

## MORPHOLOGY, DISTRIBUTION, AND HISTOCHEMISTRY OF TRICHOMES OF *THYMUS LYKAE* DEGEN & JAV. (LAMIACEAE)

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**Abstract** — Micromorphology, distribution, and histochemistry of the trichomes of *Thymus lykae* were studied using scanning electron microscopy (SEM) and confocal laser scanning electron microscopy (CLSM). The leaves, stem, and calyx bear numerous glandular and non-glandular trichomes. Two types of glandular trichomes are identified - peltate and capitate. Results of histochemical tests showed positive reactions to polysaccharides, proteins, and lipids. Yellow autofluorescence of secreted material was noticed in peltate and capitate trichomes. Strong reddish-yellow autofluorescence of the lipophilic and hydrophilic secreted material was observed with CLSM.

**Key words:** Trichomes, distribution, histochemistry, autofluorescence, *Thymus lykae*, Lamiaceae

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### INTRODUCTION

Trichomes are specialized epidermal cells found on the aerial surfaces of nearly all plants. They can take many shapes and forms. Trichomes serve a variety of functions, depending on their location. As leaf hairs, they reflect radiation, lower plant temperature, and reduce water loss (Wagner, 1991). They also provide defense against insects. Trichomes may be either unicellular or multicellular, branched or unbranched. According to secretory activities, trichomes can be glandular, consisting of a stalk terminating with a glandular head, or nonglandular, consisting of elongated tapering structures.

The family Lamiaceae is rich in aromatic species used as herbs, folk medicines, fragrances, etc., because of the essential oils produced in glandular hairs spread over the aerial vegetative and reproductive organs. In the Lamiaceae, there are two types of trichomes: peltate and capitate (Werner, 1993).

The genus *Thymus* L. belongs to the family Lamiaceae and is represented in the flora of Serbia by 30 species. It includes numerous species and vari-

eties, and their essential oil compositions have been studied intensively (Baser et al., 1992; Salgueiro et al., 2000; Miguel et al., 2004; Adelheid et al., 2005; Slavkowska et al., 2006; Rota et al., 2008). This taxonomically complex group of plants includes aromatic species, which are used for medical purposes and also as culinary herbs (Karman et al., 2001).

*Thymus lykae* Degen & Jav. is an endemic species from the central Balkans which grows on dry, rocky, sunny slopes of serpentine hills from the lowlands to the mountains. It is a small, aromatic semishrub with ovate leaves and capitate inflorescences with a purple corolla (Diklić, 1974).

This study presents the results of investigating the morphology, distribution, and histochemistry of glandular trichomes of *T. lykae*.

### MATERIAL AND METHODS

#### *Plant material*

Aerial parts of *T. lykae* were collected from plants at the flowering stage during June of 2006 in Southwest

