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Morphological characterization of fruits and seeds of *Jatropha mollissima* (Pohl) Baill. (Magnoliopsida: Euphorbiaceae)

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Abstract. *Jatropha mollissima* (Pohl) Baill. is a native plant of the Brazilian semiarid that belongs to the Euphorbiaceae family. It is a widely distributed monoecious occurring in all states of Northeast Brazil, except Maranhão, and inhabiting areas of caatinga and restinga. In order to add information about this species as well as to facilitate their identification, this study aimed to determine the biometrics, to describe and illustrate the internal and external structures of the seed and the fruit, and to determine the chemical composition of *J. mollissima* seed. It was observed that the fruit is dry, smooth, and capsular, with average length of 2.31 cm, width of 2.06 cm, and thickness of 2.01 cm, respectively. The seed is eurispermic, oval, dorsum convex, flat wrap, with caruncle, and its average length, width, and tickeness are 1.27 cm, 0.84 cm, and 0.66 cm, respectively. The cytochemical and chemical analyses revelead marked lipid (35%) and protein (19.87%) contents in the seeds of this species.

Keywords: Euphorbiaceae, Jatropha, Morphology, Chemical, Lipids.

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Introduction

The Euphorbiaceae Family includes plants with nearly 300 genera and 6,000 species, distributed mostly in tropical regions. Brazil hosts about 70 genera and 1,000 species, making it a common family in the country's flora and one of the most taxonomically complex (Souza and Lorenzi, 2008). In general, species have caustic latex that, when in direct contact with mucous membranes, especially the eyes, can cause serious injury (Shlamovitz et al., 2009). Some plants of the Euphorbiaceae Family have economic importance such as the castor bean, *Ricinus communis* L., an African species whose seeds are used in the production of biodiesel from its oil, and rubber trees of the Amazon, *Hevea brasiliensis*, important in the production of rubber and widely cultivated in Malaysia and Indonesia (Souza and Lorenzi, 2008).

In popular medicine, several species stand out because of their applications, such as *Jatropha curcas* L., whose oil extracted from the seeds is used as a drastic purgative. Jatropha plants, especially of *J. curcas*, have been cultivated in Africa for exploitation of oil seeds in

candles, soaps, and in popular medicine (Leal and Agra, 2005).

Jatropha are native plants to South America (Francis et al., 2005). They are considered very important for the semiarid due to easy cultivation, adaptation to infertile soils, and drought resistance and have become a new option for income generation (Orhan et al., 2004; Shah et al., 2004; Francis et al., 2005; Shah et al., 2005). *Jatropha mollissima* (Pohl) Baill. is a plant native to the semiarid and belongs to the Euphorbiaceae family. It is a large, fast growing shrub whose average height is 2 to 3 m. *J. mollissima* is widely employed in folk medicine of the Paraiba state savanna and is used in therapeutic indications. The latex *in natura* is used as antivenin and seeds are sold in the open markets of the region for extraction of fixed oils, which are used as a veterinary purgative (Leal and Agra, 2005). With all this potential, few studies have been done on the morphological and chemical aspects of the seeds and the little existing information is more practical than scientific. In view of the foregoing information, the present study aimed to determine the biometrics, describe and illustrate the internal and external morphology of the fruit and seed, and investigate the chemical composition of *J. mollissima* seeds.

Materials and methods

The study was conducted in the Plant Cell Biology and Seed Analysis Laboratories belonging to the Federal University of Ceará. *J. mollissima* fruit samples were collected manually at random in the city of Beberibe (4° 11' S and 38° 08' W), located 74 km from the sate capital Fortaleza. A sample of the material collected was deposited at Prisco Bezerra Herbarium/UFC under voucher number 48,950. The fruits were then taken to the Plant Cell Biology Laboratory, where the seed bags were obtained by keeping the fruits tied to prevent dispersion due its explosive dehiscence. Thereafter, morphological, biometrical, and chemical determinations of the specimens were performed.

Fruit biometrics and external morphology

50 specimens were randomly selected to so that the fruit length, width, and thickness were determined using a digital caliper with 0.01 mm resolution. The length was considered the region between the fruit's base and apex. The width and thickness were measured in the middle portion, where the width region was considered the space between the right and left sides and the thickness, the region between the the fruit's back and belly. The averages, standard deviation, coefficient of variation, and range of values were also obtained. In studies of fruit morphology, the most commonly observed aspects are size, type, color, texture, opening, and number of seeds per fruit.

Seed biometrics and external morphology

50 seeds were randomly selected for individual measurement of length, width, and thickness using a digital caliper with 0.01 mm resolution. The length was considered the region between the seed's base and apex. The width and thickness were measured in the middle portion; the width region was considered the area between the right and left sides and the thickness, the area between the seed's back and belly. The averages, standard deviation, coefficient of variation, and range of values were also obtained. In previous seed morphology studies, some external characteristics including coat size, color, and and the presence of wattle (a fleshy structure located at the hilum of some seeds that operates in the dispersion) were observed (Gonçalves and Lorenzi, 2007).

