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The role of the parenchyma sheath and PCD during the development of oil cavities in *Pterodon pubescens* (*Leguminosae-Papilionoideae*)

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ABSTRACT

Pterodon pubescens cavities are constituted by lumen and uniseriated epithelium surrounded by multiseriate parenchyma sheath. We studied the development of secretory cavities, including the role of parenchyma sheath, using light and transmission electron microscopy. A Tunel assay was performed to verify whether programmed cell death (PCD) occurs during the process. The lumen is formed by schizogeny and lysigeny occur in later developmental stages of the secretory cavities. Ultrastructurally, epithelial cells in later developmental stages become dark and with sinuous walls; the protoplast becomes retracted and the cytoplasm shows low organelle definition. Degenerated cells are released toward the lumen. Our results showed that PCD occurs during later developmental stages of cavities and plays a critical role in functioning of these glands. New cells originated from the parenchyma sheath differentiate into secretory cells and replace those degenerated ones. This fact associated to PCD guarantees epithelium renovation during the secretory cycle and the maintenance of secretory activity of cavities.

1. Introduction

Secretory spaces, i.e. canals and cavities, are inner glands in the vegetal body constituted by an epithelium of specialized secretory cells surrounding a wide lumen [1]. The occurrence of a parenchyma sheath surrounding these glands, producing new epithelial cells, has been described by some authors [2–6].

Canals and cavities are common in legume species and work as the primary sites of the synthesis, secretion and accumulation of important secondary compounds as gums, oil and oleoresins [6–8]. Leguminosae is one of the more numerous plant families and is widely spread around the world. Many economically important species are exploited by food, pharmaceutical, cosmetical and other industries [9].

Pterodon is a legume genus belonging to the Papilionoideae subfamily and comprises tree species that inhabits the east and central Brazil and east Bolivia. The species belonging to this genus are exploited by wood and medicine industries and are used as ornamentals and for reforestation [9]. *Pterodon pubescens*, commonly known as sucupira-branca, is found exclusively in the Brazilian cerrado vegetation. The oil extracted from its fruits has traditionally been used during the treatment against rheumatism [10], arthritis [11], schistosomiasis [12], and has shown anti-inflammatory [13] and analgesic [14] action.

Oil cavities have been described in fruits [15] and in pulvinus, petiole and rachis [16] of *P. pubescens*. Oil glands formation in *Pterodon* has been interpreted to occur through a schizogenous process [17,18] or, conversely, through a lysigenous process [15]. Although the variable biogenesis (schizogeny, lysigeny and schizolysigeny) of canals and cavities have been reported in legumes [6,8,15,19–22], the literature refers to the early ontogeny stages and ultrastructural descriptions of mature glands

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are scarce. Some ultrastructural evidence suggests the occurrence of PCD in legumes [21]. However, this phenomenon has not yet been confirmed in this group of plants.

In contrast to necrosis, PCD is an active, genetically controlled process that occurs during normal growth and development and during the plant response to stress factors [24–26], being reported in different situations throughout the life cycle [27–33]. Molecular and structural features such as condensation of chromatin, cleavage of DNA, disorganization of the nuclei and membrane swelling are identified as PCD signals [34]. Studies employing transmission electron microscopy and the Tunel assay [23,35] have conclusively demonstrated that PCD plays a key role in the lysigenic development of glands in different taxa.

In this work, we studied the development of secretory cavities in *Pterodon pubescens* using light microscopy and transmission electron microscopy. In situ, Tunel (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay was employed to verify whether programmed cell death (PCD) occurs during this developmental process.

2. Material and methods

2.1. Plant material

Samples were collected from the fourth internode (from the apex to the base) of shoots of *Pterodon pubescens* plants living in an area of cerrado vegetation located in the municipality of Botucatu (22° 55′ S, 48° 30′ W), in São Paulo state, Brazil. Five adult specimens were selected to this study. Vouchers were deposited in the Herbarium Irina Delanova Gemtchújnicov (BOTU) of the Institute of Biosciences of Botucatu, UNESP, under the number 24911.

Samples of the stem cortical region containing secretory cavities in different developmental stages were submitted to conventional light and electron microscopy and Tunel assay.

2.2. Light microscopy

For general anatomical characterization, the collected samples were fixed in FAA 50 [36] for 24 hours at room temperature (25 °C), and then dehydrated in an ethanol series and embedded in methacrylate resin (Leica historesin) [37]. The material was sectioned in a semi-automatic rotary microtome and the sections (3 μm thick) were stained with Toluidine blue 0.05% pH 4.3 in 0.1 M phosphate buffer [38]. Permanent slides were mounted with Permount [39].

The presence of non-cellulosic polysaccharides was detected in sections of fresh material treated with Schiff's reagent (periodic acid-Schiff's [PAS]) [40] that facility the visualization of the middle lamellae and its swelling during the schizogeny.

All specimens were examined and documented on a light microscope (BX 40, Olympus) equipped with a digital camera.

2.3. Transmission electron microscopy (TEM)

Samples were initially fixed in a 2.5%-glutaraldehyde solution in 0.1 M phosphate buffer pH 7.3 for 24 hours at 5 °C, post-fixed with 1% osmium tetroxide in the same buffer for 1 hour at 25 °C, dehydrated through an acetone series and embedded in Araldite® resin [41]. Ultra-thin sections were obtained with a Diatome diamond knife and post-stained with uranyl acetate and lead citrate [42]. The material was examined and photographed using a Philips EM 301.

2.4. Tunel assay

To assay DNA fragmentation, samples were fixed overnight in FAA (formalin-acetic acid-alcohol), dehydrated in an alcohol series and embedded in paraffin. The 10-μm sections were obtained using a rotary microtome and were attached to glass slides previous treated with poly-lysine. The sections were washed in xylol, rehydrated through a graded ethanol series, followed by rising in TBS buffer pH 7.6. The Tunel assay was performed using an *in situ* apoptosis detection kit (Calbiochem, TdT-FragEl DNA Fragmentation Detection Kit) according to manufacturer's instructions. A negative control was performed without the terminal deoxynucleotidyl transferase enzyme. The positive control was performed utilizing control slides contained in the kit. The sections were observed and photographed using a light microscope (BX 40, Olympus) equipped with a digital camera.

