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DIVERSITY AND GENETIC STRUCTURE OF NATURAL POPULATIONS OF ARAÇÁ (*Psidium guineense* Sw.)[1]

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ABSTRAT - *Psidium guineense* Sw*,* popularly known as araçá, is a fruit tree there is widely distributed in Brazil and belongs to the *Myrtaceae* family. In northeastern Brazil, araçá occurs along coast and in the Zona da Mata; its fruit looks like guava but is more acidic and has a stronger smell. There is a little information about this species, which increases the difficulty of conserving its genetic resources and exploiting araçá as an economic resource. The objective of this research was the evaluation of the genetic diversity and genetic structure of *P. guineense* from Pernambuco's Zona da Mata. One hundred and fourteen individuals and 18 isozyme loci were evaluated, showing 28 alleles. The percentage of polymorphic loci ($\hat{P}$) and the average number of alleles per locus ($\hat{A}$) were 0.5 and 1.5, respectively, in this population. The expected heterozygosity $(\hat{H}_e)$, which corresponds to the genetic diversity, ranged from 0.22 to 0.23, a high value when considering that isozymes mark access from the functional genome. The differentiation index among the population was $(\hat{\theta}_p)$ = 0.015; therefore, the populations were not different among the sampled places. The inbreeding values ($\hat{f}$) ranged from -0.549 to -0.794, indicating an absence of inbreeding and a greater-than-expected heterozygosity in all the studied populations. The estimated gene flow (N_m) for a pair of this population ranged from 3.23 to 20.77, sufficient to avoid genetic differentiation among the population and in accordance with the values of genetic divergence found in this study.

Keywords: Genetic resource. Isozyme. Native fruit. Gene flow.

DIVERSIDADE E ESTRUTURA GENÉTICA DE POPULAÇÕES NATURAIS DE ARAÇÁ (*Psidium guineense* Sw.)

RESUMO – *Psidium guineense* Sw.*,* conhecida popularmente como araçá, é uma fruteira pertencente à família *Myrtaceae* que tem ampla distribuição geográfica. Nos Estados do Nordeste Brasileiro ela ocorre principalmente na faixa litorânea e Zona da Mata. O fruto do araçazeiro tem sabor que lembra o da goiaba, ligeiramente mais ácido e de perfume mais acentuado, e sua exploração é feita de modo extrativista. As informações sobre esta espécie são escassas, o que dificulta a conservação dos recursos genéticos e sua exploração econômica. Neste trabalho foram estudadas a diversidade e estrutura genética de quatro populações de araçá, tendo sido avaliados um total de 114 indivíduos por meio de 18 loci isoenzimáticos que revelaram 28 alelos. A porcentagem de locipolimórficos ($\hat{P}$) e o número médio de alelos por loco ($\hat{A}$) nas populações foram 0,5 e 1,5, respectivamente. A heterozigosidade esperada ($\hat{H}_e$), que corresponde à diversidade gênica, variou entre 0,22 e 0,23, valores altos, principalmenteconsiderando-se que isoenzimas acessam a porção funcional do genoma. O índice médio de diferenciação ($\hat{\theta}_p$) entre as populações foi de 0,015, portanto, as populações basicamente não diferem entre os locais amostrados. O índice de fixação ($\hat{f}$) variou de -0,549 a -0,794 indicando ausência de endogamia e excesso de heterozigosidade nas populações. O fluxo gênico (N_m) estimado para os pares de populações variou de 3,23 a 20,77, valores que corroboram com a ausência de diferenciação observada entre estas.

Palavras chave: Recurso genético. Isoenzima. Fruteira nativa. Fluxo gênico.

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INTRODUCTION

Psidium guineense SW., popularly known as araçá, belongs to the Myrtaceae family, which includes approximately 100 genera and 3000 species, including trees and shrubs, and is distributed on all continents except Antarctica; it occurs more often in tropical and subtropical regions (PEREIRA; NACHTIGAL, 2003).

