Acta Iguazu
ISSN: 2316-4093

Growth, oil and protein yield of *Jatropha curcas* L. populations

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Artigo enviado em 21/01/2021, aceito em 03/03/2021.

Abstract: *Jatropha curcas* L. is an oilseed with multiple applications, especially the pharmaceutical-industrial one. Cultivation is still very low due to the lack of commercial varieties, therefore, with the need to start the genetic improvement program and select superior genotypes, it is essential to know the genetic variability of the culture. Thus, the purpose of this study was to evaluate the vegetative growth, grain yield and oil and protein content in seeds populations of *Jatropha curcas*, to support the species breeding program. The experiment was carried out at the University Federal de Viçosa, State of Minas Gerais, Brazil, using a randomized complete block split-plot design with four replications and 4-plant plots, spaced at 2.5 m. Each plot consisted of clones of six populations coming from the towns of Janaúba (J1, J2, J3, J4 and J5) and Bonfim (B1), located in Minas Gerais. The 4.5-year-old populations were evaluated for plant height, stem diameter, leaf and fruit dry matter, grain yield and oil and protein content. The populations presented different plant heights (2.97 m), stem diameters (13.49 cm) and leaf dry matter (0.818 g) amounts. The J5 population showed taller plants (3.29 m) with longer stem diameter (15.34 cm). The greatest dry leaf weight (0.864 g leaf^{-1}) was observed in the J2 population. The populations did not present genetic variability for fruit dry matter, grain yield and oil and protein content. Overall, the J5 population stood out with the highest levels of growth, grain yield and seed oil (31.7%) content.

Keywords: Genetic improvement, Grain and oil production, Biodiesel.

Crescimento e produção de óleo e proteína de populações de *Jatropha curcas* L.

Resumo: *Jatropha curcas* L. é uma oleaginosa com múltiplas aplicações, destacando-se a fármaco-industrial. O cultivo ainda é bem reduzido em virtude de não haver variedades comerciais, portanto, com a necessidade de iniciar o programa de melhoramento genético e selecionar genótipos superiores é imprescindível o conhecimento da variabilidade genética da cultura. Sendo assim, o objetivo deste estudo foi avaliar o crescimento vegetativo, produção de grãos e o teor de óleo e proteína nas sementes de populações de pinhão manso para apoiar o programa de melhoramento genético da espécie. O experimento foi conduzido na Universidade Federal de Viçosa, Estado de Minas Gerais, Brasil, utilizando um desenho de parcelas subdivididas em blocos casualizados com quatro repetições e parcelas de 4 plantas, no espaçamento de 2,5 m. Cada parcela foi constituída por clones de seis populações provenientes dos municípios de Janaúba (J1, J2, J3, J4 e J5) e Bonfim (B1), localizada em Minas Gerais. As populações de 4,5 anos de idade, foram avaliadas quanto à altura da planta, diâmetro do caule, folhas e frutos de matéria seca, produção de grãos e teor de óleo e proteína. As populações apresentaram diferentes alturas de plantas (2,97 m), diâmetro do caule (13,49 cm) e folha de matéria seca (0,818 g) valores. A população J5 mostrou plantas mais altas (3,29 m) com diâmetro caulinar (15,34 cm). O maior peso da folha seca (0,864 g folha^{-1}) foi observada na população J2. As populações não apresentaram variabilidade genética para a matéria seca, a produção de frutos secos de grãos e de óleo e teor de proteína. No geral, a população J5 destacou-se com os mais altos níveis de crescimento, rendimento de grãos e óleo de semente (31,7%) de conteúdo.

Palavras-chave: Melhoramento genético, Produção de grãos e óleo, Biodiesel.

Introduction

Jatropha curcas L., also popularly known as e physic nut, is a perennial oilseed belonging to the euphorbia family. Rapid growth, reaching up to 5 meters in height and trunk diameter of approximately 20 cm. Grain productivity increased gradually with the age of planting, starting in the first year of cultivation with 200 kg/ha with the potential to produce 6,000 kg / ha in the sixth year (Dias et al. 2007). Regarding the oil and protein contents, the grains present about 55 to 58% of oil and between 31 and 35% of protein, according to Virgens et al. (2017) and Machado e Silva (2019), as mentioned by Silva et al. (2020).

