

# Pericarp development in the macaw palm *Acrocomia aculeata* (Arecaceae)

Desenvolvimento do pericarpo em macaúba [Acrocomia aculeata (Arecaceae)]

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### Abstract

The anatomy of the pericarp of the macaw palm (*Acrocomia aculeata*) was followed during development. Ovaries of flowers collected at anthesis of the bracts as well as pericarps were evaluated at different development phases using traditional plant anatomy techniques. The ovary wall has two meristematic regions, one adjacent to the external epidermis and the other surrounding the seminal cavity. The external meristematic region gives rise to the woody exocarp, and the internal meristematic region is responsible for thickening of the oily/fibrous mesocarp as well as the hard endocarp. Sclerification of the exocarp and endocarp occurs approximately 70 days after anthesis and defines the final fruit volume. Lignification of the exocarp that are formed by the fusion of raphide-containing idioblasts. Lignification of the sclereids and their generally random arrangement confers impermeability and rigidity to the endocarp. In mature fruits, lipidic reserves are observed in parenchymatic cells of the mesocarp, and the germination pore in the endocarp is composed of parenchymatic cells.

Key words: fruit ontogenesis, lipids, sclerification.

#### Resumo

O objetivo deste trabalho foi caracterizar anatomicamente o pericarpo da macaúba (*Acrocomia aculeata*) durante seu desenvolvimento. Foram avaliados ovários de flores coletadas no dia da antese da bráctea e pericarpos em diferentes fases de desenvolvimento, por meio de técnicas usuais em anatomia vegetal. A parede ovariana apresenta duas regiões meristemáticas, uma adjacente à epiderme externa e outra que envolve a cavidade seminal. A região meristemática externa origina o exocarpo lenhoso. A região meristemática interna é responsável pelo espessamento do mesocarpo oleaginoso-fibroso e do endocarpo pétreo. A esclerificação do exocarpo e do endocarpo, que ocorre próximo dos 70 dias da antese, define o volume final do fruto. A lignificação das células do exocarpo é incompleta, conferindo porosidade à estrutura. No mesocarpo se desenvolvem numerosos canais, formados pela fusão de idioblastos contendo ráfides. No endocarpo, a lignificação e a orientação das esclereídes em várias direções, conferem impermeabilidade e rigidez. Nos frutos maduros, a reserva lipídica se localiza em células parenquimáticas do mesocarpo e observa-se a presença de poro germinativo no endocarpo, composto por células parenquimáticas.

Palavras-chave: ontogênese do fruto, lipídios, esclerificação.

# Introduction

The macaw palm [Acrocomia aculeata (Jacq.) Lodd. ex Mart.] is widely distributed throughout Brazil, and its oleaginous fruits have traditionally been used in foods and for manufacturing of soaps (CETEC 1983; Motta *et al.* 2002). The high productivity of this palm, the quality of its oil, and its drought-resistance have created interest in its agro-industrial uses, including for the production of bio-fuels (CETEC 1983; Moura *et al.* 2010).

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Pecularities in the cells of the pericarp may confer economic importance to the fruit (Sousa 2006) influencing its reproduction, use and cultivation. The protection provided by the exocarp of A. aculeata, due to its mechanical resistance and limited permeability, influences post-harvest bacterial degradation of the oils present in the mesocarp (CETEC 1983). The fibrous nature of the pulp and the presence of nutrients reflects the types of cells found in the mesocarp (Souza 2006; Vegas et al. 2008), which in turn influence the commercial value of the fruits and the types of the industrial processes required for oil extraction (CETEC 1983). The hard endocarps that are common features of palm fruits influence their relationships with disperser and predator species (Essig 1999) and can determine physical dormancy (Orozco-Segovia et al. 2003), and in the specific case of the macaw palm these structure have excellent potential for energy generation (CETEC 1983; Silva et al. 1986).

As such, the present work examined the anatomical development of the pericarp of *Acrocomia aculeata* to provide more detailed information about its structure that should aid studies examining post-harvesting storage and seed germination as well as contribute to programs of genetic improvement of this species to produce larger fruits with more oil and calorific power.

