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MAPEAMENTO DE LOCOS QUANTITATIVOS (QTLs) ASSOCIADOS À PRODUÇÃO DE MILHO TROPICAL ATRAVÉS DE MARCADORES MOLECULARES

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas para a obtenção do título de Doutor em Genética. e Biologia Molecular – área de concentração: Genética Vegetal e Melhoramento.

Orientadora: Dr[a]. Anete Pereira de Souza

Co-orientador: Dr. Cláudio Lopes de Souza Jr.

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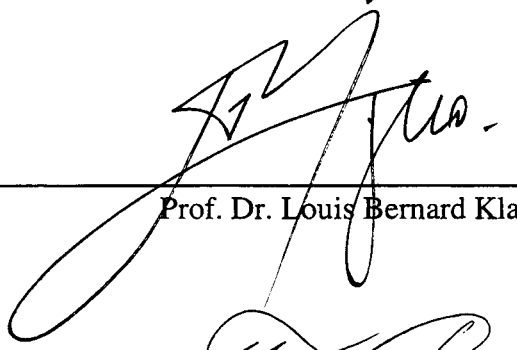

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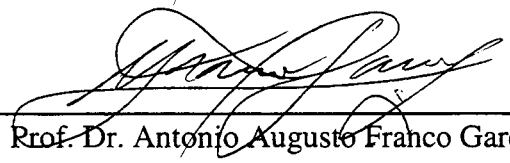

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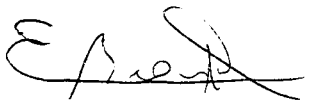

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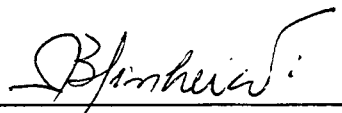

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ÍNDICE

RESUMO

A descoberta e a aplicação de polimorfismos baseados na variação de pequenas sequências repetitivas de DNA denominadas microssatelites, proporcionou grande avanço para a estudos envolvendo mapeamento molecular. Para o desenvolvimento de uma mapa genético em germoplasma de milho tropical, uma população de mapeamento foi formada por 400 plantas F_2 obtidas do cruzamento das linhagens L-08-05F (população IG-1) e L-14-4B (população BR-106). A seleção de microssatélites polimórficos e a genotipagem da população de mapeamento foi feita através de géis de agarose de alta resolução. O mapa genético obtido apresentou 117 locos, possuindo 1634 cM de extensão e 14 cM de intervalo médio entre marcas adjacentes. O Mapa foi utilizado para mapear locos de características quantitativas (QTLs) para produção de grãos (PG), altura da planta (AP), altura da espiga (AE) e teor de umidade (UM) em uma população de milho tropical. A partir das plantas F_2, 400 progênies $F_{2:3}$ foram derivadas e avaliadas em experimentos de campo com duas repetições em cinco ambientes diferentes da região de Piracicaba, SP. A localização e a magnitude dos QTLs foram determinados utilizando o método de mapeamento por intervalo composto (CIM). Foram detectados um total de 13 QTLs para os caracteres avaliados: quatro para PG, quatro para AP e cinco para AE, sendo que UM não apresentou nenhum QTL. Os QTLs foram distribuídos entre os cromossomos 1, 2, 7, 8 e 9. A porcentagem da variação fenotípica explicada pelos QTLs individuais variou de 3,09% até 11,18%. No total, os 13 QTLs detectados explicaram 32,73%, 24,76% e 20,91% da variação fenotípica, respectivamente, para PG, AP e AE. Dominância parcial e sobredominância foram os tipos predominantes de ação gênica. Uma grande proporção dos

alelos para aumento de produção de grãos, altura da planta e altura da espiga estão presentes predominantemente no genitor L-14-4B. QTLs para altura da planta e altura da espiga foram localizados nas proximidades de locos com efeitos quantitativos para produção de grãos, indicando possíveis efeitos pleiotrópicos ou de ligação entre esses locos.

SUMMARY

The discovery and application of a different polymorphism assay based on the variation of short tandemly repeated DNA sequences, termed microsatellites, revolutionized genome analysis. Microsatellites have become the most important class of markers for mapping procedures. To characterize the level of polymorphism of microsatellite loci and construct a genetic map in tropical maize, two elite inbred lines, L-08-05F and L-14-4B, were crossed to produce 400 F_2 individuals that were used as a mapping population. A survey of 859 primers pair sequences of microsatellites was used. The polymorphism screens of each microsatellite and genotype assignment were performed using high-resolution agarose gels. The final genetic map with 117 markers spanned 1634 cM in length with an average interval of 14 cM between adjacent markers. QTL for yield and yield components were characterized in a population of 400 $F_{2:3}$ lines, derived from selfing the F_2 plants, and were evaluated with two replications in five environments. QTL determinations were made in the mean of these five environments. Grain yield (GY), plant height (PH), ear height (EH) and grain moisture (GM) were assessed. Variance components for genotypes (G), environments (E) and G X E interaction were highly significant for all traits. Heritability was 0.69 for GY, 0.66 for PH, 0.67 for EH and 0.23 for GM. Using Composite Interval Mapping (CIM), we identified a total of 13 distinct QTLs: four for GY, four for PH and five for EH. No QTL was detected for GM. The QTL explained 32.73% of the phenotypic variance of GY, 24.76% of PH and 20.91% of EH. The 13 QTL displayed mostly partial dominant or overdominance gene action and mapped to chromosomes 1, 2, 7, 8 and 9. Most QTL alleles conferring high values for the traits were contributed from line

L-14-4B. Mapping analysis identified genomic regions associated with two or more traits in a manner consistent with correlation among traits, supporting either pleiotropy or tight linkage among QTL.

1. INTRODUÇÃO

Desde o início do século passado, já havia sido demonstrado que um caráter poderia ser controlado por vários genes, ao contrário da herança Mendeliana simples. A freqüência dos fenótipos obtidos a partir da segregação destes genes apresentaria uma distribuição contínua, que poderia ocorrer mesmo se poucos genes estivessem envolvidos, bastando que fatores ambientais influenciassem o caráter. Características quantitativas são influenciadas tanto por fatores genéticos quanto por fatores ambientais. Assim, contando somente com os dados fenotípicos, o mapeamento dos locos envolvidos com tais características, os denominados QTLs (Quantitative Trait Loci), torna-se praticamente impossível (Liu 1998).

Muitas características economicamente importantes em plantas, como produção de grãos, apresentam herança quantitativa. Mesmo sendo alvo principal de programas de melhoramento, quase nada se conhece a respeito de quantos locos estão envolvidos com estas características, onde estes locos estão localizados nos cromossomos, quais os seus efeitos e como eles interagem entre si (Lee 1995).

As análises de características quantitativas tiveram muitos avanços a partir do surgimento dos marcadores moleculares. A idéia básica é que a genotipagem de plantas por estes marcadores poderia ser utilizada para obter informações indiretas sobre os genótipos de determinado QTL. Portanto, esta metodologia só funciona bem se o QTL estiver localizado muito próximo do marcador. Assim, alelos do marcador, estando ligados aos alelos do QTL, podem co-segregar. O ideal seria a utilização de uma grande quantidade de marcadores para detectar a presença de um QTL em qualquer parte do genoma (Tanksley 1993).

Atualmente, há mapas genéticos altamente saturados com centenas de marcadores em milho e em outras culturas. Porém, em milho, a grande maioria dos trabalhos de mapeamento foi realizada utilizando linhagens de clima temperado (Hoisington e Coe 1990). Populações tropicais apresentam uma maior diversidade quando comparadas com germoplasma temperado e, até o momento, foram pouco estudadas (Lanza *et al.* 1997). Assim, é extremamente importante o desenvolvimento de um mapa genético para linhagens de milho tropical, tanto para aumentar o conhecimento básico sobre o material, quanto para permitir estudos sobre o mapeamento de genes ligados a características quantitativas ou qualitativas.

O trabalho aqui apresentado está inserido em uma linha de pesquisa ampla sobre estudos de QTLs em milho tropical. Estão em curso e em fase final de análise de dados, uma série de trabalhos envolvendo inúmeras medidas fenotípicas, diferentes tipos de populações (F_2, $F_{2:3}$, Retrocruzamentos) obtidas a partir de diferentes parentais. Ao final, com a grande quantidade de dados gerados, será possível obter-se um panorama sobre a localização e o comportamento dos locos de características quantitativas em linhagens tropicais de milho. Neste contexto, este trabalho teve por objetivo a identificação do número, localização cromossômica, magnitude e efeitos dos principais QTLs controlando produção de grãos, altura da planta, altura da espiga e teor de umidade.

2. REVISÃO BIBLIOGRÁFICA

2.1. Mapeamento de QTLs

Muitas características importantes no melhoramento de plantas exibem variação fenotípica e distribuição contínua. Esta distribuição resulta da ação de variáveis ambientais atuando sobre caracteres controlados por vários genes, localizados em locos conhecidos como "quantitative trait loci" (QTLs). Como os efeitos de cada um destes QTLs no fenótipo são relativamente pequenos, torna-se muito difícil o trabalho de identificá-los ou mapeá-los.

O primeiro trabalho de mapeamento de QTLs foi apresentado por Sax (1923), indicando que o tamanho das sementes (um caráter complexo) estava associado com a sua cor (um caráter monogênico). Este conceito foi aprimorado posteriormente por Thoday (1961), sugerindo que, se a segregação de um caráter monogênico simples poderia ser utilizada para detectar ligações com QTLs, então poderia eventualmente ser possível mapear e caracterizar todos os QTLs envolvidos com caracteres complexos.

Atualmente, o mapeamento de QTLs é essencialmente uma extensão deste conceito, com uma inovação importante definida por seqüências de DNA agindo como marcadores moleculares. Os recentes progressos na obtenção destes marcadores e seus mapas de ligação têm proporcionado uma excelente ferramenta para o mapeamento de locos individuais, não apenas para características monogênicas mas também para características quantitativas (Tanksley 1993).

Usualmente, a variação contínua é estudada por análises estatísticas cuja premissa é de que a variabilidade na expressão de um caráter quantitativo resulta de vários locos

segregantes que interagem com o ambiente (Fisher 1918). Usando cruzamentos controlados entre pais com fenótipos contrastantes, os melhoristas estimam a herdabilidade, o número de locos relevantes, o grau de dominância, aditividade e heterose e a magnitude das interações gene-gene e gene-ambiente (Tanksley 1993). Estes estudos focam as distribuições fenotípicas dentro das populações e suas correlações entre os fenótipos dos indivíduos relacionados. Através da análise conjunta dos resultados experimentais com modelos genéticos baseados em médias populacionais, variâncias e covariâncias, é possível estimar o número de genes, a ação gênica e a interação entre os parâmetros estudados. Os resultados de tais estudos fornecem grande quantidade de importantes informações a respeito da base genética dos caracteres quantitativos e do progresso de seleção em programas de melhoramento (Lee 1995).

Porém, estes estudos não possibilitam a análise do comportamento de QTLs individualmente. Detalhes sobre qualquer loco envolvido na expressão do caráter poligênico é muito difícil de discernir. Este problema começou a ser solucionado com o desenvolvimento de mapas cada vez mais saturados com marcadores moleculares (Phillips e Vasil 1994). A disponibilidade destes mapas para a maioria das espécies cultivadas tornou possível analisar a variação de um caráter quantitativo em fatores individuais, isto é, assinalando os locos envolvidos na variação do caráter em determinadas regiões cromossômicas e estimar seus efeitos.

Um tipo de análise de mapeamento de QTLs, denominado análise de marcas simples, envolve o teste de marcadores de DNA por todo o genoma na busca de uma provável ligação com um QTL (Liu 1998). Plantas de um população adequada (F_2, retrocruzamentos, linhagens recombinantes, etc.) são analisadas em temos de seu genótipo

para o marcador de DNA e pelas medidas fenotípicas do caráter de interesse. Para cada marcador, as plantas são agrupadas em classes de acordo com sua genotipagem pelos marcadores. Os parâmetros de médias e variâncias são calculados e comparados entre as classes. Uma diferença significativa entre as classes sugere uma provável ligação entre o marcador de DNA e o caráter de interesse, ou seja, o marcador pode estar ligado ao QTL. A disponibilidade destes mapas teve o efeito de redirecionar os estudos com caracteres poligênicos para questões a respeito da localização cromossômica, ação gênica e o papel biológico de locos específicos envolvidos em caracteres complexos (Tanksley 1995).

Enquanto o conceito de mapeamento de QTLs parece ser claro e simples, há ainda muitas limitações práticas. Muitos mapas ainda não estão suficientemente saturados com marcadores para serem usados para o mapeamento de QTLs, uma vez que mapas com poucos marcadores dispersos no genoma são um limite importante para a detecção de QTLs (Lander e Botstein 1989). Mesmo sob ótimas condições experimentais, QTLs múltiplos em um mesmo grupo de ligação são difíceis de se detectar individualmente. As populações de mapeamento precisam ser relativamente grandes para cobrir locos de pequeno efeito e o limite de significância estatística, estabelecido durante o cálculos dos parâmetros fenotípicos, precisa ser escolhido com cuidado, pois a importância biológica de determinado loco está diretamente ligada a ele (Lander e Botstein 1989; Tanksley 1993).

Desde que os caracteres de interesse são, por natureza, geneticamente complexos, fatores ambientais e a constituição genética possuem um grande potencial de impacto nos resultados obtidos. Finalmente, embora a análise das interações gene-gene e gene-ambiente seja uma das mais poderosas aplicações do mapeamento de QTLs, a metodologia consome tempo e recursos consideráveis na elaboração, condução e análise de experimentos muito

grandes. Porém, acompanhando o surgimento de novos sistemas de marcadores moleculares, novos modelos biométricos para a detecção e localização de QTLs foram se aprimorando cada vez mais. O método de mapeamento por intervalo simples (SIM - Lander e Botstein 1989) detectando um QTL dentro de uma região limitada por dois marcadores ligados, serviu de base para modelos subsequentes. Um refinamento deste método, o mapeamento por intervalo composto (CIM - Zeng 1994, Jansen e Stam 1994) condiciona o teste para um QTL em determinado intervalo a outros marcadores selecionados, para minimizar efeitos de outros QTLs no genoma. Porém, tanto SIM como o CIM detectam efeitos de QTLs em diferentes regiões genômicas separadamente. Jiang e Zeng (1995) estenderam o conceito do CIM para múltiplos QTLs e múltiplos ambientes para o estudo de pleiotropia e interações genótipo ambiente. Finalmente, efeitos epistáticos entraram no modelo através do mapeamento por intervalo múltiplo (MIM - Kao e Zeng 1997, Kao *et al.* 1999).

2.2. Mapeamento de QTLs associados à produção de grãos

Um dos primeiros trabalhos com mapeamento de QTLs utilizando marcadores moleculares envolveu o estudo do tamanho do fruto, pH e sólidos solúveis em tomate (Paterson *et al.* 1988). Este trabalho pioneiro ilustra as possibilidades do mapeamento de QTLs, demostrando que caracteres exibindo variação contínua podem ser resolvidos em fatores mendelianos discretos, utilizando um mapa de ligação elaborado com marcadores RFLP. Caracteres relacionados com frutos em tomate (Paterson *et al.* 1988) e a morfologia e caracteres agronômicos em milho (Stuber *et al.* 1992) são dois sistemas que foram bem

caracterizados através do mapeamento de QTLs. Nestes estudos, temas importantes em genética de plantas como número de genes, ação gênica, heterose, sobredominância e interações gene-ambiente foram descritos em detalhes.

Em milho, a análise de QTLs com marcadores moleculares baseados em mapas de ligação bem saturados, facilitaram a identificação de regiões cromossômicas relacionadas com uma série de caracteres morfológicos e agronômicos: resistência a fungos (Pè *et al.* 1993) e insetos (Bohn *et al.* 1997; Groh *et al.* 1998), tolerância a seca (Ribaut *et al.* 1997; Frova *et al.* 1999), altura da planta e tempo de florescimento (Khairallah *et al.* 1998), heterose, interações entre genótipo e ambiente (Stuber *et al.* 1992) e as bases genéticas da evolução do milho a partir do teosinte (Doebley e Stec 1991) são apenas alguns exemplos.

