


## Articles

### Investigation of dormancy in pyrenes of *Trithrinax acanthocoma* Drude: anatomy and histochemistry

Investigação de dormência em pirênios de *Trithrinax acanthocoma* Drude: anatomia e histoquímica

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## ABSTRACT

*Trithrinax acanthocoma* Drude is a rare palm, specifically found in Mixed Ombrophilous Forest in southern Brazil. It plays a significant role in forest ecosystems, providing habitat and food for local fauna. However, there is little information about the presence of dormancy in the pyrenes of this species, making it difficult to produce seedlings. The aim was to investigate the cause(s) of dormancy in pyrenes of *T. acanthocoma*. To do so, the following tests were carried out: soaking in methylene blue with intact and scarified pyrenes; quantification of total phenolic compounds; bioassay on lettuce seeds; morphological analysis of pyrenes and embryos; histochemical tests were also carried out to assess the presence of lipids, proteins, lignin and phenolic compounds. When soaked in methylene blue, only the scarified pyrenes were absorbed. In the morphological assessment, the embryo, despite showing some structures, is very small in relation to the endosperm, which suggests that it is underdeveloped. In the histochemical evaluation, lignin, lipids and phenols were observed; the quantification of phenols showed considerable levels in the pyrenes and the bioassay with lettuce seeds did not detect the presence of water-soluble inhibitors. It can be concluded that the probable causes of *T. acanthocoma* pyrenia dormancy are physical and morphological ones.

**Keywords:** Buriti-palito; Histochemistry; Germination inhibitors

## RESUMO

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*Trithrinax acanthocoma* Drude é uma palmeira rara, especificamente encontrada em Floresta Ombrófila Mista no sul do Brasil, desempenha um papel significativo nos ecossistemas florestais, fornecendo habitat e alimento para a fauna local. Porém, são escassas as informações a respeito da presença de dormência em pirênios da espécie, dificultando a produção de suas mudas. Objetivou-se investigar a(s) causa(s) da dormência em pirênios de *T. acanthocoma*. Para isso, foram realizados os seguintes testes: absorção em azul de metileno com pirênios íntegros e escarificados; quantificação de compostos fenólicos totais; bioensaio em sementes de alface; análise morfologia dos pirênios e embriões, também foram realizados testes histoquímicos para avaliar a presença de lipídios, proteínas, lignina e compostos fenólicos. Na embebição em azul de metileno, ocorreu a absorção apenas nos pirênios escarificados. Na avaliação morfológica, o embrião, apesar de apresentar algumas estruturas, é muito pequeno em relação ao endosperma, o que sugere que seja subdesenvolvido. Na avaliação histoquímica, foram observados lignina, lipídios e fenóis; Na quantificação de fenóis apresentou níveis consideráveis nos pirênios e no bioensaio com sementes de alface não detectaram a presença de inibidores solúveis em água. Conclui-se que as prováveis causas de dormência de pirênios de *T. acanthocoma* são físicas e morfológicas.

**Palavras-chave:** Buriti-palito; Histoquímica; Inibidores da germinação

## 1 INTRODUCTION

The seed dormancy is an adaptive mechanism with an important ecological role once it allows plants to survive in environments with adverse conditions, preventing premature germination, favoring the formation of seed banks in the soil and increasing the chances of successful emergence of the seedlings in favorable conditions (Bewley *et al.*, 2013; Baskin; Baskin, 2014a; Long *et al.*, 2014). In the context of palm trees, the dormancy plays an even more significant role, given the diversity of species and the economic and ecological importance of these plants in various regions of the world (Baskin; Baskin, 2014b).

The types of dormancy in palm trees vary, including the physical dormancy seen in species such as *Syagrus coronata* (Mart.) Becc. (Medeiros *et al.*, 2015), *Butia capitata* (Mart.) Becc. (Dias *et al.*, 2018) and *Mauritia flexuosa* L. f. (Moura *et al.*, 2019); the physiological dormancy present in *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart. (Ribeiro *et al.*, 2012), *Attalea vitrivir* Zona (Neves *et al.*, 2013) and *Butia capitata* (Mart.) Becc. (Oliveira *et al.*, 2015); and morphophysiological dormancy identified in species such as

*Pritchardia remota* (Kuntze) Becc (Pérez, 2008), *Pseudophoenix ekmanii* Burret (Visscher *et al.*, 2020) and *Phoenix canariensis* (He *et al.*, 2021) being the most common ones.

Determining the type of dormancy present in the palm seeds is crucial before applying methods to overcome it, but it is important to specify the classification system followed, so that the type of dormancy is correctly determined (Silveira, 2013). Once identified, the treatments to break dormancy can be applied efficiently (Brasil, 2009b; Piveta *et al.*, 2014). In general, different classes of dormancy require different methods to overcome it (Hilhorst, 2011).

Methods such as histochemical analysis, seed anatomy and imbibition tests are essential tools to identify the causes of dormancy and to propose appropriate strategies to overcome it. The histochemistry can reveal the presence of compounds such as lignin and phenolic compounds that can affect germination (Debeaujon *et al.*, 2007; Silva *et al.*, 2007; Carvalho; Nakagawa, 2012). The anatomical analysis of seeds helps identify physical barriers to germination, such as thick seed coats or underdeveloped embryos (Demason, 1988; Silveira *et al.*, 2012; Bewley *et al.*, 2013). Imbibition tests allow to evaluate the water absorption capacity of seeds and to detect possible blocks to germination (Baskin *et al.*, 2006).

