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Original Article

Diversity and genetic structure of yellow passion fruit in Boyacá-Colombia using microsatellite DNA markers

Diversidade e estrutura genética do maracujá-amarelo em Boyacá, Colômbia, utilizando marcadores microssatélites de DNA

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Abstract

The Yellow passion fruit belongs to the Passifloraceae family with great economic, nutritional and social importance in Colombia. It presents a great phenotypic and genotypic diversity, which has not yet been explored or used in genetic improvement programs. The objective of this study was to evaluate the genetic diversity of 84 cultivars of *Passiflora edulis* f. *flavicarpa* from nine farms in the municipality of Miraflores, Boyacá, using eight microsatellite markers (SSR). On the basis of this information, estimates of genetic diversity parameters, molecular variance analysis (AMOVA), genetic distances, and cluster of cultivars were obtained. Low levels of genetic differentiation between cultivars were observed in the Bayesian analysis using Structure software, as well as the absence of correlation between genetic and geographic distances. The observed heterozygosity (0.50) was greater than the expected heterozygosity (0.43), suggesting a significant number of heterozygous individuals. The number of alleles per locus varied from 2 to 4, with a mean 2.88. In general, SSR were classified as informative (0.36). The average value of the Shannon Index was 0.71, which shows moderate variability in this cultivar. AMOVA showed higher diversity within cultivars (98%). The gene flow (Nm=28.4) was moderate, this can be explained by the flow of pollen between the different cultivars, the reproduction system of the species, self-incompatibility and the introduction of genotypes from other sites by farmers. The genetic diversity identified in this study is sufficient to initiate breeding programs aimed at identifying cultivars with higher yields.

Keywords: genetic structuring, germplasm, *Passiflora edulis* f. *flavicarpa*, SSR, variation.

Resumo

O maracujá-amarelo pertence à família Passifloraceae e tem grande importância econômica, nutricional e social na Colômbia. Apresenta uma grande diversidade fenotípica e genotípica que ainda não foi explorada ou utilizada em programas de melhoramento genético. O objetivo deste estudo foi avaliar a diversidade genética de 84 cultivares de *Passiflora edulis* f. *flavicarpa* de nove fazendas do município de Miraflores, Boyacá, Colômbia, utilizando oito marcadores microssatélites (SSR). Com base nessas informações, foram determinadas estimativas de parâmetros de diversidade genética, análise de variância molecular (AMOVA), distâncias genéticas e conjuntos de cultivares. Baixos níveis de diferenciação genética entre cultivares foram observados na análise Bayesiana pelo *software* Structure, bem como ausência de correlação entre distâncias genéticas e geográficas. A heterozigosidade observada (0,50) foi maior que a esperada (0,43), sugerindo um número significativo de indivíduos heterozigotos. O número de alelos por locus variou de 2 a 4, com média de 2,88. Em geral, os SSR foram classificados como informativos (0,36). O valor médio do Índice de Shannon foi de 0,71, o que demonstra variabilidade moderada nesta cultivar. AMOVA apresentou maior diversidade dentro das cultivares (98%). O fluxo gênico (Nm=28,4) foi moderado e isso pode ser explicado pelo fluxo de pólen entre as diferentes cultivares, pelo sistema de reprodução da espécie, pela autoincompatibilidade e pela introdução de genótipos de outros locais pelos agricultores. A diversidade genética identificada neste estudo é suficiente para iniciar programas de melhoramento visando identificar cultivares com maiores produtividades.

Palavras-chave: estruturação genética, germoplasma, *Passiflora edulis* f. *flavicarpa*, SSR, variação.

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1. Introduction

Originating in tropical America, passion fruit belongs to the family Passifloraceae and the *Passiflora* genus (Souza et al., 2020). This genus includes around 500 species, with 150 native to Brazil, considered its center of greatest diversity. But only two species are commercially cultivated *i.e, Passiflora edulis* Sims f. *edulis* (purple passion fruit) and *Passiflora edulis flavicarpa* Degener (yellow passion fruit). Some of the other important species, such as *P. quadrangularis* L., *P. incarnate* L., *P. ligularis* Juss., *P. laurifolia* L., are cultivated in limited scale for local consumption (He et al., 2020). This genus is diploid, 2n=12, 18 or 20 chromosomes, with *P. edulis* f. 2n= 18. This is of great importance because being diploid facilities genetic breeding and hybrization between species. Furthermore, the genome size has been estimated at ~ 1230 Mb (Yotoko et al., 2011; Ferreira et al., 2020). Likewise, it is known for having cross-pollination, presenting self-incompatibility, favoring crossing and promoting genetic variability (Bugallo et al., 2023).

