

Genetic diversity in a half-sibling family of *Passiflora morifolia* mast. assessed by RAPD

Diversidade genética em família de meio-irmãos de *Passiflora morifolia* mast. acessada por RAPD

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ABSTRACT – The characterization of genetic diversity in wild *Passiflora* species is essential to support conservation and breeding programs, allowing the identification of divergent and promising genotypes. The present study aimed to evaluate the genetic diversity within a half-sibling family of *Passiflora morifolia* Mast. using RAPD markers. The experiment was conducted in a greenhouse at the Laboratory of Biotechnology and Plant Breeding, Department of BioScience, Center of Agricultural Sciences, Federal University of Paraíba, in Areia, PB, Brazil. A total of 22 individuals from a half-sibling family of the wild species *P. morifolia* were evaluated. Young leaves were collected for genomic DNA extraction using the cetyltrimethylammonium bromide (CTAB) method. The samples were quantified on 0.8% agarose gel and, after purification with RNase and proteinase K, RAPD assays were performed using 14 primers. The presence and absence of bands were coded as 1 and 0, respectively, generating a binary matrix used to estimate genetic distances among accessions based on the complement of Jaccard similarity coefficient. Based on this matrix, a dendrogram was constructed using Ward's hierarchical clustering method. RAPD reactions amplified 895 fragments, of which 96.7% were polymorphic, indicating considerable genetic variability among the 22 individuals of the *P. morifolia* half-sibling family. The results indicate the potential use of these accessions in breeding and conservation programs for the species, with accessions 6 and 22 being the most genetically divergent.

RESUMO – A caracterização da diversidade genética em espécies silvestres de *Passiflora* é fundamental para subsidiar programas de conservação e melhoramento genético, permitindo a identificação de genótipos divergentes e promissores. O presente trabalho teve como objetivo estudar a diversidade genética dentro de uma família de meio-irmãos de *Passiflora morifolia* Mast. utilizando marcadores RAPD. O experimento foi conduzido em casa de vegetação do Laboratório de Biotecnologia Vegetal, Departamento de Ciências Biológicas, do Centro de Ciências Agrárias da Universidade Federal da Paraíba. Foram avaliados 22 indivíduos de uma família de meio-irmãos da espécie silvestre de *P. morifolia*. Folhas jovens foram coletadas para extração do DNA genômico pelo método do brometo de cetiltrimetilamônio (CTAB). As amostras foram quantificadas em gel de agarose a 0,8% e após purificação do DNA genômico com RNase e proteinase K, foram conduzidos os ensaios de RAPD utilizando 14 primers. A presença e ausência de bandas foram codificadas como 1 e 0, respectivamente, gerando uma matriz binária utilizada para estimar as distâncias genéticas entre os acessos baseado no complemento do coeficiente de similaridade de Jaccard. A partir dessa matriz foi construído um dendrograma utilizando o método de agrupamento hierárquico de Ward. As reações de RAPD amplificaram 895 fragmentos, dos quais 96,7% foram polimórficos, evidenciando considerável variabilidade genética entre os 22 indivíduos da família de meio-irmãos de *P. morifolia* Mast. Os resultados indicam potencial para utilização desses acessos em programas de melhoramento genético e conservação da espécie, sendo os acessos 6 e 22 os mais geneticamente divergentes.

Keywords: Molecular marker. Genetic Resources. Germplasm characterization. Passifloraceae. Breeding.

Palavras-chave: Marcador molecular. Recursos Genéticos. Caracterização de Germoplasma. Passifloraceae. Melhoramento Genético.

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INTRODUCTION

The Passifloraceae family comprises about 18 genera and 630 species distributed mainly in the Americas and Africa. In Latin America, the *Passiflora* genus is the most representative of the Passifloraceae family, comprising approximately 400 species, of which around 150 are found in Brazil (BERNACCI et al., 2015). Species of this genus have considerable economic, ecological, and ornamental importance. Several species are cultivated for fruit production, such as *Passiflora edulis*, while others have ornamental potential due to their beautiful flowers and vigorous growth habit. In addition, some species are recognized for their medicinal properties and ecological roles, particularly as food resources for pollinators and butterflies (CERQUEIRA-SILVA et al., 2014).

The genus also exhibits a wide genetic diversity both within the genus and among cultivated species (VIANNA et al., 2019), representing an important genetic resource for breeding programs. Therefore, the characterization of genetic variation in *Passiflora* species is essential for the conservation, management, and efficient use of germplasm in breeding programs (OLUOCH; NYABOGA; BARGUL, 2018). Molecular markers have been widely used for this purpose, including RAPD, ISSR, AFLP, and SSR markers, allowing the evaluation of

genetic relationships and variability among accessions and species (CERQUEIRA-SILVA et al., 2014).

