Mapping Resistance Quantitative Trait Loci for Three Foliar Diseases in a Maize Recombinant Inbred Line Population—Evidence for Multiple Disease Resistance?

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ABSTRACT

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Southern leaf blight (SLB), gray leaf spot (GLS), and northern leaf blight (NLB) are all important foliar diseases impacting maize production. The objectives of this study were to identify quantitative trait loci (QTL) for resistance to these diseases in a maize recombinant inbred line (RIL) population derived from a cross between maize lines Ki14 and B73, and to evaluate the evidence for the presence genes or loci conferring multiple disease resistance (MDR). Each disease was scored in multiple separate trials. Highly significant correlations between the resistances and the three diseases were found. The highest correlation was identified between SLB and GLS resistance (r = 0.62). Correlations between resistance to each of the diseases and time to flowering were also highly significant. Nine, eight, and six QTL were identified for SLB, GLS, and NLB resistance, respectively. QTL for all three diseases colocalized in bin 1.06, while QTL colocalizing for two of the three diseases were identified in bins 1.08 to 1.09, 2.02/2.03, 3.04/3.05, 8.05, and 10.05. QTL for time to flowering were also identified at four of these six loci (bins 1.06, 3.04/3.05, 8.05, and 10.05). No disease resistance QTL was identified at the largest-effect QTL for flowering time in bin 10.03.

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* The *e-***X**tra logo stands for "electronic extra" and indicates that the online version contains a figure showing the Ki14 × B73 linkage map of the 10 maize chromosomes and the positions of the QTL identified in this study.

Most maize (*Zea mays* L. subsp. *mays*) disease resistance is quantitative rather than qualitative in nature (83). Qualitative disease resistance is generally controlled by one gene or a few genes with major effects, whereas quantitative disease resistance (QDR) is generally controlled by many minor genes (61,75). Although rapid progress has been made in recent years in the genetic characterization of qualitative disease resistance (11), progress in the understanding of the genetic and physiological processes underlying QDR has been limited due to their complexity and incomplete and variable expression (29,40,85).

Multiple disease resistance (MDR), in which the same locus conditions resistance to multiple pathogens, is both practically and conceptually important and yet is also poorly understood. Limited evidence is available regarding quantitative trait loci (QTL) conditioning MDR. The detection of clusters of QTL conferring resistance to multiple diseases is consistent with but does not prove the hypothesis that MDR genes are present in plants (83,84). More direct evidence for MDR is the observation of pleiotropic effects on multiple diseases shown with some induced gene mutations (17,18,23,54). Mitchell-Olds (53) studied genetic correlations among levels of disease resistance of *Brassica rapa* to three fungal pathogens: *Peronospora parasitica*, *Albugo candida*, and *Leptosphaeria maculans.* They reported heritable genetic variation for resistance to all three pathogens and positive, statistically significant genetic correlations between resistance to *L. maculans* and *P. parasitica* in populations in which selection was directed at only one of the pathogens. Recently, Balint-Kurti et al. (6) identified highly significant correlations between resistances to southern leaf blight (SLB), gray leaf spot (GLS), and northern leaf blight (NLB) in the maize intermated B73 × Mo17 (IBM) population (43), although they did not detect any disease resistance QTL associated with resistance to all three diseases. Analysis of complex trait inheritance in single population can only provide a partial understanding of its genetic architecture, however, because of the potential genetic heterogeneity of such traits across diverse germplasm. Therefore, a robust understanding of the genetic architecture of MDR requires its analysis in additional mapping populations.

SLB, causal agent *Cochliobolus heterostrophus* (Drechsler) Drechsler (anamorph = *Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker); GLS, caused by *Cercospora zeae-maydis* (Tehon and E. Y. Daniels); and NLB, causal agent *Setosphaeria turcica* (Luttr.) K. J. Leonard & Suggs (anamorph *Exserohilum turcicum*

(Pass.) K. J. Leonard & Suggs), are foliar, substantially necrotrophic, fungal pathogens of maize. All three pathogens are ascomycete fungi in the class Dothideomycetes and share some similar pathogenesis characteristics (10,38). For all these diseases, infection is initiated when spores land on the leaf surface, germinate, and penetrate either directly through the stomata or the leaf cuticle and epidermis. *S. turcica* grows intracellularly in the leaf while *Cochliobolus heterostrophus* and *Cercospora zeae-maydis* grow intercellularly during initial infection. The latent periods (period of time from infection to sporulation) for the three fungi vary from a few days for *Cochliobolus heterostrophus* to ≈2 weeks for *S. turcica* and up to 3 weeks for *Cercospora zeae-maydis* (10,38). It seems likely that loci associated with regulating aspects of the parts of the pathogenesis process shared by two or more of these pathogens may be detected as MDR QTL. This hypothesis was tested in this study.