Research in this area can significantly contribute to the advancement of knowledge in seed technology and in the development of more efficient strategies for the plant propagation in agricultural, forestry and conservation contexts (Ren-Fe *et al.*, 2023).

The lack of specific studies on the structure of the pyrene of *Trithrinax acanthocoma* Drude (Arecaceae) represents a valuable opportunity to the advance of the knowledge about dormancy in palm trees. In this context, the study of its pyrenes is of great importance, as they can present different types of dormancy. *T. acanthocoma*, popularly known as Carandaí or Burití-palito, is a rare palm tree from the Mixed Ombrophylous Forest that extends across the three southern states of Brazil and Paraguay (Carvalho, 2010; Cano *et al.*, 2013; Elias *et al.*, 2018). It is recognized by its solitary stipe, dark green

flabelliform leaves, and reproductive cycle with flowering between December and March, followed by fruiting from August to February (Lorenzi *et al.*, 2010; Soares, 2014).

Information about *Trithrinax acanthocoma* is scarce, especially regarding its morphology and anatomy, including its propagation. Knowing the causes of dormancy in *T. acanthocoma* not only provides the basic data necessary for theoretical research on the mechanisms of dormancy and seed germination, but also promotes improvements in the production and promotion and conservation of this species.

In this study, we sought to fill these research gaps by investigating the morphoanatomical and histochemical aspects of *Trithrinax acanthocoma* pyrenes to understand their structure and identify barriers that may affect germination. The central questions are: (1) Do newly benefited/mature *Trithrinax acanthocoma* pyrenes have dormancy? (2) If pyrenes are dormant, what is their dormancy type? (3) What are the roles of different pyrene structures in controlling the germination?

## 2 MATERIALS AND METHODS

The fruits of *Trithrinax acanthocoma* were collected in February 2022 in the urban perimeter of the municipality of Curitibanos - Santa Catarina state, located at latitude 27°17' and longitude 50°38', at an altitude of 1,016m. The region's climate is classified as humid temperate (Cfb), with moderately hot summers, the average annual precipitation is between 1500 and 1700mm, and the average annual temperature of 17°C (Wrege *et al.*, 2012; Alvares *et al.*, 2013).

To select the matrices for the formation of the batch, the rarity and difficulty of locating this species in native forest areas were taken into account. The trees that had fruits with desired characteristics, such as specific shade and firm consistency, were selected to ensure a representative sample of the local *T. acanthocoma* population. The fruits, harvested with the aid of a secateur, were chosen based on their greenish-yellow hue, corresponding to 5Y 7/8 and 5Y 8/6 on Munsell scale.

The pyrenes (seeds with endocarp) of *T. acanthocoma* were manually extracted after the harvest, using a knife to separate them from the pulp (epicarp + mesocarp) and during the process, the damaged pyrenes were excluded.

The determination of the water content of the pyrenes followed the oven method at  $105^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 24 hours, in accordance with the seed analysis rules (Brasil, 2009b). Two subsamples of 10.0g were used and the results were expressed as a percentage (wet basis).

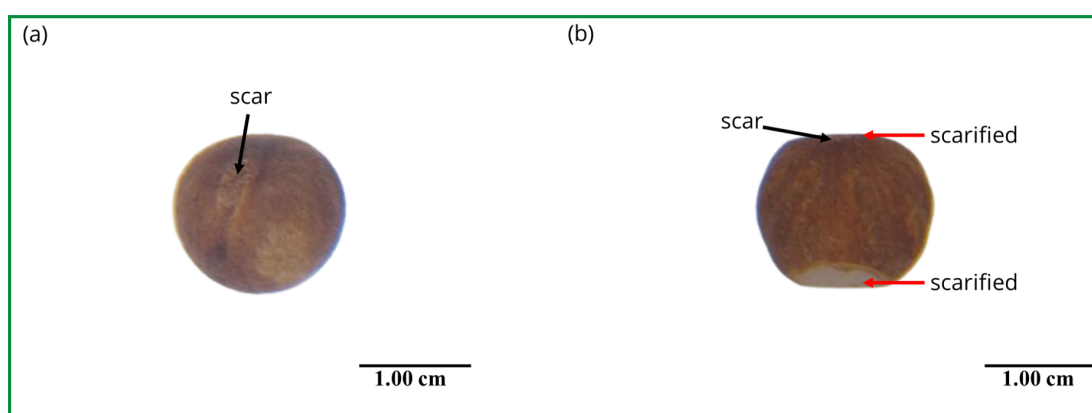
The viability of the pyrenes was determined by the germination, where the pyrenes were subjected to an initial disinfestation process, with immersion in 70% ethyl alcohol for one minute, followed by immersion in a commercial sodium hypochlorite solution with 2% active chlorine. . After stirring for 15 minutes, the pyrenes were rinsed four times with distilled water. The test was conducted in gerbox-type acrylic boxes, with four repetitions of 25 pyrenes each. The pyrenes were placed in a fine-grained vermiculite substrate, previously autoclaved. The test was carried out in a B.O.D. germination chamber. (Biochemical Oxygen Demand), maintained at a constant temperature of  $25^{\circ}\text{C}$  and under continuous light. No additional pre-germination treatments were applied to the pyrenes to ensure the accuracy in results.

