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A molecular preliminary study of Colombian maize (*Zea mays* L.) accessions by using a cpDNA region

Estudio molecular preliminar de accesiones de maíz (*Zea mays* L.) criollo e indígena Colombiano, utilizando una región de ADN cloroplástico

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Abstract

In order to preliminarily explore the genetic diversity in the 23 Colombian races of maize described by Roberts and co–workers (1957), this study evaluate 28 nuclear and chloroplast primers. From these, 14 amplified by PCR, which were sent to sequencing to Macrogen Inc. (Korea). Bioinformatics programs revealed that six of these primers had a high level of polymorphism. It was considered that the genomic chloroplast region *atpB–rbcL–1–1* showed the highest polymorphism, therefore it was used to evaluate 23 materials, representative of the 23 races, stored in the CIMMYT germplasm bank, Mexico. By analyzing sequences we could defend and confront racial groups obtained in the present study ('primitive races' three, 'probably introduced' seven, 'Colombian hybrid', 13) with those established by Roberts and co–workers at the 50´s, who showed two 'primitive', nine 'probably introduced' and twelve 'Colombian hybrid' races and the groups set by Cardona (2010), applying the strategy Ward–MLM for the same characters described by Roberts *et al.* (1957), which shows five 'primitive', seven 'probably introduced' and nine 'Colombian hybrid' races. Thus, it makes a methodological contribution to validate historical data and redefine racial groups.

Key words: cpDNA, genetic diversity, *Zea mays* L.. creole maize, creole races, Colombia.

Resumen

Se exploró en forma preliminar la diversidad genética existente en las 23 razas de maíz criollo e indígena descritas para Colombia por Roberts y colaboradores (1957), para el efecto se evaluaron 28 cebadores nucleares y cloroplásticos. Catorce de ellos amplificaron en la reacción en cadena de la polimerasa (PCR) y fueron enviados para secuenciar a Macrogen Inc. (Corea). Mediante programas bioinformáticos (BioEdit 7.1.0, ClustalW versión 1.81, EditPlus Text Editor versión 3.20 y Gblock 0.91b 8) se encontró que ocho de estos cebadores presentaron un nivel alto de polimorfismo. La región genómica cloroplástica *AtpB–1–RbcL–1* mostró el mayor polimorfismo y por tanto se utilizó para evaluar 23 materiales representativos de las 23 razas conservadas en el Banco de Germoplasma del CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) en México. Con el análisis de secuencias se revalidaron y confrontaron los grupos raciales obtenidos

en el presente estudio –tres razas primitivas, siete razas probablemente introducidas, y 13 razas híbridas colombianas– con aquellos establecidos por Roberts y colaboradores, en los cuales se encontraron dos razas primitivas, nueve probablemente introducidas y 12 híbridas colombianas y con los grupos establecidos por Cardona (2010) utilizando la metodología Ward–MLM para los mismos caracteres descritos por Roberts *et al.* (1957), donde se encontraron cinco razas primitivas, siete probablemente introducidas y nueve híbridas colombianas. Con base en lo anterior, en el presente trabajo se hizo un aporte metodológico para revalidar datos históricos y redefinir grupos raciales

Palabras clave: ADNcp, diversidad genética, *Zea mays* L., razas criollas, Colombia.

Introduction

The large diversity in South America maize keeps relation with its geography and history. The culture development of the different American populations, their migrations, the America´s discovery and subsequently, the European migrations, were decisive factors on the creation of the germplasm diversity of corn (Goodman and Bird, 1977).

The continuous interchanges of genes among populations, the later selection, both, natural and by the humans, have keep active the diversification process by which it has been selection and modification of genotypic characteristics that formed new adapted population to several weathers and soil types. Sánchez *et al.* (2000) proposed that the environment x genotype interaction and the geographical isolation also favor the geographical distribution of the new maize varieties, mainly in the high lands (Roberts *et al.*, 1957).

Anderson and Cutler (1942) proposed a natural classification of the maize ratial diversity and introduced the race concept as the set of individuals with enough number of common traits that allows its recognition as a group.

In Colombia there have been identified 23 maize races, grouped into three categories according to it probable origin, recognized by Roberts *et al.* (1957). Two primitive, nine probably introduced races and twelve hybrid races originated in Colombia, are defined. These authors identified, also, four evolution factors that contributed to the formation of those races: geographical isolation, interratial hybridation, hibridation with maize contaminated with teocintle coming from Mexico and, hibridation of maiz with it wild relative *Tripsacum*.