A identificação de QTLs envolvidos com a expressão de vários componentes de produção em milho já foi objeto de estudo de vários trabalhos (Stuber *et al.* 1987, 1992; Zehr *et al.* 1992; Beavis *et al.* 1994; Schön *et al.* 1994; Stromberg *et al.* 1994; Veldboom e Lee 1994, Veldboom *et al.* 1994; Ajmone-Marsan *et al.* 1995, 1996; Berke e Rocheford 1995; Ragot *et al.* 1995; Graham *et al.* 1997; Austin e Lee 1998; Melchinger *et al.* 1998; Sanguineti *et al.* 1999; Frova *et al.* 1999). Um resumo dos resultados destes trabalhos mostra que o número de plantas utilizadas para avaliar a produção de grãos está entre 100 a 300 e os tipos de população incluem plantas F_2, retrocruzamentos ou linhagens puras recombinantes. O número estimado de QTLs identificados está entre três a oito e a magnitude esperada para o efeito genético para qualquer QTL variou de 5 a 35% da variabilidade fenotípica. A distribuição para os efeitos genéticos estimados de QTLs de produção em todos os estudos é caracterizada por um loco ou poucos locos com grande efeito e vários outros QTLs adicionais que explicam relativamente pequenas porções na

variabilidade fenotípica.

Outra característica importante destes trabalhos envolvendo o mapeamento de QTLs é que a maioria dos artigos explora linhagens de germoplasma de clima temperado, principalmente do cinturão de milho norte-americano. Estas linhagens temperadas são oriundas de grupos heteróticos bem definidos e de estreita base genética. Em contraste, grande parte das populações de milho tropical utilizadas em programas de melhoramento são derivadas de diferentes populações e compostos, podendo serem consideradas populações de ampla base genética e com maior variabilidade do que o material de clima temperado (Lanza *et al.* 1997, Laborda *et al.* 2002).

Embora os experimentos tenham sido conduzidos em ambientes diferentes, os autores encontraram poucas indicações de interação entre genótipo e ambiente para os caracteres analisados. Porém, estas conclusões estão restritas a experimentos com material de clima temperado cultivadas em ambientes diversos, mas em condições normais de cultivo. QTLs para produção detectados por Ribaut *et al.* (1997) com material tropical em ambientes simulando três condições diferentes de estresse hídrico não foram estáveis, em termos de sua localização genômica e percentual da variância fenotípica explicada. Este resultados indicam que diferentes conjuntos gênicos podem estar relacionados com o controle da produção de grãos em diferentes ambientes.

Certa inconsistência também ocorre quando QTLs de grande efeito, responsáveis por uma alta porcentagem da variação fenotípica, são mapeados em diferentes posições genômicas: 27% da variação fenotípica explicada por QTL localizado na região 3L (Ragot *et al.* 1995), 35% em 5C (Stuber *et al.* 1992), 24% em 6C (Ajmone-Marsan *et al.* 1995), 35% em 6L (Veldboom e Lee 1994) e 23% em 9C (Beavis *et al.* 1994).

Vários fatores poderiam estar confundindo a comparação dos resultados de mapeamento de QTLs, causando esta inconsistência. Trabalhos recentes tentam identificá-los, com o objetivo de melhorar a precisão na detecção e localização de QTLs, bem como a estimação de seus efeitos. Basicamente estes trabalhos tentam aprimorar os quatro itens propostos por Tanksley (1993) que influenciariam a detecção de determinado QTL, através de marcadores moleculares: i) a taxa de recombinação entre os marcadores e o QTL; ii) a herdabilidade do caráter; iii) o delineamento experimental utilizado; iv) o tamanho da população segregante. Estes itens estão detalhados a seguir.

i) a taxa de recombinação. A taxa de recombinação entre marcadores e QTLs depende, em grande parte, do número de marcadores mapeados. Em milho o nível de saturação deste mapas já é alto (Hoisington e Coe 1990). Além dos marcadores RFLP utilizados inicialmente, surgiram os marcadores baseados em PCR, como os RAPDs, AFLPs e microssatélites, que são mais baratos, seguros e proporcionam maior número de marcas por unidade de DNA (Beckman e Soller 1990). O aumento contínuo destes marcadores polimórficos, dispersos por todo o genoma, irá aumentar proporcionalmente a consistência e a precisão de QTLs mapeados (Lee 1995).

O tipo de progênie utilizada também é importante no mapeamento de QTLs. O uso de linhagens recombinantes auxiliam no mapeamento de QTLs múltiplos, mapeados em uma mesma região cromossômica. Austin e Lee (1996) compararam os resultados do mapeamento de QTLs ligados à produção e a componentes de produção em linhagens recombinantes $F_{2:3}$ e $F_{6:7}$, originadas de uma mesma população. Uma grande região cobrindo parte de 1L estava significativamente associada com altura da planta em progênies $F_{2:3}$, sendo atribuída a um único QTL. A mesma região foi dividida em 3 QTLs em progênies

$F_{6:7}$. Estas observações sugerem que quanto maiores forem as possibilidades de recombinação entre as linhagens recombinantes utilizadas, maior será a percepção e a caracterização de QTLs.

ii) a herdabilidade do caráter. A herdabilidade do caráter pode ser melhor trabalhada através de um eficiente delineamento experimental, assim como uma medição fenotípica acurada. Não só o tamanho mas também o tipo de progênie avaliada são importantes. Medidas individuais, feitas em cada planta F_2, como as usadas por Edwards *et al.* (1987), Stuber *et al.* (1987), Doebley *et al.* (1990) e Edwards *et al.* (1992), estão sujeitas a erros de precisão. Para diminuir este tipo de erro, medidas efetuadas em linhas com plantas $F_{2:3}$, em experimentos com repetições, melhoram a precisão dos dados, pois trabalha-se com médias, reduzindo-se o erro-padrão e proporcionando uma estimativa mais acurada para o valor de cada genótipo em determinando ambiente (Cowen 1988).

iii) o delineamento experimental. Vários delineamentos experimentais têm sido propostos para gerar o desequilíbrio de ligação ente os marcadores moleculares e QTLs possibilitando assim o mapeamento (Weller e Wyler 1992). Entre os delineamentos mais utilizados estão o uso de plantas F_2, retrocruzamentos, linhagens puras recombinantes e linhagens duplo-haplóides (Tanksley 1993, Lee 1995). Como a genotipagem com marcadores moleculares é um processo relativamente mais caro do que a obtenção dos dados quantitativos, vários métodos foram propostos para aumentar o poder estatístico na detecção de um QTL segregante por indivíduo genotipado. Dentre estes métodos destaca-se a estratégia onde efetua-se a análise da segregação dos alelos utilizando-se dois conjuntos de plantas contrastantes para determinado caráter quantitativo, ao invés de toda a população segregante. Em cada conjunto ou "bulk" as plantas são selecionadas, de forma que todas

apresentem uma dada característica em comum. Isto significa que pelo menos um loco será idêntico em todos as plantas de um "bulk". No entanto, todos os outros locos segregarão aleatoriamente, uma vez que não sofreram seleção. A característica selecionada será aquela que se quer mapear. Esta estratégia desenvolvida por Milchelmore *et al.* (1991) foi denominada de BSA (Bulked Segregant Analysis).

Ainda em relação a metodologias específicas relacionadas a detecção de QTLs, outras metodologias tentam encontrar um método para detectar se mais de um QTL está presente em um mesmo cromossomo. Estimativas de localização de QTLs e de seus efeitos através do método de "mapeamento por intervalo" baseiam-se nas estimativas de máxima verossimilhança (Lander e Botstein 1989) ou máxima verossimilhança linearizada (Knapp *et al.* 1990; Haley e Knott 1992). Porém, este método consegue apenas indicar que "um ou mais que um QTL" podem estar presentes no cromossomo (Goffinet e Mangin 1998). Diferentes autores propuseram uma variedade de métodos que poderiam aumentar a resolução do mapeamento (Jansen 1993, 1994; Zeng 1993, 1994; Whittaker *et al.* 1996; Goffinet e Mangin 1998).

iv) o tamanho da população segregante. Estudos teóricos iniciais sugeriam que uma população muito grande (acima de 1000 plantas) seria necessária para identificar associações entre marcadores e caracteres quantitativos (Soller e Brody, 1976). Entretanto, resultados de experimentos iniciais mostraram que a detecção da segregação de QTLs em populações poderia ser obtida com populações com um menor número de plantas (Edwards *et al.* 1987; Stuber *et al.*, 1987; Weller *et al.* 1988). A partir destas pesquisas iniciais e, principalmente, devido à grande quantidade de trabalho e ao elevado custo para fenotipar e genotipar grandes populações, na maioria dos experimentos envolvendo mapeamento de

QTLs, o tamanho das populações varia entre 100 a 300 plantas.

2.3. Seleção Assistida por Marcadores e a criação do Maize Data Bank

Em última análise, do ponto de vista prático do melhorista, o principal objetivo dos trabalhos envolvendo o mapeamento de QTLs é a aplicação da seleção assistida por marcadores, ou "marker assisted selection" (MAS) em programas de melhoramento. MAS seria especialmente útil na seleção de caracteres que são difíceis ou muito caros para serem mensurados (Lande e Thompson 1990). Além disso, se uma grande proporção da variância genética aditiva em um caráter puder ser explicada usando marcadores moleculares, MAS poderia produzir aumentos substanciais na resposta à seleção (Hoisington *et al.* 1996).

Porém, o uso somente de QTLs relacionados à produção em um esquema de MAS seria questionável devido principalmente à sua inconsistência. Em geral, melhoristas incluem caracteres secundários de interesse para construir um índice de seleção com o objetivo de ajudar na melhoria da eficiência da seleção (Banziger e Lafitte 1997). No desenho da melhor estratégia para a utilização de MAS, caracteres e critérios adicionais têm que ser considerados. Para cada caráter de interesse, alguns critérios seriam o número de QTLs detectados, a percentagem da variação fenotípica explicada, a porcentagem total do genoma que eles representam e sua estabilidade em diferentes ambientes (Ribaut *et al.* 1997).

Na tentativa de padronizar as informações e facilitar o intercâmbio do conhecimento gerado sobre o mapeamento de QTLs para vários caracteres agronômicos em milho, foi criado um banco de dados com descrições sistemáticas sobre estudos envolvendo QTLs,

não só em milho, mas também em outras culturas (http://www.agron.missouri.edu - Byrne *et al.* 1995). Mais ainda, trabalhos começam a ser publicados propondo formulações estatísticas para otimizar a consulta neste grande volume de informação (Goffinet e Gerber, 2000).

Com um banco de dados assim estruturado e com novas ferramentas estatísticas para explorá-lo, surge uma nova abordagem para uma série de questões que, agora, podem ser melhor formuladas. Como exemplo, comparações entre resultados de diferentes trabalhos poderiam indicar se QTLs identificados para um dado caráter em uma população correspondem àqueles detectados em outras populações. Ou ainda, se QTLs identificados em uma espécie correspondem àqueles detectados em regiões correspondentes em outras espécies.

Revisões feitas nesse banco de dados sugeriram que associações entre QTLs coincidiam com agrupamentos de genes relacionados com o desenvolvimento (Khavkin e Coe 1997, 1998). Análises comparativas entre espécies revelaram a existência de QTLs homólogos para a altura da planta e o tempo de maturação dentro da família Poaceae (sorgo, milho, arroz, trigo e cevada; Lin *et al.* 1995). Observações similares para caracteres envolvidos na domesticação das culturas sugerem que poucos genes com grande efeito podem estar envolvidos com os caracteres em estudo (Paterson *et al.* 1995).

Conforme o número de trabalhos sobre a localização de QTLs aumenta, maiores são as chances para a identificação mais acurada de determinado QTL, ou seja, a identificação de determinados genes com função biológica conhecida, envolvidos no desenvolvimento ou na fisiologia do caráter. Um exemplo deste tipo de estudo tornou-se um dos artigos mais interessantes publicados em 2000. Frary *et al.* (2000) publicaram a clonagem de um QTL, o

fw2.2, envolvido com as diferenças quantitativas em relação ao peso do fruto em tomate. Clonado a partir da espécie selvagem de frutos pequenos *Lycopersicon pimpinellifolium*, o *fw2.2* foi utilizado para a transformação de uma espécie cultivada *L. esculentum* cv, melhorada para produzir grandes frutos. A novidade deste trabalho foi o uso da transformação para confirmar a clonagem pois, uma vez transformadas, as plantas da espécie cultivada apresentaram um decréscimo no peso do fruto. Notavelmente, a magnitude deste decréscimo ajustou-se exatamente com o previsto, demostrando que não havia nenhum outro QTL de efeito aditivo nas proximidades do cromossomo.

A história do clone *fw2.2* começa com um dos primeiros trabalhos sobre a localização de QTLs por marcadores moleculares (Paterson *et al.* 1988), realizados pelo grupo de Steven Tanksley da Universidade Cornell. O passo seguinte foi identificar 11 QTLs afetando o peso do fruto em tomate (Paterson *et al.* 1991), sendo o QTL *fw2.2* o de maior efeito (Alpert *et al.* 1995). Através de técnicas de mapeamento mais refinadas (Alpert e Tanksley 1996), conseguiram isolar o *fw2.2* em um YAC, possibilitando assim a clonagem.

Em milho, técnicas de mapeamento de QTLs mais refinadas foram utilizadas na região do braço curto do cromossomo 5 que possui efeitos significativos na produção de grãos em várias populações de milho (Stuber 1992, Stuber *et al.* 1992). Graham *et al.* (1997) identificaram dois QTLs de efeito maior nesta região. Outra região interessante como alvo de um mapeamento mais fino seria a região contendo o gene *Tb1*, que controla o caráter de perfilhamento em milho. Doebley e Stec (1993) mapearam QTLs que afetam a arquitetura da planta nesta mesma região do cromossomo 1, utilizando cruzamentos entre milho e teosinte. Outros trabalhos genéticos (Doebley *et al.* 1995) e moleculares (Doebley

et al. 1997) confirmaram a estreita ligação entre o gene *Tb1* e um dos QTLs relacionados com a domesticação do milho.

Todos esses trabalhos indicam que o uso da tecnologia de marcadores moleculares caminha não só para a identificação e localização de genes (ou segmentos cromossômicos) úteis, mas também, para a possibilidade de transferência destes genes de efeito quantitativo para cultivares elite. Com a automação crescente das análises em laboratório, a conseqüente redução dos custos, além do surgimento de novas perspectivas no mapeamento, através do seqüenciamento de genomas completos, as técnicas moleculares para o melhoramento de características quantitativas terão uma grande expansão no futuro (Stuber *et al.* 1999).

03. 1° ARTIGO: Molecular mapping and polymorphism analysis of tropical maize (*Zea mays* L.) using microsatellite markers

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Summary

The discovery and application of a different polymorphism assay based on the variation of short tandemly repeated DNA sequences, termed microsatellites, revolutionized genome analysis. Microsatellites have become the most important class of markers for mapping procedures. Primarily based on restriction fragment length polymorphism (RFLP) markers, several molecular genetic maps of maize (*Zea mays* L.) have been developed, mainly using temperate inbred maize lines. To characterize the level of polymorphism of microsatellite loci and construct a genetic map in tropical maize, two elite inbred lines, L-08-05F and L-14-4B, were crossed to produce 400 F_2 individuals that were used as a mapping population. A survey of 859 primers pair sequences of microsatellites was used. The polymorphism screens of each microsatellite and genotype assignment were performed using high-resolution agarose gels. The resolution of amplification products on agarose gels and the distribution of polymorphism detected by microsatellites vary between different sizes of their repeat unit. About 54% of the primer sets gave clearly scorable amplification products, 13% did not amplify and 33% could not be scored on agarose gels. A total of 213 polymorphic markers were identified and used to genotype the mapping population. Among the polymorphic markers, 40 showed loci deviating from expected Mendelian ratios; clusters of deviating markers where located in three chromosome regions. Non-Mendelian scoring was present in 19 markers. The final genetic map with 117 markers spanned 1634 cM in length with an average interval of 14 cM between adjacent markers.

Key words: tropical maize, microsatellites, molecular mapping, polymorphism.

Introduction

Extensive genome mapping, based on restriction fragment length polymorphism (RFLP) markers, has been developed in maize (Helentjaris *et al.* 1986, 1988, Burr *et al.* 1988, Weber and Helentjaris 1989, Gardiner *et al.* 1993, Coe *et al.* 1995). These maps have been used for a number of applications in genetic research and breeding, including mapping several genes contributing to agronomically relevant traits, marker-assisted selection (MAS), quantitative trait loci (QTL) detection and positional cloning (Tanksley 1993, Lee 1995, Khavkin and Coe 1997). However, there are several disadvantages regarding RFLP marker systems that make the analysis of large populations an expensive and time-consuming process. The development of the polymerase chain reaction (PCR - Mullis and Fallona 1987) and the advent of PCR-based markers, mainly microsatellites, provided new marker systems where the analyses are more efficient and inexpensive.

