Characterization of the genetic diversity in *Passiflora* spp. in the Boyacá Department, Colombia

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ABSTRACT

Passiflora is a group of species of economic importance in Colombia because of their productive potential and nutritional, pharmaceutical and industrial properties; therefore, it is necessary to study the genetic diversity of the species cultivated in principal productive departments, such as Boyacá. The use of molecular markers has become a fundamental tool for germplasm characterization, which effectively complements morphological and agronomic information. This research aimed to characterize the genetic diversity of *Passiflora* spp. grown in the Department of Boyacá using inter-simple sequence repeat (ISSR) markers. The eight ISSRs generated a total 138 bands with molecular weights between 250 and 1350 kb. With a similarity coefficient of 0.60, seven groups were formed, showing lax distribution of the individuals. The percentage of polymorphic loci was between 56% (TG) and 90% (AG). The average value of heterozygosity was 0.56 with a genetic differentiation coefficient (FST) of 0.16, which indicated great genetic diversity but without a defined population structure. The AMOVA showed that 64% of the observed genetic variation was due to the component within the groups. The results suggested levels of hierarchy and subdivision finer than those considered in this study, in addition to a complementary morphoagronomic characterization that established the total genetic diversity, which can be used to implement a breeding program for more productive cultivars that are resistant to the principal phytosanitary problems.

Key words: Fruit trees, genetic diversity, microsatellites, variability.

INTRODUCTION

The *Passiflora* genus is the most representative one of the *Passifloraceae* family, with around 520 species distributed in the tropical regions of the Americas, Asia and Africa (Araya et al., 2017). Cultivated species have great nutritional, medicinal and ornamental value since they are a source of minerals, vitamins A, C and D, alkaloids, flavonoids and carotenoids, which are beneficial for human health. These species have compounds with anxiolytic, antihypertensive, sedative and analgesic properties (Konta et al., 2014). The seeds are a source of essential fatty acids that can be used in food and cosmetic industries (Malacrida and Neuza, 2012). In spite of the economic importance and various potential uses, studies on the characterization of the diversity of the germplasm of cultivated passionflower in Colombia, specifically in the Department of Boyacá, are limited.

Colombia is the center of diversity of an important group of these species. *Passiflora edulis* Sims f. *flavicarpa* O. Deg. known as passion fruit, *P. edulis* Sims f. *edulis* (gulupa), *P. tripartita* (Juss.) Poir. var. *mollissima* (Kunth) Holm-Niels. & P. Jørg. (curuba), *P. maliformis* L. (cholupa), and *P. ligularis* Juss. (granadilla) have acquired economic importance because of their production and export potential. *Passiflora* production in the country increased 34% in the last 3 yr, reaching 227 813 t during 2018, the principal production departments include Antioquia and Meta (Agronet, 2019). Boyacá has increased area and production for *Passiflora*; by 2018, it had a planted area of 1532 ha, with a yield of 10.5 t ha^{-1}. Despite

the productive potential, a limiting factor in the development of this crop in Colombia is the incidence of pests and diseases, generating significant losses in yield (Ocampo et al., 2013). On the other hand, there is a need for breeding programs that offer cultivars with higher genetic and phytosanitary quality that respond to the needs of farmers (Ocampo et al., 2017).

The genetic diversity in the *Passiflora* genus is very wide, both within the genus and within the most cultivated species (Vianna et al., 2019), so the characterization of the genetic variation is essential for the conservation, management and efficient use of germplasm in genetic breeding programs (Oluoch et al., 2018). Traditionally, diversity in *Passiflora* is estimated by observing variations in morphological or agronomic characteristics or tolerance to biotic or abiotic factors, which does not reflect the genetic relationship between evaluated materials and is affected by environmental changes and dependence on the stage of development (Pereira et al., 2015). DNA markers overcome these limitations and are a useful tool for the evaluation and characterization of germplasm (Vieira et al., 2019).

