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Transfer cells in trichomatous nectary in *Adenocalymma magnificum* (Bignoniaceae)

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ABSTRACT

Transfer cells are specialized cells that play an important role where there are high energy costs due to facilitation of transmembrane flow of solutes. This study aimed to investigate the ontogenesis, histochemistry and ultrastructure of glandular trichomes focusing on stalk cells and their possible transfer function. Samples of prophylls of axillary buds, calyces of flowers in anthesis, and flower buds in different stages of development were collected, fixed and processed according to the common methods of microscopy. The glandular trichomes are composed of a secretory head with its cells in columnar format. The stalk is formed by two layers of cells, with the upper layer composed of cuboidal cells where the wall starts to thicken at the beginning of the pre-secretory phase. The secretion is heterogeneous, releasing glucose, other carbohydrates, lipids and phenolic compounds, with two types of release - eccrine and granulocrine. These trichomes are functionally termed as nectaries. The stalk cells appear as transfer cells since they have a thicker anticlinal wall with irregular ingrowths. The presence of transfer cells in the nectaries suggests a high specialization because it improves transport capacity of nectar and compensation in the high energy expenditure for its production and release.

Key words: glandular trichomes, nectaries, ontogenesis, secretory structures, wall ingrowths.

INTRODUCTION

Transfer cells are highly specialized cells and occur in vegetative and reproductive organs, especially in places where the demand for an intensive transport of solutes through the membrane, is high (Gunning and Pate 1974).

For most cells, the primary wall is deposited uniformly over all faces of the cell during expansion (Carpita and Gibeau 1993). When expansion is completed, some plant cells produce a thick secondary wall (Talbot et al. 2001). In

transfer cells the thickness of the secondary wall is not uniformly formed by invaginated projections, which characterize these cell types (Gunning and Pate 1974).

The deposition of these wall ingrowths amplifies the surface area of the plasma membrane, which hypothetically facilitates the transmembrane flow and exchange of solutions between apoplast and symplast (Pate and Gunning 1972). Because of this specialization, transfer cells are found in various organs, such as gametophytic cells (Diboll and Larson 1966, Cass 1972, Wilms 1981, Bohdanowicz and Turala-Szybowska 1985, 1987) and vascular tissues, specifically in the parenchyma

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cells of the xylem and companion cells of the phloem (Pate and Gunning 1969, Gunning et al. 1970, Talbot et al. 2002).

Transfer cells, due to their function of intensifying the transport of solutes over short distances, play a very important role in secretory structures that require this flow to release substances (Offler et al. 2003). These cells are common in different secretory tissues, such as nectaries, hydathodes and glands, in general (Fahn 1979a), because regions that are closely involved in secretion and absorption have this kind of cell as an integral part of their morphology (Dahiya and Brewin 2000). Research from Schnepf (1964) and Gunning and Pate (1969) revealed that the beginning of ingrowths are related to the physiological events of nectar secretion.

Nectaries are structures characterized by the secretion of a sugary and heterogeneous solution (Baker and Baker 1982, Nicolson et al. 2007). Among the compounds of this solution which require more energy for production, are lipids (Nicolson and Thornburg 2007). There are several works with nectaries in Bignoniaceae, of which the most comprehensive is that of Seibert (1948), Rivera (2000a, b), Gonzalez (2011) and Nogueira et al. (2013). Despite the large number of studies, little is known about the ontogeny and ultrastructure of these nectaries.

Previous anatomic studies in *Adenocalymma* Mart. ex Meisn. (e.g. Laroche 1974) showed the presence of a layer of cells with thickened anticlinal walls, which prompted this research. The goals of the current research were to analyze the ontogenesis, histochemistry and ultrastructure of glandular trichomes, focusing on stalk cells and their possible transfer function.

MATERIALS AND METHODS

BOTANICAL MATERIAL

Specimens of *Adenocalymma magnificum* Mart. ex DC. were collected along the road of Marudá

city (PA-318) in the state of Pará, Brazil, for herborization and procedures in plant anatomy. Fresh prophylls of axillary buds (first to fifth node), calyx of the flower in anthesis, and flower buds in different stages of development were collected and fixed. Glucose paper strips were used to analyze the presence of glucose in the secretion. Voucher specimens were prepared and deposited in the herbarium João Murça Pires (MG 203513) and the glandular trichome morphology was classified based on Nogueira et al. 2013.

