ABSTRACT

KUMP, KRISTEN LYNN. Combined Population Analyses for Mapping Loci Conditioning Resistance to Southern Corn Leaf Blight. (Under the direction of James B. Holland and Peter J. Balint-Kurti.)

Southern leaf blight (SLB) is a fungal disease that attacks the leaves of maize plants, forming tan elliptical lesions. The causal agent is *Cochliobolus heterostrophus*, a necrotrophic ascomycete, which is endemic to hot, humid maize-growing regions. Most maize hybrids have at least a moderate level of quantitative resistance to SLB, which is the primary method of control for the disease. Quantitative resistance is conditioned by small effects of many genes; thus, to make most efficient use of the genetic variation present for SLB resistance, the number of genes involved, the effects they have singly and in combinations, and their potential interactions with the environment must be elucidated. Because the maize-SLB pathosystem is an excellent model for host-necrotroph genetics, the identification of genes underlying the disease response will not only accelerate breeding progress for SLB resistance, but also for necrotrophic resistance in other crop species.

Several attempts have been made to map quantitative trait loci (QTL) responsible for conditioning resistance to SLB in biparental segregating populations; however, the precision of the positional estimates of the resultant loci does not allow for identification of genes underlying the response. In addition, the limited germplasm studied only offers the potential of examining two alleles per locus. Many more alleles exist in breeding germplasm, and for this reason, usefulness of results are limited.

The objective of the two studies presented here is to address these limitations through combination of data across distinct populations. The first study jointly analyzes data from four independently derived B73 x Mo17 populations to validate the existence of and fine map

an SLB resistance QTL in bin 3.04, the most significant QTL detected in a study of the IBM population (a set of advanced intercross lines derived from a B73 x Mo17 cross). The four populations used consisted of the IBM population, a set of recombinant inbred lines (RILs), and two sets of $F_{2:3}$ lines derived from crosses between near isogenic lines (NILs, lines genetically identical except for the 3.04 region). These $F_{2:3}$ lines allowed for validation of the 3.04 QTL in a uniform genetic background. Combined analysis of data from the four populations yielded a smaller positional confidence interval than the IBM study interval, in which 3 canidate leucine repeat kinase genes lie.

The second study utilizes the Nested Association Mapping (NAM) population to provide a global view of the genetic architecture of SLB resistance across a broad range of maize germplasm. The NAM population consists of 5000 RIL derived from crosses between B73 and 25 diverse founder inbred lines. The full set of lines was phenotyped for resistance to SLB, and linkage analysis was used to identify 32 QTL segregating for SLB resistance. Additive effects explained 79.6% of the phenotypic variation; no significant epistatic interactions were detected between pairs of additive loci. To compare efficacy of the NAM approach with previously published studies, QTL identified using NAM and previously identified QTL were positioned on the IBM2008 map. 60% of NAM QTL had been detected previously, however, 13 novel QTL were detected in this study. The advantage to the NAM approach lies in not only the increased population size and statistical power, but also the wider sampling of alleles.

Combined Population Analyses for Mapping Loci Conditioning Resistance to Southern Corn Leaf Blight

by
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BIOGRAPHY

Kristen Kump was born on Long Island, New York in 1982, to Donald and Lynn Kump. Kristen spent the first fourteen years of her childhood on Long Island, after which she and her family moved to the northwest suburbs of Chicago. Kristen attended high school in Lake Zurich, Illinois, where she was a member of the marching band and math team. Upon graduation from high school, Kristen attended Brown University in Providence, Rhode Island. During her first semester at Brown, Kristen enrolled in an Environmental Issues class, a choice that profoundly affected the course of her education. It was through this class that Kristen became aware of the great environmental impact of a burgeoning human population and the subsequent nutritional requirements imposed on the planet. Kristen became increasingly interested in this problem, and through additional course work in varied disciplines, decided to pursue a career in agriculture. During a hiatus from Brown for career exploration, Kristen volunteer interned at the Chicago Botanic Garden with their resident ornamental breeder. Through this experience, Kristen decided to become a plant breeder and complete her undergraduate education at the University of Wisconsin-Madison as a genetics major. At Wisconsin, Kristen became a member of the Women In Science and Engineering program as a peer mentor, and conducted research with the USDA cucurbit genetics and breeding program, then under the direction of Dr. Jack Staub. She received her Bachelor's of Science in Genetics in May of 2007. In June of that year, Kristen matriculated as a master's student in Crop Science at North Carolina State University in Raleigh, where she studied under the mentorship of James Holland and Peter Balint-Kurti. For the past two years, she

has studied the genetics of quantitative disease resistance in maize. During the 2008-2009 year, Kristen served as President of the Crop Science Graduate Student Association and Social Committee Chairperson of the University Graduate Student Association. After completion of this thesis, Kristen will continue her graduate education as a doctoral student in the Holland and Balint-Kurti labs, refining her knowledge of quantitative genetics and disease resistance.

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Literature Review

I. Quantitative Trait Locus Mapping- Current Methodology and Limitations

Phenotypic variation within a population can be described as qualitative or quantitative. Qualitative variation, manifested as variation between discrete phenotypic classes, is typically governed by single genes that segregate in familiar Mendelian ratios. In contrast, quantitative traits are governed by a more complex genetic architecture encompassing many genes. The individual phenotypes comprising the population assume trait values that exist along a continuous distribution with a mean and a variance (Lynch and Walsh, 1998; Mackay, 2001). Whereas many genes controlling qualitative variation have been mapped in different organisms, relatively fewer genes contributing to quantitative traits have been mapped quite so precisely (Glazier et al., 2002). The genes underlying a quantitative trait are more difficult to map for several reasons, most of which stem from the segregation of multiple loci for the trait, the small effects these individual genes have, and the interactions both within the organism's genome and between its genome and environment (Lynch and Walsh, 1998; Mackay, 2001). Additionally, these phenotypes must be evaluated or measured on a, sometimes somewhat arbitrary, numerical scale, which can be prone to errors in measurement or observation.

Several methods have been developed for locating quantitative trait loci (QTL). A quantitative trait locus can be defined as a chromosomal region affecting the expression of a quantitative trait. Several statistical methods ranging from simple single marker ANOVA and

regression analyses to more complex mixed models for multiple QTL and their interactions have been developed. However, the basic premise behind QTL mapping remains the same: if a genetic marker and a QTL co-segregate, there will exist a difference in the trait value means between the different genotypic classes (Sax, 1923; Lynch and Walsh, 1998; Mackay, 2001; Doerge, 2002).

The most straightforward method of QTL detection is single marker analysis, which does not require construction of a genetic map. Molecular markers detect allelic variation at specific loci based on differences in their DNA sequences, e.g. single nucleotide polymorphisms (SNP), restriction fragment length polymorphisms (RFLP), microsatellites, or the proteins encoded by them, e.g. isozymes (Tanksley, 1993). These physical or chemical differences can be detected via methods such as gel electrophoresis or complementary binding of a fluorescent or radioactive probe (Kwok, 2001). ANOVA or regression is used to test the null hypothesis that different genotypic class means are equal. Only one marker is fit in the model at a time; markers are tested for associations with phenotypic values separately. A t-test or an F-test can be used to evaluate the null hypothesis, and if the p-value is smaller than a threshold value, a QTL is said to be linked to that marker locus. Effects are estimated by the differences between the marker classes. The additive effect can be estimated as half the difference between the means of the two homozygous classes; the dominance effect can be estimated as the difference between the average of the means of the two homozygous classes and the mean of the heterozygous class (Edwards et al., 1987). Confounding between position and effect estimates of the QTL restricts the usefulness of this method. For example, in a set of F_2 lines, the additive effect of a QTL is biased downward by a factor of (1-2r),

where r is the recombination frequency between the marker and the QTL; the dominance effect is biased downward by a factor of $(1-2r)^2$ (Edwards et al., 1987). Thus, a QTL of small effect tightly linked to the marker and a QTL of large effect loosely linked to the marker cannot be distinguished. Because the probability that a random marker is at the same genetic locus as the QTL is very small, effect estimates are generally biased downward due to recombination between the QTL and the marker. Although single marker analysis is useful for discovering markers that cosegregate with QTL, determination of the locus's position within the genome necessitates construction of a genetic linkage map (Lander and Botstein, 1989; Lynch and Walsh, 1998; Doerge, 2002).

Constructing a genetic map commences with the creation of a mapping population that often consists of one of the following generations derived from a biparental cross: F_2 plants or F_2-derived lines, backcrosses of the F_1 to one of the parents, or recombinant inbred lines. Recombinant inbred lines are derived through several generations of selfing and single seed descent after the creation of the F_1 (Tanksley, 1993; Lynch and Walsh, 1998). These lines are then genotyped with several molecular markers that exhibit polymorphism, or difference between the two parents. A genetic map that orders these markers according to their locations along the chromosome is then inferred from the rates of recombination between parental haplotypes (Tanksley, 1993; Kearsey and Pooni, 1996).

During prophase I of meiosis, crossovers between distant loci generally occur at higher frequencies than crossovers between physically closer loci, although the relationship between physical distance and recombination frequency is not consistent either within or across genomes (Mancera et al., 2008; McMullen et al., 2009). Recombination frequencies

can be estimated based on the number of recombinant gametes observed, but are unsuitable measures of genetic distance since they are not additive in nature. Assuming diploidy and independent exchange of the four chromatids within an interval, one crossover results in the formation of two recombinant gametes, but so do two or more crossovers on average. The theoretical maximal recombination frequency is 50%, the frequency with which unlinked loci will form non-parental combinations in the gametes by chance. Under certain assumptions about crossover interference, mapping functions translate observed recombination frequencies into linear genetic distances along the chromosome that reflect the average number of crossovers per meiosis in a given interval. Map distances are measured in centiMorgans (cM) (Kearsey and Pooni, 1996; Lynch and Walsh, 1998). One cM corresponds to one crossover per 50 meioses and an observed recombination rate of .01, but the relationship between cM and recombination frequency decays over longer distances (Kearsey and Pooni, 1996). Two of the most commonly used mapping functions can be attributed to Haldane (1919) and Kosambi (1944). The principal difference between the two is Kosambi's adjustment for interference, the phenomenon by which a crossover in one region inhibits a second crossover in that same region. After recombination frequencies and corresponding map distances between each pair of markers have been estimated, the genetic markers can be assembled into linkage groups. Markers within linkage groups can then be ordered based on the map distances between them, i.e. markers with the largest intervening distance will be the farthest apart (Kearsey and Pooni, 1996).

The first method of QTL mapping to incorporate use of a genetic map was interval mapping. Interval mapping divides the genetic map into pairs of adjacent markers and tests

for marker-phenotype associations at incremental positions within each interval. Because flanking markers, with known genetic distance between them, are used in this analysis, the genotype at each tested position is inferred probabilistically according to its distance from each of the two adjacent markers. Lander and Botstein (1989) developed this method for a set of backcross lines. Lander and Botstein's model includes parameters for the mean of the lines, the effect of substituting a non-recurrent parent allele for a recurrent parent allele, and the error variance. A likelihood function estimates these parameters while accounting for the probability distribution of the unknown genotypes within the interval. The likelihoods of a model in which a QTL effect is attributed to a particular genome position and one in which no QTL is assumed are compared by taking the base 10 log of their ratio. This is called the log of odds, or LOD score, for the tested position. A LOD score of 3 is generally used as the threshold for declaring the presence of a QTL and is equivalent to saying the model with a QTL at that location is 1000 times more likely than the model with no QTL. The LOD scores for consecutive positions in an interval can be plotted, with the QTL most likely residing at the peak of the distribution. LOD maps can also be used to infer the presence of multiple QTL. A larger "ghost" peak residing between two smaller peaks indicates the possibility of linked QTL in the region (Lander and Botstein, 1989).

