Floral biology and characterization of seed germination in physic nut (*Jatropha curcas* L.)[1]

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ABSTRACT - The objective of this study was to characterize morphologically the seed germination and floral biology of *Jatropha curcas* grown in Viçosa, Minas Gerais state. The floral biology study was made on fresh inflorescences of 20 plants. For the post-seminal development study, the seeds were submitted to laboratory and greenhouse germination test. *J. curcas* has flowers of both sexes within the same inflorescence, with each inflorescence having an average of 131 flowers, being 120 male and 10.5 female flowers. Low numbers of hermaphrodite flowers were also found, ranging from 0 to 6 flowers per inflorescence. The germination of *J. curcas* begins on the third day with radicle protrusion in the hilum region. The primary root is cylindrical, thick, glabrous and branches rapidly, with about 4-5 branches three days after protrusion, when the emergence of the secondary roots begins. Seed coat removal occurs around the 8th day, when the endosperm is almost totally degraded and offers no resistance to the cotyledons that expand between the 10th and 12th day. A normal seedling has a long greenish hypocotyl, two cotyledons, a robust primary root and several lateral roots. On the 12th day after sowing, the normal seedling is characterized as phanerocotylar and germination is epigeal.

Index terms: physic nut, flowering, morphology, germination.

Biologia floral e caracterização morfológica da germinação de sementes de pinhão manso (*Jatropha curcas* L.)

RESUMO – Objetivou-se estudar a biologia floral e caracterizar morfologicamente a germinação das sementes de pinhão manso nas condições de Viçosa, MG. O estudo da biologia floral foi feito em inflorescências provenientes de 20 plantas. Para a descrição morfológica do desenvolvimento pós-seminal, as sementes foram submetidas ao teste de germinação, em laboratório e casa de vegetação. *J. curcas* possui flores dos dois sexos reunidas na mesma inflorescência. Cada inflorescência apresentou, em média, 131 flores, sendo 120 masculinas e 10,5 femininas. Flores hermafroditas também foram encontradas, porém, em número menor, que variou de 0 a 6 flores/inflorescência. A germinação da semente inicia-se no 3° dia, caracterizada pela protrusão da raiz primária na região próxima ao hilo. A raiz primária é cilíndrica, espessa, glabra, apresentando cerca de 4-5 ramificações no 3° dia após a protrusão, quando tem início o aparecimento das raízes secundárias. A liberação do tegumento ocorre por volta do 8° dia, quando o endosperma, quase totalmente degradado, não oferece resistência aos cotilédones, que se expandem entre o 10° e o 12° dia. A plântula normal apresenta hipocótilo longo, de coloração esverdeada, 2 folhas cotiledonares, raiz principal robusta e muitas raízes secundárias. Por volta do 12° dia fica caracterizada a plântula normal, fanerocotiledonar, originada de germinação epígea.

Termos para indexação: pinhão manso, floração, morfologia, germinação.

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Introduction

Jatropha curcas L., a plant species belonging to the Euphorbiaceae, and known as the physic nut, is an oilseed crop, with a large energy potential due to the oil content of the seeds (Achten et al., 2008). Its cultivation has various advantages for biodiesel production: the plant is perennial, rustic, easy to manage, producing a high-quality oil in the seeds (38% content) for biodiesel, and is suitable for intercropping with food crops (dry beans) or other energy-producing crops (peanuts), since it is a shrub suitable for wide spacing planting. Such advantages make this species appropriate for growing on small farms using family labor, which generates income and encourages people to stay in the countryside (Dias et al., 2007).Various studies have been done aiming the characterization (Senthil et al., 2008; Sunil et al., 2008; Freitas et al., 2011; Dias et al., 2012) and breeding (Popluechai et al., 2009; Spinelli et al., 2010; Bhering et al., 2012) of *J. curcas*. However, few basic regional studies of the reproductive biology and seed and seedling morphology have been carried out.

