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Genetic parameters and selection of macaw palm (*Acrocomia aculeata*) accessions: an alternative crop for biofuels

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Abstract: *This study aimed to estimate the parameters related to the genetic control of the physical characteristics of the fruits, oil content, and oil yield, and to proceed with the selection of macaw palm accessions. Forty-four macaw palm accessions of the active germplasm collection of the Federal University of Viçosa were collected for the evaluation of epicarp dry matter, pulp dry matter, endocarp dry matter, kernel dry matter, oil content, and oil yield. Narrow-sense individual heritability estimates were considered as of intermediate magnitude. The coefficient of repeatability and the accuracy in family selection was of high magnitude. Oil yield per plant presented the highest coefficient of individual genetic variation. Five different accessions contributed to the ten first individuals selected by the individual BLUP. The Mulamba and Mock's rank index allowed classifying the accessions in a sequence favorable to selection.*

Key words*: Bioenergy, Arecaceae, genetic variability.*

INTRODUCTION

Brazil is a country of typically tropical climate, and therefore, presents a considerable diversity of oil-producing species, such as macaw palm [*Acrocomia aculeata* (Jacq. Lodd. Ex. Mart.) (Arecales: Arecaceae)], a native plant to the Brazilian Cerrado. The species occurs mostly in its natural form, in almost all the national territory. Therefore, its commercial exploitation is still little (Costa et al. 2014).

Lately, the high oil yield extracted from its fruit has called the attention of industry, due to the estimated oil yield, ranging between 1,500 and 5,000 kg ha^{-1}, highlighting its great potential to be used in biodiesel production (Teixeira 2005). Motoike and Kuki (2009) reported that macaw palm has similar productive potential to that of African oil palm (*Elaeis guineensis*), which is among the highest oil-yielding plants in the world (FAO 2013).

According to Domiciano et al. (2015), macaw palm is under domestication process, and most studies involving the species aims at its breeding. The Federal University of Viçosa-Brazil has been developing studies on macaw palm breeding with the implementation of an active germplasm bank to improve the yield performance of the species. According to Nugroho et al. (2014), breeding has been one of the technologies that most contributes to the actual increase in oil yield.

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For being an incipient culture, genetic studies related to oil yield are scarce. Manfio et al. (2012) and Domiciano et al. (2015) studied the genetic control of morphological and physiological traits. Thus, knowing the genetic structure of macaw palm populations is necessary to support the species breeding, especially for the most economically important product of the culture, the oil extracted from its fruits.

Active germplasm banks are crucial in a breeding program since they conserve the diversity and constitute the source of genetic material for breeding. The characterization of preserved accessions allows estimating the genetic parameters of interest and advancing with the selection of superior genotypes. According to Farias Neto et al. (2013), breeding depends on the correct choice of the best individuals to be used as parents, and the estimate of the genetic parameters are fundamental for a successful program.

The REML/BLUP methodology is widely used in genetic studies. It is based on the estimate of the variance components by the residual maximum likelihood (REML) approach, combined with the best linear unbiased prediction (BLUP) for prediction of genetic values and selection. This methodology is the most appropriate, mainly because macaw palm is a perennial culture, which originates unbalanced data in the field experiments.

According to Carvalho et al. (2003), knowing the genetic divergence between a group of parents is essential to predict hybrid combinations of higher heterozygosity and higher heterotic effect. Thus, the study on the genetic diversity based on morphological and physiological traits provides knowledge about the best combinations for these traits. Moreover, it does not require hybrid combinations between the parents and can help establish selection strategy for the improvement of oil yield in macaw palm.

However, besides being divergent, the cross between materials that present good performance is desirable to ensure the success of the progenies (Nascimento et al. 2014). To select genotypes considering a combination of desirable traits, a selection index that enables evaluating a genotypic aggregate should be used (Freitas et al. 2013), such as the Mulamba and Mock (1978)'s rank index, which is usually applied to a simultaneous selection of traits.

This study aimed to estimate the parameters related to the genetic control of the physical characteristics of the fruits, oil content, and oil yield; and to proceed with the selection of macaw palm accessions.

MATERIAL AND METHODS

Macaw palm Active Germplasm Bank

Fruits were collected from the macaw palm Active Germplasm Bank (BAG – Macaúba) of the Federal University of Viçosa, located in the municipality of Araponga - MG (lat 20º 40'1'' S, long 42º 31'15'' W, alt ~ 980m asl). The BAG – Macaúba is registered under the number 084/2013 – SECEX/CGEN, and is made up of 253 accessions, collected in several parts of Brazil. This bank is considered as the largest macaw palm germplasm bank in the world.

