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MCM2019

PROCEEDINGS

from the

14th MULTINATIONAL CONGRESS ON MICROSCOPY

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We are honored to host for the first time the Multinational Congress of Microscopy (MCM2019) in Serbia. The aim of MCM conferences is to become a worldwide forum for discussion on different application of various microscopical techniques for both experts and young researchers. MCM conferences have always been a good instrument for establishment of new liaisons between laboratories interested in similar projects. Trade exhibitions also helped to gain insight into the newest development of microscopy

MCM2019 is jointly organized by 8 societies: Austrian Society for Electron Microscopy (ASEM), Croatian Microscopy Society (CMS), Czechoslovak Microscopy Society (CSMS), Hungarian Society for Microscopy (HSM), Italian Society of Microscopical Sciences (SISM), Serbian Society for Microscopy (SSM), Slovenian Society for Microscopy (SDM) and Turkish Society for Electron Microscopy (TEMD)

The bit of history

Extracted from the "Opening lecture" given at the 10th Multinational Congress on Microscopy (Urbino, 4-7 September 2011) by Giuseppe Arancia,, Department of Technology and Health, Italian National Institute of Health Past President and Honorary Member of the Italian Society of Microscopical Sciences.

"In 1990, some representatives of the Italian, Hungarian, Austrian, Yugoslavian and Czechoslovak Societies for Electron Microscopy began to have contacts in order to evaluate the possibility of organizing jointly a multinational congress on electron microscopy. The inspirer reasons of this idea were, mainly, the substitution of a number of small congresses in neighboring countries with a single multinational meeting with the aim of increasing the scientific level and reducing the organizing costs, and to favor interactions and exchange of information and experiences among researchers operating in different countries."

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Micromorphological traits of *Micromeria graeca* (L.) Benth. ex Rchb. (Lamiaceae) leaf glandular trichomes of *in vitro* propagated plants

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1. Introduction

Plants of the genus *Micromeria* Benth. (Lamiaceae) are perennial herbs, subshrubs and shrubs distributed throughout the temperate belt [1]. *Micromeria* species are generally aromatic due to the presence of external glandular structures that produce essential oils, which serve to protect plants against herbivores and pathogens. This natural product isolated from a variety of *Micromeria* species was shown to exhibit antimicrobial, antifungal and antioxidant activities. Due to the socio-economic importance of the essential oil production, glandular trichomes of Lamiaceae species are among the most investigated secretory structures concerning their microporphology, ultrastructure, type and mode of secretion.

M. graeca (L.) Benth. ex Rchb. subsp. *graeca* is a perennial subshrub widely distributed in the Mediterranean area. The plant is pubescent, stout, 10-50 cm in height, has ovate to linear-lanceolate leaves with revolute margins, and flowers in spring. It is used in folk medicine in the Tyrrhenian part of the Basilicata region of southern Italy. This study aimed to record micromorphology and secretion of leaf glandular trichomes of *M. graeca* plants cultured under *in vitro* environmental conditions.

2. Experiment

Plant material – Shoots of wild-growing *M. graeca* plants, dissected into one-node stem segments, were used to establish *in vitro* cultures. Surface sterilized nodal segments were transferred to Murashige and Skoog (MS) culture medium [2] supplemented with 3% (w/v) sucrose, 0.7% (w/v) agar (Torlak, Belgrade) and 0.1% activated charcoal. Shoot multiplication was carried out on the same medium, by routine subculture performed in 5-week intervals.

Scanning electron microscopy (SEM) – For SEM analyses fresh leaves isolated from shoots cultured on MS medium were used. Leaf samples were coated with a thin layer of gold and palladium in a BAL-TEC SCD 005 sputter coater. Samples were examined with a JEOL JSM-6390 LV (JEOL, Tokyo, Japan) SEM operated at 15 kV.

