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M. A. Saghai Maroof · Y. G. Yue · Z. X. Xiang
E. L. Stromberg · G. K. Rufener

Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize

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Abstract Breeding maize for gray leaf spot (GLS) resistance has been hindered by the quantitative nature of the inheritance of GLS resistance and by the limitations of selection under less than optimumal disease pressure. In order to identify the quantitative trait loci (QTLs) controlling GLS resistance, a cross was made between B73 (susceptible) and Va14 (resistant) to generate a large F_2 population. Six GLS disease assessments were made throughout the disease season for over 1000 F_2 plants in 1989, and for 600 F_2-derived F_3 lines replicated in two blocks in 1990. RFLP analysis for 78 marker loci representing all ten maize chromosomes was conducted in 239 F_2 individuals including those with the extreme GLS disease phenotypes. The GLS disease scores of the three field evaluations, each averaged over six ratings, were separately used for the interval mapping in order to determine the consistency of the QTL effects. The heavy GLS disease pressure, meticulous disease ratings, and large population size of this study afforded us the sensitivity for detecting QTL effects. QTLs located on three chromosomes (1, 4, and 8) had large effects on GLS resistance, each explaining 35.0–56.0%, 8.8–14.3%, and 7.7–11.0% of the variance, respectively. These three QTL effects were remarkably consistent across three disease evaluations over 2 years and two generations. Smaller QTL effects were also found on chromosomes 2 and 5, but the chromosome-5 effect might be a false positive because it was not repeatable even in the same location. The chromosome-1 QTLs had the largest effect or highest R^2 reported for any quantitative trait to-date. Except for the chromosome-4 gene, which was from the susceptible parent B73, the resistance alleles at all QTL were derived from Va14. The resistance QTLs on chromosomes 1 and 2 appear to have additive effects, but those on chromosomes 4 and 8 are dominant and recessive, respectively. Significant interaction between the QTLs on chromosomes 1 and 4 was detected in all three evaluations. Cumulatively, the four QTLs identified in this study explained 44, 60, and 68% of the variance in F_2, and in F_3 replications 1 and 2, respectively.

Key words Restriction fragment length polymorphism · *Zea mays* L. · Quantitative resistance · Oligogenic · Gene mapping

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M. A. Saghai Maroof (✉) · Y. G. Yue[1] · Z. X. Xiang
Department of Crop and Soil Environmental Sciences,
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia 24061, USA

E. L. Stromberg
Department of Plant Pathology, Physiology and Weed Sciences,
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia 24061, USA

G. K. Rufener
ICI Seeds, Slater, Iowa 50246, USA

Present address:
[1] Union Camp Corporation, Research and Development Division,
P.O. Box 3301, Princeton, NJ 08543, USA

Introduction

Breeding for resistant hybrids appears to be the most practical method to prevent grain losses from gray leaf spot (GLS), a disease of maize caused by *Cercospora zeae-maydis* Tehon & Daniels. The incidence of GLS has been rising in both severity and distribution in the US, largely because of reduced- or non-tillage practice in corn production (Stromberg and Donahue 1986). In areas where GLS is a problem, yield potential in diseased corn may be lost by as much as 50% due to the loss of photosynthetic area, increase in lodging, and premature death (Stromberg and Donahue 1986; Stromberg and Flinchum 1994). Latterell and Rossi (1983) identified a number of germ plasms that were resistant to GLS, ranging from reduced disease development to highly resistant. Most of the commonly used maize inbred lines, however, are highly susceptible to *C. zeae-maydis*, and the transfer of GLS resistance into these high-yielding lines has been difficult (Ayers et al. 1984).

The quantitative nature of the inheritance of GLS resistance was determined by Manh (1977) based on the con-

tinuous distribution of GLS disease ratings in F_2 and F_3 generations from a cross between a resistant line, Va14, and a susceptible line, H98. He also reported that GLS resistance in Va14 was controlled by genes that act primarily in an additive manner. Several GLS-resistant maize inbred lines have been identified by GLS ratings in inbreds per se, diallel crosses, or in both (Ayers et al. 1984; Thompson et al. 1987; Huff et al. 1988; Elwinger et al. 1990; Ulrich et al. 1990; Donahue et al. 1991; Saghai Maroof et al. 1993). Using these resistant germ plasms and based on generation means (Thompson et al. 1987; Huff et al. 1988) and diallel analysis (Ulrich et al. 1990; Donahue et al. 1991), it was concluded that the additive effect or general combining ability (GCA) was overwhelmingly important for GLS resistance. However, Elwinger et al. (1990) have argued that dominance should also be included in a model to fully explain the inheritance of GLS resistance. The broad-and narrow-sense heritabilities for GLS disease resistance have been estimated to be about 0.32 (Manh 1977). Ulrich et al. (1990) reported that genotypes explained 52% of the total variation in a diallel cross, while Donahue et al. (1991) found that only 26% of the variation was explained in their diallel set.

