Seed and seedling morphological characterization of *Jatropha mollissima* (Pohl) Baill. (Euphorbiaceae): a potential species with multiple uses[1]

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ABSTRACT

The seeds and seedlings morphological differentiation is a challenge in the early stages of plant growth. This study aims to describe the physical and morphological characteristics of seeds and seedlings of *Jatropha mollissima* to delimit differences between other species from the same genus. The seeds were placed to germinate for morphological description and their physical characteristics were measured. The *J. mollissima* presented a 1000-seed weight of 332 g, 3,008 seeds per kilogram, 6.7% of moisture, 1.29 cm length, 0.84 cm width, and 0.66 cm thickness. We verified that the seeds are oval in shape; their colour is dark brown, greyish or mixed colour; they have visible raphe and hilum, and caruncle with beige coloration. The endosperm is oleaginous and involves the embryo with foliaceous and spatulate cotyledons. The embryo is axial, and the hypocotyl-radicle axis has a short cylindrical shape. Germination is epigeal and phanerocotyledonary; leaves have dimorphism and serrated margins with simple trichomes; and their apex is cuspidate or attenuated. Our research demonstrates that it is possible to differentiate *J. mollissima* from other species of the same genus through morphological characteristics. Therefore, this work contributes to the correct identification of the species, laboratory analysis, and field recognition.

Keywords: physical analysis; Caatinga; botanical description; pinhão-bravo; semiarid.

INTRODUCTION

The Euphorbiaceae family includes about 6,300 species distributed in 246 genera spread all over the world (Carneiro-Torres *et al.,* 2017). This family presents the highest diversity of species distributed in tropical regions (Judd *et al.,* 2009). The *Jatropha* genus is known for the compounds it produces, mainly oil from the seeds, and colourful or white latex. It is important to mention that most of the *Jatropha* species found in Northeastern Brazil, especially in the Caatinga biome (Steppic Savannah or Dry Forest) (Brasil, 2019), has medicinal properties and is also used by the local population in the dry seasons as hedge, firewood, and animal fodder (Leal & Agra, 2005). In the Caatinga biome, there are 230 endemic species and 26 species which belong to this family and have been registered (Flora e Funga do Brasil, 2023).

Jatropha mollissima (Pohl) Baill. (Euphorbiaceae) is known as 'pinhão-bravo' and is classified as shrub-tree, bush, or small tree (Leal & Agra, 2005; Vasconcelos *et al.,* 2014; Lima *et al.,* 2015; INPI, 2021). The species presents latex with antiophidic properties, oil in seeds, and ornamental and medicinal potential. Such potential derives from the secondary compounds found in the leaves.

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Besides, the species also presents potential for biodiesel production because of the oil contained in the seeds (Leal & Agra, 2005; Lima *et al.,* 2015; Zegarra, 2015).

The region where the species occurs has low rainfall, high temperatures and evapotranspiration. Thus, the plants of semiarid areas such as this modify their morphological and physiological characteristics to adapt to these adverse conditions (Souza *et al.,* 2015). Considering this scenario, it is essential to know the behaviour, distribution, and morphology of the species (Cosmo *et al.,* 2010). In addition, we can evaluate the effects of environmental factors on the phenotype (Souza & Cavalcanti, 2019), which is important to obtain a better knowledge of the autecology of native species.

The morphological description of *J. mollissima* is restricted to floral biology (Santos *et al.,* 2005; Neves *et al.,* 2010; Neves *et al.,* 2011; Queiroz *et al.,* 2013), or secondary compounds for pharmacological purposes as described (Ribeiro *et al.,* 2014; Braquehais *et al.,* 2016). Regarding the morphological description of the fruits, they are superficial with little to no specification in relation to the seeds and seedlings (Leal & Agra, 2005; Vasconcelos *et al.,* 2014; Souza & Cavalcanti, 2019). In fact, the seed and seedlings characterization contribute to the evaluation of the germination test, commercialization, and identification of the forest species in the field (Felix *et al.,* 2021).

