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Molecular characterization of progenies of bacurizeiro (*Platonia insignis*) from Marajó Island, northeastern Amazon

Lígia Cristine Gonçalves PONTES[1], Elisa Ferreira MOURA[2*], Mônika Fecury MOURA[2], Simone de Miranda RODRIGUES[2], Maria do Socorro Padilha de OLIVEIRA[2], José Edmar Urano de CARVALHO[3], Josette THERRIER[4]

[1] Universidade Federal do Pará, Instituto de Ciências Biológicas, Campus Básico - Rua Augusto Corrêa, n. 01, Guamá, CEP 66075-110, Belém, Pará, Brasil

[2] Embrapa Amazônia Oriental, Laboratório de Genética Molecular, Travessa Dr. Enéas Pinheiro, s/n, Marco, CEP 66095-903, Belém, Pará, Brasil

[3] Embrapa Amazônia Oriental, Laboratório de Fruticultura, Travessa Dr. Enéas Pinheiro, s/n, Marco, CEP 66095-903, Belém, Pará, Brasil

[4] Universidade Federal Rural da Amazônia, Avenida Presidente Tancredo Neves, nº 2501 Terra Firme, CEP 66077-830, Belém, Pará, Brasil

* Corresponding author: elisa.moura@embrapa.br

ABSTRACT

The bacurizeiro (*Platonia insignis* Mart.) is a tree native to the Amazon whose fruit is much used in the gastronomy in the North and Northeast regions of Brazil. Due to its great economic potential for these regions, the species is being conserved in germplasm banks to support genetic breeding programs. The aim of this work was the molecular characterization of *P. insignis* accessions belonging to the germplasm bank of the Embrapa Eastern Amazon research unit using ISSR (Inter Simple Sequence Repeat) markers. Seventy-eight accessions of *P. insignis* belonging to 16 progenies were sampled in two different localities on Marajó Island, state of Pará, Brazil. Among the 16 progenies, seven were collected in Soure and nine in Salvaterra. The 78 accessions were genotyped with 23 pre-selected primers. We obtained 121 amplified products, of which 54 were polymorphic. The most polymorphic primers were UBC 834, UBC 899 and UBC 900. Primers UBC810 and UBC884 did not amplify polymorphic bands. Forty-nine markers out of 54 were selected for genetic analyses. AMOVA within and among progenies showed low genetic differentiation (Φ_{PT} = 0.064, P<0.001) with higher diversity within progenies (96%), low genetic differentiation among sampling localities (Φ_{PT} = 0.025, P<0.013), and higher diversity within (98%) than among localities. Clustering by UPGMA based on Jaccard similarities among pairs of accessions did not separate genotypes according to progeny or sampling localitiy. We recommend that new germplasm surveys consider a greater sampling effort within sampling localitites.

KEYWORDS: genetic diversity, germplasm bank, Clusiaceae

Caracterização molecular de progênies de bacurizeiro (*Platonia insignis*) da Ilha de Marajó, nordeste da Amazônia

RESUMO

O bacurizeiro (*Platonia insignis* Mart.) é uma espécie frutífera nativa da Amazônia muito utilizada na cultura alimentar nas regiões Norte e Nordeste do Brasil. Devido a seu grande potencial econômico regional, a espécie vem sendo conservada em bancos ativos de germoplasma (BAG) para apoiar programas de melhoramento genético. Dessa forma, o objetivo deste trabalho foi caracterizar molecularmente acessos de *P. insignis* pertencentes ao BAG da Embrapa Amazônia Oriental por meio de marcadores ISSR (*Inter Simple Sequence Repeat*). Foram coletados 78 acessos de *P. insignis* pertencentes a 16 progênies coletadas em dois locais diferentes na Ilha de Marajó, PA. Das 16 progênies, sete foram coletadas em Soure e nove em Salvaterra. Os 78 acessos foram genotipados com 23 *primers* ISSR pré-selecionados. Obteve-se 121 produtos amplificados, dos quais 54 foram polimórficos. Os *primers* mais polimórficos foram UBC 834, UBC 899 e UBC 900. Já os *primers* UBC810 e UBC884 não apresentaram bandas polimórficas. Das 54 marcas, 49 foram selecionadas para as análises genéticas. A AMOVA entre e dentro de progênies identificou baixa diferenciação genética (Φ_{PT} = 0,064, P<0,001) com maior diversidade dentro de progênies (96%), bem como baixa diferenciação genética entre os locais de coleta (Φ_{PT} = 0,025, P<0,013), com maior diversidade dentro (98%) do que entre locais. O agrupamento pelo método UPGMA, com base nas similaridades de Jaccard entre os acessos, não separou as amostras por progênie ou local de coleta. Recomenda-se que novas coletas de germoplasma considerem maior esforço de coleta em cada local amostrado.