Nowadays, factors such as the change in land use in function of the marketing, population movement for natural and social phenomena, the absence of public programs for *in situ* conservation and, some events related with evolutionary processes typical from panmitic species such as genetic drift, mutations or hibridation with improved materials, could possibly favored a geographical redistribution or loss in genetic diversity of the races identified by Roberts *et al.* (1957).

The molecular analysis with DNA sequences in creole and indigenous maize from Colombia are scarce. For the analysis of genetic diversity, Peñaranda *et al.* (2005) performed a molecular characterization with SSR markers in 22 Colombian creole maize races to determine the oil contents in the grain.

The main objective of this research was to evaluate a methodology to study the genetic diversity in Colombian creole maize, with a molecular technique on a highly polymorphic genomic region and, revalidate the racial groups proposed by Roberts *et al.* (1957) and Cardona (2010).

Materials and methods

Representative accessions of the 23 races characterized by Roberts *et al.* (1957) in Colombia were used (Table 1, Figure 1). The seeds of these accessions were taken from the collections stored at the Germplasm

Table1. Passport data of maize (*Zea mays* L.) introductions used in this study.

Order	Racial group	Department	Municipality	Race	Latitude	Longitude	Altitude (MASL)
1	Primitive	Cundinamarca	Ubaté	Pira	5,19	-73,49	2377
2		Cundinamarca	Zipaquirá	Pollo	5	-74	2570
3	Probable	N. Santander	Mutiscua	Sabanero	7,1748	-72,4447	2709
4	Introduced	Putumayo	Puerto Leguízamo	Andaquí	0,12	-74,46	185
5		Tolima	San Luis	Clavo	4,0721	-75,0517	435
6		Cundinamarca	La Palma	Harinoso Dentado	5,2152	-74,24	1549
7		Nariño	Buesaco	Pira Naranja	1,23	-77,09	1646
8		Magdalena		Güirua	10,5424	-74,0247	1397
9		Nariño	Tangua	Maíz Dulce	1,0552	-77,2313	2515
10		Córdoba	Montería	Cariaco	8,43	-75,53	17
11		Sabana Bogotá		Imbricado			
12	Colombian	N. Santander	Pamplona	Cabuya	7,2229	-72,3915	2378
13	hybrids	Antioquia	La Ceja	Montaña	6,0208	-75,2557	2144
14		Cauca	Popayán	Amagaceño	2,27	-76,36	1609
15		Valle	Pradera	Común	3,25	-76,15	1043
16		Santander	Charalá	Cacao	6,1702	-73,0905	1251
17		Tolima	Las Piedras	Yucatán	4,3244	-74,5243	387
18		Atlántico	Campo de la Cruz	Costeño	10,2244	-74,521	9
19		Magdalena	Magdalena	Negrito	11,3048	-72,5203	8
20		Cesar	Aguachica	Puya	8,19	-73,38	125
21		N. Santander	Salazar	Puyagrande	7,4705	-73	796
22		Chocó	Infiernito-Rio Nauca	Chococeño	5,3614	-77,0058	37
23		Putumayo	Sibundoy	Maíz Capio	1,10365	-78,54536	2096

Bank of the International Maize and Wheat Improvement Center (CIMMYT) in Mexico.

The seedling production was done in the Cytogenetics lab and the molecular study in the Molecular Biology lab of the Universidad Nacional de Colombia-Palmira. For that, 20 seeds per accession were used, for germination they were placed on newspaper and water. Five days after germination DNA was extracted from young, underdeveloped leaves. The DNA extraction was done with the methods proposed by Dellaporta *et al.* (1983) modified by the Molecular Biology lab of the Universidad Nacional - Palmira, Doyle and Doyle (1987) modified by CIMMYT (1996), Fulton *et al.* (1995), and the Promega dNTPs kit for plant DNA extraction, however, the method of Doyle and Doyle (1987) modified by CIMMYT (2006) was the one that allowed good quality DNA, with a concentration around 50 ng/µl in all the accessions evaluated.

To evaluate the quantity and quality of DNA 0.8% agarose gels were prepared and run in TBE 0.5X buffer (Tris-borate 0.045M; EDTA 0.001M) and dyed with ethidium bromide at final concentration of 0.5 ng/ml. the concentrations were determined by comparison with DNA of the bacteriophage Lambda at 30, 60 and 90 ng/µl. A database was made to record as much information as possible.

