

Leaf anatomy of *Qualea parviflora* (Vochysiaceae) in three phytophysiognomies of the Mato Grosso State, Brazil

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ABSTRACT

Leaves have a variety of morphological and anatomical characters mainly influenced by climatic, edaphic and biotic factors. The aim of this study was to describe the anatomical leaf traits of *Qualea parviflora* from three phytophysiognomies. The studied phytophysiognomies were Amazon Savannah on rocky outcrops (ASR), Transition Rupestrian Cerrado (TRC), and Cerradão (CDA). Freehand sections of the leaf blade were made and stained with 0.5% astra blue and with basic fuchsin. From the adaxial and abaxial leaf surface, freehand paradermal sections were made for epidermis analysis. The Jeffrey's method, with modifications, was used in the epidermis dissociation process. The samples from the TRC phytophysiognomy had relatively smaller ordinary epidermal cells, higher abundance of trichomes, and mesophyll with few intercellular spaces, in comparison to the other phytophysiognomies. The leaves from the ASR phytophysiognomy had higher stomatal index (SI = 21.02), and five to six layers of sclerenchyma surrounding the midrib vascular bundle. The secondary vascular bundles had thicker cell walls and the bundle sheath extended up to the epidermal tissue of both leaf sides. Leaves from the CDA phytophysiognomy had mesomorphic environmental traits, such as a thinner cuticle. It is concluded that trees from ASR and TRC phytophysiognomies have xeromorphic traits following the environmental conditions where they occur.

KEYWORDS: Adaptations, Amazon, Cerrado, Environmental conditions.

Anatomia foliar de *Qualea parviflora* (Vochysiaceae) em três fitofisionomias do Estado Mato Grosso, Brasil

RESUMO

As folhas são órgãos vegetativos que expressam uma variedade de características morfológicas e anatômicas influenciadas, principalmente, por fatores climáticos, edáficos e bióticos. O presente estudo objetivou levantar as características anatômicas das folhas de *Qualea parviflora* Mart. presente em três fitofisionomias: Savana Amazônica sobre afloramentos rochosos (SAR), Cerrado Rupestre de Transição (CRT) e Cerradão (CDA). Os cortes anatômicos foram realizados a mão livre e corados com azul de astra e fucsina básica 0,5%. Para a dissociação das epidermes, foi utilizado o método de Jeffrey modificado. As amostras da fitofisionomia de CRT apresentaram células epidérmicas comuns relativamente menores, maior quantidade de tricomas e mesofilo com poucos espaços intercelulares em relação as demais fitofisionomias. As amostras da fitofisionomia de SAR apresentaram maior índice estomático (IE: 21,05), maior quantidade de esclerênquima envolvendo o feixe vascular da nervura central (5 a 6 camadas), feixes vasculares secundários com células de paredes mais espessadas, com bainha que se estende até as células epidérmicas em ambas as faces. As amostras da fitofisionomia de CDA apresentaram caracteres de ambientes mesomórficos, como cutícula mais delgada. Pôde-se concluir que os espécimes que ocorrem em SAR e CRT apresentaram características xeromórficas que estão relacionados ao ambiente de ocorrência.

PALAVRAS-CHAVE: Adaptações, Amazônia, Cerrado, Condições Ambientais.

INTRODUCTION

Mato Grosso State (Brazil) has an extensive biogeographical area, with large diversity of plant species distributed among three biomes, namely: Amazon, Cerrado (Brazilian savannah), and Pantanal (Bonini *et al.* 2013). The Cerrado has different phytophysiognomies, and very few studies about its ecological plant anatomy have been found, such as those conducted by Somavilla and Ribeiro (2011), and Bieras (2009), who suggest the adaptive strategies adopted by plant species typical of savannah environments.

Despite the floristic and phytosociological information on plant diversity (Nunes-Freitas 2004), morphological and anatomical data may also show the diversity patterns found in a given ecosystem and their adaptive potential (Barros *et al.* 2006). Thus, the Cerrado may be considered to be quite diverse, because its species evolved under different selective pressures (Fowler and Duarte 1991), by adapting themselves to environments containing acidic, dystrophic soils, and prolonged dry periods (Castro *et al.* 1999).

