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Scientific Article

Genetic diversity among acerola accessions collected in different brazilian states using ISSR markers

Diversidade genética entre acessos de acerola coletados em diferentes estados do Brasil utilizando marcadores ISSR

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ABSTRACT – Commercial acerola (*Malpighia emarginata* Sessé & Moc. ex DC.) orchards in Brazil are typically established using only one or two genotypes, making them highly susceptible to biotic and abiotic stresses. Molecular characterization of available germplasm can help identify valuable alleles for the development of new cultivars. This study aimed to assess the genetic diversity of a representative sample of acerola germplasm cultivated in Brazil using inter-simple sequence repeat (ISSR) primers. Genomic DNA was extracted from 96 accessions conserved in Petrolina, Pernambuco state (PE) and amplified using 15 ISSR primers. The presence/absence of bands was scored to estimate allelic similarity based on the Jaccard index, and a dendrogram was constructed using the UPGMA method. Genetic variation within and between groups was quantified via analysis of molecular variance (AMOVA), using Genes, GenAlex and Cervus software. The accessions were grouped into 24 clusters, with ACO01 and MAR12 being the most divergent and Costa Rica and Flor Branca the most similar. The cluster analysis revealed substantial variation among individuals from the same region (81%), a result confirmed by AMOVA and the Shannon -Wiener diversity index. The ISSR primers effectively captured the genetic variation among the accessions studied. Given that the samples analyzed represent acerola germplasm cultivated across Brazil, these results provide valuable insights for the management of genetic resources and the development of acerola breeding programs.

Keywords: *Malpighia emarginata*. Genetic breeding. Molecular markers. Genetic divergence.

RESUMO – Pomares comerciais de aceroleira (*Malpighia emarginata* Sessé & Moc. ex DC.) têm sido formados utilizando-se apenas um ou dois genótipos, o que resulta em grande vulnerabilidade desses cultivos a estresses bióticos/abióticos. A caracterização do germoplasma disponível, utilizando marcadores moleculares, pode identificar alelos de interesse, úteis no desenvolvimento de novos cultivares. Diante disso, o objetivo do presente trabalho foi estimar diversidade genética de uma amostra representativa do germoplasma de aceroleira cultivado no Brasil, utilizando marcadores ISSR. O DNA genômico de 96 acessos conservados em Petrolina-PE foi extraído e amplificado utilizando 15 marcadores ISSRs. As amplificações foram anotadas para presença (1), ausência (0) de bandas, possibilitando estimar similaridade alélica utilizando o índice de Jaccard e obtenção do dendrograma (método UPGMA). A análise de variância molecular foi utilizada para quantificar a diversidade genética entre e dentro de grupos. Foram utilizados os softwares Genes, GenAlex e Cervus. Os acessos foram agrupados em 24 clusteres, sendo ACO01 e MAR12 os mais divergentes e Costa Rica e Flor Branca os mais similares. A análise de agrupamento evidenciou a existência de uma maior variação entre os indivíduos coletados em uma mesma região (81%), resultado confirmado pela AMOVA, e pelo índice de Shannon-Wiener. Os marcadores moleculares utilizados no presente estudo foram eficientes em captar variação genética presente nos acessos do Banco Ativo de Germoplasma de aceroleira da Embrapa Semiárido. Os acessos representam minimamente a distribuição da variabilidade genética da espécie no país, essa informação traz subsídios para a tomada de decisão no manejo dos recursos genéticos da aceroleira.

Palavras-chave: *Malpighia emarginata*. Melhoramento genético. Marcadores moleculares. Divergência genética.

