Molecular characterization of *Guadua angustifolia* Kunth using RAMs

Caracterización molecular de *Guadua angustifolia* Kunth mediante marcadores moleculares RAMs

Paula Andrea Rugeles-Silva[1], Andrés Mauricio Posso-Terranova[1], Ximena Londoño[2], Nancy Barrera- Marín[1], and Jaime Eduardo Muoz-Flórez[1]*

[1]Faculty of Agricultural Sciences, Universidad Nacional de Colombia - Palmira, A.A. 237, Palmira, Valle del Cauca, Colombia. [2]Colombian Society of Bamboo, Armenia, Quindío, Colombia. *Corresponding author:paularugeles@yahoo.com

Rec.: 30.09.11 Acept.: 11.12.12

Abstract

Guadua angustifolia Kunth is a neotropical bamboo species distributed in the Andean region of Colombia. It has a great industrial importance in the well-known Colombian coffee region due its use in building, industrialization and furniture. In order to know the genetic diversity of nine superior materials of *G. angustifolia* previously selected by morphologic and physic-mechanic characteristics, a molecular characterization using RAMs molecular markers was carried out. Values of *He* = 0.31 and polymorphic loci percentage of 81.03% were obtained, indicating a high genetic diversity of the evaluated materials.

Key words: Genetic diversity, genetic markers, *Guadua angustifolia*, molecular markers, Colombian coffee growing zone.

Resumen

Guadua angustifolia Kunth es un bambú del Neotrópico que se distribuye principalmente en la región Andina. En la región del Eje Cafetero colombiano esta especie presenta gran importancia por su utilización en la construcción, industrialización y fabricación de muebles y artesanías. Para conocer la diversidad genética de nueve materiales superiores de *G. angustifolia* seleccionados previamente por sus características morfológicas y físico-mecánicas se realizó una caracterización molecular usando marcadores moleculares RAMs. Se obtuvo un valor de *He* = 0.31 y un porcentaje de loci polimórfico de 81.03% lo que indica una alta diversidad genética de los materiales evaluados.

Palabras clave: Diversidad genética, eje cafetero de Colombia, *Guadua angustifolia*, marcadores genéticos, marcadores moleculares.

Introduction

Guadua, *Guadua angustifolia* Kunth, is a bamboo of the tropical regions of Central and South America. It grows on natural environments, especially along riversides forming dominant or mixed forest together with other types of trees (Kleinn and Morales-Hidalgo, 2006). In Colombia, bamboos are concentrated on the central region of the Andes (Central and Western mountain ranges) generally between 900 and 2000 MASL. Till now,

there have been reported 30 species of the genus *Guadua*; Colombia is the second diversity center of Bambusoideae after Brazil (Londoño, 1990; Londoño and Clark, 2002).

Due to the long sexual reproduction cycles, bamboo identification relies on morphological descriptors of the non-reproductive structures, limiting the resolution power of those descriptors, therefore, molecular markers are a valuable tool that helps bamboo classification and identification all over the world. Markers based on PCR are well documented, they have been used in hundreds of plant species of almost all the botanical families and it is possible to use them for species identification and cultivar characterization (Khasay and Dancik, 1996; Samec and Nainec, 1996; Raina *et al.,* 2001; Johnson *et al.,* 2003).

The molecular technique of Randomly Amplified Microsatellites (RAMs) combines several characteristics of the RAPD and microsatellites. In this technique, DNA between the distal extremes of two highly related microsatellites is amplified by PCR. Hantula *et al.*, (1996) demonstrated that RAMs are applicable on the study of genetic variation on fungi, and Muñoz-Flórez *et al.* (2008) performed different studies of genetic variation on microorganisms, animals and plants using this technique with successful results on the evaluation of genetic diversity and population structure. Additionally, RAMs allow the determination of variability among individuals since the number of detectable polymorphisms is theoretically unlimited (Mahuku *et al.,* 2002).

