Acta Biológica Catarinense
2016 Jul-Dez;3(2):149-54

# Root anatomy of seedlings of *Virola bicuhyba* (Schott) Warb.

## *Anatomia do sistema radicular de plântulas de Virola bicuhyba (Schott) Warb.*

Fernanda **KARSTEDT**[1, 3] & Karin **ESEMANN-QUADROS**[2]

Recebido: 4 set. 2016
Aceito: 19 out. 2016

**ABSTRACT**
*Virola bicuhyba* (Schott) Warb. (Myristicaceae), an arboreal basal angiosperm, had its root system questioned by empirical observations occurred when trees fell by windstorms and which showed no main root. This study aimed to verify the anatomy of the emerging root system of *V. bicuhyba* from the analysis of seedling roots, obtained by seed germination. Material for analysis was fixed, included in paraffin, sectioned, mounted in permanent slides and examined under light microscope. The first root has 16-17 strands of xylem arranged in a circle around a large central region occupied by pith; phloem consists of 16-17 strands of few conductive cells and parenchyma cells between each pole of protoxylem, characterizing a hollow vascular cylinder. In the lateral roots, the xylem have only 5-6 poles of exarch protoxylem, interspersed with 5-6 distinct phloem strands and a central region occupied by metaxylem without pith. Idioblasts containing clearly distinct substance occur associated with the xylem and close to the pericycle. *Virola bicuhyba* has a first root that does not present the typical anatomical pattern of root originated from embryo radicle but similar to the adventitious root. This combination of features is not common, but has been observed in species of Clusiaceae.
**Keywords:** becuiba; Myristicaceae; root anatomy.

**RESUMO**
*Virola bicuhyba* (Schott) Warb. (Myristicaceae), uma angiosperma basal arbórea, teve seu sistema radicular questionado por observações empíricas de árvores derrubadas por vendavais e que não apresentavam uma raiz principal. Este trabalho buscou verificar qual a anatomia do sistema radicular emergente da semente de *V. bicuhyba* pela análise de raízes de plântulas obtidas por meio de germinação. O material para análise foi fixado, incluído em parafina, seccionado e lâminas histológicas permanentes foram montadas e analisadas em microscópio de luz. A primeira raiz apresenta 16-17 cordões de xilema dispostos em círculo ao redor de grande região central ocupada por medula; o floema é formado por 16-17 cordões de poucas células condutoras e células parenquimáticas entre cada polo de protoxilema, caracterizando um cilindro vascular oco. Nas raízes laterais, o xilema tem apenas 5-6 polos de protoxilema exarco, intercalados com 5-6 cordões distintos de floema e região central ocupada por metaxilema, sem medula. Idioblastos contendo substância evidentemente distinta ocorrem associados ao xilema e próximos ao periciclo. *Virola bicuhyba* possui uma primeira raiz sem o padrão anatômico típico de raiz originada da radícula do embrião, mas de raiz adventícia. Essa combinação de características não é comum, mas já foi observada em espécies de Clusiaceae.
**Palavras-chave:** anatomia de raiz; bicuíba; Myristicaceae.

[1] Universidade Regional de Blumenau (Furb), Rua Antônio da Veiga, n. 140, sala T-226, Rua Victor Konder, CEP 89010-971, Blumenau, SC, Brasil.
[2] Universidade da Região de Joinville (Univille), Rua Paulo Malschitzki, n. 10, sala D-119, CEP 89219-710, Joinville, SC, Brasil.
[3] Autor para correspondência: fekarstedt@yahoo.com.br.

## INTRODUCTION

Much research has been done on the anatomy of the stem of tree species, most of which are descriptive; however, few studies have dealt with the root anatomy, particularly its secondary structure (CHALK, 1989; GASSON & CUTLER, 1990; MOURÃO *et al*., 2002). The little economic importance of the root of the trees and the idea that it is anatomically quite similar to the stem contribute to the little interest in its study. In addition, the difficulty in obtaining samples, mainly of roots with secondary growth, is also a limiting factor. The pioneering work of Cutler (1976) and Gasson & Cutler (1990) in this line was motivated by the occurrence of storms, which caused the fall of many trees in England, which facilitated the collection of material for analysis.

