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CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

Structure and molecular genetic diversity in natural populations and active germplasm banks of *Passiflora cincinnata* Mast.

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Received: 16 March 2022; Accepted: 19 June 2022; doi:10.4067/S0718-58392022000400628

ABSTRACT

Passiflora cincinnata Mast. is a wild passion fruit species widely distributed in Brazil, with diverse economic potential and source of resistance to biotic and abiotic stress factors. Despite this, molecular genetic studies on this species are incipient, which limits the definition of priority regions for conservation and identification of accessions to be prospected for germplasm banks and inserted in genetic improvement programs. Thus, this study aimed to characterize the structure and genetic diversity of *P. cincinnata* accessions using inter simple sequence repeat (ISSR) markers. The study was carried out using leaf samples from 213 accessions of *P. cincinnata*, with 158 accessions from six natural populations (collectively considered in this study as a hypothetical metapopulation) in the state of Bahia, Brazil, and the other 55 accessions from two collections of Embrapa Cerrados and Embrapa Cassava & Fruits active germplasm banks (AGBs). Genetic estimates were based on an analysis of the amplification profile of 12 ISSR primers. Descriptive statistics analysis and the polymorphic information content (PIC) and expected heterozygosity (He) estimates were carried out using the GENES software. Approaches based on the Bayesian model, principal coordinate analysis (PCoA), and analysis of molecular variance (AMOVA) were used to assess the genetic structure. *Passiflora cincinnata* populations/collections showed high polymorphism rates. The metapopulation showed 25.3% of private markers/alleles. The metapopulation is structured in at least three genetic groups, while AGBs in two genetic groups. AMOVA indicated higher diversity within populations, with low differentiation between them.

Key words: Conservation, genetic diversity, ISSR, passion fruit, *Passiflora cincinnata*.

INTRODUCTION

The genus *Passiflora*, with more than 500 species, is considered the most representative among the genera of the family Passifloraceae (He et al., 2020), in addition to encompassing the main species of economic importance of the family, such as *P. edulis* (Faleiro et al., 2020). In Brazil, about 87 out of approximately 150 existing *Passiflora* species are considered endemic (Bernacci et al., 2020). It makes the country one of the main centers of diversity of the genus. In the state of Bahia, located in the Northeast region of Brazil, the genus is represented by 44 species widely distributed in the three biomes of the state, Cerrado, Caatinga, and Atlantic Forest (Bernacci et al., 2020).

Passion fruits, as *Passiflora* species are popularly known, have diversified economic importance, such as medicinal, ornamental, and foodstuff uses, with fruit production and commercialization standing out among the commercial activities of this genus (Cerqueira-Silva et al., 2014a; Faleiro et al., 2020). Brazil stands out on the world stage as the largest producer and consumer of passion fruit, with most of this production concentrated in the Northeast region (64.5%), especially in the state of Bahia (28.4%) (IBGE, 2019). In this context, the species with the highest commercial expression is *P. edulis* (common passion fruit), used in more than 90% of the area destined for passion fruit production in Brazil (Faleiro et al., 2020). Despite this primacy in the passion fruit production chain, there are at least 70 species of passion fruit that produce edible fruits, therefore potentially marketable and sometimes already present in small regional popular fairs (Machado et al., 2017; Faleiro et al., 2020).

Passiflora cincinnata Mast. (Figure 1), popularly known as wild passion fruit, is among the genetic resources of the genus already commercialized regionally, whose production comes mostly from extractivism and cultivated areas on a regional scale (Araújo et al., 2020). The species stands out as a wild passion fruit that has potential for diversified use, both in the food, medicinal, and ornamental fields (Machado et al., 2017; Araújo et al., 2020). It is also well appreciated in genetic improvement programs as a source of resistance to biotic and abiotic stress factors (Araújo et al., 2020). Wild passion fruit is widely distributed in Brazil, mainly in the Northeast region, with confirmed occurrence in the North, Midwest, and Southeast regions (Bernacci et al., 2020).

Molecular genetic studies on this species, especially the characterization of natural populations, are scarce although *P. cincinnata* has ecological importance, given its wide geographic distribution in different biomes and the mentioned economic potential (Cerqueira-Silva et al., 2014a). Molecular genetic studies are essential to enable the definition of priority regions for conservation and identification of species and accessions to be prospected for germplasm banks and inserted in genetic improvement programs (Cerqueira-Silva et al., 2016; Martínez et al., 2020).

Although in recent decades an advance has been observed in the quantification of genetic characterizations of the genus *Passiflora* and diversification of the adopted molecular tools/techniques (Cerqueira-Silva et al., 2016), there is a concentration of research using species with the highest commercial interest and, mainly, accessions maintained in germplasm banks or from producing regions (Cerqueira-Silva et al., 2014b; Martínez et al., 2020). The current scenario indicates that research dedicated to the genetic characterization of natural populations of wild passion fruit is incipient (Pereira et al., 2015; Maciel et al., 2019).

