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## Leaf Anatomy of *Xylopia aromatica* (Lam.) Mart. (Annonaceae) occurring in a rocky savannah in the Brazilian Amazonian

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**ABSTRACT.** Savannas of the Amazon Region of Mato Grosso State have an unique ecological identity due to the complexity of the Cerrado-Amazon transition region, their geographical isolation and the physical-chemical properties of the soil. This study aimed to characterize the leaf anatomy of *Xylopia aromatica*, and to identify potential adaptive traits to the xeric environment. We collected adult leaves from *X. aromatica* from a Amazonian savannah located in the Nova Canãa do Norte city, Mato Grosso. The leaves were fixed and stored in 70% ethanol. Cross sections were obtained by free hand with the aid of a razor blade, stained with astra blue and basic fuchsin, and mounted on histological slides. Characters considered adaptive: thick cuticle, epidermal cells with thickened walls, the presence of silica in the epidermis, trichomes, hypostomatic leaves, stomata on the same level as the other epidermal cells, presence of hypodermis, and dorsiventral mesophyll with palisade parenchyma occupying more than 50%. The anatomical characters presented are of great importance for the establishment and development of *X. aromatica* in xeric environments. They contribute to the protection of the leaves from many biotic and abiotic factors to which they are subjected, thus ensuring the species survival in the savannic environment.

**Keywords:** Ecological anatomy; adaptive strategies; environmental conditions; xerophytes.

## Anatomia foliar de *Xylopia aromatica* (Lam.) Mart. (Annonaceae) ocorrente em uma savana rochosa na Amazônia Brasileira

**RESUMO.** A região de savana amazônica do Estado do Mato Grosso possui identidade ecológica própria, explicada pela complexa região de transição Cerrado-Amazônia, pelo isolamento geográfico e pelas propriedades físico-químicas do solo. O objetivo deste estudo foi caracterizar a anatomia foliar de *Xylopia aromatica* (Lam.) Mart, identificando possíveis características adaptativas ao ambiente xerófilo. Foram coletadas folhas adultas de *X. aromatica* em savana amazônica, localizado no município de Nova Canãa do Norte - Mato Grosso. As folhas foram fixadas em FAA<sub>50</sub> e armazenadas em etanol a 70%. Os cortes transversais foram obtidos a mão livre com auxílio de lâmina de barbear, corados com azul de Astra e fucsina básica e montados em lâminas histológicas. Caracteres aqui considerados adaptativos: cutícula espessa, células epidérmicas com paredes espessadas, presença de sílica na epiderme, tricomas, folhas hipoestomáticas, estômatos no mesmo nível das demais células epidérmicas, presença de hipoderme, mesófilo dorsiventral com parênquima paliádico ocupando mais de 50% e lacunoso bem compacto. Os caracteres anatômicos aqui apresentados são de grande importância para as espécies desenvolverem-se em ambientes xerófilos, demonstrando contribuição para a proteção das folhas a diversos fatores bióticos e abióticos aos quais esta se sujeita, garantindo a sobrevivência das espécies no ambiente savânico.

**Palavras-chave:** Anatomia ecológica; estratégias adaptativas; condições ambientais; xerófitas.

### Introduction

Savannas of the Amazon Region of Mato Grosso State have their own unique ecological identity due to the complexity of the Cerrado-Amazon transition region, their geographical isolation and the physical-chemical properties of the soil (Ferreira et al., 2015; Simioni, Eisenlohr, Pessoa, & Silva, 2017). Although distant and isolated geographically, Amazonian savannas share floristic similarities with the Cerrado of the

Brazilian Central Highlands, although with a lower level of endemism, richness and diversity of species (Barbosa, Campos, Pinto, & Fearnside, 2007; Abadia et al., 2018). Despite of the existence of several studies about the origin and the floristic diversity of natural savannas of the Brazilian Amazon (Barbosa & Fearnside, 2005; Miranda, Martins, & Santos, 2007; Barbosa, Campos, Pinto, & Fearnside, 2007; Meneses Costa & Behling, 2013), little is known about the anatomy

of the species that occur in these environments. The anatomical studies of plants have an important value in the context of taxonomy, phylogeny and ecology, since they reveal a combination of essential characters, thus contributing to a better understanding of plant adaptations to a particular environment (Somavilla & Graciano, 2011; Ferreira et al., 2015; Ariano & Silva, 2016; Simioni et al., 2017).

