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Seed morphoanatomy may confer restraints against the germination of *Pouteria glomerata* (Sapotaceae) in a Neotropical wetland

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ABSTRACT. The tree-shrub species *Pouteria glomerata* (Miq.) Radlk. (Sapotaceae) occurs in periodically flooded areas marked by periods of drought. It has the potential for use in the ecological restoration of riparian forests; however, seed germination strategies were not fully understood. In this study, we aimed to evaluate the morphology, anatomy, and physiological aspects of the seed to identify possible factors related to low germination. The morphoanatomical seed analysis was assessed according to the routine procedures. For physiological assessment, seed germination was estimated under different conditions; seeds were scarified and soaked in water to overcome dormancy. Seeds store starch, lipids, and proteins, and are covered by a hard and thick seed coat. The embryo was underdeveloped consisting of a mass of meristematic cells. Numerous laticifers and phenolic idioblasts were found, mostly on the periphery of the cotyledons. Germination was hypogeal and the seedling was cryptocotylar type. The treatments to overcome seed dormancy were not efficient to break dormancy. The morphoanatomical analysis suggested that the dormancy of *P. glomerata* seeds may be related to an impermeable seed coat and undifferentiated or immature embryo indicating a morphophysiological dormancy.

Keywords: immature embryo; Pantanal wetland; seed dormancy; seed coat; seed morphoanatomy.

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Introduction

The Brazilian Pantanal wetland is a seasonal humid area marked by alternating rainy and dry seasons. The annual flood pulse is the main driving force that determines local ecological conditions (Nunes da Cunha et al., 2021); however, a portion of the Pantanal region is fire-prone where flooding and fire significantly impacts species distribution (Damasceno-Junior et al., 2021). Among the plant species adapted to flooding and associated with riparian forests is *Pouteria glomerata* (Miq.) Radlk. (Sapotaceae) (Damasceno-Junior et al., 2005; Silva et al., 2021).

Pouteria glomerata is a tropical tree-shrub species, native to the Americas (Plants of the World Online [POWO], 2024) and occurs in the phytogeographic domains of the Amazon forest, Cerrado (Brazilian savanna), Atlantic Forest (Alves-Araújo, 2015), floodplains, igapó, terra-firme, gallery forests (CNC Flora, 2022), and riparian forests (Silva et al., 2021) in Brazil. In the Pantanal, *P. glomerata* is distributed in sandy and clayey soils of low fertility, periodically flooded, and subject to drought and occasional natural fire disturbances (Pott & Pott, 1994, 2002, 2021). The study species is a key player species in the ecosystem as a pioneer, which colonizes the riparian forests during periods of flooding in the Pantanal (Pott & Pott, 1994). Therefore, its seeds may be impacted by the flooding, drought, and occasional natural fire disturbances.

Seed germination is a physiological and metabolic process that aims to resume the embryonic axis's growth with the primary root's protrusion (Bewley & Black, 1994). This process can fail to initiate due to the lack of essential factors such as optimal temperature, water availability, or dormancy (Bewley et al., 2012). The last factor prevents or delays the beginning of the resumption of embryo development (Marcos Filho, 2005); however, it contributes to seed dispersion over time and space while increasing the chance of successful seedling establishment (Bewley et al., 2012; Baskin & Baskin, 2014). Thus, the ecological implications of seed dormancy interfere with the species distribution and ecology (Aguiar et al., 1993; Baskin & Baskin, 2014).

Seed dormancy can be a physical restriction, caused by the morpho-anatomical structure of tegument or endosperm, which confers resistance and impermeability to the seed (Bewley et al., 2012; Baskin & Baskin, 2014), or physiological restrictions as result of development of the embryo tissues, and presence of growth inhibitors, such as phenolic compounds (Bewley et al., 2012; Baskin & Baskin, 2014). In some cases, the fruits' endocarp may be stony, and their hardness may prevent the expansion of the embryo (Cardoso, 2004).

Studies focusing on the morpho-anatomical and physiological characteristics of seeds are critical for the scientific interpretation of species' reproductive strategies and adaptations in natural habitats (Baskin & Baskin, 2001) and for propagation (Bewley et al., 2012). Previous research have shown that some aquatic angiosperms and species subject to annual flooding periods develop specific reproductive strategies, like seeds with prolonged dormancy and hypogeal seedlings to improve flooding tolerance (Parolin et al., 2003; Soares et al., 2021a, 2021b).

Although *P. glomerata* plays an important ecological role and has a potential for ecological restoration, little is known about its seed biology. Thus, we aim to evaluate the germination of its seeds under different treatments, describe seed anatomy, seed and seedling morphology, and correlate these characteristics with germination responses and reproductive strategies. We hypothesize that *P. glomerata* seeds have dormancy associated with anatomical traits that hinder its germination and seedling establishment in flooded habitats; the type of seedlings combined with seed characteristics might enhance its flooding tolerance and contribute to its wide distribution in areas periodically flooded, and subject to water deficit in a markedly seasonal climate.

