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BIOTECHNOLOGY

Morphoagronomic and molecular characterization of *Euterpe oleracea* accessions from eastern Brazilian Amazon

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ABSTRACT. Açaí (*Euterpe oleracea* Mart.) - a common tropical palm has high social, economic, and environmental importance in the Amazon region. In the light of increasing exploration to obtain the fruit and heart of this palms, comprehensive studies are warranted for conservation and genetic improvement. Here, we characterized açaí accessions using phenological, morphological, and agronomic descriptors and random amplified polymorphic DNA (RAPD) molecular markers for joint selection of accessions with greater productivity. Hundred accessions were analyzed using 18 morphoagronomic descriptors and 13 RAPD markers. The spathe and inflorescence emission phases during flowering and fruiting showed seasonality. Based on the coefficient of variation and mean squared error, the accessions exhibited high variability in the tested morphoagronomic descriptors and were distributed into seven groups. Fruit, seed, and pulp weights were important descriptors for the distinction of accessions and identification of those with greater productivity. The accessions presented >85% similarity, and 85 accessions, distributed in nine subgroups, could not be differentiated using RAPD markers. There was no correlation between grouping based on morphometric descriptors and RAPD markers. Panicle weight was 3.9-9.0 kg in 15 accessions and 100-fruit pulp weight was 35-50 g in six accessions. Therefore, accessions with high productivity could be selected.

Keywords: anthocyanin; molecular marker; acai, Arecaceae, functional food, floodland forest.

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Introduction

Euterpe species (Arecaceae) have high economic, social, and environmental relevance in the Amazon region (Heijink et al., 2020). *Euterpe oleracea* Mart., popularly known as the açaí palm, grows naturally in the estuary of the Amazon River and is exploited for the production of fruit pulp, palm heart, cellulose, firewood, handicraft, brooms, ethanol, and biocomposites for use in civil construction. Moreover, various extracts are employed in folk medicine for their anthelmintic and antidiarrheal properties (Barbosa, Rebelo, Martorano, & Giacon, 2019; Cordeiro, Almeida, Rodrigues, Chaves Neto, & Machado, 2019).

Based on fruit color, three açaí types are reported: violet (immature and ripe) for the type species (‘*preto’* açaí) and the ‘*espada’* ethnovariety; green (immature and ripe) for the ‘*branco’* ethnovariety; and green when immature and violet when ripe for the ‘*tinga’* ethnovariety (Pimentel & Jardim, 2009). Due to higher oil content and lower anthocyanin and iron content, ‘*branco’* pulp tastes like avocado with milk and is easily digestible (DuVal, 2010). Furthermore, in an *in vitro* gastric system, ‘*branco’* pulp exhibited a low capacity to form nitrogen compounds and a high potential to prevent cancer development due to red meat consumption (Silveira et al., 2017).

Recent years have been marked by the rising production and consumption of açaí palm products, due mainly to the way paved by the ‘*preto’* variety in the domestic and international market following the recognition of its pulp as a functional food that is rich in anthocyanins, calories, lipids, carbohydrates, proteins, minerals, and fibers (Cedrim, Barros, & Nascimento, 2018). This increase in production has resulted from simultaneous movement for greater exploitation of native stands and expansion of planting areas (Lindolfo, Matos, Pereira, & Fernandes, 2020).

As such, the native stands are exploited by altering the number and spatial distribution of açaí palm, other palm species, and arboreal species from other botanical families. This balance optimizes the use of resources such as nutrients, physical space, solar energy, and water to increase fruit yield (Queiroz & Mochiutti, 2012). However, excessive exploitation of açaí palm in detrimental to other forest species reduces diversity in areas of natural occurrence and promote predominance of the '*preto'* variety due to selective exploitation in detrimental to the '*branco'* type (Tagore, Canto, & Sobrinho, 2018). Moreover, the establishment of cultivated stands with limited genetic diversity and intense exploitation of native populations can lead to genetic erosion.

In this context, it is important to preserve and increase species diversity, seeking optimum utilization of this variability and sustainable exploitation of resources. Planting and characterization of accessions in germplasm banks are alternatives to prevent the loss of genetic resources. Morphoagronomic descriptors, such as tillering and fruit production, as well as DNA-based molecular markers for polymorphism have been employed to characterize accessions deposited in germplasm banks and support breeding programs aimed at improved fruit yield (Galate, Mota, Gaia, & Costa, 2014; Yokomizo, Farias Neto, & Oliveira, 2016; Farias Neto, 2019). To this end, in the present study, we characterized açaí accessions using phenological, morphological, and agronomic descriptors and random amplified polymorphic DNA (RAPD) markers for joint selection of accessions with higher productivity.