The scarcity of research related to the description of the species in its early stages becomes a hindrance to seeds and seedlings identification, limiting the dissemination of information about the ecology of the species. In the Brazilian semiarid region, there are 14 species of the *Jatropha* genus (Bigio *et al.,* 2020), which can make it difficult to distinguish species by seeds or at a juvenile stage, since some of these species present morphological characteristics of seeds and seedlings very similar (Añez *et al.,* 2005; Lyra *et al.,* 2012).

This paper is important because it presents information that can assist in reaching correct identification and better results at laboratory and in the field regarding *J. mollissima*. In addition, this characterization helps us understand how this species has adapted itself so as to survive in semiarid regions. Therefore, our research aims to describe the physical and morphological characteristics of seeds and seedlings of *J. mollissima* to delimit differences between other species from the same genus.

MATERIAL AND METHODS

The fruits of *Jatropha mollissima* were collected from 10 parent trees in May 2018, municipality of Floresta, State of Pernambuco, Brazil (08°36'04'' S and 38°34'07'' W, at 316 m altitude). The number of seeds per kilogram was obtained from the measurement of the 1000-seed weight with eight samples of 100 seeds, and the seed moisture was determined by oven-drying method at 105 ± 3 °C for 24 h, with three samples of 4.5 ± 0.5 g (Brasil, 2009a). We obtained a biometric characterization of *J. mollissima* seeds from the measurement of 100 seeds for length (Figure 1A), width (Figure 1B), and thickness (Figure 1C), with the aid of a calliper (0.01 mm). The results were presented in terms of mean, standard deviation, and coefficient of variation.

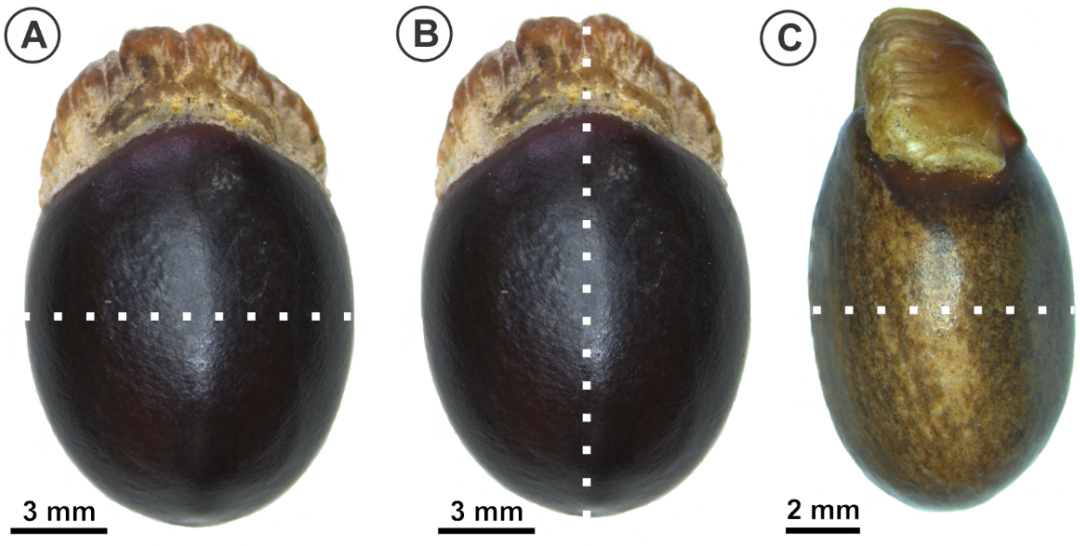

Figure 1: Measurement of length (A), width (B) and thickness (C) of *Jatropha mollissima* seeds indicated by white dashed lines.

We described the species' morphology from the visual observation of 50 seeds, with the aid of a stereoscopic magnifying glass. For the internal evaluations, we sectioned the seeds in radial, transversal, and longitudinal sections. The internal structures were identified and described concerning the presence or absence of endosperm, the position of cotyledons, the position of embryo (cotyledons + hypocotyl-radicle axis), as well as the shape and colour of these parts. The following external characteristics were evaluated: colour, texture, consistency, shape, hilum, and micropyle position. The description and determination of terms regarding morphological characteristics were described based on Barroso *et al.,* (1999), Brasil (2009b), Gonçalves & Lorenzi (2011), and Souza *et al.,* (2013).