The fruits used in this study were collected in the BAG – Macaúba, consisting of 44 accessions (half-sib families). The accessions, collected from a natural population in the states of Minas Gerais and São Paulo were established in the field in February 2009. This study used 44 accessions of the BAG – Macaúba, which produced fruits with a variable number of individuals per family, totaling 142 individuals.

Evaluation of the physical characteristics of the fruits, oil content, and oil yield

After being collected, fruits were taken to the postharvest laboratory of the Federal University of Viçosa, where they remained stored at room temperature for 30 days for postharvest oil accumulation. After this period, they were frozen in a refrigerator in order to paralyze the metabolism and to conserve the fruit.

For the evaluations, fruits were separated into epicarp, pulp, endocarp, and kernel and oven-dried at 105 °C/24 hours. After that, the epicarp, pulp, endocarp, and kernel dry matters of five fruits per plant were weighed on a precision scale.

The evaluation of pulp oil content (%) of three fruits per plant was performed by Near Infrared Reflectance - NIR (**Varian ® FT-IR 660**), and the spectra were obtained in the pulp of each fruit. Three fruits per plant were used in this analysis.

Oil yield per plant (kg) was obtained by the product between total number of fruits per plant, pulp dry matter, and oil content.

Statistical analysis via REML/BLUP

The mixed model methodology, REML (Restricted Maximum Likelihood) / BLUP (Best Linear Prediction not biased) (Resende 2007) were used to estimate the components of variance and genetic parameters.

The model associated with the BAG – Macaúba, considering the evaluation of individual plants of the several accessions, was:

$y = X\mu + Za + e$, in which:

y, μ, a, e: are vectors of data, of fixed effects (general mean), of additive genetic effects (random), and of random errors, respectively.

X and Z are the incidence matrices for b and a, respectively.

The mixed model equations are:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}((1-h^2)/h^2) \end{bmatrix} \begin{bmatrix} \hat{\mu} \\ \hat{a} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

The estimators of the variance components by algorithm EM (Expectation Maximization) are:

$$\hat{\sigma}^2_e = [y'y - \hat{b}'X'y - \hat{a}'Z'y]/[N - r(X)]$$

$$\hat{\sigma}^2_a = [\hat{a}'A^{-1}\hat{a} + \hat{\sigma}^2_e trC^{22}]/N_a$$

In which:

$r\ (X)$: is the position or the number of linearly independent columns of X.

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}\,\sigma^2_e/\,\sigma^2_a \end{bmatrix}^{-1}$$

C: is the coefficient of mixed model matrix

Na: is the number of random elements (individuals).

A: is the additive genetic relationship matrix.

tr: is the trace operator matrix, given by the sum of the diagonal elements of the matrix.

N: is the total number of data.

The methodology recommended by Resende (2007) was applied to estimate the genotypic correlation matrix, using the additive genetic values of the families for all traits.

Superior accessions were identified by the sum of ranks index, adapted from Mulamba and Mock (1978)'s rank index. Selection intensities of 10% between families and 4% between individuals in the population were adopted.

The SELEGEN-REML/BLUP software (Resende 2016) was used for the statistical analysis.

RESULTS AND DISCUSSION

Narrow-sense individual heritability estimates ranged between 22% and 46% for all traits and were classified as of intermediate magnitude (Table 1). These values confer considerable genetic control of these traits. Based on the classification proposed by Resende (2002), heritability values between 0.15 and 0.50 are considered as moderate for perennial species. Resende (2002) states that for most quantitative traits of economic importance, individual heritability accounts for approximately 20%, corroborating the results of this study, especially for oil yield, which presented 30% narrow-sense individual heritability.

Table 1. Estimate of genetic parameters for the traits: epicarp dry matter (EpDM), pulp dry matter (PuDM), endocarp dry matter (EDM), kernel dry matter (KDM), oil content (OC), and oil yield per plant (OYP), evaluated in 44 accessions of the macaw palm active germplasm bank

