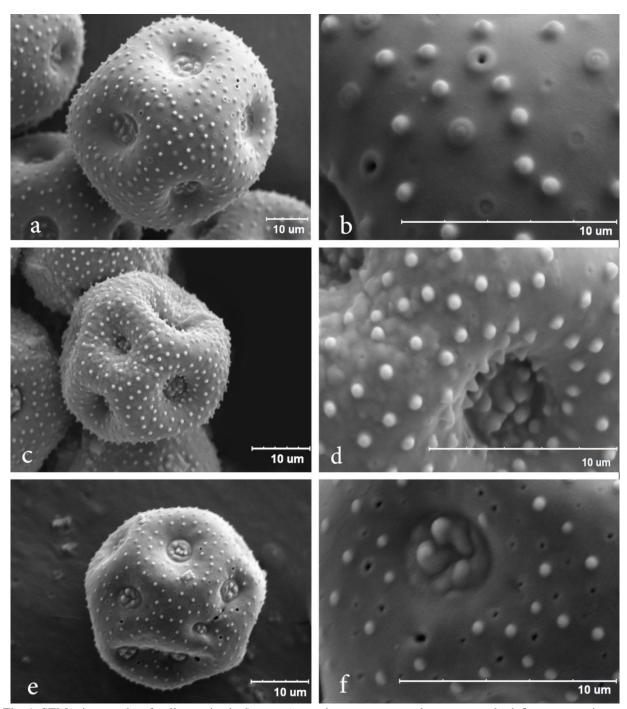


Fig. 1. SEM micrographs of pollen grains in *Saponaria* species. a, c, e, general appearance; b, d, f, ornamentations. a and b, *S. officinalis*; c and d, *S. bodeana*; e and f, *S. cerastoides*.

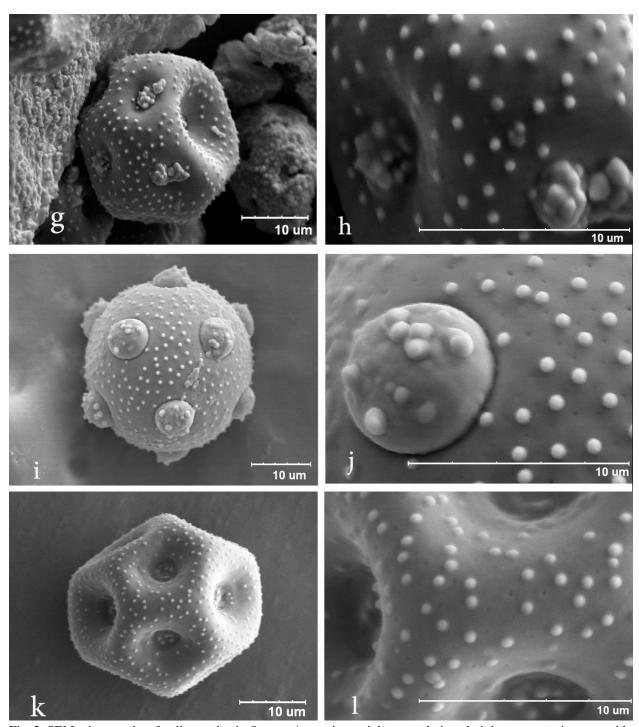


Fig. 2. SEM micrographs of pollen grains in *Saponaria* species. g, i, k, general view; h, j, l, ornamentations. g and h, *S. esfandiarii*; i and j, *S. viscosa*; k and l, *S. orientalis*.

