

Molecular characterization of guava *Psidium guajava* in Cereté, Córdoba, Colombia

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Abstract

Guava (*Psidium guajava* L.) is the most cherished cultivated fruit species in the Myrtaceae family, and it is a perennial tree native to tropical America. The objective of this study was to determine the genetic variability of *Psidium guajava* in Cereté (Colombia) using SSR markers. DNA extraction was performed using the Mini-prep method with modifications. Nine microsatellites were amplified using the PCR Touchdown technique. Genetic-population parameters such as the number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity, fixation index, Hardy-Weinberg equilibrium, and polymorphic information content were calculated using PopGene 1.31 software. The number of alleles varied between 4 for markers *mPgCIR13*, *mPgCIR20*, *mPgCIR23*, and 8 for marker *mPgCIR19*, respectively. The average value of effective number of alleles was 3.722, observed heterozygosity was 0.217, and expected heterozygosity was 0.254. The average fixation index was 0.101. Hardy-Weinberg equilibrium tests revealed significant differences in the markers. The F_{IS} coefficient had an average value of 0.385, the F_{IT} coefficient showed an average of 0.490, and the F_{ST} coefficient had a value of 0.178. Genetic distance analysis showed that Mateo Gómez was closely related to Retiro de los Indios, while Rabolargo appeared to be the most distant population. The study revealed low genetic variability within and between the populations studied, possibly, reflecting the type of asexual propagation applied in guava crops.

Keywords: genetic variability; guava; Hardy-Weinberg equilibrium; heterozygosity; SSR

Introduction

Guava (*Psidium guajava* L.; Myrtaceae family), known as the "apple of the poor," is cultivated in tropical and subtropical regions worldwide (Morton, 1987). Guava cultivation has a significant impact on the global economy, with Pakistan accounting for 22%, Brazil for 17%, India for 16%, Mexico for 15%, and Egypt for 12% of the total guava production. In the year 2021, the global production of guava reached 2,075,000 tons per year (Ministry of Agriculture, 2021).

In Colombia, guava cultivation has expanded to cover 19,277 hectares, resulting in a total production of 165,543 tons of fruits. This makes the guava sector one of the most significant contributors to the country's agricultural development. The productivity of guava cultivation has increased by 15% in the departments of

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Santander, Boyacá, and Atlántico, which together account for 70% of the national production (Ministry of Agriculture, 2021).

Guava is gaining increasing attention worldwide due to its extraordinary nutritional and health benefits, as well as its easy availability and relatively affordable prices compared to other fruits (Rajan and Hudedamani, 2019). Therefore, the lack of information about the diversity of guava genetic resources poses a significant vulnerability to the agricultural system. Additionally, it reduces the genetic base of cultivars, leading to decreased productivity and survival of the crop. This could result in inbreeding processes, loss of genetic variation, and, in the long term, an increased rate of population extinction (Robitzsch *et al.*, 2023).

Currently, the implementation of molecular techniques has been used to obtain specific genetic markers for each plant species, identify polymorphic differences, and provide essential information for species identification. In this context, molecular markers such as microsatellites have become a powerful tool for various applications (Vázquez *et al.*, 2012). Microsatellites offer several advantages, including codominance, multiallelism, and high heterozygosity. They also require a minimal amount of DNA for analysis and can precisely discriminate between closely related individuals due to their high polymorphic nature (González, 2003). These characteristics make microsatellites valuable for genetic studies, diversity assessments, and population genetics research in guava and other plant species.

Microsatellite markers are increasingly being implemented to assess the molecular-level genetic variability of cultivars and lineages. They find applications in plant breeding, marker-assisted selection, genetic map validation, as well as studies of genetic diversity and structure (Bandera and Pérez, 2015). The polymorphism of microsatellites is based on the variation in the number of tandem repeats of alleles at a locus. These markers have proven to be highly valuable for conducting diversity studies and identifying accessions within the crop (Kumar *et al.*, 2023; Ma *et al.*, 2020).

Their ability to reveal unique genetic profiles and differences between individuals or populations has made them essential tools in the field of molecular genetics and breeding. By analysing the variation in microsatellites, researchers can gain insights into the genetic diversity within guava populations and better understand the relationships between different cultivars and varieties. This information is crucial for conservation efforts, germplasm management, and the development of improved guava varieties through selective breeding.

In Córdoba, and in Colombia as a whole, there is limited knowledge regarding the genetic diversity and population structure of guava cultivars and populations. Therefore, this study aims to provide valuable insights into the genetic makeup and relationships among the guava accessions in the region. By analysing these microsatellite markers, researchers can assess the level of genetic variability and identify distinct genetic profiles within the guava population. Understanding the genetic diversity of guava in Cereté can help inform conservation strategies, enhance breeding programs, and contribute to the sustainable management of this important fruit crop in Colombia.