Given the importance of guadua in the coffee region of Colombia, the main objective of this work was to determine by randomly amplified microsatellites (RAMs) the genetic diversity of *Guadua angustifolia* materials, previously selected by their desirable characteristics for building, furniture and handicraft fabrication and industrialization, as well as, to identify the geographical areas with high genetic diversity. This will generate fundamental information for managing the conservation and resource use strategies.

Materials and methods

Nine materials of *G. angustifolia* were evaluated, they were previously selected in the departments of Quindío, Caldas and Risaralda for their outstanding morphological characteristics like culm diameter and length, thickness of the culm wall, absence of branches without thorns, internode symmetry, and physic-mechanical characteristics such as fiber parallel compression, fiber parallel cut, module of compression elasticity and water content (Muñoz-Flórez, 2011) (Table 1).

DNA extraction

For DNA extraction were selected five bamboo plants per site, young leaves were collected and preserved on liquid nitrogen for transportation and later processing in the Molecular Biology Lab of the Universidad Nacional de Colombia – Palmira. For DNA extraction was used the methodology of Dellaporta *et al.*, (1983) with the modifications described by Palacio-Mejia (2004). Quality and quantity

Table 1. Superior materials selected of *Guadua angustifolia*.

Location	Town	Department	Geographic coordinates			Uses
			Latitude N	Longitude O	MASL	
A	Montenegro	Quindío	4° 51' 83"	75° 86' 25"	1252	Industrialization
C	Montenegro	Quindío	4° 33' 16.2"	75° 48' 8.3"	1250	Building
D	Montenegro	Quindío	4° 55' 50"	75° 86' 36"	1247	Building
E	Montenegro	Quindío	4° 33' 25.6"	75° 48' 28.1"	1223	Furniture and handicrafts
H	Circasia	Quindío	4° 35' 50.5"	75° 39' 34.4"	1616	Building
M	Quimbaya	Quindío	4° 36' 25.1"	75° 44' 77"	1420	Furniture and handicrafts
W	Chinchiná	Caldas	4° 58' 6.3"	75° 39' 41.3"	1318	Building
Z	Palestina	Caldas	5° 0.1' 22.3"	75° 39' 25.5 "	1318	Industrialization
α	Pereira	Risaralda	4° 51' 54.5"	75° 45' 8.9"	1320	Building

evaluation of the extracted DNA was performed in 0.8% agarose gels run in 0.5X TBE (0.045M tris-borate; 0.001M EDTA) buffer at 80 volts for 45 minutes and dyed with ethidium bromide to a final concentration of 0.5 µg/ml. DNA concentrations were determined by comparison with known concentration of bacteriophage Lambda DNA.

RAMs molecular markers

Seven primers for RAMs were evaluated (Table 2) with the following amplification conditions: 1X buffer, 2.5 mM $MgCl_2$, 0.2mM of each dNTP, 0.625 U Taq Polimerase, 2 µM primer, 0.4 mg/ml BSA (bovine serum albumin) and 20 ng of genomic DNA, to a final volume of 25 µl.

Table 2. RAMs primers used in this study.

Primer	Sequence	Anealling temperature
CT	DBDCTCTCTCTCTCTCTC	55
CGA	DHBCGACGACGACGACGA	58
CA	DBDACACACACACACACACA	50
AG	HBHAGAGAGAGAGAGAGAG	50
TG	HVHTGTGTGTGTGTGTGT	55
CCA	DDBCCACCACCACCA	55
GT	VHVGTGTGTGTGTG	58

For the degenerated sites the following designations were used: H (A ó T ó C); B (G ó T ó C); V (G ó A ó C) and D (G ó A ó T).