The structure and organization of the leaf blade affects the processes of adjustment and the magnitude of the flow of water and CO<sub>2</sub> through the leaf (Ferreira et al., 2015). Changes in the size and shape of the leaf blade, as well as its level of sclerophylly, are characteristics that are commonly associated with the life history, distribution and resource requirements of plants species (Bieras & Sajo, 2009). Plants which exhibit the strategy of stress-avoidance possess features that enable them to minimize or compensate water loss, or to increase water uptake (Habermann et al., 2011). Indicator traits of the strategy of drought avoidance include deep roots, small or strongly lobulated leaves, small mesophyll cells, thick cell walls and cuticles, multiseriate epidermis, strongly developed palisade parenchyma, stomata sunken or in crypts, and abundant trichomes (Valladares, Gianoli, & Gómez, 2007; Rossatto, Hoffmann, & Franco, 2009; Fang & Xiong, 2015).

Known as “pindaíba” or “pimenta-de-macaco” (monkey pepper), the *Xylopia aromatica* (Lam.) Mart. (Annonaceae) is a native species of Brazil, where it is widely dispersed among the phytophysognomies of Cerrado (sensu lato), Seasonal Deciduous Forests and Amazonian savannas (Marimon-Junior & Haridassan, 2005; Lorenzi, 2008). This species is of commercial, ornamental and medicinal interest, and it can also be used in heterogeneous reforestations with the goal of restoration of permanent preservation areas that are degraded (Lorenzi, 2008; Almeida, Proença, Sano, & Ribeiro, 1998; Socolowski, Cicero, & Vieira, 2012). Although it is widely distributed and occurs in several Cerrado physiognomies, there is a lack of descriptions of its anatomy in Cerrado environments with extremely contrasting conditions in relation to those of the Cerrado of the Central Plateau of Brazil. For this reason, we sought to characterize the leaf anatomy of *Xylopia aromatica* in an Amazonian savanna on rocky outcrops in extreme northern Mato Grosso State, searching for characteristics related to xeric environments and, thus, to interpret possible adaptive strategies.

## Material and methods

### Study area

The chosen species for this study, *X. aromatica*, is part of the arboreal-shrubs stratum of an Amazonian savannah on rock outcrops of the municipality of Nova Canaã in the North of Mato Grosso State (10°53'98.7" S and 55°46'68.7" W). Located in the South Amazon depression (Tarifa, 2011), the vegetation is established on or between the cracks of rocks from sandstone origin, and it is surrounded by dense and open sub mountain Ombrophylous forests.

According to Köppen classification, the region presents a climate type Awi (tropical rainy), with pronounced dry season. The average annual temperature varies between 20°C and 38°C, with an average of 26°C (Souza et al., 2013). The annual precipitation is high, and it could reach 2.000 mm and present well defined seasonality, with a rainy period from October to April, and another of drought between May and September (Souza et al., 2013).

The soil is classified as Litolic Neosol, dystrophic, alic, extremely acid, sandy and with low concentration of nutrients. It also presents high levels of N, P, K<sup>+</sup>, S, Mn, Zn, Na<sup>2+</sup>, interchangeable Al<sup>2+</sup>, potential acidity, CTC, saturation through aluminum, organic matter and sand (Pessoa, unpublished data). As a general rule, the high levels of interchangeable aluminum, potential acidity, the percentage of organic matter and saturation through aluminum in the soil indicate dystrophic soils, with phytotoxic level of interchangeable Al<sup>2+</sup> to plants and with greater availability of macro and micronutrients.

