Anatomical and histochemical aspects of the peach palm (*Bactris gasipaes* Kunth) seed[1]

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ABSTRACT - *Bactris gasipaes* Kunth (peach palm) is a palm tree (Arecaceae) widely used by Amazon people, whose seeds have dormancy of unknown cause, which result in difficulties on producing uniform seedlings. This study aimed at identifying anatomical and histochemical aspects of peach palm seeds that may have influence on germination. Histological sections were performed with seed material embedded into hystoresin, which were then stained with toluidine blue and assessed under optical microscope to verify structural characteristics. Histological sections were manually performed to identify primary and secondary metabolites; and histochemical test were performed for fatty acids, alkaloids, starch, phenolic compounds, lignin, neutral and acidic lipids, pectins, polysaccharides, proteins, tannins, and terpenoids. Tegument is thin and contains two layers. The external layer is thicker and has vascular bundles; and the internal layer is thinner containing irregular cells replete of phenolic compounds. Endosperm is whitish in color, with cells with shapes that vary from oblong or ellipsoidal until oval; containing lipids, proteins, pectins, and polysaccharides. Embryo is relatively small as related to seed size, conical and vascularized at distal portion. On longitudinal sections, plumule appears in proximal region as three foliar primordia. At seedling protrusion region the cells contain acidic lipids, proteins, and neutral polysaccharides.

Index terms: palm tree, seed structure, primary metabolites, secondary metabolites.

Aspectos anatômicos e histoquímicos da semente de pupunha (*Bactris gasipaes* Kunth)

RESUMO - *Bactris gasipaes* Kunth (pupunha) é uma palmeira (Arecaceae) muito utilizada pelas populações da Amazônia, cujas sementes apresentam dormência de causa desconhecida, o que representa dificuldade para produção uniforme de mudas. Este trabalho visou identificar os aspectos anatômicos e histoquímicos de sementes de pupunha que possam ter influência na germinação. Para isso, foram feitos cortes histológicos de material incluso em resina, os quais foram corados com azul de Toluidina para verificar características estruturais, avaliadas em microscópio. Para identificação de metabólitos primários e secundários, foram feitos cortes manuais e realizados testes histoquímicos para ácidos graxos, alcaloides, amido, compostos fenólicos, lignina, lipídios, lipídios ácidos e neutros, pectinas, polissacarídeos, proteínas, taninos e terpenoides. O tegumento é fino e em duas camadas, a externa mais espessa, com feixes vasculares e a interna menos espessa, com células irregulares e repletas de compostos fenólicos. O endosperma é esbranquiçado, com células oblongas obovais e ovais, com conteúdo de lipídios, proteínas, pectinas e polissacarídeos. O embrião é pequeno em relação ao tamanho da semente, cônico e vascularizado na porção distal. Na região proximal, em corte longitudinal, aparece a plúmula, com três primórdios foliares. As células apresentam conteúdo de lipídios ácidos, proteínas e polissacarídeos neutros na região de protrusão da semente

Termos para indexação: palmeira, estrutura da semente, metabólitos primários, metabólitos secundários.

Introduction

Palm trees belong to the family Arecaceae comprising circa 183 genera and 2,450 species, which are mainly distributed on tropical and subtropical regions worldwide (Johnson, 2010). The peach palm (*Bactris gasipaes Kunth*) is a species of palm tree that has multiple uses, i.e., the flowers can be cooked and used as a condiment; the roots and stem

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have vermicidal action; and the trunk provides good quality wood (Mora-Urpí et al., 1997; Jesus and Abreu, 2002). However, traditionally the main way of its use is through cooked fruit, which have high nutritional value. Currently, the species has been grown for production of heart of palm, by advantages that this palm tree offers in relation to other palm trees as: early cut, high productivity, good palatability, and abundant sprouting (Neves et al., 2005).

Seeds of various palm trees species have dormancy, which may in part be overcome by using methods such as: immersion in water; hot stratification; application of gibberellins; among others (Martine, et al., 2009; Fondom et al., 2010; Nazario and Ferreira, 2010; Ribeiro et al., 2011). Nevertheless, for most species of palm trees, the causes of seed dormancy are still not known.

