Rodriguésia 72: e01832019. 2021
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202172048

Original Paper

Morphoanatomical and histochemical studies of the seed development of *Euterpe oleracea* (Arecaceae)

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Abstract

Although the consumption of *açaí* (*Euterpe oleracea*) pulp has long been an important component of the diet of the peoples from the Amazon, the *açaí* palm tree has recently attracted economic and scientific interest because of its vast array of bioactive compounds found in the fruit pericarp. The *açaí* seeds are the largest byproduct after pulp extraction and have potential for use in ethanol production, but this process is hindered by limited knowledge of seed biology, chemical composition and pattern reserve deposition during seed development. The aim of this work was to describe the morphoanatomical development of the seeds, as well as to identify the main organic compounds stored in the seeds. To achieve this goal, histological and histochemical analyses were performed on developing seeds. Results showed the seed is albuminous, bitegmic and that ingrowths of the seed coat give rise to a ruminate endosperm. Moreover, the nutritive reserves of *açaí* seeds are found in the endosperm thickened cell walls as reserve polysaccharides. Our findings provide information for future studies dealing with reproductive biology, propagation and the improvement of this profitable crop.

Key words: embryogenesis, hypostase, pachychalaza, palm trees, ruminate endosperm.

Resumo

Embora o consumo de açaí (*Euterpe oleracea*) seja há tempos um importante componente da dieta dos povos amazônicos, o açaizeiro tem recentemente atraído tanto o interesse econômico quanto científico devido à presença de uma vasta gama de compostos bioativos encontrados no pericarpo de seus frutos. As sementes de açaí representam o principal subproduto após a extração da polpa e podem ser potencialmente utilizadas para a produção de etanol, mas esse processo é dificultado pelo conhecimento limitado sobre a biologia das sementes, sua composição química e a deposição de reserva de padrões durante o desenvolvimento das sementes. O objetivo deste trabalho é descrever o desenvolvimento morfoanatômico das sementes, bem como identificar os principais compostos orgânicos armazenados nas sementes. Para atingir esse objetivo, foram realizadas análises histológicas e histoquímicas das sementes em desenvolvimento. Os resultados revelaram que a semente é albuminosa, bitegmica e que tegumento interno da semente dá origem às ruminações do endosperma. Além disso, os resultados indicam que as reservas nutritivas das sementes de açaí são encontradas nas paredes celulares espessadas pelo endosperma na forma de polissacarídeos de reserva. Nossas descobertas fornecem informações úteis para futuros estudos sobre biologia reprodutiva, propagação e melhoria de uma cultura tão lucrativa.

Palavras-chave: embriogênese, hipóstase, paquicalazal, palmeiras, endosperma ruminado

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Introduction

Euterpe oleracea Mart. (Arecaceae), popularly known as "açaí", is a palm species found in several Brazilian states and is especially important in the North and Northeast Brazil, as it is used for feeding or in the production of cosmetics, crafts and others (Paula 1975; Silva *et al.* 2006; Lorenzi 2008). Although the *açaí* fruit production as well as the consumption of its pulp is an ancient activity of the peoples from the Amazon (Salo *et al.* 2013; Oliveira & Schwartz 2018), the consumption of *açaí* beverages has spread far beyond the Amazon basin and its pulp is in increasing demand by the functional food industry (Schauss 2010; Bichara & Rogez 2011; Oliveira & Schwartz 2018). According to data from Plant Extraction and Forestry Research (Produção da Extração Vegetal e da Silvicultura), the production of *açaí* in Brazil from 2010 to 2016 has increased from 706 thousand ton to 1 million ton (CONAB 2017). This demand is being met by newly established commercial plantations and independent family growers along the Amazon River estuary. It is hoped that this fruit will become a major revenue source for small and large growers alike.

The lack of basic knowledge about the biology and biochemistry of this crop hinders both its genetic improvement and the ability to mitigate environmental problems arising from the seed surplus after pulp extraction. Because there is no significant commercial for the seeds at this time (Farinas *et al.* 2009; Monteiro *et al.* 2019), the disposal of thousands of tons of seeds annually represents a significant ecological problem. Although it is technologically feasible to use this surplus of seeds to produce ethanol, this cannot be accomplished without defining the details of the major cell wall carbohydrates of the seeds. Identifying of the enzymes used in the synthesis and deposition of the carbohydrates during seed development is also important. Acquiring this knowledge will require a comprehensive description of seed development, which will be used for the metabolomic and proteomic analysis of the relevant organs, tissues and developmental stages.

Morphoanatomical studies are fundamental for understanding embryo development and the germination process, as demonstrated for *Syagrus inajai* (Spruce) Becc. (Genovese-Marcomini *et al.* 2014). Bearing in mind that seeds are the main means of plant propagation and establishment of palm species (Genovese-Marcomini *et al.* 2013), sound knowledge of seed development is essential for understanding the reserves that sustain the germination and seedling establishment processes. Although anatomical studies have been performed on *açaí* species (both *E. oleracea*, *Euterpe precatoria* Mart. and *Euterpe edulis* Mart.) (Aguiar & Mendonça 2003; Gonçalves *et al.* 2010), previous works have not provided detailed descriptions of seed and zygotic embryo development. Thus, we offer here a morphoanatomical and histochemical analysis of seed development of *E. oleracea*.

Materials and Methods

Flowers and fruits at different development stages were collected from a commercial orchard at the municipality of São Gonçalo do Amarante - Ceará state, Brazil (3°36'26''S, 38°58'14''W). A morphological characterization of flowers and fruits based on flower length, stigma color, pericarp diameter and pericarp color was carried out to establish the developmental stages used in the anatomical study of the seed development. A refence color chart was created to illustrate the colors being used on the description of flowers and fruits (Fig. 1).

