SENRA Academic Publishers, Burnaby, British Columbia
Vol. 3, No. 3, pp. 875-885, 2009
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

CHARACTERIZATION OF THORNLESS *RUBUS GLAUCUS* IN COLOMBIA

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ABSTRACT

High phenotypical plasticity has been identified within the species *Rubus glaucus* Benth, commonly known as 'mora de castilla' or Castilla blackberry, in Colombia's coffee-growing area using AFLP molecular markers as well as morphological characters. Thornless plants have been observed among the blackberry materials grown. These plants present the same characteristics of productivity as thorny varieties are widely distributed. A first approximation to the genetic relationship between thorny and thornless materials indicated that thornless blackberry could possibly originate from the departments of Risaralda or Quindío. This work focuses on identifying the genetic and morpho-agronomic differences in thornless *R. glaucus* materials found in the coffee-growing region, especially in the department of Risaralda. Materials were collected from five different locations**:** two in the department of Risaralda, one in the department of Caldas, and two in the department of Quindío. For the morpho-agronomic characterization, 40 farmers were selected in the municipalities of Santa Rosa de Cabal and Guática, Risaralda, each farmer planting 50 plants from each of the five different collection sites, which had been multiplied *in vitro*, as well as 50 plants of thornless blackberry propagated by farmers, totaling 12,000 plants. Eight microsatellite (SSR) sequences were used to study 23 regional accessions of *Rubus*, including thorny and thornless *R. glaucus*, both cultivated and wild. Genetic and molecular differences were observed between thornless blackberry materials of different origins.

Keywords: *Rubus glaucus*, thornless blackberry, Castillo blackberry, genetic characterization, Colombia.

INTRODUCTION

American *Rubus* species are perennial shrubs that show a broad range of growth habits: from erect to semi-erect to creeping (Daubeny, 1996). In addition, their morphological and phenotypical characteristics are probably among the most well known. *Rubus* species of economic importance present perennial roots, foliage that produces biannual productive canes or branches with flower structures, and new branches, known as primary branches, which present vegetative growth and form tillers (Moore and Skirvin, 1990).

In temperate regions, the prolonged exposure of blackberries to the cold induces both vegetative and primary branches to develop flower buds or productive branches, also known as female branches, which die after producing fruit. Some species produce flowers without being exposed to the cold in addition to another very desirable characteristic—a productive primary branch capable of producing flower branches during the early growth cycle (López-Medina and Moore, 1999; Lopez-Medina *et al*., 2000). However, in the case of *Rubus glaucus*, the formation of female branches does not occur with the same mechanisms. According to popular belief, this formation is not a physiological phenomenon strongly controlled by environmental conditions, as described elsewhere in the world, but is determined rather by genetic factors.

Another interesting characteristic of most *Rubus* species is the absence of thorns or brambles. For example, commercial varieties 'Chester Thornless', 'Thornfree', and 'Thornless Evergreen' are thornless genotypes from the United States. This monogenic characteristic is controlled by a recessive gene that has been widely studied in Europe and the United States (Jennings and Ingram, 1983; Hall, 1990; Skirvin *et al.*, 2005; Strik *et al*., 2006). In Colombia, farmers of the department of Quindío discovered a thornless blackberry biotype, which has been multiplied by farmers throughout the country, without really understanding its agronomic and genetic characteristics. This thornless blackberry offers great potential for blackberry cultivation not only in the Quindío region but throughout the country.

Previous work carried out by Aguilar (Aguilar, SB. 2006) and Marulanda *et al*. (2007) revealed high phenotypical and molecular plasticity in *R. glaucus* in Colombia's coffee-growing region. Other wild species of *Rubus* found in the area and nearby lots planted to the Castilla blackberry were also submitted to morphological and molecular characterization, using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) molecular markers.

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Some of the cultivated thornless blackberry genotypes present the same productivity and same fruit size as thorny plants. Because of their interesting phenotypical characteristics and reduced production costs, these materials, commonly known as thornless blackberry, have been massively disseminated by farmers in the region using vegetative methods. Marulanda *et al*. (2007) found two possible sites of origin of thornless *R. glaucus* materials: one is located in the department of Risaralda and the other in the department of Quindío. There could be other sources of thornless *R. glaucus*, but further study is necessary.

Many studies using molecular markers, such random amplified polymorphic DNA (RAPD), AFLP, and SSR, have been conducted in *Rubus* (Graham and MacNicol, 1995; Graham *et al*., 2002, and 2004; Marulanda and Márquez, 2001**;** Marulanda *et al*., 2007; Amsellem *et al*., 2000 and 2001). However, the studies conducted with SSR markers are of particular interest because these markers are co-dominant, highly replicable, frequent in most eukaryotes, and reveal a high allelic diversity (Mohan *et al*., 1997).

