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Genetic diversity of wild and cultivated *Rubus s*pecies in Colombia using AFLP and SSR markers

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ABSTRACT - *The Andean blackberry belongs to the genus* Rubus*, the largest of the Rosaceae family and one of the most diverse of the plant kingdom. In Colombia* Rubus glaucus *Benth, known as the Andean raspberry or blackberry, is one of the nine edible of the genus out of forty-four reported species. In this study wild and cultivated genotypes, collected in the Central Andes of Colombia were analyzed by AFLP and SSR markers. Sexual reproduction seems to play an important role in maintaining the genetic variability in* R. glaucus*, and the viability of using the SSR of* Rubus alceifolius *to characterize Colombian* Rubus *species was clearly demonstrated. All species evaluated produced very specific banding patterns, differentiating them from the others. Both AFLP and SSR produced bands exclusive to each of the following species:* R. robustus*,* R. urticifolius, R. glaucus, and R. rosifolius. *The SSR markers differentiated diploid and tetraploid genotypes of* R. glaucus.

Key words: AFLP**,** SSR, genetic diversity, *Rubus* spp.

INTRODUCTION

The Colombian and Ecuadorian Andes are the natural habitat of the *Rubus* genus, with almost 700 species the largest of the *Roseaceae* family and one of the most diverse of the plant kingdom (Romoleroux 1992). The ploidy level of this interesting group of plants ranges from diploid to dodecaploid, mostly apomictic and highly heterozygous.

Rubus has been divided into 12 subgenera of which only few species have been domesticated. The subgenus *Idaeobatus* contains the "raspberries" that are distributed in the Northern Hemisphere, mainly Asia, Africa, Europe, and North America; the subgenus *Rubus* includes species found in Europe, Asia, and North America, whereas the subgenus *Orobatus* is exclusive to South America. Representatives of the subgenera *Rubus, Orobatus*, and *Idaeobatus* are found in the Colombian and Ecuadorian Andes (Ballington et al. 1993).

Rubus has spread over the tropical highlands, usually at over 800 meters above sea level. *Rubus glaucus* fruits are produced from Mexico to Ecuador; they are consumed fresh and processed for products such as jellies and beverages. Also known as the Andean blackberry, this species is widely distributed in the three Cordilleras of Colombia and combines traits of the subgenera *Idaeobatus* and *Rubus*. It is a fertile amphidiploid or allotetraploid, originated by the genome fusion of two species (Ballington et al. 1993, Jennings 1988). *Rubus glaucus* is the only native species of the genus used for commercial production in Colombia (Ballington et al. 1993). *Rubus* has a wide altitudinal

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and geographical distribution in Colombia. Vargas (2002) reports six species: *R. bogotensis*, *R. glaucus*, *R. macrocarpus*, *R. nubigenus*, *R. porphyromallus*, and *R. urticaefolius*. Romoleroux (1992) further mentions the existence of nine edible species in Colombia of the 44 species reported. Due to natural crosses, up to 500 varieties can be identified.

In the areas where *Rubus* species are grown, wild species are found in areas of secondary growth, forest margins, and along roadsides. Cultivated and wild plants have the potential to interact in several ways. Cultivars can influence the genetic diversity of natural populations through gene loss and transfer by pollen. Wild populations can also serve as host plants of pests and their natural predators. Additionally, wild populations are also a potential source of breeding material for improvement programs (Graham et al. 1997b). However, very little information is available on the nature and extent of the interactions and relationships between wild and cultivated populations of *Rubus* species (Graham et al. 1977b).

Studies on the genetic diversity of temperate *Rubus* species have been carried out, such as *R. idaeus* (Parent and Fortin 1993, Graham and Mcnicol 1995, Graham et al. 1997b) and *R. occidentalis* (Parent and Page 1998), as well as Asian species (Amsellem et al. 2000). These studies used RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Lenght polymorphism), and SCAR (Sequence Characterized Amplified Region) markers as well as SSR (Single Sequence Repeats) (Antonius-Klemola 1999). Hybrids of *R. idaeus* and *R. caesius* were also studied using Internal Transcribed Spacer (ITS) markers (Alice et al. 1997), this markers made it possible to confirm the genetic origin of the hybrids and to further phylogenetic and evolutionary studies in *Rubus* (Alice 2002). Major advances have recently been made by using molecular markers in temperate *Rubus* species, i.e., DNA fingerprinting, genotype characterization, development of linkage maps, use of marker-assisted selection, and QTL (Quantitative Trait Loci) mapping (Antonius-Klemola 1999, Graham et al. 2002).