For the stadardization of the conditions for each marker, a mix of reactives was pre-

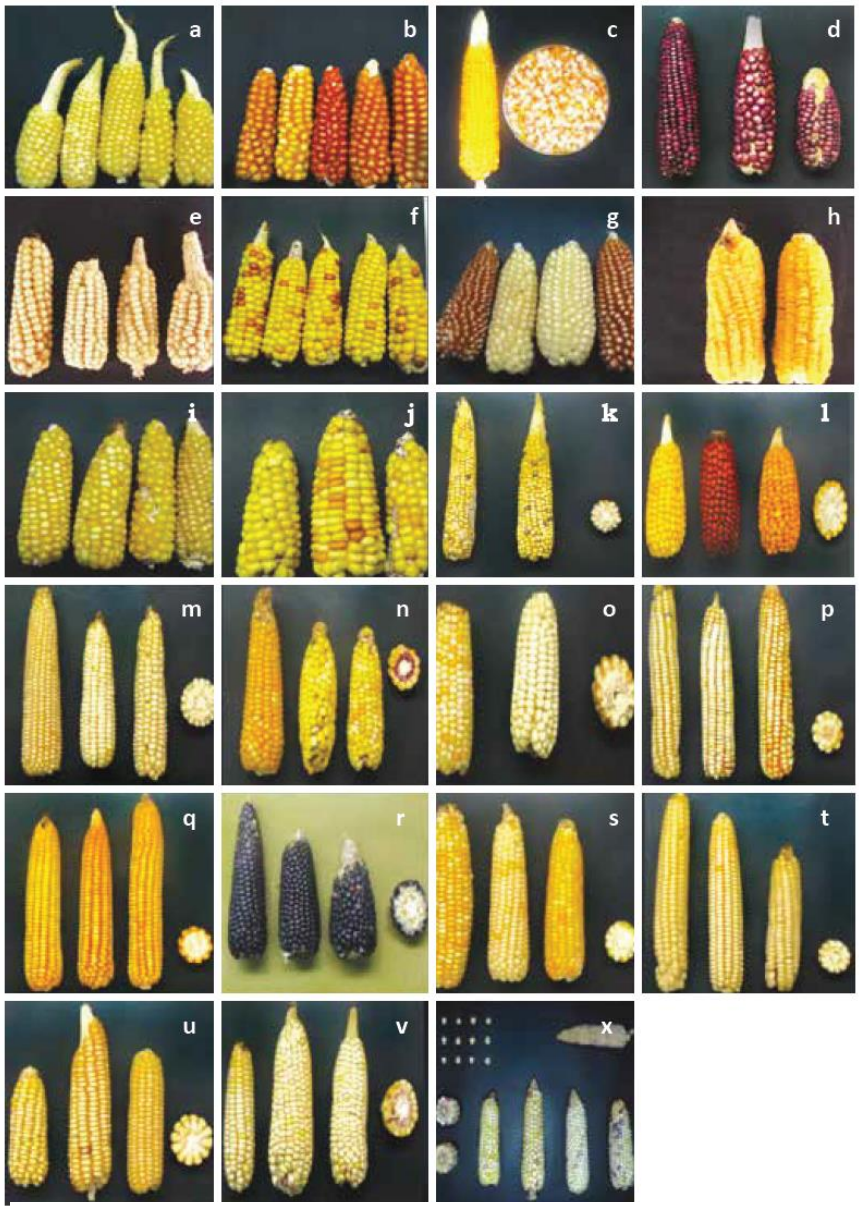

Figure 1. Creole and indigenous maize races in Colombia (Roberts *et al.*, 1957). a. Pira; b. Pollo; c. Pira naranja; d. Negrito; e. Amagaceño; f. Cabuya; g. Imbricado; h. Maíz Dulce; i. Montaña; j. Sabanero; k. Guirua; l. Cariaco; m. Común; n. Cacao; o. Costeño; p. Puya; q. Puya Grande; r. Chococeño; s. Andaquí; t. Clavo; u. Yucatán; v. Maíz Harinoso Dentado; x. Capio. (Pictures: Authors).

pared on a sterile tube for microcentrifuge (1.5 ml) for a final volume of 25 µl. 2 µl of genomic DNA at 50 ng/µl were used for PCR with final concentration of the 1X taq buffer, 0.2 mM dNTPs, 0,1 µM primer F and R, 3 mM $MgCl_2$ and 2.5 units of Taq polymerase (Table 2). For the amplification of the primers in this study the polymerase chain reaction (PCR) was used (Table 3).