Genetic parameters	EpDM (g)	PuDM (g)	EDM (g)	KDM (g)	OC (%)	OYP (kg)
σ^2_g	0.64	1.61	1.06	0.05	2.14	0.24
σ^2_{perm}	1.43	4.07	1.94	0.19	3.56	_
σ^2_e	0.27	0.92	0.66	0.10	2.67	1.06
σ^2_f	2.34	6.60	3.66	0.34	8.37	1.30
h^2_a	0.43	0.39	0.46	0.22	0.40	0.30
Repeatibility	0.88	0.86	0.82	0.70	0.68	_
Accuracy	0.88	0.86	0.89	0.8	0.89	0.8
h^2_m	0.77	0.74	0.79	0.64	0.79	0.64
CV_g (%)	15.42	19.77	13.47	13.67	2.61	44.55
Family gain (%)	11.94	14.62	10.67	8.75	2.06	28.51
Individual σ^2_g	1.92	4.82	3.17	0.14	6.43	0.73
Individual CV_g (%)	26.71	34.25	23.33	23.67	4.51	77.16
Individual gain (%)	11.56	13.19	10.66	5.20	1.83	22.82
BLUP gain (%)	23.50	27.82	21.33	13.95	3.89	51.33
Mean	5.18	6.41	7.63	1.59	56.17	1.11

σ^2_g: genetic variance among families whose components of the additive genetic variance and dominance genetic variance depend on the selfing rate; σ^2_{perm} = variance due to permanent effects; σ^2_e : residual variance; σ^2_f : individual phenotypic variance; h^2_a : narrow-sense individual heritability; h^2_m : Heritability of family means; CV_g : coefficient of genetic variation between families; Family gain: gain with selection among family; Individual σ^2_g: genetic variance among individuals; Individual CV_g: Coefficient of genetic variation among individuals; Individual gain: gain with selection of individuals within families (10% intensity). BLUP gain: gain with the selection of individuals in the population. Mean: experiment overall mean.

Families selection accuracy for all traits was of high magnitude, according to the classification proposed by Resende (2002), providing reliability for the estimated genetic values and the selection. The proximity between the predicted additive genetic values and the true genetic values of the individuals can be evaluated based on the accuracy (Resende 2002). At the early and intermediate stages of a breeding program, accuracy values of 70% or higher (Resende 2007) are desirable. Thus, accuracy values provide greater credibility to the selection of macaw palm accessions.

Oil yield per plant had the highest coefficient of individual genetic variation (77.16%), which means high genetic variation and indicates that the population has great potential for selection and obtainment of genetic gain. The coefficient of genetic variation expresses the magnitude of the genetic variation in relation to the trait mean; thus, the genetic variability can be verified and quantified (Farias Neto et al. 2013).

In perennial plants, such as macaw palm, the coefficient of repeatability is crucial since it allows determining the number of measurements required to accurately evaluate the permanent additive genetic values, genotypic values,

Table 2. Number of fruits necessary to obtain the values of coefficient of determination (r²), accuracy (ACUR) and efficiency (Ef)

Measurements	Oil content			Epicarp DM			Pulp DM			Endocarp DM			Kernel DM		
	r²	Acur	Ef	r²	Acur	Ef	r²	Acur	Ef	r²	Acur	Ef	r²	Acur	Ef
1	0.68	0.82	1.00	0.88	0.94	1.00	0.86	0.93	1.00	0.82	0.90	1.00	0.70	0.84	1.00
2	0.81	0.90	1.09	0.94	0.97	1.03	0.92	0.96	1.04	0.90	0.95	1.05	0.82	0.91	1.08
3	0.86	0.93	1.13	0.96	0.98	1.04	0.95	0.97	1.05	0.93	0.96	1.07	0.87	0.94	1.12
4	0.89	0.95	1.15	0.97	0.98	1.05	0.96	0.98	1.06	0.95	0.97	1.08	0.90	0.95	1.14
5	0.91	0.96	1.16	0.97	0.99	1.05	0.97	0.98	1.06	0.96	0.98	1.08	0.92	0.96	1.15
6	0.93	0.96	1.17	0.98	0.99	1.05	0.97	0.99	1.07	0.96	0.98	1.09	0.93	0.97	1.16
7	0.94	0.97	1.18	0.98	0.99	1.05	0.98	0.99	1.07	0.97	0.98	1.09	0.94	0.97	1.16
8	0.94	0.97	1.18	0.98	0.99	1.06	0.98	0.99	1.07	0.97	0.99	1.09	0.95	0.97	1.17
9	0.95	0.97	1.18	0.99	0.99	1.06	0.98	0.99	1.07	0.98	0.99	1.09	0.95	0.98	1.17
10	0.95	0.98	1.19	0.99	0.99	1.06	0.98	0.99	1.07	0.98	0.99	1.09	0.96	0.98	1.17

or phenotypic values of the individuals (Pires et al. 2011). The coefficient of repeatability for all the evaluated traits is considered as of high magnitude, and thus reliable. According to Resende (2002), values greater than 0.60 are considered as high for this parameter.

Manfio et al. (2011) evaluated the biometric trait of macaw palm fruits and observed coefficient of repeatability ranging between 0.68 and 0.99. Such results corroborate those detected in this work, which ranged from 0.68 to 0.88, considering only five traits.