In this sense, despite the success in the generation of some ornamental hybrids, there are incompatibility barriers in interspecific crosses that are still unclear. Therefore, it is necessary to study the reproductive systems and chromosomal homology between the *Passifloras* species that are going to be used as parents in the hybridization program (Ferreira et al., 2020). Differences have been reported between *Passiflora* species in terms of pollen quantity and viability; determining the pollen/ovule ratio and in vivo pollination is necessary for reproductive analysis; cross-pollination presents greater fertilization than self-pollination; self-incompatibility occurs in different floral structures depending on the species; Regions of self-incompatibility have been detected in the pistil by fluorescence analysis (Soares et al., 2015; Castillo et al., 2020)

Yellow passion fruit (*P. edulis f. flavicarpa*), the most important cultivated variety, accounts for about 95% of the global commercial production of passion fruit. The fruit has its origins in an ancestral form known in Colombia as *gulupa* (*P. edulis* f. *edulis* Sims.), commonly known in English as round passion fruit or purple passion fruit and distributed in the Brazilian Amazon region (Araújo et al., 2020). It is known for its wonderful aroma, flavor and medicinal as well as nutritional importance. Fruits with edible and medicinal value is rich in vitamins, amino acids, dietary fiber, among other nutrients (Fonseca et al., 2022).

Passion fruit is commercially cultivated in different countries of the world, primarily in tropical regions, like Brazil, Colombia, Peru and Ecuador representing the major producers of these fruits (Bernal Moreno and Rodríguez, 2023). In Colombia the yellow passion fruit cultivation corresponds to 35% of the most cultivated passion flower plants in Colombia. In 2022, the passion fruit crop was cultivated in the departments of Meta (20.3 t/ha), Antioquia (18.2 t/ha), and Arauca (17.9 t/ha) with a total annual production of 188.834 t (AGRONET, 2022). In Boyacá, the municipalities of Miraflores, Tenza and Covarachía were also engaged in this fruit´s production, which in 2021 reached 1,345 ha, with a production of 9.439 t and a yield of 14 t/ha for various passion fruit crops (AGRONET, 2022). In Colombia, however, the crop of yellow passion fruit is limited to small areas due to lack of planting materials. To increase the productivity, there should be availability of good quality planting material along with proper management practices (Ocampo et al., 2021).

For breeding studies and also for conservation, maintenance, and effective use of available genetic resources, it is necessary to increase the knowledge of genetic structure and diversity in populations of any species (Marques Junior et al., 2023). Studying genetic diversity in a population allows exploring different genotypic effects to obtain new and better cultivars (Morillo et al., 2023a, b). Genetic variability is of essential importance for the breeder, given that, without it, it would not be possible to identify superior genotypes (Ocampo et al., 2021) by maximizing genetic gains in characteristics of interest (Lalrinmawii et al., 2023). In the genetic improvement of passion fruit, significant advances have been made in the yield and quality of the fruit (Ribeiro et al., 2019). Given the economic and social importance of the crop, breeding programs should focus their research on obtaining new cultivars resistant to phytosanitary problems, with high yields and adapted to the different scenarios currently imposed by climate change (Ocampo et al., 2021; Bernal Moreno and Rodríguez, 2023; Morillo et al., 2023b).

However, basic information associated with agronomic evaluations and the available estimates of genetic variability remain limited for many of these cultivars in Colombia. Therefore, pre-breeding activities, such as the characterization of accessions, are required to maintain the variability in collections, and germplasm banks could be effectively used as a genetic resource allowing expansion of the genetic basis of passion fruit breeding programs (Cavalcante et al., 2023). The costs associated with all the activities involved in the pre-improvement of a species can be very high, in addition to the time required to obtain the genetic gains of desirable characters (Jesus et al., 2022).

For yellow passion fruit, by being a semi-perennial species, estimates of genetic variability, based on agronomic performance, requires a lot of time, and control of strong environmental influence (Fischer et al., 2022). In search of more efficient selection methods, the use of molecular markers is fundamentally important for enhancing the amount of information generated and for reducing the time required to obtain results (Testolin et al., 2022). In *Passifloras*, both dominant and co-dominant molecular markers are being used for purposes such as identifying hybrids, the identification of genetic variability in segregating and backcrossing populations, the characterization of germplasm, the identification of mutations, among others (Lalrinmawii et al., 2023).