The QTL effect estimate is taken as the estimate from the position at the peak of the LOD curve, eliminating the confounding effect of recombination between markers and QTL on the effect estimates. Calculating these maximum likelihood estimates is computationally difficult; however, regression methods independently developed by Haley and Knott (1992) and Martinez and Curnow (1992) provide similar results to the maximum likelihood

expectations. For purposes of illustration, consider the following scenario from Haley and Knott's paper. If two inbred parents with genotypes $A_1A_1Q_1Q_1B_1B_1$ and $A_2A_2Q_2Q_2B_2B_2$ are crossed, the F_2 population will consist of 3 QTL genotypes (denoted as Q_1Q_1, Q_1Q_2, and Q_2Q_2) with effects *m*+*a*, *m*+*d* , and m-*a*, with *m*, *a*, and *d* symbolizing the mean of the two homozygous classes, additive effect, and dominance effect, respectively (Falconer and Mackay, 1996). The recombination frequency between the two markers, *r*, can be estimated from marker data; however, the two recombination frequencies r_A (between the right marker and the QTL) and r_B (between the left marker and the QTL) are unknown. Also unknown are the genotypes of individual lines at the QTL. Conditional probabilities of these QTL genotypes are derived based on the marker locus genotypes and the values of *r*, r_A, and r_B. Each marker genotype class is comprised of three possible QTL genotypes. Haley and Knott derived the expected means of each of the nine marker genotype classes by multiplying the conditional QTL genotype probabilities by their expected values (*m*+*a*, *m*+*d*, and *m*-*a* for Q_1Q_1, Q_1Q_2, and Q_2Q_2 respectively), summing across the three probabilities and dividing by the total frequency of the marker genotype. The coefficients of *a* and *d* in the expected means are used as coefficients of the additive and dominance effect variables in a regression model. Several positions within the interval are then tested by substituting different values of r_A and r_B into the expected mean expressions. To test for significance, an F-test is constructed as the ratio of the regression sum of squares to the residual sum of squares. The position with the lowest residual sum of squares is considered the most likely position of the QTL. An approximate likelihood ratio test can also be used to test the null hypothesis of no QTL. In order to evaluate the likelihood ratio, two models must be fit: a reduced model assuming *a*

and *d* parameters are zero and a full model containing these parameters. The likelihood ratio test, distributed as a χ^2 with two degrees of freedom (one for each of the parameters omitted from the full model) is expressed as:

$$\text{likelihood ratio test} = n \ln(RSS_{reduced}/RSS_{full})$$

Fitting multiple QTL on a chromosome requires adding parameters for the additional QTL to the regression model. Epistatic interactions between QTL can also be fitted by changing the genotypic mean expectations to include additive-additive, additive-dominance, and dominance-dominance components as appropriate (Haley and Knott, 1992). An even simpler analysis of regressing phenotype directly on marker genotype was proposed by Whittaker et al. (1996) and shown to provide similar results to regressing on QTL genotype probability.

There are several advantages of using interval mapping over single marker analysis; the principal benefit is determination of the QTL position within the genome relative to the markers tested. Computationally, interval mapping is more strenuous, but provides better estimates of QTL effects and positions due to lack of confounding between position and effect estimates. Fewer progeny are needed to obtain the same precision as the single marker method (Lander and Botstein, 1989; Haley and Knott, 1992). However, there are still problems with the interval mapping model. Interval mapping cannot ascertain if a QTL detected in one interval is independent of the effect of a linked QTL in another interval. Additionally, using only two markers at a time for the test does not make use of the full complement of marker information (Zeng, 1994).

Composite interval mapping (CIM) was developed by Zeng (1993) to detect and estimate the effect of a QTL while controlling variation due to other segregating QTL in the genome. In CIM, intervals are tested, but additional markers are used as cofactors in a multiple regression model to account for genetic variation caused by QTL outside of the tested interval. Jansen (1993) also formulated a method of using multiple regression with different marker cofactors. Including information from additional markers increases the power of the test if the appropriate number of markers is fitted (Zeng, 1994).

Kao et al. (1999) further extended the idea of CIM by fitting a model with multiple QTL (using the maximum likelihood positions for all QTL rather than just one) and their interactions. Multiple interval mapping (MIM) first uses stepwise selection to identify genome positions (which may be at marker loci or inside inter-marker intervals) with effects above a specified threshold. The position with the highest likelihood ratio statistic (LRT) is chosen first and markers are added one by one to the model, provided their LRTs are above the threshold value. The most informative subset of markers is placed into a model, with additional parameters encoding for possible epistatic interactions between pairs. MIM is thought to be a more precise and powerful method for QTL mapping, as the effects of multiple QTL are estimated simultaneously and epistatic effects are not ignored (Kao et al., 1999).

II. Association Mapping

Association mapping is another method for localizing genes that contribute to quantitative traits. It is often used for fine-mapping QTL that have been detected using

traditional linkage mapping or for which candidate genes are available. Phenotype-allele associations are either tested across many genetic markers in a genome scan or at polymorphic sites within a candidate gene. These tests determine if specific phenotype-genotype combinations occur at higher frequencies than expected (Flint-Garcia et al., 2005). The resolution achieved through association mapping depends on the rate of linkage disequilibrium decay over genetic distance in the population under study. In a genetically diverse population of an outcrossing species, many generations of recombination will have broken linkages between most portions of the genome. Linkage disequilibrium generally decays to near zero over 2000 base pairs among diverse maize lines, so in theory, gene level resolution can be achieved. This potential increased resolution is one advantage that association mapping has over traditional linkage mapping (Remington et al., 2001). Other advantages include ability to use natural populations, eliminating the need to develop synthetic populations, and inclusion of more than two alleles in the study (Mackay and Powell, 2007).

Because population admixture can cause linkage disequilibrium, effects attributed to population structure must be included in the model to prevent the detection of false associations. In order to control for population structure, researchers have devised several ways to account for different levels of relatedness. One is the genomic control method, which utilizes random markers to adjust test statistics, but assumes that all loci in the analysis are affected equally. Structured association also uses random markers to estimate a population structure, but this is incorporated into the model in the form of a Q (population structure) matrix. Further precision in estimating population structure has come with the inclusion of

both Q and K (kinship) matrices in the model. The K matrix approximates the identity by descent between each pair of individuals. Using information from random markers distributed throughout the genome, the average identity by state between random members of the population is calculated. The identity by descent between a pair of individuals is then estimated by subtracting the average identity in state from the specific identity in state between the two individuals (Devlin and Roeder, 1999; Yu et al., 2006). Basically, the model for association mapping considers a trait value as a combination of effects due to genetic polymorphism, population structure (Q), kinship structure (K) and error (Yu et al., 2006). Alternatively, principal components analysis of random markers can be used to characterize population structure, and the eigenvalues of a few key principal components can be used to adjust for that structure in the association analysis (Price et al., 2006).

III. Combined Population Analyses

A single population does not provide full information about the genetic architecture, i.e., the number, identities, and effects of genes, controlling a trait. The power of QTL detection and the resolution of positional estimates in traditional linkage mapping is limited by, among other factors, population sizes. QTL are typically mapped to an interval encompassing ten to 30 cM (Kearsey and Farquhar, 1998; Salvi and Tuberosa, 2005) and only a limited number, approximately between four and eight, are detected for each trait (Beavis, 1998; Kearsey and Farquhar, 1998). Usefulness of the information obtained from linkage mapping is limited by the number of alleles under study; results can be used only as widely as the alleles sampled in the study extend. Moreover, when a biparental population is

evaluated for the trait in question, and is not segregating for a QTL, the QTL will not be detected. The parents chosen for the population are usually not randomly chosen; two parents at opposite extremes of the trait distribution generally are selected. This lack of randomness can introduce bias into the estimates of QTL effects (Xu, 1998). The scope of inference of biparental QTL studies is limited by the small sample of alleles tested (Holland, 2007). Some of the resolution, power, and relevance limitations of biparental studies can be addressed through combining data from several smaller populations or separate QTL studies into a single joint analysis. There are two fundamental ways that data can be combined across multiple linkage populations: meta-analyses compile post-analysis data from several studies and pooled analyses combine raw data.

Meta-analyses

A meta-analysis compiles the QTL position and effect estimates from different studies, then estimates the most likely position and effect estimates of shared QTL for a trait. Meta-analysis has some unique challenges that stem from use of different markers, different tests for linkage, and different criteria for determining significance (Allison and Heo, 1998). Meta-analysis, like any other combined analysis approach, necessitates the construction of a joint linkage map, a projection of markers from all of the populations onto a single consensus map.

Goffinet and Gerber (2000) suggested a method for selecting models that most accurately reflect the number of distinct QTL identified within all of the studies being analyzed. There are two reasons why the same locus may be positioned differently across

studies; either different causal genes underlie the QTL, in which case the locus in question is really two or more loci, or experimental error has interfered with the correct placement of the QTL. If *n* different QTL have been identified in all of the studies, a maximum likelihood method estimates the most likely distribution of QTL for each one. The Akaike Information Criterion is used to select among the *n* different models. Chardon et al. (2004) used this method to comprehensively evaluate flowering time QTL from 22 different studies that had detected a total of 313 different QTL. These QTL were consolidated into 62 consensus QTL with interval position estimates that were on average smaller by a factor of 1.8. The contraction of these confidence intervals allowed Chardon et al. (2004) to more accurately predict candidate genes underlying the interval. Khatkar et al. (2004) used this method in a meta-analysis of QTL mapping studies in dairy cattle and also achieved more narrow positional confidence intervals.

Additional procedures for meta-analysis of linkage mapping results have been proposed. MetaQTL, a software program, builds consensus maps that consider recombination rates from different studies as estimates from a normal distribution. The variance of the estimate is determined by the cross design, recombination rate, sample size, and informativeness of the markers. In order to model the most likely distribution of QTL in the genome, a clustering approach based on a Gaussian mixture model is used (Veyrieras et al., 2007).

Pooled Analyses

In contrast to meta-analyses, pooled analyses combine raw data from different populations segregating for the trait of interest. This approach is preferred over the meta-analysis due to the problems outlined above (Lander and Kruglyak, 1995); however, the pooled analysis approach also requires the creation of a consensus map. Certain software programs, such as JoinMap (Stam, 1993) and MCQTL (Jourjon et al., 2005), have been implemented for the task. In JoinMap (Stam, 1993), pairwise recombination frequencies between markers are obtained using a maximum likelihood approach. Estimates from different populations are combined by weighting as a function of their LOD scores and standard errors. This information is used to infer a map order. JoinMap then calculates genetic distances between markers and minimizes a weighted (by LOD) least square difference between the expected and estimated distances (Stam, 1993). The weighted least square difference is differentiated with respect to the distances between each set of markers, and the resulting set of linear equations can be solved to yield the map distances. MCQTL is another program that will map QTL incorporating data from multiple populations (Jourjon et al., 2005).

