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# Genetic Variability of Passion Fruit Multispecific Hybrids and Their Respective Wild Parents Determined by Microsatellite Markers

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**Abstract**

The *Passiflora* genus comprises more than 500 species that are used for food, industrial, ornamental, and pharmaceutical purposes. The sour passion fruit (*P. edulis* Sims) has low genetic variability for disease resistance, and the use of wild species in the cross-breeding basis is a promising alternative for introgression of resistance genes. The objective of this study was to characterize multispecific hybrids and wild materials with potential to be used as parents in passion fruit genetic breeding programs, using microsatellite markers. Genomic DNA from 33 accessions was extracted and analyzed using 23 microsatellite markers, which were used to estimate the genetic dissimilarities among accessions. The genetic dissimilarity matrices were used to perform clustering analysis by dendrogram using the Unweighted Pair-Group Method as grouping criterion and by graphic dispersion based on multidimensional scale, using the principal coordinates method. Genetic distances between accessions ranged from 0.067 to 1.00. The markers indicated genetic variability among the studied accessions and also the efficiency of the recurrent genome recovery within the backcross program. The genetic structure among the accessions shows the clustering tendency between the wild accessions of *P. hatschbachii* and *P. quadrifaria* and the accessions obtained by crossing these species. The same occurred for *P. incarnata* and *P. edulis* accessions. The knowledge generated by the molecular characterization provides information on the diversity of accessions and contributes to the work of breeders in the selection of parents.

**Keywords:** SSR, clustering analysis, passion fruit, wild species, genetic breeding

## 1. Introduction

*Passiflora* L. is the largest genus of the *Passifloraceae* family, comprising approximately 525 species (Bernacci, Vitta, & Bakker, 2003). The majority of these species is found in the Americas, especially South America, where Colombia and Brazil are the largest diversity centers with nearly 240 native species (Bernacci et al., 2003; Ulrich & MacDougal, 2004; Imig, Milward-de-Azevedo, & Cervi, 2018). Most species are popularly known as passion fruit. Some of them have commercial and pharmacological value and are cultivated on a large scale for consumption or as ornamental plants (Imig et al., 2018). The *Passiflora* genus contains the largest number of species within the *Passifloraceae* family. Its taxonomy recognizes five subgenuses: *Passiflora* sub. *Passiflora*, *P.* sub. *Decaloba* (DC) Rchb., *P*. sub. *Astrophea* (DC) Mast., *P.* sub. *Deidamioides* (Harms) Killip (Feuillet & MacDougal, 2004), and *P*. sub. *Tetrapathea* (DC) P. S. Green, included by Krosnick, Ford, & Freudenstein, (2009).

The sour passion fruit (*Passiflora edulis* L.) is an allogamous species with great variability of shapes and colors of fruits and flowers. It is the cultivated species of highest economic importance. Its edible fruits are widely used in culinary and in preparations of alcoholic beverages and juices (Vanderplank, 1996). This species also has medicinal importance in the manufacturing of calming agents. Additionally, its crushed seeds are used as a vermifuge (Bernacci et al., 2003).

However, significant losses are observed with crop expansion in Brazil due to the infection by several plant pathogens, such as *Xanthomonas axonopodis* pv. *passiflorae*, Cowpea aphid-borne mosaic virus (CABMV), *Colletotrichum gloeosporioides*, *Meloidogyne* spp., *Fusarium oxysporum* f. sp. *passiflorae*, and *F. solani*

(Junqueira, Braga, Faleiro, Peixoto, & Bernacci, 2005). As a consequence, the development of disease resistant varieties is critical for this crop since it reduces production costs, increases fruit market quality, and contributes to the environmental preservation and sustainability of passion fruit cultivation in the country (Quirino, 1998).

Considering the economic and social importance of this crop, the breeding program developed by the Brazilian Agricultural Research Corporation (Embrapa Cerrados-CPAC) and partners performs inter and intraspecific crosses to obtain disease resistant hybrids. Crosses are performed between species that are compatible to the commercial *P. edulis*, such as *P. setacea* DC, *P. quadrifaria* Vanderpl., *P. incarnata* L., *P. caerulea* L., *P. hatschbachii* Cervi, among others, aiming the introduction of desirable characteristics, especially disease resistance (Junqueira et al., 2005). The molecular characterization of accessions used in the *Passiflora* breeding program contributes to a better understanding of plant diversity and genetic background, which are crucial information for parent selection by breeders (Segura, D'Eeckenbrugge, Bohorquez, Ollitrault, & Tohme, 2002; Viana et al., 2003).

