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Antioxidant Activity, Rutin Content and Genetic Similarity Between Matrices and Progenies of *Hancornia speciosa*

Caroline de Araújo Machado[1], Leila Albuquerque Resende de Oliveira[1], Annie Carolina Araújo de Oliveira[1], Milena Nascimento Cardoso[1], Francine Ferreira Padilha[2], Bruno Trindade Cardoso[3], Ana Veruska Cruz da Silva[3] & Ana da Silva Ledo[3]

[1] Programa de Pós-graduação em Agricultura e Biodiversidade, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brazil

[2] Programa de Pós-graduação em Biotecnologia Industrial, Universidade Tiradentes, Aracaju, Sergipe, Brazil

[3] Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

[4] Embrapa Tabuleiros Costeiros, Aracaju, Sergipe, Brazil

Correspondence: Caroline de Araújo Machado, Programa de Pós-graduação em Agricultura e Biodiversidade, Universidade Federal de Sergipe, Av. Marechal Rondon s/n, São Cristóvão, Sergipe, Brazil. Tel: 55-79-999-199-119. E-mail: camachado@hotmail.com

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Abstract

Mangaba tree (*Hancornia speciosa* Gomes) is a tropical fruit species from Brazil and presents socioeconomic potential. The objective of this study was to determine antioxidant activity, rutin content and genetic similarity among *in vivo* and *in vitro* matrices and progenies of six accessions from the Mangaba Active Germplasm Bank of the Embrapa Coastal Tablelands, SE, Brazil. Young leaves of adult matrix plants and *in vitro* callus methanolic extracts obtained from young leaves resulted in differences between the accessions for the rutin content and high antioxidant activity. The Costa Azul accession, from Bahia, Brazil outstood with values above 3,000 g of dry extract/g of DPPH (2.2-diphenyl-1-picrilhydrazyl) in *in vitro* callus coming from nodal and internodal segments (3,023.73 and 3,136.80 g of dry extract/g of DPPH, respectively). Rutin was not found in extracts resulting from *in vitro* callus of mangaba tree. The difference among the values obtained for DPPH analysis are superior when compared with *in vivo* leaf extract, with the *in vitro* Costa Azul accession (nodal and internodal) outstanding, concluding that *in vitro* callus induction can interfere with chemical compounds of the plant. The markers detect genetic similarity under *in vitro* cultivation conditions. The Costa Azul accession group itself in isolation from others and EC_{50} concentrations differentiated between leaf and callus are obtained. O EC_{50} is superior in extracts deriving from *in vitro* callus, with the *in vitro* Costa Azul accession (nodal and internodal).

Keywords: HPLC, ISSR, DPPH, callogenesis

1. Introduction

The mangaba tree (*Hancornia speciosa* Gomes) is a Brazilian fruit species, belonging to the Apocynaceae family (Silva et al., 2017). It presents great market potential within the tropical fruits segment. The fruit (mangaba) is very important for the Brazilian agroindustry, especially in the northeastern region (Santos et al., 2017). Besides this, mangaba tree present potential for recovery of degraded areas, allowing its sustainable use, also serving as a source of alternative income for local populations (Oliveira et al., 2016).

According to data from IBGE (Brazilian Institute of Geography and Statistics) (2016) presented extractive production of 922 tons of mangaba and, in 2015, 663 tons, an increase of 39.1%. The total national production, Sergipe is in fourth place, this fact was caused by the 33% reduction of the mangaba collectors and due to the real estate advance in areas of natural production of this species. However, the increase in production in other states is considered due to its fruitful potential, being one of the cerrado species with greater chances of being studied aiming its conservation.

The medicinal properties of mangaba have been reported by some authors. Antihypertensive activity potential demonstrated in *in vitro* studies for the angiotensin-converting enzyme (ACE) inhibition to the ethanolic extraction of leaves (Serra et al., 2005) and endothelium-dependent vasodilatation activity, via nitric oxide, in *in*

vitro preparations of aortic rings, also for ethanolic extraction of leaves of the species (Ferreira et al., 2007). It is possible that the bioactive molecules present in the aqueous extract of *Hancornia speciosa* fruits bind to receptors of endothelial cells, inhibiting the cell migration and the activation of inflammatory mediators involved in chemotaxis and diapedesis (Torres-Rêgo et al., 2016).

