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Article

Assessment of Diversity and Genetic Variability of Guava (*Psidium guajava*) by Means of Microsatellite Markers in Montería, Córdoba-Colombia

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Abstract: Guava (*Psidium guajava* L.) is a species is widely distributed in Córdoba, despite this, there are few studies that have been carried out to understand its genetic diversity. For this reason, the objective of this research was to evaluate the genetic diversity and variability of guava (*Psidium guajava* L.) using microsatellite markers in Montería, Córdoba-Colombia. Forty-five accessions were evaluated. For DNA extraction, the CTAB 2x extraction protocol was used with modifications. The extracted DNA was amplified by the PCR technique using seven microsatellite markers. A total of 46 alleles were found with an average of 6.5 alleles per locus. A total of 28 private alleles were identified in the populations, possibly fixed by different evolutionary forces. The evaluated population presented an average expected heterozygosity of 0.572 and the observed heterozygosity was lower (0.058). The fixation index presented a total average of 0.906, indicating that in Montería there is an excess of homozygotes. The PIC values allowed us to determine that the markers mPgCIR9, mPgCIR11, mPgCIR13, mPgCIR16, mPgCIR19 and mPgCIR22 are highly informative, while mPgCIR23 is moderately informative. The guava population in Montería presented a low genetic diversity within the subpopulations, which could be related to inbreeding events such as self-pollination..

Keywords: genetic diversity; genetic variability; microsatellites; allele frequencies; population structure

1. Introduction

Guava (*Psidium guajava* L.) is a fruit tree belonging to the Myrtaceae family. It is native to the American continent, ranging from Mexico to Peru, where it can be found in the wild. However, it is currently cultivated in tropical and subtropical countries around the world [1].

Optimal conditions for the growth of these trees include soils with varying textures and drainage capabilities, pH ranges between 4.5 to 9.4, areas with high precipitation, and the ability to withstand drought conditions [2].

P. guajava possesses excellent nutritional and medicinal properties that are essential for health. It has been used as an antimicrobial, antigenotoxic, anti-inflammatory, antiallergic, antimalarial, hepatoprotective, and for the treatment of diarrhea and diabetes [3]. Likewise, its consumption has been associated with the reduction of cholesterol, triglycerides, and blood pressure [1].

In addition to its nutritional and medicinal properties, the cultivation of this species generates a socio-economic impact that benefits many families. They market this fruit in various forms, from fresh consumption to the production of jams, juices, snacks, among others, taking advantage of its well-established market in many countries. This is due to its hardiness, proliferation, high vitamin content, and significant economic [4].

P. guajava has been cultivated and utilized as a significant fruit in many parts of the world, particularly in tropical areas such as India, Indonesia, Pakistan, Bangladesh, and South America [5]. Currently, there is a wide range of cultivated varieties, and in Colombia specifically, three of these are present: common, pear, and apple [6], making it one of the most important fruit crops in the country.

In 2021, the departments of Santander, Meta, and Valle del Cauca were the main producers of this crop in Colombia. As for the department of Córdoba, the cultivation of this species for the same year covered an area of 297 hectares, with a production of 6,816 tons and a yield of 22.95 tons/ha [6].

To ensure high guava crop production in the department of Córdoba, it is necessary to determine the variability in the gene composition expressed in individuals of this species. Molecular tools are used for this purpose, with microsatellite markers being prominent among them. These markers are repetitive DNA sequences of 1 to 6 base pairs and are widely used to understand how living organisms change over time [7].

In Córdoba, and in Colombia in general, there is a lack of information regarding the genetic diversity and population structure of guava cultivars and populations. Therefore, the purpose of this study was to analyze the genetic diversity of 45 guava samples from Montería, Córdoba, using 7 microsatellite markers.

2. Materials and Methods

2.1. Area of study:

The plant material was collected in the municipality of Montería-, Córdoba (08°45'0.5" North Latitude, 75°52'59.2" West Longitude) which has an area of 3043.00 km², is characterized by having a warm-tropical climate, with a dry season and a rainy season throughout the year and has an average temperature of 28°C. Sampling was conducted in the following districts: Maracayo, Patio Bonito y Santa Lucia (Table 1).

