



Original research

Secretory structures in stems of five lianas of Paullinieae (Sapindaceae): Morphology and histochemistry



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ABSTRACT

Paullinieae lianas are noteworthy for possessing cambial variants, so it is not surprising that the structure of the vascular system has been the focus of most of anatomical studies involving these plants. However, secretory structures remain understudied in the tribe and in the family Sapindaceae as whole. This study aims to characterize the secretory structures in the stems of five lianas of Paullinieae in order to better understand their morphology and histochemistry. Stem anatomy was analysed using light microscopy and the chemical composition of their secretory structures was determined histochemically. Three secretory structures were found: idioblasts, glandular trichomes and laticifers. Tannin-containing cells (idioblasts) are distributed throughout the stems. Glandular trichomes are capitate and have a complex mixture of phenolic compounds, tannins, proteins and polysaccharides. Laticifers occur in primary tissues, and are also observed in the secondary structure of *Serjania pernambucensis* Radlk.; they occur in regular and variant phloem (cambial variant), arranged in clusters due to divisions of pre-existing laticiferous cells. This phenomenon has not been reported previously. Additionally, this is the first detailed study of laticifers in primary and secondary structure of stems of lianas of Sapindaceae. The laticifers are articulated and non-anastomosing, containing the following chemical components: rubber, lipids, proteins, polysaccharides and terpenoids. Future studies might provide a broader understanding of the morphological diversity and the role of secretory structures in the tribe.

1. Introduction

The family Sapindaceae *s.l.* comprises four subfamilies (Xanthoceroideae, Hippocastanoideae, Dodonaeoideae, and Sapindoideae) with approximately 1900 species of trees, shrubs and lianas, which are distributed mostly in the tropics and some species extending into temperate regions in Asia and North America (Acevedo-Rodríguez et al., 2017). According to the author, the tribe Paullinieae is currently included in the supertribe Paullinioidae, which contains 3 other tribes (i.e. Athyaneae, Bridgesieae, Thouinieae). In this classification, Paullinieae is circumscribed to include 6 genera (*Cardiospermum*, *Lophostigma*, *Paullinia*, *Serjania*, *Thinouia* and *Urvillea*), all of which are composed of lianas or climber-derived shrubs. There are around 475 lianas within the tribe, which makes Paullinieae the largest tribe of Sapindaceae, comprising almost a quarter of all species from the family (Coulleri et al., 2012; Acevedo-Rodríguez et al., 2017). Paullinieae is predominantly distributed in the Neotropics and several species

are native to Brazil (Acevedo-Rodríguez, 1990; Medeiros et al., 2016; Acevedo-Rodríguez et al., 2017).

A variety of secretory structures have already been reported within Sapindaceae, such as mucilaginous epidermal cells (Radlkofer, 1895; Solereder, 1908; Metcalfe and Chalk, 1950), glandular trichomes, tannin-containing cells and laticifers (Silva, 2009; Souza, 2010; Cunha Neto and Martins, 2012), as well as floral and extrafloral nectaries (Silva, 2009; Souza, 2010; Ning and Wu, 2005; Solís and Ferrucci, 2009; Zini et al., 2014; Avalos et al., 2017; Solís et al., 2017; Weryszko-Chmielewska and Chwil, 2017). Among these secretory structures, nectaries are probably the most studied type since they have been investigated in different genera of the family, e.g., *Aesculus*, *Allophylus*, *Cardiospermum*, *Dodonaea*, *Diplokeleba*, *Koeleruteria*, *Litchi*, *Melicoccus*, *Paullinia*, *Serjania*, *Urvillea* and *Thinouia* (Ning and Wu, 2005; Solís and Ferrucci, 2009; Zini et al., 2014; Avalos et al., 2016; Solís et al., 2017; Weryszko-Chmielewska and Chwil, 2017), contributing to the understanding of the variation in nectary morphology and their systematic

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importance for Sapindaceae (Zini et al., 2014; Solís et al., 2017).