The genetic diversity of *Passiflora* has been assessed by morphological descriptors (Plotze et al., 2005; Viana, Souza, Araújo, Corrêa, & Ahnert, 2010; Ramaiya, Bujang, & Zakaria, 2014; Oliveira, Faleiro, Junqueira, & Viana, 2016; Pérez & D'Eeckenbrugge, 2017), agronomic traits (Meletti, Soares-Scot, & Bernacci, 2005; Cerqueira-Silva, Moreira, Figueira, Corrêa, & Oliveira, 2008; Abreu, Peixoto, Vilela, & Figueiredo, 2009), and ecological descriptors (Segura, D'Eeckenbrugge, López, Grum, & Guarino, 2003). DNA polymorphism in *Passiflora* spp. has been detected by different types of molecular markers, such as restriction enzymes (cpDNA; Yockteng & Nadot, 2004; Paikrao et al., 2010), Inter Simple Sequence Repeats (ISRR; Santos et al., 2011, Oliveira, Faleiro, Junqueira, Fonseca, & Araya, 2019), Random Amplified Polymorphic DNA (RAPD; Fajardo et al., 1998; Aukar et al., 2002; Crochemore, Molinari, & Vieira, 2003; Bellon et al., 2007; Cerqueira-Silva et al., 2010; Oliveira et al., 2019), Amplified Fragment Length Polymorphism (AFLP; Segura et al., 2002), Internal Transcribed Spacer (ITS; Muschner et al., 2003; Mäder et al., 2010; Bellon, 2014; Ramaiya et al., 2014), and Simple Sequence Repeats (SSR/microsatellites; Oliveira et al., 2005; Cerqueira-Silva et al., 2012; Cerqueira-Silva et al., 2014; Paiva et al., 2014a; Araya et al., 2017).

Despite the high variability found in morphological and agronomic evaluations, as well as in most studies using molecular markers, the use of microsatellite markers in *P. edulis* has generally presented low levels of polymorphism (Oliveira et al., 2005; Cerqueira-Silva et al., 2012, 2014; Ortiz et al., 2012). High reproducibility and polymorphism, codominance, and multiallelism (Litt & Luty, 1989; Powell et al., 1996) are advantages of microsatellites when compared to other types of molecular markers. However, less than 200 microsatellite markers were available for genetic studies in *P. edulis* by 2017 (Oliveira et al., 2005; Oliveira, 2006; Cerqueira-Silva et al., 2014), most of them based on imperfect and compound motifs (Domaniç & Preparata, 2007; Lim, Kwoh, Hsu, & Wirawan, 2013). Currently, more than 1,000 microsatellite markers are available for *P. edulis*, considering the recent work of Araya et al. (2017), in which primer pairs for 816 perfect microsatellite regions were developed. The prefix "BrPe" was given to this new set of SSR markers. The "BrPe" microsatellites proved to be highly polymorphic, with high values for PIC (polymorphic information content), Ho (observed heterozygosity), and allele number (Araya et al., 2017). In addition, it has a high transferability to other species of the *Passiflora* genus, showing a potential to be used in genetic analyses of a wide number of *Passiflora* species.

Therefore, this work aimed to characterize 33 passion fruit accessions, which include multispecific hybrids and their respective wild parents, through microsatellite markers. The knowledge generated by the molecular characterization aims to provide information about the genotype diversity and to contribute to the work of breeders in the selection of parents.

## 2. Method

### *2.1 Plant Material and DNA Extraction*

Fresh young leaves from 33 accessions from the Embrapa Cerrados germplasm bank, Planaltina, DF, Brazil, were used for DNA extraction with the standard CTAB protocol, with modifications (Faleiro, Faleiro, Cordeiro, & Karia, 2003). The DNA concentration was estimated by 1% agarose gel electrophoresis, and the fluorescence intensities of each sample stained with ethidium bromide were compared with different Lambda DNA standards. Each sample was then diluted to the concentration of 3.0 ng/µl.

