UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

Caracterização morfofuncional de idioblastos secretores em *Ocotea pulchella* (Nees & Mart.) Mez

KARLA BIANCA DE DEUS BENTO

Tese apresentada ao Instituto de Biociências, Câmpus de Botucatu, UNESP, para obtenção do título de Doutora em Biologia Vegetal Interunidades Botucatu/Rio Claro. AC: Morfologia e Diversidade Vegetal

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Orientadora

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Sumário

Bento, KBD. Caracterização morfofuncional de idioblastos secretores em *Ocotea pulchella* (Nees & Mart.) Mez

RESUMO: Células isoladas especializadas na secreção de óleo, ou idioblastos secretores, são uma característica relevante da família Lauraceae. Em algumas espécies de Lauraceae, idioblastos secretores de mucilagem juntamente com idioblastos secretores de óleo foram reportadas em seus órgãos vegetativos. Um certo grau de complexidade nas paredes celulares e na compartimentalização da secreção de mucilagem e de óleo tem sido reportado na literatura; no entanto, estudos ontogenéticos e ultraestruturais detalhados comparando esses dois idioblastos são restritos a poucas espécies. Estudos comparativos são necessários para acessar particularidades em sua morfologia, desenvolvimento e funcionamento. Neste estudo, investigamos a distribuição, origem, desenvolvimento e ultraestrutura de idioblastos secretores de óleo e idioblasto secretores de mucilagem no ápice caulinar e folhas de *Ocotea pulchella* segundo técnicas convencionais em microscopia de luz e eletrônica. Ambos os tipos de idioblastos secretores se originam de células iniciais solitárias aparentemente idênticas, localizadas no meristema fundamental logo abaixo da protoderme. A iniciação dos idioblastos secretores é restrita ao estágio inicial de desenvolvimento dos órgãos caulinares; os idioblastos secretores têm crescimento assíncrono e podem ser encontradas em diferentes estágios de desenvolvimento durante a diferenciação do ápice caulinar e folhas, sendo que o desenvolvimento dos idioblastos secretores de óleo precede o desenvolvimento dos idioblastos secretores de mucilagem. Os idioblastos secretores de mucilagem ocorreram exclusivamente no parênquima paliçádico, enquanto que os idioblastos secretores de óleo ocorreram dispersos no mesofilo (predominantemente no parênquima esponjoso) nervura central e pecíolo. Os idioblastos secretores de mucilagem apresentam secreção de aspecto lamelar consistindo predominantemente de polissacarídeos, enquanto nos idioblastos de óleo a secreção é homogênea, brilhante e contém uma mistura de terpenos, lipídios neutros e ácidos. A abundância de ribossomos livres, retículo endoplasmático rugoso, dictiossomos e numerosas vesículas fundindo-se com a membrana plasmática, além do acúmulo extraplasmático da secreção caracterizaram os idioblastos secretores de mucilagem. A presença de retículo endoplasmático liso, frequentemente com cisternas dilatadas e plastídios polimórficos contendo inclusões osmiofílicas caracterizaram os idioblastos secretores de óleo. Presença de cúpula foi observada unicamente nos idioblastos secretores de óleo sob microscopia de luz. Lamela mediana suberizada entre duas camadas celulósicas nas paredes celulares foi detectada em ambos os idioblastos secretores; contudo, lamela de suberina com aspecto lamelar foi observado

sob microscópio eletrônico de transmissão unicamente nos idioblastos secretores de óleo. Os idioblastos secretores de mucilagem maduros eram hipertrofiados e se localizavam em posição subjacente a epiderme. Sinais de desintegração parcial da parede do idioblasto secretor e das células epidérmicas adjacentes, com extravasamento do conteúdo mucilaginoso para a superfície da folha ou para os espaços intercelulares do mesofilo foram frequentemente observados. Paredes labirínticas em padrão reticulado nos idioblastos secretores de mucilagem maduros, presença de camada protetora (aglomerados elétron-densos ramificados na face interna da parede de células do parênquima justapostas ao idioblasto secretores de mucilagem), presença de bainha de células fenólicas ao redor dos idioblastos secretores de óleo, e fusão entre idioblastos secretores óleo adjacentes foram novidades deste estudo. A presença de estruturas secretoras envolvidas na produção contínua de secreções hidrofílica e lipofílica nos meristemas e órgãos em desenvolvimento pode ser um atributo que contribui para a sobrevivência das plantas em áreas ensolaradas, como é o caso de *O. pulchella* no cerrado. Os dados deste estudo fornecem subsídios para discussões sobre a possível homologia dos idioblastos secretores de óleo e de mucilagem em Lauraceae.

Palavras-chave: idioblasto secretor de mucilagem, idioblasto secretor de óleo, Lauraceae, ontogenia, parede suberizada, secreção, ultraestrutura

ABSTRACT: Solitary cells specialized in oil secretion, or secretory idioblasts, are a prominent feature of the Lauraceae family. In some species of Lauraceae, mucilage-secreting cells together with oil cells have been reported in their vegetative organs. A certain degree of complexity in cell walls and in the compartmentalization of mucilage and oil secretion has been reported in the literature; however, detailed ontogenetic and ultrastructural studies comparing these two idioblasts are restricted to a few species. Comparative studies are needed to access particularities in their morphology, development and functioning. In this study, we investigated the distribution, ontogenesis, development and ultrastructure of oil cells and mucilage cells in the shoot apex and leaves of *Ocotea pulchella*, according to conventional methods in light and electron microscopy. Both types of secretory cells arise from seemingly identical solitary initial cells located in the ground meristem just below the protoderm. The initiation of secretory cells is restricted to the initial stage of development of the stem organs; the secretory cells have asynchronous growth and can be found at different stages of development during the differentiation of the shoot apex and leaves, with the development of oil cells preceding the development of mucilage cells. Mucilage cells occurred exclusively in the palisade parenchyma, while oil cells occurred dispersed in the mesophyll (predominantly in the spongy parenchyma), midrib and petiole. Mucilage cells have a lamellar-like secretion consisting predominantly of polysaccharides, while in oil cells the secretion is homogeneous, shiny and contains a mixture of terpenes, neutral and acidic lipids. The abundance of free ribosomes, rough endoplasmic reticulum, dictyosomes and numerous vesicles fusing with the plasma membrane, in addition to the extraplasmic accumulation of secretion characterized mucilage cells. The presence of smooth endoplasmic reticulum, often with dilated cisternae, and polymorphic plastids containing osmiophilic inclusions characterized oil cells. Dome presence was observed only in oil cells under light microscopy. Suberized median lamella between two cellulosic layers in the cell walls was detected in both secretory idioblasts; however, a lamellar-looking suberin slide was observed under transmission electron microscopy only in the oil cells. The mature mucilaginous cells were hypertrophied and located in a position underlying the epidermis. Signs of partial disintegration of the secretory cell wall and adjacent epidermal cells, with extravasation of mucilaginous content to the leaf surface or to the intercellular spaces of the mesophyll were frequently observed. Labyrinthine walls in a reticulated pattern in mature mucilaginous cells, presence of a protective layer (branched electron-dense clusters on the inner face of the parenchyma cell wall juxtaposed with the mucilaginous cell), presence of a sheath of phenolic cells around the oil cells, and fusion between adjacent oil cells were novelties of this study. The presence of secretory structures involved in the continuous production of

hydrophilic and lipophilic secretions in meristems and developing organs may be an attribute that contributes to the survival of plants in sunny areas, as is the case of *O. pulchella* in the cerrado. The data from this study provide subsidies for discussions about the possible homology of oil-secreting and mucilage-secreting idioblasts in Lauraceae.

Key words:, mucilage-secreting idioblast, oil-secreting idioblast, Lauraceae, ontogeny, suberized wall, secretion, ultrastructure

INTRODUÇÃO GERAL

A Família Lauraceae

Lauraceae Juss. (Ordem Laurales), é uma grande família pertencente a Magnoliidae, que compreende 50 gêneros e 2.500-2.830 espécies (Stevens, 2022), de distribuição pantropical, ocorrendo, por exemplo no sul da Ásia (Paleotrópico) e no Brasil (Neotrópico) (Costa et al., 2017; Sousa et al., 2019). No Brasil são registradas 467 espécies das quais 239 são endêmicas distribuídas em 27 gêneros (Flora e Funga do Brasil, 2022). O gênero *Ocotea* Aubl., em sua circunscrição atual, é o maior gênero entre as Lauraceae Neotropicais, composto por cerca de 400-450 espécies reconhecidas (Rohwer, 1986; van der Werff, 1996; 2002; 2013; 2017;Trofimov et al., 2019; Trofimov e Rohwer, 2020), das quais cerca de 90% são Neotropicais, enquanto as restantes são provenientes da Macaronésia, África e Madagáscar (Trofimov et al., 2022).

Lauraceae é composta quase exclusivamente por árvores e arbustos, embora espécies do gênero *Cassytha* L. possam apresentar formas de crescimento herbáceas ou parasitárias (Weber, 1981; Rohwer et al., 1993). A família é principalmente perene e atualmente ocupa florestas tropicais e temperadas quentes em uma faixa altitudinal significativa (van Der Werff e Richter, 1996; Reis-Avila e Oliveira, 2017) e está entre as famílias de plantas lenhosas mais frequentes em áreas tropicais úmidas (Rohwer et al., 1993; Trofimov et al., 2019; Trofimov e Rohwer, 2020). Algumas espécies desta família são conhecidas por fornecer madeiras de construção conhecidas como “coração verde” (*Chlorocardium rodiaei* (R.Schomb.) R.R.W. (Greenheart)) “madeira de ferro” (*Eusideroxylon zwageri* Teijsm. & Binn.), imbuia (*Ocotea porosa* (Nees & Mart.) Barroso), nogueira (*Endiandra palmerstonii* (F.M.Bailey) C.T.White) e a madeira sul-africana (*Ocotea bullata* (Burch.) Baill.), além de frutos comestíveis como o abacate (*Persea americana* Mill.), sementes oleaginosas (e.g. *Laurus nobilis* L. e *Litsea sebifera* Pers.)

(Gottlieb, 1972; Rohwer, 1993). Outras espécies são também fonte de substâncias aromáticas extraídas para condimentos e perfumaria (Rohwer, 1993), tais como o óleo de pau-rosa (*Aniba rosaedora* Ducke), o óleo de sassafrás brasileiro (*Ocotea odorífera* (Vell.) Rohwer), canela (*Cinnamomum verum* J.Presl) ou para fins farmacêuticos, como a cânfora (*Cinnamomum camphora* (L.) J.Presl).

As folhas de Lauraceae são geralmente verde-escuras e brilhantes em suas superfícies adaxiais e vilosas e verde-acinzentadas ou verde-azuladas em suas superfícies abaxiais, frequentemente coriáceas o que melhora seu potencial de preservação no registro fóssil (Bannister et al., 2012). A presença de idioblastos secretores no mesofilo às vezes origina pontos geralmente imperceptíveis na folha comumente chamadas de "pellucid gland dots" (Rohwer, 1993).

Idioblastos secretores de óleo constituem uma característica importante da família Lauraceae, tendo sido reportadas na raiz, caule, casca, folha, flores e frutos de representantes desta família (Metcalfe e Chalk, 1950; 1983; Chu et al., 1999; Judd et al., 2002). Estudos microscópicos reportaram além de idioblastos secretores de óleo, idioblastos secretores de mucilagem ou de ambos os tipos no mesmo órgão para algumas espécies desta família (Richter, 1981; Bakker e Gerritsen, 1989; Bakker et al., 1991; 1992; Chu e Hu, 1999; Chu et al., 1999; Chu e Hu, 2001a; Farago et al., 2005; Betim et al., 2020).

Ocotea pulchella (Nees & Mart.) Mez. é uma espécie arbórea de Lauraceae, conhecida como "canela-do-cerrado", amplamente distribuída em cerrados e floresta estacional das regiões Centro-Oeste, Sudeste e Sul do país (Lorenzi, 2002; Baitello e Marcovino, 2003). Esta espécie possui características que a recomendam para paisagismo e plantio misto em áreas degradadas (Lorenzi, 2002), além da importância de seus frutos que são muito apreciados por aves frugívoras (Baitello e Marcovino, 2003; Durigan et al., 2004).

Ocotea pulchella é uma espécie heliófita, que tolera baixas temperaturas (Carvalho, 2006). Seus representantes variam de semidecíduos à perenes, podendo atingir 30 metros de altura e até 120 centímetros de DAP (diâmetro altura de peito); possuem tronco geralmente tortuoso, com fuste de até 10 metros de comprimento; a copa é ampla, paucifoliada e estratificada, com folhagem verde-fosca. Suas folhas medem de 2 a 8 cm de comprimento e 1 a 3 cm de largura; são simples, alternas, inteiras, coriáceas, curto-pecioladas e base atenuada, com face superior geralmente glabra e face inferior com pilosidade ferrugínea característica, densa ou rala. A lâmina varia de elíptica a lanceolada, com ápice obtuso ou subacuminado. A nervação é peninérvea, densa e nitidamente reticulada em ambas as faces e, muitas vezes, a face inferior é cerulescente (Carvalho, 2006).