SLB is a widespread disease with the potential to cause yield losses in hot and humid tropical and subtropical regions, such as the southeastern United States, parts of India, Africa, Latin America, and Southern Europe (82). Resistance to SLB is quantitatively inherited and the gene action is primarily additive or partially dominant (14,34–36,46,47,55,71). Under experimental conditions, yield losses as high as 38 to 46% have been observed in maize inoculated with SLB (15,28). However, losses of this magnitude are rare, because most hybrids have some level of quantitative resistance.

GLS is one of the most significant yield-limiting diseases of maize worldwide. It has greatly increased in importance and geographical distribution in the last 25 years, primarily as a result of moves toward conservation tillage and no-till practices, which allows GLS inoculum to overwinter in debris on the soil surface (1,42,56,57). This disease is a serious threat in the Eastern United States and sub-Saharan Africa as well as in the more temperate and humid regions of Mexico, Central and South America, Europe, and Asia (49,79,82). In most cases, GLS resistance is moderately to highly heritable and additive in nature (30,37,72).

NLB can cause extensive disease in areas where temperatures are moderate (15 to 25°C) during the growing season (45). Severe NLB infection prior to flowering may cause >50% total yield loss (59,70). Historically both, qualitative and quantitative disease resistance have been important for controlling NLB. (22,82).

The objectives of this study were to map QTL associated with SLB, GLS, and NLB resistance in a Ki14 × B73 (KB) recombinant inbred line (RIL) population and to identify QTL that potentially confer MDR. Disease resistance has been shown in a number of studies to be correlated with time to maturity (83). Therefore, QTL for time to anthesis were also mapped to see whether they colocalized with any disease resistance QTL.

MATERIALS AND METHODS

Parents and RIL development. A RIL population was developed from a cross between two maize inbreds, Ki14 and B73. The parents are of tropical and U.S. Corn Belt origin, respectively. Ki14 (previously designated KUI14) was released in 1975 from Kasetsart University in Thailand. It was developed from Suwan-1 (S) C4, a high-yielding cultivar which provided resistance to infection by *Peronosclerospora sorghi* (casual agent of downy-mildew) (67,69). The Suwan-1 population was initially developed from a composite pedigree of West Indian, Mexican, and Central and South American lines, U.S. dents, *P. sorghi*-resistant lines, and other germplasm. Ki14 is more resistant than B73 to SLB, NLB, and GLS.

B73 was developed at Iowa State University (62,73). Although B73 is susceptible to many diseases and insects, its high yield potential (2,26) and excellent seed production attributes led to its widespread use in the development of public- (32) and private-sector inbred lines (52).

Individual F₂ generation plants from a KB cross were selfed for five generations to produce $F_{5:6}$ seed. These lines were designated as RIL lines KB001 to KB135. Then, 117 of the RILs were self-pollinated in Ohio to produce $F_{6:7}$ seed. The lines were again increased by self-pollination of several plants within each line and the seed was bulked to produce $F_{6:8}$ lines. The $F_{6:8}$ RILs (henceforth called the KB population) were used for screening SLB, GLS, and NLB in this study. All 117 lines were assessed for each disease although, ultimately, only 109 of these lines were suitable for use in QTL analysis (see below).

Field trials. Disease screening trials for SLB were performed in five environments: at Clayton, NC in 2004, 2005, and 2006 (CL04, CL05, and CL06); at Tifton, GA in 2005 (GA05); and one environment in Wooster, OH in 2004 (KING04), with two replications at each environment. Each trial was performed using a randomized complete block design. CL04, CL05, CL06, and GA05 plots were planted as single rows 2 m in length, with 0.97 m between rows and a 0.6-m alley at the end of each plot. Twelve seeds per entry were planted in each plot and the rows were not thinned. KING04 plots were planted as single rows 3.04 m in length, with 0.76 m between rows and a 0.46-m alley at the end of each plot. Fifteen seeds per entry were planted in each plot and rows were not thinned.

SLB inoculum for the field disease screening experiments was prepared as previously described (21), and rows were inoculated at the four- to six-leaf stage by placing ≈20 grains of *Cochliobolus heterostrophus* race O, isolate 2-16Bm-infested (*Sorghum bicolor* M.) grain in the leaf whorl (20,21). Immediately after inoculation in the late afternoon, the field was irrigated by overhead irrigation to provide free moisture to initiate fungal growth.

Disease screening trials for GLS were performed in six environments: at Andrews, NC in 2004 and 2005 (ANDW04 and ANDW05); at Salisbury, NC in 2006 (SBRY06); and two environments in 2004 and one environment in 2005 at Wooster, OH (FRY04, FRY05, and KING04), with two replications at each environment. Each trial conducted in North Carolina was performed using a randomized complete block design. Plots were planted as single rows 4 m in length, with 0.97 m between rows and a 0.6-m alley at the end of each plot. Fifteen seeds per entry were planted in each plot and the rows were not thinned. Ohio trials were planted as single rows 3.04 m in length, with 0.76 m between rows and a 0.46-m alley at the end of each plot. The FRY04 and KING04 environments were inoculated using isolates collected in the field in 2003. The inoculum was applied by placing several infested sorghum kernels in the whorl of the maize plants. Naturally occurring GLS inoculum was used for field disease screening trials for ANDW04, ANDW05, FRY05, and SBRY06. These four trials were no-till planted into corn residue from the previous year.

