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Molecular characterization of *Passiflora edulis* f. *flavicarpa* Degener with ISSRs markers

Caracterização molecular de *Passiflora edulis* f. *flavicarpa* Degener com marcadores ISSRs

A. C. Morillo[a]*, D. A. Muñoz[b] and Y. Morillo[c]

[a]Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias Agropecuarias, Grupo Competitividad Innovación y Desarrollo Empresarial – CIDE, Tunja, Boyacá, Colombia

[b]Universidad Pedagógica y Tecnológica de Colombia, Facultad de Ciencias, Grupo Competitividad Innovación y Desarrollo Empresarial – CIDE, Tunja, Boyacá, Colombia

[c]Universidad Nacional de Colombia, Palmira, Valle del Cauca, Colombia

Abstract

Passiflora edulis it is a specie widely distributed and cultivated in Colombia, with economic potential. Although there is a wide genetic and phenotypic variability, it has not yet been explored through the use of molecular techniques. This study aimed to characterize the structure and genetic diversity of *P. edulis* cultivars using ISSR markers. The study was carried out using leaf samples from 21 cultivars of *P. edulis* collected within a productive system in the department of Boyacá, Colombia, using seven ISSR primers. Genetic similarity was used to cluster by the UPGMA method, polymorphic information content (PIC), expected heterozygosity (He), Shannon index (I), gene flow (Nm), and coefficient of genetic differentiation (Gst) were estimated using POPGENE and TFPGA software. The Bayesian model and analysis of molecular variance (AMOVA) were used to assess the genetic structure. Cultivars of *P. edulis* showed high polymorphism rates. Seven ISSR produced 138 loci. The cluster analysis formed two groups according to the genetic similarity and phenotypic characteristics associated mainly with the fruit. The average value of expected heterozygosity was 0.29 for the total population and 0.27 and 0.22 for groups I and II, respectively. AMOVA indicates higher diversity within groups, but not between groups showing levels of hierarchy different from those considered in this study. Moderate genetic differentiation (Gst=0.12) and high gene flow (Nm=3.91) are observed.

Keywords: genetic diversity, genetic resource, molecular markers, passion fruit, plant breeding.

Resumo

A *Passiflora edulis* é uma espécie amplamente distribuída e cultivada na Colômbia, com potencial econômico. Embora exista uma grande variabilidade genética e fenotípica, ela ainda não foi explorada através do uso de técnicas moleculares. O presente estudo teve como objetivo caracterizar a estrutura e diversidade genética dos cultivares de *P. edulis* utilizando marcadores ISSR. O estudo foi realizado com amostras de folhas de 21 cultivares de *P. edulis* coletadas em um sistema produtivo no departamento de Boyacá, Colômbia, utilizando sete *primers* ISSR. A similaridade genética foi utilizada para agrupamento pelo método UPGMA, o conteúdo de informação polimórfica (PIC - Polymorphic Information Content), a heterozigosidade esperada (He), o índice de Shannon (I), o fluxo gênico (Nm) e o coeficiente de diferenciação genética (Gst) foram estimados usando os Programas POPGENE e TFPGA. O modelo bayesiano e análise de variância molecular (AMOVA - Analysis of Molecular Variance) foram utilizados para avaliar a estrutura genética. Os cultivares de *P. edulis* apresentaram altas taxas de polimorfismo. Sete ISSR produziram 138 loci. A análise de agrupamento formou dois grupos de acordo com a similaridade genética e características fenotípicas associadas principalmente ao fruto. O valor médio da heterozigosidade esperada foi de 0,29 para a população total e 0,27 e 0,22 para os grupos I e II, respectivamente. A AMOVA indicou uma maior diversidade dentro dos grupos, mas não entre grupos apresentando níveis de hierarquia diferentes daqueles considerados neste estudo. Observou-se diferenciação genética moderada (Gst=0,12) e alto fluxo gênico (Nm=3,91).

Palavras-chave: diversidade genética, recurso genético, marcadores moleculares, maracujá, melhoramento de plantas.

1. Introduction

Passion fruit belongs to the family Passifloraceae. The genes *Passiflora* L., considered to be the largest of this family comprising about 500 species (He et al., 2020), which present a wide phenotypic variability in flowers, stems, leaves and fruits (Martinez et al., 2020). Worldwide, Brazil is the largest producer and consumer of passion fruit, both

*e-mail: ana.morillo@uptc.edu.co
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fresh and processed (Ocampo et al., 2021). The passion fruit tree is native to tropical America (Souza et al., 2020). The botanical varieties *Passiflora edulis* f. *flavicarpa* (yellow or sour passion fruit) and *P. edulis* Sims f. *edulis* (purple passion fruit) are most important economically (Wu et al., 2020).

Most *Passiflora* species are allogamous, diploid, with 2n = 12, 18, or 20 chromosomes (Yotoko et al., 2011), with ornamental potential and medicinal properties (Belo et al., 2018; He et al., 2020), due to its contents of alkaloids, flavonoids and carotenoids, minerals and vitamins A, C, and D. The seeds contain essential fatty acids (55–66% linoleic acid, 18–20% oleic acid, and 10–14% palmitic acid), that can be used in the food and cosmetic industries (da Silva et al., 2015).

In spite of the economic importance and its various potential uses in industry and medicine of passion fruit in Colombia, there has been scanty information on the genetic diversity of cultivated germplasm in its main producing departments (Antioquia, Huila, Meta, and Valle del Cauca) (Ocampo et al., 2021). The characterization of the germplasm is fundamental for the knowledge of its genetic variability (Hashemi and Khadivi, 2020), to direct the programs of conservation, genetic improvement and potential use of the germplasm (Chavarría-Perez et al., 2020; Holanda et al., 2020). The genetic diversity of Passiflora species worldwide has been evaluated using morphological descriptors (Ramaiya et al., 2018; do Carmo et al., 2017; Ocampo et al., 2017; Pérez and d'Eeckenbrugge, 2017), agronomic traits (Galeano Mendoza et al., 2018) and physicochemical descriptors (dos Reis et al., 2018). Although the morphological characterization is the one that is carried out most frequently due to its low cost and easy to do, it presents serious limitations in terms of the inheritance of the characters, the dependence on the phenology of the plant and the vulnerability to environmental changes (Ocampo et al., 2017).