This specie is originally from South America (BRANDÃO et al., 2002), and in Brazil occurs spontaneously from the Rio Grande do Sul to the Amazon, mainly in coastal areas (BEZERRA et al., 2006). In northeastern Brazil, *P. guineense* occurs along the seaside and Zona da Mata, principally in coastal board areas (DEMATTÊ, 1997; BALOCH et al., 2006) and the Semi-arid region of Bahia (QUEIROZ, 2011; SANTOS et al., 2014).

P. guineense is a shrub with stature from 2.0 to 2.5 m and stem with smooth bark that peels off. Their fruits are similar to those of the guava but are smaller, more acidic, stronger smelling, globular (sometimes ovoid), pedunculated, and clear-yellow when ripe with a cream-colored pulp and many seeds (GONZÁLEZ et al., 2005). *P. guineense* fruit is accepted by consumers, who appreciate its exotic flavor and high C vitamin content (RASEIRA and RASEIRA, 1996); however, exploration performed through the extractivism (BEZERRA et al., 2006).

P. guineense has great importance as a genetic resource to be exploited directly in the pre-breeding process or as a source of genes to transfer to the guava, *Psidium guajava* L., by conventional crossing. Nematode gall-resistant genotypes of *P. guineense* reportedly showed promise as grafts to guava (CASTRO et al., 2012; MARTINS et al., 2013).

The conserved genetic resources of *P. guineense* are little, especially considering the wide geographic distribution of the species. The accessions of this species are maintained in collections with other species of the *Psidium* genus, including cultivated species of *P. guajava,* according to Bezerra (2006), Santos et al. (2008), and Queiroz (2011). There is little information about the diversity and genetic structure of natural populations of *P. guineense*; however, severe genetic erosion is assumed due to the devastation of ecosystems where the species occurs, as registered with other fruit native species.

Therefore, studies of the diversity and genetic structure of natural populations are very important because they could provide fundamental parameters for pre-breeding and define conservation strategies, especially those that guarantee the continuity of the evolutionary process of populations over time (RAU; HODGKIM, 2002).

Using molecular markers can get parameters on the structure and genetic diversity of populations, as well as estimate gene flow among populations (HAMRICK, 1982). Isoenzyme markers have been used in many studies of natural populations because isoenzymes access information from the functional genome and are codominant. Applying these markers were studied populations of several tropical fruits, such as cagaiteira (*Eugenia dysenterica* DC.) (TELLES et al., 2001), araticunzeiro (*Annona crassiflora* Mart.) (TELLES et al., 2003), genipapo (*Genipa americana* L.) (SEBBENN et al., 2003), pequizeiro (*Caryocar brasiliense* Camb.) (MELO JÚNIOR et al., 2004), cajazeira (*Spondias mobin* L.) (SILVA et al., 2009), mangabeira (*Hancornia speciosa* var. *speciosa* Gomes) (MARTINS et al., 2012), and other species of ecological interest.

The objective of this work was to study the diversity and genetic structure of four populations of araçá in Zona da Mata region of Pernambuco State using isoenzymatic markers, in order to provide information for use in conservation and pre-breeding programs.

MATERIAL AND METHODS

Sampling

Four natural populations of *P. guineense,* named Itamaracá, Marieta, Arariba, and Palmares, located in the municipalities of Itamaracá, Moreno, Escada, and Palmares, respectively, corresponding to the coastal lowland of the State of Pernambuco, were studied (Table 1). The Itamaracá population occurred in clearings of the remnant vegetation of the Atlantic Forest and the individuals showed different ages. The other three populations occurred in areas where sugar cane was previously cultivated, where soil conditions seemed to favor the development of the plants. The plants seemed to have similar ages, and according to reports from local people, the populations were established for around seven to 11 years.

Random sampling was conducted to accurately represent plant density and population sizes. The young leaves of sampled individuals were collected, placed in plastic bags, kept in an ice cooler, and carried to the Genetics Population Laboratory of the Biology Department of the Federal Rural University of Pernambuco, where they were stored in at -80^{0}C until isoenzyme extraction.

Table 1. Identification of araçá (*P. guineense*) populations studied in Pernambuco state.

