



Breeding of *Acrocomia aculeata* using genetic diversity parameters and correlations to select accessions based on vegetative, phenological, and reproductive characteristics

S.M. Coser¹, S.Y. Motoike¹, T.R. Corrêa¹, T.P. Pires¹ and M.D.V. Resende²

¹Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, MG, Brasil

²EMBRAPA Florestas e Departamento de Engenharia Florestal, Universidade Federal de Viçosa, Viçosa, MG, Brasil

Corresponding author: S.M. Coser
E-mail address: cosersm@gmail.com

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ABSTRACT. Macaw palm (*Acrocomia aculeata*) is a promising species for use in biofuel production, and establishing breeding programs is important for the development of commercial plantations. The aim of the present study was to analyze genetic diversity, verify correlations between traits, estimate genetic parameters, and select different accessions of *A. aculeata* in the Macaw Palm Germplasm Bank located in Universidade Federal de Viçosa, to develop a breeding program for this species. Accessions were selected based on precocity (PREC), total spathe (TS), diameter at breast height (DBH), height of the first spathe (HFS), and canopy area (CA). The traits were evaluated in 52 accessions during the 2012/2013 season and analyzed

by restricted estimation maximum likelihood/best linear unbiased predictor procedures. Genetic diversity resulted in the formation of four groups by Tocher's clustering method. The correlation analysis showed it was possible to have indirect and early selection for the traits PREC and DBH. Estimated genetic parameters strengthened the genetic variability verified by cluster analysis. Narrow-sense heritability was classified as moderate (PREC, TS, and CA) to high (HFS and DBH), resulting in strong genetic control of the traits and success in obtaining genetic gains by selection. Accuracy values were classified as moderate (PREC and CA) to high (TS, HFS, and DBH), reinforcing the success of the selection process. Selection of accessions for PREC, TS, and HFS by the rank-average method permits selection gains of over 100%, emphasizing the successful use of the accessions in breeding programs and obtaining superior genotypes for commercial plantations.

Key words: Biofuel; Plant genetic resource; Genetic parameters; Genetic diversity; Heritability; *Acrocomia aculeata*

INTRODUCTION

Awareness of the consequences of exploiting non-renewable resources for fuel production, together with rising petroleum prices and increased emission of greenhouse gases, has stimulated researchers to develop alternative sustainable energy resources, and has boosted the demand for biofuels (Gan and Li, 2013).

Macaw palm, *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. (Arecaceae), stands out as a promising genetic resource in the biofuel sector. It is a native and rustic palm tree, with great potential for oil production and is widely distributed in the tropical and subtropical Americas, with Brazil being a center of origin (Lanes et al., 2015). These features make the macaw palm a major national genetic resource that can be managed for biofuel industries. Motoike and Kuki (2009) reported that the macaw palm has similar productive potential (4500 L oil production per ha/year; Roscoe et al., 2007) to African oil palm (*Elaeis guineensis*), which is among the highest oil-yielding plants in the world (FAO, 2013). This indicates the importance of the macaw palm in the biofuel sector. In addition, the high demand and use of its derivative products add value and sustainability to the production chain.

Conversely, macaw palm is currently exploited as an extractive activity (Wandeck and Justo, 1988); therefore, domestication and breeding of this plant are essential to establish competitive plantations. In this scenario, plant breeding is a crucial step in agribusiness, since it permits the development of cultivars that meet the demands of markets and farmers permitting the establishment of these plantations.

The genetic diversity of the species, which is conserved as germplasm, is the key factor on which the success of plant breeding programs relies. The Macaw Palm Germplasm Bank of Universidade Federal de Viçosa contains 302 accessions (open-pollinated progenies) totaling 1322 plants, collected from diverse Brazilian biomes, which makes this the main Germplasm Bank registered in the world for this palm tree.

Quantifying genetic diversity and determining its magnitude by efficient methods of estimation help to successfully select the best individuals to establish the next generation,

maximizing genetic gains and efficiency in the development of new cultivars (Farias Neto et al., 2013).