For structural characterization, calyx and corolla were removed from flowers as well as the pericarp from fruits in order to isolate pistils and seeds, respectively. Pistils and seeds from five individuals were fixed in a solution of 1% glutaraldehyde and 4% formaldehyde in phosphate buffer (Karnovsky 1965). After fixation (48h), samples were washed in 0.2 M phosphate buffer at pH 7.2, dehydrated though a crescent ethanol series and embedded in hydroxyethyl methacrylate resin (Historesin, Leica) according to the manufacturer's

Figure 1 – Reference color chart for colors being used on the description of flowers and fruits of *Euterpe oleracea*.

recommendations, modified. Before embedding, samples were immersed in resin for 30 consecutive days. Each day, the flasks with samples immersed in resin were placed under vacuum during the day and in the fridge at night. The embedded material was then cross and longitudinally sectioned (5 µm thick) with the aid of an automated rotary microtome (Leica® RM 2065) equipped with tungsten carbide knives. Sections were stained with 0.05 % toluidine blue in 0.12 % borax for 10 min followed by 0.05 % basic fuchsin for 1 min (Junqueira 1990).

Sections of embedded material were used in the following histochemical tests carried out to study seed reserves: xylidine Ponceau, to detect total proteins (O'Brien & McCully 1981); ruthenium red, for pectins (Johansen 1940); lugol, for starch (Johansen 1940); Sudan III, for total lipids (Pearse 1980); Nadi reagent, for essential oils and oil/resins (David & Carde 1964) and periodic acid-Schiff (PAS), for total polysaccharides (O'Brien & McCully 1981), toluidine blue O at pH 4.4, for phenolic compounds (Ramalingam & Ravindranath 1970; Retamales & Scharaschkin 2014). Sections subjected to Sudan III, Nadi reagent were hand sectioned from fresh material only for stage S8.

Permanent slides were mounted with Tissue Mount (Tissue-TEK) and results were recorded with a digital camera (HP Photosmart R967) attached to a light microscope (Leica® DM4000) equipped with a digital system for image captures.

Results

Characterization of the flowers and definition of the seed development stages

Eight distinct colors were found to illustrate the flowers and the fruit development stages (Fig. 1). Flowers characterization and the seeds development stages are summarized in Table 1. Flower buds at pre-anthesis already present the gynoecium with reddish gynoecium at upper half of the gynoecium. The only remarkable morphological difference from flowers buds at pre-anthesis to flower at anthesis is the average length of flowers, 2 mm and 4 mm, respectively. S1 is the stage at which fruit is at the beginning of its development, the average diameter is 2 mm and gynoecium has black dry stigma and swollen ovary after fertilization. The upper half of the gynoecium is reddish, the middle portion is opaque yellow and lower portion is dark brown. At S2 and S3 stages, fruits present 4.5 mm and 9 mm diameter, respectively, and yellow-green pericarp. At S4, fruit is 10 mm wide and pericarp is shiny green. S5 presents fruits with 11 mm wide, opaque green

Table 1 – Description of flower and developing fruits selected for the developmental study of *Euterpe oleracea* seeds. F = flower at anthesis; FB = flower bud; S = stage of seed development.

Stages	Average diameter (mm)	Morphological Description*
FB	1.2	Flower bud at pre-anthesis. Reddish gynoecium, at upper half of the gynoecium.
F	1.3	Flower at anthesis. Reddish gynoecium, at upper half of the gynoecium.
S1	2	Developing young fruit. Gynoecium with dark brown dry stigma and swollen ovary after fertilization. Upper half of the gynoecium is reddish, middle portion opaque yellow and lower portion dark brown.
S2	4.5	Fruit with yellow-green pericarp.
S3	9	Fruit with yellow-green pericarp.
S4	10	Fruit with shiny green pericarp.
S5	11	Fruit with opaque green pericarp. Soft developing seed.
S6	11	Fruit with opaque green pericarp. Developing endocarp and seed harden.
S7	12	Fruit with green-purple pericarp. Developing endocarp and seed harden even more.
S8	12	Fruit with black-purple pericarp. Hard fully developed endocarp and seed.

* Refer to Figure 1 for color chart.

pericarp and soft developing seed while at S6 the size of fruit and pericarp color are the same as the previous stage, but the developing endocarp and seed begin hardening. At S7, the fruit become a little larger and the pericarp begins to turn purple in a way that the fruit is green-purple while the endocarp and seed becomes even harder. At the last stage, S8, the fruit ripens, the pericarp becomes black-purple and a hard fully developed endocarp and seed are found.

Anatomy of ovule during pre-anthesis

Ovules in *Euterpe oleracea* are bitegmic, hemianatropous with axile placentation and occupy almost the whole cavity of the locule (Fig. 2a). The funiculus is short and readily distinguished between the placentation area and the chalazal region (Fig. 2a). Tannin-containing idioblasts are found in the chalazal area opposite to the placentation (Fig. 2a). The vascular bundle penetrates through the funiculus and spreads from the massive chalaza, forming a pachychalaza (Fig. 2a). The hypostase accumulating phenolic compounds is found in the chalazal region above the embryo sac (Fig. 2a). The straight micropyle, opposite to the chalaza, is composed of the opening of both inner (endostome) and outer (exostome) integuments (Fig. 2b).

The inner and outer integuments are distinguished only at the micropylar area because of the presence of a pachychalaza (Fig. 2c). The inner integument is made up by 3–4 layers of small cells with densely stained cytoplasm and conspicuous bulky nuclei (Fig. 2b-c). On the other hand, the outer integument presents about 20 layers of larger and more vacuolated cells (Fig. 2b-c).