Populations			Geographical coordinates	
Municipalities		n*	Larger and smaller Latitude	Larger and smaller Longitude
Itamaracá	Itamaracá	28	07º 44'197'' a 07º44' 989''	034º51'312'' a 034º51'667''
Moreno	Marieta	30	08º07'113'' a 08º08'965''	035º10'986'' a 035º11'171''
Escada	Arariba	28	08º16'017'' a 08º17'968''	035º08'721'' a 035º11'196''
Palmares	Palmares	28	08º 43'266'' a 08º 46'179''	035º31'013'' a 035º31'985''

n* = number of individuals sampled

Twenty-eight to 30 individuals in each population were sampled, according to the abundance of plants and population size, in the period of January to April 2013. The minimum distance between sampled individuals was 50 meters to minimize the probability of collecting sister plants. Each tree was identified by a numbered aluminum label and georeferenced using a Global Positioning System (GPS) to facilitate if necessary, resampling or the collection of propagative parts.

Extraction and revelation of enzymes

The enzymes were extracted using the buffer no. 1 of Alfenas et al. (1998), and PVP40 (Polyvinylpyrrolidone) was added during leaf maceration. The obtained samples were stored at -80^{0}C until the isozymes were separated by horizontal electrophoresis on 13% starch gels, according to the method of Alfenas et al. (1998). The gel/electrode buffer systems used were: TCP (Tris-Borate, Citrate, pH 7.5) and LB (Lithium Borate, pH 8.5).

A total of 18 enzyme systems were tested to provide more informative band patterns: esterase - EST, shikimate dehydrogenase - SKDH, catalase - CAT, alcohol dehydrogenase - ADH, 6-phosphogluconate dehydrogenase - 6-PGDH, Leucine aminopeptidase - LAP, Isocitrate dehydrogenase - IDH, malate dehydrogenase - MDH, acid phosphatase - ACP, peroxidase - PO, glucose 6-phosphate dehydrogenase - G6PDH, isoglucose isomerase - PGI, phosphoglucomutase - PGM, malic enzyme - ME, glutamate oxaloacetate transaminase - GOT, polyphenol oxidase - PPO, superoxide dismutase – SOD, and alkaline phosphatase - AKP.

Data analysis

Zymograms were interpreted using allele frequencies and diversity indices, such as: percentage of polymorphic loci ($\hat{P}$), estimated by the ratio of the average of number of polymorphic loci and the total number of loci, considering loci whose frequency of the most common polymorphic alleles did not exceed 95%; average number of alleles by locus ($\hat{A}$), obtained by dividing the total number of alleles by the total number of loci; observed heterozygosity ($\hat{H}_o$), which was obtained by the equation $\hat{H}_o = 1 - \sum p_{ii}$, where p_{ii} = frequency of homozygous genotypes; expected heterozygosity ($\hat{H}_e$), obtained by the equation $\hat{H}_e = 1 - \sum p_i^2$, where p_i = allele frequency estimated the ith allele; and index ($\hat{f}$), estimated by the equation $\hat{f} = 1 - (\hat{H}_o/\hat{H}_e)$. The effective size ($N_e$) was estimated as reported by Vencovsky (1992), i.e., $N_e = (n/1 + \hat{f})$, where n is the number of sampled plants and $\hat{f}$ is the population inbreeding coefficient.

The estimated parameters of the genetic structure of the population were: unbiased genetic divergence among populations (F_{ST}), according to Nei (1978); genetic identity (GI); and estimates of the gene flow among populations, according to the method of Crow and Aoki (1984), which followed the equation: $N_m = \left[\left(\frac{1}{F_{ST}}\right) - 1\right]/4 \propto$, where $\propto = [n/(n-1)]^2$, and N_m = number of migrants per generation, n the number of populations, and F_{ST} is genetic divergence among populations. The coancestry coefficients were estimated using the method of Cockerham (1969), i.e., using confidence intervals with 95% probability and the bootstrap resampling method with 10000 repetitions per locus. All analyses were performed using the GDA software program (LEWIS; ZAYKIN, 2000).