PALAVRAS-CHAVE: diversidade genética, banco de germoplasma, Clusiaceae

INTRODUCTION

Platonia insignis, popularly known as bacurizeiro, is a deciduous tree of the family Clusiaceae native to the Amazon that produces one of the most widely appreciated fruits in the region, the bacuri. It occurs mainly in the states of Pará, Maranhão and Piauí, in northern Brazil (Nascimento *et al.* 2007). In Pará, it is often found on Marajó Island and in the northeastern part of the state (Carvalho 2007). Its sweet pulp is used in many processed forms, such as desserts, jams, liqueurs and ice cream. Because of its taste and texture, it has been gaining attention in gastronomy beyond the frontiers of the northern and northeastern Brazil.

Platonia insignis trees can reach up to 30 meters. They have very efficient asexual reproduction, generating new buds from the roots. In deforested areas in regions of common occurrence of *P. insignis*, buds are frequently observed shooting from the ground. Besides asexual reproduction, *P. insignis* reproduces by seeds, via cross pollination, associated with sporophytic self-incompatibility (Maués and Venturieri 1996).

Since *P. insignis* has great economic potential in agroforestry, it is necessary to select more adapted and productive genotypes. In this context, the conservation of the species in active germplasm banks is of great importance, to maintain genetic variation and provide material for genetic breeding programs. In Brazil there are germplasm banks of *P. insignis* in the states of Pará and Piauí. Each one is represented mainly by accessions collected in these states or nearby regions. Some efforts to characterize the conserved germplasm have been carried out by Carvalho *et al.* (2002, 2003, 2004) and Souza *et al.* (2016). The germplasm bank accessions of Piauí were molecularly characterized with inter simple sequence repeat (ISSR) markers, identifying genetic differentiation among sampling localities in the states of Maranhão and Piauí (Souza *et al.* 2013). However, studies of the genetic variability of accessions from Pará state are still lacking.

For plant species with no genome sequence available, the use of inter simple sequence repeat (ISSR) molecular markers is a good option (Faleiro 2007). They are dominant and represent genetic variations within microsatellite regions in the genome, detected with random primers (Faleiro 2007). They have been used to estimate genetic diversity and genetic parameters of populations of other Brazilian native fruit species, such as *Rollinia mucosa* (Lorenzoni *et al.* 2014) and *Genipa americana* (Silva *et al.* 2014), being able to identify considerable genetic variability and divergent genotypes. An additional advantage of ISSR markers is the possibility of obtaining a high number of polymorphic loci without the need for sequence knowledge (Faleiro 2007), as is the case of *P. insignis*.

The set of accessions evaluated in this study is part of the germplasm bank formed for the selection of superior clones of *P. insignis* (Carvalho *et al.* 2002) and identification of morphological variants (Carvalho *et al.* 2003). Thus, the aim of this study was to estimate the genetic variability and genetic structure of accessions of *P. insignis* from Marajó Island in Pará, preserved in the germplasm bank of Embrapa Eastern Amazon using ISSR markers.

MATERIALS AND METHODS

To estimate the genetic variability of *P. insignis* conserved in the germplasm bank of Embrapa Eastern Amazon, we selected 78 accessions belonging to 16 progenies collected on Marajó Island, Pará (Guimarães *et al.* 1992). These progenies represent open-pollinated families, and were sampled in two localities of Marajó Island: Soure and Salvaterra (Figure 1, Table 1). The accessions were established in the Quatro Bocas Experimental Field of Embrapa Eastern Amazon, in Tomé-Açu, Pará. Four to five plants per progeny were collected (Table 1).