# **Materials and Methods**

The plant material used was obtained from Acrocomia aculeata palms growing on the campus of the Universidade Estadual de Montes Claros in Minas Gerais State, Brazil, between the months of September/2008 and November/2009. The ovaries examined where removed from flowers obtained on the first day of anthesis of the bracts, and the fruit pericarps were collected every two days during the first two weeks, and then every fourth day from the third to eleventh week of development (when the lignification of the exocarp and endocarp were complete). The presence of reserve compounds was investigated using histochemical tests of mature fruits collected after natural abscission. In preparation for the anatomical evaluations, the material was fixed in FAA, stored in 70% ethanol, dehydrated in an ethanol series, and embedded in paraffin using butanol as the solvent (Johansen 1940). Transversal and longitudinal sections were made at 10 µm thicknesses in a rotary microtome, stained with astra blue and Safranin (Bukatsch 1972) as well as toluidine blue and hematoxylin (Johansen 1940), and were subsequently mounted on permanent slides using acrylic resin. The classification and description of pericarp will be according Roth (1977).

Part of the freshly collected material was transversally sectioned and submitted to histochemical tests that could identify total lipids using Sudan III (Johansen, 1940), proteins using xilidine Pounceau (O'Brien & McCully 1981), starch using lugol solution (Jensen 1962), mucilage using tannic acid (Pizzolato & Lillie 1973), and tannins using vanillin chloride (Mace and Howell 1974). The slides were observed under a Nikon 250 light microscope and photomicrographs were taken using a coupled Canon A620 digital camera.

## Results

The ovary of macaw palm is superior, tricarpellary, syncarpous and has a single ovule in each locule with axile placentation. In the carpels mesophyll it is possible to distinguish an external and a median regions, and an inner adaxial meristem (Fig. 1a). The external epidermis is uniseriate, is composed of cubical cells, and has long, multiseriate tector trichomes with elongated cells and thin cell walls (Fig. 1b). Globoid meristematic-appearing cells were observed in the external region of the mesophyll interspersed with radially elongated cell bundles (Fig. 1b). The median region of the mesophyll is predominantly composed of parenchymatic cells, and tannin-containing idioblasts as well as raphidecontaining idioblasts were seen (the latter cells being positioned more externally) (Fig. 1a,c). The vascularization, which started in the receptacle, was distributed diffusely through the mesophyll (Fig. 1a,c) and was composed of collateral vascular bundles (Fig. 1c,d). An adaxial meristem (Fig. 1a,d) correspond to the internal region of the mesophyll, present periclinal divisions. The internal epidermis was uniseriate and composed of cubical cells (Fig. 1d).

It was possible to observe at two, 20, and 70 days after anthesis the principal events that would determine the characteristics of the mature pericarp. After two days of development, the initial stages of the atrophy of two of the carpels in the fruit could be seen. As well as the differentiation of the exocarp that initiated in the external epidermis and the

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**Figure 1** — Transversal sections of the ovary of *Acrocomia aculeata*. a. Panoramic view, indicating the different anatomical regions. b. External region of the mesophyll, indicating bundles of radially elongated cells (arrow). c. Median region of the mesophyll, indicating raphide-containing idioblasts (arrow). d. Adaxial meristem, corresponding to the internal region of the mesophyll, indicating cells undergoing periclinal divisions (arrow); internal region of the adaxial meristem (box). am, adaxial meristem; ee, external epidermis; er, external region of the mesophyll; ie, internal epidermis; lo, locule of the ovary; mc, meristematic cells; mr, median region of the mesophyll; ov, ovule; ri, raphide-containing idioblast; ti, tannin-containing idioblast; tr, trichome; vb, vascular bundle.

external region of the mesophyll showing bundles of radially elongated cells (Fig. 2a). The trichomes show accumulations of tannins and the epidermis remains uniseriate (Fig. 2b). The mesocarp differentiates from the median region of the ovarian carpel mesophyll where increases in cell volume and fusion of the raphide-containing idioblasts were observed, the latter resulting in the formation of canals (Fig. 2a,c). The endocarp begins its differentiation, in the adaxial meristem, and cells could be seen there dividing in all directions; cells in the internal epidermis only divide anticlinally maintaining its uniseriate nature (Fig. 2d). At 20 days of development, continuity of the differentiation of all of the pericarp regions was observed. In the exocarp, the remaining trichomes had become lignified, and stomata could be seen in the external epidermis as well as raphide-containing idioblasts in the parenchymatic layer (Fig. 3a-b). Bundles of radial sclerenchymal fibers could be seen in their initial stages of lignification, and these were bordered by isodiametric cells. In the mesocarp/endocarp transition, the external layers of the adaxial meristem add new mesocarp elements such as parenchymatic cells, tannin and raphide containing idioblasts, vascular bundles, and bundles of



