Notas Científicas

Genetic diversity of macaúba fruits from 35 municipalities of the state of Goiás, Brazil

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Abstract – The objective of this work was to evaluate the genetic diversity of macaúba (*Acrocomia aculeata*) accessions from 35 municipalities of the state of Goiás, Brazil, in order to obtain information about the genetic variability and to provide subsidies for the commercial deployment of the culture in the Midwestern region of Brazil. The bunches were harvested with ripe fruits. The following were evaluated: fruit and almond lengths and diameters; the weights of fruits and almonds; the average number of almonds, as well as the oil contents in the almond and in the dehydrated fruit. The parameters that indicated significant contributions to the variability were fruit weight, fruit width, fruit length, and oil content in the almond.

Index terms: *Acrocomia aculeata*, agroenergy, almond quality, biodiesel, divergence, oleaginous seed.

Diversidade genética de frutos de macaúba provenientes de 35 municípios de Goiás

Resumo – O objetivo deste trabalho foi avaliar a diversidade genética de acessos de macaúba (*Acrocomia aculeata*) provenientes de 35 municípios de Goiás, a fim de obter informações sobre a variabilidade genética e fornecer subsídios para implantação comercial da cultura na região Centro-Oeste. Procedeu-se à colheita dos cachos com frutos maduros. Foram avaliados: os comprimentos e os diâmetros dos frutos e das amêndoas; os pesos dos frutos e das amêndoas; o número médio de amêndoas; bem como os teores de óleo na amêndoa e no fruto desidratado. Os parâmetros que indicaram contribuições significativas para a variabilidade foram peso do fruto, largura do fruto, comprimento do fruto e teor de óleo na amêndoa.

Termos para indexação: *Acrocomia aculeata*, agroenergia, qualidade da amêndoa, biodiesel, divergência, semente oleaginosa.

The search for viable alternatives for replacing fossil fuels by biodiesel is a growing concern, especially in relation to global climate change (Pimentel et al., 2016). The macaúba – *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. – has been used as a raw material in the production of biofuels due to its high content of oil in the fruit, production of various energy residues, besides being a native perennial plant found naturally in almost all the Brazilian territory (Pimentel et al., 2015, Evaristo et al., 2016). The oil of the macaúba mesocarp is rich in oleic acid, and is demanded by both the food and the agroenergy industry (Coimbra & Jorge, 2011).

Currently, a large part of macaúba cultivation is restricted to extractive activity, and the few plantations are not yet in full production, and others in some research centers (Oliveira et al., 2016). These research centers in Brazil have been evaluating the genetic potential of different genotypes through quantitative and qualitative analyses as well as physical and chemical analysis of macaúba fruits (Matsimbe et al., 2015).

Thus, the macaúba is considered of great interest by the scientific community, especially for its oleaginous property, in relation to the possibility of obtaining genetically superior materials. This results in the identification of accessions of macaúba trees that have peculiar characteristics that can lead to gains in production, which could be an important step to improve the cultivation of this species (Manfio et al., 2012).

Due to the absence of such scientific knowledge, especially regarding genetic diversity, the use of

macaúba on a competitively commercial scale becomes an obstacle (Manfio et al., 2011b). Thus, there is a need for studies that make it possible to make reliable technical recommendations on its cultivation, especially in the identification of genetic diversity between the different accessions based on quantitative and qualitative characters of the fruits for biodiesel production. Studies on the genetic diversity of macaúba in the state of Goiás are still incipient. Thus, the objective of this work was to evaluate the genetic diversity of macaúba accessions from 35 municipalities of Goiás, in order to obtain information on genetic variability and to provide subsidies for the commercial implantation of the crop in the Midwestern Region of Brazil.

Fruits were collected from 130 macaúba accessions in the morning, from 35 municipalities in the state of Goiás, as seen in Table 1.