*Virola bicuhyba* (Schott.) Warb. (Myristicaceae), also known as *Virola oleifera* (Schott) AC Smith (invalid name, RODRIGUES, 1998), is an arboreal species, up to 35 m in height and diameter at breast height up to 1,05 m, considered one of the tallest trees of the Dense Ombrophylous Forest (rain forest) of southern Brazil (REITZ, 1968). It is endemic to the Atlantic Forest, occurring from Rio Grande do Sul to Bahia (RODRIGUES, 2015), and with its botanical description, reproductive biology and phenology known (REITZ, 1968; CARVALHO, 1994; RODRIGUES, 2002), besides a detailed description of the germination process (MÜLLER *apud* MÖLLER, 1921; CARVALHO, 1994). Since *V. bicuhyba* is a basal angiosperm, the root system is expected to be pivotal (ESAU, 1974; CUTTER, 1986; APPEZZATO-DA-GLÓRIA *et al*., 2012; EVERT & EICHHORN, 2013). However, this fact was questioned when empirical observations showed that trees of this species, knocked down by windrows, did not present a main root (SEVEGNANI, 2004). Thus, this work sought to broaden the knowledge about the anatomical characteristics of the *V. bicuhyba* root system, by analyzing seedling roots.

## MATERIAL AND METHODS

A total of 140 *Virola bicuhyba* seeds were collected from the soil in the São Francisco de Assis Municipal Natural Park in Blumenau (SC), which were cleaned manually with fine tissue in running water to remove the aryl and placed to germinate. After germination, two to five seedlings were collected, when the root was about 1, 3, 5 and 7 cm long, respectively, fixed for 24 hours in FAA (formaldehyde 37-40%, acetic acid, ethyl alcohol 50%, 1: 1: 18, v / v) and preserved in 70% alcohol (JOHANSEN, 1940). The roots and the basal part of the stem were separated from the seedlings and the material was prepared for analysis. Segments of up to 1.0 cm were included in paraffin and sectioned on a rotating microtome. The sections were stained with double staining (1% astra blue and 1% safranin) and mounted on permanent slides with synthetic resin, according to usual microscopy techniques (GERLACH, 1984, modified KRAUS & ARDUIN, 1997). The material was analyzed in a photomicroscope (Zeiss – Axiostar plus) and the photographic record of the anatomical structures was performed with a digital camera (Canon Power Shot G10) coupled to the microscope using Axion Vision 4.8 software.

## RESULTS AND DISCUSSION

The germination of *Virola bicuhyba* followed as described by Fritz Müller (MÖLLER, 1921). The first external sign of germination was the rupture of the outer shell of the seed by the swelling of the endosperm, in the form of a crown of teeth. The root, with a diameter greater than 2 mm and without root hair, broke the thin endosperm layer and, 4-5 mm long, bent down. The hypocotyl was also externalized, greenish and with small pointed nipples, which differentiates it from the root, which is brown and without nipples. After the growth of lateral roots, the hypocotyl began a rapid growth in the form of a loop or hook, typical of epigeal seedlings in the initial stage of development (SOUZA *et al.*, 2009), releasing the seed coat with the cotyledons from the ground and raising them 8-10-10.0 cm or more above the ground.

All the analyzed roots, sectioned in the regions indicated in figure 1a, showed, in cross section, epidermis, hypodermis, cortical parenchyma, endodermis, pericycle, phloem, xylem and medulla (figures 1b-i). The epidermis is one layered, with external periclinal wall covered by cuticle. There is a hypodermic, also one layered, with cells larger than those of the epidermis. The cortical parenchyma presents 10-16 layers of cells, both in the youngest region of the root and in the region closest to the hypocotyl. The endoderm shows *striae* of Caspary and the pericycle is layered.

The first root, which emerged from the germinating seed, does not present the typical root pattern that originates from the embryo's radicle, with a single xylem cord with few poles of protoxylem exarchs and the central region occupied by metaxylem (ESAU, 1974; GABRIELLI, 1992; SCHWEINGRUBER *et al*., 2008, CUTLER *et al*., 2011; EVERT & EICHHORN, 2013), designated a solid vascular cylinder by Apezzato-da-Glória and Hayashi (2012). The first root of *V. bicuhyba* presents 16-17 poles of protoxylem exarchs with metaxylem arranged in a circle around a large central region occupied by pith (medulla); the phloem is formed by 16-17 strands of few conducting cells and parenchyma cells between each protoxylem pole, characterizing a hollow vascular cylinder (APEZZATO-DA-GLORIA & HAYASHI, 2012).

