DISTRIBUTION, MORPHOLOGY, AND HISTOCHEMISTRY OF THE SECRETORY SYSTEM IN ROOTS OF *PROTIUM HEPTAPHYLLUM* (AUBL.) MARCHAND (BURSERACEAE)

DISTRIBUIÇÃO, MORFOLOGIA E HISTOQUÍMICA DO SISTEMA SECRETOR EM RAÍZES DE *PROTIUM HEPTAPHYLLUM* (AUBL.) MARCHAND (BURSERACEAE)

Fernanda Helena Palermo, Juan de Nicolai, Diana Pacheco Seixas, Stefany Cristina de Melo Silva, Tatiane Maria Rodrigues

[1]Universidade Estadual Paulista (Unesp), Instituto de Biociências de Botucatu, Departamento de Botânica, Laboratório de Pesquisas em Anatomia Vegetal (LaPAV), Botucatu, SP, Brasil.

* Autor para correspondência: fer.helena@yahoo.com.br

ABSTRACT

Protium heptaphyllum (Aubl.) Marchand (Burseraceae) is known by the abundant production ofresin with ecological, industrial, and pharmaceutical properties. Despite this, information about the resin secretory sites in this species is scarce and restrict to the aerial axis. This study aimed to analyze the distribution, morphology, and histochemistry of the secretory system in roots of *P. heptaphyllum*. Roots samples were processed according to usual techniques in plant anatomy and histochemistry. Idioblasts and secretory canals were observed in the outer region of the phloem. The secretory canals were constituted by an uniseriate epithelium and wide lumen. Histochemical tests detected total lipids, resins, and polysaccharides in idioblasts and secretory canals; phenolic compounds were stained only in idioblasts. Our results indicate the mixed nature of the secretion in *P. heptaphyllum* and suggest the involvement of idioblasts complementing the role of the secretory canals in the interaction between plant and environment.

Keywords: Anatomy. Breu-branco. Histochemistry. Idioblasts. Roots. Secretory canals.

RESUMO

Protium heptaphyllum (Aubl.) Marchand (Burseraceae) é conhecida por sua abundante produção de resina com importância ecológica, industrial e farmacêutica. Apesar disso, informações sobre os sítios secretores de resina nessa espécie são escassas e restritas ao eixo aéreo. O objetivo deste estudo foi analisar a distribuição, morfologia e histoquímica do sistema secretor nas raízes de *P. heptaphyllum*. Amostras de raízes foram processadas segundo técnicas usuais em anatomia e histoquímica vegetal. Idioblastos e canais secretores foram observados na região externa do floema. Os canais secretores apresentaram-se constituídos por um epitélio unisseriado e lume amplo. Os testes histoquímicos detectaram lipídios totais, resinas e polissacarídeos em idioblastos e canais secretores; compostos fenólicos foram marcados apenas nos idioblastos. Nossos resultados indicam a natureza mista da secreção em *P. heptaphyllum* e sugerem o envolvimento dos idioblastos complementando o papel dos canais secretores na interação da planta com o meio ambiente.

Palavras-chaves: Anatomia. Breu-branco. Canais secretores. Histoquímica. Idioblastos. Raiz

Burseraceae species are historically known by the presence of secretory structures responsible for the production of aromatic resin [(1)]. *Protium* Burm. f. represents the main South American genus of Burseraceae and comprises 146 species [(2)], of which only 35 occur in Brazil [(3)]. The secretory structures that occur in *Protium* species are involved in terpenes production, mainly resins with important role in plant protection against herbivores and pathogens [(1,4)], in addition to their pharmaceutical and industrial importance.

Protium heptaphyllum (Aubl.) Marchand is a tree species popularly named almécega or breu-branco [(5)]. It is the most common Burseraceae species found in Brazil, occurring widespread in different environments, such as the Cerrado, Caatinga and Atlantic Forest [(5)]. This species is known

by their commercial importance, being used for manufacturing of incenses, essences, cosmetics and drugs [1,6]. Its medicinal potential is recognized and pharmaceutical studies have demonstrated the gastroprotective, antinociceptive, anti-inflammatory, hepatoprotective, analgesic, anti-ulcerogenic, antimycotic, and antioxidantactions proprieties of its resin [7, 8, 9, 10, 11].

Previous researches have demonstrated the presence of secretory canals and cavities in leaves and stems of *P. heptaphyllum* [12,13,14]. However, nothing is known on the occurrence of secretory structures in the root system of this species. Here we aimed to analyze the distribution, morphology and histochemistry of the secretory structures in roots of *P. heptaphyllum*.