The development of GLS disease is highly influenced by microclimatic conditions (Payne and Waldron 1983). Such a strong dependency on the environmental effects makes the assessment of the disease, for both inheritance studies and resistance breeding, very difficult. The presence of a heavy disease pressure is an essential prerequisite to evaluate the level of GLS resistance, but such an optimal condition is difficult to achieve through artificial inoculation or supplementary irrigation. Donahue et al. (1991) found that the GLS disease prevails in high relative humidity and with the extended leaf wetness, which varies among different locations and climatic conditions. The difficulties in disease evaluation have further limited progress in developing GLS-resistant maize hybrids.

The recent development of molecular genetic markers, such as DNA restriction fragment length polymorphism (RFLP), has made it possible to construct detailed linkage maps, and to dissect the genetic control of quantitative traits (Tanksley 1993). Identification of genes controlling a quantitative trait is critical in order to determine the effect of these genes or quantitative trait loci (QTLs), to study the molecular mechanisms of individual genes and, more directly, to facilitate the transfer of desirable traits in marker-assisted breeding programs. If selection can be made directly at the DNA level, breeding for a quantitative trait can be independent of environmental influence regardless of the level of heritability. The essential elements of molecular marker-based breeding programs are knowledge of the number of genes or QTLs, and the percentage of variance that can be explained by these genes.

Based on his estimate of the number of effective factors, Manh (1977) suggested, that the quantitative resistance to GLS might be controlled by a small number of genes. This hypothesis of a "not-too-complex" genetic control of GLS resistance agrees with the recent observations: (1) that a high level of resistance was retained in the progeny from backcrossing into a susceptible cultivar (Thompson et al. 1987), and (2) that a three-locus model derived from a diallel analysis explained 63–67% of the observed variation in inbreds and their single-cross, F_2, and backcross generations (Elwinger et al. 1990). On the other hand, based on molecular-marker analysis, QTLs with significant effects on GLS resistance have been identified on all maize chromosomes (Bubeck et al.,1993). The percentage of variation explained by individual markers was small and the QTL effects "were often inconsistent over environments resulting from G × E interactions, random environmental errors or, in some cases, false positives" (Bubeck et al. 1993).

The RFLP study of GLS resistance reported here was undertaken in 1989 and 1990. In order to increase the precision in disease assessment, we conducted a preliminary study and developed a GLS rating method (Saghai Maroof et al. 1993 and unpublished data) which is based on evaluation at a finer scale (0.25 increments in the 1.0 to 5.0 range) of single-plant observations. The objectives of the present study were to identify GLS-resistance QTLs by RFLP mapping using a large population and a "selective genotyping" strategy, and to evaluate the consistency in the detection of QTL effects in replicated field experiments. A field with a uniform naturally occurring inoculum potential maintained by continuous non-tillage, and the extremely high relative humidity associated with the location, was used in this study. The disease ratings in F_2 and two replications of the F_3 generation were analyzed individually to detect the QTL effects of RFLP markers. Because of the consistent heavy disease pressure and the improved rating methodology, we have identified major genes that account for majority of the quantitative resistance to GLS, and the effects of these QTLs were highly consistent over replicated blocks and years.

Materials and methods

Plant materials

A cross was made between two maize inbreds, Va14 (highly resistant to GLS) and B73 (susceptible). An F_2 population of over 1000 seeds was planted at Whitethorne-Kentland Plantation Farm, Blacksburg, Virginia in 1989. A total of 958 F_2 plants was scored for GLS disease reaction and each F_2 was selfed to produce an F_2-derived F_3 line. Six-hundred F_3 lines were planted as rows in two separate GLS disease blocks, designated as F_3/rep1 and F_3/rep2, in 1990. Leaf tissue samples from 239 F_2 individuals, including the extremely resistant and susceptible plants as well as those with the intermediate GLS disease expression, were used for DNA extraction and subsequent RFLP analysis. Such "selective genotyping" would maximize the power of the mapping population with limited expenditure in RFLP analysis (Lander and Botstein 1989).

GLS disease evaluations

F_2 plants and their corresponding F_3 lines were grown in 1989 and 1990, respectively, in field plots with naturally infested corn debris, and rated for their GLS expression as previously described (Saghai Maroof et al. 1993). The evaluation of over 1000 F_2 plants was made in a complete randomized design, and that of 600 F_3 lines in two rep-

lications using a complete randomized block design. A known GLS-susceptible check hybrid was planted every seven rows to assess disease uniformity within the plot area. In each F_3 replication, 20 seeds were sown as a single row, 3.4 m long and spaced 0.76 m apart, with a cone-planter equipped for non-tillage seeding. F_3 rows were hand-thinned to 12 plants per row 2 weeks after emergence.