Cytochemical analysis

In order to describe the internal morphology of the dried seeds, the specimens were cut transversely and fixed with a solution of 40 $g.L^{-1}$ paraformaldehyde in 0.1 $mol.L^{-1}$ phosphate buffer (pH 7.2) and 10 $g.L^{-1}$ glutaraldehyde for 24 h at room temperature (Karnovsky, 1965). After fixation, the samples were dehydrated in an ascending ethanol series. After dehydration, they were placed in the

pre-resin infiltration, infiltration, and then embedded into historesin (Historesin Embbeding Kit - Jung). The cuts were made at a thickness of 5 µm in a semi-automatic microtome (Slee Mainz CUT 5062) and subjected to the following cytochemical staining: 0.025% Toluidine Blue (TB) at pH 4.0 as metachromatic dye to detect anion radical (Vidal, 1977); 0.1% Xylidine Ponceau (XP) at pH 2.5 to detect the total cationic radical (Vidal, 1970); Pas (periodic acid-Schiff) reaction to detect neutral polysaccharides (Maia, 1979), and Lugol for detection of starch. The seeds were subjected to manual cuts to detect lipids, which were evidenced by 0.7% (m/v) Sudan IV test (Jensen, 1962). The slides were examined under an Olympus BX41 light microscope and UC30 camera.

Chemical determination

For determination of lipids and proteins, 20 randomly selected seeds were used, totaling 0.2 g of plant material. The samples were macerated in an Osterizer Blender grinder and the fresh and dry weights were determined. The fat content was measured from the difference of lipids after extraction with hexane according to Triebold (1946). Protein determination was carried out according to Bradford (1976) using bovine serum albumin as standard to construct the calibration curve. The protein content was quantified by reading in a spectrophotometer with a wavelength of 595 nm. The carbohydrates were extracted using a sequence of extractions with 80% ethanol and quantified by reading in a spectrophotometer with a wavelength of 620 nm according to Morris (1948) and Yemm and Willis (1954), using anthrone reagent and a reference curve from known glucose concentrations. For the determination of their fatty acids, 4 g of seed oil were put to react with 4 g of KOH in 30 mL of methanol. The mixture was maintained at reflux for 1 h. After concentrated, the material was dissolved in approximately 40 mL of distilled water, transferred to a separation funnel, and extracted with hexane (50 mL x 2). After separation of the phases, the aqueous phase pH was adjusted with 20% HCl to pH 5-6. The material was then transferred to a separation funnel and extracted with ethyl acetate (50 mL x 2). The organic phase was concentrated in a rotary evaporator and identified as saponifiable. Approximately 2 g of the saponifiable material were transferred to a 100 mL flask and 20 mL of methanol and 1 mL of concentrated HCl (12 mol/L) were added. The reaction mixture was refluxed for 1 h and concentrated in a rotary evaporator. The methyl esters were analyzed by GC-MS (gas chromatography mass spectrometry).

Results and Discussion

Biometrics and external morphology of the fruit and seeds of *J. mollissima*

The species has a dry, smooth, capsular fruit (Figure 1b). It consists of three globular cocas formed by a hard and woody dehiscent pericarp or rind. The dried fruit has an explosive dehiscence