This paper reports the results of a molecular analysis carried out with AFLP and SSR (simple sequence repeat) markers of wild and cultivated *Rubus* species collected in the Central Andes of Colombia. Strategies were established for the sustainable use and conservation of genetic resources of *Rubus glaucus* and related wild species, generating information on the current status of populations, their uses and distribution, as well as other data considered indispensable to launch an improvement program for *R. glaucus*.

MATERIAL AND METHODS

Plant material and DNA extraction

Fifty-one samples of cultivated and wild *Rubus* species were analyzed collected from 27 localities in Caldas, Quindío and Risaralda (departments in the Central Andes of Colombia, at altitudes between 1511 and 2851 m asl). Plants of the following species were analyzed: *R. glaucus* (27), *R. adenotrichos* (1), *R. bogotensis* (1), *R. robustus*, (4), *R. rosifolius*, (3), and *R. urticifolius* (15). Total genomic DNA was isolated according to the protocol described by Doyle and Doyle (1990).

Analysis with AFLP markers

The AFLP Analysis System I Kit (Invitrogen, Carlsbad, CA, USA) (Vos et al. 1995) was used for the AFLP analysis, and PCR products were electrophorized under denaturing conditions in 6% acrylamide gels (containing 7 M urea), which were silver-stained, according to the procedure described by Bassam et al. (1991). The primer-enzyme combinations with highest polymorphism were E-AGG * MCAG, E-ACT * M-CTG, and E-AAC * M-CAT (Table 1).

AFLP and SSR products were scored qualitatively (Ghosh et al. 1997). Only clear and apparently unambiguous bands were scored for AFLP and SSR. Groups of genetic diversity were determined by calculating the genetic similarity (GSij) between each genotype pair by the formulas of Dice (1945) and Nei and Li (1979), based on the proportion of shared alleles using the subprogram simqual of the NTSYS-pc version 2.02i (Rohlf 1989). The resulting distance matrix data were used to construct the dendrogram using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA), NTSys subprogram (Rohlf 1993).

SSR analysis

Eight pairs of primers, perfect and imperfect micro satellite sequences (SSR) were evaluated based on the

Table 1. Number of polymorphic bands obtained per primer combination, with AFLP

Enzyme-primer combinations	Number of total bands	Number of polymorphic bands	Polymorphism (%)
E-ACT * M-CTG	89	819	1.011
E-AAC * MCAT	75	648	5.333
E-AGG * M-CAG	65	649	8.461
Tota	12	292	09
Mean	76.333	69.666	91.601

results described by Amsellem et al. (2001) (Table 2). Amplification reactions were performed in a final volume of 25 µl, with 5 ng genomic DNA, 0.3 µM of each primer, 1X reaction buffer (10 mM Tri-HCl, pH 8.3, 50 mM KCl), 200 µM of each of the dNTP, 2 mM MgCl2 and 1.0 unit of Amplitaq DNA polymerase. The allelic diversity of the SSR was evaluated by determining the polymorphism information content (PIC) value, as described by Bonstein et al. (1980) and cited and modified by Anderson et al. (1993), as expressed below:

$$PIC_i = 1 - / \sum_{j=1}^{n} P_{ij}^2$$

Where *Pij* is the frequency of the *j*th pattern, *i* is the sum, and *n* are the patterns. The test of Mantel (Mantel 1967) was used to correlate the matrixes.

To measure the utility of the marker systems, the mean heterozygosity, expected and observed heterozygosity (Weir and Cockerham 1984) and FST-values (Wright's fixation index, Brown and Weir, 1983), were calculated using ARLEQUIN version 3.1 (Excoffier et al. 2006) with significances based on a permutation process. The genetic variation within and among the formed groups was determined by analysis of molecular variance (AMOVA) using SSR markers (Excoffier et al. 2006) with the same software.

RESULTS AND DISCUSSION

The DNA fingerprinting database was prepared using the two different PCR-based markers (SSR and AFLP) systems for 51 *Rubus* samples.

Genetic variability detected by AFLP markers

A total of 229 bands were obtained with the three primer-enzyme combinations evaluated. The band size ranged between 30 to 330 base pairs (Table 1), and 91.6% polymorphism was obtained.

