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RESEARCH

Use of an Advanced Intercross Line Population for Precise Mapping of Quantitative Trait Loci for Gray Leaf Spot Resistance in Maize

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ABSTRACT

Gray leaf spot [GLS, causal agent *Cercospora zeae-maydis* (Tehon and E. Y. Daniels)] is an important fungal disease of maize in the U.S. and worldwide. The IBM population, an advanced intercross recombinant inbred line population derived from a cross between the maize lines Mo17 (resistant) and B73 (susceptible), was evaluated in three environments (Andrews, NC in 2005, 2006, and 2007) for resistance to GLS and for days from planting to anthesis (DTA). A conventional recombinant inbred line population derived from the same two parents (the "Stuber" population) was also assessed for GLS resistance in two environments (Andrews NC, 2004 and 2005). Quantitative trait loci (QTL) for GLS resistance were detected in each population. Five significant QTL were detected in the IBM population in bins 1.05, 2.04, 4.05, 9.03, and 9.05. In each case the QTL were localized to regions less than 3 centiMorgans (cM). Two QTL for GLS resistance were identified in the Stuber population in bins 2.04 and 7.05. The GLS QTL in bin 2.04 was previously identified as a QTL for southern leaf blight resistance in the IBM population. These results were compared with results from five previous GLS QTL studies and two potential GLS QTL "hotspots" were identified in bins 1.05–1.06 and 2.03–2.05. As expected, QTL were identified with much more precision in the IBM population compared to the Stuber population and to previous studies. There was no significant correlation between disease resistance and days to anthesis. Three DTA QTL were detected in bins 4.09, 8.05, and 9.02, which did not co-localize with GLS QTL.

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Abbreviations: AIL, advanced intercross line; AUDPC, area under disease progress curve; BLUP, best linear unbiased predictor; cM, centiMorgan; DTA, Days to anthesis; GLS, gray leaf spot; IBM, intermated B73/Mo17; Imu, IBM map units; MQM, Multiple QTL mapping; QTL, quantitative trait loci; RIL, recombinant inbred line; WMD, weighted mean disease.

Gray leaf spot [GLS, causal agent *Cercospora zeae-maydis* (Tehon and E. Y. Daniels)] of maize (*Zea mays* L.) has increased in importance over the past 15 years due to increased practice of conservation tillage (Ward et al., 1999) which allows plant residue to remain on the soil surface and act as a spore reservoir. It is often prevalent in climates with regular periods of high humidity and moderate temperatures and is now the major foliar disease problem in the U.S. and in sub-saharan Africa (Pratt and Gordon, 2006). In general, resistance has been reported to be moderate to highly heritable and based largely on additive effects (Gevers et al., 1994; Gordon et al., 2006; Thompson et al., 1987). There is a report of a major gene for gray leaf spot resistance (Gevers and Lake, 1994), but a subsequent study found that, rather than a single major gene, resistance in the material was conferred by two significant QTL (Gordon et al., 2004).

Several previous studies have identified quantitative trait loci (QTL) for GLS resistance (Bubeck et al., 1993; Clements et al., 2000; Gordon et al., 2004; Lehmensiek et al., 2001; Saghai Maroof

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et al., 1996). In these cases, as with most QTL mapping using biparentally-derived populations, the QTL were defined relatively imprecisely with the support or confidence interval for a QTL position spanning 10 to 30 cM or 1 to 3% of the genome. Reasons for this level of imprecision include insufficient marker density and limited opportunities for recombination between closely linked loci because of the relatively small size of many mapping populations (often 200 or fewer lines). Increasing QTL resolution while maintaining a manageable population size can be achieved through the development of advanced intercross lines (AILs), as proposed by Darvasi and Soller (1995). The Intermated B73 × Mo17 (IBM) population is an AIL maize population developed by including four generations of random mating following the formation of the F_2 generation and before the development of inbred lines (Lee et al., 2002). The increased opportunity for recombination has had the effect of expanding the genetic map approximately fourfold compared to non-intermated, conventional, recombinant inbred line (RIL) populations (Lee et al., 2002). The IBM population consists of a relatively large number of lines (302) which have been densely genotyped with more than 2000 molecular markers (Coe et al., 2002).

The primary aim of this study was to use the superior characteristics of the IBM population (Lee et al., 2002) to precisely localize QTL for GLS resistance. While several other studies have reported GLS QTL (Bubeck et al., 1993; Clements et al., 2000; Gordon et al., 2004; Lehmensiek et al., 2001; Saghai Maroof et al., 1996), these have all used conventional $F_{2:3}$ or backcross populations. Compared to these, the B73/Mo17 advanced intercross recombinant inbred line (IBM) population provides two main advantages (i) the use of immortal inbred lines allows for replicated multi-environment trials for the genotype set and (ii) the IBM population captures much more recombination and has many more molecular markers scored on it, meaning that traits can be mapped much more precisely.

