Identification and Mapping of Quantitative Trait Loci Conditioning Resistance to Southern Leaf Blight of Maize Caused by *Cochliobolus heterostrophus* Race O

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ABSTRACT

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A random set of recombinant inbred (RI) lines (F2:7) derived from the cross of the inbred lines Mo17 (resistant) and B73 (susceptible) were evaluated for resistance to southern leaf blight (SLB) caused by *Cochliobolus heterostrophus* race O. The RI lines were genotyped at a total of 234 simple sequence repeat, restriction fragment length polymorphism, or isozyme loci. Field plots of the RI lines were inoculated artificially with an aggressive isolate of *C. heterostrophus* race O in each of two growing seasons in North Carolina. Lines were rated for percent SLB severity two (1996) or three (1995) times during the grain-filling period. Data also were converted to area under the disease progress curve (AUDPC) and analyzed using the composite interval mapping option of the PLABQTL

program. When means of disease ratings over years were fitted to models, a total of 11 quantitative trait loci (QTLs) were found to condition resistance to SLB, depending upon which disease ratings were used in the analyses. When the AUDPC data were combined and analyzed over environments, seven QTLs, on chromosomes 1, 2, 3, 4, 7, and 10 were found to come from the resistant parent Mo17. An additional QTL for resistance on chromosome 1 came from the susceptible parent B73. The eight identified QTLs accounted for 46% of the phenotypic variation for resistance. QTL $\times$ environment interactions often were highly significant but, with one exception, were the result of differences in the magnitude of QTL effects between years and not due to changes in direction of effects. QTLs on the long arm of chromosome 1 and chromosomes 2 and 3 had the largest effects, were the most consistently detected, and accounted for most of the phenotypic variance. No significant additive $\times$ additive epistatic effects were detected. These data support earlier reports of the polygenic inheritance of resistance to SLB of maize.

The most economical and environmentally acceptable means of reducing losses to diseases of maize is the use of genetically resistant hybrids. Most U.S. hybrids rely upon some form of quantitative, polygenically inherited, partial resistance to control diseases considered important in areas where they are marketed. Despite the current reliance upon quantitative forms of disease resistance in maize, our current knowledge of the genetics of this type of resistance is rudimentary. The use of molecular marker technologies has dramatically changed the study of quantitative inheritance in crop plants. In particular, it is now possible to estimate the number, chromosomal location, and the type of gene action for quantitative trait loci (QTLs) associated with a given trait, included disease resistance in maize.

The U.S. Department of Agriculture–Agricultural Research Service maize quantitative genetics program at Raleigh, NC has developed a number of mapping populations of maize to study the inheritance and manipulation of QTLs for agronomic traits, particularly grain yield. One population, a set of random recombinant inbred (RI) lines derived from the cross of the agronomically important inbred lines B73 and Mo17 (35), also is suitable for mapping QTLs conditioning partial resistance to a number of

diseases. B73 is susceptible to many foliar pathogens, whereas Mo17 has a reasonably high level of partial resistance.

Southern leaf blight (SLB) of maize, caused by the ascomycete *Cochliobolus heterostrophus* (Drechs.) Drechs. (anamorph = *Bipolaris maydis* (Nisikado) Shoemaker; synonym = *Helminthosporium maydis* Nisikado), is a serious disease of maize throughout the world where maize is grown under warm, humid conditions (42). Three races of *C. heterostrophus* have been described (34,41). Race O, considered the most common race, is the indigenous race throughout most areas where SLB occurs. Race T, the cause of the 1970 SLB epidemic in North America, is specifically virulent on Texas male-sterile cytoplasm (cmsT) maize due to its ability to produce a polyketide toxin (T toxin) to which cmsT maize is sensitive. After the use of cmsT maize was discontinued following the 1970 epidemic, the frequency of race T in North America has declined to a very low level (21,22). Race C of *C. heterostrophus*, specifically virulent on C male-sterile cytoplasm (cmsC) maize, has been reported from China, but is not known to occur elsewhere (41).

Estimates of yield losses due to SLB vary widely, depending upon the race and environment, and can be quite substantial. During the 1970 SLB epidemic, total crop failures due to race T were reported in the southern United States, while the entire U.S. maize crop in 1970 was reduced by an estimated 15% by SLB (37). Losses of up to 40% or more have been demonstrated in inoculated yield loss trials with race O (6,12,14,38).

Resistance has been the primary means of SLB control. Resistance to race T is achieved by avoiding use of cmsT hybrids.

