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Secretory cells in *Piper umbellatum* (Piperaceae) leaves: A new example for the development of idioblasts

Cristina Ribeiro Marinho[a], Adilson Ariza Zacaro[b], Marília Contin Ventrella[a,*]

[a] *Laboratório de Anatomia Vegetal, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil*
[b] *Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil*

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ABSTRACT

This work aims to investigate the origins and development of secretory cells in *Piper umbellatum* (L.) Miq. (Piperaceae) leaves as well as the course and the nature of their secretion. The results were compared with studies in oil-secreting cells of several species. Fully expanded fresh leaves were sectioned and subjected to different histochemical tests. Leaves in different developmental stages were fixed and processed for study under light and scanning and transmission electron microscopy techniques. The secretory cells show mixed secretion made up of hydrophobic (oleoresin) and hydrophilic (phenolic compounds and alkaloids) compounds. Secretory cells originate either from the protodermis or the ground meristem. The growth of these cells occurs primarily by increasing the volume of the central vacuole, which corresponds to an extraplasmatic space connected to a protuberance of the wall. Electron-opaque compounds are observed initially in leucoplasts, while electron-dense compounds occur in small vesicles in the cytoplasm. Both are accumulated in the central vacuole which is already developed. Besides the mixed chemical nature of the secretion identified in secretory cells of *P. umbellatum* leaves, these secretory cells differ from those that have already been described mainly because of the development of the central vacuole prior to the accumulation of the secretion.

Introduction

Secretory cells or secretory idioblasts have been described in several plant families, and a variety of compounds such as oils, resins, mucilage, gums and tannins have been found in the secretion of these cells (Fahn, 1990; Mauseth, 1988). Ontogenetic and ultrastructural studies have shown that there are substantial differences in both the development and the process of secretion among oil-secreting cells and mucilage-secreting cells (Baas and Gregory, 1985; Evert, 2006; Fahn, 1979).

According to the available literature, the accumulation of secretions in oil-secreting cells occurs in an extraplasmatic space similar to a large central vacuole (Maron and Fahn, 1979) also called an oil cavity (Bakker and Gerritsen, 1990) or oil sac (Postek and Tucker, 1983). This extraplasmatic space is bound by a membrane, and it develops from cupule-shaped protuberances at fixed points in the cell wall (Maron and Fahn, 1979). The cell wall of oil-secreting cells usually has a trilamellar structure made up of an outer cellulose layer or primary wall, an intermediary suberin layer, and an inner cellulose layer or tertiary wall, which usually constitute the cupule (Bakker and Gerritsen, 1990; Bakker et al., 1991; Fahn, 1979; Mariani et al., 1989; Maron and Fahn, 1979; Platt and Thomson, 1992; Platt-Aloia et al., 1983).

Conversely, the mucilage-secreting cells have only cellulosic walls, without either the formation of cupules or the deposition of suberin layers. Furthermore, the accumulation of the secretion occurs between the cell wall and the plasma membrane, over the entire periphery of the cell (Baas and Gregory, 1985; Evert, 2006; Fahn, 1979).

Therefore, several authors tend to generalize the developmental course of secretory cells once they create a pattern of development that is directly related to the nature of the secretion (Baas and Gregory, 1985; Bakker and Gerritsen, 1990; Bakker et al., 1991; Mariani et al., 1989; Maron and Fahn, 1979; Platt-Aloia et al., 1983). However, differences are likely to be found in the development of oil-secreting cells in several families such as Annonaceae, Lauraceae, Magnoliaceae and Myristicaceae (Postek and Tucker, 1983; West, 1969).

In Piperaceae, oil cells are considered diagnostic for the family (Cronquist, 1981; Judd et al., 2002). However, at present time we could not find reports on the ontogenesis, histochemistry and ultrastructure of these secretory cells of this family in the literature. In *Piper umbellatum* (L.) Miq. (Piperaceae), which has a wide popular and scientific use as a medicinal species (Atindehou et al., 2004; Braga et al., 2007; Desmarchelier et al., 1997; Ferreira-da-Cruz et al., 2000; Lorenzi and Matos, 2002; Núnez et al., 2005; Perazzo et al.,

* Corresponding author. Tel.: +55 31 38992479.
E-mail address: ventrella@ufv.br (M.C. Ventrella).

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2005; Ropke et al., 2005), secretory cells occur in vegetative and reproductive organs (Moraes et al., 1985, 1986).

Taking into account the importance of *P. umbellatum* as a medicinal plant, and prior studies on the occurrence and distribution of secretory cells in this species, *P. umbellatum* may be considered as an interesting model for the study of secretory cells in Piperaceae. Therefore, the present work aims to investigate the origins and development of secretory cells in leaves of *P. umbellatum* as well as the nature and the course of the secretion, using light microscopy, scanning and transmission electron microscopy techniques. The results were compared with studies in oil-secreting cells of species of different families, in order to determine whether the secretory cells of *P. umbellatum* follow the general pattern of development of oil-secreting cells.

Materials and methods

Plant material

Piper umbellatum (syn. *Pothomorphe umbellata* C. DC.) seedlings 30–60 cm tall were collected at a cultivation area of medicinal plants located at Sítio Violeira in the Viçosa Municipality, State of Minas Gerais, Brazil, and transplanted into plastic pots containing soil and sand (3:1). The seedlings were grown under shade net (35% shade coverage) at the Unidade de Crescimento de Plantas (UCP) of the Departamento de Biologia Vegetal, at the Universidade Federal de Viçosa (UFV), Viçosa Municipality (20°45′S, 42°55′W, average elevation 690 m), State of Minas Gerais, Brazil. Voucher material is kept at the Herbarium of the Universidade Federal de Viçosa under the number VIC 30218.