Material and methods

Sample collection

Fruits of the '*branco'* type (phenotypic) of açaí palm were collected in October 2001 from various sites in the communities of Curralinho (01°48′29″S, 49°48′17″W), Breves (01°39′46″S, 50°30′31″W), São Sebastião da Boa Vista (01°43′28″S, 49°31′52″W), Muaná (1°32′03″S, 49°12′58″W), Limoeiro do Ajurú (01°53′20″S, 49°23′05″W), and Ponta de Pedras (1°24′02″S, 48°52′06″W), located in the Marajó Archipelago, at the mouth of the Amazon River (Pará, Brazil). Seeds were used to produce seedlings in the experimental farm of the *Empresa Brasileira de Pesquisa Agropecuária* (Embrapa Amapá) research station (Macapá, Amapá). The seedlings were then planted in the experimental farm of Embrapa Amapá (Mazagão, Amapá, 00°02′33″S, 51°15′24″W) in June 2002, in a floodplain forest environment (Figure 1). The seedlings were planted ensuing 5 × 5 m spacing, with 25 plants per row. According to the Köppen classification, the climate in Mazagão is Am type, with mean annual temperature of 27.3°C and mean annual rainfall of 2,410 mm.

Access to genetic heritage was registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge of the Ministry of the Environment (SISGEN, MMA Brazil) under number AB88CF5, pursuant to Law 13,123/2015 and its follow-on regulations (Brasil, 2015).

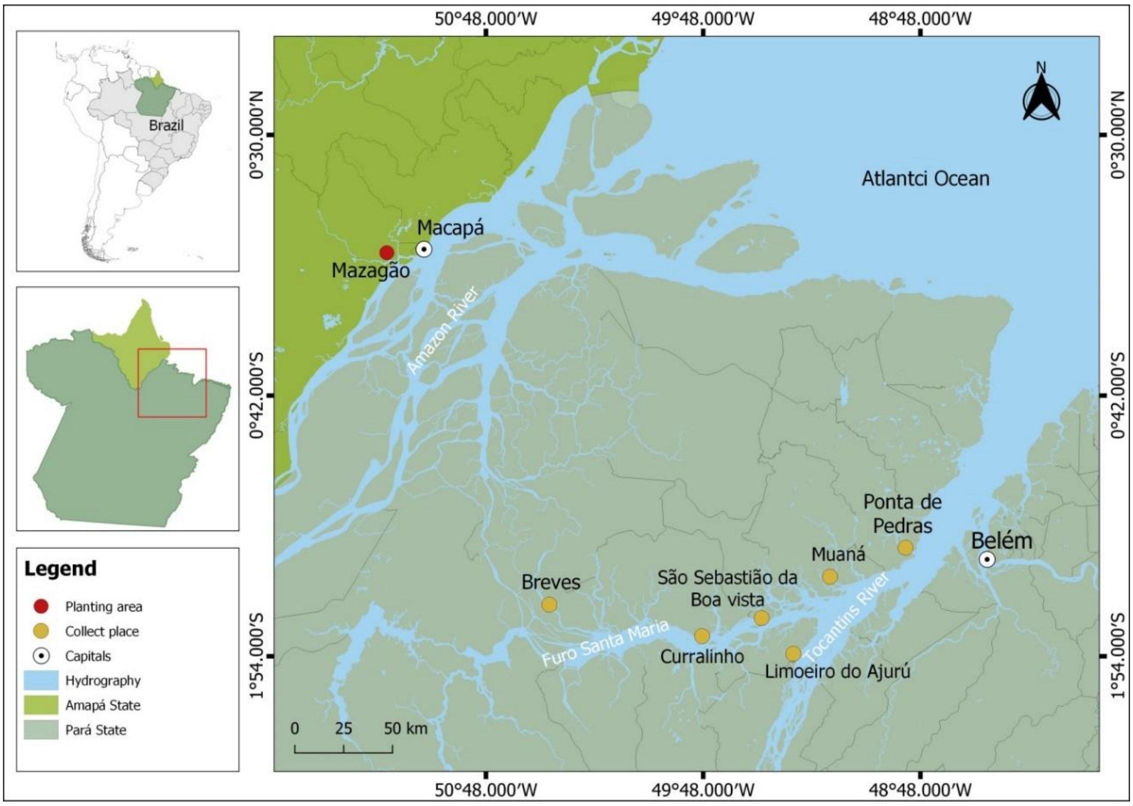

Figure 1. Açaí seed collection locations in Pará and Embrapa Amapá açaí germplasm bank of the Mazagão experimental station in Amapá.

Morphoagronomic characterization

For morphoagronomic characterization of the selected accessions, performed between January and December 2019, 18 descriptors were used (Table 1).