LIGHT MICROSCOPY AND HISTOCHEMICAL TESTS

For the anatomical analysis over light microscopy, samples were fixed in 50% FAA and neutral buffered formalin for 24 and 48 h, respectively, dehydrated in a butyl series (tertiary butyl alcohol; Johansen 1940), and embedded in Paraplast (Leica Microsystems Inc., Heidelberg, Germany). Cross and longitudinal sections (8–13µm) were made with the assistance of a rotary microtome and later stained with Safranin and Astra Blue (Gerlach 1984). The following histochemical tests were performed in fresh hand-cut sections: PAS reaction, to detect the presence of carbohydrates (McManus 1948); Sudan Black B, for lipids (Pearse 1980); Ferric Chloride, for phenolic compounds (Johansen 1940); and NADI for terpenoids (David and Carde 1964).

ELECTRON MICROSCOPY

The material designated for transmission electron microscopy (TEM) analysis was fixed in 2.5% glutaraldehyde for 24 h, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 h, pH 7.2, dehydrated in an acetone-solution series and embedded in Spurr resin (Roland 1978). Ultrathin sections were made and counterstained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963) for further analysis on a Zeiss EM 900 electron microscope.

The samples designated for scanning electron microscopy (SEM) analysis were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, dehydrated in an ethanol series, and critical-point dried (Robards 1978). The dried material was placed over aluminum stubs and examined using a LEO 1450 VP scanning electron microscope.

For confocal microscopy analysis, the samples were fixed in 50% FAA, clarified with sodium hypochlorite, and stained with 0.01% Acridine Orange (D. Demarco, personal communication).

RESULTS

NECTARY STRUCTURE

The glandular trichome found here is classified as patelliform, due to its disc-shaped morphology. The patelliform trichome occurs on vegetative and reproductive organs in all stages of development (Fig. 1a-b). Since the glucose paper test strips revealed the presence of glucose in the secretion of these trichomes, the nectary may be classified as a functional nectary.

The nectary is structurally a glandular trichome formed by one secretory head and stalk. The head consists of a secretory epidermis bi- or multi-stratified, presenting column format with a thin wall, while the stalk is formed by two layers of cells, in which the upper layer is comprised of cuboids cells with thick walls. The trichome is on the surface of the organ (Fig. 1c), with ordinary epidermal cells surrounding these trichomes (Fig. 1d-g).

ONTOGENESIS AND SECRETION

The nectary presents asynchronous development and has protodermic origin. It begins from one cell which undergoes anticlinal division, forming a series of cells with columnar shape (Fig. 1c). Concomitantly, periclinal divisions occur, which form a row corresponding to the stalk cells (Fig. 1d-f). The stalk cells undergo asymmetric anticlinal division, originating a short stalk with wide cells

and thin anticlinal walls. The anticlinal wall remains thin in order to begin the pre-secretory phase, thereafter these cells thicken.

The nectary, due to its protodermic origin, does not have direct vascular supply although phloem strands are observed two layers bellow of the stalk cell (Fig. 1g). In the secretory phase, the cuticle stretches in the central portion of the nectary and the anticlinal walls of stalk cells become thick (Fig. 1h-i).

The histochemistry analysis demonstrates the presence of carbohydrates, lipids and phenolic compounds on the nectariferous tissue (Fig. 1j-l).

ULTRASTRUCTURAL ASPECTS

The ultrastructure of secretory cells indicates that the vacuome is dispersed and has lipid drops (Fig. 2a), mainly in the lower and upper region of the cell. Mitochondria, endoplasmic reticulum and leucoplasts (Fig. 2b) are abundant in these cells and are related to the secretory process. The dictyosomes (Fig. 2c) are not as representative at this stage when found close to the vesicles and cell wall.

Hydrophilic substances may have different release modes depending on the nectary region and are commonly released by projections in the wall cell (Fig. 2d); however, in the central region of the nectary was observed only disruption of the cuticle. Lipids and phenolic compounds are surrounded by a membrane, forming vesicles that fuse with the plasma membrane (Fig. 2e). The nectary remains intact after the release of both secretions.

The basal cells of the stalk show ultrastructural features of cytoplasm similar to those of the ordinary epidermal cells. However, plasmodesmata were seen between stalk cells, indicating that there was a route of substances via symplast (Fig. 3a).

