

 **UNIVERSIDADE FEDERAL DE UBERLÂNDIA**
INSTITUTO DE GENÉTICA E BIOQUÍMICA
PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

MAPEAMENTO E VALIDAÇÃO DE LOCI DE CARACTERES QUANTITATIVOS
(QTL) PARA RESISTÊNCIA A *Cercospora zeae-maydis* EM MILHO TROPICAL
(*Zea mays* L.)

ALUNO: Gilberto Pozar

Orientador: Prof. Dr. Julio Cesar Viglioni Penna

Co-Orientador: Dr. Heyder Diniz Silva

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ÍNDICE

APRESENTAÇÃO	1
CAPÍTULO 1	3
1. FUNDAMENTAÇÃO TEÓRICA.....	3
1.1.Mapeamento de QTLs de resistencia a <i>C. zea-maydis</i> e sua interação com o ambiente	3
1.2.Base genética da resistencia a <i>C. zea-maydis</i>	7
1.3.Influência do patógeno na interação QTL x ambiente	8
1.4.Validação dos QTLs de resistência por meio do uso de Linhagens Quase Isogênicas.....	10
1.5.Referencias bibliográficas.....	12
CAPÍTULO 2	16
2. MAPPING AND VALIDATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO <i>Cercospora zea-maydis</i> INFECTION IN TROPICAL MAIZE.....	16
RESUMO	17
ABSTRACT	18
2.1.INTRODUCTION	19
2.2.MATERIALS & METHODS	21
2.2.1. QTL mapping	21
2.2.1.1. Generation and evaluation of the segregating population	21
2.2.1.2. <i>C. zea-maydis</i> Severety Evaluation	21
2.2.1.3. Genotyping of F _{2:3} progenies	22
2.2.1.4. Construction of linkage groups and QTL mapping	22
2.2.1.5. QTL x environment interaction	23
2.2.2. Production and evaluation of the NILs	23
2.2.2.1. Backcross program.	23
2.2.2.2. Evaluation of NILs	24
2.2.2.3. Statistical analysis.....	24
2.2.3. Production and evaluation of the NIHS.....	25

2.3. RESULTS	27
2.3.1. QTL mapping for <i>C. zea-maydis</i> resistance	27
2.3.1.1. Incidence of <i>C. zea-maydis</i>	27
2.3.1.2. Linkage groups	27
2.3.1.3. QTL mapping results	27
2.3.1.4. Epistatic Interactions	28
2.3.1.5. QTL x location interaction	28
2.3.2. Evaluation of the NILs	28
2.3.2.1. NIL genotypes	28
2.3.2.2. Estimate of individual and epistatic QTL effects	29
2.3.3. Evaluation of the NIHs	30
2.3.3.1. Effect of QTL on <i>C. zea-maydis</i> incidence	30
2.3.3.2. QTL effects on grain moisture	30
2.3.3.3. QTL effects on stalk lodging	30
2.3.3.4. QTL effects on grain yield	30
2.4. DISCUSSION	32
2.5. CONCLUSÕES	37
2.5.1. Mapeamento dos QTL de resistência a <i>C. zea-maydis</i>	37
2.5.2. Avaliação das Linhagens Quase Isogênicas (LQIs)	37
2.5.3. Avaliação dos Híbridos Quase Isogênicos (HQIs)	38
2.6. REFERENCES	40

2.7. TABELAS E FIGURAS	44
TABLE 1 – <i>Cercospora zeae-maydis</i> resistant QTL mapped by the Multiple Interval Mapping method, based on phenotypic evaluation of disease incidence using 1-to-9 score scale at four locations, under natural disease occurrence. 2001/02 growing season.....	44
TABLE 2 – ANOVA for the incidence of <i>C. zeae-maydis</i> based on the 1-to-9 scale, evaluated by Near Isogenic Lines. Iraí de Minas and Mineiros – 2002/2003 growing season.	45
TABLE 3 – Estimates of the effects of the QTL for <i>C. zeae-maydis</i> resistance based on the 1-to-9 scale, obtained by regression analysis through the evaluation of the Near Isogenic Lines. Iraí de Minas and Mineiros – 2002/2003 growing season.	46
TABLE 4 – ANOVA for the incidence of <i>C. zeae-maydis</i> based on the 1-to-9 scale, evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.	46
TABLE 5 – ANOVA for Grain Moisture at harvest evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.	47
TABLE 6 – Estimates of the effects of the QTL for <i>C. zeae-maydis</i> resistance on Grain Moisture, obtained by regression analysis by Near Isogenic Hybrids evaluation. Iraí de Minas and Mineiros – 2004/2005 growing season.....	47
TABLE 7 – ANOVA for stalk lodging evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.	48
TABLE 8 – ANOVA for grain yield corrected for 15.5% moisture, evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.	48
TABLE 9 – ANOVA for grain yield corrected for 15.5% moisture, evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.	49
TABLE 10 – Summary of QTL effects for <i>Cercospora zeae-maydis</i> severity evaluated on a 1-to-9 scale (Cz), Grain Moisture (GM), Stalk Lodging (SL) and Grain Yield (GY) evaluated in the NIHS in two locations and joint analysis over locations.....	50

FIGURE 1 - Esquema resumido mostrando passo a passo o mapeamento dos QTL de resistência a <i>C. zea-maydis</i> e o desenvolvimento das Linhagens e Híbridos Quase Isogênicos (LQIs e HQIs).....	51
FIGURE 2 – Single Marker Analysis Mapping Results showing the markers, Pr(F), distance (cM) and bin for the 10 chromosomes; and genotype of the two progenies selected for the backcrossing program Subject	52
FIGURE 3 – Near Isogenic Lines (NIL) genotype, genotype code, number of NIL evaluated per genotype and average percentage of recurrent parent recovered in the NIL	53
FIGURE 4.- Frequency distribution of <i>Cercospora zea-maydis</i> severity based on 1-9 scale, of 187 F _{2:3} progenies as average of four locations	54

APRESENTAÇÃO

O milho (*Zea mays L.*) foi cultivado em 13,6 milhões de hectares no Brasil na safra 2006/2007 (CONAB, 2007). A ampla exposição da cultura aos mais variados estresses edafo-climáticos e biológicos, torna improvável que o potencial genético máximo para produtividade de grãos seja atingido por qualquer cultivar híbrida, mesmo que altamente adaptada, qualquer que seja o sistema de produção adotado.

As últimas duas décadas foram marcadas por profundas modificações na tecnologia de produção utilizada no Brasil. Dentre elas, o aumento da área de safrinha e de plantio direto provocaram grande alteração na dinâmica de dispersão das doenças. Se por um lado a safrinha permite o aumento da renda dos agricultores pelo uso mais intensivo do solo e se tornou prática amplamente difundida e incorporada no sistema de produção de várias regiões, por outro tem favorecido o aumento da severidade de doenças que encontram condições favoráveis para se desenvolver durante o ano todo. O plantio direto, uma importante prática de conservação do solo e largamente difundida no país, pode também favorecer o aumento do potencial de inoculo dos patógenos que sobrevivem nos restos da cultura anterior deixados sobre a superfície do solo.

Praticamente todos os programas de melhoramento genético do milho objetivam o aumento da produtividade e estabilidade da produção de grãos nos vários ambientes de cultivo. O progresso genético histórico obtido pelo melhoramento do milho em vários países, foi função principalmente do aumento do nível de resistência ou tolerância tanto aos estresses bióticos como abióticos, relacionados com as mudanças nos sistemas de produção, principalmente, a maior resposta ao suprimento de nutrientes no solo e aumento na densidade de plantio. No Brasil, embora nenhum estudo tenha sido realizado nesse sentido, a maior causa da descontinuidade de híbridos comerciais no mercado, se deve ao desenvolvimento em nível epidêmico, de novas doenças ou de raças fisiológicas de doenças pré-existentes, antes consideradas sem importância econômica, ocasionadas principalmente pelo cultivo de híbridos suscetíveis e às citadas mudanças nos sistemas de produção. Tal foi o caso das doenças causadas por *Bipolaris maydis* raça T no início dos anos 70, da *Phaeospharia maydis* na década

de 80 e do complexo de enfezamento do milho (“corn stunt” e “maize bushy stunt”) na década de 90.

A partir de 2000, a cercosporiose do milho, causada pelo fungo *Cercospora zeae-maydis* (Cz), de ocorrência endêmica na forma de pequenas e esparsas lesões foliares, começou a assumir proporções epidêmicas em várias regiões do país. Esta doença foi responsável pela descontinuidade de vários híbridos comerciais suscetíveis de alto potencial produtivo, que chegaram a atingir níveis de até 60% de redução na produtividade de grãos. Hoje, a incorporação de resistência genética a Cz está entre os principais objetivos dos programas de desenvolvimento de híbridos para as regiões onde essa doença é prevalente.

O mapeamento de Loci de Caracteres Quantitativos (ou em inglês Quantitative Trait Loci – QTL) possibilita o rápido desenvolvimento de linhagens e híbridos resistentes via seleção assistida por marcadores moleculares. A estimação da magnitude dos seus efeitos individuais e de possíveis interações epistáticas entre eles é importante na definição de estratégias que maximizem o ganho genético e a utilização dos recursos disponíveis para o programa de melhoramento. A presente pesquisa teve por objetivos: 1) o mapeamento de QTL associados com a resistência à Cz em germoplasma tropical, 2) a validação desses QTL pela utilização de Linhagens Quase Isogênicas (LQI), e 3) a estimação dos seus efeitos sobre três características agronômicas importantes, produtividade de grãos, porcentagem de plantas quebradas e porcentagem de umidade dos grãos na colheita.

CAPÍTULO 1

1. FUNDAMENTAÇÃO TEÓRICA

1.1. Mapeamento de QTL de resistência a *Cercospora zeae-maydis* e sua interação com o ambiente

A maior parte das pesquisas de mapeamento de QTL de resistência a Cz foram conduzidas em ambiente de clima temperado (EUA – Estados Unidos da América) ou sub-tropical (RAS – República da África do Sul) (Bubeck et al, 1993; Saghai-Marooft et al, 1996; Clements et al, 2000; Gordon et al, 2004; Lehmensiek et al, 2001). Apenas a pesquisa de Pedrosa (2002) foi conduzida em ambiente tropical (Brasil).

No decorrer dessa pesquisa, para a caracterização dos QTL adotou-se como medida da magnitude do seu efeito, a proporção da VFT explicada por ele, a qual corresponde aos valores de R^2 . Assim, QTL de *grande efeito* (“major QTL”) são aqueles cujo efeito explica mais de 10% da VFT e os de *pequeno efeito* (“minor QTL”), menos de 10%. Outra nomenclatura usada para descrever o QTL é a que se refere à sua consistência ou estabilidade através de ambientes. QTL *consistentes* normalmente são os de grande efeito mapeados em todas ou na maioria dos ambientes de teste. Os *inconsistentes* normalmente são os de pequeno efeito e são ambientalmente sensíveis (Collard et al, 2005).

QTL de resistência a Cz foram mapeados nos 10 cromossomos. O estabelecimento de consenso entre os resultados de diferentes pesquisas tem por objetivo a validação dos QTL visando a sua utilização em programas de melhoramento monitorados por marcadores. Nesse sentido, os cromossomos 1 e 4 parecem ser os que mais se aproximam de um consenso na determinação da resistência a Cz, pelo menos em regiões temperadas dos EUA e sub-tropical da RAS. Três pesquisas (Saghai-Marooft et al, 1996; Clements et al, 2000; Lehmensiek et al, 2001) mapearam QTL consistentes no cromossomo 1 localizadas nos bins 1.05 e 1.06, que explicaram entre 20% e 56% da VFT. Pela proximidade dos marcadores envolvidos, Clements & White (2000) inferiram a possibilidade de se tratar do mesmo QTL, e portanto comum às linhagens 061,

Va14, NC250A e ADENT. Bubeck et al (1993) mapeou um QTL em uma região próxima desta no bin 1.03/1.04, mas que não foi consistente através dos ambientes. Um QTL consistente através de três ambientes tropicais também foi mapeado nesse cromossomo (Pedrosa, 2002), mas não se pode inferir sobre a sua localização já que os marcadores utilizados não foram apresentados. Gordon et al (2004) não detectaram QTL nessas regiões em nenhum dos dois ambientes de avaliação, EUA e RAS.