Seeds of *Bactris gasipaes* germinate slowly and irregularly and have different degrees of dormancy between plants, as well as between fruit maturation stages (Bovi et al., 1994; Ferreira, 2005). These seeds are recalcitrant (Villalobos et al., 1992) and their tolerance to desiccation will vary with the seed lot and/or its source (Bovi et al., 2004).

Among factors influencing germination and seed dormancy are the structural aspects, such as number of cells, cell wall thickening of their wraps (Debeaujon et al., 2007) and the differentiation and maturation of embryo (Hilhorst, 2007). Chemical seed composition may also influence germination; once products of primary metabolism, such as proteins, lipids and carbohydrates are synthesized by the seeds as reserve materials to be used by the embryo during germination and formation of new cellular structures. Nevertheless, among these compounds may be present some substances that act in controlling germination of the seeds, as some fatty acids of the triacylglycerols, as well as some sugars (Ulbright et al., 1982; Buckeridge et al., 2004).

Secondary metabolites can be divided into three major groups: terpenes, phenols and nitrogenous compounds. In plants these compounds are generally associated to defense and, despite of not presenting apparent function on processes of growth and development, some of them present some positive or negative correlation in the seed germination process (Taiz and Zeiger, 2009).

Anatomical study of the seed may help on identifying mechanical and physical barriers affecting germination, and also on assessing the differentiation and maturation of the embryo structures (Baskin and Baskin, 2000; Cardoso, 2008). Information on structure of the *Bactris gasipaes* seeds, such as the histochemical, and anatomical aspects, are important to development and improvement of techniques to its *in vitro* propagation, as well as on identifying structures and compounds that may prevent or inhibit germination of the seeds, or only of the embryos.

In face of the foregoing this study aimed at identifying the anatomical aspects of *Bactris gasipaes* seeds and detecting the presence of ergastic substances (primary and secondary metabolites) that may influence the germination of this palm seeds.

Material and Methods

The seeds used in the experiments were collected on a peach palm plantation located in the Centro de Pesquisa do Instituto de Saúde e Biotecnologia da Universidade Federal do Amazonas (ISB/UFAM) (Research Center of Institute of Health and Biotechnology, Federal University of Amazon), located on the road linking the municipalities of Coari and Itapeuá, within the municipality of Coari, Amazon State, Brazil. According to Köppen's classification, the climate in the region is type Afi humid tropical, with mean annual rainfall of 2,250 mm and mean annual temperature of 26 ºC (Vieira and Santos, 1987). Bunches containing the ripe fruits, i.e., presenting epicarp completely yellow or red, were collected from 14 different palm trees.

After collection, fruits were removed from the inflorescences and then the tegument of each fruit has been longitudinally sectioned for seed removal, with aid of a scalpel. Cleaning has consisted on manually rubbing the seeds on a sieve under tap water for removal of remaining residues; what was performed with the tip of a knife. From each inflorescence 30 seeds were removed and then fixed in 50% FAA [formaldehyde (5%); ethanol (90%); and acetic acid (5%)], for 72 hours, and stored in 70% ethanol (Kraus and Arduin, 1997).

To embedding the seeds in histological resin (hystoresin), these seeds were fragmented into smaller pieces, which were dehydrated in an increasing ethanol series (from 70% to 95%) and then infiltrated with 2-hydroxyethyl methacrylate (Hystoresin Leica®, prepared according to instructions of manufacturer). Fragments were sectioned with aid of a rotary microtome, which has been adapted to cut sections of 5 μm thicknesses. The sections for structural analysis were stained with toluidine blue (O'Brien et al., 1964) and fixed in permanent slides with the Permount® mounting medium.

Histochemical tests for analysis of organic compounds present in the embryos were carried out with tissue samples embedded in hystoresin and sectioned with a rotary microtome; and for analysis of these compounds present in the tissues of the tegument and endosperm, the cuts were manually performed with the aid of a razor blade and with the tissue sections fixed in permanent slides only with Permount®. Results of histochemical tests can be seen on Table 1.

Assessment of the slides containing the sectioned material as well as the photomicrographs were performed under an optical microscope (brand Zeiss, model Primo Star) with a digital camera (brand Canon, model Powershot A 650 IS) coupled to it.

Table 1. Organic compounds studied in seed tissues of the palm tree *Bactris gasipaes* (peach palm), and substances used in their detection (reagent), as well as the expected color of the cells after positive reaction with the dye.