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

Study zone	Sampled individuals	Latitude	Longitude	Altitude
Maracayo	15	8° 24' 34" N	75° 53' 27" W	35 mamsl
Patio Bonito	15	8° 47' 37" N	75° 56' 4" W	16 mamsl
Santa Lucia	15	8° 37' 25" N	75° 52' 15" W	17 mamsl

2.2. Sample collection

Forty-five accessions of *P. guajava* were collected from the three sampling zones, with 15 accessions from each population. These samples were placed in resealable bags with silica gel, properly labeled, and kept at room temperature during transportation to the Genetics Laboratory at the University of Córdoba, Colombia.

2.3. DNA Extraction

For the DNA extraction from the field-collected samples, the CTAB 2x extraction protocol with modifications proposed by Pardo et al., (2021) was employed. Briefly, leaf fragments of *P. guajava* were macerated in liquid nitrogen. Subsequently, 45-50 mg of the macerated material was placed in a reaction tube and 900 µL of extraction buffer was added. To each sample, 100 µL of 10% PVP and 20 µL of beta-mercaptoethanol were added. The mixture was then incubated in a water bath at 65°C for 60 minutes with intermittent vortexing every 10 minutes. After the incubation period, it was removed from the water bath and allowed to rest for 5 minutes. Next, 900 µL of chloroform-isoamyl alcohol solution (24:1) was added, and the mixture was gently inverted for 10 minutes and then centrifuged at 7000g for 5 minutes. The supernatant was transferred to a 1.5 ml reaction tube, and 120 µL of 3M sodium acetate and 500 µL of absolute ethanol were added. The solution was vortexed, cooled at -20°C for 1 hour, and then centrifuged at 13,000 RPM for 10 minutes, with the supernatant being discarded. Following this, 600 µL of 70% ethanol was added to clean the pellet. The tubes were

centrifuged again at 13,000 RPM for 3 minutes, the supernatant was discarded, and the tubes were left to air-dry for 30 minutes inverted on a paper towel. The purity and concentration of the DNA were determined using both 2.5% (w/v) agarose gels and the NanoDrop® ND-1000 spectrophotometer. Finally, a rehydrating solution was added, and the extracted DNA was stored at 4°C until further use.

2.4. Polymerase Chain Reaction

The microsatellite markers (Table 2) were amplified using the PCR technique.

Table 2. Primers of *Psidium guajava* L [8].

Marker	Primer forward 5' – 3'	Primer reverse 5' – 3'
<i>mPgCIR9</i>	GCGTGTCGTATTGTTTC	ATTTTCTTCTGCCTTGTC
<i>mPgCIR11</i>	TGAAAGACAACAAACGAG	TTACACCCACCTAAATAAGA
<i>mPgCIR13</i>	CCTTTTTCCCGACCATTACA	TCGCACTGAGATTTTGTGCT
<i>mPgCIR16</i>	AATACCAGCAACACCAA	CATCCGTCTCTAAACCTC
<i>mPgCIR19</i>	AAAATCCTGAAGACGAAC	TATCAGAGGCTTGCATTA
<i>mPgCIR22</i>	CATAAGGACATTTGAGGAA	AATAAGAAAGCGAGCAGA
<i>mPgCIR23</i>	GTCTATACCTAATGCTCTGG	CCCAGGAAAATCTATCAC

The reaction mixture had a final volume of 25 µl (Table 3), following the proposed specifications [8].

Table 3. PCR conditions for a volume per individual of 25 µl.

Reagents	Stock concentration*	Individual concentration	Volume x one tube (µl)
DNA	25 ng/ µL	5 ng/ Ml	5
Enzyme	5U/ µL	0.05 U/ Ml	0.25
Primer Forward	10 µM	0.5 Mm	1.25
Primer Reverse	10 µM	0.5 Mm	1.25
dNTPs	10 mM**	0.2 Mm	0.5
Buffer	10 X	1 X	2.5
Water	-----	-----	14.25
Total	-----	-----	25

The PCR reaction (Table 4) was carried out in a Bio-Rad T100™ thermocycler (BIO-RAD, Hercules, USA) using the technique of PCR Touchdown. The PCR results were analyzed, and genetic variations were identified using the CEQ™ 8800 XL Capillary Genetic Analysis System from Beckman Coulter, Fullerton, CA. The analyses were repeated a minimum of two times in order to ensure that the results were consistent and reproducible.

Table 4. PCR cycles performed for the amplification of the 7 molecular markers used.