The genetic groupings and distances that produced the polymorphic bands are shown in the dendrogram (Figure 1), which differentiates six similarity groups. When compared with other species species *R. bogotensis* (34), group 6 of the dendogram, stood out as well as several individuals (genotypes 42, groupe3; genotype 5, groupe 4; and genotype 45, group 6) because of large differences. Two main groups were formed, with 28% similarity. A first group (1) consisted of *R. urticifolius* plants (genotypes 3, 103, 107, 67, 37, 55, 82, 47, 79, and 52), *R. glaucus* (genotypes 35, 90, 87, 99, 101, 7, 22, and 10), *R. robustus* (genotypes 18 and 33), *R. adenotrichos* 85, and *R. rosifolius* 63.

A second group (2) contained three *R. glaucus* plants (genotypes 3, 13, and 18) with one of *R. robustus* (88) separated from the main group. The rest of the group comprised mainly *R. glaucus* plants (genotypes 9, 54, 100, 80, 46, 83, 102, 86, 95, 97, 50, 65, 21, and 5) with higher similarity indices (50 and 100%). The species *R. urticifolius* was also represented (genotypes 41, 107, 68, 64, and 44) as well as *R. rosifolius* (32 and 59) and *R. robustus* (36).

The lowest similarity values, however, were found in the first group (1). The highest similarity indexes in the second group (2) were observed between *R. urticifolius* 107 and *R. glaucus* 100 (70%), *R. glaucus* 54 and *R. robustus* 36 (90%), *R. glaucus* 83 and *R. rosifolius* 59 (95%), *R. glaucus* 9 and *R. urticifolius* 41 (70%), and *R. urticifolius* 68 and *R. glaucus* 83 (80%). The results of this study are similar to those reported by Kollmann et al. (2000), who studied the influence of reproduction on the variability of European *Rubus* species, using AFLP markers.

Kollmann et al. (2000) concluded that genetic variability in *Rubus* is determined by the plant propagation system and demonstrated that there is an effect of cross-pollination between polyploid *Rubus* species. This type of crossbreeding influences seed and

Table 2. SSR loci and primers used in *Rubus* species and respective number of alleles, product size and PIC (Polymorphic Information Content)

Locus	Microsatellite structure	Mean PIC value per microsatellite	Gene bank accession Number	Number of alleles in different *Rubus* species						Product size (pb)
				glaucus	*urticifolius*	*robustus*	*rosifolius*	*adrenotrichos*	*bogotensis*	
mRaCIRRI1D3	(GA)26		AF205115	NA	NA	NA	NA	NA	NA	161-308
mRaCIRRI1G3	(GA)28	0.499166	AF205116	1-3	1-3	1-2	1-2	1	3	195-265
mRaCIRRI1H3	(GT)15(GA)17	0.385842	AF205117	2-5	2- 4	2	1	5	NA	160-226
mRaCIRRI2B5	(GA)19		AF205118	NP	NP	NP	NP	NP	NP	222-272
mRaCIRRIV2A8	(CA)12(CT)11	0.378691	AF261693	3-5	1-3	1-2	1	NA	2	191-237
mRaCIRRV1C10	(CTT)8(CT)6		AF261694	NA	NA	NA	NA	NA	NA	153-203
mRaCIRRV2F4	(CT)8(CA)17(CT)11		AF261695	NP	NP	NP	NP	NP	NP	180-242
mRaCIRRIV1E8	(CTT)33		AF261696	NP	NP	NP	NP	NP	NP	197-355

NA: No amplification.

fruit quality positively, whilst increasing the ploidy levels and taxonomic proximity.

This phenomenon could also occur with *Rubus* species in the Central Andes of Colombia and may explain the very high similarity indexes observed between different species such as *R. urticifolius* and *R. glaucus, R. glaucus* and *R. robustus,* and *R. rosifolius* and *R. robustus*, which belong to different subgenera, and between *R. glaucus* and *R. rosifolius*, which belong to the same subgenera. In cases of very high similarities between different individuals of *R. glaucus* with individuals of other species, such as *R. robustus* and *R. urticifolius*, these genotypes could be hybrids between both species or share a common ancestor, as reported by Kollmann et al. (2000) in pollination studies with *R. armeniacus*. Hybridization in *Rubus* occurs mostly between closely related species and, in some instances, between subgenera (Gustafsson 1942, Jennings 1978, Weber 1995, Alice et al. 1997, Alice and Campell 1999).

AFLP markers also produced several bands or private alleles, which were exclusive to each species and serve to quickly identify genotypes or interspecific hybrids of interest in the early stages of an improvement program (Table 3).

