Mapping QTL Controlling Southern Leaf Blight Resistance by Joint Analysis of Three Related Recombinant Inbred Line Populations

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ABSTRACT

Southern leaf blight (SLB) is a foliar necrotrophic disease of maize (*Zea mays* L.) caused by the ascomycete fungus *Cochliobolus heterostrophus* (Drechs.) Drechs. It is particularly important in warm humid parts of the world where maize is cultivated, such as the southern Atlantic coast area of the United States and parts of India, Africa, and Western Europe. Quantitative trait loci (QTL) for resistance to SLB disease caused by *C. heterostrophus* race O were identified in three maize recombinant inbred populations assessed in two environments: Clayton, NC, in the summer and Homestead, FL, in the winter. The three populations were derived from the crosses B73 × CML254, CML254 × B97, and B97 × Ki14. Each of these populations was derived from a cross between a temperate maize line (B73 or B97) and a tropical maize line (Ki14 or CML254). Quantitative trait loci were identified by separate analysis of each population and by joint connected and disconnected analyses of all the populations. The most significant QTL identified were on chromosomes 3, 8, 9, and 10. Joint analysis led to more precise position estimates than separate analysis in each case. Results are discussed in the context of previous SLB QTL analysis studies and a recent flowering time QTL study that used the same populations. The chromosome 8 and 9 QTL colocalized with previously identified flowering time QTL, which suggested that the perceived effect on SLB resistance at these QTL may have been mediated through an effect on flowering time.

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Abbreviations: BIC, Bayesian information criterion; NAM, nested association mapping; QTL, quantitative trait locus (loci); RIL, recombinant inbred line; sAUDPC, standardized area under disease progress curve; SLB, southern leaf blight; SNP, single nucleotide polymorphism.

Cochliobolus heterostrophus (Drechs.) Drechs. [anamorph = *Bipolaris maydis* (Nisikado) Shoemaker; synonym = *Helminthosporium maydis* Nisikado] is a necrotrophic plant pathogen and the causal agent of southern leaf blight (SLB). The most devastating crop disease epidemic in modern U.S. agricultural history was the SLB epidemic of the early 1970s, which resulted in a yield loss of 20 to 30% and highlighted the vulnerability of the U.S. maize (*Zea mays* L.) crop due its limited genetic diversity (Hooker, 1972; Ullstrup, 1972). That epidemic was caused by Race T of *C. heterostrophus*, which is highly virulent to maize with male-sterile T type cytoplasm (Dewey et al., 1988). T cytoplasm was used widely in maize hybrids at the time, but has since been removed from production. The currently predominant form of *C. heterostrophus* is Race O, which can cause yield losses of up to 40% (Byrnes et al., 1989; Fisher et al., 1976; Gregory et al., 1979). *C. heterostrophus* Race O is a particular problem in East Asia and Africa, where maize is an important component of food security (Ngoko et al., 2002). The improvement of quantitatively inherited resistance to

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SLB disease can contribute to stabilized maize production in vulnerable regions and avoid the use of costly and environmentally damaging fungicides.

Most of the genetic resistance to SLB is quantitative and can be additive or recessive in effect (Balint-Kurti et al., 2008; Burnette and White, 1985; Holley and Goodman, 1989; Lim and Hooker, 1976; Scott and Futrell, 1975; Zwonitzer et al., 2010; Kump et al., 2010), though one recessive, major-effect gene, *rhm*, has been identified (Zaitlin et al., 1993). Quantitative trait loci (QTL) for field resistance to SLB in maize have been identified in several studies (Balint-Kurti and Carson, 2006; Balint-Kurti et al., 2006, 2007, 2008; Carson et al., 2004; Jiang et al., 1999; Zwonitzer et al., 2009, 2010, Kump et al., 2011). With few exceptions (Jiang et al., 1999; Zwonitzer et al., 2010), these studies used populations derived from crosses between two temperate adapted lines. Tropical lines tend to be more resistant than temperate lines to SLB and a number of other diseases due, presumably, to disease being a much more important selection pressure in tropical environments (Goodman, 1999; Kraja et al., 2000). Furthermore, with the exception of Kump et al. (2011), each study reported results from analyses of single populations involving the segregation of alleles from two parents.

