Stem base and root anatomy of four young trees of Legume species from Cerrado

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Abstract

Fabaceae is among the most representative families of the Cerrado, which is the second largest phytophysiognomy in South America and has the presence of fire. Thus, the anatomy of species present in the Cerrado is intrinsically related to their survival after a fire event. The objective of this study was to carry out an anatomical description of the base of the stem and root of four species of tree legumes present in the Cerrado, at 6 and 18 months of age. For this, the seedlings were grown in a greenhouse and then the base of the stem and the root were fixed and dehydrated. Some samples were cut using a sliding microtome, and others were sectioned using a rotating microtome. The presence of starch was checked using Lugol. The four species showed secondary growth, and in general there are no major differences between the ages of 6 and 18 months. The species have a large number of fibers and parenchymatic rays, which are generally uniseriate. In some organs it was possible to verify the presence of residual cortex and evident vascular cambium. A large amount of starch was found in the species analyzed, especially in the secondary xylem. Buds were observed at the stem base in *Albizia niopoides*, at both ages, and in *Senegalia polyphylla*, at 6 months. These characteristics can be advantageous in providing the persistence of these species in the Cerrado, especially after a fire event.

Introduction

Cerrado is the second largest phytophysiognomy in South America, occupying an area of more than 2 million square kilometers (Nascimento 2001), representing approximately 22% of Brazil's land surface (Oliveira-Filho & Ratter 2002). It contains the greatest biodiversity on the continent in terms of endemic species e and is characterized by a seasonal climate, with a well-defined dry season (Eiten 1982). The biome also has the recurrent presence of fire, a determining element for vegetation (Miranda et al. 2002). Most of the Cerrado's flora are species adapted to fire, involving not only tolerance to it, but also dependence on this element, that is, fire is part of the Cerrado's ecology (Coutinho 1990). According to Oliveira-Filho & Ratter (2002), the woody flora of Cerrado has typical characteristics of vegetation that suffers periodic fires. Furthermore, they have a bud bank that allows their post-fire resprout (Pausas et al. 2018, Vesk & Westoby 2004).

Fabaceae (Leguminosae) is among the most representative families in Cerrado (Filgueiras 2002; Miranda et al. 2002), and stands out among the Angiosperms for being the third largest in number of species, having around 770 genera and more than 19,500 species (LPWG 2017). In Brazil, with confirmed occurrence in all regions, legumes currently have 254 genera accepted in the country, totaling 3,038 species, 1,590 of which are considered endemic (Flora and Funga do Brasil 2023). Its species have a cosmopolitan distribution, being present from tropical forests to deserts (Doyle & Luckow 2003), thus having a great environmental, morphological and physiological diversity (Simon et al. 2009). Also, it is one of the botanical families that presents species with adaptations related to fire (LPWG 2013).

Albizia niopoides (Spruce ex Benth.) Burkart, *Inga laurina* (Sw.) Willd. and *Senegalia polyphylla* (DC.) Britton & Rose are arboreal legumes belonging to the subfamily Caesalpinioideae (mimosoid clade)

(LPWG 2017), while *Erythrina crista-galli* L. belongs to the subfamily Papilionoideae (Lorenzi 1998). The first three species can reach around 20 m in height and up to 60–70 cm in diameter (Lorenzi 1992; 1998) and are excellent for use in projects to recover degraded areas and in urban afforestation (Lorenzi 1992; 1998; Carvalho 2008). *E. crista-galli* reaches up to 10 m in height and has a diameter of 30 to 40 cm, and, despite being a thorny plant, it is quite ornamental when in flower, being used in the afforestation of parks and gardens (Lorenzi 1998).

