Journal of Multidisciplinary Engineering Science Studies (JMESS)
ISSN: 2458-925X
Vol. 7 Issue 5, May - 2021

Characterisation of Some Epidermal Structures on the Leaves of In Vitro Ocimum Basilicum L. Plantlets

Jonida Balla
Department of Biomedical and Human Discipline Faculty of Rehabilitation Science,
Sports University
Tirana, Albania
Jonidab87@live.it

***Abstract*—*Ocimum basilicum L*. as an aromatic plant of economic and medicinal importance was cultivated *in vitro* in MS basal media supplemented with BAP 1 mg l^{-1} and NAA 0.1 mg l^{-1}. The aim of this study was the analysis at the histological and cytological level of the epidermis microstructures at *Ocimum basilicum L.* var. *minimum* leaves cultivated *in vitro*. The use of these techniques at the *in vitro* grown plantlets allows a faster and an earlier characterization of variations between varieties and subspecies of aromatic plants. Stomata and trichomes as growth-sensitive structures have been analyzed in plant leaves collected from 4 weeks old plants cultivated *in vitro*. The microscopic specimens were analyzed with stereomicroscope and light optical microscope. For the evaluation of the stomata and trichomes, we prepared the microscopic slides with the nail polish colloidal technique. Stomata are located both on upper and lower leaf epidermis characterizing the leaves as amphistomatic. Stomata were generally characterized as diacytic type. A distinctive feature was the presence of high-frequency stomata clusters, a phenomenon specific for *in vitro* grown plantlets. Trichomes are very well structured even on this early development stage of these *in vitro* plantlets and are present on either side of the leaf. We distinguished three types: one type of non-glandular trichomes and two types of glandular trichomes: peltate and capitate.**

Keywords*—*aromatic plant, capitate trichomes; in vitro; peltate trichomes; stomata;

I. INTRODUCTION

Ocimum basilicum L. of the family Lamiaceae is a herbaceous species rich in aromatic essential oils. Basil is not only valued for its pharmaceutical properties but also for the aromatic oil it yields. *Ocimum* species have a major importance not just as an aromatic or ornamental plant which utilized for many purposes, additionally as a medicinal plant because of its high substances from volatile oils and numerous secondary metabolites such as rosmarinic acid, flavonoids and anthocyanins [1]. Many *in vitro* studies have been conducted on Lamiaceae species, including the *Ocimum* genus, using different explants, initial from *in vitro* germinated seeds. The ordinary technique for proliferation of this family is through seeds. Seeds were rinsed three times with sterile distilled water and inoculated in MS medium [2]. The cytogenetic studies on the *in vitro* - derived plants of *Ocimum basilicum* L. are demonstrated from [3]. Histological and cytological studies in the *in vitro* plants of *Ocimum basilicum* L., as to our knowledge are not conducted in Albania before. *In vivo* epidermal structures of the leaf on *Ocimum basilicum* L. plant are studied and characterized by [4] ; [5] ; [6]. This study evaluated the effects of MS medium on the epidermal structure of the leaf (stomata and trichomes) at *Ocimum basilicum* L. var. *minimum* plantlets cultivated *in vitro*. Evaluation of those structures in the early stages of development for *in vitro* cultivated plantlets is the most important aim of this study.

II. MATERIALS AND METHODS

We have analyzed leaves collected from 4 weeks old plantlets cultivated *in vitro* in MS basal media supplemented with BAP 1 mg l^{-1} and NAA 0.1 mg l^{-1}.

As initial explants were used apical and lateral shoots. These explants were sterilized using HgCl2 0.01 % for 10 min., followed by a treatment with ethanol 70% for 2 min. After that, the explants were rinsed three times with sterile distilled water and inoculated in MS medium.

Fresh, hand-cut sections and fixing sections were used for investigating specimen with stereomicroscopy and light microscopy. Fixing sections were prepared by fixing in FAA for 48 hours. Nail polish technique and cut sections are used for characterization of stomata and trichomes.