Considering the reality posed for wild passion fruit species, the application of molecular biology tools, such as Inter Simple Sequence Repeat (ISSR) molecular markers, allows a fast and low-cost characterization, especially useful for still poorly studied species (Turchetto-Zolet et al., 2017). Thus, this study aimed to characterize the structure and genetic diversity of *P. cincinnata* accessions present in natural populations and maintained in germplasm banks using ISSR markers.

Figure 1. *Passiflora cincinnata*, the wild passion fruit, in the native environment of occurrence (A), with emphasis on flowers and fruits in the process of development (B). Personal collection.

MATERIALS AND METHODS

Biological material

The study was carried out using samples from 213 accessions of *Passiflora cincinnata* Mast., with 158 accessions from natural populations (Table 1) and 55 accessions from active germplasm banks (AGBs) (Table 2). Samples associated with natural populations were obtained from young and healthy leaves of plants collected in six municipalities in Bahia (Anagé, Encruzilhada, Caetité, Caculé, Malhada de Pedra, and Ibiassucê), Brazil (Table 1). A specimen collected in Anagé was deposited in the State University of Southwest Bahia (UESB) herbarium under the registration HUESBVC9162. In turn, the samples related to AGBs were obtained from Embrapa Cassava & Fruits (Embrapa Mandioca e Fruticultura), located

Table 1. Populations of *Passiflora cincinnata* sampled in six municipalities in the state of Bahia, with data on collection site, geographic coordinates, altitude, precipitation, and temperature.

Site	Geographical coordinates	Altitude	Precipitation	Temperature
		m a.s.l.	mm	°C
Encruzilhada	15°31′38.8″ S, 40°59′02.6″ W	814	649	17-26
Anagé	14°38′50.7″ S, 41°03′49.2″ W	745	595	18-28
Malhada de Pedras	14°14'55.9" S, 42°35′24.9" W	639	781	15-31
Ibiassucê	15°15'06.0" S, 40°15′10.5′ W	481	717	18-28
Caculé	14°29'00.7" S, 42°15'.66.3" W	676	672	18-28
Caetité	14°06'24.1" S, 42°35'24.9" W	732	982	17-27

Table 2. Description and identification of *Passiflora cincinnata* accessions used in the study according to the active germplasm bank (AGB) of origin.

Active germplasm bank (AGB)	Code	Number of plants	Origin
Embrapa Mandioca e Fruticultura	BGP200	3	São Paulo
	BGP300	3	Bahia
	BGP-483	2	Mato Grosso do Sul
	BGP297	2	Bahia
	BGP422	3	Bahia
	BGP421	3	Bahia
	BGP268	3	Bahia
	BGP-279	3	Bahia
	BGP-481	3	Bahia
	BGP398	3	Bahia
	BGP-246	3	Bahia
	BGP-243	3	Bahia
	BGP-276	3	Bahia
	BGP-239	3	Bahia
	BGP016	1	Alagoas
Subtotal		41	
Embrapa Cerrados	CPAC MJ -26-03 (PL1)	1	PMGP CPAC
	CPAC MJ -26-03 (PL2)	1	
	CPAC MJ -26-03 (PL3)	1	
	CPAC MJ -26-03 (PL4)	1	
	CPAC MJ -26-03 (PL5)	1	
	CPAC MJ -26-03 (PL6)	1	
	CPAC MJ -26-03 (PL7)	1	
	CPAC MJ -26-03 (PL8)	1	
	CPAC MJ -26-03 (PL9)	1	
	CPAC MJ -26-03 (PL10)	1	
	CPAC MJ -26-03 (PL11)	1	
	CPAC MJ -26-03 (PL12)	1	
	CPAC MJ -26-03 (PL13)	1	Minas Gerais
	CPAC MJ -26-03 (PL14)	1	Minas Gerais
Subtotal		14	
Total		55	

CPAC: Cerrado Agricultural Research Center (Embrapa Cerrados).

in Cruz das Almas, Bahia, totaling 41 accessions, and the Cerrado Agricultural Research Center (Embrapa Cerrados – CPAC), located in Brasília-DF, totaling 14 accessions.

The genomic DNA of accessions from natural populations were isolated from leaf samples following the protocol adapted from Doyle and Doyle (1990) and stored at -20 °C at the Laboratory of Applied Molecular Genetics (LGMA), located at UESB (Campus Juvino Oliveira) in the municipality of Itapetinga-Bahia. The DNA samples of the accessions from AGBs were obtained from the LGMA genomic DNA bank.