In conjunction with the germination test, the tetrazolium test was conducted, where the pyrenes were cut lengthwise and soaked in water for 12 hours. Subsequently, the embryos were extracted with the aid of tweezers and a scalpel, and immersed in a solution of 0.1% 2,3,5-triphenyl tetrazolium chloride with pH 7.1, for six hours, and 0.1% of tetrazolium at  $25^{\circ}\text{C}$ , in a B.O.D. germination chamber. Four (4) replications of 25 embryos were used, and the results were expressed as a percentage of viable embryos.

To verify the permeability of pyrenes, the absorption method in 1% methylene blue was used (Orozco *et al.*, 2007). The intact pyrenes (Figure 1A) and the scarified pyrenes (Figure 1B) were used in the scar region and behind the scar (Figure 1B), with the aid of #40 sandpaper until the endosperm was visible. The pyrenes were

immersed in the methylene blue solution and evaluated every 72 hours until the end of the experiment. The evaluation consisted of longitudinally sectioning the pyrenes and analyzing their absorption under a stereomicroscope and a table magnifying glass with LED lighting.

Figure 1 – Preparation of pyrenes from *Trithrinax acanthocoma* Drude for absorption experiment in methylene blue: a) intact pyrene; b) scarified pyrene in the upper and lower regions of the scar



Source: Authors (2023)

To evaluate the presence of chemical inhibitors, a bioassay and the quantification of total phenolic compounds were carried out, both using extracts obtained from pyrene (endocarp, tegument, endosperm and embryo).

## 2.1 Bioassay

The extracts for the bioassay were prepared with four grams of crushed pyrenes, which were added separately to water, in proportions of 1:1 and 1:2 (extract: water). Next, a germination test was conducted with lettuce seeds (*Lactuca sativa*), used as a test species. The test was carried out in a B.O.D. germination chamber, maintained at 20°C, with four replications of 50 seeds each (Brasil, 2009b). The substrate used was filter paper, moistened 2.5 times the weight of the paper with the extracts.

Control tests were also carried out, in which lettuce seeds germinated in substrates moistened with distilled water. The first germination count was carried out

at 4 days, and the final count at 7 days after the start of the test, according to Brasil (2009b). The *Lactuca sativa* seeds used in the test had a germination percentage of 98% (provided by the manufacturer). The results were expressed as percentage of normal seedlings.

## 2.2 Quantification of Total Phenolic Compounds

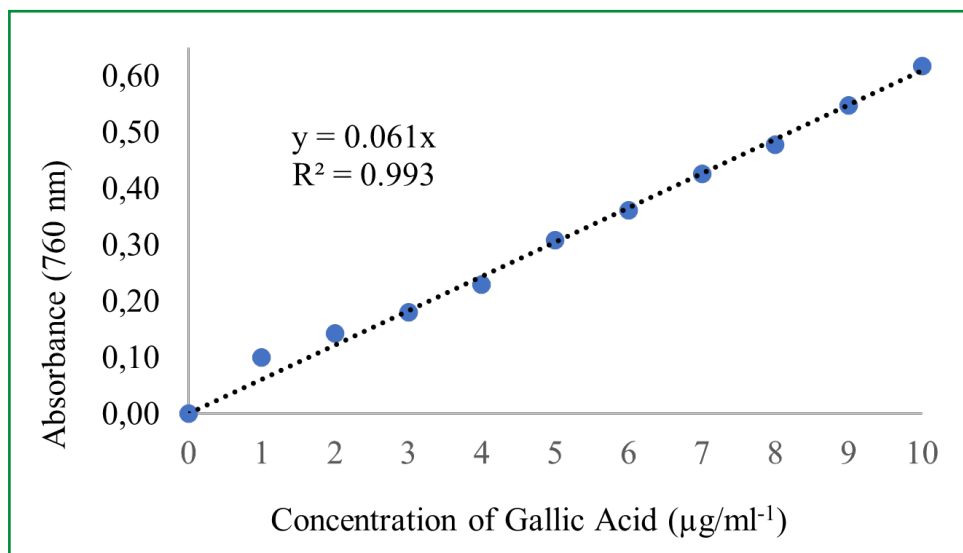
The quantification of total phenolic compounds was carried out according to the methodology proposed by Swain and Hillis (1959) and Singleton *et al.* (1999). A sample of four grams of pyrenes was ground in an electric coffee grinder and then deposited in 50 mL Falcon tubes, to which 25 mL of methanol was added. This mixture was placed in a water bath for 10 minutes and, subsequently, the tubes were wrapped with aluminum foil and kept in a dark environment, at room temperature, for 24 hours. After this period, the extracts were filtered and volumetric in 25 mL volumetric flasks, stored in an amber bottle and used as crude extracts for the quantification of total phenols.

For the total quantification of phenols in the extract, 100  $\mu\text{L}$  of the extract were collected, to which 500  $\mu\text{L}$  of Folin-Ciocalteu reagent and 200  $\mu\text{L}$  of 15% sodium carbonate were added. The absorbance of the liquid fraction was determined at 760 nm in a UV-vis spectrophotometer, using white as a reference. A gallic acid calibration curve was used for the quantification of total phenols (Figure 2). The quantification of phenolic compounds in pyrene extracts was carried out in triplicate, and the results were expressed in gallic acid equivalents ( $\text{mg. g}^{-1}$ ).

To study the morphology of the pyrenes, longitudinal and transverse sections were made. In these segments, the color, the consistency and shape of the seed coat, endosperm and embryo were observed with the aid of a magnifying glass and microscope.



