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Selection of clones and parents in full-sibling families of *Jatropha curcas*

Abstract – The objective of this work was to estimate some genetic parameters of *Jatropha curcas*, in order to predict which productive characteristics could show the greatest genetic gain to identify which family would contribute the most to the selection of clones and parents, and to identify the best genotype for selection. Six full-sibling families with 18 individuals each were evaluated. Five production traits were evaluated in the third year after planting. Data obtained were analyzed through mixed models. The restricted maximum likelihood (REML) and the best linear unbiased prediction (BLUP) procedures were used to estimate the genetic parameters and to predict the selection gains, respectively. Environmental effects predominated over genetic effects for most evaluated traits. A hundred-seed press cake weight showed high additive heritability estimates. The BLUP analysis individual components, in the selection of the best progenies for use as potentials parents, showed significantly similar values to those of the selection of the best progenies for use as clones. The family F is the most relevant in the selection of potential parents and clones by the evaluated characters. The F9 progeny is the best individual for use as a parent and for vegetative propagation (clone).

Index terms: physic nut, heritability, biodiesel, oil content.

Seleção clonal e de família de irmãos completos de *Jatropha curcas*

Resumo – O objetivo deste trabalho foi estimar alguns parâmetros genéticos em *Jatropha curcas*, para predizer quais características produtivas apresentariam maior ganho genético para identificar qual família mais contribuiria para a seleção de clones e genitores, e identificar o melhor genótipo para a seleção. Seis famílias de irmãos completos, com 18 indivíduos cada, foram avaliadas. Cinco características de produção foram avaliadas no terceiro ano após o plantio. Os dados obtidos foram analisados por meio de modelos mistos. Utilizaram-se os procedimentos de máxima verossimilhança restrita (REML) e a melhor predição linear não viesada (BLUP), para estimar os parâmetros genéticos e predizer os ganhos de seleção, respectivamente. Os efeitos ambientais predominaram sobre os efeitos genéticos na maioria das características avaliadas. A massa da torta de cem sementes apresentou altas estimativas de herdabilidade aditiva. Os componentes individuais da análise BLUP, na seleção das melhores progênies para uso como potenciais genitores, apresentaram valores significativamente semelhantes aos da seleção das melhores progênies para uso como clones. A família F é a mais relevante na seleção de potenciais genitores e clones por meio dos caracteres avaliados. A progênie F9 é o melhor indivíduo para uso como genitor e para propagação vegetativa (clone).

Termos para indexação: pinhão-manso, herdabilidade, biodiesel, teor de óleo.

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Introduction

The search for alternative sources of energy and for cleaner fuels has been gaining ground worldwide (Borges et al., 2016). Biofuels are promising alternatives to replace petroleum-based fuels, which are largely responsible for greenhouse gas emissions (Vidal, 2019). Biofuels favor Brazil, as the country holds the main competitive advantage in terms of green energy. The vast availability of arable land in Brazil and the favorable climatic conditions for biofuel crops are some of the advantages (Medeiros et al., 2021).

Jatropha curcas L. is an oilseed species that grows well in dry tropical regions and arid soils, withstanding long periods without rain (Heller, 1996). The semiarid region of northern Minas Gerais presents such conditions. This oilseed can be a good alternative for agricultural production in that region. The potential of *J. curcas* as a biofuel crop is directly linked to its high productivity, rapid growth, and high energy content (Alherbawi et al., 2021).

The extraction of oil from its seeds also generates an important byproduct known as press cake, yielding approximately 2 Mg per Mg of extracted oil (Gomes et al., 2018). The press cake can be used for production of biogas. It can also be used as animal feed due to its high nutritional value. Its crude protein content varies significantly among genotypes, but it is generally a protein-rich byproduct (Mélo Neto, 2023). Ahluwalia et al. (2020) found 41.98% of crude protein in fresh press. The possibility of using all parts of *J. curcas* fruits contributes to the sustainability of the production chain of this species (Witt et al., 2023).

Despite of the wide range of uses and the commercial potential of this species, most commercial plantations of *J. curcas* consist of genetically unknown materials, resulting in high variation among plants within the same stand (Laviola & Alves, 2011; Rocha et al., 2012). Therefore, developing breeding programs focused on highly productive materials is essential to improve its farming.

