AGRARIAN SCIENCES

Genetic structuring of segregating populations of *Psidium* spp resistant to the southern root-knot nematode by Bayesian approach as basis for the guava breeding program

RAIANE M. SANTOS, ALEXANDRE P. VIANA, EILEEN A. SANTOS,
RICARDO M. DE SOUZA, ODIMAR F. DE ALMEIDA, VICENTE M. GOMES,
DANIELE L. RODRIGUES & PAULO RICARDO DOS SANTOS

Abstract: There are no guava cultivars resistant to the *Meloidogyne enterolobii*; for this reason, genetic breeding has been performed by introgressing genes into the current cultivars through interspecific hybridization. We used 33 microsatellite markers for the genetic-molecular characterization of segregating populations of *Psidium* resistant to *M. enterolobii*, aiming at selection within and between populations for generation advancement in the guava breeding program. The average number of alleles per locus ranged from 1.60 to 2.09. Populations 1 (*P. guineense* × *P. cattleyanum*) and 5 (*P. guajava* × *P. cattleyanum*) obtained the greatest genetic diversity, which can be confirmed by the higher observed-heterozygosity values (0.422 and 0.312, respectively). Bayesian analysis showed that the populations were subdivided into three groups, agreeing with the number of groups observed by Nei's genetic distance. The population obtained from the *P. guineense* × *P. cattleyanum* cross differed from the others with a clear structuring, whereas the *P. guajava* × *P. cattleyanum* and *P. cattleyanum* × *P. guineense* populations were the most similar between each other. The SSR markers were efficient in discriminating the populations, and individual 80 may be employed in future crosses with guava, allowing generation advancement in the guava breeding program aimed at resistance to *M. enterolobii*.

Key words: Araçá, DNA markers, Genetic diversity, Guava decline, Interspecific hybridization, *Psidium guajava*.

INTRODUCTION

The genus *Psidium*, of the family Myrtacea, comprises 183 species among which the guava (*P. guajava* L.) and the araçá fruits (*P. cattleyanum* and *P. guineense*) stand out, originating from tropical regions of America (Flora do Brasil 2020).

India, Pakistan, Brazil, Colombia, and Mexico are the biggest guava producers in the world. This crop is amidst the 19 most produced fruits in Brazil (Pommer & Murakami 2014). The annual production of red guava in Brazil is approximately 578.608 t, with an estimated production worth around BRL 195.31 (IBGE 2019). Guava is found in practically the entire Brazilian territory, with the states of São Paulo, Pernambuco, Minas Gerais, and Rio de Janeiro concentrating the most part of national production (Pommer & Murakami 2014). Cultivars Paluma, Rica, Pedro Sato, Kumagai, Sassaoka, Ogawa, Yamamoto, and Século XXI are those most commonly grown in the country (Pommer & Murakami 2014).

However, the crop has been affected by a disease caused by the nematode *Meloidogyne*

enterolobii, responsible for the appearance of galls in large quantities and yellowing of the leaf edges, ultimately leading to total defoliation of the shoots (Gomes et al. 2014). A matter of an even greater concern is the synergistic association between *M. enterolobii* and the fungus *Fusarium solani*, since root rots occur only when two pathogens are involved in the process. This disease in association is known as the 'guava decline', which eradicates numerous orchards of this fruit (Gomes et al. 2011). This result in huge economic impacts for producers, once the losses are estimated to reach over $ 70 million (Gomes et al. 2014).

Practices for the control of this nematode have limited or zero effectiveness. There are no reports of genetic resistance to *M. enterolobii* identified in *P. guajava* (Castro et al. 2012, Freitas et al. 2014) and no guava cultivar resistant to the nematode has been registered thus far. Therefore, studies conducted by many researchers identified the araçás of the species *P. cattleyanum* (Miranda et al. 2011, Biazatti et al. 2016) and *P. guineense* (Costa et al. 2012) as sources of resistance to this pathogen.

However, the araçás have limited or full incompatibility when used as rootstock for guava (Castro et al. 2012, Robaina et al. 2012). For this reason, hybrids between guava and araçá have been generated as an alternative to prevent losses and the devastation of guava orchards across Brazil. Interspecific *Psidium* hybrids have already been obtained and assessed for resistance to the nematode (Gomes et al. 2017, Costa et al. 2016). Gomes et al. (2017) performed crosses between *P. cattleyanum* (resistant araçá) and *P. guajava* (susceptible guava) and selected 30 hybrids including immune and resistant types that will be backcrossed with guava to retrieve the desired agronomic traits and for the later release of a new cultivar.

Gomes et al. (2017) and Costa et al. (2016) obtained diverging results for inheritance of resistance to *M. enterolobii*. Costa et al. (2016) evaluated 22 hybrids from the *P. guajava* × *P. guineense* cross and found that 226 plants were immune (Reproduction Factor [RF] = 0) and 16 were susceptible (RF between 0.003 and 0.322), suggesting simple inheritance with full dominance. Gomes et al. (2017), by contrast, evaluated 367 hybrids obtained from the cross between *P. guajava* and *P. cattleyanum* and identified 18 immune, 46 resistant, and 303 susceptible genotypes, discarding the hypothesis of monogenic inheritance. However, it should be stressed that the resistant araçá species used in both studies are different, which may lead to conflicting results. In order for monogenic inheritance to occur, as suggested by Costa et al. (2017), the species *P. guineense* must be homozygous for the alleles that provide resistance to *M. enterolobii*, which is not common in allogamous species like the araçás.

Results obtained by Gomes et al. (2017) led to the generation of many progenies through crosses between *P. guineense* × *P. cattleyanum*, *P. guajava* × *P. cattleyanum*, and *P. cattleyanum* × *P. guineense*, which were evaluated for both resistance to *M. enterolobii* and genetic diversity through morphological traits (Almeida 2017). However, the phenotypic expression is influenced by several external factors such as environmental conditions, plant age, among others. Studies based on molecular methods, in turn, have a lower environmental effect and have been increasingly used in breeding programs, since, in addition to providing direct information on the genome of each individual, they improve the genotype selection efficiency and guide breeders in the choice of crosses.

To save time and accelerate the response of the programs, breeders make use of auxiliary tools such as molecular markers. Among the

many classes of molecular markets available today, microsatellites stand out for their informative power and wide spread across the genome, allowing for good sampling in genetic studies (Oliveira et al. 2010). Other features of these markers are codominant inheritance, multi-allelic nature, and high reproducibility. Microsatellites are a class of more-promising markers for broad use in breeding programs. Of their several applications, noteworthy cases are studies of genetic diversity (Tuler et al. 2015), selection of parents (Fernández & Pelea 2015), fingerprinting (Naga Chaithanya et al. 2015), assisted selection (Rivero et al. 2017), recovery of the recurrent parent (Ramos et al. 2014), confirmation of hybrids in interspecific crosses (Costa et al. 2012), and cultivar protection (Nunes de Jesus et al. 2009).