On the other hand, there are several references indicating the occurrence of tannin-containing cells which are prevalent among the genera (Solereider, 1908; Metcalfe and Chalk, 1950), while few anatomical studies have explored in detail the morphology of other secretory structures of the family. Mucilaginous epidermal cells, for example, is only mentioned to several *Paullinia* species by Radlkofer, (1895), whereas glandular trichomes have been reported in the classical works of Solereider (1908), Radlkofer (1895;1931–1934) and Metcalfe and Chalk (1950), and more recently they were studied in detail for a few species of *Paullinia*, such as *P. cupana* Kunth, *P. carpopoda* Cambess., *P. rubiginosa* Cambess. (Areia, 1966; Areia et al., 1973; Ferraz and Costa, 1985; Souza, 2010) and *Serjania* as described in the species *S. pernambucensis* Radlk., *S. pygmaea* (Radlk.) Ferrucci & Medina, *S. rzedowskiana* Ferrucci & V. W. Steinm. (Silva, 2009; Ferrucci and Medina-Lemos, 2013; Ferrucci and Steinmann, 2016), as well as *Sapindus saponaria* L. (Albiero et al., 2001) and *Aesculus hippocastanum* L. (Chwil et al., 2013). However, the chemical composition of the exsudates produced by these trichomes has been studied only in *P. rubiginosa* Cambess. (Souza, 2010). In fact, this species is probably the most known representative of the family regarding the occurrence of secretory structures since Souza (2010) performed a thorough investigation on its aerial vegetative organs, carrying out an ontogenetic study, including a histochemical characterization of idioblasts, glandular trichomes, laticifers and nectaries.

Within the tribe Paullinieae, *Paullinia* and *Serjania* representatives are particularly noteworthy for the presence of laticifers, where several species are known to have milky white exsudates (Radlkofer, 1895; Ferraz and Costa, 1985; Acevedo-Rodríguez, 1993; Somner, 2001; Acevedo-Rodríguez et al., 2011). Nevertheless, laticifers have been described in detail only in a few species within the genera. They were recorded in the leaves of *P. cupana* (Areia, 1966; Areia et al., 1973) and *P. carpopoda* (Ferraz and Costa, 1985), in the flowers and stems of *S. pernambucensis* (Silva 2009; Cunha Neto and Martins 2012, respectively), in the ovary wall of *P. alata* G. Don, *P. clavigera* Schltdl., *P. obovata* (Ruiz & Pav.) Pers., *P. pachycarpa* Benth., *P. caloptera* Radlk., *P. dasystachya* Radlk., *Cardiospermum halicacabum* L., *S. altissima* (Poepp.) Radlk., *Urvillea ulmacea* Kunth (Weckerle and Rutishauser, 2005) and studied ontogenetically and histochemically only in young leaves and shoot apices of *P. rubiginosa* (Souza, 2010). Therefore, detailed information on laticifers in the secondary plant body of species of Sapindaceae are lacking.

Thus, the goal of this work was to study the distribution, morphology and exsudate of idioblasts, glandular trichomes and laticifers present in the stems of five Paullinieae lianas belonging to the genera *Paullinia* and *Serjania*. In particular, this study aimed at investigating the occurrence and distribution of laticifers in the secondary structure of *S. pernambucensis*, and to describe an unreported process of laticifer cell formation.

2. Materials and methods

2.1. Plant material

Five lianas endemic to Brazil from the genera *Paullinia* and *Serjania* (tribe Paullinieae) were studied. The specimens were collected from natural populations in different environments of the Atlantic Forest in the states of Bahia and Rio de Janeiro, Brazil. The following species were studied: *Paullinia micrantha* Cambess. (RBR 39198 – S 22°55'31.8"S, 42°26'38.3"W); *P. pseudota* Radlk. (RBR 39201 – 22°55.501'S, 42°26.759'W; RBR 39202 – 22°01'37"S, 43°11'32"W), *P. trigonia* Vell. (RBR 39199 – 22°32'37.2"S, 42°18'13.5"W), *P. weinmanniifolia* Mart. (RBR 39200 – 22°57'50.1"S, 42°53'40.1"W; RBR 34430 – 22°55'31.8"S, 042°26'38.3"W) and *Serjania pernambucensis* Radlk. (HURB 556 – 12°39'35.5"S, 39°05'07.4"W; HURB 11524 and HURB 11525 – 12°39'43.2"S, 39°04'38.2"W). Vouchers were deposited

