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Growth and yield of *Jatropha curcas* L. (Euphorbiaceae) plants cultivated in different spacing and treated with kinetin

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Abstract

Recommendations for *J. curcas* plants were studied for adequate crop spacing, and to increase the number of female and hermaphrodite flowers and fruit yields. The study was carried out in an experimental field in Brazil,using four-year-old adult plants in a 5 x 3 factorial in a completely randomized design with three replications. Treatments included five doses of the growth regulator kinetin (0, 3, 6, 9 and 12 mg L^{-1} at the volume of 330 ml per plant) in a single application to six plants and three planting spaces (3×3, 3×2, 3×1 m). The results showed that the applied kinetin doses did not interfere in the production index of *J. curcas*, but population density exerted a significant influence on the vegetative and reproductive development of the *J. curcas* plants in the 3x3 spacing, where competition for essential growth resources (water, light and nutrients) seems to have been less intense and the plants were more vigorous. Cropping *J. curcas* plants in 3 × 3 spacing is recommended to obtain vigorous plants with a high number of branches, inflorescences and high grain yield. Applying kinetin at concentrations lower than 12 mg L^{-1} did not interfere in the sexual expression of *J. curcas* flowers. Further studies with higher kinetin doses in 3 × 3 m spacing are necessary to elucidate and recommend new management practices.

Keywords: Vegetable oil, biofuel, flower biology.

Abbreviations: pH_hydrogen ionic potential, M.O._ organic matter, P_phosphorus, K_potassium, Ca_calcium, Mg_magnesium, Al_ aluminum, H+Al_Hidrogen plus aluminum, CTC_ cataion exchange capacity, V%_ base saturation, Aw_wet tropical climate, IN_inflorescence number, NMF_number of male flowers, NFF_number of female flowers, NBF_number of bisexual flowers, HP_plant height, NB_number of branches, DC_cup diameter, SD_stomatal density, Prod_ productivity, 100S_mass of 100 seeds, INMET_national institute of meteorology, PCA_ principal component analysis.

Introduction

Growing world concern with the environmental impacts caused by fossil fuels drives the search for renewable energy sources. Biofuel is an important alternative for substituting petrodiesel to minimize pollutant emission (Muller et al., 2015).

Plant oil production increased in Brazil as result of the creation of the Brazilian National Program for Biofuel Production and Use (PNPB) in 2004 and legislation regulating the percentage of biofuel added to commercialized petrodiesel. Plant oil production has increased so that in 2016 the installed capacity in Brazil was about 271 thousand m^3 and 39% of this production was located in the Central Western region of Brazil (Anp, 2016). The main raw materials for biofuel production in Brazil are soy bean, bovine fat, cotton and other waxy materials with contributions of 72.24%, 20.13%, 2.81% and 4.83%, respectively (Anp, 2016).

Brazil has favourable conditions for biofuel production, including extensive territory, climatic diversity and wide biodiversity of potential species adapted to different climates and biomes (Costa et al., 2013). In this context, *J. curcas* (Euforbiaceae), native to Central America and found under the most diverse edaphoclimatic conditions, has significant economic value because of the high oil content in the seed (20 to 45%) that has excellent physical chemical quality (Sousa et al., 2012; Matos et al., 2013).

The *J. curcas* species has not yet been domesticated t and there are few agronomic data, such as spacing recommendations, for plant densities that ensure high yields (Surwenshi et al., 2011).

Adequate spacing is essential because it determines the economically viable yield. Various spacings are usually adopted, from high adensed density (2×2 m) to low density (2×5 m) according to the soil fertility and physical conditions, climate and how the plants are conducted (Arruda et al., 2004; Dias et al., 2007; Cassiano et al., 2013). However, because it is a bush, care should be taken when choosing the population density to minimize the effects of competition for abiotic resources and prevent a microclimate propitious for pest and disease development (Drumond et al. 2010; Brasileiro et al. 2012; Horschutz et al. 2012). Plant density, in addition to interfering in competition for water, nutrients, solar radiation and micro-climate establishment in the canopy, is also determinant in the sexual expression (Negussie et al., 2016).

The species *J. curcas* is a perennial and monoecious that produces few female flowers that results in low fruit set. The female flowers are located in the median portion of the inflorescence and are surrounded by male and bisexual flowers. The inflorescences produce ca. 100-300 flowers and ca. 10 fruits (Rao et al., 2008). *J. curcas* seed production is also low, insufficient to meet the biofuel demand. The low yield is due to the low female/male flower ratio of 01:29 and 01:13, respectively (Matos et al., 2013). Increase in the female and/or bisexual flowers seems to be fundamental to improve yield. Recent research has indicated that applying growth regulators improved the yield of several species (Doorn et al., 2011; Greene et al., 2011; Purgatto and Colli, 2008; Cassiano et al., 2013; Muller et al., 2015; Costa et al., 2016).