Nos cerrados da região centro-oeste do estado de São Paulo, os indivíduos de *O. pulchella* medem entre 4-10 metros de altura (Fig. 1a). Na lâmina das folhas jovens podem ser observadas áreas circulares a ovaladas de tamanhos variados, de coloração verde-claro, dispersas nas áreas internervurais (Fig. 1b-c), que correspondem as "glândulas pelúcidas".

No decorrer de um estudo sobre a anatomia foliar de espécies de *Ocotea* em diferentes fisionomias de Cerrado (Bento et al., não publicado), encontramos situações indicativas da co-ocorrência de idioblasto de óleo e idioblasto de mucilagem em ápices caulinares e folhas jovens. Com base nesses achados, discutimos a necessidade de aprofundar os estudos a fim de distinguir esses dois tipos de idioblastos secretores. Consideramos que *O. pulchella* oferece um excelente modelo para estudos comparativos sobre a distribuição, ontogênese, desenvolvimento e ultraestrutura dos idioblastos secretores.

Idioblastos secretores e suas secreções

Células solitárias especializadas em secreção, denominadas células secretoras ou idioblastos secretores, foram relatadas em diferentes famílias de plantas, incluindo Lauraceae

(Solereder, 1908; Metcalfe e Chalk, 1950; Fahn, 1979; Roshchina e Roshchina, 1993). Na maioria dos casos, os idioblastos secretores constituem os principais locais de síntese e armazenamento de uma variedade de substâncias especializadas, como óleos essenciais, resinas, mucilagem e substâncias fenólicas (Fahn, 1990; Evert, 2006). Os metabolitos secundários desde sempre despertaram grande interesse na comunidade científica não só pelas atividades biológicas que desempenham, como também constituem uma fonte única de produtos com diversas aplicações nas áreas farmacêutica, alimentar, agronômica, perfumaria e outras (Ascensão, 2006; Ramakrishna e Ravishankar, 2011).

As secreções vegetais também desempenham uma função importante nas interações da planta com os ambientes biótico e abiótico (Harbone, 1993), muitas vezes atuando como um mecanismo de defesa das plantas contra herbívoros e patógenos (Roshchina e Roshchina, 1993). Os óleos essenciais, por exemplo, consistem em uma mistura complexa de compostos, geralmente de 20 a 60, em diferentes concentrações, sendo os terpenos os seus principais constituintes (Guimarães et al., 2019). Os terpenos desempenham importantes funções no metabolismo vegetal como constituintes da membrana, pigmentos fotossintéticos, substâncias de crescimento e reguladores vegetais (Taiz et al., 2017). Além disso, agem como transportadores de glucosil em reações de glicosilação e estão envolvidos na regulação do crescimento celular (Taiz et al., 2017), além da comprovada atividade antimicrobiana e defesa da planta contra herbívoros e patógenos (Gershenzon e Croteau, 1992). A importância comercial dos terpenos, por exemplo, como aromatizantes de alimentos e na indústria de perfumes, inseticidas naturais, entre outros é amplamente reconhecida (Guimarães et al., 2019).

A mucilagem é uma matriz de compostos com alto peso molecular secretada na forma de um gel viscoelástico rico em polissacarídeos e ácidos urânicos, solúvel em água, presente em diferentes partes das plantas incluindo folhas e gemas (Sasse et al., 2018; Mukherjee et al., 2019). Diversas funções têm sido atribuídas à mucilagem como adesão, lubrificação (Galloway

et al., 2020), neutralização de agentes químicos e poluentes (Alvikar Annapurna et al., 2017) e retenção de água (Mollenhauer e Morré, 1966). Por sua propriedade bioadesiva, a mucilagem apresenta potencial comercial emergente na agricultura, alimentos, cosméticos, biomedicina e produtos farmacêuticos devido às suas propriedades não tóxicas e biodegradáveis. Além de suas propriedades funcionais, a mucilagem também pode ser utilizada para a produção de nanocarreadores (Tosif et al., 2021).

Além dos potenciais papéis dos idioblastos secretores durante um estágio particular e sensível do desenvolvimento da planta, é importante destacar a importância da presença e do tipo de estruturas secretoras em estudos taxonômicos, na distinção de ordens, tribos, gêneros e até mesmo espécies (Metcalfe e Chalk, 1950; Robson, 1977; Metcalfe e Chalk, 1979; Robson, 1981; Metcalfe e Chalk, 1983).

Estudos sobre a ocorrência, morfologia e valor taxonômico de idioblastos secretores de óleo e de mucilagem são numerosos no final do século 19 e início do século 20, como mostram as compilações de diferentes autores (Metcalfe e Chalk, 1950; Fahn, 1979; Evert, 2006). Idioblastos de mucilagem ocorrem dispersos no tecido fundamental dos diferentes órgãos em muitas famílias (magnoliídeas e eudicotiledôneas), incluindo Annonaceae, Cactaceae, Lauraceae, Magnoliaceae, Malvaceae e Tiliaceae (Mollenhauer, 1967; Metcalfe e Chalk, 1979; Gregory e Baas, 1989). Idioblastos de óleo ocorrem em Calycanthaceae Lauraceae, Magnoliaceae, Winteraceae e Simaroubaceae (Metcalfe e Chalk, 1979; Bass e Gregory, 1985).

A ideia que idioblastos secretores de óleo e idioblastos secretores de mucilagem são homólogas, uma vez que podem ter iniciais idênticas e que poderiam substituir-se mutuamente nas Lauraceae foi proposta por Janssonius (1926, 1934). Este autor se baseou em observações da casca de *Cinnamomum verum* (sinonímia *Cinnamomum zeylanicum* Nees). Posteriormente, ao estudar a madeira e casca de numerosas espécies de Lauraceae com microscopia de luz, Richter (1981) documentou casos de espécies estreitamente relacionadas com idioblastos

secretores de óleo, idioblastos secretores de mucilagem ou ambos os tipos de idioblastos secretores. Em uma importante revisão sobre a ocorrência de idioblastos secretores de óleo em eudicotiledôneas, com especial ênfase na ocorrência conjunta (idioblasto de óleo e idioblasto de mucilagem) ou aparente substituição mútua desses dois tipos de idioblastos secretores, Baas e Gregory (1985) ressaltam a possível homologia desses idioblastos.

Em algumas famílias basais de angiospermas como Calycanthaceae, Lauraceae, Magnoliaceae, Piperaceae, Simaroubaceae e Winteraceae as células de óleo, quando maduras, apresentam três camadas parietais distintas, sendo as camadas externa e interna celulósica e a intermediária caracterizada pela presença de suberina (lamela de suberina). No protoplasto destas células, forma-se um compartimento revestido pela membrana plasmática ("cavidade de óleo") que se liga à camada parietal mais interna por uma cúpula, que nada mais é que uma protrusão desta parede (Fahn, 1979).

As células de mucilagem e de óleo compartilham vários aspectos nas magnoliídeas (Magnoliales e Laurales), sendo a presença de uma camada suberizada (lamela de suberina) na parede celular mediana um dos mais importantes (Bakker e Gerritsen, 1989; 1990; Bakker et al., 1991; Bakker e Gerritsen,1992). Já as eudicotiledôneas teriam perdido a capacidade de depositar uma camada suberizada em suas células de mucilagem (Bakker e Baas, 1993). Na maioria dessas plantas, a mucilagem é depositada pelo citoplasma entre a parede celular própria e a membrana plasmática. Apenas em casos excepcionais (por exemplo, raízes de *Symphytum* Tourn. ex L.; células de mucilagem com ráfides) considera-se que a mucilagem é produzida no, ou, em direção ao vacúolo (Tschirch, 1889; Gregory e Baas, 1989).

O desenvolvimento e a ultraestrutura dos idioblastos secretores em Lauraceae foi foco de estudos nas décadas de 1970-1980 (Tabela 1).

Os estudos indicam que os idioblastos secretores de óleo e de mucilagem apresentam uma diversidade considerável em sua morfologia, desde globular, alongada a ramificada, sendo

ambos os tipos de idioblastos geralmente maiores do que as células circundantes do tecido onde estão inseridos (Baas e Gregory, 1985). Certos critérios têm sido usados para distinguir esses dois tipos de células secretoras, principalmente a compartimentalização da secreção e especializações da parede celular como a presença de parede suberizada e formação de cúpula (Maron e Fahn, 1979; Baas e Gregory, 1985; Bakker e Gerritsen, 1989, 1990; Bakker et al., 1991; Bakker e Gerritsen, 1992; Chu et al., 1999; Chu e Hu, 2001a, 2001b).

Com relação aos idioblastos secretores de óleo, as principais características no nível ultraestrutural incluem a deposição de uma camada mediana de suberina entre duas camadas celulósicas caracterizando uma estrutura trilamelar e a formação da cúpula na qual o saco de óleo está ligado (Mariani et al., 1989; Bakker et al., 1991; Chu e Hu, 2001a). A gota de óleo é frequentemente anexada a uma protusão da camada parietal mais interna (cúpula) com o formato de um sino envolto em plasmalema; os plasmodesmos são numerosos e estão concentrados no local da fixação da cúpula na parede (Maron e Fahn, 1979; Mariani et al., 1989; Bakker e Gerritsen, 1990; Platt-Aloia e Thomson, 1992; Chu e Hu, 2001a). Em seu estudo com *Cinnamomum cassia* Presl. (Lauraceae) Geng et al. (2012) observaram de 2 a 4 cúpulas ligadas a um único saco de óleo, localizadas em qualquer lado da célula; os autores sugerem que a cúpula pode desempenhar um papel importante no apoio ou fixação do saco de óleo em um local específico no lúmen celular. Estudos também reportaram que em alguns táxons falta uma cúpula característica (Postek e Tucker, 1983; Bakker e Gerritsen, 1989). O óleo é sintetizado principalmente em plastídios modificados desprovidos de tilacóides e são compostos de terpenos, gorduras e agliconas (Hegnauer, 1966; Fahn, 1979, 1988). Na maturidade, ocorre degeneração completa do protoplasto localizado na região periférica da célula e todo o volume celular é ocupado pelo saco de óleo misturado a detritos citoplasmáticos, sendo isolado pela parede celular especializada (Geng et al., 2012). Especula-se que a suberificação da parede tem como função impedir o extravasamento da secreção, protegendo

as células adjacentes de compostos tóxicos do óleo, impedindo o transporte apoplástico do conteúdo celular (Bakker e Gerritsen, 1989; Bakker e Baas, 1993).

Nos idioblastos secretores de mucilagem, a secreção é produzida nos corpos de Golgi, cujas vesículas, cheias de polissacarídeos, se movem em direção a membrana plasmática e com ela se fundem liberando o conteúdo no espaço extraplasmático; o acúmulo da secreção ocorre entre a parede celular e a membrana plasmática, em toda a periferia da célula; devido à deposição contínua de mucilagem neste espaço, o citoplasma é empurrado para o interior da célula (Mollenhauer, 1967; Hyde, 1970; Trachtenberg e Fahn, 1981; Bakker e Gerritsen, 1989). De um modo geral, os idioblastos secretores de mucilagem frequentemente possuem unicamente paredes celulósicas, sem formação de cúpulas ou deposição de camada de suberina; no entanto, a presença de cúpula e lamela suberizada tem sido reportada em células de mucilagem em alguns representantes de Magnoliales e Laurales incluindo Annonaceae e Lauraceae respectivamente (Baas e Gregory, 1985; Mariani et al., 1989; Bakker e Gerritsen, 1989; Bakker et al., 1991; Bakker e Bass, 1993). Para certos autores, a lamela de suberina nos idioblastos secretores de mucilagem desses taxons pode ser interpretada como uma relíquia ancestral, considerando-se que estes evoluíram a partir de idioblastos secretores de óleo e os substituem em certas linhagens basais (Baas e Gregory, 1985; Bakker et al., 1991). A partir dos idioblastos secretores de mucilagem sem paredes suberificadas teriam se desenvolvido as 'bolsas' (cavidades secretoras) e os canais de mucilagem, ou alternativamente, estes poderiam ter surgido "*de novo*" várias vezes durante a evolução das eudicotiledôneas (Bakker e Baas, 1993).

