

TRICHOMES ON *SALVIA POMIFERA* (LAMIACEAE) IN TURKEY

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Abstract

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Morphology and localization of glandular and non-glandular trichomes on vegetative and reproductive organs of *Salvia pomifera* were investigated by light and scanning electron microscopy. This species is endemic to the east Mediterranean region. Five distinct types of glandular trichomes (one peltate and four capitate) were determined on the organs of this species. The peltate trichomes were characterized by a short stalk cell, a basal cell and a large spherical two to four-celled head. The capitate trichomes consisted of one to four-celled stalk, one-celled neck and one to two-celled head. On the leaves and calyx peltate trichomes were abundant, while on the stem and petiole they were rare. The capitate glandular and non-glandular trichomes were widely observed on vegetative and reproductive organs of *S. pomifera*.

Keywords: trichomes, morphology, localization.

INTRODUCTION

Glandular trichomes are found on the organs of many *Salvia* species. These trichomes are the main secretory parts of the plants, and their structures can vary widely among species (WERKER et al., 1985 a, b; SERRATO-VALENTI et al., 1997). Two main types of glandular trichomes – capitate and peltate – are known on *Salvia* species. They can be distinguished by head size and stalk length. The capitate trichomes are very variable in stalk length, head shape and can be subdivided into I–IV types (WERKER et al., 1985 a; CORSI & BOTTEGA, 1999). Generally, they consist of one to two-celled head supported by one to four stalk cells. The peltate trichomes are composed of a short stalk, a basal cell and a large two to four-celled head. In the Lamiaceae family, the morphology, localization and frequency of glandular trichomes are used as distinguishing taxonomic characters (ASCENSAO et al., 1995).

The essential oil (secretion material) is secreted by glandular trichomes of *Salvia* species. *S. pomifera*, *S. officinalis* L. and *S. fruticosa* Miller are important essential oil sources of the *Salvia* genus. Generally, the essential oil is composed of hydrocarbons, flavonoids, diterpenoids and triterpenoids (BAŞER et al., 1993; TOPÇU et al., 1994; SERRATO-VALENTI et al., 1997; KAROUSOU et al., 1998; PITAROKILI et al., 2002). Therefore, it is significant for pesticide, pharmaceutical, flavouring, perfumery, fragrance and cosmetic industries (PITAROKILI et al., 1999; ZEYBEK & ZEYBEK, 2002).

S. pomifera is the most popular medicinal wild plant species. The leaves of *S. pomifera* have a strong odour and flavour, resembling lavender and common sage. They are used as commercial food flavouring and to make tea both in Greece and Turkey (PITAROKILI et al., 1999). The tea is regarded as a good general tonic also beneficial in cases of tonsillitis, sore throats, bronchitis and respiratory infections. It has sedative effect on stomach and nervous system. The leaves are also used

internally in the treatment of menstrual problems, rheumatic pains, influenza, coughs, nervous tension and depression (BOWN, 1995). This species bears large green galls produced by wasps on the stem. These galls are made into a kind of conserve or sweetmeat by crystallizing them in sugar, and the Greeks regard this as a great delicacy. *S. pomifera* is grown in parks and gardens as an ornamental plant in some areas of Turkey and is known as native only in western Anatolia, İzmir and Aydın provinces (DAVIS, 1982).

Morphologically, *S. pomifera* resembles both *S. fruticosa* and *S. officinalis*. As it is distributed together with *S. fruticosa* in some areas of western Anatolia and with *S. officinalis* in some areas of Greece and Italy, these three species are often confused by researchers (DAVIS, 1982; KAROUSOU & KOKKINI, 1997; CORSI & BOTTEGA, 1999). This study was carried out to clarify the confusion between these three species morphologically.

The purpose of this research was to determine the morphology and localization of glandular and non-glandular trichomes on vegetative and reproductive organs of *S. pomifera*. It was also aimed to distinguish *S. pomifera* from other *Salvia* species (particularly *S. officinalis* and *S. fruticosa*) by applying the morphology and localization properties of trichomes.