Matos et al. (2013) showed that in Brazilian Cerrado (Savannah) region, the total number of male flowers produced by the inflorescences is always greater than the number of female flowers, resulting in low yield, so that increase in female and/or bisexual flowers seems to be essential to reach satisfactory *J. curcas* fruit yields.

Lopes et al. (2012) reported that the male - female flower ratio and the number of bisexual flowers can be managed using growth regulator. The inflorescences have about 100-300 flowers with a ten-fruit yield (Rao et al., 2008). The production and productivity of *J. curcas* seeds are low, insufficient to meet any demand for vegetable oil production, due to the low female-male flower ratio, approximately one female flower for 29 male flowers (Tewari et al., 2007). Since management practices should be developed and e agronomic information should be generated to ensure high yields of biofuel plants in the field, the objective of the present study was to recommend adequate spacing for cropping and to increase the number of female and hermaphrodite flowers and fruit yield in *J. curcas* plants.

Results and Discussion

Table 2 shows the summary of the analysis of variance for plant height, number of branches, canopy diameter, stomata density, yield and mass of 100 seeds. The variables analyzed did not fit the linear or quadratic regression models for the kinetin doses applied. The canopy diameter, number of branches and yield presented values directly proportional to the increases in spacing and were on average 68%, 47% and 78% higher in the 3x3 m spacing compared to the 3x1 m spacing, respectively. Spacing variation resulted in significant differences in the vegetative growth and reproductive development. The higher number of branches and bigger canopy diameter in plants cropped in bigger spacing (3x3 m) indicated more vigorous vegetative growth in these plants.

Although *J. curcas* plants have high potential for water and nutrient extraction, the exploitation of a greater soil volume permits the capture of more growth-sustaining resources. The bigger canopy diameter of the *J. curcas* plants in 3 x 3 m spacing was directly related to the bigger productive capacity of these plants because they contained more source organs. The results did not correlate with those found by Cassiano et al. (2013) who reported no difference in growth in *J. curcas* plants cropped in 3x3 m, 3x2 m and 3x1 m spacings in the Brazilian Cerrado. Vigorous plant growth and high assimilation capacity are requirements to obtain an adequate number of flowers and fruits.

Table 3 shows the summary of the analyses for the number of inflorescences and female, masculine, asexual and bisexual flowers. The variables analyzed did not fit the linear or quadratic regression models for the kinetin doses applied. The number of inflorescences and the number of male and female flowers increased as the spacing was increased and were on average 68%, 80% and 69% higher in the 3 x 3m spacing compared to the 3 x 1m spacing, respectively. The summary of the multiple regression analysis for the weight of the variables on grain yield is shown in Table 4. The model explained 44% of the variance in *J. curcas* yield. In this regression model, the variable number of inflorescences most contributed to grain yield.

Sex expression in *J. curcas* flowers is regulated by genetic aspects and interactions of environmental factors such as temperature, light period, nutrient and hormone availability (Ramírez, 2013). Increase in the number of female and bisexual flowers has been indicated as an important strategy for increasing seed yield in *J. curcas* plants. In spite of positive reports regarding the use of growth regulators in the sexual expression of flowers (Brito et al., 2015; Costa et al., 2016), the present study reported no significance of the kinetin concentration on any of the variables analyzed. Very possibly the type of cytokinin applied (kinetin) was different from that used in other research (benzyladenine) (Pan et al., 2011; Matos et al., 2013) and played a fundamental role in the sexual expression.

The principal components analysis (PCA) showed that axis 1 explained 40% of the variation in the data and axis 2, 11.89%. The most productive treatments were located to the left of axis 1. The variables that were determinant to obtain the different groups were number of branches, canopy diameter, number of flowers, number of male and female flowers (Figure 2). Axis 2 was not influenced by any of the variables analyzed. This result was confirmed by the PERMANOVA analysis which showed data significant at $P<0.01$.