Plant anatomy is an important tool that assists in the identification and classification of species (Maiti et al. 2012), even helping to establish affinities between genera of uncertain taxonomic status (Metcalfe & Chalk 1950). Anatomical microscopic methods are also widely used in the botanical identification of commercial samples of medicinal plants, wood and fibers, among others (Metcalfe & Chalk 1950). For Fabaceae, it has already been shown that wood anatomy, for example, is useful in exploring the systematics and evolution of the family (Baretta-Kuipers 1981, Gasson 2000). Furthermore, the anatomy of species present in Cerrado is intrinsically related to their survival after a fire event, since one of the main strategies is the ability to resprout from the underground organs or the base of the stem (Hoffmann & Moreira 2002) and accumulate of reserves (Franco 2002).

Therefore, the aim of this study was to carry out an anatomical description of the base of the stem and root of four species of tree legumes present in Cerrado, at 6 and 18 months of age. Thus, it could provide subsidies for plant systematics and for future studies of the relationship between these species and the fire present in Cerrado.

Material and methods

Plant material

The seedlings of four species of Fabaceae, native to Brazil, were provided by CESP (Companhia Energética de São Paulo) (Table 1) and grown in a greenhouse at UNESP/FEIS - Ilha Solteira until they reached 6 and 18 months, ages at which they were analyzed.

Table 1
Species of Fabaceae selected for study (Flora and Funga do Brasil 2023).

Species	Popular name	Phytogeographic domains
Albizia niopoides (Spruce ex Benth.) Burkart	Farinha-seca	Amazônia, Cerrado, Atlantic Forest, Pampa
Erythrina crista-galli L.	Suinã; cortiçeira	Cerrado, Atlantic Forest, Pampa, Pantanal
Inga laurina (Sw.) Willd.	Ingá-miúdo	Amazônia, Caatinga, Cerrado, Atlantic Forest
Senegalia polyphylla (DC.) Britton & Rose	Monjoleiro	Amazônia, Caatinga, Cerrado, Atlantic Forest, Pantanal

Anatomical studies

For each species and age, the region of the stem base and root was analyzed. The vegetative material was fixed in a mixture of formaldehyde, glacial acetic acid and 70% alcohol (FAA70%) (Johansen 1940) and subsequently dehydrated and stored in 70% alcohol. A portion of the samples was sectioned at 30µm thickness using a Leica SM2010R sliding microtome, clarified with 20% sodium hypochlorite and washed in distilled water. Some sections were stained in safranin and alcian blue (Bukatsch 1972), and mounted in 50% glycerin (semi-permanent slides). The presence of starch was checked using Lugol (Berlyn & Miksche 1976) and the slides were mounted with the reagent itself.

Other samples were dehydrated in an ethyl series, included in hydroxy-ethyl-methacrylate (Leica Historesin) and the blocks obtained were sectioned at 8–10 µm thickness using a Leica RM2245 rotary microtome. The slides were stained with 0.05% toluidine blue in phosphate buffer and citric acid pH 4.5 (Sakai 1973) and mounted in Entellan synthetic resin (Merck, Germany).

The photomicrographs of the materials arranged on slides were taken using a photomicroscope (Zeiss Primo Star with attached camera model AxioCam ERc5s), with the micrometric scales photographed and magnified under the same optical conditions that were used.

Results

Stem base and root anatomy

In the four species studied, the stem base and the root showed secondary growth at both ages. The lining is made by the periderm, and the phloem and secondary xylem are present. At 6 months, the phellogen at the base of the shoot was more evident in *E. crista-galli* and *I. laurina*, while in *A. niopoides* and *S. polyphylla* this layer appeared more degraded. Even at this age, phellogen was more evident in the roots of *A. niopoides* and *I. laurina*, and less evident in *E. crista-galli* and *S. polyphylla*. At 18 months, phellogen was evident in all species.

A. niopoides had a stem base with a phellem of an average of five layers and a phelloderm with flattened cells distributed in two layers, and there was the presence of residual cortex. The secondary phloem, in its outermost part, had more parenchyma cells. In the internal portion, close to the cambium, it was possible to observe a large amount of fibers. The parenchymatic rays began uniseriate, but then appeared dilated and with more voluminous cells. At 6 months of age, the vascular cambium was evident and had 8–11 layers, while at 18 months this region was not so evident. In the secondary xylem, the rays also had a single layer of cells and the vessel elements, for the most part, were isolated, but they could also appear in groups. Around the vessel elements, it was possible to visualize the presence of axial parenchyma. There was a large amount of fiber throughout the secondary xylem, even in the outermost portion.

