Scientia Horticulturae 226 (2017) 372–378

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Genetic diversity and the quality of Mangabeira tree fruits (*Hancornia speciosa* Gomes – Apocynaceae), a native species from Brazil

Priscilla Santana Santos[a], Lisiane dos Santos Freitas[a,*], Josefa Grasiela Silva Santana[a], Evandro Neves Muniz[b], Allívia Rouse Carregosa Rabbani[c], Ana Veruska Cruz da Silva[b]

[a] Federal University of Sergipe, Av. Marechal Rondon, s/n, 49100-000, São Cristovão, SE, Brazil
[b] Embrapa Coastal Tablelands, Av. Beira Mar, 3250, 49025-040, Aracaju, SE, Brazil
[c] Federal Institute of Bahia, Rod. BR 367, km 57, 5.Rua José Fontana, s/n, 45810-000, Porto Seguro, BA, Brazil

ARTICLE INFO

Keywords:
Hancornia speciosa gomes
Molecular markers
Native fruits
Post-harvest

ABSTRACT

The mangaba is a native Brazilian species of socioeconomic importance and with a great appeal for the conservation of its genetic resources. The fruit is rich in nutrients, especially ascorbic acid. Because the species' areas of natural occurrence have decreased rapidly, the Embrapa Coastal Tablelands has maintained it in the Mangaba Active Germplasm Bank (AGBMangaba), in Itaporanga D'Ajuda, Sergipe, Brazil, since 2006, in an attempt to conserve its genetic resources. The objective of the present study was to estimate the genetic diversity and fruit quality of six bank accessions (BI, CA, LG, PR, PT, and TC) from the states of Bahia and Sergipe, aiming at the selection of promising material for genetic improvement. Twenty ISSR markers were tested, and 12 were selected to evaluate genetic similarity, which allowed the identification of three distinct groups. The longitudinal and transversal diameters were measured, and fresh mass content, the number of seeds, pH, soluble solids content, titratable total acidity, and vitamin C content were evaluated. The quality of these accessions was evident; the BI, CA, PR, and PT accessions showed the highest values of vitamin C content, which is a characteristic of interest in both genetic improvement programs and agroindustry.

1. Introduction

Mangabeira (*Hancornia speciosa* Gomes) is a native Brazilian fruit and lactiferous species that belongs to the Apocynaceae family. It is found in the Cerrado biome, mainly in the Northern, Southeastern, and Midwestern regions of Brazil, and in the Coastal Tablelands and Coastal Lowlands (Vieira Neto et al., 2009).

The plant adapts well in areas with an average temperature of 25 °C, rainfall of 750–1600 mm/year, and altitudes of up to 1500 m. It has good tolerance to water stress and presents good vegetative development during high-temperature seasons (Ferreira and Marinho, 2007). Parts of Mangabeira fruits are used in folk medicine in some regions of Brazil (Santos et al., 2012).

The fruit (mangaba) is very important for the Brazilian agroindustry, especially in the northeastern region. It is presented as yellow berries with small and big red spots, have a sweet-acidulous pulp, and mature from October to March (Lorenzi et al., 2006). Despite that the tree produces latex, the fruits are the main product considered as a good source of iron, manganese, zinc, and ascorbic acid (Pereira et al., 2006). They are marketed as fresh fruits and, among other forms, as processed jams, jellies, cookies, juices, and ice cream (Guilherme et al., 2007; Clerici and Silva, 2011).

Exogenous factors such as intense market of lands, production of monocultures, construction of touristic infrastructures, and agriculture have contributed to a reduction of areas of natural Mangabeira occurrence (Mota et al., 2008). Although the extractive activity is threatening the species, it has a strong economic and social influence on many families in the Northeastern region, who usually generate an income as Mangabeira pickers (Schmitz et al., 2009). More than 5000 families practice the extractive collection of Mangabeira fruits in the State of Sergipe during the fruit season; the Brazilian production exceeds 800 tons/year (IBGE, 2016).

