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Ontogenesis and secretion mechanism of *Morinda citrifolia* L. (Rubiaceae) colleters

S.K. de Paiva Pinheiro [a], F.B.S. Teófilo [a], A.K.M. Lima [a], B.V. Cordoba [a], T.B.A.R. Miguel [b], E. de Castro Miguel [a,*]

[a] Central Analítica, Departamento de Física, Centro de Ciências, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil
[b] Laboratório de Materiais Funcionais Avançados - LaMFA, Departamento de Física, Centro de Ciências, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil

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ABSTRACT

Colleters are secretory structures present in more than 60 families of angiosperms, whose function is protecting the leaf primordium and meristem against biotic and abiotic damage. The colleters are developed from protoderm and ground meristem and the secretory process begins during cell differentiation. The secretion is a complex phenomenon of synthesis, separation and isolation of substances, as well as release or extracellular elimination. This research describes the ontogenesis of *Morinda citrifolia* L. colleters and the mechanism of exsudate externalization. For this purpose, various microscopy techniques, including scanning electron microscopy, light microscopy (including histochemical tests) and confocal scanning laser microscopy were used. Standard type colleters are attached on the stipule adaxial surface. During development, the colleters pass through four developmental stages defined by structural and anatomical changes: undifferentiated, pre-secretory, secretory and senescent. In the undifferentiated stage, the secretory structures are characterized by small protuberances. In the pre-secretory stage, the secretory epithelium begins its differentiation. In the following stages, the colleters present a parenchymatic central axis covered by palisade epidermal cells. Secretion occurs by the rupture of the cuticle. At the end of the secretory process, the colleters begin the senescent stage, characterized by deformation, color change and cuticle wrinkling. This work evidences the section passage mechanism by cuticle rupture, accumulating knowledge in the still poorly understood process of molecules passages by the external periclinal wall in Rubiaceae colleters.

1. Introduction

Rubiaceae is the fourth largest family in the plant kingdom (Robbrecht, 1988), with a great diversity of species. The genus *Morinda* comprises about 40 species, distributed mainly in pantropical regions (Kesonbuaa and Chantaranothai 2013). Among the species described so far, *Morinda citrifolia* L. stands out for the antioxidant properties (Zin et al. 2002) in its roots (Hemwimon et al. 2007) and fruits (Krishnaiah et al. 2015).

This plant is popularly known as "Noni" being distributed in Pacific Islands, tropical Asia, and India. The medicinal properties of *Morinda citrifolia* have been known for more than 200 years and are exploited by indigenous people for diseases treatment in primitive communities. Such medicinal properties help in the fight against heart disease, AIDS, ulcers, arthritis and cancer (Wang et al. 2002). Flowers, leaves, bark, stems, roots and fruits are all used (Dixon et al. 1999; Deng et al. 2007).

Like most species of Rubiaceae, *Morinda citrifolia* has colleters on the stipule adaxial surface (Pinheiro et al. 2015), whose function is to protect the leaf primordium and developing structures. Colleters are secretory structures present in more than 60 angiosperm families (Thomas 1991). Such secretory structures can also occur in various organs of the plant, varying among the botanical families. In Rubiaceae, they are mainly on the stipule's adaxial surface (Thomas 1991), but may also be present on bracts and sepals (Barreiro and Machado 2007). In Aquifoliaceae, colleters were found in the base and teeth of the leaf and nodal area (Gonzalez and Tarragó 2009). In the Orchidaceae, colleters were found in bracts, sepals and ovary wall, the first record for all these structures (Cardoso-Gustavson et al. 2014). The fully expanded colleter has an elongate axis and short base (Thomas and Dave 1990), with the central axis constituted of parenchymatic cells, covered with palisade secretory cells (Da Cunha and Vieira 1993; Barreiro and Machado 2007; Gonzalez and Tarragó 2009; Martins et al. 2010; Tullii et al. 2013; Vitarelli et al. 2015).

Based on morphology and anatomy, such secretory structures are classified as: reduced standard, standard, filiform, dendroid, intermediate, brush type (Thomas 1991; Simões et al. 2006), digitiform (Paiva and Machado, 2006a), conical, hemispheric Gonzalez and Tarragó 2009), lachrymiform (Miguel et al. 2010) and claviform (Machado et al., 2015). Standard type is the most common shape of colleter present in

* Corresponding author at: Universidade Federal do Ceará, Campus do Pici, Departamento de Física/Central Analítica, CEP 60455-900 Fortaleza, Ceará, Brazil.
E-mail address: emilio.decastromiguel@gmail.com (E. de Castro Miguel).

