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ORIGINAL PAPER

Mapping and validation of quantitative trait loci for resistance to *Cercospora zeae-maydis* infection in tropical maize (*Zea mays* L.)

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Abstract Breeding for resistance to gray leaf spot, caused by *Cercospora zeae-maydis* (Cz) is paramount for many maize environments, in particular under warm and humid growing conditions. In this study, we mapped and characterized quantitative trait loci (QTL) involved in the resistance of maize against Cz. We confirmed the impact of the QTL on disease severity using near-isogenic lines (NILs), and estimated their effects on three major agronomic traits using their respective near isogenic hybrids (NIHs), which we obtained by crossing the NILs with an inbred from a complementary heterotic pool. We further validated three of the four QTL that were mapped using the Multiple Interval Mapping approach and showed LOD values >2.5. NILs genotype included all combinations between favorable alleles of the two QTL located in chromosome 1 (Q_1 in bin 1.05 and Q_2 in bin 1.07), and the allele in chromosome 3 (Q_3 in bin 3.07). Each of the three QTL separately significantly reduced the severity of Cz. However, we found an unfavorable epistatic interaction between Q_1 and Q_2: presence of the favorable allele at one of the QTL allele effectively nullified the effect of the favorable allele at the other. In contrast, the interaction between Q_2 and Q_3 was additive, promoting the reduction of the severity to a greater extent than the sum of their individual effects. When evaluating the NIH we found significant individual effects for Q_1 and Q_3 on gray leaf spot severity, for Q_2 on stalk lodging and grain yield, and for Q_3 on grain moisture and stalk lodging. We detected significant epitasis between Q_1 and Q_2 for grain moisture and between Q_1 and Q_3 for stalk lodging. These results suggest that the combination of QTL impacts the effectiveness of marker-assisted selection procedures in commercial product development programs.

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Introduction

When compared to temperate growing areas, tropical to sub-tropical environments often require crops with higher level of defensiveness, that is, a better tolerance to multiple biotic and abiotic stresses, to produce profitable grain yields. An important component of this needed defensiveness is adequate resistance to diseases. Outbreak of new diseases or the presence of preexisting physiological races frequently force commercial seed companies to remove varieties and hybrids from the market. 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. Brazil, with most of the 8.5 million hectares cultivated with hybrid maize (*Zea mays* L.) grown between 10° and 30° south has many such environments (CONAB—Companhia Nacional de Abastecimento, http://www.conab.gov.br/).

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 in 2000, in particular at altitude above 700 m in the highlands of the states of Mato Grosso, Goiás, and Minas Gerais. Several high-yielding hybrids susceptible to Gray Leaf Spot had to be removed from the market. Currently, the incorporation of resistance to infection by Cz ranks among the most important objectives of maize breeding programs and the utilization of molecular markers has led to the mapping and characterization of several quantitative trait loci (QTL) related to its resistance.

Quantitative trait loci for resistance to infection by Cz have been mapped in all ten maize chromosomes (Bubeck et al. 1993; Saghai Maroof et al. 1996; Clements et al. 2000; Lehmensiek et al. 2001; Pedrosa et al. 2002; 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 were 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 that analysis were those reported by Bubeck et al. (1993), Saghai Maroof et al. (1996), Clements et al. (2000), Lehmensiek 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 percentage is due to 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 and 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.

The goals of the present study were (1) to map QTL associated with resistance to infection by Cz in tropical maize germplasm, (2) to validate these QTL using near isogenic lines (NILs), and (3) to estimate the effects of these QTL on three important agronomic traits using near isogenic hybrids (NIHs).

Materials and methods

QTL mapping

Generation and evaluation of the segregating population

During the 2001 growing season, 187 testcrosses of $F_{2:3}$ families 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 four locations: Iraí de Minas-MG (951 m altitude, 19°00′S and 47°05′W) as summer (s) and winter (w) crops, (IR_s and IR_w, respectively), Montividiu-GO (821 m altitude, 17°04′S and 51°02′W) (MV_s), and Jataí-GO (708 m 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} P_2O_5 and 100 kg ha^{-1} K_2O, 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}).

Cz severity evaluation

The disease reaction was evaluated visually by means of a class number ranging from 1 to 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).

Genotyping of $F_{2:3}$ families

Genotyping involved the removal of leaf tissue samples from ten 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 et al. (1983).