Comparing results across studies is often complicated by the confounding effects of different environments and experimental procedures used. It is also often difficult to interpret discrepancies in QTL identified in different mapping families, as observed differences may be due to genetic heterogeneity (true genetic differences) or sampling (Holland, 2007). Combined analysis of multiple mapping populations, connected by common parents, evaluated in common environments using a common set of DNA markers minimizes these problems, increases mapping power and precision, and provides directly comparable allele effect estimates for multiple founder parents (Blanc et al., 2006; Coles et al., 2010).

The objectives of this study were to map SLB resistance QTL in populations derived from crosses between temperate-adapted (B97 and B73) and tropical-adapted (CML254 and Ki14) maize lines and to compare results derived from separate analysis of individual populations with those derived from joint analysis over the three linked populations. Here we report the results of QTL mapping of SLB resistance using three such populations derived from the following crosses: B73 × CML254, CML254 × B97, and B97 × Ki14.

MATERIALS AND METHODS

Plant Material

Three recombinant inbred line (RIL) populations developed at North Carolina State University were used for this study (Coles et al., 2010). These three RIL populations are comprised of 120, 126, and 214 RILs derived from crosses between B73 × CML254, CML254 × B97, and B97 × Ki14, respectively. B73 and B97 were both developed at Iowa State University (Hallauer et al., 1994; Russell, 1972) and represent two distinct maize germplasm groups for U.S. Corn Belt Dent maize: Stiff Stalk temperate (B73) and non–Stiff Stalk temperate (B97) (Liu et al., 2003). CML254 was developed by the International Maize and Wheat Improvement Center (CIMMYT) in Mexico (Srinivasan, 2001) and Ki14 by Kasetsart University in Thailand (Chutkaew et al., 1997). CML254 and Ki14 are both tropical, but they represent distinct subgroups of tropical germplasm (Liu et al., 2003).

Field Experiments

The three populations were planted in the summer of 2007 in Clayton (here referred to as CL07) in one replication as randomized blocks and at Homestead, FL, in the winter of 2007–2008 (here referred to as FL07) using complete randomized block design with two replications. All plants were inoculated with the 2-16Bm isolate of *C. heterostrophus* at the six- to eight-leaf stage as described previously (Carson, 1998). Irrigation was immediately applied after inoculation to foster fungal growth. *Cochliobolus heterostrophus* is endemic to both North Carolina and southern Florida, as such, inoculum most likely constituted a mixed isolate population. A 1 to 9 scale was used for scoring disease severity, with 1 denoting a symptomless plant and 9 denoting a dead plant (Balint-Kurti et al., 2006, Kump et al., 2011). Values were recorded in half unit increments. Southern leaf blight severity was scored starting approximately 1 mo after inoculation at approximately 2-wk intervals on two occasions in Homestead and on three occasions in Clayton.

Phenotypic Data Analysis

The standardized area under disease progress curve (sAUDPC) was calculated for each replicate in each environment in the following way: the average value of two consecutive ratings was obtained and multiplied by the number of days between the ratings. Values were then summed over all intervals, and then divided by the number of days of evaluation to determine the weighted average (Campbell and Madden, 1990; Shaner and Finney, 1977). In cases in which the data were collected only twice, sAUDPC is effectively the same as the mean of the ratings. The sAUDPC values for SLB were used for analysis of variance (ANOVA) using PROCGLM of SAS version 9.2 (SAS Institute, Cary, NC); PROC CORR was used to estimate the Pearson correlation coefficients. Heritability for SLB was estimated using PROC MIXED procedure of SAS, as described previously (Holland et al., 2003).

Mapping QTL

For CL07 the sAUDPC values were used for mapping. For FL07, the least square means of the sAUDPC values over the two replicates were used for mapping. For the average QTL, the least square means over both environments were used. Least square means were calculated using PROC MIXED in SAS (version 9.2; SAS Institute). We mapped QTL for SLB using the consensus genetic map developed using 1339 loci (Coles et al., 2010), which is available at http://www.genetics.org/cgi/content/full/genetics.109.110304/DC1 (verified 27 Apr. 2011). We were able to use the consensus map since the order of markers was largely

consistent among individual-population maps (Coles et al., 2010). Using the consensus map rather than individual-population maps allowed us to directly compare mapping results from the three populations and to compare these results with the previous studies (Coles et al., 2010; Zwonitzer et al., 2010).