For seedlings description, we selected 15 germinated seeds to evaluate their development until the primary leaf. We placed seeds to germinate in transparent acrylic boxes (gerbox®) using two sheets of paper towels as substrate (germitest®). After germination, the seeds were allocated in trays with a commercial substrate based on pine bark for growth monitoring, remaining in a BOD (Biochemical Oxygen Demand) incubator at 25 °C and under constant lighting.

The images were obtained with a Sony camera (Cyber-shot Dsc-w630, 16.1 megapixels) and a Leica magnifying glass (model DM300) was used for the monitoring and description of the seeds and seedlings. The figures were edited in the software CorelDRAW Graphics Suite 2020 – version 22.

RESULTS AND DISCUSSION

According to the Instructions for Analysis of Seeds of Forest Species (Brasil, 2013) and based on the results of the 1000-seed weight, the verified weight was 332 g, which corresponds to 3,008 seeds per kilogram. The seed lot of *J. mollissima* should have a maximum of 250 kg; the average sample should contain 1,000 seeds; the purity analysis should be performed with 800 seeds; and the germination test should be performed with 400 seeds. This information is important for marketing and evaluation of seed germination when the species is not listed in official documents.

The species in this study has specific characteristics that can be found in the Euphorbiaceae family and in the *Jatropha* genus (Table 1). We could observe that *J. mollissima* is more rounded than other species, if compared, for instance, to *Jatropha curcas* L., and *Jatropha elliptica* is smaller.

Jatropha mollissima seed coat colour varies between black, brown, greyish, or mixed colour (Figure 2A-D). Seed shape is oval and estenospermic, with little variation. The coat is smooth and glossy, with a chartaceous consistency. The seeds present linear raphe, which goes from the hilum to seed base, with the presence of a caruncle,

Table 1: Comparation between biometrics and morphological characters of seeds and seedlings of *Jatropha* genus species

Jatropha mollissima						
Width	**Length**	**Thickness**	**Colour**	**Endosperm**	**Germination**	**Reference**
0.84 cm	1.29 cm	0.66 cm	Diverse	White, embedded in the embryo, spatulated, axial	Epigeal-phanerocotyledonary type	
Jatropha curcas						
1.09 cm	1.75 cm	0.87 cm	Dark brown - uniform	White, embedded in the embryo, spatulated, axial	Epigeal-phanerocotyledonary type	Pimenta *et al.* (2014)
Jatropha elliptica						
0.43 cm	0.81 cm	0.37 cm	Diverse	White, embedded in the embryo, spatulated, axial	Epigeal-phanerocotyledonary type	Añez *et al.* (2005)
Jatropha ribifolia						
3.82 cm	7.46 cm	0.04 cm	Diverse	White, embedded in the embryo, spatulated, axial	hypogeal- cryptocotylar	Lyra *et al.* (2012)
Jatropha peiranoi						
0.46 cm	0.91 cm	0.35 cm	Diverse	Endosperm insert in a short and straight embryonic axis	unspecified	Paterlini *et al.* (2019)