Materials and Methods

Study area

The plant material was collected in the municipality of Cereté, Córdoba (08°55'5.5" North Latitude, 75°48'7.2" West Longitude) with an average temperature of 26 °C. Sampling was conducted in the following districts: Mateo Gómez, Rabolargo, Retiro de los Indios, Manguelito, and Severá (Table 1).

Table 1. Coordinates of the study areas and number of individuals sampled in the municipality of Cereté, Córdoba

Study zone	Sampled individuals	Latitude	Longitude
Mateo Gómez	11	8° 50' 39.1" N	75° 49' 51.5" W
Rabolargo	10	8° 57' 18.6" N	75° 44' 28.8" W
Retiro de los Indios	12	8° 51' 45.8" N	75° 48' 35.6" W
Manguelito	10	8° 55' 17.1" N	75° 47' 20.3" W
Severá	12	8° 53' 32.5" N	75° 52' 53.1" W

Sample collection

Young leaves from 55 guava individuals (*Psidium guajava* L.) were collected. The collected material was placed in resealable bags containing silica gel, appropriately labeled, and stored at room temperature. The samples were transported to the Genetics Laboratory at the University of Córdoba, Colombia.

DNA extraction

For DNA extraction, the Mini-prep method with modifications was used following the protocol below: each sample was macerated with liquid nitrogen, and then 25 mg of the macerated material was transferred to a 2 ml reaction tube. To this tube, 450 μ L of CTAB buffer and 450 μ L of water were added, followed by 100 μ L of 10% PVP (polyvinylpyrrolidone) and 20 μ L of β -mercaptoethanol. The mixture was then heated in a water bath at 65 °C for 1 hour with agitation every 15 minutes. After this time, 900 μ L of chloroform-isoamyl alcohol (24:1) was added, and the mixture was inverted and agitated for 5 minutes. Subsequently, it was centrifuged for 5 minutes at 7500 g, and the supernatant was transferred to a 1.5 ml Eppendorf tube. Then, 90 μ L of 0.3M sodium acetate and 500 μ L of absolute ethanol were added, and the solution was cooled at -20 °C for one hour. After this time, it was centrifuged at 13000 RPM for 10 minutes, the supernatant was discarded, and 600 μ L of 70% ethanol was added. Another centrifugation step was performed for 3 minutes at 13000 RPM, repeating the previous step. Afterwards, the eluted DNA samples underwent an RNase treatment. The DNA's purity and concentration were assessed using both 2.5% (w/v) agarose gels and the NanoDrop® ND-1000 Spectrophotometer. Finally, 100 μ L of rehydration solution was added, and the mixture was heated at 65 °C for 30 minutes in a water bath and stored at -20 °C for later use.

Polymerase Chain Reaction

The microsatellite markers (Table 2) were amplified using PCR end point. The reaction mixture had a final volume of 25 μ L, which included 12.5 μ L of Taq polymerase enzyme, 0.75 μ L of each forward and reverse primer, 4 μ L of genomic DNA, and 7 μ L of sterile water to reach the total volume. The PCR reaction (Table 3) was performed in a Bio-Rad T100™ Thermal Cycler (BIO-RAD, Hercules, USA) using the Touchdown PCR technique, which consisted of an initial denaturation phase at 95 °C for 3 minutes, followed by 35 cycles distributed as follows: denaturation at 95 °C for 30 seconds, two annealing cycles for each temperature ranging from 60 °C to 56 °C for 30 seconds, and 23 additional annealing cycles at 56 °C for 30 seconds. Extension of the DNA strands was carried out for 1 minute in all cycles. Finally, there was a final extension step at 72 °C for 5 minutes, followed by a hold time at 6 °C. The PCR products were analysed, and the polymorphisms were determined using the CEQ™ 8800 XL Capillary Genetic Analysis System from Beckman Coulter, Fullerton, CA. The analyses were repeated at least two times to ensure the reproducibility of the results.

Table 2. Characteristics of the evaluated SSR markers (Risterucci *et al.*, 2005)

Locus	Sequence	Annealing temperature (°C)	Range in bp
<i>mPgCIR 7</i>	F: ATGGAGGTAGGTTGATG R: CGTAGTAATCGAAGAAATG	55	148–160
<i>mPgCIR 9</i>	F: GCGTGTCGTATTGTTTC R: ATTTTCTTCTGCCTTGTC	55	156–176
<i>mPgCIR 11</i>	F: TGAAAGACAACAAACGAG R: TTACACCCACCTAAATAAGA	55	298–314
<i>mPgCIR 13</i>	F: CCTTTTTCCCGACCATTACA R: TCGCACTGAGATTTTGCT	55	240–260
<i>mPgCIR 16</i>	F: AATACCAGCAACACCAA R: CATCCGTCTCTAAACCTC	55	268–296
<i>mPgCIR 19</i>	F: AAAATCCTGAAGACGAAC R: TATCAGAGGCTTGCATTA	55	258–280
<i>mPgCIR 20</i>	F: TATACCACACGCTGAAAC R: TTCCCCATAAACATCTCT	55	270–298
<i>mPgCIR 22</i>	F: CATAAGGACATTTGAGGAA R: AATAAGAAAGCGAGCAGA	55	236–252
<i>mPgCIR 23</i>	F: GTCTATACCTAATGCTCTGG R: CCCAGGAAAATCTATCAC	55	184–198

