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Floral nectaries of basil (*Ocimum basilicum*): Morphology, anatomy and possible mode of secretion

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Abstract

Floral nectaries of *Ocimum basilicum* L. were studied using light, fluorescence and scanning electron microscopy. Each nectary forms an asymmetrical four-lobed disc at the base of the outer surface of the ovary. The three large lobes were found to be functional, whereas the smallest lobe is lacking in the modified stomata. Nectary anatomy is characterized by three major zones: a uniseriate epidermis, sub-epidermal secretory tissue and vascular tissue. The nectary epidermis of three functional lobes is covered with a very thin cuticle, and contains many modified stomata involved in the exudation process. They are diffusely distributed and lie in the same plane as the epidermal cells. The secretory tissue is composed of small cells with thin walls, relatively large nuclei, dense granular cytoplasm and small vacuoles. Most of the cells of the nectariferous tissue contain calcium oxalate crystals of different sizes. The nectary diameter, the size of the secretory cells and the thickness of the epidermis and cuticle increased during nectary ontogeny. The nectary is vascularized exclusively by phloem originating from vascular bundles destined for the gynoecium.

Keywords: Modified stomata; Nectary structure; *Ocimum basilicum*

1. Introduction

Floral nectar is secreted by distinct glandular organs, the nectaries, whose anatomy and morphology vary greatly upon the taxon. These glands, which produce and secrete nectar may perform a vital function in sexual plant reproduction by attracting pollinators. Because the gland is usually well vascularized, its product may also be basically regarded as a derivative of phloem sap. However, it is modified during the secretion process, which is a complex physiological accomplishment controlled by anthesis. Except in rare cases where the nectariferous disc continues secreting for a while after the corolla is shed (*Lamium*), nectar production is confined to anthesis. Usually it starts shortly before the flower unfolds (Vogel, 1983).

There is a considerable literature on the structure and ultrastructure of nectaries in the Lamiaceae (Gulyás, 1967; Fahn, 1979; Dafni et al., 1988; Zer and Fahn, 1992). Members of the Lamiaceae usually have disc glands at the base of the ovary (Fahn, 1979).

Besides the morpho-anatomical studies of the floral nectary of *Ocimum basilicum* undertaken here, a comprehensive investigation of nectar production, including ecophysiological aspects of this process, as well as pollination biology, is necessary to estimate the contribution and significance of this melliferous plant to bee pasture. Like other species of the Lamiaceae, basil is entomophilus, predominantly bee-pollinated (Faegri and Van der Pijl, 1979). Basil is a cross-pollinating protandrous plant, in which stamens are developed before the stigma becomes receptive (Ichimura and Noguchi, 2004). The structure of the basil nectary was studied to obtain a better understanding of the possible mechanism of nectar secretion, as well as to identify the anatomical alterations during flower ontogeny, which has been compared with other Lamiaceae.

Abbreviations: ca, calyx; co, corolla; e, epidermis; lnl, large nectary lobe; nl, nectary lobe; ol, ovary lobe; pa, receptacle parenchyma; ph, phloem; sc, secretory cells; snl, smal nectary lobe; s, stomata; st, style; ucl, upper calyx lip; vb, vascular bundles.

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2. Materials and methods

Basil (*O. basilicum* L., Lamiaceae) plants were grown from seeds in an experimental outdoor plot without fertilizing and were watered as required. Nectaries were investigated during budding (two days before anthesis) and at the full flowering stage (anthesis). Flowers were sampled from May until October, 2003. Five flowers of each of these two stages of growth from five individual plants were taken.

2.1. Light microscopy

Flowers were prepared by removing the floral parts near the gland (the apical portions of the sepals, petals, stamens). The remaining receptacles with the ovaries were fixed in FAA for 24 h and post-fixed in 70% ethanol, dehydrated through a graded series of ethanol and then xylol before embedding in paraffin wax and dissected to various degrees. Both transverse and longitudinal sections (3–5 μm thick) were cut on a LEICA SM 2000 R microtome, mounted serially and stained with alcian blue-safranin.

Measurements of some of the anatomical features of the nectaries, including the width and diameters of secretory cells were made on 25 slides and 100 measurements of each character per stage. The samples were examined by light microscopy and documented by digital camera. Measurements were done using the software program IM 1000.

To identify the chemical nature of the crystals, chloridric acid was utilized with the analysis of solubility (Howart and Warne, 1959).