MATERIALS AND METHODS

Taxonomic description of this species was made according to DAVIS (1982). The specimens of *S. pomifera* were collected from natural populations in İzmir and Aydın in 2005 on the following localities, altitudes and dates: C1 İzmir: Samsun Mountain, rocky areas, altitude 1–50 m, leg. et det. N. Kandemir, No. 430, on 14 May 2005. C1 Aydın: 5 km north of Kuşadası, scrub and rocky areas, altitude 10 m, leg. et det. N. Kandemir, No. 431, on 20 May 2005. The laboratory investigations were performed in 2006.

Scanning electron microscopy (SEM). To investigate organs, both scanning electron and light microscopy were applied. The small pieces of plant parts (stem, petiole, leaf and flower) were fixed for 24 h in FAA (formalin 5: acetic acid 5: 50 % ethyl alcohol 90) at room temperature and subsequently dehydrated in a graded ethanol series (SERRATO-VALENTI et al., 1997). Then, these plant parts were dried to a critical point with liquid CO₂ and coated with gold. They were viewed by scanning electron microscope at an acceleration voltage of 20 kV and photographed at different magnification.

Light microscopy (LM). Fresh plant parts were

fixed in a 70 % alcohol solution. Both alcohol and fresh materials were prepared as follows: plant parts (stem, petiole, leaf and flower) were fixed with FAA for 24 h, dehydrated in a graded ethanol series. Then, longitudinal and cross-sections of these materials were taken using a microtome equipped with a glass knife. Longitudinal and cross-sections were investigated by LM. The whole leaf and calyx were investigated by using stereomicroscope with illumination from below. The number of glandular and non-glandular trichomes per area unit of the organ surface was estimated by counting trichomes of both types directly on SEM images at a magnification of ×200. The areas were chosen randomly and multiple counts of organ surfaces were made. The numbers of basal, stalk and head cells of glandular and non-glandular trichomes, morphology and localization were determined. Glandular and non-glandular trichomes were classified according to WERKER et al. (1985 a).

RESULTS

Glandular trichomes

Both peltate and capitate glandular trichomes were found on the vegetative and reproductive organs of *S. pomifera*.

Morphology and localization of peltate trichomes

Peltate trichomes were composed of a short stalk cell, a basal cell and a large head with two to four secretory cells, seven to eight peripheral cells ranged in a circle around it. The anticlinal wall of the stalk cell was cutinized. The basal and stalk cells of the peltate trichomes were similar to each other. The cells of the secretory head had thick cuticle. On calyx and leaves, these trichomes were lifted by strong distension of the basal cell (Fig. 1–3). The secretion materials of the peltate trichomes were accumulated between the outer wall of the head cells and the cuticle. The secretion material exuded after the cuticle had ruptured. Cuticular rupture of peltate trichomes was observed by SEM (Fig. 2, 4).

Peltate trichomes were seen on the vegetative and reproductive organs, mainly on leaves and calyx. They were particularly abundant in grooves of the calyx and the upper and lower surfaces of leaves (Fig. 1–9). However, these trichomes were rare on the veins of the leaves and the calyx. Though peltate trichomes on the calyx were generally with short stalk and four secretory head cells, they were mainly with short stalk and two to four secretory head cells on the leaves. Peltate trichomes with two and four secretory head cells were rare on the stem and the petiole as well.

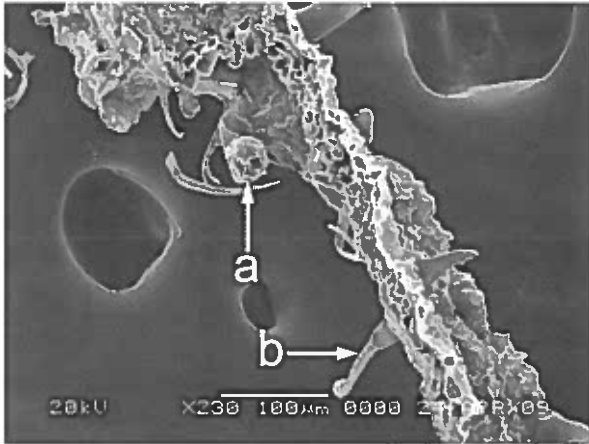


Fig. 1. Glandular peltate (a) and type II capitate (b) trichomes in the groove of calyx

