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GENETIC DIVERGENCE AND MORPHO-AGRONOMIC PERFORMANCE OF *JATROPHA CURCAS* L. CLONES FOR SELECTION OF CLONAL VARIETIES[1]

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ABSTRACT - The knowledge about genetic diversity of jatropha crop is important for genetic conservation resources and breeding of this species. The aim of this study was to evaluate the genetic diversity and performance of jatropha clones through morphological characterization to selection of clonal varieties for biofuels production. The clones were obtained through shoot cuttings from previous selection in a population of half-sibs progenies. The morphoagronomic analyses of clones was carried out at 180 days after transplantation and were evaluated plant height, stem diameter, number of primary branches and number of secondary branches, number of bunches and number of fruits per plant. Evaluating clones performance, significant results were found for the number of secondary branches. About analysis of genetic diversity, the measures of dissimilarity genetic varied from 0.62 to 13.11, this way, the UFRBPR14 and UFRBPR15 clones were more divergent. The Tocher method was efficient to verify formation of four groups. The characteristics that most contributed to the divergence among clones were branches number, height and number of bunches, and, stem diameter had lower contribution. The jatropha clones differed only in the secondary branches number and multivariate analysis showed divergence among the jatropha clones with formation of four groups. Also, branches number, plant height and number of bunches were characteristic that contributed to genetic divergence.

Keywords: *Jatropha curcas*.L.. Genetic breeding. Genetic resources.

DIVERGÊNCIA GENÉTICA E DESEMPENHO MORFOAGRONÔMICO DE CLONES DE PINHÃO MANSO VISANDO SELEÇÃO DE VARIEDADES CLONAIS

RESUMO – A necessidade do conhecimento da diversidade genética da cultura do pinhão manso é importante para conservação dos recursos genéticos e melhoramento genético dessa espécie. O objetivo desse trabalho foi avaliar a divergência genética e o desempenho e de clones de pinhão manso, através de caracteres morfoagronômicos, visando a seleção de variedades clonais produtivas para a produção de biocombustíveis. Os clones foram obtidos, através de estaquia, a partir da seleção em uma população de progênies meio-irmãos. A caracterização morfoagronômica dos clones foi realizada aos 180 dias após o transplante e foram avaliados a estatura da planta, diâmetro do caule, número de ramificações primárias e ramificações secundárias, número de cachos e número de frutos por planta. Quanto ao desempenho, resultados significativos foram encontrados para o número de ramos secundários. Quanto às análises de divergência genética as medidas de dissimilaridade ge-nética apresentaram uma variação de 0,62 a 13,11, assim, os clones UFRBPR14 e UFRBPR15 foram mais divergentes. Pelo método de Tocher verificou-se a formação de quatro grupos. Os caracteres que mais contribuíram para a divergência entre os clones foram número de ramos secundários, estatura e número de cachos, a que menos contribuiu foi o diâmetro do caule. Os clones de pinhão manso diferiram apenas quanto ao número de ramos secundários, a análise multivariada detectou divergência entre os clones de pinhão manso com formação de quatro grupos. Além disso, caracteres que mais contribuíram para a divergência genética foram número de ramos secundários, estatura da planta e número de cachos.

Palavras-chave: *Jatropha curcas* L.. Melhoramento genético. Recursos genéticos.

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INTRODUCTION

The increased world demand for liquid fuels, problems due to global warming, energy security and political will for agricultural, social and energy development are the motive forces responsible for the renewed interest in the production of biofuels from oil plants (DABDOUB; BRONZEL, 2009). Therefore, studies on the use of renewable energy sources in recent years were intensified, especially on the use of natural resources that are based on the choice and improvement of producing lipid sources used for biodiesel production (TEIXEIRA et al., 2010).

Brazil stands out with potential for production of oil plants, by its great extent of agricultural lands, irrigation potential and edaphoclimatic conditions for cropping various species that has potential to be used as raw material for biofuels (DARCE, 2005), including the *Jatropha curcas* L. (DRUMMOND et al., 1984). This species produces a viable alternative raw material, complementary for biodiesel production, since it is a low cost, non-food crop of good geographical adaptation (DIAS et al., 2007, SCHIAVO et al., 2010, OLIVEIRA et al., 2013). The castor bean, sunflower and corn are also among the species that can be used for biofuel production.

