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BIOMETRICS AND POST-SEMINAL DEVELOPMENT OF *COURATARI MACROSPERMA* A.C. SMITH (LECYTHIDACEAE) SEEDS

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ABSTRACT
Couratari macrosperma is widely distributed in Amazonia. This tree has economic value due to its wood. For the identification of individuals, biometrics uses data on the species and morphology facilitates the recognition of seedlings in the field. This work aimed to study the biometrics of seeds and the post-seminal development of *C. macrosperma*. Length, width, thickness and matter of seeds were evaluated and the germination phases were described. We concluded that *C. macrosperma* has variability as to biometrical features and its germination, which is epigeal, may end at 30 days, the seedling being phanerocotylar.

KEYWORDS: Biometrical characteristics, morphological characterization, tauari.

INTRODUCTION

Couratari macrosperma belongs to the family Lecythidaceae, which comprises around 282 species distributed into 17 genera of woody plants native to South America, Madagascar, Southeast Asia and West Africa, and has higher diversity in the Neotropical region (Procopio and Secco, 2008). In Brazil, there are 14 genera with around 100 species mainly in Amazonian forests and in the Atlantic Forest of the Northeast Region (Muller *et al*., 1995). Most of the representatives are trees or shrubs, but some species are lianas. The wood from *Couratari macrosperma* has been used in the manufacture of handcrafts and furniture and in architecture (procópio and Secco, 2008).

There is an increasing interest in understanding the biology of native forest species aimed at their domestication and reproduction (Monteiro and Ramos, 1997). This has been proven by the great quantity of studies on native species during the last years (Carvalho, 1994; Paula and Alves, 1997; Monteiro and Ramos, 1997; Lorenzi, 1998; Nascimento; Oliveira, 1999 and Procópio and Secco, 2008). However, forest resources have been threatened over time both by deforestation for agriculture and livestock raising purposes and by extraction of raw material to supply the different demands of industries. For regions where such resources have already been excessively exploited, forest plantings are the solution and seeds constitute the starting point for seedling production (Ibama, 1998).

The observation of seedling development allows differentiating between very similar taxonomic groups and helping regeneration studies. For the modern systematic, which is based, among other aspects, on the largest number of characteristics for comparison, the morphological study of seedlings constitutes another element for identification (Pereira, 1988), such as the studies performed by Lima (1985), Fernandes (2007), Santos et al. (2006), and Melo et al. (2007).

The biometric characterization of fruits and seeds may provide important information to differentiate between species of one same genus. Seed biometrics is also related to aspects of dispersion and seedling set, and has been used to differentiate between pioneer and non-pioneer species in tropical forests (Cruz *et al*., 2001). Studies such as those of Fontenelle et al. (2007) and Carpanezzi and Marques (1981) have demonstrated the importance of biometrics for genus and species differentiation.

Information on seedling development and morphology is essential for nursery workers to plan the production of forest seedlings. A long germination period and/or a slow initial development of seedlings are frequently typical of these species, although little known and therefore not considered in the planning and production process (Leonhardt *et al*., 2008).

In Brazil, despite the increasing number of works due to the richness of its flora, there is still a lack of studies on native species, especially those at initial development stages, which could provide information for programs of recovering and management of natural areas. Thus, the aim of this work was to describe the biometrics and the post-seminal development stages of *Couratari macrosperma* seeds.

MATERIALS AND METHODS

This work was carried out in the Seed Lab. of Mato Grosso State University - UNEMAT, Alta Floresta Campus, Mato Grosso State, Brazil.

Couratari macrosperma seeds were obtained from 10 trees located in Alta Floresta Municipality (9°30'22.5"S 65°22'34.6"W), and vouchers were prepared and stored in the Herbarium of UNEMAT (HERBAN, record no. 1235). Fruits were collected by using a trimmer

at the beginning of operculum opening (October 2008) and kept in the sun (around 7 days) until complete opening, when seeds were removed, placed on a tray in the shadow for 48h and stored in plastic bags kept in a refrigerator. Evaluations were performed from December 2008 to March 2009.

Biometrics included 300 randomly selected seeds. The length, width and thickness of each seed were measured by using a digital caliper (0.1mm precision), considering length the distance from the base to the apex, and measuring width and thickness at the median line of seeds. Seed matter was obtained by weighing 50 seeds in an analytical balance (Marte, AL-500 model, 0.001g precision).