Microsatellites, also termed as simple sequence repeats (SSRs) (Litt and Lutt 1989) or short tandem repeats (STRs) (Edwards *et al.* 1991), are genomic regions which are comprised of highly variable tandem repeats of two to six DNA nucleotides. Microsatellite markers are highly polymorphic, co-dominants, widely disperse in diverse genomes, and mapped to a single locus. Moreover, they are easy to be assayed by polymerase chain reaction methodology and their dissemination is straightforward among laboratories, making them very valuable genetic markers for mapping analysis (Powel *et al.* 1996). Similar to human and other mammalian genomes (Jacob *et al.* 1995, Dib *et al.* 1996, Dietrich *et al.* 1996), microsatellite markers have been developed and integrated into several existing plant linkage maps, such as rice (Chen *et al.* 1997), wheat (Röder *et al.*

1998), potato (Milbourne *et al.* 1998), *Eucalyptus* (Brondani *et al.* 1998), soybean (Cregan *et al.* 1999), and sorghum (Kong *et al.* 2000). In maize, microsatellites were very useful as genetic markers for determining genetic similarities and relationships (Smith *et al.* 1997, Senior *et al.* 1998) and for genetic mapping (Senior and Heun 1993, Senior *et al.* 1996, Taramino and Tingey 1996, Davis *et al.* 1999).

However, molecular markers mapped in such works, RFLPs or microsatellites, helped to further characterize the temperate maize genome, mainly representing a range of U.S. Corn Belt germplasm. Few studies have dealt with mapping on tropical maize material (Bohn *et al.* 1997, Ribaut *et al.* 1997, Groh *et al.* 1998, Khairallah *et al.* 1998). The U.S. maize crop has a relatively narrow germplasm base because of the high levels of relatedness among the parents of current elite cultivars (Smith 1988). In contrast, tropical maize populations have a broad genetic base, formed usually by composites with larger variability than temperate synthetic materials (Lanza *et al.* 1997). The development of a second-generation genetic map for tropical maize germplasm, based on a wide set of microsatellite markers in a large F_2 mapping population, should provide more information on tropical maize genome and the level of similarities and differences between divergent genetic backgrounds.

The common approach employed for the analysis of microsatellites is radioactive labeling of the PCR product, followed by electrophoresis on denaturing polyacrilamide gels. An alternative for radioactive labeling is the silver staining of polyacrilamide gels. The most recently method is the use of fluorescent dyes for the automated detection of alleles. These methods offer the best resolution as single repeat differences can be resolved and thus all possible alleles detected, but they require special equipment and/or quite a lot

of manipulation, which makes the process time-consuming for large experiments, expensive and impossible to be carried out in non-radioactive laboratories.

For a quick polymorphism screen of each microsatellite and later genotyping, the approach employed for allele detection in our study was the high-resolution agarose gel system stained with ethidium bromide (Senior and Heun 1993, Senior *et al.* 1998). This method has a lower resolution, but makes the process of scanning a large number of loci, in a large mapping population, faster and less expensive.

Objectives of our study were: (I) the development of a second-generation genetic map based on microsatellite markers in a maize F_2 mapping population; (II) to determine the level of polymorphism of microsatellite markers in tropical maize genetic base; (III) to provide more information in terms of usefulness on the least costly and most widely available agarose gel system to solve microsatellite polymorphisms.

Material and methods

Plant Material

Two maize inbred lines showing contrasting behavior for grain yield were used: L-08-05F and L-14-4B. These inbreeds belong, respectively, to IG-1 and BR-106 maize populations and this crossing was selected because of differences between parents in terms of plant height, kernel type, maturity and yield. In addition, their cross produces a highly heterotic hybrid. By crossing these lines, F_1 plants were obtained which were selfed, generating the F_2 populations. The combined set of 400 F_2 plants from four F_1 plants generated the mapping population.

DNA extraction

Leaf material from parents, F_1 and F_2 generations were collected, freeze-dried, ground to powder and stored at -20°C in individually labeled vials. DNA extraction followed the procedures described in Hoisington *et al.* (1994).

Microsatellite primer selection

The accumulated information on molecular markers in maize allows the designation of a set of core RFLP markers evenly spaced throughout the genome. These markers have facilitated the dissection of the maize genome into 100 evenly spaced "bins" of

approximately 20 cM each (Gardiner *et al.* 1993). Initially, two or three microsatellites per bin were chosen for polymorphism screen. Next microsatellite selections were addressed to (1) bins where no polymorphic marker was found after tests; and (2) bins that contained polymorphic microsatellites but presenting erratic amplification, fuzzy or indistinct bands during the genotyping of the mapping population.

Sequences of 859 primer pairs to amplify maize microsatellites were obtained from the Maize Data Bank (2001), and synthesized by Gibco BRL (São Paulo, SP). When available, tetranucleotides repeats or even a greater number of repeats, were preferably selected to relate to di- or trinucleotide ones. Independently of the repeat type, microsatellite loci with higher repeat counts and perfect motif were the first choice.

By means of agarose gels, the primer pairs were screened against the parents and the four F_1 plants, which F_2 progenies were used. Microsatellites showing the clearest polymorphism among the parents were then used for genotyping. Locus designation, chromosome location, primer sequence information, description of microsatellite motif, and size of PCR product amplified in reference lines, can be found in the Maize Data Bank (2001).

Amplification Conditions

The reactions were carried out in 96-well microtitier plates using a PTC-100 Thermal Cycler (MJ Research, Watertown, MA). The amplification consisted of a 2 min denaturation step at 94°C followed a by modified "touchdown" program (Don *et al.* 1991). This program began with two 1 min cycles at 94°C, 1 min at 65°C and 2 min at 72°C. The

annealing temperature was then reduced by 1°C every two cycles until a final annealing temperature of 55°C was reached. The last cycle was repeated 20 times and was terminated with a continuous cycle at 15°C. The 20 μL reaction mix consisted of 1X reaction buffer (20 mM Tris-HCl, 50 mM KCl; pH 8.4), 2 mM $MgCl_2$, 100 μM of each dNTP, 0.5 unit *Taq* DNA polymerase (GIBCO BRL, São Paulo, SP), 0.2 μM of each primer, 50 ng of template DNA and ddH_2O. Reactions were stopped with 2 μL loading-dye (500 μL/mL glycerol, 20 mM EDTA, 0.6 mg/mL bromophenol blue). After amplification, a total of 22 uL of the mix was loaded into the wells of a 1 mm wide comb and products were separated by electrophoresis in a horizontal gel system (HORIZON 20:25, GIBCO BRL, Gaithersburg, MD) using 0.5X TBE (Sambrook *et al.* 1989) on a 4% agarose gel (2% ultra pure agarose, GIBCO BRL, Gaithersburg, MD : 2% Metaphor agarose; FMC BioProducts, Rockland, ME). The gel ran at approximately 170V for 1.5 h and was stained with 0.5 μg/mL ethidium bromide. Two different workers independently performed the visual score of the gel. Agarose gels were reused at least three times after running by remelting.

When the primers failed to amplify using the "touchdown" program, the amplification was performed with low stringency annealing temperatures. This program began with a 2 min denaturation step at 94°C, followed by 30 1 min cycles at 94°C, 1 min at 48°C to 52°C (according to each primer pair optimized annealing temperature) and 2 min at 72°C, and terminated with a continuous cycle at 15°C. When banding patterns were difficult to score accurately on agarose gels, the electrophoresis was conducted at a lower voltage (100V) and at an increased running time (5 to 8h).

Segregation and Genome Composition

Segregation at each marker locus was checked for deviations from Mendelian segregation ratio 1:2:1 by chi-square tests. Taking into account that multiple tests were performed, appropriate type I error rates were determined by the sequential rejective Bonferroni procedure (Rice 1989). Estimates of the proportion of parental genome for each individual were obtained according to Paterson *et al.* (1991).

Linkage Analysis

Linkage analysis was performed using the MAPMAKER/EXP v.3.0 program (Lander *et al.* 1987, Lincoln *et al.* 1992). Linkage was inferred using a LOD ($\log_{10}$ of the likelihood 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 in order to determine maximum likelihood recombination frequency, and linkage groups were established using the "group" command. Three-point linkage analyses were then conducted to determine the most likely order of loci within groups using "compare" command for smaller groups. For large linkage groups, a framework was established and the "ripple" command was used to confirm marker order as determined by multipoint analysis. Data quality was checked using "error detection" command, and unlikely double crossovers, due to possible genotyping errors, were corrected by rechecking the data. Crossover units were converted into map distances (centiMorgans, cM) by applying the Kosambi function (Kosambi 1944).

Results

Polymorphism analysis

Of the 859 microsatellites screened against the genomic DNA from the two parents and four F_1 individuals, 213 (25%) yielded a good amplification pattern, showed polymorphism between the parents and were selected for further study; 251 (29%) were not polymorphic; 113 (13%) failed to produce any amplified products even after the optimization of annealing temperature and re-amplification; and 282 (33%) lacked resolution on agarose gels (Figure 1a). The "touchdown" program was able to amplify 66% of the primer pairs. For the remaining 34%, the amplification was performed with low stringency annealing temperatures. Among the 464 (54%) microsatellites which were solved on agarose gels, 85% of the molecular weight of amplification products varied between 50 to 200 pb. For 15% of these microsatellites, electrophoresis was conducted at a lower voltage and at an increased running time to separate fragments weighing over 200 pb.

Among the 213 polymorphic microsatellites, 119 (56%) were useful for mapping analysis, 35 (16%) have not shown bands clearly distinguishable during genotyping and could not be scored; 40 (19%) presented segregation distortion after genotyping; and 19 (9%) showed non-Mendelian scoring with absence of a parental band in one or more F_1 plants tested (Figure 1b). All microsatellites mapped appear to be single locus markers, producing only one set of segregating bands.

Repeat type

These 859 microsatellites were grouped in five classes according to the repeat type: 392 dinucleotides, 230 trinucleotides, 66 tetranucleodites, 45 penta- or hexanucleotides and 26 non-perfect repeats (imperfect, interrupted and/or compound microsatellites). For 100 microsatellites no information was found on the repeat type in this research. The level of polymorphism and resolution on agarose gels vary widely among such classes (Table 1).

Polymorphism: similar levels of polymorphism were demonstrated between di- tetra- or non-perfect microsatellites. Penta- or hexanucleotides repeats had the lowest polymorphism. The proportion of non-polymorphic microsatellites for each class was directly related to the size of their repeat unit. Dinucleotides had the lowest proportion, penta- or hexanucleotides had the highest proportion of non-polymorphic microsatellites. Non-perfect repeats microsatellites showed intermediate proportion of non-polymorphic microsatellites.

Resolution: microsatellites with tetranucleotides or more had the best resolution on agarose gels. Dinucleotide repeats have the lowest resolution. Trinucleotides and non-perfect microsatellites had intermediate levels of resolution between these two classes. Despite the repeat type differences, every class revealed similar levels of amplification failure (Figure 2a to 2f).

Segregation

By applying the sequential rejective Bonferroni procedure, the critical threshold value for chi-square tests went up of 5.99 (alpha = 0.05, 2 df) for 16.31 (for 170 tests). A total of 119 markers segregated into a 1:2:1 ratio among the F_2 individuals in agreement with the segregation of single locus co-dominant alleles. Deviation from the expected 1:2:1 genotype frequency was significant for 40 markers scored and they were not used in subsequent mapping analysis. Between these 40 markers seven had presented inferior values of chi-square test to the critical threshold (1.00-umc1354, 1.06-umc1396, 2.01/2.02-umc1518, 2.03-bnlg0469, 2.03-bnlg0381, 5.02/5.03-umc1226 and 9.01-umc1867) however, they had been excluded therefore presented great deviation with only one small part of the genotyped population. Twenty-three markers with distorted segregation ratios tended to cluster in four specific genomic regions on chromosomes 2, 4, 9 and 10. Adjacent loci in bins 2.03 and 10.07 had a much greater frequency of heterozygous and homozygous of L-08-05F alleles associated to a decrease of homozygous of L-14-4B alleles. In bin 4.07-4.08, a locus with a deficiency of homozygous L-08-05F genotypes and an excess of homozygous for L-14-4B alleles, was observed. In bin 9.01, there was a deficiency of homozygous L-14-4B genotypes and an excess of homozygous of L-08-05F alleles (Table 2).

Markers per Bin

The 859 microsatellites assayed were distributed for all 100 bins. No polymorphic

markers or problems were found during genotyping, maintaining 17 bins uncovered. The 119 polymorphic microsatellite loci useful in mapping analysis fall into 83 bins. Chromosome 5 contains the largest number of microsatellite/bin with four microsatellite loci found in bin 5.06 (Figure 3). To reach these 119 microsatellites, 2 to 10 markers were tested in order to find one polymorphic marker for 62 bins, and 11 to over 20 markers were tested to find one polymorphic marker for 21 bins (Table 3). On average, 5.57 microsatellites per bin were tested in order to find a polymorphic marker useful for mapping analysis, that is to say, a polymorphic marker with no problems during genotyping of the mapping population.

Genetic map

The 119 loci data set was evaluated using MAPMAKER/EXP, resulting in 2 unlinked loci excluded in the map: bnlg2057 in bin 1.06 and bnlg1863 in bin 8.04, and 11 distinct linkage groups. Eleven linkage groups were obtained due to the failure in an attempt to establish linkage between the four most distal markers on chromosome 2 (umc1560, umc1633, umc1230 and bnlg1520) located in bins 2.07 to 2.09 and umc1396 in bin 2.06. The microsatellite linkage map of the F_2 population with 117 microsatellites, covered 1634 cM and the average distance between the markers on the map was 14 cM. The marker order on this F_2 map was in good agreement with the microsatellite loci mapped, which have been published for temperate maize germoplasm on Maize Data Bank (2001). A few exceptions were observed: (1) the initial linked markers umc1106 (bin 1.00) and umc1177 (bin 1.01) on chromosome 1 and phi420701 (bin 8.00) and umc1139 (bin

8.01) on chromosome 8 were reversed in an order based on the mapping analysis carried out in this work, (2) on chromosome 4, the marker bnlg589 (bin 4.11) was mapped onto a position close to the marker umc1989 (bin 4.09), but was assigned to the lower end of chromosome 4 in previous maps, (Figure 3).

Although the number of microsatellite markers mapped onto individual chromosomes is roughly proportional to their cytogenetic length (Coe *et al.* 1995), some genomic regions do not have complete coverage. For example, all attempts to identify more polymorphic loci in the 76.6 cM interval on chromosome 2 failed. Therefore, the two linkage groups were placed together based on previously published maps on Maize Data Bank (2001). Also, due to the lack of polymorphic loci, relatively little coverage was obtained for chromosomes regions 4, 5, 9, and 10.

Genome composition

According to the genotyping of 117 microsatellites mapped, the average percent heterozygosity per plant in the F_2 population was 52.09 ± 4.6%, with a range from 35% to 60%. The average percent homozygosity was 23.0 ± 3.2% (range: 13.50-29.25%) for L-08-05F/L-08-05F genotypes and 23.6 ± 4.0% (range 13.50-32.25%) for L-14-4B/L-14-4B genotypes. The parental genome ratio was 49.03% L-08-05F and 49.64% L-14-4B, which places the genotypic class mean values within the expectations for F_2 populations.

Discussion

The first genetic map of the tropical maize genome based on microsatellite markers is presented here. The 117 mapped loci in the genetic map cover 83% of the bins from maize genome. However, some genomic regions present large intervals among markers. The intervals up to 40 cM on chromosomes 4 and 5 are related to bins without polymorphic markers: bins 4.02, 4.03 and 4.08 on chromosome 4 and bins 5.01 and 5.08 on chromosome 5. The interval of 76.6 cM on chromosome 2, between bnlg1396 (2.06) and umc1560 (2.07), two markers mapped on adjacent bins, is uncommon. Since the average size of each bin is 20 cM, if two linked markers were mapped on opposite extremes of adjacent bins, theoretically, the distance could reach 40 cM. This fact could explain the interval ranges from 30 to 40 cM along the genome. However, the greatest interval on chromosome 2 reveals one region where a great rate of crossing-over could hinder linkage. The rate of crossing-over is known to differ from one species to another, as well as among genotypes and among genomic regions (Tanksley *et al.* 1992, Lukaszewski and Curtis 1993). Other markers mapped on this interval could solve this question.

