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Population Genetics of Polyploid Complex *Psidium cattleyanum* Sabine (Myrtaceae): Preliminary Analyses Based on New Species-Specific Microsatellite Loci and Extension to Other Species of the Genus

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Abstract

Polyploidy is a phenomenon that alters the genetic diversity of populations and has been reported as one of the most important evolutionary forces for plant diversification. The *Psidium cattleyanum* complex comprises a group of wild populations with several ploidy levels reported in the literature. The multiple cytotypes, associated with its wide distribution area, make this species a potential key model for understanding evolutionary processes related to polyploidization. In this study, we isolated and characterized nuclear microsatellite markers of *P. cattleyanum* and tested their transferability to other nine species of the genus. We performed a preliminary analysis of genetic diversity and population structure in three populations of *P. cattleyanum*. The three populations analyzed had different chromosome numbers, being polyploid cytotypes ($2n = 6x = 66$, $2n = 7x = 77$ and $2n = 8x = 88$). We designed 46 primer pairs and successfully amplified 37 markers, from which the 10 best were selected for analysis. Considering both the PIC and DP values, most of markers were highly informative. The new SSR markers were used to assess the levels of genetic diversity of the populations and detected one population with predominance of sexual reproduction. DAPC analysis pointed the formation of three groups, which corresponded to the populations analyzed. The markers were successfully amplified in related species, with some species presenting 80% transferability. By producing this panel of polymorphic microsatellites, we contribute to the understanding evolution in groups of natural polyploids for future studies.

Keywords Araçá · Cattley guava · Cytotype · Cross-amplification · Genetic structure · Polyploidy · SSR

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Introduction

Polyploidization has been reported as an evolutionary mechanism that promotes plant diversity (Soltis et al. 2009, 2010, 2014). The use of molecular biology techniques has revolutionized the study of polyploidy, revealing that a single species can frequently form polyploids (Soltis et al. 2014). Polyploidy arises from the multiplication of a single genome (autopolyploidy) or the combination of two or more different genomes, followed by the multiplication of this new hybrid genome (allopolyploidy) (Chen and Ni 2006; De Wet 1980). The existence of multiple origins of polyploidy and the possibility of gene flow between populations (diploids and polyploids) and between polyploids with different origins (auto and allopolyploids) demonstrate that polypolidization is a mechanism that causes genetic variation (Schifino-Wittmann 2004). Higher genetic diversity in polyploid organisms allows a species to expand its geographic distribution (Otto and Whitton 2000; Soltis and Soltis 2000), whereas the effects of polyploidy on plant growth, epigenetics and physiology is likely to lead to niche shift between the different ploidy levels (Baniaga et al. 2019; te Beest et al. 2011).

The neotropical genus *Psidium* L. (Myrtaceae) includes species of high economic importance, like *P. guajava* L. (common guava), and other species with great economic potential, with promising futures for their fruits, such as *P. cattleyanum* Sabine (Cattley guava) and *P. guineense* Sw. (Brazilian guava) (Franzon et al. 2009; Mani et al. 2011). In this genus, polyploidization events are recurrent, with records of diploid, triploid, tetraploid, hexaploid, and octaploid species (Marques et al. 2016), and also species with two or more ploidy level (Costa and Forni-Martins 2006).

Psidium cattleyanum presents several ploidy levels, which characterizes it as a polyploid complex. The chromosomal numbers reported in the literature include $2n = 33, 44, 46, 48, 55, 58, 66, 77, 82, 88, 99, 110$, and 132 (Atchison 1947; Costa and Forni-Martins 2006; Costa 2009; de Souza et al. 2015; Hirano and Nakazone 1969; Machado 2016; Raseira and Raseira 1996; Singhal et al. 1984; Souza-Pérez and Speroni 2017). Diploid individuals of *P. cattleyanum* ($2n = 22$) have not yet been observed and the origin of polyploid (either auto or allopolyploidy) in this taxon remains unknown. Two hypotheses regarding the origin of polyploidy in *P. cattleyanum* have been discussed: based on in situ hybridization studies (*FISH*), Costa (2009) raised the hypothesis that the tetraploid *P. cattleyanum* could have originated by the hybridization of two species with $2n = 22$ and differentiated genomes; meanwhile Medina (2014) and Machado (2016) suggested the hypothesis of an autopolyploid origin due to the repetition of a genome $x = 11$, based on observations of fluorochromes CMA/DAPI banding and mapping DNAr sites (using *FISH*).

