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ARTICLE

Anatomical study of some *Silene* L. species of *Lasiostemones* Boiss. section in Iran

M. Keshavarzi[1]*, M. Mahdavinejad[1], M. Sheidai[2], A. Gholipour[3]

[1]Biology Department, Alzahra University, Tehran, Iran, [2]Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran, [3]Biology Department, Payamenour University Sari Branch, Mazandaran, Iran

ABSTRACT *Silene* (Caryophyllaceae) composed of 110 species in Iran from which 35 species are endemic. *Lasiostemones* section is one of *Silene* sections with 10 species in Iran. In present study leaf and stem anatomical structure were considered for the first time. In order to study the anatomical variations of stem and leaf, 36 populations of 7 species of *Silene* (*Lasiostemones* section) were collected from different habitats of Iran. In leaf anatomy vascular bundle shape, shape of dorsal surface of mid vein, cortex diameter, hair presence in dorsal and ventral surface of leaf, mid rib diameter, cuticle upper and lower thickness, fiber presence in mid rib, stomata cell shape, stomata index and hair frequency show significant differences among studied species. In stem anatomy features as shape of cross section, hair type, cortex and xylem and phloem diameter were of diagnostic value in species separation. **Acta Biol Szeged 58(1):15-19 (2014)**

KEY WORDS

Silene
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The genus *Silene* L. (Caryophyllaceae) based on *Flora Iranica* is composed of 110 species in Iran, form which 35 species are endemic (Melzheimer 1980). One of *Silene* sections is *Lasiostemones* Boiss with 10 species in Iran (Melzheimer 1980). This section is distinguished from other sections by perennial form of life, raceme inflorescences, white flowers, short, obconical to cylindrical and hairy calyx and nerves of calyx producing reticulate, scabrous filaments (Melzheimer 1980).

Relationships of *Silene* species have been discussed mainly by morphological data sets. There are no extensive anatomical studies in this section. Chalk and Metcalfe (1950) provide general anatomical features of carnation family. Kilic (2009) studied stem and leaf anatomical structure of 8 species of Silene in Turkey. Fathi et al. (2010) studied stem anatomy of 10 species of *Silene* of four sections. Jafari et al. (2008) studied epidermis features of *Silene* as diagnostic ones in taxonomic studies. Yildiz and Minareci (2008) specified *S. urvillei* for its glandular hairs and stomata in leaf both surface.

Evaluation the anatomical structure of leaves of some *Silene* species in Pakistan showed a considerable variation in anatomical structure and the importance of shape and size of epidermal cells, hairs and crystals in species separation (Sahreen et al. 2010).

Diacytic stomata type is a diagnostic feature in Caryophyllaceae family. In *Silene* the main stomata type is diacytic but there are also anisocytic and anomocytic. In present study leaf and stem anatomical features of some *Silene* (*Lasiostemones* section) species have been studied for the first time in order to find valuable characters for taxonomic purposes.

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*Corresponding author. E-mail: Keshavarzm@alzahra.ac.ir

Materials and Methods

In order to study the leaf and stem anatomical features, 36 populations of 7 *Silene* species of *Lasiostemones* section were collected from different parts of Iran (Table 1). Ten individuals from each species have been studied. Form each individual, 3 leaves were sampled form the uppermost internode. Handmade sections were done for basal leaves and lower parts of stems. Double coloration by methyl green and Congo red was used. Dorsal epidermis was prepared for study by tissue removal method. A camera bearing Olympus DP12 microscope was used in this research. Altogether, 21 qualitative and quantitative anatomical features were measured and evaluated (Table 2).

For stomata index, the number of stomata and epidermal cells present in a leaf unit area were calculated using a micrometer. The following formula was used:

$$\text{Stomatal Index (\%)} = \frac{\text{Stomatal density x 100}}{\text{Stomatal density + epidemal cell density}}$$

Vouchers are deposited at Herbarium of Shahid Beheshti University (HSBU) and Herbarium of Payamenour University – Sari Branch (PNUSH).

In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, we have used cluster analysis and principal

Table 1. Population details of studied *Silene* species (♦ leaf, ■ epidermis and ● stem anatomical studies).