Organic Compounds	Reagent	Cell Coloration	Reference
Terpenoids with carbonyl group	2,4-Dinitrophenylhydrazine	Red-orange	Ganter and Jollès (1969; 1970)
Phenolic compounds	Ferric trichloride	Deep-green, magenta, blue, or black	Johansen (1940)
Tannins	Vanillin–HCl	Red	Mace and Howell (1974)
Fatty acids	Copper acetate/rubeanic acid	Deep-green	Ganter and Jollès (1970)
Lipids	Sudan IV	Red	Pearse (1980)
Acidic lipids and neutral lipids	Nile blue sulphate	Neutral lipids – pink Acidic Lipids - blue	Cain (1947)
Proteins	Xilidine Ponceau	Red	Vidal (1977)
Pectins	Ruthenium red	Pink	Johansen (1940)
Neutral polysaccharides	Periodic acid-Schiff (PAS)	Pink	Feder and O'Brien (1968)
Starch	Lugol's solution	Purple	Johansen (1940)
Lignin	Phloroglucinol (cyclohexane-1,3,5-trione)	Red	Johansen (1940)

Results and Discussion

The palm *Bactris gasipaes* has the seeds with the tegument slender, as previously described by Aguiar and Mendonça (2003) in the palm tree commonly known in Brazil as "Açaí" (*Euterpe precatoria*); and within this tegument it is possible to observe two strata or layers, termed as external and internal (Figures 1-A and 1-B). The outer layer is pluristratified and consists on circa 10 layers of cell, which have shapes that may vary from rounded to oval, forming a vascularized tissue (Figure 1-C).

The presence of terpenoids has been observed in the region of external layer of tegument (Table 2 and Figure 2-A). With the increase on their concentration and time of seed exposure, the compounds of the terpenes family may have inhibitory effect on seed germination, as already observed by Dudai et al. (1999). Terpenes present in the leaf tissues, flowers and fruits of *Tagetes minuta* are able to inhibit seed germination of some competing species (López et al., 2009). However, in palm trees there are still no studies correlating presence of terpenes to seed germination.

The outermost layer is replete of acidic lipids (Table 2 and Figure 2-H); and presence of lipids in the tegument suggests that seed is coated with wax or suberin (Table 2 and Figure 2-E). These substances are responsible for regulating the inflow and outflow of water into seeds, and hence may cause hindrance to seed germination (Laboriau, 1983; Taiz and Zeiger, 2009); however, the function of a tegument containing free fatty acids is exactly regulating the entry of water into seed, with no role on the germination metabolism.

The inner stratum of tegument has from two to four layers of rounded shaped cells (Figures 1-A and 1-B). Phenolic compounds were present in all layers of tegument, with greater intensity in the cells that forms the inner layer; what was evidenced by the black staining to reagent ferric trichloride (Figure 2-B). The staining test with Vanillin–HCl has been positive, thus confirming presence of tannins in the inner stratum of seed tegument (Figure 2-C). The function of the testa (seed coat) containing water soluble inhibitors, such as some phenolic compounds, is to prevent germination of the non-dormant embryos until favorable environmental conditions occur (Weber et al., 2005). When present in the testa, besides preventing germination, some of these compounds are able to slow seedling growth (Möise et al., 2005). The thin thickness of tegument, does not offer mechanical resistance to seeds, possibly due to presence of hard endocarp in the fruits of this palm species (Tomlinson, 1990). In other species of palm trees as *Acrocomia aculeata*, the lack of resistance of seed tegument was also attributed to the presence of a sclerified endocarp (Moura et al., 2010).

On seeds of peach palm the proteins are present in all cell layers of tegument (Figures 3 -A, 3 -B); however, pectins were also found in the structure of cellular walls of the seed coat (Figure 3 -C). The inner epidermis of tegument is characterized by a layer of isodiametric cells (equally expanded on all sides) with rectangular shape, and with periclinal elongated walls (Figure 3 -D).