RESULTS AND DISCUSSION

From the 18 isoenzymatic systems tested, nine revealed band patterns on all individuals of the four studied populations, which were used for analysis (Table 2). The nine systems showed 18 loci and 28 alleles. The ACP1, EST1, GOT1, GOT2, PGI1, SKDH1, 6-PGDH1, and AKP1 loci revealed only one allele that was present in the four studied populations. The other loci (LAP1, LAP2, ACP2,

EST2, PGI2, 6PGDH2, AKP2, ASK2, ADH1, and ADH2) revealed two alleles each, with frequencies ranging from 0.017 to 1,000, showing variation among and within populations.

Changes in allele frequencies in finite populations can indicate genetic drift resulting from gamete sampling in each generation (HEDRINK, 2005). Analyses of allele frequencies are of great importance because they reflect the stochastic effects better than most of the other parameters used in studies of population genetics (BOTREL and CARVALHO, 2004). In the Arariba, Marieta, and Palmares populations, the changes in allele frequencies may be more associated with genetic drift because the populations are new, formed after the interruption of sugar cane cultivation in areas of steep topography; in contrast, in the Itamaracá population, the glades are open and booked sporadically because of anthropic action and due to the regeneration of the vegetation by secondary and tertiary plants that restrict the permanence of *P. guineense*, and therefore young plants are the most prevalent.

Table 2. Frequency of alleles observed at each locus in the four populations of araçá (*P. guineense*) studied.

		Populations			
Loco	Alleles	Itamaracá	Marieta	Arariba	Palmares
Acp1	1	1.000	1.000	1.000	1.000
Acp2	1	0.607	0.600	0.428	0.535
	2	0.392	0.400	0.571	0.464
Lap1	1	1.000	0.648	0.875	0.818
	2	0.000	0.351	0.125	0.181
Lap2	1	0.660	0.616	0.500	0.410
	2	0.339	0.383	0.500	0.589
Est1	1	1.000	1.000	1.000	1.000
Est2	1	0.464	0.150	0.303	0.500
	2	0.535	0.850	0.696	0.500
Got1	1	1.000	1.000	1.000	1.000
Got2	1	1.000	1.000	1.000	1.000
Pgi1	1	1.000	1.000	1.000	1.000
Pgi2	1	0.500	0.766	0.410	0.607
	2	0.500	0.233	0.589	0.392
Skdh1	1	1.000	1.000	1.000	1.000
Skdh2	1	0.500	0.483	0.500	0.500
	2	0.500	0.516	0.500	0.500
6Pgdh1	1	1.000	1.000	1.000	1.000
6Pgdh2	1	0.500	0.350	0.500	0.589
	2	0.500	0.650	0.500	0.410
Akp1	1	1.000	1.000	1.000	1.000
Akp2	1	0.410	0.350	0.464	0.392
	2	0.589	0.650	0.535	0.607
Adh1	1	0.982	1.000	1.000	1.000
	2	0.017	0.000	0.000	0.000
Adh2	1	0.410	0.500	0.500	0.456
	2	0.589	0.500	0.500	0.543

The percentage of polymorphic loci ($\hat{P}$) was 0.5, and the average number of alleles per locus (Â) was 1.5 (Table 3). The regularity of these parameters can be associated with the characteristics of this species, which behaves as a pioneer in the Zona da Mata area of Pernambuco and shows the capability of establishing itself in areas where sugar cane cultivation was disrupted. As the populations are new, only seven to 11 years old, not enough time has passed for selective factors to work in the populations. The behavior of *P. guineense* as a pioneering plant was reported by Barnett et al. (2002). Furthermore, one should also consider that the primary dispersal mode of this species is by birds carrying the seeds, which usually have large displacements and probably are the same birds that disseminate guava seed because all araçá populations occur with guava plants (ARAÚJO, 2014).

Table 3. diversity index in studied populations of araçá (*P. guineense*) based on the 18 allozyme loci.

