

Silene thymifolia Sibth. et Sm. (Caryophyllaceae) – A vulnerable species in Romania: Anatomical aspects of vegetative organs

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Abstract

Silene thymifolia (Caryophyllaceae) is considered a rare, vulnerable species in Romania, growing only on Black Sea coastal dunes. Anatomical aspects of the root, stem and leaf of this species are presented in this study. Root, stem and leaves were analysed by brightfield microscopy, with specific staining. The root in cross section exhibited a secondary structure, generated by the activity of phellogen, and vascular cambium, with thick cork, continuous secondary xylem rings, no pith rays, but with cortical air lacunes. The stem had a primary structure with a number of collateral vascular bundles arranged on a single circle, with thick cortical schlerenchymal layers and oxalate crystals in pith cells. The epidermis showed stomata and glandular hairs. The leaf was amphistomatic leaf, with a heterogeneous mesophyll (equifacial type) and embedded vascular bundles, with a rounded mid rib surrounded by a parenchyma sheath. The spongy tissue has some large intercellular air spaces with druses. The mechanical tissue is well developed in the root and stem and poorly developed, represented by few collenchyma elements in the mesophyll, being represented by a continuous schlerenchyma fibers band present in root and stem and collenchyma in the leaf. The secretory histological elements were represented by epidermal glandular hairs and oxaliferous cells (druses) present in stem and leaf.

Keywords: anatomy; leaf; root; *Silene thymifolia*; stem

Introduction

Silene thymifolia Sibth. et Sm. (syn. *Silene pontica* Brandzá) is one of the 700 species of *Silene*, the largest genus of the Caryophyllaceae family (Bernasconi *et al.*, 2009). *Silene thymifolia* Sibth. et Sm. is a perennial plant with branched, woody stock. Stems are numerous, 15-20 cm, procumbent or ascending to erect, scabrid-puberulent (Figure 1). Leaves are small, fleshy, 8-20 mm long, ovate, rarely elliptic, conspicuously pubescent. The flowers are grouped in small numbers, in a dichasial inflorescence. Flower has a glandular-villous calyx, narrowly clavately, 12-15 mm, and a corolla with white bifid petals, neck ligulae. Capsule (8- 9 mm) is slightly longer than carpophore (7 mm; Săvulescu, 1953; Tutin, 1972).

Silene thymifolia is a vulnerable (Vu) coastal dune plant species (Dihoru and Negrean, 2009) in Romanian Black Sea Coast. Concerning its zoological status, it is also considered as a rare (R) species (Dihoru and Dihoru, 1994) and near-threatened (NT) species (Oltean *et al.*, 1994). On its ecology it is known that *Silene thymifolia* Sibth. et Sm. is a hemicryptophyte (H) characteristic on littoral dunes with spreading on

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North-West Black Sea sandy seashores. According to Flora Europaea it is present on terrestrial maritime sands from Northern and Western coasts of Black Sea in Bulgaria, Romania, Russia (W) and Turkey (Tutin, 1972).

It is a heliophile species, spread in warm areas, with dry to moderately moist soils, neutral pH (Ciocârlan, 2009; Sârbu *et al.*, 2013). Vegetal associations cited to contain *Silene thymifolia* are: *Secali sylvestris-Alysetum borzeani* (Borza, 1931); *Secali sylvestris-Brometum tectorum* (Hargitai, 1940); *Alyso borzeani – Ephedretum distachyae* (Tzonev *et al.*, 2005). These associations are part of two Natura 2000 natural habitat types: 2110 Embryonic shifting dunes, 2130 Fixed coastal dunes with herbaceous vegetation ('grey dunes'; Doniță *et al.*, 2005; Gafta and Mountford, 2008).



Figure 1. *Silene thymifolia*
(original photo Jianu L.D.)

Because of its habitat preference specificity (it grows only on marine sand dunes) the studied species is extremely rare so its current distribution in Romania includes only the Agigea Natural Reserve (Jianu and Făgăraș, 2015). The current conservation status of the *Silene thymifolia* regarding species distribution, population status and its future population perspectives have a totally inadequate and unfavourable conservation status. The habitat of local populations was heavily affected by the intensified human activities in the Romanian coastal area over the past 50-60 years (Jianu, 2014). Without the application of urgent protection and conservation measures the extinction of *Silene thymifolia* species from the Romanian flora is possible (Jianu, 2014) despite the effort to preserve small populations in the natural protected area Agigea Natural Reserve (according to its Management Plan).

