



# Asian Journal of Plant Sciences

ISSN 1682-3974

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## A Biosystematical Investigation on *Silene* L. Species in North-East of Iran

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**Abstract:** In present research anatomical and palynological studies carried out seven species of *Silene* which growing in north-east of Iran. These species belonged to four sections including *Sclerocalycinae*, *Melandrifformes*, *Auriculatae* and *Conoimorpha*. For comparative anatomy studying, cross sections from stem and leaf were prepared using microtome and differential staining. The characters of secondary xylem and epidermal cell in leaf were studied. For palynology studying, after extraction of the pollen and acetolysis, the ornamentation of the pollen were investigated. The results of anatomy showed, presence of sclerid and fiber, spiral and reticulate vessel. The arrangement of vessel was radial chain pore, solitary and cluster. The arrangement of mesophyll was dorsi-ventral and isolateral. The pollen was spheroid, pantaporate, inaperture, tectate. Finally, a correspondence between morphology and palynology characters was observed.

**Key words:** Anatomy, Caryophyllaceae, morphology, palynology, pollen, *Silene*

### INTRODUCTION

*Silene* belongs to Silenoideae subfamily, Caryophyllaceae family, which composes of 700 species around the world (Jurgens *et al.*, 2002; Jurgens, 2004). This genus distributed in Turkey, Russia, Italy, Iraq, Iran, east of Mediterranean, Europe, Japan, Spain and England (Boissier, 1884; Tutin, 1964; Zohary, 1966; Groshkova *et al.*, 1970; Davis, 1965-1985; Anzalone, 1982; Melzheimer, 1988). The number of *Silene* L. species in Iran is 98 (Melzheimer, 1988), only seven species are found and distributed in North-East of Iran. In present study, stem and leaf anatomy in cross section were investigated. The aim of this present investigation was study of variation in internal structure and identification of *Silene* species on the basis of micromorphology and internal structure because morphologically identification of this species is difficult. The previous anatomy studying on

Caryophyllaceae had been done by Metcalfe and Chalk (1983). Also, the stem anatomy had been carried out *Saponaria* (Ataslar, 2004). In this part of investigation, the type of sclerenchyma tissue, stoma, arrangement of leaf mesophyll and vessel studied.

The earlier palynology investigation had been done of sect. *Auriculatae*, *Siphonomorpha* by Ghazanfar (1984). In the present research, anatomy of stem, leaf and the pollen ornamentation of *Silene* species growing in North-East of Iran were studied.

### MATERIALS AND METHODS

As for the anatomic study, the examined species were collected from the localities in North-East of Iran during May-June 2005-2006 (Table 1). For preparing of cross section of stem and leaf, base of stem and basal leaves

Table 1: The localities of studied *Silene* species

Section	Species	Localities
<i>Sclerocalycinae</i> Boiss.	<i>S. bupleuroides</i> L. subsp. <i>bupleuroides</i>	Mashad, Moghan to Maj- Gusouleh, 2000 m, Zangooi and Faghilnia, 23637
	<i>S. bupleuroides</i> L. subsp. <i>ramosa</i>	Mashhad, north-west of Kardeh, Balghour, 1750 m, Zangooi and Fagihian, 27544
<i>Auriculatae</i> Boiss.	<i>S. swertifolia</i> Boiss.	Mashhad, Moghan village, 1750 m. Jafari and Fathi, 34
	<i>S. indeprensia</i> Schischk	Between Mashhad and Kalat. Sandough Shekan, 1700 m, Zangooi and Faghilnia, 20870
	<i>S. gertraudiae</i> Melzh	Mashhad to Torbat-Heidarieh, Robat Sefid, 2000 m, Fathi, Azizzadeh, 25
<i>Melandrifformes</i> Boiss.	<i>S. latifolia</i> Poir. subsp. <i>ericalycinae</i> (Boiss) Greuter and Burdet	70 km Mashhad to Kalat, 1600 m, Zangooi and Faghilnia, 24683
	<i>S. latifolia</i> Poir. subsp. <i>alba</i> (Mill.) Greuter and Burdet	Mashhad, Zoshk, 1500 m, Safavi and Joharchi, 12575
	<i>S. latifolia</i> Poir. subsp. <i>persica</i> (Boiss and Buhse) Melzh.	Mashhad, Moghan. Mountain, 1900 m, Faghilnia and Zangooi 20382
	<i>S. noctiflora</i> L.	South-west of Mashhad, Azghad, 1600 m, Hosseinzade and Zangooi, 24262
<i>Conoimorpha</i> Otth.	<i>S. conoidae</i> L.	Mashhad, north-west of Kardeh- Balghour. 1750 m, Faghilnia and Zangooi, 27532

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were selected from 6-7 specimens. The living materials were fixed in FAA then they were dehydrated with ethanol and later, some slices were prepared with microtome. Twelfth- microns thickness was stained with Safranin and Fast-green (Johnson, 1940; Chamberlain, 1990). For the stem maceration, this organ was placed in Jeffery solution for 4 h to soften.