Among the different molecular markers used in plant characterization, microsatellites or Simple Sequence Repeats (SSR) stand out for their high polymorphism, co-dominance, multi-allelic nature loci with independent segregation (not linked) that are distributed along the genome and their high reproducibility and resolution (Cavalcante et al., 2023; Sagar et al., 2024). A wide range of microsatellite markers are available for passion fruit and have been used in important conservation, characterization,

and germplasm improvement studies (Cerqueira-Silva et al., 2014a; Marques Junior et al., 2023; Testolin et al., 2022). Studies on population genetic structure are generally estimated by applying microsatellite markers. A Bayesian approach has recently been used in genetic diversity and population structure studies, implementing the Structure program, a model based on attributing individuals to subgroups (Pritchard et al., 2000).

No study has yet been carried out to estimate to the magnitude of genetic structure and variability in yellow passion fruit in Boyacá, Colombia, with SSR. There are some characterization studies on the *Passifloras* germplasm in the country which show the genetic and productive potential of these species (cultivars and wild) under tropical agroclimatological conditions (Martínez et al., 2020; Ocampo et al., 2021; Morillo et al., 2023a, b), in this context, we characterized *P. edulis* f. *flavicarpa* cultivars based on molecular markers to identify variability and genetic structure as a first step toward selecting genotypes with desirable genetic characteristics.

2. Material and Methods

2.1. Plant material

The 84 genotypes of *Passiflora edulis* f. *flavicarpa* evaluated were collected from nine farms in the municipality of Miraflores (Table 1), located in the southeast of Boyacá department, at 1432 meters above sea level. In this area the average temperature varies from 15-32 °C, depending on the location of the farm, the relative humidity is 86% and the photoperiod is 12:12.

2.2. Molecular characterization using SSR markers

For molecular characterization, three to four young leaves were collected per plant for the extraction of total genomic DNA using the protocol of Dellaporta et al. (1983), modified by Muñoz et al. (2008), regarding the time and repetition of some steps, especially those related to DNA resuspension and purification. Total DNA was visualized on 0.8% agarose gels in a Maxicell Primo EC-340 electrophoresis chamber. The concentration was determined by spectrophotometry in a Biotek EPOCH|2 equipment, subsequently diluted using HPLC water for a total volume of 100 µl to 10 ng/µl, and stored at -20 °C.

Eight microsatellites were selected that showed to be highly polymorphic, with expected heterozygosities greater than 0.7 and a high number of alleles per locus (> 6), in the genotyping of passion fruit germplasm in the work carried out by Oliveira et al. (2005, 2013), Castro et al. (2016) and Ocampo et al. (2017) (Table 2).

The SSRs amplification reaction was prepared in a final volume of 25 µL. The reaction mixture included 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1U Taq polymerase, 2 µM primer, and 10 ng of genomic DNA. Amplification was carried out in a PTC 100 programmable thermal controller thermocycler (M.J. Research, Inc.). The amplification conditions consisted of initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 seconds; for hybridization the temperatures depended on the primers used (Table 2), and extension at 72 °C for 1.5 min. Amplified products were separated on 2.5% high-resolution agarose gels at 200 volts for two hours in a Maxicell Primo EC-340 Electrophoresis Gel System chamber and stained with Z-Vision, and then visualized under transilluminator.

2.3. Analysis of data

The banding pattern generated in the evaluation of the 84 passion fruit genotypes with the eight microsatellite loci were recorded in a numerical matrix where they were assigned to each of the alleles found per locus, and each individual was assigned a maximum of two values by locus, depending on the genotype (homozygous-heterozygous). From this matrix, a dendrogram was constructed with the NTSYS-pc TREE Program (NTSYS-pc Numerical Taxonomy System for Personal Computer) and the cophenetic correlation coefficient was calculated, which is a measure between the similarity values of the dendrogram and those originated by the similarity matrix, through the COPH and MXCOMP programs of the NTSYS-PC statistical program. The genetic diversity parameters, such as number of alleles per locus (*Na*), gene flow (*Nm*), effective number of alleles (*Ne*), expected (*He*) and observed (*Ho*) heterozygosity

Table 1. Sites of origin of the genotypes of *Passiflora edulis* f. *flavicarpa* molecularly characterized with SSR markers.

Genotypes	Farm	Location	Georeferencing	
1-21	1	Finca Jorge Molina	05°13.140'N	073°09.130'W
22-31	2	Finca Florecita	05°13.240'N	073°09.205'W
32-38	3	Finca el Recuerdo	05°13.188'N	073°09.265'W
39-46	4	Finca Sandra	05°13.192'N	073°09.227'W
47-54	5	Finca Carlos Leguizamón	05°31.306'N	073°21.698'W
55-63	6	Finca Carlos Leguizamón	05°31.306'N	073°21.698'W
64-71	7	Finca San Calletano	05°13.033'N	073°09.446'W
72-79	8	Finca las Brisas	05°13.097'N	073°09.498'W
80-84	9	Finca Raúl Bernal	05°13.097'N	073°09.497'W

Table 2. SSR markers used to determine genetic diversity in passion fruit cultivars.