Taxon	Studies	Population details
S. claviformis Litv.	♦■	Kerman, Chatroud, Gholipour, 86070 PNUSH
S. longipetala vent.	♦	Chaharmahal and Bakhtiari, Gholipour, 8500251 HSBU
S. longipetala	♦■	Western Azerbaijan, Naghadeh, Oshnaviyeh, Gholipour, 8500250 HSBU
S. marschallii C.A.Mey.	♦	Lorestan, Borojerd, Gholipour, 87018 PNUSH
S. marschallii	♦	Isfahan, Khonsar, Golestan Kuh, Gholipour, 8500245 HSBU
S. marschallii	♦	Tehran, Firouz Kuh Road, Gholipour, 8500236 HSBU
S. marschallii	♦●	Zanjan, Anguran Mount., Gholipour,86095 PNUSH
S. marschallii	♦	Markazi, Arak, Gholipour, 87024 PNUSH
S. marschallii	♦	Western Azerbaijan, Ashk Island, Gholipour,--
S. marschallii	♦	Mazandaran, Chalous to Kandovan, Gholipour, --
S. marschallii	♦	Tehran, Firouzkouh, Gholipour, 8686PNUSH
S. marschallii	♦	Mazandaran, Damavand Peak, Gholipour, 8500243HSBU
S. marschallii	♦	Mazandaraan, Tonekabon, Chagoul, Gholipour, 86140PNUSH
S. marschallii	♦	Tehran, Touchal Highlands, Gholipour, --
S. marschallii		Zanjan, Zanjan, Gholipour, 86026PNUSH
S. marschallii	♦	Eastern Azerbaijan, Tabriz, Khoy, Gholipour, 87040PNUSH
S. marschallii		Semnan, Khonar Fields, Gholipour, -
S. marschallii	♦■	Eastern Azerbaijan. Tabriz to Kaleybar, Gholipour, 8500249SBU
S. parrowiana Boiss. & Hausskn. ex Boiss.	♦■	Kermanshah, Bisotun, Pero, Gholipour, 86102 PNUSH
S. propinqua Schischk.	♦	Kurdistan, Divandare to Sanandaj, Gholipour, 86097 PNUSH
S. propinqua	♦	Western Azerbaijan, Uromiyeh, Khalil Kuh, Gholipour, 87053 PNUSH
S. propinqua	♦■●	Western Azerbaijan, Uromiyeh, Khoy, Gholipour, 87097 PNUSH
S. ruprechtii Schischkin	♦■●	Eastern Azerbaijan, Tabriz, Ahar, Gholipour, 85012 PNUSH
S. tenella A.Huet ex Schenk	♦	Tehran, Firouzkuh, Gholipour, 86087 PNUSH
S. tenella	♦	Eastern Azerbaijan, Sahand Mount, Gholipour, 87030 PNUSH
S. tenella	♦	Western Azerbaijan, Uromiyeh, Khalil Kuh, Gholipour, 87055 PNUSH
S. tenella	♦	Western Azerbaijan, Ziveh, Gholipour, 900862 PNUSH
S. tenella	♦	Ardebil, Gholipour, 86111 PNUSH
S. tenella	♦	Firouzkouh, Gadouk defile, Gholipour, 86087 PNUSH
S. tenella	♦	Guilan, Gholipour, 86138 PNUSH
S. tenella	♦	Ardebil, Neor Lake, Gholipour, 8500228HSBU
S. tenella	♦	Mazandaran, Nour, Gholipour,--
S. tenella	♦	Ardebil, Sabalan, Gholipour, 86110 PNUSH
S. tenella	♦	Mazandaran, Damavand Mount, Gholipour, 86080 PNUSH
S. tenella	♦	Mazandaran, Balade, Gholipour, 900558 PNUSH
S. tenella	■	Tehran, Firouzkuh, Gadouk defile, Gholipour, 8500230HSBU

component analysis (PCA) (Ingrouille 1986). For multivariate analysis, the mean of the quantitative characters was used, while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of anatomical data. In order to determine the most variable characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 19 software was used for statistical analysis.

Results

Analysis of variance showed that nerve number for all studied species is constant and observed variation in quantitative features as cuticle thickness at adaxial and abaxial surface and average vascular bundle diameter are not significant. Observed variation in other studied characters are significant and can be used as diagnostic features (Table 3). Post hoc tests used for qualitative features revealed that form of central vein, hair on the ventral and dorsal surface, collenchymas presence at central vein, form of dorsal surface of the mid rib and fiber condition in the central vessel are of significance.

Leaf cross section

For leaf anatomical observations 34 populations of seven *Silene* species were studied. In all studied species mid rib shape is ellipsoid, orbicular or triangular, with one vascular bundle. Collenchymas are present under dorsal epidermis and crystals are present in mesophyll. Dorsal surface of mid rib is smooth, round, dome-shaped or acute. All studied species has oxalate crystals in mesophyll. In leaf cross section of *S. claviformis* mid rib dorsal surface is smooth and vascular bundle is rounded with sclerenchymas fiber (Fig. 1.c). *S.*

Table 2. Qualitative and quantitative anatomical features used in present research.

