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Genetic diversity pattern of *Passiflora* spp. in Boyacá, Colombia

Abstract – The objective of this work was to characterize the genetic diversity, using ISSR markers, of 70 genotypes of five species of *Passiflora* spp. in Boyacá, Colombia. For molecular characterization, samples of young leaves were collected from 11 municipalities of the Boyacá department. Genetic similarity was used to cluster the genotypes by the UPGMA method, and genetic structure was evaluated by the Bayesian model. Eight ISSR primers produced 138 loci. The formed cluster consists of two populations, with most individuals of the same species but from different geographic origins. The percentage of polymorphic loci is higher than 80%. The average value of heterozygosity is between 0.29 and 0.36 for population I and II, respectively, and the values of polymorphic information content are low. A moderate genetic differentiation (0.16) and high gene flow (3.35) are observed.

Index terms: germplasm, ISSR molecular markers, passion fruit, plant breeding, variability.

Padrão de diversidade genética de *Passiflora* spp. em Boyacá, Colômbia

Resumo – O objetivo deste trabalho foi caracterizar a diversidade genética, por meio de marcadores ISSR, de 70 genótipos de cinco espécies de *Passiflora* spp. em Boyacá, Colômbia. Para a caracterização molecular, foram coletadas folhas jovens em 11 municípios do departamento de Boyacá. A similaridade genética foi utilizada para o agrupamento dos genótipos pelo método UPGMA, e a estrutura genética foi avaliada pelo modelo Bayesiano. Oito iniciadores ISSR produziram 138 locos. O agrupamento formado consiste de duas populações, com a maioria dos indivíduos da mesma espécie mas de diferentes origens geográficas. A percentagem de locos polimórficos é superior a 80%. O valor médio de heterozigosidade fica entre 0,29 e 0,36 para a população I e II, respectivamente, e os valores de conteúdo de informação polimórfica são baixos. Observa-se diferenciação genética moderada (0,16) e alto fluxo gênico (3,35).

Termos para indexação: germoplasma, marcadores moleculares ISSR, maracujá, melhoramento de plantas, variabilidade.

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Introduction

The Passifloraceae family is divided into two tribes; among them is the *Passiflora* genus, which contains the largest number of species – passion fruits are native to the New World and their highest diversity is in the north of the Andes, especially in Colombia and Ecuador (Rodríguez Castillo et al., 2020). Plants of this genus have high economic, nutritional, medicinal and ornamental value with benefits for human

health (Ramaiya et al., 2018). The main cultivated fruit species are *Passiflora alata* Dryander, *P. edulis* Sims, *P. ligularis* Juss., *P. maliformis* L., *P. popenovii* Killip, *P. quadrangularis* L., and *P. tripartita* var. *mollissima* (Kunth) Holm-Niels. & P. Jørg. (Ocampo et al., 2017).

Studies on plant breeding and characterization and conservation of the germplasm in Colombia are scarce (Ocampo et al., 2004, 2017; Martínez et al., 2020). In recent years, the classification of passion fruit was based on morphological characteristics such as locality, leaf/flower/flesh color, growth habit and other characteristics of plants. Although morphological classification is commonly performed due to its low cost and to the fact that it is easy to do, it has a complex inheritance pattern, dependency to development of plant growth phase, and vulnerability to environmental changes (Dias et al., 2020).

Genetic characterization research in passion fruit species has been carried out for decades using different molecular markers (Pérez et al., 2020). In Colombia, genetic diversity studies with ISSR markers in *P. ligularis* accessions (sweet granadilla) showed that the cultivated germplasm has high variability with a slight genetic structure (Bernal-Parra et al., 2014). The results were similar to those obtained by Ocampo et al. (2017) when evaluating the genetic diversity and population structure of 51 Colombian passion fruit accessions (*P. edulis*) using microsatellite markers.