Table 1. Phenological, morphological, and agronomic descriptors used for açaí accession characterization in this study.

Descriptors	Methodology
Fruit color (FC)	Presence or absence of anthocyanin in the epicarp (violet or green)
Stipe type (ST)	Presence or absence of tillering (solitary stipe)
Number of spathes emitted (NSE)	Obtained by counting spathes emitted during the study period
Number of flowering inflorescences (NFI)	Obtained by counting inflorescences emitted during the study period
Plant height (PH)	Measured in the oldest stipe, from the insertion of the leaves to the roots
Circumference (CIRC)	Measured in the oldest stipe at the breast height (1.30 m)
Length of internodes (INL)	Measured from five internodes (leaf scars) of the oldest stipe, starting from the breast height (1.30 m)
Longitudinal diameter (LFD)	Average of 50 fruits, determined in the longitudinal direction, from the insertion of the chalice and corolla (floral remains) until the trace of stigma
Transversal diameter (TFD)	Average of 50 fruits, determined in the transverse direction
Hundred-fruit weight (HFW)	Mass of 100 fruits from each harvested panicle
Hundred-seed weight (HSW)	Mass of 100 seeds, after the removal of epicarp and mesocarp, from each harvested panicle
Hundred-fruit pulp weight (HFPW)	Obtained as the difference between HFW and HSW
Pulp yield (PY)	Obtained as the ratio of HFPW and HFW, expressed as a percentage
Total panicle weight (TPW)	Average mass of panicles harvested during the study period
Fruit weight per panicle (FWPP)	Average fruit mass per panicle
Number of rachillas per panicle (NRPP)	Average number of rachillas from panicles harvested during the study period
Length of rachillas of the panicle (LRP)	Average length of the rachis per panicle harvested during the study period
Fruit yield per panicle (FYPP)	Obtained as the ratio of FWPP and TPW, expressed as a percentage.

Stipe type (ST) and fruit color (FC) are shown as frequency. The numbers of spathes emitted (NSE) and flowering inflorescences (NFI) per clump are shown as mean and standard error of mean as a function of months of the year. Plant height (PH), circumference (CIRC), and internode length (INL) are shown as dispersion (coefficient of variation and minimum, mean, and maximum values). Other data were subjected to analysis of variance (ANOVA) with a generalized linear model (GLM) because of different number of replicates. The experimental design was completely randomized, with accessions being considered the treatments and number of panicles the replicates. Pulp yield (PY) and fruit yield per panicle (FYPP) data were submitted to $\sin^{-1}\sqrt{x}$ transformation, and the number of rachillas per panicle (NRPP) was subjected to $\sqrt{x}$ transformation. In addition, data were normalized and subjected to principal component analysis (PCA). Based on the results of PCA, eight variables that explained 86% of the total variance were selected and submitted to cluster analysis using Euclidean distance and unweighted pair group method with arithmetic mean (UPGMA).

Molecular characterization

For molecular characterization, performed at the Molecular Biology Laboratory of Embrapa Amapá, young leaves were collected in December 2018. Total DNA was extracted from leaflets macerated in liquid nitrogen using the Plant/Fungi DNA Isolation Kit (Norgen Biotek Corp., Thorold, Ontário, Canadá). The obtained product was diluted in water (1:20) for use as template in polymerase chain reaction (PCR). The reaction mixture contained 1× buffer (200 mM Tris–HCl [pH 8.0] and 500 mM of KCl), 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 0.3 µM of primer, 1 µL of Tween 20 (2%), 1 U of *Taq* polymerase, and 2 µL of template, in a final volume of 25 µL.

PCR was performed in a thermocycler (AMPLITHERM® TX96+G), under the following conditions: initial denaturation at 94°C for 1 min., followed by 35 cycles of denaturation at 92°C for 1 min, annealing at 35°C for 1 min, and extension at 72°C for 2 min., and final extension at 72°C for 5 min. The amplified products were separated using agarose gel (1.5% w v^{-1}) electrophoresis at 120 V for 120 minutes. The gels were stained with ethidium bromide, visualized, and photographed under ultraviolet light.

The profiles observed in the RAPD gels were converted into binary matrices of presence (1) and absence (0). This matrix was used to calculate similarity by employing the Jaccard coefficient, and clustering was determined using UPGMA. The polymorphic information content (PIC) was calculated as described by Pandey et al. (2019).

Finally, the Mantel correlation between the morphoagronomic and molecular data was determined. All analyses were performed using Past (Hammer, Harper, & Ryan, 2001) and RStudio (R Core Team, 2015).