Temperature (°C)	Time	Number of cycles
95°	3 min	1
61°-55°	30 seg	10
56°	30 seg	23
72°	1 min	
72°	5 min	1

2.5. Data analysis

Genetic-population parameters such as allelic frequencies, Private Alleles, Number of alleles (Na), Effective Number of alleles (Ne), Observed Heterozygosity (Ho), Expected Heterozygosity (He), Fixation Index (F), Hardy-Weinberg Equilibrium (HWE), Wright's F-statistics, and genetic distances were calculated using GenAlEx 6.5 software. Polymorphic Information Content (PIC) was determined with Cervus 3.0.7 software. The construction of the dendrogram based on the results was performed using Mega X software. For the analysis of population genetic structure, Structure 2.3.4 software was used.

3. Results

The allelic frequencies of the 7 microsatellite markers used to assess the genetic diversity of *P. guajava* in Montería can be observed in Table 5. A total of 46 alleles were found in the populations, with an average of 6.5 alleles for each of the analyzed loci. According to these data, it was possible to determine that the markers *mPgCIR9* and *mPgCIR13* had the highest number of alleles, each with 9, while the marker *mPgCIR23* had the lowest number with 3 alleles.

The highest allelic frequencies were observed in allele 237 of marker *mPgCIR19* and allele 203 of marker *mPgCIR23*, with a frequency of 1.000 in the Santa Lucia and Patio Bonito subpopulations, respectively. It is worth noting that these alleles were the only ones fixed in the population.

Table 5. Allelic frequencies of microsatellite markers used to assess genetic diversity in *P. guajava* in Montería-Córdoba.

Locus	N° Allele	Allele	Patio Bonito	Maracayo	Santa Lucia
<i>mPgCIR9</i>	9	161	0.000	0.143	0.000
		163	0.000	0.143	0.000
		166	0.167	0.143	0.000
		168	0.417	0.143	0.125
		170	0.083	0.429	0.625
		173	0.000	0.000	0.250
		181	0.083	0.000	0.000
		186	0.083	0.000	0.000
		189	0.167	0.000	0.000
<i>mPgCIR11</i>	6	275	0.000	0.000	0.500
		277	0.000	0.429	0.250
		280	0.250	0.143	0.000
		283	0.250	0.429	0.250
		286	0.250	0.000	0.000
		295	0.250	0.000	0.000

		224	0.000	0.250	0.000
		227	0.083	0.063	0.000
		229	0.167	0.188	0.000
		232	0.500	0.375	0.167
<i>mPgCIR13</i>	9	235	0.000	0.000	0.111
		236	0.167	0.000	0.444
		239	0.083	0.063	0.000
		241	0.000	0.063	0.222
		244	0.000	0.000	0.056
		240	0.375	0.000	0.000
		246	0.000	0.857	0.000
		254	0.000	0.000	0.800
<i>mPgCIR16</i>	7	256	0.125	0.000	0.000
		270	0.500	0.000	0.000
		274	0.000	0.000	0.200
		281	0.000	0.143	0.000
		237	0.000	0.000	1.000
		250	0.000	0.500	0.000
<i>mPgCIR19</i>	5	254	0.333	0.333	0.000
		257	0.667	0.111	0.000
		269	0.000	0.056	0.000
		229	0.000	0.000	0.500
		233	0.000	0.000	0.375
		238	0.250	0.125	0.000
<i>mPgCIR22</i>	7	241	0.000	0.625	0.000
		252	0.000	0.000	0.125
		254	0.250	0.000	0.000
		259	0.500	0.250	0.000
		203	1.000	0.250	0.143
<i>mPgCIR23</i>	3	206	0.000	0.750	0.714
		210	0.000	0.000	0.143

A total of 28 private alleles were identified in the Montería population, with Santa Lucia having the highest number at 11 alleles, followed by Patio Bonito with 9 and Maracayo with 8 alleles (Table 6).

Table 6. Private Alleles Identified in the *P. guajava* populations in Montería-Córdoba.