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INTRODUCTION

The acerola tree (*Malpighia emarginata* Sessé & Moc. ex DC.), a member of the *Malpighiaceae* family, is native to Central America and northern South America. Its broad adaptability to tropical and subtropical regions and high fruiting capacity have contributed to the widespread cultivation of acerola in the Americas, Asia and Africa (OLĘDZKI; HARASYM, 2024). The fruit, commonly referred to as "Barbados Cherry" or "Western Island Cherry", is recognized for its considerable social and economic importance, primarily due to its high ascorbic acid content and the presence of other bioactive compounds such as anthocyanins and polyphenols, which contribute to its considerable antioxidant potential (MISKINIS; NASCIMENTO; COLUSSI, 2023; VILVERT et al., 2024).

Brazil is the world's largest producer and exporter of acerola (SANTOS; LIMA, 2020), facilitated by the country's favorable climate for its cultivation. As a result, Brazil maintains the largest cultivated acerola area globally, reaching 7,724 ha and yielding 143,000 tons of fruit in 2017 (BARROS et al., 2020; MALEGORI et al., 2017; IBGE 2018).

However, commercial acerola orchards are typically composed of a limited number of genotypes, often just one or two clones, which significantly narrows

the crop's genetic base and increases vulnerability to biotic and abiotic stresses (SOUZA et al., 2017). This genetic uniformity poses a risk, since the emergence of unforeseen stressors can severely affect production. A rational strategy to mitigate this risk is to increase genetic variability in orchards by simultaneously cultivating a larger number of genotypes (SOUZA, 2013). In this context, evaluating existing germplasm can support the development of new cultivars and expand the supply of new commercial materials. Therefore, studying genetic diversity is fundamental to understanding the variability within the species in order to implement strategies for its conservation and genetic improvement (HOBAN et al., 2021).

Among the tools available for assessing genetic diversity, inter-simple sequence repeat (ISSR) markers have proven effective in a variety of plant species, including *Teucrium stocksianum* Boiss. (KAMALI et al., 2023), Salvia spp. (HEJAZI, 2023), *Sorghum bicolor* (MEDRAOUI et al., 2023) and *Euphorbia resinifera* O. (ABD-DADA et al., 2023). ISSR markers offer a rapid, reproducible and efficient means of characterizing genetic profiles, with minimal influence from environmental factors (CAIXETA et al., 2016).

Embrapa Semiárido maintains a germplasm collection of 96 acerola accessions. These genotypes were obtained through exchanges with universities and research institutions and from collections in older commercial orchards established from seed-propagated seedlings in Brazil's main acerola-producing areas (MARÇAL, 2024). As such, these accessions provide a representative sample of the species' genetic variability since the early stages of its cultivation in the country. In light of the above, this study aimed to assess the genetic diversity of a representative sample of acerola germplasm cultivated in Brazil using ISSR markers.

MATERIAL AND METHODS

Samples of young healthy leaves were collected from 96 acerola (*Malpighia emarginata*) accessions (Table 1), maintained in the largest Active Germplasm Bank (AGB) for the species in Brazil (09°09' S, 40°22' W; altitude: 376 m). This AGB is located at the experimental field of Embrapa Semiárido, within the irrigated Bebedouro project in Petrolina, Pernambuco state (PE). The orchard is managed in line with standard agronomic recommendations for acerola cultivation under local environmental and agricultural conditions. The plants are maintained in the field without a specific experimental design, with two plants representing each accession; however, only one plant per accession was sampled for DNA (deoxyribonucleic acid) extraction. Leaf samples were placed in labeled paper bags, stored in Styrofoam boxes with ice, and transported to the Biotechnology Laboratory, where they were stored at -80°C until DNA extraction.

Table 1. Acerola accessions from the Embrapa Semiárido AGB used for DNA extraction and subsequent genotyping.