No cromossomo 4, Saghai-Maroofo et al (1996) e Gordon et al (2004) mapearam um QTL consistente nos bins 4.05 e 4.08, que representaram 9 a 14% e 20 a 24% da VFT, respectivamente. Bubeck et al (1993) também detectou esse QTL no bin entre 4.05 e 4.07, que embora tenha sido considerado inconsistente, apresentou efeito bastante pronunciado em dois dos três ambientes de avaliação, e explicou até 20% da VFT. As pesquisas de Clements et al (2000) Lehmsiek et al (2001) e Pedrosa (2002) não mapearam QTL nessas posições.

No cromossomo 2, Bubeck et al (1993) e Clements et al (2000) mapearam QTL bem próximos, nos bins 2.04 e 2.06, que explicaram 9% e 14% da VFT, respectivamente. Gordon et al (2004) também mapeou um QTL nesse cromossomo entre os bins 2.08 e 2.09, com efeito, bastante pronunciado, que explicou de 20 a 23% da VFT. Esses dados sugerem que as linhagens 061, NC250A e ADENT compartilham essa região genômica (Clements e White, 2000). Um QTL no braço curto do cromossomo 2 também foi mapeado por Saghai-Maroofo et al (1996), mas não foi consistente através dos ambientes. Assim, Va14 parece não compartilhar a mesma região genômica do cromossomo 2 das outras linhagens (Clements e White, 2000). Nas pesquisas de Lehmsiek et al (2001) e Pedrosa (2002), essas regiões não foram associadas com QTL.

Dois QTL consistentes foram mapeados no cromossomo 5 nos bins 5.03/5.04 e 5.05/5.06 por Clements et al (2000) e Lehmsiek et al (2001). Cada um desses QTL respondeu por 10% da VFT, embora os QTL mapeados nessas posições por Bubeck et al (1993) e Saghai-Maroofo et al (1996) tenham se mostrado inconsistentes. Esse último autor o considerou como um falso positivo por não ter se repetido nem mesmo no mesmo local em diferentes anos, embora Clements e White (2000) argumentem que a similaridade da região genômica da linhagem 061 com as identificadas na Va14, NC250A e ADENT, indiquem que a

sua significância no cromossomo 5 provavelmente não é o resultado de um falso positivo. Pedrosa (2002) também mapeou um QTL inconsistente no cromossomo 5, mas não se pode inferir quanto a sua localização.

No cromossomo 7, Clements et al (2000) mapeou um QTL consistente no bin 7.01, que respondeu por 5 a 11% da VFT. Bubeck et al (1993) o detectou na população com a linhagem NC250A, mas não com a ADENT. Clements e White (2000) argumentam que as linhagens 061 e NC250A provavelmente apresentam uma região comum no cromossomo 7, compartilhando genes de resistência que são diferentes dos identificados nas linhagens Va14 e ADENT. Nenhuma outra pesquisa detectou associações significativas nesse cromossomo.

No cromossomo 8, Saghai-Maroofo et al (1996) mapearam um QTL consistente localizado no bin 8.06 que contribuiu com 8 a 11% da VFT. Esse mesmo QTL também foi identificado na posição bin 8.05 por Bubeck et al (1993) e Clements et al (2000), mas se mostrou inconsistente nessas pesquisas. Esse QTL foi responsável pela resposta tardia à infecção observada por Clements et al (2000).

Outros QTL inconsistentes foram mapeados nos cromossomos 3 (Bubeck et al, 1993, Clements et al, 2000, e Lehmensiek et al, 2001) nos bins entre 3.04/3.05 e 3.07, e nos cromossomos 6 (Bubeck et al, 1993 e Clements et al, 2000), 9 e 10 (Bubeck et al, 1993) nos bins 6.05/6.08, 9.01 e 10.06/10.07, respectivamente.

A análise da origem genética das fontes de resistência utilizadas nessas pesquisas, explicam as conclusões de Clements & White (2000) com respeito ao compartilhamento de diversas regiões cromossômicas contendo os QTL de resistência nas populações usadas para mapeamento. Quase todas utilizaram QTL de resistência originados de germoplasma tropical. Bubeck et al (1993) utilizaram ADENT que é composta de 75% de Amarillo Dentado e NC250A por 25% de Composto Nigéria A-Rb. A linhagem 061 utilizada por Clements et al (2000) é um germoplasma tropical originado do Brasil. A linhagem VO613Y utilizada por Gordon et al (2004) inclui germoplasma da RAS e teosinte. Embora a pesquisa conduzida por Pedrosa (2002) não cite a origem do germoplasma

utilizado, é plausível assumir que se trata também de germoplasma tropical ou com alta percentagem de germoplasma tropical.

Wisser et al (2006) consolidaram o mapa de consenso para diversas doenças de milho baseados em pesquisas publicadas até julho de 2005. No caso específico de Cz, os resultados foram baseados nas pesquisas de Lehmensiek et al (2001), Saghai-Maroofo et al (1996), Bubeck et al (1996), Clements et al (2000) e Gordon et al (2004). Baseados em várias premissas entre as quais a de que todos os QTL declarados nas diversas pesquisas foram considerados sem levar em conta a magnitude dos seus efeitos (proporção da VFT explicada), e de que foram construídos intervalos de confiança de 95% baseados no marcador molecular de maior significância determinaram a ocorrência de QTL nos segmentos entre os bin 1.02 a 1.08, 2.01 a 2.09, 3.04 a 3.09, 4.03 a 4.09, 5.03 a 5.06, 6.01 a 6.05; 7.01 a 7.05, 8.01 a 8.06, 9.01 e 9.03, e 10.03 a 10.06. Considerando que os cromossomos de 1 a 10 tem 12, 10, 10, 11, 9, 8, 6, 9, 8 e 7 bins, respectivamente, e que cada bin mede em média 20 cM, estima-se que essa amplitude representa em média 62,4% do genoma. Para os autores, a larga distribuição dos QTL dentro do genoma pode refletir tanto a baixa precisão e exatidão no mapeamento, como o grande número de genes envolvidos na interação patógeno x hospedeiro, uma vez que genes de resistência podem ser condicionados por genes que afetam o crescimento e desenvolvimento da planta.

1.2. Base genética da resistência a *C. zeae-maydis*

Todas as pesquisas relacionadas com a determinação do tipo de ação gênica envolvida na resistência a Cz indicam a predominância da ação gênica aditiva (Thompson et al, 1987, Elwinger et al, 1990, Ulrich et al, 1990, Donahue et al, 1991, Gevers & Lake, 1994, Coates & White, 1998, Verma, 2001, Menkir & Ayodele, 2005). Efeitos dominantes significativos também foram descritos por Elwinger et al (1990), Gevers & Lake (1994), Coates & White (1998), Verma (2001), Menkir & Ayodele (2005). As pesquisas realizadas com mapeamento de QTL para resistência corroboram em grande parte esses resultados. Efeito gênico aditivo foram relacionados com QTL consistentes mapeados por Bubeck et al

(1993), Clements et al (2000) e Lehmensiek et al (2001), para os QTL mapeados nos cromossomo 1 e 2 por Shagai Maroof et al (1996) e no cromossomo 4 por Gordon et al (2004). Ação gênica recessiva foi observada por Shagai Maroof et al (1996) para o QTL mapeado no cromossomo 8 e para o do cromossomo 4 mapeado por Gordon et al (2004). Ação gênica dominante foi observada por Shagai Maroof et al (1996) para o alelo do QTL mapeado no cromossomo 4 originado do parental suscetível B73. Esse QTL é o mesmo mapeado por Gordon et al (2004) ocupando exatamente a mesma posição no cromossomo, bin 4.08, com ação gênica dominante, porém nesse caso, se originou do parental resistente VO613Y. Em resumo, a ação gênica aditiva prevaleceu em todas as pesquisas realizadas.

Com relação a avaliação de efeitos epistáticos entre QTL, Clements et al (2000) detectaram epistasia entre os QTL mapeados no cromossomo 1 e 4. Shagai-Marooof et al (1996) verificaram que o QTL mapeado no cromossomo 4 da linhagem B73 tinha pouco ou nenhum efeito quando o QTL no cromossomo 1 era homozigoto para o alelo da linhagem Va14. Calborg & Haley (2004) discutem sobre a pouca ênfase dada pelas pesquisas em geral, na detecção de epistasia entre QTL. Segundo esses autores, o nível de estringência usado nas pesquisas de mapeamento de QTL para se evitar o erro tipo I, ou seja, evitar falsos positivos, é muito alto. Assim, somente QTL de grande efeito são considerados, aumentando-se o risco de que os locos individuais de menor efeito não sejam detectados, ou que efeito dos QTL mapeados seja severamente viesado.

1.3. Influência do patógeno na interação QTL x ambiente

Tanto nos EUA como no Brasil, ocorrem duas espécies de *Cercospora*: *C. sorghi f. sp. maydis* e *Cz*. As duas espécies normalmente são isoladas das lesões de hospedeiros suscetíveis. A primeira espécie provoca apenas lesões pequenas circundadas por um halo clorótico, e por isso é considerada muito pouco eficiente na colonização dos tecidos. A *Cz* por sua vez, é representada por dois grupos distintos (I e II) (Dunkle & Levy, 2000; Carson et al, 2002; Brunelli, 2004). A similaridade genética entre os dois grupos determinada por marcadores de AFLP (Amplified Fragment Length Polimorphism) foi de 40% nos EUA, com sintomas

idênticos produzidos pelos dois grupos (Dunkle e Carson, 1999), e de 35% no Brasil, avaliada pelo coeficiente de Dice (Brunelli, 2004). O grupo II se caracteriza por apresentar taxa de crescimento mais lenta e por não produzir cercosporina em meio de cultura quando comparada ao grupo I (Dunkle & Levy, 2000; Brunelli, 2004). Existe ainda predominância de um grupo sobre o outro de acordo com a geografia considerada. O grupo I é o mais prevalente nos EUA (Dunkle & Levy, 2000) e no Brasil (Brunelli, 2004). Comparações entre isolados dos EUA e África mostraram a ocorrência do grupo II naquele continente (Dunkle & Levy, 2000), e no Brasil esse grupo parece ser predominante no estado de Goiás (Brunelli, 2004).

Apesar das diferenças genéticas observadas entre os grupos I e II e da existência de uma amplitude de agressividade dentro de cada grupo, nenhum estudo encontrou diferenças que pudessem separá-los como duas raças fisiológicas diferentes, baseados na ausência de resposta diferencial de hospedeiros a esses grupos (Dunkle e Carson, 1999 e Gordon et al, 2004).

Thompson et al (1987) comentam sobre a estabilidade da resistência de linhagens antigas através dos anos. Apesar disso, a avaliação de híbridos através de ambientes normalmente tem indicado interação genótipo x ambiente significativa. No entanto, grande parte da interação parece ser devida ao aumento de magnitude nas diferenças entre os genótipos (interação simples) mais do que mudança na sua ordem relativa, principalmente para aqueles genótipos de resistência intermediária nos locais onde os níveis de doenças são mais altos (Carson et al, 2002). A não mudança no sinal dos efeitos aditivos através dos ambientes é uma indicação de que nas interações QTL x ambiente, estão associadas mudanças na magnitude e não na classificação relativa dos QTL (Clements et al, 2000). Brunelli (2004) hipotiza também a possibilidade de variação na expressividade dos fatores de resistência nos diversos ambientes.

