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Genetics and plant breeding

Molecular characterization of a species in the genus *Rubus* in Boyacá, Colombia

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Abstract – Colombia is home to blackberry genetic resources which present a morphological diversity. The relevant characteristics related to its diversity are the presence of prickles, the shape of its leaves, the number and color of its fruits, and its enormous agro-industrial potential due bioactive compounds such as polyphenols. These plants can grow between 1,700 and 3,400 m asl and are cultivated in the central region of the country. The study evaluated 13 wild and cultivated plants from the genus *Rubus*. A molecular characterization was carried out using 16 SSR microsatellite markers, all of which produced positive amplification generating 23 loci and 26 alleles. The AMOVA indicated a molecular genetic differentiation of 23% between the groups which corresponded to the geographic location of the sample. The greatest contribution to variance is found within the groups (76%), possibly because each of them is composed of different cultivated species and wild relatives of the genus *Rubus*. This suggests that the grouping of the genotypes studied doesn't necessarily correspond to geographical origin. However, the findings show high genetic variation, with an F_{st} value of 0.27. This may be useful in breeding programs where genetic diversity, morphological characteristics of the fruits, and the molecular identification of the fruits are taken into account.

Index terms: SSR, genetic diversity, variability, heterozygosity, and alleles.

Caracterização molecular de espécies do gênero *Rubus* em Boyacá, Colômbia

Resumo –A Colômbia conta com recursos genéticos de amora que apresentam ampla diversidade morfológica. Entre os caracteres mais relevantes relacionados com sua diversidade, encontram-se a presença de aguilhões, a forma das folhas, o número e a cor dos frutos, e seu enorme potencial agroindustrial devido a seu dulçor e a seus compostos bioativos, como os polifenóis. Estas plantas podem crescer entre 1.700 e 3.400 metros acima do nível do mar. No presente estudo, avaliaram-se 13 materiais silvestres e cultivados do gênero *Rubus*. Realizou-se sua caracterização molecular mediante 16 marcadores microssatélites SSR, dos quais, todos produziram amplificação positiva, gerando 23 lócus e 26 alelos. A AMOVA indicou uma diferenciação genética molecular de 23% entre os grupos, os quais correspondem à localização geográfica da amostra, e o maior aporte à variância está no interior dos grupos (76%), possivelmente devido a que cada um deles está composto por diferentes espécies cultivadas e parentes silvestres do gênero *Rubus*. Isto sugere que a agrupação dos genótipos estudados não necessariamente corresponde à origem geográfica. Não obstante, as descobertas evidenciam alta variação genética, com o valor F_{st} de 0.27, a qual pode ser útil em futuros programas de fitomelhoramento, nos quais se tenham em conta a diversidade genética, as características morfológicas dos frutos e a identificação molecular das cultivares.

Termos para indexação: SSR, diversidade genética, variabilidade, heterocigocidade e alelos.

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Introduction

There are between 750 and 800 species of the genus *Rubus*, including raspberry, blackberry, some native species, and other species considered invasive (CLARK et al., 2013; MORENO-MEDINA et al., 2018). However, this genus of plants is difficult to identify due to its frequent hybridization and apomixis. This leads to cultivars of similar morphology which makes testing for SSR markers (Simple sequence repeats) in this genus of plants of enormous interest (LÓPEZ et al., 2019). This procedure has contributed to the selection of species that present agronomic potential, provide reliable identification, and allow the genetic diversity of populations to be measured (ROA et al., 2014; MARULANDA et al., 2012).

In Colombia, the most important commercial cultivar is the blackberry (*R. glaucus*), however, there are other species such as *R. alpinus*, *R. alutaceus*, *R. floribundus*, *R. bogotensis*, *R. giganteus*, *R. megalococcus*, and *R. nubigenus* which can grow between 1,700 and 3,400 meters above sea level. Departments such as Antioquia, Boyacá, Caldas, Cauca, Cundinamarca, Santander, and Valle del Cauca all contribute to the national production of blackberries throughout the year; however, most of the crops established in these regions do not have suitable agronomic management. These problems include adjustments to the growth and fruiting habits of the species (MORENO-MEDINA et al., 2016) and the occasional lack of pre and postharvest management with the purpose of improving yields or increasing the quality of the fruit (MORALES; VILLEGAS, 2012; MORENO – MEDINA; DEAQUIZ, 2016; MORENO – MEDINA et al., 2020). Colombia has valuable genetic resources for blackberries in its cultivars and natural populations. This includes a wide morphological diversity such as prickles, the shape of the leaves, the number and color of the fruits, and an enormous agro-industrial potential due to its sweetness and phenolic compounds.

The genetic diversity and identification of any species can be measured through morphological descriptors and molecular markers. Morphological descriptors have some limitations including their complexity, access to taxonomic keys, subjective results due to plant phenology and environmental factors, and inaccurate information. (CASTILLO et al., 2010). Molecular markers represent a fast, objective method, use simple techniques, are not affected by environmental conditions, are used in plants regardless of their phenology, and are efficient when discriminating genotypes in the same species (FAQIR et al., 2017). Using molecular markers for the characterization of a species are a useful instrument to describe morphologically similar individuals with agronomic potential that present a certain capacity to adapt to climate change (EL KADRI et al., 2019).