NLB disease screening trials were performed at six environments: at Clayton, NC in 2006 (CL06); and two environments in 2004 and one environment in each of 2005, 2006 and 2007 at Wooster, OH (FRY04, KING04, SHFTR05, SHFTR06, and OARDC07), with two replications planted at each environment, except for OARDC07, with one replication. Trials for CL06, FRY04, and KING04 were planted as described above. All other plots at Ohio were planted as single rows 4.6 m in length, with 0.76 m between rows and a 0.61-m alley at the end of each plot.

The CL06 NLB inoculation was conducted using infected sorghum grain produced in the same way as the SLB inoculum (21). Rows were inoculated at the four- to six-leaf stage by placing ≈20 grains of NLB inoculum (sorghum grain culture) in the leaf whorl. The NLB inoculum contained a mixture of isolates with various race-specificities (*Setosphaeria turcica* race 0, race 1, race 23, and race 23N). The remaining five environments were inoculated using the following procedures. *E. turcicum* inoculum was produced from an isolate obtained from infected maize leaves from Licking County, OH. Aseptic cultures were produced from

sporulating conidia and maintained in petri plates containing lactose casein hydrolysate agar (37.5 g of lactose, 3 g of casein hydrolysate, 1 g of KH_2PO_4, 5 g of $MgSO_4$, 2 ml of micro-elements, and 15 g of agar dissolved in 1 liter of deionized water). Colonized media sections from the culture were placed onto sorghum kernels as described above. Cultures were subsequently handled according to the protocol described by Hakiza et al. (31). Before inoculation, race-specific virulence of the isolates was established by inoculating known differentials of maize: inbred lines H4460, H4460*Ht1*, H4460*Ht3*, A619, A619*Ht* and A619*Ht2* grown in the greenhouse at the Ohio Agricultural Research and Development Center (OARDC). Based on the specific reactions of these differentials, isolates were confirmed to be race 1. Neither Ki14 nor B73 have any known *Ht* genes and, therefore, are assumed to have no qualitative resistance to any of the NLB isolates used. Plots were inoculated twice by placing several kernels of NLB inoculum (sorghum grain culture) in the leaf whorl at the five-leaf stage and then again at the seven-leaf stage.

Rating of field trials. All field trials were scored for resistance on a per-plot basis using a 1-to-9 scale in increments of 0.5, with 1 = symptomless, 3 = small lesions on the ear leaf, 5 = confluent necrosis on the ear leaf, 7 = the ear leaf and the leaf above it almost completely dead, and 9 = completely dead. The field trials in Clayton, Salisbury, and Andrews were rated multiple times at intervals of 10 days to 2 weeks, while the trials in Ohio were only rated once, late during the growing season after flowering but before leaf senescence. Because more than two disease ratings were taken in some environments the weighted mean disease (WMD) was calculated as previously described (8). WMD rating values were calculated for each replication in each environment by taking the average value of two consecutive ratings and multiplying by the number of days between the ratings. Values were then summed over all the intervals and divided by the number of days between the first and last evaluations. WMD is equivalent to standardized area under the disease progress curve (sAUDPC) (16,68).

Days to anthesis (DTA) was measured for the SLB CL04, CL05, and CL06 trials and for the GLS ANDW05 trial. A row was deemed to have reached anthesis when half the plants in the row were shedding pollen. Because of the strong influence of temperature on DTA, measurements were converted to growing degree days to anthesis (GDDTA) following McMaster and Wilhelm (50), with 10 and 30°C as minimum and maximum temperatures, respectively. Growing degree day is a measurement of the cumulative average daily heat units that an experimental plot receives from the time of planting

DNA extraction and molecular markers. For simple-sequence repeat (SSR) analyses, DNA was extracted using a modified Saghai-Maroof et al. (63) cetyltrimethylammonium bromide extraction procedure previously described (87). Two parents, B73 and Ki14, and 117 lines from the KB population

were screened with ≈200 SSR primer pairs and 1,536 SNP markers to identify polymorphic markers for the KB population. Amplification of SSRs on the parents and the KB population were screened as previously described (66). SSR detection was performed as described by Robertson-Hoyt et al. (60). SNP genotype data were generated using the Illumina Golden Gate genotyping assay (27). Of the initial 1,536 SNPs, 771 SNPs failed, were homozygous for the KB population, or showed >30% hetero-zygosity, resulting in data available for 765 SNPs. In addition, 106 polymorphic SSR markers were identified. Based on the genotypic data, eight lines were identified as likely or possible outcrosses and were removed from the data set. Therefore, the statistical analyses, linkage mapping, and QTL mapping were performed using 109 RILs.

