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Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae)

A. Kahraman *, F. Celep, M. Dogan

Middle East Technical University, Department of Biological Sciences, 06531 Ankara, Turkey

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Abstract

The anatomy, palynology, morphology and distribution of the trichomes on the aerial parts of *Salvia chrysophylla* Stapf, an endemic species in Turkey, were studied in order to understand the usefulness of these characteristics for systematic purposes. Some anatomical characters such as (1–)2–24-rowed pith rays in roots, dorsiventral leaves, obviously larger upper epidermal cells, and two to three large vascular bundles in the center and two to four small subsidiary bundles in the wings of petiole provide information of taxonomical significance. Three main types of trichomes were observed on the stem, inflorescence axis, leaf and calyx surfaces of *S. chrysophylla*. They are peltate, capitate glandular and non-glandular. Capitate glandular and non-glandular trichomes were further subdivided into several kinds. Glandular trichomes are present in abundance on the inflorescence axis and calyx, but non-glandular ones were mainly situated on the leaf and stem. Scanning Electron Microscopy (SEM) studies on the pollen grains have revealed that they are oblate-spheroidal and their exine ornamentation is bireticulate-perforate.
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Keywords: Anatomy; Glandular and non-glandular trichomes; Palynology; *Salvia chrysophylla*

1. Introduction

Salvia L., the largest genus of Lamiaceae, is composed of nearly 1000 species distributed extensively in three regions of the world: Central and South America (500 spp.), western Asia (200 spp.) and eastern Asia (100 spp.) (Walker and Sytsma, 2007). Anatolia is a major diversity center for *Salvia* in Asia (Hedge, 1982). Turkey is home to 95 *Salvia* species, 49 (52%) of which are endemic (Kahraman et al., 2010-a).

Salvia species are used in traditional medicines all around the world, possessing antioxidant, antidiabetic, antibacterial, anti-tumor, antiplasmodial and anti-inflammatory features (Ulubelen, 2003; Kamatou et al., 2008). Many *Salvia* species are used as herbal tea and in food, cosmetics, perfumery and the pharmaceutical industry (Chalchat et al., 1998; Baylac and Racine, 2003). In addition, *Salvia* species are also grown in parks and gardens as ornamental plants (Marin et al., 1996).

There are a number of studies on anatomy (Metcalfe and Chalk, 1972; Kahraman et al., 2009; Kahraman et al., 2010-a,b; Kahraman and Dogan, in press), trichome morphology (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Kaya et al., 2003; Siebert, 2004; Kamatou et al., 2007; Ozkan, 2008) and palynology (Henderson et al., 1968; Cantino et al., 1992) of the genus. The usefulness of the structure of the vascular bundles in petioles for species identification in the family Lamiaceae has been demonstrated (Metcalfe and Chalk, 1972). The taxonomic significance of the structure of trichomes is well known in the Lamiaceae and related families (Metcalfe and Chalk, 1972). Pollen morphology has been pointed out to be useful in systematics of the Lamiaceae (Abu-Asab and Cantino, 1994). However, the anatomical, palynological and trichome structure of most *Salvia* species in Turkey have not yet been investigated.

Salvia chrysophylla Stapf is a Mediterranean element which grows on limestone slopes and grassy meadows at an altitude of 1300–2300 m in the southwest part of Turkey (Fig. 1). It does not appear to be closely related to any other *Salvia* species in

* Corresponding author.
E-mail address: ahmetk@metu.edu.tr (A. Kahraman).

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Fig. 1. *Salvia chrysophylla* plant in bloom.

Turkey. It is a perennial herb with a height of approximately 10–60 cm. The plant has erect stems, paniculate inflorescence axes, oblong to lanceolate and serrulate-denatate leaves, tubular-campanulate to infundibular calyces, lilac upper lip and yellow lower lip of corolla. The essential oil has important antistaphylococcal activity on some strong microorganisms, such as *Staphylococcus aureus* and *Cowan liyofi* (Arslan and Celik, 2008). In addition, the species can be used as an alternative to traditional food preservatives, eliminating or reducing the growth of important foodborne pathogens and spoilage bacteria (Arslan and Celik, 2008). Antioxidant activity of eight *Salvia* species was studied by Bozan et al. (2002). They found that the highest antioxidant activity was in *S. chrysophylla*.

Anatomy, trichome morphology and palynology of *S. chrysophylla*, a Turkish endemic species, have not been studied previously and also such investigations on *Salvia* species are rather limited. Therefore, the present study aims to investigate anatomical and palynological features of *S. chrysophylla*, to determine the various types of trichomes and their distribution on the aerial parts and to evaluate the usefulness of these characters for systematic purposes.