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Resistance to race O may be inherited in as simple a manner as one (*rhm1*) (10,33) or two (*rhm1* and *rhm2*) (8) linked recessive genes on chromosome 6 (45), a few unlinked recessive nuclear genes (16,36), or many genes acting in a mostly additive manner (4,23,24,27,32). The *rhm1* gene has been of limited use in commercial maize hybrids in the United States due to its recessive inheritance and its ineffectiveness in providing effective control after anthesis. Most hybrids rely on some form of polygenic partial resistance for control of the disease. This type of SLB resistance is generally of medium to high heritability and is easily selected for in breeding programs. Although virulence to these nuclear genes has not been observed in nature, Kolmer and Lenard (19) were able to increase the aggressiveness of *C. heterostrophus* race O to a partially resistant maize inbred line through recurrent selection. Their selection increased not only the level of general aggressiveness of *C. heterostrophus* to all maize inbreds tested, but also specific virulence to the partially resistant inbred line on which the recurrent selection was practiced. They speculated that the reason or reasons such selection has not been observed in nature may be due to rare occurrence of sexual reproduction in *C. heterostrophus*, the low heritability of virulence, and the constantly changing genetic makeup of maize hybrids being grown.

The subject of this mapping study was resistance to SLB, caused by *C. heterostrophus* (anamorph = *B. maydis*), race O. Earlier studies of partial resistance in maize to SLB have used approaches such as diallel and generation mean analysis, or others, to study its inheritance (4,23,24,27,32,36). Moon et al. (26) identified a major QTL for SLB resistance in a set of RI lines, but the technique did not allow mapping this QTL to a specific chromosomal location. Jiang et al. (17) identified a single QTL for resistance to SLB on chromosome 3 in a cross between an inbred line derived from a tropical lowland population and one derived from a tropical highland population. Their analysis was based on data from natural infection with multiple pathogens at a single location where fungicide applications were used. We believe our article is the first detailed report of a study whose specific goal was to map QTLs for partial resistance to SLB.

MATERIALS AND METHODS

Plant materials. In all, 158 random, recombinant F2:7 RI lines, derived from the cross of maize inbred lines B73 and Mo17, were produced by successive generations of self-pollination with no selection for agronomic traits. This RI population initially was created by C. W. Stuber in the late 1980s to map QTLs for agronomic traits in germ plasm that is representative of that used in many commercial maize hybrids.

Field trials. The RI lines were evaluated for levels of partial resistance to SLB (*C. heterostrophus*, race O) in field trials at the North Carolina State University Central Crops Research Station located at Clayton, NC in the summers of 1995 and 1996. Plots were machine planted on 15 April 1995 and 17 April 1996. Experimental units consisted of single-row plots arranged in randomized complete blocks with two replications. Twenty seed were planted in rows which were 3.1 m long and spaced 0.97 m apart. Plots were not thinned.

Inoculations. Experimental plots were inoculated at the four- to six-leaf growth stage by placing 20 to 30 grains of a sorghum grain culture of *C. heterostrophus* race O (isolate 2-16Bm) in the leaf whorl. This isolate was originally isolated from a commercial maize field in Wilkes County, NC in 1985, is of the *mat1-2* mating type, and is of race O pathogenicity (7). The isolate is consistently one of the more aggressive isolates that we have tested. The pathogen was stored in 15% glycerol at -80°C and cultured on a reduced-strength potato dextrose agar (10 g of potato dextrose broth/liter). Conidia were washed from the surface of 2-week-old cultures of *C. heterostrophus* using sterile water. The resulting conidial suspension (20 ml) was used to

inoculate 1-liter flasks of sterilized sorghum seed. The sorghum seed flasks were prepared by soaking sorghum seed overnight in water, draining off the excess water, filling the flasks with the moistened seed, and then autoclaving for 1 h. The sorghum grain cultures were grown at room temperature for 2 weeks, then stored at 4°C until use.

Ratings. Whole plots were visually rated three (1995) or two (1996) times for percent SLB severity beginning at 2 weeks postanthesis and at 2-week intervals. A severe spider mite infestation prevented taking a third SLB rating late in the 1996 growing season. SLB severity data were used to calculate area under the disease progress curve (AUDPC) for each plot (43). Data on days to anthesis (midsilk stage) were taken from plots in the 1996 trial only.

Molecular markers. The RI lines were fingerprinted at 234 restriction fragment length polymorphism, isozyme, and simple sequence repeat marker loci covering most of the maize genome. A map was generated using Mapmaker (20) software. Five percent of marker data was missing from the dataset.

Statistical analyses. Disease severity data initially were analyzed using analysis of variance (ANOVA). Estimates of genotypic (σ^2_g), error (σ^2_e), and genotype $\times$ environment (σ^2_{ge}) variance components were calculated from the ANOVA and used to calculate heritabilities on an entry mean basis and their standard errors as described by Hallauer and Miranda (15): $h^2 = \sigma^2_g / \sigma^2_g + \sigma^2_{ge} + \sigma^2_e$.