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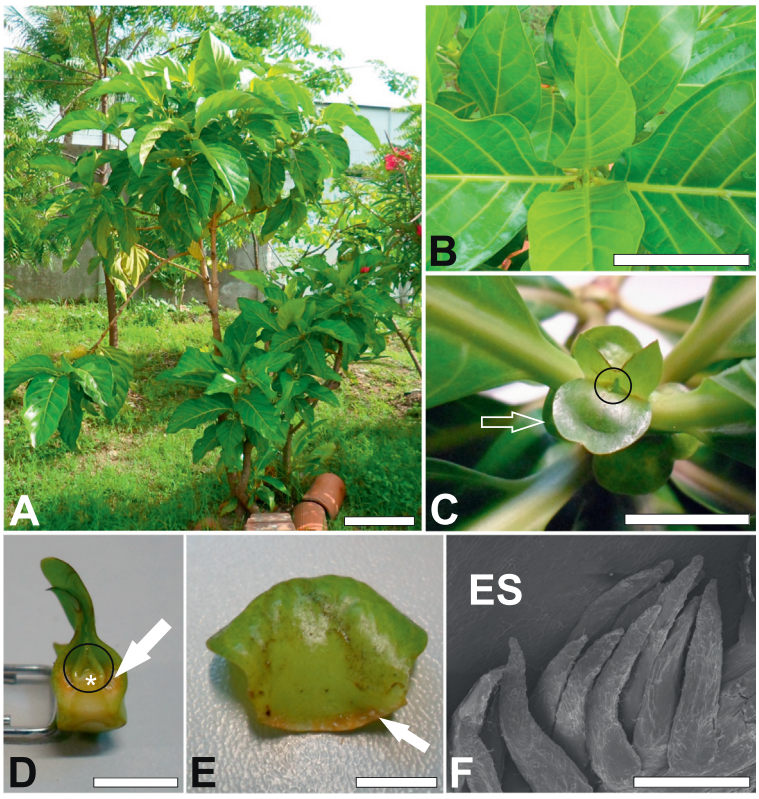

Fig. 1. Habit of *Morinda citrifolia* L. A, *Morinda citrifolia* plant; B, Shoot apex showing opposite, decussate leaves; C, Detail of the vegetative apex. Note stipules (open arrow) protecting the leaf primordium (circle); D, Longitudinal section of the shoot apex, stipules with colleters (closed arrow) protecting the leaf primordium. Note the presence of secretion near the leaf primordium (*); E, Detail of the adaxial surface of stipule with colleters in the base (closed arrow). Bars: A – 30 cm; B – 15 cm; C – 2,5 cm; D – 1,1 cm; E – 1,4 cm; F – 500 μm.

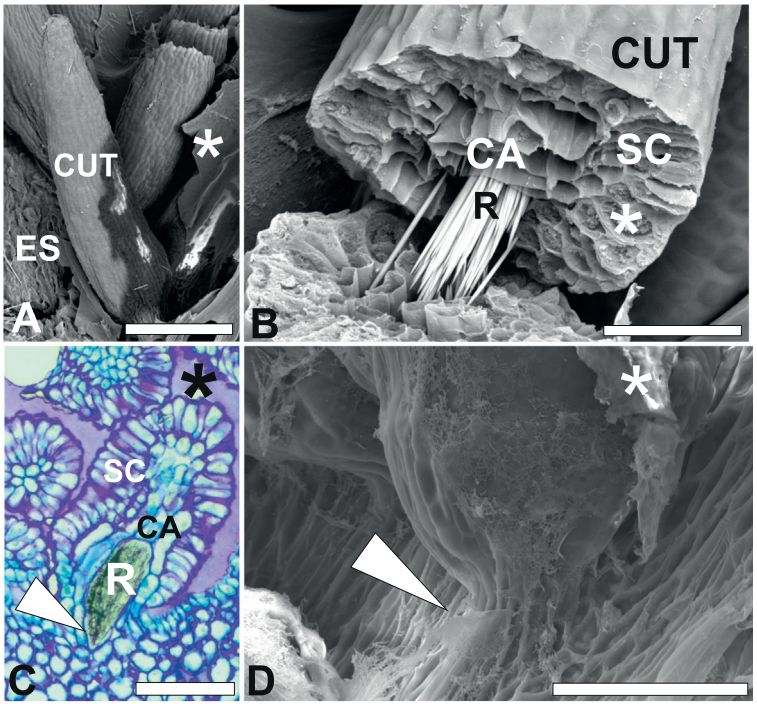

Fig. 2. Scanning electron micrographs (A, B, D), light micrograph (C) of *Morinda citrifolia* colleters; A, Detail of colleter with standard type morphology in stipule base; B, Colleter with ruptured cuticle showing morphological details; C, Colleter showing elongate central axis (CA) with raphides (R), surrounded by secretory cells (SC) axially disposed; D, Colleter exhibited base constriction next to the secretory cells (CS). Bars: A – 500 μm; B – 250 μm; C – 30 μm; D – 50 μm.