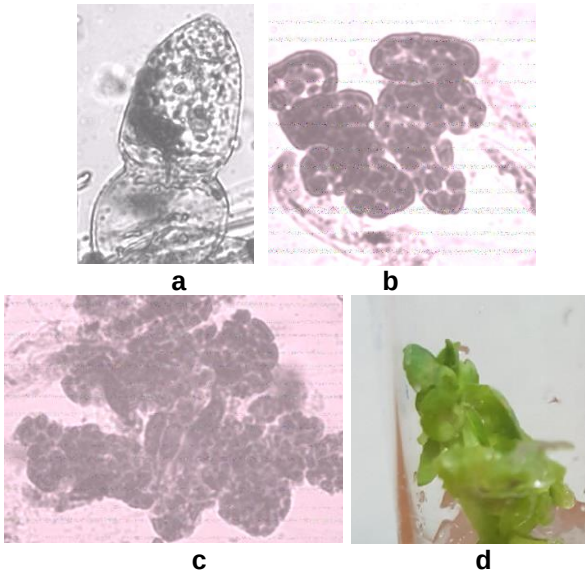
This was examined with Olympus light microscope and phase contrast microscope. The photos are taken with Paralux camera and Samsung telephone camera.

a b

Fig. 1: *Ocimum basilicum var. minimum cultivated in vitro, 4 weeks old (a). Phase contrast microscope (b).*

III. RESULTS AND DISCUSSION

Fig. 2. a,b,c, presents the first stages of in vitro development of the initial explants which developed the 4-week-old plants used for the analysis (Fig. 2. d).



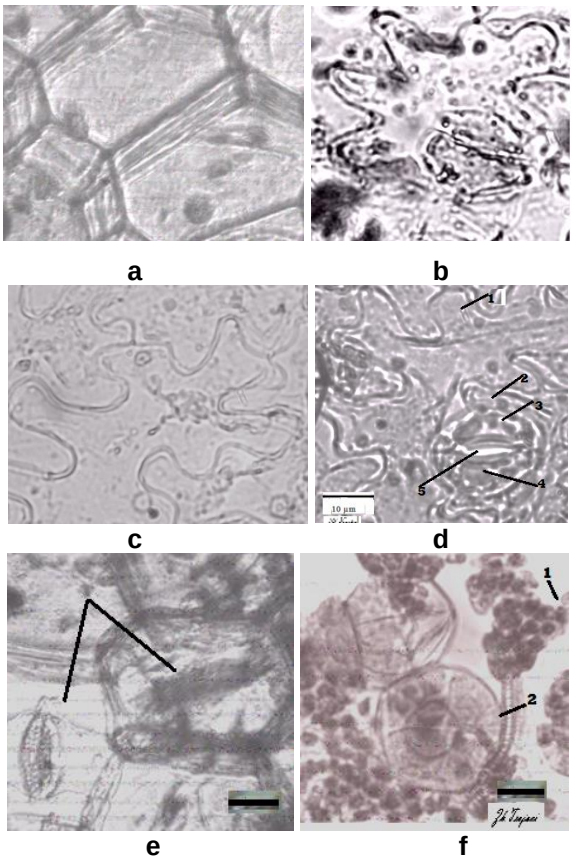
a b c d

Fig. 2: *Explants in the first stage of growth (a,b,c,). 4-week-old plant with 2 leaves (d).*

The leaf epidermis of the analyzed plants show :

1- The presence of two types of epidermal cells, prismatic cells near the leaf nerves and curved epidermal cells in the leaf area (Fig. 3. d, f). The epidermal cells of both types are composed of the cell wall and cytoplasm as clearly shown in Fig. 3. a, b, c.

