

Genetic mapping for resistance to gray leaf spot in maize

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Abstract The molecular marker technology has been used on mapping of quantitative trait loci (QTLs) associated with plant resistance. The objectives of this research were to estimate genetic parameters and to map genomic regions involved in the resistance to gray leaf spot in maize. Ninety F₃ families from the BS03 (susceptible) and BS04 (resistant) cross were used. Field trials were performed using a 10 × 10 square lattice design with three replications. Data from 62 SSR markers were used for linkage analysis. The locations of the QTLs on the linkage groups were determined by composite interval mapping method and the phenotypic variance explained by each marker was determined by regression analysis. Several QTLs

associated to disease resistance were identified in the population BS03 × BS04. Some QTLs showed significant effects over the different environments studied. The existence of significant QTLs in common among different environments indicates these genomic regions as possible new tools for marker-assisted selection in maize breeding programs.

Keywords *Zea mays* L. · QTLs ·
Cercospora zae-maydis · Molecular marker

Introduction

Maize (*Zea mays* L.) is one of the most important plant species. Currently it occupies a distinguished position in the national market, Brazil being the third greatest grower, coming right after the United States of America and China. In the 1999/2000 crop the world production reached 605.7 million tons (Brandalitze 2001). The diseases in maize have increased in number and incidence due to, above all, increased acreage on the Brazilian savannah vegetation in recent years. Therefore, many diseases cause decrease in grain yield, which can reach 60% of reduction in highly susceptible genotypes. Among the major diseases present in Brazil are: white spot or *Phaeosphaeria* leaf spot (*Phaeosphaeria maydis*, *Phylosticta maydis*, *Phoma sorghina* and *Pantoea ananatis*), common rust

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(*Puccinia sorghi*) and, more recently, gray leaf spot (GLS) (*Cercospora zea-maydis*; Juliatti 2009).

The fungus *Cercospora zea-maydis* Tehon and E. Y. Daniels was identified for the first time on specimens collected in southern Illinois, near the Mississippi river, in 1924 (Ward et al. 1999). In Brazil, even though the GLS was reported as being a secondary disease (Fernandes and Oliveira 1997), there was an epidemic outbreak in maize in the 1999/2000 crop in Brazilian Central Region (Rio Verde, Montividiu, Mineiros and Jataí), which caused a loss of 20–50% in yield, varying according to the genetic material or hybrid grown (Juliatti and Brandão 2001).

The ideal method for disease control in maize is to employ resistant cultivars, considering that every other method increases the costs and, in most cases, is not accessible to a great number of farmers (Vieira 1972). According to Ferreira and Grattapaglia (1998), plant breeding tends to integrate classic breeding techniques to those generated by biotechnology.

In many economically important plant pathosystems, disease is controlled by breeding for genetic resistance in the host plant. Plant pathologists and breeders recognize two general types of resistance: qualitative and quantitative. Qualitative resistance typically confers a high level of resistance, is usually race-specific, and is based on single dominant or recessive genes. In contrast, quantitative resistance in plants is typically partial and race-nonspecific in phenotype, oligogenic or polygenic in inheritance and is conditioned by additive or partially dominant genes. Although a great number of traits in plant species are controlled by qualitative genes, most of the economically important heritable characteristics are quantitatively natured, which means, they result from a joint action of many genes. The phenotype resultant presents a continuous distribution, instead of phenotypic discrete classes. The loci associated with the genetic control of these traits are named QLT—“Quantitative trait locus”. These traits are studied by statistical analysis to identify the QTLs associated with molecular markers, which may provide more precise information about the architecture of the quantitative trait loci (Melo 2000). The QTL mapping is based on the association between molecular markers and phenotypic data, using genetic-statistical models that involve variance analysis, simple and multiple linear regression, partial regression with or

without flanking markers and maximum likelihood methodology. The QTL mapping methodologies have permitted the estimation of number and localization of genes that control the phenotypic variance of a trait, the magnitude of their effects and the interactions with other QTLs. It allows a better comprehension of quantitative traits, besides tracking the genomic regions where the loci of interest are located.

Wisser et al. (2006) published a genetic architecture of disease resistance in maize. The confidence intervals of the QTL were distributed over all ten chromosomes and covered 89% of the genetic map to which the data were anchored. Visual inspection indicated the presence of clusters of QTL for multiple diseases. On these maps are also included the QTLs for resistance to GLS. The genetic resistance to GLS has been demonstrated to be ruled by a small number of quantitative loci, with five or more genes involved, which are additively, inherited (Bubeck et al. 1993; Saghai Maroof et al. 1996; Derera et al. 2008). There is limited information about the mode of inheritance for GLS resistance in regionally adapted germplasm. Additive effects were more important than nonadditive gene action.