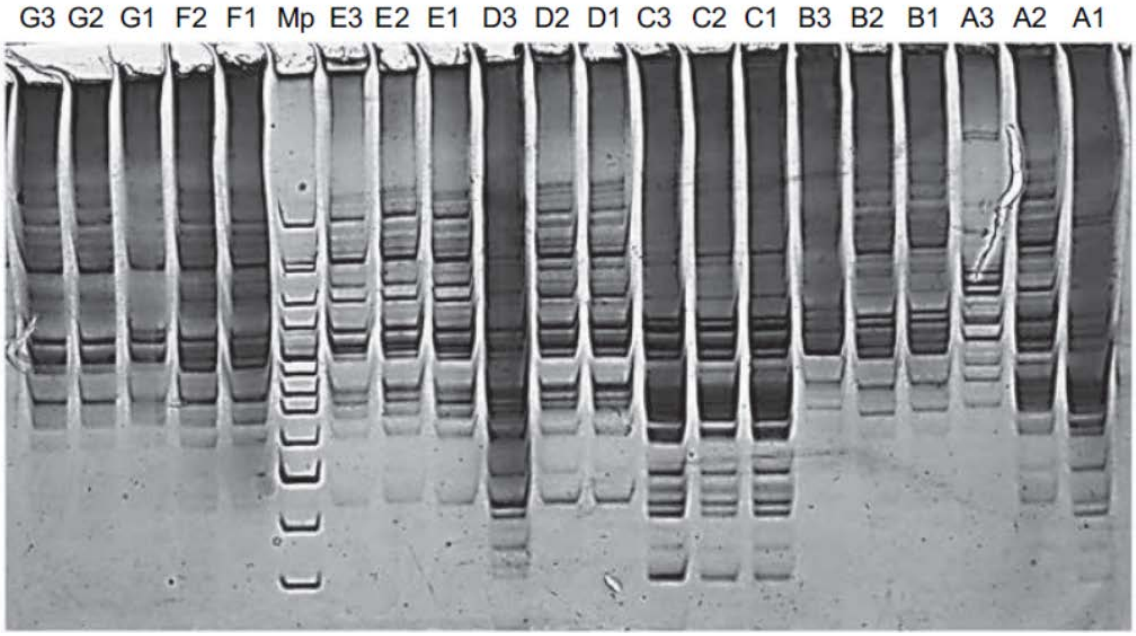
Hybridization temperatures were established according to the primer used (Table 2). The program for amplification consisted on an initial denaturation at 95 °C for 5 min, followed by 37 cycles of denaturation at 95 °C for 30 s, an annealing phase at 50-58°C for 45 s (according to the primer used, Table 2) and an extension at 72 °C for 2 min. a final extension at 72 °C for 7 min was done. Amplified products were visualized on 7% polyacrylamide gels (37:1 acrylamide bisacrylamide) run at 160 vaults for 1 h and dyed with ethidium bromide and silver salts, as described in standard protocols (Sambrook *et al.*, 1989).

Information of band pattern was registered in a binary matrix of presence (1) and absence (0). For polymorphic bands selection it was considered as polymorphic locus that has a frequency less than 95% for the most common allele. From this matrix and with the SIMQUAL from NTSYS- pc (Numerical Taxonomy System for Personal Computer) software and the TFPGA (Tools for Population Genetic Analysis) software (Miller, 1997) were performed the following statistical analyses: (1) Genetic similarity estimated by Dice coefficient (Nei, 1978); (2) Grouping analysis done with the software *SAHN* of NTSYS pc (version 2.02g, 1998) using UPGMA, graphical method of pair grouping that uses the unweighted arithmetical average; (3) Dendogram that was built with the software *TREE* of of NTSYS pc (version 2.02g, 1998); (4) To estimate genetic diversity the parameters of average expected heterozygosity (*He*) and percentage of polymorphic loci (*P*) were used, that were estimated over all the loci and their average according to the unbiased formula of Nei (1973).

Results and discussion

Concentrations between 50 and 300 ng/µl of DNA with good quality for PCR were found, indicating that the used protocol was suitable to extract DNA from *G. angustifolia*. In the analysis were used six of the seven RMA primers because they showed some degree of polymorphism (Picture 1). The GT primers did not amplified in the evaluated samples. The six primers used generated a 116 loci pattern with molecular weight between 1400 and 200 bp. The number of loci per primer varied between 16 for AG and 24 for CT (Table 3).