The production of secondary metabolites employing plant cell culture has been extensively studied, constituting a suitable system to produce important pharmacological compounds (Morais et al., 2012). The *in vitro* culture of plant cells presents advantages for the supply of bioactive compounds with defined production system and short culture periods, ensures a continuous supply of metabolites of interest, and the cells are free of diseases and are not exposed to seasonal variations (Castro et al., 2016). Manipulations of *in vitro* nutritional components and physical factors are the main factors for the culture productivity optimization (Fazal et al., 2016). Studies on the chemical composition, antioxidant activity and volatile compounds of mangaba are scarce (Lima et al., 2015). Some studies demonstrated high antioxidant activity in mangaba fruits, those observed in other fruit species such as *Anacardium occidentale* (St. Hilaire)*, Spondias tuberosa* (Arr. Cam.) and *Euterpe oleracea* (Mart) (Rufino et al., 2010).

The reproductive system of the tree species is one of the most decisive factors for the maintenance of genetic variability among individuals of a population. Populations that present cross-fertilization have greater possibilities of increasing the genetic variability without adding new genes when compared with the population of individuals that perform self-fertilization (Borges et al., 2009). In addition, the phenotypic variation is strongly influenced by environmental components such as anthropological level, soil condition, climate, age of plants and genetic differences between individuals. While the species are not yet fully domestic, variability is an important factor to enable the plant selection for the establishment of orchards with greater uniformity and specific characteristics (Yokomizo et al., 2017). Thus, was aimed with this study to evaluate the antioxidant activity, the rutin content and genetic similarity between *in vivo* and *in vitro* matrices and progenies of six accessions from the Mangaba Active Germplasm Bank of the Embrapa Coastal Tablelands, SE, Brazil.

2. Material and Methods

2.1 Local and Plant Material

The experiments were conducted in the laboratories of Plant Tissue Culture and Molecular Biology of Embrapa Coastal Tablelands and in the laboratory of Biomaterials from Center of Colloidal System StudyTechnological Institute of Research, Tiradentes University, in Aracaju, Sergipe, Brazil.

Six accessions were selected from the Mangaba Active Germplasm Bank, located in Itaporanga d'Ajuda, SE, Brazil (11°06′40″S and 37°11′15″W). All plant matrices were in the same environmental conditions, and the fruits were collected in the summer for seed excision. After 30 days the seeds grown *in vitro* were germinated, to obtain callus deriving from leaf, nodal and intermodal segments. For the *in vivo* analysis of mangaba leaves, 50g of leaves of 3 plants of each accession (Table 1).

Table 1. Accessions evaluated from the Mangaba Active Germplasm Bank. Embrapa Coastal Tablelands, Aracaju, Sergipe, Brazil

Accessions	Origin (Municipality-State)	Geographic Coordinates
AB	Água Boa-Sergipe	0°46′31″ S; 48°31′05″ W
CA	Costa Azul-Bahia	11°33′32″ S; 47°47′06″ W
CJ	Itaporanga d'Ajuda-Sergipe	11°06′40″ S; 37°11′15″ W
BI	Barra do Itariri-Bahia	11°48′39″ S; 37°36′40″ W
PT	Indiaroba-Sergipe	11°31′10″ S; 37°30′47″ W
TC	Terra Caída-Sergipe	0°46′31″ S; 37°30′47″ W

Callogenesis was induced in nodal, internodal and foliar segments derivated from *in vitro* seedlings, cultivated in MS medium supplemented with 10 mg L^{-1} of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 5.0 mg L^{-1} of benzylaminopurine (BA) (Adapted from: Prudente et al., 2016; Soares et al., 2011; Machado et al., 2016). After 60 days, the callus was extracted.

2.2 Preparation of Extracts

Young leaves and callus obtained *in vitro* were dehydrated in ventilated oven (Marconi®, MA035/1152) at 38-40 °C for 24 h. Then, methyl alcohol solvent, 2 g of dry leaf in 20 mL of methanol (MeOH) (Vetec®) and 100 mg of dry callus in 10 mL of the same solvent were added and placed on ultrasound (Ultracleaner™ 1400, Unique, Indaiatuba, São Paulo, Brazil) for 30 min. After that, they were filtered and kept at room temperature until complete evaporation of the solvent.