Como se observa na literatura, a maioria dos estudos ontogenéticos e ultraestruturais sobre idioblastos secretores de óleo ou de mucilagem é restrita a poucas espécies de Lauraceae (Tabela 1). De modo geral, os diversos autores tendem a generalizar o padrão de desenvolvimento e características dos idioblastos secretores em diferentes famílias associando-

os com a natureza química da secreção, isto é, hidrofílica ou lipofílica (Marinho et al., 2011). Estudos ultraestruturais detalhados têm revelado relatos conflitantes especialmente com relação às características dos idioblastos secretores de óleo (Postek e Tucker, 1983), além de diferenças substanciais em certos aspectos da ontogênese e no curso de desenvolvimento desses idioblastos. As contradições dizem respeito às generalizações sobre a presença obrigatória de uma camada parietal suberizada e da cúpula em idioblastos secretores de óleo "típicas". A literatura mostra que poucos estudos compararam o desenvolvimento e a ultraestrutura de idioblastos secretores de óleo e idioblastos secretores de mucilagem ocorrendo lado a lado em determinado órgão da mesma espécie, exceto o estudo detalhado realizado por Bakker et al. (1991) em *Cinnamomum burmanni* (Nees & T.Nees) Blume, um representante de Lauraceae. Segundo esses autores, estudos ultraestruturais sob o ponto de vista do desenvolvimento são fundamentais para contribuir com argumentos a favor ou contra a hipótese de Janssonius (1926, 1934) de uma possível homologia entre os idioblastos secretores de óleo e os idioblastos secretores de mucilagem uma vez que acumulam metabólitos especializados muito diferentes.

OBJETIVOS

Este trabalho teve como objetivo investigar comparativamente os idioblastos secretores de mucilagem e idioblastos secretores de óleo no ápice caulinar e folhas de *Ocotea pulchella* a fim de distinguir as peculiaridades morfológicas e funcionais desses dois tipos de idioblastos secretores. Tal abordagem é necessária para caracterizar adequadamente os dois tipos de idioblastos secretores coexistindo lado a lado. Além do mais, os resultados obtidos poderão contribuir com as discussões sobre possível homologia desses dois tipos de idioblastos secretores em Lauraceae.

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FIGURA E LEGENDA

Figura 1. Morfologia de *Ocotea pulchella*. **a**. Indivíduo com aproximadamente quatro metros de altura, mostrando copa ampla, paucifoliada e estratificada. **b**. Ramo com folhas em diferentes estágios de desenvolvimento. Note áreas circulares mais claras (setas) dispersas nas áreas internervurais das folhas jovens. **c**. Detalhe da lâmina foliar com áreas mais claras denominadas "glândulas pelúcidas" que correspondem aos idioblastos secretores.

Figura 1.

TABELA

Tabela 1. Idioblastos secretores estudados em diferentes espécies e órgãos de representantes de Lauraceae. * Estudo de Idioblasto secretor de mucilagem e idioblasto secretor óleo ocorrendo no mesmo tecido/órgão.

ESPÉCIES	IDIOBLASTO SECRETOR	ÓRGÃO	REFERÊNCIA BIBLIOGRÁFICA
Persea americana Mill.	Idioblasto secretor de óleo	Fruto (mesocarpo)	Platt-Aloia e Thomson, 1981
Persea americana Mill.	Idioblasto secretor de óleo	Fruto (mesocarpo)	Platt-Aloia et al., 1983
Cinnamomum burmanni (Nees & T. Nees) Blume e *Cinnamomum verum* J.Presl	*Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	Folha	Bakker e Gerritsen, 1989
Cinnamomum burmanni (Nees & T.Nees) Blume	Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	Ápice e as folhas jovens	Bakker et al., 1991
150 espécies de *Cinnamomum* Schaeff.	*Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	Folha	Bakker et al., 1992
Persea americana Mill.	Idioblasto secretor de óleo	Fruto, cotilédone, folha e raiz.	Platt-Aloia e Thomson, 1992
Cinnamomum Schaeff.	*Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	Folha	Bakker e Bass, 1993
13 espécies de Lauraceae Juss.	Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	Folha	Chu et al., 1999

Cinnamomum longepaniculatum (Gamble)	*Idioblasto secretor de mucilagem e Idioblasto secretor de óleo	----	Chu e Hu, 2001a
Litsea pungens Hemsl.	Idioblasto secretor de óleo	Parte aérea	Chu e Hu, 2001b
Cinnamomum cassia Presl.	Idioblasto secretor de óleo	Casca de árvore	Geng et al., 2012

APRESENTAÇÃO DO CAPÍTULO

Conforme estabelecido pelo Programa de Pós-Graduação em Biologia Vegetal Interunidades Botucatu/Rio Claro, os resultados obtidos durante a execução deste Projeto de doutorado foram reunidos em um artigo científico, redigido de acordo com as normas de periódicos da área (Comitê de Biodiversidade da Capes).

O artigo intitulado "Oil and mucilage idioblasts co-occur in the vegetative organs of *Ocotea pulchella* (Lauraceae): Comparative development, ultrastructure and secretions" foi redigido segundo normas do periódico Protoplasma (A2).

Oil and mucilage idioblasts co-occur in the aerial vegetative organs of *Ocotea pulchella* (Lauraceae): Comparative development, ultrastructure and secretions

ABSTRACT: Solitary oil-secreting cells, or oil idioblasts, are a critical feature of the Lauraceae. In some species, including in *Ocotea pulchella* – the object of this study, the oil and mucilage idioblasts occur together in the vegetative organs. In this study, we undertake a comparative analysis of the two types of secretory idioblast, with a view to identifying their individual morphological and functional peculiarities. Both types of idioblast originate from solitary cells located in the ground meristem, just below the protoderm. The initiation of both types of idioblast is restricted to the early stages of organ development, but their growth is asynchronous, with the oil idioblasts developing first. Secreting idioblasts occurred in the shoot apex and in leaf primordia, as well as in young internodes, petioles and the laminae of expanded leaves. Mucilage idioblasts in the leaves occur exclusively in the palisade parenchyma, while oil idioblasts are scattered through the mesophyll, midrib and petiole. Mucilage idioblasts exhibit a lamellar secretion, consisting predominantly of polysaccharides, while the secretion of the oil idioblast was homogeneous in appearance, shiny and contained a mixture of terpenes and lipids. Cupules (cell wall protuberances) occurred only in the oil idioblasts. Suberized layers occurred in both types of idioblast. The new findings in this study include: the fusion of adjacent, young oil idioblasts; the presence of labyrinthine walls in a reticulate pattern in the mature mucilage idioblasts; a protective layer in the adjacent parenchyma cells; and a phenolic cell sheath around the oil idioblasts. Contrary to previous reports, the two types of secretory idioblast can be identified in the early stages of their development.

Keywords: Lauraceae, mucilage-secreting cells, oil-secreting cells, ontogeny, secretion, suberized layer, ultrastructure

1. INTRODUCTION

Solitary secretory cells or secreting idioblasts have been reported in numerous plant families, including in the 'Laurels' – Lauraceae Juss. In most cases, secretory idioblasts constitute the primary sites for synthesis and/or storage of various specialized substances, such

as essential oils, resins, mucilage and phenolic compounds (Fahn 1988; Evert 2006). For a long time, secondary metabolites have aroused the interest of the scientific community for their biological activities and also as unique sources of products with a diversity of applications in the pharmaceutical, food, agronomic, perfumery and other industry sectors (Ascensão 2006; Ramakrishna and Ravishankar 2011).

The Lauraceae are mostly tropical in distribution (Costa et al. 2017; Sousa et al. 2019), and the family is particularly well-represented in Brazil, with 467 registered species of which 239 are endemic. These are distributed across 27 genera (Flora e Funga do Brasil 2022). The Lauraceae species are particularly well-known for their production of aromatic substances, often extracted for perfumery or pharmaceutical purposes (Rohwer 1993; Judd et al. 2002; Teles et al. 2019). Oil-secreting idioblasts are a critical feature in the vegetative and reproductive organs of the Lauraceae species (Metcalfe and Chalk 1950; Chu et al. 1999; Judd et al. 2002). In taxonomic studies, the presence of 'pellucid gland dots' in the leaves is often used as a diagnostic character of the Lauraceae (Hammel 1986; Rohwer 1993). Although oil idioblasts are a critical feature in Lauraceae, previous studies have also documented the occurrence of mucilage idioblasts or both types of secretory idioblasts in the same organ (Baas and Gregory 1985; Bakker and Gerritsen 1989). Despite the taxonomic and commercial value of these plant secretions, there have been few studies on the ontogeny, development and ultrastructure of secretory idioblasts in the Lauraceae species, particularly on their oil-secreting and mucilage-secreting cells (Baas and Gregory 1985; Bakker and Gerritsen 1989; 1990; Bakker et al. 1991; Bakker and Gerritsen 1992).

Oil and mucilage idioblasts display considerable diversity in their morphology, from globular, to elongated, to branched (Foster 1956; Baas and Gregory 1985), with both kinds of idioblast being larger than the cells surrounding the tissue in which they are embedded (Baas and Gregory 1985). Specific criteria have been used to distinguish between oil-secreting and

mucilage-secreting idioblasts, most notably their cell wall specializations and the compartmentalization of their secretions (Maron and Fahn 1979; Baas and Gregory 1985; Bakker and Gerritsen 1989; 1990; Bakker et al. 1991; Bakker and Gerritsen 1992; Chu et al. 1999; Chu and Hu 2001a, 2001b). However, there are conflicting reports on some characteristics of oil and mucilage idioblasts. These relate to the presence of a suberized parietal layer and the cupule in 'typical' oil idioblasts, whereas such a suberized wall layer is lacking in mucilage idioblasts (Maron and Fahn 1979). However, the presence of a cupule and suberized lamella has been reported in mucilage-secreting idioblasts in representatives of the Magnoliales and Laurales (Baas and Gregory 1985; Mariani et al. 1989; Bakker and Gerritsen 1989; Bakker et al. 1991). According to these authors, the suberin lamella in the mucilage cells of these taxa can be interpreted as an ancestral relic, as these evolved from oil idioblasts, replacing them in certain primitive families (Baas and Gregory 1985; Bakker et al. 1991). It is remarkable that few studies have attempted to compare oil-secreting cells and mucilage-secreting cells that occur side-by-side in the leaves of the same species. The dearth of such comparative studies can lead to errors in interpreting these secretory structures (Bakker et al. 1991).

In a previous study with Lauraceae species distributed in the Brazilian Cerrado domain (a tropical savannah), we repeatedly observed situations indicative of the co-occurrence of oil and mucilage idioblasts in the leaves of *Ocotea pulchella* (Nees & Mart.) Mez. (Bento et al., unpublished). Based on these findings, we considered it appropriate to discriminate between these idioblasts. We consider this species offers an excellent model for comparative studies on the origins, development, lifespan and secretion dynamics of oil and mucilage idioblasts occurring together, in the same organ.

This study compares mucilage- and oil-secreting idioblasts in the shoots and leaves of *O. pulchella* with the aim of elucidating their ontogeny and development and their morphological and functional peculiarities. This approach should usefully contribute to

discussions on the possible homologies between these two types of secretory idioblasts in Lauraceae.

2. MATERIAL AND METHODS

2.1 Study site and plant material

Materials were collected from adult individuals (n = 5) of *O. pulchella* occurring in a cerradão fragment located in Fazenda Palmeira da Serra (coordinates 220° 48′ S, 480° 44′ W) in the municipality of Pratânia, State of São Paulo, Brazil. It is a forest formation in the Cerrado with sclerophyllous characteristics (Ribeiro et al. 1983). Cerradão has a continuous canopy and the tree cover ranges from 50 to 90% - higher in the rainy season, lower in the dry season. The average height of the tree layer varies from 8 to 15 m, providing light conditions that favor the formation of differentiated shrubs and herbaceous strata (Ribeiro et al. 1983). Voucher specimens were deposited in the Herbarium "Irina Delanova de Gemtchujnicov" (BOTU) under the registration number BOTU (36216).

Shoot apices and leaves at different developmental stages, from young (<10 mm) to fully-expanded leaf blades (~70 mm) growing in the periphery of the canopy, were collected from the five, previously selected trees. The samples were dissected immediately, and small pieces were promptly immersed in appropriate fixative solutions for light and electron microscopy.

2.2 Anatomical and histochemical studies

For general histological characterization under a light microscope (LM), shoot apex and leaf samples at different stages of development were fixed in 2.5% glutaraldehyde in 0.05 M sodium phosphate buffer for 24 h. These samples were dehydrated in an ethanol series and

processed following the conventional methods of (2-hydroxyethyl)-methacrylate historesin embedding (Leica Microsystems, Nussloch/Heidelberg, Germany). Cross- and longitudinal sections, 4–6 μm thick, were cut using a semiautomatic microtome (Leica RM2245), stained with 1% toluidine blue in 1% aqueous sodium tetraborate solution (O'Brien et al. 1964) and mounted in synthetic resin (Entellan, Merck KGaA, Darmstadt, Germany). Alternatively, fixed material was cut with a hand microtome (Ranvier). Sections (13−15 μm) were clarified in 50% sodium hypochlorite, double-stained with 1% aqueous solution of Astra blue (Roeser 1972) and Safranin (Kraus and Arduin 1997), dehydrated in ethanol series and n-butyl acetate, and sealed with Entellan® (Merck KGaA, Darmstadt, Germany) synthetic resin on permanent slides. To elucidate the nature of the 'pellucid gland dots' on the leaf blade, cross- and longitudinal sections were taken through these structures.