The concentration and quality of DNA samples were estimated by spectrophotometry using a spectrophotometer (BioDrop μLITE, Biochrom, Cambridge, UK) and electrophoresis in 1% agarose gel (m/v) for 1 h at 90 V, with running solution 0.5X TBE (Tris-boric acid-EDTA), visualized and photo-documented on an L-PIX EX photo-documentation system (Loccus, Cotia, São Paulo, Brazil) under ultraviolet light using a nucleic acid gel stain (GelRed, Biotium, Fremont, California, USA) intercalator and a Kodak camera. The molecular weight marker Lambda (undigested Lambda DNA) (Invitrogen, Carlsbad, California, USA) was adopted as a standard to estimate the DNA concentration (ng μL^{-1}), according to the specifications described by the manufacturer.

Amplification reactions

The amplification reactions to characterize the 213 accessions of *P. cincinnata* were performed using 12 previously selected inter simple sequence repeat (ISSR) primers (Table 3) (Dias et al., 2020). Reactions were carried out in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, California, USA), with a final volume of 16 μL containing 8 ng DNA, 1.7 μL 10X PCR buffer (20 mM Tris-HCl [pH 8.4] and 50 mM KCl), 1.0 μL $MgCl_2$ (50 mM), 1.0 μL 2.5 mM dNTP mix, 0.11 unit Taq DNA polymerase (Invitrogen), Milli-Q water, and 1.0 μL 0.8 mM primer. The amplification program involves initial denaturation at 94 °C for 5 min, followed by 34 cycles (94 °C for 50 s, 48 °C for 60 s, and 72 °C for 60 s) and a final extension at 72 °C for 5 min.

Aliquots of the amplification products were stained with GelRed intercalator (Biotium) and subjected to electrophoresis in a 2% (m/v) agarose gel for 2 h at 120 V and visualized in an L-PIX EX photo-documentation system (Loccus) under UV light. The molecular weight marker 1 kb Plus DNA Ladder (Invitrogen) was used as a pattern of generated marks.

Analysis and estimates of structure and genetic diversity

Analyses of the amplification profile of the different ISSR primers were conducted by two evaluators. The obtained marks were used to construct a matrix of binary data in Excel, where zero (0) was assigned for the absence of markers, one (1) for their presence, and nine (9) for inconclusive data. Then, descriptive statistical analyses were carried out, such as number of markers, percentage of polymorphic markers, number of rare markers/alleles (with an occurrence equal to or less than 5% of accessions in a population/AGB), and number of private markers/alleles (only present in one population/AGB).

Table 3. Description of 12 ISSR primers, number of observed markers, estimates of polymorphic information content (PIC), and expected heterozygosity (He) from the genetic characterization of 213 *Passiflora cincinnata* accessions.

Code	Sequence (5′ → 3′)	Number of markers*	PIC	He
DiGA3′T	$(GA)_8$ T	11	0.29	0.37
DiCA3′G	$(CA)_8$ G	14	0.24	0.30
DiCA3′RG	$(CA)_8$ RG	10	0.28	0.35
DiCA3′YG	$(CA)_8$ YG	12	0.31	0.39
TriCAC3′YC	$(CAC)_5$ YC	11	0.33	0.42
TriCAC5′CY	$(CAC)_5$ CY	11	0.25	0.30
TriGTG3′YC	$(GTG)_5$ YC	13	0.23	0.29
TriACG3′RC	$(ACG)_5$ RC	13	0.24	0.30
TriCGA3′RC	$(CGA)_5$ RC	15	0.28	0.35
TriGAC3′RC	$(GAC)_5$ RC	12	0.30	0.38
TriGCA3′RC	$(GCA)_5$ RC	11	0.25	0.32
TriGCC3′RC	$(GCC)_5$ RC	9	0.32	0.40
Total		142	0.28	0.35

*100% polymorphic markers considering the evaluation of 213 *Passiflora cincinnata* accessions.

Estimates of polymorphic information content (PIC) and expected heterozygosity (He) were carried out using the Genes software (Cruz, 2006); PIC was calculated according to the formula proposed by Botstein et al. (1980):

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$$

where P_{ij} is the frequency of allele j at marker i.

According to the authors, indices below 0.25 are slightly informative; between 0.25 and 0.50, informative; and above 0.50 highly informative, where 0.50 is the maximum value reached in dominant markers such as ISSR (Botstein et al., 1980).

The He was calculated as proposed by Nei (1987), applying the following formula:

$$He = 1 - \sum_{i=1}^{k} x_i^2$$

where x_i is the frequency of allele i and k is the number of alleles.

The Bayesian analysis of population structure estimation was performed using the STRUCTURE software version 2.3.4 (Pritchard et al., 2000). The admixture model, which admits genetic flow between samples, was assumed in these analyses, and the possibility of the existence of one to 10 genetic groups (K) was evaluated. Five replicates were performed for each K estimate, and a burn-in period of 50 000 was considered in each replicate/estimate, followed by 500 000 replicates. The estimation of the number of genetic groups that best represents the distribution of diversity was performed using the online tool STRUCTURE Harvester (Earl and vonHoldt, 2012), using the method proposed by Evanno et al. (2005).