Natural populations of *P. cattleyanum* are distributed in eastern Brazil, from Bahia to Rio Grande do Sul (Wikler 2007), and in Uruguay (Souza-Pérez and Speroni 2017). The species was introduced to other tropical regions, where it became an aggressive invader, suppressing native vegetation and causing ecological disturbance (Wikler 2007) and continues to invade new areas, being

considered one of the top 100 invasive plants of the world according to the Global Database of Invasive Species (2020). This highly invasive potential can be related to its reproduction strategies, among other factors.

The occurrence of asexual reproduction (i.e., apomixis and clonal reproduction) is strongly associated with polyploidy (Kólar et al. 2017; Van Drunen and Husband 2019). Recently, Souza-Pérez and Speroni (2017) detected apomixis in two cytotypes of *P. cattleyanum*. Regarding sexual reproduction, studies showed that the species can be autogamous (Raseira and Raseira 1996), and allogamous as well, with this strategy varying from individual to individual (Normand and Habib 2001). The occurrence of root sprout regeneration is also possible for this species (Global Database of Invasive Species 2020; Machado, personal observation).

The several ploidy levels recorded in *P. cattleyanum* reveal its potential as a model for understanding evolutionary processes in groups with polyploidy. Despite this potential, there are no data about the genetic diversity of *P. cattleyanum* populations. Most genetic analyses of *Psidium* have focused on investigating the genetic variability among common guava cultivars (Coser et al. 2012; Kherwar et al. 2018; Noia et al. 2017), whereas only few attempts have been made to assess the genetic diversity of indigenous *Psidium* species, using dominant markers (Mani et al. 2011; Sharma et al. 2005) and SSR (Simple Sequence Repeats) markers transferred from *P. guajava* (Tuler et al. 2015, 2019).

Due to their high polymorphism, molecular microsatellite (or SSR) markers are useful for evaluating the genetic similarity between closely related individuals or taxa (Hodel et al. 2016). However, the use of markers transferred from related species is associated with selection of the most polymorphic loci, which may lead to a bias in genetic diversity parameters (Queirós et al. 2015). Hence, despite the large panel of SSR markers developed for *P. guajava* (Noia et al. 2017; Tuler et al. 2015), analyses using species-specific microsatellites could be more accurate for measuring genetic variability in *P. cattleyanum*.

Therefore, the purpose of this study was to develop and characterize genomic microsatellite markers for *P. cattleyanum* and test the cross-amplification of these markers in other species of *Psidium*. Additionally, we performed preliminary analysis of genetic diversity and population structure in three wild populations of *P. cattleyanum* from Brazil. We also used three cytotypes to develop the libraries and tested whether the SSR markers are transferred to individuals of *P. cattleyanum* with other ploidy levels.

Materials and Methods

DNA Extraction and Ploidy Level Assessment

For DNA extraction of all individuals analyzed in this study (Tables 1, 5), we followed a modified method based Tel-Zur et al. (1999) using approximately 20 mg of leaves dehydrated in silica gel of each sample. We quantified the genomic DNA with a Nanodrop spectrophotometer.