2.3 Chromatographic Conditions

For chromatographic analysis in HPLC and UHPLC-High Performance/High Pressure Liquid Chromatography-chromatograph (Thermo Scientific™ UltiMate™ 3000, USA), 10 mg of extract were dissolved in 10 mL of dimethyl sulfoxide-DMSO (Vetec®). The mobile phase used was MeOH: H_2O in the proportion of 1:1 and vacuum filtered. The column used was the C18 (250 mm × 4.6 mm; 5 μm), the wavelength was 210 nm, at room temperature, with a flow of 1 mL/min. The concentration of diluted extract was determined by interpolation of the area of each sample in a standard curve of rutin with concentration ranging from 10 to 100 ppm (10, 25, 50, 75, 100 ppm). The rutin mass of the diluted extract was calculated by the formula: m Rutin = CDE × VDE, where m Rutin = rutin mass of the specimen, CDE = rutin concentration of the diluted extract and VDE = volume of diluted extract. Then, the plant mass (m plant) required to produce 10 mg of the extract was calculated through the formula: m plant = m extract/yield. The rutin content in the leaf was calculated by the method: Rutin content (%) = m rutin/mplant. The calibration curve generated was validated by the analysis of a solution from the rutin pattern on concentration of 45 ppm. The concentration generated in the analysis was 43.97 ppm. Both *in vivo* leaves and callus were submitted to the same conditions of HPLC extraction and injection.

2.4 Total Antioxidant Activity

In vitro callus extracts and *in vivo* leaves were used. The Brand-Williams et al. (1995) method adapted by Rufino (2006) was applied. The absorbance readings were held after 180 to 220 min of spectrophotometer reaction of 515nm wavelength (Thermo Scientific™ GENESYS 10S UV-Vis Spectrophotometer, USA). Absorbance decline of specimens related to loss of absorbance of the control (Ac) results in percentage of free radicals scavenging (% FRS). The results were expressed in g of callus extract/g DPPH (2,2-diphenyl-1-picrilhydrazyl). The antioxidant capacity was expressed in required antioxidant concentration to reduce the original amount of free radicals in 50% (EC_{50}).

Determination of the EC_{50}, the sample concentration or pattern that causes 50% inhibition of the initial DPPH concentration, was obtained by linear regression of the plotted plot of the dilutions of the extracts (40, 20 and 10 μg/ml). For plotting the points, the values of the means obtained from triplicates were used for each of the tests.

2.5 Analysis of Genetic Diversity

Young leaves from each genotype were used for DNA extraction (J. J. Doyle & J. L. Doyle, 1987) with modifications and quantified in a spectrophotometer (Thermo Scientific NANODROP 2000c) at 260 and 280 nm. The evaluation of the DNA quality was performed by agarose gel electrophoresis 1% (m/v), and visualized in Gel doc L-pix HE photo-documentation equipment (Loccus Biotecnologia, Brazil).

The specimens were diluted in 100 μL of TE (10 mM Tris-HCl, pH 8.0, 1 mMEDTA) and stored at -20 °C for subsequent use in ISSR (Inter-Simple Sequence Repeat) reactions. Eighteen ISSR (University of British Columbia, Vancouver, Canada) primers were used from (Table 2).

Table 2. Synthesis initiators selected with their respective sequences and temperature

Primers	Sequence	Temperature (°C)
807	5' AGA GAG AGA GAG AGA GT 3'	47.0
809	5' AGA GAG AGA GAG AGA GG 3'	57.2
810	5' GAG AGA GAG AGA GAG AT 3'	54.8
811	5' GAG AGA GAG AGA GAG AC 3'	46.8
812	5' GAG AGA GAG AGA GAG AA 3'	54.8
815	5' CTC TTC TCT CTC TCT CTG 3'	47.6
816	5' CAC ACA CAC ACA CAC AT 3'	54.8
817	5' CAC ACA CAC ACA CAC AA 3'	54.8
818	5' CAC ACA CAC ACA CAC AG 3'	57.2
823	5' TCT CTC TCT CTC TCT CC 3'	57.2
825	5' ACA CAC ACA CAC ACA CT 3'	54.8
826	5' ACA CAC ACA CAC ACA CC 3'	57.2
828	5' TGT GTG TGT GTG TGT GA 3'	54.8
834	5' AGA GAG AGA GAG AGY T 3'	56.5
848	5' CAC ACA CAC ACA CAC ARG 3'	58.8
855	5' ACA CAC ACA CAC ACY T 3'	56.5
856	5' ACA CAC ACA CAC ACA CYA 3'	56.5
888	5' BDB CAC ACA CAC ACA CA 3'	56.4

Regarding PCR tests, the total volume of the reaction was 20 µL, containing: 2 µL of the genomic DNA solution; 1.0 µL of each initiator along with a mix composed of 2 µL of 10X PCR buffer; 0.4 µl of dNTP (10 mM); 0.6 µL of $MgCl_2$ (50 mM); 0.2 µL of Taq DNA polymerase (5 U/µL) Ludwig®, and 12.8 µL of ultrapure water.

