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Genetic Diversity and Transferability of Rubus Microsatellite Markers to South American *Rubus* Species

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1. Introduction

Rubus L. (Rosaceae) is grown extensively worldwide, in altitudes ranging from 0 to 4500 m above sea level. Found in six continents, this genus is reported to contain approximately 800 species due to biological processes such as hybridization, apomixis, and polyploidy that weaken species boundaries (Thompson, 1995). *Rubus* has been divided into 12 subgenera of which only a few species have been domesticated (Table 1).

Subgenus	Number of species
Anoplobatus (Focke) Focke	6
Chamaebatus (Focke) Focke	5
Chamaemoras (Hill) Focke	1
Comaropsis (Rich.) Focke	2
Cylactis (Raf.)Focke	14 (4 series)
Dalibarda (L.) Focke	5
Dalibardastrum Focke	4
Idaeobatus (Focke) Focke	117 (9 sections)
Lampobatus Focke	10
Malachobatus (Focke) Focke	115 (7 sections)
Orobatus (Focke)	19
Rubus L. (= *Eubatus* Focke)	132 (6 sections)

Table 1. Subgenera and number of species of *Rubus* (Focke, 1910, 1911, 1914).

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

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exclusive to South America. Species representative of the subgenera *Rubus, Orobatus*, and *Idaeobatus* are found in the Colombian and Ecuadorian Andes (Ballington et al., 1993). Vargas (2002) reported six species in Colombia's central Andes: *R. bogotensis*, *R. glaucus*, *R. macrocarpus*, *R. nubigenus*, *R. porphyromallus*, and and *R. urticifolius*. Romoleroux (1992) also reported the existence of nine edible species in Colombia of a total of 44 species reported, and that due to natural crossing, up to 500 varieties can be identified. Besides its ecological relevance, for example as an invasive weed, this genus is an economically important fruit crop for small farmers in the northern Andes, mainly because of the production of *Rubus glaucus* L., commonly known as the Andean blackberry (Fig. 1).

Fig. 1. Plants of *Rubus glaucus* L. growing on the Andes.

Of flowering plant species, the genus *Rubus* is one of the most complexes because of its hybridization, polyploidy, agamospermy, and loss of the concept of species (Alice & Campbell, 1999). Polyploidy and hybridization prevails in the subgenus *Rubus.* Only subgenera *Idaeobatus, Dalibarda*, and *Anoplobatus* are predominantly diploid, whereas *Dalibardastrum, Malachobatus*, and *Orobatus* are exclusively polyploid (Thompson, 1995, 1997). Hybridization occurs in *Rubus* between closely related species (Kraft et al., 1996; Naruhashi, 1979, 1990; Steele & Hodgdon, 1963, 1970) and, in some cases, between subgenera (Alice et al., 1997; Gustafsson, 1942; Jennings, 1978; Weber, 2003).

This species presents traits of two subgenera—*Idaeobatus* and *Rubus*—possibly being a fertile amphidiploid or allotetraploid (n=7, 4x=28) (Delgado et al., 2010; Thompson, 1997). Sympatrically with *R. glaucus*, some other wild *Rubus* species are found in the Andean cordillera along with the introduced and cultivated Eurasian *R. idaeus* L. Because hybridization is a common process that affects species of any genus (Randell et al., 2004), it is reasonable to believe that gene flow is currently taking place between species of this genus.

Studies on the genetic diversity of *Rubus* have been carried out in temperate species, such as *Rubus idaeus* (Graham & Mcnicol, 1995; Graham et al., 1997; Parent & Fortin, 1993) and *Rubus occidentalis* (Parent & Page, 1998), and Asian species (Amsellem et al., 2000). These

works used RAPD, RFLP, and SCAR markers as well as SSR (Antonius-Klemola, 1999). ITS are also been used to study hybrids of *R. idaeus* and *R. caesius* (Alice et al., 1997) .These markers made it possible to confirm the genetic origin of the hybrids and further phylogenetic and evolutionary studies in *Rubus* (Alice, 2002). Recently, major advances have been achieved worldwide in the use of molecular markers in temperate species of *Rubus*, such as DNA fingerprinting to study and characterize genotypes, development of linkage maps, marker-assisted selection, and mapping of QTLs (Antonius-Klemola, 1999; Graham et al., 2002).