Once GLS QTL were mapped in the IBM population, we were able to compare and contrast these results to GLS QTL we had identified in a conventionally-derived Mo17 × B73 RIL population, to previously published GLS QTL, and to QTL for southern leaf blight resistance that we had previously identified in the IBM population. All these comparisons are reported below.

MATERIALS AND METHODS

Plant Materials

Phenotypic data were collected from two populations. The IBM mapping population is comprised of 302 $F_{7:8}$ recombinant inbred lines (RILs) derived from the cross of maize inbred lines B73 (relatively susceptible parent) and Mo17 (relatively resistant parent). This population had been intermated four times subsequent to the F_2 stage before inbred lines were derived (Lee et al., 2002). In this study, 288 IBM lines rather than the full 302 were used because of seed shortages for 14 lines. The other population used was also an RIL population derived from a B73/Mo17 cross, but in this case no intermating had occurred subsequent to the F2 stage, rather, selfing was performed directly from the F_2 generation (C. Stuber; personal communication, 2004). This population is here referred to as the 'Stuber population'. A set of 204 of these $F_{2:7}$ RILs were used in this study.

Field Trials

All experiments were performed in Andrews NC. Experiments were performed in a field with a history of severe GLS development that lay in a mountain valley that had regular morning mists and heavy dews, conditions that favor disease development. The field was planted with corn every year. Plant debris from the previous year was routinely left on the soil surface, which provided a good source of inoculum. Consequently, artificial inoculation was not necessary. For the IBM population, field resistance was evaluated in three different years, 2005, 2006, and 2007. For the Stuber population, resistance was evaluated in 2004 and 2005.

Each experiment consisted of two replicates plus parental lines (B73 and Mo17) in complete randomized blocks. Experimental units in each case consisted of single-row plots. Plots were 4 m in length with a 0.6-m alley at the end of each plot. Inter-row spacing was 0.97 m. Fifteen seeds per plot were planted and rows were not thinned. At least two plots of inbred border were planted on all sides of the experiment.

Ratings

Entries in each environment were rated on a plot basis. In 2004, two ratings were made at 82 and 97 d after planting. In 2005, two ratings were made at 74 and 85 d after planting. In 2006, three ratings were made at 77, 84, and 94 d after planting. In 2007, three ratings were taken at 81, 89, and 96 d after planting. These dates generally corresponded to the period from 1 to 3 wk after pollen shed. Plots were rated visually on a one to nine scale, in increments of 0.5, with one being a symptomless plant and nine being a completely dead plant. Thus, a one unit difference in rating represented an approximately 12.5% difference in disease severity. DTA was determined for the IBM population as the number of days after planting when half the plants in the row were shedding pollen. DTA was not recorded for the Stuber population.

Statistical Analyses

For individual environments, weighted mean disease rating (WMD) values were calculated for each replication in each environment. To do this, 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. WMD is functionally equivalent to an area under disease progress curve (AUDPC) rating and has been called a "Standardized AUDPC" rating in other publications (Campbell and Madden, 1990; Shaner and Finney, 1977). To account for the rare (less than 4%) occasions when a line was represented in only one replication within an environment, least square means were calculated using the PROC

GLM procedure of SAS (SAS Institute, Cary, NC) to obtain average ratings over the two replications for each line for each environment. These least square mean values were used for the individual environment QTL analyses. Gray leaf spot QTL were identified across environments using "estimated" best linear unbiased predictions (BLUPs, see Littell et al. (2006) for a description of BLUPs) of the set of genotypes evaluated. For both WMD and DTA, BLUPs were determined—henceforth known as GLSBLUPs and DTABLUPs, respectively—using PROC MIXED in SAS v 9.1 (SAS Institute, Cary, NC), considering all model terms as random except for the overall mean. BLUPs were also determined for GLS WMD ratings of the Stuber population—henceforth known as StuberGLSBLUPs:

$$Y_{ijk} = \mu + E_i + R(E)_{ji} + G_k + GE_{ki} + \varepsilon_{ijk,}$$

where μ = overall mean; E_i = effect of environment i; $R(E)_{ji}$ = effect of replication j within environment i; G_k = effect of genotype k; GE_{ki} = effect of interaction between genotype k and environment i; and ε_{ijk} = effect of experimental error on plot containing genotype k in replication j and environment i.