Studies have been reported involving molecular markers in the genus *Psidium* for development and application of SSR markers in guava (Rivero et al. 2017); germplasm characterization (Sharma et al. 2010); construction of genetic linkage maps (Lepetre et al. 2010); estimation of genetic diversity (Pessanha et al. 2011); and transferability of primers to fructiferous species of the genera *Eugenia*, *Campomanesia*, *Myrciaria*, and *Syzygium* (Nogueira et al. 2015). However, there are no reports involving the use of SSR markers in interspecific *Psidium* hybrids resistant to *M. enterolobii* to support breeding programs.

The combination of breeding methods, statistical methodologies, and molecular technologies brings new prospects for genetic knowledge and for the acceleration of breeding programs. In this regard, the objectives of the present study were: i) to undertake genetic characterization, estimating genotypic indices for the quantification and structuring of the genetic variability of the segregating *Psidium* populations studied; and ii) to select genotypes

genetically closer to *P. guajava* for generation advancement in the guava breeding program aiming at resistance to *M. enterolobii*.

MATERIAL AND METHODS

Genetic material

Ninety-four (94) individuals originating from five segregating populations of *Psidium* resistant to the nematode *M. enterolobii* and their respective parents were evaluated (Table I). The populations were obtained from an interspecific hybridization of *P. cattleyanum* (resistant genotype), *P. guajava*, and *P. guineense* (susceptible genotypes) and evaluated for resistance to *M. enterolobii* (Gomes et al. 2017).

Genomic DNA extraction and quantification

Total genomic DNA was extracted from young leaves collected individually from each hybrid plant and parents, using the CTAB method with modifications (Doyle & Doyle 1990).

Next, the DNA was quantified by analysis on 1% agarose gel with TAE 1X buffer (Tris, sodium acetate, EDTA, pH 8.0), using the 100-pb lambda (λ) marker (100 ng/µL^{-1}) (Invitrogen, USA) by comparing the bands. For this procedure, samples were stained using a mixture of Gel Red™ and Blue Juice (1:1), and the image was captured by the Mini Bis Pro (Bio-Imaging Systems) imaging system. Subsequently, DNA samples were diluted to the working concentration of 10 ng/µL^{-1}.

Primer screening

The DNA of the parental genotypes was initially used for a screening of 100 microsatellite primers developed for *P. guajava* (Risterucci et al. 2005, GuavaMap 2008) aiming to identify SSR loci capable of differentiating the parents. After tracking, a set of 33 polymorphic primers was selected for the amplification reactions (Table II).

Table I. *P. guineense, P. cattleyanum*, and *P. guajava* genotypes used in the interspecific crosses to obtain segregating populations of *Psidium* spp. and number of resistant hybrid plants evaluated in each population with the parents.

Populations	Genitors	Number of Plants	Code of hybrid genotypes
1	*P. guineensi* (P36)[1] x *P. cattleyanum* (P11)[2]	20	3-20
2	*P. guajava* (13.4 II)[1] x *P. cattleyanum* (P33)[2]	16	23-36
3	*P. cattleyanum* (CV8)[2] x *P. guineensi* (CV11)[1]	21	39-57
4	*P. cattleyanum* (CV1)[2] x *P. guineensi* (CV11)[1]	20	60-77
5	*P. guajava* (13.4 II)[1] x *P. cattleyanum* (P53)[2]	17	80-94
	Total genotypes	94	

[1] = **Susceptible genotypes to *M. enterolobii* (Miranda et al. 2012); [2] = Resistant genotypes *M. enterolobii* (Miranda et al. 2012).**

Polymerase chain reaction (PCR)

PCR reactions were performed in thermocyclers (Applied Biosystems/Veriti 96 well) in a 35-cycle program under the following temperatures and times: 94 °C for 4 min (initial denaturation); 94 °C for 2 min (cyclic denaturation); specific temperature of each primer, in °C, for 1 min (annealing); 72 °C for 2 min (cyclic extension); 72 °C for 10 min (final extension); and 4 °C forever. The final volume was 13 µL from each sample, containing 2 µL DNA (10 ng/µL), 1.50 µL 10X (NH_4SO_4) buffer, 1.5 µL $MgCl_2$ (25 mM), 1.5 µL dNTPs (2 mM), 1 µL primer (R+F) (5 µM) and 0.12 µL Taq-DNA polymerase (5 U/µL) (Invitrogen, Carlsbad, California, USA).

The PCR products were diluted at a ratio of 4 µl of sample to 20 µL of buffer E from the DNF 900 kit and dis and subjected to a capillary electrophoresis system (Fragment Analyzer - AATI) in which amplified fragments of 35 to 50 pb are separated with a resolution of approximately 2bp. Each run lasted 2h20min at a voltage of 8 kw.

Statistical analysis

Estimation of genetic diversity of SSR markers

The data obtained from the amplification of the SSR primers were converted to numeric code for each allele per locus. This numerical matrix was developed by assigning values from 1 to the maximum number of alleles in the locus, as described next: a locus presenting three alleles was represented by 11, 22, and 33 for the homozygous forms A1A1, A2A2, and A3A3; and 12, 13, and 23 for the heterozygous forms A1A2, A1A3, and A2A3. Based on this numerical matrix, we calculated the genetic distance between the studied genotypes using the GENES software (Cruz, 2016) adopting the Weighted Index; and between the populations based on Nei's genetic distance (Peakall & Smouse 2009). The cluster analysis of the individuals and populations via dendrogram was performed by the UPGMA (Unweighted Pair-Group Method Average) method using the Mega software version 6 (Kumar et al. 2009), and the graphic dispersion between populations was accomplished based on the principal coordinate analysis (PCA), using the Genalex 6.3 software (Peakall & Smouse

2009). The optimal number of markers was estimated with the GENES software (Cruz 2016).

From the molecular variables, we estimated the genetic diversity between the *Psidium* spp populations with the Genalex 6.3 software (Peakall & Smouse 2009). The following parameters were estimated: number of alleles per locus (NA), genetic variance (Vg), genotypic coefficient of determination (H^2), polymorphic information content (PIC), probability of identity (PI), observed heterozygosity (Ho), expected heterozygosity (He), and fixation index (*f*).