Table 3. PCR cycles performed for the amplification of the 7 molecular markers used

Temperature (°C)	Time	Number of cycles
95°	3 min	1
60°-56°	30 seg	10
56°	30 seg	23
72°	1 min	
72°	5 min	1

Data analysis

The estimation of allele frequencies, as well as measures of genetic diversity, including expected heterozygosity (H_e), observed heterozygosity (H_o), fixation index (F), genetic differentiation coefficient (G_{ST}), gene flow (Nm), Hardy-Weinberg equilibrium, and genetic distance between populations were determined using the PopGene 1.31 software (Yeh *et al.*, 1999). The genetic structure of the populations was calculated using the FSTAT v. 2.9.3.2 program (Goudet, 2002). The polymorphic information content (PIC) of each microsatellite was determined using the CERVUS v. 3.021 software (Kalinowski *et al.*, 2021). The dendrogram representing the estimated genetic distance values was constructed using the Neighbor-Joining method with the MEGA 11.0.13 program (Tamura *et al.*, 2021). Molecular analysis of variance (AMOVA) was determined using GenAIEx 6.503 software (Peakall and Smouse, 2012). The number of population groups and the delta K value were determined using the software STRUCTURE 2.3.4 (Pritchard *et al.*, 2000).

Results

The allelic frequencies of the markers used to assess genetic variability in guava populations are displayed in Table 4. The highest number of alleles (Table 4 and Table 5) was found for marker *mPgCIR20* with eight alleles, while markers *mPgCIR 7*, *mPgCIR 16*, and *mPgCIR 20* and *mPgCIR 23* exhibited six, five and four

alleles and the marker with the lowest number of alleles was *mPgCIR11* with two and an average number of 4.3 alleles. The highest frequency was allele 298 of marker *mPgCIR11*, with a frequency of 0.938 in the Mateo Gómez population. As for the lowest frequencies, they were observed for marker *mPgCIR20* with values ranging between 0.056 and 0.063. Furthermore, marker *mPgCIR11* exhibited fixation of allele 298 in four of the populations.

The genetic parameters of the nine microsatellite markers used to assess genetic diversity in guava populations in Cereté are presented in Table 5. For observed heterozygosity (H_o), the results obtained ranged from 0.152 to 0.310, corresponding to markers *mPgCIR 19* and *mPgCIR 16*, respectively, with an average of 0.217. Expected heterozygosity (H_e) values fluctuated between 0.188 (*mPgCIR 19*) and 0.388 (*mPgCIR 16*), with an average of 0.254. The average number of effective alleles (N_e) was 3.722, with marker *mPgCIR 19* showing the highest effective number of alleles at 4.723. The fixation index (F) presented a variation from 0.363 to -0.192 for markers *mPgCIR 11* and *mPgCIR 13*, respectively (Table 5). The polymorphic information content (PIC) for the population ranged between 0.578 (*mPgCIR 11*) and 0.759 (*mPgCIR 19*), with these values corresponding to markers with the lowest and highest number of alleles. The average PIC was 0.678 (Table 5), and in this study, all markers can be considered highly informative ($PIC > 0.05$). The population showed a departure from Hardy-Weinberg equilibrium ($p < 0.05$) in 7 markers, while the remaining 2 loci did not exhibit significant differences (*mPgCIR 23*, *mPgCIR 16*) (Table 5).

Table 4. Allelic frequencies of microsatellite markers assessed in *P. guajava* L. populations in Cereté. Córdoba