Construction of linkage map. JoinMap 3.0 (76) was used to estimate locus orders, identify linkage groups, and transform estimated recombination frequencies to centimorgans using the Haldane mapping function. Linkage mapping was performed on a composite map produced by combining the four mapping populations, including the KB population, as previously described (25). In addition to the KB population, the other three RIL populations used to produce the linkage map were developed by crossing B73 × CML254, CML254 × B97, and B97 × Ki14. The KB linkage map (25) was produced by combining the SSR data from each of the four mapping populations, plus SNP data from the two populations which had B73 as a parent (i.e., the KB population and B73 × CML254), because these were the only populations from that study with available SNP genotypic data at the time of our analysis. In regions where the marker order of the KB map differed greatly from the intermated IBM reference maps (http://www.maizegdb.org), the MAPMAKER/EXP 3.0 (41,48) COMPARE command was used to finalize marker order. The final linkage map produced by MAPMAKER/EXP agreed closely with the order defined by the commonly used IBM2 2005 Neighbors (IBM 2005) reference map (http://www.maizegdb.org).

Statistical analyses. Genotype least square means (LSM) for SLB WMD, GLS WMD, and NLB WMD were estimated across

TABLE 2. Pearson correlation coefficients between least square means for southern leaf blight measured at five environments: at Clayton, NC in 2004, 2005, and 2006 (CL04, CL05, and CL06); at Tifton, GA in 2005 (GA05); and at Wooster, OH in 2004 (KING04)

Location	CL05	CL06	GA05	KING04
CL04	$r = 0.88$ $P < 0.0001$	$r = 0.85$ $P < 0.0001$	$r = 0.80$ $P < 0.0001$	$r = 0.36$ $P = 0.002$
CL05	…	$r = 0.89$ $P < 0.0001$	$r = 0.82$ $P < 0.0001$	$r = 0.37$ $P = 0.001$
CL06	…	…	$r = 0.83$ $P < 0.0001$	$r = 0.28$ $P = 0.024$
GA05	…	…	…	$r = 0.31$ $P = 0.011$

TABLE 1. Variance component estimates (Var.), their standard errors (std err), and *P* values of the random effects in mixed-models analysis of southern leaf blight (SLB), gray leaf spot (GLS), and northern leaf blight (NLB) disease ratings obtained across all environments for the Ki14 × B73 recombinant inbred line population[a]

Random factor[b]	SLB		GLS		NLB	
	Var. (SE)	*P* value	Var. (SE)	*P* value	Var. (SE)	*P* value
Env	0.57 (0.42)	0.0002	0.3 (0.2)	0.0001	2.11 (1.36)	<0.0001
Rep (Env)	0.03 (0.02)	<0.0001	0.02 (0.02)	<0.0001	0.04 (0.04)	0.0027
Line	0.45 (0.07)	<0.0001	0.27 (0.05)	<0.0001	0.44 (0.09)	<0.0001
Env × Line	0.16 (0.03)	<0.0001	0.22 (0.03)	<0.0001	0.38 (0.08)	0.0001
Error	0.29 (0.02)	…	0.35 (0.02)	…	1.25 (0.08)	…

[a] Disease screening trials for SLB were performed at five environments (Clayton, NC: CL04, CL05, and CL06; Tifton, GA: GA05; and Wooster, OH: KING04). Disease screening trials for GLS were performed at six environments (Andrews, NC: ANDW04 and ANDW05; Salisbury, NC: SBRY06; and Wooster, OH:FRY04, FRY05, and KING04). NLB disease screening trials were performed at six environments (Clayton, NC: CL06; and Wooster, OH: FRY04, KING04, SHFTR05, SHFTR06, and OARDC07).

[b] Env = environment and Rep = replication.

all environments for each disease and were used as the trait values for QTL analysis. In addition, LSM for GDDTA were calculated across the four environments in which this trait was measured (see above). LSM were calculated using PROC MIXED in SAS (version 9.1.3; SAS Institute, Cary, NC) with line as a fixed-effect factor and all other factors (environment, environment–line, and replication within environment) considered as random effects. The PROC CORR procedure in SAS was used to calculate all phenotypic correlations. Estimates of heritability, environment, replication within environment, line, and environment–line interaction were considered random in PROC MIXED (33). Significance of random-effect variation was tested using the type III F test (PROC MIXED, method = type3).