In the palynological study, the pollen was extracted from the anther and dehydrated by glacial acetic acid, then acetolised, coated with sputter finally studied by LM Olympus and SEM LEO1450VP (Erdtman, 1952; Moore *et al.*, 1991). The pollen terminology was adapted from Punt *et al.* (1994).

### RESULTS AND DISCUSSION

**Anatomic characters:** The results from the anatomic studies showed:

- Some (several) parenchymatous layers with small cells under the epidermis.
- Some sclerenchymatous layers with small cells
- Vascular bundles which the arrangement of vessel was variable for example solitary- radial chain pore in *S. bupleuroides*, *S. latifolia* subsp. *ramosa*, radial chain pore in *S. noctiflora*, *S. bupleuroides*, *S. latifolia* subsp. *ericalycinae*, *S. conoidae* (Fig. 1), radial chain pore- cluster in *S. latifolia* subsp. *alba*, *S. latifolia* subsp. *persica*, *S. swertifolia* (Fig. 2) and cluster in *S. indepressa*, *S. gertraudiae* (Fig. 3). The sclerid layers observed in *S. bupleuroides* and *S. swertifolia* while in *S. gertraudiae* existed only fiber. In the rest of studied species both of the cells type observed. The results from stem maceration confirmed the presence of vessel with simple perforation plate.

The results of leaf anatomy showed, the arrangement of mesophyll in perennial species, i.e., *S. swertifolia*,

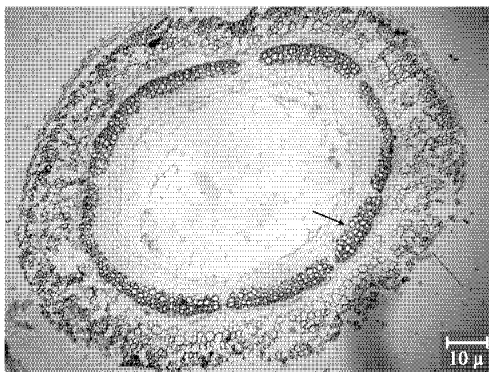


Fig. 1: Stem cross section of *S. noctiflora*. The arrow showing radial chain pore (x416)

*S. gertraudiae*, *S. indepressa*, *S. bupleuroides* were isolateral (Fig. 4) but in annual and biennial species i.e., *S. latifolia*, *S. noctiflora*, *S. conoidae* were dorsi-ventral (Fig. 5).

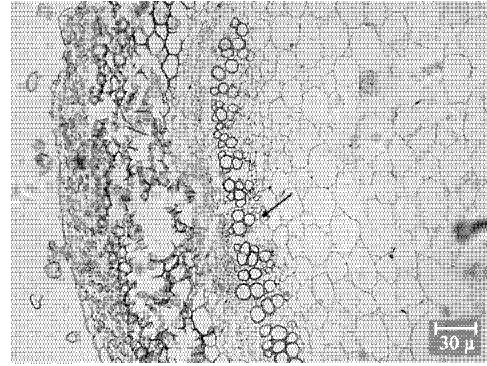


Fig. 2: Stem cross section of *S. swertifolia*. The arrow showing Radial chain pore with cluster (x416)

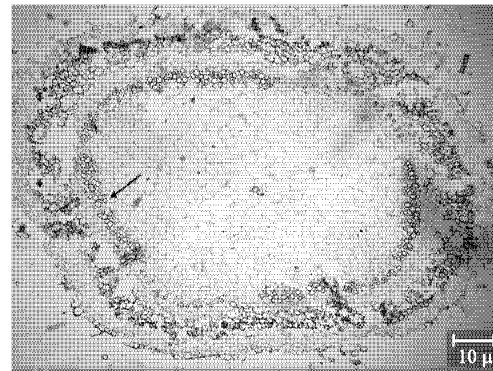


Fig. 3: Stem cross section of *S. indepressa*. The arrow showing cluster (x416)

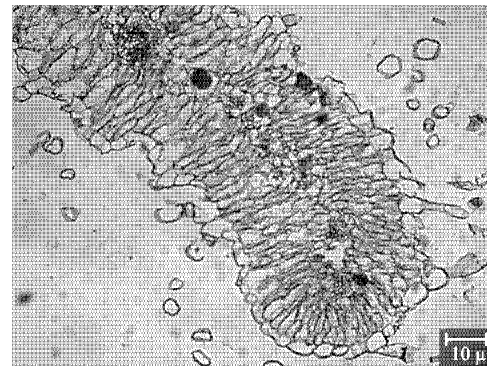


Fig. 4: Leaf cross section of *S. gertraudiae*. Isolateral mesophyll (x416)