Analysis of the structure and clustering of *Psidium* spp. populations

Based on the information obtained from the polymorphic primers, a molecular analysis of variance (ANOVA) was performed to evaluate the differences between and within the populations by Wright's F statistics, where the genetic structure of a population is characterized by obtaining three distinct parameters: FIS (inbreeding coefficient among individuals in the subpopulations), FST (degree of genetic differentiation among the subpopulations), and FIT (degree of genetic differentiation among the total populations), using the Genalex 6.3 software (Peakall & Smouse 2009).

Analyses of population structure were performed with the Bayesian method using the STRUCTURE software, version 2.3.4 (Pritchard et al. 2000). Considering that the present study was conducted using populations (comprising plants from controlled crosses), we used the no admixture model and correlated allele frequencies in each population. The burn-in period and replication numbers were set to 10,000 and 50,000 respectively, for each run. The number of groups (K) was systematically varied from 1 to 10, and 20 simulations were performed to estimate each K. We used the ΔK ad hoc method described by Evanno et al. (2005)

and implemented in the online tool Structure Harvester Earl & Vonholdt (2012) to estimate the most likely K in each group of *Psidium*. The membership probabilities threshold of 0.60 was used as the maximum membership probability among subgroups. Based on the posterior probability of membership (q) of a given accession belonging to a given group compared to the total number of groups (K), we classified individuals with q > 0.60 as a member of a given cluster, whereas for clusters with membership (q) values ≤0.60, the accession was classified as admixed (Cerqueira-Silva et al. 2014).

RESULTS

Genetic-molecular characterization of *Psidium* segregating populations by SSR markers

A total of 100 microsatellite primers were used to distinguish the segregating populations of *Psidium* spp resistant to *M. enterolobii* at molecular level. Of these, 70 produced amplified DNA fragments, 33 of which were polymorphic and thus used in this study (Table II). These SSR loci generated 89 alleles; the number of alleles per locus ranged from two to four, averaging 2.70 alleles per locus, considering the entire dataset (Table III).

For most of the analyzed loci, Ho was higher than He and ranged from 0.000 to 0.871, averaging 0.288. Expected heterozygosity, in turn, ranged from 0.033 to 0.508, averaging 0.216. As for the fixation index, the variation between the loci was −0.082 to 1. According to the F statistics, perhaps the most disseminated statistics in population genetics, FIS values ranged from −0.049 to 1, while FIT values went from −0.100 to 1 and FST from 0.003 to 0.932 (Table III).

The parameters Na, Ho, He, *f*, and PIC were also estimated at population level (Table IV). Na ranged from 1.60 to 2.09, with a total average of 1.86 alleles between populations. Expected

Table II. Sequence of the 33 pairs of microsatellite primers used in the analysis of the 94 genotypes originating from five populations obtained from interspecific crosses of *Psidium* spp.

Locus	Sequence (5' 3')	Annealing temperature	Reference
mPgCIR027	F: AGCACTTAGGGACAAATTCA		
	R: CTCACTCTCCTCCATTCAAG	54°C	GuavaMap 2008
mPgCIR02	F: AGTGAACGACTGAAGACC		
	R: ATTACACATTCAGCCACTT	52°C	Risterucci et al. 2005
mPgCIR029	F: CTCGCTTCAATCTCCATCTA		
	R: AGCGACACAGACTCTTCATT	54°C	GuavaMap 2008
mPgCIR038	F: AGCCTGTTTTACGCCTTC		
	R: CGGCTGCTCTATTGTTATTT	53°C	GuavaMap 2008
mPgCIR040	F: TGAATCTCCAGTGTCTTATCG		
	R: TGATTTCAACTGCGTATGTC	53°C	GuavaMap 2008
mPgCIR042	F: CTCACCCAAATCTACACAAG		
	R: AAGGGACTGGACGATGTT	52°C	GuavaMap 2008
mPgCIR18	F: TAAGCTGCATGTGTGC		
	R: ATGGCTTTGGATGAAA	52°C	Risterucci et al. 2005
mPgCIR22	F: CATAAGGACATTTGAGGAA		
	R: AATAAGAAAGCGAGCAGA	56°C	Risterucci et al. 2005
mPgCIR94	F: CAACCTTCCCGTGATTATT		
	R: CTAGCTTCTTCAGTGGGAAC	53°C	GuavaMap 2008
mPgCIR99	F: TCAAAGTCCAAAACTCATGC		
	R: GGGATGGAGTAAAGATGAAA	50°C	GuavaMap 2008
mPgCIR102	F: AATTGGTGTAGCATCTGGA		
	R: GCCTACCATGAACAGAGAAA	53°C	GuavaMap 2008
mPgCIR108	F: AGGACCTCACAGAAGTTCAC		
	R: CGCTGTTACACTGTCGTT	56°C	GuavaMap 2008
mPgCIR131	F: GAGGTTGAGAGTTCAAGGT		
	R: GGTTTGCTCTTGAAATCACTC	53°C	GuavaMap 2008
mPgCIR148	F: CATACAGAGTCGGATGGTTT		
	R: GCTGCTGGTCTTAAAGCTAA	52°C	GuavaMap 2008
mPgCIR157	F: AACCACCAACCATACACC		
	R: CGACCAACCCTACATTCTG	50°C	GuavaMap 2008
mPgCIR159	F: GGTTAAGAGGCTTCAGTTCA		
	R: CAGCAAGGACAGGTTAAGAG	52°C	GuavaMap 2008
mPgCIR166	F: CTTTCCATCAAAACGTAAG		
	R: CCAATTCATGCACTTAGACA	50°C	GuavaMap 2008
mPgCIR172	F: CACCCTTAACTTCTGCTTTG		

Table II. (continuation)

