

KARYOTYPIC SIMILARITY AMONG DIFFERENT CULTIVARS OF *Musa* SPP FROM QUINDÍO-COLOMBIA

Similaridad cariotípica entre diversas variedades de *Musa* spp del Quindío-Colombia

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ABSTRACT. Identification of *Musa* spp genotypes has had a high degree of difficulty by the small size of the chromosomes and by the small number of cells in prometaphase that are obtained. Cytogenetic characterization of the cultivars is a useful tool for its productive improvement or resistance increase to diseases and pests. For this reason cytogenetic characterization of 9 cultivars of *Musa* collected from Quindío-Colombia was proposed. Triploid condition was found in all cultivars, where 'Dominico', 'Dominico-Hartón' and 'Hartón' were AAB; 'Popocho', 'Guayabo', and 'Guineo' ABB and 'Bananas' were AAA; all had $2n = 3x = 33$ chromosomes. Three type of chromosomes sizes were found; A (1.951 – 2.790 μm); B (1.134 – 1.950 μm) and C (0.307- 1.141 μm). A wide variability was found between chromosomes number by type: A chromosomes: 9 and only in 'Dominico-Hartón'; B chromosomes: between 0 and 18; C chromosomes: between 6 and 33. Also were found significant differences in chromosome size and genome size among cultivars: 'Hartón' being the smallest, and 'Dominico-Hartón' with the largest chromosome and genome size. In conclusion all *Musa* spp, evaluated in Quindío, have three types of chromosomes, differ in chromosomes number in each type and genome length, but all they are triploid hybrids (AAB, ABB, AAA). This study contributes to the advance of the genetics of *Musa* spp for future improvement.

Key words: cytogenetic, chromosome number, plantain, genome size, genetic diversity

RESUMEN. La identificación de genotipos de *Musa* spp ha tenido un alto grado de dificultad, debido al pequeño tamaño de los cromosomas y al reducido número de células que se obtienen en prometafase. La caracterización citogenética de los cultivares es una herramienta útil para su mejoramiento productivo o aumentar su resistencia a plagas, por esta razón se propuso la caracterización citogenética de nueve cultivares de *Musa paradisiaca* colectadas en Quindío-Colombia. Se comprobó la condición triploide de todos los cultivares, donde 'Dominico', 'Dominico-Hartón' y 'Hartón' fueron AAB; 'Popocho', 'Guayabo' y 'Guineo' ABB y los 'Bananas' fueron AAA; todos tenían $2n=3x=33$ cromosomas. Se encontraron tres tamaños de cromosomas; cromosomas tipo A (1,951 - 2,790 μm); tipo B (1,134- 1,950 μm) y tipo C (0,307- 1,141 μm). Se encontró significativa variabilidad en el número de cromosomas por tipo, cromosomas tipo A: 9 y solo en 'Dominico-Hartón'; cromosomas tipo B: entre 0 y 18; C cromosomas: entre 6 y 33. También se encontró variabilidad en el tamaño cromosómico y el tamaño del genoma entre los cultivares siendo el más pequeño el 'Hartón' y el 'Dominico-Hartón' el de mayor tamaño cromosómico y de genoma. En conclusión todos los cultivares de *Musa* spp evaluados en el Quindío-Colombia, tienen tres tipos de cromosomas, difieren en el número de cromosomas en cada tipo y en la longitud del genoma, pero todos ellos son híbridos triploides (AAB, ABB, AAA). Este estudio contribuye al avance de la genética de *Musa* spp para su futuro mejoramiento.

Palabras clave: citogenética, número de cromosomas, plátano, tamaño del genoma

INTRODUCTION

Musa genus belongs to the *Musaceae* family and is more than 50 million years old, it is included in diets and a source of energy for millions of people, especially those living in the tropics (1).

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This family has three genera; *Musa*, *Ensete* and *Musella*. The *Musa* genus is the most widely distributed and embraces four cultivars within it, *Callimusa*, *Australimusa*, *Eumusa* and *Rhodochlamys*, which differ according to number of chromosomes and morphological patterns (2,3). During the first half of the 20th century, cytogenetics research on the *Musa* cultivars, led to determination of the number of chromosomes in wild and cultivated species (4). In this regard, it was demonstrated that - the first two- *Callimusa* and *Australimusa* have 10 chromosomes ($2n=2x=20$), comprising important ornamental species; while *Eumusa* and *Rhodochlamys* have 11 chromosomes ($2n=2x=22$), cultivars with edible fruits and greater commercial value (1,3).