3. Results

3.1. Anatomical aspects

Observation of longitudinal and cross-sections of young stem revealed that the initiation and developmental processes of the secretory cavities in *Pterodon pubescens* are precocious and are already observed subjacent to the apical meristem, continuing in the young internodes of the shoot. Secretory cavities in different developmental stages occur side by side in the outer region of the cortex of the stem in primary state of growth (Fig. 1A). Cavities are still differentiating when shoot primary tissues maturation finishes.

Based on light microscopy observations, the ontogeny of the secretory cavities in the internodes can be divided into four sequential stages: the initial cell cluster stage (Figs. 1B and C), the intercellular space-forming stage (Fig. 1D), the lumen expanding stage (Figs. 1E and F) and the mature stage (Fig. 1G). The initial of the secretory cavities is originated from a single cell of the ground meristem. Initially, this single cell undergoes a periclinal division resulting in a pair of initial cells (Fig. 1B). The initial cells are distinguishable from neighboring cells by the dense cytoplasm and voluminous nucleus (Fig. 1B). These cells divide in an oblique direction and form a globular cluster of 6–8 cells of different sizes (Fig. 1C). In each cluster, voluminous cells are pyramidal in shape and show conspicuous nuclei and abundant and dense cytoplasm (Fig. 1C). These will become the epithelial cells. The

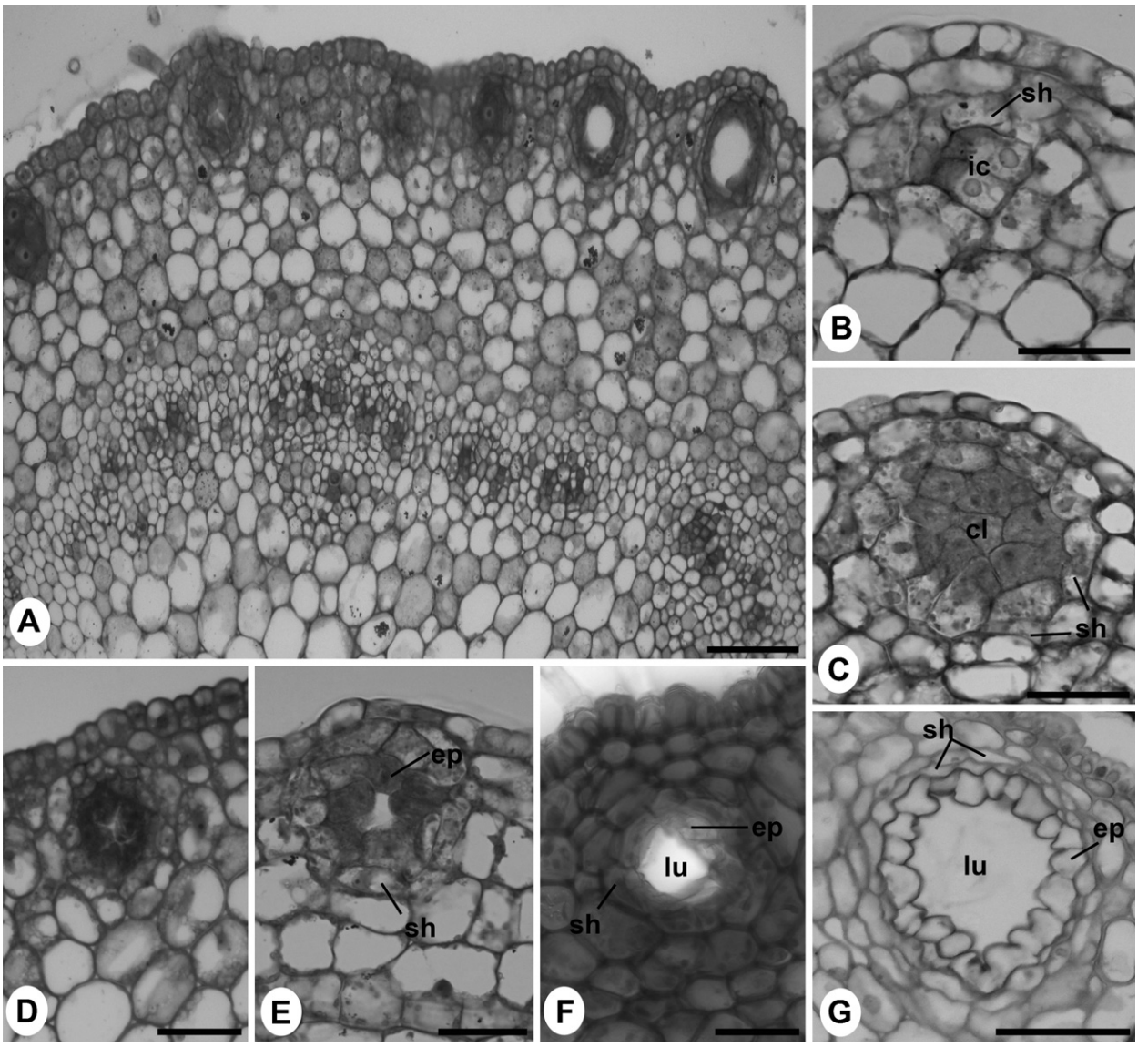

Fig. 1. Cross-sections of *Pterodon pubescens* primary stem. A. General view showing secretory cavities in different stages of development in the outer cortex. B. Initial stage of gland formation, showing a cluster of two initial cells and evident sheath cells. C. Cluster compound by several cells with dense cytoplasm and surrounded by the parenchyma sheath. D. Initial stage of lumen formation by the cell wall separation. E. Young secretory cavity showing small lumen and epithelium surrounded by the parenchyma sheath. F. Secretory cavity with moderately wide lumen and epithelium with voluminous cells. G. Mature secretory cavity with wide lumen and uniseriate epithelium (cl: cluster; ep: epithelial cells; ic: initial cells; lu: lumen; sh: sheath cells). Scale bars = 150 μm (A), 50 μm (B–G).

parenchyma cells around this cluster become tangentially elongated and divide periclinally (Fig. 1E) and will become the parenchyma sheath (Figs. 1F and G).

In this stage, the middle lamellae and anticlinal walls among the central cells of the cluster become swollen (Fig. 1C) and stain strongly with PAS, showing the presence of non-cellulosic polysaccharides. In a following developmental stage, the middle lamella in the apical region of the cells break up (Fig. 1D) and gives rise to small intercellular spaces signaling the initiation of lumen cavity (Fig. 1E).

The future epithelial cells become radially expanded and the central intercellular space increases rapidly (Figs. 1E and F) developing the isodiametric lumen by schizogeny (Fig. 1G). Additionally, the process of middle lamellae dissolution progresses centrifugally (Fig. 1F).