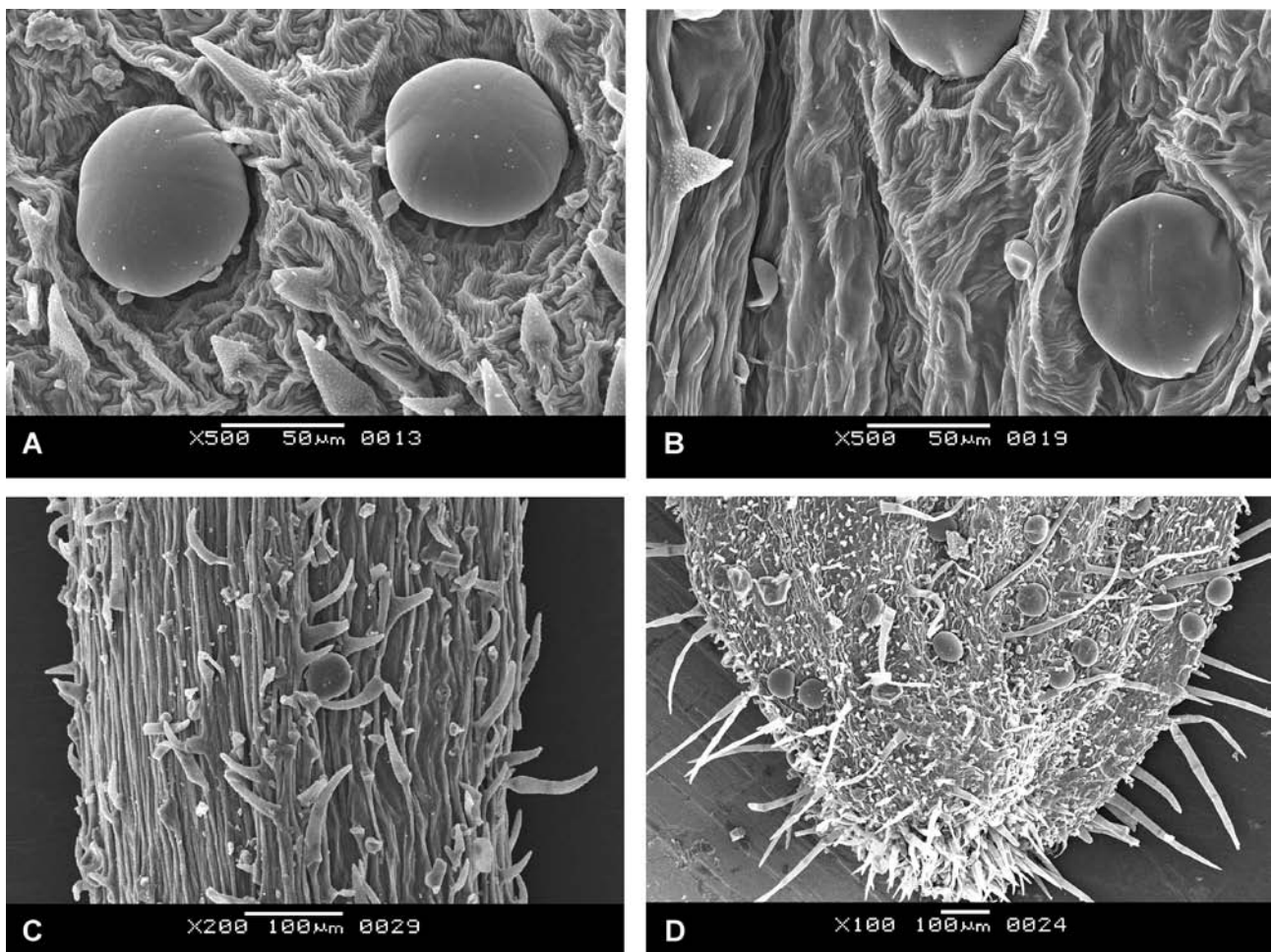
Serbia (Studenica) and Western Serbia (Ibar-Kula). Voucher specimens were deposited in the herbarium of the Institute of Botany, Faculty of Biology, University of Belgrade.

#### Scanning electron microscopy (SEM)

Leaves, stem, and calyces were fixed in glutaraldehyde (3% with buffer solution at pH 7.4), dehydrated in an alcohol and acetone series, dried to the critical point, and coated with a thin layer of gold (ion sputtering coating) in a BALTEC-SCD 005 sputtering device, after which observations were carried out on a JEOL JSM 6460 LV scanning electron microscope at 20 KV.

Histochemical analyses were performed on hand-fixed sections using the following tests: periodic acid-Schiff's reagent (PAS) for polysaccharides, osmium tetroxide for unsaturated lipids, and Page Blue for proteins (Jensen, 1962). Standard control procedures were carried out simultaneously. Observations were made under a LEITZ DMRB (Leica) microscope and a Leica Diastar microscope with Canon Powershot S40 equipment.

Hand sections of fresh or dry material were examined with a light fluorescence microscope of the LEICA DMLB type equipped with I3 blue filter, and also with a CLSM 510 instrument from Carl