Few studies have estimated the genetic parameters of *A. aculeata* (Manfio et al., 2012; Berton et al., 2013). However, no studies have investigated individual heritability and average heritability levels related to traits such as precocity (PREC), total of spathes (TS), diameter at breast height (DBH), height of the first spathe (HFS), and canopy area (CA), which are main features in the initial stage of selection for the crop ideotype.

Therefore, this study aimed to generate information for the breeding of *A. aculeata* by quantifying genetic diversity, estimating genetic parameters related to the genetic control of the traits, and identifying superior genotypes from the Macaw Palm Germplasm Bank in Universidade Federal de Viçosa. Accessions with favorable features were selected for the development of cultivars for commercial plantations, contributing to the rational exploitation of the species as a renewal biofuel source.

MATERIAL AND METHODS

Germplasm bank

Evaluations were conducted using the Macaw Palm Germplasm Bank (BAG-Macaúba), registration No. 084/2013, SECEX/CGEN, from Universidade Federal de Viçosa, located in Araponga, State of Minas Gerais. Fifty-two accessions represented open-pollinated progenies, 305 plants collected across Minas Gerais and São Paulo (Table 1) were introduced in 2009 and evaluated during the 2012/2013 season. Genotypes were arranged in a completely randomized design with variation from 2 to 10 plants per accession, accordingly, using the plants available for each accession in the germplasm bank.

Table 1. Site of collection/source of the accessions from BAG- Macaúba.

Accession	Region	Accession	Region
BGP 1	Campos das Vertentes - MG	BGP 17	Belo Horizonte - MG
BGP 3	Campos das Vertentes - MG	BGP 21	Belo Horizonte - MG
BGP 5	Campos das Vertentes - MG	BGP 28	Belo Horizonte - MG
BGP 6	Campos das Vertentes - MG	BGP 29	Belo Horizonte - MG
BGP 14	Campos das Vertentes - MG	BGP 33	Belo Horizonte - MG
BGP 15	Campos das Vertentes - MG	BGP 36	Belo Horizonte - MG
BGP 24	Campos das Vertentes - MG	BGP 38	Belo Horizonte - MG
BGP 43	Campos das Vertentes - MG	BGP 48	Belo Horizonte - MG
BGP 53	Campos das Vertentes - MG	BGP 52	Belo Horizonte - MG
BGP 16	Centro - MG	BGP 45	Noroeste - MG
BGP 20	Centro - MG	BGP 19	Norte - MG
BGP 22	Centro - MG	BGP 25	Norte - MG
BGP 27	Centro - MG	BGP 30	Norte - MG
BGP 31	Centro - MG	BGP 49	Norte - MG
BGP 32	Centro - MG	BGP 26	Oeste - MG
BGP 37	Centro - MG	BGP 23	No identification
BGP 40	Centro - MG	BGP 41	No identification
BGP 46	Centro - MG	BGP 34	Sudeste - SP
BGP 50	Centro - MG	BGP 35	Sudeste - SP
BGP 54	Centro - MG	BGP 39	Sudeste - SP
BGP 2	Belo Horizonte - MG	BGP 42	Sudeste - SP
BGP 4	Belo Horizonte - MG	BGP 47	Sudeste - SP
BGP 7	Belo Horizonte - MG	BGP 51	Sudeste - SP
BGP 8	Belo Horizonte - MG	BGP 12	Triangulo Mineiro - MG
BGP 11	Belo Horizonte - MG	BGP 9	Zona da Mata - MG
BGP 13	Belo Horizonte - MG	BGP 44	Zona da Mata - MG

MG: Minas Gerais region; SP: São Paulo region.

Evaluations

The evaluated traits allowed information to be added to a species ideotype. The following vegetative biometric traits were considered: HFS (in meters, measured with a Haglof EC II digital clinometer), DBH (in centimeters, measured with a caliper rule), and AC (in square-meters, measured through an aerial photo documentation technique). Phenological and reproductive traits such as PREC, which was determined by the age of the plant (days after planting) when the first spathe was emitted, and TS, which were counted every 15 days during the productive cycle season, were also calculated.

Statistical analyses

All analyses were performed using the software Selegen-REML/BLUP [Sistema Estatístico de Seleção Genética Computadorizada via restricted estimation maximum likelihood (REML)/best linear unbiased predictor (BLUP)], 2014 version (Resende, 2002).

