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Floral biology and reproductive system of physic nut (*Jatropha curcas* L., Euphorbiaceae) in 'Recôncavo da Bahia', Brazil

LUIS F. DE-FARIAS, SIMONE A. SILVA, LIDYANNE Y.S. AONA & FAVÍZIA F. DE OLIVEIRA

Abstract: Understanding the floral and reproductive biology of botanical species is crucial for the development of strategies in plant breeding systems. *Jatropha curcas* L. is a promising species for the manufacture of biofuels, being previously studied mainly in genetic improvement to develop characteristics suitable to biofuels. In order to contribute with data for hybridization and breeding programs, this paper studies the floral biology and reproductive system in two experimental populations of different ages of *Jatropha curcas* in Cruz das Almas/BA. Both of them were examined about their anthesis, durability, number of flowers, stigma receptivity, P:O ratio, and reproduction tests. As observed, *Jatropha curcas* is a monoecious species and its flowering occurs between September and April. Inflorescences are composed of unisexual flowers with daytime anthesis (♂: 05-06h; ♀: 07-08h), where the staminates last 10h and pistillates 60h. The physic nut is self-compatible, forming fruits by self-fecundation and cross-pollination, although the greatest number of fruits/seeds is generated by natural pollination. Experiment 02, presented a larger number of flowers, probably due to the plant's age and physiology. Performing artificial pollination between 08:00h and 09:30h is recommended for larger production since the stigma is receptive and the flowers have a large amount of pollen available.

Key words: flowering, pollination ecology, geitonogamy, xenogamy, cross-pollination.

INTRODUCTION

The Euphorbiaceae is one of the families with the greatest richness of plant species, there are approximately 6.547 ones (The Plant List 2013), among which are included the physic nut (*Jatropha curcas* L.) and the castor bean (*Ricinus communis* L.). These two species have great potential for the production of biofuels (Neves et al. 2011). Therefore, given that the physic nut is considered one of the most promising plants for biofuels production, it has been extensively researched mainly about genetic improvement, since their oil has excellent quality and presents stability to oxidation, low viscosity, and a low flow point (Sujatha et al. 2005, Bailis & McCarthy 2011). However, cultivating the physic nut may be challenging as it is an undomesticated species. Several Asian and American institutions have sought domestication through genetic improvement (Durães et al. 2011, Silva et al. 2017).

The physic nut can be found throughout the world, but it is believed that the region that comprises Mexico and Central America is the most likely center of origin (Heller 1996, Dias et al. 2012). The studies on this species have been mainly focused on the genetic improvement of the seeds and the oil productivity, usually taking descriptors for this approach (Durães et al. 2009). Notwithstanding, flowering is extremely important for the crop, once seed and oil production depends directly on phenological aspects (Juhász et al. 2009, Nietsche et al. 2014).

In this sense, understanding the floral biology and the reproductive system of this species is an important strategy as it can be crucial for genetic improvement, especially when coupled with the controlled pollination studies (Brasileiro et al. 2012). These studies may help in the development of strategies aimed to increase productivity, for example, in the management of efficient pollinators in the crops, generating a greater number of fruits and consequently, increasing the income of the producers of this crop (Rincón-Rabanales et al. 2016).

There are gaps in the knowledge regarding the floral and reproductive biology of the genus *Jatropha* (Santos et al. 2005, Neves et al. 2011) and its species in Brazil (Juhász et al. 2009, Paiva-Neto et al. 2010). In this way, little is known about the influence of the form of reproduction on the size, weight, and fruits/seeds production in *J. curcas*. Given that, in order to contribute with important data for future hybridization and genetic improvement programs, the objective of this work was to know the characteristics of floral biology and the reproductive system of *J. curcas*, based on collecting data from two experimental populations of different ages in tropical conditions, implanted in the 'Recôncavo' of Bahia, northeastern Brazil.

MATERIALS AND METHODS

Characterization of study area

The present work was carried out between April 2017 and May 2018. There were two experimental populations of *Jatropha curcas* L. set up in the Experimental Field of the Genetic Improvement and Biotechnology Program (Núcleo de Melhoramento Genético e Biotecnologia – NBIO) part of the Center for Agrarian, Environmental, and Biological Sciences (Centro de Ciências Agrárias, Ambientais e Biológicas) of the Universidade of Recôncavo da Bahia (UFRB), located in Cruz das Almas, Bahia (12° 39'44.2 "S and 39° 05' 04.0"W) at an altitude of 220 m.

The topography of the study area is flat, and the soil is classified as Cohesive Argisolic Yellow Latosol. The average annual temperature is 24.2°C. According to Köppen's classification, the climate is tropical, hot, and humid (AW to AM). The average annual precipitation is 1,206 mm and varies between 1,000 and 1,300 mm per year (Melo-Filho et al. 2007). The first population of the study was established in 2014 (Exp. 1, approximately 170 individuals), and the second one in 2017 (Exp. 2, approximately 180 individuals). The experiments are apart by 20m. The temperature, humidity, and rainfall data were obtained from the Inmet (2018) system.