The accessions used in the present study are described in Table 1. These accessions represent a group of 18 interspecific hybrids; 11 plants of different species used as parents (*P.* aff. *amethystina* "macrocarpa", *P. caerulea* L., *P. edulis Sims*, *P. hatschbachii* Cervi, *P. incarnata* L., *P. quadrifaria* Vanderpl, *P. setacea* DC, and *P. tholozanii* Sims); 1 accession of the MSC matrix ("Marília Seleção Cerrado"); and 3 different accessions of *P.*

*edulis* "roxo típica". The *P. edulis* "roxo típica" is an autogamous variety of little-known phylogeny that produces small purple peel fruits. All 33 accessions were genotyped with 23 microsatellite markers developed by Araya et al. (2017). These microsatellites were chosen based on their amplification quality and on the PIC obtained by Araya et al. (2017).

Table 1. Passion fruit (*Passiflora* spp.) accessions from the Embrapa Cerrados Germplasm Bank genotyped with microsatellite markers

| Number | Accession | Genetic origin |
|---|---|---|
| 1 | PL 3 × GA (T4R4PL1) | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis* (BC3)/CPAC experimental field |
| 2 | ML 1 | (*P. edulis* × *P. amethystina*) × *P. edulis* (BC5)/CPAC Greenhouse |
| 3 | ML 2 | [(*P. edulis* × *P. amethystina*) × *P. edulis* (BC5)/CPAC Greenhouse |
| 4 | PL 2 | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis* (BC1)/CPAC Greenhouse |
| 5 | MEAM-3 | (*P. edulis* × *P. amethystina*) × *P. edulis* (BC3)/CPAC Greenhouse |
| 6 | GX DF | *P. edulis*/CPAC Greenhouse |
| 7 | GX SB | *P. edulis*/CPAC Greenhouse |
| 8 | PL 6 | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis* (BC2)/CPAC Greenhouse |
| 9 | PL 1 MEAM-2 | [(*P. edulis* × *P. amethystina*) × *P. edulis* (BC1)] × *P. incarnata*/CPAC Greenhouse |
| 10 | PL 4 (Longão flor azul) | (*P. edulis* × *P. incarnata*) × *P. edulis* (BC3)/CPAC Greenhouse |
| 11 | PL 3 | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis* (BC1)/CPAC Greenhouse |
| 12 | PL 5 | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis* (BC2)/CPAC Greenhouse |
| 13 | LD3 | *P. edulis*—flowers with 3 stigmas/BAG 162 |
| 14 | 325 | *P. caerulea* × *P. edulis* (BC4)/BAG167 |
| 15 | LD4 | *P. edulis*—flowers with 4 stigmas/BAG 72 |
| 16 | Vermelhão | *P. caerulea* × *P. edulis* (BC5)/CPAC Greenhouse |
| 17 | *P. caerulea* | Wild species/BAG 111 |
| 18 | MEAM-1 | [(*P. edulis* × *P. amethystina*) × *P. edulis* (BC1)] × *P. incarnata*/BAG |
| 19 | *P.* aff. *amethystina* | wild species/CPAC Greenhouse |
| 20 | *P. edulis* × *P. incarnata* | (*P. edulis* × *P. incarnata*) × *P. edulis* (BC2)/BAG line 10 |
| 21 | *P. incarnata* | Wild species/BAG |
| 22 | Rosa Púrpura × *P. edulis* | [(*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*] × *P. edulis*/CPAC Greenhouse |
| 23 | Rosa Púrpura | (*P. hatschbachii* × *P. quadrifaria*) × *P. incarnata*/BAG 84 |
| 24 | *P. setacea* × *P. edulis* | *P. setacea* × *P. edulis*/CPAC experimental field |
| 25 | *P. setacea* | Wild species/CPAC experimental field |
| 26 | *P. hatschbachii* | Wild species/BAG 131 |
| 27 | *P. hatschbachii* × *P. quadrifaria* | *P. hatschbachii* × *P. quadrifaria*/BAG |
| 28 | *P. quadrifaria* | Wild species/CPAC experimental field |
| 29 | *P. tholozanii* | BAG 97/Manaus-Amazonas/ Brazil |
| 30 | MSC | *P. edulis*/CPAC experimental field |
| 31 | *P. edulis* "roxo típica" (PL1) | Wild species/CPAC experimental field |
| 32 | *P. edulis* "roxo típica" (PL2) | Wild species/CPAC experimental field |
| 33 | *P. edulis* "roxo típica" (PL3) | Wild species/CPAC experimental field |

