Scientific Paper

Effect of the reproduction method on the morphological and productive traits of *Jatropha curcas* L.

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Abstract

Objective: To determine the effect of the reproduction methods on the morphological and productive traits of *Jatropha curcas* L.

Materials and Methods: In a randomized block design, planting by seed (T1) and by stake (T2) was evaluated regarding seed emergence, propagule sprouting, seedling survival, number of fruits per raceme, flowering start, height, stem diameter, number of branches, crown projection, quantity of formed racemes, number of fruits per raceme, quantity of seeds per tree, weight of 100 seeds, seed length and width. The data were analyzed through descriptive and inferential statistics for two independent samples, and the means were compared at a significance level of $p \leq 0,05$.

Results: More sprouted propagules (94) than emerged plants (71) were found. With T2, the highest height, stem diameter and number of branches were reached in the nursery. In the establishment, the crown projection was higher for T1. Meanwhile, T2 was more precocious in the beginning of flowering and produced a higher quantity of formed racemes, as well as higher number of fruits per racemes and quantity of seeds per tree. For the other variables no differences were found between the treatments.

Conclusions: Through reproduction by propagules morphologically higher *J. curcas* seedlings are obtained in the nursery stage (higher height, stem diameter and number of branches). In the productive stage higher quantity of fruits and seeds was achieved per tree, which were stable or had a slight increase each year.

Keywords: propagation materials, nursery, establishment

Introduction

Jatropha curcas L. is a plant that belongs to the family Euphorbiaceae (Tsuchimoto, 2017). Since more than a decade ago, in several countries its potential to utilize the oil from its seeds as biofuel is studied (Araiza-Lizarde *et al*., 2016), although its multiple uses in the cosmetic, pharmaceutical and food industries are also acknowledged (Campuzano-Duque *et al*., 2016; Zavala, 2016).

The species is adapted to a large variety of soils, even to those with low nutrient content (Borah *et al.,* 2018). Regarding texture, it prefers the light and well-drained ones, although it can also grow on arid and semiarid soils (Lozano *et al*., 2017).

Its reproduction can occur by seeds as well as by propagules. The sexual way is the most widely used one for the establishment of commercial crops (Zavala *et al.,* 2015), because the plants from seeds are robust and drought-resistant, of higher longevity, with a taproot system, of higher capacity to explore the soil (Evangelista-Lozano *et al.,* 2018) and its seed production is abundant (Pérez-Vázquez *et al*., 2014). Nevertheless, Diédhiou *et al*. (2017) refer that the seed when newly collected shows a germination percentage of approximately 80 % and begins its emergence between 7 and 10 days after seeding (Eras-Chacho and Pintado-Muy, 2018).

The plants from stakes have a shorter life cycle and a fasciculated root system. They are vulnerable to strong winds and tropical storms, but the offspring has the genetic traits of the mother plant and requires less time to reach its maximum production (Mejía *et al.,* 2015).

J. curcas is an allogamous, that is, cross-pollinated, species. Thus, the plants that come from reproduction by seeds do not inherit the same characteristics as the mothers and this can influence their productivity (Díaz-Chuquizuta *et al*., 2017). That is why vegetative propagation is recommended, when the specific attributes of the mother plants are to be preserved or when adult plants are required at short term. In this sense, Mejía *et al.* (2015) determined that the plants from stakes have precocious development and higher fruit production than the ones from seeds.

Regarding the above-explained facts, it is considered that the studies related to the influence of

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the reproduction method on the survival, development and seed production in the species *J. curcas*, are insufficient, important aspect to obtain better yields and stability in the long term, fundamentally because *J. curcas* has a production cycle higher than 20 years (Tavecchio *et al.,* 2016). Considering these arguments, the objective of this study was to determine the effect of reproduction method on the morphological and productive traits of *J. curcas*.

Materials and Methods

Location. The study was conducted in the cooperative of agricultural production (CPA, for its initials in Spanish) Abel Santa María, located between 22°57′08″ North latitude and 80°55′00″ West longitude, at 20 m.a.s.l., in the Martí municipality, belonging to Matanzas province, Cuba.

