

Morphoanatomical characterization of *Brassavola tuberculata* Hook's (Orchidaceae) leaf and root upon the rocky outcrop

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Abstract

Orchids have morphological and anatomical adaptations that make them able to occupy the most different environments with the most varied conditions. These adaptations, together with the physiological attributes are essential for the establishment, growth, reproduction, and colonization of orchids. Therefore, this study was developed to describe the leaf and root anatomy of *Brassavola tuberculata* Hook found in a phorophyte located on a rocky outcrop in Campestre, southern Minas Gerais. The collected leaf and root samples were freehand cut and stained with astra blue and safranin. The histochemical tests applied were: sudan III for lipids and lugol for starch. The leaves are subulate, succulent and grooved containing a continuous longitudinal cannula. The epidermis has a thick cuticle, stomata with a suprastomatal chamber on both leaf faces and the mesophyll is homogeneous with aquifer parenchyma in the central portion. In the root, the velamen and cortical tissue are composed of several cell layers that allow it to store water. There were starch grains in the leaf and root. These xeromorphic characters found allow to the species to survive in the restricted conditions found in the phorophyte on rocky outcrops.

Keywords: Plant anatomy. Ecological Anatomy. Leaf anatomy. Root anatomy. Orchid.

Introduction

The Orchidaceae family is home to 7 % of the planet's flora species, with 26,454 species cataloged and distributed in 735 genera (CHASE *et al.*, 2015). In Brazil, the family is represented by 217 genera and 2,443 species, of which more than 60 % are endemic (FLORA DO BRASIL, 2021). The species of this family are distributed in almost all regions of the planet with the exception of the polar and desert regions, concentrated in humid forests within tropical and subtropical regions, mainly in Asian and American tropical forests (DRESSLER, 2005). They have terrestrial, rupicolous, epiphytic, or saprophytic habits (MATTIUZ; RODRIGUES; MATTIUZ, 2006).

The family is recognized for its flowers that display a uniform arrangement along their floral whorls among most species. However, they vary in

terms of size, shape, and color (PABST; DUNGS, 1975). In addition, they exhibit highly specialized characteristics that serve to attract insects and favor cross-pollination (DRESSLER, 1993). Unlike flowers, the organization of its vegetative parts is notoriously diverse (DRESSLER, 1993), favoring the variety of growth forms (PABST; DUNGS, 1975) and providing ample adaptive power for different environments to this family, mainly aerial (BENZING *et al.*, 1982).

All plant species basically use the same resources: light, carbon dioxide (CO₂), water, and nutrients. Therefore, ecological differences between species arise from different ways of acquiring and using the same resources (WESTOBY *et al.*, 2002). This difference is only possible due to the functional traits that are anatomical determined and physiological characteristics. They can command the organism's responses to the environment (STERCK *et al.*,

2011). Safe to say, the considerable richness of species and wide distribution find explanations in the various specialized strategies that these plants have developed.

The genera *Maxillaria* (FOLDATS, 1970), *Vanda*, and *Epidendrum* (OLIVEIRA; SAJO, 2001), for example, developed pseudobulbs, which are water and carbohydrate storage organs and are generally associated with highly developed floral organs. The genus *Pleurothallis* (OLIVEIRA; SAJO, 1999) does not have pseudobulbs and its leaves are responsible for water and nutrient storage functions. In addition to these reserve structures, orchids have other adaptations to xeric environments such as the velamen which has the ability to absorb water and nutrients, reduce transpiration, and provide mechanical protection (PRIDGEON, 1986). They have associations with endomycorrhizal fungi and Crassulacean Acid Metabolism (CAM) (OLIVEIRA; SAJO, 2001). Such attributes favor survival in limiting conditions (NOGUERA-SAVELLI; JÁUREGUI, 2012).

The genus *Brassavola*, originated in America, is among the main ones in the Orchidaceae family. Its distribution occurs from northern Mexico to northern Argentina (NOGUERA-SAVELLI; JÁUREGUI, 2011). This genus is characterized by having epiphytic plants, cylindrical stems (FIGURE 1B) with a single subulate succulent leaf and inflorescences, and racemose with one to three flowers. Most plants have similar aspects in their vegetative and floral characteristics which has caused conflicts in the delimitation of species and in the proposed classification systems (NOGUERA-SAVELLI; JÁUREGUI, 2011).