Outros fatores podem contribuir para a interação QTL x ambiente. A presença de parcelas adjacentes pode influenciar a acurácia da avaliação fenotípica da reação à Cz. Thompson et al (1987) observou que a linhagem resistente H99 obteve nota 1.9 (em uma escala de 1 = resistente a 5 = suscetível) quando estava afastada de uma fonte suscetível, e 2.9 quando estava ao lado de plantas suscetíveis, chamando a atenção para a importância do manejo da área

experimental. Para genótipos de reação intermediária, a influência de parcelas adjacentes ainda é maior, o que pode contribuir grandemente para a interação QTL x ambiente. Uma característica do patógeno que pode estar relacionada com a influência de parcelas pareadas é o fenômeno da conidiação microcíclica, que é a formação de esporos secundários sem a fase vegetativa intermediária, a partir da germinação de um conídio, normalmente após um estresse ambiental (Lapaire & Dunkle, 2003). Esses conídios são facilmente dispersos dentro de um campo de milho por pequenas turbulências de ar (Meredith, 1973). Vinte e seis por cento dos isolados pertencentes ao grupo I de Cz estudados por Brunetti (2004) apresentaram conidiação microcíclica. Isolados do grupo II não apresentaram essa característica.

A época de avaliação da doença é outra variável a ser considerada no mapeamento de QTL. Bubeck et al (1993) constatou que o aumento no número de dias entre duas avaliações para Cz diminuiu a correlação entre elas, indicando que genes diferentes podem agir no início e final do processo da doença. Clements et al (2000) mapeou QTL associados à resistência em uma avaliação duas semanas após o florescimento, nos cromossomos 3 (marcador asg10, bin = 3.07), 6 (npi373, bin = 6.05) e 7 (umc113, bin = 7.02), e quatro a cinco semanas após o florescimento, nos cromossomos 3 (umc102, bin = 3.05), 4 (agrr321, bin = 4.06) e 8 (umc89, bin = 8.05). Essa diferença poderia ser explicada pela associação entre severidade da doença e a maturidade do genótipo. Nessa mesma pesquisa, seis QTL de resistência foram associados com a maturidade, associação que já havia sido detectada por Bubeck et al (1993), Coates & White (1998) e Saghai Maroof et al (1996).

1.4. Validação dos QTL de resistência por meio do uso de Linhagens Quase Isogênicas (LQI)

A necessidade de validação de QTL decorre do fato de que, com os tamanhos das populações normalmente usadas nos trabalhos de mapeamento, o seu poder de detecção é baixo, e a estimativa de seus efeitos viesada (Melchinger et al, 1998), além da limitação da análise de variância em detectar interações (Wade, 1992 apud Doebley & Gustus, 1995). Doebley & Gustus (1995) não

detectaram epistasia entre dois QTL quando baseados em estimativas a partir de populações F_2 , mas por meio de teste específico delineado para essa finalidade, concluíram que os efeitos epistáticos foram superiores aos efeitos individuais de cada QTL.

A determinação da função do QTL é fundamental para maximizar o ganho genético e a otimização de recursos disponíveis. Várias regiões do genoma do milho são duplicadas e portanto, QTL podem estar duplicados. Os bins 1.06/1.07 são duplicações da região próxima aos bins 9.04/9.05 (McMullen & Simcox, 1995). Embora QTL para resistência a *Sethosphaeria turcica* tenham sido mapeados nessas duas regiões, não foram detectados nas três populações avaliadas por Welz & Geiger (2000), sugerindo que não se trata do mesmo QTL. Também a região cromossômica bin 8.05/8.06 é homoeóloga das regiões 3.06/3.07 e 5.06/5.07 (McMullen & Simcox, 1995). Muitos probes de DNA da região bin 8.05/8.06 se hibridizaram nas regiões 3.06/3.07 e 5.06/5.07 nas três populações estudadas por Welz & Geiger (2000), e como os QTL mapeados nessas regiões apresentaram a mesma magnitude de efeitos, os autores concluíram que esses resultados sugerem a origem comum desses QTL, e portanto, a mesma função (Welz & Geiger, 2000). Duplicação de QTL também foi observado em soja (Lee et al, 1999).

Existem fortes indícios de que os genes de resistência a doenças se organizam em grupos ("clusters") distribuídos ao longo do genoma, com várias doenças diferentes podendo ser mapeadas na mesma região do cromossomo (bin). Seis regiões cromossômicas contêm agrupamento de genes de resistência para mais de uma doença: região ao redor do bin 1.04 no cromossomo 1 próximo à região do centrômero, no cromossomo 3 na região bin 3.04, no braço curto do cromossomo 4 entre os bins 4.01 e 4.04, na região bin = 6.01 no cromossomo 6 e no braço longo do cromossomo 8 no bin 8.06 (McMullen & Simcox, 1995). Wisser et al (2006) identificaram dois "clusters" em cada cromossomo 1 e 3, e um em cada cromossomo 2, 4, 5 e 10. QTL's de resistência a Cz foram mapeados próximos a esses clusters nos cromossomos 1 (Bubeck et al, 1993, Saghai Maroof et al, 1996, Clements et al, 2000 e Lehmensiek et al, 2001), no cromossomo 3 (Bubeck et al, 1993, Clements et al, 2000 e Lehmensiek et al, 2001) e no 8 (Bubeck et al, 1993, Shagai Maroof et al, 1996 e Clements et al,

2000). McMullen & Simcox (1995) especulam sobre a existência de um significado funcional no agrupamento de genes de resistência a doenças, ou se é uma consequência da organização do genoma. Esses genes poderiam representar famílias envolvidas em um sinal de transdução ou outros passos que levam a uma interação incompatível entre patógenos e hospedeiro, ou ainda poderiam representar arranjos ancestrais de genes de resistência que foram selecionados durante a evolução. Os autores ainda chamam a atenção para o fato de que os grupamentos (“clusters”) nos cromossomos 1 e 3 estão próximos da região do centrômero, enquanto que do cromossomo 6, está próximo da região organizadora do nucléolo. Essas regiões são supressoras de recombinação e portanto com potencial reduzido de recombinação, podendo formar blocos de ligação. Como nos programas de melhoramento em regiões tropicais a obtenção de resistência múltipla é fundamental, a combinação não só de diferentes QTL's de resistência para a mesma doença, como também para doenças diferentes deve ser considerada.

O uso de linhagens isogênicas ou quase-isogênicas (LQI) é uma ferramenta útil e de fácil execução para a validação de QTL. LQI para características de resistência a doenças são desenvolvidas pela transferência dos QTL de resistência do parental resistente (doador) para a linhagem parental suscetível (recorrente), por meio de retrocruzamentos sucessivos assistidos por marcadores moleculares. No final do processo, as LQI se contrastam somente pelas regiões cromossômicas contendo os QTL de resistência, sendo o restante do seu genoma, da linhagem parental suscetível. Além de se contrastarem para cada QTL separadamente, as LQIs também podem conter todas as combinações entre os QTL em estudo, o que permite uma estimativa mais direta de efeitos epistáticos. LQIs tem sido usadas para a validação de QTL's em várias culturas como o tomate (Bernacchi et al, 1998), cevada (van Berloo et al, 2001) e arroz (Wissuwa & Ae, 2001).

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CAPÍTULO 2

2. MAPPING AND VALIDATION OF QUANTITATIVE TRAIT LOCI FOR RESISTANCE TO *Cercospora zea-maydis* INFECTION IN TROPICAL MAIZE

RESUMO

Resistência à infecção por *C. zea-maydis* (Cz) está entre os objetivos mais importantes dos programas de melhoramento no Brasil. Os objetivos da presente pesquisa foram mapear e caracterizar Quantitative Trait Loci (QTL) relacionados com a resistência a Cz, validar as estimativas dos seus efeitos na severidade de Cz com uso de linhagens quase isogênicas (LQIs), e estimar os seus efeitos sobre três caracteres agrônômicos importantes usando os seus respectivos híbridos quase isogênicos (HQIs), obtidos pelo cruzamento das LQIs com uma linhagem de um grupo heterótico complementar. Foram mapeados quatro QTL com $LOD > 2.5$ dos quais três foram avaliados em LQIs. Foram avaliados dois QTL mapeados no cromossomo 1 em fase de repulsão, Q1 localizado no bin 1.05 e Q2 no bin 1.07, e um no cromossomo 3, Q3 localizado no bin 3.07. A avaliação das LQIs mostraram efeitos individuais altamente significativos para os três QTL na redução da severidade de Cz. Uma interação epistática desfavorável para a reação a Cz foi detectada entre Q1 e Q2, na qual o efeito de um dos alelos foi efetivamente anulado pelo outro. Alternativamente, a interação entre Q2 e Q3 foi positiva, promovendo a redução na severidade de Cz a um nível maior do que a soma de seus efeitos individuais. A avaliação das HQIs revelou efeitos individuais significativos para Q1 e Q3 na redução da severidade de Cz, de Q2 sobre a porcentagem de plantas quebradas e produtividade de grãos, e de Q3 sobre a porcentagem de umidade dos grãos na colheita e porcentagem de plantas quebradas. Interações epistáticas significativas foram detectadas entre Q1 e Q2 para umidade dos grãos na colheita e entre Q1 e Q3 para porcentagem de plantas quebradas. A combinação de QTL impacta na efetividade de procedimentos de seleção monitorados por marcadores em programas de melhoramento para o desenvolvimento de produtos comerciais.

Palavras-chave: Loci de Caracteres Quantitativos (QTL em inglês); Linhagens Quase Isogênicas (LQI); Híbridos Quase Isogênicos (HQI); Mapeamento de QTL; Validação de QTL; Epistasia

ABSTRACT

Resistance to infection by *Cercospora zeae-maydis* (Cz) ranks among the most important objectives of breeding programs in Brazil. The objectives of this research were to map and characterize Quantitative Trait Loci (QTL) related to resistance to Cz, to validate the estimates of their effects on disease severity using Near-Isogenic Lines (NILs), and to estimate their effects on three major agronomic traits using their respective Near Isogenic Hybrids (NIHs) obtained by crossing the NILs with an inbred of complementary heterotic pool. Four QTL with LOD values > 2.5 were mapped using the Multiple Interval Mapping approach. Three of them were evaluated in this study. NILs genotype included the two QTL allele located in chromosome 1 in repulsion phase, Q1 in bin 1.05 and Q2 in bin 1.07, and one in chromosome 3, Q3 in bin 3.07. NIL evaluation showed that individually, the three QTL significantly reduced the severity of Cz. An unfavorable epistatic interaction for the reaction to Cz was detected between Q1 and Q2, in which one QTL allele effectively nullified the effect of the other. Alternatively, the interaction between Q2 and Q3 was positive, promoting the reduction of the severity to a greater extent than the sum of their individual effects. NIH evaluation revealed significant individual effects for Q1 and Q3 on Cz severity, for Q2 on stalk lodging and grain yield, and for Q3 on grain moisture and stalk lodging. Significant epistatic interactions were also detected between Q1 and Q2 for grain moisture and between Q1 and Q3 for stalk lodging. The combination of QTL impacts the effectiveness of marker-assisted selection procedures in commercial product development programs.

Key words: Quantitative Trait Loci (QTL); Near Isogenic Line (NIL); Near Isogenic Hybrid (NIH); QTL mapping; QTL validation; Epistasis.

2.1.INTRODUCTION

Approximately 8.5 million hectares are cultivated with hybrid maize (*Zea mays L.*) in Brazil (<http://www.conab.gov.br/>). The maize growing area is located between 10 and 30 degrees south. Environmental conditions vary from tropical to sub-tropical where the required level of defensiveness to produce profitable grain yields is much higher than in temperate growing areas. An important component of the needed defensiveness is adequate resistance to diseases. For commercial companies, an important reason for removing commercial hybrids from the seed market is the outbreak of new diseases or the presence of preexisting physiological races. This disease pressure is caused by alterations in the pathogen dispersion dynamic, which can be due to the utilization of susceptible hybrids, to changes in the cropping system or both.