Jatropha is a multifunctional crop, as the plant is used as a hedge and in recovery of degraded areas, while the oil contained in the grains is used in the manufacture of soap, paints and as a raw material for the production of medicines and biofuel (Virgens et al., 2017). The pie obtained from the pressing of the grains is an excellent organic fertilizer, rich in nitrogen, phosphorus and potassium, and since it has a high protein content, the pie can be used in animal feed after detoxification (Laviola, 2019; Richetti and Santos, 2019). Briquettes obtained from the stem and/or peel of the fruits, can be used as thermal energy for burning in industrial boilers, bakery and pizzeria ovens, and in fireplaces and residential heaters, according to Dias et al. (2012) and cited by Rocha (2019).

Originally from Central America, Jatropha was introduced to the African and Asian continents by the Portuguese after verifying its medicinal potential (Virgens et al., 2017; Laviola et al., 2019). It is abundantly found vegetating in tropical regions which, due to its genetic variability, adapts well to different climatic conditions.

Bearing in mind that Jatropha still has no commercial variety, genetic variability is the main tool used to start a genetic improvement program, since it will be possible to select genotypes with characters of agronomic interest (Santos et al., 2016; Cardoso et al., 2018; Laviola et al., 2019; Tomaz et al., 2020).

With information about the genetic variability of the germplasm bank, according to Laviola et al. (2019), the next step is the formation of the breeding population, which in turn consists of combinations of breeding, genetic design of the crosses to obtain the progenies and selection methods to identify the superior genotics.

The phenotypic characterization of the jatropha culture has revealed the existence of genetic varibability for several traits of interest to breeding programs, such as: grain production and weight, size, oil content, plant architecture, resistance to powdery mildew, absence of forbol ester (Laviola, 2019). In addition to these, studies also reveal the existence of genetic varibality for the characters related to plant morphology, germination and growth (Freitas et al., 2011; Cardoso et al., 2018; Pinto et al., 2018).

Therefore, the objective of this study was to evaluate vegetative growth, grain yield and oil and protein content in populations of *J. curcas*, in order to subsidize the species improvement program.

Material and methods

Experiment location and climate and soil characteristics

The experiment was conducted on 4.5-year-old *Jatropha curcas* plants cultivated in 2.5x2.5 m spaces at the Federal University of Viçosa (UFV) (lat 20° 45' 58" S, long 42° 52' 06" W and 676m alt), in Viçosa, Minas Gerais state, Brazil. The soil of the area was classified as Oxisol according to Embrapa (2018) soil classification. According to Köppen classification, the climate type is Cwa, hot and humid and characterized by cold dry winters, with lowest temperatures below 10 °C. The temperature during the experiment period ranged from 15.5 to 33.4 °C, and the accumulated rainfall was 844.5 mm (Figure 1).