State of Character	Character	
Presence/ absence	Calcium oxalate	
Round/ elliptic/ triangular	Mid rib shape	
Presence/ absence	Hair at leaf dorsal surface	Qualitative characters
Presence/ absence	Hair at leaf ventral surface	
Presence/ absence	Collenchyma at mid rib	
Round/ smooth/angled/ pointed	Dorsal shape of mid rib	
Presence/ absence	Fiber at mid rib vascular bundle	
Rectangular/ jigsaw puzzle shaped/ oblong	Epidermal cell shape	
Smooth/ undulate	Epidermal cell wall shape	
Quantitative characters		
Cortex thickness	Number of hairs per leaf area	Epidermal cells length
Vascular bundle number	Stomata index	Epidermal cells width
Width at middle of leaf blade	Stomata width	Stomata length
Leaf adaxial cuticle thickness	Leaf abaxial cuticle thickness	Average vascular bundle diameter

Table 3. The quantitative data of the epidermis in different *Silene* species.

Species	Stomata Length (µm)	Stomata width (µm)	Epidermis length (µm)	Epidermis width (µm)	Hair no.	Stomata index
S. claviformis	33.43	26.13	82.05	48.31	4.00	.37
S. longipetala	49.33	37.72	52.12	43.73	11.00	.33
S. marschallii	33.06	20.30	94.50	15.92	23.00	.15
S. tenella	30.75	26.06	37.54	57.75	17.00	.21
S. ruprechti	31.20	21.84	36.53	41.06	.00	.42
S. propinqua	32.25	25.00	67.77	51.21	10.00	.37
S. parowiana	31.80	20.40	36.40	42.30	2.00	.15

ruprechtii and *S. parrowiana* are similar in leaf anatomical features. Both species show angled mid rib, rounded vascular bundle, bilateral phloem without sclerenchymatous fibers (Fig. 1b & d).

In *S. longipetala* leaf cross section, mid rib is rounded and the central vascular bundle is ellipsoid, with bilateral phloem and sclerenchymatous fibers (Fig. 1 h). In *S. propinqua* mid rib is rounded to angled, main vascular bundle is rounded, sclerenchymatous fiber is not present, phloem is bilateral. Hairs are thicker than other studied species (Fig. 1 g). Populations of *S. marschallii* and *S. tenella* show great intra-specific variations in leaf cross sections features as mid rib and vascular bundle shape and phloem condition (Fig. 1 a, e & f).

Leaf epidermis

Epidermal cells in all studied taxa are rectangular except in *S. pa*rrowiana and *S. ruprechtii. S. marschallii* has elongated rectangular epidermal cells. Cell walls are smooth except in *S. parrowiana* and *S. ruprechtii* that undulated cell walls have been observed. Main stomata type in *Silene* is diacytic but in *S. parrowiana* and *S. propinqua* there are also anisocytic type (Fig. 2 a - g)

Stem cross section

Four species are studied for their stem cross sections. In *S. marschallii* and *S. tenella* stem general shape is round, in *S. ruprechtii* ellipsoid and in *S. propinqua* quadrangular. Among studied species, *S. propinqua* has long single celled and multicellular hairs on stem, *S. ruprechtii* has no hairs and two other species have small single-celled hairs. Presence of continuous sclerenchyma in cortex and oxalate crystals in pith parenchymas are common features in studied species (Fig. 3).

Discussion

In order to clarify the species relationships cluster analysis by WARD method was done. In phenogram two main clusters are observed. In first main cluster *S. parowiana* and *S. ruprechtii* are grouped while in second cluster there are two sub-clusters. *S .longipetala* has a separate and isolate position. Four species are grouped in two subsets. *S. marschallii* and *S. tenella* in one set and *S. claviformis* and *S. propinqua* in another one show more similarities (Fig. 4). Cluster analysis and PCA ordination of the studied species of *Silene*, based on both quantitative and qualitative anatomical characters, have produced similar results (Fig. 5).