População	n	P	$\hat{A}$	$\hat{H}_0$	$\hat{H}_e$	$\hat{f}$	*Ne*	*Ne/n*
Itamaracá	28	0.50	1.50	0.39	0.22	-0.794	135.92	4.85
Marieta	30	0.50	1.50	0.34	0.22	-0.549	66.52	2.22
Arariba	28	0.50	1.50	0.40	0.23	-0.754	113.82	4.06
Palmares	28	0.50	1.50	0.39	0.23	-0.678	86.96	3.10
Média	28,5	0.50	1.50	0.38	0.22	-0.693	100.80	3.54

Number of individuals sampled in each population (n), percentage of polymorphic loci (P), average of allele per locus number ($\hat{A}$), observed heterozygosity ($\hat{H}_0$), expected heterozygosity ($\hat{H}_e$), fixation index ($\hat{f}$), effective size (*Ne*), and relationship between effective size and real size (*Ne/n*)

The average observed heterozygosity ($\hat{H}_o$), which ranged from 0.34 to 0.40, and the average expected heterozygosity ($\hat{H}_e$), which corresponds to the gene diversity, basically did not vary, with values from 0.22 to 0.23 (Table 3); Itamaracá and Marieta had the same value (0.22) and Araiba and Palmares had the same value (0.23). Therefore, more heterozygotes were observed than expected by the Hardy-Weinberg Equilibrium. This diversity level (from 22 to 23%) is considered high or intermediate in population studies of isoenzymes, mainly considering that isozyme markers use the functional portion of the genome; other isoenzyme-based studies of wild species populations showed similar diversity values. Kageyama et al. (2003) obtained $\bar{H}_e$ values that ranged from 0.255 to 0.480 for five forest species (*Trema micrantha*, *Cecropia pachystachya*, *Cariniana legalis*, *Maytenus aquifolia,* and *Esenbeckia leiocarpa*) in different successional phases. Martins-Corder et al. (2009) obtained values that ranged from 0.203 to 0.276 to *Euterpe edulis Martius*, under three different environmental conditions. Martins et al. (2012) observed values from 0.300 to 0.420 when studying *Hancornia speciosa* variety *speciosa* in northeastern Brazil. The genetic diversity and absence of differentiation observed among populations of *P. guineense* in the Zona da Mata of Pernambuco State indicate that, for germplasm conservation or to perform comparison studies with materials from other areas, only one of the studied populations is necessary to represent this region.

The fixation index ($\hat{f}$) ranged from -0.549 to -0.794 (Table 3). Negative values indicate high heterozygosity, according to $\hat{H}_o$, and the obtained $\hat{H}_e$ values suggested natural reproduction by allogamy. High levels of genetic diversity provide a large number of combination genotypes in sexually-reproducing species, which increases the evolutionary potential as a consequence of the great adaptability to environmental changes (SEBBENN et al., 2000). The observed uniform diversity of the four studied populations can also be related to the pioneering behavior of *P. guineense* that promotes the founding of plant populations with similar ages. Furthermore, because *P. guineense* is dispersed mainly by birds, with distances between populations ranging from 16 to 130.3 km (Table 4), these populations could be created by seeds of the same origin, principally the Arariba, Marieta, and Palmares populations.

The genetic differentiation among populations (F_{ST}) and genetic identity (GI) in pairs of populations (Table 4) showed that there is no difference between populations. Indirect estimates of gene flow (Nm) showed values that ranged from 3.23 to 20.77 (Table 4), considered high and sufficient to avoid differentiation among populations; according to Elltsrad (2003), values higher than 1.0 prevent genetic differentiation among the four studied populations. These results indicate that all the studied populations are representatives to *ex situ* or *in situ* conservation, as well as to perform *in situ* conservation because the four have the same representative genetic potential.

Table 4. Genetic identity (*GI*), estimated genetic differentiation (F_{ST}) according to Nei (1978), geographical distance, and gene flow (*Nm*) for each pair of population of araçá (*P. guineense*) in the Zona da Mata of Pernambuco.