Locus	Sequence (5' 3')	Annealing temperature	Reference
	R: GTCTCTATTTCCCTCCGTTC	52°C	GuavaMap 2008
mPgCIR173	F: CGTGTTCTTTACATCCGTCT		
	R: TACCAGCAACACCAATGC	52°C	GuavaMap 2008
mPgCIR178	F: TACAGCATTCTATCCCCTA		
	R: TCACGATGACAAGATAAAGC	53°C	GuavaMap 2008
mPgCIR180	F: CATGGATTCAACTCTTGTCG		
	R: CTACATTGGAAGCAGAATGG	53°C	GuavaMap 2008
mPgCIR184	F: AAGCTACAATCGACGAAAAC		
	R: CACTATTAGCGAACCTGCAT	54°C	GuavaMap 2008
mPgCIR187	F: AACGCCTAATAATGCGGAAGT		
	R: TCTTTCCCAGGATGGAGTA	52°C	GuavaMap 2008
mPgCIR188	F: TACAGCGATTCTATCCCCTA		
	R: TTGTGGGGAAGAAACTACTG	51°C	GuavaMap 2008
mPgCIR191	F: GACCCTCCACTTATATTTTG		
	R: AAGCTGACATAACAGTCGAA	53°C	GuavaMap 2008
mPgCIR193	F: GAACGTGGGTTACATACCAT		
	R: ATCACCGTCCTCCTAAATCT	52°C	GuavaMap 2008
mPgCIR197	F: CACCCAACTCTATACCCAAC		
	R: GTAGCTCACCAAGCTGAAAC	53°C	GuavaMap 2008
mPgCIR204	F: GTCGGATCATGGAGAGATCA		
	R: GCGGCTAAAGAAATCTGG	53°C	GuavaMap 2008
mPgCIR207	F: CAAGATTTGCCTCAAGAAAC		
	R: AACTAAATAGCCTGCTGGTG	53°C	GuavaMap 2008
mPgCIR210	F: CTACGAGGGTGTAACGAAAA		
	R: CTACAACAGCCAACGTGAG	53°C	GuavaMap 2008
mPgCIR227	F: GGGATGCTCAAAACTGTAAG		
	R: CCTGTTACATTGACGAATCA	53°C	GuavaMap 2008
mPgCIR230	F: CACATTTGCTCCTGATTTTC		
	R: GCTCTTCAACGACCATCTT	53°C	GuavaMap 2008
mPgCIR249	F: TTTGTCTGGTCGTCCTAGTT		
	R: CTTCAGTCCATCAGCAAAAT	52°C	GuavaMap 2008

heterozygosity varied from 0.14 to 0.26, averaging 0.21, whereas observed heterozygosity ranged from 0.21 to 0.42, averaging 0.28 (Table IV). Like heterozygosity, PIC can also be employed to quantify the genetic polymorphism of each population. The highest PIC value was observed for population I (*P. guineensi* × *P. cattleyanum* - 0.22), and the lowest for populations 3 and 4 (0.11), originating from the *P. cattleyanum* × *P. guineense* cross, similarly to what was observed

for heterozygosity (Table IV). The fixation index, which estimates the inbreeding coefficient of the populations, had its lowest estimated value in population 1 (−0.29), which was expected, since this population exhibited the highest values for PIC and He. The highest *f* value, in turn, was observed in population 2 (*P. guajava* × *P. cattleyanum*) (0.27) (Table IV).

Of the 33 loci analyzed, just 15 would be sufficient to estimate the genetic diversity of the populations (Figure 1). The ranking of PI (probability of identity) values obtained allowed us to highlight the most informative loci; i.e. those that most contributed to the differentiation of the populations (Table V). Most of these loci are associated with high Vg and H^2 values, which ranged from 0.1432 to 156.70265 and from 24.19284 to 99.80875, respectively (Table V).

The individual analysis clearly showed the formation of three large groups (Figure 2). The hybrids originating from the *P. guineense* × *P. cattleyanum* cross and their parents formed a group highlighted in blue that differed from the others. Hybrids whose female parent was *P. guajava*, in turn, were genetically closer to each other and remained in the same group highlighted in red. The hybrids resulting from the *P. cattleyanum* × *P. guineense* cross, which have one parent in common, displayed greater similarity with each other and also formed a single group highlighted in green (Figure 2). However, greater emphasis should be placed on the red group, which comprises the parents and hybrids of the *P. guajava* × *P. cattleyanum* cross, in which the genotypes genetically closer to *P. guajava* can be used as parents in future crosses with guava (Figure 2).

Genetic structure and diversity among populations of *Psidium* spp.

The Bayesian approach indicated that the *Psidium* populations were clustered into three genetic groups. According to the criterion of Evanno et al. (2005), the optimal ΔK was obtained when K=3, suggesting that maximum structuring was observed when the sample was divided into three groups (Figure 3). With a high probability of adhesion, the evaluated populations were grouped as follows: group 1 consisted of the genotypes originating from the *P. guineense* × *P. cattleyanum* cross, which corresponds to population 1; group 2 was formed by the hybrids from the *P. cattleyanum* × *P. guineense* cross, representing populations 3 and 4; and group 3 allocated the individuals resulting from the *P. guajava* × *P. cattleyanum* cross, corresponding to populations 2 and 5 (Figure 3). The Bayesian clustering method agrees with the number of groups observed in the dendrogram based on Nei's distance (Figure 3) and by the principal coordinate analysis (PCA) (Figure 4). The first two coordinates explained 99.98% of the total variation (PC1: 91.25%; PC2: 8.72%), and the largest genetic distance was observed between populations 1 and 2 (Figure 4). In addition, AMOVA indicated that approximately 80% of the variance originated from the differentiation between these populations and only 20% of the total variation was observed within the populations (Table VI).
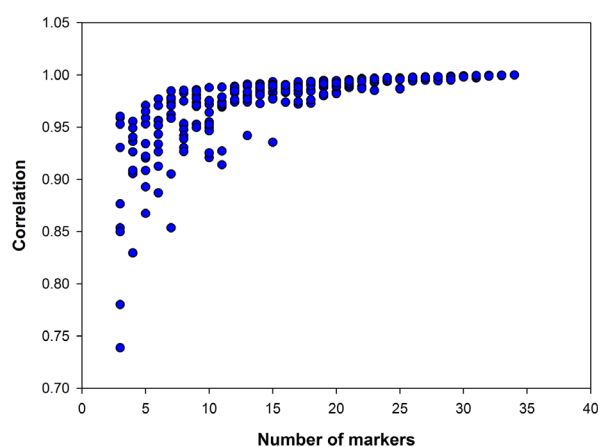
Figure 1. Optimal number of microsatellite markers analyzed in 94 individuals originating from five populations obtained from interspecific crosses of *Psidium* spp.

Table III. Number of alleles per locus (Na), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (f), coefficient of inbreeding among individuals in the subpopulations (FIS), degree of genetic differentiation among the total populations (FIT), and degree of genetic differentiation among the subpopulations (FST) of microsatellite primers evaluated in 94 individuals originating from five populations obtained from interspecific crosses of *Psidium* spp.

