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Full Length Research Paper

Fruit maturation and *in vitro* germination of macaw palm embryos

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***Acrocomia aculeata* (macaw palm) is oil producing palm tree with significant agro-industrial potential. Seed dormancy in palm species may be due to embryo immaturity, which could result from delayed embryogenesis. We evaluated the correspondence between the visual characteristics of maturing fruits and their physiological aspects and the *in vitro* germination capacity of the embryos. 11 fruit bunches in different stages of maturity were collected and classified in terms of the degree of maturation of the endosperm, the color of the exocarp, and the occurrence of abscission. The water and oil contents of the mesocarp and seed were determined, and lipids and proteins were identified through histochemical analyses of the mesocarp, endosperm, and embryo. The embryos from each fruit bunch were cultivated *in vitro* in 75% Murashige and Skoog (1962) media with added organic compounds. The water contents of the seeds varied from 71.2 to 21.1% among the different stages of fruit ripening and were related to the visual markers of fruit maturation (exocarp color ranging from dark green to brown). Lipid accumulation in the mesocarp occurred later than in the endosperm, and only occurred in fruits from bunches showing signs of abscission. Embryos from bunches in different stages of maturation showed similar germinative capacities, as well as similar patterns of lipid and protein storage. Embryogenesis in *A. aculeata* is precocious, and the embryos of immature fruits can be utilized for *in vitro* cultivation.**

Key words: *Acrocomia aculeata*, embryo culture, embryogenesis, water content, oil content.

INTRODUCTION

The macaw palm tree [*Acrocomia aculeata* (Jacq.) Lodd. ex. Mart.] is very common in the “Cerrado” (Brazilian savanna) biome (Lorenzi et al., 2010). Due to the large quantities of high-quality oil in the mesocarps and seeds of these plants and their adaptation to a dry tropical climate, this species has significant economic potential, especially in terms of biofuel production (Hiane et al., 2005; Moura et al., 2010).

The establishment of commercial cultivations of macaw palm palms has been limited, by their seed dormancy, which results in slow germination speeds and low germination rates (taking up to four years) (Ribeiro et al., 2011). Dormancy in palms is often related to the imamturity of their embryos (Baskin and Baskin, 1998; 2004; Orozco-Segovia et al., 2003), which reflects delayed embryogenesis in relation to fruit development (Werker, 1997; Hartmann et al., 2002).

In vitro cultivation of palm tree embryos offers the possibility of overcoming dormancy in many species and facilitating studies of embryogenesis and germination (Bhojwani and Razdan, 1996; Raghavan, 2003; Pechy Aké et al., 2004; 2007). Ribeiro et al. (2011) reported high germination percentages of macaw palm embryos derived from mature fruits, although there are no published reports that relate embryo development and germinative capacity to fruit maturation.

The development of *Acrocomia aculeata* fruits is slow,

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requiring about 13 months (Figures 1A to C) (Scariot and Lleras, 1991). A better understanding of the patterns of mesocarp, endosperm, and embryo maturation, as well as the biochemical profile of the seeds, could help optimize strategies for industrial harvesting and for obtaining the best embryos for *in vitro* propagation (DeMason, 1988; Panza et al., 2004; Moura et al., 2010).

Considering the economic importance of macaw palm and the scarcity of studies focusing on palm diaspores (Orozco-Segovia et al., 2003), the present study evaluated the biochemical and histochemical characteristics of their fruits in different stages of maturation, as well as the *in vitro* germinability of their embryos to address the following questions: i) are there easily identifiable visual characteristics that can be associate with the water contents and metabolic reserves of fruits and seeds? ii) Are there differences in the *in vitro* germinative capacities of embryos from fruits at different stages of maturation? iii) What are the relationships of embryogenesis with fruit development?