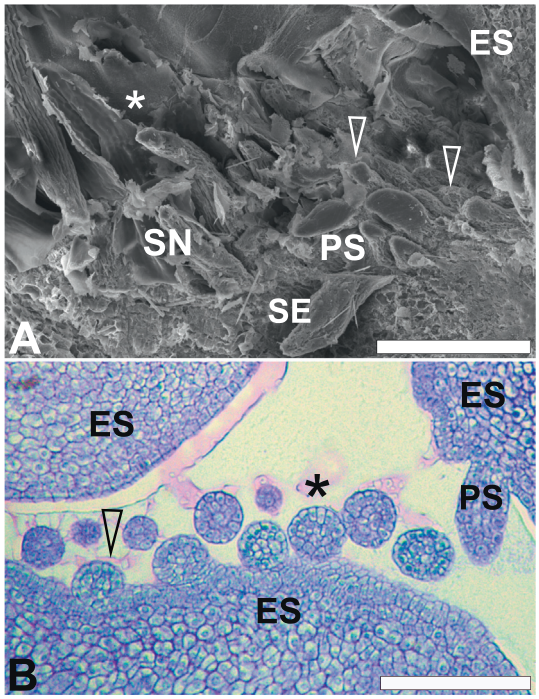

Fig. 3. Scanning electron micrograph (A) and light micrograph (B) of *Morinda citrifolia* colleters at various stages of development. A, Overview of the stipule adaxial surface (ES) exhibited undifferentiated colleters (arrowhead), pre-secretory colleters (PS); secretory colleters (SE) and senescent colleters (NS) covered by secretion (*); B, Colleters in undifferentiated stage (arrowhead) and pre-secretor (PS) with secretion (*). Bars: A – 200 μm; B – 300 μm.

Rubiaceae (Klein et al. 2004; Mayer et al. 2013; Miguel et al. 2016; Tresmondi et al. 2015, 2017). The disposition of the colleters, as well as their number can vary. *Simira pikia* (K.Schum.) Steyerm. colleters are distributed in line on the stipule base, while the colleters of *Simira glaziovii* (K.Schum.) Steyerm. form two triangles on the stipule base (Klein et al. 2004).

The secretory activity begins in young stipules. The secretion protects the leaf primordia chemically and physically (Thomas 1991; Klein et al. 2004; Paiva and Machado, 2006b; Miguel et al. 2010). The colleters begin to secrete before the expansion of the foliar primordia as observed in *Alibertia sessilis* K.Schum. (Rubiaceae) (Barreiro and Machado 2007). The secretion is basically composed of lipids (Appezzato-Da-Glória and Estelita, 2000; Paiva 2009), proteins (Klein et al. 2004; Miguel et al. 2006) and mucilage (Klein et al. 2004; Coelho et al. 2013; Miguel et al. 2010, 2016). Some studies revealed the secretion externalization by cuticle rupture as described for *Caryocar brasiliense* Cambess. (Paiva and Machado, 2006a) and *Tocoyena bullata* (Vell.) Mart. (Miguel et al. 2016). However, in *Bathysa nicholsonii* K. Schum. (Rubiaceae), there is no cuticle rupture, the passage of secretion occurs in six stages, through the cell wall (Miguel et al. 2017). After the secretory process, colleters enter into a senescent stage (Thomas and Dave 1989; Thomas 1991; Miguel et al. 2006, 2009).

Colleters cells in the senescent stage are more sinuous than those in other stages (Coelho et al. 2013) and have a wrinkled cuticle (Miguel et al. 2016). At this stage, the colleters change color from yellow to dark-staining, indicating that there has been tissue necrosis (Thomas and Dave 1990). The secretion at this stage of development acquires a yellowish crusty appearance (Gonzalez 1998). Senescence is a natural process controlled by plant genetic programming (Miguel et al. 2016). This research describes the ontogenesis of *Morinda citrifolia* colleters and the mechanism of exsudate externalization.

2. Material and methods

2.1. Plant material

Morinda citrifolia shoot apex containing stipules at various developmental stages were collected in Maracanaú (3°52′27.5″S 38°36′29.2"W), Ceará-Brazil and voucher specimes deposited in the Prisco Bezerra herbarium (EAC) with number 59806. Samples were immediately taken to Central Analítica of Universidade Federal do Ceará and investigated. For scanning electron microscopy and light microscopy, the stipules were chemically fixed and dehydrated in the laboratory. The identification of stipules was started from the oldest node to the close to the apical meristem.

2.2. Scanning electron microscopy

Samples at various stages were fixed in a solution of 4.0% formaldehyde, 2.5% glutaraldehyde and 0.05 M sodium cacodylate buffer, pH 7.2, at room temperature for 24 h. The material was then rinsed three times in sodium cacodylate buffer for 45 min for each rinse and post-fixed with 1.0% osmium tetroxide in 0.05 M cacodylate buffer, pH 7.2 for one hour at room temperature. Subsequently, the samples were dehydrated for 45 min with increasing acetone series. After dehydration, the material was dried with hexamethyldisilizane (HDMS) and placed on stubs, sputter-coated with 20 nm gold, and observed with a digital scanning electron microscope Quanta FEG 450 (FEI).

2.3. Light microscopy

Stipule fragments were fixed, post-fixed and dehydrated as described for scanning electron microscopy. The fragments were infiltrated with epoxy resin (EponPolibed), using a series of increasing resin in propanone. Thin sections (2.0 μm) were stained with 1% toluidine blue. Glass slides were sealed with varnish composed of acrylic resin. Observations were made with a photomicroscope Olympus UC30 and recorded using a UC30 model digital camera and software for Cell B image analysis.