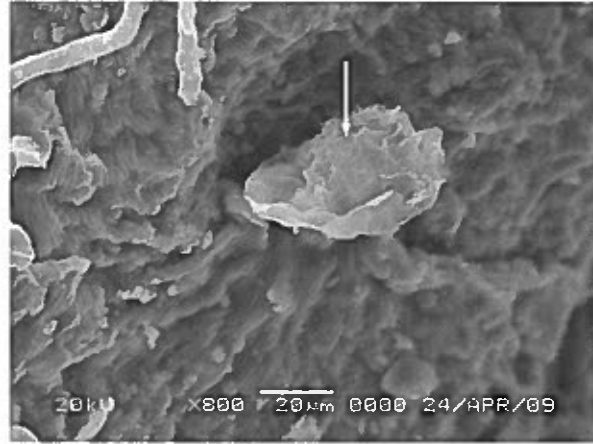


Fig. 4. Sunken glandular peltate trichome in the groove of calyx



Fig. 2. Glandular peltate trichome in the groove of calyx



Fig. 5. Glandular peltate trichomes on the upper surface of leaf

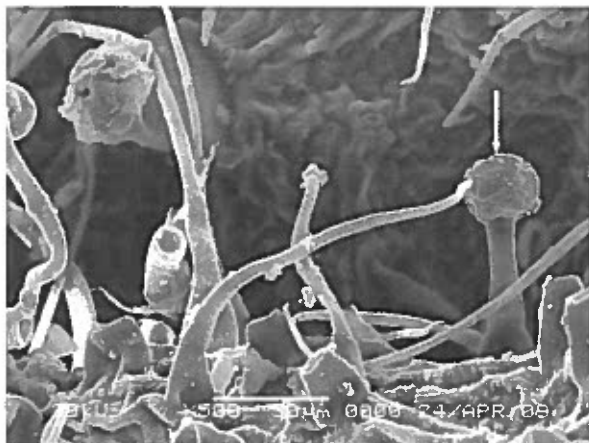


Fig. 3. Glandular peltate trichome erected because of strong distension of basal cell

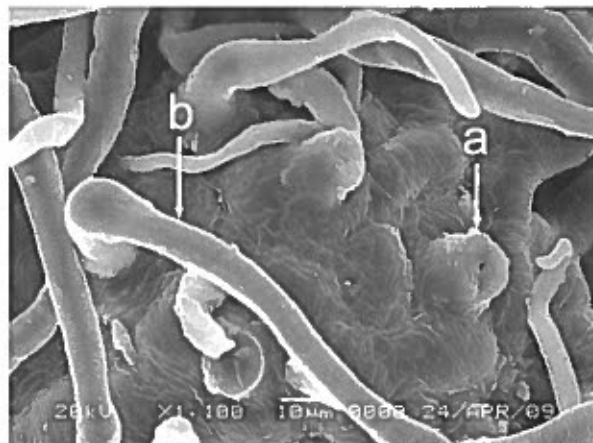


Fig. 6. Juvenile glandular peltate (a) and non-glandular (b) trichomes on the upper surface of leaf



Fig. 7. Glandular peltate (a) and non-glandular (b) trichomes on the upper surface of leaf

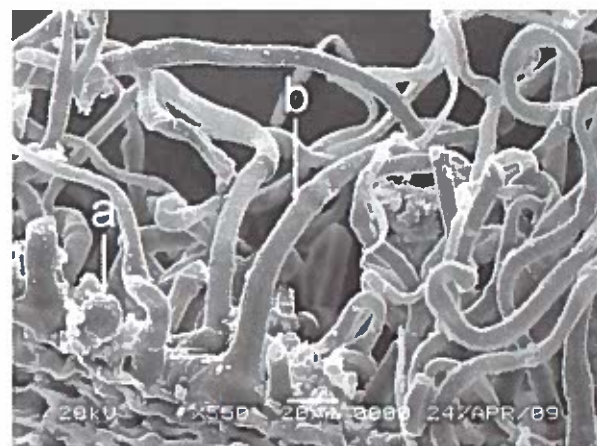


Fig. 9. Glandular peltate (a) and non-glandular (b) trichomes on the lower surface of leaf

