

Phenotypic characterization and genetic diversity of macauba (*Acrocomia aculeata*) accessions based on oil attributes and fruit biometrics

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Abstract

Macauba is a Brazilian native palm widely distributed in environments with different edaphoclimatic characteristics. Its fruits have a great economic potential because the presence of oil in the pulp and kernel, that can be used in the biofuel, cosmetic and food companies. Macauba is a non-domesticated species, and their genetic variability is an important characteristic to be evaluated, aiming the selection of superior genotypes to start a pre-breeding of the palm. In this context, the objective of this work was evaluating the genetic variability in macauba genotypes from different Brazilian locations. For this, 50 accessions were evaluated for following phenotypic trait: oil attributes and fruit biometry. The genotypes distributed in the accessions, were divided into three populations according to the state of origin: Pop1 was formed with accessions from Mato Grosso do Sul and São Paulo, Pop2 from Pará and Pernambuco, and Pop3 from Minas Gerais. The pulp oil was extracted to obtain the oil attributes: pulp oil content, fatty acid composition, carotenoid content, and pulp oil color. Fruit biometrics were performed to obtain total fruit weight and dry mass of husk, pulp, endosperm and kernel. Results showed that Pop1 presented the smallest fruits and the highest content of carotenoids, while Pop2 and Pop3 presented the largest fruits and lower carotenoid content. The highest oil content was observed in Pop3. Genetic diversity showed that genotypes clustered according to their geographic origin. Our results reveal that macauba had high variability for the traits evaluated and potential to be genetically improved.

1. INTRODUCTION

Macauba, *Acrocomia aculeata* (Jacq.) Lodd. ex Martius, is a palm tree of the tropical and subtropical regions of the American continent, and its oleaginous fruits are the main product of interest to the agriculture. The oil can be found in the pulp and in the kernel of the fruits. Pulp oil has a high content of oleic acid (a monounsaturated fatty acid) and has a lot of characteristics similar to the olive oil, while kernel oil is rich in lauric acid (a saturated fatty acid), which can be used in the pharmaceutical-cosmetic sector. (Hiane et al. 2005; Bora and Rocha 2004; Ciconini et al. 2013; Berton et al. 2013). Macauba pulp oil has high levels of carotenoids, mainly β -carotene, which are biomolecules with antioxidant action, i.e. nutraceutical utility (Coimbra and Jorge, 2011; Ramos et al. 2008). Carotenoids are pigments found in nature and are responsible for the red, orange or yellow colors in many plants. Its quantification in food is important due to its high biological value (Coimbra and Jorge, 2011; Ramos et al. 2008), being that colorimetry technique has been used to be efficient for the characterization and quantification of pigments in plant materials (Montes et al. 2005; Ribeiro et al. 2014; Sinnecker et al. 2002).

Beside the macauba oils, the residual brans, husk and endocarp can be used by other sectors, such as thermal-based energy and human and animal food industries (Pires et al. 2013). This occurs because the fruits are even rich in fibers and proteins. The possibility to use all the parts of the fruit confers value to the macauba chain and its sustainable character possibility its exploitation as an agricultural crop.

In Brazil, macauba is found in the five regions of the territory, being greater density in the Southeast and Midwest areas (Henderson et al. 1995). Large oligarchic populations are generally found in the Brazilian

cerrado, transition cerrado - caatinga, seasonal semideciduous forest, and tropical forest ecoregions. The palm is characterized by its tolerance to high irradiance and temperature, fire and drought. Macauba also colonize areas of active or degraded pastures. (Scariot et al. 1991; 1995; Lorenzi et al. 2010; Arkcoll 1990; Motoike and Kuki, 2009; Pires et al. 2013). Because macauba is a species with wide geographic distribution in Brazil, and still subjected to different edaphoclimatic conditions, it has a great phenotypic heterogeneity, including those related to oil yield and fatty acid profile. (Motoike et al. 2013; Conceição et al. 2015). Thus, it is expected that the different environmental characteristics where macauba grows in nature, together with the characteristic of cross-pollination of the species, act in a selective pressure for genetic diversity in the species.

In a plant breeding program, the study of genetic diversity allows the breeders to have information about the genetic variability of the evaluated populations. From this information, the selection of the most contrasting parents is facilitated, and the crossings are better oriented, maximizing the genetic gains and the efficiency in the development of new cultivars (Cruz et al. 2011; Farias Neto et al. 2013). In the last 10 years, research on macauba has grown exponentially, and scientific articles published have increased knowledge of the genetic diversity of different characteristics of the fruit, as well as more specific information about the life cycle of this species (Manfio 2012; Lanes et al. 2015; Domiciano et al. 2015; Mengitsu et al. 2016; Coser et al. 2016). However, genetic diversity studies related to the qualitative attributes of macauba pulp oil are still incipient. Thus, the objectives of this work were: 1) to characterize the phenotypic variability of oil quality and productivity attributes of 50 macauba accessions and 2) evaluate the genetic diversity between these accessions to understand whether the variability between them is related to their geographic origin. These macauba fruit assessments were made by measuring the fruit biometry, pulp oil content and the composition in the oil of fatty acids, carotenoids content, and color. We believe that this is the most comprehensive and informative work about the phenotypic variability of qualitative attributes of macauba pulp oil.

2. MATERIAL AND METHODS

2.1. Plant material and sample collection

The study was carried out in the years of 2017 and 2018 using 197 macauba plants from 50 accessions cultivated in the Macauba Germplasm Bank (BAG-Macauba) from the Federal University of Viçosa (UFV). The experimental station is located in Araponga (20° 39'S, 42° 31'W, 1040 m altitude), Minas Gerais, Brazil. The selection of the 197 plants was based on the most productive plants of the evaluation year, and in individuals from different ecogeographic regions. Accessions were considered half-sib progenies originated from five Brazilian states: Minas Gerais (MG), São Paulo (SP), Mato Grosso do Sul (MS), Pará (PA) and Pernambuco (PE). The accessions were subdivided into three populations based on the geographic coordinates (latitude and longitude) of their matrices (Fig. 1). Populations were obtained by applying the K-means algorithm (Hartigan and Wong, 1979), in the R software (R Core Team 2018). From the calculated K-means algorithm, populations were divided as: Population 1 (Pop1) consisted of 13

accessions from MS and SP states, Population 2 (Pop2) consisted of 3 accessions from PA and PE states, and Population 3 (Pop3) consisted of 34 accessions from MG state (Table 1).

Table 1

Identification of macauba genotypes from the BAG-Macauba: Code of the accessions, states of origin and source populations