The SSR of related species has been used in diversity and genetic variability studies (Stafne *et al*., 2005) and several have demonstrated the success of this strategy, which is based on the transferability of SSR primers between species and between genera of the Rosacea family (Ashley *et al*., 2003; Cipriani *et al*., 1999; Decroocq *et al*., 2003; Dirlewanger *et al*., 2002; Graham *et al*., 2002; Lewers *et al*., 2005; Marulanda *et al*., 2007).

For example, Stafne *et al*. (2005) used SSR developed from Glen Moy red raspberries (*R. idaeus* ssp. *idaeus*) reported by Graham *et al*. (2002, 2004). On the other hand, Amsellem *et al*. (2001) used SSR from wild blackberry (*R. alceifolius*) from Asia; Lewers et al. (2005) used SSR from strawberry (*Fragaria* × *ananassa*), Ashley *et al*. (2003) used SSR from *Fragaria virginiana*; and James *et al*. (2003) SSR from *Fragaria vesca* to evaluate parental genotypes of North American raspberry and blackberry and determine the level of polymorphism present in parental lines. Stafne *et al*. (2005) found 60 SSRs available for evaluating American blackberries and raspberries.

This study therefore aims to (1) identify the genetic and morpho-agronomic differences of thornless *R. glaucus* materials grown in the department of Risaralda and (2) characterize cultivated and wild *Rubus* materials found in Colombia's coffee-growing region both genetically and morphologically, using SSR molecular markers.

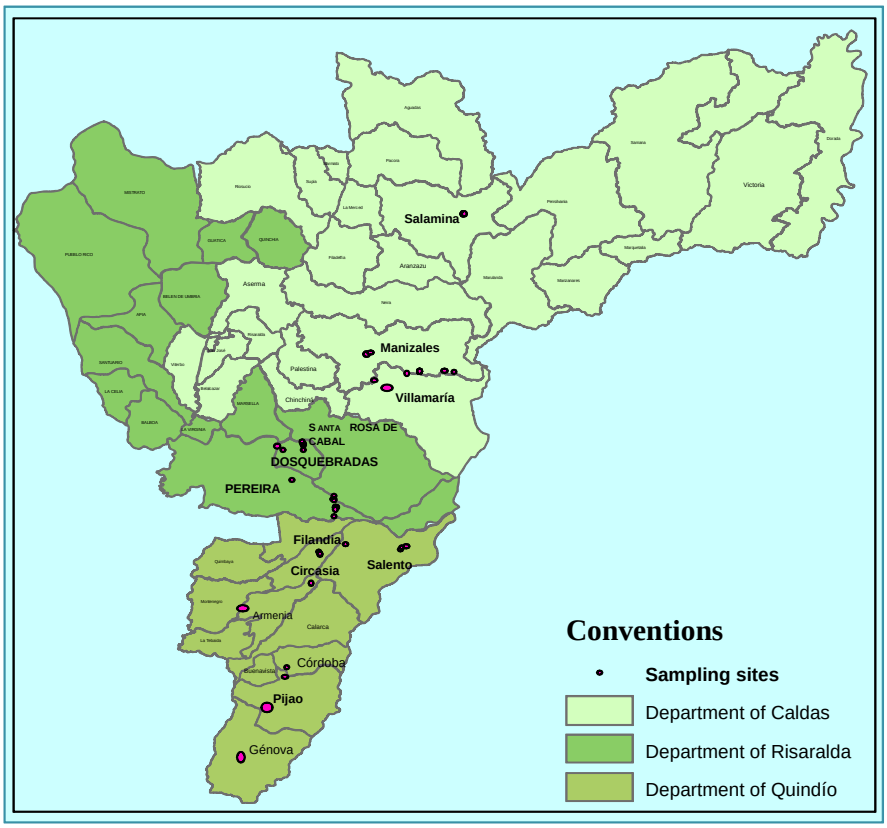

Fig. 1. Collection sites of cultivated and wild blackberries in Colombia's coffee-growing region.

MATERIALS AND METHODS

Collection of Study Materials

From 2005 to 2008, plants of cultivated and wild blackberry species were collected in several municipalities of Colombia's coffee-growing region where blackberry is grown (Fig. 1).

Morpho-agronomic Characterization

Thornless *R. glaucus* accessions were collected from five different collection sites in three different departments of Colombia (Risaralda, Caldas, and Quindío). Each accession was assigned a code beginning with the letter P and numbered 1 to 5. Table 1 lists the different sampling sites and the identification code assigned to each material**.** Blackberry plants were multiplied in vitro and, after hardening, a total of 50 plants from each of the five collection sites (P1, P2, P3, P4, P5) were delivered to 20 farmers in each of the two municipalities of Risaralda (Santa Rosa de Cabal and Guática). Thornless plants from Santa Rosa de Cabal propagated by the farmers (P6) were used as control. Overall, each farmer established 300 plants, totaling 6,000 plants per municipality and 12,000 plants for the entire experiment. Fifteen morpho-agronomic variables were evaluated as follows: number of female and male branches, number of runners, length of internodes of female and male branches, width and length of folioles on female and male branches, stem diameter on female and male branches, plant height at weeks 15 and 32 after planting, number of flower buds, and days to flowering.