We used histochemical assays on sections (10–12 μm) of fresh material cut with a hand microtome (Ranvier) to record the *in situ* locations of major compounds in the cell walls and protoplasts of secretory cells. We also used sections (4–6 μm) of fixed (FAA 70) and embedded (methacrylate resin) material. The sections were treated with Sudan IV for lipids (Johansen 1940); Nile blue A for acid and neutral lipids (Cain 1947); Lugol's reagent for alkaloids and starch grains (Johansen 1940); Nadi's reagent (α-naphthol + dimethyl-p-phenylenediamine) for essential oils and oleoresins (David and Carde 1964); ferric trichloride for phenolic compounds (Johansen 1940); phloroglucinol for lignins (Jensen 1962); Ruthenium red for pectins (Jensen 1962); mercuric bromophenol blue for proteins (Mazia et al. 1953); iodine-zinc chloride for cellulose (Jensen 1962); and tannic acid and iron chloride for mucilage (Pizzolato and Lilie 1973). The control samples were tested, following the specifications of each test. All observations and documentation were performed using a light microscope (Leica DMR) with a digital camera (Leica DFC 500).

An additional histochemical probe for the detection of lipids using fluorochrome neutral red was developed (Kirk 1970). Free-hand sections of fresh material were treated with a 0.1% neutral red solution in 0.1% sodium phosphate buffer (pH 6.5) for 20 min at room temperature. The stained sections were washed and mounted in distilled water under a glass coverslip for observation (Machado and Rodrigues 2021). Images were acquired using a laser scanning confocal microscope (Leica TCS SP5) with LS AF software, using a 488/550 nm laser for excitation.

2.3 Ultrastructural studies

To characterize the leaf surface micromorphology, samples (~0.25 cm^2) from fully-expanded leaves were cut with a razor blade and immediately fixed in 2.5% glutaraldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7.2). Samples were then dehydrated in an ethanol series to 100% (2 h in each), critical-point dried (Bal-Tec CPD 030), mounted on a metallic support with adhesive and sputtered coated with gold (10 nm) (Bal-Tec SCD 050) for 160 s at voltage of 50 mA. The material was then examined with a scanning electron microscope (Quanta 200, Fei Company, FEI, Gräfelfing, Germany) at 20 kV.

To characterize the subcellular organization of the secretory idioblasts, samples of shoot apices and leaves at different stages of development were fixed with 2% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 50 mM sodium phosphate buffer (pH 7.4) and left overnight at 4°C. The material was post-fixed with 1% (w/v) osmium tetroxide solution in the same buffer for 2 h at ambient temperature (25°C), dehydrated in an ethanol series, and embedded in Araldite resin (Araldite 502, Electron Microscopy Sciences, Hatfield, USA). Ultrathin sections (70 nm) were contrasted with 4% (w/v) uranyl acetate and lead citrate solutions (Reynolds 1963). The materials were examined with a transmission electron microscope (TEM) Tecnai Spirit (FEI Company, Germany) at 80 kV.

3. RESULTS

3.1 Distribution, cytological and histochemical features of the secretory idioblasts

Secreting idioblasts were present in the shoot apices and leaf primordia, as well as in young internodes (Fig. 1a–c), and the petioles (Fig. 1d) and laminae of expanded leaves (Fig. 1e, f). These cells were conspicuous and usually had spherical to ovoid shapes (Fig. 1d, f). In large-diameter veins and petioles, these secretory structures are located mainly in the outer cortex (Fig. 1b–e), whereas, in the leaf blade, they are immersed in the mesophyll on both the adaxial and abaxial surfaces (Fig. 1f). Under the LM, secretory idioblasts were distinguishable from the neighboring cells based on their larger size and distinct contents (Fig. 1d, f). Mature mucilage and oil idioblasts occurred side-by-side and differed in the size, shape and appearance of their protoplasts and metachromatic reactions with toluidine blue dye (Figs. 1d and 2a), as described below.

Mucilage idioblasts

Mature mucilage idioblasts were located in a zone underlying the epidermis of the fully-expanded leaf, and embedded mainly in the palisade tissue (Fig. 2). The larger size and spherical-to-ellipsoidal shape (Fig. 2a, b), the thickened primary cell walls (Fig. 2d, h) and the translucent appearance of the protoplast (Fig. 2h, i) allowed easy recognition of mature mucilaginous idioblasts embedded within neighboring cells. In fresh samples, under transmitted light, mature mucilage idioblasts exhibited a lamellar appearance of the protoplast and translucent contents (Fig. 2 h, i) that overflowed shortly after collection, making it hard to observe these structures. Moreover, fresh specimens discharge copious secretions upon being cut, making it difficult to process these specimens for anatomical studies.

In the sections of material embedded in methacrylate resin and stained with toluidine blue, the cell contents were pink, indicating the presence of acid mucopolysaccharides (Fig. 2a, b). Mature mucilage idioblasts exhibited a broad extraplasmic space filled with secretions surrounding the cytoplasm (Fig. 2a, b) that occurred as a dense core in the central region of the idioblasts with strands attached to the cell walls (Fig. 2a).

Large mucilage idioblasts with broken walls and secretion through the mesophyll filling the intercellular spaces were often noted (Fig. 2c). Bulky mucilage idioblasts with thickened outer walls appressed against the epidermis (Fig. 2d) were often noted, as well as epidermal cells detached along their anticlinal walls (Fig. 2e) or even with broken external periclinal walls (Fig. 2f, g).

The cell walls of the mucilage idioblasts were stained red with ruthenium red, confirming the presence of polysaccharides in their composition (Fig. 2h, i). The cell contents were stained black when treated with tannic acid and an iron chloride reagent (Fig. 2j), indicating the presence of mucilage. No staining was observed for phloroglucinol, which is specific for lignin (Fig. 2k). Treatment with Sudan IV resulted in the detection of a thin layer in the cell wall (Fig. 2l). Probing with neutral red, produced a uniform fluorescence pattern over the cell walls and confirmed the presence of a suberized layer (Fig. 2m).

Under the scanning electron microscope (Fig. 3), several holes scattered on the adaxial surface of mature leaves were observed (Fig. 3a). Each hole was surrounded by elongated epidermal cells positioned radially to form a rosette (Fig. 3b). Occasionally, holes contained deposits of an unknown material (Fig. 3c). In addition, in particular regions of the leaf surface, the epidermis was raised and partially detached (Fig. 3d) or exhibited broken epidermal cells with an ample accumulation of amorphous material, probably the residues of secretory products (Fig. 3e–g). These images were consistent with the light microscope observations (Figure 2f, g) and were evidence of the release of secretion through the epidermis.

Oil idioblasts

Oil-secreting idioblasts occurred in the cortical parenchyma of the petiole and midrib (Fig. 1d, e) and were scattered in the mesophyll eventually in the palisade tissue (Fig. 4a), but mainly among the cells of the spongy parenchyma (Fig. 4a, b). Under the light microscope, mature oil idioblasts were characterized based on their smaller size than that of mucilage idioblasts and variable shapes, ranging from spherical, oval and rectangular to polygonal with roughly angular outlines (Fig. 4a–c).

In sections of material embedded in methacrylate resin and stained with toluidine blue, the oil idioblast content was stained purple or blue (Fig. 4a–c). Mature oil idioblasts exhibited a peripheral cytoplasm around a translucent central region (oil sac) filled with secretions (Fig. 4c, d). The appearance of the protoplast allowed us to distinguish between mucilage and oil idioblasts occurring together (Fig. 4c).

In freshly-sliced sections not exposed to any dye or reagent, under transmitted light, the area surrounding the oil sac was translucent, and the center of the oil sac contained deposits of opaque amorphous material and electron-lucent drops (Fig. 4d). The extrusion of secretory products was not observed in any situation.

Using the Sudan IV test to detect lipids, proved particularly effective in identifying oil idioblasts in the expanded leaves (Fig. 4e–g). The cell contents of the mature oil idioblasts was red-stained with Sudan IV (Fig. 4e), indicating neutral lipids, and the oil sac attached to the cupule (Fig. 4g) was visible. The cell contents were reddish-stained with Nile blue (Fig. 4h), revealing acidic lipids. Phenolic compounds, detected with the iron chloride test, were abundant in the parenchyma cells arranged as a sheath around the oil idioblasts (Fig. 4i). When treated with Nadi's reagent, part of the cell contents stained purple (Fig. 4j-k), revealing a mixture of resins and essential oils. Probing with neutral red lipid-fluorochrome produced strong green fluorescence in the center of the protoplast and the cell wall (Fig. 4l, m), confirming the presence of the oil sac (Fig. 4l, m) and a suberized layer in the cell wall, respectively (Fig. 4l).

3.2 Ontogenesis and development of secretory idioblasts

Histological sections throughout the shoot apex and young leaves revealed secretory cells at different developmental stages, ranging from early stages to full maturity occurring side-by-side (Fig. 5a). Both the oil and mucilage idioblasts originated from the ground meristem just below the protoderm on both the adaxial and abaxial sides (Fig. 5a). On the abaxial surface of the leaf primordium and young leaves, secretory cells appeared to differentiate earlier than those on the adaxial surface (Fig. 5a). The expanding secretory cells were full of secretory products when the other leaf tissues were still poorly differentiated.

The characteristics of the protoplast and cell growth enabled us to distinguish mucilage and oil idioblasts under a light microscope in the shoot apex and undeveloped leaves (Fig. 5b). In the early stages of differentiation, mucilage idioblasts exhibited numerous small vacuoles (Fig. 5c). The vesicular appearance of young mucilage idioblasts was observed in the fresh material (Fig. 5d). Later, the vacuoles merged, and a more extensive extraplasmatic region between the cell wall and cytoplasm was evident (Fig. 5e). This region was filled with mucilage from the hypertrophied vesicles/vacuoles (Fig. 5f). During the subsequent stages of differentiation, the cytoplasm became darker with a split appearance (Fig. 5f). Due to continuous mucilage deposition in the extraplasmatic space, the cytoplasm was gradually pushed toward the idioblast's central region, and where it appeared as darker strands (Fig. 5g). In contrast, the young oil idioblasts have vacuoles that coalesce to form a large central vacuole (Fig. 5h), which gradually expands, causing the nucleus (Fig. 5i) and cytoplasm to be restricted to areas close to the walls (Fig. 5j). A translucent zone, i.e., 'oil sac', was observed in the central region of the oil idioblast. Oil droplets gradually accumulated in the oil sac, pushing the cytoplasm to the periphery of the cells (Fig. 5j). Mature oil idioblasts, filled with oil, were devoid of cytoplasm (Fig. 5k). Young oil idioblasts, highly variable in shape and size occurred clustered in areas of large-diameter veins and petioles (Fig. 1c-e). Juxtaposition (Fig. 5l) and

coalescence (Fig. 5m) of several oil idioblasts forming conspicuous secretory cells were frequently observed.

3.3 Ultrastructure of the secretory idioblasts

Mucilage idioblasts

Under a transmission electron microscope (Fig. 6), young mucilage idioblasts were recognized based on the presence of an extensive extraplasmatic region between the cell walls and cytoplasm (Fig. 6a). Numerous vacuoles containing fibrillar materials, plastids with prominent starch grains (Fig. 6a), well-developed dictyosomes with adjacent vesicles (Fig. 6b), extensive rough endoplasmic reticulum, and free ribosomes (Fig. 6c) were the main features of the initial stages of mucilage cell differentiation. As differentiation progressed, multilamellar structures were formed through the cytoplasm (Fig. 6d, e), in addition to numerous dense vesicles in the peripheral cytoplasm or close to the plasmalemma (Fig. 6e). Dense fibrillar materials were observed in the extraplasmatic space (Fig. 6f). As the mucilage idioblasts expanded, the cytoplasm appeared somewhat fragmented, and the scattered fragments had a coarse appearance (Fig. 6g). When sectioned tangentially, the cell wall exhibited small electron-lucent ingrowths in a reticulate pattern (similar to labyrinthine walls, Fig. 6h). Due to the continued deposition of mucilage, the cytoplasm was pushed inwards (Fig. 6i), and the collapsed cytoplasm in the central region of the mucilage idioblast contained hypertrophied vesicles (Fig. 6j). Often, the cytoplasm of mature cells filled with secretion was folded during microtome sectioning (Fig. 6k). In fully-expanded cells, the remnants of the degenerate cytoplasm were embedded entirely in the mucilage (Fig. 6l). A distinct intermediary suberized layer (Fig. 6m) without typical lamellation was observed over the entire surface of the mucilage cell. The inner cellulosic wall layer facing the mucilage deposits had a loose appearance (Fig.

6n). The inner wall of parenchyma cells facing mucilage cells was covered internally with ramified clusters of pectin compounds, featuring a protective layer (Fig. 6n).