2.2. Fluorescence microscopy

To examine the cuticle and xylem, freshly sectioned material was monitored for autofluorescence using a LEICA DMLS epifluorescence microscope (filter I3 BLU 450–490 nm). Corolla lips were observed using UV light (340–380 nm wavelength) to determine a possible presence of the nectar guides.

2.3. Magnifying glass and scanning electron microscopy (SEM)

Receptacles with the ovaries were dissected from fully-developed flowers of plants taken from the experimental field. Receptacles were studied using both a magnifying glass and a JOEL JSM-35 scanning electron microscope on fresh material without prior preparation procedure.

3. Results

The floral nectary has the form of a prominent ring, encircling the base of the ovary (Figs. 1A, 2A and 3A). Nectary lobes prominently rise at the junction of the ovary lobes. SEM investigation and serial sectioning demonstrated that the floral nectaries are slightly convex and asymmetrically four-lobed, with three large lobes facing the lower corolla lip and the smallest facing the upper corolla lip (Fig. 1A).

The nectary consists of three distinct regions: a uniseriate epidermis, sub-epidermal nectar-producing parenchyma cells and vascular tissue (Figs. 2C, 3C and D). The floral nectary is vascularized by phloem alone, originating from vascular bundles destined for the gynoecium. Each individual nectary lobe has its own traces of phloem (Fig. 3D). Sieve elements

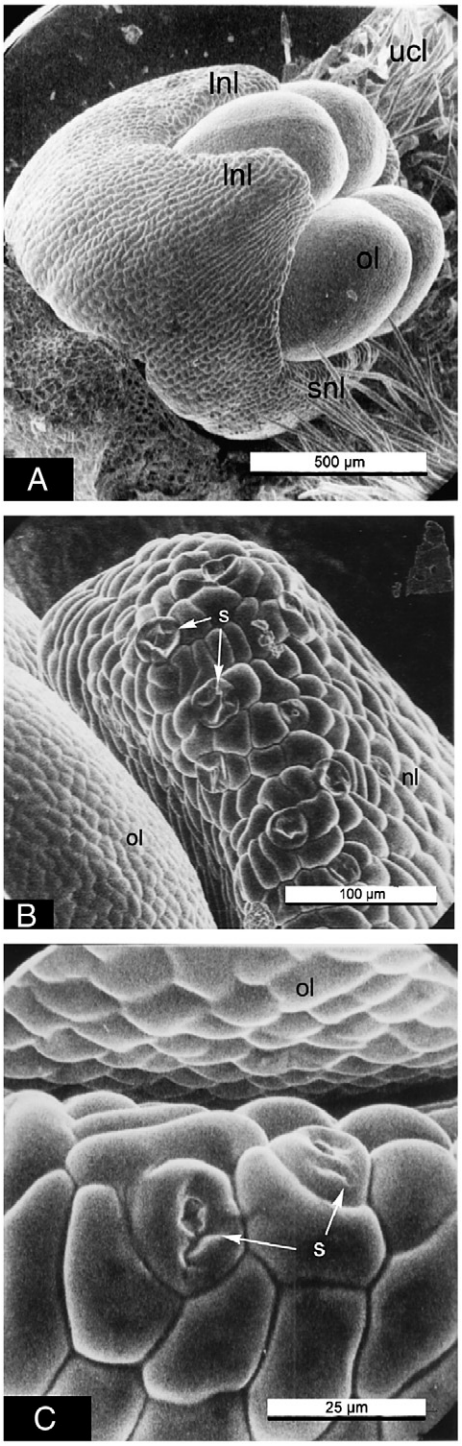

Fig. 1. Scanning electron micrographs of the nectary surface at anthesis: (A) Unequal-sized nectary lobes with extensions between ovary lobes. Three larger secretory lobes are visible, and the fourth, facing the calyx (as corolla is removed), is the smallest one. Part of calyx is evident; (B) On the upper part of the nectary large lobe and surface facing the ovary, many modified stomata are visible; (C) Tip of the large nectary lobe, bearing two modified stomata.

(Fig. 3D) can be detected in the glandular tissue. Phloem is present at the nectary base as sieve tubes. Larger parenchyma cells of the receptacle are located below the nectary tissue. Nectar is secreted to the outside surface through modified stomata, which are always open (Fig. 1B and C). Most of the nectar accumulates between the base of the nectary and the inner surface of the corolla base.