The establishment of germplasm banks is the first step for the implementation of a breeding program; one of the main requirements is the ability to identify superior genotypes and segregating populations. Molecular genetics techniques have become an important tool set for the identification, characterization, and genetic mapping of species; these tasks are being carried out more safely, quickly and efficiently

* Corresponding author.
E-mail addresses: Brazil.priscilla-santana1987@bol.com.br (P.S. Santos), lisiane_santos_freitas@yahoo.com.br (L. dos Santos Freitas), grasi_agronomia@hotmail.com (J.G. Silva Santana), enmuniz@hotmail.com (E.N. Muniz), alliviarouse@hotmail.com (A.R.C. Rabbani), ana.veruska@embrapa.br (A.V.C. da Silva).

http://dx.doi.org/10.1016/j.scienta.2017.09.008
Received 22 May 2017; Received in revised form 25 August 2017; Accepted 8 September 2017

than before (Bered et al., 1997).

In Brazil, there are three important gene banks for the conservation and maintenance of Mangabeira species; the oldest belongs to the Pernambuco Agricultural Research Company (IPA), which began in 1970 with 125 accessions (Bezerra et al., 1993); the second oldest belongs to the Agricultural Research of Paraíba State Company – EMEPA, established in 1991 and currently with 220 accessions (Aguiar Filho et al., 1998; Souza et al., 2007); and the third belongs to the Embrapa Coastal Tablelands (AGBMangaba), was established in 2006 in the municipality of Itaporanga D'Ajuda in Sergipe State, has 271 accessions, and is located in a resting area with 4.7 ha (Costa et al., 2011; Embrapa, 2013).

The studies carried out at the AGBMangaba underscore the low genetic diversity found in the first implanted accessions (Costa et al., 2011). In 2013, the first fruits blossomed from these accessions; their quality attributes were evaluated by Silva et al. (2015) and the BI access was identified with the highest level of vitamin C.

The use of effective techniques for the identification and characterization of genotypes is essential for genetic improvement programs. The characterization of quality attributes in isolated fruits is not efficient for estimating available genetic variability because environmental factors can contribute to the chemical composition variation in the evaluated genotypes.

Molecular markers are important tools for the detection of variability; they can be used to indicate polymorphism at the DNA level and establish links between the presence or absence of markers and genes that control a particular trait (Oliveira et al., 1996).

The correlation between the physicochemical and genetic characterization of AGBMangaba genotypes in the Embrapa Coastal Tablelands has not been evaluated. Thus, the objective of this study was to estimate the genetic diversity and quality attributes of mangabeira fruits from six AGBMangaba accessions aiming at the selection of promising material for genetic improvement.

2. Material and methods

2.1. Plant material

The plant material originated from the mangabeira Active Bank of Germplasm (AGBMangaba) was implemented in the Experimental Field of the Embrapa Coastal Tablelands in the restinga area without irrigation, in the Municipality of Itaporanga D'Ajuda, SE (11°06′40″S and 37°11′15″W), with soil classified as Humiluvic Spodosol. It is currently composed of 271 genotypes, which represent 22 accessions from different origins and ages. Fruits that were present at the 2015 harvest were selected for the study, collected at maturation stage “ripe”, totaling 36 genotypes from six accessions (Table 1). The fruits were harvested at this stage to maintain uniformity in the analyses since chemical composition can be altered according to the maturation stage in mangaba fruits.

Table 1
Mangabeira accessions (*Hancornia speciosa* Gomes) from the Active Germplasm Bank of Embrapa Coastal Tablelands.

