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Structural investigation of the glandular trichomes of *Salvia argentea*

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Abstract: The morphology, anatomy and distribution of glandular trichomes on the aerial organs of *Salvia argentea* L. has been investigated. Two morphologically distinct types of glandular trichomes were determined. Capitate glandular trichomes forming a base 1–7 celled, a stalk 1–5 celled or no stalk and a head uni- or bicellular had various types. In capitate trichomes, the neck cell that has an important role especially for xeroformic plants, acting to prevent the backflow of secreted substance through the apoplast has been distinctively observed in the investigated species. The capitate trichomes were present abundantly on all aerial organs of *S. argentea*. Peltate glandular trichomes had a large secretory head forming 1–5, 8 central and 8–10, 12, 14 peripheral cells. Peltate trichomes are present on all aerial organs, except petiole, being the most abundant on calyx and corolla. Results were shown by tables and photographs.

Key words: *Salvia argentea*; Lamiaceae; glandular trichome

Introduction

The essential oil produced by glandular trichomes is one of the characteristic features of the *Lamiaceae* family (Ascensão et al. 1995). Glandular trichomes that develop from epidermal cells are generally considered as the site of biosynthesis or accumulation of essential oils (Nishizawa et al. 1992; Turner et al. 2000). Volatile oil produced by glandular trichomes is important for pesticide, pharmaceutical, flavouring, fragrance and cosmetic industries (Serrato-Valenti et al. 1997; Zeybek & Zeybek 2002). These glandular trichomes are widely distributed over the aerial reproductive and vegetative organs of the plants of Lamiaceae. They are the primary secretory organs of these plants and their structures can vary widely among species (Serrato-Valenti et al. 1997). In almost all species studied, two main types of glandular trichome-peltate and capitate, which can be distinguished by head size and stalk length occur. As a rule, in a capitate trichome, the length of the stalk should be more than half the height of the head, whereas peltate trichomes are short with a uni- or bicellular stalk and a large secretory head with 4 to 18 cells arranged in one or two concentric circles (Ascensão & Pais 1998). Capitate trichomes are very variable in stalk length, head shape and secretion process, and can be subdivided into various types (Werker et al. 1985a).

Plant trichomes (trichomes) are of great interest to descriptive and experimental botanists and indumenta are routinely included in many types of studies. As simple morphological tools, trichomes are useful due to the ease with which they are examined and their almost universal occurrence, particularly among the ferns and flowering plants (Jurišić Grubešić et al. 2007). The great diversity of plant trichomes interests botanists because of their adaptive and taxonomic value. In the Lamiaceae family, the morphology, distribution and frequency of glandular trichomes are used as discriminative characters at the subfamiliar level (Ascensão et al. 1995). Most of the aromatic genera of Lamiaceae are in the subfamily Nepetoideae, which also includes the genus of *Salvia* (Bisio et al. 1999). A volatile oil that gives a special fragrance is characteristic for many species of *Salvia* (Stace 1991). *Salvia* is the genus that has the richest glandular trichome of the family (Metcalfe & Chalk 1972).

Salvia, the largest genus of the family *Lamiaceae*, represents an enormous and cosmopolitan assemblage of nearly 1000 species displaying a remarkable range of variation. The genus comprises 500 spp. in Central and South America, 250 spp. in Central Asia/Mediterranean, and 90 spp. in Eastern Asia (Walker et al. 2004). *Salvia* species are an important group of useful plants, which have not lost their importance since ancient times. The genus is named "Salvia" derived from "Salveo" which means "to save, to recover" in Latin (Hamlyn 1969). *Salvia*, commonly known as sage, has multiple uses such as condiment, food additive, seasoning, spice and herbal tea (Demirci et al. 2005). In addition to that, *Salvia* species have medicinal value and they are also grown in parks and gardens as ornamental plants (Nakipoğlu 1993). The antibacterial, antituberculous and antiphlogistic activities of the constituents of *Salvia* species are well established (Ulubelen et al. 2001). The antimicrobial and antioxidant activities of *S. argentea* have been recently

compared to those of other *Salvia* species by Salah et al. (2006). Historically, *Salvia argentea* leaves have been used against wounds, probably as a haemostatic. The appearance of the young leaves, which are very hairy, may have played a role in the folk 'perception' of the external medical properties of the species (Pieroni et al. 2004). In view of the potential medical and commercial value of this species, the work described in this paper was carried out to characterize the structure and morphology of its glandular trichomes. Of the great number of *Salvia* species which have not been analysed in point of the structural properties of their glandular trichomes, we aimed to investigate the morphology, cell number and distribution of glandular trichomes of *Salvia argentea* in the present study. The classification of glandular trichomes is made according to Werker et al. (1985a) and Özdemir & Şenel (2001).