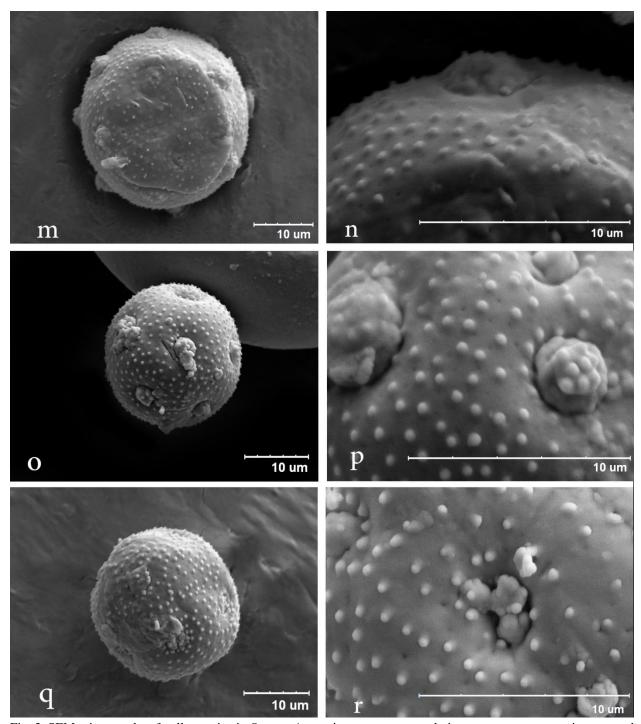


Fig. 3. SEM micrographs of pollen grains in *Saponaria* species. m, o, q, general view; n, p, r, ornamentations. m and n, *S. iranica*; o and p, *S. floribunda*; q and r, *S. makranica*.

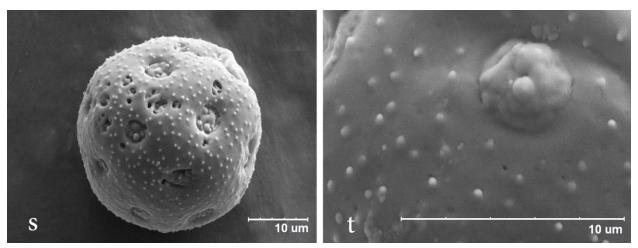


Fig. 4. SEM micrographs of pollen grains in S. kermanensis. s, general view; t, ornamentations.

Ornamentations

Pollen grain ornamentations were microechinatepunctate in all studied taxa except in S. esfandiarii with microechinate ornamentation. The lack of puncta was only observed in S. esfandiarii. Two different pore ornamentations, including protuberant and deeply excavated could be found in the genus Saponaria. Protuberant pollen grains were observed in S. iranica, S. floribunda, S. makranica and S. viscosa, while species of S. esfandiarii, S. orientalis, S. officinalis, S. bodeana, S. kermanensis and S. cerastioides had deeply excavated pore ornamentations (figs. 1-4 and table 3).

PCA and Cluster Analysis

In PCA, characters were grouped in two components. Based on these two components, the spatial arrangement of studied taxa is shown in fig. 5. The comparison of the variance between 13 quantitative and qualitative characters is presented in table 4. The characters with the highest values in each component were considered as the most effective ones. In the first component, most values were related to equatorial axis (0.971), polar axis (0.965) and the number of echini in 10 microns (0.796). In the second main component, diameter of the pores without the annulus and diameter of the pores with the annulus had the highest variances with the values of 0.961 and 0.937, respectively.

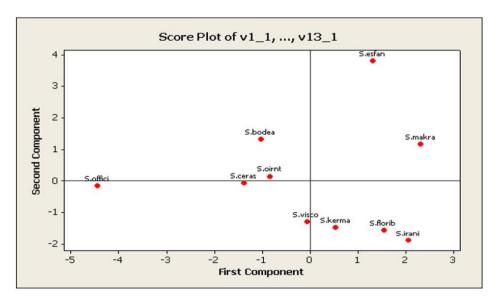


Fig. 5. PCA analysis of the pollen data of Saponaria species.

Table 4. The comparison of the variance between 13 quantitative and qualitative characters by use of Component Analysis extraction method.

Rotated Component Matrix^a.