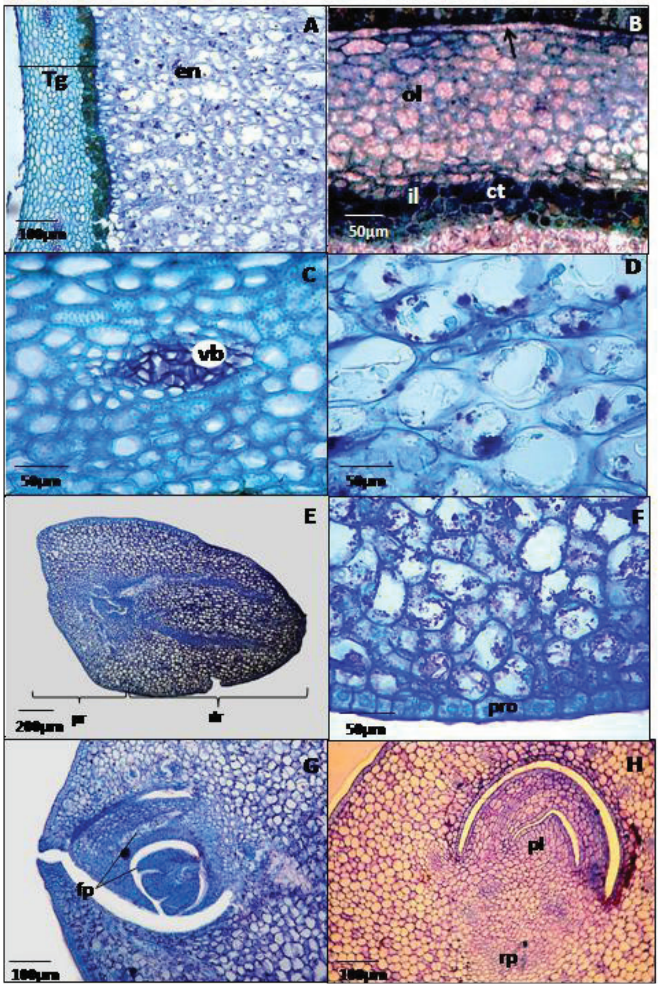

Figure 1. Photomicrographs showing structural aspects of tegument (A, B, and C); endosperm (D); and embryo (E, F, G, and H) of seed of the palm *Bactris gasipaes* (peach palm). Tg = tegument; ol = outer layer; il = inner layer; ct = content of tannin; en = endosperm; vb = vascular bundle; pr = proximal region; dr = distal region; pro = protoderm; fp = foliar primordia; pl = plumule; rp = root primordium.

The endosperm has a solid consistency and is whitish in color; it is homogeneous and composed of multidimensional cells with shapes varying from oblong to elliptic or ovoidal longitudinally; and transversely these cells are rounded and occupy almost all inner part of seed (Figure 1-D). In seeds of the palm trees *Oenocarpus minor* (Mendonça et al., 2008), *Euterpe precatoria* (Henderson et al., 1995; Aguiar and Mendonça, 2003) and *Euterpe edulis* (Belin-Depoux and Queiroz, 1971) it was observed that endosperm is also homogeneous and also occupies almost the entire interior of the seed. Besides the homogeneous endosperm, the palm trees may also have similar ruminate endosperms, as observed by Paula (1975) for the palm tree *Euterpe oleracea*. However, this feature can not be used to distinguish larger groups, once there are variations within same genus (Tomlinson, 1990).

Table 2. Organic compounds found through histochemical tests applied to histological sections performed on tissues of three different components of the seeds of palm *Bactris gasipaes* (peach palm).

Compound	Tegument	Endosperm	Embryo
Terpenoids with carbonyl group	+	-	-
Phenolic compounds	+	-	-
Tannins	+	-	-
Fatty acids	-	+	-
Lipids	+	+	+
Acidic lipids	+	+	+
Neutral lipids	-	+	-
Proteins	+	+	+
Pectins	-	+	+
Neutral Polysaccharides	-	+	+
Starch	-	-	-
Lignin	-	-	-

In monocots the endosperm is reserve tissue for the embryo formation during seed germination process and establishment of the seedlings (Davide and Silva, 2008). Tests for lipids detection in the content of the endosperm cells were positive (Figure 2-F); thus making it possible to observe as much acidic lipids as neutral lipids (Figures 2-G and 2-H). These lipids are found as oleic bodies arranged within cells, where free fatty acids were found in cells of the endosperm (Figures 2-D and 2-E).