In pre-secretory nectaries, the epidermis is formed by a single cell layer. It consists of small, uniform and cylindrical cells. Epidermal cells are characterized by dense cytoplasm and prominent nuclei (Fig. 2C). The cuticle covering the epidermis is continuous (Fig. 2D). It exhibits typical auto-fluorescence when observed by epifluorescence microscopy (Fig. 2D). The epidermis in both stages was covered on the outside surface with a cuticle. However, the overlying cuticle located over the bud nectary epidermal cells was slightly thinner (for 0.27 μm or about 20%) compared with the cuticle of epidermal cells of the nectary at anthesis (Table 1.).

The region of secretory tissue at bud stage is approximately six to eight parenchyma cells wide. The superficial layers consist of small, more or less isodiametric cells. The cytoplasm of these cells is dense and the nuclei are relatively large.

In mature nectaries the epidermal cells are not uniform. They differ in shape and show convex outer cell walls, but exhibiting similar characteristics as determined for the bud stage. Epidermal cells bear a cuticle, which maintains its continuity during the nectar secretion phase. The cuticle is variable in thickness, ranging from 0.7 μm to 3.0 μm.

Modified stomata are located among these epidermal cells (Fig. 1B and C). The stomata lack subsidiary cells and consist of two guard cells, surrounding a large pore. When observed by SEM, stomata were found to occur along a circular line on the upper part of the nectary and were always open. They are confined to the apical part of the upper and inner surfaces of the three large nectary lobes. The apical part of the nectary surface possessed an average of 12±4 modified stomata per large nectary lobe. However, the type (according to Mauseth, 1988) and size of the modified stomata clearly differed from leaf stomata. The modified stomata are characterized as anomocytic with an average length of the guard cells of 11 μm±1, whereas the leaf stomata as diacytic and of the much higher size (30 μm±3).

Secretory cells resemble the ordinary parenchyma cells. The nectariferous tissue is presumably formed of up to ten layers of relatively isodiametric cells (Fig. 3C and D). These cells differ

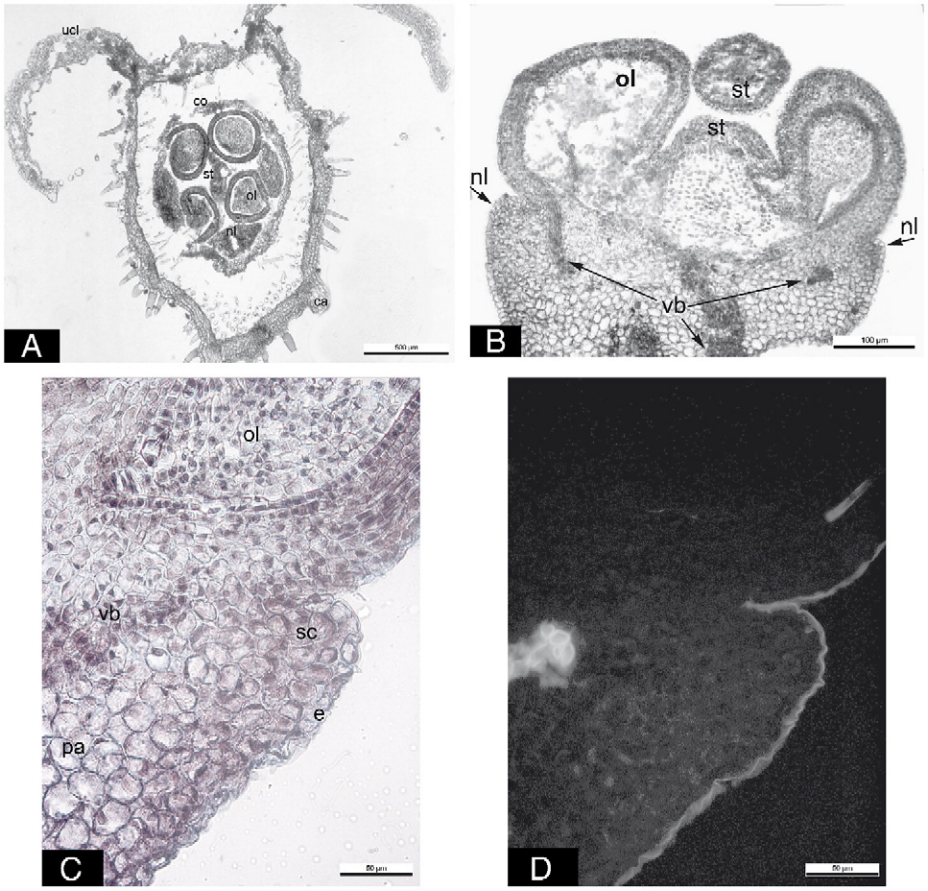

Fig. 2. Light and fluorescence micrographs of the bud section: (A) Cross section of bud base showing four ovary lobes and three lobes of glandular tissue. The 4th lobe facing the upper corolla lip is undeveloped. ×50; (B) Median L.S. of bud through the style, two ovary lobes and two nectary lobes. Note vascular bundle leading to ovary base. ×200; (C) Partial longisection of a bud showing the ovary and nectariferous tissue. ×400; (D) Fluorescence microscopy (the same section as in C) demonstrating autofluorescence of the xylem and the cuticle of the covering epidermal cells. ×400.

