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Analysis of transferability of microsatellite primers (SSR) in wild *Passiflora* species and intraspecific genetic diversity in *Passiflora alata*

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ABSTRACT. The genus *Passiflora* L. is the most representative of Passifloraceae, with over 500 known species, among which 150-200 originated from Brazil. In addition to the great commercial importance of this genus for the fruit market, many of the species have exotic flowers with a huge diversity of colors and can thereby be exploited as ornamental plants. This study was aimed at investigating the transferability of microsatellite primers in wild *Passiflora* species (*P. cacao*, *P. cincinnata*, *P. glandulosa*, *P. gibertii*, and *P. mucronata*) and characterizing 29 *P. alata* accessions using microsatellite primers that were previously developed in a library enriched with microsatellites from *P. edulis* f. *flavicarpa* for *P. alata*. The interspecies cross-amplification rate varied, and *P. cacao* exhibited the highest rate of amplification, suggesting a greater degree of proximity to *P. edulis*. The study of intraspecific accessions in *P. alata* found genetic similarity, with values ranging from 0.47 to 1.00 and an average similarity

of 0.74. Hence, this study revealed the intraspecific genetic variability of *P. alata* in the Universidade Estadual de Santa Cruz's Active Germplasm Bank and will lead to the adoption of mating strategies between accessions; thus making their use more suitable for breeding purposes.

Key words: Genetic diversity; Passion flowers; Microsatellite markers; Transferability of primers

INTRODUCTION

Passiflora is the most represented genus in the Passifloraceae family, with a broad geographical distribution and species occurring both in tropical and warm temperate regions (Cervi, 2006). Despite the large representation of *Passiflora*, this genus encompasses about 520 species (Cervi, 2005); yet, many of the species are still being discovered and characterized (Vitta and Bernacci, 2004; Bernacci and Souza, 2012). The representatives of the genus *Passiflora* are distributed in tropical forests stretching from North America to South America, with endemic species of the Brazilian flora. The economic importance of passion flowers stems chiefly from its fruit. *P. alata* (sweet passion fruit) and *P. edulis* f. *flavicarpa* (passion fruit) are the most commercialized species, which are generally directed at the food industry. Other qualities are assigned to species of this genus, such as medicinal use - where *P. incarnata* stands out because of its anxiolytic and cosmetic effect (Dhawan et al., 2004). *P. caerulea*, *P. incarnata*, and several interspecific hybrids cultivated mainly in Europe and the USA stand out in the ornamental market (Rushing, 2003). The variables determining the potential for the ornamental market are shape, size, color, and beauty; this goes as much for floral pieces as it does for vegetative parts (Abreu et al., 2009). Despite *Passiflora* being little recognized as ornamental plants in Brazil (Abreu et al., 2009), many studies aimed at obtaining cultivars directed at the soils and climate of Brazil are in progress (Abreu, 2008; Santos et al., 2008; Viana, 2009).

The characterization of cultivars and wild species maintained in germplasm banks cover the morphoagronomic (Souza et al., 2008), cytogenetic (Peñaloza and Pozzobon, 2007), botanic (Guen et al., 2002), and molecular markers (Crochemore et al., 2003). Among the molecular markers, the simple sequence repeat (SSR) and SSR-polymerase chain reaction (PCR) microsatellites are widely used in studies of inter- and intra-specific diversity and population inferences and thus help to outline conservation strategies (Pádua, 2004). Among the advantages of SSR markers, one may mention the codominant nature of the observed polymorphism, as well as the multiallelism and high polymorphism information content (Ferreira and Grattapaglia, 1996). On the other hand, the biggest technical bottleneck of SSR-PCR is the necessity of prior knowledge of microsatellite sequences to design specific primers for SSR loci. Nevertheless, the potential transferability of primers between species of the same genus has been reported in several plant groups, allowing the application of the SSR-PCR technique in other species (Ferreira and Grattapaglia, 1996; Oliveira et al., 2005).

Studies involving the molecular approach with microsatellite markers can be considered incipient because of the scarcity of these markers for species of the genus *Passiflora*. SSR markers are only available for three species of the genus: *P. edulis* f. *flavicarpa*, *P. alata* (Pádua et al., 2005; Oliveira, 2006), and *P. cincinnata* (Cerqueira-Silva et al., 2012). Using 25 microsatellite primers developed for *P. edulis* f. *flavicarpa*, cross-amplification has provided different transfer rates (Cerqueira-Silva et al., 2008a; Conceição et al., 2009).