In histochemical tests, performed by using the tissue staining with Nile blue sulphate method, the differential staining is due to presence of two substances in this dye: an oxazone (red color), which reacts with the neutral lipids, or fats; and an oxazine (blue color), which reacts with the carbonyl groups of the free fatty acids and orthophosphoric acid residues originating from phospholipids (Cain, 1947). The synthesis of triacylglycerols is directly related to the presence of enzyme diacylglycerol acyltransferase (DAGAT), once the greater the amount of this enzyme in tissues, the greater the neutral lipids production for functioning as reserve of lipids (Ohlrogge and Jaworski, 1997).

It has been found a large concentration of lipid bodies in the endosperm; which indicates that this is the main reserve compound in the seeds of species typical for palm oil production. On palm trees species such as *Acrocomia aculeata* (Moura et al., 2010) and *Euterpe edulis* (Panza et al., 2004) lipids also appear in great abundance and likewise stored in lipid bodies. Lipids that are present in the endosperm are fat reserves that will be used as much during germination process as on development of the seedlings (Buckeridge et al., 2004).

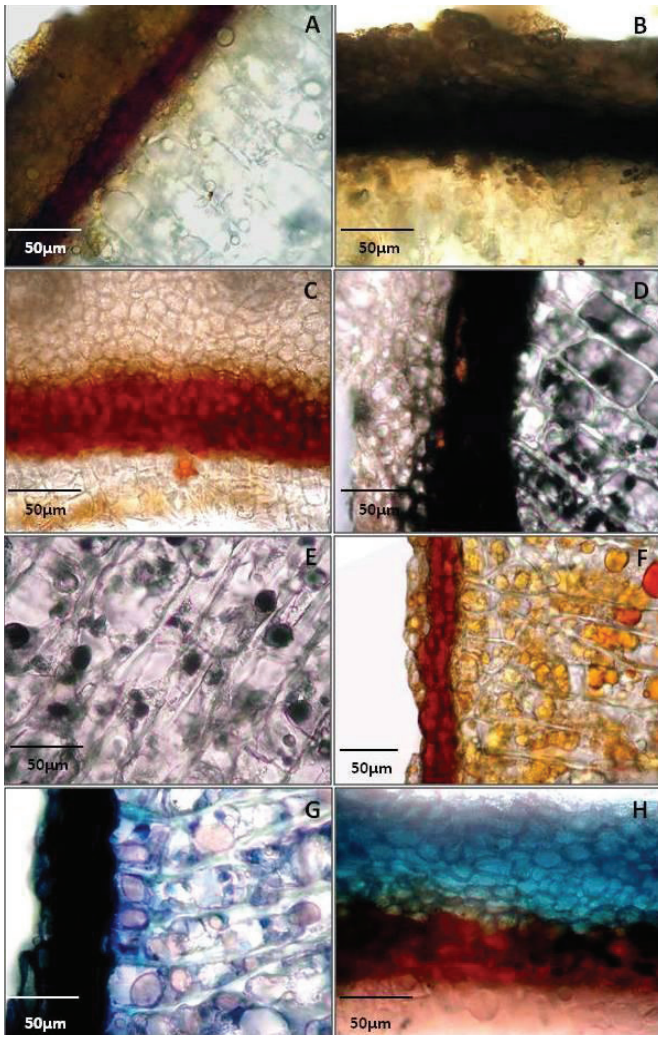

Figure 2. Photomicrographs of the results of the histochemical tests performed in tissues of the tegument and endosperm of seeds of the palm tree *Bactris gasipaes* (peach palm) showing presence of terpenoids (A), phenolic compounds (B), tannins (C), fatty acids (D and E), lipids (F), acidic lipids(G); and neutral lipids (H).

Stored as protein bodies, this compound was likewise detected in the endosperm cells (Figures 3-A and 3-B). In seeds of palm tree *Euterpe oleracea*, Gonçalves et al. (2010) suggest that the major source of nitrogen supplied for embryo germination comes from proteins that are translocated from the reserve tissue. The pectins and the polysaccharides that are present in the endosperm appear as cellular content (Figures 3-C and 3-D). Similar result was also observed in fruits of the palm tree *Acrocomia aculeata* (Moura et al., 2010). Pectins are cell wall component polysaccharides, which usually contain acidic and neutral sugars; and frequently also contain calcium as one of their structural component. Alterations in structure of pectins may change its configuration as well as modify its links within cell wall. When pectin is formed within the cellular walls (or: within cellular content), the fatty acids of the esters groups that compose its structure may be translocated from the cellular wall; which increases its ability to form a rigid gel (Taiz and Zeiger, 2009). Buckeridge et al. (2000) suggest that the galactan, a sugar present as a side chain of the pectin, is actually a polysaccharide of primary cell wall that has been enlarged and adapted to be storage and that will be mobilized during germination.