*Note.* CPAC: Embrapa Cerrados; BC: backcross; BAG: Germplasm Genetic Bank.

### *2.2 Microsatellite Marker PCR Assays*

Multiplex panels for simultaneous evaluation of microsatellite markers were designed using the Multiplex Manager program (Holleley & Geerts, 2009). PCR assays were performed in a final volume of 5 µl containing 5 ηg of genomic DNA, 1X QIAGEN Multiplex PCR Kit Master Mix (QIAGEN), 0.5X Q-Solution (QIAGEN), and 0.2 µM of each primer. Reactions were performed on a Veriti™ Thermal Cycler (Applied Biosystems, USA) using the following amplification program: 95 °C for 15 minutes; 35 cycles at 94 °C for 30 seconds; 55, 57 or 60 °C for 90 seconds; 72 °C for 60 seconds; and a final extension step at 60 °C for 60 min. Afterwards, 9 µl of Hi-Di ™ Formamide (Applied Biosystems, USA) and 1 µl of ROX-labeled internal size standard were added to 1 µl of the PCR product. Samples were then subjected to denaturation at 94 °C for 5 minutes. The denatured product was injected into an ABI3730 automated sequencer (Applied Biosystems, USA). Identification of the alleles according to size and genotyping was performed using the GeneMapper® software v. 4.1 (Applied

Biosystems, USA). Automated allelic binning was performed with the Tandem software (Matschiner & Salzburger, 2009).

Polymorphism, number of alleles, expected and observed heterozygosities (He and Ho, respectively), PIC values, and other statistics were estimated by the CERVUS program (Marshall, Slate, Kruuk, & Pemberton, 1998). *He* values were determined as the probability of an individual being heterozygous at a locus according to its allele frequencies.

The generated genotyping data were converted into a binary matrix data. The genetic dissimilarity among accessions was estimated based on the complement of the Nei and Li's similarity coefficient, using the Genes software (Cruz, 2013). The genetic dissimilarity matrix was used for clustering analysis by dendrogram using the Unweighted Pair-Group Method (UPGMA) as a clustering criterion. In addition, graphical dispersion was generated based on multidimensional scale, using the principal coordinates method, and with the aid of the SAS (SAS Institute Inc., 2008) and Statistica (StatSoft Inc, 2005) programs.

## 3. Results and Discussion

The 23 microsatellite markers were polymorphic and allowed the detection of 147 alleles (Table 2). The number of alleles observed for all microsatellite markers ranged from 2 to 10, with a mean of 6.39 alleles per locus. Marker He varied between 0.36 and 0.87, with a mean value of 0.65. Ho values ranged from 0.09 to 0.87, with a mean of 0.35. PIC varied from 0.32 to 0.83, with a mean value of 0.59 (Table 2). PIC values above 0.5 are classified as highly informative; values ranging from 0.25 to 0.5 are considered as moderately informative; those below 0.25 are classified as less informative (Botstein, White, Skolnick, & Davis, 1980). The amplicon size produced by each microsatellite marker is shown in Table 2.