According to Openshaw (2000), the *J. curcas* oil can be used for biodiesel production, but its potential is not exploited because the lack of research. Souza et al. (2012) stated that the *J. curcas* yield is very variable and dependent on the region, cultivation practices, precipitation and soil fertility. Drummond et al. (2010) found yields ranging from 330 kg ha^{-1} in dry conditions and 1,200 kg ha^{-1} in irrigated areas, confirming the *J. curcas* yield dependence on water regimens.

Despite the lack of basic technical information, *J. curcas* crops are being disseminated and implemented in several regions of Brazil (NERY et al., 2013), however, the number of *J. curcas* accessions introduced in Brazil is low, leading to the occurrence of related accessions from different regions of the country, indicating a low genetic divergence for this species (ROSADO et al., 2010; BORGES et al., 2014).

The *J. curcas* materials that are being cropped are genetically unknown, not uniform and have no minimum guarantees regarding environmental adaptability and yield for each genotype (LAVIOLA, 2010). Hamrick, (1983) and Cui et al. (2001) reported the importance and need of the knowledge on the genetic diversity within and between populations to support the conservation of genetic resources and genetic improvement of this species. Therefore, the identification of the diversity by morpho-agronomic characteristics is widely used to identify crossings to obtain segregating populations, reduce the crop vulnerability to diseases and improve the genetic progress for certain characteristics.

In this context, the objective of this work was to evaluate the performance and genetic divergence of *J. curcas* clones through its morpho-agronomic characteristics, aiming to select clonal varieties for biofuel production.

MATERIAL AND METHODS

Developing of clonal saplings and transplanting of clones

The clones were acquired from selection in a population of half-sib progenies of *J. curcas* through asexual reproduction (cuttings). The parental selection was based on works and assays carried out since May 2008 in the experimental field of the Breeding and Biotechnology Unit (NBIO) of the Agricultural Environmental and Biological Sciences Center (Reconcavo of Bahia Federal University) (12º39'657"S, 39º05'062"W). In these experiments, the performance of the progenies was evaluated through morpho-agronomic characterization and genetic diversity, using the RAPD marker. These works allowed the selection of the superior parents UFRBPR03, UFRBPR05, UFRBPR08, UFRBPR09, UFRBPR11, UFRBPR13, UFRBPR14 and UFRBPR15, which are the most divergent and promising, with better production performance regarding the characteristics evaluated (number of fruit clusters per plant, number of fruits per plant, number of seeds per plant, fruit weight per plant, seed weight per plant).

Young, healthy and vigorous branches of *J. curcas* (diameter average of 2.0 cm and length of 20-30 cm) were taken from half-sib plants in January 2014, cleaned, treated with fungicide (Thiophanate Methyl 4 g L^{-1}) for five minutes and planted in black plastic bags containing a substrate (Veracel®) consisting of coconut shell fiber, vermiculite, organic compound, purified single superphosphate, and other macronutrients and micronutrients (quantitative information not provided by the company). The saplings were placed in a greenhouse (50% shading) and water was supplied daily, or as needed.

Weekly irrigation with a nutrient solution (HOAGLAND; ARNON, 1950) were performed to prevent nutritional deficiency in plants (LANA et al., 2009; MORAES et al., 2010) 40 days after planting the cuttings (DAP). The volume needed for each plant was calculated by weighing the bags and applying the amount needed to reach 100% of field capacity when the they were with 80%. The maximum retention capacity found was 200 mL per cutting.

The saplings were transplanted to a field area of the NBIO at 150 DAP. The soil of the area was a dystrophic Red-Yellow Latosol and the region's climate was characterized by a transition zone between areas Am (hot and humid with short dry

season) and Aw (hot and humid with summer rains), according to the Köppen classification.