SSR markers (Simple Sequence Repeats) are small sequences of DNA repeated in tandem which are widely used in plants to perform a genetic diversity analysis. These markers have codominant characteristics and a high level of allelic diversity at different loci (KALIA et al., 2011). In the case of the *Rubus* genus, microsatellites (SSR) have been used to perform a polymorphism analysis to evaluate the genetic diversity of raspberry cultivars with different fruit colors using structural genes and regulators of flavonoid biosynthesis (LEBEDEV et al., 2019). Studies were performed to identify the genetic diversity of cultivars in different locations in Venezuela (ROA et al., 2014) and from 32 *Rubus* cultivars in Europe and the United States with differences in ploidy and berry color. Additionally, LÓPEZ et al. (2019) recently evaluated the genetic diversity of parental plants in Colombia, which are used in the process of reproduction or development of parental populations, based on their morphology and tolerance to *C. gloeosporioides*.

The objective of this study is geared towards the molecular characterization of the species in the genus *Rubus* located in Colombia. The genetic information provided by wild and cultivated blackberries species found in province of Alto Ricaurte, in Boyacá, Colombia would provide numerous benefits. These benefits include promising production alternatives, recovering cultivation areas, increasing employment in rural areas, and establishing species with adequate agronomic management and minimum environmental impact.

Materials and methods

Processing and analysis of samples: The 13 samples used in this characterization were taken from the completely expanded leaves of cultivated and wild blackberry species collected in the municipalities of Arcabuco and Gachantivá, Colombia (Table 1). The samples were conserved on silica gel for processing and were sent to the Plant Biotechnology Laboratory at the Universidad Tecnológica de Pereira. Two more samples corresponding to the species *Rubus glaucus* Benth were added as an external group (outgroup).

Table 1. Samples processed for the molecular characterization of wild and cultivated species of the genus *Rubus* including location, altitude, and common name.

Code	Locality	Coordinates	Altitude (masl)	Species
A-1BL	Gachantivá	5° 45’ 11.62’’N 73° 32’ 15.04’’W	2504	*R. alutaceus*
A-2GL	Gachantivá	5° 44’ 21.7’’N 73° 50’ 23.1’’W	2507	*R. glaucus*
A-3GL	Gachantivá	5° 46’ 11.5’’N 73° 30’ 46.5’’W	2674	*R. glaucus*
A-4AL	Gachantivá	5° 44’ 30.4’’N 73° 33’ 69.4’’W	2399	*R. alpinus*
A-5AL	Gachantivá	5° 77’ 10.8’’N 73° 33’ 53.7’’W	2740	*R. alpinus*
B-6AL	Arcabuco	5° 44’ 1.84’’N 73° 29’ 2.30’’W	2496	*R. alpinus*
B-7AL	Arcabuco	5° 44’ 1.96’’N 73° 29’ 1.04’’W	2544	*R. alpinus*
B-8GL	Arcabuco	5° 44’ 0.10’’N 73° 29’ 2.55’’W	2486	*R. glaucus*
B-9GL	Arcabuco	5° 44’ 1.96’’N 73° 29’ 1.04’’W	2544	*R. glaucus*
A-10BS	Gachantivá	5° 46’ 11.4’’N 73° 27’ 8.65’’W	2568	*R. alutaceus*
A-11S	Gachantivá	5° 46’ 27.55’’N 73° 30’ 2.88’’W	2723	*Rubus* sp.
A-12R	Gachantivá	5° 45’ 11.62’’N 73° 32’ 15.04’’W	2504	*R. alpinus*
B-13S	Arcabuco	5° 44’ 9.56’’N 73° 29’ 4.44’’W	2429	*Rubus* sp.
UTP1	*Rubus glaucus* - Outgroup			
UTP7	*Rubus glaucus* - Outgroup			

DNA extraction was performed exclusively on healthy leaf tissue using the commercial Plant DNeasy Mini kit (QIAGEN, Venlo, Netherlands) following the manufacturer’s instructions.

Amplification: Homologous SSR markers developed by the Universidad Tecnológica de Pereira for the *R. glaucus* species and heterologous SSR markers developed for other species of the *Rubus* genus were used. The amplification reactions were carried out using the conditions described by MARULANDA et al. (2012) and a “touchdown” amplification profile of 32 cycles with denaturation at 95°C for 1 minute. The annealing procedure lasted 1 minute, with a decrease in temperature of 1°C every two cycles from 63°C to 58°C and with 10 cycles at 59°C and 10 cycles at 58°C. The elongation procedure was carried out at 72°C for one minute. The amplified products were separated by electrophoresis in 6% denaturing polyacrylamide gels, which were run in a vertical Sequi-Gen Sequencing Cell chamber (BioRad, Hercules, California, USA) at 110 W and 50°C for an hour. The gels were stained with silver nitrate according to the protocol developed by BENBOUZA et al. (2006).

Microsatellite markers: The markers used for this characterization are shown in table 2. Seven of them were homologous and coded by CL and Rg, used by LÓPEZ et al. (2019); MARULANDA et al. (2012) and developed by the Universidad Tecnológica de Pereira. Four microsatellite markers were used from the “Rubus” series, developed by CASTILLO (2006) for the species *R. idaeus* and the cultivar called Marion corresponding to a *Rubus* hybrid. Three microsatellite markers from the RhM and RiM series developed by GRAHAM et al. (2002, 2004) were also used for *R. idaeus*. Finally, 2 microsatellite markers from the mRaCIRRI series developed by AMSELLEM et al. (2001) were used for the species *R. alceifolius*.