State	Municipality	Accession	Number of genotypes
Bahia	Jandaíra	Costa Azul (CA)	6
	Conde	Barra de Itariri (BI)	6
	Mata de São João	Lagoa Grande (LG)	6
Sergipe	Indiaroba	Terra Caída (TC)	6
		Preguiça (PR)	6
		Pontal (PT)	6

2.2. DNA extraction and ISSR amplification

Young leaves from each genotype were used for DNA extraction (Doyle and Doyle, 1987) with modifications and quantified in a spectrophotometer (*Thermo Scientific* NANODROP 2000c) at 260 and 280 nm wavelength. The DNA quality was evaluated by agarose gel electrophoresis (0.8%, m/v) and visualized on the Gel doc L-pix HE photo documentation equipment (Loccus Biotechnology, Brazil). The quantified samples were diluted in TE [10 mM Tris-HCl, pH 8.0, 1 mM EDTA] at the concentration of 25 ngμL^{-1} and stored at −20 °C for subsequent use in the ISSR reactions. Twenty ISSR primers (UBC – University of British Columbia, Vancouver, Canada) were chosen to test the accessions (Vieira Neto et al., 2009). The polymerase chain reactions (PCR) were performed in 20 μL volume containing: 2 μL of the genomic DNA solution, 2 μL of each primer, and a mix composed of 2 μL of 10X PCR buffer; 0.4 μL dNTP (10 mM); 0.6 μL $MgCl_2$ (50 mM); 0.2 μL Invitrogen® Taq DNA polymerase (5 U/μL), and 12.8 μL of ultrapure water. The amplification cycles included one denaturation cycle at 95 °C for 5 min and 45 amplification cycles of denaturation at 94 °C for 1 min, annealing at different temperatures for 45 s, and a final extension at 72 °C for 2 min.

Two microliters of loading buffer (0.01% bromophenol blue, 40% glycerol) were added to 20 μL of each PCR reaction volume after amplification; 17 μL were loaded onto 2% agarose gel (dissolved in 1X TBE – 89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) and subjected to horizontal electrophoresis at 200 V, 200 mA, and 100 W for about one hour and thirty-five minutes. The gels were stained in a solution containing ethidium bromide (0.02 μL/mL water) for 60 min for visualization under ultraviolet light. The 1 kb molecular weight marker (Promega, Madison, South Dakota, EUA) was used as the molecular band size standard.

The visualization of results was carried out in the Loccus L-pix HE gel photo documentation equipment (Loccus Biotecnologia, Brazil).

The electrophoretic profile of each ISSR primer was transformed into a binary matrix. The presence of a fragment was represented by 1 and the absence by 0. The binary data were used to perform all subsequent analyses. The binary matrix was used to obtain genetic similarity estimates with the aid of the FreeTree software using the Jaccard coefficient (1908) to carry out the analysis of genotype clustering through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in the TreeView software and 10,000× bootstraps.

The Principal Coordinates Analysis (PCoA) was also carried out based on genetic similarity using the Genalex version 6.3 software (Peakall and Smouse, 2006).

The Shannon index (I) (Brown and Weir, 1983) and genetic diversity (He) estimated on the basis of expected heterozygosity were calculated as described by Lynch and Milligan (1994) and Maguire et al. (2002) using the Genalex software version 6.3 (Peakall and Smouse, 2006). The polymorphic information content (PIC) is a parameter that provides an estimate of the discrimination power of the molecular marker per primer and was calculated according to Ghislain et al. (1999). The marker index (MI) was determined as a PIC product and number of polymorphic fragments per test unit as described by Zhao et al. (2007).

Inferences about the structure within Mangabeira genotypes were carried out using the Structure version 2.2. software (Falush et al., 2007; Pritchard et al., 2000). To estimate K, the number of Reconstructed Panmictic Populations (RPPs) of individuals was calculated using values ranging from 1 to 10 and assuming that the sampled genotypes were from anonymous plants of unknown origin. In addition, five replicates for each estimated K value were used, each one consisting of a burning period length of 15,000 steps, followed by the 100,000 replicas of Markov chain Monte Carlo. The Structure software estimates the most probable number of clusters (K) by calculating the probability of data log for each value of K. According to Evanno et al. (2005), the alteration of the second order of the likelihood function