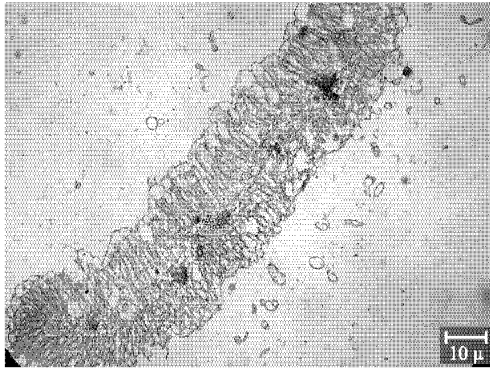


Fig. 5: Leaf cross section of *S. conoidae*. Dorsi-ventral mesophyll (x416)

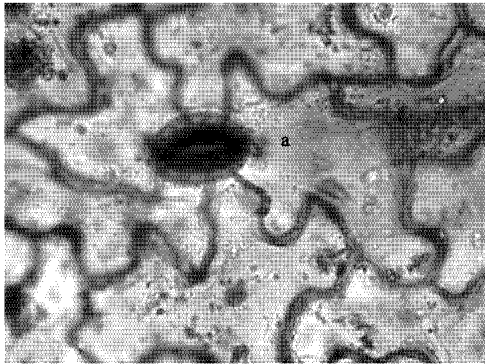


Fig. 6: Crenate subsidiary cell in *S. latifolia*

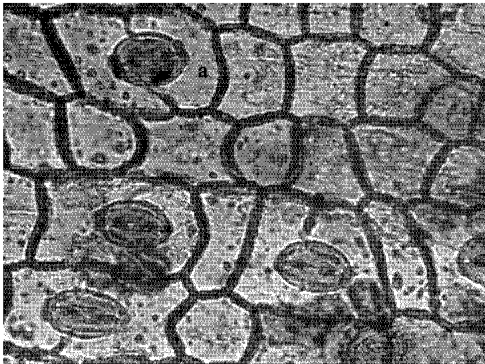


Fig. 7: Entire subsidiary cell in *S. bupleuroides*

The subsidiary cell in *S. latifolia*, *S. noctiflora*, *S. conoidae* were crenate (Fig. 6) and entire in *S. indepressa*, *S. swertifolia*, *S. gertraudiae*, *S. bupleuroides* (Fig. 7).

**Palynology analysis:** The pollen was spheroid minuta and media size, tectate or semi-TECTATE, pantaporate or inaperture reticulate, scabrate-granulate, occasionally muri with microechinate (Fig. 8-11). The characters were shown in Table 2.