DNA was quantified by diluting 20 μL of DNA solution in 980 μL of TE 0.1× 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): Ultra-PureTM Agarose 1000 (Invitrogen), with 2∝L EtBr (ethidium bromide), TBE 1x buffer, at 160–170 V for 3–3.5 h. An extra amount of EtBr (8∝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 1–10, respectively.

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_woftware/) (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) (Jensen 1992, 1993; Zeng 1993, 1994), which threshold was determined with 1,000 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).

QTL × environment interactions

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

Production and evaluation of the NILs

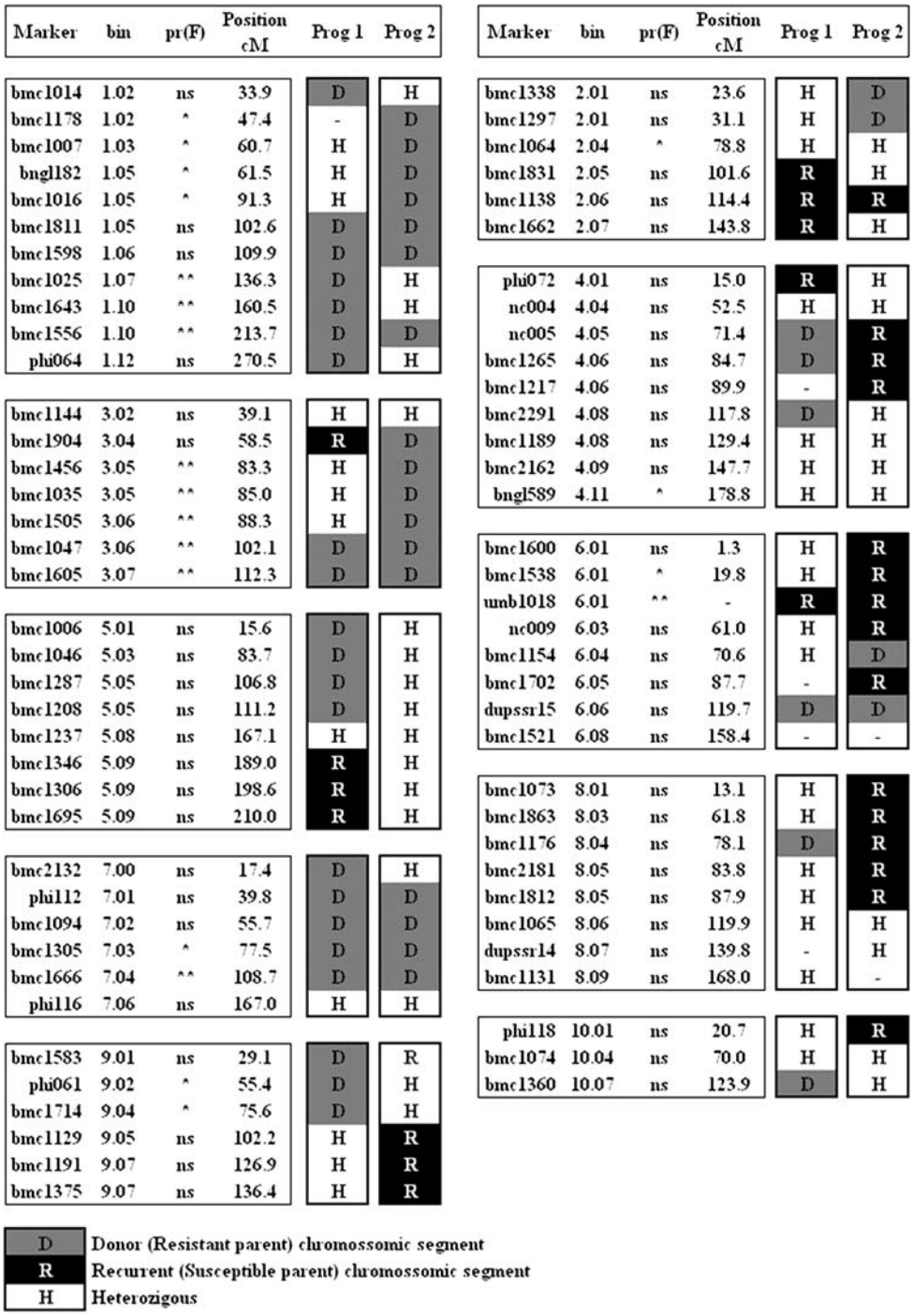
Backcross program

Production of the NILs began with a preliminary mapping through Single Marker Analysis (SMA) and using only SSR markers (Fig. 1). 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 $F_{2:3}$ families evaluated, two were selected as the most promising to initiate the BC program (Fig. 1). In the summer of 2001, the F_3BC_1 generation was synthesized by backcrossing ten individual plants from each of the selected family 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. For the introduction of Q_1, Q_2, Q_3, Q_4, and Q_5, chromosome segments measuring about 45.3, 219.2, 95.2 95.2, and 99 cM, respectively, were selected from the recurrent parental line.