Four genomic segments with segregation ratios that are distorted in favor of heterozygous and L-08-05F alleles (bins 2.03 and 10.07), L-08-05F alleles (bin 9.01), and L-14-4B alleles (bins 4.07-4.08) were observed (Table 2). Deviations from expected Mendelian segregation ratios of molecular markers have been reported in maize (Bentolila *et al.* 1992, Murigneux *et al.* 1993, Gardiner *et al.* 1993, Pereira and Lee 1995), as well as in many other plant species. The reasons for this distortion may result from a selection process during gametogenesis, fertilization or germination (Lyttle 1991). Errors in marker

genotyping may also cause systematic deviations from the expected segregation ratio. However, this could lead to misscoring of only a single marker. In contrast, if segregation distortion is caused by segregation-distortion loci (SDL), all markers in the vicinity of the SDL will be affected (Vogl and Xu 2000). Pre or post zygotic selection at one or more genes in the neighborhood of these loci is a possible explanation for these clusters with distorted ratios.

The frequency of non-Mendelian scoring was low: 9% among the polymorphic microsatellite tested, but was greater than the frequencies that have been observed for microsatellite markers in maize. Smith *et al.* (1997), checking the scoring and the inheritance of polymorphisms of 131 microsatellite loci among 13 sets of triplets (a progeny line and both parents), found that 2.2% of microsatellites segregated in a non-Mendelian fashion. Such non-Mendelian scoring is generally attributed to residual heterozygosity remaining within an inbred line used as parents, outcrossing or a mutation within the binding site for a DNA primer. The absence of the PCR product of one homologue in the heterozygous made this marker unsuitable for correct genotyping and the marker was discarted from the mapping experiment.

Due to the large mapping population and high number of microsatellites, a simple methodology for amplification and detection of all primer pairs ("touchdown" program and agarose gels), with little optimization of these conditions, was adopted. The aim was the quick polymorphism screen and genotype assignment of each marker. Certainly, among the 113 microsatellites for which non-amplification was observed, a number of them could be recovered by modifying concentrations of the PCR components or amplification program. However, considerable time had to be spent in this optimization for each microsatellite. It

was observed that 87% of the microsatellites tested amplified either by "touchdown" program (66%), or simply by decreasing annealing temperature (34%) showing that this methodology was effective.

Senior and Heun (1993) reported that Metaphor agarose was effective in separating microsatellite alleles. The same approach was used for Senior *et al.* (1998) for determining genetic relationships in maize using microsatellites. Preliminary tests in our lab (data not shown) showed those 4% gels with 2% ultra-pure agarose: 2% Metaphor has a degree of resolution equal to that of 4% gels with only Metaphor agarose. This way, a mix of 2% ultra pure agarose and 2% Metaphor agarose, was used. This methodology was able to solve PCR products for 62% (464 microsatellites) of the 746 microsatellites that amplified. For 38% (282 microsatellites), the agarose gel methodology was not effective to solve the PCR products. High-resolution agarose gels have a resolution limit of 4 pb, and alleles are more difficult to solve when the total allele length exceeds 200 bp. This limitation could be overcome by selecting microsatellites the amplification products of that are easier to score on agarose gels.

Microsatellite polymorphism is due to mutation events that change the number of copies of repeat units. These mutations might be generated by polymerase slippage during DNA replication (Levinson and Gutman 1987, Schlotterer and Tautz 1992, Weber and Wong 1993). The mutation rate varies substantially among microsatellite loci and depends on structural factors of repeat unit as size, type, and composition. Weber (1990) found that the polymorphism of (CA)n markers increased when increasing the average number of repeats. For a given size of repeat unit, the presence of non-perfect repeats (imperfect repeats with one or more interruptions and/or compound repeats with adjacent tandem

repeats of different sequences) is correlated with the stability of a microsatellite, reducing mutation rates (Goldstein and Clark 1995, Petes *et al.* 1997). Finally, it has been observed that dinucleotide repeats showed mutation rates higher than tetranucleotides, with trinucleotides intermediate between the di e tetra (Chakraborty *et al.* 1997).

Based on these results, the best microsatellites for agarose gel systems are the perfect tetranucleotides (or greater) repeats, the sizes of which had good allele separation after a long run. However, the most common microsatellite found in genomes is the dinucleotide repeat. Trinucleotide repeats are less common than dinucleotides and tetranucleotide repeats are the less abundant microsatellite (Wang *et al.* 1994, Gupta *et al.* 1996). Moreover, our results on agarose gels showed that the number of polymorphic microsatellites was inversely related to the size of their repeat unit. It means that tetranucleotides were less polymorphic than tri- or dinucleotides, and the results are in good agreement with the estimates of Chakraborty *et al.* (1997).

Thus, in order to explore the microsatellite polymorphism on agarose gels, a balance between the abundance of the repeat type and their resolution in this genotyping system, must be considered. Tetranucleotides microsatellites or greater, have the best resolution, but are less abundant and less polymorphic. Dinucleotides have the worst resolution, but are the most abundant and are more polymorphic. According to the results here, if equal amounts of di- and tetranucleotides are assayed, the number of polymorphic microsatellites resolved on agarose gels will be approximately the same.

Conclusion

Microsatellites have several features that make them increasingly useful as DNA markers. What limits their widespread use is the need of sequence information for the development of microsatellite primers, which is an expensive and labor intensive process. However, once developed, the use of specifically designed primers based upon the flanking sequences allows the easy dissemination of these sequences from public databases. With the primer sequences available and the relatively low price of custom-made oligonucleotides, more attention can be drawn to a faster and less expensive microsatellite detection. The agarose gel system has been demonstrated to be effective to solve amplification products of microsatellites, thus avoiding the radioactive or fluorescent labeling or the development of high throughput automation systems. This simple methodology has become an important option for analysis for a large number of samples in laboratories unable to undertake expensive and laborious processes.

The comparisons of maps in different genetic backgrounds confirm that microsatellites are highly reproducible between laboratories and maintain the marker order of loci mapped on previous maps. Although 117 microsatellite loci have been mapped, this genetic map represents the first step towards the development of a more fully saturated map of the tropical maize genome based on microsatellite markers. Work is presently under way to map additional microsatellite markers to close the gaps on poorly represented chromosome regions and to provide more information about the segregating distortion loci and genomic regions presenting high levels of crossing-over events discovered.

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Figure 1. Frequency distribution of amplification results detected using high-resolution agarose gels. (**A**) Results after polymorphism screen of 859 microsatellites against the parents and the four F_1 individuals. (**B**) Results among 213 polymorphic microsatellites after genotyping the mapping population.

Figure 2. Frequency distribution of amplification results after polymorphism screen among the five classes of known repeat types. (**A**) Distribution of repeat types among the 859 microsatellites tested. (**B**) to (**F**): results of dinucleotides, trinucleotides, tetranucleotides, penta- or hexanucleotides and non-perfect repeats (imperfect, interrupted and/or compound microsatellites), respectively.

Figure 3. Genetic map of tropical maize showing the locations of 117 microsatellite loci based on 400 F_2 individuals from a cross between L-08-05F e L-14-4B. Loci names are to the right of each chromosome with the corresponding maize bin. Map distances between adjacent markers, on the left side of the chromosomes, are in centiMorgans (cM) calculated using the Kosambi function. Dark circles indicate estimated centromere positions, obtained from Coe et al. (1995). Underlined marker loci were mapped to different position than those reported in literature. Map length is 1634 cM and average interval length is 14 cM. Linkage analysis was carried out using MAPMAKER/EXP v.3.0 program. All markers are linked to the map with LOD scores of 3.0 or greater. Total centiMorgan length for each chromosome is indicated below the chromosome number.

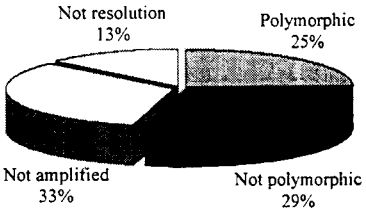
Table 1. Comparisons of microsatellites amplification results as detected in five classes of repeat types and among a number of microsatellites in which repeat type is unknown.

Table 2. Microsatellite loci showing distorted segregation.

Table 3. Number of microsatellites tested per bin to find the 119 polymorphic microsatellite loci useful for mapping analysis.

Figure 1.

A) Polymorphism screen of 859 microsatellites



B) Genotyping of 213 polymorphic microsatellites

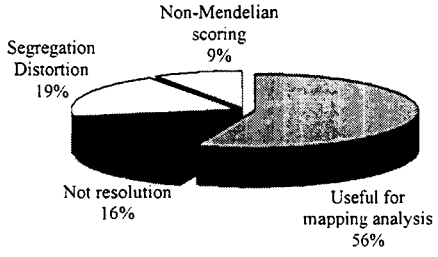


Figure 2.

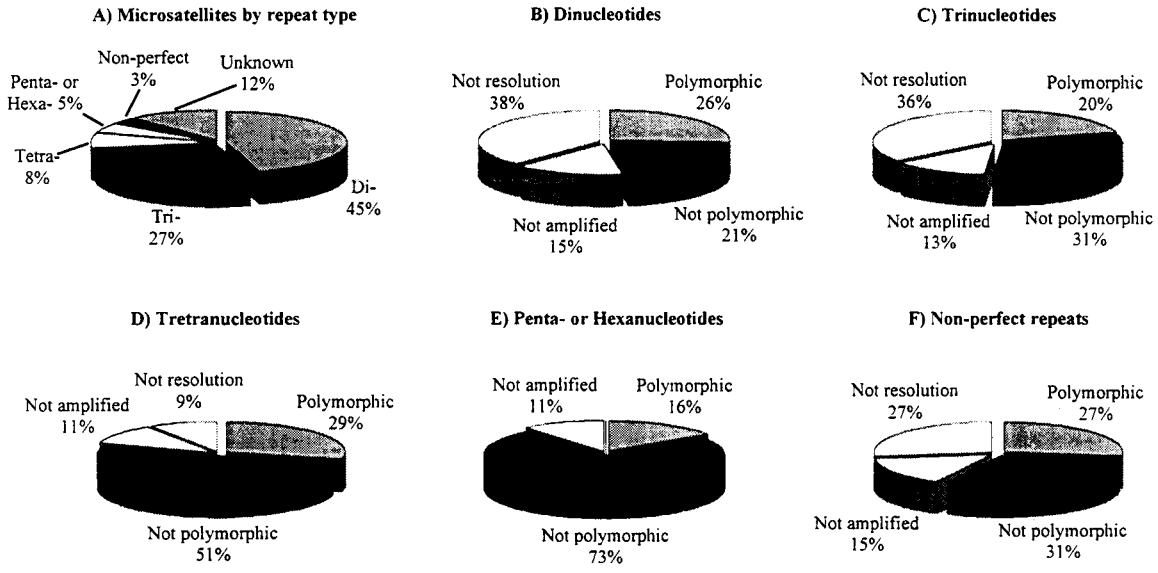

A) Microsatellites by repeat type
Non-perfect 3%
Unknown 12%
Penta- or Hexa- 5%
Tetra- 8%
Di- 45%
Tri- 27%
B) Dinucleotides
Not resolution 38%
Polymorphic 26%
Not amplified 15%
Not polymorphic 21%
C) Trinucleotides
Not resolution 36%
Polymorphic 20%
Not amplified 13%
Not polymorphic 31%
D) Tretranucleotides
Not resolution 9%
Not amplified 11%
Polymorphic 29%
Not polymorphic 51%
E) Penta- or Hexanucleotides
Not amplified 11%
Polymorphic 16%
Not polymorphic 73%
F) Non-perfect repeats
Not resolution 27%
Polymorphic 27%
Not amplified 15%
Not polymorphic 31%

Figure 3.

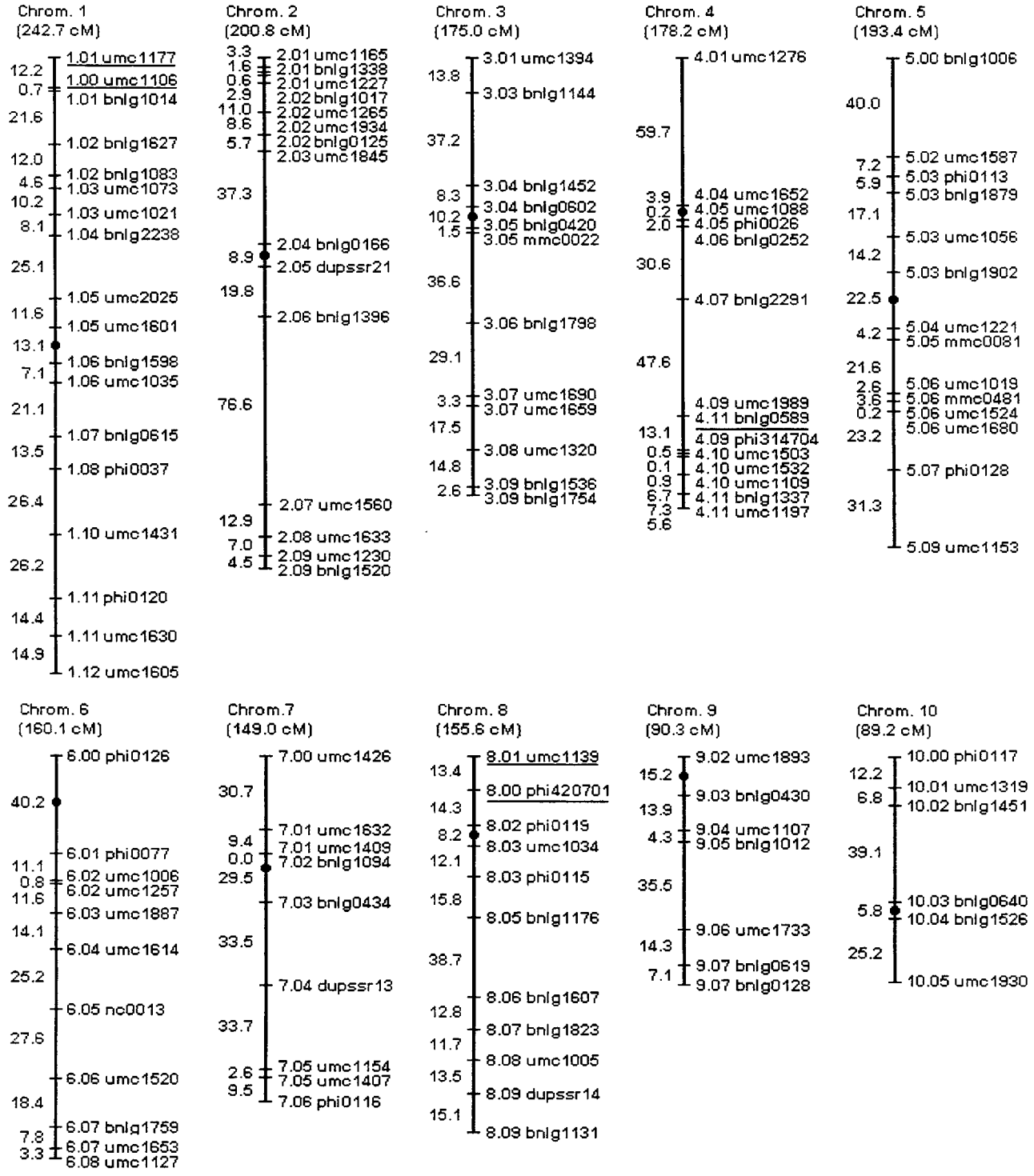

Chrom. 1
(242.7 cM)
1.01 umc1177
1.00 umc1106
1.01 bnlg1014
1.02 bnlg1627
1.02 bnlg1083
1.03 umc1073
1.03 umc1021
1.04 bnlg2238
1.05 umc2025
1.05 umc1601
1.06 bnlg1598
1.06 umc1035
1.07 bnlg0615
1.08 phi0037
1.10 umc1431
1.11 phi0120
1.11 umc1630
1.12 umc1605
Chrom. 2
(200.8 cM)
2.01 umc1165
2.01 bnlg1338
2.01 umc1227
2.02 bnlg1017
2.02 umc1265
2.02 umc1934
2.02 bnlg0125
2.03 umc1845
2.04 bnlg0166
2.05 dupssr21
2.06 bnlg1396
2.07 umc1560
2.08 umc1633
2.09 umc1230
2.09 bnlg1520
Chrom. 3
(175.0 cM)
3.01 umc1394
3.03 bnlg1144
3.04 bnlg1452
3.04 bnlg0602
3.05 bnlg0420
3.05 mmc0022
3.06 bnlg1798
3.07 umc1690
3.07 umc1659
3.08 umc1320
3.09 bnlg1536
3.09 bnlg1754
Chrom. 4
(178.2 cM)
4.01 umc1276
4.04 umc1652
4.05 umc1088
4.05 phi0026
4.06 bnlg0252
4.07 bnlg2291
4.09 umc1989
4.11 bnlg0589
4.09 phi314704
4.10 umc1503
4.10 umc1532
4.10 umc1109
4.11 bnlg1337
4.11 umc1197
Chrom. 5
(193.4 cM)
5.00 bnlg1006
5.02 umc1587
5.03 phi0113
5.03 bnlg1879
5.03 umc1056
5.03 bnlg1902
5.04 umc1221
5.05 mmc0081
5.06 umc1019
5.06 mmc0481
5.06 umc1524
5.06 umc1680
5.07 phi0128
5.09 umc1153
Chrom. 6
(160.1 cM)
6.00 phi0126
6.01 phi0077
6.02 umc1006
6.02 umc1257
6.03 umc1887
6.04 umc1614
6.05 nc0013
6.06 umc1520
6.07 bnlg1759
6.07 umc1653
6.08 umc1127
Chrom.7
(149.0 cM)
7.00 umc1426
7.01 umc1632
7.01 umc1409
7.02 bnlg1094
7.03 bnlg0434
7.04 dupssr13
7.05 umc1154
7.05 umc1407
7.06 phi0116
Chrom. 8
(155.6 cM)
8.01 umc1139
8.00 phi420701
8.02 phi0119
8.03 umc1034
8.03 phi0115
8.05 bnlg1176
8.06 bnlg1607
8.07 bnlg1823
8.08 umc1005
8.09 dupssr14
8.09 bnlg1131
Chrom. 9
(90.3 cM)
9.02 umc1893
9.03 bnlg0430
9.04 umc1107
9.05 bnlg1012
9.06 umc1733
9.07 bnlg0619
9.07 bnlg0128
Chrom. 10
(89.2 cM)
10.00 phi0117
10.01 umc1319
10.02 bnlg1451
10.03 bnlg0640
10.04 bnlg1526
10.05 umc1930

Table 1. Comparisons of microsatellites amplification results as detected in five classes of repeat types and among a number of microsatellites in which repeat type is unknown.