2-Since the first stages of investigation on implemented explants for the in vitro plantlets, we observed the development of epidermal structures (stomata, trichomes). In the upper and lower epidermis of the leaves of the analyzed plants, we noticed the presence of stomatal complex as a structure composed of: epidermal cell, 2-stomata, 3-guard cell, 4-chloroplast and 5-stomatal pore.



a b c d e f

Fig. 3: *Ocimum basilicum var minimum in vitro. 10µm scale. Epidermal structures at an earlier stage of development of in vitro O. basilicum var. minimum explants. Phase contrast microscope 100x. Ocimum basilicum var minimum in vitro epidermal cell (a,b,c,e). Prismatic epidermal cell (a,e). Curved epidermal cell (b,c) Stomata (d,e). Glandular trichomes 1-capitate, 2-peltate trichomes (f). Stomatal complex 1-epidermal cell, 2-stomata, 3-guard cell, 4-chloroplast, 5-stomatal pore (g). Stomata, nail polish technique(e)*

Stomata are present on both side of the leaf characterizing the leaves as amphistomatic. Stomata were generally characterized as diacytic type with two epidermal cells located perpendicular to the cells of the stomata as described by [7].

A distinctive feature for *in vitro* grown plantlets was the presence of high-frequency stomatal clusters. Stomatal cluster are collections of two or more stomata that according to [8] appear in specific conditions of plant growth.

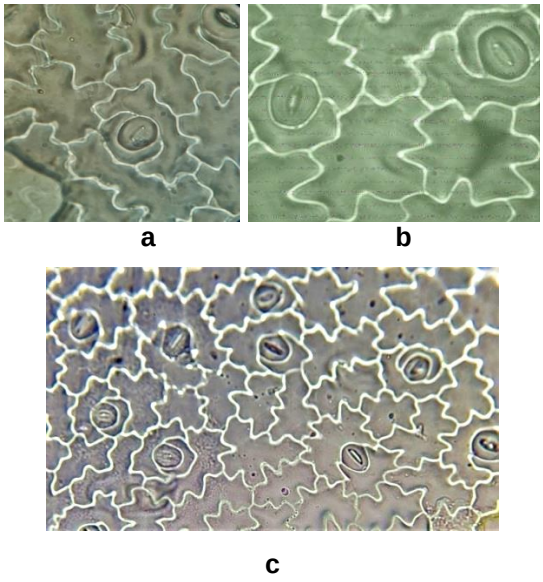


Fig. 4: *Ocimum basilicum var minimum 4 week old in vitro. Nail polish method. Optical light microscope. Stomata in the adaxial leaf surface. Magnification 40x (a), magnification 100X (b), and stomatal clusters (c).*

Trichomes are very well structured even on this early development stage of these *in vitro* plantlets. There are non-glandular trichomes and glandular which are peltate and capitate type (Fig. 5. a,b,c).

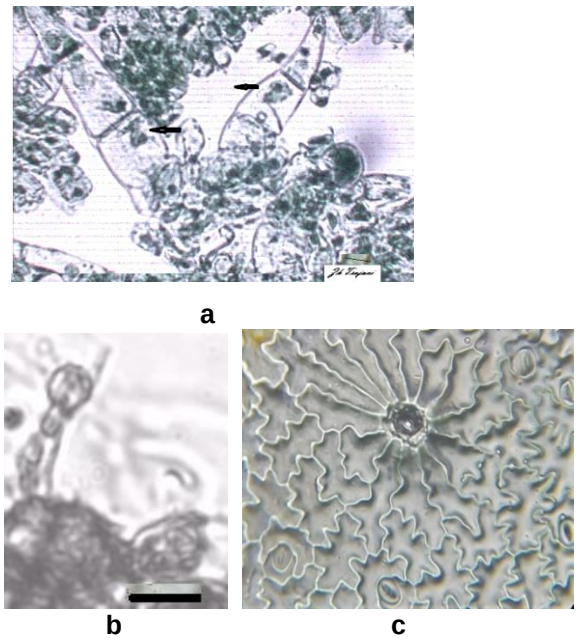


Fig. 5: *Ocimum basilicum var minimum in vitro. 10µm scale. Phase contrast microscope, photo with Paralux camera (a,b). Non-glandular trichomes (a), capitate glandular trichomes (b) and peltate glandular trichomes (c).*

Non-glandular trichomes are often found at different regions on leaves and are unoserial (2-3 cells). The cytology is described on Fig. 6.c.