The results of the evaluated characteristics were subjected to descriptive analysis, which provided the respective means, minimum value, maximum value, coefficient of variation, and standard error of the mean.

To evaluate post-seminal development, two subsamples of 25 seeds were allowed to germinate in paper towel rolls kept at 25°C in a BOD incubator. To facilitate the test procedures, the circumferential wing of seeds was manually cut with scissors. Seeds underwent asepsis with 1% sodium hypochlorite solution for 30 min, followed by washing in tap water for 5 min and in distilled water for 2 min. They were also treated with the fungicide Cercobin (Thiophanate-methyl) at 0.6g per liter for 30 min and then washed in distilled water for 2 min in order to remove the excess of the product (Dal Bem, 2006).

Paper towel rolls were moistened with distilled water at a quantity three-fold larger than the weight of the dry substrate; moistening was repeated by using 30ml distilled water every two days during 10 days. After this period, seeds were transferred to plastic flasks (400mL capacity) containing sand (autoclaved at 120 ºC for 90 min) and kept in the germinator at 25 ºC for 30 days; 30ml water was added to the flasks every two days during the 30-day evaluation period.

Daily observations were done to morphologically describe seeds and seedlings at different development stages. For seeds, integument coloration, texture and consistency, as well as the embryo format, were considered. For seedlings, which were collected in sequential stages, the characters were described in order to evidence primary root development, secondary root emergence, cotyledon emergence, first leaf and conspicuous apical bud initial growth, and eophyll expansion.

Germination was characterized as to type, and seedlings as to format, coloration, texture and indumentum of protophylls and leaves. Then, seeds and seedling development stages were manually illustrated with the naked eye. In general, descriptions followed the criteria and terminologies adopted by Beltrati (1992) and Duke (1965).

RESULTS AND DISCUSSION

Couratari macrosperma seeds greatly vary in dimension, with length, width and thickness ranging from 44.11 to 117.56 mm, 13.68 to 32.89 mm and 0.04 to 2.39 mm, respectively (Table 1). The mean values 84.41mm length, 22.24mm width and 1.13mm thickness (Table 1) were close to those detected by Dal Bem (2006) for *Couratari guianensis* Aubl., the mean values of which were 87.80mm length, 18.60mm width and 1.29mm thickness.

Biometrical data for other Lecythidaceae seeds indicate great variation among species, as described by Camargo et al. (2003) for *Cariniana micrantha* Ducke seeds with lower mean length (55mm) and width (13mm); for *Cariniana legalis* (Martius) Kuntze, however, Rego (2001) reported 24.6mm mean length and 9.0mm mean width − lower values than those obtained for *C. macrosperma*. The 1000-seed weight detected for *C. macrosperma* (290g) was higher than that reported by Dal Bem (2006) for *C. guianensis* (204.9g) and that obtained by Camargo et al. (2003) for *Cariniana micrantha* Ducke (190g), which indicates *C. macrosperma* had the largest seeds among the already studied species of the same genus.

The thickness of *C. macrosperma* seeds had higher coefficient of variation than the remaining measures (Table 1) and probably has expressive influence on seed matter. Seed matter is known to likely influence germination percentage and the future seedling development.

According to Mesquita et al. (1990), the matter and size of bean seeds present genetic control, with heritability estimate higher than 65%. Alves (2002) stated that the characters associated with the reproductive organs in plants are usually more stable than the vegetative ones. This can be explained by the higher genetic control and selection pressure on the reproductive characters during evolution.

Most seeds (87.33%) had 63.00–100.99mm length (Figure 1A), 18.00–27.99mm width (82.33%) (Figure 1B), 0.60–1.79mm thickness (98.33%) (Figure 1C) and 0.013–0.020g matter (40%) (Figure 1D).

Baskin and Baskin (1998) stated that the difference in seed size within a species is correlated to variations in the environment of the parent plant. The latter produces seeds of varying size, which leads to different germination responses, whenever its energy resource is limited due to temperature, soil structure, solar radiation and other environmental factors during the seed development period.

