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Analysis of morpho-agronomic and climatic variables in successive agricultural years provides novel information regarding the phenological cycle of *Jatropha* in conditions of the Brazilian cerrado

GISELE P. DOMICIANO¹, ALEXANDRE A. ALVES¹,
BRUNO G. LAVIOLA¹ and JULIO C. ALBRECHT²

¹Embrapa Agroenergia, Parque Estação Biológica/PqEB, Avenida W3 Norte (Final), s/n, 70770-901 Brasília, DF, Brasil

²Embrapa Cerrados, BR-020, Km 18, 73310-910 Planaltina, DF, Brasil

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ABSTRACT

Phenological studies can provide information that enables the understanding of the dynamics of plants and how these dynamics are related to the biotic and abiotic environment. In order to study the phenological phases of *Jatropha* during two agricultural years, agronomic and climatic variables, such as temperature and rainfall, were evaluated. Data for each variable in each year and each genotype were subjected to analysis of variance (ANOVA) and the differences were tested at 5% probability by F test. In addition, the correlation of growth behavior and reproductive development of two *Jatropha* accessions (CNPAE-102 and CNPAE-169) as a function of time elapsed after the start of the phenological cycle with climatic variables were analyzed through Pearson's correlation. It was found that: (i) the resuming of plant growth by producing new branches and flowers of both genotypes coincides with the start of the rainy season, (ii) the flowering may be related to the increase in temperature and rainfall; (iii) the number of inflorescences per plant and number of female flowers determine the number of green fruits, (iv) the environmental changes are responsible for the delimitation of phenophases; and finally that (v) the responses to phenological changes are genotype-dependent.

Key words: *Jatropha curcas*, flowering, growth, yield.

INTRODUCTION

The search for renewable fuels has recently been one of the most prominent subjects within the scientific community. Regarding the production of biodiesel, which is a biofuel obtained from (trans) esterifying (vegetable) oils and/or animal fats, it has been sought to identify plant species capable of producing, in the same area, an amount of oil of three to ten times higher than that obtained from

soybeans (currently, soybean oil yield is around 500kg/ha). Among the many species that stand out, *Jatropha* (*Jatropha curcas* L.) is one of the most promising (Durães et al. 2011). The oil content in its seeds can reach 40% (Dias et al. 2007, Rocha et al. 2012) and grain yield per hectare may reach 4.000 kg, which corresponds to potentially 1.500 kg of oil per hectare (Drumond et al. 2010).

In Brazil, *Jatropha* has been receiving governmental support due to its promising potential as feedstock for biodiesel production (Laviola et al.

Correspondence to: Bruno Galveas Laviola
E-mail: bruno.laviola@embrapa.br

2010). Several studies have been carried out in order to domesticate it. Results of these studies (Bhering et al. 2013, Bhering et al. 2013, 2012, Rocha et al. 2012, Laviola et al. 2010, 2011a, b, 2012a, b, Rosado et al. 2010, Spinelli et al. 2010, Drumond et al. 2010, Juhász et al. 2009) confirm that the species has a high potential to be exploited commercially. Parallel to this work, attempts to introduce this species in various regions in Brazil are being carried out, such as in the Cerrado (a savannah-like biome) and in the semiarid regions. However, a successful implementation of this crop in these regions requires the use of superior genetic material and the adoption of appropriate management practices (Durães et al. 2011). In addition, the preparation and conduction of studies on phenological behavior of different genotypes in different regions is also necessary. Phenological studies are usually extremely useful for (i) understanding the dynamics of plant communities, (ii) perceiving the regeneration and reproduction of plants, (iii) comprehending the dynamics of biotic and abiotic factors that may affect plant development (Talora and Morellato 2000), (iv) planning of crop practices, such as choosing cultivars, season and location, for future predictions of management practices. However, such studies are still scarce in what regards to *Jatropha*. Belonging to the family *Euphorbiaceae*, it is known that *Jatropha* is a fast-growing shrub, deciduous, which can reach more than 5 m in height. Fruits are ovoid-shaped capsules, with 1.5 to 3.0 cm in diameter, trilocular, containing 3 seeds, and one seed per locule (Laviola et al. 2011b). However, little is known about (i) the behavior of the species in specific environments (e.g. when the peak of growth, flowering, and fruiting occur at a given location); (ii) the related physiological parameters of the species (e.g. gas exchange, source-drain ratio, among others); and (iii) the genetic and environmental influence on such parameters. Gurgel et al. (2011), when

studying three morphologically distinct accessions of *J. curcas* at their young stage at the Brazilian Cerrado biome, which has two quite remarkable climatic seasons, hot and humid season and a cold and dry one (Sarmiento 1984), observed that over a one-year term (August 2009 to July 2010) the accessions differed in growth rates, height increments and final heights and earliness. The authors also found that the minimum temperature was the climatic variable that adversely determined, to a greater degree, the number of inflorescences and consequently the productivity. Some other studies were carried out with other species of the genus *Jatropha* regarding the phenology of plants under evaluation. For instance, Neves et al. (2010) studied the phenology of *Jatropha molissima* (Pohl) Baill., *Jatropha mutabilis* (Pohl) Baill. and *Jatropha ribifolia* (Pohl) Baill. From July 2005 to June 2007, they found that the three species showed high water storage capacity, which ensured the occurrence of phenological events during the drought season.

Since works addressing repetitive events regarding the behavior of species/genotypes of *Jatropha* over agricultural years are scarce (Gurgel et al. 2011) and quite conclusive for the Brazilian reality (due to the changing environment, results may not be extrapolated), this study aimed to analyze the phenological patterns of two distinct accessions of *Jatropha* in the Brazilian cerrado, over two successive agricultural years.