Much of the cultivars of edible *Musa* in the world are hybrids of diploid lineages of *Musa acuminata Colla* and *Musa balbisiana Colla* which contributed to the A and B genomes respectively (5). Hybridization among several subspecies of *Musa acuminata Colla* and/or *Musa balbisiana Colla* results in triploid cultivars ($2n=3X=33$) (2,6-8).

Most AAA cultivars are edible raw when ripe, while the AAB (plantain) or ABB (bananas that require cooking) cultivars require some form of cooking before being eaten (9).

Plantain is one of the most important crops in the world, being a staple in the diet of millions of people, mainly in Africa and America including Colombia, where there is a consumption estimate of 155 kg/year per capita. This crop has become an element of great economic importance for the country, by employment generation and food security perspective. Plantain production in Colombia has had great ups and downs, in 2013 the plantain crops area showed a reduction of 6,1 % compared to 2012, similarly, production was reduced by 32 % compared to the same year. In Quindío, a region with large plantain crops, between 2000 and 2010, the yield was 8,2-10 ton/ha (9,5 ton/ha average), similar to Venezuela's production for 2009 (8,6 ton/ha) (10); However, production in the region has now dropped to 7,5 ton/ha. This reduction in production may be due to a wide variety of phytosanitary problems, inadequate agricultural management, inappropriate cultural practices, irrational use of agrochemicals and use of low production clones (11), among other difficulties affecting Quindío and other regions dedicated to plantain farming.

In this respect, it has been demonstrated that the genotypes of *Musa balbisiana* spp (genome B), to provide resistance to pests, diseases and droughts (12), while *Musa acuminata* (genome A) genotypes are rich in phytochemical compounds use in many diseases treatment in traditional medicine (13). Thus, the use of crop genetic diversity could be an appropriate option to improve production through the use of more productive genotypes (14).

Despite the commercial and food security significance that plantain crops have to Quindío, the genome of these plants is poorly understood especially in terms of chromosomal charge, as far as is known, the cultivars karyotype of Quindío region has not been described in number and size. For this reason, the aim of this study was to determine the similarities in the karyotypes of different cultivars of *Musa spp* from Quindío region, with the purpose of contributing to characterization, expanding possibilities for intervention, and subsequent use of these genotypes characterized in the evaluation of their productive capacity or resistance to diseases and pests in other works.

MATERIALS AND METHODS

VEGETAL MATERIAL

Nine samples of *Musa spp.* from several municipalities of Quindío region were collected (Table I). The cultivars were classified according to the knowledge of the farmer (native nomenclature) and identified with a unique access code.

CYTOLOGICAL OBSERVATIONS

Preparation of vegetal material was carried out following the methodology described by Adeleke et al and D'Hont et al. (7,15), with some minor modifications, (material was radicular meristems instead anthers and enzyme cocktail was different in percentage), briefly: Radicular meristems were obtained from horns (sprouts), radicular apices of approximately 1 cm long were cut from these meristems, obtained from previously established vegetative propagules. Some samples were directly taken from the plant. The cutting was made between 8:00 am-12:00 pm. Apices were placed in amber bottles with 2 ml of 8- Hydroxyquinoline 0,02 % solution for 24 hours at 8 °C. Fixation was performed with ethanol/ glacial acetic acid 3:1 v/v), for 24 hours at 11-13 °C. Subsequently, they were stored at -20 °C until use. The samples were divided into two, one for conventional staining and one for DAPI staining.

Table I. *Musa spp* accessions, geographical location and chromosomes technical identification

Municipio	Origen de la Plantacion	Nomenclatura nativa de <i>musa spp</i>	Código de accesoión	Técnica de análisis Convencional DAPI*	
Buenavista	Alsacia La Maravilla	'Dominico-Hartón'	D-H-1	X	X
		'Hartón'	H-1	X	X
		'Dominico'	D-1	X	X
		'Banano común'	B-c-1	X	
		'Popocho'	P -1	X	X
		'Africano'	A -1	X	
		'Guineo'	Gu-1	X	X
Pijao	Buenos Aires Bajo	'Guayabo'	G-2		X
		'Popocho'	P -2	X	
La Tebaida	Santa María	'Dominico-Hartón'	D-H-2	X	
		'Africano'	A-3	X	
		'Guayabo'	G-3	X	
		'Guineo'	Gu	X	X
Montenegro Vereda la Cabaña	La Fortuna	'Dominico-Hartón'	D-H-4	X	X
		'Banano común'	B-c-4	X	X
		'Hartón'	H-4	X	X
Calarcá	Bella la Nubia	'Dominico-Hartón'	D-H-5	X	X
Calarcá Quebrada Negra	Villa Laura	'Dominico-Hartón'	D-H-5	X	
		'Hartón'	H	X	X
		'Africano'	A-5		X
		'Banano sapo'	B-s-6	X	X
Calarcá Vereda Bohemia	Villa Gabriela	'Banano enano'	B-e-6	X	X
		'Dominico'	D-6	X	X
		'Dominico-Hartón'	D-H-6	X	X
Pueblo Tapao	La Aurora	'Dominico-Hartón'	D-H-7	X	

