ANATOMY AND TRICHOME MICROMORPHOLOGY OF STACHYS SCARDICA (GRISEB.) HAYEK (LAMIACEAE)

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Abstract – The anatomy and micromorphology of the vegetative organs and calyx of Stachys scardica (Griseb.) Hayek were investigated using light (LM) and scanning electron microscopy (SEM). The analysis of the anatomical structure of stem, leaf and leaf petiole showed the presence of an additional adaxial phloem in the vascular bundle of the petiole that was recorded exclusively in species belonging to the subgenus Betonica. On the surface of studied plant parts, three types of trichomes were found: simple nonglandular uniseriate multicellular, nonglandular branched elongated and glandular peltate trichomes. The present study shows that certain micromorphological and anatomical features of Stachys taxa are valuable taxonomic characters.

Key words: Stachys scardica; LM; SEM; glandular trichomes; petiole anatomy.

INTRODUCTION

The genus Stachys L., consisting of about 300 species, is one of the largest genera of the Lamiaceae (Mabberley, 1997). It is a subcosmopolitan genus centered in the warm temperate regions of the Mediterranean and South West Asia (Bhattacharjee, 1980). The Balkan Peninsula is one of two main centers of diversity in number of species. The majority of the species prefers alpine and subalpine habitats and grows under various ecological conditions, in habitats such as rocky places, mainly on limestone and other basic rocks, mountain steppes, stream banks or sometimes in forest growing in rocky places. Saxatile habit condition has evidently an influence of general habit and life form (Salmaki et al., 2009).

Some species of this genus have long been used in folk medicine as sedatives, antispasmodics, diuretics and emmenagogues (Lewis, 1977; Hartwell, 1982; Duke, 1986). S. palustris L. and S. sylvatica L. are approved for healing wounds, treating abdominal pains and as disinfectant, antispasmodic and anti-fever agents (Gruenwald et al., 2000). Many studies of this genus have shown various biological activities such as antiinflammatory (Maleki et al., 2001; Khanavi et al., 2005; Kukic et al., 2007), antianxiety (Rabbani et al., 2003), antibacterial (Digrak et al., 2001; Grujić Jovanović et al., 2004; Sonboli et al., 2005), antinephritic (Hayashi et al., 1994), anticancer (Amirghofran et al., 2006, 2007), anti-Helicobacter pylori (Stamatis et al., 2003) and antioxidant (Aydenet al., 2006; Kukic et al., 2006; Matkowski and Piotrowska, 2006; Erdemoglu et al., 2006; Vundac et al., 2007; Conforti et al., 2009).

Stachys scardica is an erect, hirsute, perennial herb with ovate and oblong-lanceolate leaves. The leaf margin is crenate-serrate or dentate. Leaf indumentum is composed of multi-branched nonglandular
trichomes and peltate glandular trichomes. It grows on mountain and subalpine meadows and scrub, on serpentine or limestone. Distribution on the Balkan Peninsula includes Albania, Bulgaria, Greece and the southern parts of ex Yugoslavia (Ball, 1972).

In spite of the large species diversity of *Stachys*, investigation of the anatomy, morphology, palynology, trichome diversity and distribution, was only conducted on a small number of species (Demisew and Harley, 1992; Dinç and Öztürk, 2008; Muhittin and Ozturk, 2008; Giuliani et al., 2008; Giuliani and Maleci Bini, 2008, 2012; Salmaki et al., 2009; Rezakhanlo and Talebi, 2010; Erdoğan et al., 2011; Venditti et al., 2013).

According to the available literature data, the anatomy and trichome micromorphology of *S. scardica* have not yet been examined. In this work, we investigated the anatomy of the vegetative organs, and morphology and distribution of trichomes on the stem, leaves and calyx of *S. scardica* using a light microscope (LM) and scanning electron microscope (SEM). The type of indumentum is described and classified. The general classification scheme and the terminology follow Radford et al. (1974) and Roe (1971).

**MATERIALS AND METHODS**

**Plant material**

Aerial parts of the analyzed plants were collected at the flowering stage in July 2010 on Mt. Kopaonik. A voucher specimen has been deposited in the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade, Faculty of Biology, Serbia (BEOU), voucher No. 16828.

**Light microscopy (LM)**

Small segments of stem, leaf and calyx were fixed in FAA and further processed using the standard paraffin method (Jensen, 1962). For the light microscopy analysis, 5µm-thick sections were cut on a Reichert microtome and stained with safranin and alcian blue. The sections were then observed on a LM LEICA DMSL and photographed using a LEICA DC300 digital camera.

**Scanning electron microscopy (SEM)**

Small segments of dry stem, leaves and calyx were coated with a thin layer of gold (ion sputtering coating) in a BALTEC-SCD 005 Sputtering Device. Observations were carried out on a JEOL JSM 6460 LV scanning electron microscope at 20 kV.