Locus	No. of alleles	Alleles	Mateo Gómez	Rabolargo	Retiro de los Indios	Mangelito	Severá
<i>mPgCIR11</i>	2	298	0.938	1.000	1.000	1.000	1.000
		314	0.063	0.000	0.000	0.000	0.000
<i>mPgCIR07</i>	6	154	0.000	0.000	0.000	0.200	0.000
		157	0.000	0.000	0.000	0.200	0.167
		160	0.222	0.000	0.200	0.500	0.833
		164	0.278	0.000	0.000	0.000	0.000
		167	0.333	1.000	0.800	0.000	0.000
		176	0.167	0.000	0.000	0.100	0.000
<i>mPgCIR 9</i>	4	158	0.187	0.427	0.000	0.551	0.108
		162	0.000	0.202	1.000	0.111	0.090
		168	0.552	0.371	0.000	0.208	0.651
		172	0.261	0.000	0.000	0.130	0.151
<i>mPgCIR19</i>	3	260	0.778	0.714	0.900	1.000	1.000
		270	0.222	0.286	0.000	0.000	0.000
		278	0.000	0.000	0.100	0.000	0.000
<i>mPgCIR20</i>	8	270	0.000	0.786	1.000	0.125	0.000
		280	0.000	0.000	0.000	0.750	0.929
		284	0.889	0.143	0.000	0.000	0.000
		290	0.000	0.071	0.000	0.000	0.000
		297	0.000	0.000	0.000	0.063	0.000
		300	0.056	0.000	0.000	0.063	0.000
		302	0.056	0.000	0.000	0.000	0.000
305	0.000	0.000	0.000	0.000	0.071		
<i>mPgCIR 22</i>	3	240	0.000	0.108	0.395	0.000	0.688
		244	0.000	0.474	0.605	0.531	0.000
		248	1.000	0.418	0.000	0.469	0.312
<i>mPgCIR23</i>	4	185	0.000	0.000	0.800	0.200	1.000
		188	1.000	0.929	0.200	0.700	0.000
		199	0.000	0.071	0.000	0.000	0.000
		201	0.000	0.000	0.000	0.100	0.000
<i>mPgCIR13</i>	4	240	1.000	0.643	0.200	0.857	1.000
		242	0.000	0.286	0.000	0.000	0.000
		245	0.000	0.000	0.800	0.143	0.000
		255	0.000	0.071	0.000	0.000	0.000

<i>mPgCIR16</i>	5	270	0.214	0.000	0.584	0.301	0.263
		272	0.000	0.000	0.201	0.124	0.301
		276	0.325	1.000	0.000	0.403	0.278
		282	0.114	0.000	0.111	0.172	0.000
		286	0.347	0.000	0.104	0.000	0.158

Table 5. Genetic parameters calculated in the population of *Psidium guajava* L. from Cereté

Locus	Na	Ne	Ho	He	F	PIC	HW
<i>mPgCIR 23</i>	4	3.898	0.214	0.243	0.119	0.701	0.278 ns
<i>mPgCIR 19</i>	3	2.723	0.152	0.188	0.191	0.615	0.000***
<i>mPgCIR 16</i>	5	3.717	0.310	0.388	0.201	0.757	0.213 ns
<i>mPgCIR 20</i>	8	5.095	0.262	0.277	0.054	0.759	0.249
<i>mPgCIR 22</i>	3	1.657	0.167	0.227	0.264	0.691	0.000***
<i>mPgCIR 13</i>	4	3.119	0.292	0.245	-0.192	0.701	0.000***
<i>mPgCIR 11</i>	2	1.420	0.156	0.245	0.363	0.578	0.004**
<i>mPgCIR 7</i>	6	4.485	0.185	0.213	0.131	0.669	0.007**
<i>mPgCIR 9</i>	4	3.181	0.240	0.286	0.161	0.629	0.000***
Average	4.3	3.252	0.217	0.254	0.129	0.678	

Na: Number of alleles; Ne: Effective alleles; Ho: Observed heterozygosity; He: Expected heterozygosity; F: Fixation index; PIC: Polymorphic Information Content; H-W: Hardy-Weinberg equilibrium <<* P<0.05. Ns: not significant>>

The F statistics obtained are shown in Table 6, where the values of F_{IS} range from -0.192 for marker *mPgCIR 13* to 0.363 for marker *mPgCIR 11*, with an average value of 0.144. As for the F_{IT} values, it obtained an average of 0.490, with values ranging from 0.321 for marker *mPgCIR 9* to 0.613 for marker *mPgCIR 16*. Regarding the F_{ST} statistic, it had an average value of 0.404, with values ranging from 0.191 for marker *mPgCIR 9* to 0.603 for marker *mPgCIR 13* (Table 6). The gene flow values ranged from 1.2 to 6.6 for markers *mPgCIR 11* and *mPgCIR 20*, respectively, with an average Nm value of 3.8.

Table 6. F statistics and G_{ST} for each locus in the population of *Psidium guajava* L. in Cereté. Córdoba

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
<i>mPgCIR 23</i>	0.119	0.572	0.514	3.8
<i>mPgCIR 19</i>	0.191	0.454	0.325	5.6
<i>mPgCIR 16</i>	0.201	0.613	0.516	4.5
<i>mPgCIR 20</i>	0.054	0.328	0.290	6.6
<i>mPgCIR 22</i>	0.264	0.606	0.464	5.3
<i>mPgCIR 13</i>	-0.192	0.527	0.603	4.1
<i>mPgCIR 11</i>	0.363	0.493	0.204	1.2
<i>mPgCIR 7</i>	0.131	0.328	0.226	1.5
<i>mPgCIR 9</i>	0.161	0.321	0.191	2.0
Average	0.144	0.490	0.404	3.8

F_{IS} : Fixation index of an individual within a subpopulation. F_{IT} : Fixation index of an individual within the total population. F_{ST} : Fixation index of a subpopulation within the total population. Nm: gene flow.