To visualize the PCR products under UV light, 1.2% agarose gel dyed with ethidium bromide was used on a horizontal electrophoresis device CBS Scientific SGE-00-02 and a digital equipment for image acquisition Uvitec. The gel tray was prepared with adhesive tape, closing the open sides reinforcing the sides and corners of the gel tray. 1.2 g of agarose were added per

Table 2. Volume for PCR mix.

Sample	[ ] initial	[ ] final	Volume (µl)	Total (µl * n)
TAQ	5u / µl	1,25 Mm	0.25	2.5
dNTP's	1,25 mM	0.2 mM	4	40
$MgCl_2$	25 mM	3 mM	3	30
Primer F	10 µM	0,1 µM	0.25	2.5
Primer R	10 µM	0,1 µM	0.25	2.5
Buffer TAQ	10X	1X	2.5	25
H_2O	—	—	13.75	137.5
DNA	10 - 100 ng/µl	10 - 100 ng/µl	1	—
Vol. total	—	—	25	240

TAQ = *Thermus aquaticus* (Taq polymerase), dNTPs= deoxyribonucleotides triphosphate.
$MgCl_2$ = Magnesium chloride. Primer F: forward. R: reverse.

150 ml of 0.5X TBE buffer prepared. It was placed at the microwave until the solution was totally dissolved and ethidium bromide (1 µl) was added for each 100 ml of agarose solution prepared. Then, it was cooled down until it polymerizes under the fume hood. The DNA + run buffer mix were added on the gel wells submerged in the electrophoresis chamber. The electrophoresis was run for 40 min at 80 volts. Finally, the bands were observed on a camera with UV light and pictures were taken to the nucleic acid bands excited by the ethidium bromide. The sequencing was done in Macrogen Inc. (Korea) together with DNA purification.

For the bioinformatic analysis, the sequences were edited and assembled with the program BioEdit 7.1.0, a software for Windows 95/98. Each DNA region evaluated was aligned using the program ClustalW v. 1.81, with the standard parameters of Larkin *et al.* (2007). The alignments were manually modified in EditPlus Text Editor v. 3.20 for Windows 7/vista/2000/ 2003/XP. For the identification of non-informative blocks and divergent regions of the alignment, the program Gblock 0.91b 8 Castresana (2000) was used. Each one of the sequences based on the NCBI database with the BLASTn algorithm to estimate the score and confirm the species under study was checked.

To evaluate the markers, five criteria were used: (1) easy amplification markers were identified and 5 individuals were selected per marker to evaluate the quality of the sequences; (2) for each loci the number of haplotypes, polymorphic sites number and Nei haplotype diversity (Nei, 1973) was calculated using the DNAsp program (Rozas and Rozas, 1999); (3) models of nucleotide substitution from the individuals representing the maize creole Colombian races were evaluated, with the goal of describing the existent consanguinity; (4) with the best marker able to discriminate the racial group a genetic distance tree using the MEGA v. 5 program (Tamura *et al.*, 2011) was constructed; and (5) the marker selected as highly informative, with all the sequences to observe its efficiency in discriminating species and racial groups was evaluated. The bootstrap value (BS) for each individual clade by 500 iterations was calculated. All the positions with gaps or missing data were eliminated from the database.

Results and discussion

Among the evaluated methods for DNA extraction in maize, the one from Doyle and Doyle (1987) modified by CIMMYT (2006) allowed the extraction of good quality DNA, with an average concentration of 50 ng/µl

Table 3. Primers explored in this study.