The GLS rating of F_2 and F_3 plants was made on a disease severity index of 1–5 in increments of 0.25, where 1=no symptoms; 2=moderate lesion development below the leaf subtending the ear; 3=heavy lesion development on and below the leaf subtending the ear with a few lesions above it; 4=severe lesion development on all but the uppermost leaves, which may have a few lesions; and 5=all leaves dead. F_2 plants were rated for disease reaction six times at 3-day intervals after disease symptoms were first observed. Similarly, F_3 rows were rated six times on a single-plant basis at weekly intervals. The six individual GLS ratings of each F_2 plant were averaged as a mean F_2 disease score. At each rating, a disease-index estimate was made for each individual F_3 plant in every row, and then averaged as an F_3 row rating. For each F_3 replication, the six ratings of each F_3 row were also averaged as the F_3 disease score. Thus, three sets of GLS disease scores, one for the F_2 and two for the F_3 (designated as F_3/rep1 and F_3/rep2), were available for QTL mapping with RFLP markers.

RFLP analysis

RFLP analysis was conducted using DNA samples prepared from leaf tissues of the parental lines and F_2 individuals. DNA samples were digested with appropriate restriction enzymes: *Eco*RI, *Hin*dIII, *Eco*RV, or *Dra*I. Agarose-gel electrophoresis and Southern blotting were performed using standard protocols (Dudley et al. 1991; Zhang et al. 1993).

Previously mapped RFLP clones of maize were obtained from Brookhaven National Laboratory (bnl), University of Missouri at Columbia (umc), and Native Plants Inc. (npi). DNA inserts from these clones were labeled with [^{32}P]dCTP by random hexamer priming, and hybridized to Southern blots made from parental or F_2 DNA samples.

Interval mapping for QTL

GLS disease indices estimated for F_2 plants and F_2-derived F_3 progeny rows at six different dates throughout the disease season were used in statistical analyses. Because of the high correlation among disease ratings of the same plants or lines at different stages (Saghai Maroof et al. 1993, and unpublished data), averaged GLS disease indices of F_2 plants or F_3 families over all six ratings were used in QTL mapping.

Construction of a maize RFLP linkage map from the F_2 data was based on the Mapmaker computer program (Lander et al. 1987). All linkage groups were scanned for the presence of a QTL effect at a log-likelihood (LOD) threshold of 3.0 in every 2.0-cM interval using Mapmaker/QTL at a free model (Lincoln et al. 1992). Because of "selective genotyping" using individuals having extreme GLS disease phenotypes, a large portion of the F_2 population has a GLS disease score but no RFLP data. These individuals were included in the interval mapping by designating their RFLP marker genotypes as missing data, according to Lander and Botstein (1989).

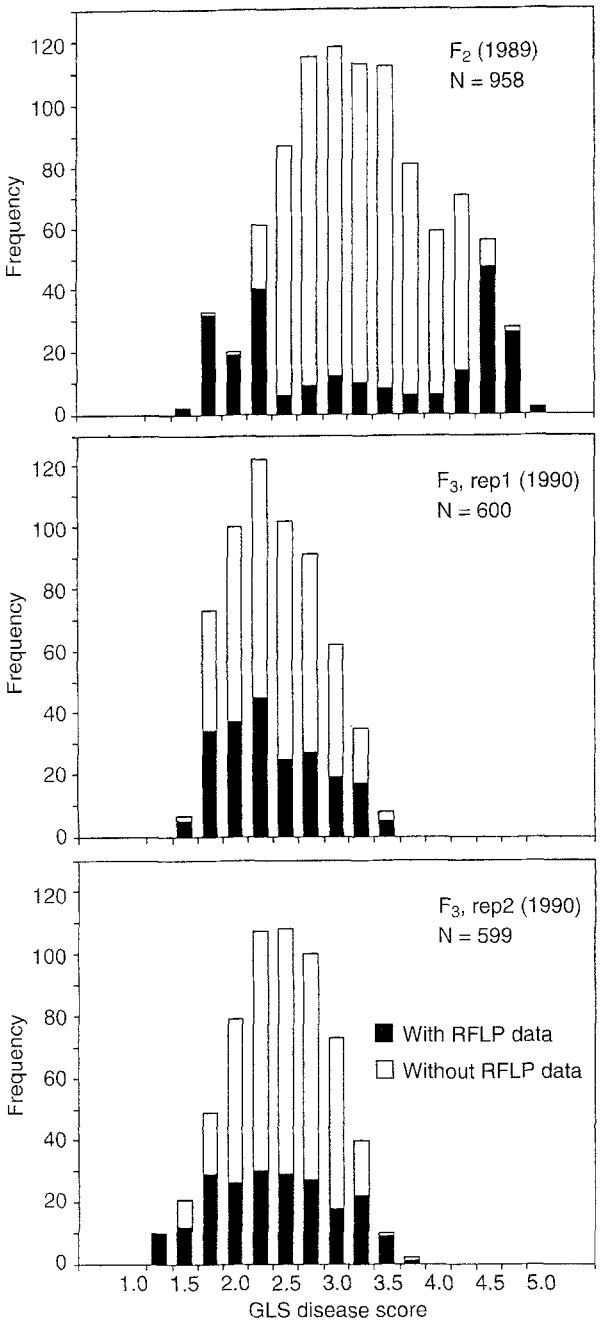

Fig. 1 Continuous, normal distributions of GLS disease scores, averaged from six ratings made throughout each growing season, in 958 F_2 plants from B73×Va14, and 600 and 599 F_2-derived F_3 families in two replications of F_3 disease evaluations, respectively. A "selective genotyping" was used by determining the RFLP marker genotypes only in the 239 F_2 plants (shown in *closed bars*) that include the most resistant, the most susceptible, and some intermediate types.