Total genomic DNA was extracted according to a procedure similar to that of Doyle and Doyle (1990). Leaves were macerated with liquid nitrogen, and then polyvinylpyrrolidone (PVP) and 3 mL of cetyl trimethylammonium bromide (CTAB) extraction buffer (2% CTAB, 5 M NaCl, 0.5 M EDTA, PVP, 1 M Tris-HCl, and sterile water) were added to the macerate. The macerate was homogenized and incubated in a hot water bath at 65°C for 1 h. Afterwards, chloroform:isoamyl alcohol (24:1) was added followed by homogenization, and the samples were centrifuged for 10 min at 10,000 rpm. Three milliliters of 95% ethyl alcohol were added to the supernatant to precipitate the DNA, and the samples were again centrifuged for 10 min at 10,000 rpm. Next, the precipitate was washed with 70% ethyl alcohol for 10 min and centrifuged at 5,000 rpm. DNA samples were resuspended in 300 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and RNAse. DNA was quantified on 1% agarose gel using lambda phage DNA as a standard, at different concentrations (50, 100 and 200 ng μL^{-1}).

Samples were genotyped with 23 ISSR primers (University of British Columbia, Vancouver, Canada). Four randomly selected accessions were used to test and select annealing temperatures for each primer (Table 2). PCR was performed in a final volume of 20 µL, containing 10 ng of genomic DNA, 75 µM of each dNTP, 2.0 µM of primer, 1.0 mg mL^{-1} BSA (bovine serum albumin), reaction buffer containing 1.2 mM $MgCl_2$ and 0.2 U Taq DNA polymerase (Invitrogen, Brazil). Reactions were carried out in 0.2 mL microtubes and amplified in an Amplitherm TX96 thermocycler programmed for 35 cycles. First, there was a denaturation phase at 95 °C for 5 min. Then, each cycle consisted of DNA denaturation at 95 °C for 1 min, primer annealing at temperatures from 50 – 62 °C (depending on the primer, Table 2) for 45 s and

elongation at 72 °C for 2 min. After the 35 cycles, there was final extension at 72 °C for 5 min.

Reaction products were run on 1.5% agarose gel (Invitrogen, Brazil) prepared with 1.0X TBE buffer (0.45 M Tris-borate and 0.01 M EDTA). Gels were run in a horizontal electrophoresis unit containing 1.0X TBE at constant voltage of 80 V for 3:30h. Gels were visualized with an ultraviolet light transilluminator and images were digitally captured. Bands with the same run pattern were considered from the same locus, and the presence of a band was scored as (1) and absence as (0), generating a binary matrix. The fragments were compared with the molecular marker 1Kb DNA ladder (Invitrogen). Only polymorphic bands were analyzed.

Table 1. List of 78 accessions of bacurizeiro (*Platonia insignis*) from 16 progenies sampled on Marajó Island, Pará, Brazil maintained in the germplasm bank of Embrapa Eastern Amazon and characterized with ISSR markers.

Order	Accession	Progeny	Plant	Sampling locality
1	101-1	1	1	Soure
2	101-2	1	2	Soure
3	101-3	1	3	Soure
4	101-4	1	4	Soure
5	101-5	1	5	Soure
6	102-1	2	1	Soure
7	102-2	2	2	Soure
8	102-3	2	3	Soure
9	102-4	2	4	Soure
10	102-5	2	5	Soure
11	103-1	3	1	Soure
12	103-2	3	2	Soure
13	103-3	3	3	Soure
14	103-4	3	4	Soure
15	103-5	3	5	Soure
16	104-1	4	1	Soure
17	104-2	4	2	Soure
18	104-3	4	3	Soure
19	104-4	4	4	Soure
20	104-5	4	5	Soure
21	105-1	5	1	Soure
22	105-2	5	2	Soure
23	105-3	5	3	Soure
24	105-4	5	4	Soure
25	105-5	5	5	Soure
26	106-1	6	1	Soure
27	106-2	6	2	Soure
28	106-3	6	3	Soure
29	106-4	6	4	Soure
30	106-5	6	5	Soure
31	207-1	7	1	Soure
32	107-2	7	2	Soure
33	207-3	7	3	Soure
34	107-4	7	4	Soure
35	107-5	7	5	Soure
36	108-1	8	1	Salvaterra
37	108-2	8	2	Salvaterra
38	108-3	8	3	Salvaterra
39	108-4	8	4	Salvaterra
40	108-5	8	5	Salvaterra
41	209-1	9	1	Salvaterra
42	209-2	9	2	Salvaterra
43	209-3	9	3	Salvaterra
44	209-4	9	4	Salvaterra
45	209-5	9	5	Salvaterra
46	210-1	10	1	Salvaterra
47	210-2	10	2	Salvaterra
48	110-3	10	3	Salvaterra
49	210-5	10	5	Salvaterra
50	211-1	11	1	Salvaterra
51	211-2	11	2	Salvaterra
52	211-3	11	3	Salvaterra
53	211-4	11	4	Salvaterra
54	211-5	11	5	Salvaterra
55	212-1	12	1	Salvaterra
56	212-2	12	2	Salvaterra
57	112-3	12	3	Salvaterra
58	212-4	12	4	Salvaterra
59	212-5	13	5	Salvaterra
60	213-1	13	1	Salvaterra
61	113-2	13	2	Salvaterra
62	213-3	13	3	Salvaterra
63	113-4	13	4	Salvaterra
64	213-5	13	5	Salvaterra
65	214-2	14	2	Salvaterra
66	214-3	14	3	Salvaterra
67	114-4	14	4	Salvaterra
68	114-5	14	5	Salvaterra
69	215-1	15	1	Salvaterra
70	215-2	15	2	Salvaterra
71	215-3	15	3	Salvaterra
72	215-4	15	4	Salvaterra
73	215-5	15	5	Salvaterra
74	216-1	16	1	Salvaterra
75	216-2	16	2	Salvaterra
76	216-3	16	3	Salvaterra
77	216-4	16	4	Salvaterra
78	216-5	16	5	Salvaterra