Results and discussion

A total of 100 accessions (50 pairs of half-sibs) were characterized in the present study. For accessions IB-336 and IIB-01, data on panicles and fruits could not be obtained because of premature fruit abortion. Regarding ST, tillering was absent in 11 accessions and all presented a solitary stipe. Clump formation is a unique trait of *E. oleracea* among all *Euterpe* species. Nevertheless, the presence of tillering has previously been reported in *E. edulis* (Silva, Ahnert, & Silva, 2014). In another study, the percentage of interspecific hybridization between *E. edulis* and *E. oleracea* presenting tillering was higher when the latter was used as the female parent, indicating that this trait may have a maternal origin (Bovi, Godoy Júnior, & Sáes, 1987). The presence of tillering allows the selection of genetic material for sustained exploitation of the plant (harvesting fruits and heart of the palm) by eliminating older stipes. Meanwhile, the presence of a solitary stipe enables more combinations in terms of spacing and plant age for plantation renewal.

Although all plants were derived from the seeds of the '*branco'* type, 46 accessions produced green fruits ('*branco'* type), 46 produced violet fruits ('*preto'* type), and 1 produced greenish-brown fruits (Figure 2). In five accessions, panicles emitted from different stipes of the same plant bore fruits of different colors (green and violet). To the best of our knowledge, this is the first report of such a variation in fruit color in a single plant. Unfortunately, little is known regarding the genetic determinants of açaí fruit color (DuVal, 2010).

Spathe emission and flowering inflorescence formation were more frequent in the first half of the year, coinciding with the rainy season (Figure 3), while fruiting (harvest) occurred in the second half, corroborating the observations of Lindolfo et al. (2020). However, accession IIB-02 presented flowering inflorescences throughout the year, and accession IB-37 emitted spathes throughout the year, except in October. In addition, the tested accessions showed great variability in PH, CIRC, and INL of the main stipe, and there were significant differences in all analyzed descriptors, except FYPP, among the tested accessions (Table 2). For 50% of the variables with significant differences, a ratio of >5 was observed between the mean and mean squared error values of the accessions. The highest coefficient of variation was recorded for fruit weight per panicle (FWPP) and total panicle weight (TPW). In another study, significant differences in 17 morphoagronomic descriptors were observed among 129 açaí palm progenitors (Galate et al., 2014).

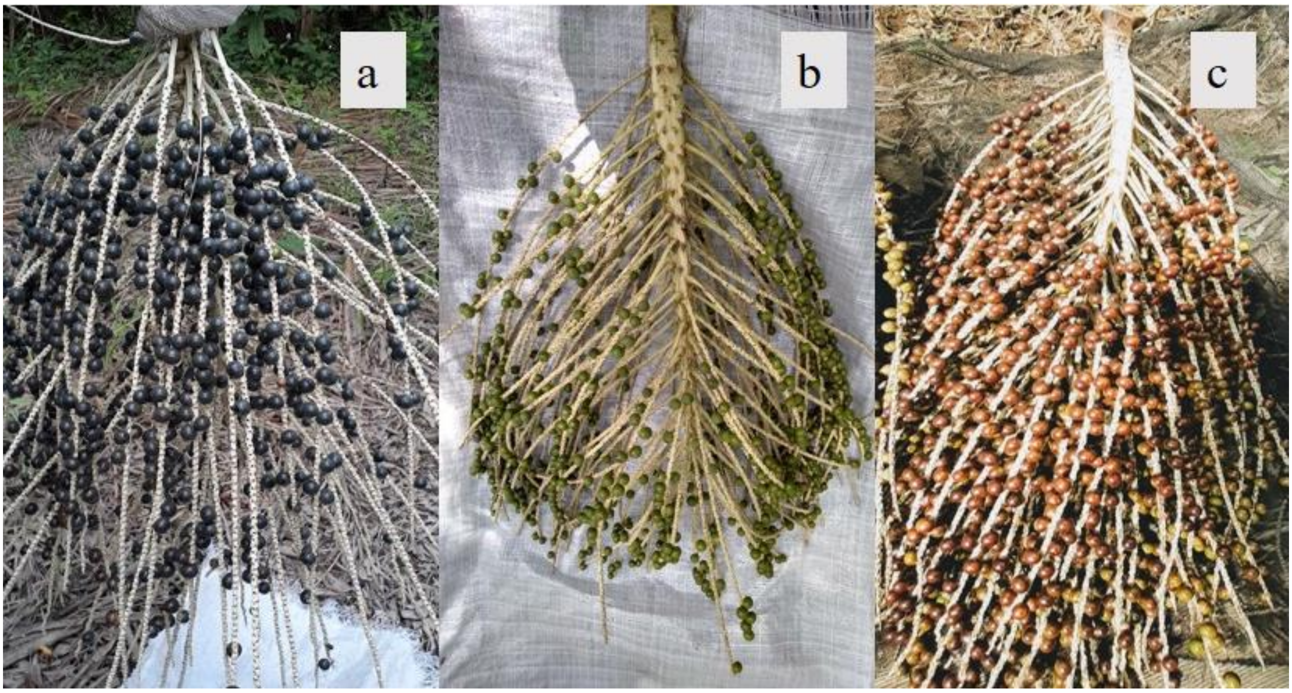

Figure 2. Ripe fruit color: violet ('*preto'* type) (A), green ('*branco'* type) (B), and greenish-brown (C) fruits observed in accessions from the Embrapa Amapá açaí germplasm bank of the Mazagão experimental station, Amapá.