Among these markers, RAPD (Random Amplified Polymorphic DNA) has been frequently applied in diversity studies due to its simplicity, low cost, and ability to detect polymorphisms without prior genomic information (VIANA et al., 2003). RAPD markers have already been successfully used to estimate genetic diversity in 33 *Passiflora* species, for the characterization and confirmation of six hybrids, and for the genetic mapping of *P. edulis*, among other applications revealing significant levels of inter- and intraspecific variation (CERQUEIRA-SILVA et al., 2014).

Passiflora morifolia Mast. is a wild species native to Brazil, popularly known as purple passion fruit, hairy passion fruit or curly passion fruit (BERNACCI et al., 2015). This species occurs naturally in several countries of the Americas, including Mexico, Guatemala, Venezuela, Colombia, Ecuador, Peru, Bolivia and Argentina (MILWARD-DE-AZEVEDO; BAUMGRATZ, 2004). In Brazil, it is mainly found in the Southeast region, where it occurs in Cerrado areas and submontane rainforest environments. The species does not show differentiation in leaf morphology and presents medium-sized white and purple flowers. *P. morifolia* has several characteristics that favor its use as an ornamental plant, such as intermediate plant size, lush foliage, beautiful flowers, and the ability to grow in pots and indoor environments (JUNGHANS, 2024). The ornamental use of native species and hybrids of the genus *Passiflora* as ornamental plants represents a market with great potential for expansion in Brazil and other Latin American countries (BUGALLO et al., 2014).

Despite the growing number of molecular studies in the genus, information regarding the genetic diversity of *P. morifolia* remains limited. Understanding the genetic variability within this species is essential for supporting strategies of germplasm conservation and for identifying genetically divergent individuals that may be useful in breeding programs. In this context, the evaluation of genetic diversity in half-sibling families can provide important information about the distribution of genetic variability and assist in the selection of promising genotypes and potential parents.

Therefore, this study aimed to evaluate the genetic diversity among 22 accessions of a half-sibling family of *P. morifolia* using RAPD markers.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Laboratory of Biotechnology and Plant Breeding, Department of Biosciences, Center of Agricultural Sciences, Federal University of Paraíba (CCA-UFPB), located in Areia, Paraíba, Brazil (06°58'12" S, 35°41'15" W; approximately 575 m altitude). The region has a tropical climate with dry summer (As, Köppen classification), with an average annual temperature ranging from 22 to 24 °C and mean annual rainfall between 1,200 and 1,400 mm. The study was carried out under greenhouse conditions with natural photoperiod.

Seeds of the parental plants were obtained from the fruit germplasm bank of the Fruit Science sector at the

Federal University of Viçosa (UFV). Seeds from 22 accessions belonging to a half-sibling family, obtained from open-pollinated mother plants (with a common female parent and unknown male parents), were sown with five replicates per accession (100 observations) in tubes filled with the commercial substrate Plantmax® (Brazil). Ten plants died before the analyses, resulting in 90 tubes evaluated.

After germination, the seedlings were transferred to 8-L pots, staked, and maintained in the greenhouse. Plants were fertilized using a nutrient solution composed of the commercial fertilizer DripSol Produção® supplemented with calcium nitrate and iron chelate. DripSol Produção® contains 12% total nitrogen (N), 6% phosphorus (P₂O₅), 40% potassium (K₂O), 1% sulfur (S), 0.03% boron (B), and 0.005% molybdenum (Mo). The nutrient solution was applied at a concentration of 250 mg L.

For molecular analyses, 200 mg of fresh leaf tissue per accession were collected and subjected to genomic DNA extraction using the CTAB protocol, as described by Ferreira and Grattapaglia (1998). The method used was Cetyltrimethylammonium Bromide (CTAB) and analyzed 0.8% agarose gel, with visualization under ultraviolet light.

The amount of DNA was analyzed on a 0.8% agarose gel, and the concentration of the samples was estimated by visually comparing the fluorescence intensity of the DNA bands with those of the known standard.

For DNA purification, the samples were incubated in a water bath at 65 °C for 30 min. Subsequently, 600 µL of CIA (chloroform-isoamyl alcohol) was added, and they were centrifuged for 10 min at 13,400 rpm. The supernatant was removed to new microtubes and 500 µL of CIA was added, then they were centrifuged at 13,400 rpm for 5 min, transferred to new microtubes with 40 µL of buffer added. After homogenization, 500 µL of CIA was added and centrifuged again. 280 µL of the supernatant was transferred to a new microtube and 185 µL of isopropanol was added and centrifuged. After discarding the supernatant, 1 mL of 70% ethanol was added and left on the bench for 10 min, after this the supernatant was discarded, a procedure that was repeated twice, then 1 mL of ethanol was added and centrifuged at 13,400 rpm for 2 min.

