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Microsatellite-dense genetic map: towards genome coverage in a tropical maize (*Zea mays* L.) population[1]

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ABSTRACT – (Microsatellite-dense genetic map: towards genome coverage in a tropical maize (*Zea mays* L.) population). Dense molecular genetic maps are used for an efficient quantitative trait loci (QTL) mapping and in the marker-assisted selection programs. A dense genetic map was generated with 139 microsatellite markers using 256 F_2 plants generated by the crossing of two tropical maize inbred lines (L-02-03D and L-20-01F). This map presented 1,858.61 cM in length, where 10 linkage groups were found spanned, with an average interval of 13.47 cM between adjacent markers. Seventy seven percent of the maize genetic mapping bins were covered, which means an increase of 14% coverage in relation to the previous tropical maize maps. The results provide a more detailed and informative genetic map in a tropical maize population representing the first step to make possible the studies of genetic architecture to identify and map QTL and estimate their effects on the variation of quantitative traits, thus allowing the manipulation and use in tropical maize breeding programs.

Key words - dense genetic map, genome coverage, microsatellites, tropical maize, *Zea mays* L.

RESUMO – (Mapa genético saturado de microssatélite: caminhando para uma cobertura genômica em uma população de milho tropical (*Zea mays* L.). Mapas genéticos saturados são utilizados para um eficiente mapeamento de caracteres de interesse agronômico (QTL) e nos programas de seleção assistida. Este trabalho gerou um mapa genético saturado utilizando 139 marcadores moleculares do tipo microssatélites em 256 plantas F_2 geradas pelo cruzamento de duas linhagens de milho tropical (L-02-03D e L-20-01F). O mapa obtido teve uma extensão total de 1.858,61 cM, ao longo de 10 grupos de ligação, com intervalo médio entre os marcadores de 13,47 cM. Setenta e nove percento dos "bins" do mapa genético de milho foram cobertos, com um acréscimo de 14% de cobertura em relação aos mapas de milho publicados. Os resultados mostram um mapa genético mais detalhado e informativo em uma população de milho tropical representando uma primeira etapa que possibilitará desenvolver estudos da arquitetura genética para a identificação e mapeamento de QTL e a estimativa de seus efeitos sobre a variação de um caráter quantitativo, permitindo assim a sua manipulação e utilização em programas de melhoramento do milho.

Palavras-chave - cobertura genômica, mapa genético saturado, microssatélites, milho tropical, *Zea mays* L.

Introduction

The use of a highly dense molecular genetic map using informative markers is very important for efficient QTL mapping for potential applications in marker-assisted selection and introgression programs capable of increasing breeding efficiency and to overcome some limitations of conventional breeding methods (Young 1999). Thus, the accuracy of QTL analysis is related to the occurrence of a linking between molecular marker genotypes and phenotypic values of individuals or lines, which allows the detection and location of the loci that affect quantitative traits (Dekkers & Hospital 2002). Simulations show that for accurate QTL location, a large population with linkage disequilibrium and a high number of informative markers, with a maximum distance of 5 cM between a marker and the QTL should be used (Moreau *et al.* 1998).

Several dense molecular genetic maps were developed in the last few years for economically important plant species (Falque *et al.* 2005, Sargent *et al.* 2006, Wang *et al.* 2006, Truco *et al.* 2007, Varshney *et al.* 2007). These dense maps facilitate the positional cloning of important genes, allow genetic dissection of quantitative trait loci, and provide an ordered scaffold on which complete physical maps can be assembled. The usefulness of genetic maps thus largely depends on their density (Meksem *et al.* 2001, Servin & Hospital 2002).

Many genetic maps have been reported for temperate maize (*Zea mays* L.) germplasm (Lee *et al.*

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2002, Sharopova *et al.* 2002, Wei *et al.* 2004, Falque *et al.* 2005). Most of these maps were largely developed to map QTL using F_2, immortalized F_2, BC_1, double haploid (DH) and recombinant line (RIL) populations (Edwards *et al.* 1992, Ajmone-Marsan *et al.* 1995, Austin & Lee 1996, Veldboom & Lee 1996, Davis *et al.* 1999). Few genetic maps are available involving different populations of tropical maize germplasm. Ribault *et al.* (1996) developed a RFLP genetic map for QTL mapping and used a crossing from two inbred lines derived from Mexican races of maize for the lowland tropics (Tuxpeño-based germplasm). Sibov *et al.* (2003) and Mangolin *et al.* (2004) constructed microsatellite-genetic maps in tropical maize populations derived from Brazilian and Mexican germplasm, however both of the genetic maps presented some genomic regions with large distances between markers that might be reducing the number of QTL mapped. Currently, no molecular dense genetic map has been published for tropical maize population.