Table 7 displays the values of genetic distance (Nei, 1972) between pairs of populations, and the Neighbor-Joining tree obtained from Nei's genetic distance (1972) for the different populations. The results revealed that Mateo Gómez is more closely related to Retiro de los Indios, while Rabolargo appeared as the most distant population (Figure 1).

Table 7. Genetic Distance Matrix Nei's (1972) for pairwise populations

	Severá	Mangelito	Rabolargo	Mateo Gómez	Retiro de los Indios
Severá	---				
Mangelito	0,174	---			
Rabolargo	0,512	0,207	---		
Mateo Gómez	0,180	0,273	0,374	---	
Retiro de los Indios	0,418	0,568	0,399	0,095	---

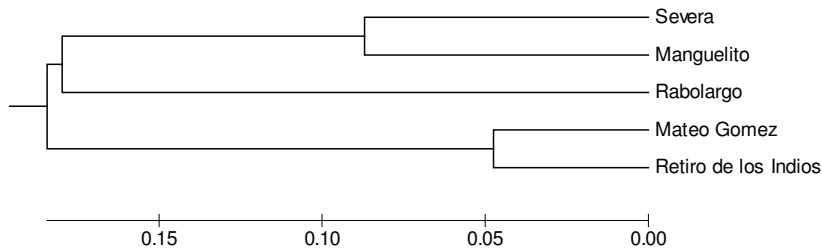


Figure 1. UPGMA Dendrogram constructed from the genetic distances (Nei, 1972) of guava (*Psidium guajava* L.) populations from Cereté, Córdoba

Regarding population structure, Delta K values are shown in Figure 2. Indicating the likelihood of different K (genetics groups), being K=2 the value with higher likelihood.

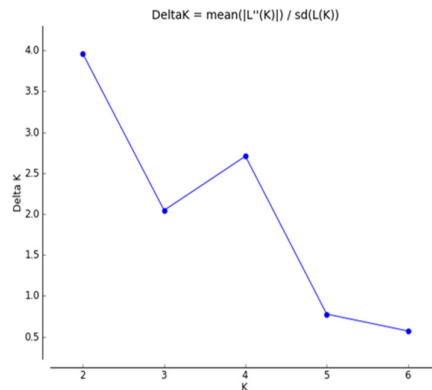


Figure 2. Estimating population structure in the population Cereté using the modal value Δk

Regarding genetic similarity two groups were evidently generated: Severá, Mangelito and Rabolargo populations correspond to the first group as shown in Figure 3, whereas Mateo Gómez and Retiro de los Indios populations correspond to the second genetic group. K, the number of genetic groups represented on each column by colors red and green (Figure 3).

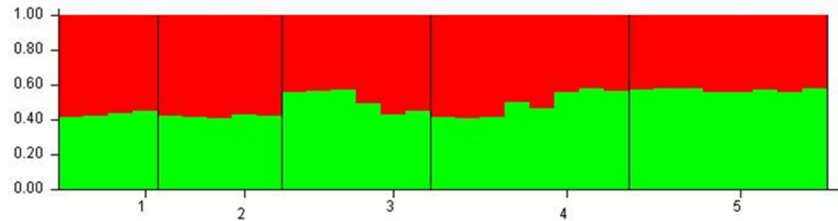


Figure 3. Genetic structure from 5 population de guava from Cereté: (1: Severá, 2: Manguelito, 3: Rabolargo, 4: Mateo Gómez, 5: Retiro de los Indios), based on a Bayesian analysis, considering $K = 2$, obtained by the ΔK method, from 20 independent simulations for each number of possible clusters (k).

Discussion

In the studied populations, the utilized markers showed an average of 4 alleles per locus, which were lower than those reported by De Oliveira *et al.* (2022), Chiveu (2018), Mehmood *et al.* (2016) and higher than those reported by Kumar *et al.* (2023), Oliveira *et al.* (2022), Kumar *et al.* (2020), Kherwar *et al.* (2018), and similar to that reported by Chaithanya *et al.*, 2014. The total number of guava alleles found was 39, a result similar to that obtained by Kumar *et al.* (2023), which could be attributed to Wahlund effect (Martín, 2017), the self-pollinating behavior of the species due to possessing perfect flowers, which aids in self-fertilization (Kumari *et al.*, 2018), and to the extent of the populations used in each of the studies (Díaz *et al.*, 2022). Primers *mPgCIR11*, *mPgCIR19*, and *mPgCIR22* exhibited low diversity indices and, at the same time, had a lower number of alleles per locus. Furthermore, it is important to note that marker *mPgCIR11* was fixed in four out of the five populations, suggesting that these loci may be closely linked to a favourable trait. The average value of the Effective Number of Alleles was 3.2, lower than the reported by Sitther *et al.* (2014) and higher than the obtained by Tapia and Legaria, 2007.