The pathogenic fungus *Cercospora zeae-maydis* Tehon & E. W. Daniels (Ward *et al* 1999) (Cz) began to assume epidemic proportions in various regions of Brazil starting in 2000, with predominance in highlands, altitude above 700 m, located at Mato Grosso, Goiás and Minas Gerais states. It was responsible for the discontinuance of several high-yielding but susceptible hybrids. Currently, the incorporation of resistance to infection by Cz ranks among the most important objectives of hybrid development programs and the utilization of molecular markers has led to the mapping and characterization of several Quantitative Trait Loci (QTL) related to its resistance.

QTL for resistance to infection by Cz have been mapped in all 10 maize chromosomes (Bubeck *et al*, 1993; Saghai-Maroo *et al*, 1996; Clements *et al*, 2000; Lehmensiek *et al*, 2001; Pedrosa, 2002 and Gordon *et al*, 2004). Establishing a consensus among the results found in the literature should help validate the QTL discovered, enabling their more routine use in marker-assisted breeding (MAB) programs. Wisser *et al* (2006) looked for consensus QTL-resistance positions for several corn diseases based on results described in published research. All declared-QTL reported had been considered regardless of the magnitude of their effects. The authors also constructed 95% confidence intervals based on the molecular marker of the highest significance. The QTL for

resistance to Cz used in this analysis were those reported by Bubeck et al (1996), Saghai-Maroo et al (1996), Clements et al (2000), Lehmsiek et al (2001) and Gordon et al (2004). Based on those results, it can be estimated that these QTL covered about 60% of the maize genome. According to Wisser et al (2006), this high level of coverage of the maize genome to a certain extent reflects both the low precision and accuracy of QTL mapping, as well as the large number of loci involved in the genotype x host interaction. The genotype x host interaction includes genes related to the plant development that can impact resistance. Moreover, epistatic interactions among QTL have not been effectively exploited either in basic mapping research or in MAB. When one utilizes a very high degree of stringency for QTL detection, it is unlikely that epistatic interactions among minor effect QTL can be detected (Carlborg & Haley, 2004) or even considered for MAB. Thus, the validation of QTL becomes necessary to maximize genetic gains and to make feasible the use of available resources in MAB.

Considering the complexity of the issues and the importance of Cz to commercial product development in Brazil, we conducted research with the objectives of 1) mapping QTL associated with resistance to infection by Cz in tropical maize germplasm; 2) validating the QTL using Near Isogenic Lines (NILs), and: 3) estimating the effects of QTL on three important agronomic traits using Near Isogenic Hybrids (NIHs).

2.2. MATERIALS & METHODS

2.2.1. QTL mapping

2.2.1.1. Generation and evaluation of the segregating population.

During the 2001 growing season, 187 testcrosses of $F_{2:3}$ progenies derived from a cross of two Monsanto inbred lines contrasting for reaction to Cz, MON323 (37.5% Tropical Dent, 62.5% Stiff Stalk) and MON402 (100% Tropical Flint), were evaluated at 4 locations: Iraí de Minas–MG (951m altitude, 19°00'S and 47°05'W) as summer (s) and winter crops (w), (IR_s and IR_w, respectively), Montividiu–GO (821m altitude, 17°04'S and 51°02'W) (MV_s), and Jataí–GO (708m altitude; 17°52'S and 51°42'W) (JT_s), the last two locations as summer crops. The tester was a Full Tropical Flint inbred line, but unrelated to MON402. The experimental design utilized consisted of a completely randomized block with two replications. The plots were comprised of two 5 m long rows. Both sowing and harvesting were mechanical. Soil fertilization was attained with 45 kg ha⁻¹ N, 80 kg ha⁻¹ P₂O₅ and 100 kg ha⁻¹ K₂O, and side dressing of 138 kg ha⁻¹ N applied 30 days after sowing (DAS). Weed control was accomplished with the spraying of 3 L ha⁻¹ of a mixture of the herbicides atrazine (200 g L⁻¹) and metalachlor (300 g L⁻¹).

2.2.1.2. Cz severity evaluation. The disease reaction was evaluated visually by means of a class number ranging from 1 through 9 (1-to-9 scale), representing the percentage of infected foliar area (IFA) as follows: 1 = 0% IFA and absence of symptoms; 2 = < 1% IFA with a few and sparse lesions; 3 = 1% - 20% IFA; 4 = 20% - 40% IFA; 5 = 40% - 50% IFA with lesions reaching the ear leaf and a few lesions in the leaves above the ear; 6 = 50% - 60% IFA, with lesions reaching leaves above the ear; 7 = 60% - 75% IFA; 8 = 75% - 90% IFA and 9 = > 90 % IFA with premature plant death before reaching physiological maturity (black-layer formation).

2.2.1.3. Genotyping of $F_{2:3}$ progenies. Genotyping involved the removal of leaf tissue samples from 10 plants for each $F_{2:3}$ progeny within the isolated detasseled corn plots used for synthesis of the top-crosses. DNA extraction followed the methodology presented by Dellaporta & Hicks (1983).

DNA was quantified by diluting 20 μ L of DNA solution in 980 μ L of TE 0.1x in a spectrophotometer. The material was then prepared for PCR amplification for either SSR or SNP markers. SSR markers were individually amplified using PCR and marker genotype was visualized using electrophoresis in 2.8% agarose gel (3:1 Metaphor® agarose (Cambrex Corporation): UltraPure™ Agarose 1000 (Invitrogen), with 2 μ L EtBr (ethidium bromide), TBE 1x buffer, at 160-170V for 3 to 3.5 h. An extra amount of EtBr (8 μ L) was added to the TBE in the electrophoresis bowl at the positive pole for contrasting. SNP markers were genotyped using the ABI Prism 7700 Sequence Detection System (TaqMan®), available from Applied Biosystems, Foster City, California, per manufacturer's specifications. A total of 138 markers were used: 68 SSR (Single Sequence Repeats) and 70 SNP (Single Nucleotide Polymorphisms) distributed in numbers of 30, 12, 16, 15, 12, 10, 13, 11, 13 and 6 markers amongst chromosomes 1 through 10, respectively.

2.2.1.4. Construction of linkage groups and QTL mapping. The linkage groups were determined utilizing the QTL/MAPMAKER v3.0 software (http://www.broad.mit.edu/genome_wofware/) (Lander et al, 1987) with the Haldane mapping function and a minimum of 44 individuals and codominant markers. QTL mapping was performed with the QTL/CARTOGRAPHER (<http://www.statgen.ncsu.edu/qtlcart/WQTLCart.htm>) v2.5 software (Wang, 1999). The Multiple Interval Mapping method (MIM) (Kao et al, 1999) was used, assuming as the level of significance a value of LOD score > 2.5. LOD is the logarithm of odds which is equal to the logarithm of the likelihood ratio test. The initial model for the selection of markers was based on the QTL mapped by the Composite Interval Mapping method (CIM) (Jansen, 1992, 1993; Zeng, 1993 and 1994), which threshold was determined with 1000 permutations, at a walk speed of 2 cM. The models were tested for additive, dominant and epistatic effects. QTL mapping was performed for each location individually and across location means. In addition to the adoption of the QTL characterization system proposed by Collard et al (2005),

QTL mapped within a distance of 20 cM whose additive effects had the same signal, were declared as same locus (Melchinger et al, 1998).

2.2.1.5. QTL x environment interaction. The interaction between QTL and environment was evaluated adapting the linear regression model proposed by Eberhart & Russel (1966) to evaluate the stability of genotypes, using the additive effects of the markers associated with the QTL.

2.2.2. Production and evaluation of the NILs

2.2.2.1. Backcross program. Production of the NILs began with a preliminary mapping through Single Marker Analysis (SMA) and using only SSR markers (Figure 2). Markers presenting the lowest p-value were located in chromosome 1, bmc1007 (bin 1.03) and bmc1643 (bin 1.10); chromosome 2, bmc1064 (bin 2.04); chromosome 3, bmc1456 and bmc1035, both in bin 3.05, and bmc1505 and bmc1047, both in bin 3.06; chromosome 4, bnlg589 (bin 4.11); chromosome 6, umc1018 (bin 6.01); chromosome 7, bmc1666 (bin 7.04); and in chromosome 9, bmc1714 (bin 9.04). The region of resistance associated to marker bmc1007 in chromosome 1 originated from the susceptible parent. All the others originated from the resistant parent. Of the 187 $F_{2:3}$ progenies evaluated, two were selected as the most promising to initiate the BC program (Figure 2). In the summer of 2001, the F_3BC_1 generation was synthesized by backcrossing the selected progeny to the susceptible (recurrent) parent. The chromosomal regions selected were the two from chromosome 1 and those located on chromosomes 2, 3 and 7, which were confirmed as linked to Cz resistance by Multiple Interval Mapping approach.

In the winter of 2002, the F_3BC_1 generation was planted in a nursery and genotyped. The segregating plants were selected for the genotype of the donor parent (resistant) in the chromosomal regions encompassed by the mapped QTL, while for the other regions, the selection of markers was for the recurrent parent (susceptible). In the end of the process, the F_3BC_2 plants were selfed and 1417 segregating for the five QTL were obtained, which were genotyped for confirmation

purposes at the chromosomal regions recovered by BC with 5, 2, 3 and 2 SNP (Single Nucleotide Polymorphism) markers in chromosomes 1, 2, 3 and 7, respectively. The QTL regions in chromosomes 2 and 7 were not considered in the NILs selection because they did not present segregates with QTL combinations suitable for the objectives of this research.

2.2.2.2. Evaluation of NILs. In the summer of 2002, 18 NILs, carrying all QTL combinations (000 to 111 for Q1, Q2, and Q3) (Figure 3), were evaluated *per se* for Cz reaction in Mineiros–GO and Iraí de Minas–MG. The experimental design used was a complete randomized block (CRD) with three replications in a 3x2 factorial scheme (three QTL, presence and absence). Planting was done mechanically and the plots comprised of one 3m-long row. Spacing among rows was 0.8 m and planting density was 90,000 plants ha⁻¹. The same amount of nutrients was applied as for the top-crosses. At every two plots, a row of a highly susceptible inbred was intercalated for ease of disease dissemination, so that each experimental plot was paired with the disseminating inbred line. This inbred was also used as a border row, at both sides of the experiment. The severity of Cz was evaluated at 99 DAS in Mineiros and at 95 DAS in Iraí, both based on the 1-to-9 scale. Each genotype was represented by two to three NILs. Due to the high natural incidence of the pathogen, artificial inoculation was not required at any locality.

2.2.2.3. Statistical analysis. SAS 9.1 proc GLM was used to run the statistical analysis. The model used for the analysis was:

$$y_{ij} = \mu + b_j + \beta_1 Q_{1i} + \beta_2 Q_{2i} + \beta_3 Q_{3i} + \beta_4 (Q_{1i} \times Q_{2i}) + \beta_5 (Q_{1i} \times Q_{3i}) + \beta_6 (Q_{2i} \times Q_{3i}) + e_{ij}$$

in which:

y_{ij} is the value observed in the j -th repetition ($j=1,2,3$) of the NIL i ($i=1,2...18$);

b_j is the effect of the repetition j ;

μ is a constant inherent to all the observations;

β_k are the individual effects of the QTL and of the epistatic interactions between them;

Q_{ki} are the “dummy” variables indicating the presence or absence of the QTL k ($k=1,2,3$) in the NIL i ($i=1,2..18$); and

e_{ij} is the random error associated to the observation y_{ij} , $e_{ij} \sim N(0, \sigma^2)$. All the other parameters were considered as fixed.

