MORPHOLOGY AND ANATOMY OF SERJANIA MILL. (SAPINDACEAE) SEEDLINGS WITH EMPHASIS ON VASCULARIZATION

MORFOLOGIA E ANATOMIA DE PLÂNTULAS DE SERJANIA MILL. (SAPINDACEAE) COM ÔNFASE NA VASCULARIZAÇÃO

DOI: 10.56083/RCV3N5-048
Recebimento do original: 26/04/2023
Aceitação para publicação: 30/05/2023

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ABSTRACT: In the forest remnants of the Maringá region, Brazil, it is common the occurrence of Serjania Mill. (Sapindaceae) species, which have a lianescent habit; the genus has apicultural importance and ichthyotoxic property. Seedlings of Serjania caracasana (Jacq.) Willd., S. fuscifolia Radlk. and S. laruoetteana Cambess. were examined morphologically and anatomically in order to contribute with features for the separation of species
and characterization of the genus. Seedlings were grown in a Petri dish and germination chamber, fixed in glutaraldehyde, embedded in historesin and sectioned in a rotation microtome. The venation pattern was analyzed in diaphanized eophylls. Seedlings of *S. fuscifolia* and *S. laruotteana* are phanerocotylar whereas *S. caracasana* has cryptocotylar seedling, all species with thick cotyledons and trifoliolate compound eophylls. Seedlings exhibit diarch primary root, short hypocotyl with root-stem transition structure, unilacunar cotyledonal node with double trace, and trilacunar eophyll node. Venation pattern is craspedodromous in *S. fuscifolia*, camptodromous brochidodromous in *S. laruotteana*, and camptodromous eucamptodromous in *S. caracasana*. The seedlings show a lot of morphoanatomical similarity, but some characters, such as germination and venation types, can be useful to separate some species within the genus.

**KEYWORDS:** Diarch Root, Hypocotyl, Leaf Node, Seedling Type, Venation Pattern.

**RESUMO:** Nos remanescentes florestais da região de Maringá, Brasil, é comum a ocorrência de espécies de *Serjania* Mill. (Sapindaceae), que possuem hábito lianescente; o gênero tem importância apícola e propriedade ictiotóxica. Plântulas de *Serjania caracasana* (Jacq.) Willd., *S. fuscifolia* Radlk. e *S. laruotteana* Cambess. foram examinadas morfológica e anatomicamente a fim de contribuir com caracteres para a separação de espécies e caracterização do gênero. Plântulas foram cultivadas em placa de Petri em câmara de germinação, fixadas em glutaraldeído, emblocadas em historesina e secionadas em micrótomo de rotação. O padrão de venação foi analisado em eofilos diafanizados. Plântulas de *S. fuscifolia* e *S. laruotteana* são fanerocotiledonares, enquanto *S. caracasana* possui plântula criptocotiledonar, todas espécies com cotilédones espessos e eofilos compostos trifoliolados. As plântulas apresentam raiz primária diarca, hipocótilo curto com estrutura de transição raiz-caule, nó cotiledonar unilacunar com traço duplo e nó do eófilo trilacunar. O padrão de venação é craspedódromo em *S. fuscifolia*, camptódromo broquidódromo em *S. laruotteana* e camptódromo eucamptódromo em *S. caracasana*. As plântulas apresentam muita similaridade morfoanatômica, mas alguns caracteres, como tipos de germinação e nervação, podem ser úteis para separar algumas espécies dentro do gênero.

**PALAVRAS-CHAVE:** Hipocótilo, Nó Foliar, Padrão de Venação, Raiz Diarca, Tipo de Plântula.
1. Introduction

One of the most vigorous neotropical genera of Sapindaceae is *Serjania* Mill., whose circumscription has changed considerably over the past century, due to a better understanding of the relationships between its closely related species and genera (STEIMANN et al., 2022). The genus is a diverse group of about 240 species, and it belongs to the Paullinieae tribe, Paulliniodae supertribe, characterized by having a climbing or shrubby habit derived from the climbing habit, compound leaves, stipulated, and a pair of tendrils at the base of the inflorescence axis (ACEVEDO-RODRIGUES et al., 2017).