Table 2. List of SSR markers used for the molecular characterization of species of the genus *Rubus* in two municipalities of Boyacá, Colombia.

SSR Markers	Motif	Primer Pairs	GenBank Accession Number	Reference
CL1891.Contig3_All_166_1	(GCA)7	F-GAGGGAGAGATTTGGAGATGAAT R-GTGCCATAAGCTTACAGGTTCAG	MH516344	LÓPEZ., et al, 2019
CL1366.Contig2_All_134_1	(GAA)6	F-AAGGATGATTGTCACGTATGAGG R-ACTCGGCAATCCATTCTCTATTT	MH516340	LÓPEZ, et al., 2019
CL2455.Contig1_All_192_1	(AGC)6	F-AGCTTGGACTGTGAACAAGGAT R-CAACAATCACCAACCCAAGAC	MH516347	LÓPEZ, et al., 2019
CL2455.Contig1_All_193_1	(AAG)7	F-CAGATTTCAGCCAAGAAGAGGTT R-CGATCTCCTTCTTCTTCCTCTTT	MH516348	LÓPEZ, et al., 2019
CL2364.Contig3_All_186_1	(GTGGTA)4	F-CCAAACATGAAATCAGTAGGGAA R-TCATAAGAGGGCCATAAGAATGA	ND	LÓPEZ, et al., 2019
CL274.Contig3_All_22_1	(TGT)6	F-CTGTTGTTATCGCTGTTGTTGAT R-AGAGACCTTGTGAAGGAGTGGTT	ND	LÓPEZ et al., 2019
Rubus 105b	(AG)8	F-GAAAATGCAAGGCGAATTGT R-TCCATCACCAACACCACCTA	ND	CASTILLO, 2006
RiM017	(TG)6	F-GAAACAGGTGGAAAGAAACCTG R-CATTGTGCTTATGATGGT TTCG	FJ194453	GRAHAM et al., 2002, 2004
mRaCIRRIV2A8	(CA)12(CT)11 F	F-TAAAAAGGCGCAACAGTCG R-AGACACAGAAACAGGCATCG	AF205117	AMSELLEM et al., 2001
mRaCIRRIH3	(GT)15(GA)17	F- CTGGATGTGTGGGTGTGTATC R- CCTGGATATGTTTACCCTGACC	AF205117	AMSELLEM et al., 2001
RgA12-2	(AC)8	F-GCGGGCATTCTCTTGCTTAC R-GCGGTTCGTGACTCAGACAG	HQ637525	MARULANDA et al., 2012
Rubus251A	(GA)10	F-GCATCAGCCATTGAATTTCC R-CCCACCTCCATTACCAACTC	ND	CASTILLO, 2006
Rubus107A	(AG)8	F-GCCAGCACCAAAAACCTACA R-TTTCACCGTCAAGAAGAAAGC	ND	CASTILLO, 2006
Rubus98d	(GAA)5-(GA)10F	F-GGCTTCTCAATTTGCTGTGTC R-TGATTTGAAATCGTGCGGTTA	AF205116	CASTILLO, 2006
RhM011	(TC)18	F-AAAGACAAGGCGTCCACAAC R-GGTTATGCTTTGATTAGGCTGG	FJ194446	GRAHAM et al., 2002, 2004
RhM021	(TC)6	F-CAGTCCCTTATAGGATCCAACG R-GAACTCCACCATCTCCTCGTAG	FJ194448	GRAHAM et al., 2002, 2004

Statistical Analysis

Measures of diversity, variability, and genetic distance were determined using the GenAlEx 6.5 program (PEAKALL and SMOUSE, 2012). A cluster analysis was performed using the paired group algorithm while the Dice similarity coefficient was calculated using the PAST tool (Paleontological Statistics Software Package for Education and Data Analysis) (HAMMER et al., 2001). A population analysis was carried out while taking into account that the samples belong to two different localities (A- Gachantivá and B- Arcabuco).

Results and discussion

The molecular markers showed positive amplification with the 16 SSRs used, generating 23 loci and 26 alleles, seven of which amplified two loci. These samples were analyzed using SSR markers from other species of the *Rubus* genus (heterologous markers) and showed positive polymorphic amplification. The average number of alleles was 4.2 and approximately 3 effective alleles were present (table 3). The results are similar to what was found

by LÓPEZ et al. (2019) who reported similar genetic diversity parameters in blackberry (*R. glaucus*) cultivars. These authors found 29 loci and 58 alleles, with 3.4 effective alleles which were very close to those found in this study. LEBEDEV et al. (2019) analyzed the genetic diversity of 21 raspberry cultivars (*R. idaeus* and *R. occidentalis*) with SSR. A total of 26 alleles with an amplification of up to nine alleles per locus with an average of 3.7 alleles were found from their study. The similarity of results from previous studies, the current study, and those reported by CASTILLO et al. (2010) and MARULANDA et al. (2012) suggest that there is an adequate transferability or positive response of the markers (SSR) used to characterize the polymorphisms of wild and cultivated species of the *Rubus* genus in different geographic locations. These findings may be used in genetic improvement programs to encourage agronomic success.