Accession	Collection site	Accession	Collection site	Accession	Collection site
MAR01	Maranguape - CE	ACO35	Petrolina - PE	CARP02	Carpina - PE
MAR02	Maranguape - CE	ALHA03	Alhandra - PB	CARP03	Carpina - PE
MAR03	Maranguape - CE	ALHA04	Alhandra - PB	CARP04	Carpina - PE
MAR05	Maranguape - CE	ALHA05	Alhandra - PB	CARP05	Carpina - PE
MAR06	Maranguape - CE	ALHA06	Alhandra - PB	CARP06	Carpina - PE
MAR07	Maranguape – CE	ALHA09	Alhandra - PB	CARP07	Carpina - PE
MAR08	Maranguape - CE	BRS Apodi	Pacajus - CE	CARP08	Carpina - PE
MAR09	Maranguape - CE	BRS Cereja	Pacajus - CE	CARP09	Carpina - PE
MAR10	Maranguape - CE	BRS Frutacor	Pacajus - CE	RECI01	Recife - PE
MAR11	Maranguape- CE	BRS Jaburu	Pacajus – CE	Natália	Londrina - PR
MAR12	Maranguape - CE	BRS Roxinha	Pacajus - CE	RECI02	Recife - PE
BRS_Sertaneja	Petrolina - PE	BV01	Pacajus - CE	LAG01	Lagarto - SE
Coopama Nº1	Petrolina - PE	BV07	Pacajus - CE	LAG03	Lagarto - SE
Costa Rica	Petrolina - PE	Clone47/1	Pacajus - CE	LAG04	Lagarto - SE
Flor Branca	Petrolina - PE	Clone71/2	Pacajus - CE	LAG05	Lagarto - SE
Junko	Petrolina - PE	FP19	Pacajus - CE	LAG06	Lagarto - SE
Nikki	Petrolina - PE	Mineira	Pacajus - CE	LAG07	Lagarto - SE
Okinawa	Petrolina - PE	Monami	Pacajus - CE	LAG08	Lagarto - SE
ACO01	Petrolina - PE	CAMTA	Pacajus - CE	LAG09	Lagarto - SE
ACO03	Petrolina - PE	Barbados	Pacajus - CE	Olivier	São Paulo - SP
ACO05	Petrolina - PE	BRS_Cabocla	Cruz das Almas - BA	Carolina	Londrina - PR
ACO07	Petrolina - PE	BRS_Rubra	Cruz das Almas - BA	Dominga	Londrina - PR
ACO08	Petrolina - PE	Morena	Cruz das Almas - BA	Lígia	Londrina - PR
ACO09	Petrolina - PE	Mulata	Cruz das Almas - BA	Luisa	Londrina - PR
ACO10	Petrolina - PE	Tropicana	Cruz das Almas - BA	Manoela	Londrina – PR
ACO13	Petrolina - PE	Florida Sweet	Cruz das Almas - BA	Neusa	Londrina – PR
ACO14	Petrolina - PE	Florida Sweet x BRS_Cabocla	Cruz das Almas - BA	Valéria	Londrina - PR
ACO15	Petrolina - PE	ACO30	Petrolina - PE	Samurai	Londrina - PR
ACO17	Petrolina - PE	ACO31	Petrolina - PE	UEL01	Londrina – PR
ACO18	Petrolina - PE	ACO33	Petrolina - PE	UEL03	Londrina - PR
ACO19	Petrolina - PE	ACO20	Petrolina - PE	Eclipse	Londrina - PR
ACO34	Petrolina - PE	CARP01	Carpina - PE	LOND01	Londrina - PR

Genomic DNA was extracted following the protocol described by Doyle and Doyle (1990), with some modifications. For each accession, 5g of leaf tissue was macerated using an L-Beader 24-cell and tissue disruptor under the following conditions: three 45-second cycles, with 5-second interval between cycles, at an agitation speed of 6:30 m/s. The material was processed in 2 ml microtubes containing seven 2 mm spheres and 950µL of 2% CTAB supplemented with β-mercaptoethanol solution. Next, 950 µL of chloroform:isoamyl alcohol (24:1) was added. The DNA was then resuspended in 50 µL of ultrapure water. Genomic DNA was quantified by electrophoresis on 1% agarose gel stained with ethidium bromide, using λ phage DNA of known concentrations (100, 50 and 25 ng/µL). Electrophoresis was performed at 100W for 1 hour. Following quantification, part of the DNA of each accession was diluted to a working concentration of 25ng/µL.