MATERIALS AND METHODS

The experiments were carried out in the *Jatropha* active germplasm collection (third year) maintained by Embrapa Agroenergy at the experimental station of Embrapa Cerrados (Planaltina-DF) (15°35'30"S and 47°42'30"W, 1007 m asl) (Laviola et al. 2011b). According to Köppen classification, the climate that prevails in the region is tropical wet and dry or savannah (Aw), characterized by prolonged period of drought season and rainfall concentration during

the summer. The climatic variables monitored by a weather station during the evaluated period were: (i) maximum temperature, (ii) minimum temperature, (iii) average temperature, (iv) relative humidity and (v) season rainfall. The soil was classified as latosol (oxisol) containing high levels of aluminum. In order to achieve soil correction, application of lime was carried out to achieve 60% base saturation. In each agricultural year, 400 grams of NPK 20:5:20 fertilizer was used in four applications, which occurred in January, April, October and November. Management practices, control of pests and diseases were routinely carried out in the germplasm bank as previously described (Dias et al. 2007).

The germplasm bank was constituted based on a series of collection of samples of isolated *J. curcas* plants and seeds collected between December 2007 and May 2008, at different locations, from collections and plantations in Brazil (Laviola and Alves 2011, Rosado et al. 2010). For this study, two morphologically distinct accessions were evaluated: CNPAE-102 (toxic, 30.35 mg/g of phorbol esters in grains), CNPAE-169 (non-toxic, without phorbol ester in grain) (Laviola et al. 2011b). Accessions with undetectable levels of phorbol ester were considered as non-toxic (Devappa et al. 2010a, Makkar et al. 2010, Liu et al. 2010, Devappa et al. 2010b). The experiment was set following a randomized block design with five replications and treatments were arranged in a split-plot system. The genotypes and the assessment period represented the plots and subplots, respectively. During the years of 2010, 2011 and 2012 (assessment periods - August 2nd 2010 to July 12th 2011; and September 27th 2011 to July 2nd 2012) the following morpho-agronomic traits were assessed: (i) plant height, the highest branch measured from the soil level (height, m); (ii) number of inflorescences per plant (NIP) from the appearance of the first inflorescence; (iii) number of male flowers (NMF); (iv) number of

female flowers (NFF) per inflorescence; (v) ratio of female and male flowers (RFM); and (vi) relative growth rate (RGR) (growth in cm per day).

Data for each variable, in each year and each genotype, were subjected to analysis of variance (ANOVA) and the differences were tested at 5% probability by the F test. The evaluated variables were correlated with climatic variables by means of the Pearson correlation coefficient. All analyses were performed using Genes software (Bhering et al. 2011, Cruz 2006a, b, 2010, Cruz et al. 2004).

RESULTS

ASSESSMENT OF CLIMATIC VARIABLES IN TWO SUCCESSIVE AGRICULTURAL YEARS

The monitoring of climatic variables is essential in any phenological study. Thus, in this research the following variables were monitored: maximum, minimum and average temperature; maximum, minimum and average relative humidity; rainfall and radiance during two years (2010/2011 and 2011/2012). In the evaluated agricultural years, the maximum temperatures were 32°C and 30°C, and the mean maximum temperatures were 27.7°C and 28°C, respectively. The minimum temperatures were 12°C and 15°C, and the mean minimum temperatures in the assessed periods were 16.2°C and 17.0°C. The mean temperature, in both agricultural years was 21.5°C. The relative humidity ranged from 43 to 85%, maintaining an average of 68.5% in both agricultural years. The average rainfall in both years was 1.178 mm. The average radiance during both periods was 17.2 and 13.9, being the maximum and minimum values 22.8 and 3.0, respectively. Besides the mean values, it is important to observe the fluctuations of these variables over the agricultural years as shown in Figures 1a, 1b, 1c and 1d, respectively. In general, the variables related to temperature and humidity reach higher values from October to May, which is a period that favors plant growth and reproduction.