Roots samples with 1,5 mm of diameter underinitial secondary growthwere collected from individuals of *Protium heptaphyllum* growing in a greenhouse at the Department of Botany, São Paulo State University (UNESP), Institute of Biosciences (IBB), Botucatu city, São Paulo State, Brazil.

For conventional light microscopy, root samples were fixed in FAA 50% [15], dehydrated in an ethanol series, and embedded in resin methacrylate [16]. The samples were sectioned in a rotary microtome and the longitudinal and transverse sections (6 µm) were stained with 0,05% Toluidine Blue, pH 4,7 [17]. Permanent slides were mounted with synthetic resin [16].

For the histochemical tests, root samples fixed in FNT [18] were sectioned by hand using a razor blade and thesections were treated with Sudan IV for detection of total lipids [15] and cupric acetate solution 10% for resins [15]. Samples fixed in FAA 50% [15] were sectioned and treated with ruthenium red 0,02% for polysaccharides and pectins [19], ferric chloride 10% for phenolic compounds[15] and Lugol's reagent for starch and alkaloids [15]. The slides were analyzed under light microscope and the relevant results were documented using an Olympus BX41 photomicroscope coupled to a digital camera.

Idioblasts and secretory spaces occur in the outer region of the phloem of *P. heptaphyllum* (Fig. 1A). The idioblasts are rounded or oval in shape, and are commonly organized in groups; some solitary idioblasts are also observed.

The secretory spaces are constituted by an uniseriate epithelium and wide lumen (Fig. 1B). The epithelial cells exhibit shape varying from rounded to pyramidal, thin walls, dense cytoplasm, and conspicuous nucleus. Cells in periclinal division are observed in the epithelium and in the subjacent cell layer (Fig. 1C), suggesting the meristematic potential of these cells and their involvement in the epithelium renovation [20]. In the longitudinal sections, the secretory spaces are elongated in shape (Fig. 1B), characterizing secretory canals.

Lipophilic and hydrophilic compounds are histochemically identified in idioblasts and secretory canals (Table 1). The histochemical tests detected phenolic compounds only in idioblasts (Fig. 2A). Secretory canals and idioblasts contain polysaccharides (Fig. 2B), total lipids (Fig. 2C-D), and resin. Lipids and phenolic substances have been commonly associated with plant protection against herbivores and pathogens [1, 4], while hydrophilic substances, like polysaccharides, are related to water retention in protoplasm cells, being an important factor to maintenance of water potential in plant tissues [21].The presence of phenolic compounds in the idioblasts and their absence in the secretory canals of the roots is opposite to the findings in the secretory canals of *P. heptaphyllum* leaves [13]. Considering that phenolics compounds are considered to be directly related to plant protections against UV radiation [22], the lower abundance of this substance in roots can be associated to the environment that this organs has developed.

Our results evidenced the mixed nature of secretion in roots of *P. heptaphyllum* and suggest the involvement of idioblasts complementing the role of the secretory canals in the interaction between plant and environment.

