Seed and pollen morphology of some *Silene* species in Iran

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Abstract. With about 110 species in the geographic area of Iran, genus *Silene* (*Caryophyllaceae*) is expanding. Of these, about 35 are endemic species. This seed morphological study was carried out into 15 populations of three species from this section, in different regions of Iran, using 15 qualitative and quantitative characteristics. Statistical results have confirmed that such features as shape, length and width of seed, size and coat decoration cells indicate the greatest diversity in the studied populations. In the morphological studies of pollen grains, 22 populations of six species were considered for the first time. Pollen grains were spherical, monad and polypantoporate. Diagnostic values of seed and pollen features have been discussed.

Key words: *Lasiostemones*, morphology, pollen, seed, *Silene*

Introduction

Silene L. (*Caryophyllaceae, Silenoideae*) has 110 species on the Iranian Plateau, 35 of which are endemic (Melzheimer 1988). The *Lasiostemones* Boiss. section, with 10 species in Iran, is distinguished from other sections by the perennial form of life, raceme inflorescence, white flowers, short cylindrical, hairy calyx with indistinct convergent nerves, and scabrous filaments (Melzheimer 1988).

Rohrbach (1869) studied *Silene* seeds for the first time and described their peripheral and lateral surfaces. Chowdhuri (1957) drew attention to the special diagnostic forms of seeds significant in the section separation. Melzheimer (1987) used SEM micrographs of seed testa cells for distinguishing the *Silene* species. He mentioned that seed shape and hillum features are non-variant. El-oqlah (1990) believed that seed testa features are important, but insufficient in distinguishing the species, due to certain similarities in the seeds of different sections. Diagnostic value of seed features has been proved in different taxonomic ranks for *Silene* (Ghazanfar 1983; Yildiz & Cirpici 1998; Hong & al. 1999; Zareh 2005; Fawzi & al. 2010; Camelia 2011).

Ghazanfar (1984) studied pollen morphology of 44 taxa from *Siphonomorpha* and *Auriculata* sections of *Silene*. He considered the ektexine ornamentations of some species. Prentice (1987) described most diagnostic features of the *Silene latifolia* pollen grains. Taia (1994) studied 21 *Silene* species of Egypt and confirmed pore differences in the studied pantoporate pollen grains. Yildiz (2001, 2006a,b) and Yildiz & al. (2009) studied pollen grains of different *Silene* species in Turkey. They pointed out that pollen grains are spheroid and polypantoporate with microechinate-microperforate ornamentations. Sahreen & al. (2008) considered 16 *Silene* species from Pakistan. In the present study, seed micromorphological features in three species and pollen morphology of six species are studied for the first time in Iran, in order to find diagnostic characters helpful for species differentiation.

Material and methods

In order to study seed micro-morphological features, 15 accessions of three species have been collected and analyzed for 13 qualitative and quantitative features (Tables 1, 2 & 3). We have used a Dinolite digital stereomicroscope (AM413T) and scanning electron microscope (SEM) (EM3200 in KV25). Punt & al (2007) terminology was applied.

Table 1. Voucher details of studied taxa (* – pollen studies, ■ – seed studies, PNUSH – Herbarium of Payame Nour University, sari branch, HSBU – Herbarium of Shahid Beheshti University).