Lehmensiek et al. (2001) using Bulked segregant analysis identified 11 polymorphic markers amplified fragment length polymorphism markers (AFLPs) linked to quantitative trait loci (QTLs) involved in the resistance to GLS in maize. They converted to sequence-specific PCR markers and five of the 11 converted AFLPs were linked to three GLS resistance QTLs in the maize chromosomes 1, 3 and 5. They studied linkage maps of two commercially available recombinant inbred-line populations. Shi et al. (2007) suggested that the combination of meta-analysis of gray leaf spot in maize and sequence homologous comparison between maize and rice could be an efficient strategy for identifying major QTLs and corresponding candidate genes for the gray leaf spot. The integration QTL map for gray leaf spot resistance in maize was constructed by compiling a total of 57 QTLs available with genetic map IBM2 2005 neighbors as reference. Twenty-six “real QTLs” and seven consensus QTLs were identified by refining these 57 QTLs using overview and meta-analysis approaches. Seven consensus QTLs were found on chromosomes 1.06, 2.06, 3.04, 4.06, 4.08, 5.03, and 8.06, and the map coordinates were 552.53, 425.72, 279.20,

368.97, 583.21, 308.68 and 446.14 cM, respectively. Pozar et al. (2009) suggest that the combination of QTL impacts the effectiveness of marker-assisted selection procedures in commercial product development programs.

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 GLS 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, 81.9 cM; CIMMYT: <http://www.aizegdb.org/cgi-bin/qtlocosummarytable.cgi?sortby=8>). For many authors QTL covered about 60% of the maize genome (Bubeck et al. 1993; Saghai Maroof et al. 1996; Clements et al. 2000; Lehmensiek et al. 2001; Pedrosa 2002; Gordon et al. 2004; Pozar et al. 2009). 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 \times host interaction. 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 \times host interaction (Wisser et al. 2006). The genotype \times 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 MAS (marker-assisted selection). 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 MAS. Results found by Pozar et al. (2009) demonstrate the utility and level of complexity that needs to be considered when using QTL to improve GLS infection resistance in a commercial breeding program.

The objective of this work was to identify molecular markers and genomic regions associated with GLS resistance in a maize population in three environments.

Materials and methods

Field experiments

In this study, 90 F_3 families from a cross between the lines BS03 (Syngenta seeds susceptible inbred line) and BS04 (Syngenta seeds resistant inbred line) were employed. The line BS03 is susceptible and BS04 is resistant to common rust, white spot (*Phaeosphaeria* leaf spot) and GLS. These two lines were developed by Syngenta Seeds, Maize Breeding Program, at Uberlândia, MG, Brazil. Sowing was performed in October/2000.

The 90 families plus the two parents and eight controls, totally 100 entries were evaluated for GLS resistance in a 10×10 square lattice with three replications at three different locations in Minas Gerais state: Uberlândia, Ipiacaçu and Campo Florido. These locations were chosen due to existing reports of GLS occurrence. Each plot consisted of ten 6-m long lines, 0.75 m between lines and six seeds per meter. The field trials were maintained under regular cultural practices for maize growth. The regions where the trials were established are known to present high levels of incidence and severity of the disease.

A 1–9 scale (Table 1) was used to score family reaction to GLS. The flowering plants (55-day-old seedling) were examined visually for leaf infection. Families were independently scored by two different evaluators at the time of maximum severity of diseases (maturity) under natural infection epidemic from GLS. Five plants per plot were sampled by two people and each plant was scored individually. Then the mean score for each plot was calculated.

Statistical analysis

In the experiments where the relative efficiency of the lattice design, compared with randomized complete block design was below 105%, the randomized complete block design was adopted. The broad sense heritability for each location was also estimated using the estimates of the variance components. Combined analysis of variance for a maize population in three locations, based on the GLS severity (SAS Institute 1989).

The following statistical model, presented by Viana (1993), was adopted for the individual intra-blocks

Table 1 Scale of scores used in the assessment of the reaction to gray leaf spot (*Cercospora zea-maydis*)

Scores	Severity-leaf area infected (%)
1	0
2	1
3	10
4	20
5	30
6	40
7	60
8	80
9	80–100

Source: Agroceres (1994)

analysis of the lattice: $Y_{i\ell(j)} = \mu + r_j + (b/r)_{\ell(j)} + t_i + e_{i\ell(j)}$, in which: $Y_{i\ell(j)}$ is the observed value of treatment i ($i = 1, 2, \dots, v = k^2$), in the incomplete block ℓ ($\ell = 1, 2, \dots, k$), of repetition j ($j = 1, 2, \dots, r$); μ is a constant inherent to all observations; r_j is the effect of replication j ; $(b/r)_{\ell(j)}$ is the effect of incomplete block ℓ inside replication j ; t_i is the effect of treatment i ; $e_{i\ell(j)}$ is the random error associated with observation $Y_{i\ell(j)}$. The treatment (entry) sum of squares were decomposed through orthogonal contrast into families, parents, parents versus families and other (checks, checks vs. families, checks vs. parents). The random model was adopted with variance components due to progeny effects ($\hat{\sigma}_g$), residual ($\hat{\sigma}^2$) and phenotypic ($\hat{\sigma}_f^2$) estimated according to Silva's (1997) indications.