To date, molecular markers such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) (Marulanda et al., 2007), and microsatellites (simple sequence repeats, SSRs) (Marulanda et al., 2011) have been used to study the genetic diversity of the Andean blackberry. Previous work carried out by Marulanda et al. (2007) and Marulanda & López (2009) on the genetic diversity of Colombian blackberries identified high phenotypic and molecular plasticity in the *R. glaucus* species known as the "Castilla" blackberry in Colombia's central Andean area. Other wild *Rubus* species present in the Andean region are found near farms where the "Castilla" blackberry is commercially grown. These plants were also submitted to morphological, agronomic, and molecular characterizations using AFLP and SSR molecular markers (Marulanda & López, 2009). A genomic library enriched for microsatellite sequences was recently developed for *R. glaucus.*

This chapter presents the results of the molecular characterization of wild and cultivated *Rubus* species collected in the central Andes of Colombia using SSR markers from other *Rubus* species available in Genbank, together with 11 microsatellite markers isolated from *R. glaucus* and characterized in 39 samples of *Rubus* (Table 2). Intra- and inter-specific differences between *R. glaucus* and its wild relatives were established, generating not only information on the current status of populations, their uses, and distribution, but also information considered crucial to launch a breeding program for *R. glaucus*.

2. Materials and methods

2.1 Plant material and DNA extraction

A total of 39 *Rubus* samples were collected at altitudes ranging from 1800 to 2455 m above sea level in the central Andes of Colombia (between 1° 42´10.7 ´´ and 6° 99¨44´´N and 72° 92´80´´ and 76° 25´ 35.9´´ W), and placed on silica gel (1:10, plant tissue: silica gel) (Table 2). DNA was isolated using the Plant DNAeasy Miniprep kit (QIAGEN®), following the manufacturer's instructions. Several samples did not show any DNA after the isolation procedure so it was necessary to reprocess these samples following the Doyle & Doyle (1990) protocol. In all cases, samples were purified using the protocol described by Castillo (2006).

2.2 Analysis with SSR markers

A total of 36 microsatellite sequences from other *Rubus* species, *R. idaeus* (23 primer pairs) (Series RhM, RiM and Rubus) and *R. occidentalis* (2 primer pairs) (Series mRaCIRRI), and 11 microsatellites from *R. glaucus* were used (Table 3). The microsatellite named as "Rg" was

developed using a genomic library enriched for microsatellite sequences from a cultivated genotype of *R. glaucus,* following the protocol described by Billotte et al. (1999).