The value of H_o ranged from 0.152 to 0.292, with an average value of 0.217, which was higher than that reported by Espín (2018), Kumari *et al.* (2018), Sitther *et al.* (2014), and Viji *et al.* (2010), and lower than that reported by De Oliveira *et al.*, 2022. The results revealed higher values of expected heterozygosity (H_e) compared to observed heterozygosity (H_o), indicating a high number of homozygous individuals, which could be attributed to the species' reproductive behavior, suggesting the presence of autogamy processes limiting the dispersion of genetic variability through pollen (Díaz-Cruz, 2016). The average expected heterozygosity (H_e) value was 0.254, indicating low genetic variability in the population (Nei, 1978). This value was higher than that reported by Viji *et al.* (2010), lower than that recorded by Kanupriya *et al.*, 2011, and similar to the values obtained by Naga *et al.* (2015).

The average number of effective alleles (N_e) was 3.2, which was lower than that obtained by Mehmood *et al.* (2016), higher than that reported by Kumar *et al.* (2023), Oliveira *et al.* (2022) and similar to that obtained by Sitther *et al.* (2014).

According to Botstein *et al.* (1980), all nine markers used can be considered highly informative and suitable ($PIC > 0.5$) for determining the genetic variability of the guava population in Cereté. The polymorphic information content (PIC) ranged from 0.759 for the locus *mPgCIR19* to 0.578 for marker *mPgCIR11*, with an average PIC value of 0.608, which was higher than that reported by Kumar *et al.* (2020), and Kherwar *et al.* (2018), and lower than that recorded by Kanupriya *et al.*, 2011, and similar to that reported by Mehmood *et al.* (2016).

Regarding the fixation index of 0.129, the positive values found indicate a high number of homozygous individuals, which could be attributed to the species' high rate of self-pollination, reaching up to 75% (Díaz-

Cruz, 2016). This limits the dispersion of genetic variability through pollen among plant populations in *Psidium guajava* L., increasing the likelihood of allele loss for future generations (Ellegren and Galtier, 2016).

Two of the markers did not show significant differences (NS) with respect to Hardy-Weinberg equilibrium, while the remaining seven showed deviations ($p < 0.05$), which could be attributed to an excess of homozygous individuals (Allendorf and Luikart, 2007) and may be related to processes of inbreeding and conventional techniques of asexual propagation applied in guava, which significantly reduce the heterozygous nature of the species (Rai *et al.*, 2010). Additionally, a continuous process in nature, where certain environmental factors determine which traits or variations within a population are more successful, can also lead to a deviation from the Hardy-Weinberg equilibrium (Postma and Van Noordwijk, 2005). Furthermore, this lack of Hardy-Weinberg equilibrium may be due to the action of genetic drift, which operates more strongly in small populations, such as those of guava in Cereté (Martin *et al.*, 2023).

Regarding the Wright's F statistics, the F_{IS} coefficient showed an average value of 0.144, indicating a moderate level of homozygosity in the analysed populations, which could be associated with a high degree of inbreeding, suggesting a limited genetic variation within the population. As for the F_{IT} statistic, with an average value of 0.490, it also reveals a high percentage of homozygosity with respect to the total population. On the other hand, the F_{ST} value for the subpopulations exhibited an average of 0.304, expressing a moderate level of genetic differentiation among the examined populations. Furthermore, these values are similar to those reported by De Oliveira *et al.* (2022). The value obtained for the genetic flow (Nm) indicates the existence of a movement of more than three migrant individuals per generation between populations, which could explain the limited differentiation between them.

The close relationship between the studied populations, as revealed by the dendrogram obtained, is likely attributed to their geographical proximity, continuous migratory flows, or agricultural exchange in the area.

The Bayesian clustering analysis based on genetic similarity reveals that it is highly probable that all individuals from the five populations share two genetic clusters, indicating extensive genetic ex-change among these population samples.

Conclusions

The results of the study on the guava (*Psidium guajava* L.) population in the municipality of Cereté, using microsatellite markers, revealed low genetic variability, which may be associated with processes of inbreeding and conventional techniques of asexual propagation applied in guava crops. The microsatellites used were highly informative, making them suitable for determining the genetic diversity among guava accessions.