Knowledge of species floral structures and reproductive biology are fundamental for genetic improvement programs since they provide information for developing controlled pollination techniques. Several studies of *J. curcas* floral biology have been done over the last few years (Abdelgadir et al., 2008; Juhász et al., 2009; Paiva Neto et al., 2010). The variations found in flowering patterns can be attributed to species genetic variability and to the effects of environmental factors, principally photoperiod and temperature. Considering that the transition from the vegetative to the reproductive phase involves both environmental factors, as well as those inherent in the plant, such as genetic and hormonal characteristics (Gomes et al., 2001; Silva et al., 2001), studies done in localities with different environments are justified.

Seed and seedling morphology have been analyzed as part of morpho-anatomical studies with the aim of increasing knowledge of a certain species or plant group, or for seedling recognition and identification in a region with an ecological focus (Oliveira and Pereira, 1987). Based on the morphological studies of seeds and seedlings, information on germination, storage, viability and sowing methods can also be obtained (Matheus and Lopes, 2007). The objective of this study was to describe the floral biology and the germination morphology of *J. curcas* in the conditions of Viçosa, Minas Gerais state.

Material and Methods

The study on floral biology was made between December 2010 and February 2011. Plants from the germplasm bank of the Federal University of Viçosa, which was established in an experimental area of the Department of Crop Science (Latitude: 20°45'14"south; Longitude: 42°52'54" west; Altitude: 648 m), were used. Observations were made early in the morning and the floral morphology was described using 30 inflorescences taken from 20 plants. Then, the material was sent to the laboratory for counting and flower characterization, which was made using a stereo microscope.

To quantify the reproductive success from free pollination under natural conditions, another 30 inflorescences were tagged during anthesis and the number of fruits formed and seeds per fruit were counted later. The proportion of fruits per female flower was calculated according to the method adopted by Silva et al. (2010).

To characterize the seedling and describe the morphology of post-seminal development, the seeds were submitted to laboratory and greenhouse germination test, according to the methodology used by Vanzolini et al. (2010). Four sub-samples of 25 seeds were distributed on two sheets of paper towel previously moistened with a volume of water three times the weight of the dry paper. The paper towel sheets were then turned into rolls and taken to a germinator at 25 ºC. At the same time, another test with four sub-samples of 25 seeds was done in greenhouse using sand as the substrate. Seed germination was evaluated daily until 12 days after sowing to observe seedling development for both substrates.

Real size illustrations were made manually with the help of a stereo microscope and using the seedlings obtained from the paper roll test. The morphological characteristics of the structures of each developmental stage were described from daily observations of the most vigorous seedlings. The developmental and differentiation processes of all stages were described in order to characterize all the germination process, i.e. protrusion of the radical, development of the hypocotyls-radicle axis, emission of the cotyledons and the plumule. The terms used here are the same as those adopted by Vidal and Vidal (2003) and Gonçalves and Lorenzi (2007).

Results and Discussion

Floral biology

J. curcas has male and female flowers in terminal

corymbose cyme inflorescences (Figure 1A), with the normal occurrence of a central female flower surrounded by male flowers (Figure 1B). The inflorescences of the study population had a mean of 135 flowers, where the number of male flowers varied from 42 to 241 and female flowers from 3 to 31 (Table 1). Similar results were obtained by Juhász et al. (2009), with the number of male flowers varying from 87 to 222, but with fewer female flowers, varying from 4 to 12 flowers per inflorescence. In India, Raju and Ezradanam (2002) stated that an inflorescence can produce from 1 to 5 female flowers and from 25 to 93 male flowers. Also in India, Bhattacharya et al. (2005) observed a ratio of from 2 to 19 female flowers to 17 to 105 male flowers per inflorescence. There were hermaphrodite flowers but in much lower numbers, varying from zero to six flowers per inflorescence. However, Juhász et al. (2009) and Paiva-Neto et al. (2010) did not find hermaphrodite flowers in *J. curcas* in North Minas Gerais and in Mato Grosso do Sul states, respectively. The occurrence of hermaphrodite flowers and the variation in the ratio of male to female flowers may be related to genetic and environmental factors among other things (Bhattacharya et al., 2005).