Evaluation of NILs

In the summer of 2002, 18 NILs, carrying all QTL combinations (000–111 for Q_1, Q_2, and Q_3) (Fig. 2), were evaluated per se for Cz reaction in Mineiros-GO and Iraí de Minas-MG. The experimental design used was a randomized complete block (RCBD) with three replications in a 3×2 factorial scheme (three QTL, presence and absence). Planting was done mechanically and the plots comprised of



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

Marker	bin	pr(F)	Position cM	Prog 1	Prog 2
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

Fig. 1 Single marker analysis mapping results showing the markers, pr(F), distance (cM) and bin for the 10 chromosomes; and genotype of the two $F_{2:3}$ families selected for the Backcross Program

one 3-m-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.

Statistical analysis

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

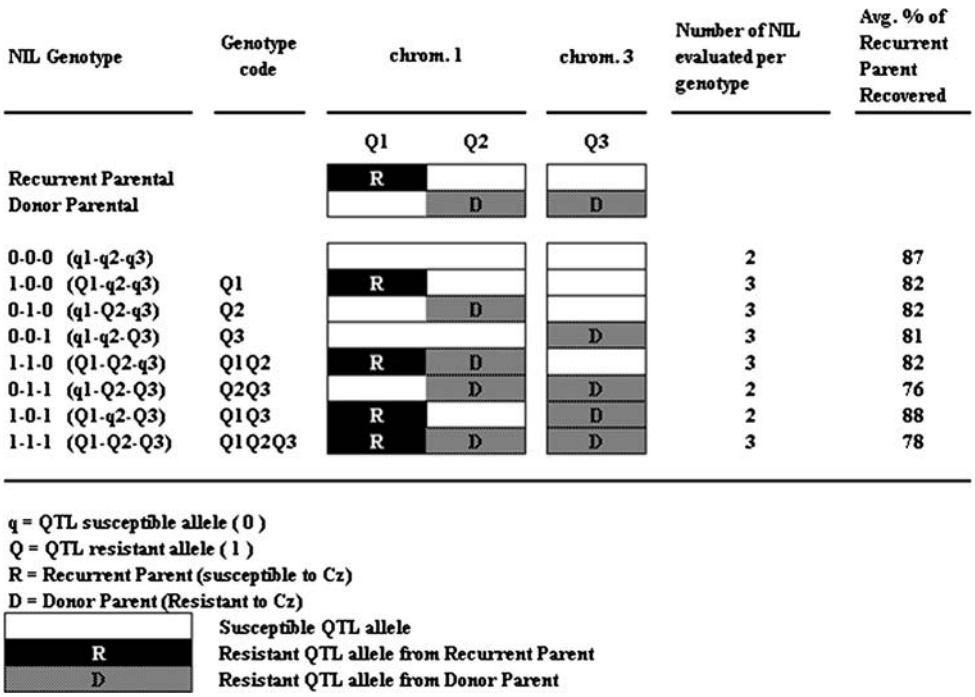


Fig. 2 Near isogenic line (*NIL*) genotype, genotype code, number of NIL evaluated per genotype and average percentage of Recurrent Parent Recovered in the NIL

$$y_{ij} = \mu + b_j + \beta_1 Q_{1i} + \beta_2 Q_{2i} + \beta_3 Q_{3i} + \beta_4 (Q_{1i} \times Q_{2i}) + \beta_5 (Q_{1i} \times Q_{3i}) + \beta_6 (Q_{2i} \times Q_{3i}) + \beta_7 (Q_{1i} \times Q_{2i} \times Q_{3i}) + e_{ij}$$