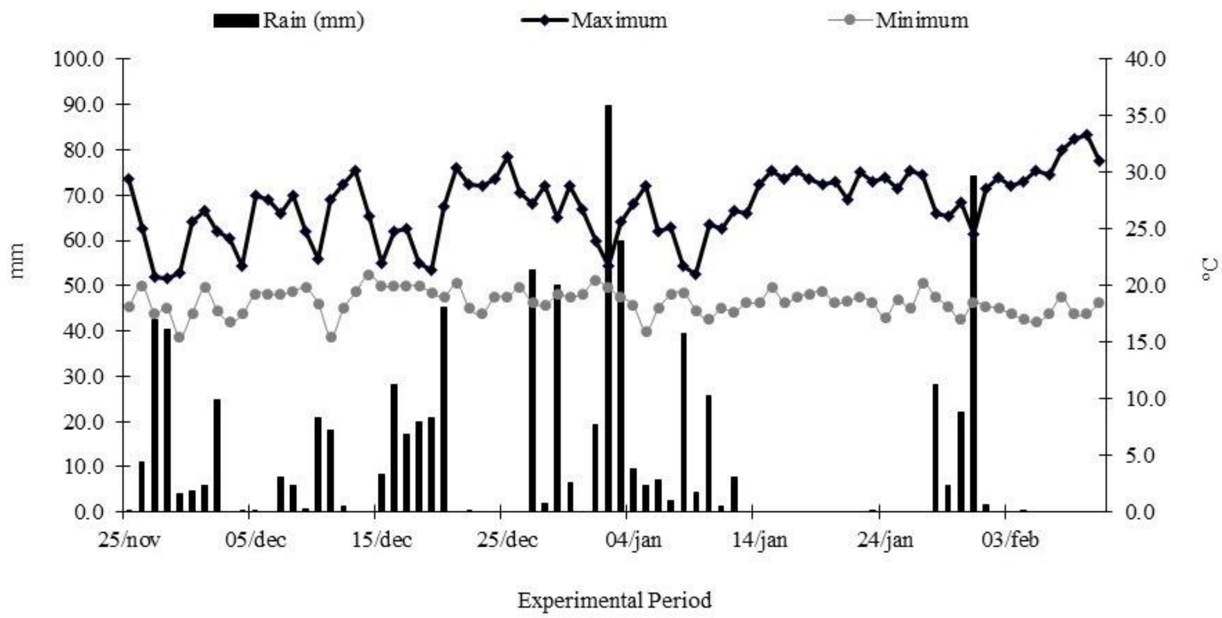

Figure 1. Meteorological data on precipitation (mm) and maximum and minimum temperature (°C) during the experiment period at Viçosa Federal University, Viçosa, MG.

The chemical fertilization was carried out according to Dias et al. (2007) by applying 400 g plant^{-1} of NPK, 20-10-15 formula in the beginning of the experiment cycle. The experiment was conducted under rain-fed system and protected against weeds, pests and diseases. Before data recording, soil samples were collected from the 0-20 and 20-40 cm layers for chemical analysis (Table 1).

Table 1. Chemical analysis of soil from the experiment area at 0-20 and 20-40 cm layers.

Chemical Characteristics	Layer (cm)	
	0–20	20–40
pH (H_2O)	4.50	4.56
OM (dag kg^{-1})	2.93	2.00
P (mg dm^{-3})	2.6	0.9
K (mg dm^{-3})	37	25
Ca^{2+} (cmol$_c$ dm^{-3})	0.99	0.97
Mg^{2+} (cmol$_c$ dm^{-3})	0.37	0.37
Al^{3+} (cmol$_c$ dm^{-3})	0.59	0.49
H + Al (cmol$_c$ dm^{-3})	6.9	5.5
BS (cmol$_c$ dm^{-3})	1.45	1.40
CTC$_{effective}$ (cmol$_c$ dm^{-3})	2.04	1.89
CTC$_{total}$ cmol$_c$ dm^{-3}	8.35	6.90
V (%)	17.4	20.3
m (%)	28.9	25.9
Zn (mg dm^{-3})	1.39	1.14
Fe (mg dm^{-3})	47.2	42.6
Mn (mg dm^{-3})	17.0	12.4
Cu (mg dm^{-3})	1.98	2.14
B (mg dm^{-3})	0.25	0.27
S (mg dm^{-3})	22.4	21.7

pH in water. OM: Organic Matter = C.org x 1.724 – Walkley – Black. P, K, Zn, Fe, Mn, Cu: Mehlich^{-1} extractor. Ca^{2+}, Mg^{2+} and Al3+: KCl 1 mol L^{-1} extractor. H + Al: 0.5 mol L^{-1} calcium acetate extractor. BS: bases sum (Ca^{2+} + Mg^{2+} + K^{+}). CTCeffective = BS + Al^{3+}. CTCtotal = BS + (H + Al). V: base saturation: (BS/CTCtotal) × 100. m: Al. B saturation index (Hot water); S (NH_4O Acc. 0.5 mol L^{-1} and HO Acc. 0.25 mol L^{-1}).