Fonseca-Trujillo et al. (2009) characterized cultivated gulupa (*P. edulis* f. *edulis*) materials collected in the Departments of Boyacá, Cundinamarca and Huila using ISSR markers, finding high genetic diversity, probably as a result of the method of propagation, site of origin and short time of crop establishment. Ortiz et al. (2012) evaluated purple passion fruit (*Passiflora edulis* Sims f. *edulis*) genetic variability in individuals from commercial plantations in Colombia with AFLP, founding low genetic variability. In the Department of Boyacá, Martínez et al. (2020) characterized the genetic diversity of *Passiflora* spp. using ISSRs, and their results indicated a great genetic diversity but without a defined population structure.

The characterization and quantification of the genetic variability of germplasm is important, since it allows to analyze which is the genetic basis of the breeding programs and, thus, maintain the variability found during the recurrent cycles in order to maximize genetic gains and decrease the effects of inbreeding that are common in different species of the *Passiflora* genus (Ho et al., 2021). The results of this research will provide scientific information for identification, conservation and breeding purposes regarding passion fruit growing areas in Boyacá, Colombia.

The objective of this work was to characterize the genetic diversity, using ISSR markers, of 70 genotypes of five species of *Passiflora* spp. in Boyacá, Colombia.

Materials and Methods

Young leaf tissue samples of *Passiflora* spp. belonging to five species were collected in the main producing municipalities in the Department of Boyacá (11 municipalities and 19 farms were sampled). Identification of the species was made in situ according to the information provided by the farmers and was corroborated by the herbarium of the Universidad Pedagógica y Tecnológica de Colombia (Table 1). Photos of the main passion fruit (*Passiflora* spp.) species cultivated in Colombia are shown in Figure 1.

Molecular characterization was carried out in the molecular biology research laboratories of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja. Total DNA was extracted for each plant from dried passion fruit leaves using the protocol of Dellaporta et al. (1983). DNA quality was evaluated in a 1% agarose gel and stained with GelRed dye (Biotium, USA). The concentration was determined by a fluorometer (Dyna Quant 200, Hoefer, Holliston, Massachusetts, USA), and was diluted in HPLC water to a total volume of 100 mL at 10 ng mL^{-1} and stored at -20°C.

A total of 8 ISSR primers synthesized by Technologies, Inc., Bioneer Corporation (Alameda, California, USA) were used (Table 2). 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 reactions were performed with 1X buffer, 1.5 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTPs, 1 U Taq Polymerase, 2 µmol L^{-1} primer and 10 ng genomic DNA for a final volume of 25 µL. The PCR cycles consisted of an initial denaturation at 95°C for 5 min, followed by 37 cycles at 95°C for 30 s, annealing temperature 58°C (GT), 50°C (AG, CA, ACA), and 55°C (CCA, TG, CT, CGA) for 45 s, extension at 72°C

Table 1. Collection sites for *Passiflora* spp. in the municpalities of Department of Boyacá, Colombia, during the years 2018 to 2019.