Material and methods

Seed collection

Fruits of *P. glomerata* were collected from wild stands of a single population occurring along the Paraguay River, municipality of Corumbá (Coordinates: Latitude 19º1"39'S Longitude 57º38"1'W), in the Pantanal wetland region, Mato Grosso do Sul State, Brazil, in February of 2009. Ripe fruits, no matter the shape, with little or no apparent damage were collected from trees and on the ground, and mixed in one batch. Fruits can vary independently of the individual plant (Figure 1). Seeds were manually extracted, mucilage was removed by running water, and the seeds were dried superficially on absorbent paper under shade.

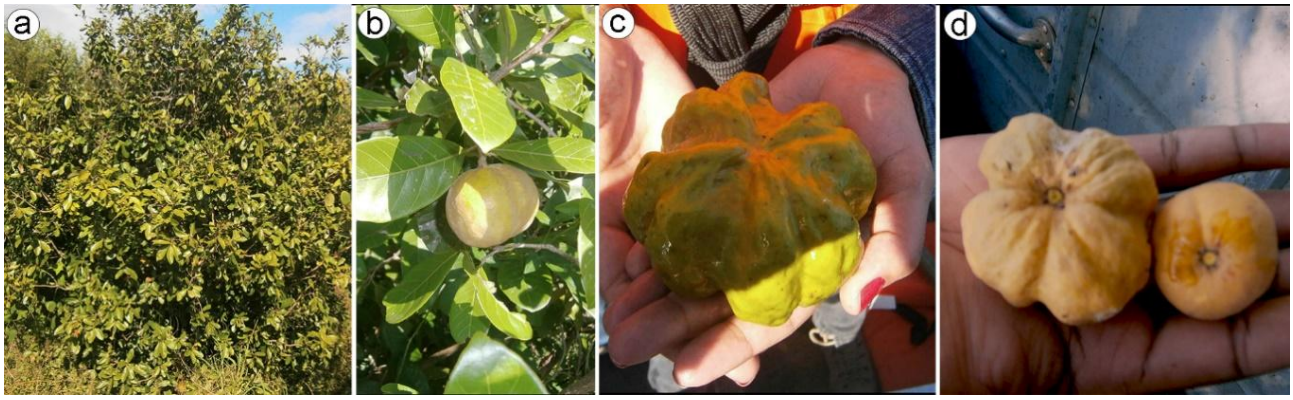

Figure 1. *Pouteria glomerata* (Sapotaceae). (a) plant habit; (b) detail of branches with leaves and fruit; (c-d) variation in fruit morphology and size. (Images: C. S. Farias).

Seed characterization and germination tests

After cleaning and drying the seeds, 1000 seeds were weighed according to the Brazilian Rules to Seed Testing (Regras para Análise de Sementes, 2009) and the International Seed Testing Association (ISTA, 2024), and 100 seeds were used to determine biometrics (length, width, and thickness).

To assess seed dormancy, three tests were carried out in the laboratory using four replicates of 20 non-scarified seeds (total =100 seeds each). The tests were carried out in a germination incubator under the alternative temperature of 20-30ºC (photoperiod of 12 hours with light at 30 ºC and 12 hours without light at 20ºC) and light (1) sealed in transparent plastic bags, or dark (2) by using black plastic bags. This alternative temperature simulates the common temperature range in the species' natural habitat during the fruit

dispersion period, which occurs from January to August (Pott & Pott, 1994). For the germination tests, seeds were placed between two paper towels moistened with distilled water equivalent to 2.5 times the weight of the dry paper (optimal amount for most tropical seeds in Brazil) according to the Brazilian Rules to Seed Testing (Regras para Análise de Sementes, 2009), and rolled up forming rolls. Then, the rolls were placed together inside plastic bags 0.04 mm and sealed with an elastic to avoid desiccation. Germinated seeds in the dark treatments were counted under green light according to Amaral-Baroli and Takaki (2001). The germination criterion was the radicle protrusion counting daily, and the germination percentages were determined at 60 and 90 days after sowing.

In the third test (seedling emergence test), the seeds were placed in plastic trays containing expanded vermiculite and maintained in the laboratory at room temperature (~ 25 °C under 16 hours light and 8 hours dark; 60% RH). The substrate was replenished with water daily as needed. The germination criterion was the emergence of seedlings when the emerged epicotyl was visible on the surface of the substrate. The emerged seedlings were counted at 60, 90, and 270 days after sowing. Results were expressed as a percentage of emerged seedlings.

Treatments to overcome seed dormancy

The results of the germination tests showed that the seed exhibit dormancy. Thus, considering the environments *P. glomerata* species occurs, subject to periodic flooding, and following recommendations by Regras para Análise de Sementes (2009) and Manual de Análise Sanitária de Sementes (2009), we defined two treatments to overcome dormancy: (1) soak seeds in a 0.5% sodium hypochlorite solution for 1 minute at room temperature (25 ºC) (Regras para Análise de Sementes, 2009; Manual de Análise Sanitária de Sementes, 2009), and then drying on the bench for 4 hours followed by scarification with 220 grit wood sandpaper at the end opposite the hilum (Regras para Análise de Sementes, 2009); and (2) soak seeds in water at 40 ºC for 24 hours (Regras para Análise de Sementes, 2009) followed by 1 minute in 0.5% sodium hypochlorite solution (Regras para Análise de Sementes, 2009; Manual de Análise Sanitária de Sementes, 2009). We performed mechanical scarification by removing the seed coat tissues completely until exposing the embryonic content. Next, we set up germination tests following the methods above (see seed characterization and germination tests section).

