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Analyzing the genetic diversity of *Guadua* spp. in Colombia using rice and sugarcane microsatellites

Marta Leonor Marulanda[1*], Ana María López[1], and Jose Luis Claroz[1]

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ABSTRACT - *To study the genetic diversity of* Guadua angustifolia *Kunth, the world's third largest bamboo species, 55 accessions were evaluated using microsatellite sequences of rice and sugarcane, crops genetically related to this species. Amplifications were obtained with 27 rice and 10 sugarcane microsatellite sequences. Six similarity groups were obtained. The first is formed mainly by individuals of* G. angustifolia *and biotypes from the coffee-growing region, as well as* G. uncinata *and* G. macrospiculata*. The second brings together other* G. angustifolia *accessions and biotypes from Antioquia, Cundinamarca, and Nariño. The third consists of* G. angustifolia *plants from Antioquia, Meta, and Santander and* Guadua *sp. 1. A fourth group is formed by* G. angustifolia *accessions from Santander, Antioquia, and Caldas and* G. superba *from the Amazon. A fifth group is formed by* G. amplexifolia *accessions.* G. angustifolia *from Meta was grouped separately and presented the lowest similarity values.*

Key words: Giant bamboo, SSRs, synteny

.INTRODUCTION

Bambusoideae is one of the five or six grass subfamilies (Poaceae) that include true rice and bamboo. Bamboo includes 90 genera, and at least 1100 species are distributed in the tropics and subtropics of Asia and America (Clark 1995). To date there are 20 woody bamboo genera in the Neotropics, distributed from Mexico down to Chile. Soderstrom et al. (1998) and Clark (1990) consider that the areas of greatest diversity and endemism in the New World are Brazil, the northern and central Andes, and Mexico. Woody bamboo is a common and sometimes dominant element of the landscape in neotropical forests, mainly in areas of secondary vegetation. Seven genera and approximately 130 species of woody bamboo are distributed in the northern and central Andes (Clark 1995).

The seven genera of Andean woody bamboo are *Arthrostylidium*, *Aulonemia*, *Chusqea*, *Elytrostachys*, *Guadua*, *Neurolepis*, and *Rhipidocladum*, which all grow in Venezuela, Colombia, and Peru. Colombia, however, with 59 species, has the greatest diversity of Andean species (Clark 1995). None of the genera is exclusive of the Andes but approximately 90% of the species are endemic (Gentry 1982, Londoño 1989). The *Guadua* genus is distributed from Mexico down to Chile and is composed of approximately 33 species that grow in the lowlands of South America, such as in the Amazon

[1] Laboratorio de Biotecnología Vegetal, Universidad Tecnológica de Pereira, La Julita, OB, Colombia.* E- mail: ubioteve@utp.edu.co

jungle, the forests along the Atlantic Coast, gallery forests, and the Brazilian *cerrados*.

The species *Guadua angustifolia* Kunth—the third largest bamboo in the world—is distributed throughout Colombia, Ecuador, and Venezuela, and has been successfully introduced into several countries of Central America, the Caribbean, and Asia. It is surpassed in height only by two Asian species that reach heights of 30 m and a diameter of 22 cm. Three varieties have been identified in Colombia: *G. angustifolia* var. *angustifolia*, *G. angustifolia* var. *bicolor* Londoño, and *G. angustifolia* var. *nigra* Londoño (Judziewicz et al. 1999, Londoño and Clark 1998).

Commonly known as giant bamboo, this species shows major genetic variability at the phenotypic level and can be differentiated on the basis of its morphological and taxonomic characteristics and by observation of local communities. Based on this information, different biotypes of *G. angustifolia* have been characterized, and accordingly named 'macana', 'castilla', 'cebolla', 'cotuda', and 'grandicaula' (Gómez et al. 2001). According to Londoño (1989) and Londoño and Clark (1998), all the above biotypes, except 'grandicaula', present a higher response to changes in soil and climate conditions than to taxonomic variations.