All phenotypic correlation calculations were made using the PROC CORR procedure of SAS. Heritability was estimated for each trait using the PROC MIXED procedure of SAS, as described by Holland et al. (2003). PROC MIXED was also used to estimate the variance components attributable to environment, replication within environment, line, and line × environment interactions.

QTL analyses were performed using MapQTL5 (Van Ooijen, 2004). Multiple QTL mapping (MQM, also known as composite interval mapping or CIM) was performed with cofactors initially identified by simple interval mapping and subsequently by initial rounds of MQM. The 95% threshold level for calling QTL was determined by permutation tests (1000 permutations in each case). Multiple interval mapping was performed using Windows QTL cartographer version 2.5 (Wang et al., 2004) as described previously (Balint-Kurti et al., 2006), to examine possible epistatic interactions. Publically available genotypic data for 1345 markers spaced over the genome was used for the QTL analysis of the IBM population. Map distances are based on the IBM2 map (http://www.maizegdb.org/ verified 30 June 2008). Similarly for the Stuber population, publically available data for 234 markers spaced over the genome (Carson et al., 2004) was used for QTL analysis.

Since the units of distance in the IBM population are not, strictly speaking, centiMorgans (cM), IBM map units (Imu) are used as a measure of genetic distance. Broadly speaking, 1 cM ≈ 4 Imu (Falque, 2005; Lee et al., 2002; Winkler et al., 2003).

RESULTS AND DISCUSSION

Populations Analyzed

Two different populations, the IBM and the Stuber population, were used in this study. Both were derived from a B73/Mo17 cross. Overall, the data for the IBM population was substantially superior to that for the Stuber population, since the IBM population was larger (288 vs. 204 lines) and was assessed in more environments (3 vs. 2). In addition, the IBM population captures much more recombination than the Stuber population, due to the fact that it was intercrossed four times at the F_2 stage, which increased the effective map size about four-fold (Lee et al., 2002). Genotypic data for the IBM population was also much superior (1345 vs. 234 markers used in this study). For these reasons this paper emphasizes the results derived from analysis of the IBM population, although results from analysis of the Stuber population are also presented and discussed.

Disease and Anthesis Ratings

In the IBM population, phenotypic correlations between years for WMD and DTA ratings were moderate to high with pairwise Pearson correlation coefficients for WMD ranging from 0.63 to 0.81 and for DTA from 0.34 to 0.57 ($P < 0.0001$ in each case, see Table 1). In the Stuber population the Pearson correlation coefficient between the two environments for WMD was 0.64 ($P < 0.0001$). DTA was not rated for the Stuber population. Correlations between DTA and WMD in the IBM population were 0.07 (not significant), 0.14 ($P = 0.015$) and 0.24 ($P < 0.0001$) in 2005, 2006, and 2007, respectively. The correlation between the overall IBM WMD BLUP values (here referred to as GLSBLUPs) and the overall IBM DTA BLUP values (DTABLUPs) was 0.21 ($P = 0.0005$). The heritabilities for WMD and DTA in the IBM population were 0.77 (S.E. 0.02) and 0.57 (0.03), respectively. In the Stuber population, the heritability for WMD was 0.69 (0.03). These GLS resistance heritablities are broadly in line with previous reports (Clements et al., 2000; Gordon et al., 2006). GLSBLUPs and DTABLUPs followed an approximately normal distribution, with some evidence of transgressive segregation, especially for DTABLUP (Fig. 1).

In the IBM and Stuber populations, genotype and genotype-by-environment effects were the main significant contributors to phenotypic variance in WMD and (for the IBM population) DTA (Table 2). The standard error of the environmental variance estimate for the IBM DTA was large and the effect was consequently not significant. DTA is generally quite sensitive to environmental variation (e.g., Veldboom and Lee, 1996). Flowering was delayed by an average of approximately 5 d in 2007

Table 1. Pearson correlation coefficients between weighted mean disease (WMD) gray leaf spot ratings (scored on a one to nine scale) and days to anthesis (DTA) for the maize IBM population obtained in three environments (Andrews NC, 2005, 2006, and 2007, denoted as IBM05, IBM06, and IBM07 in the table). WMD correlations are the upper values, DTA correlations are the lower values in italics. All correlations are significant at $P < 0.0001$.