Table 3. Genetic diversity parameters in wild and cultivated species of the genus *Rubus*, from the 16 evaluated SSR markers. **N:** Number of alleles; N_a: Number of polymorphic alleles; N_e**:** Number of effective alleles; H_o**:** Observed heterozygosity; H_e**:** Expected heterozygosity; F_{st}**:** Fixation index.

SSR Marker	Number of Loci	N	N_a	N_e	H_o	H_e	F_{st}
CL1891.Contig3_All_166_1	1	4.3	4.7	3.4	0.7	0.5	0.351
CL1366.Contig2_All_134_1	1	5.0	3.7	3.0	0.8	0.6	0.241
CL2455.Contig1_All_192_1	1	5.0	3.3	2.9	1.0	0.6	0.253
CL2455.Contig1_All_193_1	1	3.3	2.7	2.0	0.4	0.5	0.278
CL2364.Contig3_All_186_1	2	4.0	2.0	1.6	0.3	0.3	0.296
CL274.Contig3_All_22_1	2	4.5	3.5	3.0	0.8	0.6	0.176
Rubus 105b	2	4.5	3.3	2.8	0.8	0.6	0.140
RiM017	2	5.0	4.0	3.2	0.9	0.7	0.185
mRaCIRRIV2A8	2	4.7	4.0	3.5	0.6	0.5	0.387
mRaCIRRIH3	2	4.2	3.2	2.4	0.7	0.5	0.287
RgA12-2	1	4.0	3.7	2.7	0.7	0.6	0.204
Rubus251A	2	3.8	3.3	2.7	0.7	0.5	0.321
Rubus107A	1	3.7	4.0	3.4	0.7	0.5	0.423
Rubus98d	1	4.0	2.7	2.3	0.9	0.6	0.115
RhM011	1	3.7	4.3	3.5	0.6	0.5	0.436
RhM021	1	4.0	3.3	2.9	0.6	0.5	0.273
Average		**4.275**	**3.435**	**2.812**	**0.696**	**0.538**	**0.268**

The observed heterozygosity (H_o) showed values between 0.333 and 1.0, while the expected heterozygosity (H_e) ranged between 0.305 and 0.662 with an average of 0.697 and 0.538 respectively. The H_o values for these markers were greater than the H_e values which coincide with what was reported by CASTILLO et al, (2010), LÓPEZ et al, (2019), and MARULANDA et al, (2012) who found the same trend for cultivars and wild species of the genus *Rubus*. The findings from the current study differ from those found by WANG et al. (2017) and LEBEDEV et al. (2020), which found that the H_e was higher than H_o in two types of wild Chinese brambles of the genus *Fragaria* and *Rubus*, in 32 cultivars from Europe and the United States respectively. Consequently, it can be inferred that there is excess genetic variability in the evaluated individuals since there is a higher value in the observed heterozygosity, compared to the expected heterozygosity. This information can be used to understand the morphological diversity of the species in the area where the studied samples come from, particularly in relation to the number of leaves and the color of the fruits.

With the use of the marker RhM011, the greatest contribution to genetic differentiation was observed with an F_{st} value of 0.436. This indicates its importance in the search for differences between the genotypes studied. This result agrees with the study from DOTOR-ROBAYO et al. (2016) who found a marker (F_{st} 0.53) that was useful to differentiate the ecotypes collected in seven municipalities in Boyacá, Colombia, using RAMs. In the current study, the fixation index (Fst) obtained when evaluating cultivated and wild materials from the localities of Arcabuco and Gachantivá had an average value of 0.273. This is an indicator of high genetic diversity as mentioned by WRIGHT. (1978). Some of the plant material collected for the current study is asexual which almost guarantees low genetic diversity (Hu et al., 2012). The wild and cultivated ecotypes collected from the study area must be conserved and taken into account for future plant breeding programs.

The estimation of genetic diversity using the Dice index allowed the construction of a dendrogram, represented in Figure 1. The 15 genotypes studied including the reference groups (UTP-UTP7), were grouped without strictly depending on the collection site. The groups of plant material included cultivated and wild species whose similarity ranged between 35 and 80%. This indicates that there is genetic diversity among the tested materials which is necessary in various genetic improvement programs. Research carried out by LÓPEZ et al. (2019) in six departments of Colombia reported similarities ranging between 32 and 88%. Research carried out by MARULANDA et al. (2012) found molecular similarities of between 49 and 100% with the species from the genus *Rubus* in eight Colombian departments.

The cluster analysis separated the genotypes into three groups, which were analyzed from the bottom up in figure 2. The first group was made up of four cultivated species (A-5AL; A-3GL and A-1BL) and one wild species (A -10BS). The color of fruit was different with a node of similarity close to 83% with species that produce a yellow-whitish fruit (A-1BL and A-10BS). The second group consisted of five genotypes (A-4AL; B-7AL; B-6AL; A-12R and A-11S), of which four correspond to locally grown plant materials (*R. alpinus*) and one wild species. The node with the greatest genetic similarity in this group is represented by samples A-12R and B-6AL even though they differ in the color of the branches (red and purple). The third group is made up of three cultivars (*R. glaucus*) and a wild species (B-12S; B-9GL; B-8GL and A-2GL). The node with the greatest similarity in this group is made up of cultivated species with similar morphological characteristics (B-9GL and B-8GL) which had a similarity of 73%.