Light microscopy (LM)

For the histochemical study, fresh samples of the middle region of the first fully expanded leaf were cross-sectioned on a table-microtome (LPC, Rolemberg e Bhering Comércio e Importação Ltda, Belo Horizonte, Brazil). The sections were subjected to different dyes and reagents: Sudan III (Johansen, 1940), Sudan red B (Brundrett et al., 1991) and Sudan black B (Pearse, 1980) for lipids; Nile blue A (Cain, 1947) for acid and neutral lipids; osmium tetroxide (Ganter and Jollés, 1969, 1970) for unsaturated lipids; NADI reagent (David and Carde, 1964) for essential oils and oleoresins; antimony trichloride (Hardman and Sofowora, 1972; Mace et al., 1974) for steroids; concentrated H_2SO_4 (Geissman and Griffin, 1971) and Abraham reaction (Caniato et al., 1989) for sesquiterpenic lactones; 2,4-dinitrophenylhydrazine (Ganter and Jollés, 1969, 1970) for terpenoids with carbonyl group; ferric trichloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for phenolic compounds; vanillin–hydrochloric acid (Mace and Howell, 1974) for tannins; aluminium trichloride and lead neutral acetate (Charriére-Ladreix, 1976) for flavonoids; phloroglucinol (Jensen, 1962) for lignins; Wagner and Dittmar reagents (Furr and Mahlberg, 1981) for alkaloids; periodic acid–Schiff (PAS) reagent (Feder and O'Brien, 1968) for neutral polysaccharides; lugol (Johansen, 1940) for starch; Ruthenium red (Johansen, 1940) for pectins; Alcian blue (Pearse, 1980) for acid mucopolysaccharides; and Xylidine-Ponceau (Vidal, 1977) for proteins. When required, standard control procedures were carried out in parallel with the tests employed.

For the ontogenetic study, samples of leaf primordia and leaves (leaf blade and petiole) at different stages of development were fixed in 2.5% glutaraldehyde in 0.05 M sodium phosphate buffer for 24 h and rinsed three times, for 10 min each, in the same buffer. The samples were dehydrated in ethanol series and embedded in methacrylate (Historesin – Leica), according to the manufacturer's recommendations. Cross and longitudinal sections 5 μm thick of the material were made with a rotary microtome. Slides were stained with Toluidine blue (O'Brien et al., 1964) and mounted on synthetic resin (Permount). Sudan red B, Ruthenium red and PAS tests were also applied in embedded-material in order to monitor the accumulation of secretion of the secretory cells in development.

Scanning electron microscopy (SEM)

Samples of fully expanded leaves of *P. umbellatum*, measuring about 0.25 cm^2, were fixed in FAA_{50} for 48 h and stored in ethanol 70% (Johansen, 1940). The samples were dehydrated in ethanol series and subjected to critical point drying in CO_2 using appropriate equipment (CPD 030, Bal-Tec, Balzers, Liechtenstein). Samples were mounted on stubs with double-face tape, and covered in gold using a Sputter Coater equipment (FDU 010, Balzers Union, Balzers, Liechtenstein). Observation and image capture were performed in a scanning electron microscope (1430VP, LEO, Cambridge, UK).

Transmission electron microscopy (TEM)

For the ultrastructural characterization of secretory cells, samples of approximately 1 mm^2 of leaf primordia and leaves at different stages of development were fixed in 2.5% glutaraldehyde in 0.05 M sodium phosphate buffer, pH 7.0, for 24 h and rinsed in the same buffer six times, for 10 min each. The material was then post-fixed in 1% osmium tetroxide in 0.05 M phosphate buffer for 3 h at room temperature and rinsed in the same buffer six times, for 10 min each. After dehydration in ethanol series, samples were embedded in epoxy resin (Spurr). Ultrathin sections (60–90 nm) were made on an ultramicrotome (Porter-Blum MT2-B, DuPont-Sorvall, Newtown, USA), collected on copper grids covered with FormVar and contrasted with uranyl acetate and lead citrate. The sections were examined and photographed in a transmission electron microscope (EM 109, Zeiss, New York, USA) of 80 kV.

Results

Structure and histochemical analysis of the secretory cells

In the early stages of leaf primordium development (Fig. 1A), secretory cells are already present. These cells are conspicuous, have a spherical to oval shape, and are abundant in *P. umbellatum* leaves (Fig. 1B–F). In the leaf blade, the secretory cells occur among the epidermal cells on both adaxial and abaxial surface (Fig. 1C, D), lodged in a slight depression or at the same level of the neighboring cells. In paradermal section observations, the secretory cells are surrounded by elongated epidermal cells arranged radially to form a rosette (Fig. 1E). In the large-diameter veins and petioles, these structures are located mainly in the ground parenchyma, but also exist among the epidermal cells (Fig. 1B).

Cross-sections of fresh leaves, not subjected to any kind of dye or reagent, present secretory cells with a translucent secretion of oily appearance (Fig. 2A), which in few hours after the collection change color, varying from dark orange (Fig. 2B) to brown (Fig. 2C). According to histochemical tests, it was found that the secretion of the secretory cells is rich in lipids, which are orange, red, and black when stained with Sudan III, Sudan red B (Fig. 2D), and Sudan black B (Fig. 2E), respectively. The secretion of these structures is dominated by neutral lipids, which are red stained with Nile blue (Fig. 2F), and unsaturated lipids, black stained with osmium tetroxide (Fig. 2G). Part of the lipid material is characterized as resin acids, indicated by the reddish color produced by the NADI reagent (Fig. 2H). The secretion of some secretory cells shows purple color when exposed to the NADI reagent, indicating a mixture of resins