There were highly significant ($P < 0.01$) differences among RI lines for reaction to SLB, and replication effects were small; therefore, RI line means from each experiment were used in analysis of marker data. Putative QTLs controlling SLB resistance were detected with a composite interval mapping (CIM) approach using PLABQTL software (39). The map was scanned at 2-centimorgan (cM) intervals. A model that included both additive genetic effects and additive $\times$ additive epistatic effects was fitted to the data. Cofactor markers were selected using the cov SELECT option with a log of the likelihood (LOD) score of >3.0 to enter into the model. The data were analyzed over the two environments and QTL $\times$ environment effects were estimated. Correlations between disease measures and days to anthesis were calculated. The location of putative QTL corresponded to the location (in centimorgans) of peak LOD scores in the scan of individual chromosomes. Putative QTL were designated by the corresponding chromosome bin in which they were found. Chromosome bins divide the genetic map of the 10 chromosomes of maize into 90 segments of approximately 20 cM between two fixed core markers (13). The segments or bins are designated with the chromosome number followed by a two-digit decimal (e.g., 1.00, 1.01, 1.02, and so on). A bin is the interval that includes all loci from the leftmost or top core marker to the next core marker. Placement of a locus to a bin is dependent on the precision of mapping data and increases in certainty as markers increase in number or populations increase.

RESULTS

Disease development. There was excellent disease development in 1995. At 2 weeks postanthesis, the average percent SLB severity on the susceptible inbred B73 was 40% (Table 1). By the last rating date, disease severity had reached 93% on B73 and several RI lines were 100% blighted. In contrast, disease development was much slower in 1996, with B73 reaching only 10% disease severity 2 weeks postanthesis. The hot, dry conditions during grain filling were conducive to a severe spider mite infestation in the plots, which prevented accurate assessment of late-season disease ratings.

There were highly significant differences in SLB reaction among RI lines at all rating dates (Table 2). Genotype $\times$ environment interactions also were a significant source of variation in the

combined analyses. Heritability of SLB reactions ranged from 0.52 to 0.79, depending on the rating date. Correlations between disease severity measures were highly significant and positive, although the correlation between early (SLB1) and late (SLB3) ratings in 1995 was less than 0.50 (Table 3). All SLB ratings were significantly negatively correlated with date of anthesis (midsilk), although none of the correlations were less than -0.20.

Highly significant QTLs for resistance to SLB were detected on both arms of chromosome 1, near the centromere of chromosome 2, on the long arms of chromosomes 3 and 4, the short arms of chromosomes 5 and 7, and the long arms of chromosomes 9 and 10 (Table 4). Up to almost 47% of the phenotypic variation in SLB reactions could be attributed to the QTLs detected. All the QTLs for resistance came from the resistant parent Mo17 except for one of the QTLs on the short arm of chromosome 1, the QTL on the short arm of chromosome 5, and the QTL on the long arm of chromosome 9. Three QTLs, all from the resistant parent Mo17, found on chromosomes 1 (bin 1.09/1.10), 2 (bin 2.04), and 3 (bin 3.03/3.04) were associated with relatively large, consistent effects on SLB reactions over years and rating methods. A single QTL on the long arm of chromosome 1 centered at 196 cM accounted for up to 24.9% of the variation in SLB resistance among the RI lines. Markers in this region of chromosome 1 include phi055, umc107a, umc161a, and phi120. The major QTL on chromosome 2 at 82 cM accounted for up to 19.8% of the phenotypic variation in SLB reaction. Markers in this region of chromosome 2 include bnl2.09, bngl108, nc131, and umc34. The major QTL on chromosome 3 at 52 cM accounted for up to 26.6% of the variation in phenotypic variation for SLB in early ratings. Markers in this region of chromosome 3 include asg48 and phi36.

In addition to the three major QTLs discussed above, several other QTLs for SLB reaction were detected when certain SLB ratings were considered, particularly AUDPC. QTLs on the short arm of chromosome 1, the long arm of chromosome 4, the short arm of chromosome 7, and the long arm of chromosome 10 were detected when AUDPC ratings were used. Although the QTL detected in chromosome bin 1.01 was consistently associated with an allele for SLB susceptibility from Mo17, the additive effect of the QTL was never statistically significant, and in no case ac-

counted for more than 2% of the phenotypic variation. The QTL in chromosome bin 1.02 was consistently associated with SLB resistance from Mo17; however, its additive effect was judged statistically significant only in the combined analysis and accounted for only 3.2% of the phenotypic variation. The additive effects of QTLs on chromosomes 4 and 10 were consistently statistically significant and accounted for up to 6 and 7.7% of the phenotypic variation, respectively.