Silene genus is a very complex group of plants in terms of taxonomic because of its fairly uniform external appearance (Coode and Cullen, 1967). In literature, there are many studies regarding the morphological features, used mostly to establish the relationships among *Silene* species (Greuter, 1995; Yıldız, 2002; Kilic and Ozcelik, 2008). Few data are known about the anatomy of *Silene* species vegetative organs. Succinct general information concerning anatomical characteristics of the Caryophyllaceae family (including *Silene* as well) belong to Metcalfe and Chalk (1950). Recent anatomical studies concerning a number of *Silene* species belong especially to Kilic (2009), Yıldız and Minareci (2008), Fathi *et al.* (2010), Jafari *et al.* (2008), Keshavarzi *et al.* (2014), Bağcı and Biçer (2015), but no detailed anatomical study for *Silene thymifolia*.

The purpose of this paper is to highlight anatomical features of *Silene thymifolia* and to contribute with more information to the knowledge concerning this taxon.

Materials and Methods

Biological material

For anatomical analysis of the studied species, fresh plant material was collected from a small population found on Agigea Natural Reserve marine sand dunes (Constanța County, southeastern Romania). The biological material was represented by root, herbaceous stem and stem leaves, collected from plants at full maturity. Taking into account the status of endangerment of this plant, only fragments were collected from vegetative organs necessary for the respective anatomical sections.

Anatomical analyses and micrographs

Small pieces of root, stem and leaf were fixed in FAA (formalin: glacial acetic acid: alcohol 5:5:90). The cross sections were performed by freehand technique (Bercu and Jianu, 2003). The samples were stained with alum-carmin and iodine green and mounted with gelatine-glycerine to make permanent slides. Anatomical observations and micrographs were performed with a BIOROM-T bright-field microscope, equipped with a TOPICA 6001A video camera.

Results and Discussion

The cross section of the root, showed a thick cork (8-10 cell layers) generated by the cork cambium. Beneath the cork cambium there is the phelloderm, composed of a number of layers of living parenchyma cells. Some cells contain calcium oxalate crystals – druses. Rare air spaces are present (Figure 2A, B). The vascular cambium, composed of 2-3 layers of flattened cells, generates outwards the secondary phloem and inwards the secondary xylem. Phloem consists of phloem vessels, companion cells and phloem parenchyma. The secondary xylem (xylem vessels and xylem parenchyma) is more developed than phloem forming a continuous ring. The vessels are few in the center, more numerous towards the cambium, where the difference between spring and autumn wood tissue is clearly marked. The numerous xylem vessels are separated by small, compact, thin-walled cells. Medullary rays are absent. In the secondary xylem, toward the pith, a thick schlerenchyma ring (7-8 sclerenchymatous cells) is present (Figure 2A, C). The presence of intra-annual fiber bands in the xylem is the characteristic of Caryophyllaceae species growing at lower altitudes (Schweingruber, 2007).

Cross section of the stem has a circular-sinuous shape (Figure 3A). The outermost single-layered epidermis is represented by slightly tangentially elongated rectangular cells with thick internal and external walls and covered by a thick cuticle. The epidermis shows stomata and densely multicellular (4-5 cells) glandular hairs (Figure 3B). Some of them are grouped in pairs.

The cortex, located just below the epidermis, consists of 4-5 cell layers, some of them with chloroplasts and others (fewer) with druses, followed by several layers of sclerenchyma cells, occupying a large area (9-10 layers) of the stem (Figure 3A, B). These findings, like those of Fathi *et al.* (2010) and Keshavarzi *et al.* (2014) in several other *Silene* species, place the thick, continuous ring of schlerenchymal fibers in the cortex. However, Bağcı and Biçer (2015) suggest that it may belong to the stele. The presence of the sclerenchyma ring in the stem cortex and oxalate crystals in the pith parenchyma cells are common features in *Silene* species stem (Keshavarzi *et al.*, 2014).

The vascular system is represented by a single ring of 9 open collateral vascular bundles. Phloem tissue consists of sieve cells, companion cells and phloem parenchyma. Xylem vessels are radially arranged, placed in a cellulosic parenchyma (Figure 3C). The vascular bundles are separated by rays of cells with cellulosic walls. The cambium zone is not easily distinguishable, a situation similar to that reported by Yıldız and Minareci (2008) for *Silene urvillei* stem.