Principal coordinate analysis (PCoA) and analysis of molecular variance (AMOVA), with 999 bootstraps (random permutations), were performed to observe the genetic variation and its distribution/structuring. The analyses were performed using the GenALEX v.6.5 software (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

Amplification reactions from the 12 ISSR primers, considering the 213 accessions of *P. cincinnata*, produced 142 markers, all polymorphic, with a mean of 11.8 markers per primer (Table 3). Primers TriCGA3`RC and TriGCC3`RC produced the lowest (nine) and highest (15) number of markers, respectively (Table 3). The PIC values ranged from 0.23 (TriGTG3`YC) to 0.33 (TriCAC3`YC) (mean of 0.28), most of them classified as informative (DiGA3`T, DiCA3`RG, DiCA3`YG, TriCAC3`YC, TriCAC5`CY, TriCGA3`RC, TriGAC3`RC, TriGCA3`RC, and TriGCC3`RC) (Table 3).

The descriptive analyses showed differences for all the variables (i.e., number of markers, percentage of polymorphism, private alleles, rare alleles, and PIC) in the six natural populations in both germplasm banks and, mainly, between accessions of natural populations and germplasm banks (Table 4). The number of markers ranged from 100 (Ibiassucê) to 137 (Anagé) for natural populations and 72 (Embrapa Cerrados) to 103 (Embrapa Cassava & Fruits) for AGBs (Table 4). Together,

Table 4. Descriptive analyses, estimates of polymorphic information content (PIC) and expected heterozygosity (He), obtained from the characterization of 213 *Passiflora cincinnata* accessions based on ISSR primers.

Population/Collection	Number accessions	Number markers	Private alleles	Rare alleles	Polymorphism percentage	PIC	He
Anagé	26	137	7	4	78.1	0.21	0.28
Encruzilhada	26	109	0	3	83.5	0.26	0.33
Caetité	30	108	0	0	86.1	0.26	0.33
Caculé	28	114	0	0	78.0	0.24	0.30
Malhada de Pedra	30	115	0	0	77.4	0.24	0.30
Ibiassucê	18	100	0	0	77.0	0.24	0.30
Metapopulation	158	142	36	2	97.9	0.28	0.35
Embrapa Mandioca e Fruticultura	41	103	26	3	87.4	0.23	0.30
Embrapa Cerrados	14	72	3	0	75.0	0.30	0.38
Active germplasm banks	55	106	0	3	95.2	0.27	0.33

the six natural populations (considered in this study as a hypothetical metapopulation) totaled 142 markers, while 106 markers were observed in the two germplasm banks (Table 4). The percentage of polymorphism was ≥ 75% in all populations and banks, the highest percentage being observed for the Embrapa Cassava & Fruits collection (87.4%), and the lowest for the Embrapa Cerrados collection (75%) (Table 4). The PIC estimates showed mean values ranging from 0.21 (Anagé) to 0.30 (Embrapa Cerrados). The He estimates between populations/AGBs showed values ≥ 0.28, ranging from 0.28 (Anagé) to 0.38 (Embrapa Cerrados) (Table 4).

The percentages of polymorphism found at the species level (100%) (Table 3) and between populations/collections (≥ 75%) (Table 4) are similar to the data available in the literature for different species of the genus *Passiflora* based on ISSR primers (Costa et al., 2012; Pereira et al., 2015; Sousa et al., 2015; Fonseca et al., 2017; Vianna et al., 2019; Maciel et al., 2019; Martínez et al., 2020; Ho et al., 2021) with percentages ranging from 75 (Martínez et al., 2020) to 100% (Ho et al., 2021). The observed polymorphism rates may be associated with the flower anatomy, which, although presenting anatomical characteristics of complete flowers with the two reproductive organs, gynoecium and androecium, present self-incompatibility due to floral morphology. Thus, they require cross-pollination by floral visitors, which contributes to the increase in the genetic variability of the species of the genus, especially *P. cincinnata* (Kiill et al., 2010; Araújo et al., 2020).