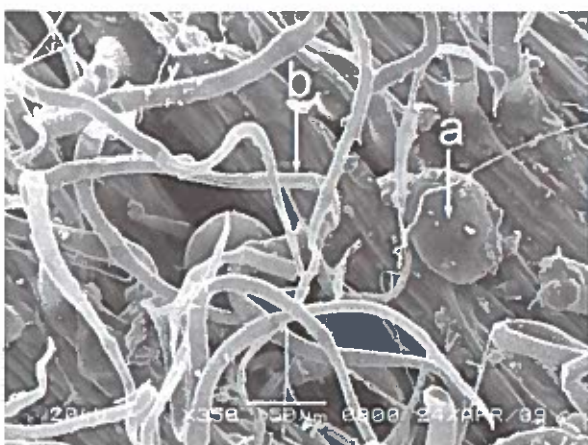


Fig. 8. Glandular peltate with two-celled head (a) and non-glandular (b) trichomes on the lower surface of leaf



Fig. 10. Glandular type I (a), III (b), IV (c) capitate and non-glandular (d) trichomes on the upper surface of leaf

Morphology and localization of capitate trichomes

Capitate trichomes were found on four types of vegetative and reproductive organs of *S. pomifera*. They generally consisted of one to four-celled stalk, one-celled neck and one to two-celled head.

Type I. It consisted of one-celled short stalk, one-celled basal embedded between epidermis cells and a large two-celled secretory head (Fig. 10). The cuticle was thin. The secretion material was exuded through the cuticle when the cuticle had been ruptured.

Type II. The trichomes had a large basal cell, two-stalk cells, a short neck cell and a globose one-celled secretory head (Fig. 1, 11). The secretion material came out from pores on the cuticle. It was observed by SEM.

Type III. The trichomes were long. They had one to four stalk cells, a narrow neck cell, cup-shaped one-celled secretory head (Fig. 10, 12, 13). The secretion

material was collected on the cup-shaped head (Fig. 13). The rupture of cup-shaped head was observed by SEM.

Type IV. The trichomes were long and capitate. They consisted of a long slender stalk (3 to 4-celled), one-celled neck, and wide and cutinized one-celled head (Fig. 10, 14).

Capitate trichomes of type I were rare on the teeth and in the grooves of calyx, whereas they were abundant on the stem, the petiole and especially in grooves on both upper and lower surfaces of leaves (Fig. 9, 10). Capitate trichomes of type II were rarely observed on the petioles, veins of the calyx and the leaves, the corolla lips. Trichomes of type III were observed on the upper and lower surfaces of leaves (particularly on the veins) and on the surface of calyx (Fig. 10, 12, 13). Trichomes of type IV were found only on the upper surface of leaves (Fig. 10, 14).

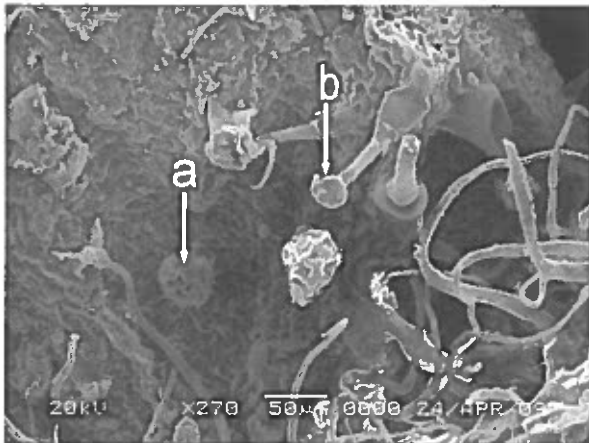


Fig. 11. Glandular peltate (a) and type II capitate (b) trichomes on the petiole



Fig. 13. Glandular type III capitate trichome with cup-shaped head on the lower surface of leaf



Fig. 12. Glandular type III capitate trichome on the ribs of calyx



Fig. 14. Glandular type IV capitate (a) and non-glandular (b) trichomes on the upper surface of leaf

Morphology and localization of non-glandular trichomes

Two types of non-glandular trichomes on the vegetative and reproductive organs of *S. pomifera* were distinguished: multicellular-uniseriate and unicellular papillae (Fig. 7–10, 14–20). The multicellular-uniseriate non-glandular trichomes had two to seven-celled stalks and a swollen basal cell, unbranched and acute apex. The density of multicellular-uniseriate non-glandular trichomes varied on different organs. These trichomes were more scattered on the calyx and the upper surface of leaves, whereas they were denser on stem, petiole, the lower surface (particularly on the veins and the margins) and side of the leaves. They were bent and contorted. The calyx was covered with many long multicellular-uniseriate non-glandular trichomes. The trichomes were abundant on the veins