Obtaining segregating populations from inbred lines is not feasible for some allogamous species, including *J. curcas,* due to their long juvenile phase (Arockiasamy et al., 2021). It is necessary to use genetic designs involving outcrossing populations, such as full-sibling families.

Identifying genetic variability within a germplasm is an important step in breeding programs, typically focusing on selecting genotypes as parents for targeted crosses (Moraes et al., 2017; Silva et al., 2021). Studies have shown significant selection gains with half-sibling families, with productivity and stability over time (Spinelli et al., 2015; Peixoto et al., 2018). In this sense, it is also considered relevant to analyze the use of full-sibling families for genetic improvement of the species in question.

In this context, estimating genetic parameters is important for the genetic improvement of *J. curcas*, especially for traits related to seed. The genetic improvement of the species is still in the beginning, with limited results in the programs carried out (Kumar & Kamari, 2020). There are few studies evaluating full-sibling families of *J. curcas*, resulting in lack of information on the effects of inbreeding on genetic gains.

The objective of this work was to estimate genetic parameters in six full-sibling families of *J. curcas* in order to predict which productive characteristics present the greatest genetic gain, to identify which family contributes the most to the selection of clones and parents, and to identify the best genotype for selection.

Materials and Methods

The experiment was carried out in the facilities of the Universidade Estadual de Montes Claros (Unimontes) – the experimental farm and the biotechnology laboratory –, in the municipality of Janaúba, in the state of Minas Gerais, Brazil (15°48'13"S, 43°19'3"W, at 510 m altitude). The climate of the region is classified as Aw, tropical savanna, with rainy summers and dry winters, according to the classification of Köppen-Geiger (1948). The soil of the experimental area is classified as a Typic Hapludox (Santos et al., 2018).

The parents used in the present study were selected based on their high seed production and oil yield. They originated from artificial hybridizations through six biparental crosses, resulting in six full-sibling families, each with 18 individuals (Juhász et al., 2009) (Table 1).

Seed were collected and sown in 5 L pots, which were filled with a substrate composed of cattle manure, soil, sand, and mineral fertilizer (N-P-K). The pots were placed in a nursery installation under shade screen. In April 2015, six months after sowing, the seedlings were taken to the field and planted 4 m x 2 m apart, in the experimental farm of the Unimontes.

The trial consisted of six families (A, B, C, D, E, and F) of full-siblings, in a randomized block design, with three replicates, and six plants per plot (experimental unit is one plant). A total of 108 genotypes was evaluated. Irrigation was performed weekly, using a microsprinkler system, with one microsprinkler per plant, and a flow rate of 70 L per hour, for two hours.

In the field, liming was performed to increase the base saturation (V) to 60%. Fertilization in the planting hole consisted of 300 g of granulated simple superphosphate and 5 L of cattle manure. In the first year, two topdressings were performed, one 30 days after planting, and another one 120 days after planting. The first topdressing consisted of 30 g per plant of the N-P-K formula 20-0-20; and the second one, of 40 g per plant of the same formula. In the second year, two topdressings were performed, one at flowering and another one 30 days after flowering. A dose of 80 g per plant of N-P-K formula 20-5-20 was used in each topdressing. In the third year, two topdressings were performed, one at flowering and the other 30 days later, and the dose was 100 g per plant of the same N-P-K formula of the second year in each top dressing, as recommended by Rocha (2011).

Formative pruning was performed at 50 cm height in a cup shape. Production pruning consisted of eliminating 20 cm from the tip of each branch. When necessary, the controls of pests, diseases, and weed were performed following the recommendations by Rocha (2011).

The following production traits were evaluated in the third year after planting (2018): total fruit yield (kg ha^{-1}), estimated by multiplying the fruit production per plant by the number of plants per hectare, based on the planting spacing adopted; oil content extracted from 100 seed (100-seed oil content, g), determined by the methodology proposed by Zambrano et al. (2015); total oil yield (kg ha^{-1}), estimated by multiplying the oil content extracted from seed of all fruit harvested per plant by the number of plants per hectare; 100-seed press cake weight (g), weight of a press cake composed of the remaining residues of 100 seed after the oil extraction; total press cake yield (kg ha^{-1}), corresponding to press cake yield per hectare, estimated by multiplying the press cake weight after oil extraction from seed of all fruit harvested per plant by the number of plants per hectare.