Table 2. Descriptive statistics of *Passiflora edulis* microsatellite markers

| # | Marker | Primer Sequences 5'-3' | Repeat Motif | Ta (°C) | Allele Number | Allele Size (bp) | He | Ho | PIC |
|---|---|---|---|---|---|---|---|---|---|
| 1 | BrPe0032 | F:TTGCACAATGACCAATGTTGT<br>R:CTGAGCACCTTGTCAAAATACA | (AT)13 | 60 | 7 | 134-152 | 0.71 | 0.23 | 0.67 |
| 2 | BrPe0028 | F:CAAAAGGAACAGGGAAGA<br>R:GAAAGAGAGAAAGACAGAGA | (TA)6 | 55 | 8 | 91-105 | 0.81 | 0.41 | 0.77 |
| 3 | BrPe0024 | F:CCCTACCTTTCTCTGCTT<br>R:CATCTCCTCTATCTCCTTC | (TC)7 | 55 | 4 | 221-231 | 0.63 | 0.11 | 0.55 |
| 4 | BrPe0031 | F:AGGTCGGTGGGTGTGTTTAG<br>R:CATTCAACTCCCCAAAAGGT | (TA)9 | 60 | 8 | 130-152 | 0.66 | 0.52 | 0.61 |
| 5 | BrPe0014 | F:AATATGGCTGGGGAAAAC<br>R:TTCCTGTCTTTGGACCTT | (AG)7 | 57 | 3 | 214-222 | 0.36 | 0.19 | 0.32 |
| 6 | BrPe0033 | F:GCCATGAGAGACTTGGGAGA<br>R:CGGTTGCCAAAAAGAAGAGA | (AT)8 | 60 | 6 | 232-252 | 0.77 | 0.12 | 0.71 |
| 7 | BrPe0038 | F:TTTCAACTTTTCGTGTGTGC<br>R:TGTTGTTGCTTGGAAGGATG | (AT)6 | 60 | 6 | 152-168 | 0.65 | 0.23 | 0.60 |
| 8 | BrPe0042 | F:CATGCATTCATTTGTTTTTCTTG<br>R:GATGCTGGGAAAAAGAGTGC | (AT)8 | 60 | 8 | 133-167 | 0.66 | 0.21 | 0.59 |
| 9 | BrPe0003 | F:CTTTCTCTCCCTATACCC<br>R:CCCTCCATAATCACATAAC | (TC)11 | 55 | 8 | 273-295 | 0.77 | 0.30 | 0.72 |
| 10 | BrPe0043 | F:TCATACATGGATGTCAAATCGATAC<br>R:GCGGACCAAGAAAATTCAAA | (AT)8 | 60 | 3 | 199-207 | 0.47 | 0.39 | 0.41 |
| 11 | BrPe0006 | F:AAGGAAAAGAACAGCCTCA<br>R:CGCTCTCAAATCAGTCAAA | (TC)10 | 55 | 9 | 183-199 | 0.83 | 0.23 | 0.79 |
| 12 | BrPe0002 | F:AAAGCCCAGATGAAGTGAA<br>R:GGCTCCAATCAGAAGTGT | (AG)12 | 55 | 6 | 160-182 | 0.72 | 0.34 | 0.67 |
| 13 | BrPe0021 | F:ACTTCCTCATCATTCG<br>R:GCTATGCCTCTTTTTG | (TA)7 | 55 | 8 | 147-171 | 0.66 | 0.22 | 0.61 |
| 14 | BrPe0023 | F:AGATACCACACCCAATAG<br>R:TTGGAGTTGTTGGGGA | (CT)7 | 55 | 3 | 116-120 | 0.48 | 0.09 | 0.42 |
| 15 | BrPe0037 | F:TGATAATGCAGCGAAAGAGC<br>R:TCACACTCCATTTGCTCTGC | (TG)6 | 60 | 9 | 214-238 | 0.80 | 0.87 | 0.76 |
| 16 | BrPe0010 | F:GAAGAAAAAAGGGCTTG<br>R:GTTAGGGTTTGGAGGA | (TC)9 | 55 | 10 | 189-209 | 0.75 | 0.35 | 0.72 |
| 17 | BrPe0001 | F:GTTGAGAGGATTGTGTTTG<br>R:ATGGTAGAGGAGGAGAGA | (CT)14 | 55 | 5 | 149-159 | 0.61 | 0.56 | 0.52 |
| 18 | BrPe0008 | F:TTTTCAGCCTCCACTCTT<br>R:TACACCACCAACACTCAC | (AG)9 | 55 | 6 | 262-274 | 0.70 | 0.75 | 0.63 |
| 19 | BrPe0025 | F:CAAGGAACCAGAACAAGAAGAA<br>R:GAAGAACAAGCCAGCCCA | (GA)6 | 55 | 9 | 108-130 | 0.87 | 0.75 | 0.83 |
| 20 | BrPe0039 | F:GCTGCTCCACTGTGAATGTC<br>R:AACCTAGCCCCGTCACAGTA | (AT)6 | 60 | 2 | 195-197 | 0.43 | 0.14 | 0.33 |
| 21 | BrPe0050 | F:TCAAGGGTATCTTTGGTGCTG<br>R:AGCTTCAGCGAGACAAAACC | (TG)7 | 60 | 7 | 194-212 | 0.58 | 0.21 | 0.54 |
| 22 | BrPe0013 | F:GATCGAGGTGAGGTACTG<br>R:GGTTTGGCTTTAATGGAGG | (AG)8 | 55 | 7 | 161-179 | 0.55 | 0.62 | 0.51 |
| 23 | BrPe0020 | F:TAAAGCATCAGGTCAG<br>R:TAGATAGATTTGACGGG | (GT)7 | 55 | 5 | 287-299 | 0.56 | 0.33 | 0.49 |
| | Mean | | | | 6.39 | | 0.65 | 0.35 | 0.59 |