Table 1 *Psidium cattleyanum* populations used to validate the microsatellite-enriched library (SSR)

Population	Number of individuals	Ploidy level	Proportion of ploidy	Collection site	Latitude	Longitude	Voucher UEC
PR-MX	15	Mixed, 7x and 8x	93.3% 2n=77; 6.6% 2n=88	Curitiba—PR	− 25.445917	− 49.233472	*R.M. Machado 1*
SP-6X	15	6x	100% 2n=66	Cunha—SP	− 23.138069	− 44.901639	*R.M. Machado 3*
RJ-8X	15	8x	100% 2n=88	Paraty—RJ	− 23.064306	− 44.678722	*R.M. Machado 4*

Ploidy levels of the three individuals used in the SSR-enriched library development were previously assessed by Machado (2016), whereas those of the studied populations of *P. cattleyanum* (PR-MX, RJ-8X and SP-6X from Table 1) were determined here based on direct chromosomal counts, following the protocol proposed by Guerra (2002). For each population, we sampled all 15 individuals for PR-MX, 10 individuals for RJ-8X and nine individuals for SP-6X and counted at least five cells per individual (Online Resource 3). Vouchers of all populations were deposited in the Herbarium UEC of the University of Campinas (Table 1).

Construction of the SSR-Enriched Library

To construct the *P. cattleyanum* microsatellites library, individuals of three ploidy levels were selected: $2n = 4x = 44$ (L1 = Bertioga, SP—Voucher: *R.M. Machado 2* (UEC)); $2n = 8x = 88$ (L2 = Curitiba, PR—Voucher: *R.M. Machado 1* (UEC); and $2n = 12x = 132$ (L3 = Uruguaiana, RS—Voucher: *A.V. Scatigna 670* (UEC), cultivated in a public park). We built the three microsatellite libraries (L1, L2 and L3) according to a modified protocol of Billotte et al. (1999). The total DNA was digested using the *Afa* I (10 u/µL) restriction enzyme. After digestion, we performed a connection reaction to the Rsa21 (10 µM) (5′CTCTTGCTTACGCGTGGACTA3′) and Rsa25 (10 µM) (5′TAGTCCACGCGTAAGCAAGAGCACA3′) adaptors to facilitate the enrichment step. In the enrichment step, we used probes (magnetic beads—Invitrogen-Dynal, Lillestrøm, Norway) to capture the DNA fragments containing microsatellite sequences, via hybridization with oligonucleotides Biotin-IIIII(CT)8 and Biotin-IIIII(GT)8.

The captured fragments were amplified by PCR and the resulting products were cloned in a pGEM-T vector (Promega, Madison, WI, USA). Afterwards, they were inserted into a competent *E. coli* cell (Agilent Technologies, Stratagene Products Division, La Jolla, CA, USA) via electroporation. We cultured the competent cells in petri dishes with agar and selected 94 positive colonies from each plate for transformation, then we extracted the vectors from them and sequenced a total of 282 recombinant clones.

Subsequently, we removed the adapters and performed trimming to eliminate contamination by the vectors using the Geneious v.9 platform (https://www.geneious.com). We also used this program to obtain the consensus sequences and searched for vectors using the VecScreen tool (https://www.ncbi.nlm.nih.gov/tools/vecscreen/). The consensus sequences were aligned using the NCBI nonredundant (Nr) database, through the BLASTN algorithm with an e-value cutoff of 1e−06 using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We identified the sequences containing microsatellites with the PHOBOS plugin (https://www.rub.de/ecoevo/cm/cm_phobos.htm) and designed the primers using Prime3Plus on Geneious v.9. All the primer pairs were synthesized with the sequence m13-5′CACGACGTTGTAAAACGA3′ (Schuelke 2000).

Validation of the Library and Statistical Analysis

To validate the microsatellite library, we used 45 individuals from three *P. cattleyanum* populations (15 individuals for population) (Table 1).