Seed size has great influence on the establishment and dispersal of species and is related to competition, predation and spatial distribution. Large seeds present lower restrictions to establish in distinct microsites under natural conditions, which gives them higher adaptive advantages (Lusk and Kelly, 2003). This condition results from the relation between seed size and seedling size, which affects the initial establishment in the field, named "effect of reserve size" (Leishmann *et al*., 2000). On the other hand, smaller seeds are usually produced in larger quantities and are more easily dispersed, exploring places that are not occupied by larger seeds.

Table 1. Mean dimensions of Tauari (*Couratari macrosperma* A.C. Smith) seeds.

Parameters	Length (mm)	Width (mm)	Thickness (mm)	Matter (g)
Mean	84.41	22.24	1.13	0.25
Minimum value	44.11	13.68	0.04	0.14
Maximum value	117.55	32.89	2.39	0.41
Coefficient of variation (%)	15.53	16.31	34.67	27.50
Standard error of the mean (s)	0.75	0.21	0.02	0.01

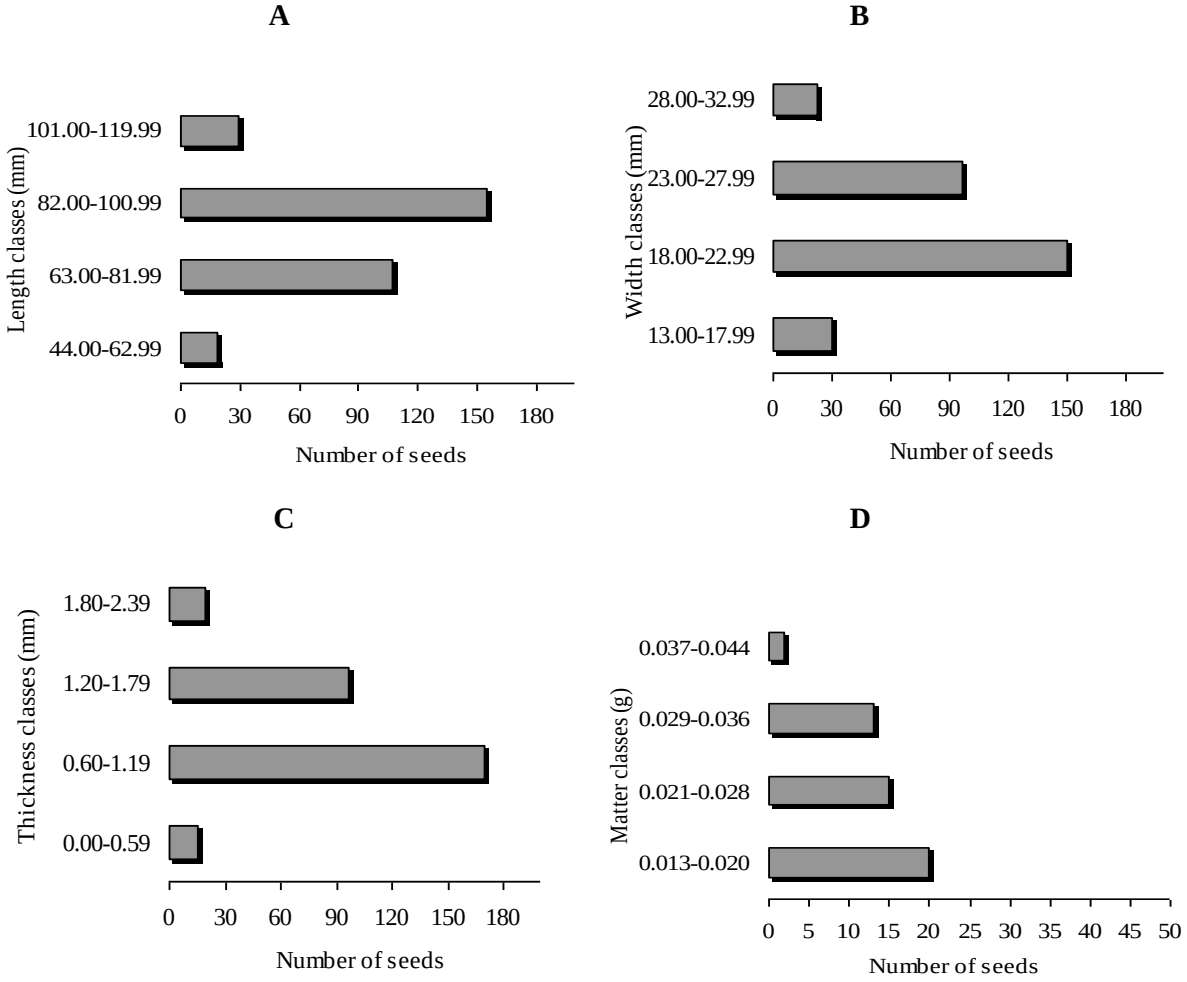

FIG. 1 Frequencies of length (A), width (B), thickness (C) and matter (D) of Tauari (*Couratari macrosperma* A.C. Smith) seeds.

The present results show that *C. macrosperma* seeds are morphologically very similar to those of *Couratari guianensis* Aubl, based on the description of Dal Bem (2006), likely making difficult their identification, especially due to other taxonomic characteristics too, including fruit and plant aspects. This can be proven by the great confusion in the identification of these materials in herbariums. In the field, these species have also been confused with each other and even with other *Cariniana* species, as demonstrated by Procópio and Secco (2008).

C. macrosperma seeds have a funiculus located in the center of the circumferential wing (Figure 2A), differently from *Cariniana legalis* (Mart.) Kuntze seeds which, as described by Rego (2001), have unilateral wing. The integument is brown and has a slightly wrinkly surface of brittle consistency (Figure 2A). The endosperm is thin and yellowish. The embryo is beige, opaque and extremely curved, with radicle and hypocotyl juxtaposed to the foliaceous cotyledons (Figure 2B and C), similar in format and coloration to the embryo reported by Camargo et al. (2003) for *Cariniana micrantha* Ducke. Schoenberg (1983) described the same type of foliaceous plicate cotyledon for *Couroupita guianenses* Aubl.

Germination started at 10 days after sowing through integument disruption, and the root was white cylindrical smooth glabrous (Figure 3A). According to Floss (2004), this is the most common process, with the radicle as the first organ to emerge from the integument during germination.