The differentiation of species of the *Guadua* genus has been achieved by the molecular characterization of its genetic variability using amplified fragment-length polymorphisms (AFLP). Fifty-five accessions of the germplasm bank of the 'Juan María Céspedes' Botanical Garden, located in Tulua in central Valle del Cauca, Colombia, have been accordingly characterized using AFLP, which differentiated and identified *G. angustifolia* accessions. Inter-specific variability was relatively low when compared with that found in other species studied. No band pattern could be related to the species, biotypes or varieties (Marulanda et al. 2002).

Microsatellite sequences (SSRs) are highly polymorphic and replicable markers, easily purchased and, considering the information they generate, of relatively low cost. These markers are characterized by having a high degree of molecular variability and are obtained by DNA amplification using PCR. The sequences resulting from this amplification follow a simple Mendelian heredity with a codominant trait, and are distributed regularly throughout the genome of the analyzed species (Djé et al. 2000, Karp et al. 1997), successfully replacing most of the molecular markers known to date and being frequently used to study plant species of commercial interest (Djé et al. 2000).

The genetic proximity of different crops is evidenced in grasses and has been clearly demonstrated in different studies conducted on monocotyledons, using the homology of DNA bases in sequence regions flanked by microsatellites and reported in the germplasm bank as criterion. Elements of highly replicable and conserved regions have now been identified in the case of rice. These sequences are capable of amplifying different loci in maize and bamboo, making the identification of genera and species possible (Hernández et al. 2001, Ishii and McCouch 2000, Kresovich et al. 1995, Zhao and Kochert 1992).

Based on the above, this study was aimed to learn more about the genetic diversity existing in the giant bamboo genome; evaluate the usefulness of microsatellite sequences obtained from crops genetically related to giant bamboo, such as rice and sugarcane, in the identification and estimation of the variation existing in a group of 55 giant bamboo genotypes; and, according to laboratory analyses, identify major genetic differences within and between giant bamboo species or biotypes using these markers.

Rice microsatellite sequences taken from the database (Gramene Cornell microsatellite RM markers) were used. Microsatellite sequences, obtained from a sugarcane genomic library, were also assessed and used as genetic diversity marker probes, evaluating the group of accessions representative of the genetic diversity of giant bamboo in Colombia.

MATERIAL AND METHODS

Plant material and DNA extraction

Fifty-five accessions of *Guadua* spp., from all over the country, were characterized. Of these, 37 were collected from the germplasm bank of the 'Juán María Céspedes' Botanical Garden, located in Tuluá, Colombia (Table 2). The remaining 18 were representative samples of natural populations collected in the country's coffee-growing region in the central Andes (Table 2).

The extraction protocol used was that of Doyle and Doyle (1990). The DNA concentration was calculated by spectrophotometry at 260 nm, using a Shimadzu UV-1601 spectrophotometer. DNA integrity was observed in 0.8% agarose gel.

Table 1. Rice microsatellite sequences (Gramene Cornell microsatellite RM markers)