Locus	Na	Ho	He	f	FIS	FIT	FST
mPgCIR027	2	0.267	0.200	-0.335	-0.335	0.442	0.582
mPgCIR02	2	0.490	0.279	-0.731	-0.756	-0.100	0.373
mPgCIR029	2	0.010	0.033	0.487	0.699	0.979	0.932
mPgCIR038	2	0.402	0.231	-0.342	-0.737	-0.216	0.300
mPgCIR040	2	0.289	0.262	-0.082	-0.104	0.275	0.343
mPgCIR042	2	0.430	0.283	-0.332	-0.519	0.101	0.408
mPgCIR18	3	0.180	0.172	0.456	-0.049	0.653	0.669
mPgCIR22	4	0.241	0.216	0.191	-0.115	0.653	0.689
mPgCIR94	4	0.000	0.102	1.000	1.000	1.000	0.846
mPgCIR99	2	0.000	0.082	1.000	1.000	1.000	0.830
mPgCIR102	3	0.012	0.056	0.817	0.788	0.982	0.913
mPgCIR108	4	0.338	0.256	-0.272	-0.319	0.391	0.538
mPgCIR131	2	0.180	0.138	0.400	-0.304	0.639	0.724
mPgCIR148	3	0.281	0.300	0.040	0.063	0.565	0.535
mPgCIR157	3	0.712	0.508	-0.392	-0.402	-0.398	0.003
mPgCIR159	3	0.410	0.265	0.030	-0.549	0.187	0.475
mPgCIR166	2	0.023	0.044	0.314	0.489	0.953	0.907
mPgCIR172	2	0.000	0.046	1.000	1.000	1.000	0.903
mPgCIR173	3	0.439	0.310	-0.270	-0.418	-0.108	0.219
mPgCIR178	4	0.350	0.310	-0.136	-0.131	0.459	0.522
mPgCIR180	3	0.278	0.231	-0.133	-0.206	0.486	0.574
mPgCIR184	2	0.095	0.174	0.476	0.455	0.792	0.619
mPgCIR187	3	0.570	0.349	-0.127	-0.634	0.095	0.446
mPgCIR188	3	0.495	0.298	-0.512	-0.660	-0.318	0.206
mPgCIR191	2	0.130	0.141	0.029	0.081	0.735	0.711
mPgCIR193	2	0.871	0.480	-0.797	-0.813	-0.763	0.028
mPgCIR197	4	0.554	0.328	-0.699	-0.690	0.194	0.523
mPgCIR204	2	0.190	0.100	-0.905	-0.905	-0.105	0.420
mPgCIR207	2	0.503	0.290	-0.730	-0.733	-0.336	0.229
mPgCIR210	3	0.000	0.078	1.000	1.000	1.000	0.879
mPgCIR227	3	0.183	0.227	0.419	0.194	0.706	0.636
mPgCIR230	3	0.022	0.056	0.484	0.609	0.966	0.914
mPgCIR249	3	0.578	0.306	-0.890	-0.889	0.061	0.503
Average	2.70	0.288	0.216	0.018	-0.088	0.393	0.558

Table IV. Number of individuals (N), average number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (f), and polymorphic information content (PIC) estimated for the five populations obtained from interspecific crosses of Psidium spp.

Populations	N	Na	Ho	He	f	PIC
1- *P. guineensi* x *P. cattleyanum*	20	1.939	0.422	0.264	−0.209	0.227
2- *P. guajava.* x *P. cattleyanum*	16	2.091	0.276	0.269	0.272	0.209
3- *P. cattleyanum* x *P. guineensi*	21	1.667	0.220	0.145	−0.056	0.117
4- *P. cattleyanum* x *P. guineensi*	20	1.606	0.213	0.143	−0.030	0.112
5- *P. guajava.* x *P. cattleyanum*	17	2.030	0.312	0.263	0.087	0.205
Total average	-	1.867	0.289	0.217	0.018	0.174
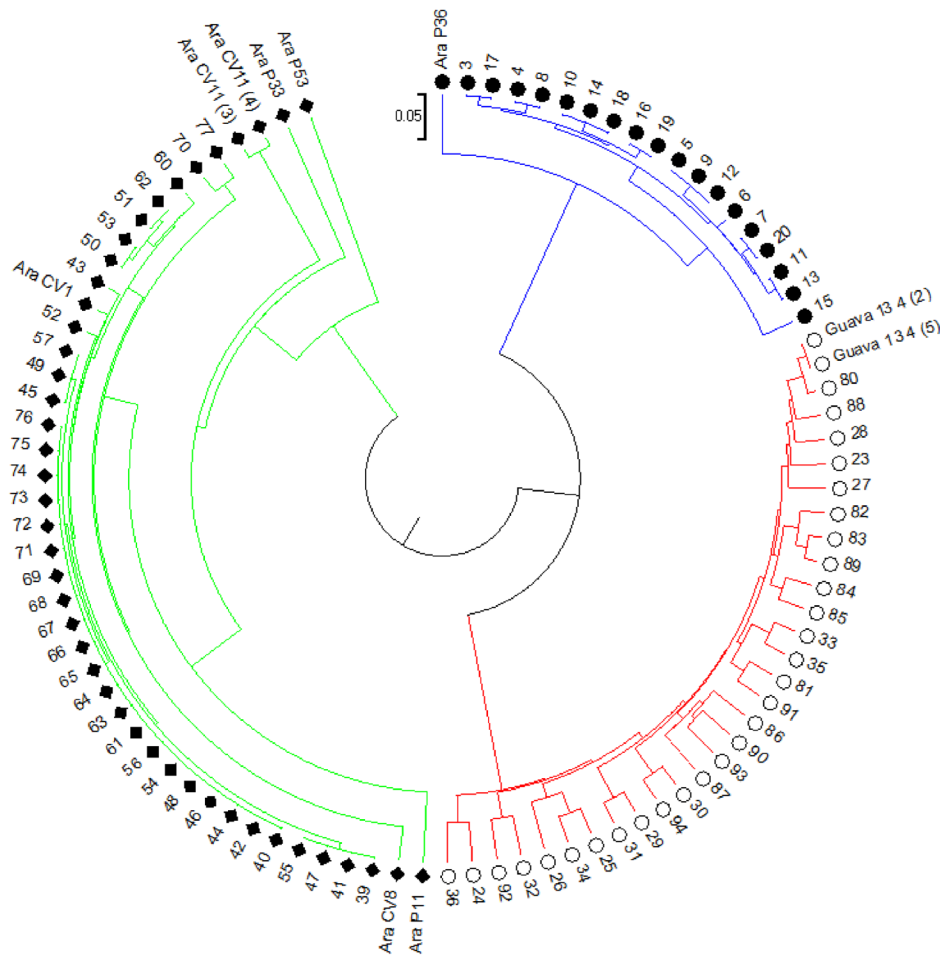
Figure 2. Dendrogram generated by the UPGMA method based on the distance established by the Weighted Index for 94 individuals originating from five populations obtained from interspecific crosses of *Psidium* spp. Blue group: formed by genitor Ara P36 and P. guineense (P36) × P. cattleyanum (P11) hybrids; red group: genitors Guava 13 4 (2), Guava 13 4 (5) and P. guajava (13.4II) × P. cattleyanum (P33) and P. guajava (13.4II) × P. cattleyanum (P51) hybrids; and green group: genitors Ara P11, Ara CV8, Ara CV1, Ara CV11 (3), CV11 (4), Ara P33 and Ara P53 and P. cattleyanum (CV8) × P. guineense (CV11) and P. cattleyanum (CV1) × P. guineense (CV11) hybrids. Cophenetic correlation = 0.96.