*DAPI= 4',6-diamino-2-fenilindol

CONVENTIONAL STAINING

The following method was used for conventional chromosomal analysis: meristematic tissue was hydrolyzed in HCl 1N at 60 °C for 15 minutes. Next, the apices were washed to remove excess of HCl. Staining was performed by embedding the apices in hematoxylin-chromic lacquer at 60 °C for 3 hours, followed by the squashing or crushing. The size and chromosome number for each sample was estimated in five to twenty prometaphases for each material using negatives of enlarged photos.

DAPI STAINING

Radicular apices were obtained and fixed as described above, washed three times with distilled water for 5 minutes each wash.

Subsequently they were digested in 10 ul of a 2 % cellulase and 20 % pectinase cocktail in humid chamber for 2 hours at 37 °C. The excess of enzyme cocktail was removed and a drop of 45 % acetic acid was added to macerate and homogenize. Meristems were extracted, squashed and brought to liquid nitrogen. After removal of coverslips, the laminae were air dried for two hours at room temperature. Subsequently the laminae were stained with DAPI 10 ul 2,5 ug/ml for 30 minutes at room temperature in dark chamber. After five washes for 5 minutes with phosphate buffer (PBS-1X), there were added 10ul of 50 % glycerol, then it was performed a mild pressure of coverslip over laminae and the edges of coverslip were sealed with clear smalt.

CHROMOSOME VARIABLES

The following variables were measured on chromosomes:

- ◆ *Absolute chromosome length (microns)*: taken as the result of adding the short and long arms longitude.
- ◆ *Genome total length (microns)*: taken as the result of adding each chromosome length ($n=11 \times 3$) representing 100 %.
- ◆ *Relative chromosome length*: measured as the proportion of each chromosome with respect to the total genome length ($n=11$), taken this as 100 %.
- ◆ *Genome haploid length (microns)*: taken as the result of adding each chromosome length ($n=11$) representing 100 %.
- ◆ *Chromosomal classification*: given the impossibility to define the centromere, classification was arbitrary and thus defined: group A chromosomes- medium-large size (1,951 – 2,790 μm); group B chromosomes- small-medium size (1,134 – 1,950 μm) and group C chromosomes - very small (0,307- 1,141 μm).
- ◆ *Genome uniformity*: defined as the difference in absolute length between chromosomes of greater and lesser length.

DATA ANALYSIS

Digital images for observation of chromosomes (40 and 100 X) with conventional staining, were captured using Nikon Eclipse 80i microscope with photographic device and with the NIS-Elements F2.30 program in a Leica QWIN® Image Analysis System. For DAPI staining, the laminae were observed under an EVOS FL fluorescence microscope with a 340-380 nm filter, with 60X magnification. ImajeJ software (<https://imagej.nih.gov/ij>) was used for identification and cutting of chromosomes, and MicroMeasure software version 3.3, to measure length of each chromosome. The image that offered better dispersion and condensation of chromosomes was used for morphological identification and description.

The values of the size of the chromosomes, the total haploid genome and the genome length (taken in triplicate), are expressed as mean values with confidence intervals at 95 %. To determine the differences between the average lengths in the cultivars studied, a way ANOVA and Tukey test was performed. A K-means cluster analysis was performed using a Tukey Test, taking genome haploid length as variable; for all analysis SPSS v 20 was used (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

RESULTS AND DISCUSSION

From the cytogenetic study performed it was possible to establish and compare the number, size and other chromosomal characteristics of plantain crops from Quindío-Colombia.