As an alternative, molecular marker utilizations for plant identification have been widely accepted due to several advantages, such as unlimited number, unaffected by environment and growing conditions, easy interpretation, and reliable repeatable results (Antunes et al., 1997). Several studies have been reported on the genetic diversity of *Passiflora* species with random amplified polymorphic DNA (RAPD) (Vieira et al., 2019), amplified fragments length polymorphism markers (AFLP) (Ortiz et al., 2012), simple sequence repeat markers (SSR) (Araya et al., 2017; Grisi et al., 2019), inter-simple sequence repeats (ISSR) (Martinez et al., 2020) and sequence-related amplified polymorphism (SRAP) markers (Oluoch et al., 2018). Among these, the ISSR method is preferred for use in plants with limited prior genetic knowledge, as it is a simple, fast, relatively cheap technique, requires minimal laboratory skills, small amounts of DNA, and generates a large number of fragments in each reaction (Vianna et al., 2019).

In Colombia, some studies have been carried out on intra- and interspecific genetic variability in the genus *Passiflora* L using morphological descriptors (Ocampo et al., 2021), molecular markers such as amplified fragment length polymorphism (AFLP) (Ocampo et al., 2004); microsatellites (Bernal-Parra et al., 2014) and Intersimple Sequence Repeat (ISSR) (Morillo et al., 2023). These studies have established the distances between cultivated species and their wild relatives as a strategy for the exploration and conservation of genetic resources (Ocampo et al., 2021). 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 (Martinez et al., 2020).

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 (Silva et al., 2022; Morillo et al., 2023). Thus, this study aimed to characterize the genetic diversity of *P. edulis* f. *flavicarpa* cultivars present in natural populations and maintained in farms of passion fruit producers in the municipality of Miraflores, Boyacá in Colombia, using ISSR markers, with a view to proposing conservation strategies and genetic improvement of the species.

2. Material and Methods

2.1. Plant material

Leaf samples of twenty-one passion fruit cultivars were collected from farmer´orchard with the greatest tradition in this crop in the municipality of Miraflores. The cultivars were selected based on the differences observed in terms of clearly distinguishable morphological descriptors (Ocampo et al., 2021) (Figure 1), Boyacá located at 5°11'47"NL and 73°08'40"WL, at an altitude of 1,432 masl, with an average annual temperature of 16°C and a relative humidity of 87% on the sidewalk Pueblo and Cajón. The harvested samples and stored to -80°C until use.

2.2. DNA extraction

Genomic DNA was obtained from approximately 200 mg of healthy young plant leaf tissue macerated in liquid nitrogen using the Dellaporta et al. (1983) protocol modified. The DNA integrity was tested by electrophoresis on 0.8% agarose gel, stained with Gelred dye (Biotum, USA). The concentration was determined in a spectrophotometer (Biotek EPOCH|2 device) in the absorbance ratios in ng/µL to A260/A280. They were diluted in HPLC water for a total volume 100 µL to 10 ng/µL, stored at -20°C.

2.3. ISSR amplification

For each sample, seven ISSRs primers were amplified (Table 1). The ISSRs were selected from a database of ISSRs applied in previous based on their high polymorphic content and broad coverage of the passion fruit genome (Ferreira et al., 2021). The PCR amplification reaction consisted of 20 ng of DNA, 2 µmol of the primers, 1 U of Taq DNA Polymerase, 0.2 mM of dNTPs, 1.5 mM of $MgCl_2$ and 1X buffer in 25 µL reaction. The amplification program was 5 minutes at 95°C (initial denaturation), followed by 37 cycles at 95°C for 30 s, annealing temperature 50-58°C (depending of primer, Table 1) for 45 s, 72°C for 2 min;

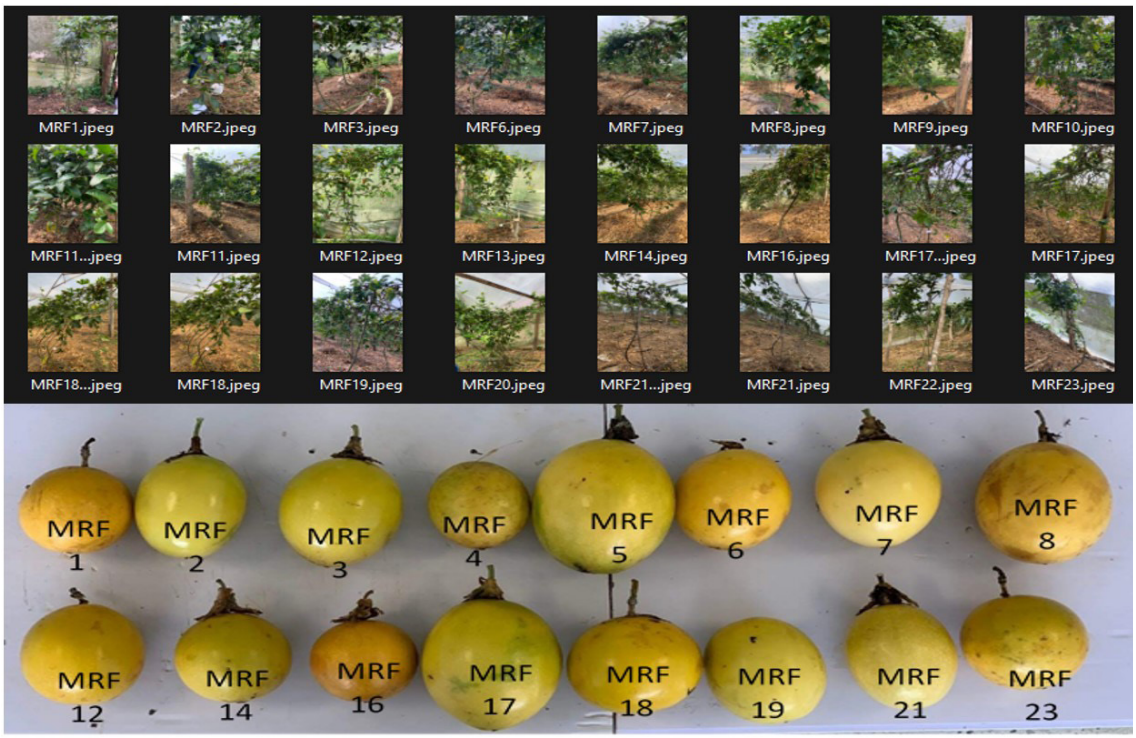

Figure 1. Some phenotypic characteristics in plant and fruit that define the 21 cultivars used in the study. MRF: Corresponds to each of the different passion fruit cultivars that were evaluated in the study.

Table 1. ISSR markers used to determinate the genetic diversity in the passion fruit cultivars.