Histochemical tests were performed with tannic acid and ferric chloride for mucilage (Pizzolato & Lillie, 1973), with periodic acid-Schiff (PAS) reagent for neutral polysaccharides (Jensen, 1962), Sudan black for total lipids (Pearse, 1980), Xylidine ponceau (XP) for proteins (Vidal, 1970), Lugol's reagent (Jensen, 1962) and potassium dichromate for phenols (Gabe, 1968). Control samples were treated in the same way as other samples for each test.

2.4. Confocal scanning laser microscopy

Vegetative shoot apices were collected with the use of blades and taken immediately to the laboratory. After the separation of stipules, fragments containing colleters were immersed in 1% auramine for 10 min, the fragment was washed three times in distilled water for cuticle observation. Samples were observed in the laser confocal microscope (LM 710 Zeiss) using an excitation laser with a wavelength of 405 nm and an emission of 455 nm.

3. Results

3.1. Morinda citrifolia morphology

Morinda citrifolia plants average 2 m in height (Fig. 1A) and have simple, opposite, decussate, lanceolate and petiolate leaves, with undulate margin and pinnate venation (Fig. 1B). The stipules are free and interpetiolar, and protect the leaf primordia. The secretion found in the leaf primordium made it difficult to separate (Fig. 1C). The secretion covered the entire leaf primordium, was sticky and translucent, and became vitreous when in contact with the environment (Fig. 1D). *Morinda*

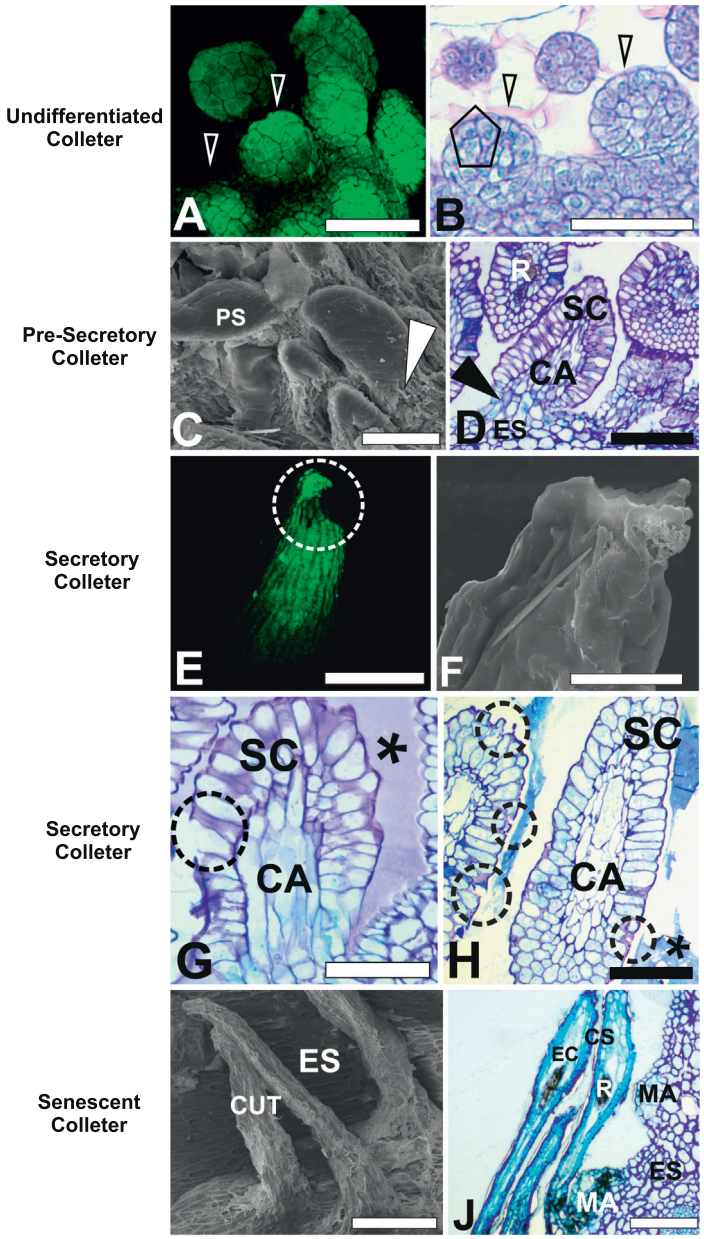

Fig. 4. Scanning electron micrographs (C, F, I), light micrographs (B, D, G, H, J) and confocal scanning laser micrographs (A, E) of *Morinda citrifolia* colleters at various stages of development; A, Colleter in early developmental stage (open arrowhead); B, Colleter in longitudinal section at the initial stage of development (open arrowhead). Note the cell division and nucleus at the beginning development of colleter (Pentagon); C, Detail of pre-secretory colleter (PS). Observe base constriction (White arrowhead); D, Longitudinal cut of pre-secretory (PS) colleters. Note secretion (*) accumulated in secretory cells (SC); E, Secretory colleter with ruptured cuticle (CUT) (dotted circle). Note slightly wrinkled cuticle, indicating the beginning of the secretory process; F, Detail of colleter showing the ruptured cuticle (CUT) at the apex (dotted circle); G, Colleter in longitudinal section covered by secretion (*) with ruptured cuticle (dotted circle); H, Ruptured cuticle at various points of the colleter (dotted circle); I, Overview of colleters at the stipule base, all covered by secretion (*). Note that the colleters decrease in volume at the senescent stage (SN); J - Senescent colleter (SN) and abscission meristem (AM) on the stipule surface. At this stage, the secretory cells are disorganized (SC). Bars: A, B, C – 50 μm; D, H, J – 60 μm; E, I – 200 μm; F, G – 30 μm.

citrifolia colleters were found at the base of the adaxial surface of the stipule and were arranged in a single line (Fig. 1E, F).