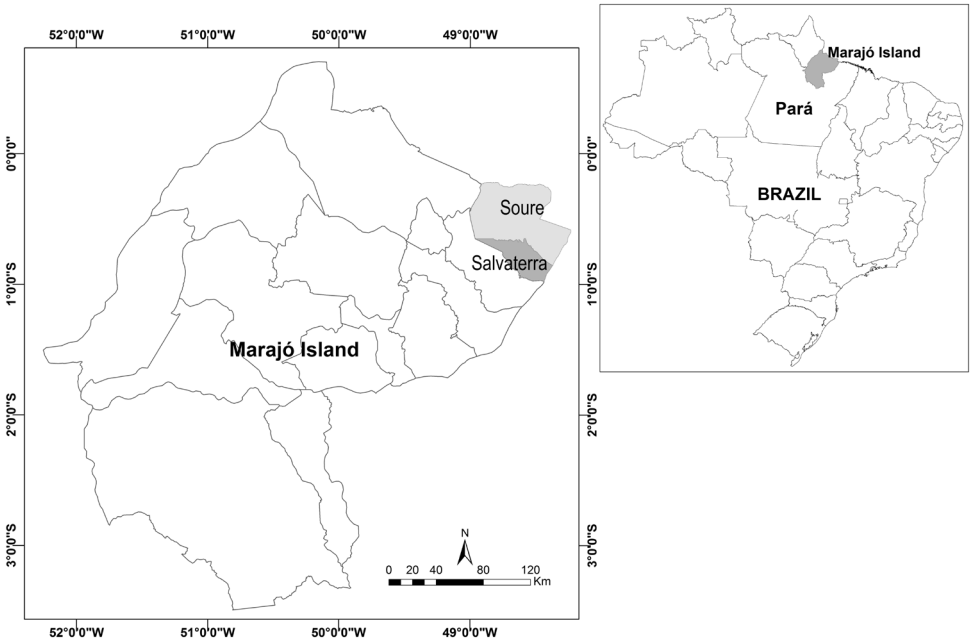

Figure 1. Map of sampling localities of accessions of bacurizeiro (*Platonia insignis*) on the Marajó Island, Pará, Brazil.

Table 2. Identification of the 23 ISSR primers used in the genotyping of 78 accessions of bacurizeiro (*Platonia insignis*) sampled on Marajó Island, Pará, Brazil, and their respective annealing temperatures, sequence, number of loci, total number of polymorphisms and polymorphism rates.