The significant genotype $\times$ environment interaction seen in the combined analysis of the RI lines also was reflected in significant QTL $\times$ environment interactions (Table 4). The three major QTLs, as well as the QTLs on chromosomes 9 and 10, interacted significantly with environments over the 2 years of the study. In all these significant QTL $\times$ environment interactions, the interaction arose from changes in magnitude of QTL effects between years and not from changes in direction of the effects. In one instance, the effect of a QTL on chromosome 7 changed direction between years; being significantly negative in 1995 and positive but nonsignificant in 1996. However, this QTL $\times$ environment effect was not significant. Estimates of QTL effects were much lower in 1996 versus 1995. No significant epistatic (additive $\times$ additive) effects for SLB resistance were detected in any of the analyses.

DISCUSSION

Our results are consistent with most previous studies of the inheritance of partial resistance to SLB of maize in that additive gene action is of primary importance, with little evidence of epistasis; heritabilities are medium to high; and a relatively small number of loci appear to control resistance. In our study, three major QTLs from the resistant parent Mo17 found on chromosomes 1, 2, and 3 were associated with relatively large, consistent effects on SLB reactions and accounted for most of the genetic variation seen among the RI lines. The major QTL on chromosome 3 is in the same area where a QTL for SLB resistance was found in tropical germ plasm by Jiang et al. (17).

The expression of quantitative resistance in plants often is influenced strongly by the environment. Genotype $\times$ environment interactions are a common feature of genetic studies of resistance

TABLE 1. Means and ranges of percent southern leaf blight (SLB) severities, and area under the disease progress curve (AUDPC) of 158 recombinant inbred (RI) lines derived from the cross Mo17 $\times$ B73

Disease ^a	1995					1996				
	RI line		Mo17	B73	F1	RI line		Mo17	B73	F1
	Mean	Range				Mean	Range			
SLB1	26	5–50	7	40	30	4	0.5–15	1	10	3
SLB2	50	12–90	25	68	40	22	2–60	5	40	27
SLB3	82	30–100	30	93	68	...	...	...	...	...
AUDPC ^c	1,357	455–2,145	569	1,739	1,154	275	27–787	63	525	320

^a Disease assessment; SLB1, SLB2, and SLB3 are expressed as percent disease severity.

^b No disease severity data were recorded on the third rating date in 1996 due to a severe spider mite infestation in the plots.

^c Area under the disease progress curve is in percent-day units.

TABLE 2. Estimates of variance components and heritabilities for resistance to southern leaf blight (SLB) in 158 recombinant inbred lines of maize from the B73 $\times$ Mo17 population measured over 2 years

Trait ^b	Variance component ^a			Heritability (h ²)
	σ^2_g	σ^2_{gxe}	σ^2_e	
SLB1	23.4**	31.2**	24.9	0.52 $\pm$ 0.12
SLB2	137.0**	30.4**	79.6	0.79 $\pm$ 0.11
AUDPC ^c	40,466**	19,685**	18,605	0.74 $\pm$ 0.12

^a ** Indicates variance component is significant at the 0.01 level of probability.

^b Disease assessment; SLB1 and SLB2 are expressed as percent disease severity.

^c Area under the disease progress curve is in percent-day units.

TABLE 3. Phenotypic correlation coefficients between southern leaf blight (SLB) disease ratings and mid-anthesis date (SILKDATE) among 158 recombinant inbred lines from the B73 $\times$ Mo17 population^a

Trait	SLB2	SLB3	AUDPC	SILKDATE
SLB1	0.83**	0.48**	0.92**	-0.15**
SLB2	...	0.63**	0.92**	-0.19**
SLB3	...	...	0.78**	-0.16*
AUDPC	...	...	...	-0.19**

^a Data are based on individual plot ratings over two years except for SLB3 and SILKDATE, which were measured in a single year. AUDPC = area under the disease progress curve; * and ** indicate correlation coefficient is significant at the 0.05 or 0.01 probability level, respectively.

to foliar diseases of maize, including SLB. The significant QTL $\times$ environment interactions for several of the QTLs arose from changes in the magnitude of QTL effects between years. The environment in the growing seasons of the 2 years of our study were very different. The 1995 growing season was very conducive for SLB development, with consistent rains and high humidity, whereas 1996 was very hot and dry, limiting disease development. Because SLB severities were much higher in 1995 than 1996, the magnitudes of any QTL effects also would be much higher and probably gave rise to the QTL $\times$ environment interactions observed.

Because we mapped QTLs using largely homozygous RI lines, only the additive and additive $\times$ additive effects of QTL for SLB resistance could be estimated. Previous inheritance studies of SLB resistance have shown the importance of partial dominance of resistance in certain crosses, although the amount of variation

due to dominance generally was small in relation to additive genetic effects (4,23,24,27,32). In this study, we had no means of measuring dominant QTL effects. However, we found the mean SLB ratings of the F1 to be approximately midway between that of the two parent inbred lines, indicative of mostly additive gene action. Epistasis, although sometimes found to be statistically significant, has not been found to be an important source of variation in previous studies of the inheritance of partial resistance to SLB. In this study, we detected no significant additive $\times$ additive epistasis among the resistance QTLs detected.