For amplification of the reactions, the thermal cycler (Thermo Scientific™, MA, USA) was programmed in such a way that the samples were denatured at 95 °C for five minutes, followed by 45 cycles of amplification. Each cycle the specimens suffered a chain reaction of denaturation at 94 °C for one minute, girdling at various temperatures for 45 seconds and, finally, extension at 72 °C for two minutes (Soares et al., 2016).

In each microtube containing 20 µL of amplified DNA, 2 µL of specimen buffer (0.01% bromophenol blue; 40% glycerol) were added. From this mixture, 17 µL were arranged in channels of 2% agarose gel (dissolved in TBE 1X-Tris 89 mM, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and subjected to horizontal electrophoresis in voltage of 200 V, 200 mA, power of 100 W for approximately one hour and thirty-five minutes.

After electrophoresis, the gels were stained in ethidium bromide solution (0.02 µL/mL of water) for approximately 60 min for viewing under ultraviolet light. To measure the banding pattern, the 1Kb molecular weight marker (Promega, Madison, South Dakota, USA) was used. The visualization of the gels was made in Loccus L-pix HE equipment (Loccus Biotecnologia, Brazil).

2.6 Statistical Analysis

To detect the differences between the treatments for total antioxidant activity and rutin content, were applied the variance analysis (ANOVA) and the Tukey's Test using the software SAS. For performing the grouping, the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method was used in the construction of phylogenetic relations and the dendrogram generated from the Jaccard similarity coefficient, obtained by the Ward method, with the electrophoretic profiles data coded to a binary matrix submitted to GENES program (Cruz, 2013) and the dendrogram by the statistical package STATISTICA 7.0, STATSOFT, Inc., 2004.

3. Results

3.1 Identification of Phenolic Compounds in in vivo Leaves and Callus

The generated calibration curve was validated by the analysis of a 45 ppm routine standard solution. The concentration generated in the analysis was 43.97 ppm, which represents a 2% error of the measured value in relation to the expected value. The concentration of the diluted extract was determined by interpolating the area of each sample on a routine standard curve showing different values for concentration of the accessions to rutin mass and content in *in vivo* leaves (Table 3).

Table 3. Chromatographic analysis of rutin mass (mg) and content (%) in leaf (*in vivo*) extracts of *Hancornia speciosa*. Accessions: AB (Água Boa), BI (Barra do Itariri), CA (Costa Azul), CJ (Itaporanga), TC (Terra Caída) and PT (Pontal)

Accessions	Rutin Concentration (mg/L)	M Rutin (mg)	M plant (mg)	Content (%)
AB	55.20±6.95ab	0.55±0.07ab	75.81±7.35a	0.73±0.13a
BI	64.26±11.22 a	0.64±0.11a	93.17±7.85a	0.68±0.07a
CA	59.27±11.04 ab	0.59±0.11ab	83.40±10.93a	0.71±0.09a
CJ	67.26±13.14 a	0.67±0.13a	89.12±14.81a	0.78±0.25a
PT	35.56±7.67 b	0.35±0.08b	79.41±10.52 a	0.45±0.16a
TC	42.75±5.76 ab	0.42±0.06 ab	72.01±4.55a	0.59±0.07a
VC (%)	17.35			

Note. * Same lower case letters in a column do not differ significantly by the Tukey test ($p < 0.05$).

The BI and CJ accessions reached greater rutin concentration (64.26 and 67.26 mg of rutin/L of extract) among the accessions studied. Differences in rutin concentration between accessions can be related to the phenolic compounds of plants. There was no detection of rutin peaks in callus obtained from *in vitro* culture of leaf segments.