No.	Species	Municipality	Geographic location	No.	Species	Municipality	Geographic location
			Population I				
1	*Passiflora edulis* f. *edulis*	Firavitoba	5°40'08"N,72°59'38"W	8	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
2	*P. edulis* f. *edulis*	Firavitoba	5°40'08"N,72°59'38"W	9	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
3	*P. edulis* f. *edulis*	Firavitoba	5°40'08"N,72°59'38"W	10	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
4	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W	11	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
5	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W	12	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
6	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W	13	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W
7	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W				
			Population II				
14	*P. edulis* f. *edulis*	Ramiriquí	5°25'1"N,73°19'59"W	43	*P. edulis* f. *edulis*	Buenavista	5°30'60"N,73°57'59"W
15	*P. ligularis*	Ramiriquí	5°25'1"N,73°19'59"W	44	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
16	*P. ligularis*	Ramiriquí	5°25'1"N,73°19'59"W	45	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
17	*P. maliformis* red flower	Úmbita	5°13'1"N,73°28'1"W	46	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
18	*P. maliformis* red flower	Úmbita	5°13'1"N,73°28'1"W	47	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
19	*P. maliformis* white flower	Úmbita	5°13'1"N,73°28'1"W	48	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
20	*P. maliformis* red flower	Úmbita	5°13'1"N,73°28'1"W	49	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
21	*P. maliformis* red flower	Turmequé	5°19'20"N,73°29'21"W	50	*P. edulis* f. *edulis*	Miraflores	5°13'1"N,73°8'47"W
22	*P. maliformis* red flower	Turmequé	5°19'20"N,73°29'21"W	51	*P. edulis* f. *edulis*	Miraflores	5°13'1"N,73°8'47"W
23	*P. maliformis* red flower	Turmequé	5°19'20"N,73°29'21"W	52	*P. edulis* f. *edulis*	Miraflores	5°13'1"N,73°8'47"W
24	*P. maliformis* white flower	Turmequé	5°19'20"N,73°29'21"W	53	Graft congolo-cholupa	Miraflores	5°13'1"N,73°8'47"W
25	*P. ligularis*	Turmequé	5°19'20"N,73°29'21"W	54	Graft congolo-cholupa	Miraflores	5°13'1"N,73°8'47"W
26	*P. ligularis*	Turmequé	5°19'20"N,73°29'21"W	55	Graft congolo-cholupa	Miraflores	5°13'1"N,73°8'47"W
27	*P. tripartita* var. *mollissima* common pink	Turmequé	5°19'20"N,73°29'21"W	56	*P. edulis* purple fruit	Miraflores	5°13'1"N,73°8'47"W
28	*P. tripartita* var. *mollissima* common	Turmequé	5°19'20"N,73°29'21"W	57	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
29	*P. edulis* f. *edulis*	Sutamarchán	5°38'3"N,73°37'12"W	58	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
30	*P. edulis* f. *edulis*	Sutamarchán	5°38'3"N,73°37'12"W	59	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
31	*P. edulis* f. *edulis*	Sutamarchán	5°38'3"N,73°37'12"W	60	*P. edulis*	Miraflores	5°13'1"N,73°8'47"W
32	*P. tripartita* var. *mollissima* common pink	Sutamarchán	5°38'3"N,73°37'12"W	61	*P. edulis*	Briceño	5°43'25"N,73°56'24"W
33	*P. tripartita* var. *mollissima* common pink	Sutamarchán	5°38'3"N,73°37'12"W	62	*P. edulis*	Briceño	5°43'25"N,73°56'24"W
34	*P. tripartita* var. *mollissima* common pink	Sutamarchán	5°38'3"N,73°37'12"W	63	*P. edulis*	Briceño	5°43'25"N,73°56'24"W
35	Graft *P. maliformis* (cholupa) *P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54"N,74°38'53"W	64	*P. edulis*	Tununguá	5°43'59"N,73°55'59"W
36	Graft *P. maliformis* (cholupa) *P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54"N,74°38'53"W	65	*P. edulis*	Tununguá	5°43'59"N,73°55'59"W
37	Graft *P. maliformis* (cholupa) *P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54"N,74°38'53"W	66	*P. edulis*	Tununguá	5°43'59"N,73°55'59"W
38	Graft congolo-cholupa	Tinjacá	5°34'54"N,74°38'53"W	67	*P. edulis* purple fruit	Tununguá	5°43'59"N,73°55'59"W
39	*P. edulis* f. *edulis*	Tinjacá	5°34'54"N,74°38'53"W	68	Graft congolo-cholupa	Nuevo Colón	5°21'30"N,73°27'38"W
40	*P. ligularis*	Tinjacá	5°34'54"N,74°38'53"W	69	Graft congolo-cholupa	Nuevo Colón	5°21'30"N,73°27'38"W
41	*P. edulis* f. *edulis*	Buenavista	5°30'60"N,73°57'59"W	70	Congolo in vitro	Nuevo Colón	5°21'30"N,73°27'38"W
42	*P. edulis* f. *edulis*	Buenavista	5°30'60"N,73°57'59"W	-	-	-	-

for 2 min, and a final extension at 72°C for 7 min. The DNA fragments were separated on 2% agarose gel run at 100 volts for 3 h using 0.5% TBE, and stained with 0.5 µg mL^{-1} GelRed dye loading buffer, and then visualized under transilluminator.