2. Material and methods

Plant specimens were collected during the flowering period from natural populations in Antalya (in the vicinity of Goyne, 36° 34′ 432″ N 29° 36′ 432″ E, June 2007, FCelep 1327) and Mugla (Eren mountain, 36° 44′ 890″ N 29° 36′ 477″ E, June 2007, FCelep 1330) provinces in the Mediterranean region of Turkey. Voucher samples are stored in the Herbarium of Faculty of Science, Ankara University, Turkey (ANK).

Anatomical investigations were performed using an average of 30 fresh specimens kept in 70% alcohol. The paraffin wax method was applied for preparing cross-sections of middle parts of mature roots, stems, leaves. All sections are made from the midrib and the margin of the leaf blades. The sections were stained with safranin-fast green (Johansen, 1944) with some modifications relating to staining time and amount of additions to the stains, and then they were mounted on slides using entellan. Slides were viewed and photographed with a Leica DM1000 light microscope.

Trichomes were obtained from stems, inflorescence axes, leaf blades, petioles and calyces and studied with a stereomicroscope and a light microscope. Sections were made with a Leica RM2125RT rotary microtome using the paraffin wax method and by hand using commercial razor blades. 50 measurements for each type of trichome were taken. They were studied using a Leica DM1000 light microscope with 400× to 1000× magnifications. The types and distributions of trichomes are described. The general trichome terminology follows Metcalfe and Chalk (1972), Payne (1978) and Navarro and El Oualidi (2000).

Pollen material was obtained from herbarium specimens. For Light Microscopy (LM) studies, pollen grains were first treated with 70% alcohol to remove oily substances, and then embedded in glycerine jelly stained with basic fuchsin, following the method of Wodehouse (1935). Measurements and observations were made using a Leica DM1000 light microscope. The polar length, equatorial length, colpus length, exine and intine thickness for 30 pollen grains were measured under the light microscope (×1000) and *P*/*E* ratios were calculated. For Scanning Electron Microscopy (SEM), the pollen grains were directly placed on aluminum stubs using double-sided adhesive tape and sputter-coated with gold a using a Hummer VII gold-coating apparatus. They were observed and photographed at a magnification of ×2000 and ×10,000 with a JEOL-6060 scanning electron microscope to determine their exine ornamentation. The general pollen terminology follows Punt et al. (2007).

3. Results

3.1. Anatomy of the root, stem and leaf

In cross-sections taken from the root, stem, leaf blade and petiole of *S. chrysophylla*, the following significant properties were observed below.

The pith rays in the root are composed of rarely 1 and often 2–24-rowed rectangular cells (Figs. 2–3). 2–8-layered collenchyma tissue is located at the corners of the stem while 2–4-layered chlorenchyma tissue is found between the corners (Fig. 4). The cortex consists of 6–12 layers of oval or rectangular parenchymatic cells. The phloem is surrounded by 2–5 layers of sclerenchymatic cells (Fig. 5). The leaf blade is dorsiventral and amphistomatic. The upper epidermis cells (25–55 × 20–48 μm) are larger than lower epidermis cells (13–22 × 7–12 μm). The mesophyll comprises 2–4-layered palisade and 1–3-layered spongy (Fig. 6). The midrib is adaxially flat and abaxially convex (Fig. 7). In the petiole, the adaxial surface is almost flat to concave and the abaxial surface is convex. There are two to three large vascular bundles in the center and two to four small subsidiary bundles in the petiolar wings (Figs. 8–9). The vascular tissues lie along a shallow arc and their arrangement is collateral. The xylem is surrounded by parenchyma.

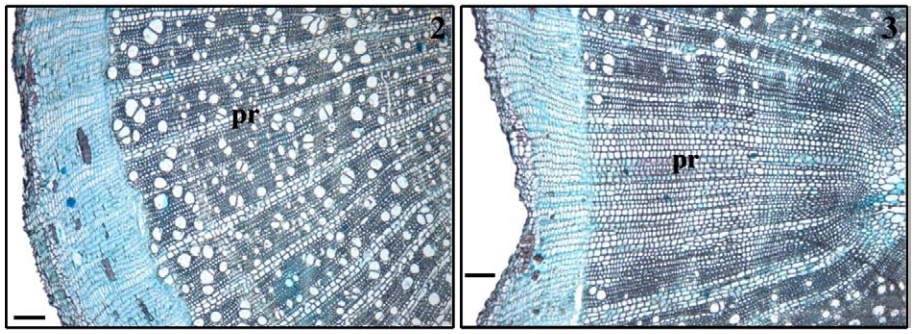

Fig. 2–3. Cross-sections of the root of *Salvia chrysophylla*. pr: pith rays. Bars = 100 μm.