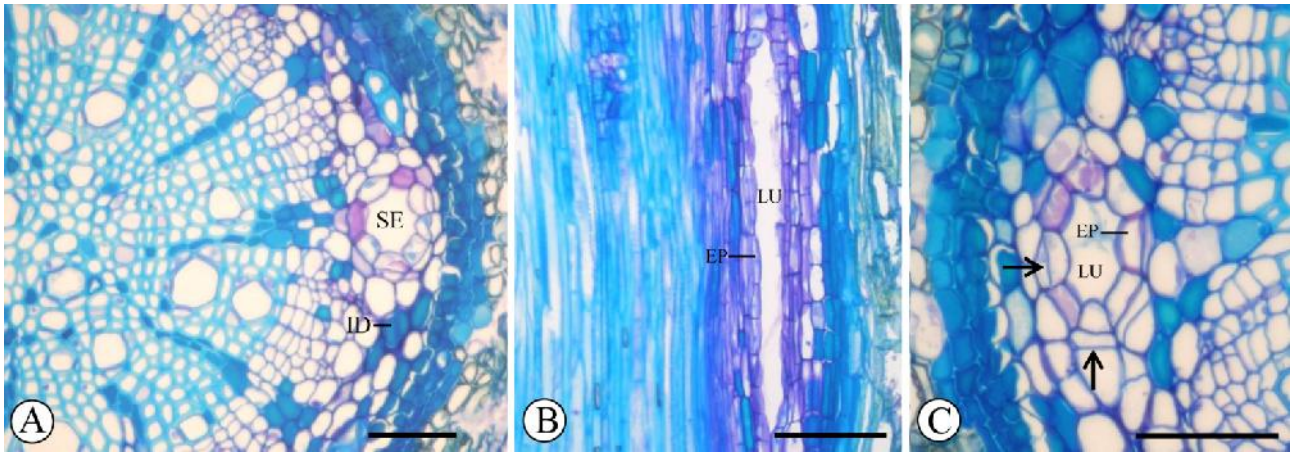

Figure 1. Light micrographies of roots in *Protium heptaphyllum*. A, Cross section showing idioblasts (ID) and secretory canals (SE) in the phloem. B, Longitudinal section showing secretory canal with uniseriate epithelium (EP) and elongated lumen (LU). C, Cross section showing secretory canal with lumen (LU) and uniseriate epithelium (EP) constituted by cells with variable shapes. The arrows indicate dividing cells in the epithelium and subjacent layer.Scale bars = 50µm (A and B), 20 µm (C).

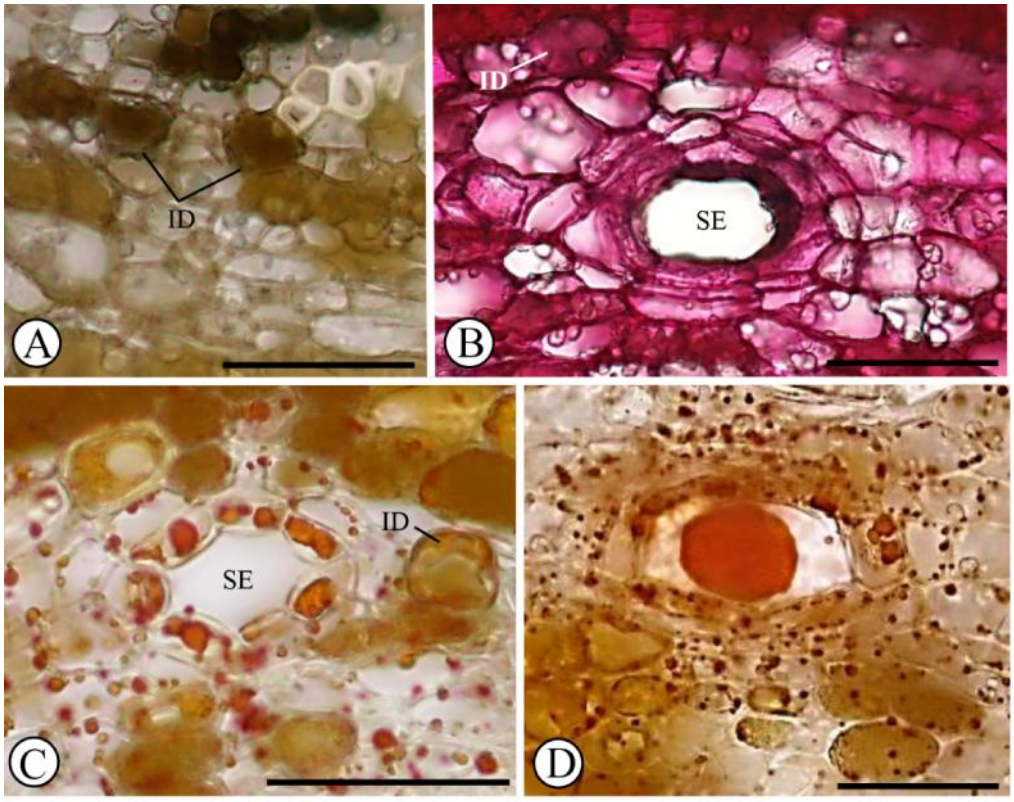

Figure 2. Histochemical tests in idioblasts and secretory canals of *Protium heptaphyllum* roots. A, Ferric Chloride. B, Ruthennium red. C-D, Sudan IV. **ID**: idioblasts; **SE**: secretory canals. Scale bars= 150 μm.

Table 1. Histochemical tests in idioblasts and secretory canals of *P. heptaphyllum* roots.

Reagent	Idioblasts	Secretory canals
Lugol's Reagent	-	-
Sudan IV	+	+
Ferric Chloride	+	-
Cupric acetate	+	+
Ruthenium Red	+	+

(+): positive; (-): negative.

REFERENCES

(1) LANGENHEIM, J.H. Plant resins: chemistry, evolution, ecology and ethnobotany. **Timber Press**, Portland, Cambridge, p. 586 2003.

(2) MELO, M.F.F., MACEDO, S.T., DALY, D.C. Morfologia de frutos, sementes e plântulas de nove espécies de *Protium* Burm. f. (Burseraceae) da Amazônia Central, Brasil. **Acta Botânica Brasileira**, p. 503-520. 2007.