The *Brassavola tuberculata* Hook species, popularly known as “silver rain, chives, mouse tail, and lady-of-the-night orchid” has abundant and lasting blooms (June to September). Thus, have a high ornamental value. Its inflorescences of five to seven flowers are white, slightly yellow (FIGURE 1C), and exude a slightly sweet odor at

dusk (RECH; ROSA; MANENTE-BALESTIERE, 2010). This plant has epiphytic or rupicolous habits and a wide geographic distribution occurring in the five regions of Brazil's geographic division. We find them in the phytogeographic domains of the Caatinga, Cerrado, and Atlantic Forest and are components of the Cerrado vegetation (lato sensu), Riparian Forest, Semideciduous Seasonal Forest, Ombrophilous Forest, Restinga and of the Rocky Outcrops vegetation (FLORA DO BRASIL, 2021).

Rock outcrops, also known as inselberg (“rock island”) are territorial fragments that present geographic isolation, peculiar soil and climate conditions, and a relative degree of specific endemism (POREMBSKI; BARTHLOTT, 2000). Rocky outcrops are frequent in Brazil (ESGARIO *et al.*, 2008) and have severe environmental characteristics, such as poor soil, high insolation, and large temperature fluctuations between day and night (POREMBSKI *et al.*, 1998). Low water and nutrients retention, few alternatives for fixing seeds, propagules, or roots of adult plants tend to accentuate exposure to wind, light, and heat (LARSON *et al.*, 2005). This set of environmental characteristics makes the present flora within the peculiar and distinct environment surroundings (LIMA, 2018).

The anatomical characterization of plants acquired great importance for taxonomy over time (NOGUERA-SAVELLI; JÁUREGUI, 2012). Along with morphological and/or molecular characterization, it provides a better basis for unraveling phylogenetic relationships and understanding how environmental factors influence the distribution and functioning of plants (BENZING, 2000).

It is known that environmental factors can influence the morphology, anatomy, and physiology of plants. Thus, anatomical and ecophysiological studies contribute to understanding the dynamics of species and the strategies they present that enable them to survive in certain environments. Such information