QTL detection and estimation. In the current study, detected QTL are reported for each SLB, GLS, NLB, and GDDTA, with all environments combined for each disease. QTL Cartographer version 2.5 (78) was used for QTL mapping. Composite interval mapping (CIM) was used to create an initial model for each disease separately. CIM was performed using a 1.0-centimorgan (cM) walk speed, a window size of 10 cM, and the CIM Model 6 with forward and backward regression, using a probability of 0.05 to include or exclude a QTL from the model.

Multiple interval mapping (MIM) was initiated from the CIM models using a logarithm-of-odds (LOD) threshold of 2.6 and a minimum distance of 10 cM between QTL. These values were chosen to reduce the number of QTL used in the initial model, because the default settings included too many QTL in the initial model. The search for the best MIM model was completed in an iterative, stepwise manner, searching for new QTL, testing for their significance after each cycle of searching for new QTL, and optimizing QTL positions when new QTL were added to the model. New models were accepted if they decreased the Bayesian information criterion (BIC) (58). Use of the BIC is the best choice if the experimental objectives are to deduce genetic parameters such as the number of QTL (86). Using the BIC gives preference to models with higher likelihoods; however, it also includes a penalty for each additional parameter that is added to the model to prevent overfitting the model (4,60,74). When no additional QTL could be added to the model while decreasing the BIC, each pair of QTL in the model was tested for epistatic interactions. An additional approach used to prevent overfitting QTL models was to exclude models in which the proportion of the total phenotypic variation accounted for by the QTL exceeded the entry mean heritability. If the model was overfit, the QTL with the smallest effect was dropped from the model and the effects of the remaining QTL were reestimated. Additive effects and QTL interactions were reported when the LOD score was >2.0. These threshold values were set to limit the number of QTL reported such that several QTL of very small effect identified in the final MIM analysis were not reported.

RESULTS

Phenotypic ratings. The variance components for all random-effect factors (environment, replication within environment, line, and environment–line interaction) for the three diseases were significantly different from zero (Table 1). The high level of variation associated with environment likely reflects the fact that each disease was assessed in relatively diverse environments in northern Ohio and in central North Carolina. Pairwise Pearson correlations of SLB WMD scores were significant between all pairs of environments of 0.28 to 0.89 (Table 2). The correlations between CL04, CL05, CL06, and GA05 for SLB were highly significant ($P < 0.0001$), with correlation coefficients of 0.80 to 0.89. Pearson correlations of GLS WMD scores were highly significant between all but one pair of environments, FRY04 and SBRY06 (Table 3). Pairwise NLB WMD Pearson correlation coefficients ranged from highly significant (SHFTR05 and SHFTR06, $r = 0.56$ and $P < 0.0001$) to not significant (OARDC07–KING04, $r = 0.1$, $P = 0.1881$) (Table 4) but most pairwise correlations (10 of 15) were significant to at least the $P < 0.05$ level. Correlations between overall LSM of the three diseases were statistically significant; the highest correlation was identified between SLB and GLS ($r = 0.62$, $P < 0.0001$) (Table 5). The entry mean heritability across all environments was 0.85 ± 0.02 for SLB, 0.75 ± 0.04 for GLS, and 0.67 ±0.05for NLB.

In addition to disease resistance ratings, GDDTA was measured in the SLB CL04, CL05 and CL06 trials and for the GLS ANDW05 trial. The heritability for GDDTA across these four environments was 0.68 ± 0.04. Pairwise correlations between overall LSM for the three diseases and GDDTA ranged from –0.24 to –0.51 and were statistically significant (Table 5). In other

TABLE 5. Pearson correlation coefficients between overall least square means for three diseases: southern leaf blight (SLB), gray leaf spot (GLS) northern leaf blight (NLB), and growing degree days to anthesis (GDDTA)

Disease	GLS	NLB	GDDTA
SLB	$r = 0.62$ $P < 0.0001$	$r = 0.25$ $P = 0.0075$	$r = –0.51$ $P < 0.0001$
GLS	…	$r = 0.32$ $P = 0.0005$	$r = –0.47$ $P < 0.0001$
NLB	…	…	$r = –0.24$ $P = 0.012$

TABLE 3. Pearson correlation coefficients between least square means for gray leaf spot measured at six locations: at Salisbury, NC in 2006 (SBRY06); at Andrews, NC in 2004 and 2005 (ADWS04 and ADWS05); and two environments in 2004 and one environment in 2005 at Wooster, OH (FRY04, FRY05, and KING04)

Location	ADWS04	ADWS05	FRY04	FRY05	KING04
SBRY06	$r = 0.46, P < 0.0001$	$r = 0.59, P < 0.0001$	$r = 0.05, P = 0.6694$	$r = 0.43, P < 0.0001$	$r = 0.31, P = 0.0085$
ADWS04	…	$r = 0.61, P < 0.0001$	$r = 0.34, P = 0.0041$	$r = 0.52, P < 0.0001$	$r = 0.47, P < 0.0001$
ADWS05	…	…	$r = 0.32, P = 0.0070$	$r = 0.57, P < 0.0001$	$r = 0.36, P = 0.002$
FRY04	…	…	…	$r = 0.45, P = 0.0001$	$r = 0.45, P = 0.0001$
FRY05	…	…	…	…	$r = 0.52, P < 0.0001$