Results

Heavy GLS disease pressures were observed, both in the susceptible check hybrid and in the segregating F_2 and F_2-derived F_3 populations, in the three disease evaluation experiments conducted in 1989 and 1990. Six GLS disease ratings throughout the growing season were completed in a total of 958 F_2 plants, and six single-plant-based ratings were made for each of the 600 F_3 rows in F_3/rep1 and F_3/rep2. The GLS disease scores averaged over the six ratings had a normal distribution in the F_2 population and in the two replications of the F_3 (F_3/rep1 and F_3/rep2) population (Fig. 1). The variances in the F_3 generation (0.45 in F_3/rep1 and 0.50 in F_3/rep2) were somewhat smaller than

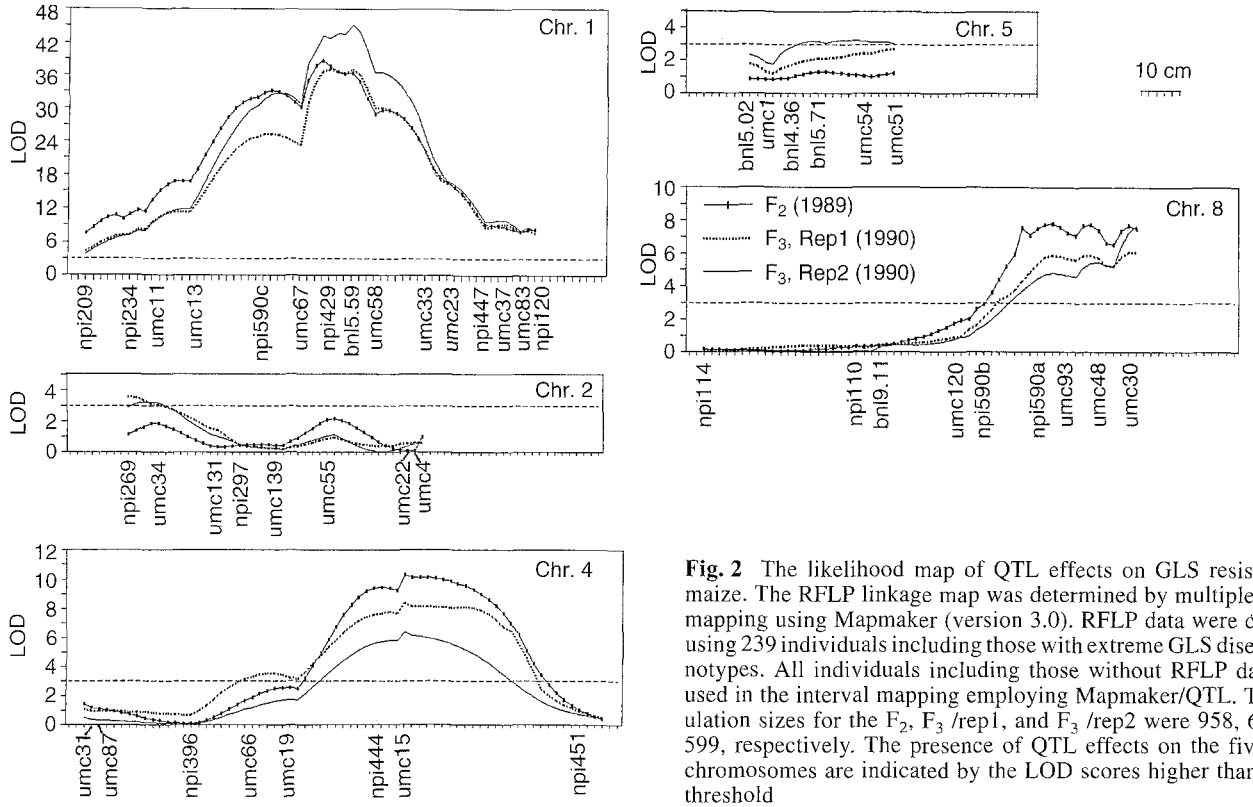

Fig. 2 The likelihood map of QTL effects on GLS resistance in maize. The RFLP linkage map was determined by multiple linkage mapping using Mapmaker (version 3.0). RFLP data were collected using 239 individuals including those with extreme GLS disease phenotypes. All individuals including those without RFLP data were used in the interval mapping employing Mapmaker/QTL. The population sizes for the F_2, F_3 /rep1, and F_3 /rep2 were 958, 600, and 599, respectively. The presence of QTL effects on the five maize chromosomes are indicated by the LOD scores higher than the 3.0 threshold

those in the F_2 (0.75). The GLS ratings in the F_3 generation were more accurate because they can be replicated, and they are averaged from single-plant estimates within each F_3 row. Such a large population size and replicated GLS disease assessments, coupled with heavy disease pressures, afford the sensitivity to detect most, if not all, QTLs controlling resistance to the GLS disease.