Despite the proven efficiency of the use of RFLP marker in the development of genetic maps, this methodology very costly in time and money, also not providing a balanced coverage of the genome in some cases, which is an important requirement for efficient QTL mapping. The use of the microsatellites, or SSR (Simple Sequence Repeats), as molecular markers helps mapping studies due to the quick and easy data obtention, which allows the automation of the methodology in laboratory. The advantage of using these markers is that they are specific loci, multiallelic and highly polymorphic. Besides, microsatellites are codominant genetic markers with Mendelian inheritance, like the RFLP, and are distributed randomly in the genomes of many eukaryotes (Ellegren 2000, Schlötterer 2004).

Several authors developed microsatellites using a temperate maize germplasm, which is available in the Maize Genetics and Genomics Database (MaizeGDB – http://www.maizegdb.org/ssr.php). In this website all the information on these markers is available, such as the primer sequences, the locus designation, the description of microsatellite motifs, the size of the PCR product amplified in reference lines, and the chromosome location based on "bin boundaries" (Gardiner *et al.* 1993).

In this context, the objective of this study was to reach greater genome coverage to obtain a dense genetic map of an F_2 population of tropical maize from two contrasting inbred lines, to use molecular markers microsatellite developed from temperate maize populations, and also determine the efficiency and the level of polymorphism of the those markers. Only a high-resolution and marker-dense genetic map will provide a better understanding of genetic architecture for future QTL mapping studies that can be used in the tropical maize breeding programs.

Materials and methods

Genetic materials – The plant material used in this study has already been described in detail (Mangolin *et al.* 2004). Briefly, the parental inbred lines L-20-01F and L-02-03D were derived from the Brazilian early-flowering maize populations IG-1 (orange flint kernels) and IG-2 (yellow dent kernels), respectively. These populations and their respective inbred lines were in different heterotic groups and they were developed by the Maize Breeding Program of the Department of Genetics (ESALQ – Universidade de São Paulo). Both populations were derived only from tropical germplasm. The inbred lines were crossed deriving the F_1 plants which were self-pollinated to generate the 256 F_2 plants. Three F_1 plants were previously screened against the inbred parental lines with microsatellite markers to access their genetic identity, in order to discard F_1 plants produced with pollen from other genotype.

Microsatellite genotyping – Young leaves from F_1, F_2 and parental lines were collected, freeze-dried and ground. The material was stored at -20 °C in individually labeled vials. DNA extraction followed the procedure which uses CTAB described by Hoisington *et al.* (1994). The amplification conditions followed the process described by Sibov *et al.* (2003), who adopted two amplification programs: the Touchdown PCR (Don *et al.* 1991) and the one that uses specific annealing temperature (TA), calculated by the Marmur & Doty (1962) formula. All amplifications were carried out using a PTC-100 thermalcycler (MJ Research, Inc.). PCR products were separated in either 0.5·TBE buffer (Sambrook *et al.* 1989), high-resolution agarose gel (agarose/MetaPhor 1:1), 6% polyacrylamide gels, based on the genotyping difficulty of each microsatellite. Horizontal electrophoresis was conducted at 170 V for 1,5 hours in HORIZON 20:25 gel system (GIBCO BRL) and stained with ethidium bromide (0.5 μg mL^{-1}) for 0,33 hours. For the documentation and analyses of the gel, the equipment GeneGenius (Syngene Ltda.) was used. Vertical electrophoresis were conducted at 90 W for 2,0 h using a Model S2001 Sequencing Gel Electrophoresis Apparatus (Life Technologies – GIBCO BRL), and samples were detected by silver staining according to Creste *et al.* (2001).

Microsatellite primer selection – In order to fill the regions still not covered in the map developed by Mangolin *et al.* (2004) that used the same mapping population, 392 microsatellite primers were choosen and synthesized by Invitrogen™. From those, 72 microsatellites were polymorphic based on the existing allelic polymorphism between the parental and the segregating population and these were selected for the work. All information regarding primers used can be

found in MaizeGDB (http://www.maizegdb.org/ssr.php). From the 72 selected microsatellites 8 were later discarded due to problems that occurred during the genotyping of the population, resulting in a 64 microsatellites base which the main information are described in table 1.