Primer	Sequence (5´to 3´)
CCA	$DDB(CCA)_5$
CGA	$DHB(CGA)_5$
GT	$VHV(GT)_5G$
AG	$HBH(AG)_7A$
CT	$DYD(CT)_7C$
TG	$HVH(TG)_7T$
CA	$DBDA(CA)_7$

The following designations are used for the generative sites: H (A or T or C); B (G or T or C); V (G or A or C) and D (G or A or T).

and a final extension at 72°C for 7 min. All reaction was carried out with the PTC 1000 programmable thermal controller thermocycler (M.J. Research, Inc). PCR product was then separated by electrophoresis in 2% agarose gel, with running TBE 0.5X at 100 volts for approximately 3 h in a Maxicell Primo EC-340 Electrophoresis Gel System chamber and stained with Gelred dye, and then visualized under transilluminator.

2.4. Statistical analysis

The analyzes were carried out only with those bands that presented a clear amplification. A binary matrix was generated where one indicates the presence and zero the absence. Cluster analysis was performed by using Unweighted Pair Group Method with the Arithmetic mean (UPGMA). The SIMQUAL program was used to calculate the Jaccard's coefficients by using NTSYS-pc 2.1 (Rohlf, 2000). The dendrograms were constructed using the algorithm with the SAHN module. The cut-off values of dendrograms were then determined based on calculation method described by Jamshidi and Jamshidi (2011). Genetic similarity (GS) was estimated for all cultivars pairs using the following equation (Nei and Li, 1979):

$$Gs_{ij} = \frac{2N_{ij}}{2N_{ij}} + N_i + N_j \quad (1)$$

where Gs_{ij} represents the similarity estimated between the genotypes i and j, based on the ISSR data, N_{ij} is the total number of bands common to i and j, and N_i and correspond to the number of bands found in genotypes i and j, respectively. Cophenetic correlation coefficient (CCC) between similarity matrix and dendrogram cophenetic values was estimated to validate the dendrogram in relation to the original similarity estimates and the binary data matrix analyzed using COPH and MXCOMP programs in NTSYSpc.

The Nei´s genetic distance (H), Shannon information index (I), coefficient of genetic differentiation (CGD), number and percentage of polymorphic loci, and gene flow (GF) (McDemott and McDonald, 1993), heterozygosity were estimated with the statistical package POPGENE version 3.2 and TFPGA (Tools for Population Genetic Analysis,

version 1.3, Miller, 1997). Polymorphic information content (PIC) was calculated according to the formula proposed by Botstein et al. (1980):

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

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; an above 0.50 highly informative, where 0.50 is the maximum value reached in dominant markers such as ISSR (Botstein et al., 1980).

The genetic structure analysis was done based on Bayesian model (Hubisz et al., 2009) with STRUCTURE program version 2.3.4. The runs for K values ranging from 1 to 10 were executed with a burn-in length of 100,000 tailed by 1,000,000 Monte Carlo Markov Chain (MCMC) interactions using admixture model. The number of subpopulations was determined using the Delta K (ΔK) ad hoc method proposed by Evanno et al. (2005) and implemented in the online tool Structure Harvester (Earl and vonHoldt, 2012) to estimate the most likely *K* in each set of passion fruit cultivars. The difference between and within the groups was evaluated by molecular variance analysis AMOVA, using GenAlex 6.5 program.

3. Results

Amplification reactions from the seven ISSR primers, considering the 21 cultivars of *P. edulis*, produced 225 bands, 87% polymorphic, with a mean of 28.2 per primer (Table 2). Primers CA and CT, AG produced the lowest (21) and highest (38) numbers of bands, respectively (Table 2). The estimated PIC values ranged from 0.20 to 0.25, according to Equation 2, for ISSRs AG and CT, respectively. The dendrogram based on UPGMA cluster analysis constructed from seven ISSR markers among the passion fruit cultivars were grouped into two sub-groups (Figure 2) as reveled by population structure in Figure 3 and Figure 4.

The Bayesian clustering method with linear and generalized mixed model indicated that the 21 passion fruit cultivars were clustered into two genetic groups (K=2) (Figure 3 and Figure 4). Figures 2 and 4 shows the estimated population structure based on Delta K (ΔK) when it reaches its maximum value following the *ad-hoc* method and subpopulation clusters (K) that are represented by different colors, respectively. The structuring in two genetic groups show a predominance of a genetic group was represented by the red color for the different cultivars (approximately 71%), while the second genetic group was represented by the green color for the rest of the cultivars evaluated (29%).The groupings corresponded mainly to phenotypic characteristics such as leaf shape, fruit shape, presence or absence of tendrils, among others. Cophenetic correlation coefficient was 94%, showed a good relationship between the genetic distance and the groups formed.

Shannon diversity index of *Passiflora edulis* observed here was 0.4 (Table 2), while in the first group it was 0.39 and in group two 0.34. Mean expected heterozygosity (He) from the analyzed total population of passion fruit were 0.29 (Table 2). In genetic group I, the expected heterozygosity values ranged from 0.21 to 0.32 with an average of 0.27; and in the second, this parameter was between 0.13 and 0.30 with an average of 0.22.

The genetic differentiation (Gst) were 0.12 according to Equation 1, showed that 12% of the total genetic variation were between population. The gene flow represented in the Nm coefficient was 3.91 on average for the total population, being low for the 21 passion fruit cultivars evaluated. AMOVA revealed that the diversity among groups and within individuals of passion fruit cultivars were 22% and 78%, respectively (Table 3).

Table 2. Genetic parameters measured in the total population of *Passiflora edulis* cultivars and in the clusters formed in the conglomerates and structure analyses.

	Total Population							Group I		Group II	
Primer	**N° Loci Polymorphic**	**% Polymorphic loci**	**He**	**I**	**Gst**	**PIC**	**Nm**	**He**	**I**	**He**	**I**
AG	30	79	0.26	0.39	0.13	0.20	3.45	0.32	0.46	0.16	0.26
CA	19	90	0.27	0.41	0.14	0.22	3.05	0.30	0.45	0.19	0.31
CCA	18	100	0.37	0.55	0.15	0.29	2.94	0.21	0.31	0.24	0.36
CGA	37	93	0.29	0.45	0.06	0.23	7.36	0.30	0.44	0.27	0.41
CT	30	79	0.32	0.46	0.14	0.25	3.12	0.24	0.36	0.30	0.44
GT	22	92	0.30	0.46	0.14	0.24	2.99	0.25	0.36	0.26	0.41
TG	23	72	0.20	0.31	0.10	0.24	4.45	0.25	0.37	0.13	0.21
Average	**25.57**	**86.43**	**0.29**	**0.43**	**0.12**	**0.24**	**3.91**	**0.27**	**0.39**	**0.22**	**0.34**

He: Expected heterozygosity; I: Shannon Index; Gst: Coefficient of genetic differentiation; PIC: Polymorphic Information Content; Nm: Gene flow.