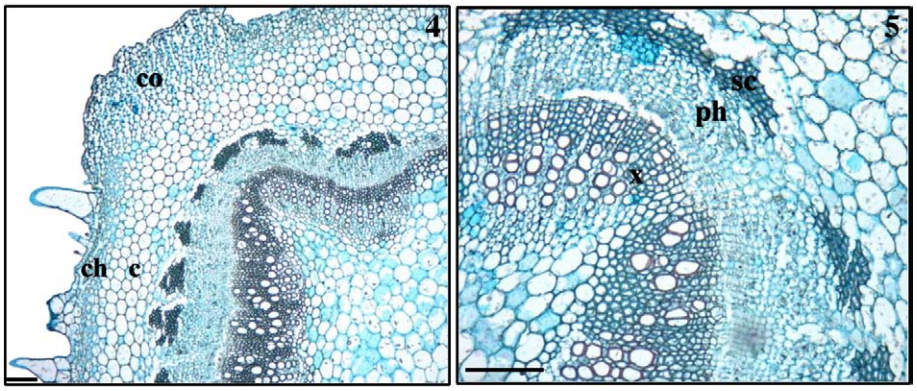

Fig. 4–5. Cross-sections of the stem of *Salvia chrysophylla*. c: cortex; ch: chlorenchyma; co: collenchyma; ph: phloem; pi: pith; sc: sclerenchyma tissue; x: xylem. Bars = 100 μm.

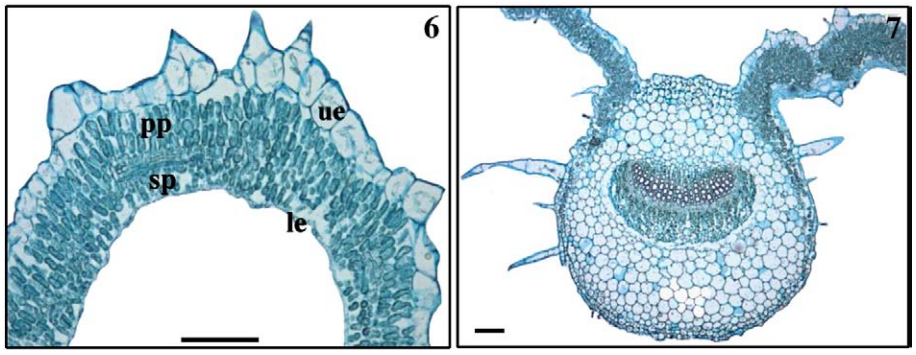

Fig. 6–7. Cross-section of the leaf blades of *Salvia chrysophylla*. le: lower epidermis; pp: palisade parenchyma; sp: spongy parenchyma; ue: upper epidermis. Bars = 100 μm.

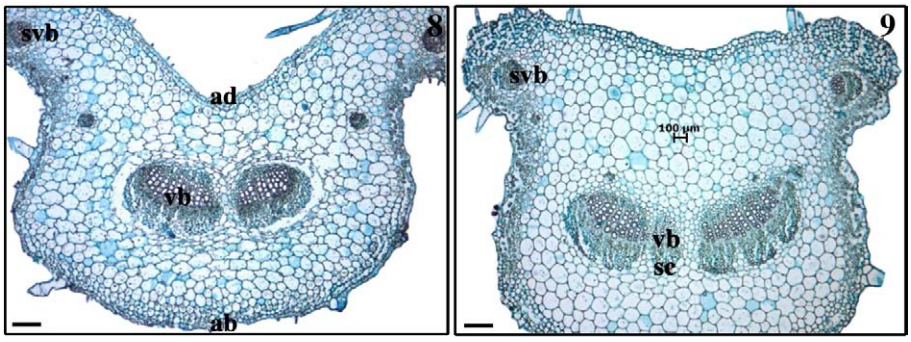

Fig. 8–9. Cross-section of the petiole of *Salvia chrysophylla*. ab: abaxial epidermis; ad: adaxial epidermis; sc: sclerenchyma tissue; svb: subsidiary vascular bundles, vb: vascular bundles. Bars = 100 μm.

No difference was observed in root, stem and leaf anatomy of the specimens collected from the two different regions.

3.2. Trichome morphology

3.2.1. Types of trichomes

Three different trichome types on the stems, inflorescence axes, leaf blades, petioles and calyces of *S. chrysophylla* were observed: peltate, capitate glandular and non-glandular trichomes (Figs. 10–27). Moreover, four subtypes of capitate glandular and three subtypes of non-glandular trichomes were recognized. Types of trichomes observed and their distribution are given in Table 1.

Type I is the typical peltate glandular trichome and consists of a basal epidermal cell, a very short monocellular stalk and a broad, round multicellular secretory head consisting of four or twelve cells (one or four central cells surrounded by four or eight peripheral cells) in a single shield (Figs. 10–13).