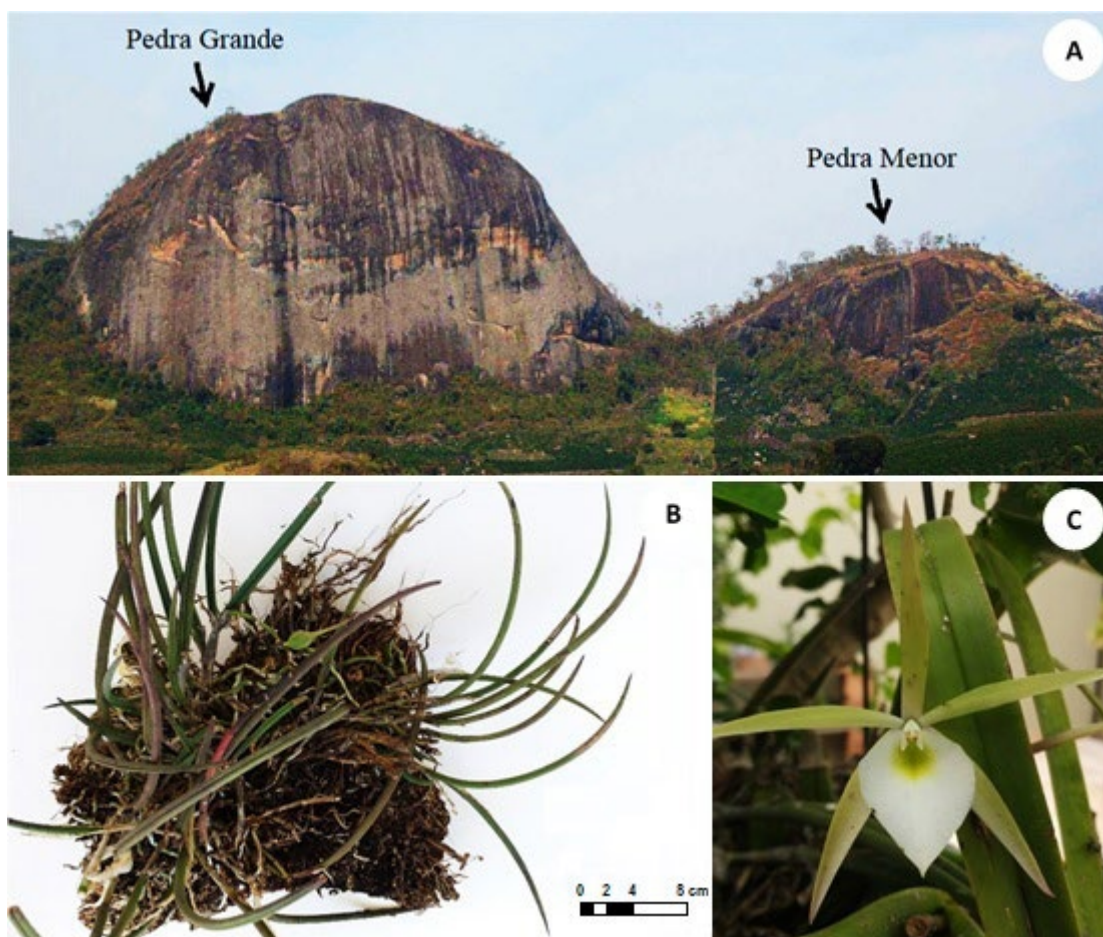
is of great importance for the conservation of plant biodiversity as it allows the expansion of knowledge about the mechanisms adopted by the species, in the face of environmental changes increasing the possibilities of preserving them. Therefore, this developed work has the objective of describing the leaf and root anatomy of *Brassavola tuberculata* Hook found in a rocky outcrop of Serra da Pedra Grande in Campestre-MG serving as a basis for future ecophysiological studies on the adaptive strategies of this species.

Material and methods

We collected leaf and root samples of the *Brassavola tuberculata* Hook species belonging

to the Orchidaceae family. The samples were obtained from a phorophyte located on the rocky outcrop called Pedra Menor (FIGURE 1A). A component of the small mountain range, Serra da Pedra Grande, which is located on the banks of the Vital Brasil/BR-267 highway, 15 km from the municipality of Campestre that flows towards the municipality of Machado, in the south of Minas Gerais, Brazil. The region is part of the Atlantic Forest biome (IBGE, 2004) and the local landscape is predominantly agricultural and severely fragmented, with only 9 % covering the original forest and 51 % of pastures (OLIVETTI, 2015). The vegetation in the region is classified as Montana Seasonal Semideciduous Forest (IBGE, 2004).

Figure 1. Characterization of the studied area and species. **A.** Work area, Pedra Menor, seen from the side of the Vital Brasil highway; **B.** Leaves and roots on periderm of the phorophyte of *Brassavola tuberculata* Hook and **C.** Flower of *Brassavola tuberculata* Hook.



Source: Prepared by the authors (2021).

After collection, the leaf blade samples were subjected to measuring the length with the aid of a ruler using a caliper. The smallest and largest diameters of the regions of the base, middle third, and 5 cm from the apex were measured. After the measurements, the slides were mounted to perform the anatomical analyzes of the leaf and root. All procedures were performed at the Biotechnology Laboratory of the Federal Institute of the South of Minas Gerais (IFSULDEMINAS) – Campus Machado.

The samples were selected from the root and the middle portion of the leaf blade of adult leaves and fixed in FAA 70 (JOHANSEN, 1940) for 48 hours and then stored in 70 % ethanol. Freehand histological sections and paradermal sections stained with Astra Blue and Safranin were obtained from these samples (ROESER, 1962). The histochemical tests performed were lugol for starch (BÜCHERL, 1962) and Sudam III for lipids (JOHANSEN, 1940). The results were recorded using photomicrographs, obtained using a Leica EC3 photomicroscope and Leica LAS EZ software.