in the Herbaria of the Universidade Federal Rural do Rio de Janeiro (RBR) and Universidade Federal do Recôncavo da Bahia (HURB).

For the structural study, shoot apices, including samples from the first, second and third internode, as well as from more basal portions of the stem, were collected from all individuals of all species. The middle portion of each of the specified internodes was investigated in all specimens. Developed stems (ca. 10–30 mm in diameter) were also used for the study of laticifers in the secondary structure of *Serjania pernambucensis* (see Section 2.3 for histochemical tests). Stem samples were fixed in FAA (formalin, acetic acid, 70% ethanol, 1:1:18 v/v/v) for 24 h, at room temperature (Johansen, 1940) or in 1% glutaraldehyde and 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2 (McDowell and Trump, 1976) for 24 h under vacuum conditions, dehydrated in a graded ethanol series and stored in 70% ethanol.

2.2. Anatomical analysis

For the anatomical study, part of the stem samples were dehydrated in a graded ethanol series and embedded in 2-hydroxyethyl-methacrylate (Historesin, Leica), sectioned with a rotary microtome (Leica RM2245), at variable thicknesses (ca. 3–7 µm), and stained with 0.05% toluidine blue (O'Brien and McCully, 1981). Other samples were softened in boiling water with 50% glycerine and then embedded in increasingly more concentrated solutions of polyethylene glycol 1500 (Rupp, 1964), beginning with 10% and reaching 100%, allowing one day in each solution (Barbosa et al., 2010). Histological sections of variable thickness (ca. 12–20 µm) were obtained with a slide microtome (Leica SM2000R) following the methods described in Barbosa et al. (2010), and double-stained in 1% astra blue and 2.5% safranin (Bukatsch, 1972).

Sections were photographed from permanent slides using a photomicroscope Olympus BX50 with a digital camera Media Cybernetics CoolSNAP-Pro.

The lumen diameter of laticifers was investigated for the species *S. pernambucensis* and *P. weinmanniifolia* in the different regions of the stem. All measurements were performed using the free software Image Pro-Plus 6.01 (Media Cybernetics).

2.3. Histochemical tests

For the histochemical analyses, fresh recently collected samples were cut with hot razor blades and sectioned using a cryomicrotome (Model CM1850; Leica Microsystems), at the laboratory of Plant Anatomy of the Federal University of Recôncavo of Bahia (UFRB). Sections recovered from historesin-embedded samples were also used. The performed tests are listed in Table 1. For each analysed metabolite group, standard negative-control procedures were carried out simultaneously, as recommended in the reference of the respective histochemical test.

In this study, histochemical tests for the contents of idioblasts were performed for all species, while glandular trichomes and laticifers were studied histochemically only in the species *S. pernambucensis* and *P. micrantha*, due to availability of fresh plant material.

2.4. Terminology notes

Some anatomical terms were adopted in this study that are related to the presence of successive cambia (a kind of cambial variant) in the secondary growth of stems: the term 'regular cambium' refers to the ordinary vascular cambium, which produce 'regular xylem' and 'regular phloem'; the terms 'variant xylem' and 'variant phloem' are used in regard to the vascular tissues originated from the 'successive cambium'.

Since *P. pseudota* has priority here, because *P. racemosa* was already taken, and *P. pseudota* was used earlier by Radlkofer (Acevedo-Rodríguez, P. pers. comm.), we used the name *Paullinia pseudota* Radlk. instead of *P. racemosa* Wawra.

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