Material and methods

Plant samples were collected between 2003–2004 from the following locations:

B1 İzmir : Kemalpaşa Nif mountain, road side, 1500m, 06.2003 (Baran 015).

B1 Manisa : Spil mountain, Horse area, rocky place, 1500m, 06.2003–2004 (Baran 016).

Taxonomical description of the plant followed Davis (1982). The paraffin method was applied for preparing cross-sections of the plant organs using microtom (Algan 1981). The cross-sections taken by hand were stained in Sartur reactive (Baytop 1981). Results were presented in photographs and tables. The classification of glandular and eglandular trichomes was made according to Werker et al. (1985a) and Özdemir & Şenel (2001).

Results

Glandular trichomes were of two main types, peltate and capitate, which were different in size, structure and mode of secretion.

Peltate trichomes: Peltate glandular trichomes had a base including epidermal cells and a very short stalk cell and a large secretory head forming the central and peripheral cells. They had 1–5, 8 central and 8–10, 12, 14 peripheral cells. A large space, in which the secreted material accumulated, developed at the time of secretion by the elevation of the cuticle together with the outermost layer of the secretory cell walls. Two different ways of the release of the secreted product were observed. They were present at the stem, leaf, pedicel, corolla and calyx, except petiole (Fig. 1 A–F; Table 1).

Capitate trichomes: Capitate trichomes consisted of a base including 1–7 epidermal cells, a stalk 1–5 celled usually with a short neck cell or no stalk and a uni- or bicellular head. The thickened walled neck cell was adjacent to the head and usually short in length and appeared to be filled with the secreted product. We observed three types of capitate trichomes. Type I capitate trichomes had a round-like cell and droplets on the cuticle (Fig. 2 A–H). Type II had a pear-like or round-like head and larger subcuticular space filled with secreted material and subsequent broken cuticle. Type II, which was of round-like and larger head cell, had an obviously elevated cuticle forming a large subcuticular space surrounding the cell up to its base (Fig. 2 I–R, Fig. 3 A–C). Type III had a cup-like head cell and a broken cuticle (Fig. 3 D–O). Type I, II and III capitate trichomes were all together on stem, leaf, petiole, pedi-