The genetic diversity in *Passiflora* has been studied with different molecular techniques, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and sequence-related amplified polymorphism (SRAP) (Oluoch et al., 2018; Vieira et al., 2019); however, simple sequence repeat (SSR) markers are the most used techniques for the characterization of germplasm in different crops since they are highly polymorphic, reproducible and co-dominant, with wide genome coverage (Gramazio et al., 2018). Among the microsatellite markers, inter-simple sequence repeat (ISSR), which combines characteristics of the RAPD and microsatellites techniques, facilitates multilocus genomic analysis with a single primer based on regions of SSR, presents good reproducibility, has a low cost, is simple, does not need previous genome information (Morillo et al., 2018), and has proven to be polymorphic and informative in studies on genetic diversity in *Passiflora* (Santos et al., 2011; Sousa et al., 2015; Batista et al., 2017; Oliveira et al., 2019; Vianna et al., 2019).

In Colombia, genetic diversity studies conducted by Santos et al. (2011) with ISSR markers in *P. edulis* (purple and yellow type) and *P. alata* have shown that there is no structure in the evaluated populations; however, these studies provided information for genetic improvement. Other research conducted in Colombian on *P. ligularis* accessions (sweet granadilla) suggests that the cultivated germplasm has high variability (He = 0.96), with a slight genetic structure (Bernal et al., 2014). Ocampo et al. (2017) studied the genetic diversity and population structure of 51 Colombian passion fruit accessions (*P. edulis* Sims f. *flavicarpa* O. Deg.) using microsatellite markers, where there was little geographic structuring and high differentiation between individuals of the same accession. Galeano et al. (2018) conducted an agronomic evaluation of 60 accessions of *P. edulis* Sims from the Agrosavia germplasm bank, where 30 promising accessions were identified; however, this information must be complemented with biochemical and molecular data. Fonseca-Trujillo et al. (2019) characterized cultivated gulupa materials (*P. edulis* f. *edulis*) 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, provenance and short time of crop establishment. Studies of genetic diversity in the *Passiflora* spp. germplasm in the department of Boyacá are necessary for the sustainable use of its genetic resource with a view to increasing the profitability of the crop by identifying elite materials that have good yields, with tolerance to biotic and abiotic factors and adapted to local conditions.

In this context, it is necessary to continue with genetic studies in order to gather more information on the current genetic diversity of *Passiflora* and the relationships between individuals of the germplasm. For this reason, the objective of this research was to evaluate the genetic diversity in 71 cultivated *Passiflora* materials from the Department of Boyacá in order to provide the information for the management, conservation and efficient use of this plant genetic resource.

MATERIALS AND METHODS

Plant material

The sampling of the *Passiflora* spp. was carried out in the main producing municipalities in the Department of Boyacá (Firavitoba, Ramiriquí, Umbita, Turmeque, Sutamarchán, Tinjacá, Buenavista, Miraflores, Briceño, Tunungua and Nuevo Colón), in total 11 municipalities and 19 farms were sampled. In each of the municipalities, production farms with a tradition in cultivation were visited, where taking into account sanitary quality and phenotypic variation, four plants were selected at random per species. Table 1 shows the specific geographical location of the sampling points according to the species sampled and the selected plants. The 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. Collection sites for *Passiflora* spp. in the Department of Boyacá.