Order	Primers	Sequence (5 '- 3')	Source
1	*rpS16x2F2*	AAA GTG GGT TTT TAT GAT CC	Shaw et al. 2007
	trnK(UUU)	TTA AAA GCC GAG TAC TCT ACC	
2	*psbA3'f*	GTT ATG CAT GAA CGT AAT GCT C	Kress et al. 2005
	trnHf	CGC GCA TGG TGG ATT CAC AAT CC	
3	*trnL(UAG)*	CTG CTT CCT AAG AGC AGC GT	Shaw et al. 2007
	rpL32-F	CAG TTC CAA AAA AAC GTA	
4	*rbcLa_rev*	GTA AAA TCA AGT CCA CCY CG	Kress et al. 2005
	rbcLa_for	ATG TCA CCA CAA ACA GAG ACT AAA GC	
5	*ITS4*	TCC TCC GCT TAT TGA TAT GC	Whithe et al. 1990
	ITS5A	CCT TAT CAT TTA GAG GAA GGA G	
6	*Adhc-P1*	CTG CKG TKG CAT GGG ARG CAG GGA AGC C	Small et al. 1998
	Adhc-P2	GCA CAG CCA CAC CCC AAC CCT G	
7	*GPDX7F 3*	GAT AGA TTT GGA ATT GTT GAG G	Strand et al. 1997
	GPDX9R 3	AAG CAA TTC CAG CCT TGG	
8	*trnQ(UUG)*	GCG TGG CCA AGY GGT AAG GC	Shaw et al. 2007
	rpS16x1	GTT GCT TTY TAC CAC ATC GTT T	
9	*TabC*	AAT TAG CGA CGG ACG CTA CG	Taberlet et al. (1991)
	TabF	ATT ACT TGA GCA AG ACG GGT	
10	*rpL32-R:*	CCA ATA TCC CTT YYT TTT CCA A	Shaw et al. 2007
	ndhF:	GAA AGG TAT KAT CCA YGM ATA TT	
11	*nad1-BF2*	GGA GGC AAG AAC CAT GCT TTC A	
	nad1-BF3	GAA AGG GCT GTA GGT GAT GGT G	
12	*trnG2G-F*	GCG GGT ATA GTT TAG TGG TAA AA	
	trnG-R	GTA GCG GGA ATC GAA CCC GCA TC	
13	*atpB-1:*	ACA TCK ART ACK GGA CCA ATA A	
	rbcL-1	AAC ACC AGC TTT RAA TCC AA	
14	*TabE*	GGT TCA AGT CCC TCT ATC CC	
	TabF	ATT ACT TGA GCA AG ACG GGT	

for all the evaluated materials. This agrees with the reports by Wang *et al.* (1993), Weir *et al.* (1996), Henry (1997), Jewit *et al.* (1998) among others, that have used CTAB to isolate DNA from mature leaves to characterize species by RAPD.

The success rate of the primers in the PCR amplification showed that six of them had problems to amplify. These include: *ITS4–ITS5A, Adhc–P1–Adhc–P2, GPDX7F–GPDX9R, TabC–TabF, rpL32–R–ndhF* and *nad1–BF2–nad1–BF3*. The primers that amplified were *rpS16x2F2–trnK(UUU)x, psbA3'f–trnHf, trnL(UAG)–rpL32–F, rbcLa–rbcLb, trnQ(UUG)–rpS16x1, trnG2G–F–trnG–R, TabE–TabF* and *atpB–1–rbcL–1* (Figure 2).

Preeliminar test for sequence variation
The descriptive statistic for the eight evaluated primers is shown in Table 4. The marker *AtpB–1–RbcL–1* showed the largest number of polymorphic sites (182), being the most informative among the ones evaluated. Then, with this pair of primers evaluations on the 23 accessions used in this study were made.

Sustitution and nucleotide frequency model For the evaluation, all the positions with gaps of missing data were deleted. In total 24 models of nucleotide substitution from the representatives of the maize Colombian races were evaluated. According to the criteria for nucleotide substitution, the model with the lowest BIC (Bayesian

