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Allelic database and divergence among *Psidium* accessions by using microsatellite markers

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ABSTRACT. This study aimed to investigate the genetic variability among guava accessions and wild *Psidium* species of the Embrapa Semiárido germplasm collection by using microsatellite loci to guide genetic resources and breeding programs, emphasizing crosses between guava and other *Psidium* species. DNA was extracted using the 2X CTAB method, and polymerase chain reaction products were analyzed on 6% denatured polyacrylamide gels stained with silver nitrate. The unweighted pair-group method using arithmetic average dendrogram generated from the distance matrix of the Jaccard coefficient for 183 alleles of 13 microsatellite loci was used for visualization of genetic similarity. The number of base pairs was estimated using inverse mobility method based on the regression of known-size products. Analysis of molecular variance was performed using total decomposition between and within guava accessions. The accessions showed similarity from 0.75 to 1.00, with the dendrogram presenting cophenetic value of 0.85. Five groups were observed: the first included guava accessions; the second, *P. guineense* accessions; the third, one accession of *P. friedrichsthalianum*; and the last 2 groups, *P. cattleianum*. The genetic similarity among *P.*

guineense and some guava accessions were above 80%, suggesting greater possibility to obtain interspecies hybrids between these 2 species. The genetic variability between the accessions was considered to be high (Φ_{ST} = 0.238), indicating that guava genetic variability is not uniformly distributed among the 9 Brazilian states from where the accession were obtained. Obtaining a greater number of accessions by Brazilian states is recommended in order to have greater diversity among the species.

Key words: *Psidium*; Active germplasm bank; Breeding; Dendrogram; AMOVA

INTRODUCTION

In his overview, Gonzaga Neto (1999) expressed that De Candolle, when studying the guava origin, started by eliminating the old world and arrived at the conclusion that the guava originated in America and only the region it originated needed to be determined. According to De Candolle, the origin of the guava could have been Mexico, Colombia, Peru, or Brazil. According to Risterucci et al. (2005), the guava is native to the north of South America since it is abundantly distributed throughout the tropical American regions.

At present, guava has a well-established market in over 60 countries due to its rustic, prolific character; high level of vitamin C; and great economic return (Negi and Rajan, 2007). The species is abundantly distributed in the tropics and subtropics that people from different countries consider it native to the region (Singh, 2007). Brazil is the third largest producer of commercial guava, possessing edaphoclimatic conditions that favor the production of the fruit. Its cultivation is favored due to the nutrient and functional elements present in the fruit, besides the fact that it is possible to consume the fruit *in natura* or in the form of sweets and jams, resulting in a great economic return for the producers due to the versatility of its uses (São José et al., 2003; IBGE, 2011).

Among the traditional species of the Myrtaceae family, the guava and araçazeiro are the most important. Although the latter does not have the same economic importance as guava, they are of interest for research because their fruits show desirable characteristics with an exotic flavor and high levels of vitamin C. Furthermore, they are being studied as a source of tolerance to *Meloidogyne enterolobii*, a pest that has decimated guava orchards (Raseira and Raseira, 1996; Souza et al., 2006). Guava is a species that shows a high genetic diversity, due to the mixed reproductive system, as well as the use of seeds originating from heterozygous genitors for the production of seedlings (Alves and Freitas, 2007; Pessanha et al., 2011).

Pessanha et al. (2011) used the random amplified polymorphic DNA (RAPD) marker to evaluate the genetic diversity among 20 accessions of *Psidium*. Similarly, Erig et al. (2003) studied the genetic diversity among 24 accessions of araçazeiros by using RAPD markers and separated the genotypes into 4 groups in which the first showed 40% of similarity with the others, while the largest proximity was found between the last two. However, this marker has a low reproducibility and is dominant (Esselink et al., 2003). Corrêa et al. (2011) studied and compared the genetic similarity of 62 guava and 24 araçazeiro accessions by using amplified fragment length polymorphism (AFLP) markers and separated the genotypes into 2 groups, one formed by guava accessions and another by araçazeiro accessions with the inclusion of some guava accessions, with a similarity ranging from 28 to 98%.

Valdés-Infante et al. (2007) were the first to report and characterize Cuban guava accessions by using microsatellites. The authors used 7 microsatellite loci that generated 34 different alleles, of which 10 were considered to be rare. Aranguren et al. (2010) identified a high diversity in 31 Venezuelan guava accessions when genotyped with 16 microsatellite loci. Sánchez-Teyer et al. (2010) reported a similarity of 0.64 to 0.97 in 57 Mexican guava accessions genotyped with 6 microsatellite loci. Studies involving the use of microsatellite loci for the characterization of guava germplasm have not yet been reported in Brazil.