Light microscopy (LM) – Micromorphological and histochemical analyses were performed on hand-sections of fresh leaves. Histochemical test using Sudan IV dye was applied for *in situ* detection of total lipids [3]. Sections were examined and photographed using a Zeiss Axiovert light microscope (Carl Zeiss GmbH, Göttingen, Germany).

3. Results and Discussion

Nodal segments of *M. graeca* cultured on MS medium developed non-branched axillary shoots (Fig. 1). SEM and LM investigations of regenerated plantlets indicated that two types of glandular trichomes, peltate and capitate, were present on their leaf surfaces (Figs. 2-10).

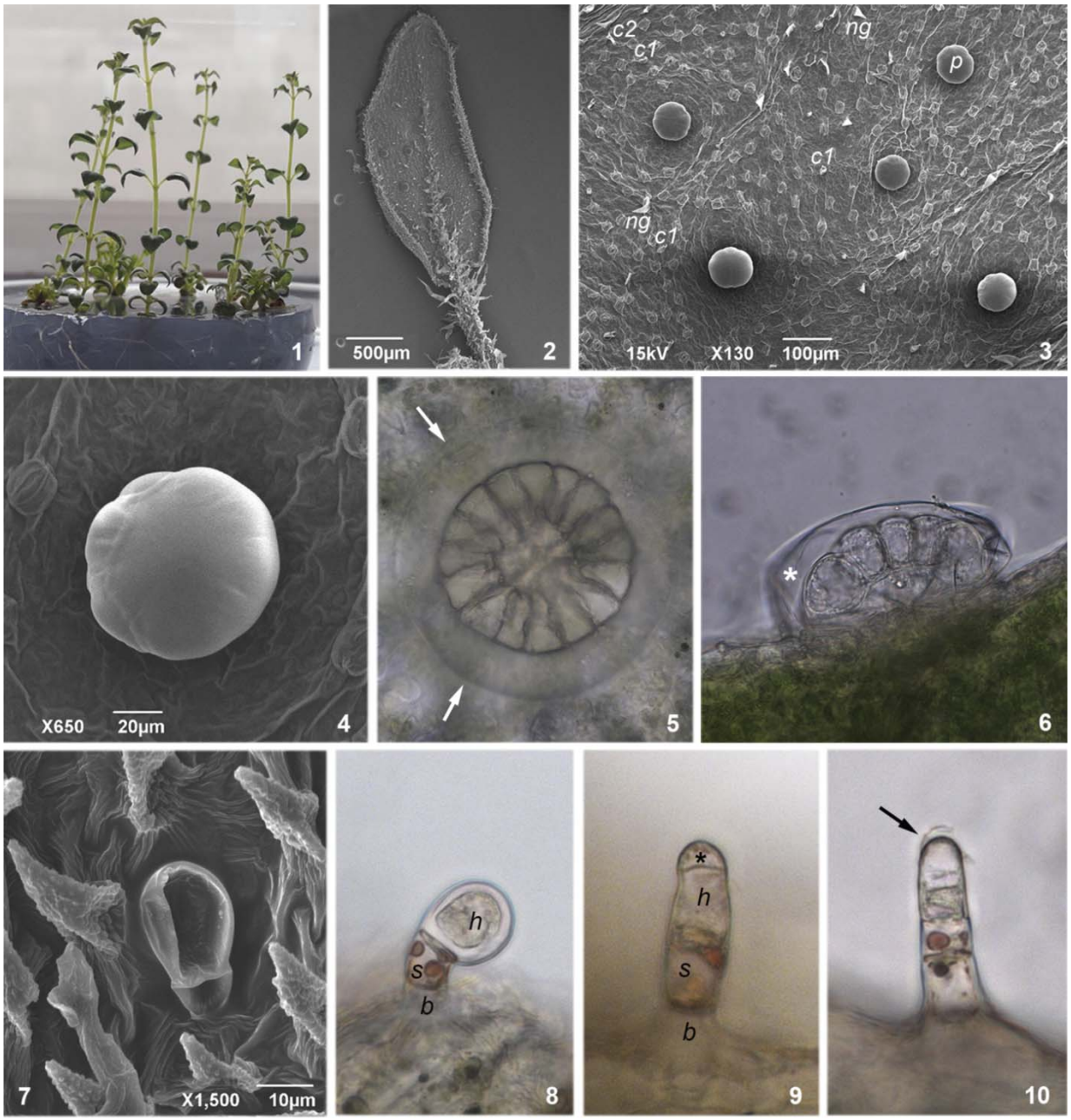

Figure 1. *In vitro* plantlets cultured on MS medium for 5 weeks, used for trichome characterization. Figure 2. Sparsely pubescent young lanceolate leaf with revolute margins. Figure 3. Micromorphology of the abaxial leaf surface of *in vitro* plantlets. Note sharply pointed non-glandular (*ng*) trichomes and two types of glandular trichomes, peltate (*p*) and capitate (*c1*, *c2*). Figure 4. Glandular head of developing peltate trichome on mature *in vitro* leaf, with its cuticle firmly attached to the secretory cell walls. Figure 5. Upper view of mature peltate trichome, with secretory cells arranged in two circles: peripheral, consisting of 16 cells, and central, composed of four cells. Note cuticular cap (*arrows*) detached from the head cell lateral walls. Figure 6. Mature peltate trichome with subcuticular storage cavity (*asterisk*), formed by detachment of the cuticle from the upper cell walls. Figure 7. Upper view of type I capitate trichome clinging to the leaf surface. Figure 8. Type I capitate trichome, with basal epidermal cell (*b*) and short unicellular stalk (*s*) subtending an oblong unicellular secretory head (*h*). Note lipophilic droplets within the stalk cell, after staining with Sudan IV. Figure 9. Type II capitate trichome, with conical basal cell (*b*), elongated stalk (*s*) and cylindrical unicellular secretory head (*h*). Note well-developed round subcuticular storage cavity (*asterisk*) atop the secretory cell. Figure 10. Type II capitate trichome with broken cuticle of the secretory cell. Note remnants of broken cuticle (*arrow*) attached to the secretory cell.

