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Molecular characterization of cajá, *Spondias mombin* (Anacardiaceae), by RAPD markers

A.T.B. Lima[1], V.A.B. de Souza[1], R.L.F. Gomes[2] and P.S.C. Lima[1]

[1]Embrapa Meio-Norte, Teresina, PI, Brasil
[2]Departamento de Fitotecnia, Centro de Ciências Agrárias,
Universidade Federal do Piauí,
Campus Universitário Ministro Petrônio Portella, Teresina, PI, Brasil

Corresponding author: P.S.C. Lima
E-mail: sarmanho@cpamn.embrapa.br / paulosarmanho@yahoo.com.br

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ABSTRACT. The arboreal species *Spondias mombin* L. (Anacardiaceae) is widely distributed in Brazil, where the fruits, known by the common name of cajá, are an important commercial commodity. We evaluated genetic variability among 32 cajá accessions of the Germplasm Collection of Embrapa Meio-Norte using RAPD technique. Reaction conditions for efficient RAPD amplifications were optimized in preliminary tests, and primers were selected from a set designed by the University of British Columbia on the basis of high levels of polymorphism and adequate band resolution. The 21 primers employed in the final analysis produced 145 fragments, 79% of which were polymorphic. Based on the RAPD data, a dendrogram was constructed using the unweighted pair group method with arithmetic mean clustering technique. The 32 cajá accessions were classified into three main groups with a mean genetic similarity of 68.8%. Group I comprised 26 accessions (74.1% similarity), and group II included five accessions (74.0% similarity), while group III consisted of one accession (BGC 06), which exhibited the lowest similarity coefficients. Accessions BGC 06 and BGC 31 were the most unrelated and, hence, most suitable for initial crossings in order to obtain

high levels of segregation. We concluded, based on the repeatability and reproducibility tests, that the RAPD technique is reliable and efficient for revealing the genetic diversity of cajá accessions, which will be useful for genetic improvement programs.

Key words: Cajá; Genetic variability; Plant improvement

INTRODUCTION

The genus *Spondias* (family Anacardiaceae) comprises a number of economically important trees, including *Spondias mombin* L., *S. tuberosa* Arr. Câm. (umbu), *S. purpurea* L. (ciriguela), *S. dulcis* Park. (cajarana or cajá-mango), and *Spondias* sp (umbu-cajá and umbuguela) (Sacramento et al., 2008). *S. mombin* originates from tropical America where it is known as jobo in Mexico, yellow mombin in the Caribbean islands and gully plum in Jamaica. The species is distributed throughout Brazil where the exotic fruits are known by a wide variety of regional names including taperebá in the Amazon, small cajá in the southeast, cajá-mirim in the south, or simply cajá in the northeast (Bosco et al., 2000). Cajá fruits are rich in vitamins A, B1, B2, and C, calcium, phosphorus, potassium, carotenoids, and tannins, and can be eaten raw or in the form of pulp, nectar, sorbet, jam, or liqueur. The wood of cajá is employed in the furniture manufacturing industry, while the bark and leaves are used in popular medicine (Sacramento and Souza, 2000).

Although no statistical data are available for cajá, the fruits are considered to be of great importance in the northeastern region of Brazil because of the growing demand for the products derived from them and the increasing interest in improving cultivation of the species. In the State of Piauí, however, cajá cultivation is currently restricted to small farmers and is generally carried out in a rudimentary manner without the application of advanced technology (Leal, 2005).

Knowledge regarding the genetic variability of any species is an essential prerequisite for its preservation and for success in breeding programs. In the case of cajá, a high genetic variability among cultivars has been confirmed by a number of studies involving morphological (Soares et al., 2006; Mendonça et al., 2008), isoenzyme (Gois et al., 2009) and molecular (Silva, 2009) markers.

Recently, studies in plant breeding have focused on the search for molecular markers linked to different economic traits, which would allow the indirect selection of desirable characteristics in early segregating generations. According to Oliveira et al. (2007), the use of such markers has advanced the development of genetics and breeding mainly by offering the possibility of access to the genotype rather than just the phenotype, thus allowing the study of genetic mapping, genetic diversity, phylogeny and indirect marker-assisted selection.

Among the various molecular techniques based on the polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD) markers are advantageous because they can detect polymorphisms in a very straightforward manner. Furthermore, RAPD markers can be used in studies concerning genetic divergence since they are efficient in distinguishing minimal differences between individuals, populations and species (Brammer, 2000). Intraspecific characterization and evaluation of the genetic variability of accessions with the aid of molecular markers facilitate the selection of the most suitable accessions for cultivation and/or plant improvement, and reduce the time spent on this stage of the process.

The aim of the present study was to determine the genetic variability among the cajá accessions in the Germplasm Collection of Embrapa Meio-Norte using RAPD markers in order to select genotypes suitable for use in commercial cultivation and in future breeding projects.