Analysis of variance was performed for each location separately. The analysis was combined because the variances were homogeneous. The reason between the higher and the lower level values was smaller than seven (Banzatto and Kronka 1995). Since the error are homogeneous, combined analysis was performed across the three locations. For the combined analysis the flowing model was adopted: $Y_{i\ell(j)(k)} = \mu + \alpha_k + r_{j(k)} + (b/r)_{\ell(j)(k)} + t_i + \alpha t_{ik} e_{i\ell(j)(k)}$, in which: $Y_{i\ell(j)(k)}$ is the observed value of treatment i ($i = 1, 2, \dots, v = k^2$), in the incomplete block ℓ ($\ell = 1, 2, \dots, k$), of replication j ($j = 1, 2, \dots, r$) at location k ($k = 1, 2, 3$); μ is a constant inherent to all observations; α_k is the location k effect; $r_{j(k)}$ is the effect of replication j within location k ; $(b/r)_{\ell(j)(k)}$ is the effect of incomplete block ℓ inside replication j within location k ; t_i is the effect of treatment i ; αt_{ik} is

the treatment I by location k interaction effect; and $e_{i\ell(j)(k)}$ is the random error associated with observation $Y_{i\ell(j)(k)}$. As in the individual analysis, treatment and treatment \times location sum of squares were decomposed by orthogonal contrasts. The random model was adopted with variance components due to progeny effects ($\hat{\sigma}_g$), family \times location $\hat{\sigma}_{ga}$, residual ($\hat{\sigma}^2$) and phenotypic ($\hat{\sigma}_f^2$) estimated according to Silva's (1997) indications.

Identification of QTLs associated to resistance to gray leaf spot

The experiment was conducted in the Molecular Genetics Laboratory of Syngenta Seeds Ltd in Uberlândia, MG, Brazil. For plant DNA extraction, the MINIPREP method (Doyle and Doyle 1990) was used, with some modifications. About 2 g of young leaves were randomly sampled in each family, collected from 15 plants. These leaves were lyophilized in 96-well plates and then macerated. The powder obtained was re-suspended in 300 μ l of extraction buffer containing 700 mM NaCl, 100 mM Tris HCl, pH 8.0, 10 mM EDTA, 10% CTAB, 100% BME, pH between 8.0 and 9.0, favorable to enzyme action. Then, the samples were incubated in 65°C water bath for 40 min, with intermittent agitation every 10 min, which permitted the homogenization of the solution. After incubation, the plates were cooled on ice for 10 min and then 400 μ l of chloroform organic solvent: isopropanol (24:1) were added. The plates were then reversed for 2 min, put on ice for another 15 min and centrifuged at 3,200 rotations per minute (rpm) for 20 min. This way, proteins and cellular fragments were separated from DNA and the supernatant was transferred to another plate. This step was repeated to make sure enough amount of DNA will be obtained. Precipitation was accomplished with 300 μ l of previously cooled isopropanol. One more time, plates were centrifuged in the same conditions described above. The supernatant was discarded and the pellet washed twice using ethanol 70%, employing the same procedure as for centrifugation. After precipitation, DNA was re-hydrated in 100 μ l of buffer TE (10 mM Tris HCl, pH 7.5 and 1 mM EDTA).

After extraction, DNA quality was analyzed by electrophoresis in common agarose gel with a

constant voltage of 170 V during 60 min. Then, the DNA samples were quantified in a spectrophotometer, for absorbance to be read at 260 nm.

Initially, 600 SSR primers were assessed between the parental lines, for selection of the polymorphic loci. The microsatellite amplification reactions consisted of 5.0 μ l of genomic DNA, 0.32 μ l of each primer, 0.75 μ l of dNTPs (5 mM), 1.5 μ l of PCR 10 \times buffer (20 mM MgCl₂), 7.28 μ l of sterile H₂O and 0.15 μ l of Taq polymerase enzyme (5 U/ μ l) in a total volume of 15 μ l. The amplifications were done in the Thermocycler PTC-200. There was a first step of 2 min at 94°C followed by forty 15-min cycles at 94°C, then 45 s at 60°C, and a last step of 72°C for 2 min.

The amplified products were separated by horizontal electrophoresis in high resolution 3% agarose gel, using Tris-Borate EDTA (TBE) 0.5 \times buffer. For DNA fragment visualization, ethidium bromide was used in a 0.5 μ l/ml concentration. After running 60 min in the electrophoresis cubes at 170 V, the fragments were visualized in a UV transilluminator and the images were scanned through a software named Multi-Analyst[®].