3.2. Colleter anatomy

Morinda citrifolia colleters are of the standard type (Fig. 2A), characterized by an elongate central axis, covered with palisade secretory cells (Fig. 2B, C). No vascular traces were observed in the central axis, but raphide bundles were evident (Fig. 2C). Colleters with this characteristic are basally constricted (Fig. 2D), forming a region of non-secretory cells.

3.3. Ontogenesis

The development of *Morinda citrifolia* colleters did not occur synchronously in different nodes. Thus, the colleter development was described according to the stages of development.

Colleters of all stages of development can be present on the same stipule. Using morphological characteristic, colleters were classified as undifferentiated, pre-secretory, secretory and senescent (Fig. 3A, B). Colleters in the initial developmental stage were named undifferentiated. Such secretory structures were characterized by small protuberances on the base of the stipule adaxial surface (Fig. 4A). The colleter cuticle was smooth (Fig. 4A).

Table 1
Histochemical characterization of the main chemical compounds of colleter and secretion in *Morinda citrifolia*.

			Lipids	Phenolic compounds	Polysaccharides	Proteins	Starch	Mucilage
Undifferenciated colleter	Protoderm	Cell wall	+	–	–	–	–	–
		Cytoplasm	–	–	ND	–	–	–
		Nucleus	–	–	–	++	–	+
	Fundamental meristem	Cell wall	–	–	ND	–	–	–
		Cytoplasm	–	–	+	–	–	–
		Nucleus	–	–	–	++	–	+
Pre secretory colleter	Central axis	Cell wall	–	–	–	+	–	+
		Vacuole	–	–	–	–	–	–
		Cytoplasm	–	–	+	+	–	+
	Secretory cells	Cell wall	++	–	–	+	–	+
		Vacuole	–	–	–	–	–	–
		Cytoplasm	–	–	+	++	–	+
		Nucleus	–	–	–	ND	–	ND
Secretory colleter	Central axis	Cell wall	–	–	+	+	–	+
		Vacuole	–	–	–	–	–	–
		Cytoplasm	–	–	+	+	–	+
	Secretory cells	Cell wall	++	–	++	+	–	+
		Vacuole	–	–	–	+	–	–
		Cytoplasm	–	–	++	+	–	+
		Nucleus	–	–	–	++	–	+
	Secretion		–	–	+++	+	–	+++
Senescent colleter	Central axis	Cell wall	–	–	++	–	–	+
		Vacuole	–	–	–	–	–	–
		Cytoplasm	–	–	+	–	–	+
	Secretorycells	Cell wall	++	–	++	–	–	+
		Vacuole	–	–	–	–	–	–
		Cytoplasm	–	–	++	–	–	+
		Nucleus	–	–	–	++	–	ND
	Secretion		–	–	+++	++	–	+

Key: Weak reaction (+), Moderate reaction (++), Strong reaction (+++), No rection (−), No detected (ND).

Longitudinal sections of stipules exhibited isodiametric protodermal cells, with few difference in shape between the fundamental meristem cells and protoderm cells (Fig. 4B). Anticlinal and periclinal divisions that occurred in the protodermis and divisions in the fundamental meristem formed a protuberance in the stipule basal region. Intense mitotic activity was observed in the cells, with periclinal walls being formed (Fig. 4B). Cells of the meristematic epidermal and protodermis differentiated, forming the colleter in the pre-secretory stage.

Pre-secretory colleters have a smooth cuticle (Fig. 4C). The secretory cells at this developmental stage were turgid (Fig. 4D), and no secretion was detected outside cells. The cuticle was attached to the cell wall (Fig. 4D). As the developmental process continued, colleters went into the secretory stage.

Morinda citrifolia colleters in the secretory stage undergo cuticular rupture, which is a physical process for exudate release (Fig. 4E). As exudate is released to the external environment, the cuticle became wrinkled (Fig. 4F). The cuticle rupture occurred along the entire structure (Fig. 4G), causing cuticle discontinuity (Fig. 4G). The ruptures occur at several places in the cuticle, optimizing the secretory process (Fig. 4H). As the secretory phase proceeds, the cuticle becomes strongly wrinkled, beginning the senescent phase.

At the end of secretory stage, colleters enter the senescent phase. It is common that at this stage, the secretion covers the colleter surface (Fig. 4I). The constriction becomes less evident in the base (Fig. 4J). An abscission meristem forms at the base of senescent colleter, with collapsed secretory cells of irregular shape (Fig. 4N).