TABLE 4. Pearson correlation coefficients between least square means for northern leaf blight measured at six environments: at Clayton, NC in 2006 (CL06); and two environments in 2004 and one environment in each of 2005 and 2006 at Wooster, OH (FRY04, KING04, SHFTR05, SHFTR06, and OARDC07)

Location	FRY04	KING04	OARDC07	SHFTR05	SHFTR06
CL06	$r = 0.21, P = 0.0819$	$r = 0.38, P = 0.0017$	$r = 0.49, P < 0.0001$	$r = 0.35, P = 0.0003$	$r = 0.31, P = 0.0013$
FRY04	…	$r = 0.19, P = 0.1193$	$r = 0.24, P = 0.0463$	$r = 0.20, P = 0.0961$	$r = 0.31, P = 0.0084$
KING04	…	…	$r = 0.16, P = 0.1881$	$r = 0.33, P = 0.0061$	$r = 0.17, P = 0.1494$
OARDC07	…	…	…	$r = 0.29, P = 0.0027$	$r = 0.31, P = 0.0012$
SHFTR05	…	…	…	…	$r = 0.56, P < 0.0001$

words, a longer time to anthesis was associated with higher resistance for all the diseases.

Linkage mapping. A linkage map was constructed using 871 molecular markers (SSR and SNP markers) covering a total map length of 1,697.3 cM. The average distance between mapped markers was 1.9 cM. The markers dupssr14 and PHM14046.9 on chromosome 8 bin 8.08 are inverted in the linkage map constructed in this study compared with the marker order reported in the intermated IBM reference map (IBM2 2005 Neighbors map). The construction of this map is further detailed in Coles et al. (25).

QTL mapping. QTL mapping, using LSM values derived from combined data over all environments for each disease and for GDDTA, detected 9 QTL for SLB; 8 for GLS; 6, with 1 QTL–QTL interaction, for NLB (Table 6); and 10 for GDDTA.

Of the nine SLB QTL detected, the three that accounted for the largest amount of phenotypic variation were located in bins 3.04, 9.03, and 10.05, accounting for 26.0, 9.0, and 10.6%, respectively. For GLS, the three QTL that accounted for the largest amount of phenotypic variation were located in bins 2.02-2.03, 7.02, and 10.05, accounting for 11.2, 9.9, and 16.0% respectively. The three NLB QTL that accounted for the largest amount of phenotypic variation were located in bins 1.06, 8.02, and 8.05 and accounted

for 18.7, 12.8, and 8.3%, respectively. In each of these cases, with the exception of the GLS QTL in bin 8.07-8.09, resistance was conferred by the allele derived from Ki14. The largest-effect QTL for GDDTA were located in bins 3.04/3.05 and 10.03, accounting for 11.6 and 23% of the phenotypic variation, respectively.

Several QTL were associated with resistance to more than one disease. In bin 1.06, QTL for SLB, GLS, and NLB all colocalized at approximately the same map position (<5 cM difference between the QTL peaks for the three diseases). QTL for SLB and GLS resistance colocalized in bins 1.08-1.09, 3.04, and 10.05. One QTL in bin 8.05 colocalized for SLB and NLB resistance and one QTL in bin 2.02-2.03 colocalized for GLS and NLB resistance. For our purposes, QTL were considered colocalized if they were within 20 cM of each other (19,51,77). QTL for GDDTA were also identified at four of these six loci (bins 1.06, 3.04/3.05, 8.05 and 10.05). However, no disease resistance QTL were identified at the largest-effect GDDTA QTL at bin 10.03.

DISCUSSION

The significant correlations between resistances and the three diseases assessed (SLB, GLS, and NLB) (Table 5) were observed, and the six QTL associated with resistance to two or more

TABLE 6. Chromosomal location in centimorgans (cM) and parameters associated with quantitative trait loci (QTL) detected in the 109 Ki14 × B73 recombinant inbred lines (KB population) for resistance to southern leaf blight (SLB), gray leaf spot (GLS), and northern leaf blight (NLB) and for growing degree days to anthesis (GDDTA) over all environments in which each trait was measured[a]