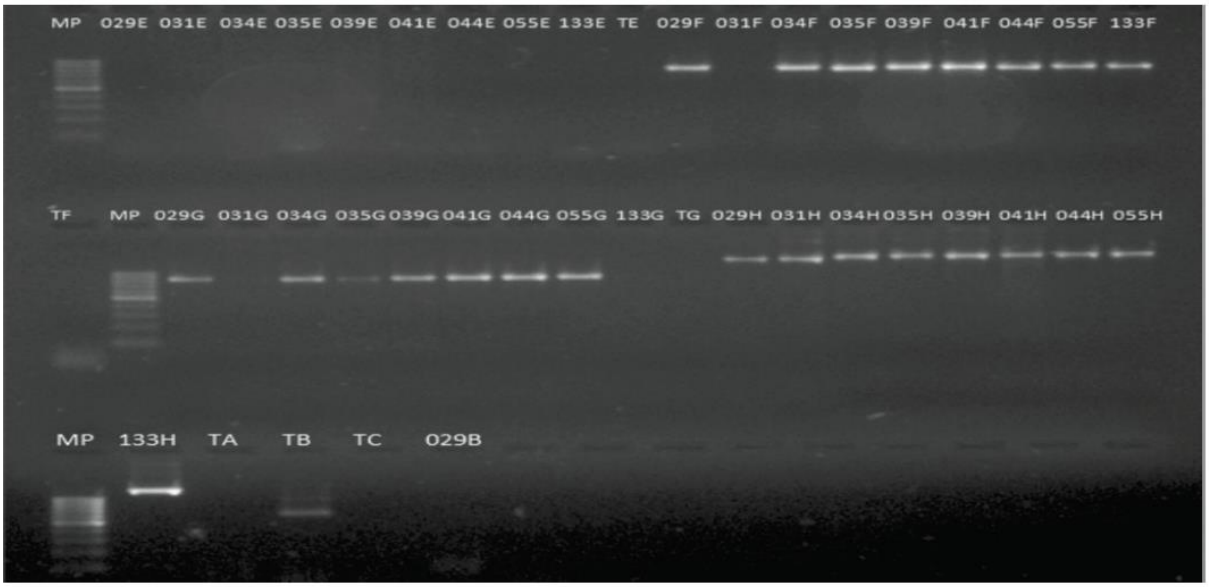

Figure 2. Electroforesis of the amplified products with the evaluated primers in this study, the size marker was 100 bp.

Table 4. Descriptive statistics for the eight primers evaluated in *Zea mays* L.

Order	Primer	S	Hd
1	*rpS16x2F2-trnK(UUU)x1*	4	0.972
2	*psbA3'f-trnHf*	12	0.83
3	*trnL(UAG)-rpL32-F*	33	0.72
4	*rbcLa_rev-rbcLa_for*	15	0.66
8	*trnQ(UUG)-rpS16x1*	19	0.86
12	*trnG2G-F-trnG-R*	4	0.400
13	*AtpB-1- RbcL-1*	182	0.143
14	*TabE-TabF*	45	0.677

S: Polymorphic sites, Hd: Haplotipic diversity.

Information Criteria) score is considered the most suitable to describe the existent consanguinity between individuals and to create a phylogenetic tree for research. In this study the model T92 (Tamura 3-parameter) got as BIC 4338.8 being the lowest value among the 24 studied models.

The nucleotide frequency estimated the adenine (A), cytosine (C), guanine (G) and thiamine (T) contents. For the *AtpB–1–RbcL–1* was observed a high A and T contents with 67.56% frequency, respectively. However, for C and G the frequency of 32.44% was lower than the A-T ratio. For the evaluation a total of 897 positions in the final data set was estimated (Table 5).

Descriptive analysis

According to the markers evaluation, *AtpB–1–RbcL–1* offered better results when doing the phylogenetical study for *Z. mays* species, this because of its easy amplification and larger number of informative sites in comparison to the other evaluated markers. The phylogenetic tree was constructed to observe the consanguinity level. According to the dendogram, based on the Tamura 3-parameter, a clear pattern of separation at the racial level was observed, considering the three racial groups proposed by Roberts *et al.* (1957): 'Primitives', 'Probably Introduced' and 'Colombian Hybrids' (Figure 3); however, differences in the location of

Table 5. Model for nucleotide substitution and frequency in the region *AtpB-1-RbcL-1*.