Mukherjee *et al.* (2010) performed a study of the genetic relations between 22 bamboo taxa using Inter Simple Sequence Repeats (ISSR) and random EST-Based primers and obtained 216 bands in total, from which five were monomorphic and 211 were polymorphic. Marulanda and López (2010) characterized 55 accessions of *Guadua* spp. with AFLP molecular markers and got 771 bands, from which 382 were polymorphic. Potosi (2005) estimated the genetic diversity of guadua in the department of Cauca, Colombia, using RAPDs, having the highest polymorphism when obtaining 42 loci in total with 40 polymorphic loci. When comparing the number of loci found in the other studies and the one obtained in the present study, it can be con



Picture 1. Polyacrylamide gel with the CCA primer dyed with silver salts.

clude that 116 bands is a good number that gives confidence for establishing genetic diversity parameters.

The expected heterozigosity (*He*) estimates the probability that two alleles randomly extracted from population gene pool of genes could be different and it is an estimator of genetic diversity. The highest values for *He* for superior materials were found with the primers CCA (0.37), CA (0.35) and TG (0.33), whereas the lowest ones were found with the primers CT (0.18) and CGA (0.19). He for the nine superior materials was 0.31, indicating high allele diversity and that the selected materials correspond to different genotypes and not to genetically uniform clones. This diversity is important because ensures the sustainability of the materials through the time.

Polymorphism values found in the superior materials (94.11% and 81.25%) with the TG and AG primers, respectively, allow concluding that these primers were the ones that contribute the most to genetic diversity of the superior materials of *G. angustifolia*. The percentage of polymorphic loci confirms the high diversity of the nine selected materials and indicates that in 81.03% of the cases the most repetitive allele has a frequency less than 95%, meaning that there is a high diversity due to the presence of different alleles.

Descriptive analysis of genetic diversity

Superior materials were grouped with a similarity index of 74% (Figure 1). Group **A** is composed with most of the superior materials,

Table 3. Loci number, expected heterozygosity (*He*) and percentage of polymorphic loci for the primers used.

Primer	Total bands	Polymorphic bands	*He*	Percentage
AG	16	13	0.31	81.25
CT	24	12	**0.19**	50.00
CCA	22	20	**0.37**	59.09
CGA	20	20	0.19	**35.00**
CA	17	17	0.35	76.58
TG	17	17	0.33	**94.11**
Total	116	99	0.31	81.63

while the group **B** has the materials of the site Z (Palestina) and two individuals of the site M (Quimbaya). Guadua from the site α (Pereira) did not differ genetically, which is explained by the possibility of a vegetative reproduction in these individuals. The site Z is differentiated from the other groups below 75%, indicating that guaduas of this site diverge significantly with respect to other areas.

In general, there was a grouping by geographical zone, however the dendogram indicated variation within each site, probably because of sexual reproduction that can happen twice a year (Londoño and Peterson, 1992; Giraldo and Sabogal, 1999) and produce between 96 and 344 seeds per culm when flowering, that under controlled conditions have a high germination percentage (Muñoz-Flórez, 2011).

Conclusions

- A high genetic diversity was found in the superior materials of *G. angustifolia* from the coffee area of Colombia that were evaluated.
- In general there was grouping by geographical origin, however, there is diversity within each group, which can be explained by crossing reproduction, this confirms the results of previous research (Muñoz-Flórez, 2011).
- The high genetic diversity found proposed the sustainability of the selected materials over time and ensures the conservation of variability required for establishment of future genetic breeding programs of this species.