Holes of 40 x 40 x 50 cm, spaced 3 x 2 m were dug in a mowed area, in which the vegetation cover was maintained to avoid excessive heating of the soil and water loss and affect the establishment of the saplings transplanted. Planting fertilization was performed with 40 g of P_2O_5 (single superphosphate 18% P_2O_5). An additional fertilization and an application of 40 g of potassium chloride (60% K_2O) and 40 g of urea (45% N) per plant were performed 60 DAT.

An organic fertilization was performed 120 DAT, using 2 kg of cattle manure per plant. Fertilizer distribution was performed on the soil under the canopy of the clones.

Morpho-agronomic characteristics

The morpho-agronomic characteristics of the eight *J. curcas* clones obtained from superior parents was assessed at 180 DAT (early ripening of fruits). The variables used as agronomic descriptors were the plant height (HGT), measured with a millimeter tape, stem diameter (SD), measured with a digital caliper, and the number of primary branches (NPB), secondary branches (NSB), fruit clusters per plant (NCP) and fruits per plant (NFP).

A randomized block experimental design was used with four replications, with experimental plots consisting of three plants.

Data analysis

The data were subjected to univariate analysis of variance to assess the existence of genetic variability among clones and then to multivariate analysis of variance for analyze and evaluate the global genetic variability among them. These analyzes were performed using the software GENES (CRUZ, 2013) and treatment averages were compared by the Scott-Knott cluster test (1974) at 5% probability.

The genetic diversity of the clones was determined based on the cluster analysis and canonical variables presented by Cruz et al. (2004). The Mahalanobis distance was used as dissimilarity measure.

The group delimitation was performed applying the Tocher optimization technique, keeping the intragroup average distance below any average distance intergroup. The relative importance of characteristics was evaluated by the method of Singh (1981).

The cophenetic correlation coefficient (CCC), obtained from 1,000 simulations and analyzed by "t" test through the GENES software (CRUZ, 2013), was calculated to assess the accuracy of the clusters.

RESULTS AND DISCUSSION

The analysis of variance showed genetic difference among clones only for the characteristic of number of secondary branches (NSB) (Tables 1 and 2). The other characteristics (plant height, stem diameter, number of primary branches, number of fruit clusters per plant and number of fruits per plant) were similar among clones at 180 DAT.

Table 1. Analysis of variance of the morpho-agronomic characteristics of *J. curcas* clones produced from superior parents, in its first production cycle, performed six months after transplantation (180 DAT).

Source of variation	GL	Mean Square					
		HGT	SD	NPB	NSB	NCP	NFP
Block	3	0.0109	18.1297	0.0222	6.3591	25.4221	480.6198
Clones	7	0.0102^{ns}	5.3900^{ns}	0.0870^{ns}	2.4512^{*}	8.0761^{ns}	83.5123^{ns}
Average		1.37	52.47	2.40	4.55	8.35	19.48
CV (%)		7.12	6.46	10.94	22.07	30.28	44.88

HGT = plant height (m); SD = stem diameter (cm); NPB = number of primary branches, NSB = number of secondary branches; NCP = number of fruit clusters per plant; NFP = number of fruits per plant. * = significant at 5% probability; ^{ns} = no significant at 5% probability.

According to Saturnino et al. (2005), the number of secondary branches is one of the most interesting production characteristic for breeding programs, since *J. curcas* is a culture that produces inflorescences in terminal buds of branches grown in the same year (characteristic of perennial and semi perennial crops), thus, the production of fruit depends on the number of branches. No relation between the number of secondary branches and fruit production was found in the present work, since only the first variable was significantly different among the clones.

Albuquerque et al. (2009) evaluated the initial growth of *J. curcas* and found significant results for plant height, but similar results regarding stem diameter. Drumond et al. (2009) evaluated different genotypes of *J. curcas* and found no significant differences for height and stem diameter in 3-month-old plants, however, after 9 months these variables resulted in significant data. Juhász et al. (2010) evaluated five *J. curcas* populations and found that the plant age may influence the results, since significant results in 3 and 6-month-old were found. Souza et al. (2013) evaluated saplings of *J.*

curcas and found no significant differences in stem diameter.