3.2 Antioxidant Activity

There was a significant effect of the mangaba tree accessions on the values obtained for the EC_{50} in *in vivo* leaves and *in vitro* callus ($P < 0.05$). The EC_{50} was calculated from the 29.3 µmol/mL concentration of DPPH and methanol, using 50% of the absorbance of that dilution. For the antioxidant activity of the *in vivo* accessions from leaf extracts, the BI and CA obtained higher capacity in capturing the free radicals (EC_{50} = 110.09 and 176.03 g dry extract/g DPPH) when compared with the others (Figure 1). The presence of rutin in these accessions (BI and CA) was identified and quantified with approximate values of 51.6 mg of rutin/L of extract and 52.9 mg of rutin/L of extract, respectively.

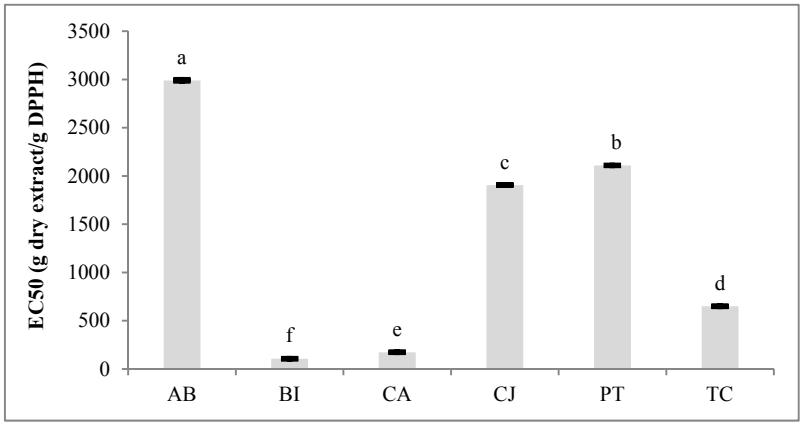

Figure 1. Antioxidant activity by DPPH method in *in vivo* leaf of six mangaba accessions from the Mangaba Germplasm Bank. Embrapa Coastal Tablelands, Sergipe, Brazil. AB (Água Boa), BI (Barra do Itariri), CA (Costa Azul), CAJU (Itaporanga), TC (Terra Caída) and PT (Pontal). Same letters do not differ significantly by the Tukey test ($p < 0.05$)

Since no previously published articles determine the antioxidant activity using DPPH method for mangaba leaves and *in vitro* callus, it is only possible to compare the results related by Rufino et al. (2010) in mangaba fruits with EC_{50} of 3345 g/g DPPH. Thereby, the antioxidant activity of the callus extracts expresses the ability of eliminating free radicals in DPPH tests, which can be correlated with the total amount of compounds present in callus. Therefore, in the same study the values of other tropical fruits such as Carnaúba (*Copernicia prunifera*) and Cajá (*Spondias mombin*) obtained higher Ec50, that is, with lower antioxidant capacity. Thus the

establishment of antioxidant activity level will assist in future studies comparing with different parts of the plant and performing other chemical analyzes is relevant.

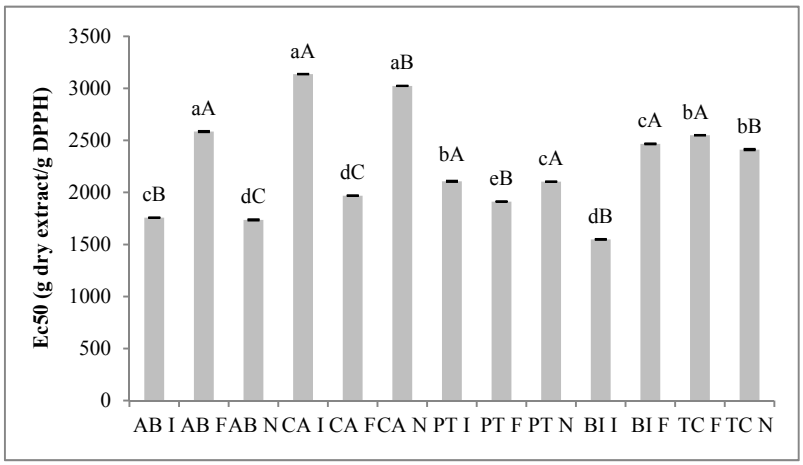

Figure 2. Antioxidant activity by DPPH method of *in vitro* callusof six mangaba accessions from the Mangaba Germplasm Bank. Embrapa Coastal Tablelands, Sergipe, Brazil. AB (Água Boa), BI (Barra do Itariri), CA (Costa Azul), CJ (Itaporanga), TC (Terra Caída) and PT (Pontal). (I: Internodal; F: Leaves; N: Nodal). Same lower case letters (for accessions) and capital letters (explant types) do not differ significantly by the Tukey test ($p < 0.05$)

The highest values for EC_{50} (3000 g dry extract/g DPPH) were observed in callus of the CA accession coming from nodal and internodal segments (Figure 2), suggesting the lower antioxidant capacity between the accessions. Extracts from AB (nodal and internodal segments) and BI (internodal segment) accessions presented lower antioxidant activity (1554.81 g of dry extract/g of DPPH) when compared to the others accessions.