Finally the supernatant was discarded and the microtubes were kept at room temperature for total evaporation of the ethanol, and the precipitate was suspended with 50 µL of extraction buffer (EB).

DNA concentration and purity were determined using an ultra-micro UV-Vis spectrophotometer (NanoDrop One, Thermo Fisher Scientific, Waltham, MA, USA). Later, high quality DNA was diluted to appropriate concentrations for PCR amplification, and the remaining DNA was stored at -20 °C.

To evaluate the genetic diversity among the 22 accessions, 14 RAPD primers were used (Table 1).

RAPD amplifications were performed following the protocol described by Viana et al. (2003). The DNA amplification reaction was performed with a final volume of 25 µL, being 23 µL of Master Mix (TBE buffer 0.5x 60 µL + MgCl₂ 36 µL + dNTP 12 µL + primer 60 µL + Taq DNA polymerase 4.8 µL + H₂O DDA 379.2 µL) and 2 µL of genomic DNA from the sample, at a concentration of 5 ng.µL⁻¹.

Table 1. Sequences of the oligonucleotide primers used for DNA amplification of the *P. morifolia* Mast. half-sibling family.

Primer	Base sequence (5' – 3')
UB – 01	AGACGGCTCC
UB – 02	GTTCGGAACC
UB – 03	GGGCGACTAC
UB – 04	GTGCGCAATG
UB – 05	TCGCATCCAG
UB – 06	CAGAAGCGGA
UB – 07	CACAGCGACA
UB – 08	CAAAGCGCTC
UB – 10	TGCGGGTCCT
UB – 11	CAGGATTCCC
UB – 12	GTGGAGTCAG
UB – 13	AAGTCCGCTC
UB – 14	CAGCACTGAC
UB – 15	GACAGGAGGT

Amplification was performed in a thermocycler (Techne TC-Plus, Bibby Scientific Ltd., Stone, Staffordshire, UK), with the following schedule: 94 °C for 5 minutes, 30 cycles; 94 °C for 1 min; 36 °C for 1 min; 72 °C for 2 min and finally the last cycle at 72 °C for 5 min. Immediately after amplification, the samples were stored at -20 °C until use.

To make the electrophoresis gel, an aliquot of 10 µL of the products amplified in the RAPD reaction was used and transferred to Eppendorf tubes, and 2 µL of loading buffer was added (4 g of glucose + 0.025 g of bromophenol blue + 0.025 g of xylene cyanol + 10 µL of distilled water).

Then, 10 µL were applied with a pipette to each well of the 1.5% agarose gel in mL 10^x TBE buffer; 0.06 µL of ethidium bromide (10 mg/mL). It was used as a DNA molecular weight marker, with 5 µL of DNA Ladder – 1 kb (Ludwig Biotec) as the molecular weight standard. The electrophoresis run condition was at 70 volts for approximately two hours. The gel was visualized on a T26M UV 302 nm transilluminator, and the image was captured in the Easy Doc 200 photodocumentation system.

A polymorphic locus was defined as one in which the most common allele has a frequency equal to or less than 0.95 (NEI, 1973). Assuming that the RAPD marker is a dominant marker, data were calculated as absence (0) and presence (1) of bands. Therefore, the genetic diversity among accessions was estimated using a genetic dissimilarity matrix generated by the *Genes* program (CRUZ, 2013), based on the complement of Jaccard (1908) similarity coefficient, calculated using Equation 1:

$$S_j = a/(a + b + c) \quad (1)$$

where:

a - presence of the band in both accessions *i* and *j*;

b - presence of the band in accession *i* and absence in accession *j*; and

c - absence in accession *i* and presence in accession *j*.

The dendrogram cutoff point and group definition were generated by the hierarchical method described by Mojena (1977), which suggests a procedure based on the relative size

of the merger levels (distances) in the dendrogram. The proposal was to select the number of groups in step *j* that satisfy the inequality presented in Equation 2, where the cutoff reference value was calculated according to Equation 3:

$$a_j > q_k \quad (2)$$

where:

a_j - dissimilarity value at the *j*-th fusion step (*j* = 1, 2, ..., g^{-1});

q_k - cutoff reference value, given by:

$$q_k = a + ks_a \quad (3)$$

where:

a - average;

S_a - standard deviation of the values of *a*;

k - constant whose value to be adopted as a rule for defining groups is 1.25.

