



Morphoanatomical shifts and energy storage: Unlocking the survival strategy of a disturbance-prone violet of the *Pombalia lanata* complex (Violaceae)

Anelise Gabriela Grotto^a , Juliana de Paula-Souza^{a,*}, Marcelo Rodrigo Pace^b, Ana Claudia Rodrigues^a, Makeli Garibotti Lusa^a, Fernanda Maria Cordeiro de Oliveira^a

^a Departamento de Botânica, Universidade Federal de Santa Catarina (UFSC), R. Eng. Agrônomo Andrei Cristian Ferreira 216, Carvoeira, Florianópolis, 88040-535, Brazil

^b Departamento de Botánica y Herbario Nacional de México, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM), Circuito Zona Deportiva s/n, Alcaldía Coyoacán, Mexico City, 04510, Mexico

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ABSTRACT

Several herbaceous species develop thickened underground systems of root, stem, or mixed origin, which may exhibit morphological and anatomical traits associated with resistance to drought and fire, as well as tolerance of nutrient-poor soils. Despite their ecological importance, the diversity and functional significance of these structures remain poorly understood. This is particularly relevant for the *Pombalia lanata* complex, a clade endemic to the Cerrado, the second largest Brazilian domain, whose species commonly develop specialized underground systems. Here, we describe the morphoanatomy of the underground system of *Pombalia velutina*, a species occurring in southern Brazil. Underground systems of three individuals were analyzed. Samples were fixed, dehydrated in an ethanol series, sectioned either fresh or embedded in methacrylate. Histochemical tests were performed to identify the main storage and structural compounds. Two underground morphologies were found: (1) a proximal region of stem nature (sobole) associated with a taproot tuber; (2) a vertically oriented underground stem bearing roots distally. Both stem-derived regions exhibited numerous buds. Acidic polysaccharides, pectins, proteins and lipids were detected within the vessel elements of the underground vertical stem, as well as proteins in the radial parenchyma of the sobole and in the vessel elements, ray parenchyma, and periderm of the taproot tuber. Numerous inulin spherocrystals occurred predominantly in the cortical parenchyma of the taproot tuber. These findings expand knowledge and contribute to understanding of the structural and functional diversity of underground systems in the group, while highlighting the ecological importance of these traits in Cerrado vegetation dynamics.

1. Introduction

The Cerrado extends across approximately 23% of the Brazilian territory, encompassing all of its geopolitical regions (IBGE, Coordination of Natural Resources and Environmental Studies, 2019). The southern limit of this domain is located in Southern Brazil (Eiten, 1972), where the landscape is characterized by broad expanses of grasslands interspersed with patches of riparian forests, thickets of Araucaria Forest, and savannas (Cerrado s. str.), known as Campos Gerais (Bodziak Jr. and Maack, 1946; Behling, 1997; Carmo et al.,

2012).

The Campos Gerais region presents high species diversity and a considerable degree of endemism (Uhlmann et al., 1998; Von Linsingen et al., 2006) and, although geographically it is treated as one of the phytogeographies of the Atlantic Forest domain, its vegetation exhibits great floristic similarity with the central areas of the Cerrado domain (Ritter et al., 2010). Like most grassland areas in southern Brazil, Campos Gerais has a long history of human occupation, resulting in alterations and reduction of natural environments (Dillehay et al., 1992; Behling, 1997), most drastically from the 18th century onwards,

* Corresponding author.

E-mail addresses: anelise.gabriela.grotto@gmail.com (A.G. Grotto), j.paula.souza@ufsc.br (J. de Paula-Souza), marcelo.pace@ib.unam.mx (M.R. Pace), ana.rodrigues@ufsc.br (A.C. Rodrigues), makeli.lusa@ufsc.br (M.G. Lusa), fernanda.m.oliveira@ufsc.br (F.M.C. Oliveira).

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due to systematic disturbances caused by agricultural practices and the invasion of exotic forest and forage species (Ziller and Galvão, 2002; Rocha and Santana, 2016). Naturally restricted to small fragments, the savannas of Campos Gerais therefore constitute a severely threatened ecosystem.

Although the Violaceae is not as represented in the Cerrado as in the Amazon and Atlantic Forest, this domain is particularly rich in *Pombalia* species (Paula-Souza, 2025), especially those belonging to the *Pombalia lanata* complex, which occurs exclusively in savannas and associated grassland formations (Paula-Souza et al., 2011, 2021). This complex includes six species that are consistently grouped in a well-supported clade in phylogenetic analyses: *Pombalia cristalina* Paula-Souza, *P. insignis* Paula-Souza, *P. lanata* (A.St.-Hil.) Paula-Souza, *P. poaya* (A. St.-Hil.) Paula-Souza, *P. strigoides* (Taub.) Paula-Souza and *P. velutina* (Schulze-Menz) Paula-Souza (Paula-Souza et al., 2021). The group is predominantly distributed in the central area of the Brazilian Cerrado, with half of its species - *P. cristalina*, *P. insignis*, and *P. strigoides* - being narrow endemics. The exception is *Pombalia velutina*, which has a disjunct distribution in sparse areas at the southernmost limits of this phytogeographic domain in Brazil and Paraguay (Paula-Souza et al., 2011) (Fig. 1).

Previous analyses of herbarium material (Schulze, 1936; Paula-Souza et al., 2011) revealed that the species within the *P. lanata* complex possess a thickened underground system with a high capacity for resprouting. These underground systems have tentatively been called xylopodia, but this terminology is based only on the morphological analysis and is not supported by recent anatomical studies (Gonçalves et al., unpubl. res.). Thickened underground organs are characteristic of many perennial herbaceous and subshrub species in the Cerrado (Filgueiras, 2002). They function as nutrient storage, facilitate vegetative propagation (Carvalho and Dietrich, 1993; Asega and Carvalho, 2004), and are fundamental for survival in environments subject to prolonged periods of drought and natural fire events (Rizzini and Heringer, 1961; Hayashi and Appezzato-da-Glória, 2007). Considering the broad structural diversity of underground systems and the relevance

of their characterization within the context of the *Pombalia lanata* complex, the present study aims to investigate the morphoanatomy of the underground system of *Pombalia velutina* to identify the nature of its underground organs and structural traits potentially associated with resistance to environmental stressors.

2. Material and methods

2.1. Sampling and voucher specimens

Samples from three individuals of *Pombalia velutina* were collected from natural populations in Vila Velha Park, Paraná, Brazil, during November 2023. Voucher specimens (FLOR 77227 - late flowering/early fruiting, 77231 - sterile, and 77232 - sterile) were deposited in the Herbarium of the Department of Botany of the Federal University of Santa Catarina (FLOR).

2.2. Morphological and anatomical analyses

For morphological analyses, the underground organs were excavated and photographed in situ. Illustrations were produced digitally using Sketchbook software on a Galaxy Tab S6 Lite. Morphological terminology followed Pausas et al. (2018) and Appezzato-da-Glória (2015).