Code	Latitude (N)	Longitude (W)	Cultivated/Wild	Altitude (m.a.s.l.)	Characteristics
CVM 1	4°48′06′′	74°24′49′′	*R. glaucus* Cultivated	2455	Thorny
CVM 2	4°47′37′′	74°25′27′′	*R. glaucus* Cultivated	2337	Thorny
CVM 3	4°79′33′′	74°42′68′′	*R. glaucus* Cultivated	2288	Thorny
CVM 4	4°09′09′′	74°23′27′′	*R. glaucus* Cultivated	2007	Thorny
CVM 6	6°99′44′′	72°98′80′′	*R. glaucus* Cultivated	2157	Thorny
CVM 7	6°99′44′′	72°98′80′′	*R. glaucus* Cultivated	2157	Thornless
CVM 8	6°59′39.1′′	72°59′13′′	*R. glaucus* Cultivated	2176	Thorny
CVM 9	7°00′38′′	72°58′39′′	*R. glaucus* Cultivated	2357	Thorny
CVM 10	4°40′00.8′′	76°16′06.6′′	*R. glaucus* Cultivated	2049	Thorny
CVM 11	4°13′23.8′′	76°25′35.9′′	*R. idaeus* Raspberry	2100	Thornless
CVM 12	4°13′23.8′′	76°25′35.9′′	*R. glaucus* Cultivated	2380	Thorny
CVM 13	4°13′23.8′′	76°25′35.9′′	*R. glaucus* Cultivated	2380	Thorny
CVM 15	6°14′52.3′′	75°24′08.5′′	*R. glaucus* Cultivated	2150	Thorny
CVM 18	6°09′15.4′′	75°23′00.1′′	*R. glaucus* Cultivated	2000	Thornless
CVM 19	6°09′15.4′′	75°23′00.1′′	*R. glaucus* Cultivated	2000	Thorny
CVM 20	6°09′15.4′′	75°23′00.1′′	*R. glaucus* Cultivated	2000	Thorny
CVM 22	1°57′45.2′′	76°14′17.4′′	*R. glaucus* Cultivated	1840	Thorny
CVM 25	1°51′41.8′′	76°21′21.9′′	*R. glaucus* Cultivated	2140	Thorny
CVM 26	1°53′42.8′′	76°18′45.5′′	*R. glaucus* Cultivated	1850	Thorny
CVM 27	1°42′10.7′′	76°12′08.3′′	*R. glaucus* Cultivated	2000	Thorny
CVM WILD	_____	_____	*R. glaucus* Wild	_____	Wild
CVM A	4° 44′45.1′′	75° 36′39.6′′	*R. glaucus* Cultivated	2000	Thornless
CVM B	4° 39′7′′	75° 35′26.3′′	*R. glaucus* Cultivated	2014	Thorny
CVM C	4°38′36′′	75°28′41,5′′	*R. glaucus* Cultivated	2300	Thornless
CVM D	4°48′99.2′′	75°41′86′′	*R. glaucus* Cultivated	1950	Thorny
CVM E	5° 2′2.7′′	75° 27′10.5′′	*R. glaucus* Cultivated	1800	Thorny
95	4°21′22.5′′	75°42′10.1′′	*R. glaucus* Wild	1805	Wild
107	4° 33′51.9′′	75° 39′14.7′′	*R. urticifolius*	1800	Wild
106	4°39′7′′	75° 35′26.3′′	*R. urticifolius*	1574	Wild
97	4° 41′23.3′′	75°37′33.8′′	*R. glaucus* Wild	1997	Wild
86	4° 38′36′′	75° 29′9.5′′	*R. glaucus* Wild	2430	Wild
22	5° 4′ 35.8′′	75° 32′ 31.1′′	*R. glaucus* Cultivated	1947	Thorny
37	5° 0′50.3′′	75° 32′11.7′′	*R. urticifolius*	1777	Wild
44	4° 45′ 00.2′′	75° 36′ 39′′	*R. urticifolius*	1879	Wild
MSA 1	4°52′15.0′′	75°37′32.4′′	*R. glaucus* Cultivated		Thornless
MSA 2	4° 44′45.1′′	75° 36′39.6′′	*R. glaucus* Cultivated	1850	Thornless
MSA 3	4°38′36′′	75°28′41.5′′	*R. glaucus* Cultivated		Thornless
MSA 4	4°11′36.1′′	75°48′14.6′′	*R. glaucus* Cultivated	2000	Thornless
MSA 5	5°0′50.3′′	75°32′11.7′′	*R. glaucus* Cultivated	1825	Thornless

Table 2. Samples of *Rubus* species, accessions of *R. glaucus*, and collection sites.

Amplification reactions were performed in a final volume of 12.5 µl, with 5 ng genomic DNA, 0.3 µM of each primer, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl), 200 µM of each dNTP, 2 mM $MgCl_2$ and 1.0 unit of Taq DNA polymerase. The PCR was performed according to the following parameters: 94 °C for 4 min, 10 cycles of 94 °C for 30 sec, 65 °C (−1 °C/cycle) for 30 sec and 72 °C for 1 min; 35 cycles of 94 °C for 15 sec, annealing temperature (°C) for 30 sec and 72 °C for 1 min; and 72 °C for 5 min.

2.3 Statistical analysis

The allelic diversity of the SSR was evaluated by determining polymorphism information content (PIC) value, as described by Bonstein et al. (1980) and cited and modified by Anderson et al. (1993), as described in Equation 1:

$$_{n}PIC_{i} = 1 - / \sum_{j=1} P_{ij^2} \quad (1)$$

Where P_{ij} is the frequency of the *j*th pattern, *i* is the sum, and *n* are the patterns. To measure the utility of the marker systems, average heterozygosity and expected and observed heterozygosity were calculated. The partitioning of genetic variation within and among the groups by the SSR marker system was achieved by analysis of molecular variance (AMOVA) using the same software. Analyses were performed in GenAlex (Peakall & Smouse, 2006) and Arlequin v.3.5 (Excoffier & Lischer, 2010).