For the joint analysis of the data, the following fixed model was used:

$$y_{ija} = \mu + l_a + b_j(l_a) + \beta_1 Q_{1i} + \beta_2 Q_{2i} + \beta_3 Q_{3i} + \beta_4 (Q_{1i} \times Q_{2i}) + \beta_5 (Q_{1i} \times Q_{3i}) + \beta_6 (Q_{2i} \times Q_{3i}) + \beta_7 Q_{1i} \times l_a + \beta_8 Q_{2i} \times l_a + \beta_9 Q_{3i} \times l_a + \beta_{10} (Q_{1i} \times Q_{2i}) \times l_a + \beta_{11} (Q_{1i} \times Q_{3i}) \times l_a + \beta_{12} (Q_{2i} \times Q_{3i}) \times l_a + e_{ija}$$

in which:

y_{ija} is the value observed in the j -th repetition ($j=1,2,3$) of the NIL i ($i=1,2..18$), at location a ($a=1,2$);

l_a is the effect of location a ;

$b_j(l_a)$ is the effect of repetition j in location a ;

μ is a constant inherent to all the observations;

β_k are the effects of the individual QTL and of the epistatic interactions between them;

Q_{ki} are the “dummy” variables indicating the presence or absence of the QTL k ($k=1,2,3$) in the NIL i ($i=1,2..18$); and

e_{ija} is the random error associated to the observation y_{ija} , $e_{ij} \sim N(0, \sigma^2)$.

2.2.3. Production and evaluation of the NIHS

The effects of the QTL on grain yield, grain moisture percent at harvest and stalk lodging (breakage) percent were assessed using NIHS. In the summer of 2004, 21 NILs (Figure 3) were crossed with a complementary heterotic group inbred derived from Tropical Dent x Lancaster. The tester, albeit not susceptible to

Cz *per se*, displays a neutral behavior in hybrid combinations, with the reaction of the hybrids depending on the level of susceptibility of the other parental line. It is also resistant to *Phaeosphaeria maydis* (Henn.) Rane, Payak, & Renfro (anamorph = *Phoma maydis*, synonym = *Leptosphaeria zea-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnelt) (PLS) which is crucial to prevent premature loss of foliar area due to this disease, which would impair the Cz evaluation since the two diseases occur simultaneously in these environments. The experiments were conducted in Mineiros-GO and Iraí de Minas-MG, using CRDs with three replications. The plots consisted of two 5m-long rows spaced apart 0.8 m and were planted and harvested mechanically. At both locations, the final plant population average was 80,000 plants ha⁻¹. Fertilizers were applied at the rate of 40 kg ha⁻¹ of N and 100 kg ha⁻¹ P₂O₅ and K₂O, with a complementary side-dressing of 90 kg ha⁻¹ N at 30 DAS. In addition to the GY, GM and SL variables, the reaction to Cz infection was assessed based on the 1-to-9 scale at 95 DAS. The data was analyzed using the same models as those described for the NILs.

Figure 1 summarizes all steps followed for QTL mapping and NIL and NIH production.

2.3. RESULTS

2.3.1. QTL mapping for Cz infection resistance

2.3.1.1. Severity of Cz. The highest severity of Cz occurred in JT_s with average of 7.1 ± 0.2 evaluated on a 1-to-9 scale. The lowest occurred in IR_s with average of 4.7 ± 0.2 (Table 1). No artificial inoculation was needed in any location. The frequency distribution of Cz severity based on a 1-to-9 scale for the average of the four locations is presented in Figure 3.

2.3.1.2. Linkage groups. Of the 138 markers used for mapping the QTL, 117 were grouped and associated with the 10 maize chromosomes (85%), encompassing a total length of 1576 cM or 89% coverage of the genome, with an average of 11.7 markers per chromosome and standard deviation (SD) = 7.3. The average and SD for intermarker distance were 14.7 cM and 13 cM, respectively. Chromosomes 5, 6, 8 and 10 had the fewest associated markers, with an average coverage of 58.5% of the total used for these chromosomes. The other chromosomes showed an average of 95.8% of marker coverage.

2.3.1.3. QTL mapping results. Four QTL with LOD values > 2.5 (Table 2) were mapped using Multiple Interval Mapping. In MV_s, two were mapped in chromosome 1 in the repulsion phase and one in chromosome 3. The QTL allele located in bin 1.05 with LOD value = 3.2 (Q1) originated from the susceptible parental inbred, with an additive effect explaining 8% of the phenotypic variance (PV). The QTL allele located in bin 1.07 with LOD value = 3.2 (Q2) originated from the resistant parental inbred, with an additive effect explaining 11% of the PV. The effects of these two QTL were predominantly additive and were not mapped in the other locations or in the combined analysis of locations. The third QTL allele (Q3) originated from resistant parental line was mapped in chromosome 3 in bin 3.07 in JT_s, IR_w and in the combined analysis of locations, with LOD values = 4.8, 2.5 and 7.4, with additive effects explaining 26 and 27% of the PV, respectively. In MV_s, this QTL was mapped in bin 3.06 with LOD value = 2.6, its additive effect explaining 6% of the PV. In IR_s it was also mapped in position bin 3.07, but with

LOD value = 1.9 for the dominance effect, which explained 7% of the PV. At this same location, this QTL presented LOD value = 6.3 in the Composite Interval Mapping (CIM) analysis, explaining 24% of the PV (data not shown). The fourth QTL allele (Q4) originated from resistant parental line was mapped in IR_s and in the combined analysis of locations in bin 7.03, with LOD value = 2.6 and 3.5, respectively, and each one with additive effects explaining 8% of the PV. In JT_s and in the combined analysis of the four environments, a fifth QTL allele (Q5) originated from the susceptible parent, inconsistent and of minor effect, was mapped in chromosome 9 in bin 9.04. The LOD values were 1.5 and 2.2 and the additive effects explained 6% and 7% of the PV, respectively. This QTL is presented in Table 2 because of its epistatic interaction with Q3, as will be discussed later.

2.3.1.4. Epistatic Interactions. Several epistatic interactions of minor effects were detected in the MIM (Multiple Interval Mapping) analysis at IR_s, MV_s and in the combined analysis of the locations. In IR_s, the LOD values varied from 0.6 to 0.9 and explained from 2% to 4% of the PV. In MV_s, only one additive x additive interaction between Q3 and Q5 with LOD = 1.7 explained 5% of the PV. In the combined analysis of the four environments, the LOD values varied from 0.9 to 1.7, explaining from 1 to 3% of the PV.

2.3.1.5. QTL x location interaction. No significant QTL versus environment interactions were detected for any of the QTL mapped. The results of this analysis are therefore not presented.

2.3.2. Evaluation of the NILs

2.3.2.1. NIL genotypes. The average percentage of the recurrent (susceptible) genotype recovered after the second BC was $81.5 \pm 2.5\%$, with total amplitude varying from 73 to 90%. Thirty percent of the NILs fell within the class of 86 to 90% of the genotype of the recovered recurrent parental line, 15% between 81 and 85%; 40% between 76 and 80%, and 15% between 70 and 75%. All the genotypic combinations among the three QTL were represented in the NILs and

the chromosomal regions containing the QTL were recovered almost entirely in the backcrossing process. Even Q1 and Q2 occurring in the repulsion phase, both alleles were recovered in the same NIL.

2.3.2.2. Estimate of individual and epistatic QTL effects. The three QTL had highly significant effects on the reduction of the severity of Cz evaluated by the 1-to-9 scale (Table 3). At Iraí, the average reductions in the severity estimated by regression analysis were 3.4 units on the 1-to-9 scale for Q1, 2.3 for Q2 and 1.3 for Q3 (Table 4). These reductions represented mean score values of 4.4, 5.5 and 6.5 for the NILs containing Q1, Q2 and Q3, respectively. The epistatic interaction Q1*Q2 increased the severity by 3.2 units, with the average score of the NILs containing these two QTL equaling 5.3 on the 1-to-9 scale (Table 4). In Mineiros, the effects of the three QTL were also highly significant, with average reductions of 2.4, 1.8 and 1.1 units in the severity (Table 4). The average scores of the NILs containing Q1, Q2 and Q3 were 4.7, 5.3 and 6.0, respectively. The epistatic interaction Q1*Q2 was also highly significant at this locality, with an average increase of 1.7 units on the 1-to-9 scale. The average score of the NILs containing the two QTL was 4.6 (Table 4). In the combined analysis of the locations, the reduction in the severity was 2.9, 2.0 and 1.2 units on the 1-to-9 scale for Q1, Q2 and Q3, with average scores of 4.5, 5.4 and 6.2, respectively (Table 4). The epistatic interaction Q1*Q2 promoted an average increase of 2.4 units in the severity (Table 4). The average score of the NILs containing the two QTL was 4.9. The epistatic interaction Q2*Q3 was also significant (Table 3), leading to the reduction of one score on the 1-to-9 scale (Table 4). This effect, however, was not significant in the individual analyses of the locations. The average score of the NILs containing these QTL was 3.2, i.e., a lower value than the sum of their individual effects. As Q1*Q2*Q3 epistatic interaction effect was not significant at any location, it was not included in the final analysis. No QTL interacted significantly with any locations.

2.3.3. Evaluation of the NIHs

2.3.3.1. Effect of QTL on severity of Cz. Highly significant effects of Q1 and Q3 were found on the severity of Cz evaluated by means of NIHs for Q1 and Q3 (Table 5). Q1 promoted a reduction in score of 0.5 units in Iraí and 0.8 in Mineiros, with average scores of 4.6 and 5.3. Q3 reduced 0.6 and 1 units, with average scores of 4.5 and 5.1 in Iraí and Mineiros, respectively. The effect of Q2 was significant only in Iraí. Significant epistatic or QTL x location interactions were not detected. The regression analysis did not indicate significance for any effect evaluated (data not shown).

2.3.3.2. QTL effects on grain moisture. A significant effect was detected of Q3 on grain moisture (Table 6), although it was not detected by the regression analysis (Table 7). The average increase of GM on the NIHs containing Q3 was 0.6%. The epistatic interaction Q1*Q2 was highly significant (Table 7). The joint occurrence of these QTL led to an increase of 1.5% in the GM. No QTL x location interaction was significant for this trait.

2.3.3.3. QTL effects on stalk lodging. Q2 and Q3 presented significant effects on stalk lodging (Table 8). These results are similar to those of Iraí, since the effects of none of these QTL was significant in Mineiros. The average SL in this location was 1.6%, while in Iraí it was 6%. On average, there was a 4.3% reduction in SL in the NIHs containing Q2. The reduction promoted by Q3 was 2.6%. A significant epistatic Q1*Q3 interaction was detected in the joint analysis of locations. Significance was also detected in Iraí for the Q1*Q2 interaction. Q1 and Q2 both interacted significantly with locations, so reflecting their dissimilar performance in such locations.

2.3.3.4. QTL effects on grain yield. The effects of QTL on grain yield corrected to 15.5% moisture were evaluated only in Iraí (Table 9). Q2 was the only QTL that had a significant effect on yield, increasing the yield by 444 kg ha⁻¹. No significant epistatic interaction was detected.

A summary of the QTL effects for all agronomic traits evaluated in the NIHS is presented in Table 10.

2.4. DISCUSSION

The average recovery percentage of the recurrent genotype after two BC cycles was 81.5%. This value would have been higher if Single Mapping Analysis (SMA) had more precision and more precision would have allowed for the selection of a smaller chromosome segment for backcrossing. For the introduction of Q1, Q2, Q3, Q4 and Q5, chromosome segments measuring about 45.3 , 219.2, 95.2 95.2, and 99 cM, respectively, were selected from the recurrent parental line, segments big enough to carry other genes than ones under selection. Regions in chromosome 2 were also selected, but their QTL detected by SMA were not confirmed by MIM. Therefore, no significant differences are expected among NILs as a function of differences in genetic background, and hence, among NIHs for the regions in Chromosome 2.

Considering the values of the proportions of phenotypic variance (PV) explained by the additive effects of the QTL estimated by MIM (Table 2), Q3 is the only QTL that can be classified as exerting a strong effect. It explained 27% of the PV in the combined analysis of the locations and was the only one mapped in 3 out of the 4 locations. This QTL can therefore be classified as consistent (Collard et al, 2005). Q1 and Q2 can be considered as exerting only minor effects and as inconsistent. These QTL were mapped in only one out of the four localities and each one explained less than 10% of the PV.