Primer	Temperature °C	Sequence (5'-3')	N loci	N polymorphic loci	Polymorphism rate (%)
UBC 807	57	$(AG)^7GT$	5	3	60
UBC 808	57	$(AG)^8C$	5	3	60
UBC 809	57	$(AG)^8G$	4	1	25
UBC 810	53	$(GA)^8T$	4	0	0
UBC 811	54	$(GA)^8C$	6	4	66.6
UBC 817	53	$(CA)^8$	5	1	20
UBC 825	54	$(AC)^7$	5	3	60
UBC 826	59	$(AC)^8C$	5	1	20
UBC 827	59	$(AC)^8G$	4	1	25
UBC 834	53	$(AG)^8YT$	10	5	50
UBC 840	54	$(GA)^8YT$	5	1	20
UBC 842	52	$(GA)^8YG$	3	1	33.3
UBC 856	59	$(AC)^8YA$	8	4	50
UBC 866	58	$VDV(CT)^7$	4	3	75
UBC 868	58	$(GAA)^6$	3	1	33.3
UBC 884	57	$HBH(AG)^7$	8	0	0
UBC 888	59	$BDB\ (CA)^7$	7	1	14.2
UBC 889	57	$DBD\ (AC)^7$	5	1	20
UBC 890	59	$VHV\ (GT)^7$	5	3	60
UBC 891	59	$HVH\ (TG)^7$	6	4	66.6
UBC 899	57	CATGGTGTTGG TCATTGTTCC	5	5	100
UBC 900	53	ACTTCCCCACAG GTTAACACA	6	5	83.3
TOTAL			121	57	

The matrix of genetic similarity was generated with the PAST program (Hammer et al. 2001) based on Jaccard's coefficient:

$$sg_{ij} = \frac{a}{a + b + c}$$

Where:

a = number of events where the band occurred in both genotypes;

b = number of events where the band occurred only in genotype *i*;

c = number of events where the band occurred only in genotype *j*.

Based on the genetic similarity matrix, a dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA). The relation between similarity matrix and dendrogram was estimated by the cophenetic correlation coefficient (CCC), according to Sokal and Rohlf (1962).

The genetic structure was estimated by analysis of molecular variance – AMOVA (Excoffier *et al.* 1992) using the GenAlEx 6.501 program (Peakall and Smouse 2012). Two approaches considering two hierarchical levels were used. First the variance within and among the sixteen progenies was analyzed. Then, partition of variance was analyzed based on the sampling localities of the progenies (Salvaterra and Soure).

RESULTS

We amplified 121 products with the 23 primers used, with an average of 5.0 bands per primer (Table 2). Among the 121 amplified products, 54 were polymorphic, which corresponds to a polymorphism rate of 44.62%, with an average of 2.35 polymorphic bands per primer. The most polymorphic primers were UBC 834, UBC 899 and UBC 900 (five polymorphic bands each). All bands amplified by UBC 899 were polymorphic. On the other hand, UBC 810 and UBC 884 did not amplify polymorphic bands. Due to the lower quality data, five polymorphic bands (one each amplified by UBC 808, 825 and 900 and two amplified by UBC 856) were discarded, so further analyses were performed with 49 polymorphic bands.

The genetic similarity based on Jaccard's coefficient varied from 0.51 to 0.98, with an average of 0.79. The least similar accessions were 213-2 and 101-2 (gs_{ij} = 0.51) and the most similar pairs were 209-2 and 102-3 and 209-2 and 104-4 (gs_{ij} = 0.98). Despite the high genetic similarity among 209-2, 102-3 and 104-4, these three belong to different progenies. Also, these accessions were sampled in different localities (Table 1).

The dendrogram formed by UPGMA and Jaccard's genetic similarities among the 78 accessions showed no clustering according to progenies or sampling localities (Figure 2). Accessions 101-1, 101-2, 103-5, 216-1 and 103-4 were the most divergent and clustered separately from the other accessions.

Based on AMOVA, there was a significant genetic differentiation among progenies (Φ_{PT} = 0.064, P<0.001). We found that 6% of total variation was among progenies and 94% was within progenies of *P. insignis* (Table 3). When AMOVA was performed considering partition of variance between sampling localities, there was low but significant genetic variation between Soure and Salvaterra (Φ_{PT} = 0.02, P<0.011). Genetic variation among sampling localities was 2% and within 98% (Table 3).

Table 3. Analysis of molecular variance (AMOVA) of genetic structure among and within 16 progenies of bacurizeiro (*Platonia insignis*) from Marajó Island, Pará, Brazil, genotyped with ISSR markers. DF= degrees of freedom; *P*= probability based on 1000 random permutations across the full dataset; Φ^{PT}= estimate of population genetic differentiation.