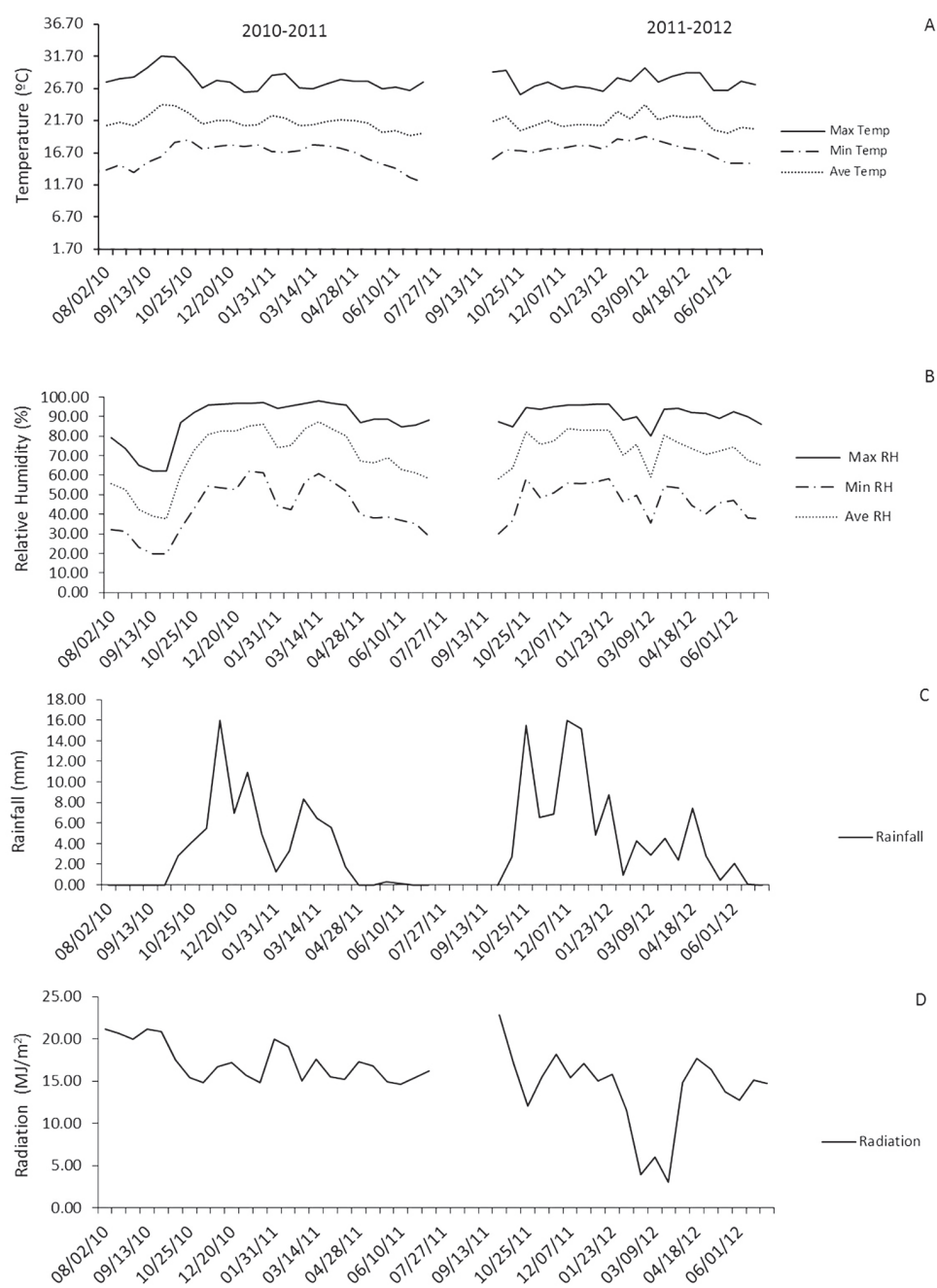


Figure 1 - Fluctuation of climatic variables **(a)** temperature, **(b)** relative humidity, **(c)** rainfall **(d)** radiation during the two successive agricultural years in the Brazilian cerrado region (Planatina, DF - 15°35'30"S and 47°42'30"W, 1007 m asl)

ASSESSMENT OF MORPHO-AGRONOMIC VARIABLES IN TWO SUCCESSIVE AGRICULTURAL YEARS

The variations in the growth (height) of accession CNPAE-102 and accession CNPAE-169 in the years 2010/2011 and 2011/2012 are shown in Figure 2a.

It is important to mention that in August 2011 a maintenance pruning was performed, which influenced the reduction in the height of the accessions (Figure 2a). The height of CNPAE-102 ranged from 2.55 m to 3.26 m in the year of 2010/2011, with a

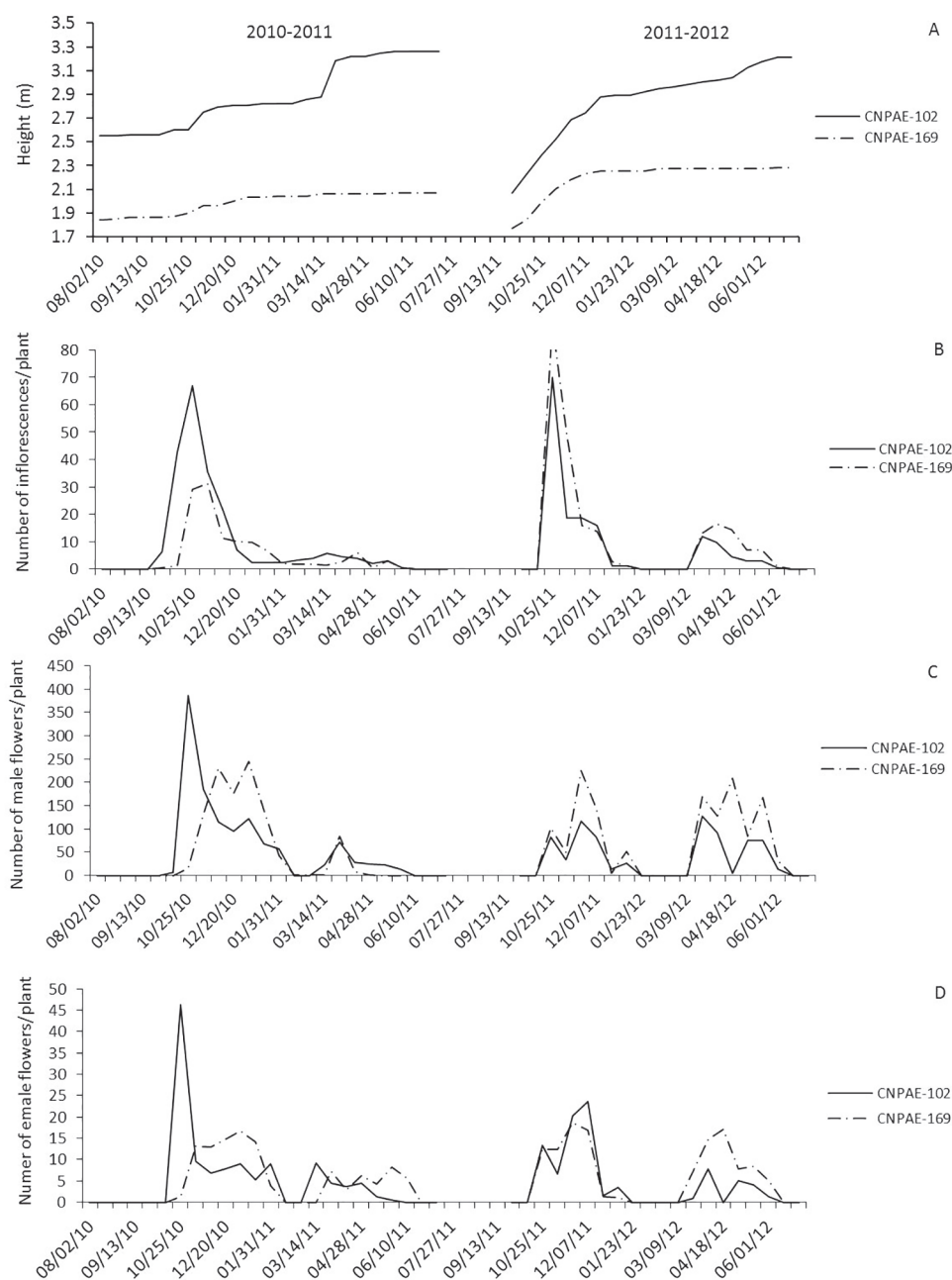


Figure 2 - Growth behavior and reproductive development of two *Jatropha* accessions (CNPAE-102 and CNPAE-169) as a function of time elapsed after the start of the phenological cycle. **(a)** height, **(b)** number of inflorescences per plant, **(c)** number of male flowers and **(d)** number of female flowers.