Floral biology

The stigma receptivity tests were performed (n=20 flowers) with the use of 3% hydrogen peroxide every 2 hours, during a specific time set from 6:00h to 18:00h (Dafni, 1992). The quantification of the pollen grains/flower was performed based on ten pre-anthesis flower buds, using a Neubauer's Chamber, and considering the methodology proposed by Maêda (1985) and Zambon et al. (2014). The pollen/ovule ratio (P: O) was estimated according to Cruden's (1977). The inflorescence durability, number of pollen grains per flower/anther, number of ovules per flower, pollen: ovule ratio (P: O), and stigma receptivity were evaluated in individuals of experiment 2 since this population presented greater flowering.

Reproductive system

The reproductive tests were performed in each experiment (Experiments 1 and 2), evaluating 30 flowers per crossing. As treatments, the floral buds were submitted to crosses and bagged (organza bags) until the flower fall or the fruit maturation. Treatments performed were: I) artificial self-pollination (ASP), in which the staminate flowers were removed from the inflorescences (emasculation) and the stigma received pollen grains from three staminate flowers of another inflorescence from the same plant; II) spontaneous self-pollination (SSP), occurs the bagging of the pre-anthesis floral buds, without emasculation; III) artificial cross-pollination (ACP), in which the inflorescences were emasculated and the pollen grains of three staminate flowers from another individual were deposited on the stigma of the flower; IV) apomixis (APO), with the emasculation of the inflorescences and the bagging pistillate flowers in the pre-anthesis (previous day); and V) natural pollination (NAP), that the flower buds were marked and exposed to the pollinators, without manipulation or bagging the flowers. Artificial pollination (ACP, ASP) are the flowers pollinated by hand with pollen from other inflorescences (from the same or different plants - ACP and ASP), and then bagged again to avoid manipulation by visitors.

After the treatments, the yield components obtained for each treatment were: initial fruiting, quantified after 15 days of pollination (IF %), effective fruiting, quantified after fruit collection and drying, performed between 45 and 60 days (EF %), number of seeds per fruit (NSF), seed length (SL), seed weight (SW), and number of fruits/seeds formed (NF/ NS). The fruits and seeds were measured with the aid of a digital caliper and weighed with a precision analytical balance.

The results of the reproductive tests were used to determine the self-incompatibility index (ISI = division of the percentage of effective fruiting of artificial self-pollination by the percentage of effective fruiting of artificial cross-pollination) and the reproductive efficacy index (RE = division of the percentage of effective fruiting of natural pollination by the percentage of effective fruiting of artificial cross-pollination), according to the work by Zapata & Arroyo (1978) and modifications suggested by Freitas & Oliveira (2002). The abortion rate for each reproductive system was also calculated (value obtained for the initial fruiting, subtracted by the result of the effective initial fruiting).

Data analysis

Descriptive data was the chosen method to analyze the results of studies of flowering/ fruiting phenology, anthesis, stigma receptivity, inflorescence/flower durability (pistillate and staminate), floral opening pattern, pollen quantification, and P: O ratio. The number of flowers per inflorescence (total, pistillate and staminate) were evaluated by analysis of variance (ANOVA). The averages of the experiments were compared using the Tukey test with a 5% probability rate in the R software (R Core Team 2018).

The reproductive biology data, which has a binomial character (fallen fruit = 0 and viable fruit = 1), were analyzed by Kruskal-Wallis, because they did not meet the assumptions of variance analysis, and the averages were compared with Dunn's test at 5%. These analyses were evaluated through the PAST software version 3.20 (Hammer et al. 2001).

RESULTS

Floral biology

The physic nut is a perennial species, monoecious, shrubby, and deciduous. Its inflorescences are racemes with unisexual, dichlamydeous, pentamerous flowers and there are five nectariferous glands at the base of the reproductive structures of the pistillate and staminate flowers. The flowering of *Jatropha curcas* occurs practically all year long (September to April) and the greatest number of flowers occurs between November and February (Peak). Flowering was not observed between June and August/17, when there was presented high humidity and low temperatures (Humidity = 83.97%, 86.98%, 81.07%; Temperature = 23.07 °C, 21.17 °C, 22.42 °C, respectively). In May, the inflorescence was observed only at the final stage of senescence (one to five flowers staminate by inflorescence).

The development of the inflorescences started between the end of August and the beginning of September/17, in which the first inflorescences formed were composed only of staminate flowers (September-October/17). This behavior of floristic production may be a strategy of the plant to increase the attraction of bees (its pollinators). This may be related to the high temperatures in sun periods, in which the bees end up leaving to forage earlier, which would increase the pollen flow at the moment when the staminate flowers are open. Furthermore, the occurrence of inflorescences (n= 4) composed only of pistillate flowers was observed during the peak of flowering.