The root of *A. niopoides* had a four-layered phellem and a phelloderm with two layers of rectangular to flattened cells. At 18 months, it was possible to check the presence of lenticels. At 6 months, the

parenchymatic rays of the phloem were uniseriate and presented dilation and an increase in cell volume, while at 18 months the parenchymatic rays presented one to two layers of cells. In this species, the phloem had well-distributed fibers, but more concentrated in the innermost region, even forming a layer of fibers just before the vascular cambium, which, at 6 months, presented 5–8 layers of cells, while at 18 months it was little evident and formed by few layers of cells.

In the secondary xylem, there was a large number of fibers, and the parenchymatic rays were mostly uniseriate, but at 18 months they might have two layers of cells. The vessel elements were largely isolated, especially in the outermost region. It was possible to see some of them obliterated and/or interrupted, possibly by gums or resins secreted by this species. There was the presence of axial parenchyma and a large number of fibers in the primary xylem region, often organized in bands. At 18 months it was possible to observe the origin of secondary roots. In younger individuals, the root had several arches of protoxylem maturation, therefore it was considered a polyarch; In older individuals, some had four arches of protoxylem maturation, and others had five, so the roots varied from tetrarch to polyarch.

E. crista-galli presented a stem base with a reduced periderm, formed by well-condensed suber layers and with the presence of an extensive residual cortex area in 6-month-old individuals, while 18-month-old individuals presented this reduced area. More internally, it was possible to observe the development of a second periderm, with cortical parenchyma and the presence of sclereids in the shape of alters and clusters of fibers just below the phelloderm, which also appeared condensed. The parenchymatic rays were organized continuously along the entire length of the xylem and secondary phloem. The secondary xylem presented several thick layers of fibers at both ages. In 18-month-old individuals, a tangential area was found in the secondary xylem, where after it, the vessel elements reversed their growth direction, changing from axial to radial.

In the roots, the covering made by the periderm was similar to the stem base, maintaining the area of residual cortex, but appearing to have fewer sclereids and fibers. The vascular cambium was composed of several layers and the secondary xylem presented denser vessel elements in the center. The secondary phloem was formed by parenchymatic rays organized in groups of 2 to 4 rows of cells and clusters of fibers between the parenchymatic rays, showing no pattern of organization in 6-month-old individuals, while in 18-month-old individuals, they were organized into small bands of 2 to 3 layers perpendicular to the parenchymatic rays and aligned with each other.

In *I. laurina*, at the stem base, the suber was presented with many layers organized in an irregular manner. In the secondary phloem, as well as throughout the cortical parenchyma, it was possible to observe the presence of many idioblasts, while the highly lignified secondary xylem showed scattered vessel elements and parenchymatic rays organized in a row of cells that dilated at the periphery shortly after the cambium. No presence of residual cortex was identified. The vascular cambium was narrow and the parenchymal medulla was characterized by starch storage.

In the roots, the suber also presented irregularly organized layers and the cortex was reduced with a dense layer of fibers closer to the phelloderm. The anatomy of the secondary phloem and xylem was very close to the organization observed at the stem base at both ages.

At the stem base of *S. polyphylla*, the suber presented rectangular to tabular cells and phelloderm with 2–3 layers of cells. At 18 months, the presence of lenticels was noted. In the secondary phloem, there was not a large number of fibers, when compared to other species. The parenchymatic rays were uniseriate and the vascular cambium was not so evident in this region, at both ages. In the secondary xylem, the rays continued to have only one layer of cells, and the vessel elements appeared both isolated and grouped. At 18 months there was the presence of branches, similarly to self-grafting.