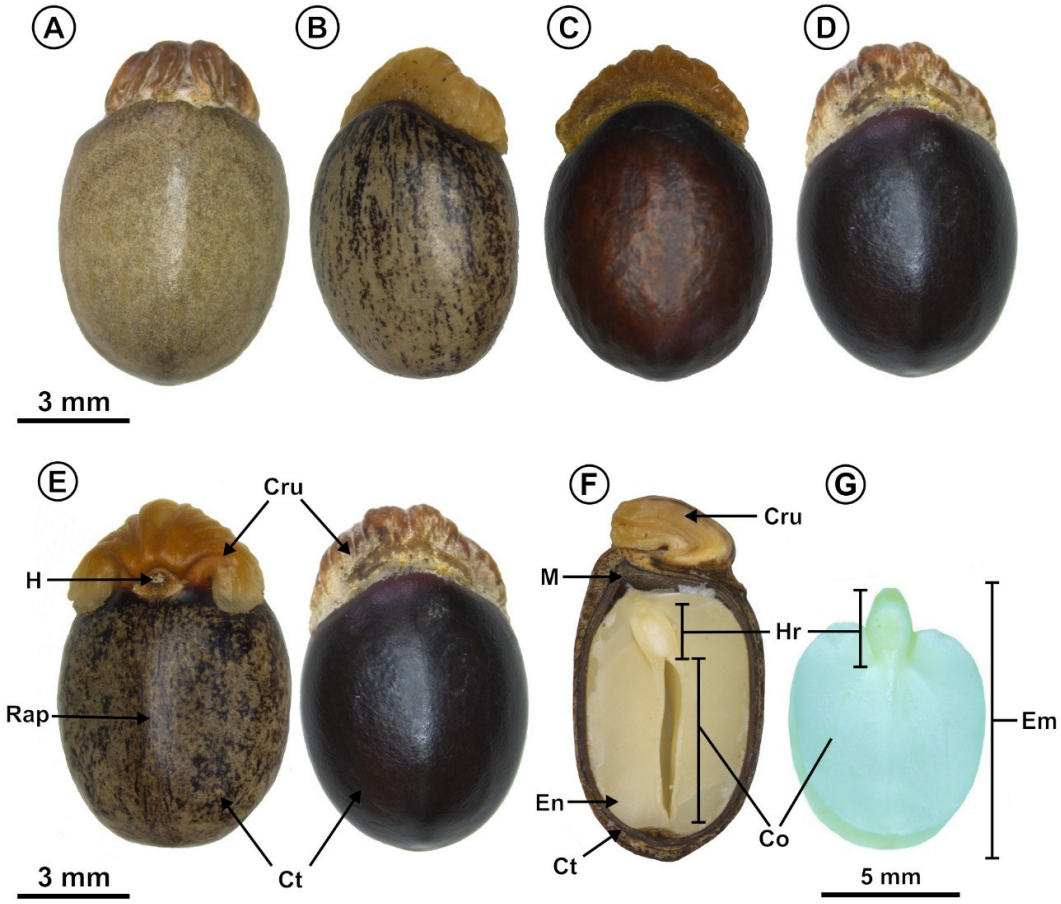

Figure 2: Greyish (A), mixed (B), brown (C) and black (D) seeds, external morphology (E), internal structures in the radial longitudinal section (F) and detail of the embryo of *Jatropha mollissima* (G). Cru: caruncle; Ct: coat; Rap: raphe; H: hilum; Hr: hypocotyl-radicle axis; Co: cotyledons; En: endosperm; M: micropyle; Em: embryo.

a structure derived from the external coat, which is beige. The hilum has an oval shape, with a raised surface, and it is at the intersection of the caruncle with the integument, in the apical position (Figure 2E). The micropyle is below the caruncle (Figure 2F).

These different seed colours influence animal attraction according to the location of parent trees. Seed coloration is an essential factor in species ecology because it is attractive for dispersing agents since primary dispersion is autochoric, and secondary is zoochoric, with ants working with this dispersion, for example (Hernández-Nicolás *et al.,* 2017). Darker coloration in more open environments will draw the attention of dispersers and predators more than lighter colours. Differences between the same genus species can be observed in seed colour. For instance, if compared to *J. mollissima*, *J. curcas* has darker brown coloration and it is more uniform. Differences concerning colour and size can also be observed between *J. elliptica* and *J. peiranoi* Lourteig & O'Donnell seeds (Añez *et al.,* 2005; Pimenta *et al.,* 2014; Paterlini *et al.,* 2019). Differences between collecting sites can also interfere in seed size and species behaviour (Hernández-Nicolás *et al.,* 2017), since changes in soil type or precipitation influence seed production. Another aspect that can attract dispersers and predators is the presence of caruncle, which is a succulent excrescence of the oil-rich tegument (Paterlini *et al.,* 2019) and a type of aryl (Brasil, 2019b). Neves *et al.,* (2011) say that *J. mollissima* seeds can easily attract ants because they present a larger caruncle.

We observed in the internal morphology of *J. mollissima* seeds (Figure 2F) a continuous, homogenous, oleaginous, yellowish-white, and thick endosperm, which is embedded in the embryo (cotyledons + hypocotyl-radicle axis). It was not possible to identify the plumule at the seeds. The embryo is spatulated, foliaceous, axial, and erect. Cotyledons have leafy consistency, with visible

nervures, rounded shape, and yellowish-white coloration, similar to the endosperm. The hypocotyl-radicle axis has a short cylindrical shape (Figure 2G).