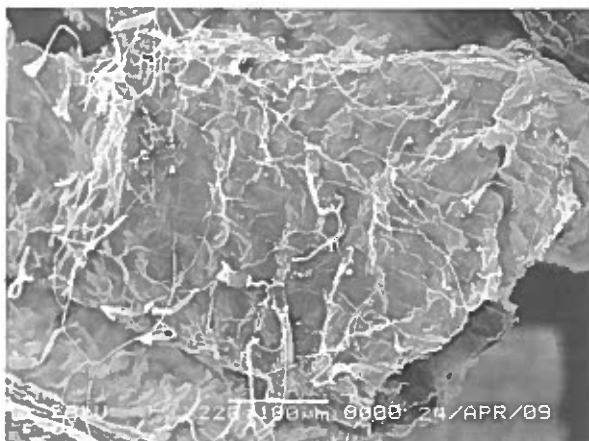


Fig. 15. Non-glandular trichomes on the surface of corolla

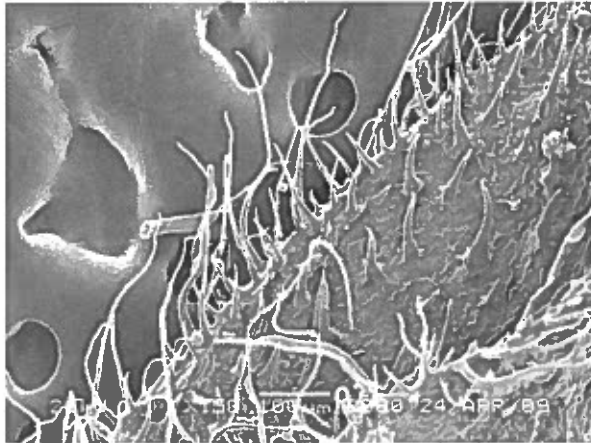


Fig. 16. Non-glandular trichomes on the petiole



Fig. 19. Non-glandular trichomes on the lower surface of leaf

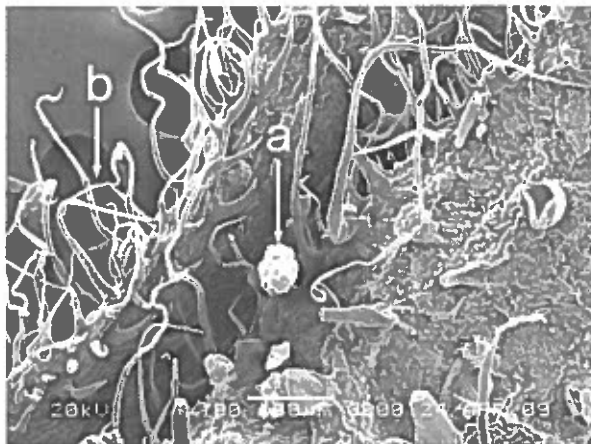


Fig. 17. Glandular peltate (a) and non-glandular (b) trichomes on the petiole

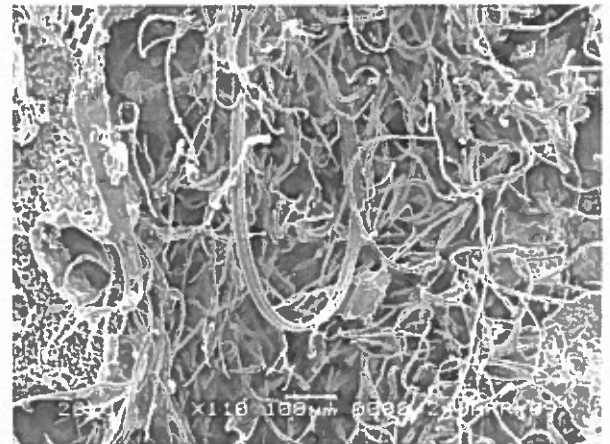


Fig. 20. Non-glandular trichomes on the margin and veins of leaf



Fig. 18. Non-glandular trichomes on the upper surface of leaf

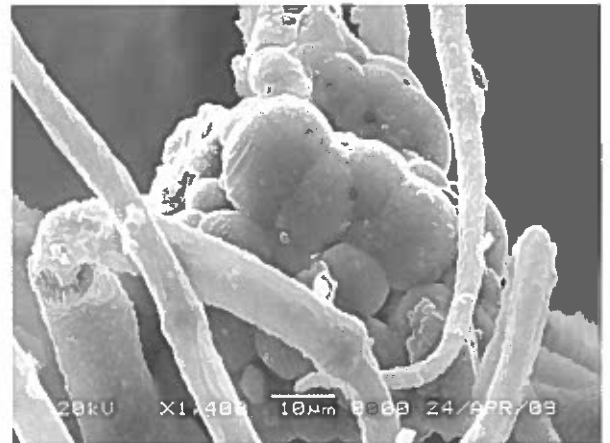


Fig. 21. Crystals on the upper surface of leaf



Fig. 22. Crystals on the upper surface of leaf



Fig. 23. Crystals on the lower surface of leaf

of calyx, but they were rare in the grooves of calyx. The multicellular-uniseriate non-glandular trichomes were rare on the upper and lower lips of corolla, the anthers. The unicellular papillae consisted of a swollen basal epidermal cell and one to three-celled stalks. The apices of these trichomes were acute. They were observed only on the lower surface of leaves. However, the glandular and non-glandular trichomes were not seen on the filament, pistil and at the base of corolla of *S. pomifera*. In SEM investigation, the crystals were observed only on the lower and upper surfaces of leaves (Fig. 21–23).

DISCUSSION

The species of the *Lamiaceae* family are characterized by the presence of glandular trichomes. Since the morphology and localization of these trichomes have taxonomic value, they have been examined by many researchers (WERKER et al., 1985 a; b; KANDEMIR, 2003; ASCENSAO et al., 1995; CORSI & BOTTEGA, 1999; SERRATO-VALENTI et al., 1997; BISIO et al., 1999).