The assumptions of linearity, normality, homoscedasticity, and independence of errors for each trait were verified. Linearity was checked using residues plot (residuals vs fitted). The software Selegen (Resende, 2016) was used to generate the plot. Shapiro-Wilk's test was used to check the normality. Levene's test was used to check homoscedasticity. Durbin-Watson's statistic was used to check the independence of residues. For the tests, the R software (R Core Team, 2020) was used. All assumptions were met. The significance of the random effects of the statistical model was tested using the likelihood ratio test (LRT), as described by Rao (1973), defined as follows:

$$LRT = -2[\log_e L_{p+1} - \log_e \log_p],$$

where L_{p+1} and L_p represent the maximum points of the restricted likelihood function, associated to the complete model (L_{p+1}) and to the reduced one (L_p). Significance was tested using the chi-square statistics, with 0.5 degree of freedom for traits with estimates inside the parameter space, and 1.0 degree of freedom for the other traits (Stram & Lee, 1994). The probability threshold used for type I error was equal to 5%.

Data were analyzed using a mixed model. As recommended by Resende (2002), the restricted maximum likelihood (REML) was used to estimate genetic parameters, and the best linear unbiased

Table 1. Description of crosses conducted in the field to obtain the six full-sibling families of *Jatropha curcas*, in the municipality of Janaúba, in the state of Minas Gerais, Brazil, 2024.

Family	Parent/cross	Municipality of origin of parents	Progeny identification
A	Accession 73♀ × Accession 64♂	Janaúba × Araçuaí	A1 to A18
B	Accession 27♀ × Accession 23♂	Janaúba × Janaúba	B1 to B18
C	Accession 27♀ × Accession 74♂	Janaúba × Janaúba	C1 to C18
D	Accession 74♀ × Accession 64♂	Janaúba × Araçuaí	D1 to D18
E	Accession 23♀ × Accession 27♂	Janaúba × Janaúba	E1 to E18
F	Accession 23♀ × Accession 56♂	Janaúba × Carbonita	F1 to F18

prediction (BLUP), to predict selection gains (BLUP). Model 147 of the Selegen software was applied, as follows:

$y = X_r + Z_g + W_p + e$,

where, y is the data vector (observations); r is the vector of replicate effects (fixed effects) plus the overall mean; g is the vector of individual genotypic effects (random effects); p is the vector of plot effects (random effects); e is the vector of errors or residues (random effects); and X, Z, and W are the incidence matrices for the respective effects.

The variance components (individual REML analysis) were the following: genotypic variance among full-sibling progenies (Vg), equivalent to half of the additive genetic variance plus one quarter of the dominance genetic variance, assuming no epistasis; environmental variance among plots (Vplot); residual variance within plots (Vwithin); individual phenotypic variance (Vp); individual narrow-sense heritability ($h^2a = h^2$), calculated by disregarding one quarter contribution of the dominance genetic variance; coefficient of determination of plot effects ($c^2plot = c^2$); heritability of the mean of full-sibling progenies, assuming complete survival (h^2mp); accuracy of progeny selection, assuming complete survival (Ac_{prog}); additive heritability within plots (h^2ad), calculated by disregarding one quarter contribution of the dominance genetic variance; and overall mean of the experiment

Individuals exhibiting the highest genetic gains in the selection were selected for further BLUP analysis to predict selection gains.

Results and Discussion

Considering the five production traits evaluated, the 100-seed press cake weight showed the highest contribution to genetic variation (Table 2). The within-plot residual variance contributed the most to individual phenotypic variance, with 63.91% for fruit yield, 69.70% for 100-seed oil content, and 60.77% for total oil yield.

Oil is the most important byproduct extracted from *J.* curcas. It is a high quality oil and easier to be processed than other raw materials used as biofuel (Kumar & Choudhary, 2023). In an experiment conducted in the Oio region, Guinea-Bissau, Baldini et al. (2014) evaluated the oil extraction performance of *J. curcas* seed. The best operational results were

Table 2. Estimates of variance components (individual REML analysis) for production traits of six full-sibling families of *Jatropha curcas*, in the municipality of Janaúba, in the state of Minas Gerais, Brazil, 2024.