### Material collection and identification of the species

The identification of the species was performed in a floristic and phytosociological survey carried out in the studied area. This step is made up of morphological analysis, consultation with specific literature and experts in the field, and comparisons with the files of the Herbarium of the Southern Amazon (HERBAM) and the herbarium of Nova Xavantina (NX), both belonging to the *Universidade do Estado do Mato Grosso*. The botanical classification was performed based on the APG III (2009) and the name of the species was verified on the database available on the website of the Brazilian Flora (Reflora, 2015). From this identification, we collected the botanical material, that was taken to the Laboratory of Plant Biology of the *Universidade do Estado do Mato Grosso* - UNEMAT, University Campus of Alta Floresta, Mato Grosso States, Brazil, for the realization of the anatomical analysis.

Three full expanded leaves were collected from three different individuals. The samples were fixed in FAA50 for 48 hours and preserved in ethanol 70% (Johansen, 1940).

#### Preparation of the blades

The leaf blades and petioles were sectioned transversely by the free hand. The sections were submitted to clarification in sodium hypochloride and they were washed in distilled water. Subsequently, the leaves were stained in Astra blue and basic fuchsin (Kraus & Arduin, 1997) and mounted on glycerinated gelatin. For the analysis of leaf epidermis, the leaves were dissociated through the method of Jeffrey modified (Kraus & Arduin, 1997), where the leaf portions were placed in test tubes with hydrogen peroxide (Volume 30) and glacial acetic acid in a ratio of 1:1, and then, the portions were kept in an oven at 60°C for about 48 hours. After this period, the samples were washed in distilled water and in ethanol 50%. With the aid of a brush, the two epidermis surfaces were separated, which were stained with basic fuchsin (Roeser, 1962) and mounted on glycerinated gelatin.

The images were obtained by means of the zvr images, coupled with the microscope trinocular photonic Leica ICC50, with the aid of the software LAZ EZ V1.7.0 in the Laboratory of Plant Biology (UNEMAT).

#### Results

Leaves of *X. aromatica* are hypostomatic with paracytic stomata that appear at the same level as the epidermal cells (Figure 1A, B and I). In frontal view, the outline of the walls of the epidermal cells is straight and silica is present in the adaxial face (Figure 1A, B and I).

The epidermis of the midrib is unistratified with thickened cell walls on both faces, and it is covered by a thick cuticle (Figure 1D and E). In the region of the leaf edge, the cuticle is thicker and the epidermal cells have periclinal external walls and anticlinal thickening. (Figure 1J). Pluricellular non-glandular trichomes are present on both faces, and they are uniseriate with acute apices (Figure 1C, D, F and 2B).

Dorsiventrally, the mesophyll comprises three layers of palisade parenchyma and two layers of lacunous parenchyma (Figure 1G, H and J), while

secretory cavities are present between the cells of the palisade parenchyma (Figure 1G and J). The vascular bundles are collateral and have the sclerenchymatic sheath, whose extension reaches both epidermal faces (Figure 1H and J). A layer of hypodermis occurs below the epidermis only on the adaxial face; the hypodermic cells are voluminous and feature thickened cell walls (Figure 1G and H).

In the petiole, the epidermis is unistratified with thickened walls covered by a thick cuticle and many non-glandular trichomes (Figure 2A and B). The cortex possesses parenchyma cells, groups of fibers, secretory cavities and druses (Figure 2C and D). The vascular bundle is composed of three collateral bundles with fibers in the two poles, with the secretory cavities located close to the region of protoxylem (Figure 2C and D).