	Component		
	1	2	
V1=Polar axis (P)	.965	086	
V2=Equatorial axis (E)	.971	042	
V3=P/E	.057	337	
V4=Number of pores in equatorial surface (N)	204	336	
V5=Exine thickness=Pores Diameter with annulus (D)	.040	.937	
V6=Diameter of pores without annulus (R)	052	.961	
V7=Diameter of the annulus (AD)	.067	.188	
V8=Shortest distance between the pores (d)	.755	.449	
V9=D/d	471	.424	
V10=Number of echini in 10 microns (n)	796	.018	
V11=Pollen shape	480	.120	
V12=Pollen ornamentation	419	.449	
V13=Proce ornamentation	306	.030	

The dendrogram obtained from cluster analysis is shown in fig. 6. In 0-5 taxonomical distance, firstly, five species were divided into two groups: *Saponaria floribunda*, *S. iranica* and *S. viscosa* were categorized in the first group and *S. cerastoides* and *S. kermanensis*

were placed in the second group. In 10-20 taxonomical distance, three species including *S. officinalis*, *S. esfandiarii* and *S. makranica* were separated from others. Finally, in 20-25 taxonomical distance, *S. officinalis* completely categorized in a separate group.

Dendrogram using Average Linkage (Between Groups)

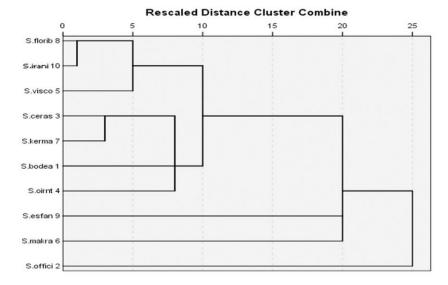


Fig. 6. Cluster dendrogram based on quantitative and quantitative traits of pollen grains of Saponaria.

DISCUSSION

Caryophyllaceae pollen grains are suboblate, subprolate, spherical or rounded and polyhederal. The exine is tectate and the tectum is punctate-gillate or occasionally anulopunctate, rarely reticulate, and finally spinulose (Brittrich 1993).

The results showed that the pollen grains of Saponaria species are peroblate, polyhederal and spheroidal. The studied species have polyporate pollen grains with many pores, spinulose-scabrate to punctate tectum and echini ornamentation observed on the surface of reported species. Three types of echini arrangement (irregular, regular and rather regular) have been reported, while three type of echini density (medium, dense and very dense) were observed. Also, four puncta status, distinct, almost indistinct, indistinct, and no puncta were seen. Ataslar (2004) studied pollen grains of S. kotschyi Boiss. and concluded that pollen grains are spheroidal in shape, with a diameter of 36 µm, periporate with 12 pores, which is somewhat similar to the results of Saponaria species in Iran (Dashti 2013). All ornamentation on the surface of the pollen grains of the genus Saponaria are small and punctate, except for S. esfandiarii, which has no puncta on the surface of grain (fig. 2 g, h). Rechinger (1988), demonstrated that S. oreintalis and S. viscosa are morphologically very similar, but the results of our study showed that the pollen grains shape of these species are quite different, as S. viscosa has a spheroidal pollen grain with protuberant ornamentation and S. orientalis has polyhedral pollen grains with deeply excavated ornamentation. Dashti & al. (2014) stated that, S. makranica, S. kermanensis and S. floribunda, all have short tubular-calyx or cups that are somewhat similar to the genus Gypsophila. However, the pollen grains of all three mentioned species were different according to the result of this work.

Pollen morphology of some *Saponaria* species has been studied by Arkan & Inceoglu (1992). Among the 16 species of *Saponaria* which they examined, the number of pores were between 9 to 14, except in *S. pumilia* Boiss., in which it was 7-8. The absence of spinules and fewer pores in *S. pumilia* were considered primitive pollen characteristic by the authors. In our study, the number of pores varies between 8-24.

Our results showed that although some of studied taxa have morphological similarities, but the pollen morphological characters separate them completely.

Two perennial taxa including *S. officinalis* and *S. bodeana* and two annual species including *S. makranica* and *S. kermanensis* are the best examples.

Considering our results, pollen morphological studies have had noticeable role in distinguishing *Saponaria* species.

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