



Morphology and anatomy of the seedling and the tirodendro of *Calophyllum brasiliense* Cambess. (Clusiaceae)

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ABSTRACT. *Calophyllum brasiliense* Cambess. is a tree species that is presented as an alternative to replace endangered species of hardwood. The morphology and anatomy of the seedling of this species is the object of the present study. Seedlings at different stages of development were obtained in greenhouse and analyzed fresh and fixed in FAA (Formalin-Acetic-Alcohol) 50. The anatomical analysis was done by the freehand and microtome sections, according to standard techniques in plant anatomy. The seedling and/or tirodendro is cryptocotylar and hypogeal, has cataphylls, and presents eophylls and metaphylls simple with venation pinnate craspedodromous simple. The root is polyarch, the hypocotyl is very short, the cotyledons have an oily and starchy reserve, the epicotyl has stem structure, and eophylls and metaphylls are dorsiventral. The seedling may be classified in the *Horsfieldia* type/subtype.

Keywords: seedling axis, cotyledon, eophyll, *Calophyllum*, structure.

Morfoanatomia da plântula e do tirodendro de *Calophyllum brasiliense* Cambess. (Clusiaceae)

RESUMO. *Calophyllum brasiliense* Cambess. é uma planta arbórea que se apresenta como possível alternativa para substituir espécies em extinção com madeira nobre. A morfologia e anatomia da plântula desta espécie é objeto do presente trabalho. As plântulas, em diferentes fases de desenvolvimento, foram obtidas em casa de vegetação e analisadas a fresco e fixadas em FAA 50. A análise anatômica foi feita mediante seções a mão livre e microtômicas, de acordo com técnicas usuais em anatomia vegetal. A plântula e/ou tirodendro é criptocotiledonar e hipogéia, possui catafilos, e apresenta eófilos e metafilos simples de venação pinada craspedódroma simples. A raiz é poliarca, o hipocótilo é muito reduzido, os cotilédones possuem reserva amilácea e oleaginosa, o epicótilo tem natureza caulinar e os eófilos e metafilos são dorsiventrais. A plântula enquadra-se no tipo e subtipo *Horsfieldia*.

Palavras-chave: eixo da plântula, cotilédono, eófilo, *Calophyllum*, estrutura.

Introduction

Clusiaceae (Lindl.) includes 50 genera and 1,200 species distributed in tropical regions as well in northern temperate zones of the globe. This family includes trees, bushes, lianas and herbs of economic interest by timber production, edible fruits, chemical derivatives of pharmaceutical interest and others. Among the genera, there is *Calophyllum* L., which produces hardwoods (GASPAROTTO JÚNIOR et al., 2005).

Calophyllum brasiliense Cambess., commonly known as guanandi, olandi, olandim, galandim, Jacareúba, gulande-carvalho, guanandi-carvalho, cedar-guanandi, landim, among others, is an arboreal species which ranges from 20 to 30 m, it has diameter between 40 and 60 cm, wood density of 0.62 g cm⁻³, and metaphylls with a length ranging

from 10 to 13 cm and width from 5 to 6 cm (LORENZI, 1992). This is a climax species (SANTOS et al., 2008) which occurs naturally from Mexico to southern Brazil; it occurs in Brazil from the Amazon region to the north of Santa Catarina State, and it is found mainly in atlantic rain forest (CARVALHO, 1994; LORENZI, 1992; NERY et al., 2007; REIS et al., 2009).

Calophyllum brasiliense has major importance by presenting substances used to treat rheumatism, varicosities, hemorrhoids and chronic ulcers (GASPAROTTO JÚNIOR et al., 2005; NOLDIN et al., 2006). In addition to chemistry and pharmacology importance, is a species recommended for riparian forest restoration in places subject to periodic flooding from medium to long term duration (CARVALHO, 1994). This

species presents good quality wood and similar characteristics to the mahogany and cedar (NERY et al., 2007), providing a possible alternative to replace these hardwood species in extinction endangered.

Plant's first vegetative stage after seed germination is known as seedling, and it has enormous value at the population dynamics study, forestry, seeds storage, works in nurseries and in forest preservation and restoration (SOUZA, 2003). A failure in the adaptive process at the seedling stage may lead to species extinction (AMO-RODRIGUES; GOMEZ-POMPA, 1976). As a result, many studies about seedling have deserved researchers' attention, especially those related to its morphology and seeds germination, which provide subsidies for useful work in nurseries and forest regeneration (NG, 1973). Seedlings' morphological study is also important for its recognition in the woods, for the population dynamics establishment in forests and silvicultural management.