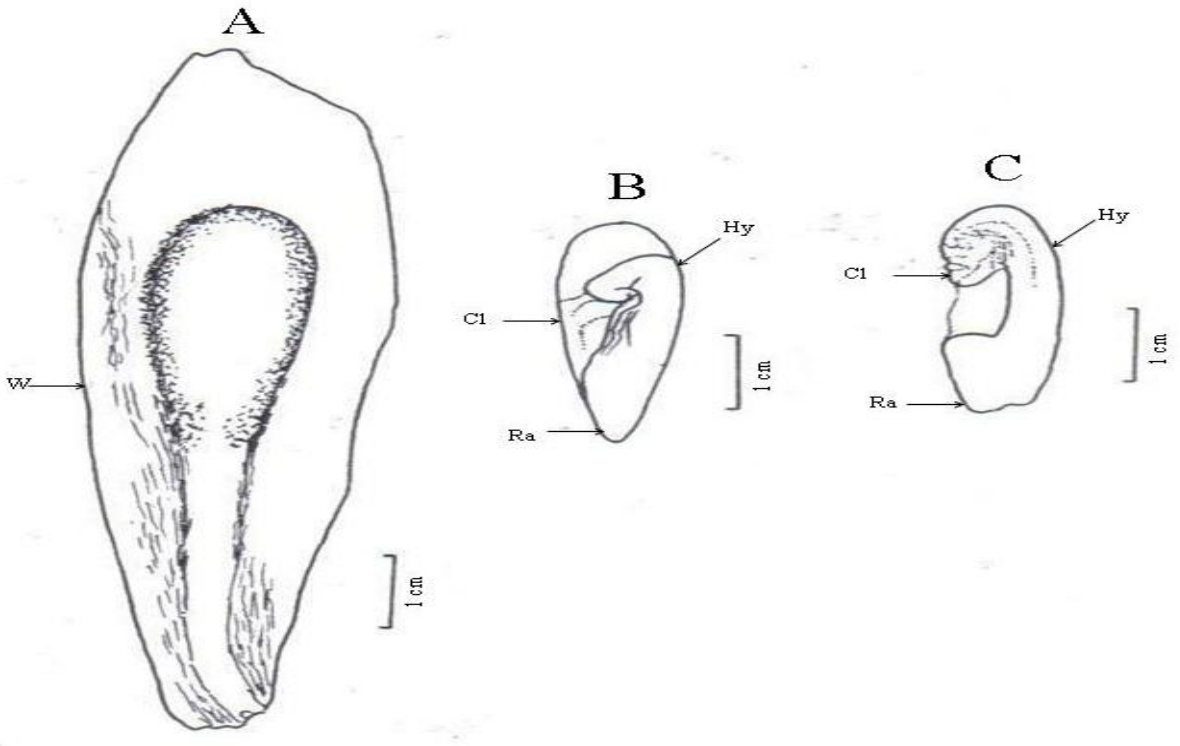

Fig. 2 Aspect of seed (A) and embryo (B and C) of Tauari (*Couratari macrosperma* A.C. Smith). W – Seed wing, Cl – Cotyledonary leaf, Ra – Radicle, Hi – Hypocotyl. *Illustration L.F. Braga*.

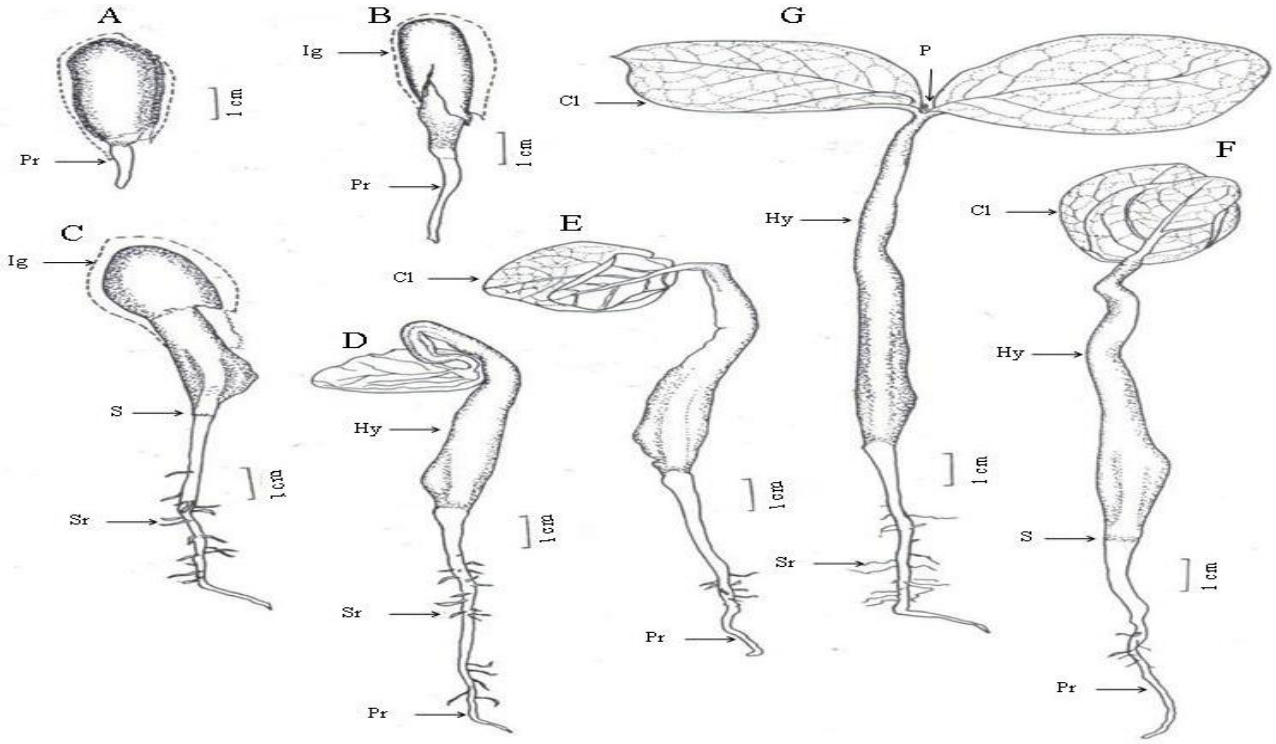

Fig. 3 Germination phases of Tauari (*Couratari macrosperma* A.C. Smith) seeds. A. Primary root protrusion; B. Emergence; C. Hypocotyl elongation; D. Foliaceous cotyledon exposure; E. Cotyledon expansion; F. Hypocotyl straightening; G. Leaf expansion and plumule exposure. Ig – Integument, Pr – Primary root, Sr – Secondary root, S – Stem, Hy – Hypocotyl, Cl – Cotyledonary leaves, P – Plumule. *Illustration L.F. Braga*.