Once identified the polymorphic loci between the parents, evidencing the existence of segregation in the population, 62 primers were selected for family genotyping.

As the markers based on microsatellite amplification have co-dominant expression, meaning that it is possible to identify heterozygous and homozygous genotypes in each studied locus, data were tabled as A, B, H and (–), being A, resistant parent, B, susceptible parent, H, heterozygous and (–) missing data. If the number of missing data for a given primer exceeded 10% of the obtained data, this primer was discarded.

The linkage genetic map using the polymorphic SSR markers was obtained through the program MAPMAKER 3.0 (Lander et al. 1987; Lander and Botstein 1989), using a lod score of 1.62 and the maximum distance of 80 cM between two adjacent markers, for the construction of the linkage groups. A lod score indicates, essentially, how much more likely it is for linkage between two loci to happen, than the non-existence of the linkage between them (Lander and Botstein 1989). Haldane's mapping function (Haldane 1919) was employed in order to calculate the genetic distances in centimorgans.

After the construction of the linkage map, QTL mapping for GLS resistance assessed in the

experiments was accomplished, using the program “Cartographer for Windows”. The composite interval mapping method (CIM) was applied (Zeng 1993, 1994), using a lod score of 2.5, in order to consider a QTL associated with the genetic control of the analyzed trait, in a determined position of the genome.

The composite interval mapping mathematical model is the following: $y_i/(M_j M_{j+1}) = \beta_0 + \beta_j X_{ji} + \sum_{l=j,j+1}^K \beta_l X_{li} + \varepsilon_i$; where: y_i is the observed value for individual i ; $(M_j M_{j+1})$ is the genotype of the individual i for markers j and $j+1$; β_j is the effect of the QTL placed between markers j and $j+1$; X_{ji} is an encoder dummy variable of individual i 's genotypes for the markers j and $j+1$; β_l is the partial regression coefficient for the trait in question on marker l ; X_{li} is the representative dummy variable of the individual i 's genotype, for marker l ; and ε_i is the random error associated with observation i , $\varepsilon_i \sim N(0, \sigma^2)$.

After identifying the QTLs involved in GLS genetic control, a multiple regression model was adjusted. The phenotypic variance proportion explained by the QTLs, all together, was estimated by the determination coefficient of the adjusted model and the phenotypic variance proportion explained for each QTL was estimated by the partial determination coefficient for each QTL. QTLs were identified using Mapmaker/QTL (Lander et al. 1987) and the regressions was determined using SAS (SAS Institute 1989).

Qualitative analysis study of generations F₂ and F₃ in relation to phenotypic segregation and genetic control

In possession of the populations' frequency distribution for the pathogen/disease, the score for resistant parent and susceptible parent was established as the truncating point. This way, the progenies with a disease severity score smaller than or equal to the resistant parent's score were considered resistant. The individuals disease severity counted between the resistant parent [BS04—severity (score 1.0)] and the susceptible parent's score [BS03—severity (score 6.0)] were considered moderately resistant. The individuals with scores equal to or higher than the susceptible parent were considered as susceptible. With this analysis, three phenotypic classes were established and the Chi-square test (χ^2) was done, according to Ramalho et al. (1989).

Results and discussion

Phenotypic analysis

Most progenies in Uberlândia has a moderate resistance to GLS, with scores between 4 and 5. In Ipiaçu most progenies are moderate resistance with scores between 3 and 4 and Campo Florido between 2 and 3 (resistants). The averages at the three locations ranged from 2.44 (Campo Florido) to 4.03 (Uberlândia) (Fig. 1). The coefficient of variation were between 16.65% (Uberlândia) and 26.41% (Campo Florido) and the heritability between 61.44% (Ipiaçu) and 72.84% (Campo Florido). In a QTL study associated to resistance to *Cercospora zeaе-maydis* done by Clements et al. (2000), similar values were found.

Significant differences between parents and progenies were observed at all three locations studied (Uberlândia, Ipiaçu and Campo Florido; Table 2). For the contrast between parents and progenies, significant differences were observed only in Uberlândia, which was also the location with the highest disease pressure, with an average score of 4.03 in the 1–9 scale.