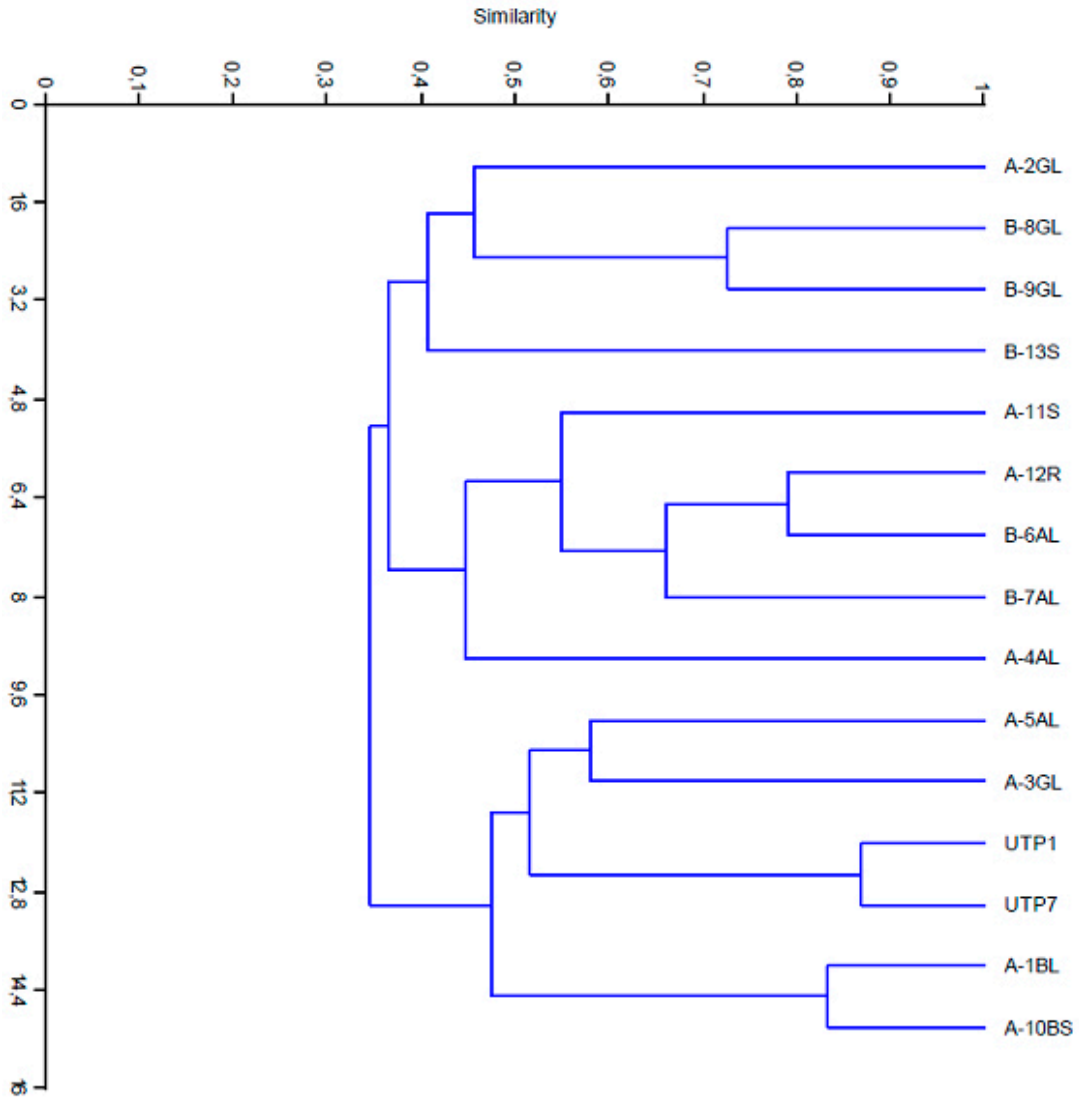

Figure 1. Cluster analysis with a paired algorithm for the species of the genus *Rubus* studied using the Dice distance index. Two outgroups are included as reference samples.

As described above, the mixture of the genotypes studied, the distribution of the groups, and the behavior of the nodes with greater genetic similarity could be explained through the exchange of plant material propagated by asexual methods which occurs in the area of this study. This occurs as a result of geographical proximity which is similar to what was reported in other departments of Colombia as mentioned by LÓPEZ et al. (2019). It is interesting to note the contribution of wild species to the genetic diversity of each of the groups formed. In figure 1, the samples of the wild species: A-10BS, A-11S, and B13S are located in the three groups contributing to the differentiation of the studied populations and their genetic relationships. This suggests that the genetic diversity of cultivars can be influenced by wild populations which is important due to its wide distribution in the studied localities and can stimulate the transfer of genes through pollination (DOTOR-ROBAYO et al., 2016).

Reference samples UTP1 (resistant to *Colletotrichum gloeosporioides*) and UTP7 (moderately resistant to *C. gloeosporioides*) highlight the differences that exists between the plants cultivated in the department of Boyacá with those found in the regions such as Risaralda and Quindío. These results may be useful for recommendations to progenitors and to initiate plant breeding processes at the national level. This is especially important since genetic distance and morphological characteristics such as the presence or absence of prickles, fruit qualities, and tolerance to phytopathogens must be considered before the development of cultivars in various areas of the country can begin (LÓPEZ et al., 2019).