Thirteen ISSR primers were selected from an initial set of 25 (Table 2). PCR (polymerase chain reaction) amplifications were performed in a Gene Amp® PCR System 9600 thermal cycler (Applied Biosystems), in a final reaction volume of 6.25 µL, containing: 0.5 µL of genomic DNA (25 ng/µL); 0.94 µL of primer (4 mM, Eurofins Genomics); 1.25 µL of 5X buffer; 0.5 µL of dNTPs (2 mM); 0.05 µL of Taq DNA polymerase (5 U, Ludwig); 0.5 µL of $MgCl_2$ (25 mM), and 2.51 µL of ultrapure water.

Table 2. ISSR primers used to amplify genomic DNA from acerola accessions.

Locus ISSR	Sequence	Annealing temperature (ºC)	Size (pb)
DiGA3´C	5'-GAGAGAGAGAGAGAGAC-3'	50	190-1000
DiGT5´CR	5'-CRGTGTGTGTGTGTGTGT-3'	50	500-1100
DiGT5'CY	5'-CYGTGTGTGTGTGTGTGT-3'	50	300-1100
TriCAC3'YC	5'-CACCACCACCACCACYC-3'	50	190-900
TriCAC'5CR	5'-CRCACCACCACCACCAC-3'	50	400-1200
TriCAC5'CY	5'-CYCACCACCACCACCAC-3'	50	200-1000
TriCAG	5'-CAGCAGCAGCAGCAG-3'	50	500-1200
TriCAG3'RC	5'-CAGCAGCAGCAGCAGRC-3'	50	190-1200
TriCAG3´YC	5'-CAGCAGCAGCAGCAGYC-3'	50	400-1300
TriACA3'RC	5'-ACAACAACAACAACARC-3'	50	250-1000
TriACG3'RC	5'-ACGACGACGACGACGRC-3'	50	300-1000
DiCA5'CY	5'-CYCACACACACACACACA-3'	50	230-700
DiCA5'G	5'-GCACACACACACACACA-3'	50	300-900

The amplification program consisted of an initial denaturation step at 94ºC for 3 minutes, followed by 35 denaturation cycles at 94ºC for 1 minute, annealing at 50ºC for 30 seconds, and extension at 72ºC for 75 seconds. A final extension step was performed at 72ºC for 5 minutes. The reaction products were then maintained at 4ºC. Amplified fragments were separated on 2% agarose gels stained with ethidium bromide and run at a constant voltage of 75 V for 3 hours. Band visualization was performed under ultraviolet light and images were captured using a photo documentation system (Loccus Biotecnologia). Fragment sizes were estimated using a 100 bp molecular weight ladder (Cellco) as the standard.

The data were scored based on the presence (1) or absence (0) of bands recorded for each locus. Based on the binary data generated from the dominant ISSR markers, Analysis of Molecular Variance (AMOVA) was performed using GenAlEx software, version 6.2 (SMOUSE; PEAKALL; GONZALES, 2008), considering a hierarchical structure in which accessions were grouped by their collection location (ten locations, excluding São Paulo). Genetic diversity among accessions was further assessed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm, based on Jaccard's similarity coefficient. A dendrogram was constructed to represent the genetic relationships among accessions, and the goodness-of-fit between the similarity matrix and the dendrogram was evaluated using the cophenetic correlation coefficient (CCC), calculated in Genes software, version 2021.1.9 (CRUZ, 2016).