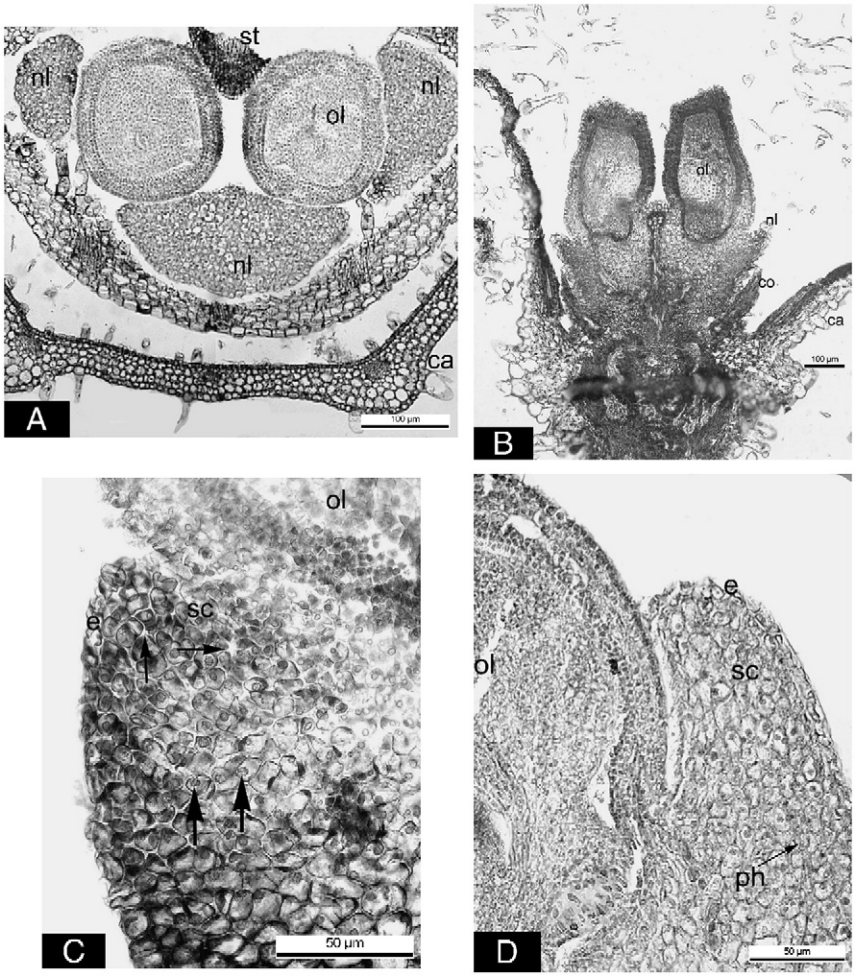

Fig. 3. Micrographs showing nectary structure at anthesis: (A) Cross section, three large nectary lobes ×200; (B) Longitudinally section of flower showing ovary and nectary lobes; (C) Secretory cells with intercellular spaces (smaller arrows) in flower longisection. Crystals occur in almost all cells of nectariferous tissue of the large nectary lobe (large arrows). Each of these cells contains a single calcium oxalate crystal ×400; (D) Longitudinal section of a large nectary lobe showing the secretory tissue and vascular supply (phloem only-indicated by arrow) ×400.

from those of the neighboring tissues by their size. They are more or less small and closely packed (Figs. 2C, 3C and D). Numerous intercellular spaces occur between the parenchyma secretory cells (Fig. 3C).