	SSR	Primer Forward	Primer Reverse	Motive
1	AY768782	ATGCTTTTGGAAATCCGTTT	TGCTCATGCAAAGTCACTGG	(TG)4T(TG)5
2	AY768785	TGCTCATTGATGGTGCTTG	TCGTCTCTTCTCCTCCTTCA	(AG)22
3	AY768786	TCTAATGAGCGGAGGAAAGC	CCGGATACCCACGCATTA	(GTTGTG)4
6	AY768790	CAGGATAGCAGCAGCAATGA	AGCCAAATGTCAAACTGAAC	(GT)7
8	PE07	TGCTCATTGATGGTGCTTG	TCGTCTCTTCTCCTCCTTCA	(GA)23
9	PE15	ACCGTTAAATCCAAGCAAGT	AAATGCAAAAGAATGATATGTTA	(CTTTAGC)5
10	PE90	TCAGGAAGATTGCATGTTAGT	CTGGGTTTTGTTTATGTTGC	(AGC)5
11	PE16	CGCATGTTGTTTTCCTTCTG	CAGTCCAAAGCTCGTTCTCC	(TG)23

per locus, Polymorphic Information Content Index (*PIC*), Shannon Information Index (*I*), Fixation indices (*F*) and Pairwise genetic distance comparisons among subgroups (*NeiDST*), were determined using POPGENE, GenAlex 6.5 and Microsatellite Tool Kit.

To determine the existence of a population structure that described the 84 passion fruit accessions, the grouping methods based on the Bayesian model and a discriminant analysis of principal components (DAPC) were used with the Structure program, version 2.3.4 (Pritchard et al., 2000). A mixed model of correlated allelic frequencies was used, evaluating between 1 and 7 subpopulations (K) with 10 repetitions and 100.000 burn-in iterations and 1.000.000 steps of Markov Monte Carlo (MCMC) chains for each number of groups in the analysis (*K*). The r parameter was evaluated to determine whether the information on the localities was informative. This optimal value was determined by observing the graphs of the logarithmic probability of the data (Ln*P* (*K*)) and the Δ*K* values. Analysis of molecular variance (AMOVA) was performed to determine the distribution of variation between and within the groups formed, using the program GenAlex 6.5.

3. Results

In this study, evidence of population structure was found in passion fruit cultivars from the municipality of Miraflores-Boyacá. Analysis using the STRUCTURE program (Pritchard et al., 2000) showed the peak in delta K at K = 2, suggesting the presence of two main populations (Figure 1 and Figure 2).

The analysis using the Nei and Li (1979) coefficient differentiated the yellow passion fruit accessions into seven groups (Figure 3). However, the groups were not established according to the collection area or place of origin.

The genetic diversity values between the two groups identified in STRUCTURE are presented in Table 3. The expected heterozygosity was low for both groups with a value of 0.43. The results showed low diversity or genotypic variability within each subpopulation analyzed. The percentage of polymorphic loci was 100%. The fixation indices in the two populations were less than 0.03, which shows that there is no differentiation between the general population and the subpopulations. The same number of private alleles were present with an average of 0.12 and a higher Shannon's fixation index in group A of 0.72 compared to B with 0.68. Pairwise comparisons of the Nei coefficient showed that the two groups are significantly interrelated with each other with a genetic distance of 0.025 (Table 3).

Table 3. Genetic diversity parameters for the two population subgroups formed by the clustering analyzes in STRUCTURE.

	Group A (n= 25)	Group B (n= 59)
Allelic diversity		
Mean *Na*	2.75	2.75
Private alleles	0.12	0.12
Shannon's Information Index	0.72	0.68
% of Polymorphic Loci	100%	100%
Fixation Index	0.03	-0.13
Expected heterozygosity	0.43	0.42
Pairwise genetic distance comparisons among subgroups (NeiDST)		
Group A	****	0.025
Group B	0.025	****

***Distance between individuals belonging to the same group.

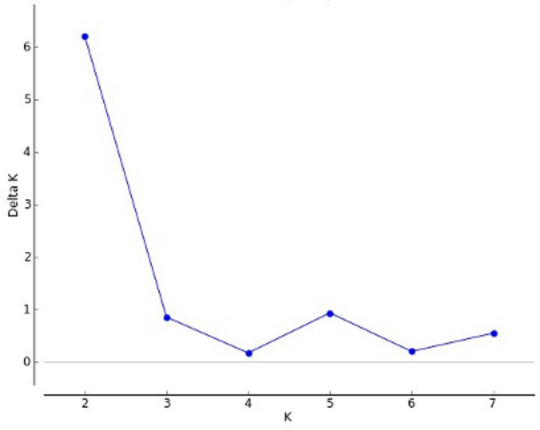

Figure 1. Delta K values obtained from Harvester Structure, estimated as the mean of the probability of K divided by the standard deviation of the probability of K. Evanno´s method with an optimal model of K=2.