The harvesting of the bunches was performed when the fruits were physiologically ripe, using as a subjective criterion the pericarp color, that is, when 50% of the fruits had mature maturation stage – totally yellow fruits with no whitish spots (Amaral et al., 2011).

After harvest, the fruits were removed from the bunches and stored in polypropylene bags with capacity of 50 kg which were properly identified and later packed in paper boxes to avoid insolation and dehydration. The boxes were transported in a truck without refrigeration until the reception area of the genetic resources laboratory of the Universidade Federal de Goiás/Regional Jataí. In the genetic resources laboratory, 10 fruits without mechanical injuries were selected from each accessed site.

With the aid of a digital caliper, the following characters were measured (in millimeters): fruit length (FL), dry almond length (DA), fruit diameter (FD), and dry almond diameter (DD). The fruit weights (FW) and average weights of the almonds (AW), expressed in grams, were obtained with a precision scale. For the destructive analyses, the fruit parts were separated in bark, pulp, endocarp and almond, and dried in a greenhouse at 105ºC for 24 hours, and then the dry mass (g) of the bark, pulp, endocarp and almond were individually weighed on a precision scale. Ten fruits per plant were used in this evaluation. To determine the oil content, the methodology 032/IV with adaptations was used (Zenebon et al., 2008), the mesocarp and almond were pressed in a hydraulic press for extraction of the oil and then its residues were washed in an extractor of oils and greases to extract the oil, using n-hexane as the organic solvent.

The oil content (OC) in percentage was calculated by the equation: $OC\ (\%) = (W1 - W2 / W1 - W) \times 100$, in which: W is cartridge weight in g; W1 is weight of the cartridge + dry sample before extraction of the oil in g; and W2 is weight of the cartridge + sample after extraction of the oil in g.

For the statistical analysis of data, the study first obtained the averages of each parameter grouped by municipality. Then the genetic divergence between the municipalities was evaluated, and their classification into groups of similarity was performed, using Ward's multivariate agglomerative hierarchical clustering method (Ward Jr., 1963). The dendrogram of the agglomerative clustering was generated with the values of the square of the semipartial correlation.

To adopt the appropriate number of groups, the Pseudo T^2 statistic (PST^2) was used (Duda & Hart, 1973). After the definition of the groups, the variance analysis (univariate) was performed in a completely randomized design, using the GLM procedure of the SAS program, following the analysis structure used by Alvares et al. (2012), in order to evaluate the effects of the groups on each parameter studied. Then, comparisons were made between the means of the groups, for each parameter, using the Tukey test, at a 5% probability level. All analyses were performed using the SAS software version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

The formation of 5 groups through the dendrogram of the grouping of 35 municipalities in the state of Goiás was observed, as a function of the square of the semipartial correlation (Figure 1), which indicate that the municipalities constituting the first junctions in the dendrogram are similar to each other, for having small values of dissimilarity, and the municipalities of Fania and Goiás show high similarity.

The municipalities were not grouped in their entirety according to their geographical location, and each of the five groups had distinct proportions of geographically close municipalities within the same group. The distribution of the geographically close municipalities within the groups was 28.5, 40.0, 75.0, 28.5, and 41.0%, respectively, for groups 1, 2, 3, 4, and 5. This result corroborates those obtained by Manfio et

al. (2012), which report a certain tendency of grouping macaúba accessions according to their geographic origin, regarding states of Minas Gerais and São Paulo.

Conceição et al. (2015), when studying genetic divergence in natural populations of macaúba in some regions (– in state of Minas Gerais: municipalities of Montes Claros, Alto Paranaíba, and Lavras; state of Goiás: – municipality of Formosa; and state of Tocantins: – municipality of Combinado), observed the formation of five groups regarding the characteristics: physical aspects of the fruits and oil content.