Table 1
Measurements of anatomical features of nectaries of *Ocimum basilicum* at the bud and anthesis (in µm)

Anatomy feature	Bud	Anthesis	*F*	*p*
Nectary diameter	986.7±55.2 a	1159.4±36.8	88.7607	***
Height of the large lobes	562.9±115.9 a	715.0±71.4 b	20.0535	**
Height of the small lobe	297.4±25.3 a	419.1±19.4 c	155.3221	***
Epidermis width	12.0±3.1	13.2±2.1	2.2037	n.s.
Epidermis height	11.3±2.7 a	6.8±1.4 c	107.3564	***
Diameter of secretory parenchyma cells	14.1±4.8 a	17.5±3.9 c	17.3905	***
Cuticle thickness	1.0±0.4 a	1.3±0.5 b	20.9027	***
Crystal diameter	6.8±1.4	7.3±1.0	1.4195	n.s.

Numbers followed by different letters significantly differ, where a:b means $p<0.05$, and a:c $p<0.001$.

In nectariferous tissue of both developmental stages, some cells contain crystals (Figs. 2C and 3C). Each crystal forming cell contains only one, polygonally-shaped calcium oxalate crystal. The size of these crystals ranged between 6.8 and 10.2 µm, and from 7.3 to 9.9 µm at bud stage and anthesis,

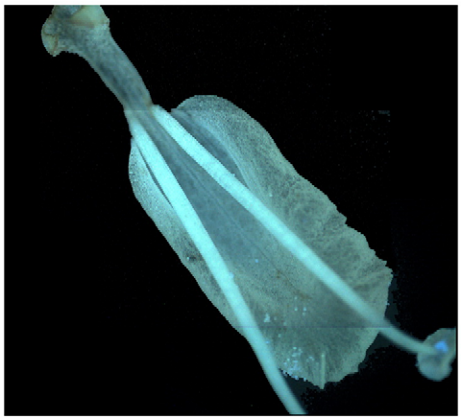

Fig. 4. Lower corolla lip lacking in nectar guides and stamens in UV light.

respectively, but such differences were not statistically significant (Table 1). Sieve elements can be detected in the glandular tissue (Fig. 3D). Phloem resides only centrally as separate bundles.

Nectar guides were not observed at the base of the corolla using the UV light. However, the stamens laying at the lower corolla lip, in contrast to most of the other Lamiaceae, in which fertile parts are situated below the upper corolla lip (Huck, 1992), exhibit strong autofluorescence (Fig. 4A), indicating a possibility for directing a pollinator toward the nectaries.

4. Discussion

As in many other genera of Lamiaceae (Fahn, 1979), a conspicuous nectariferous annular and unequally lobed disc surrounds the base of the ovary in *O. basilicum*. In *Clerodendrum tracyanum* they are irregularly scattered over the entire abaxial leaf surface, whereas in *Faradaya splendida* they appear on both sides of the leaf midvein (Blüthgen, 2003).

Floral nectaries in basil have not been investigated so far. Our results indicate that they belong to the toral nectary type that develops in the receptacle, and that is common in the Lamiaceae (Dafni et al., 1988). In most of studied Lamiaceae the nectary is asymmetrical, and its thicker part, containing the modified stomata, faces the lower corolla lip (Fahn, 1979; Dafni et al., 1988; Mačukanović-Jocić, 2006). A special basil nectary morphology, compared with the other investigated Lamiaceae, is related to the existence of the three functional lobes of the same size and the distinctly smaller lobe facing the upper corolla lip, lacking in the modified stomata. Such differences might be explained with a phylogenetic position of basil, belonging to the tribe Ocimeae, which is monophyletic and easily diagnosable with morphological synapomorphies (Paton et al., 2004). Ocimeae are usually considered to have stamens pressed against the anterior, or lower side of the flower (declinate) and deliver pollen to the ventral surface of visitors (sternotribic) (Huck, 1992). The size of the mature nectary of basil, reaching about 1.2 mm, was similar as in *Salvia exserta*, *S. summa*, *S. oaxacana* and *S. henryi* (Wester, 2007). Like in the other studied Lamiaceae, hairs were found neither on the nectary surface nor near the nectary, in contrast to *Satureja thymbra* (Dafni et al., 1988), *Salvia africana-lutea* and *S. lanceolata* (Wester, 2007), in which the dense fields of long hairs were found in the short distance to the nectary.

The anatomy of the basil floral nectary is generally similar to previous reports of many other Lamiaceae (Dafni et al., 1988; Zer and Fahn, 1992; Mačukanović-Jocić, 2006). Secretory tissue of the nectary consists of a one cell-layer thick epidermis and nectar-producing parenchyma below, in the form of several layers of approximately isodiametric cells.

Some alterations in anatomical structure of the basil floral nectaries occur during floral development. It was found that characters, such as nectary diameter, the height of the larger lobes and the small lobe, epidermis height, diameter of secretory parenchyma cells and cuticle thickness differed significantly between the two developmental stages (Table 1). The size of secretory parenchyma cells, the width of epidermal cells and cuticle thickness increased during nectary ontogenesis, whereas the height of epidermal cells decreased.