MATERIALS AND METHODS

Six individual palm trees were selected among a population of *A. aculeata* (Figure 1A) in the municipality of Montes Claros, Minas Gerais, Brazil. 11 fruit bunches (Figure 1B) in different stages of maturity were selected and classified based on their visual characteristics: 1) fruits with dark green exocarp, endosperm not completely developed; 2) fruits with green exocarp and developed endosperm; 3) fruits with brownish exocarp; and 4) fruits with brown exocarp, extracted from bunches in which the abscission started (Figures 1D to G).

Four samples of ten fruits were chosen at random from each bunch for evaluation of their water and oil contents. After rupturing the exocarp (using a wooden mallet), the mesocarps were removed using scalpels and the seeds were then extracted from the endocarp (after breaking it in a vise). The fresh weights of the mesocarps and seeds were determined for each sample. The dry weights of the mesocarps and seeds were determined separately after drying at 105ºC for 24 h; their water contents were calculated considering the differences between their fresh and dry weights. The oil contents of the mesocarps and seeds were determined by extracting the dehydrated samples in a Soxhlet extractor using petroleum ether as the solvent (AOAC, 2005). Regression analysis was used to adjust the curve indicative of the variation of the seed water contents as a function of the maturation statge of the fruit bunches (SAS Institute, 1990).

The presence of reserve compounds in the mesocarp, endosperm, and embryo of five fruits from nine different bunches were determined by histochemical analyses. The fruit and seed samples were fixed in FAA_{50} (Johansen, 1940) or Karnovsky solution (Karnovsky, 1965) for 24 h, conserved in 70% alcohol, and dehydrated in an ethanol series for embedding in hydroxyethyl-methacrylate (Leica®) following the protocol described by Paiva et al. (2011). These samples were sectioned transversely (10 µm) and stained with Sudan IV to indicate the presence of lipids (Foster, 1949) and with bromophenol blue to indicate the presence of proteins (Mazia et al., 1953). The slides were examined using a Nikon Eclipse E200 optical microscope.

The effect of the degree of fruit maturation on the *in vitro* germinability of palm embryos was evaluated using seeds from the fruits of the 11 bunches. The seeds were removed using a bench vise and disinfected in 6% sodium hypochlorite for 10 min; the embryos were subsequently extracted using scalpels while working in a laminar flow cabinet and then placed in a 100 mg L^{-1} solution of ascorbic acid. After disinfecting the embryos in 0.5% chlorine for 10 min, followed by three rinses in autoclaved distilled water, the embryos were inoculated into 7.5 x 1 cm test tubes containing 2 mL of 75% strength MS medium (Murashige and Skoog, 1962) with 0.4 mg L^{-1} thiamine; 1 mg L^{-1} pyridoxine; 0.5 mg L^{-1} nicotinic acid; 100 mg L^{-1} myo-inositol; 0.5 g L^{-1} hydrolyzed casein; 3 g L^{-1} activated charcoal; 30 g L^{-1} sucrose; 6 g L^{-1} agar; the pH was adjusted to 5.7 (Ribeiro et al., 2012). The experiment was established in a randomized design with 11 different treatments (fruits at different stages of maturation) and five repetitions of 10 tubes with one embryo each. After inoculation with the embryos, the tubes were wrapped in aluminum foil and maintained in the dark at 30°C. After 30 days, the emissions of the primary roots and the leaf sheaths were determined, as well as the dry weights of the haustorium, cotyledon petiole, roots, and leaf sheaths (Figure 1H) after drying at 105°C for 24 h.

The data concerning the emissions of roots and leaf sheaths was registered by counting the number of embryos or plantlets showing those events and then transforming those numbers into percentages, which were subsequently arc sine transformed $[(x/100)^{0.5}]$ for comparison. Analysis of variance was used for each variable, and the Tukey test used for comparisons between the averages, after significant differences were determined among the different treatments using the F test; regression analysis was used to define the tendency curve (SAS Institute, 1990).