*Note*. F: forward primer; R: reverse primer; Ta: annealing temperature; Ho: observed heterozygosity; He: expected heterozygosity; PIC: polymorphic information content.

Genetic dissimilarities ranged from 0.067 to 1.00. The maximum distance (1.00) was observed between an accession of *P. edulis* "roxo típica" and *P. caerulea* (Table 1; accessions 33 and 17, respectively); and between accession 'GX SB', 'PL1 MEAM-2', and *P. incarnata* in relation to the *P. hatschbachii* accession (Table 1,

accessions 7, 9, 21, and 26, respectively). The lowest distance occurred between the hybrids 'PL3 × GA (T4R4PL1)' (accession 1) and 'ML2' (accession 3) in the BC2 and BC5 backcrossing stage, respectively (Table 1). The analysis of accessions with different degrees of dissimilarity is evidenced by the amplitude of the genetic distance values. Similar results were reported by Araya et al. (2017) in a collection of 28 accessions of six species of *Passiflora* (*P. edulis*, *P. alata*, *P. maliformis*, *P. nitida*, *P. quadrangularis*, and *P. setacea*), based on SSR markers.

Clustering and graphical dispersion analyses evidenced the divergence among accessions (Figure 1). Clustering analyses also showed the formation of four similarity groups and considered the genetic distance of 0.75 as the cutoff point.

The cophenetic correlation measures the fit degree between the dissimilarity matrix and the matrix obtained by the clustering method. A coefficient of cophenetic correlation of good magnitude (r = 0.85) was observed and evidenced the consistency in the adjustment between the graphic representation of the genetic similarity and its original matrix. Also, it ensures that inferences be made by visual evaluation of the dendrogram (Figure 1A).

The first group comprises passion fruit accessions originated from selection among and within progenies. By its turn, these progenies were obtained from interspecific crosses followed by backcrosses with commercial *P. edulis*. Therefore, plants from group 1 are genetically close to the recurrent parent (Table 1, accessions 1 to 16, 18, and 20; Figure 1). This result suggests that the backcrossing cycles led to recurrent genome recovery. The SSR markers evidenced the genetic variability among accessions. This first group also included the MSC accession, a commercial *P. edulis* variety originated from mass selection, and the *P. incarnata* and *P. caerulea* accessions (Table 1, accessions 30, 21, and 17, respectively; Figure 1). Muschner et al. (2003) had already clustered *P. edulis*, *P. incarnata*, *P. setacea*, and *P. caerulea* by phylogeny molecular analyses in *Passiflora* species using polymorphisms of nrITS, trnL-trnF, and rps4. Paiva, Viana, Santos, Silva, & Oliveira (2014b) also reported molecular similarity between *P. edulis* and *P. setacea* and between *P. edulis* and *P. caerulea* when using morphological and quantitative descriptors.