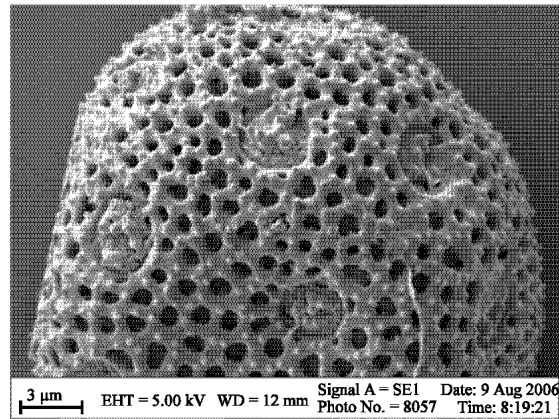


Fig. 8: The pollen of *S. latifolia*. The muri with microechinate

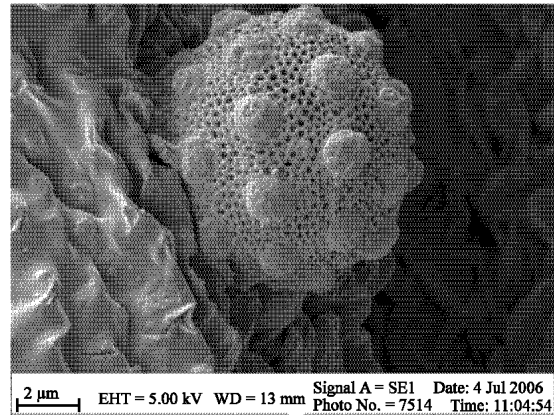


Fig. 9: The pollen of *S. noctiflora*

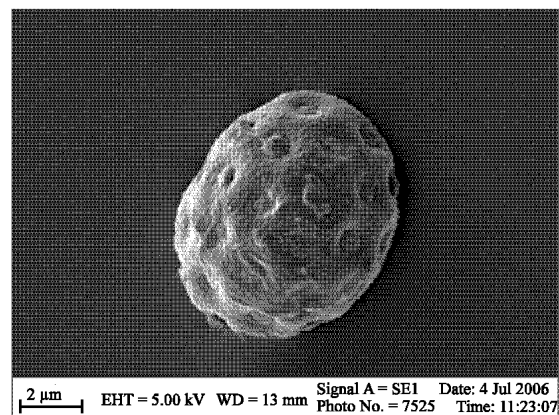


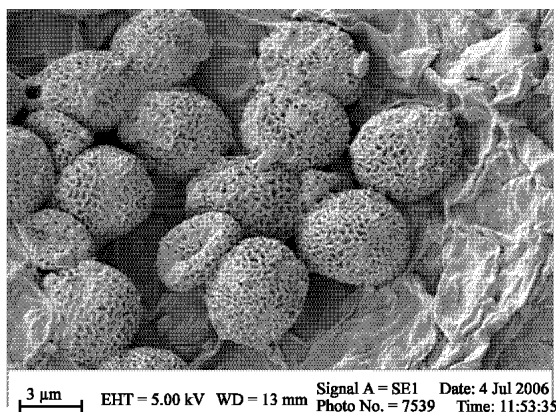
Fig. 10: The pollen of *S. conoidae*

The anatomy results showed that the glabrous and pubescent species had sclerid and fiber, respectively. The arrangement of mesophyll in annual and biennial species of *Melandrifomes* and *Conoimorpha* sections growing

Table 2: The characters of the pollen of studied *Silene* species

Species	Tectum	Aperture shape	Aperture position	Pollen ornamentation	Microechinate on the muri
<i>S. latifolia</i>	Tectate-perforate (semi-ectate)	Forate	Pantaporate	Reticulate	+
<i>S. noctiflora</i>	Tectate-perforate (semi-ectate)	Forate	Pantaporate	Reticulate	-
<i>S. conoidae</i>	Tectate-perforate (semi-ectate)	Forate	Pantaporate	Scabrate-granulate	-
<i>S. swertifolia</i>	Tectate-perforate (semi-ectate)	Forate	Pantaporate	Scabrate-granulate	-
<i>S. gertauidae</i>	Tectate-perforate (semi-ectate)	Inaperture	Inaperture	Reticulate	-
<i>S. indepressa</i>	Tectate-perforate (semi-ectate)	Forate	Inaperture	Reticulate	+
<i>S. bupleuroides</i>	Tectate-inperforate	Forate	Inaperture	Scabrate-granulate	-
subsp. <i>bupleuroides</i>					
<i>S. bupleuroides</i>	Tectate-inperforate	Forate	Inaperture	Scabrate-granulate	-
subsp. <i>ramosa</i>					

+: Present, -: Absent

Fig. 11: The pollen of *S. gertrauidae*

around the river is dorsi-ventral, but that in perennial species of *Auriculatae* and *Sclerocalycinae* sections which growing in stony ground is isolateral. Druse crystals in *Saponaria* like *Silene* have been observed by Ataslar (2004). The morphology of pollen in *Auriculatae* and *Siphonomorpha* sections has been reported by Ghazanfar (1984). In *Siphonomorpha* section, ectexine is punctuate (tubulifer/spinolose) but it is composed of punctuate, reticulate, semi- tectate (tubulifer/spinolose) types in *Auriculatae*. The origin of reticulate ectexine is punctuated. The pollen of some Caryophyllaceae species, like *S. noctiflora*, has been observed (Punt and Hoen, 1995). Besides, the pollen of *S. latifolia* subsp. *alba* has also been investigated (Moore *et al.*, 1991). Their results are similar to present research study. In present research, the ectexine in *Sclerocalycinae* and *Conoimorpha* sections is scabrate- granulate, tectate, while that in *Melandriformes* and *Auriculatae* sections is semi- tectate and reticulate. Two of studied species i.e., *S. indepressa* and *S. latifolia* had muri with microechinate. Morphologically, *S. latifolia* is similar to *S. noctiflora*, also their pollen are similar, but the former species differs from another for having muri with microechinate. Phylogenetically, tectate- perforate gradually changes to form lumina and muri with semi- tectate (Ghazanfar, 1984).

The *Melandriformes* and *Auriculatae* sections having semi- tectate and reticulate exine are more advanced than *Conoimorpha* and *Sclerocalycinae* sections. In conclusion the results showed that the similarity of their ornamentation to some extant related to their morphology. The sections could be recognized on the basis pollen ornamentation.

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