In *Cariniana legalis* (Mart.) Kuntze, as reported by Rego (2001), germination was later when vermiculite was used as substrate, starting at 25 days after sowing, and the root was long with white short thin shining hairs.

Germination is epigeal and phanerocotylar (Figure 3B and C), according to the terminology of Duke (1965). This type of germination is common for the genera *Couratari* and *Cariniana* but different from that of *Bertholletia excelsa* Humboldt and Bonpland, which belongs to the same family and has germination classified as cryptocotylar and hypogeal, with no cotyledon distinction, as observed by Santos *et al*. (2006).

Primary root (beige) elongation, with slight distinction from the stem region occurs between the 10th and the 17th day (Figure 3B). Such a period is compatible with that observed by Camargo *et al*. (2003) for *Cariniana micrantha* Ducke: root emergence and elongation at 14 days of germination. On the 20th day, the stem is well delimited, separating the primary root from the hypocotyl, which is flat, glabrous, smooth and violaceous near the stem. The first secondary roots are also observed at this stage. Concomitantly, the integument starts sagging and being eliminated (Figure 3C) due to the expansion of cotyledonary leaves, which are simple, opposite, dark green and ovate, presenting round base and full, glabrous and smooth margin (Figure 3D and E). These characteristics are different from those described for *Cariniana micrantha* Ducke, the cotyledonary leaves of which are alternate, petiolate, membranous and lanceolate, with crenulate margins, obtuse base, acuminate apex, glabrescent-to-glabrous abaxial surface and glabrous adaxial surface (Camargo *et al*., 2003).

At 22 days, seed integument disrupts and releases the cotyledonary leaves, which are plicate and light green; hypocotyl is flat and bent in its upper portion (Figure 3D). At 24 days, there is an increase in cotyledonary leaves, with a slight change in the coloration of hypocotyl and cotyledonary leaves, which presented darker green coloration and clear brochidodromous venation on the abaxial surface (Figure 3E). In *Cariniana legalis* (Mart.) Kuntze (Rego, 2001), hypocotyl is also bent in its upper portion, but cylindrically, elongating after 40 days from sowing, a longer process compared to that observed for *C. macrosperma.* At 27 days from germination (Figure 3F), hypocotyl is green-rose and completely straight, cotyledonary leaves are ready to open and secondary roots are located along the whole primary root. At 29 days, cotyledonary leaves are fully open, dark green, showy and present a small plumule in their center (Figure 3G).

The second pair of opposite leaves emerges between 50-55 days (Figure 4A) and presents dark green coloration, elliptical shape, attenuate base and acuminate apex after expansion of the blade, which has brochidodromous venation (Figure 4B). In this period, seedlings presented on average 18 to 27cm length, pivoting root system, thin irregular axial root, characterized by a thinning in the extremity, with secondary roots presenting few short and thin lateral ramifications.