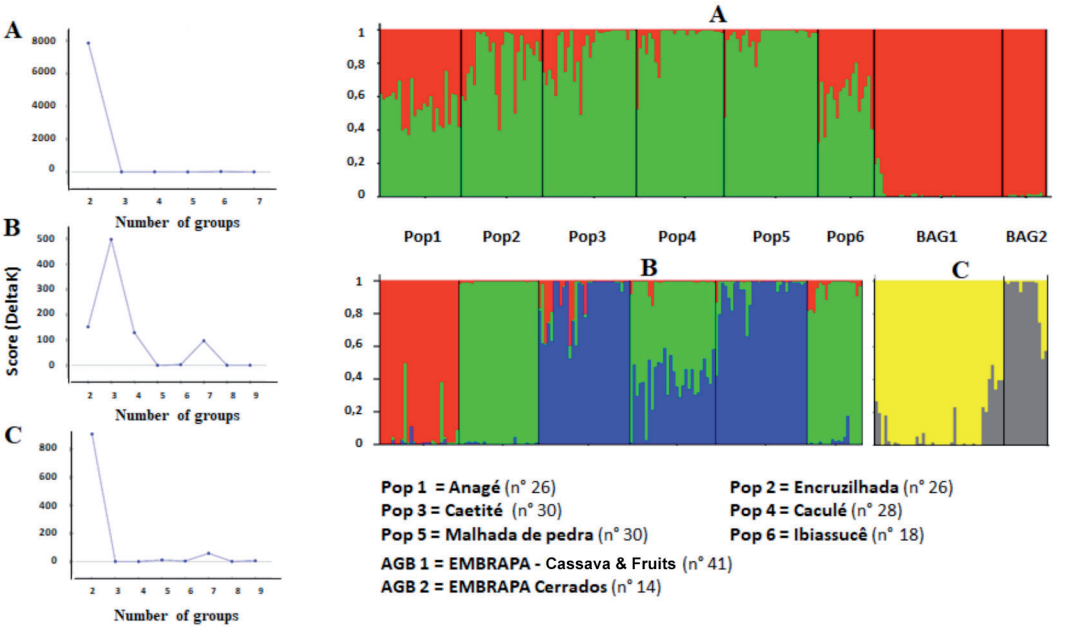
Thirty-six private alleles were identified in natural populations when considering the hypothesis of the existence of a metapopulation compared to AGBs (Table 4). In turn, a total of 29 private alleles/markers were observed among germplasm banks, most of them (26) in the Embrapa Cassava & Fruits AGB. Seven private alleles/markers were identified among natural populations, all of them present in the Anagé population. In turn, regarding the identification of rare alleles/markers, considering the metapopulation hypothesis, only two rare alleles were observed in the set of 158 accessions. Three rare alleles/markers were observed when the two AGBs were evaluated, all of them present in the Embrapa Cassava and Fruits AGB. Considering only the natural populations, seven alleles/markers were identified, four in the Anagé population and three in the Encruzilhada population.

Of the observed 142 markers, 25.3% were alleles present only in the metapopulation (private alleles) (Table 4), that is, they were not detected in both AGBs. All private alleles/markers among natural populations were found in the Anagé population, and one of them was also rare. The presented scenario emphasizes the importance of characterizing natural populations of *P. cincinnata* so that the prospection strategies for accessions to be conserved in AGBs and/or inserted in genetic improvement programs and the indication of areas to be conserved are better targeted. Although *P. cincinnata* is widely distributed in Brazil (Bernacci et al., 2020) and does not present extinction risk assessments (IUCN, 2021), most of the populations studied here are present in anthropized environments, such as roadsides, and most of the fruit commercialization is based on extractivism, factors that together can jeopardize the species survival. Studies that characterize the diversity of natural populations become even more important (Cerqueira Silva et al., 2014b; 2016) when considering the presence of private and rare markers/alleles, given that they may be linked to genes of agronomic interest (Reis et al., 2011).

The Bayesian analysis indicated the existence of two genetic groups (Figure 2A) in the natural populations and germplasm banks of *P. cincinnata* as the most likely. The structuring in two genetic groups showed a predominance of a genetic group represented by the green color for the metapopulation accessions (approximately 72%), while the second genetic group was represented by the red color for the AGB accessions (100%) (Figure 2A).

The structure evaluation indicated the existence of three genetic groups as the most likely when considering only the metapopulation hypothesis (Figure 2B). The first genetic group was formed by 24 out of the 26 accessions of the Anagé population and one accession of the Caculé population (represented by the red color in the histogram); the second genetic group was formed by all 44 accessions of the Encruzilhada and Ibiassucê populations and three accessions of the Caculé population (represented by the green color in the histogram); and the third genetic group was composed of 58 out of the 60 accessions from the Caetité and Malhada de Pedra populations (represented by the blue color in the histogram). In turn, 24 out of the 28 accessions of the Caculé population, together with four accessions of the other populations, were identified as a mixture of the second and third genetic group, as they did not reach the minimum percentage of 70% of a single genetic group in their composition. Accessions belonging to AGBs were grouped into two distinct subgroups when evaluated separately from natural populations (Figure 2C). The first subgroup was composed of 36 out of the 41 Embrapa Cassava & Fruits accessions (represented by the yellow color in the histogram), whereas the second subgroup was composed of 12 out of the 14 Embrapa Cerrados accessions (represented by the gray color in the histogram). The other seven accessions were classified as a mixture, adopting the same criteria used in natural populations.