Considering the literature, few studies have been registered on morphoanatomy of Clusiaceae's seedlings, may be mentioned those of Hzn (1972) referring to the description *Calophyllum inophyllum* L., *Calophyllum soulattri* Burm. f., and *Garcinia parviflora* (Miq) Miq. seedlings; of Vogel (1980) allusive to the description of *Mammea odorata* (Raf.) Kosterm. seedlings; of Mourão and Beltrati (1995) on *Platonia insignis* Mart. germination and seedling morphology and of Mourão and Beltrati (2001) on *Mammea americana* L. and *Vismia guianensis* (Aubl.) Choisy. seedling morphology.

Therefore, given the economic, medicinal and ecological importance of *Calophyllum brasiliense*, and the lack of morphoanatomical studies on brazilian native species' seedlings, especially Clusiaceae, this study aims to analyze the seedling's structure and tirodendro of this species.

Material and methods

Species seeds were collected in April of 2008, from an artificial forest stands, containing trees between four and six years old, in the city of Monte Alto, São Paulo State, Brazil.

The seedlings and tirodendros were obtained in non-acclimatized greenhouse, located at the Center for Applied Research in Agriculture (Nupagri). Seed germination and the development of seedling and tirodendros occurred in plastic trays containing coarse sand as substrate, at a minimum temperature of 7 and maximum of 44°C. The trays containing

the species under study were watered twice a day, in the early morning and late afternoon. Both development phases, seedling and tirodendro, were analyzed and described according to Souza (2003), who considers seedling as the phase which covers the plant, from consummated seed germination until the first leaf or eophyll formation; the following phase, called tirodendro, extends up to the moment when the first metaphyll appears. Were morphologically described the root, hypocotyl, cotyledons, epicotyl, eophylls and metaphyll, adopting Rizzini (1977), Souza (2003) and Souza et al. (2009) terminologies. Seedling and tirodendro classification was based on Vogel (1980) and Garwood (1996).

The seedling and tirodendro anatomical analysis was made on fresh material and in FAA (Formalin-Acetic-Alcohol) 50 fixed material, and stored in 70% ethanol (JOHANSEN, 1940). The fresh material was sectioned transversal and paradermal, colored with Safranin and Atrá Blue, and mounted in glycerol at 33% (SOUZA et al., 2005). Fixed botanical material was submitted to dehydration in ethyl alcohol series, included in hystoresin Leica, according to specified product orientation, and sectioned in a rotary microtome. After, the obtained sections were colored with toluidine blue (O'BRIEN et al., 1965).

Histochemical tests were conducted to detect different substances in different parts of seedling/tirodendro, using specific reagents: lugol to starch, ammonia vapor to anthocyanin, phloroglucinol in acid to lignin and Sudam IV to lipophilic substances (JOHANSEN, 1940).

Venation study on eophyll and metaphyll was done by diaphanization technique of leaves dyed with 1% safranin alcoholic solution, considering Foster technique (SOUZA et al., 2005). Classification of venation standard was based on Hickey (1979).

The species development stages of seedling and tirodendro were illustrated through digital photographs. Seedling illustration was also made in stereoscopic microscope Leica EZ4D with digital camera fitted, and later on computer image capture.

The anatomical illustration was done by photomicrographs. These were obtained by image capture with Canon Power Shot A95 digital camera (Zoom Browser EX 4.6). The scales relating to the illustrations were obtained with a micrometer slide under the same optical conditions used in each case.

Results and discussion

Morphology of the seedling and the tirodendro

The seedling (Figure 1) is cryptocotyledonary and hypogeal. Presents ramified taproot, very small hypocotyl, two cotyledons of reserves, without chlorophyll, petiolate and orbicular, and glabrous green epicotyl with variable number of scales. The scale-like leaves are alternate and under-opposite, being the proximal reduced and the distal shows slightly green limbus. In seedling there are two simple eophylls, opposite or under-opposite, green, petiolate, oblong to lanceolate, retuse apex, rounded or acute, obtuse to cuneate base, and simple craspedodromous venation (Figure 2A). Tirodendro phase (Figure 1) is very short, with lanceolate metaphyll, acute base and apex, and the venation is also simple cross-venate (Figure 2B).