At anthesis, secretory tissue can be easily recognized from the surrounding non-glandular parenchyma, with a less visible distinction at the bud stage. During the period of nectar production, i.e. in fully-developed flowers, secretory parenchyma cells enlarge, as shown by Fahn (1979), whereas epidermal cells increase in width.

Vasculature of the floral nectary of *O. basilicum* consisted of phloem strands, which ascended into the nectary from vascular bundles, presumably destined for the gynoecium. Although these bundles were collateral, as earlier studies of some other species indicated (Davis et al., 1996; Razem and Davis, 1999), phloem alone departed these fascicles toward the floral nectary. Only phloem supplied the interior of each lateral nectary lobe, corroborating the findings of Zer and Fahn (1992). Nectary lobes received a small supply of phloem only to the base. This was also observed in *Rosmarinus officinalis*, *Salvia judaica*, *S. hierosolymitana* and *S. thymbra*, in difference to *Phlomis viscosa*, *Salvia fruticosa* and *Stachys aegyptiaca*, in which both phloem and xylem elements occurred in the nectariferous tissue (Dafni et al., 1988). Using the fluorescence microscopy, in *Lavandula officinalis* and *Salvia officinalis* the xylem was noticed in the vicinity of nectariferous tissue, whereas in *Hyssopus officinalis*, *Melissa officinalis* and *Salvia sclarea* tracheary elements were found to enter the secretory tissue (Mačukanović-Jocić, 2006). As Ca-oxalate crystals appear more frequently and are more abundant adjacent to vascular tissues, especially the phloem (Paiva and Machado, 2005), they have been found to be widely distributed within secretory parenchyma cells in the basil nectaries both at bud stage and anthesis.

It has been established that nectar leaching can occur in different ways: through modified stomata, across a permeable, porous or ruptured cuticle (including specialised trichomes), or by cellular degeneration (Fahn, 1979; Findlay, 1988).

Pores of structures which are unable to contract their apertures and lose the capacity to close completely are designated as »modified stomata« (Rachmilevitz and Fahn, 1973; Davis and Gunning, 1992). However, Carr and Carr (1987) postulated that these structures are able to open and close in the case of *Eucaliptus stellulata* nectary, and may regulate nectar flow through them.

Stomata of the basil nectaries are distributed in a circular line on the upper third of the nectary surface and on the inner side facing the ovary, as seen in scanning electron micrographs. Nectar is released from the subepidermal secretory cells by modified stomata. In this case, the nectar is transported from the parenchyma via an intercellular pathway.

Unlike the other studied Lamiaceae, (Dafni et al., 1988; Zer and Fahn, 1992; Mačukanović-Jocić, 2006) where the modified stomata are present on the thicker side of the disc only, in basil they were found on each of the three large lobes. The modified stomata of the basil nectary are formed of the two guard cells, in difference to *Lamium* spp., where the aperture is surrounded by more than two guard cells (Gulyás, 1967).

The level of the stomata in relation to the neighboring, ordinary epidermal cells, differs among species of the Lamiaceae. In basil,

the modified stomata occur at the same level as the neighboring epidermal cells, as found for *P. viscosa*, *R. officinalis*, *S. fruticosa*, *S. judaica* and *S. aegyptiaca*, (Dafni et al., 1988). Nevertheless, in *Corydothymus capitatus*, *M. officinalis*, *S. thymbra*, the stomata were elevated above the nectary surface (Dafni et al., 1988), while in *Salvia hierosolymitana* (Dafni et al., 1988) and *S. sclarea* (Mačukanović-Jocić, 2006) the stomata were sunken The modified stomata of the basil nectary have been characterized as anomocytic, unlike the diacytic and much larger stomata obtained for its leaves.

Some results have outlined the development, anatomy and fine structure of the modified stomata of the floral nectary and the potential use of stomatal number and distribution on nectaries as indicators of increased nectar–sugar production (Davis and Gunning, 1992). Nevertheless, there is insufficient information on the molecular, genetic and environmental factors that may control the stomatal features of nectaries.

The present study pointed out that the morphology of basil nectaries, characterized by the presence of three functional lobes, differed from the other studied Lamiaceae in which only the thickest lobe was functional.

Future research should be focused on the interspecific differences of nectary structure within the Lamiaceae, that might be correlated with floral nectar production.

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