Although classified as *P. edulis*, the accessions of *P. edulis* "roxo típica" (Table 1; accessions 31, 32, and 33), did not cluster within the *P. edulis* group (Figure 1). In contrast, they were associated with a second group formed with the *P. setacea* accession (Table 1; accession 25). Similar results were recorded by Araya et al. (2017). Clustering of the accessions *P. edulis* Sims "flavicarpa" and *P. edulis* "roxo típica" were verified when using chloroplast DNA sequences (Belon, 2014). However, phylogenetic differences between these accessions were detected when ITS sequences were analyzed (Belon, 2014). These results suggest that future investigations should comprise a larger sample of *P. edulis*, including *P. edulis* "flavicarpa" and wild *P. edulis* Sims accessions. Recent analyses of the reproductive system of *P. edulis* "roxo típica" indicate that these accessions are preferentially autogamous, while those of *P. edulis* are mostly allogamous. These findings could probably explain the genetic distance among the accessions of passion fruit (Araya et al., 2017).

The third group included accessions of *P. quadrifaria*, *P. hatschbachii*, *P. tholozanii*, the *P. hatschbachii* × *P. quadrifaria* hybrid, and the BRS Rosa Púrpura cultivar (Table 1, accessions 28, 26, 29, 27, and 23, respectively). This result confirms the hybridization and genealogy of these hybrids. The species *P. tholozanii* and *P. quadrifaria* belong to the *Distephana* supersection. The systematic review study performed by Feuillet & MacDougal (2003) incorporated the subgenus *Distephana*, currently considered a supersection, to the subgenus *Passiflora*.

The fourth and last group is formed by a single accession of the species *P.* aff. *amethystina* "macrocarpa". The phylogenetic tree of 43 *Passiflora* species based on chloroplast and ITS DNA sequences obtained by Bellon (2014) grouped three accessions of *P. amethystina* (*P. amethystina* "verdadeira", *P. amethystina* "SP", and *P. amethystina* "macrocarpa"). An accession of *P. caerulea* was also grouped within this phylogenetic *Passiflora* subgenus group. In this work, although *P. amethystina* "macrocarpa" and *P. caerulea* accessions did not cluster together, the latter was one with highest similarity to the *P. amethystina* accession (0.78), together with the accessions 'MEAM-1' and *P. tholozanii*.