Thus, we have Equation 4:

$$\bar{a} = \frac{1}{g-1} \sum_{j=1}^{g-1} a_j \text{ and } S_a = \sqrt{a_j^{g-1} - \frac{1}{g-1} \sum_{j=1}^{g-1} a_j^2} / g-2 \quad (4)$$

Based on the dissimilarity matrix, a dendrogram was constructed using the one proposed by Ward (1963). This method takes into account the individuals who provide the smallest sum of squares of the deviation. *Bootstrap* analysis was performed to verify and provide statistical support to the internal nodes of the dendrogram generated using the Ward clustering method. The fit between the dissimilarity matrix and the dendrogram was estimated by the cophenetic correlation coefficient (*r*), according to Sokal and Rohlf (1962), through the *Genes* program (CRUZ, 2013). The cutoff point for group definition was established at a genetic distance of 0.60, corresponding to the selected fusion level in the dendrogram. This threshold was defined according to the criterion proposed by Mojena (1977), adopting *k* = 1.25, in

Equation 2.

For each primer, the number of amplified bands, number of polymorphic loci, number of monomorphic loci, polymorphic information content (PIC), marker index (MI), resolving power (Rp) and expected heterozygosity (He) were estimated. To ensure reproducibility, all RAPD reactions were performed at least twice, and only clear, consistent, and reproducible bands were considered for analysis. Negative controls (without DNA template) were included in each amplification set to verify the absence of contamination. PIC was calculated using the formula described by Serrote et al. (2020): $PIC = 1 - (p^2 + q^2)$, where PIC is the polymorphic information content of the primer, p is the frequency of the present bands, and q is the frequency of the absent bands. For dominant markers, the maximum value for PIC is 0.5 for $p = 0.5$ (SERROTE et al., 2020). MI and Rp were estimated as proposed by Prevost and Wilkinson (1999), $MI = PIC \times \text{polymorphic bands}$ and $Rp = \sum Ib$, where Ib represents the band information, estimated as follows: $Ib = 1 - (2 \times |0.5 - p|)$, where p is the proportion of accessions containing band I. The percentage of polymorphic loci (PPL) was also estimated.

Expected heterozygosity was estimated considering Hardy-Weinberg equilibrium ($2pq$), where p is the frequency of the present bands and q is the frequency of the absent bands. Allelic diversity analyses were performed using Microsoft® Excel for Mac version 15.25.

RESULTS AND DISCUSSION

The CTAB method proved to be efficient in extracting genomic DNA from the 22 accessions of the half-sibling family, with good quality and quantity. The fourteen RAPD primers produced 895 DNA bands, with an average of 63.93 bands amplified per primer (Table 2). The efficiency of primers in detecting polymorphisms in different regions of the genome is a fundamental aspect for genetic diversity studies. The number of polymorphic loci directly reflects the ability of primers to capture relevant genetic variations, since polymorphisms at distinct loci indicate genetic differences within a population (BIDYANANDA et al., 2024).

Table 2. Molecular markers, their sequences, number of amplified bands (NAB), number of polymorphic loci (NPL), number of monomorphic loci (NML), percentage of polymorphic loci (PPL), polymorphic information content (PIC), resolving power (Rp), marker index (MI) and expected heterozygosity (He).

Marker	Sequence (5'-3')	NAB	NPL	NML	PPL (%)	PIC*	R _p	MI	He
UB-05	TCGCATCCAG	92	7	1	87.5	0.2335	8.3636	1.868	0.490
UB-02	GTTCGGAACC	144	8	0	100	0.2242	13.0909	1.793	0.297
UB-11	CAGGATTCCC	85	7	0	100	0.2686	7.7273	1.880	0.495
UB-04	GTGCGCAATG	78	6	0	100	0.4146	7.0909	2.488	0.484
UB-07	CACAGCGACA	65	6	0	100	0.2831	5.9091	1.698	0.491
UB-03	GGGCGACTAC	88	5	0	100	0.2992	8.0000	1.496	0.320
UB-15	GACAGGAGGT	83	6	0	100	0.3037	7.5455	1.822	0.467
UB-01	AGACGGCTCC	79	4	0	100	0.1581	7.1818	0.632	0.184
UB-06	CAGAAGCGGA	43	4	0	100	0.2820	3.9091	1.128	0.498
UB-08	CAAAGCGCTC	14	4	0	100	0.2583	1.2727	1.033	0.268
UB-10	TGCGGGTCCT	10	3	0	100	0.2507	0.9091	0.752	0.257
UB-12	GTGGAGTCAG	47	3	0	100	0.3815	4.2727	1.142	0.410
UB-13	AAGTCCGCTC	23	2	1	66.7	0.2934	2.0909	0.880	0.454
UB-14	CAGCACTGAC	44	3	0	100	0.4380	4.0000	1.314	0.444
Total		895	68	2					
Average		63.9	4.9	0.14	96.7	0.2921	5.8117	1.424	0.398

*PIC is equivalent to observed heterozygosity, when the molecular marker is dominant (SERROTE et al., 2020).