This study aimed to assess the rate of transferability of SSR primers from five wild species kept at the Active Germplasm Bank of Universidade Estadual de Santa Cruz (UESC). At the same time, the genetic diversity among accessions of *P. alata* was analyzed for future use in conservation and breeding programs.

MATERIAL AND METHODS

The analysis of transferability of SSR primers involved five wild species of the genus *Passiflora*: *P. cacao*, *P. cincinnata*, *P. gibertii*, *P. glandulosa*, and *P. mucronata* (Table 1). The germplasm used in this study was obtained from samples collected in fragments of the Atlantic Forest from southern Bahia, and accessions granted by research institutions of Brazil. All of the species were identified and deposited at the Agronomic Institute of Campinas, São Paulo, and Universidade Federal do Paraná, Curitiba, Paraná. During analysis, the species were kept in the Active Germplasm-Passifloras of UESC, Ilhéus, BA, Brazil (39°10'W, 14°39'S, altitude 78 m), in a rustic greenhouse measuring 6 x 7 x 39 m (the semi-arc type), covered with plastic plus additives against ultraviolet rays, and coated with 30% shade cloth.

Table 1. *Passiflora* species analyzed by SSR and kept at the Active Germplasm Bank of UESC.

Species	Accession number	Origin	Acquisition
P. alata Curtis	101, 102, 104, 242, 244, 245, 317, 53	No information	Donation
P. alata	54, 55, 78, 79, 80, 81, 82, 83, 86, 88, 109, 114, 115	Camacan, Serra Bonita, BA	Collection
P. alata	25, 39, 312	Instituto Plantarum	Donation
P. alata	59, 60, 85, 87, 359	Fazenda Ouro Verde, Una, BA	Collection
P. cacao Bernacci and Souza	344, 346, 347, 348	Serra Bonita, Camacan, BA	Collection
P. cincinnata Mast.	199	No information	Donation
P. cincinnata	42, 52	Pato de Minas, MG	Donation
P. cincinnata	334	Fazenda Ouro Verde, Una, BA	Collection
P. gibertii N.E. Brown	171, 172, 173, 174	Embrapa Cerrados	Donation
P. glandulosa Cav.	G02, G03, G04, G05, G06, G07	Fazenda Ouro Verde, Una, BA	Collection
P. mucronata Lam.	127, 128, 129, 130	Praia do Aeroporto, Ilhéus, BA	Collection

Genomic DNA was extracted in duplicate from young leaves using the methodology proposed by Doyle and Doyle (1990). SSR amplifications were performed using 31 microsatellite primer pairs, where 10 primers, pe01, pe02, pe03, pe04, pe05, pe06, pe07, pe08, pe09, and pe10 (Oliveira et al., 2005), and 14 primers, pe11, pe27, pe28, pe37, pe38, pe42, pe54, pe58, pe59, pe60, pe64, pe66, pe75, and pe90 (Oliveira, 2006), were previously developed for *P. edulis* f. *flavicarpa*. The primers A01BP3, A01FP3, A03AP3, A06FP1, A07FP1, A08GP1, and A08FP1 were developed for *P. alata* (Pádua et al., 2005). The SSR-PCR amplifications were performed according to Oliveira et al. (2005), Oliveira (2006), and Pádua et al. (2005).

The tests assessing transference from primers of *P. alata* and *P. edulis* f. *flavicarpa* to different species were made in four accessions of each species as a means to amplify specific loci and adjust the reaction conditions. The amplification products were subjected to gel electrophoresis on 2.5% agarose gels and 6% denaturing polyacrylamide gels and were then detected by staining with silver nitrate ($AgNO_3$) according to Creste et al. (2001).

The same primers used in the analysis of transferability were used in the analysis of intraspecific genetic diversity in *P. alata*, as represented by 29 accessions (Table 1). The products generated by the amplification of microsatellite primers were considered to be domi-

nant markers, and the presence and absence of SSR loci were evaluated. The amplified bands specific to SSR loci were analyzed and used in the construction of a binary matrix wherein 1 (one) was the value denoting the presence of the band while 0 (zero) denoted absence. Binary data served as the basis for calculating the similarity matrix for all accessions using Dice's similarity coefficient (Dice, 1945). Analyses were performed using unweighted pair group method with an arithmetic mean (UPGMA) and the sequential agglomerative hierarquic nonoverlapping procedure. Likewise, a principal coordinate analysis (PCO) generated using Dice's similarity coefficient was conducted. In order to estimate the significance of the correlation between the similarity matrix and the grouping matrix, the test of matrix comparison of Mantel (1967) was applied with a thousand permutations and observed from the cophenetic correlation (r). All analyses were performed using the NTSYSpc 2.0 software (Rohlf, 2000).