Locus	Primer sequence F (5´- 3´)	Primer sequence R (5´-3´)	Status
RM 2	ACGTGTCACCGCTTCCTC	ATGTCCGGGATCTCATCG	NA
RM 7	TTCGCCATGAAGTCTCTCG	CCTCCCATCATTTCGTTGTT	A
RM 13	TCCAACATGGCAAGAGAGAG	GGTGGCATTCGATTCCAG	A
RM 17	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	A
RM 19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	A
RM 27	TTTTCCTTCTCACCCACTTCA	TCTTTGACAAGAGGAAAGAGGC	A
RM 31	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC	A
RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC	A
RM 48	TGTCCCACTGCTTTCAAGC	CGAGAATGAGGGACAAATAACC	NA
RM 84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC	NA
RM 87	CCTCTCCGATACACCGTATG	GCGAAGGTACGAAAGGAAAG	NA
RM 110	TCGAAGCCATCCACCAACGAAG	TCCGTACGCCGACGAGGTCGAG	NA
RM 135	CTCTGTCTCCTCCCCCGCGTCG	TCAGCTTCTGGCCGGCCTCCTC	A
RM 138	AGCGCAACAACCAATCCATCCG	AAGAAGCTGCCTTTGACGCTATGG	NA
RM 140	TGCCTCTTCCCTGGCTCCCCTG	GGCATGCCGAATGAAATGCATG	A
RM 142	CTCGCTATCGCCATCGCCATCG	TCGAGCCATCGCTGGATGGAGG	A
RM 154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	A
RM 167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC	A
RM 178	TCGCGTGAAAGATAAGCGGCGC	GATCACCGTTCCCTCCGCCTGC	NA
RM 201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	A
RM 204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	NA
RM 206	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	A
RM 210	TCACATTCGGTGGCATTG	CGAGGATGGTTGTTCACTTG	NA
RM 211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	NA
RM 214	CTGATGATAGAAACCTCTTCTC	AAGAACAGCTGACTTCACAA	NA
RM 220	GGAAGGTAACTGTTTCCAAC	GAAATGCTTCCCACATGTCT	NA
RM 222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	NA
RM 224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	A
RM 225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	NA
RM 234	ACAGTATCCAAGGCCCTGG	CACGTGAGACAAAGACGGAG	A
RM 240	CCTTAATGGGTAGTGTGCAC	TGTAACCATTCCTTCCATCC	A
RM 245	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG	A
RM 253	TCCTTCAAGAGTGCAAAACC	GCATTGTCATGTCGAAGCC	A
RM 254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	A
RM 261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC	A
RM 288	CCGGTCAGTTCAAGCTCTG	ACGTACGGACGTGACGAC	NA
RM 290	ACCCTTATTCCTGCTCTCCTC	GTGCTGTAGATGGAAGGGAG	A
RM 293	TCGTTGGGAGGTATGGTACC	CTTTATCTGATCCTTGGGAAGG	NA
RM 295	CGAGACGAGCATCGGATAAG	GATCTGGTGGAGGGGAGG	NA
RM 300	GCTTAAGGACTTCTGCGAACC	CAACAGCGATCCACATCATC	NA
RM 301	TTACTCTTTGTGTGTGTGTGAG	CTACGACACGTCATAGATGACC	NA
RM 309	GTAGATCACGCACCTTTCTGG	AGAAGGCCTCCGGTGAAG	A
RM 315	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG	A
RM 325	GACGATGAATCAGGAGAACG	GGCATGCATCTGAGTAATGG	A
RM 332	GCGAAGGCGAAGGTGAAG	CATGAGTGATCTCACTCACCC	A
RM 333	GTACGACTACGAGTGTCACCAA	GTCTTCGCGATCACTCGC	NA
RM 400	ACACCAGGCTACCCAAACTC	CGGAGAGATCTGACATGTGG	NA

NA: No amplification. A: Amplification.

Table 2. List of samples with their respective site of collection and collection number