Evaluated traits

Plant height, stem diameter, leaf and fruit dry matter, grain yield and oil and protein contents were determined in six *Jatropha curcas* populations. The plant height was determined by measuring the length from the ground to the highest branch apex, whereas the stem diameter was obtained by measuring the plant structure at approximately 5 cm from the ground with a caliper. The same values were determined for all the plants in the plot. The leaves were collected during the crop cycle, starting at flowering and ending when the fruits were dry. The fruits were harvested when they were unripe, ripe and dry. Leaf and fruit sampling was accomplished by collecting them in the upper, middle and lower sections of plants.

As soon as these vegetative structures were collected, they were taken to UFV Agroenergy Laboratory, rinsed in deionized water and forced dried in an oven at 70 °C, until they reached constant weight. Next, the samples were weighed for dry weight. As for the leaves, fully expanded limbs were collected between the sixth and eighth leaves below the inflorescence or infructescence, amounting to 48 leaves from each plant in the plot. Only leaves with no apparent nutritional deficiency and /or pest attack and diseases were collected. Fruit collection too was carried out in different sections of the plant, though in smaller quantity, 18 fruits from each plant stratum in the plot. Also, these organs were collected with no visible signs of nutritional disorder, pest attack and/or diseases.

In order to measure productivity, all ripe and dry fruits from all the plants in the plot were harvested. After threshed, the grains were weighed, and exceeded the amount usually obtained in 1 hectare.

The grain oil content was obtained through magnetic nuclear resonance imaging, read on an Oxford Instruments device. For this measurement, a block of grains was first heated at 40 °C, and then the samples were put in the test tubes and weighed. Each tube bears a magnetic ray range, and its function is to indicate the amount of grains used in the analysis. On average, 100.5 g grains were used for each sample, totaling 5 g per sample. A previously calibrated instrument recorded the weight, which was then inserted into the reader, and after 20 seconds, the oil content was determined in percentage (%).

The seed protein contents were obtained by reading the measurements on a near-infrared spectrophotometer (FT-NIR). The sample preparation methodology was the same as that for the oil content resonance, except that the reading was done with the test tube placed over the infrared ray-emitting lens. The FT-NIR, too, needs previous calibration for the analysis. For both the oil and protein quantification, grains of six fruits from each plot stratum were used.

Experimental arrangement

The randomized complete block design was used with six populations of *Jatropha curcas* clones from the Minas Gerais state towns of Janaúba (J1, J2, J3, J4 and J5) and Bonfim (B1). Four replications were accomplished on 4 per plant plots, totaling 96 plants.

Statistical analyses

The data obtained in the evaluations were submitted to variance analysis, after normality and variance homoscedasticity tests. As mathematical expectation from mean squares for adjustment of F testers, the fixed model was used. Treatment averages, in turn, were compared through Tukey test at 5% probability.

Results and discussion

Plant height and stem diameter

The populations showed significant differences in plant height and stem diameter (Table 2). In terms of height, the J5 population had the highest average (3.29 m), whereas populations B1 and J4 obtained the lowest ones (2.70 and 2.83 m, respectively). Studies carried out in several parts of the world, evaluating samples from different sources, showed variability in plant height and stem diameter. Gohil and Pandya (2008), while evaluating the vegetative growth of nine genetically diverse 1.5-year-old Indian samples, found differences of over 22% in plant height and 27% in stem diameter. In another study carried out in India, Sunil et al. (2011) recorded plant height ranging from 91 to 225.3 cm and stem diameter, from 3.72 to 10.72 cm. In this experiment, 34 two-year-old samples from different regions of India were analyzed. When analyzing the vegetative growth of 34 samples in India, though in plants aged one and two years old, Saikia et al. (2009) found that in the first year, the plant height varied from 25-119 cm and stem diameter, from 0.38-3.14 cm. In the second year this range was much broader, 72-280 cm of height and 1.75-8.59 cm of diameter. In Brazil, Freitas et al. (2011) also observed variation among 75 eight-month-old samples as to plant height and stem diameter. The average values ranged from 5 to 105 cm in height and from 0.30 to 5.87 cm in diameter. Drumond et al. (2009) observed differences in plant height and stem diameter between 10 to 12-month-old populations analyzed. The averages ranged from 2.4 to 2.7 m in height and 5.3 to 6.3 cm in diameter. The differences in plant height and stem diameter between the Janaúba and Bomfim populations were attributed to genetic differences.