Authors' Contributions

Conceptualization, E.P.; Investigation; methodology, E.P. and T.C.; statistical analysis, K.H.; writing—original draft preparation, Software E.P. and K.H.; writing—review and editing, E.P., K.H. and T.C. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Allendorf F, Luikart G (2007). Conservation and the Genetics of Populations. Blackwell Publishing, Malden, Massachusetts, USA.
- Bandera E, Pérez L (2015). Mejoramiento genético de guayabo (*Psidium guajava* L.). Cultivos Tropicales 36:96-110
- Botstein D, White R, Skolnick M, Davis R (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314-331
- Chaithanya MN, Dinesh MR, Vasugi C, Reddy DL, Sailaja D, Aswath C (2014). Assessment of genetic diversity in guava (*Psidium guajava*) germplasm using microsatellites. Journal of Horticultural Sciences 9(2):117-125. <https://doi.org/10.24154/jhs.v9i2.180>
- Chiveu J (2018). Assessment of genetic and nutritional diversity, and salinity tolerance of Kenyan guava (*Psidium guajava* L.): an underutilized naturalized fruit species. PhD Thesis. University of Göttingen, Germany.
- De Oliveira Bernardes C, Tuler AC, Canal D, Carvalho MS, Ferreira A, Da Silva Ferreira MF (2022). Genetic Diversity and Population Structure of *Psidium* Species from Restinga: A Coastal and Disturbed Ecosystem of the Brazilian Atlantic Forest. Biochemical Genetics 60(6):2503-2514 <https://doi.org/10.1007/s10528-022-10222-7>
- Díaz-Cruz JA (2016). Diversidade e estrutura genética de populações de *Psidium guajava* L. (Myrtaceae) oriundas do Brasil e do México. MSc Dissertation. Universidad Estadual de Ponta Grossa. Paraná, Brazil.
- Ellegren H, Galtier N (2016). Determinants of genetic diversity. Nature Reviews Genetics 17:427-433. <https://doi.org/10.1038/nrg.2016.58>
- Espín A (2018). Diversidad genética de la guayaba (*Psidium guajava*) en la Isla Isabela. MSc Dissertation. Universidad San Francisco de Quito. Ecuador.
- González E (2003). Microsatélites: sus aplicaciones en la conservación de la biodiversidad. Graellsia 59:377-388.7. <https://doi.org/10.3989/graellsia.2003.v59.i2-3.253>
- Goudet J (2002). FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. Journal of Heredity 86:485-486
- Kalinowski ST, Taper ML, Marshall TC (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1106. <https://doi.org/10.1111/j.1365-294X.2007.03089.x>
- Kanupriya PM, Aswath C, Reddy L, Padmakar B, Vasugi C, Dinesh MR (2011). Cultivar identification and genetic fingerprinting of guava (*Psidium guajava*) using microsatellite markers. International Journal of Fruit Science 11(2):184-196. <https://doi.org/10.1080/15538362.2011.578521>
- Kherwar D, Usha K, Amitha SV, Singh B (2018). Microsatellite (SSR) marker assisted assessment of population structure and genetic diversity for morpho-physiological traits in guava (*Psidium guajava* L.). Journal of Plant Biochemistry and Biotechnology 27:284-292. <https://doi.org/10.1007/s13562-017-0438-2>
- Kumar C, Kumar R, Singh SK, Goswami AK, Nagaraja A, Paliwal R, Singh R (2020). Development of novel g-SSR markers in guava (*Psidium guajava* L.) cv. Allahabad Safeda and their application in genetic diversity, population structure and cross species transferability studies. PLoS One 15(8):e0237538. <https://doi.org/10.1371/journal.pone.0237538>