The high number of amplified bands is an indicative of the efficiency of the primers in revealing genetic variation within the half-sibling family of *P. morifolia*. The high percentage of polymorphic loci (96.7%) also indicates considerable genetic variability among the studied accessions, demonstrating the presence of substantial intrafamilial genetic diversity. Similar levels of polymorphism have been reported for other *Passiflora* species, such as *Passiflora edulis*, *Passiflora cincinnata* (CERQUEIRA-SILVA et al., 2014) and *Passiflora setacea* (BARBOSA et al., 2021), in which ISSR and RAPD markers revealed polymorphism rates frequently above 80%. In a comparative study involving six

species of the genus (*P. morifolia* Mast.; *P. foetida* var. *foetida* L.; *P. micropetala* Mart.; *P. coriacea* Juss.; *P. palmeri* var. *subanceolata* Rose; and *P. suberosa* L.), a high level of polymorphism was observed among species, contrasting with a lower level of intraspecific polymorphism, indicating greater genetic differentiation at the interspecific level (VIANNA et al., 2019). This pattern suggests that the high level of polymorphism is not exclusive to *P. morifolia* Mast., but rather represents a common characteristic of *Passiflora* species, likely associated with their predominantly allogamous reproductive system and self-incompatibility mechanisms, which promote cross-pollination, gene flow

among individuals, and high recombination rates, thereby contributing to the maintenance of broad genetic diversity and adaptive potential (RIBEIRO et al., 2024). In this context, the genetic variability detected among the accessions highlights their potential for use in breeding programs and germplasm conservation strategies, particularly for the identification of genetically divergent parents and the formation of superior combinations in future crosses (CERQUEIRA-SILVA et al., 2014).

Similar patterns have been reported for species of the genus *Passiflora*, where substantial genetic diversity has been observed among wild and cultivated accessions, reinforcing the importance of this variability for adaptation, germplasm conservation, and its potential use in genetic breeding programs (CERQUEIRA-SILVA et al., 2014; VIANNA et al., 2019). In this regard, the results obtained with *P. morifolia* suggest that the species has a robust genetic base, with a wide variability, crucial for their adaptation and survival under different environmental conditions.

The bands were distributed across 70 loci, of which 68 are polymorphic (96.7%). The number of polymorphic loci ranged from 2 (UB-13) to 8 (UB-02). The percentage of marker polymorphism ranged from 66.7% (UB-13) to 100% (UB-02, UB-11, UB-04, UB-07, UB-03, UB-15, UB-01, UB-06, UB-08, UB-10, UB-12 and UB-14). Estimates of primer resolving power ranged from 0.91 (UB-10) to 13.09 (UB-02) (Table 2). Among the evaluated primers, UB-02 showed the highest resolving power (Rp), indicating a greater capacity to discriminate among different accessions of *P. morifolia* Mast. High resolving power indicates a greater ability of a primer to discriminate among genotypes, as this parameter reflects the capacity of a marker to detect polymorphic patterns among individuals (PREVOST; WILKINSON, 1999). The Rp value is widely used as indicator of evaluating primer efficiency in detecting genetic variation, representing the ability of a primer to clearly separate distinct genotypes within a population (HANCI; PAŞAZADE, 2025). These results highlight the potential applicability of these primers, particularly UB-02, in genetic diversity assessments and in supporting breeding programs and germplasm conservation strategies for *Passiflora* species. This pattern is consistent with recent findings in *Passiflora edulis*, where ISSR markers revealing high levels of polymorphism enabled robust discrimination of genetic structure and diversity within germplasm collections (MORILLO et al., 2023). The use of primers such as UB-02, which demonstrate high efficiency in discriminating genetic variations, is especially relevant in species with great genetic diversity, such as *P. morifolia*, where accurate identification of polymorphisms can facilitate the identification of molecular markers useful for genetic improvement, in addition to contributing to genetic conservation strategies (COSTA et al., 2017). In this context, evaluating the informativeness of molecular markers becomes essential to determine their effectiveness in detecting genetic variability among accessions.