Soil. It is classified as humic and calcic brown, carbonated, according to the classification proposed by Hernández-Jiménez *et al*. (2015).

Climate conditions. The trial lasted two consecutive years, from 2014 to 2016. Table 1 shows the performance of the climate variables and rainfall, temperature and mean relative humidity in the study period are indicated. These data were obtained in the meteorological station of La Salina Bidos, 2 km away from the above-referred areas.

Treatments. The treatments were made up by the reproduction forms: T1) by seeds and T2) by propagules in the nursery, field establishment and seed production stages.

Experimental procedure. Three stages were considered for the study: nursery, establishment in field and seed production. *J. curcas*, Cape Verde provenance, was used. The plant material was taken from the germplasm bank of the Pastures and Forages Research Station Indio Hatuey. Spot-free, black and preferably shiny seeds, without damage, with a size of 16 mm and weight higher than 600 mg, approximately, were selected. The propagules were from healthy plants, and were selected from the mid part of the branches. They measured between 20 and 30 cm of length, with width between 3 and 4 cm (Pérez-Vázquez *et al*., 2014).

The procedure followed in each stage is described below:

Nursery. In March, 2014, the nursery planting was carried out. Perforated, 20 x 12 cm, black polyethylene bags, were used, in which a substrate composed by 70 % soil (manually sieved) and 30 % organic matter (sugarcane filter cake) was deposited. Sixty bags were planted in each treatment, arranged in randomized blocks (20 bags with three replicas). In each one two seeds or propagules were placed, according to the treatment. Sprinkler irrigation was done in the mornings.

Establishment in field. Before this stage, the soil was prepared with the conventional method (plowing, harrowing, crossing, harrowing and furrowing) and the preceding crop and degree of infestation by weeds were considered.

The plants were taken to the definite site in June, 2014. For planting, holes 20 x 20 cm of width and depth, respectively, were dug in the soil, and thus it was guaranteed that the roots were not affected. The planting distance used was 6 x 3 m (for a density of 555 plants ha^{-1}). All the plants were placed in the field with a complete randomized block design, which was in correspondence with the previous stage. The experimental plots were made up at a rate of 324 m^2 and 20 plants. For the sampling six plants were used per block, which were also measured in the nursery for a total of 18, and were placed at the center of each plot, in an area of 108 m^2.

When the species started the flowering period, it was considered the end of this stage. During this stage *Manihot esculenta* Crantz was planted between the tree rows, in order to make a better utilization of the soil between the rows, in a frame of 0,70 between rows and between plants, at a distance from *J. curcas* of 1,50 m. Afterwards, on several occasions, cultivation works were carried out.

Seed production. It started with the first fructification of the plants. Later, at the end a formation pruning was performed, at 50 cm over the soil surface, to guarantee the emission of productive branches, according to the criterion expressed by Moreira-González *et al.* (2019). Next, they started a period of new regrowths, stem thickening,

Table 1. Performance of the climate variables during the study.

Year	Rainfall, mm	Mean temperature, ^{0}C	Mean relative humidity, %
2014	1 398	24,10	78
2015	1 462	23,86	80
2016	1 198	24,41	79

growth and new branches during eight months. Afterwards, the emergence of flowers and fruits, between December and February, was repeated in 2016 between July and September.

Measurements per stages

Nursery. Thirty days after planting, the seed emergence (SE) or propagule sprouting (PS) was recorded by counting the total detected plants. Afterwards, thinning was carried out and one seedling was left per bag. At 90 days, six plants were randomly selected in each replica (18 per treatment) to measure height (H), stem diameter (SD) and number of branches (NB). Then, they were all taken to the field. At the moment of transplant they showed a height of 0,70-0,80 m approximately, according to the recommendation by Pérez-Vázquez *et al*. (2014).