The stability analysis detected no significance for the three QTL x environment interactions. The fact that a QTL was mapped at some environments but not at others may be related to factors such as the low detection power of the analysis, as well as the QTL x environment interaction. A MAB program based solely on MIM results might fail to consider Q1 and Q2 for selection, since the premises for their efficacy are the magnitude of the effects and the stability of the QTL over environments (Hittalmani et al, 2002).

The validation of Q1 and Q2 by means of NILs, however, indicated highly significant main effects for both (Tables 2 and 3). Individually, Q1, Q2 and Q3 reduced the severity by 2.9, 2.0 and 1.2 units on the 1-to-9 scale, with average scores of 4.5, 5.4 and 6.2, respectively (Table 4). The classification of Q1 and Q2 as QTL with minor effects was not confirmed by the analysis of the NILs. Q3,

which presented the highest effect in MIM, obtained a lower value through the use of NILs. In addition to the effect of years, the differences between the results of MIM and NILs may be attributed to the difference in genetic background of the top-crosses versus the NILs.

Epistatic interactions among QTL have not been effectively explored in either basic mapping research or in MAB programs. The degree of stringency utilized for the detection of QTL is normally very high, increasing the risk that loci of minor effects presenting epistatic interactions are not detectable (Carlborg & Haley, 2004) or even considered for selection in a MAB program. In our research the MIM estimates of epistatic interactions presented low LOD values and minor effects. The interaction of highest effect was detected between Q3 and Q5, with a LOD value = 2.2 and an additive x additive effect explaining 5% of the PV. Simulation studies have indicated that the power of detection of epistatic interactions in F_2 populations, with effects varying from 1 to 5% of the PV, varies from 50 to 80% in populations with sizes of 200 to 400 individuals (Verona et al, 2001 *apud* Carlborg, 2004). Alternatively, the analyses of NILs detected significant epistatic interactions for the severity of Cz (Tables 2 and 3). Individually, Q1 and Q2 reduced 2.9 and 2.0 units in the 1-to-9 scale, respectively (Table 4). The simultaneous occurrence of Q1 and Q2, however, increased the score by 2.4 units.

These results indicate that with the simultaneous presence of these QTL alleles, the effect of one of them was nullified. The average score of Cz with the joint presence of Q1 and Q2 was 4.9, while the individual presence of Q1 and Q2 led to a score of 4.5 and 5.4, respectively. The combined analysis of locations also detected a significant and positive epistatic interaction between Q2 and Q3 (Table 3). The score of the average severity with the simultaneous occurrence of the two QTL was 3.2, while the individual scores for Q2 and Q3 were 5.4 and 6.2, respectively (Table 4). This interaction was not significant in the individual analysis of locations. The Q1*Q3 interaction was not significant, although the average additive effect of these QTL in the same NIL showed a score of 3.3, i.e., practically the same value as that obtained with Q1 and Q3. The highest efficacy in reducing the severity is therefore achieved through the combination of either Q1 and Q3 or Q2 and Q3. Shagai-Maroo et al (2000) verified that the QTL allele mapped on chromosome 4 originating from the inbred B73 had little to no effect when the QTL

on chromosome 1 was homozygous for the allele derived from the inbred Va14. Knowledge of the epistatic effects is crucial in a MAB program, for it enables one to maximize not only the genetic gain but also the available resources to obtain inbred lines with the best QTL combination. Thus, the best cost-effective choice would be the combination of either Q2 and Q3 or Q1 and Q3.

These results also appear to indicate the greater efficiency of NILs in detecting epistatic interactions when compared to MIM. NILs have been applied to validate QTL in several crops (Glover et al, 2004; Van Berloo et al, 2001; Bernacchi et al, 1998), allowing for direct comparisons not only of the effects of individual QTL but also of their combinations, thus facilitating the estimation of epistatic interactions. Li et al (1997), working with rice, and Eshed & Zamir (1996) with tomatoes, demonstrated that epistatic interactions among loci apparently presenting no individual main effects can influence important quantitative traits. The latter authors worked with NILs, which have allowed for more frequent detection of epistatic interactions.

Ignoring epistatic interactions may lead to biased estimates of detected QTL effects and to increase the risk of individual locus going undetected (Carlborg & Haley, 2004). Also according to these authors, traditional protocols have focused on estimating the average genetic effect of the genotype of the QTL, ignoring the influence of the genetic background. Doebley et al (1995) have detected a strong influence of the genetic background on the expression of various QTL even affecting the degree of dominance at some loci.

In this research, the mapping and estimation of the QTL effects by MIM were based on the evaluation of top-crosses of $F_{2:3}$ progenies rather than on the progenies themselves. Similarly, the NIHs were NILs crossed with a test line. The correlation between NILs and NIHs for the severity of Cz based on the average of the two locations was highly significant (0.83), even though they were evaluated in different years. The same correlations within locations in the two years were also highly significant, i.e., 0.78 and 0.75 for Mineiros and Iraí, respectively. Nevertheless, in the evaluation of the NIHs, no significance was detected for the effect of Q2 in Mineiros or for the epistatic interactions between any combinations of QTL (Table 5).

The discrepancy between these results can be attributed both to the effect of years and to the tester used in the production of the NIHs. Distinct responses among testers in experiments involving mapping and estimation of QTL effects have been reported by Melchinger et al (1998) and Austin et al (2000). Even the use of NILs *per se* for estimating QTL effects may be influenced by the genetic background of the recurrent inbred. The development of NILs is based on the substitution of the chromosomal regions of a susceptible inbred by corresponding segments containing the QTL for resistance of a resistant parental line. In this research, Q1, originating from the susceptible (recurrent) parental inbred, interacted significantly with Q2 (Tables 2 and 3), which originated from the resistant (donor) parental line. The interaction QTL x genetic background is crucial in programs aimed at the development of hybrids, for selected inbreds are crossed with different testers of distinct genetic backgrounds.

Wisser et al (2006) found a low but significant correlation between date of inflorescence, a measure of plant maturity, and disease-resistant QTL. The association between resistance to infection to Cz and maturity has been described in various studies (Bubeck et al 1993; Coates & White, 1998; Saghai-Marooof et al 1996; Clements et al 2000). The strong link of Q3 with some QTL related to maturity or a possible pleiotropic effect of this allele must be considered. Some QTL related with increased plant maturity are mapped close to the position occupied by Q3. Three QTL that increase the number of days for pollination were mapped in bin 3.05 (92.4 cM, 90.4 cM and 81.9 cM) (CIMMYT-<http://www.maizegdb.org/cgi-bin/qtllocisummarytable.cgi?sortby=8>). One of them was associated to grain moisture (Melchinger et al, 1998), and the others to the number of days to flowering at bin 3.06 (Khairallah et al, 1998) and bin 3.08 (Abler et al, 1991). The highly significant epistatic interaction between Q1 and Q2 alleles (Tables 5 and 6) indicate the need for choosing the best combination of QTL in a program that also selects for earliness. The simultaneous occurrence of these QTL alleles increased the grain moisture by 1.5%, although their individual effects were not significant.

Correlated responses are also of paramount concern and should be taken into account in improvement programs. In the evaluation of the NIHs, Q2 and Q3 had a significant effect on the reduction of stalk lodging (Table 8). Diseases that

reduce the plant photosynthetic rate, as in the case of Cz, by reducing the plant leaf area through necrosis of leaf tissues, therefore interfering in the source-sink relations (Dodd, 1980). Because grains monopolize the consumption of photosynthesized products after flowering, the reduction in the post-flowering photosynthetic rate causes redistribution of the sugars from the stalk to the grains. The result is accelerated senescence of the stalk tissues, rendering them more susceptible to infection by rot-inducing pathogens and predisposing the plant to early death and stalk breaking. Thus, the reduction of leaf tissue loss promoted by Q2 and Q3 was probably reflected in a higher photosynthetic rate for the plant, with a positive response on stalk lodging. This combination of QTL was the one that also promoted the greatest reduction in the severity of the disease (Table 4). In Iraí and in the joint analysis of the locations, a significant epistatic interaction was detected between Q1 and Q3 for stalk lodging (Table 8), although the regression analysis failed to detect it (data not shown).

The ultimate objective of any genetic improvement program for resistance to diseases infection in maize is to attain increases in grain yield. Although the evaluation of the NIHS revealed that Q1 and Q3 had highly significant effects on the reduction of the severity of Cz (Table 5), their effects on grain yield were not significant (Table 9). Conversely, Q2 had a significant effect on the reduction of severity in Iraí (Table 5) and a significant, positive effect on grain yield (Table 9). However, it should be stressed that the observed results, which were obtained mainly for the agronomic traits, can be associated with the residual genotypic differences between NIHS, 18% on average (Figure 3), than the Cz resistant QTL.

Our results demonstrate the utility and level of complexity that needs to be considered when using QTL to improve Cz infection resistance in a commercial breeding program.

2.5. CONCLUSÕES

2.5.1. Mapeamento dos QTL de resistência a *C. zea-maydis*

Como rotina para programas de melhoramento assistidos por marcadores moleculares, a Análise de Marcas Simples (AMS) se mostrou eficiente quando comparado ao Mapeamento por Intervalo Múltiplo (MIM).

Nenhum QTL interagiu significativamente com o ambiente, mesmo Q1 e Q2 que foram mapeados com valores $LOD < 2.5$ apenas em uma das quatro localidades (Montividiu safra de verão).

2.5.2. Avaliação das Linhagens Quase Isogênicas (LQIs)

A porcentagem média de recuperação do genótipo recorrente após dois ciclos de retrocruzamentos foi de $81.5 \pm 2.5\%$. O valor teórico esperado com seleção via marcador para apenas um QTL é de aproximadamente 90-95% (Collard et al, 2005). Vários fatores podem ter contribuído para essa discrepância: a) Baixa precisão do mapeamento. Os tamanhos dos segmentos cromossômicos transferidos principalmente no primeiro ciclo de retrocruzamento foram grandes (45.3 cM, 219.2 cM, 95.2 cM, 95.2 cM e 95.0 cM para Q1, Q2, Q3, Q4 e Q5, respectivamente); b) O QTL mapeado pela AMS no cromossomo 2 e recuperado nos retrocruzamentos, não foi confirmado posteriormente pelo MIM (tabela 2); c) Foram manipulados cinco segmentos cromossômicos ao mesmo tempo. O recomendado é se trabalhar no máximo com três QTL por vez, mesmo assim, com a seleção dos marcadores baseada em mapas de alta densidade (Collard et al, 2005).

O background genético do parental recorrente das LQIs pode interagir com o QTL e afetar o valor de sua estimativa. É o caso da interação epistática observado entre Q1*Q2 (tabelas 3 e 4). O alelo Q1 se originou do parental recorrente, enquanto que o Q2, do parental doador.

As diferenças observadas entre as estimativas dos QTL obtidas pela MIM e LQIs podem em parte ser explicadas pelo efeito de anos (as LQIs foram avaliadas no ano agrícola 2002/03, enquanto que as HQIs no de 2004/05), e em

parte pelo efeito do background genético do testador usado na produção dos testcrosses, uma vez que o testador pode conter QTL interagindo epistaticamente com os QTL em estudo.

O uso de LQIs foi mais eficiente do que o Mapeamento por Intervalo Múltiplo para a estimativa de interações epistáticas entre QTL.

O conhecimento das interações epistáticas entre os QTL permite estabelecer estratégias mais eficientes e econômicas para programas de melhoramento assistidos por marcadores. As combinações Q1*Q2 ou Q2*Q3 foram as que representariam a maior eficiência e o menor custo efetivo. A combinação Q1*Q2 não apresentou vantagem na redução da severidade da doença (tabelas 3 e 4), e aumentou a maturidade das HQIs avaliada pela % de umidade dos grãos em 2.8% (tabela 7).