Results and discussion

Leaf characterization

The studied *Brassavola tuberculata* leaf is subulate, succulent, and furrowed, having a continuous longitudinal cannula (FIGURE 1B), whose limb has the following average dimensions: 22.3 cm long, base diameter 0.50 cm x 0.39 cm (largest and smallest diameters, respectively); middle third diameter 0.52 cm x 0.41 cm (larger and smaller diameter, respectively); and its apex 0.34 cm x 0.26 cm (larger and smaller diameter, respectively).

Anatomical analysis revealed that the leaves are amphistomatic (FIGURES 2C, 2D), with most stomata of the tetracytic type, randomly distributed in the epidermis, with ostioles (pores) always aligned parallel to the largest axis of the leaf

(FIGURE 2E). When considering the subgroups of the Orchidaceae family, generally, the leaves are hypostomatic, having stomata only on the abaxial face (SILVA; MILANEZE-GUTIERRE, 2004), but some species of *Neuwiedia* and *Apostasia*, considered primitive within the family, also have amphistomatic leaves (STERN *et al.*, 1993). In the study by Noguera-Savelli and Jáuregui (2011), on the genus *Brassavola*, all the species studied had amphistomatic leaves. As for the arrangement of subsidiary cells, Withner *et al.* (1974) concluded that the typical stomata of Orchidaceae are anomocytic. But the results observed is consistent with those of Stern and Carlswald (2009), who pointed out the tetracytic type as the most common in Laeliinae.

No type of trichome was observed in *B. tuberculata*, corroborating the study by Noguera-Savelli and Jáuregui (2011) who did not find trichomes in any of the species of the genus. The analysis of the cross-sections showed that the dermal system is composed of isodiametric, unistratified, rectangular epidermal cells with straight, and thin anticlinal walls, slightly convex and thin external periclinal walls (FIGURE 2C).

On the leaf epidermis of *B. tuberculata* there is a thick smooth cuticle (FIGURE 2D). This characteristic, in most species, is considered a xeromorphic character (FAHN; CUTLER, 1992) and is determined by the degree of exposure of the organ to the sun, which is, then, one of the characteristics of orchids in sunny environments (WITHNER *et al.*, 1974; OLIVEIRA; SAJO, 1999; SILVA; MILANEZE-GUTIERRE, 2004).

Analyzes of cross-sections of the leaf of *B. tuberculata* showed that the cuticle forms an expressive prominence over the stomatal pore contributing to the formation of a suprastomatic chamber (FIGURE 2D) similar to those observed by Oliveira and Sajo (1999) and Silva and Milaneze-Gutierrez (2004). For Rasmussen (1987), this chamber is common in epiphytic orchids that face high temperatures and low

