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Genetic variability in accessions of the acerola germplasm bank of Universidade Federal Rural de Pernambuco, Brazil

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ABSTRACT. Brazil is the world's largest producer of acerola, *Malpighia emarginata* (Malpighiaceae); the Northeast is responsible for 60% of the national production. The culture of acerola in Brazil has great genetic variability; plantings have high phenotypic diversity and are not very productive, often originating from propagation by seed. We evaluated the genetic diversity of 42 accessions from the Acerola Active Germplasm Bank of Universidade Federal de Pernambuco. Using 15 RAPD primers, 182 markers were obtained, of which 166 were polymorphic and 16 were monomorphic. We found high genetic variability among the accessions ($\hat{H}_E$ = 0.29), with no redundancy. Considering the accessions from the states of Pernambuco, Bahia and Pará as distinct groups, there was greater diversity in accessions from Bahia than from the other two states.

Key words: Molecular markers; RAPD; *Malpighia emarginata*

INTRODUCTION

Acerola (*Malpighia emarginata* DC), also known as "Barbados Cherry" and "West Indian Cherry", is a shrub found naturally on several Caribbean islands and in Central America and parts of Amazonia (Carrington and King, 2002). The acerola crop in Brazil began in the mid-twentieth century with the introduction of the first seeds from the Antilles and the United States. In the 1980s, this crop gained great attention due to the high content of ascorbic acid in their fruits, which can be as high as 5 g/100 g pulp, corresponding to 80 times the amount found in oranges and lemons (Junqueira et al., 2002; Lima et al., 2003).

There are few varieties of acerola available to Brazilian producers, limiting the crop's potential. The orchards are often established using materials of unknown origin, which results in poor uniformity of crops and fruits (Salla et al., 2002).

Much of the genetic variety of this crop in Brazil is represented in an active germplasm bank (AGB) consisting of 42 accessions from different growing regions, established in 1998 at Experimental de Cana-de-açúcar de Carpina (EECAC)/UFRPE in the municipality of Carpina, PE (Figure 1), belonging to Universidade Federal Rural de Pernambuco (UFRPE). The implementation of this AGB aimed to preserve the genetic variability of acerola, as well as to provide evaluation and indication of promising genotypes (Lima et al., 2003).

Molecular markers are a powerful tool for rapid and efficient access of genetic variability and have been used in germplasm banks and breeding programs of various crop species, and the random amplified polymorphic DNA (RAPD) technique is a good alternative for studying the genetic diversity of crop species with little molecular genetics research (Nybom, 2004; Wünsch and Hormaza, 2007).

Figure 1. UFRPE acerola's AGB and three Brazilian origin states.

In this study, we analyzed the genetic diversity and relationships of the Acerola Germplasm Collection of UFRPE using RAPD markers with the following objectives: 1) to assess the level and distribution of genetic diversity of the collection, 2) to determine the genetic distance of the accessions within the collection, and 3) to use the information for future breeding programs as well as to devise sampling strategies for collections of the acerola germplasm.

MATERIAL AND METHODS

Plant material

The accessions of the Acerola Active Germplasm Bank evaluated are listed in Table 1, which also contains the data source and morpho-agronomic characteristics. The ABG is maintained at the Estação Experimental de Cana de Açúcar de Carpina (EECAC) in the municipality of Carpina (7°51'4"S and 35°14'27"W), Zona da Mata of Pernambuco State.

Table 1. Identification and origin of the 42 accessions from the UFRPE Acerola's AGB.

Accession	Procedence	Accession	Procedence
002-SPE	Pernambuco	026-CMF	Bahia
003-APE	Pernambuco	027-CMF	Bahia
004-RPE	Pernambuco	028-CMF	Bahia
005-APE	Pernambuco	029-CMF	Bahia
006-TPA	Pará	030-CMF	Bahia
007-TPA	Pará	031-CMF	Bahia
008-CPA	Pará	032-CMF	Bahia
011-BPA	Pará	033-CMF	Bahia
012-CPA	Pará	034-CMF	Bahia
013-CPA	Pará	035-CMF	Bahia
014-CPA	Pará	036-CMF	Bahia
015-CPA	Pará	037-CMF	Bahia
016-CMF	Bahia	038-CMF	Bahia
018-CMF	Bahia	039-CMF	Bahia
019-CMF	Bahia	040-CMF	Bahia
020-CMF	Bahia	041-CMF	Hawaii/EUA
021-CMF	Bahia	042-CMF	Bahia
022-CMF	Bahia	043-UFRPE	Pernambuco
023-CMF	Bahia	044-APE	Pernambuco
024-CMF	Bahia	045-APE	Pernambuco
025-CMF	Bahia	049-APE	Pernambuco

Genomic DNA extraction

DNA was extracted from approximately 1 g leaves, which were macerated in liquid nitrogen, according to the method proposed by Doyle and Doyle (1990). All DNA samples were diluted to a final concentration of 20 ng/μL.