Genetic map – Sixty-four microssatélites were grouped with the 75 microsatellites obtained from Mangolin *et al.* (2004), resulting in a 139 base, and used to develop the dense genetic map in tropical maize population. Each marker locus was checked for deviation from the expected Mendelian segregation ratio (1:2:1) by standard χ^2 analysis ($P < 0.05$ and $P < 0.01$). Because multiple tests were performed, appropriate type I error rates were determined by the sequentially rejective Bonferroni procedure (Rice 1989). The dense genetic map was constructed based on linkage analyses using the MAPMAKER/EXP version 3.0b program (Lincoln *et al.* 1993). Linkage was inferred using a LOD (log10 of the odds ratio) threshold of 3.0 and maximum distance between two loci of 50 cM for forming linkage groups. Two-point linkage analysis was conducted to estimate the maximum likelihood recombination frequency and linkage groups were established with the ''group'' command. The Kosambi (1944) mapping function was used to convert recombination frequencies into map distances (cM). Linkage analyses were then conducted to determine the most likely order of loci within groups using the "compare" command for all groups and the likelihood of all possible orders were compared. Once the most likely order of the subset is chosen, the remaining marks were inserted by means of a "try" command. The consistency of the orders was verified by means of a "ripple" command determined by multipoint analyses. The linkage groups with markers distances higher than 50 cM (r = 0.38) were grouped using the previous information of the microsatellite location (bin) on the maize chromosomes, which is available in the MaizeGDB. This procedure was also used by Sibov *et al.* (2003) and Mangolin *et al.* (2004), and it allowed a graphic representation of the map.

Table 1. Primers selected and used in the tropical maize map.

Nº	Bin[a]	Locus designation[b]	Microsatellite motifs[c]	Primers sequence: forward (left end) / reverse (right end)	Amplification conditions[d]
1	1.02	bnlg1007	(AG)15	GATGCAATAAAGGTTGCCGT / ATGTGCTGTGCCTGCCTC	TA (55 °C)
2	1.02	bnlg1178	(AG)16	ACTACAGTTGAACGCCCCTG / GCTCATGTGCAAATGCAAGT	TA (55 °C)
3	1.03	bnlg1203	(AG)17	GACCCGTCTCTCTTGAGTGC / GTCTGTCTGCACCCGTTTTT	TD
4	1.03	bnlg1953	(AG)17	CCTCGGAGCTCGATTTACAC / AACATTTAACCGCCGTCATC	TD
5	1.04	umc1558	(AG)7	GAGGTTGAGAGCAGCATATGAAAAA / AAGGTGGAGAACCAGAAGAGGAAG	TD
6	1.04	umc1917	(CTG)6	ACTTCCACTTCACCAGCCTTTTC / GGAAAGAAGAGCCGCTTGGT	TA (54 °C)
7	1.05	umc2025	(AGCT)4	CGCCGTAGTATTTGGTAGCAGAAG / TCTACCGCTCCTTCGTCCAGTA	TA (55 °C)
8	1.06	umc1919	(CT)8	TAAATCTGAGCCAGTCATAAGGGC / AGCAGAATAAAGTACGGTAGAGGTGG	TA (60 °C)
9	1.10	umc1737	(AGA)7	ATGCTTCTCTTCAGAAGCCATCC / TAGCTAGGTAGTGATGTGCGTGCT	TD
10	1.11	umc1630	(ATGGG)4	CAGACCTTCGAGGGCAAGAACT / AGTTTTGGCTTCTTCTCCCAAGTC	TA (60 °C)
11	1.12	umc1797	(AC)20	TCAAGTGAAATGCATAGCTTGCTC / ACTGTTGGTAAACCCTGCATGACT	TA (60 °C)
12	2.01	umc1227	(AGG)4	CAAGTTGGTGAGATGGATCTGTTG / GCTCCTGGGTCTTCCTCTCC	TD
13	2.02	bnlg1017	(AG)18	ATTGGAAGGATCTGCGTGAC / CAGCTGGTGGACTGCATCTA	TA (55 °C)
14	2.02	umc1542	(AG)10	TAAAGCTATGATGGCACTTGCAGA / CATATTTGCCTTTGCCCTTTTGTA	TD
15	2.03	mmc0111	(GA)34	TACTGGGGATTAGAGCAGAAG / AATCTATGTGTGAACAGCAGC	TD
16	2.07	bnlg2077	(AG)33	GACCAGAGGATGGGGAAATT / GTAGGCACATGCACATGAGG	TD
17	2.07	umc1042	(GA)17	AAGGCACTGCTACTCCTATGGCTA / CTGACCTTTGAATTCTGTGCTCCT	TD
18	2.07	umc1560	(GC)6	CGTTCGTCTCTGGGTAGCGTAG / TATAACAGCCTGCTGCTGCTTG	TA (54 °C)
19	2.07	umc1946	(GCTGCT)	GAAACGACCAGCACAGCACAT / GCACCACACCATCAGATCCAG	TA (55 °C)
20	2.08	bnlg1329	(AG)14	ATAGAATGGGATGTGGGCAA / TCCGATCATATCGGGAGATC	TA (52 °C)
21	2.08	bnlg1662	(AG)18	GCACCCACATGAAGTATCCC / TTGTTTTTGCAGTGCCTCAG	TA (52 °C)
22	2.08	bnlg1721	(AG)23	ACGACTTTCATGCCTCGTCT / ATTTCTTTTGCCACCTCAGC	TA (54 °C)
23	2.08	umc1516	(TA)12	CAATAACAGATCAGCATGGAGACG / TAGCGACCTGTTATCCGAAAGAAC	TD
24	3.04	bnlg1647	(AG)23	CGTCGTCTGTGGACGTACTG / AGAAGCTCACAAGCCTGCTC	TA (55 °C)