Brazilian *Serjania* species occur in the Amazon, “Caatinga”, “Cerrado”, Atlantic Forest and “Pantanal” (FLORA E FUNGA DO BRASIL, 2020), which exhibit alternate and compound leaves, appendicular petals and flowers with nectaries (HARRINGTON et al., 2005; SILVA et al., 2018). About 119 Brazilian species of *Serjania* are registered, 65 of which are considered endemic (REFLORA, 2000); in the state of “Paraná”, the genus has about 24 species (ROSADO; SOUZA, 2022).

*Serjania* species have medicinal, apicultural and ichthyotoxic importance (GUARIM-NETO et al., 2000; MORETI et al., 2009), in which the fruit type (GENTRY, 1991; COULLERI et al., 2012; SPRENGER-LIMA; REZENDE, 2013; ALVES et al., 2014), and cambial variants (CABALLÉ, 1993) are features that have taxonomic value. The fruits are winged and can be dispersed by ants (PINHEIRO et al., 2009).

Seedling is considered a plant phase that begins with the germination of the seed and extends until the expansion of the first eophyll (SOUZA et al., 2009; SOUZA, 2016), and can reveal morphological and anatomical characters that can be employed in the recognition of species in the field and species identification. The seedling literature provides features which are of taxonomic value at the specific level (COMPTON, 1912; SOUZA et al., 2009; GURGEL et al., 2012). The morphoanatomical investigation of seedlings in
Serjania is very scarce, highlighting the work by Lopes & Souza (2015), on Serjania communis Cambess.

The vascular system of the root, hypocotyl and cotyledons can be considered as a unit (ESAU, 1959). The vascularization of the seedling axis can vary between species, but it can also vary within the same species or individual. The number of protoxylematic poles in the root, for example, can vary from 2 to 5 in seedlings of various species and genera, but within Vismia guianensis (Aubl.) Choisy, Clusiaceae, it varies from triarch to tetrarch (SOUZA, 2009). The vasculature of the root-stem transition zone may also vary between species, leading Compton (1912) to formulate types of transition zone for Leguminosae seedlings. The vascular structure of the cotyledonary nodes generally consists of a lacuna with two distinct traces, but there may be variation, as in Asteraceae, which has trilacunar cotyledonary nodes (SOUZA, 2009). For Takhtajan (1980), the unilacunar node of leaves is secondary in phanerogams, having originated from a basic type of tri-pentalacunar node. The venation pattern of cotyledons and eophylls can also be a useful tool in the taxonomy of angiosperm groups (SOUZA et al., 2009).

Three lianescent species of Serjania (S. caracasana (Jacq.) Willd., S. fuscifolia Radlk. and S. laruoetteana Cambess.) that are abundant in forest fragments of Paraná, Brazil, were selected for the morphoanatomical study of seedlings. The present paper is mainly devoted to the comparative vasculature of the axis and eophylls of seedlings of the three species, in order to evaluate features of potential taxonomic significance.
2. Material and methods

Seed collection, germination and obtaining seedlings – Seeds (diaspores) were collected from some randomly selected plants of *Serjania caracasana*, *S. fuscifolia* and *S. laruotteana* at forest fragments of Maringá and region, Paraná state, Brazil.

The seeds were washed in a solution of sodium hypochlorite and distilled water and dried at room temperature. The seeds were placed to germinate in Petri dishes with moist filter paper in a TE 400 Tecnal germination chamber, with controlled light and temperature (30ºC), until the appearance of the first two eophylls still inside the germination chamber, in the Petri dish.

Seedling morphology - The seedling stages of the three species were analyzed and morphologically described, according to the terminology of Vogel (1980) and Souza et al. (2009), and photographed with a common camera.

Vascular system of the seedling axis - The vascular system of the root, hypocotyl, cotyledonary node, epicotyl and eophyll node was investigated through cross-sections of seedlings fixed in glutaraldehyde, obtained in a rotation microtome. To obtain these sections, the fixed seedlings were embedded in Leica historesin (GUERRITS; HOROBIN, 1991), and the sections stained with toluidine blue (O'BRIEN et al., 1964). The sections were photographed using a Leica ICC50 light microscope with a digital camera and subsequently processed using LAS 50 software.