Accession code	Selected genotypes	State of origin	Population	Accession code	Selected genotypes	State of origin	Population
BGP4	4, 5	MG	3	BGP80	1, 2	MG	3
BGP6	1, 2, 5	MG	3	BGP81	1, 2, 3, 4	MG	3
BGP7	1, 2, 4, 5, 6	MG	3	BGP83	1, 2	MG	3
BGP8	1, 2, 4, 5, 6, 7, 9	MG	3	BGP88	1, 2, 4	MG	3
BGP9	1, 3, 5, 6, 7, 8	MG	3	BGP132	2, 4, 9	MG	3
BGP12	1, 2, 5, 7, 8, 9, 10	MG	3	BGP134	2, 3, 9, 18	MG	3
BGP14	1	MG	3	BGP136	1, 2, 5, 7, 8, 11, 15, 16, 18	MG	3
BGP15	1, 2, 3, 4, 5, 7, 8	MG	3	BGP137	2, 3, 6, 7	MG	3
BGP20	3, 4, 7	MG	3	BGP35	1, 2	SP	1
BGP26	1, 5, 6, 7, 8, 9, 10	MG	3	BGP47	1, 3, 4	SP	1
BGP33	1, 4, 5, 6, 7	MG	3	BGP92	1, 2, 3, 4, 5	MS	1
BGP36	1, 3, 5, 6, 8	MG	3	BGP94	1	MS	1
BGP37	2, 7, 9	MG	3	BGP100	1, 2	MS	1
BGP38	3, 4, 5	MG	3	BGP101	1, 2, 3	MS	1
BGP43	3, 4, 6	MG	3	BGP102	1, 2, 4	MS	1
BGP48	1, 2, 3, 4	MG	3	BGP104	1, 2, 3, 4	MS	1
BGP50	3, 5, 6, 8, 10	MG	3	BGP105	2, 3, 4	MS	1
BGP52	1, 2, 3, 4	MG	3	BGP106	1, 2, 3	MS	1
BGP53	2, 3, 4, 6	MG	3	BGP114	1, 2	MS	1
BGP64	1, 7, 8, 9	MG	3	BGP119	1, 2, 3, 4	MS	1
BGP65	1, 2, 4, 5, 6, 8, 9	MG	3	BGP120	2, 3	MS	1

Accession code	Selected genotypes	State of origin	Population	Accession code	Selected genotypes	State of origin	Population
BGP67	9	MG	3	BGP82	1, 4, 5, 6, 7, 8, 9	PE	2
BGP69	1, 2, 3, 5, 6, 7	MG	3	BGP99	1, 3	PA	2
BGP70	1, 3, 4, 5, 6, 7, 8, 9	MG	3	BGP133	1, 2, 3	PA	2
BGP74	1, 6, 8, 9, 10	MG	3				
BGP75	1, 2	MG	3				

At harvest time, between the months of December of 2017 and April of 2018 year, a sample of ripe fruits were collected directly from the bunches of each studied genotypes. After this stage, the fruits were sent to the Post-Harvest Laboratory of the Department of Agronomy at UFV, where they were cleaned and sub-sampled for subsequent quantitative and qualitative analysis.

2.2. Fruit traits evaluation

To evaluated the fruits from the different accessions, variables were divided into (i) pulp oil attributes being them fatty acid profile, carotenoid content and oil color; and (ii) productivity traits being them pulp oil content and fruit biometry.

The fruits subsampled for the biometric analysis were kept frozen at -20°C until the moment of evaluation. In contrast, fruits subsampled to analyze pulp oil attributes and pulp oil content were stored for 10 days at ~ 23°C in a large room with air circulation, in order to allow postharvest oil accumulation in the pulp (Goulart 2014). After this step, the fruits were frozen at -20°C.

2.2.1 Pulp Oil Attributes

To obtain the pulp oil attributes, about 50 fruits from each genotype were manually depulped using a knife. The pulps were removed from the fruits and separated. After this step, the pulps were kept in a drying oven at 65°C for 16 hours. Then, the oil was extracted by cold pressing process and stored at -20°C until the analysis.

Fatty acid profile

The fatty acid profile was analyzed by gas chromatography, according to the methodology described by Lieb et al. 2017, using a SHIMADZU chromatograph equipment, model 2010, equipped with automatic injector, flame ionization detector and SP-2560 capillary column, with 0.14 µm thick film, 75 m long and 0.18 mm internal diameter. Fatty acids were identified by comparing their retention times with analytical

standards (SUPELCO, composed of a mixture of 37 fatty acid methyl esters). Quantification in each genotype was done by comparing the peak area of fatty acid from the genotype, with the total area of all fatty acids analyzed in the same genotype. Values were presented as a percentage number.

Total carotenoids

The quantification of total carotenoids was carried out according to the method proposed by Rodriguez-Amaya (2001). In this method, 0.15 grams of thawed oil was weighed in a 10 millimeters volumetric flask and then solubilized in petroleum ether. The solution was transferred to a glass cuvette and read in an UVmini-1240 spectrophotometer (Shimadzu, Japan) at a 450 nm wavelength. The absorptivity coefficient of the number 2592 was considered in the analysis and the results were expressed as equivalent to the β -carotene.

The total concentration (Ct) of carotenoids (μg of carotenoids per g of oil) was calculated according to Eq. 1:

$$Ct \left(\frac{\mu\text{g}}{\text{g}} \right) = \frac{Abs \times Vol \times 10^4}{A_{1\text{cm}}^{1\%} \times m} \text{ (Eq. 1)}$$

where Abs is the absorbance at 450nm, Vol is the final volume of the sample (10 mL), $A_{1\text{cm}}^{1\%}$ is the absorption coefficient of β -carotene in petroleum ether (2592) and m is the sample weight g.

Oil colorimetric analysis

Pulp oil color was determined using a Colorquest XE Colorimeter (Hunter Lab, Reston, VA) with direct reading of coordinate values L^* , a^* and b^* , from the CIE $L^*a^*b^*$ color model. For the reading, the thawed oil sample was poured in a 10 mL cuvette. The tonality (Hue) and saturation (chroma) parameters, of the CIE $L^*C^*h^*$ color model, were calculated from the values of a^* and b^* , as follow:

$$\text{Hue} = \arctan(b^*/a^*); \text{chroma} = (a^{*2} + b^{*2})^{1/2}.$$

2.2.2. Productivity traits

To study the productivity traits, six fruits per genotypes were used, being three fruits used in the oil content analysis and the other three fruits in the biometry analysis.

Oil content

Pulp oil content (grams of oil per grams of dry matter in percentage) was quantified in the pulp fruits per genotypes using near infrared spectrometry (NIR) based on the partial least squares (PLS) model. Spectra were collected using an Antaris II FT-NIR analyzer (Thermo Fisher Scientific, USA). The NIR-PLS model has the advantage of being a low-cost, faster and more reliable method than traditional methods (Pasquini 2018).

Biometric assessment was performed on three fruits per genotypes, to ensure a coefficient of determination above 90% (Manfio et al. 2011). To obtain the dry matter the parts of the fruit (husk, pulp, endosperm and kernel) was kept in a drying oven until it reached constant weight. The evaluated variables were: transversal and longitudinal fruit diameter (TD and LD, respectively) in centimeters; dry matter (DM) of the fruit and its components - husk, pulp, endosperm and kernel - in grams. The diameter was measured with digital caliper and the dry matter was measured with precision scale.

2.3. Statistical analysis

A randomized block design with information within plots was used, with two blocks for the variables associated with the pulp oil attributes and three blocks for the variables associated with the productivity attributes. The number of plants ranged from 1 to 9 per genotype, according to the availability of productive plants for the selected progenies of the BAG-Macauba.

The populations mean for each variable studied (fatty acids, total carotenoids, pulp oil color, fruit biometrics and oil content) was compared by the t test at 5% probability.

Pearson's correlation analysis was also performed between all variables evaluated.

All the statistical analyzes described were performed using the Genes software (Cruz 2013).

2.4. Genetic diversity

Genetic diversity analyzes were performed using multivariate statistical procedures. These analyses were made dividing the variables in two groups: (i) oil characteristics, that included fatty acids content (palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids content) and carotenoid content; and (ii) productivity characteristics, that included biometric traits of the fruits and pulp oil content. For the cluster analysis, the Mahalanobis distance was used as a dissimilarity measure and for the delimitation of groups, the Tocher optimization method and the hierarchical UPGMA method (Unweighted Pair-Group Average) were used. The relative contribution of traits to diversity was performed using the Singh criterion (1981) based on the Mahalanobis distance. Genetic diversity analyses were performed using the Genes software (Cruz 2013).