	IBM05	IBM06
IBM06	0.63	
	0.34	
IBM07	0.64	0.81
	0.57	*0.47*

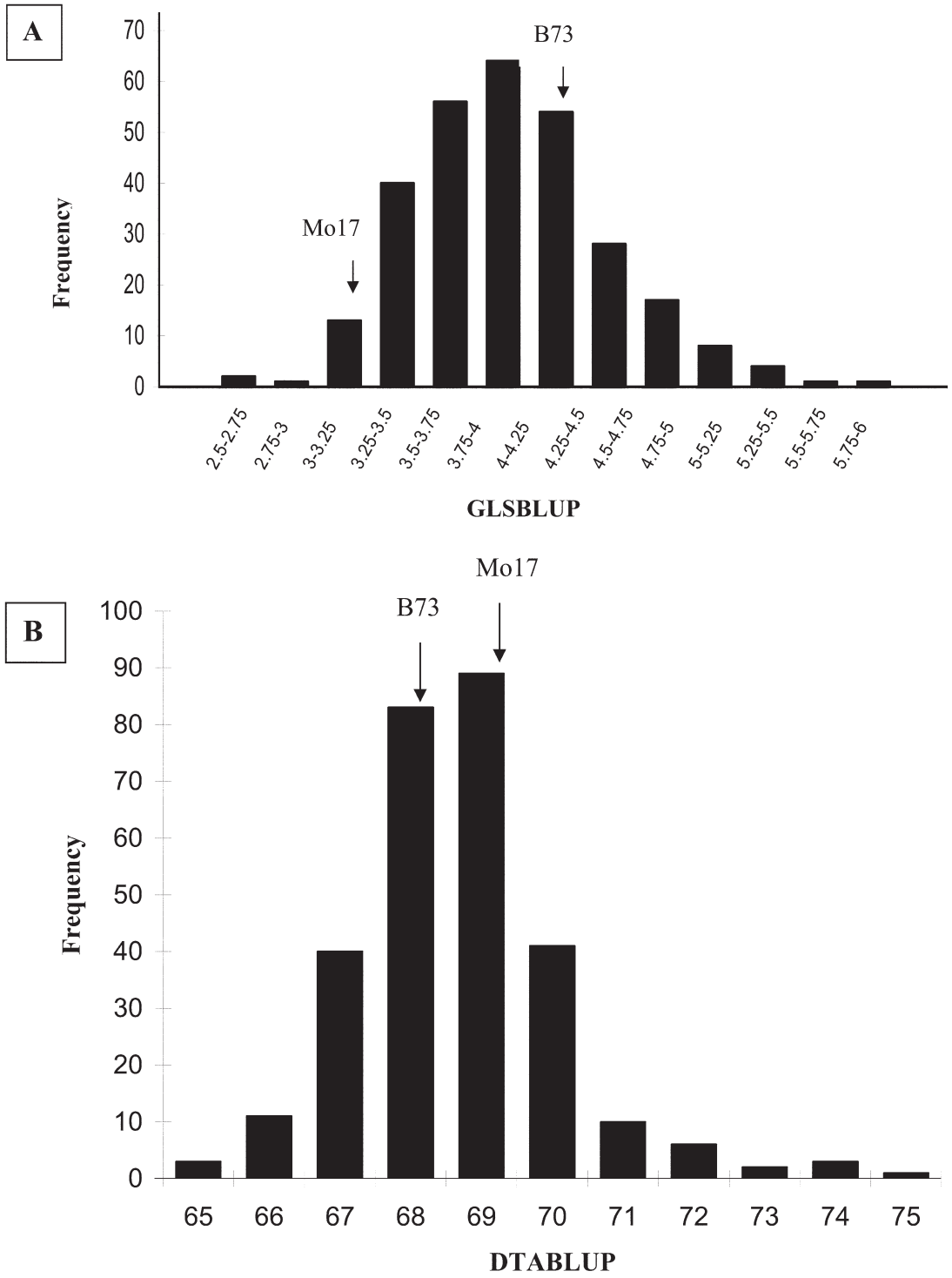

Figure 1. The distribution of best linear unbiased predictors (BLUPs) for weighted mean disease ratings for gray leaf spot of maize (GLSBLUP) and days from planting to anthesis (DTABLUP) in the IBM populations consisting of 288 lines, scored in 2005, 2006, and 2007 in Andrews, North Carolina. Plants were scored for disease resistance on a 1 to 9 scale with 1 being complete resistant and 9 being dead. DTA, the day when half the row had started shedding pollen, was recorded. In each case the average scores of the parental lines are indicated. (A) GLSBLUP (B) DTABLUP.

compared to previous years (data not shown), probably because of extremely low rainfall early in the season.

The fact that natural rather than artificial inoculum was used in this study may have exacerbated the environmental variation observed. The amount of natural inoculum present at the start of the season varies depending on a number of factors. The humidity level would affect the amount of sporulation and the infection efficiency of the pathogen, while the conditions over the previous winter and, most importantly, whether or not the field had been plowed, would affect how much inoculum was able to over-winter (Payne and Waldron, 1983). Several of the other GLS QTL

Table 2. Variance component estimates and standard errors for weighted mean disease (WMD) for gray leaf spot resistance and days to anthesis (DTA) for two recombinant inbred line populations, the IBM and Stuber populations, consisting of 288 and 204 lines, respectively. The IBM population was scored in 2005, 2006, and 2007, the Stuber population in 2004 and 2005. The Stuber population was not scored for DTA. All experiments were performed in Andrews, North Carolina.