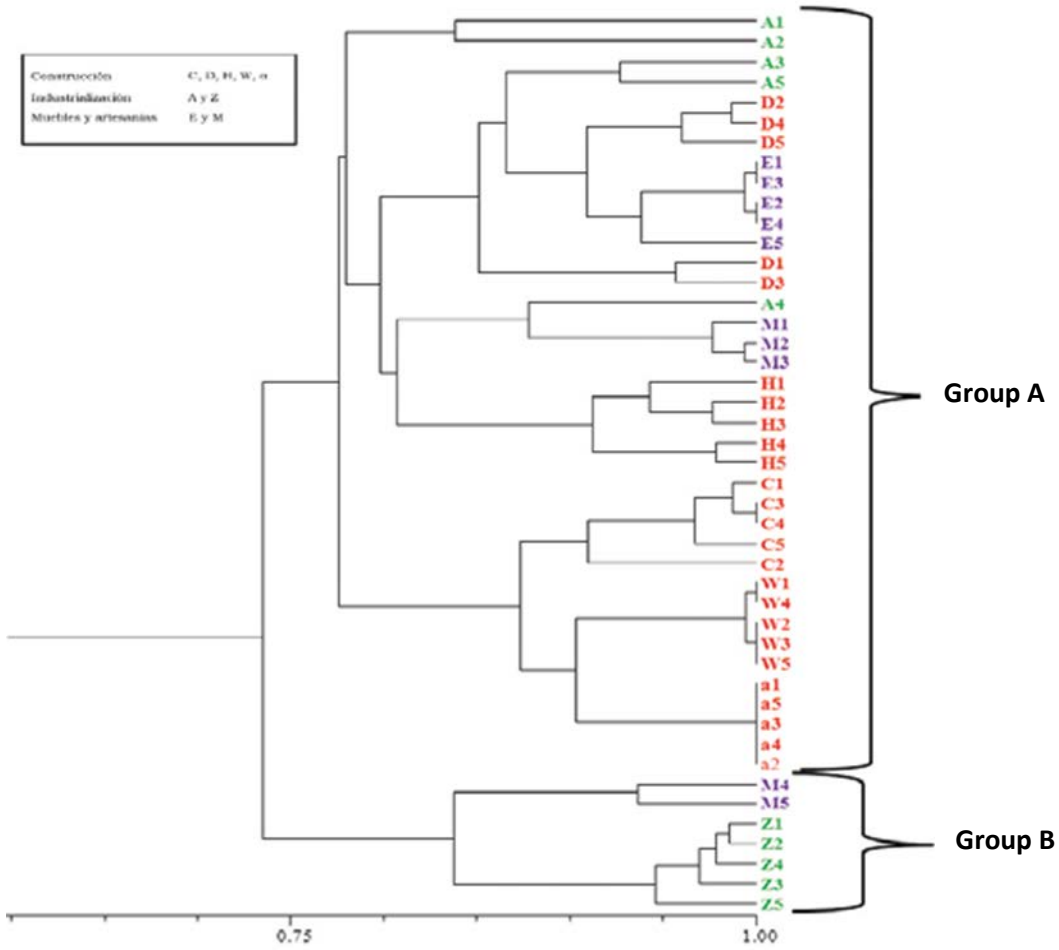


Figure 2. Dendogram of similarity of the superior materials of *Guadua angustifolia.*

Acknowledgments

To the Ministry of Agriculture and Rural Development (MADR) for financing this research through the project 2008M6336-3616.

References

Dellaporta, S. L.; Wood, J.; and Hicks, J. B. 1983. A plant DNA minipreparation: Versión II. Plant Molec. Biol. Rep. 1(4):19 - 21.

Giraldo, E. and Sabogal, A. 1999. Una alternativa sostenible: La Guadua. Corporación Autónoma Regional del Quindío-CRQ. 192 p.

Hantula, J.; Dusabenyagasani, M.; and Hamelin, R. C. 1996. Random Amplified Microsatellites (RAMs) - a novel method for characterizing genetic variation within fungi. Europ. J. Forest Pathol. 26(3):159 - 166.

Johnson, E. L.; Saunders, J. A.;Mischke, S.; Helling, C. S.; and Emche, S. D. 2003. Identification of *Erythroxylum* taxa by AFLP DNA analysis. Phytochem. 64(1):187 - 197.