Eophyll venation pattern - The eophyll diaphanization technique was based on Foster (1950). The technique consisted of drying the leaves in 5% NaOH, dehydration in an ethanol series and staining in 1% aqueous safranin. Then, the leaves were photographed using a Leica EZ4D stereoscopic microscope and the venation pattern was described according to Hickey's terminology (1979).
Figure 1 - Seedling morphology of Serjania caracasana (A), S. fuscifolia (B) and S. laruotteana (C). (eo=eophyll; ep=epicotyl; hy=hypocotyl; pr=primary root). Scale bars: 7mm.
3. Results

Seedling morphology – Seedlings (Figure 1B,C) of Serjania fuscifolia and S. laruoetteana are phanerocotylar, although it is possible to observe for some time after germination one of the cotyledons still surrounded by the seed coat (Figure 1B). The exposed cotyledons of these two species remain close to the substrate surface, because the hypocotyl is very small. The S. caracasana seedlings are cryptocotylar, in which the cotyledons remain enveloped in the seed coat.

All seedlings have axial and long primary root, short hypocotyl, and both thick cotyledons. The epicotyl is long, green and cylindrical, and the petiolute eophylls are opposite trifoliate (Figure 1). The leaflets have indentations on the edge, being superficial (dentate) in Serjania laruoetteana (Figure 1C) and much deeper (split leaves) in S. fuscifolia (Figure 1B) and S. caracasana.

Seedling anatomy and vasculature – Seedling primary roots are diarch (Figure 2A,B), and they consist of hairy (unicellular hairs) uniseriate epidermis, cortex with exodermis, parenchyma and endodermis, and vascular cylinder which exhibits two strands of primary xylem and phloem, and secondary vascular tissues (Figure 2A). In the root in secondary growth, the cortex and epidermis are eliminated and Figure 2A shows this region and tissue already separated from the central cylinder.

The short hypocotyl exhibits root-stem transition region along its entire length in which the tracheary elements of the central primary xylem strand move away, giving rise to a parenchymatic pith (Figure 2C), still in the root, close to the collet; each primary phloem strand is subdivided into two other primary phloem strands. The vascular cylinder (tracheary elements arranged around the pith and four strands of phloem) thus persists in the hypocotyl up to the vicinity of the cotyledonary node, when the cotyledonary traces (double traces with a single leaf gap for each cotyledon) are formed, these
traces being subopposite. In the region of the cotyledonary node, there are also epicotyledonary traces (vascular tissues located between the double traces of the two cotyledons) (Figure 2D), which vascularize the epicotyl. The node of the opposing eophylls is trilacunar with three traces in the three species (Figure 2E).

Figure 2 - Structure of root, hypocotyl (root-stem transition region), cotyledonary node and eophyll node of *Serjania laruotteana* (A,B), *S. fuscifolia* (C,D) e *S. caracasana* (E), in cross-sections. A,B – Root and central cylinder detail (white arrow indicates protoxylem). C – Beginning of the root-stem transition region; V-shaped groups of xylem, and formation of the pith (white arrow indicates protoxylem). D – Cotyledonary node (subopposite cotyledons). E – Eophyll node (opposite eophylls). (dt=double traces; ll=cotyledonary leaf lacuna; lt=leaf traces of the eophyll). Scale bars: 100µm (B), 200µm (A,C,E), 300µm (D).
The epicotyl has a tetralobed outline, with a stem structure of an eustelic nature, that is, with collateral vascular bundles in secondary growth, arranged around the parenchymal pith (Figure 3A). The epicotyl is composed of uniseriate epidermis with stomata and sparse hairs, collenchymatous and parenchymal cortex, layers of perivascular fibers that completely envelop the central tetralobed cylinder (Figure 3A,B).

Figure 3 - Epicotyl structure, and diaphanized leaves (central leaflet) of Serjania caracasana (A,D), S. laruotteana (B,E) and S. fuscifolia (C). A,B – Epicotyl, and anatomical detail of the lobe. C,D - Leaves showing venation pattern. (pf=perivascular fibers; pv=primary vein). Scale bars: 70µm (B), 200µm (A).

The eophyll venation is pinnate with a single primary vein and secondary veins, but different subtypes are distinguished in the three investigated species. The central eophyll of Serjania fuscifolia has simple craspedodromous venation type with the secondary veins terminating at the
margin (Figure 3C). In *S. laruotteana* the venation is camptodromous brochidodromous in which the secondary veins do not end at the margin and they joined together in a series of prominent arches (Figure 3D). Finally, the *S. caracasana* venation is camptodromous eucamptododromous with the secondary veins do not terminate at the margin; the secondaries upturned and gradually diminishing apically inside the margin.