The roots of *S. polyphylla* had a suber with rectangular and flattened cells, and a phelloderm with two layers of cells. At 18 months, lenticels were present. The parenchymatic rays of the phloem generally had a single layer of cells, but could have two. No increase in volume of cells in the outermost portion was observed. The fibers were well distributed throughout the phloem region, sometimes forming bands. The vascular cambium was evident at both ages, presenting three to six layers of cells. In the secondary xylem, there was a large number of fibers, both in the inner and outer portions, and at 18 months these cells appeared more lignified. As in the phloem, the vast majority of parenchymatic rays were uniseriate, and most of the vessel elements were isolated, especially in the outermost region. At both ages it was possible to observe the origin of secondary roots, and the roots varied from tetrarch to polyarch.

In *A. niopoides*, the presence of buds was found at the stem base, in both species studied. At 6 months, the bud was observed at an initial stage, while at 18 months it was already formed and protected by the periderm. Buds protected by the periderm were also observed at the stem base of *S. polyphylla* at 6 months of age.

Presence of starch

In *A. niopoides*, starch was observed throughout the entire structure of the root collar, at both ages. It was present in the secondary phloem and xylem, including starch grains within the vessel elements, and in the medullary parenchyma. The periderm had few starch grains. In the root, at 6 months, fewer starch granules were found in the secondary xylem when compared to 18-month-old individuals, which, in turn, had the entire secondary xylem region with a great reaction to Lugol. The reaction was negative in the axial parenchyma and periderms.

In the stem base of *E. crista-galli*, at the two ages studied, the presence of starch in the periderm and secondary phloem was not found. At 6 months, the entire xylem reacted positively to starch, while at 18 months, the starch grains were organized into bands, mainly in and around the parenchymatic rays. A similar situation was found at the root. At 6 months, the amount of starch in the xylem was visibly greater than at 18 months, the age at which the concentration occurred in the parenchymatic rays.

For *I. laurina*, the pattern was repeated. There was no presence of starch in the periderm and phloem in any of the structures analyzed, at both ages. At the root, 18-month-old individuals showed a more evident

reaction in the secondary xylem, mainly in the parenchymatic rays.

The presence of starch was evident in both regions of *S. polyphylla.* At the stem base, at both ages, the place with the least reaction was the periderm, and the xylem was the region with the most starch grains. In the root, at 6 months, starch was present in the secondary phloem and secondary xylem, but the reaction was more evident in the most central portion of the secondary xylem. At 18 months, the xylem also showed a reaction to Lugol, but with a smaller quantity of starch grains, when compared to the younger age. The periderm showed no reaction.

Discussion

It was observed that the four species studied showed secondary growth, at 6 and 18 months. The presence of this growth in thickness is related to some conditions, such as the mechanical support of the plants (Rajput et al. 2012), and it could be evident even in young plants. Duarte and Krentkowski (2015) found incipient secondary structures in young portions of the stem of Erythrina falcata, in addition to the presence of periderm, although the epidermis still persisted. The presence of secondary growth was also observed in the roots of *I. laurina* by Hayashi (2005). The anatomical description carried out here corroborates what was found by the author, who also observed a phloem full of bands of condensed fibers, a coating made by periderm and starch present in the parenchymatic rays. However, according to (Hayashi 2005), the roots are described as geminiferous with buds of exogenous origin, formed from the phloem meristem, which was not observed in the present study.

According to Longui et al. 2012, *Caesalpinia echinata* presents, in the root and stem, diffuse, solitary vessels in multiples of two or more, and parenchymatic rays in 2 homogeneous series. In the four studied species, the parenchymatic rays varied between one and two layers of homogeneous cells. These cells act in the storage and radial transport of substances between the xylem and phloem (Longui et al. 2012) and it is common to have a funnel shape, with the width increasing in the secondary phloem (Kraus and Basconsuelo 2009), as it was observed at the stem base and root of *A. niopoides* and *I. laurina* and root of *S. polyphylla*. Plants with superficial roots and from seasonal environments may have wider parenchymatic rays, thus guaranteeing the supply of nutrients during periods of drought (Goulart and Marcati 2008).