Establishment in the field. Thirty days after transplant, plant survival (SP) was measured. In addition, the crown projection (CP), height, stem diameter and number of primary branches, were evaluated every three months. The month in which flowering started (FS) for each treatment was also considered.

Seed production. Three harvests were taken into consideration, defined in three moments: harvest 1) December, 2014, to February, 2015, harvest 2) December, 2015, to February, 2016, and harvest 3) August to October, 2016. The variables quantity of formed racemes (QFR), number of fruits per raceme (NFR), quantity of seeds per tree (QST), weight of 100 seeds (WS), seed length (SL) and width (SW), were measured.

Mathematical analysis. For the interpretation of the results the maximum value obtained in each treatment for the SE, PS, SP and NFR. For the FS, the month of appearance of the first flowers was determined, for which descriptive statistics was used. In the other variables inference analysis for two independent samples was applied from a t-test, with significance level of 0,05. The statistical package Infostat®, free version, was used.

Results and Discussion

Table 2 shows the morphological variables evaluated during the nursery stage. There were more sprouted propagules (94) than plants that emerged from the seeds (71), aspect that is corroborated in the study conducted by Mejía *et al.* (2015).

It is known that this species can be propagated vegetatively and by seeds (Dasumiati *et al.,* 2018). However, when stakes are used higher production precocity is obtained and the traits of the mother plant are reproduced with higher fidelity. In addition, the plants established from seeds show higher genetic variability with regards to the mother plant. They are vigorous, but start production at a later time.

It is important to state that with none of the evaluated treatments 100 % of emerged or sprouted seedlings was obtained. In the case of the seeded ones, this could be explained by seed quality, because the nursery conditions were adequate, in terms of humidity and radiation. Regarding the plants propagated by stakes, it is assumed that they should be determined by the reserve present in each one to start the development process of a new plant. That is why different hormonal or rooting treatments which are inoculated in the propagules are frequently used (Hamilton-de-Souza *et al*., 2016).

Zavala *et al.* (2015) consider that the main way of establishing plantations is through botanical seed. These authors stated that to ensure germination and the development of vigorous plants the seed must have high physiological quality. In addition, they indicate that its germination capacity is also affected by the high oil quantity, for which it is advisable not to store it for a long time after harvest, in order to prevent it from passing to dormancy state (Díaz-Chuquizuta *et al*., 2017). Méndez-López *et al*. (2020) reported that seed quality comprises genetic, phytosanitary, physical and physiological aspects, and that inherent attributes can be defined which determine its germination potential and growth traits.

When using propagules as reproduction method (treatment 2) the highest values were reached in terms of plant height (H), stem diameter (SD) and number of branches (NB). This treatment differed significantly from 1 (P < 0,05). The favorable

Table 1. Performance of the climate variables during the study.

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performance since early ages of the plant, like the nursery stage, is highly important from the functional point of view. Similar results to these ones were found by López-García (2011).

The values obtained in favor of the reproduction by propagules in each of the morphological variables indicate that this method was more efficacious for the plant to reach adequate development in the nursery stage, in a period of 90 days.

When transplanting the seedlings to the field, 100 % of survival was achieved for each one of the treatments (figure 1). They did not differ statistically between them, which indicates that the seedlings were rapidly recovered from the initial stress caused by the transplant, and restarted their growth and development. This performance could have been given by the adequate management during this stage, and also by the favorable environmental conditions present during this period, because the rainfall and temperatures were in the optimum range indicated by literature for the development of this species (Laviola *et al*., 2017), and the agricultural works conducted in the associated crop (cassava) favored the tree.

López-García (2011) reported contrasting results, when studying reproduction by stakes in *J. curcas*. This author obtained 67,36 % of plants that survived, lower values than the ones in this study.

Table 3 shows the morphological indicators of *J. curcas* during the establishment in the field. The crown projection was higher in T1, and significantly differed from T2 ($p < 0,05$).