According to our investigations, *S. pomifera* has one type of peltate trichomes, in which broad secretory head is composed of two to four central cells and seven to eight peripheral cells. Peltate trichomes on the organs of *Salvia* species generally include a large secretory four or sometimes two-celled head and seven to twelve peripheral cells, whereas in the *Lamiaceae* family they generally consist of a large secretory four-celled head and six to fourteen peripheral cells (WERKER et al., 1985 a; WERKER, 1993). It has been ascertained for other species such as *Salvia officinalis*, *S. fruticosa* and *S. blepharophylla* (WERKER et al., 1985 a; CORSI &

BOTTEGA, 1999; BISIO et al., 1999). Our findings (cell number of peltate trichomes) are in agreement with the results mentioned above, whereas in *S. aurea*, the number of head cells varies from six to eight (SERRATO-VALENTI et al., 1997).

On *S. pomifera* peltate trichomes are found on vegetative organs, particularly on leaves, whereas on reproductive organs they prevail on calyx. Peltate trichomes are observed abundantly in the grooves of leaves and calyx, but they are rare on stem, petiole, the veins of leaves and calyx. These trichomes were observed abundantly on the leaves and calyx of *S. officinalis* by CORSI & BOTTEGA (1999) and the leaves of *S. aurea* by SERRATO-VALENTI et al. (1997).

On leaves and calyx, peltate trichomes are elevated by a strong distension of a basal cell. It is not clear whether distension of the basal cell is due to hormonal factors, osmosis or both. But CORSI & BOTTEGA (1999) have supposed that distension in the basal cell may occur because of high potassium concentrations. We assume that this phenomenon in *S. pomifera* could be caused by high potassium concentrations in the basal cell and strong distension of the basal cell, as proposed by CORSI & BOTTEGA (1999).