continue

continuation

Nº	Bin[a]	Locus designation[b]	Microsatellite motifs[c]	Primers sequence: forward (left end) / reverse (right end)	Amplification conditions[d]
25	3.04	bnlg1904	(AG)21	AGGAGCATGCACTTGGTTCT / ACTCAACTGATGGCCGATCT	TA (55 °C)
26	3.04	umc1025	(CT)11	GCTCCACTTCCACCCTGATATG / CGCTAATGTCCCCATTGATGAT	TD
27	3.07	umc1528	(TGCG)6	AGTTCAACTGCTTAAGATCCGGTG / GTCTGTCGTTGTGTGCCAGTG	TD
28	3.07	umc1659	(GA)6	CAAGCTTGCTACTGTGATTTCTCG / AACTTCTCGGTGATCTTGTCCATC	TA (60 °C)
29	3.09	bnlg1496	(AG)18	CTGGGCAGACAGCAACAGTA / AGCCAAAGACATGATGGTCC	TD
30	3.09	umc1052	(AAC)5	GTGTACAACACCAGCAACAGCTTC / GTAGCTCCCCATCTTGTCGAAC	TD
31	3.09	umc1639	(TGTCC)4	CTAGCCAGCCCCCATTCTTC / GCAAGGAGTAGGGAGGACGTG	TD
32	3.10	umc1641	(TCGCC)4	CTCCCTTCGTCTCCCGACTC/ CAGATCGGCTCAGCCACAAC	TA (55 °C)
33	4.07	bnlg1189	(AG)12	CGTTACCCATTCCTGCTACG / CTTGCTCGTTTCCATTCCAT	TA (55 °C)
34	4.08	umc1086	(CT)12	CATGAAAGTTTTCCTGTGCAGATT / GGGCAACTTTAGAGGTCGATTTATT	TD
35	4.09	umc1939	NA	CAAATACACCTCCAGCATCAGTTG / GATCCCATTTGTTGTTGCTGCT	TA (55 °C)
36	4.10	umc1532	(AAAT)4	ATCTAGAAAATTATAATGGCATGGATTC / CTCTCGGTTTTGGACTCTGCT	TD
37	4.10	umc1738	(CGCT)5	CCAGACATTCCCCAAACCCTA / CGTCGGTGTCGTACTGGTTG	TD
38	5.02	bnlg105	NA	GACCGCCCGGGACTGTAAGT / AGGAAAGAAGGTGACGCGCTTTTC	TD
39	5.03	bnlg1208	(AG)10	GCTGTGATGGTGAGACGAGA / GCAGGCACTACTAAAACCGC	TA (55 °C)
40	5.03	bnlg1660	(AG)19	ACCAAGGTTCTTGGAGGCT / ACCATTGTATTTTCCTAGAGAATCG	TA (55 °C)
41	5.03	bnlg1879	(AG)14	TGCTCTCACAAGATGGTGGA / CCACAGGATAAAATCGGCTG	TA (55 °C)
42	5.07	umc2013	NA	GGGACGAGAGTCTGTTGTTGTTG / GTTGATGCATGTGACTCTGGAAAC	TA (55 °C)
43	5.08	umc1792	(CGG)5	CATGGGACAGCAAGAGACACAG / ACCTTCATCACCTGCAACTACGAC	TD
44	6.00	bnlg1600	(AG)21	CGATCAGTGCGTGGAGAGTA / TAGGCATGCATTGTCCATTG	TD
45	6.04	mmc0523	(CT)24	CCCTCTCTTCACCCCTT / AGACCCTGCTGCTAGCTAG	TD
46	6.04	umc2006	NA	AGTCCATCACCATCCCTGGC / GCAGAACTATTGTCAGTTAACCTTGCAT	TD
47	6.06	umc1762	(TC)7	CTTACTCCAGGCACTCCATACCAT / ATCCAGGTGAATGGTGTTTACGAT	TD
48	6.07	umc1653	(GAAA)24	GAGACATGGCAGACTCACTGACA / GCCGCCCACGTACATCTATC	TA (60 °C)
49	7.01	umc1066	(GCCAGA)5	ATGGAGCACGTCATCTCAATGG / AGCAGCAGCAACGTCTATGACACT	TA (52 °C)
50	8.08	umc1069	(GGAGA)6	AGAGAATCCCCAAGCAAACAAAC / CTTCATCGGAGCCATGGTGT	TA (55 °C)
51	9.01	bnlg1583	(AG)14	ATCAAGCTTATCGAGAGAGAGAGAG / CGACGGTGGAAAGACTGC	TA (55 °C)
52	9.01	bnlg1810	(AG)14	ATGCTCCTCCTCTCCTCCAT / GCGATGATGAGCTGCAAGTA	TA (55 °C)
53	9.01	umc1040	(CT)11	CATTCACTCTCTTGCCAACTTGA / AGTAAGAGTGGGATATTCTGGGAGTT	TA (55 °C)
54	9.02	umc1037	(CT)16	GTGCGCGATTCCTTAGTTTGC / CTTCTTCGTAAAGGCATTTTGTGC	TD
55	9.02	bnlg1082	(AG)11	AAAGATCATGGGCGTACCAG / CAGGAACCTGATGACCACCT	TD
56	9.02	bnlg1401	(AG)22	CACTCGGTTTTTGCTTAGCC / GTGTCGTCGAGTGCATGC	TD
57	9.07	bnlg1525	(AG)25	AGGAATTGCGAGTCTTCCAA / CAACCCCCAAAATGAACAAA	TD
58	9.07	bnlg0279	NA	GCATGCGTACCTTCAAGCTA / TGTGTTCATCGGCAATTTTG	TD
59	10.05	umc1827	(GAC)6	GCAAGTCAGGGAGTCCAAGAGAG / CCACCTCACAGGTGTTCTACGAC	TA (55 °C)
60	10.05	umc2043	(TCC)4	GAGGCATACGGCATACCATACC / GTAGGAGAAACAGGTGCTGGTGTC	TA (55 °C)
61	10.06	umc1993	(GCCT)	CTTTTCTGCTACTCCTGCCTGC / CTAGCTGATGGAGGCTGTAGCG	TD
62	10.07	umc1038	(CT)15	CGTCACACTCCTCTGCCACTT / GAGGATTCAGAACTCGACTCGG	TA (55 °C)
63	10.07	umc2021	(TGG)4	AAACTCAAGCTCGGAATGTACTGC / CGATACTGATCTACTTCACGCTGG	TD
64	10.07	umc1569	(GCA)4	GCAGCTCCAAGTACAGAGGTGAG / CACTGCAGACACGTAAAATCCAAG	TA (53 °C)