The objective of this study was to analyze the genetic variability of guava and wild *Psidium* (araçazeiros) accessions of the Embrapa Semiárido germplasm collection by using microsatellite markers in order to obtain subsidies for the genetic improvement and resource program for facilitating guava crossing with other species of the genus *Psidium*.

MATERIAL AND METHODS

The material was obtained from the Embrapa germplasm collection (BAG) of guava and araçazeiros (Table 1) located at the experimental field at Bebedouro, Embrapa Semiárido, Petrolina, PE. The BAG is divided into 2 blocks consisting of 118 guava and 40 araçazeiro accessions that were collected from 10 Brazilian States: Maranhão, Sergipe, Piauí, Pernambuco, Goiás, Bahia, Roraima, Rondônia, Amazonas, and Rio Grande do Sul. Each accession is represented by 6 plants with 4.0 x 4.0 m spacing. The accessions are irrigated 3 times per week by drip irrigation.

Table 1. Origin of guava accessions and araçazeiros of Embrapa Semiárido *Psidium* germplasm collection evaluated with 13 SSR loci.

Accession	Origin	State	Accession	Origin	State
GUA132 RR	Iracema	RR	GUA117 GO	Morrinhos	GO
GUA138 RR	Boa Vista	RR	GUA120 GO	Goiás Velho	GO
GUA133 RR	Iracema	RR	GUA121 GO	Goiás Velho	GO
GUA135 RR	Iracema	RR	GUA124 GO	Santa Isabel	GO
GUA136 RR	Rorainópolis	RR	GUA127 GO	Mimoso de Góias	GO
GUA137 RR	Caracaraí	RR	GUA128 GO	Mimoso de Góias	GO
GUA34 PE	Ibimirim	PE	GUA87 AM	Iranduba	AM
GUA38 PE	Pesqueira	PE	GUA88 AM	Iranduba	AM
GUA36 PE	Pesqueira	PE	GUA90 AM	Iranduba	AM
GUA33 PE	Ibimirim	PE	GUA92 AM	Manacapuru	AM
GUA39 PE	Belo Jardim	PE	GUA97 AM	Autazes	AM
GUA161 PE	Petrolina	PE	GUA98 AM	Autazes	AM
GUA51 SE	Capela	PE	GUA62 BA	Antonio Gonçalves	BA
GUA61 SE	Riachão dos Dantas	SE	GUA146 BA	Valença	BA
GUA55 SE	Pirambu	SE	GUA147 BA	Pateroá	BA
GUA52 SE	Capela	SE	GUA150 BA	Nilo Peçanha	BA
GUA53 SE	Japoratuba	SE	GUA151 BA	Nilo Peçanha	BA
GUA59 SE	Umbamba	SE	GUA155 BA	Igrapiúna	BA
GUA03 MA	Coelho Neto	SE	GUA106 RS	Pelotas	RS
GUA02 MA	Caxias	MA	GUA109 RS	Pelotas	RS
GUA26 MA	Paraibano	MA	GUA110 RS	Pelotas	RS
GUA05 MA	Buriti	MA	GUA104 RS	Pelotas	RS
GUA07 MA	Mata Roma	MA	ARA138 RR	Boa Vista	RR
GUA06 MA	Mata Roma	MA	ARA140 RR	Boa Vista	RR
GUA67 RO	Jaru	MA	ARA153 BA	Ituberá	RR
GUA68 RO	Buritis	RO	ARA105 RS	Pelotas	RS
GUA72 RO	Monte Negro	RO	ARA55 RS	Pelotas	RS
GUA73 RO	Ariquemes	RO	ARA58 RS	Pelotas	RS
GUA81 RO	Porto Velho	RO	ARA Costa Rica	-	-
GUA82 RO	Porto Velho	RO	Paluma	-	PE

Extraction and quantification of DNA

New and healthy leaves of 61 *Psidium* accessions were collected in paper bags, duly identified, and conditioned in a freezer at -80ºC until DNA extraction.