The fruit formation was concomitant with the flowering, starting at the end of October/17 until the middle of May/18. Fruit production was greater in December and March. The development of the fruits began between eight and fifteen days after the pollination of the flowers. The fruit is a schizocarp, with explosive dehiscence, and usually has three seeds. When immature they are green, and as they mature, they acquire a brownish color. The fruit maturation occurred between 42 and 62 days after the anthesis (Average = 50.2 ± 4.53 days).

The staminate and pistillate flowers have visibly distinctly morphologies (Figs. 1a-b). The staminate flowers have ten diadelphous anthers

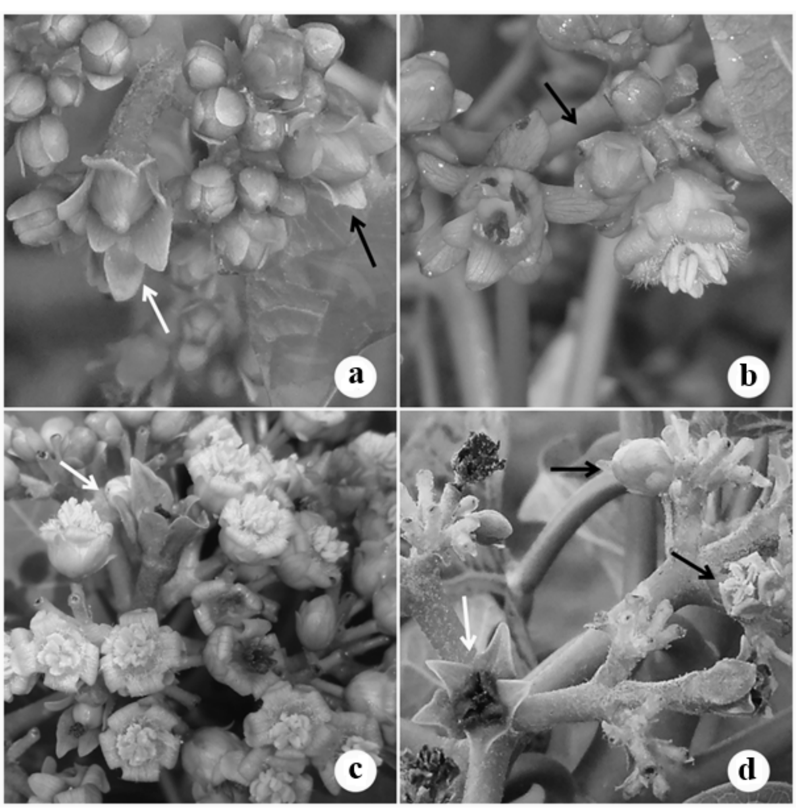

Figure 1. **Durability/Phenology of the inflorescence of *Jatropha curcas* L. (Euphorbiaceae). a) Inflorescence demonstrating sexual differentiation in pre-anthesis; (white arrows: pistillate flowers, black arrows: staminate flowers); b) Inflorescence in anthesis (white arrows: pistillate flowers, black arrows: staminate flowers); (black arrows: staminate flowers); c) Inflorescence with staminate in anthesis and pistillate in fruit formation; (white arrows: pistillate flowers); d) Inflorescence with the opening of the last staminate flower. (white arrows: pistillate flowers, black arrows: staminate flowers).**

(five free lower and five superior united), round shape, and occur in greater number in the inflorescences than the pistillate ones (Fig. 1c), which have three bifid styles, superior ovary, tricarpel, and trilocular. The pistillate flowers (approximately 2 mm long) have a conical shape and usually located in the center of the inflorescences, surrounded by the staminate flowers (Figs. 1a-c). Moreover, the pistillate flowers are larger than the staminate ones (approximately 1 mm long), with longer sepals and larger peduncles.

In the staminate flowers, the anthesis occurred between 05:00 and 06:00h (Figs. 1b, 2a), however, the rimose dehiscence of the anther occurred only between 07:30 and 09:30h (Fig. 2b), characterized by the detachment of the anthers' grains. After 12:00h, flowers started to change their appearance and fall (Fig. 2c), hence it is possible to visualize the scarcity of the pollen grains in the anther until the end of the afternoon, which probably may have occurred due to the intense activity of floral visitors (Figs. 2c-e). Thus, the floral senescence occurred after 16:00h (10h after the opening of the flowers), which is noticeable for the whitish coloration of the anthers (Fig. 2d), and most of the flowers fell after this period (16h). Nevertheless, when the staminate flowers remained in the plant, they had dark yellow anthers (Fig. 2e) with an appearance of the absence of pollen and oxidation.