Walling et al. (2000) applied a pooled analysis approach to fatness data from seven different F_2 swine populations. Using Haley-Knott regressions, varying scenarios were tested. The first model examined whether there existed a QTL with the same effect in each population. The second model tested whether there were different effects in each population by fitting unique additive and dominance effects. A third model assumed different effects according to breed. Most of the QTL detected did not show the same effect across all

populations, and most of the QTL exhibited similar effects within breed classes. The joint analyses provided stronger evidence of QTL in terms of LOD scores, as well as narrower confidence intervals (Walling et al., 2000). Guo et al. (2006) extended this method by including marker cofactors in a combined analysis of two $F_{2:3}$ soybean populations segregating for resistance to soybean cyst nematode. Although their least square composite interval mapping approach produced higher LOD scores when QTL were shared among populations, surprisingly, there was no significant change in the size of the position confidence intervals (Guo et al., 2006).

Some pooled analyses consider multiple interrelated populations that share common parents; this idea was first suggested by Gilbert (1985). An advantage of these designs is derived from the ability to consider fewer allele effects compared to unrelated populations. When parents are shared, the number of allele effect parameters is reduced relative to the total number of parents in common (Rebai and Goffinet, 1993; Blanc et al., 2006). Due to the decrease in parameters to be estimated, the power to detect QTL is increased relative to unrelated population analysis. This increase in power is evidenced by the results of Blanc et al. (2006), who evaluated six sets of $F_{2:3}$ families derived from a diallel between four maize inbreds for silking date, grain moisture, and grain yield. When accounting for shared parent alleles, forty-six QTL were detected, an increase in fifteen QTL over a model with nested allelic effects. Position estimates were also more precise over nested allele and single population analyses (Blanc et al., 2006).

Simulation studies have determined the optimal designs and numbers of parents and families needed in multiple interconnected populations. Muranty (1996) showed that if the

total experiment size were fixed, the number of parents used in the design affected the power for detection, but the design employed (either diallel, factorial, cyclic, or single-pair mating) did not matter. Wu and Jannink (2004) discovered that designs with many parents had the most accurate QTL estimates, but designs with fewer parents had better detection power. Verhoeven et al. (2006) found that if the number of F_2 individuals in a family was held constant, designs with more families, e.g. single and double round robin partial diallels, had higher QTL detection power; thus, a specific QTL was more likely to be identified when more families were employed. Fewer and larger families were also preferred over more and smaller families (Verhoeven et al., 2006). In choosing designs, diallels in which all combinations of parents are crossed directly are most efficient for contrasting allele effects; however, if many fixed allele effects need to be fitted, a design in which a reference parent is mated to the other parents may be used instead (Wu and Jannink, 2004).

IV. From QTL to Gene

Although numerous publications report QTL every year, very few genes underlying quantitative traits have been identified. Salvi and Tuberosa (2005) compiled a list of only a dozen cloned genes controlling quantitative traits. Fortunately, recent advances in sequencing and bioinformatics have increased the cloning success rate, and are expected to continue to do so.

Positional cloning is the most common strategy used to clone QTL. Positional cloning commences with mapping the QTL to as a small an interval as possible (Salvi and Tuberosa, 2005; Bortiri et al., 2006). Generally, a higher resolution mapping population is constructed

through crossing near isogenic lines (NILs). NILs are two lines that differ for only a small chromosomal segment, and are produced through backcrossing (Patterson et al., 1990; Tanksley, 1993). The next steps of positional cloning are anchoring the nearest markers to the physical map of the species, and then searching for candidate genes within the identified physical interval (Salvi and Tuberosa, 2005; Bortiri et al., 2006). Candidate genes are selected based not only on physical position, but also previous physiological or molecular knowledge of the trait (Pflieger et al., 2001). Other methods of cloning exist; for example, transposon tagging was used to identify *tb1*, a regulator of plant architecture responsible for apical dominance in maize (Doebley et al., 1997). Transposons are DNA sequences that are excised from the genome and then reinserted in novel locations along the chromosome. Upon reinsertion into a coding region, gene function is disrupted. Transposon tagging can be either directed or undirected. Directed tagging requires crossing a line containing a recessive allele of the gene to a line that harbors an active transposon. If the transposon inserts into the gene of interest, only the recessive phenotype will be visible among the progeny. Because most of the transposons used in tagging reinsert into closely linked genes, it is imperative that the transposon is linked to the dominant allele of the gene of interest. Undirected tagging involves assembling a collection of lines in which transposons have disrupted the functioning of many different genes. Lines are then screened for phenotypic differences caused by transposon insertions. Large populations must be screened to find the few in which a transposon insertion has disrupted the phenotype of interest (May and Martienssen, 2003).

Functional validation is important to confirm the identity of the cloned QTL. Association mapping may be performed to statistically associate polymorphisms within

candidate genes to phenotypes within a set of diverse germplasm. Testing of candidate genes may be accomplished through transformation, complementation testing, or mutagenesis. The functional allele can be transformed into the plant or introduced sexually through crossing to a plant with a nonfunctional allele. RNA interference could be used to abrogate the function of an allele (Pflieger et al., 2001; Salvi and Tuberosa, 2005). Mutagenesis is yet another option. A transposon insertion library, a collection of lines in which transposons have disrupted the functioning of numerous genes, can be screened via polymerase chain reactions (PCR). Primers incorporating both transposon and candidate gene sequences are used to find insertions within genes of interest (May and Martienssen, 2003). Targeting Induced Local Lesions IN Genomes, or TILLING, is another approach in which point mutations have been induced to disrupt gene functioning in a set of lines (McCallum et al., 2000). Primers designed to amplify candidate gene sequences are used in PCR testing to determine which plants in a collection carry mutations in those candidate genes. PCR products are digested with a restriction endonuclease such as Cel I, which digests DNA at mismatches. A mismatch between wild-type and mutagenized DNA indicates that a point mutation has occurred in the gene of interest, and warrants further investigation (Stemple, 2004). If a mutation does alter the plant phenotype, this offers significant proof that the gene is causally related to the phenotype under consideration.

Despite the focus on genes as the subjects of cloning, some causal loci contributing to flowering time and inflorescence architecture in maize are not genes. *Vgt1*, vegetative to generative transition 1, is actually a noncoding enhancer located 70 kb upstream of a transcription factor gene (Salvi et al., 2007). Fine mapping of QTL in the region of *tb1*

revealed a cis non-coding enhancer locus between 58 and 69 kb upstream of the gene (Clark et al., 2006).

V. Genetic Basis of Disease Resistance- Quantitative vs. Qualitative

Disease resistance in plants can be divided into two broad categories: qualitative and quantitative. Whereas qualitative resistance is complete and typically governed by single genes, quantitative resistance is partial, and attributed to the synergistic action of several loci (van der Plank, 1968). Qualitative resistance is conditioned by dominant, race-specific resistance (R) genes. Every R-gene product recognizes a unique avirulence protein in the pathogen. A resistance response occurs only when the R-gene product and avirulence (Avr) protein meet; if either partner is missing or functionally compromised, the response is absent. The one-for-one relationship between R-gene and avirulence protein is termed "gene-for-gene", and was first recognized by Flor (1942). In the absence of the corresponding R-gene, an Avr protein may contribute to the virulence of the pathogen. In this case, by losing functionality of the Avr protein, the pathogen loses a degree of aggressiveness, but escapes R-gene mediated resistance (Flor, 1971; White et al., 2000; Jones and Dangl, 2006; Bent and Mackey, 2007).

The resistance reaction triggered by activated R-genes is usually the hypersensitive response (HR) (Heath, 2000; Jones and Dangl, 2006; Bent and Mackey, 2007). HR is usually defined as a rapid cell death of the host around the point of pathogen ingress. It is a form of programmed cell death, and is often associated with an oxidative burst, loss of membrane integrity, mitochondrial cyctochrome-c release, activation of cysteine proteases, and DNA

laddering (Heath, 2000; Keon et al., 2007). Some qualitative resistance genes, however, do confer alternate mechanisms of defense. For example, the *Hm1* gene in maize detoxifies the toxin produced by *Cochliobolus carbonum* (Johal and Briggs, 1992).

Recent evidence suggests some R-gene products do not recognize their specific Avr targets directly (Mackey et al., 2002; Axtell et al., 2003; Axtell and Staskawicz, 2003; Mackey et al., 2003; Kim et al., 2005). In contrast to the traditional view of R-gene and avirulence product interacting in the lock and key sense, the guard hypothesis was formulated to describe an indirect interaction between the Avr and R-gene products. This model postulates an avirulence factor-host target interaction that activates the R-gene product through modification of the host target activity or structure (Jones and Dangl, 2006). In susceptible genotypes, the effectors' depression of target function promotes infection. For example, the *Arabidopsis* RIN-4 protein is a target of several avirulence factors from *Pseudomonas syringae*. AvrRpm1 and AvrB induce phosphorylation of RIN-4, which activates the gene product of the R-gene, *Rpm1* (Mackey et al., 2002). AvrRpt2, a third effector, is a cysteine protease that cleaves RIN-4 at two sites (Axtell et al., 2003; Kim et al. 2005). The cleavage products activate the product of the *RPS2* R-gene (Axtell and Staskawicz, 2003; Mackey et al., 2003). Another example of an interaction not involving R-gene product and corresponding Avr protein is the transcriptional activation of the R-gene *BS3* in pepper upon binding of the *Xanthomonas campestris pv. vesicatoria* protein AvrBs3 to its promoter (Romer et al., 2007).

Although six classes of R-genes exist, most cloned to date fall into the nucleotide-binding leucine rich repeat (NB-LRR) class; as the name suggests, these genes are

characterized by nucleotide binding sites at their amino termini, with several leucine rich repeats at their carboxy termini (Ellis et al., 2000; Tan et al., 2007) Interestingly, the subclass of NB-LRR genes that also contain Toll-Interleukin 1 receptors at their amino termini have not been found in monocots, including maize (Ellis et al., 2000).

Despite the efficacy of R-genes in combating plant diseases, their usefulness in breeding is often limited by their transient effectiveness. If an R-gene is widely deployed, a selective advantage is bestowed upon those pathogens carrying a mutation in the corresponding Avr gene that prevents its detection by the R-gene. In this case, the mutants lacking the R-gene-specific Avr protein come to dominate the population, and the R-gene is "defeated" (Jones and Dangl, 2006; Bent and Mackey, 2007). In agricultural systems, crops that are dependent on R-genes for protection from a pathogen often go through boom and bust cycles in which R-genes are deployed, defeated, and replaced with new R-genes in a repeating cycle. Because of the existence of multiple loci conditioning quantitative resistance, the smaller effects of these individual loci, and their non-race-specific nature, quantitative resistance has been hypothesized to be more durable. Not only will more mutations have to occur in order to overcome resistance, but also, each mutation will have less of a selective advantage. It is also unlikely that single mutations would allow pathogens to overcome quantitative resistance, as some of the hypothetical mechanisms responsible for this resistance would not rely on specific recognition of pathogen effectors. For these reasons, quantitative resistance should be favored over R-gene mediated resistance in most breeding programs (McDonald and Linde, 2002).