In addition, the following diversity parameters were estimated: total number of amplified bands (NTB), number of different alleles (Na), effective number of alleles (Ne), Shannon-Wiener diversity index (I), percentage of polymorphic loci (%P), calculated as the ratio of polymorphic loci to the total number of loci analyzed, and expected heterozygosity (He), estimated as the average heterozygosity across loci (NEI, 1987). The parameters were calculated using Cervus software, version 3.0.7 (KALINOWSKI; TAPER; MARSHALL, 2007).

RESULTS AND DISCUSSION

The genetic similarity analysis, based on the 13 ISSR primers and calculated using Jaccard's similarity coefficient (1901), indicated that the ALHA05 accession appeared most frequently in the most dissimilar pairs. The greatest genetic dissimilarity (d) was observed between accessions ACO01 and MAR12 (d = 100%), followed by ACO01 and ALHA04 (d = 80.70%). These findings suggest that crosses between these pairs may produce progeny with greater variability, making them promising candidates for breeding programs.

The most genetically similar accessions were Costa Rica and Flor Branca (d = 7.96%), followed by FP19 and CARP09 (d = 8.70%), indicating that crossing these pairs could result in progeny with a narrow genetic base, which is

typically viewed as less advantageous for generating variability. However, if each accession exhibits distinct desirable traits, such as disease resistance in one and market-preferred characteristics in another, crossing them may allow the combination of these traits into a single genotype.

An example is the pair Flor Branca and Junko, which showed high similarity based on ISSR analysis. Both are commercial clones, with well-defined, market-accepted traits. Flor Branca is known for its year-round flowering and soft-fleshed fruits, while Junko produces firmer, more durable fruits but is sensitive to temperature fluctuations (SOUZA, 2013). A cross between these two accessions could therefore generate segregating populations from which genotypes with more stable production and firmer fruit can be selected. Additionally, since both are commercial clones, the resulting progeny are less likely to exhibit undesirable traits due to linkage drag, reducing the need for intensive selection and culling.

Analysis of the UPGMA dendrogram Figure 1 further confirmed the presence of genetic divergence among the accessions. When a cut-off point of 50% genetic distance was applied, 24 distinct groups were formed, some of which were further subdivided. Group 1 included 10 accessions: Costa Rica, Flor Branca, Junko, Clone71/2, LAG03, LAG04, LOND01, Clone47, Dominga and Eclipse, with Costa Rica and Eclipse being the most divergent (12.4%) (Table 3). The accessions in Group 1 account for 10.42% of the total and originate from three distinct regions (Petrolina, Pernambuco state (PE); Pacajus, Ceará state (CE), and Londrina, Paraná state (PR)). Group 3 comprised 21.88% of the accessions, including: CARP06, CARP08, BRS Cabocla, CARP02, BRS Sertaneja, CARP09, FP19, Florida Sweet x BRS Cabocla, CARP03, LAG08, CARP05, Florida Sweet, LAG07, BRS Roxinha, BRS Rubra, ALHA06, Barbados, BRS Apodi, ALHA05, ALHA09 and BRS Jaburu. Of these, CARP06 and BRS_Jaburu were the most divergent (10.1%) (Table 3). In general, the accessions within each group often originated from multiple collection sites, suggesting a history of genetic exchange, resulting in shared alleles among regions.

Analysis of Table 3 indicates that even within the same genetic group, some accessions display substantial internal divergence. If selected based on desirable traits, these contrasting accessions can be recombined to aggregate favorable alleles, thereby generating superior genotypes for commercial cultivation.

Table 3. Genetic distances between the most divergent accessions within each group.