Figure 2 – Standard Curve of Gallic Acid at 760 nm (Folin-Ciocalteu) for the Quantification of Total Phenolic Compounds ( $R^2 = 0.993$ )



Source: Authors (2023)

For the anatomical study of pyrenes, the fresh material was cut longitudinally, removing part of the endosperm close to the embryo, without exposing it completely, leaving it covered by a thin layer of endosperm. Then, the pyrenes were fixed in 70% FAA (formalin, acetic acid and ethyl alcohol) according to Kraus and Arduin (1997). After the fixation, the pyrenes were dehydrated in grades of ethyl alcohol (80-100%) and embedded in 2-hydroxyethyl methacrylate (Historesin® Leica), and subsequently blocked according to the manufacturer's instructions.

The blocks were then sectioned into anatomical sections with a thickness of 5 to 7  $\mu\text{m}$ , using disposable blades on a Leica rotating microtome (HistoCore BIOCUT). The sections were stained with 0.5% toluidine blue in citrate buffer, pH 4.0 (O'Brien *et al.*, 1964), and the slides were prepared in water (Genovese-Marcomini *et al.*, 2013).

The parameters used for the morphoanatomical descriptions and the terms applied to the structures are in accordance with the studies by Martin (1946) and Dransfield *et al.* (2008).

After the sections were made, they were stained with specific reagents for each substance: Lugol's solution for the starch detection (Johansen, 1940); Sudan III



for the lipid detection (Pearse, 1972); Coomassie Brilliant Blue (Fisher, 1968); Ferric chloride for the detection of general phenolic compounds (Johansen, 1940); finally, acidic Phloroglucin for the detection of lignin (Johansen, 1940). The material was then examined under a microscope and the images were recorded. Subsequently, the material was examined under a microscope and the images were recorded.

The observation and photomicrographs relating to the anatomical and histochemical study were obtained using the Leica DM750 optical microscope, together with the Leica Application Suite EZ program.

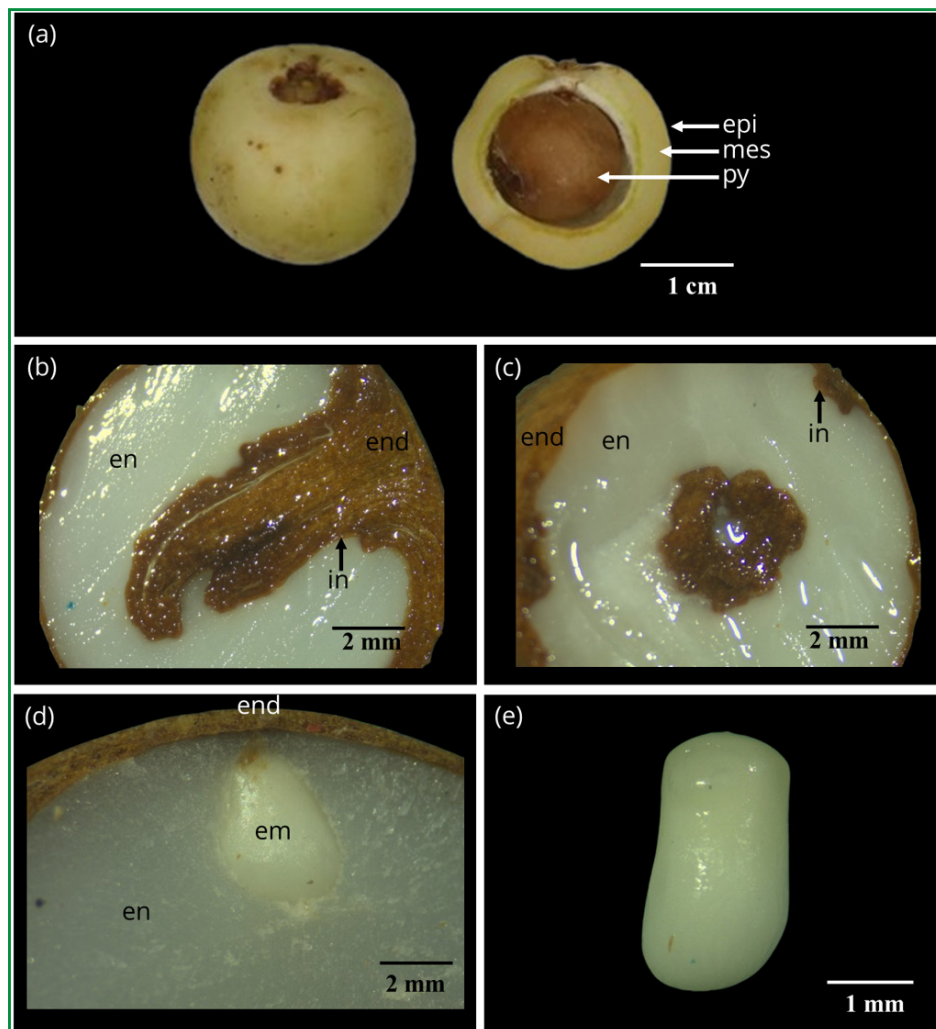
### 3 RESULTS AND DISCUSSIONS

The fruits of *Trithrinax acanthocoma* have a globose shape, with an average diameter of 24.76 mm, greenish-yellow color (5Y 7/8 and 5Y 8/6), and an average mass of 9.69 g. Structurally, the fruits are made up of the epicarp (outermost layer), fleshy mesocarp, and the pyrene, which includes the endocarp, tegument, endosperm and embryo (Figure 3a).