SLB often is considered a late-season disease of maturing maize plants, usually not developing until the postanthesis, grain-filling period (42). Because of this, there is concern that late-season disease ratings may be confounded by differences in plant maturity. In this study, we found a consistently significant, albeit low negative correlation between SLB ratings and days to mid silk among

TABLE 4. Location of and parameters associated with major quantitative trait loci (QTLs) for resistance to southern leaf blight (SLB) of maize, caused by *Cochliobolus heterostrophus* race O, in the B73 $\times$ Mo17 recombinant inbred line mapping population^a

Location, parameter ^c	SLB1			SLB2			SLB3	AUDPC ^b		
	1995	1996	Comb.	1995	1996	Comb.	1995	1995	1996	Comb.
1.01 (bn15.62a)										
<i>a</i>	...	...	...	...	...	...	...	24.6	9.2	22.1
<i>R</i> ²	...	...	...	...	...	...	...	0.7	0.3	1.9
<i>a</i> $\times$ E	...	...	...	...	...	...	...	...	...	NS
1.02 (umc157)										
<i>A</i>	...	...	...	...	-0.9	...	...	-37.2	-17.5	-31.4*
<i>R</i> ²	...	...	...	...	0.3	...	...	1.4	0.8	3.2
<i>a</i> $\times$ E	...	...	...	...	...	...	...	...	...	NS
1.07 (amp1)										
<i>a</i>	...	...	...	...	...	...	-4.4**	...	...	...
<i>R</i> ²	...	...	...	...	...	...	11.2	...	...	...
1.09/1.10 (umc107a)										
<i>a</i>	...	...	...	-6.7**	-5.1**	-5.9**	-5.3**	-142.4**	-60.5**	-81.3**
<i>R</i> ²	...	...	...	22.9	13.7	22.2	12.9	23.5	11.9	24.9**
<i>a</i> $\times$ E	...	...	...	...	...	NS	...	...	...	...
2.04 (bn12.09)										
<i>a</i>	-2.7**	-1.0	-1.9**	-5.5**	-5.1**	-5.3**	-4.6**	-118.4**	-65.4**	-71.5**
<i>R</i> ²	7.9	7.9	9.5	17.9	14.8	19.6	13.1	16.9	14.0	19.8**
<i>a</i> $\times$ E	...	...	NS	...	...	NS	...	...	...	...
3.03/3.04 (asg48)										
<i>a</i>	-5.6**	-0.8**	-3.1**	-6.4**	-4.0**	-5.2**	-3.8**	-132.5**	-46.6**	-49.1**
<i>R</i> ²	26.6	5.0	21.6**	19.2	8.1	15.9	6.9	21.6	8.2	11.2**
<i>a</i> $\times$ E	...	...	...	...	...	NS	...	...	...	...
4.09 (npi449b)										
<i>a</i>	...	...	...	...	...	...	...	-45.1*	-38.0**	-28.2*
<i>R</i> ²	...	...	...	...	...	...	...	3.3	6.0	4.3
<i>a</i> $\times$ E	...	...	...	...	...	...	...	...	...	NS
5.01 (umc147a)										
<i>a</i>	...	...	...	3.9**	1.7	2.9**	...	...	...	...
<i>R</i> ²	...	...	...	8.5	1.5	5.7	...	...	...	...
<i>a</i> $\times$ E	...	...	...	...	...	NS	...	...	...	...
7.02 (umc116a)										
<i>a</i>	...	...	...	...	...	...	...	-47.0*	3.4	-18.4
<i>R</i> ²	...	...	...	...	...	...	...	3.5	0.1	1.8
<i>a</i> $\times$ E	...	...	...	...	...	...	...	...	...	NS
9.05 (csu93a)										
<i>a</i>	2.1*	0.5	1.3*	2.2*	3.1**	2.7**	...	...	...	...
<i>R</i> ²	4.1	1.7	3.7**	3.2	5.4	5.4	...	...	...	...
<i>a</i> $\times$ E	...	...	...	...	...	NS	...	...	...	...
10.04 (umc44a)										
<i>a</i>	...	...	...	...	...	...	...	-65.2**	-32.7*	-44.0**
<i>R</i> ²	...	...	...	...	...	...	...	5.2	3.5	7.7*
<i>a</i> $\times$ E	...	...	...	...	...	...	...	...	...	...
Total <i>R</i> ²	35.6	19.2	32.7	44.7	31.6	43.4	36.7	46.9	33.2	45.8
Epistasis	...	...	NS	...	...	NS	NS	...	...	NS

^a Data are from models fitted to means over 2 years, with the exception of SLB3 which was measured only in 1995.