Oil idioblasts

Under a transmission electron microscope (Fig. 7), the very young oil-secreting idioblasts were identified by their thick walls, conspicuous nucleus, dense cytoplasm rich in free ribosomes, mitochondria (Fig. 7a), plastids (Fig. 7a, b), and cytoplasmic oil drops (Fig. 7c). The plastids exhibited large osmiophilic bodies or small globules but lacked thylakoids (Fig. 7b). Starch grains were absent. The differentiating oil idioblasts were irregular in shape, and larger than the neighboring chlorenchymatous cells, and cytoplasm exhibited low electron density and prominent plastids (Fig. 7d). As the oil idioblasts expanded, the walls became thicker and irregular in outline and appeared to push and compress the neighboring parenchyma cells (Fig. 7e). Immature oil idioblasts showed a thin electron-lucent line between the two walls, indicating the presence of a suberized lamella in the median position (Fig. 7f). Immature oil idioblasts with irregular outlines were observed, appearing to have intrusive growth, (Fig. 7g). During differentiation, an electron-transparent zone became apparent in the central region of the oil cells (Fig. 7h). Large plastids and nuclei with heterochromatin were observed at the periphery of the cells (Fig. 7h). Smooth endoplasmic reticulum in a circular arrangement forming circumvolutions at the edge of the oil idioblast cytoplasm were typical at this stage (Fig. 7i). The oil cells were surrounded by a sheath of tangentially elongated cells containing voluminous nuclei, chloroplasts (Fig. 7i) or modified plastids, surrounded by smooth endoplasmic reticulum profiles (Fig. 7j). During the deposition of the inner cellulosic layer, small protuberances with electron-dense materials became evident in the wall (Fig. 7i). However, wall protuberances (cupules) with characteristic structures were not observed using TEM. The initially limited oil sac, then increased in volume and finally occupied the central

part of the oil idioblast (Fig. 7k). At this stage of differentiation, the cytoplasm gradually degenerates and the structural integrity of the organelles is lost. At maturity, the oil cell cytoplasm was reduced to a thin layer lining the cell wall and devoid of cytoplasmic contents (Fig. 7l). The cell wall of the mature oil idioblast had a tripartite structure seen successively from the outside to the inside of the oil cell with an outer denser fibrillar layer, an intermediate electron-lucent layer, i.e., suberized layer, and an inner layer with a loose texture (Fig. 7m). The suberized layer with a typical lamellar structure was distinct over the entire cell surface.

4. DISCUSSION

This study presents a structural and functional characterization of mucilage and oil idioblasts located side-by-side in leaves of *Ocotea pulchella*, a Lauraceae species. The co-occurrence of mucilage and oil-secretory idioblasts in the same organ has previously been reported for other Lauraceae species, such as *Cinnamomum burmanni* (Nees & T.Nees) Blume (Bakker et al. 1991), *Ocotea puberula* (Rich.) Nees (Farago et al. 2005) and *Ocotea nutans* (Nees) Mez (Betim et al. 2020). However, we found some uniquenesses in the distribution, ontogenesis and cytological features of these idioblasts. Our comparative study contributes to a broadening of the discussion on the structure-function relationships of secretory idioblasts, as introduced here. The results may also contribute to discussions of the possible homology between these two types of secretory idioblast in the Lauraceae.

4.1 Distribution, ontogenesis and lifetime of the secretory idioblasts

Under the LM, these two types of secretory idioblast are easily distinguished in *O. pulchella* by their localizations, shapes, sizes, cell appearances and contents. The idioblasts secreting mucilage occur only in the mesophyll, and exclusively in the palisade parenchyma,

whereas the oil idioblasts are distributed throughout the mesophyll (principally in the spongy parenchyma), and in the midrib and petiole. The preferential distribution of the mucilage idioblasts is in the adaxial leaf epidermis. More rarely they are to be found in the leaf abaxial epidermis, the mesophyll, the midrib cortex and the petiole. These results are consistent with a previous study (Gregory and Baas 1989). It is worth emphasizing that the exclusive location of mucilage cells in the palisade parenchyma is being described here for the first time for Lauraceae. This character may help in their distinction. As demonstrated histochemically, the mucilage idioblasts present a lamellar secretion consisting mainly of polysaccharides, while oil idioblasts contain a homogeneous and shiny mixture of terpenes, neutral lipids and acid lipids. In *O. pulchella*, secretion of both mucilage and oil continues throughout leaf differentiation until leaves are fully expanded.

Our ontogenetic analyses show that both types of secretory idioblasts originate from solitary initials, located in the ground meristem just below the protoderm. Secretory cell initiation is restricted to the leaves' early developmental stage. Their growth is asynchronous, and can be observed at all stages of leaf expansion. The oil idioblasts develop first. In the fully expanded leaf, only mature idioblasts (oil and mucilage) are present. Histochemical analyses show that both mucilage and oil secretory idioblasts initiate secretion quite early and remain metabolically active throughout leaf development. This finding is consistent with previous studies suggesting prolonged metabolic activity of secretory structures throughout organ development (Machado et al. 2017). Evidence for fusion between secretory structures, such as observed in the cortex of the petiole and in central vein of *O. pulchella* has been recorded for different secretory systems (Rocha et al. 2011; Palermo et al. 2018; Canaveze et al. 2019). However, the cellular and molecular bases of this phenomenon are poorly understood and are worth closer study.

4.2 Cytological events associated with the development of secretory idioblasts

Our ultrastructural analyses provide evidence allowing early distinction between mucilage idioblasts and oil idioblasts. The absence of osmiophilic deposits in the vacuoles and the presence of typical plastids containing starch grains are both satisfactory criteria for recognizing future mucilage idioblasts, as noted in a previous comparative study (Bakker et al. 1991). The subcellular machinery of mucilage idioblasts in *O. pulchella,* indicated in the abundance of free ribosomes, a rough endoplasmic reticulum, dictyosomes, and numerous vesicles merging with the plasmalemma are all consistent with a hydrophilic secretion (Fahn 1988). In addition, there is the extraplasmatic accumulation of the secretion, featuring mucilage-secreting idioblasts (Bakker and Gerritsen 1989). The smooth endoplasmic reticulum and modified plastids containing osmiophilic inclusions, (probably lipids and terpenes/phenols) identified histochemically in the oil idioblasts in the leaves of *O. pulchella,* are associated with lipophilic secretions (Fahn 1988).

In *O. pulchella* leaves, a suberized layer was identified in the cell walls of both the mucilage and the oil idioblasts. Although comparative studies using TEM indicate that the morphology of the suberized layer in the cell walls of mucilage cells is identical to that in the oil cells (Bakker and Gerritsen 1989; Bakker et al. 1991), our study showed that the morphology of the suberized layer of these secretory cells was slightly different. In the oil idioblasts, the lamellar pattern can be due to a distinct degree of osmication of the subsequent thin layers of suberin and wax present in the suberized layer (Bakker and Gerritsen 1989). For both types of secretory idioblasts, we observed that under TEM, the suberin layer was already present before oil and mucilage secretion had begun. This follows a pattern similar to that described for some different species of Lauraceae (see Bakker et al. 1991). It has been speculated that wall suberization prevents secretion and extravasation, protects adjacent cells from toxic compounds

and prevents apoplastic transport of cell contents (Bakker and Gerritsen 1989; Bakker and Baas 1993).

The differences in the subcellular apparatus of the mucilage and oil idioblasts in *O. pulchella* are primarily associated with the chemical natures of the two secretions - i.e., hydrophilic vs. lipophilic (Fahn 1988). However, these two cell types share some characteristics, such as a suberized layer in the cell wall, extraplasmatic accumulation of secretion and degeneration of the cytoplasm in maturing mucilage cells. Such similarities between the oil and mucilage idioblasts were emphasized by Bakker et al. (1991). According to these authors, these characters lend strong support the hypothesized homology and mutual replacement of these two types of secretory idioblasts, as previously proposed by many (including Tschirch 1889; Janssonius 1926, 1934; Bakker and Gerritsen 1989; Bakker et al. 1991).

In *O. pulchella*, a wall protuberance, the so-called cupule (Bakker et al. 1991), was observed in the oil secretory cells only under LM, despite numerous efforts to detect it in ultrathin sections examined under TEM. We could not verify the occurrence of protuberances or cupule-like wall structures in our mucilage idioblasts using LM and TEM. In fact, cupules are less frequently recorded in mucilage cells (Bakker et al. 1991).

Our study reports a particularly noticeable wall structure specialization in the mucilage cells, characterized by a reticulate architecture featuring labyrinthine walls. Labyrinthine walls are normally characteristic of transfer cells (Evert 2006) which are usually located at key sites throughout the plant body and would seem to support particularly high transport fluxes of nutrients between apo- and symplastic compartments (Xia et al. 2017). Such sites function in nutrient acquisition across cell: cell interfaces to regulate nutrient partitioning between competing sinks (Pate and Gunning 1972). The membrane transport capacities of transfer cells are proportional to the amplification (up to 20-fold) offered by their transporter-enriched

plasmalemma surface areas (Xia et al. 2017). Therefore, we speculate that these wall projections in the mucilage idioblasts provide a capability of amplifying apoplast and symplast compartments, as discussed for transfer cells (Pate and Gunning 1972; Xia et al. 2017). In addition, the presence of a distinct layer in the parenchyma cells facing the mucilage idioblasts, called a 'protective layer' (O'Brien 1970) or a 'secondary cellulosic layer' (Czaninskiy 1973), could increase water/nutrient movement via the apoplast (Barnett et al. 1993) and facilitate hydroactive mechanisms at the interface between the parenchyma cell and the secretory idioblast. Similar wall specializations have been reported for the parenchyma cells of secondary xylem (Barnett et al. 1993; Chaffe 1974; Wisniewski and Davis 1989), and in leaf vascular bundle sheaths (Sajo and Machado 2001).

Phenolic compounds are a large class of secondary metabolites widely distributed in higher plants, including in *Ocotea* Aubl. species (Coutinho et al. 2006; Farago et al. 2005; Brustulim et al. 2020). The cells are filled with phenolic compounds around the oil idioblasts, as observed in this study. They could perform exert critical roles in disease resistance and in protection against damage from ultraviolet radiation, microbes and herbivores (Zhang et al. 2022). In addition, sheath cells with phenolic compounds can help maintain the structural integrity of the secreting idioblasts. The role of phenolic compounds in the osmoregulation and osmoprotection of cells subjected to severe water deficits is well known (Ennajeh et al. 2009).

4.3 The link between oil and mucilage secretions and the environment

The shoot apex is essential for plant building, so damage is highly detrimental to plant survival (Coley and Barone 1996). Therefore, apices could well be associated with specific morphological and chemical features that would increase protection or resistance against harmful agents and influences – such as from herbivory, desiccation, excess solar radiation/ultraviolet (Fahn and Cutler 1992; Scariot et al. 2005; Freeman and Beattie 2008). In

this circumstance, the importance of secretory structures to evolutionary success must be highlighted, if by producing a wide variety of biologically active substances a plant species might have a competitive edge (Fahn 1988). Thus, the presence of mucilage in the aerial organs could be interpreted as an adaptation to a harsh environment associated with water retention and ionic balance, prevention of desiccation and favoring organ growth and also as a protective mechanism against herbivory and pathogens (Lusa et al. 2014). Mucilage may also be important as a reserve carbohydrate for use in lean times of the year, as highlighted by Gregory and Baas (1989). Lastly, it is worth considering that (based on our own observations) mucilage idioblasts can release their contents onto the leaf surface, thus changing the water-permeability properties of the leaves (Paiva et al. 2022). Conversely, the secretory products of the oil idioblasts accumulate and are retained inside the oil sac, without leakage. Here, it is important to emphasize that the essential oils produced by Lauraceae species are recognized for their role in increasing resistance to fungal decay (Kew Science 2023) and a defensive role against herbivorous insects (Rodriguez-Saona and Trumble2003).

Summarizing, it's fair to conclude that the continuing production of hydrophilic and lipophilic secretions in the meristems and developing organs are an adaptation that contributes to the survival of these plants in sunny and harsh areas, such as *O. pulchella* in the Cerrado. The Cerrado is characterized by a seasonal tropical climate, with dry winters (May to September) and rainy summers (October to April). Average precipitation is 1,500 mm. In drought periods air humidity can fall to 15%, usually in July and August (Ratter et al. 1997). The high level of insolation throughout the year and frequent fires are also characteristic of the Cerrado (Coutinho 1978), and plants have specialized structural or functional attributes determined by climatic and edaphic conditions, and by the effects of fire that allow them to survive and reproduce in these conditions (Scariot et al. 2005).

4.4 Conclusions and perspectives

The differential distribution of mucilage and oil idioblasts in leaves of *O. pulchella*, and the subcellular structures linked to the development of these secretory cells, helps us distinguish between the two types of secretory idioblasts. Unlike previous studies, and other Lauraceae species (see Bakker et al. 1991), the mucilage and oil idioblasts in *O. pulchella* already show distinct characteristics in the initial stages of differentiation. The data gathered in this study will contribute to discussions on the possible homology between these two types of secretory idioblasts in Lauraceae.