MATERIAL AND METHODS

Plant material and extraction of genomic DNA

Thirty-two cajá accessions were available in the Germplasm Collection of Embrapa Meio-Norte, and these originated from Teresina, Altos, Eliseu Martins, Demerval Lobão, and Itaueira in the State of Piauí, and Timon in the State of Maranhão (Table 1). Young healthy leaves were collected from each accession, wrapped separately in paper towels, placed in plastic bags and transported to the laboratory in a box filled with ice. DNA extraction was carried out immediately with the aid of Invisorb Spin Plant Mini kits (Invitek, Berlin, Germany) or DNeasy Plant Mini kits (Qiagen, Valencia, CA, USA), according to manufacturer instructions. Sample leaves (100 mg) were macerated separately in a Precellys® 24 tissue homogenizer and grinder (Bertin, Montigny-le-Bretonneux, France) using 2-mL tubes with five 3-mm glass beads and buffers from the appropriate extraction kit. Quantification of genomic DNA was performed by comparison with λ DNA standards (50, 100 and 150 ng) following electrophoresis on 0.8% agarose gel in Tris-borate-EDTA (0.5X TBE) buffer and staining with SYBR® Safe DNA Gel Stain (10,000X; Invitrogen, Carlsbad, CA, USA). The quality of DNA was determined with the aid of a Nanodrop (Wilmington, DE, USA) model 2000 spectrophotometer using 2-µL aliquots of the extracts. DNA samples were diluted in Tris-EDTA (TE) buffer to a final concentration of 15 ng/µL and stored in the freezer at -20°C until required for RAPD reactions.

Table 1. Origins of the accessions of cajá (*Spondias mombin* L.) in the Germplasm Collection of Embrapa Meio-Norte.

Origin	Accessions
Timon (MA)	*BGC02*, *BGC 03*, *BGC 13*
Itaueira (PI)	*BGC 17*
Eliseu Martins (PI)	*BGC 08*
Altos (PI)	*BGC 04*, *BGC 05*, *BGC 06*
Teresina (PI)	*BGC 01*, *BGC 07*, *BGC 09*, *BGC 10*, *BGC 11*, *BGC 12*, *BGC 14*, *BGC 15*, *BGC 16*, *BGC 18*, *BGC 19*, *BGC 20*, *BGC 21*, *BGC 22*, *BGC 23*, *BGC 24*, *BGC 25*, *BGC 26*, *BGC 27*, *BGC 28*, *BGC 29*, *BGC 30*
Demerval Lobão (PI)	*BGC 31*, *BGC 32*

MA = State of Maranhão; PI = State of Piauí.

RAPD reactions

PCR amplifications were carried out in 0.2-mL microtubes using a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) according to the general protocol of Williams et al. (1990). However, in order to optimize the PCR assays, preliminary tests were performed under various reaction conditions that included $MgCl_2$ concentrations of 1.5, 2.0, 2.5, or 3.0 mM, dNTP concentrations of 0.25, 0.40, 0.50, or 0.75 mM, annealing temperatures of 32° or 35°C, and 40 or 45 reaction cycles. Optimized reaction mixtures comprised 1X Invitrogen buffer (20 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, pH 8.0), 3.0 mM $MgCl_2$ (Invitrogen), 0.75 mM dNTP (Invitrogen), 1 U Taq DNA polymerase (Cenbiot-Enzimas, Porto Alegre, RS, Brazil), 0.2 µM primer, 1 µL DNA template (~15 ng) and ultrapure

distilled water to a final volume of 20 µL. Optimized reaction conditions were: initial denaturation step at 92°C for 1 min, 45 cycles comprising denaturation at 92°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 2 min, and a final extension step at 72°C for 5 min. In these assays, primers A10 and M03 (with melting temperatures of 22.26° and 26.36°C, respectively) were employed in order to assess their behavior under the reaction conditions.

With the purpose of selecting the most suitable primers for the subsequent RAPD reactions, DNA samples from cajá accessions BGC 17 and BGC 29 were amplified using 100 primers developed by the University of British Columbia. Twenty-one primers (Table 2) were chosen on the basis of resolution and high levels of polymorphism, and these were used in amplification reactions involving DNA samples from each of the 32 cajá accessions. For each assay, amplicons were separated by electrophoresis on 1.5% agarose gel in 0.5X TBE at 100 V for 4 h. Gels were stained with SYBR Safe DNA Gel Stain (10,000X), visualized under a UV transilluminator and photographed. DNA ladders of 100 bp and 1 kb (Invitrogen) were used as molecular weight markers.

Table 2. Primers employed in the random amplified polymorphic DNA reactions of the accessions of cajá (*Spondias mombin* L.) .