total increment in plant height of 0.71 m. For the year 2011/2012, height ranged from 2.06 to 3.21 m, with a total increment of 1.15 m. For CNPAE-169, height ranged from 1.84 to 2.07 m in 2011, and from 1.77 to 2.28 cm in 2012 (i.e. increments of 0.23 and 0.55 cm,

respectively). The greatest height in the agricultural year of 2011/2012 is mostly due to pruning, which causes rapid plant growth. As expected, due to genetic differences (Laviola et al. 2011b), the accessions showed significant differences for heights in both

years (Tables I and II). In general, increment in height of CNPAE-169 occurred more gradually, while CNPAE-102 presented a steepened growth curve (Figure 2a). In the year 2010/2011, the largest increment in height occurred at the end of the phenological cycle, while in 2011/2012 there was a trend for higher growth in early phenological cycle. It is probable that, the practice of maintenance pruning directly interfered in the difference in the growth curves in both crop years.

Regarding the relative growth rate (RGR) (Figure 2b), there was no significant difference between accessions, but there was a significant interaction between accessions and the dates of evaluation in 2010/2011 (Table I). The significant interaction indicates that the accessions had different growth patterns in 2010/2011, showing growth peaks at different times (Figure 1c).

RGR for CNPAE-102 reached a maximum of 2.05 cm/day in 2010/2011, with a sharp peak

TABLE I

Analysis of variance of plant height (m), number of inflorescence per plant (NIP), number of male flowers (NMF), number of female flowers per plant (NFF), ratio of female and male flowers (RFM) and relative growth rate (RGR), evaluated in the agricultural year of 2010/2011.

VARIATION SOURCE	MEAN SQUARE					
	Height	NIP	NMF	NFF	RFM	RGR
Genotype	48.2317**	1061.2011*	2814.671 ^{ns}	52.698551 ^{ns}	0.343908 ^{ns}	0.047024 ^{ns}
ERROR A	0.182945	62.750116	5991.641	26.597233	0.489114	0.037863
Assessment Season	0.313498**	0.313498 ^{ns}	57516.868**	416.59844*	1.061331**	0.66349**
INTERACTION	0.103869**	103869 ^{ns}	32256.147**	416.413092 ^{ns}	0.86648**	0.486111**
ERROR B	0.014723	0.014723	4995.366	41.413768	0.413709	0.038745
MEAN	2.434792	11.086894	76.0282	6.94216	0.199758	0.144863
PLOT CV	17.567	71.4492	101.8117	74.2888	350.1064	134.3236
SUBPLOT CV	4.9836	56.7539	92.9626	92.6996	321.9898	135.8786

** Significant at 1% probability by the F test; * Significant at 5% probability by the F test; and ^{ns} Not significant by the F test. CV - coefficient of variation. NIP - number of inflorescence per plant. NMF - number of male flowers, NFF - number of female flowers, RFM - ratio female and male flowers, RGR - relative growth rate

TABLE II

Analysis of variance of plant height (m), number of inflorescence per plant (NIP), number of male flowers (NMF), number of female flowers per plant (NFF), ratio of female and male flowers (RFM) and relative growth rate (RGR), evaluated in the agricultural year of 2011/2012.

VARIANCE SOURCE	MEAN SQUARE					
	Height	NIP	NMF	NFF	RFM	RGR
Genotype	21.19656**	496.13333 ^{ns}	79825.20833**	285.208333*	0.020449 ^{ns}	0.146733 ^{ns}
ERROR A	0.153474	165.925	1910.204167	15.041667	0.010797	0.1185
Assessment Season	0.540407**	4608.615152**	25607.971**	401.299242**	0.066661*	0.952232*
INTERACTION	0.085413**	96.642424 ^{ns}	8671.3174**	87.753788**	0.035406 ^{ns}	0.03812 ^{ns}
ERROR B	0.005278	91.306818	1524.335985	20.814394	0.015804	0.070519
MEAN	2.51935	15.216667	87.858333	8.708333	0.106835	0.180843
PLOT CV	15.5499	84.6518	49.7459	44.5362	97.2598	190.3513
SUBPLOT CV	2.8837	62.796	44.4383	52.3898	117.6725	146.8417

** Significant at 1% probability by the F test; * Significant at 5% probability by the F test; and ^{ns} Not significant by the F test. CV - coefficient of variation. NIP - number of inflorescence per plant. NMF - number of male flowers, NFF - number of female flowers, RFM - ratio female and male flowers, RGR - relative growth rate

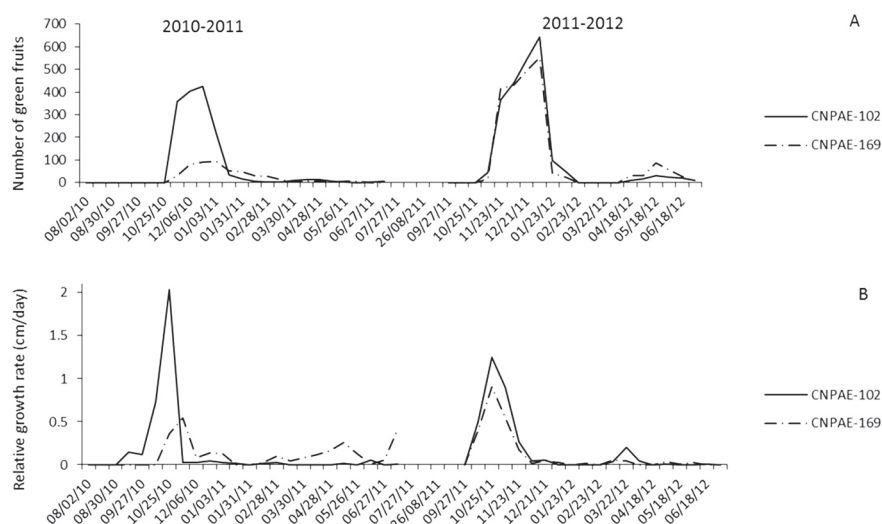


Figure 3 - Growth behavior and reproductive development of two *Jatropha* accessions (CNPAE-102 and CNPAE-169) as a function of time elapsed after the start of the phenological cycle. **(a)** Number of green fruits and **(b)** relative growth rate.