Table 1. Characterization of the municipalities with natural occurrence of macaúba (*Acrocomia aculeata*)

Municipalities[(1)]	Latitude (S)	Longitude (W)	Altitude (m)
Acreúna (1)	17°16'32"	50°14'36"	500
Araguapaz (2)	15°06'10"	50°37'13"	329
Cachoeira Alta (3)	18°50'17"	50°51'13"	491
Cachoeira Dourada (4)	18º28'5"	49°26'13"	595
Caçu (5)	18°36'4"	51°08'4"	539
Campos Belos (6)	13°00'41"	46°38'16"	616
Divinópolis de Goiás (7)	13°15'18"	46°24'46"	684
Edéia (8)	17°22'37"	49°51'53"	531
Faina (9)	15°20'44"	50°24'34	322
Firminópolis (10)	16°33'29"	50°19'37"	655
Formosa (11)	15°29'24"	47°20'28"	753
Formoso (12)	13°36'33"	48°49'40"	403
Goiânia (13)	16°42'8"	49°20'8"	768
Goiás (14)	15°53'42"	50°06'23"	566
Inaciolândia (15)	18°30'42"	49°54'53"	478
Indiara (16)	17°06'26"	49°54'48"	577
Iporá (17)	16°24'46"	51°10'57"	638
Itaberaí (18)	16°01'5"	49°51'50"	707
Itarumã (19)	18°46'18"	51°18'58"	496
Itumbiara (20)	18°23'59"	49°19'6"	692
Jataí (21)	17°53'6"	51°42'10"	670
Montividiu do Norte (22)	13°07'27"	48°36'38"	450
Nova Crixás (23)	14°07'52"	50°21'7"	290
Palmeiras de Goiás (24)	16°46'52"	49°56'18"	651
Palminópolis (25)	16°48'2"	50°10'50"	629
Pontalina (26)	17°32'59"	49°25'56"	585
Porangatu (27)	13°24'15"	49°07'11"	379
Posse (28)	14°06'8"	46°20'29	822
Santa Helena (29)	17°48'29"	50°35'46"	545
São Domingos (30)	13°25'24"	46°19'43"	727
São João da Paraúna (31)	16°46'43"	50°22'35"	639
São Luís de Montes Belos (32)	16°27'35"	50°23'18"	493
Serranópolis (33)	18°19'34"	51°58'10"	635
Trombas (34)	13°31'28"	48°45'5"	404
Turvânia (35)	16°35'49"	50°11'6"	588

[(1)]Numbered list of the municipalities used in the survey.

Manfio et al. (2012) evaluated the formation of 7 groups in a study on the genetic divergence of macaúba progenies (51) from states of Minas Gerais (45) and São Paulo (6), associated with the morphological characteristics: growth and vigor of the species. Domiciano et al. (2015) analyzed 3 groups when evaluating the genetic diversity of macaúba progenies (15) from some states (Minas Gerais, 12; Goiás, 1; and Distrito Federal, 2) and found that there was variability for the morphological and not for the physiological characteristics.

Manfio et al. (2011a), in their work on genetic variability between 145 matrices of macaúba in the states of Minas Gerais, São Paulo, Mato Grosso do Sul, Pará, Maranhão and Pernambuco, observed that this diversity is associated to the thickness of the endocarp, fruit diameter, volume of almond, and fruit weight.

Regarding the relative contribution, the parameters that contributed least to the increase in variability were almond weight and number of almonds, which may have been slightly affected by the environmental variables (Table 2). On the other hand, the parameters that most contributed to the variability were fruit weight, fruit width, fruit length and percentage of oil in the almond, which had the values of, respectively, 31.57; 23.86; 17.65; and 16.81% of contribution to the morphological variation, which may have been more influenced by the environmental variables such as soil pH, nutrients, water, photoperiod and thermoperiod. The dissimilarity between the municipalities ranged from 0.258 to 2.89, with averages of 1.34. The lowest values were recorded in the municipalities of Faina and Goiás. On the other hand, the municipalities of Palmeiras de Goiás and Turvânia were the most divergent ones. The lower value of dissimilarity observed between the municipalities of Faina and Goiás can be justified by the fact that the accessions collected in these municipalities had similar values for the variables studied according to multivariate statistical analysis.