Table V. Probability of identity (PI), genetic variance (Vg), and genotypic determination coefficient (H^2) estimated for 33 loci analyzed in 94 individuals originating from five populations obtained from interspecific crosses of *Psidium* spp.

Locus	PI	Vg	H^2
mPgCIR027	**0.602716873**	41.97137	94.52649
mPgCIR02	**0.618206600**	5.11445	94.49821
mPgCIR029	**0.596622579**	35.77267	99.80875
mPgCIR038	**0.695545945**	0.1432	63.3445
mPgCIR040	**0.673962294**	6.13368	94.12912
mPgCIR042	**0.616323227**	28.39501	99.38077
mPgCIR18	0.579355142	26.91365	95.22583
mPgCIR22	0.447075859	156.70265	99.05381
mPgCIR94	0.466190125	156.56025	98.74012
mPgCIR99	**0.601036804**	22.58317	94.67371
mPgCIR102	0.484105523	98.38357	99.25399
mPgCIR108	0.560845960	129.87167	97.81555
mPgCIR131	0.594075284	25.77403	97.26393
mPgCIR148	0.485258277	48.42876	98.68225
mPgCIR157	0.584962079	2.73774	81.41414
mPgCIR159	0.581018923	106.92739	99.57495
mPgCIR166	**0.625022864**	32.37442	98.92227
mPgCIR172	**0.631224581**	30.35242	99.55321
mPgCIR173	**0.668312815**	1.22907	78.44428
mPgCIR178	0.497084892	113.24974	96.58938
mPgCIR180	0.578758663	28.04802	97.58626
mPgCIR184	0.615940082	35.51784	96.95175
mPgCIR187	0.478766598	17.92915	95.45898
mPgCIR188	**0.687178606**	0.17105	24.19284
mPgCIR191	**0.596684862**	29.45576	97.85715
mPgCIR193	**0.596622579**	2.48484	85.91457
mPgCIR197	0.450520043	73.66933	93.94008
mPgCIR204	**0.830680701**	0.18889	99.69952
mPgCIR207	**0.685762965**	0.18765	28.28196
mPgCIR210	0.475428004	74.24832	99.35794
mPgCIR227	0.503708104	20.64271	93.99346
mPgCIR230	0.47980167	71.38173	99.27513
mPgCIR249	0.514957842	29.89038	95.15053

The loci that most contributed to the differentiation of populations are in bold.

Table VI. Analysis of molecular variance (AMOVA) in segregating populations of *Psidium* spp. by simple sequence repeat (SSR) analysis.

Source	df	SS	MS	Estimated variance	Percentage of molecular variance
Among Pops	4	1832.883	458.221	24.120	80%
Within Pops	89	531.798	5.975	5.975	20%
Total	93	2364.681		30.095	100%

d.f. = degrees of freedom; SS = sum of squares; MS = mean square.

DISCUSSION

Genetic diversity is essential to sustain the productivity of a crop, since it provides 'new genes' for yield, adaptation, and disease resistance. The disease known as 'guava decline' has caused a strong economic impact for the guava crop, with losses estimated at over 90 million dollars (Gomes et al. 2014). Therefore, identifying resistant genotypes is a measure of paramount importance to increase yields. Interspecific crosses involving guava and araçá have been made as an alternative to attain success, since no variability exists for resistance to the nematode in the commercial species *P. guajava* (Gomes et al. 2017, Costa et al. 2017). However, the genetic breeding of perennial species is a long process, and thus markers have been used as an alternative to reduce the time and resources employed throughout the program until the release of a cultivar.

Genetic-molecular characterization of *Psidium* segregating populations by SSR markers

The microsatellites developed for *P. guajava* were efficiently transferred to the guava and araçá segregant populations. This can be corroborated by the work of Tuler et al. (2015), who investigated the transferability of microsatellite markers developed for *P. guajava* into another 12 *Psidium* species including *P. cattleyanum* and *P. guineense* and detected a high percentage of transferability, which evidences the narrow phylogenetic relationship between these species, considering that transferability occurs through the preservation of microsatellites and anchoring regions.

The maximum number of alleles per locus observed in *Psidium* populations was four. This result would be expected if the parents involved in the crosses were diploids and heterozygotes for different alleles. However, the araças are known polyploids, which would allow finding more than 4 alleles per locus, such result was not observed in the present study. Costa & Santos (2013) found seven to 22 alleles per locus in the 13 SSR analyzed for 61 accessions of genotyped *Psidium*. Aranguren et al. (2010) evaluated 31 accessions of native Venezuelan guavas and detected a total of 111 alleles and three to 11 alleles per locus in 16 SSR analyzed. Tuler et al. (2015) found one to six alleles per locus in 31 SSR markers used for the identification of 13 indigenous *Psidium* species of the Atlantic Forest. The highest number of alleles found in these studies is due to the genotypes studied by the authors belonging to germplasm banks of guava and araçá that have greater genetic variability.