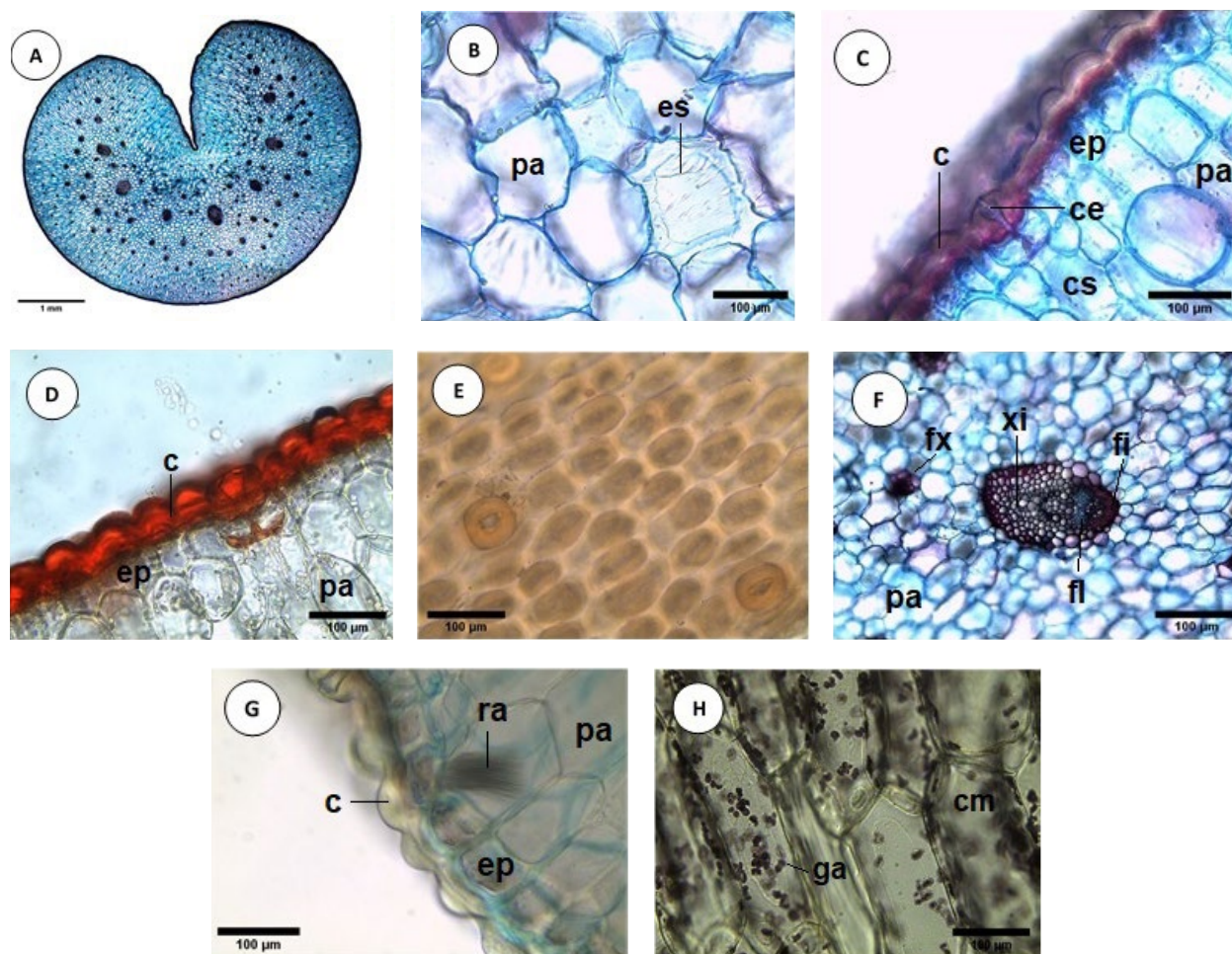
water availability contributing to the reduction of leaf transpiration.

The leaf has a homogeneous mesophyll composed of 4-8 layers of polyhedral parenchyma cells and 18-22 layers of isodiametric parenchyma cells (FIGURE 2A). Some of these cells have thickening bars (FIGURE 2B). According to Pridgeon (1986), thickened cells are common in orchids and do not have pseudobulbs. They have the function of storing water and preventing tissue collapse during drought, thus characterizing the

occurrence of aquifer parenchyma. Noguera-Savelli and Jáuregui (2012) observed this adaptation in several species of *Brassavola* (*B. acaulis* Lindl. & Paxton, *B. ceboletta* Rchb.f. and *B. grandiflora* Lindl.), however, they did not find it in *B. tuberculata*.

The mesophyll is also transpassed by a large number of bundles of extravascular fibers (FIGURE 2F) arranged both on the abaxial and adaxial sides of the leaf. Noguera-Savelli and Jáuregui (2012) also observed that the 11 species

Figure 2. Anatomical sections of a leaf of *Brassavola tuberculata* Hook located in an arboreal phorophyte on a rocky outcrop in Serra da Pedra Grande, Campestre, Minas Gerais.



Abbreviations: A. Entire leaf limb; B. Mesophyll aquifer parenchyma cells with thickening; C. Epidermis showing stomata; D. Cuticle stained with Sudam III; E. Stomata in paradermal section, stained with Sudam III; F. Mesophyll and vascular bundle; G. Raphides; H. Lugol-stained starch grains; pa = aquifer parenchyma; es = thickening; c = cuticle; ep = epidermis; ce = suprastomatic chamber; cs = substomatic chamber; fx = extravascular fiber; fi = fibers; xi = xylem; fl = phloem; ra = raphides; cm = mesophyll cell; ga = grain of starch.