Genetic diversity and principal component analysis

Cluster analyses based on multivariate genotypic divergence were achieved by Mahalanobis distance with clusters delimited by Tocher's optimization method. Principal component analysis was based on the standardized genotypic values, permitting the identification of major variables by the highest absolute scores in the first components (Sokal and Rohlf, 1995).

Genetic correlation

Genotypic correlations were obtained through multivariate analyses between pairs of variables.

Genetic parameters and selection index

Estimations of variance components, genetic parameters, and genetic values were obtained by mixed-model methodology, REML/ BLUP procedures, following the model:

$$y = Xb + Zg + e \quad (\text{Equation 1})$$

where y , b , g , and e represent data vector, fixed effects (general mean), additive genetic effects (random), and random error, respectively. X and Z represent the incidence matrices for b and g , respectively.

Mixed model equation

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}((1-h^2)/h^2) \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix} \quad (\text{Equation 2})$$

where $h^2 = \hat{\sigma}_g^2 / (\hat{\sigma}_g^2 + \hat{\sigma}_e^2)$ represent the narrow-sense heritability.

Variance components estimators by EM algorithm

$$\hat{\sigma}_e^2 = [y'y - \hat{b}'X'y - \hat{g}'Z'y] / [N - r(X)] \quad (\text{Equation 3})$$

$$\hat{\sigma}_g^2 = \left[\hat{g}'A^{-1}\hat{g} + \sigma_e^2 \text{tr} C^{22} \right] / N_g \quad (\text{Equation 4})$$

where $r(X)$ represents the rank or number of linearly independent columns of X, and C^{22} is:

$$\begin{bmatrix} C^{11} & C^{12} \\ C^{21} & C^{22} \end{bmatrix} = \begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z + A^{-1}(\sigma_e^2 / \sigma_g^2) \end{bmatrix}^{-1} \quad (\text{Equation 5})$$

where N_g is the number of random elements (individuals), A is the genetic additive kinship matrix, Tr is the trace operator of a matrix, given by the sum of diagonal elements of the X matrix, and N is total number of observations.

Predicted additive genetic values were used to calculate the selection index based on the rank-average (Mulamba and Mock, 1978) for the accessions. The following traits were considered for the selection process: PREC, TS, and HFS. The selection process aimed to associate favorable traits resulting in improved crops for all traits considered simultaneously. Thus, selected plants that have earlier production (PREC), with a greater number of spathes, are considered to yield high production, and with shorter height for spathe emission, thereby simplifying the harvesting and management of bunches.

RESULTS

Genetic diversity and principal component analysis

Cluster analyses of the 52 Macaw palm accessions using Tocher's method (Table 2) resulted in four main groups, from which the first group (G1) brought together 92% of the accessions. Two accessions from Minas Gerais grouped together in a separate group (G2) followed by two accessions collected in the São Paulo region, which formed two distinct clusters (G3 and G4).

Table 2. Cluster analyses of 52 *Acrocomia aculeata* accessions established by Tocher's method based on the Mahalanobis distance.

Group	Accessions
G1	1 2 3 4 5 6 7 8 9 11 13 14 15 16 17 19 20 21 22 23 24 25 27 28 29 30 31 32 33 35 36 37 38 39 40 41 42 43 44 45 46 48 49 50 51 52 53 54
G2	12 26
G3	34
G4	47

Principal component analyses are able to distinguish the relevance of each character in relation to the total variation presented by the genotypes. CA was the trait that contributed most to the discrimination among accessions, showing the highest absolute score for the first and second principal components, followed by HFS, which showed the highest score in the third component. Together, CA and HFS were responsible for 92% of the observed variation (Table 3).

Table 3. Estimation of eigenvectors associated with the principal components, obtained by the genetic correlations between five traits of *Acrocomia aculeata*.

Components	PREC	TS	DBH	CA	HFS	Accumulated variation (%)
PC1	0.5301	0.3621	0.2365	-0.6968	0.2155	44.79
PC2	-0.4902	-0.4198	-0.2939	-0.7038	-0.0419	74.38
PC3	-0.4067	0.6005	0.2108	-0.1247	-0.6434	92.55
PC4	-0.206	0.5721	-0.6603	0.0519	0.4378	97.5
PC5	0.5205	-0.0686	-0.6142	-0.0301	-0.5884	100

PREC = precocity; TS = total spathe; DBH = diameter at breast highness; CA = canopy area; HFS = height of the first spathe.