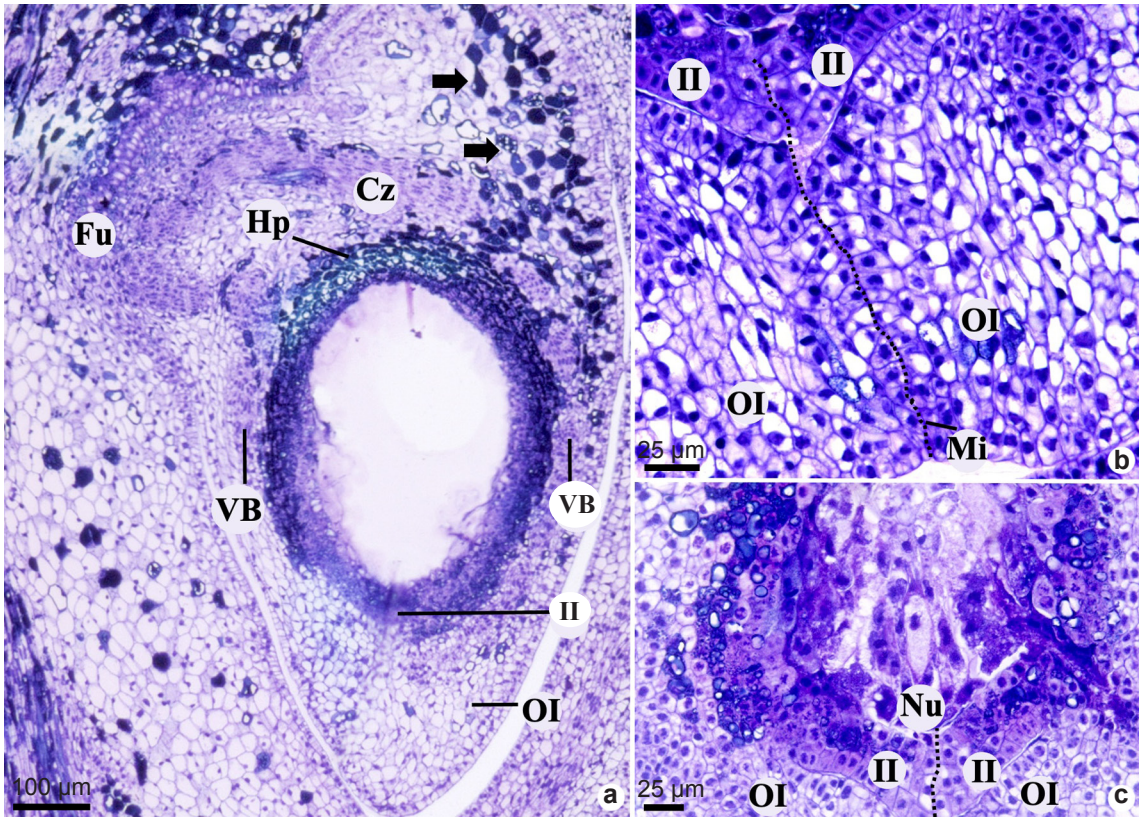

Figure 2 – a-c. Anatomy of *Euterpe oleracea* ovule during pre-anthesis in longitudinal sections – a. general aspects of the fully formed ovule [note the idioblasts with phenolic compounds (arrows)]; b. detail of the micropyle (dashed line), particularly of the outer integument; c. detail of the micropyle (dashed line), particularly of the inner integument. Cz = Chalaza; Fu = Funiculus; VB = Vascular bundle; Hp = Hypostase; Nu = Nucellus; Mi = Micropyle; OI = Outer integument; II = Inner integument.

Fruit and seed development

After the fertiization, during the seed development stage S1 (Tab. 1, Fig. 3), the young fruit of about 2 mm holds a developing seed with the seed coat occupying most of the seed volume (Fig. 3a-b). The seed coat cells present phenolic compounds while the raphe does not bear such substances (Fig. 3b). At stage S1, seed coat ingrowths have already begun to develop. Both the zygote (Fig. 3c) and a nuclear endosperm (Fig. 3d) are observed. At this stage, the zygote undergoes its first periclinal division.

At stage S2 (Tab. 1), the pericarp is shiny and yellow-green while the seed coat greatly develops, and its ingrowths reach the central cavity (Fig. 3e-f). The pro-embryo is composed of three cells