The pyrenes vary from oval to globose in shape. The endocarp is striated, light brown in color (10YR 6/6), with a hard and dense consistency. The endosperm is ruminated, with a hard consistency and a whitish color (Figure 3b-c). The average mass of 1000 pyrenes is 2.54 kg, with a water content of 41.82%. The *T. acanthocoma* embryo is immersed in the hard and massive endosperm, located close to the endocarp scar. Compared to the total volume of the endosperm, the embryo is relatively small, measuring on average 4.45 mm in length (Figure 3d-e).

In the morphological evaluation, it was found that the *T. acanthocoma* embryo is undivided, oblong, lateral (Baskin; Baskin, 2014b) and rudimentary (Martin, 1964; Brasil, 2009a) (Figure 4a). Pérez (2008) reports that the embryos of the palm tree *Pritchardia remota* (Kuntze) Beck, measuring around 4.0 mm, require more time for their growth, to break the tegument and eventually produce a radicle, which should grow to around 1.5 and 3.0 times their initial length, and are therefore considered morphologically dormant.

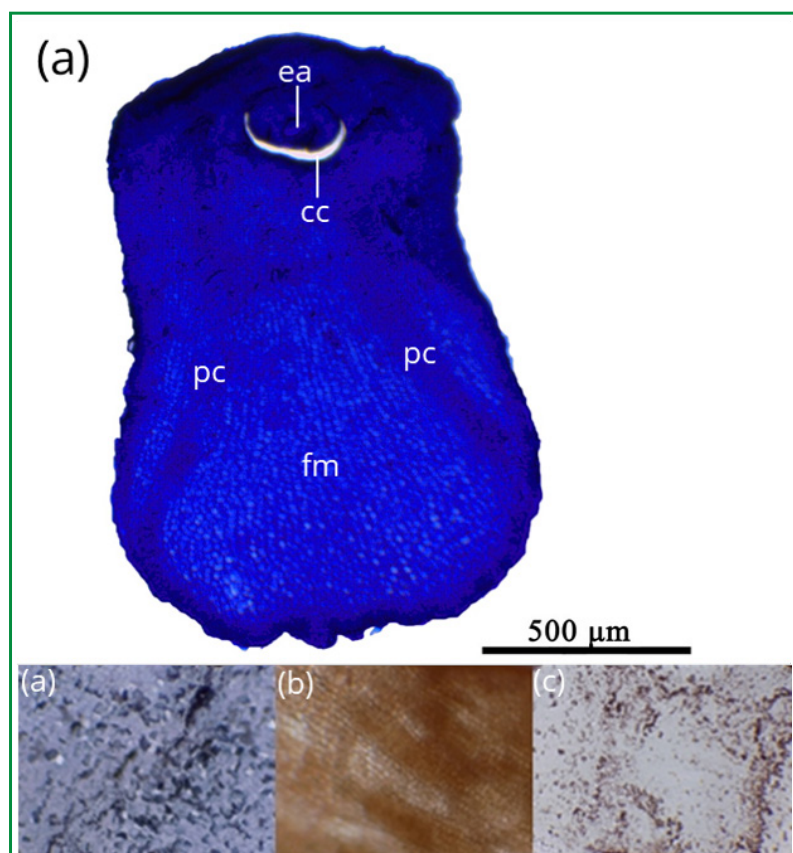
Figure 3 – Morphological aspects of *Trithrinax acanthocoma* Drude. (a) freshly harvested fruit; (b) longitudinal section of the pyrene; (c) pyrene cross section; (d) longitudinal section of the pyrene with embryo; (e) embryo



Source: Authors (2023)

In where: en: endosperm; em: embryo; end: endocarp; epi: epicarp; in: integument; mes: mesocarp; py: pyrene.

The procambial cells are elongated and narrow, intensely stained with toluidine blue. These cells begin above the embryonic axis, passing along its side, and are distributed in an orderly manner. As they move away from the embryonic axis, the procambial cells branch, becoming peripheral close to the haustorium and remaining so throughout their distal region. The parenchyma cells are approximately isodiametric (Figure 4a). The embryonic axis is axial and curved.

Figure 4 – Anatomical aspects of the *Trithrinax acanthocoma* Drude embryo

Source: Authors (2023)

In where: (a) Longitudinal anatomical sections included in methacrylate and stained with toluidine blue. Result of histochemical tests on the embryo; (b) Proteins (blue coloring); (c) Lipids (orange color); (d) Starches (brown color); ea: embryonic axis; cc: cotyledonary cavity; fm: fundamental meristem; pc: procambial cords.

Nazário *et al.* (2013) verified the set of cotyledon, plumule and root structures in *Bactris gasipaes* Kunth, indicating that, at the time of dispersion, the embryo is differentiated and mature, with no morphological dormancy. However, even observing some of these structures, it cannot be said that the *T. acanthocoma* embryo is completely developed. The embryo is reduced in relation to the endosperm, suggesting that it needs to grow significantly to overcome the barriers of the integument and endocarp, which indicates an underdeveloped state. According to Baskin and Baskin (2014a), an embryo can be considered underdeveloped even if it has developed cotyledons, radicle and endosperm, if it is small in relation to the endosperm.

The decisions about assigning morphological dormancy should be based on the results of isolated seed responses and not just on seeds within the dispersal unit (Baskin; Baskin, 2014b). It is likely that the pyrenes of *T. acanthocoma* present morphological dormancy. However, new studies that evaluate the development of the embryo during maturation and germination are necessary to confirm the presence or the absence of dormancy in the species.