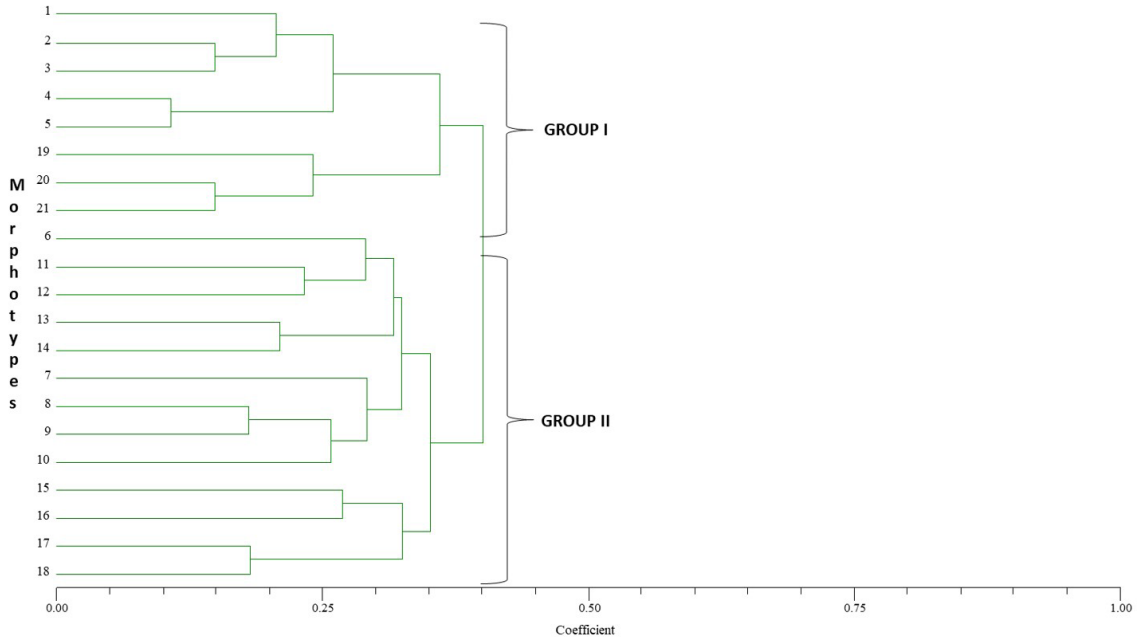

Figure 2. Dendrogram of passion fruit cultivars based on the Nei-Li similarity coefficient and estimated with seven ISSR markers with UPGMA, SAHN and TREE classification methods of NTSYS-pc, version 1.8 (Exeter Software, NY, USA).

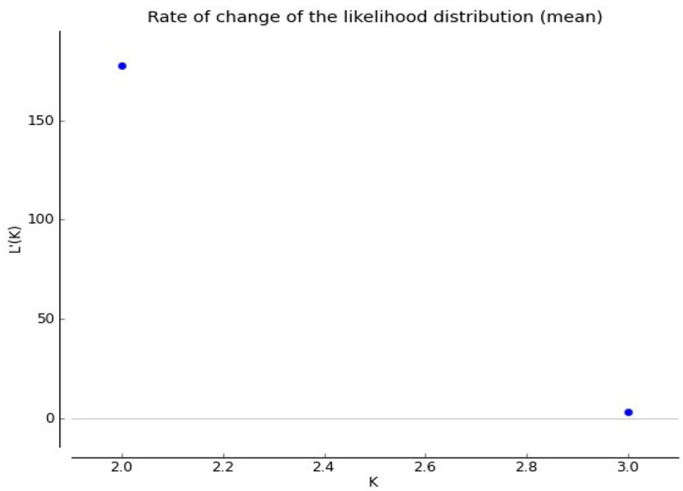

Figure 3. Delta K values obtained from Harvester Structure, estimated as the mean of the probability of K divided by the standard deviation of the probability of K. Evanno´s method with an optimal model of K=2.

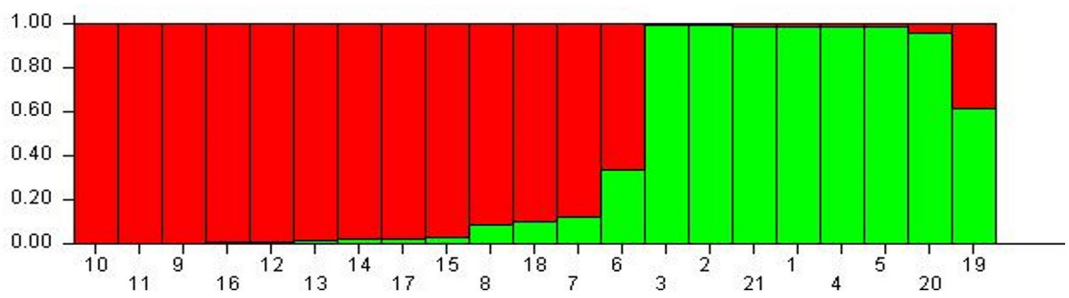

Figure 4. Population genetic structure of 21 passion fruit cultivars analyzed, calculate using the Structure software. Each vertical bar represents an individual sample, and the color of the bar indicates the probability that an individual will be assigned to one of the identified groups.

Table 3. Analysis of molecular variance (AMOVA) among and within groups using seven ISSR markers.

Source	df	Sum of squares	Variance Components	Total variation %	p-value
Among Groups	1	120.959	8.927	22%	0.001
Within Groups	19	618.279	32.541	78%	0.01*
Total	20	739.238	41.468	100%	

*Significance test after 1000 permutations; df: Degree of freedom.

4. Discussion

The seven ISSR markers used to characterize the genetic diversity of the 21 passion fruit cultivars generated 255 loci, which is considered appropriate for conducting a genetic study, results similar to those found in other characterizations of *Passiflora* germplasm (Ho et al., 2021; Silva et al., 2022; Morillo et al., 2023). The percentage of polymorphism found in the evaluation of the 21 cultivars was higher than 80% (Table 2) are similar to the data available in the literature for different species of the genus *Passiflora* based on ISSR markers (Maciel et al., 2019; Martinez et al., 2020; Ho et al., 2021). The values found here were higher than those observed in *P. cincinnata* for Embrapa Cassava & Fruits Collection (87.4%) and in the Embrapa Cerrados Collection (Silva et al., 2022).

The percentage of polymorphism may be due to morphological and/or anatomical characteristics of the flower that lead to the self-incompatibility of the species (Table 2). Therefore, cross-pollination mediated by external agents is required, which contributes to the increase of the genetic variability of these species, for example, in *P. cincinnata* (Silva et al., 2022). Sousa et al. (2020) using 31 ISSR markers in 25 wild passion fruit species and Sousa et al. (2015) observed that 20 primers had polymorphic loci. In view of the studies with the same primers in different species of the genus, it was observed that these markers, in addition to enabling molecular studies, also inform the divergence between species, whose characteristic makes this marker a potential tool for the selection of taxa in wild species of *Passiflora* L.