RESULTS

Transferability rate of microsatellite primers in wild *Passiflora* species

Pairs of specific primers for SSR loci were previously developed for two economically important species of the genus *Passiflora*: *P. edulis* Sims f. *flavicarpa* (24 primers) (Oliveira et al., 2005; Oliveira, 2006) and *P. alata* (7 primers) (Pádua et al., 2005). These 31 primers were tested in five wild *Passiflora* species to verify the transferability percentage of SSR primers from the respective species. The results revealed that a minimum of 7 and a maximum of 18 SSR primers were transferred per species (Tables 2 and 3). Among the 7 primers that were previously developed for *P. alata*, the highest rate of amplification was observed in *P. cacao* and *P. gibertii* (28.5%). On the other hand, no amplification was observed in the species *P. glandulosa* and *P. mucronata*. The transferability of the 24 primers developed for *P. edulis* f. *flavicarpa* revealed a higher amplification percentage (62.5%) in *P. cacao*. The remaining species, in turn, had a lower percentage of transferability (37.5%), with the exception of *P. glandulosa*, whose transfer rate was lower (29.1%) (Table 3).

For each primer pair that was used in the transferability tests, the percentage of amplification of all of the five species studied was obtained (Table 4). The amplification rate of primers remained between 14.2 and 42.8%, with null amplification in some of the primers. The primer pairs pe07 and pe75 showed a high percentage of transferability (87.7%). The primers A07FP1 and A08FP1 exhibited transferability rates of 71.4 and 57.1%, respectively.

Table 2. Cross-amplification results reported in the 31 loci analyzed for the five wild *Passiflora* species on 2.5% agarose gel.

Primers	*P. cacao*	*P. cincinnata*	*P. gibertii*	*P. glandulosa*	*P. mucronata*
A01BP3	-	-	-	-	-
A01FP3	-	-	-	-	-
A03AP3	-	-	-	-	-
A06FP1	+	-	-	-	-
A07FP1	+	+	+	-	-
A08GP1	-	-	-	-	-
A08FP1	+	-	+	-	-
T (%)	28.5	14.4	28.5	0	0

T (%) = transferability rate; (-) = no amplification; (+) = observed amplification. Primers developed for *P. alata* (Pádua et al., 2005).

Table 3. Cross-amplification results reported in the 31 loci analyzed for the five wild *Passiflora* species on 2.5% agarose gel.

Primers	*P. cacao*	*P. cincinnata*	*P. gibertii*	*P. glandulosa*	*P. mucronata*
pe01	+	+	-	+	-
pe02	+	-	-	+	-
pe03	-	-	-	-	-
pe04	+	-	-	-	+
pe05	-	-	-	-	+
pe06	+	-	-	+	+
pe07	+	+	-	+	+
pe08	-	+	-	-	-
pe09	-	-	+	-	-
pe10	+	-	-	-	-
pe11	+	-	-	-	+
pe27	-	+	-	-	-
pe28	-	-	-	+	-
pe37	+	-	+	-	+
pe38	+	-	+	+	-
pe42	+	-	-	-	-
pe54	+	-	+	-	+
pe58	+	-	+	+	+
pe59	+	-	-	-	-
pe60	-	-	-	-	-
pe64	-	+	-	-	-
pe66	+	-	-	-	+
pe75	+	+	+	-	+
pe90	+	-	+	-	-
T (%)	62.5	37.5	37.5	29.1	37.5

T (%) = transferability rate; (-) = no amplification; (+) = observed amplification. Primers developed for *P. edulis* f. *flavicarpa* (Oliveira et al., 2005; Oliveira, 2006).

Table 4. Transferability rates observed in the presence of PCR products on agarose gel with primer pairs designed from *Passiflora edulis* f. *flavicarpa* and *P. alata* in wild *Passiflora* species.