Peltate trichomes (Figs. 4-6) were more frequent on the abaxial leaf surface. They consisted of a broad basal cell, one wide stalk cell, and a glandular head comprising 12-16 peripheral and 4 centrally located secretory cells. On leaves of *in vitro* plantlets, mostly immature peltate trichomes, with cuticle firmly attached to the secretory cell walls, were observed (Fig. 4). During maturation, a storage cavity was formed by the separation of the cuticle from the secretory upper cell walls, rendering these trichomes spherical shape, characteristic of a peltate gland (Figs. 5, 6).

Two types of capitate trichomes could be distinguished on *M. graeca* leaves. Type I capitate trichomes were found on both adaxial and abaxial leaf side, positioned at an angle to the leaf surface (Figs. 7, 8). They were composed of one large basal epidermal cell, cutinized unicellular stalk and unicellular ellipsoidal head (Fig. 8). Cutinization of the stalk cell walls is presumed to prevent apoplastic backflow of trichome-produced compounds, which can be autotoxic to other parts of the plant.

Type II capitate trichomes (Figs. 9, 10) were observed on both adaxial and abaxial leaf surface, but appeared to be less frequent comparing to peltate and type I capitate trichomes. Type II capitate trichomes were composed of one conical basal cell, a stalk comprising two cells, and unicellular secretory head. In young trichomes small subcuticular storage cavity was present (Fig. 9). On mature leaves, their glands commonly had ruptured cuticle (Fig. 10).

Histochemical analysis revealed scarce lipophilic secretion of both peltate and capitate trichomes under *in vitro* conditions. Further optimization of *in vitro* culture conditions is needed in order to increase the production of secondary metabolites in *M. graeca* plantlets.

5. References

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