Order	Model	BIC	Freq A	Freq T	Freq C	Freq G
1	T92+G+I	4338.9	0.3378	0.3378	0.1622	0.1622
2	HKY+G+I	4356.8	0.3472	0.3284	0.1586	0.1658
3	HKY+I	4362.5	0.3472	0.3284	0.1586	0.1658
4	TN93+G+I	4364.1	0.3472	0.3284	0.1586	0.1658
5	JC+G+I	4428	0.25	0.25	0.25	0.25
6	K2+G+I	4437.2	0.25	0.25	0.25	0.25
7	GTR+I	4447.4	0.3472	0.3284	0.1586	0.1658
8	T92+G	4507.2	0.3378	0.3378	0.1622	0.1622
9	HKY+G	4525.4	0.3472	0.3284	0.1586	0.1658
10	TN93+G	4531.6	0.3472	0.3284	0.1586	0.1658
11	GTR+G	4549.2	0.3472	0.3284	0.1586	0.1658
12	GTR+G+I	4559.1	0.3472	0.3284	0.1586	0.1658
13	JC+G	4603.5	0.25	0.25	0.25	0.25
14	K2+G	4613	0.25	0.25	0.25	0.25
15	TN93+I	4870.9	0.3472	0.3284	0.1586	0.1658
16	T92	4908.5	0.3378	0.3378	0.1622	0.1622
17	T92+I	4918.4	0.3378	0.3378	0.1622	0.1622
18	HKY	4927.2	0.3472	0.3284	0.1586	0.1658
19	TN93	4930.6	0.3472	0.3284	0.1586	0.1658
20	GTR	4950.9	0.3472	0.3284	0.1586	0.1658
21	JC+I	4971.6	0.25	0.25	0.25	0.25
22	K2+I	4978	0.25	0.25	0.25	0.25
23	JC	5008	0.25	0.25	0.25	0.25
24	K2	5017.4	0.25	0.25	0.25	0.25

BIC: Bayesian Information Criterion.
Freq: Frequency. A: Adenina. T: Timina. C: Citocina. G: Guanina.
Abbreviatures: GTR: General Time Reversible; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter; JC: Jukes-Cantor.

some maize introductions were found in comparison to the initial proposal of Roberts *et al.* (1957).

The first cluster identified the consanguinity level among Imbricado, Pollo y Pira, classifying them as primitive. A second cluster grouped Clavo, Güirúa, Cabuya, Yucatán, Costeño and Cariaco as probably introduced races. At last, there was a group composed by Harinoso Dentado, Puya Grande, Chococeño, Común, Sabanero, Montaña, Amagaceño, Pira Naranja, Negrito, Puya, Maíz Dulce, Capio and Cacao maize as Colombian hybrid race. The Bootstrap value for the phylogenetical tree conformation was 500 iterations (Figure 3).

Cardona (2010) using the Ward-MLM (Franco and Crossa, 2002) strategy, classified the racial groups according to quantitative statistical methods taking as reference the work by Roberts *et al.* (1957). This study compared the three classifications, were similarities and differences can be observed. Roberts *et al.* (1957) clasified the Pollo and Pira races as primitives, whereas Cardona (2010) expanded that group with three more races: Pira Naranja, Clavo and Imbricado; however, in this study Pollo, Pira and Imbricado were identified as primitive races (Table 5).

In the second racial group, according to Roberts *et al.* (1957), seven races are probably introduced: Güirúa, Andaquí, Pira Na