Repeatability analysis provides information about the minimum number of fruits to be evaluated to achieve accurate information for genetic values prediction (Table 2). One fruit is enough to evaluate epicarp, pulp, and endocarp dry matter since the fruit provides coefficients of determination of 88, 86, and 82%, and selective accuracies of 94, 93, and 90%, respectively.

For oil content and kernel dry matter, the ideal is to sample three fruits to obtain greater efficiency, which increases from 1.00 to 1.13, with 93% accuracy and 86% determination for oil content. For kernel dry matter, efficiency increases from 1.00 to 1.12, with 94% accuracy and 87% determination, respectively.

One of the purposes of the coefficient of repeatability is the possibility of determining how many phenotypic observations should be carried out in each individual to optimize the selection of genotypes and reduce costs and labor (Cruz et al. 2012). Manfio et al. (2011) also found a minimum number of evaluations between one and four fruits for biometric traits of macaw palm fruits. The increase in the number of measurements, when repeatability is high, little influences the increase of accuracy when compared with a single observation of an individual (Cruz et al. 2012).

The highest genotypic correlations (Table 3) were observed for the variables endocarp dry matter (EDM) and epicarp dry matter (EpDM) (0.65), followed by endocarp dry matter (EDM) and kernel dry matter (KDM) (0.62), and oil yield per plant (OYP) and pulp dry matter (PuDM) (0.62). Therefore, selection for PuDM leads to positive changes in OYP.

Non-significant traits or with low correlation estimate evidence the independence among traits, as observed for all the estimates that correlate oil content to the other traits. The same was observed with the genotypic correlation of oil yield with epicarp dry matter, which was considered as of low correlation, despite being significant. The low genetic correlation between oil content and oil production per plant (0.18) (Table 3) in this population is due to the low coefficient of genetic variation for oil content (2.62 at the family level and 4.51 at the individual level) (Table 1). Therefore, the production of oil per plant depends more on the number of fruits per plant than on the content of oil in the pulp.

The genetic correlation between traits denotes the degree of genetic association between them (Pires et al. 2011) and allows the breeder to perform indirect selection when a trait presents favorable and significant correlation with another trait of interest and of easy evaluation (Nascimento et al. 2014).

Five different accessions (8, 37, 49, 43 and 26) represented the top ten selected individuals for oil yield per plant (kg) (Table 4). Top five individuals belong to the family of accession 8. Accession 8 plant 7 stood out for its phenotypic value (f) of 8.43 kg and additive genetic value of 3.13 (Table 4).

The selection of the 30 best individuals for the establishment of a seed orchard will lead to a genetic gain of 0.67 kg

Table 3. Genotypic correlation matrix for the traits: epicarp dry matter (EpDM), pulp dry matter (PuDM), endocarp dry matter (EDM), kernel dry matter (KDM), oil content (OC), and oil yield per plant (OYP), evaluated in 44 accessions of the macaw palm active germplasm bank

Traits	**EpDM**	**PuDM**	**EDM**	**KDM**	**OC**	**OYP**
EpDM		0.57**	0.65**	0.41**	0.00	0.35*
PuDM			0.60**	0.47**	-0.03	0.62**
EDM				0.62**	-0.04	0.27
KDM					0.24	0.44**
OC						0.18
OYP						

Table 4. Phenotypic values (f), additive effects (a), additive genetic values (u + a), predicted genetic gains, and new mean with the selection of the 30 best individuals of the accessions of the macaw palm active germplasm bank for oil yield per plant (kg)