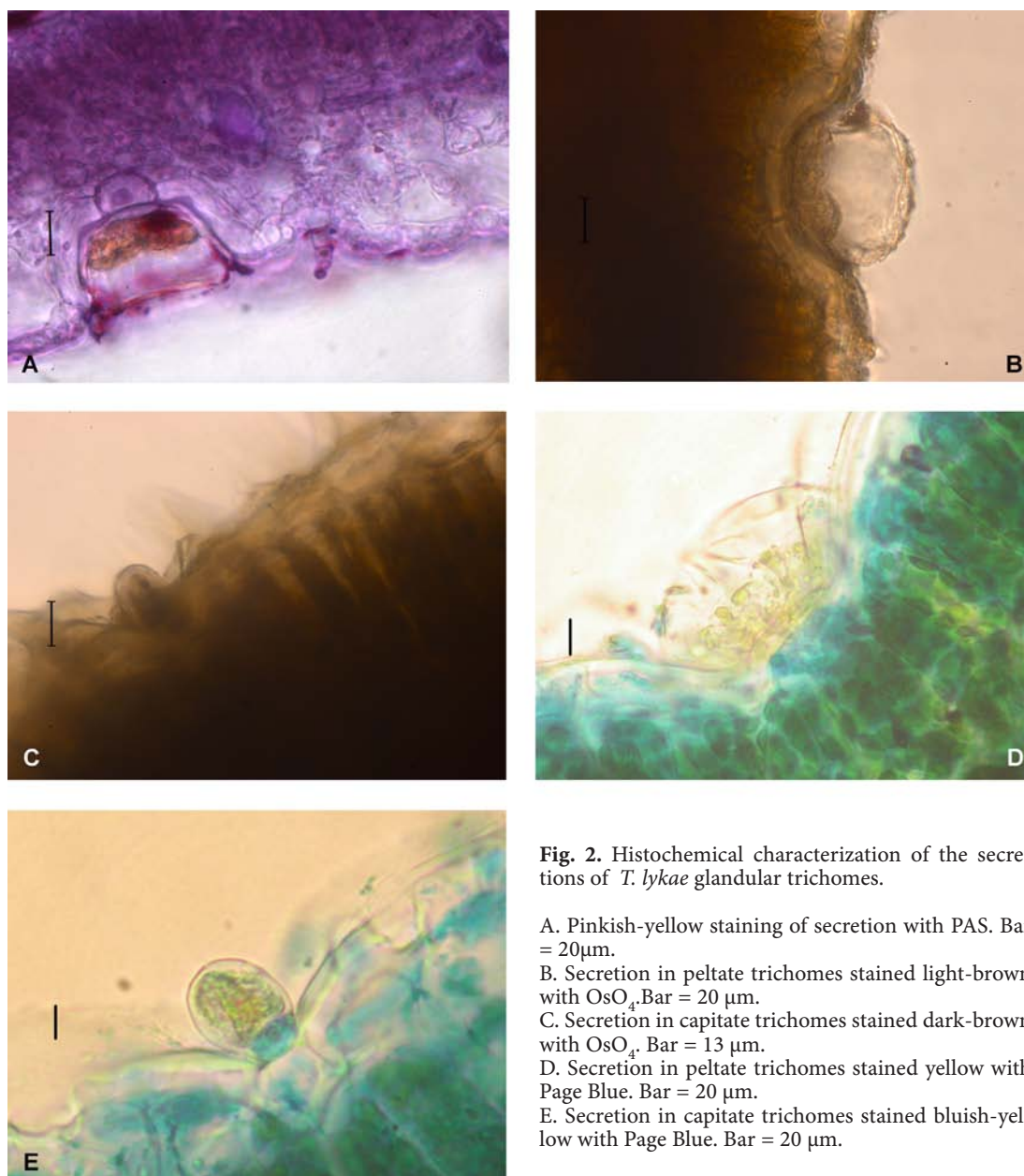
**Fig. 1.** Scanning electron micrographs of *T. lykai* – leaves, stem, and calyx. A. Mature peltate trichomes on adaxial leaf surface. Bar = 50  $\mu$ m. B. Peltate and capitate glandular trichomes on abaxial leaf surface. Bar = 50  $\mu$ m. C. Glandular and non-glandular trichomes on stem. Bar = 100  $\mu$ m. D. Glandular trichomes on outer calyx surface. Bar = 100  $\mu$ m.

Zeiss and an Axioskop FS2mot microscope. Plan-Apochromat 20x /0.75 objective lenses were used at excitation wavelengths of 488 and 543 nm.