In the pistillate flowers, the anthesis occurred between 07 and 08:00h (Figs. 1b, 3a), and the stigma presented a green coloration. However, over the days, the stigma gradually lost color/brightness, and its reduced stigma size in relation to the anthesis (Fig. 3c). There was also a color change of the petals from green to brown, thus characterizing the floral senescence

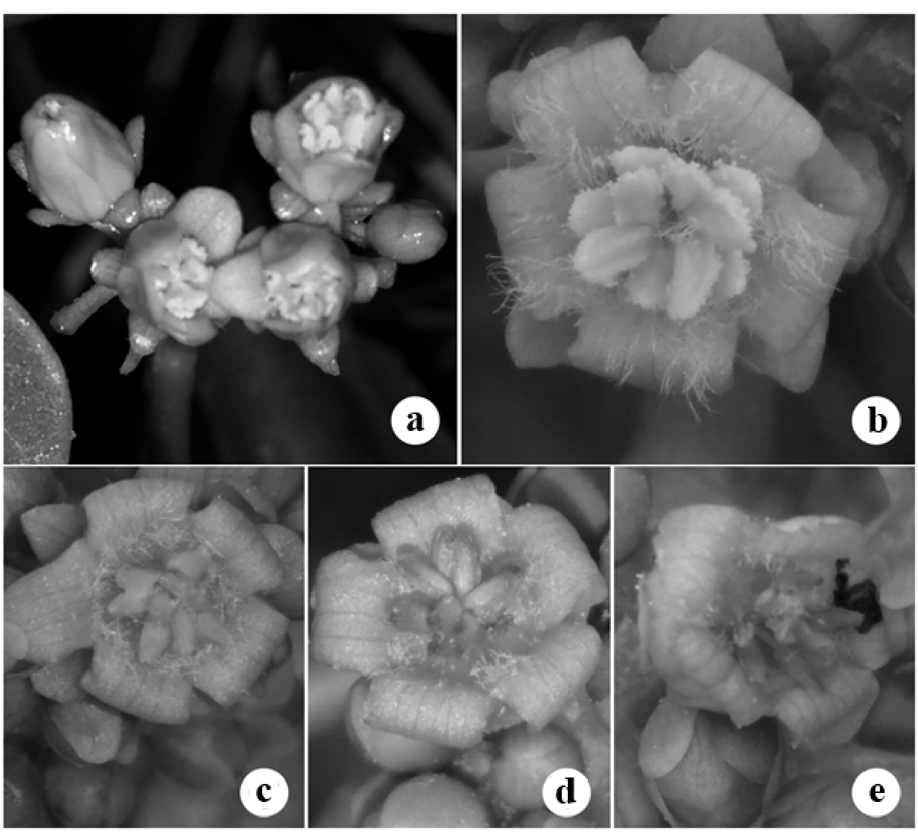

Figure 2. Durability/Phenology of the staminate flower of *Jatropha curcas* L. (Euphorbiaceae). a) Anthesis of the staminate flower (06 h); b) Anther pollen dehiscence (09 h); c) Decrease of pollen amount of the anther in the middle day (12 h); d) Decrease of pollen amount in anther in the late afternoon - senescence (16 h); e) Senescent flower on the second day (08 h).

(Figs. 3d-e). The senescence began on the second day, 48h after the anthesis (Fig. 3d), but most of the pistillate flowers were senescent at the end of the third day, 60h after the anthesis (Fig. 3e). Thus, through morphological data, it was found that the flowers that had the green stigma surface were more viable than those that had the darkest stigma surface. This fact was confirmed by the stigma receptivity test with hydrogen peroxide, through which the greatest reaction was observed in the pre-anthesis flowers (12h before the anthesis) and on the first day of anthesis. There was a receptivity dropped along the days in which at the end of the third day, there was no further stigma reaction.

In the first days, the inflorescences do not have many open flowers. The flower opening peak occurred between the sixth and ninth days. The inflorescences remained between 12 and 20 days in the plant, with average durability of 15.95 ± 2.16 days. Generally, the staminate flowers open first, between one and three days before the pistillate (protandry), although there are inflorescences that occur the opposite way (protogyny). Furthermore, when the protogyny occurs, there was a staminate flower in the same plant or nearby plants, this pattern can be considered a phenological strategy, probably with chemical signaling, to ensure the pollination. Therefore, soon after the opening of the first flowers, there is synchrony of floral types, especially in the peak phase, with staminate and pistillate flowers opened in the same inflorescence (Fig. 1b). However, the staminate peak oftentimes occurred after the senescence of the pistillate flowers (Fig. 1c), and because of the large number of staminate flowers observed in the inflorescences, these are the first to open and are the last to fall (Fig. 1d), this may be a strategy of the plant to ensure the cross-pollination.