STATISTICAL ANALYSIS

A multivariate analysis was performed and groups were identified by cluster analysis, using the SAS program. Information on origins and sites was compared and 12 combinations were identified for the cluster analysis (Table 2). Data were supported by the results of the multiple correspondence analysis.

Evaluating Thorny and Thornless Accessions using SSR Markers

Twenty-three microsatellite sequences developed by Amsellem (2001) and Graham *et al*. (2002 and 2004) were evaluated and, of these, eight SSRs were selected for their polymorphism and amplification quality. Twenty-three regional accessions of *Rubus*, including thorny and thornless *R. glaucus*, both cultivated and wild, were analyzed as follows.

- 9 wild and cultivated accessions of thornless *R. glaucus*
- 4 wild accessions of thorny *R. glaucus*
- 2 cultivated accessions of thornless *R. glaucus*
- 1 accession of *Rubus rosifolius*
- 6 accessions of *Rubus urticifolius*
- 1 accession of *Rubus robustus*

To determine groups of genetic diversity, the genetic similarity (GSij) was calculated based on the formulas of

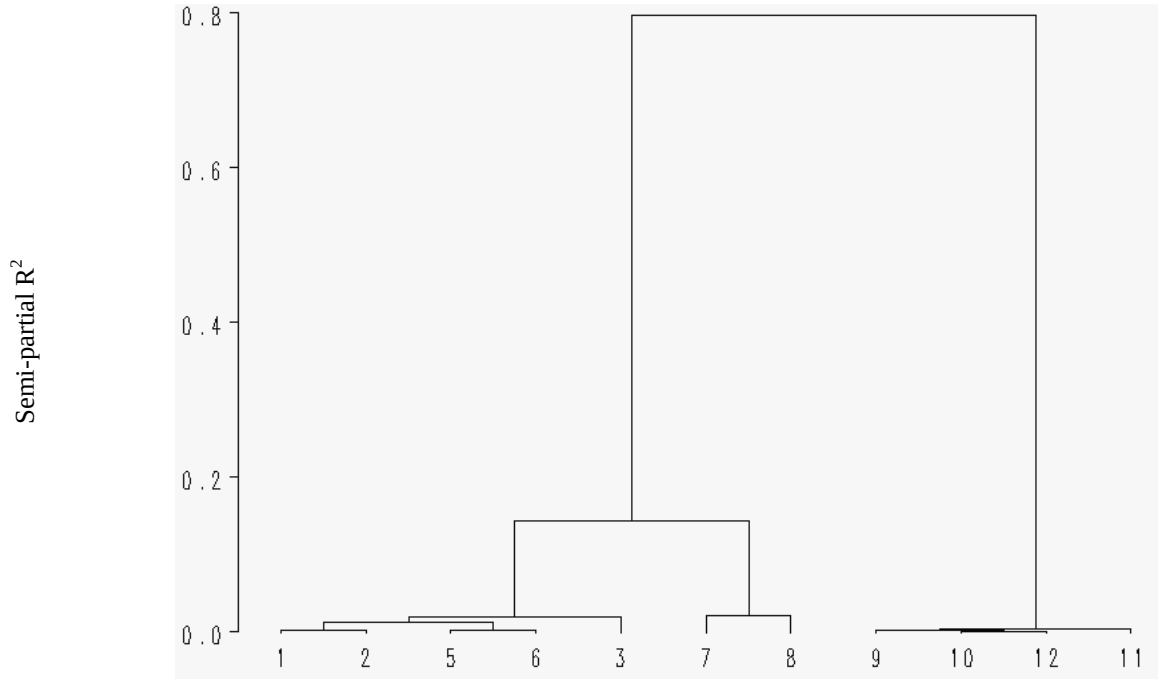

Fig. 2. Dendogram and cluster analysis based on morpho-agronomic characterization.
Treatments* see table 2

Dice (1945) and Nei and Li (1979). Genotypes were grouped based on dissimilitude values (1-GSij) between all genotype pairs (Sneath and Sokal, 1973), using the unweighted pair-group method with arithmetic average (UPGMA) with the statistical package NTSys PC version 2.02i (Rolhf, 1998).