The layer of stalk cells adjacent to the secretory cells is peculiar in this species, because these cells present thick anticlinal walls formed by ingrowths and present plasmodesmata in the periclinal walls, which connect the stalk cells to the secretory

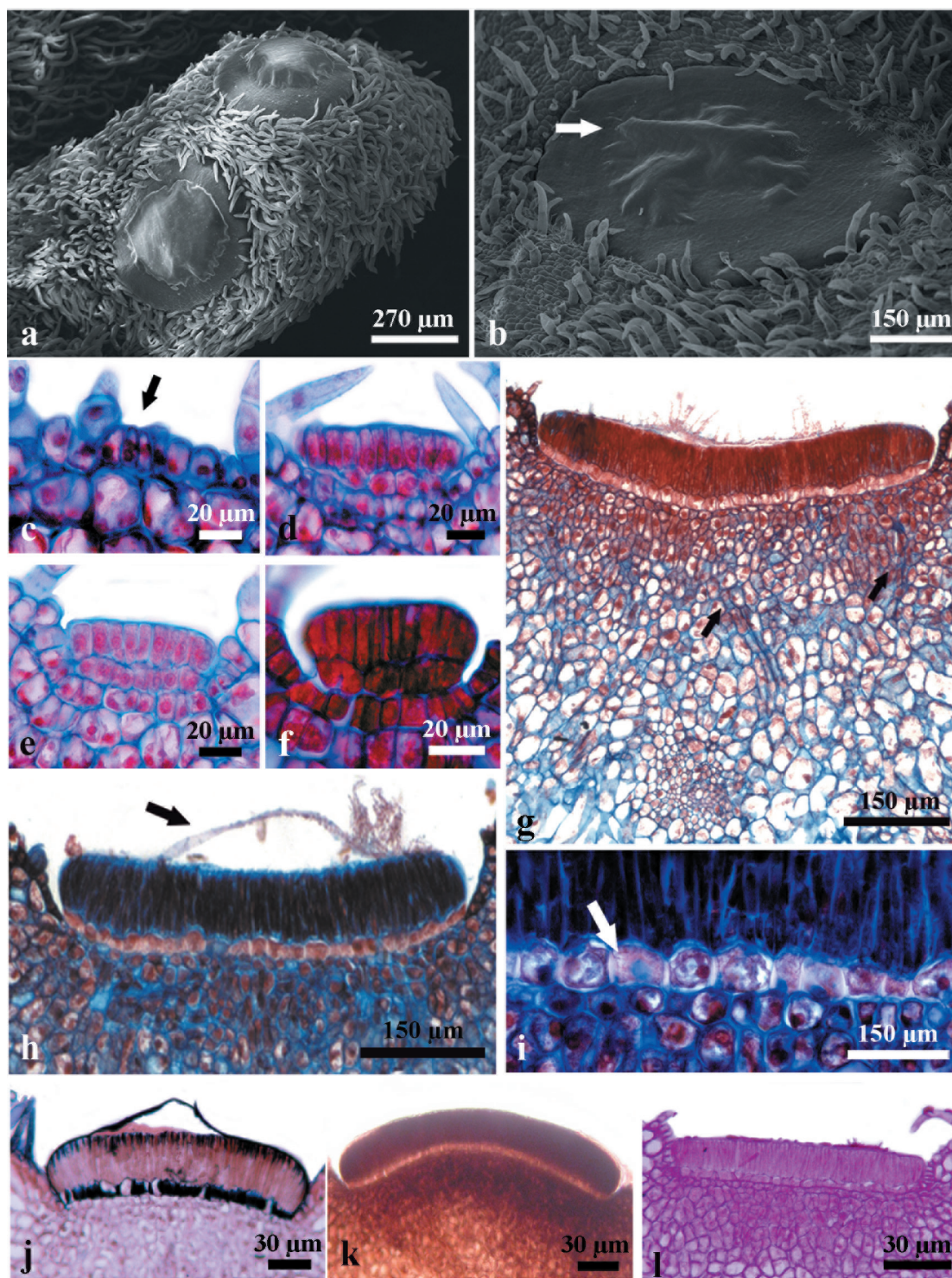


Figure 1 - Structure of the phases of development of the nectaries of *Adenocalymma magnificum*. (a,b) scanning electron microscopy; (a) nectary morphology on prophylls of axillary buds; (b) calyx of flower in anthesis, detail of the nectary in secretory phase (arrow). (c-f) development phases; (c) first phase; (d-f) intermediate phase showing anticlinal divisions of the stalk cells. (g-i) Adult nectary; (g) detail of phloem strands (arrow); (h) detail of disruption of the cuticle in the central portion (arrow); (i) detail of thicker anticlinal wall. (j-l) histochemistry tests; (j) sudan Black B; (k) ferric chloride; (l) PAS reaction (see the colors in the online version).