Code	Collection[a]	Species	Site of collection	Altitude (m a.s.l.)
1	XL 076	*G. angustifolia*	Sasaima (Cundinamarca)	1191
2	XL 109	*G. uncinata*	Morelia (Caqueta)	500
3	XL 144	*Guadua* sp_1	Florencia (Caqueta)	450
4	XL 206	*G. angustifolia*	Pto. Caicedo (Putumayo)	250
5	XL 214	*Guadua* sp. 1	Mocoa (Putumayo)	595
6	XL 233	*G. angustifolia*	Ricaute (Nariño)	1181
7	XL 235	*G. angustifolia*	Ricaute (Nariño)	1181
8	XL281	*G.* aff. *angustifolia*	Acacias (Meta)	450
9	XL 289	*G. angustifolia*	San Juan de Arama (Meta)	450
10	XL 291	*Guadua* sp. 2	Río Guejar (Meta)	450
11	XL 303	*G. angustifolia*	Cumaral (Meta)	450
12	XL 343	*G. angustifolia*	Puente Nacional (Santander)	1625
13	XL 344	*G. angustifolia*	Curiti (Santander)	1491
14	XL 345	*G. angustifolia*	Bucarica (Santander)	1125
15	XL 366	*G.* aff. *angustifolia*	Encizo (Santander)	1580
16	XL 375	*G. angustifolia*	Mercaderes (Cauca)	1167
17	XL 392	*G. angustifolia*	Neira (Caldas)	1969
18	XL 397	*G. angustifolia*	Rio Armas (Caldas)	-
19	XL 399	*G. angustifolia*	Abejorral (Antioquia)	2125
20	XL 413	*G. amplexifolia*	Cáseres (Antioquia)	85
21	XL 416	*G. angustifolia*	Sabaneta (Antioquia)	1600
22	XL 418	*G. angustifolia*	Valparaiso (Antioquia)	1358
23	XL 542	*G. superba*	Leticia (Amazonas)	96
24	JA 1003	*G. amplexifolia*	Monteria (Córdoba)	18
25	JA 1006	*G. amplexifolia*	Atlantic Coast	-
26	JA 1012	*G. angustifolia*	Venecia (Antioquia)	1335
27	JA 1013	*Guadua* sp. 3	Bolombolo (Antioquia)	1335
28	JA 1016	*G. angustifolia*	Cañas gordas (Antioquia)	1300
29	JA 1019	*G. angustifolia*	Mutata (Antioquia)	66
30	JA 1026	*Guadua* sp. 4	Aipe (Huila)	390
31	JA 1027	*G. angustifolia*	San Juan de Rioseco (Antioquia)	1303
32	JA 1028	*G. angustifolia*	Guaduas (Cundinamarca)	992
33	JA 1029	*G. angustifolia*	Rio Negro (Antioquia)	2120
34	JA 1031	*G. angustifolia*	Florencia (Caldas)	1460
35	wn_1	*G. angustifolia*	Ingenio Providencia (Valle)	1101
36	wn	'macana'	El Eden, Quebradanegra (Quindio)	1536
37	JA 1010	*G.* aff. *amplexifolia*	Turbana (Bolivar)	1294
38	wn	*G. angustifolia* var. *bicolor*	Comfamiliar, Cerritos (Risaralda)	1411
39	wn	'castilla'	Maracaibo, Caicedonia (Valle)	1291
40	wn	'cebolla'	Santa Lucia, La Tebaida (Quindio)	1808
41	wn	'cebolla'	La Hungria, Cerritos (Risaralda)	1411
42	wn	'cebolla'	La Hungría, Cerritos (Risaralda)	1411
43	wn	'cotuda'	Santa Lucia, La Tebaida (Quindio)	1291
44	wn	'criolla'	Napoles, Montenegro (Quindio)	1291
45	wn	'grandicaula'	Santa Lucia, La Tebaida (Quindio)	1411
46	XL 536	*G. macrospiculata*	La Esmeralda, Montenegro (Quindio)	96
47	wn	'macana'	Napoles, Montenegro (Quindio)	1294
48	wn	'macana'	La Hungria, Cerritos (Risaralda)	1411
49	wn	'macana'	Club Campestre, Cerritos (Risaralda)	1411
50	wn	'macana'	Club Campestre, Cerritos (Risaralda)	1411
51	wn	*G. angustifolia* var. *nigra*	Napoles, Montenegro (Quindio)	1291
52	wn	*G. angustifolia* var. *nigra*	La Sonora, Combia (Risaralda)	1411
53	wn	'pepina'	La Gaucha, Morelia (Risaralda)	1411
54	XL 887	*G. angustifolia*	San Calixto (North of Santander)	1125
55	wn_2	*G. angustifolia*	La Esmeralda, Montenegro (Quindio)	1294

[a]. XL= X. Londoño; JA = J. Adarve; wn = without number

Analysis of microsatellite sequences (SSRs)

Forty-eight microsatellite sequences developed to prepare the genetic map of rice (Gramene Cornell microsatellite RM markers) were evaluated (Table 1), as well as 10 highly polymorphic microsatellite sequences for sugarcane (CIR), developed by the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD): CIR 23, CIR 27, CIR 38, CIR 39, CIR 51, CIR 52, CIR 53, 278 CS, 334 BS, and 1557 CL.