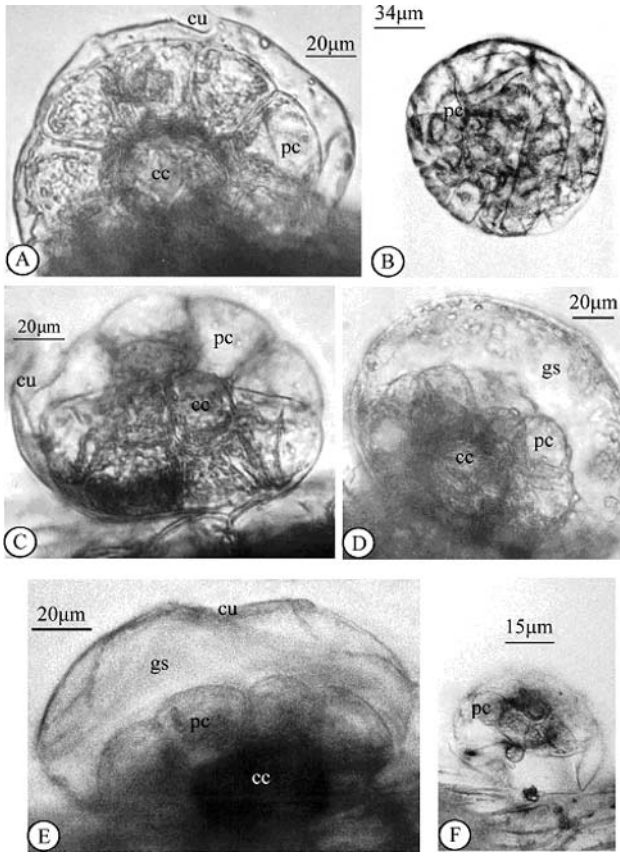

Fig. 1. Photographs of peltate glandular trichomes of *Salvia argentea* L. (A–F), hc – head cell, nc – neck cell, sc – stalk cell, cu – cuticle, bc – base cell, gs – glandular space, pc – periphery cells, cc – central cells.

Table 1. Cell numbers of parts of peltate trichomes found on organs of *Salvia argentea* L.

	Peltate trichomes				
	Centre	Periphery		Centre	Periphery
Stem	4	10	Corolla	1	8
	4	12		2	8
				4	10
Leaf	1	8		8	14
	1	10			
	3	8	Pedicel	1	8
	3	9		2	8
	3	10		4	10
				8	14
Calyx	4	10			
	4	12	Petiole	–	–
	5	10			