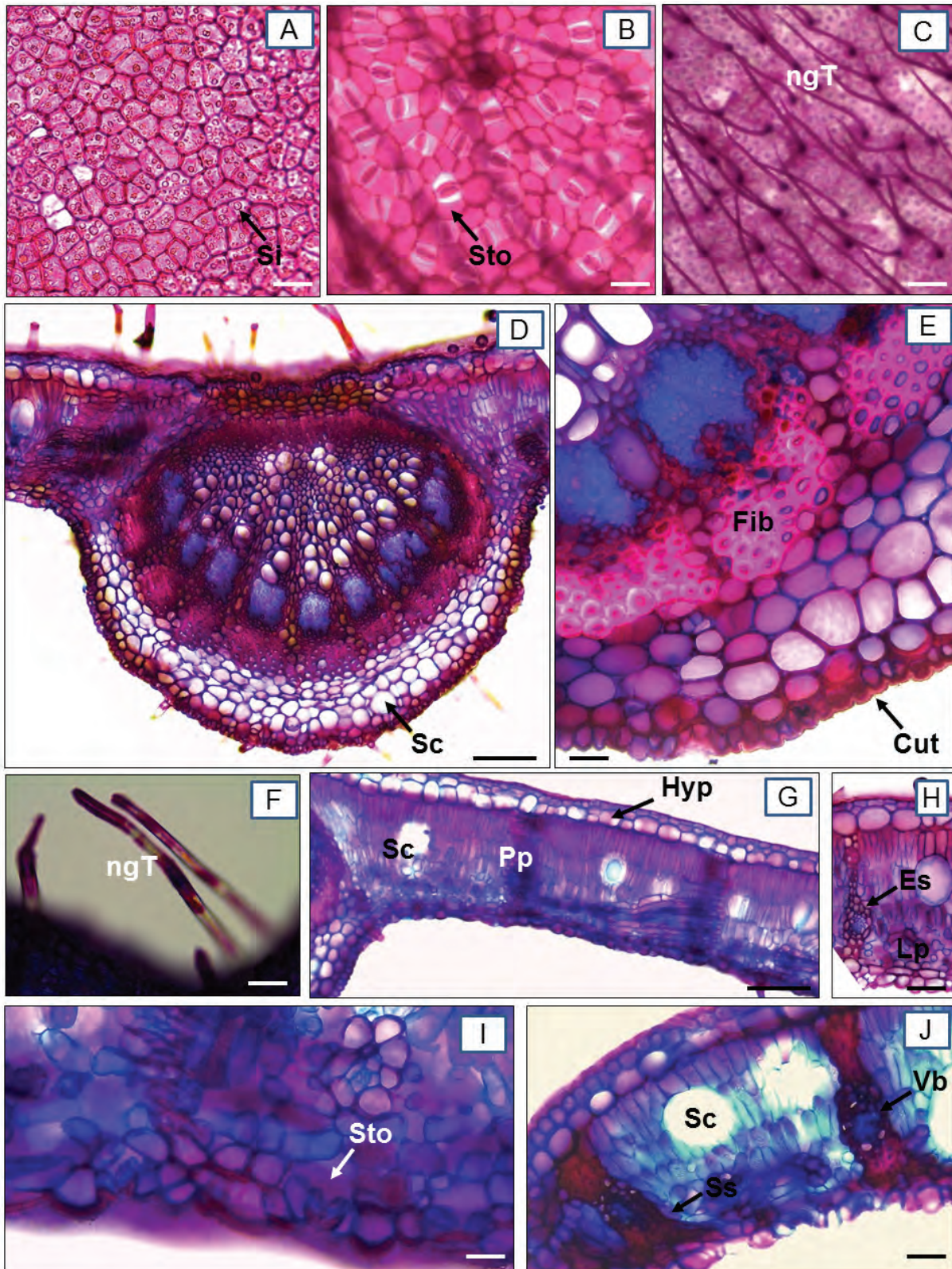
#### Discussion

In the midrib the epidermis is unistratified, with thickened cell walls on both faces and it is covered by a thick cuticle. These characteristics were also found by Bieras and Sajo (2009), Somavilla and Graciano (2011), Ariano and Silva (2016) and Simioni et al. (2017), and are common for leaves of plant species of the Amazonian savannah and Cerrado. According to Fahn and Cutler (1992), thick and lignified epidermal cell walls highlight the presence of mechanical components, further development of palisade parenchyma and projections of the cell walls and the cuticle onto the stomata. As well as the presence of a cuticle, these characteristics are adaptive strategies for xeric environments.

