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The role of fibres and the hypodermis in Compositae melanin secretion

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ABSTRACT

Melanins are dark, insoluble pigments that are resistant to concentrated acids and bleaching by oxidising agents. Phytomelanin (or phytomelan) is present in the seed coat of some Asparagales and in the fruits of some Compositae. In Compositae fruits, melanin is deposited in the schizogenous spaces between the hypodermis and underlying fibrous layer. Phytomelanin in Compositae is poorly understood, and there are only speculations regarding the cells that produce the pigment and the cellular processes involved in the secretion and polymerisation of phytomelanin. This report describes the cellular processes involved in the secretion of phytomelanin in the pericarp of *Praxelis diffusa*, a species with a structure typical of the family. The ovaries and fruits at different stages were fixed and processed according to the standard methods of studies of light microscopy and transmission electron microscopy. Hypodermal cells have abundant rough endoplasmic reticulum and mitochondria, and the nuclei have chromatin that is less dense than other cells. These characteristics are typical of cells that synthesise protein/amino acids and suggest no carbohydrate secretion. The fibres, however, have a dense cytoplasm rich in the Golgi bodies that are associated with vesicles and smooth endoplasmic reticulum, common characteristics of carbohydrate secretory cells. Our results indicate that the hypodermal cells are not responsible for the secretion of phytomelanin, as previously described in the literature; in contrast, this function is assigned to the adjacent fibres, which have an organisation typical of cells that secrete carbohydrates.

1. Introduction

Melanins are dark, insoluble pigments that are resistant to degradation by concentrated acids and bleaching by oxidising agents (Nicolaus et al., 1964). These substances can be found in plants, animals and microorganisms (Bell and Wheeler, 1986; Kotob et al., 1995; Hill, 1997; Gibson and George, 1998; Prota et al., 1998; Sarna and Swartz, 1998). In plants, a compound known as phytomelanin (or phytomelan) is present in the seed coat some taxa of Asparagales (Dahlgren and Clifford, 1982) and the fruits of certain Compositae (Pandey and Singh, 1982; Pandey et al., 1989; Marzinek et al., 2008, 2010; Marzinek and Oliveira, 2010).

Fruits with phytomelanin are found in approximately 5400 species in 460 genera, including 11 tribes, and form a monophyletic group called the Phytomelanin Cypsela Clade (PCC), with neotropical representatives accounting for approximately one quarter of the species of Compositae (Panero and Funk, 2008). Beyond the PCC, the presence of phytomelanin was confirmed in fruits of subtribe Sipolisiinae (Vernonieae; Loeuille, 2011). It is believed that the adaptive value of phytomelanin is related to the protection of the fruit against predation (Johnson and Beard, 1977). However, it is possible that the occurrence of phytomelanin has been underestimated in the family, as there are recent reports of its presence in the vegetative organs of tribe Cardueae (Fritz and Saukel, 2011).

Despite its importance in the family, the processes involved in forming the phytomelanin layer remain unclear; indeed, several authors have debated for a century about which pericarp tissue is responsible for its production, what the cellular mechanisms are and how the polymerisation occurs. Hanausek (1912) suggested that phytomelanin is formed by the modification of cell wall fibres. Sárkány (1947) proposed that the layer of phytomelanin may be produced by the hydration of the inner wall of the hypodermis, whereas Knowles (1978) indicated that this layer would be formed by lysis of the fibres. Rogers et al. (1982) suggested that the phytomelanin layer may be the result of the lysis of hypodermal cells discharging their content into the fibres. De Vries (1948), Misra (1964, 1972), Pandey and Singh (1982, 1983) and Pandey et al.

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(1989) proposed that the layer phytomelanin is secreted by the hypodermis. Pandey et al. (1989) also observed the occurrence of endoplasmic reticulum (apparently smooth) in the hypodermis, thus enhancing the secretory potential of hypodermal cells. According to certain authors, phytomelanin precursors are synthesised in the hypodermis and polymerised by enzymatic activity in the cell wall of the fibres. However, there are no reports of secretion in the hypodermis or fibres, and the cellular processes involved in melanin production in Compositae remain unknown.

Here, we describe the cellular processes involved in the production of phytomelanin in Compositae and clarify the role of the hypodermis and fibres in the synthesis and deposition of this compound. In addition, we discuss the possible pathways of secretion, as compared with other groups of plants that produce this pigment.

2. Material and methods

Praxelis diffusa (Rich.) Pruski plants were collected in ruderal areas in the region of Botucatu, São Paulo, Brazil (22°53′11,4″S, 48°26′07,8″W). A voucher specimen was deposited at the Herbarium BOTU (Holmgren et al., 1990) under accession number 25,555.

For light microscopy (LM), the ovary and fruit at different stages were fixed in FAA 50 for 48 h (Johansen, 1940) and stored in 70% ethanol (Jensen, 1962). The samples were dehydrated through an ethanol series and embedded in methacrylate (Leica), according to the manufacturer's instructions. Transverse and longitudinal sections (8 μm) were stained with toluidine blue (0.05% in 0.1 M acetate buffer, pH 4.7) (O'Brien et al., 1964 modified), mounted in synthetic resin and observed using an Olympus BX41 light microscope.

For transmission electron microscopy (TEM), the samples were fixed in glutaraldehyde (2.5% in 0.1 M phosphate buffer, pH 7.3) for 24 h, post-fixed in osmium tetroxide (1% in 0.1 M phosphate buffer, pH 7.3) incubated in uranyl acetate (0.5% aqueous solution), dehydrated through an acetonic series and embedded in Araldite. Ultrathin sections (50 nm) were contrasted using a saturated solution of uranyl acetate and lead citrate (Reynolds, 1963) and examined using a TEM Philips EM 100 microscope at 80 kV.