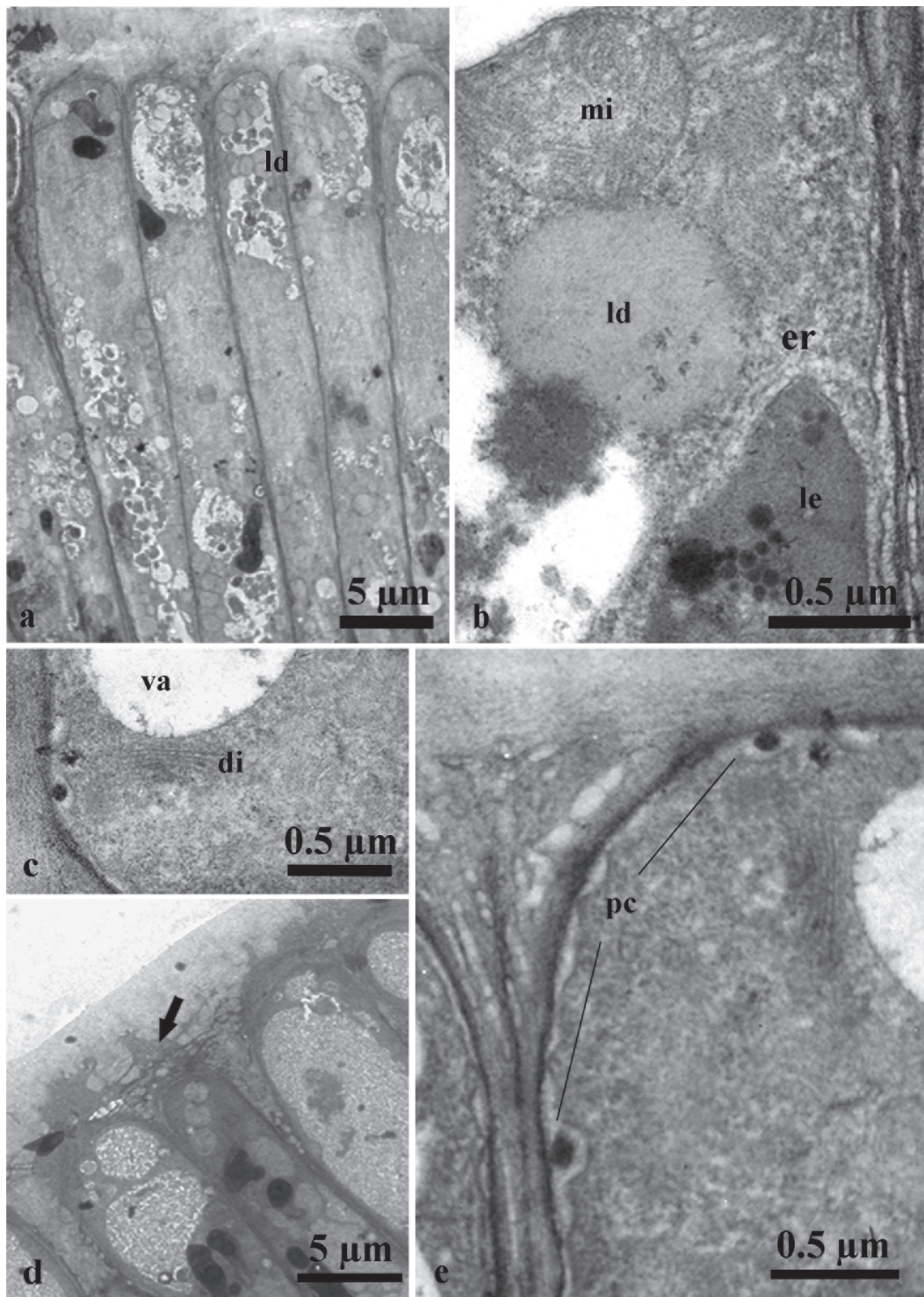


Figure 2 - Aspects of the ultrastructure of nectaries of *Adenocalymma magnificum*. Secretory cells. (a) secretory cells in palisade, showing lipid droplets; (b) most representative organelles in these cells, mitochondria, endoplasmic reticulum and leucoplasts; (c) dictyosomes close to the vesicles; (d) projections of the wall cell into the cuticle (arrow); (e) vesicles releasing phenolic compounds. Abbreviations: (di) dictyosomes, (er) endoplasmic reticulum, (ld) lipid droplets, (le) leucoplasts, (mi) mitochondria, (pc) phenolic compounds, (va) vacuole.