Primers	T (%)
pe07, pe75	87.7
A07FP1	71.4
A08FP1, pe37, pe58	57.1
pe01, pe02, pe04, pe06, pe11, pe27, pe54, pe66	42.8
pe08, pe38, pe59, pe64	28.5
A06FP1, A08GP1, pe03, pe10, pe28, pe42, pe90, pe05	14.2
A01BP3, A01FP3, A03AP3	0.0

T (%) = transferability rate.

Analysis of intraspecific genetic diversity in accessions of *P. alata*

The intraspecific study analyzing accessions of *P. alata* was carried out with the use of six microsatellite primer pairs, revealing a total of 18 polymorphic alleles. The average similarity obtained was 0.74, whereas the observed maximum similarity was 1.00 and the minimum was 0.47. The correlation coefficient (r) of 0.78 suggests a good fit between the graphical representations of distances generated by the UPGMA grouping method. The cophenetic distance was high by the Mantel test (p[Zrdmd ≤ Zobs] = 1000 / *t*-test = 10,2716), since values ≥ 0.56 are considered to be optimal (Vaz Patto et al., 2004). The lowest similarity coefficient of SSR loci (0.47) was observed between the accessions 39 and 59 and between 27 and 109. On the other hand, the highest similarity coefficient (1.00) was observed between genotypes 102 and 244, 53 and 312, 54 and 55-82-87, and 55 and 82-87 (Table 5), showing an overall genetic similarity among SSR loci.

Table 5. Dice's similarity coefficient of *Passiflora alata* accessions based on SSR markers.

Acc.	104	101	102	245	244	242	317	25	39	312	53	54	55	59	60	78	79	80	81	82	83	359	85	86	87	88	109	114	115
104	*****																												
101	0.7692	*****																											
102	0.7143	0.9333	*****																										
245	0.7692	0.8571	0.9333	*****																									
244	0.7143	0.9333	1.0000	0.9333	*****																								
242	0.6250	0.8235	0.8889	0.8235	0.8889	*****																							
317	0.6667	0.8750	0.9412	0.8750	0.9412	0.9474	*****																						
25	0.7143	0.8000	0.7500	0.6667	0.7500	0.7778	0.8235	*****																					
39	0.7692	0.8571	0.8000	0.7143	0.8000	0.7059	0.7500	0.9333	*****																				
312	0.7692	0.7143	0.6667	0.7143	0.6667	0.7059	0.7500	0.9333	0.8571	*****																			
53	0.7692	0.7143	0.6667	0.7143	0.6667	0.7059	0.7500	0.9333	0.8571	1.0000	*****																		
54	0.8333	0.6154	0.7143	0.7692	0.7143	0.7500	0.8000	0.7143	0.6154	0.7692	0.7692	*****																	
55	0.8571	0.6667	0.7500	0.8000	0.7500	0.7778	0.8235	0.7500	0.6667	0.8000	0.8000	1.0000	*****																
59	0.6250	0.5882	0.5556	0.5882	0.5556	0.7000	0.6316	0.5556	0.4706	0.5882	0.5882	0.6250	0.6667	*****															