Regarding to Botstein et al. (1980), the average value of the PIC values according to Equation 2 for the evaluated ISSR markers was 0.24, the equations were mentioned within the results, which indicates that they are slightly informative (Table 2). Ho et al. (2021) suggested that ISSR marker is slightly more informative than RAPD, due to ISSR markers are longer and require higher annealing temperature, resulting in higher consistency. In general terms, low or low PIC values may be influenced not only by the type of molecular marker selected, but also by the way the species reproduces, for which reason Chepkoech et al. (2020) report average PIC values of 0.65, attributing said values to the fact that it is the producers themselves who select their seeds and carry out sexual seed multiplication, in addition to the fact that this influences the distribution of allelic frequencies in the cultivated populations (Pérez and d'Eeckenbrugge, 2017; Morillo et al., 2023).

The dendrogram generated from the UPGMA analysis using the seven ISSR markers revealed two groups of passion fruit cultivars with group II being the most diverse compared to group I (Figure 2). Cophenetic correlation coefficient was 82%, demonstrating a relationship between the genetic distances and the groups. The grouping of cultivars into different groups may be the result of exchange of planting material between producing regions and/or due to hybridization, gene flow, propagation system, origin, among others. The similarity or dissimilarity observed between the passion fruit cultivars is possibly due to the processes of conservation and domestication of the germplasm (Chepkoech et al., 2020; Morillo et al., 2023). Genetic variability within a population of a species is affected by a number of factors comprising the seed dispersal, gene flow, natural selection, geographic range, and the center of diversity (Hamrick and Godt, 1989). On the other hand, some authors point out that the selection of markers is essential to achieve greater precision in the identification of passion fruit cultivars, since the combination of markers affects the results (Ocampo et al., 2021; Ho et al., 2021).

The cluster analysis was executed to deduce the genetic structure (Figure 4), estimate the lineage and presence of possible populations of the sample cultivars. The ISSRs studied using Bayesian groping generated two clusters $\Delta k = 2$ from 21 passion fruit cultivars, observed a small mixture between cultivars probably due to gene flow and continuous planting processes cycle after production cycle (Martinez et al., 2020). The 21 cultivars were more defined by their phenotypic characteristics associated with the fruit than by geographical origin, in addition to their type of reproduction, self-incompatibility processes, cross-pollination and the absence of directed selection processes (Ocampo et al., 2021). Admixture is considered because of exchange of plant material between the areas and/or hybridization (Chepkoech et al., 2020).

The results of two analysis performed (UPGMA cluster and Bayesian model-based method) agreed with existence of clusters or subpopulations. In spite of minor differences, the results were largely consistent. The results of the analyzes showed that the cultivars present reasonably heterogeneous phenotypic and genotypic characteristics, showing a small correspondence with the selection and domestication processes to which the species has been subjected in passion fruit-producing municipalities in Colombia (Ocampo et al., 2017; Morillo et al., 2023). Other studies have reported great genetic heterogeneity for cultivated accessions of *P. edullis* f. *flavicarpa* (Chepkoech et al., 2020; Ho et al., 2021); *P. cincinnata* (Silva et al., 2022) and *P. setacea* (Barbosa et al., 2021). In contrast, Ortiz et al. (2012) reported a high genetic homogeneity in cultivated material of purple passion fruit

(*P. edulis* f. *edulis*) with microsatellite markers and AFLP in Colombia. However, it is possible that an inadequate selection plan has not been developed in the department of Boyacá, for passion fruit, as farmers inconsistently select the finest fruits in each harvest, without considering cross-pollination (Ocampo et al., 2017). In general, ISSR marker may be a valuable tool for studying intraspecific genetic diversity in *Passiflora*, mainly by grouping accessions according to genetic origin (Costa et al., 2012).

Regarding the expected heterozygosity values (He), these ranged between 0.20 (TG) and 0.37 (CCA) with an average value of 0.29 for the entire population (Table 2). Results similar to those obtained by Morillo et al. (2023) when evaluating the pattern of genetic diversity of *Passifloras* in Colombia. In group I and group II, average heterozygosity values of 0.27 and 0.22 were obtained, respectively (Table 2), lower values than those (He=0.56) reported in diversity studies in *Passiflora* in Colombia and other countries with ISSR markers (Maciel et al., 2019; Martinez et al., 2020). Reis et al. (2011) when studying populations of two cycles of recurrent selection in *P. edulis* obtained He = 0.200. However, they were higher than those found by Pereira et al. (2015) in 12 populations of *P. setacea* distributed in three agroecological zones within the state of Bahia, Brazil, using ISSR. The low molecular variability can be attributed to the loss and fixation of the alleles by the selection process directed towards agronomically favorable genotypes. The variability reported in *Passifloras* may be associated with allogamy and self-incompatibility (Ocampo et al., 2021). In general terms, genetic diversity depends on the reproduction system, seed dispersal, genetic drift, the coevolution process of the species, and the habitat (Maciel et al., 2019).

The Shannon diversity index found for the 21 passion fruit cultivars evaluated with the seven ISSRs, 0.43 (Table 2) indicating a high genetic diversity. These results are similar to those reported in other cross-pollinated species (Castañeda et al., 2020). Pereira et al. (2015) found similar values with inter simple sequence repeats (ISSR) and resistance genes analogs (RGA) markers (I = 0.51 and 0.34, respectively). This represents an advantage for crop improvement because species with high genetic diversity allow the selection of genotypes with agronomically favorable alleles (Bernardes et al., 2020).

According to Yeh (2000), estimates of genetic divergence between 0.151 and 0.250 represent a high level of differentiation; therefore, the results obtained with the GST (0.12), according to Equation 1, were low compared to the high levels of GST detected in other genetic diversity studies using SSR, ISSR and RGA markers (Gst = 0.36, 0.38, 0.42, respectively) on populations of *P. setacea* (Cerqueira-Silva et al., 2014; Pereira et al., 2015). Barbosa et al. (2021) reported mean genetic divergence between populations (Gst) was 0.221, indicating high levels of genetic differentiation in *P. setacea* in different regions of Brazil.

Analysis of Molecular Variance (AMOVA) revealed that the highest percentage of variation occurs within (78%) and not between groups (22%) (Table 3). Silva et al. (2022) indicated higher diversity within (99%) populations, with low differentiation between them (1%) in *P. cincinnata*. 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. These data suggest high genetic variability within plant populations, which may be due to sexual reproduction, somatic cell mutation, selection, genetic flow, genetic drift, and environmental change (Araújo et al., 2020).