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:

$$y_{ija} = \mu + l_a + b_j(l_a) + \beta_1 Q_{1i} + \beta_2 Q_{2i} + \beta_3 Q_{3i} + \beta_4 (Q_{1i} \times Q_{2i}) + \beta_5 (Q_{1i} \times Q_{3i}) + \beta_6 (Q_{2i} \times Q_{3i}) + \beta_7 Q_{1i} \times l_a + \beta_8 Q_{2i} \times l_a + \beta_9 Q_{3i} \times l_a + \beta_{10} (Q_{1i} \times Q_{2i}) \times l_a + \beta_{11} (Q_{1i} \times Q_{3i}) \times l_a + \beta_{12} (Q_{2i} \times Q_{3i}) \times l_a + \beta_{13} (Q_{1i} \times Q_{2i} \times Q_{3i}) \times l_a + e_{ija}$$

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_k 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 with the observation y_{ija}, $e_{ij} \sim N(0, \sigma^2)$

A 0.05 P value was adopted for all models and effects. To obtain better QTL effect estimates all non significant effects were dropped from the initial models.

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 (Fig. 2) were crossed with a complementary heterotic group inbred derived from Tropical Dent × 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

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

Location	Average[b]	Range[a]		SD
		Min	Max	
IR_s	5.65 ± 0.09	2	8	1.3409
JT_s	7.06 ± 0.11	3	9	1.5747
MV_s	5.32 ± 0.10	2	8	1.4678
IR_w	4.72 ± 0.08	2	7	1.1490

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

IR_w = Iraí de Minas winter crop

SD Standard deviation

[a] Range of *Cercospora zeae-maydis* severity evaluated on a 1-to-9 scale with the Minimum and the Maximum values

[b] Mean ± standard error

to *Phaeosphaeria maydis* (Henn.) Rane, Payak, & Renfro (anamorph = *Phoma maydis*, synonym = *Leptosphaeria zeae-maydis* Saccas; *Metasphaeria maydis* (Henn.) Höhnel) (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 5-m-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} P_2O_5, and K_20, 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 were analyzed using the same models as those described for the NILs.

Results

QTL mapping for Cz infection resistance

Severity of Cz

The highest severity of Cz occurred in JT_s with average of 7.1 ± 0.11 evaluated on a 1-to-9 scale. The lowest occurred in IR_s with average of 4.7 ± 0.08 (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.

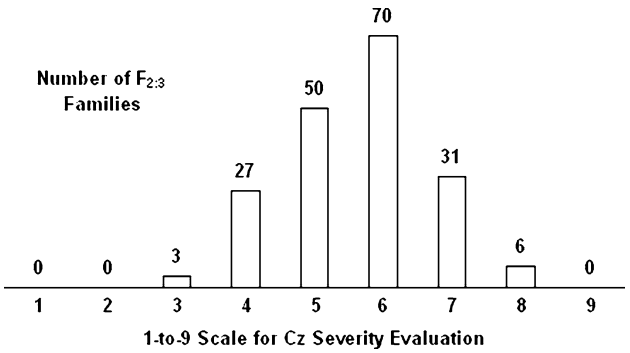


Fig. 3 Frequency distribution of *Cercospora zeae-maydis* severity of 187 $F_{2:3}$ families evaluated by 1-to-9 scale based on the average over locations

Linkage groups

Of the 138 markers used for mapping the QTL, 117 were grouped and associated with the ten 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 distances were 14.7 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.

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 (Q_1) 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 (Q_2) 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 (Q_3) 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 (Q_4) originating 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 (Q_5) originating 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 Q_3, as will be discussed later.

Epistatic interactions

Some epistatic interactions of minor effects were detected in the multiple interval mapping (MIM) analysis at IR_s, MV_s and in the combined analysis of the locations. In IR_s, an additive x additive interaction between bmc1505 bin 3.06 * bmc1094 bin 7 with LOD = 0.7 explaining 3.6% of the PV. In MV_s, one additive × additive interaction between Q_3 and Q_5 with LOD = 1.8 explained 5.2% 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 (bmc1598 bin 1.06 * bmc1094 bin 7.02 and bngl182 bin 1.05 * bmc1129 9.05, both additive x dominant; and bmc1598 bin 1.06 * bmc1714 bin 9.04, additive x additive).

QTL × 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.