Taxon	Locality
Silene claviformis	Kerman, Chatroud, Bandar village, Paye Sib, Gholipour, 86070-PNUSH*
S. longipetala	Chahar Mahalo Bakhtiyari, Gholipour, 8500251-HSBU *
*S. marschallii**	Lorestan, Borojerd, 87018-PNUSH*, Isfahan, Khonsar, Golestan Kouh, Gholipour, 8500245-HSBU*, Tehran, Firouz kouh Road, Gholipour 8500236-HSBU*■, Zanjan, Angouran Mt., Gholipour, 86095-PNUSH■, Markazi, Arak, Gholipour, 87024-PNUSH*, WS Azerbaijan, Ashk Island, Gholipour*■, Chalous to Kandovan Tunnel, Gholipour*, Tehran, Firouz Kouh, Ghoipour, 8686-PNUSH■, Tehran, Lar, Damavand Peak, Gholipour, 8500243-HSBU■*, Tonekabon, Chagol, Gholipour, 86140-PNUSH■, Zanjan, Zanjan, Gholipour, 86026-PNUSH*, E. Azerbaijan, Tabriz to Khoy, Gholipour, 87040-PNUSH*■, Semnan, Khonar fields, Gholipour,■, Tehran, Polour, Larijan, Gholipour, 8500234-HSBU*, Gilan, Manjil, Gholipour, 8500242-HSBU■, Zanjan, Soltaniyeh, road to Masouleh, Gholipour, 86026-PNUSH■, W. Azerbaihjan, Uromiyeh, Khoy, Pastak village, Gholipour, 87040-PNUSH■
S. propinqua■	Kurdistan, Divan Dare to Sanandaj, Gholipour, 86097-PNUSH■, W. Azerbaijan, Eromiyeh Khalil Kouh, Gholipour, 87053- PNUSH*, W. Azerbaijan, Piranshahr to Naghadeh, Gholipour, 890270*■, W. Azerbaijan, Marmishou, Gholipour, 900823-PNUSH*■
*S. ruprechtii**	E. Azerbaijan, Tabriz to Kaleybar, Gholipour, 8500238-HSBU*, E. Azerbaijan, Tabriz to Ahar, Gholipour, 8501-PNUSH*
*S. tenella**	W. Azerbaijan, Ziveh, Gholipour, 900862-PNUSH*, Gilan, --, Gholipour, 86138-PNUSH■, Ardebil, Neor lake, Gholipour, 8500228-HSBU■, Mazandaran, Nour, Gholipour*, Sabalan Mt., Gholipour, 86110-PNUSH■, Tehran, Damavand peak, Gholipour, 86080-PNUSH■, Mazandaran, Kiasar, Shahdej, Gholipour 85058-PNUSH*■, Sahand Mt., Gholipour,*, Mazandaran, Savadkouh, Gholipour*, Semnan, Gholipour ■, Ardebil, Meshkinshahr, Gholipour 900712-PNUSH■, Ardebil, Sarab, Bozghosh Mt., Gholipour 86111-PNUSH■
*S. claviformis**	Kerman, Taf Mt., Gholipour, 8604-PNUSH*

For the pollen morphological studies, 22 populations of six species were used (Table 1). Pollen grains were taken without any treatment and studied for 19 qualitative and quantitative characteristics by light microscopy and SEM (Table 2). Measurements were made with Image tool ver. 3 software.

Table 2. Qualitative features of the studied *Silene* species.

Character	State of character	Character	State of character
Reniform/cordate-angular/reniform-rounded/cordate/ reniform-angular	Seed shape	Ribbed/ not so	Seed lateral surface
Light/ dark	Seed color	Regular/ irregular	Seed width
Evidence/ not so	Seed peripheral rib	v- shaped/ s- shaped	Anticlinal cell walls in seed
Micro-perforate/ micro-echinate – micro-perforate	Pollen exine sculpture	Spheroid/ non-spheroid	Pollen shape
Tectate/ atectate	Pollen exine type	polypantoporate/ polypantoporate	Pollen type

Table 3. Quantitative features of the studied *Silene* species.