Two-hundred and thirty nine F_2 plants, including the most resistant and susceptible, were chosen from the population of 958 for "selective genotyping" at RFLP marker loci (Fig. 1). A total of 78 RFLP markers segregated in the F_2 population. A maize linkage map was constructed based on 71 RFLP marker loci, while seven loci remained unlinked. Each of the ten maize chromosomes was represented in the 811-cM map. The average distance between markers, as calculated by dividing the total distance by the number of markers, is 11.4 cM.

The likelihood map of the QTL effects on GLS resistance in the F_2 and the two replications of the F_3 (F_3/rep1 and F_3/rep2), computed using the disease data of their entire corresponding populations with the RFLP data of the selected extreme individuals, is shown in Fig. 2. Despite the marked difference in the distribution of GLS disease scores among F_2, F_3/rep1, and F_3/rep2, the three resultant LOD curves for each chromosome were strikingly parallel to each other, indicating that the genetic factors contributing to GLS resistance are highly repeatable. The QTL effects on chromosomes 1, 4, and 8 are remarkably consistent across three disease evaluations. Chromosome 2 has a QTL effect (QTL2) shown in both replications of the F_3 in 1990, but not in the F_2 generation in 1989, suggesting that QTL2 may be generation- or environment-dependent. A LOD peak higher than 3.0 was found on chromosome 5 in replication 1 of the F_3 but not in the other replication in the same year at the same location. Its non-reproducibility indicates this marginally significant QTL effect (QTL5) may be a false positive. Thus, QTLs located on four different chromosomes (1, 2, 4, and 8) control the quantitative resistance to GLS in this mapping population.

The highest LOD score for GLS resistance was observed for the chromosome-1 QTL. These LOD values were: 33.8 at the umc67-npi429 interval, 37.1 at the npi429-bnl5.59 interval, and 45.2 at the bnl5.59-umc58 interval based on the disease data from the F_2, F_3/rep1, or F_3/rep2, respectively (Table 1 and Fig. 2). LOD values higher than the 3.0 threshold were found over the entire linkage-group 1 (108 cM), suggesting that more than one QTL may be present on this chromosome. Re-scanning of chromosome 1 by fixing one QTL at the LOD peaks showed a significant increase in LOD value, but the increase was largest in the close vicinity of the fixed QTL. Thus, the positions of any additional QTLs on chromosome 1 remain to be determined by saturating the chromosome with more markers, or by using near-isogenic lines (Paterson et al. 1991). For simplicity, we assume the QTL effect on chromosome 1 is controlled by a single gene designated as "QTL1". QTL1 explained 35.0% of the variance in the F_2, 50.0% in F_3/rep1, and 56.0% in F_3/rep2 (Table 1). The

Table 1 The chromosomal locations of the QTLs controlling resistance to gray leaf spot (GLS) of maize, and the percentage of variance explained by them

QTLs	F_2 (1989)				F_3 rep1 (1990)				F_3 rep2 (1990)			
	Interval[a]	Pos.[b]	Var. exp.[c]	LOD	Interval	Pos.	Var. exp.	LOD	Interval	Pos.	Var. exp.	LOD
QTL1 (chr 1)	umc67 (7.5) npi429	6.0	35.0%	38.8	npi429 (2.2) bnl5.59	0.0	50.0%	37.1	bnl5.59 (7.1) umc58	2.0	56.0%	45.2
QTL2 (chr 2)	–	–	–	–	npi269 (6.3) umc34	0.0	7.7%	3.6	npi269 (6.3) umc34	6.0	4.8%	3.2
QTL4 (chr 4)	npi444 (3.5) umc15	2.0	8.8%	10.3	npi444 (3.5) umc15	2.0	14.3%	8.4	npi444 (3.5) umc15	2.0	9.0%	6.4
QTL5 (chr 5)	–	–	–	–	–	–	–	–	bnl5.71 (10.9) umc54	6.0	5.7%	3.2
QTL8 (chr 8)	npi590A (4.7) umc93	0.0	7.7%	7.8	umc48 (6.9) umc30	6.0	9.6%	6.1	umc48 (6.9) umc30	6.0	11.0%	7.7
Cumulative effect			44.0%	54.7			60.0%	49.1			68.0%	64.5

[a] Each interval is defined by two RFLP marker loci that have a distance (cM) in between as shown in parenthesis
[b] The distances (cM) from the topmost one of the two marker loci for the corresponding intervals
[c] Percentage of variance explained by individual QTLs, or by all multiple QTLs acting together (cumulative effect)

resistance allele was from Va14, and acted in near additive fashion in all three GLS experiments (Table 2). The additivity of this major QTL is consistent with results from previous field studies based on classical quantitative genetic analyses (Huff et al. 1988; Elwinger et al. 1990; Thompson et al. 1990; Ulrich et al. 1990; Donahue et al. 1991).