In relation to the quantity of flowers, there was a greatest number of total flowers (Exp1: 108.80 ± 28.05 flowers, varying between 58 |-| 164; Exp2: 198.00 ± 35.97 flowers, 132 |-| 282; F=107.2, p= <0.01), staminate flowers (Exp1: 105.87 ± 27.18, varying between 58 |-| 162; Exp2: 190.80 ± 35.78, 129 |-| 268; F=107.1, p= <0.01) and, pistillate flowers (Exp1: 2.83 ± 1.88, ranging from 1 |-| 8; Exp2: 7.27 ± 3.89, 1 |-| 16; F= 31.66, p= <0.01) in

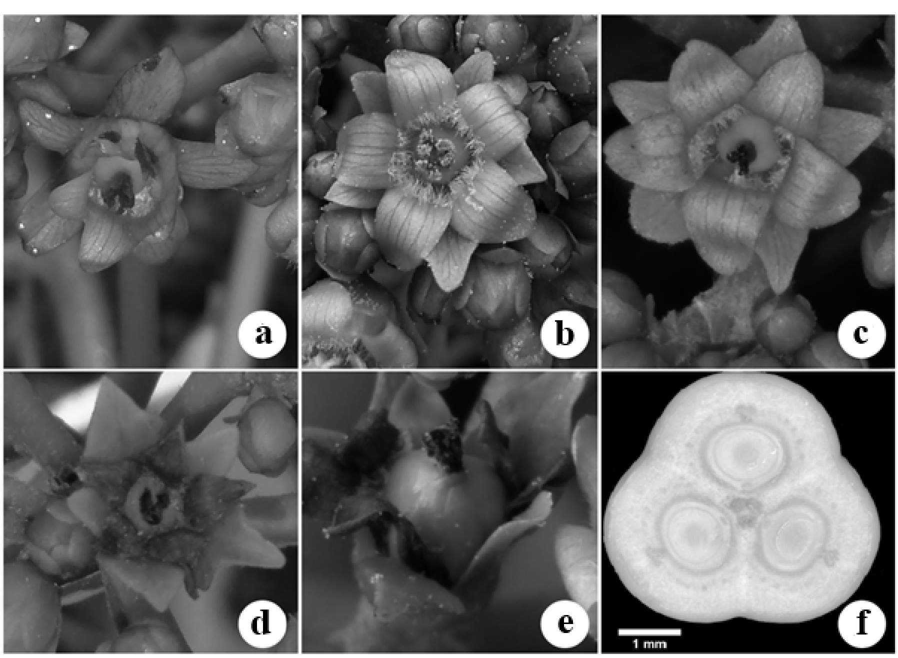

Figure 3. Durability/ Phenology of pistillate flower of the *Jatropha curcas* L. (Euphorbiaceae). a) Flower after anthesis (08 h); b) Flower with pollen on stigma after intense visitation (10h); c) Flower on the second day (one day after the anthesis, 24h); d) Flower on the third day (two days after the anthesis, 48h); e) Flower at the end of the third day - floral senescence (two days after the anthesis, 60h); f) three locules with three ovules (one ovule in each locule).

experiment 2 in relation to experiment 1. This can be related to the difference in age and the physiology of the populations studied (Exp. 1: four years; Exp. 2: one year). Thus, experiment 2 presented a higher number of flowers, it also presented a smaller pistillate/staminate ratio (Exp1: 1:37.41 flowers; Exp2: 1:26.24 flowers).

Reproductive system

There was a statistical difference ($p < 0.05$) between the evaluated reproductive systems for all variables (Table I). Fruit formation was verified in all reproductive systems (except the apomixis in experiment 2), including the artificial pollination (ACP, ASP), thus showing that the plants are self-compatible (Table I). Although this study observed an apomictic fruit formation, it is fair to presume that the seeds generated by this treatment will not germinate and produce healthy seedlings because the seeds formed are smaller and much lighter compared to the seeds of the other treatments evaluated.

Natural pollination (NAP) was the system that obtained better reproductive success and a higher number of seeds per fruit in both experiments. The treatments that obtained the heavier seeds were different among the experiments, and no pattern for weight was observed (Exp.1, NAP; Exp.2, ASP and SSP). The longer seeds were obtained mainly in self-pollination (Exp.1, APA, NAP and SSP; Exp.2, APA and APE). The different results in the reproduction tests between the experiments (NSF, SL, SW) may have occurred due to genetic origin, age, planting form, and plant physiology (Table I). In all treatments there were aborted fruits, however, the highest abortion rate (between 10 and 17%) occurred in artificial pollination (ASP and ACP), probably because they received more manipulation (Table I).

Each staminate flower produced approximately 2.250 grains of pollen, and the pistillate had three ovules (one ovule in each locule), with a pollen/ovule ratio of 791.66 (Fig. 3f). Therefore, according to Cruden's (1977) classification, *Jatropha curcas* is a species that presents facultative allogamy, a fact confirmed by reproductive tests.

Table I. Initial fruiting (IF), effective fruiting (EF), number of seeds per fruit (NSF), seed length (SL), seed weight (SW), and number of fruits/seeds formed (NF/NS) in experimental populations of *Jatropha curcas* L. (Euphorbiaceae), in Cruz das Almas, Bahia.