Genetic variability with SSR markers

Twenty loci and positive amplification were obtained with the following SSR markers: mRaCIRRI1H3, mRaCIRRI1G3, mRaCIRRI2B5, mRaCIRRIV2A8, mRaCIRRV2F4, and mRaCIRRIV1E8. Amplification was not positive with the following markers: mRaCIRRI1D3 and mRaCIRRV1C10. SSRs with highest polymorphism were mRaCIRRI1H3, mRaCIRRI1G3, and mRaCIRRIV2A8 (Table 2). A mean of 8.167 polymorphic loci per primer were detected, out of the 20 total loci. The number of polymorphic bands was highest in *R. urticifolius* with 16, followed by *R. glaucus* with 14, *R. robustus* with 13, and *R. rosifolius* with 6 (Table 4).

The bands obtained were similar in weight and number to those obtained by Amsellem et al. (2001) in Asian species. SSR also made it possible to differentiate groups within *R. glaucus* (intraspecific variability) as well as between *Rubus* species (interspecific variability). Seven private or exclusive alleles were detected for several subgenera, providing a molecular profile for genotypes based on their banding pattern. Amsellem et al. (2000) observed the amplification of three to four

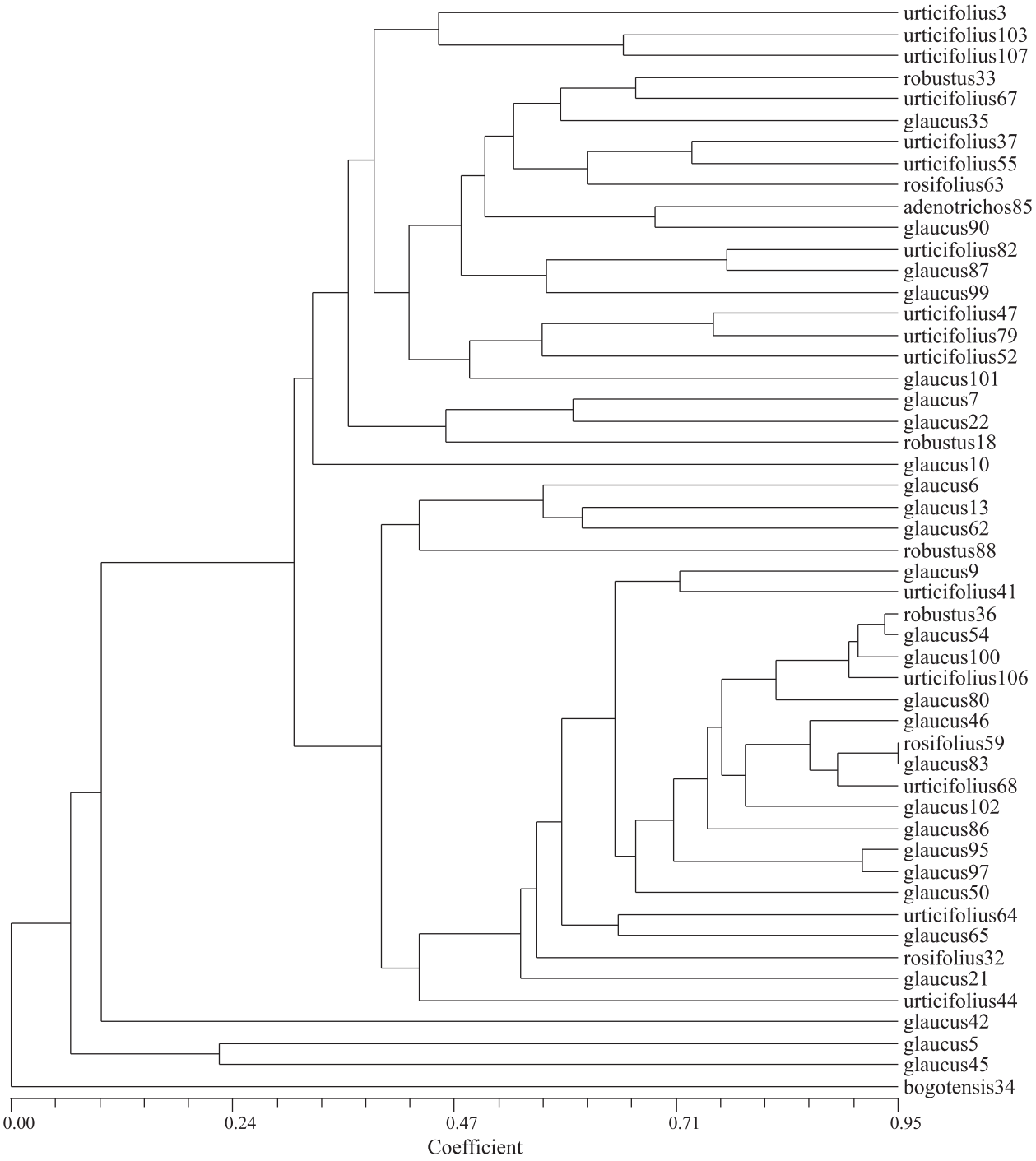

Figure 1. Cluster analysis of 51 accessions of *Rubus* sp., based on the matrix of genetic similarity calculated based on AFLP markers. The UPGMA method was the grouping criterion

Table 3. Exclusive markers for *Rubus* species obtained with AFLP

Species	E-ACT * M-CTG	E-AAC * MCAT	E-AGG * M-CAG
R. glaucus	3	4	8
R. urticifolius	3	2	12
R. robustus	2	0	3
R. rosifolius	0	0	2

Table 4. Genetic diversity in populations of *Rubus* species

Rubus species	Nr. of accessions	Nr. of loci	P*	Hs	Dst	Ht	Gst
R. robustus	4	20	13	0.038	-0.020	0.31286	-7.03
R. adenotrichos	1	20	0	0.000	0.31286	0.31286	1
R. bogotensis	1	20	0	0.000	0.31286	0.31286	1
R. glaucus	27	20	14	0.27863	0.03423	0.31286	0.1
R .rosifolius	3	20	3	0.200	0.11286	0.31286	0.3607
R. urticifolius	15	20	15	0.25333	0.05953	0.31286	0.190

* Polymorphic loci (P), Expected heterozygosity (Hs), Genetic diversity within population (Dst), Total heterozygosity (Ht), Differentiation at the locus level (Gst)