Type II is a capitate glandular trichome composed of a basal epidermal cell, unicellular to multicellular stalk of variable length (15–1000 μm), a neck cell (7–18 μm) and a large, cutinized, unicellular or bicellular secretory head (Figs. 14–22). These trichomes can be subdivided into four subtypes. *Subtype IIA*: A globose unicellular or bicellular head and a stalk of one to four cells (30–900 μm) (Figs. 14–16). A large percentage of these trichomes have one head cell. *Subtype IIB*: A cup-shaped unicellular head and one to five-celled stalk (35–1000 μm) (Figs. 17–19). *Subtype IIC*: A hemispherical unicellular head and a unicellular or bicellular stalk, rarely three-celled on the inflorescence axis (35–500 μm) (Figs. 20–21). *Subtype IID*: An oblong unicellular head and a short unicellular stalk (15–90 μm), sometimes bicellular stalk (up to 150 μm) on both surfaces of the leaves (Fig. 22).

Type III is a non-glandular trichome composed of one basal epidermal cell. It is unicellular to multicellular, uniseriate, unbranched (Figs. 23–27). They are quite variable in length (50–3000 μm). These trichomes might be subdivided into three subtypes. *Subtype IIIA*: Unicellular to multicellular (of up to seven cells) acicular trichomes in a single order (Figs. 23–25). Especially, unicellular trichomes are thick-walled and densely covered by micro-papillae. These trichomes vary in length between 50 and 1000 μm. Multicellular trichomes are curved or straight at the tip. *Subtype IIIB*: Multicellular (of up to thirteen cells), uniseriate flagelliform trichomes with the distal end of the terminal cells delicate and much elongated (Fig. 26). Their lengths are 1500–3000 μm. *Subtype IIIC*: Multicellular (of up to five to eight cells) trichomes with ridges and marked internodes (Fig. 27). These trichomes are between 500 and 1400 μm long.

3.2.2. Distributions of trichomes

Trichome distribution on the stem, inflorescence axis, leaf blade, petiole and calyx of *S. chrysophylla* is shown in Table 1.

3.3. Palynological characteristics

S. chrysophylla has hexacolpate, radially symmetrical and isopolar pollen grains. Polar axis (*P*) is 46.11±2.2 μm and

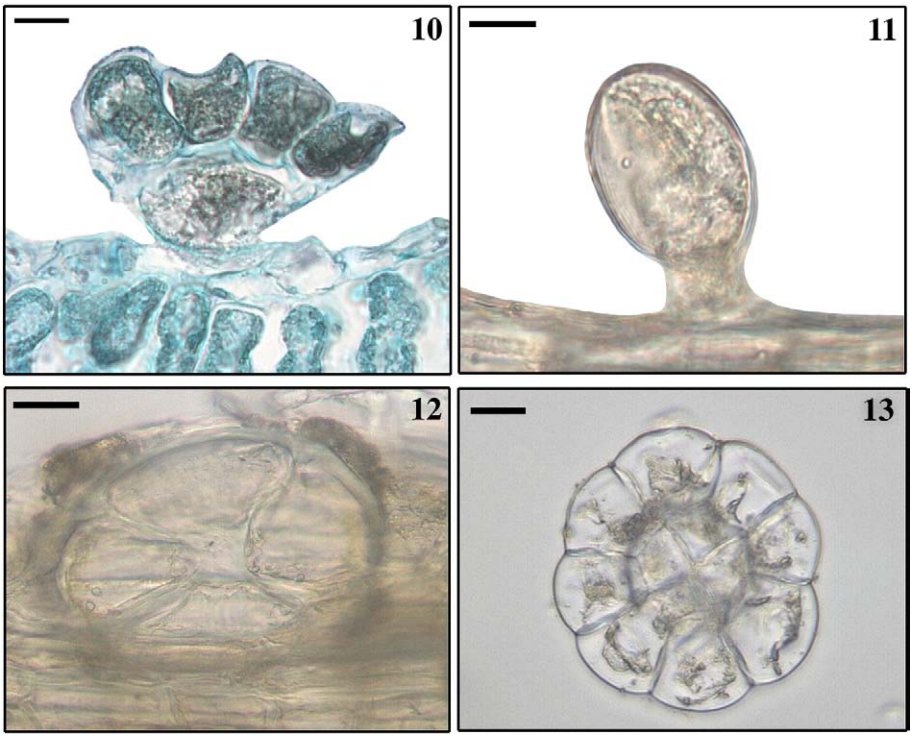

Fig. 10–13. Light micrographs of Type A peltate glandular trichomes of *Salvia chrysophylla* on the: (10) leaf abaxial surface, (11) stem, (12) inflorescence axis, and (13) calyx. Bars=10 μm.