In this study, as in previous studies with Lauraceae, no anatomical characteristics were observed that would justify the use of the term 'pellucid glands'. Conceptually, glands are multicellular structures, while isolated secretory cells are typical secretory idioblasts (Fahn 1988). Finally, our data points to the need for a reevaluation of some terms and concepts in relation to secretory structures, which have been used widely and mainly in taxonomic studies of Lauraceae, especially of 'pellucid gland dots' in the leaves and that in fact correspond to secretory idioblasts.

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FIGURE LEGENDS

Figure 1. Secretory idioblasts distribution in the vegetative organs of *Ocotea pulchella*. a. Longitudinal section. b–f. Cross-sections. a–b. Secretory idioblasts in the shoot apex and young leaves. Note secretory idioblasts in the cortical and medullary regions of the young internode. c. Secretory idioblasts in the cortex of the young stem. d. Secretory idioblasts in the cortex of the petiole. e–f. Expanded leaves. e. Main vein. f. Mesophyll. Symbols: sc, secretory cells.

Figure 2. Structural and histochemical characterization of the mucilage-secreting idioblasts in the expanded leaves of the *Ocotea pulchella*; cross-sections. a–b. Mucilage-secreting idioblasts and oil secreting idioblastsoccurred side-by-side (a). Mature mucilage-secreting idioblasts with broad extracellular space filled with secretion surrounding the cytoplasmic strands (arrows) that remain attached to the cell walls (a–b). c. Cells with broken walls (arrowheads) and intercellular spaces in the mesophyll filled with secretions. d. Expanding secretory cells with thickened outer cell walls toward the adaxial epidermis. e–f. Broken external periclinal walls (arrowheads) of the mucilage-secreting idioblasts and epidermal cells. G. Residues of secretion in the leaf surface. h–i. Red-stained pectin with Ruthenium red. Note the lamellar appearance of the protoplast, reduced cytoplasm (arrow), and positive reactions of pectin in the cell walls (marker). j. Black-stained mucilage stained with tannic acid and iron chloride. k. Absence of lignin in the walls of mucilage-secreting idioblaststreated with phloroglucinol. Note red-stained sclerenchyma cell walls. l. Sudan IV reaction. A red-stained thin median layer in the cell wall (marker). m. Lipids displaying green fluorescence (marker) with neutral red staining under a confocal microscope. Symbols: ep, epidermis; oc, oil cell; mc, mucilage cell; cw, cell wall; and se, secretion.

Figure 3. Scanning electron micrographs of the adaxial surface of the expanded leaves of *Ocotea pulchella*. a–b. Holes (arrowheads) are scattered in the epidermis. Note epidermal cells positioned radially to each hole (arrowhead in b). c. A hole containing deposits of an unknown material. d. Epidermis raised and partially detached. e–g. Broken epidermal cells showing accumulation of amorphous material (secretion residues).

Figure 4. Structural and histochemical characterization of the oil secreting idioblastss in the leaf of the *Ocotea pulchella*; cross-sections. A–c. Mature mucilage-secreting idioblastsand oil secreting idioblasts are embedded in the mesophyll. Note that oil secreting idioblasts are smaller than mucilage-secreting idioblasts. D. Oil sac filled with translucent material in a freshly sliced section. Note cupule (arrowhead). e–g. Red-stained lipids stained with Sudan IV. Note the oil sac attached to the cupule (f, g, arrowhead). h. Red-stained acidic lipids stained with Nile blue inside the oil sac. i. Black-stained phenolic compounds with ferric trichloride in the sheath cells surrounding the oil secreting idioblast. j–k. Nadi (α-naphthol + dimethyl-p-phenylenediamine) reaction. The purple color reveals a mixture of resins and essential oils. l-m. Lipids displaying green fluorescence with neutral red staining under a confocal microscope. l. Green fluorescence in the cell wall (marker) indicates a suberized layer. m. Green fluorescence inside the oil sac. Symbols: mc, mucilage cell; oc, oil cell; os, oil sac; and sh, sheath cells.

Figure 5. Secretory idioblast origin and development in the leaf of *Ocotea pulchella*. a. Oil secreting idioblastsand mucilage-secreting idioblasts differentiate earlier on the abaxial surface of leaf primordium than on the adaxial surface. b. Young oil secreting idioblastsand mucilage-secreting idioblaststogether. c–g. mucilage-secreting idioblast development. c–d. Young mucilage-secreting idioblast with numerous small vacuoles. d. The vesiculate

appearance of the protoplast in fresh material. e. Extraplasmatic region and coalescence of vacuoles. f. Expanding mucilage-secreting idioblast showing dark cytoplasm with a split impression (arrow) and mucilage material in the extraplasmatic space. g. Mature mucilage wall showing dark cytoplasmic strands (arrow) in the central region of the cell and broad extraplasmatic space filled with mucilage. h–m. oil secreting idioblast development. h. Young cells with a peripheral nucleus and cytoplasm and large vacuoles. Note the fusion of vacuoles. i. In a later stage of differentiation, the oil sac is overly large, and the nucleus and cytoplasm remain restricted to a thin peripheral layer (arrow). j. Translucent zone (oil sac) in the central region of the cell and cytoplasm (arrow) degeneration. Note sheath cells around the oil secreting idioblast. k. Mature oil secreting idioblastwith considerably reduced cytoplasm and large oil sac. l–m. Juxtapositions (l) and coalescence (m) of adjacent oil secreting idioblasts in the central vein. Symbols: cw, cell wall; ex, extraplasmatic region; mc, mucilage cell; nu, nucleus; oc, oil cell; os, oil sac; sh, sheath cell; and va, vacuole.

Figure 6. Transmission electron micrographs of mucilage-secreting idioblastsin the leaf of *Ocotea pulchella*. a–c. Young mucilage-secreting idioblasts. a. A cell containing numerous vacuoles, plastids with starch grains, and mitochondria. Note the extensive extraplasmatic region between the outer cell wall and plasmalemma. b. Dictyosomes with adjacent vesicles, plastids with starch grains, and mitochondria. c. Rough endoplasmic reticulum and free-ribosomes. d–h. Differentiation in progress. d. Expanding mucilage cells containing multilamellar structures in the cytoplasm. e. Dense vesicles in the peripheral cytoplasm and close to plasmalemma. f. Extensive accumulations in extraplasmatic space. g. Fragmented cytoplasm with a coarse appearance. h. Tangential view of the cell wall showing small electron-lucent protuberances labyrinthine-like walls. i–n. Mature mucilage-secreting idioblast. i. Mucilage deposits in extracellular space and collapsed cytoplasm in the central region of the

cell (dotted rectangle). j. Details of the dotted area showing hypertrophied vesicles. Note the fusion of vesicles with the plasmalemma. k. Cytoplasmic strands and abundance of mucilage. l. Fully expanded cell with degenerating cytoplasm embedded in mucilage secretion. M–n. The tripartite structure of the cell wall: inner cellulosic wall layer, intermediary suberized layer, and outer wall layer underlying the middle lamella. N. Details of the cell wall with ramified clusters of pectin compounds featuring a protective layer underlying the cytoplasm of the parenchyma cell. Symbols: cs, cytoplasmic strand; ct, cytoplasm; cw, cell wall; di, dictyosome; ex, extraplasmatic region; iw, inner wall layer; lm, multilamellar structure; lw, labyrinthine wall; mi, mitochondria; ml, middle lamella; mu, mucilage; ow, outer wall layer; pa, parenchyma cell; pl, plastid; pr, protective layer; rer, rough endoplasmic reticulum; sl, suberized layer; va, vacuole; and ve, vesicle.

Figure 7. Transmission electron micrographs of the Oil secreting idioblasts in the leaf of the *Ocotea pulchella*. a–c. Young oil secreting idioblasts. a. Irregularly thickened cell walls, conspicuous nucleus, and dense cytoplasm with mitochondria and plastids containing large osmiophilic inclusions. Note the angular cell outline. b. Plastids are devoid of thylakoids containing small osmiophilic globules. c. Free ribosomes, dilated profiles of smooth endoplasmic reticulum, vesicles, and oil drop. d–k. Differentiation in progress. d. Expanding cell showing cytoplasm with low electron density. Note sheath cells with black content surrounding the oil secreting idioblast. e. Oil secreting idioblast with thicker walls. Note adjacent parenchyma cell is irregular in outline. f. Immature oil secreting idioblastswith a thin electron lucent line between the two polysaccharide cell walls. Note large plastid. g. Oil secreting idioblastwith a starry outline showing the residues of degenerated cytoplasm. h. Expanding oil secreting idioblastwith a peripheral nucleus, big plastids, and small electron transparent oil sac in the central region. i. Two immature oil secreting idioblastshow an

electron-lucent line (suberin layer) in the wall. Note the inner cellulosic layer wall with dense, small protuberances. j. Part of the oil secreting idioblastshows abundant smooth endoplasmic reticulum with a circular arrangement in the cell periphery. Note a sheath cell containing a voluminous nucleus and modified plastids surrounded with smooth endoplasmic reticulum profiles. k. The final stage of differentiation of the oil secreting idioblast. Note residual degenerated cytoplasm in the cell periphery and large oil sac occupying most of the cell. l. Mature oil secreting idioblastwith reduced cytoplasm forming a thin layer lining the cell wall. m. Tripartite cell wall organization in the mature oil secreting idioblast. Note the outer denser layer, an intermediate electron-lucent layer with a lamellar structure (suberized layer), and an inner fibrillar layer with a loose texture. Symbols: cw, cell wall; iw, inner wall layer; mi, mitochondria; ml, median lamella; nu, nucleus; oc, oil cell; ol, oil; os, oil sac; ow, outer wall layer; pa, parenchyma cell; pl, plastid; ser, smooth endoplasmic reticulum; sh, sheath cell; and sl, suberin layer.

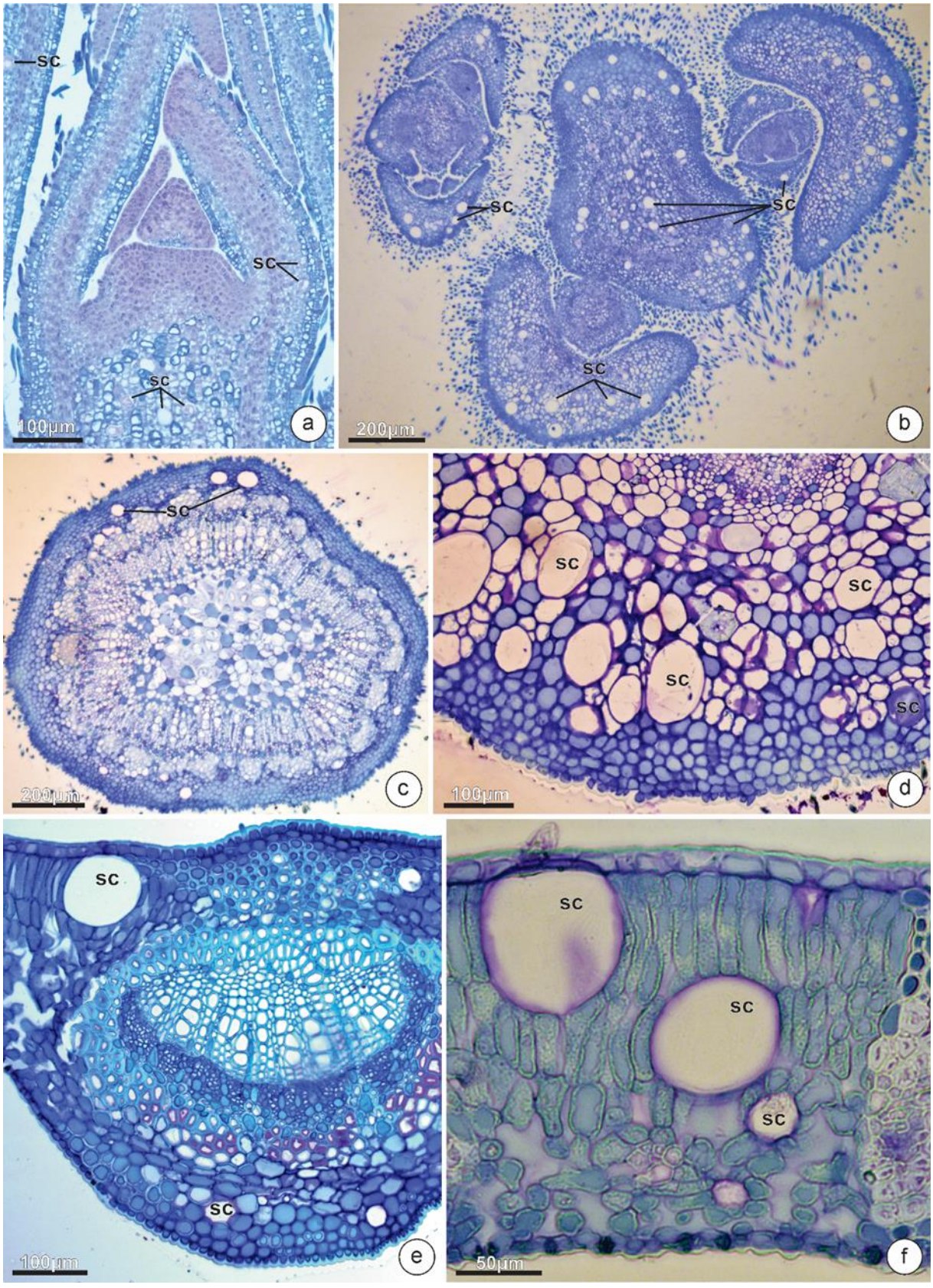
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Figure 1.