cells (Fig. 3a). Having this morphology, they are functionally called transfer cells. The transfer cells present a dispersed vacuome with electron-

dense content (Fig. 3b) and many mitochondria concentrated along the outer periclinal wall next to the secretory cells (Fig. 3c).

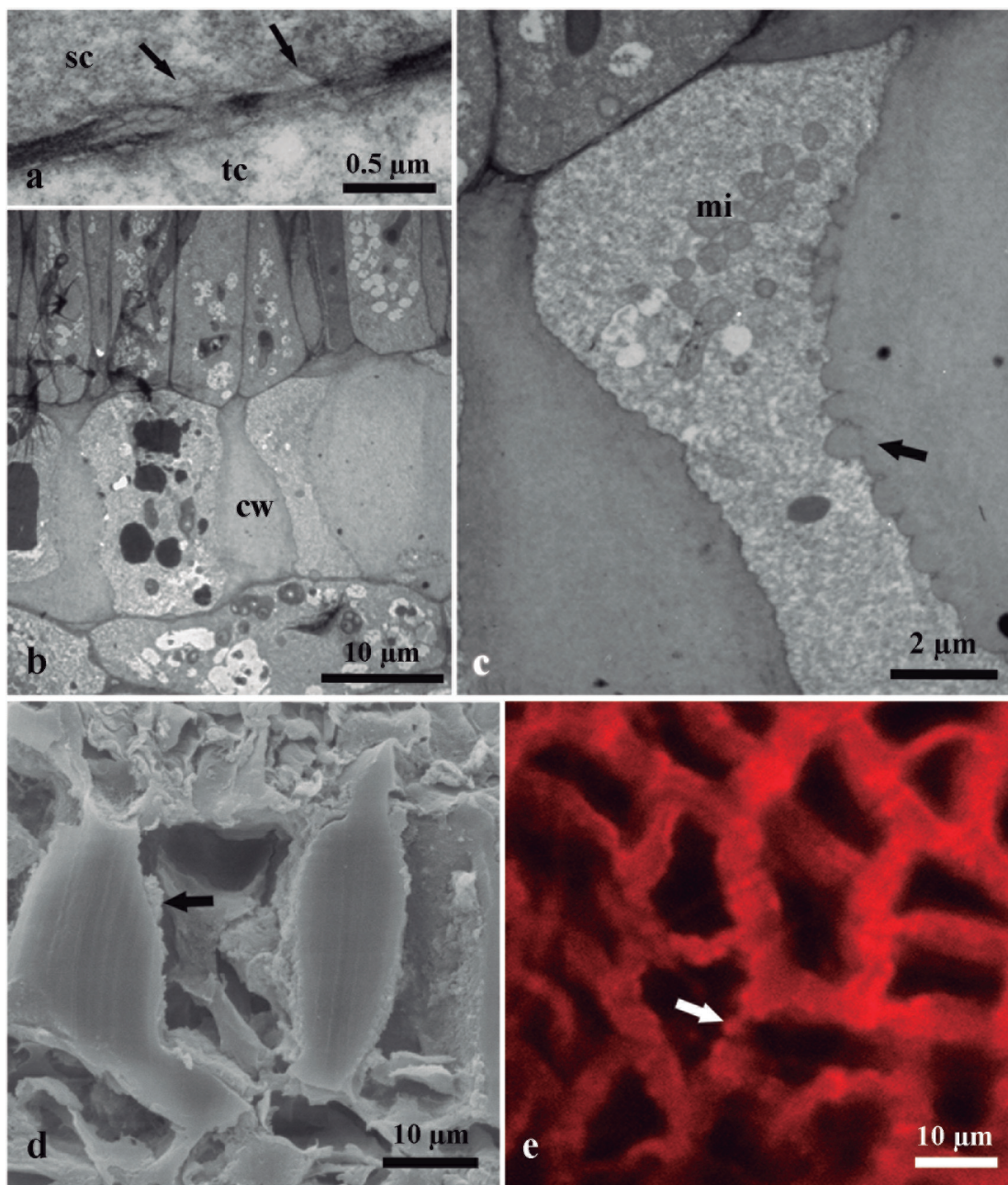


Figure 3 - Aspects of the structure of transfer cells of *Adenocalymma magnificum*. (a-c) Transmission electron microscopy; (a) plasmodesmata between stalk cells and secretory cells (arrow); (b) dispersed vacuome with electron-dense content; (c) agglomerate of mitochondria in transfers cells, detail of wall ingrowths (arrow); (d) scanning electron microscopy of wall ingrowths formed by small papillae (arrow); (e) overview of papillae in transfer cell walls using confocal microscopy (arrow). Abbreviations: (cw) cell wall, (mi) mitochondria, (sc) secretory cell, (tc) transfer cell (see the colors in the online version).

INGROWTH CHARACTERISTICS

The wall ingrowths are characterized as reticulate, as deposition begins with the formation of small papillae, which can present different sizes according to their development. Those occurring in *A. magnificum* emerge from the secondary wall and occasionally merge with neighboring papillae, but this process does not culminate in a complex labyrinthine network (Fig. 3d, e).

DISCUSSION

The presence of glucose in the secretion of trichomes of *A. magnificum* makes this structure be a nectary, as seen in another species of Bignoniaceae (e.g. Belmonte et al. 1994, Rivera 2000, Lopes et al. 2002, Nogueira et al. 2013). However, in *A. magnificum* different events occur simultaneously, which were understood by the manner in which a heterogeneous secretion was released in the presence of chemical substances produced by various metabolic pathways, unique to secretory structures. The sugars from phloem are largely transferred by the stalk cells and then released by wall projections. The molecules cross the plasma membrane of the secretory cells as a result of a concentration gradient or active process (Fahn 1979b), which also occurs in *Gasteria* and in some other Liliaceae (Schnepf 1964, Lüttge and Schnepf 1976). This possibility of active transport of sugars into the nectary of *Abutilon* was discussed by Findlay et al. (1971).

In *A. magnificum* the lipid substances are secreted by vesicles into the external environment by exocytosis, this mode of transport is classified as granulocrine secretion. The process of fusion between membranes and plasmalemma was observed by Fahn (1988).