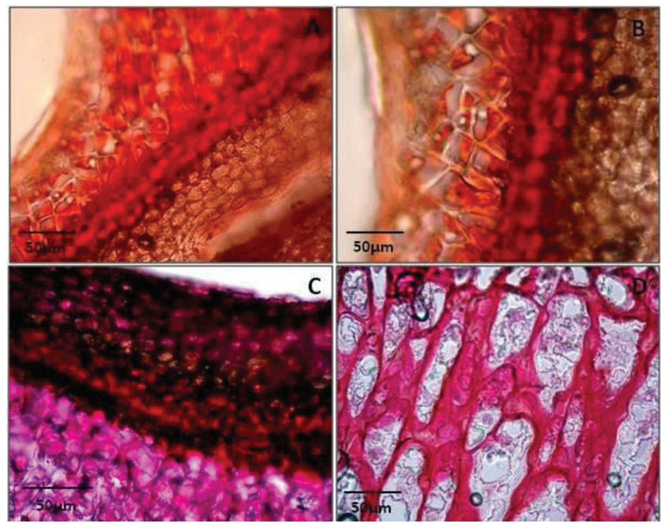

Figure 3. Photomicrographs of result of the histochemical tests performed on tissues of the tegument and endosperm of the seeds of the palm tree *Bactris gasipaes* (peach palm) showing presence of proteins (A and B), pectins (C), and polysaccharides (D).

The embryo is laterally located; is conical and have a homogeneous coloration. It is composed by a wider proximal region in relation to germinative pore, which is formed by the embryonic axis; and the distal portion (which is narrower) is formed by the cotyledon (Figure 1-E). Distal and proximal regions are morphologically distinct. Embryos with this same configuration were likewise observed on seeds of the palm trees *Euterpe precatoria* (Aguiar and Mendonça, 2003) and *Archontophoenix alexandrae* (Charlo et al., 2006). On seeds of the palm *Oenocarpus minor*, the proximal region of the embryo, which is geared to the germinative pore has a light-yellow color; and the distal region has a very pale yellow color (Oliveira et al., 2010).

On longitudinal sections performed on the proximal region of germinative pore, the cells of the embryo have an oval shape; and the protoderm is composed by cells that are smaller and round-shaped (Figure 1-F). On longitudinal sections performed on proximal region of germinative pore, the embryo cells have an oval shape; and the protoderm is composed by cells that are smaller and round-shaped (Figure 1-F). The protoderm has a slit in the center of proximal region that connects the embryonic axis to external environment. The embryonic axis, that is short and has apical location at a right angle with the proximal protoderm, is constituted by the cells that will form the plumule, which is formed by three leaf primordia and by the precursor cells of the radicle and that are located in a 180º angled at opposite side of this region, is characterized by a cluster of smaller cells with dense cytoplasm and shaped as a hood (Figure 1-G and 1-H). Plumule formed by three leaf primordia were already observed in other species of palms such as *Euterpe precatoria* (Aguiar and Mendonça, 2003), *Oenocarpus minor* (Oliveira et al., 2010) and *Acrocomia aculeata* (Ribeiro et al., 2012).

Cross sections performed in the distal region of germinative pore showed multidimensional cells with shapes that varied from rounded; ellipsoidal or rectangular (Figure 4-A and 4-B). Cells of protoderm also vary in shape, and the cotyledonal tissue is formed by a parenchyma with bulky protoplasmic content, in which vascular concentric bundles that extends from the embryonic axis until distal end may be observed (Figures 4-A and 4-B). The presence of this set of structures shows that, at moment of dispersal, the embryo is completely differentiated and mature, indicating that there is no morphological dormancy in seeds of *Bactris gasipaes* (Baskin and Baskin, 2004); what explains physiological maturity of the seeds of this palm tree species circa one month before dispersal of fruits (Ferreira, 2005).