During DNA extraction, the 2X CTAB of Doyle and Doyle (1990) was used, with the following modifications: A) mechanical maceration was performed in the presence of liquid nitrogen until a fine powder was obtained; B) the macerated leaves of each sample were transferred to duplicated 2-mL Eppendorf tubes, each containing 950 µL 2X CTAB; C) samples were put in a water-bath at 60°C for 30 min and were gently inverted every 10 min; D) after 30 min, 950 µL chloroform:isoamyl alcohol (24:1) was added, followed by centrifugation at 6000 rpm for 10 min; E) 700 µL supernatant was transferred to new Eppendorf tubes; F) 467 µL chilled isopropyl alcohol was added next, and the tubes were gently inverted and maintained on ice for 20 min; G) after 20 min, the samples were centrifuged at 10,000 rpm for the formation of a "pellet" at the bottom of the tube; H) the pellet was re-suspended in 30 µL Tris-EDTA and kept in a refrigerator for 24 h to completely dissolve the pellet; I) the co-extracted RNAs were removed using 10% RNAse for 45 min in a water bath at 37°C.

The DNA was quantified on 0.8% agarose gel stained with ethidium bromide by visually comparing the intensity of the DNA bands extracted with those of bands of Lambda phage DNA. The samples were diluted to 10 ng/µL and stocked at -20°C.

Reaction and amplification of DNA and resolution on polyacrylamide gels

All 16 SSR loci, suggested by Briceño et al. (2010), were evaluated for guava diversity studies: mPgCIR227, mPgCIR228, mPgCIR229, mPgCIR233, mPgCIR236, mPgCIR242, mPgCIR243, mPgCIR246, mPgCIR247, mPgCIR249, mPgCIR251, mPgCIR252, mPgCIR253, mPgCIR255, mPgCIR256, and mPgCIR257. The PCR amplification was carried out for a final volume of 10 µL, containing 30 ng DNA, 0.2 µL of each primer, 1X Taq DNA polymerase buffer, 2.5 mM $MgCl_2$, 0.8 mM dNTPs, and 0.75 U enzyme Taq DNA polymerase. The amplification program consisted of denaturation of the initial cycle at 94°C for 4 min; 30 cycles at 94°C for 45 s, 52°C for 60 s, and 72°C for 60 s; and one stage of final extension at 72°C for 5 min.

Half of the volume of the denaturing buffer of 98% formamide (10 mM EDTA, pH 8.0; 1 mg/mL xylene cyanol; and 1 mg/mL bromophenol blue) was added to the PCR mixture, followed by complete denaturation at 94°C for 5 min in a thermocycler. Amplified PCR products were separated on 6% polyacrylamide gels for approximately 3 h, with constant 40 W power. A pre-run of 30 min at 45 W was performed before the application of the PCR samples. The molecular marker 50-bp DNA Ladder (Fermentas, USA) was loaded in the lateral extremities of each gel. The gels were stained with silver nitrate, as per the procedure described by Creste et al. (2001).

The 61 accessions were genotyped on 2 plates with polyacrylamide gels: one plate containing 54 accessions and the other containing the remaining accessions. On the first plate, at least 1 accession was identified and represented a genotype or allelic combination to be used as an allelic reference on the second gel plate for each microsatellite.

Annotation and analysis of microsatellite data

The size estimate in bp for each allele for the construction of allelic patterns for each accession was obtained by the inverse mobility method based on regression of products of known size of the 50-bp molecular marker (Fermentas).

The microsatellites were analyzed for the presence (1) versus absence (0) of alleles to construct a similarity matrix of the Jaccard index. The dendrogram with distances of the accessions was designed based on the unweighted pair-group method using arithmetic average method. The adjustment of the dendrogram was evaluated using the cophenetic correlation, or more specifically, the correlation between the real distances and those represented graphically. For these analyses, the NTSYSpc (Rophlf, 1989) computer application was used. The frequency of the allele number, genotype number, gene diversity, heterozygosity, and polymorphic information content (PIC) for each microsatellite was estimated using the Power Marker (Liu and Muse, 2005) program.

RESULTS

Of the 16 microsatellite loci used, only 13 showed polymorphic amplifications of an easy interpretation: mPgCIR227, mPgCIR233, mPgCIR242, mPgCIR243, mPgCIR246, mPgCIR247, mPgCIR249, mPgCIR251, mPgCIR252, mPgCIR253, mPgCIR255, mPgCIR256, and mPgCIR257. Aranguren et al. (2010) studied the accession variability of 31 Venezuelan guava and reported that all 16 microsatellite loci were polymorphic. This reduction in the number of SSRs to detect polymorphisms can be used because, in the present study, these microsatellites were used for a joint evaluation of guava and araçazeiro belonging to *P. guineense*, *P. cattleianum*, and *P. friedrichsthalianum* species.