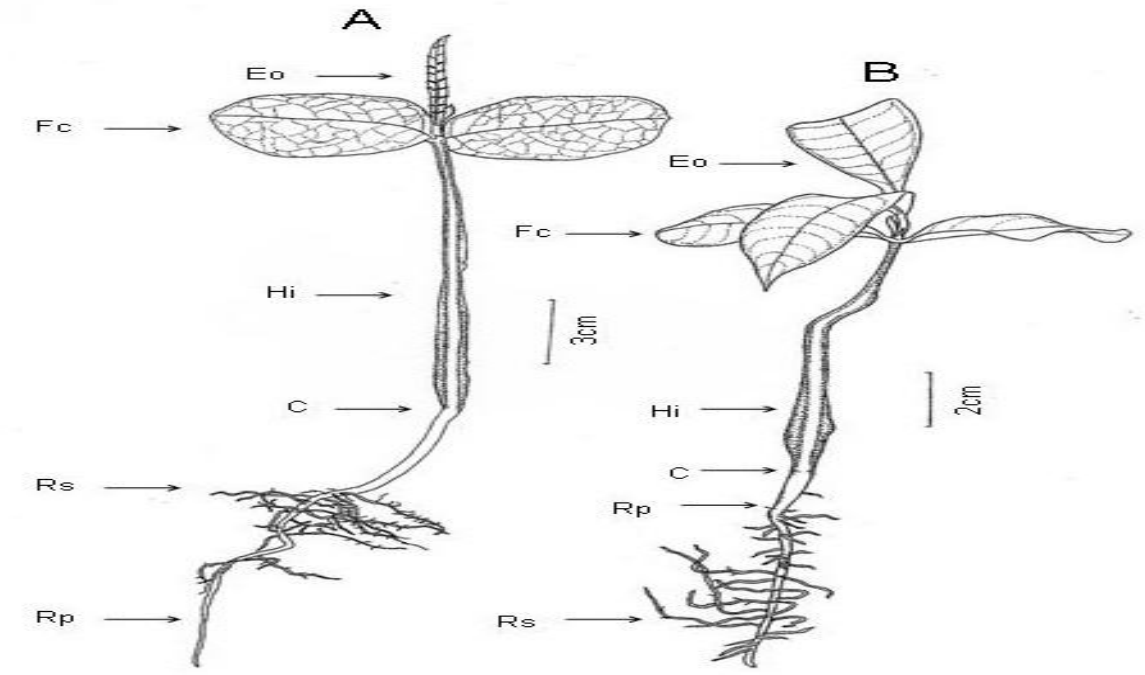

Fig. 4 Germination phases of Tauari (*Couratari macrosperma* A.C. Smith) seeds. A. Second pair of leaves; B. Expansion of the second pair of leaves. Pr – Primary root, Sr – Secondary root, S – Stem, Hy – Hypocotyl, Cl – Cotyledonary leaves, Eo – Eophyll. *Illustration L.F. Braga.*

According to Ribeiro *et al*. (1999), there are no obvious vegetative characters in the family Lecythidaceae, which usually presents simple, alternate, full or serrated leaves, without glands or other useful signs for identification. Those same authors stated that the most useful vegetative characters for the identification of species are venation types (brochidodromous or eucamptodromous), rhytidome pattern and living bark coloration.

The present results for Lecythidaceae species indicated typical characteristics of this family, as observed for

Cariniana micrantha Ducke, the seedlings of which have alternate and simple first leaves with elliptical-to-obovate shape and crenulate margins (Camargo *et al*., 2007; Camargo *et al*., 2003). It must be emphasized that, in the present study, *C. macrosperma* has brochidodromous venation, as observed by Santos *et al*. (2006) for *Bertholetia excelsa* Humboldt and Bonpland. Procópio and Secco (2008) adopted venation to differentiate between commercial species, such as tauari; the genus *Couratari* had first- and second-order venation, and *Cariniana* had second- and third-order venation. Those authors reported eucamptodromous venation for *Cariniana decantra* Ducke and *Cariniana micrantha* Ducke, and brochidodromous venation for *Couratari guianenses* Albul., *Couratari oblongifolia* Ducke and Knuth., *Couratari stellata* A.C. Smith. and *Couratari tauari* Berg. Seedling malformations are shown in appendix A, and the post-seminal development of *Couratari macrosperma* can be also observed in appendix B.

CONCLUSIONS

Couratari macrosperma seeds greatly varied as to biometrical parameters, including length, width, thickness and matter, which indicates a difference during their dispersal. Since they are dispersed by wind, the larger and softer the seed is, the further it will be taken from the parent plant. However, very soft seeds may present defective embryos and originate weak seedlings; thus, ideal seeds are consistent and present great circumferential wing.

The post-seminal development may end at 30 days, when the seedling has the first pair of expanded leaves with brochidodromous venation. In this stage, the most vigorous seedlings can be detected. The temperature 25ºC mostly favored seedling development. Of the studied substrates, the paper towel roll led to a faster germination.

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