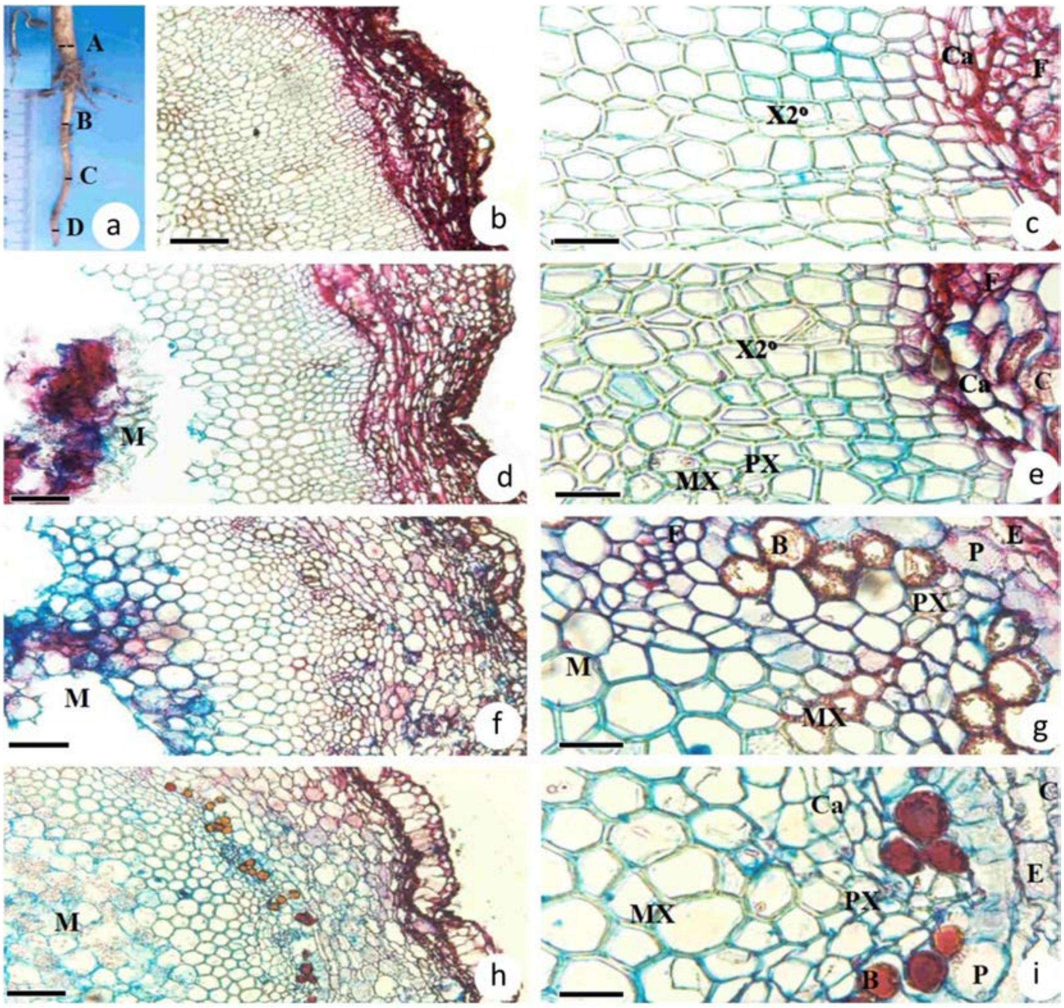


**Figure 1** – **a**: Seedling of *Virola bicuhyba* with indication of the position of the cross sections b-I; **b**: Section of hypocotyl (region A); **c**: Detail; **d-i**: Sections of the first root (regions B, C and D); **d**: Section of the region B; **e**: Detail; **f**: Section of the region C; **g**: Detail; **h**: Section of the region D; **i**: Detail. Structures: **B** – Parenchyma cells with bicuibotanic acid; **C** – Cortex; **Ca** – Cambium; **E** – Endoderm; **F** – Phloem; **M** – Medulla; **MX** – Metaxylem; **P** – Pericycle; **PX** – Protoxylem; **X2º** – Secondary xylem. Bars: **b**, **d**, **f**, **h**: 100 µm; **c**, **e**, **g**, **i**: 25 µm.