3.4. Histochemical tests

Histochemical tests were performed on *Morinda citrifolia* colleters and secretions. Various chemical substances were identified during the development of these structures. The staining varied in intensity as a function of the amount of chemical compounds present in each region of the secretory structure. Secretion is composed mainly of proteins, mucilage and polysaccharides (Table 1). Histochemical tests using XP showed proteins in secretory cells and cytoplasm in the pre-secretory stage (Fig. 5B). XP revealed proteins in the nucleus in the undifferentiated, secretory and senescent stages (Fig. 5A, C, D). Proteins were identified in the secretion in three stages of development, except in the undifferentiated stage (Fig. 5A).

No reaction was observed with the cell nucleus using periodic-Schiff acid reagent (PAS) to detect polysaccharides in any stage of development. In undifferentiated stage colleter, there was a weak reaction on the external periclinal wall (Fig. 5E), and in subsequent stages there was a strong reaction on the external periclinal wall (Fig. 5F, G, H). Using tannic acid/ferric chloride to detect mucilage, a positive reaction was observed in the cell nucleus in undifferentiated (Fig. 5I), pre-secretory (Fig. 5J) and secretory stages (Fig. 5K). No reaction was observed in the cell nucleus in the senescent stage (Fig. 5L). Reaction with secretions were observed in all stages.

Histochemical tests for lipid detection using Sudan Black, revealed a positive reaction with the cuticle and cuticular extracts, with intense staining in this region in all developmental stages (Fig. 5M, N, O, P). No lipids were detected in the colleter secretion, however. To detect starch, an iodine solution was used, but no positive reaction was observed in secretory cells and colleter secretions (Table 1). With potassium dichromate, no phenol was detected in the secretion and secretory cells of the colleter. The same result was shown by cell nucleus and central axis.

4. Discussion

4.1. Colleter morphology

Morinda citrifolia colleters were characterized with light microscopy, scanning electron microscopy and confocal scanning laser microscopy. Such tools allowed observation of the colleters at various developmental stages, including secretion mechanisms. Colleters were found at the base of the stipule adaxial surface, arranged as single lines. This agrees with the observations of several authors (Thomas and Dave 1990; Klein et al. 2004; Pinheiro et al. 2015; Miguel et al. 2016, 2010).