Mature glands, in both cross- and longitudinal sections, consist of a wide isodiametric lumen and a uniseriate secretory epithelium surrounded by a 2–3 layered sheath of tangentially elongated parenchyma cells (Fig. 1G). The epithelial cells are of different sizes and shapes, varying from rounded to pyramidal. The outer periclinal walls of the epithelial cells vary from straight to convex or concave according to the secretory stage of the cell.

3.2. Ultrastructural aspects

Ultrastructurally, the cluster cells have features typical of meristematic cells (Figs. 2A–C). The walls of these cells are very thin and exhibit conspicuous plasmodesmata (Fig. 2B). In the central region of the cluster, small intercellular spaces (Fig. 2A) are formed and coalesce to give rise to a wide lumen (Fig. 2C). In the beginning of the lumen expansion stage, a rearrangement of the cluster cells is remarkable. The outermost cells of the cluster

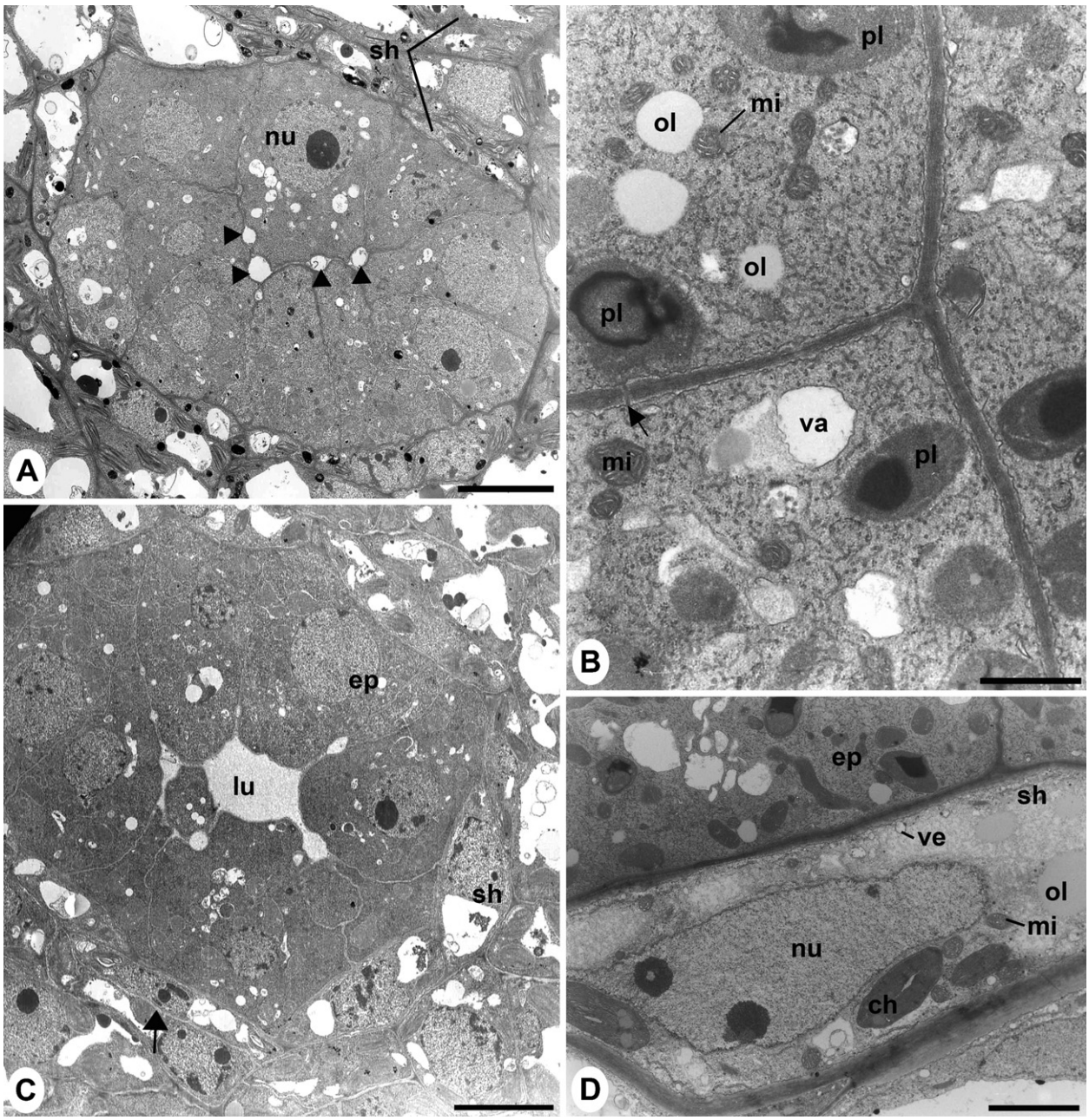

Fig. 2. Electron micrographs (TEM) of *Pterodon pubescens* secretory cavities in the initial stages of development. A. Cluster constituted by secretory cells with conspicuous nuclei and dense cytoplasm. Observe the small intercellular spaces (arrowheads) in the central region of the cluster and the parenchyma sheath surrounding the cluster. B. Detail of cluster cells showing thin walls with plasmodesmata (arrow), sinuous plasma membrane and dense cytoplasm with polyribosomes, globular and oval plastids, mitochondria with voluminous cristae, small vacuoles and oil droplets. C. Secretory cavity in the early stage of development showing a central lumen and smaller intercellular spaces along the anticlinal faces of the epithelial cells. Note the occurrence of cell division (arrow) in the sheath. D. Detail of one sheath cell showing conspicuous nucleus, chloroplast with well-structured grana, mitochondria, oil droplets and vesicles near the plasma membrane (cl: chloroplast; ep: epithelial cells; lu: lumen; mi: mitochondria; nu: nucleus; ol: oil droplets; pl: plastids; sh: sheath cells; va: vacuoles; ve: vesicles). Scale bars = 4.6 μm (A, C), 2.6 μm (B), 0.5 μm (D).

penetrate the radial walls of the innermost cells and become part of the uniseriate epithelium that surrounds the central intercellular space (Fig. 2C).