According to Reynertson et al. (2005) the intensity of antioxidant activity can be measured from the effective concentration (EC_{50}), using the results of antioxidant activity of dilutions in series. EC_{50} is the concentration of extract needed to reduce 50% of radical DPPH and the lower the value of EC_{50} the greater the antioxidant activity. Extracts with EC_{50} below 50 µg m/L indicate elevated activity, from 50 µg/mL to100 µg/mL indicate moderate activity, from 100 µg/mL to 200 µg/mLactivity and above 200 µg/mL are considered inactive (Reynertson et al., 2005). Despite the high value found, it was observed the change in the coloration of the solutions with DPPH and extract from 60 minutes, this change in color indicates the occurrence of antioxidant activity.

Callus of *Harpagophytum procumbens* Burch. achieved higher EC_{50} (46.7 µg/mL) on MS medium with 0.2 mg/L of NAA (naftalen acetic acid) and 1 mg/L BA (Grabkowsa et al., 2016). On the other hand, Giri et al. (2012) reported that on callus of *Habenaria edgeworthii* the total phenolic content varied significantly in callus grown on BA, and ranged from 10.33 to 14.30 mg gallic acid equivalent (GAE)/g dry weight (DW) and antioxidant activity showed maximum activity in callus grown on 3.0 µM BA containing medium.

In studies with *in vitro Passiflora pofli*icallus, Simão et al. (2016) observed greater antioxidant activity in ethanolic extract on primary and secondary roots. Fazal et al. (2016) obtained no significant differences for the antioxidant activity in *P. vulgaris* callus. The difference between the amount and growth regulators used on the induction of callus modify the antioxidant activity (Bonfil et al., 2014). Another factor to be considered is the presence of chemical constituents in the extract, those responsible for antioxidant activity (Sadeghi et al., 2015).

It was not possible to determine the antioxidant activity in the CJ accession due to *in vitro* callus formation, there was oxidation of the tissue, requiring adjustment of protocol of callus induction. This result reinforces the genotype-dependence of *Hancornia speciosa* Gomes to callus formation. According to reports by Benderradji et al. (2012), genetic factors are considered one of the main contributors to the *in vitro* response. Thereby, there is difference in the callus production and plant regeneration, depending on the genotype and source of the explants (Ganesha et al., 2006).

3.3 Molecular Analysis

The 17 ISSR initiators resulted in 39 fragments, of which 24 were polymorphic (52.07%) (Table 4). The allogamy of mangaba tree and its self-incompatibility makes the plants derived from highly divergent seeds among themselves and in relation to the mother plant (Vieira Neto, 2002; Darrault & Schlindwein, 2006).

Table 4. Number of bands formed using different primers in mangaba accessions.

Primers	Number of fragments	Number of polymorphic fragments	% of Genetic similarity
811	2	0	0
807	2	2	100
815	3	1	33
816	1	0	0
817	4	3	75
855	3	2	66
823	5	4	80
825	4	1	25
826	3	3	100
888	2	2	100
856	2	1	50
810	2	0	0
816	1	0	0
848	5	5	100
Total	39	24	52.07

Therefore, inside the germplasm bank progenies hybridization may occur due to natural pollinator agents. Soares et al. (2016) carried out the test with 155 mangaba individuals of natural species from Sergipe, concluding 100% of polymorphism presenting high genetic variability among populations. The rate of genetic similarity of this study was considered low when compared with the study on natural species, the fact of the matrices belonging to the BAG, factors such as geographical proximity of the accessions and the position of the matrices in the experimental field, interfere in genetic diversity between matrix/progeny. Therefore, when comparing the matrices and the progenies of our study, we concluded that the genetic similarity was maintained in all accessions to seeds germinated *in vitro*.