3. Results and discussion

3.1 Genetic diversity and variability

A total of 41 loci and 133 alleles were detected. The number of alleles observed for each locus ranged from 2 to 6, with an average of 4.6 alleles per locus. The PIC value varied between 0.07 and 0.61 (average 0.48), and the discriminating power (D) ranged from 0.05 to 0.52 (average 0.27). Observed heterozygosities (Ho) were 0.078–1.0 (average 0.84) and expected heterozygosities (He) were 0.07-0.582 (average 0.473).The highest PIC value (0.61) was found in the Rg-D7 locus, which presented a high number of alleles (5). To compare the efficiency of markers to identify varieties, the D value of each primer was estimated. The highest D value (0.52) was also found in the Rg-D7 locus (Table 3).

Similar results were reported by Castillo (2006), who used 12 SSRs to analyze an extensive collection of North American *Rubus* (raspberry) germplasm. Results indicated from 3 to 16 alleles per locus, with an average of eight alleles per locus and a total number of alleles of 96. More recently, Flores et al. (2010) isolated 12 microsatellites from SSR-enriched genomic libraries of *R. idaeus.*

Another measure of genetic variability is the presence of exclusive alleles per loci and genotype (Tables 4 and 5). The genotypes presenting the highest number of exclusive alleles are listed in Table 4, with *R. idaeus* genotype CVM11 ranking highest, which could be attributed to the fact that most of the SSRs used are derived from *R. idaeus*. Of the evaluated microsatellites, those of the series "Rubus" were one of the most polymorphic groups and detected the highest number of alleles in the study population. It should be mentioned that exclusive alleles also appear in wild genotypes and in genotypes 106 and 107, which belong to the species *R. urticifolius*. The loci in which the private alleles were detected are very important for genotype differentiation, particularly in the case of the thornless genotypes.

Fig. 2 presents the results of the principal coordinates analysis. There is no clear differentiation of genotypes based on collection sites; however, the genotypes belonging to the species *R. urticifolius* (37, 44, 106, and 107) are separate from the species *R. glaucus*, both cultivated and wild. Likewise, the species *R. idaeus* is separate from the species *R. urticifolius*