The lateral roots of *V. bicuhyba* originate in front of a protoxylem pole, corroborating Guttenberg (1940 *apud* ESAU, 1974), Gabrielli (1992), Appezzato-da-Glória & Hayashi (2012). These lateral roots, originating in front of a protoxylem pole (figure 2a), present the xylem with only 5-6 poles of exarch protoxylem, interspersed with 5-6 distinct phloem strands and metaxylem-occupied central region, without pith (medulla) (figures 2c-d). According to Appezzato-da-Glória & Hayashi (2012), what determines the presence or absence of pith is the number of poles of protoxylem, that is, roots with a few protoxylem poles may not present pith, even if it is present in the root which gave rise to them.

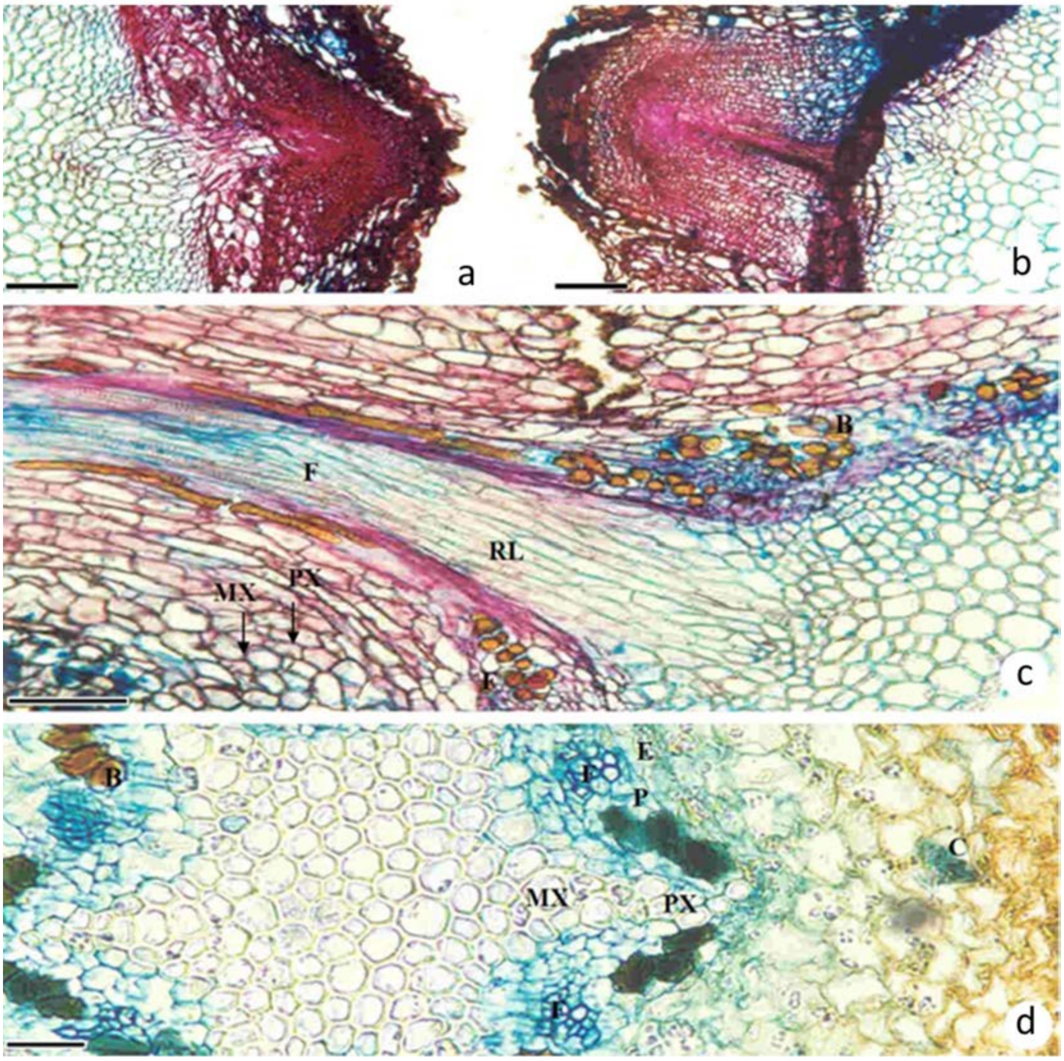


**Figure 2** – Cross sections of the root of *Virola bicuhyba.* **a-c**: First root, showing origin and side root growing (**RL**) in front of a protoxylem pole (**PX**); **d**: Lateral root. **B** – Idioblasts with bicuibotanic acid; **C** – Cortex; **E** – Endoderm; **F** – Phloem; **MX** – Metaxylem; **P** – Pericycle; **PX** – Protoxylem. Bars: **a-b**: 100 µm; **c-d**: 25 µm.

Another relevant aspect observed in these *V. bicuhyba* roots is the presence of evidently distinct substance-containing idioblasts, which are associated with the xylem cord (s) and close to the pericycle (figures 1g-i; figures 2c-d). Pio Correa, cited by Reitz (1968), indicates that this specific substance is myristicico acid, with medicinal properties and that possibly acts as a defense against herbivory.

Thus, it was verified that *Virola bicuhyba* possesses a first root that does not present the typical anatomical pattern of root originated from the radicle of the embryo, but of adventitious root, making the supposition that the whole root system is adventitious. This combination of characteristics is not common, but has already been observed in other *taxa,* such as Clusiaceae, for example, in which the origin of the root system is also not of the radicle of the embryo, as described for *Garcinia* por Souza *et al.