**RESULTS**

*S. scardica* is a perennial herb with a four-sided stem and ovate-lanceolate leaves. The indumentum of vegetative organs of *S. scardica* comprised three types of trichomes: simple nonglandular uniseriate multicellular trichomes, nonglandular branched elongated trichomes (stellate – branched), and glandular peltate trichomes. Nonglandular branched trichomes were found on all vegetative organs of *S. scardica*, playing a protective role against herbivores. Glandular peltate trichomes were observed on the stem, leaves and calyx. SEM micrographs of *S. scardica* aerial parts are presented in Fig. 2. The capitate glandular trichomes were not found on the surface of *S. scardica* organs.

**Stem anatomy and indumentum**

The herbaceous stem of *S. scardica* in cross section has a square form with pronounced angles. The stem is covered with a one-layered epidermis and thin cuticle. Collenchyma is single-layered among the angles, but 6–7 layers of collenchyma are observed below the epidermis at the angles. The rest of the cortex is composed of several layers of compact parenchyma. The pericycle is cylindrical, made of sclerenchyma elements, interrupted in a few places by several parenchyma cells. The cambium is in the form of a ring and produces the phloem and xylem elements, also in a ring form. The central part of the stem is built up of large parenchymatous cells with small intercellulars (Fig. 1. A). On the stem surface, simple multicellular uniseriate trichomes are present, as well as specific branched trichomes – multicellular dendroid hairs,
Fig. 1. Anatomy of *S. scardica*. A - cross section of stem of *S. scardica*; B – cross section of petal leaf; C, D – cross section of leaf; E, F – cross section of petioles.
Fig. 2. Micromorphology of *S. scardica* (SEM). A, B – stem of *S. scardica*; C, D – abaxial leaf surface; E – adaxial leaf surface; F, G, H – calyx.
with three multicellular uniseriate branches above the base (Fig. 2. A, B). Glandular peltate trichomes have a short one-celled stalk and head of four cells, of various diameters (Fig. 2. B). Cuticle rupture was not observed using SEM, and secretion seems to remain trapped in the intact subcuticular spaces unless external factors cause their rupture.

**Leaf anatomy and indumentum**

*S. scardica* has ovate-lanceolate cauline leaves, with a crenate margin, cordate at the base, green above, grayish-green below. The mesophyll is differentiated into palisade and spongy tissues. The palisade parenchyma consists of two layers of densely packed, elongated cells. Spongy globular-shaped parenchyma cells are 3-4 layered with small intercellular spaces (Fig. 1. D). Vascular bundles are collateral, without cambium, surrounded by a parenchymatic bundle sheath. The main vein is well developed with pronounced lower side and has one-layered collenchyma located below the upper and lower epidermis (Fig. 1. C).

The leaves of *S. scardica* are petiolate, with the petiolo up to 10 mm long. The adaxial surface of the petiolo is plane with a visible curve on the ends (V shaped) and the abaxial surface is convex (Fig.1. E.). The transverse section of petiolo of *S. scardica* shows a one-layered epidermis, subepidermal collenchyma cells in the angles, numerous densely packed parenchymatous cells, one large central vascular bundle and two lateral vascular bundles. Epidermal round-shaped cells are covered with a thin cuticle. Simple nonglandular uniseriate multicellular trichomes and nonglandular branched elongated trichomes are present on the epidermis. Ground tissue consists of dense parenchyma cells, with the largest cells around the central vascular bundle. The vascular bundle is collateral, without cambium, containing xylem and two phloem, of which the adaxial phloem is more developed (Fig. 1. F). Tracheal elements are very well developed. The sclerenchyma enveloped the adaxial phloem and xylem (Fig. 1. F). Small lateral vascular bundles contained xylem and phloem and were surrounded by sclerenchyma more developed around the phloem.

The leaf showed dorsiventral symmetry with larger adaxial and smaller abaxial rectangular epidermal cells covered with a thin cuticle (Fig. 1. D). Both leaf sides bear numerous nonglandular trichomes and peltate glandular trichomes (Fig. 2. C, D, E). Multicellular uniseriate trichomes, as well as branched trichomes, are situated on both leaf sides. On the abaxial leaf surface, branched stellate trichomes are situated along the veins, and between the veins (Fig. 2. C). The base of stellate trichome is large and different numbers of multicellular uniseriate branches are divided from the base. The peltate trichomes with 4-celled heads, as well as stomata, are observed on the abaxial leaf surface (Fig. 2. D). The adaxial leaf surface is covered with branched nonglandular and peltate trichomes. From the base of the branched trichome mostly unicellular branches emerge (Fig. 2. E). The peltate trichomes have 4-celled heads (Fig. 2. E).