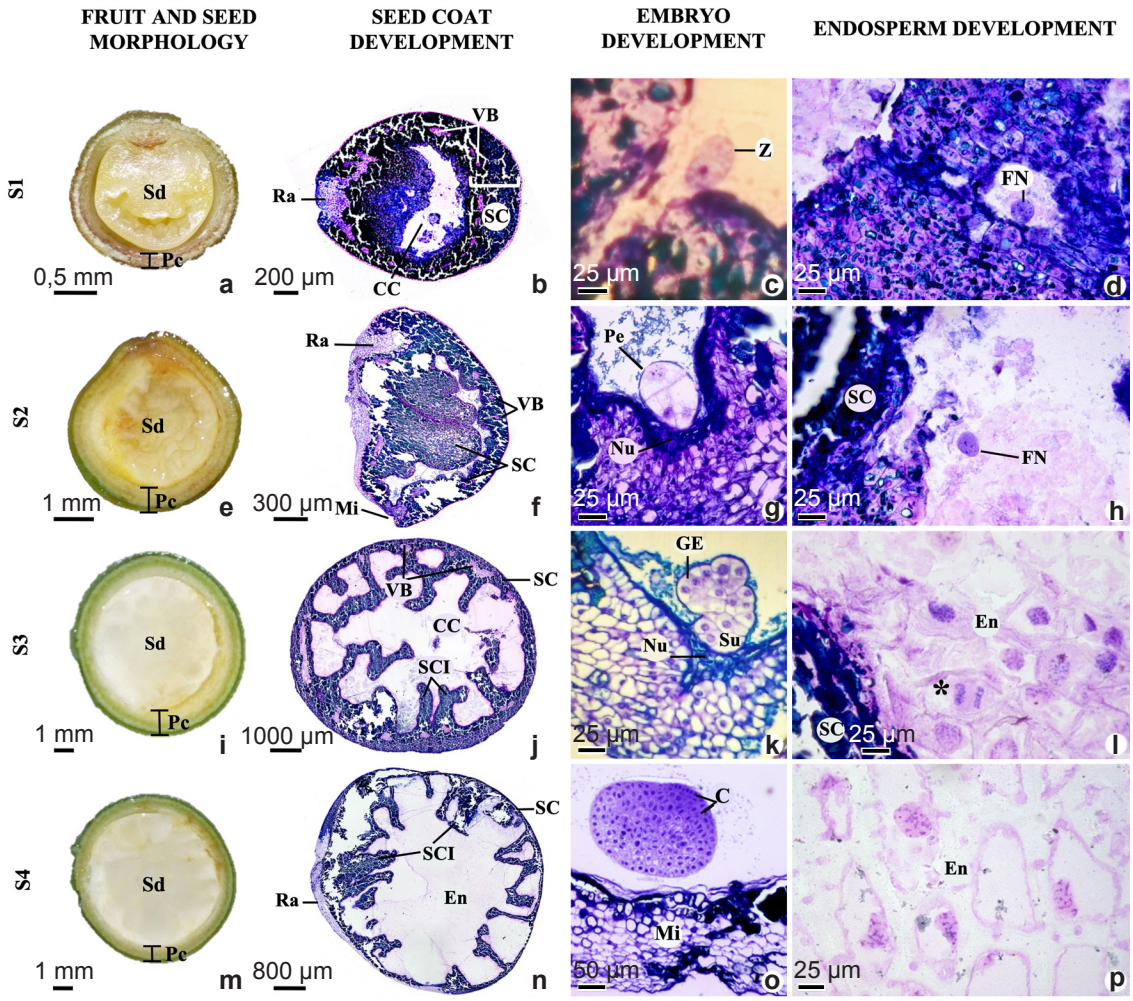

Figure 3 – a-p. Fruit and seed development of *Euterpe oleracea* in longitudinal sections, stages S1-S4 – a-d. stage S1 – a. developing fruit and seed (2 mm); b. general anatomical view of the seed; c. detail of zygote; d. free nuclear endosperm (note the free nucleus); e-h. stage S2 – e. developing fruit and seed (4.5 mm); f. initial development of the ruminate endosperm; g. pro-embryo made up by three cells; h. free nuclear endosperm; i-l. stage S3 – i. developing fruit and seed (9 mm); j. note the ruminate endosperm; k. early globular embryo; l. cellular endosperm [note that some cells are still dividing (asterisk)]; m-p. stage S4 – m. developing fruit and seed (> 10 mm); n. note the ruminate endosperm and thin seed coat; o. late globular embryo (note the expansion of the future cotyledon); p. endosperm with thickened cell walls and peripheral nuclei. C = cotyledon; Cc = central cavity; GE = globular embryo; En = endosperm; FN = free nucleus; Mi = micropyle; Nu = nucellus; Pc = pericap; Pe = pro-embryo; Ra = raphe; SC = seed coat; SCI = seed coat ingrowths; Sd = seed; Su = suspensor; VB = vascular bundles; Z = zygote.

(Fig. 3g), as the basal cell already went through an anticlinal division. The free nuclei of the endosperm are more evident (Fig. 3h).

At stage S3 (Tab. 1), there is an increase in the fruit diameter (Fig. 3i). In longitudinal sections it is possible to observe prominent ingrowths of the seed coat which correspond to the endosperm ruminations towards the central cavity (Fig. 3j). The pro-embryo displays the suspensor attached to a thin layer of nucellar cells (Fig. 3k). At this time, the endosperm has a jelly-like appearance in seeds longitudinally sectioned and it is possible to anatomically observe the beginning of the cellularization process (Fig. 3l). Such cells present thin walls and show active division (Fig. 3l). At stage S4 (Tab. 1), the pericarp structure is similar to S3 (Fig. 3m) while the seed coat becomes thinner (Fig. 3n) as a result of the endosperm cells enlargement. A remarkable difference at this stage is the embryo with initial organization of a lateral cotyledon and a shoot apex (Fig. 3o). Moreover, the endosperm presents thickened-wall cells with large peripheral nuclei (Fig. 3p).

Fruits with opaque green pericarps are observed at stage S5 (Fig. 4a). The layers of seed coat ingrowths are slightly thinner than in the former stage (Fig. 4b). The embryo shows a cotyledonary primordium expanding around the shoot apex (Fig. 4c). At this point, the endosperm has cells with thicker cell walls, where several primary pit fields are observed (Fig. 4d).

The fruits still have opaque green pericarps at stage S6 (Fig. 4e), while the seed is hard. The seed coat is similar to the previous stage (Fig. 4f). In the embryo, the shoot apical meristem is observed within the cotyledonal cavity, which forms because of growth of the upper side of the cotyledon. The root apical meristem is also differentiated (Fig. 4g). The endosperm cells display thicker walls and cytoplasmic inclusions (Fig. 4h).

At stage S7 (Fig. 4i), the embryo presents greater development of the upper side of the cotyledon (Fig. 4j-k), which occupies most of the embryonic cavity. The endosperm is well developed, the cell walls become thicker and cytoplasmic inclusions are still persistent (Fig. 4l). Finally, at stage S8, the fruit shows a purple pericarp and fully developed seed (Fig. 4m-n). The minute conic and linear embryo (Fig. 4o) occupies the whole embryonic cavity. Radicle and shoot apical meristems are fully developed. The proximal region of the embryo, at the cotyledon base, corresponds to the cotyledonary petiole. The upper side of the cotyledon, at the distal region, is richly vascularized by procambial strands (Fig. 4o), corresponding to the haustorium. The endosperm displays irregular-shaped cells with thickened walls full of primary pit fields and cytoplasmic inclusions (Fig. 4p).