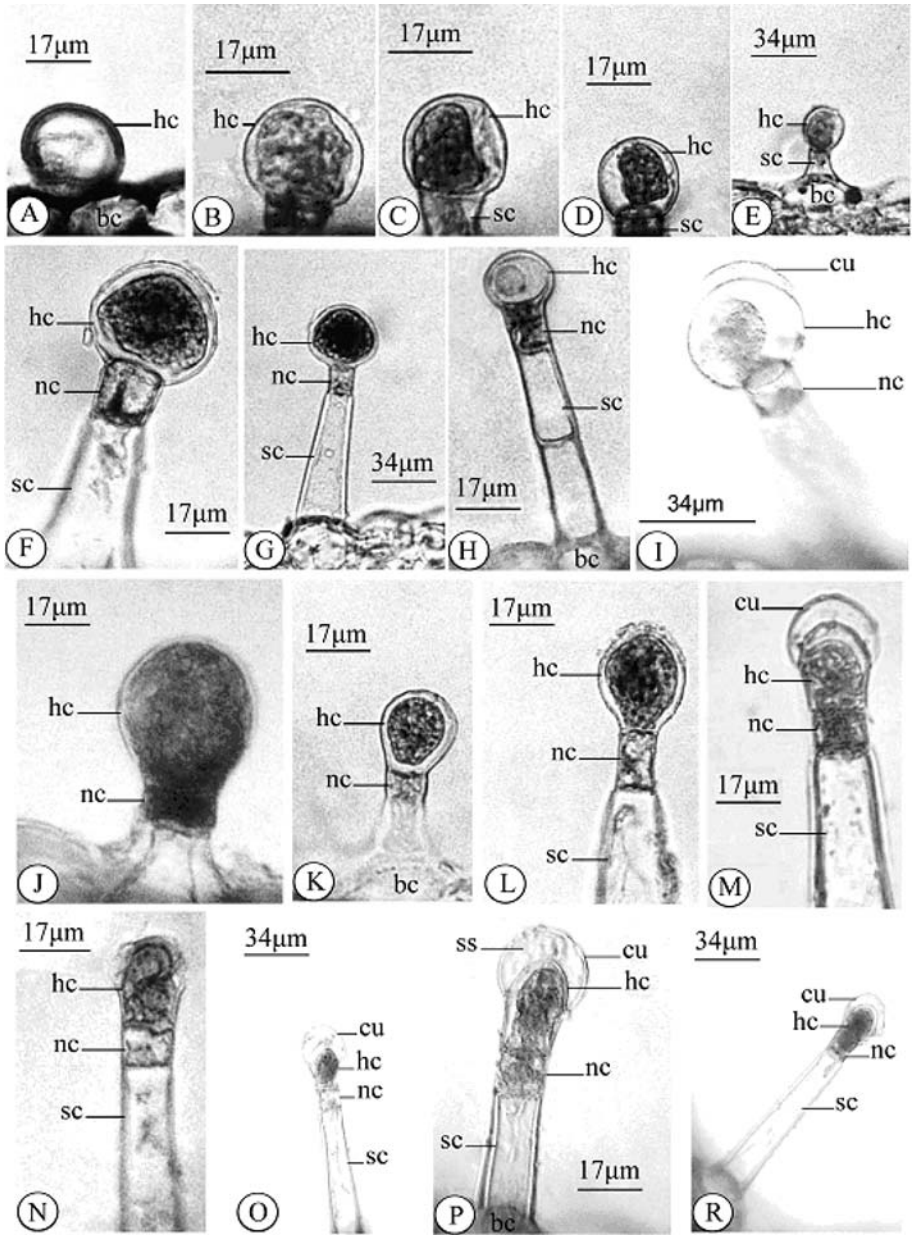

Fig. 2. Photographs of glandular trichomes of *Salvia argentea* L. A–H – Type I capitate trichomes; I–R – Type II capitate trichomes; hc – head cell, nc – neck cell, sc – stalk cell, cu – cuticle, bc – base cell.

Table 2. Cell numbers of parts of capitate trichomes found on organs of *Salvia argentea* L.

	Capitate trichomes								
	Type I			Type II			Type III		
	Base	Stalk	Head	Base	Stalk	Head	Base	Stalk	Head
Stem	1 – 4	0 – 5	1 – 2	1 – 2	1 – 3	1	1 -3	1 – 5	1
Petiole	1 – 2	1 – 3	1	1 – 2	2 – 3	1	1 – 2	0 – 4	1
Leaf	1 – 2	0 – 4	1	1 – 4	1 – 3	1	1 – 4	2 – 5	1
Pedicel	1–2, 4	0, 2–5	1 – 2	1	1	1	1 – 4	2 – 5	1
Calyx	1 – 3	0 – 3	1	1 – 2	1 – 3	1	1–3, 5	1, 3–5	1
Corolla	1 – 5	0 – 4	1	1, 3	2 – 3	1	1–4, 6, 7	1 – 5	1

cel, calyx and corolla, but type II capitate trichomes with a round-like and larger head were only present on stem and pedicel. Capitate trichomes had 1–2 head cells and usually a short neck cell adjacent to the head and 1–5 stalk cells or no stalk cell and 1–7 base cells (Table 2).