Nr	Species	Municipality	Geographic coordinates
1	*Passiflora edulis* f. *edulis*	Firabitoba	5°40'08" N, 72°59'38" W
2	*P. edulis* f. *edulis*	Firabitoba	5°40'08" N, 72°59'38" W
3	*P. edulis* f. *edulis*	Firabitoba	5°40'08" N, 72°59'38" W
4	*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
6	*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
8	*P. edulis* f. *edulis*	Ramiriquí	5°25'1" N, 73°19'59" W
9	*P. edulis* f. *edulis*	Ramiriquí	5°25'1" N, 73°19'59" W
10	*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
12	*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
14	*P. edulis* f. *edulis*	Ramiriquí	5°25'1" N, 73°19'59" W
15	*P. ligularis*	Ramiriquí	5°25'1" N, 73°19'59" W
16	*P. ligularis*	Ramiriquí	5°25'1" N, 73°19'59" W
17	*P. maliformis* red flower	Umbita	5°13'1" N, 73°28'1" W
18	*P. maliformis* red flower	Umbita	5°13'1" N, 73°28'1" W
19	*P. maliformis* white flower	Umbita	5°13'1" N, 73°28'1" W
20	*P. maliformis* red flower	Umbita	5°13'1" N, 73°28'1" W
21	*P. maliformis* red flower	Turmeque	5°19'20" N, 73°29'21" W
22	*P. maliformis* red flower	Turmeque	5°19'20" N, 73°29'21" W
23	*P. maliformis* red flower	Turmeque	5°19'20" N, 73°29'21" W
24	*P. maliformis* white flower	Turmeque	5°19'20" N, 73°29'21" W
25	*P. ligularis*	Turmeque	5°19'20" N, 73°29'21" W
26	*P. ligularis*	Turmeque	5°19'20" N, 73°29'21" W
27	*P. tripartita* var. *mollisima* common pink	Turmeque	5°19'20" N, 73°29'21" W
28	*P. tripartita* var. *mollisima* common	Turmeque	5°19'20" N, 73°29'21" W
29	*P. edulis* f. *edulis*	Sutamarchán	5°38'3" N, 73°37'12" W
30	*P. edulis* f. *edulis*	Sutamarchán	5°38'3" N, 73°37'12" W
31	*P. edulis* f. *edulis*	Sutamarchán	5°38'3" N, 73°37'12" W
32	*P. tripartita* var. *mollisima* common pink	Sutamarchán	5°38'3" N, 73°37'12" W
33	*P. tripartita* var. *mollisima* common pink	Sutamarchán	5°38'3" N, 73°37'12" W
34	*P. tripartita* var. *mollisima* common pink	Sutamarchán	5°38'3" N, 73°37'12" W
35	Graft *P. maliformis* (cholupa)-*P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54" N, 74°38'53" W
36	Graft *P. maliformis* (cholupa)-*P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54" N, 74°38'53" W
37	Graft *P. maliformis* (cholupa)-*P. edulis* f. *edulis* (gulupa)	Tinjacá	5°34'54" N, 74°38'53" W
38	Graft congolo-cholupa	Tinjacá	5°34'54" N, 74°38'53" W
39	*P. edulis* f. *edulis*	Tinjacá	5°34'54" N, 74°38'53" W
40	*P. ligularis*	Tinjacá	5°34'54" N, 74°38'53" W
41	*P. edulis* f. *edulis*	Buenavista	5°30'60" N, 73°57'59" W
42	*P. edulis* f. *edulis*	Buenavista	5°30'60" N, 73°57'59" W
43	*P. edulis* f. *edulis*	Buenavista	5°30'60" N, 73°57'59" W
44	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
45	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
46	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
47	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
48	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
49	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
50	*P. edulis* f. *edulis*	Miraflores	5°13'1" N, 73°8'47" W
51	*P. edulis* f. *edulis*	Miraflores	5°13'1" N, 73°8'47" W
52	*P. edulis* f. *edulis*	Miraflores	5°13'1" N, 73°8'47" W
53	Graft congolo-cholupa	Miraflores	5°13'1" N, 73°8'47" W
54	Graft congolo-cholupa	Miraflores	5°13'1" N, 73°8'47" W
55	Graft congolo-cholupa	Miraflores	5°13'1" N, 73°8'47" W
56	*P. edulis* f. *flavicarpa* purple fruit	Miraflores	5°13'1" N, 73°8'47" W
57	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
58	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
59	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
60	*P. edulis* f. *flavicarpa*	Miraflores	5°13'1" N, 73°8'47" W
61	*P. edulis* f. *flavicarpa*	Briceño	5°43'25" N, 73°56'24" W

Continuation Table 1.

Nr	Species	Municipality	Geographic coordinates
62	*P. edulis* f. *flavicarpa*	Briceño	5°43'25" N, 73°56'24" W
63	*P. edulis* f. *flavicarpa*	Briceño	5°43'25" N, 73°56'24" W
64	*P. edulis* f. *flavicarpa*	Tunungua	5°43'59" N, 73°55'59" W
65	*P. edulis* f. *flavicarpa*	Tunungua	5°43'59" N, 73°55'59" W
66	*P. edulis* f. *flavicarpa*	Tunungua	5°43'59" N, 73°55'59" W
67	*P. edulis* f. *flavicarpa* purple fruit	Tunungua	5°43'59" N, 73°55'59" W
68	Graft congolo-cholupa	Nuevo Colón	5°21'30" N, 73°27'38" W
69	Graft congolo-cholupa	Nuevo Colón	5°21'30" N, 73°27'38" W
70	Congolo in vitro	Nuevo Colón	5°21'30" N, 73°27'38" W