Germination and seedling morphology

To understand the reproductive adaptive strategy of the species, we described the type of germination and seedling by sowing 50 seeds in plastic trays with wet expanded vermiculite, to monitor the germination process, and recording it by botanical illustration of the seed and seedlings. The seedlings were classified as normal or abnormal according to Regras para Análise de Sementes (2009) and the Association of Official Seed Analysts (AOSA, 1992). The terms used were based on Duke (1969), Oliveira (1993), Ferreira et al. (2001), Souza (2003), and Camargo et al. (2008). The illustrations were made manually and finished with Indian ink to create the botanical illustration boards.

Morphoanatomical description of the seeds

For the seed morphoanatomical description, observations were carried out under a stereomicroscope. Color, texture, shape, size, consistency of the seed coat, position of the hilum, and micropyle were identified. Illustrations of the seed were made by hand and using a magnifying glass. In a sample of 100 seeds, the length, width, and thickness of the seeds were measured using a caliper. The methods and terms used were based on Joly (1993), Damião-Filho (1993), Barroso et al. (1999), Vidal and Vidal (2000), Ferreira et al. (2001), Souza (2003) and Camargo et al. (2008). To describe the internal structures, seeds were hydrated in water for 24 hours to facilitate the removal of seed coat. These seed traits were analyzed: presence and shape of the embryo, morphology of the cotyledons, and type of reserve according to histochemical analyses.

Light microscopy analysis

After fruit collection, 20 mature seeds were immediately fixed in buffered neutral formalin for 48 hours (Johansen, 1940), washed with water, dehydrated in ethanolic series, and stored in ethanol 70% (Kraus & Arduin, 1997). Seeds were infiltrated in paraffin, sectioned in a rotary microtome (10-15µm, Leica RM-2145; Leica Microsystem, Germany), stained with safranin and Astra blue, and permanently mounted in synthetic

resin. All slides were analyzed under light microscopy (Olympus CX-41F). Photomicrographs were obtained with a Leica DMLB (Leica Microsystem, Germany) coupled to an image capture system and camera (Leica DC 300F, Leica Suite Application v.3.0.8). Histochemical tests were performed on free-hand sections with Sudan IV to identify lipophilic substances (Pearse, 1972), IKI (iodine potassium iodine) for starch grains (Johansen, 1940), bromophenol blue to identify proteins (Mazia et al., 1953), phloroglucinol and hydrochloric acid to recognize lignified cell walls (Johansen, 1940), and ferric chloride for phenolic compounds (Johansen, 1940). Control slides were prepared following protocols. For scanning electron microscopic (SEM) analyses, seed fragments of about 0.5cm were fixed on stubs, and coated with a thin layer of gold (Dentum Vaccum Desk IV Standard Sputter Coater, LLC). Observations and images were obtained using a JEOL JSM-6880LV scanning electron microscope (JEOL, Japan).

Results

Seed characterization and germination tests

Biometric analysis showed that seeds were 18.8 ± 1.1 cm long, 14.9 ± 1.0 cm wide, and 13.1 ± 0.8 cm thick. The individual seed weight was estimated at 2.29 grams and the average weight of 1000 seeds was 2,295.4 ± 90.6 grams. Non-scarified seeds under light (test 1) or dark (test 2) conditions did not germinate during the evaluating period. In the third test (emergency test), only 4% of seedlings emerged after 270 days of sowing (Table 1).

Table 1. Percentage of germinated seeds and emerged seedlings of *Pouteria glomerata* (Sapotaceae), after 60, 90, and 270 days of sowing. Untreated seeds (controls) and subjected to pre-germination treatments (mechanical scarification with sandpaper and soaking in water at 40 ºC for 24 hours) were evaluated. Germination tests were conducted at 20-30 °C (12h light and 12h dark) and 20-30 °C (24h dark) and emergence test at room temperature (~25°C). The dash indicates the test was not continued to this date.

	Controls			Scarification			Soaking in water		
	60	90	270	60	90	270	60	90	270
Germination test at 20-30 °C (12h light 12h dark)	0	0	-	0	16	-	0	4	-
Germination test at 20-30°C (24h dark)	0	0	-	0	0	-	0	0	-
Emergence test at 25 °C	0	0	4	0	-	-	0	-	-

The methods to overcome dormancy were not efficient resulting in a maximum of 16% of germination. We did not observe water imbibition in non-germinated seeds after the germination tests, probably indicating the presence of dormancy. Scarified and soaked in water seeds were removed after 90 days of sowing in the emergence test, as the seeds had fungal contamination. Statistical analyses were not carried out due to the low to zero germination percentage. The tetrazolium test was not performed in non-germinated seeds to evaluate if they were viable, due to the laborious identification and handling of embryos of *P. glomerata*.