In all, 183 alleles were detected in the 13 microsatellites analyzed, and the number of alleles per locus ranged from 7 to 22, with an average of 14.07 alleles per microsatellite in the 61 accessions of genotyped *Psidium*. The size of the alleles ranged from 129 bp in mPgCIR33 to 802 bp in mPgCIR247 (Table 2).

The largest number of genotypes was observed with microsatellite mPgCIR256, whereas the largest diversity of alleles was observed with microsatellite mPgCIR253 (Table 3); this finding is different from that reported by Aranguren et al. (2010), who used the same microsatellite set and found the largest diversity in microsatellite mPgCIR255.

The PIC values, which reflect the allelic diversity and frequency rate between accessions, were not uniform for all the microsatellite loci tested. The PIC average was 0.709, with the largest and smallest values observed in loci mPgCIR253 (0.862) and mPgCIR233 (0.227), respectively (Table 3).

The average heterozygosity was 0.695 with loci mPgCIR227 and mPgCIR249 showing the largest values (1.000) and the locus mPgCIR233 showing the smallest (0.104) value (Table 3), indicating that the microsatellites showed a large variability detection capacity. The PIC and heterozygosity showed the existence of variability because each individual diploid could have up to 2 alleles per locus (Weir, 1996).

The identification of accessions with reference alleles for each microsatellite and its inclusion in the second gel polyacrylamide plate allowed a correct comparison and allelic identification of the remaining accessions. This strategy was adopted by Dos Santos Ribeiro et al. (2012), who evaluated 103 mango accessions by using 12 microsatellites.

The cophenetic correlation was 0.85, which indicates that the dendrogram (Figure 1) presented a good adjustment in grouping *Psidium* accessions, with 183 alleles of 13 microsatellite loci analyzed. The similarity among accessions ranged from 0.75 to 1.00, reflecting the existence of genetic variability in the accessions studied. Rodríguez-Medina et al. (2010) found similarity ranging from 0.40 to 1.00 among 43 accessions of a Cuban guava collection, which was evaluated using 7 microsatellite markers. High genetic variability was also found and reported by Corrêa et al. (2011) for 88 *Psidium* accessions of the same collection, evaluated using AFLP markers.

Table 2. Allelic pattern, in base pairs, estimated for 61 accessions of *Psidium*, genotyped with 13 microsatellite markers (Petrolina, 2012).