	RT	IF%	EF %	NSF (n)	SL (mm)	SW (g)	NF/NS (n)
Experiment 1 (four years)	ASP	66,67b	50,00bc	2,13b	16,45a	0,52b	15/32
	SSP	36,67c	33,33c	2,00b	15,74a	0,40c	10/20
	APO	10d	3,33d	3,00*	15,43*	0,16*	1/3
	ACP	73,33ab	60,00b	2,22ab	15,65b	0,43bc	18/40
	NAP	93,33a	90,00a	2,67a	16,20a	0,67a	27/72
Experiment 2 (one year)	ASP	70,00bc	53,33b	1,88b	16,00a	0,62a	16/30
	SSP	53,33c	46,67b	2,36ab	15,85a	0,59ab	14/33
	APO	20,00d	0,00c	-	-	-	0/0
	ACP	80,00ab	70,00b	2,29ab	15,00c	0,49c	21/48
	NAP	100,00a	93,33a	2,61a	15,49b	0,57b	28/73

The averages followed by the same letter do not differ statistically from each other in the column by the Dunn test at 5% probability. Post hoc comparisons were performed for each experiment separately.
Reproductive tests (RT): ACP = Artificial cross-pollination; APO = Apomixis; ASP = Artificial self-pollination; NAP = Natural pollination; SSP = Spontaneous self-pollination.
***Due to APO having generated only one fruit, this treatment was not included in the analysis.**

The index of self-incompatibility (ISI) and reproductive efficiency (RE) were high in both experiments (Exp. 1: ISI = 0.83; RE = 1.5; Exp. 2: ISI = 0.76; RE = 1.33). Hence, according to the criteria of Oliveira & Gibbs (2000), one can conclude that *J. curcas* is self-compatible, with the possibility of reproduction by cross-pollination and self-pollination (ISI). Furthermore, there was no shortage of pollinators in this work because the fruiting of artificial cross-pollination (ACP) did not exceed the rate obtained by natural pollination (NAP), thus showing the importance of pollinators for the reproduction of the species (RE).

DISCUSSION

The physic nut is a plant with annual flowering, where the flowering occurred between September to April and the peak phase occurred between November/17 and February/18. The flowering of *J. curcas* occurs after the winter, the phase when the leaf abscission occurs (Saturnino et al. 2005, Juhász et al. 2009), once this plant is a deciduous shrub (Raju & Ezradanam 2002, Luo et al. 2007b). This deciduous characteristic is considered an evolutionary strategy of the species, once the fall of the leaves leads to energy savings, increasing the chances of plant survival in the colder seasons (Dias et al. 2012). In addition, dormancy occurs in a period of low temperature and high humidity. Henceforth, it is perceived that flowering is influenced by climatic factors, which was also observed by Domiciano et al. (2014) and Nietsche et al. (2014).

In *J. curcas* there are some morphological factors to ensure the reproductive success and pollination mediated by biotic factors such: the highest occurrence of protandry, the synchronization of staminate and pistillate flowers in the plants, spatial separation of the flowers (monoecious plant), a predominance of staminate flowers around the pistillate ones, anthesis in the first hours of the morning (Staminate: 05 - 06 h; Pistillate: 07 - 08 h), stigma receptivity during 60 hours, a long durability of the inflorescence (15.95 ± 2.16 days).

It is also important to emphasize that the highest number of staminates is a strategy for the higher pollen flow in the population, the chance of cross-pollination increases (Neves et al. 2011) and thus genetic variability between plants is also greater. The development of only staminate flowers in September and October can be a process to attract and accustom the pollinators (*Nannotrigona testaceicornis* (Lepeletier, 1836), *Scaptotrigona xanthotrica* Moure, 1950 and *Trigona spinipes* (Fabricius, 1793) to visit the plants of *J. curcas,* causing a slight floral fidelity (L.F. De-Farias et al., unpublished data)). In this sense, Hoeltgebaum et al. (2018), reports that the occurrence of long flowering during the year makes the resources predictable, increasing the number of visitors (mainly bees - Apidae) in search of resources (nectar and pollen) and, consecutively, increase the pollen flux between the plants.

The dioecy, i.e., presence of only staminate flowers in some individuals was also visualized for other *Jatropha* species (Neves et al. 2011). The functional dioecy is when occurs the presence of only one floral type in a monoecious plant species (Cruden 1988). Furthermore, sometimes in *J. curcas,* during the peak phase (November-February) the functional dioecy was observed in certain plants, in which only staminate flowers were found in these plants, while most individuals had staminate and pistillate flowers. Therefore, this process has the function of decreasing the occurrence of geitonogamy and increasing the chances of xenogamy (Cruden 1988).

It is believed that artificial pollination (ASP and ACP) should be performed between 8 and

9 h to obtain higher production of fruits and seeds because this period has high receptivity of stigma and a large number of pollen available, a fact confirmed by previous studies (Luo et al. 2007a, Singh et al. 2010). However, this fact should be studied with more attention, analyzing pollen viability by histochemical tests, germination in culture media, and joint evaluation of the stigma receptivity by hydrogen peroxide and alpha-naphthyl acetate solution.