Amplified products were scored as present (1) or absent (0) to construct a binary matrix. Genetic similarity (GS) was estimated for all genotype pairs using the following equation (Nei & Li, 1979):

$$GS_{ij} = \frac{2N_{ij}}{2N_{ij}} + N_i + N_j$$

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 N_j correspond to the number of bands found in genotypes i and j, respectively. The matrix generated with GS estimates was used to cluster the genotypes in a dendrogram obtained by the unweighted pair group method with arithmetic mean (UPGMA) using Numerical Taxonomy System for Personal Computer NTSYSpc statistical software (version 2.02 PC, Setauket, New York, USA). 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 statistical package POPGENE version 3.2 was used to estimate the Nei's genetic diversity (H), Shannon information index (I), coefficient of genetic differentiation (CGD), number and percentage of polymorphic loci, and gene flow (GF) using the equation GF = 0.5(1-CGD)/CGD (McDermott & McDonald, 1993). The parameters of genetic diversity like polymorphic information content (PIC) and heterozygosity were estimated by the TFPGA program (Tools for Population Genetic Analysis, version 1.3, 1997).

The genetic structure analysis was done based on Bayesian model (Hubisz et al., 2009) as implemented

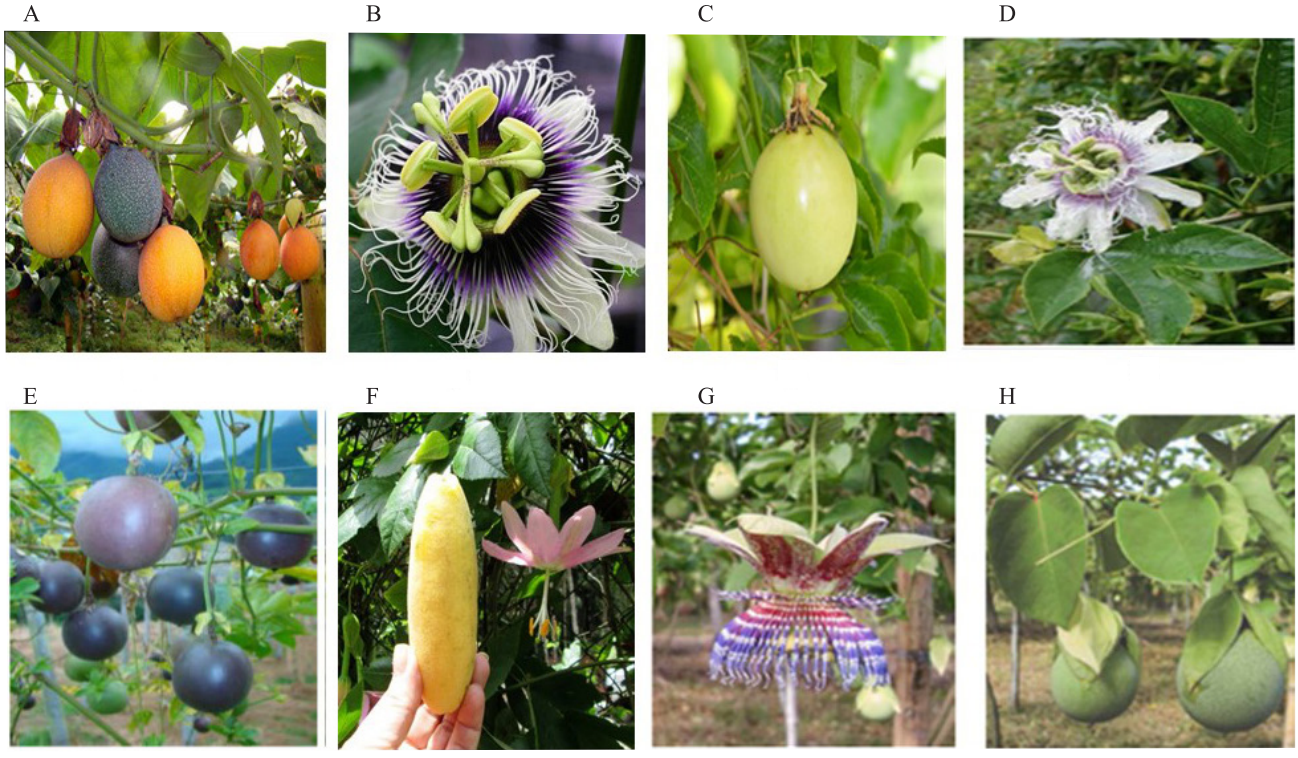

Figure 1. Main species of *Passiflora* cultivated in the Colombia: granadilla (*Passiflora liguris*) (A); passion fruit (*P. edulis*) (B and C); gulupa (*P. edulis* f. *edulis*) (D and E); curuba (*P. tripartita* var. *mollissima*) (F); and cholupa (*P. maliformis*) (G and H). Photos by María Antonia Martínez Camargo.