RESULTS

The fruits of *A. aculeata* are globose drupes with a fibrous exocarp, a fibrous mesocarp having a mucilaginous and oily aspect, and a hard endocarp. The seed was covered by a thin tegument layer and had an oily endosperm and small embryo (Figure 1C). The water content of the mesocarp was greater than 70% in all of the studied stages of fruit maturation, so that this parameter cannot be used as an indicator of fruit maturity (Figure 2). The water content of the seeds, however, was more closely related to the visual characteristics of fruit maturation. Oil accumulation occurred in the seeds before being observed in the mesocarp. Fruits with brownish exocarps were found to have considerable quantities of oil in the endosperm, while significant increases in the lipid contents of the mesocarps were only seen in fruits of the bunches where the abscission started. These seeds contained developed embryos that could be extracted with relative ease, independent of the maturation statges of the fruits (Figure 1C).

Histochemical evaluations did not indicate the presence of protein in any significant quantities in the mesocarps during any of the fruit maturation stages evaluated (Table 1). In relation to lipids, higher concentrations of lipidic reserves in mesocarps from fruit bunch showing signs of abscission was noted (Table 1; Figure 3A). Variations were observed in terms of the presence of lipids in the endosperm, and fruit bunches with the lowest seed water contents had the greatest lipid reserves (Table 1; Figure 3B). High concentrations of proteins were also identified in the endosperms of fruit bunches that were in the most advanced states of maturation (Table 1; Figure 3C).