Longui et al. (2018) analyzed 10-year-old individuals of *I. laurina*, and observed that the roots had vessels with a larger diameter than the stems, which, added to the large proportion of starchy parenchyma in the roots. The abundant presence of starch parenchyma in the roots was observed, which may be related to the ability to resprout after the passage of fire (Hayashi & Appezzato-da-Glória 2009). A large number of fibers was observed in the secondary phloem and xylem, especially in the innermost region, in all species analyzed, except in the secondary phloem at the stem base of *S. polyphylla*, which was visibly the region that presented fewer fibers. The presence of sclerenchymatic fibrous cells, whether individual or in groups, is common in the cortex, secondary phloem, and secondary xylem in Fabaceae (Tekin and Yilmaz

2015). Silva et al. 2013 found, in the stem of *Erythrina velutina*, groups of fibers forming a band over the phloem, as seen in some of the four studied species

The presence of lenticels was verified in *A. niopoides* and *S. polyphylla* at 18 months. Although lenticels generally form during secondary growth (Dickison 2000), they were not visualized at 6 months, probably because, at that age, this adaptation for gas exchange and/or direct water acquisition was not yet necessary for the species.

It was observed buds at the stem base in *A. niopoides*, at both ages, and *S. polyphylla*, at 6 months. As these species are present in the Cerrado, a biome that has the recurrent presence of fire, the presence of buds may be essential for the survival of individuals. The stem base, also called the root crown, is a transition zone, and it is from the buds located there that many woody plants resprout after fire events (Pausas et al. 2018). Bud location and protection are key drivers of post-fire resprout (Clarke et al. 2013; Charles-Dominique et al. 2015), and buds positioned at or below ground level during fires have the advantage of being protected by characteristics of the plant, such as the bark, and also by the soil, due to its low thermal conductivity (Clarke et al. 2013). Under frequent fire regimes, juvenile woody species that regularly suffer complete shoot loss and do not show the ability to resprout can be suppressed and even eliminated from the environment (Hoffmann et al. 2012).

A large amount of starch was also found in the species analyzed, especially in the secondary xylem. Starch is the most abundant storage carbohydrate in underground organs of Cerrado species, and plays an essential role as an energy source for these species (Alonso & Machado 2007). According to the authors, starch also appears to be related to the formation of buds, which helps with subsequent regrowth. In the four studied species, this accumulation may be related to tolerance to environmental stresses in the Cerrado, such as prolonged droughts and fires, thus functioning as a survival strategy for the species to adverse environmental conditions, as cited by Franco 2002.

Therefore, it was concluded that the four species have common anatomical traits of the Fabaceae species, and that there are, in general, no major differences between the ages of 6 and 18 months. Some important features were highlighted, such as the presence of buds in *A. niopoides* and *S. polyphylla* and the large amount of starch in all species, which, when associated with the fact that they are present in Cerrado and susceptible to the passage of fire, it can be advantageous characteristics to provide the persistence of these species in the environment.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

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Authors' contributions

All authors contributed to the conception and design of the study. TCP, JOM and MAdA developed anatomical analysis and data collection. The first draft of the manuscript was written by TCP and JOM, and all authors commented on later versions. ARM contributed to the guidance, critical reading and final editing of the manuscript. All authors read the final manuscript and approved the submission.