The data on clone characteristics found (Table 1) differed from that observed by Saturnino et al. (2005), who evaluated the use of cuttings of *J. curcas* and reported that this procedure may limit its soil exploitation, reduce its longevity, and generate plants that are more susceptible to damping off, pests and diseases. The non-significant results found this work (plant height, stem diameter, number of primary branches, number of fruit clusters per plant and number of fruits per plant), denote that the production of clones by cuttings can standardize the plants and consequently the cultural practices, fruit production and harvest.

Table 2. Averages of the morpho-agronomic characteristics of *J. curcas* clones produced from superior parents, in its first production cycle, performed six months after transplantation (180 DAT).

Clone	HGT	SD	NPB	NSB	NBP	NFP
UFRB-CPM 3	1.35a	51.56a	2.24a	3.58b	7.16a	17.91a
UFRB-CPM 5	1.37a	54.55a	2.33a	4.75a	6.58a	12.58a
UFRB-CPM 8	1.40a	51.88a	2.25a	4.66a	9.25a	19.75a
UFRB-CPM 9	1.33a	51.16a	2.42a	3.58b	7.00a	16.41a
UFRB-CPM 11	1.33a	52.89a	2.25a	5.41a	9.50a	19.87a
UFRB-CPM 13	1.41a	53.47a	2.50a	5.25a	10.33a	28.50a
UFRB-CPM 14	1.30a	51.60a	2.58a	3.83b	7.58a	21.71a
UFRB-CPM 15	1.45a	52.77a	2.58a	5.33a	9.42a	19.08a

HGT = plant height (m); SD = stem diameter (cm); NPB = number of primary branches, NSB = number of secondary branches; NCP = number of fruit clusters per plant; NFP = number of fruits per plant. Means not followed the same letter differ by the Scott-Knott test at 5% probability.

The genetic divergence analysis, evaluated through the result of hierarchical clustering using the method of UPGMA, from the matrix containing the values of the generalized Mahalanobis distance (D^2), showed the limit value of 0.38 (Genetic Distance) for the characterization of the groups (Table 3). The genetic dissimilarity varied from 0.62 to 13.11, indicating a great genetic divergence among the clones analyzed, according to Benitez et al. (2011).

The UFRB-CPM14 and UFRB-CPM15 clones were the most divergent (D^2 = 13.11), followed by the UFRB-CPM3 and UFRB-CPM15 (D^2= 9.03). The UFRB-CPM3 and UFRB-CPM9 clones were the least divergent (D^2= 0.62), followed by the UFRB-CPM14 and UFRB-CPM9 (D^2= 2.08).

Table 3. Distance estimates of *J. curcas* clones with the lowest and highest distances, based on the generalized Mahalanobis distances (D^2), evaluated in its first production cycle, performed six months after transplantation (180 DAT).

Clone	Lowest D^2	Nearest clone	Highest D^2	Farthest genotype
UFRB-CPM3	0.62	9	9.03	15
UFRB-CPM5	2.86	9	6.66	14
UFRB-CPM8	2.96	11	8.40	14
UFRB-CPM9	0.62	3	8.08	15
UFRB-CPM11	2.96	8	8.80	14
UFRB-CPM13	3.18	8	5.36	15
UFRB-CPM14	2.08	9	13.11	15
UFRB-CPM15	3.42	8	13.11	14

The UFRB-CPM14 and UFRB-CPM15 clones were the most divergent, found in four combinations. These genotypes can be used in bi-parental crossings, since their contrast can potentiate the obtaining of highly segregating populations and possibly obtaining transgressive genotypes for multiple characteristics (BELETE et al., 2011).

According to Ferrão et al. (2002), studies on genetic diversity are important in plant breeding, by provide parameters to identify parents that enable the rise of superior genetic materials when crossed, and knowledge on the genetic basis of the population evaluated.

The lower genetic distance, the greater the similarity among the groups, thus, four groups (I, II, III and IV) were formed. Figure 11 shows that UFRB-CPM3 and UFRB-CPM9 clones had greater similarity, considering the characteristics evaluated, denoted by the lower distance. The groups I and IV stood out, with the highest distance, and the UFRB-CPM14 clone with the farthest distance from the UFRB-CPM15 clone.