Another factor that limits the use of R-genes in breeding is the existence of necrotrophs, pathogens that colonize dead plant tissue. R-gene mediated resistance is mainly effective against biotrophic fungi, i.e., species that must parasitize living plant tissue in order to survive. Because R-genes cause invaded cells to 'commit suicide', the spread of biotrophs is curtailed. Necrotrophic fungi live on dead tissue and thus, rather than being thwarted by programmed cell death, they might actually thrive (Glazebrook, 2005). Recent evidence suggests that some necrotrophs actually produce toxins that trigger the HR response to create a more hospitable environment for infection (Lorang et al., 2007; Nagy et al., 2007).

Although use of quantitative resistance in breeding programs has been touted as a solution to many disease problems, the mechanisms and causal genes behind quantitative disease resistance have not been as well characterized as those behind R-gene based resistance. Several different theories exist as to how gene products might condition these responses based on theories of pathogenesis and evidence from QTL mapping, positional cloning, and experimentation with mutants (Poland et al., 2009).

Disease is the product of an intricate series of host-pathogen interactions, but these processes can be generalized into a few steps. Each of these steps could, in theory, be manipulated to contribute to a quantitative resistance phenotype. In order to cause disease, fungi and bacteria must first gain access to the intercellular space by either utilizing existing openings in plant tissue, such as stomata, or by actively penetrating the cuticle (Mendgen et al., 1996). Plants with thicker cuticles or fewer stomata might be able to limit penetration of pathogens (Melotto et al., 2006). For example, rose cultivars highly resistant to blackspot have a different cuticular composition than susceptible cultivars (Goodwin et al., 2007).

Other differences in the epidermal layers of plants might similarly contribute to quantitative resistance. Trichome differences in potatoes have been associated with differential resistance levels to *Phytophthora infestans* (Lai et al., 2000). Morphological differences in plants may also affect the access of bacterial cells or spores to plant surfaces. For example, canopy structure, in terms of plant height and stem density, can affect development of *Ascochyta* blight severity on spring pea (Le May et al., 2005). Another example of plant architecture contributing to resistance is the reduced susceptibility of wheat cultivars with erect leaves to septoria leaf blotch compared to those with droopy leaves (Joshi and Chand, 2002).

Once the pathogen has penetrated the plant, it must surmount the general surveillance system. Plants have a basal level of immunity to pathogens because they possess receptors that are able to recognize broadly conserved pathogen motifs such as chitin, flagellin, and elongation factor-Tu (Nurnberger et al., 2004; Chisholm et al., 2006). These motifs are also referred to as PAMPs, or pathogen associated molecular patterns. Once the plant has identified a pathogen attack, it signals for a defense response to be mounted (Nurnberger et al., 2004; Chisholm et al., 2006; Jones and Dangl, 2006; Bent and Mackey, 2007). Plants lacking these PAMP receptors appear more susceptible to pathogens than wild-type plants. *Arabidopsis* plants mutant for FLS2, a flagellin receptor, are more susceptible to colonization by *Pseudomonas syringae* (Zipfel et al., 2004). Similarly, when LysM RLK1, a protein functioning in chitin recognition and signal transduction in *Arabidopsis*, is knocked out, plants become more susceptible to infection by *Alternaria brassicicola*, a necrotrophic fungus (Wan et al., 2008).

In addition to the basal defense PAMP receptors, plants also can recognize elicitors that are specific to certain pathogens via R-genes. Although R-genes typically have been associated only with qualitative resistance, quantitative resistance also may be conditioned by R-genes. Some R-genes are known to provide incomplete resistance to pathogens e.g., a mutant form of the *Xa21* R-gene for resistance to rice blast disease (Andaya and Ronald, 2003). A cloned major effect QTL for resistance to anthracnose stalk rot of maize, Rcg1, proved to be an NB-LRR protein (Broglie et al., 2006). Several authors have shown that defeated R-genes still function in providing low level resistance to pathogens. This has been shown to be the case for the rice *Xa4* gene conferring resistance to infection by *Xanthomonas oryzae pv. oryza* (Li et al., 1999). Other examples exist in the wheat-powdery mildew and wheat-stem rust pathosystems (Nass et al., 1981; Brodny et al., 1986).

Once a pathogen has been detected, a complex network of signaling pathways is activated. Detection of avirulence factors, and activation of the corresponding R-gene, is usually followed by a MAP kinase cascade, leading to the induction of a transcriptional response via WRKY or TGA transcription factors (Eulgem, 2005). The hormonal balance of the plant is adjusted based on the type of pathogen detected; the salicylic acid pathway is typically induced in response to biotrophs, whereas the jasmonate and ethylene pathways generally are induced in response to necrotrophs (Kunkel and Brooks, 2002; Garcia-Brugger et al., 2006). These pathways do not exist in isolation, and substantial crosstalk occurs among them (Kunkel and Brooks, 2002). A multitude of proteins play roles in the defense signaling process, and in theory, a level of quantitative resistance could be conferred by any slight change in the sequence or expression of any of the protein players (Poland et al., 2009). A

mutated MAP kinase in *Arabidopsis* was responsible for enhanced disease susceptibility to *Pseudomonas syringae pv. tomato* and *Peronospora parasitica* (Petersen et al., 2000). Plants mutant for NPR1, a downstream protein in the salicylic acid pathway that interacts with TGA transcription factors, showed increased susceptibility to *Pseudomonas syringae, Peronospora parasitica,* and *Erysiphe* species (Glazebrook et al., 1996; Reuber et al., 1998). Plants deficient in salicylic acid or jasmonate due to downregulated production or upregulated degradation are compromised in defense (Glazebrook et al., 1996; Reuber et al., 1998; Nawrath and Metraux, 1999; Xu et al., 2001; Jensen et al., 2002). Conversely, *cev1* mutants accumulate higher than normal levels of jasmonate and show increased resistance to *Erysiphe* species (Ellis and Turner, 2001).

The transcriptional response engendered by the defense signaling pathways sometimes includes production of defensins and phytoalexins, antibiotic compounds that limit pathogenic growth. Quantitative resistance phenotypes could be caused by differential levels of these compounds. One study showed *Arabidopsis* plants that produced higher amounts of camalexin, a phytoalexin, were more resistant to colonization by *Botrytis cinerea* (Kliebenstein et al., 2006). Solanaceous plants produce a defensin, NAD1, that retards growth of *Botrytis cinerea* and *Fusarium oxysporum* through permeabilization of the fungal cell membranes (Lay et al., 2003; van der Weerden et al., 2008). Presumably, plants with defective *NAD1* genes would not be as resistant to these fungi. On the other hand, plants can also increase production of compounds that reduce or inactivate toxic compounds produced by pathogens. A major effect QTL for rice blast disease was found to encode a cluster of twelve germin-like proteins, formerly referred to as oxalate oxidases. The function of these

germin-like proteins is not known, although their activity seems to be related to generation of reactive oxygen species. Some family members bear resemblance to the superoxide dismutases. When these germin-like proteins were targeted by RNA interference, rice plants became more susceptible to rice blast and sheath blast pathogens (Manosalva et al., 2009). Additionally, glutathione S-transferases (GSTs) colocalize with disease resistance QTL, and reduced glutathione was implicated as the cause of disease susceptibility in phytoalexin deficient mutants (Wisser et al., 2006; Schlaeppi et al., 2008). GSTs function in protecting cells by detoxifying organic hydroperoxides (Marrs, 1996).

It is likely that loci functioning in quantitative disease resistance will include not only the gene classes mentioned above, but also genes with novel characteristics and functions.

VI. The Biology and Genetics of Southern Leaf Blight Resistance

Southern leaf blight (SLB) is a fungal foliar disease of maize that occurs worldwide, but is more prevalent and destructive in warm temperate and tropical regions. *Cochliobolus heterostrophus,* the causal agent of SLB, is a necrotrophic member of the phylum Ascomycota. When infected by *C. heterostrophus,* maize develops tan rectangular lesions delimited by the leaf vasculature. Three races of *C. heterostrophus* have been characterized: race O, race T, and race C. Race O, the most common race, is responsible for the majority of current SLB outbreaks. Race T produces the T-toxin, which is toxic only to corn containing Texas male-sterile cytoplasm (cms-T). Seed companies relied widely on cms-T in producing hybrids, creating a paucity of cytoplasmic diversity that invoked

selection and prosperity of race T (Carson, 1999). The epiphytotic of 1970-71 highlighted not only the perils of SLB, but also the dangers of genetic uniformity (Commitee on Genetic Vulnerability of Major Crops, 1972). The third race, C, discovered in 1987, only afflicts cytoplasmic male-sterile C corn, and to date has been found only in China (Hooker, 1972).

The biology and genetics of the maize- *C. heterostrophus* interaction have several implications for control of SLB. While the 50% yield loss that southern cms-T hybrids suffered during 1970-71 is the exception, even hybrids with moderate genetic resistance to SLB have been shown to suffer yield losses of 0.7-0.8% for every 1% increase in affected leaf area between 0 and 25% (Hooker, 1972; Byrnes and Pataky 1989). Losses can be attributed not only to the blight, but also to the higher incidence of associated *Fusarium*, *Diplodia*, *Giberella*, anthracnose, and charcoal stalk rots (Dodd, 1980). SLB overwinters as mycelia and conidia from the previous year's crop. Primary infection occurs when these conidia are dispersed by wind currents and water droplets. These primary lesions sporulate, spawning secondary infections. Crop rotation and tilling under of debris can provide a moderate level of control through truncation of the infection cycle; however, deployment of genetic resistance is the favored control method (Carson, 1999).

Genetic resistance to SLB, as well as pathogens in general, falls under the quantitative or qualitative classification. Qualitative resistance often refers to an "all-or nothing" disease response that is conditioned by the presence or absence of a single major gene. Although a single gene, *rhm* (**r**esistance to ***H***elminthosporium ***m***aydis), imparts a qualitative-like resistance to SLB Race O, it is inherited recessively. This recessive mode of inheritance implies lack of functional protein, and thus Avr recognition is not likely the mode

of action of this gene. Furthermore, juvenile *rhm* plants exposed to SLB exhibit a chlorotic flecking reaction, but post-anthesis, *rhm* is only partially effective at deterring disease symptoms. An additional two resistance genes have been identified in the inbred NC250 that are not allelic to *rhm*; this resistance is maintained in mature plants (Thompson and Bergquist, 1984).

In 1968, the *rhm* resistance phenotype was identified in Nigerian germplasm. Craig and Fajemisin at first hypothesized that the source of resistance was two linked recessive genes (Craig and Fajemisin, 1969). A later study by Smith and Hooker showed resistance in F_2 families segregating in a 3 resistant: 1 susceptible ratio, indicating only one recessive gene was responsible for the resistance (Smith and Hooker, 1973). Zaitlin et al. constructed a map based on RFLPs that placed the *rhm* gene on the tip of chromosome 6 (Zaitlin et al., 1993). The one v. two gene controversy reemerged when transposon tagging was used to map the location of *rhm* and an unusually high mutation frequency could be accounted for only by the presence of two recessive genes, *rhm1* and *rhm2* (Chang and Peterson, 1995). The mode of action of *rhm* has not been identified. Global transcript profiling revealed no consistent difference in expression of 8,000 to 13,000 genes between *rhm* mutants and wild-type infected plants 24 hours post-infection (Simmons et al., 2001).