Evaluation of the NILs

NIL genotypes

The average percentage of the recurrent (susceptible) genotype recovered after the second BC was 81.5 ± 2.5%,

Table 2 *Cercospora zeae-maydis* resistant QTL mapped by the multiple interval mapping method

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	Q_3	Additive	3	bmc1605	73.36	3.07	0.03	0.1032	1.2
IRA_s	Q_3	Dominant	3	bmc1605	73.36	3.07	1.94	−1.2856	7.0
IRA_s	Q_4	Additive	7	bmc1305	63.26	7.03	2.61	0.6371	7.7
IRA_s	Q_4	Dominant	7	bmc1305	63.26	7.03	0.06	−0.1244	0.3
JAT_s	Q_3	Additive	3	bmc1605	95.36	3.07	4.82	1.2121	25.6
JAT_s	Q_3	Dominant	3	bmc1605	95.36	3.07	0.43	0.4625	3.4
MV_s	Q_1	Additive	1	bmc1811	152.93	1.05	3.16	−0.6973	7.8
MV_s	Q_1	Dominant	1	bmc1811	152.93	1.05	0.01	0.0415	0.1
MV_s	Q_2	Additive	1	bmc1025	225.65	1.07	3.18	0.7187	10.6
MV_s	Q_2	Dominant	1	bmc1025	225.65	1.07	0.03	0.0989	0.1
MV_s	Q_3	Additive	3	bmc1047	61.99	3.06	2.59	0.6502	6.1
MV_s	Q_3	Dominant	3	bmc1047	61.99	3.06	0.09	0.1572	0.0
IRA_w	Q_3	Additive	3	bmc1605	102.36	3.07	2.52	0.6675	12.0
IRA_w	Q_3	Dominant	3	bmc1605	102.36	3.07	0.00	−0.0009	0.0
ALL	Q_3	Additive	3	bmc1605	98.36	3.07	7.41	0.7880	27.1
ALL	Q_3	Dominant	3	bmc1605	98.36	3.07	0.02	0.0434	0.3
ALL	Q_4	Additive	7	bmc1305	63.18	7.03	3.53	0.4367	7.8
ALL	Q_4	Dominant	7	bmc1305	63.18	7.03	0.16	0.1152	−0.6
ALL	Q_5	Additive	9	bmc1714	55.72	9.04	2.17	−0.4128	6.8
ALL	Q_5	Dominant	9	bmc1714	55.72	9.04	0.13	−0.1145	0.3

Based on phenotypic evaluation of disease severity using 1-to-9 score scale at four locations, under natural disease occurrence 2001/2002 growing season

[a] Interval between two markers determined by "Maize Mapping Project" (www.maizemap.org) (Gardiner et al. 1993)

[b] Likelihood odds ratio (significant threshold LOD = 2.5)

[c] Alleles provided by Suceptible (−) and Resistant (+) parent

Table 3 F statistic *P* value for *Cercospora zeae-maydis* severity evaluation (based on 1-to-9 scale) by both near isogenic lines (NIL) and near isogenic hybrids (NIH); and for Grain moisture (%), stalk lodging (%) and grain yield (ton/ha), evaluated by NIH in two locations

Source	*df*	Pr > F												
		Near isogenic lines[a]			Near isogenic hybrids[b]									
		Cercospora zeae-maydis severity						Grain moisture (%)			Stalk lodging (%)			Grain yield (ton/ha)
		IM	MN	JA	IM	MN	JA	IM	MN	JA	IM	MN	JA	IM
Q_1	1	<.0001	<.0001	0.0002	0.0307	0.0382	0.0038	0.222	0.246	0.723	0.704	0.178	0.324	0.329
Q_2	1	<.0001	<.0001	<.0001	0.0338	0.3782	0.0629	0.941	0.514	0.550	0.000	0.741	0.002	0.030
Q_3	1	<.0001	<.0001	<.0001	0.0022	0.0053	<.0001	0.006	0.481	0.039	0.023	0.968	0.044	0.349
$Q_1 \times Q_2$	1	<.0001	0.004	0.0002	0.6643	0.9708	0.7992	0.027	0.037	0.003	0.024	0.309	0.127	0.903
$Q_1 \times Q_3$	1	0.463	0.065	0.4804	0.7335	0.3497	0.3269	0.130	0.446	0.148	0.028	0.486	0.022	0.632
$Q_2 \times Q_3$	1	0.116	0.091	0.2657	0.1676	0.2002	0.0699	0.212	0.280	0.115	0.129	0.584	0.109	0.118
$Q_1 \times Q_2 \times Q_3$	1	0.539	0.559	0.7035	0.7122	0.3336	0.5179							
$Q_1 \times$ Loc	1			0.3109			0.4911			0.102			0.748	
$Q_2 \times$ Loc	1			0.0895			0.7247			0.603			0.001	
$Q_3 \times$ Loc	1			0.1063			0.3955			0.380			0.040	
$Q_1 \times Q_2 \times$ Loc	1			0.7688			0.8483							
$Q_1 \times Q_3 \times$ Loc	1			0.2577			0.5257							
$Q_2 \times Q_3 \times$ Loc	1			0.5193			0.6909							
$Q_1 \times Q_2 \times Q_3 \times$ Loc	1			0.4162			0.3061							