3. RESULTS

3.1. Oil attributes

The main fatty acids found in macauba pulp oil were, in descending order, oleic (C18:1), palmitic (C16:0), linoleic (C18:2), palmitoleic (C16:1), stearic (C18:0), and linolenic (C18:3). The mean fatty acid content observed for each accession is shown in Supplementary Table 1. There was a wide range of variation in fatty acid contents based on the lowest and highest values observed (Table 2), indicating a large phenotypic variability among the genotypes studied. Oleic acid followed by palmitic acid were the most

abundant, based on the overall mean (Table 2). Monounsaturated fatty acids (MUFA - C16: 1 and C18: 1) formed most of the profile, equivalent to 65.28%, comparing on the overall mean, followed by saturated fatty acids (SFA - C16: 0 and C18: 0) and by polyunsaturated fatty acids (PUFA - C18: 2 and C18: 3) corresponding to 20.24% and 14.27%, respectively. In addition, there was a variation in the mean of fatty acids between populations (Table 2). The main differences in fatty acids occurred between Pop1 and Pop3. Pop1 had higher MUFA content and lower SFA and PUFA content, while the Pop3 had higher SFA and PUFA content and lower MUFA content.

The carotenoid content showed a diversified value, ranging from 27.09 to 2682.87 µg of carotenoids per gram of oil (Table 2). The wide range of variation is due to the high pigment content in Pop1 genotype, which had a higher mean than the other two populations. Among the Pop1 genotypes, the accessions from Mato Grosso do Sul stand out with a greater mean, than those from São Paulo, from the same population (Supplementary Table 1).

Table 2

Means of fatty acids, color parameters and carotenoids in pulp oil macauba fruits from Pop1, Pop2 and Pop3. .

	Overall mean	Lowest value	Highest value	Pop1	Pop2	Pop3
Fatty acids (%)						
Palmitic (C16:0)	18.64	0.41	37.18	13.30 b	17.84 ab	20.04 a
Palmitoleic (C16:1)	4.26	0.44	17.73	3.52 a	3.17 a	4.54 a
Stearic (C18:0)	1.61	0.50	8.03	2.06 a	1.82 a	1.48 a
Oleic (C18:1)	61.02	34.71	86.23	68.58 a	63.02 ab	58.96 b
Linoleic (C18:2)	13.20	1.01	26.45	10.12 b	12.91 ab	13.99 a
Linolenic (C18:3)	1.08	0.54	4.81	1.44 a	1.00 b	0.99 b
SFA	20.24	2.58	38.04	15.36 b	19.66 ab	21.51 a
MUFA	65.28	43.16	91.53	72.10 a	66.19 ab	63.50 b
PUFA	14.27	2.38	31.23	11.56 b	13.91 ab	14.98 a
Color parameters						
L*	46.24	29.33	68.75	44.67 a	37.22 b	47.21 a
a*	16.35	1.29	39.22	30.16 a	7.35 c	14.40 b
b*	35.00	6.92	69.88	32.37 a	20.03 b	36.61 a
Chroma	39.84	8.38	70.06	45.07 a	21.91 b	40.18 a
Hue	62.74	25.90	88.92	45.61 b	66.80 a	65.67 a
Carotenoids (µg/g)	226.50	27.09	2682.87	622.36 a	149.78 b	133.76 b

*Means followed by the same letter on the horizontal do not differ statistically from each other by the t test at 5% probability.

The color of the macauba pulp oil was diverse among the different genotypes, ranging from red to green; to different shades of yellow and orange, based on the visual aspect (Fig. 3). The mean value of the color parameters for each accession is shown in Supplementary Table 1. The different colors observed in this

study reflect the large amplitude presented for the values of the color parameters (Table 2). Despite this variation, it is noteworthy that all values of the a^* and b^* parameters were positive, implying that the oil colors are found at various points between red and yellow, according to the CIE $L^*a^*b^*$ model. The same can be concluded by the Hue parameter, which values ranged from 25.90 to 88.92, indicating that the colors of all examined oils are in the first color quadrant of the CIE $L^*C^*h^*$ model, between pure red and pure yellow.

The estimated mean of the color parameters per population demonstrates aspects that were visually perceived in the different oils. Pop1 had the highest value for the a^* parameter and the lowest value for the Hue parameter, suggesting that plants from this population tend to produce an oil towards a more reddish tone than the others. Pop2 had the lowest value for L^* , a^* and b^* parameters. The combination of these parameters resulted in oils with a greenish appearance. Pop3's plants showed, in general, greater intensity of yellow (b^*) than red (a^*), generating oils with shades between yellow and orange.

3.2. Oil content

The oil content in the pulp of macauba fruits showed variable values among the accessions (Supplementary Table 2). The plant with the highest oil content presented a value about six times higher than the plant with the lowest oil content, demonstrating the wide variation in this trait (Table 3). There was also variation between the mean of the populations. Pop3 had the highest oil content mean and Pop1 and Pop2 did not differ statistically from each other.

Table 3

Means of fruit biometry and pulp oil content of macauba in Pop1, Pop2 and Pop3. Transversal (TD) and longitudinal (LD) fruit diameter; dry mass (DM) of husk, pulp, endosperm and kernel.

	Overall mean	Lowest value	Highest value	Pop1	Pop2	Pop3
Oil content (%)	44.15	10.63	71.31	27.62 b	35.83 b	48.96 a
TD	4.28	2.87	5.62	3.58 b	4.43 a	4.44 a
LD	4.32	2.30	5.93	3.74 b	4.43 a	4.46 a
Fruit DM	24.20	7.80	49.70	14.26 b	25.45 a	26.58 a
Husk DM	5.22	0.90	13.60	2.70 b	5.46 a	5.83 a
Pulp DM	9.17	1.70	25.60	5.08 b	10.01 a	10.12 a
Endosperm DM	8.20	3.00	17.10	5.35 b	8.08 a	8.92 a
Kernel DM	1.64	0.50	4.90	1.13 b	1.90 a	1.75 a

*Means followed by the same letter on the horizontal do not differ statistically from each other by the t test at 5% probability.

3.3. Fruit biometrics

Fruits of different genotypes showed great variability in size, weight, husk color, pulp color, kernel and pulp thickness, based on the visual appearance (Fig. 4). This variability can be also observed in the mean values of the accessions (Supplementary Table 2) and in the values presented by the fruit biometric traits (Table 3). Pop1 had a lower value than Pop2 and Pop3 for all fruit biometric parameters, while these two populations did not differ statistically from each other.

3.4. Phenotypic correlation

The phenotypic correlations are shown in Table 4. The values were identified as low (0 to 0.39), medium (0.4 to 0.69) and high (0.7 to 1). Among fatty acids, the high positive correlations were observed between palmitic acid and SFA (0.98), oleic acid and MUFA (0.95), and linoleic acid and PUFA (1.00). These high correlations arise because palmitic, oleic and linoleic acids are those that occur in greater proportion among SFA, MUFA and PUFA, respectively. The high negative correlations occurred between the traits SFA and MUFA (-0.88), and MUFA and PUFA (-0.79). These values indicate that in plant breeding is difficult attempts to simultaneously increase these correlated variables, since when one increases, the other decreases.

Considering the pulp oil color parameters, there was a high correlation between L^* and b^* (0.99), L^* and Chroma (0.87), and b^* and Chroma, suggesting that the higher is the luminosity, more yellow and saturated is the color of the oil sampled. a^* and Hue showed high negative correlation (value of -0.85), because the properties of each model, since the red intensity is associated with higher values of a^* in the CIE $L^*a^*b^*$ model, and lower values of Hue in the CIE $L^*C^*h^*$ model.