Many inbred maize lines, especially those developed closer to the equator, exhibit moderate to high levels of quantitative resistance to SLB race O (Pratt and Gordon, 2006). This resistance appears as difference in lesion size, number, and time required for lesion development. The first study that described quantitative inheritance to SLB was conducted by Pate and Harvey (1954). Crosses between susceptible and resistant inbreds yielded

progeny that were always intermediate in resistance, suggesting partial dominance of this trait (Pate and Harvey, 1954). Lim and Hooker (1976) studied general and specific combining ability among four double cross parents in a diallel. General, but not specific, combining ability was highly significant, indicating that a parent's genetic contribution to resistance was consistent across all combinations, and independent of its other partner in a cross. Thus, the predominant mode of inheritance for SLB resistance in this set of crosses was additive (Lim and Hooker, 1976). Results from subsequent studies confirmed the importance of additive over dominance inheritance and general over specific combining ability effects (Thompson and Bergquist, 1984; Burnette and White, 1985). In an evaluation of tropical inbreds and their hybrid progeny, Holley and Goodman (1989) identified both additive and recessive forms of gene action; however, recovery of moderately resistant progeny from crosses involving inbreds with recessive sources of resistance indicated that epistatic interactions could contribute to resistance phenotypes.

As most of the resistance phenotypes associated with SLB are quantitative in nature, QTL mapping has been used to locate the causal genetic loci. The introduction of molecular markers to QTL mapping provided the first glimpse at the genetic architecture underlying SLB resistance. Bubeck (1991) mapped QTL conferring SLB resistance utilizing three populations of $F_{2:3}$ lines derived from the following crosses: ADENT x B73rhm, B73rhm x NC250A, and NC250A x B73. Lines were genotyped with about 80 RFLP's, too small a number to provide complete genome coverage. Bubeck (1991) identified seven QTL in each of the populations with limited consistency of QTL among populations.

The implementation of SSR markers in maize genotyping greatly increased the resolution of genetic maps. Carson et al. (2004) genotyped a mapping population of 158 $F_{6:7}$ recombinant inbred lines developed from a cross between B73 and Mo17 with a combination of 234 SSR markers, RFLPs and isozymes. Over years and ratings, QTL with highly significant effects were mapped to bins 1.09/.10, 2.04, and 3.03/.04 (Carson et al., 2004). Balint-Kurti and Carson (2006) characterized this same population for juvenile resistance to SLB scored in the greenhouse. QTL conditioning significant resistance to SLB were localized to bins 1.08/.09, 2.09, 3.04, 6.00, 7.02, and 8.03. The only QTL imparting both juvenile and adult plant resistance were those in bins 1.08/.09 and 3.04. Balint-Kurti and Carson (2006) also examined whether any QTL were isolate-specific, but detected no significant line-by-isolate interactions. Subsequent to these studies, QTL for SLB resistance were mapped in a similar population, the intermated B73 x Mo17 (IBM) population. The IBM population is composed of 302 $F_{7:8}$ recombinant inbred lines (RIL) that were allowed to intermate four times prior to the derivation of inbred lines. This intermating presents more opportunities for recombination; the resultant greater precision in mapping is reflected by the fourfold expansion of the IBM map over traditional RIL maps (Sharopova et al., 2002). The use of over 2000 markers also enhanced the resolution of estimated QTL positions over previous studies. Composite and multiple interval mapping methods identified four QTL with significant effects in all environments: two in bin 3.04, one in bin 1.10, and one straddling the intersection of bins 8.02 and 8.03. Other smaller effect QTL were detected only in a subset of the environments. QTL from the Carson et al. (2004) study were resolved to

smaller intervals due to the greater number of lines, recombination opportunities, and number of markers used (Balint-Kurti et al., 2007).

Additional RIL populations, derived from crosses between B104 and NC300, H99 and B73, as well as B73 and B52 have been studied. In the B104 x NC300 population, the 3.04 QTL was identified, as was one in bin 9.03-.04. Other QTL associated with subtle effects were localized to chromosomes 1, 2, 3, 6, and 9. Notably, this was the first population in which a substantial resistance effect was attributed to a QTL in the 9.03-.04 region (Balint-Kurti et al., 2006). QTL in bin 3.04, 6.01, and 8.05 were identified in the H99 population, and one QTL was identified in bin 2.07 in the B52 population (Balint-Kurti et al., 2008).

The analyses consistently associated two regions with resistance: bins 1.10 and 3.04. Both of these bins have been identified as regions conditioning resistance to multiple diseases. These associations could reflect clusters of genes that function in plant defense, or imply the existence of genes that impart resistance to multiple diseases (Wisser et al., 2006). To determine which of these possibilities is correct, finer-scale mapping will be required to distinguish pleiotropy from tight linkage. In order to elucidate the underlying molecular basis of quantitative resistance to SLB and diseases in general, the causal genes must be mapped and cloned.

VII. Objectives

Although several studies have mapped QTL in various segregating populations, the molecular genetic basis of the resistance response is still obscured by the imprecision inherent in linkage mapping. Traditional mapping is limited by small population sizes, low

density genotyping, and a narrow genetic base. Small population sizes contain fewer recombinants between parental genomes, and fail to reveal QTL with small effects due to inadequate statistical power. Linkage of QTL with molecular markers, the basis of all QTL mapping, necessitates small distances between adjacent markers; otherwise, recombination between the nearest marker locus and the QTL will confound results (Doerge, 2002). Typical RIL mapping populations are derived from a two inbred parent cross with at most two alleles at the QTL; thus, QTL identified cannot necessarily be applied to other populations for use in marker assisted selection or marker aided backcrossing (Holland, 2007). Because of these restrictions, QTL are often identified at low resolution, with loci encompassing hundreds to thousands of genes. As a result, the molecular bases of these QTL remain a mystery.

The objective of this research is to provide precise estimates of QTL positions to aid in the identification of the genes underlying quantitative resistance to Southern leaf blight. Two approaches have been taken to this end. One is use of the Nested Association Mapping population, a set of RILs derived from crosses between inbreds B73 and 25 other diverse founder inbred lines (Yu et al., 2008). This population is the largest-scale public mapping resource of any species and has been shown to provide high power to detect QTL of relatively small effects segregating in diverse germplasm (Buckler et al., 2009; McMullen et al., 2009; Yu et al., 2008). The quantitative resistance response to Southern leaf blight was phenotyped on nearly 5000 NAM lines, and QTL associated with this response were mapped. Second, a novel approach was developed to analyze jointly four independent populations derived from a B73 by Mo17 cross to enhance precision in the positional estimate for the

QTL identified at bin 3.04 in the IBM population. The position of this QTL was validated in two F2:3 mapping populations derived from crosses between near isogenic lines.

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-CHAPTER II-

Joint Analysis of Near Isogenic and Recombinant Inbred Line Populations Yields Precise Positional Estimate for Southern Leaf Blight Resistance QTL

by

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Abstract

Near isogenic lines (NILs) are typically constructed to fine-map quantitative trait loci (QTL) after QTL have been identified in initial mapping experiments. Available data for lines segregating at the QTL of interest and throughout the genome are usually ignored once NILs have been developed. Combining data from the original recombinant inbred line (RIL) studies with new data collected from NIL experiments increases the number of recombination events sampled and may lead to greater precision of position and effect estimates. Southern leaf blight (SLB), a fungal foliar disease of maize, is controlled partially by host quantitative resistance. Several QTL have been mapped for this disease in biparental segregating populations, including the IBM (intermated B73 x Mo17) population. The most significant QTL in the IBM population was previously identified in bin 3.04, within a less than 1-cM interval. Near isogenic lines were constructed and used to create two distinct populations of $F_{2:3}$ families, each of which was segregating only for this QTL, for purposes of validation and fine mapping this QTL in homogenized genetic backgrounds. Combining data across studies in a joint analysis yielded a narrower QTL confidence interval, in which three candidate leucine repeat kinase genes reside. Although other studies have jointly analyzed data from multiple populations, this is the first combined QTL analysis across discrete generations segregating for a common pair of alleles.

I. Introduction

The utility of quantitative trait locus (QTL) mapping to identify specific genes affecting complex traits is limited by a lack of precision of QTL position estimates and biased estimates of their effects (Dekkers and Hospital, 2002; Holland, 2007). Increasing the number of lines sampled, the number of markers genotyped, or number of replications grown will reduce these problems (Lynch and Walsh, 1998; Beavis, 1998). Precision of QTL position estimates can also be increased by sampling lines enriched for recombination events (Balint-Kurti et al., 2007; Boddhireddy et al., 2009). In addition, an overriding difficulty of QTL analysis is the simultaneous segregation of multiple QTL within a test population, resulting in reduced detection power, and inflated effect estimates of those QTL detected, a problem that becomes severe in small population samples (Beavis, 1998).

To study more precisely the effect and position of a specific QTL, a uniform genetic background, differing only for the target QTL, should be constructed to eliminate all other sources of genetic variation. Near isogenic lines (NILs), pairs of lines that are identical except for a single genomic segment, are ideal for this purpose (Tanksley et al., 1993; Szalma et al., 2007). NILs can be derived through repeated backcrossing to a recurrent parent. Molecular markers are used to select lines with the donor parent allele at the QTL and to maximize contribution of the recurrent parent background outside of the QTL region (Patterson et al., 1990; Tanksley et al., 1993; Szalma et al., 2007). "Mendelizing" QTL by isolating their differences from background genomic segregation in NILs has been used as a key step in identifying causal sequence variation at some QTL (Frary et al., 2000; Yano et al., 2000; Takahashi et al., 2001; McMullen, 2003; Salvi et al., 2007).

Another option used to derive NILs is identification of heterogeneous inbred families (HIFs) from recombinant inbred line (RIL) populations. Although the majority of the genetic background in a RIL is fixed, a small percentage of loci will be segregating. A RIL that is segregating at the QTL can be selfed and a set of HIFs, lines differing for alleles at that QTL with an otherwise homogeneous genetic background, can be developed (Tuinstra et al., 1997).

Estimation of a QTL effect in a homogenized genetic background also incorporates the effects of any epistatic interactions between the QTL alleles and the background, which may be of particular importance if that QTL is to be deployed in that genomic context as a cultivar (Tanksley and Nelson, 1996; Stuber et al., 1999). Although significant epistatic effects are often not detected in QTL studies, it is unclear whether this is due to epistasis having only a minor role in the inheritance of complex traits or to statistical limitations hindering their detection (Carlborg and Haley, 2004; Holland, 2007). The small sample sizes used in typical mapping studies do not provide sufficient statistical power to reliably detect and estimate epistatic interactions. As the number of interacting loci increases, the chance of all relevant combinations being present becomes too small to reliably detect differences between them (Tanksley, 1993; Carlborg and Haley, 2004). Studies employing NILs have identified more epistatic interactions because fewer allele combinations are considered (Doebley et al., 1995; Eshed and Zamir, 1996; Melchinger et al., 2007). A homogenized genetic background will eliminate confounding of epistatic with main effects of loci.