In the secretory cells, some organelles were quite evident, such as mitochondria, which is involved in energy production (Nepi 2007), and leucoplasts and endoplasmic reticulum, which are

the two organelles involved in the production of lipids of *A. magnificum*. Although the endoplasmic reticulum is involved in this production, it is possible that it is also related to the transport of nectar inside the cell. According to Heinrich (1975), this organelle actively participates in the transport of nectar in *Aloe*, where there is high ATPase activity, nucleoside diphosphatase, and glucose-6-phosphatase in the endoplasmic reticulum of nectaries and generally no activity of these phosphatases in the plasma membrane. The same is supported by Fahn (2000) and Stpiczyńska et al. (2005), who state that the predominance of smooth reticulum is associated with lipid metabolism. Another organelle noticed was the dictyosome, which are associated with the release of nectar (Fahn 1979b, Kronstedt-Robards et al. 1986, Sawidis et al. 1987, 1989).

Studies have indicated that transfer cells exist in these nectaries, which facilitate the transport of solutes over short distance by transmembrane flow (Pate and Gunning 1972). Transfer cells are apparently restricted to situations where the area-volume correlations are not equivalent (i.e., the area is smaller than the volume) or when the solute transported is accompanied by a minimum flow of solvent. The presence of these specialized cells suggests that selection pressures of a physiological nature may have shaped the evolution of the transfer cell (Gunning and Pate 1969).

Morphologically, transfer cells found in this study appear with thin anticlinal walls at certain times and with thick walls at posterior times. The onset of wall thickening occurs when the glandular cells are in the pre-secretory phase. This information corroborates the conclusions of Schnepf (1964), who states that the proliferation of the cell ingrowths is related to physiological events in nectar secretion of *Gasteria*, in which cell wall thickening begins when it is in the pre-secretory phase and ends in the secretory phase, followed by degeneration and reabsorption in the post-secretory phase.

Observations about the secretory cells of the extrafloral nectary of *Vicia faba* L. lead to the conclusion that invaginations are not formed until the start of sugar secretion (Gunning and Pate 1969). In contrast, the post-secretory stage cell wall underwent redifferentiation in *Gasteria* and *Aloe*; ingrowths disappeared and the cell wall became covered by a “third layer” (Schnepf and Pross 1976).