Repeat Type	Polymorphic	Not Polymorphic	Not Amplified	Not Resolution	Total	%
Di-	102	84	57	149	392	45
Tri-	47	71	30	82	230	27
Tetra-	19	34	7	6	66	8
Penta- or Hexa-	7	33	5	0	45	5
Non-perfect	7	8	4	7	26	3
Unknown	31	21	10	38	100	12
Total	213	251	113	282	859	100

Table 2. Microsatellite loci showing distorted segregation.

Bin	Locus	L08/L08[a] Obs.	L08/L08[a] %	L08/L14[a] Obs.	L08/L14[a] %	L14/L14[a] Obs.	L14/L14[a] %	Total	χ^2	p-value
1.00	umc1354	60	(38)	85	(-2)	29	(-33)	174	11.14	3.81×10^{-3}
1.06	umc1396	36	(-30)	121	(17)	50	(-3)	207	7.81	2.01×10^{-2}
1.06	umc1508	65	(-35)	226	(13)	109	(9)	400	16.44	2.70×10^{-4}
2.01-2.02	umc1518	73	(9)	149	(12)	45	(-33)	267	9.47	8.78×10^{-3}
2.02	bnlg2277	98	(5)	216	(16)	58	(-38)	372	18.28	1.10×10^{-4}
2.02	umc1823	68	(-23)	222	(25)	64	(-28)	354	22.97	1.10×10^{-5}
2.03	**bnlg0469**	55	**(-26)**	180	**(20)**	64	**(-14)**	299	12.99	1.51×10^{-3}
2.03	**dupssr27**	87	**(-4)**	217	**(20)**	57	**(-37)**	361	19.75	5.00×10^{-5}
2.03	**mmc0111**	88	**(4)**	199	**(17)**	52	**(-39)**	339	17.91	1.30×10^{-4}
2.03	**bnlg0381**	79	**(11)**	158	**(11)**	48	**(-33)**	285	10.12	6.35×10^{-3}
2.03	**bnlg2248**	112	**(12)**	225	**(13)**	62	**(-38)**	399	19.05	7.00×10^{-5}
2.03	**umc1776**	112	**(12)**	224	**(12)**	64	**(-36)**	400	17.28	1.80×10^{-4}
2.07	umc1637	41	(-42)	134	(-5)	106	(51)	281	30.67	2.20×10^{-7}
2.08	umc1464	53	(-37)	176	(5)	106	(27)	335	17.63	1.50×10^{-4}
2.08	umc2005	96	(-4)	238	(19)	66	(-34)	400	18.94	8.00×10^{-5}
3.10	bnlg1098	57	(-39)	211	(13)	105	(13)	373	18.79	8.00×10^{-5}
3.10	umc1136	70	(13)	175	(42)	2	(-97)	247	80.39	3.50×10^{-18}
4.07	dupssr34	66	(-33)	203	(3)	124	(26)	393	17.55	1.50×10^{-4}
4.08	**bnlg2162**	56	**(-43)**	228	**(16)**	110	**(12)**	394	24.56	4.00×10^{-6}
4.08	**umc1086**	53	**(-41)**	195	**(9)**	111	**(24)**	359	21.42	2.00×10^{-5}
4.08	**bnlg2244**	63	**(-37)**	220	**(10)**	117	**(17)**	400	18.58	9.00×10^{-5}
4.08	**umc1051**	57	**(-42)**	212	**(8)**	122	**(25)**	391	24.40	1.00×10^{-5}
5.02-5.03	umc1226	49	(-30)	160	(15)	70	(0)	279	9.19	1.01×10^{-2}
5.04	dupssr10	123	(59)	118	(-24)	69	(-11)	310	36.48	1.20×10^{-8}
5.05-5.06	bnlg0278	106	(6)	161	(-20)	133	(33)	400	18.86	8.00×10^{-5}
6.02	bnlg1371	87	(-13)	163	(-19)	150	(50)	400	33.54	5.20×10^{-8}
9.01	**umc1867**	41	**(46)**	58	**(4)**	13	**(-54)**	112	14.14	8.50×10^{-4}
9.01	**umc1040**	51	**(82)**	42	**(-25)**	19	**(-32)**	112	25.29	3.20×10^{-6}
9.01	**phi0028**	115	**(52)**	154	**(2)**	33	**(-56)**	302	44.65	2.00×10^{-10}
9.02	**bnlg1401**	119	**(19)**	220	**(10)**	61	**(-39)**	400	20.82	3.00×10^{-5}
9.02	**dupssr06**	136	**(38)**	203	**(3)**	55	**(-44)**	394	33.67	4.90×10^{-8}
9.02	**umc1170**	136	**(39)**	202	**(4)**	52	**(-47)**	390	36.69	1.10×10^{-8}
10.05	umc1506	121	(27)	206	(8)	54	(-43)	381	26.09	2.20×10^{-6}
10.07	**umc1084**	62	**(22)**	119	**(17)**	22	**(-57)**	203	21.80	2.00×10^{-5}
10.07	**umc1344**	72	**(20)**	142	**(18)**	26	**(-57)**	240	25.70	2.60×10^{-6}
10.07	**umc1569**	103	**(16)**	214	**(21)**	37	**(-58)**	354	40.08	2.00×10^{-9}
10.07	**umc1038**	113	**(22)**	214	**(16)**	43	**(-54)**	370	35.58	1.90×10^{-8}
10.07	**umc1196**	115	**(18)**	231	**(18)**	45	**(-54)**	391	37.96	5.70×10^{-9}
10.07	**umc2021**	119	**(27)**	212	**(13)**	43	**(-54)**	374	37.57	6.90×10^{-9}
10.07	**bnlg1839**	121	**(30)**	205	**(10)**	46	**(-51)**	372	34.12	3.90×10^{-8}

[a] Number of individuals observed and the percent of the deviations from the expected ratio are shown for the three genotypic classes: homozygous for L-08-05F alleles (L08/L08), heterozygous (L08/L14) and homozygous for L-14-4B alleles (L14/L14).

Obs.: Numbers in black and shaded strings highlight the clusters of segregation distortion loci.

Table 3. Number of microsatellites tested per bin to find the 119 polymorphic microsatellite loci useful for mapping analysis.

Number of microsatellites tested	Number of bins covered
from 1 to 5	27 (33%)
from 6 to 10	35 (42%)
from 11 to 15	14 (17%)
from 16 to 20	4 (5%)
more than 20	3 (4%)
Total[a]	83 (100%)

[a] Not polymorphic markers found or problems during genotyping maintained 17 bins uncovered. The remainder 83 bins are covered by one or more polymorphic microsatellites.

04. 2° ARTIGO: Genetic Mapping of Quantitative Trait Loci (QTL) for Grain Yield and Yield-Components in Tropical Maize (*Zea mays* L.) using microsatellites markers

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Summary

Two tropical maize inbred lines, L-08-05F and L-14-4B, were crossed to produce 400 F_2 plants that were used as a mapping population. A genetic map containing 117 microsatellite loci was constructed for Quantitative Trait Loci (QTL) mapping. The final genetic map spanned 1634 cM in length with an average interval of 14 cM between adjacent markers. QTL for yield and yield components were characterized in a population of 400 $F_{2:3}$ lines, derived from selfing the F_2 plants, and were evaluated with two replications in five environments. QTL determinations were made in the mean of these five environments. Grain yield (GY), plant height (PH), ear height (EH) and grain moisture (GM) were assessed. Variance components for genotypes (G), environments (E) and G X E interaction were highly significant for all traits. Heritability was 0.69 for GY, 0.66 for PH, 0.67 for EH and 0.23 for GM. Using Composite Interval Mapping (CIM), we identified a total of 13 distinct QTLs: four for GY, four for PH and five for EH. No QTL was detected for GM. The QTL explained 32.73% of the phenotypic variance of GY, 24.76% of PH and 20.91% of EH. The 13 QTL displayed mostly partial dominant or overdominance gene action and mapped to chromosomes 1, 2, 7, 8 and 9. Most QTL alleles conferring high values for the traits were contributed from line L-14-4B. Mapping analysis identified genomic regions associated with two or more traits in a manner consistent with correlation among traits, supporting either pleiotropy or tight linkage among QTL.

Key words: tropical maize, microsatellites, molecular mapping, QTL.

Introduction

The development of high-density linkage maps based on molecular markers in maize and other major crops have been allowed the investigations on the genetic basis of quantitative traits, commonly referred to as quantitative trait loci (QTL). Coupled with statistical procedures, along with the availability of computer software (e.g. Lander and Botstein 1989, Zeng 1994), these maps provide information on the location and genetic effects of genomic regions affecting economically important quantitative traits. In maize, studies have reported several chromosome regions for QTLs such as resistance to biotic and abiotic stresses, morphological characters and grain yield (Stuber *et al.* 1992, Edwards *et al.* 1992, Koester *et al.* 1993, Beavis *et al.* 1994, Ajmone-Marsan *et al.* 1995, Lee 1995).

Grain yield is one of the most important traits to maize breeding programs, but its evaluation and improvement are difficult and expensive to assess due its complex plant biology, environmental interactions, and low heritability (Hallauer and Miranda Filho 1988). These characteristics have become grain yield the primary trait of interest to QTL mapping studies for marker-assisted selection (MAS - Tanksley 1993, Lee 1995). However, these studies for the detection of QTLs have been done mainly with temperate germplams. Similar studies with tropical germplasm are fewer (Bohn *et al.* 1997, Groh *et al.* 1998, Khairallah *et al.* 1998). Tropical maize germplams have a broad genetic base with greater variability than temperate synthetic materials (Lanza *et al.* 1997), and are exposed to a wide range of environmental stress, higher in the tropics than in temperate zones (Ribaut *et al.* 1997). Analysis of QTL on tropical maize germplams could identify novel genomic regions that have not yet been defined by alleles with quantitative effects.

Restriction fragment length polymorphisms (RFLP) have become the molecular marker widely used in the genetic analysis of quantitative traits in maize (Coe *et al.* 1995). However, the use of RFLPs in QTL analysis represents excessive labor and costs for genotyping large populations. The development of the polymerase chain reaction (PCR) (Mullis and Fallona 1987) have made possible the arising of highly informative markers for genetic mapping, including microsatellite sequences (Litt and Lutt 1989). Microsatellites markers, also called simple sequence repeats (SSR), are highly polymorphic, co-dominant, wide dispersion in diverse genomes, and mapped to a single locus (Powell *et al.* 1996). The type of DNA polymorphism could be detected after PCR amplification and separation on gel electrophoresis. The simple experimental procedures by which they can be detected reducing time and cost to construction of high-density linkage maps (Senior and Heun 1993, Senior *et al.* 1998).

Utilizing the great number of PCR primers for maize microsatellites obtained through both database searches and random screening of genomic libraries, and available at MaizeDB (2001), we developed a genetic map in a tropical maize population. After that, we evaluated 400 $F_{2:3}$ lines from the cross of two tropical maize inbred lines with replicated trials over five environments. Our objectives were (i) the detection of QTLs for grain yield and its components in tropical maize germplasm that show consistency in expression across environments using the composite interval mapping (CIM) approach; (ii) determine the number, genomic positions, and gene effects of QTLs involved in variation of grain yield (GY), plant height (PH), ear height (EH) and grain moisture (GM) in this material.

Material and methods

Plant Material

Two maize inbred lines showing contrasting behavior for grain yield were used: L-08-05F and L-14-4B. These inbreeds belong, respectively, to IG-1 and BR-106 maize populations and this crossing was selected because of differences between parents in terms of plant height, kernel type, maturity and yield. In addition, their cross produces a highly heterotic hybrid. By crossing these lines, F_1 plants were obtained which were selfed, generating the F_2 populations. The combined set of 400 F_2 plants from four F_1 plants generated the mapping population. By selfing individual F_2 plants, 400 $F_{2:3}$ lines were obtained. The lines were sib-mated by using 20 plants of the same line to increase seed for the evaluations of the lines.

Field Trials

Field trials were conducted at the Research Farms of Departamento de Genética, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP) at Piracicaba, SP (Southeast Brazil) in 1999 and 2000. $F_{2:3}$ lines were evaluated in field experiments in five environments: Areão (1999 and 2000), Caterpillar (1999 and 2000), and Departamento (2000). Each site-year combination was treated as an environment in subsequent statistical analysis. The 400 $F_{2:3}$ lines were grown in four 10 x 10 lattice design with two replications. Plots consisted of single rows, 0.8 m apart and 4 m

long. Plots were overplanted and later thinned to a final plant density of 10 plants m^{-2} with a total of 20 plants per row, corresponding to a planting density of 62.500 plants per hectare. At each location, plot size and management were in accordance with local practice.

Shortly after flowering, plant height (PH) and ear height (EH) were measured on five plants per plot. PH: as the distance (cm) from the ground to the point of insertion of the flag leaf. EH: as the distance (cm) from the ground to the node of attachment of the primary ear. After the plots were harvested, grain yield (GY) and grain moisture (GM) were measured. GY: the total weight (g) of hand harvested, shelled grain adjusted to 150 g kg^{-1} grain moisture. GM: moisture of a 200 g kernel sample from shelled grain of the plot's total measured by electronic equipment and converted to g H_2O kg^{-1}.

Field Data Analysis

For all traits, means, standard deviations, and skewness of trait distribution were calculated. For those traits, that were not approximately normally distributed, log-transformation was used to improve the normality of the trait distribution. Analyses of variance were performed on the data from each environment. For each trait, the adjusted entry means of the five trials were averaged to give trait values for the mean environment used in the QTL analysis. From the combined analysis of variance across environments, estimates of variance components σ^2_g (genotypic variance), $\sigma^2_{g \times e}$ (genotype-by-environment interaction variance) and σ^2_e (error variance) of $F_{2:3}$ lines and their confidence intervals lines were calculated. Broad sense heritabilities (h^2) were estimated as described by Hallauer and Miranda Filho (1988). Exact 90% confidence intervals of h^2 were calculated according to

Knapp *et al.* (1985). All these analyses were performed using the analysis tools of SAS software (SAS Institute, 2001).

Molecular Marker Assays and Linkage Map

Microssatellite assays and the linkage analysis of the marker loci were previously described in Sibov *et al.* (2002). The microsatellite data and marker linkage map given by these authors were also used in the QTL analyses presented here. A total of 117 microsatellite marker loci were used to genotype the parental F_2 individuals of all 400 $F_{2:3}$ lines. Locus designation, chromosome location, primer sequence information, description of microsatellite motif, and size of PCR product amplified in reference lines, can be found in the MaizeDB (2001). A molecular linkage map was constructed using the Mapmaker/Exp version 3.0 program (Lander *et al.* 1987, Lincoln *et al.* 1992). A LOD threshold of 3.0 was used to declare linkage in two-point analyses. Three-point linkage analyses were then conducted using "compare" command for smaller groups. For large linkage groups, a framework was established and the "ripple" command was used to confirm marker order as determined by multipoint analysis. Crossover units were converted into map distances (centiMorgans, cM) by applying the Kosambi function (Kosambi 1944). Microsatellite loci positions were noted on "bins", genomic regions of approximately 20 cM each that facilitated the dissection of the maize genome in collection points for mapped genetic loci (Gardner *et al.* 1993).