The PCR used 4 µl of genomic DNA (20 ng), 0.75 µl 10×Buffer (50 mM KCl, 20 mM Tris–HCl (pH 8.4)), 0.3 µl MgCl2 (50 mM), 0.4 µl dNTP (2.5 mM), 0.8 µl BSA (2.5 mg/ml), 0.1 µl of each primer (10 mM), 0.1 µl of fluorophore 700 ,or 800, 0.3 µl of Taq DNA Polymerase 5 U/µl (Roche®) and water to complete a final volume of 10 µl. The amplification conditions were based on those described by Tuler et al. (2015), with the specific annealing temperature of each primer pair (Table 2). After the optimization phase of the PCRs, the amplified products were checked on 6.5% acrylamide gel using the Li-Cor device (4300 DNA Analyzer) to confirm if the fragments were polymorphic. We also tested whether the primers designed for each cytotype ($2n = 4x = 44$, $2n = 8x = 88$ and $2n = 12x = 132$) were transferred to ploidy levels that differed from those of the original library. After the tests, we performed the amplification and genotyping of the studied populations (Table 1).

In the present study, we treated the microsatellites as dominant markers. The data were visually scored based on the presence (1) and absence (0) matrix for the bands on the acrylamide gel. This effectively reduces the information of the markers content and precludes analyses that consider observed heterozygosity of individuals or allele frequency distributions. However, polyploid microsatellite genotyping is difficult due to stutter peaks and/or allele overlap caused by multiple copies of the same size.

The polymorphism content (PIC—Botstein et al. 1980) and the discriminating power (DP—Tessier et al. 1999) (Table 3) were used to evaluate and characterize each marker. We estimated the Shannon index (Shannon 1948), Simpson index (Simpson 1949), and Nei diversity index (Nei 1978) to evaluate the genetic diversity of individuals, using the *poppr* (Kamvar et al. 2014) and *polysat* (Clark and Jasieniuk 2011) packages in the R 3.4.0 platform.

Since apomixis can occur in *P. cattleyanum* (Souza-Pérez and Speroni 2017), we decided to estimate the number of multilocus genotypes (MLG) and the eMLG, which is the MLG value of the smallest sample. Both of which are indirect inferences of asexual reproduction. The *poppr* package (Kamvar et al. 2014) in the R 3.4.0 platform was also used for these analyses.

We used a Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010), which uses a nonparametric approach, free from Hardy–Weinberg constraints, to detect the population structure and grouping of the individuals studied. DAPC combines PCA, K-means clustering and discriminant analysis to detect and visualize population structure and can be applied to polyploid datasets (Meirmans et al. 2018). This analysis was implemented in the R package *adegenet*. Two approaches were conducted: (1) the first DAPC analysis was performed, providing the information of three groups (populations of *P. cattleyanum*), and (2) the number of clusters was assessed using the function *find.clusters*. We assumed 15 as the maximum number of clusters. The optimal number of clusters was estimated using the Bayesian information criterion (BIC), both DAPC results were presented as scatter plots and the second approach was presented as a barplot.

Table 2 Characteristics of the 10 primer pairs designed for the microsatellites of the *Psidium cattleyanum* genome