	Variance component estimates (standard error)and *P*-values					
	IBM population				Stuber population	
Parameter	WMD†	*P*-value	DTA‡	*P*-value	WMD	*P*-value
Environment	0.17 (0.2)§	ns¶	10.01 (10.26)	ns	0.10 (0.38)	ns
Replication within environment	0.06 (0.05)	ns	0.30 (0.25)	ns	0.29 (0.29)	ns
Line	0.27 (0.03)	< 0.01	2.92 (0.38)	< 0.01	0.35 (0.05)	< 0.05
Environment by line	0.13 (0.01)	< 0.01	2.67 (0.26)	< 0.01	0.06 (0.02)	< 0.05
Residual	0.10 (0.004)	< 0.01	3.11 (0.14)	< 0.01	0.27 (0.02)	< 0.05

†Weighted mean disease.

‡Days to anthesis.

§Standard Error.

¶ns, not significant.

studies reported have used artificial inoculum for at least a portion of the reported experiments (Clements et al., 2000; Gordon et al., 2004; Lehmensiek et al., 2001).

QTL Analyses

Since there were significant genotype × environment effects, QTL analyses are presented for each of the three environments in which the IBM population was assessed (Table 3) as well as for the overall GLSBLUPs. Five significant QTL were identified using the GLSBLUPs, in bins 1.05, 2.04, 4.05, 9.03, and 9.05. The resistance allele for two of those QTL (bins 4.05 and 9.05) was derived from B73, the more susceptible of the parents, while in the other three cases the resistance allele was derived from Mo17. The QTL with the largest effect was in bin 9.03 explaining 12.0% of the total variation. Among the QTL, no significant epistatic interactions were detected. QTL identified in the individual environments were all similar to GLSBLUP QTL, but only the QTL in bin 4.05 was detected in all three environments and only in 2006 were all of the five GLSBLUP QTL detected (Table 3). A previous study, also conducted in part in Andrews NC, also found that GLS QTL were inconsistent over environments (Bubeck et al., 1993). However QTL were quite consistent over environments in some other GLS QTL studies (Gordon et al., 2003; Saghai Maroof et al., 1996). In one case (Gordon et al., 2003) similar QTL were detected in different environments as diverse as South Africa and the U.S. corn belt.

Only two QTL were detected from analysis of the GLS WMD BLUPs derived from the Stuber population (here referred to as StuberGLSBLUPs). One of these was in bin 2.04 at the same location as the corresponding GLSBLUP, but the other one was in bin 7.05, unlinked to any detected GLSBLUP (Table 3). Both of these QTL were identified in both the environments in which the Stuber population was assessed (data not shown).

The Stuber and the IBM populations were derived from the same parental cross, so it would be expected that the same major QTL would be detected in both. The IBM population should have more power to precisely map QTL, to detect smaller QTL, and to differentiate closely linked QTL because of the population attributes previously discussed. These expectations were generally fulfilled in a previous study which compared QTL for southern leaf blight in the two populations (Balint-Kurti et al., 2007). In the present study the comparison is less straightforward. The QTL in bin 2.04 was detected in both populations and, as expected, the GLSBLUP QTL is much more precisely defined. The 2-LOD interval for the GLSBLUP QTL spanned just 10 IBM map units (Imu) while the corresponding StuberGLSBLUP QTL spanned 63 Imu. The other four GLSBLUP QTL were not detected in the Stuber population. The linked GLSBLUP QTL in bins 9.03 and 9.05 had opposite effects (i.e., their resistance alleles were derived from different parents). The lower level of recombination may have meant that in the Stuber population these two QTL could not be resolved and they effectively cancelled each other out. Inspection of the QTL analysis results also showed that closely linked to the GLSBLUP QTL in bin 4.05, there was an effect in the opposing direction (Fig. 2). While this effect was not strong enough to result in the detection of a significant QTL in the IBM population, it may have reduced the apparent effect of the 4.05 QTL below significant range in the Stuber population. The non-detection of a StuberGLSBLUP QTL in bin 1.05 may simply have been due to the more limited power of the Stuber population. Indeed, there were nonsignificant effects detected in the 1.05 region in the Stuber population and it is possible that, were this population bigger, this QTL might have risen to the level of significance.