Qn QTL effect, *Qn* × *Qn* epistatic interactions between the corresponding QTL, *Qn* × *Loc* QTL × Location interaction, *IM, MN and JA* Iraí de Minas, Mineiros and joint analysis over location, respectively

[a] Evaluated in 2002/2003 growing season

[b] 2004/2005 growing season, respectively

with total amplitude varying from 73 to 90%. Thirty percent of the NILs fell within the class of 86–90% 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 Q_1 and Q_2 occurring in the repulsion phase, both alleles were recovered in the same NIL.

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 Q_1, 2.3 for Q_2, and 1.3 for Q_3 (Table 4). These reductions represented mean score values of 4.4, 5.5, and 6.5 for the NILs containing Q_1, Q_2, and Q_3, respectively. The epistatic interaction $Q_1 \times Q_2$ 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. 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 Q_1, Q_2, and Q_3 were 4.7, 5.3, and 6.0, respectively. The epistatic interaction $Q_1 \times Q_2$ 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. 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 Q_1, Q_2, and Q_3, with average scores of 4.5, 5.4, and 6.2, respectively (Table 4). The epistatic interaction $Q_1 \times Q_2$ promoted an average increase of 2.4 units in the severity. The average score of the NILs containing the two QTL was 4.9. The epistatic interaction $Q_2 \times Q_3$ 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 (Table 3). 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 $Q_1 \times Q_2 \times Q_3$ epistatic interaction effect was not significant at any location, it was not included in the final analysis. No QTL interacted significantly with any locations.

Table 4 Estimates of the effects of the QTL for *C. zeae-maydis* infection resistance based on the 1-to-9 scale, obtained by regression analysis by the evaluation of near isogenic lines; and for Grain moisture (%) obtained by near isogenic hybrid evaluation in two locations

Source	*Cercospora zeae-maydis* severity						Grain moisture (%)	
	IM[a]		MN[a]		JA[a]		JA (IM + MN)[b]	
	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
Intercept	7.8	<0.0001	7.1	<0.0001	7.4	<0.0001	23.21	<0.0001
Q_1	−3.4	<0.0001	−2.4	<0.0001	−2.9	<0.0001	−0.30	0.520
Q_2	−2.3	0.000	−1.8	0.000	−2.0	<0.0001	−0.79	0.093
Q_3	−1.3	0.028	−1.1	0.030	−1.2	0.002	−0.06	0.906
$Q_1 \times Q_2$	3.2	<0.0001	1.7	0.004	2.4	<0.0001	1.52	0.006
$Q_1 \times Q_3$	0.4	0.557	0.9	0.091	0.7	0.124	−0.62	0.259
$Q_2 \times Q_3$	−1.1	0.116	−0.9	0.091	−1.0	0.022	0.73	0.191

Qn QTL effect, *Qn* × *Qn* epistatic interactions between the corresponding QTL, *IM, MN and JA*́ Iraí de Minas, Mineiros and Joint Analysis over location, respectively

[a] Evaluated in 2002/2003 growing season

[b] 2004/2005 growing season, respectively

Evaluation of the NIHs

Effect of QTL on severity of Cz

Highly significant effects of Q_1 and Q_3 were found on the severity of Cz evaluated by means of NIHs for Q_1 and Q_3 (Table 3). Q_1 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. Q_3 reduced 0.6 and 1 units, with average scores of 4.5 and 5.1 in Iraí and Mineiros, respectively. The effect of Q_2 was significant only in Iraí. Significant epistatic or QTL × location interactions were not detected. The regression analysis did not indicate significance for any effect evaluated (data not shown).