#### RESULTS AND DISCUSSION

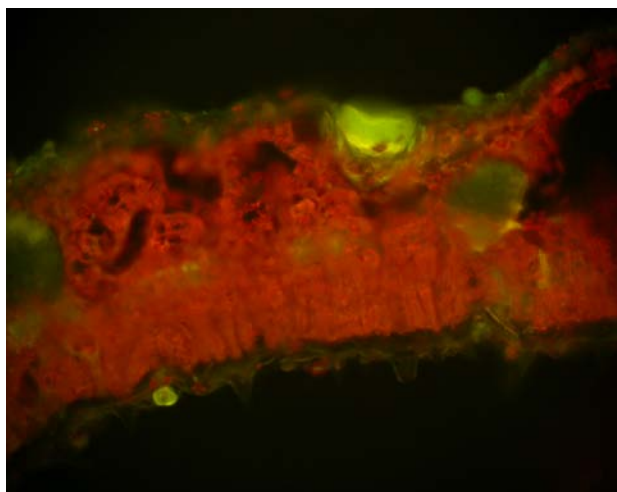
The leaves of *T. lykae* are covered with a thick cuticle on the adaxial leaf side. The epidermis was a unicellular layer on the abaxial and adaxial surface. Two

types of glandular trichomes — peltate and capitate — were found, as well as short and elongated unbranched nonglandular trichomes. Glandular trichomes had a cuticular covering thicker than that of the epidermis. The peltate trichomes, located in epidermal depressions, were composed of one basal cell, a short stalk cell, and a rounded head. The head of mature trichomes consists of eight secretory cells

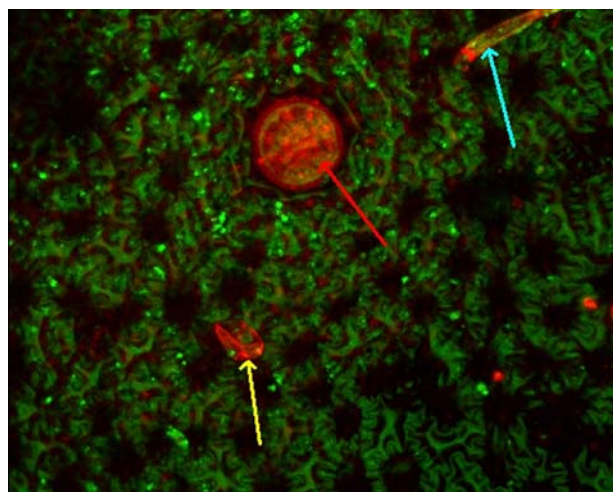


**Fig. 2.** Histochemical characterization of the secretions of *T. lykae* glandular trichomes.

- A. Pinkish-yellow staining of secretion with PAS. Bar = 20  $\mu$ m.  
 B. Secretion in peltate trichomes stained light-brown with  $\text{OsO}_4$ . Bar = 20  $\mu$ m.  
 C. Secretion in capitate trichomes stained dark-brown with  $\text{OsO}_4$ . Bar = 13  $\mu$ m.  
 D. Secretion in peltate trichomes stained yellow with Page Blue. Bar = 20  $\mu$ m.  
 E. Secretion in capitate trichomes stained bluish-yellow with Page Blue. Bar = 20  $\mu$ m.



**Fig. 3.** Autofluorescence of secreted materials of trichomes of *T. lykae*. Strong yellowish-green in peltate trichomes, weak yellow in capitate and nonglandular trichomes. Strong red autofluorescence in mesophyll cells. 20X.



**Fig. 4.** CLSM Autofluorescence of secreted materials of trichomes of *T. lykae*. Strong reddish-yellow autofluorescence in peltate and capitate trichomes, and yellowish-green autofluorescence in nonglandular trichomes. 20X.

(Fig. 1A). Peltate glandular trichomes were distributed on both leaf sides, on the stem (Fig. 1C), and on the outer calyx surface (Fig. 1D). Capitate glandular trichomes were found on both leaf surfaces, especially on the abaxial surface (Fig. 1B). They are composed of one basal cell, a short stalk cell, and a unicellular head.

The stem, leaves, and calyx bore numerous short and elongated nonglandular trichomes. Elongated nonglandular trichomes were especially densely distributed on the stem (Fig. 1C) and on the margins of the leaves, while short nonglandular trichomes were densely distributed on the adaxial side (Fig. 1A) and on the main vein of the *T. lykae* leaf.

The results of histochemical analysis of products secreted by the glandular trichomes are presented in Table 1. For histochemical analysis of the secreted material, several staining methods were used.

Staining with PAS for polysaccharides gave a positive reaction, the color being pinkish-yellow in peltate and pink in capitate trichomes. Situated in the neck of peltate trichomes, the nucleus was also stained pink (Fig. 2A).