Source: Prepared by the authors (2021).

of *Brassavola* had bundles of extravascular fibers on both leaf surfaces, differing from the present study in terms of quantity and arrangement. These sclerified cells, together with the others, provide mechanical resistance in case of dehydration (OLIVEIRA; SAJO, 1999), contributing to the leathery appearance of the leaves, exerting the function of supporting the leaf blade (SILVA; MILANEZE-GUTIERRE, 2004).

The vascular system is composed of closed collateral bundles arranged in a central arch. The main bundles and the four arches of secondary vascular bundles from which three are subjacent and one overlying the central arch are zig-zag spread across the mesophyll (FIGURE 2A). The xylem is oriented towards the adaxial surface and the abaxial phloem, and fiber sheath developed more next to the phloem (FIGURE 2F). This type of vascular bundle, surrounded by fibers is also commonly found in many orchid species observed by Pridgeon (1982), Oliveira and Sajo (1999), Stern and Judd (2001), and Silva and Milaneze-Gutierrez (2004).

Cellular inclusions of the calcium oxalate crystals type present in the raphids form were observed in cells close to the epidermis (FIGURE 2G). According to Silva and Milaneze-Gutierrez (2004), this is another very common feature among orchids, but its functions are still unknown and it is believed that the crystals act as a protection mechanism against animals or, alternatively, as a removal mechanism of oxalic acid excess from the plant system.

The lugol test showed the presence of starch in the mesophyll parenchyma cells (FIGURE 2H). Viera (2005) in his study observed this same characteristic in orchids of the genus *Pleurothallis* found in similar conditions.

Root characterization

The studied root is adherent and has an irregular surface (FIGURE 3A). The roots

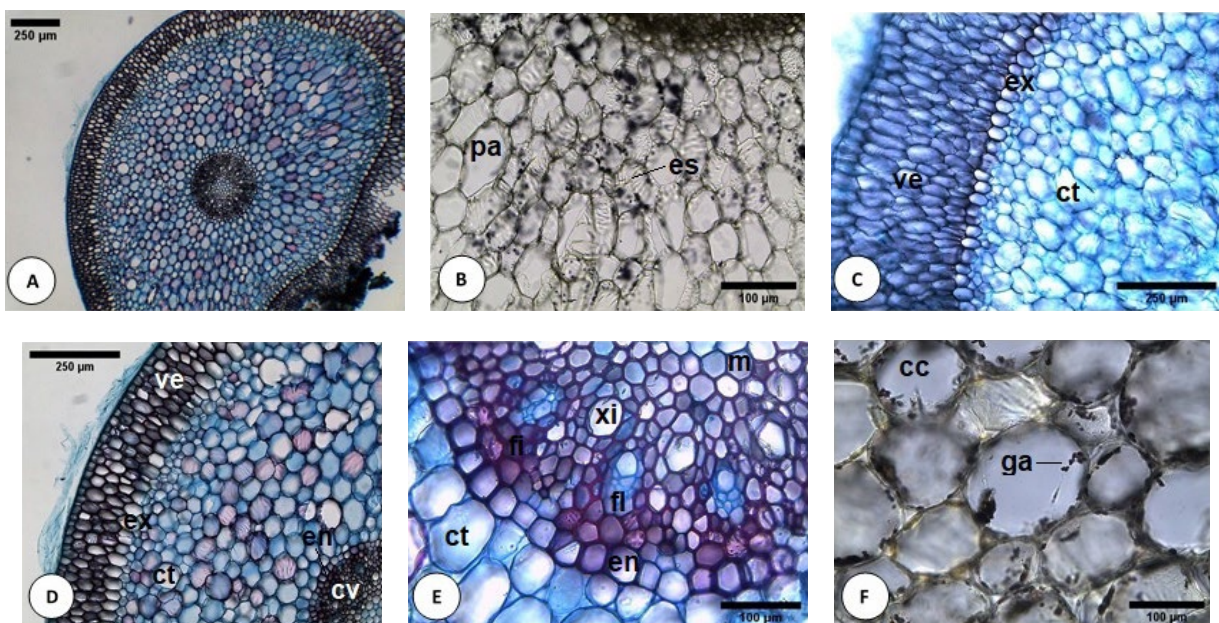
are relatively long, thick and very branched (FIGURE 1B). From the periphery to the central portion, the root of *B. tuberculata* is composed of: i) velamen, ii) cortex with clearly differentiated exoderm and endoderm, and iii) central cylinder surrounded by the pericycle, which was also differentiated (FIGURE 3A). This tissue organization is common to other orchid species, as verified by Benzing *et al.* (1982), Pridgeon (1987), and Morales *et al.* (2002).