Molecular characterization

The molecular characterization was carried out in the Molecular Biology research laboratories of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Tunja. The protocol of Dellaporta et al. (1983) was used for the DNA extraction, which was visualized in 0.8% agarose gels prepared with 0.5% TBE in an electrophoresis gel system (EC 340, Maxicell Primo, Thermo Fisher Scientific, Waltham, Massachusetts, USA). The concentration was determined with a fluorometer (Dyna Quant 200, Hoefer, Holliston, Massachusetts, USA), diluted in HPLC water to a total volume of 100 mL at 10 ng mL^{-1} and stored at -20 °C. For the ISSR analysis, eight primers synthesized by Technologies Inc. Bioneer Corporation (Alameda, California, USA) were selected (Table 2) because they are highly polymorphic and efficient in determining intra and interspecific genetic diversity in genetic diversity in plants like *Passiflora* spp. (Oliveira et al., 2019; Vianna et al., 2019).

For the amplification, the cocktail was prepared in a sterile microcentrifuge tube (1.5 mL) for a final volume of 25 µL. Reaction components are indicated as final concentration. The reaction mixture was prepared with 1X buffer, 1.5 mM MgCl$_2$, 0.2 mM dNTPs, 1 U Taq Polymerase, 2 µM primer and 10 ng genomic DNA. The amplification was carried out in a programmable thermal controller thermal cycler (PTC-100, MJ Research, St. Bruno, Canada). The initial denaturation was 95 °C for 5 min; 37 cycles of denaturation at 95 °C for 30 s, and hybridization: 58 °C (GT, CGA), 50 °C (AG, CA, ACA), and 55 °C (CCA, TG, CT) for 45 s, with an extension of 72 °C for 2 min. The final extension was at 72 °C for 7 min. The amplification products were separated using electrophoresis in 3% high resolution agarose gels prepared with 1% TBE, at 100 V for 3 h and displayed on a transilluminator.

Statistical data analysis

A binary matrix of presence (1) and absence (0) was generated. The genetic similarity between the evaluated materials was calculated with the coefficient of Nei and Li (1979). Clustering analysis was performed using the UPGMA method, and a dendrogram was generated using the NTSYS statistical package (Numerical Taxonomy System for Personal Computer, version 2.02 PC, Setauket, New York, USA). The parameters of genetic diversity were estimated with the TFPGA program (Tools for Population Genetic Analysis, version 1.3, 1997, Miller, Northern Arizona University, Flagstaff, Arizona, USA), where the percentage of polymorphic loci and the heterozygosity were unbiased. The unbiased statistical "F" value was determined with a 95% confidence interval. The molecular variance analysis (AMOVA, Excoffier, Rutgers University, New Jersey, USA) was done with the GenAlEx 6.5 program (Peakal, Australian National University, Canberra, Australia).

Table 2. Primers used in the ISSR technique.

Primer	Sequence (5' to 3')
CCA	DDB(CCA)$_5$
CGA	DHB(CGA)$_5$
GT	VHV(GT)$_5$G
AG	HBH(AG)$_7$A
CT	DYD(CT)$_7$C
TG	HVH(TG)$_7$T
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 analysis with the Nei-Li coefficient (Nei and Li, 1979) at a similarity level of 0.60 showed that the first group mainly included the gulupa materials from the municipalities Firabitoba and Ramiriquí. No grouping by species was observed since the other gulupa materials from the municipality Miraflores were distributed in other groups (Figure 1). Group II, at a similarity of 0.50, was made up of eight materials, including granadilla, curuba red flower, gulupa and passion fruit, and was highly variable. Also, at a similarity of 0.50, Group III mainly contained the gulupa species from Miraflores and the calabash species from Miraflores and Turmeque, in addition to the white and red flower curuba. Group IV had all the passion fruit materials from the municipality of Tunungua, the white and red flower curuba of Umbita and Turmeque and the granadilla of Tinjacá. At a similarity of 0.60, Group V included the most passion fruit materials from different geographical origins (Miraflores, Briceño and Tunungua). Group VI had the most materials, especially gulupa and curuba from Turmequé, Sutamarchán and Tinjacá, at a similarity of 0.55. Finally, Group VII contained the materials that presented a lower degree of similarity (0.40), 15 (granadilla), 64 (maracuyá) and 41 and 42 (gulupa) from Ramiriquí, Tunungua and Buenavista, probably because the origin was wild or spreading distribution.