RESULTS AND DISCUSSION

Morpho-agronomic Characterization

Table 3 presents the cluster grouping of the 12 combinations described, while table 4 presents the average, minimum, and maximum values, standard error, and coefficient of variation for each morpho-agronomic variable, regardless of the site and of the material. Data on plant height at weeks 15 and 32 after planting present coefficients of variation of 85.9% and 58.2%, respectively. This variation can be attributed to the difference in vegetative growth presented by plants, which is strongly influenced by agricultural tasks. The variable "number of runners" presents a coefficient of variation of 82.6%, with a maximum value of 3, a minimum value of 0, and an average value of 1 runner, which can be attributed to the difficulty in identifying these branches because they are easily confused with thin branches.

According to principal component analysis, the variables described are classified into six components, in such a way that those contributing the greatest variation with regard to total variation are located in the first component and those contributing the smallest variation in the sixth component (Table 5).

Variables presenting the greatest variation were, in descending order: length of foliole on the male branch, width of foliole on the male branch, stem diameter on the male branch, number of runners, and number of male branches. The number of male branches indirectly determines the productive capacity of a material because, after pruning, male branches can become female branches.

The contribution of variables related to the productive capacity, such as number of female branches and number of flower buds, to total variation was smaller, respectively ranking fifth and sixth, which indicates that these variables alone have a smaller capacity to differentiate materials. The variable "number of flower buds" is directly related to fruit production and presents the smallest contribution to total variation, meaning that it is highly unlikely that this variable can be used to differentiate materials.

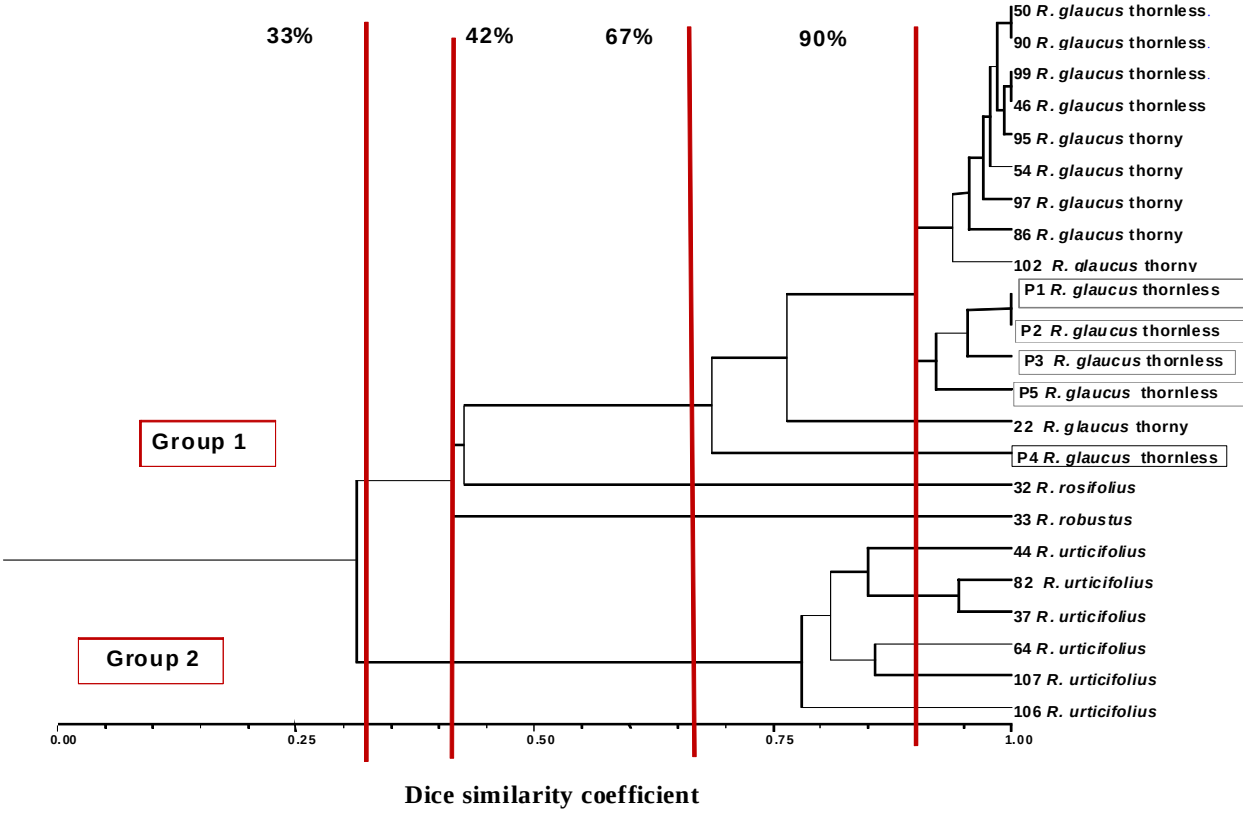

Fig. 3. Dendrogram of blackberries estimated by Dice similarity index (1945), based on SSR markers.