in the 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 & vonHoldt, 2012) to estimate the most likely *K* in each set of passion fruit accessions. Finally, an analysis of molecular variance (AMOVA) was computed using GenAlEx 6.5 program to identify the proportion of variation between and within the groups established according to the population structure analysis.

Table 2. Primers used in the ISSR technique for molecular characterization of *Passiflora* spp. germplasm from the municipalities of the Department of Boyacá, Colombia.

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	$DBDA(CA)_7$
ACA	$BDB(ACA)_5$

The following designations are used for the degenerate 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).

Results and Discussion

The ISSR markers used to characterize the genetic diversity of 70 *Passiflora* genotypes from the Department of Boyacá produced a total of 138 loci, ranging from 14 (CT) to 24 (GT) bands per primer, with an average of polymorphic loci greater than 80% being highly variable according to the species studied (Table 3). These results were similar to other genetic diversity studies in *Passiflora* (Martínez et al., 2020; Ferreira et al., 2021). Based on the ISSR results, it was shown that, of the eight primers used, the ones with di- and trinucleotide repeat produced the highest number of scorable loci, results contrary to the ones reported by Ho et al. (2021).

ISSR results revealed genetic relatedness between the 70 genotypes; and at about 70% genetic distance, the dendrogram generated using Nei's coefficient resulted in three groups (Figure 2), considering that only one allele per locus is used in the analysis, due to the dominant nature of the marker. The genotypes from Group A were composed of *P. edulis* f. *edulis*, which was collected in Ramiriquí, Firatova, Sutamarchán; graft *P. maliformis* (cholupa); *P. edulis* f. *edulis* (gulupa); and *P. edulis*, which was collected in Miraflores. Most genotypes in this group belong to *P. maliformis* red and white flower; *P. edulis* (Miraflores, Briceño, Tunungua); graft congolo-cholupa (Miraflores); and *Passiflora edulis* f. *edulis* (Tinjacá and Buenavista). The remaining genotypes were clustering in group C, *P. ligularis*

Table 3. *Passiflora* spp. diversity obtained by the ISSR polymorphism analysis of primers[(1)] in 2018 and 2019 in Boyacá, Colombia[(1)].

Primer	Population I (representing two municipalities)						Population II (representing nine municipalities)						CGD	GF
	No. loci	No. polymorphic loci	% polymorphic loci	He	I	PIC	No. loci	No. polymorphic loci	% polymorphic loci	He	I	PIC		
ACA	18	15	83	0.33	0.49	0.27	18	18	100	0.38	0.57	0.29	0.09	4.96
AG	20	19	95	0.31	0.48	0.23	20	20	100	0.39	0.57	0.30	0.07	6.70
CA	10	10	100	0.39	0.57	0.30	10	10	100	0.37	0.56	0.28	0.38	0.02
CCA	18	14	78	0.30	0.44	0.23	18	17	94	0.34	0.51	0.19	0.14	2.94
CGA	16	12	75	0.24	0.37	0.19	16	16	100	0.34	0.52	0.19	0.15	2.81
TG	18	13	72	0.18	0.28	0.11	18	18	100	0.38	0.56	0.29	0.21	1.85
CT	14	12	86	0.34	0.51	0.28	14	14	100	0.40	0.58	0.37	0.12	3.70
GT	24	21	88	0.30	0.46	0.23	24	24	100	0.32	0.50	0.26	0.12	3.80
Mean	138	116	84	0.29	0.44	0.23	138	137	99	0.36	0.54	0.27	0.16	3.35

[(1)]PIC, polymorphic information content; He, expected average heterozygosity; I, Shannon information index; CGD, coefficient of genetic differentiation; and GF, gene flow.