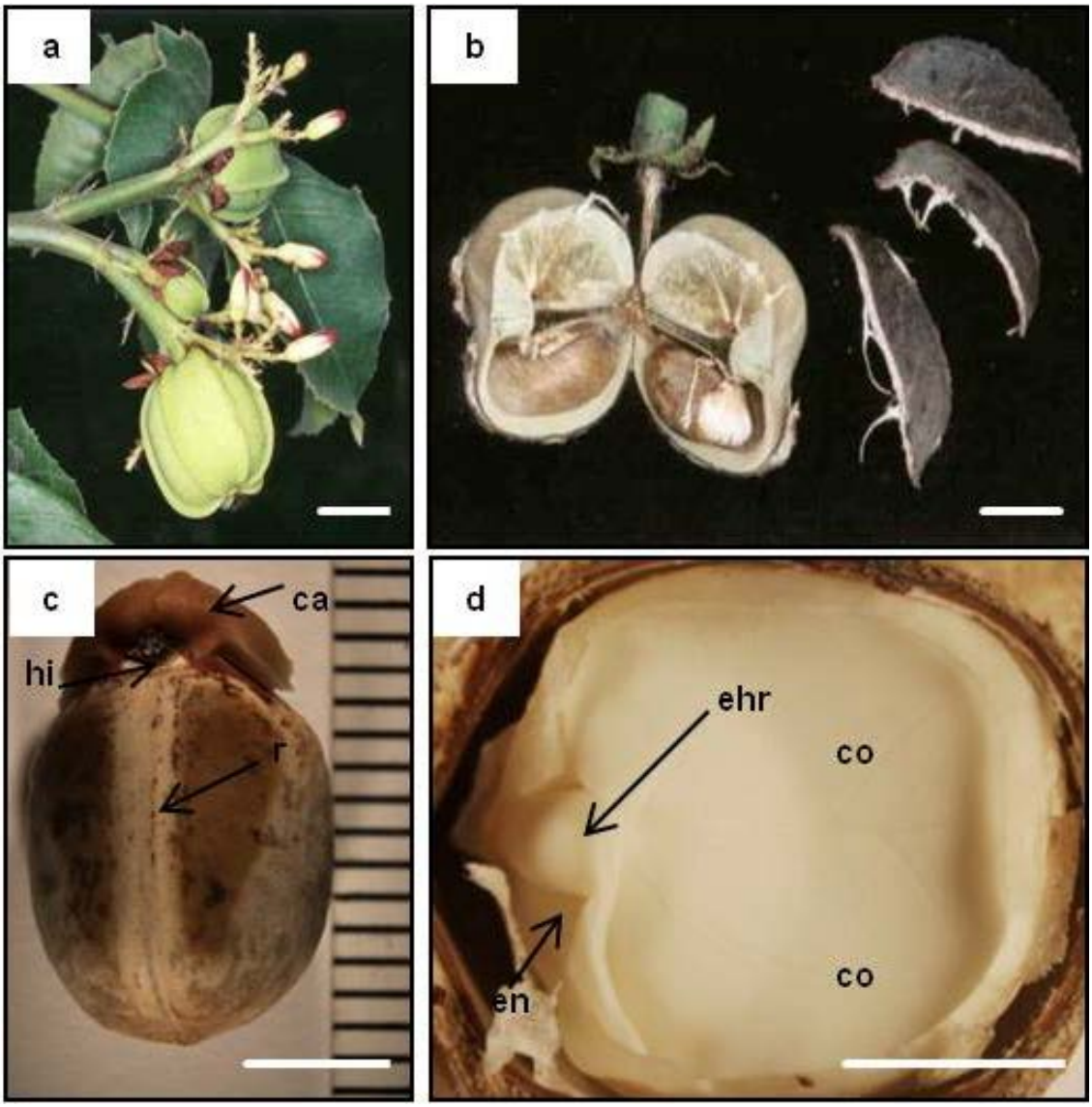

Figure 1. Morphological appearance of fruits and seeds of *J. mollissima.* **a**) The fruit; **b**) Detail of dry fruit; **c**) Seed; **d**) Detail of the seed cut longitudinally. Legend: ca: caruncle; co: cotyledon; en: endosperm; hi: hilo; r: raphe; hra: hypocotyl-radicle axis. Bar: 1 cm.

mechanism, which causes the cocas to show a fissure on their sides that creates an opening in order to release the seeds. The pericarp has two distinct areas: the exocarp, the thinner layer, and the endocarp, the thicker one. Fruit color varies with the degree of maturation from green (young fruit) (Figure 1a) to yellow and eventually dark brown (ripe fruit). The fruit usually has three seeds. Through biometric determinations, it was found that the average fruit length, width, and thickness are 2.31 cm, 2.06 cm, and 2.01 cm, respectively.

Regarding the fruits, the data in this study are similar to those reported by Saturnino et al. (2005). When working with *J. curcas*, those authors found that the fruit is about 2.3 cm to 4 cm long and 2 cm to 2.5 cm wide.

The seed is eurispermic and oval in shape, exhibits a convex back and a smooth wrap, has a coloration varying from pale brown when dry to black when hydrated, showing a caruncle (Figure 1c), located near the micropyle, stuck in the ventral portion. The hilum is visible at the base and the raphe is well marked along the seed

(Figure 1c). The seeds are, on average, 1.27 cm long, 0.84 cm wide, and 0.66 cm thick.

The albumen or endosperm is located within the seed (Figures 1d and 1e) and is white colored, tender, and rich in oil. The embryo of *J. mollissima* is provided with two foliaceous cotyledons (Figure 1e), which are very broad, but thin. The outline of the cotyledons is oval with pronounced veins and the hypocotyl-radicle axis is cylindrical and straight (Figure 1e).

Cytochemical analysis of *J. mollissima* seed

Through the cytochemical analyzes, the location of the constituents was determined in cotyledon cells. TB staining at pH 4.0 revealed the presence of anionic radicals in pectic substances of the seeds, staining the cell walls of the seed coat and endosperm with a blue color. In the endosperm cells, the metachromasia phenomenon was evidenced by the purplish shade, which indicates a greater availability of anionic radicals (Figure 2a).