The adaptive features are still little known in the three Cerrado phytophysiognomies (Coutinho 2002), namely: Rupestrian Cerrado, Amazonian Savannah and Cerradão. The Transition Rupestrian Cerrado (TRC) presents high endemic-degree flora (Alves and Kolbek 2010) and several endangered species (Mendonça and Lins 2000), which may occur in continuous landscapes but generally along with other mosaic plant formations (Ribeiro and Walter 2008).

The Amazonian Savannah (ASR) is an enclave occurring on soils with rocky outcrops (Zappi *et al.* 2011). These areas have sites where it is possible to see the formation of ecosystems typical of Cerrado. However, they are embedded within an entirely different nature domain and show peculiar floristic elements from the Amazon Biome (Bernasconi *et al.* 2009). Nonetheless, the Amazonian Savannah area on rocky outcrops has been threatened by the nearby exploratory activities, such as hydroelectric plants.

The Cerradão (CDA), which is one of the herein studied areas, corresponds to a more closed and shaded forest vegetation. It occurs both on dystrophic and mesotrophic soils, and its varying flora composition is related to soil fertility (Araujo and Haridasan 1988).

Ratter *et al.* (1996) analyzed the floristic composition of 98 Cerrado and Amazonian Savannah areas and found *Qualea parviflora* Mart. (Vochysiaceae: Myrtales) in 71% and 60% of the studied areas, respectively. The authors discussed the great importance of *Q. parviflora* to these biomes. This importance was confirmed by the phytosociological survey conducted in Cerrado phytophysiognomies in Nova Xavantina County (Marimon *et al.* 1998).

The leaf anatomy is an important tool that helps understanding the species' adaptation to the environment a plant lives in. According to Metcalfe and Chalk (1983), leaf growth and organization are highly influenced by environmental factors such as temperature, light intensity and water availability. These factors may just influence the phenotypic variation in the short term. However, in the long term, they may also work in the evolution selection process, by showing favoritism towards the anatomical features that enable higher fitness to plants. The aims of the current study were to describe the anatomical features of *Qualea parviflora* leaves and to relate the possible differences in the anatomical features and in the environmental conditions of each region where the species occurs.

MATERIALS AND METHODS

Qualea parviflora Mart. leaves were collected in three different phytophysiognomies in Mato Grosso State, namely: Transition Rupestrian Cerrado (13°00'09.8"S and 51°45'12.5"W), Cerradão (14°41'00"S and 52°20'00"W), and Amazonian Savannah on rocky outcrops (10°53,987"S and 55°46,687"W). Transition Rupestrian Cerrado is one of the phytophysiognomies found in the Cerrado biome. It is an area of rocky outcrops located in a transition region between two biomes - Cerrado and Amazon - in Ribeirão Cascalheira County-MT. Cerradão is a sedimentary soil area predominant in typical Cerrado, and it is located in Nova Xavantina County-MT. The Amazonian Savannah is a savannah area in the Amazon biome, in Nova Canaã do Norte County-MT.

Twelve plants were collected from each phytophysiognomy and their leaves were stored in the Southern Amazon Herbarium (HERBAM) at UNEMAT / Campus Alta Floresta, in Nova Xavantina Herbarium, UNEMAT / Campus Nova Xavantina, and in the Plant Biology Laboratory / Campus Alta Floresta / MT. After collection, the material was fixed in FAA₅₀ and transferred to 70% ethanol after 48 hours, for anatomical analysis (Johansen 1940). Freehand cross sections were made in the median region of the leaves using razor blades, and they were stained with 0.5% astra blue and with basic fuchsin (Kraus *et al.* 1998).

Freehand paradermal sections were made in the adaxial and abaxial leaf surface using razor blades, for epidermis analysis. Jeffrey's dissociation method (Kraus and Arduin 1997) - with modifications - was used and the leaf portions were placed in test tubes containing hydrogen peroxide (30 volumes) and glacial acetic acid at the ratio of 1:1. The portions were kept at 60 °C for approximately 48 hours. After this period, the samples were washed in distilled water and in 50% alcohol. The two epidermal surfaces were separated using paintbrushes and they were stained with 0.5% basic fuchsin

(Roeser 1962) and mounted in Haupt's jelly glycerin (Kraus and Arduin 1997).