Pop	Locus	Allele	Frecuencia
Patio Bonito	<i>mPgCIR9</i>	181	0.083
Patio Bonito	<i>mPgCIR9</i>	186	0.083
Patio Bonito	<i>mPgCIR9</i>	189	0.167
Patio Bonito	<i>mPgCIR11</i>	286	0.250
Patio Bonito	<i>mPgCIR11</i>	295	0.250
Patio Bonito	<i>mPgCIR16</i>	240	0.375

Patio Bonito	<i>mPgCIR16</i>	256	0.125
Patio Bonito	<i>mPgCIR16</i>	270	0.500
Patio Bonito	<i>mPgCIR22</i>	254	0.250
Maracayo	<i>mPgCIR9</i>	161	0.143
Maracayo	<i>mPgCIR9</i>	163	0.143
Maracayo	<i>mPgCIR13</i>	224	0.250
Maracayo	<i>mPgCIR16</i>	246	0.857
Maracayo	<i>mPgCIR16</i>	281	0.143
Maracayo	<i>mPgCIR19</i>	250	0.500
Maracayo	<i>mPgCIR19</i>	269	0.056
Maracayo	<i>mPgCIR22</i>	241	0.625
Santa Lucia	<i>mPgCIR9</i>	173	0.250
Santa Lucia	<i>mPgCIR11</i>	275	0.500
Santa Lucia	<i>mPgCIR13</i>	235	0.111
Santa Lucia	<i>mPgCIR13</i>	244	0.056
Santa Lucia	<i>mPgCIR16</i>	254	0.800
Santa Lucia	<i>mPgCIR16</i>	274	0.200
Santa Lucia	<i>mPgCIR19</i>	237	1.000
Santa Lucia	<i>mPgCIR22</i>	229	0.500
Santa Lucia	<i>mPgCIR22</i>	233	0.375
Santa Lucia	<i>mPgCIR22</i>	252	0.125
Santa Lucia	<i>mPgCIR23</i>	210	0.143

Based on the results of the population genetic parameters (Table 7), an overall average of 3,286 alleles (N_a), 2,482 effective alleles (N_e), 0.058 observed heterozygosity (H_o), 0.572 expected heterozygosity (uH_e), and 0.906 fixation index (F) were determined by marker in each of the subpopulations.

The subpopulation that exhibited the highest values in terms of observed heterozygosity and expected heterozygosity was Patio Bonito, with an average of 0.107 and 0.621, respectively. On the other hand, Santa Lucia was the subpopulation with the lowest values, with an average of 0.016 and 0.501 for the two parameters.

Regarding the fixation index, the highest value was obtained in the Santa Lucia subpopulation, with an average of 0.974, while the Patio Bonito subpopulation had the lowest average value at 0.815.

In relation to Hardy-Weinberg equilibrium (HWE), the Patio Bonito subpopulation was the only one that exhibited markers in equilibrium, with a total of 4 markers.

Table 7. Population genetic parameters assessed in the populations of Montería-Córdoba.

Population	Locus	N_a	N_e	H_o	uH_e	F
	<i>mPgCIR9</i>	6.000	4.000	0.333	0.818	0.556 ^{ns}
	<i>mPgCIR11</i>	4.000	4.000	0.000	0.857	1.000 ^{ns}
Patio	<i>mPgCIR13</i>	5.000	3.130	0.167	0.742	0.755 ^{**}
Bonito	<i>mPgCIR16</i>	3.000	2.462	0.250	0.679	0.579 ^{ns}
	<i>mPgCIR19</i>	2.000	1.800	0.000	0.533	1.000 ^{ns}
	<i>mPgCIR22</i>	3.000	2.667	0.000	0.714	1.000 [*]
	<i>mPgCIR23</i>	1.000	1.000	0.000	0.000	-----

Average		3.429	2.723	0.107	0.621	0.815
	<i>mPgCIR9</i>	5.000	3.769	0.000	0.791	1.000**
	<i>mPgCIR11</i>	3.000	2.579	0.000	0.659	1.000**
	<i>mPgCIR13</i>	6.000	4.000	0.250	0.800	0.667**
Maracayo	<i>mPgCIR16</i>	2.000	1.324	0.000	0.264	1.000**
	<i>mPgCIR19</i>	4.000	2.656	0.111	0.660	0.822**
	<i>mPgCIR22</i>	3.000	2.133	0.000	0.567	1.000**
	<i>mPgCIR23</i>	2.000	1.600	0.000	0.429	1.000*
Average		3.571	2.580	0.052	0.596	0.927
	<i>mPgCIR9</i>	3.000	2.133	0.000	0.567	1.000**
	<i>mPgCIR11</i>	3.000	2.667	0.000	0.714	1.000*
Santa	<i>mPgCIR13</i>	5.000	3.447	0.111	0.752	0.843**
Lucia	<i>mPgCIR16</i>	2.000	1.471	0.000	0.356	1.000*
	<i>mPgCIR19</i>	1.000	1.000	0.000	0.000	-----
	<i>mPgCIR22</i>	3.000	2.462	0.000	0.633	1.000**
	<i>mPgCIR23</i>	3.000	1.815	0.000	0.484	1.000**
Average		2.857	2.142	0.016	0.501	0.974
Total		3.286	2.482	0.058	0.572	0.906

Na: Number of alleles; Ne: Effective number of alleles; Ho: Observed heterozygosity; uHe: Unbiased expected heterozygosity; F: Fixation index; HWE: Hardy-Weinberg Equilibrium (ns: not significant *: P-Value < 0.05 **: P-Value < 0.01).