60	0.7692	0.6154	0.7143	0.7143	0.7143	0.7500	0.8000	0.7143	0.6154	0.7143	0.7143	0.9231	0.9333	0.5882	*****														
78	0.6667	0.5000	0.5882	0.6250	0.5882	0.6316	0.6667	0.5882	0.5000	0.6250	0.6250	0.9333	0.8235	0.5263	0.8750	*****													
79	0.7500	0.5882	0.6667	0.7059	0.6667	0.7000	0.7368	0.6667	0.5882	0.7059	0.7059	0.8750	0.8889	0.6000	0.9412	0.8421	*****												
80	0.9231	0.7143	0.6667	0.7143	0.6667	0.5882	0.6250	0.6667	0.7143	0.7143	0.7143	0.7692	0.8000	0.5882	0.7143	0.6250	0.8235	*****											
81	0.6667	0.6154	0.5714	0.6154	0.5714	0.6250	0.6667	0.6667	0.5714	0.7143	0.7143	0.6667	0.7143	0.8000	0.6154	0.5333	0.7500	0.7692	*****										
82	0.8571	0.6667	0.7500	0.8000	0.7500	0.7778	0.8235	0.7500	0.6667	0.8000	0.8000	1.0000	1.0000	0.6667	0.9333	0.8235	0.8889	0.8000	0.7143	*****									
83	0.8000	0.6250	0.7059	0.7500	0.7059	0.7368	0.7778	0.7059	0.6250	0.7500	0.7500	0.9333	0.9412	0.6316	0.8750	0.7778	0.9474	0.8750	0.8000	0.9412	*****								
359	0.6667	0.6250	0.7059	0.7500	0.7059	0.7368	0.7778	0.7059	0.6250	0.7500	0.7500	0.8000	0.8235	0.5263	0.7500	0.6667	0.8421	0.7500	0.7500	0.8235	0.8889	*****							
85	0.6250	0.5882	0.6667	0.7059	0.6667	0.7000	0.7368	0.6667	0.5882	0.7059	0.7059	0.7500	0.7778	0.6000	0.8235	0.7368	0.9000	0.7059	0.7059	0.7778	0.8421	0.8421	*****						
86	0.5882	0.5556	0.6316	0.6667	0.6316	0.6667	0.7000	0.6316	0.5556	0.6667	0.6667	0.7059	0.7368	0.5714	0.7778	0.7000	0.8571	0.6667	0.6667	0.7368	0.8000	0.8000	0.9524	*****					
87	0.8571	0.6667	0.7500	0.8000	0.7500	0.7778	0.8235	0.7500	0.6667	0.8000	0.8000	1.0000	1.0000	0.6667	0.9333	0.8235	0.8889	0.8000	0.7143	1.0000	0.9412	0.8235	0.7778	0.7368	*****				
88	0.8000	0.6250	0.7059	0.7500	0.7059	0.6316	0.6667	0.5882	0.6250	0.6250	0.6250	0.8000	0.8235	0.5263	0.8750	0.7778	0.9474	0.8750	0.6667	0.8235	0.8889	0.7778	0.8421	0.8000	0.8235	*****			
109	0.6667	0.5000	0.5882	0.6250	0.5882	0.5263	0.5556	0.4706	0.5000	0.5000	0.5000	0.6667	0.7059	0.6316	0.6250	0.5556	0.7368	0.7500	0.8000	0.7059	0.7778	0.6667	0.6316	0.6000	0.7059	0.7778	*****		
114	0.8000	0.6250	0.7059	0.7500	0.7059	0.6316	0.6667	0.5882	0.6250	0.6250	0.6250	0.8000	0.8235	0.6316	0.7500	0.6667	0.8421	0.8750	0.8000	0.8235	0.8889	0.7778	0.7368	0.7000	0.8235	0.8889	0.8889	*****	
115	0.9231	0.7143	0.8000	0.8571	0.8000	0.7059	0.7500	0.6667	0.7143	0.7143	0.7143	0.9231	0.9333	0.5882	0.8571	0.7500	0.8235	0.8571	0.6154	0.9333	0.8750	0.7500	0.7059	0.6667	0.9333	0.8750	0.7500	0.8750	*****