	Locus	Motif	GenBank accession no.	Primer sequences (5´- 3´)	Size (bp)	T (°C)	Number of loci	PIC*	A*	D*	He*	Ho*
1	RhM018[3]	$(CTT)_6$	FJ194447	F-CACCAATTGTACACCCAACAAC R-GATTGTGAGCTGGTGTTACCAA	379	52	1	0.3510	2	0.5094	0.279	0.435
2	RhM023[3]	$(CAT)_5$	FJ194449	F-CGACAACGACAATTCTCACATT R-GTTATCAAGCGATCCTGCAGTT	196	52	1	0.4864	2	0.3388	0.463	0.835
3	RhM011[3]	$(TC)_{18}$	FJ194446	F-AAAGACAAGGCGTCCACAAC R-GGTTATGCTTTGATTAGGCTGG	280	58	2	0.5419	3	0.1448	0.530	0.969
4	RhM001[3]	$(CA)_7$	FJ194444	F-GGTTCGGATAGTTAATCCTCCC R-CCAACTGTTGTAAATGCAGGAA	232	50	1	0.1328	3	0.2148	0.117	0.078
5	RhM021[3]	$(TC)_6$	FJ194448	F-CAGTCCCTTATAGGATCCAACG R-GAACTCCACCATCTCCTCGTAG	282	50	1	0.5461	3	0.219	0.536	1.000
6	RhM003[3]	$(TG)_{10}$	FJ194445	F-CCATCTCCAATTCAGTTCTTCC R-AGCAGAATCGGTTCTTACAAGC	200	48	1	0.5151	3	0.047	0.512	1.000
7	RiM017[3]	$(TG)_6$	FJ194453	F-GAAACAGGTGGAAAGAAACCTG R-CATTGTGCTTATGATGGTTTCG	194	58	1	0.5869	3	0.2148	0.556	0.897
8	RiM015[3]	$(ATC)_5$	FJ194452	F-CGACACCGATCAGAGCTAATTC R-ATAGTTGCATTGGCAGGCTTAT	350	58	1	0.4462	2	0.4451	0.367	0.610
9	Rubus 76b[2]	$(CT)_5(CT)_4$	___	F-CTCACCCGAAATGTTCAACC R-GGCTAGGCCGAATGACTACA	190-210	55	1	0.6004	4	0.2463	0.567	0.897
10	Rubus 16a[2]	$(AT)_8(GT)_{11}$	___	F-TGTTGTACGTGTTGGGCTTT R-GGGTGTTTGCCAGTTTCAGT	169	55	3	0.3461	3	0.0588	0.533	1.000
11	Rubus 116a[2]	$(CT)_{12}(T)_{10}$	___	F-CCAACCCAAAAACCTTCAAC R-GTTGTGGCATGGCCTTTTAT	299	55	2	0.5297	3	0.053	0.520	0.969
12	Rubus 105b[2]	$(AG)_8$	___	F-GAAAATGCAAGGCGAATTGT R-TCCATCACCAACACCACCTA	165 -173	55	2	0.5924	6	0.2171	0.571	0.948
13	Rubus 137a[2]	$(TG)_8(TA)_4$	___	F-TGTGAGCAGAGTGAAGGAGCTA R-AGCATTATTCGCGCAGTTTT	198	55	1	0.4995	2	0.0465	0.496	0.969
14	Rubus 259f[2]	$(CT)_4\ (AG)_8$	___	F-TGGCACAAGAAGCCTGTAAC R-TCCCATATCCCTCAGCATTC	265	55	1	0.5717	4	0.1727	0.552	1.000
15	Rubus 98d[2]	$(GAA)_5(GA)_{10}$	___	F-GGCTTCTCAATTTGCTGTGTC R-TGATTTGAAATCGTGCGGTTA	198	55	2	0.5880	4	0.2558	0.544	0.861
16	mRaCIRRI1G3[1]	$(GA)_{28}$	AF205116	F-CTCTACAAAAGGATCTGCATGA R-CAGCAAAAGTGAAATGGTTCA	195-265	55	2	0.5473	3	0.2566	0.524	0.881
17	mRaCIRRIV2A8[1]	$(CA)_{12}(CT)_{11}$	AF261693	F-TAAAAAGGCGCAACAGTCG R-AGACACAGAAACAGGCATCG	191-237	55	2	0.5604	3	0.23	0.548	0.959
18	Rubus 285a[2]	$(TC)_9$	___	F-TCGAGAAGCTTGCTATGCTG R-GGATACCTCAATGGCTTTCTTG	138-167	55	1	0.5497	4	0.2462	0.543	1.000