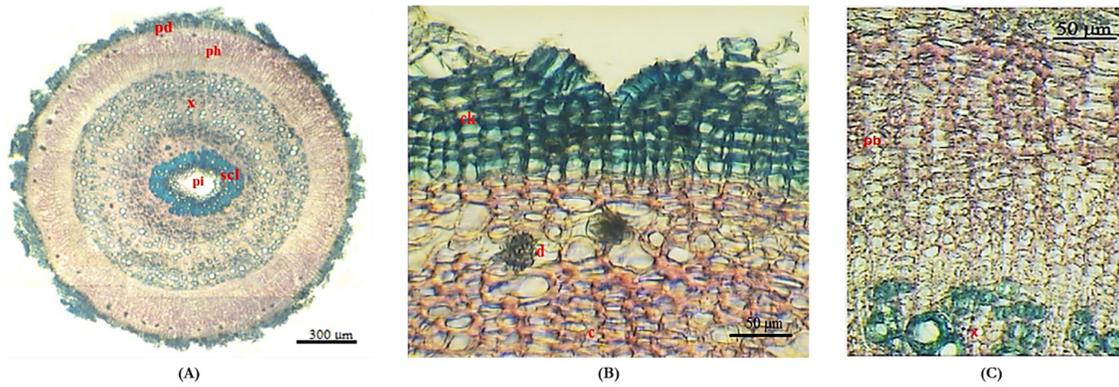


Figure 2. Cross section of the root; (A) Ensemble; (B) Portion with cork and cortex; (C) Portion of the stele

cb- cambium, ck- cork, d- druse, pd- periderm, ph- phloem, pi- pith, scl- sclerenchyma, x- xylem

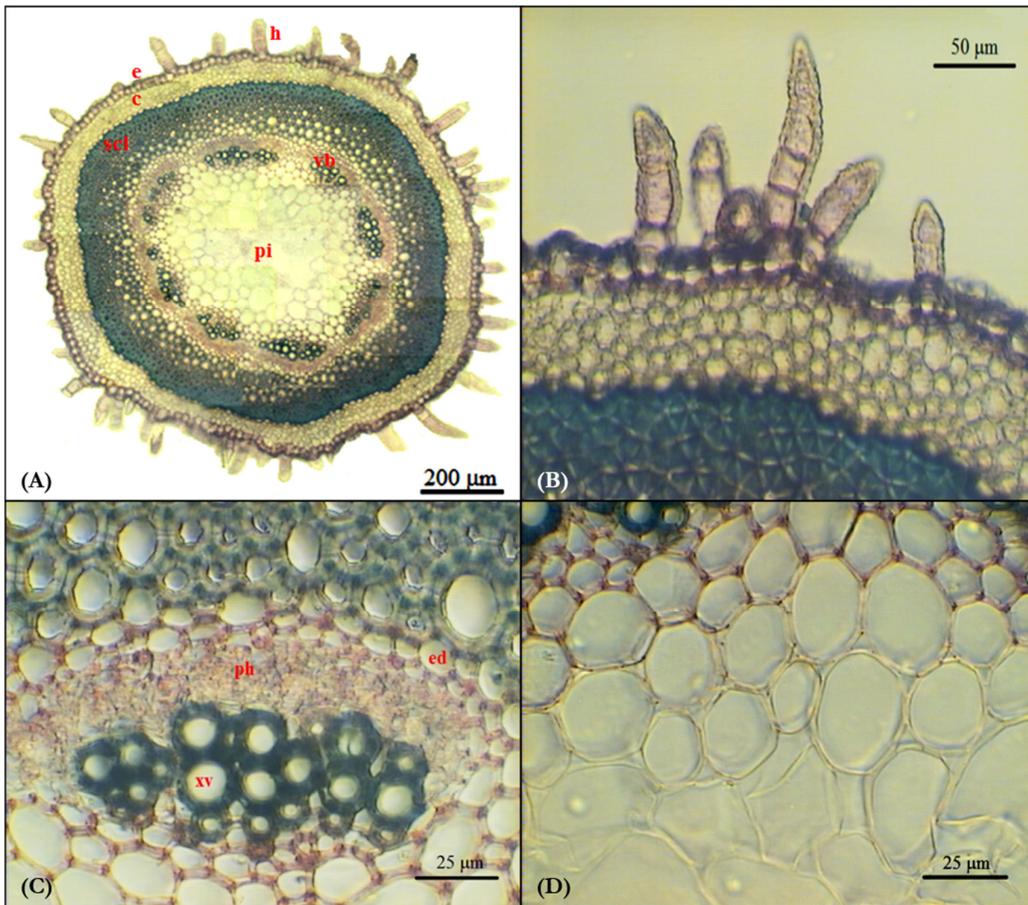


Figure 3. Cross section of the stem; (A) Ensemble; (B) Portion with cortex; (C) A vascular bundle of the stele; (D) Pith

c- cortex, e- epidermis, ed- endodermis, h- hair, pi- pith, ph- phloem, scl- sclerenchyma, vb- vascular bundle, xv- xylem vessel

The centrally located pith is composed of parenchyma cells, some of them possessing calcium oxalate crystals. Some empty spaces occur due to pith cells disorganization, this area being gradually replaced by a pith lacuna (gap) in mature stages (Figure 3D).