Primer	Size of amplified fragments (bp)	Numbers of fragments	
		Total	Polymorphic
OPA03	600-2036	10	8
OPA05	600-2036	7	5
OPA08	506-3054	8	6
OPA10	600-3054	7	7
OPA14	320-2036	5	5
OPF02	700-2036	8	8
OPF03	1100-1500	3	2
OPF04	300-1636	9	7
OPF15	300-3054	9	8
OPG02	350-3054	8	5
OPG08	390-3000	10	9
OPG17	510-4072	9	8
OPG18	870-3000	7	3
OPL01	1100-2100	6	3
OPL03	1100-1500	4	3
OPL14	600-1500	8	7
OPM07	1150-4072	8	7
OPN14	1100-1300	2	1
OPN16	650-3054	5	4
OPN18	900-2100	5	3
OPT12	700-2100	7	6
Total		145	115

Data analysis

Initially, a visual examination of the gel photographs was performed in order to determine the number of polymorphic bands generated by each primer, taking into account the resolution and degree of amplification. Only bands with medium and strong intensities were included in the investigation. Each band was considered as representing a single character, and a binary matrix was created in which 1 indicated the presence of the marker and 0 its absence. This matrix was used to estimate the genetic similarity between the cajá accessions using Jaccard coefficients, and on this basis a dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) clustering technique. The cophenetic

correlation coefficient (r) was calculated from the similarity matrix and the dendrogram. The bootstrap confidence index was also evaluated from the binary matrix of amplified fragments generating a dendrogram from 1000 permutations. Analyses were performed with the aid of the PAST version 1.34 program (Hammer et al., 2001).

Repeatability and reproducibility of RAPD amplifications

Three different tests using primer OPG17 were carried out to determine the reliability of the RAPD method: (i) repeatability - amplification reactions involving DNA samples from each of the 32 cajá accessions were repeated exactly as described above using Cenbiot-Enzimas Taq DNA polymerase; (ii) reproducibility with different thermocyclers - the amplifications outlined in (i) were performed using a Techne® Endurance TC412 Thermal Cycler (Bibby Scientific, Stone, UK), and (iii) reproducibility with different Taq DNA polymerases - the amplifications outlined in (i) were performed using Invitrogen Taq DNA polymerase.

RESULTS

Extraction of the 32 cajá accessions provided genomic DNA that was suitable for RAPD amplifications. The absorbance ratio at 260 and 280 nm of the extracted DNA varied from 1.24 to 1.96 (mean of 1.72), while the concentrations of the DNA solutions were in the range 15 to 150 ng/µL (mean of 63.1 ng/µL). The conditions selected for the PCRs, namely 3.0 mM $MgCl_2$, 0.75 mM dNTP, 35°C annealing temperature and 45 cycles, generated bands of good definition and with high resolution. The 21 primers selected for the RAPD analyses produced 145 fragments, of which 115 (79.3%) were polymorphic (Table 3). The average number of bands produced per primer was 6.8, with an average of 5.4 bands per primer being polymorphic, and the size of the amplicons varied between 300 and 4072 bp. Figure 1 shows the degree of polymorphism of the RAPD bands generated by primers OPG08 (Panel A) and OPF15 (Panel B).

The dendrogram (Figure 2) indicating the relationships among the 32 cajá accessions showed a mean genetic similarity of 68.8%. The accessions could be separated into three main clusters. Group I comprised 26 accessions and had a mean similarity of 74.1% (62.2-85.2%), and group II included five accessions with a mean similarity of 74.0% (61.4-85.2%), while group III consisted of a single accession (BGC 06), which displayed the lowest similarity value (45.5%) of all accessions. The highest similarity (85.2%) was determined for the accessions BGC 13 and BGC 14, which had been collected in the States of Maranhão and Piauí, respectively. The reliability of the data and the consistency of the nodes are demonstrated by the bootstrap values shown in Figure 2, and by the cophenetic correlation coefficient of 92.4%. Comparison of the band patterns of the 32 accessions generated by RAPD amplifications with those obtained in the first validation test employing primer OPG17 indicates that repeatability was 100% (Figure 3A and B). The reproducibility of the band patterns generated in the second test in which two different thermal cyclers were used was also highly satisfactory (91.7%; Figure 3B and C). In the third test, in which amplification reactions were carried out using Taq DNA polymerases from Cenbiot-Enzimas and Invitrogen were compared, reproducibility was estimated at 83.3% (Figure 3A and D).

Table 3. Similarity coefficients for the accessions of cajá (*Spondias mombin* L.) in the germplasm collection of Embrapa Meio-Norte determined by RAPD analysis.