For both the most similar and the most divergent municipalities, it was found that they are located geographically close to each other, demonstrating that the region where they are located has much of the variability contained in the species. This is in keeping with the reports made by Evaristo et al. (2016), who affirm that macaúba plants in their natural environment have great genetic variability, and there is great

dissimilarity of production between the individuals present in these areas.

For the univariate analyses of variance, there was a significant effect of groups at 5% and 1% probability, and the 5% probability level was found only in the characteristics of percentage of oil in the almond and percentage of oil in the dry fruit (Table 2). This result, according to Santos-Garcia et al. (2012), indicates that the clustering analysis was able to classify the municipalities into dissimilarity groups, which ratify their divergences even when the variables are analyzed independently.

The group 5 stood out for all the evaluated characteristics. As for group 3, it showed the best average values for almond weight, percentage of oil in the almond and percentage of oil in the dry fruit. Group 4 showed means intermediate between groups 5 and 3. In contrast to this, group 1 had lower mean

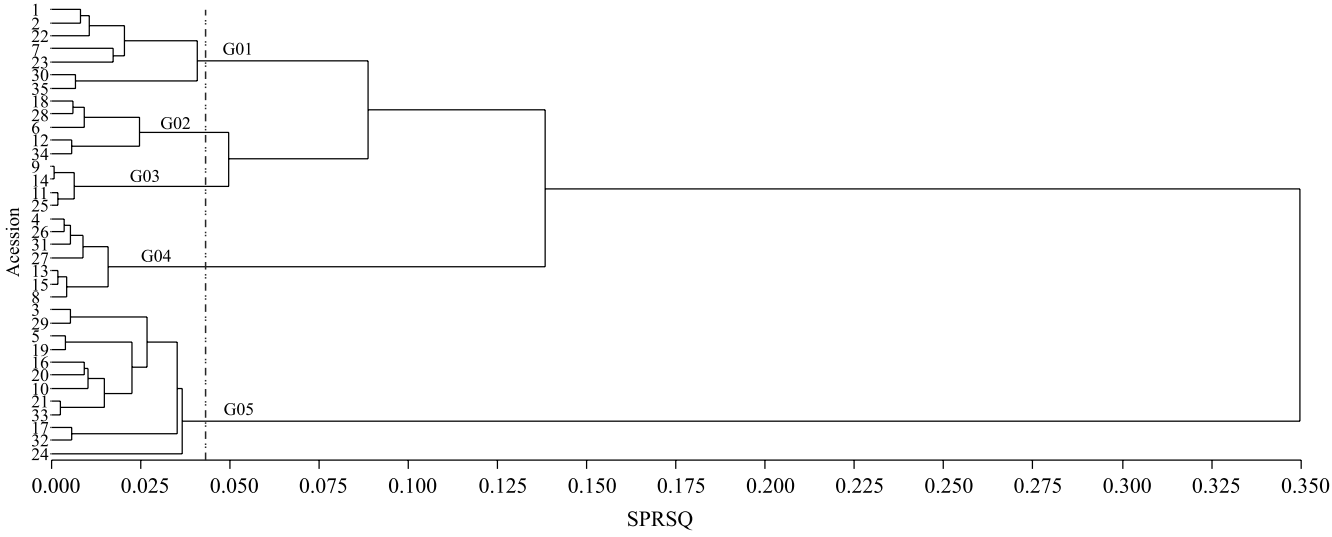

Figure 1. Dendrogram of the grouping of 35 municipalities, by the Ward method, using nine variables, in relation to the semi-partial R-squared value (SPRSQ). The vertical line represents the stopping point of the algorithm for the formation of the five formed groups. Municipalities: 1, Acreúna; 2, Araguapaz; 3, Cachoeira Alta; 4, Cachoeira Dourada; 5, Caçu; 6, Campos Belos; 7, Divinópolis; 8, Edeia; 9, Faina; 10, Firminópolis; 11, Formoso; 12, Formosa; 13, Goiânia; 14, Goiás; 15, Inaciolândia; 16, Indiara; 17, Iporá; 18, Itaberaí; 19, Itarumã; 20, Itumbiara; 21, Jataí; 22, Montividiu do Norte; 23, Nova Crixás; 24, Palmeiras de Goiás; 25, Palminópolis; 26, Posses; 27, Pontalina; 28, Porangatu; 29, Santa Helena; 30, São Domingos; 31, São João da Paraúna; 32, São Luís de Montes Belos; 33, Serranópolis; 34, Trombas; and 35, Turvânia.

Table 2. Mean data for relative contribution of variables analyzed for variability (S.j), univariate analysis of variance (Anova), and comparison of means of similarity groups, obtained from cluster analysis by the Ward method.

Variable	Relative contribution S.j (%)	Anova test			Comparison of groups' averages[(1)]				
		Average	CV (%)	SQ	1	2	3	4	5
Fruit length	17.65	38.43	4.31	307.06**	35.66B	35.54B	36.41B	37.59B	42.41A
Fruit width	23.86	38.22	7.06	307.07**	36.13B	35.25B	35.52B	37.08B	42.24A
Fruit weight	31.57	15.97	9.23	630.9**	11.83C	11.39C	12.40C	16.1B	21.40A
Almond length	2.92	10.37	7.98	43.79**	9.39CD	12.22A	10.69BC	8.87D	10.93AB