Histochemical analyzes revealed the presence of protein bodies (Figure 4b), lipids (Figure 4c) and starch grains (Figure 4d). Proteins are present throughout the embryo, while lipids and starch are present only in the haustorium region. The presence of proteins and lipids is a common characteristic of palm trees of different species such as *Washingtonia filifera* (L. Linden) H.Wendl (Demason, 1988), *Euterpe edulis* Mart. (Panza *et al.*, 2004), *Bactris gasipaes* Kunth. (Nazário *et al.*, 2013) *Bactris maharajá* Mart. (Rodrigues *et al.*, 2015), and *Syagrus oleraceae* (Mart.) Becc. (Silva Cardoso *et al.*, 2019), and both subsidize the growth of the embryo during the germination process as a food reserve. As for the presence of starch grains in palm embryos, it is generally not common, with few reports, such as in *Euterpe edulis* Mart. (Panza *et al.*, 2004), *Butia capitata* (Mart.) Becc. (Oliveira *et al.*, 2013) and *Syagrus oleraceae* (Mart.) Becc. (Silva Cardoso *et al.*, 2019), functioning as an additional source of energy.

No germination was recorded during the experiment period, which lasted 158 days. This lack of germination raises the hypothesis that pyrenes are experiencing some type of dormancy, a very common phenomenon in palm trees.

In the literature, it is in fact reported that germination for palm trees can be characterized by a slow, irregular and low percentage process (Tomlinson, 1990; Ferreira *et al.*, 2010). The seeds require a long period to begin the germination process, which makes seedling production difficult, as observed in species such as *Euterpe oleraceae* Mart. 57 days (Bovi; Cardoso, 1976); *Euterpe edulis* Mart. 97 days (Bovi, 1990); *Archontophoenix alexandrae* (F.Muell.) H.Wendl and Drude 68 days for germination (Charlo *et al.*, 2006); *Syagrus oleracea* Becc. - 90 days (Nascente *et al.*, 2007); *Phoenix*

*roebelenii* O'Brien - 50 days (Iossi *et al.*, 2007); *Astrocaryum acaule* Mart 130 days (Côrrea *et al.*, 2019); *Oenocarpus Bataua* Mart. 105 days (Bastos *et al.*, 2021); and *Bactris maraja* Mart., 152 days (Rodrigues *et al.*, 2022).

The results of this experiment corroborate this trend, since even after 158 days of observation, there was no evidence of germination. However, when evaluated by the tetrazolium test, they were 86% viable. This disconnect between the viability of the embryos and the lack of germination reinforces the hypothesis that the pyrenes are dormant.

The long period for the start of germination may be associated with the presence of seed dormancy (Orozco-Segovia *et al.*, 2003). Baskin and Baskin (2014a) report that viable seeds that do not germinate for up to 4 weeks are considered dormant, even when placed in environmental conditions favorable to germination (water, temperature and light). The dormancy of seeds from the Arecaceae family is commonly related to physical dormancy, due to the structures that surround the embryo (Schlindwein *et al.*, 2013).

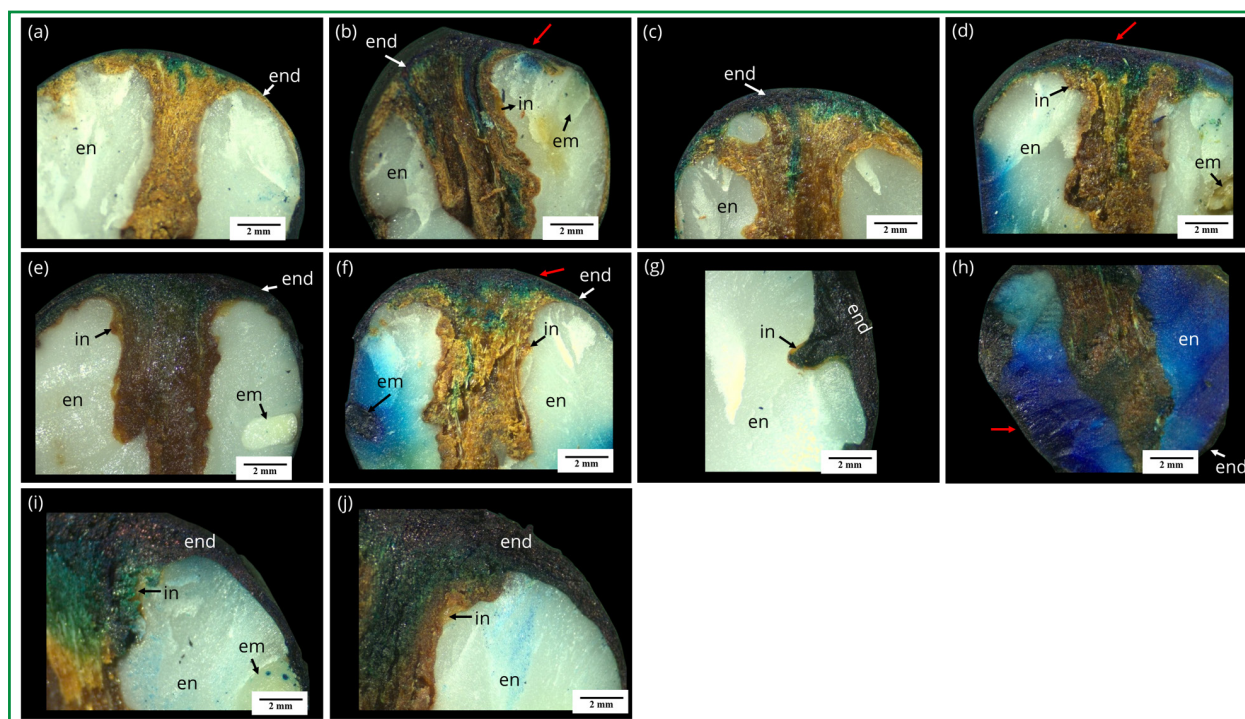
Observing the results of absorption in 1% methylene blue in intact pyrenes, it was noted that even after 2880 hours (120 days), there was no imbibition in the endosperm (Figure 5j). Throughout the experiment, the blue infiltration was restricted to the endocarp (Figure 5b, e, f, i, j), characterizing the impermeability of the integument. This impermeability suggests the presence of physical dormancy, since the physical barrier imposed by the integument prevents the water absorption.