Groups	Number of accessions per group	First and last acession	Distance (d) (%)
1	10	Costa Rica - Eclipse	12.4
2	5	Carolina - CARP04	10.1
3	21	BRS_Cabocla - BRS_Jaburu	18.8
4	5	Luisa- Lígia	11.6
5	1	MAR11	-
6	2	BV07 - CANTA	15.3
7	3	LAG06 - MAR08	18.2
8	6	MAR06 - MAR09	24.8
9	1	MAR09	-
10	5	BRS_Frutacor - LAG 01	10.4
11	2	ACO 31 - ACO 34	9.3
12	2	ACO 01 - ACO 03	17.1
13	4	ACO 19 - ACO 35	15.7
14	10	ACO 08 - ACO 07	15.1
15	1	ACO 18	-
16	1	ACO 20	-
17	5	UEL 01 - UEL 03	20.3
18	2	Okinawa - Reci 02	21.7
19	2	Olivier - Reci 01	16.7
20	2	Monami - Morena	17.1
21	3	Natalia - Mineira	21.7
22	1	Neusa	-
23	1	Mulata	-
24	1	MAR12	-

Further analysis of the dendrogram (Figure 1) revealed that certain individuals from the same collection site, which were expected to group together, formed separate clusters instead. For instance, Group 11 included accessions ACO31 and ACO34 (2.08%), group 12 ACO01 and ACO34 (2.08%), and group 14 ACO08, ACO30, ACO09, ACO13, ACO10, ACO15, ACO17, ACO14, ACO05 and ACO07 (10.42%). This may be explained by the limited time some of these individuals remained at their respective locations, possibly restricting allele exchange and preventing genetic equilibrium from being established. These results also suggest the presence of significant intra-population variation, highlighting the potential for within-population selection as a viable strategy in breeding programs.