For anatomical analyses, fresh samples were fixed in formalin-acetic acid-alcohol (FAA 50) (Johansen, 1940). Freehand transverse sections were prepared using razor blades, stained with a 9:1 (v/v) solution of 1% Astra Blue and 1% aqueous Safranin (Bukatsch, 1972), and mounted in glycerinated gelatin (Kaiser, 1880). Additionally, samples from different regions of the underground system underwent gradual alcohol dehydration and were infiltrated with methacrylate (Leica Historesin®), following the slow infiltration protocol described by Paiva et al. (2011). Serial sections (3–5 µm thick) were obtained using a rotary microtome (RM 2125 RT Leica), stained with 0.05% Toluidine Blue (citrate-phosphate buffer pH 4.0) (Feder and O'Brien, 1968) and mounted with Entellan or Permount.

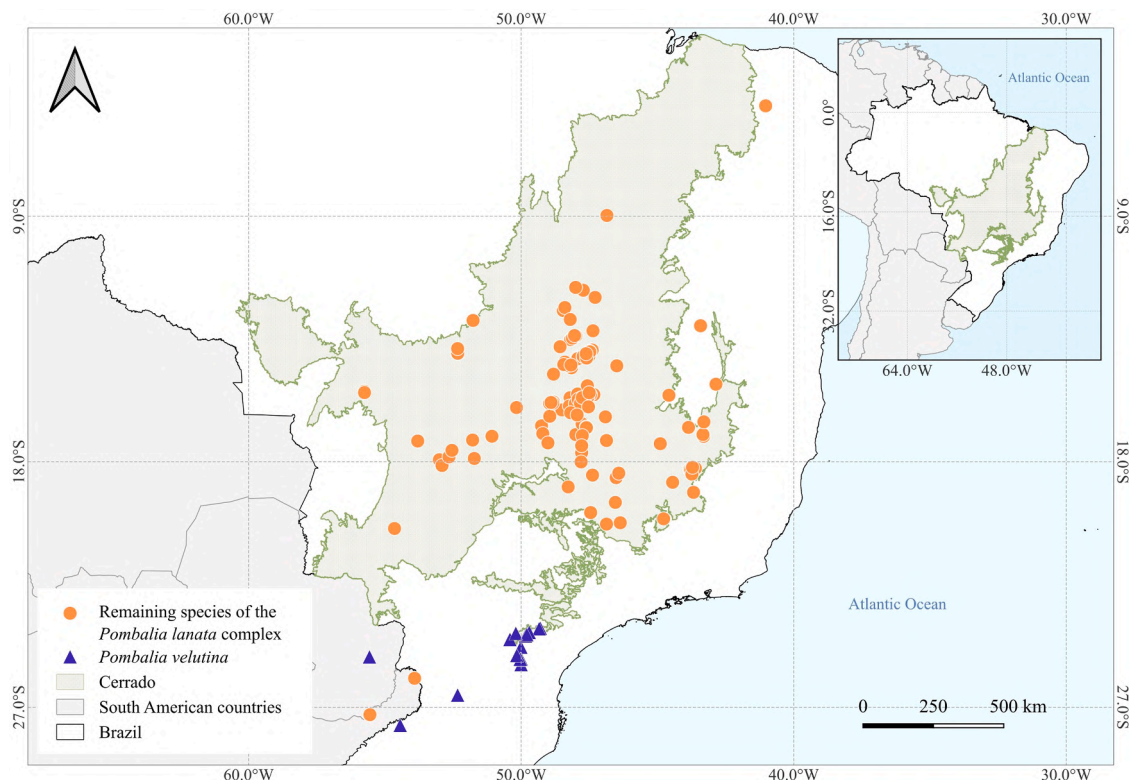


Fig. 1. Geographic distribution of the species of *Pombalia lanata* complex. Cartographic bases: IBGE 2021, 2024.

Histochemical tests for the detection of specific compounds were performed on freehand sections of fixed materials: Sudan Black B for total lipids (Pearse, 1985); 2% Lugol's solution for starch (Johansen, 1940); Ferric Chloride for phenolic compounds (Johansen, 1940); Ruthenium Red for pectic substances (Johansen, 1940); Alcian Blue for acidic polysaccharides (Pearse, 1985); Xylidine Ponceau for total proteins (O'Brien and McCully, 1981). Polarized light was used for the detection of crystals. Standard control procedures were carried out simultaneously for each test.

The slides were observed and described under a light microscope (Zeiss Lab A1 Axio model) and photographed using the RisingView software and Leica DM 2500 microscope with camera coupled with Leica Application Suit (LAS) software. The anatomical features of the secondary xylem and bark were aided by the IAWA List of Features for Hardwood Identification (IAWA Committee, 1989) and IAWA List of Microscopic Bark Features (IAWA Bark Committee, Angyalossy et al., 2016).

3. Results

3.1. Morphology of the underground system

Pombalia velutina individuals displayed an underground system composed of axes varying between 20 and 98 cm in length (Fig. 2). Among the three individuals collected, two distinct morphologies could be identified (Fig. 3):

- Type 1 - underground system with a proximal region of stem nature, referred to as a sobole (woody rhizome), horizontally arranged and forming an underground runner with numerous buds. The distal region consists of a taproot tuber (a tuberous structure of root origin, non-woody with a soft consistency), vertically oriented in the soil, from which lateral roots of different diameters emerge (Fig. 2A-B and 3A). In the proximal region of the root portion, some protuberances and contraction zones are observed along the axis (Figs. 1C and 3A).
- Type 2 - underground system with a thickened stem structure, vertically oriented in the soil, from which new shoots emerge from its proximal portion, and new shoots and adventitious roots emerge from its distal portion (Fig. 2D-E).

3.2. Anatomy of the underground system

This section presents the anatomical characteristics of each underground organ. We chose to separate the two distinct morphologies and describe them starting from the proximal region in relation to the soil surface, moving to the most distal region.

3.2.1. Type 1 - sobole (woody rhizome) and taproot tuber

The sobole (Fig. 3A and 4A-H) has a single periderm (Fig. 4B-C), originated from subepidermal or cortical origin (Fig. 4D), and its lenticels are homogeneous (i.e., filling tissue nonstratified Fig. 4B-C). The cortical portion shows parenchyma cells of different sizes. Some parenchymal cells increase in size and undergo belated scleriosis becoming sclereid cells (Fig. 4D-E). Pericyclic fibers occur at the limits of the vascular system (Fig. 4D-E), forming a ring of discrete fiber strands embedded in parenchymatous ground tissue. In some portions, the buds on the sobole develop aerial stems and their bases undergo self-grafting (Fig. 4B). In these same regions there is no presence of gaps. The vascular system is formed by secondary phloem with either solitary sieve tube elements, in small groups, in short radial rows, or in clusters (Fig. 4F), with one or two associated companion cells (Fig. 4F). In the secondary xylem of portions with more advanced secondary growth, growth rings vary from semi-porous to occasionally diffuse. The growth layers are delimited by vessel elements of larger diameter, the radial flattening of fibers, and a greater presence of parenchyma cells (Fig. 4B). Vessels can be solitary, in radial multiples of 2–8, or in clusters (Fig. 4B). The fibers are thin walled (Fig. 4B, G). Some vessels present tyloses (Fig. 4G). Parts of the rays and some axial parenchyma cells are non-lignified (Fig. 4B). The stem nature was confirmed by the presence of endarch xylem (Fig. 4H). The pith presents dividing parenchyma cells and sclerified cells in some portions (Fig. 4B, H).