Each cluster cell has a voluminous nucleus with evident nucleoli and abundant cytoplasm (Figs. 2A and C) rich in polyribosomes, mitochondria, dictyosomes with many cisterns and adjacent vesicles, abundant smooth and rough endoplasmic reticulum, oil droplets and plastids (Fig. 2B). Plastids are spherical or oval, with granular stroma and osmiophilic inclusions and lack thylakoids (Fig. 2B). The vacuoles are small and contain membranes or electron dense bodies (Fig. 2B).

The sheath parenchyma cells that encircles each cluster are characterized by thicker walls, conspicuous nuclei with

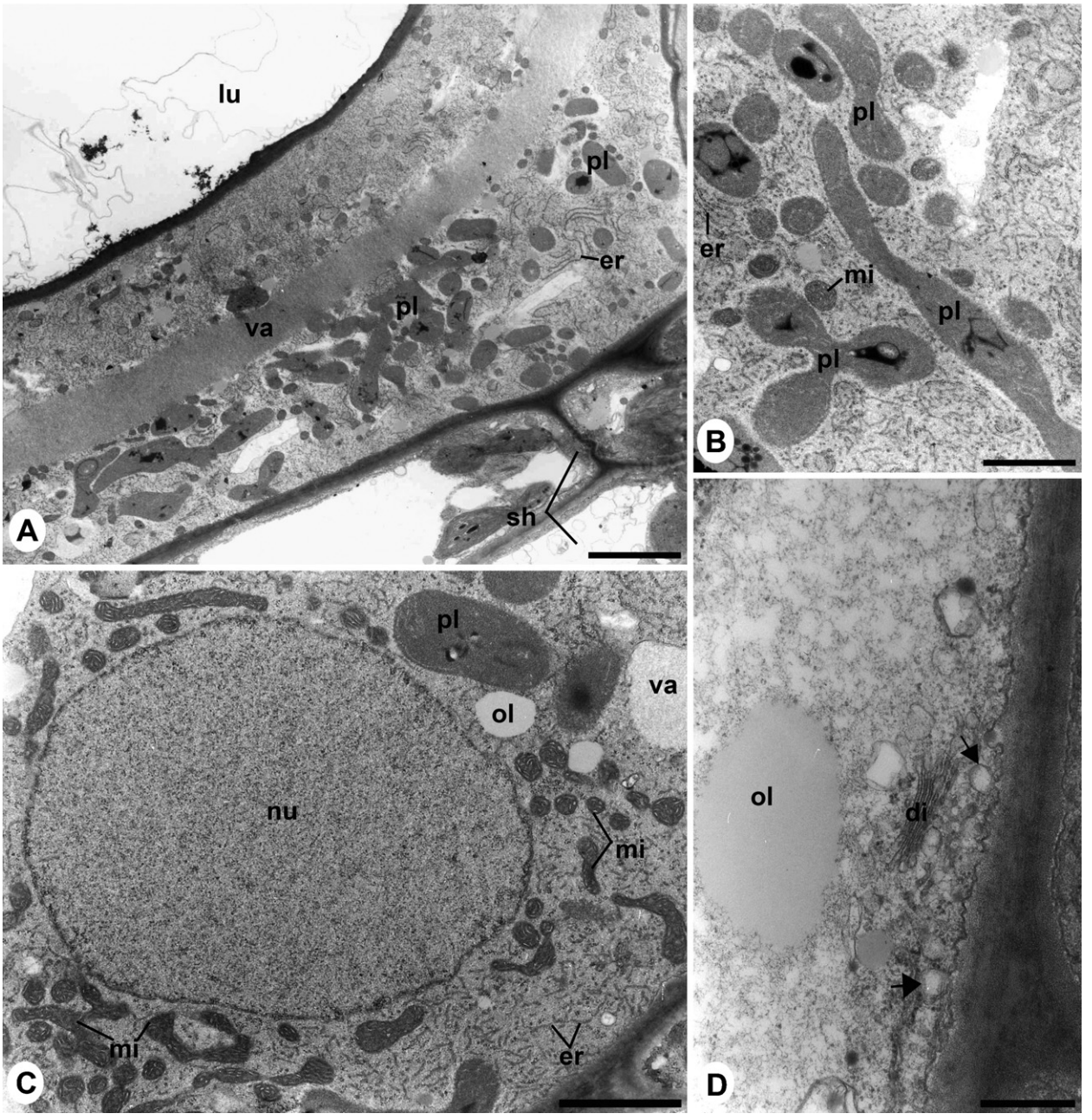

Fig. 3. Electron micrographs (TEM) of mature secretory cavities of *Pterodon pubescens*. A. Flattened epithelial cell showing proliferation of polymorphic plastids, extensive endoplasmic reticulum and elongated vacuole. Note the sheath cells and the presence of cell remnants in the lumen. B. Detail of the previous figure showing polymorphic plastids, polyribosome, endoplasmic reticulum and mitochondria. C. Part of an epithelial cell showing conspicuous nucleus, plastids, abundance of mitochondria, proliferation of endoplasmic reticulum, vacuole and oil droplets in the cytoplasm. D. Detail showing sinuous plasma membrane, dictyosome, vesicles (arrows) and oil droplet in the cytoplasm of epithelial cell (di: dictyosome; er: endoplasmic reticulum; lu: lumen; mi: mitochondria; nu: nucleus; ol: oil droplets; pl: plastid; sh: sheath cells; va: vacuole). Scale bars = 3.4 μm (A), 1.0 μm (B), 2.5 μm (C), 0.4 μm (D).