alleles per individual in *R. alceifolius*, which confirmed the hypothesis that this is a tetraploid species. In this paper, *R. robustus*, *R. rosifolius*, and *R. bogotensis* produced between one and two alleles, while *R. glaucus* produced between three and five alleles, and *R. urticifolius* between two and three alleles, whereas *R. adenotrichos* amplified five alleles. These results suggest that the last three species have higher ploidy levels than the others. In phylogeny studies with internal transcribed spacers (ITS) *Rubus* Alice and Campbell (1999) describe *R. robustus* and *R. rosifolius* as diploid species and subgenus *Orobatus* as hexaploid. In contrast, the ploidy level in the *Rubus* subgenus ranges from diploid to tetraploid, while the subgenus *Idaeobatus* presents both diploid and tetraploid species. According to morphological and molecular descriptions made by the abovementioned authors on other *Rubus* species, the ploidy level among *Rubus* genotypes can vary greatly. The results presented here, using two different molecular markers, agree with the highly variable ploidy level found in other *Rubus* species.

With some SSR markers, e.g., mRaCIRRI1H3 and mRaCIRRI1G3, *R. glaucus* amplified only two alleles, as in the case of the genotypes 5, 6, 21, 22, 35, 42, 45, 46, 50, 54, 65, 80, 83, 86, 87, 95, 97, and 102 while the *R. glaucus* genotypes 7, 9, 10, 13, 90, 99, 100, and 101 amplified three, four and five alleles. Several *R. urticifolius* genotypes (3, 64, 103, 106, and 107) amplified more than two alleles with these same SSR markers, suggesting that there are both diploid and tetraploid genotypes of *R. glaucus* and *R. urticifolius* in the wild and cultivated germplasm. According to Jennings (1988) and Jennings and McGregor (1988), *R. glaucus* is a species that combines traits of *Idaeobatus* (leaf morphology, plant growth habit and chemical fruit characteristics) with the external traits of fruits and inflorescences of subgenus *Rubus*. The morphological description of *R. glaucus* at the molecular level is quite similar to results of AFLP as well as SSR markers, which evidences the great inter-relatedness between species at the subgenus level.

mRaCIRRI1H3 amplified six loci, one of which is exclusive to *R. rosifolius* 32. The other five loci were shared by all species, except *R. bogotensis*. *Rubus adenotrichos* as well as *R. glaucus* (genotypes 99, 100, and 101) produced amplification in five of the six loci.