The taproot tuber (Fig. 5A-J) has a single periderm as covering tissue (Fig. 5B), with characteristics similar to the stem portion. In regions with more advanced secondary growth, the cortex shows large-volume parenchymatic cells, some of which are undergoing division and sclereids are observed (Fig. 5C). In some portions of the root axis, phelloderm cells are also sclerified (Fig. 5B). Adjacent to the phloem, fibers can be seen forming caps (Fig. 5C-D). The vascular system is externally composed of a thin layer of conducting secondary phloem with sieve

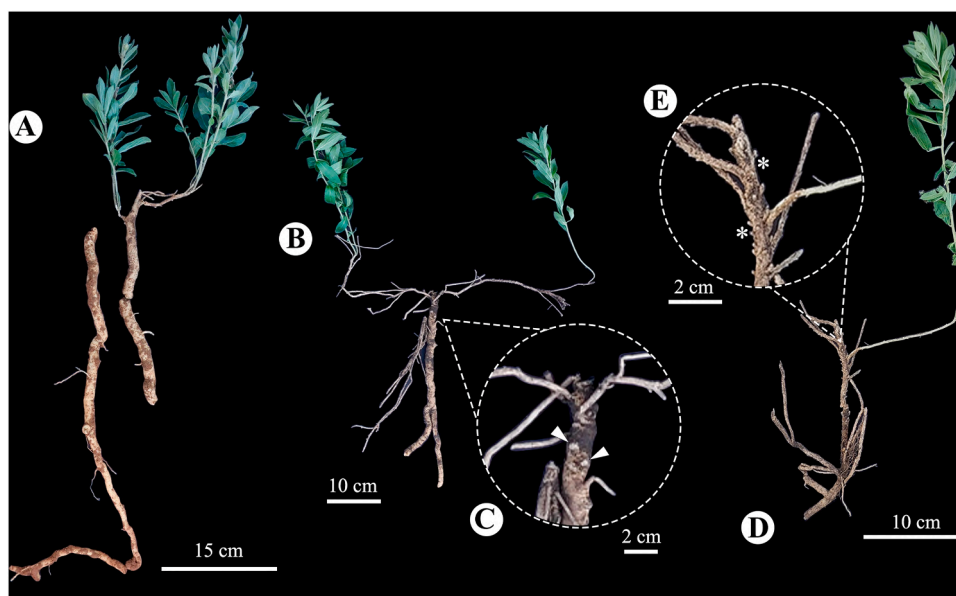


Fig. 2. Morphology of *Pombalia velutina*. (A-B) General view of morphological type 1. (C) Detail of the proximal region of the taproot tuber showing the emergence of new shoots and some protuberances (arrowhead). (D) General view of morphological type 2. (E) Detail of the proximal region of the vertical stem highlighting buds (asterisk).

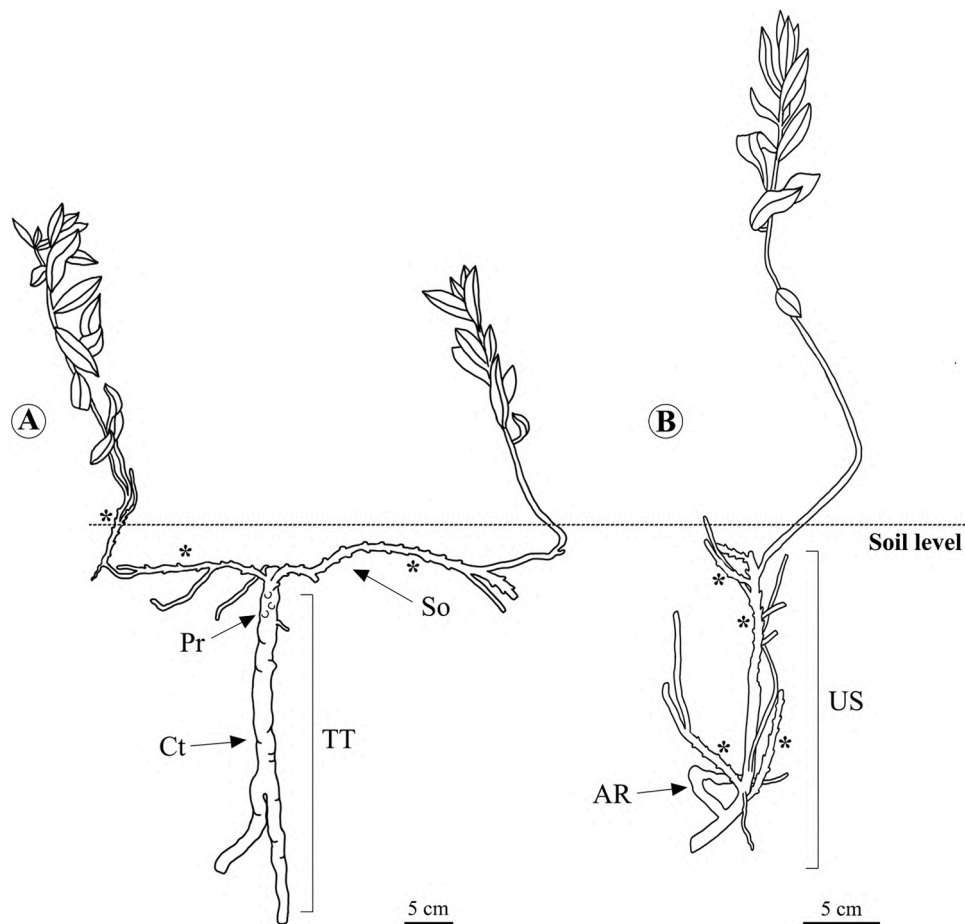


Fig. 3. Illustration of the two distinct underground morphologies found in *Pombalia velutina*. (A) Type 1 - underground system consisting of a sobole and a taproot tuber. (B) Type 2 - underground system consisting of a thickened vertical axis of stem nature and an adventitious root. AR = adventitious root; asterisk = buds; Ct = contractions; Pr = protuberances; So = sobole; TT = taproot tuber; US = underground stem.

tube elements, which are solitary or in clusters (Fig. 5D). The secondary xylem, there are indistinct growth rings and diffuse porosity, the vessel elements can be solitary, radial multiples of 2–6, or in clusters (Fig. 5C, E). Some vessel elements show tyloses (Fig. 5F). The rays are unligified and appear dilated due to an increase in cell volume and anticlinal and periclinal divisions (Fig. 5E). Especially in portions where secondary growth is more pronounced, the parenchymatic cells are larger in volume and sometimes occupy the central portion (Fig. 5I). In some portions of the axis protuberances (natural self-grafting) was observed (Fig. 5H, J). The root structure was confirmed by the presence of exarch xylem (Fig. 5G).