A validação dos QTL é fundamental na implantação de um programa de melhoramento genético assistido por marcadores. Além de permitir a estimação mais acurada do efeito dos QTL, também permite obter estimativas diretas de interações epistáticas, o que possibilita maximizar os recursos disponíveis ao programa.

2.5.3. Avaliação dos Híbridos Quase Isogênicos (HQIs)

As diferenças observadas entre os resultados obtidos para as estimativas dos QTL e das interações epistáticas entre eles para a severidade da *C. zea*- *maydis* avaliadas nas LQIs e HQIs, podem em parte ser explicadas pelo pequeno número de ambientes de avaliação, efeito de anos e de possíveis efeitos de dominância e interações epistáticas entre os QTL avaliados e os do testador.

Somente Q2 teve efeito significativo na produtividade (tabela 9), promovendo acréscimo de 444 kg/ha. Q3 e a interação Q1*Q2 teve efeitos significativos no aumento na umidade dos grãos (tabelas 6 e 7) e à menor severidade da doença (tabelas 5), o que promove menor índice de morte prematura e conseqüentemente quebramento do colmo, todas características correlacionadas com o aumento da produtividade.

Os resultados observados principalmente para as características agrônômicas, podem estar associadas com as diferenças genotípicas residuais

entre os HQIs (18% em média) e não aos efeitos dos QTL de resistência a *C. zea-maydis*.

A interação QTL x Local para a severidade da *C. zea-maydis* não foi significativa em nenhuma fase da pesquisa (mapeamento do QTL, avaliação da LQIs e das HQIs).

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2.7. TABELAS E FIGURAS

Table 1 - Statistics on *Cercospora zeae-maydis* severity evaluated on a 1-to-9 scale on 187 F_{2:3} progenies in four locations – 2000/2001 growing season.

Location	Average	Range ^a		SD
		Min	Max	
IR_s	5,65 ± 0,19	2	8	13,409
JT_s	7,06 ± 0,23	3	9	15,747
MV_s	5,32 ± 0,21	2	8	14,678
IR_w	4,72 ± 0,16	2	7	11,490

^a Range of Cz severity evaluated on a 1-to-9 scale

IR_s, JT_s, MV_s = Iraí de Minas, Jatí and Montividiu summer crop

IR_w = Iraí de Minas winter crop

SD = Standard Deviation

TABLE 2 – *Cercospora zeae-maydis* resistant QTL mapped by the Multiple Interval Mapping method, based on phenotypic evaluation of disease severity using 1-to-9 score scale at four locations, under natural disease occurrence. 2001/02 growing season.

Location	QTL	Type of Gene Action	Chromosome	Marker	Position (cM)	Bin ^a	LOD ^b	Genetic Effect ^c	Genetic Effect as a Percent of Total Variance (%)
IRA_s	Q3	additive	3	bmc1605	73.36	3.07	0.03	0.1032	1.2
IRA_s	Q3	dominant	3	bmc1605	73.36	3.07	1.94	-1.2856	7.0
IRA_s	Q4	additive	7	bmc1305	63.26	7.03	2.61	0.6371	7.7
IRA_s	Q4	dominant	7	bmc1305	63.26	7.03	0.06	-0.1244	0.3
JAT_s	Q3	additive	3	bmc1605	95.36	3.07	4.82	1.2121	25.6
JAT_s	Q3	dominant	3	bmc1605	95.36	3.07	0.43	0.4625	3.4
MV_s	Q1	additive	1	bmc1811	152.93	1.05	3.16	-0.6973	7.8
MV_s	Q1	dominant	1	bmc1811	152.93	1.05	0.01	0.0415	0.1
MV_s	Q2	additive	1	bmc1025	225.65	1.07	3.18	0.7187	10.6
MV_s	Q2	dominant	1	bmc1025	225.65	1.07	0.03	0.0989	0.1
MV_s	Q3	additive	3	bmc1047	61.99	3.06	2.59	0.6502	6.1
MV_s	Q3	dominant	3	bmc1047	61.99	3.06	0.09	0.1572	0.0
IRA_w	Q3	additive	3	bmc1605	102.36	3.07	2.52	0.6675	12.0
IRA_w	Q3	dominant	3	bmc1605	102.36	3.07	0.00	-0.0009	0.0
ALL	Q3	additive	3	bmc1605	98.36	3.07	7.41	0.7880	27.1
ALL	Q3	dominant	3	bmc1605	98.36	3.07	0.02	0.0434	0.3
ALL	Q4	additive	7	bmc1305	63.18	7.03	3.53	0.4367	7.8
ALL	Q4	dominant	7	bmc1305	63.18	7.03	0.16	0.1152	-0.6
ALL	Q5	additive	9	bmc1714	55.72	9.04	2.17	-0.4128	6.8
ALL	Q5	dominant	9	bmc1714	55.72	9.04	0.13	-0.1145	0.3

^a Interval between two markers determined by “Maize Mapping Project” (www.maizemap.org) (Gardiner al, 1993)

^b Log of the Likelihood odds ratio (significant threshold LOD = 2.5)

^c Alleles provided by Susceptible (-) and Resistant (+) parent.

TABLE 3 – ANOVA for *C. zea-maydis* severity based on the 1-to-9 scale, evaluated by Near Isogenic Lines. Iraí de Minas and Mineiros – 2002/2003 growing season.

Source	df	Iraí de Minas		Mineiros		Joint Analysis	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Q1	1	32.6667	<.0001	13.5000	0.001	44.0833	<.0001
Q2	1	17.6333	0.001	24.3000	<.0001	41.6667	<.0001
Q3	1	30.3343	<.0001	13.5565	0.001	42.2241	<.0001
Q1*Q2	1	33.0917	<.0001	9.0008	0.004	38.3045	<.0001
Q1*Q3	1	0.7758	0.463	3.5030	0.065	3.7879	0.080
Q2*Q3	1	3.6465	0.116	2.9192	0.091	6.5455	0.022
Q1*LOCATION	1					2.0830	0.192
Q2*LOCATION	1					0.2666	0.639
Q3*LOCATION	1					1.6666	0.242

Q*Q = epistatic interactions between the corresponding QTL.

Q*LOCATION = QTL x location interactions.

TABLE 4 – Estimates of the effects of the QTL for *C. zea-maydis* resistance to infection based on the 1-to-9 scale, obtained by regression analysis by the evaluation of the Near Isogenic Lines. Iraí de Minas and Mineiros – 2002/2003 growing season.

	Iraí de Minas			Mineiros			Joint Analysis		
	Estimate	Error	Pr > t	Estimate	Error	Pr > t	Estimate	Error	Pr > t
Intercept	7.8	0.5063	<.0001	7.1	0.4203	<.0001	7.4	0.3780	<.0001
Q1	-3.4	0.5514	<.0001	-2.4	0.4577	<.0001	-2.9	0.3601	<.0001
Q2	-2.3	0.5514	0.000	-1.8	0.4577	0.000	-2.0	0.3601	<.0001
Q3	-1.3	0.5860	0.028	-1.1	0.4864	0.030	-1.2	0.3827	0.002
Q1*Q2	3.2	0.6552	<.0001	1.7	0.5438	0.004	2.4	0.4278	<.0001
Q1*Q3	0.4	0.6579	0.557	0.9	0.5461	0.091	0.7	0.4296	0.124
Q2*Q3	-1.1	0.6579	0.116	-0.9	0.5461	0.091	-1.0	0.4296	0.022

Q*Q = epistatic interactions between the corresponding QTL.

TABLE 5 – ANOVA for *C. zea-maydis* severity based on the 1-to-9 scale, evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.

SOURCE	df	Iraí de Minas		Mineiros		Joint Analysis	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Q1	1	3.267	0.029	8.438	0.038	11.102	0.004
Q2	1	3.146	0.032	1.471	0.378	4.459	0.062
Q3	1	6.889	0.002	15.838	0.005	21.809	<.0001
Q1*Q2	1	0.126	0.662	0.003	0.971	0.082	0.799
Q1*Q3	1	0.076	0.733	1.658	0.349	1.223	0.326
Q2*Q3	1	1.294	0.164	3.135	0.200	4.229	0.069
Q1*LOCATION	1					0.602	0.490
Q2*LOCATION	1					0.157	0.724
Q3*LOCATION	1					0.918	0.394

Q*Q = epistatic interactions between the corresponding QTL.

Q*LOCATION = QTL x location interactions

TABLE 6 – ANOVA for Grain Moisture evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.

SOURCE	df	Iraí de Minas		Mineiros		Joint Analysis	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Q1	1	0.8402	0.222	2.0167	0.246	0.1268	0.723
Q2	1	0.0030	0.941	0.6328	0.514	0.3615	0.550
Q3	1	4.4494	0.006	0.7356	0.481	4.4017	0.039
Q1*Q2	1	2.8406	0.027	6.7066	0.037	9.1384	0.003
Q1*Q3	1	1.3011	0.130	0.8613	0.446	2.1398	0.148
Q2*Q3	1	0.8758	0.212	1.7455	0.280	2.5470	0.115
Q1*LOCATION	1					2.7301	0.102
Q2*LOCATION	1					0.2743	0.603
Q3*LOCATION	1					0.7833	0.380

Q*Q = epistatic interactions between the corresponding QTL.

Q*LOCATION = QTL x location interactions

TABLE 7 – Estimates of the effects of the QTL for *C. zea-maydis* resistance to infection on Grain Moisture, obtained by regression analysis by Near Isogenic Hybrids evaluation. Iraí de Minas and Mineiros – 2004/2005 growing season.

Join Analysis			
	Estimate	Error	Pr > t
Intercept	23.21	0.4222	<.0001
Q1	-0.30	0.4645	0.520
Q2	-0.79	0.4645	0.093
Q3	-0.06	0.4645	0.906
Q1*Q2	1.52	0.5450	0.006
Q1*Q3	-0.62	0.5450	0.259
Q2*Q3	0.73	0.5519	0.191

Q*Q = epistatic interactions between the corresponding QTL.

TABLE 8 – ANOVA stalk lodging evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.

SOURCE	df	Iraí de Minas		Mineiros		Joint Analysis	
		MS	Pr>F	MS	Pr>F	MS	Pr>F
Q1	1	2.8976	0.704	11.1364	0.178	12.6976	0.324
Q2	1	282.9000	0.000	0.6624	0.741	128.0923	0.002
Q3	1	109.8116	0.023	0.0100	0.968	53.8627	0.044
Q1*Q2	1	106.8084	0.024	6.3090	0.309	30.6000	0.127
Q1*Q3	1	101.6458	0.028	2.9495	0.486	69.6126	0.022
Q2*Q3	1	47.3409	0.129	1.8169	0.584	33.8534	0.109
Q1*LOCATION	1					1.3364	0.748
Q2* LOCATION	1					155.4701	0.001
Q3* LOCATION	1					55.9589	0.040

Q*Q = epistatic interactions between the corresponding QTL.

Q*LOCATION= QTL x location interactions

TABLE 9 – ANOVA for grain yield corrected for 15.5% moisture, evaluated by Near Isogenic Hybrids. Iraí de Minas and Mineiros – 2004/2005 growing season.

SOURCE	df	MS	Pr>F
Q1	1	52.9596	0.329
Q2	1	271.8976	0.030
Q3	1	48.5569	0.349
Q1*Q2	1	0.8147	0.903
Q1*Q3	1	12.6553	0.632
Q2*Q3	1	137.4732	0.118

Q*Q = epistatic interactions between the corresponding QTL.

Table 10 – Summary of QTL effects for *Cercospora zeae-maydis* severity evaluated on a 1-to-9 scale (Cz), Grain Moisture (GM), Stalk Lodging (SL) and Grain Yield (GY) evaluated in the NIHS in two locations and joint analysis over locations.