The tree crown is the area of the photosynthetic current where the solar energy is absorbed and utilized. Its main function is to sustain the photosynthetic tissue, and besides to provide regrowths (leaves and branches) for the continuity of this activity. Thus, it should be expected that trees with large crowns grow faster than others of the same species with smaller crowns (Bender *et al.,* 2015). In studies conducted by these authors it was proven that stem diameter, plant height and stem volume were highly correlated to the crown width. These aspects could explain the performance observed in

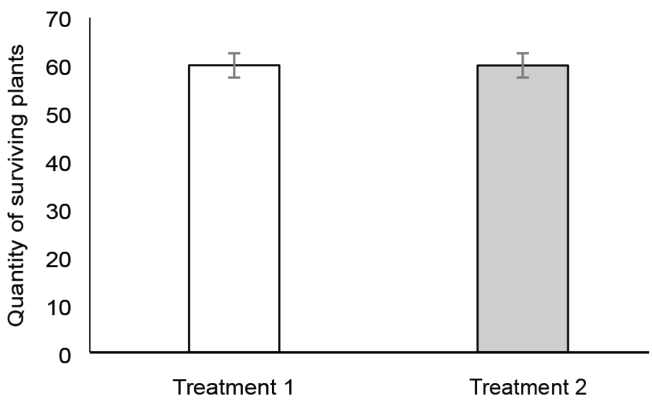

Figure 1. Quantity of surviving plants per treatment after transplant. SE: ± 2,54

Table 2. Performance of the morphological variables evaluated in the nursery stage.

Indicator	Treatment		SE ±	P - value
	Seed	Propagule		
Seed emergence	71	-	-	-
Seedling sprouting	-	94	-	-
Height, cm	70,4	84,1	1,204	<0,0001
Stem diameter, cm	0,5	0,6	0,025	0,0158
Number of branches	2	4	0,255	<0,0001

this study. It is deduced that the plants from seeds had an accelerated growth after the transplant in the field. This can be asserted because no significant differences were found between the treatments for the other variables (H, SD and NB) in this stage, which indicates the efficiency of solar capture and photosynthetic activity provided by the crown for the development of such morphological indicators.

In contrast, Machado (2011) in an evaluation of different *J. curcas* provenances from seeding or planting, determined that in those from stakes height was higher (between 115 and 184 cm) after one year of being established. In this regard, Mejía *et al.* (2015) referred that 56 % of the plants, the seeded as well as the planted ones, showed different heights at 180 days since establishment.

Regarding the beginning of *J. curcas* flowering, it was corroborated that the plants from propagules were more precocious than those in which the seeds were used for sowing. The time passed between transplant and beginning of flowering was 120 days approximately, and coincided with one of the propitious periods in Cuba for the development of this stage in *J. curcas*, which occurs twice a year. The first one takes place from May to September, stage that was not evaluated in this study because the transplant was carried out in June, and the second occurs from October to February, at the end and the beginning of the year, respectively (Toral *et al*., 2008).

The fast response of the plants from propagules could have been given by the accumulated reserve, because they are part of adult plants, and this propitiates the early development of inflorescences (Laviola *et al.,* 2018).

Table 4 shows the productive variables of *J. curcas* in each harvest. For the quantity of formed racemes (QFR) the highest mean was obtained with treatment 2 in each of the harvests that were done, which differed significantly from treatment 1 (p < 0,05). This could have been given because the plants from vegetative reproduction started flowering earlier, which could have propitiated higher pollination activity by certain insects or by climate incidence. Nevertheless, other studies are required to assert these arguments.

Table 3. Quantity of the morphological indicators of *J. curcas*, according to the propagation method during the stage of establishment in field.

Indicator	Treatment		SE ±	P - value
	Seed	Propagule		
Crown projection, m	0,5	0,4	0,021	0,0083
Height, m	1,2	1,2	0,311	0,2874
Stem diameter, cm	0,8	0,9	0,122	0,0630
Number of branches	8	10	1,811	0,3110

Table 4. Productive variables of *J. curcas* at different harvest moments.