Combinations	*GI*	F_{ST}	Geographical distance (km)	*Nm*
Itamaracá x Arariba	0.997	0.003	72.7	20.77
Itamaracá x Marieta	0.981	0.019	56.1	3.23
Itamaracá x Palmares	0.997	0.003	130.3	20.77
Palmares x Arariba	0.997	0.003	57.6	20.77
Palmares x Marieta	0.985	0.015	74.2	4.10
Marieta x Arariba	0.985	0.015	16.6	4.10

The genetic differentiation among populations (F_{ST}) and genetic identity (GI) in pairs of populations (Table 4) showed that there is no difference between populations. Indirect estimates of gene flow (Nm) showed values that ranged from 3.23 to 20.77 (Table 4), considered high and sufficient to avoid differentiation among populations; according to Elltsrad (2003), values higher than 1.0 prevent genetic differentiation among the four studied populations. These results indicate that all the studied populations are representatives to *ex situ* or *in situ* conservation, as well as to perform *in situ* conservation because the four have the same representative genetic potential.

Table 5. Average inbreeding for each locus in all populations ($\hat{F}$), average of inbreeding for each locus within populations ($\hat{f}$), and coefficient of coancestrality ($\hat{\theta}_p$) in four natural populations of *P. guineense* on 18 allozyme loci.

Loci	$\hat{F}$	$\hat{f}$	$\hat{\theta}_p$
Acp1	-0.615	-0.690	0.044
Acp2	-0.610	-0.690	0.050
Lap1	-0.654	-0.720	0.040
Lap2	-0.622	-0.600	0.044
Est1	-0.615	-0.690	0.044
Est2	-0.630	-0.690	0.036
Got1	-0.615	-0.690	0.044
Got2	-0.615	-0.690	0.044
Pgi1	-0.615	-0.690	0.044
Pgi2	-0.627	-0.691	0.038
Skdh1	-0.615	-0.690	0.044
Skdh2	-0.566	-0.650	0.050
6Pgdh1	-0.615	-0.690	0.044
6Pgdh2	-0.619	-0.696	0.045
Akp1	-0.615	-0.690	0.044
Akp2	-0.621	-0.705	0.050
Adh1	-0.616	-0.691	0.044
Adh2	-0.580	-0.662	0.050
Média	-0.691	-0.617	0.015

The average estimates of the coancestry coefficients of Cockerham (1969) showed the absence of inbreeding for the populations ($\hat{F}$= -0.691) and excess heterozygosity within these populations ($\hat{f}$=-0.617), confirming the tendency of populations toward allogamy (Table 5). Inbreeding due to genetic drift, by a foundation effect from a small number of individuals, may occur because of the fixation of deleterious alleles, causing inbreeding depression and reducing the adaptability of species in cases where overdominance gene interaction prevails (FRANKHAM et al., 2006).

The average divergence among populations ($\hat{\theta}_p$) was very low (1.5%) (Table 5), confirming the F_{ST} parameter results, and seems to be a specific characteristic of *P. guineense* due to its dispersion mode, allogamy reproduction system, and pioneer specie status.

The genetic representativeness of individuals evaluated by estimating the effective population size (Ne) showed estimated values higher than the number of individuals sampled (Table 3), confirming the absence of inbreeding in the populations. According to Moraes and Derbyshire (2002), this parameter evaluates the impact due to genetic drift. Table 3 also shows the relationship between the effective size and the actual population size (Ne/n), which allows the estimation of the minimum viable population size needed to maintain the population's genetic integrity. According to Raposo et al. (2007), this relationship is fundamental to establishing conservation strategies.

CONCLUSIONS

The araçá (*P. guineense*) populations at the Zona da Mata of Pernambuco are not different from one another in terms of genetic diversity.

The genetic diversity within populations of araçá in the Zona da Mata of Pernambuco is high.

There is no restriction on gene flow among araçá populations in the Zona da Mata of Pernambuco, sufficient for avoiding differentiation among then.

The absence of endogamy in populations suggests that reproduction predominantly occurs by allogamy in *P. guineense* in the Zona da Mata of Pernambuco.

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