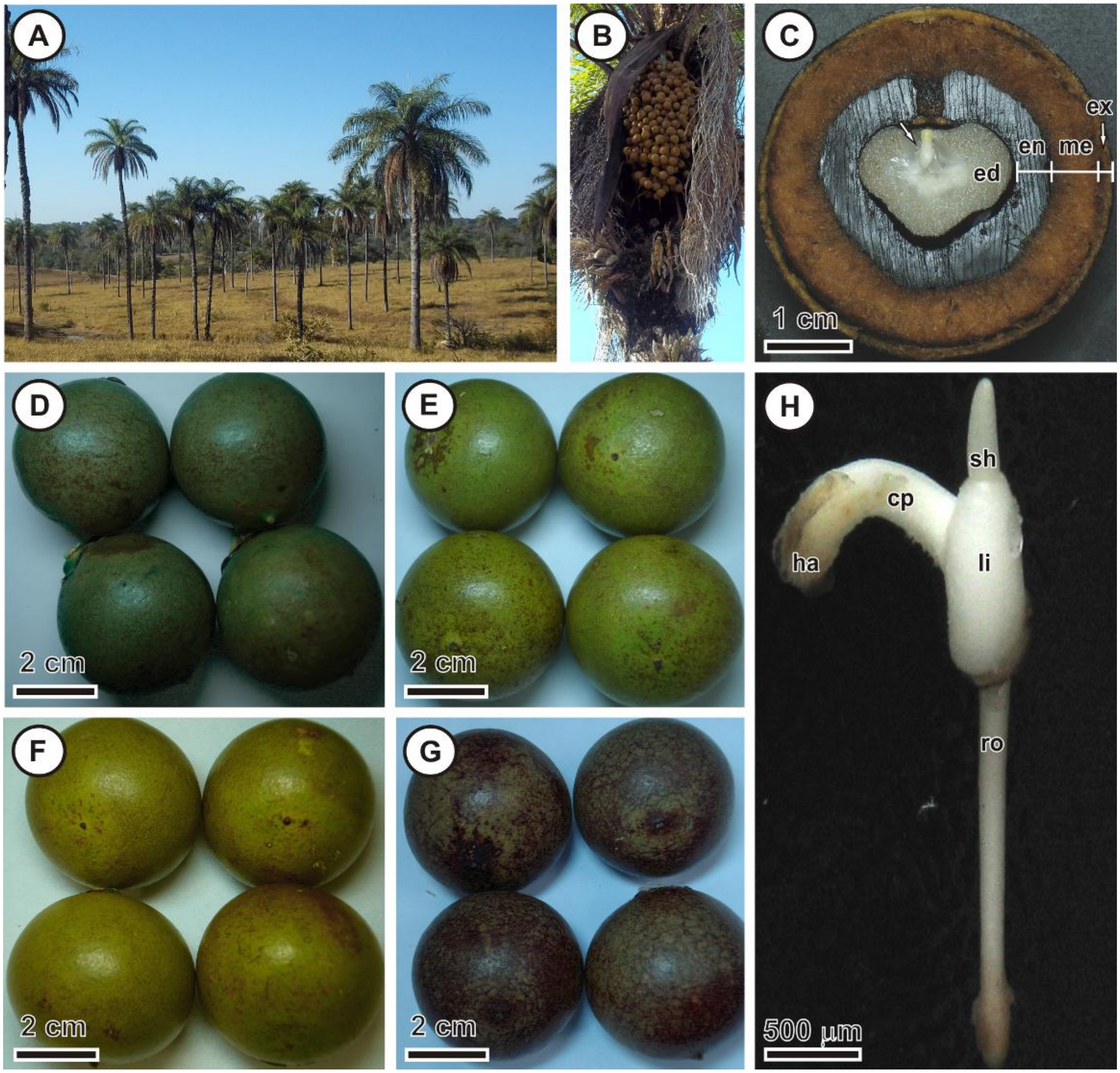

Figure 1. Individuals, fruits, and plantlets of *A. aculeata*: **A)** A natural population in northern Minas Gerais State, Brazil. **B)** Fruit bunch nearing abscission**. C)** Mature fruit indicating its contents and the embryo (arrow). **D)** Fruits with dark green exocarps in which the endosperms are not completely developed. **E)** Fruits with greenish exocarps. **F)** Fruits with brownish exocarps. **G)** Fruits with brown exocarps that have abscised. **H)** Plant with after 30 days of *in vitro* cultivation*.* **cp,** Cotyledon petiole; ed, endosperm; en, endocarp; ex, exocarp; ha, haustorium; li, ligule; me, mesocarp; ro, root; sh, leaf sheath.

Abundant reserves of proteins and lipids were observed in all of the embryos, but no large variations were noted among fruits at different stages of maturation (Table 1; Figure 3B, D).

The results of the *in vitro* cultivation of embryos indicate that all fruits contained embryos capable of germinating independent of their state of maturation (Figure 1H). Nonetheless, the level of fruit maturity did affect the capacity of the plantlets to emit roots and leaf sheaths. Plantlets obtained from embryos from extremely mature or immature fruits showed the highest capacity to emit leaf sheaths, while fruits of intermediate age showed a greater capacity to emit roots (Figure 4). Embryos from fruits in their initial or final stages of maturation yielded plantlets; higher capacity to produce both leaf sheaths and roots. Embryos from the most immature fruits (having seeds with water contents of 71.2%) had the same capacity to produce plantlets with leaf sheaths and roots as did embryos from seeds from bunches with abscising fruits.

It was not possible to identify any definitive tendency in relation to influence of the stages of maturity of the fruit