Accession	Locus mPgCIR												
	227	233	242	243	246	247	249	251	252	253	255	256	257
GUA092 AM	468/474	144/144	468/468	275/280	388/398	429/444	518/520	557/566	616/616	562/568	604/611	607/610	362/365
GUA087 AM	596/618	144/144	468/468	275/280	388/398	429/444	503/505	516/524	616/616	562/568	604/611	607/610	362/362
GUA087 AM	596/618	144/144	468/468	275/280	388/398	429/444	503/505	516/524	616/616	562/568	618/625	607/610	362/362
GUA098 AM	596/618	144/144	468/468	341/347	295/322	424/429	537/543	524/532	616/616	562/568	604/611	571/573	362/362
GUA088 AM	596/618	144/144	468/468	250/275	388/398	429/429	503/505	516/524	616/616	494/499	604/611	607/610	362/362
GUA097 AM	435/446	144/144	468/468	250/275	295/322	429/444	518/520	524/532	616/616	529/535	604/611	607/610	299/362
GUA090 AM	435/446	144/144	468/468	250/275	295/322	429/444	503/505	524/532	616/616	562/568	604/611	607/610	362/362
GUA062 BA	435/446	144/144	458/458	250/275	295/303	429/444	503/505	516/524	616/616	535/545	618/625	571/573	299/299
GUA150 BA	435/446	144/144	458/468	329/335	295/322	723/732	503/505	516/524	616/616	529/535	618/625	571/573	299/299
GUA147 BA	596/618	144/144	506/506	329/335	295/303	429/444	503/505	516/524	623/623	562/568	618/625	567/570	362/365
GUA147 BA	596/618	144/144	506/506	329/335	295/303	429/444	503/505	516/524	623/623	562/568	618/625	571/573	362/365
GUA155 BA	435/446	144/144	458/468	275/280	295/303	429/444	503/505	516/524	616/616	529/535	618/625	571/573	299/299
GUA151 BA	435/446	144/144	468/506	250/275	295/322	429/444	503/505	516/524	616/616	529/535	604/611	571/573	299/365
GUA151 BA	435/446	144/144	468/506	250/275	295/322	429/444	503/505	516/524	616/616	529/535	618/625	567/570	299/365
GUA146 BA	435/446	144/144	468/468	341/347	303/303	429/444	503/505	516/524	616/616	529/535	618/625	567/570	299/365
GUA146 BA	435/446	144/144	468/468	296/301	303/303	429/444	503/505	516/524	616/616	529/535	618/625	567/570	299/365
GUA061 SE	697/706	144/144	468/468	275/280	388/398	424/424	503/505	516/524	623/623	562/568	604/611	571/573	459/554
GUA055 SE	435/446	144/144	458/458	250/275	303/303	429/444	503/505	516/524	616/616	529/535	618/625	571/573	299/299
GUA052 SE	435/446	144/144	468/468	275/280	295/303	424/424	503/505	516/524	616/616	562/568	618/625	571/573	459/554
GUA059 SE	435/446	144/144	458/468	341/347	295/303	424/424	503/505	516/524	616/623	529/535	618/625	571/573	459/554
GUA059 SE	435/446	144/144	458/468	341/347	295/303	424/424	503/505	516/524	616/623	529/535	604/611	571/573	459/554
GUA051 SE	435/446	144/144	468/468	341/347	295/303	429/444	503/505	516/524	616/616	529/535	618/625	571/573	362/365
GUA053 SE	633/641	144/144	458/468	341/347	295/303	429/444	503/505	516/524	616/616	562/568	618/625	571/573	299/299
GUA138 RR	435/446	144/144	458/468	341/347	295/295	424/444	503/505	516/524	616/616	562/568	618/625	571/573	299/299
GUA137 RR	441/451	144/144	468/468	341/347	295/295	424/429	503/505	532/557	616/616	562/568	618/625	571/573	409/413
GUA132 RR	596/618	144/144	468/468	250/275	412/422	424/429	537/543	532/557	616/616	494/499	618/625	571/573	409/413
GUA133 RR	435/446	144/144	468/468	341/347	295/303	424/424	503/505	524/532	616/616	529/535	604/611	571/573	362/365
GUA135 RR	435/446	144/144	468/468	341/347	412/422	424/424	537/543	524/532	616/616	562/568	618/625	614/614	409/413
GUA136 RR	596/618	144/144	468/468	222/231	412/422	429/429	503/505	524/532	616/616	562/568	604/611	567/614	409/413
GUA081 RO	480/486	144/144	468/468	341/347	295/322	429/429	503/505	524/532	616/616	562/568	618/625	614/614	362/365
GUA073 RO	435/446	144/144	506/506	341/347	422/428	424/429	503/505	532/557	616/616	545/562	618/625	614/614	409/413
GUA067 RO	441/451	144/144	468/468	341/347	295/303	429/429	518/520	524/532	616/616	545/562	618/625	571/614	362/365
GUA082 RO	596/618	144/144	458/468	275/280	499/511	429/429	503/505	524/532	616/616	529/535	618/625	614/614	299/299
GUA072 RO	480/486	144/144	506/506	296/301	422/428	429/429	503/505	524/532	616/616	562/568	618/625	614/614	299/299
GUA072 RO	480/486	144/144	506/506	296/301	422/428	429/429	513/516	524/532	616/616	562/568	618/625	614/614	299/299
GUA068 RO	480/486	144/144	468/468	250/275	422/428	429/444	503/505	524/532	616/616	499/499	611/631	614/614	418/423
GUA036PE	441/486	144/144	458/468	341/347	295/303	424/424	537/543	524/532	623/623	494/499	618/625	571/573	362/365
GUA033 PE	435/446	144/144	506/506	286/291	318/331	791/802	503/505	532/532	623/623	529/535	660/668	571/573	400/404
GUA038 PE	435/446	144/144	458/458	275/280	295/322	429/444	503/505	524/532	616/616	529/535	638/646	605/605	299/299
GUA039 PE	435/446	144/144	458/458	275/280	295/303	429/444	503/505	524/532	616/616	529/535	638/646	571/573	299/299

Continued on next page

Table 2. Continued.