mRaCIRRI1G3 amplified seven loci, two of which were exclusive to *R. robustus* 18. One band was shared by *R. urticifolius* 82 and *R. robustus* 33 only and another band was shared by *R. robustus* 33 and *R. urticifolius* (82 and 52). A final band was shared by *R. glaucus* genotypes (5, 6, 7, 9, 10, 13, 21, 22, 65, 83, 87, 95, 99, 100, 101, and 102) and *R. urticifolius* genotypes (37, 64, 67, and 103).

mRaCIRRIV2A8 amplified seven loci, one of which was exclusive to *R. glaucus* genotypes. Two loci were almost completely exclusive to *R. glaucus* and present

in only two plants of *R. urticifolius* (106 and 107). One locus amplified only in *R. robustus* 18, *R. bogotensis* 34, and *R. urticifolius* 37. The SSRs achieved cumulative polymorphism information content (PIC) of 0.4056466, and individual values for each locus between 0.27332005 and 0.499166 (Table 2). In comparison, Ishii and McCouch (2000) obtained mean PIC values of 0.267 when evaluating rice SSRs and Cordeiro et al. (2000) obtained PIC values between 0.48 and 0.8 when evaluating sugarcane SSRs. These PIC values demonstrated that, in spite of the low number of primers used, the SSR were sufficiently polymorphic and informative.

Apart from demonstrating that *R. alceifolius* SSR can be used to study and evaluate the diversity of Colombian *Rubus* species, our results evidenced that the SSR markers developed by Amsellem et al. (2001) can be a powerful tool to help classify *Rubus* species.

All study species produced very specific banding patterns, differentiating them from the other species, besides seven private alleles for recognition at the subgenus and species level. Molecular results were consistent with the taxonomic description of Aguilar (2006), whose data were based on morphological traits.

The dendrogram (Figure 2) shows the major genetic differences between *R. robustus* (genotype 18 of group 6 and genotype 33 of group 7) and *R. urticifolius* (genotype 82 of group 7 and genotype 52 of group 8). *Rubus rosifolius* 32 (group 9) was isolated from all other species, with only 12% similarity.

Three closely related groups were formed, with high similarity indexes. The first group (1) contained *R. urticifolius* (3, 41, 44, 47, and 68) and *R. adenotrichos* 85. The second group (2) was formed by 20 individuals of *R. glaucus* from different origins, with high similarity indexes, together with *R. urticifolius* 79. A third group (3) united *R. robustus* 36, *R. urticifolius* (55, 106, and 107), *R. glaucus* (42 and 50), and *R. rosifolius* (59 and 63) a most peculiar species combination.

The *R. glaucus* genotypes 13, 86, and 97 presented the lowest similarities values of all species and form a fourth group (4). A fifth group (5), separated from the other four, joins *R. bogotensis* 34, *R. urticifolius* (37, 64, 67, and 107), *R. glaucus* (83, 87, 88, and 102), and *R. robustus* 88, with similarity indexes between 70 and 100%.

Genetic diversity within and among populations

The analysis of molecular variation (AMOVA) revealed differences in partitioning variation within and among groups. SSR markers showed greater variance within than among groups (Table 5). The variance found within groups for *Rubus* with SSR (80.4) is quite similar to that obtained by Saini et al. (2004) for rice populations (76.93). As described by Brown and Weir (1983), mean expected heterozygosity (He) and total heterozygosity (Ht) were estimated as a measure of polymorphism and usefulness of the marker systems used in this study (Table 4). Mean total heterozygosity (Ht) was 0.31286. The mean expected heterozygosity within populations varied from 0.00000 to 0.33333 for *R. robustus,* which presented the highest heterozygosity. *Rubus glaucus*, *R. rosifolius*, and *R. urticifolius* showed similar values: 0.27863, 0.20000, and 0.25333, respectively. The genetic diversity (Dst) or polymorphism within each population (Nei 1987) varied from 0.020 to 0.31286 (Table 4).

The differentiation of a simple locus is defined by Nei (1973) as gst, whereas the relative magnitude of differentiation between populations is measured as GST. Nei (1987) recommends the estimation of GST when calculating the differentiation for the whole genome. In this study with *Rubus,* the mean GST was 0.4042, a rather high value that evidences the high differentiation between populations or, in this case, between species (Table 4).