Amplification reactions using RM and CIR microsatellite sequences carried out in a MJ Research thermocycler (model PTC 100), in a final volume of 25 µl reaction buffer at 1X concentration (10 mM Tri HCl, 50 mM KCl, and 0.1% Triton X 100), 1.44 mM $MgCl_2$, 0.6 mM of each dNTP, 0.5 U Taq polymerase, 200 nM of each primer, and 8 ng DNA μL^{-1}. Rice and sugarcane DNA amplifications were used as positive controls.

The amplification profile included an initial denaturation stage at 94 °C for 3 minutes, followed by 30 cycles at 94 °C during 30 seconds, 52 °C for 45 seconds for rice microsatellite sequences and 53 °C for 45 seconds for sugarcane microsatellite sequences, 1 minute at 72 °C, and then a final extension at 72 °C during 5 minutes.

Amplified products were run in polyacrylamide gel at 6%, in BioRad Sequi-Gen GT Sequencing Cell vertical electrophoresis chambers, at 110W and a temperature of 50 ºC, and subsequently stained with silver nitrate.

Data analysis and estimate of genetic variability

Groups of genetic diversity were determined by calculating the genetic similarity (GS_{ij}) between each pair of genotypes using the Dice (1945) and Nei and Li (1979) formulas. A dendrogram, based on similarity coefficient, was generated using the Unweighted Pair-group Method with Arithmetic Average (UPGMA). The software NTSYS-pc version 2.02i was used for cluster analysis. The statistical confirmation of diversity groups, determined by cluster analysis, was performed by re-sampling (1,000 times) using the bootstrap method with the WinBoot statistical software.

The three-dimensional graph, based on Multiple Correspondence Analysis, was obtained using the spin platform of the JMP program in SAS.

RESULTS AND DISCUSSION

Transferability of microsatellite sequences and their amplification patterns

The present study demonstrated the usefulness of rice and sugarcane microsatellite sequences to establish genetic relationships between genotypes, varieties, and species of the *Guadua* genus.

Positive amplification was obtained with 27 of the 48 RM (Table 1) and with 10 of the sugarcane microsatellite sequences. The high degree of homology in DNA sequences between species of the Poaceae family was therefore demonstrated.

The RM 31, RM 309, and RM 332 microsatellite sequences presented polymorphism in the band patterns of the 55 accessions of the *Guadua* germplasm bank. Several co-dominant bands were obtained with the RM 309 and RM 332 microsatellite sequences, as can be expected with these markers (Karp et al. 1997), suggesting the presence of different alleles for the same locus.

Only one of the 10 sugarcane microsatellite sequences (CIR 23) presented polymorphism during the evaluation of the 55 accessions of the *Guadua* germplasm bank. The following genotypes presented polymorphism in one of the bands with the CIR 23 sugarcane microsatellite sequences: *G. uncinata, G. superba, G. amplexifolia*, *G. angustifolia* accessions from Caldas and Antioquia, and *Guadua* sp. 1 (Putumayo) and *Guadua* sp. 2 (Meta).

The analysis of *G. angustifolia* with microsatellite sequences developed for species of the same family was proposed in accordance with the phylogenetic proximity existing between grass species belonging to the Orizaoideae and Bambusoideae subfamilies (Clark et al. 1995, Gielis 1995, Zhao and Kochert 1992).

Clark et al. (1995), who used 45 chloroplast sequences of the ndhF gene to study the phylogenetic relationships in grasses, following a strict consensus, derived from parsimony analysis. Based on the phylogenetic data obtained for Oryzaoideae and Bambusoideae, these are considered as subfamilies that are very close. Zhao and Kochert (1992), cited by Gielis (1995), states that many of the 200,000 copies of repetitive DNA evaluated in rice were also found in bamboo.