Values of correlation between the content of carotenoids and the parameters a^* (value of 0.84) and Hue (value of -0.86), showed that redder oils had higher content of carotenoids. Medium and high correlations were also observed between fruit biometry traits and a^* color parameters, these being negative values, and Hue, positive values. These values suggest that plants that have redder pulp oil also have smaller fruits (usually belonging to Pop1). Therefore, plants with a higher content of carotenoids in the oil - which are those with a reddish color - have smaller fruits (or vice-versa), according to the negative correlations between carotenoids content and fruit biometric variables. The content of carotenoids and the intensity of red in the oil are also correlated with the oil content of the plants. The high negative correlation between oil content and carotenoid content (-0.74) and the medium correlation between oil content and a^* (-0.61) indicate that the higher is the carotenoid content, the lower is the content of oil. On the other hand, the greater is the intensity of yellow, the greater is the oil content, as indicated by the average positive correlation between oil content and Hue (0.64).

The correlations between the fruit biometric variables were all positive and, in general, of high intensity among them (Table 4). Regarding oil content, all fruit biometric variables had a medium and positive correlation, meaning that larger fruits tend to have higher oil content. In summary, the correlation analysis showed that, in general, plants with sizeable fruits (larger TD and LD) also have higher dry mass values of

all fruit components, higher oil content, lower content of carotenoids and lower intensity of red in oil. In contrast, plants with smaller fruits have lower dry mass for the fruit and its parts, lower oil content, higher carotenoid content and, consequently, higher intensity of red in the oil.

3.5. Genetic diversity

The dendrogram generated by the UPGMA method based on the Mahalanobis distance showed the formation of 3 groups with the 50 accessions (Fig. 5). The first group consisted of 32 accessions, 29 from Pop3, 2 from Pop2 and 1 from Pop1. The second group consisted of 14 accessions, being 8 from Pop1, 1 from Pop2 and 5 from Pop3. The third group consisted of 4 accessions, all belonging to Pop1.

In the grouping by the Tocher optimization method, the accesses were separated into eight groups (Table 5). Group 1 consisted of 31 accesses, being 27 from Pop3, 3 from Pop2 and 1 from Pop1. Group 2 consisted of 5 accessions from Pop1 and 2 accessions from Pop3. Group 3 consisted of 4 accessions from Pop3 and 2 from Pop1. Groups 4, 5, 7 and 8 were formed by accessions exclusively from Pop1 and group 6 by 1 accession from Pop3.

In general, in both methodologies, the accessions tended to reunite according to their geographic origin. However, because of the high variability presented by the data, some accessions showed greater similarity with genotypes from different provenances.

Table 5

Grouping of 50 macauba accessions by the Tocher optimization method based on the Mahalanobis distance using the variables palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acid content and carotenoid contend.

Group	Accessions
1	BGP64 BGP74 BGP7 BGP26 BGP133 BGP48 BGP75 BGP8 BGP65 BGP36 BGP81 BGP82 BGP6 BGP38 BGP33 BGP88 BGP4 BGP52 BGP37 BGP69 BGP15 BGP43 BGP134 BGP9 BGP53 BGP70 BGP35 BGP12 BGP99 BGP137 BGP20
2	BGP102 BGP106 BGP100 BGP47 BGP80 BGP50 BGP120
3	BGP14 BGP67 BGP136 BGP114 BGP83 BGP104
4	BGP101 BGP105
5	BGP92
6	BGP132
7	BGP119
8	BGP94

The relative contribution of traits to genetic diversity is shown in Table 6. The most important variable for the differentiation of accessions was oleic acid, which contributed with 76.40% to the total dissimilarity.

The second most important variable was palmitoleic acid, showing a relative contribution of 17.90% to the total dissimilarity.

Table 6
Relative contribution of the characters palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids content and carotenoid content to genetic diversity among macauba accessions, based on Singh statistics (1981).

Variable	Sj	Relative contribution (%)
Palmitic acid (C16:0)	0	0
Palmitoleic acid (C16:1)	49840066.55	17.90
Stearic acid (C18:0)	13269596.01	4.77
Oleic acid (C18:1)	212682013.89	76.40
Linoleic acid (C18:2)	0	0
Linolenic acid (C18:3)	0	0
Carotenoids	2604645.21	0.94
Negative Sj values were considered null		

Multivariate analysis based on biometric traits of the fruits and pulp oil content also grouped accessions according to their geographic origin. The dendrogram generated by the UPGMA method showed the formation of 3 groups with the 50 evaluated accessions (Fig. 6). Group 1 consisted of 17 accessions, mostly belonging to Pop1. There were 12 accessions from Pop1, 1 from Pop2 and 4 from Pop3. Group 2 consisted of 29 accesses, 28 from Pop3 and 1 from Pop1. Group 3 consisted of 4 accessions, 2 from Pop2 and 2 from Pop3.

On the other hand, the Tocher method showed 12 groups (Table 7). Group 1 consisted of 10 accessions, all belonging to Pop1. Group 2 consisted of 21 accessions, all belonging to Pop3. Group 3 consisted of 5 accessions, 4 from Pop3 and 1 from Pop2. Group 4 consisted of 4 accessions, 3 from Pop3 and 1 from Pop1. Group 5 consisted of 2 accessions from Pop2. Group 6 consisted of 2 accessions, 1 from Pop1 and 1 from Pop3. Groups 7, 9, 10, 11 and 12 were formed by accessions from Pop3 and group 8, by 1 accession from Pop1.

Table 7

Grouping of 50 macauba accessions from BAG-Macauba by the Tocher optimization method based on the Mahalanobis distance using the variables of fruit biometry (transversal and longitudinal fruit diameter, dry mass of fruit, husk, pulp, endosperm and kernel) and pulp oil content.

Group	Acessions
1	BGP92 BGP114 BGP119 BGP102 BGP104 BGP120 BGP100 BGP94 BGP101 BGP105
2	BGP36 BGP38 BGP15 BGP50 BGP74 BGP20 BGP6 BGP7 BGP137 BGP4 BGP81 BGP65 BGP33 BGP52 BGP83 BGP132 BGP48 BGP70 BGP69 BGP12 BGP88
3	BGP134 BGP136 BGP9 BGP82 BGP53
4	BGP26 BGP80 BGP35 BGP8
5	BGP99 BGP133
6	BGP47 BGP75
7	BGP14
8	BGP106
9	BGP67
10	BGP43
11	BGP64
12	BGP37

The relative contribution of fruit biometric traits and pulp oil content to genetic diversity is shown in Table 8. The most important variable for the differentiation of accessions was the pulp oil content, which contributed with 41.85% to the total dissimilarity. The second most important variable was the dry matter of the fruit, that showed a relative contribution of 20.76% to the total dissimilarity.

Table 8
Relative contribution of transversal and longitudinal fruit diameter, dry mass (DM) of fruit, husk, pulp, endosperm, kernel and pulp oil content for genetic diversity among 50 macauba accessions, based on Singh statistics (1981).

Variable	Sj	Relative contribution (%)
Transversal diameter	15550.94	9.84
Longitudinal diameter	16621.37	10.52
Fruit DM	32796.07	20.76
Husk DM	13229.33	8.38
Pulp DM	12633.05	8.00
Endosperm DM	0.00	0.00
Kernel DM	1025.45	0.65
Oil Content	66104.63	41.85
Negative Sj values were considered null		

Cluster analysis was performed to assess the similarity between the three populations studied based on oil characteristics (fatty acid profile, oil color and carotenoid content) and on the biometric traits of fruits and pulp oil content (Fig. 7). For both sets of variables, a group was formed with Pop2 and Pop3 and another group with Pop1, indicating that there is greater genetic similarity between plants from Pop2 and Pop3.