Additionally, the greatest pollinators activity in the study area occurred in the first hours of the morning, between 6h and 11h (L.F. De-Farias et al., unpublished data), when the daily anthesis of new staminate flowers allows the occurrence of pollination in this species during the morning period. Furthermore, although the stigma being receptive during 60h (from the anthesis to the senescence), the pollination permeated by Meliponini bees (*N. testaceicornis, S. xanthotricha,* and *T. spinipes*) only occurs between 6h and 13h of the first day since there were no visits to the pistillate flowers after this period (L.F. De-Farias et al., unpublished data). Moreover, from 13 h, there were fewer resources in the staminates, possibly due to the intense activity of the visitors, being that similar results were observed by Rincón-Rabanales et al. (2016) and Paiva-Neto et al. (2010).

In this study, the physic nut was considered a facultative allogamy plant (ratio P:O of 791.66) according to Cruden's classification (1977). This result was similar to that obtained by other studies on genus *Jatropha* (Santos et al. 2005, Singh et al. 2010, Kaur et al. 2011, Neves et al. 2011). In this way, the reproduction can occur by self-pollination (Exp.1: 50%; Exp.2: 53.33%) or by cross-pollination (Exp.1: 60%; Exp. 2: 70%), a fact proven by artificial pollinations (ASP and ACP) in *J. curcas* (Table I). Therefore, there is no evidence of a preference for geitonogamy (self-pollination) or xenogamy (cross-pollination) with the species, indicating the occurrence of a mixed reproductive system.

On the other hand, pollination permeated by biotic vectors (natural pollination - NAP) generates higher production (fruits and seeds) and the number of seeds per fruit than other treatments. The importance of pollinators is even more evident when the effective fruiting rate (EF%) of flowers excluded from visitation (SSP - Exp.1: 33.33%; Exp.2: 46.67%) is compared to fruit set of flowers that have received visits freely (NAP - Exp.1: 90%. Exp.2: 93.33%), a fact also observed by Paiva-Neto et al. (2010).

Furthermore, the difference in reproductive rate between natural pollinations (NAP) and artificial pollinations (ASP and ACP) may be related to the number of visits, once natural pollination (NAP) may receive several visits during the day due to the supply of periodic resources from flowers. Thus, in artificial pollination, the plant was pollinated just once which may be one of the factors that influenced this lower reproductive rate. Thus, there was no pollinator shortage in the populations studied because the natural fruiting rate was higher than the artificial cross-pollination (ACP) rate. Moreover, the large number of open flowers may encourage the pollinators to visit more flowers and, consequently, result in a higher rate of cross-pollination by biotic vectors (Cruden 1988).

It is worth mentioning that the occurrence of fruit formation by self-pollination can be important in environments where there is a small rate of pollinators, moreover, due to the plant being monoecious, the presence of vectors is essential for the reproduction of this species. Nevertheless, the fruiting rate of artificial pollinations in *J. curcas* in previous studies showed higher values than the obtained in the present work, with a range between 82 and 96% for artificial cross-pollination (ACP) and between 77 and 90.5% for artificial self-pollination (ASP)

(Raju & Ezradanam 2002, Juhász et al. 2009, Paiva-Neto et al. 2010, Negussie et al. 2014). This may be related to differences in climatic/abiotic factors, soil type, genetic material, age, plant fitness, cultural practices, and planting form (Juhász et al. 2009, Palupi et al. 2014, Rincón-Rabanales et al. 2016).

Although previous studies have reported wind inefficiency as a pollen vector in *Jatropha* (Luo et al. 2007a), average fruiting was found in the treatment in which the inflorescences were isolated (bagged) and emasculated (Exp. 1: 33.33%; Exp. 2: 46.67%). Thus, since *J. curcas* is a monoecious plant, it is believed that pollination in this specific case, must have happened through the wind action, by the movement of the flowers inside the organza bag. Regarding reproduction by apomixis, it has been verified that reproduction without fecundation in the genus is rare, and when it occurs it is low (Santos et al. 2005, Neves et al. 2011), generating infertile light seeds (Rincón-Rabanales et al. 2016), therefore, it is believed that this group of plants cannot form fruits by this mechanism.

The high abortion rate in this study (IF-EF), especially on artificial pollination (ASP, ACP), may have occurred due to greater manipulation in treatments, genetic factors, plant physiology, or even the age of plants (Exp.1: four years; Exp.2: one year). These factors may have influenced the reproduction of the species since the plants in experiment 1 produced the smallest number of total flowers, staminate, and pistillate flowers. However, the abortion of immature flowers and fruits is a common mechanism in angiosperms (Stephenson 1981, Bawa & Webb 1984, Hoeltgebaum et al. 2018).