QTL effects on grain moisture

A significant effect was detected of Q_3 on grain moisture (Table 3), although it was not detected by the regression analysis (Table 4). The average increase of GM on the NIHs containing Q_3 was 0.6%. The epistatic interaction $Q_1 \times Q_2$ was highly significant (Table 3). The joint occurrence of these QTL led to an increase of 1.5% in the GM. No QTL × location interaction was significant for this trait.

QTL effects on stalk lodging

Q_2 and Q_3 presented significant effects on stalk lodging (Table 3). 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 Q_2. The reduction promoted by Q_3 was 2.6%. A significant epistatic $Q_1 \times Q_3$ interaction was detected in the joint analysis of locations. Significance was also detected in Iraí for the $Q_1 \times Q_2$ interaction. Q_1 and Q_2 both interacted significantly with locations, thus reflecting their dissimilar performance in such locations.

QTL effects on grain yield

The effects of QTL on grain yield corrected to 15.5% moisture were evaluated only in Iraí (Table 3). Q_2 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.

Discussion

The average recovery percentage of the recurrent genotype after two BC cycles would have been higher if single mapping analysis (SMA) was more precise and more precision would have allowed for the selection of a smaller chromosome segment for backcrossing. For the introduction of the QTL, chromosome segments from the recurrent parental line, large enough to carry other genes than ones under selection were selected. 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), Q_3 is the only QTL that can

be classified as exerting a strong effect. It was the only one mapped in three out of the four locations. This QTL can therefore be classified as consistent (Collard et al. 2005).

Q_1 and Q_2 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 × 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 × environment interaction. A MAB program based solely on MIM results might fail to consider Q_1 and Q_2 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 Q_1 and Q_2 by means of NILs, however, indicated highly significant main effects for both (Tables 3, 4). The classification of these QTL as exerting minor effects was not confirmed by the analysis of the NILs. Q_3, 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 and 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. 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 200–400 individuals (Varona 2001 *apud* Carlborg and Haley 2004). Alternatively, the analyses of NILs detected significant epistatic interactions for the severity of Cz (Tables 3, 4).

Individually, Q_1 and Q_2 reduced 2.9 and 2.0 units in the 1-to-9 scale, respectively (Table 4). The simultaneous occurrence of Q_1 and Q_2, 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 Q_1 and Q_2 was 4.9, while the individual presence of Q_1 and Q_2 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 Q_2 and Q_3 (Table 3). The score of the average severity with the simultaneous occurrence of the two QTL was 3.2, while the individual scores for Q_2 and Q_3 were 5.4 and 6.2, respectively (Table 4). This interaction was not significant in the individual analysis of locations. The $Q_1 \times Q_3$ 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 Q_1 and Q_3. The highest efficacy in reducing the severity is therefore achieved through the combination of either Q_1 and Q_3 or Q_2 and Q_3. Shagai-Maroof 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 Q_2 and Q_3 or Q_1 and Q_3.

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 and 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 and 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 $F_{2:3}$ families 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 2 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 Q_2 in Mineiros or for the epistatic interactions between any combinations of QTL (Table 3).

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, Q_1, originating from the susceptible (recurrent) parental inbred, interacted significantly with Q_2 (Tables 2, 3), which originated from the resistant (donor) parental line. The interaction QTL × 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 and White 1998; Saghai Maroof et al. 1996; Clements et al. 2000). The strong link of Q_3 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 Q_3. Three QTL that increase the number of days for pollination were mapped in bin 3.05 (92.4, 90.4 and 81.9 cM) (CIMMYT: http://www.maizegdb.org/cgibin/qtllocisummarytable.cgi?sortby=8). One of them was associated to grain moisture (Melchinger et al. 1998), and others to the number of days to flowering at bin 3.08 (Abler et al. 1991). The highly significant epistatic interaction between Q_1 and Q_2 alleles (Tables 3, 4) 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, Q_2 and Q_3 had a significant effect on the reduction of stalk lodging (Table 3). 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 with 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 Q_2 and Q_3 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 Q_1 and Q_3 for stalk lodging (Table 3), 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 Q_1 and Q_3 had highly significant effects on the reduction of the severity of Cz, their effects on grain yield were not significant (Table 3). Conversely, Q_2 had a significant effect on the reduction of severity in Iraí and a significant, positive effect on grain yield (Table 3). 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 (Fig. 2), 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.

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