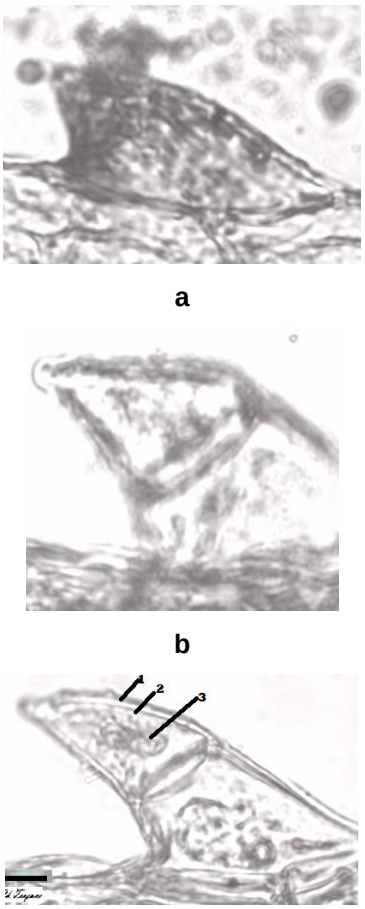


Fig.6: Evolution of non glandular trichomes on *Ocimum basilicum L. var minimum in vitro* culture. 10µm scale. Non glandular trichomes: a- unoseriale with one cell, b-unoseriale with two cells and c-cellular structure of non glandular trichome: 1-cell wall, 2-cytoplasm, 3-nucleus.

Glandular trichomes consisted of peltate and capitate trichomes. We distingushed three different types of capitate hairs Fig. 7.

The first type (1) (Fig. 7. a, b) composed of a bicellular body and a bicellular head; the second type (2) (Fig.7. c) have one-cell formed body with an unicellular elongated head and a basal cell as described by [6] and the third type (3) one basal cell, 2-3 body cells and one elongated cell head not similar with [6].

On *O. basilicum,* the type 2 is more frequent, with larger glandular cells [6].

Type 3 is very rare.

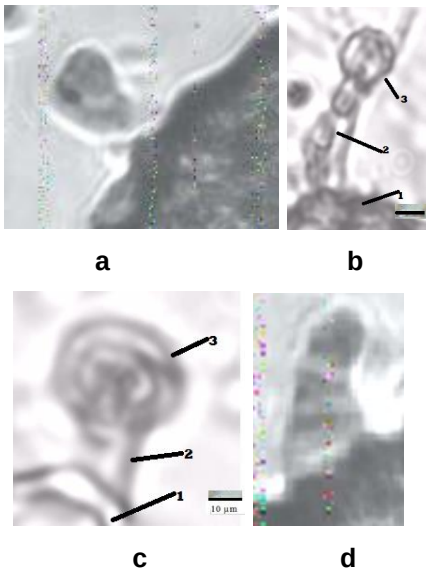


Fig. 7: Glandular trichomes of Ocimum basilicum L. minimum in vitro culture. 10µm scale. Phase contrast microscope.Glandulare capitate trichomes (a, b, c, d,). Capitate hair trichome, Type-1 made of these parts: 1-one basal cell, 2-two body cell, and 3-two head cell (b). Capitate hair type-2 made of these parts: 1-one basal cell, 2- one body cell- and 3- one cell head (c)

The peltate hairs (Fig. 8) are present on both leaf surface but with difference in distribution as they appear more frequent on adaxial leaf surface than on abaxial. Peltate trichomes comprised of four-celled head, a very short, almost non-existent stalk cell and a large basal cell.