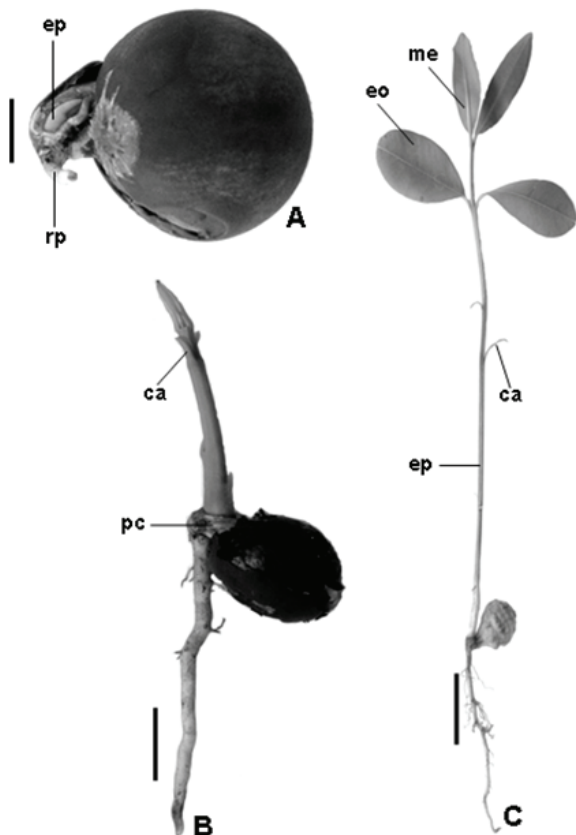


Figure 1. Seedling morphology of *Calophyllum brasiliense*. A and B – seedlings; C – tirodendro (ca = cataphyll, eo = eophyll, ep = epicotyl, me = metaphyll, pc = cotyledon petiole, rp = primary root). Scale = 0.50 cm (A), 1 cm (B) and 2 cm (C).

The seedling of *Calophyllum brasiliense* presents the general morphological characters recorded for the family, as cryptocotyledonary germination, unilateral cotyledons and supracotyledonary

cataphylls. With reference to the seedlings morphology of Clusiaceae species, which have been already investigated, some differences considered relevant are recorded, as phanerocotylar seedling, epigeal and foliaceous cotyledons in *Vismia guianensis* (MOURÃO; BELTRATI, 2001); undeveloped cotyledons in *Garcinia parviflora* (HZN, 1972); and cataphylls in opposite arrangement in *Calophyllum inophyllum* (HZN, 1972), *Rheedia edulis* (Seem.) Triana and Planchon (AMO-RODRIGUES, 1979), *Mammea odorata* (VOGEL, 1980) and *Platonia insignis* (MOURÃO; BELTRATI, 1995).

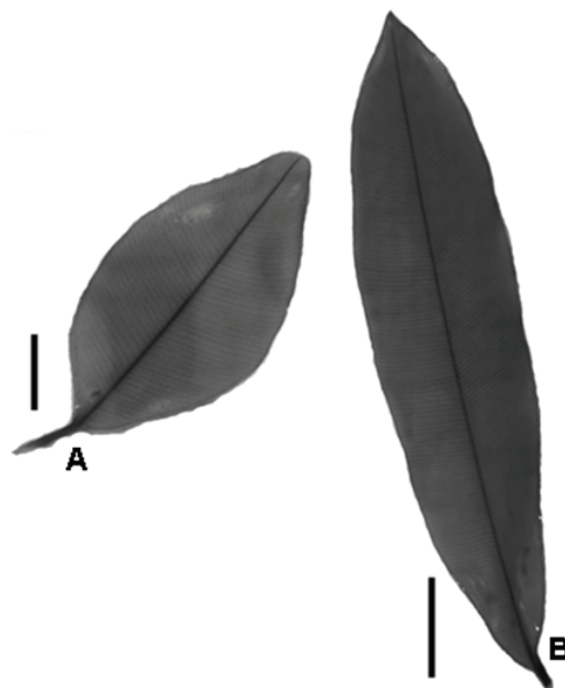


Figure 2. Eophyll (A) and metaphyll (B) of *Calophyllum brasiliense*, presenting venation pattern. Bar = 2 and 4 mm.

Eophylls venation of the pinned camptodromous-broquidodromous type seems to be common in species from different families (SOUZA et al., 2009), including the Clusiaceae species studied, as *Mammea americana*, *Platonia insignis* and *Vismia guianensis* (MOURÃO; BELTRATI, 1995; MOURÃO; BELTRATI, 2001). However, in the case of *Calophyllum brasiliense*, the venation is simple craspedodromous pinnate.