The use of synteny in molecular studies on grass species has been extensively reported. Selvi et al. (2003) used maize microsatellite sequences for the molecular

analysis of sugarcane, evaluating 34 primers pairs, of which 14 presented replicable amplification in *Saccharum* clones and in related commercial hybrids and genera such as *Erianthus*.

Cluster analysis with rice and sugarcane microsatellite sequences

The genetic groupings and distances that produced the polymorphic bands are indicated in the dendrogram and in the Multiple Correspondence Analysis (Figures 1 and 2).

Figure 1 differentiates six similarity groups, relatively close to each other, according to the Dice index. A first group is formed mainly by individuals of *G. angustifolia* and the 'macana' and 'criolla' biotypes. The species *G. uncinata* from Caquetá and *G. amplexifolia* from the Atlantic coast also appear, as well as the accessions of *G. angustifolia* from Cundinamarca, Norte de Santander, and Caldas. A second group is formed by other *G. angustifolia* accessions and the 'cebolla', 'cotuda', 'castilla', 'grandicaula', and 'bicolor'

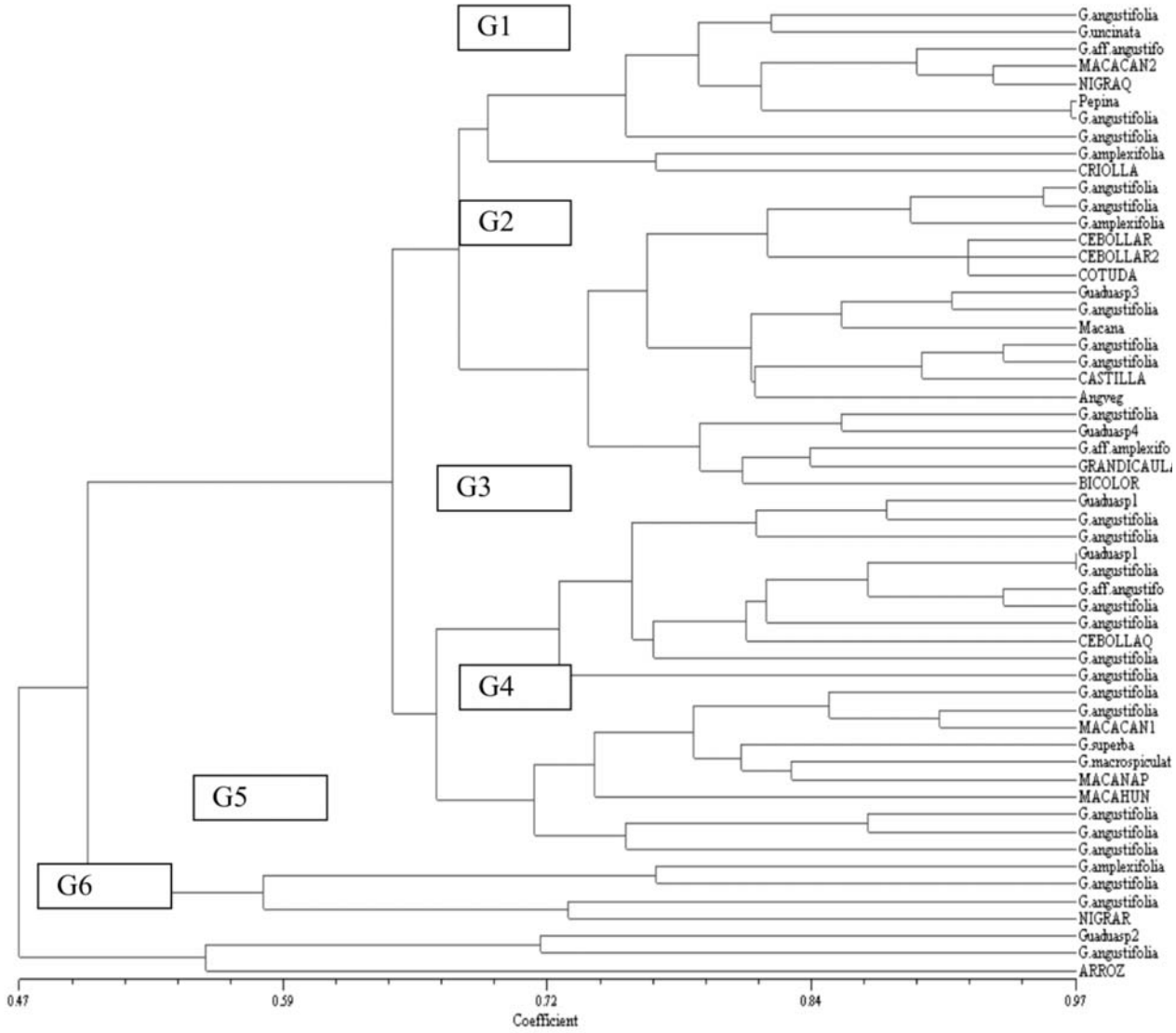

Figure 1. Dendrogram estimated with the Dice similarity index

biotypes, and accessions of *G. angustifolia* from Antioquia, Cundinamarca, and Nariño. A third group consists of *G. angustifolia* plants from Antioquia, Santander, Meta, and Putumayo, and *Guadua* sp. 1 from Putumayo and Caquetá. A fourth group is formed by *G. angustifolia* accessions from Santander, Antioquia, and Caldas, and by the 'macana' biotype from Risaralda and *G. superba* from the Amazon. A fifth group comprises *G. amplexifolia*, accessions of *G. angustifolia* from Antioquia, and the Nigra variety from Risaralda. This group has lower similarity indexes compared