Considering the results of the Polymorphic Information Content (PIC), it was determined that the marker *mPgCIR22* had the highest value at 0.795, indicating that it is the most informative marker. Conversely, the marker *mPgCIR23* had the lowest value at 0.443 (Table 8).

Table 8. Polymorphic Information Content (PIC) estimated for each marker in the Montería-Córdoba population.

Locus	PIC
<i>mPgCIR9</i>	0.738
<i>mPgCIR11</i>	0.740
<i>mPgCIR13</i>	0.787
<i>mPgCIR16</i>	0.732
<i>mPgCIR19</i>	0.713
<i>mPgCIR22</i>	0.795
<i>mPgCIR23</i>	0.443

According to the results of Wright's F statistics (Table 9), it was determined that the lowest values of F_{IS} and F_{IT} were observed in the marker *mPgCIR13*, with values of 0.753 and 0.781, respectively. In contrast, the highest values were found in markers *mPgCIR11*, *mPgCIR22*, and *mPgCIR23*, each with a value of 1.000.

Regarding F_{ST} , it was determined that the marker contributing the most differentiation between the evaluated subpopulations is *mPgCIR19* with a value of 0.522, while the marker *mPgCIR13* contributes the least differentiation, with a value of 0.110.

As for the number of migrants (N_m), the lowest value was observed in the marker *mPgCIR19* with a value of 0.229, and the highest value was found in the marker *mPgCIR9* with a value of 1.591.

Table 9. Wright's F statistics used to assess *P. guajava* populations in Montería-Córdoba.

Locus	F _{IS}	F _{IT}	F _{ST}	N _m
<i>mPgCIR9</i>	0.835	0.857	0.136	1.591
<i>mPgCIR11</i>	1.000	1.000	0.166	1.256
<i>mPgCIR13</i>	0.753	0.781	0.110	2.024
<i>mPgCIR16</i>	0.784	0.895	0.514	0.236
<i>mPgCIR19</i>	0.896	0.950	0.522	0.229
<i>mPgCIR22</i>	1.000	1.000	0.294	0.600
<i>mPgCIR23</i>	1.000	1.000	0.495	0.255
Media	0.895	0.926	0.320	0.885

F_{IS}: individual fixation index with respect to subpopulations within a population;
F_{IT}: individual fixation index with respect to the total population.; F_{ST}: genetic differentiation coefficient among subpopulation; N_m: number of migrants per generation.

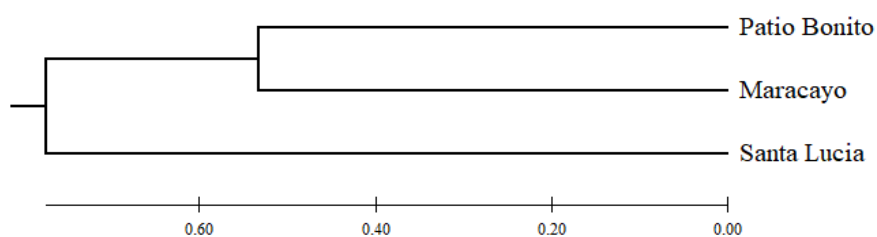
In Table 10, the Nei genetic distance matrix among the studied subpopulations can be observed. Based on this matrix, it was determined that Patio Bonito and Santa Lucia are the subpopulations with the greatest differences (2.000).

Table 10. Nei genetic distance matrix evaluated among the studied populations.

	A	B	C
A	0.000		
B	1.066	0.000	
C	2.000	1.096	0.000

A: Patio Bonito; B: Maracayo; C: Santa Lucia

In the dendrogram (Figure 1), you can observe the genetic relationships that exist between the studied subpopulations, showing that Patio Bonito and Maracayo are genetically closer, while Santa Lucia is more distant from the other populations.