For the aforementioned reasons, NILs are typically constructed after QTL have been identified in initial mapping experiments. Available data for lines segregating at the QTL of

interest and throughout the genome are usually ignored once NILs have been developed. Here, we demonstrate a method to combine data from initial RIL studies with new data collected from NIL experiments to increase the number of recombination events sampled in the QTL region, leading to greater precision of position and effect estimates. Combined analyses, including approaches such as multiple population analyses, meta-analyses and pooling of original data, have resulted in more precise positional estimates for QTL (Walling et al., 2000; Chardon et al., 2004; Khatkar et al., 2004; Blanc et al., 2006; Guo et al., 2006; Coles et al., 2009). To present, however, we are aware of no previous combined analysis of distinct generations of lines segregating for a common pair of alleles.

Southern leaf blight (SLB), a foliar disease of maize caused by the necrotrophic fungus, *Cochliobolus heterostrophus*, is controlled partially by host quantitative resistance (Carson, 1999). Several QTL have been mapped for this disease in biparental segregating populations, including the IBM (intermated B73 x Mo17) population (Lee et al., 2002). The highest effect QTL identified in the IBM lies in bin 3.04 (Davis et al., 1999) between 164 and 166 IBM cM (IcM; Balint-Kurti et al., 2007) units on the chromosome 3 IBM map (Schaeffer et al., 2008). This QTL has also been detected in several other studies; however, its existence has not been validated and fine mapped in a homogenized genetic background (Bubeck, 1991; Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al., 2009). Near isogenic lines were constructed and crossed to obtain two distinct populations of $F_{2:3}$ families segregating for this QTL. The objectives of this research were: to validate the 3.04 QTL and estimate its effects in uniform genetic backgrounds, and

to analyze combined data from these $F_{2:3}$ families with two previously characterized B73 × Mo17 populations in attempt to more precisely position the QTL.

II. Materials and Methods

Derivation of Populations Studied

A set of 204 B73 × Mo17 $F_{6:7}$ RILs were derived by C. Stuber and colleagues as described in Carson et al. (2004) and are here referred to as the Stuber RILs. The IBM population is a set of 302 advanced intercross lines derived from the cross between parental inbreds B73 and Mo17. These lines are recombinant inbred lines, but four cycles of intermating were conducted prior to derivation of the RILs for increased chance of recombination, expanded map length, and therefore greater resolution in mapping (Lee et al., 2002; Sharopova et al., 2002). A highly significant QTL in bin 3.04 was detected in both Stuber RIL and IBM populations (Carson et al., 2004; Balint-Kurti et al., 2007). Based on this result, populations segregating only for the 3.04 region were created for validation and fine mapping studies.

A set of families segregating for the Mo17 resistance allele at bin 3.04 in an otherwise mostly homozygous B73 background was created. After the initial cross of B73 to Mo17, progeny underwent five cycles of marker assisted backcrossing. At each backcross generation, offspring heterozygous at the 3.04 region were selected via genotyping with the following simple sequence repeat (SSR) markers flanking the IBM 3.04 SLB resistance QTL confidence interval (Balint-Kurti et al., 2007): umc1886, bnlg1447, umc1030, and umc1495; www.maizegdb.org, verified 17 May 2009). Outside of the 3.04 region, these lines are expected to be 98.4% homozygous for B73 alleles. BC_5F_1 lines were selfed to create the BC_5F_2 generation. Six BC_5F_2 heterozygous lines, 863-8, 874-1, 878-5, 880-4, 895-1, and 895-

8, were selected and selfed to create six subpopulations of $BC_5F_{2:3}$ lines segregating for the 3.04 QTL. This set of 209 lines are referred to here as the B73 NIL $F_{2:3}$'s.

A separate mapping population was also developed by intercrossing a HIF pair polymorphic at the QTL. One of the original Stuber RILs, segregating at the 3.04 QTL, was identified by genotyping at markers asg48 and phi036 (www.maizegdb.org, verified 17 May 2009). This line was selfed and progeny were genotyped at four SSR markers flanking the IBM 3.04 SLB resistance QTL confidence interval (Balint-Kurti et al., 2007; umc1886, bnlg1447, umc1030, bnlg1452; www.maizegdb.org, verified 17 May 2009) to assess fixation for either the B73 or Mo17 allele. Two sublines, 844-1 and 844-6, were identified as homozygous for the Mo17 and B73 alleles, respectively, in the target region. Lines 844-1 and 844-6 were crossed, and the F_1 was selfed to the $F_{2:3}$ generation. This set of 144 $F_{2:3}$ families are referred to here as the Stuber NIL $F_{2:3}$'s.

Phenotyping for Southern Leaf Blight Resistance

Details of phenotyping the Stuber RIL and IBM populations are presented in Carson et al. (2004) and Balint-Kurti et al. (2007). Two experiments encompassing the set of 209 B73 and 144 Stuber NIL $F_{2:3}$'s were grown in Clayton, NC during the summer of 2008. Lines were randomized into incomplete blocks of ten plants in an alpha lattice design with three complete replications using the software Alphagen (Scottish Agricultural Statistics Service, Edinburgh, UK). In the Stuber NIL $F_{2:3}$'s experiment, B73, Mo17, P39, and parental sublines 844-1 and 844-6 were included once in each replication as checks. In the B73 NIL $F_{2:3}$'s

experiment, B73, Mo17, and P39 were included three times in each replication as checks. Ten seeds were planted per 2.44-m row, with 0.96 m between rows.

All plants were inoculated with the 2-16Bm isolate of *C. heterostrophus* at the six to eight leaf stage (Carson, 1998). Inoculum was prepared according to Carson et al. 2004. Approximately twenty grains of infected sorghum were placed in the whorl of each plant. Irrigation was immediately applied after inoculation to foster fungal growth. *C. heterostrophus* is endemic to North Carolina, and as such, inoculum most likely constituted a mixed isolate population. Plants were rated for symptoms of SLB once per week for four weeks starting approximately one month after inoculation. A 1-9 scale was used, with 1 denoting a symptomless plant and 9 denoting a dead plant (Balint-Kurti et al., 2006). Values were recorded in half units. Days to anthesis (DTA) were measured on each plot as the number of days from planting to 50% pollen shed.

Deriving Best Linear Unbiased Predictors (BLUPs) for Marker-Association Tests

In the original analysis of the Stuber RIL population, Carson et al. (2004) calculated AUDPC (area under the disease progress curve) from raw percent disease severity data taken three times during 1995 and two times during 1996; these AUDPC values were used for QTL mapping. Raw data were used in calculating AUDPC values due to small replication effects and highly significant entry effects ($p<.0001$) (Carson et al., 2004). IBM lines were rated three times during 2005 and four times during 2006 on the 1-9 scale. These ratings were used to calculate a weighted mean disease (WMD) score. Weighted mean disease is comparable to

a weighted AUDPC value (Wilcoxson et al., 1974). Least square means of these WMD scores were estimated and used for QTL mapping (Balint-Kurti et al., 2008).

Data for the two segregating near-isogenic populations were analyzed with a multivariate mixed model that treated the four disease scores taken on different dates on each plot as dependent variables using ASREML (Gilmour et al., 2002). Random effects in the model were included for replications, blocks within replications, and entries. Separate error variances and covariances were fit for each disease score. Diagonal variance-covariance matrices were modeled for replication and blocking effects, allowing each disease score to have separate variances due to effects for replications and blocking, but constraining covariances to zero to obtain model convergence. In addition, an unstructured variance-covariance matrix was modeled for entry effects, permitting each disease score to have a unique genetic variance and each pair of rating dates to have a unique genetic covariance.

SLB symptoms increase most rapidly during and after flowering; consequently, earlier-flowering plants generally exhibit disease sooner than later-flowering plants, and are scored higher. DTA was included as a cofactor in the analysis to minimize confounding between maturity effects and disease resistance. Because DTA and SLB score do not follow a perfectly linear relationship, a multiple regression analysis was performed using Proc Mixed in SAS software version 9.1.3 (SAS Institute, Inc., 2004). SLB scores from the first through fourth weeks of evaluation were modeled as linear, quadratic, cubic, and quartic polynomials, and Akaike Information Criterion (AIC) values were compared (Table 1). The quadratic plus linear polynomial model best accounted for the relationship between disease score and DTA, without exhausting degrees of freedom for increasingly minor contributions

from the higher order polynomials. DTA was included in the model as linear plus quadratic fixed covariates for all scoring dates. Predictions were made for each entry at each of the four scoring dates. A BLUP-weighted mean disease (WMD) index was calculated for each entry through use of the following formula:

$$WMD = \frac{\left[\frac{(BLUP1 + BLUP2)}{2} * d_{1,2} + \frac{(BLUP2 + BLUP3)}{2} * d_{2,3} + \frac{(BLUP3 + BLUP4)}{2} * d_{3,4}\right]}{d_{1,2} + d_{2,3} + d_{3,4}}$$

in which BLUP*n* refers to the BLUP for the SLB score of the entry in the *n*th week of evaluation, and $d_{n,n+1}$ is the number of days that elapsed between scores in consecutive weeks.

In order to minimize differences in phenotype values across different populations due to different scoring methods and different environments, BLUP-WMD values for the two near isogenic populations were standardized by subtracting the population mean from the BLUP-WMD value and dividing by the population standard deviation (Walling et al., 2000). The resulting value is referred to as STWMD.

Heritability of family means for BLUP-WMD was estimated for an index in which BLUPs were weighted according to their relative contributions in the BLUP-WMD calculation. These index weights were 1/6, 1/3, 1/3, and 1/6 for BLUP1, BLUP2, BLUP3, and BLUP4, respectively. The following formula was used to calculate heritability:

$$h^2 = \mathbf{b'Gb / b'Pb}$$

where **b** is the vector of index coefficients, and **G** and **P** are the genetic and phenotypic variance-covariance matrices (Lin and Allaire, 1977). The phenotypic variance-covariance matrix was derived by summing the genetic variance covariance matrix and the residual variance-covariance matrix and dividing by the harmonic mean of the number of replications in which entries were scored (Holland et al., 2003). Family mean heritabilities were also calculated for individual scores by dividing the genotypic by phenotypic variance for that scoring date. Student's t-tests were used to assess significance of BLUP-WMD differences between parent and inbred checks.