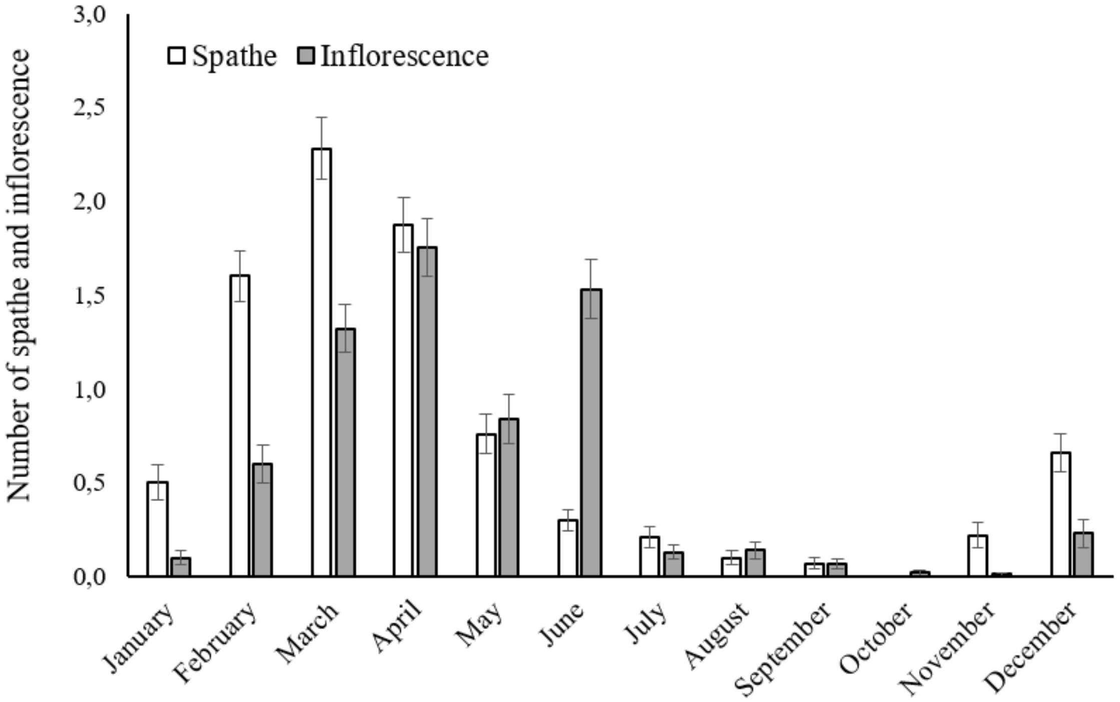

Figure 3. Mean number of spathes emitted (NSE) and flowering inflorescences (NFI) per clump as a function of months of the year in the tested açaí accessions. The columns represent the mean, and the bars represent the standard error of mean.

Table 2. Minimum (min), mean, and maximum (max) values; coefficient of variation (CV); and summary of analysis of variance for 14 morphoagronomic descriptors of the açaí tested accessions.

Descriptor	Mean squared		CV (%)	Values		
	Accessions	Error		Min	Mean	Max
PH	-	-	13.30	6.48	9.77	13.21
CIRC	-	-	13.39	23.00	37.40	50.00
INL	-	-	17.18	27.50	61.53	92.00
LFD	1.2835**	0.1336	4.92	12.69	14.77	17.03
TFD	1.0753**	0.1379	5.25	11.43	12.91	14.91
HFW	1453.1**	169.10	13.74	109.74	180.07	266.04
HSW	1181.5**	121.35	14.39	101.72	153.33	233.04
HFPW	146.90**	76.93	28.88	9.53	34.80	85.75
PY	0.0039*	0.0028	25.33	5.25	19.35	39.55
TPW	6.5156**	2.7768	45.84	0.48	4.39	11.61
FWPP	5.5027**	2.1462	51.54	0.13	3.52	9.79
NRPP	1.3757**	0.2286	16.40	36	91.27	139
LRP	108.66**	29.93	14.78	5	51.10	74
FYPP	0.0054^{ns}	0.0049	13.25	13.16	77.31	98.62