Quantitative trait loci mapping in individual RIL populations was conducted using MCQTL4.0 (Jourjon et al., 2005). The genome-wide LOD threshold level for each trait at $\alpha = 0.05$ was determined by permutation analysis (Churchill and Doerge, 1994), 1000 permutations in each case. The automated iterative QTL mapping (iQTLm) procedure (Charcosset et al., 2000) with the 5-cM walking speed option was used to detect QTL for each trait. A 2-LOD support interval, corresponding to a conservative 95% confidence interval in a RIL population, was used to delimit the region around each QTL (Ooijen, 1992). Significant QTL were declared when LOD scores exceeded the genome-wide $\alpha = 0.05$ threshold level. For each trait, individual R^2 and allelic effects at each QTL were estimated. Southern leaf blight resistance QTL were mapped in three ways:

1. In each of the three RIL populations separately. QTL were identified in each environment separately and over both environments together.
2. Using disconnected joint analysis of the three populations simultaneously in each of the environments and over both environments. The disconnected additive model analyzes the three populations jointly in a single analysis but allows founder allele effects to vary across populations, so we estimated six allele by population effects for each QTL.
3. Using connected joint analysis of all three RIL populations simultaneously, using the connected additive model of MCQTL. The connected additive model assumes that the founder parental allele effects are consistent across populations, so that four allele effects were estimated (with 3 df) at each QTL (Blanc et al., 2006). The fact that the three populations are connected by common parents makes the joint connected analysis possible as the relative effects of alleles that are not segregating against each other in a population can be determined by reference to their segregation against a common allele.

The connected and disconnected additive models were compared using Schwarz's Bayesian information criterion (BIC), and the model with the lower BIC value was selected (Coles et al., 2010).

RESULTS AND DISCUSSION

Population Characteristics

As expected, the two temperate parents (B73 and B97) were more susceptible and earlier-flowering in both environments than the two tropical parents (CML254 and Ki14, see Table 1). The population means fell between the means of the parents in each case. Environment and genotype had significant effects on the variance, while the effects of replications within environment and genotype by environment were not significant (Table 2). This result is largely in line with previous observations for this trait where genotype and environment had much larger effects than replication or genotype by environment (e.g., Balint-Kurti et al., 2006, 2008; Zwonitzer et al., 2009, 2010). In these previous cases, however, the genotype by environment effect was still significant. It is not clear why it was not significant in this study.

The phenotypic distribution of the SLB scores averaged over the two locations closely followed a normal distribution and some transgressive segregation was observable (Fig. 1). Correlations for sAUDPC were significant between environments ($r = 0.58$, $P < 0.0001$) and between replications within an environment (0.73, $P < 0.001$ for FL07). Within each population, the Pearson correlation coefficients for sAUDPC between the two environments was 0.65, 0.59, and 0.67 (all $P < 0.0001$) for B73 × CML254, CML254 × B97, and B97 × Ki14 RIL populations, respectively. The heritability on a plot basis (0.60 ± 0.03) and family mean basis (0.66 ± 0.03) was also moderately high. These observations are also in line with our previous observations for this trait. (e.g., Balint-Kurti et al., 2006, 2007, 2008; Zwonitzer et al., 2009, 2010).

QTL Mapping

Since environment was a significant contributor to variance, we mapped QTL based on the two individual environments as well as the overall average scores. All QTL detected by analysis of individual populations are reported in Table 3.

In the B73 × CML254 population we detected a QTL in bin 3.04 in both environments and for the average over-environment values. A QTL was also detected in bin 8.06 for the CL07 environment and at a closely linked locus in bin 8.05 for the average values. A QTL in bin 1.03 was detected in FL07 but not in CL07 or in the over-environment analysis.

In the CML254 × B97 population, significant SLB resistance QTL were identified in bin 2.03 and 9.04 in the FL07 environment and at very closely linked positions using the average data (in bins 2.02 and 9.05). No significant QTL were detected in the CL07 environment.