**Figure 1.** Dendrogram of the genetic relationships among the *P. guajava* populations in Montería-Córdoba.

In Figure 2, you can find the Delta K values, which helped determine the different genetic groups, indicating that a value of K=2 is the number of genetic groups that best fit the data.

Similarly, you can observe two genetic groups represented by the colors red and green. In the first genetic group (green), the subpopulations of Patio Bonito and Maracayo are included, as they

are like each other. Santa Lucia is placed in the second genetic group (red), distinct from the other subpopulations.

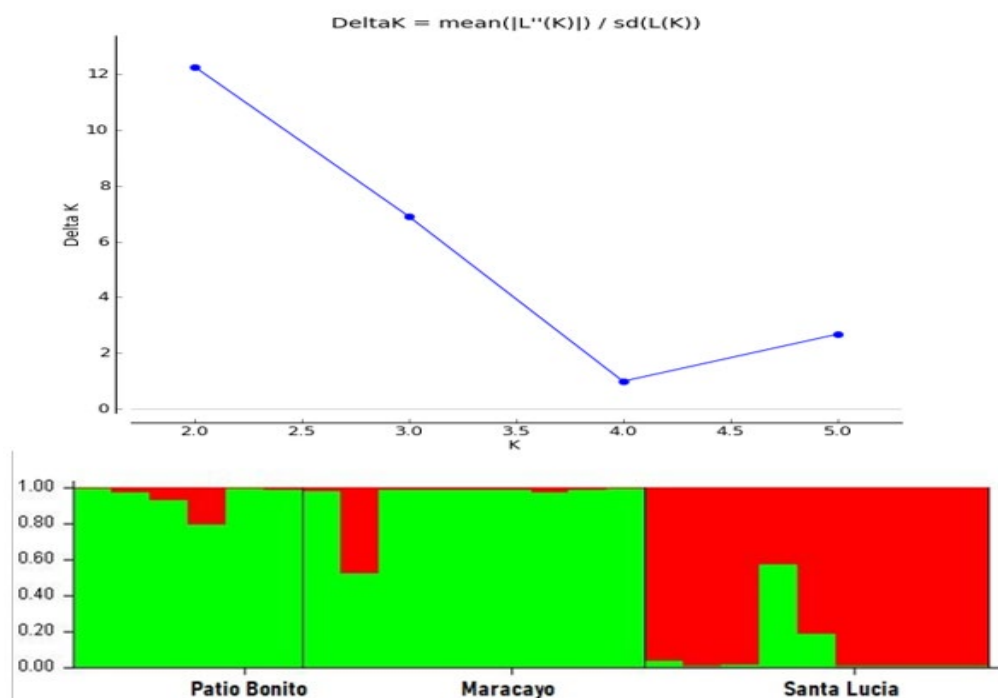


Figure 2. Bayesian analysis of the genetic structure of *P. guajava* populations using Harvester Structure. A. Chart with Delta K values for two populations. B. Population genetic structure for $k = 2$.

4. Discussion

In the studied populations, the allelic frequencies had an average of 6.5 alleles per locus (Table 5). The allele frequency values found in this research are higher than those already reported by other researchers [9–12]. However, the allelic frequencies obtained in this study are lower than those previously reported by other researchers [13–16]. The difference in results may be due to the limited number of individuals used in the present study. A total of 46 alleles were identified, a figure that aligns with results already obtained [9]. This similarity could be attributed to the characteristic phenomenon of self-pollination in the species, that has perfect flowers favoring self-fertilization [17], as well as to the diversity of the populations investigated in both studies [13].

Private alleles are alleles exclusive to each subpopulation that have become fixed due to different evolutionary forces. This condition allows these subpopulations to respond favorably to environmental conditions as generations progress [18]. Additionally, the presence of these alleles indicates high genetic diversity. The number of private alleles detected in the current study (Table 6) differs from those already reported, which found a total of three private alleles on Isla Isabela [19]. Although this study analyzed a larger number of individuals, it found a lower richness of private alleles compared to what was reported in the municipality of Montería.

The evaluated guava population had an average expected heterozygosity (uHe) of 0.572, and the observed heterozygosity (H_o) was lower, with a value of 0.058 (Table 7). These values were lower than others already reported [13,17,19]. The results of the guava accessions in Montería indicate low genetic diversity in the evaluated accessions, which can be explained by the effects of inbreeding resulting from the self-pollination of the species.