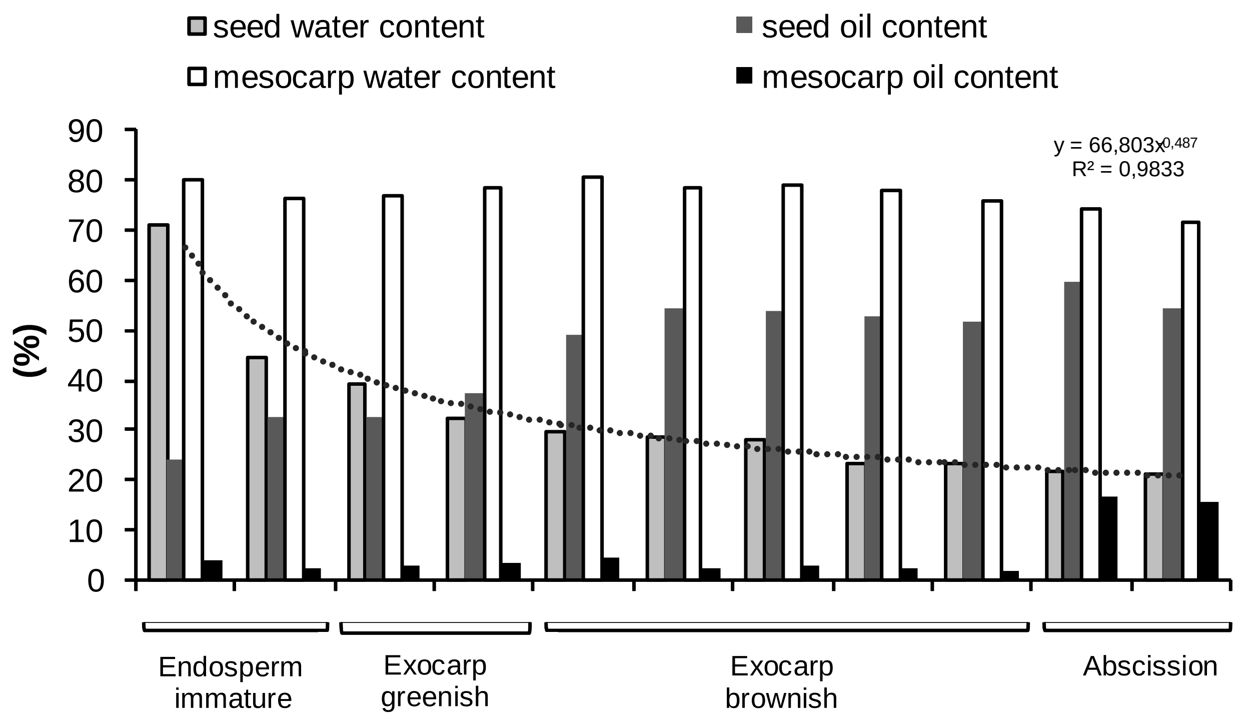

Figure 2. Water and oil contents of the mesocarp and seeds of *A. aculeata* that were removed from fruits with different visual characteristics indicating maturity.

bunches in plantlet development (Figure 5), as dry weights of their structures did not vary greatly in plantlets obtained from fruits with greater or lesser degrees of maturity. The bunches with seeds having the lowest water contents gave rise to seedlings with more developed haustoria (except for one bunch with fruits having brown exocarps). No differences were observed in the weights of the leaf sheaths among fruits derived from bunches of differing maturities.

DISCUSSION

The water content of macaw palm seeds can be used as an indicator of fruit maturity. The water content of the mesocarp, however, was not found to be related to fruit maturity, as had been observed in *Butia capitata* (Mart.) Becc. (Arecaceae) (Neves et al., 2010). Iossi et al. (2007), on the other hand, reported that both seeds and the mesocarps of *Phoenix roebelenii* O'Brien (Arecaceae) fruits undergo significant reductions in their water contents during maturation.

Moura et al. (2010) noted the abundance of protein and lipidic reserves in the endosperms and embryos of mature *A. aculeata* seeds. The high lipidic concentrations in the mesocarps of fruits in bunches that showed signs of abscission, as well as the precocious accumulation of this same reserve substance in their endosperms is in agreement with the results found for *Elaeis guineensis* Jacq. (Cheang et al., 1985; Tranbarger et al., 2011). In this latter species, the oil content of the mesocarp tripled in the three weeks before abscission; the oils found in their seeds also increased precociously (and to a greater extent) near the halfway-point of fruit development.

Seed development usually involves phases of morphogenesis, maturation (time during which reserves are accumulated), and then desiccation (Bewley and Black, 1994). The synchrony of embryo development in relation to the other seed components can vary, however, resulting in dry seeds with embryos in diverse states of development, depending on the species considered (Werker, 1997). In the present study, we determined that reserve accumulation occurs relatively early with macaw palm embryos, indicating that embryogenesis in this plant is precocious in relation to fruit development.