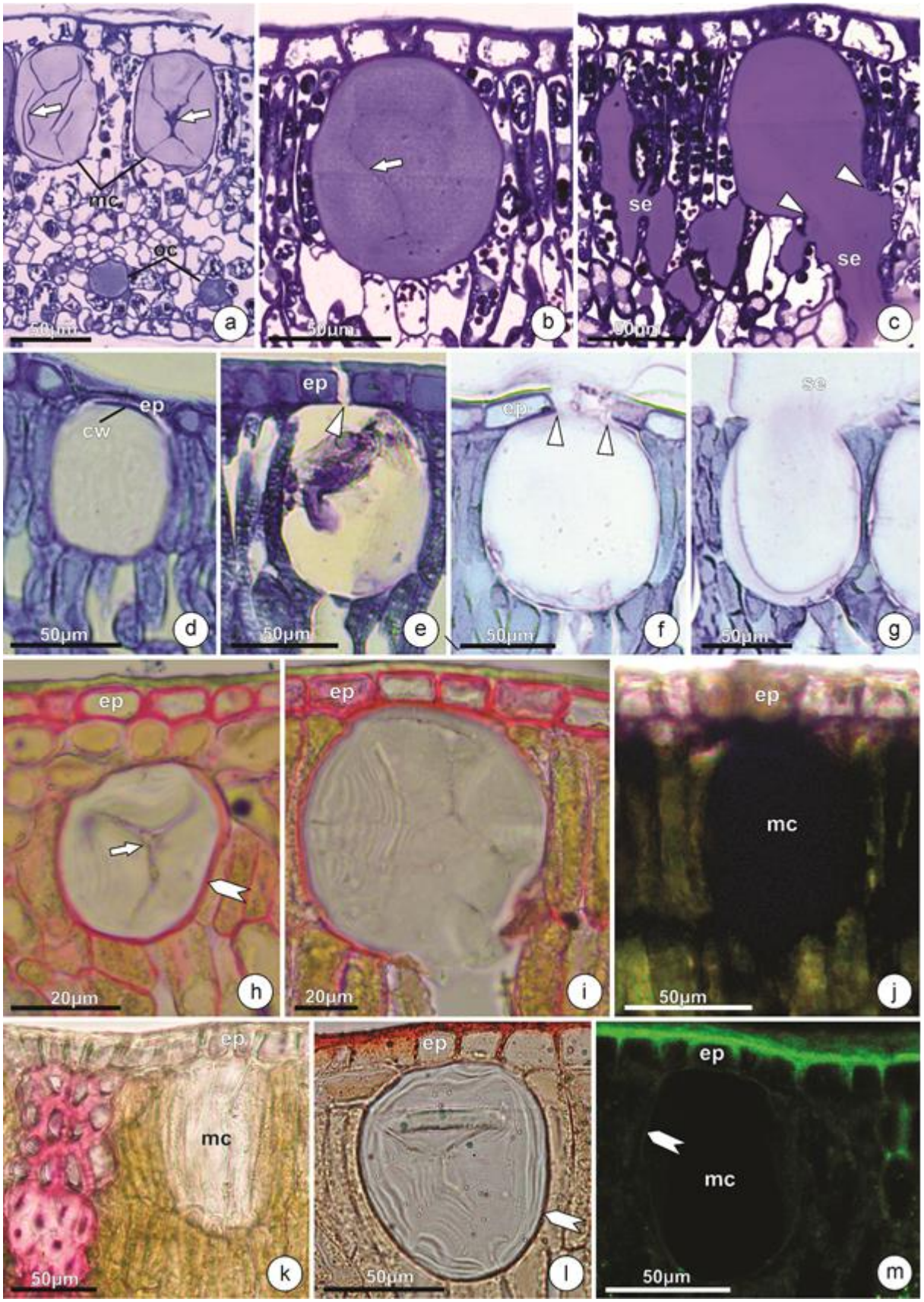
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Figure 2.

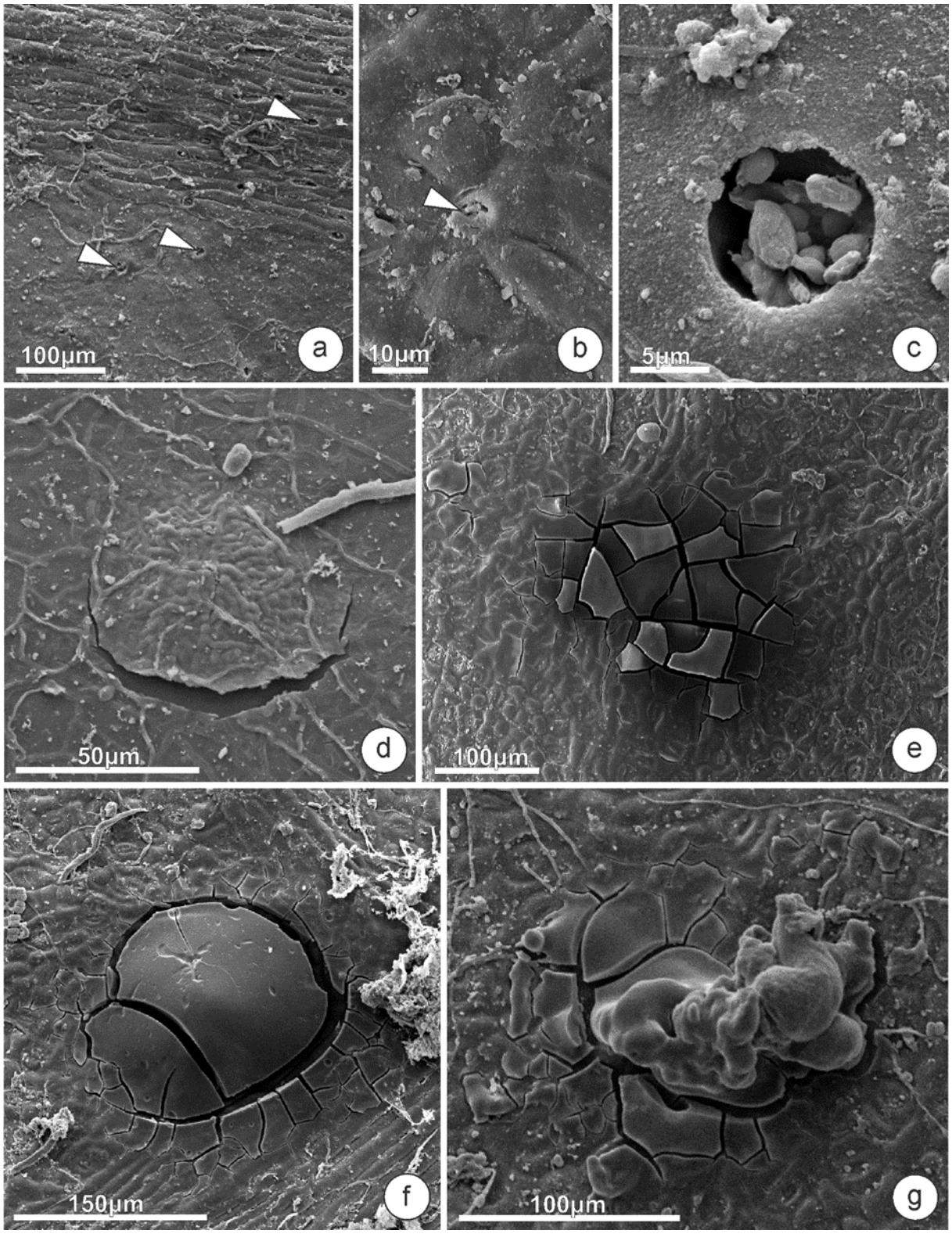

Figure 3.

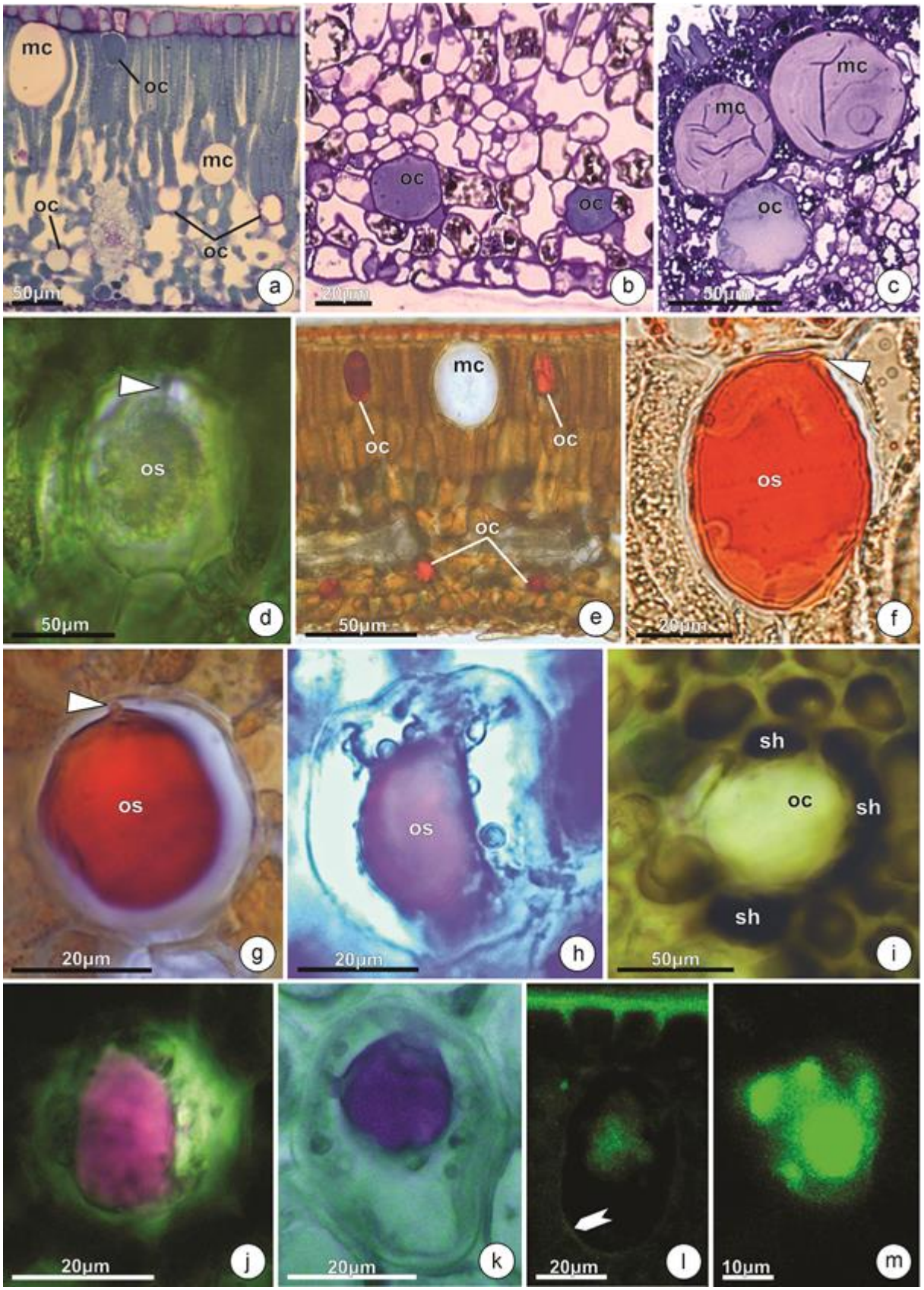
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Figure 4.