In general, the genetic distance among the studied accessions was large, since there is a genotypic and phenotype difference between accessions. However, the genetic diversity between matrix and progeny was low. In CA, CJ and PT accessions we observed higher values for genetic distance between the matrix and progeny (Figure 3).

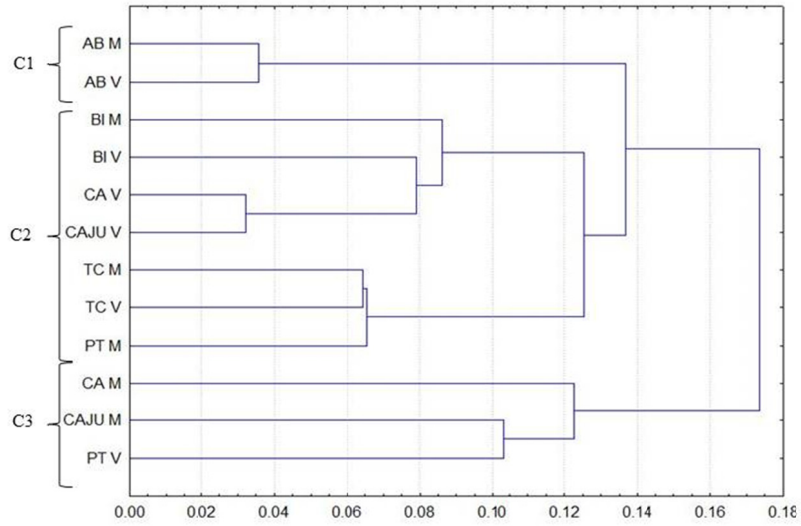

Figure 3. Dendrogram generated from the Jaccard similarity coefficient, obtained by the Ward method, using 14 primers through the ISSR technique among 12 mangaba individuals belonging to the Mangaba Germplasm Bank of Embrapa Coastal. Accessions: AB (Água Boa), BI (Barra do Itariri), CA (Costa Azul), CAJU (Itaporanga), TC (Terra Caída) and PT (Pontal). (M: Matrix; V: *in vitro*)

We highlight the CA accession, which had greater genetic distance between matrix/progeny, and EC50 data were different between leaf and callus.

4. Discussions

4.1 Identification of Phenolic Compounds in in vivo Leaves and Callogenesis

Callus induced from *Gynochthdes umbellata* leaves, obtained higher concentration of anthraquinone (18.18±0.58) on MS medium supplemented by 1 mg/L of 2,4-D after 60 days of cultivation (Anjusha & Gangaprasa, 2017).

According to Chavan et al. (2015), different concentrations of auxins and cytokinins change in the quantification of the studied compound in *in vitro* callus cultures. In the study conducted by Khattab et al. (2014), using of phytoregulators in callogenesis of *Pilosocereus robinii*, were detected the minimum concentrations of the malic acid phenolic compound. Other factors may be considered on the *in vitro* production of secondarymetabolitessuch as abiotic factors, temperature, humidity, light, water supply, minerals and CO_2. They influence plant growth and directly affect the biochemical ways (Akula & Ravishankar, 2011), and the genetic factors interfere in the synthesis of these compounds (Bai et al., 2017). Fazal et al. (2016) suggest that the use of LED lamps is a promising approach to the antioxidant secondary metabolites production in *Phaseolus vulgaris* callus cultures.

Rutin was not decteted in extract from callus obtained from leaf explants. Similar results of absence of flavonoids were obtained by Szopa et al. (2016), in *in vitro*callus of *Schisandra chinensis* (Turcz.), popularly known as Chinese Magnolia. In opposite, a study conducted by Castro et al. (2016), showed the potential of *in vitro* Murici-do-cerrado (*Byrsonomia verbascifolia* Rich.) callus as a new source of total phenolic compounds. Changes in injection or extraction protocol, as well as in the concentrations and types of growth regulators, are essential for optimizing the *in vitro* rutin production from callus.

4.2 Antioxidant Activity

The values of EC_{50} obtained in this study are higher than those observed by Damiano et al. (2017) with methanolic extract of *Ziziphus jujuba* Mill. leaves with results of EC_{50} was 25 µg/mL, concluding higher antioxidant activity when compared to the results obtained in this study. The DPPH analysis in extract of *Olea europaea* leaves held by Difonzo et al. (2017) obtained antioxidant potential, and in the chromatographic profile the rutin compound was identified. Torres-Rêgo et al. (2016) observed the anti-inflammatory effect of rutin and chlorogenic acid extracted from mangaba fruits in animal models. Thus, these results corroborate our study, due to the identification of different rutin concentrations among accessions on *in vivo* mangaba leaves, concluding that this chemical compound is related to all analyses carried out in this research.