Order	Accession	Plant	f	a	u+a	Gain	New mean
1	8	7	8.43	2.03	3.13	2.03	3.13
2	8	5	4.33	1.48	2.58	1.75	2.86
3	8	4	3.34	1.34	2.45	1.62	2.72
4	8	1	1.36	1.08	2.19	1.48	2.59
5	8	2	1.22	1.06	2.17	1.40	2.50
6	37	3	4.52	1.01	2.11	1.33	2.44
7	8	8	0.48	0.96	2.07	1.28	2.39
8	49	3	3.92	0.83	1.94	1.22	2.33
9	43	1	3.68	0.76	1.86	1.17	2.28
10	26	7	3.04	0.74	1.85	1.13	2.23
11	26	5	2.73	0.70	1.80	1.09	2.20
12	26	10	2.50	0.67	1.77	1.05	2.16
13	43	2	2.75	0.63	1.74	1.02	2.13
14	26	6	2.11	0.62	1.72	0.99	2.10
15	47	2	3.02	0.56	1.66	0.96	2.07
16	26	9	1.54	0.54	1.65	0.94	2.04
17	43	6	1.65	0.48	1.59	0.91	2.02
18	36	2	3.43	0.48	1.58	0.89	1.99
19	47	6	2.25	0.45	1.56	0.86	1.97
20	43	4	1.23	0.43	1.53	0.84	1.95
21	26	3	0.50	0.40	1.51	0.82	1.93
22	36	9	2.66	0.37	1.48	0.80	1.91
23	38	3	3.46	0.37	1.48	0.78	1.89
24	47	1	1.62	0.37	1.48	0.76	1.87
25	50	5	2.75	0.36	1.47	0.75	1.85
26	43	3	0.69	0.35	1.46	0.73	1.84
27	47	3	1.23	0.32	1.42	0.72	1.82
28	50	3	2.30	0.30	1.41	0.70	1.81
29	27	1	2.35	0.27	1.38	0.69	1.79
30	36	1	1.88	0.27	1.38	0.67	1.78

f: individual phenotypic value or field measurement;
a: predicted additive genetic effect;
u + a: predicted additive genetic value.

on the overall mean (1.11 kg), and the mean of improved population in the next generation of planting will be of 1.78 kg, providing a genetic gain of 61%. This fact confirms the great potential of the breeding of the macaw palm population.

By the Mulamba and Mock (1978)'s rank index, considering the rank's mean for the evaluated traits, accessions were classified in a sequence favorable to selection (Table 5). The lowest value of the rank's indicates a more favorable combination of the evaluated traits, and the highest value indicates an unfavorable combination. The selected accessions 43, 8, 37, 35, and 6 were allocated to the top positions in the rank's mean. This fact indicates that these accessions have the best performance for physical characteristics of fruits when they are combined with oil yield. Therefore, they are the most suitable for selection.

By observing the results previously reported, in relation to the divergence of the accessions of the germplasm bank, and taking into account the best genotypes according to the rank's mean, based on the Mulamba and Mock (1978)'s rank index, the best parents can be chosen to be crossed in a breeding program aimed at improving oil yield.

This work is the first genetic study on the active germplasm bank of macaw palm of the Federal University of Viçosa, considering the oil yield traits, and is essential to the domestication and integration processes of macaw palm in the economic sector, particularly regarding the breeding of the species.

Table 5. Classification of the 20 best accessions according to the rank's mean based on the Mulamba and Mock's rank index, in relation to the evaluated traits: epicarp dry matter (EpDM, g), pulp dry matter (PuDM, g), endocarp dry matter (EDM, g), kernel dry matter (KDM, g), oil content (OC, %), and oil yield per plant (OYP, kg)

Rank	Accession	*Rank*- EpDM	*Rank* - PuDM	*Rank* - EDM	*Rank* - KDM	*Rank* - OC	*Rank* - OYP	*Rank*- Mean
1	43	18	2	0	4	22	5	10.2
2	8	42	4	0	1	8	1	11.2
3	37	43	5	0	2	6	2	11.6
4	35	16	27	0	14	1	12	14.0
5	6	31	6	0	3	21	13	14.8
6	25	23	12	0	8	16	23	16.4
7	36	26	24	0	12	14	7	16.6
8	15	36	3	0	26	3	16	16.8
9	31	6	15	0	10	17	40	17.6
10	52	33	13	0	16	9	17	17.6
11	53	2	42	0	5	4	35	17.6
12	47	5	18	0	30	30	6	17.8
13	49	21	14	0	39	13	4	18.2
14	50	1	26	0	41	18	8	18.8
15	17	20	19	0	18	5	36	19.6
16	38	27	23	0	32	2	14	19.6
17	16	12	16	0	35	27	9	19.8
18	27	8	7	0	37	42	10	20.8
19	29	35	21	0	20	10	21	21.4
20	1	15	30	0	7	36	20	21.60

CONCLUSIONS

The accessions of the macaw palm active germplasm bank present genetic variability, which means the possibility of a successful macaw palm breeding program for oil yield.

The traits epicarp dry matter, pulp dry matter, endocarp dry matter, kernel dry matter, oil content, and oil yield per plant can be evaluated with three fruits per individual without losing selection efficiency.

Pulp dry matter and oil yield per plant presented good positive correlation. Thus, indirect selection of fruits with higher pulp dry matter will increase oil yield per plant.

The ten best individuals for oil yield per plant belong to five different accessions.

Accessions 43, 8, 37, 35, and 6 stood out at the top positions by the Mulamba and Mock's rank index.

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