In the combined analysis (Table 3) of the three different locations, significant differences were observed between parents, between progenies, locations and progenies × locations. No significant differences were found between parents and progenies,

Table 2 Variance analysis for gray leaf spot in three different locations

SV	DL	MS		
		Uberlândia	Ipiaçu	Campo Florido
Replication	2	6.33**	0.25 NS	2.94**
Block	27	2.35**	0.59 NS	2.10**
Treatment	99	1.67**	1.17**	1.64**
Between parents	1	3.71**	4.26**	6.80**
Parents vs. progenies	1	3.90**	0.02 NS	0.26 NS
Progenies	89	1.47**	1.13**	1.60**
Rest	8	3.44	1.34	1.63
Residue	171	0.45	0.42	0.42
Average		4.03	2.97	2.44
CV (%)		16.66	21.79	26.42
h ²		69.83	61.44	72.4

parents × location and parents × progenies × location. The significant interaction of progenies × location and the absence of interaction between parents and locations demonstrated that, despite the resistant parent’s stability, the progenies originating from this cross did not show the same resistance stability. Possibly, the modifier genes in the susceptible parent cancel the resistance stability effect of the resistant parent. The absence of significant interaction between

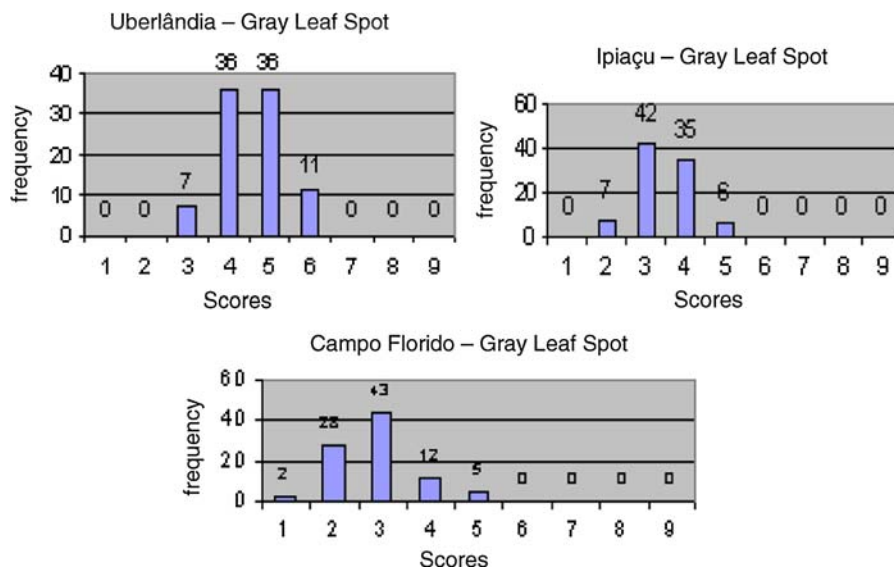


Fig. 1 Frequency distribution for gray leaf spot in the F₃ progenies in the maize population [BS03(resistant parent score 1) × BS04 (susceptible parent score 6)] in Uberlândia, Ipiaçu and Campo Florido

Table 3 Combined analysis of variance for a maize population in three locations, based on the severity of gray leaf spot

SV	Degrees of freedom	Gray leaf spot MS
Location	2	196.25**
Rep. (local)	6	3.18**
Block (location × rep)	81	1.68**
Treatment	99	3.36**
Between parents	1	14.51**
Parents vs. progenies	1	2.33 NS
Progenie	89	3.12**
Rest	8	4.67
Treatment × location	198	0.57**
Parents × location	2	0.13 NS
Progenie × location	178	0.54*
Parents vs. progenie × location	2	1.86 NS
Residue	509	0.43
Average		3.15
CV (%)		20.8
h^2		81.43

parents and the places analyzed permit inferring about the same race in different locations for these trials.

Mapping for population

Since the progeny × location interaction was significant, QTL mapping is discussed for each location separately. Four QTLs were identified for GLS resistance in Uberlândia (Fig. 2). The first one was

located on chromosome 1, at 445.26 cM from its beginning (lod 2.7). The second and the third ones were located on chromosome 3, the second one at 74.43 cM (lod 3.8) and the third one at 83.07 cM (lod 4.1) from the beginning of the chromosome. The fourth, located on chromosome 5, was at 84.38 cM from the beginning of the group (lod 2.5). Lehmen-siek et al. (2001) also identified four QTLs for gray leaf spot, one QTL located on chromosome 1, placed on bin 1.05/06 with wide resistance effect against GLS, which explained 37% of phenotypic variance, two on chromosome 5, on bins 5.03/04, explaining 11% of the phenotypic variance and one QTL on chromosome 3, on bin 3.04, with phenotypic variance of 8–10%. Clements et al. (2000), also studying QTLs associated to resistance to *Cercospora zeaemaydis*, found five of them located on chromosomes 1, 2, 5 and 7. In the present study were found QTLs located on chromosomes 1, 3 and 5 for the population BS03 × BS04 (Table 4). For Uberlandia and Campo Florido trials were identified N516 e N566 QTLs markers in the chromosome 1. Ipiacu location was found N38, N247 e N566 in the chromosome. In the chromosome 3 were found the markers N310 and N904 for Uberlandia and Campo Florido. For Uberlandia and Campo Florido the N338 were found in the both locations.