QTL Mapping

QTL were determine on the adjusted entry means of the mean environment by the composite interval mapping method (CIM) described by Zeng (1993, 1994) with the software QTL Cartographer version 1.15 (Basten *et al.* 1994, 1999). CIM is an extension of interval mapping (Lander and Botstein 1989) and tests the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while statistically accounting for the effects of additional segregating QTL using multiple regression on markers outside the tested interval. The likelihood-ratio (LR) test statistic is $-2\ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) to the alternative hypothesis (there is a QTL in the interval).

We used Model 6 of the Zmapqtl procedure of QTL Cartographer, stipulating an F_2 population, scanning intervals of 1 cM between markers and putative QTLs with a window size of 10 cM. The number of marker cofactors for the background control was set by forward-backward stepwise regression. A genome-wide critical threshold value for the experimentwise Type I error rate (alpha = 0.10) was set for each trait independently by randomly permuting the line means among genotypes 500 times and using the empirical permutation false positive rate (Churchill and Doerge 1994). Stringent controls on the Type I error greatly increases the probability of reject a true QTL (a Type II error). Then, an alpha level of 0.10 was chosen to reduce the probability of Type II error. The estimation of the position, Genetic effects and percentage of phenotypic variation of the QTL was made at the significant LR peak in the region under consideration.

The phenotypic variance explained by a single QTL was estimated by the square of the partial correlation coefficient (R^2). Estimates of the R^2 value, the additive and

dominance effects of a QTL were estimated by the Zmapqtl procedure and obtained from the output of the program QTL Cartographer. The total proportion of phenotypic variance explained were obtained by fitting a model including all QTL for the respective trait simultaneously, according to the procedures described by Bohn *et al.* (1996). The sign of the estimates was used to identify the favorable alleles contributed by each parent. For QTL main effects, positive and negative signs of the estimates indicate that L-14-4B and L-08-05F, respectively, contributed the higher value alleles for the trait. Average levels of dominance were calculated as the ratio $|d|/|a|$ with the dominance effects, *d*, being the dominance effects estimated for the F_2 population. Gene action was determined on the basis of the average level of dominance by using the criteria of Stuber *et al.* (1987): additive (A) = 0 to 0.20; partial dominance (PD) = 0.21 to 0.80; dominance (D) = 0.81 to 1.20; and overdominance (OD) > 1.20.

Results

Linkage map

The survey of microsatellite markers on the mapping population of 400 F_2 plants identified a total of 117 markers that were polymorphic between the parents. Mapmaker analysis at LOD 3.0 resolved the markers into 10 linkage groups that had a total length of 1634 cM with an average interval length of 14 cM. The marker order on this F_2 map was in good agreement with published genetic maps (MaizeDB, 2001), only few inversions were observed on bins 1.00-1.01, 4.09-4.11 and 8.00-8.01 (Figure 1). More details about the linkage map were described in Sibov *et al.* (2002).

Field Data Analysis

For all the evaluated character, the confidence intervals and the coefficients of variation had shown that the estimates for means, variance components and heritabilities of the $F_{2:3}$ lines had been gotten with good accuracy (Table 1). The means for GY, PH and EH were continuously distributed as expected for a quantitative trait, but GM had significant deviations from a normal distribution and the data was log-transformed. In combined analyses across environments, GY, PH and EH have highly significant differences among the lines and highly significant genotype x environment interactions. (Table 1). But GM showed limited variability and the genotypic variance was 25 times lower than the error variance. Because the measurements were taken in five environments with two replications,

the estimated broad-sense heritabilities were high due to decreased both the experimental errors and genotype-by-environment interaction. These heritabilities estimated by the values from the mean environment from the 400 $F_{2:3}$ lines were high for GY (h^2 = 0,69), PH (h^2 = 0,66) and EH (h^2 = 0,67) and low for GM (h^2 = 0,23) indicating the low genetic variability (Table 1).

QTL Analysis

The gene actions, the directions of response and the QTLs detected using CIM for the traits are listed in Table 2 and illustrate in Figure 2. By applying permuting the line means among genotypes 500 times, a genome-wide critical threshold value for each trait was calculated (alpha = 0.10). These values were 16.01 for GY, 16.31 for PH, 15.78 for EH and 32.44 for GM. Determinations of QTL in the mean of the five environments yielded 13 "stable" QTLs which affected GY, PH and EH. The parent lines were not significantly different for GM, the trait did not segregate in the mapping population and none QTL were mapped. The QTL were located on chromosomes 1, 2, 7, 8, and 9 (Table 2, Figure 2).

Grain Yield

For GY, 4 QTL were detected on chromosomes 2 (*Gy2*), 7 (*Gy7*) and 8 (*Gy8a, Gy8b*) (Figure 2, Table 2). With these 4 QTL explains altogether 32.73% of phenotypic variation. Individual QTL accounted for 5.22-11.18% of phenotypic variation (Table 2). For two of the QTLs (*Gy8a* and *Gy8b*), alleles from L-08-05F contributed to an increase of

the trait values. For the other two QTLs (*Gy2 and Gy7*), alleles from L-14-4B were in direction of increasing the trait value. Dominance effects reached significance at three QTL whereas only 1 QTL (*Gy8a*) displayed additive gene action.

Plant Height

Four QTLs were resolved for PH on chromosomes 1 (*Ph1a*, *Ph1b*), 2 (*Ph2*) and 7 (*Ph7*) (Figure 2, Table 2) explaining 24.76% of phenotypic variation. Individual QTL accounted for 5.86-8.76% of phenotypic variation (Table 2). For three of these QTL, alleles from L-14-4B were in direction of increasing the trait values. While the QTL on chromosome 1 (*Ph1a*), the allele from L-08-05F contributed to the increase of the trait score. The QTLs displayed significant dominant gene action with the QTL on chromosome 1 (*Ph1a)*, showing a very large dominance effect. Only 1 QTL displayed additive gene effects for PH (*Ph1b)* (Table 2).

Ear Height

Five QTLs were detected for EH on chromosomes 1 (*Eh1*), 7 (*Eh7a, Eh7b, Eh7c*) and 9 (*Eh9*) (Figure 2, Table 2) explaining 20.91% of phenotypic variation. Individual QTL accounted for 3.66-5.61% of phenotypic variation (Table 2). At the majority of the QTLs, alleles from L-14-4B were in the direction of increasing ear height. For only one of these QTL, alleles from L-08-05F were in direction of increasing the trait values. Additive gene

action occurred at two QTL. The remaining QTLs displayed significant dominant gene effects.

QTLs Groups

Taken together, only three QTLs (*Ph1a*, *Eh7a* and *Eh9)* were not mapped close to other QTL (Figure 2). The remaining 10 QTLs were detected in the same chromosomal regions, forming four groups of QTLs. The highest concentrations of QTLs were found on the marker interval dupssr13-umc1154 on chromosome 7 where QTLs for all three traits were detected. Each group of QTL has the same parental alleles contributing to increase trait values. Alleles from L-08-05F contributed to an increase of the trait values for the group on chromosome 8 (*Gy8a-Gy8b*). Alleles from L-14-4B contributed to an increase of the trait values for the remaining groups on chromosomes 1 (*Eh1-Ph1b*), 2 (*Gy2-Ph2*) and 7 (*Eh7b-Ph7-Eh7c-Gy7*).

Discussion

In this study, a total of 13 QTLs had been identified for GY, PH, EH in tropical maize lines through the use of the mean environment by replicated field trials and evaluations in five environments. Results for the QTLs detection in maize (Veldboom and Lee 1996a, 1996b, Austin and Lee 1998) had shown that the use of the average phenotypic value of the characters in different tested environments was an efficient approach to QTL detection, therefore consider those only most steady ones. According to Knapp *et al.* (1990), the average between environments reduces the error standard of the values of the evaluated traits, increasing the precision and magnifying the power of detection and characterization of QTLs.

The estimates of the heritabilities for GY, PH and EH had been relatively high, reflecting the use of averages of lines and the great amount of replications and environments tested, reducing the variance components of genotype-by-environment interaction and error. Moreover, low heritabilities for GM can also be explained for the absence of genetic variability for the trait in that crossing. The observed differences among the trials for GM should have been caused mainly by experimental errors, providing strong influence in the estimate the variability and the accuracy in the detention of QTLs.

The parental lines L-08-05F and L-14-4B have contrasting behavior for plant height, kernel type, maturity and yield. L-14-4B is the higher, with more delayed cycle and bigger production line that L-08-05F. This contrast was demonstrated in the results, L-14-4B contributed alleles that increased the trait value at 9 of the 13 QTL for GY, PH and EH (Table 2). Gene action for all QTL for the traits evaluated was mainly dominance effects.

Of these QTL, 31% exhibited partial dominance, 15% dominance, 23% overdominance, and the remainder 31% exhibited additive effects (Table 2). Quantitative PH and EH is generally highly heritable and additive gene action or partial dominance prevailed for QTL affecting these traits depending on the materials and experimental conditions employed (Veldboom *et al.* 1994, Ragot *et al.* 1995, Austin and Lee 1996, Khairallah *et al.* 1998). Gene action for EH is in agreement with former studies, but overdominant gene action for PH was surprising. This result is due mainly to a QTL (*Ph1a*) which had dominance effects several times larger than the corresponding additive effects (Table 2). This kind of gene action may reflect the effects of several QTLs within the genomic interval under study, a situation that would tend to result in overestimation of dominance.

In diverse studies for the mapping of QTLs associates to the yield and yield components, individual QTLs had explained great percentage of the phenotypic variation, with a range between 25% to 35% (Ragot *et al.* 1995, Stuber *et al.* 1992, Ajmone-Marsan *et al.* 1995, Veldboom and Lee 1994, Beavis *et al.* 1994). QTLs with sharp effect had not been detected in our studies, possibly because we are mapping the QTLs most steady for the average of environments. For all the characters, the majority of the QTLs detected possess small effect, explaining less than 10 % of the phenotypic variation. Only a QTL for GY possess bigger effect, explaining 11.18% of the variation. In the total, the ratio of the phenotypic variation explained by all the QTLs detected for each character was of 32.73% for GY, 24.76% for PH and 20.91% for EH. The phenotypic variation not explained by the QTLs detected in this population can be due for QTLs in regions not mapped in the genome, QTLs with small effect not detected or epistatic effect between QTLs.

Genomic regions significantly involved with the evaluated features had been detected in five of the ten chromosomes of the maize. An important consideration in the detention of QTLs is if the location and the estimated effect of a QTL detected in a population can be observed in other populations (Lee 1995). The direct matching of results of mapping of QTLs between different studies becomes difficult, for the differences in methodology, in the size and the type of the mapping populations, for the lack of marker loci in common and for different tested environments (Lee 1995, Beavis 1994). Moreover, in this work was used germplasm tropical, while that the majority of the other works in maize used crossings between temperate material. Considering all these limitations, little QTLs had been detected next to mapped regions already in other works. For PH and EH, the QTLs mapped more close had been located for Veldboom *et al.* (1994) on chromosomes 1 (*Eh1-Ph1b*), 2 (*Ph2*) and 7 (*Eh7c-Ph7*).

The distribution of the QTLs for the genoma showed one high concentration of QTLs in few chromosomic regions. Such concentration in the distribution of QTLs already had been observed in previous studies (Abler *et al.* 1991, Stuber *et al.* 1992, Veldboom and Lee 1994, Austin and Lee 1998). PH and EH possess two genomic regions in common on chromosomes 1 and 7 (Figure 2). These morphologic characters also mapped in regions very close to QTLs for GY in chromosome 2 (*Gy2-Ph2*) and 7 (*Ph7-Eh7c-Gy7*) demonstrating that QTL for correlated traits were commonly associated within the same genomic regions (Figure 2). According to Aastveit and Aastveit (1993), there are three primary causes of correlation among traits: pleiotropy, linkage, and environmental effects. Because the QTLs in this population were identified in the mean environment, environmental effects would be improbable. If the same QTL controls more than a different

character or if QTLs, each one specific one for determined character, is narrowly on, only future studies, using more robust statistical analyses, will allow distinguishing.

The mapping of QTLs in the mean environment, allowed the identification of QTLs with effect on the production of grains, height of the plant and height of the ear in of tropical maize germplasm. The QTLs detected consistently had been identified on environments favoring its use in future programs for marker-assisted selection (MAS). However, new methods of analysis will be necessary to magnify sensitivity in the mapping of QTLs, incorporating in the model, more precise tests for the detention of pleiotropy and linkage, or the quantification of epistatic effects. Thus, the study of the quantitative characters will go to provide safe tools to answer basic questions considering the genetic base of these characters in the maize in general, and in tropical germplasm in particular.

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Figure 1. Genetic map of tropical maize showing the locations of 117 microsatellite loci based on 400 F_2 individuals from a cross between L-08-05F e L-14-4B. Loci names are to the right of each chromosome with the corresponding maize bin. Map distances between adjacent markers, on the left side of the chromosomes, are in centiMorgans (cM) calculated using the Kosambi function. Dark circles indicate estimated centromere positions, obtained from Coe et al. (1995). Underlined marker loci mapped to different position than those reported in literature. Map length is 1634 cM and average interval length is 14 cM. Linkage analysis was carried out using MAPMAKER/EXP v.3.0 program. All markers are linked to the map with LOD scores of 3.0 or greater. Total centiMorgan length for each chromosome is indicated below the chromosome number.

Figure 2. Genetic map of chromosomes 1, 2, 7, 8 and 9 in a population of tropical maize showing the location of the 13 QTLs associates with grain yield (GY), plant height (PH), ear height (EH). The vertex of each triangle points with respect to the chromosomic position with bigger value of the LR test for analyses using CIM. The size of each triangle is proportional to the values of the explained phenotypic variation (R^2) for each QTL. It is only indicated the names of the locos that flanking the interval where the QTL was detected. Triangles to the right of each chromosome indicate contribution of alleles of the parental L-08-05F. Triangles to the left of each chromosome indicate favorable alleles of the parental L-14-4B.

Table 1. Estimates of means, variance components and heritabilities of the $F_{2:3}$ lines for grain yield (GY), plant height (PH), ear height (EH) and grain moisture (GM), taken from the corrected means of all trials.

Table 2. Chromosomic location, effects and types of individual gene action of QTLs for grain yield (GY), plant height (PH) and ear height (EH) taken from the corrected means of the $F_{2:3}$ lines evaluated in five environments.

Figure 1.