The  $\text{OsO}_4$  test showed light-brown staining of

secretory material in the head of peltate trichomes (Fig. 2B) and dark-brown staining in the head of capitate trichomes (Fig. 2C).

With the Page Blue procedure, the reaction was positive in both peltate and capitate trichomes, indicating the presence of protein compounds. Peltate trichomes were stained yellow (Fig. 2D), whereas capitate trichomes stained bluish-yellow (Fig. 2E).

The examined stains gave slightly different colors in peltate and capitate trichomes, but we cannot deduce the exact differences without detailed chemical analyses.

Peltate trichomes showed strong yellow autofluorescence of the lipophilic and hydrophilic secreted material inside the head cells, whereas the subcuticular space showed a strong yellowish-green autofluorescence. Weak yellow autofluorescence is noticed in the head of capitate glandular trichomes and in nonglandular trichomes. Mesophyll cells were colored red because of the presence of chlorophyll (Fig. 3).

Strong reddish-yellow autofluorescence of secreted substances can be seen in both peltate and capitate trichomes, whereas yellowish-green autofluorescence is obtained on nonglandular trichomes observed with

**Table 1.** Histochemistry of secreted material of glandular trichomes of *Thymus lykae*.

Staining procedure	Target compounds	Peltate trichomes	Capitate trichomes
PAS	Polysaccharides	pinkish-yellow	pink
Osmium tetroxide	Unsaturated lipids	light-brown	dark-brown
Page Blue	Proteins	yellow	bluish-yellow

CLSM (Fig. 4). The autofluorescence observed in this study was also noted by other authors in the family Lamiaceae (Ascensao et al., 1999; Bisio et al., 1999).

The presence of peltate and capitate glandular trichomes is a characteristic feature of Lamiaceae species. Heterogenous secreted material was temporarily stored in the subcuticular space in mature peltate trichomes and released by rupture of the cuticle, while in capitate trichomes it was probably released through micropores.

Their secretions may be involved in chemical defense of the plants or serve to attract pollinators, but the specific function of these trichomes is not known. The non-glandular trichomes on vegetative and reproductive organs presumably collaborate with glandular trichomes in mechanical defense. Many Lamiaceae species have been investigated because of their high content of essential oils, which are widely used in pharmaceutical preparations, perfumery, and cosmetics (W e r k e r, 1993).

Taxonomically, *Thymus* is a very complex genus owing to the polymorphism of a number of species and the absence of intrageneric incompatibility. Interspecific and unprogressive hybridization between related species is a very common feature of this genus and the main cause of variation. The resulting hybrids often have intermediate or mixed morphological characteristics and chemical compounds (H o r w a t h et al., 2008).

Data obtained from histochemical tests revealed that the secreted material in the glandular trichomes of *Thymus lykae* is of heterogeneous composition, containing polysaccharides, unsaturated lipids, and proteins.

Comparison with previously published results shows many similarities of morphology, distribution, and histochemical contents of glandular trichomes

with *Thymus lykae* and other investigated species of the family Lamiaceae. Further research will include more detailed histochemical, ultrastructural, and chemical analyses to clarify differences among the species of this taxonomically complex group of plants.

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## МОРФОЛОГИЈА, ДИСТРИБУЦИЈА И ХИСТОХЕМИЈА ТРИХОМА *THYMUS LYCAE* DEGEN ET JAV. (LAMIACEAE)

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Микроморфологија, дистрибуција и хистохемијска анализа трихома код врсте *Thymus lycae* урађена је коришћењем скенинг електронског микроскопа и конфокалног ласерског скенинг микроскопа. Нежлездане, негранате и два типа жлезданих трихома - пелтатне и капитатне описане су у овом раду. Бројне нежлездане и жлездане трихоме налазиле су се како на листовима (и на лицу и на наличју) тако и на стаблу и на чашици.

Резултати хистохемијских истраживања показала су позитивне реакције на полисахариде, липиде и протеине. Секреторни производи унутар пелтатних трихома имали су јако жуту аутофлуоресценцију, док је у капитатним трихомама запажена слабија аутофлуоресценција. Коришћењем CLSM уочена је јарко црвено-жута аутофлуоресценција липофилних и хидрофилних секреторних материја у жлезданим трихомама.