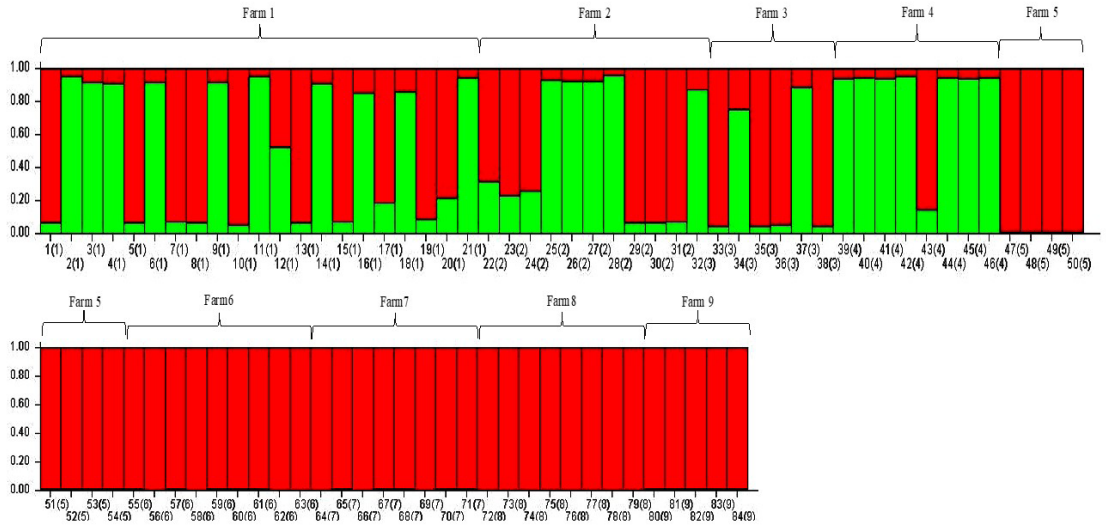

Figure 2. Population structure of 84 yellow passion fruit genotypes, grouping the genotypes into groups A (red) and B (green). Each vertical bar represents an individual sample and the color of the bar indicates the probability of an individual being assigned to one of the identified groups.