3.2.2. Type 2 - underground system composed of a vertical stem with pronounced secondary growth and an adventitious root

The vertically arranged underground stem in secondary growth (Fig. 6A–G) presents numerous buds (Fig. 6C). It is covered by a single periderm (Fig. 6B), which originates in the cortex. The cortical region exhibits cells of different sizes, some of which are sclerified (Fig. 6B). At the limits of the vascular system, associated with the non-conducting phloem, scattered fibers occur (Fig. 6B, D). The vascular system is composed of secondary phloem with sieve tube elements solitary or in clusters (Fig. 6E), with one or two associated companion cells (Fig. 6E). In the secondary xylem, the rings are semi-porous, showing growth layers delimited by larger-diameter vessel elements and radially flattened fibers (Fig. 6B, F). The vessels can be solitary, in radial multiples of 2–8, or occasionally in clusters. The rays have lignified and unligified portions (Fig. 5B), and in the unligified, innermost portions of the secondary xylem, they become dilated (Fig. 6B). The stem structure was

confirmed by the presence of endarch xylem (Fig. 6G). The pith is sclerenchymatous in some portions.

The adventitious root observed in the distal portion of the stem axis (Fig. 6H) exhibits characteristics similar to the taproot tuber of the other individuals, except for the ray dilation, which occurs mainly in its median portion, giving it a convex aspect (Fig. 6H). The root structure was confirmed by the presence of exarch xylem.

3.3. Storage reserves and other compounds in the underground system

Based on the histochemical tests and observations under polarized light, we identified several storage reserves and other compounds (Fig. 7A–Q and Table 1). Idioblasts with calcium oxalate crystals of diverse shapes are present in both the stems, sobole (Fig. 7A–C) and vertical stem, and the root organs, tuberous (Fig. 7G) and adventitious. They can be observed in the cortex, the axial parenchyma of the secondary xylem, the ray parenchyma, and the pith. Idioblasts with druses occur in fewer quantities in the pith of the sobole. Inulin spherocrystals are present in the vertical stem and sobole (Fig. 7D) largely associated with the vessel elements. While in the tuberous roots, they are found in the cortical parenchyma (Fig. 7F) but can also occur associated with axial and radial parenchyma. The composition of the crystals as inulin was confirmed by observing their typical spheroidal shape under polarized light.

For the histochemical tests performed on the type 1 underground system, positive results were obtained only for proteins. In the ray parenchyma of the sobole (Fig. 7E), and in the tuberous root portion, especially in the ray parenchyma (Fig. 7I–J), but in smaller quantities in

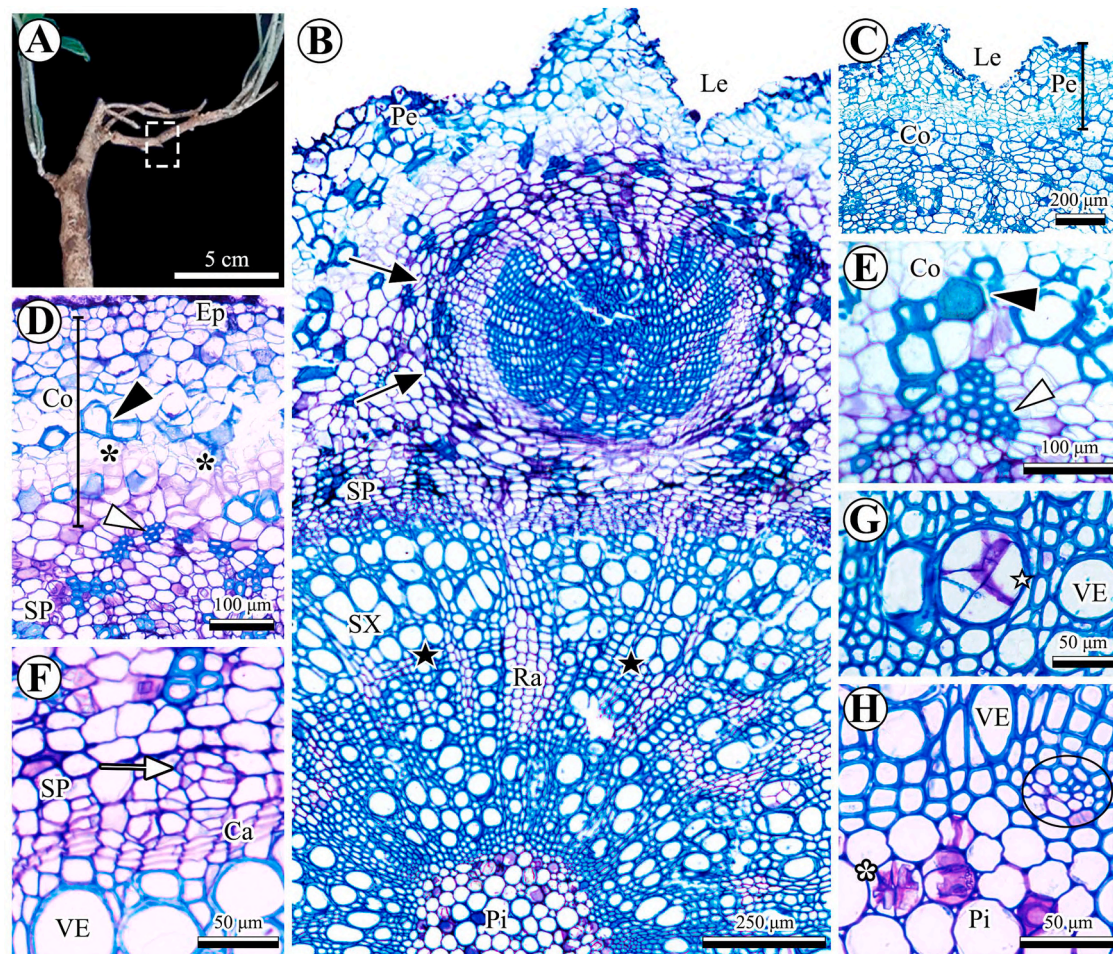


Fig. 4. Morphoanatomy of the type 1 underground system of *Pombalia velutina*. (A) General morphology of the proximal portion of the underground system, detail of the sobole (dashed line). (B-H) Cross sections. (B) Showing a natural self-grafting (black arrow) and highlighting the secondary xylem with a growth ring (black star). (C) Detail of the periderm and a lenticel with homogeneous filling tissue. (D) Cortical region showing cell divisions (black asterisk) indicating the origin of the periderm. (E) Sclerified cells in the cortex (black arrowhead) and pericyclic fibers (white arrowhead). (F) Clusters sieve tube elements of the secondary phloem (white arrow). (G) Detail of a vessel element of the secondary xylem with a tylosis (white star). (H) Pith detail showing protoxylem (black circle), and a druse (white asterisk). Ca = vascular cambium; Co = cortex; Ep = epidermis; Le = lenticel; Pe = periderm; Pi = pith; Ra = ray; SP = secondary phloem; SX = secondary xylem; VE = vessel elements.

the periderm (Fig. 7K) and inside the vessel elements of the secondary xylem (Fig. 7L).