Trichomes are important structures for adaptation to xeric environments since they maintain an atmosphere around the leaf saturated in water vapor (Fahn & Cutler, 1992; Larcher, 2000).

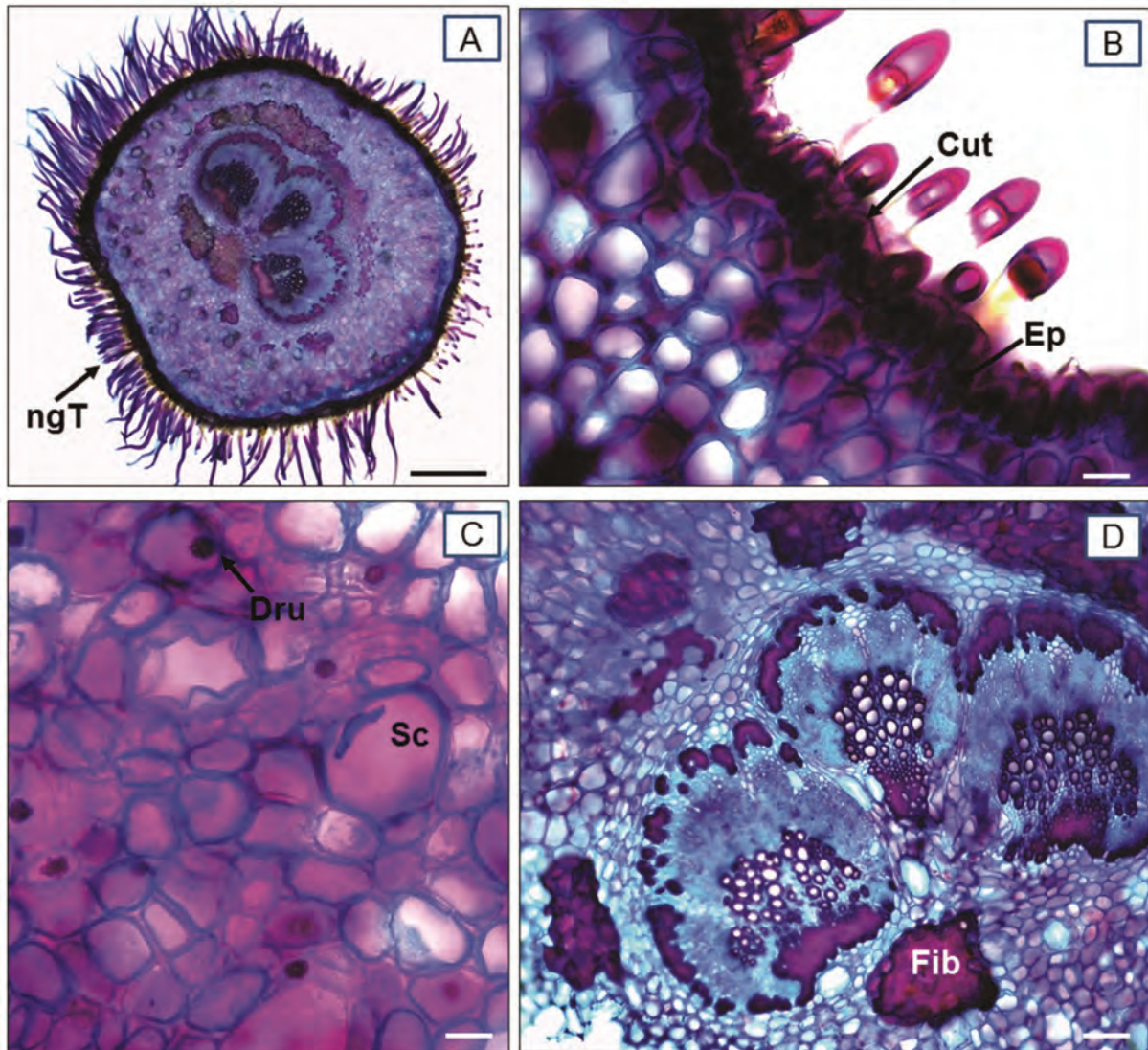
In addition, these structures can also influence, indirectly, in water conservation by plants by means of regulation of temperature and reflection of solar radiation that reaches the leaves (Larcher, 2000). Thus, trichomes may represent a morphological adaptation that favors the maintenance of CO<sub>2</sub> assimilation, because they promote the reduction of leaf temperature during periods of high temperature and low water availability (Ehleringer & Mooney, 1978).