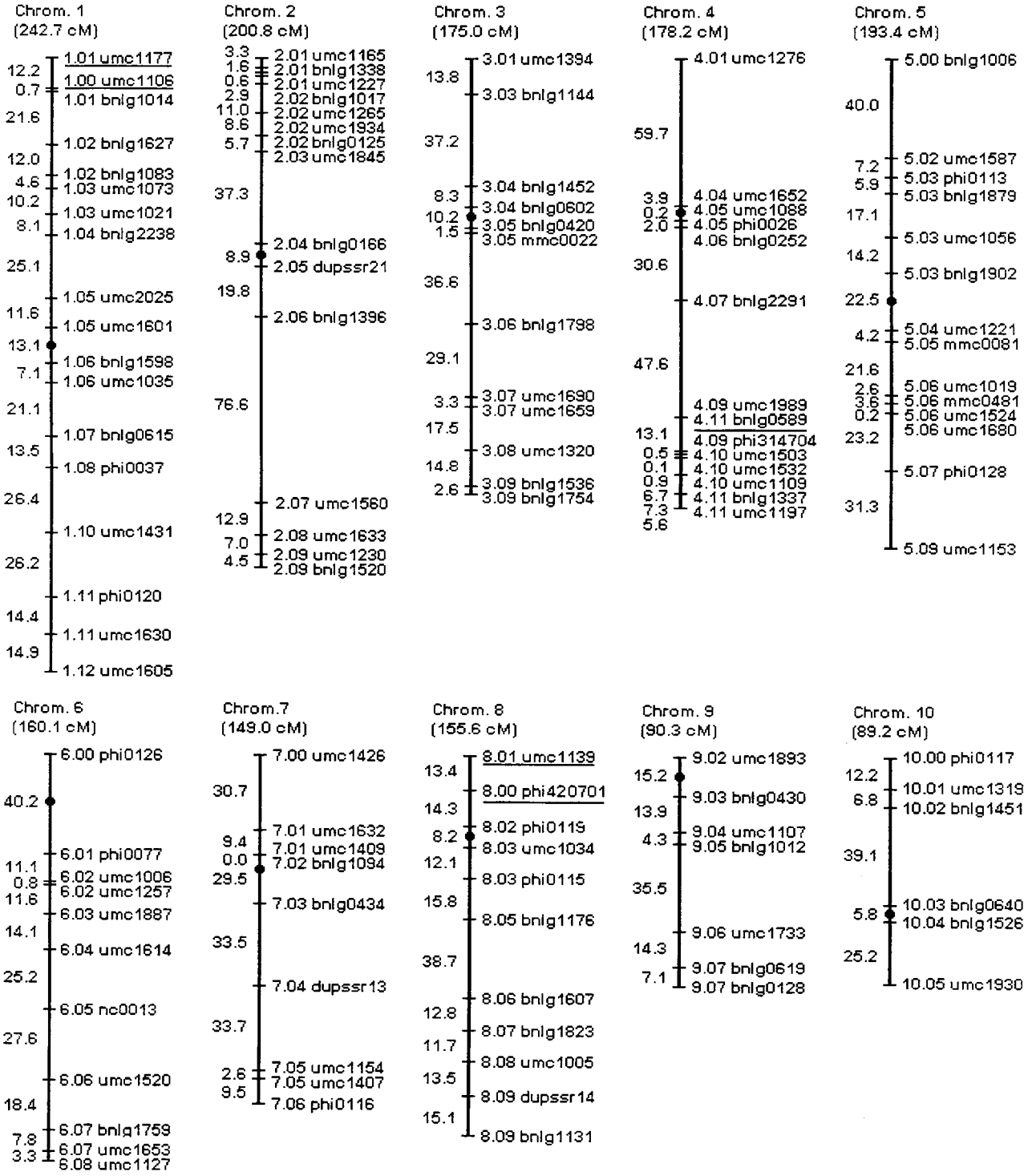
Chrom. 1
(242.7 cM)
1.01 umc1177
1.00 umc1106
1.01 bnlg1014
1.02 bnlg1627
1.02 bnlg1083
1.03 umc1073
1.03 umc1021
1.04 bnlg2238
1.05 umc2025
1.05 umc1601
1.06 bnlg1598
1.06 umc1035
1.07 bnlg0615
1.08 phi0037
1.10 umc1431
1.11 phi0120
1.11 umc1630
1.12 umc1605
Chrom. 2
(200.8 cM)
2.01 umc1165
2.01 bnlg1338
2.01 umc1227
2.02 bnlg1017
2.02 umc1265
2.02 umc1934
2.02 bnlg0125
2.03 umc1845
2.04 bnlg0166
2.05 dupssr21
2.06 bnlg1396
2.07 umc1560
2.08 umc1633
2.09 umc1230
2.09 bnlg1520
Chrom. 3
(175.0 cM)
3.01 umc1394
3.03 bnlg1144
3.04 bnlg1452
3.04 bnlg0602
3.05 bnlg0420
3.05 mmc0022
3.06 bnlg1798
3.07 umc1690
3.07 umc1659
3.08 umc1320
3.09 bnlg1536
3.09 bnlg1754
Chrom. 4
(178.2 cM)
4.01 umc1276
4.04 umc1652
4.05 umc1088
4.05 phi0026
4.06 bnlg0252
4.07 bnlg2291
4.09 umc1989
4.11 bnlg0589
4.09 phi314704
4.10 umc1503
4.10 umc1532
4.10 umc1109
4.11 bnlg1337
4.11 umc1197
Chrom. 5
(193.4 cM)
5.00 bnlg1006
5.02 umc1587
5.03 phi0113
5.03 bnlg1879
5.03 umc1056
5.03 bnlg1902
5.04 umc1221
5.05 mmc0081
5.06 umc1019
5.06 mmc0481
5.06 umc1524
5.06 umc1680
5.07 phi0128
5.09 umc1153
Chrom. 6
(160.1 cM)
6.00 phi0126
6.01 phi0077
6.02 umc1006
6.02 umc1257
6.03 umc1887
6.04 umc1614
6.05 nc0013
6.06 umc1520
6.07 bnlg1759
6.07 umc1653
6.08 umc1127
Chrom.7
(149.0 cM)
7.00 umc1426
7.01 umc1632
7.01 umc1409
7.02 bnlg1094
7.03 bnlg0434
7.04 dupssr13
7.05 umc1154
7.05 umc1407
7.06 phi0116
Chrom. 8
(155.6 cM)
8.01 umc1139
8.00 phi420701
8.02 phi0119
8.03 umc1034
8.03 phi0115
8.05 bnlg1176
8.06 bnlg1607
8.07 bnlg1823
8.08 umc1005
8.09 dupssr14
8.09 bnlg1131
Chrom. 9
(90.3 cM)
9.02 umc1893
9.03 bnlg0430
9.04 umc1107
9.05 bnlg1012
9.06 umc1733
9.07 bnlg0619
9.07 bnlg0128
Chrom. 10
(89.2 cM)
10.00 phi0117
10.01 umc1319
10.02 bnlg1451
10.03 bnlg0640
10.04 bnlg1526
10.05 umc1930

Figure 2.

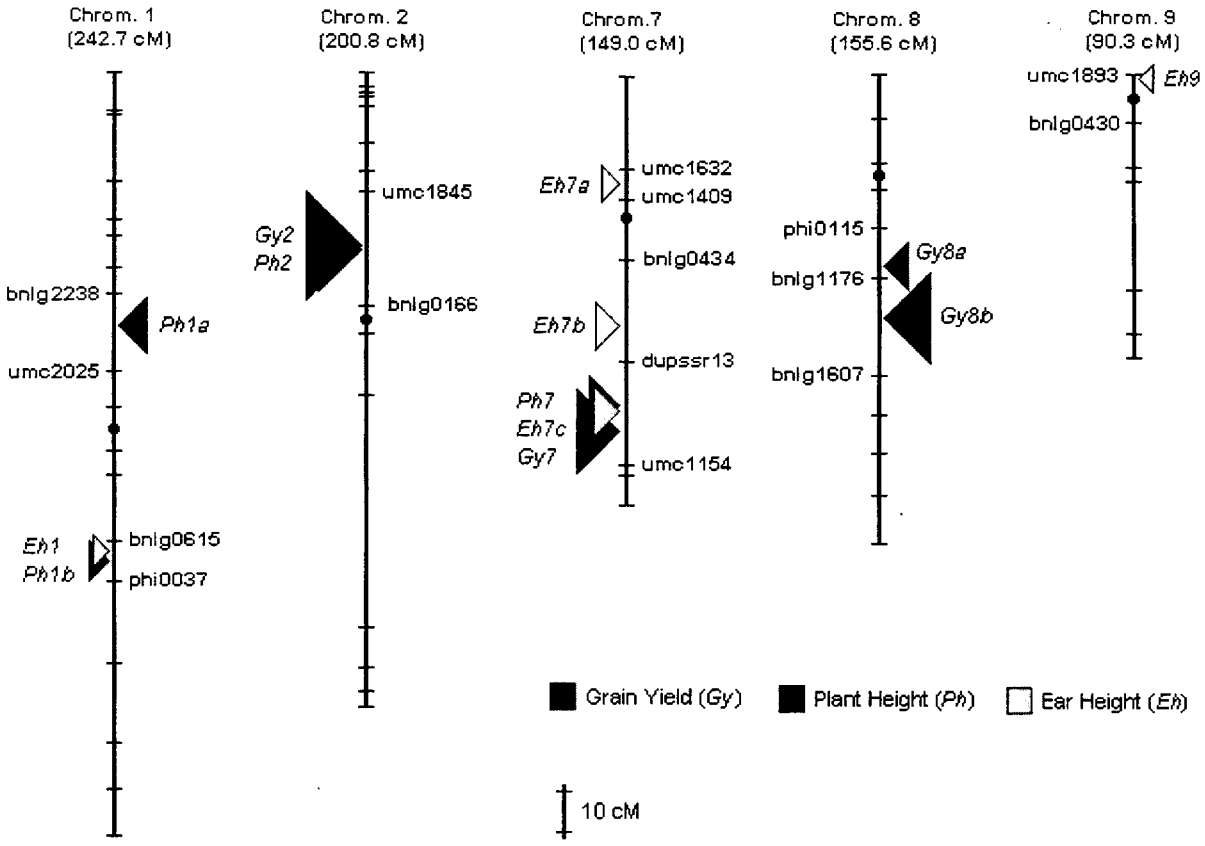
Chrom. 1
(242.7 cM)
Chrom. 2
(200.8 cM)
Chrom.7
(149.0 cM)
Chrom. 8
(155.6 cM)
Chrom. 9
(90.3 cM)
umc1893
Eh9
bnlg0430
umc1632
Eh7a
umc1409
bnlg0434
Eh7b
dupssr13
Ph7
Eh7c
Gy7
umc1154
phi0115
Gy8a
bnlg1176
Gy8b
bnlg1607
umc1845
Gy2
Ph2
bnlg0166
bnlg2238
Ph1a
umc2025
Eh1
Ph1b
bnlg0615
phi0037
Grain Yield (Gy)
Plant Height (Ph)
Ear Height (Eh)
10 cM

Table 1. Estimates of means, variance components and heritabilities of the $F_{2:3}$ lines for grain yield (GY), plant height (PH), ear height (EH) and grain moisture (GM), taken from the corrected means of all trials.

Parameters	Grain yield (g/plant)	Plant height (cm)	Ear height (cm)	Grain moisture (gH_2O/Kg)
Means [a] $F_{2:3}$ lines	57,69 ± 13,58	171,72 ± 9,84	85,60 ± 6,82	10,76 ± 0,70
Variances				
σ^2_g	82,29 [67,84; 105,69]	62,19 [50,77; 77,96]	27,52 [22,54; 34,35]	0,0103 [0,0096; 0,0110]
$\sigma^2_{g\,x\,e}$	93,09 [80,23; 109,32]	78,18 [68,39; 90,26]	29,78 [25,64; 35,01]	0,0442 [0,0437; 0,0448]
σ^2_e	184,39	160,00	73,05	0,25
C.V.(%)	23,54	7,37	9,98	4,65
Heritabilities[b] h^2 (90% I.C)	0,69 [0,63; 0,73]	0,66 [0,60; 0,71]	0,67 [0,621; 0,72]	0,23 [0,15; 0,28]

[a] With standard errors.
[b] Exact 90% confidence intervals of h^2 were calculated according to Knapp *et al.* (1985).

Table 2. Chromosomic location, effects and types of individual gene action of QTLs for grain yield (GY), plant height (PH) and ear height (EH) taken from the corrected means of the $F_{2:3}$ lines evaluated in five environments.

	QTL position				Genetic effect[b]			Gene action[c]		
QTL	Bin[a]	cM	Marker Interval	LR	R^2	a	d	|d|/|a|	Type	Direction
Grain yield										
Gy2	2.03-2.04	52.88	umc1845-bnlg0166	28.20	11.18	85.47	-96.76	1.13	D	L-14
Gy7	7.04-7.05	125.20	dupssr13-umc1154	26.38	8.35	80.39	61.39	0.76	DP	L-14
Gy8a	8.03-8.05	57.94	phi0115-bnlg1176	23.23	5.22	-77.62	-6.54	0.08	A	L-08
Gy8b	8.05-8.06	74.70	bnlg1176-bnlg1607	23.39	7.98	-91.46	29.30	0.32	DP	L-08
				Total[d]:	**32.73**		**Total[e]:**	**0.67**	**DP**	
Plant height										
Ph1a	1.04-1.05	79.38	bnlg2238-umc2025	17.55	5.87	-0.42	-5.08	12.08	SD	L-08
Ph1b	1.07-1.08	153.38	bnlg0615-phi0037	18.48	4.27	3.25	-0.54	0.17	A	L-14
Ph2	2.03-2.04	53.88	umc1845-bnlg0166	21.23	8.76	3.49	-4.29	1.23	SD	L-14
Ph7	7.04-7.05	117.20	dupssr13-umc1154	18.70	5.86	2.86	2.81	0.98	D	L-14
				Total:	**24.76**		**Total:**	**3.56**	**SD**	
Ear height										
Eh1	1.07-1.08	150.38	bnlg0615-phi0037	17.38	3.73	1.98	0.56	0.28	DP	L-14
Eh7a	7.01	35.70	umc1632-umc1409	16.95	3.66	1.69	-1.11	0.66	DP	L-14
Eh7b	7.03-7.04	91.70	bnlg0434-dupssr13	17.05	4.82	2.04	-0.41	0.20	A	L-14
Eh7c	7.04-7.05	119.20	dupssr13-umc1154	19.47	5.61	2.21	0.44	0.20	A	L-14
Eh9	9.02-9.03	1.01	umc1893-bnlg0430	16.11	3.09	-0.84	-2.42	2.89	SD	L-08
				Total:	**20.91**		**Total:**	**0.69**	**DP**	

[a] Bin locations are designated by an X.Y code, where X is the linkage group containing the bin and Y is the location of the bin within the linkage group (Gardiner *et al.* 1993).

[b] Additive (a): effect of the substitution of L-08-05F allele by L-14-4B allele. A negative value indicates that L-08-05F allele diminishes the value of the trait. Dominance (d): effect of L-08-05F allele on L-14-4B allele. Positive values indicates that the heterozigous possess upper phenotypic values than the average of the two homozigous. Negative values indicates that the heterozigous possess inferior phenotypic values than the average of the two homozigous.

[c] Gene action was determined on the basis of the average level of dominance calculated as the ratio $|d|/|a|$ by using the criteria of Stuber *et al.* (1987): additive (A) = 0 to 0.20; partial dominance (PD) = 0.21 to 0.80; dominance (D) = 0.81 to 1.20; and overdominance (OD) > 1.20.

[d] Total proportion of phenotypic variance explained were obtained by fitting a model including all QTL for the respective trait simultaneously, according to the procedures described by Bohn *et al.* (1996).

[e] Total gene action of all QTL detected for the trait: weighed mean between the ratio |d|/|a| and the phenotypic variance explained by each QTL (R^2).

5. CONCLUSÕES

- A utilização das sequências de "primers" para a amplificação de microssatélites disponíveis no MaizeDB e a comparação entre mapas genéticos já desenvolvidos confirmou a alta reprodutibilidade deste marcador entre laboratórios, e a manutenção na ordem dos locos no genoma do milho em diferentes germoplasmas.

- A utilização de géis de agarose de alta resolução na seleção de microssatélites polimórficos e na genotipagem da população de mapeamento demostrou ser um sistema barato e efeciente para a resolução de produtos da amplificação de microssatélites, tornando-se importante opção para análises envolvendo grande número de amostras.

- O mapa genético desenvolvido com uma população de mapeamento formada por 400 plantas F_2 apresentou 117 locos microssatélites, possuindo 1634 cM de extensão e 14 cM de intervalo médio entre marcas adjacentes. Também foram identificados quatro locos envolvidos com desvios de segregação e um loco com alta frequência de recombinação no germoplasma de milho tropical em estudo.

- Foram detectados 13 QTLs: quatro para produção de grãos, quatro para altura de planta e cinco para altura de espiga, distribuídos entre os cromossomos 1, 2, 7, 8 e 9. Nenhum QTL foi detectado para teor de umidade. Os QTLs foram consistentemente identificados sobre ambientes favorecendo seu uso em futuros programas de seleção assistida por marcadores.

- QTLs para altura da planta e altura da espiga foram localizados nas proximidades de locos com efeitos quantitativos para produção de grãos, indicando possíveis efeitos pleiotrópicos ou de ligação entre esses locos.

6. PERSPECTIVAS

Os 13 QTLs detectados por CIM utilizando a média dos cinco ambientes e os limites estabelecidos pelas permutações, representam as localizações mais seguras sobre locos de características quantitativas nas linhagens tropicais em estudo. Esta segurança no mapeamento será muito importante para futuras comparações entre QTLs detectados em trabalhos envolvendo as mesmas características, utilizando as mesmas linhagens parentais, mas com dados de plantas F_2 e Retrocruzamentos.

Porém, o número de QTLs não é necessariamente um bom indicador do número total de locos explicando determinado caráter, dado que QTLs com pequenos efeitos ou ligados em repulsão geralmente permanecem não detectados. Além disso, genes não atuam independentemente ou somente por ação aditiva para produzir um fenótipo. Dados recentes sugerem que fenótipos complexos são determinados pela sobreposição e interação de inúmeras rotas bioquímicas.

No momento, a maioria dos métodos biométricos para o mapeamento de QTLs ainda dificulta o estudo da magnitude de interações não aditivas. Nestes modelos, a variação atribuída para um par de locos é a soma da variação explicada por cada loco individualmente. Porém, o efeito de um alelo pode variar dependendo das interações com outros locos e com o ambiente. Assim, o passo seguinte à detecção de QTLs por CIM, seria a utilização de modelos mais robustos através de métodos de análise múltipla para aumentar a precisão do mapeamento e, principalmente, melhor estimar os efeitos das interações entre alelos como pleiotropia, ligação, epistasia e interação com o ambiente.