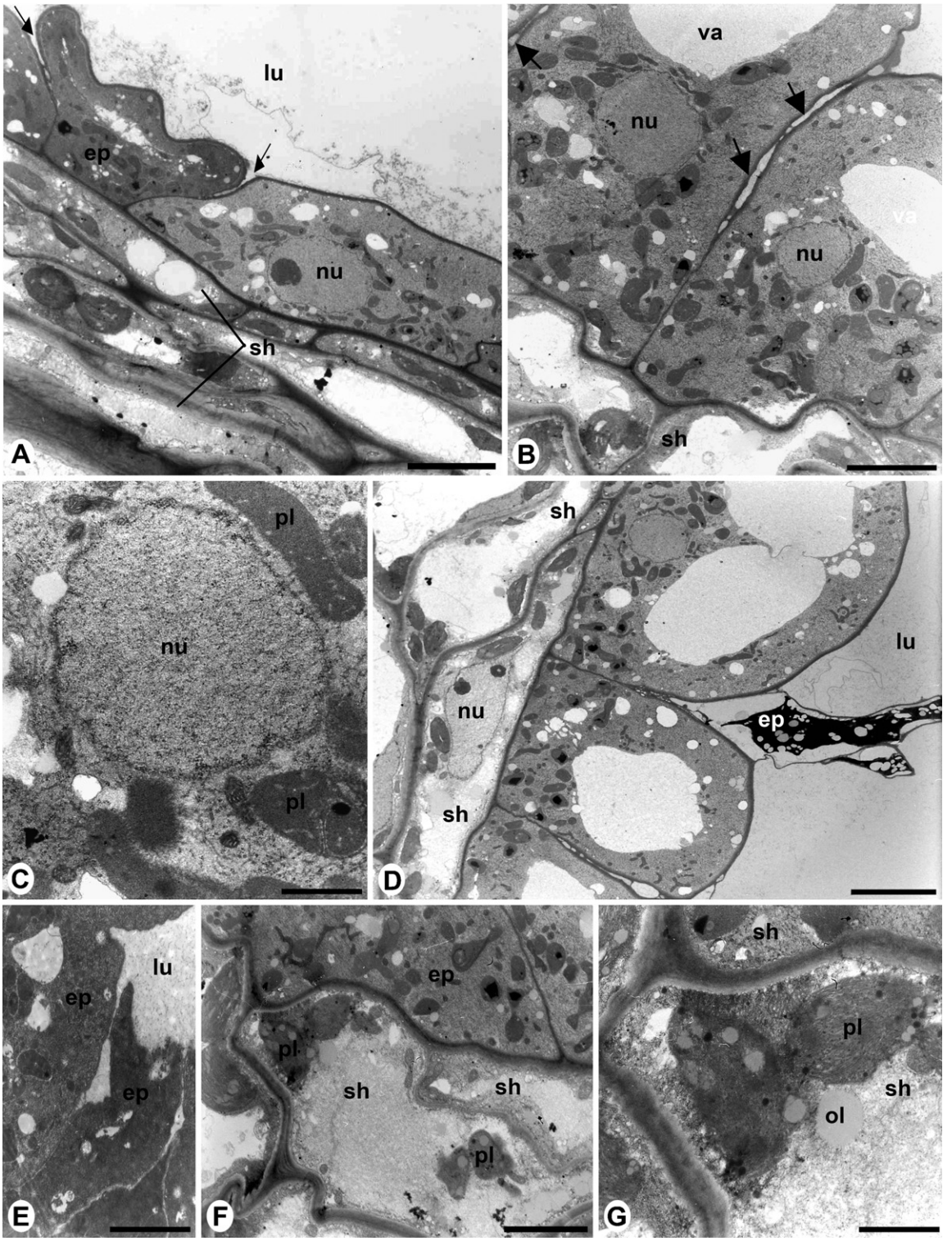

Fig. 4. Electron micrographs (TEM) of mature secretory cavities in *Pterodon pubescens*. A. Epithelial cells with different shapes, sizes and electron-density side-by-side. Note cell wall disruption between epithelial cells (arrows) and cell remnants in the lumen. Observe the radial flattened sheath cells. B. Detail of neighboring senescent epithelial cells showing cell wall disruption (arrows) along the anticlinal walls, reduced nuclei with irregular contours and large vacuoles. C. Detail of the previous figure showing nuclei with irregular contour and vesiculated nuclear envelope. D. Protrusion of the degenerated epithelial cell toward the lumen. Observe the apical growth of sheath cells. E. Epithelial cells in different developmental stages. Note the ruptured protoplast of the cell on the right. F. Sheath cells showing intrusive growth and plastids with oil droplets. G. Detail of the previous figure showing sheath cells with plastids with oil droplets and osmiophilic inclusions. Oil droplets are also seen scattered in the cytoplasm (ep: epithelial cells; lu: lumen; nu: nucleus; ol: oil droplets; pl: plastids; sh: sheath cells; va: vacuole). Scale bars = 4.0 μm (A), 3.4 μm (B), 1.0 μm (C, G), 2.6 μm (D, E), 1.5 μm (F).

evident nucleoli, abundant cytoplasm and small vacuoles (Figs. 2A, C and D). In these cells occur typical chloroplasts, mitochondria with well-developed cristae, oil droplets and numerous vesicles near the plasma membrane (Fig. 2D). These cells are tangentially elongated, compactly arranged (Figs. 2A and C) and can divide periclinally (Fig. 2C). Plasmodesmata connect these cells among themselves and to the adjacent cluster cells.

In the mature cavities, cell remnants are seen in the lumen of the secretory cavities (Fig. 3A). In the cytoplasm of intact epithelial cells, abundance of polymorphic plastids without tylakoids is a remarkable feature (Figs. 3A and B). The endoplasmic reticulum becomes proliferated (Figs. 3A and B). Abundant elongated mitochondria with well-developed cristae are seen agglomerated around the nucleus (Fig. 3C). Oil droplets become abundant inside the protoplast (Figs. 3B–D) and can cross the plasma membrane and the cell wall and reach the lumen of the cavity. The dictyosomes are well developed and produce several vesicles that can be seen in the peripheral cytoplasm near the sinuous plasma membrane (Fig. 3D). Images suggesting fusion between vesicles and the plasma membrane are common in this developmental stage (Fig. 3D).

During the secretion process, active intact epithelial cells exhibiting high levels of metabolism and cells showing senescence signals are seen side by side in the same epithelium (Fig. 4A). Senescent epithelial cells show loose sinuous walls and retracted protoplast (Fig. 4A). The nucleus undergoes retraction (Fig. 4B) and the nuclear envelope becomes irregular, discontinuous and vesiculated (Fig. 4C). Mitochondria are proliferated, well-developed and their cristae are swollen (Figs. 4B and C). In these cells, degradation of the middle lamella starts at the anticlinal walls and progresses toward the whole cell extension (Figs. 4A and B). The cytoplasm of such cells become very dark and retracted and the visualization of organelles become impossible (Figs. 4D and E). These changes culminate with the protrusion of the degenerated epithelial cell toward the lumen (Fig. 4D) or with the cell fragmentation (Fig. 4E). In fact, cell debris are seen in the lumen of the mature cavity (Figs. 3A and 4A).

The sheath of parenchyma cells continues well delimited around the mature cavities (Figs. 3A and 4A). The sheath cells located just bellow the senescent epithelial cells are characterized by a larger nucleus, dense and abundant cytoplasm and very small vacuoles (Fig. 4D) indicating their intense metabolism. Their chloroplasts gradually change from typical ones (Fig. 2D) to leucoplasts (Figs. 4F and G). Interestingly, these cells show intrusive growth toward the epithelium (Figs. 4A, D and F). In a posterior stage, such cells are incorporated to the epithelium and replace the degenerated epithelial ones.