Germination and seedling morphology

Germination was hypogeal, and the seedling was the cryptocotylar type (Duke, 1969) (Figure 2), or crypocotylar-hypogeal-reserve according to Garwood (1996), in which the cotyledons are not withdrawn from within the seed coat during germination. Seedling hypocotyl was short (Figure 2c) and lengthened while the primary root elongated. Epicotyl grew and pushed the plumule above the ground (Figure 2d, e). Germination was completed with the elongation of radicle, which passed through the seed coat near the hilar area. The primary root was thick and rigid (Figure 2c) and the aerial part (epicotyl and plumule) began to elongate through a protrusion of the seed coat and had many fine and short trichomes along the stem (Figure 2c, d). It grew slowly, erect, and bore buds (Figure 2e). The radicle came out after 90 days of sowing and the eophylls appeared approximately after 30 days. Eophylls were opposite, petiolate, simple craspedodromous venation, lanceolate limb with an acute apex and obtuse base, and smooth margins (Figure 2f).

Seed morphoanatomy and histochemistry

The shape of the seeds was ellipsoid, with a hard, slightly wavy envelope and light brown color (Figure 3a-b, 2a-c). The basal hilum was large (Figure 3a) with a slight thickening at the edge (Figure 3d). One side of the hilum had a contour line with a slightly oblong shape, not deep but visible, and presented a slight protrusion at the end opposite the hilum (Figures 3a, 2a).

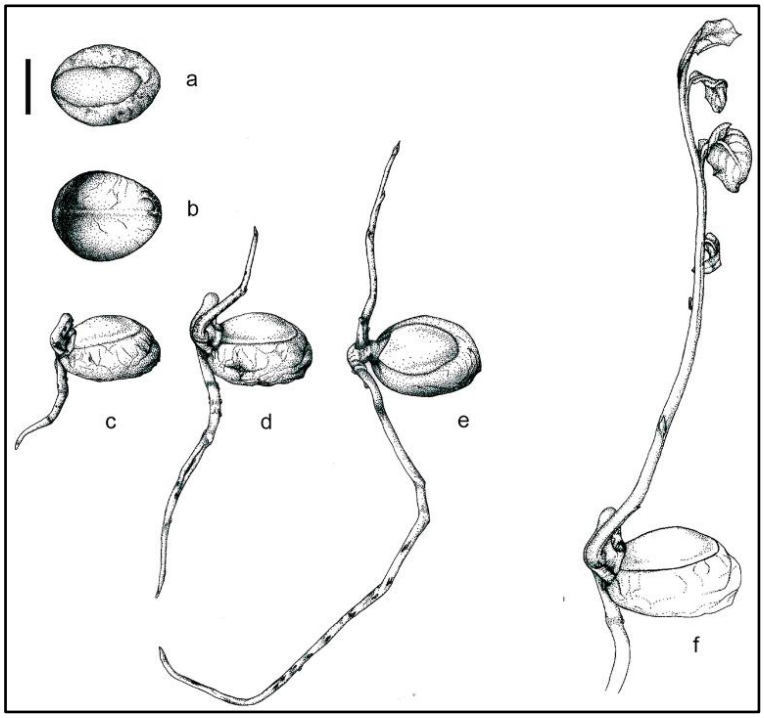

Figure 2. Seedling development phases, from the protrusion of the primary root to the formation of the eophylls of *Pouteria glomerata* (Sapotaceae). (a, b) seed; (c-f) seedling development phases, from the protrusion of the primary root to the formation of the eophylls. Scale bar: 1cm. (Illustration by Lima, L.B.).

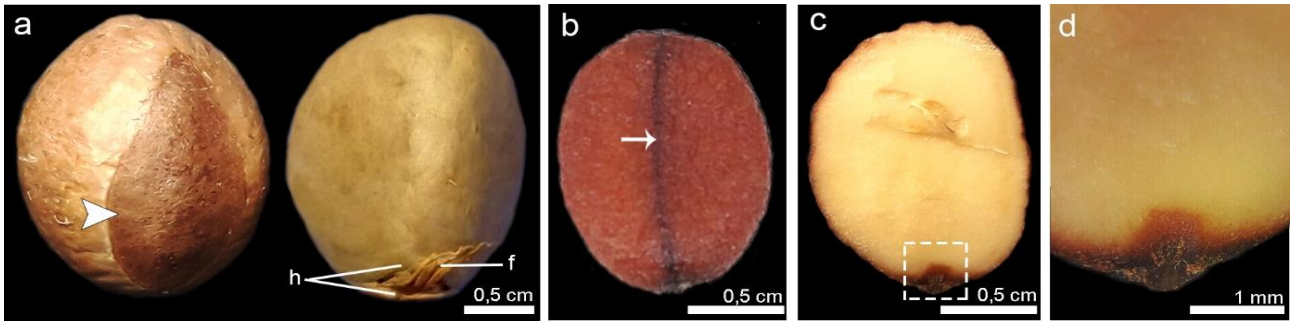

Figure 3. Features of *Pouteria glomerata* (Sapotaceae) seeds. Images under a stereomicroscope. (a) seeds in frontal and back view showing the seed coat surface and hilum, the white arrowhead indicating the dark brown region of seed coat, corresponding to the raphe; (b) seed after seed coat removal, observe the natural reddish-brown color of the seed, white arrow indicating the delimitation of the two cotyledons; (c) the internal side of the seed (after the longitudinal section) evidences the large cotyledon (light yellow structure) and the portion of the hypocotyl-radicle axis of the embryo (in the square); (d) detail of the hypocotyl-radicle axis region**.** Abbreviations: h = hilo, f = funicle.