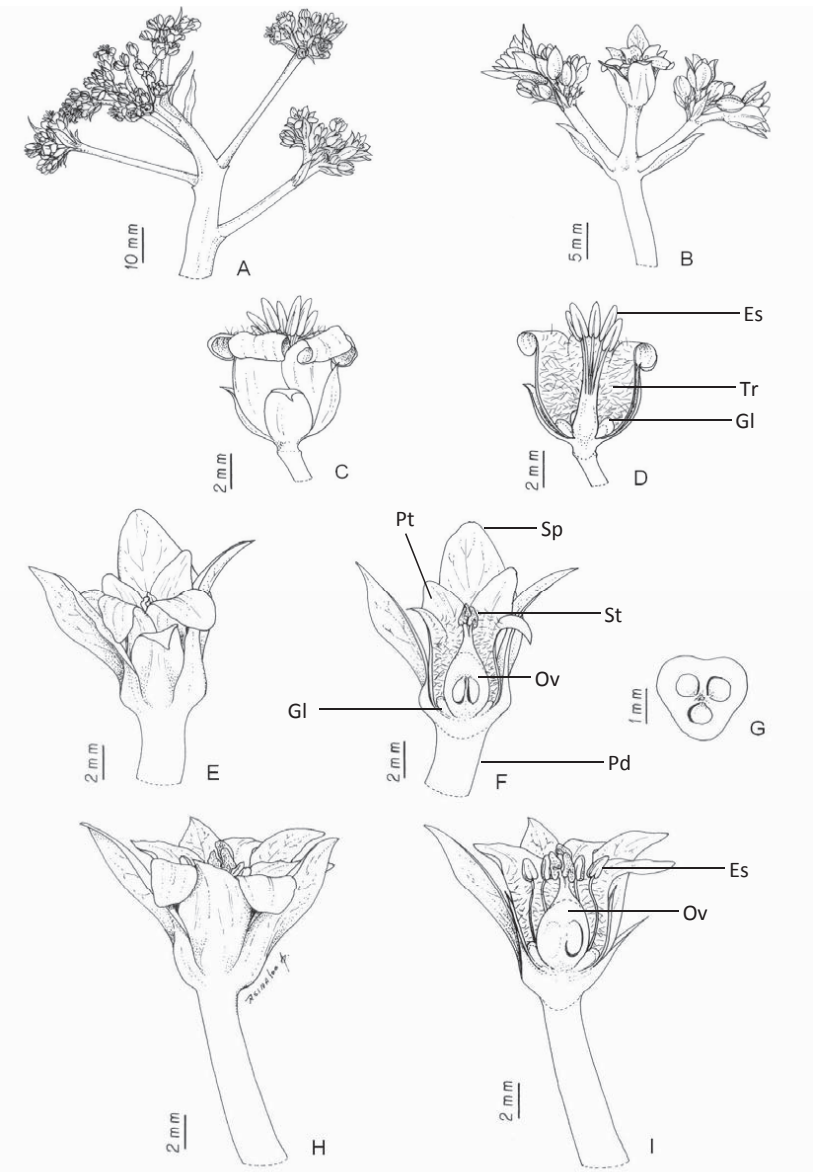

Figure 1. *Jatropha curcas* L. A and B = inflorescence; C and D = male flower; E and F = female flower; G = ovary (transversal section); H and I = hermaphrodite flower. Es-stamen; Gl-gland; Ov-ovary; Pt-petal; Pd-peduncle; Sp-sepal; St-stigma; Tr-trichomes. Illustration: R. Pinto.

Table 1. Floral characteristics of *Jatropha curcas* L.