**Figure 1 A-J.** Leaf anatomy of *Xylopia aromatica* in transverse and paradermal sections. A - Frontal view of the adaxial face; B, C - frontal view of the abaxial face with paracytic stomata and trichomes; D, E - midrib with details of cuticle, secretory cavities and fibers; F - non-glandular trichomes; G, H, I - dorsiventral mesophyll; J - detail of leaf edge with vascular bundle and sclerenchymatous sheath. Legend: Ss - sclerenchymatous sheath; Sc - secretory cavities; Cut - cuticle; Sto - stomata; Vb - vascular bundle; Fib - fibers; Hyp - hypodermis; Pp - palisade parenchyma; Lp - lacunous parenchyma; Si - silica; ngT - non-glandular trichomes. Bars: A, B, C, E, F, H, I, J = 40  $\mu$ m; D, G = 150  $\mu$ m.





**Figure 2 A-D.** Anatomy of the petiole of *Xylopia aromatica* in transverse sections A - General appearance of the petiole; B - petiole showing the cuticle and thicker epidermis with trichomes; C - cortex with the presence of secretory canals and collenchyma; D - detail of the position of the vascular bundle with the presence of fibers. Legend: Sc - secretory cavities; Cut - cuticle; Dru - druses; Ep - epidermis; Fib - fibers; ngT - non-glandular trichomes. Bars: A = 150  $\mu\text{m}$ ; B, C, D = 40  $\mu\text{m}$ .

The hypodermis is related to tissue water storage and the restriction of transpiration, thereby performing adaptive functions (Bieras & Sajo, 2009; Araújo, Azevedo, Silva, & Meira, 2010; Ariano & Silva, 2016). Another structural adjustment is the presence of more than one cell layer of palisade parenchyma, which may be related to environments with high luminosity indexes (Rabelo et al. 2013), where these tissues would act to more evenly distribute light to the foliar mesophyll, thereby maximizing the uptake of light by chloroplasts (Volgemann & Martin, 1993). Several authors believe that the number of layers of mesophyll can vary, and may even differ among populations of the same species that occur in

locations with different environmental conditions (Bieras & Sajo, 2009; Rossatto & Kolb, 2010).

*Xylopia aromatica* exhibited a sclerenchymatous sheath extending to the epidermis, which is a common characteristic of plants from savanna-like environments (Ferreira et al., 2015). This characteristic facilitates the filtration and transfer of the incident light, which is enriched mainly with the wavelengths of blue and red light, therefore increasing the availability of light in the photosynthetically active range to the inner layers of mesophyll; this phenomenon is more accentuated in compact and thick leaves (Karabourniotis, Bornman, & Nikolopoulos, 2000).

Ferreira et al. (2015) and Simioni et al. (2017) performed anatomical studies of woody species of savanna areas. The characteristics of a thickened cuticle, presence of trichomes, dorsiventral mesophyll, well developed palisade parenchyma, presence of a hypodermis, extended bundle sheaths, hypostomatic leaves, and stomata at the same level of other epidermal cells were common to the species of these studies. Thus, we can infer that species that occur in Amazonian savannas manifest characteristics that promote its survival in xeric environments.

## Conclusion

The anatomical characters presented here are of great importance for the establishment and development of *X. aromatica*, and other species, in xeric environments by contributing to the protection of leaves from the various biotic and abiotic factors to which they are subjected, thus ensuring the survival of the species in the savannic environment.

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