In general, the clusters reflected the phenotypic variability observed in these species (Torres et al., 2019). The similarity rates were not so high since they are allogamous species, with sexual reproduction preferred, so there was manifest variability with no consistent formation of groups in terms of geographical origin or species, but there was lax distribution of the materials in terms of the evaluated municipalities, with inter- and intraspecific variability that can be used to obtain new planting materials. The lack of group structuring according to the geographical origin or species probably derive from the self-incompatibility found in most *Passiflora* species and the cross-pollination (Ocampo et al., 2017). The presence of exotic species such as gulupa or curuba also made a great contribution to the total genetic diversity observed in each group (Figure 2).

The gulupa (*Passiflora edulis* Sims f. *edulis*) is a neotropical species, known as purple passion fruit. In Colombia it is cultivated between 1800 and 2400 m a.s.l. and its fruit is considered promising, with high potential in the Colombian agricultural and export market. Its flavor, appearance, nutritional value, availability, accessibility and medicinal properties, in addition to its exotic connotation, make it a product in high demand in the international market (Checa et al., 2011;

Figure 1. Dendrogram for the 70 *Passiflora* spp. genotypes based on the Nei-Li (1978) similarity coefficient and calculated with eight ISSR markers with the UPGMA, SAHN and TREE of NTSYs-pc (version 2.02g, 1998) classification methods.
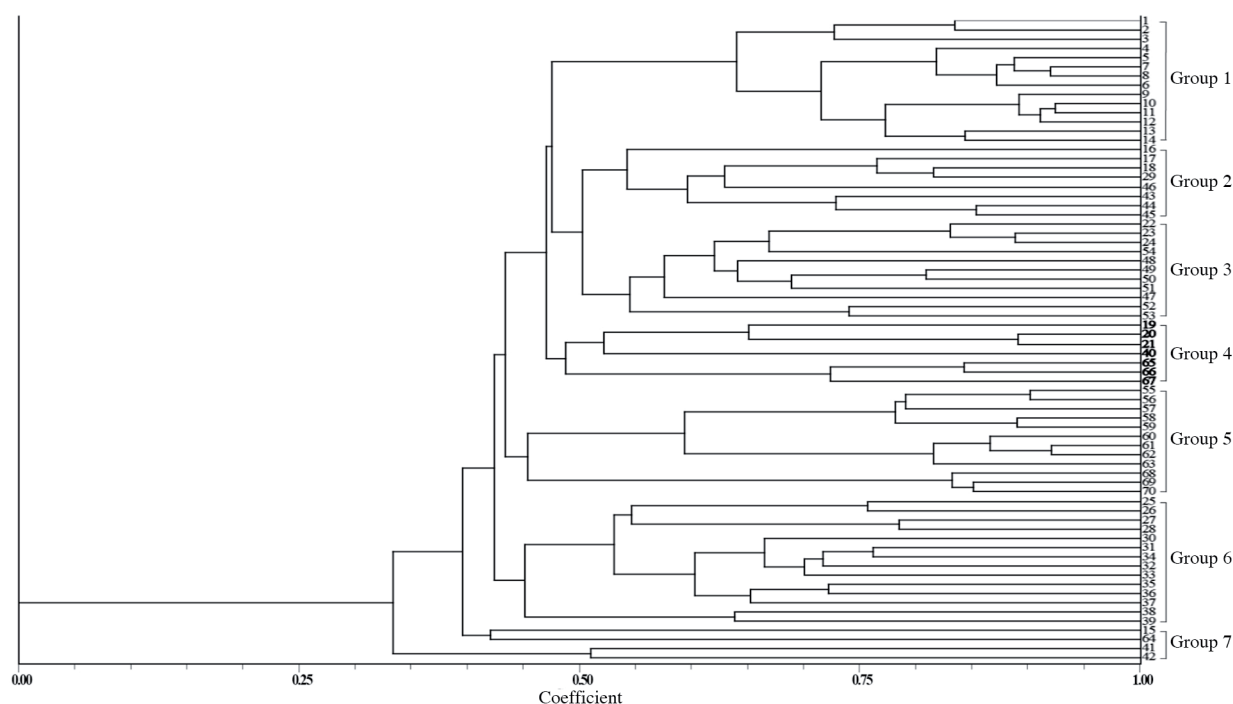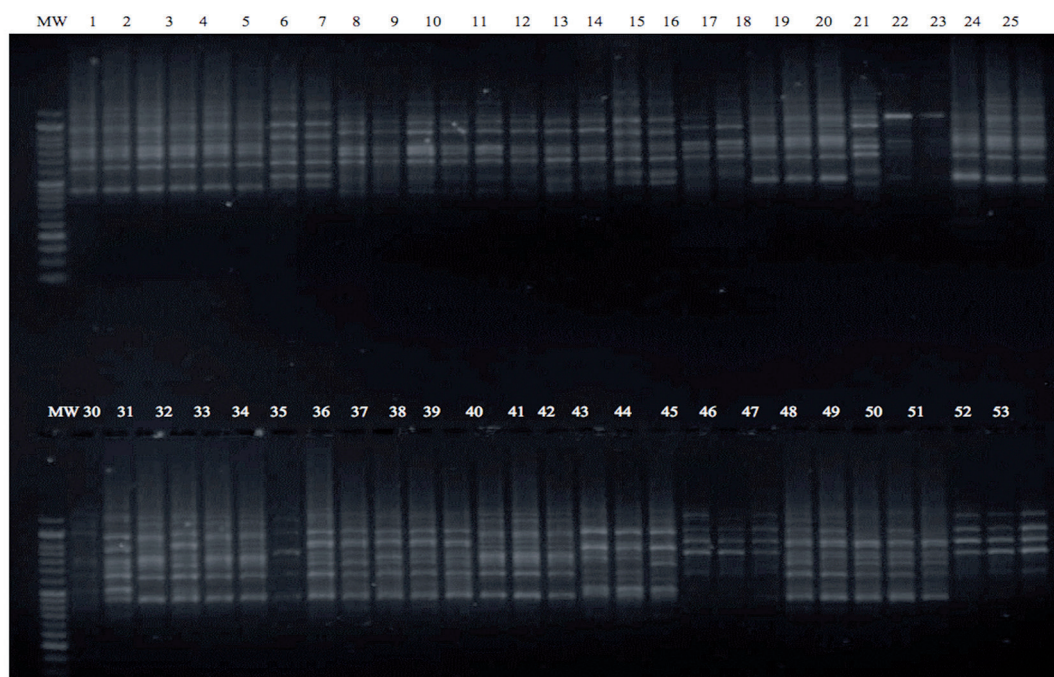
Figure 2. Amplification patterns generated by the ISSR TG in which inter and intraspecific variation in *Passiflora* material evaluated.