Histochemical tests for detecting terpenoids, phenolic compounds, tannins, fatty acids, starch, and lignin were negative. However, in the test for detecting lipids the dye reacted with the neutral and acidic lipids present within embryo cells (Figure 5-A), which were found in the form of oleic bodies (Figure 5-B). Proteins were also found within the embryo cells (Figure 5c). Synthesis of some proteins is generally higher in dormant seeds than in non-dormant seeds; nevertheless, it was not possible establishing a correlation between the presence or absence of protein and dormancy (Bewley, 1997). Polysaccharides were observed only in the protrusion region of the seed (Figure 5-D), demonstrating that this region is where synthesis of sugars occur; possibly with function of providing energy to start germination process.

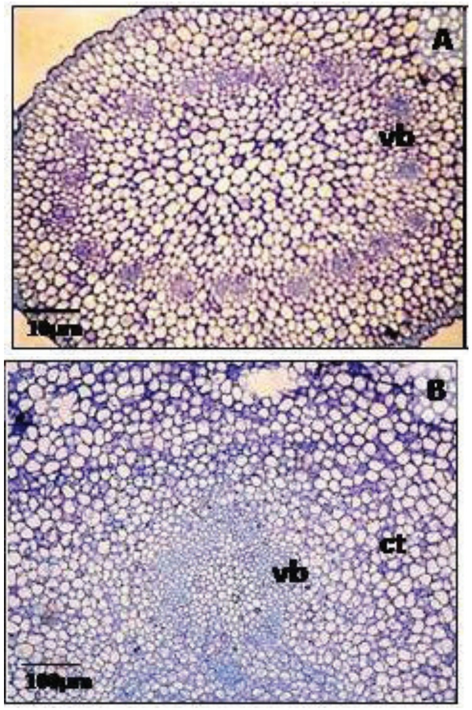

Figure 4. Photomicrographs showing structural aspects of embryo of seeds of palm *Bactris gasipaes* (peach palm). vb = vascular bundle; Co = cotyledonal tissue.

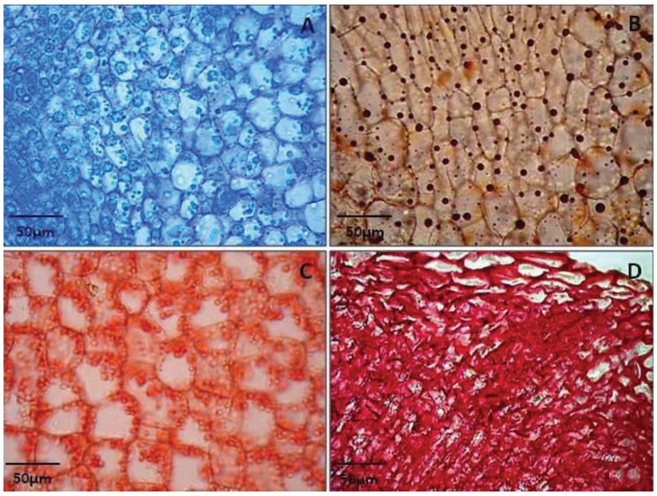

Figure 5. Photomicrographs of the results of the histochemical tests performed on tissues of the embryo of seeds of the palm tree *Bactris gasipaes* (peach palm) showing presence of acidic lipids and neutral lipids (A), total lipids (B), proteins (C); and polysaccharides (D).

Conclusions

The tegument of the palm tree *Bactris gasipaes* seed is thin and has two different layers. The outer layer is thicker, with presence of vascular bundles and contain terpenoids and acidic lipids; the inner layer is thicker and contains cells of irregular shapes and replete of tannins.

The endosperm of the seed of *Bactris gasipaes* occupies most of the seed; is whitish in color and contains cells with shapes that may vary from oblong or ellipsoid until oval; containing lipids, proteins, pectins, and polysaccharides.

The embryo of the palm tree *Bactris gasipaes* is small in relation to seed size and is conical and vascularized on distal portion to the germinative pore. In longitudinal sections, the plumule is located in the proximal region and contains three foliar primordia; indicating that, on the moment of dispersal, the embryo is fully differentiated and mature, thus eliminating the possibility of morphological dormancy in these seeds. The cells in the protrusion region of the seed contain acidic lipids, proteins, and neutral polysaccharides.

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