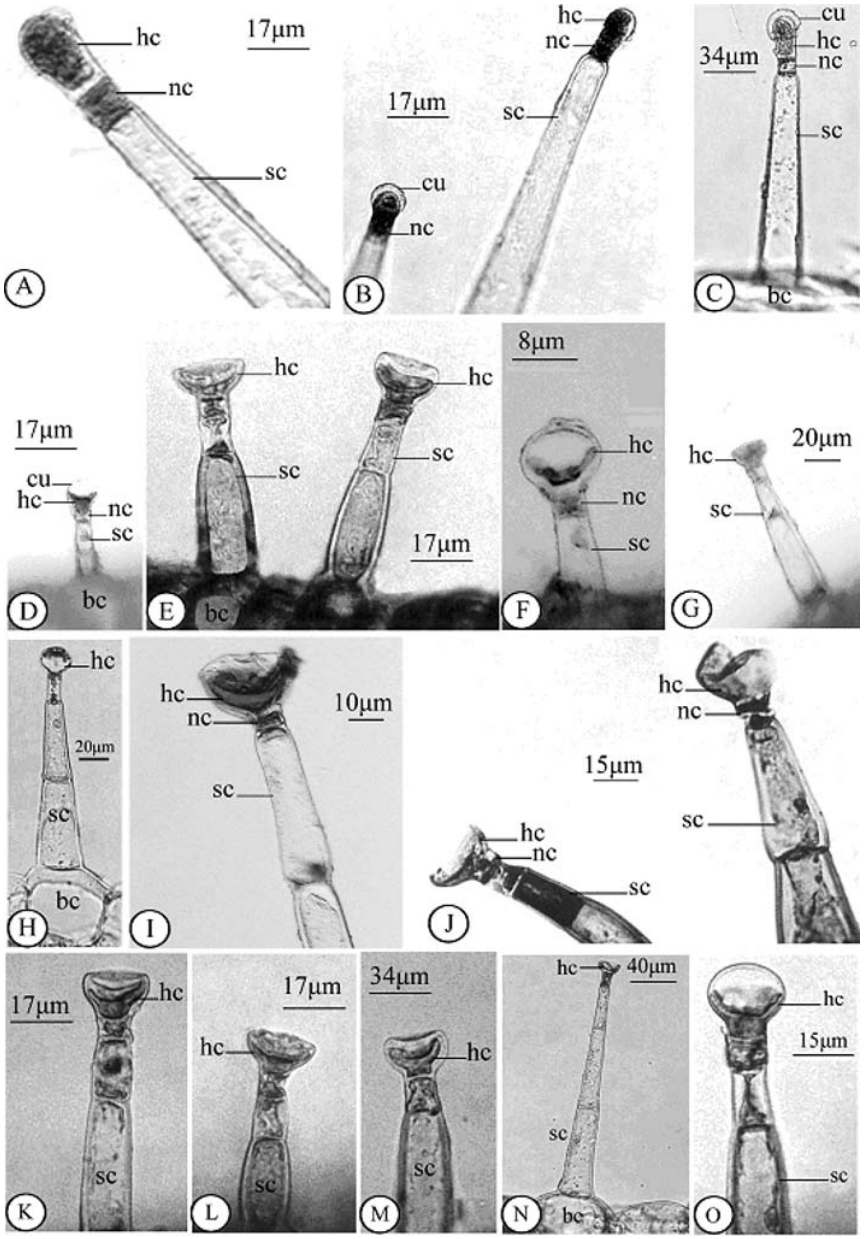

Fig. 3. Photographs of glandular trichomes of *Salvia argentea* L. A–C – Type II capitate trichomes; D–O – Type III capitate trichomes; hc – head cell, nc – neck cell, sc – stalk cell, cu – cuticle, bc – base cell

Discussion

Many researchers observed peltate and various capitate trichomes on some *Salvia* species (Werker et al. 1985a,b; Kesercioğlu & Nakipoğlu 1992; Serrato-Valenti et al. 1997; Bisio et al. 1999; Özdemir & Şenel 1999, 2001; Özkan & Soy 2007). As in plants of most *Lamiaceae* species, the aerial parts of *S. argentea*, especially the leaves and flowers, densely carried glandular trichomes, including the two main types, peltate and capitate trichomes, which differed in structure and secretion process.

The heads of peltate trichomes were made up of 1–5, 8 central and 8–10, 12, 14 peripheral secretory cells arranged in two concentric circles, which is a typical feature in the Lamiaceae. There is a similar arrangement in *Origanum* species (Bosabalidis & Tsekos 1984), *Satureja thymbra* (Bosabalidis 1990), *S. forskahlei* and *S. sclarea* (Özdemir & Şenel 1999; 2001) and different species examined by Werker et al. (1985a,b), but in other species of the same family, such as *Leonotis leonurus* (Ascensão et al. 1995), *Ocimum basilicum* (Werker et al. 1993), a small number of head cells are arranged in a single circle.

Capitate trichomes are widespread in the Lamiaceae, but they vary greatly in structure and size. According to the present observations, the capitate trichomes of *S. argentea* displayed variation in morphology: type I had a round-like head uni- or bicellular and droplets on the cuticle, without subcuticular space; type II had a pear-like head cell with a large subcuticular space filled with secreted material, of which head cell was sometimes round-like and larger; type III had a cup-like head cell with a broken cuticle. Those kinds

of capitate glandular trichomes of *S. argentea* observed here (capitate type I, II, III) corresponded to three types of capitate trichomes described by Werker et al. (1985a) in their study of some Lamiaceae species including *Salvia*. Serrato-Valenti et al. (1997) observed two types of capitate glandular trichomes in *S. aurea*, which occurred in *S. argentea* as type I and III. In *Nepeta racemosa* L. (Bourett et al. 1994) and *S. aurea* (Serrato-Valenti et al. 1997), small capitate glands with two head cells were found. In our study, the capitate trichomes with a bicellular head had a 1–3 celled stalk or no stalk cell, while bicellular headed capitate trichomes in *S. sclarea* (Özdemir & Şenel 1999) had no stalk cell and those in *S. forskahlei* (Özdemir & Şenel 2001) had a 1–2 celled stalk. Özdemir & Şenel (1999, 2001) are the first authors to report the base cell number of capitate glandular trichomes in the *Salvia*. In the present study, we also determined the base cell number of capitate glandular trichomes of *S. argentea*. The capitate trichomes of *S. argentea* had 1–7 celled base, while they had 1–2 celled base in *S. sclarea* and *S. forskahlei* (Özdemir & Şenel 1999, 2001).