Many descriptive measures have been employed in the search for the quantification of the genetic diversity between populations, making it possible to infer about the structure of the population in addition to the informative and discriminatory ability of the many classes of molecular markers in the process of genotypic identification and diversity analysis (Tuler et al.
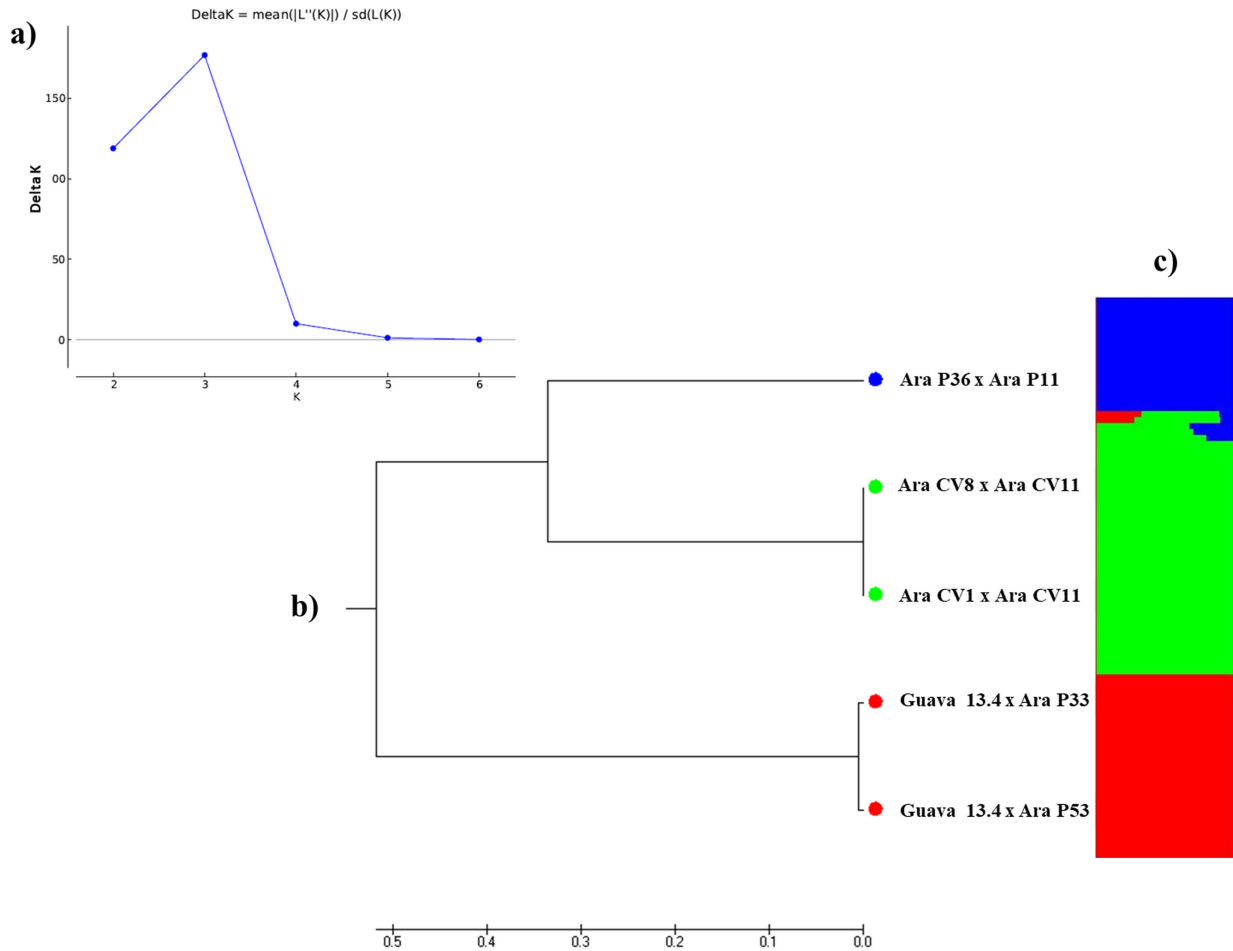
Figure 3: a) Delta K (ΔK) values for the respective numbers of groups (K) in five populations obtained from interspecific crosses of *Psidium* spp; **b)** Dendrogram generated by the UPGMA method based on Nei's genetic distance for the five populations obtained from interspecific crosses of *Psidium* spp; **c)** Clustering by Bayesian inference in five populations obtained from interspecific crosses of *Psidium* spp. with k = 3 groups.

2015, Pena et al. 2016). To this end, most research studies have adopted diversity parameters such as observed and expected heterozygosity and PIC.

In this study, the observed (Ho) and expected (He) heterozygosity values varied largely across the loci, indicating that the microsatellites were efficient in detecting variability between populations. Mean values for Ho (0.288) and He (0.216) were close, suggesting that the studied populations have medium genetic diversity inasmuch as the observed frequency of heterozygotes (Ho) is near the expected

level. Although the mean values for Ho and He obtained in the current study were not the expected for allogamous populations, especially those obtained from interspecific crosses, these findings are similar to that found by Tuler et al. (2015), who observed an average Ho of 0.15 using 23 SSR primers in 13 *Psidium* species. By contrast, P.G.O. Pessanha et al. (unpublished data) evaluated intraspecific hybrids of *P. guajava* using 10 SSR primers and obtained higher mean He (0.46) and Ho (0.58) values than those obtained here.
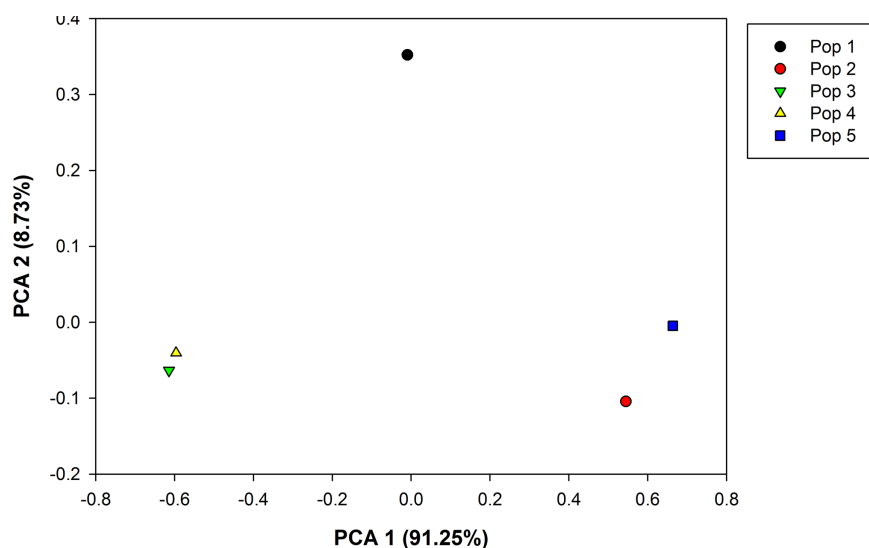
Figure 4. Scatter plot by Principal Coordinate Analysis based on Nei's genetic distance for the five populations obtained from interspecific crosses of *Psidium* spp.