On the other hand, the scarification of the pyrenes allowed the subition of methylene blue (Figure 5c, d, g, h). The soaking process began after 720 hours (30 days) of immersion (Figure 5d), and after 1,944 hours (81 days), the endosperm was completely dyed (figure 5h). These results prove the barrier imposed by the stretching in the poles.

The physical numbness in the palm seeds has been mainly attributed to the rigid endocarp, which limits water and gases flows. (Reis *et al.*, 2012). This phenomenon was verified in several species, such as *Syagrus schizophylla* (Mart.) Glass. (Pivetta *et al.*, 2005), *Astrocaryum aculeatum* meye (Ferreira; Gentil, 2006) and *Areca Triandra* Roxb. Ex Buch-Ham (Yang *et al.*, 2007).



Figure 5 – Longitudinal section of intact (a,c,e,g,i,j) and scarified (b,d,f,h) pyrenes of *Trithrinax acanthocoma* Drude after a-b: 72 hours; c-d: 720 hours; e-f: 1440 hours g-h: 1944 hours i: 2160 hours j: 2880 hours of absorption in 1% methylene blue solution



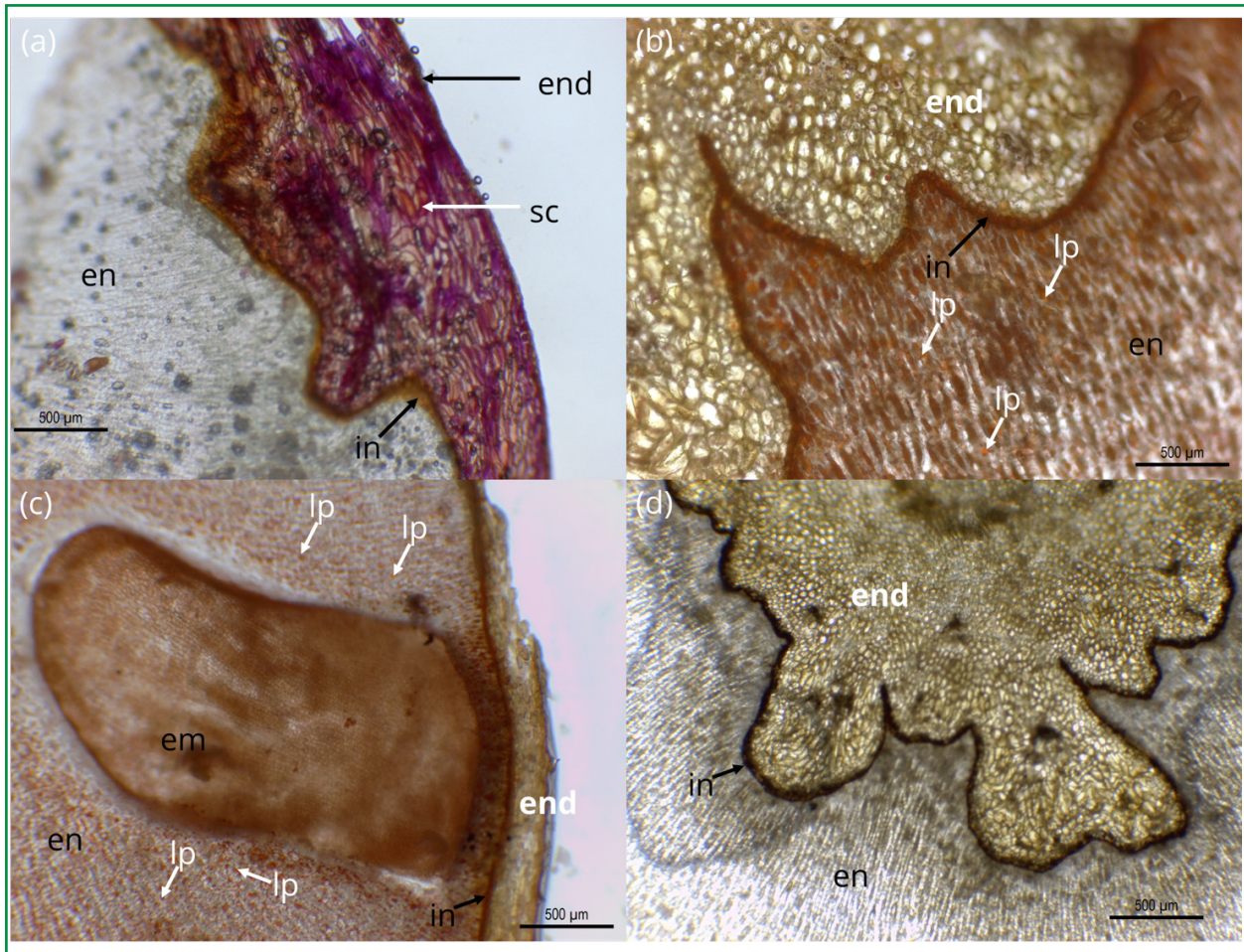
Source: Authors (2023)

In where: en: endosperm; em: embryo; end: endocarp; in: integument. Arrow red: scarified regions.