Genetic correlation

The traits PREC and TS, DBH and HFS, and DBH and CA showed correlation values considered satisfactory for indirect selection (Table 4), with values higher than 0.5, which can be used in breeding programs when seeking to obtain gains in one trait by the selection of another.

Table 4. Genetic correlation matrix for five traits of *Acrocomia aculeata*.

Trait	PREC	TS	DBH	CA	HFS
PREC	1	-0.7499	-0.1116	-0.077	0.4385
TS	-0.7499	1	0.0422	0.0358	-0.3564
DBH	-0.1116	0.0422	1	0.5326	-0.6042
CA	-0.077	0.0358	0.5326	1	0.0377
HFS	0.4385	-0.3564	-0.6042	0.0377	1

PREC = precocity; TS = total spathe; DBH = diameter at breast highness; CA = canopy area; HFS = height of the first spathe.

Genetic parameters and selection index

According to Resende (2002), individual heritability values lower than 0.15 are classified as “low”, values from 0.15 to 0.50 are classified as “moderate”, and values higher than 0.5 are classified as “high”. For the estimated genetic parameters (Table 5), narrow sense heritability (h^2_a) presented moderate values for PREC, TS, and CA, and high values for DBH and HFS.

According to Resende (2002), accuracy values between 0.40 and 0.70 are classified as “moderate” and values higher than 0.70 are classified as “high”. The traits PREC and CA showed moderate accuracy values for progeny selection, while the traits TS, DBH, and HFS showed high values.

Table 5. Estimates of genetic parameters obtained for 52 *Acrocomia aculeata* accessions, related to the traits precocity (PREC), total spathe (TS), diameter at breast highness (DBH), canopy area (CA), and height of the first spathe (HFS).

Parameters	PREC	TS	DBH	CA	HFS
Vg	4923.74	5.28	25.44	3.41	0.57
Ve	20976.54	14.21	27.46	18.88	0.60
Vf	25900.29	19.49	52.90	22.29	1.17
CVg	4.48	26.78	12.67	12.82	34.79
h ² a	0.34 ± 0.14	0.48 ± 0.17	0.85 ± 0.19	0.27 ± 0.10	0.87 ± 0.22
h ² mp	0.44	0.55	0.77	0.46	0.72
Accuracy	0.66	0.74	0.88	0.68	0.85
M	1567.82	8.58	39.81	14.40	2.17

Vg = genetic variance among accessions; Ve = residual variance; Vf = individual phenotypic variance; CVg = coefficient of genetic variation; h²a = heritability of individual plants in the narrow sense; h²mp = heritability at progeny means level; M = overall mean.

By the average-rank selection index (Mulamba and Mock, 1978), based on genotypic values (Table 6), accessions were ranked for selection of the traits PREC, TS, and HFS. The lower rank-average values indicate a more favorable combination of traits established for the selection in the classification rank (Corrêa et al., 2015).

For the proposed ideotype, selection of the top 20 accessions resulted in selection gains higher than 100%. Two accessions corresponding to progenies from São Paulo were selected (35 and 47), and the others corresponded to the Minas Gerais region.

DISCUSSION

Diversity of plant genetic resources provides plant breeders the opportunity to develop new and improved cultivars with desirable characteristics, which include both farmer- and breeder-preferred traits (Govindaraj et al., 2015).

In the present diversity study, cluster analysis showed the formation of four main groups (Table 2). The first group (G1) contained the greatest proportion of the accessions (92% of the total), from which, 67% collected in São Paulo were grouped together with 96% of the accessions from Minas Gerais.