A graphical representation of the group of individuals clearly formed three groups that were obtained from the cut-off point calculated by the average similarity = 0.74 (Figure 1) (Silva et al., 2008; Amorim et al., 2009). Group I was formed by 17 accessions and includes most of the accessions collected in Camacan and Una, BA. Group II, in turn, consists of 10 accessions and exhibited individuals whose genotype data are identical for the loci analyzed: accessions 102 and 244, and 312 and 53. Individuals from this group are the ones whose places of collection have not been reported, and all accessions were granted by Instituto Plantarum. Group III only consisted of two accessions. This group significantly differed from the other groups (Figure 1). In some of the groups, no relationship was observed between the place or origin of collection and genetic similarity. This fact was observed in group III, which consisted of two accessions of different origins and whose similarity values were low when compared with those of groups I and II (Figure 1).

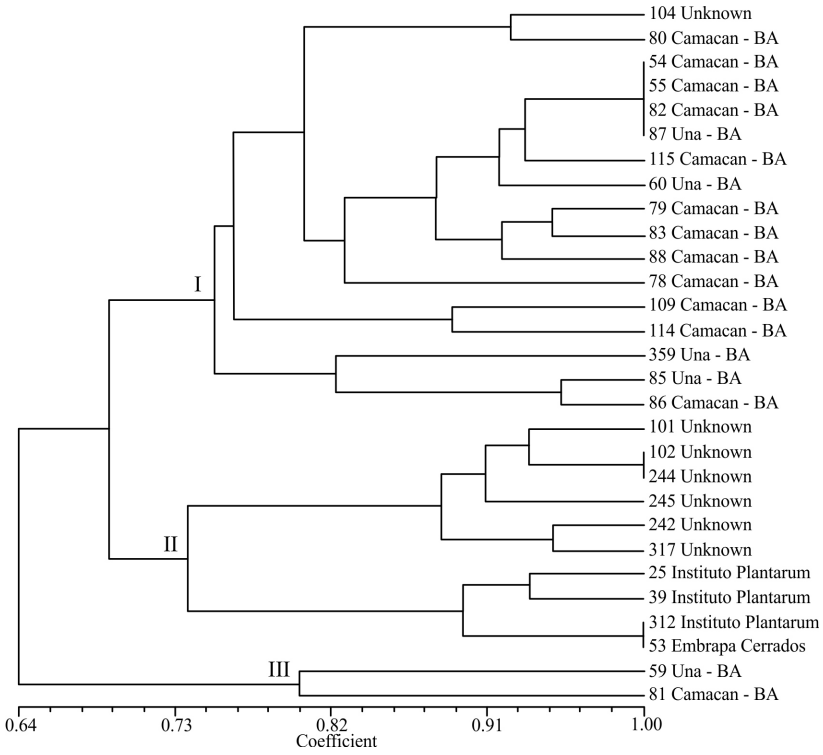

Figure 1. Dendrogram resulting from the grouping analysis of the 29 *Passiflora alata* genotypes, obtained by UPGMA using the Dice's similarity coefficient. The cophenetic correlation coefficient is 0.84.

Besides the statistical grouping by UPGMA, a PCO analysis was carried out using Dice's similarity coefficient. PCO analysis showed that the distribution of accessions of *P. alata* was similar to that observed in the dendrogram generated by UPGMA. Three distinct groups were formed on the axes C1 and C2. Furthermore, various other accessions from *P. alata*, such as 59, 80, 81, 109, and 245, have an isolated distribution (Figure 2).

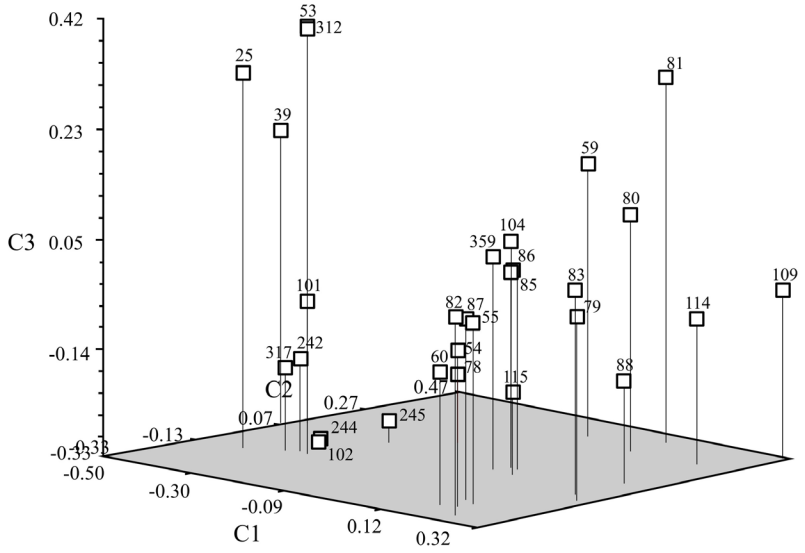

Figure 2. Analysis of principal coordinates generated using the Dice's similarity coefficient.

DISCUSSION

Previous studies addressing the transferability of SSR primers in species of the genus *Passiflora* have been reported in the literature (Pádua, 2004; Cerqueira-Silva et al., 2008a; Conceição et al., 2009). The transferability analysis of SSR primers that were designed from the library enriched with microsatellites of *P. alata* and *P. pohlii* was carried out in 80 wild *Passiflora* species and showed a higher transfer (78.75%) for the primer A08FP1 that was developed for *P. alata* (Pádua, 2004). In this study, the primer pair A08FP1 exhibited a high transferability rate (71.4%). The transferability rate of 25 primer pairs developed for *P. edulis* f. *flavicarpa* (Oliveira et al., 2005; Oliveira, 2006) in wild *Passiflora* species (*P. cincinnata*, *P. coccinea*, *P. kermesina*, *P. gardneri*, *P. rubra*, *P. capsulares*, *P. misera*, *P. suberosa*, *P. nitida*, *P. watsoniana*, *P. bahienses*, *P. eichleriana*, and *P. setacea*) were between 32 and 76%; in addition, nine of the 13 species showed amplification exceeding 52% (Cerqueira-Silva et al., 2008a). Using the same primers, similar results were obtained for other *Passiflora* species (*P. foetida* var. *foetida*, *P. galbana*, *P. amethystina*, and *P. sublanceolata*), indicating a transfer rate of 40-44% (Conceição et al., 2009). The observed cross-amplification in *P. gardneri*, *P. edulis* f. *flavicarpa*, *P. watsoniana*, and *P. bahiensis* using seven pairs of primers developed for *P. alata* (Pádua et al., 2005) showed different amplification rates among the species, in the order of 86, 86, 57, and 29%, respectively (Cerqueira-Silva et al., 2008b).