Table 2. Means of plant height (H), stem diameter (SD), leaf and fruit dry matter (LDM and FDM, respectively), grain yield (GY) and oil (OC) and (PC) protein content of six populations of *Jatropha curcas* L.

Population	H (m)	SD (cm)	LDM (g leaf^{-1})	FDM (g fruit^{-1})	GY (kg ha^{-1})	OC (%)	P C (%)
J1	3.07 ab	14.57 ab	0.751 b	1.952 a	100.54 a	31.25 a	20.38 a
J2	2.93 ab	13.46 ab	0.864 a	2.013 a	148.99 a	31.05 a	20.49 a
J3	2.99 ab	12.55 ab	0.821 ab	2.025 a	132.72 a	31.57 a	20.88 a
J4	2.83 b	12.09 b	0.814 ab	1.952 a	122.65 a	31.32 a	21.16 a
J5	3.29 a	15.34 a	0.836 ab	2.083 a	173.20 a	31.69 a	20.22 a
B1	2.70 b	12.95 ab	0.822 ab	2.027 a	151.22 a	31.42 a	21.17 a
Mean	2.97	13.49	0.818	2.008	138.22	31.38	20.71
CVe (%)	11.46	20.22	15.72	18.32	41.71	4.87	9.44
CVf (%)	6.85	9.21	4.56	2.49	18.30	0.73	1.97

CVe % = Experiment coefficient of variation. CVf % = Phenotypic coefficient of variation. Means followed by the same letter in the column do not differ among themselves according to Tukey test at 5% probability.

Leaf and Fruit dry matter

Only the leaf dry matter presented differences between populations (Table 2). Despite not differing from the other populations, the ones that presented greater and smaller means were J2 (0.864 g leaf^{-1}) and J1 (0.751 g leaf^{-1}). The amount of leaf biomass produced is an important parameter because it allows for evaluation of the nutritional (Santos et al., 2017), hydric (Silva et al., 2014) and physiological status of the culture (Santos et al., 2018).

As for fruit dry matter, though no significant effect was observed in the studied populations (Table 2), the values approached those found by Cremonez et al. (2017), who obtained fruits with 2.24 g of dry matter, whereas in this study the average dry weight was 2.00 g fruit^{-1}.

Grain yield

The evaluated populations did not differ statistically from one another (Table 2), and all of them produced much less than expected. The low temperature in the region and a less intensive management in terms of fertilization and liming in the previous years may explain this low grain yield. The ideal temperature range for the development of *J. curcas* lies between 18 and 28 °C.

Drumond et al. (2009) also found statistically similar grain yield when evaluating 10 *J. curcas* populations in Brazil. However, these researchers obtained much higher yields, between 2,853 and 3,542 kg ha^{-1}. This high grain yield was explained by the fact that the plants had weekly dripping irrigation, with an average application of 3.3 mm of water per plant and, in the four hottest months of the year, with two weekly applications. Another factor that also increased productivity was the planting location, as this was done in a semiarid region of Pernambuco state characterized by high light rate and optimum temperature for the growth and development of *J. curcas*. Also in Brazil, Spinelli et al. (2014) and (2015) evaluated 16 half-sib families of *J. curcas* in Porto Velho, Rondônia State, and found grain yield mean of 830 kg ha^{-1}, in the third crop year, under non-irrigation conditions. Freitas et al. (2016), evaluating 77 half-sib families of *J. curcas* in Araponga, MG, found mean grain yield per plant of 377.9 g (ranging from 169.8 to 772.1 g), after 52 months in the field. Rao et al. (2008) evaluated 29 populations from different locations in India and found yields from 40.67 to 293.30 kg ha^{-1}.