Figure 2. Histograms (based on Delta K values) representing the distribution of probable genetic groups for the 213 *Passiflora cincinnata* accessions representative of six natural populations and two active germplasm banks (AGB) collections of Embrapa Cerrados and Embrapa Cassava & Fruits (A); substructure considering the analysis of the metapopulation (B) and two AGBs (C). The colors used in the histograms represent the most likely ancestry of the group from which the individuals were derived.



The formation of an exclusive genetic group, represented by the red color, for accessions of the Anagé population may be related to the fact that 7 markers/alleles were exclusive to this population. The second genetic group was mostly shared by the Encruzilhada and Ibiassucê accessions. The genetic similarity seems to indicate that human interference has occurred in the distribution of the species despite the distance of about 258 km between the municipalities. Natural dispersion and selection processes may not have been the main force shaping the genetic structure since environmental factors are also different between environments. The third genetic group shared between the Malhada de Pedras and Caetité accessions can be explained by the predominance of the Caatinga biome in these municipalities. Larger geographic distances associated with smaller genetic distances can be observed between populations located within the same geo-environmental unit (Pereira et al., 2015). Conserving populations sampled in the widest possible range of ecogeographic conditions should maximize conserved genetic diversity (Thormann et al., 2016).

The scatter plot obtained by PCoA (Figure 3) allowed the observation of a wide genetic diversity among populations/collections. The results obtained through PCoA corroborate the sub-structuring hypothesis obtained by the Bayesian analysis in three and two genetic groups for the metapopulation and AGBs, respectively (Figures 2B and 2C), showing a grouping of populations/collections that share the same genetic group. The groups formed in the PCoA graph show the separation between metapopulation and AGBs, with the genetic composition of each population/collection. The AGBs differed from each other in terms of the polymorphism rate (Table 3) and genetic group composition (Figures 2C and 3), which demonstrates the importance of maintaining the species in different AGBs.

The implementation of an AMOVA revealed that the highest percentage of variation occurred within (99%) and not between populations (1%) (Table 5). Pereira et al. (2015) found similar results when evaluating the use of ISSR markers in 259 accessions of *P. setacea* distributed in 12 municipalities in the state of Bahia. The higher genetic diversity found within populations and not between populations (Table 4) may also be related to self-incompatibility due to floral morphology observed in *Passiflora* species (Araújo et al., 2020), which would lead to an increase in the polymorphism rate of species from the same population, thus increasing genetic variability. Although the flowers of *P. cincinnata* are hermaphroditic, they present the strategy of herkogamy, thus reducing inbreeding depression (Kiill et al., 2010).

Figure 3. Scatter plot based on principal coordinate analysis (PCoA). The pie charts represent a mean of the total gene composition of each evaluated population/collection.

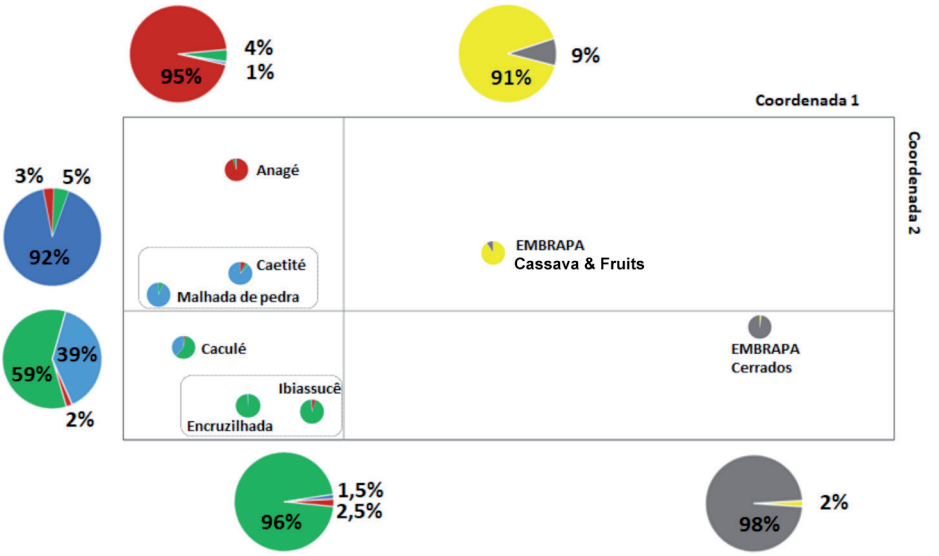


Table 5. Analysis of molecular variance (AMOVA) based on ISSR primers for the 213 *Passiflora cincinnata* accessions.