(3) DALY, D.C., FINE, W.A. A new Amazonian section of *Protium* (Burseraceae) including both edaphic specialist and generalist taxa. Studies in Neotropical Burseraceae XVI. **Sistematic Botany**, p. 939-949. 2011.

(4) BOWERS, W.S. EVANS, P.H., VENABLE, D.L., BECERRA, J.X. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. **American Zoologist**, p. 865-876. 2001.

(5) LORENZI, H., MATOS, F.J.A. **Plantas medicinais no Brasil: nativas e exóticas**. Nova Odessa: Plantarum, 2002. 512 p.

(6) TOLERA, M., MENGER, D., SASS-CLAASSEN, U., STERCK, F.J., COPINI, P., BONGERS, F. Resin secretory structures of *Boswellia papyrifera* and implications for franckincense yield. **Annals of Botany**, p.1-8. 2012.

(7) REVILLA, J. **Plantas da Amazônia: oportunidades econômicas e sustentáveis**. Manaus: SEBRAE-AM/INSPA, 2001.

(8) SUSUNAGA, G.S. Estudo químico e biológico da resina produzida pela espécie *Protium heptaphyllum* March.(Burseraceae). (Dissertação de Mestrado). Univ. do Amazonas, Manaus. p. 163. 1996.

(9) SIANI, A.C., RAMOS, M.F.S., MNEZES-DE-LIMA, O., SOARES, R.O.A., ROSAS, E.C., SUSUNAGA, G.S., GGUIMARÃES, A.C., ZOGHBI, M.G.B., HENRIQUES, M.G.M.O., Evaluation of antiinflamatory related activity of essential oils from the leaves Andrés in of species of *Protium*. **Journal of Ethnpharmacology**, p. 57-69. 1999.

(10) BANDEIRA, P. N., PESSOA, O. D. L., TREVISAN, M. T. S., LEMOS, T. L. G. Metabólitos secundários de *Protium heptaphyllum* March. **Química Nova**, p. 1078-1080. 2002.

(11) OLIVEIRA, F.A., COSTA, C.L.S., CHAVES, M.H., ALMEIDA, F.R.C., CAVALCANTE, I.J.M., LIMA, A.F., LIMA Jr., R.C.P., SILVA, R.M., CAMPOS, A.R., SANTOS, F.A, RAO, V.S.N. Attenuation of capsaicin-induced acute and visceral nociceptive pain by alpha and beta-amyrin, a triterpene mixture isolated from *Protium heptaphyllum* resin in mice. **Life Science**, p. 2942-2952. 2005.

(12) BIERAS, A.C., SAJO, M.G. Leaf structure of the cerrado (Brazilian savanna) woody plants. **Trees**, p. 451-471. 2009.

(13) SOUZA, L.R., TRINDADE, F.G., OLIVEIRA, R.A., COSTA, L.C.B., GOMES, V. M., CUNHA, M. Histochemical characterization of secretory ducts and essential oil analysis of *Protium* species (Burseraceae). **Journal of Essential Oil Research**, p. 166-171. 2016.

(14) RODRIGUES, M.I.A. Anatomia do eixo vegetativo aéreo de *Protium heptaphyllum* Aubl. (Marchand) (Burseraceae) com ênfase no sistema secretor e ambiente com influência das marés. (Tese de Doutorado). Instituto de Biociências, Universidade Estadual Paulista (Unesp), São Paulo, p. 116. 2017.

(15) JOHANSEN, D.A. **Plant microtechnique**. New York: McGraw-Hill, 1940. 523 p.

(16) GERRITS, P.O. **The application of glycol methacrylate in histotechnology: some fundamental principles**. Netherlands: Department of Anatomy and Embryology State University Groningen, 1991. 80p.

(17) O'BRIEN, T.P., FEDER, N., McCULLY, M.E. Polychromatic staining of plant cell walls by toluidine blue. **Protoplasma**, p. 368-373. 1964.

(18) CLARK, G. **Staining procedures**. 3rd ed. Baltimore: The Williams & Wilkins, 1973. 422 p.

(19) JENSEN, W.A. **Botanical histochemistry: principles and practice**. San Francisco: W. H. Freeman and Company, 1962. 408 p.

(20) RODRIGUES, T.M., MACHADO, S.R. Oil gland in *Pterodon pubescens* Benth. (Leguminosae-Papilionoideae): distribution, structure and secretion mechanisms. **International Journal of Plant Sciences**, p. 984-922. 2012.

(21) ASCENÇÃO, L., MOTA, L., CASTRO, M.M. Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: morphology, distribution and histochemistry. **Annals of Botany**, p. 437–447. 1999.

(22) NACZK, M., SHAHIDI, F. Extraction and analysis of phenolics in food. **Journal of Chromatography A**., p. 95–111. 2004.