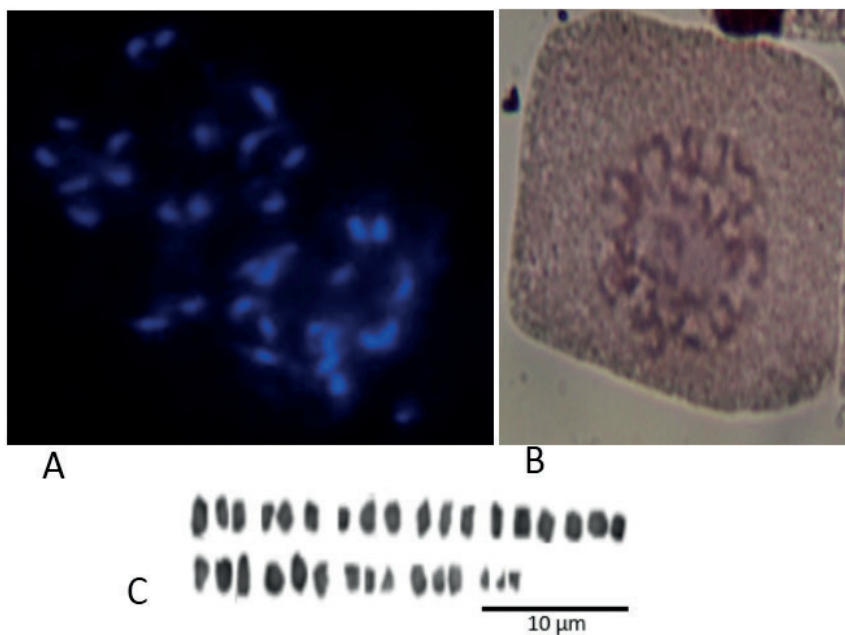
There were found cells in various mitotic states and between five to twenty prometaphase cells per cultivars were analyzed.

Results show that chromosomal number was not consistent in all preparations for the same cultivars. In the case of 'banano enano' (sample B-e-6, Table I), count ranged between 26 and 44 (data not shown). However, detailed somatic chromosomal analysis of all genotypes studied using conventional staining or DAPI staining, showed a somatic average number of $2n=3x=33$ chromosomes in 99 % of cultivars, where $x=11$. These results confirm that these cultivars are triploid species with $n=11$ (3,7,16).

For these cultivars, staining with DAPI (A) was not superior to traditional staining (B), as shown in Figure 1. Chromosomes organization in triplets was made difficult by the size of the same ones (C). Figure 1 shows one of the best images of the chromosomes of the nine cultivars.

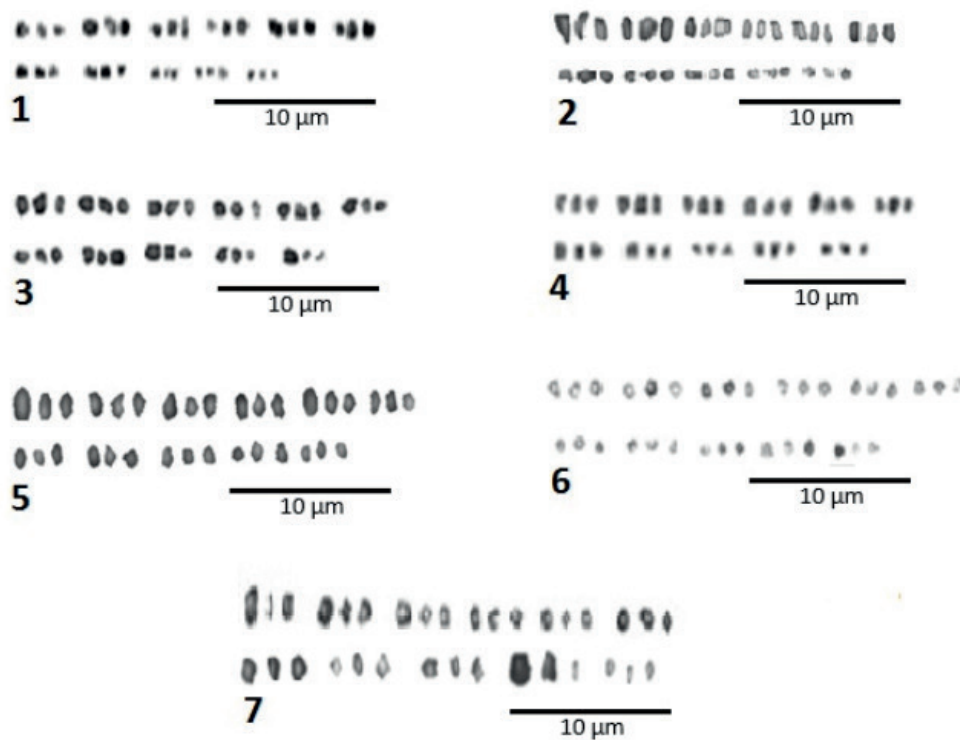
In past decades, it had been determined that the basic number of chromosomes in the *Musa* genus was 11 (3,5) and found that the cultivated types have three levels of natural ploidy: $2n=2x=22$, $2n=3x=33$, $2n=4x=44$ chromosomes (7,17). From our results, the regional cultivars studied belong to ploidy level $2n=3x=33$, and include hybrids AAB, ABB, AAA. This type of banana and plantain has been described as interspecific triploid hybrids of *Musa acuminata* (genome A) and *Musa balbisiana* (genome B) (18). Polyploidy or alteration of the basic number of chromosomes is considered one of the determining factors in evolution of species, as demonstrated by Vichiato *et al.* for *Dendrobium nobile* (19), but our results show that the morphological differences observed among studied cultivars, are not related to changes in the ploidy level.