The average effective number of alleles (N_e) was 2,482, this value being lower than that already reported in Pakistan [16], higher than what was reported in India [9], and similar to that obtained in the United States [20].

The fixation index (F) had an overall average of 0.906 (Table 7), indicating that there is an excess of homozygotes in Montería. This could be attributed to the high rate of self-pollination in the species,

which can reach up to 75% [21]. Inbreeding in populations increases the risk of losing alleles in subsequent generations, which, in turn, leads to a reduction in the genetic diversity of species over time [22].

According to the Hardy-Weinberg equilibrium (HWE), it was determined that the microsatellite markers of the Maracayo and Santa Lucia subpopulations exhibit significant differences (P -Value < 0.05 and P -Value < 0.01) between observed and expected genotypes. Similarly, in Patio Bonito, the markers mPgCIR13 and mPgCIR22 showed significant differences (Table 7). This reaffirms the deficit of heterozygotes in the studied population. This condition may be related to the species' mode of reproduction, as *P. guajava* tends to undergo self-pollination, leading to a significant reduction in the number of heterozygotes and, in turn, causing a loss in the genetic variability of a population [23].

PIC values greater than 0.50 indicate highly informative markers, values between 0.50 and 0.25 indicate moderately informative markers, and values less than 0.25 indicate slightly informative markers [24]. According to this classification, the markers mPgCIR9, mPgCIR11, mPgCIR13, mPgCIR16, mPgCIR19, and mPgCIR22 are highly informative (Table 8), while the marker mPgCIR23 is moderately informative. Estos resultados son similares a los ya reportados en Pakistán con valores superiores a 0,50 para los mismos marcadores [16].

Regarding Wright's F statistics, the fixation index of individuals with respect to subpopulations within a population (FIS) and the fixation index of individuals with respect to the total population (FIT) showed values of 0.895 and 0.926, respectively (Table 9). With these positive values, it can be indicated that there is a deficiency of heterozygotes in the studied population, this could be related to significant levels of consanguinity, indicating limited genetic variation in the population [25]. These results are similar to those reported in Córdoba, where values for FIS and FIT were detected, revealing a deficit of heterozygotes and high levels of homozygosity [26].

For the genetic differentiation index among populations (F_{ST}), an average value of 0.320 was obtained (Table 9). Values from 0.25 to 1 indicate very high genetic differentiation [27], suggesting that there is very high genetic differentiation in Montería. These results are similar to those reported in Córdoba, where it was determined that the studied municipalities exhibited a high degree of genetic differentiation [26]. Concerning the number of migrants (N_m), the average value was 0.885 (Table 9), indicating that there is a limited number of individuals moving between populations. In other words, there is little genetic flow or reproduction between the studied subpopulations of *P. guajava*. These results are similar to those described in Tierralta, Córdoba, where an average of 1,010 migrants is reported, revealing a limited flow of individuals [28].

With respect to the genetic structure, it was found that the best grouping of individuals occurs when there are two genetic groups ($K=2$). According to this result, Patio Bonito and Maracayo share the same genetic group in relation to Santa Lucia, as this subpopulation belongs to a different genetic group (Figure 1). These results are consistent with the genetic distance matrix and the dendrogram (Table 10; Figure 2), where it is evident that Patio Bonito and Maracayo are the subpopulations with shorter distances (1.066) and are genetically closer. These findings align with what has been reported in the Galapagos Islands, where genetic differentiation is observed when there are two genetic groups [29].

5. Conclusions

The assessment of genetic diversity and variability in *P. guajava* in the municipality of Montería, Córdoba, using seven microsatellite markers, revealed that the guava population exhibited low genetic diversity within the studied subpopulations. This was evident through a high number of homozygotes and low levels of heterozygosity, likely resulting from inbreeding due to self-pollination. Additionally, it was determined that the markers *mPgCIR9*, *mPgCIR11*, *mPgCIR13*, *mPgCIR16*, *mPgCIR19*, and *mPgCIR22* were highly informative, making them recommended for future research. Furthermore, it was observed that *P. guajava* populations did not follow the Hardy-Weinberg equilibrium in the subpopulations of Maracayo and Santa Lucia. Likewise, it was established that the Patio Bonito and Maracayo subpopulations are genetically closer to each other

compared to Santa Lucia. These results can be attributed to their geographic locations, as the first two subpopulations are closer to each other in terms of territory.

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