[a] Microsatellite location of the maize chromosomes based on the concept of "bins" (Gardiner *et al.* 1993).
[b] Microsatellite primers developed in University of Missouri/EUA (umc) and Brookhaven National Laboratory/EUA (bnlg) and available in the MaizeGDB (http://www.maizegdb.org/).
[c] NA: microsatellite motifs not available in the MaizeGDB (http://www.maizegdb.org/).
[d] TD: Touchdown PCR amplification program (Don *et al.* 1991); TA: specific annealing temperature program (Marmur & Doty 1962).

Results

Polymorphism analyses – On what regards the allelic polymorphism from the 392 microsatellites tested, 72 (18%) were polymorphic, 255 (65%) were non polymorphic and the remaining 65 (17%) did not amplify, even with annealing temperature optimization (TA). All the polymorphic microsatellites were loci specific, producing just one set of segregating bands.

Forty-four (61%) polymorphic microsatellites evaluated presented dinucleotide sequence motifs, 12 (16%) showed trinucleotide motifs and six (8%) displayed tetranucleotide sequence repeats. The most abundant dinucleotide sequence motif found was (AG)n. The best resolution in terms of allele separation was found in the dinucleotide motif during the visualization of the alleles in high-resolution agarose gel. Null alleles, characterized by the non-observation of the allele of one parental inbred line in the F_1 generation were observed in 4% of the F_2 plants. These loci were not used in the genetic map.