On the vertical stem of the type 2 (Fig. 7M-Q), positive results were obtained for acidic polysaccharides (Fig. 7N), pectin (Fig. 7O), lipids (Fig. 7P), and proteins (Fig. 7Q) inside the vessel elements of the secondary xylem. Furthermore, vessel elements filled with a granular substance that did not react to the histochemical tests were observed (Fig. 7M-O, Q). More detailed morphological observations revealed that some regions of the stem axis exhibited a deteriorated appearance, and it was precisely in these regions that the vessel elements were more filled with these substances. We hypothesize that this may indicate the presence of pathogens, perhaps fungi.

4. Discussion

We observed two distinct morphologies in the underground systems of the analyzed *Pombalia velutina* individuals: one consisting of a sobole and a tuberous taproot, and the other of a vertically oriented thickened stem and adventitious roots. These contrasting morphologies reveal structural diversity in the underground system of *P. velutina* and provide a basis for discussing their potential roles in storage, resprouting, and persistence under environmental stress.

Regarding the underground stem observed in type 1, we decided to

designate it as a sobole. Soboles represent a specialized underground stem, described by Pausas et al. (2018) as woody and elongated stems that grow horizontally beneath the soil surface and produce new aerial shoots from buds located along their axis or at the nodes that connect them. The authors consider the term sobole as synonymous with woody rhizome. Unlike sobole, non-woody rhizomes (rhizomes s.s.) do not exhibit secondary growth and can be classified as fleshy or dry and rigid (Pausas et al., 2018). Our observations are consistent with the report by Bennett (1972) regarding underground rhizomes in hybanthoid species in Australia - a country known for the occurrence of intense seasonal fires - and with the records of specialized underground organs in the *P. lanata* clade, native to the central area of the Brazilian Cerrado (Paula-Souza et al., 2011, 2021; Gonçalves et al., unpubl. res.).

In contrast to the stem portion, the root tuberization observed in type 1 occurs primarily due to volume increase and cell proliferation in the cortical region and the radial parenchyma, which confers a fleshy consistency to the underground organ. Regarding the dilation of the xylem rays, particularly pronounced in organs of root nature studied here, we hypothesize that this feature is associated with the accumulation of storage compounds, such as proteins and inulin, detected in our analyses. The combination of cell enlargement and proliferation observed in portions of these rays may increase the volume of living parenchyma available for the storage of these compounds, potentially contributing to