The first QTL (Fig. 2) stands between the markers N566 and N516, at 343.95 cM from the first one and 4.74 cM from the second one. The next QTL stands between markers N904 and N310, at 3.73 cM from the first one and 4.74 cM from the second one.

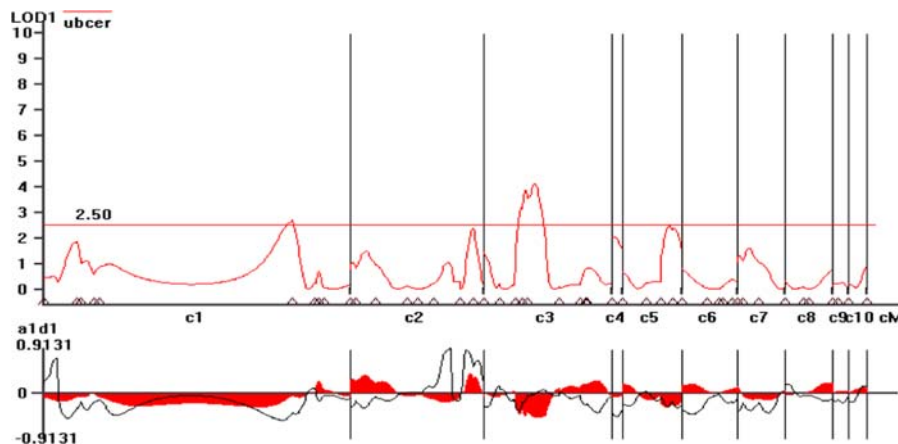


Fig. 2 Mapping, additive (fulfilled lines) and dominance effects (black lines) of QTLs for gray leaf spot in the maize population BS03 × BS04 grown in Uberlândia MG

Table 4 Location of QTLs found for gray leaf spot resistance in Uberlândia, Campo Florido and Ipiaçú using a maize population BS03 × BS04

Chromosomes	Location	Marker
1	Uberlândia	N566 e N516
1	Campo Florido	N556 e N516
1	Ipiaçú	N38 e N566
1	Ipiaçú	N247 e N38
3	Uberlândia	N904 e N310
3	Campo Florido	N904 e N310
3	Uberlândia	N310 e N1121
5	Uberlândia	N912 e N338
5	Campo Florido	N338 e N369

The third QTL stands between markers N310 and N1121, at 3.90 cM from the first one and 52.79 cM from the second one, while the fourth QTL stands between markers N912 and N338, at 14.88 cM from the first one and 5.83 cM from the second one. All QTLs identified have negative additive effect (Table 4).

In Ipiaçú, two QTLs were identified (Fig. 3), the first one located on chromosome 1, placed at 88.69 cM from the beginning of the group (lod 4.8), between markers N247 and N38, at 22.05 cM from the first one and 1.85 cM from the second one. The other QTL, also on chromosome 1, was placed at 99.34 cM from the beginning of the chromosome (lod 4.2), between markers N38 and N566, at

8.80 cM from the first one and 1.97 cM from the second one. Both QTLs have negative additive effect.

The mapping done for GLS in Campo Florido identified three QTLs (Fig. 4). The first one located on chromosome 1, at 445.27 cM from the beginning of the group (lod 2.5), placed between the markers N566 and N516, at 343.96 cM from the first marker and 1.43 cM from the second one. The next QTL, located on chromosome 3 at 74.46 cM from its beginning (lod 2.5), was placed between the markers N904 and N310, at 3.76 cM from the first marker and 4.71 cM from the second one. The third QTL, located on chromosome 5 at 102.76 cM from the beginning of the group (lod 2.6), was placed between the markers N338 and N369, at 12.55 cM from the first marker and 5.10 cM from the second one. All QTLs identified have negative additive effect. This means that for all these loci the resistance allele originates from the resistant parent.

Superimposed graphics of all mappings done for the reaction to *Cercospora zae-maydis* for the population studied, showing the regions with the greatest possibilities of identification of the QTLs on different locations, can be observed in Fig. 5. QTLs were observed on chromosomes 1, 3 and 5. On chromosome 1, the same QTL was identified in Uberlândia and Campo Florido, located between markers N556 and N516. Also on chromosome 3 a QTL was identified for two different locations (Uberlândia and Campo Florido), located between the markers N904 and N910. QTLs identified in Uberlândia and Campo Florido