Size	Pollen	Perforation diameter	Pollen
Exine diameter		Pore number	
Pollen diameter		Pore diameter	
Spinule length on operculum		Mesoporium	
Spine number per area		Operculum diameter	
Spine length		Spinule number on operculum	
Distance between spines		Distance between perforations	
Number of micro-perforations per area			
Maximum testa length	Seed	Seed length	Seed
Number of lateral testa cell rows		Seed width	
Minimum testa length		Seed length-to-width ratio	
		Seed thickness	

Results

Seed morphology

Seed shape in the studied taxa has shown some variations. Different accessions of *S. marschallii* manifested intra-specific variation in the seed features. In this species, reniform, heart-shaped, angled, semi-spheroid and intermediate forms have been observed (Fig. 1), while *S. tenella* has shown reniform-rounded, and *S. propinqua has shown* reniform-angled shapes (Fig. 2). Peripheral surface was ribbed in *S. marschallii* and *S. tenella.*

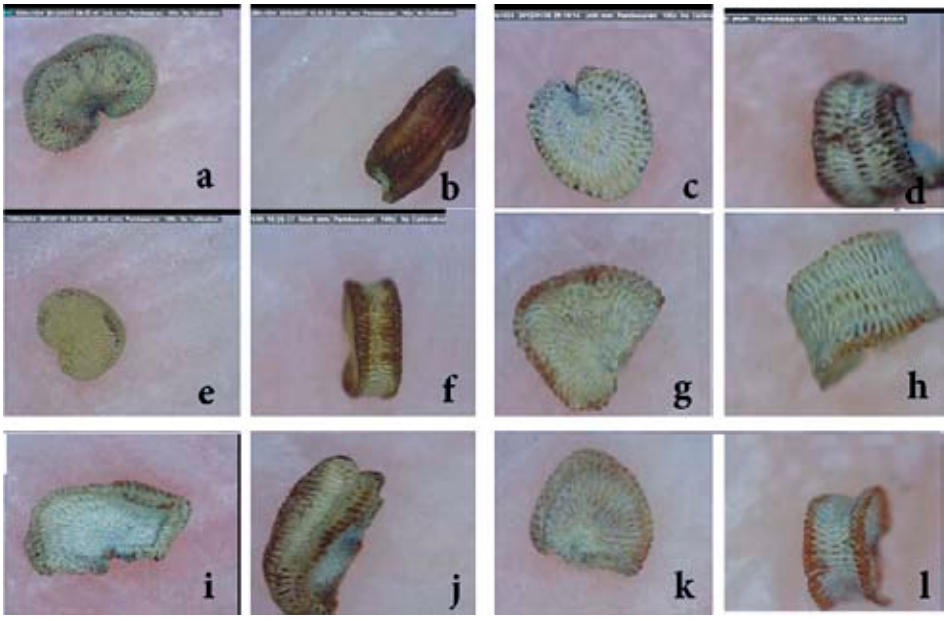

Fig. 1. Seed shape in *S. marschallii*: **a** & **b** – reniform shape in the Firouzkouh population; **c** & **d** – cordate shape in the Khoy population; **e** & **f** – Ashk island population, reniform-cordate shape; **g** & **h** – cordate-angled shape in the Damavand population; **i** & **j** – reniform-angled shape in the Manjil population; **k** & **l** – semi-spheroid shape in the Pesak population.

In order to show the relationships between populations, a cluster analysis by the WARD method was applied. In the WARD phenogram, two main clusters were formed. In the first cluster, *S. tenella* and *S. marschallii* were grouped near each other. *S. propinqua* was set separately (Fig. 3).

The studied species have shown variation in the seed shape, size, number of testa cell rows and their shape. *S. propinqua* had smooth testa cell walls, but in *S. marschallii* lateral surface had granulate-perforate to granulate testa ornamentations (Fig. 4). *S. tenella* showed rough to micro-perforate testa cell ornamentations. Peripheral surface in the three studied species manifested differences in the cell row number, shape and size of testa cells (Fig. 5).