Calophyllum brasiliense seedling is classified into the *Horsfieldia* type, *Horsfieldia* subtype, according to Vogel (1980) classification, considered by the author as a common type among tropical woody dicots. At the Garwood (1996) classification, the seedlings of the species in question can be classified into the CHR type (Cryptocotylar, Hypogeal, Reserve storage).

Anatomy of the seedling and the tirodendro

The root primary growth (Figure 3A and B) shows irregular epidermis with thin-wall cells and unicellular hairs; cortex with unilateral or bisseriate exodermis of suberized wall cells, loose parenchymatic tissue with secretory ducts, and endodermis with Casparian strips; and central cylinder with parenchymatous uniseriate pericycle, phloem strands alternating with an equal number of xylem strands, provided with six to ten protoxylematic poles (polyarcroot) and parenchymatous pith. The variation in the number of protoxylem poles varies along the primary root. The root on secondary growth (Figure 3C and D) that is reduced in the seedling and tirodendro stages presents cambium, secondary phloem and xylem, and periderm of pericyclic origin. Epidermis and cortex (Figure 3C and D) are maintained during secondary growth through anticlinal division and cells tangential elongation; parenchymatous pith shows in this stage cells with thick secondary walls and simple pits. Secondary growth is similar to verified type for most of the dicots roots, cambium with procambium, and phellogen that also origins from pericycle.

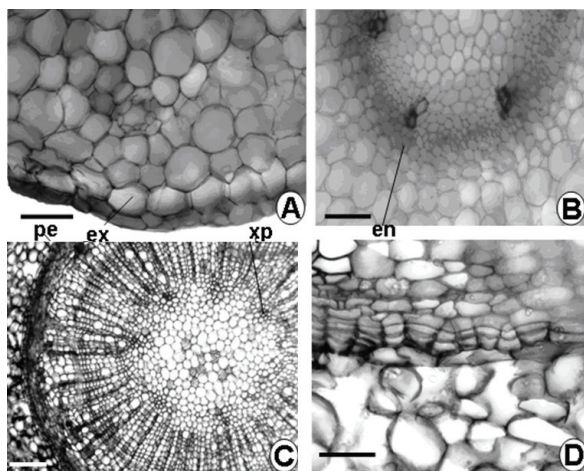


Figure 3. *Calophyllum brasiliense* root structure in cross sections. A and B – root on primary structure, showing epidermis/cortex and cortex/central cylinder; C and D – root on secondary structure, in overview and detail showing region periderm region (en = endoderm, ex = exodermis, pe = periderm, xp = primary xylem). Bar = 50 μ m (A, B and D) and 150 μ m (C).

Polyarc condition of primary root, seen in *Calophyllum brasiliense* differs from triarch or tetrarch condition registered to the root of *Vismia guianensis*, and the common pattern of seedlings' primary roots, which is diarch or triarch (EAMES, 1961). Number variation of protoxylem poles may occur among species (EAMES; MacDANIELS, 1953) and along of a seedling primary root (SOUZA, 2009).

Adventitious monocotyledon roots are generally polyarchies, but there exists a correlation between the vascular cylinder diameter and number of protoxylem groups, and the presence or absence of pith (FAHN, 1990). In the case of *Calophyllum brasiliense*, the polyarc condition and the pith presence are, probably, due to the bigger diameter of the primary root. Polyarchies primary roots have also been registered in Bignoniaceae species (SOUZA et al., 2007; SOUZA, 2009).

Close to the cotyledonary node occurs the transition zone root-stem, which is much reduced. In this region, tracheary elements of each primary xylem strand deviate and are distributed on the parenchymatic pith periphery (Figure 4A). The transition from the exarch condition of primary xylem of root to endarch of stem is very fast, and occurs at cotyledon node.