	DF	SS	MS	Var	%
Among	7	4.453	0.636	0.006	1
Within	205	99.105	0.483	0.483	99
Total	212	103.559		0.489	100

PhiPT = 0.012 P (rand ≥ data) 0.001

DF: Degrees of freedom; SS: sum of squares; MS: mean squares; Var: estimated variation; PhiPT: value for genetic variability for dominant markers, P (rand ≥ data): Probability for PhiPT based on standard permutation across the entire data set.

CONCLUSIONS

Passiflora cincinnata populations showed high polymorphism rates. The metapopulation showed 25.3% of private markers/alleles. Natural populations are structured in at least three genetic groups, while active germplasm banks (AGBs) are in two genetic groups. Analysis of molecular variance indicated higher diversity within populations, with low differentiation between them.

The knowledge about the variability and genetic structure of *P. cincinnata* presented in this study is important regarding the elaboration of collection strategies and expeditions to compose germplasm banks and make in situ and ex situ conservation programs more efficient. A large proportion of the variability present within populations suggests that fewer populations will be needed for a representation of *P. cincinnata* diversity. Material collection from the Anagé population is suggested to be prioritized since 7 alleles are exclusive of this population.

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Research Support of the State of Bahia (FAPESB TO PIE 0014/2016 and TO APP005/2016) and CNPQ (No. 431493/2016.2), and AUXPPI/UESB Program (TO 24/2019); the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Graduate Programs (PPGCA/UESB and PMBqBM/UESB) of the State University of Southwest Bahia (UESB) for granting scholarships to promote the research.

REFERENCES

Araújo, F.P., Melo, N.F., Aidar, S. de T., Faleiro, F.G., and Jesus, O.N. 2020. Maracuyá de la Caatinga: *Passiflora cincinnata* Mast. In Carlosama, A.R., Faleiro, F.G., Morera, M.P., Costa, A.M. (eds.) PASIFLORAS especies cultivadas en el mundo. ProImpress, Brasília, D.F., Brasil.

Bernacci, L.C., Nunes, T.S., Mezzonato, A.C., Milward-de-Azevedo, M.A., Imig, D.C., and Cervi, A.C. (in memoriam). 2020. *Passiflora* in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. Available at http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB12506 (accessed September 2020).

Botstein, D., White, R.L., Skolnick, M., and Davis, RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics 32:314-331.

Cerqueira-Silva, C.B.M., Faleiro, F.G., Jesus, O.N., Santos, E.S.L. and Souza, A.P. 2016. The genetic diversity, conservation, and use of passion fruit (*Passiflora* spp.) In Ahuja, M.R., and Jain, S.M. (eds.) Genetic diversity and erosion in plants. Sustainable Development and Biodiversity, vol 8. Springer, Cham, Switzerland. doi:10.1007/978-3-319-25954-3_5.

Cerqueira-Silva, C.B.M., Nunes, O., Santos, E.S.L., Corrêa, R.X., and Souza, A.P. 2014a. Genetic breeding and diversity of the Genus *Passiflora*: Progress and perspectives in molecular and genetic studies. International Journal of Molecular Sciences 15:14122-14152. doi:10.3390/ijms150814122.

Cerqueira-Silva, C.B.M., Santos, E.S.L., Jesus, O.N., Vieira, J.G.P., Mori, G.M., Corrêa, R.X., et al. 2014b. Molecular genetic variability of commercial and wild accessions of passion fruit (*Passiflora* spp.) targeting *ex situ* conservation and breeding. International Journal of Molecular Sciences 15(12):22933-22959. doi:10.3390/ijms151222933.

Costa, J.L., Jesus, O.N.D., Oliveira, G.A.F., and Oliveira, E.J.D. 2012. Effect of selection on genetic variability in yellow passion fruit. Crop Breeding and Applied Biotechnology 12:253-260. doi:10.1590/S1984-70332012000400004.

Cruz, C.D. 2006. Programa Genes: Análise multivariada e simulação. Editora da Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

Dias, N.D.S.C., de Souza, L.N.B., Santana, V.D.O., Meira, M., Santos, E., Faleiro, F., et al. 2020. Characterization and selection of ISSR molecular markers in species of *Passiflora* spp. Multi-Science Journal 3(3):17-22. doi:10.33837/msj.v3i3.1290.

Doyle, J.J., and Doyle, J.L. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Earl, D.A. and vonHoldt, B.M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14:2611-2620.

Faleiro, F.G., Junqueira, N.T.V., de Jesus, O.N., Cenci, S.A., Machado, C.F., Rosa, R.C.C., et al. 2020. Maracuyá: *Passiflora edulis* Sims. In Carlosama, A.R., Faleiro, F.G., Morera, M.P., Costa, A.M. (eds.) PASIFLORAS especies cultivadas en el mundo. ProImpress, Brasília, D.F., Brasil.