3.3. Tunel assay

The nuclei of some epithelial cells of mature cavities were Tunel-positive, showing brown deposits suggesting chromatin fragmentation. Marked and unmarked cells were observed side by side in a same epithelium of a mature cavity. No sheath cell was Tunel-positive (Fig. 5).

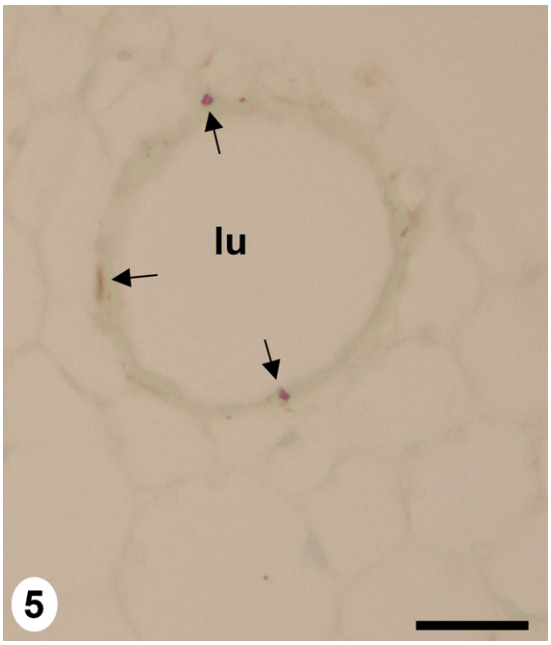

Fig. 5. *In situ* detection of nuclear DNA fragmentation by TUNEL assay in *Pterodon pubescens* mature secretory cavity. Observe TUNEL-positive in some epithelial cells (arrows) (lu: lumen). Scale bar = 25 μm.

4. Discussion

In the stem of *Pterodon pubescens*, oil cavities are distributed exclusively in the outer cortical region [43] suggesting the protective importance of these glands to the young shoot. Besides, the position and type of glands may be a significant taxonomic character within Fabaceae [19,22,44].

In *P. pubescens*, the secretory cavities are derived from a cluster of initial cells in the ground meristem, as seen in others taxa [6,22,23,45,46].

In the present work, it is evident that the initiation and expansion of the cavity lumen in stem of *P. pubescens* occur by schizogeny and the lysigeny occur in mature cavity releasing the secretion to the lumen. Lysis of epithelial cells in late stages of development was also observed in *Myrtus communis* [47]. To some authors, the lysigeny is of particular interest, since the autolysis of glandular protoplast releases secretion products into the lumen of the cavity and this may be considered a true programmed cell death phenomenon [23,48].

These data are complementary to the information found in the literature, where secretory cavities in *Pterodon* are classified as being of schizogenic origin by Solereder [17] and by Metcalfe and Chalk [18] or of lysigenic origin by Paiva et al. [15]. The issue of whether cavity formation is a schyzigenous, lysigenous, or a overlapping of both processes is controversial in the literature and contradictory views are expressed in different studies for a same species or a same organ [49–55]. In a critical review, Turner et al. [49] suggested that lysigenesis could be a misinterpretation of artifacts or insufficient gland sampling. Besides, different methods of material preparation and

variations in the mounting media can affect the interpretation of the formation process of glands [50].

The cellular features of *P. pubescens* secretory cavities, namely the presence of polymorphic plastids with lipophilic droplets, abundance of smooth endoplasmic reticulum and scattered oil drops, are common to oil-secreting glands [1,15,22,52,55–59]. Although the smooth endoplasmic reticulum has been associated to the transportation of oil droplets from plastids to parietal cytoplasm [57], in *P. pubescens* no evidence about this was detected, as observed by Bennici and Tani [52] in *Citrus* species. The abundance of polyribosomes and rough endoplasmic reticulum in the *P. pubescens* epithelial cells can be attributed to intense, enzymatic synthesis needed for cell metabolism and cell wall degradation during the schizolysigenic development of secretory cavity. In fact, the activation of pectinases and cellulases that participate in middle-lamellae dissolution and cell wall degradation processes was cytochemically demonstrated in *Citrus* fruits by Liang et al. [54].

Our cytological observations as dark cytoplasm with low definition of the organelles, swollen membranes, nuclei with irregular contours and vesiculated envelope, sinuous walls and loss of adherence to neighboring cells, seem to be a typical form of PCD, which occurs during the normal plant development [30]. On the bases of ultrastructural studies plus the Tunel assay result to *P. pubescens* mature cavities, we conclude that lysis is a natural process and is related to programmed cell death (PCD), as reported by Liu et al. [23] to *Gossypium hirsutum* pigment glands.

The presence of a well-developed parenchyma sheath, surrounding the epithelium, which acts as a meristem providing new cells that replace the damaged ones is a remarkable feature of *P. pubescens* secretory cavities. Some features of the sheath cells, such as voluminous nuclei, dense cytoplasm and abundant lipid content, indicate the ability of these cells to secrete oils; however, this secretory activity is more conspicuous in the inner cell layer of sheath and diminishes towards the sheath periphery, as observed in *Porophyllum lanceolatum* by Monteiro et al. [56]. We hypothesize that the plastids with a well-developed internal membranes and starch grains present in the sheath cells are able to perform photosynthesis and provide the energy and precursors necessary for biosynthesis of secretion compounds, as observed in glandular trichomes of different species [55,57].

The presence of several layers of flattened parenchyma cells around the secretory cavities has been described in different taxa [4,5,52,56,57]. In *P. pubescens*, a parenchyma sheath formed by thick-walled cells is evident since the initial stages of gland development. The most peripheral cells can have a protective function as suggested by Bosabalidis and Tsekos [57] and Bennici and Tani [52] while the inner one perform secretory activity, as Bennici and Tani [52]. In this work, we registered a continuous differentiation from parenchyma cells into epithelial cells during the oil cavity maturation. Remarkably, the parenchyma sheath cells in contact with the senescent epithelial cells progressively acquire secretory features. The main cell changes along this process include intrusive growth, chloroplast to leucoplasts differentiation and accumulation of oil droplets. This process constitutes an open model of gland development [5,6,56].