The dendrogram in figure 2 shows the grouping of materials depending on the combination of site of origin and planting site. Two groupings are possible: the first separates the materials originating from Villamaría (Caldas) and Génova (Quindío) and planted in Santa Rosa de Cabal and Guática from the others. The second conserves the distance from these combinations, but separates materials originating from SENA-Manizales (Caldas) and Pereira (Risaralda) and planted in Guática. Overall, the dendrogram shows that the materials originating from Villamaría (Caldas) and Génova (Quindío) are promising and different, regardless of the site where they are planted.

Based on the dendrogram, the three groups were submitted to cluster analysis (Table 3), resulting in the following combination of materials and planting sites:

Cluster 1: Material originating from Santa Rosa de Cabal and planted in Santa Rosa de Cabal; material from Santa Rosa de Cabal propagated by farmers and planted in Santa Rosa de Cabal (control); material originating from

Table 1. Collection sites of blackberry materials to be evaluated in the field and assigned code.

Collection site	Department	Assigned ID code
Santa Rosa de Cabal	Risaralda	P1
SENA-Manizales	Caldas	P2
Alto Manzano, Pereira	Risaralda	P3
Cumaral, Génova	Quindío	P4
Papayal, Villamaría	Caldas	P5
Material from Santa Rosa de Cabal propagated by farmers (control)	Risaralda	P6

Table 2. Combinations used in the cluster analysis, origin of materials and evaluation site.

Combination	Origin	Evaluation site
1	Santa Rosa de Cabal	Santa Rosa de Cabal
2	Santa Rosa de Cabal (control)	Santa Rosa de Cabal
3	Santa Rosa de Cabal	Guática
4	Santa Rosa de Cabal (control)	Guática
5	SENA-Manizales	Santa Rosa de Cabal
6	Pereira	Santa Rosa de Cabal
7	SENA-Manizales	Guática
8	Pereira	Guática
9	Génova	Santa Rosa de Cabal
10	Villamaría	Santa Rosa de Cabal
11	Génova	Guática
12	Villamaría	Guática

Table 3. Materials evaluated at two sites and group identification by cluster analysis.

Combination	Origin	Site	Cluster 1	Cluster 2
1	Santa Rosa de Cabal	Santa Rosa de Cabal	1	1
2	Santa Rosa de Cabal (control)	Santa Rosa de Cabal	1	1
3	Santa Rosa de Cabal	Guática	1	1
4	Santa Rosa de Cabal (control)	Guática	1	1
5	SENA-Manizales	Santa Rosa de Cabal	1	1
6	Pereira	Santa Rosa de Cabal	1	1
7	SENA-Manizales	Guática	3	1
8	Pereira	Guática	3	1
9	Génova	Santa Rosa de Cabal	2	2
10	Villamaría	Santa Rosa de Cabal	2	2
11	Génova	Guática	2	2
12	Villamaría	Guática	2	2

Santa Rosa de Cabal and planted in Guática; material originating in SENA-Manizales and planted in Santa Rosa de Cabal; material originating in Pereira and planted in Santa Rosa de Cabal.

Cluster 2: Material originating in Génova and planted in Santa Rosa de Cabal; material originating in Villamaría and planted in Santa Rosa de Cabal; material originating in Génova and planted in Guática, and material originating in Villamaría and planted in Guática.

Cluster 3: Material originating in SENA-Manizales and planted in Guática; material originating in Pereira and planted in Guática.

This grouping corroborates the data presented in the dendrogram—that materials originating from Villamaría and Génova can be differentiated, regardless of the planting site (Cluster 2). The cluster analysis for the three groups and Duncan's multiple comparison test at 5%, indicate that the only variable that differentiates the three clusters is "plant height at week 32". Whereas cluster analysis for any two groups and the results of the minimum difference test, at 5%, indicate that the only variable that differentiated any two groups was the number of male branches.

Based on the results obtained, materials originating from Santa Rosa de Cabal, SENA-Manizales, and Pereira

Table 4. Values obtained for morpho-agronomic variables.

Variable	Average	Minimum	Maximum	Standard error	C.V. (%)
Number of female branches	10.6	4	28	0.51	43.9
Number of male branches	2.4	0	4	0.09	35.3
Number of runners	0.98	0	3	0.09	82.6
Length of internodes of female branches	6.62	4.5	8.5	0.09	13.0
Length of internodes of male branches	7.28	3.0	10.0	0.12	14.6
Width of folioles on female branches	21.04	15.0	29.5	0.30	12.6
Length of folioles on female branches	16.35	12.0	21.0	0.21	11.5
Width of folioles on male branches	21.29	11.0	26.0	0.30	12.6
Length of folioles on male branches	16.60	8.5	19	0.22	12.2
Stem diameter on female branches	4.30	2.0	8.0	0.14	28.5
Stem diameter on male branches	4.80	2.5	8.0	0.11	21.5
Plant height at week 15	23.81	3.0	99.0	2.28	85.9
Plant height at week 32	145.30	16.0	354	9.40	58.2
Number of flower buds	32.31	11	95	1.37	37.1
Days to flowering	168.9	143	190	1.80	9.24

Table 5. Variables identified in each component.