4. DISCUSSION

Results in this work revealed the presence of genetic variability for the characteristics of oil quality, pulp oil content and biometrics of macauba fruits. The variability reflects the high genetic diversity among the accessions, that occurred because the large representativeness of the evaluated individuals, coming from different ecogeographic regions. Other factor that explain the genetic diversity is the allogamic trait of the species, that possibility the alleles recombination (Lanes et al. 2015, 2016, Coelho et al. 2018). Macauba has an ability to survive in heterogeneous environments (Ciconini et al. 2013). Thus, the variability of the data also reflects the different adaptation strategies used by the species in response to contrasting environments (Lanes et al. 2015). The occurrence of variability in the individuals studied here, indicate the possibility to select promising genotypes, which is an essential condition for the establishment of a genetic improvement program (Manfio et al. 2012). In this sense, once the macauba is a non-domesticated species, the study of genetic variability open insights in the selection of the best genotypes to the interest traits, to build a first breeding cycle.

The composition of fatty acids present in vegetable oils was directly related to their physicochemical characteristics, and their applicability to various industrial sectors. The fatty acids found in this study

were the oleic (C18:1), palmitic (C16:0) and linoleic (C18:2). This composition agrees with other reports in the literature for mean values of the main fatty acids (Hiane et al. 1990; Coimbra and Jorge 2011b; Nunes et al. 2015; Barreto et al. 2016; Favaro et al. 2017). However, as far as we know, there are no records in the literature of the existence of genotypes with the range of variation of fatty acids as found in this work (Table 2). Such variability creates a range of possibilities for applications of macauba pulp oil and for the selection of superior genotypes.

Oxidation resistance is an important factor in vegetable oils, which is related to their nutritional quality. The susceptibility of certain oils to oxidation can limit their use in the food, cosmetics and pharmaceutical industries, reducing their commercial value (Coimbra and Jorge 2011b; Barreto et al. 2016). The high content of monounsaturated fatty acids (MUFA) in macauba pulp oil is an important characteristic that makes it desirable for food and biofuel industries, once it increases the oxidative stability of the oil. In addition, MUFA in macauba pulp oil combine nutritional quality and operability at low temperatures in the industry (Colombo et al. 2018; Nunes et al. 2015).

The predominance of high levels of oleic and palmitic acid in macauba oil is an important factor in the fractionation process and expands its uses, as it allows the production of two main industrial products: (i) olein, a liquid fraction, rich in monounsaturated fatty acids, and (ii) stearin, a solid fraction, rich in saturated fatty acids (Nunes et al. 2015). In addition, its fatty acid composition gave it the nickname “olive oil from the Brazilian cerrado” (Nunes et al. 2015), due to its similarity to olive oil, which has an average of 55–83% oleic acid and 7.5–20% palmitic acid (Mapa 2012), reinforcing its potential in food use.

Despite the predominance of MUFA and SFA, the identification of genotypes with more than 30% PUFA is an important factor for the improvement of macauba. Linoleic and linolenic acids (omega 6 and 3, respectively) are essential for the formation of compounds that act to mediate immunological and inflammatory diseases (Lescano et al. 2015). Thus, the adequate consumption of macauba pulp oil can help enrich the diet with these fatty acids, highlighting their importance for human health.

Carotenoids content observed in pulp oil showed large variation among the evaluated macauba genotypes. We observed genotypes with lower and higher values than the means found in the literature, generally around 350 µg/g (Coimbra and Jorge 2011; Nunes et al. 2015; Trentini et al. 2016). The presence of carotenoids in macauba oil is a very relevant feature, once they play an important role in preventing oil oxidation (Favaro et al. 2017). Therefore, to the human health, carotenoids are biomolecules with antioxidant, anti-inflammatory and chemopreventive action (Ramos et al. 2008; Hiane et al. 2006). In addition, carotenoids such as β -carotene, present in macauba, can be converted into vitamin A and can be used in food fortification of populations in developing countries. In Brazil, hypovitaminosis A represents one of the main nutritional problems in the population (Ambrósio et al. 2006). According to research did by Ramos et al. (2007), the β -carotene from macauba oil pulp is highly bioavailable, compared to pure β -carotene, and if the oil is consumed, the bioavailability is greater.

Colorimetry is a technique that can be used to characterize the color of different pigments, such as chlorophyll (Sinnecker et al. 2002) and anthocyanins (Montes et al. 2005). In our work, it was observed that there is a strong correlation between the content of carotenoids and the presence of the red color in the oil. This correlation is a useful tool for technological, once it allows the establishment of equations that can predict the content of carotenoids through routine color parameters, as demonstrated by Ribeiro et al. (2014).

In Brazil, there is no consensus on the number of species that form the group of palm trees identified as macauba. Some authors believe that the group is formed by three species, which differ by morphological characters and their distribution in the territory (Lorenzi et al. 2010; WFO 2021, Viana et al. 2017). They are: *Acrocomia aculeata*, with wide distribution in the Brazilian territory but with greater concentration in the state of Minas Gerais; *Acrocomia totai*, predominant in the states of Mato Grosso do Sul, São Paulo and southern states of Brazil; and *Acrocomia intumescens*, which occurs mainly in the northern and northeastern regions of Brazil. Other authors believe that these three species are actually three ecotypes of the *Acrocomia aculeata* species, namely: Sclerocarpa, Totai and Intumescens ecotypes, respectively (Pimentel et al. 2011).

In our work, macauba was considered a single species, to which the phenotypes were an expression of existing ecotypes (Lima et al. 2020). The studied plants were subdivided into three populations based on their geographic distribution, which coincide with the three ecotypes mentioned by Pimentel et al. (2011): Pop1 – Totai ecotype; Pop2 – Intumescens ecotype and Pop3 – Sclerocarpa ecotype. Great phenotypic variability was observed between and within populations, and these observations differentiated the plants from different ecogeographic regions.

Regarding the fruit biometry, Pop1 had plants with smaller fruits and less dry mass, while Pop2 and Pop3 plants had larger fruits and greater dry mass. According to Viana et al. (2017), the three macauba ecotypes (considered as three species by the authors) can be distinguished based on the mean size and mean dry mass of the fruits: the fruits of Sclerocarpa ecotype have greater size and dry mass, followed by Intumescens ecotype and by Totai, with smaller size and fruit dry mass. In our study, there was no significant difference in fruit biometry between Pop2 and Pop3 (referring to Intumescens and Sclerocarpa ecotypes, respectively). This fact can be associated with the low representation of Pop2's genotypes in our material. Regarding the mean pulp oil content, the value found for the three populations is similar to that reported in the literature for the three macauba ecotypes: Sclerocarpa ecotype (referring to Pop3) showed the highest pulp oil content, followed by Intumescens (referring to Pop2) and Totai (referring to Pop1) with lower pulp oil contents (Pimentel et al. 2011; Viana et al. 2017). According to Pimentel et al. (2011), macauba ecotype Totai has lower pulp oil content probably due to the predominance of starch and sugar formation in the pulp at the expense of oil formation. This characteristic results in a sweetened pulp, more suitable for the production of flour and human food.

Genetic diversity analyzes with the two groups of variables showed that accessions tend to group according to their geographic origin. Other studies on macauba genetic diversity reported the same trend

using quantitative variables (Manfio et al. 2012; Conceição et al. 2015) and molecular data (Lanes et al. 2015). Here, the most important variables for the differentiation of accessions were the oleic acid content among the oil quality variable, and the oil content followed by fruit dry mass, and oil content variables. The same variables were also identified as an important factor for the divergence of macauba genotypes from the states of Minas Gerais, Goiás, Tocantins and Distrito Federal (Conceição et al. 2015).