The primary veins of eophylls of the three species are straight in course, without any noticeable deviation, change in course, or more primary ramifications (Figure 3C,D,F). The secondary veins have an almost uniform angle of divergence and the ramifications that originate from them are characterized by being transverse, that is, the ramifications are oriented through the intercostal area (those portions of the leaf blade lying between the secondary veins).

4. Discussion

The morphological analysis of the seedlings of *Serjania fuscifolia* and *S. laruotteana* revealed very similar characters, such as thick exposed cotyledons and semi-epigeal, taproot, short hypocotyl, long epicotyl, and trifoliate compound eophylls. The seedling of *S. caracasana* has a morphological pattern similar to the seedlings of the other two species, but unlike these species, it maintains the cotyledons surrounded by the seed coat. The phanerocotylar seedling of *S. communis* (LOPES; SOUZA, 2015), which is a liana that also occurs in the same forest remnants of the species investigated here, has a comparatively very different morphology, with a long hypocotyl, leaf-like cotyledons and simple eophylls. Therefore, *Serjania* can present species with phanerocotylar and cryptocotylar seedlings, which shows that the germination type character does not seem to be taxonomically conservative in the genus, as demonstrated for other groups of plants by Wright et al. (2000).
Seedlings of *Serjania fuscifolia* and *S. laruotteana* belong to the “Sloanea” type, “Palaquium” subtype of Vogel (1980), which is characterized by having exposed cotyledons from the seed envelope, a short hypocotyl and eophylls with opposite phyllotaxis. On the other hand, *S. caracasana* seems to fit better in the “Horsfieldia” type, “Horsfieldia” subtype, recorded by the author in Sapindaceae and other families, which exhibits characters such as cryptocotylar seedlings, with a short hypocotyl and opposite eophylls. *Serjania communis* (LOPES; SOUZA, 2015) has a Macaranga-type seedling (VOGEL, 1980), suggesting that *Serjania* may have morphological diversity of seedlings. In the functional morphology classification of seedlings by Garwood (1996) the species *S. fuscifolia* and *S. laruotteana* can be considered as belonging to the PER (Phanerocotylar, Epigeal and Reserve cotyledons) type, whereas the seedling type of *S. caracasana* is CER (Cryptocotylar, Epigeal and Reserve cotyledons) type. According to Garwood (1996), Neotropical sites have more species with PEF seedling types than Paleotropical sites, but fewer species with CER type.

The two types of *Serjania* seedlings found in our work (phanerocotylar and cryptocotylar with succulent cotyledons) were also recorded by Hzn (1972) who analyzed seedlings of seven tree species of Sapindaceae from Asia, belonging to the genera *Allophylus* L., *Dodonaea* Mill., *Erioglossum* Blume, *Ganophyllum* Blume, *Pometia* J.R. Forst. & G. Forst., and *Schleichera* Willd. Likewise, Vogel (1980) investigated seedlings of several families of some angiosperms from Malaysia, including six species of Sapindaceae from the genera *Cubilia* Blume, *Dimocarpus* Lour., *Harpullia* Roxb., *Lepisanthes* Blume and *Nephelium* L., finding the same types of seedlings and cotyledons. Our study and the investigations of seedlings by both authors mentioned above indicate that Sapindaceae shows variation in seedling types within the same genus and family.

The three *Serjania* species have diarch primary root and hypocotyl that exhibits root-stem transition region that are similar to seedling of *S.*
The root-stem transition structure (root exarch condition to stem endarch) can be considered of the intermediate type of Compton (1912), which starts at the collet or just below the root and ends near the cotyledonary node. The structural nature of the transition between root and stem of the hypocotyl deserved attention from Compton (1912) under the phylogenetic aspect. For the author, the hypocotyl with this transitional structure it seems to be best considered, not as a stem-nor as root-structure, nor as a compound organ partly composed of stem partly of root, nor as plant-member “sui generis” but as a specialized region of a primitively (ancestral) undifferentiated axis.