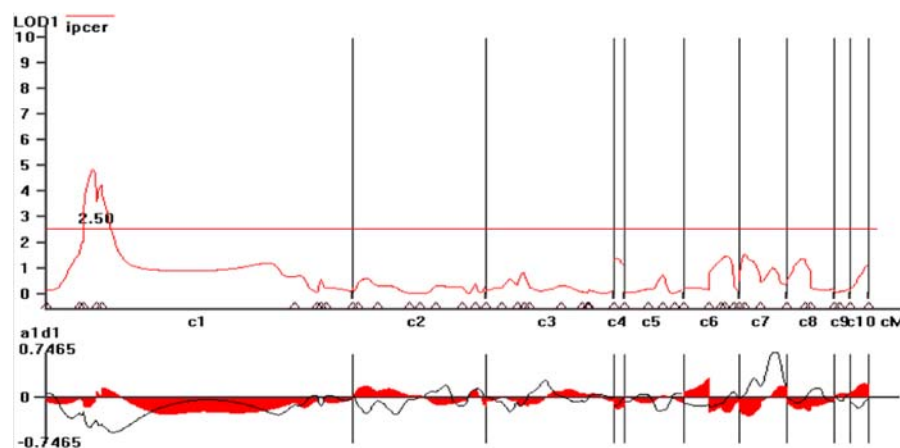


Fig. 3 Mapping and additive (*fulfilled lines*) and dominance effects (*black lines*) of QTLs for gray leaf spot in Ipiaçú in the maize population BS03 × BS04

Fig. 4 Mapping and additive (*fulfilled lines*) and dominance effects (*black lines*) of QTLs for gray leaf spot in Campo Florido in the maize population BS03 × BS04

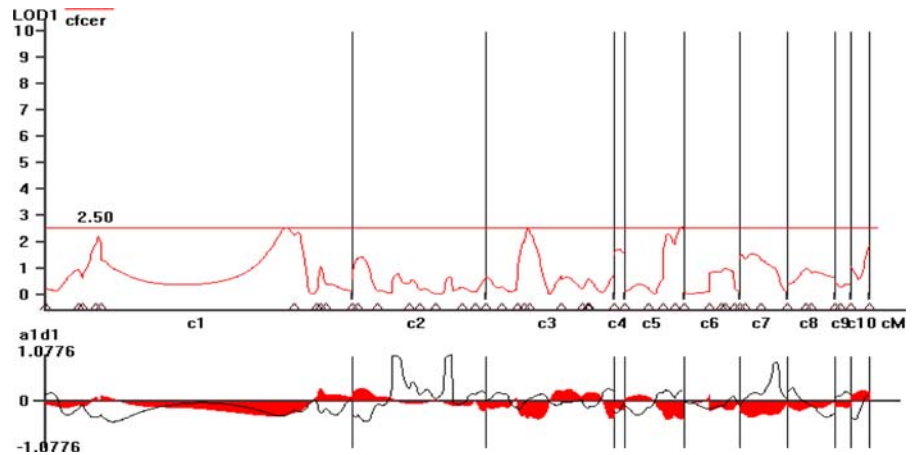
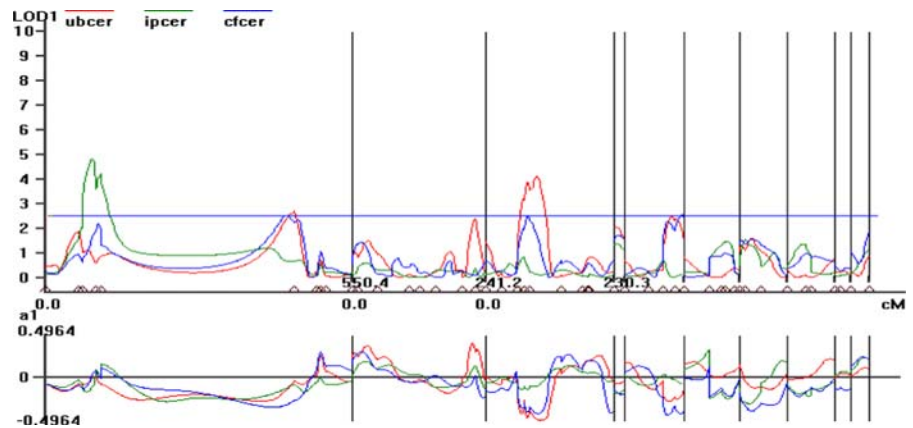


Fig. 5 Mapping and additive effects of QTLs for gray leaf spot in the three different locations in a maize population (BS03 × BS04)



were very close to each other on the chromosome, considering that one was between markers N912 and N338 and the other was between markers N338 and N369 (Table 4).

In the regression analysis for GLS (Table 5) in Uberlândia, the effect of the QTL associated to marker N310 was the one that better explained the phenotypic variation (14.02%). The three markers together explained 14.26% of phenotypic variation. At the second location (Ipiacu), the phenotypic variance was better explained by the effect of the QTL associated to the marker N38, with 14.73%. All markers together explained 16.06% of phenotypic variation. At the third location (Campo Florido), the phenotypic variance was better explained by the effect of the QTL associated with marker N369, with 11.28% and the two markers together explained 22.14% of the variation.