RAPD analysis

We conducted the amplification reactions in a final volume of 25 μL containing: 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 100 mM each dNTP, 1 U Taq DNA poly-

merase, 25 ng DNA and 0.4 mM primer. The DNA samples were amplified in a thermocycler programmed for 40 cycles, as follows: at 94°C for 15 s, 35°C for 30 s, 72°C for 60 s. The amplification products were separated by horizontal electrophoresis, 100 V for 1.5 h, on 1.2% agarose gels containing 8 µg/mL ethidium bromide and immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0).

The polymorphism obtained by the RAPD technique was tabulated as the presence or absence of the bands. Each RAPD band was considered a single bi-allelic locus with one amplifiable and one null allele. The NTSYSpc program (Rohlf, 2000) was used to calculate the Nei (1972) standard genetic distances. It was also used to perform the sequential agglomerative hierarchical nested cluster analysis (SAHN), using the unweighted pair group method with arithmetic average (UPGMA). In the analysis considering the origin of the accessions, the accessions were divided into three groups, both Pernambuco and Pará with eight accessions and Bahia with 26. Accession 041-CMF, from Hawaii, USA, was included in Bahia group. Nei's genetic diversity (H_E), total heterozygosity parameters (Ht), the mean heterozygosity within groups (Hs), and an average coefficient of differentiation between the groups (G_{ST}), according to Nei (1978), were obtained using POPGEN32 software (Yeh et al., 1999).

RESULTS

The 15 primers generated 4322 RAPD fragments distributed in 182 loci. The average number of loci per primer was 12.13, ranging from 6 (D0142A01) to 17 (D0142A09) (Table 2). Of all loci observed, 16 (8.8%) were monomorphic and 166 (91.2%) showed polymorphism, thus revealing high genetic variability between the accessions. Salla et al. (2002) used 37 RAPD primers and obtained an average of 4.4 fragments per primer, distributed in 164 loci. $\hat{H}_E$ was relatively high in all groups (Table 3). Accessions from Bahia showed the highest variability (0.27), followed by accessions from Pará (0.24), and Pernambuco (0.21), which demonstrated reasonable genetic variability in the germplasm bank (Ht = 0.29) (Table 4). The analysis of the distribution of genetic variability showed that 20% of the genetic variability occurred between populations (G_{ST} = 0.20) and 80% within populations.

Table 2. List of primers, sequences, number of loci, and polymorphic loci.

Primer	Sequence 5'-3'	No. of loci	Polymorphic loci
D0142A01	AGTCAGCCAC	6	2
D0142A02	GTGATCGCAG	11	10
D0142A03	CAATCGCCGT	16	15
D0142A04	TCGGCGATAG	12	12
D0142A05	CAGCACCCAC	11	8
D0142A06	TTCCGAACCC	11	11
D0142A07	GACCGCTTGT	14	13
D0142A08	AGGTGACCGT	12	11
D0142A09	CTGCTGGGAC	17	16
D0142A10	GTGTGCCCCA	14	13
D0142A11	TCAGGGAGGT	11	11
D0142A12	CACCAGGTGA	10	10
D0142B01	GAGCCCTCCA	14	11
D0142B02	AGCGTGTCTG	12	12
D0142B03	GTGACGTCAC	11	11
Total		182	166

Table 3. Diversity estimates of 42 active Acerola Germplasm Bank accessions from three Brazilian regions.

Origin	N	N_A	N_E	$\hat{H}_E$	Npl	Ppl
Bahia	26	1.82 (0.38)	1.46 (0.35)	0.27 (0.18)	150	82.42%
Pará	8	1.67 (0.47)	1.42 (0.39)	0.24 (0.20)	122	67%
Pernambuco	8	1.47 (0.5)	1.31 (0.39)	0.18 (0.21)	86	47.25%
Total	42	1.94 (0.23)	1.48 (0.34)	0.29 (0.17)	172	94.51%

Data are reported as means (standard deviation). N_A = number of observed alleles; N_E = effective number of alleles; $\hat{H}_E$ = Nei's genetic diversity; Npl = number of polymorphic loci; Ppl = percent of polymorphic loci.

Table 4. Genetic structure of Acerola's Germplasm Bank.

	*H*t	*H*s	G_{ST}	*N*m
Mean	0.29	0.23	0.20	1.98
Standard deviation	0.03	0.02	-	-

*H*t = total genetic heterozygosity; *H*s = mean heterozygosity within population; G_{ST} = coefficient of population differentiation; *N*m = allelic flow.

The UPGMA dendrogram (Figure 2) showed a similarity range of 68 to 89% with Nei's (1978) genetic identity matrix for the accessions sampled. Nei's (1978) genetic identity between groups showed greater similarity between accessions of Pernambuco and Bahia (0.949) and lower between Pará and Pernambuco (0.848). Similarity was intermediate between Bahia and Pará (0.905).