The average value of Nm was 3.91, thus showing a high gene flow, results similar to those reported for the germplasm of Colombia (Martinez et al., 2020; Morillo et al., 2023). Barbosa et al. (2021) in *P. setacea* reported gene flow moderate (1.179, based upon Fst) and congruent with the high population structuring, results similar to those obtained in populations of *P. setacea* from southern Bahia, Brazil with ISSR and RGA markers Nm were low (0.86 and 0.68, respectively) (Pereira et al., 2015). Factors such as pollen and seed dispersal determine the gene flow of populations, playing an essential role in its structure. It is important to keep in mind that most *Passifloras* species present self-incompatibility, which favors cross-pollination and therefore gene flow (Ocampo et al., 2017). In addition, it must be considered that the majority of *Passifloras* have a nocturnal habit, requiring external pollinating agents, which transport the pollen grain over long distances (Barbosa et al., 2021), thus promoting an increase in the flow and a decrease in genetic differentiation between populations (Anderson et al., 2022).

Passifloras producers in Colombia are the ones who carry out the selection processes of the phenotypes cycle after sowing cycle based on the standards required by the national or international market (Ocampo et al., 2017), which directly or indirectly influences diversity. The above is observed in the great phenotypic diversity of the cultivars within the same producers' farms, since they do not carry out any pollination control, they only select the fruits to extract their seed for the next cycle considering the production characteristics and in some cases resistance to phytosanitary problems.

Genetics of these crops. Generally, the genus *Passiflora* have a very extensive genetic variability both within the genus and within the most cultivated species (Ocampo et al., 2017). The results obtained in this research show the phenotypic variation expected in allogamous species. However, this does not present very high values compared to other studies of *Passifloras* since some producers have opted for vegetative propagation which makes the cultivars more homozygous and homogeneous. Despite the existence of genetic variability, it is necessary to implement directed selection processes that lead to obtaining new and better cultivars that meet the needs of farmers, producers, and consumers.

The passion fruit cultivars showed high values of polymorphism. Low values of heterozygosity, low genetic structure, high gene flow and the conformation of at least two genetic groups. Analysis of molecular variance indicated higher diversity within populations, with low differentiation between them. The ISSRs markers allowed the identification of variation at the intraspecific level. 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.

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References

ANDERSON, J.D., VIDAL, R.F., BRYM, M., STAFNE, E.T., RESENDE JUNIOR, M.F., VIANA, A.P. and CHAMBERS, A.H., 2022. Genotyping-by-sequencing of passion fruit (*Passiflora* spp.) generates genomic resources for breeding and systematics. *Genetic Resources and Crop Evolution*, vol. 69, no. 8, pp. 2769-2786. http://dx.doi.org/10.1007/s10722-022-01397-4.

ANTUNES, M.S., VASCONCELOS, M.J.V. and NETTO, D.A.M., 1997. RAPD analysis of pearl millet cultivars. *International Sorghum and Millets*, vol. 38, no. 7, pp. 146-150.

ARAÚJO, F.P., MELO, N.F., AIDAR, S.T., FALEIRO, F.G. and JESUS, O.N., 2020. Maracuyá de la Caatinga: *Passiflora cincinnata* Mast. In: A.R. CARLOSAMA, F.G. FALEIRO, M.P. MORERA and A.M. COSTA, eds. *PASIFLORAS especies cultivadas en el mundo*. Brasília: ProImpress, pp. 170-186.

ARAYA, A., MARTINS, A.M., JUNQUEIRA, N.T.V., COSTA, A.M., FALEIRO, F.G. and FERREIRA, M.E., 2017. Microsatellite marker development by partial sequencing of the sour passion fruit genome (*Passiflora edulis* Sims). *BMC Genomics*, vol. 18, no. 2, pp. 549. http://dx.doi.org/10.1186/s12864-017-3881-5. PMid:28732469.

BARBOSA, N.C., BATISTA, K.R., SILVEIRA, M.L., DE JESUS, C. and SELBACH, A., 2021. Genetic diversity of *Passiflora setacea* in different regions of Bahia, Brazil, through SSR markers. *Comunicata Scientiae*, vol. 12, no. 2, pp. 3654. http://dx.doi.org/10.14295/CS.v12.3654.

BELO, G.O., SOUZA, M.M., SILVA, G.S. and LAVINSCKY, M.P., 2018. Hybrids of *Passiflora: P. gardneri* versus *P. gibertii*, confirmation of paternity, morphological and cytogenetic characterization. *Euphytica*, vol. 214, no. 1, pp. 1-13. http://dx.doi.org/10.1007/s10681-017-2021-2.

BERNAL-PARRA, N., OCAMPO-PÉREZ, J. and HERNÁNDEZ-FERNÁNDEZ, J., 2014. Characterization and analysis of the genetic use of granadilla (*Passiflora ligularis* Juss.) in Colombia using microsatellite markers. *Revista Brasileira de Fruticultura*, vol. 36, no. 3, pp. 586-597, 2014. [In Spanish]. https://doi.org/10.1590/0100-2945-251/13.

BERNARDES, P.M., NICOLI, C.F., ALEXANDRE, R.S., SOLER, J., MIRANDA, M., FERREIRA, A. and DA SILVA, M., 2020. Vegetative and reproductive performance of species of the genus *Passiflora*. *Scientia Horticulturae*, vol. 265, pp. 1-6. http://dx.doi.org/10.1016/j.scienta.2020.109193.

BOTSTEIN, D., WHITE, R.L., SKOLNICK, M. and DAVIS, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, vol. 32, no. 3, pp. 314-331. PMid:6247908.

CASTAÑEDA, C.C., MORILLO, Y. and MORILLO, A.C., 2020. Assessing the genetic diversity of *Dioscorea alata* and related species from Colombia through inter-simple sequence repeat (ISSR) markers. *Chilean Journal of Agricultural Research*, vol. 80, no. 4, pp. 608-616. http://dx.doi.org/10.4067/S0718-58392020000400608.

CERQUEIRA-SILVA, C.B.M., NUNES, O., SANTOS, E.S.L., CORRÊA, R.X. and SOUZA, A.P., 2014. Genetic breeding and diversity of the Genus *Passiflora*: progress and perspectives in molecular and genetic studies. *International Journal of Molecular Sciences*, vol. 15, no. 8, pp. 14122-14152. http://dx.doi.org/10.3390/ijms150814122. PMid:25196515.