Library	Name	GenBank		5′–3′ sequence	Motif	SZ	AT°C
L1	Pca-UNICAMP01	MN047794	F	GACTTGACAAGGGCAAAGTC	(CT)14(CT)8	206–148	55
			R	TAAAGGTGCATTTGTCTGCG			
L1	Pca-UNICAMP02	MN047795	F	AAGTTGGCAGGTCTAGTTCC	(TG)9	294–280	60
			R	TCAAGCTAGGTATGCTTCCC			
L1	Pca-UNICAMP04	MN047797	F	CCTTTTACACATTAGCTCTCTC	(CT)22	182–130	55
			R	GACCTGGGGTGTCATAACAA			
L1	Pca-UNICAMP05	MN047798	F	CAAAGTAGGTATGCTGCGTG	(CT)16(CA)10	100–94	63
			R	GCAAGTTAAACCGATCTGCA			
L2	Pca-UNICAMP06	MN047799	F	GACTTGACAAGGGCAAAGTC	(CT)16(CT)8	190–148	60
			R	CTGCGTGTGCTAGACCTTAA			
L2	Pca-UNICAMP07	MN047800	F	ACTAATGACGGTCCTTGAGAC	(GT)8(CT)20	214–210	51
			R	TTGTTGAGACTGCATGCATG			
L2	Pca-UNICAMP08	MN047801	F	GCACGTGCAAGAAAGAGAG	(AG)31(CT)26(TC)16(TG)9	234–200	60
			R	GTTCACACAGCACGCTAATT			
L2	Pca-UNICAMP09	MN047802	F	CATGAAAAATGAGTAGGCTCTC	(CT)15(AG)23	222–192	60
			R	CTCAGCTGGTTGTGCATAAC			
L2	Pca-UNICAMP10	MN047803	F	ACAACCCTTCTTTGCCCTAA	(TC)15(AC)11(CT)21	242–174	60
			R	ACAGATGTCATCAGAAGACACT			
L2	Pca-UNICAMP11	MN047804	F	CGTTATCTCCTTCCTCCGAG	(CT)11(TTC)8	290–176	60
			R	ATCGCCGATCAACTTCGAG			

SZ Size of the amplified fragment. *AT°C* specific annealing temperature of the primer pairs

Table 3 Description of the SSR markers developed for *Psidium cattleyanum*

Locus' name	NM	PIC	DP	Exclusive bands		
				PR-MX	SP-6X	RJ-8X
Pca-UNICAMP01	8	0.79	0.79	4	0	0
Pca-UNICAMP02	4	0.58	0.61	1	1	0
Pca-UNICAMP04	15	0.91	0.83	7	0	3
Pca-UNICAMP05	2	0.37	0.31	0	0	0
Pca-UNICAMP06	9	0.79	0.77	6	2	0
Pca-UNICAMP07	3	0.51	0.74	0	0	0
Pca-UNICAMP08	10	0.88	0.84	3	0	5
Pca-UNICAMP09	6	0.74	0.81	2	1	1
Pca-UNICAMP10	14	0.89	0.84	6	4	1
Pca-UNICAMP11	10	0.85	0.85	4	1	1
		Total exclusive bands		33	9	11

NM number of bands, *PIC* polymorphism content, *DP* discriminatory power and exclusive bands for each population

Cross-amplification of the *P. cattleyanum* Library

We tested the transferability of the SSR-enriched library developed for *P. cattleyanum* to nine species of the genus (one sample for each species): *P. acutangulum* DC., *P. australe* Cambess, *P. brownianum* Mart. ex DC., *P. friedrichsthalianum* (O. Berg) Nied., *P. gaudichaudianum* Proença & Faria, *P. guajava* L., *P. myrtoides* O. Berg, *P. oblongatum* O.Berg, and *P. rombeum* O.Berg. Dehydrated leaves of these nine species were provided by Prof. Marcia Flores da Silva Ferreira, who cultivates these plants at the Universidade Federal do Espírito Santo. PCR parameters were the same used herein for *P. cattleyanum*.

Results and Discussion

This is the first effort to assess genetic diversity and mating system in *P. cattleyanum* using species-specific SSR marker, which are also herein developed, and spanning different ploidy levels. Below, we present and discuss our results of the characterization of the microsatellite library, its cross-amplification to other nine species of *Psidium,* and the preliminary analysis of population genetics in natural populations of *P. cattleyanum*.

The population from Paraná (PR-MX) presented two cytotype, $2n = 77$ and $2n = 88$, but the first was predominant (14/15 samples); the population from São Paulo (SP-6X) presented only hexaploid specimens ($2n = 66$); and the population of Rio de Janeiro (RJ-8X) presented only octaploid specimens ($2n = 88$) (Table 1; Online Resource 3).