Moreover, due to the development of seeds and fruits is a process with great energy expenditure, the plant can perform abortion as a low-cost selection strategy (Oliveira & Maruyama 2014), in which one discards ovules fertilized by low aptitude pollen, or due to this plant presenting limited resources for the development of the fruit (Stephenson 1981, Oliveira & Maruyama 2014). Other elements have also been reported in the literature as influencers in the abortion of flowers and fruits, such as the antagonistic action of insects, the genetic divergence of lineages, late-acting self-incompatibility, and age of plants (Kiill & Costa 2003, Silva et al. 2007, Palupi et al. 2014). Given that, the development of future studies to evaluate these factors becomes essential in order to better elucidate the interference in the formation of viable fruits.

Thus, although *J. curcas* is an exotic plant, it has been considered an important species in the recovery of degraded areas, besides being economically promising for use in biofuel manufacture (Saturnino et al. 2005, Durães et al. 2011). Nevertheless, in Brazil, the studies carried out in *J. curcas* have focused on the evaluation of vegetative and reproductive characters (Saturnino et al. 2005, Durães et al. 2009, Almeida et al. 2016). Therefore, the data on the floral and reproductive biology of physic nut become necessary requirements to begin genetic improvement work on this species for commercial exploitation (Kaur et al. 2011), mainly in controlled crossing programs.

In this sense, information on phenology, floral biology, and pollination can assist in the development of artificial-controlled pollination in hybridization and breeding programs. The reproductive systems evaluated here may be used for specific purposes and form new plants with differentiated genetic constitutions, mainly, aiming to increase the genetic base of the species. Additionally, the reproduction techniques carried out may be used to obtain half-sibling populations (NAP), obtaining pure lineages (ASP and SSP), hybrids formation with characters of interest for the diallel system

(ACP). However, if the goal is only to obtain a greater number of seeds and heavier seeds, natural pollination (NAP) is recommended.

In summary, *Jatropha curcas* is characterized for being a perennial, shrubby, deciduous species that present flowering during a great part of the year, except in the months with lower temperatures (July-August). The physic nut has a mixed reproductive system (self-compatible), in which reproduction can occur either by cross-pollination or by self-pollination, with similar seed production. However, the species is monoecious with spatial separation of flowers, therefore the reproduction is dependent on the action of biotic vectors for pollination and seed production. The greatest reproductive success occurs by natural pollination (NAP), mainly by Meliponini bees (*N. testaceicornis, S. xanthotrica,* and *T. spinipes*).

For conducting the artificial pollinations, it is suggested that it be performed the first hours of the morning, especially between 8 and 9:30h, in order to obtain a greater number of fruits, because the staminate and pistillate flowers will be open and with receptive stigma and a large amount of pollen available. There were more flowers on the plants of experiment 02, it is probable that the age and physiology (fitness) of the plants are the main influencers of this result. The results of the phenology (flowering and fruiting phase), floral biology (anthesis, flower durability, anther dehiscence, receptivity), and reproductive system of *J. curcas* (seed production in each system) are valuable information that will contribute to the development of hybridization and genetic improvement programs for this species. Although the studied plant is an exotic species, it constitutes a plant with important economic potential for Brazil, and particularly the Northeastern states.

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LUIS F. DE-FARIAS [1,2,*]
https://orcid.org/0000-0002-9099-8569

SIMONE A. SILVA [1,2]
https://orcid.org/0000-0002-3017-6133

LIDYANNE Y.S. AONA [1,3]
https://orcid.org/0000-0001-8477-5791

FAVÍZIA F. DE OLIVEIRA [4]
https://orcid.org/0000-0003-4366-5005

[1]Universidade Federal do Recôncavo da Bahia, Centro de Ciências Agrárias, Ambientais e Biológicas, Rua Rui Barbosa, 710, Centro, 44380-000 Cruz das Almas, BA, Brazil
[2]Universidade Federal do Recôncavo da Bahia, Núcleo de Melhoramento Genético e Biotecnologia, Rua Rui Barbosa, 710, Centro, 44380-000 Cruz das Almas, BA, Brazil
[3]Universidade Federal do Recôncavo da Bahia, Herbário do Recôncavo da Bahia, Rua Rui Barbosa, 710, Centro, 44380-000 Cruz das Almas, BA, Brazil
[4]Universidade Federal da Bahia, Instituto de Biologia, Laboratório de Bionomia, Biogeografia e Sistemática de Insetos, Rua Barão de Jeremoabo, 668, Ondina, 40170-290 Salvador, BA, Brazil

Correspondence to: **Luis Fernando de Farias**
E-mail: luisfernandodefarias@gmail.com
**Part of the Master's thesis of the first author.*

Author contributions

Designed the study: LFF, SAS, LYSA. Performed the experiments and analyzed the data: LFF. Contributed to reagents, materials, and equipment: SAS, LYSA, FFO. Wrote the article: LFF, SAS, LYSA, FFO.