For the joint analysis, data from both NIL populations plus the Stuber RIL and IBM populations were combined. To estimate the effects of genome positions specifically within the 3.04 QTL, data from the RIL and IBM populations were adjusted for the effects of QTL outside the 3.04 QTL interval. SLB AUDPC or WMD values for each RIL from Balint-Kurti et al. (2007) and Carson et al. (2004) were used, but adjusted according to their genotypes at the QTL detected in these populations. Adjusted RIL phenotype values from each population were derived by fitting a multiple interval mapping model (MIM) in Windows QTL Cartographer version 2.5 (Wang et al., 2007) with the QTL that had been previously detected in these populations. For a population containing *n* QTL, a line's adjusted phenotypic value, *p*, was estimated with the following equation:

$$p = \mu + \sum_{i=1}^{n} Q_i \alpha_i + \varepsilon$$

in which μ is the population mean, Q_i is a QTL genotype indicator variable that takes on the value of 0 if the genotype at the *i*th QTL is homozygous for the B73-derived allele and 1 if

the genotype at the *i*th QTL is homozygous for the Mo17-derived allele, α_i is the effect of the homozygous Mo17 genotype in reference to the B73 genotype at the *i*th QTL, and ε is a residual term including error and all non-genetic variation. The adjusted genotype values should reflect as little of the segregating variation as possible other than the 3.04 QTL, so all of the $Q_i\alpha_i$'s besides the one for the 3.04 QTL were included in the adjustment equation. Separate equations were modeled for the Stuber RIL and IBM populations, based on the QTL mapped in those populations individually.

Because the QTL genotypes are unknown, the genotype at the closest flanking marker was used as an approximation. In the case of missing data, the probability that a RIL was homozygous for the Mo17 allele at the QTL was estimated according to the genotypes at the nearest flanking markers for which data were available and the genetic distance between these markers (Lander and Botstein, 1989). This probability was multiplied by the Mo17 allele effects at the QTL and used in the summation portion of the phenotypic adjustment equation. Finally, these adjusted values were standardized by subtracting the population mean from the Stuber RIL AUDPC or IBM WMD value and dividing by the population standard deviation. The resulting value on this standardized scale is referred to as STWMD.

DNA Extraction and Genotyping

IBM line genotypes were obtained from MaizeGDB (www.maizegdb.org/ibm302scores.html, verified 17 May 2009). Duplicate sets of tissue of the B73 and Stuber NIL F_2 plants were collected from the winter nursery in Homestead, FL. One set of tissue was sent to DuPont Crop Genetics Research for preliminary genotyping in

the 3.04 region at the proprietary SNP loci PHM8477, PHM12576 and PHM4145. Additional background screening at markers flanking highly significant QTL conditioning resistance to SLB in the IBM population (bins 1.10, 3.04, 8.02/.03; Balint-Kurti et al. 2007) was also undertaken with additional proprietary SNP markers (PHM5586, PHM11071, PHM16795, PHM14098, PHM13823, PHM6836, PHM12861, PHM13725, PHM5158, and PHM6523). DNA was extracted according to a modified CTAB protocol (Doyle and Doyle, 1987) and SNP data were generated using a PCR and probe-based technology at Pioneer Hi-Bred International.

A duplicate set of leaf tissue of the B73 and Stuber NIL F_2 plants was extracted using the Doyle and Doyle (1987) CTAB protocol with the addition of 1.0% PVP w/v to the CTAB buffer (John, 1992). Tissue was extracted in sets of eight 1.1-ml strip tubes arranged in a 96 well format. A #4 stainless steel shot (washed with 1X TE buffer) was added to each well, and plates were suspended in liquid nitrogen. Grinding was performed using a Retsch Mixer Mill MM301 Retsch GmbH & Co. (Haan, Germany).

F_2 plants were genotyped with the following SSRs in the 3.04 QTL region: umc1772, umc1425, umc2000, umc2158, umc1495, and umc1392 (www.maizegdb.org, verified 17 May 2009). These six SSR markers had already been genotyped in the IBM populations, but not in the Stuber RIL population. Therefore, tissue of the Stuber RILs was obtained from seedlings germinated in greenhouse pots, extracted according to the protocol referenced above, and genotyped at these six SSR loci. Genomic DNA was diluted 1:5 with sterilized distilled water. For each 17.5-µl PCR reaction, 5 µl of diluted DNA was added to 1 µl each of .25 µM forward and reverse primer, 1.9 µl of 15 mM $MgCl_2$, .6 µl of dNTP's (with dATP,

dCTP, dGTP, and dTTP at .25 mM), 3.78 μl of 5 M betaine, 1.82 μl of 1:2 .04 % (v/v) cresol red: glycerol, 1.9 μl of Tris-KCl buffer (.5 M Tris [pH=8.4], 1 M KCl), and 1.5 U of Taq polymerase. PCR reactions were performed on the 384-well Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) according to the following protocol: 94 C for 4 minutes, 50 cycles of 94 C for 25 seconds, 55 C for 25 seconds, and 72 C for 35 seconds, followed by 72 C for 5 minutes. 15 μl of each PCR product was loaded on to a 4.0% SFR TBE agarose gel and electrophoresed at 130 V in TBE buffer for 2 hours on the Life Technologies Gibco BRL Sunrise 96 Gel Electrophoresis Apparatus (Life Technologies, Carlsbad, CA). Genotyping data indicating a double recombination in a small interval or manifesting inconsistencies with the preliminary SNP genotyping were discarded.

Single Marker Regression Analyses- Single Population and Combined Analyses

For use in regression analysis, genotype data for all of the populations were converted to indicator variables for additive and dominance effects according to the following scheme:

$$a = \begin{cases} 0, \text{ if homozygous for B73 allele} \\ 1, \text{ if heterozygous} \\ 2, \text{ if homozygous for the Mo17 allele} \end{cases}$$

$$d = \begin{cases} 0, \text{ if homozygous for B73 allele} \\ 2, \text{ if heterozygous} \\ 0, \text{ if homozygous for the Mo17 allele} \end{cases}$$

Missing marker data were imputed if right and left flanking marker data were available. Marker order was assumed to be equivalent to the IBM2008 Neighbors Map (Schaeffer et al., 2008). SNP marker positions were provided by DuPont Crop Genetics and validated by prior genetic and physical mapping. To conduct interval mapping of the SLB QTL (Lander and Botstein, 1989), virtual markers were placed at the midpoint between two markers. For these virtual markers, *a* and *d* values were averaged, as any recombination would produce the genotype at the left flanking marker half of the time and the genotype at the right flanking marker half of the time. Proc GLM of SAS software v. 9.1.3 (SAS Institute, Inc. 2004) was used to model STWMD as a function of the additive and dominance variables at both markers and virtual markers. The LOD score at each test position was calculated according to Haley et al. (1994):

$$LOD = \frac{n \ln(SSE_{reduced} / SSE_{full})}{2 \ln 10}$$

The location of the QTL was taken to be the marker or virtual marker at which the highest LOD score occurred. 2-LOD support intervals were identified as the smallest interval between two positions surrounding the LOD peak for which the LOD score dropped at least two points below the interval maximum (van Ooijen, 1992). Because LOD scores are affected by the number of observations, only lines with a full complement of marker data, either by genotyping or imputation, were included in LOD score calculations. The additive effect was estimated as the partial regression coefficient of *a*.

A combined data set consisting of the data from the B73 NIL $F_{2:3}$, Stuber NIL $F_{2:3}$, IBM, and Stuber RIL populations was constructed. This data set was analyzed according to the same protocol used for individual populations.

Searching for Candidate Genes

To find candidate genes, the B73 genome sequence (www.maizesequence.org, verified 17 May 2009) between the two markers flanking the confidence interval was examined. CLUSTALW (www.ebi.ac.uk/Tools/clustalw2/index.html, verified 17 May 2009) was used to align the amino acid sequences of the three candidate genes identified (Larkin et al., 2007). BLAST searches of these three candidate genes against proteins in the non-redundant NCBI database were also performed (www.ncbi.nlm.nih.gov, verified 17 May 2009).

III. Results

Variation among entries was significant for both B73 NIL $F_{2:3}$ and Stuber NIL $F_{2:3}$ populations (Tables 2 and 3). 844-1 and 844-6, the Stuber NIL $F_{2:3}$ parental lines, had BLUP-WMD values of 4.2 and 6.1 and were significantly different ($p < 0.0001$) from one another. B73 (BLUP-WMD=4.2) had the same level of resistance as 844-1. 844-1, 844-6, and B73 were all significantly different ($p < 0.0001$) from Mo17 (BLUP-WMD=2.0, Figure 1).

Heritability was estimated to be 89.4% in the B73 NIL $F_{2:3}$ experiment and 86.6% in the Stuber NIL $F_{2:3}$ experiment. For individual scoring dates, heritability estimates were 81.3%, 82.5%, 76.0%, and 82.9% for the first through fourth weeks, respectively, in the B73 NIL $F_{2:3}$ population. For the Stuber NIL $F_{2:3}$ population, these individual scoring date heritability estimates were 76.8%, 79.0%, 82.0%, 84.4% While these heritability estimates are high for a disease controlled only by quantitative resistance, they are consistent with previous SLB studies (Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al. 2009).

In the single population marker association analysis, the B73 NIL $F_{2:3}$ population exhibited a peak r^2 value ($r^2 = 0.58$) at PHM12576 (165 IcM); however, its peak LOD score (26.0) occurred at umc2000 (166.9 IcM) (Tables 4 and 5). This inconsistency is most likely due to the omission of lines with missing marker data from the LOD calculation. At this QTL, the estimated additive effect was $a = -0.96$ ($p < 0.0001$) and the dominance effect was $d = 1.3$ on the standardized scale ($p < 0.0001$; Table 4). On the original 1-9 scale, the additive effect was $a = -0.38$ and the dominance effect was $d = 0.52$. Thus, at the bin 3.04 SLB resistance QTL, the Mo17 homozygote is on average 0.76 points more resistant than the B73

homozygote. The significant dominance effect indicates that heterozygotes for the QTL are less resistant than the average of the B73 and Mo17 homozygotes, and 0.90 points less resistant than Mo17 homozygotes. In other words, the Mo17 SLB resistance allele in bin 3.04 is largely recessive to the B73-derived susceptibility allele.

The Stuber NIL $F_{2:3}$ population similarly exhibited a peak r^2 value ($r^2 = 0.47$) at PHM12576 (165 IcM, Table 4). Neither the B73, nor Stuber NIL $F_{2:3}$ populations, was segregating for the four markers tested in bins 1.10 or 8.02/.03; however, the Stuber NIL F_2 plants, as well as two of six B73 NIL BC_5F_2 lines, were segregating in the region of a second, smaller effect SLB resistance QTL in bin 3.04 that was previously reported in the IBM population (Figure 2). This second QTL was identified in the Stuber NIL $F_{2:3}$ population, where single marker analysis revealed a local maximum r^2 value at 260 IcM, 94 IcM from the main 3.04 QTL, with B73 contributing the more resistant allele. Although both $F_{2:3}$ populations were derived from near-isogenic parents, a smaller proportion of the phenotypic variation in the Stuber NIL $F_{2:3}$ population was explained by PHM12576 because of segregation at this additional bin 3.04 QTL. The peak LOD score for the major 3.04 QTL in the Stuber NIL $F_{2:3}$ population (14.2) occurred at umc1425, which also maps to 165 IcM (Table 5). This result is consistent with the position for the maximum r^2 value. For this population, the additive effect of the major QTL was estimated as -0.87, and the dominance effect was estimated as 0.76 (Table 4). Back transformed to the original 1-9 scale, the estimates were $a = -0.43$ and $d = 0.38$.

In terms of position and allelic effects, this result is consistent with a QTL detected in the IBM population between 217-258 IcM (Balint-Kurti et al., 2007). No significant

dominance effect was detected for this second, smaller effect QTL at 260 IcM (p=.8649). Although two of six B73 NIL F_2 subpopulations were segregating at this smaller effect QTL, its effect was not significant (Table 4, PHM6836), likely due to limited sample size of lines carrying the Mo17 allele (Table 4).