Variable	Harvest	Treatment		SE ±	P - value
		Seed	Propagule		
Quantity of formed racemes	1	11	16	2,412	0,032
	2	15	18	1,571	0,005
	3	14	20	2,94	0,041
Number of fruits per raceme (Range)	1	1-6	1-6	-	
	2	1-9	1-9	-	
	3	1-8	1-8	-	
Quantity of seeds per tree	1	186	274	11,263	<0,0001
	2	354	480	14,530	<0,0001
	3	246	475	12,712	<0,0001

Harvest 1: December, 2014, to February, 2015; Harvest 2: December, 2015, to February, 2016; Harvest 3: August to October, 2016

These results are in agreement with the ones reported by Lima *et al*. (2006), who when evaluating several multiplication forms determined that the propagation by stakes produced more fruits and was more precocious. In addition, these authors stated that such performance is given by the presence of reproductive buds in the propagule, which are easily activated.

The number of fruits per raceme (NFR) varied for each treatment according to the harvest moment, and reached maximum values of 6 and 8 for T1, and 6 and 9 for T2. Similar results were obtained by Machado and Brunet (2014), when evaluating the morphological performance of different *J. curcas* provenances, among them Cape Verde.

Regarding the quantity of seeds per tree (QST), treatment 2 obtained the highest mean and differed significantly from T1 for each of the harvests that were evaluated. Thus, 274, 480 and 475 seeds/tree were achieved in harvest 1, 2 and 3, respectively. Also Machado (2011) and Mejía *et al*. (2015) determined higher seed production in plants that came from vegetative reproduction.

In these results higher uniformity and stability in the values found for the plants from stakes is required, aspect that was also indicated by Chakrabarty *et al*. (2019). These authors consider that the plantations established by seeds are more heterogeneous (for their condition of allogamous plant) than the ones sown by stakes, regarding the aspects related to the phenotypic and genetic characteristics (plant uniformity, stage and flowering type, vegetative vigor, grain and oil yield).

For the seed weight, length and width no significant differences were found between the treatments (table 5). The recorded values in each variable were in the ranges reported by Machado (2011), when evaluating different provenances reproduced by stakes or by seeds. This variable was estimated and the weight of one hundred seeds was a highly important component.

In general, it is considered that asexual reproduction is an efficient method to produce *J. curcas*. Through it obtaining plants genetically equal to the mothers is possible. In addition, the development and growth of the crop is guaranteed, which is precociously reproduced with good production and stability in fruit emission.

It is concluded that through the reproduction by propagules, morphologically higher *J. curcas* seedlings are obtained in the nursery stage (higher height, stem diameter and number of branches). In the productive stage higher quantity of fruits and seeds, which were stable or high a slight increase every year, is achieved per tree.

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Authors' contribution

- Yolai Noda-Leyva. Conceptualization of the idea, data taking, preparation of the manuscript for its publication.

Table 5. *J. curcas* seed weight, length and width at the different harvest moments.

Variable	Harvest	Treatment		SE ±	P - value
		Seed	Propagule		
Seed weight	1	81,4	79,3	4,692	0,0634
	2	74,9	75,2	5,022	0,7510
	3	84,3	81,5	7,144	0,3913
Seed length	1	1,85	1,79	0,081	0,0925
	2	1,89	1,84	0,160	0,1211
	3	1,86	1,82	0,112	0,1184
Seed width	1	1,09	1,11	0,121	0,1015
	2	1,04	1,06	0,093	0,0914
	3	1,08	1,08	0,102	0,1212

Harvest 1: December, 2014, to February, 2015; Harvest 2: December, 2015, to February, 2016; Harvest 3: August to October, 2016

- Giraldo Jesús Martín-Martín. Conceptualization of the research idea.
- Rey Leovigildo Machado-Castro. Research supervision.
- Julio Brunet-Zulueta. Conduction of the experiments and data collection.
- Héctor Santana-Armas. Conduction of the experiments and data collection.

Conflict of interests

There is no conflict of interests among the authors.

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