In the B97 × Ki14 population, three significant SLB resistance QTL were detected in the CL07 environment in bins 1.10, 9.03, and 10.04. The QTL in bin 1.10 was not significant in the FL07 environment, but the 9.03 (10 cM distant in the adjacent bin 9.04) and 10.04 QTL were again detected, together with a QTL in bin 3.03. The 3.03, 9.03/4, and 10.04 QTL were also detected in the over-environment analysis. The QTL in bin 3.03 detected in this population was close to the bin 3.04 QTL detected in the B73 × CML254 population and the bin 9.04 QTL was very close to the bin 9.04/5 QTL identified in the CML254 × B97 population. In every case for all three populations analyzed separately, the allele from the temperate parent (B73

Table 1. Overall southern leaf blight score means of the parents of the populations and for the population means used in this study. Southern leaf blight resistance was scored on a 1–9 scale with 1 being the most resistant and 9 the most susceptible.

Parents	sAUDPC†
B73	3.95
B97	4.55
CML254	2.70
Ki14	1.80
B73 × CML254	3.03
CML254 × B97	3.06
B97 × Ki14	2.44

†sAUDPC, standardized area under disease progress curve.

Table 2. Analysis of variance for southern leaf blight (SLB) resistance scored on three recombinant inbred line populations at Clayton, NC, in 2007 and Homestead, FL, in 2007.†

Source	df	MS
Environment	1	93.98***
Rep(environment)	1	3.52ns
Genotype	489	1.28***
Genotype × environment	367	0.68ns
Error	438	1.19

***Significant at $\alpha = 0.001$.

†df, degree of freedom; MS, means sum of squares; ns, not significant.

or B97) conferred susceptibility relative to the allele derived from the tropical parent (CML254 or Ki14).

It should also be noted that the fact that a QTL is not declared in one environment while it is in another, does not necessarily mean that there is no effect at this locus in the environment in which the QTL is not declared. For example, for the bin 9.04/5 QTL that is found in the CML254 × B97 population in FL07 but not in CL07, there is a peak of 3.2 in the LOD graph at that locus in the CL07 analysis, but it just does not rise above the threshold level of 3.5 so it is not declared as a QTL. Nevertheless there is almost certainly an effect at this locus in CL07. This explains why, for instance, the Pearson correlation coefficient between the two environments for the CML254 × B97 population is moderately high (0.59) despite them ostensibly sharing no QTL.

We performed both connected and disconnected joint QTL analyses of the three populations simultaneously. We found that the number, positions, and confidence intervals of the QTL detected did not differ significantly between these two types of joint analysis (Table 4 and Table S1). However, the BIC of the combined analysis was superior (i.e., lower) for the connected analysis for all traits compared with the disconnected analysis (Table S2). Thus, QTL allele effects were consistent across genetic backgrounds.

Joint analysis assumes that the founder parental allele effects are consistent across populations, so that four allele effects are estimated. This assumption is not necessarily valid in every case of course. For instance two linked resistance genes might be present in B97, one of them segregating in the B97 × CML 254 population and the other in the B97 × Ki14 population. In this case the joint analysis might conflate the effects of these two distinct genes. The advantages of joint analysis with connected populations (i.e., populations that are connected by shared parents) are that allele effects of a wide range of alleles can be compared. Also the larger total population size often leads to more precise positional estimates (e.g., Coles et al., 2010).

Table 4 shows the results of the joint analysis. The results are largely consistent with the individual population analyses. If one assumes that QTL within 20 cM represent the same underlying allele (which is not unreasonable given that the 2-LOD support intervals are often larger than 20 cM) all the SLB resistance QTL detected by joint analysis on the average phenotypic values were also detected in at least one of the individual population analyses of average phenotypic values (compare Tables 3 and 4), and only one QTL detected in the individual population analyses of average phenotypic values was not detected in the joint analysis (the bin 2.02 QTL from the CML254 × B97 individual analysis). As expected, the 2-LOD support intervals of each QTL were smaller in the joint analysis compared to the individual population analyses. For example the 2-LOD intervals for the bin 9.04/5 QTL were ~54 cM and ~37 cM for the CML254 × B97 and the B97 × Ki14 populations, respectively, compared to ~30 cM for the joint analysis.