Groups of factors	Variable
First factor	Width of folioles on male branches Length of folioles on male branches Number of runners Stem diameter of male branches Number of male branches
Second factor	Width of folioles on female branches Length of folioles on female branches
Third factor	Stem diameter on female branches Plant height at 15 weeks after planting Plant height at 32 weeks after planting Days to flowering
Fourth factor	Length of internodes on female branches (cm)
Fifth factor	Number of females branches Length of internodes on male branches (cm)
Sixth factor	Number of flower buds

differ, in general, from materials originating from Génova and Villamaría for all study variables and individually (univariate analysis) for the variables "number of male branches" and "plant height at week 32".

"Days to flowering" was another variable presenting a low coefficient of variation because flowering is closely linked to a unified physiological condition of blackberry, regardless of origin of the material or planting site. Foliole length and width on both male and female branches are variables with unique characteristics for thornless blackberry and are also important in differentiating materials. Variables presenting the highest coefficient of variation were "plant height at 15 and 32 weeks after planting", for which plants presented differences in their vegetative growth associated with fertilization and weed control, as well as the variable "number of runners" because some farmers found it difficult to identify these branches in the field, confusing them with thin branches or they want to attribute the lack of crop vigor to the presence of these branches.

Evaluating Thornless Blackberry Accessions using SSR Markers

A total of 57 alleles with eight loci were obtained as a result of the evaluation of 23 genotypes belonging to four *Rubus* species: *R. glaucus*, *R. urticifolius*, *R. robustus*, and *R. rosifolus*. The genotypes of *R. glaucus* included in the study belonged to two groups: thorny and thornless. The number of alleles per SSR marker ranged from 3 to 11 (Table 6). Polymorphic bands were obtained in thornless *R. glaucus* genotypes, evidencing that genetic variability does exist among these materials, which were considered to be very uniform because of their limited genetic origin and their massive multiplication by cuttings and widespread distribution by blackberry farmers in the region over the last five years.

The dendrogram in figure 3 shows 67% similarity for Group 1, which includes all *R. glaucus* accessions. The material differing most from the others was the P4 thornless blackberry from Génova, Quindío—an outlier from the rest of the group due to molecular differences.

At 90% similarity, two groups are formed that separate the remaining *R. glaucus* accessions. One group gathers thornless blackberry materials P1, P2, P3, and P5; P1 and P2, which are 100% similar with the SSR markers used. Both P4, originating from Génova (Quindio), and P5, originating from Villamaría (Caldas) show large differences at the molecular level. The dendrogram also shows other thornless blackberry genotypes that do not differ significantly from cultivated genotypes such as 50, 90, 99, and 46.

Analyses using SSR Molecular Markers

The SSRs used differentiated the studied genotypes by specie, producing exclusive bands for each and separating

Table 6. Description of the SSRs used for genetic characterization of *Rubus* species.

Locus[1]	SSR	Average PIC[2] value	*Rubus* species[4]					Expected band size (pb)
			Thornless *R. glaucus*	Thorny *R. glaucus*	*R. rosifolius*	*R. urticifolius*	*R. robustus*	
mRaCIRRIV2A8[1]	$(CA)_{12}$ $(CT)_{11}$	0.08678	1-3*	1-2*	1	1	1	191-237
mRaCIRRIV2F4[1]	$(CT)_8$ $(CA)_{17}$ $(CT)_{11}$	0.38143	6-11*	8-11*	2	3	3	180-242
mRaCIRRI1G3[1]	$(GA)_{28}$	0.43543	5-6*	2-6*	1	1-2*	2	195-265
Rubus 105b[2]	$(AG)_8$	0.4444	1-3	1-3	1-4	1-2*	1-2	165/173/181
Rubus 98d[2]	$(GAA)_5$ $(GA)_6$	NA[3]	1-2	1-2	1	1	1	173
Rubus 76b[2]	$(CT)_5$ $(CT)_4$	0.30215	1-3*	1-2	1	1	1-2	190-210
Rubus 16a[2]	$(AT)_8$ $(GT)_{11}$	0.31519	4	4-6*	4	2-4*	NAmp[5]	169
Rubus 116a[2]	$(CT)_{12}$ $(T)_{10}$	0.79717	2-5*	2	4	3-5*	5	299

1 1 = Derived from *Rubus alceifolius* (Amsellem *et al*., 2001); 2 = Derived from *Rubus* (red raspberry) hybrid species (Graham *et al*., 2002, 2004).
2 PIC = Polymorphism index content.
3 NA = non-available.
4 Presence of polymorphism.
5 NAmp = No amplification.