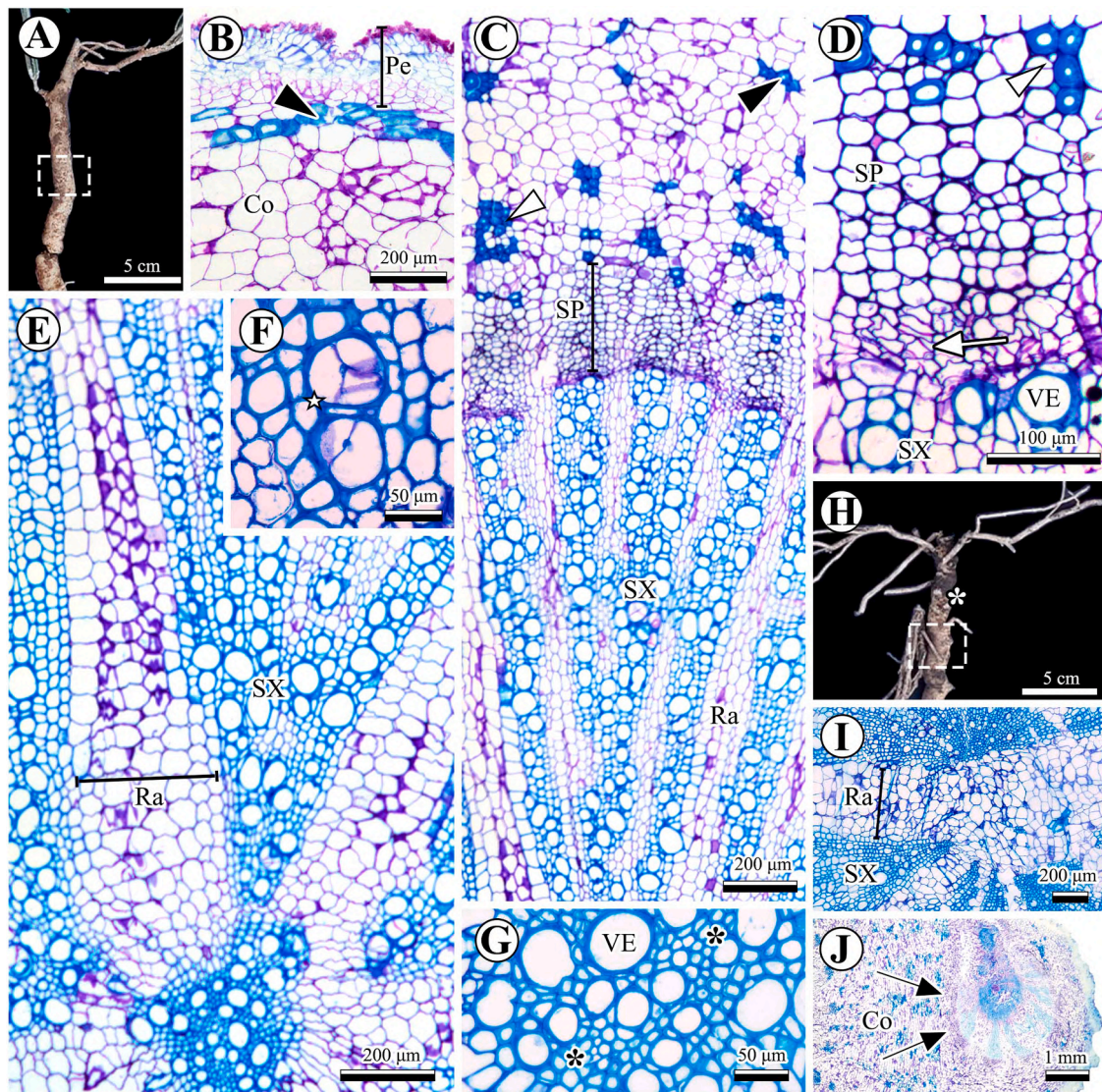


Fig. 5. Morphoanatomy of the type 1 underground system of *Pombalia velutina*. (A) General morphology of the proximal portion of the taproot tuber (dashed line), whose anatomy is illustrated in images B–G. (B) Sclerified phelloderm cells (black arrowhead). (C) Sclerified cells in the cortex (black arrowhead) and pericyclic fibers (white arrowhead). (D) Detail of the secondary phloem with pericyclic fibers (white arrowhead) and sieve tube elements (white arrow). (E) Secondary xylem with non-lignified dilated rays in the central portion. (F) Detail of vessel elements with tyloses (white star). (G) Detail showing protoxylem poles (black asterisk). (H) General morphology of the proximal portion of taproot tuber (dashed line) and protuberances (natural self-grafting) (white asterisk), whose anatomy is illustrated in images I and J. (I) Detail of the proliferation of parenchymatous cells occupying the central portion of the root. (J) Detail of the self-grafting region (black arrow). Co = cortex; Pe = periderm; Ra = ray; SP = secondary phloem; SX = secondary xylem; VE = vessel elements.

the expansion of the tuberous root. A comparable pattern has been reported in the tuberous roots of Cucurbitaceae species, in which starch grains accumulate in the radial parenchyma (Burrows and Shaik, 2015), supporting a potential role for this tissue in storage during root thickening.

Underground systems composed of tuberous roots are commonly recorded in other Cerrado species, in families such as Asteraceae and Apocynaceae (Appezato-da-Glória and Estelita, 2000; Vilhalva and Appezato-da-Glória, 2006; Hayashi and Appezato-da-Glória, 2007; Abdalla et al., 2016), and are associated with the storage of reserves that aid in the resprouting process (Moreira et al., 2012). Contraction zones and protuberances, identified as natural self-grafting, were also observed in this portion of the root. Root contractions have been recorded in other perennial herbaceous species, related to the process of plant self-burial, a mechanism that allows the displacement of buds to deeper regions of the soil (Hayashi and Appezato-da-Glória, 2007; Appezato-da-Glória, 2015). Based on these characteristics, and

according to Pausas et al. (2018), we consider the taproot tuber to be the most appropriate terminology for the organ observed in type 1.

The type 2 underground system is characterized by a vertically oriented underground stem axis, with adventitious roots emerging from its distal portion, and by a well-pronounced secondary growth. These features share similarities with rhizophores, specialized structures that can compose underground systems. Rhizophores are characterized by a bipolar system of stem branching, where there is an aerial stem with negative geotropism and an underground stem with positive geotropism (Menezes, 2007). The primary root interrupts its development, and all roots are formed from this underground structure (Appezato-da-Glória, 2015). The use of the term rhizophore in angiosperms was established following a study by Menezes et al. (1979), to designate underground stem structures with root emission in *Vernonia* species (Asteraceae). According to Pausas et al. (2018), the term rhizophore is currently used to designate underground structures present in species from fire-prone environments, and refers to non-woody, slightly fleshy, and leafless

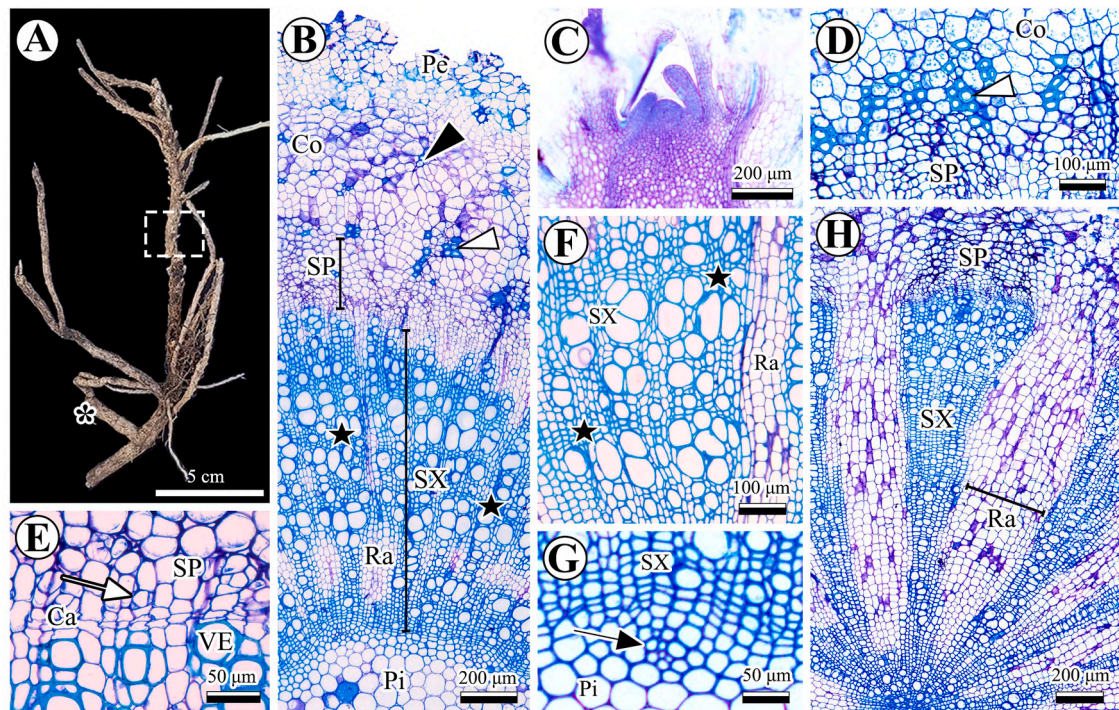


Fig. 6. Morphoanatomy of the type 2 underground system of *Pombalia velutina*. (B-H) Cross sections. (B-G) Median stem portion. (H) Adventitious root. (A) General morphology of the underground system. In detail, the median stem portion (dashed lines) and the distal portion with an adventitious root (white asterisk). (B) Overview evidencing sclerified cells in the cortex (black arrowhead), pericyclic fibers (white arrowhead), and growth rings (black star). (C) Detail of a bud. (D-E) Detail of the secondary phloem. (D) Pericyclic fibers (white arrowhead). (E) Sieve tube elements and companion cells (white arrow). (F) Detail of the secondary xylem with growth rings distinct (black star) and a dilated ray in its innermost, nonlignified portion. (G) Pith detail showing protoxylem (black arrow). (H) Highlighting dilated rays in the median portion. Ca = cambium; Co = cortex; Pe = periderm; Pi = pith; Ra = ray; SP = secondary phloem; SX = secondary xylem.

rhizomes. Although the underground system of type 2 shares some similarities with these structures, especially due to the vertical arrangement of the stem axis and the emergence of an adventitious root at the distal end, it differs by presenting a well-pronounced secondary growth, a characteristic that is inconsistent with the rhizophore described by Pausas et al. (2018). Therefore, given the absence of ontogenetic studies clarifying the nature of this structure, we chose to refer to it more generically as an underground stem.