Accession	*BGC01*	*BGC02*	*BGC03*	*BGC04*	*BGC05*	*BGC06*	*BGC07*	*BGC08*	*BGC09*	*BGC10*	*BGC11*	*BGC12*	*BGC13*	*BGC14*	*BGC15*	*BGC16*
BGC01	1	0.802	0.824	0.583	0.807	0.541	0.647	0.790	0.696	0.789	0.702	0.779	0.774	0.764	0.771	0.736
BGC02	0.802	1	0.793	0.587	0.731	0.604	0.626	0.746	0.676	0.773	0.629	0.763	0.744	0.734	0.695	0.733
BGC03	0.824	0.793	1	0.588	0.783	0.532	0.612	0.797	0.702	0.825	0.750	0.785	0.810	0.813	0.733	0.742
BGC04	0.583	0.587	0.588	1	0.584	0.607	0.702	0.576	0.544	0.586	0.577	0.574	0.573	0.528	0.573	0.596
BGC05	0.807	0.731	0.783	0.584	1	0.500	0.622	0.738	0.670	0.764	0.719	0.726	0.722	0.754	0.675	0.683
BGC06	0.541	0.604	0.532	0.607	0.500	1	0.536	0.471	0.510	0.491	0.477	0.466	0.479	0.463	0.514	0.538
BGC07	0.647	0.626	0.612	0.702	0.622	0.536	1	0.600	0.573	0.623	0.575	0.598	0.597	0.578	0.626	0.664
BGC08	0.790	0.746	0.797	0.576	0.738	0.471	0.600	1	0.686	0.821	0.720	0.797	0.764	0.754	0.718	0.698
BGC09	0.696	0.676	0.702	0.544	0.670	0.510	0.573	0.686	1	0.728	0.649	0.644	0.669	0.661	0.706	0.670
BGC10	0.789	0.773	0.825	0.586	0.764	0.491	0.623	0.821	0.728	1	0.746	0.795	0.762	0.766	0.730	0.782
BGC11	0.702	0.629	0.750	0.577	0.719	0.477	0.575	0.720	0.649	0.746	1	0.680	0.760	0.736	0.683	0.720
BGC12	0.779	0.763	0.785	0.574	0.726	0.466	0.598	0.797	0.644	0.795	0.680	1	0.724	0.756	0.691	0.699
BGC13	0.774	0.744	0.810	0.573	0.722	0.479	0.597	0.764	0.669	0.762	0.760	0.724	1	0.852	0.702	0.724
BGC14	0.764	0.734	0.813	0.528	0.754	0.463	0.578	0.754	0.661	0.766	0.736	0.756	0.852	1	0.720	0.701
BGC15	0.771	0.695	0.733	0.573	0.675	0.514	0.626	0.718	0.706	0.730	0.683	0.691	0.702	0.720	1	0.718
BGC16	0.736	0.733	0.742	0.596	0.683	0.538	0.664	0.698	0.670	0.782	0.720	0.699	0.724	0.701	0.718	1
BGC17	0.756	0.754	0.807	0.552	0.732	0.496	0.590	0.746	0.622	0.758	0.672	0.792	0.730	0.790	0.711	0.677
BGC18	0.756	0.739	0.778	0.587	0.702	0.543	0.685	0.746	0.771	0.819	0.757	0.733	0.744	0.720	0.770	0.811
BGC19	0.772	0.727	0.779	0.542	0.748	0.474	0.675	0.748	0.681	0.732	0.730	0.779	0.746	0.764	0.727	0.694
BGC20	0.746	0.686	0.782	0.539	0.667	0.496	0.553	0.764	0.681	0.762	0.717	0.767	0.720	0.738	0.714	0.694
BGC21	0.609	0.571	0.600	0.674	0.556	0.586	0.646	0.575	0.558	0.558	0.618	0.573	0.585	0.566	0.630	0.609
BGC22	0.769	0.667	0.760	0.589	0.688	0.478	0.587	0.758	0.661	0.742	0.711	0.760	0.728	0.719	0.723	0.689
BGC23	0.795	0.721	0.817	0.551	0.770	0.496	0.615	0.798	0.675	0.797	0.782	0.758	0.768	0.772	0.721	0.744
BGC24	0.748	0.702	0.769	0.530	0.724	0.474	0.608	0.752	0.655	0.779	0.809	0.726	0.750	0.740	0.717	0.740
BGC25	0.760	0.686	0.738	0.580	0.721	0.509	0.647	0.736	0.681	0.734	0.746	0.724	0.720	0.724	0.744	0.708
BGC26	0.758	0.742	0.839	0.583	0.734	0.487	0.607	0.776	0.653	0.760	0.730	0.824	0.803	0.764	0.685	0.721
BGC27	0.779	0.719	0.800	0.616	0.814	0.504	0.612	0.797	0.702	0.766	0.736	0.756	0.738	0.742	0.748	0.685
BGC28	0.752	0.721	0.730	0.551	0.714	0.483	0.576	0.770	0.690	0.811	0.738	0.772	0.754	0.758	0.707	0.758
BGC29	0.744	0.728	0.806	0.562	0.734	0.496	0.598	0.789	0.656	0.746	0.744	0.736	0.773	0.737	0.674	0.736
BGC30	0.595	0.615	0.559	0.693	0.556	0.624	0.614	0.537	0.500	0.545	0.589	0.586	0.571	0.579	0.586	0.624
BGC31	0.694	0.661	0.672	0.540	0.656	0.455	0.567	0.726	0.685	0.738	0.735	0.713	0.669	0.662	0.703	0.697
BGC32	0.698	0.664	0.661	0.731	0.702	0.579	0.636	0.621	0.568	0.631	0.652	0.633	0.658	0.650	0.649	0.629

Continued on next page

Table 3. Continued.