Recent studies corroborate the importance of primers with a high rate of polymorphic loci, since they allow the detection of a wider range of genetic variations, providing a more robust analysis of the genetic structure of populations (PIMENTA et al., 2022). Primers with a high number of polymorphic loci, like UB-02, which had a 100% polymorphism, have shown greater effectiveness in revealing genetic variability, being essential instruments for genomic

studies aimed at the characterization and management of genetic resources (COSTA et al., 2017). Polymorphic information content (PIC) ranged from 0.16 (UB-01) to 0.44 (UB-14) and the marker index from 0.63 (UB-01) to 2.49 (UB-04). Expected heterozygosity ranged from 0.18 (UB-01) to 0.49 (UB-05). These parameters (PIC, MI and He) are widely used in genetic diversity studies because they allow the evaluation of the informativeness and discriminatory power of molecular markers in population analyses (PREVOST; WILKINSON, 1999). In general, the most informative primers for assessing genetic diversity among the 22 accessions of the half-sibling family of *P. morifolia*, were UB-02, UB-14, UB-04 and UB-05, as they had the highest estimates of polymorphic loci, PIC, Rp, MI and He, while UB-10 and UB-01 were the least informative for the genetic diversity evaluated in this study and may be excluded from future analyses.

According to current standards, molecular markers with polymorphic information content (PIC) values above 0.5 are considered highly informative. This threshold is widely accepted in recent genetic diversity studies involving microsatellite markers. In recent *Passiflora* studies employing SSR markers, mean PIC values above 0.6 have been reported, with individual loci reaching values as high as 0.76, indicating that SSR primers with high PIC are highly informative for distinguishing genetic variation among accessions (RÊGO et al., 2025).

Although none of the primers evaluated in the present study reached the value of 0.5, UB-14 showed a value close to this threshold, suggesting its potential application in genetic diversity studies of *P. morifolia* Mast. The marker index, in turn, was not considered an appropriate measure of the primer discriminatory capacity for distinguishing genotypes of *Ximenia Americana* L., according to Rêgo et al. (2025). Although marker index has been used for this purpose, Prevost and Wilkinson (1999) reported that there is little or no relationship between marker index and the ability of a primer to distinguish genotypes, indicating that this index may have limitations when used as the sole parameter for evaluating marker efficiency.

Nevertheless, the PIC values observed demonstrate the usefulness of RAPD markers in detecting genetic variability. Future studies could further enhance marker informativeness by increasing the number of primers and by integrating other molecular marker systems with higher reproducibility and resolution, such as ISSR, SSR, or SNP markers.

The expected heterozygosity is a key parameter for assessing genetic diversity within populations (NEI, 1973). In the present study, primer UB-05 demonstrated a higher capacity to detect genetic variation, indicating its greater discriminatory power (Table 2). Therefore, its use may be prioritized in future studies aimed at the selection of genetically divergent accessions and the assessment of genetic diversity in *P. morifolia* Mast. The exclusion of primers with low informativeness contributes to the optimization of resources and enhances the efficiency of future genetic analyses. In the present study, primers such as UB-10 and UB-01, which showed the lowest estimates of polymorphism and heterozygosity, may be considered less informative and could be excluded from future analyses. Recent studies employing optimization algorithms for microsatellite panels have shown that the removal of markers with low polymorphic information content and low heterozygosity increases the

accuracy of diversity estimates while reducing operational costs, without compromising the representativeness of the genetic data (RASOARAHONA et al., 2023). Pairwise genetic dissimilarity analysis allowed the identification of more or less divergent individuals among the evaluated accessions (Table 3). The estimated dissimilarity values ranged from 0.127 to 0.683. The lowest value was observed

between genotypes 12 and 16, indicating high genetic similarity between these accessions. In contrast, the highest dissimilarity values were observed between genotypes 6 and 22 (0.683), 15 and 22 (0.633), 18 and 22 (0.628), 3 and 11 (0.621), and 4 and 22 (0.619), indicating the presence of highly divergent genotypes within the studied population.