Source of variation	DF	Variance	Genetic variation (%)	*P*	Φ^{PT}
Among progenies	15	0.31	6	0.001	0.064
Within progenies	62	4.52	94		
Total	77	4.82	100		
Between sampling localities	1	0.07	2	0.013	0.025
Within sampling localities	76	2.67	98		
Total	77	2.74	100		

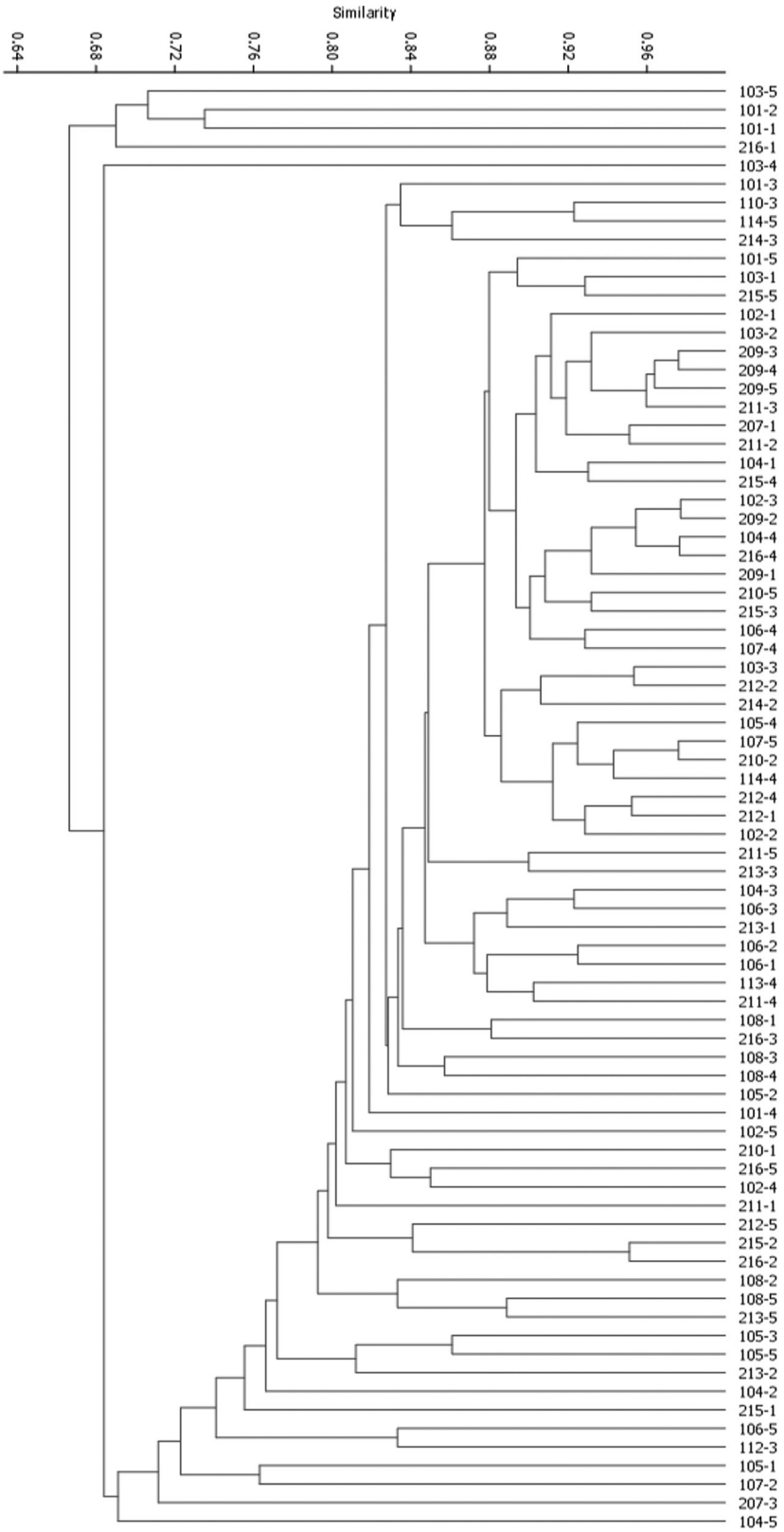

Figure 2. Cluster analysis of 78 accessions of bacurizeiro (*Platonia insignis*) based on 49 ISSR markers. The dendrogram was generated using UPGMA based on the similarity coefficient of Jaccard. Cophenetic correlation coefficient = 0.80.