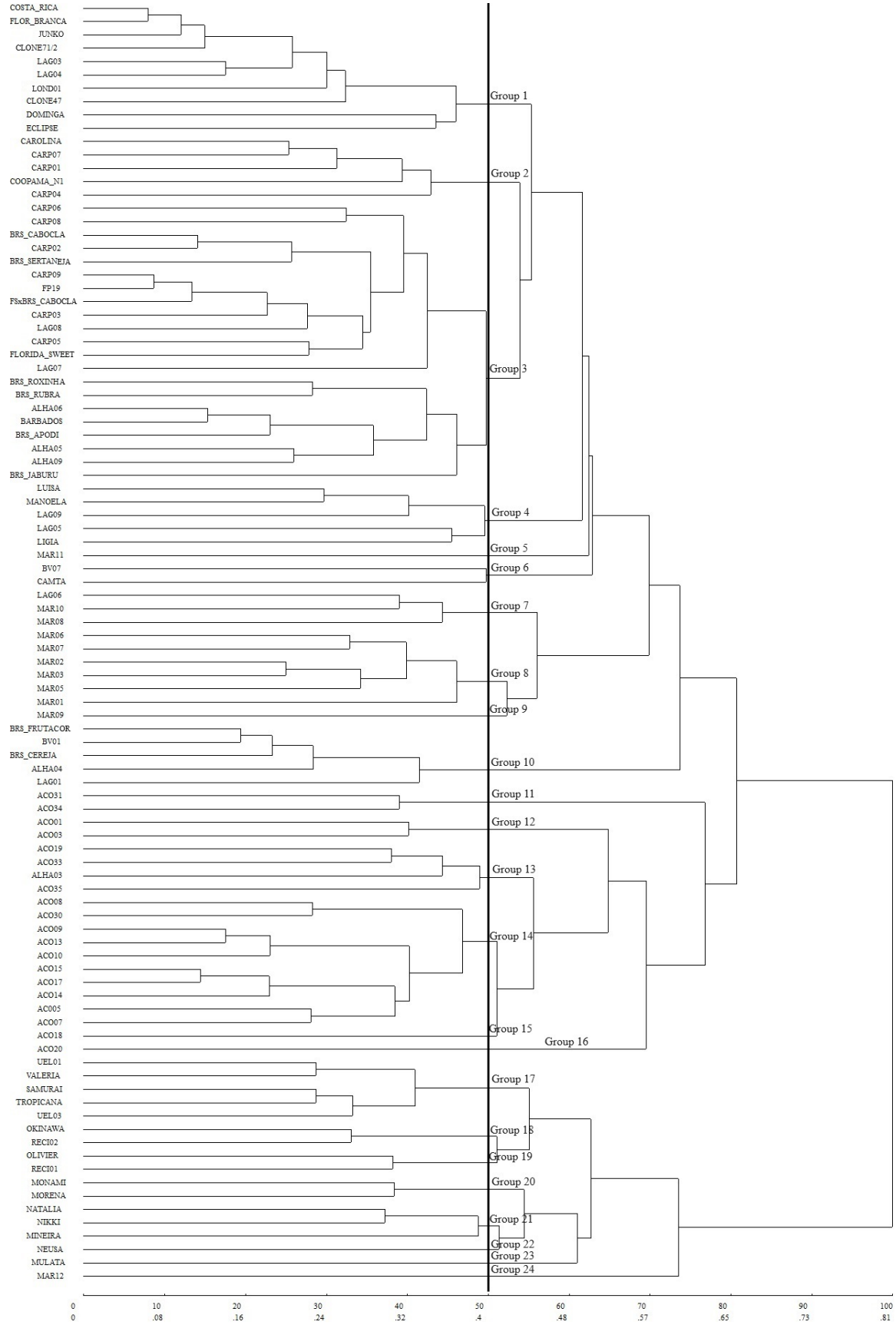

Figure 1. Dendrogram representing genetic dissimilarity among 96 acerola accessions, generated using the unweighted pair group method with arithmetic mean (UPGMA), with a cophenetic correlation coefficient (CCC) of 88%.

Additionally, several groups consisted of single accessions only, namely Groups 5, 9, 15, 16, 22, 23 and 24, which included accessions MAR11, MAR09, ACO18, ACO20, Neusa, Mulata and MAR12, respectively. Each of these groups represented 1.04% of the total accessions (Figure 1). These accessions demonstrated exceptional genetic distinctiveness, since most other individuals collected from the same site were clustered together. This result indicates that these isolated accessions harbor alleles not shared with the rest of the collection, which may be linked to traits of interest. The presence of such distinct genotypes reinforces the importance of ongoing germplasm collection and characterization efforts. It is worth noting that this work represents the first molecular characterization of these particular accessions

The dendrogram generated by the UPGMA method was consistent with the genetic similarity values obtained using Jaccard's index. For example, the Costa Rica and Flor Branca accessions, which showed the highest similarity (d = 7.96%), were clustered together in Group 1, while the greatest genetic distance was observed between ACO01 and MAR12 (d=100%), which were placed in separate groups. This alignment between the dendrogram and the similarity matrix is supported by the satisfactory cophenetic correlation coefficient (r= 88%), indicating a high degree of accuracy in the dendrogram's graphical representation of the data matrix. In addition to the dendrogram analysis, AMOVA was performed to quantify the genetic diversity both between and within groups. The results showed that 19% of the total genetic variation was attributable to differences between groups and 81% within groups (Table 4). This suggests that genetic diversity is greater within collection sites than between them, indicating that selection within populations may be more effective. These findings are consistent with the dendrogram results, which also suggested substantial variation within the regions where the accessions were collected. This may reflect a broader genetic base within each site.

Table 4. Analysis of Molecular Variance (AMOVA) based on ISSR markers, considering the collection site of acerola accessions.

Source of variation	[1]DF	SS	VC	% Variation
Between collection sites	8	338.138	2.956	19
Within collection sites	86	1079.756	12.555	81
TOTAL	94	1417.895	15.511	-

[1]Degrees of freedom (DF), sum of squares (SS), variance component (VC).