(Ramiriquí and Turmeque); *P. tripartita* var. *mollisima* (Turmeque); *P. tripartita* var. *mollisima* common pink (Sutamarchán); graft congolo-cholupa (Tinjacá, Nuevo Colón); *P. edulis* purple fruit (Tunungua); and congolo in vitro (Nuevo Colón).

The foregoing can be explained by the allogamous nature of the species, the asexual propagation and the continuous exchange of seeds that exists between the producers of *Passiflora* in Colombia, which is influencing in one way or another the genetic diversity of the species within productive systems.

The Bayesian clustering method with admixed model indicated that the 70 passion fruit genotypes were clustering into two genetic groups (K= 2). Figure 3 shows the estimated population structure based on Delta K when it reaches its maximum value following the ad-hoc method, and two subpopulations clusters (k) that were represented by different colors. Taking the above into account, the population genetic analyses were made taking into account the two populations formed as follows: population I, which is made up of the 11 individuals that belong to *P. edulis* f. *edulis* (Table 1); and the most diverse population II, comprising of 59 individuals. All genetic parameters were estimated in the two populations.

In general, the cluster analyses reflected a high phenotypic variability observed in these species, compared to other studies of various passion fruit accessions (Torres et al., 2019). The differences can be attributed to the higher genotypic variation and to the technique employed (Ho et al., 2021). The clustering trend was by species rather than by geographic origin, and ISSR markers did not allow the complete separation of different forms of *P. edulis* (Figures 2 and 3), showing high genetic similarity between these forms – results are similar to the ones reported by Ho et al. (2021). The lack of a grouping pattern may be due to the reproductive factors of the species, its sexual propagation, the exchange of seeds between farmers, self-incompatibility and the presence, among the evaluated genotypes, of exotic species such as gulupa or curuba (Ocampo et al., 2017; Martínez et al., 2020).

Cophenetic correlation coefficient was 74%, demonstrating a relationship between the genetic distances and the groups – these results were similar to the ones obtained in different studies of genetic diversity in *Passiflora* spp. (Ferreira et al., 2021). In