Bin, parameters[b]	Flanking markers[c]	SLB	GLS	NLB	GDDTA[d]
1.02	PZA02393.2-bnlg1953	…	…	57.0	…
A		…	…	0.18	…
LOD		…	…	2.7	…
r^2		…	…	6.3	…
1.05-06	PZA01041.2-bnlg1057	118.6	121.4	**123.6**	110.2
A		0.15	0.18	**0.32**	−10.3
LOD		3.1	3.7	**8.0**	6.6
r^2		5.1	8.7	**18.7**	9.7
1.08-1.09	Bz2.2-PHM14475.7	175.2	171.6	…	…
A		0.18	0.16	…	…
LOD		4.1	3.4	…	…
r^2		6.1	7.7	…	…
2.02-2.03	PZA03699.1-PZA03228.4	…	**68.6**	48.4	…
A		…	**0.22**	0.2	…
LOD		…	**6.9**	3.5	…
r^2		…	**11.2**	7.8	…
2.04	PZA02058.1-PZA02168.1	100.0	…	…	…
A		0.20	…	…	…
LOD		5.0	…	…	…
r^2		7.1	…	…	…
2.09	bnlg1520-PZB10103.1	189.4	…	…	…
A		0.18	…	…	…
LOD		4.2	…	…	…
r^2		8.4	…	…	…
3.02	PZA03678.1-PZA00100.10	26.7	…	…	…
A		0.14	…	…	…
LOD		2.4	…	…	…
r^2		2.8	…	…	…

(*continued on next page*)

[a] SLB field trials were performed at Clayton, NC in 2004, 2005, and 2006; Tifton, GA in 2005; and Wooster, OH in 2004. GLS field trials were performed in Salisbury, NC in 2006; Andrews, NC in 2004 and 2005; and at two environments in 2004 and one environment in 2005 at Wooster, OH. Field trials for NLB were performed at Clayton, NC in 2006 and two environments in 2004 and one environment in each of 2005 and 2006 at Wooster. GDDTA was measured in the SLB 2004, 2005, and 2006 trials and in the GLS 2005 trial in Andrews, NC.

[b] Chromosome bin location of QTL peak on one of the 10 chromosomes of the maize genome. Bins divide the genetic map into 100 approximately equal segments. 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). The marker order determined for the population used in this experiment largely follows the marker order shown in the standard maize genetic map (the B73 × Mo17 map). Numbers following the bin number indicate chromosomal position of the predicted QTL in centimorgans (cM). A = additive effect of the QTL. A positive number indicates that the allele for resistance or for fewer GDDTA was derived from Ki14. A negative number indicates that the allele for resistance or fewer GDDTA was derived from B73. LOD = logarithm-of-odds value at the position of peak likelihood of the QTL and r^2 estimates the proportion of phenotypic variance (percentage) explained by the QTL. QTL with $r^2 > 10\%$ are indicated in bold.

[c] Markers flanking the QTL peak positions.

[d] GDDTA explaining <5% of the variation and not colocalizing with any disease resistance QTL are not shown for space considerations.

[e] QTL interactions. Type of QTL interaction (AA) identified and determined by QTL Cartographer (AA is an additive–additive interaction).

[f] R^2 estimates the proportion of phenotypic variance (percentage) explained by the full model (all of the detected QTL) for each of the three diseases.

diseases (Table 6) would seem to provide good evidence that MDR loci, either carrying a single gene conferring resistance to two or more diseases or two closely linked genes conferring disease-specific resistances to different diseases, are segregating in the KB population. However, significant correlations between resistance and each disease and GDDTA were also observed, and four of the six putative MDR loci also had an effect on GDDTA. Furthermore, in each of these four cases, the Ki14 allele conferred both later maturity and increased disease resistance. This is what would be expected if the MDR was derived from an effect on maturity rather than on disease resistance itself. Of the remaining two putative MDR loci that did not colocalize with GDDTA QTL in bins 1.08/1.09 and 2.02/2.03 (Table 6), the former comprises SLB and NLB QTL that are 20 cM apart—the arbitrarily chosen maximum distance used here for declaring colocalizing QTL. Although QTL mapping with a relatively small population is imprecise, it is somewhat unlikely that the same gene would underlie QTL 20 cM apart.

All three of the diseases assessed here are substantially necrotrophic diseases that tend to progress most rapidly after anthesis, when resources start to be directed toward grain fill rather than growth and defense. Therefore, it would seem that a significant proportion of the observed correlation between disease resistances may be due to the common effect of variation in maturity on resistance ≈ to the different diseases. It should be noted that the

association between flowering time and disease resistance, while strong, is not absolute. The largest flowering time QTL in bin 10.03, explaining 23% of the variation in this trait, was not associated with resistance to any of the diseases assessed. It is also worth noting that the largest-effect QTL for both SLB and GLS in bins 2.02-2.03 and 3.04, respectively, are not within 20 cM of any flowering time QTL but are also relatively distant from other disease resistance QTL (20 and 15 cM distant, respectively).