DISCUSSION

Despite the strong potential of *P. insignis* for commercial fruit production, there are few studies about the genetic variation of this species, and fewer considering molecular aspects (Almeida *et al.* 2007; Souza *et al.* 2013). In this study, we characterized 78 accessions of *P. insignis* with ISSR markers, which employ universal primers and can be used to study species with no genomic information.

The mean genetic similarity among accessions could be considered high ($\bar{x}$ = 0.79) compared to other studies of genetic diversity of Brazilian fruit species (Santana *et al.* 2011; Lorenzoni *et al.* 2014). Besides this, the percentage of polymorphic loci was 42%, even though a high number of ISSR primers were used (23). In a previous study to evaluate the genetic diversity of 28 accessions of *P. insignis* from the Pará germplasm bank, high similarity levels among some accessions were detected with RAPD markers (Almeida *et al.* 2007). Souza *et al.* (2013) analyzed 72 accessions of *P. insignis* from a germplasm collection formed by accessions from northeastern Brazil using 18 ISSR primers and obtained 221 polymorphic loci and mean genetic similarity of 0.52. Those authors analyzed samples from ten different localities in the states of Maranhão and Piauí, which may have contributed to the higher detection of genetic variation.

The analysis of molecular variance showed that the highest amount of genetic variation was contained within progenies or sampling localities (Table 3), which was expected for allogamous species and explained by the higher efforts to collect samples within places. However, the work of Souza *et al*. (2013) showed that higher variation can be obtained with samples in a wider geographical range, which can enrich genetic breeding programs. Perhaps the low genetic differentiation between sampling localities was an effect of the geographical proximity between Soure and Salvaterra, since higher values of genetic differentiation were detected by Souza *et al*. (2013). The effect of sampling of *P. insignis* in different localities was observed in morphological and chemical variation of fruits (Silva *et al.* 2009; Carvalho-Saraiva *et al.* 2014).

We observed high genetic similarity among accessions from different progenies and sampling localities. On the other hand, the least similar accessions were from different sampling localities, besides belonging to different progenies. This can be an effect of pollen dispersion from different origins, since these progenies are half-sib families. The clustering of accessions in the dendrogram was not associated with the progeny or sampling localities, which was reflected in the AMOVA analyses. Cophenetic correlation of the dendrogram with the similarity matrix was r = 0.88, which is high and confirms the reliability of the results. Considering genetic partition within and among sampling units, Souza *et al.* (2013) also identified higher genetic variation within populations from Maranhão and Piauí (71.82%), but genetic differentiation among populations was higher (28.18%) than in this study. This might be an effect of collecting samples from more distant areas. Since *P. insignis* is an allogamous species with sporophytic self-incompatibility (Maués and Venturieri 1996) the higher portion of genetic variation within populations or progenies was expected. Again, the geographical proximity likely favoured a more frequent gene flow among trees of our two sampling localities.

CONCLUSIONS

The genetic variation of *Platonia insignis* from two localities on the Marajó Island (Pará state, northeastern Amazon) was higher within than among progenies and sampling localities, which means that sampling efforts for germplasm enrichment should consider a higher sampling effort within localities. The low genetic differentiation between geographically close sampling places probably was a result of the allogamous behavior of *P. insignis* trees.

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REFERENCES

Almeida, H.J.S.; Costa, J.T.A.; Benbadis, A.K.; Innvecco, R.; Aloufa, M.A.I.; Carvalho, A.C.P.P. 2007. Aplicação de marcador molecular (RAPD) para estudos da diversidade genética em bacurizeiro. In: Lima M. da C. (Ed.). *Bacuri: Agrobiodiversidade*, Instituto Interamericano de Cooperação para a Agricultura, São Luís, Amazonas, p.157-170.

Carvalho, J.E.U. 2007. Aspectos botânicos, origem e distribuição geográfica do bacurizeiro. In: Lima M. da C. (Ed.). *Bacuri: Agrobiodiversidade,* Instituto Interamericano de Cooperação para a Agricultura, São Luís, Amazonas, p.17-27.

Carvalho, J.E.U. de; Alves, S.M.; Nascimento, W.M.O.; Müller, C.H. 2002. Características físicas e químicas de um tipo de bacuri (*Platonia insignis* Mart.) sem sementes. *Revista Brasileira de Fruticultura*, 24: 573-575.