CHAVARRÍA-PEREZ, L.M., GIORDANI, W., DIAS, K.O.G., PORTUGAL, Z., ALBUQUERQUE, C., BENEDETTI, A., CAUZ-SANTOS, L., SILVA, G., BACHEGA, J., FRANCO, A. and CARNEIRO, M., 2020. Improving yield and fruit quality traits in sweet passion fruit: evidence for genotype by environment interaction and selection of promising genotypes. *PLoS One*, vol. 15, no. 5, pp. 1-20. http://dx.doi.org/10.1371/journal.pone.0232818. PMid:32407352.

CHEPKOECH, E., ROTICH, F. and ALKAMOI, B., 2020. Assessment of genetic variability of passion fruit using simple sequence repeat (SSR) markers. *Journal Agricultural Science Practice*, vol. 5, no. 1, pp. 202-211. http://dx.doi.org/10.31248/JASP2020.230.

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*, vol. 12, no. 4, pp. 253-260. http://dx.doi.org/10.1590/S1984-70332012000400004.

DA SILVA, M.A., DE SOUSA, C.B., DA SILVA, R.M., CAGNIN, C., SIQUEIRA DE LIMA, M. and MORAES, R., 2015. Physical and chemical characteristics and instrumental color parameters of passion fruit (*Passiflora edulis* Sims). *African Journal of Agricultural Research*, vol. 10, no. 10, pp. 1119-1126. http://dx.doi.org/10.5897/AJAR2014.9179.

DELLAPORTA, S.L., WOOD, J. and HICKS, J.B., 1983. A plant DNA minipreparation: version II.-. *Plant Molecular Biology Reporter*, vol. 1, no. 4, pp. 19-21. http://dx.doi.org/10.1007/BF02712670.

DO CARMO, T.V.B., MARTINS, L.S.S., MUSSER, R.D.S., DA SILVA, M.M. and SANTOS, J.P.O., 2017. Genetic diversity in accessions of *Passiflora cincinnata* mast. based on morphoagronomic descriptors and molecular markers. *Revista Caatinga*, vol. 30, no. 1, pp. 68-77. http://dx.doi.org/10.1590/1983-21252017v30n108rc.

DOS REIS, L.C.R., FACCO, E.M.P., SALVADOR, M., HICKMANN, S. and DE OLIVEIRA, A., 2018. Antioxidant potential and physicochemical characterization of yellow, purple and orange passion fruit. *Journal of Food Science and Technology*, vol. 55, no. 7, pp. 2679-2691. http://dx.doi.org/10.1007/s13197-018-3190-2. PMid:30042584.

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*, vol. 4, no. 1, pp. 359-361. http://dx.doi.org/10.1007/s12686-011-9548-7.

EVANNO, G., REGNAUT, S. and GOUDET, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, vol. 14, no. 8, pp. 2611-2620. http://dx.doi.org/10.1111/j.1365-294X.2005.02553.x. PMid:15969739.

FERREIRA, A.F.N., KRAUSE, W. and CORDEIURO, M.H.M., 2021. Multivariate analysis to quantify genetic diversity and family selection in sour passion fruit under recurrent selection. *Euphytica*, vol. 217, no. 11, pp. 11. http://dx.doi.org/10.1007/s10681-020-02740-5.

GALEANO-MENDOZA, C.H., CÉRON-SOUZA, I. and ARANGO, L.V., 2018. Agronomic evaluation of a Colombian passion fruit (*Passiflora edulis* Sims) germplasm collection. *Agronomy Research (Tartu)*, vol. 16, no. 4, pp. 1649-1659. http://dx.doi.org/10.15159/AR.18.190.

GRISI, M.D.M., FALEIRO, F.G., JUNQUEIRA, N.T.V. and OLIVEIRA, J.D.S., 2019. Genetic variability of passion fruit multispecific hybrids and their respective wild parents determined by microsatellite

markers. *Journal of Agricultural Science (Toronto)*, vol. 11, no. 10, pp. 302-312. http://dx.doi.org/10.5539/jas.v11n10p302.

HAMRICK, J.L. and GODT, M.J.W., 1989. Allozyme diversity in plant species. In: A.H.D. BROWN, M. T. CLEGG, A.L. KAHLERAND and B.S. WEIR, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, Mass: Sinauer Press, pp. 43-63.

HASHEMI, S. and KHADIVI, A., 2020. Morphological and pomological characteristics of white mulberry (*Morus alba* L.) accessions. *Scientia Horticulturae*, vol. 259, no. 3, pp. 1-9. http://dx.doi.org/10.1016/j.scienta.2019.108827.

HE, X., LUAN, F., YANG, Y., WANG, Z., ZHAO, Z., FANG, J., WANG, M., ZUO, M. and LI, Y., 2020. *Passiflora edulis*: an insight into current researches on phytochemistry and pharmacology. *Frontiers in Pharmacology*, vol. 11, pp. 617. http://dx.doi.org/10.3389/fphar.2020.00617. PMid:32508631.

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*, vol. 53, no. 1, pp. 1-14.

HOLANDA, D.K.R., WURLITZER, N.J., DIONISIO, A.P., CAMPOS, A.R., MOREIRA, R.A., MACHADO, P., SOUSA, E., VASCONCELOS, P., FRADE, M. and COSTA, A.M., 2020. Garlic passion fruit (*Passiflora tenuifila* Killip): assessment of eventual acute toxicity. anxiolytic, sedative, and anticonvulsant effects using *in vivo* assays. *Food Research International*, vol. 128, no. 4, pp. 108813. http://dx.doi.org/10.1016/j.foodres.2019.108813.

HUBISZ, M.J., FALUSH, D., STEPHENS, M. and PRITCHARD, J.K., 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, vol. 9, no. 5, pp. 1322-1332. http://dx.doi.org/10.1111/j.1755-0998.2009.02591.x. PMid:21564903.

JAMSHIDI, S. and JAMSHIDI, S., 2011. NTSYSpc 2.02e implementation in molecular biodata analysis (Clustering, screening and individual selection). In: *International Conference on Environmental and Computer Science*, Singapore, 2011, Hong Kong: APCBEES, pp. 165-196.

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*, vol. 13, no. 7, pp. 1083-1093. http://dx.doi.org/10.21475/ajcs.19.13.07.p1545.

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*, vol. 80, no. 3, pp. 342-351. http://dx.doi.org/10.4067/S0718-58392020000300342.

MCDEMOTT, J.M. and MCDONALD, B.A., 1993. Gene flow in plant pathosystems. *Annual Review of Phytopathology*, vol. 31, pp. 353-373. https://doi.org/10.1146/annurev.py.31.090193.002033.

MILLER, M.N., 1997 [accessed 3 September 2023]. *Tools for population genetic analysis (TFPGA)* [online]. Available from: https://www.scienceopen.com/document?vid=91146e4d-17b1-42e6-b5e0-7717b8d6600d.