Studies with *Pueraria lobata* (Fabaceae) showed values of expected heterozygosity between 0.290 and

Table 5. Partitioning of variance derived from the analysis of molecular variance (AMOVA) with SSR markers

Source of Variation	Degrees of freedom	Sum of Squares	Variance components	Percentage of variation
Among Populations	5	34.520	0.66031 Va	19.60
Within populations	45	121.911	2.70914 Vb	80.40
Total	50	156.431	3.36945	

Fixation Index FST: 0.19597
Significance tests (1023 permutations)

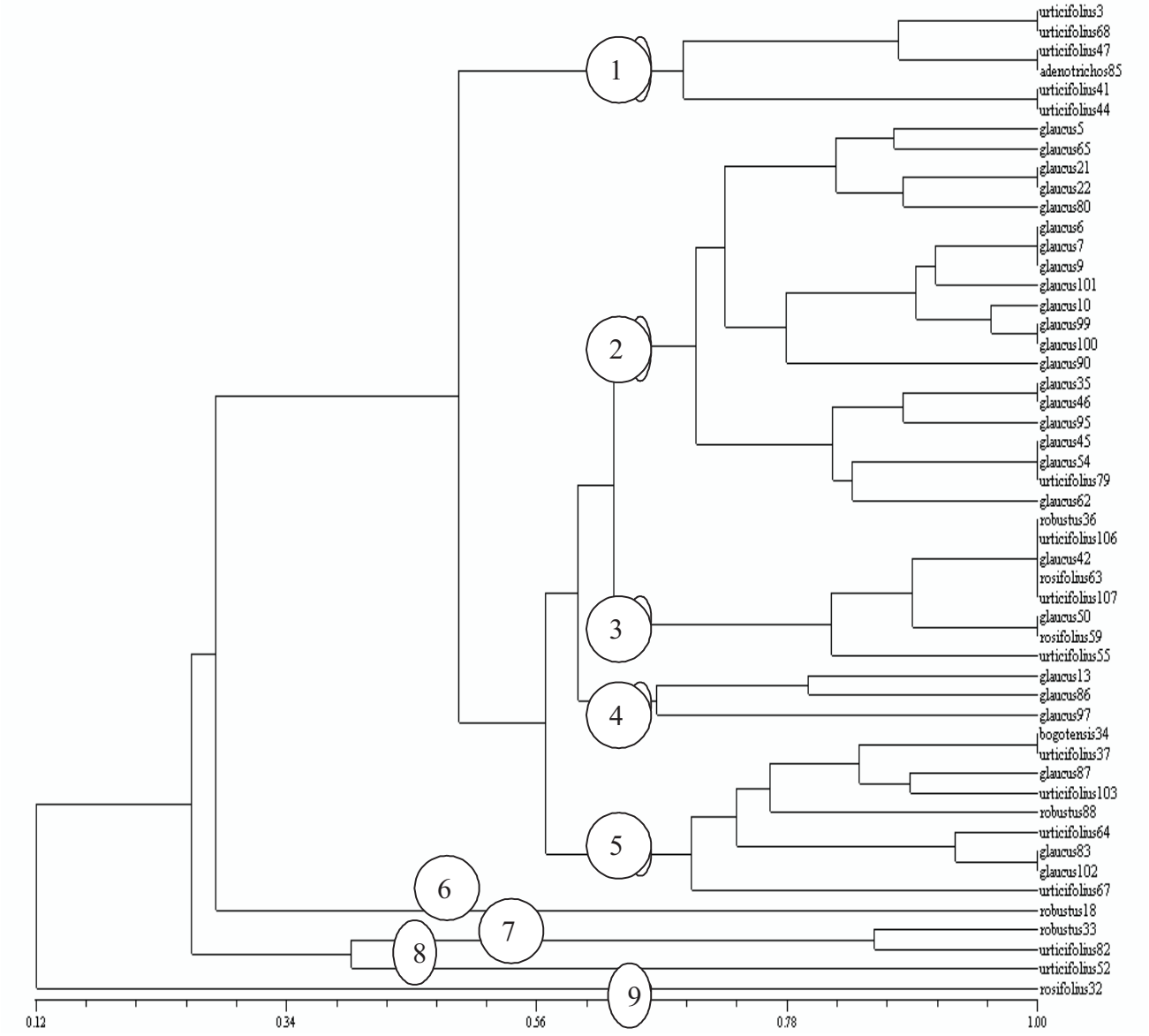

Figure 2. Cluster analysis of 51 accessions of *Rubus* sp., based on the matrix of genetic similarity calculated based on SSR markers. The UPGMA method was the grouping criterion

0.213 (Pappert et al. 2000). Selander (1976) found mean values of polymorphic loci (P) and heterozygosity (H) of 0.344 and 0.078 for allogamous plants. The respective values were 0.405 and 0.312 for *Rubus*. In contrast, in studies with Lycopodiaceae conducted by Frankel et al. (1995), the following values were obtained: Ht, 0.071; Hs, 0.051; and GST, 0.284, with four sub-populations and 13 loci. These values are relatively low compared with those obtained in *Rubus*—a very interesting result because once again the reproductive pattern of this plant group affects the genetic variability.