Plants frequently cope up with the rapid fluctuations and adversity of environmental conditions because of their intrinsic metabolic capabilities (Simontacchi et al., 2015). Variations in the outside environment could put the plant metabolism out of homeostasis (Foyer & Noctor, 2005), and create necessity for the plant to harbor some advanced genetic and metabolic mechanisms within its cellular system (Apel & Hirt, 2004; Gill & Tuteja, 2010). Plants possess an array of protective mechanisms acquired during the course of evolution to combat adverse environmental situations (Yolcu et al., 2016).

4.3 Molecular Analysis

The similarity between accessions of nearby regions such as Sergipe and Bahia can be explained by the movement of genes within plant populations that are conditioned by the degree of genetic compatibility between the individuals of the population (Silva et al., 2011). Khattab et al. (2014) used the ISSR-PCR technique to prove the genetic polymorphism of the explants submitted to MS medium supplemented with TDZ and 2,4-D in *Pilosocereus robinii*, determining that abiotic factors may interfere with DNA.

There was low genetic divergence between the matrix and the progenies, with the exception of PT, CA and CJ, in which the distance was approximately 0.06. This fact suggests that the alogamy of the matrices in the Caju reserve can cause a greater genetic distance between the progeny and the mother plant. The results of genetic divergence corroborate with the results obtained by Cheruvathur et al. (2012), where the analysis of ISSR data in nine plants regenerated *in vitro* from *R. nasutus* callogenesis and the mother plant, the use of 12 ISSR markers indicated low genetic variability. In a study with ISSR markers in plants regenerated *in vitro* from the callogenesis, Perera et al. (2015) concluded that these are useful in detecting polymorphisms in *Miscanthus* × *Giganteus* and can be used for generation and screening of mutants leading to the development of new cultivars.

The divergence data were compared with previous successful studies in individuals of the native population of *Hancornia speciosa* Gomes (Santos et al., 2017; Silva et al., 2017; Soares et al., 2016; Amorim et al., 2015; Costa et al., 2015; Jimenez et al., 2015; Silva et al., 2013) and BAGs (Silva et al., 2011; Costa et al., 2011; Luz, 2016; Vitória, 2017), with the use of molecular markers.

In the present study, the use of molecular markers has been used in a number of studies, including the use of molecular markers (Silva et al., 2011). The results of genetic divergence corroborate the results obtained by Cheruvathur et al. (2012), where the analysis of ISSR data in nine plants regenerated *in vitro* from callogenesis of *R. nasutus* and mother plant, the use of 12 ISSR markers indicated low genetic variability. The combination of phytochemical studies and genetic similarity favors the identification of more divergent individuals with characteristics of interest. The results will collaborate on the conservation strategies of BGMangaba and future breeding programs.

5. Conclusions

The rutin concentrations in the BI and CJ accessions were 64.26 and 67.26 mg/L of extract. The antioxidant activity of the *in vivo* accessions from BI and CA leaves extracts was higher (EC_{50} = 110.09 and 176.03 g of dry extract/g of DPPH). The antioxidant activity of the *in vitro* accessions from different explant extracts showed lower antioxidant capacity. Rutin is detected in different amounts of *in vivo* leaf extract. However, the rutin is not detected in extracts from *in vitro* callus of mangaba tree. DPPH concentration is superior in extracts from *in vitro* callus, with the *in vitro* CA accession (internodal and nodal). There is low diversity among the evaluated mangaba accessions; however, there is high genetic similarity between matrices and their progenies. The CA accession groups itself in isolation from others and EC_{50} concentrations differentiated between leaf and callus are obtained.

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Abbreviations

AA: ascorbic acid; BAP: 6-benzylaminopurine; IAA: indole-3-acetic acid; IBA: indole-3-butyric acid; ISSR: inter simple sequence repeats; MS: Murashige and Skoog's medium; NAA: naphthalene acetic acid; HPLC: high performance liquid chromatography; DPPH: 2.2-diphenyl-1-picrilhydrazyl; Accessions: AB (Água Boa), BI (Barra do Itariri), CA (Costa Azul), CJ (Itaporanga), TC (Terra Caída) and PT (Pontal).