in growth between the beginning of the cycle (in late September) and late January (Figure 3b). This period coincides with the period of greatest height (Figure 2). With the decrease of RGR, a smaller increment in height also occurred. In the case of CNPAE-102, almost at the end of the physiological cycle, no increase in RGT was observed (Figure 3b), which resulted in height increment (Figure 2a). The increase in RGR and in plant height coincided with the increase in incident rainfall and radiation (Figures 1c and 1d), although CNPAE-169 did not respond to it. In 2011/2012, synchronized growth peak (RGR) between the accessions was observed, resulting in no significant interaction between genotype by assessment period (Table II).

The number of inflorescence per plant (NIP) differed significantly between genotypes only in 2010/2011 (Table I). In this agricultural year, NIP was significantly higher for CNPAE-102, reaching 66.8 inflorescences per plant, 63.3% higher than CNPAE-169, whose maximum number of inflorescences per plant reached only 31.2 (Figure 2b). This fact is probably reflected in an increased amount of green fruit for CNPAE-102 in comparison

with CNPAE-169 (Figure 3a). There was no significant difference of assessment periods, or of their interaction. In 2011/2012, there was a significant difference only for genotype by assessment periods, indicating that the accessions did not necessarily flower in the same period.

When evaluating the number of male flowers (NMF) and number of female flowers (NFF) in the inflorescences of the genotypes evaluated, it was noted that NMF was, as expected, superior to NFF (Figures 2c and 2d). Furthermore, even though the NMF and NFF did not differ between the accessions in 2010/2011, in 2011/2012 these differences were significant (Tables I and II). In the case of the NMF, while CNPAE-169 presented an average of 224.6 flowers, CNPAE-102 had an average of only 127. In the case of the female flowers, CNPAE-169 had 22% less flowers than CNPAE-102. For both types of flowers, regardless of growing season, considerable differences in the assessment period and the interaction between season and accession was noted, indicating that the number of flowers varies differently along the phenological cycle in the two accessions (Tables I and II). This different behavior can clearly be seen in Figures 2c and 2d.

In relation to the ratio between female and male flowers (RFM), there was significant difference only regarding the assessment periods and their interaction in 2010/2011, and only for the assessment period in 2011/2012 (Tables I and II).

CORRELATION BETWEEN MORPHO-AGRONOMIC VARIABLES AND CLIMATIC VARIABLES IN TWO SUCCESSIVE AGRICULTURAL YEARS

In the evaluation of the relationship between climatic variables and morpho-agronomic variables, the occurrence of significant correlations for different pairs of variables was verified, which indicates how the environment influences the growth and reproduction of *Jatropha* (Tables III and IV).

In general, the correlations between variables of growth/reproduction and climatic variables are different between accessions, as well as from one year to another. Such observations are expected due

to genetic differences among accessions and their interactions with the environment. Furthermore, the correlations between pairs of variables, although significant, present low to medium magnitude, indicating that not only one, but a set of variables including some which were not evaluated (e.g. soil chemistry), influence the phenological responses. Therefore, *Jatropha* cultivation presents a seasonal phenological dynamics in the Brazilian cerrado, with steady growth during drought season. Both accessions coincided in the increment in height and highest growth rate, starting from the second half of September to early October, in both years. Furthermore, the onset of flowering culminates with the increase of height and growth rate for both accessions in the two years assessed, coinciding with the rainy season. It was also observed that the accessions have 2 growth stages during the year. The first growth stage was observed from

TABLE III
Pearson correlation analysis (CNPAE-102) of plant height (m), number of inflorescence per plant (NIP), number of male flowers (NMF), number of female flowers per plant (NFF), ratio of female and male flowers (RFM) and relative growth rate (RGR), with climatic variables evaluated in the agricultural years of 2010/2011 and 2011/2012).

2010/2011	CNPAE-102											
	NMF	NFF	RFM	RGR	Tmax	Tmin	Tave	RHave	Rad	Rainfall	RHmax	RHmin
Height	-0.14 ^{ns}	-0.17 ^{ns}	-0.04 ^{ns}	-0.26**	-0.46**	-0.18*	-0.50**	0.29**	-0.56**	-0.11 ^{ns}	0.38**	0.21**
NIP	0.64**	0.70**	-0.03 ^{ns}	0.81**	0.35**	0.51**	0.46**	0.16 ^{ns}	-0.23*	0.27**	0.20*	0.11 ^{ns}
NMF		0.88**	-0.04 ^{ns}	0.62**	-0.02 ^{ns}	0.45**	0.17 ^{ns}	0.33**	-0.30**	0.35**	0.31**	0.32**
NFF			0.07 ^{ns}	0.77**	0.04 ^{ns}	0.47**	0.21*	0.30**	-0.25**	0.26**	0.28**	0.27**
RFM				-0.02 ^{ns}	-0.11 ^{ns}	0.16 ^{ns}	-0.01 ^{ns}	0.19*	0.03 ^{ns}	0.11 ^{ns}	0.16 ^{ns}	0.21*
RGR					0.40**	0.34**	0.41**	-0.03 ^{ns}	-0.12 ^{ns}	0.02 ^{ns}	0.02 ^{ns}	-0.08 ^{ns}
2011/2012	CNPAE-102											
	NMF	NFF	RFM	RGR	Tmax	Tmin	Tave	RHave	Rad	Rainfall	RHmax	RHmin
Height	0.01 ^{ns}	-0.21*	-0.17 ^{ns}	-0.43**	-0.14 ^{ns}	-0.04 ^{ns}	-0.06 ^{ns}	0.14 ^{ns}	-0.36**	-0.24*	0.02 ^{ns}	0.10 ^{ns}
NIP	0.37**	0.46**	0.28**	0.66**	-0.38**	0.02 ^{ns}	-0.26**	0.36**	-0.06 ^{ns}	0.52**	0.32**	0.40**
NMF		0.63**	0.20*	0.12 ^{ns}	-0.20*	0.14 ^{ns}	-0.10 ^{ns}	0.40**	-0.11 ^{ns}	0.24*	0.40**	0.40**
NFF			0.68**	0.21*	-0.29**	0.02 ^{ns}	-0.19*	0.41**	0.17 ^{ns}	0.53**	0.43**	0.40**
RFM				0.17 ^{ns}	-0.28**	0.03 ^{ns}	-0.21*	0.36**	0.14 ^{ns}	0.52**	0.36**	0.34**
RGR					-0.22*	-0.01 ^{ns}	-0.17 ^{ns}	0.15 ^{ns}	-0.01 ^{ns}	0.33**	0.13 ^{ns}	0.19 ^{ns}