According to Barroso *et al.,* (1999) and Zegarra (2015), it is typical of the family Euphorbiaceae to present abundant endosperm and rich oilseed resources. The endosperm of *J. mollissima* is similar to others of the *Jatropha genus* (Table 1), which commonly has white colour, involving the embryo completely, axial, and sparse. Other authors observed these characteristics in seeds of *J. elliptica, J. curcas, J. macrocarpa* Griseb., *J. peiranoi,* and *Gymnanthes klotzschiana* Müll. Arg. (Añez *et al.,* 2005; Cosmo *et al.,* 2010; Loureiro *et al.,* 2013; Pimenta *et al.,* 2014; Vasconcelos *et al.,* 2014; Tavecchio *et al.,* 2018; Paterlini *et al.,* 2019).

Seed germination occurs from the fifth day after sowing, in the root protrusion (Figure 3). The radicle is emitted through the micropyle region, located below the caruncle, so it is possible to see the radicle going through the caruncle. In the present study, we observed the simultaneous emission of more than one radicle, which pointed out that it is not always possible to identify a differentiation between primary and secondary roots in the seedling phase.

The root system of *Jatropha* seedlings comprises the primary and secondary axial roots (Alves *et al.,* 2008; Lyra *et al.,* 2012; Silva *et al.,* 2016). Brito *et al.* (2019), in a study with *J. curcas, J. multifida* L., *J. podagrica* Hook., and *J. gossypiifolia* L. seeds, observed four additional meristematic regions in the hypocotyl-radicle region, besides the apical meristem that will form the main root. Still according to the authors, they classified it as further radial primary roots, which was also observed by Soares *et al.* (2017). This conclusion leads us to believe that the *Jatropha* genus presents a differentiated pattern of germination concerning radicle protrusion. This behaviour can be understood as an adaptation of the species due to the region where it is located and as a way of improving root performance to absorb water, mainly, and nutrients that are in low availability in the soil.

An indication of the adaptivity of the species is the emission of more than one adventitious root, which allows the species to grow and develop in stressful environments, as observed in *J. curcas* (Brito *et al.,* 2019). This root behaviour is classified as branched, where it is not possible to define the primary root, emphasizing that site conditions in which the species occurs and genetic characteristics are factors that have a considerable influence on root growth (Gonçalves & Mello, 2000).

The germination is epigeal and of the phanerocotyledonary type. Cotyledons are elevated above the substrate with hypocotyl growth and detached from the coat, completely expanded (Figure 4). The cotyledons are photosynthetic and persistent, providing nutrients to the seedling until the emission of the first leaves (indicated in figure 4 by primary leaf), because even after the emission of the first leaves the seedling is still attached. In some seeds, we observed that cotyledons do not detach from the coat. However, they were not considered abnormal seedlings because the primary leaf emission occurred normally without harming the development of the seedling.

Jatropha mollissima follows the same pattern of germination seen in other species of the Euphorbiaceae family, as observed by Añez *et al.* (2005), Cosmo *et al.* (2010), Pimenta *et al.* (2014), Vasconcelos *et al.,* (2014),