Genetic map – With the objective of allowing integrated mapping, the maize genome was divided into 100 parts or bins (99 evenly spaced). Each bin was bounded by an RFLP "core marker", with approximately 20 cM each (Gardiner *et al.* 1993). Based on this concept of bins, Mangolin *et al.* (2004) map that used the same mapping population obtained a 65% coverage of the tropical maize genome. In this work a 79% coverage was obtained presenting a 14% increase.

According to the first law of Mendel, segregation occurs in a manner wherein a gamete has an equal probability to carry either of the segregating alleles. However, rare deviations from the expected segregation have been known for a long time. These so-called distorters can alter transmission ratio in specific cases (Lyttle 1991). Only one marker displayed segregation distortion in this work which occurred with the umc1516 (bin 2.08) marker, but once not rejected at the 1% significance level, it was used in the genetic map. The map presented a length of 1,858.61 cM with an average interval of 13.47 cM between markers (figure 1). Ten linkage groups were generated, which were equivalent to 10 maize chromosomes. The linkage group that presented the smallest length was chromosome 10 with 103.62 cM, and the greatest was chromosome one with 303.36 cM. The length between mapped markers ranged from 0.39 cM (chromosome one) to 103.78 cM (chromosome four). The number of markers in each linkage group ranged from 22 to seven in chromosomes one and seven, respectively. The average number of markers per chromosome was 13.90 with an average distance of 13.47 cM between markers (table 2). Three microsatellites did not link in its respective linkage groups (umc1177, bnlg1370 and phi116), and it is bins data were

Table 2. Results of 10 linkage groups from tropical maize genetic map of 256 F_2 plants and their distances in centimorgans (cM).

Linkage group	Microsatellite markers nº	Linkage group distance	Average between microsatellites			Microsatellites unlinked*
			Average distance	Maximum distance	Minimum distance	
1	22	303.36	14.45	54.08	0.59	umc1177 (1.01)
2	19	225.64	12.54	37.66	2.57	
3	18	245.33	14.43	40.88	0.59	
4	14	265.94	20.46	103.78	0.78	bnlg1370 (4.00)
5	13	163.43	13.62	28.46	3.38	
6	11	136.33	13.63	27.31	3.39	
7	7	167.03	27.84	66.33	2.37	phi116 (7.06)
8	10	119.02	13.22	22.52	2.38	
9	15	128.90	9.21	36.41	1.97	
10	10	103.62	11.51	25.83	1.18	
Total	139	1,858.61	13.47			

* Denomination of microsatellite loci with their location of based on the concept of "bins" between brackets.