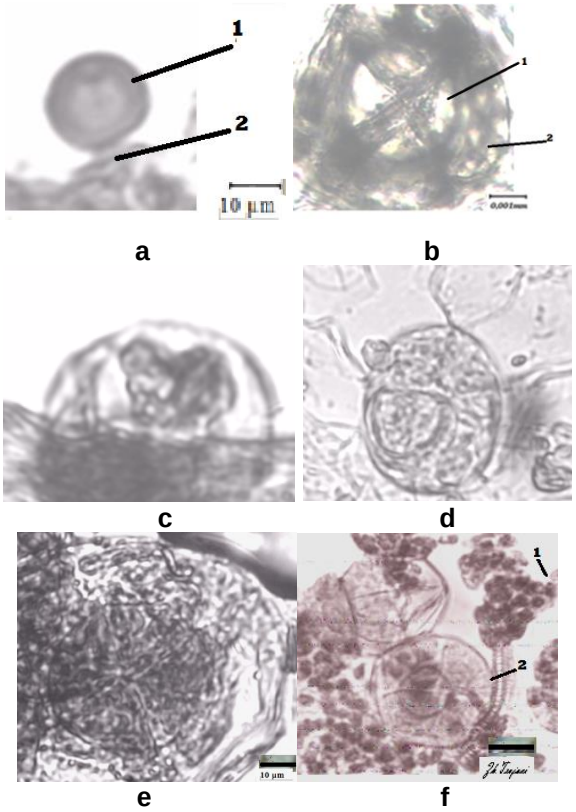


Fig. 8: 10µm scale. *Peltate hairs with 1-four cells head and 2-one cellbody are present on the leaves (a). Top view of peltate trichomes 1- four cell head and 2- one short cell body (b). Different view of peltate trichomes (c, d,e,f).*

Glandular trichomes: peltate and capitate are secretory structure. The highest density of the peltate hairs was noticed on lower epidermis of O. basilicum var. minimum. The preliminary test showed the presence of the phenolic and lipophilic composition. Dedection of phenolic and lipophilic compounds at an earlier stage of growth for vitro explants of *O. basilicum* var. *minimum* (Fig. 9).

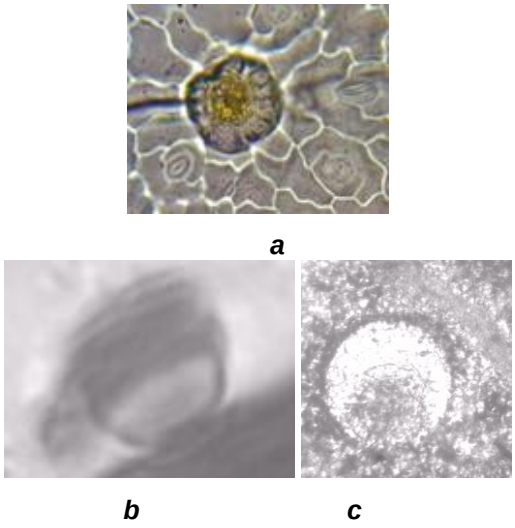


Fig. 9: Ocimum basilicum var minimum. Peltate trichomes (a,b,c). Peltate glandular trichomes with phenolic compounds (b) and peltate trichomes with lipophilic compounds (c).

IV. CONCLUSIONS

During in vitro growth of O. basilicum var. minimum that is cultivated in Albania is noticed that:

The epidermal structures of the leaves, stomata and trichomes, develop at an early stage with all their structural (cellular characteristic) and functional characteristics (production of secondary metabolites) for in vitro cultivated plantlets.

We noticed the presence of three type of capitate trichomes. Type 3 which is not noted in other author. Only one type of peltate trichomes is present.

No structural deviations of these epidermal leaf structures are observed. In vitro cultivation of this species indicates the presence of a high frequency of stomatal clusters, due to specific growing conditions.

Preliminary data indicate the presence of secondary metabolites in small quantity. It is worth noting for a more in-depth study of the function of glandular trichomes, in terms of detecting the type of metabolites and their amount for in vitro growth conditions.

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