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Figures

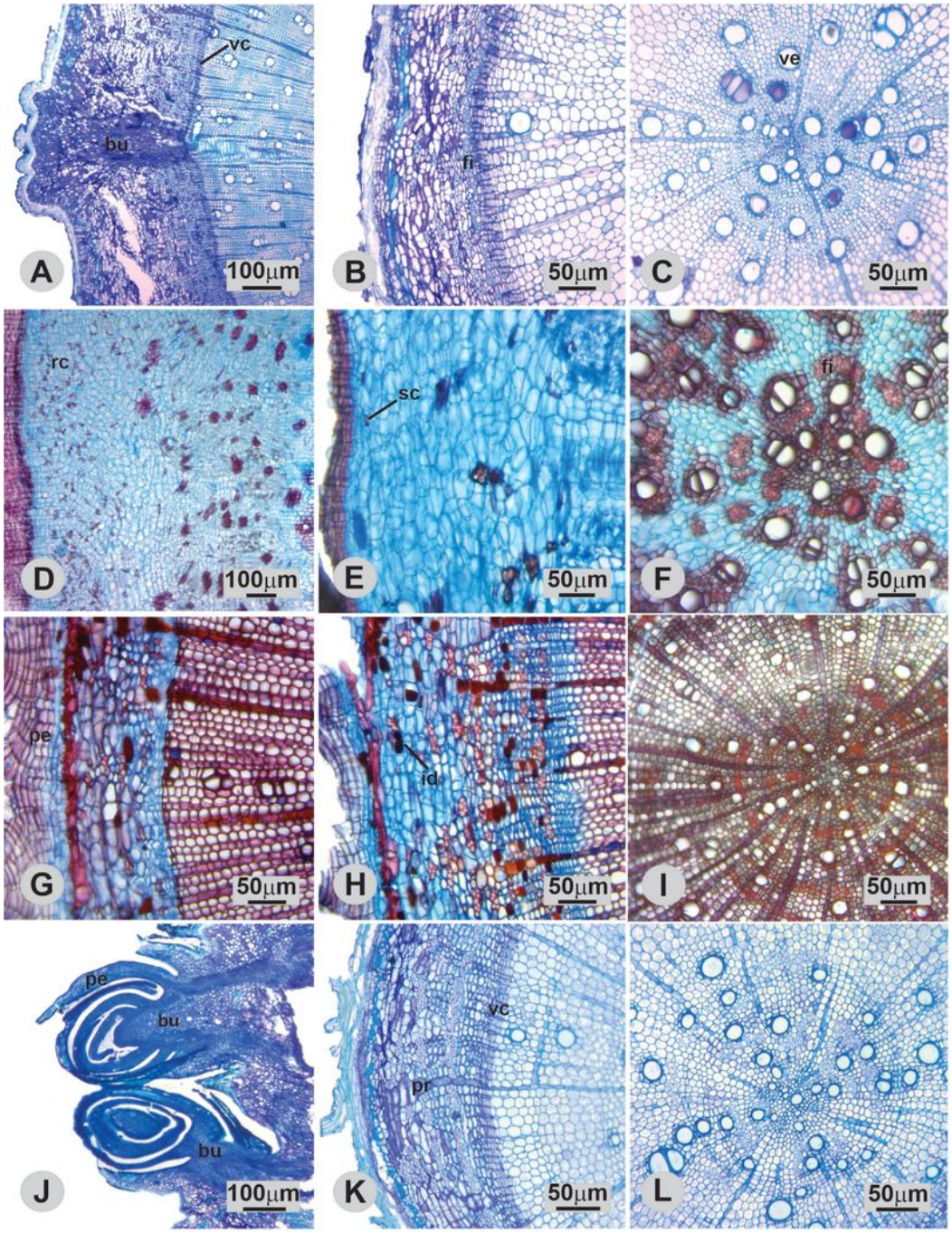

Fig. 1 Anatomy of the stem base (A, D, G, J) and root (B, C, E, F, H, I, K, L) of four legume species at 6 months of age. **A-C:** *A. niopoides*, with the beginning of the formation of a bud in A. **D-F:** *E. cristagalli*. **G-I:** *I. laurina*. **J-L:** *S. polyphylla*, with buds evident in J. Legend: bu: bud; fi: fiber; id: idioblasts; pe: periderm; pr: parenchymatic radius; rc: residual cortex; sc: sclereides; vc: vascular cambium; ve: vessel element.

Figure 1

See image above for figure legend.