** Significant at 1% probability by the F test; * Significant at 5% probability by the F test; and ^{ns}Not significant by the F test.

NMF - number of male flowers, NFF - number of female flowers, RFM - ratio female and male flowers, RGR - relative growth rate, Tmax - maximum temperature, Tmin - minimum temperature, Tave - average temperature, RHave - average relative humidity, Rad - radiation, RHmax - maximum relative humidity and RHmin - minimum relative humidity.

TABLE IV
Pearson correlation analysis (CNPAE-169) of plant height (m), number of inflorescence per plant (NIP), number of male flowers (NMF), number of female flowers per plant (NFF), ratio of female and male flowers (RFM) and relative growth rate (RGR), with climatic variables evaluated in the agricultural years of 2010/2011 and 2011/2012.

2010/2011	CNPAE-169											
	NMF	NFF	RFM	RGR	Tmax	Tmin	Tave	RHave	Rad	Rainfall	RHmax	RHmin
Height	0.06 ^{ns}	0.10 ^{ns}	-0.06 ^{ns}	0.07 ^{ns}	-0.46**	0.06 ^{ns}	-0.36**	0.47**	-0.49**	0.12 ^{ns}	0.52**	0.41**
NIP	0.39**	0.34**	0.37**	0.42**	0.05 ^{ns}	0.48**	0.23**	0.32**	-0.32**	0.37**	0.31**	0.31**
NMF		0.81**	0.01 ^{ns}	0.09 ^{ns}	-0.29**	0.41**	-0.01 ^{ns}	0.49**	-0.26**	0.70**	0.40**	0.54**
NFF			0.02 ^{ns}	0.08 ^{ns}	-0.28**	0.39**	0.01 ^{ns}	0.44**	-0.26**	0.50**	0.37**	0.49**
RFM				0.06 ^{ns}	0.19*	0.41**	0.30*	0.19*	-0.18*	0.13 ^{ns}	0.21*	0.17 ^{ns}
RGR					-0.11 ^{ns}	0.02 ^{ns}	-0.11 ^{ns}	0.14 ^{ns}	-0.27**	0.05 ^{ns}	0.18*	0.10 ^{ns}
2011/2012	CNPAE-169											
	NMF	NFF	RFM	RGR	Tmax	Tmin	Tave	RHave	Rad	Rainfall	RHmax	RHmin
Height	0.13**	0.06**	-0.01 ^{ns}	-0.36**	-0.19 ^{ns}	0.17 ^{ns}	0.01 ^{ns}	0.32**	-0.39**	-0.02 ^{ns}	0.18 ^{ns}	0.30**
NIP	0.34**	0.44**	0.44**	0.63**	-0.37**	0.01 ^{ns}	-0.26**	0.36**	-0.05 ^{ns}	0.53**	0.33**	0.40**
NMF		0.78**	0.36**	0.04 ^{ns}	-0.11 ^{ns}	0.10 ^{ns}	-0.06 ^{ns}	0.37**	0.04 ^{ns}	0.24*	0.38**	0.33**
NFF			0.74**	0.20*	-0.14 ^{ns}	0.04 ^{ns}	-0.10 ^{ns}	0.39**	0.17 ^{ns}	0.38**	0.44**	0.35**
RFM				0.35**	-0.26	-0.11 ^{ns}	-0.25*	0.31**	0.12 ^{ns}	0.30**	0.39**	0.27**
RGR					-0.20*	-0.04 ^{ns}	-0.17 ^{ns}	0.11 ^{ns}	0.02 ^{ns}	0.32**	0.09 ^{ns}	0.14 ^{ns}

** Significant at 1% probability by the F test; * Significant at 5% probability by the F test; and ^{ns}Not significant by F test.

NMF - number of male flowers, NFF - number of female flowers, RFM - ratio female and male flowers, RGR - relative growth rate, Tmax - maximum temperature, Tmin - minimum temperature, Tave - average temperature, RHave - average relative humidity, Rad - radiation, RHmax - maximum relative humidity and RHmin - minimum relative humidity.

the second half of September until late November to early December. The second growth stage was observed in January until the first half of February, continuing through the second half of April to early May. The complete cycle of formation, development and maturation of fruit extends for approximately 120 days. All these details are very important for deciding which pesticide to apply, when to start irrigation, or the fruit harvest planning.

DISCUSSION

Jatropha has attracted the attention of investors and researchers interested in bioenergy. Given its high oil productivity, the species has been suggested as an alternative of complementing soybean in the market of vegetable oils for biodiesel production (Laviola and Alves 2011, Durães et al. 2009). Recent results have shown that the potential of Jatropha for commercial production can be increased with the possibility of selecting superior genotypes and/

or desirable characteristics, e.g. increased yield, plant architecture or fruit non-toxicity (Drumond et al. 2010, Bhering et al. 2012, 2013, Rocha et al. 2012, Laviola et al. 2010, 2011a, b, 2012b, Laviola and Alves 2011, Rosado et al. 2010). Even though studies on genetic improvement are crucial for the release of commercially viable crop species, works related to the development of production systems suitable for small, medium and large establishments are also required. Thus, it is noteworthy that the cerrado region is a major area of potential crop expansion. This biome covers an area of more than 200 million hectares, which represents about 20% of the Brazilian territory (Gurgel et al. 2011). Given the importance of this biome for growing Jatropha on a long term, the development of studies to elucidate the phenology of the species and specific genotypes in this region is necessary. In addition, the successful implementation of Jatropha in this region depends upon the adoption

of appropriate management practices and the use of uniform and superior genetic material (Durães et al. 2011). Phenological studies can provide data that enable the understanding of the dynamics of plants and how these dynamics are related to the occurrence and range of biotic and abiotic factors that may affect plant development (Talora and Morellato 2000). Furthermore, the knowledge of the phenological stages of this crop may allow one to adopt the most appropriate management measures to each phenophase in order to ensure a satisfactory production.