Embryos derived from immature fruits, without completely developed endosperms, are capable of producing seedlings with fully formed roots and leaf sheaths – confirming that embryogenesis is precocious in *A. aculeata* and that immature fruits can be used as sources of embryos for *in vitro* cultivation, as was seen in the palm trees *Astrocaryum ulei* Mart. (Pereira et al., 2006) and *B. capitata* (Neves et al., 2010). The reduced capacity of fruits in intermediate stages of development to produce normal seedlings (with leaf sheaths and roots), as observed in the present work, is compatible with reports from other species. A number of studies have demonstrated variations in the levels of abscisic acid (ABA) that restrict germination during embryo development. ABA is often associated with preventing vivipary

Table 1. Presence and accumulation of lipids and proteins in the mesocarps, endosperms, and embryos of *A. aculeata* as evaluated by histochemical tests from fruits with different seed water contents.

Seed water content (%)	Mesocarp		Endosperm		Embryo	
	Lipid	Protein	Lipid	Protein	Lipid	Protein
71.2	+	-	++	++	++	+++
44.3	+	-	++	++	++	+++
39.1	+	-	+	++	++	+++
29.9	+	-	++	++	+++	+++
28.7	+	-	+	++	++	+++
28.2	+	-	++	++	+++	+++
23.2	+	-	+	++	++	+++
21.4	++	-	++	+++	++	+++
21.1	+++	-	+++	+++	++	+++

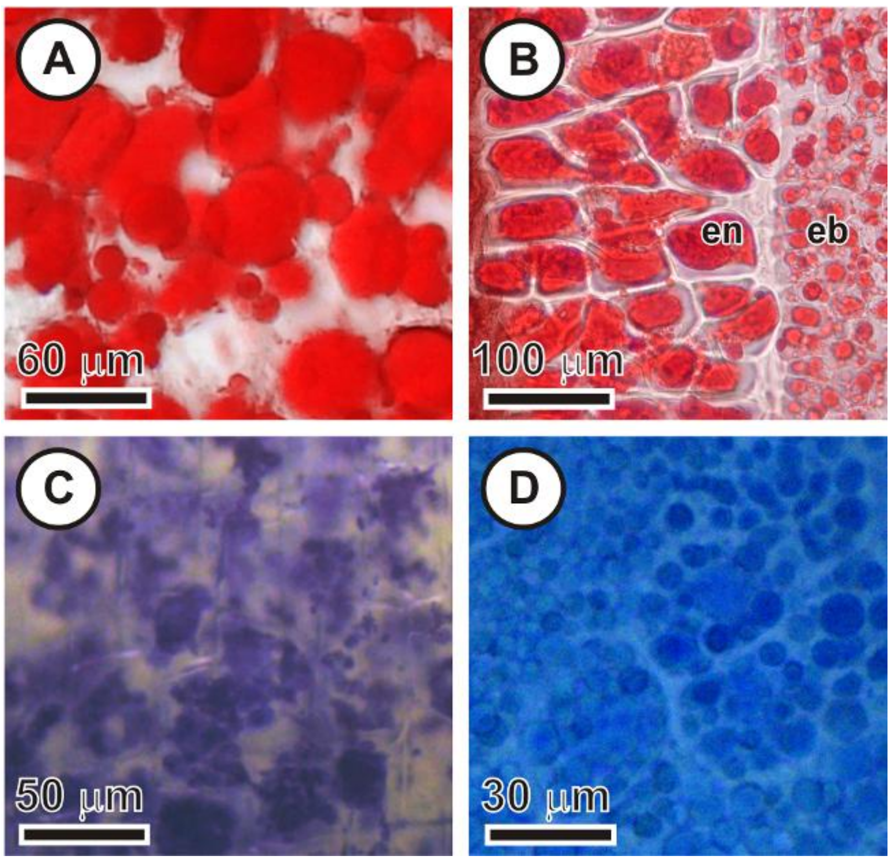

Figure 3. Histological sections of the mesocarp, endosperm, and embryo of *A. aculeata* submitted to histochemical analyses: **A)** Mesocarp of an abscising fruit stained with Sudan IV, indicating an abundance of lipids (red color). **B)** Endosperm and embryo of an abscising fruit, indicating the abundance of lipids (red color). **C)** Endosperm of an abscising fruit, stained with bromophenol blue, indicating the presence of proteins (blue color). **D)** Embryo of a fruit with a greenish exocarp, indicating the presence of proteins (blue color). **eb,** embryo; **en**, endosperm.