A consistent, though generally relatively low, association between disease resistance and time to maturity has been observed in a number of recent mapping studies and meta-analyses (3–8,13,24,81,83). In a series of studies mapping QTL for resistance to SLB, GLS, and NLB in the widely used maize IBM population, pairwise correlations between resistances to the different diseases and between resistance to each disease and time to flowering were all significant but were substantially lower than those observed in this study (5,6,8). Furthermore, in these studies, only a single QTL was identified that conferred resistance to more than one disease; a QTL in bin 2.04 that was associated with resistance to both SLB and GLS. The parents of the IBM population, B73 and Mo17, are both temperate inbreds and flower at approximately the same time under a variety of conditions. The parents of the Kb population, Ki14 and B73, are tropical and temperate inbreds, respectively, and B73 flowers ≈2 to 3 weeks

TABLE 6. (*continued from preceding page*)

Bin, parameters[b]	Flanking markers[c]	SLB	GLS	NLB	GDDTA[d]
3.04-3.05	PZA00508.2-PZA02474.1	**57.3**	71.9	…	**81.7**
A		**0.36**	0.15	…	**−13.2**
LOD		**15.4**	3.7	…	**10.1**
r^2		**26.0**	7.0	…	**11.6**
4.05	PZA00445.22-PZA00057.2	…	82.1	…	…
A		…	0.19	…	…
LOD		…	5.3	…	…
r^2		…	6.7	…	…
6.05	PHM13020.1-PZA003745.1	…	…	52.2	61.0
A		…	…	0.21	10.6
LOD		…	…	3.6	7.1
r^2		…	…	8.0	8.0
7.02	PHM4818.15-PZA00132.17	…	14.3	…	…
A		…	0.19	…	…
LOD		…	5.1	…	…
r^2		…	9.9	…	…
8.02	PHM9695.8-PZA02955.3	…	…	**26.3**	
A		…	…	**0.27**	
LOD		…	…	**4.7**	
r^2		…	…	**12.8**	
8.05	PHM3993.28-PZA03612.2	72.6	…	76.2	68.4
A		0.16	…	0.19	−11.5
LOD		4.0	…	2.8	8.3
r^2		3.5	…	8.3	7.6
8.07-8.09	dupssr14-PHM14046.9	…	137.9	…	…
A		…	-0.16	…	…
LOD		…	3.7	…	…
r^2		…	5.3	…	…
9.03-9.04	wx1.1-PZA01819.1	51.0	…	…	66.8
A		0.22	…	…	−6.2
LOD		6.5	…	…	2.7
r^2		9.0	…	…	3.1
10.03	PZA03491.1-PZA01877.2	…	…	…	**46.3**
A		…	…	…	**−15.5**
LOD		…	…	…	**10.8**
r^2		…	…	…	**23**
10.05	bnlg1074-bnlg1250	95.5	94.5	…	98.4
A		0.24	0.25	…	-6.3
LOD		6.7	7.8	…	2.2
r^2		10.6	16.0	…	7.3
1.06 × 8.02[e]	…	…	…	…	…
A		…	…	−0.18	…
LOD		…	…	2.6	…
r^2		…	…	5.5	…
R^{2f}		0.80	0.73	0.68	0.83

earlier than Ki14 in the summer in NC. Therefore, the fact that flowering time had little association with disease resistance in the IBM population is likely a consequence of the relatively small amount of variation for this trait in the IBM population compared with the KB population. It should also be noted that the relatively small population size used in this study likely resulted in failure to detect some QTL, particularly those with relatively small effects, as well as possible overestimation of the effects of those QTL detected (9,65).

Most of the SLB QTL identified in the current study (Table 6) colocalize with SLB QTL identified previously in other populations (3–6,12,21,39,87). In particular, the large-effect SLB QTL identified in bin 3.04 colocalized with the largest-effect QTL identified in most of these previous studies. Several QTL for resistance to other diseases and major virus disease resistance genes have also been identified in this region (83). The GLS QTL in bin 1.06 is of particular interest because it colocalized with QTL identified for SLB and NLB in this study and, furthermore, large-effect GLS QTL have been detected in this region in several other studies with different populations (13,24,44,64). However, flowering time was measured in only one of these studies (13) and, in that case, as in the present study, an association was observed between the 1.06 region and flowering time. Four of the six NLB QTL identified in the current study, in bins 1.06, 2.02-2.03, 6.05, and 8.02, colocalized with previously reported NLB resistance QTL (80). None of the sources of resistance are shared between this study and these previous studies.

Our initial hypothesis was that, because the causal organisms of the three diseases assessed share certain aspects of their pathogenesis processes, one might expect to find MDR QTL that affected these shared aspects. Based on this study as well as our previous work (5,6,8), this has not been borne out. Our unpublished work as well as the previous findings suggest that MDR QTL likely exist, though they may be QTL of relatively small effect—possibly below the level of detection in these studies. In the cases we have studied, the larger-effect QTL tend to be disease specific. Furthermore, these results emphasize the fact that, when MDR QTL are identified, one must be aware that they may be confounded with the pleiotropic effects of loci affecting maturity.

ACKNOWLEDGMENTS

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