Pouteria glomerata seeds have a lignified seed coat, which detaches from the surface of mature seeds (Figure 4a). The seed coat was formed by two main layers: an external, thin, and soft layer, and an internal extremely hard with lignified cell wall layer. The external seed coat presented 2-3 cell layers with thin primary cell walls, containing mucilage and phenolic compounds (Figure 4b). The lignified seed coat had many layers with strongly thickened and lignified sclereids arranged in various directions (Figure 4c-e). The cells that form the sclerified layer were also characterized by having phenolic compounds (Figure 4e, f).

The embryo was composed of two storage cotyledons and the hypocotyl-radicle axis (Figure 5). Mature seeds did not have endosperm as storage tissue. The storage cotyledons were extremely rigid in consistency and had different sizes: a larger and a slightly wider one that partially covered the smaller one (Figure 5a, b). The epidermis was one-layered and formed by cells with primary walls. These cells contained phenols and were covered by a thin cuticle (Figure 5c–e). Cells under the epidermis also contained phenolic compounds (Figure 5). The mesophyll of the cotyledons was filled with storage parenchyma formed by cells with rigid walls and full of reserved material. Cells of storage tissue had thick primary cell walls. The cotyledons' reserve tissue consists mainly of starch grains and proteins accumulated in cells with thickened cell walls (Figure 5f-g). Numerous phenolic idioblasts and laticifers were dispersed throughout the cotyledonary tissue (Figure 5h, i). The vascular system of cotyledons was composed of vascular bundles with well developed phloem and few proxylem cells (Figure 5j, k).

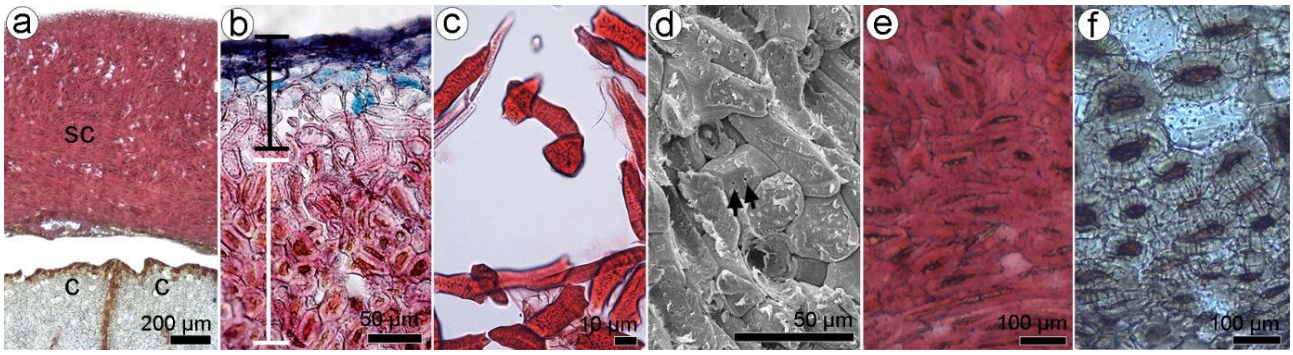

Figure 4. Seed coat of *Pouteria glomerata* (Sapotaceae). Under light microscopy (a-c, e-f) and scanning electronic microscopy - SEM (d). (a) cross-section of the seed coat and part of cotyledons; (b) detail of the seed coat: external (black line) and internal part (woody stratum: white line); (c) detail of the seed coat, isolated cells; (d) detail of sclereids with pits in the secondary cell walls (black arrows); (e) lignified sclereids with histochemical test; (f) lumen of sclereids containing phenolic compounds. Abbreviations: c = cotyledon, sc = seed coat.