Floral characteristics	Mean ± Standard Deviation
Mean number of flowers/inflorescence	135.4 ± 60.7
Mean number of male flowers/inflorescence	123.7 ± 57.7
Mean number of female flowers/inflorescence	11.2 ± 6.1
Mean number of hermaphrodite flowers/inflorescence	0.4 ± 1.2
Mean number of fruits/inflorescence	6.4 ± 2.9
Number of seeds/fruit	3 ± 0
Reproductive success	57%

Male flowers have a greenish perianth, a dialisepalous calyx with 5 sepals, a gamopetalous corolla with 5 petals and an androecium with 10 stamens, with 5 stamens united in a central tube and 5 free. The anthers are yellow, dithecal and dorsally fixed (Figure 1C-D). The female flowers are also greenish, with a dialisepalous calyx with 5 sepals and a corolla with 5 petals (Figure 1E-F). The ovary is superior, trilocular, tricarpellate, triovular with apical placentation (Figure 1G). The female flowers are much bigger and, compared to the male flowers, are approximately twice the diameter of the peduncle (2 and 1 mm, respectively). The hermaphrodite flowers are similar to the female flower in shape and size, with the main difference being the presence of nine stamens (Figure 1H-I). The disposition of the reproductive structures favors pollinator access to all the stamens, and later to the stigma of the female flowers, during each visit.

There are five glands at the base of the stamens in male flowers (Figure 1D) and at the base of the ovary in female flowers (Figure 1F) and hermaphrodites (Figure 1I). Glandular trichomes are present inside the petals in the three flower types, probably releasing a sweet, bland odor on the corolla of the inflorescences studied. Since the plants are cross-pollinated and entomophilous, these glands probably have a direct relationship with nectar production, attracting pollinators (Heller, 1996; Raju and Ezradanam, 2002; Saturnino et al., 2005).

In the study population, the female flowers opened before the male flowers (Figure 2A), in the same inflorescence. This was also observed by Saturnino et al. (2005) and Juhász et al. (2009), but not by other authors, who reported the opposite, i.e. that the male flowers opened before the female ones (Raju and Ezradanam, 2002; Chang-Wei et al., 2007; Dnissa and Paramathma, 2007). This non-uniform pattern of anthesis of the male and female flowers favors cross fecundation and may be related to environmental conditions (Heller, 1996; Chang−Wei et al., 2007).

Figure 2. Inflorescences of *J. curcas*. A- Anthesis of female flowers; B- Bee pollination (*Apis mellifera* L.); C- Visit by ant. Fh = Hermaphrodite flower; Ff = Female flower. Photos: B. G. Brasileiro.

Raju and Ezradanam (2002) listed ants, bees, flies, thrips and other insects as the principal pollinators, being the importance of each type of insect dependent on the locality. In the current study, bees were often observed, but so were ants and other insects that visited the flowers (Figure 2B-C). Bhattacharya et al. (2005) considered that bees were the efficient pollinators of *J. curcas*.

The availability of floral resources (nectar and pollen) for visitors to insect-pollinated plants may regulate flower visits, maintain the visiting population and result in pollination and, consequently, in the formation of the fruit and seeds, as a reward. The flower visitors depend on nectar (intra or extrafloral) and pollen for their nutrition.The lack of floral resources may result in a weak interaction between plants and their specialist pollinators, which may limit pollination success (Bhattacharya et al., 2005).

The morphological differences of the pistillate and staminate flowers help in their recognition and in the emasculation necessary for the crossing procedure in improvement programs.

The natural reproductive success was 57% of completely formed fruits, all containing three formed

seeds. The characteristics involved in the reproductive mechanisms of *J. curcas*, i.e.fruits with 100% of the seeds formed/fruit, in natural pollinations, and maintenance of the fruits with seeds from crossed pollination, reflect the high reproductive success of *J. curcas* population.

Morphological aspects of germination:

From a morphological point of view, the germination of physic nut starts on the 3rd day, characterized by the protrusion of the radicle, which breaks the integument in the region next to the hilum (Figure 3D-E). The primary root is white, cylindrical, thick, glabrous and branches rapidly, with around 4-5 branches on the3rd day after protrusion. At the same time as the formation of the rooting system, the whitish-green hypocotyl develops (Figure 3F). Soon after this period (6th-7thday), the first secondary roots also start to appear (Figure 3G). The root development process is similar to that observed in *J. elliptica (*Añez et al*.,* 2005), but the average time for radicle emission was less in *J. curcas*. The complete removal of the integument occurs around the 8th day, making the endosperm visible, which totally envelops the cotyledons (Figure 3H). The cotyledons expand between the 10th and 12th day, when the endosperm has almost completely disintegrated (Figure 3I). The normal seedling has a long, greenish hypocotyl, two cotyledonous leaves, a strong main root and many secondary roots. The region of the stem base is characterized by a slight narrowing compared to the diameter of the hypocotyls. On the 12th day, the normal seedling, phanerocotyledonous, is characterized, which is originated from epigeal germination (Figure 2J). As for most species of this family, the seeds have phanerocotyledonous germination, but exceptions to this pattern were seen in *Hevea* sp., *Huracrepitans* L. and *Omphaleadiandra* Vell., which have cryptocotyledonous germination (Oliveira and Pereira, 1987).