From the consensus sequences, we designed a total of 46 primer pairs (Online Resource 1) for the microsatellites' nuclear flanking regions, obtaining 18 primer pairs from the library of cytotype $2n = 4x = 44$, 12 from the library of cytotype $2n = 8x = 88$, and 16 from the library of cytotype $2n = 12x = 132$. We found the annealing temperatures for 37 pairs of primers, which are listed in Online Resource 1. Nine were discarded from the analysis because they did not amplify fragments under the various conditions tested.

Even if the primer pair was designed from the sequence of a specific cytotype library, we observed no amplification problems across different ploidy levels. Thus, we resumed the analysis considering only one general library for the species. We identified six pairs of primers with monomorphic products and 31 with polymorphic microsatellites, but 21 primer pairs had amplification artifacts (i.e., overlapping fragments or non-specific amplification resulting in several bands), which made genotyping difficult. Therefore, we selected the 10 pairs that resulted in ideal genotyping conditions, clear fragments, and easy interpretation on the gel (Table 2).

The PIC values obtained for the 10 markers ranged from 0.37 to 0.89, with an average of 0.73 (Table 3). According to the Botstein classification (Botstein et al. 1980), PIC values ≥ 0.5 are highly informative. Thus, nine loci were highly informative and only Pca-UNICAMP05 was moderately informative, with a PIC of 0.37. DP values ranged from 0.31 to 0.85, with an average of 0.74 (Table 3). Considering both the PIC and DP values found for each locus, four markers showed the highest levels of informativeness: Pca-UNICAMP04, Pca-UNICAMP10, Pca-UNICAMP08 and Pca-UNICAMP11 (Table 3).

For the 10 loci analyzed, the number of bands per locus ranged from 2 to 15 for *P. cattleyanum*, with an average of 8.2 (Table 3). Most of these bands were exclusive, i.e., bands observed in only one population; only two loci showed no exclusive bands (Pca-UNICAMP05 and Pca-UNICAMP07). The population with the highest number of exclusive bands was PR-MX, with a total of 33 bands. The exclusive bands represent a simple measurement of a population's genetic distinction (Oliveira et al. 2016).

The highest diversity levels (Table 4) were observed for SP-6X population (Shannon index: 2.52 and Simpson index: 0.91). Meanwhile, we found that the

Table 4 Genetic diversity estimates for the *Psidium cattleyanum* populations (Pop) evaluated in this study

Pop	N	MLG	eMLG	Shannon Index	Simpson Index	Nei Index
PR-MX	15	3	3	0.8820	0.551	0.279
SP-6X	15	13	13	2.5230	0.916	0.119
RJ-8X	15	10	10	2.1760	0.871	0.117
Total	45	26	11.3	2.9590	0.926	0.326

Population code according to Table 2

N number of individuals per population, *MLG* number of multilocus genotypes, *eMLG* expected number of MGL, considering the smallest sample size, *Shannon Index* Shannon index (Shannon 1948), *Simpson Index* Simpson diversity index (Simpson 1949), *Nei Index* Nei diversity index (Nei 1978)

population with two cytotypes (PR-MX) showed the lowest levels of genetic diversity (Shannon index: 0.88 and Simpson index: 0.551), when compared to populations with one cytotype only.

The PR-MX population also presented a low number of multilocus genotypes (Table 4), only three were detected, suggesting that this population was formed by a small number of generating individuals (founder effect), and was probably propagated via apomictic seeds. This hypothesis explains the low genetic diversity of the population indicated by the Shannon and Simpson indices, reinforced by the higher Nei diversity index (Nei 1978), showing the highest heterozygosity in the PR-MX population (0.279). One consequence of apomixis is the preservation of heterozygosity of the mother plant (Cruz et al. 1998). Apomixis has already been detected for Uruguayan *P. cattleyanum* populations, in the heptaploid and octaploid cytotypes (Souza-Pérez and Speroni 2017). Once the levels of genetic diversity in different cytotypes depend on many factors, i.e., changing mating system, geographical distribution, and environmental niche (Baduel et al. 2018; Baniaga et al. 2019; Meirmans et al. 2018), a more in-depth population analysis encompassing a larger number of individuals sampled can help to elucidate these results.