The cophenetic correlation coefficient (CCC) shows how faithfully the matrix is to make clusters. This correlation, evaluated with repetition, was 61% ($r = 0.61$, $P<0.0001$, 1000 simulations), considering an appropriate value, with a good fit between the graphical representation of the genetic similarity and the matrix of similarity. Moreira et al. (2010) found CCC (r) of 0.92, evaluating *J. curcas*. According to Vaz Patto et al. (2004), values of $r>0.56$ are considered ideal, denoting consistence with the genetic similarity values.

These results show the importance of early characterization of *J. curcas* clones for an early establishment of the distinction between the originating materials and the main morpho-agronomic characteristics to be used for improve the species and select clonal varieties.

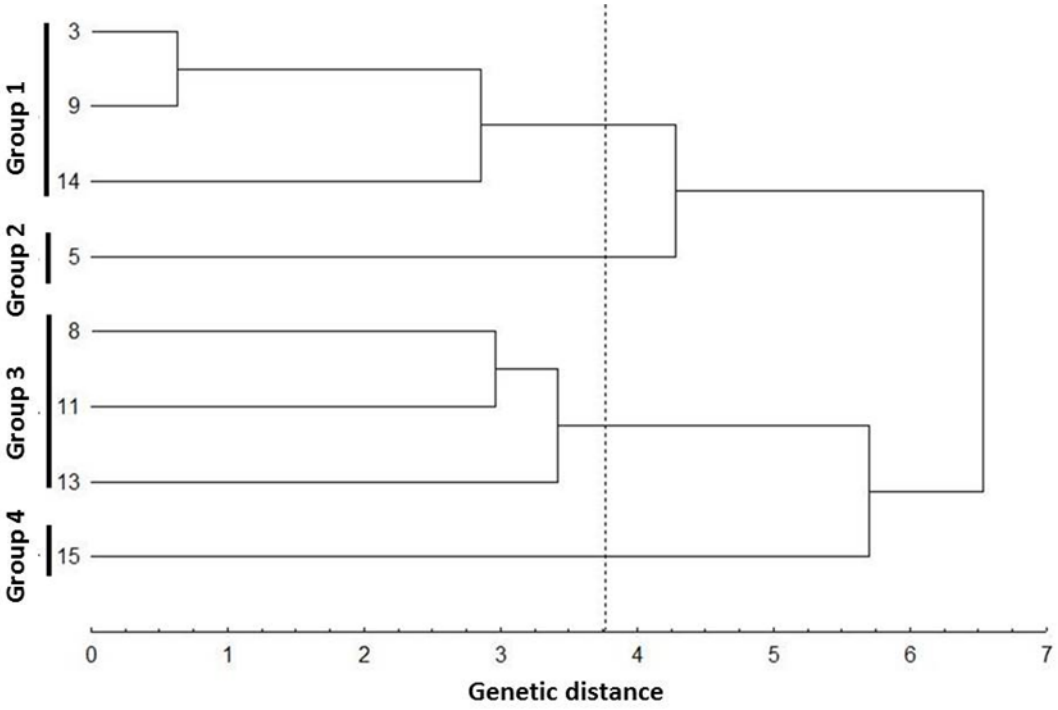

Figure 1. Dendrogram developed from quantitative data with the generalized Mahalanobis distance and by the UPGMA clustering method for the eight *J. curcas* clones. Dotted line: Cut on the dendrogram.

The clustering by the Tocher method showed the formation of four groups, from six characteristics, group I (UFRB-CPM3, UFRB-CPM9 and UFRB-CPM14), group II (UFRB-CPM5), group III (UFRB-CPM8, UFRB-CPM11 and UFRB-CPM13) and group IV (UFRB-CPM15) (Table 4). The intergroup distance was 10.07 and the intragroup was 2.11 for group I.

The intergroup analysis showed greatest genetic divergences among groups I and IV (10.07) and I and III (6.10), and greater genetic similarity among groups I and II (4.28), inferring that the offspring from crossing clones of groups I and IV would have a broader genetic base than those from crossing clones of groups I and II (Table 5).