The cross section of the leaf blade exhibited an upper and lower single-layered epidermis with tangential slightly elongated cells and covered by a thick cuticle. Both epidermises show stomata with substomatal cavities of various sizes (amphistomatic leaf) and hairs. The same type of glandular hairs on the stem are present, especially on the lower epidermis, more abundant close to the mid rib zone (Figure 4A). Hairs placed close to the mesophyll margins are longer than the others. The upper epidermis cells are larger than those of the lower one. The lower and upper epidermis are slightly prominent in the mid rib zone.

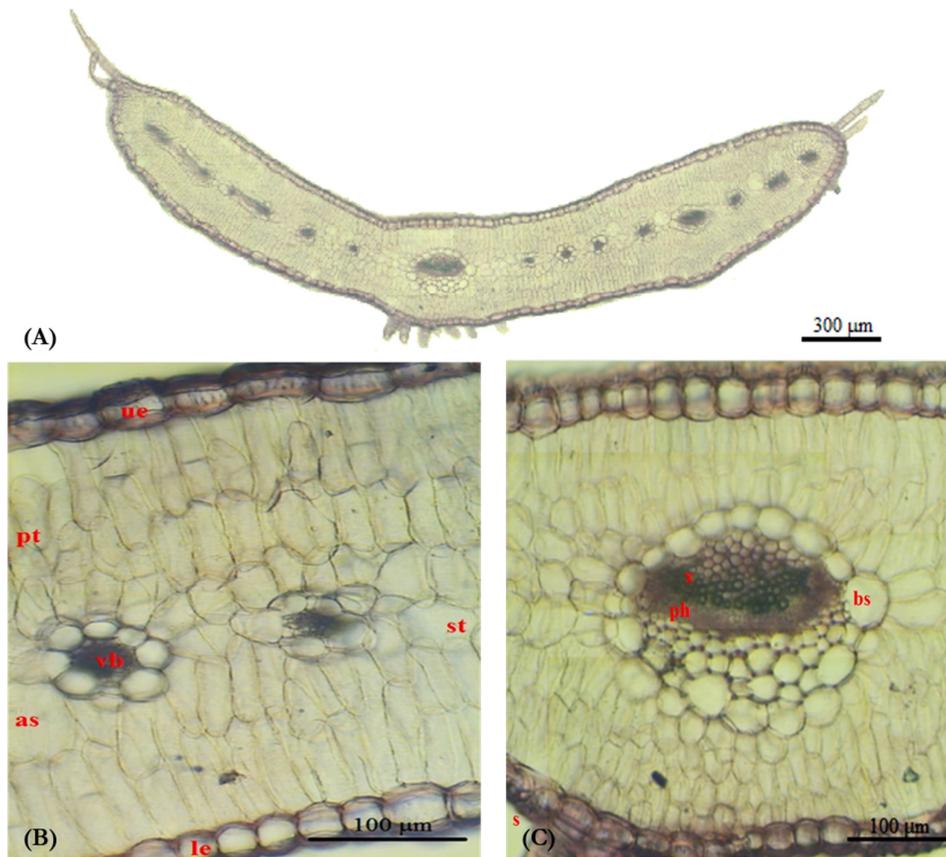


Figure 4. Cross section of the leaf; (A) Ensemble; (B) Portion with mesophyll and secondary veins; (C) Portion of the mid rib zone
 as- air space, bs- bundle sheath, le- lower epidermis, ph- phloem, pt- palisade tissue, sp- spongy tissue, ue- upper epidermis, vb- vascular bundle, x- xylem

The mesophyll has both palisade and spongy tissue. The palisade tissue is placed below the upper and lower epidermis (equifacial type; Figure 4B, C). The mesophyll had druses in its large lacunes. The round-shaped mid rib is represented by a collateral vascular bundle surrounded by a parenchymatous bundle sheath (Figure 4C). The secondary vein bundles possess few conductive elements and a single-layered bundle sheath around them (Figure 4B).

Conclusions

The root has a secondary structure due to the activity of the phellogen and vascular cambium, without pith rays and with some air spaces in the cortex. The stem has a primary structure with a number of collateral vascular bundles arranged on a single circle. The leaf is amphistomatic with an equifacial mesophyll and a rounded mid rib surrounded by a parenchyma sheath. The spongy tissue has some large intercellular air spaces with druses.

The secretory histological elements are represented by epidermal glandular hairs and oxaliferous cells (druses) present in stem and leaf. The mechanical tissues are represented by a continuous sclerenchyma fibres band present in root and stem and collenchyma in the leaf.

Authors' Contributions

Conceptualization: LDJ and RB; Data curation: RB and DRP; Formal analysis: RB; Investigation: LDJ and RB; Methodology: LDJ and RB; Software: DRP; Supervision: RB; Validation: LDJ and RB; Visualization: DRP; Writing - original draft: RB; Writing - review and editing: DRP. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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