Generally, transfer cells are highly polarized and develop only in areas where there is intensive transmembrane transport activity, e.g., areas bordering xylem elements or adjacent to sieve tubes, companion cells, in addition to adjacent intercellular spaces (Pate and Gunning 1972). Due to the functionality promoted by ingrowths, it is common to find them in secretory cells. Several studies of nectaries have reported the presence of these cells, e.g., perigonal nectaries of Fritillary (Stpiczyńska et al. 2012) and septal nectaries of *Tillandsia* (Fiordi and Panandri 1982), *Gasteria*, *Aloe* (Schnepf and Pross 1976), *Strelitzia* (Kronstedt-Robards and Robards 1987) and at the nectariferous parenchyma of *Eccremocarpus scarber* from Bignoniaceae (Belmonte et al. 1994).

According to Gunning (1977), almost all invaginate cell walls are closely associated with mitochondria, suggesting the involvement of high ATPase activity during transport of solutes across the membrane (Maier and Maier 1972, Joshi et al. 1993). Thus, the efficient transport of substances by transfer cells in *A. magnificum* is confirmed by the presence of several mitochondria found within these cells, which are supposed to produce energy to pump the secretion into the secretory cell. The hypothesis that these cells are involved only in the solution transport with sugar is strongly supported in a study by Schnepf (1969) which showed that the invaginations are rarely found in systems where the secretion occur by vesicles. However, ingrowths supporting eccrine secretions often occur (Schnepf 1961, 1969).

Descriptive observations of transfer cells in many species revealed different types of ingrowth morphologies (Gunning 1977), these types are grouped in two categories, called “flange” and “reticulate” (Talbot et al. 2002). In most transfer cells, the formation of ingrowth occurs by the appearance of small papillae on the wall, which then develop branches and merge, forming complex networks; this pattern is called reticulate (Gunning and Pate 1969, Talbot et al. 2002). However, there is another method of deposition of ingrowths in which these invaginations are apparently similar to ridge projections, such as sided cords (Davis et al. 1990) or flanges, which generally resemble secondary wall thickenings of some tracheary elements (Zee and O’Brien 1971). In *A. magnificum* the type of deposition is reticulate, although the invaginations do not develop interconnected networks with wall material, which is similar to the initial stages of deposition of transfer cells in other species, such as transfer cells of xylem parenchyma in the roots of *Vicia faba* L. (Talbot et al. 2002).

The presence of transfer cells is described here for the first time in nectaries of Bignoniaceae. These transfer cells are the same stalk cells which eventually assumed the role of transporting sugar through the transmembrane flow. It was also seen that, concomitantly with the nectar secretion, lipophilic compounds occur, classified as heterogeneous secretion, with two types of release - eccrine and granulocrine. The production of lipophilic compounds is generated from a high energy outflow of the plant (Nicolson and Thornburg 2007), justifying the presence of the ingrowths in the cell wall, and producing specialized cells (Stpiczyńska et al. 2012).

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RESUMO

As células de transferência são células especializadas que desempenham um papel importante onde há altos custos de energia devido a facilitação do fluxo transmembranar de solutos. Esse estudo objetivou investigar a ontogênese, histoquímica e ultraestrutura dos tricomas glandulares focando nas células do pedúnculo e suas possíveis funções de transferência. Amostras de perfis da gema axilar, cálices das flores em antese, e botões florais em diferentes estágios de desenvolvimento foram coletados, fixados e processados de acordo com os métodos usuais de microscopia. Os tricomas glandulares são compostos de uma cabeça secretora com suas células em formato colunar. O pedúnculo é formado por duas camadas de células, a camada superior compreende células cuboidais, onde a parede começa a se espessar no início da fase pré-secretora. A secreção é heterogênea, liberando glicose, outros carboidratos, lipídeos e compostos fenólicos, com dois tipos de liberação – écrina e granulócina. Estes tricomas são funcionalmente denominados nectários. As células do pedúnculo são consideradas células de transferência por possuírem parede anticlinal mais espessa com invaginações irregulares. A presença de células de transferência nos nectários sugere uma alta especialização, pois isso aumenta a capacidade de transporte de néctar e a compensação do alto gasto de energia para a produção e liberação dessa substância.

Palavras-chave: tricomas glandulares, nectários, ontogênese, estruturas secretoras, invaginação na parede.

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