Variance component(1)	Production trait(2)				
	FW	100SOC	TOY	100SCW	TPCY
Vg	143.05	0.03	16.33	62.66	134.04
Vplot	27,774.93	2.43	1,861.15	21.08	13,991.03
Vwithin	49,459.02	5.66	2,908.87	39.01	18,123.16
Vp	77,377.01	8.12	4,786.36	122.76	32,248.24
$h^2a = h^2$	0.0036	0.0084	0.0068	1.0208	0.0083
$c^2plot = c^2$	0.35	0.29	0.38	0.17	0.43
h^2mp	0.01	0.02	0.02	0.87	0.02
Ac_{prog}	0.10	0.17	0.14	0.93	0.15
h^2ad	0.00	0.00	0.00	1.60	0.00
Overall mean(2)	470.85	14.69	104.67	43.27	294.51

Production traits: FW, total fruit yield per hectare; 100SOC, 100-seed oil content; TOY, total oil yield per hectare; 100SCW, 100-seed press cake weight; TPCY, total press cake yield per hectare. (1)Vg, genotypic variance among full-sibling progenies, equivalent to half of the additive genetic variance plus one quarter of the dominance genetic variance, assuming no epistasis; Vplot, environmental variance among plots; Vwithin, residual variance within plots; Vp, individual phenotypic variance; $h^2a = h^2$, individual narrow-sense heritability, calculated by disregarding one quarter of the dominance genetic variance; c^2plot = c2, coefficient of determination of plot effects; h^2mp, heritability of the mean of full-sibling progenies, assuming complete survival; Acprog, accuracy of progeny selection, assuming complete survival; h^2ad, additive heritability within plots, calculated by disregarding one quarter of the dominance genetic variance. (2)Overall mean: average of all plants for each of the variables.

obtained at a seed feeding rate of 25.7 kg h^{-1}, producing approximately 8 kg h^{-1} of oil (31.12%).

Fruit yield and oil content showed a great environmental effect. That happens because of their quantitative nature, and the large number of genes involved in their expression, requiring long periods in the field to be observed (Almeida, 2017). Likewise, the 100-seed press cake weight showed a greater contribution of genetic effects than environment effects (Table 2).

The 100-seed press cake weight had the best genetic parameters for selection purposes. This trait showed the highest narrow-sense heritability and a significant genotypic variance, denoting the potential for relatively high additive genetic gains. These results are significant for breeding programs focused on improving press cake yield.

The genotypic variance (Vg) showed the highest contribution (51.04%) to the individual phenotypic variance (Vp), with heritability estimates of 102.0% for individual narrow-sense heritability, 87.0% for heritability of the mean of full-sibling progenies, and 160.0% for additive heritability within plots. The high accuracy of progeny selection (93.0%) and the medium level of the coefficient of determination of plot effects (17.0%) denote a moderate environment effect among plots and within blocks (Table 2).

The 100-seed press cake weight is the trait that showed the highest individual gain, becoming a candidate criteria to be considered for genotype selection. The best progenies and individuals, with potential for use as clones or parents for the subsequent recombination cycle, were identified based on the highest genetic gains using a selection index of 10%, 15%, and 30% for the selected trait (Table 3). This result highlights the promising use potential of this trait for the clone selection process, since the chances of finding individuals within these populations that contribute with favorable alleles to this trait are increased (Silva, 2015).

Four families (A, B, E, and F) were selected, using a selection index of 10% for 100-seed press cake weight. Eleven individuals stood out with the highest genetic gains (Table 3). Family F yielded three selected individuals, and individual F9 showed the highest selection gain. Sixteen individuals were selected from families A and F, when using a selection index of 15%. Family F contributed 50% of individuals with the highest selection gains. Five families (A, B, C, E, and F) were selected using a selection index of 30%. Again, family F contributed 50% of the best individuals, followed by families B (25%) and A (12.5%).

Traits that provide higher genetic gains are valuable for clone selection. The individuals selected in the present study can be propagated asexually through

Table 3. Gain estimate (individual BLUP analysis) for selection of clones of *Jatropha curcas* on the basis of the selected production trait (100-seed press cake weight) evaluated in the third year after planting, in the municipality of Janaúba, in the state of Minas Gerais, Brazil, 2024.