Almond width	5.64	13.86	9.03	77.29**	11.62C	13.98AB	12.33BC	14.05AB	15.50A
Almond weight	0.12	1.06	19.56	1.38**	0.81B	1.01AB	1.14AB	0.90B	1.31A
Almond number	0.15	1.34	14.32	2.22**	1.08B	1.07B	1.14B	1.73A	1.46A
% almond oil	16.81	49.28	5.76	128.78*	47.04B	46.41B	52.23A	50.15AB	50.30AB
% dry pulp oil	1.26	3.85	17.11	14.83*	2.67B	3.64AB	4.7A	4.23A	4.14A

[(1)]Means followed by equal letters in the rows do not differ by Tukey's test, at 5% probability. * and **Significant at 5% and 1% probability, respectively.

values for all characteristics evaluated. It can be verified that groups 3, 4 and 5 showed statistically different averages for pulp weight and statistically similar means for percentage of oil in the fruit, which indicates that the selection based on fruit weight may increase oil yield (in quantity).

The knowledge of genetic diversity between a group of parents is important for plant breeding, especially to identify hybrid combinations of higher heterozygosity and greater heterotic effect, since it allows knowing the available germplasm (Domiciano et al., 2015). In addition, it has the advantage of dispensing the need for obtaining hybrid combinations between the parents, and may support the selection strategy for the improvement of oil production in macaúba. For Nugroho et al. (2014), genetic improvement has been one of the technologies that have most contributed to the real increase in oil production for some oilseed crops. Thus, the results obtained may provide subsidies for future macaúba harvesting in the municipalities that make up the groups, once the breeder has information about where he can find contrasting macaúba accessions to compose future works and collections of germplasm banks.

By observing the previously reported results, on the genetic divergence found between the accessions of the 35 municipalities in this study for macaúba fruits, it is possible to justify the choice of the best accessions to compose a study collection and possible exploitation of the genetic resource, aiming at improvement of oil production. The genetic improvement depends on the correct choice of the best individuals that will be used as parents, and the genetic parameters estimation is the indispensable basis for the success of the breeding program, according to Farias Neto et al. (2013).

Thus, this is a pioneer work in the study of genetic diversity carried out in the state of Goiás, Brazil, considering the characteristics that were previously demonstrated, and is important for the process of domestication and integration of macaúba in the economic sector for commercial production in the Midwestern Region of Brazil, especially regarding the improvement of the species.

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