Apart from the endosperm ruminations, the outer layers of the seed coat form a homogeneous tissue that becomes thinner during seed development (Fig. 3b, 3f, 3j, 3n, 4b, 4f). Only at the raphe-hilar region does the seed coat homogeneity change and the slight mound formed by the raphe and hilar wound can be readily distinguished (Fig. 3n, 4b, 4f).

Histochemical study of the seed reserves

The histochemical analysis showed that protein accumulation is gradual during endosperm development. Protein bodies are well observed in the endosperm cells (Fig. 5a) and embryo (Fig. 5b). The presence of pectin is observed within the cytoplasm of the endosperm cells (Fig. 5c) and embryo (Fig. 5d). The thickening in the cell walls of the endosperm is not due to pectin depositions, as the ruthenium red dye did not bind to the wall. Starch is only found in the embryo (Fig. 5e) near the shoot and radicle apical meristems. Total lipids (Fig. 5f) and a mixture of essential oils/resins (Fig. 5g) are detected only within the endosperm cells.

At stage S4, the PAS reaction shows the development of the endosperm cell wall thickening (Fig. 6a-c). Cells adjacent to the inner integument have thin walls (Fig. 6a-b). The farther the endosperm cells are from the inner integument (*i.e.* closer to the central cavity), the thicker they become (Fig. 6a, 6c). These cell wall thickenings display a more homogenous thickness at stage S7 (Fig. 6d). At stage S8, the endosperm cells are homogenously stained with PAS (Fig. 6e) and cells bordering the embryonic cavity start to be digested (Fig. 6e).

Discussion

The ovules in *Euterpe oleracea* are hemianatropous. But this is a variable condition in members of Arecaceae, which may also exhibit anatropous, campylotropous and orthotropous ovules (Uhl & Moore 1971; Genovese-Marcomini *et al.* 2013; Mazzottini-dos-Santos *et al.* 2015; Castaño *et al.* 2016). Moreover, although bitegmic ovules are widely distributed in the family, the micropyle may be formed by both the integuments, as observed in *E. oleracea*, by the inner integument, or by the outer integument (Genovese-Marcomini *et al.* 2013; Mazzottini-dos-Santos *et al.* 2015)