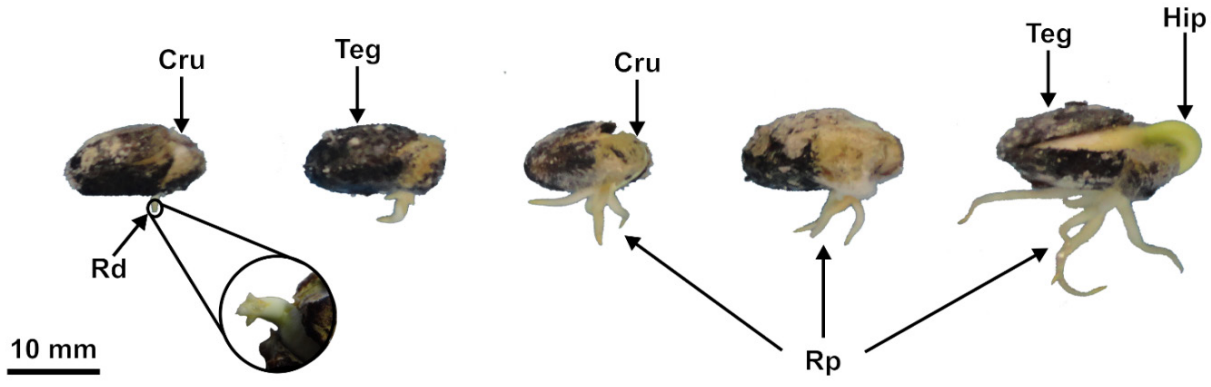

Figure 3: Germination of *Jatropha mollissima* seeds. Cru: caruncle; Teg: coat; Hip: hypocotyl; Rd: radicle; Rp: secondary roots.

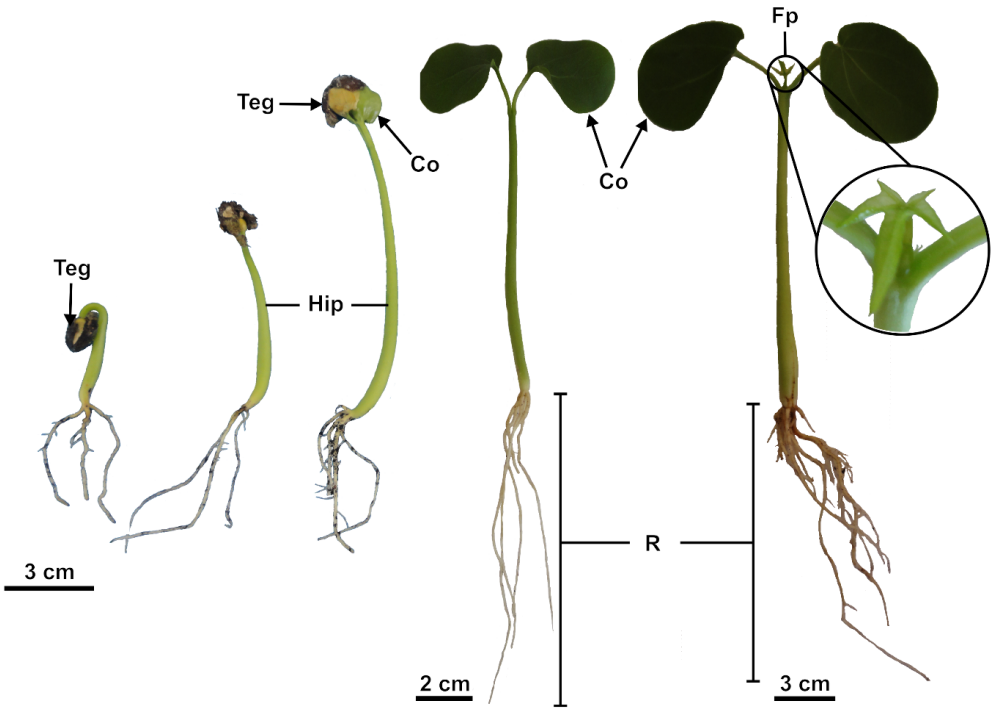

Figure 4: Development of *Jatropha mollissima* seedlings. In detail primary leaf. Teg: Coat; Co: cotyledons; Hip: hypocotyl; R: roots; Fp: primary leaf.

Lima *et al.* (2015), Virgens *et al.* (2017), Tavecchio *et al.* (2018), Brito *et al.* (2019), and Souza & Cavalcanti (2019). We also observed that its roots are superficial in the early stages, indicating the species' preference to shoot growth to the detriment of root growth. This behaviour can be explained due to the region in which the species occurs, since Caatinga soils are sandy and water infiltration occurs quickly, causing plants to have maximum water use capacity.

We observed that the species' leaves present different characteristics when young and mature. Initially, they have a simple, palmatilobate blade with five lobes (Figure 5A-D), culminated or attenuated apex, and a sagittal base. Mature leaves have three to five lobes, although some leaves remain with five lobes and others with only three. In both young and mature leaves, we observed attenuated or acuminated apex. The margin is serrated, both on the young and mature leaves, with simple trichomes at the tips (Figure 5E-G). It is typical of the genus *Jatropha* to have palmatilobate blade leaves with three to seven lobes (Abdulrahaman *et al.,* 2014). Leal & Agra (2005) and Sátiro & Roque (2008) described the number of leaf lobes, indicating that the leaves have five lobes and that this is one of the differences between the species of the *Jatropha* genus. The differences in the numbers of leaf lobes happen due to the plant's age, as well as climatic factors.