The additive phenotypic effects of the QTL identified in both analyses (Tables 3 and 4) were generally somewhat smaller than those reported in previous studies (Balint-Kurti and Carson, 2006; Balint-Kurti et al., 2006, 2007, 2008; Carson et al., 2004; Jiang et al., 1999; Zwonitzer et al., 2009, 2010). It is not clear why this might be, but it may be due to the specific environments used in this study. Most of the effects identified were in the region of 0.1 on the 1 to 9 scale. In the RIL populations these effects would essentially be doubled as loci are in a homozygous state. But even so, an effect of ~0.2 is very hard to differentiate visually.

We have shown previously (see in particular Zwonitzer et al., 2010) that flowering time and SLB resistance phenotypes are correlated. We believe that this is because resistance to SLB, as with many necrotrophic diseases, decreases rapidly after flowering as the plant devotes its resources to grain fill. Thus an SLB resistance QTL that colocalizes with a flowering time QTL may really be caused by effects on flowering time rather than on disease resistance per se. Flowering time QTL for these populations have been reported previously (Coles et al., 2010) using, in part, data from the same environments we use here. In the previous study, a large-effect QTL for flowering time was identified in bin 10.04 in long-day environments such as CL07 but not in short-day environments such as FL07. The bin 10.04 SLB resistance QTL, however, was detected in both short-day and long-day environments (Table 4), which is not what would be expected