The stem structures that compose the underground systems of *P. velutina* exhibited a large number of buds. The underground allocation of buds, in addition to favoring vegetative propagation, represents an effective protection strategy, as the soil acts as an efficient thermal insulator (Auld and Bradstock, 1996), reducing extreme temperature variations (Tromp, 1983). This set of dormant buds is known as a bud bank, and its presence enables the regeneration of perennial plants after fires, herbivory, drought, agricultural management, or other environmental disturbances (Klimesová and Klimes, 2007; Ott et al., 2019).

Aside from the *P. lanata* complex, there are few references to specialized underground systems within the family. The underground organs of the Australian species *Pigea calycina* (DC.) F.Muell., *Pigea debilissima* (F.Muell.) P.I.Forst., *Pigea volubilis* (E.M.Benn.) P.I.Forst., and *Pigea monopetala* (Schult.) Ging. were designated as underground rhizomes (Bennett, 1972). Although the same underground organ was reported for all above-mentioned hybanthoid species, there is no evidence that they constitute a morphologically or phylogenetically distinct group, as occurs in the *Pombalia lanata* complex. An additional case is *Paypayrola arenacea* Aymard & G. A. Romero, a small dwarf tree native to the shrub savannas of Venezuela (Aymard et al., 2014), in which the underground organ was termed a lignotuber. However, since the descriptions of the vegetative structures of all the species described above were based on herbarium specimens and no anatomical analysis was performed to substantiate the terminologies used by the authors, the nature of these organs still needs to be confirmed.

Regarding the histochemical tests, the presence of pectic and lipid compounds in the vessel elements of the secondary xylem may be associated with the storage and reallocation of energy reserves or with protection against pathogens. Pectins are a complex group of hydrophilic polysaccharides located mainly in the middle lamella and the primary cell wall of plants. They also constitute a major component of mucilage, which are associated with water absorption and contribute to desiccation tolerance (Fahn, 1982). In an investigation of *Platanus x acerifolia* seedlings exposed to the vascular fungus *Ceratocystis fimbriata* f. sp. *platani* (Clérivet et al., 2000), the authors reported that the formation of tyloses and the deposition of gels rich in phenolic compounds and polysaccharides, such as pectin and cellulose, represent defense responses associated with metabolic changes in vessel associated cells. This response appears to be efficient in compartmentalizing the fungus, restricting its spread.

Beyond pectic compounds, lipids are commonly synthesized by specialized secretory tissues and serve multiple physiological roles, including energy and carbon storage, as well as structural functions in cell membranes (Fahn, 1982; Dickson, 2000; Taiz et al., 2017). Recent studies have also identified lipids in the xylem sap, forming a thin layer along the inner walls of xylem elements after cell death (Schenk et al., 2017; Levionnois et al., 2022; Huang et al., 2024). The lipid coatings may, in some cases, contribute to the prevention of gas embolism by stabilizing nanobubbles within the xylem sap (Schenk et al., 2017).

The proteins that were present in large quantities in the taproot tuber of the species analyzed here have previously been detected in the roots of other members of the *Pombalia lanata* complex (Pinto et al., 2018; Gonçalves et al., unpubl. res.). Their presence is generally associated with defense mechanisms against pathogens, stress responses, or nitrogen storage (Staswick, 1994; Corre et al., 1996; Bewley, 2002; Adamczyk et al., 2010). Violaceae is particularly rich and diverse in cyclotides, small circular proteins that exhibit intense biological activity against microorganisms and have been reported in various *Pombalia*