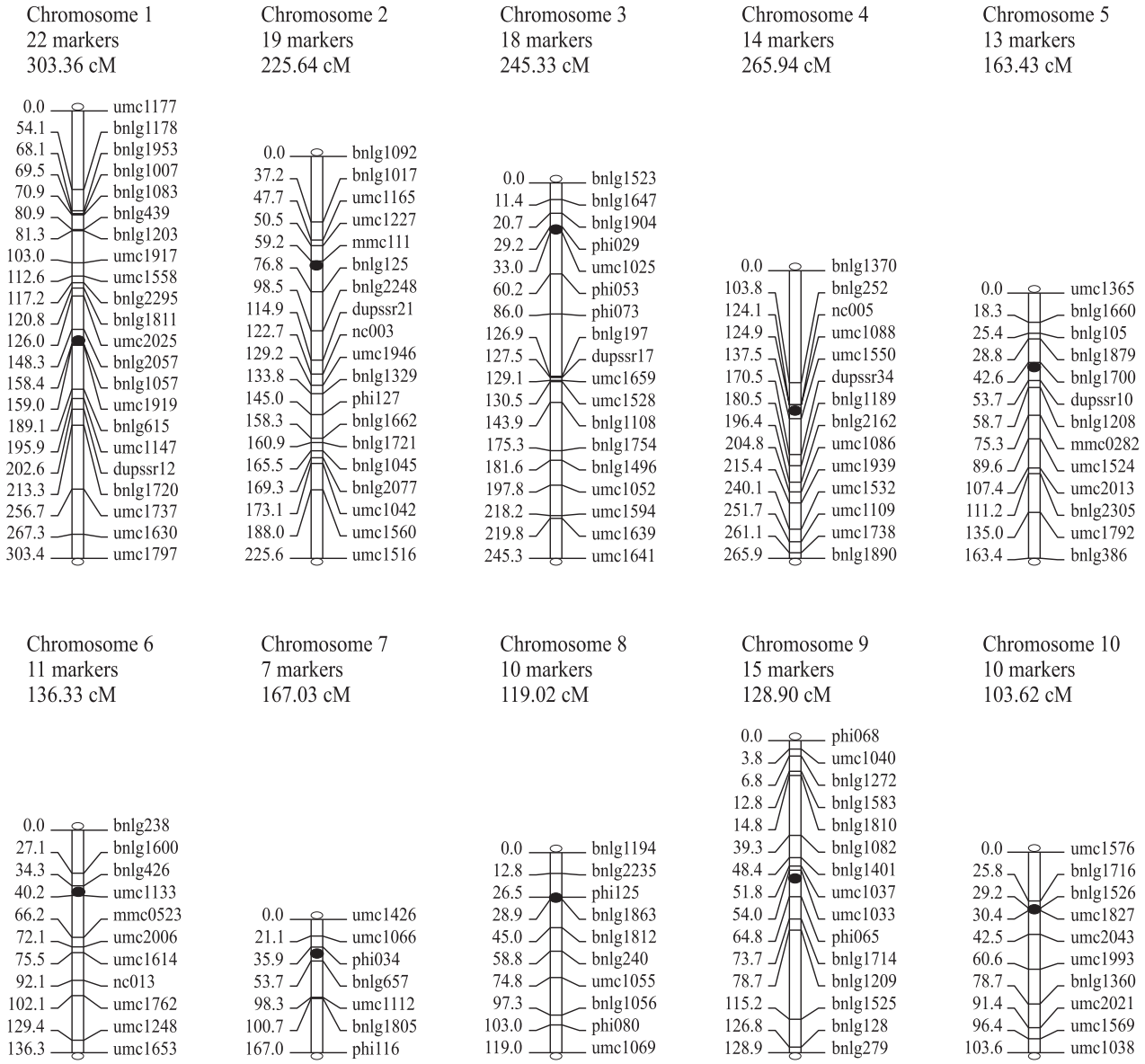

Figure 1. Dense genetic map of tropical maize, constructed with 139 microsatellite markers, based on 256 F_2 plants derived from crossing L-02-03D and L-20-01F parental lines. The name of each marker is identified on the right side of each chromosome; on the left are their lengths in centimorgans (cM). Dark circles indicate the estimated centromeric region positions, obtained by Gardiner *et al.* (1993). The map covered 1,858.61 cM with an average interval of 13.47 cM between markers.

replaced by previous developed information found in the MaizeGDB, procedure previously used by other authors (Sibov *et al.* 2003; Mangolin *et al.* 2004).

Discussion

The absence of amplification for several microsatellites suggests that there might have occurred an annealing failure with the sequence of the temperate maize germplasm. This annealing failure can be due to differences in base sequences of the loci evaluated, between the temperate maize germplasm, for which the primers were designed, and the tropical germplasm under study. Working with Expressed Sequence Tag (EST) library from tropical maize endosperm, and also with EST library from USA and Europe, Verza *et al.* (2005) used bioinformatic tools (CAP3 software) and observed that the software identified three consensus sequences for each gene. This showed the high polymorphism among tropical maize inbred lines.

The most frequent sequence motifs found were the dinucleotide (61%). These results are aligned with those obtained by Wang *et al.* (1994) in temperate maize and Sibov *et al.* (2003) in tropical maize. Chin *et al.* (1996) analyzing microsatellites available in MaizeGDB and EMBL databases derived from known genes, cDNAs and ESTs of temperate maize, showed that the trinucleotide is the most frequent (44.5%) motifs, followed by tetra (27%) and di (15.5%). The same authors suggested that when microsatellites obtained from non-genic regions are analyzed, the occurrence and characteristic of the motifs can differ.

Results presented by Grupta *et al.* (1996), which reported that the most abundant microsatellite motif in plants is the (GA)n disagree with those presented in this work, where (AG)n was found to be the most abundant. On the other hand, Levinson & Gutman (1987) commented that the differences between motifs are partially due to methylation patterns in different species.

Although the dinucleotide motif has shown the best resolution in terms of allele separation, Taramino & Tingey (1996) observed that in temperate maize, the best resolution and facility to separate using high-resolution agarose gel would be tri and tetranucleotide microsatellites motifs, even with a single repetition unit of difference between the alleles. The same authors commented that null alleles could be nucleotide sequence polymorphisms occurring at the primer sites.