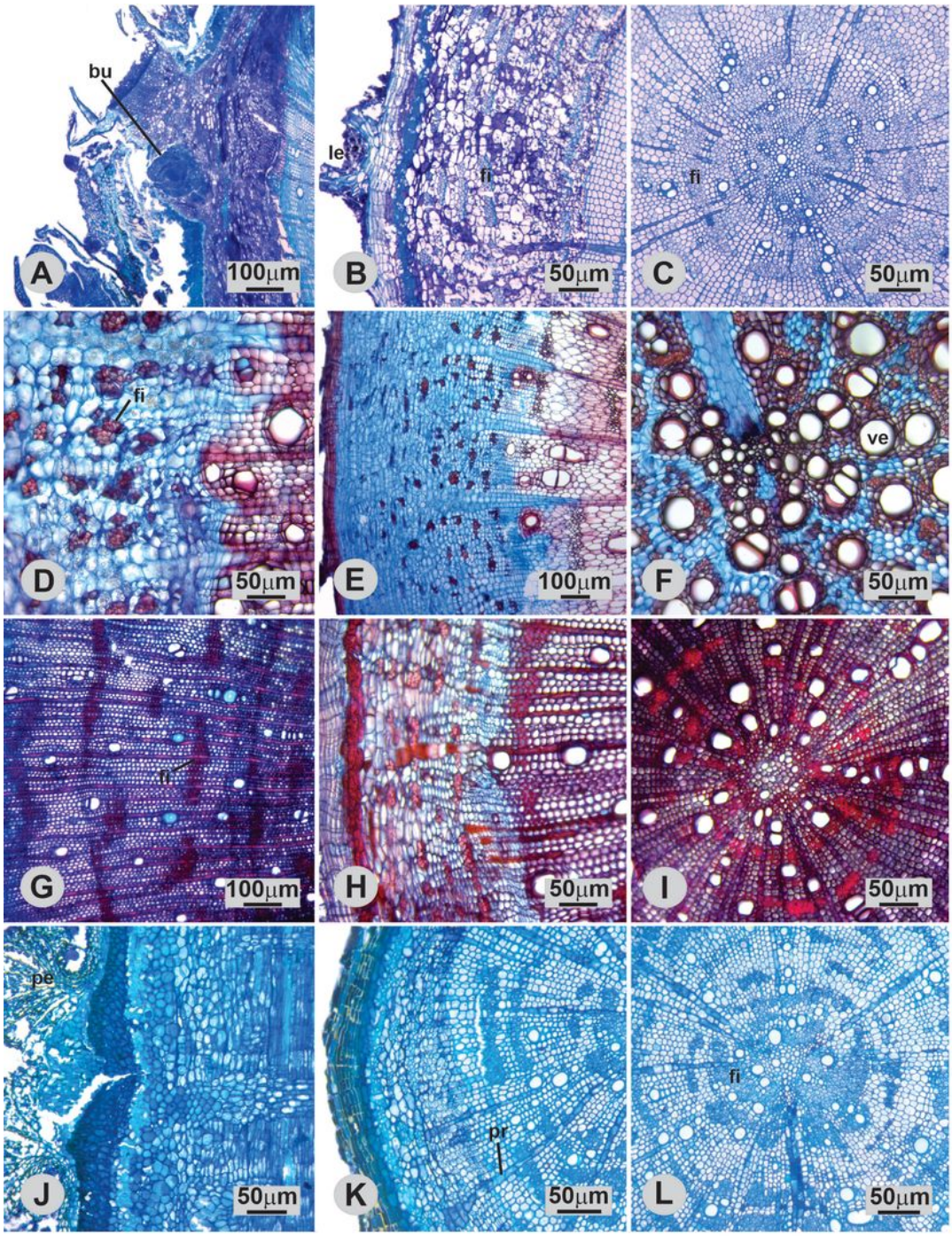

Fig. 2 Anatomy of the stem base (A, D, G, J) and root (B, C, E, F, H, I, K, L) of four legume species at 18 months of age. **A-C:** *A. niopoides*, with evident bud in A. **D-F:** *E. crista-galli.* **G-I:** *I. laurina*. **J-L:** *S. polyphylla.* Legend: bu: bud; fi: fiber; le: lenticel; pe: periderm; pr: parenchymatic radius; ve: vessel element.