Analysis of the parameters obtained using ISSR molecular markers (Table 4) showed that the total number of amplified bands (NTBA) per population ranged from 101 (site 9) to 846 (site 1). Lima et al. (2015) evaluated 20 ISSR primers across 56 acerola accessions and reported a total of only 186 amplified fragments, a considerably lower number than that observed in the present study. This result highlights the presence of significant polymorphism even among accessions from the same collection site. From a breeding perspective, this genetic diversity represents essential raw material for cultivar development, including alleles that may confer tolerance or resistance to biotic and abiotic stresses, which are critical for the commercial cultivation of acerola.

A possible explanation for the observed polymorphism of individuals is the reproductive biology of acerola, which is predominantly allogamous, facilitated by pollinators of the Centridini bee tribe. Cross-pollination enhances the genetic breeding process by contributing to increased genetic variation in progeny, promoting the generation of superior genotypes.

With respect to the number of different alleles (Na) (Table 5), the highest values were recorded at collection sites 1 and 8 (1.15 and 1.19, respectively). This aligns with the clustering pattern in the dendrogram (Figure 1), where some individuals appeared as outliers within the groups. All the groups showed effective number of allele (Ne) values greater than one, with sites 4 and 8 standing out at 1.22 and 1.28, respectively.

Table 5. Genetic diversity parameters obtained using dominant ISSR primers in acerola populations.

Groups	[1]N	NTBA	Na	Ne	I	He	%P
1	26.00	846	1.15	1.21	0.21	0.13	57.40
2	5.00	119	0.39	1.09	0.08	0.05	15.98
3	14.00	391	0.94	1.16	0.16	0.10	44.97
4	7.00	236	0.97	1.22	0.21	0.17	47.34
5	9.00	226	0.49	1.08	0.08	0.05	20.71
6	8.00	238	0.61	1.14	0.13	0.07	27.81
7	11.00	454	0.85	1.20	0.18	0.12	38.46
8	13.00	522	1.19	1.28	0.27	0.18	58.58
9	2.00	101	0.50	1.10	0.08	0.06	13.61

[1]N=number of individuals per population, NTBA= total number of amplified bands, Na= number of different alleles, Ne= effective number of alleles; I= Shannon-Wiener diversity index, He= expected heterozygosity, %P= percentage of polymorphic loci.

The highest Shannon-Wiener diversity index (I) values were obtained in populations from sites 1, 4 and 8, with values of 0.21, 0.21, and 0.28, respectively (Table 4). According to Bolstein et al. (1980), this index ranges from 0 to 1, with values closer to 1 indicating greater genotypic diversity within a group. Based on this criterion, the results obtained here indicate low diversity between groups, consistent with the patterns observed in the dendrogram and AMOVA (Table 4), which also showed greater variation within collection sites. Expected heterozygosity (He) was low across all populations, ranging from 0.05 to 0.18 (Table 5). This can be explained by the dominant nature of the ISSR markers, which cannot distinguish between heterozygous and homozygous dominant genotypes, thereby underestimating true heterozygosity.

The percentage of polymorphic loci (%P) is another useful parameter in the effective detection of polymorphism. In this study, %P values ranged from 13.63 to 57.40, with an average of 36.09 (Table 5). According to Felix et al. (2020), molecular markers with %P above 0.5 are considered highly informative, between 0.25 and 0.5 moderately informative, and below 0.25 poorly informative. Based on these criteria, the primers used in this study demonstrated high informativeness in sites 1 (57.40) and 8 (58.58), moderate in sites 3 (44.97), 4 (47.34), 6 (27.81) and 7 (38.46), and low in the remaining locations.

CONCLUSIONS

The molecular primers used in this study proved effective in capturing the genetic variation present among the acerola accessions in the Embrapa Active Germplasm Bank. Given that these accessions represent a sample of the acerola germplasm cultivated in Brazil since the species' introduction, the results can be considered a preliminary reflection of the genetic variability of *Malpighia emarginata* in the country. Furthermore, the findings provide valuable information to support decision-making regarding the management of acerola genetic resources, particularly in the selection of parent plants aligned with the objectives of breeding programs.

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