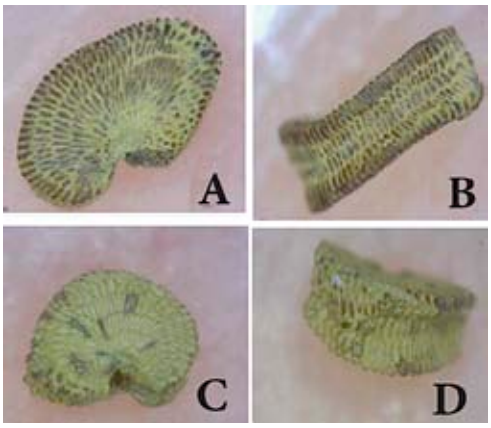

Fig. 2. Seed lateral and peripheral shape: **A** & **B** – *S. propinqua* of the Divandare population, and **C** & **D** – *S. tenella* of the Meshkinshahr population.

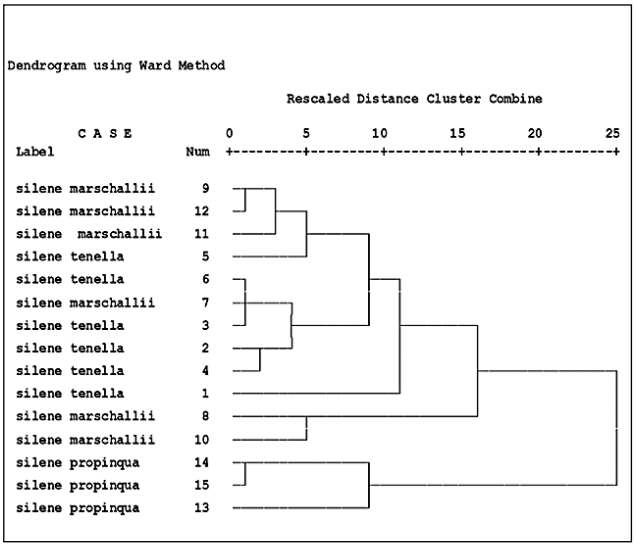

Fig 3. WARD phenogram based on seed characters.

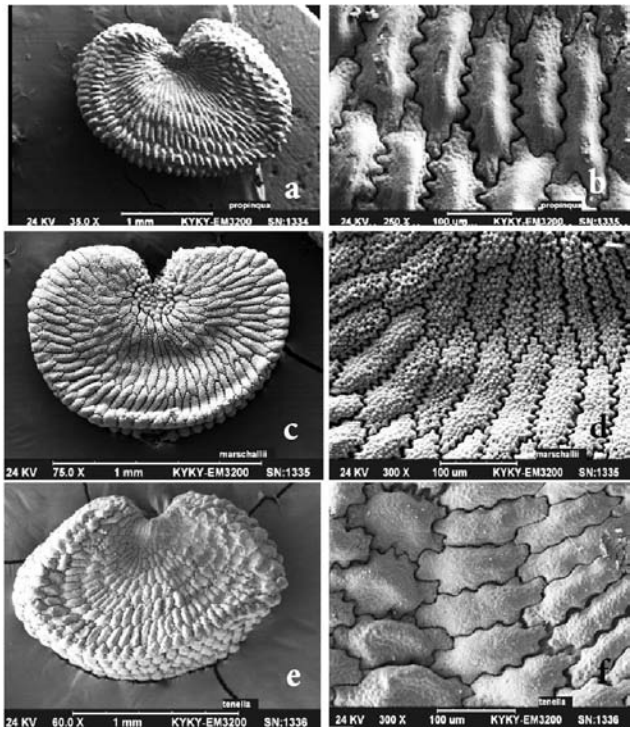

Fig. 4. Seed electromicrographs in: **a** & **b** – *S. propinqua*, **c** & **d** – *S. marschallii*, **e** & **f** – *S. tenella*. Left column: seed general shape; right column: testa cell shape and ornamentations.