^b AUDPC = area under the disease progress curve; NS = not significant.

^c Chromosomal location: bin and nearest marker. Chromosome bin location of putative QTL on 1 of the 10 chromosomes of the maize genome. Bins divide the genetic map into 90 segments of approximately 20 centimorgans between two fixed core markers. The segments are designated with the chromosome number followed by a two-digit decimal (e.g., 1.00, 1.01, 1.02, and so on). A bin is the interval that includes all loci from the leftmost or top core marker to the next core marker. Placement of a locus to a bin is dependent on the precision of mapping data, and increases in certainty as markers increase in number or populations increase. Parameters: *a* = additive genetic effect of the putative QTL and E = additive genetic effect $\times$ environment (year) interaction.

the RI lines. Because days to mid silk was measured in only 1 year, and the range in maturities among the RI lines and between the two parents was low, no attempt was made to map QTLs for this trait in this population. The low negative correlation between maturity and SLB ratings suggests that few, if any, of the QTLs for SLB resistance are associated with plant maturity.

Genes for resistance to different pests and pathogens often are clustered in the maize genome (25). When the three major QTLs identified in this study are compared with previously mapped disease and pest resistance loci, several associations are noticed. Resistance to the sugarcane borer (2) has been mapped to the same region of chromosome 1 (bin 1.10) where we found a major QTL for SLB resistance. QTLs for resistance to gray leaf spot (3) and Gibberella stalk rot (28) have been mapped to the same region of chromosome 2 where another major QTL for SLB resistance was found. Interestingly, the locus for the pathogenesis-related protein (*prp2*) (9) also is located in the same region of chromosome 2. Located in the same region of chromosome 3 as the third major QTL for SLB resistance is the *Rp3* locus for resistance to *Puccinia sorghi* (30), and QTLs for resistance to Gibberella stalk rot (28) and sugarcane mosaic virus (44). There are also other pathogen or pest resistance loci in the same chromosomal regions as minor QTLs for SLB resistance found in our study. A QTL for resistance to gray leaf spot (3) is located in the same chromosomal bin (1.02) as a QTL for SLB resistance in our study. QTLs for resistance to Gibberella stalk rot (28) and *Peronosclerospora sorghi* (1) are located in the same region (bin 1.07) of chromosome 1 as another minor QTL for SLB resistance. A QTL for SLB susceptibility in Mo17 is in the same bin on chromosome 5 as a QTL for resistance to northern leaf blight (11). The QTL for susceptibility to SLB on chromosome 9 is in the same bin as a QTL for resistance to southwestern corn borer (18). The QTL for SLB resistance on chromosome 10 is in the same bin as QTLs for resistance to second brood European corn borer (31), sugarcane borer (2), and ear worm (5). No known pathogen or pest resistance loci are known in the chromosomal regions associated with the SLB resistance QTLs on chromosomes 4 and 7. Although there is some evidence of clustering resistance genes in the maize genome, it is not clear how many of these "clusters" are due simply to random chance versus some underlying genetic principle or mechanism. As the number of QTLs for pathogen and pest resistance being mapped increases, so does the probability that resistance gene clusters will be found purely due to chance. QTLs in these clusters will need to be cloned and sequenced before any conclusions about the origin of these clusters can be drawn.

Robertson has hypothesized that QTLs often are alleles at major gene loci controlling the same trait (29). In our study, we found no evidence of QTLs for SLB resistance at or near the major gene locus for SLB resistance, *rhml* on chromosome 6. This does not preclude the possibility that QTLs for SLB resistance might be located at other major gene loci for SLB resistance that have yet to be mapped, such as those described by Thompson and Berquist (36).

Phenotypic selection for SLB resistance is easy and economical with existing artificial inoculation and rating methods. Marker-assisted selection of the major QTLs found in this study might be useful if selection would be conducted in an environment that was not conducive for SLB development or a breeder wanted to pyramid different QTLs from different sources of resistance into a single inbred line.

Development of near isogenic lines (NILs) via marker-assisted backcrossing (MAB) may prove to be a valuable tool in the attempt to isolate and measure the effects of individual QTLs for partial resistance to SLB, such as been done with QTLs for partial resistance to leaf rust in barley (40). The transfer of single chromosomal segments associated with the major QTLs found in this study into a susceptible inbred line via MAB would be relatively

easy and should confer a measurable level of partial SLB resistance. Development of NILs in different genetic backgrounds also would allow the study of the effects of those backgrounds on expression of these QTLs. MAB also could be used to attempt to transfer some of the QTLs with minor or inconsistent effects found in this study to determine if those effects are real. The development of NILs, along with additional fine mapping of these major QTLs, may prove helpful in the eventual cloning of a QTL for SLB resistance.