Accession	*BGC17*	*BGC18*	*BGC19*	*BGC20*	*BGC21*	*BGC22*	*BGC23*	*BGC24*	*BGC25*	*BGC26*	*BGC27*	*BGC28*	*BGC29*	*BGC30*	*BGC31*	*BGC32*
BGC01	0.756	0.756	0.772	0.746	0.609	0.769	0.795	0.748	0.760	0.758	0.779	0.752	0.744	0.595	0.694	0.698
BGC02	0.754	0.739	0.727	0.686	0.571	0.667	0.721	0.702	0.686	0.742	0.719	0.721	0.728	0.615	0.661	0.664
BGC03	0.807	0.778	0.779	0.782	0.600	0.760	0.817	0.769	0.738	0.839	0.800	0.730	0.806	0.559	0.672	0.661
BGC04	0.552	0.587	0.542	0.539	0.674	0.589	0.551	0.530	0.580	0.583	0.616	0.551	0.562	0.693	0.540	0.731
BGC05	0.732	0.702	0.748	0.667	0.556	0.688	0.770	0.724	0.721	0.734	0.814	0.714	0.734	0.556	0.656	0.702
BGC06	0.496	0.543	0.474	0.496	0.586	0.478	0.496	0.474	0.509	0.487	0.504	0.483	0.496	0.624	0.455	0.579
BGC07	0.590	0.685	0.675	0.553	0.646	0.587	0.615	0.608	0.647	0.607	0.612	0.576	0.598	0.614	0.567	0.636
BGC08	0.746	0.746	0.748	0.764	0.575	0.758	0.798	0.752	0.736	0.776	0.797	0.770	0.789	0.537	0.726	0.621
BGC09	0.622	0.771	0.681	0.681	0.558	0.661	0.675	0.655	0.681	0.653	0.702	0.690	0.656	0.500	0.685	0.568
BGC10	0.758	0.819	0.732	0.762	0.558	0.742	0.797	0.779	0.734	0.760	0.766	0.811	0.746	0.545	0.738	0.631
BGC11	0.672	0.757	0.730	0.717	0.618	0.711	0.782	0.809	0.746	0.730	0.736	0.738	0.744	0.589	0.735	0.652
BGC12	0.792	0.733	0.779	0.767	0.573	0.760	0.758	0.726	0.724	0.824	0.756	0.772	0.736	0.586	0.713	0.633
BGC13	0.730	0.744	0.746	0.720	0.585	0.728	0.768	0.750	0.720	0.803	0.738	0.754	0.773	0.571	0.669	0.658
BGC14	0.790	0.720	0.764	0.738	0.566	0.719	0.772	0.740	0.724	0.764	0.742	0.758	0.737	0.579	0.662	0.650
BGC15	0.711	0.770	0.727	0.714	0.630	0.723	0.721	0.717	0.744	0.685	0.748	0.707	0.674	0.586	0.703	0.649
BGC16	0.677	0.811	0.694	0.694	0.609	0.689	0.744	0.740	0.708	0.721	0.685	0.758	0.736	0.624	0.697	0.629
BGC17	1	0.711	0.742	0.702	0.578	0.656	0.750	0.690	0.688	0.770	0.734	0.736	0.742	0.564	0.625	0.598
BGC18	0.711	1	0.817	0.774	0.615	0.752	0.810	0.776	0.744	0.756	0.748	0.795	0.756	0.615	0.733	0.635
BGC19	0.742	0.817	1	0.760	0.609	0.754	0.810	0.792	0.760	0.787	0.779	0.795	0.772	0.581	0.694	0.628
BGC20	0.702	0.774	0.760	1	0.607	0.833	0.783	0.736	0.691	0.775	0.683	0.740	0.732	0.593	0.752	0.600
BGC21	0.578	0.615	0.609	0.607	1	0.574	0.617	0.596	0.682	0.609	0.614	0.576	0.587	0.689	0.595	0.640
BGC22	0.656	0.752	0.754	0.833	0.574	1	0.777	0.730	0.727	0.754	0.718	0.748	0.700	0.631	0.675	0.678
BGC23	0.750	0.810	0.810	0.783	0.617	0.777	1	0.815	0.783	0.780	0.758	0.833	0.766	0.576	0.758	0.623
BGC24	0.690	0.776	0.792	0.736	0.596	0.730	0.815	1	0.750	0.706	0.740	0.785	0.748	0.569	0.784	0.644
BGC25	0.688	0.744	0.760	0.691	0.682	0.727	0.783	0.750	1	0.746	0.797	0.754	0.732	0.607	0.708	0.627
BGC26	0.770	0.756	0.787	0.775	0.609	0.754	0.780	0.706	0.746	1	0.793	0.766	0.800	0.595	0.680	0.628
BGC27	0.734	0.748	0.779	0.683	0.614	0.718	0.758	0.740	0.797	0.793	1	0.772	0.764	0.573	0.699	0.704
BGC28	0.736	0.795	0.795	0.740	0.576	0.748	0.833	0.785	0.754	0.766	0.772	1	0.766	0.603	0.758	0.636
BGC29	0.742	0.756	0.772	0.732	0.587	0.700	0.766	0.748	0.732	0.800	0.764	0.766	1	0.561	0.709	0.619
BGC30	0.564	0.615	0.581	0.593	0.689	0.631	0.576	0.569	0.607	0.595	0.573	0.603	0.561	1	0.526	0.726
BGC31	0.625	0.733	0.694	0.752	0.595	0.675	0.758	0.784	0.708	0.680	0.699	0.758	0.709	0.526	1	0.562
BGC32	0.598	0.635	0.628	0.600	0.640	0.678	0.623	0.644	0.627	0.628	0.704	0.636	0.619	0.726	0.562	1