- Kumar S, Singh A, Yadav A, Bajpai A, Singh NK, Rajan S, Mala T, Muthukumar M (2023). Identification and validation of novel genomic SSR markers for molecular characterization of guava (*Psidium guajava* L.). South African Journal of Botany 155:79-89. <https://doi.org/10.1016/j.sajb.2023.02.005>
- Kumari S, Arumugam N, Singh R, Srivastav M, Banoth S, Mithra AC, Arun M, Kumar G, Khan Y (2018). Diversity analysis of guava (*Psidium guajava*) germplasm collection. Indian Journal of Agricultural Sciences 88:489-497.
- Martin CA, Sheppard EC, Illera JC, Suh A, Nadachowska-Brzyska K, Spurgin LG, Richardson DS. (2023). Runs of homozygosity reveal past bottlenecks and contemporary inbreeding across diverging populations of an island-colonizing bird. Molecular Ecology 32(8):1972-1989. <https://doi.org/10.1111/mec.16865>
- Martín D. (2017). Somatic embryogenesis: a biotechnological tool for the in vitro propagation of guava. Biocología Vegetal 17(4):209-220.
- Ma Z, Liu S, Liang Z, Xu S, Hu W (2020). Analysis of genetic diversity of 45 guava germplasm evaluated using SSR markers. International Journal of Fruit Science 20(3):385-393. <https://doi.org/10.1080/15538362.2019.1640168>
- Mehmood A, Luo S, Ahmad NM, Dong C, Mahmood T, Sajjad Y, Jaskani MJ, Sharp P (2016). Molecular variability and phylogenetic relationships of guava (*Psidium guajava* L.) cultivars using inter-primer binding site (iPBS) and microsatellite (SSR) markers. Genetic Resources and Crop Evolution 63:1345-1361. <https://doi.org/10.1007/s10722-015-0322-7>
- Ministry of Agriculture and Rural Development (2021). Cadena de la guayaba. Retrieved 2023 February 22 from: <https://sioc.minagricultura.gov.co/Guayaba/Documentos/2021-03-31%20Cifras%20Sectoriales.pdf>.
- Morton JF (1987). Guava (*Psidium guajava* L.). In: Julia F (Ed). Morton Publisher. Fruits of Warm Climates. Miami, FL, USA pp 356-363.
- Naga MV, Sailaja D, Dinesh MR, Vasugi C, Lakshmana DC, Aswath C. (2015). Microsatellite-based DNA fingerprinting of guava (*Psidium guajava*) genotypes. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 87:859-867. <https://doi.org/10.1007/s40011-015-0660-4>
- Nei, M. (1972). Genetic distance between populations. American Naturalist 106:283-292. <https://doi.org/10.1086/282771>
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-90. <https://doi.org/10.1093/genetics/89.3.583>
- Oliveira, JAVS, Santos EA, Viana AP, Walter FHB, Ribeiro RM (2022). Genetic molecular characterization in guava full-sib progeny. Bragantia 81:e3322. <https://doi.org/10.1590/1678-4499.20210267>
- Postma E, AJ Van Noordwijk (2005). Genetic variation for clutch size in natural populations of birds from a reaction norm perspective. Ecology 86(9):2344-2357.
- Pritchard JK, Stephens M, Donnelly P (2000). Inference of population structure using multilocus genotype data. Genetics 155(2):945-959. <https://doi.org/10.1093/genetics/155.2.945>
- Rai MK, Asthana P, Jaiswal VS, Jaiswal U (2010). Biotechnological advances in guava (*Psidium guajava* L.): recent developments and prospects for further research. Trees 24:1-12. <https://doi.org/10.1007/s00468-009-0384-2>
- Rajan S, Hudedamani U (2019). Genetic Resources of Guava: Importance, Uses and Prospects. In: Rajasekharan P, Rao V (Eds). Conservation and Utilization of Horticultural Genetic Resources. Springer, Singapore. https://doi.org/10.1007/978-981-13-3669-0_11
- Risterucci AM, Duval MF, Rohde W, Billotte N (2005). Isolation and characterization of microsatellite loci from *Psidium guajava* L. Molecular Ecology Notes 5:745-748. <https://doi.org/10.1111/j.1471-8286.2005.01050.x>
- Robitzch V, Saenz-Agudelo P, Alpermann TJ, Frédéric B, Berumen ML (2023). Contrasting genetic diversity and structure between endemic and widespread damselfishes are related to differing adaptive strategies. Journal of Biogeography 50(2):380-392. <https://doi.org/10.1111/jbi.14540>
- Sitther V, Zhang D, Harris DL, Yadav AK, Zee FT, Meinhardt LW, Dhekney S (2014). Genetic characterization of guava (*Psidium guajava* L.) germplasm in the United States using microsatellite markers. Genetic Resources and Crop Evolution 61:829-839. <https://doi.org/10.1007/s10722-014-0078-5>
- Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology Evolution 38:3022-3027. <https://doi.org/10.1093/molbev/msab120>
- Tapia D, Legaria J.P. (2007). Variabilidad genética en cultivares de guayabo (*Psidium guajava* L.). Revista Fitotecnia Mexicana 30(4):391-401. <https://doi.org/10.35196/rfm.2007.4.391>

- Vázquez A, Molina F., J Farfán, Figueroa M 2012. Potencial de los marcadores moleculares para el rescate de individuos de *Theobroma cacao* L. de alta calidad. Biotecnología 16:36-56.
- Viji G, Harris DL, Yadav AK (2010). Use of Microsatellite Markers to Characterize genetic diversity of selected accessions of guava (*Psidium guajava*) in the United States. Acta Horticulturae 859:169-176. <https://doi.org/10.17660/ActaHortic.2010.859.20>
- Yeh FC, Yang RC, Mao J, Ye Z, Boyle TJB (1999). POPGENE, the Microsoft Windows-based user-friendly software for population genetic analysis of co-dominant and dominant markers and quantitative traits. Department of Renewable Resources, University of Alberta, Edmonton, Alta.



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