Accession	Locus mPgCIR												
	227	233	242	243	246	247	249	251	252	253	255	256	257
GUA034 PE	435/446	144/144	458/458	275/280	318/331	429/444	503/505	524/532	616/616	529/535	638/646	605/605	342/342
GUA121 GO	435/446	144/144	468/468	341/347	295/295	424/424	503/505	524/532	616/616	562/568	618/625	571/573	362/365
GUA117 GO	435/446	144/144	468/468	341/347	295/303	424/444	503/505	524/532	616/616	529/535	660/668	571/573	299/299
GUA127 GO	435/446	144/144	458/468	275/280	295/295	424/444	503/505	524/532	616/616	529/535	618/625	571/573	362/365
GUA128 GO	596/618	144/144	468/468	341/347	295/303	424/424	503/505	532/557	616/616	562/568	618/625	571/573	362/365
GUA124 GO	435/446	144/144	458/458	341/347	295/303	424/424	503/505	532/557	616/616	529/535	638/646	571/573	362/365
GUA124 GO	435/446	144/144	458/458	341/347	295/303	424/424	503/505	532/557	616/616	529/535	618/625	571/573	362/365
GUA120 GO	596/618	144/144	468/468	341/347	295/295	424/424	503/505	524/532	616/616	562/568	638/646	571/573	362/365
GUA005 MA	435/446	144/144	506/506	275/280	295/303	429/444	503/505	524/532	616/623	418/540	638/646	571/573	299/299
GUA003 MA	435/446	144/144	458/458	275/280	499/511	429/444	530/532	369/369	616/616	529/535	638/646	571/573	299/299
GUA006 MA	435/446	144/144	458/506	275/280	303/303	444/444	503/505	557/566	623/623	418/540	504/509	605/605	299/299
GUA006 MA	435/446	144/144	458/506	275/280	303/303	444/444	503/505	557/566	623/623	418/540	638/646	605/605	299/299
GUA007 MA	697/706	144/144	458/458	275/280	295/303	429/444	530/532	369/369	616/616	418/540	504/509	571/573	299/299
GUA026 MA	435/446	144/144	458/458	250/275	295/303	429/444	503/505	524/532	616/616	529/535	638/646	571/573	299/299
GUA002 MA	435/446	144/144	463/463	275/280	303/303	429/444	518/520	524/532	623/623	418/540	638/646	605/605	459/554
ARA140 RR	457/463	144/163	458/468	191/196	307/314	444/460	503/505	455/455	613/613	446/454	638/646	571/593	358/358
ARA140 RR	457/463	144/163	458/468	191/196	307/314	444/460	503/505	455/455	613/613	446/454	618/625	571/593	358/358
ARA138 RR	468/474	144/176	448/468	191/196	318/318	429/444	503/505	394/394	650/650	446/454	631/631	593/593	358/358
ARA153 BA	468/474	144/163	458/468	191/191	295/303	429/444	503/505	524/532	631/638	437/446	638/646	593/593	299/299
ARA153 BA	435/446	144/163	458/468	191/191	295/303	429/444	503/505	524/532	631/638	680/688	638/646	593/593	299/299
ARA105 RS	-	126/141	385/406	163/171	311/225	390/419	535/540	-	-	680/688	-	587/587	299/299
ARA105 RS	-	126/141	411/415	163/171	311/225	439/450	503/505	-	-	680/688	-	587/587	299/299
GUA110 RS	468/474	144/144	468/468	275/280	295/303	424/429	503/505	524/532	631/631	529/535	638/646	571/573	299/299
GUA106 RS	468/474	144/144	468/468	275/280	295/303	424/429	503/505	524/532	631/631	529/535	638/646	571/573	299/299
GUA109 RS	468/474	144/144	458/458	275/280	295/303	483/483	503/505	557/566	631/631	529/535	631/631	573/573	299/299
GUA104 RS	468/474	144/144	458/458	250/275	295/303	483/483	503/505	557/566	631/631	529/535	631/631	573/573	299/299
GUA161 PE	516/528	144/144	443/458	275/280	295/303	483/525	503/505	394/394	631/642	545/562	618/625	573/567	305/308
Paluma	516/528	144/144	443/458	275/280	295/303	483/525	503/505	394/394	631/642	545/562	618/625	573/567	305/308
Pedro Sato	516/528	144/144	458/458	341/347	295/303	483/483	503/505	557/566	634/634	529/535	618/631	573/573	299/299
ARA Costa Rica	457/463	138/144	439/453	-	310/323	-	503/505	-	-	651/658	504/537	583/585	-
ARA Costa Rica	510/522	138/144	439/453	-	310/324	-	503/505	-	-	651/658	504/537	583/585	-
ARA055 RS	425/430	129/129	406/411	-	311/225	414/419	503/505	-	-	363/363	-	561/563	259/259
ARA055 RS	425/430	129/129	385/402	-	311/225	414/419	525/527	-	-	363/363	-	569/570	259/259
ARA058 RS	425/430	129/129	385/402	-	311/225	414/419	503/505	-	-	363/363	-	569/570	259/259
ARA058 RS	425/430	129/129	406/411	-	311/225	414/419	525/527	-	-	363/363	-	561/563	259/259