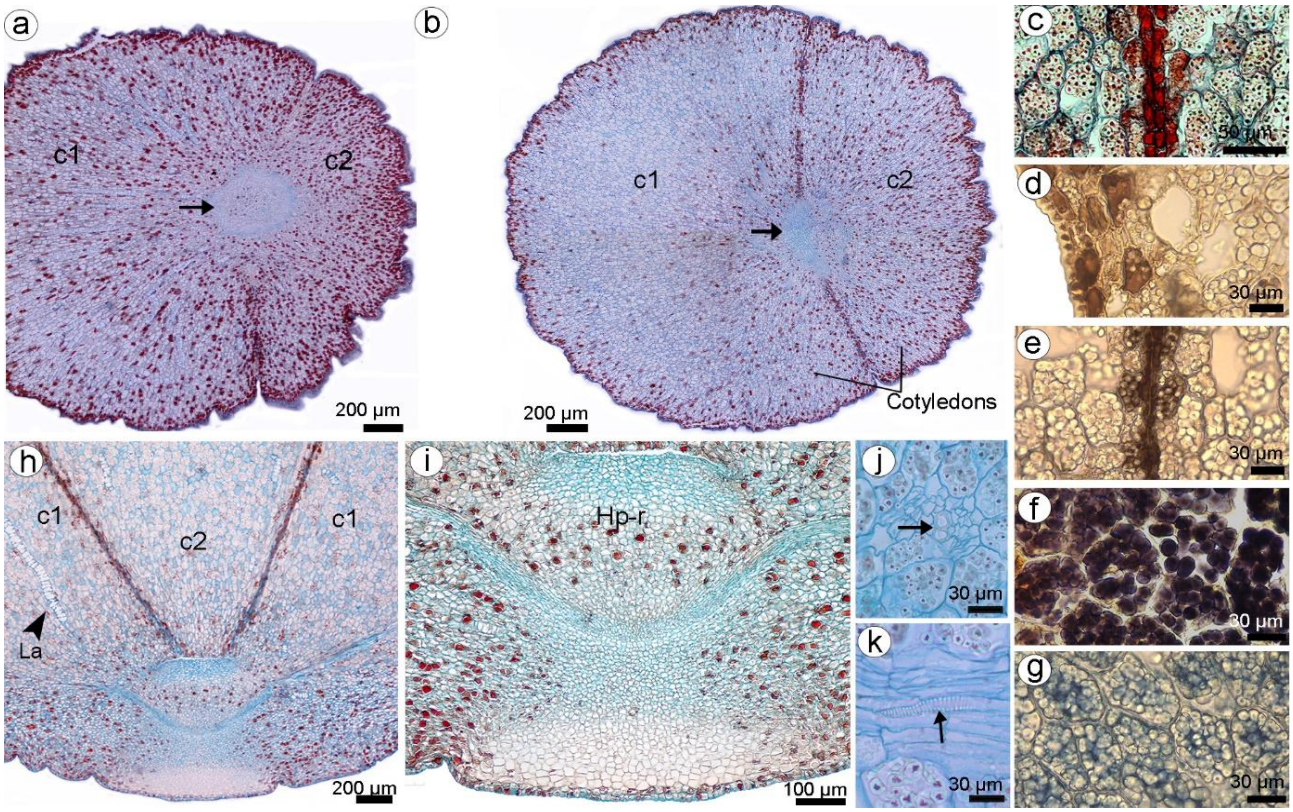

Figure 5. Seed anatomy of *Pouteria glomerata* (Sapotaceae), in cross sections (a-g) and longitudinal sections (h-i). (a-b) cotyledons with different dimensions (c1 and c2) and hypocotyl-radicle axis (black arrow); (c) part of cotyledon epidermis in detail showing phenolic compounds in epidermal cells (in red) and starch grains stored; (d-e) detection of phenolic compounds with ferric chloride reagent in epidermis and mesophyll; (f) detection of starch grains with IKI; (g) reaction with Blue bromophenol to identify proteins in the cotyledon cells; (h) longitudinal section of the seed showing laticifer (arrowhead); (i) detail of the hypocotyl radicle axis (Hp-r) showing only meristematic cells; (j-k) detail of the vascular system of cotyledons, in cross and longitudinal section, respectively (black arrows indicate protoxylem cells). Abbreviations: c1 = cotyledon 1, c2 = cotyledon 2, La = laticifers, Hp-r = hypocotyl-radicle.

The hypocotyl radicle axis was short and wide, circular in cross-section (Figure 5a, b), and formed by a mass of small cells with no organogenetic differentiation (Figure 5h); it was also achlorophyllous and straight (Figure 5h, i) and located between the two cotyledonary leaves (Figure 5a, b). In the axis the procambium tissue is related to the cotyledon vascularization (Figure 5a). The apical shoot meristem was flat at the top. Besides the cotyledonary leaves, no other protective structures are observed covering this region (e.g., leaf primordium, Figure 5h, i). The meristematic cells were small, compact, and presented thin cell walls, with no differentiation into primary meristematic tissues (Figure 5i). The basal area, corresponding to the radicle, was conical and composed of meristematic cells with no differentiation (Figure 5h, i). The primary root did not possess a protective structure (e.g., root cap) (Figure 5i). Phenolic idioblasts were observed between the area of the shoot apical meristem and the basal area corresponding to the radicle and around this region (Figure 5i).

Discussion

Germination tests

Untreated seeds and the methods applied to overcome seed dormancy in *P. glomerata* resulted in zero to a low germination percentage. Baskin & Baskin (1998) consider lots with dormant seeds when they do not reach 75% germination in four weeks. Since there was no noticeable imbibition in non-germinated treated seeds, the low germination after pre-treatments, and the absence of germination in untreated (control) seeds indicated the presence of dormancy in the study species. An environment with seasonal flooding, such as the areas where *P. glomerata* occurs, can favor or hinder the germination and establishment of its seedlings. The presence of dormancy can constitute an adaptive advantage for many species in various environments (Bewley et al., 2012; Aguiar et al., 1993; Piña-Rodrigues & Fortes, 1996).

The temperature of 20-30 °C and light conditions combined with other treatments did not overcome the dormancy of *P. glomerata seeds*. On the other hand, seeds of a relative of the study species had different germination behavior. Andrade et al. (2002) found that *Pouteria campachiana* Kunth (Baehni) seeds showed the highest germination percentage (89%) after 63 days of sowing. These authors also applied mechanical scarification, which did not result in higher germination after 84 days. Similarly, Amoakoh et al. (2017) showed that 57% of untreated seeds of *P. campechiana* from a wild African population germinated. They also pointed out that scarification treatments increased germination by up to 67%.