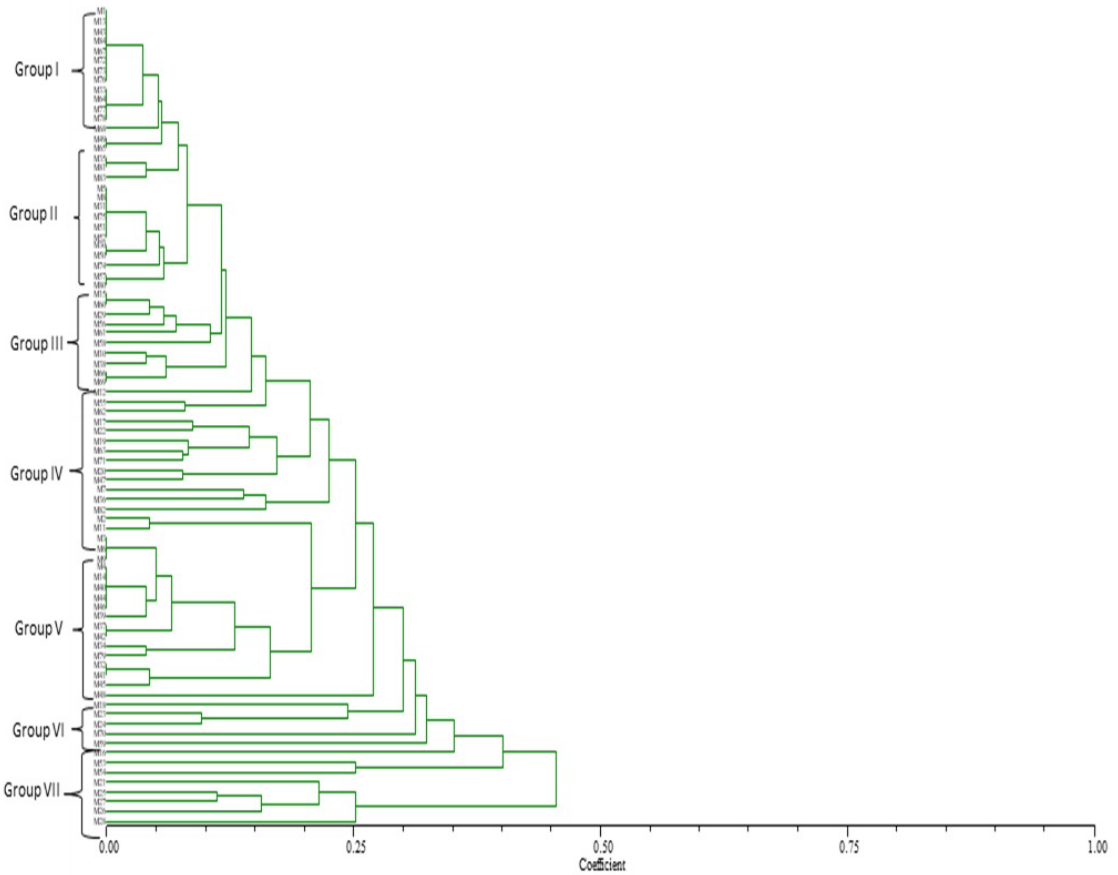

Figure 3. Dendrogram of yellow passion fruit cultivars based on the Nei and Li (1979) similarity coefficient and calculated with eight SSR markers with the UPGMA, SAHN and TREE classification methods of NTSYS-pc, version 1.8 (Exeter Software, NY, USA).

Analysis of molecular variance (AMOVA) showed that most of the genetic variation is in accordance with the differences observed within populations (98%). In comparison, the variability between populations was 2% (Table 4).

The evaluation of the genetic diversity of the 84 passion fruit genotypes from the municipality of Miraflores, using eight microsatellite markers, showed that these were moderately informative and produced 23 alleles, with a range of 2 to 4 alleles per locus (mean = 2.88). The markers with the highest number of alleles were the locus AY768782 and AY768785 with four alleles each, while the markers with the lowest number of alleles were AY768786, PE07 and PE16 with 2 alleles, respectively (Table 5). The evaluation of the effectiveness of the markers was also observed through the average value corresponding to the number of effective alleles per polymorphic locus (*Ne*). This value was 1.93, ranging between 2.99 (AY768785) and 1.10 (PE16). Markers AY768782 and AY768785 made the largest contribution to the observed variation with an Fst of 0.025. These two markers were also the most informative microsatellites, with PIC values of 0.59 (AY768785), 0.53 (AY768782), plus another marker with PIC value of 0.43 (AY768790). The average value of gene flow (*Nm*) was 28.43, thereby showing that the number of individuals that migrate per generation is approximately 28% (Table 5).

The total observed heterozygosity was 0.50, with values ranging between 0.05 and 0.92, with the most informative SSR being AY768782. While the expected heterozygosity for the evaluated markers varied between 0.09 and 0.67, with a mean of 0.43 (Table 5). These results suggest a high genetic diversity compared to other genetic studies carried out in the *Passifloras* germplasm in Colombia.

Table 4. Analysis of molecular variance (AMOVA) for passion fruit populations obtained through SSR allele analysis.

Source	df	SS	MS	Est. Var.	%
Among Pops	2	3.902	3.902	0.035	2%
Within Pops	84	169.500	2.018	2.018	98%
Total	167	290.173		2.053	100%

df = Degrees of freedom; *SS* = Suma of squares; *MS* = Var Components of variance; *Est. Var* = Variance estimation; *%* Percentage of variance.

Table 5. Genetic parameters evaluated in the total population in the 84 genotypes of *Passiflora edulis* f. *flavicarpa*.

SSR	*Na*	*Ne*	*Ho*	*He*	*I*	*Nm*	*PIC*	*Fst*
AY768782	4	2.56	0.92	0.61	1.08	9.77	0.53	0.025
AY768785	4	2.99	0.52	0.67	1.16	9.82	0.59	0.025
AY768786	2	1.35	0.05	0.26	0.43	50.29	0.23	0.005
AY768790	3	2.02	0.61	0.50	0.84	13.84	0.43	0.018
PE07	2	1.96	0.86	0.49	0.68	45.78	0.37	0.005
PE15	3	1.99	0.82	0.50	0.74	61.70	0.38	0.004
PE90	3	1.50	0.21	0.33	0.60	11.19	0.29	0.022
PE16	2	1.10	0.10	0.09	0.19	25.02	0.08	0.010
Average	2.88	1.93	0.50	0.43	0.71	28.43	0.36	0.014

Na = Number of alleles per locus; *Ne* = Number of effective alleles per locus; *Ho* = Observed heterozygosity; *He* = Expected heterozygosity; *I* = Shannon information index; *Nm* = Gene flow; *PIC* = Polymorphic Information Content; *Fst* = Coefficient of genetic differentiation.