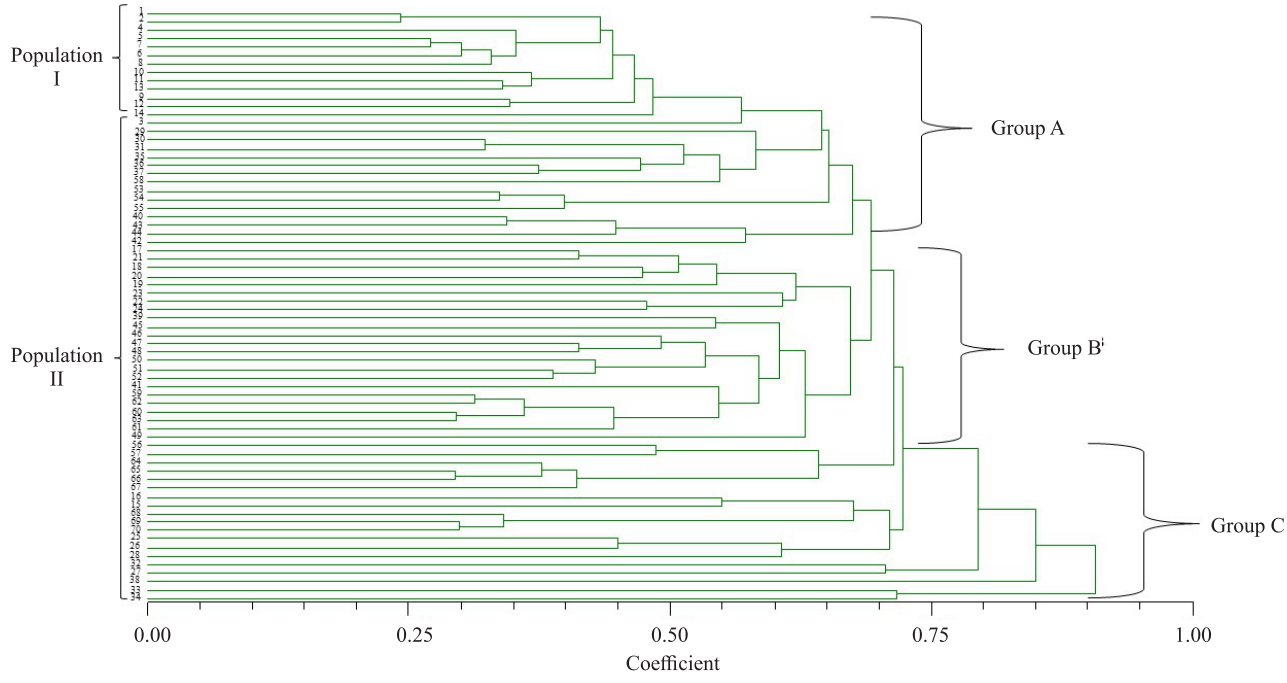

Figure 2. Dendrogram for the 70 *Passiflora* genotypes based on the Nei-Li (1979) similarity coefficient (%) and estimated with eight ISSR markers, with UPGMA, SAHN, and Tree of NTSYS-pc version classification methods. Molecular characterization was carried out in germplasm from Boyacá, Colombia, in the period of 2018 to 2019.

the population I, Shannon's information index at the ISSR level ranged from 0.28 (TG) to 0.57 (CA), with an average value of 0.44 (Table 3). The population II obtained values ranging from 0.50 (GT) to 0.58 (CT), with an average value of 0.54. These data are similar to the ones of previous studies of other allogamous species, such as *Chenopodium quinoa* (Abd El-Moneim et al., 2021), and *Dioscorea alata* (Castañeda-Cardona et al., 2020).

In the population I, the highest values of expected heterozygosity and PIC were obtained for the locus CA (He = 0.39, PIC = 0.30), and in the population II (He = 0.40; PIC = 0.37), for the CT primer. Nei's genetic diversity or expected heterozygosity (He) was 0.29 and 0.36 for population I and II, respectively, and the medium values were lower than those (He = 0.56) reported in genetic diversity studies in *Passiflora* in Colombia and in other countries (Martínez et al., 2020) with loci microsatellites (ISSR). 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.

Regarding the polymorphic information content (PIC), Botstein et al. (1980) defined this parameter as highly informative when PIC is greater than 0.5; reasonably informative when PIC is between 0.25 and 0.50; and slightly informative when PIC values are lower than 0.25). The loci TG and CGA (PIC = 0.11 and PIC = 0.19, respectively) were slightly informative; and the CA (PIC = 0.30) and CT (PIC = 0.37) loci were the most informative among all the markers evaluated in the populations I and II, respectively (Table 3). Overall ISSR values were classified as reasonably informative for genotypes evaluated.

The genetic differentiation (CGD) between the populations was 0.16, based on Shannon's diversity index, indicating that 16% of the total genetic variation was between populations. A similar level of differentiation between the populations was shown by AMOVA, with 88% of the variation apportioned within populations and 12% between populations (Table 4). These values show a pattern of strong homogeneity within populations and strong variation between populations. The average value of GF was 3.35, where values greater than one show a high genetic flow. Studies in several plant species that were based on the analysis of isozymes and RAPD loci have estimated values of genetic divergence ranging from 0.05 to 0.34 between natural or non-domesticated populations (Zimback et al., 2004). According to Yeh (2000), estimates of genetic divergence between 0.151 and 0.250 represent a high level of differentiation, meaning that natural or non-domesticated populations of *Passiflora* spp. show a high level of divergence.