Plant height (PH), circumference (CIRC), internode length (INL, mean of five values) of the main stipe of each clump, longitudinal diameter (LFD, mean of 50 fruits), transverse diameter (TFD, mean of 50 fruits), hundred-fruit weight (HFW), hundred-seed weight (HSW), hundred-fruit pulp weight (HFPW), pulp yield (PY), total panicle weight (TPW), fruit weight per panicle (FWPP), number of rachillas per panicle (NRPP), length of rachillas of the panicle (LRP), and fruit yield per panicle (FYPP) are shown. ^{ns}: not significant. * and **: significant at 5 and 1% probability by F-test, respectively.

The dendrogram generated based on the analyzed morphoagronomic descriptors revealed the formation of seven distinct groups (Figure 4A). Of these, three groups included a single accession each and the others included 6-42 accessions (Table 3). Hundred-fruit weight (HFW), hundred-seed weight (HSW), and hundred-fruit pulp weight (HFPW) were in the range of 142-178 g, 118-156 g, and 21-43 g in group 6; 153-194 g, 131-167 g, and 28-37 g in group 7; 180-223 g, 147-192 g, and 24-49 g in group 3; and 217-247 g, 180-218 g, and 35-50 g in group 5, respectively. Fruit diameter is related to PY. In previous studies in Pará, TPW, NRPP, INL, tillering, and FWPP have been successfully used to select accessions with the highest fruit weight and hence productivity (Teixeira, de Oliveira, Gonçalves, & Nunes, 2012; Yokomizo et al., 2016; Farias Neto, 2019). Owing to its high heritability, HFW is an important trait to select accessions for use in genetic improvement programs (Teixeira et al., 2012). Standing out from the rest, accessions in group 5 presented HFPW in the range of 35-50 g and accessions in group 7 presented TPW in the range of 3.9-9.0 kg.