Khasa, P. D.; and Dancik, B. P. 1996. Rapid identification of white-Engelmann spruce species by RAPD markers. Theor. Appl. Gen. 92(1):46 - 52.

Kleinn, C. and Morales-Hidalgo, D. 2006. An inventory of Guadua (*Guadua angustifolia*) bamboo in the Coffee Region of Colombia. Europ. J. Forest Res. 125(4):361 - 368.

Londoño, X. 1990. Aspectos sobre la distribución y la ecología de los bambúes de Colombia (Poaceae:Bambusoideae). Caldasia 16(77):139 - 153.

Londoño, X. and Peterson, P. 1992. *Guadua chacoensis* (Poaceae: Bambusoideae), its taxonomic identity, morphology and relationships. Novon 2:41 - 47.

Londoño, X. and Clark, L. G. 2002. Three new taxa of Guadua (Poaceae:Bambusoideae) from South America. Novon 12(1):64 - 76.

Mahuku, G. S.; Henríquez, M. A.; Muñoz, J.; and Buruchara, R. A. 2002. Molecular markers dispute the existence of the Afro-Andean group of the bean angular leaf spot pathogen, *Phaeoisariopsis griseola*. Phytopathology 92(6):580 - 589.

Marulanda, M. L. and López, A. M. 2010. Estimación de la variabilidad genética en especies y genotipos del género Guadua en Colombia, con marcadores moleculares AFLP. In: Biodiversidad y Biotecnología de la *Guadua angustifolia* Kunth. Universidad Tecnológica de Pereira. p.57 - 76.

Miller, M. P. 1997. Tools for population genetic analysis. Version 1.3. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Mukherjee, A. K.; Ratha, S.; Dhar, S.; Debata, A. K.; Acharya, P. K.; Mondal, S.; Panda, P. C.; and Mahapatra, A. K. 2010. Genetic relationships among 22 taxa of bamboo revealed by ISSR and EST-based random cebadors. Bioch. Gen. 48:1015 - 1025.

Muñoz-Flórez, J. E.; Morillo-Coronado, A.; and Morillo-Coronado, Y. 2008. Microsatélites amplificados al azar (RAM) en estudios de diversidad genética vegetal. Acta Agronómica 57(4):219 - 226.

Muñoz-Flórez, J. E. 2011. Diversidad genética, estructura poblacional y selección de clones superiores de *Guadua angustifolia* Kunth en la eco-región cafetera de Colombia. Doctoral thesis. Universidad Nacional de Colombia sede Palmira. 151 p.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. 70(12):3321 - 3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583 - 590.

Palacio-Mejía, J. D. 2004. Estudio comparativo de la diversidad genética y divergencia evolutiva entre la especie vulnerable *Colombobalanus excelsa* (Lozano *et al.*) Nixon & Crepet y el roble común *Quercus humboldtii* Bondpl: implicaciones para la biología de la conservación. Master thesis. Universidad Nacional de Colombia Sede Palmira. 108 p.

Potosí, C. T. 2005. Variabilidad de la guadua (Guadua angustifolia Kunth) en la cuenca Cauca, Departamento del Cauca. Thesis. Universidad Nacional de Colombia. Palmira. 53 p.

Raina, S. N.; Rani, V.; Kojima, T.; Ogihara, Y.; Singh, K. P.; and Devarumath, R. M. 2001. RAPD and ISSR finger prints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome 44(5):763 - 772.

Sambrook, J.; Fritsch, E.F.; and Maniatis, T. 1989. Gel electrophoresis of DNA. In: Sambrook, J.; Fritsch, E.F.; y Maniatis, T. (eds.). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Nueva York. p. 3 -60.

Samec, P. and Našinec, V. 1996.The use of RAPD technique for the identification and classification of *Pisum sativum* L. genotypes. Euphytica 89(2):229 - 234.