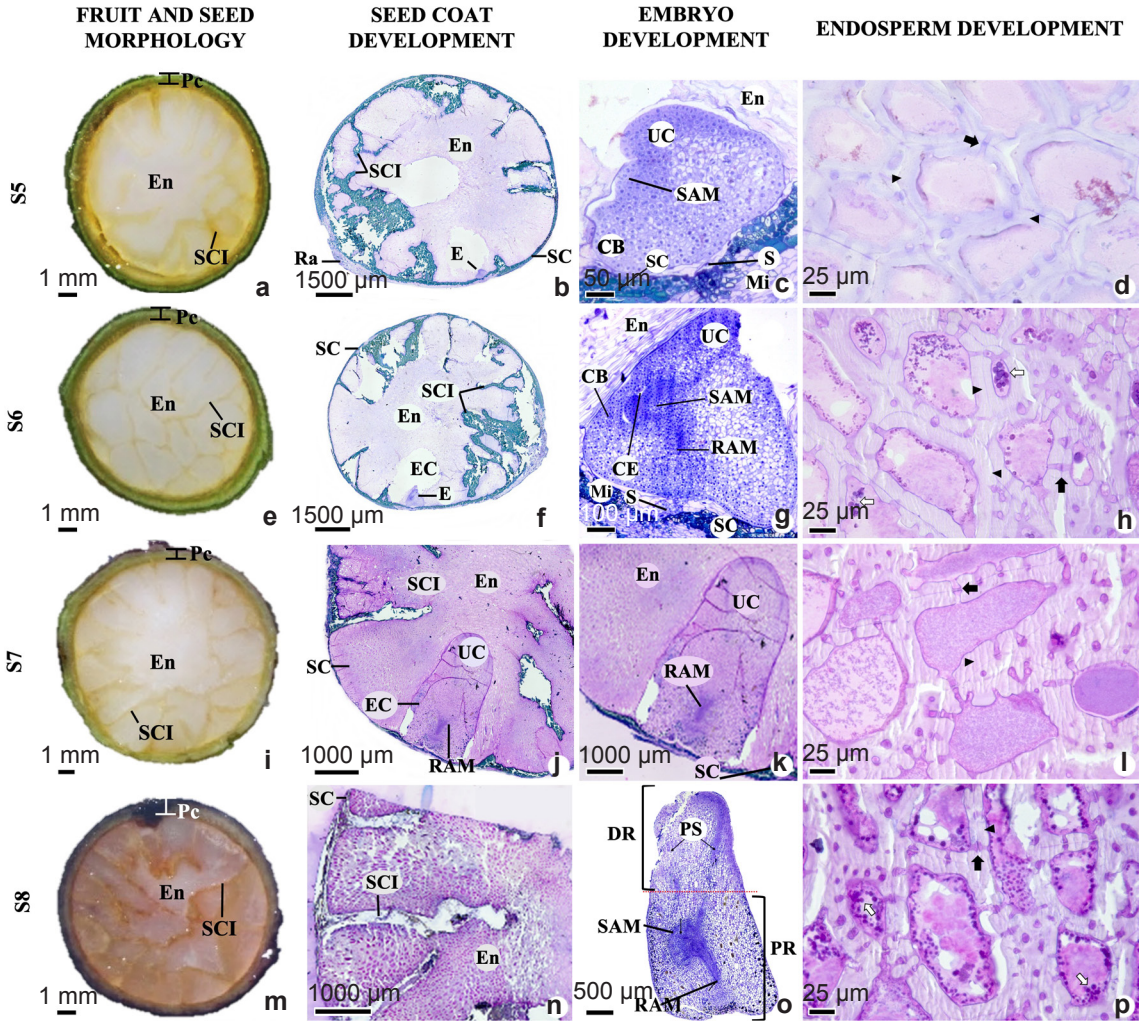

Figure 4 – a-p. Fruit and seed development of *Euterpe oleracea* in longitudinal sections, stages S5-S8 – a-d. stage S5 – a. developing fruit and seed (11 mm, opaque green pericarp and soft seed); b. general view of an anatomical section from the developing seed; c. young embryo showing the cotyledonary base and the upper part of the cotyledon; d. endosperm with thickened cell walls (black arrowheads) [note the primary pit fields (black arrow)]; e-h. stage S6 – e. developing fruit and seed (11 mm, opaque green pericarp and hard seed); f. general view of seed anatomy; g. detail of the embryo; h. endosperm with thickened cell walls (black arrowheads) and cytoplasmic inclusions (white arrows) [note the primary pit fields (black arrow)]; i-l. stage S7 – i. developing fruit and seed (12 mm, green-purple pericarp and hard seed); j. general view of seed anatomy with ruminate endosperm (note the development of the upper side of the cotyledon); k. detail of cotyledonal embryo; l. detail of endosperm cells with cytoplasmic inclusions, thickened cell walls (black arrowheads), and primary pit fields (black arrow); m-p. stage S8 – m. fruit and seed (12 mm, purple pericarp and hard fully developed seed); n. mature seed (note the ruminate endosperm); o. mature embryo; p. detail of endosperm with thickened cell walls (black arrowheads), primary pit fields (black arrow), and cytoplasmic inclusions (white arrows). CB = Cotyledonary base; CE = cotyledonary edge; DR = distal region; E = embryo; EC = embryo cavity; En = endosperm; Mi = micropyle; PC = procambium strands; Pc = pericarp; PR = proximal region; PS = procambium strand; Ra = raphe; RAM = root apical meristem; S = suspensor; SAM = shoot apical meristem; SC = seed coat; SCI = seed coat ingrowths; UC = upper cotyledon.