Table 3. Genetic dissimilarity matrix of *P. morifolia* Mast. accessions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	0.00																					
2	0.46	0.00																				
3	0.38	0.44	0.00																			
4	0.56	0.30	0.58	0.00																		
5	0.38	0.43	0.53	0.52	0.00																	
6	0.44	0.49	0.41	0.55	0.40	0.00																
7	0.48	0.34	0.53	0.38	0.35	0.47	0.00															
8	0.41	0.34	0.58	0.42	0.26	0.43	0.35	0.00														
9	0.50	0.30	0.54	0.41	0.35	0.42	0.28	0.15	0.00													
10	0.40	0.52	0.54	0.45	0.47	0.47	0.36	0.43	0.42	0.00												
11	0.48	0.30	0.62	0.42	0.37	0.52	0.36	0.19	0.19	0.50	0.00											
12	0.52	0.32	0.58	0.42	0.39	0.51	0.44	0.19	0.22	0.51	0.16	0.00										
13	0.36	0.36	0.41	0.44	0.30	0.35	0.33	0.27	0.30	0.30	0.38	0.37	0.00									
14	0.42	0.37	0.47	0.50	0.48	0.52	0.41	0.44	0.47	0.40	0.48	0.52	0.28	0.00								
15	0.52	0.43	0.56	0.57	0.33	0.51	0.46	0.36	0.35	0.54	0.30	0.32	0.40	0.50	0.00							
16	0.50	0.31	0.59	0.45	0.43	0.52	0.45	0.27	0.29	0.54	0.15	0.12	0.41	0.50	0.23	0.00						
17	0.48	0.40	0.59	0.45	0.50	0.47	0.50	0.38	0.41	0.57	0.37	0.36	0.37	0.52	0.46	0.38	0.00					
18	0.54	0.42	0.59	0.40	0.43	0.46	0.32	0.36	0.42	0.42	0.46	0.43	0.31	0.35	0.48	0.49	0.37	0.00				
19	0.52	0.33	0.59	0.38	0.43	0.51	0.37	0.32	0.31	0.49	0.28	0.27	0.33	0.44	0.33	0.27	0.25	0.36	0.00			
20	0.37	0.37	0.55	0.44	0.32	0.40	0.41	0.22	0.31	0.43	0.33	0.33	0.26	0.37	0.33	0.34	0.25	0.28	0.21	0.00		
21	0.37	0.46	0.46	0.52	0.44	0.43	0.47	0.34	0.34	0.43	0.42	0.41	0.32	0.41	0.44	0.45	0.34	0.39	0.34	0.22	0.00	
22	0.47	0.57	0.61	0.61	0.59	0.68	0.56	0.48	0.47	0.57	0.54	0.56	0.52	0.47	0.63	0.59	0.59	0.62	0.54	0.48	0.42	0.00

Ward's cluster analysis separated the 22 accessions into three distinct groups. Group I comprised accessions 1, 3, and 6. Group II included accessions 5, 8, 9, 11, 12, 15, and 16, while Group III consisted of accessions 2, 4, 7, 10, 13, 14, 17, 18, 19, 20, 21, and 22. Groups II and III contained the largest number of individuals (Figure 1). The formation of these groups indicates the presence of considerable genetic variability among the 22 evaluated accessions.

Genetic dissimilarity between accessions can be explained by factors such as geographic isolation, different selective pressures or even by different reproduction strategies within the species (NEL, 1973). Recent studies have demonstrated that geographic isolation plays a key role in the evolution of reproductive isolation and divergent morphology, with divergence not solely explained by molecular genetic variation (WORSHAM et al., 2017). In studies of genetic diversity, high dissimilarity values, such as those observed between genotypes 6 and 22 (0.683), are indicative of potential genetic variability for breeding programs, as they provide rich genetic material for the selection of desirable agronomic characteristics (NIU et al., 2019).

Ward's cluster analysis, an efficient method for

identifying clustering patterns based on the minimization of intragroup variation (WARD, 1963), allowed the separation of accessions into three groups. The formation of group III, which includes 12 of the 22 accessions, indicates a certain level of genetic relatedness among these individuals, possibly associated with their geographic origin or similar management practices. However, the presence of a smaller group (group I) suggests the existence of genetically distinct accessions, corroborating the high genetic variability observed among the *P. morifolia* accessions. Similar clustering patterns were reported by Campos et al. (2019) in studies with *Passiflora edulis* Sims. using RAPD molecular markers, in which 24 genotypes were subdivided into five groups, reflecting the genetic diversity present among the evaluated accessions. Such genetic diversity can be combined with the agronomic performance of genotypes under field conditions to guide breeding programs aimed at developing high-yielding and disease-resistant genotypes, since genetically distinct accessions may respond differently to environmental factors such as climate change and disease pressure (SHAW et al., 2025).