In the case of *Jatropha* species, some studies were carried out for this purpose (Gurgel et al. 2011). However, few works have evaluated the behavior of distinct and contrasting genotypes of *Jatropha* for more than one growing season (Neves et al. 2010). Phenological cycles of tropical plants are complex, with irregular patterns which are difficult to recognize, especially in short-term studies (Bencke and Morellato 2002). This study was carried out to obtain information about the behavior of a high yield toxic accession (CNPAE-102) and a non-toxic accession (CNPAE-169) considered unproductive. Grain yield needs to be considered as one of the most important features that will determine the crop viability in the cerrado region; thus, it is important to verify how these genotypes behave in terms of (i) size; (ii) growth rate; (iii) flowering season; (iv) number of inflorescence per plant; and (v) female flowers, since these characteristics are crucial in yield analysis. It is known that *Jatropha* produces inflorescences in terminal buds of branches grown in the current year, with the production of new inflorescences which are dependent on the continuous flow of vegetative growth (Gurgel et al. 2011). Therefore, plants which are larger and/or present higher growth rate may produce a greater number of inflorescences and fruits than smaller plants. This study showed that CNPAE-102 is larger than CNPAE-169, although the growth rate was not different. Similar results were obtained by Gurgel et al. (2011). As the soils

of the Brazilian cerrado region are characterized by high acidity and low fertility (Lopes 1994) (mainly presenting low phosphorus content), the vigor and nutritional efficiency of genotypes may be one of the factors related to their size, as biomass production demands high concentrations of nitrogen and phosphorus (Foidl et al. 1996). Recently, Amaral et al. (2012) demonstrated that the absorption of phosphorus differed among *Jatropha* genotypes, and that the greater the availability of phosphorus in the soil, the greater was the vegetative growth and dry matter accumulation in *Jatropha*. As *Jatropha* is an oilseed crop that responds well to fertilizer application in order to obtain satisfactory fruit production (Laviola and Dias 2008), yearly fertilization is necessary to provide enough nutrients for vegetative growth and reproductive development, avoiding depletion of soil nutrients.

It is interesting that the resumption of plant growth by producing new branches and inflorescences of both genotypes coincides with the rainy season (October). The period before the resumption of growth, is characterized by a long dry period, when the *Jatropha* plants remain in the rest period. Many functional mechanisms are often triggered by plants in order to survive under conditions of water stress caused by drought, many of which are anatomical morphological changes, such as deep root growth, decrease in leaf size, stem growth and leaf abscission (Trovão et al. 2007). Some of these modifications, such as the abscission, appear to be a major mechanism used by *Jatropha curcas* to survive during the rest period, since at this stage the plants practically lose all their leaves (Saturnino et al. 2005). With the decomposition of leaves in the soil, the nutrients are released and reabsorbed by the root system. Furthermore, the nutrients contained in the leaves which are movable in the phloem may be taken to growth points and/or fruits before leaves fall (Epstein and Bloom 2006). Arruda et al. (2004) reported that the dormancy of *Jatropha* is induced by climate change in particular temperature/luminosity.

The period in which genotypes CNPAE-102 and CNPAE-169 remained at rest coincided with the drop of average temperature, rainfall and radiation. Arruda et al. (2004) also reported that *Jatropha* is a plant of low water requirement and that it tolerates drought, heat or cold. The same author relates that in conditions of extreme drought, the loss of leaves works as a mechanism to conserve moisture in their tissues. However, the loss of leaves results in stoppage of growth and the plants survive with the water and organic reserves stored in their stems and roots during the previous phenological cycle. *Jatropha* plants, besides being able to get enough nutrients and produce enough assimilates to support flowering and fruiting, should also be able to re-translocate nutrients and assimilates to the composition of rootstocks, as was observed by Santos et al. (2010). This might occur at the end of the cycle, as observed in this study, since even after the end of flowering, plants of both genotypes continued to grow. As further seen in this study, the growth rate of both genotypes is not constant. In the case of genotype CNPAE-169, growth appears to be more constant, while the growth of genotype CNPAE-102 occurs in peaks. Therefore, the new branches produced at the end of the cycle, after the emission of inflorescences, should serve as a source of assimilates for translocation and rootstock. Later, with leaf emergence in the next cycle, translocation should cause greater drain, increasing growth and providing the leafiness and the emergence of inflorescences.

Regarding the flowering season, the results of this study show that under the conditions of the Brazilian cerrado, flowering is concentrated along one or two seasons. This phenological stage is one of the major stages related to oil production in *Jatropha* since the number of female flowers and their fertilization determine how many fruits and seeds will be developed (Juhász et al. 2009). As previously reported, *Jatropha* produces inflorescences in terminal buds of branches grown during the current year. Thus, the production of new inflorescences

ultimately depends on the vegetative growth of the plants. This study demonstrates that the flowering periods coincide with periods of greatest vegetative growth of both genotypes. This result is extremely important from the crop science standpoint: since the flowering is concentrated in one or two seasons, harvest may also occur in concentrated form and not continuously, which certainly reduces production costs. As anticipated (Durães et al. 2011), during the growth/flowering period, plant management is important in order to favor high growth rates and avoid the occurrence of stresses, whether biotic or abiotic, since decreases in growth rate would evidently result in decreases in yield.