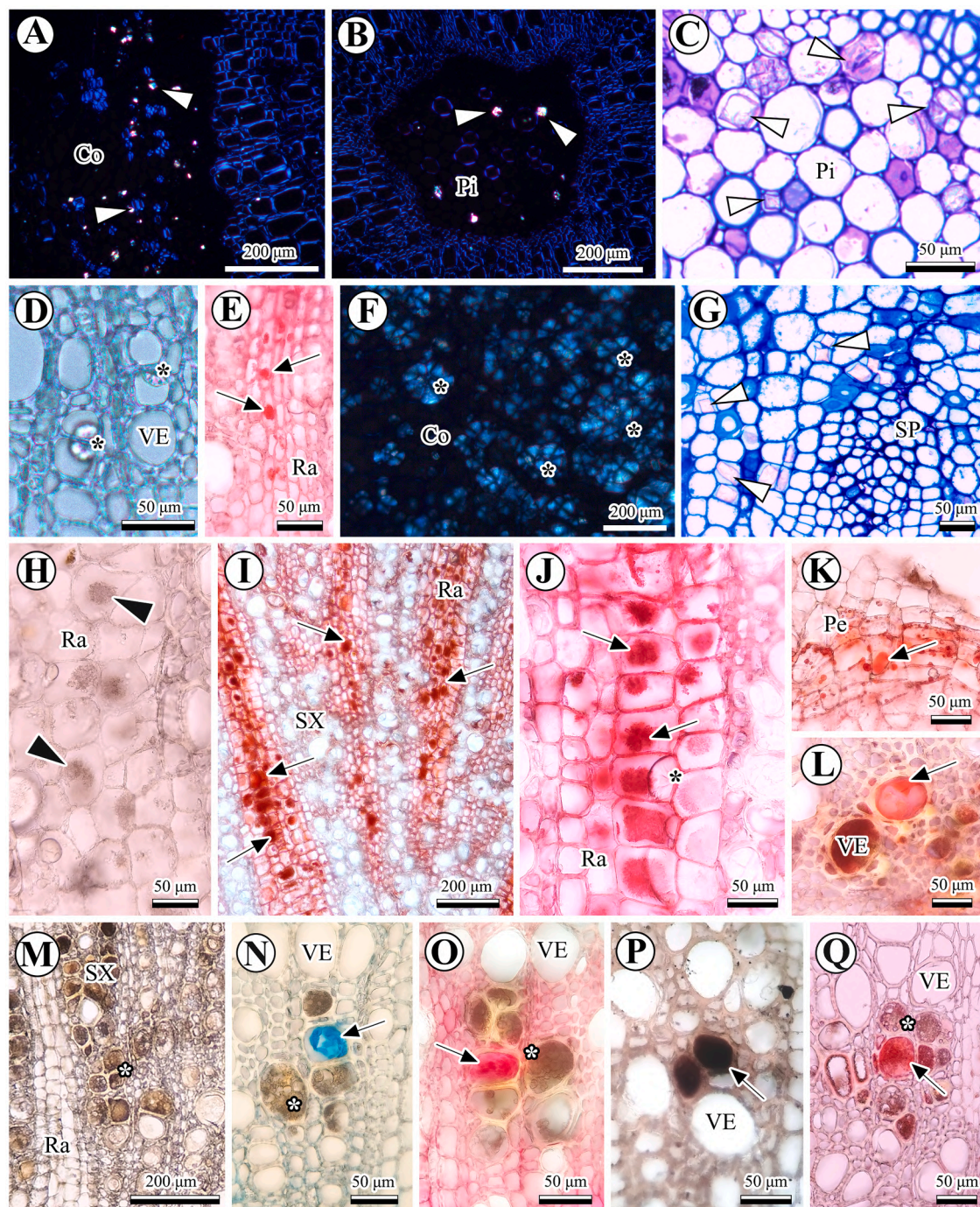


Fig. 7. Transverse sections of the sobole (A-E), the proximal portion of the taproot tuber (F-L), and the vertical stem (M-Q) of *Pombalia velutina*. (A-B) Oxalate crystals (white arrowhead) under polarized light in the cortex (A) and in the pith (B). (C) Detail of the pith with oxalate crystals (white arrowhead). (D) Detail of inulin spherocrystals inside the vessel elements in fresh material (black asterisk). (E) Positive reaction (black arrow) for proteins in parenchymatic ray cells. (F) Inulin spherocrystals in the cortex (black asterisk). (G) Prismatic crystals associated with the secondary phloem (white arrowhead). (H) Control section. (I-J) Positive reaction (black arrow) for proteins and inulin spherocrystal (black asterisk) in the parenchymatic ray cells. (K-L) Positive reaction for proteins (black arrow) in the periderm (K), and inside vessel element of the secondary xylem (L). (M) Control section. (N-Q) Positive reaction (black arrow) for acidic polysaccharides (N), pectic substances (O), lipids (P), and proteins (Q). Co = cortex; Pe = periderm; Pi = pith; SP = secondary phloem; SX = secondary xylem; Ra = ray; VE = vessel elements.

species (Burman et al., 2015; Fernández-Bobey et al., 2023). The analysis carried out in this study did not test the nature of the proteins found in *P. velutina* roots, but cyclotides have already been identified in *P. lanata* leaves (Cunha et al., 2016) and in *P. calceolaria* (L.) Paula-Souza roots (Pinto et al., 2018), the sister group to the *P. lanata* clade. Considering the longstanding medicinal use of other species in the clade and the potential for the discovery of new biomolecules of interest to the agrochemical and pharmaceutical industries, this can be

considered a promising field for future exploration.

In addition to the compounds mentioned previously, observations under polarized light revealed inulin spherocrystals in all analyzed individuals. Although this is an uncommon record for Violaceae, the presence of inulin spherocrystals in the roots of *Ionidium ipecacuanha* (L.) Vent. (= *Pombalia calceolaria*) had already been reported by Solereder (1908) and Pontes (2016). In *P. velutina*, the inulin spherocrystals were observed in both the stem and root portions, predominantly

Table 1
Histochemical tests with positive results in different portions of the underground systems of *Pombalia velutina*. VE = vessel element; RP = ray parenchyma; Pe = periderm; - = weak or absent reaction; + = moderate reaction; ++ = strong reaction.

Reagent	Metabolite Groups	Morphological type 1				Morphological type 2 Underground vertical stem VE
		Sobole	Taproot tuber			
		RP	RP	VE	Pe	
Alcian Blue	Acidic polysaccharides	-	-	-	-	+
Ruthenium Red	Pectins	-	-	-	-	+
Sudan Black B	Lipids	-	-	-	-	+
Xylidine Ponceau	Proteins	+	++	+	+	+

distributed in the cortical parenchyma, but also visible in different tissues of the vascular cylinder. According to Pontes et al. (2016), in the roots of *P. calceolaria*, inulin appears to be more concentrated in the cortical parenchyma.

Inulin-type fructans are among the most abundant carbohydrates and represent one of the main reserve compounds in flowering plants (Hendry and Wallace, 1993). They are primarily described in species of Asteraceae, Amaranthaceae, and Poaceae (Tertuliano and Figueiredo-Ribeiro, 1993; Vieira and Figueiredo-Ribeiro, 1993; Appezzato-da-Glória and Cury, 2011; Abdalla et al., 2016; Joaquim et al., 2018; Carbone et al., 2019). In addition to functioning as reserve substances, fructans also promote drought resistance, cold tolerance, and maintenance of plant growth during periods of limited water availability (Hendry, 1993) and interact with cell membranes to promote their stabilization under stress conditions (Hinch et al., 2007). Future functional studies could help elucidate the role of these compounds in the resistance of *P. velutina* to adverse environmental conditions.

Although the Campos Gerais region is also susceptible to natural fires in late winter/early spring, these fires are not as frequent or intense as those in the Central Plateau, due to much less pronounced pluviometric seasonality and weaker winds during the dry season (<https://pt.weatherspark.com/> © Cedar Lake Ventures, Inc.). Thus, it is reasonable to infer that lower average temperatures and, particularly, the frequent occurrence of severe frosts constitute more limiting factors than fire for *P. velutina* populations, as also observed in Old World savanna ecosystems (Davies et al., 2016; Finckh et al., 2016). In this context, similar to other plant groups (Proteaceae, Lamont et al., 2017; Poaceae, Schat et al., 2025), the species's ability to cope with freezing temperatures could be interpreted as a shift from the pre-existing condition of drought and/or fire resistance observed in other species of the clade and in the clade's sister species, *P. arenaria* (Ule) Paula-Souza, native to the northern portion of the Cerrado and the Caatinga (dry forest), and *P. calceolaria*, widely distributed in the Cerrado, the Caatinga, and in dry environments of the Atlantic Forest and the Amazon, extending to Mexico. It is worth to note that to confirm this hypothesis, future studies integrating phylogenetic, ecological, and morphoanatomical data is needed and certainly will help to clarify the evolutionary history of underground systems within the *P. lanata* complex. Nevertheless, the data obtained in this study contribute to our understanding of the structural diversity of underground systems, but the evolutionary processes underlying the origin and maintenance of these characteristics still remain unclear.

5. Conclusion

This study provides the first comprehensive analysis of the underground morphoanatomy of *P. velutina*, revealing the nature of an

underground system with mixed origins composed of storage structures and numerous buds. These features highlight the structural complexity of the underground systems and provide a basis for understanding their role in resprouting and the persistence of herbaceous species in the Campos Gerais savannas. Finally, considering the morphological variation observed among individuals, we suggest carrying out complementary research that addresses ecological and ontogenetic aspects, aiming to improve the understanding of these complex structures.

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CRediT authorship contribution statement

Anelise Gabriela Grotto: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Juliana de Paula-Souza: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. Marcelo Rodrigo Pace: Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition. Ana Claudia Rodrigues: Writing – review & editing. Makeli Garibotti Lusa: Writing – review & editing. Fernanda Maria Cordeiro de Oliveira: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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