Finally, we can conclude that the formation of new epithelial cells from the parenchyma sheath plus the PCD in *P. pubescens* oil cavities ensures the epithelium renovation and the maintenance of the secretory activity in oil glands.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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References

[1] A. Fahn, Secretory tissues in plants, Academic Press, London, 1979.
[2] G.H. Wittler, J.D. Mauseth, The ultrastructure of developing latex ducts in *Mammilaria heyderi* (Cactaceae), Am. J. Bot. 71 (1984) 100–110.
[3] G.M. Fueyo, Ontogenia de las glandulas foliares e involucrales de *Tahetes minuta* (Compositae), Bol. Soc. Arg. Bot. 24 (1986) 403–410.
[4] W.R. Monteiro, M.M. Castro, A. Fahn, W. Caldeira, Observations on the development of the foliar secretory cavities of *Porophyllum lanceolatum* (Asteraceae), Nord. J. Bot. 15 (1995) 69–76.
[5] S.R. Machado, S.M. Carmello-Guerreiro, Estrutura e desenvolvimento de canais secretores em frutos de *Schinus terebinthifoloius* Raddi (Anacardiaceae), Acta Bot. Bras. 15 (2001) 189–195.
[6] T.M. Rodrigues, S.P. Teixeira, S.R. Machado, The oleoresin secretory system in seedlings and adult plants of copaiba (*Copaifera langsdorffii* Desf., Leguminosae-Caesalpinioideae), Flora 206 (2011) 585–594.
[7] J.H. Langenheim, Plant resins: chemistry, evolution, ecology and ethnobotany, Timber Press, Portland, Cambridge, 2003.
[8] T.M. Rodrigues, S.R. Machado, Developmental and structural features of secretory canals in root and shoot wood of *Copaifera langsdorffii* Desf. (Leguminosae–Caesalpinioideae), Trees 23 (2009) 1013–1018.
[9] G.P. Lewis, B. Schrire, B. Mackinder, M. Lock, Legumes of the World, The Royal Botanic Garden, Kew, 2005.
[10] M.P. Correa, Dicionário de plantas úteis do Brasil e das exóticas cultivadas, Ministério da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro, 1984.
[11] M.G.P. Coelho, P.R. Marques, C.R.M. Gayer, L.C.A. Vaz, J.F. Nogueira, K.C.C. Sabino, Subacute toxicity evaluation of a hydroalcoholic extract of *Pterodon pubescens* seeds in mice with collagen-induced arthritis, J. Ethnopharmacol. 77 (2001) 159–164.
[12] N. Katz, D. Santos Filho, S.J. Sarti, N.M. Mendes, P.A. Rocha Filho, N. Araújo, Atividade quimioprofilática na esquistossomose mansoni de sabonetes contendo óleo essencial de frutos de *Pterodon pubescens*, Rev. Inst. Med. Trop. São Paulo 35 (1993) 183–191.
[13] E.A. Nunan, M.G. Carvalho, D. Piló-Veloso, R.M.M. Turchetti-Maia, D.L. Ferreira-Alves, Furane diterpenes with anti- and pro-inflammatory activity, Braz. J. Med. Biol. Res. 15 (1982) 450.
[14] I.D. Duarte, D.L. Ferreira-Alves, D.P. Veloso, M. Nakamura-Craig, Evidence of the involvement of biogenic amines in the antinociceptive effect of vouacapan extracted from *Pterodon polygalaeflorus* Benth, J. Ethnopharmacol. 55 (1996) 13–18.
[15] E.A.S. Paiva, D.M.T. Oliveira, S.R. Machado, Anatomy and ontogeny of the pericarp of *Pterodon emargiatus* Vogel (Fabaceae, Faboideae) with emphasis on secretory ducts, An. Ac. Bras. Cienc. 80 (2008) 455–465.