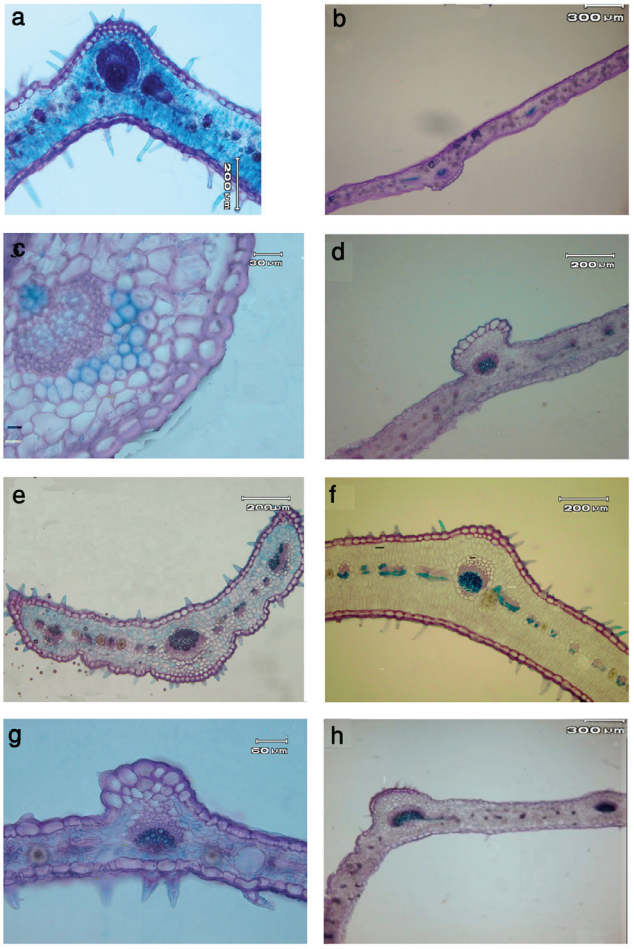

Figure 1. Leaf cross section structure in different studied *Silene* species. a: *S. marschallii*, b: *S. parrowiana*, c: *S. claviformis*, d: *S. ruprechtii*, e & f: *S. tenella*, g: *S. propinqua*, h: *S. longipetala*.

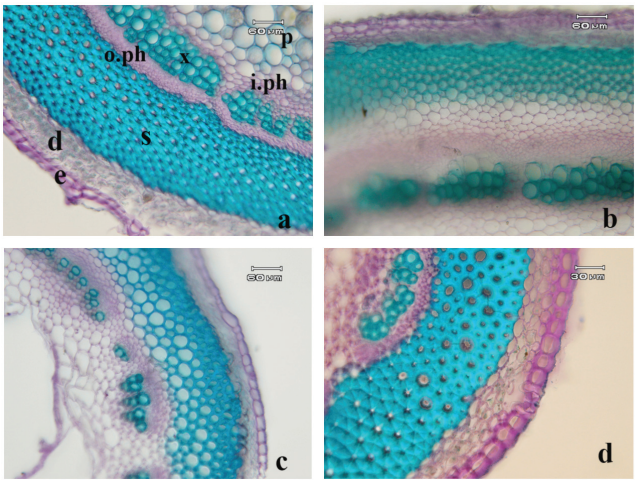

Figure 3. Stem cross sections in studied *Silene* species. a: *S. marschallii*, b: *S. tenella*, c: *S. ruprechtii*, d: *S. propinqua*. (e: epidermis, d: cortex, s: sclerenchyma, o.ph: outer phloem, i.ph: inner phloem, x: xylem, p: pith parenchyma).

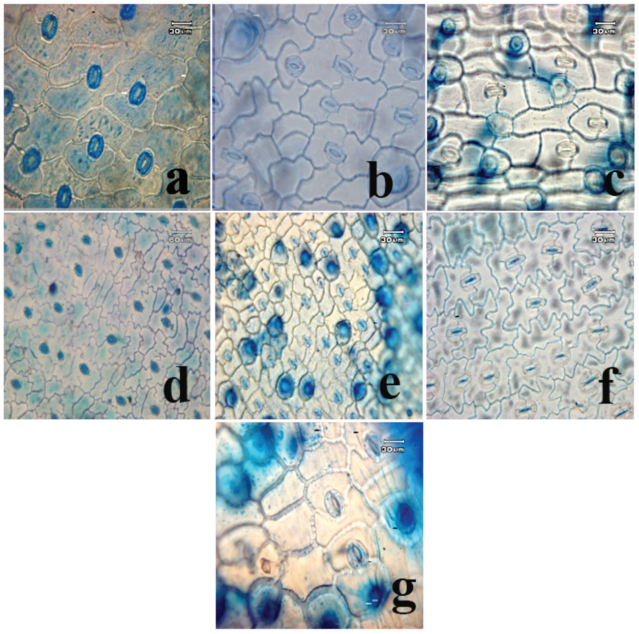

Figure 2. Leaf epidermis in studied *Silene* species. a: *S. claviformis*, b: *S. longipetala*, c: *S. marschallii*, d: *S. ruprechtii*, e: *S. tenella*, f: *S. parrowiana*, g: *S. propinqua.*