SI (%)	Rating	Family	Individual	g	μ+g	SG (%)
10	1	F	09	24.59	67.87	36.23
	2	E	01	24.25	67.53	36.07
	3	F	03	23.50	66.77	35.78
	4	B	14	19.27	62.54	34.60
	5	F	01	19.10	62.38	33.84
	6	B	08	18.91	62.19	33.30
	7	E	02	17.95	61.23	32.76
	8	B	06	17.56	60.84	32.29
	9	B	05	16.89	60.16	31.85
	10	A	07	15.43	58.71	31.33
	11	F	02	14.95	58.23	30.85
15	16	F	01	12.93	56.20	28.95
30	32	F	04	7.31	50.58	24.00
100	100	A	10	-80.81	-37.23	0.45

SI: selection index; g, predicted genotypic effect; μ+g, predicted genotypic value; SG, selection gain.

cloning, ensuring that the desirable genotypic characteristics of the parent plant are fully transmitted to the offspring.

The population-level selection yielded lower genetic gains for the evaluated production traits than the individual selection. Families F and B showed the highest selection gains for 100-seed press cake weight (Table 4). These results indicate the potential of these families for use as clones and crossbreeds.

The individual BLUP components for selecting the best progenies – for use as parents in relation to 100-seed press cake weight – were highly similar to those for selecting the best progenies for use as clones (Table 5).

The ranking of the top individuals within each family showed consistency with those identified for use as clones (Table 3). Three families (A, B, and E) were selected using a selection index of 10%. Giving the selected individuals, 81.82% were similar to those identified for use as clones, except for individuals F18 and F14.

Family F contributed 68.75% of the superior individuals, when using a selection index of 15% (Table 5). However, the selection index of 30% resulted in a selection of four families (A, B, E, and F), in which family F contributed 53.12% of the individuals, followed by families B (28.62%), A (12.50%), and E (6.25%). Therefore, the selection results of the best progenies for use as clones can be applied to the selection of the best progenies for use as parents, in subsequent recombination programs, except for family C.

High genotypic values should be prioritized in breeding programs, since they represent the true values to be predicted. The use of selection indices, as employed in the present study, is an effective strategy to improve selection effectiveness, by ranking breeding materials with the highest predicted gains based on multiple traits simultaneously (Anshori et al., 2022). The results of this study enable the identification

Table 4. Gain estimate (individual BLUP analysis) for the selection of the best families of *Jatropha curcas* on the basis of selected production trait (100-seed press cake weight) evaluated in the third year after planting, in the municipality of Janaúba, in the state of Minas Gerais, Brazil, 2024.

Rating	100-seed press cake weight		
	Family	g(1)	SG (%)(2)
1	F	11.48	20.96
2	B	6.33	17.05
3	D	-2.83	10.33
4	C	-3.10	6.40
5	A	3.39	3.75
6	E	-8.46	0.00

(1)g: predicted genotypic effect. (2)SG: selection gain.

Table 5. Gain estimate (individual BLUP analysis) for selection of the best progenies of *Jatropha curcas* on the basis of the selected production trait (100-seed press cake weight) evaluated in the third year after planting, in the municipality of Janaúba, in the state of Minas Gerais, Brazil, 2024(1).

SI (%)	Rating	Family	Individual	g	μ+g	SG (%)
10	1	F	09	20.84	64.12	32.51
	2	E	03	20.06	63.34	32.09
	3	F	13	16.92	60.20	30.82
	4	B	14	15.57	58.85	29.78
	5	F	08	15.32	58.60	29.08
	6	B	01	14.90	58.18	28.52
	7	E	06	14.35	57.63	28.03
	8	B	02	13.96	57.24	27.59
	9	B	05	13.87	57.15	27.24
	10	A	18	13.71	56.99	26.93
	11	F	14	13.30	56.57	26.63
15	16	F	11	12.01	55.29	25.31
30	32	A	09	5.56	48.83	21.11
100	100	A	10	-58.69	-15.42	0.45

(1)SI, selection index; g, predicted genotypic effect; μ+g, predicted genotypic value; SG, selection gain.

of potential clones of *J. curcas* and the best parents at individual-level and family-level selections. This information is valuable for breeding programs of this species and can be used for selections or crossbreeding.

The conclusions of the present study were based solely on one year of data for the evaluated traits. As mentioned before, the environment effects are predominant given the type of the traits, which would demand more years of evaluation to confirm or not the obtained results. Because of the lack of published articles on the subject, the authors believe that is worthwhile publishing the manuscript as it is, in order to support future studies.

Conclusions

1. The 100-seed press cake weight is a recommended trait for selection of *Jatropha curcas*.
2. Family F contributes the most for potential clones and parents of *J. curcas*.
3. The progeny F09 is the best individual to be explored as parent and for cloning of *J. curcas*.

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