19	Rubus 145a[2]	$(GT)_7$	——	F-TGTCCCAGCTTTCTGGTTTC R-GGCATCTGTGCGGTAAAAAT	131	55	1	
20	Rubus 262b[2]	$(AG)_{15}$	——	F-TGCATGAAGGCGATATAAAGG R-TCCGCAAGGGTTGTATCCTA	217-225	55	1	
21	Rubus 107a[2]	$(AG)_8$	——	F-GCCAGCACCAAAAACCTACA R-TTTCACCGTCAAGAAGAAAGC	179	55	1	
22	Rubus 251a[2]	$(GA)_{10}$	——	F-GCATCAGCCATTGAATTTCC R-CCCACCTCCATTACCAACTC	157-283	55	1	
23	Rubus 270a[2]	$(GA)_{10}$	——	F-GCATCAGCCATTGAATTTCC R-CCCACCTCCATTACCAACTC	167-185	55	1	
24	Rubus 2a[2]	$(GT)_{12}$-G-$(GT)_8$	——	F-TGAGGGAAGAAGAGGCAAGA R-CACGTGTGACCCCAATGATA	175-180	55	1	
25	Rubus 194h[2]	$(GA)_{12}$	——	F-TGTGTTGTTCTCTGCAACCA R-AGCCCTTACTTTTCCTGCAA	100-110	55	1	
26	Rg-A6[4]	$(GAA)_4$	——	F-AGCGCAAGGACTTCTACC R-TTCAGCTCCGGTAGTAGC	282-293	55	1	
27	Rg-A12-1[4]	$(GA)_{23}$	HQ637525	F-TCATGTTCATGTTCGTGTG R-CTAACACGCGATAGAATAGG	387-432	48	1	
28	Rg-A12-2[4]	$(AC)_8$	HQ637525	F-GCGGGCATTCTCTTGCTTAC R-GCGGTTCGTGACTCAGACAG	179-194	58	1	
29	Rg-B7[4]	$(CA)_{11}$	HQ637519	F-TACGCGTGGCACTAACAC R-TCAGAGCACTCCAAGAAGG	150-171	58	2	
30	Rg-B8[4]	$(AG)_{23}$	HQ637493	F-CTGATGAGTAGCTGGTTTCTC R-GTCTGAAATAGCACATGGAC	185-230	52	1	
31	Rg-B9[4]	$(CA)_9$	HQ637500	F-TTCAGAACCTCATGCAGAGCC R-TTCACTCAGGAGGAGGAGGTG	348-367	57	1	
32	Rg-D2[4]	$(GA)_{16}$	HQ637534	F-AAACCCTAATTTGTTGAC R-CCCAAGCTAGATTTCTCTC	418-449	48	1	
33	Rg-D7[4]	$(GA)_{25}$	HQ637494	F-AACCATCGGTGTCGACCTC R-GGCTCTTGACCGGTAACTTAG	61-110	55	1	
34	Rg-F3[4]	$(GA)_{17}(GT)_9$	HQ637516	F-GCCAATGAAACGGAAAGAC R-GCCTTCACTCATATCATTCTCC	210-265	60	1	
35	Rg-F7[4]	$(CT)_{21}$	HQ637527	F-GCAGGTGTGTCTATGTGATATG R-ATTCCCAACGACTACATAATTC	290-331	55	1	
36	Rg-F8[4]	$(GA)_{14}$	HQ637499	F-CATGCTGGCTTTGCTGCTC R-TGCTTACGCGTGGACTAACAG	267-294	60	1	

[1] *R. alceifolius* (Amsellem et al., 2001); [2] *R. idaeus* (Graham et al., 2002, 2004) ; [3] *R. idaeus* and blackberry "Marion" (Marulanda et al., 2010 (data not published).
* PIC = polymorphic information content; A = allele number; D = discrimination power; He = expected heterozy heterozygosity.

Table 3. Microsatellite sequences and characteristics of each SSR used to evaluate *Rubus* materials.

and from cultivated and wild genotypes of *R. glaucus*. Genotype CVM Wild of the species *R. glaucus* is notably separate from the genotypes of the same species.

Locus	Private alleles	Frequency
RhM003	2	0.125
RhM001	2	0.250
Rubus 76b	1	0.125
Rubus 98d	1	0.333
Rubus 98d	1	0.333
mRaCIRRI1G3	2	0.125
mRaCIRRIV2A8	5	0.125
Rubus 128a	3	0.125
Rubus 262b	3	0.125
Rubus 107a	5	0.250
RgA12-1	4	0.125
RgB7	2	0.125
RgB7	1	0.125
RgB7	2	0.125
RgF3	1	0.250
RgF7	1	0.250
Rubus 116a	1	0.250
Rubus 105b	2	0.100
Rubus 105b	5	0.100
Rubus 105b	4	0.200
RgA12-1	2	0.100
Rubus 98d	3	0.222

Table 4. Summary of private alleles.

Sample	No. of loci with private alleles	Loci with private alleles
R. glaucus cultivated (CVM 10)	1	RgB7
R. idaeus (CVM 11)	14	RhM003, RhM001, Rubus 76b, Rubus 98d, Rubus 98d, mRaCIRRI1G3, mRaCIRRIV2A8, Rubus 128a, Rubus 262b, Rubus 107a, RgA12-1, Rg B7, RgF3, RgF7
R. glaucus cultivated (CVM 13)	1	RgB7
R. glaucus wild (CVM WILD)	3	Rubus 116a, Rubus 105b, Rubus 105b
R. glaucus cultivated (22)	1	RgA12-1
R. urticifolius (107)	1	Rubus 98d
R. urticifolius (106)	1	Rubus 98d

Table 5. List of samples with one or more private alleles.