Meneses et al., 2019). On the other hand, curuba (*P. tripartita* var. *mollissima*) materials are characterized by a fruit tree native to the northern Andes, introduced to countries such as Chile, Mexico, New Zealand, Australia, and USA, classified as one of the best edible *Passifloras* spp. for its appetizing organoleptic characteristics and its high nutritional content. Additionally, it has nutraceutical importance given its high antioxidant potential (Meneses et al., 2019).

The coefficient of cofenetic correlation was 75%, demonstrating a relationship between the genetic distances and the groups. Similar results were obtained by Batista et al. (2017) when evaluating genetic diversity in *P. cincinnata* accessions based on morphological descriptors and molecular markers and by Oliveira et al. (2019) when evaluating the genetic diversity of *Passiflora* spp. using ISSR and RAPD markers. Sousa et al. (2015) used ISSR markers in wild species of *Passiflora* spp. as a tool for the selection of taxa in the improvement of ornamentals and found that, despite a high coefficient of cofenetic correlation (r = 0.94) that suggested fidelity in the representation of the dendrogram, some taxa were distributed without taxonomic support; therefore, the species were separated into different groups.

The average value of Nei-Li genetic diversity was 0.40, consistent with that expected for cross-pollinated species and with the values observed in studies on genetic diversity in *Passiflora* (Santos et al., 2011; Oluoch et al., 2018; Oliveira et al., 2019; Vianna et al., 2019). Ocampo et al. (2017), when evaluating the diversity and genetic structure of *P. edulis* f. *flavicarpa* in Colombia with microsatellites, found an average genetic distance of 0.683 and that the structuring of the groups was not consolidated and could vary, just as in this study it was observed that the internal branches of the dendrogram were moderately compatible (bootstrap ≥ 50%) and ratified the grouping between some materials of the same department. The relationship between individuals of the same species was low and did not present a geographical pattern according to the origin of each material. In most cases, this instability occurs when some individuals show intermediate similarity values compared to other groups when they are grouped and, therefore, are not assigned to a specific one and can belong to several compound branches (Oliveira et al., 2019).

The eight ISSR markers (Table 2) used to characterize the genetic diversity of *Passiflora* materials from the Department of Boyacá produced a total of 138 bands. The number of bands per primer varied from 10 for CA to 24 for GT, with molecular weights between 250 and 1320 kb (Table 3). The number of bands obtained in this study was adequate for estimating genetic parameters when compared to other studies on genetic diversity in *Passiflora* where these markers have been used (Sousa et al., 2015; Oliveira et al., 2019; Vianna et al., 2019). Considering the high rate of dinucleotide SSR describe in others studies for *Passiflora* spp. (Santos et al., 2011; Sousa et al., 2015) we chose the ISSR markers

Table 3. Percentage of polymorphic loci and average expected heterozygosity (He) for the eight ISSRs evaluated in *Passiflora* materials.

Primer	Number of bands	Polymorphic loci %	He	FST	Standard deviation
ACA	18	72	0.56	0.41	0.04
AG	20	90	0.58	0.21	0.01
CA	10	70	0.28	0.35	0.02
CCA	18	61	0.51	0.43	0.04
CGA	16	50	0.57	0.41	0.04
CT	14	71	0.38	0.38	0.02
TG	18	56	0.54	0.47	0.03
GT	24	79	0.48	0.30	0.02
Total	138	75	0.56	0.16	0.01

FST: Genetic differentiation coefficient.

to characterize the genetic diversity present in the evaluated materials. The percentage of polymorphic loci for the eight ISSR primers was between 56% (TG) and 90% (AG), with an expected average heterozygosity of 0.17 to 0.38 for the CGA and AG primers, respectively (Table 3). The first TG showed a greater genetic differentiation between the materials, with a genetic differentiation coefficient (FST) of 0.47, which means that it can be useful for studies on genetic diversity in *Passiflora* (Figure 2).

For the total population, the percentage of polymorphic loci and the expected average heterozygosity (He) were 75.4% and 0.56, respectively (Table 3), because of the genetic nature of the materials, the type of marker, and the genome coverage, among others. The FST obtained when evaluating the *Passiflora* materials with the eight ISSR markers was 0.16, with a standard deviation of 0.01 (Table 3). According to Wright (1978), values greater than 0.25 show great genetic differentiation, so there was not a population genetic structure in the evaluated materials.

The AMOVA showed that 64% of the variation observed in the evaluated *Passiflora* materials was explained by the component within the groups, and 36% was explained by the differentiation between the groups, so it is necessary to consider subdivision levels and hierarchy greater than those contemplated in this study, in addition to the high levels of variation at the intraspecific level, which should be used for management and conservation strategies of this plant genetic resource (Table 4), which has also been reported in other genetic diversity studies on *Passiflora* using this marker (Ocampo et al., 2017; Oluoch et al., 2018; Fonseca-Trujillo et al., 2019). In interspecific analyses, a higher polymorphism rate in combination with a higher average number of bands amplified per primer is indicative of high genetic diversity (Cerqueira-Silva et al., 2012). However, there was also high intraspecific genetic diversity among the evaluated *Passiflora* materials.

In studies on genetic diversity in *Passiflora*, it has been found that ISSR markers are highly polymorphic and informative (Batista et al., 2017; Oliveira et al., 2019; Vianna et al., 2019); in addition, heterozygosity values greater than those found in this study have been reported by Lougon et al. (2014) when evaluating the genetic diversity in the *Passiflora* genus with SSR markers, finding an average heterozygosity of He = 0.57. These values show broad genetic variability in the *Passiflora* genus. Similar values were found by Cerqueira-Silva et al. (2012), who evaluated *P. cincinnata* accessions and obtained He = 0.51, and Cazé et al. (2012), who used seven microsatellite loci to study the genetic structure.