Once the karyotypes were obtained, size of chromosomes based on its longitudinal axis (absolute length in microns) and their relative length representing the ratio of each chromosome with respect to the genome total length ($n=11$) were analyzed. Chromosomes were arbitrarily grouped by size and sorted by triplets based on the absolute size (Figure 2). Under this arrangement three types of chromosomes were found; type A chromosomes-medium-large size (1,951 - 2,790 μm); type B chromosomes-small-medium size (1,134 - 1,950 μm); type C chromosomes- very small (0,307- 1,141 μm).



A = DAPI staining; B = eosin Hematoxylin staining; and C = Chromosomal size and number from 'Dominico-Hartón'. 50 μm scale for A, 100X augmentation for B and 10 μm for C

Figure 1. 'Dominico-Hartón' cultivar karyotype



1: 'Hartón', 2: 'Guayabo', 3: 'Popocho', 4: 'Guineo', 5: 'banano enano', 6: 'banano común' and 7: 'banano sapo'. Digital imagines for chromosomes observations with DAPI staining were 40 y 60 X, and with conventional staining were 40 and 100 X

Figure 2. Cultivars karyotype

There were found differences in chromosomal size among cultivars, hence 'Hartón' cultivar had the lowest average chromosomal (0,595 μm) and genome size (19,656 μm), while the 'Dominico-Hartón' had the greater average chromosomal (2,326 μm) and genome size (57,946 μm). Wide variability was found between chromosomes number by type, e.g. type A chromosomes only was found in 'Dominico-Hartón' and 'banano enano': nine and three chromosomes respectively; type B chromosomes between zero ('Hartón') and twenty four ('Dominico-Hartón'). Except 'Dominico-Hartón', all accessions had type C chromosomes, between six and thirty-three. Genome length also had a wide variability ranging from 19,273 μm ('Hartón') to 57,946 μm . Table II shows the results.

With the purpose of developing efficient strategies for improving *Musa* cultivars, several authors have tried to define chromosomal number, type and size of *Musa* species through cytogenetic and/or molecular studies (7,20,21); although the number of chromosomes ($n=11$) is currently known (16), these chromosomes have not been fully numbered and identified, partly due to technical difficulties such as difficulty in obtaining good quality cellular samples, time and effort to find the correct mitotic states, rigidity of cell wall, poor staining capability of chromosomes in prophase stage and small size of chromosomes in these species (7).

Table II. Chromosomal size of *Musa* spp. cultivars

Accesión	Tipo de cromosoma	Cantidad	Tamaño promedio (μm)	Intervalo (μm)	Tamaño del genoma (μm)	Genoma relativo %	Longitud del haploide (μm)
'Dominico-Hartón'	A	9	2,326	(1,961 - 2,790)	57.946	36,12	19,273*
	B	24	1,542	(1,134 - 1,950)		63,88	
	C	0	-	-		-	
'Banano Enano'	A	3	1,967	(1,961 - 1,998)	46.058	12,89	15,397
	B	24	1,414	(1,167 - 1,805)		73,72	
	C	6	1,028	(0,829 - 1,109)		13,40	
'Popocho'	A	0	-	-	40.646	-	13,541
	B	21	1,362	(1,136 - 1,733)		70,41	
	C	12	1,002	(0,855 - 1,132)		29,59	
'Guayabo'	A	0	-	-	37.529	-	12,462
	B	15	1,400	(1,151 - 1,644)		55,96	
	C	18	0,918	(0,533 - 1,097)		44,04	
'Banano Sapo'	A	0	-	-	37.054	-	12,341
	B	12	1,536	(1,267 - 1,863)		49,75	
	C	21	0,8866	(0,600 - 1,141)		50,25	
'Banano común'	A	0	-	-	36.540	-	12,174
	B	15	1,284	(1,151 - 1,480)		52,72	
	C	18	0,959	(0,808 - 1,113)		47,28	
'Dominico'	A	0	-	-	35.791	-	11,930
	B	12	1,449	(1,152 - 1,854)		48,57	
	C	21	0,876	(0,550 - 1,104)		51,43	
'Guineo'	A	0	-	-	29.189	-	9,731
	B	3	1,146	(1,141 - 1,174)		11,78	
	C	30	0,869	(0,562 - 1,107)		88,2	
'Hartón'	A	0	-	-	19.656	-	6,552
	B	0	-	-		-	
	C	33	0,595	(0,307 - 0,928)		100	