An important finding which contributes to the wealth of knowledge about the genetic resources available in the Alto Ricaurte Province in Boyacá is based on the group with a high genetic similarity (Figure 2) created by cultivated *R. alutaceus* (A-1BL) and wild (A-10BS). This species was reported for the first time by MORENO-MEDINA et al. (2020) and has specific morphological characteristics that are unique to other materials grown conventionally or present in the wild. The fruits of this species are yellow-whitish in color, with a sweetness of 8.8 ° Brix. According to MORENO-MEDINA et al. (2020), this species is intended to be projected as a production line of agricultural interest for Boyacá.

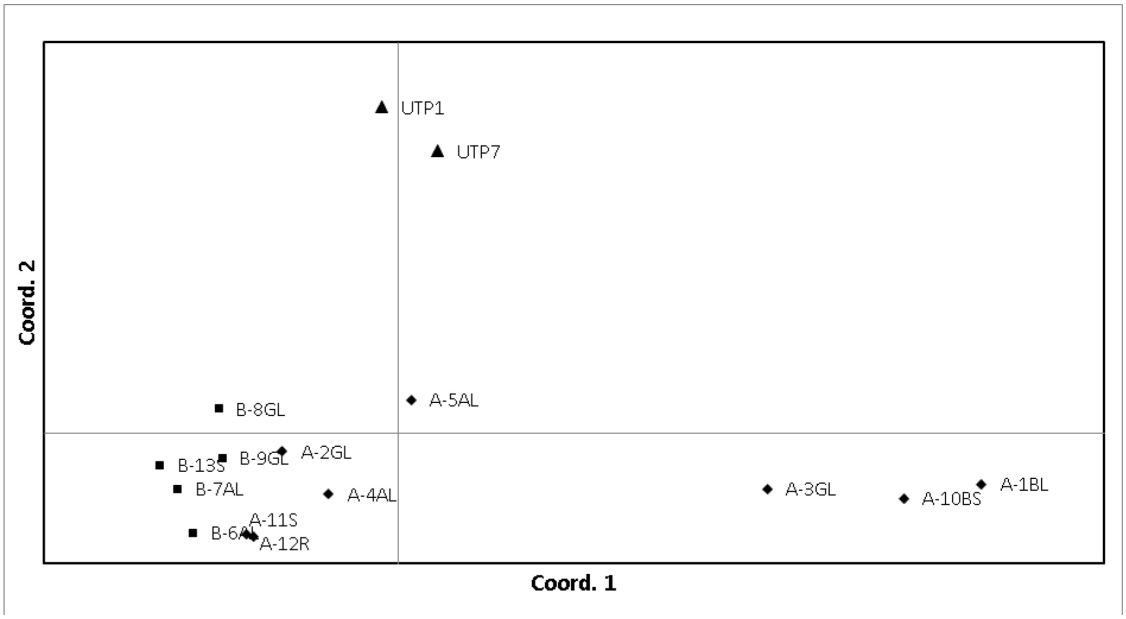

Figure 2. Analysis of principal coordinates for the species of the genus *Rubus* studied using the Dice distance index (includes Outgroup). The description of each code is found in Table 1.

The principal coordinate analysis was explained using two components which represented 39.21% of the total genetic variability. Some similarities were observed in relation to the cluster analysis in figure 1 including the weak grouping of species depending on the collection site. In figure 2, three groups can be seen with the first one located in the lower right part of the graph representing the greatest genetic distance. Additionally, within the morphological characteristics of the species that make up this group are purple fruits and wild and cultivated species that have yellow-whitish fruits (*R. alutaceus*). The second group includes a wild species and the cultivated genotypes characteristic of the area known until now as *R. alpinus*. The third group consists of cultivated plants from *R. glaucus* and a wild species. It is important to highlight that the reference groups (Outgroup) used in the current trial correspond to the *R. glaucus* species that are resistant to *Colletotrichum gloeosporioides* and moderately resistant to *C. gloeosporioides*. The current and former studies on this topic allow the consolidation of a group of cultivars that can be utilized to create progenitors in the future to obtain plant populations with high genetic gain (LÓPEZ et al., 2019).

Table 4. Results of the analysis of molecular variance for cultivated and wild species of the genus *Rubus*.

Source	df	SS	MS	Est. Var.	(%)
Between groups	2	86.45	43.225	5.658	24
Within groups	12	219.95	18.329	18.329	76
Total		306.4		23.987	100

In order to calculate the level of genetic differentiation between the species collected in Arcabuco and Gachantivá, a hierarchical analysis of molecular variance (AMOVA) was performed. Table 4 demonstrates that there is molecular genetic differentiation of 23% between the groups corresponding to the geographical location of the sample. In addition, it was observed that the greatest contribution to variance is within the groups (76%), possibly because each of them is composed of different cultivated species and wild relatives of the *Rubus* genus.

Based on the results of Table 4, the greatest genetic variation is possibly due to the fact that the individuals studied were collected at altitudes between 2,399 and 2,800 meters above sea level. These regions are characterized by having mountains, streams, rivers, and some roads that can act as corridors that determine the behavior and genetic differentiation within the groups or species evaluated (GARRIDO-GARDUÑO; VAZQUEZ-DOMINGUEZ, 2013).