The root velamen of *B. tuberculata* is made of 4 to 5 cell layers with irregularly thickened walls, thus configuring the longitudinal and sometimes intertwined striations (FIGURE 3C), common to many orchid species (POREMBSKI; BARTHLOTT, 1988). Just below the velamen, the exodermis is formed by longitudinally elongated cells with thickening in the anticlinal and external periclinal walls forming a thickening wall in the shape of a "U" (FIGURE 3C). This velamen-exodermis complex has the function of mechanical protection of the roots and prevention of water loss by the cortex (DYCUS; KNUDSON, 1957). Benzing *et al.* (1982) evidenced the contribution of this complex in mineral nutrition in epiphytic species when they reported the velamen's ability to imbibe with solutions deposited on the roots. The solutes are able to pass through the cells and through the exodermis.

Below the exodermis, forming the cortex, there are 10 to 13 layers of rounded cells of varying sizes with thin walls (FIGURE 3D). Some of these cells have cellulose-thickening bars (FIGURE 3B). Using the Lugol test, the presence of starch in cortical cells was detected (FIGURE 3F), thus evidencing the reserved character of the root. Vieira *et al.* (2010) in their thesis also describe this character in *Acianthera teres* (Lindl.) Borba and *Epidendrum xanthinum* Lindl, which are found in the altitude field of Serra do Brigadeiro State Park (MG).

The endoderm is formed by groups of 4 to 5 cells with "O" thickening, alternating with

Figure 3. Anatomical sections of the root of *Brassavola tuberculata* Hook located in an arboreal phorophyte on a rocky outcrop in Serra da Pedra Grande, Campestre, Minas Gerais.



Abbreviations: **A.** Whole root; **B.** Cells with thickening bars; **C.** Velamen; **D.** Cortex; **E.** Vascular cylinder; **F.** Lugol stained starch grains; pa = aquifer parenchyma; es = thickening; ve = velamen; ex = exoderm; ct = cortex; in = endoderm; cv = vascular cylinder; fi = fibers; xi = xylem; fl = phloem; m = marrow; cc = cortical cell; ga = grain of starch.

Source: Prepared by the authors (2021).

2 to 3 thin-walled cells, called endodermal passage cells (SILVA; MILANEZE-GUTIERRE, 2004), positioned on the pericycle cells which are also non-sclerified, and on xylem elements (FIGURE 3E). Morales *et al.* (2002) diagnosed this alternating pattern of cells around the central cylinder in all orchid species analyzed. However, they emphasized that secondary wall depositions can also occur in the passage cells with the maturation of the roots, thus preventing their distinction from the others.

The central cylinder of *B. tuberculata* roots has 12 to 15 groups of phloem cells interspersed with xylem cells, thus configuring a polyarch root. In the central portion of the root the medullary tissue is formed by thin-walled cells (FIGURE 3E). Orchids are characterized by polyarch roots (SILVA; MILANEZE-GUTIERRE, 2004). Therefore, the roots of *B. tuberculata* contribute to the economy of water by viewing the various layers of velamen and cortical tissue.

Final considerations

The following xeromorphic characteristics were observed in the leaf and root of *B. tuberculata*: succulent leaf, stoma with suprastomatal chamber, thick cuticle, large amount of fiber bundles in the mesophyll, aquifer parenchyma with thickening in the leaf and root, vascular bundles involved by fibers and the pluristratified velamen.

These characteristics allow the survival of *B. tuberculata* in the restricted environmental conditions of the rocky outcrop.

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