4. Discussion

Studies of genetic diversity in the *Passiflora* germplasm have shown a high genetic diversity which within the productive system is influenced by the type of reproduction, considering it is an allogamous species, with high levels of self-incompatibility and with a sexual or asexual propagation system (Ferreira et al., 2020). This can be clearly observed in the UPGMA grouping and population structure analyzes where individuals do not present a clearly defined grouping pattern, but rather a loose distribution of them across the different groups (Figures 2 and 3).

We can also observe the degree of admixture that exists between the passion fruit cultivars evaluated, reaffirming the existence of a continuous exchange of seeds between farmers from the different producing areas of the region, thus favoring continuous genetic flow.

There is little genetic distance between the cultivars, due to the process of vegetative propagation and exchange of planting material between the same producers in the region. Results similar to those are found in other studies of genetic diversity in *Passifloras* germplasm in Colombia (Ocampo et al., 2017; Martinez et al., 2020; Morillo et al., 2023b). In general terms, the cluster analyzes using the UPGMA method (Figure 3) and Bayesian analysis demonstrated that the genetic diversity of yellow passion fruit genotypes grown in Boyacá does not have a specific geographic pattern and that there are other biological and evolutionary forces that govern their diversity in the natural environment (Figure 2).

The Bayesian approach indicated the yellow passion fruit population was grouped into two genetic groups. According to the criterion of Evanno et al. (2005), the optimal ΔK was obtained when K=2, which showed that the maximum structuring was verified when the sample was divided into two groups (Figure 1). The Bayesian clustering showed that there was no clear structuring among the yellow passion fruit cultivars evaluated, which suggests that individuals share the same alleles with a slight variation in their frequency for some genotypes (Figure 2).

On the other hand, the Bayesian population structure analysis shows that most of the individuals from farms 1 to 4 belong to the green group, while those from 5 to 9 belong to the red group (Figure 2). This result may be mainly due to the exchange of seed between the same producers in the region, since the production systems are located at different altitudes and share certain agroclimatological characteristics (Morillo et al., 2023 a).

AMOVA showed that the most of genetic variation was in agreement with the differences observed within groups (98%, Table 4). In comparison, the variability between the groups was 2%, and the population differentiation was significant (PhipT = 0.14, $p < 0.001$). The AMOVA in other *Passifloras* revealed that the greatest genetic diversity occurred within and not between populations (Barbosa et al., 2021; Silva et al., 2022; Morillo et al.,

2023a, b). These results suggest a high genetic diversity within plant populations, which may be due to asexual reproduction, somatic cell mutation, selection, genetic flow, genetic drift, and environmental change (Maciel et al., 2019; Barbosa et al., 2021).

In this study, the number of alleles per locus varied from 2 to 4, with a mean equal to 2.88, totaling 23 alleles among the genotypes for the eight loci (Table 5). Similar values of number of alleles per locus have been found in genetic diversity studies. Maciel et al. (2019) evaluated genetic diversity in passion fruit at different altitudes using SSR markers. One to three alleles were detected by microsatellite locus in the three *Passiflora* species, totaling 29 alleles in *P. edulis* Sims; 28 in *P. edulis* Sims f. *flavicarpa* Deg. and 24 in *P. alata*. Barbosa et al. (2021) evaluated genetic diversity of *Passiflora setacea* in different regions of Bahia, Brazil, with SSR markers, and found that the number of alleles per locus ranged from two (loci PE08 and mPe-UNICAMP02) to five (loci mPs-UNICAMP09 and PE90). Cavalcante et al. (2023) evaluated 95 genotypes belonging to 20 half-sib families of sour passion fruit with 170 microsatellites, where the number of alleles per locus had a mean to 2.22, totaling 20 alleles for nine loci evaluated.

Different levels of chromosome number have been reported in *Passifloras*, which may be associated with the high degree of cross-pollination, self-incompatibility, and natural selection, among others, which is why in studies of genetic diversity, this is reflected in a greater diversity of alleles (Bugallo et al., 2023).

Regarding the polymorphic information content (*PIC*), Botstein et al. (1980) defined this parameter as highly informative (*PIC* greater than 0.5); reasonably informative (*PIC* between 0.25 and 0.50); and slightly informative (*PIC* values less than 0.24). Overall, SSRs were classified as informative for all cultivars evaluated. Higher PIC values are related to the distribution and balance of allelic frequencies in the population in which the selected markers are reliable to detect genetic diversity (Maciel et al., 2019). The microsatellite markers with a higher *Fst* were AY768782 and AY768785, which may be useful to discriminate genotypes in intrapopulation studies (Morillo et al., 2023a).