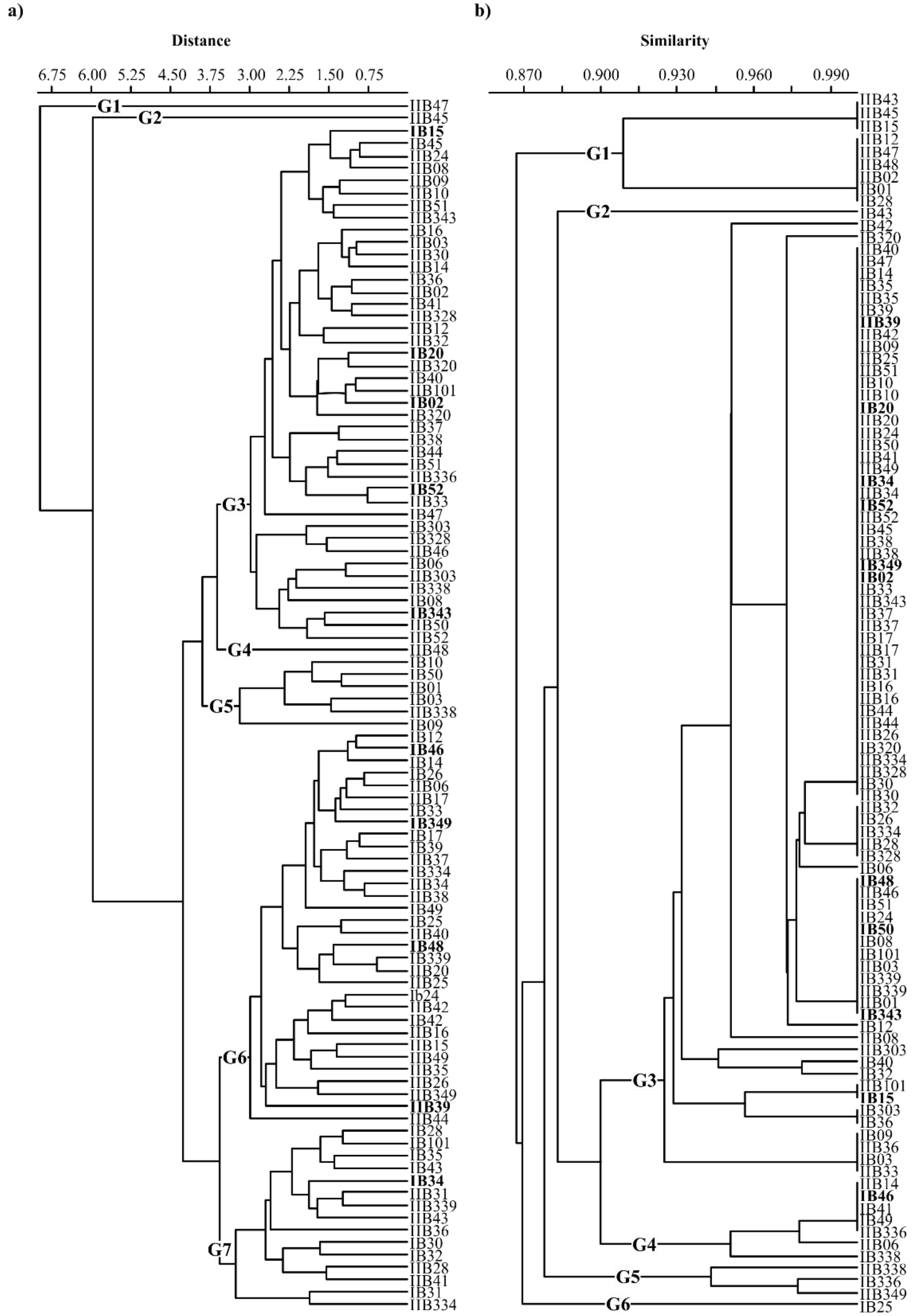

Figure 4. Dendrograms of distance and similarity among the tested açaí accessions. Dendrogram generated based on the Euclidean distance and unweighted pair group method with arithmetic mean (UPGMA) of morphoagronomic descriptors (A). Dendrogram generated based on the Jaccard similarity index and UPGMA of random amplified polymorphic DNA markers (B). The morphoagronomic descriptors included the number of rachillas per panicle (NRPP), hundred-fruit weight (HFW), hundred-seed weight (HSW), stipe circumference (CIRC), hundred-fruit pulp weight (HFPW), total panicle weight (TPW), longitudinal fruit diameter (LFD), and transverse fruit diameter (TFD). Accessions without tillering are indicated in 'bold' (solitary stipe).

Table 3. Mean values of total panicle weight (TPW), number of rachillas per panicle (NRPP), hundred-fruit weight (HFW), hundred-seed weight (HSW), hundred-fruit pulp weight (HFPW), longitudinal fruit diameter (LFD), transverse fruit diameter (TFD), and stipe circumference (CIRC) in the tested açaí accessions by morphoagronomic groups formed in cluster analysis (n = number of accessions in each group).

Group	TPW	NRPP	HFW	HSW	HFPW	LFD	TFD	CIRC
	(kg)			(g)		(mm)		(cm)
G1 (n=1)	2.91	36.00	200.41	139.40	68.08	15.23	13.52	27.00
G2 (n=1)	0.95	68.50	142.85	115.89	29.39	13.24	12.04	23.00
G3 (n=42)	4.26	86.79	195.48	166.37	37.70	15.21	13.31	36.24
G4 (n=1)	3.70	90.50	204.42	176.01	56.47	15.20	13.23	45.00
G5 (n=6)	3.44	87.27	232.40	199.15	40.89	16.26	14.17	32.83
G6 (n=32)	3.91	89.50	160.12	135.59	30.95	14.23	12.40	38.80
G7 (n=15)	6.92	113.18	173.61	147.92	32.53	14.57	12.73	39.81

A total of 50 fragments were amplified in the RAPD assay, of which 76% were polymorphic (Table 4). In açaí palm, RAPD markers have been reported to show polymorphism rates between 43% (Costa, Oliveira, & Moura, 2001) and 100% (Oliveira, Amorim, Santos, & Ferreira, 2007). Despite the high polymorphism observed in the present study, however, PIC remained low, ranging from 0 to 0.23 per indicator. Based on this assay, the accessions were distributed in six groups, with 87% similarity (Figure 4B). The number of accessions was 1 in groups 2 and 6, 3 in group 5, 9 in groups 1 and 4, and 79 in group 3. A total of 85 accessions, distributed in nine groups, presented 100% similarity, of which 28 were formed by 14 half-sib pairs. In a study by Costa, Oliveira, & Ohaze (2004), mean similarity between accessions producing violet and green fruits (‘*branco’*) from plants grown in the municipalities of Breves and Cametá (Pará) was 36.88% based on RAPD markers.

Table 4. Random amplified polymorphic DNA markers used, total number of fragments (NTF), total number of polymorphic fragments (NTP), percent polymorphism (P%), and polymorphic information content (PIC) of the tested açaí accessions.