R. glaucus from other *Rubus* species, thus proving to be powerful markers for inter- and intra- specific studies.

Polymorphic bands were obtained in thornless *R. glaucus* genotypes, evidencing genetic variability within these materials traditionally considered highly homogenous because of their restricted genetic origin, their massive multiplication by stakes, and their broad distribution by farmers in the region over the past five years. The morpho-agronomic data obtained are highly consistent with those obtained with molecular data.

Badjakov (2007) characterized 48 raspberry accessions from Bulgaria, using nine SSR markers developed by the Scottish Crop Research Institute by Graham *et al*. (2002 and 2004). He obtained 59 alleles. The number of alleles per locus varied from 4 to 10, with an average of 6.5 per locus. The SSRs allowed him to calculate the genetic distance between the materials under study as well as analyze their heterozygosity, thanks to the co-dominant character of the SSRs. Of the nine SSRs used by Badjakov (2007), two of them—Rubus 76b and Rubus 98d—were also used in this study to evaluate different *Rubus* species as well as thorny and thornless accessions of *R. glaucus* collected in Colombia (Tables 6 and 7).

The species *R. urticifolius* was characterized by the presence of eight exclusive bands; *R. rosifolius* by the presence of four exclusive bands; *R. robustus* by the presence of two exclusive bands; and *R. glaucus* by the presence of nine exclusive bands. When using SSR Rubus 116a, thornless blackberry genotypes P4 and P5 were characterized by the presence of 1 exclusive band or allele and, when SSR Rubus 116a was used, thornless blackberry genotypes P1, P2, P3, P4, and P5 were characterized by the presence of 2 exclusive bands or alleles. All thornless *R. glaucus* genotypes were differentiated by the SSRs mRaCIRRIV2F4 and mRaCIRRIV2A8 (Table 7).

The highest number of single alleles was observed in *R. glaucus*, followed by *R. urticifolius*. The SSRs used allowed the *Rubus* species under study to be differentiated by their band patterns and the presence of bands exclusive to each species (Table 7). The presence of single bands for thornless *R. glaucus* genotypes (P1, P2, P3, P4, and P5) allowed the molecular characterization and differentiation of thornless blackberry genotypes. DNA profiles made it possible to clearly differentiate these materials from other *R. glaucus* genotypes (Table 7).

Of the 60 microsatellite markers described and used by Stafne *et al*. (2005) to evaluate the diversity of North American *Rubus* species, four were selected for this study: Rubus 98d, Rubus 105b, Rubus 116a, and Rubus 16a (Graham *et al*., 2002, 2004). The molecular marker mRaCIRRIV2A8 of *Rubus alceifolius* (Amsellem et al., 2001) was used also to evaluate Colombian *Rubus* species and genotypes (Tables 6 and 7).

When the results of this study were compared with those obtained by Stafne *et al*. (2005), who found Rubus 98d to be monomorphic and Rubus 105b, Rubus 116a, and mRaCIRRIV2A8 to be polymorphic, our results differed regarding the number of alleles identified per each primer. When used to analyze Colombian species and genotypes, the SSRs produced the following results: Rubus 98d (3 alleles), Rubus 105b (7 alleles), Rubus 116a (11 alleles), and Rubus 16a (7 alleles) (Tables 6 and 7).

The highest number of alleles per locus was obtained with the SSR markers Rubus 116a and mRaCIRRIV2F4, with

Table 7. Exclusiveness of markers in *Rubus* materials analyzed with SSRs.

Marker	Alleles specific to species/genotype (no.)	*Rubus* species/genotype
Rubus 105b	3	*R. urticifolius*
Rubus 105b	1	*R. rosifolius* and *R. robustus*
mRaCIRRIV2A8	2	*R. glaucus* (thornless)
mRaCIRRIV2A8	2	*R. glaucus*
mRaCIRRIV2F4	1	*R. glaucus* (thornless)
mRaCIRRIV2F4	6	*R. glaucus*
Rubus 98d	1	*R. urticifolius*
Rubus 98d	1	*R. glaucus*
Rubus 76b	4	*R. glaucus* (thornless)
mRaCIRRI1G3	3	*R. glaucus*
Rubus 16a	2	*R. urticifolius*
Rubus 116a	1	*R. glaucus* (P4 and P5)*
Rubus 116a	2	*R. glaucus* (P1, P2, P3, P4, P5)*
Rubus 116a	2	*R. rosifolius*
Rubus 116a	1	*R. urticifolius*

11 alleles each (Table 7). Markers mRaCIRRIV2F4 and Rubus 76b presented the highest number of polymorphic bands in thornless *R. glaucus* genotypes. Other markers that produced a high number of alleles per locus were Rubus 16a (9 alleles), mRACIRRI1G3 (6 alleles), Rubus 105b (7 alleles), mRaCIRRIV2A8, (5 alleles), and Rubus 76b (5 alleles).