* (2009). In *Clusia criuva* the root system is formed by subterranean and aerial roots. Each tree has several aerial roots, which originate from the stem at various heights. Analyzing six seedlings collected in tree sand bank, Esemann-Quadros (2001) observed that the roots originated from the stem; the analysis showed the presence of medulla and eleven poles of protoxylem, on average, in all of them. The main root, originating from the embryo's radicle, was not found in any of the seedlings, and must have degenerated at some early stage of its development. Based on these results, it was verified that the entire root system is adventitious in these seedlings, originated from the basal region of the stem.

A similar situation was also shown by Rezende (1998), in *Caryocar brasiliense* trees (Caryocaraceae). In this last species, the author analyzed the development of seedlings and demonstrated that the main root grows a few centimeters and then its apex undergoes degeneration. The author complements that the lateral roots originate from the remaining part of the main root.

As studies of roots of tree species are scarce in literature, it is likely that situations such as those observed in Clusiaceae and *Caryocar brasiliense* species, are more common than is believed for tree angiosperms, raising the question of whether there are other species that also present this singularity. It is suggested that studies of root development in other woody species should be carried out to prove this hypothesis.

## ACKNOWLEDGEMENTS

The authors thank the PIPe Program / 170 Article – Furb, for Scientific Initiation funding the first author, to Neli Branco de Miranda of Pathological Anatomy Laboratory (Furb) and to Professor Luciano Soares, of Pharmagnosis Laboratory (Univille), for the use of the facilities and equipment.

## REFERÊNCIAS

Appezzato-da-Glória, Beatriz; Adriana Hissae Hayashi & Sandra Maria Carmello-Guerreiro (Eds.). Anatomia vegetal. 3. ed. Viçosa: Editora UFV; 2012. p. 249-261.

Carvalho, Paulo Ernani Ramalho. *Virola oleifera* (Schott) A. C. Smith: bicuíba. In: Carvalho, Paulo Ernani Ramalho. Espécies florestais brasileiras: recomendações silviculturais, potencialidades e uso da madeira. Colombo: Embrapa-CNPF; Brasília: Embrapa-SPI; 1994. p. 518-522.

Chalk, Lawrence. Roots of woody plants. In: Metcalfe, Charles Russel & Lawrence Chalk. Anatomy of the dicotyledons. Wood structure and conclusion of the general introduction. 2. ed. Oxford: Oxford University Press; 1989. v. II.