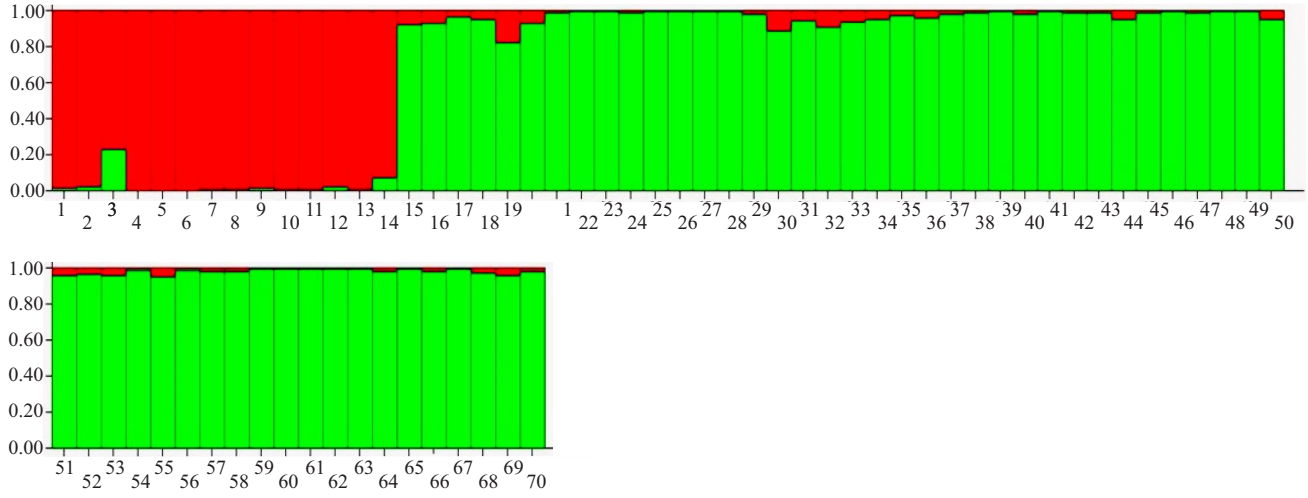

Figure 3. Population structure of the 70 *Passiflora* genotypes obtained by the structure program based on ISSR markers for K = 2. Each color represents a subpopulation (red = population I; and green = population II), and the length of the colored segment shows the estimated membership proportion of each sample per designed group. Molecular characterization was carried out in germplasm from Boyacá, Colombia, in the period of 2018 to 2019.

Table 4. Molecular analysis of variance for *Passiflora* spp. groups formed with the eight ISSR markers[1].

Source	DF	SS	MD	SD	TV (%)
Between populations	1	121.593	121.593	4.311	12
Within populations	68	2,061.592	30.318	30.318	88
Total	69	2,183.186		34.629	100

[1]DF, degrees of freedom; SS, sum of squares; MD, Middle Square; SD, standard deviation; TV, total variation.

Genetic differentiation between populations with GF < 1.0 may result from limited gene flow due to high selective pressure or discontinuous distribution (Slatkin, 1987). In this study, GF of *Passiflora* genotypes was higher than that reported by Pereira et al. (2015) in *Passiflora setacea*, indicating some degree of spatial isolation and genetic differentiation between populations. The value of GF = 3.35 found here indicates high gene flow, which is possibly due to seed dispersal mechanisms, pollen movement and individuals, and the allogamic nature of the species promotes constant gene flow between genotypes; consequently, there are no crossability barriers or high differentiation between the populations (Aquino & Amela García, 2019).

ISSR fingerprints provide a useful tool for establishing a rapid and rational approach to study the diversity and genetic relationships between alfa populations. These markers can be used to evaluate the variability of the populations analyzed (Ferreira et al., 2021). However, it must be considered that only one allele per locus is being analyzed.

The results found in this study contribute to the knowledge of national germplasm, which is essential to establish conservation, use and management strategies at the same time at a global level to know the processes of coevolution of the species and the factors that are determining its diversity (Ocampo et al., 2017).

The study findings demonstrate the existence of genetic variability between passion fruit varieties grown in different regions of Boyacá, Colombia. This suggests the potential application of these varieties in breeding programs by exploiting the use of molecular markers for selection of specific traits.

Conclusions

1. There is a moderate genetic differentiation of 0.16 and a high gene flow of 3.35 between the studied *Passiflora* spp. populations.

2. The eight evaluated primers produced 138 loci and can be used to analyze the genetic diversity in ISSR between *Passiflora* spp. from different regions of Colombia.

3. The formed Cluster shows two populations, with most individuals of the same species but not of the same geographic origin.

4. The percentage of polymorphic loci is high (>80%) and the polymorphic information content values are low for the *Passiflora* spp. genotypes.

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