Additionally, four accessions stand out for their high dissimilarity, and formed separate groups, where two genotypes from São Paulo grouped separately in G3 and G4, showing they have high divergence from the other accessions and from each other. Even with the high similarity among the genotypes collected in Minas Gerais, two stood out, forming a separate group (G2), where both were collected in different and specific regions of Minas Gerais as compared with the other accessions (Table 1). Oliveira et al. (2012) and Lanes et al. (2015) also reported that subpopulations exist among *A. aculeata* accessions collected in the Minas Gerais region, based on molecular markers, strengthening the existence of genetic diversity among accessions collected in this region. Manfio et al. (2012) found a good correlation among geographic collection sites and genetic divergence of *A. aculeata* genotypes for juvenile traits in cluster analysis. Principal component analysis revealed that the trait contributing most to the formation of the observed clusters was CA, which had the highest score in the first and second principal components (Table 3), and was responsible for 74% of the observed variation.

Correlation calculations were based on genotypic estimates; components of which are only the genetic factors of the genotypes (Table 4). Genetic correlations of moderate magnitude were found between PREC and TS (-0.7499), DBH and HFS (-0.6042), and DBH and CA

Table 6. Classification based on the rank sum related to traits precocity (PREC), total spathe (TS), and height of the first spathe (HFS) for 52 *Acrocomia aculeata* accessions.

Order	Accession	PREC	TS	HFS	Average-rank
1	36	3	6	2	3.7
2	44	10	4	3	5.7
3	4	2	2	16	6.7
4	2	5	7	9	7.0
5	35	6	11	10	9.0
6	11	7	18	4	9.7
7	3	13	9	8	10.0
8	30	12	3	18	11.0
9	23	9	8	17	11.3
10	47	4	1	32	12.3
11	6	8	24	12	14.7
12	49	11	13	20	14.7
13	14	14	20	11	15.0
14	15	18	16	14	16.0
15	26	1	5	43	16.3
16	31	23	17	15	18.3
17	8	16	15	25	18.7
18	13	15	38	5	19.3
19	29	21	33	6	20.0
20	43	30	10	23	21.0
21	42	29	12	29	23.3
22	1	20	34	24	26.0
23	7	17	30	31	26.0
24	9	44	21	13	26.0
25	21	19	31	30	26.7
26	5	35	45	1	27.0
27	52	39	37	7	27.7
28	27	25	14	45	28.0
29	39	22	23	42	29.0
30	19	34	22	34	30.0
31	33	27	36	28	30.3
32	28	31	27	35	31.0
33	50	24	29	40	31.0
34	38	49	25	22	32.0
35	48	37	32	27	32.0
36	41	36	26	41	34.3
37	51	33	28	44	35.0
38	46	28	43	39	36.7
39	40	42	19	50	37.0
40	53	50	40	21	37.0
41	25	46	35	33	38.0
42	54	45	44	26	38.3
43	12	26	39	51	38.7
44	24	40	41	38	39.7
45	17	52	51	19	40.7
46	22	38	42	49	43.0
47	34	32	50	48	43.3
48	37	47	47	36	43.3
49	45	48	48	37	44.3
50	20	43	46	46	45.0
51	16	41	52	52	48.3
52	32	51	49	47	49.0

PREC = precocity; TS = total of spathe; HFS = height of the first spathe.

(0.5326). Information on correlations between traits is important for breeding programs, since it provides an understanding of how selecting a trait can cause simultaneous changes in other traits, in addition to informing on the pleiotropic action of genes (Farias Neto et al., 2008), for which selection strategies need to account for.

The negative association between PREC and TS indicates that the more precocious the accession, the greater the number of spathes, as the sooner production begins, the longer the available period to accumulate spathes during the productive season.

Thus, selection for precocious individuals would result in positive gains for total spathe. Breeding programs for perennial plants are expensive and involve long cycles, making the selection of superior genotypes an important activity, which should be carried out with all possible rigor for efficient experiments and accurate selection (Massaro et al., 2010). If positive associations are found between the number of spathes and production traits in future studies, it would be possible to proceed with early selection for the precocity trait.

The negative correlation between DBH and HFS indicates that selection for either of them could conversely affect the other, such that, the larger the DBH, the lower the HFS. Conversely, the positive correlation between DBH and CA indicates that selection for either of these traits can favorably affect the other, that is, the larger the DBH, the greater the CA.