It is less clear why the StuberGLSBLUP QTL in bin 7.05 was not detected in the IBM population. It was detected in both single environments where the Stuber population was assessed (2004 and 2005, data not shown), but not detected in any single environment for the IBM

population, including 2005 when the two populations were planted side by side (i.e., they were essentially in the same environment). This may have been caused by a sampling issue, whereby independent samples from essentially the same population can detect different QTL, often with inflated estimates of the effects of the detected QTL (Melchinger et al., 1998). Another possibility is that the parents of the two populations were not completely identical after all.

Table 3. Parameters associated with major quantitative trait loci (QTL) for gray leaf spot (GLS) resistance and days to anthesis (DTA) identified in a maize B73/Mo17 advanced intercross recombinant inbred line population comprising 288 lines (the IBM population) evaluated in three different environments (2005, 2006, and 2007 in Andrews, NC). Also shown are parameters associated with GLS resistance QTL identified in a conventional RIL population derived from a B73/Mo17 cross comprising 204 lines (the "Stuber population") assessed in two environments (2004 and 2005 in Andrews NC).

Bin, *parameters*	GLSBLUP†	GLS2005‡	GLS2006§	GLS2007¶	StuberGLSBLUP#	DTABLUP††	Flanking markers‡‡
1.05§§	412–417¶¶		419–430	404–412			asg3-umc1515
a	−0.12##		−0.23	−0.16			
LOD	5.52†††		7.02	5.3			
R^2	0.06‡‡‡		0.09	0.08			
2.04	284–294		294–302		243–306§§§		mmp167-mmp91
a	−0.11		−0.18		−0.16		
LOD	5.03		4.36		4.19		
R^2	0.06		0.05		0.10		
4.05	288–292	283–288	288–292	288–292			csu509-bnl15.45
a	0.13	0.20	0.20	0.13			
LOD	7.16	5.37	5.79	3.97			
R^2	0.08	0.11	0.07	0.05			
4.09						5.66–5.80	lim44b-php10025
a						−0.37	
LOD						5.24	
R^2						6.5	
7.05					188–274		bnlg398-bnlg657
a					−0.14		
LOD					3.31		
R^2					0.07		
8.05						3.60–3.67	ufg80-bnlg666
a						0.5	
LOD						8.6	
R^2						11	
9.02						1.46–1.62	
a						−0.4	
LOD						6.1	
R^2						8	
9.03	243–249		240–248	240–248			umc1271-umc20
a	−0.16		−0.26	−0.18			
LOD	9.88		9.81	6.75			
R^2	0.12		0.12	0.08			
9.05	349–362		329–367				ufg64-umc2095
a	0.11		0.17				
LOD	3.87		3.83				
R^2	0.05		0.04				

†Over-environment best linear unbiased predictors (BLUPs) for weighted mean disease (WMD) ratings for the IBM population.

‡Weighted mean disease ratings for GLS determined for the IBM population in 2005.

§Weighted mean disease ratings for GLS determined for the IBM population in 2006.

¶Weighted mean disease ratings for GLS determined for the IBM population in 2007.

#Over-environment best linear unbiased predictors (BLUPs) for weighted mean disease (WMD) ratings for the Stuber population.

††Over-environment best linear unbiased predictors (BLUPs) for days to anthesis (DTA) ratings for the IBM population.

‡‡When multiple QTL map to the region in question, markers flanking the GLSBLUP QTL are indicated.

§§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). The marker order determined for the population used in this experiment largely follows the marker order shown in the standard maize genetic map, the IBM map (Lawrence et al., 2005).

¶¶The positions that define the two LOD intervals around the position of peak likelihood for the QTL. All values are in IBM map units (Imu) and are based on the IBM2 map.

##The additive effect of the QTL. For disease ratings this is in terms of the one to nine scale employed. For days to anthesis this is terms of days. A positive number indicates that the allele for resistance (or late anthesis) was derived from B73.

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

‡‡‡R^2 estimates the proportion of phenotypic variance (%) explained by the detected QTL.

§§§The 2 LOD interval for StuberGLSBLUP is based on the IBM2 map distances, not the distances from the original B73/Mo17 RIL population which are much smaller. The IBM2 map distances were inferred from markers common to both maps.