3. Results

3.1. Flower buds

The outer ovarian epidermis is uniseriate, with cuboids cells. The mesophyll features two regions: a hypodermis with two layers (slightly elongated cells) and an inner region with four to five layers (highly elongated cells). The inner epidermis is uniseriate, also with highly elongated cells (Fig. 1a and b).

The early development of the phytomelanin layer is characterised by the formation of intercellular spaces between the hypodermis and underlying layer (Fig. 1c), as evidenced by a degraded middle lamella (Fig. 1d). The cytoplasm of cells of the hypodermis and underlying layer is restricted to the periphery (Fig. 1c–f) and contains Golgi bodies, mitochondria, plastids with plastoglobulin (Fig. 1e), a large central vacuole (Fig. 1c–f) and a nucleus with condensed chromatin (Fig. 1f).

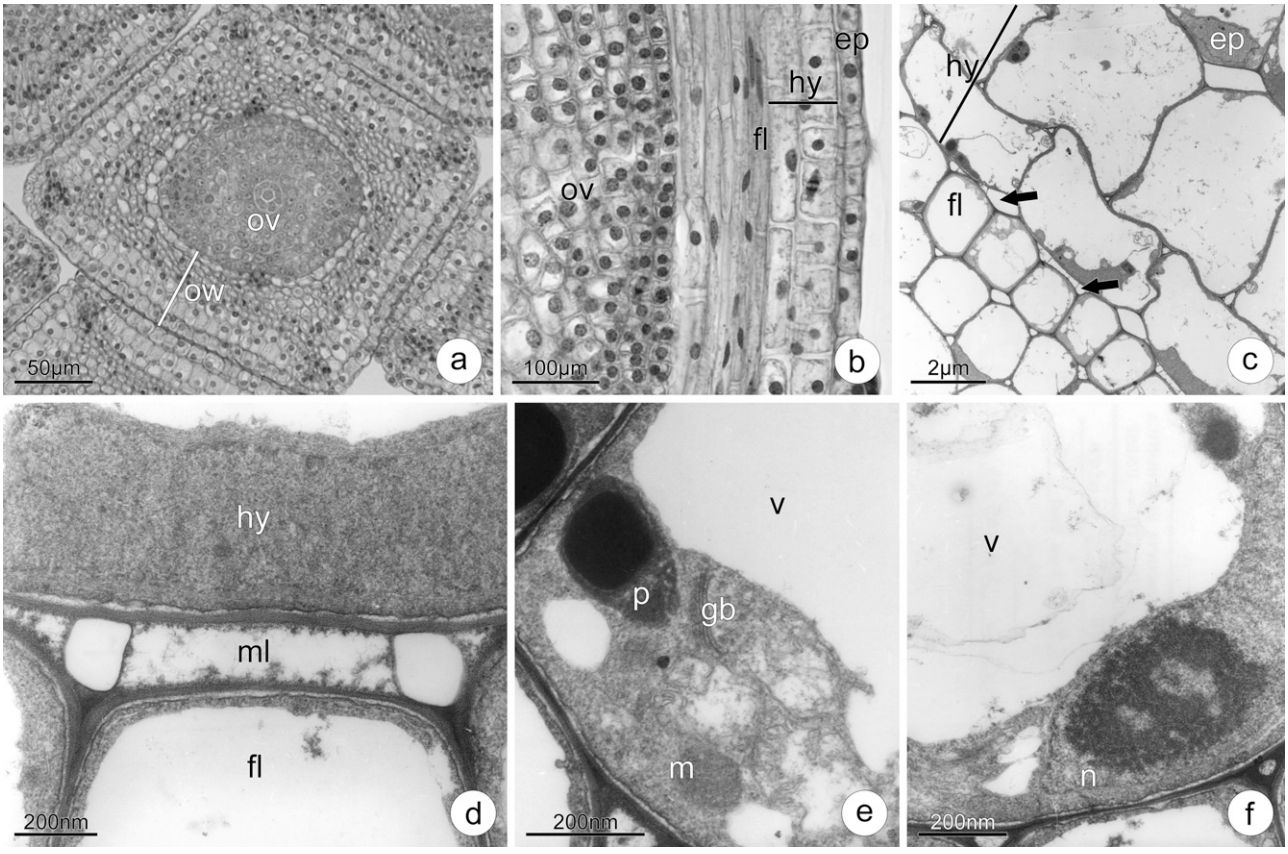

Fig. 1. Flower buds of *P. diffusa* during the early development of the schizogenous space where the phytomelanin layer will be deposited. (a and b light micrographs): (a) Ovary transversal section; (b) Detail of the ovary wall in longitudinal section; (c–f TEM micrographs from transverse sections): (c) Overview of the ovary wall showing the epidermis, two layers of hypodermis, a layer of undifferentiated fibres and the remaining undifferentiated mesophyll, note the beginning of the formation of the schizogenous space (arrow); (d) Detail of the previous panel, showing the separation of the hypodermis and undifferentiated fibres; (e) Details of undifferentiated fibres showing the organelles; (f) Detail of undifferentiated fibres showing the nucleus with condensed chromatin (ep: epidermis; fl: fibre layer; gb: Golgi bodies; hy: hypodermis; m: mitochondria; ml: middle lamella; n: nucleus; ov: ovule; ow: ovary wall; p: plastid; v: vacuole).