VELJKOVIĆ et al. (2019), mentions that in the genus *Rubus*, altitude plays an important role in the genetic separation of the populations of raspberries (*R. idaeus*). It should also be taken into consideration that the species of the genus *Rubus* are characterized by having a taxa with a wide and successful distribution and can be found in different parts of the world and present morphologically different individuals. It can be inferred that apomixis, allogamy, and polyploidy have maintained a permanent flow of genes between wild, invasive, and cultivated species (SOCHOR et al., 2015). This also extends to causing important adaptive strategies based on environmental conditions. Despite the number of edaphoclimatic conditions previously described in Arcabuco and Gachantivá, both share borders and some of the locations from where samples were taken are close. The genetic variation can therefore be attributed to the fact that the distance between collection sites is short, corroborating the fact that geographical distance is a factor that influences the genetic diversity of plant populations, as stated by WRIGHT, (1943).

Conclusions

The grouping of the species studied does not necessarily correspond to the geographical origin of the genotypes.

The principal and cluster coordinate analysis show the greatest genetic distance for the cultivated and wild species that produce yellow-whitish fruits. It also demonstrates the potential related to genetic diversity and the logical morphological characteristics that can be consolidated in this species as an interesting production line in the department of Boyacá.

The molecular analysis of variance (AMOVA) revealed greater variability between species than between the studied populations.

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References

AMSELLEM, L.; DUTECH, C.; BILLOTTE, N. Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir. (Rosaceae), an invasive weed in La Réunion island. **Molecular Ecology Notes**, Oxford, v.1, n.1-2, p.33-35, 2001.

BENBOUZA, H.; JACQUEMIN, J.M.; BAUDOIN, J.P.; MERGEAI, G. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. **Biotechnologie, Agronomie, Société et Environnement**, Gembloux, v.10, n.2, p.77-81, 2006.

CASTILLO, N,R.; REED, B,M.; GRAHAM, J.; FERNANDEZ-FERNANDEZ, F.; BASSIL, N,V. Microsatellite markers for raspberry and blackberry. **Journal of the American Society for Horticultural Science**, Alexandria, v.135, n.3, p. 271–278, 2010.

CLARK, L.V.; EVANS, K.J.; JASIENIUK, M. Origins and distribution of invasive *Rubus fruticosus* L. agg. (*Rosaceae*) clones in the Western United States. **Biological Invasions**, Dordrecht, v.15, n.6, p.1331–1342, 2013.

DOSSETT, M.; BASSIL, N.V.; LEWERS, K.S.; FINN, C.E. Genetic diversity in wild and cultivated black raspberry (*Rubus occidentalis* L.) evaluated by simple sequence repeat markers. **Genetic Resources and Crop Evolution**, Dordrecht, v.59, n.8, p.1849-1865, 2012.

DOTOR-ROBAYO, M.; GONZÁLEZ-MENDOZA. L.; CASTRO, M.; MORILLO-CORONADO, A.; MORILLO-CORONADO, Y. Análisis de la diversidad genética de la mora (*Rubus* spp.) en el departamento de Boyacá. **Biotecnología en el Sector Agropecuario y Agroindustrial**, Popayan, v.14, n.2, p.10-17, 2016.

EL KADRI, N.; MIMOUN, M,B.; HORMANZA, J,I. Genetic diversity of Tunisian male date palm (*Phoenix dactylifera* L.) genotypes using morphological descriptors and molecular markers. **Scientia Horticulturae**, New York, v.253, n.6, p.24–34, 2019.

FAQIR, N.; MUHAMMAD, A.; HYDER, M.Z. Diversity assessment and cultivar identification in date palm through molecular markers- a review. **Turkish Journal of Agricultura-Food Science and Technology**, Sivas, v.5, n.5, p.1516–1523, 2017.

GARRIDO-GARDUÑO, T.; VAZQUEZ-DOMINGUEZ E. Métodos de análisis genéticos, espaciales y de conectividad en genética del paisaje. **Revista Mexicana de Biodiversidad**, México, v.84, n.3, p.1031-1054, 2013.

GRAHAM, J.; SMITH, K.; WOODHEAD, M.; RUSSELL, J. Development and use of simple sequence repeat SSR markers in *Rubus* species. **Molecular Ecology Notes**, Oxford, v.2, n.3, 3, p.250-252, 2002.

GRAHAM, J.; SMITH, K.; MACKENZIE, K.; JORGENSON, L.; HACKETT, C.; POWELL, W. The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp idaeus based on AFLPs, genomic-SSR and EST-SSR markers. **Theoretical and Applied Genetics**, Berlin, v.109, n.4, p.740–749, 2004.

HAMMER, O.; HARPER, D.; RYAN, P. PAST: paleontological statistics software package for education and data analysis. **Palaeontologia Electronica**, College Station, v.4, n.1, p.1-9, 2001.

HU, J.; PAN, L.; LIU, H.; WANG, S.; WU, Z.; KE, W.; DING, Y. Comparative analysis of genetic diversity in sacred lotus (*Nelumbo nucifera* Gaertn.) using AFLP and SSR markers. **Molecular Biology Reports**, Dordrecht, v.39, p.3637–3647, 2012.