The images were obtained through image capture equipment coupled to a light microscope (Leica Microsystems, Wetzlar, Germany), and through Leica IM50 software at the Plant Biology Laboratory (UNEMAT). Micrographs were generated to show the specimens' anatomical pattern in each region.

The stomata were counted and the stomatal index (SI) was calculated in the Anati Quant 2° UFV software (Aguiar *et al.* 2007). Three individuals from each phytogeography were analyzed; four sections per individual, thus totaling 12 sections. One-way multivariate variance analysis (MANOVA) was used, and it was followed by Tukey's test at 0.05% significance level, in order to evaluate which anatomical characters differed in each phytogeography.

RESULTS

According to the frontal view, the *Qualea parviflora* leaves in the three phytogeographies (Figure 1A-C) have straight anticlinal walls on both sides of the ordinary epidermal cells. The leaves of the Transition Rupestrian Cerrado (TRC) specimens have larger epidermal cells and thicker cell walls than the leaves from the Amazonian Savannah on rocky outcrops (ASR) and from Cerradão (CDA), which present relatively smaller cells.

The cross sections allowed us to observe that the leaf blade (Figure 3A-E) is covered by a smooth and thin cuticle and that it is especially thicker on the adaxial surface. Regarding the CDA samples (Figure 3A, 3D), the cuticle is even thinner than that found in samples from the other collection sites. The epidermis is always uniseriate and presents thickened and lignified cell walls. Stomatal cristae are covered by the cuticle (Figure 3G-I).

The leaves are hypostomatic (Figure 1D-E) and present kidney-shaped guard cells. The leaves collected in the CDA

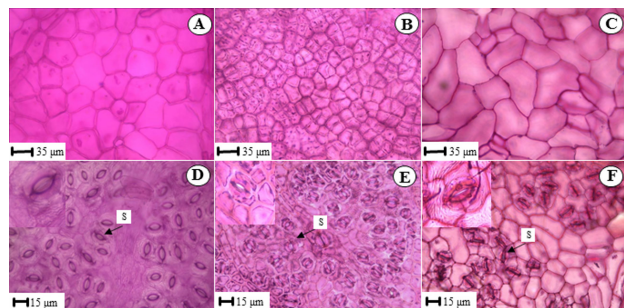


Figure 1. Frontal view of the adaxial and abaxial surfaces of *Qualea parviflora* Mart. leaves from the Amazonian Savannah on rocky outcrops (A and D, respectively), Transition Rupestrian Cerrado (B and E, respectively), and from Cerradão (C and F, respectively). (S-stomata). This figure is in color in the electronic version.

have more elongated guard cells than the leaves collected in the other phytogeographies. The stomata are anomocytic in all samples, but anisocytic stomata may also be found in the CDA. Both are at the same level or slightly above the other epidermal cells, and they are heterogeneously distributed in higher amounts in the ASR (stomatal index (SI) = 21.02) (Figure 1D), and in smaller amounts in TRC (SI = 17.43), as well as in the CDA specimens (SI = 19.59).

The non-glandular trichomes of the midrib (Figure 2A, 2C, 2E) are distinct in the three phytogeographies. They are just found on the adaxial surface and in lesser amount in the