However, for *T. acanthocoma*, the endocarp does not restrict the water inlet. In this case, the function can be attributed to mechanical protection (Tomlinson, 1990). The sclerified cells present in the endocarp (Figure 6a) provide this protection to the seeds.

In the histochemical test, the presence of lignin was observed only in the endocarp (Figure 6a), corroborating the protection function of the seed, since the presence of lignin gives rigidity, which provides resistance. Although lignin also gives impermeability, its presence in the endocarp was not so expressive as to constitute a barrier. In addition, the endocarp cells have intercellular space, which can contribute to the permeability of the tissue.

Figure 6 – Result of histochemical tests in *Tithrinax acanthocoma* drude poles. (a) lignin (pink color); (B-C) Lipids (orange color); (D) Phenolic compounds (black color)



Source: Authors (2023)

In where: en: endosperm; em: embryo; end: endocarp; in: integument; ld: lipid droplet; sc: sclerified cells.

The endocarp protection function for palm seeds was also observed for other species, such as *Acrochomy aculeata* (jacq.) Lodd. Mart. (Moura *et al.*, 2010), *Bactris gasipaes* Kunth (Nazário *et al.*, 2013), *Bactris maraja* Mart (Rodrigues *et al.*, 2015). The impermeability of the integument can be attributed to the deposition of substances such as waxy cuticle, suberin, lignin, palisadic tissue, cutum and mucilage in the forehead, pericarp or nuclear membrane of the seeds (Perez, 2004; Bewley *et al.*, 2013). According to Esau (1977), the compact arrangement of seed integument cells is directly related to the degree of impermeability.



In histochemical tests for total lipid detection, the presence of lipids in the integument, endosperm and embryo (Figure 6a-d) was identified. Lipids in the integument (Figure 6b-c), probably in the form of suberin, contribute to the barrier to the water of the integument. In the endosperm, there is a large accumulation of lipid droplets, especially in the region near the embryo (Figure 6c), which may explain the slow absorption of methylene blue in the endosperm of the scarified poison (Figure 5c, d, g, h) of *T. acanthocoma*.

The presence of phenolic compounds in the endocarp, integument and endosperm was found, evidenced by the black color to the reagent (Figure 6d). These phenolic compounds can act as germination inhibitors, controlling the oxygen input, as they fix oxygen that the seed is absorbing, preventing its arrival inside (Dietrich, 1986). The presence of germination process inhibitors conditions the occurrence of physiological type numbness, which has been associated with phytones, especially with abscisic acid (ABA), and secondary metabolism products such as terpenes and phenolic compounds (Taiz; Zeiger, 2013).

In the quantification of phenols in *T. acanthocoma* poison, they had a concentration of 1.97 mg.g<sup>-1</sup>. This presence may eventually be inhibiting the development of the embryo, as reported by Mireski *et al.* (2018), which quantified the total polyphenols in *Ilex Paraguariensis* seeds A. St. Hil., Using ethanolic and aqueous extracts. They observed that ethanolic extracts resulted in higher polyphenol content and noted that the presence of these phenolic compounds in seeds interfered with the germination of lettuce seeds. According to Come (1973), the phenolic compounds present in the seed wrap up oxygen, which could limit its supply to the embryo during germination, causing numbness.

In assessing the inhibitory effect of sarcotest extracts on lettuce seed germination and by quantifying phenolic papaya compounds, Tokuhisa *et al.* (2007) reported that these compounds can exert control over the papaya seed germination. They observed an inhibitory effect on the lettuce seed germination, mainly interfering with the root growth.

In the present paper, the aqueous extract of the *T. acanthocoma* pyrenes showed no inhibitor effect on germination or the formation of abnormal seedlings on lettuce seeds (Table 1).

Table 1 – Germination (%) of *Lactuca sativa* seeds sowed on substrate moistened with aqueous extract of *Tithrinax acanthocoma* drude poles

Treataments	Germination (%)	
	1st score (4 days)	Final score (7 days)
Witness	97	100
Polyan extract (1:1)	89	94
Polyan extract (1:2)	92	97

Source: Authors (2023)

This suggests the absence of water-soluble inhibitors. However, it is important to consider that this absence of effect may be due to differences in extract concentration or the extraction method. It is suggested to test hydroalcoholic extracts of *T. acanthocoma* pyrenes to evaluate the presence of possible inhibitors as complementary tests.

4 CONCLUSIONS

Based on the results obtained, the probable causes of numbness in *T. acanthocoma* pyrenes can be characterized as physical and morphological. It is recommended to exploit treatments involving hydroalcoholic extracts of *T. acanthocoma* pyrenes to investigate the possible presence of inhibitor agents. This study offers significant contributions to understand the dormancy mechanism in *T. acanthocoma*. These conclusions can serve as the basis for the development of strategies aimed at improving the propagation of this species.

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