with the other four groups. However, the lowest similarity indexes are found in the sixth group, where two individuals of *G. angustifolia* from Meta are separate from the rest of the *G. angustifolia* individuals. These intraspecific variations related to distant geographical areas, such as Meta, Santander, Antioquia, Caquetá, and Putumayo, suggest the existence of gene pools of *G. angustifolia* other than those of the coffee-growing region. The giant bamboo stands from which the samples were collected should be considered priority for the conservation of the genetic variability of this species in Colombia.

Multiple Correspondence Analysis (Figure 2) differentiated eight groups, which are discriminated in Table 3. Group 1 comprises most of the accessions of *G. angustifolia,* the biotypes 'castilla', 'cebolla', 'cotuda', 'macana', 'criolla', and 'grandicaula' from the coffee-growing area in the central Andes of Colombia, and the species *G. macrospiculata* and *G. uncinata.* A second group includes the *Guadua* genus with unclassified species, such as *Guadua* sp. 1 and *Guadua* sp. 2, and several accessions of *G. angustifolia* from Meta, Putumayo, Santander, and Antioquia. Group 3 gathers *G. amplexifolia* and *G. angustifolia* var. Nigra from Risaralda. Several genotypes of *G. angustifolia* from Antioquia, Meta, and Santander are located in groups 4, 5, and 6. The species *Oryza sativa* is located in group 7, and the species *G. superba* in group 8.

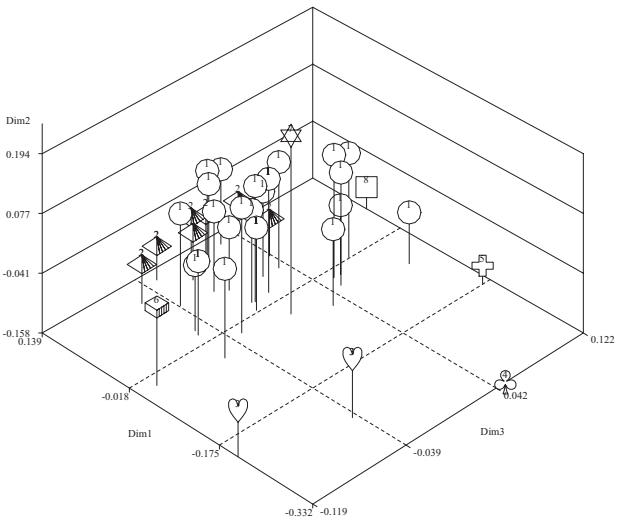

Figure 2. Three-dimensional graph based on Multiple Correspondence Analysis, plotted using the spin platform of the JMP program in SAS. Group 1 (balloons), Group 2 (pyramids), Group 3 (hearts), Group 4 (clovers), Group 5 (crosses), Group 6 (cubes), Group 7 (stars), and Group 8 (squares)