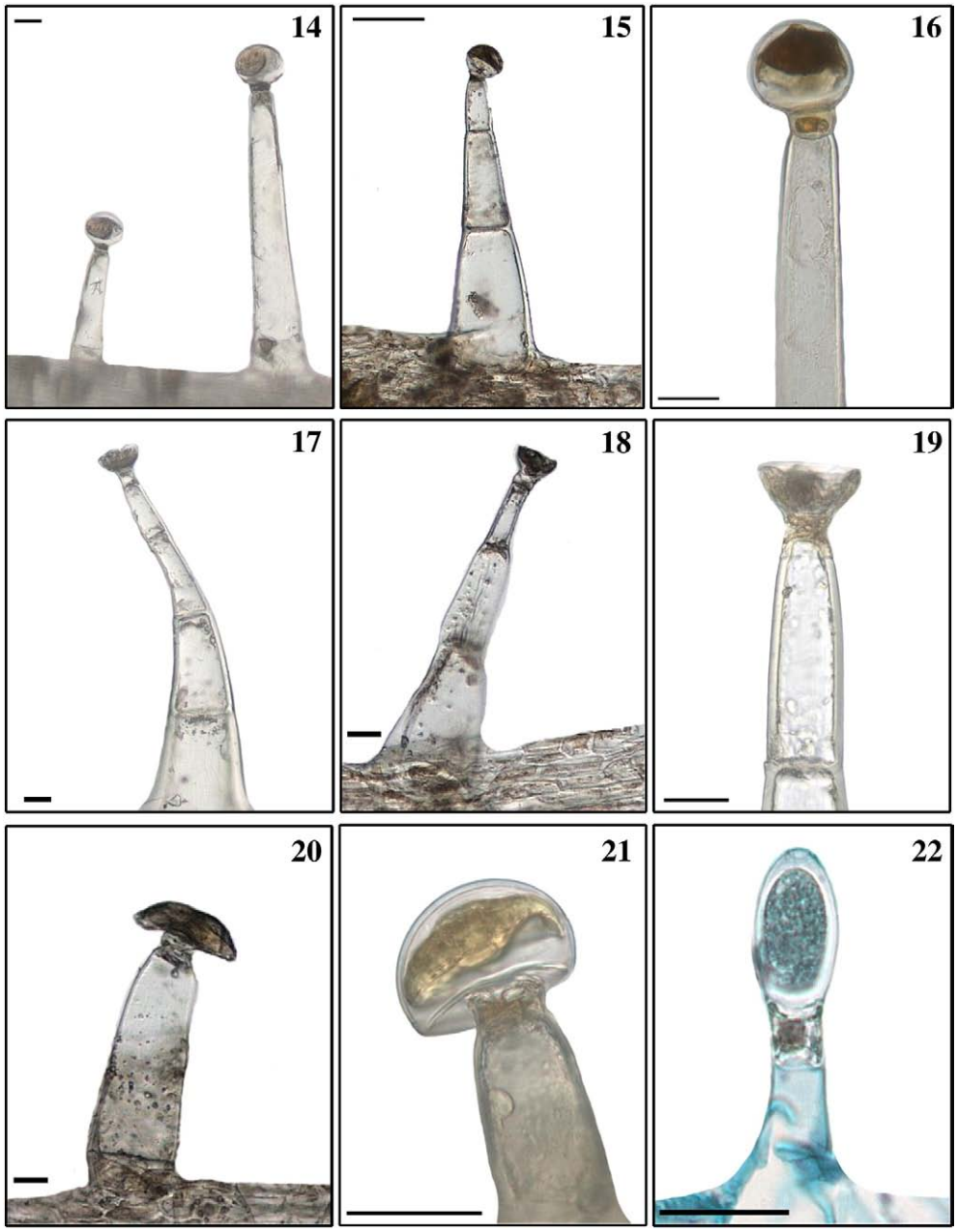

Fig. 14–22. Light micrographs of Type B capitate glandular trichomes of *Salvia chrysophylla*: (14) Type B1 on the stem, (15–16) Type B1 on the inflorescence axis, (17–19) Type B2 on the inflorescence axis, (20–21) Type B3 on the calyx, and (22) Type B4 capitate glandular of the leaf adaxial surface. Bars = 30 μm.

equatorial axis (*E*) is 50.46±2.6 μm. The ratio of *P*/*E* is 0.91. The pollen grains are oblate-spheroidal in shape (Fig. 28). The exine thickness is 0.78±0.2 μm and the intine thickness is 0.62±0.15 μm. Colpus length is 37.9±1.8 μm and colpus width is 4.16±0.9 μm. The exine ornamentation is bireticulate-perforate (Fig. 29). Lumina of the primary reticulum are angular and lumina number of the secondary reticulum is 5 to 8.

4. Discussion

The present study sought to provide useful information on the anatomy, palynology and trichome morphology of *S. chrysophylla*. This is the first report on the examined characteristics of the species.