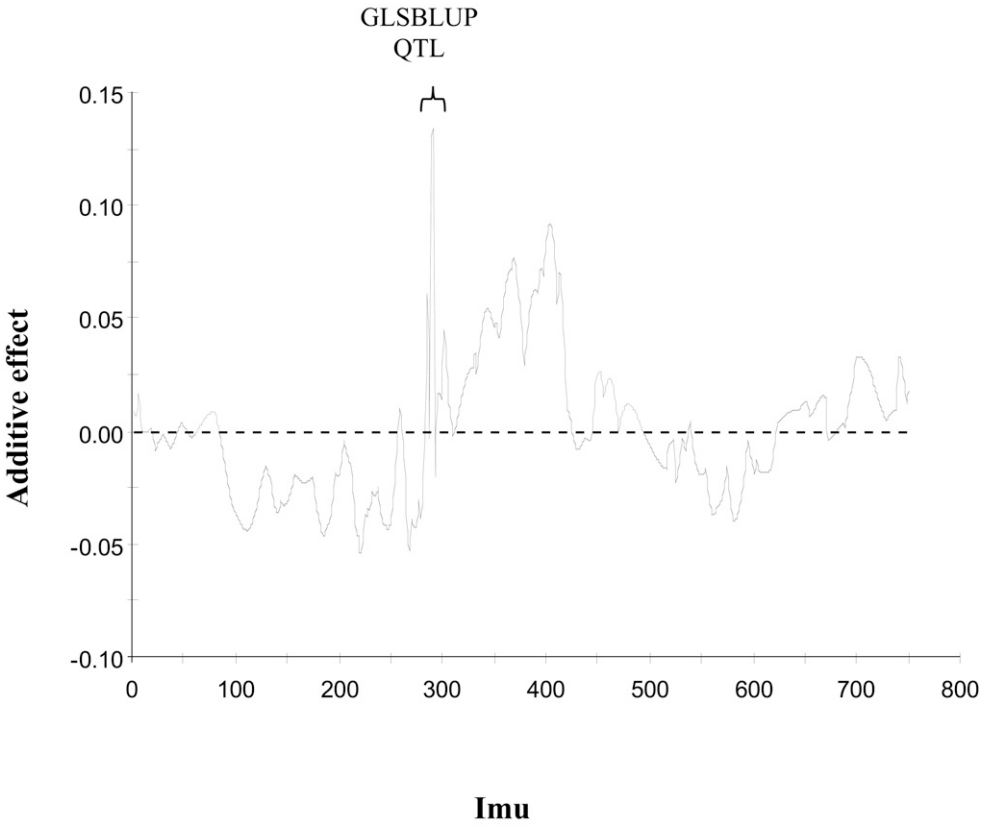

Figure 2. The additive effects for best linear unbiased predictors (BLUPs) for weighted mean disease ratings for gray leaf spot of maize in the IBM population (GLSBLUP) along the length of maize chromosome 4. The position of the GLSBLUP QTL identified in bin 4.05 is identified. While the QTL has a positive effect, closely linked opposing effects can be observed which may make the QTL undetectable in the Stuber population which has less recombination. Map positions are in IBM map units (Imu).

Identically named lines maintained by different breeding programs and institutions often vary somewhat, as determined by marker analysis (Gethi et al., 2002).

GLS symptoms generally develop rapidly after anthesis. Furthermore, there have been reports of variation in plant maturity being associated with GLS resistance (Bubeck et al., 1993; Clements et al., 2000). In this study, the correlation between GLSBLUPs and the DTABLUPs was moderate to low ($r = 0.21$). DTABLUP QTL were detected in bins 4.09, 8.05, and 9.02, none of which coincided with GLSBLUP QTL (Table 3). These three QTL were also detected in each of the three environments in which the IBM population was grown (data not shown). Only the bin 8.05 QTL was detected for DTA in both Andrews NC and in the previous study performed in Clayton NC (Balint-Kurti et al., 2007). This is in the region of the *vgt1* gene, a major QTL involved in floral transition (Salvi et al., 2002). Segregation distortion has previously been noted in the IBM population in this region (Fu et al., 2006).

Comparison with Previous GLS QTL Studies

Table 4 summarizes GLS QTL detected in six other populations that have been reported in the literature (Bubeck et al., 1993; Clements et al., 2000; Gordon et al., 2004; Lehmensiek et al., 2001; Saghai Maroof et al., 1996). Two loci appeared to be 'hotspots' for GLS QTL. QTL were detected in the bin 1.05/06 in four different populations. In three of these populations (PG, FR1141 × 061, and B73 × Va14) it was the major QTL detected with R^2 values ranging from 0.2 to 0.5. The other notable hotspot was in the region spanning bins 2.03 to 2.05. QTL were detected in this region in all four populations in which B73 was one of the parents (Table 4) and in each case the resistance allele was derived from the non-B73 parent. This suggests that a particularly weak allele, a "GLS susceptibility" allele, exists at the locus in B73. This was also the only GLS QTL that was detected in both the IBM and Stuber populations.

It was notable that more than half of the GLS QTL detected over all these studies were identified in only a single population. This diversity of detected QTL may reflect the complex nature of the genetic architecture underlying GLS resistance. It may also reflect environmental interactions which may lead to some QTL not being detected in some environments.