Carvalho, J.E.U. de; Nazaré, R.F.R.; Nascimento, W.M.O. 2003. Características físicas e físico-químicas de um tipo de bacuri (*Platonia insignis* Mart.) com rendimento industrial superior. *Revista Brasileira de Fruticultura*, 25: 326-328.

Carvalho-Saraiva, R.V.; Correia-Albuquerque, P.M.; Girnos, E.C. 2014. Floral and vegetative morphometrics of three *Platonia insignis* Mart. (Clusiaceae) populations, a native tree from the Brazilian Amazon. *Plant Biosystems*, 148: 666-674.

Cavalcante, P.B. 1996. *Frutas comestíveis da Amazônia.* Cejup, Belém. 279p.

Doyle, J.J.; Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.

Excoffier, L.; Smouse, P.E.; Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics,* 131: 479-491.

Faleiro, F.G. 2007. *Marcadores genéticos moleculares aplicados a programas de conservação e uso de recursos genéticos*, Embrapa Cerrados, Planaltina, Distrito Federal, 102p.

Guimarães, A.D.G.; Mota, M.G. da C.; Nazaré, R.F.R. de. 1992. Coleta de germoplasma de bacuri (*Platonia insignis* Mart.) na Amazônia I. Microrregião Campos do Marajó (Soure/Salvaterra). *Boletim de Pesquisa*, 132, Embrapa Amazônia Oriental, Belém, Pará, 23p.

Hammer, O.; Harper, D.A.T.; Ryan, P.D. 2001. PAST: paleontological statistics software package for education and data analysis. *Paleontologia Electronica*, 4: 1-9.

Homma, A.K.O.; Carvalho, J.E.U; Menezes, A.J.E.A. 2010. Bacuri: fruta amazônica em ascensão. *Ciência Hoje*, 46: 40-45.

Lorenzoni, R.M.; Soares, T.C.B.; Santiago, V.F.; Silva, J.A.; Coelho, R.I. 2014. Utilização de marcadores ISSR na avaliação da divergência genética entre acessos de biribazeiro. *Revista Brasileira de Fruticultura*, 36: 251-257.

Maués, M.M.; Venturieri, G.C. 1996. Ecologia da polinização do bacurizeiro (*Platonia insignis* Mart.) Clusiaceae. *Boletim de pesquisa* 170, Embrapa Amazônia Oriental, Belém, Pará. 24p.

Nascimento,W.M.O. do; Carvalho, J.E.U.; Muller, C.H. de. 2007. Ocorrência e distribuição geográfica do bacurizeiro (*Platonia insignis* Mart.) *Revista Brasileira de Fruticultura*, 29: 657-660.

Santana, I.B.B.; Oliveira, E.J.; Soares-Filho, W.S.S.; Ritzinger, R.; Amorim, E.P.; Costa, M.A.P.C.; Moreira, R.F.C. 2011. Variabilidade genética entre acessos de umbu-cajazeira mediante análise de marcadores ISSR. *Revista Brasileira de Fruticultura*, 33: 868-876.

Silva, A.V.C.; Freire, K.C.S.; Ledo, A.S.; Rabbani, A.R.C. 2014. Diversity and genetic structure of jenipapo (*Genipa americana* L.) Brazilian accessions. *Scientia Agricola* 71: 345-355.

Silva, R.G.; Chaves, M.C.L.; Arnhold, E.; Cruz, C.D. 2009. Repetibilidade e correlações fenotípicas de caracteres do fruto de bacuri no Estado do Maranhão. *Acta Scientiarum Agronomy***,** 31: 587-591.

Sokal, R.R.; Rohlf, F.J. 1962. The comparison of dendrograms by objective methods. *Taxon,* 11: 30-40.

Souza, I.G.B.; Souza, V.A.B.; Lima, P.S.C. 2013. Molecular characterization of *Platonia insignis* Mart. (Bacurizeiro) using inter simple sequence repeat (ISSR) markers. *Molecular Biology Reports*, 40: 3835-3845.

Souza, I.G.B.; Souza, V.A.B.; Silva, K.J.D.; Lima, P.S.C. 2016. Multivariate analysis of 'bacuri' reproductive and vegetative morphology. *Comunicata Scientiae*, 7: 232-240.

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