The results don't support the use of these markers for assisted selection, but they give an indication of

Table 5 Summaries of the multiple regression analysis for the reaction to GLS in a maize population (BS03 × BS04)

Markers	DF	Proportion of phenotypic variance	SE	Partial R ²
Uberlândia MG				
N516	1	0,0158	0,1790	0,0002
N904	1	0,0670	0,2697	0,0022
N310	1	0,2979	0,2247	0,1402
R ² = 0,1426				
Ipiacu MG				
N247	1	0,0111	0,1615	0,0001
N38	1	0,1998	0,2132	0,1473
N566	1	0,1171	0,1753	0,0132
R ² = 0,1606				
Campo Florido MG				
N310	1	0,3731	0,1855	0,1086
N369	1	0,3485	0,1654	0,1128
R ² = 0,2214				

the hot regions to be explored. The regions have to be saturated and the size of the population increased in order to enable a fine mapping and then work on a saturated mapping.

Phenotypic analysis vs. QTL mapping

The phenotypic analysis of the behavior of this population demonstrates that the resistance genes against GLS were not established in the progenies, for evidence was found of a dominant gene for resistance at Uberlândia and Ipiacu (segregation 1:1) and dominant and recessive epistasis for two genes (13:3) at the third location (Campo Florido) (Table 6). Considering that the disease is recent in Brazil in terms of epidemic losses in maize, the breeding programs were fast and this study showed an evolution in allelic frequency fixed in breeding lines for hybrids development in Brazil. QTLs located on chromosomes 1, 3 and 5 were found for this population. In another study, the QTLs were mapped to maize chromosomes 1, 3 and 5 using existing linkage maps of two commercially available recombinant inbred-line populations (Lehmensiek et al. 2001). The association between marker N310 located on chromosome 3 for resistance to GLS occurred, although it explained only 15% of the variation.

Comparing the results between the Chi-square test and the study of QTL mapping, we found that many loci associated to diseases detected by QTL were not detected by the Chi-square test. This occurs because the Chi-square test is less discriminating, compared with the mapping method, capturing only loci of pronounced effect. Wissner et al. (2006) published a set of rules to enable the placement of these loci on a single consensus map, permitting analysis of the distribution of resistance loci identified across a variety of maize germplasm for a number of different

diseases. The confidence intervals of the QTL were distributed over all ten chromosomes and covered 89% of the genetic map to which the data were anchored. Visual inspection indicated the presence of clusters of QTL for multiple diseases. Derera et al. (2008) found that both general combining ability (GCA) and combining specific ability (SCA) effects were highly significant ($P < 0,01$), but the predominance of GCA for GLS resistance (86%) was obtained. This indicates that additive effects for GLS resistance were more important than nonadditive effects. Then in the field conditions for about GLS natural infection (inoculum), additive effects for this disease resistance were more important than nonadditive effects. In the future the results will be confirmed with the artificial inoculation, saturating the mapped regions with more markers and increasing the population size. Although the Chi-square test is performed assuming two genes controlling the trait while QTL mapping assumes more genes, the QTL mapping identified the genes (modification genes that act on the trait) while the Chi-square test could not do it. QTL to improve GLS infection resistance in a commercial breeding program is very complex utility to be considered. Pozar et al. (2009) showed that none significant epistatic between location and interactions were not detected. In the present work epistatic effects were found in Campo Florido location (13:3). In this model a significant epistatic interaction was detected between dominant and recessive alleles. For the same authors in Irai, MG, Brazil using the joint analysis of the locations, a significant epistatic interaction was detected between Q_1 and Q_3 for stalk lodging although the regression analysis failed to detect it (Pozar et al. 2009). According to Wissner 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 \times host interaction.

Table 6 Phenotypic analyses from BS03 \times BS04 population (90 progenies– F_3 families) with the severity reaction to GLS

Location	1:1 Model	3:1 Model	9:7 Model	13:3 Model	15:1 Model
Uberlândia	0,16 NS	–	–	–	–
Campo Florido	–	–	–	0,01 NS	–
Ipiacu	0,16 NS	–	–	–	–

Chi-square test (χ^2) was done, according to Ramalho et al. (1989)

Conclusions

1. Seven QTLs were identified in the maize population for resistance to GLS.
2. The heritability values (61,4–72,8%) found were high for the GLS resistance in BS04 inbred line in the three locations (Uberlândia, Ipiáçu and Campo Florido).
3. The heritability values found were high for the traits assessed.
4. The selection of resistant lines through the QTLs found in different locations would make possible a reduction of environments that need to be tested.
5. The existence of significant QTLs in common between different locations indicates these genomic regions as possible new tools for marker-assisted selection in maize breeding programs, targeting the selection of lines that would make possible production in many environments.

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