Table 3. Genetic parameters estimated for 13 microsatellites in 61 *Psidium* accessions (Petrolina, PE, 2012).

SSR	Allelic frequency	No. of genotypes	No. of alleles	Genetic diversity	Heterozygosity	PIC
mPgCIR227	0.233	12	22	0.869	1.000	0.858
mPgCIR233	0.873	6	7	0.233	0.120	0.227
mPgCIR242	0.453	14	13	0.696	0.373	0.652
mPgCIR243	0.239	10	17	0.859	0.971	0.844
mPgCIR246	0.313	14	19	0.814	0.840	0.794
mPgCIR247	0.329	14	15	0.771	0.671	0.737
mPgCIR249	0.407	7	14	0.666	1.000	0.608
mPgCIR251	0.351	8	8	0.773	0.881	0.741
mPgCIR252	0.679	9	8	0.509	0.104	0.481
mPgCIR253	0.193	12	17	0.874	0.933	0.862
mPgCIR255	0.239	9	12	0.846	0.957	0.829
mPgCIR256	0.273	15	15	0.840	0.733	0.824
mPgCIR257	0.404	13	16	0.782	0.452	0.762
Average	0.384	11	14.1	0.733	0.695	0.709

PIC = polymorphism information content.

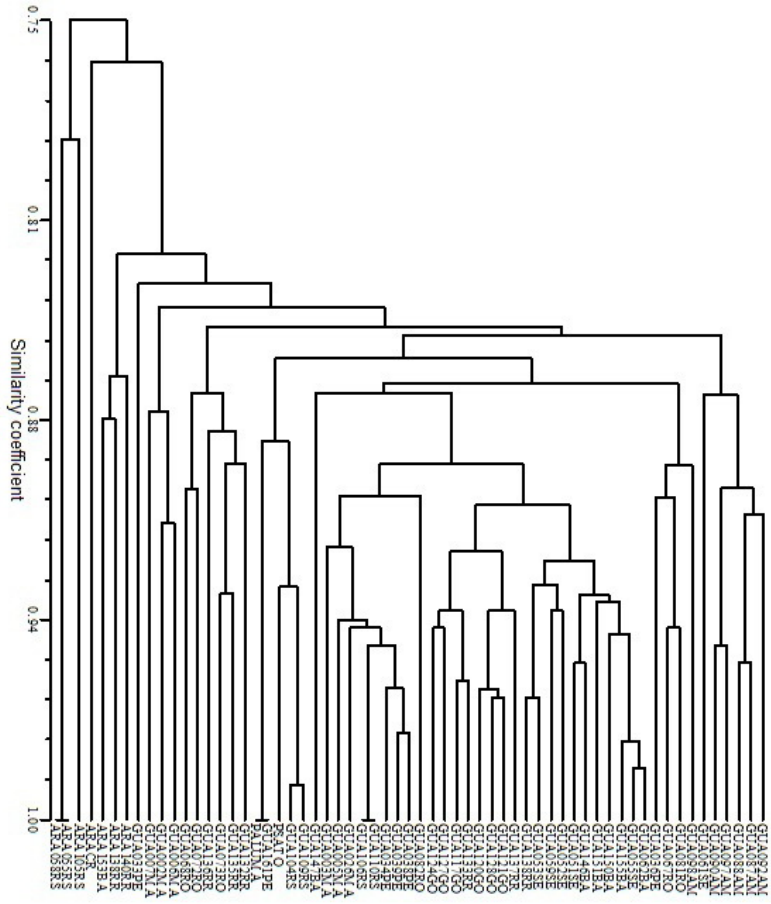

Figure 1. UPGMA dendrogram of the Jaccard coefficient among 61 accessions of *Psidium* of the Embrapa Semiárido *Psidium* germplasm collection sampled in nine Brazilian states and analyzed with 13 microsatellite loci. Cophenetic correlation = 0.85.