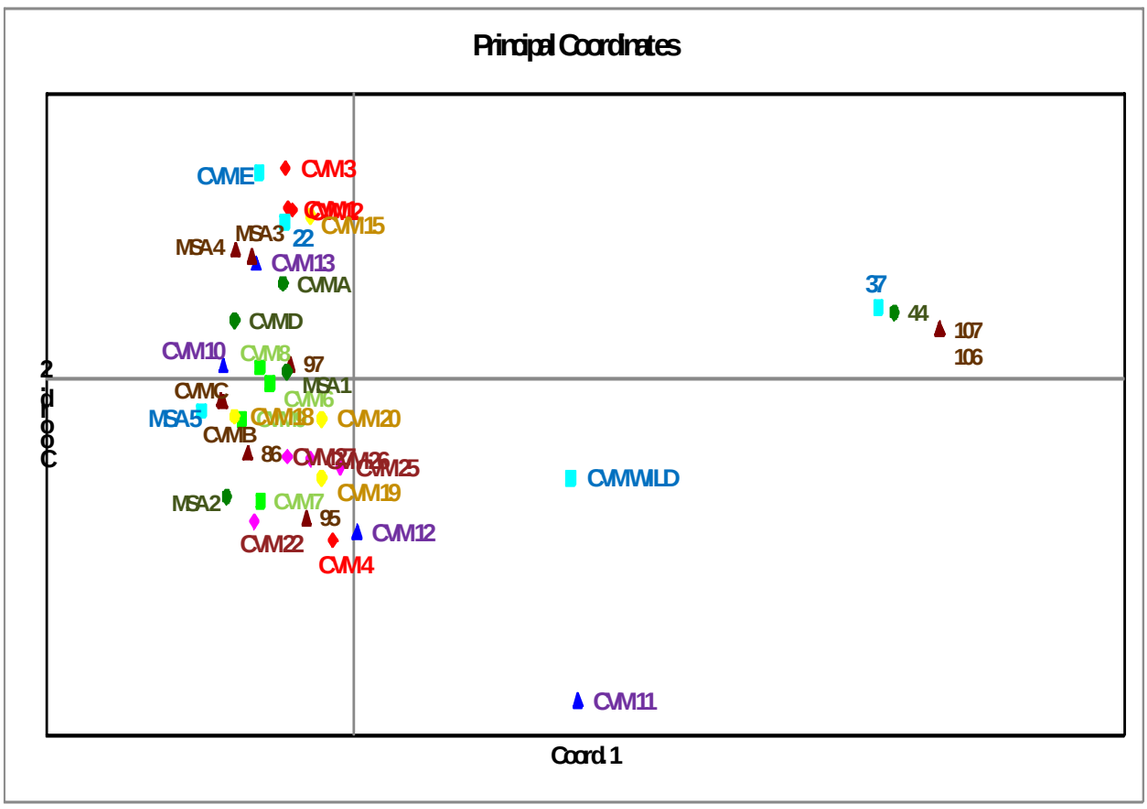

Fig. 2. Principal coordinates analysis among *Rubus* genotypes based on genetic distance .

In addition to the genetic diversity measurements already mentioned, the analysis of molecular variation (AMOVA) revealed 98%variability among all genotypes and 2% variation between populations (Michalakis & Excoffier, 1996) (Table 6). These data agree with those observed in the principal coordinates analysis, where variation is mostly attributed to individuals variation. 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 fruit quality positively, while increasing ploidy levels and taxonomic proximity.

Source of variation	df*	Sume of squeres	Variance components	Estandar Variation	% of variation
Among populations	7	18.067	2.581	0.054	2%
Within populations	31	72.005	2.323	2.323	98%
Total	38	90.071		2.377	100%

*df= degrees of freedom

Table 6. Summary of molecular variation (AMOVA) among *Rubus* genotypes.