Cutler, David. F. Variation in root wood anatomy. Leiden Botanical Series. 1976; 3:143-156.

Cutler, David F.; Ted Botha & David Watson Stevenson. Anatomia vegetal: uma abordagem aplicada. Porto Alegre: Artmed; 2011. 304 p.

Cutter, Elizabeth G. Anatomia vegetal. 2. ed. São Paulo: Roca; 1986. 304 p.

Esau, Katherine. Anatomia das plantas com sementes. São Paulo: Blucher/Edusp; 1974. 293 p.

Esemann-Quadros, Karin. Anatomia do lenho de *Clusia criuva* Camb. (Clusiaceae): órgãos, hábitos e ambientes [Tese de Doutorado]. São Paulo: Universidade de São Paulo; 2001.

Evert, Ray Francis & Susan Elizabeth Eichhorn. Biology of plants. 8. ed. New York: W. H. Freeman and Company; 2013. 727 p.

Gabrielli, Antonio Carlos. Contribuição ao estudo anatômico da raiz de *Pyrostegia venusta* (Ker) Miers – Bignoniaceae. Revista Brasileira de Botânica. 1992; 15:95-104.

Gasson, Peter E. & David F. Cutler. Root anatomy of 17 genera growing in the British Isles. IAWA Bulletin. 1990; 11(1):3-46.

Gerlach, Dieter. Botanische Mikrotechnik: eine Einführung. Stuttgart: Georg Thieme Verlag; 1984. 298 p.

Johansen, Donald Alexander. Botanical microtechnique. New York: McGraw-Hill Book; 1940. 523 p.

Kraus, Jane Elizabeth & Marcos Arduin. Manual básico de métodos em morfologia vegetal. Seropédica: Edur; 1997. 198 p.

Möller, Alfred. Fritz Müller – Werke, Briefe und Leben. Jena Gesammenlte Schriften. 1921; (1):1124-1127.

Mourão, Kathia Socorro Mathias; Daniela Dias-Pinto; Luiz Antônio de Souza & Ismar Sebastião Moscheta. Morfo-anatomia da plântula e do tirodendro de *Trichilia catigua* A.Juss., *T. elegans* A. Juss. e *T. pallida* Sw. (Meliaceae). Acta Scientarum. 2002; 24(2):601-610.

Reitz, Raulino. Miristicaceas. Itajaí: Herbário Barbosa Rodrigues; 1968. 15 p.

Rezende, Maria Helena. Anatomia dos órgãos vegetativos, da flor e estruturas secretoras de *Caryocar brasiliense* Camb. (Caryocaraceae) [Tese de Doutorado]. São Paulo: Universidade de São Paulo; 1998.

Rodrigues, Willian Antônio. Myristicaceae. In: Jardim Botânico do Rio de Janeiro. Lista de espécies da flora do Brasil. Rio de Janeiro; 2015 [acesso em: fev. 2015]. Disponível em: http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB10198.

Rodrigues, Willian Antônio. Myristicaceae. In: Wanderley, Maria das Graças Lapa; George John Shepherd; Ana Maria Giulietti (Eds.). Flora fanerogâmica do estado de São Paulo. São Paulo: Fapesp / Hucitec; 2002. v. 2. p. 209-212.

Rodrigues, Willian Antônio. Reabilitação nomenclatural e taxonômica de *Virola bicuhyba* (Schott) Warb. (Myristicaceae). Acta Botanica Brasílica. 1998; 12:249-252.

Schweingruber, Fritz Hans; Annett Börner & Ernst-Detlef Schulze. Atlas of woody plant stems. Berlin Heidelberg: Springer-Verlag; 2008. 229 p.

Sevegnani, Lucia. Raízes de *Virola bicuhyba*. Blumenau, 20 out. 2004. Comunicação pessoal.

Souza, Luiz Antônio; Ismar Sebastião Moschetta; Kathia Socorro Mathias Mourão; Adriana Lenita Meyer Albiero; Daniel Rodrigo Montanher & Adelita Aparecida Sartori Paoli. Morfologia da plântula e do tirodendro. In: Souza, Luiz Antônio (Org.) Sementes e plântulas: germinação, estrutura e adaptação. Ponta Grossa: Toda Palavra; 2009. p. 119-190.