In Colombia, genetic diversity studies on *Passiflora*, such as those carried out by Fonseca-Trujillo et al. (2009), who used ISSR markers, have characterized the cultivated gulupa materials collected in the Departments of Boyacá, Cundinamarca and Huila, finding that these molecular markers were efficient at detecting polymorphisms in this species (89%), evidencing broad genetic diversity that is distributed in the areas where gulupa is grown. The gulupa materials evaluated in this study showed high genetic diversity, probably because of the propagation method, origin and short time of establishment of these crops in this country.

Table 4. Molecular variance analysis for groups formed with the eight ISSR markers.

Source	DF	SS	MS	SV	%
Between group	10	677.33	67.73	8.62	36
Within group	59	909.15	15.41	15.40	64
Total	69	1586.48		24.05	

DF: Degrees of freedom; SS: sum of squares; MS: middle square; SV: standard variation.

Ocampo et al. (2017), in the study on the diversity and genetic structure of passion fruits in Colombia using microsatellite markers, found that six microsatellite loci amplified a total of 58 alleles, including nine unique and 31 rare ones. The diversity indices showed a polymorphic information content (PIC) of 0.74, an average observed heterozygosity of Ho = 0.52 and an expected He = 0.78, evidencing a poor geographical structuring and high diversity in the accessions, which could be explained by the allogamy and the constant exchange of seeds among farmers.

Studies on collection and morphoagronomic characterizations in *Passiflora* in Colombia have demonstrated the wide phenotypic variation that exists in cultivated and wild materials, as reported by Checa et al. (2011), who collected and morphoagronomically characterized 91 materials of the subgenus *Tacsonia* and eight materials of the subgenus *manicata*, finding that the characteristics that contributed most to the phenotypic variation were associated with the fruit and the phytosanitary state of the materials. Similar results were obtained by Ocampo et al. (2013) in the exploration of the genetic variability of passion fruits as a basis for a breeding program in Colombia, in which quality parameters were taken into account and eight elite accessions from Caldas, Valle del Cauca and Antioquia were identified. The classification analysis showed high variability, with little structuring by geographical origin. Galeano et al. (2018), when characterizing the production and physicochemical variables of 60 passion fruit accessions conserved at the Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), obtained results from the grouping and principal components that showed broad genetic diversity in the entire collection without any population substructure, as seen in this study 30 promising accessions were identified as parental for the first cycle of recurrent selection. These results are the basis for initiating a process of genetic improvement from the superior genotypes of the elite accessions identified. However, these materials have not been identified for the Department of Boyacá, which is why this study, together with morphoagronomic and quality data, is vital to starting a breeding program that leads to new and better materials.

In this study, it was found that there is high genetic diversity among the *Passiflora* materials grown in the Department of Boyacá. Most of these materials showed different genetic backgrounds and were probably derived from a number of introductions from Hawaii (USA) and Brazil that started in the 1960s, along with the flow of genes through seed exchanges between farmers with different geographical origins and the allogamy of these species, resulting in an increase in allele distribution between different accessions; as well as its way of reproduction, since in general terms the species belonging to the genus Passiflora are spread asexually through grafts, cuttings and tissue culture in vitro; and sexual route from the seed, however the latter is the most used method in most of these species in Colombia (Faleiro et al., 2019). This is consistent with the results found in others, such as *P. peruviana* (Morillo et al., 2018), who argued that genetic polymorphism can be associated with the allogamous nature of the species and the exchange of seeds between producers, which tend to favor the conservation of a high percentage of heterozygous genotypes.

Knowledge on inter- and intraspecific genetic diversity provides useful information for effective conservation, management of plant genetic resources and breeding programs (Oluoch et al., 2018). Variability between genotypes is important for breeders in the selection of high-yield varieties, so the existence of variability among individuals is a prerequisite for the production of new varieties aimed at improving crop productivity, with the ability to resist the impacts of biotic or abiotic stress (Oliveira et al., 2019).

CONCLUSIONS

The ISSR markers allowed to determine the existence of an important genetic variability in the *Passiflora* spp. material collected in the department of Boyacá, which is presented mainly at the intraspecific level, which suggests higher levels of hierarchy and subdivision than those considered in this study.

There is a genetic potential in the evaluated material that must be exploited in conservation and plant breeding strategies of *Passiflora* spp. in the main producing municipalities of the department of Boyacá.

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