the embryo is well-developed and the dehydration level of the seed is not sufficient to inhibit germination (Bewley and Black, 1994; Hartmann et al., 2002; Nambara et al., 2010). Another possibility is that variations of the equilibrium between auxins and cytokinins that control organogenesis during fruit maturation can favor either root or aerial portion development (Taiz and Zeiger, 2002). Future studies quantifying hormone contents during ma-

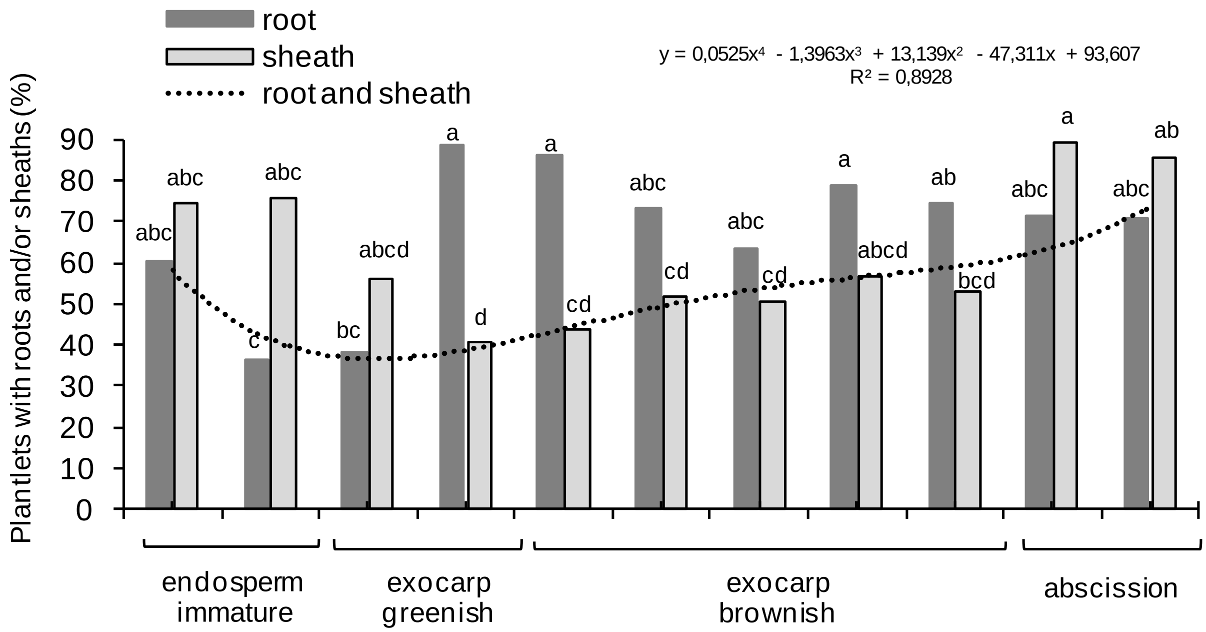

Figure 4. Percentages of plantlets of *A. aculeata* that emitted roots and foliar leaf sheaths that were obtained by the *in vitro* germination of embryos derived from fruits with different visual characteristics indicative of maturation. The dotted line indicates the tendency to produce plantlets producing both roots and leaf sheaths during the process of fruit maturation.