The group of populations based on oil characteristics, fruit biometrics and oil content showed a greater genetic proximity between Pop 2 and 3, and greater distance between these last and Pop 1 (Fig. 7a and 7b). The same observation was made by Viana et al. (2017) where genetic diversity was evaluated among the *Sclerocarpa*, *Totali* and *Intumescens* ecotypes based on biometric traits of the fruit (diameter, length, fresh and dry weight). The authors found greater proximity between the *Sclerocarpa* and *Intumescens* ecotypes and separation of these from the *Totali*. The separation of Pop 1, observed in this study, may be the result of a possible speciation process that the species is undergoing (Lanes et al. 2015). The Pantanal of Mato Grosso do Sul, which is the region of origin for most of the Pop1 genotypes, is a sedimentary basin surrounded by a large hydrographic network and plateaus with altitudes between 600 and 700 m. These geographic barriers associated with the restricted dispersion of the pollen (due to the limited flight range of pollinators) and the seeds (which often remain close to the mother plant due to barochory) can restrict the gene flow of accessions in the region, isolating the population and thus, creating a favorable scenario for speciation (Lanes et al. 2015).

According to Lanes et al. (2015) the São Francisco River, which rises in the central region of the state of Minas Gerais, probably plays an important role in the dispersion of macauba seeds in that state. However, when leaving the state of Minas Gerais, the São Francisco River follows its route through four more Brazilian states, all in the northeast region of the country (Bahia, Pernambuco, Sergipe and Alagoas). In this way, the river can also possibly act as a vector in the dissemination of seeds from the state of Minas Gerais to the states of northeastern Brazil, helping the gene flow between populations in the two regions. This factor can influence the greatest genetic similarity observed between Pop 2 and 3.

The macauba diversity is well documented and associated to a high phenotypic plasticity that allows this palm to explore a wide variety of ecoregions (Lanes et al. 2015). Two main findings emerge from this study: (a) The observed variability in the fruit attributes of the studied accessions is linked to their origin, to which the particularities of environmental conditions exert selective pressure; (b) Because of this phenotypic/genotypic variability, particularly pulp oil attributes, macauba breeding programs can be directed accordingly to the market demand.

CONCLUSION

Macauba presents wide genetic variability for the evaluated characters. There are strong indications that this variability is related to the geographic distribution and the adaptations associated with different colonized environments. Our results suggest that Pop3 is the most suitable for selecting genotypes with high pulp oil content and higher yield of co-products (husk, pulp, endosperm and kernel brans), while Pop

1 presented genotypes with the highest carotenoids contents. New studies to estimate genetic parameters will allow greater knowledge of the genetic control of the evaluated traits opening doors to the selection of superior genotypes to start a pre-breeding that concentrate several traits of interest.

Declarations

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Competing interests

The authors declare that they have no competing interests

Author contribution

The first draft of the manuscript was written by Débora D.C. Madeira and to publish, all authors read and approved the final manuscript. The author contribution by each one is presented below:

Débora D.C. Madeira: Conceptualization, Methodology, Formal analysis, Data Curation, Investigation, Writing, Visualization, Project administration; *Guilherme F. Simiqueli*: Validation, Formal analysis, Writing, Review & Editing; *Kacilda N. Kuki*: Conceptualization, Writing; *Samuel de M. Goulart*: Methodology, Validation, Investigation; *Pedro Tiago S. Nogueira*: Methodology, Investigation; *Amanda da S. Castro*: Methodology, Investigation; *Evellyn G.O. Couto*: Writing, Review & Editing; *Sérgio Y. Motoike*: Conceptualization, Validation, Resources, Supervision; Funding acquisition;

Data Availability

The datasets generated during current study are not publicly available due to other research that is in progress with these data, but they are available from the corresponding author on reasonable request.

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Tables

Table 4 is available in the Supplementary Files section.

Figures

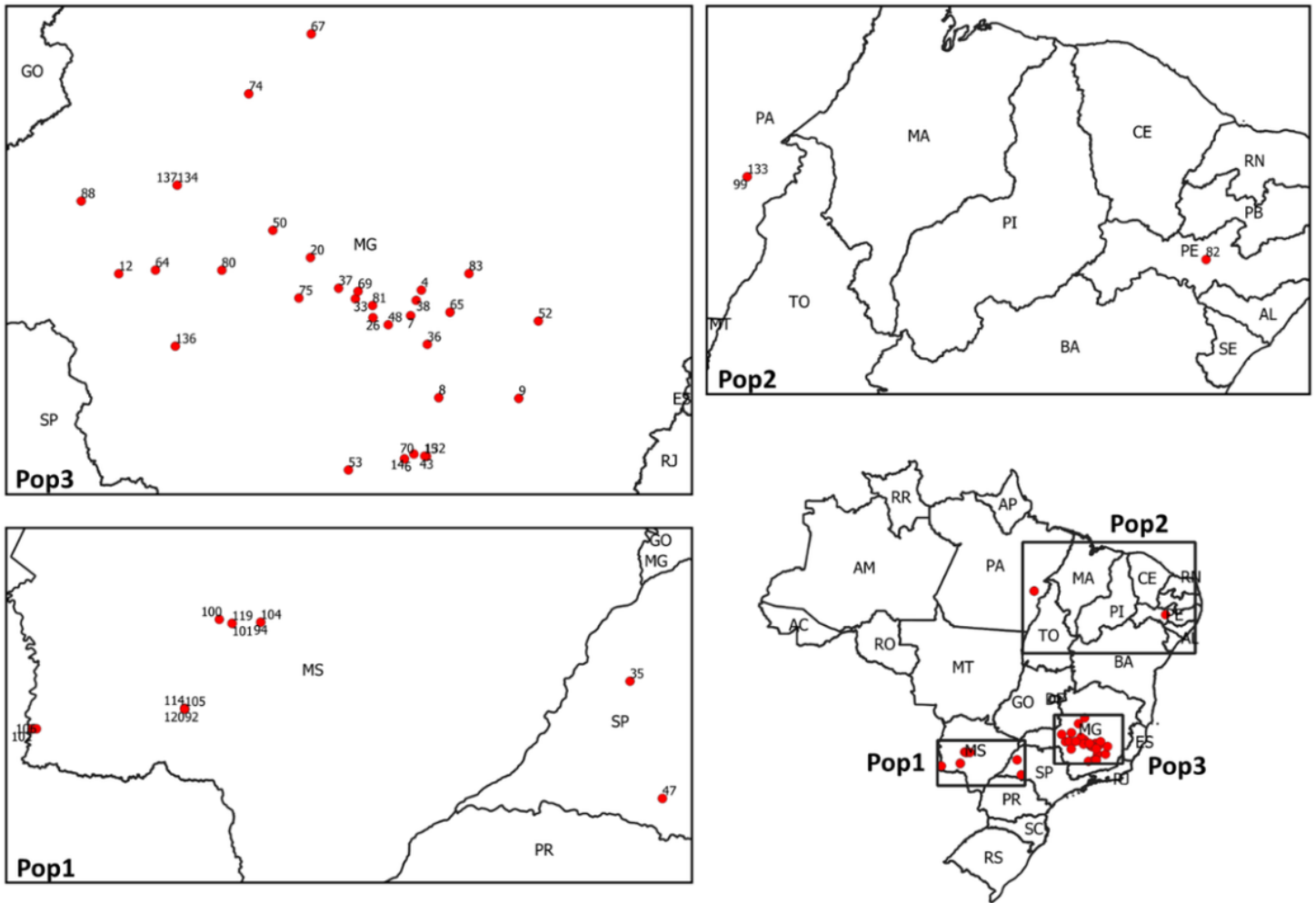


Figure 1

Geographical identification of the 50 accessions used in this study and their respective populations