*There are significant differences in the genome haploid length among all cultivars, according to ANOVA test and Tukey with a factor of $p \leq 0.5$

However, for the first time, this study establishes a difference in genome size among different cultivars of *Musa* from the region of Quindío. This allowed, although arbitrarily, to establish the presence of three types of chromosomes by size, the genome total length and genome haploid length for the studied cultivar. Except for the cultivar called 'Dominico-Hartón', which has a zero type C chromosomes, this type of chromosomes were found in all cultivars, with wide variability in terms of number of chromosomes; on the other hand, genome length varied considerably among cultivars. A detailed analysis of chromosome types reveals the dominance of type C chromosomes, between six ('banano enano') and thirty three ('Hartón'); such differences in the number of chromosomes in a subtype have been described for other plants where diploid or haploid number is maintained, but there is variability in the size of chromosomes in each subtype (22,23).

Comparison of chromosomes average length among cultivars showed that these can be placed into five groups according to increasing chromosomes average length: Group 1: 'Hartón'; Group 2: 'Guineo'; Group 3: 'Dominico', 'banano común', 'banano sapo', 'Guayabo' and 'Popocho'; Group 4: 'banano enano' and Group 5: 'Dominico- Hartón', with significant differences ($p=0,000$).

Subsequently, K-means cluster analysis allowed to place cultivars into six groups: Group 1: 'Hartón'; Group 2: 'Guineo'; Group 3: 'Dominico', 'banano común', 'banano sapo' and 'Guayabo'; Group 4: 'Popocho'; Group 5: 'banano enano'; Group 6: 'Dominico- Hartón'; with significant differences in genome haploid length between groups ($p=0,000$).

Statistical analysis, with Tukey Test, using haploid genome uniformity as variable, places cultivars into three groups: Group 1: 'Guineo', 'banano común', 'Hartón' and 'Popocho'; Group 2: 'banano enano', 'Guayabo', 'Dominico' and 'banano sapo'; Group 3: 'Dominico-Hartón', with significant differences ($p=0,000$) between groups.

The analysis of genetic relationships among species is important for breeding programs and to provide information on the genetic diversity and evolution of cultivars as well as on the effect of environmental conditions over this species (9,24).

Although characterization using molecular markers may be more accurate for the study of genetic variability of plantain cultivars (25), this study provides an approach consisting in grouping regional plantain cultivars by chromosome type, size and genome length.

This is important for the future to carry out the evaluation of genotypes against production, resistance to pests and diseases or production of metabolites for use in alternative medicine (13,26)

Unfortunately, the quality of preparation and chromosome size itself did not allow to differentiate centromeres or arms on chromosomes despite using two different staining methods, concluding that DAPI staining did not provide higher resolution to the preparations used in this work, but without ignoring the advantages of this staining method for other preparations. This inability to characterize chromosomes in a conclusive manner has already been described by Adeleke *et al.* (7) who proposed a new method to characterize them, however, although the procedure substantially improves the visibility of chromosomes, difficulties related to the quality of preparations did not allow to fully characterize them.

CONCLUSION

Studied cultivars of *Musa spp*, in the region of Quindío, have three types of chromosomes according to its size, they differ in the number of chromosomes and length of genome for each type, which allows to group cultivars, but they are all triploid hybrids ($2n=3x=33$), (AAB, ABB, AAA), confirming the use of clones in this region. This study contributes to the advance of the genetics of *Musa spp* for future breeding program.

RECOMMENDATIONS

Study results serve to improve the cytogenetic techniques and to apply to other cultivars.

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