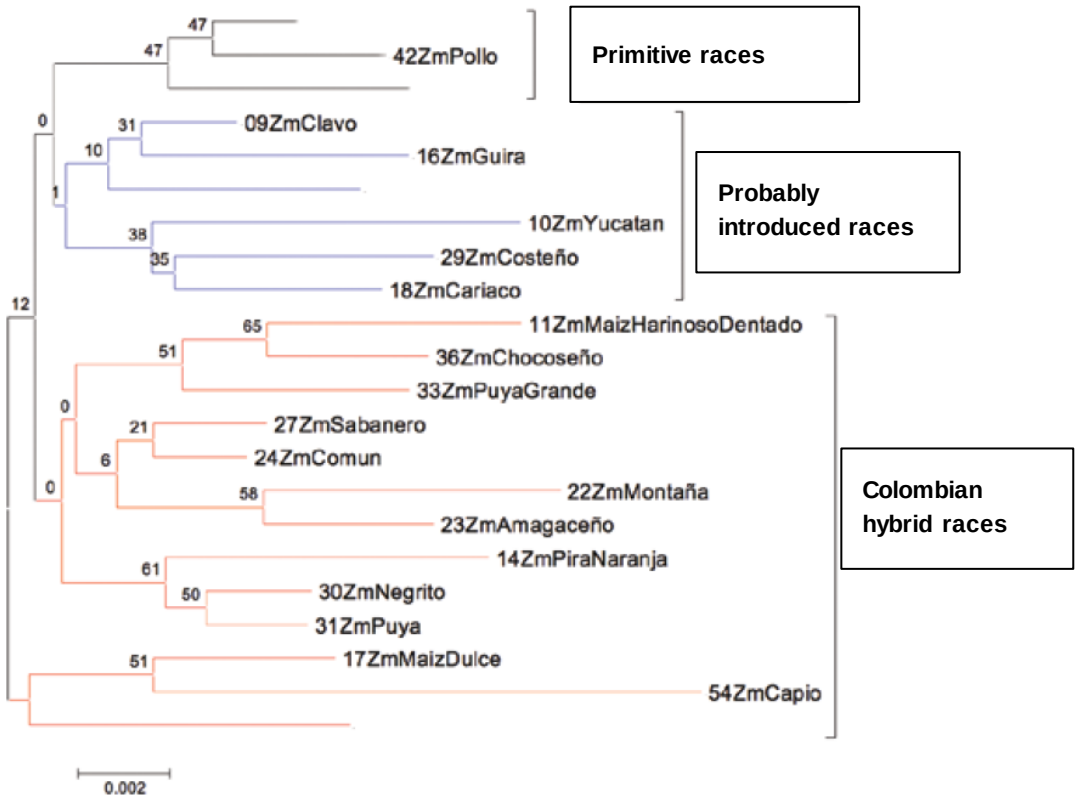

Figure 3. Genetic structure constructed by the method Tamura 3 parameter, by the chloroplast region *AtpB-1–RbcL-1*.

ranja, Cariaco, Clavo, Imbricado and Sabanero. Cardona (2010), on the other hand, composed this group with the same number of races (7) but, only two (Güirúa and Andaquí) are in the same group proposed by Roberts *et al.* (1957), the other five are Amagaceño, Común, Yucatán, Cacao and Puya Grande. In this research, compared with the one of Roberts *et al.* (1957), have in common the races Güirúa, Clavo and Cariaco. The group is completed with the Yucatán, Costeño and Cabuya races (Table 5).

The third and last racial group, according to Roberts *et al.* (1957) consists on fourteen races (Montaña, Cabuya, Capio, Costeño, Negrito, Puya, Chococeño, Amagaceño, Común, Yucatán, Puya grande, Cacao, Harinoso Dentado, Maíz Dulce) and according to this study there is similarity of 11 races with respect to the reference. At the same time, the study by Cardona (2010) only showed similarity with five races (Montaña, Capio, Negrito, Puya and Chococeño) (Table 6).

Conclusions

- The analysis among racial groups showed similarities and differences according to the studies made by Roberts *et al.* (1957) with the morphologic characterization and Cardona (2010) using the Ward–MLM strategy (Franco and Crossa 2002). However, the three initially described groups by Roberts *et al.* (1957) are preserved, although with variation in the races belonging to each group.
- According to the evaluation performed in this study at the marker level, *AtpB–1–RbcL–1* of chloroplast origin, showed efficiency in the identification of the racial groups levels, showing its potential as marker for future experiments of maize genetic diversity.

Table 6. Groups proposed by Roberts *et al.* (1957) and Cardona (2010), by the WardMLM strategy, compared to the ones of this study obtained with CpDNA.

Racial group	Roberts *et al.* (1957) (races)	Cardona (2010) (races)	cpDNA (races)
Primitive	Pollo	Pollo	Pollo
	Pira	Pira	Pira
	—	Imbricado	Imbricado
	—	Pira Naranja	—
	—	Clavo	—
Probably introduced	Clavo	—	Clavo
	Guirúa	Guirúa	Guirúa
	Andaquí	Andaquí	—
	Pira Naranja	—	—
	Cariaco	—	Cariaco
	Imbricado	—	—
	Sabanero	—	—
	—	Amagaceño	—
	—	Común	—
	—	Yucatán	Yucatán
	—	Cacao	—
	—	—	Costeño
	—	—	Cabuya
	—	Puya Grande	—
	—	—	—
Colombian hybrids	Montaña	Montaña	Montaña
	Cabuya	Cabuya	—
	Capio	Capio	Capio
	Costeño	Costeño	—
	Negrito	Negrito	Negrito
	Puya	Puya	Puya
	Chococeño	Chococeño	Chococeño
	Yucatán	—	—
	—	—	Pira Naranja
	—	Sabanero	Sabanero
	Amagaceño	—	Amagaceño
	Común	—	Común
	Puya Grande	—	Puya Grande
	Cacao	—	Cacao
	Harinoso Dentado	—	Harinoso Dentado
	Maíz Dulce		Maíz Dulce
	—	—	—
	—	—	—
		Cariaco	

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