Markers	Sequence (5′-3′)	NTF	NFP	P%	PIC
OPB-01	GTTTCGCTCC	4	4	100	0.09
OPB-10	CTGCTGGGAC	4	4	100	0.16
OPB-18	CCACAGCAGT	4	4	100	0.23
OPBA-01	TTCCCCACCC	3	2	66.6	0.01
OPBA-02	TGCTCGGCTC	2	2	100	0.02
OPBA-03	GTGCGAGAAC	5	4	80	0.02
OPBA-05	TGCGTTCCAC	5	5	100	0.02
OPBA-06	GGACGACCGT	5	5	100	0.05
OPBA-07	GGGTCGCATC	6	1	16.66	0.00
OPBA-08	CCACAGCCGA	2	0	0	0.00
OPBA-09	GGAACTCCAC	4	4	100	0.08
OPBA-10	GGACGTTGAG	4	1	25	0.00
OPF-1	ACGGATCCTG	2	2	100	0.06
Total		50	38	-	-
Mean		3.84	2.92	76.02	-

In clustering based on morphoagronomic descriptors, accessions showed no tendency to form groups according to their origin. The variability observed based on morphoagronomic descriptors was not correlated with the variability revealed by RAPD markers (Mantel test, $r = -0.01506$, $p \leqslant 0.5356$). Morphoagronomic descriptors are mainly quantitative, controlled by multiple genes, and subject to environmental influence. In contrast, the RAPD markers are associated with dispersed regions throughout the genome (Fajardo, Silva, Chagas, & Vieira, 2018; Rohini et al., 2020). In the present study, the accessions presented high variability based on morphoagronomic descriptors, but low variability based on RAPD markers. Similar results have been reported in other plant species, such as *Arachis* sp. (Choudhary et al., 2019; Ma et al., 2020) and *Momordica charantia* (bitter melon, among many other common names) (Pandey et al., 2019).

Since fruit color is the only criterion adopted for the collection of seeds for deposition in the germplasm bank and since the collection region represents a center of diversification of the species, high morphoagronomic variability was expected. At Embrapa Amapá, such a variability has proven extremely important for the development of more productive cultivars and establishment of germplasm bank to realize efficient conservation. Meanwhile, the variability observed based on RAPD markers was significantly lower than that previously reported for accessions of this species (Oliveira et al., 2007).

In a study of the *'branco'* variety in a rural community in Amapá, DuVal (2010) reported the presence of a dynamic seed system among family farmers. In this system, the farmers employed vegetative propagation (tillers, vegetative cuttings) for the use and development of varieties; planted *'branco'* seedlings spatially separated from *'preto'* seedings to prevent cross-pollination for seed production and storage under local conditions; and preferably used their own seeds or seeds provided by other farmers as the seed exchange mechanism rather than using seeds from the local market or the formal seed market of registered cultivars. With the snowball method, in which a farmer being interviewed recommends the one to be interviewed next, the author observed that *'branco'* açaí had been introduced in the community in one or two events; as such, over 45 years, 36 farmers grew the material, and via gradual selection, many farmers adopted the improved material and disseminated it to five other communities.

In the present study, we did not record the origin of the seeds or seedlings used by the farmers to cultivate the progenitor plants. Nonetheless, plants established in agroforestry yards by smallholders typically come from previous progenitors selected by them (Oliveira, Ferreira, & Santos, 2007). Additionally, based on the lower frequency of occurrence of the *'branco'* type, greater preference of many local consumers for this type, and the higher price for its pulp than for that of the *'preto'* açaí, it is reasonable to assume that the low genetic diversity observed might be related to the selection system and exchange of *'branco'* açaí seeds by farmers in the region. Microsatellite markers developed for *E. edulis* have been tested in other species of the genus and have been shown to represent a viable alternative for identifying accessions with greater genetic diversity (Oliveira, Santos, Amorim, & Ferreira, 2010; Azêvedo et al., 2017).

Conclusion

The tested açaí accessions presented low variability based on the RAPD markers but high variability based on the 18 morphoagronomic descriptors evaluated. Such a variability allows the selection of accessions for introduction in improvement programs. Additionally, accessions in morphoagronomic groups 5 and 7 stood out from the rest in terms of HFPW and TPW and are thus recommended for use in improvement programs.

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In memoriam

On March 9, 2020, the author Silas Mochiutti died due to H1N1 infection after 17 days in the ICU of Belém Adventist Hospital. Since 2013, Silas had been undergoing chemotherapy to treat multiple myeloma and fight laboratory relapse, and his immune system had weakened. Silas was 56-year-old. During his academic career, he stood out for his dedication and published works and was always recognized by his professors and peers as a brilliant student and researcher. Silas was a pioneer member of the group studying *Euterpe oleracea* in the Amazon region, and for the past 20 years, he was a leader in this theme. As a researcher, he made significant contribution to the sustainable use of the species and thus the generation of income in various communities in the Amazon Estuary. He will be greatly missed by his family, friends, colleagues, and the scientific community for his kindness and meritorious contributions.

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