The low germination of the study species may be related to the impermeability of the embryo's tissues to water, otherwise, viable scarified seeds would have germinated. In the case of the dormancy imposed by the coat, the removal of the tissues surrounding the embryo would be sufficient for the successful completion of germination (Bewley et al., 2012). The inhibition of germination caused by integument tissues interferes with water absorption and gas exchange, especially oxygen, and prevents the exit of inhibitors and mechanical containment of the embryo, which may be a physical, chemical, or mechanical cause or a combination of all (Bewley et al., 2012). These results suggested that *P. glomerata* seed dormancy may have more than one cause. Thus, we found that seeds display physical dormancy, due to the impermeability of the envelope to water or gases, and a mechanical dormancy caused by the mechanical containment promoted by the seed envelope (Baskin & Baskin, 2001).

Seed dormancy is the result of one or more factors that impact germination. They can be inhibitors related to the embryo, the seed coat, or both; it may reflect the physical, mechanical, or chemical inhibition of germination, due to the covering/protection layers of the embryo, undifferentiated or immature embryo, or due to metabolic restrictions and chemical inhibitors (Bewley et al., 2012). These inhibitors are established during seed ontogeny (Bradford & Nonogaki, 2007), and the timing of germination restriction varies between species. Scarification methods weaken the seed coat by removing physical barriers such as the presence of impermeable and rigid structures (whether seed coat, fruit, or part of the fruit) in dormant seeds (Marcos Filho, 2015), but these methods are not always efficient.

Germination and seedling morphology

According to Bawa et al., (1989), in tropical forests, most seedlings belong to two common types, seedlings with vertical and leafy cotyledons and another group with large seeds and seedlings with cotyledons that remain at ground level. Based on Figure 2 and the information described by Bawa et al., (1989) and Duke (1969), the germination of *P. glomerata* seed was categorized as cryptocotylar hypogeal. Our result corroborates with Costa et al. (2014) who found similar types of germination and seedling in *P. glomerata* populations from the Amazon forest, however, their seeds showed a higher percentage of germination.

Costa et al. (2014) also described hypogeal germination and cryptocotylar seedlings for *P. glomerata* collected near Manaus (AM), Brazil, in the Amazon forest. The authors observed that the cotyledons remained for 6 months in the seedling, and the germination started 84 days after sowing. In addition, the germination did not exceed 50% after 9 months of monitoring. According to Bawa et al. (1989), cryptocotylar seedlings occur in 41% of species in semi-deciduous forests in the Americas. These seedlings typically have robust, storage cotyledons that remain at or above ground level, extensive leaf tissue, and long roots (Figure 2).

Cryptocotylar hypogeal germination has been found in species from fire-prone savanna habitats in different regions including in the Pantanal wetlands (Jackson, 1974; Onyekwelu, 1990; Soares et al., 2021a). In this morphological type, cotyledons do not leave the seed coat after germination, remaining close to the soil surface or protected belowground, thus new shoots may arise from soil following fire or flooding

disturbances (Duke, 1969; Jackson, 1974; Garwood, 1996). The percentage of cryptocotylar species may increase along a moisture gradient from dry thorn to evergreen rainforest, according to Duke (1969). Hence, cryptocotylar hypogeal germination has been considered an efficient regeneration strategy in adverse environmental conditions, providing resources to support seedling establishment (Kitajima, 2003; Baraloto & Forget, 2007). However, hypogeal germination seems to confer delayed germination, but subsequently faster seedling growth- an ecological adaptative advantage for seedling recruitment compared to epigeal emergence in Amazonian floodplains (Parolin et al., 2003).

Bawa et al. (1989) pointed out that heavy and large seeds of forest species, which come from equally large fruits, dispersed by vertebrates, had high-water content, and did not exhibit dormancy. Our study corroborated only the first two characteristics - seed size and weight - for *P. glomerata*. Its dormant seeds did not display the pattern described for most forest species. The seed size and seedling morphology of a species can help to predict the seeds' preferred habitat and light requirements; in this case, the seedlings are adapted to survive a long period in deep shade and low rainfall (Bawa et al., 1989). Nevertheless, the areas where *P. glomerata* occurs in the Pantanal wetland are not characterized as closed forests. Besides, the study species can behave as pioneers in floodable fields (Pott & Pott, 1994), occupying open areas. An environment with seasonal flooding, such as the areas where *P. glomerata* occurs, can favor or hinder the germination and establishment of its seedlings. For Maurenza et al. (2009), the high phenotypic plasticity in *P. glomerata* seedlings can be characterized as morphophysiological adaptations to partial and total flooding, as the species tolerated up to six months of flooding, which justifies its occurrence in floodplains and other floodable areas. However, seedlings developed better in the non-flooded conditions, with the highest values of height, number of leaves, and incorporation of biomass than in the seedlings under flooded treatments (Maurenza et al., 2009). In the Amazon forest, adults individuals of *P. glomerata* showed higher tolerance to flooding by maintaining the leaves even with complete submergence, by reduction in photosynthesis rates, a process thought to be related to stomatal closure (Maurenza et al., 2012). However, seedlings developed better in the non-flooded conditions, with the highest values of height and number of leaves and greater incorporation of biomass than in the seedlings under flood treatments (Maurenza et al., 2012).