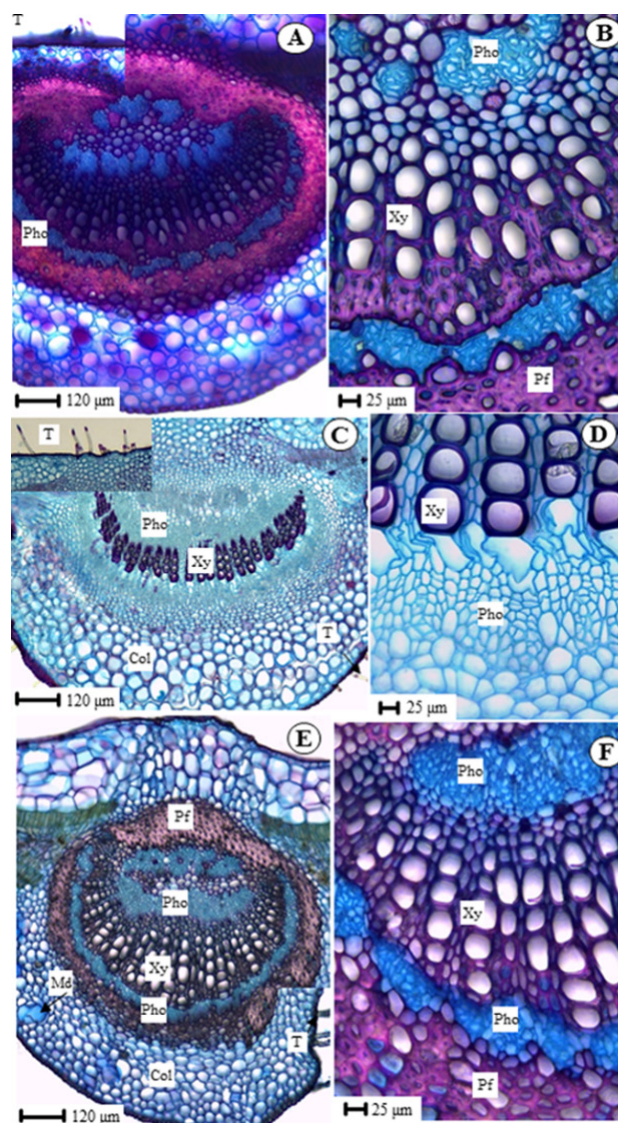


Figure 2. Midrib of *Qualea parviflora* Mart. leaf from Amazonian Savannah on rocky outcrops (A and B), Transition Rupestrian Cerrado (C and D), and from Cerradão (E and F). (Col-Colenchyma, Xy-xylem, Pho-phloem, Pf-Percyclic fibers, T-trichomes and Md-mucilage ducts). This figure is in color in the electronic version.

ASR samples. They are also found on both sides of the RCT samples and just on the abaxial surface of the CDA samples.

A lacunar collenchyma may also be observed in the midrib (Figure 2A-E), near the epidermis, on both sides, with stronger presence in the TRC samples. Some secretory canals are found between the collenchyma cells in the CDA samples (Figure 2E), differently from the ASR and the TRC phytogeographies' samples.

A larger amount of sclerenchyma is found in the midrib vascular bundle of the ASR samples (from 5 to 6 layers) (Figure 2A) in comparison to those of the TRC (from 3 to 4 layers) and the CDA samples. The midrib vascular system has bicollateral arrangement (Figure 2A, 2C, 2E). The phloem almost completely covers the xylem, with interruptions seen in the samples from the ASR and CDA phytogeographies; the xylem is completely surrounded by the phloem in the TRC samples (Figure 2C).

The leaf blade mesophyll is dorsiventral (Figure 3A-E). There are two to three hypodermis layers under the adaxial epidermis and above the palisade parenchyma, and large cells are found on the adaxial surface. The palisade parenchyma is just found near the adaxial surface and it consists of one or two cell layers. The spongy parenchyma presents three to four layers and few intercellular spaces, especially in the samples from the TRC phytogeography (Figures 3B, 3E).