The morphology of glandular hairs is often related to the kind of secreted product. Some Lamiaceae secretion from capitate trichomes contains mostly polysaccharides and only small quantities of essential oil. On the other hand, the peltate trichomes are considered the site of production and storage of essential oil (Bini Maleci et al. 1983; Werker et al. 1985a). Furthermore, Werker et al. (1985b) reported that the different morphological structure of some capitate trichomes might correspond to the production of the different materials. From a functional point of view, Werker (1993) classified the glandular hairs into two types, according to different mode and timing of secretion; a) short-term glandular trichomes, which start and end secretion rapidly; b) long-term glandular trichomes, in which secretory material is accumulated in a large subcuticular space.

In the peltate trichomes of *Salvia argentea*, the secretory product remained trapped between the cuticular sheath and the head cells, following a pattern already described for other aromatic Lamiaceae (Bosabalidis & Tsekos 1982; Werker et al. 1985a,b; Bruni & Modenesi 1983; Bourett et al. 1994). The rupture of the cuticular sheath is likely, in accordance with Ascensão et al. (1995), who stated that external factors such as high temperatures, low air humidity or animal aggression may cause the cuticular rupture. Another less common mechanism of release has been observed in peltate trichomes of *Salvia argentea*, as the droplets of secretory material were observed on the external surface of the head, which appeared to pass through the cuticular layer.

In type I capitate trichomes of *Salvia argentea*, droplets of secreted material were observed on the head cell, which was released by cuticular exudation. A similar phenomenon was observed in the secretory trichomes of *Inula viscosa* (Werker & Fahn 1981), *Majorana striaca* (Werker et al. 1985a) and *S. aurea* (Serrato-Valenti et al. 1997), *S. blepharophylla* (Bisio et al. 1999). In type II capitate trichomes of *S. argentea*, accumulation of the secreted product into an extended subcuticular space by cuticular elevation and subsequently the rupture of cuticle was observed as reported in *S. officinalis* (Corsi & Bottega 1999), *S. blepharophylla* (Bisio et al. 1999), *S. dominica, S. sclarea* (Werker et al. 1985b), *S. officinalis, S. fruticosa* and some other species of Lamiaceae (Werker et al. 1985a). In type III capitate trichomes of *S. argentea*, the collapse of the head cell wall, which gave the collapsed cell the shape of a cup, was observed just as in the trichomes of *S. officinalis* (Corsi & Bottega 1999) and in the study of Werker et al. (1985a), the secretory product of which was eventually released by the rupture of the cuticle. Volcano crater-like headed capitate glandular trichomes, the secretory product of which is extruded by a single large pore in the cuticle, have not been observed in *S. argentea,* which was firstly described in *S. officinalis* by Corsi & Bottega (1999).

In many studies on Lamiaceae, a neck cell has been described in capitate glandular trichomes. This neck cell has an important role, acting to prevent the backflow of secreted substances through the apoplast (Bruni & Modenesi 1983; Fahn 1988; Serrato-Valenti et al. 1997). In general, a short neck cell with thickened side walls, appearing filled with the secreted material, was clearly visible in the capitate glandular trichomes of *S. argentea*.

Our anatomical investigations showed that *S. argentea* was very rich from the point of glandular trichome diversity and quantity. The glandular trichomes greatly varied in structure, size, proportions, occurrence on plant organs and manner of secretion. Furthermore, we think that the features like abundance and diversity of glandular trichomes on plant organs, presence or absence of neck cells, the thickness of their side walls, and the stalk length, have been showing variation according to xeromorphic character of plants. The abundant and diverse glandular trichomes and long-length stalks and the thickened walled neck cells of *S. argentea* have corresponded to xeromorphic character of the investigated plant species that exist at high altitudes and mountain habitats.

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