Chromosomes 4 and 8 were also important for GLS resistance in all three trials, while the QTL effect of chromosome 2 was detected only in the F_3 generation. The LOD curve on chromosome 4 peaked at 10.3, 8.4 and 6.4 at the same position in the npi444-umc15 interval in the F_2, F_3/rep1, or F_3/rep2, respectively (Table 1 and Fig. 2). The QTL on chromosome 4, QTL4, accounted for 8.8%, 14.3%, and 9.0% of the variance in F_2, F_3/rep1, and F_3/rep2, respectively. B73 is highly susceptible to GLS disease as reported by Thompson et al. (1987), Donahue et al. (1991) and Saghai Maroof et al. (1993). In the present RFLP study, however, we found that it was the partially dominant B73 allele at QTL4 that contributed to GLS resistance (Table 2). On chromosome 8, LOD values reached 7.8 at the npi590a-umc93 interval, and 6.1 and 7.7 at the umc48-umc30 interval, in the F_2, F_3/rep1, and F_3/rep2, respectively (Table 1 and Fig. 2). The chromosome 8 QTL, designated as QTL8, explained 7.7%, 9.6%, and 11.0% of the variance in these three GLS experiments. The QTL effect of chromosome 2 was not significant in the F_2 but in F_3 replications 1 and 2, with LOD scores of 3.6 and 3.2, respectively, it was significant at the npi269-umc34 interval (Table 1 and Fig. 2). This QTL (QTL2) explained 7.7% and 4.8% of the variation in the two F_3 replications (Table 2). Both QTL8 and QTL2 derive their resistance alleles from Va14, but they have different gene actions: QTL8 was recessive and QTL2 was additive (Table 2).

Table 2 The effect of QTLs on GLS disease score, either by acting individually or in the combination of multiple QTLs, in the three disease evaluations

QTLs	model[a]	F_2 1989		F_3 rep1 1990		F_3 rep2 1990	
		a[b]	d[c]	a	d	a	d
QTL1	Ind.	–0.61	–0.17	–0.42	–0.09	–0.52	–0.01
	Multi.	–0.58	–0.13	–0.39	–0.07	–0.49	–0.01
QTL2	Ind.	–	–	–0.18	0.00	–0.15	0.07
	Multi.	–	–	–0.17	–0.05	–0.13	0.02
QTL4	Ind.	0.27	–0.22	0.22	–0.08	0.20	–0.09
	Multi.	0.21	–0.18	0.10	–0.02	0.08	–0.03
QTL5	Ind.	–	–	–	–	–0.16	0.06
	Multi.	–	–	–	–	–0.14	0.01
QTL8	Ind.	–0.23	0.25	–0.14	0.17	–0.15	0.07
	Multi	–0.20	0.23	–0.06	0.09	–0.10	0.09

[a] Two models were used to compute the effect of each QTL: each QTL acting individually in a single-QTL model (ind.) or all QTLs acting together in a multiple-QTL model (multi.)
[b] Additive gene effect of a Va14 allele, a=(vv–bb)/2
[c] Dominance, d=bv–(vv + bb)/2