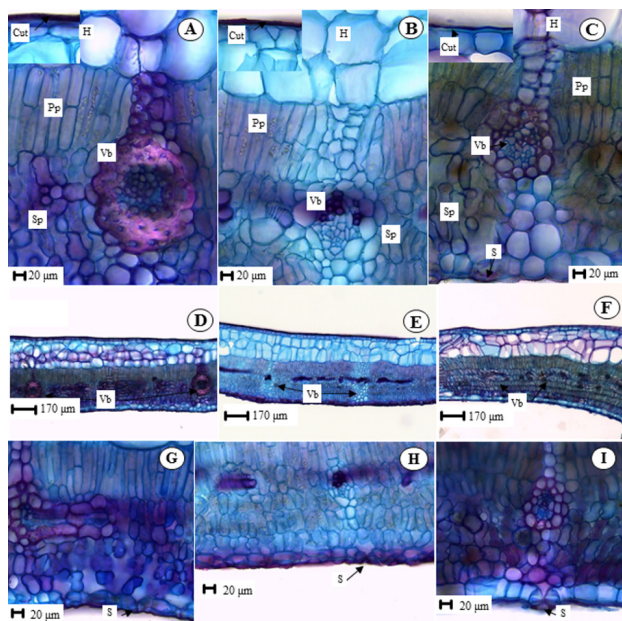


Figure 3. *Qualea parviflora* Mart. leaf mesophyll from Amazonian Savannah on rocky outcrops (A, D, and G), Transition Rupestris Cerrado (B, E, and H) and from Cerradão (C, F, and I). (Cut-cuticle, Vb-vascular bundles, Pp-palisade parenchyma, Sp-spongy parenchyma, S-stomata, and H-hypodermis). This figure is in color in the electronic version.

The vascular bundles are collateral (Figure 3G, 3H) and the pericycle presents thickened cell wall in the ASR samples in comparison to those of the RCT and CDA samples. The sheath extends up to the epidermal cells on both leaf surfaces. There is a single series of vascular bundles alternating between larger and smaller bundles along the blade.

DISCUSSION

Clear differences were observed among the three phytogeographies during the analysis. The ASR phytogeography presented higher amount of sclerenchyma in the midrib, and thickened pericycle in the vascular bundles. The TRC phytogeography samples had trichomes on both sides, thicker cuticle, and mesophyll with few intercellular spaces. Such features are consistent with xeromorphic environments. However, the plants of the CDA phytogeography had mesomorphic environment features, such as thinner cuticle.

Leaves are plant organs responsible for capturing solar energy and for performing gas exchange, as well as photosynthesis. Their anatomical variations may be interpreted as an adaptation strategy to the different environments where plants occur (Sakita and Kolb 2014). The diversity of anatomical and morphological features results from several adaptations leaves may present in response to climatic, edaphic and biotic factors (Esau 1976; Givnish 1984).

The leaf anatomy is highly specialized for light absorption (Castro *et al.* 2007). The vegetative adaptations of organs or structures prevent or reduce water loss, which is common in plants from xeric environments or in those living on rocks (Aoyama and Mazzoni-Viveiros 2006).

It is believed that these distinctions in the size of the epidermal cells and cell walls of plants from the Transition Rupestris Cerrado (TRC) phytogeography are possibly related to the species' specific ability to adapt to the environment. Since TRC has lower rainfall than the other studied regions and since it stands on rocky outcrops, these species probably have thicker cell walls, which are responsible for water storage in longer drought periods, thus preventing water loss.

According to Castro *et al.* (2007), the adaptations of common and specialized epidermal cells are fundamental to the plant adaptation to different environmental conditions. This adaptation process occurs through the optimization of the gas exchange between water loss - through transpiration - and CO₂ absorption, which is necessary for photosynthesis. Several studies, such as those conducted by Bieras and Sajo (2009) and Rossatto (2011), have demonstrated that the epidermis modification is distinct in different species, especially regarding changes in radiation levels.

The stomata are extremely important in anatomical studies involving different environments and radiation levels, since the increased stomatal frequency in leaves exposed to high irradiance may be an important adaptive mechanisms in drier environments (Abrams and Mostoller 1995). *Qualea parviflora* has large stomata and they are distributed in higher amounts in the ASR samples. The leaf takes advantage of the limited time of high relative humidity in order to perform gas exchanges under xeric conditions (Medri and Lleras 1980), which might be more efficient the higher the useful stomatal area is. Stomatal movement, density, distribution, and size are species-specific features that might change in response to environmental conditions (Camargo and Marengo 2011; Marengo *et al.* 2014). Thus, *Q. parviflora* presented higher amounts of stomata in the ASR samples as a strategy to increase the photosynthetic rate in higher relative humidity periods, since this phytophysognomy has open vegetation with abundant sunlight. A distinct strategy may be observed in the TRC phytophysognomy, where plants have less stomata, but more trichomes.