The number of branches is directly correlated with the quantity of inflorescences. Since in this species the inflorescences are on the branch tips, a larger number of branches almost always results in more inflorescences, as reported in the present study. The positive correlation between number of branches and inflorescences is common in other studies on *J. curcas* (Cassiano et al., 2013; Oliveira, 2016). The quantity of branches with inflorescences and sexual expression of the flowers are determinant for grain yield (Matos et al., 2013; Pan et al., 2011) and the number of inflorescences, of all the variables analyzed, and interfered most positively in grain yield, as exemplified in the analysis of multiple regression. Furthermore, it is pointed out that cropping in bigger spacing not only resulted in plants with more vigorous and reproductive vegetative growth, but also in higher numbers of male and female flowers, and consequently higher grain yield. Axis 1 of the principal components analysis placed the treatments in two groups and ratified the difference between the treatments. Thus the plants cropped in the 3 x 3m spacing with bigger values

Table 1. Summary of soil chemical analysis at depths of 0 to 20 cm and 20 to 40 cm.

Depth	pH em $CaCl_2$	M.O. (g.dm^{-3})	P-Mehlich (mg.dm^{-3})	Sorbic Complex (cmol.dm^{-3})						V%
				K	Ca	Mg	Al	H+Al	CTC	
0 a 20	5.6	33.0	7.0	0.20	3.4	1.3	0.0	3.5	8.4	58.3
20 a 40	5.3	37.0	8.8	0.25	4.2	1.6	0.0	3.7	9.8	62.0

M.O. = Organic matter; V% = base saturation; CTC = cation exchange capacity.

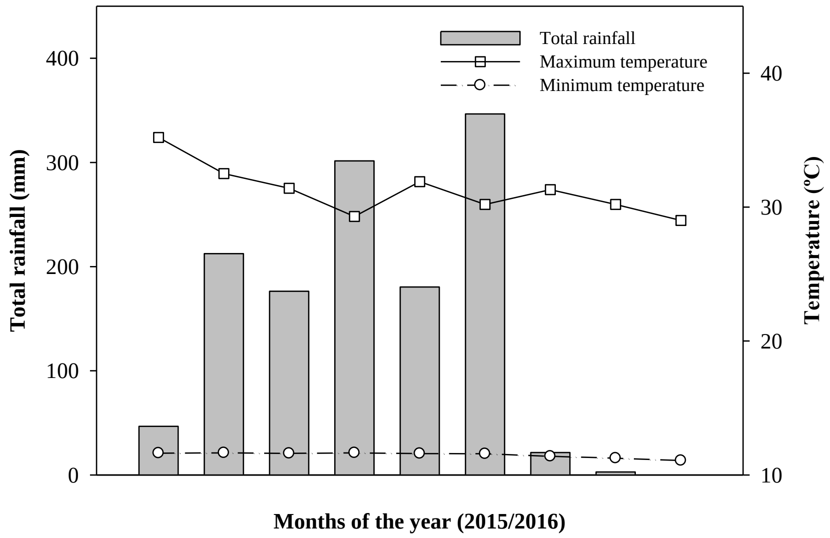

Fig 1. Monthly climatic data of total rainfall, maximum and minimum temperature collected at the local meteorological station during the experiment - October 2015 to June 2016 (INMET, 2016).

Table 2. Analysis of variance and mean test for plant height (HP), number of branches (NB), cup diameter (DC), stomatal density (SD), productivity (Prod) and mass of 100 seeds (100S) in plants of *Jatropha curcas* grown at different spacings (3x3, 3x2 and 3x1) and treated with doses of kinetin (0.0, 3.0, 6.0, 9.0, 12.0 mg L^{-1}).

Medium Squares							
Source of Variation	DF	HP (m)	NB	DC (m)	SD (mm^2)	Prod. (Kg ha^{-1})	100S (g)
Spacing	2	0.11^{ns}	892.82**	4.97**	0.01^{ns}	1.01**	43.87^{ns}
Hormone	4	0.05^{ns}	9.33^{ns}	0.09^{ns}	0.03^{ns}	0.78^{ns}	77.07*
S x H	8	0.06^{ns}	9.18^{ns}	0.08^{ns}	0.03^{ns}	1.01^{ns}	39.86^{ns}
Error	30	0.04	26.67	0.04	0.18	4.39	27.88
CV (%)		7.12	23.44	10.83	4.43	18.79	7.78
Spacing		Means					
3x3		3.01a	22.8a	2.42a	159.0a	571.18ª	68.71a
3x2		2.95a	16.0b	1.93b	162.0a	218.66b	69.04a
3x1		2.84a	7.4c	1.28c	147.0a	125.69c	65.93a
Hormone		Means					
0.0		2.95	13.77	1.84	162.46	262.75	68.92
3.0		2.96	16.22	1.71	189.05	189.39	64.12
6.0		2.99	15.22	1.96	171.60	279.20	65.78
9.0		2.88	16.22	1.93	171.60	391.78	69.19
12.0		2.99	15.77	1.94	117.01	402.75	71.47
R^2 Linear		2.56^{ns}	38.57^{ns}	42.45^{ns}	49.65^{ns}	70.73^{ns}	30.18^{ns}
R^2Quadratic		7.57^{ns}	63.17^{ns}	42.53^{ns}	74.67^{ns}	78.68^{ns}	83.01^{ns}

** significant at 1% probability; * significant at 5% probability; ns = not significant by the test F. Means followed by the same lowercase letter within the column do not differ from each other at 5% probability by the Newman-Keuls test; Coefficient of determination (R^2) linear and quadratic in response to different concentrations of Kinetin according to the regression analysis. All values of R^2 are significant $P \leq 0.05$; "ns" indicates not significant.