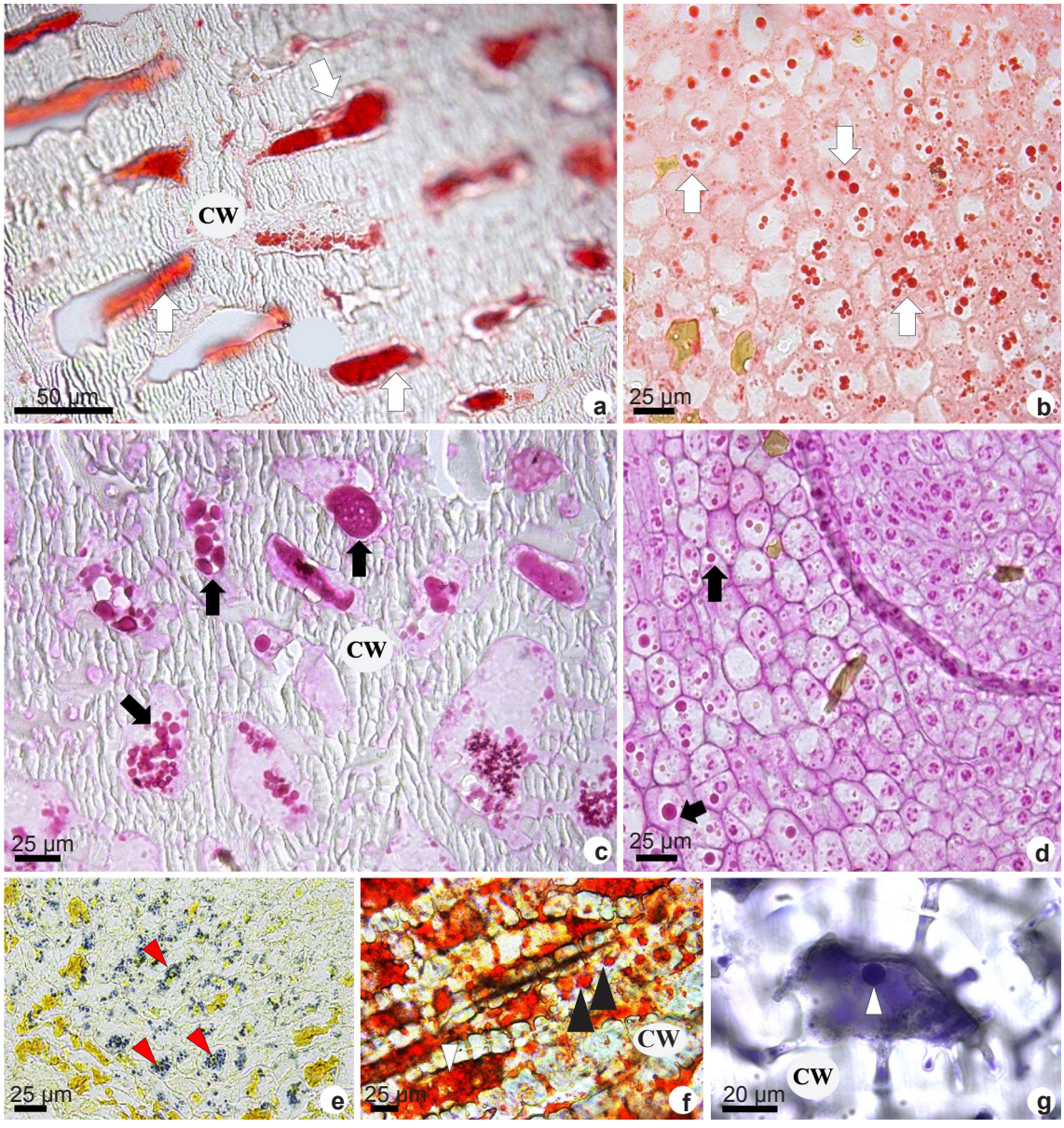

Figure 5 – a-g. Histochemical study of the seed reserves of *Euterpe oleracea* at stage S8 when fruit has black-purple pericarp and hard fully developed endocarp and seed – a-b. total proteins indicated by the orange/red color of cytoplasmatic contents stained with xylidine Ponceau (white arrows = protein bodies) – a. endosperm in fully developed seed (12 mm fruit with purple pericarp); b. embryo; c-d. pectins as indicated by the pink color when stained with ruthenium red (black arrows = pectin); c. endosperm cells; d. embryo; e. starch grains within the embryo as indicated by the black color when stained with lugol (red arrowheads = starch); f. total lipids in the endosperm as indicated by the orange/red color when stained with Sudan III (black arrowheads = lipids); g. oils in the endosperm as indicated by the purple color when stained with NADI reagent (white arrowheads = oil droplet). CW = cell wall.

(Johri *et al.* 1992). The pachychalaza, as reported in *E. oleracea*, is formed by intercalary growth of chalaza, which becomes massive and well-vascularized (Boesewinkel & Bouman 1984). It has been proposed that this allows a more efficient transfer of nutrients to the embryo (von Teichman & van Wyk 1994). However, the presence of a minute embryo does agree with such proposition. Interestingly, the presence of a conspicuous well-developed endosperm full of reserves in the seeds would agree with the pachychalaza playing a role in a more efficient transfer of nutrients to the endosperms instead. Pachychalazal ovules/seeds were previously described in Arecaceae only in *Syagrus inajai* (Genovese-Marcomini *et al.* 2013) and *Acrocomia aculeata* Lodd. *ex* Mart. (Mazzottini-dos-Santos *et al.* 2015). It is likely that pachychalaza has been overlooked in the family due to lack of embryological studies.

The accumulation of phenolic compounds throughout the chalaza, hypostase and seed coat may be a functional adaptation to protect the embryo during its development, as these compounds are usually related to protection against pathogens because of their antimicrobial activity (Roshchina & Roshchina 1993; Bhattacharya *et al.* 2010). Moreover, the hypostase is important for the stabilizing the water balance of resting seeds during the dormancy, on facilitating the rapid transport of nutrients to the embryo sac or even on producing enzymes or hormones that may play a protective role in mature seeds (Bhojwani & Bhatnagar 2008).

The seed morphoanatomy of *Euterpe* species has been described in previous reports (Aguiar & Mendonça 2002, 2003; Panza *et al.* 2004; Araújo 2005), but we highlight this is the first

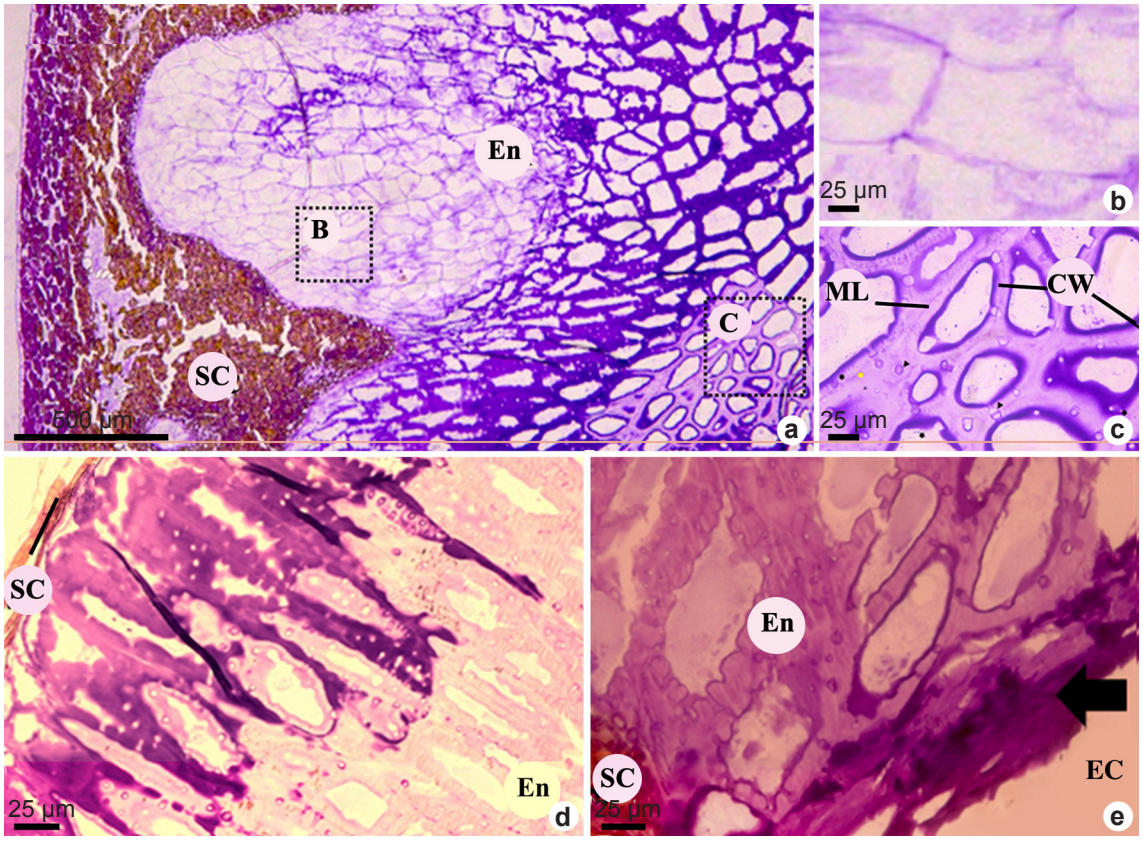

Figure 6 – a-e. Centrifugal maturation of the endosperm in the seeds of *Euterpe oleracea*. Total polysaccharides stained in shades of purple by periodic acid Schiff – a-c. stage S4 – a. general view of the endosperm showing two distinct areas (B and C); b. cells near the seed coat with thin cell walls; c. cells with thickened cell walls at innermost areas of the endosperm; d. stage S7. Fully developed endosperm (note that all cells present thickened walls); e. stage S8. Degradation of endosperm cells bordering the embryonic cavity. CW = cell wall; EC = embryonic cavity; En = endosperm; ML = middle layer; SC = seed coat; black arrowhead = digestion zone.