The transferability rates presented in this study were satisfactory in most of the species used and primer pairs. Hence, results demonstrate that the transferability of SSR loci from *P. alata* and *P. edulis* to wild *Passiflora* species was successful because there was amplification in all of the species analyzed. Nevertheless, different transferability rates were observed. The successful transfer of SSR primer pairs between species depends upon the coexistence of the

annealing site of SSR primers. With a view to save time, financial, and human resources for the development of SSR markers for *Passiflora*, transferability is seen as a promising technique for the genomic analysis of species of this genus.

In the analysis of amplifications with primers obtained from a library enriched with microsatellites of *P. edulis* f. *flavicarpa*, *P. cacao* was the species with the highest amplification rate. This event can be attributed to some morphological similarities and the genetic proximity between *P. cacao* and *P. edulis* (Viana, 2009) because they exhibit high crossability rates. Other studies associated the rate of amplification with the taxonomic affinity of some species (Primmer et al., 2005; Wang et al., 2005; Barbará et al., 2007). Instances of successful use of heterologous primers were obtained from species of the family Meliaceae, which were subjected to amplification tests using primers specific for *Swietenia humilis* Zucc. Similar to the *Passiflora* species observed in this study, the highest percentage of amplification occurred in species of the genus *Swietenia* (White and Powell, 1997).

Despite the high transfer rate of some primers, it has been noted that primers A01BP3, A01FP3, and A03AP3 (developed for *P. alata*) did not amplify products in any of the species studied. The lack of conservation of primer annealing sites may have prevented the amplification of microsatellite loci, namely null alleles (Garner, 2002), and this condition is mostly observed in the event of primer transfer between different species, which usually affects all individuals to the same degree. In the germplasm of *Triticum dicoccon* Schrank, it was noted that 10 of the 29 SSR primers that were used resulted in null alleles (Teklu et al., 2007).

The analysis of results obtained from the intraspecific variation of *P. alata* showed a high cophenetic value ($r = 0.84$), denoting good agreement with the values of genetic distance. The dendrogram generated by UPGMA distributed most of the accessions into two major groups (I and II). The analyses of similarity coefficient, grouping via UPGMA, and PCO analysis showed that some accessions of *P. alata* can indicate duplicate genotypes. Accessions 312 and 53 were granted by different institutions, but they showed the same genotype, and the places of their collections were unknown. Therefore, one may assume that these accessions are from the same place or that there may have been an exchange of germplasm between the two institutions. In assessing the diversity of some genotypes of *Theobroma cacao* with the use of microsatellite primers, redundant accessions were found because of the presence of hybrids from the same cross (sibling seeds). This result improved the representation of the collection (Irish et al., 2010).

The groupings made by UPGMA and PCO confirmed that the polymorphism observed by SSR loci amplification in accessions of *P. alata* was sufficient to distribute the accessions of this species. In *Vaccinium* spp (blueberry), only three pairs of microsatellite primers were sufficient to form groupings consistent with the reality of the collection (Silva et al., 2008). The results obtained in this study suggest that the accessions of *P. alata* can be useful for cross-breeding purposes because they are genetically distant in relation to the SSR loci analyzed, particularly between accessions from the groups I (80 and 104) and III (59 and 81). Likewise, the greatest genetic distance between accessions from groups I and II can be used to select genotypes that are aimed at the genetic improvement of *P. alata.* The identification of redundant accessions with high genetic similarity prevents crosses between genotypes with high similarity at the SSR loci; this fact would allow for the narrowing of the genetic basis of progenies obtained via intraspecific crossing.

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