The results of the two DAPC analyses are presented in Figs. 1 and 2. The screen plots of eigenvalues of discriminant analysis and the amount of variation contained in the different principal components are presented in Online Resource 2. In the first approach, DAPC showed the formation of three groups (Fig. 1), which corresponded to the populations analyzed in this study: PR-MX = orange dots; SP-6X = gray dots; RJ-8X = dark blue dots. In the second analysis, we assumed no prior information about groupings of the individuals evaluated. Using the K-means algorithm and inspection of BIC values, six clusters were revealed to explain the variance in these groups of individuals. Therefore, the data were divided into six clusters (Fig. 2), with no clear delimitation of groups within our dataset. The barplot (Fig. 2c) showed a subdivision (structuring) in the populations analyzed. Both results of the DAPC analyses showed that the microsatellite markers (SSRs) developed for *P. cattleyanum* can detect genetic structure of

Fig. 1 Scatterplot of DAPC using the three populations of *Psidium cattleyanum* as predetermined groupings. Dots represent individuals and different colors represent the populations: PR-MX = orange dots; SP-6X = gray dots; RJ-8X = dark blue dots (Color figure online)
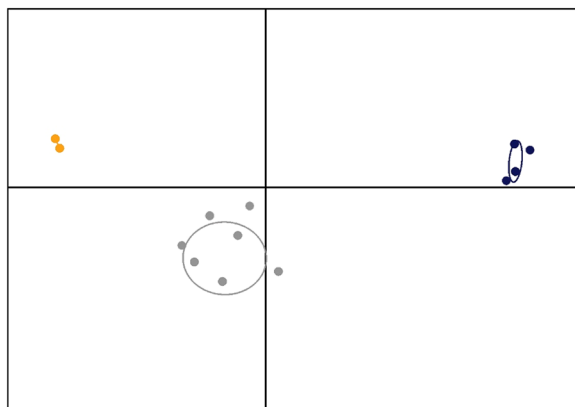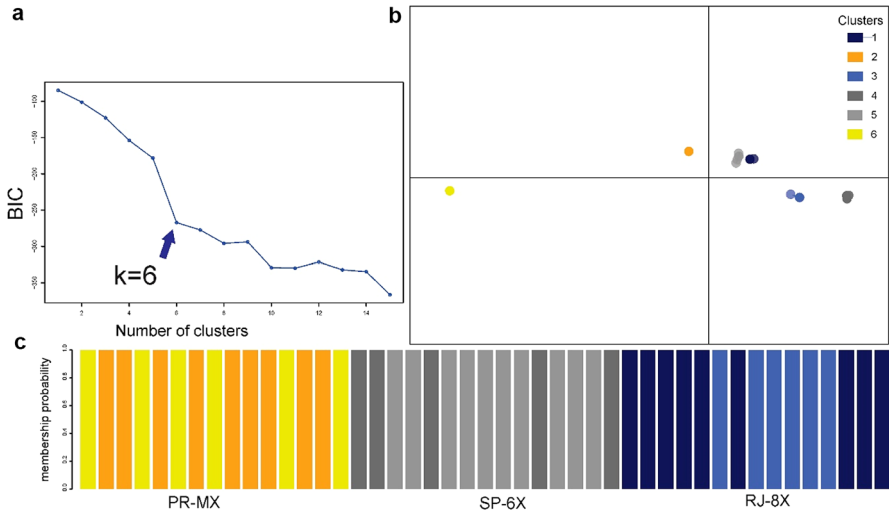
Fig. 2 DAPC scatterplots based on the K-means algorithm; **a** k number is selected based on BIC value for clusters up to k = 15; **b** scatter plot shows genetic patterns of SSR data; **c** bar plot showing the probabilities of assignment of individuals to k = 6 genetic DAPC clusters. PR-MX = yellow and orange dots/bars; SP-6X = gray and dark gray dots/bars; RJ-8X = blue and dark blue dots/bars (Color figure online)

populations. The ability to detect population structure with a clustering approach is an important part of genetic studies (Meirmans et al. 2018).