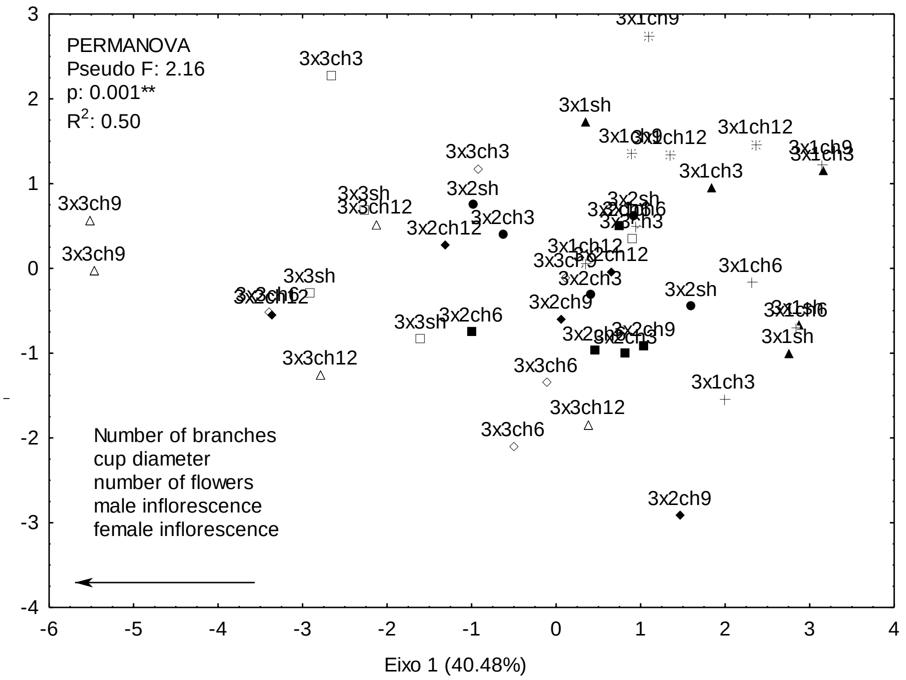

Fig 2. Score ranking principal components analysis (PCA) for the morpho-physiological data submitted to different treatments. The arrow indicates the direction in which each variable increases in relation to the axis selecting with contribution above 80%. The treatments are identified initially by the spacing (3x3, 3x2 and 3x1 m) followed by letters that represent hormone (ch) and without hormone (sh) and finally the hormone dose applied (0. 3; 6; 9; 12 mg L^{-1}).

Table 3. Analysis of variance and mean test for inflorescence number (IN), Number of male flowers (NMF), number of female flowers (NFF), number of bisexual flowers (NBF) in plants of *Jatropha curcas* grown at different spacings (3x3, 3x2 and 3x1) and treated with doses of kinetin (0.0; 3.0; 6.0; 9.0; 12.0 mg L^{-1}).

		Medium Squares			
Source of Variation	GL	IN	NMF	NFF	NBF
Spacing	2	3.105**	3.44**	3.10**	0.010^{ns}
Hormone	4	0.228^{ns}	0.743^{ns}	0.057^{ns}	0.187^{ns}
S x H	8	0.895^{ns}	2.083^{ns}	0.122^{ns}	0.225^{ns}
Error	30	4.13	5.23	4.13	1.18
CV (%)		22.55	25.65	22.55	25.02
Spacing			Means		
3x3		37.0a	325.0a	241.0a	7.0a
3x2		23.0b	162.0b	173.0ab	3.0a
3x1		12.0b	67.0b	76.0b	1.0a
Hormone			Means		
0.0		21.2	260.7	153.7	4.77
3.0		20.0	117.4	203.5	1.11
6.0		20.7	119.0	134.0	3.00
9.0		29.4	227.8	183.6	2.66
12.0		28.1	199.0	141.5	6.55
R^2 Linear		66.91^{ns}	0.10^{ns}	5.68^{ns}	14.89^{ns}
R^2Quadratic		72.11^{ns}	48.46^{ns}	14.29^{ns}	82.52^{ns}

** Statistical analysis according to Table 2.

for number of branches, canopy diameter, number of male and female inflorescences and yield were almost totally to the left of axis I, while the other treatments (3x2 m and 3x1 m) were to the right. Competition for water, light and nutrients among the *J. curcas* plants was not sufficient to interfere in the vegetative growth of the species, but, with the development of one more drain, especially, the strongest of the plants, the fruits, the competition for abiotic resources in the more adensed plants contributed to reducing yield per plant.