3.2 Transferability of microsatellite sequences

Microsatellites RhM and RiM developed from *R. idaeus* showed cross-species amplification in *R. glaucus* genotypes and in other two wild species of *Rubus,* with 2 and 3 alleles per locus. High D values were found for loci RhM018 (0.5094) and RiM015 (0.4451). The "Rubus" microsatellite markers, also from *R. idaeus*, were the most polymorphic and presented the highest number of alleles per locus: Rubus 105b with 6 alleles and Rubus 107a with 5. The microsatellite markers from *R. alceifolius* showed 3 alleles and D values ranging from 0.23 to 0.2566. The *R. glaucus* microsatellites (Rg series) amplified for other wild species, with the number of alleles ranging from 2 to 5 per locus.

The cross-species amplification data suggested that microsatellites developed for *R. idaeus*, *R. alceifolius*, and *R. glaucus* can be potentially useful for genetic diversity studies of different *Rubus* species. In the case of conservation programs, they should prove useful for characterizing natural populations and germplasm collections, as well as for determining the degree of relatedness between individuals or groups of accessions.

The microsatellites developed by Amsellem et al. (2001) to study *R. alceifolius*, subgenus *Malachobatus,* that grows in Southeast Asia were used for the characterization of Andean *Rubus*, the transferability and applicability of microsatellites of *R. alceifolius* to study and evaluate the diversity of *Rubus* species in the American Andes were demonstrated; results were similar to those obtained in Asian species.

Amsellem et al. (2001) also observed amplification from 3 to 4 alleles per individual in the species *R. alceifolius*, confirming the suspicion that this is a tetraploid species. Based on the analysis carried out by Amsellem et al. (2001), the present study produced between 3 and 5 alleles for *R. glaucus* and between 2 and 4 alleles for *R. urticifolius*., suggesting that both cultivated and wild materials of *R. glaucus* have ploidy levels greater than those of *R. urticifolius*. This polyploidy was also described by Hall (1990), who explained that *Rubus* species used in plant breeding programs have produced euploid and aneuploid hybrids and that diploid, triploid, tetraploid, hexaploid, septaploid, octaploid, and nonaploid cultivars have been selected, most of them tetraploids.

4. Conclusion

Wild forms are also usually found at sites where *Rubus* species are cultivated, particularly in forest clearings, along roadsides, and on hillsides. Both cultivated and wild forms have the potential for interacting in different ways with cultivated materials. Cultivars can influence the genetic diversity of natural populations through gene transfer by pollen and wild populations are a potential source of genetic material for improvement programs.

This evaluation of the status of genetic resources of the species *R. glaucus* and related wild species serves to provide guidelines for conservation and breeding efforts aiming to promote the development of cultivated species important for the rural economies of South America's Andean region.

Using microsatellites from other *Rubus* species has proven to be a very useful strategy to differentiate between wild and cultivated *R. glaucus* genotypes, as well as between thorny and thornless cultivars.

The development of a genomic library enriched with microsatellites and the design of microsatellite sequences for the Andean specie *Rubus glucus,* is allowing a deeper comprehension of the genetic variability existing among cultivated and wild genotypes as well as the relationships between the cultivated specie and the wild relatives.

The Analysis of molecular variation (AMOVA) showed a higher variability distributed between genotypes than between populations, which agrees with the results obtained in the principal coordinates analysis.

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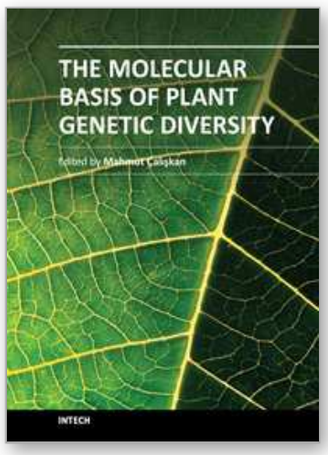

The Molecular Basis of Plant Genetic Diversity

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The Molecular Basis of Plant Genetic Diversity presents chapters revealing the magnitude of genetic variations existing in plant populations. Natural populations contain a considerable genetic variability which provides a genomic flexibility that can be used as a raw material for adaptation to changing environmental conditions. The analysis of genetic diversity provides information about allelic variation at a given locus. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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