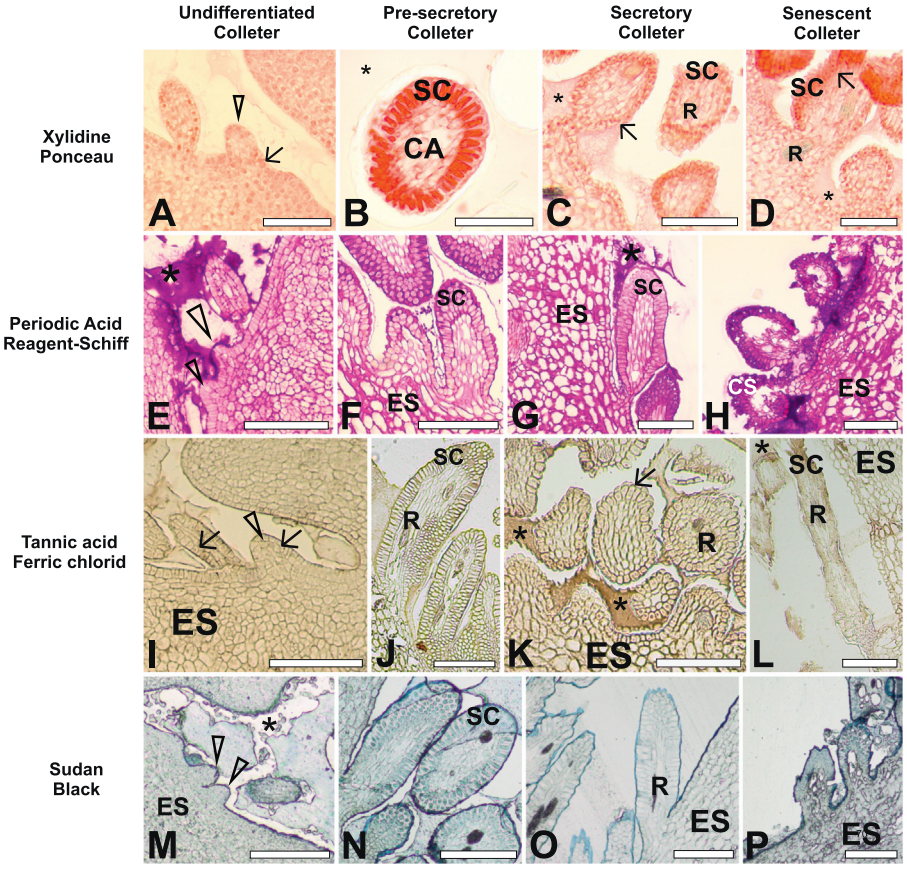

Fig. 5. *Morinda citrifolia* colleter at various developmental stages, using light microscopy. Material included in historesin (A–M) submitted to histochemical test. Test using XP for proteins (A, B, C, D); PAS for polysaccharides (E, F, G, H); Ferric chloride/Tannic acid for mucilage (I, J, K, L); Sudan black for total lipids (M, N, O, P). A, Undifferentiated Colleter (Arrowhead). Red staining indicates positive reaction for proteins. Notice the strongly colored cell nucleus (arrow); B, Colleter at pre-secretory stage, showing strong reaction with secretory cells (SC); C, Colleter at secretory stage. Positive reaction with secretion (*), cytoplasm and cell nucleus (Arrow); D, Colleter at senescent stage. Observe stained secretory cells (SC) and nucleus (Arrow) indicating a positive reaction; E, Colleter at undifferentiated stage. Pink staining indicates the presence of polysaccharides, note secretion (*) rich in polysaccharides; F, Colleter at pre-secretory showing positive reaction with secretory cells (SC); G, Colleter at secretory stage. Positive reaction with secretory cells (SC), secretion (*); H, Colleter at senescent stage. Strong reaction with secretory cells. No reaction was observed with the cell nucleus; I, Colleter at undifferentiated stage, showing positive reaction only with nucleus (Arrow); J, Colleter at pre-secretory stage showing positive reaction with secretory cells (SC). No reaction was observed with the nucleus K, Colleter at secretory stage. Weak reaction with the cell nucleus (arrow), however, strong reaction with secretion(*) indicating the presence of mucilage; L, Colleter at senescent stage (SN). No reaction was observed with the cell nucleus (arrow); M, N, O, P, Positive reaction for lipids at all stages of development of the colleter. Strong reaction with cuticle and cuticular extracts. No reaction was observed with the nucleus and secretion. Bars: A, D, G, J, L – 70 μm; B – 50 μm; C – 40 μm; E, F, H, I, M, O, P – 60 μm.

The secretion produced in the *Morinda citrifolia* colleter was translucent, was observed on the stipule base and covered the vegetative apex, as described in *Bathysa gymnocarpa* K.Schum. and *Bathysa stipulata* (Vell.) C. Presl. (Miguel et al. 2010).

4.2. Colleter anatomy

The standard type of colleters found in *Morinda citrifolia* is common in the Rubiaceae, being described in *Gardenia lucida* Roxb. (Dave et al., 1988), *Simira glaziovii* (Klein et al. 2004), *Bathysa nicholsonii* (Miguel et al. 2006) and various Rubiaceae species from tropical forest and savanna (Tresmondi et al. 2015, 2017). In addition to Rubiaceae, these characteristics were observed in other families such as Apocynaceae (Rio et al. 2002), Aquifoliaceae (Gonzalez and Tarragó 2009) and Euphorbiaceae(Martins et al. 2016). The mature and differentiated colleter has a long central axis and a short peduncle (Miguel et al. 2009).

4.3. Ontogenesis

The development of *Morinda citrifolia* colleters occurs asynchronously, presenting various stages of development in the same stipules pair. Other species have the same characteristics, as observed in *Hymenaea stigonocarpa* Mart. ex Hayne. (Paiva and Machado, 2006b) and *Tocoyena bullata* (Miguel et al. 2016). Different stages of development ensure secretion throughout the development of the inflorescence, as observed in *Dalechampia meridionalis* Müll.Arg. (Gagliardi et al. 2016). Colleter cells at the beginning of development are undifferentiated and of mixed origin, originating from the fundamental tissue and protoderm (Klein et al. 2004; Paiva and Machado, 2006a). *Morinda citrifolia* colleters had the same characteristic. At the beginning of colleter development, the cells that are going to give rise to protoderm and the fundamental tissue re still undifferentiated. Both cells at the beginning of development are isodiametric (Klein et al. 2004). In the protoderm, as in the fundamental meristem periclinal and anticlinal divisions occur forming a lump, that with the development of secretory structures becomes elongate (Dave et al. 1988). These successive divisions have fundamental importance for cell differentiation, where cells become specialized. In addition to Rubiaceae, colleters of mixed origin have been described for Apocynaceae (Appezzato-Da-Glória and Estelita, 2000). After differentiation, the colleters of *Morinda citrifolia* enter the pre-secretory stage.

At the pre-secretory stage, the cells are already differentiated, with a central axis and secretory epidermis (Vitarelli and Santos 2009; Miguel et al. 2016). The secretory cells of *Morinda citrifolia* colleters are turgid with the secretion occupying most of the cellular content, as has been observed in other species of Rubiaceae, such as *Pentas lanceolata* K.

Schum. (Muravnik et al. 2014) and *Tocoyena bullata* (Miguel et al. 2016). After the pre-secretory stage, the colleter releases secretion into the external environment.