study to describe both the embryo and endosperm development in the *açaí* genus. The embryogenesis of *E. oleracea*, as in other palm species, results in a linear embryo, surrounded by the abundant endosperm. The embryo axis is microscopic and inserted within the cotyledonary petiole (Haccius & Philip 1979; Genovese-Marcomini *et al.* 2013; Mazzottini-dos-Santos *et al.* 2015). Although embryo development seems to be uniform throughout the family (Haccius & Philip 1979; Genovese-Marcomini *et al.* 2013), more studies are needed for verification. In this sense, our results bring novel information about the embryo development of Arecaceae, and such data is a starting point to improve our understanding about the germinative process, the establishment, and propagation of palms.

The ruminate endosperm observed in *E. oleracea* have also been reported in other species of Arecaceae (Paula 1975; Reddy & Kulkarni 1985; Zona 1992; Charlo *et al.* 2006). It seems to occur in at least 51 genera within the family and may have evolved independently many times in the evolutionary history of the group (Zona 1992). It is characterized by an uneven endosperm surface, due to ingrowths or infoldings of the seed coat (Bayer & Appel 1996). These authors summarize several hypotheses that have been given to explain the presence of ruminate endosperm in seeds, such as: less seed palatability due to the presence of phenolic compounds in the seed coat, facilitation of seed imbibition during germination and enlargement of the contact area between storage tissue and seed coat, increasing the nutrient intake, and gases and water for the embryo and endosperm.

The endosperm in monocots has a fundamental role in embryo development, the germination process and the establishment of seedlings, as it is the main nutritive tissue in seeds (Oliveira *et al.* 2013; Mazzottini-dos-Santos *et al.* 2018). As for *açaí*, other palm seeds also bear thickened-wall endosperm cells occupying the entire seed cavity when mature (Belin-Depoux & Queiroz 1971; Henderson *et al.* 1995; Aguiar & Mendonça 2003; Mendonça *et al.* 2008; Moura *et al.* 2010; Nazário *et al.* 2013; Mazzottini-dos-Santos *et al.* 2017).

Proteins and lipids present in the endosperm of *açaí* are usually found in the endosperm of other palm trees (Panza *et al.* 2004; Gonçalves *et al.* 2010; Nazário *et al.* 2013), where they are used as sources of carbon and nitrogen for the growing seedling (Gonçalves *et al.* 2010; Oliveira *et al.* 2010; Bicalho *et al.* 2016). The protein bodies found within the endosperm cells of palm trees may be related to the accumulation of hydrolytic proteins, activated during the mobilization of the endosperm reserves. Similarly, the occurrence of pectins within the endosperm cells of *açaí* have also been reported for other palm species (Moura *et al.* 2010; Nazário *et al.* 2013; Rodrigues *et al.* 2015; Mazzottini-dos-Santos *et al.* 2017, 2018). Pectins are structural polysaccharides that usually compose the cell wall. When found within the cell they may be the result of structural changes as it is translocated from the cell wall in a way that pectins actually become a gel within the cell that may be metabolized during germination (Rodrigues *et al.* 2015; Taiz *et al.* 2017).

In line with observations on other palm trees, no significant amount of starch was found during the endosperm development of *E. oleracea* seeds, thus confirming the notion that starch is not the nutritive reserve of the endosperm in the Arecaceae (Panza *et al.* 2004; Gonçalves *et al.* 2010; Moura *et al.* 2010; Nazário *et al.* 2013). Starch was observed near the embryo axis at the radicle area however, as has been reported in palms (Nazário *et al.* 2013; Rodrigues *et al.* 2015). Starch is one of the first molecules to be catabolized in order to provide energy for the most common anabolic reactions during germination (Nelson *et al.* 2013). This starch also plays an important role in the control of geotropism within the root cap (Taiz *et al.* 2017).

Our results indicate the nutritive reserve of the *açaí* is located in the thickened cell walls. Reserve polysaccharides in the cell wall of the endosperm are common among the Arecaceae (Panza *et al.* 2004; Moura *et al.* 2010; Nazário *et al.* 2013; Oliveira *et al.* 2013; Pinho *et al.* 2014). During seed germination, the presence of enzyme endo-β-mannanase near the area where the endosperm is being consumed (*i.e.* adjacent to the embryo/haustorium) has already been reported (Mazzottini-dos-Santos *et al.* 2017). In addition to being one of the reserves for the developing embryo, mannans may provide mechanical resistance as the embryo is protected from damage during the long germination of palm seeds (Buckeridge *et al.* 2000; Mazzottini-dos-Santos *et al.* 2018).

Conclusions

In this study, we presented a detailed analysis of the development of *açaí* seeds and highlighted defining characteristics of the seed coat, embryo, and endosperm, relating them to fruit development. Our data show that the nutritive reserves of *açaí*

seeds are found in the thickened cell walls as reserve polysaccharides. This analysis will help in defining the seed tissues to be analyzed in order to obtain transcriptomic and proteomic data to assess the feasibility of using the seeds as a source of raw material to produce second-generation ethanol.

Acknowledgements

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. We also thank the Brazilian National Research Council (CNPq) for financial support.

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Area Editor: Dra. Simone Teixeira
Received in November 06, 2019. Accepted in April 27, 2020.