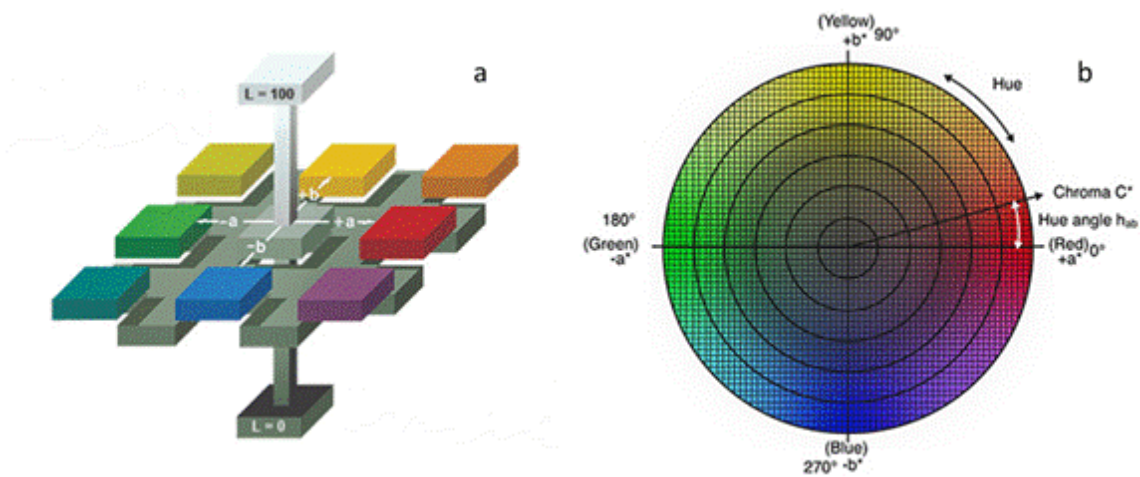


Figure 2

Colors models from CIE L*a*b* (a) e CIE L*C*h* (b). Sources: a) Modified from HunterLab; b) Konica Minolta

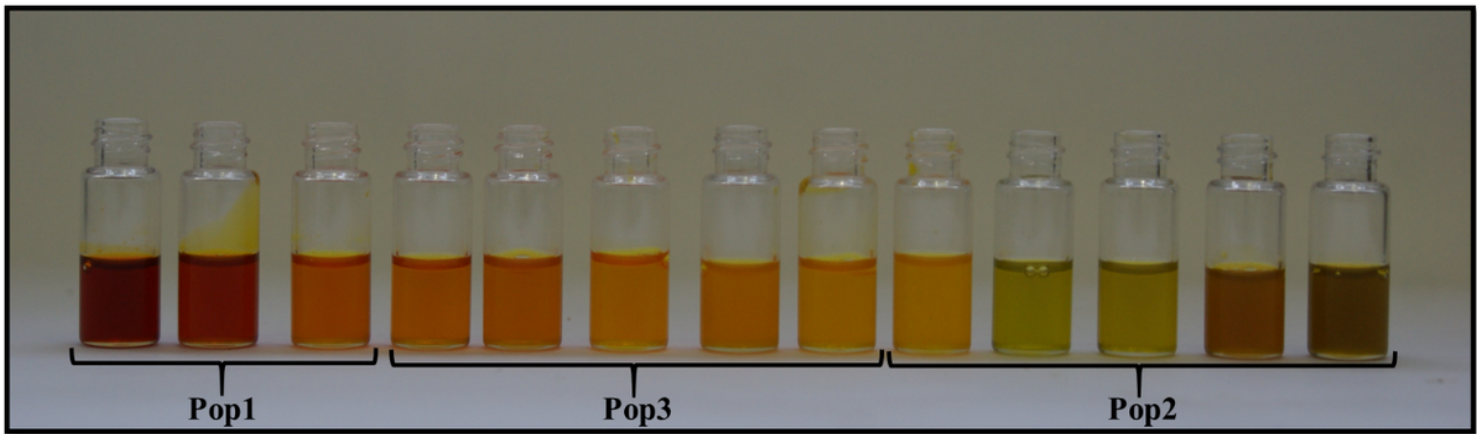


Figure 3

Illustrative diversity of macauba pulp oil colors from genotypes from different Brazilian origins and their original populations



Figure 4

Illustrative morphometrical diversity of macauba fruit from genotypes of different accessions. The number next to each fruit indicates its population of origin (Pop1, Pop2 and Pop3)

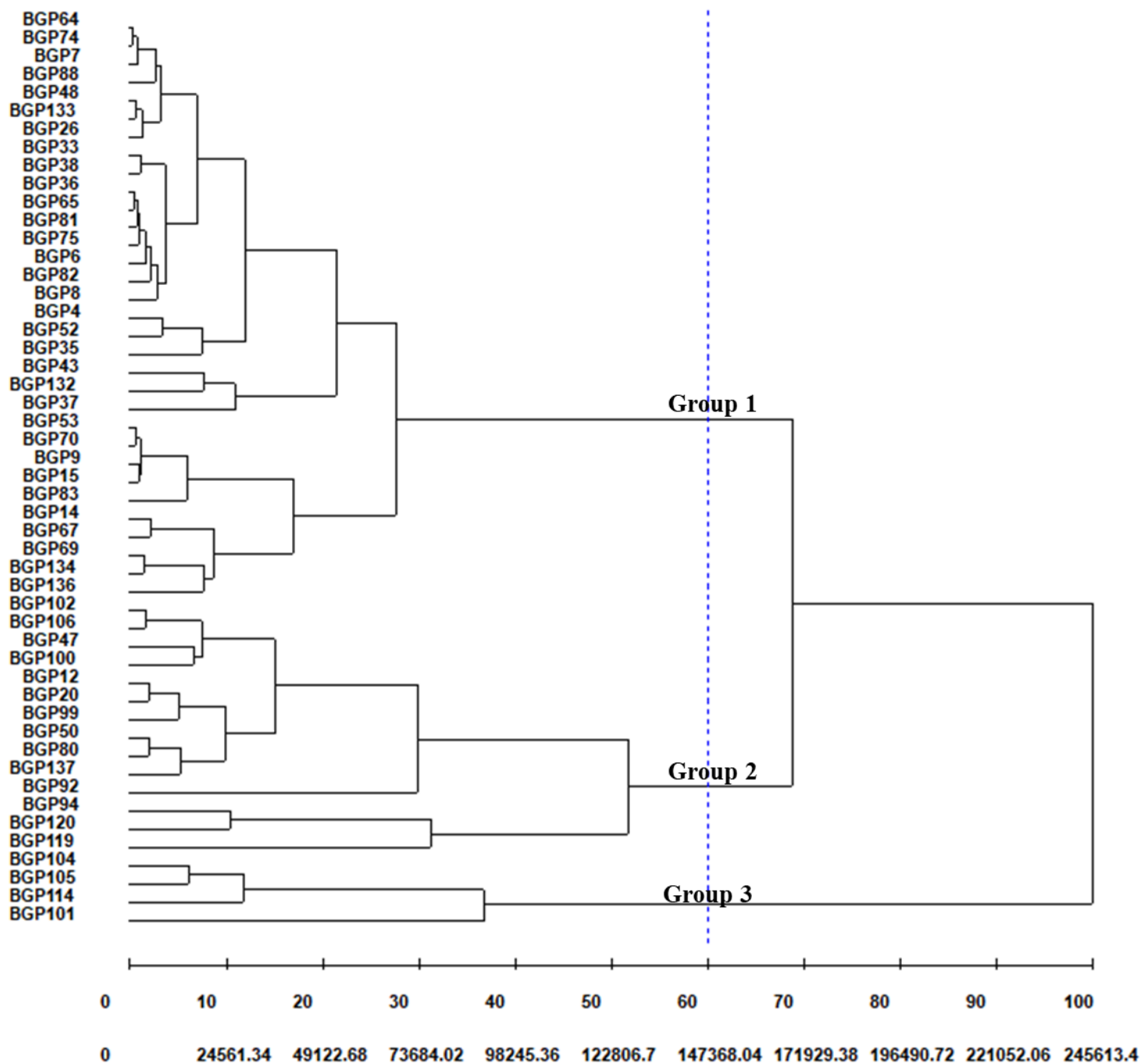


Figure 5

Dendrogram of 50 macauba accessions from BAG-Macauba, generated by the UPGMA method based on the Mahalanobis distance, using the biometrical variables of the fruits (transversal and longitudinal fruit diameter, dry mass of fruit, husk, pulp, shell and kernel) and pulp oil content