Table 3. Similarity groups according to multiple correspondence analysis

Group	Accessions
1	*G. angustifolia, G. aff. angustifolia, G. macrospiculata, G. uncinata, G. angustifolia* Varieties 'bicolor' from Risaralda and 'Nigra' from Quindío, and biotypes 'castilla', 'cebolla', 'cotuda', 'macana', 'criolla', and 'grandicaula' from the coffee-growing area of the central Andes.
2	*Guadua* sp. 1, *Guadua* sp. 2, *G. angustifolia* (from Meta, Putumayo, Santander, and Antioquia)
3	*G. amplexifolia* and *G. angustifolia* var. Nigra from Risaralda
4	*G. angustifolia* (Antioquia)
5	*G. angustifolia* (Meta)
6	*G. angustifolia* (Santander)
7	*Oryza sativa*
8	*G. superba*

Cluster analysis did not differentiate *G. macrospiculata* and *G. uncinata* as clearly as Marulanda et al. (2002) reported when using AFLPs; however, individuals of *G. amplexifolia* from the Atlantic coast and *G. superba* from the Amazon could be differentiated.

The use of molecular markers in the study of genetic diversity of bamboo is quite recent; main studies are related to the characterization of bamboo germplasm, phylogeny, and evolution of *Phillostachys* using RFLP markers (Friar and Kochert 1991, 1994) and to the evaluation of enzymatic polymorphism in bamboo species (Heng et al. 1996). Nuclear RFLP markers have been used in phylogeny studies of bamboo worldwide as well as to conduct evolution and phylogenetic studies in *Chusquea* and Bambusiodeae (Kelchner and Clark 1997, Kobayashi 1997).

Loh et al. (2000) used AFLP markers to study the genetic relationships between species of the Bambusiodeae subtribe. The molecular studies by Marulanda et al. (2002) on American bamboo are well known. The use of microsatellite sequences to study bamboo has not been reported to date.

The present study confirms the hypothesis that the DNA sequences of phylogenetically related plant crops can be used to characterize little studied species, such as the American giant bamboo, and further showed the existence of different gene pools from those identified in the coffee- growing region of the central Andes of Colombia. These results are important for the future conservation of the species.

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Análise da diversidade genética da *Guadua* spp. da Colômbia através de microssatélites de arroz e cana-de-açúcar

RESUMO - *Para estudar a diversidade genética da espécie* Guadua angustifolia *Kunth, o terceiro maior bambu do mundo, se avaliaram 55 acessos utilizando seqüências microssatélites de arroz e cana-de-açúcar, culturas geneticamente bem relacionadas com a espécie. Obteve-se amplificação com 27 seqüências microssatélites de arroz e com 10 seqüências microssatélites de cana-de-açúcar, formaram-se 6 grupos de similaridade, O primeiro constituído principalmente por indivíduos de* G. angustifolia *e os biótipos "macana" e "criolla" da região cafeeeira, incluídas também as espécies* G. uncinata *e* G. macrospiculata. *No segundo reuniram-se outros biótipos de* G. angustifolia *provenientes de Antioquia, Cundinamarca e Nariño. O grupo 3 reuniu indivíduos de* G. aungustifolia *de Antioquia, Meta e Santander e* Guadua *sp1. Um quarto grupo formado por accesos de* G. angustifolia *provenientes de Santander, Antioquia e Caldas e* G. superba *do Amazonas. O quinto grupo foi formado pelos accesos de* G. amplexifolia *e separado de estes grupos, com índices de similaridade mais baixos encontrou-se* G. angustifolia *proveniente de Meta.*

Palavras chaves: bambu gigante, SSRs, sintenia.

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