The distinct location of non-glandular trichomes on *Q. parviflora* leaves surfaces in the three phytophysognomies might be related to the species adaptation. The plants of the TRC phytophysognomy had the highest amount of trichomes, which is probably a phenotypic plasticity response that helps protecting the leaves and avoids excessive water loss during the dry periods. Cutler and Fanh (1992) mention that the non-glandular trichomes are important structures to plant adaptation to xeric environments, because they keep the atmosphere around the leaf saturated with water vapor.

The fact that only the *Q. parviflora* leaves from the TRC samples present trichomes on both sides might be related to the typical features of the region, such as lower rainfall and intense luminosity, since TRC is an open area where there is high solar incidence throughout the year. It corroborates Lin and Ehleringer (1983), who stated that the non-glandular trichomes work in light reflection. Additionally, Sandquist and Ehleringer (1997) state that the non-glandular trichomes reduce the transpiration rate, either by the increase in solar radiation reflection, which reduces the temperature, or by the thickness of the air layer trapped above the leaf, which works as barrier to water loss. Manetas (2003) also proved that the trichomes might protect the tissues against damages caused by UV-B rays.

Differently from the plants of the ASR and TRC phytophysognomies - which are open areas exposed to more extreme environmental conditions, the thin cuticle found in the cross section of the CDA samples may be interpreted as an adaptation to Cerradão, a forest area with good shading rates. The cuticle is a hydrophobic structure responsible for cuticular transpiration reduction and for nutrient leaching. It

also protects the leaf from the wind and from fungal invasion (Turner 1994); it gets thicker as the ambient light intensity increases, as it was noticed by Bieras and Sajo (2009) in their anatomical studies on Cerrado species. A study performed by Martinez and Medri (1985) found that the cuticle is thicker in the leaves located at the upper extracts, in which they are more exposed to light.

The fact that the mesophyll has few intercellular spaces, especially in the TRC phytophysognomy samples, points towards a typical aspect of xeromorphic plants. The apoplast decrease hinders the water vapor dissipation, thus leading the species to better adapt to it (Cutler and Fahn 1992).

The environmental factors affect the dimensions and even the arrangement of the vascular elements (Alves and Angyalossy-Alfonso 2000). Thus, the high amount of pericyclic fibers in the *Q. parviflora* midrib vascular system and the vascular bundles with thickened pericycle in the ASR sample may be working as adaptation strategies to long drought periods by performing a mechanical leaf protection. Leaves with more numerous fibers and thicker walls in the phloem poles are commonly found in Cerrado (Bieras and Sajo 2009). According to Rudall (1986), these are considered to be xeromorphic features.

Rossatto and Kolb (2012) also observed clear anatomical distinctions among Cerrado phytophysognomies, such as different leaf traits to cope with the environmental changes plants are subjected to. Similarly, the herein found anatomical feature distinctions are very important because they show that the species anatomical differentiation is deeply linked to the abiotic conditions of different environments. It demonstrates plasticity and helps protecting the leaves against the several biotic and abiotic factors they are subjected to. Therefore, it ensures the species survival.

CONCLUSION

The *Qualea parviflora* leaf anatomy presents typical xeromorphic structures that provide leaf mechanical protection for the species adaptation in its natural environment. The ASR and TRC phytophysognomies present xeric features evidenced by the herein observed characters, namely: trichomes on both sides, thicker cuticle, mesophyll with few intercellular spaces (TRC phytophysognomy), as well as large amounts of sclerenchyma tissues in the central vein, and thickened pericycle in the vascular bundles (ASR phytophysognomy), which protect the leaves from the abiotic and biotic factors found in the environment. The CDA phytophysognomy had mesomorphic environment features and thinner cuticle.

ACKNOWLEDGMENTS

The authors are grateful to State University of Mato Grosso (Universidade do Estado de Mato Grosso), Campus Alta Floresta, and especially to the Post-Graduation Program in Environmental Sciences (Programa de Pós graduação em Ciências Ambientais) (PPGBioagro) and to the Plant Biology Laboratory (Laboratório de Biologia Vegetal). We also thank the Biologist Marcos José Gomes Person, for all the support he gave us, especially in the species collection in the three phytogeographies. The authors acknowledge Glenn Hawes, M.Ed. English, the University of Georgia, USA, for editing this manuscript.

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Recebido em 15/10/2015

Aceito em 20/11/2015