The AMOVA analysis reveals that high genetic variability does exist among and within *Rubus* species in the study population. In addition, the *R. glaucus* genotypes studied showed high genetic variability, where the greatest differences were observed in wild genotypes. The SSRs produced bands that were exclusive to *R. robustus*, *R. urticifolius, R. glaucus*, and *R. rosifolius*. SSR markers differentiated the subgenera *Rubus* and *Idaeobatus*, and distinguished two groups between *R. glaucus* and *R. urticifolius*. Further research will indicate whether the samples of *R. glaucus* and *R. urticifolius* with high similarity index, as determined via AFLP, are potentially interspecific hybrids.

The differentiation among populations (Nei 1986), is determined by the fixation index (FST) which can vary from 0 to 1. Close-to-zero values indicate a larger number of heterozygotes while higher values indicate a larger number of homozygotes. FST values of 0.282 were reported in studies carried out with other vegetable species such as Lycopodeaceae, whereas values of 0.6 were reported for crustaceans. The FST value of 0.19597 obtained in *Rubus* therefore evidences the presence of a high number of heterozygotes in the study population.

The Mantel test (1967) did not show a statistically significant positive correlation for the AFLP and SSR matrixes, which can be attributed to the fact that most widely adopted marker technologies, such AFLP and SSR, amplify different regions of the genome. The advantages and disadvantages of each technology should be carefully assessed before being effectively deployed in diversity analysis (Saini et al. 2004). The markers target different genomic fractions involving repeat and/or unique sequences, which are differentially evolved or preserved in the course of natural or human selection (Saini et al. 2004). Virk et al. (2000) reported differences between AFLP and ISSR marker techniques when classifying 42 rice accessions, which agree with reports of Parsons et al. (1997).

Saini et al. (2004) emphasize, on the other hand, the fact that marker-based differences in genetic relationships among rice genotypes indicate the need to use a combination of different marker systems for comprehensive genetic analysis. Furthermore, the hypervariability in loci usually observed with SSR can be attributed to a mechanism of replication slippage, which occurs more frequently than point mutation and insertion/deletion events in AFLP polymorphism (Tautz et al. 1986). The usefulness of the SSR technique for co-dominant, mapped and publicly available microsatellite sequences will increase in the near future (Saini et al. 2004). AFLP and SSR were found to be complementary because their joint analysis provided additional elements to explain the complex inter-relationship between wild and cultivated *Rubus* species in a region of high genetic diversity for this plant group, such as the Colombian Andes.

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Diversidade genética de espécies cultivadas e silvestres de *Rubus* na Colômbia.

RESUMO - *A amora pertence ao gênero Rubus, o maior da família das Rosaceae e é um dos mais diversos tipos do reino vegetal. Na Colômbia, a espécie* Rubus glaucus *Benth conhecida como amora dos Andes ou amora de Castilha é uma das nove espécies comestíveis deste gênero, de 44 espécies reportadas. No presente trabalho se realizou uma análise molecular com*

marcadores AFLP e SSR de materiais silvestres e cultivados, cuja colheita foi feita na região cafeeira colombiana. O estudo permitiu concluir que a variabilidade genética observada em R. glaucus *deve-se a que a reprodução sexual cumpre um importante papel mantendo a variabilidade. Demonstrou-se a aplicabilidade dos microsatélites de* Rubus alceifolius *na caracterização de Rubus spp. Colombianas. Todas as espécies estudadas deram padrões de banda muito específicos, diferenciando-as das demais. Os microsatélites deram como resultado bandas exclusivas por espécie para* R. robustus*,* R. urticifolius*,* R. glaucus *e* R. rosifolius*. Os SSR diferenciaram dois grupos de* R. glaucus*: genótipos diplóides e tetraplóides.*

Palavras-chave: AFLP, Microsatélites, Diversidade genética, *Rubus spp.*

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