LITERATURE CITED

1. Agrama, H. A., Moussa, M. E., Naser, M. E., Tarek, M. A., and Ibrahim, A. H. 1999. Mapping of QTL for downy mildew resistance in maize. *Theor. Appl. Genet.* 99:519-523.
2. Bohn, M., Khairallah, M. M., Gonzalez-de-Leon, D., Hoisington, D. A., Utz, H. F., Deutsch, J. A., Jewell, D. C., Mihm, J. A., and Melchinger, A. E. 1996. QTL mapping in tropical maize: genomic regions affecting leaf feeding resistance to sugarcane borer and other traits. *Crop Sci.* 36:1352-1361.
3. Bubeck, D. M., Goodman, M. M., Beavis, W. D., and Grant, D. 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Sci.* 33:838-847.
4. Burnette, D. C., and White, D. G. 1985. Inheritance of resistance to *Bipolaris maydis* race 0 in crosses derived from nine resistant inbred lines of maize. *Phytopathology* 75:1195-1200.
5. Byrne, P. F., McMullen, M., Wiseman, B. R., Snook, M. E., Musket, T., Theuri, J., Widstrom, N. W., and Coe, E., Jr. 1998. Maize silk maysin concentration and corn earworm antibiosis: QTLs and genetic mechanisms. *Crop Sci.* 38:461-471.
6. Byrnes, K. J., Pataky, J. K., and White, D. G. 1989. Relationships between yield of three maize hybrids and severity of southern leaf blight caused by race 0 of *Bipolaris maydis*. *Plant Dis.* 73:834-840.
7. Carson, M. L. 1998. Aggressiveness and perennation of isolates of *Cochliobolus heterostrophus* from North Carolina. *Plant Dis.* 82:1043-1047.
8. Chang, R. Y., and Peterson, P. A. 1995. Genetic control of resistance to *Bipolaris maydis*: one gene or two genes? *J. Hered.* 86:94-97.
9. Chao, S., Baysdorfer, C., Heredia-Diaz, O., Musket, T., Xu, G., Coe, E. H. 1994. RFLP mapping of partially sequenced leaf cDNA clones in maize. *Theor. Appl. Genet.* 88:717-721.
10. Craig, J., and Fajemisin, J. M. 1969. Inheritance of chlorotic lesion resistance to *Helminthosporium maydis* in maize. *Plant Dis. Rep.* 53:742-743.
11. Dingerdisen, A. L., Geiger, H. H., Lee, M., Schechert, A., and Welz, H. G. 1996. Interval mapping of genes for quantitative resistance of maize to *Setosphaeria turcica*, cause of northern leaf blight, in a tropical environment. *Mol. Breed.* 2:143-156.
12. Fisher, D. A., Hooker, A. L., Lim, S. M., and Smith, D. R. 1976. Leaf infection and yield loss caused by four *Helminthosporium* leaf diseases of corn. *Phytopathology* 66:942-944.
13. Gardiner, J., Coe, E., Jr., Melia-Hancock, S., Hoisington, D., and Chao, S. 1993. Development of a core RFLP map in maize using an immortalized-F2 population. *Genetics* 134:917-930.
14. Gregory, L. V., Ayers, J. E., and Nelson, R. R. 1978. Predicting yield losses in corn from southern corn leaf blight. *Phytopathology* 68:517-521.
15. Hallauer, A. R., and Miranda, J. B. 1988. *Quantitative Genetics in Maize Breeding*, 2nd ed. Iowa State University Press, Ames, IA.
16. Holley, R. N., and Goodman, M. M. 1989. New sources of resistance to southern corn leaf blight from tropical maize derivatives. *Plant Dis.* 73:562-564.
17. Jiang, C., Edmeades, G. O., Armstead, I., Lafitte, H. R., Hayward, M. D., and Hoisington, D. 1999. Genetic analysis of adaptation differences between highland and lowland tropical maize using molecular markers. *Theor. Appl. Genet.* 99:1106-1119.
18. Khairallah, M., Bohn, M., Jiang, C. Z., Deutsch, J. A., Jewell, D. C., Mihm, J. A., Melchinger, A. E., Gonzalez-de-Leon, D., and Hoisington, D. 1998. Molecular mapping of QTL for southwestern corn borer resistance, plant height and flowering in tropical maize. *Z. Pflanz.* 117:309-318.
19. Kolmer, J. A., and Leonard, K. J. 1986. Genetic selection and adaptation of *Cochliobolus heterostrophus* to corn hosts with partial resistance. *Phytopathology* 76:774-777.
20. Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E., and Newburg, L. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174-181.