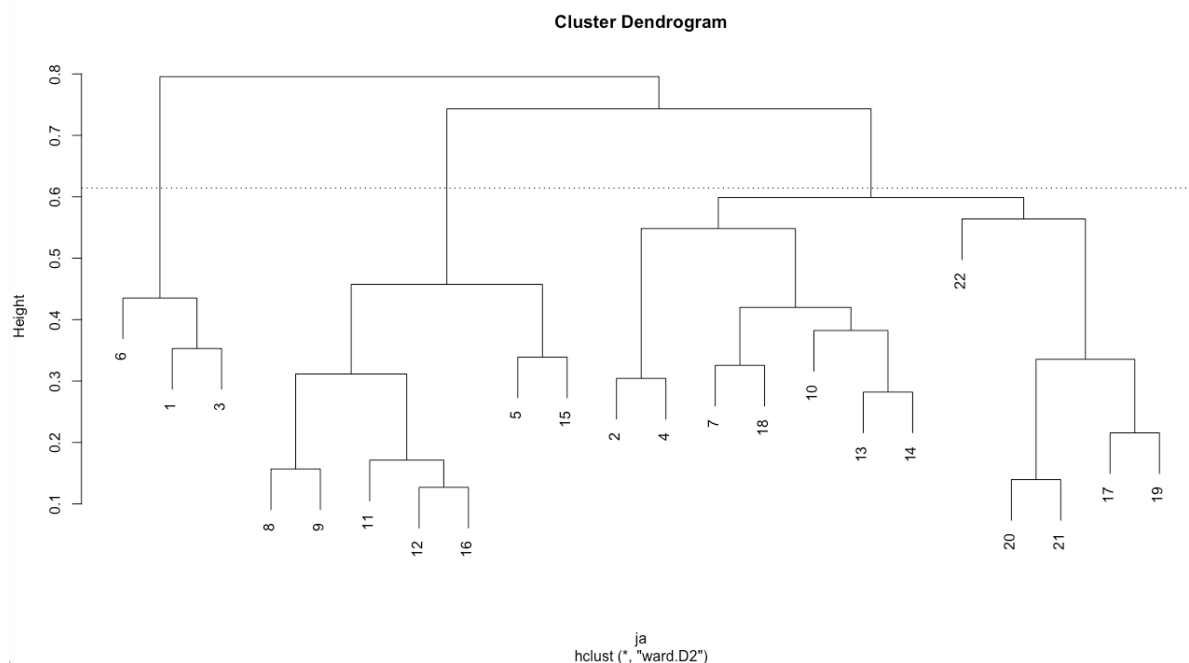


Figure 1. Dendrogram constructed by Ward's method based on Jaccard's similarity coefficient in half-sibling family of 22 *Passiflora morifolia* Mast. accessions based on 14 RAPD molecular markers. The dotted line represents the cutoff point based on Mojena (1977), forming three groups. Group I comprised accessions 1, 3, and 6. Group II included accessions 5, 8, 9, 11, 12, 15, and 16, while Group III consisted of accessions 2, 4, 7, 10, 13, 14, 17, 18, 19, 20, 21, and 22.

The cophenetic correlation coefficient was 0.98, indicating an excellent fit between the dissimilarity matrix and the dendrogram. The correlation was highly significant ($t = 19.22$; $df = 229$; $p < 0.001$). The low distortion value (2.22%) and the acceptable stress level (14.89%) further support the reliability of the hierarchical clustering and the consistency of the grouping pattern obtained. Ward's clustering technique, combined with dissimilarity analysis, provides a robust overview of genetic diversity, allowing the identification of accessions with potential for conservation and use in breeding programs. The formation of distinct groups among the accessions confirms the genetic richness within the species, highlighting the importance of conservation strategies to preserve this variability and ensure the sustainable use of *P. morifolia* in future research and breeding initiatives.

To date, studies evaluating the genetic diversity of *Passiflora morifolia* using RAPD markers are still scarce, which highlights the relevance of the results presented here as a first contribution to the molecular characterization of this species. The genetic variability detected among the 22 accessions indicates considerable divergence within the evaluated material, representing a favorable condition for breeding programs. These results will support the establishment of a base population for the breeding program of the Laboratory of Biotechnology and Plant Breeding (UFPB), enabling the identification of genetically divergent individuals with potential use as parental lines in hybridization strategies. In addition, the molecular information generated may assist in defining conservation strategies, including the maintenance of genetically divergent accessions in germplasm collections and the establishment of ex situ conservation actions aimed at preserving the genetic variability of the species. Based on these results, a base population will be established to initiate a *Passiflora* breeding program.

CONCLUSION

The RAPD markers revealed genetic variability within the half-sibling family of *Passiflora morifolia* Mast. Primer UB-02 showed the highest resolving power ($R_p = 13.09$) and 100% polymorphic loci, indicating a higher capacity to discriminate among genotypes. The greatest genetic dissimilarity was observed between accessions 6 and 22, indicating their potential use as parents in breeding programs aimed at increasing genetic variability. These findings provide relevant information for parental selection and for the strategic management of genetic resources in *Passiflora* breeding.

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