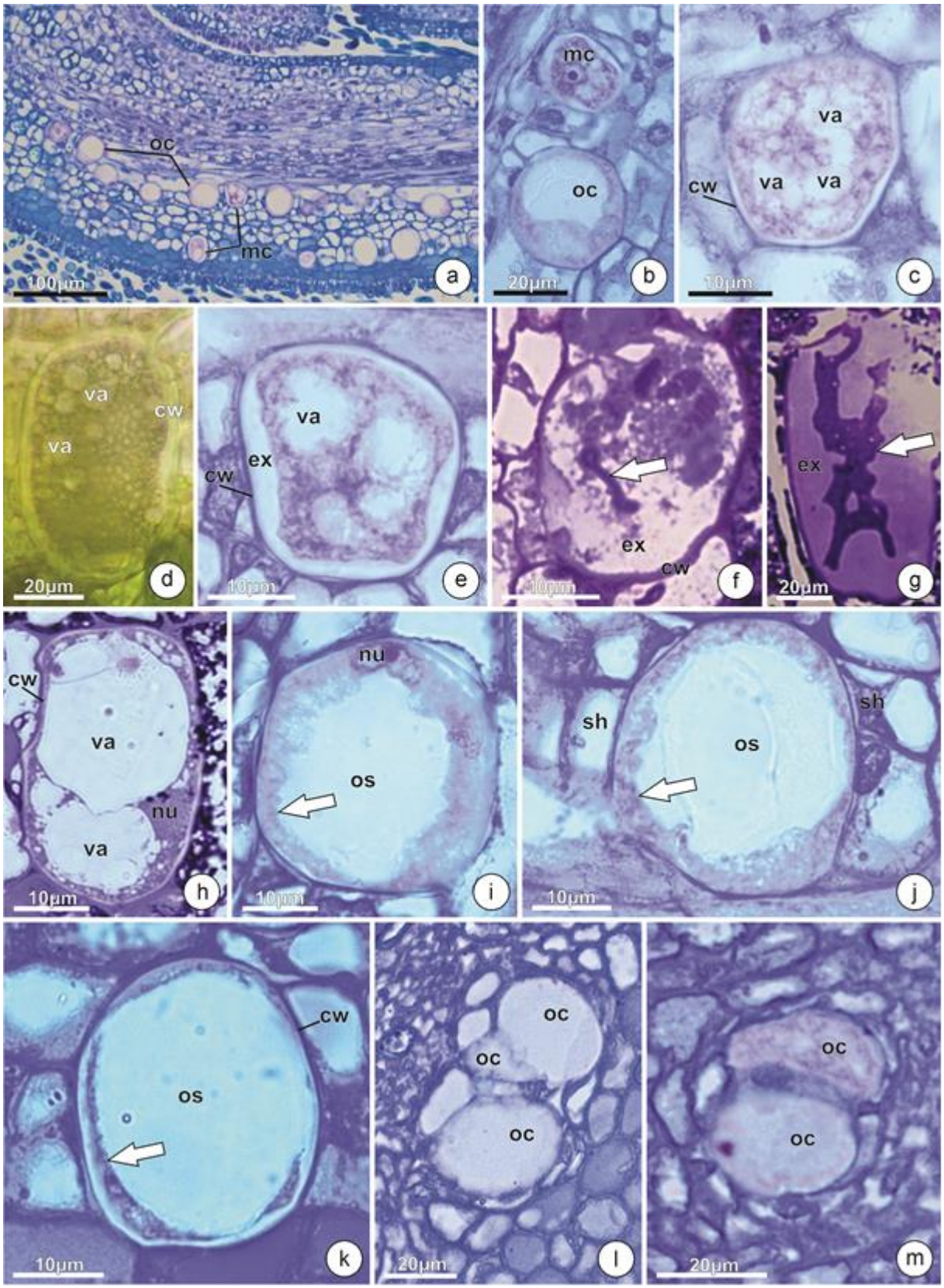
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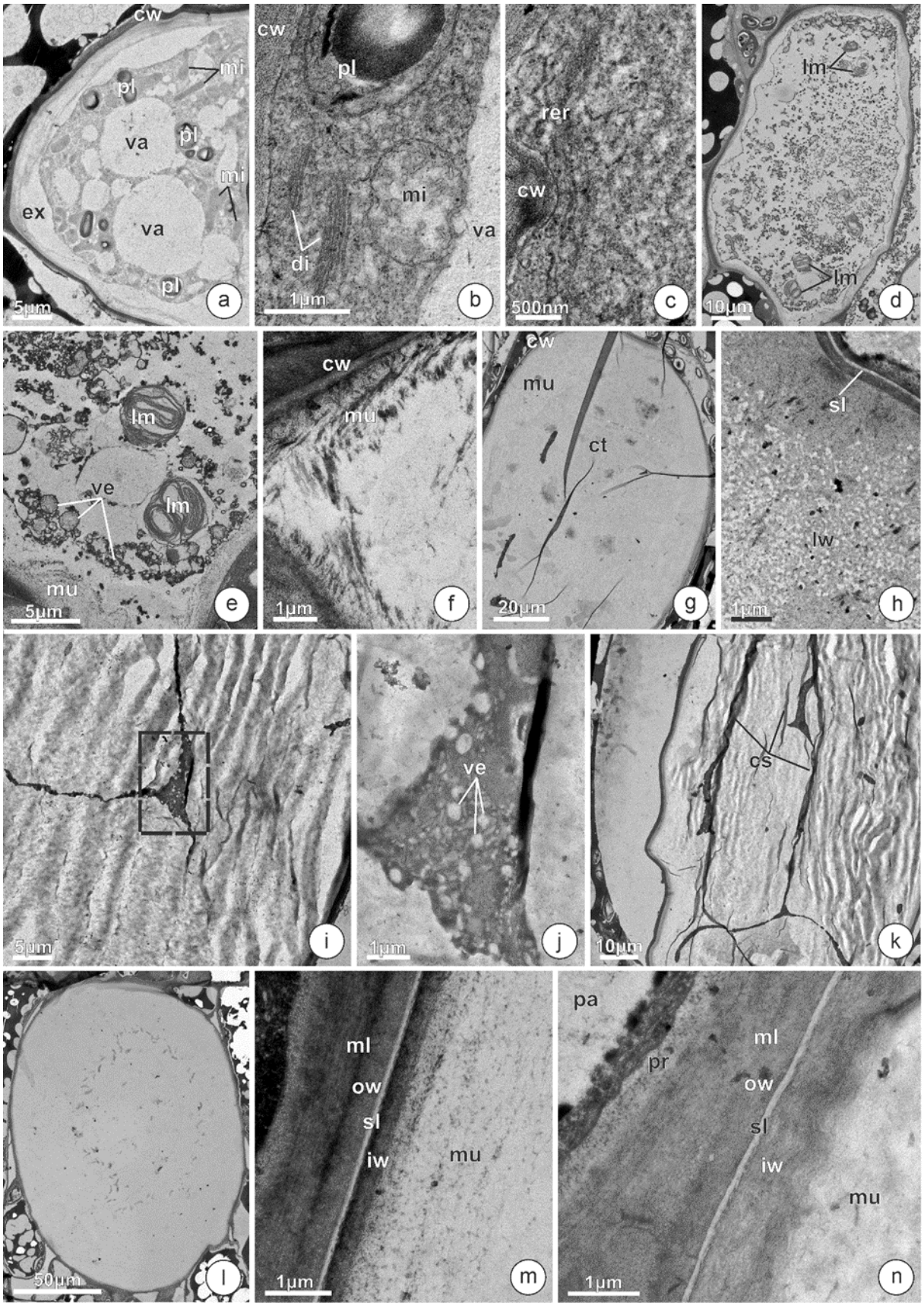
Figure 5.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mi
pl
va
pl
mi
ex
va
pl
5µm
a
cw
pl
mi
va
di
1µm
b
rer
cw
500nm
c
lm
lm
10µm
d
lm
ve
lm
mu
5µm
e
cw
mu
1µm
f
cw
mu
ct
20µm
g
sl
lw
1µm
h
5µm
i
ve
1µm
j
cs
10µm
k
50µm
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ow
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1µm
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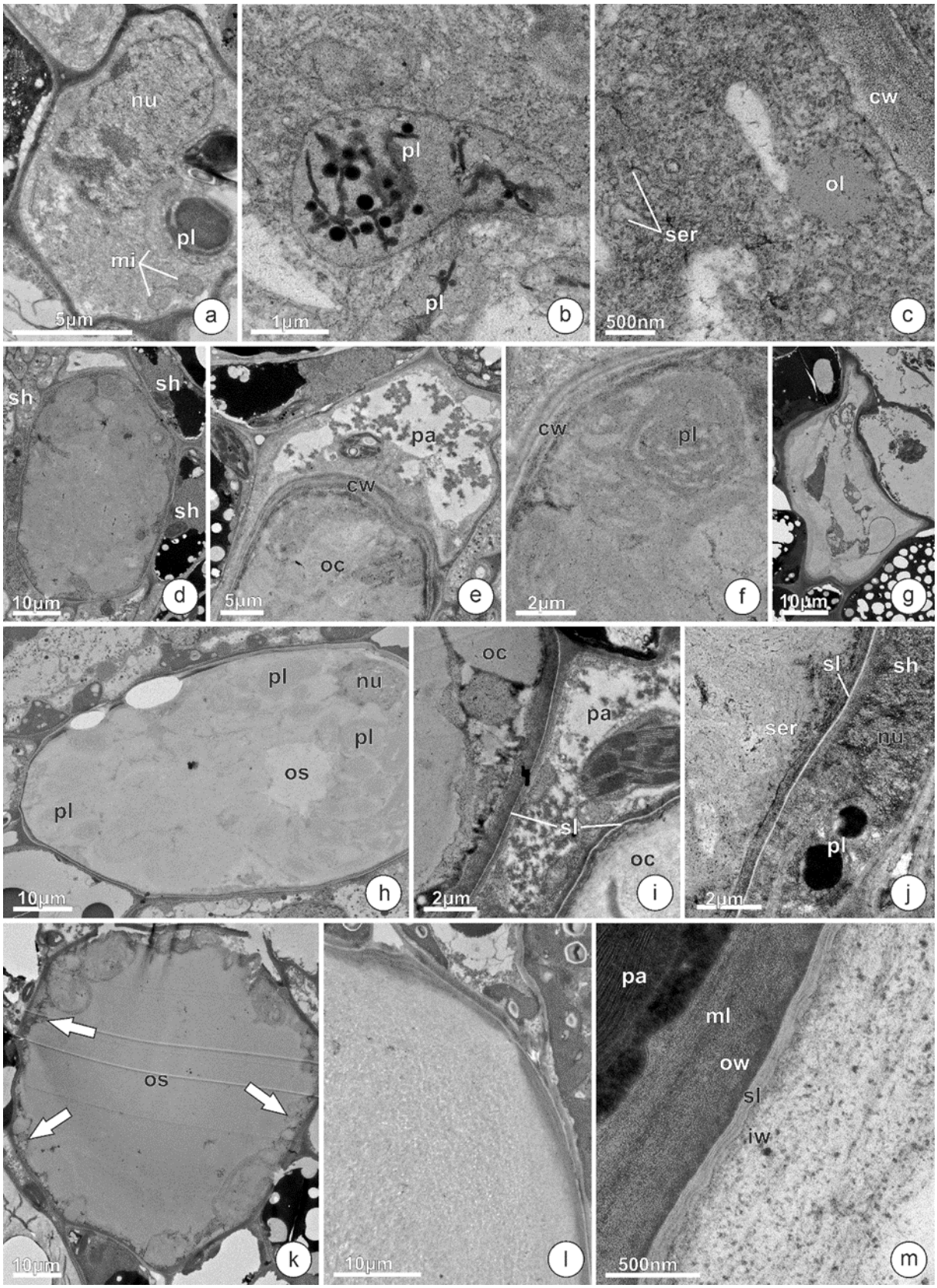
ml
ow
sl
iw
mu
1µm
n

Figure 6.


nu
pl
mi
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500nm
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pa
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oc
5µm
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cw
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2µm
f
10µm
g
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nu
pl
os
pl
10µm
h
oc
pa
sl
oc
2µm
i
sl
sh
ser
nu
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os
10µm
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10µm
l
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500nm
m

Figure 7.

CONSIDERAÇÕES FINAIS

A combinação de métodos em microscopia de luz e microscopia eletrônica de transmissão permitiu distinguir claramente idioblastos de óleo e idioblastos de mucilagem ocorrendo lado a lado em folhas de *Ocotea pulchella*. Além disso, os resultados obtidos a partir dessas análises expandiram a compreensão sobre a ontogênese, desenvolvimento e o funcionamento de idioblastos envolvidos na secreção de diferentes substâncias (lipofilicas e hidrofílicas) em Lauraceae.

Algumas dificuldades técnicas no preparo das amostras para estudos microscópicos devem ser ressaltadas, especialmente devido ao extravasamento das secreções produzidas nos idioblastos de mucilagem. Essa dificuldade foi contornada pela repicagem das amostras fixadas e sua imediata inclusão em resina metacrilato e Araldite ®, utilizadas, respectivamente, para estudos anatômicos e ultraestruturais.

Nossos dados mostram a necessidade de reavaliação de alguns termos e conceitos em relação às estruturas secretoras, que têm sido amplamente utilizados principalmente em estudos taxonômicos em Lauraceae, especialmente em relação às chamadas ‘glândulas pelúcidas” (pellucid gland dots). Neste estudo, como em outros estudos anteriores com Lauraceae, não foram observadas características anatômicas que justificassem o uso do termo "glândulas pelúcidas". Neste estudo, ficou demonstrado que tais glândulas são, na realidade, idioblastos secretores. Conceitualmente, as glândulas são estruturas multicelulares, enquanto idioblastos são células secretoras isoladas.