KALIA, R.K.; RAI, M.K.; KALIA, S.; SINGH, R.; DHAWAN, A.K. Microsatellite markers: an overview of the recent progress in plants. **Euphytica**, Netherlands, v.177, p.309–334, 2011.

LEBEDEV, VG.; SUBBOTINA, N.; MALUCHENKO, O.; KRUTOVSKY, K.; SHESTIBRATOV, K. Assessment of genetic diversity in differently colored raspberry cultivars using SSR markers located in flavonoid biosynthesis genes. **Agronomy**, Basel, v.9, n.9, p.518, 2019.

LEBEDEV, V.G.; SUBBOTINA, N.; MALUCHENKO, O.; LEVEDEVA, T.; KRUTOVSKY, K.; SHESTIBRATOV, K. Transferability and polymorphism of SSR markers located in flavonoid pathway genes *in Fragaria* and *Rubus* species. **Genes**, Basel, v.11, n. 1, p.11, 2020.

LÓPEZ, A.; BARRERA, C.; MARULANDA, M. Evaluation of SSR and SNP markers in *Rubus glaucus* Benth progenitors selection. **Revista Brasileira de Fruticultura**, Jaboticabal, v.41, n.1, p.1-14, 2019.

MARULANDA, M.; LÓPEZ, A.; URIBE, M. Molecular characterization of the Andean blackberry, *Rubus glaucus*, using SSR markers. **Genetics and Molecular Research**, Ribeirão Preto, v.11, n.1, p.322-33, 2012.

MORALES, C.; VILLEGAS, B. Mora (*Rubus glaucus* Benth). *In*: FISCHER, G. (ed.). **Manual para el cultivo de frutales en el trópico.** Bogotá: Produmedios, 2012. p.728-754.

MORENO-MEDINA, B.; CASIERRA-POSADA, F.; BLANKE, M. Índices de crecimiento en plantas de mora (*Rubus alpinus* Macfad) bajo diferentes sistemas de poda. **Revista Colombiana de Ciencias Hortícolas**, Tunja, v.10, n.1, p.28-39, 2016.

MORENO-MEDINA, B.L.; DEAQUIZ, Y. Caracterización de parámetros fisicoquímicos en frutos de mora (*Rubus alpinus* Macfad). **Acta Agronómica**, Palmira, v.65, n.2, p.130-136, 2016.

MORENO-MEDINA, B.L.; CASIERRA-POSADA, F.; CUTLER, J. Phytochemical composition and potential use of *Rubus* species. **Gesunde Pflanzen**, Frankfurt, v.70, n.2, p.65-74, 2018.

MORENO-MEDINA, B,L.; CASIERRA–POSADA, F.; ALBESIANO, S. *Rubus alutaceus* (Rosaceae), a new species for Colombia with agronomic potential. **Revista Brasileira de Fruticultura**, Jaboticabal, v.42, n.2, p.e-542, 2020.

PEAKALL, R.; SMOUSE, PE. GenAlEx 6.5: Genetic Analysis in Excel. Population genetic software for teaching and research-an update. **Bioinformatics**, Oxford, v.28, n.19, p.2537-2539, 2012.

REJMANEK, M. Invasion of *Rubus praecox* (Rosaceae) is promoted by the native tree *Aristotelia Chilensis* (Elaeocarpaceae) due to seed dispersal facilitation. **Gayana Botánica**, Concepción, v.72, n.1, p.27-33, 2015.

ROA, S.; FERNÁNDEZ, H.; ANGULO, L.; USECHE, N.; MUÑOZ, Y. Caracterización molecular de genotipos de *Rubus* mediante marcadores microsatélites. **Agronomía Tropical**, Maracay, v.64, n.1-2, p.61-72, 2014.

SOCHOR, M.; VAŠUT, R.J.; SHARBEL, TF.; TRÁVNÍČEK, B. How just a few makes a lot: speciation via reticulation and apomixis on example of European brambles (*Rubus* subgen. *Rubus*, Rosaceae). **Molecular Phylogenetics and Evolution**, San Diego, v.89, p.13–27, 2015.

VELJKOVIĆ, B.; ŠOŠTARIĆ ,I.; DAJIĆ-STEVANOVIĆ, Z.; ZLATKO L.; ZLATKO Š. Genetic structure of wild raspberry populations in the Central Balkans depends on their location and on their relationship to commercial cultivars. **Scientia Horticulturae**, New York, v.256, p.108606, 2019.

WANG, Y.; WEN, HE.; ZHANG, J.; CHEN, T.; CHEN, Q.; TANG, H-R.; LUI, L.; WANG, Genetic Relationship between *Rubus parvifolius* and *R. coreanus* (*Rubus*, Rosaceae) based on simple sequence repeat markers. *In:* INTERNATIONAL CONFERENCE ON BIOLOGICAL SCIENCES AND TECHNOLOGY (BST 2017), 2., 2017. **Proceendigs** […]. Disponivel em: Doi:10.2991/bst-17.2018.16. Acesso em: 8 mar. 2020.

WRIGHT, S. Isolation by distance. **Genetics**, Rockville, v.28, n.2, p.114-138, 1943.

WRIGHT, S. **Evolution and the genetics of populations, variability within and among natural populations**. Chicago: University of Chicago Press, 1978. p.566.