MORILLO, A.C., MARTÍNEZ, M.A. and MORILLO, Y., 2023. Genetic diversity pattern of *Passiflora* spp. in Boyacá, Colombia. *Pesquisa Agropecuária Brasileira*, vol. 58, pp. 03062. http://dx.doi.org/10.1590/S1678-3921.pab2023.v58.03062.

NEI, M. and LI, W.H., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 1, pp. 5269-5273. http://dx.doi.org/10.1073/pnas.76.10.5269. PMid:291943.

OCAMPO, J., ACOSTA-BARON, N. and HERNANDEZ-FERNANDEZ, J., 2017. Variability and genetic structure of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) in Colombia using microsatellite DNA markers. *Agronomia Colombiana*, vol. 35, no. 2, pp. 135-149. http://dx.doi.org/10.15446/agron.colomb.v35n2.59973.

OCAMPO, J., COPPENS D'EECKENBRUGGE, G., OLANO, C.T. and SCHNELL, R.J., 2004. AFLP analysis for the study of genetic relationships among cultivated *Passiflora* species of the subgenera *Passiflora* and *Tacsonia*. *Proceedings Interamerican Society Tropical Horticultural*, vol. 48, no. 4. pp. 72-76. http://dx.doi.org/10.13140/RG.2.1.4623.8167.

OCAMPO, J., MARÍN, V. and URREA, R., 2021. Agro-morphological characterization of yellow passion fruit (*Passiflora edulis* f. *flavicarpa* Degener) reveals elite genotypes for a breeding program in Colombia. *Agronomia Colombiana*, vol. 39, no. 2, pp. 156-176. http://dx.doi.org/10.15446/agron.colomb.v39n2.91622.

OLUOCH, P., NYABOGA, E.N. and BARGUL, J.L., 2018. Analysis of genetic diversity of passion fruit (*Passiflora edulis* Sims) genotypes grown in Kenya by sequence-related amplified polymorphism (SRAP) markers. *Annals of Agrarian Science*, vol. 16, no. 4, pp. 367-375. http://dx.doi.org/10.1016/j.aasci.2018.08.003.

ORTIZ, D.C., BOHÓRQUEZ, A., DUQUE, M.C., TOHME, J., CUÉLLAR, D. and VÁSQUEZ, T.M., 2012. Evaluating purple passion fruit (*Passiflora edulis* Sims f. *edulis*) genetic variability in individuals from commercial plantations in Colombia. *Genetic Resources and Crop Evolution*, vol. 59, no. 1, pp. 1089-1099. http://dx.doi.org/10.1007/s10722-011-9745-y.

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*, vol. 59, pp. 12-21. http://dx.doi.org/10.1016/j.bse.2014.12.020.

PÉREZ, J.O. and D'EECKENBRUGGE, G.C., 2017. Morphological characterization in the genus *Passiflora* L.: an approach to understanding its complex variability. *Plant Systematics and Evolution*, vol. 303, no. 4, pp. 531-558. http://dx.doi.org/10.1007/s00606-017-1390-2.

RAMAIYA, S.D., BUJANG, J.S. and ZAKARIA, M.H., 2018. Nutritive values of passion fruit (*Passiflora* species) seeds and its role in human health. *Journal of Agriculture Food and Development*, vol., vol. 4, no. 1, pp. 23-30. http://dx.doi.org/10.30635/2415-0142.2018.04.4.

REIS, R.V., OLIVEIRA, E.J., VIANA, A.P., SANTANA, T.N., GONZAGA, M. and DE MORAIS, M., 2011. Genetic diversity in recurrent selection of yellow passion fruit detected by microsatellite markers. *Pesquisa Agropecuária Brasileira*, vol.46, no.1., pp. 51-57. http://dx.doi.org/10.1590/S0100-204X2011000100007.

ROHLF, F.J., *NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Version 2.1*. New York, USA: Exceter Software; 2000.

SILVA, T.S.S., MEIRA, M.R., VIEIRA, J.G.P., SANTOS, E.S.L., DE JESUS, O.N., FALEIRO, F.G. and CERQUEIRA-SILVA, C.B.M., 2022. Structure and molecular genetic diversity in natural populations and active germplasm banks of *Passiflora cincinnata* Mast. *Chilean Journal of Agricultural Research*, vol. 82, no. 4, pp. 628-637. http://dx.doi.org/10.4067/S0718-58392022000400628.

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*, vol. 14, no. 4, pp. 18534-18545. http://dx.doi.org/10.4238/2015.December.23.41. PMid:26782501.

SOUSA, N.D., BARBOSA, L.N., DE OLIVEIRA, S., SILVEIRA, L.A., MEIRA, M.R., LISBOA, E.S., FALEIRO, F.G. and CERQUEIRA-SILVA, C.B.M., 2020. Characterization and selection of ISSR molecular markers in species of *Passiflora* spp. *Multi-Science Journal*, vol. 3, no. 3, pp. 17-22. http://dx.doi.org/10.33837/msj.v3i3.1290.

VIANNA, L.S., PEREIRA, T.N.S., SANTOS, E.A., VIANNA, A.P., PEREIRA, H.C.C. and ROSSI, A.A.B., 2019. ISSR and SSR markers for determining genetic relationships among three wild species of *Passiflora*. *Genetics and Molecular Research*, vol. 18, no. 1, pp. 1-10. http://dx.doi.org/10.4238/gmr18040.

VIEIRA, A., CAMPOS, S., PEIXOTO, J.R. and FALEIRO, F.G., 2019. Molecular characterization and genetic diversity of yellow passion fruit based on RAPD markers. *Journal of Agricultural Sciences*, vol. 11, no. 3, pp. 575-580.

WU, Y., TIAN, Q., HUANG, W., LI, J., XIA, X., YANG, X. and MOU, H., 2020. Identification and evaluation of reference genes for quantitative real-time PCR analysis in *Passiflora edulis* under stem rot condition. *Molecular Biology Reports*, vol. 47, no. 4, pp. 2951-2962. http://dx.doi.org/10.1007/s11033-020-05385-8. PMid:32215779.

YEH, F.C., 2000. Population genetics. In: A. YOUNG, D. BOSHIER and T. BOYLE, eds. *Forest conservation genetics: principles and practice.* Collingwood, Australia: CSIRO Publishing, pp. 21-37.

YOTOKO, K.S., DORNELAS, M.C., TOGNI, P.D., FONSECA, T.C., SALZANO, F.M., BONATTO, S.L. and FREITAS, L., 2011. Does variation in genome sizes reflect adaptive or neutral processes? New clues from *Passiflora*. *PLoS One*, vol. 6, no. 3, pp. 18212. http://dx.doi.org/10.1371/journal.pone.0018212.