The drop in the number of new inflorescences after the period of greatest flower production can be explained by the development of the fruits. As the fruits develop quickly, they directly compete with the formation of new inflorescences by assimilates produced by the adjacent leaves. Gurgel et al. (2011) suggest that a drastic change may occur in the metabolic drain, causing the fruits information to have priority on the partition of assimilates. Ultimately, this might restrict vegetative growth, since the developing fruits begin to receive the majority of assimilates from adjacent leaves, as well as from more distant parts, causing a sharp reduction in growth (Abdelgadir et al. 2009).

It is highlighted that in both genotypes the number of male flowers is much higher than the number of female flowers. This fact is already known, and it had been previously reported that the number of male flowers can be 80% higher than female flowers (Aker 1997). Some authors suggest that this difference may be a strategy of the plant to attract pollinating insects, as they visit more male than female flowers (Solomonraju and Ezradanam 2002).

In terms of environmental conditions, it was observed that the number of inflorescences per plant is positively correlated with the minimum temperature (in one of the agricultural years for genotype CNPAE-102, and in both agricultural

years for genotype CNPAE-169). This indicates that flowering in *Jatropha* is related to the occurrence of low temperatures. Gurgel et al. (2011) had already observed this trend. Apparently, in the Brazilian cerrado regions, the nights are cooler than the days, and the drop in night temperature may be one of the factors that triggers flowering. As the temperature amplitude range of the agricultural year of 2010/2011 was higher than that of the agricultural year of 2011/2012, and as significant correlation between flowering and minimum temperature for genotype CNPAE-169 in both years, was verified, this genotype should have greater adaptability to temperature conditions than genotype CNPAE-102. Associated with temperature, rainfall also seems to have primary influence on flowering as a positive correlation between rainfall and number of inflorescences per plant in both genotypes and years, was observed. Thus, flowering in *Jatropha* seems to depend on the increase in rainfall levels. Considering the fact that inflorescence is produced in terminal buds of young branches, the positive correlation may reflect the fact that as the rainfall increases after a long period of dry conditions, plants immediately start to grow, and shooting of new branches occurs constantly. As previously mentioned, the increase in rainfall is also an important factor for the resumption of plant growth after the rest period and reversal in the source-sink relationship. In accordance with that, Larcher (2004) mentions that in tropical and subtropical regions, where there are dry and rainy seasons, phenophases are related to periodic changes in water availability. In addition to these factors, light seems to work as a sign that can trigger and induce flowering, as the flowering of both genotypes occurred in seasons that are typically brighter (September-December and March-May). Coherently, it has been recently demonstrated that light actually functions as a sign in *Jatropha* circadian cycle. Studies performed by Yang et al. (2011) using cDNA libraries of *Jatropha curcas* identified that the gene *JcDof3* is

related to flowering. According to the authors, this is a circadian clock regulated gene, and may be involved in flowering regulation in *J. curcas*.

After flowering, plants start the fruiting stage. As the number of fruits per plant is ultimately dependent on the number of inflorescences and on the number of female flowers, genotypes with greater numbers of flowers tend to have higher yield. Indeed, in the agricultural year in which the number of inflorescences per plant and the number of female flowers were significantly higher for genotype CNPAE-102, this genotype produced a greater amount of green fruit, when compared to genotype CNPAE-169. In the crop year in which neither the number of inflorescences nor the number of female flowers differed significantly between genotypes, the number of green fruits in both genotypes was quite similar as expected. This suggests that, both the number of inflorescences and the number of female flowers, are in fact the main factors in determining yield. On the other hand, determining the number of inflorescences per plant and the number of female flowers both depend upon environmental factors, such as genetic factors. As this study demonstrated, flowering and also the determination of the flower type are influenced by the occurrence of low temperatures and high rainfall. Thus, in agricultural years when these conditions are met, there is a high possibility of obtaining higher grain yield. However, it is known that plant hormones participate in the activation of genes responsible for the development of floral primordia (Pan and Xu 2011). Thus, the identification and selection of genotypes which are more likely to produce a greater number of inflorescences and female flowers in the same environmental condition than in others should provide higher yields. In fact, Kiill and Costa (2003) had already mentioned that the knowledge of the floral biology and reproductive behavior of a species in a given region is of fundamental importance, both to support genetic improvement programs, and for the appropriate crop management, since the

production system should consider all information of plant phenology, at the same time it favors the appropriate initial establishment and high growth rates during the rainy season.

In summary, the results of this study indicate that environmental changes are responsible for the delimitation of phenophases, given the fact that the growth is resumed only after the onset of the rainy season, and flowering depends on brightness and temperature. However, the results of this research also demonstrate unequivocally that responses to phenological changes are genotype-dependent, since genotype has great influence on the expression of agronomic characteristics.

RESUMO

Estudos fenológicos podem fornecer informações que possibilitam compreender a dinâmica das plantas e sua relação com os fatores bióticos e abióticos do ambiente. Assim, buscando estudar as fases fenológicas do pinhão-manso, durante dois anos agrícolas foram avaliadas as seguintes cinco características agrônômicas e variáveis climáticas como temperatura e precipitação. Os dados de cada variável, em cada ano e cada genótipo, foram submetidos a análise de variância (ANOVA) e as diferenças testadas a 5% de probabilidade pelo teste F. Além disso, analisou-se a correlação do comportamento vegetativo e reprodutivo de dois acessos de *Jatropha* (CNPAC-102 e CNPAC-169) em função do tempo decorrido após o início do ciclo fenológico com as variáveis climáticas por meio de correlação de Pearson. Verificou-se que: (i) a retomada do crescimento das plantas com a produção de novas ramificações e inflorescências de ambos os genótipos coincide com o início do período chuvoso; (ii) o florescimento pode estar relacionado a aumento da temperatura e da precipitação; (iii) número de inflorescências por plantas e número de flores femininas determinam o número de frutos verdes; (iv) variações ambientais são responsáveis pela delimitação das fenofases e (v) as respostas às mudanças fenológicas são genótipo dependentes.

Palavras-chave: *Jatropha curcas*, florescimento, crescimento, produção de grãos.

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