When Colombian *Rubus* species and accessions were evaluated using with eight SSRs, a total of 57 alleles were obtained. The SSRs produced Rubus 76b (6 alleles) and Rubus 98d (3 alleles). Of the eight SSRs used, five belonged to the microsatellite series obtained in *Rubus idaeus* by Graham *et al*. (2002 and 2004) and three SSRs were obtained in *Rubus alceifolus* by Amsellem *et al*. (2001).

These results corroborate the efficient transferability and usefulness of the *R. alceifolius* markers described by Amsellem et al. (2001), as well as the markers described by Graham *et al*. (2002 and 2004) developed in red raspberries. The eight SSRs were highly polymorphic in Colombian *Rubus* species and accessions.

Furthermore, according to the results of Stafne *et al*. (2005), who evaluated eight SSR markers developed by Amsellem et al. (2001) in *R. alceifolius*, amplification was only obtained with mRaCIRRI1D3 and mRaCIRRIV2A8, which turned out to be polymorphic. In the current study, 22 alleles of a total of 57 were obtained with the SSR of *R. alceifolius.* The Colombian *Rubus* species evaluated shared 38.59% of the alleles of *R. alceifolus*; of a total of 57 alleles, 42 were obtained with markers obtained from red raspberry. Colombian *Rubus* species shared 62.68% of the alleles of *R. idaeus*.

In other studies carried out by Marulanda *et al.* (2007) that evaluate other Colombian *Rubus* genotypes and species, amplification was obtained in six of the eight SSR markers used by Amsellem (2001). These results contrast with those obtained by Stafne *et al*. (2005), who only obtained amplification with two of these markers, perhaps because of the divergent evolution of the study species and because *R. alceifolius* is an Asian type of the subgenus *Rubus*, probably more related phylogenetically with *R. glaucus*, which means that *R glaucus* shares 75% of the SSRs of *R alceifolius* (Stafne *et al*., 2005; Marulanda *et al*., 2007).

In the evaluation of 96 *Rubus* accessions of the Oregon germplasm bank, Castillo (Castillo, NRF. 2006) obtained 12 pairs of SSR primer derived from *Rubus* and several from *R. idaeus* that generated between 3 and 16 alleles per locus, for a total of 98 alleles and an average number of eight alleles per primer. These results are very similar to those obtained in the evaluation of Colombian *Rubus* species and accessions in which eight SSRs generated between 3 and 11 alleles per primer, for a total of 57 alleles and an average of 7.25 alleles per locus.

CONCLUSIONS

The analysis allows the differentiation of materials originating from Villamaría (P5) and Génova (P4) and planted in both Santa Rosa and Guática and those originating from SENA-Manizales (P2) and Pereira (P3) and planted in Guática. The most promising materials are those originating from Villamaría (P5) and Génova (P4), regardless of the planting site.

The variables with the lowest coefficient of variation were days to flowering and length and width of foliole. These variables are valuable because of their characteristics unique to thornless blackberry materials and their important contribution in the differentiation of materials.
The variables with the highest coefficient of variation were plant height at week 15 after planting, plant height at week 32 after planting, and number of runners.

The variables that contribute most to the differentiation of materials were length of folioles on the male branch, width of folioles on the male branch, stem diameter on the male branch, number of runners, and number of male branches.

The variables that contribute less to the differentiation of materials were those related to productive capacity, such as number of female branches and number of flower buds, indicating that these variables, when used independently, are less capable of differentiating materials.

The number of flower buds—a variable directly related to fruit production—contributes the least to total variation. In other words, it is highly unlikely that this variable serves to differentiate materials.

Polymorphic bands and bands exclusive to certain genotypes were obtained from thornless *R. glaucus*, indicating that genetic variability does exist within these materials.
The SSRs grouped the *Rubus* species studied, and exclusive bands were identified for genotyping each species.

The SSR markers of *R. alceifolius* and *R. idaeus* (red raspberry) were highly polymorphic in Colombian *Rubus* species and accessions.

ACKNOWLEDGEMENTS

Our special thanks to the blackberry farmers of the department of Risaralda. Our sincere appreciation to the Governor's Office of the department of Risaralda and COLCIENCIAS for co-financing this project; to Juan

Manuel Vásquez, undergraduate agronomy student for collecting field data; to Dr. César Sierra of the Colombian Agriculture and Livestock Institute (ICA) for monitoring the health of evaluation plots and accompanying the field evaluations; to Dr. Esther Cecilia Montoya for her support in experiment design and statistical analyses; to Juliana Arias Villegas, environment administrator, for planning and managing the present project. Our sincere thanks also go to the laboratory and nursery staff of the Biodiversity and Biotechnology Group, Universidad Tecnológica de Pereira.

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Received: Nov 6, 2008, Revised: Aug 27, 2009; Accepted: Aug 29, 2009