As expected, the IBM population provides much more precise localization of QTL compared to the other populations. The two-LOD intervals of the five GLSBLUP QTL are between 4 and 10 Imu (equivalent to about 1–3 cM), whereas the equivalent confidence intervals of GLS QTL identified in the other populations are at least five-fold larger.

QTL for Multiple Disease Resistance

A previous study assessed the IBM population for southern leaf blight resistance (Balint-Kurti et al., 2007). Southern leaf blight and GLS are both necrotrophic foliar fungal pathogens and it might be expected that some resistance mechanisms might be effective against both pathogens. The Pearson correlation coefficient between the BLUPs for GLS and southern leaf blight WMD ratings in the IBM population was 0.42 ($P < 0.0001$). However there is only one QTL, in bin 2.04, which both co-localizes and has the same-direction effect between the two diseases. This is the QTL with the putative "GLS susceptibility" allele derived from B73 (see above). It could be that it is actually a "multiple disease susceptibility" allele. In addition, the GLSBLUP

QTL in bin 1.05 is closely linked to a same-direction effect identified for southern leaf blight resistance in the previous study (Balint-Kurti et al., 2007), though the two QTL do not precisely co-localize. Loci conferring multiple disease resistance are of obvious utility in plant breeding using marker-assisted selection, where multiple traits can be followed "for the price of one."

CONCLUSIONS

As far as we are aware, this study represents only the third published use of the full IBM population to map QTL in maize. The two previous studies (Balint-Kurti et al., 2007; Hazen et al., 2003), like this one, were able to map QTL to quite precisely defined loci. With the imminent release of the B73 genome sequence, these data will become useful for identifying genes associated with these traits.

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Table 4. A comparison of the locations of gray leaf spot (GLS) resistance quantitative trait loci (QTL) identified in RIL populations derived from seven different crosses. In each case where a QTL was detected in a specific bin, the identity of the line from which resistance is derived is shown. Underlined values indicate that the resistance QTL explained more than 10% of the total phenotypic variation.

	Proprietary germplasm[†]	VO163Y x Pa405[‡]	FR1141 x 061[§]	B73 x Va14[¶]	B73 x NC250A[#,††]	ADENT x B73rhm [‡‡,§§]	B73 x Mo17[††]
1.03[¶¶]						ADENT	
1.05/1.06	PG		061	Va14			Mo17
1.08					NC250A		
2.03/04				Va14	NC250A		Mo17
2.04/2.05						ADENT	
2.06/2.08			061		NC250A		
2.09		VO163Y					
3.04/5	PG					B73	
4.05							B73
4.06/4.08		VO163Y		B73		ADENT	
5.03/04	PG		061		NC250A		
5.04/05	PG		061				
5.05/5.06					NC250A		
6.07						ADENT	
7.02/7.03			061		NC250A		
7.05							Mo17
8.05						B73	
8.06				Va14			
9.01						ADENT	
9.03					NC250A		Mo17
9.05							B73
10.06						B73	

[†]In this study proprietary germplasm was used and there is no information on the identity of the alleles conferring resistance (Lehmensiek et al., 2001).

[‡](Gordon et al., 2003).

[§]Only the five environment- and maturity-independent QTL are reported in this table, six maturity-dependent QTL are also reported in the original paper (Clements et al., 2000).

[¶]In this case, only QTL detected in at least two of the three environments are shown (Saghai Maroof et al., 1996).

[#]In this study two $F_{2:3}$ populations derived from very similar crosses, B73 x NC250A and B73rhm x NC250A (B73rhm is a near-isogenic derivative of B73 carrying the rhm gene for SLB resistance), were examined. For the sake of simplicity we are reporting the QTL identified in the overall analysis of both populations (Bubeck et al., 1993).

[††]An $F_{2:3}$ population derived from and ADENT × B73rhm cross (Bubeck et al., 1993). ADENT is an inbred line derived from the cross (Amarillo Dentado 2 × (A632 × B14A)) × Amarillo Dentado 2. Amarillo Dentado is a synthetic population derived from the International Maize and Wheat Improvement Center (CIMMYT, Mexico). Only regions detected as significant for the over-environment analysis are reported here.

[‡‡]This study was carried out in 1990 before molecular markers that comprehensively covered the maize genome were available. Therefore the maps consist of only ~80 markers and QTL are poorly defined compared to more modern studies and the confidence intervals often span several bins. Here, in the cases where this occurs, we have assigned the QTL to the most probable bins.

[§§]This study, including QTL from both the IBM and Stuber populations.

[¶¶]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).

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