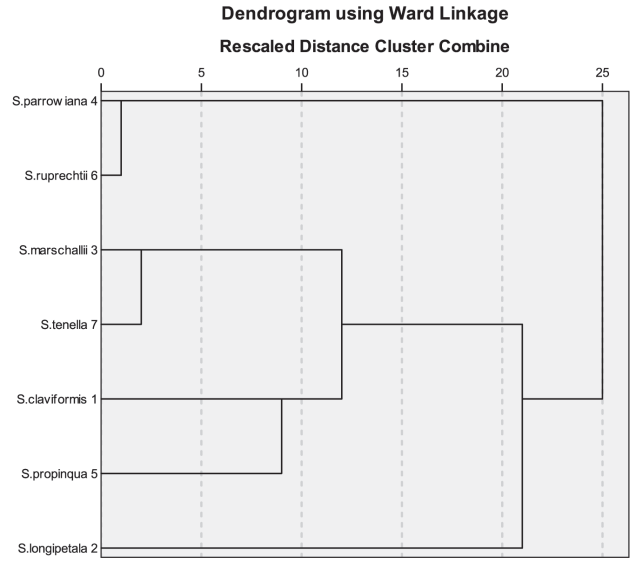

Figure 4. Cluster analysis by WARD method for studied species by leaf anatomical data.

It is the first leaf anatomical study of *Lasiostemones* species. Selected features of leaf anatomy appeared to be of taxonomic importance and could clearly separate the species. In leaf cross section a close relationship is found between *S. ruprechtii* and *S. parrowiana*. These species are morphologi-

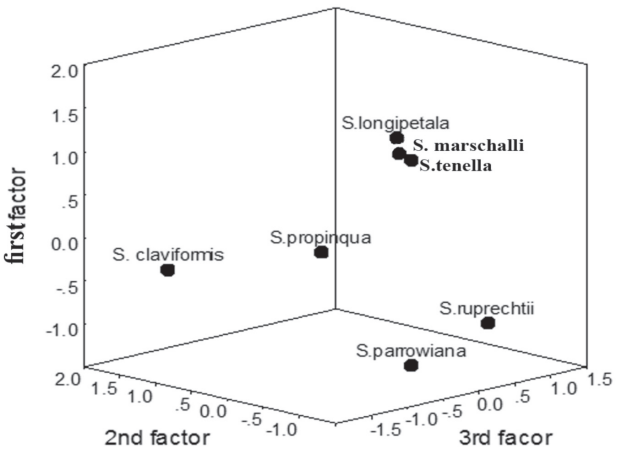

Figure 5. PCA scatter diagram by leaf anatomical features for studied species.

cally similar too. There are similarities between *S.marschallii* and *S. tenella* and *S. claviformis* and *S. propinqua* in the other hand. Most diagnostic features are mid rib shape, vascular bundle diameter, fiber presence in central vascular bundle and phloem position. Other diagnostic features like hair types, epidermal hairs and cuticle and cortex diameter are in concordant with previous studies (Jafari et al. 2008; Kilic 2009).

Studying epidermis shows its diagnostic importance and in concordant with Chalk and Metcalfe (1950) results. Sahreen et al. (2010) emphasized on diagnostic importance of epidermal features for 12 species of *Silene*. Epidermis in *S. clavformis* and *S. propinqua* show similarities. There are also similarities between *S. ruprechtii* and *S. parrowiana* which is in concordant with our leaf anatomical results. An identification key based on leaf dorsal epidermis is presented:

1a- Epidermal cells irregular	2
b- Epidermal cells regular	3
2a- Epidermis without hair	*S. ruprechtii*
b- Epidermis hairy	*S. parrowiana*
3a- Epidermal cell shape elongated rectangle	*S. marschallii*
b- Epidermal cell shape not elongated	4
4a- Stomata type diacytic	5
b- Stomata type diacytic and anisocytic	*S. propinqua*
5a- Few hairs in epidermis surface	*S. claviformis*
b- Frequent hairs in epidermis surface	6
6a- Epidermis cell length 30-40 micrometer	*S. tenella*
b- Epidermis cell length 45-60 micrometer	*S. longipetala*

Stem anatomical study of some *Lasiostemones* species in present study show some diagnostic features which are of taxonomic importance. These findings are in concordant with some previously published results (Jafari et al. 2008; Kilic 2009; Fathi et al. 2010). In all studied taxa there was a continuous cylinder of sclerenchymas fiber in cortex as was pointed by Fathi et al (2010). *S. marschallii* and *S. tenella* show similarities in their stem anatomy too.

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