**Figure 2**—Longitudinal sections of the pericarp of *Acrocomia aculeata*, after two days of development. a. Panoramic view, showing the exocarp (epidermis and external mesocarp), mesocarp and endocarp (internal mesocarp and epidermis); the arrow indicates a canal. b. Exocarp indicating trichomes with accumulations of tannins (arrow). c. Mesocarp, indicating the formation of canals through the fusion of raphide-containing idioblasts. d. Endocarp (originated from the adaxial meristem and epidermis) indicating dividing cells (arrow). ca, canals; ee, external epidermis; en, endocarp; ex, exocarp; ie, internal epidermis; me, mesocarp; ri raphide-containing idioblast; ti, tannin-containing idioblast; tr, trichome; vb, vascular bundle.

sclerenchymal fibers (Fig. 3c). The cells of the internal layers of the adaxial meristem divide in all directions, contributing to the expansion of the endocarp (Fig. 3d). An accumulation of tannins was observed in the cell layer adjacent to the internal epidermis. The epidermal cells divided anticlinally and thus did not contribute to the thickening of the endocarp.

At 70 days of development, the majority of trichomes are falled. In the parenchymatic region, of the exocarp that is located adjacent to the epidermis, canals are formed from the fusion of raphide-containing idioblasts. Internally, regions with lesser

degrees of lignification occur due to the presence of parenchymatic cells and sclereids among the radial fiber bundles (Fig. 4a). Increases in the volumes of the mesocarp canals could also be noted (Fig. 4b). The external layers of the adaxial meristem, located in the transition region between the mesocarp and the endocarp continued in activity, and continued to thicken the mesocarp (Fig. 4c). In the endocarp the differentiation of sclerids could be seen, with evident pits, and oriented in many different directions, and these structures serve to harden it. The cells of the internal layers of the endocarp accumulate tannins Pericarp development in the macaw palm



**Figure 3**— Transversal sections of the pericarp of *Acrocomia aculeata* after 20 days of development. a. Exocarp, indicating bundles of radial sclerenchymal fibers in their initial stages of lignification (arrow) and isodiametric cells (head arrow). b. Exocarp, indicating the parenchymatic layer. c. External layers of the adaxial meristem, in the transition region of the mesocarp-endocarp giving rise to new mesocarp elements (arrow). d. Endocarp, indicating dividing cells (arrow); a layer with accumulated tannins (head arrow), and epidermal cells undergoing anticlinal divisions (asterisk). ca, canal; ee, external epidermis; en, endocarp; il, internal layers of the adaxial meristem; pa, parenchyma; rf, radial fiber bundles; ri, raphide-containing idioblasts; sf, sclerenchymal fiber bundles; st, stomata; ti, tannin-containing idioblast; tr, trichome.

and cease any meristematic activity (Fig. 4d). The volume of the fruit approximates that of the mature fruit in this phase of development, its expansion being restricted by the sclerification of both the exocarp and endocarp.

At approximately 380 days after anthesis of the bracts, the pericarp of the mature fruit is very similar to that seen at 70 days, with a woody exocarp, a fibrous mesocarp, and a hardened endocarp with a germination pore composed of fibrous tissue (Fig. 5a). The fibrous/oily mesocarp shows lignification of the sclerenchymal fiber bundles and tracheal elements (Figs. 5b), as well as the accumulation of oils in the parenchymatic cells (Figs. 5c) and mucilage in the canals. A discrete accumulation of proteins was observed in the parenchymatic cells, but no starch deposition was evident during any of the developmental phases. The hard nature of the endocarp is determined by the presence of sclereids, with thick and highly lignified cell walls.



**Figure 4** — Longitudinal sections of the pericarp of *Acrocomia aculeata* after 70 days of development. a. Exocarp, indicating regions with lesser degrees of lignification (arrows). b. Mesocarp, indicating canals with increasing volumes. c. Endocarp, indicating the external layers of the adaxial meristem responsible for mesocarp thickening (arrow). d. Endocarp, indicating the meristematic region that adds cells to the endocarp (arrow); and cells with accumulations of phenolic compounds (head of arrow). ca, canal; en, endocarp; ie, internal epidermis; il, internal layers of the endocarp; me, mesocarp; pa, parenchyma; pi, pits; sc, sclereids; ti, tannin-containing idioblast; vb, vascular bundle.