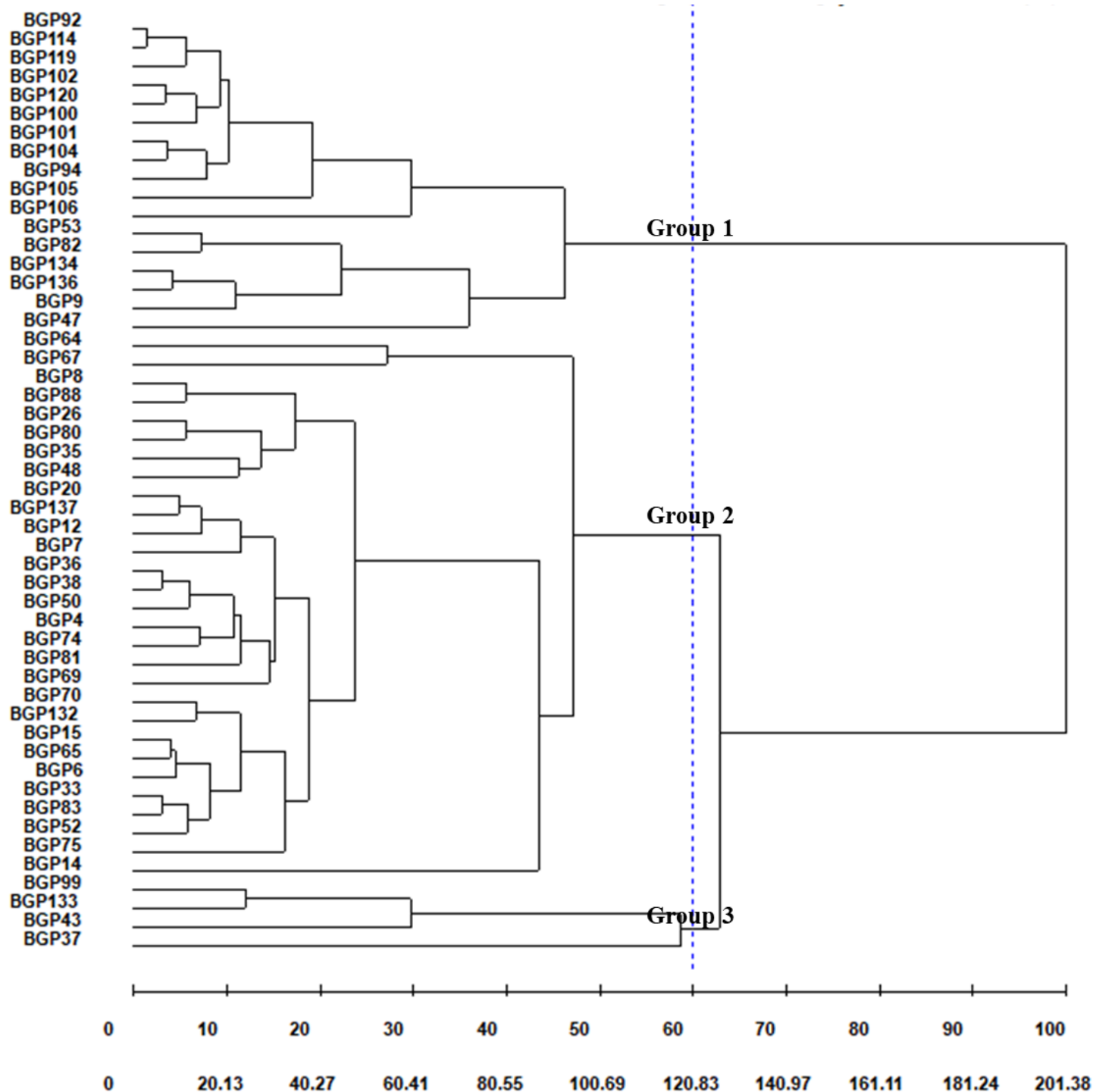


Figure 6

Dendrogram of 50 macauba accessions from BAG-Macauba, generated by the UPGMA method based on the Mahalanobis distance, using the biometrical variables of the fruits (transversal and longitudinal fruit diameter, dry mass of fruit, husk, pulp, endosperm and kernel) and pulp oil content

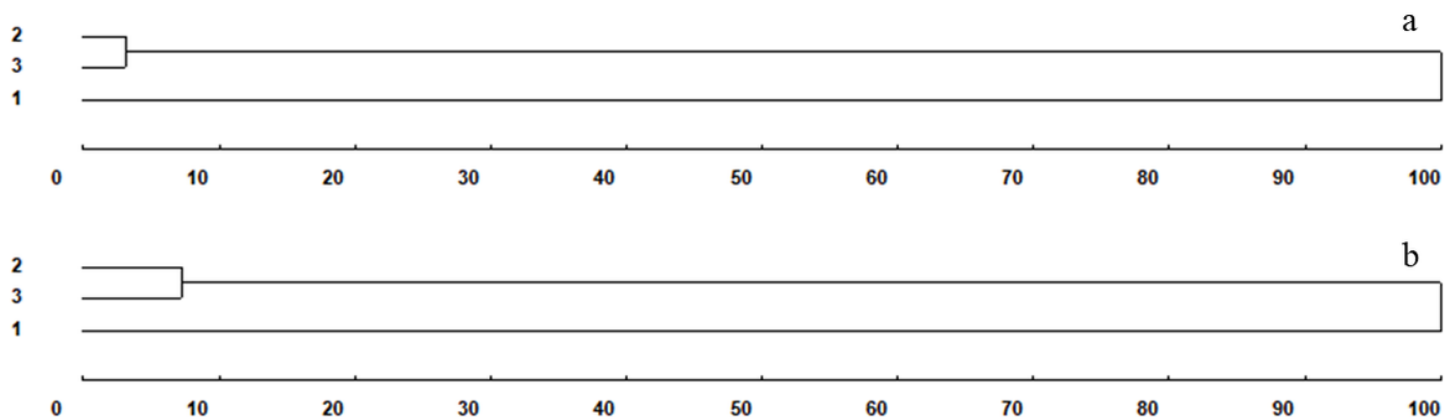


Figure 7

Dendrograms of 3 macauba populations, generated by the UPGMA method based on the Mahalanobis distance, using (a) the variables palmitic, palmitoleic, stearic, oleic, linoleic, linolenic acids and carotenoid content and (b) the variables of fruit biometrics (transverse and longitudinal diameter of the fruit, dry mass of fruit, husk, pulp, endosperm and kernel) and pulp oil content

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterial.pdf](#)
- [Table4.docx](#)