DBH is a simple and fast trait to be measured, along with high heritability (85%); therefore, the correlation permits the selection of one of these traits when the other is difficult to select due to low heritability, and/or, measuring and identification problems, optimizing the process of the selection gain for this trait. In this case, due to the correlation between CA and HFS, where both traits are difficult to measure, DBH can be used as a trait for indirect selection.

Heritability is the genetic parameter of greatest importance for application in plant breeding programs (Corrêa et al., 2015). Its relevance is related to its ability to show how the genetic effects are presented in the individual's phenotype, since it is the genetic value that interests and influences the next generation (Falconer and Mackay, 1996). Narrow-sense heritability measures the proportion of the genetic variance that can be attributed to the additive genetic variance, which is associated with allelic effects that are transmitted to the next generation.

According to Resende (2002), the majority of quantitative characters of economic importance in perennial plants have individual heritability values of approximately 20%. The h^2_a values found in the present study were classified as moderate for the traits PREC, TS, and CA, and high for the traits DBH and HFS (Table 5), indicating that the traits have satisfactory genetic control and great potential to be transmitted to future generations, enabling selection of the best individual among families, as a selection unit.

The values for heritability at the progeny means level were similar in the present study and were classified as being of moderate (CA and PREC) and high (TS, DBH and HFS) magnitude. This demonstrates that the average phenotypic value of the progenies is a good predictor of genetic values and the phenotypic selection among progenies can be effective, allowing selection between families, which can be considered at the first selection stage as an option for the breeding program.

Heritability values of 38, 36, and 45% were found in previous studies for the trait number of bunches in oil palm, açai palm, and peach palm, respectively (Lopes et al., 2012; Farias Neto et al., 2008, 2013), consistent with the heritability value for TS found in the present study. In addition, Lopes et al. (2012) reported a heritability value of 75% for the trait height of the first bunch, which is classified as high, as the value found for HFS.

Genetic variability can also be confirmed and quantified by the coefficient of genetic variation (Cvg), which expresses the magnitude of genetic variation in relation to the trait average (Farias Neto et al., 2013). The estimated Cvg values in the present study reinforce the

genetic variability present among accessions (Table 5), as shown by cluster analysis (Table 2) and by the heritability values presented above. This confirms the potential of the accessions to establish a breeding population, since considerable genetic gain is expected by applying appropriate selection procedures.

The selection accuracy shows that there is a correlation between the real and predicted genetic values, and the higher the value, the more reliable the individuals' evaluation. According to the classification of heritability and accuracy in terms of magnitude and their associations as proposed by Resende (1997), the values found in this study were considered to be of moderate and high magnitude, indicating that there are advantages of performing selection using the evaluated traits. According to Resende (2002), values of this magnitude are appropriate in the early stages of breeding programs.

The selection process acts by promoting changes in the allelic frequencies at loci that control the character under selection, leading to changes in the genotypic average of the population in the desired direction. The selection method by rank-average index permits the selection of genotypes considering more than one character in the same selection cycle. This enables the selection to be optimized, resulting in simultaneous gains for all characters.

Considerable gains can be obtained through the selection of accessions for the proposed traits. The selection gains of the top 20 accessions exceeded 100%. Among those accessions, two from São Paulo were selected (BGP 35 and 47), and the remaining were from the Minas Gerais region, from where the top three accessions originated (BGP 36, 44 and 4).

This was the first study to investigate genetic diversity, genetic parameters, and correlations for vegetative, phenological, and reproductive traits in *A. aculeata* aiming to aggregate these traits to the final ideotype of the crop. For future studies, it will be necessary to investigate the correlation between these traits with the productivity and oil content to verify the possibility of early selection or indirect gain in the early stage of the breeding program for these productive traits.

In conclusion, accessions from BAG-Macaúba exhibit great genetic variability, as evidenced by diversity analyses, estimated heritability, and genetic variation coefficients. Such variability is essential for breeding programs of this species. The traits presented moderate-to-high narrow-sense heritability coefficients, revealing strong genetic control, which facilitates the breeding process. Correlations between traits permit genetic gains by indirect and early selection. Therefore, genetic selection gains considering all traits can be achieved, especially with the contribution of the accessions from Minas Gerais and São Paulo.

Conflicts of interest

The authors declare no conflict of interest.

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