Regarding the intragroup distance, group I (2.12) had the lowest distance among accessions from this group and the higher distance intragroup was found in the group III (3.27). According to Vasconcelos et al. (2007), the Tocher method presents the average distance within the groups always lower than the average distance among the groups, thus, there will be more consistency among the accessions of same group than the accessions of different groups.

Table 4. Genetic similarity groups of *J. curcas* clones, established by the Tocher method from the matrix of the Mahalanobis distances considering six characteristics, developed in its first production cycle, evaluated six months after transplantation (180 DAT).

GROUP	CLONES (UFRB-CPM)
I	3; 9; 14
II	5
III	8; 11; 13
IV	15

Table 5. Intergroup analysis of *J. curcas* clones, established by the Tocher method from the matrix of the Mahalanobis distances considering six characteristics, developed in its first production cycle, six months after transplantation (180 DAT).

GROUP	I	II	III	IV
I	2.12	4.28	6.10	10.07
II		-	4.93	4.58
III			3.27	5.70
IV				-

The characteristics that contributed to the divergence among the eight clones *J. curcas* from half-sib progenies are shown in Table 6. The characteristics number of secondary branches, plant height and number of fruit clusters per plant presented 26.04%, 24.35% and 21.57% of contribution, respectively. The variable that least contributed to the divergence was stem diameter, which had variation of 1.48%.

Similar results were found by Santana et al. (2013), who found the number of secondary branches among the descriptors that most contributed to the divergence among nine hybrids of *J. curcas*, and stem diameter as the least contributor (2%). Laviola et al. (2011) also found similar results evaluating young stages of *J. curcas* plants from the germplasm bank of Planaltina DF, in an experimental area in partnership with the Embrapa Agroenergy and Embrapa Cerrado.

Table 6. Relative importance (S.j) of agronomic characteristics for the study of genetic diversity of eight *J. curcas* clones assessed in its first production cycle, evaluated six months after transplantation (180 DAT).

Characteristics	S.j	Value (%)
HGT	36.70004	24.3525
SD	2.233543	1.4821
NPB	19.27639	12.791
NSB	39.24543	26.0415
NCP	32.51904	21.5782
NFP	20.72872	13.7547

HGT = plant height (m); SD = stem diameter (cm); NPB = number of primary branches, NSB = number of secondary branches; NCP = number of fruit clusters per plant; NFP = number of fruits per plant.

The results of the present work are important for further studies on *J. curcas* performances in different soil and climatic conditions, since this plant has varied agronomic expressions. The variable stem diameter, for example, despite of its low contribution to breeding experiments, may indicate a water stress condition and acts as a buffer (MAES et al., 2009), increasing the extraction of water and nutrients from the soil, which are needed to maintain a vigorous vegetative growth of *J. curcas* (SOUZA et al., 2013). According to Arruda et al. (2004), the agronomic characteristics of *J. curcas* can vary greatly

depending on the growing region, production method, cultural practices, crop age, precipitation and soil fertility, factors that may have influenced the results of the present work.

The identification of descriptors that are little or no influenced by the environment are essential to differentiate and protect future populations of *J. curcas*, since the quantitative descriptors such as plant height, stem diameter, number of primary and secondary branches, number of fruit clusters and number of fruits per plant are greatly influenced by genotype x environment interactions. Therefore, multivariate analyzes are essential for detection and evaluation of descriptors in plant breeding, since the univariate analysis showed differences in only one characteristic (branch number), analyzing the characteristics independently, while the multivariate analysis showed genetic divergence among clones, analyzing the characteristics as a group.

CONCLUSIONS

Considering evaluations in the first production cycle in these crop conditions, the characteristic number of secondary branches has potential to detect variability among *J. curcas* clones;

The multivariate analysis detected a divergence among the *J. curcas* clones, forming four similarity groups;

The characteristics number of secondary branches, plant height and number of fruit clusters per plant showed a higher contribution to the genetic divergence in the *J. curcas* clones evaluated after the multivariate analysis.

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