Phenotypic plasticity is the ability of a species to present differences in its morphological or physiological characteristic, depending on environmental conditions (Tucić *et al.,* 2018; Fenollosa & Munné-Bosch, 2019; Laitinen & Nikoloski, 2019). These differentiations in leaf apex shape and number of lobes are characterized as phenotypic plasticity, since different behaviours can be observed, both in the same individual and in different individuals. Differences between young and adult plants are noticeable. However, the scarcity of information makes it difficult to identify the species at its early stages in the field. For this reason, the diffusion of studies with indications about different phases of the plant is essential to help field work.

The scarcity of research related to the descriptions of the species at its early stages is one of the difficulties found in this study. The knowledge about morphological characterization at different growing stages is important for a better identification of this species in the field and for laboratory analysis. Specifically in *J. mollissima*, we can identify seedlings with emission of more than one root at the same time, for example.

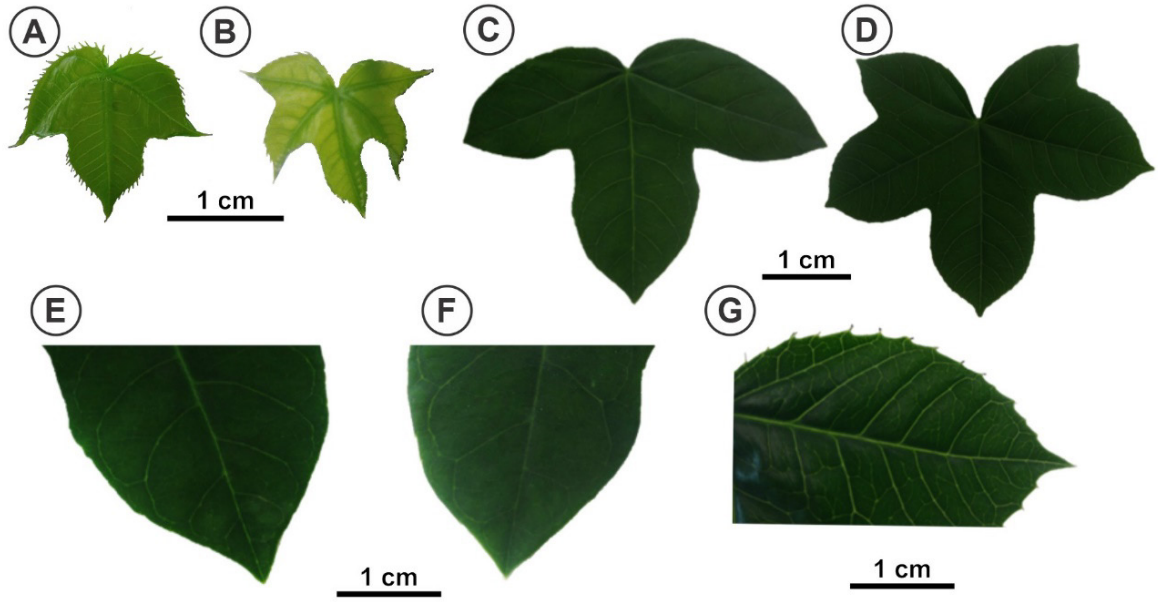

Figure 5: Young and mature leaves with three lobes (A, C) and young and mature leaves with five lobes (B, D); sharp (E) and acuminate (F) apexes and serrated margin (G) of *Jatropha mollissima* leaves.

CONCLUSIONS

Our research demonstrates that it is possible to differentiate *J. mollissima* from other species of the same genus through morphological characteristics. We also identified standard characteristics, such as the emission of more than one adventitious root, in another species from the same genus.

This study shows the deficit of information about species from Brazil's semiarid region and how they could actually help identify species in the field and in the laboratory. Therefore, this study contributes to the correct identification of the species, laboratory analysis, and field recognition.

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