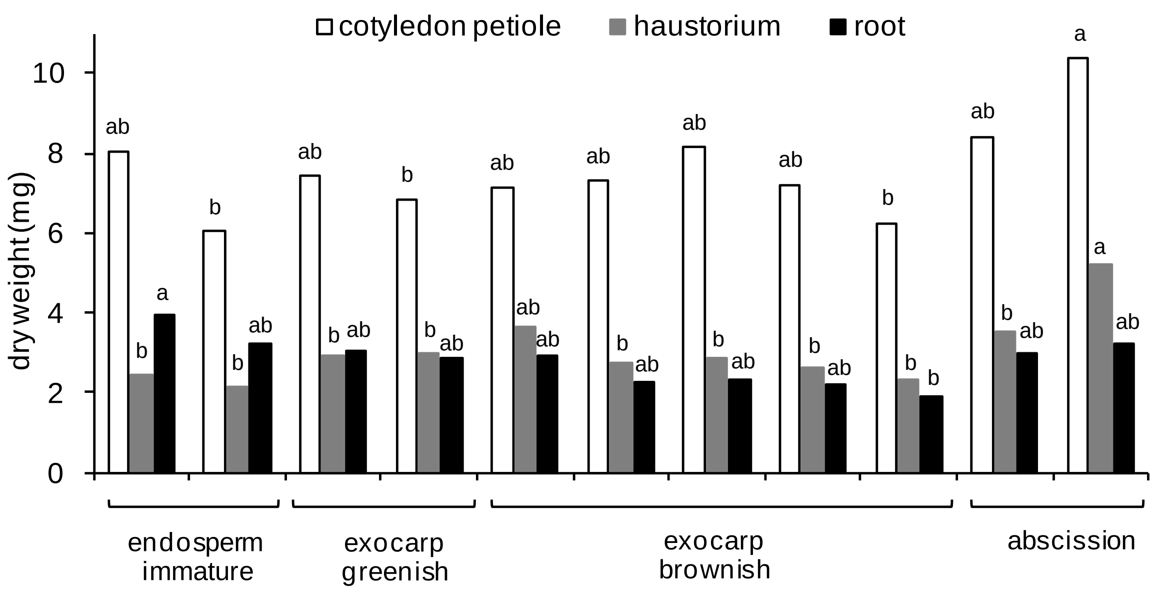

Figure 5. Average dry weights of the petiole cotyledons, haustoria, and roots of plantlets of *A. aculeata* obtained by the *in vitro* germination of embryos derived from fruits having different visual characteristics indicative of maturity.

caw palm fruit development could contribute to a better understanding of the factors controlling the germinative capacity of embryos during their maturation.

There was no well-defined effect of fruit bunch maturation on plantlet development as measured by the average dry weight of the cotyledon petiole, roots, and leaf sheaths; indicating that the growth capacity of the plantlets must be heavily influenced by their genotype. Nonetheless, the more mature fruit bunches yielded more developed haustoria than most of the others. The haustoria function in the absorption of endosperm reserves in the post-germinative period (Orozco-Segovia et al., 2003), and it is possible that the developmental capacity of this structure increases in fruits approaching abscission.

Many palm tree species have been described as showing morphological dormancy (Baskin and Baskin, 1998) related to the immaturity of the embryo (Orozco-Segovia et al., 2003). Evidence presented by Ribeiro et al. (2011), however, such as the rapid germination of both isolated embryos cultivated *in vitro* and of seeds with their opercular tegument removed and treated with gibberellic acid, indicates a type of physiological dor-mancy in macaw palm seeds. Precocious embryogenesis in relation to fruit development and the ability of macaw palm embryos derived from immature fruits to germinate (as demonstrated in the present study) reinforce the supposition that dormancy in this species is not related to embryo immaturity due to morpho-anatomical factors.

Form the results of this study, it can be concluded that: 1) the water content of the seeds is associated with visual indicators of fruit maturity; 2) the embryos of immature fruits have accumulated reserves and high germinative capacity, and can be used for *in vitro* propagation; and 3) embryogenesis is precocious in relation to fruit development.

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