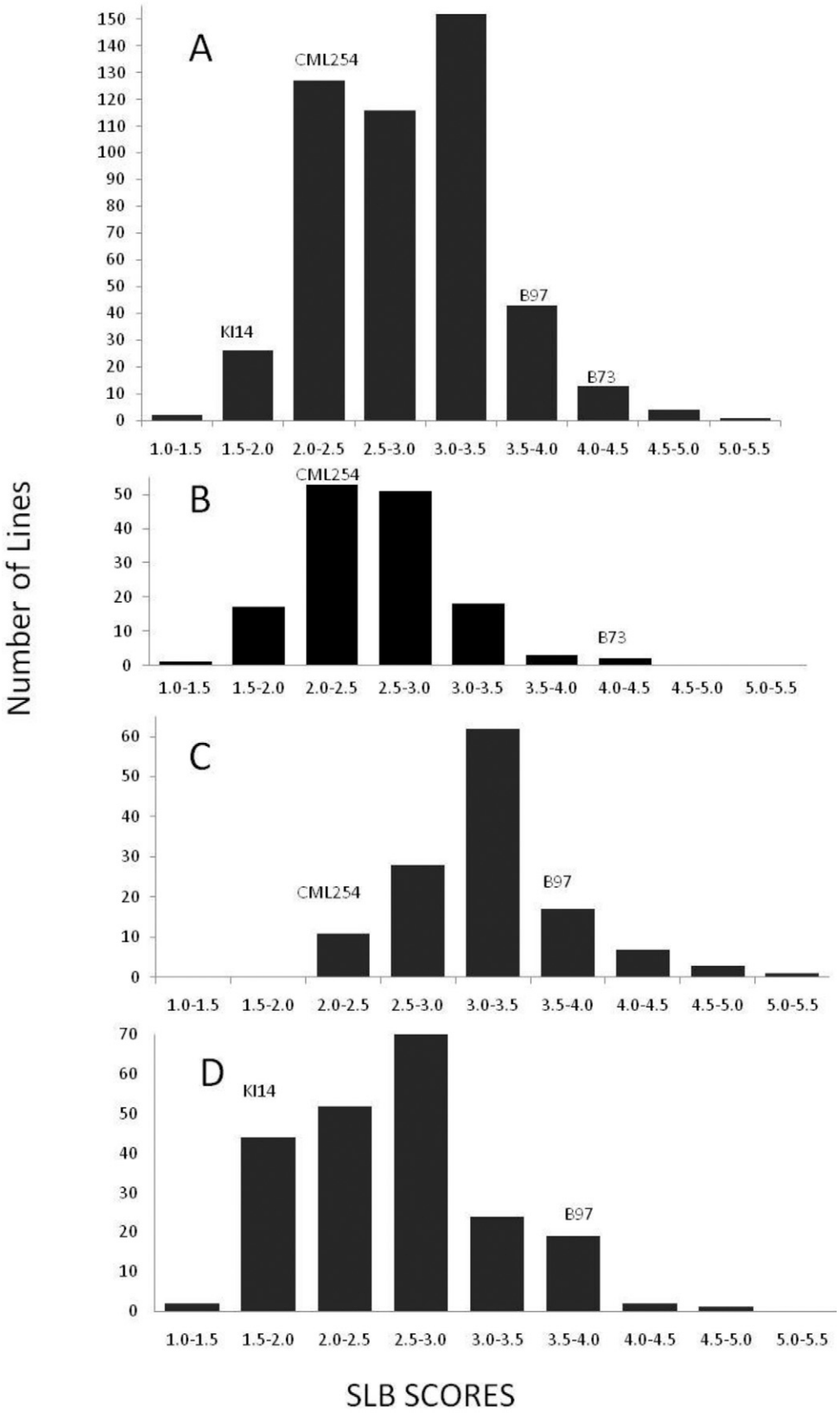

Figure 1. Phenotypic distribution of southern leaf blight (SLB) least square mean scores over two locations for the three maize recombinant inbred line populations used in this study. Resistance was scored on a 1–9 scale with 1 being resistant 9 being highly susceptible. The total combined population is shown (A) together with the individual populations, (B) B73 × CML354, (C) CML254 × B97, and (D) B97 × Ki14. The resistance levels of all the parents are indicated.

Table 3. Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for standardized area under disease progress curve (sAUDPC) ratings for southern leaf blight of maize in three recombinant inbred line (RIL) populations: B73 × CML254, CML254 × B97, and B97 × Ki14, scored in Clayton in the summer of 2007 (CL07) and Florida in the winter of 2007/08 (FL07). Quantitative trait loci for individual environments as well as overall ratings averaged over replications and years are shown. Quantitative trait loci were mapped separately for each population.

Population/ environment	Chromosome	Peak position†	2-LOD support interval‡	Marker at the peak	Peak LOD §	BIN ¶	R^2 (%)#	Additive effects††	
B73 × CML254								CML254	B73
CL07	3	71.8	61.5–132.4	PHM4145	6.97	3.04	20.0	–0.11	0.11
	8	98.5	50.4–118.1	bnlg2289	5.80	8.06	19.8	–0.11	0.11
FL07	1	103.3	58.8–149.8	PZA02292	3.96	1.03	12.0	–0.08	0.08
	3	71.8	65.2–84.9	PHM4145	8.48	3.04	25.2	–0.14	0.14
Average	3	71.8	64.7–78.1	PHM4145	9.97	3.04	28	–0.13	0.13
	8	87	40.8–116.2	PHM448	5.93	8.05	18.7	–0.09	0.09
CML254 × B97								CML254	B97
CL07	No significant QTL detected								
FL07	2	53.4	12.1–64.7	PZA03559	4.31	2.03	14.3	–0.14	0.11
	9	102.8	92.9–134.3	PZA00225	5.08	9.04	15	–0.12	0.14
Average	2	50.20	11.7–63.8	zfl2_9	3.66	2.02	12.0	–0.11	0.12
	9	117.8	83.8–137.5	bnlg1270a	4.13	9.05	10.0	–0.09	0.11
B97 × Ki14								Ki14	B97
CL07	1	271.8	262.3–283.7	PZB00018	5.41	1.10	14.2	–0.11	0.11
	9	95.40	86.1–171.8	PZA01062	4.03	9.03	11.4	–0.10	0.10
	10	61.7	11.3–139.9	PZA02219	3.82	10.04	8.9	–0.09	0.09
FL07	3	53.6	9.7–144	PZA00508_4	2.8	3.03	5.2	–0.07	0.07
	9	105.7	19.5–134.7	PZA03470	4.52	9.04	9.0	–0.09	0.09
	10	61.70	50.6–83.8	PZA02219	9.65	10.04	17.2	–0.14	0.14
Average	3	53.6	44.7–74.05	PZA00508_4	4.6	3.03	8.3	–0.08	0.08
	9	93.60	20–171.8	PZA03085	4.97	9.03	9.80	–0.08	0.08
	10	61.70	49.6–83.4	PZA02219	9.04	10.04	16.70	–0.11	0.11

†Position of peak LOD value on composite maps described previously (Coles et al., 2010).