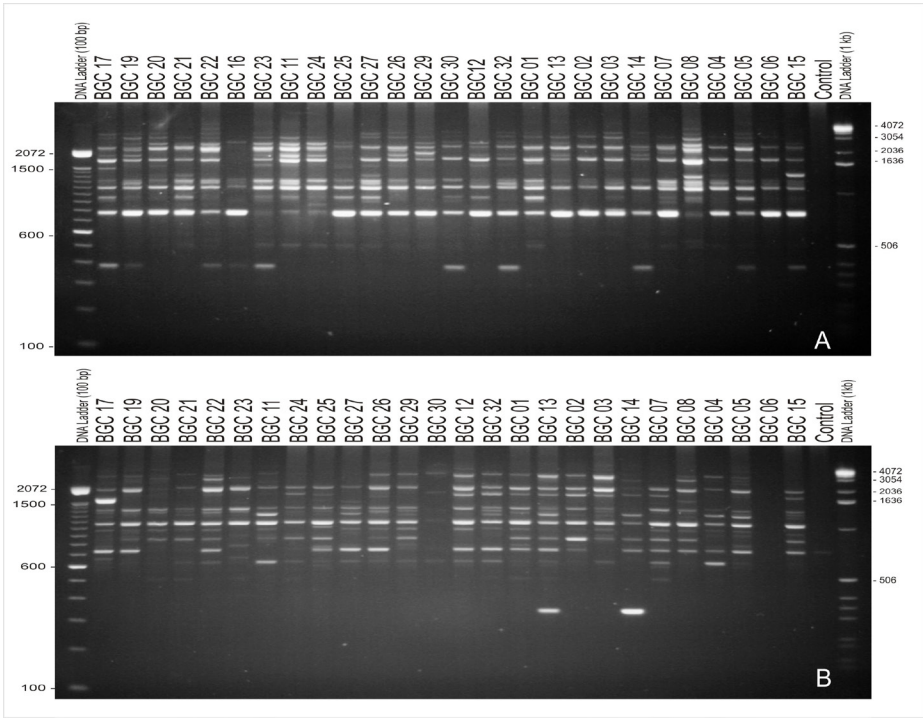

Figure 1. Electrophoretic profiles of RAPD amplifications of DNA samples from 27 accessions of cajá (*Spondias mambin* L.) generated using primers OPG08 (Panel **A**) and profiles from 26 accessions generated using primers OPF15 (Panel **B**).