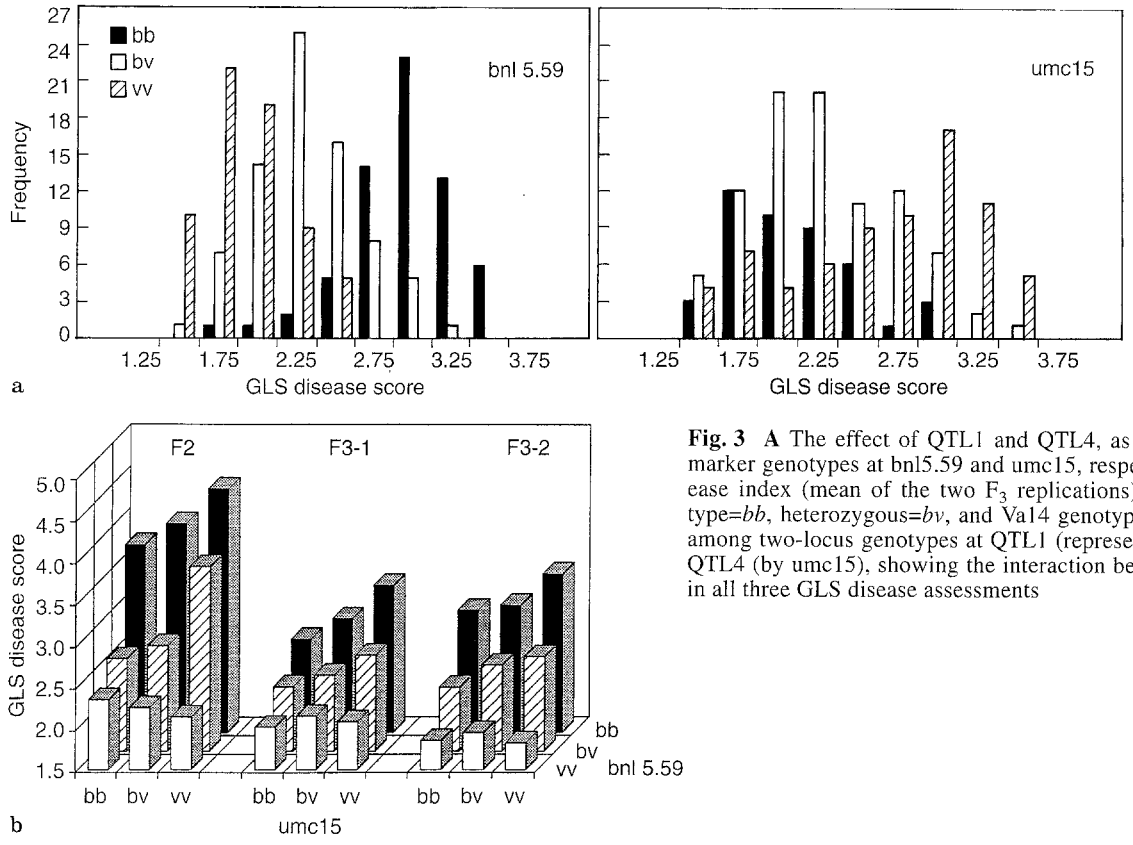

Fig. 3 **A** The effect of QTL1 and QTL4, as represented by their marker genotypes at bnl5.59 and umc15, respectively, on GLS disease index (mean of the two F_3 replications). B73 marker genotype=*bb*, heterozygous=*bv*, and Va14 genotype=*vv*. **B** Comparison among two-locus genotypes at QTL1 (represented by bnl5.59) and QTL4 (by umc15), showing the interaction between the two QTLs in all three GLS disease assessments

Cumulatively, the three major QTLs (QTL1, 4, and 8) explained 44.0%, 55.0%, and 64% of the variance in the F_2, F_3/rep1, and F_3/rep2, respectively. If the putative QTL2 is included in the F_3 analysis, up to 60.0% and 68.0% of the variation in F_3/rep1 and F_3/rep2, respectively, can be explained by the full models. The cumulative percentages of variance explained by the full models, with or without QTL2, were less than the sum of variations explained by each of the component QTLs (Table 1). The effects of QTL4 and 8 on GLS disease score in the multi-QTL full models were also smaller than their effects in single-QTL models (Table 2), indicating some possible epistatic interactions of these two QTLs on others. QTL1, however, had virtually the same effect in the multiple- as in the single-QTL model. The effects of QTL1 and 4, as represented by bnl5.59 and umc15, respectively, on GLS disease score are illustrated in Fig. 3A. Both markers had noticeable effects, despite the fact that their effects on the GLS phenotypic value were opposite. A two-factor analysis of the same two QTLs showed that the effect of QTL4 is epistatic to QTL1, i.e., QTL4 had no or little effect on GLS resistance when the QTL1 locus was homozygous resistant (Fig. 3B). Such epistatic relationship can be useful in marker-assisted breeding by eliminating the need to transfer QTL4 since it has a negligible effect on GLS resistance provided that the QTL1 resistance allele is in a homozygous state.

Discussion

Genetic control of quantitative resistance to GLS

Consistent heavy disease pressure is required to accurately assess the potential of plant genotypes to resist the onset and progress of GLS, and to determine the magnitude of the genetic factors that contribute to resistance. Because the development of GLS disease is extremely sensitive to environmental conditions, artificial inoculation cannot induce consistent disease reactions. Field plots used in this study have all the elements favored by the pathogen, including: high relative humidity, heavy morning dews, and continuous non-tillage production with *C. zeae-maydis*-infested debris from previous seasons to serve as a primary inoculum. The heavy disease pressure maintained in field plots, combined with replicated disease evaluation experiments and as many as six disease ratings throughout each growing season, have made it possible to assess the level of GLS resistance in the segregating population.

Selective genotyping at RFLP marker loci using individuals of extreme phenotypes was suggested by Lander and Botstein (1989). Such a strategy allows analysis of the genetic control of resistance to GLS in a large population. The GLS disease data in a large F_2 population and replicated disease evaluation in a large F_3 population provided us with the sensitivity to detect all QTL effects. Interval

mapping at the LOD threshold of 3.0 indicated that, in the genetic materials used in this study, resistance to the GLS disease was controlled by a small number of QTLs, or oligogenes. QTLs showing large and consistent effects on GLS resistance in all three evaluations were detected on chromosomes 1, 4 and 8, while putative QTLs with limited effects were found on chromosomes 2 and 5. The finding that GLS resistance is regulated by a relatively small number of QTLs agrees with an inheritance study by Thompson et al. (1987) who reported that the genetic control of GLS resistance was not very complex.