The family Lamiaceae has the root composed of 2–12 or more rowed pith rays, parenchymatous mesophyll and midrib surrounded by collenchymatous cells. In many genera and species of the Lamiaceae, the stems are quadrangular and consist of well-defined groups of collenchyma occupying a broad area of the corners (Metcalfe and Chalk, 1972). They also pointed out that the structure of the vascular bundles of the petiole is a taxonomically significant character. Anatomical properties of *S. chrysophylla* with *Salvia* species previously examined (Kahraman et al., 2009; Kahraman et al., 2010-a,b; Kahraman and Dogan, in press) are compared in Table 2. Our

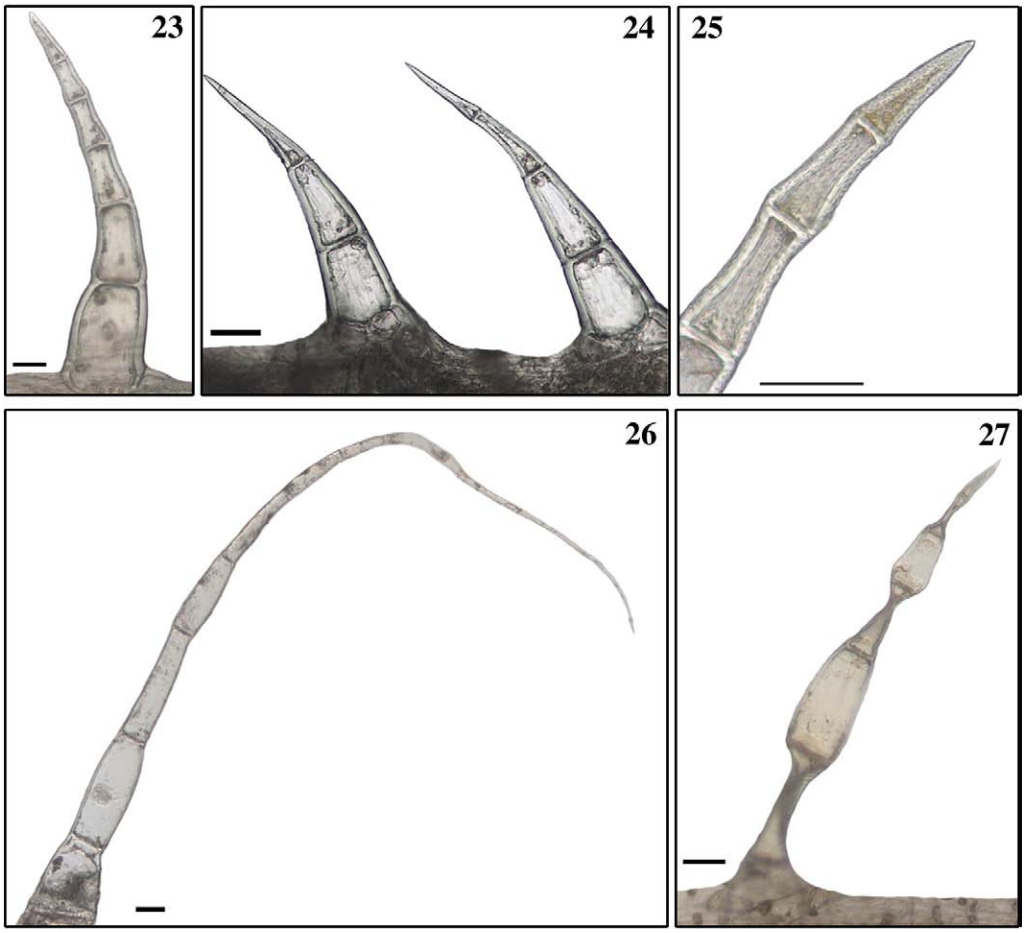

Fig. 23–27. Light micrographs of Type C non-glandular trichomes of *Salvia chrysophylla*: (23) Type C1 on the stem, (24) Type C1 on the calyx, (25) Type C1 on the petiole, (26) Type C2 on the inflorescence axis, and (27) Type C3 on the stem. Bars = 50 μm.

findings agree on those of previous studies in the Lamiaceae. Since the number of rows of pith rays significantly varies among *Salvia* species, this feature can be used to distinguish the species. Moreover, sizes of upper and lower epidermis cells in the lamina and presence of sclerenchymatic tissue outside of the phloem and xylem in the petiole were determined to be taxonomically useful characteristics. From the root anatomy point of view, *S. chrysophylla* has pith rays composed of more rows of

Table 1
Distribution of peltate, capitate glandular and non-glandular trichomes on different parts of *Salvia chrysophylla*.