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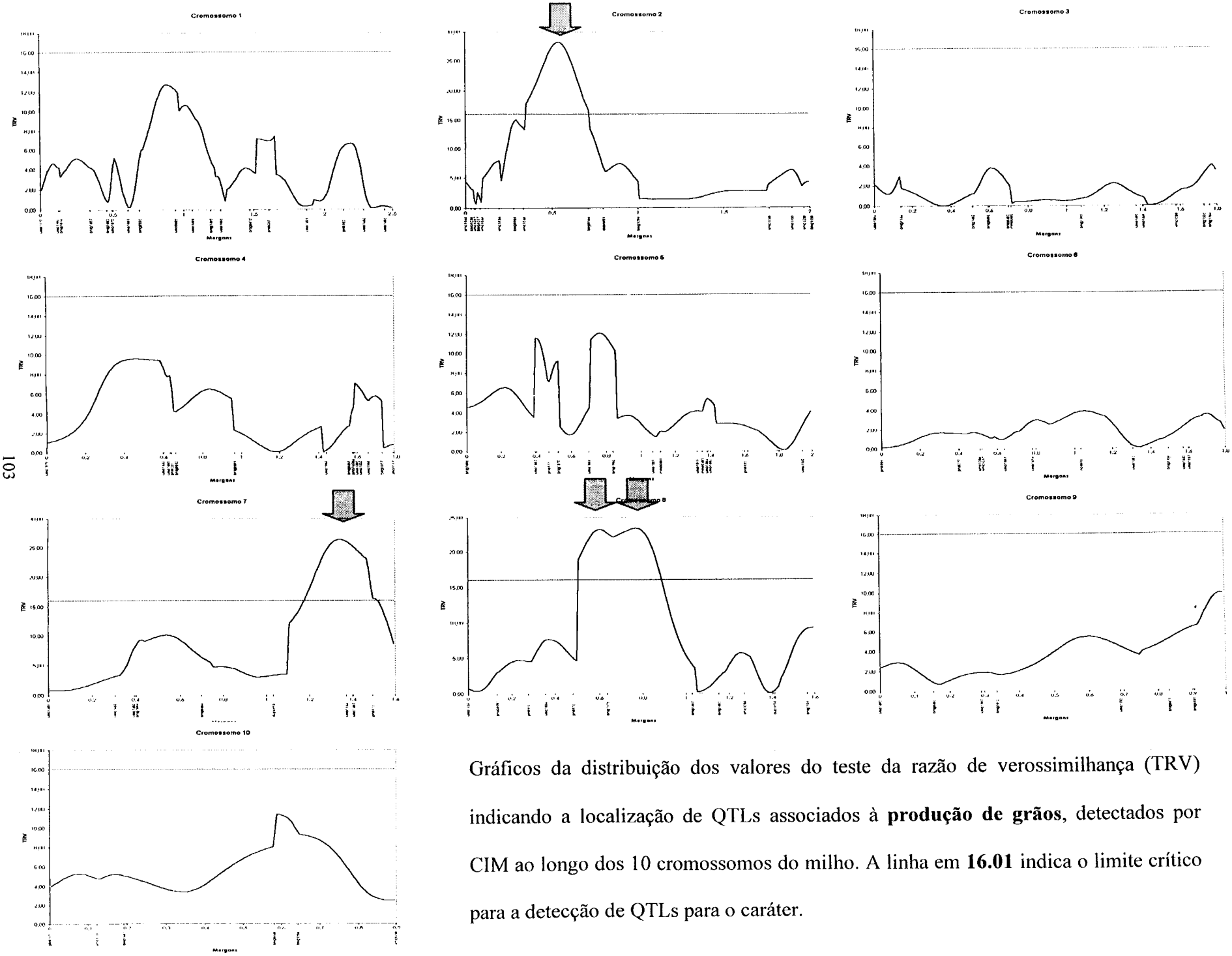
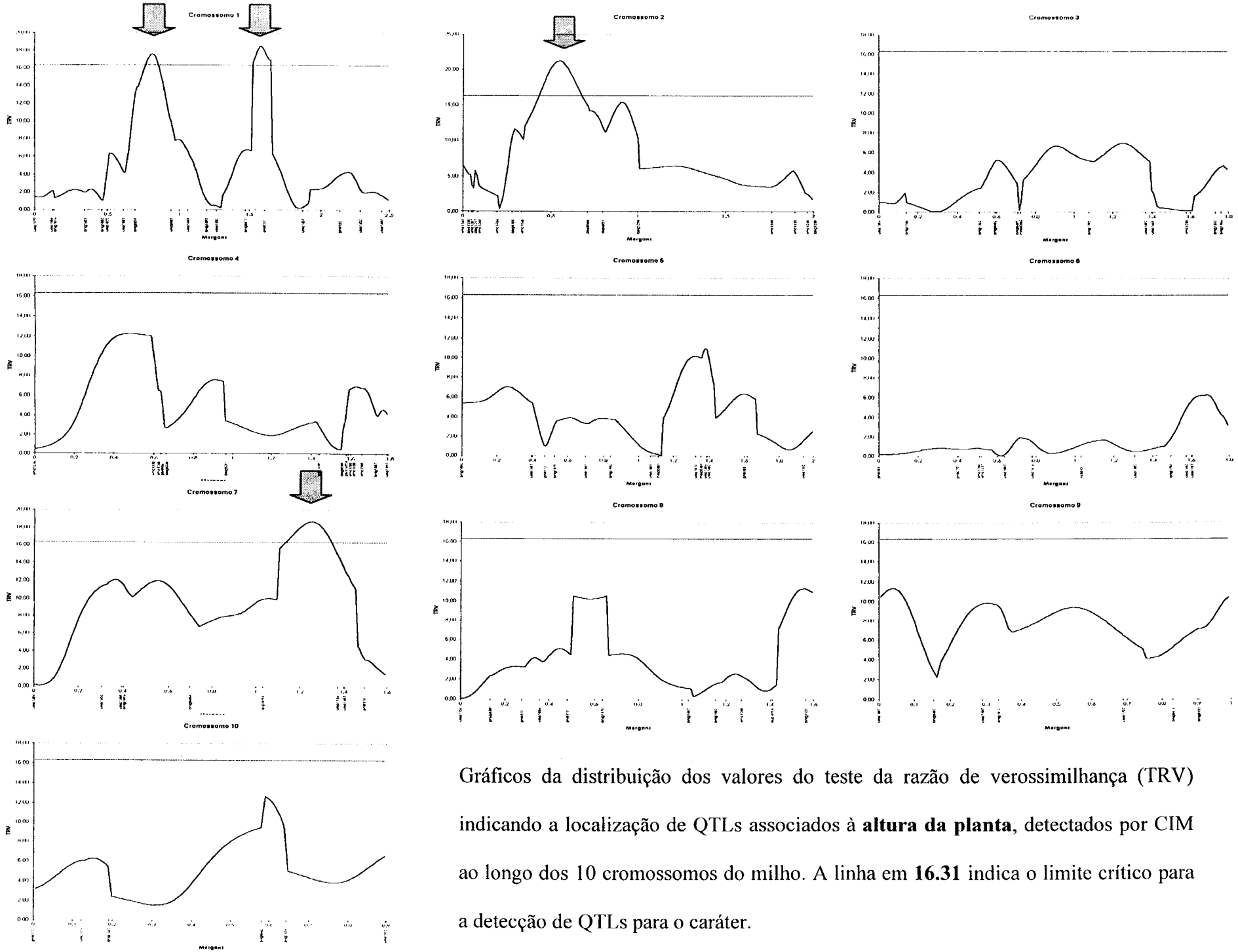
ANEXO I

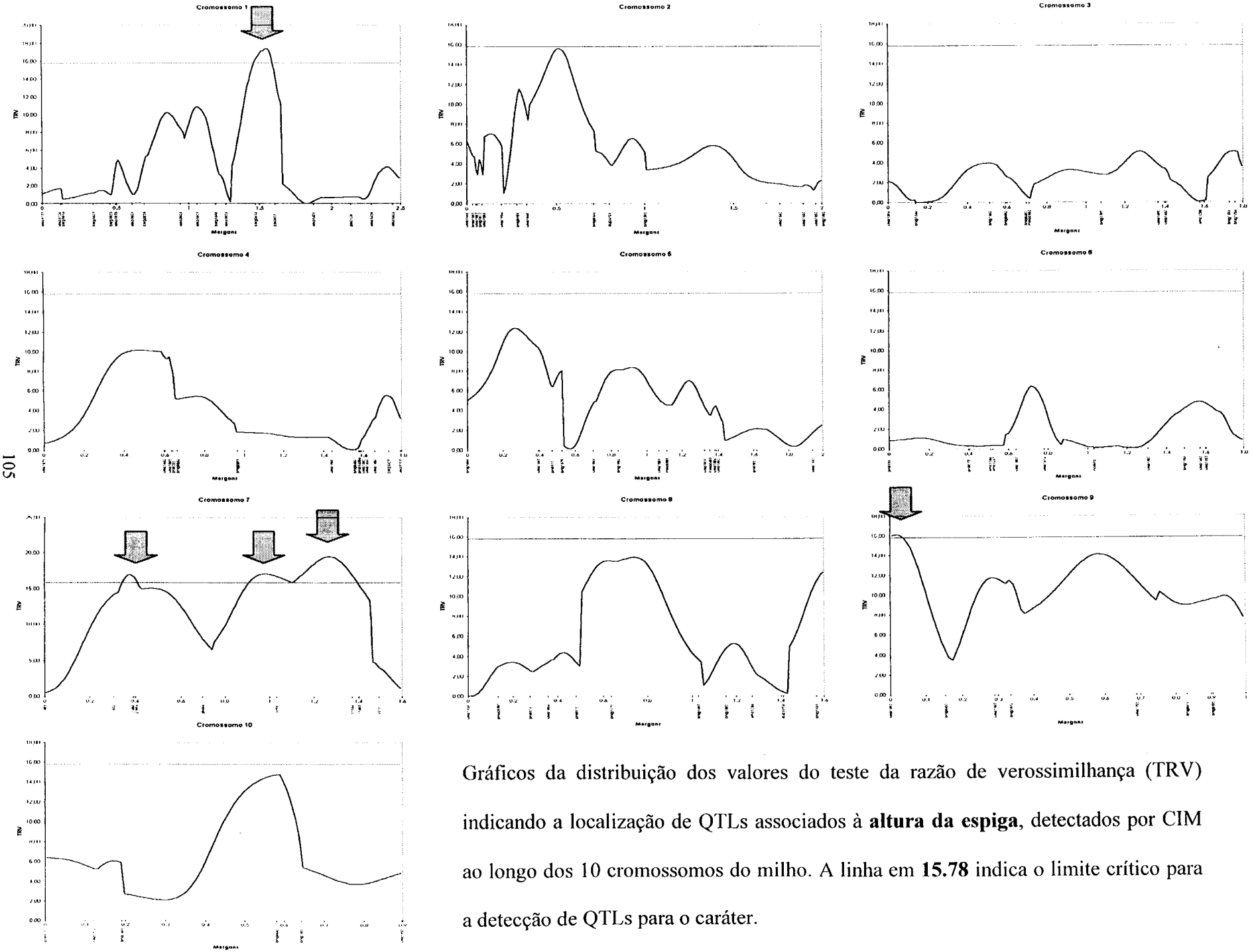
Gráficos da distribuição dos valores do teste da razão de verossimilhança (TRV) indicando a localização de QTLs para produção de grãos, altura da planta, altura da espiga e teor de umidade.



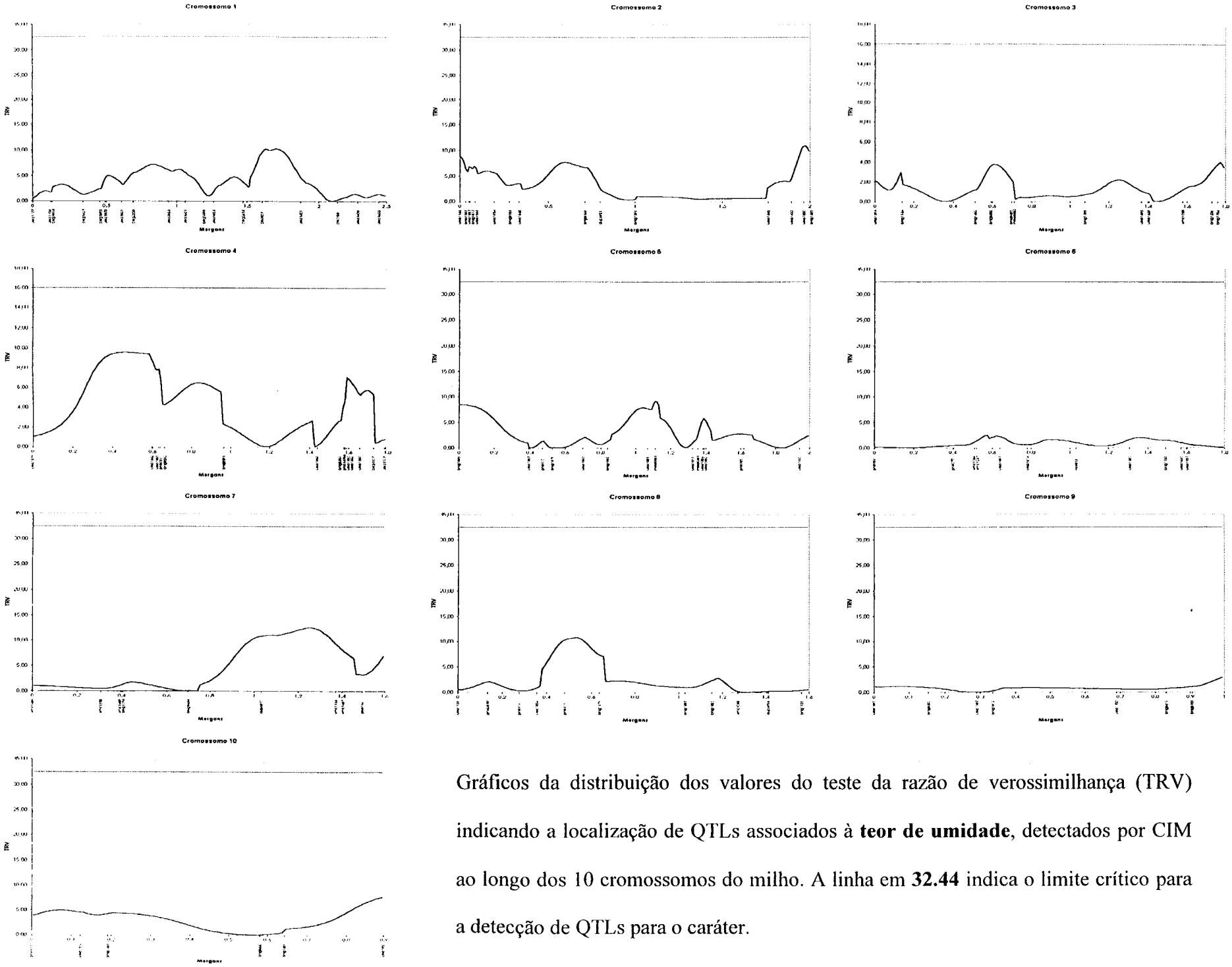
Gráficos da distribuição dos valores do teste da razão de verossimilhança (TRV) indicando a localização de QTLs associados à **produção de grãos**, detectados por CIM ao longo dos 10 cromossomos do milho. A linha em **16.01** indica o limite crítico para a detecção de QTLs para o caráter.



Gráficos da distribuição dos valores do teste da razão de verossimilhança (TRV) indicando a localização de QTLs associados à **altura da planta**, detectados por CIM ao longo dos 10 cromossomos do milho. A linha em **16.31** indica o limite crítico para a detecção de QTLs para o caráter.



Gráficos da distribuição dos valores do teste da razão de verossimilhança (TRV) indicando a localização de QTLs associados à **altura da espiga**, detectados por CIM ao longo dos 10 cromossomos do milho. A linha em **15.78** indica o limite crítico para a detecção de QTLs para o caráter.



Gráficos da distribuição dos valores do teste da razão de verossimilhança (TRV) indicando a localização de QTLs associados à **teor de umidade**, detectados por CIM ao longo dos 10 cromossomos do milho. A linha em **32.44** indica o limite crítico para a detecção de QTLs para o caráter.