Figure 2

See image above for figure legend.

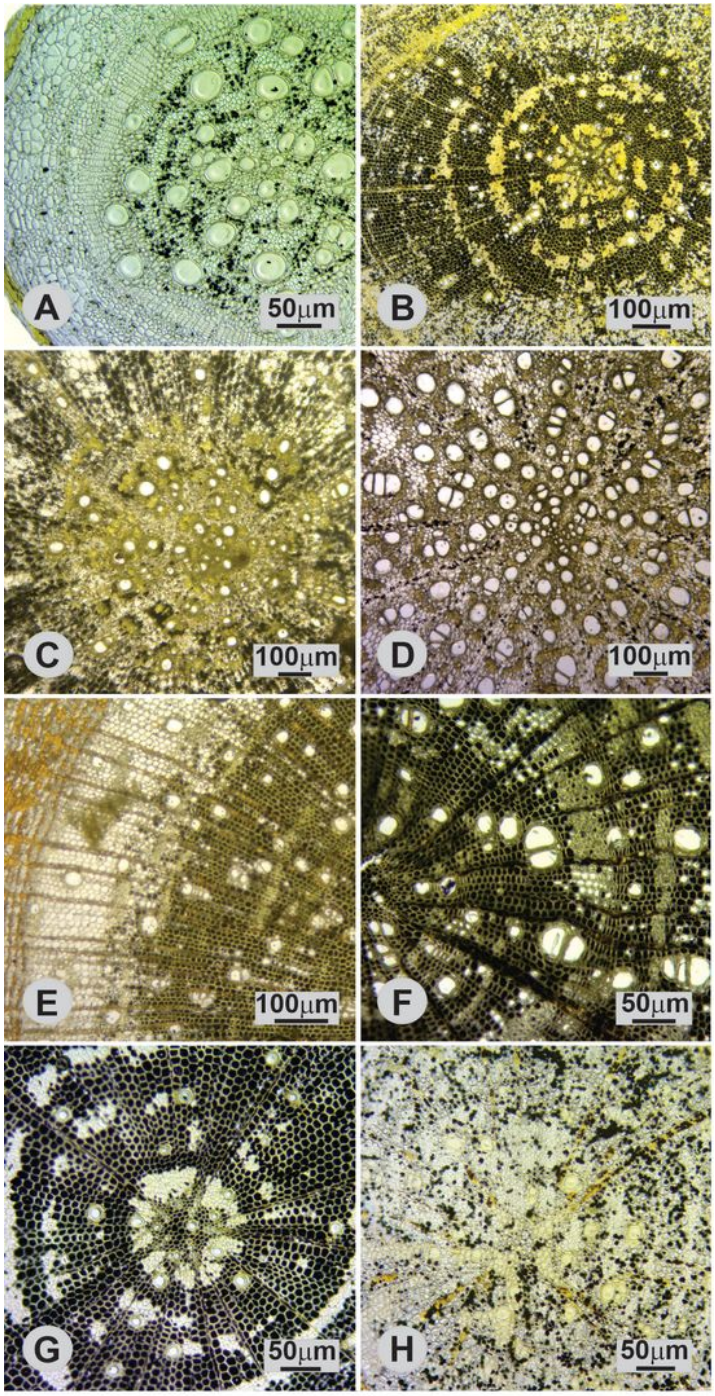

Fig. 3 Histochemical test with Lugol reaction to starch in roots of four legume species at 6 months (A, C, E, G) and 18 months (B, D, F, H) of age. **A-B:** *A. niopoides*. **C-D:** *E. crista-galli*. **E-F:** *I. laurina*. **G-H:** *S. polyphylla*.

Figure 3

See image above for figure legend.