Plant material	Peltate	Capitate														Non-glandular		
	Type I	Type IIA (Stalk 1–4 cells)				Type IIB (Stalk 1–5 cells)					Type IIC (Stalk 1–3 cells)			Type IID (Stalk 1 or 2 cells)		Type IIIA	Type IIIB	Type IIIC
		1	2	3	4	1	2	3	4	5	1	2	3	1	2			
Stem	+	+	+	−	−	++	+	+	−	−	+	−		−	−	+++	+	+
Inflorescence axis	++	+++	++	+++	+	+++	+++	+++	++	+	+++	+	+	−	−	+	++	+
Adaxial leaf surface	−	+	−	−	−	+	−	−	−	−	−	−	−	+++	+	+++	−	−
Abaxial leaf surface	+++	+	−	−	−	+	−	−	−	−	−	−	−	+++	+	+++	−	−
Petiole	+	−	−	−	−	++	+	+	+	−	−	−	−	++	−	+++	+	−
Calyx	+++	+++	++	+	−	+++	++	+	−	−	++	+	−	−	−	++	−	−

Type I. Peltate trichomes. Type II. Capitate trichomes, subtype IIA: Head globose unicellular or bicellular, stalk 1–4-celled; subtype IIB: Head cup-shaped unicellular, stalk 1–5-celled; subtype IIC: Head hemispherical unicellular, stalk 1–3-celled; subtype IID: Head oblong unicellular, stalk 1–2-celled. Type III. Non-glandular trichomes, subtype IIIA: Unicellular to multicellular acicular trichomes; subtype IIIB: Multicellular flagelliform trichomes; subtype IIIC: Multicellular trichomes with ridges and marked internodes. Symbols shows (−) absence of trichomes, (+) a few trichomes, (++, +++) increasing presence of trichomes.

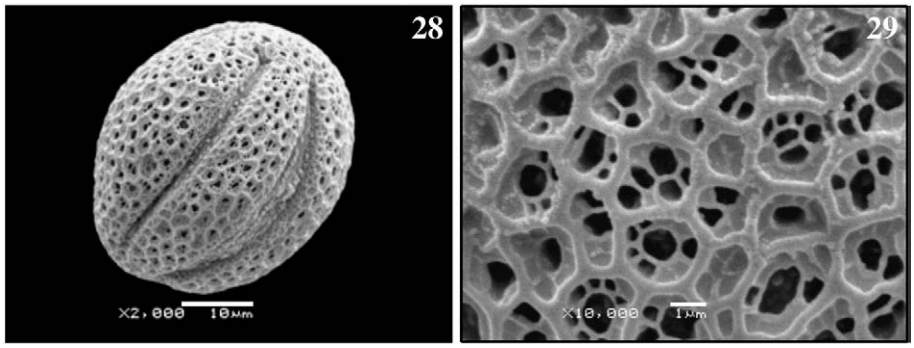

Fig. 28–29. SEM micrographs of the pollen of *Salvia chrysophylla*: (28) General appearance, (29) Exine ornamentation in detail.

cells than the other species previously studied. *S. chrysophylla* and *S. indica* are dorsiventral leaf blades while the other species are isobilateral ones. According to the petiole anatomy, the number of vascular bundles is similar in *S. chrysophylla* and *S. palaestina*, but it significantly differs in the others. Moreover, in *S. limbata* vascular bundles are ensheathed by a sclerenchymatic bundle sheat whereas in *S. chrysophylla*, *S. palaestina* and *S. macrochlamys* only the phloem is surrounded by the sclereanchymatic tissue. However, *S. ballsiana* does not include scleranchyma cells encircling vascular bundles.

Like other members of the Lamiaceae, *S. chrysophylla* carries both peltate and capitate glandular trichomes, as well as non-glandular ones. Whereas the inflorescence axis and calyx possess numerous glandular trichomes, the stem, leaf blade and petiole bear many non-glandular trichomes. Peltate trichomes of the Lamiaceae often comprise a broad head of several secretory cells (up to 16), a wide short stalk and a basal epidermal cell (Hallahan, 2000). *S. chamelaeagnea* (Kamatou et al., 2006) have the peltate trichomes, with up to sixteen head cells. The present study showed that *S. chrysophylla* has the peltate trichomes (Type I) composed of a four or twelve-celled head in a single circle which is agreement with previous studies (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Hallahan, 2000; Kamatou et al., 2006, 2007). However, in other species of the same family, such as *Origanum* species (Bosabalidis and Tseko, 1984) and *Satureja thymbra* (Bosabalidis, 1990), a higher number of head cells are arranged in two concentric circles.

Capitate glandular trichomes constitute a significant taxonomic character of the Lamiaceae and form part of the floral specialized properties for pollination (Navarro and El Oualidi, 2000). Like the peltate trichomes, the capitate ones are very common in many species of *Salvia* (Corsi and Bottega, 1999; Siebert, 2004). However, they greatly vary in structure and size. According to present observations, the capitates trichomes of *S. chrysophylla* showed some variation in morphology, and thus they were divided into four subtypes: IIA, B, C and D. Three different subtypes (IIA, B and D) trichomes observed in *S. chrysophylla* were described also in *S. officinalis* (Corsi and Bottega, 1999). Subtype IIIC was not found in *S. officinalis*. Subtypes IIA and B in *S. chrysophylla* were observed in *S. aurea* (Serrato-Valenti et al., 1997) and *S. recognita* (Ozkan, 2008).