Segregation distortion from expected Mendelian segregation ratios of molecular markers have been reported in maize (Bentolila *et al.* 1992, Gardiner *et al.* 1993, Murigneux *et al.* 1993, Pereira & Lee 1995), as well as in many other plant species. Using IBM and LHRF temperate maize populations of intermated recombinant inbred lines (IRILs), Falque *et al.* (2005) found high rates of segregation distortion. Sharopova *et al.* (2002) observed even higher rates (43%) with 983 loci (mainly SSRs) mapped on IBM, and Lee *et al.* (2002) and Lu *et al.* (2002) found also high proportions of distorted loci in nonfixed intermated recombinant inbred lines (RILs) maize populations from B73 X Mo17 and LH200 X LH216 crosses. Xu *et al.* (1997) found more segregation distortion in RILs than in doubled-haploid, backcross, or F_2 rice populations, and Lu *et al.* (2002) also stated that segregation distortion accumulates with additional generations of meiosis, which may be explained by the fact that more generations result in a stronger effect of selection. No segregation distortion was observed in any marker used in our work, which is an indication that the molecular marker system was correct, that the type and size of the population (F_2 population) was appropriate, and that no specific biologic event occurred, such as gametic variability differentiation during gametogenesis fertilization or germination. Coe *et al.* (1995) report that it is not unusual to find loci with a segregation distortion in populations where a high number of markers have been analyzed and the results are probably an artifact of sampling. Gardiner *et al.* (1993) showed that, in mapping studies with two populations derived from the same maize parental crossing, some regions presented segregation distortion in one population but not in the other.

The microsatellite map position was in accordance, but some alterations in marker positions were observed for chromosomes two, three, four, five and nine, when compared to their locations per bins with the Mangolin *et al.* (2004) map using the same tropical germplasm. In chromosome two, small modifications occurred: (i) the bnlg1017 marker (bin 2.02) positioned itself before the umc1165 and umc1227 markers (both in bin 2.01) and (ii) between the umc1946 and bnlg1045 markers (both in bin 2.07) there was the positioning of a block of markers from bin 2.08 (bnlg1329, phi127, bnlg1662 and bnlg1721). In chromosome three, there was an inversion between the bnlg1523 (bin 3.03) and bnlg1647 (bin 3.02) markers. In chromosome four, there was an inversion in the position of the bnlg252 (bin 4.06), nc005 (bin 4.05), umc1088 (bin 4.05) markers and the umc1550 (bin 4.03) marker. In chromosome five, there was an inversion between bnlg105 and bnlg1879 markers (both in bin 5.02) and the bnlg1660 (bin 5.03) marker. In chromosome nine, there was an inversion between the markers of bin 9.01 (phi068 and umc1040) with those from bin 9.00 (bnlg1272). All the inversions in the genetic map can be considered small and expected as they vary in relation to only one or two bins. Gardiner *et al.* (1993) commented on the common occurrence of small inversions when the markers are very close, separated by few centimorgans.

The map length presented in this study is the greatest when compared to the already published tropical maize maps. Analyzing each chromosome individually, the extensions were also greater. Besides that, the map showed the lower average interval between markers (13.47 cM), comparing with the 19.18 cM and 14.00 cM found for Mangolin *et al.* (2004) and Sibov *et al.* (2003), respectively. This lower average interval between markers will allow a more precise QTL mapping.

Some regions presented genetic distance values above 50 cM between the markers, which is the case of the telomeric regions of chromosomes one, four and

seven, which were not possible to saturate, although we managed to reach a satisfying resolution for telomeric region of chromosomes two and 10, reducing the distance between the markers. In the genetic map of Mangolin *et al.* (2004), these large distances were found in the telomeric regions of chromosomes two, four and 10. An explanation would be the increase in the recombination level observed in the telomeric regions, which generated great distances, despite the physical region being considered small (Davis *et al.* 1999).

Dense genetic maps are determinants in QTL mapping and marker-assisted selection programs, contributing towards physical mapping and, ultimately, gene cloning. This work generated a dense genetic map for a F_2 population for tropical maize, using microsatellite markers, providing more information about the genome of this species. A complete genetic map, with all the bins covered, still needs to be constructed. A strategy to reach this goal would be the development and use of specific microsatellite, cloned from tropical germplasm maize genome.

Despite the fact that the sequences currently available in MaizeGDB have been originally developed from the temperate maize germplasm, they were efficient in constructing a tropical maize genetic map. This generates an expectation that the complete map will be reached with the use of microsatellites available in the databases, with a more specific emphasis on the regions that are not yet covered. Apart from this, each year a large amount of new sequences are deposited. Together with this, different molecular markers can also be used in the high-density genetic maps.

The results obtained in this work establish the basis for new studies on genetic architecture of tropical maize towards the identification and mapping of QTL allowing its manipulation and use in maize breeding programs.

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