Sepal leaves, about 8-10 mm long, are also covered with different types of trichomes on both sides (Fig. 2. F, G, H). The calyx is characterized by the presence of unusual branched trichomes (Fig. 2. G); it is very hairy and covered with simple unicellular short trichomes (Fig. 2. F, H) and densely distributed branched trichomes (Fig. 2. G). The observed number of branches was two, six, or eight. The branches are multicellular, uniseriate, warted, with pointed apex. The outer surface of the calyx is densely covered by numerous stellate hairs and by small peltate glandular trichomes. From the inner side of the calyx very long simple uniseriate trichomes were observed to emerge (Fig. 2. H).

**DISCUSSION**

The herbaceous stem of *S. scardica* showed the typical four-angled form and structure of Lamiaceae members, covered with a one-layered epidermis with trichomes, well-developed collenchyma at the angles of the stem, cambium and conducting tissues in ring form and central parenchymatous tissue. The leaves are ovate-lanceolate, comprising the epidermis covered with trichomes, mesophyll and vascular bundles. The petiole is triangle-shaped, with epidermis,
not differentiated mesophyll and vascular bundles surrounded by sclerenchyma. The petiole anatomy showed variability in different Lamiaceae species (Gupta and Bhambie, 1980; Akçin et al., 2011). The distinct feature of *S. scardica* and *S. officinalis*, which belong to the subgenus Betonica, is the presence of an additional adaxial phloem in the central vascular bundle. This type has not been found in any investigated species from the *Stachys* subgenus (Bhattacharjee, 1980). Various types of trichomes were observed on all analyzed organs of *S. scardica*, which is a characteristic feature of Lamiaceae members. In Lamiaceae, nonglandular and glandular trichomes are distinguished, with peltate and capitate trichomes as the basic types of glandular trichomes (Cantino, 1990; Fahn, 2000; 2002). The glandular trichomes’ structure can vary widely among species, as does the composition of the essential oils they produce for protection of the plant aerial parts against herbivores and pathogens (Werker, 1993). The function of nonglandular trichomes depends on their morphology, the organ on which they are situated and their direction and orientation. When nonglandular trichomes are densely spread all over the plants, they may serve as a mechanical barrier (Werker, 2000). Apart from having different functions in plants, the trichomes’ microcharacters could be of taxonomical significance. Some authors, in studying the micromorphology of *Stachys* trichomes, considered that the characters of taxonomic interest were the presence of glandular and nonglandular trichomes, thickness of the cell walls, number of cells, presence of few-armed or branched, or vermiform trichomes, orientation of trichomes in relation to the epidermal surface, presence of papillae on trichome surface, etc. (Salmaki et al., 2009).

*S. scardica* has three types of trichomes: simple nonglandular uniseriate multicellular trichomes, nonglandular stellate trichomes with elongated branches, and glandular peltate trichomes.

The other investigated species of *Stachys* varied in presence and types of nonglandular and glandular trichomes. Falciani et al. (1995), Giuliani and Maleci Bini (2008), Giuliani et al. (2008), Giuliani and Maleci Bini (2012) and Venditti et al. (2013) have studied the micromorphology and distribution of trichomes of *Stachys* species grown in Italy and described different glandular and nonglandular trichomes. The typical peltate trichomes with large heads of variable numbers of secreting cells and small capitate trichomes (Subsessile) are present on the leaves and calyces of *S. officinalis* subsp. *officinalis*, *S. alopecuroides* subsp. *alopecuroides* and *S. annua*. *S. germanica* subsp. *germanica* and *S. germanica* subsp. *salviifolia* possess peculiar peltate trichomes with well-developed stalk on the leaves and calyces (Giuliani and Maleci Bini, 2008). In *S. recta* subsp. *serpentini* and *S. recta* subsp. *recta* only one type of glandular trichome was found, a short-neck capitate trichome (Giuliani et al., 2008). *S. alopecuroides* subsp. *divulsa* had peltate and two types of capitate trichomes (Venditti et al., 2013). In the study of differential characters between *Stachys* subgenus *Betonica* and *Stachys* subgenus *Stachys*, Giuliani and Maleci Bini (2012) found that *Stachys* subgenus *Betonica* possessed only peltate, while *Stachys* subgenus *Stachys* has different types of large capitate hairs, which was confirmed in our study. In addition, the peltate trichomes of the subgenus *Betonica* show an unusual secretion composed of flavonoids and essential oils (Giuliani and Maleci Bini, 2012; Marin et al., 2004), which can also support the view of various authors that within the *Stachys* genus two subgenera should be recognized (*Betonica* and *Stachys*) or even treated as a separate genus.