# Discussion

The superior, tricarpellary, syncarpous ovary of macaw palm, with a single ovule with axile placentation are typical characteristic of the Arecaceae (Uhl & Moore 1971; Mercadante-Simões *et al.* 2006; Dransfield *et al.* 2008; Judd *et al.* 2009). Raphide-containing idioblasts have been seen in the ovaries of palm species of the genera *Licuala* Thunb. (Barfod *et al.* 2003) and *Chamaedorea* Willd. (Askgaard *et al.* 2008). The process of canal formation from raphidecontaining idioblasts has been reported in three species of *Astrocaryum* G. Mey. (Arecaceae) (Vegas *et al.* 2008) and tannin-bearing idioblasts were reported in the flowers of *Geonoma interrupta* Mart. (Stauffer *et al.* 2002) and in the fruits of *Pelagodoxa* Becc. (Chapin *et al.* 2001) and *Oenocarpus* Mart. (Mendonça *et al.* 2008). Ovaries of palms are often seen to abort during the first phases of fruit development, characterizing a pseudo-monomerous condition (Uhl & Moore 1971; Orozco-Segovia *et al.* 2003) that gives rise to drupes with a single seed in the pyrene (Souza 2006). In the case of the macaw palm, the fruits Pericarp development in the macaw palm



**Figure 5**—Transversal sections of the mature fruit of *A*. *aculeata*. a. General view, indicating the germination pore (arrow). b. Mesocarp indicating sclerenchymatic fibers. c. Mesocarp, indicating lipid containing cells. ca, canals; ed, endosperm; em, embryo; en, endocarp; ex, exocarp; me, mesocarp; sf, bundle of sclerenchymal fibers.

can be considered drupoids, due to the occasional occurrence of more than one seed in the pyrene (Souza 2006).

The lignification of the exocarp in palms is due to the presence of sclerenchymal fiber bundles

and sclereids in the subepidermal region (Essig 1999; Vegas *et al.* 2008). The brittle nature of the woody exocarp of the macaw palm results from the presence of groups of sclereids interspersed in the fiber bundles that generate lines of weakness (a characteristic of the species of the subtribe Linospadicinae) (Essig 2002). This type of anatomy may favor the penetration of bacteria that can degrade the mesocarp oils - and this represents an important post-harvest problem with the fruits of the macaw palm (CETEC 1983).

The fibrous nature of the macaw palm mesocarp (Lorenzi *et al.* 2004) is due to the conspicuous presence of sclerenchymal fibers, as was also observed in species of *Astrocaryum* G. Mey. (Vegas *et al.* 2008). The presence of mucilage in the fruit pulp probably contributes to maintaining its water content, but will influence the extraction and quality of its oil. The accumulation of lipids in palm mesocarp is quite common (Sambanthamurthi *et al.* 2000; Mendonça *et al.* 2008), and a 16.5% oil content has been reported for this structure in the macaw palm (Hiane *et al.* 2005).

Fruit development in *Acrocomia aculeata* is very slow (Scariot & Lleras 1991) and the formation of oils occurs only very near to the moment of abscission. As this oil is susceptible to degradation (CETEC 1983; Hiane *et al.* 2005) additional studies will be needed that can associate the development phases of the fruit with the accumulation of lipids; this information could help define harvesting strategies appropriate to maintaining the post-harvest quality of the fruit/oil.

The mixed origin of the macaw palm endocarp from the internal mesophyll and internal epidermal regions requires the use of the term endocarp sensu lato for the covering of the woody pyrene but not sensu stricto, as origin is not exclusively epidermal (Roth 1977). Endocarp sensu lato is also observed in Oenocarpos minor Mart. that is composed of layers of mesocarpic fibers adhering to the seed integument (Mendonça et al. 2008). The high specific weight of the macaw palm endocarp makes it attractive as a potential source of renewable energy (CETEC 1983; Silva et al. 1986) and its density is a consequence of the intense lignification of the cell walls of the sclerenchymal fibers and their orientation in many directions. The endocarps of fruits of species of Astrocaryum of the tribe Linospadicinae (Essig

2002) have fibers and brachysclereids (Vegas *et al.* 2008). In spite of references to the hard endocarp as being the cause of dormancy in palm tree seeds through the limitation of the fluxes of water and gases to the seed, is possible that are no severe restrictions to the fluxes of these substances in *Acrocomia aculeata* as the endocarp was not sclerified in the region of the germination pore.

Our observation indicated that the ovary of the macaw palm is very specialized, and there were precocious indications of those regions that would differentiate into the different tissues of the pericarp. The final volume of the fruit is defined by sclerification of the exocarp and endocarp approximately 70 days after anthesis. The porosity of the exocarp is the result of alternating regions with different degrees of lignification, and it woody nature is due to the compact arrangement of the sclerids. Abundant lipidic reserves develop during the final phases of fruit development and are stored in the parenchymatic cells of the mesocarp.

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