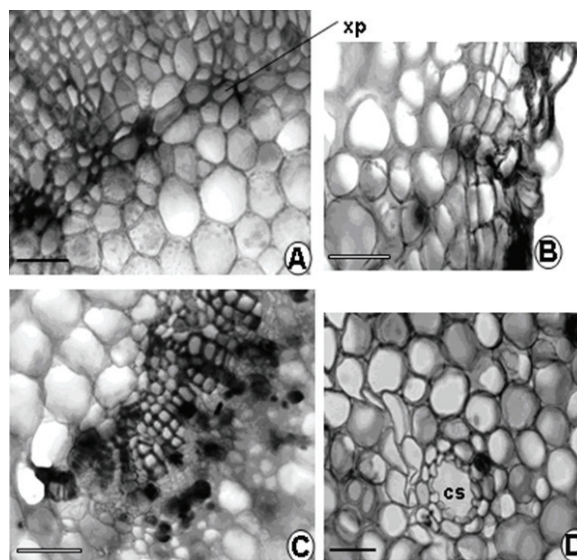


Figure 4. Hypocotyl structure (root-stem transition region) and the *Calophyllum brasiliense* cotyledon in cross sections. A – hypocotyls showing primary and secondary xylem, and pith; B – adaxial region of cotyledonary petiole with periderm; C – cotyledonary vascular bundle; D – cotyledonary mesophyll (cs = secretory ducts; xp = primary xylem). Bars = 50 μ m.

Cotyledon presents petiole with uniseriate epidermis provided of cuticle and thick lipophilic cuticle layer. Periderm may occur (Figure 4B) in certain regions of petiole's adaxial and abaxial surface, from subepidermal origin. Petiole presents also, parenchyma and a collateral single central bundle, with cambium and reduced amount of secondary vascular tissue (Figure 4C). Cotyledonary limbus is thick with starchy reserves and oleaginous, presents uniseriate epidermis, glabrous, with cuticle and cuticle layer similar to the petiole; epidermal cells vary from cuboidal to short prismatic. Mesophyll (Figure 4D) is homogeneous, with more

or less isodiametric cells, may occurring more elongated cells subepidermally; in mesophyll occurs secretory ducts in large numbers and idioblasts with druses.

Epicotyl has uniseriate epidermis, glabrous, with thick cuticle and external periclinal wall (Figure 5A and B). Cortex is parenchymatous with secretory ducts (Figure 5B) and there is no evidence of endodermis with Casparian strips nor starchy sheath. Central cylinder (Figure 5A) shows on the periphery groups of not lignified fibers, primary phloem and scarce secondary, cambium, reduced amount of secondary xylem, and primary xylem. There is pith of parenchymatic nature with secretory ducts (Figure 5A).

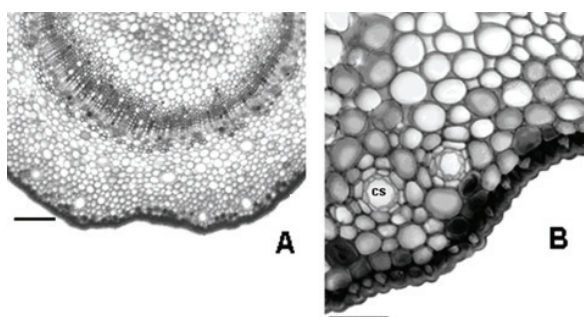


Figure 5. Epicotyl's structure of the *Calophyllum brasiliense* in cross section. A – overview; B – epidermis and cortex details (cs = secretory ducts). Bars = 150 μ m (A) and 50 μ m (B).

Calophyllum brasiliense epicotyl presents stem structure similar to that reported by Metcalfe and Chalk (1957) for young stem of Clusiaceae species. It was not observed, however, sclerified cells in the cortex, common character in the family, as indicated by the authors; maybe, this absence is due to the epicotyl's development phase still little advanced.

Eophylls have petiole with uniseriate epidermis, glabrous, with cuticle and thick cuticle layer. Under the epidermis occurs parenchyma and collenchyma, both with secretory ducts. Throughout the petiole there is only one collateral vascular bundle in the U form. In the distal, lateral and adaxial region there is laminar vascularized expansion composed of collenchymatous tissue with secretory ducts. Leaf lamina presents in epidermis central vein, collenchyma in small amounts on both sides, parenchyma, secretory ducts and a single collateral vascular bundle, also in the U form (Figure 6C). Limbus shows glabrous and hipostomatic epidermis, with epidermal cells ordinary of sinuous anticlinal walls on the adaxial side and slightly sinuous in abaxial; stomata are paracytic (Figure 6A and B). Eophylls are dorsiventral (Figure 6D) with two

layers of palisade parenchyma and spongy parenchyma; in mesophyll occurs cells with drusen and secretory ducts. The margin has sclerenchyma and secretory duct. Small veins, immersed in the mesophyll (Figure 6D), have bundle sheath (endoderm), with extension of parenchymatic or sclerenchymatic nature.