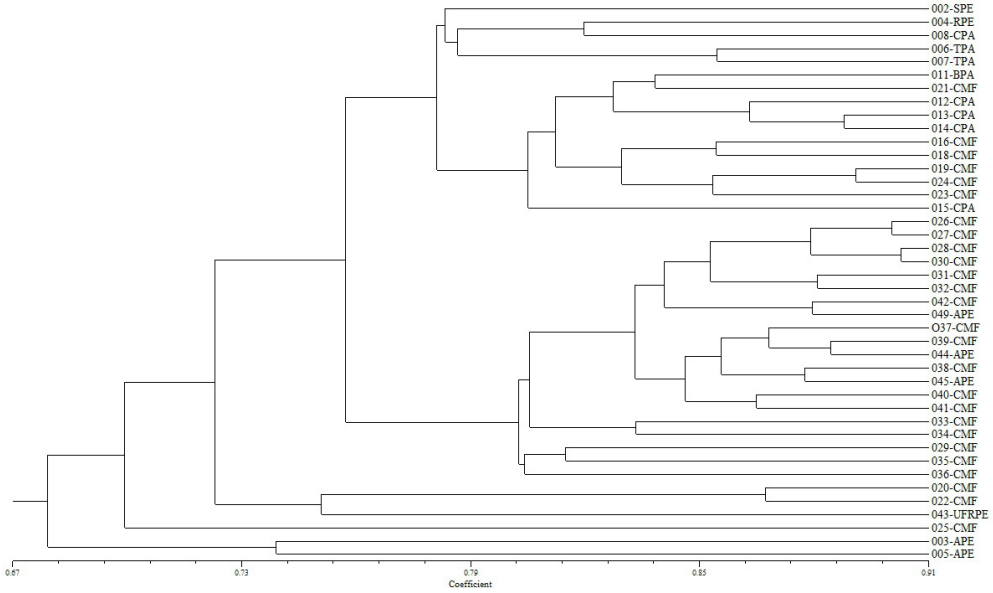

Figure 2. UPGMA dendrogram of 42 Acerola's AGB accessions from three Brazilian regions.

DISCUSSION

The percentage of polymorphic loci has been used as a genetic diversity measure in plant populations through the use of dominant markers (Xia et al., 2007; Julio et al., 2008; Kumar et al., 2010; Oliveira et al., 2012). The highest values were observed in accessions from Bahia State (82.42%), followed by Pará (67%) and Pernambuco States (47.25%). Considering all accessions, the percentage of polymorphic loci was 94.51%, indicating high genetic variability in the Acerola Active Germplasm Bank of UFRPE.

Xia et al. (2007) observed that the percentage of polymorphic loci ranged from 21.9 to 48.8% in natural populations of *Rhodiola chrysanthemifolia*, while Zimback et al. (2004) reported much higher estimates of polymorphism of 90.3 to 97.3% in populations of *Trichilia pallida* Swartz. Salla et al. (2002) observed 90.8% polymorphism in 24 acerola genotypes from Paraná State, a much closer value to that obtained in the present study.

The H_E values in the present study were similar to those in studies with natural populations, e.g., 0.27 to 0.33 for *T. pallida* (Zimback et al., 2004), 0.25 to 0.29 for *Polylepis australis* (Julio et al., 2008), 0.11 to 0.26 for *Hancornia speciosa* (Costa et al., 2011) and 0.25 to 0.42 for *Acrocomia aculeata* (Oliveira et al., 2012), suggesting a strong genetic basis.

The G_{ST} values obtained in this study (20%) were close to the expected average for outcrossing species <19%, and species with a mixed mating system 21.2-24.0% (Hamrick and Godt, 1996). We observed an *Nm* value of 1.98. Reis (1996) states that Nm is commonly above 1.0 for tropical trees. Wright (1949) had stated that an Nm value higher than 1 is sufficient to prevent the effects of the genetic drift due to gene flow, suggesting the occurrence of historical gene flow between the accessions analyzed.

The genetic similarity between accessions of Pernambuco and Bahia (0.949) and greater divergence between Pará and Pernambuco (0.165) suggest a relation of geographical distance and genetic identity between the accessions (Table 5).

Table 5. Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) among Acerola's AGB accessions from three Brazilian regions.

Region	Pará	Bahia	Pernambuco
Pará	-	0.9047	0.8479
Bahia	0.1001	-	0.9491
Pernambuco	0.1650	0.0523	-

Our results showed the absence of redundancy in the UFRPE Acerola Active Germplasm Bank, and its 42 accessions were found to be genetically distinct. RAPD analysis showed high genetic diversity, which is consistent with the results obtained by Musser (2001), who observed high agronomic, morphological and physico-chemical diversity within the 42 accessions. Nevertheless, more studies must be carried out with molecular and morphological markers to obtain information that can be used to establish conservation and breeding programs for this species.

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