Turkish species *S. balansae* and *S. carduchorum* (sect. Eriostomum) possess unbranched nonglandular trichomes, unicellular or multicellular and capitate and peltate glandular trichomes (Erdogan et al., 2011). The micromorphological study of the species *S. cydni* and *S. yildirimilii* showed that the type of trichomes may contribute to the separation these species. The branch number of the trichomes and cell number of the branches differ significantly between the two species (Dink and Ozturk, 2008).

In an investigation of the trichome morphology of the Lebanese *Stachys* species, Beyrouthy et al. (2009) found 21 types of trichomes on different organs of six species, but only four types were found on
all studied species. Among these types, the unicellular warted nonglandular trichomes were mentioned, as was found in the *S. scardica* calyx. They suggest that nonglandular trichomes are more important than glandular trichomes in the differentiation of *Stachys* species.

In the comprehensive study of Iranian *Stachys* taxa, Salmaki et al. (2009) observed numerous types of trichomes that showed considerable variability among different species, but were constant among different populations of one species, and therefore, afford valuable characters in the delimitation of sections and species. Rezakhanlo and Talebi (2010) described 24 trichome types in Iranian *S. lavandulifolia*. The obtained results confirmed the usefulness of indumentum characters in taxonomic identification at different infrageneric levels, the importance of which was previously recognized (Abu Asab and Cantino, 1987). Trichome morphology is an essential character for delimitation of the section *Ambleia* Bentham and Zietenia Gled. (Benth) in the genus *Stachys*, while the section *Ambleia* is characterized by dendroid indumentum and is isolated from other sections of *Stachys* by this feature (Bhattacharjee, 1980). The morphology and distribution of glandular and nonglandular trichomes may be considered as distinctive characters at the subspecies level. This is consistent with the taxonomic classification of *S. recta* subsp. *serpentini* as a subordinate taxon of *S. recta* (Giuliani et al., 2008).

The pubescence of *S. scardica* may contribute to its adaptation to the environmental conditions where this species usually grows, and protection from various external factors such as extensive light, UV irradiation, extreme temperatures and herbivores. Analyzing the results obtained from the investigation of trichomes of other *Stachys* species, *S. scardica* was the only one to possess branched stellate nonglandular trichomes compared to other species from the subgenus *Betonica*. In addition, the capitate glandular trichomes are absent from the vegetative organs of this species. Results of an investigation into essential oil and flavonoids (Grujic Jovanovic et al., 2004; Marin et al., 2004) are in accordance with previous investigations and classification of this genus (Bhattacharjee, 1980).

Throughout the history of the classification of *Stachys*, different opinions have been expressed about the status of *Betonica*. The *Betonica* group is characterized by prominent sterile rosettes and usually unbranched flowering shoots. The leaf margins are deeply crenate to serrate. The only constant diagnostic morphological feature of the subgenus *Betonica* is the presence of sessile flowers and bracteoles with a hardened base. Among anatomical features, the presence of adaxial phloem in the petiole, and nutlets without pitted thickenings in the sclerenchyma are constant characteristics of the subgenus *Betonica* (Bhattacharjee, 1984). The structure of the vascular bundles of the petiole in species belonging to the subgenus *Betonica*, sections *Betonica* and *Macrostachya* was specific. The adaxial phloem that was recorded exclusively in the species *S. officinalis* and *S. scardica* appeared as a valuable differential character, since it did not occur in the vascular bundles of the petiole of species from the subgenus *Stachys*. Leaf anatomy provides valuable characters that are useful in subgeneric classification as well as species discrimination in *Stachys*. The most important diagnostic characters are the shape of leaf transverse section, length of ventral and dorsiventral axis, number of median bundles in the petiole, number of cell layers of palisade and spongy parenchyma, type and thickness of collenchyma as well as trichome type (Salmaki et al., 2011). Delimitation of the *Stachys* from its related taxon, *Betonica*, has been a comprehensive investigated. Bentham (1848), Boissier (1879), Reichinger (1982) and Krestovskaya and Vassilyeva (1997) excluded Betonica from *Stachys*. Some other authors included it in the genus (Brriet, 1897; Ball, 1972; Bhattacharjee, 1980). Bhattacharjee (1980) presented a new infrageneric classification for the genus in the Old World.

The results of indumentum investigation support the classification of *Betonica* and *Stachys* as distinct subgenera or even genera, rather than the classification of some authors who consider them to be sections, at the same level as other sections proposed.
within the genus *Stachys*. From our investigation, it was revealed that the anatomical structure of the leaf petiole supports the delimitation of these two related taxa and, consequently, could be regarded as a valid taxonomic character.

**Acknowledgments** - The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 173029) for the financial support.

**REFERENCES**