In SEM analysis four different types of capitate trichomes (I–IV) were observed on vegetative and reproductive organs of *S. pomifera*. As the capitate trichomes carry different secretion materials, they are abundant on the vegetative organs of plants. Hence, they are responsible for mechanical (type I, II and IV) and chemical (type II) defences (SERRATO-VALENTI et al., 1997; CORSI & BOTTEGA, 1999). In *S. pomifera* capitate trichomes are observed on stem, petiole, the veins of leaves and calyx, in grooves on the upper and lower surfaces of leaves and the lips of corolla. The

trichomes of type I are widespread on stem, petiole, in grooves on both upper and lower surfaces of leaves. They are rarely observed on the upper and lower lips of corolla. The trichomes of type II are seldom observed on petiole, calyx ribs, the veins of leaves and corolla lips. The trichomes of type III are detected on the veins of leaves, the upper and lower surfaces of leaves and calyx. The trichomes of type IV are identified only on the upper surface of leaves of *S. pomifera*. Capitate trichomes of types I, II and III have been found widely in many *Salvia* species (especially *S. officinalis* and *S. fruticosa*) (SERRATO-VALENTI et al., 1997; WERKER et al., 1985 a; b; BISIO et al., 1999), whereas that of type IV has been noted on organs of *S. officinalis* by CORSI & BOTTEGA (1999). However, capitate trichome of the same type is not observed on the organs of *S. fruticosa* (WERKER et al., 1985 a). Therefore, *S. pomifera* is easily distinguished from *S. fruticosa* morphologically. On the other hand, the morphologic properties of the type IV capitate trichomes of *S. pomifera* and *S. officinalis* are quite similar. The type IV capitate trichomes of both species consist of a long slender stalk, a celled neck, wide and cutinized one-celled head. The type IV capitate trichomes are observed only on the veins of the calyx of *S. officinalis* and on the upper surface of leaves of *S. pomifera*. Therefore, *S. pomifera* and *S. officinalis* can be distinguished from each other by the localization of the type IV capitate trichomes. We claim that the presence, morphology and localization properties of the type IV capitate trichomes can be used as a taxonomic character to distinguish *Salvia* species.

According to our investigations, there are two types of non-glandular trichomes of *S. pomifera*: multicellular-uniseriate and unicellular papillae. These trichomes have a swollen basal cell, one to three or two to seven-celled stalk and acute apex. They are abundant on the vegetative and reproductive organs of the species. In addition, the glandular and non-glandular trichomes have not been seen on the filament, the pistil and at the base of corolla of *S. pomifera*. The crystals have been observed only on the lower and upper surface of *S. pomifera* leaves. These structures have been found on the leaves of *S. blepharophylla* by BISIO et al. (1999).

In conclusion, the glandular and non-glandular trichomes on the vegetative and reproductive organs of *S. pomifera* are similar to the glandular and non-glandular trichomes of other *Salvia* species. However, some differences can be determined in the morphology and localization of the trichomes of *S. pomifera*. We consider that these trichomes can be used as distinguishing taxonomic characters in taxonomy of

S. pomifera, since the morphology and localization of these trichomes are of taxonomic value.

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PLAUKELIAI ANT TURKIJOJE APTINKAMOS *SALVIA POMIFERA* (LAMIACEAE) ORGANŲ

Nezahat KANDEMİR

Santrauka

Straipsnyje nagrinėjama ant vieno iš populiariausių vaistinių augalų – *Salvia pomifera* L. vegetatyvinių ir generatyvinių organų esančių liaukinių ir neliaukinių plaukelių tipai, funkcijos ir paplitimas. *S. pomifera* yra endeminė Viduržemio regiono rūšis. Tyrimai atlikti šviesinės ir skenuojamosios elektroninės mikroskopijų metodais. Nustatyti penkių tipų (vienas – skydiškų, keturi – galvutės pavidalo) liaukiniai plaukeliai. Skydiškus plaukelius sudaro viena trumpa kotelio,

viena pamatinė ląstelė ir 2–4 didelės skydelio ląstelės. Galvutės pavidalo plaukeliai susideda iš 1–4 kotelio, vienos kaklelio ir 1–2 galvutės ląstelių. Nustatyta, kad skydiškų plaukelių yra gausu ant lapų ir taurelės, o ant stiebo ir lapkočių jų pasitaiko retai. Galvutės pavidalo ir neliaukiniai plaukeliai išplitę ant visų vegetatyvinių ir generatyvinių *S. pomifera* organų. Šie plaukeliai apsaugo augalo organus nuo biotinių ir abiotinių veiksnių.