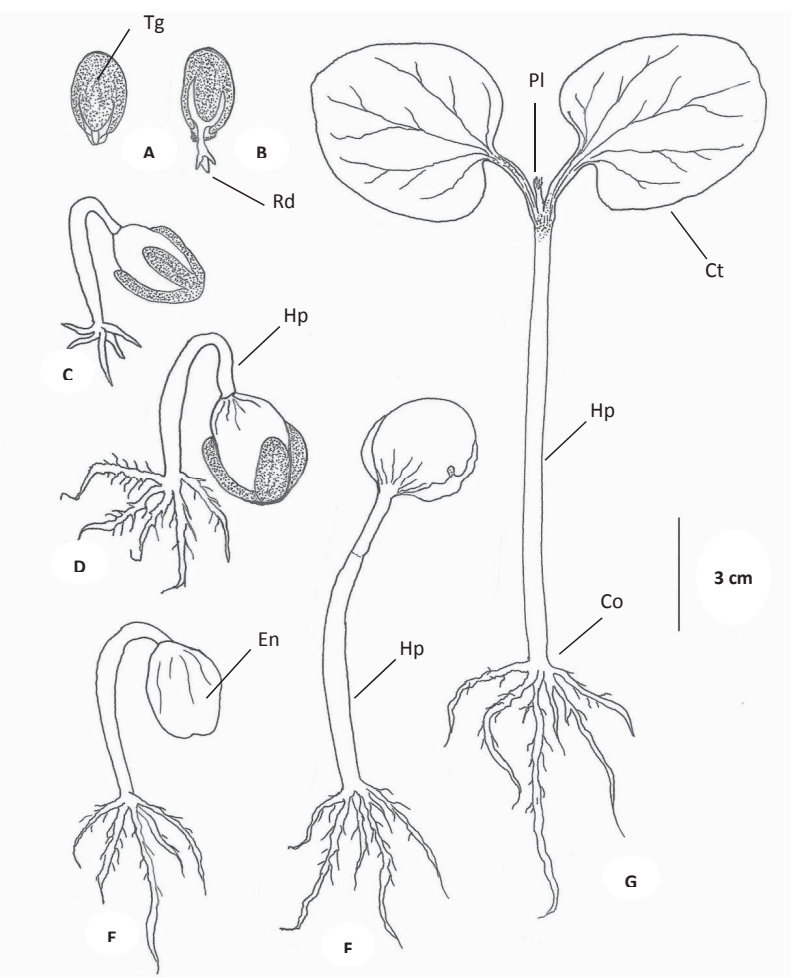

Figure 3. Morphological aspects of germinative process of *Jatropha curcas* L. (Euphorbiaceae). Legend: Co = stem base; Ct = cotyledon; En = endosperm; Hp = hypocotyl; Pl= plumule; Rd = radicle; Rf= rafe. Illustration: B.G. Brasileiro.

Conclusions

J. curcas shows a 12:1 ratio of male to female flowers under the conditions of Viçosa, Minas Gerais state. Hermaphrodite flowers are also present but in far fewer numbers, varying from 0 to 6 flowers per inflorescence.

A description of the floral biology of *J. curcas* provides characteristics and information, which can help in future research with this species.

The germination of *J. curcas* is epigeal and phanerocotyledonous, and the morphology described and illustrated in this study is sufficiently homogeneous and reliable to characterize and identify the germination and the first development stages of this species.

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