Seed morphoanatomy

The structure observed in the seeds of *P. glomerata* is similar to that described for the seeds of *P. campechiana*, whose germination showed effective results only after mechanical removal by scarification of the lignified layer (Pérez-Barcena et al., 2021). The germination of *Chrysophyllum gonocarpum* (Mart. & Eichl.) Engl. seeds (Sapotaceae family) as well as those of *P. glomerata*, were slow and uneven, characteristics that may be related to the presence of the thick and lignified envelope that contributes to physical dormancy, reducing the germination index (Felippi et al., 2010). The lignification of cell walls also results in greater stability and regulates water permeability, acting as a protective mechanism for the seeds, potentially strengthened by the incorporation of lignin (Steck et al., 2022). In addition to the presence of lignin in secondary cell walls, several other components are water-repellent, such as cutin, quinones, suberin, waxes, callose, and phenolic compounds, that contribute to different degrees of preventing germination (Bewley et al., 2012). Studies reveal that phenolic compounds, particularly flavonoids, contribute to the germination-inhibiting effects imposed by seed coats (Bradford & Nonogaki, 2007).

Lignin and other phenolic compounds also increase the hardness of the seed coat, limit gas exchange, and may act as a biochemical inhibitor (Wada et al., 2011; Briggs et al., 2005; Debeaujon et al., 2007). Studies reveal that phenolic compounds, particularly flavonoids, contribute to the germination-inhibiting effects imposed by seed coats (Bradford & Nonogaki, 2007). The presence of phenolic compounds in the tissue covering the seed may be an important trait in defense against biotic or abiotic agents, such as pathogens and ultraviolet radiation (Sharma et al., 2019). We highlight in this study that these compounds might constitute an adaptive advantage for the study species, considering the abiotic conditions of the floodable areas of the Pantanal wetland, with periods of extreme drought common in the region.

Sapotaceae species vary in the amount of endosperm, which can be abundant, deficient, or absent (Bokdam, 1977). The genus *Pouteria* was described as having seeds without endosperm (Barroso, 1978), which was reported for American species (95% of the total). On the contrary have been found for most species from Asia and the Pacific (Monteiro et al., 2007) corroborating with the results obtained here for *P. glomerata*. Regarding the consistency and appearance of the cotyledons, variations were observed for the genus *Pouteria*. Vasconcelos et al. (2021) described that *P. glomerata* seeds had a poorly developed embryo, with two very rigid

cotyledons that overlap at the margins while in *P. franciscana* Baehni, the cotyledons were plano-convex, fleshy and greenish to violet. In this study, we observed cotyledons are extremely rigid, clear, and shiny with a poorly developed embryonic axis, traits that differ from those described for the family and genus (Bokdam, 1977; Barroso, 1978). Starch grains in abundance in the constituent cells of the cotyledons, in addition to proteins and lipids, were also reported for other species of the genus (Felippi et al., 2010, Agama-Acevedo et al., 2023).

The embryo of *P. glomerata* seeds was not fully developed; the two cotyledons were rigid, overlapping at the margins, and with a lignified coating, which may explain its resistance and remain as seedling for 6 months after germination according to Costa et al. (2014). Seeds of *P. glomerata* did not present endosperm, a seed trait commonly found in hypogeal cryptocotylar seedlings with reserves in the cotyledons (Pennington, 2006; Soares et al., 2021a).

The incomplete differentiation of the embryonic hypocotyl-radicle axis observed for *P. glomerata,* resulted in embryos with the hypocotyl-radicular axis and the plumule poorly developed, which must complete their development for the seeds to germinate (Baskin & Baskin, 2001; Bewley et al., 2012). Whether embryo dormancy, removal of the coat would not permit such embryos to germinate normally, therefore, the block of germination should be more profound than in seeds with coat-imposed dormancy (Bewley et al., 2012). Thus, the presence of an impermeable envelope associated with an immature embryo may have led a low germination percentage. However, immature embryos can develop after dispersal, so that could be a strategy used by *P. glomerata* in natural habitats. For instance, embryos of *Viburnum furcatum* Blume ex Hook. F & Thomson increased their length by approximately 320% from their dispersal period to the completion of embryo growth before radicle emergence, which may indicate the presence of morphological dormancy (Phartyal et al., 2014). Our results ensured that dormancy in *P. glomerata* seeds is related to these factors: undeveloped or undifferentiated embryo and the impermeable, lignified, and extremely hard envelope rich in phenolic compounds. Therefore, seeds can be classified as morphophysiological dormancy (MPD) (Baskin & Baskin, 2004; Marcos Filho, 2015). Dormant seeds containing germination inhibitors to the entry of water and oxygen, and post-dispersal embryonic immaturity can warrant germination and seedling establishment in suitable areas distant from the mother plant. Furthermore, seeds with underdeveloped epicotyl or dormant epicotyl maintain this structure in the soil for a relatively long period before seedlings emerge fully with roots and shoots (Phartyal et al., 2014). The flooding areas seem to provide a suitable environment to remove the dormancy imposed by the envelope while allowing time for the embryo to complete its development. The germination and morphological aspects of seed and seedling of *P. glomerata* may be critical traits to its establishment success in the Pantanal wetland region, especially in areas subject to flooding.

Conclusion

The results indicate that *Pouteria glomerata* seeds showed a morphophysiological dormancy related to an impermeable and hard envelope, the presence of phenolic compounds, and an undifferentiated or immature embryo. The seed has hypogeal germination and the seedling is cryptocotylar type. The treatments applied to overcome dormancy did not improve germination.

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