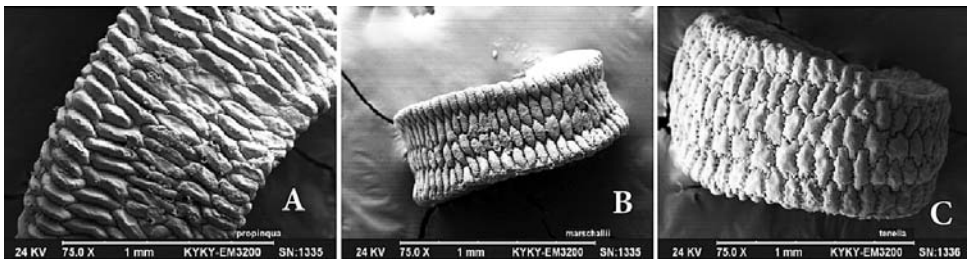

Fig. 5. Peripheral surface in: **A**) *S. propinqua,* **B**) *S. marschallii* and **C**) *S. tenella.*

Pollen morphology

Pollen grains of all studied species were observed by light microscopy. General shape of the pollen grains was evident. For better resolution, we used SEM micrographs. All studied species have shown monad, spheroid and polypantoporate pollen grains. Pores had opercules (Fig. 6). Exine sculpture was of the microechinate – micro-perforate type in all studied species. Operculum was sunken in *S. claviformis* but swollen in the other species. Operculum surface was microechinate in all studied species and composed of simple spines and bifurcating spinules. We have observed the smallest pollen grains in *S. tenella,* with the lowest pore number and less micro-perforate ornamentation on the pollen surface (Table 4), but *S. claviformis* has shown the greatest number of micro-echinate ornamentations on the exine and operculum surface.

Table 4. Average value of the pollen grains micro-morphology in this project.

Taxon	Pollen size	Pollen diameter	mesoporium	Number of spines	Spine length	Distance between spines	Diameter of perforations	Distance between perforations	Perforation number per surface pollen area	Spinule number on operculum	Length of operculum spinule
S. propinqua	43	9.8	7	20	0.31	1.25	0.39	1.87	8	14	1.34
S. marschallii	43	8.98	7.65	21	0.42	1.23	0.48	2.25	9	8	1.17
S. ruprechtii	35	5.5	7.25	30	0.4	0.9	0.96	1.42	10	8	0.65
S. tenella	33	4.7	5.7	24	0.46	0.84	0.25	2.7	1	8	0.8
S. claviformis	33.3	5.5	5.6	45	0.35	0.7	0.3	1.1	8	20	0.9

In order to demonstrate the relationships between species based on pollen features, a cluster analysis by the WARD method was applied. In the obtained phenogram (Fig. 7), two main clusters are formed.