Consistency in mapping QTLs for GLS resistance

The results of QTL mapping for GLS resistance using disease data from three experiments, F_2, F_3/rep1 and F_3/rep2, were remarkably consistent. Not only the main effects of QTL1, 4, and 8, but also the epistatic interactions between QTL1 and 4, were identical in all three experiments conducted in 2 different years. The minor QTL on chromosome 2 was significant in both F_3 experiments but not in the F_2, which may be due to the difference in disease pressure between the 2 years of the present study. A putative QTL effect was detected on chromosome 5 in one of the F_3 experiments but not in the other, nor in the F_2. The use of replicated GLS blocks enabled us to conclude that the non-reproducible QTL effect on chromosome 5 may be a false positive, rather than an environment-specific gene.

Although numerous instances of genotype-by-environment interactions has been detected by classical quantitative genetic analysis, recent results from RFLP mapping tentatively suggest very little environment-by-QTL interaction (Tanksley 1993). In maize, Stuber et al. (1992) determined the chromosomal location of QTLs contributing to grain yield by testing a segregating population in six diverse environments, and found little or no genotype×environment interaction. Similarly, QTL mapping for three other agronomic traits in maize was consistent over four environments (Schon et al. 1994). In tomato, Paterson et al. (1991) mapped QTLs affecting fruit size, soluble-solids concentration, and pH in three different environments, and found that some QTLs can be detected in all three environments while others were significant in only one or two environments, suggesting that these latter QTLs may be environmentally sensitive.

In a diallel study, Donahue et al (1991) found that environmental conditions had a significant effect on GLS, and high relative humidity and extended leaf wetness is required for GLS disease. The expression of GLS reaction is extremely sensitive to experimental conditions. The consistency in detecting the same QTLs in the three experiments of the present study is largely due to the abundant natural inoculum and unique edaphic conditions along a river-bottom valley, ensuring a uniform GLS onset in our experimental plots. Of the four QTLs detected in this study, QTLs 1 and 2 had additive effects. At QTL4, a partially dominant resistance component was contributed by the susceptible parent B73. Similarly, a favorable GLS factor contributed by chromosome 4 of B73 was also observed by Bubeck et al. (1993). Furthermore, they reported that umc15, one of the QTL4 markers identified in the present study, was associated with days-to-silking and GLS means over environments. Late maturing lines appear to be more resistant than early lines because GLS lesions often do not begin to expand until several days after flowering. Thus, QTL4 could be either an entity directly contributing to GLS resistance, or a confounding effect caused by a factor controlling late maturity on chromosome 4 near umc15. QTL8 had a recessive effect requiring its inclusion in both parental lines for attaining higher levels of GLS resistance in hybrids. The QTL information from the RFLP mapping experiments reported in this paper was subsequently validated in a marker-assisted breeding program (Rufener, unpublished) resulting in hybrids with higher levels of GLS resistance.

Linkages between GLS resistance QTLs and other resistance genes in maize

The separation of quantitative resistance into a few QTLs enabled us to examine their relationship to previously identified maize genes conferring qualitative resistance to other diseases. The chromosomal location of a *Cochliobolus carbonum* race-1 resistance gene, *hm1*, which has been previously cloned by transposon tagging (Johal and Briggs 1992), coincides with QTL1 which had the largest effect on GLS resistance. QTL8 is closely linked to two *Exserohilum turcicum* (Syn. *Helminthosporium turcicum*) resistance genes, *Ht2* (Zaitlin et al. 1992) and *Htn1* (Simcox and Bennetzen 1993). These three genes appear to belong to a resistance gene cluster since neither *Ht2* nor *Htn1* per se contributes to GLS resistance. Quantitative resistance to several maize diseases has been reported. Significant effects of RFLP markers on resistance to *Gibberella zeae* were found on chromosomes 1, 2, 4, 5 and 10, according to Pe et al. (1993). Among them, the putative QTL-associated markers on chromosomes 1 (umc23), 2 (php10-00338), and 4 (bnl15.45) are in the vicinity of the GLS resistance QTLs on these chromosomes. Freymark et al. (1993) reported that chromosomes 2, 4, and 8 each had at least one marker with some effect on resistance to *E. turcicum* at the 0.05 significance level. Among these markers, umc158 and npi203 on chromosome 4 are in the vicinity of QTL4, and bnl9.08 and bnl7.08a on chromosome 8 are closely-linked to QTL8 for GLS resistance in our mapping population.

The probe npi590 detects multiple loci, among which three segregated in the cross B73×Va14. Of these three loci, npi590c is at the region of the major QTL1, whereas npi590a and npi590b are located on chromosome 8 which had highly significant effects on GLS resistance. The linkage of independent QTLs to members of a multigene family once again supports the hypothesis of a common origin for QTLs controlling resistance to a disease.

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