‡The positions that define the two LOD interval around the position of peak likelihood for the QTL.

§The log of odds (LOD) value at the position of peak likelihood of the QTL.

¶Chromosome bin location of QTL peak on 1 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)—see Davis et al. (1999).

#R^2 estimates the proportion of RIL mean variance (%) explained by the detected QTL.

††The additive effect of the QTL in terms of the 1 to 9 scale employed. A positive number indicates that the allele for susceptibility was derived from the line indicated and a negative number means that the allele for resistance was derived from the line indicated.

if it was associated with the long day–specific flowering time QTL. This suggests therefore that there may be a bona fide SLB resistance gene residing at this locus. The bin 8.05 and 9.04 SLB resistance QTL detected here do, however, appear to colocalize with various flowering time–related QTL identified in both long- and short-day environments in the previous study (Coles et al., 2010), and it is therefore not clear whether or not they are primarily affecting flowering time with secondary effects on disease resistance. An SLB resistance QTL was identified in bin 8.05 previously in a H99 × B73 RIL population (Balint-Kurti et al., 2008) with the resistance allele derived from H99. In this case there was not a colocalizing flowering time QTL. Similarly, a QTL for SLB resistance was detected in bin 9.04 in a B104 × NC300 RIL population with resistance derived from NC300 with no colocalizing flowering QTL (Balint-Kurti et al., 2006).

The SLB resistance QTL detected in bin 3.04 does not colocalize with major flowering time QTL detected in these populations. Bin 3.04 is in fact a hotspot for SLB resistance QTL as has been noted previously (Balint-Kurti et al., 2007, 2008; Zwonitzer et al., 2009, 2010, Kump et al., 2011). In almost every case where B73 has been one of the parents of the population examined, with the exception of a population derived from the cross between B73 and the line B52 (Balint-Kurti et al., 2008), an SLB resistance QTL has been detected in bin 3.04 and the susceptibility allele has been derived from B73. It appears therefore that B73 carries a relatively rare susceptibility allele or set of linked susceptibility alleles at this locus. We furthermore have shown that this susceptibility allele is dominant with respect to the alleles from the lines Mo17 and NC250 (Kump et al. (2010) and PBK (unpublished data, 2009).

We previously mapped SLB resistance QTL in a population derived from a cross between the maize lines B73 and Ki14 population (Zwonitzer et al., 2010), both of which are parents of different populations used in this study. While the results of the previous study could not

Table 4. Chromosomal locations and parameters associated with the quantitative trait loci (QTL) for standardized area under disease progress curve (sAUDPC) ratings for southern leaf blight of maize in three recombinant inbred line (RIL) populations, B73 × CML254, CML254 × B97, and B97 × Ki14, scored in Clayton in the summer of 2007 (CL07) and least square mean ratings for Florida in the winter of 2007/08 (FL07). QTL for average of the two environments were detected using least square means. QTL were calculated by connected additive model of joint analysis of all the populations simultaneously.

Environment	Chr	Peak position†	2-LOD support interval‡	Marker at the peak	Peak LOD§	Bin¶	R^2 (%)#	Additive effect††			
								B73	CML254	B97	Ki14
SLBCL07	1	272.2	261.2–287.6	PZA01978_23	7.46	1.1	8.5	0.01	−0.01	0.10	−0.09
	3	71.8	21.2–132.11	PHM4145_18	6.21	3.04	7.7	0.16	−0.07	−0.003	−0.08
	8	78.5	41.4–80.7	bnlg1176a	8.3	8.05	10	0.1	−0.1	0.05	−0.06
	9	95.4	87.2–118.8	PZA01062	7.36	9.03	8.9	0.06	−0.03	0.08	−0.12
	10	56.3	41.7–115.9	LHY	6.55	10.03	8	0.01	−0.02	0.19	−0.08
SLBFL07	3	71.8	66–76	PHM4145_18	9.04	3.04	8.5	0.19	−0.09	0.01	−0.11
	9	105.8	92.7–122.7	PZA00015_5	7.66	9.04	7	0.03	−0.02	0.09	−0.1
	10	60.1	49.2–81.3	PHM12990_15	12.48	10.04	11.5	0.07	−0.03	−0.18	0.08
Average	3	71.8	67.5–75.5	PHM4145_18	9.69	3.04	9	0.19	−0.06	−0.01	−0.14
	8	78.5	39.21–111.61	bnlg1176a	5.14	8.05	5.1	0.07	−0.09	0.04	−0.02
	9	105.8	88.7–118.77	PZA00015_5	9.05	9.04	8.6	0.05	−0.01	0.08	−0.12
	10	60.1	53.05–67.9	PHM12990_15	14.66	10.04	13.3	−0.03	−0.02	0.16	−0.10

†Position of peak LOD value on composite interval mapping described previously (Coles et al., 2010).