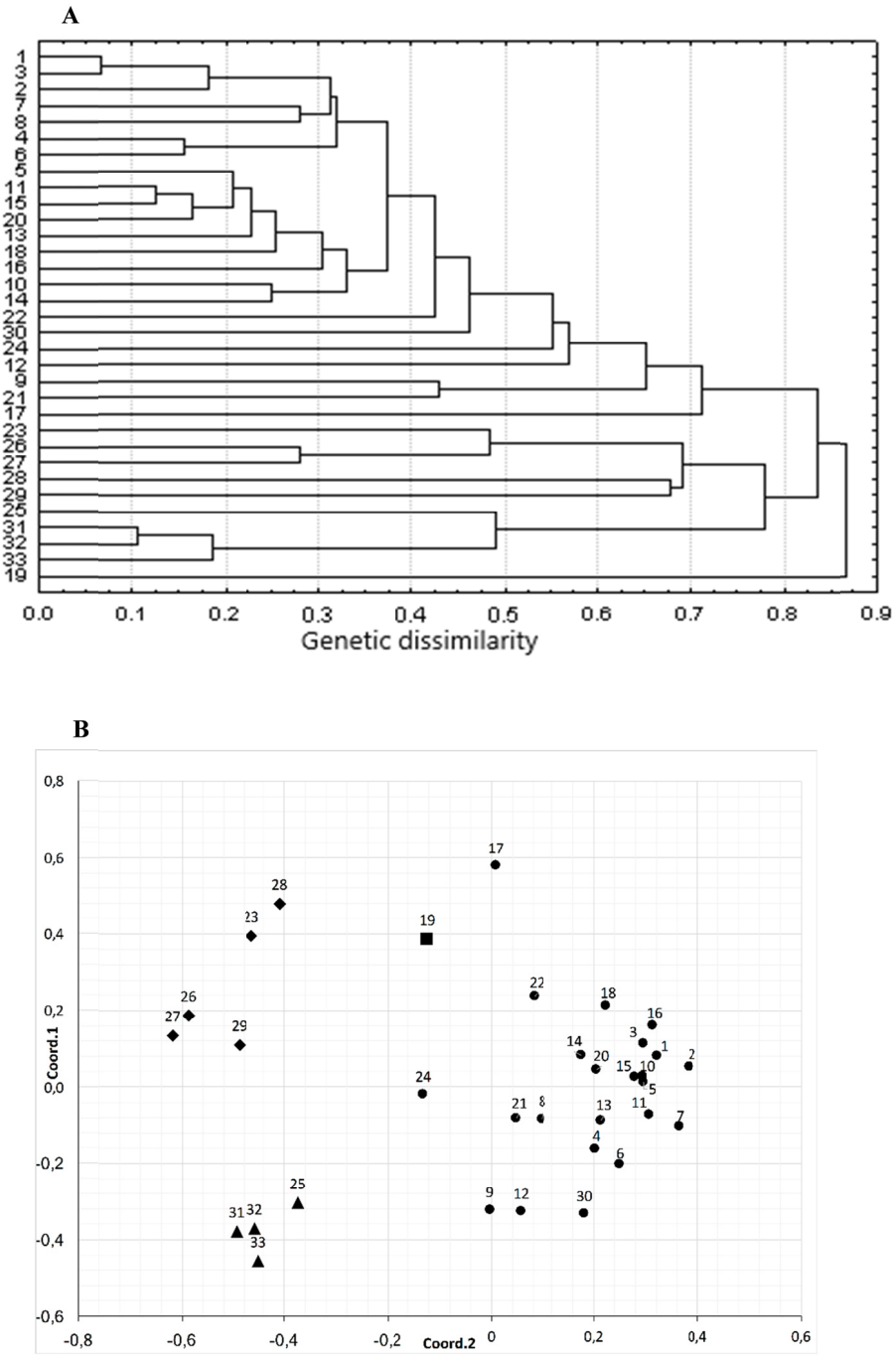


Figure 1. Clustering (A) and graphical dispersion (B) analyses of 33 *Passiflora* spp. accessions based on the genetic dissimilarity matrix and using 23 SSR markers. The UPGMA method was used as a clustering criterion. The principal coordinates method was used in graphical dispersion analysis. The coefficient of cophenetic correlation (r) was 0.85. Legend: 1-PL 3 × GA, 2 (T4R4P1); 2-ML 1; 3- ML2; 4-PL2; 5-MEAM-3; 6-GX DF; 7-GX SB; 8-PL6; 9-PL 1 MEAM-2; 10-PL 4 (Longão flor azul); 11- PL 3; 12-PL 5; 13-LD3; 14-325; 15-LD4; 16-Vermelhão; 17-*P. caerulea*; 18-MEAM-1; 19-*P.* aff. *Amethystina*; 20-*P. edulis* × *P. incarnata;* 21-*P. incarnata*; 22-Rosa Púrpura × *P. edulis*; 23-Rosa Púrpura; 24-*P. setacea* × *P. edulis*; 25-*P. setacea*; 26-*P. hatschbachii*; 27-*P. hatschbachii* × *P. quadrifaria*; 28-*P. quadrifaria*; 29-*P. tholozanii;* 30-MSC; 31-*P. edulis* "roxo típica" (PL1); 32-*P. edulis* "roxo típica" (PL2); 33-*P. edulis* "roxo típica" (PL3).
Group 1 (●); group 2 (▲); group 3 (♦); and group 4 (■)

## 4. Conclusions

The SSR markers demonstrated high genetic variability among the studied accessions and indicated the efficiency of the recurrent genome recovery in the backcross program.

The genetic structure among the accessions showed the clustering tendency between the wild accessions of *P. hatschbachii* and *P. quadrifaria* and the accessions obtained from crossings involving these species. Similar results were observed for *P. incarnata* and *P. edulis* accessions. Therefore, the hybridization and genealogy of these accessions were confirmed.

The knowledge generated in this study provides information on the diversity of the multispecific hybrids and their respective wild parents. Therefore, it will contribute to breeding programs when selecting the best crosses.

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