Unicellular to multicellular, uniseriate and unbranched non-glandular trichomes are divided into three more subtypes. The non-glandular trichomes of *S. chrysophylla* displayed some variation in morphology. Therefore they were divided into three subtypes: IIIA, B and C. Subtype IIIA are very common in the *Salvia* species studied previously (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Siebert, 2004; Kamatou et al., 2007). Subtype IIIB was described in *S. officinalis* (Corsi and Bottega, 1999). In *Teucrium fragile*, Navarro and El Oualidi (2000) observed large, thin-walled, multicellular trichomes with marked internodes, which were quite similar to those classed as non-glandular subtype IIIC in *S. chrysophylla*.

Erdtman (1945) classified the family Lamiaceae into two subfamilies on the basis of palynological characteristics. Cantino et al. (1992) revised the classification of all genera in the Lamiaceae and placed it within the subfamily Nepetoideae since the genus *Salvia* has hexacolpate pollen grains. Pollen morphology has been proved to be useful in systematics of the Lamiaceae (Abu-Asab and Cantino, 1994). Comparison of palynological features of *S. chrysophylla* with *Salvia* species previously studied (Hamzaoglu et al., 2005; Kahraman et al., 2009, Kahraman et al., 2010-a,b; Kahraman and Dogan, in press) is presented in Table 2. *S. limbata* has the largest pollen grains in terms of their dimensions. The basic shape of the pollen in most species (*S. chrysophylla*, *S. limbata*, *S. macrochlamys*, *S. anatolica* and *S. bracteata*) is oblate-spheroidal, but prolate-spheroidal and suboblate are observed in *S. palaestina* and *S. indica*, respectively. The pollen of *S. ballsiana* is suboblate to oblate-spheroidal. Based on pollen exine ornamentation, bireticulte is the most common type in *Salvia* species examined. The shapes of lumina of the primary reticulum are angular in *S. chrysophylla*, *S. palaestina* and *S. limbata*, and extended angular in *S. macrochlamys* and *S. indica*. Lumina number of the secondary reticulum of *S. chrysophylla* is 5 to 8 while that of *S. palaestina* and *S. limbata* are is more than 10.

Acknowledgement

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Table 2
Comparison of anatomical and palynological characteristics of *S. chrysophylla* with *Salvia* species studied previously.

	S. chrysophylla	*S. palaestina* (Kahraman and Dogan, in press)	*S. limbata* (Kahraman and Dogan, in press)	*S. macrochlamys* (Kahraman et al., in press-a,b)	*S. ballsiana* (Kahraman et al., in press-a,b)	*S. indica* (Kahraman et al., 2009)	*S. anatolica* (Hamzaoglu et al., 2005)	*S. bracteata* (Hamzaoglu et al., 2005)
The root anatomy								
Number of pith ray rows	(1–)2–24	1–8(–10)	2–6	1–4	1–3(–4)			
The blade anatomy								
Mesophyll structure	Dorsiventral	Isobilateral	Isobilateral	Isobilateral	Isobilateral	Dorsiventral		
Size of upper and lower epidermis cells	Larger upper epidermis	Equal to each other	Larger upper epidermis	Equal to each other	Mainly equal to each other	Larger upper epidermis		
The petiole anatomy								
Number of vascular bundles	2–3 in the center and 2–4 in the petiolar wings	2–3 in the center and 4 in the petiolar wings	4 in the center and 8 in the petiolar wings	1 in the center and 2 in the petiolar wings	1 in the center and 4–6 in the petiolar wings			
Presence of sclerenchymatic tissue outside of the xylem and phloem	Only the phloem	Only the phloem	Both	Only the phloem	Absent			
Pollen morphology								
Size (μm)	46.11±2.2× 50.46±2.6 μm	47.72±3.85× 45.40±3.73 μm	52.41±4.69× 59.82±4.89 μm	47.77±3.63× 52.87±5.23 μm	47.39±5.28× 54.39 ± 5.33	44.98±4.22 52.24±4.41 μm	47.5±2.75× 48.5±1.70 μm	47.6±0.42× 48.2±0.39 μm
Shape	Oblate-spheroidal	Prolate-spheroidal	Oblate-spheroidal	Oblate-spheroidal	Suboblate to oblate-spheroidal	Suboblate	Oblate-spheroidal	Oblate-spheroidal
Exine sculpturing	Bireticulate-perforate	Bireticulate-perforate	Bireticulate-perforate	Bireticulate-perforate	Reticulate	Bireticulate-perforate	Eurireticulate	Suprareticulate
Shape of lumina of the primary reticulum	Angular	Angular	Angular	Extended-angular		Extended-angular		
Lumina number of the secondary reticulum	5 to 8	More than 10	More than 10					

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