The 183 alleles of the 13 microsatellites were sufficient to separate the guava accessions from araçazeiro accessions. The cutting point at 83% of similarity led to the formation of 5 groups (Figure 1): Group I, from GUA92 to GUA33 PE; group II, from ARA140 RR to ARA153 BA; group III, formed exclusively by the accession ARA Costa Rica; group IV, formed only by the accession ARA105 RS; and group V, formed by accessions ARA055 RS to ARA058 RS. Group I predominantly included guava accessions, while group II included only accessions of the species *P. guineense*. Groups IV and V included *P. cattleianum*, while group III included *P. friedrichsthalianum*. The external localization of the araçazeiro accessions in relation to the guava group was an indication for the adequacy of the dendrogram generated.

DISCUSSION

Largest similarities (100%) were observed between ARA55 RS and ARA58 RS and GUA110 RS and GUA106 RS accessions, probably because they were sampled at the same BAG, at Pelotas, RS. The GUA161 PE accession and Paluma cultivar, besides showing morphologically divergent characteristics, were genetically equal to the loci analyzed, with an observed similarity of 100% (Figure 1). The GUA161 PE accession was considered to be an amphidiploid (data not shown), which might explain the 100% similarity. Additional analyses with other microsatellites and further cytogenetic studies are indicated to elucidate this similarity between GUA161 PE and Paluma.

Guava accessions belonging to the States of Bahia, Sergipe, and Goiás in group I were almost positioned sequentially in the branches of the dendrogram (Figure 1), suggesting a remarkable genetic similarity between the accessions. The 7 araçazeiro accessions - ARA140 RR, 138 RR, and ARA153 BA (*P. guineense*); ARA105 RS, 55 RS, and 58 RS (*P. cattleianum*); and ARA of Costa Rica (*P. friedrichsthalianum*) - were positioned at the base of the dendrogram, suggesting greater similarity among them. Briceño et al. (2010) found the same separation pattern among Venezuelan guava accessions and other species of *Psidium*, such as *P. guineense*, all of which were evaluated using microsatellite markers. Hernández-Delgado et al. (2007) analyzed 52 *Psidium* accessions of a Mexican collection and reported a dendrogram with 2 groups: the first comprising *P. cattleianum* and *P. friedrichsthalianum* accessions and the second comprising *P. guajava* accessions.

Crossing is possible among the ARA140 RR, ARA138 RR, and ARA153 BA accessions and guava accessions that showed 82.4% similarity. These data are supported by the results that were obtained by da Costa et al. (2012), who reported successes with interspecies hybrids between *P. guajava* and *P. guineense* and unsuccessful hybridization of other species of *Psidium* with guava accessions.

Variability among guava accessions was 0.238 (Φ_{ST}; Table 4), with a genetic differentiation that was considered to be high, indicating a high variability among the accessions analyzed. Although guava is considered to have a tendency of a high cross-pollination rate (Alves and Freitas, 2007), the gene flow among the accessions collected from 9 Brazilian states was small and considered to be restricted, probably due to the limited flow of germplasm for those accessions that were not cultivated on a commercial scale. Higher results of Φ_{ST} = 0.355 were also reported by Sanabria et al. (2006) for 53 accessions of 9 Colombian guava populations.

Table 4. Analysis of molecular variance (AMOVA) for 51 guava accessions collected in nine Brazilian states and evaluated with 183 SSR alleles.

Variation source	d.f.	SS	MS	Variation total*	Statistical Φ	P
Between accessions	8	217.3	27.1	24%	$\Phi_{ST} = 0.238$	<0.001
Within accessions	43	417.5	9.7	76%	$1- \Phi_{ST} = 0.762$	<0.001
Total	51	634.8	-	100%		

*Probability based on 1000 permutations.

The dendrogram (Figure 1) and AMOVA (Table 3) results suggested that the genetic variability in guava was not uniformly dispersed among the 9 Brazilian states, indicating that geographical barriers, edaphoclimatic conditions, predominance of self-mating in guava, or even cutting-free germplasm dispersion have limited the exchange of alleles among guava orchards. Therefore, a greater number of accessions should be sampled by Brazilian states in order to have greater diversity among guava species.

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