XP staining (Figure 2b) showed a large amount of protein bodies, while the chemical analysis determined a content of approximately 19.87% soluble proteins, uniformly distributed in the cytoplasmic content of endosperm cells stained with this dye. The amounts of proteins found in *J. mollissima* are similar to those found for *J. curcas* (20.95%) (Souza et al., 2009), both species belonging to the same family. The presence of proteins as source of reserves in the seed were detected using the XP dye in other species such as *Moringa oleifera* Lam. (Gallão et al., 2006), *Carthamus tinctorius* L. (Abud et al., 2010), and *J. curcas* (Alencar et al., 2015).

The PAS reaction, which is used to detect neutral polysaccharides like starch, cellulose, and hemicellulose (Vidal, 1977), lacks grains stained, which indicates the absence of neutral polysaccharides in the cytoplasm. Thus, it can be inferred that this seed does not have starch as an abundant constituent of its reserves. This was confirmed by the reaction with Lugol, which did not indicate the presence of starch. Chemical determination indicated approximately 0.21% of free sugars.

The test with Sudan IV (Figure 2c) showed that these seeds have fat stores aplenty, confirmed by chemical analysis that detected 35% of lipids. This species is hence considered an oilseed, whose values of fixed oil found in this study are similar to literature results in species with high economic value belonging to the same *J. curcas* family (40.33%) (Souza et al., 2009).

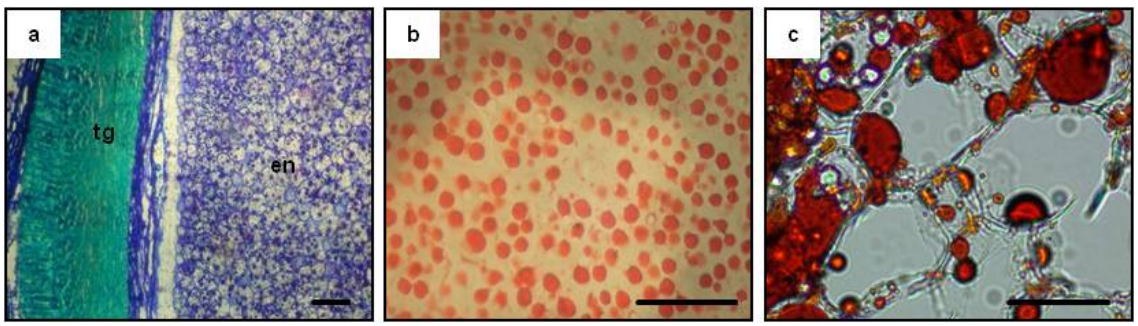

Figure 2. Cross sections in seeds of *J. mollissima* stained with: **a**) TB at pH 4.0, reserve tissue (endosperm) and testa; **b**) XP at pH 2.5, presence of protein bodies in the cytoplasm of the endosperm cells; **c**) Test with Sudan IV, showing lipid bodies in the cytoplasm of cells of the endosperm. Legend: en: endosperm; tg: tegument. Bar: 50 μm.

Table 1. Percentage amounts of fatty acids in *Jatropha mollissima* seed compared with *J. curcas*.

Fatty acid	% in the sample	
	J. mollissima	*J. curcas*
Palmitic acid	19.52	14.50
Linoleic acid	38.92	30.70
Oleic acid	22.11	48.00
Stearic acid	12.14	6.40

In Table 1, the percentage amounts of fatty acids of *J. mollissima* and percentage amounts of the fatty acids identified by Rao et al. (2009) in *J. curcas* are shown. It can be observed that linoleic acid is found in greater amounts (38.92%) in *J. mollissima* and oleic acid (48.0%) is the major constituent in the seed oil of *J. curcas*. Lipids are an important reserve component of cottonseed, peanut, sunflower, and castor bean (Marcos Filho, 2005), *Carthamus tinctorius* (Abud et al., 2010), and jatropha (Souza et al., 2009). A significant percentage of lipids observed in *J. mollissima* is a good indication for its use in biofuel production.

Conclusions

Through biometric determinations obtained, the average length, width and thickness of the fruit are on average 2.31 cm, 2.06 cm, and 2.01, while the seed's average length, width and thickness are 1.27 cm, 0.84 cm, and 0.66 cm, respectively. Regarding cytochemistry, the main reserve of *J. mollissima* are lipids (35%), followed by proteins (19.87%).

Due to the high lipid content, the seeds of this species is considered oilseeds, whose values presented in this study are similar to literature results in species with high economic value belonging to the same family. Further studies are needed to contribute to the possible use of *J. mollissima* in the production of biofuel.

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Conflict of interest statement

Authors declare that they have no conflict of interests.

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