[16] T.M. Rodrigues, S.R. Machado, Anatomia comparada do pulvino, pecíolo e raque de *Pterodon pubescens* Benth (Fabaceae-Faboideae), Acta Bot. Bras. 18 (2004) 381–390.
[17] H. Solereder, Systematic anatomy of the dicotyledons, Claredon Press, Oxford, 1908.
[18] C.R. Metcalfe, L. Chalk, Anatomy of the Dicotyledons, Claredon Press, Oxford, 1950.
[19] G. Turner, Comparative development of secretory cavities in the tribes Amorpheae and Psoraleeae (Leguminosae: Papilionoideae), Am. J. Bot. 73 (1986) 1178–1192.
[20] C.R. Marcati, V. Angyalossy-Alfonso, L. Benetati, Anatomia comparada do lenho de *Copaifera langsdorffii* Desf. (Leguminosae-Caesalpinioideae) de floresta e cerradão, Rev. Bras. Bot. 24 (2001) 311–320.
[21] E.A.S. Paiva, S.R. Machado, Structural and ultrastructural aspects of ontogenesis and differentiation of resin secretory cavities in *Hymenaea stigonocarpa* (Fabaceae-caesalpinioideae) leaves, Nord. J. Bot. 24 (2007) 423–431.
[22] S.P. Teixeira, J.F. Rocha, Oil glands in the Neotropical genus *Dahlstedtia* Malme (Leguminosae, Papilionoideae, Millettieae), Rev. Bras. Bot. 32 (2009) 57–64.
[23] W.-Z. Liu, Y.-F. Zhou, X. Wang, Z.-J. Jiao, Programmed cell death during pigment gland formation in *Gossypium hirsutum* leaves, Plant Biol. 12 (2010) 895–902.
[24] J.T. Greenberg, Programmed cell death: a way of life for plants, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 12094–12097.
[25] R.I. Pennell, C. Lamb, Programmed cell death in plants, Plant Cell 9 (1997) 1157–1168.
[26] A. Danon, V. Delorme, N. Mailhac, P. Gallois, Plant programmed cell death: a common way to die, Plant Physiol. Biochem. 38 (2000) 647–655.
[27] A.H.L.A.N. Gunawardena, J.S. Greenwood, N.G. Dengler, Cell wall degradation and modification during programmed cell death in lace plant, *Aponogeton madagascariensis* (Aponogetonaceae), Am. J. Bot. 94 (2007) 1116–1128.
[28] A.H.L.A.N. Gunawardena, D.M.E. Pearce, M.B. Jackson, C.D. Hawes, D.E. Evans, Rapid changes in cell wall pectic polysaccharides and closely associated with early stages of aerenchyma formation, a spatially localized form of programmed cell death in roots of maize (*Zea mays* L.) promoted by ethylene, Plant Cell Environ. 24 (2001) 136–1375.
[29] H Fukuda, Programmed cell death of tracheary elements as a paradigm in plants, Plant Mol. Biol. 44 (2000) 245–253.
[30] K.P. Gaffal, G.J. Friedrichs, S. El-Gammal, Ultrastructural evidence for a dual function of the phloem programmed cell death in the floral nectary of *Digitalis purpurea*, Ann. Bot. 99 (2007) 593–607.
[31] S. Gan, R.M. Amasino, Making sense of senescence, Plant Physiol. 113 (1997) 313–319.
[32] A. Levine, R.I. Pannel, M.E. Alvarez, R. Palmer, C. Lamb, Calcium-mediated apoptosis in a plant hypersensitive disease resistance response, Curr. Biol. 6 (1996) 427–437.
[33] M. Katsuhara, Apoptosis-like cell death in barley roots under salt stress, Plant Physiol. 38 (1997) 1091–1093.
[34] E. Simeonova, A. Sikora, M. Charzynska, A. Mostowska, Aspects of programmed cell death during leaf senescence of mono- and dicotyledonous plants, Protoplasma 214 (2000) 93–101.
[35] C. Ying, W. Hong, Programmed cell death in the schizolysigenous formation of the secretory cavity in *Citrus sinensis* L. (Osbeck), Chin. Sci. Bull. 55 (2010) 2160–2168.
[36] D.A. Johansen, Plant michrotechnique, McGraw-Hill, New York, 1940.
[37] P.O. Gerrits, The application of glycol methacrylate in histotechnology: some fundamental principles, Department of Anatomy and Embryology State University Groningen, Netherlands, 1991.
[38] T.P. O'Brien, N. Feder, M.E. McCully, Polychromatic staining of plant cell walls by toluidine blue O, Protoplasma 59 (1964) 368–373.
[39] G. Gerlach, Botanische Mikrotecnik eine Einfuhrung, Gorg Thieme, Stuttgard, 1969.
[40] N. Feder, T.P. O'Brien, Plant microtechnique: some principles and new methods, Am. J. Bot. 55 (1968) 123–142.
[41] S.R. Machado, T.M. Rodrigues, Anatomia e ultra-estrutura do pulvino primário de *Pterodon pubescens* Benth. (Fabaceae-Faboideae), Rev. Bras. Bot. 27 (2004) 135–147.
[42] E.S. Reynolds, The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, J. Cell Biol. 17 (1963) 208–212.
[43] T.M. Rodrigues, Espaços secretores em *Copaifera langsdorffii* Desf. e *Pterodon pubescens* Benth.: ontogênese, estrutura e secreção, PhD Thesis, Universidade Estadual Paulista, Botucatu, Brazil, 2008.
[44] N.R. Lersten, J.D. Curtis, Leaf anatomy in *Caesalpinia* and *Hoffmannseggia* (Leguminosae, Ceasalpinioideae) with emphasis on secretory structures, Plant Syst. Evol. 192 (1994) 231–255.
[45] J.D. Curtis, N.R. Lersten, Internal secretory structures in *Hypericum* Clusiaceae: H. *perforatum* L. and *H. balearicum* L., New Phytol. 114 (1990) 571–580.
[46] D. Ciccarelli, A.C. Andreucci, A.M. Pagni, Translucent glands and secretory canals in *Hypericum perforatum* L. (Hypericaceae): morphological anatomical and histochemical studies during the course of ontogenesis, Ann. Bot. 88 (2001) 637–644.
[47] D. Cicarelli, F. Garbari, A.M. Pagni, The flower of *Myrtus communis* (Myrtaceae): secretory structures, unicellular papillae, and their ecological role, Flora 203 (2008) 85–93.
[48] J.L Dangl, R.A. Dietrich, H. Thomas, Senescence and Programmed Cell Death, in: B. Buchanan, W. Gruissem, R. Jones (Eds.), Biochemistry and Molecular Biology of Plants, ASPP Press, Rockville, 2000, pp. 1044–1100.
[49] G.W. Turner, A.M. Berry, E.M. Gifford, Schizogenous secretory cavities of *Citrus limon* (L.) Burm. F. and a reevaluation of the lysigenous gland concept, Int. J. Plant Sci. 159 (1998) 75–88.
[50] G.W. Tuner, A brief history of the lyisgenous gland hypothesis, Bot. Rev. 65 (1999) 76–88.
[51] D. Ciccarelli, A.M. Pagni, A.C. Andreucci, Ontogeny of secretory cavities in vegetative parts of *Myrtus communis* L. (Myrtaceae), Israel J. Plant Sci. 51 (2003) 193–198.
[52] A. Bennici, C. Tani, Anatomical and ultrastructural study of the secretory cavity development of *Citrus sinensis* and *Citrus limon*: evaluation of schizolysigenous ontogeny, Flora 199 (2004) 464–475.
[53] D. Kalachanis, G.K. Psaras, Structure and development of the secretory cavities of *Myrtus communis* leaves, Biol. Plant. 49 (2005) 105–110.
[54] S. Liang, H. Wang, M. Yang, H. Wu, Sequential actions of pectinases and cellulases during the secretory cavity formation in *Citrus* fruits, Trees 23 (2009) 19–27.
[55] A. Fahn, Secretory tissues in vascular plants, New Phytol. 108 (1988) 229–257.
[56] W.R. Monteiro, A. Fahn, W. Caldeira, M.M. Castro, Ultrastructural observations on the foliar secretory cavities of *Porophyllum lanceolatum* DC. (Asteraceae), Flora 194 (1999) 113–126.
[57] A. Bosabalidis, I. Tsekos, Ultrastructural studies on the secretory cavities of *Citrus deliciosa* Ten. I. Early stages of the gland cells differentiation, Protoplasma 1112 (1982) 55–62.
[58] G. Henrich, Elektrinemmikroslopische beobachtungen zur entstehungsweise der exkretbehälter von *Ruta graveolens*. Citrus limon und Poncirus trifoliate, Österr. Bot. Z. 117 (1969) 397–403.
[59] G. Henrich, Elektrinemmikroslopische beobachtungen an den drüsenzellen von *Poncirus trifoliate*; zugleich ein beitrag zur wirkung ätherischer öle auf pflanzenzellen und eine methode zur unterscheidung flüchtiger von nicht-flüchtigen lipophilen komponenten, Protoplasma 69 (1970) 15–36.