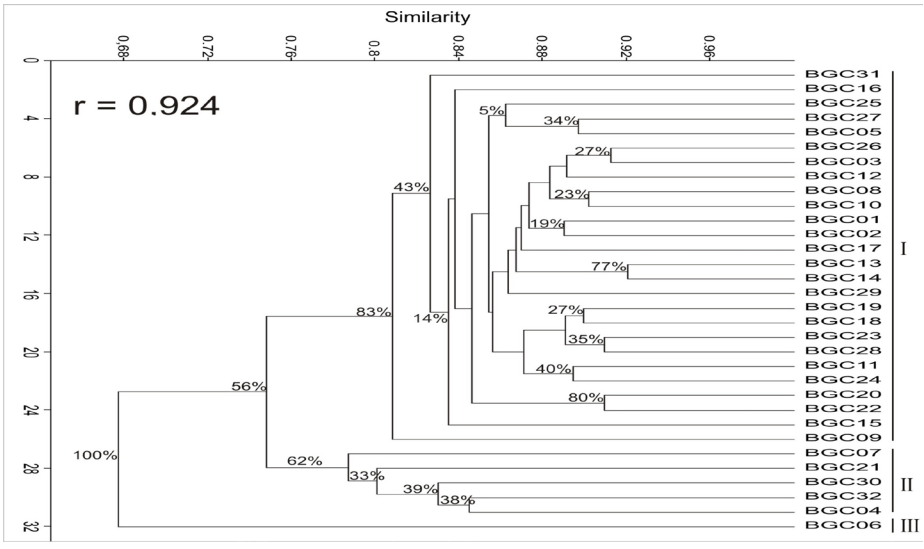

Figure 2. UPGMA dendrogram based on 21 RAPD polymorphic markers showing similarity relationships between 32 accessions of cajá (*Spondias mombin* L.).

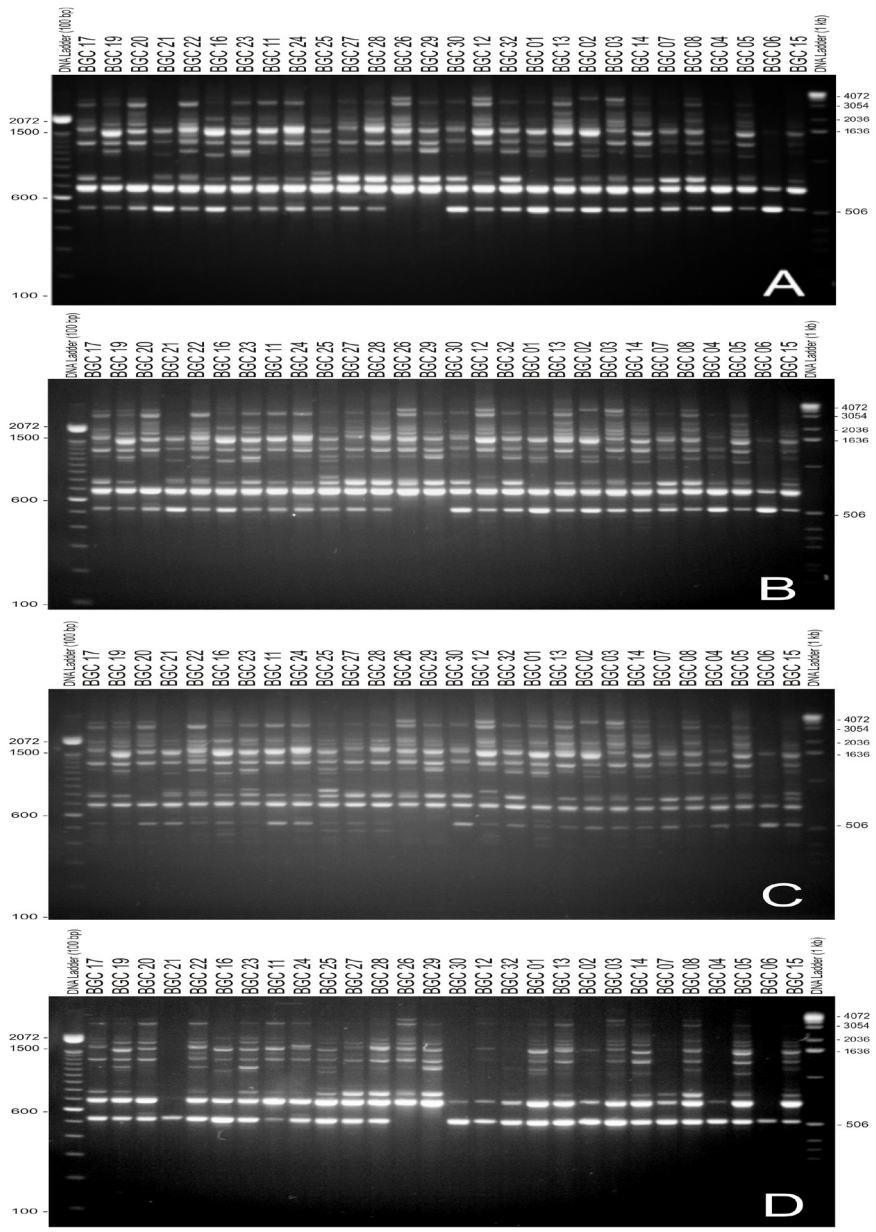

Figure 3. Electrophoretic profiles of RAPD amplifications of DNA samples from 28 accessions of cajá (*Spondias mombin* L.) generated using primer OPG17 and showing: (i) repeatability of band pattern using the same brand of Taq DNA polymerase (Panels **A** and **B**); (ii) reproducibility of band pattern using a different thermal cycler (in Panel **C** a Techne® Endurance TC412 Thermal Cycler was employed), and (iii) reproducibility of band pattern using a different brand of Taq DNA polymerase (in Panel **D** an Invitrogen polymerase was employed). The amplification conditions were identical in all experiments, and Cenbiot-Enzimas Taq DNA polymerase and Veriti 96 Well Thermal Cycler were employed throughout, unless stated otherwise.