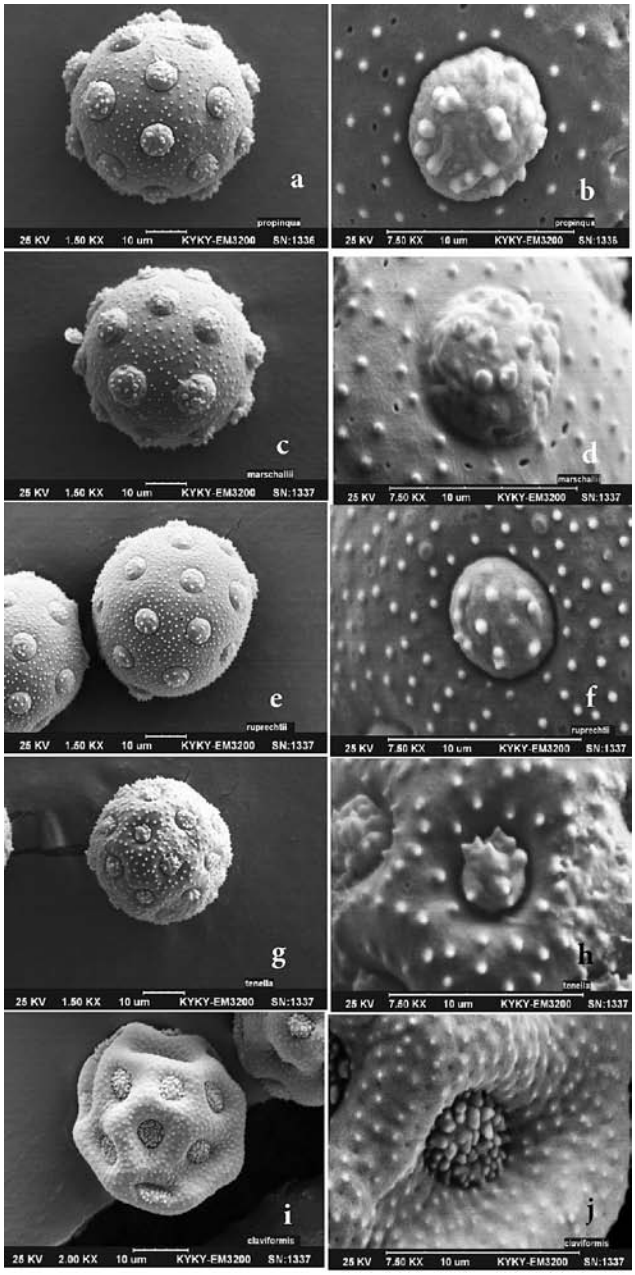

Fig.6. SEM micrographs of pollen grains in the studied species: **a** & **b** – *S. propinqua,* **c** & **d** – *S. marschallii*, **e** & **f** – *S. ruprechtii*, **g** & **h** – *S. tenella,* **i** & **j** – *S. claviformis*.

S. propinqua and *S. ruprechtii* show more similarity to each other, as well as *S. marschallii* and *S. tenella*. *S. longipetala* and *S. claviformis* form a separate set.

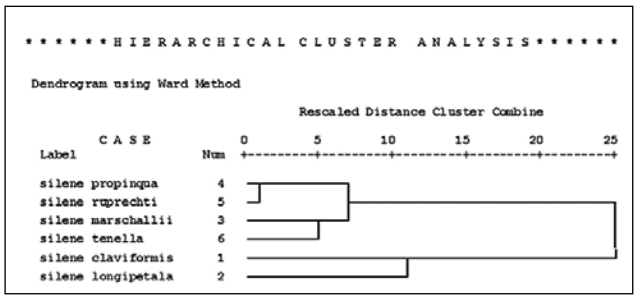

Fig.7. WARD phenogram based on the pollen grain features of the studied *Silene* species.

Discussion

A study of seed surface in the studied species of the *Lasiostemones* section has revealed that seed shape, size and testa features are of diagnostic value. This is in concord with the other authors' results (Chowdhuri 1957; Melzheimer 1987; El-oqlah 1990; Yildiz 1998, 2002, 2006a,b; Hong & al. 1999; Fawzi & al. 2010, Camelia 2011). Ghazanfar (1983) classified the *Lasiostemones* seed as type one (testa without protruding cell or if present rounded). Our results for *S. tenella*, *S. propinqua* and *S. marschallii* agree with his findings.

Pollen grains sculpture of the studied *Silene* species in the *Lasiostemones* section coincide with the findings of Taia (1994), Yildiz (2001, 2006a,b), Sahreen & al. (2008), Kilic (2009), and Yildiz & al. (2009, 2010) in other species of this genus. Yildiz & al. (2011) considered *S. marschallii* and *S. longipetala* pollen grains and our findings in this project are in concordance with their results.

In the *Caryophyllaceae* family, there is a general similarity between pollen grains – most of them are polypantoporate and spheroid. Granulate–microechinate to microperforate ornamentation type is also frequent in this family. We have used some pollen features in this project as diagnostic characters and their significance was proved earlier (Ataslar & al. 2009; Camelia 2011; El-ogqlah 1990; Erozpoyraz & Ataslar 2010). Similarity between *S. marschallii* and *S. tenella* is confirmed by pollen and seed features.

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