Oil and protein content

The populations did not show genetic variability for seed oil and protein content (Table 2). The average oil content was 31.38%, very close to the average levels found in the majority of the populations analyzed in several countries (Table 3). The six populations evaluated in this study had minimum oil content of 26.52%, which was higher than the Indian the Tatikonda et al. (2009) and Sunil et al. (2011), the Brazilian Ferrari et al. (2009) and Freitas et al. (2011), and the Mexican Ovando-Medina et al. (2011) populations, as shown in Table 3. The highest level found in populations was 37.33%, very close to that found by the maximum levels of most populations evaluated in other countries (Table 3).

Table 3. Minimum, medium and maximum oil levels in *Jatropha curcas* L. populations evaluated in several parts of the world.

Country	Oil content (%)			Number of populations evaluated	References
	Minimum	Medium	Maximum		
India	29.85	33.67	37.05	32	Rao et al. (2008)
India	19.00	31.63	39.40	48	Tatikonda et al. (2009)
India	17.50	29.20	36.70	34	Sunil et al. (2011)
Brazil	11.29	25.49	39.70	27	Ferrari et al. (2009)
Brazil	15.99	30.88	45.55	78	Freitas et al. (2011)
Brazil	30.00	36.21	39.61	77	Freitas et al. (2016)
Mexico	12.09	29.08	44.27	121	Ovalando-Medina et al. (2011)

The seed average protein content was 20.71% (Table 2), close to 20.95% found by Souza et al. (2009), studying a population from Dourados, MS, and well above 16.11% found by Ferrari et al. (2009), analyzing the grain protein content of 27 populations of *J. curcas* from different regions of Brazil. Comparing the seed protein contents obtained in this study with those found by Makkar et al. (1997), in populations from a number of countries (Table 4), it was found that all genetic material, except for those from Costa Rica, presented higher levels. It is noteworthy that the protein contents of the populations described in Table 4 were obtained by using only the seed kernel. Overall, except for the protein found by Ferrari et al. (2009), which was 16.11%, those obtained by Souza et al. (2009) and Makkar et al. (1997) were close, indicating the existence of low variability among *J. curcas* populations.

Table 4. Seed protein contents in *Jatropha curcas* L. populations from several origins evaluated by Makkar et al. (1997).

Country	City	Protein content (%)
Cape Verde	Fogo	25.6
Senegal	Santhie Ram	25.1
Senegal	Nioro du Rip	28.9
Ghana	Nyankpala	31.1
Benin	Cotonou	30.1
Burkina Faso	Kongoussi	28.1
Kenya	Kitui	25.0
Tanzania	Mombo	29.3
Burma	Sink Gaing and Mandalay	29.6
India	Kangra	24.1
India	Kangra	23.2
Costa Rica	Rio Grande	19.0
Mexico	Vera Cruz	23.7
India	Nasik	23.0
Nicaragua	(Cape Verde – population cultivated in Managua)	22.2
Nicaragua	(Nicaragua – population cultivated in Managua)	25.6
Nigeria	Ife	27.7
Mexico	Papantla	27.2
Mean		26.02

Analysis of the populations for the set of evaluated traits (Table 2) showed that population J5 stood out for presenting the highest averages, even though the fruit dry matter, grain yield, and oil content values did not show statistical difference. It is noteworthy that these six clonal populations evaluated were selected in a restricted geographic area by a pioneering producer in the cultivation and marketing of *J. curcas.* It is noticed that, up to 4.5 years of cultivation, these populations have narrow genetic variability for the studied traits. It is necessary to continue evaluating all of them for two to three consecutive harvests, under favorable climate, to decide on their genetic value. Anyway, it seems reasonable that the high oil yield will be reached when genetic variation is enlarged.

Conclusions

Population J5 presented taller plants with longer stem diameter.

The greatest leaf dry matter weight was found in population J2.

Populations evaluated did not present genetic variability in terms of fruit dry matter, grain yield, and oil and protein contents.

Overall, population J5 stood out among the others for presenting the highest average growth, grain yield and oil content.

Acknowledgements

Thanks to the CAPES for granting the research scholarship. Thanks are due to CNPq and FAPEMIG.

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