DISCUSSION

According to Freitas (2005), 15-mer oligonucleotide primers require annealing temperatures between 40° and 50°C, whereas lower annealing temperatures (around 35°C) are recommended for the shorter primers, generally used in the RAPD technique. In the present study, however, the primers used in the preliminary tests (A10 and M03) did not influence the selection of the annealing temperature used in the experiments.

The number of bands produced by RAPD primers appears to be independent of the size of the genome, with an average number of five bands per reaction (Ferreira and Grattapaglia, 1998). Hence, the average number of bands per primer (6.8) obtained in the present study was considered to be satisfactory, especially since the degree of polymorphism of the fragments was high.

The location from which the accessions had been collected had no influence on their position within the genotypic groups (Figure 2). For example, accessions BGC 04, BGC 05 and BGC 06 from Altos (State of Piauí) showed an average similarity of 56.4%, although they were classified in different groups. In a similar manner, accessions BGC 31 and BGC 32 from Demerval Lobão (State of Piauí) exhibited 56.2% similarity but were classified in different groups. Such results indicate that these accessions derived from different progenitors and that there is large genetic diversity within the species *S. mombin.* Interestingly, the accessions collected in Teresina and Timon (States of Piauí and Maranhão, respectively) displayed average genetic similarities equivalent to 70.3 and 78.2%, respectively, and the great majority were uniformly distributed within group I.

Based on an estimate of the genetic diversity of 12 cajá accessions using inter-simple sequence repeat (ISSR) markers, Silva (2009) claimed that there was no correlation between genetic similarity and the origin of the accessions. However, the mean similarity between these accessions was much lower (27.45%) than that reported herein, indicating that the diversity among the cajá accessions of the Germplasm Collection of Embrapa Meio-Norte is somewhat restricted. This view is supported by the RAPD marker study of Gois et al. (2008) in which 17 cajá plants collected in the State of Sergipe exhibited a genetic similarity of 46.77% (range 21-78%). Since *S. mombin* is a native species that is still in the process of domestication, significant divergence between accessions would be expected. It is likely that the level of homogeneity observed in the germplasm analyzed in the present study was a result of selection during sampling. Moreover, growth of the accessions was carried out via vegetative propagation (grafting), and thus, there is variation between individuals of the same accession. Nevertheless, the values of average similarity (68.8%) and similarity between most accessions indicated there is variability in the germplasm analyzed. In this context, Mendonça et al. (2008) carried out a physicochemical analysis (weight of fruits, weight of stones, amount of pulp, total soluble solids, total titratable acidity, etc.) of the fruits from 18 of the Embrapa cajá accessions and verified the occurrence of genetic diversity within the collection. Based on the physicochemical characteristics of the fruits, Soares et al. (2006) also demonstrated the presence of diversity among 14 cajá genotypes distributed in the municipality of Teresina, and stressed the possibility of selecting superior individuals for direct use in the improvement of commercial orchards. Genetic diversity in populations of *S. mombin* has also been observed in studies involving isoenzyme markers in which the observed heterozygosity was greater than that expected (Gois et al., 2009; Silva et al., 2009).

According to some researchers (Oliveira et al., 2007), one of the main limitations of the RAPD technique is the low level of repeatability of the band pattern observed when amplification reactions have not been optimized. In the present study, repeatability and reproducibility tests revealed that the amplification reactions were carried out under adequate and optimized conditions that generated consistent RAPD bands. High levels of repeatability in RAPD loci have also been reported for the frog *Physalaemus cuvieri* (85-99%; Telles, 2005) and for a Cerrado plant *Tibouchina papyrus* (85%; Ramos et al., 2008). Some studies have shown that different brands of Taq DNA polymerase can produce dissimilar results in PCRs (Evangelopoulos et al., 2001; Verweij et al., 2004; Al-Soud et al., 2005), and in several cases enhancers such as bovine serum albumin, casein, formamide, and polyvinylpolypyrrolidone are required to facilitate amplification. Moreover, some types of Taq DNA polymerase are more susceptible to inhibition than others, and this may cause differences in the band patterns obtained (Wilson, 1997).

The results presented herein reveal that genetic variability is present in the cajá accessions in the Germplasm Collection of Embrapa Meio-Norte and can be exploited in future breeding programs. The accessions BGC 06 and BGC 31 were the most unrelated and, therefore, the most suitable for initial crossings in order to obtain high levels of segregation. It is concluded from the results of the repeatability and reproducibility tests that the RAPD technique is reliable and efficient in revealing the genetic diversity of cajá accessions.

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