Location	Trait	Cz			GM			SL			GY		
	QTL Effect	Q 1	Q 2	Q 3	Q 1	Q 2	Q 3	Q 1	Q 2	Q 3	Q 1	Q 2	Q 3
Iraí de Minas	Q1	*	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns
	Q2		*	ns		ns	ns		**	ns		*	ns
	Q3			**			**			*			ns
Mineiros	Q1	*	ns	ns	ns	*	ns	ns	ns	ns			
	Q2		ns	ns		ns	ns		ns	ns			
	Q3			**			ns			ns			
Joint Analysis	Q1	**	ns	ns	ns	**	ns	ns	ns	*			
	Q2		ns	ns		ns	ns		**	ns			
	Q3			**			*			*			
	QTL* Location	ns	ns	ns	ns	ns	ns	ns	**	*			

*, ** Pr>F significant at 1% and 5% of probability, respectively

ns = non significant

QTL*Location = QTL*Location Interaction

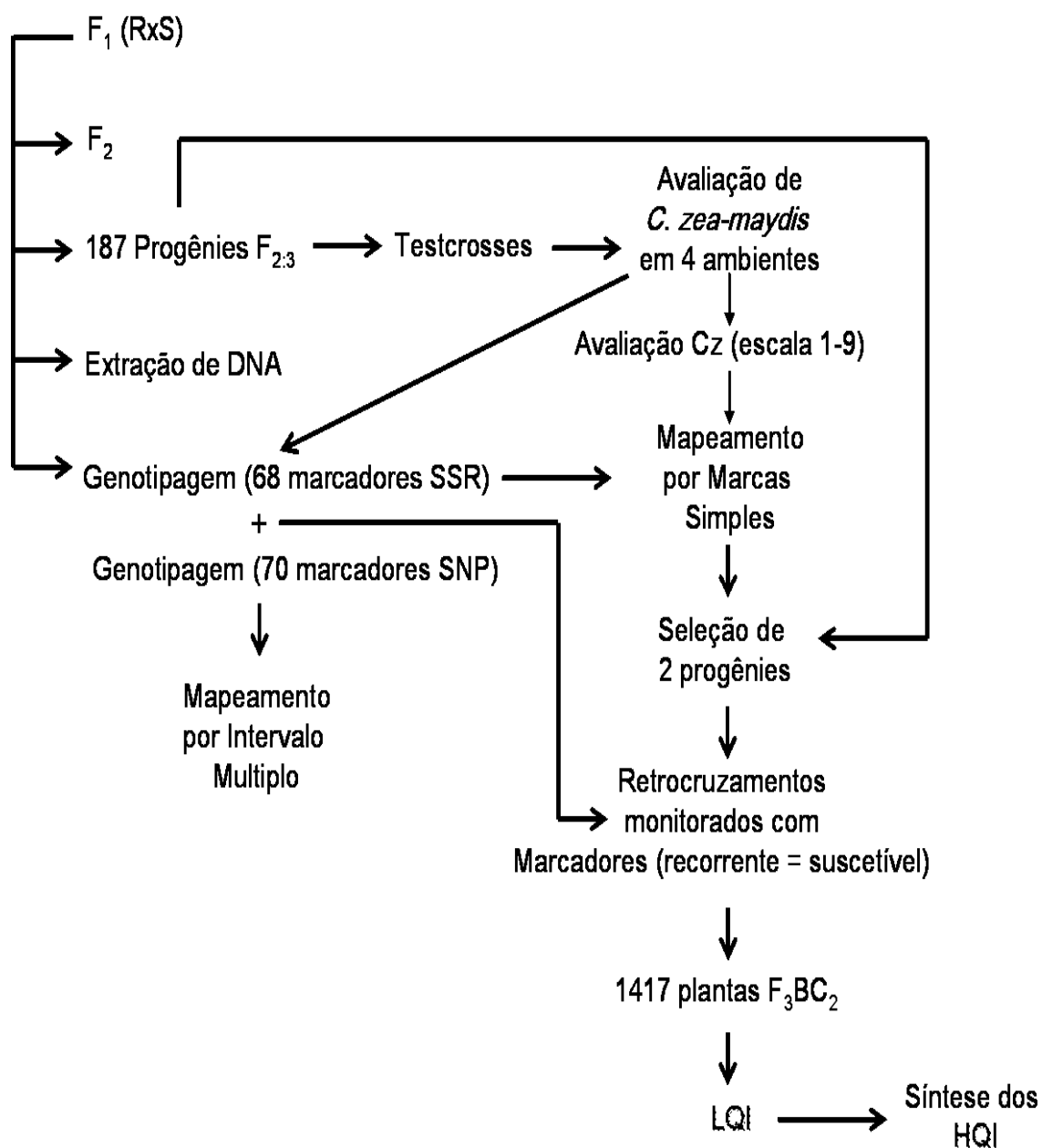


Figura 1 – Esquema resumido mostrando passo a passo o mapeamento dos QTL de resistência a *C. zeae-maydis* e o desenvolvimento das Linhagens e Híbridos Quase Isogênicos (LQIs e HQIs).

Marker	bin	pr(F)	Position cM	Prog 1	Prog 2
bmc1014	1.02	ns	33.9	D	H
bmc1178	1.02	*	47.4	-	D
bmc1007	1.03	*	60.7	H	D
bngl182	1.05	*	61.5	H	D
bmc1016	1.05	*	91.3	H	D
bmc1811	1.05	ns	102.6	D	D
bmc1598	1.06	ns	109.9	D	D
bmc1025	1.07	**	136.3	D	H
bmc1643	1.10	**	160.5	D	H
bmc1556	1.10	**	213.7	D	D
phi064	1.12	ns	270.5	D	H
bmc1144	3.02	ns	39.1	H	H
bmc1904	3.04	ns	58.5	R	D
bmc1456	3.05	**	83.3	H	D
bmc1035	3.05	**	85.0	H	D
bmc1505	3.06	**	88.3	H	D
bmc1047	3.06	**	102.1	D	D
bmc1605	3.07	**	112.3	D	D
bmc1006	5.01	ns	15.6	D	H
bmc1046	5.03	ns	83.7	D	H
bmc1287	5.05	ns	106.8	D	H
bmc1208	5.05	ns	111.2	D	H
bmc1237	5.08	ns	167.1	H	H
bmc1346	5.09	ns	189.0	R	H
bmc1306	5.09	ns	198.6	R	H
bmc1695	5.09	ns	210.0	R	H
bmc2132	7.00	ns	17.4	D	H
phi112	7.01	ns	39.8	D	D
bmc1094	7.02	ns	55.7	D	D
bmc1305	7.03	*	77.5	D	D
bmc1666	7.04	**	108.7	D	D
phi116	7.06	ns	167.0	H	H
bmc1583	9.01	ns	29.1	D	R
phi061	9.02	*	55.4	D	H
bmc1714	9.04	*	75.6	D	H
bmc1129	9.05	ns	102.2	H	R
bmc1191	9.07	ns	126.9	H	R
bmc1375	9.07	ns	136.4	H	R
bmc1338	2.01	ns	23.6	H	D
bmc1297	2.01	ns	31.1	H	D
bmc1064	2.04	*	78.8	H	H
bmc1831	2.05	ns	101.6	R	H
bmc1138	2.06	ns	114.4	R	R
bmc1662	2.07	ns	143.8	R	H
phi072	4.01	ns	15.0	R	H
nc004	4.04	ns	52.5	H	H
nc005	4.05	ns	71.4	D	R
bmc1265	4.06	ns	84.7	D	R
bmc1217	4.06	ns	89.9	-	R
bmc2291	4.08	ns	117.8	D	H
bmc1189	4.08	ns	129.4	H	H
bmc2162	4.09	ns	147.7	H	H
bngl589	4.11	*	178.8	H	H
bmc1600	6.01	ns	1.3	H	R
bmc1538	6.01	*	19.8	H	R
umb1018	6.01	**	-	R	R
nc009	6.03	ns	61.0	H	R
bmc1154	6.04	ns	70.6	H	D
bmc1702	6.05	ns	87.7	-	R
dupssr15	6.06	ns	119.7	D	D
bmc1521	6.08	ns	158.4	-	-
bmc1073	8.01	ns	13.1	H	R
bmc1863	8.03	ns	61.8	H	R
bmc1176	8.04	ns	78.1	D	R
bmc2181	8.05	ns	83.8	H	R
bmc1812	8.05	ns	87.9	H	R
bmc1065	8.06	ns	119.9	H	H
dupssr14	8.07	ns	139.8	-	H
bmc1131	8.09	ns	168.0	H	-
phi118	10.01	ns	20.7	H	R
bmc1074	10.04	ns	70.0	H	H
bmc1360	10.07	ns	123.9	D	H

D	Donor (Resistant parent) chromossomic segment
R	Recurrent (Susceptible parent) chromossomic segment
H	Heterozigous

Figure 2 – Single Marker Analysis Mapping Results showing the markers, pr(F), distance(cM) and bin for the 10 chromosomes; and genotype of the two $F_{2:3}$ progenies selected for the backcross program.

NIL Genotype	Genotype code	chrom. 1		chrom. 3	Number of NIL evaluated per genotype	Avg. % of Recurrent Parent Recovered
		Q1	Q2	Q3		
Recurrent Parental		R				
Donor Parental			D	D		
0-0-0 (q1-q2-q3)					2	87
1-0-0 (Q1-q2-q3)	Q1	R			3	82
0-1-0 (q1-Q2-q3)	Q2		D		3	82
0-0-1 (q1-q2-Q3)	Q3			D	3	81
1-1-0 (Q1-Q2-q3)	Q1 Q2	R	D		3	82
0-1-1 (q1-Q2-Q3)	Q2 Q3		D	D	2	76
1-0-1 (Q1-q2-Q3)	Q1 Q3	R		D	2	88
1-1-1 (Q1-Q2-Q3)	Q1 Q2 Q3	R	D	D	3	78

q = QTL susceptible allele (0)
 Q = QTL resistant allele (1)
 R = Recurrent Parent (susceptible to Cz)
 D = Donor Parent (Resistant to Cz)

	Susceptible QTL allele
R	Resistant QTL allele from Recurrent Parent
D	Resistant QTL allele from Donor Parent

Figure 3 – Near Isogenic Line (NIL) genotype, genotype code, number of NILs evaluated per genotype and average percentage of the recurrent parent recovered in the NILs.

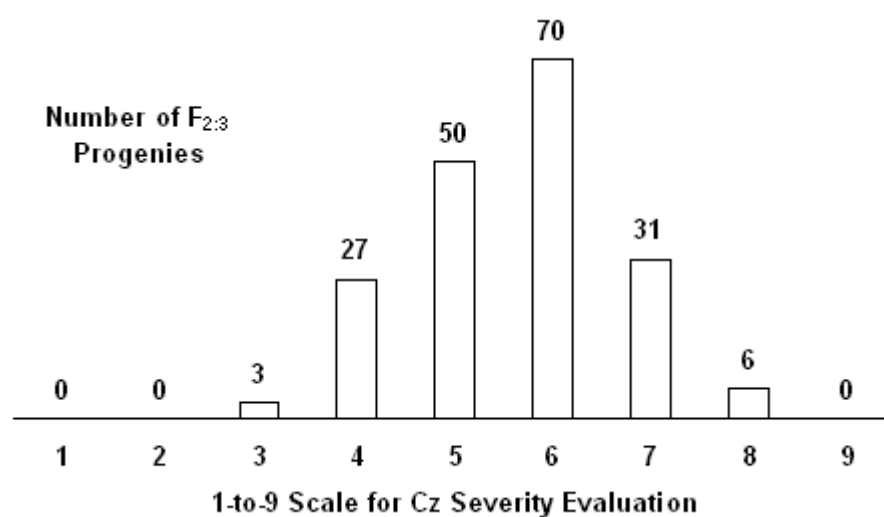


Figure 4 – Frequency distribution of *Cercospora zeae-maydis* severity based on 1-9 scale, of 187 $F_{2:3}$ progenies as average of four locations.