Of the nine species used in the transferability tests of the SSR library developed for *P. cattleyanum* (Table 5), seven showed amplification of at least two markers: *P. acutangulum* (30%), *P. australe* (10%), *P. brownianum* (70%), *P. friedrichsthalianum* (60%), *P. gaudichaudianum* (80%), *P. guajava* (20%), and *P. oblongatum* (70%). Only two species (*P. rhombeum* and *P. myrtoides*) presented no amplification of any marker. It is unclear whether there was no amplification due to the quality of extracted DNA or because the loci mutated or were lost in the differentiation of these species.

Many factors could be related to the low transferability rate found for some species, such as the genetic distance between groups and/or the difference in ploidy between *P. cattleyanum* and the other species tested in this study. Some studies have evaluated the relationships between *Psidium* species using the SSR library developed for *P. guajava* to compare and delimit them (Noia et al. 2017; Tuler et al. 2015). The transferability results demonstrate its potential for identifying groups in the genus based on the amplification patterns of the transferred library (de Oliveira Bernardes et al. 2018; Noia et al. 2017; Tuler et al. 2015). In both studies (Noia et al. 2017; Tuler et al. 2015), the *P. cattleyanum* species was grouped with *P. myrtoides*; however, the SSR markers developed for *P. cattleyanum* were not amplified for this species. These conflicting results demonstrate the importance of developing a species-specific library for detailed studies of genetics and population structure, with the development of the *P. cattleyanum* library being an important step.

Table 5 Transferability of the *Psidium cattleyanum* species' SSR library to other species of the *Psidium* genus

Species	Pca-UNI-CAMP01	Pca-UNI-CAMP02	Pca-UNI-CAMP04	Pca-UNI-CAMP05	Pca-UNI-CAMP06	Pca-UNI-CAMP07	Pca-UNI-CAMP08	Pca-UNI-CAMP09	Pca-UNI-CAMP10	Pca-UNI-CAMP11	% amplified loci
P. acutangulum DC	–	+	–	–	+	–	+	–	–	–	30
P. australe Cambess	–	–	–	–	+	–	–	–	–	–	10
P. brownianum Mart. ex DC	+	–	+	+	+	–	–	+	+	+	70
P. friedrichsthalianum (O. Berg) Nied	+	–	+	+	–	+	–	+	–	+	60
P. gaudichaudianum Proença & Faria	+	+	–	+	+	+	+	+	–	+	80
P. guajava L	–	+	–	–	+	–	–	–	–	–	20
P. myrtoides O. Berg	–	–	–	–	–	–	–	–	–	–	0
P. oblongatum O.Berg	+	–	+	+	+	+	–	+	+	–	70
P. rhombeum O.Berg	–	–	–	–	–	–	–	–	–	–	0
Total	4	3	3	4	6	3	2	4	2	3	

The loci characterized herein were successfully cross-amplified, suggesting that these markers can be used in genetic studies for other species of the genus. In addition, these newly developed SSR markers can be used to develop future breeding programs, since some species have fruits that are highly appreciated by local populations, i.e., *araçá* (*P. cattleyanum*), or global ones, i.e., guava (*P. guajava*). Additionally, markers can detect asexual reproduction, with apomixis being a huge advantage for commercial development, producing uniform *P. cattleyanum* offspring.

In the future, we aim to estimate the genetic diversity of additional natural *P. cattleyanum* populations by relating it to the amount of cytotypes and ploidy levels within each population. Furthermore, our markers could help with invasive species control programs that target *P. cattleyanum*. Finally, the SSR markers are an important contribution to research involving groups with natural polyploids.

Compliance with Ethical Standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethics Approval This article does not include studies about humans or animals performed by any of the authors.

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