All *Serjania* species, including *S. communis* (LOPES; SOUZA, 2015), have a unilacunar cotyledonary node with a double trace. The unilacunar trace condition was considered by some authors to be phylogenetically ancestral in angiosperms (TAKHTAJAN, 1980), but this author, based on the studies of Benzing (1967), emphasizes that the anatomy of cotyledonary nodes does not necessarily reflect ancestral conditions.

Eophyll nodes of *Serjania fuscifolia*, *S. laruotteana* and *S. caracasana* are trilacunar with three traces, whereas *S. communis* (LOPES; SOUZA, 2015) has unilacunar node with one trace. Takhtajan (1980) extensively discusses the evolution of leaf traces in angiosperms, and finally concludes that the unilacunar type of nodal structure is secondary in phanerogams, probably originating from the basic tri-pentalacunar type.

The three species of *Serjania* have eophylls with pinnate venation of the craspedodromous (*S. fuscifolia*) and camptodromous (*S. caracasana* and *S. laruotteana*) types. Valente et al. (1984), using different terminology from Hickey (1979), investigated the venation of nomophylls of 26 *Serjania* species from the state of Janeiro, Brazil, including the three species studied here; in this work *S. fuscifolia* was also considered to have a venation pattern of the craspedodromous type, but used the same pattern of venation (brochidodromous) for *S. caracasana* and *S. laruotteana*. These two types of
Hickey's (1979) venation pattern, craspedodromous and camptodromous, found in the eophylls of *Serjania*, appear to be common in nomophylls of many other genera of Sapindaceae (CAO et al., 2014).

Table 1 shows some comparative features of *Serjania fuscifolia*, *S. laruotteana* and *S. caracasana* seedlings, including those recorded in *S. communis* by Lopes and Souza (2015). The features reveal a lot of similarity between the seedlings of *S. fuscifolia* and *S. laruotteana*, and that may be useful to separate these species from those of *S. caracasana*. The features also show that the seedlings of *S. fuscifolia*, *S. laruotteana* and *S. caracasana* have significantly different morphoanatomy when compared to those of *S. communis*.

Table 1 – Morphoanatomical features of the three *Serjania* species investigated compared with those of *Serjania communis* (Lopes & Souza 2015).

<table>
<thead>
<tr>
<th>Species/ Features</th>
<th>Serjania caracasana</th>
<th>Serjania fuscifolia</th>
<th>Serjania laruotteana</th>
<th>Serjania communis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling type</td>
<td>Cryptocotylar</td>
<td>Phanerocotylar</td>
<td>Phanerocotylar</td>
<td>Phanerocotylar</td>
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<tr>
<td>Hypocotyl</td>
<td>Short</td>
<td>Short</td>
<td>Short</td>
<td>Long</td>
</tr>
<tr>
<td>Cotyledons</td>
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<td>Thick</td>
<td>Thick</td>
<td>Foliaceous</td>
</tr>
<tr>
<td>Eophylls</td>
<td>Trifoliate compound</td>
<td>Trifoliate compound</td>
<td>Trifoliate compound</td>
<td>Simple leaves</td>
</tr>
<tr>
<td>Eophyll margin</td>
<td>Split margin</td>
<td>Split margin</td>
<td>Dentate margin</td>
<td>Serrate margin</td>
</tr>
<tr>
<td>Eophyll node</td>
<td>Trilacunar with 3 traces</td>
<td>Trilacunar with 3 traces</td>
<td>Trilacunar with 3 traces</td>
<td>Unilacunar with 1 trace</td>
</tr>
<tr>
<td>Eophyll venation</td>
<td>Camptodromous eucamptodromous</td>
<td>Craspedodromous simple</td>
<td>Camptodromous brochidodromous</td>
<td>-</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

We thank CNPq (National Council of Scientific and Technological Development, Brazil) for the support granted to the accomplishment of this study.

COMPETING INTERESTS

The authors declare no conflict of interest.

FUNDING

The research was funded (scholarship for the last author; equipment and material used in the manufacture of slides) by CNPq (National Council of Scientific and Technological Development) and the State University of Maringá, Brazil.

LEGAL AND ETHICAL ASPECTS

Authors declare that they did not violate or omit ethical or legal norms in this research.
References


Steinmann, V.W.; Ferrucci, M.S.; Maya-Lastra, C.A. Phylogenetics of Serjania (Sapindaceae-Paullinieae), with emphasis on fruit evolution and the description of a new species from Michoacan, Mexico. Systematic and