Colleters in the secretory phase produce a large amount of exudate, as is their main function, to protect the leaf primordium and young leaves during their development. This protection function is unanimous in literature (Thomas 1991; Miguel et al. 2010; Muravnik et al. 2014; Tresmondi et al. 2015, 2017). The secretion of *M. citrifolia* is translucent and covers the shoot apex, which suggests a mechanism of protection for meristematic tissue. The accumulation of secretion occurs in the subcuticular space, causing the cuticle to distend and rupture, which is a characteristic described by other authors for the Rubiaceae (Dave et al. 1988; Paiva and Machado, 2006a; Vitarelli et al. 2015; Miguel et al. 2016), characteristics observed in other families as well as Fabaceae (Paiva 2009), Orchidaceae (Mayer et al. 2011) and Rhizophoraceae (Sheue et al. 2013). However, in *Morinda citrifolia*, an accumulation of secretions in the subcuticular space was not observed, as is the case in *Psychotria carthagenensis* Jacq. (Vitarelli and Santos 2009). The cuticle rupture in *Morinda citrifolia* occurs in any region of the colleter, however, in *Tocoyena bullata*, the secretion accumulates in the apex causing cuticle pressure, leading to a rupture in this region (Miguel et al. 2016). After releasing the secretion, colleters enter the senescent stage.

Senescent colleters were characterized by having wrinkled surfaces, with secretions still present on the surface. At this stage, the vesiculation of the protoplast occurs, indicating that dictyosomes and endoplasmic reticulum are producing lytic enzymes, which suggests programmed cell death (Paiva 2012). At this stage, the formation of the abscission meristem at the colleter base occurs (Miguel et al. 2016). After the colleters fall, scars can be observed (Fernandes et al. 2016; Macedo et al. 2016), a common characteristic observed in *Morinda citrifolia*. In *Cariniana estrellensis* Kuntze (Lecythidaceae), phenolic compounds were described in abscission area (Paiva 2012), a characteristic not observed in *M. citrifolia.*

4.4. Histochemical test

Morinda citrifolia produces secretions consisting of many chemical components. The chemical composition of the colleter secretion is complex, and commonly includes proteins (Klein et al. 2004; Coelho et al. 2013; Mayer et al. 2013; Vitarelli et al. 2015), lipids (Paiva 2009; Martins et al. 2016) and mucilage (Rio et al. 2002; Machado et al. 2015). In *M. citrifolia*, proteins are present mainly in secretory cells and nuclei, with less in the central axis and secretions. In colleters of *Zanthoxylum minutiflorum* Tul.(Rutaceae), proteins were described mainly in the secretory cells (Macedo et al. 2016). In *Croton glandulosus* L.(Euphorbiaceae) proteins were observed in the colleter's secretion (Machado et al. 2015), However in *Casearia sylvestris* Sw. (Salicaceae), there were no proteins in the colleter structure nor the secretions (Fernandes et al. 2016).

Histochemical tests using PAS have revealed that polysaccharides are common in several species. In *Casearia sylvestris* (Salicaceae) polysaccharides were observed in secretory cells and secretions (Fernandes et al. 2016). In *Bathysa cuspidata* (Rubiaceae) polysaccharides were observed in secretions and secretory cells (Coelho et al. 2013), as is found in *Morinda citrifolia*. Polysaccharides and proteins were found in the secretions and secretory cells of *M. citrifolia*, whose function is to protect developing tissues from desiccation and damage during growth. The same function was reported in *Pentas lanceolata* K. Schum. (Rubiaceae) (Muravnik et al. 2014).

The mucilage present in *Morinda citrifolia* colleters is important for the protection of developing structures. The hygroscopic nature of mucilage maintains the hydration of meristematic regions (Silva et al. 2012). The chemical composition of mucilage involves a complex mixture of sugars, formed from units of sugar and uronic acid (Bhatia et al. 2014), however, in *Arabidopsis* seeds, the compound in the highest concentration in the mucilage is pectin. Pectin and an acid polysaccharide form a gel in the extracellular matrix (Western et al. 2000).

Structural lipids that were present in the external periclinal wall reacted strongly with Sudan black. No lipids were detected in the *Morinda citrifolia* secretion, however, in colleters of *Zanthoxylum minutiflorum* (Rutaceae) lipids were observed in the secretion of the colleter (Macedo et al. 2016). Starch was not detected in the secretion of *M. citrifolia* colleters. This also occurs in other species such as *Bathysa cuspidata* (Rubiaceae) (Coelho et al. 2013) and *Dalechampia alata* Klotzsch ex Baill. (Euphorbiaceae) (Martins et al. 2016). Starch constitutes an energy reserve formed in plastids of the higher plants, and is synthesized in the leaves, where it serves as a temporary carbohydrate reserve accumulated in the chloroplasts during the day and serving as the main source for the synthesis of cytosolic sucrose at night (Vandeputte and Delcour 2004; Tester et al. 2004). Phenolic compounds were not observed in the secretion of *M. citrifolia*, a common feature found in *Bathysa cuspidata* colleters (Coelho et al. 2013).

5. Conclusions

This paper describes the ontogenesis, maturation and mechanism of exudate release of *Morinda citrifolia* colleters. Four developmental stages of colleters occur simultaneously on stipules, ensuring the production of secretion during all leaf primordia development. In early developmental stages of colleters these structures are stipular projections, formed by protoderm and fundamental parenchyma. After complete development, the secretion passes to the outside of the cell wall through cuticle rupture.

Declaration of interest

The authors declare that they have no conflict of interest.
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