Therefore, the results together show that the kinetin doses applied did not interfere in the production index of *J. curcas* but the population density exerted a significant influence on the vegetative and reproductive development of the *J. curcas* plants.I In the 3x3 spacing, the competition for essential growth resources (water, light and nutrients) seems to have been less intense and plants were more vigorous.

Materials and methods

The experiments were carried the experimental field at the State University of Goiás, (17067'90" S, 48019'59"W and 805 m altitude), Ipameri, Goiás, Brazil. The region has a tropical climate with a dry winter and wet summer (Aw), according to the Köppen classification. The annual rainfall is 1,447 mm, average temperature of 21.9°C, and average air relative humidity ranges from 58 to 81%. The region has two distinct seasons, a wet season from October to March, and a dry season from April to September. The soil in the experimental area with 2% slope is classified as oxisol (Embrapa Solos 2013). After chemical analysis (Table 1), the soil pH was corrected and fertilized following technical recommendations for the crop (Laviola e Dias et al., 2008; Matos et al., 2014).

During the experimental period (October to June 2016) the climatic data, total rainfall, maximum and mean temperature (Figure 1) were collected every 15 days at the INMET meteorological station located in the municipality of Ipameri, GO, Brazil, at 1500 m from the experimental field.

The experiment was set up using four-year-old adult plants in a completely randomized design in a 5 x 3 factorial scheme with five doses of kinetin growth regulator (0, 3, 6, 9 and 12 mg L^{-1}) at the volume of 330 ml per plant in a single application, cropped in three spacings (3x3, 3x2, 3x1 m), with three replications and plots consisting of two useful plants. Application was made three days after inflorescence emission so that all the reproductive structures were reached during spraying (Matos et al., 2013).

The following variables were analyzed 60 days after applying growth regulator: number of male, female or hermaphrodite and asexual flowers per inflorescence, chlorophyll and total carotenoid leaf concentrations, plant height, stem diameter, canopy diameter and number of branches. The fruits were collected at 100, 120 and 150 days and the yield and mass of 100 seeds were analyzed.

Growth variables

Plant height and stem diameter were measured with a graduated ruler and a digital caliper, respectively. The canopy diameter was measured using a graduated measuring tape between the two side extremities. The number of leaves was measured by counting all the leaves on each plant. The number of branches was obtained by counting all the ramifications from the base of the main stem. For chlorophylls ($a + b$) and total carotenoids, analyses were determined spectrophotometrically from the above extracts according to Lichthenthaler (1987). Leaf chlorophylls and carotenoids concentration was expressed per unit mass.

A replication of the abaxial and adaxial surfaces of the leaves was removed with colorless varnish in the region of the middle third of previously hydrated leaves. The stomata were counted in the replication under an optical microscope equipped with a light chamber. Stomata density was determined by counting the stomata situated in a 1 mm² area, giving the number of stomata/area (Borges et al. 2014).

Productive variables

The number of female, male and bisexual flowers was obtained by counting in each inflorescence as they opened, following recommendations by Pereira et al. (2011). The mass of 100 seeds was weighed on precision scales (0.001g). The yield per plant was quantified by weighing the seeds (kg ha^{-1}) according to each spacing used.

Statistical procedures

The variables were submitted to analysis of variance and linear or quadratic regression according to a completely randomized block design with five treatments and six replications. Multivariate analyses were made using the principle components technique with a correlation matrix and the axes selection criteria by Broken Stick coupled to multivariate analysis of variance by permutation - PEMANOVA (Anderson, 2001). Multiple regression analysis was used to assess yield using the *forward stepwise* selection model (Sokal and Rolf, 1969). The analyses were performed by the R software (R Core Team, 2018).

Conclusion

Cropping *J. curcas* plants in 3 x 3 spacing is recommended to obtain vigorous plants with a high number of branches, inflorescences and high grain yield. Applying kinetin at concentrations lower than 12 mg L^{-1} did not interfere in the sexual expression of *J. curcas* flowers. Further studies are needed with higher doses of kinetin in 3 x 3 m spacing to elucidate and recommend new management practices.

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