21. Leonard, K. J. 1977. Races of *Bipolaris maydis* in the southeastern US from 1974-1976. Plant Dis. Rep. 61:914-915.
22. Leonard, K. J. 1977. Virulence, temperature optima, and competitive abilities of isolines of races T and O of *Bipolaris maydis*. Phytopathology 67:1273-1279.
23. Lim, S. M. 1975. Heterotic effects of resistance in maize to *Helminthosporium maydis* race 0. Phytopathology 65:1117-1120.
24. Lim, S. M., and Hooker, A. L. 1976. Estimates of combining ability for resistance to *Helminthosporium maydis* race 0 in a maize population. Maydica 21:121-128.
25. McMullen, M. D., and Simcox, K. D. 1995. Genomic organization of disease and insect resistance genes in maize. Mol. Plant-Microbe Interact. 8:811-815.
26. Moon, H. G., Brewbaker, J. L., and Lu, X. W. 1999. Major QTL for disease resistance and other traits identified in recombinant inbred lines from tropical maize hybrids. Maydica 44:301-311.
27. Pate, J. R., and Harvey, P. H. 1954. Studies on the inheritance of resistance in corn to *Helminthosporium maydis* leaf spot. Agron. J. 46:442-445.
28. Pe, M. E., Gianfranceschi, L., Taramino, G., Tarchini, R., Angelini, P., Dani, M., and Binelli, G. 1993. Mapping quantitative trait loci (QTLs) for resistance to *Gibberella zeae* infection in maize. Mol. Gen. Genet. 241:11-16.
29. Robertson, D. S. 1985. A possible technique for isolating genic DNA for quantitative traits in plants. J. Theor. Biol. 117:1-10.
30. Sanz-Alferez, S., Richter, T. E., Hulbert, S. H., and Bennetzen, J. L. 1995. The Rp3 disease resistance gene of maize: Mapping and characterization of introgressed alleles. Theor. Appl. Genet. 91:25-32.
31. Schon, C. C., Lee, M., Melchinger, A. E., Guthrie, W. D., and Woodman, W. L. 1992. Mapping and characterization of quantitative trait loci affecting resistance against second-generation European corn borer in maize with the aid of RFLPs. Heredity 70:648-659.
32. Scott, G. E., and Futrell, M. C. 1975. Reaction of diallel crosses of maize in T and N cytoplasms to *Bipolaris maydis* race T. Crop Sci. 15:779-782.
33. Smith, D. R., and Hooker, A. L. 1973. Monogenic chlorotic-lesion resistance in corn to *Helminthosporium maydis*. Crop Sci. 13:330-331.
34. Smith, D. R., Hooker, A. L., and Lim, S. M. 1970. Physiologic races of *Helminthosporium maydis*. Plant Dis. Rep. 54:819-822.
35. Stuber, C. W., Lincoln, S. E., Wolff, D. W., Helentjaris, T., and Lander, E. S. 1992. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. Genetics 132:823-839.
36. Thompson, D. L., and Bergquist, R. R. 1984. Inheritance of mature plant resistance to *Helminthosporium maydis* race 0 in maize. Crop Sci. 24:807-811.
37. Ullstrup, A. J. 1972. The impacts of the southern corn leaf blight epidemics of 1970-71. Annu. Rev. Phytopathol. 10:37-50.
38. Ullstrup, A. J., and Miles, S. R. 1957. The effects of some leaf blights of corn on grain yield. Phytopathology 47:331-336.
39. Utz, H. F., and Melchinger, A. E. 1996. PLABQTL: A program for composite interval mapping of QTL. J. Quant. Trait Loci. On-line publication: vol. 2, paper 1. Sponsored by the Crop Science Society of America.
40. van Berloo, R., Aalbers, H., Werkman, A., and Niks, R. E. 2001. Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Mol. Breed. 8:187-195.
41. Wei, J., Lui, K., Chen, J., Luo, P., and Lee-Stadelmann, O. Y. 1988. Pathological and physiological identification of race C of *Bipolaris maydis* in China. Phytopathology 78:550-554.
42. White, D. G., ed. 1999. Compendium of Corn Diseases. 3rd ed. The American Phytopathological Society, St. Paul, MN.
43. Wilcoxson, R. D., Atif, A. H., and Skovmand, B. 1974. Slow rusting of wheat varieties in the field correlated with stem rust severity on detached leaves in the greenhouse. Plant Dis. Rep. 58:1085-1087.
44. Xia, X., Melchinger, A. E., Kuntze, L., and Lubberstedt, T. 1999. Quantitative trait loci mapping of resistance to sugarcane mosaic virus in maize. Phytopathology 89:660-667.
45. Zaitlin, D., Demars, S. J., and Ma, Y. 1993. Linkage of *rhm*, a recessive gene for resistance to southern corn leaf blight, to RFLP marker loci in maize (*Zea mays*) seedlings. Genome 36:555-564.