The reduced estimates for heterozygosity found in this study may be a result of the presence of null alleles, a problem inherent to codominant markers (Ramos et al. 2014). This problem can be overcome by excluding these markers, which would not compromise the analysis, considering that of the 33 loci used in this study, 15 would be sufficient to estimate the genetic diversity of the populations. Most markers with a high level of information for the differentiation of the populations showed the highest genetic-variance values and are associated with those which had the highest H^2 values, which corresponds to broad-sense heritability.

Seventeen of the 33 loci showed negative fixation coefficients (*f*). This indicates that the alleles for these loci are not being fixed by either inbreeding or any other factor that might lead the population to a distancing from the Hardy-Weinberg Equilibrium, as expected for allogamous populations under random crosses. Negative estimates for the inbreeding coefficient are common when observed heterozygosity values are greater than the expected heterozygosity, as observed in this study, suggesting excessive loci in heterozygosis

(Cruz et al. 2011). However, in four loci, the estimated Ho was 0 and *f* was consequently 1, indicating that, for these loci, there is a greater deficit of heterozygotes than expected under an equilibrium condition, similarly to the reports of Tuler et al. (2015) for different *Psidium* species.

The analysis of diversity by Wright's F statistics is useful to determine how much each locus influences the diversity of studied populations (Cruz et al. 2011). As stated by Hernan (2009), FST values can range from 0 (no differentiation) to 1 (complete differentiation) between a specific group and the subgroups derived from it. Therefore, the high level of differentiation between the populations was confirmed by the high mean values of FST (0.55), contrasting with the low Fit values (−0.08), which indicate a greater occurrence of heterozygotes than homozygotes in the studied populations. These findings are expected, considering the intense gene flow taking place in cross-pollination species like guava and araçá. Similar results were found by Rajesh et al. (2008), who observed an elevated level of genetic differentiation (FST = 0.42) in coconut landraces using 14 SSR markers.

At population level, Ho and He were very close, and the fixation coefficients were low and mostly negative. According to Loiola et al. (2016), close or non-significant Ho and He values suggest a predominance of panmixia; i.e. the occurrence of random crosses in the populations. The populations would thus be under Hardy-Weinberg equilibrium. This cannot considered in the current study, though, since the evaluated populations were obtained from directed crosses between unrelated heterozygous parents. Populations 1 and 5 obtained the greatest genetic diversity, which can be corroborated by the higher PIC and He values. This result suggests the presence of more heterozygotes in these populations than expected under Hardy-Weinberg equilibrium (HWE). By contrast, populations 3 and 4 had the lowest PIC and He values, indicating that the observed level of polymorphism was low, generating variability within them.

Though formed exclusively based on the molecular profiles, the three groups showed some peculiar traits in relation to resistance to *M. enterolobii*. Considering all evaluated populations, the *P. cattleyanum* (CV1) × *P. guineense* (CV11) cross showed the lowest values for the reproduction factor, making it the population with the highest level of resistance and *P. guajava* (13.4 II) × *P. cattleyanum* (P51) the cross with the highest values (Gomes et al. 2017). However, with respect to the guava × araçá crosses, population *P. guajava* (13.4 II) × *P. cattleyanum* (P33) presented the lowest amplitude for reproduction factor (0 to 3.60) (Gomes et al. 2017). In this way, genotypes like 24, 29, and 33, with a reproduction factor of 0, can be selected to be used as parents in future crosses with guava.

Genetic Structure and diversity among populations of *Psidium* spp.

The Bayesian approach based on the criterion of Evanno et al. (2005) as well as the approach based on Nei's distance suggested the formation of three well-structured groups. Populations 3 and 4, which shared the same female parent *P. cattleyanum*, formed a single group and so did populations 2 and 5, which shared the same female parent *P. guajava* These last ones were the most distant from populations 1, 3, and 4, which originate from araçá × araçá crosses. A population derived from an interspecific cross is normally expected to have greater polymorphism; however, if the parents share most of the analyzed anatomical regions, this does not occur. The loci analyzed in this study may be in well-preserved regions of the parents' genome, with a low mutation rate (Tuler et al. 2015, Paiva et al. 2014), which may also explain the low number of polymorphic markers and the fact that populations 2 and 5 (*P. guajava* × *P. cattleyanum*) and 3 and 4 (*P. cattleyanum* × *P. guineense*) were grouped with each other.

This approach has been used in fruit trees to analyze the genetic structuring of 26 varieties of *Vitis* (Santana et al. 2012); for the genetic-molecular characterization of papaya genotypes obtained from three backcross generations and to check the genetic relationships between coconut accessions collected in Brazil and in different geographic regions of the world (Loiola et al. 2016). This is the first study under the Bayesian approach characterizing *Psidium* populations.

The SSR markers were efficient in discriminating the populations, which may help in new stages of the guava breeding program aimed at resistance to *M. enterolobii*. Hybrid 33, obtained from the *P. guajava* × *P. cattleyanum*, can be used as a parent in future crosses with the guava for the generation of the first backcross

(RC$_1$). Because this genotype is genetically closer to *P. guajava*, it has a larger number of alleles shared with this parent, which will certainly promote a faster recovery of the guava genome and the release of a cultivar resistant to the nematode.

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RAIANE M. SANTOS
https://orcid.org/0000-0002-0120-6049

ALEXANDRE P. VIANA
https://orcid.org/0000-0002-2475-4910

EILEEN A. SANTOS
https://orcid.org/0000-0001-8336-9839

RICARDO M. DE SOUZA
https://orcid.org/0000-0002-2076-0336

ODIMAR F. DE ALMEIDA
https://orcid.org/0000-0002-1187-7516

VICENTE M. GOMES
https://orcid.org/0000-0002-5027-2392

DANIELE L. RODRIGUES
https://orcid.org/0000-0002-9008-3705

PAULO RICARDO DOS SANTOS
https://orcid.org/0000-0002-5968-5636

Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Plant Breeding Laboratory, Center for Agricultural Sciences and Technologies (CCTA), Av. Alberto Lamego, 200, 28013-602 Campos dos Goytacazes, RJ, Brazil

Correspondence to: **Eileen Azevedo Santos**
E-mail: eileenbiologa@gmail.com

Author contributions

Conceptualization: Santos R.M. and Viana A.P.; Writing – Original Draft: Santos R.M. and Santos E.A.; Writing – Review and Editing: Santos R.M and Santos E.A.; Data acquisition: Santos R.M, Almeida O.F, Gomes V.M, Rodrigues D.L., Santos P.R.; Resources: Viana A.P.; Supervision: Santos E.A. and Viana A.P.