‡The positions that define the two LOD interval around the position of peak likelihood for the QTL.

§The log of odds (LOD) value at the position of peak likelihood of the QTL.

¶Chromosome bin location of QTL peak on one of the ten 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)—see Davis et al. (1999).

#R^2 estimates the proportion of RIL mean variance (%) explained by the detected QTL.

††The additive effect of the QTL in terms of the one to nine scale employed. A positive number indicates that the allele for susceptibility was derived from the line indicated and a negative number means that the allele for resistance was derived from the line indicated.

be incorporated into the joint analysis performed here because different environments were used, it is informative to determine whether the QTL detected previously agree with what would be predicted by the joint analysis we present here (Table 4). In the joint analyses, strong contrasting allelic effects for B73 and Ki14 were identified in bins 3.04 and 9.05 (effect differences of 0.25 and 0.17, respectively). Significant effects were indeed detected in the Ki14 × B73 population in the vicinity of both these QTL although the QTL detected in these two studies do not precisely colocalize. The QTL detected in the Ki14 × B73 population map to bin 3.04 with a maximum LOD value at 57.3 cM (about 14 cM from the position of the maximum LOD value detected in this study) and to bin 9.03/04 with a maximum LOD value at 51 cM (48 cM away from the maximum value detected in this study). The large distance between the positions of the chromosome 9 QTL detected in the two studies make it unclear whether the same QTL was being detected in both cases.

Kump et al. (2011) recently reported SLB resistance QTL identified in the maize nested association mapping (NAM) population. The NAM population comprises 25 families of 200 RILs each. Each family is derived from a cross between B73 and one of 25 diverse "founder lines." B97 (but not CML254 or Ki14) was included as a NAM founder. The large sample size (5000 lines in total) and dense map of the NAM population (McMullen et al., 2009) make it possible to identify QTL with unprecedented precision and sensitivity (Buckler et al., 2009). Joint linkage QTL analysis identified 32 QTL and a separate genome-wide association analysis identified 51 single nucleotide polymorphisms (SNPs) expected to be tightly linked to causal genes (Kump et al., 2011). Quantitative trait loci or associated SNPs were identified at positions overlapping or close to the four QTL identified in the current study (Table 4). However, no obvious candidate genes were identified at any of these loci (Kump et al., 2011). The NAM QTL at bin 3.04 was identified by standard linkage analysis. In this case the B97 allele conferred a substantial level of resistance relative to the B73 allele. This is congruent with the results reported here (Table 4). This implies that our estimates of the relative values of the B73 and B97 alleles by joint connected analysis were accurate, despite the fact that they were not segregating directly against each other in any population.

Quantitative trait loci in bins 8.05, 9.04, and 10.04 were not identified in the NAM study; however, SNPs associated with SLB resistance were identified in these bins by the separate genome-wide association analysis. In each of these three cases, B97 carries the same SNP as B73. For the bin 8.05 and 9.04 QTL, this is precisely what we would expect since in the present study there was essentially no difference detected between the effects of the B73 and B97 alleles at these loci (Table 4). However, for the bin 10.04 QTL, the B97 allele confers a substantial level of resistance relative to the B73 allele in the present study. It is quite conceivable that in this case, the SNP identified by Kump et al. (2011) is not the causative SNP, but that it is in linkage disequilib-

rium with the causative SNP in most of the founder lines and in B97 this linkage disequilibrium is broken.

In summary, we report here on SLB resistance QTL derived from two tropical lines identified in three segregating populations. Joint analysis of these populations has enabled us to refine somewhat the positions of these QTL. These data will be useful for breeders working on the incorporation of disease resistance from tropical germplasm into elite varieties.

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