The Shannon Information Index (*I*) varied from 0.19 (PE16) to 1.16 (AY768785) with a mean value of 0.71 (Table 5), which shows moderate variability in these cultivars, but enough to begin a breeding program (Ocampo et al., 2021; Bernal Moreno and Rodriguez, 2023; Morillo et al., 2023a, b). When Shannon Information Index (*I*) is closer to one, the more diverse the population. Cavalcante et al. (2023) in the study of the characterization of the genetic diversity of passion fruit found that the Shannon Index varied between 0.06 (UN06) to 1.08 (PE46), thus showing a low variability in the analyzed population. Santos et al. (2019) observed an index of information ranging from 0.30 to 1.25, indicating the high variability of the genotypes evaluated. Shannon diversity index of *P. setacea*,0.49, indicated high genetic diversity (Barbosa et al., 2021). These results can be an advantage for crop improvement because species with high genetic variability allow selection for traits of interest to be more effective (Bernardes et al., 2020).

Results obtained in other genetic diversity studies worldwide with dominant microsatellites such us ISSR (Inter Simple Sequence Repeat) in *P. edulis* f. *flavicarpa* found lower values of He = 0.29, similarities to those reported in other research of genetic diversity of *Passifloras* in Colombia (Martinez et al., 2020; Morillo et al., 2023a, b). However, in this study high values of the average observed (*Ho*=0.50) and expected heterozygosity (*He*=0.43) were found. Overall, *Ho* and *He* presented similar means, although the heterozygosity observed was higher than the expected, which suggests a high number of heterozygous individuals. The results show that these cultivars are not in Hardy-Weinberg equilibrium and reveal the action of the environment on them, as well as gene flow, natural selection and geographical distribution (Cerqueira-Silva et al., 2014a, b; Oluoch et al., 2018). This agrees with the results obtained in other investigations of genetic diversity of the Passifloras germplasm, where values of *Ho* = 0.25-0.41 and *He* = 0.31-0.36 have been found (Barbosa et al., 2021).

High levels of heterozygosity have been reported in several studies of SSR markers from different *Passifloras* (Silva et al., 2022, Testolin et al., 2022), because this is a common characteristic of the genus. This behavior can be related because these species are self-incompatible, a mechanism that induces allogamy and maintains a high degree of heterozygosity, the constant exchange of seeds between producers, natural selection, the propagation method, among others (Pereira et al., 2015; Pallavi et al., 2022; Cavalcante et al., 2023).

When we used the microsatellite markers to evaluate the intrapopulation genetic diversity between the two groups formed in the Structure analysis we found values of 100% polymorphic loci (100%), and expected heterozygosity (*He*= 0.43, group A and *He*= 0.42, group B) (Table 4) higher than those reported in other studies of genetic diversity of *Passifloras* germplasm in Colombia (Martinez et al., 2020; Morillo et al., 2023a, b). The previous results were corroborated by the fixation index, Shannon index and genetic distances (0.025). The fixation index (*F*) obtained varied between -0.13 to 0.03 (Table 5). Negative values show high values of *Ho* in relation to *He* (Table 5). Positive values of *F* were seen in the group A (0.03), which indicates the existence of inbreeding for this locus. This low level of inbreeding can be explained for the complex method of self incompatibility in passion fruit, characterized by rejecting self-pollen (Madureira et al., 2014).

Estimated gene flow was moderate and congruent with the population structure; this can be explained by the flow of pollen between the different cultivars, the reproduction system of the species, self-incompatibility, and the introduction of genotypes from other sites by farmers. Furthermore, the allogamous nature of the species tends to favor the presence of heterozygous individuals (Bernal-Parra et al., 2014).

The genetic parameters determined the existence of genetic variability which may be due to the coevolution processes of the species in its natural environment, reproduction system, exchange of seeds between the same producers, lack of selection and certified planting material,

among others factors to consider when defining strategies for the improvement of the species (Ocampo et al., 2021; Pallavi et al., 2022; Bernal Moreno and Rodríguez, 2023; Cavalcante et al., 2023; Morillo et al., 2023a, b; Rosário et al., 2022).

These results contribute to the knowledge of the germplasm and its use in conservation and genetic improvement strategies, aimed at identifying genotypes with good agronomic and genetic characteristics of interest that can supply both national and international demand and also significantly improve the competitiveness of yellow passion fruit in the country.

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