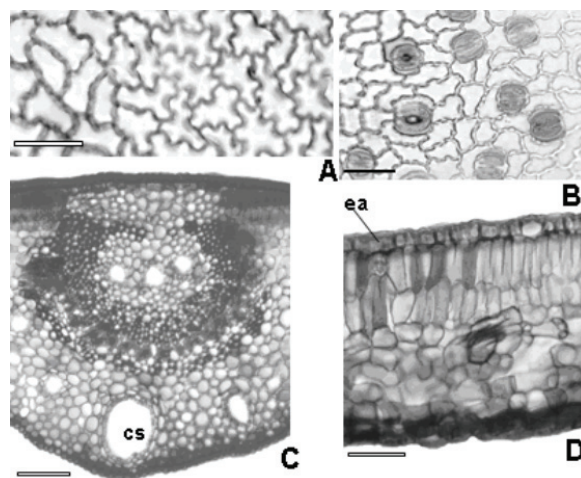


Figure 6. Eophyll's structure of *Calophyllum brasiliense*, in paradermic sections (A and B) and transverse (C and D). A and B – epidermis frontal view of the adaxial and abaxial sides; C – central vein of limbus's basal region; D – internodal region (cs = secretory duct, ea = adaxial epidermis). Bars = 30 μ m (A and B), 50 μ m (C) and 100 μ m (D).

The metaphyll's petiole shows similarity with the eophyll, except for the presence of trichomes, periderm on both sides (mainly at the base and on the adaxial side), idioblasts with druses, and primordium fibers on the phloematic region. Referring to the limbus, epidermis has cells with anticlines walls whose sinuosity not differ on both sides, and paracytic stomata (Figure 7A and B); metaphyll is also dorsiventral, but has a single palisade parenchyma layer (Figure 7C). The margin (Figure 7D) is similar to eophyll. In metaphyll's central vein (Figure 7E) there is more amount of collenchyma, especially on the adaxial surface, the vascular bundle has V format and there are only a few differentiated sclerenchyma cells at the phloematic side.

Calophyllum brasiliense eophylls and first metaphyll have characters, as dorsiventral leaves, paracytic stomata, calcium oxalate crystals and secretory ducts, which are indicated for the Clusiaceae nomofilo (METCALFE; CHALK, 1957). However, hypodermis uni or multiseriate of the adaxial lamina surface, which is recorded especially for the genus, was not observed in *Calophyllum brasiliense*.

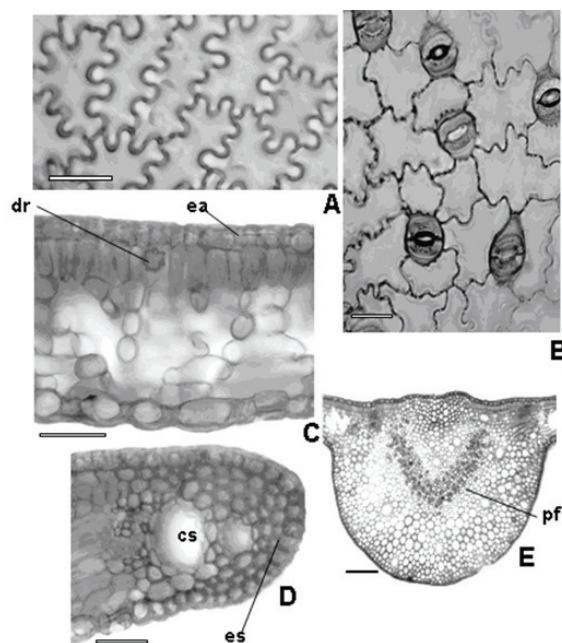


Figure 7. Metaphyll's structure of *Calophyllum brasiliense*, in paradermic sections (A and B) and transverse (C to E). A and B – epidermis frontal view of the adaxial and abaxial sides; C – internodal region; D – margin; E – central vein (cs = secretory duct, dr = drusen, ea = adaxial epidermis, es = sclerenchyma, mp = primordium fibers). Bars = 30 μm (A and B), 40 μm (D), 50 μm (C) and 150 μm (E).

Conclusion

The results presented in this work indicate that the seedling and/or tirodendro is cryptocotylar and hypogeal, has cataphylls, and presents eophylls and metaphylls simple with venation pinnate craspedodromous simple. The root is polyarch, the hypocotyl is very short, the cotyledons have an oily and starchy reserve, the epicotyl has stem structure, and eophylls and metaphylls are dorsiventral. The seedling may be classified in the *Horsfieldia* type/subtype.

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