Fonseca, K.G.D., Faleiro, F.G., Junqueira, N.T.V., Barth, M., and Feldberg, N.P. 2017. Morphoagronomic and molecular characterization of ornamental passion fruit cultivars. Pesquisa Agropecuária Brasileira 52(10):849-860.

He, X., Luan, F., Yang, Y., Wang, Z., Zhao, Z., Fang, J., et al. 2020. *Passiflora edulis*: An insight into current researches on phytochemistry and pharmacology. Frontiers in Pharmacology 11:617. doi:10.3389/fphar.2020.00617.

Ho, V.T., Ngo, T.K.A., Phan, T.H.T., Ta, T.T.T., and Tran, T.K.P. 2021. Genetic diversity among passion fruit (*Passiflora edulis*) accessions of southern Vietnam using RAPD and ISSR markers. SABRAO Journal of Breeding and Genetics 53(1):1-14.

IBGE. 2019. Produção agrícola municipal. Instituto Brasileiro de Geografia e Estatística (IBGE), Rio de Janeiro, Brasil. Available at http://www.cnpmf.embrapa.br/Base_de_Dados/index_pdf/dados/brasil/maracuja/b1_maracuja.pdf (acessed August 2020).

IUCN. 2021. Lista vermelha de espécies ameaçadas da IUCN. Versão 2021-2. Available at https://www.iucnredlist.org (accessed 20 August 2021).

Kiill, L.H.P., Siqueira, K.M.M., de Araújo, F.P., Trigo, S.P.M., Feitoza, De A.E., e Lemos I.B. 2010. Biologia reprodutiva de *Passiflora cincinnata* Mast. (Passifloraceae) na região de Petrolina (Pernambuco, Brasil). Oecologia Australis 14 (1):115-127.

Maciel, K.S., de Lima, P.A.M., Madalon, F.Z., Ferreira, M.F.S., Alexandre, R.S., and Lopes, J.C. 2019. Genetic diversity in passion fruit plants at different altitudes. Australian Journal of Crop Science 13(7):1083-1093. doi:10.21475/ajcs.19.13.07.

Machado, C.F., Faleiro, F.G., Junqueira, N.T.V., Araujo, F.P. de, Costa, A.M., Junghans, T.G. 2017. Espécies silvestres de maracujazeiro comercializadas em pequena escala no Brasil. In Junghans, T.G., e Jesus, O.N. de (eds.) 2017. Maracujá do cultivo à comercialização. Embrapa, Brasília, D.F., Brasil.

Martínez, M.A., Morillo, A.C., and Reyes-Ardila, W. 2020. Characterization of the genetic diversity in *Passiflora* spp. in the Boyacá Department, Colombia. Chilean Journal of Agricultural Research 80:342-351. doi:10.4067/S0718-58392020000300342.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, USA.

Peakall, R., and Smouse, P.E. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. Bioinformatics 28:2537-2539.

Pereira, D.A., Corrêa, R.X., and Oliveira, A.C. 2015. Molecular genetic diversity and differentiation of populations of 'somnus' passion fruit trees (*Passiflora setacea* DC): Implications for conservation and pre-breeding. Biochemical Systematics and Ecology 59:12-21. doi:10.1016/j.bse.2014.12.020.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Reis, R.V., Oliveira, E.J., Viana, A.P., Pereira, T.N.S., Pereira, M.G., e Silva, M.G.M. 2011. Diversidade genética em seleção recorrente de maracujazeiro amarelo detectada por marcadores microssatélites. Pesquisa Agropecuaria Brasileira 46(1):51-57. doi:10.1590/S0100-204X2011000100007.

Sousa, A.G.R., Souza, M.M., Melo, C.A.F., and Sodré, G.A. 2015. ISSR markers in wild species of *Passiflora* L. (*Passifloraceae*) as a tool for taxon selection in ornamental breeding. Genetics and Molecular Research 14(4):18534-18545. doi:10.4238/2015.December.23.41.

Thormann, I., Reeves, P., Reilley, A., Engels, J.M.M, Lohwasser, U., Börner, A., et al. 2016. Geography of genetic structure in barley wild relative *Hordeum vulgare* subsp. *spontaneum* in Jordan. PLOS ONE 11(8):e0160745. doi:10.1371/journal.pone.0160745.

Turchetto-Zolet, A.C., Turchetto, C., Zanella, C.M., e Passaia, G. (organizadores). 2017. Marcadores moleculares na era genômica: Metodologias e aplicações. Sociedade Brasileira de Genética, Ribeirão Preto, Brasil. Available at https://www.lume.ufrgs.br/bitstream/handle/10183/206114/001056131.pdf?sequence=1.

Vianna, L., Pereira, T., Santos, E., Vianna, A., Pereira, M., Ramos, H., et al. 2019. ISSR and SSR markers for determining genetic relationships among three wild species of *Passiflora*. Genetic and Molecular Research 18:1-10. doi:10.4238/gmr18040.