After accounting for the segregation of previously detected QTL other than the major effect QTL in bin 3.04, the IBM and Stuber RIL populations both had maximum r^2 values (0.22, 0.10) and LOD scores (15.3, 3.6) for SLB resistance at umc1425 (Tables 4 and 5). Their estimated additive effects were -0.49 and -0.32, respectively (Table 4). On the 1-9 scale, the IBM additive effect was -0.33 points. The Stuber RILs were rated on a percent disease severity scale; this value was used to calculate an AUDPC value. On this scale, the additive effect for the Stuber RIL population was -50.3. Dominance effects could not be estimated reliably in these largely homozygous RIL populations. The results of the IBM and Stuber RIL analyses are consistent with the original studies of these populations; however, the r^2 values are higher due to adjustment for effects of other segregating QTL in these populations.

Of the four individual population analyses, only the IBM population had a 2-LOD support interval that did not extend past the positions tested in this study. The 2-LOD support interval for the IBM spanned 163.5 to 166.9 IcM (Table 5). LOD scores increased when data were combined across all four populations in the joint analysis. The maximum LOD score (41.2) in the joint analysis occurred at PHM12576 (Table 5). This position also had the maximum r^2 value (0.25; Table 4). The 2-LOD support interval of the QTL in the combined

analysis encompassed 164.25 to 165.95 IcM. The standardized additive effect estimated across all four populations was -0.54; the dominance effect was 0.74 (Table 4).

Because the joint analysis reduced the 2-LOD support interval around the bin 3.04 SLB QTL to 0.425 cM, the genome sequence underlying this region is sufficiently delimited to permit an efficient search for candidate genes. The B73 physical map and genome sequence between the two markers flanking this interval, umc1772 (163.5 IcM) and umc2000 (166.9 IcM), were examined for predicted and verified genes (maizegdb.org). This interval is comprised of 1.8 Mb of sequence predicted to contain sixty genes. The only obvious candidate genes representing previously identified classes of recessive resistance genes within this interval were three different genes each encoding ATP binding sites, leucine rich repeats, and serine threonine kinase domains (GRMZM2G164024, GRMZM2G164030, and GRMZM2G010917, www.maizesequence.org, verified 17 May 2009). GRMZM2G164024 (1029 residues) and GRMZM2G164030 were 63% identical (723 residues), GRMZM2G164024 and GRMZM2G010917 (673 residues) were 65% identical, and GRMZM2G164030 and GRMZM2G010917 were 100% identical along the length of GRMZM2G010917. All three of these LRR-kinase genes possessed significant homology (E=0) to a putative *Xa21* gene in rice (dbj BAB03631.1) (Altschul et al., 1997). The putative *Xa21* gene was 56%, 61%, and 58% identical to GRMZM2G164024, GRMZM2G164030, and GRMZM2G010917, respectively.

IV. Discussion

Despite our original hope to Mendelize the response to SLB conferred by the target QTL in bin 3.04, the three genotypic classes of lines did not fall into discrete resistance phenotypes; there was significant overlap among genotypic classes (Figures 3 and 4). The Mo17 allele at the 3.04 QTL imparts a moderate effect on resistance when segregating against the B73 allele. At the time of flowering, the difference between plants homozygous for the Mo17 3.04 allele and plants homozygous for the B73 3.04 allele is almost imperceptible, but grows to approximately 1.5 points on the 1-9 scale by the time of senescence. Because WMD averages these scores, the additive effect is a modest estimate of the 3.04 Mo17 allele's contribution to SLB resistance over the plant's lifetime. This averaging of scores, in combination with recombination between marker and QTL locus, sampling error in segregating families, and environmental variation, may have somewhat obscured differences in resistance among the different genotypes. Nevertheless, we validated the 3.04 QTL in the uniform genetic backgrounds of two $F_{2:3}$ populations derived from crosses between NIL pairs. In these populations, the QTL explained 58% (B73 NIL $F_{2:3}$'s) and 47% (Stuber NIL $F_{2:3}$'s) of the otherwise limited genetic variation for SLB resistance.

The multivariate analysis using individual scores was thought preferable to performing ANOVA with WMD values. At scoring dates later in the season, there was a relatively high level of missing data due to the inability to distinguish leaf death caused by disease from that caused by senescence. For the B73 NIL $F_{2:3}$ experiment, 10.0% of the data were missing across both scores, and for the Stuber NIL $F_{2:3}$ experiment 2.4% of the data across both scores were missing. WMD cannot be calculated for plots with missing values;

thus, multivariate analysis makes most efficient use of the available information by permitting prediction of plot values even at time points where data were missing for that plot. Information sharing across time points was extensive in this experiment due to the high correlations among genotypic effects at different scoring dates (r = 0.90 to 1.0; Tables 6 and 7) and residual error effects at different dates (r = 0.22 to 0.74, Tables 8 and 9). In general, correlations among genetic effect estimates decreased as time increased between scoring dates in both populations (Tables 2, 3, 6, and 7). As a result, in both experiments, heritability was greater for BLUP-WMD values than for disease scores at any single time point.

Combining data across the four populations yielded a higher LOD score (41.2 vs. 26.0, 14.2, 15.3, and 3.6 for the B73 NIL $F_{2:3}$, Stuber NIL $F_{2:3}$, IBM, and Stuber RIL populations, respectively, Table 4) at the QTL peak than when populations were analyzed individually. The joint analysis provided higher statistical significance for the QTL peak because of the larger sample size obtained from pooling information from multiple independent families. In addition, the QTL support interval was also smaller for the combined data set than the single data sets. The QTL had previously been mapped to 164-166 IcM in the IBM population (Balint-Kurti et al., 2007), whereas the joint analysis identified a 2-LOD support interval encompassing 164.25 to 165.95 IcM. This represents a reduction in 0.3 IcM, or 15% over the previously published interval. Because mapping units in the IBM are based on multiple meiotic generations, the joint analysis interval of 1.7 IcM encompasses approximately 0.425 cM (Winkler et al., 2003). The larger number of recombination events sampled in the joint analysis provides the basis for greater precision in localizing QTL.

Reductions in QTL support interval sizes were observed in previous meta-analyses and pooled analyses of families derived from multiple parents (Walling et al., 2000; Chardon et al., 2004; Khatkar et al., 2004; Blanc et al., 2006; and Coles, 2009). However, this is the first study of which we are aware to combine data across discrete generations segregating for a common pair of alleles. Because only two alleles were segregating per locus, our method of analysis avoided several issues that complicate other combined analysis approaches. Allelic effects are known to be shared across generations in our design, leading to greater power of detection compared to analyses of nested allelic effects within populations. Common markers can be used across all generations, simplifying map construction, and reducing the potential for differential recombination among populations to confound results. In our design, common QTL reflect common underlying genes, whereas multiple population analyses may fuse distinct but tightly linked QTL segregating in different populations.

Our method not only is unimpeded by the pitfalls of other combined analysis approaches, but also is applicable to many sets of existent experimental data. RIL and NIL data from the same founder cross could be retrospectively joined in a combined analysis as proposed here to provide higher resolution mapping results. Our method addresses differences in segregating QTL, experimental environments, phenotyping protocols, and availability of marker information among populations. Adjustment of RIL phenotypic data based on initial QTL effect estimates and line genotypes allows for minimization of variation due to additional segregating QTL in RIL populations. Subtraction of the experiment mean and division by the standard deviation accounts for potential differences in experimental

environments and phenotyping protocols among populations. Differences in availability of genotypic data between populations are addressed through imputation of marker genotypes.

Analysis of the two new fine-mapping populations identified a positive dominance effect, indicating the resistance imparted by the Mo17 allele at the 3.04 QTL is recessive. The only known locus providing a qualitative-like resistance to SLB, *rhm*, is also inherited in a recessive manner. *rhm* provides a high level of resistance to juvenile maize plants, but its effect wanes as plants mature (Thompson and Bergquist, 1984). The causal locus and mode of action of *rhm* have not yet been discovered, but it is located on chromosome 6 (Zaitlin et al., 1993). Recessive gene action may imply existence of a dysfunctional gene product in resistant plants.

Iyer-Pascuzzi and McCouch (2007) hypothesized that recessive resistance may be caused by mutations at loci that otherwise normally condition susceptibility to the pathogen via interaction of their gene products with pathogen effector proteins in a gene-for-gene manner. Dominant genes for susceptibility exist in many plants infected by pathogens that produce host-selective toxins (HSTs; Wolpert et al., 2002; Friesen et al., 2008a). HSTs are pathogen effectors that, when introduced into the susceptible genotype of the host, induce tissue necrosis and disease (Wolpert et al., 2002; Friesen et al., 2008a). Most HSTs are produced by fungal necrotrophs (pathogens that parasitize dead plant tissue) of the order Pleosporales, of which *Cochliobolus heterostrophus* is a member (Eriksson, 2006; Friesen et al., 2008a). Race O of *C. heterostrophus* has been hypothesized to produce toxin(s) based on the results of culture filtrate experiments (Lim and Hooker, 1971). Susceptibility to HSTs can be conditioned by single genes, many of which exhibit dominance, e.g. *Vb* in oats

(*Cochliobolus victoriae*/victorin; Litzenberger, 1949); however, recessive forms of toxin susceptibility genes sometimes condition only quantitative resistance depending on genetic background, e.g. wheat genes *snn1* (Liu et al., 2004), *snn2* (Friesen et al., 2007), *snn3* (Friesen et al. 2008b) for resistance to *Stagonospora nodorum*, and *tsn1* (Friesen et al., 2006; Singh et al., 2008) and *tsc2* (Friesen and Faris, 2004) for resistance to *Pyrenophora tritici-repentis*. It is therefore possible that the dominant, B73-derived allele of the major 3.04 QTL is quantitatively conditioning susceptibility to a toxin produced by *C. heterostrophus*.

The most well-characterized gene-for-gene interactions occur between plant R-genes and pathogen avirulence effectors. R-genes are best known for conditioning qualitative resistance to pathogens in a dominant, race-specific manner. When the pathogen's avirulence protein activates an R-gene, a resistance reaction, typically the hypersensitive response (HR), a type of programmed cell death, occurs. If either partner is missing or functionally compromised, the response is absent (Flor, 1971; Jones and Dangl, 2006). In the absence of the R-gene, this avirulence protein often contributes to the virulence of the pathogen (White et al., 2000; Jones and Dangl, 2006). R-gene mediated resistance is most effective against biotrophs, pathogens that exclusively parasitize living plant tissue. Current models postulate that plants modulate their responses to pathogens via hormonal signaling, inducing salicylic acid pathways for biotrophs, and jasmonate and ethylene pathways for necrotrophs, colonizers of dead tissue (Kunkel and Brooks, 2002; Glazebrook, 2005). R-genes could act as dominant susceptibility genes, in the manner of the B73 allele at the bin 3.04 SLB resistance QTL mapped in this study, by allowing necrotrophic pathogens to capitalize on resistance responses designed to combat biotrophic pathogens. *Botrytis cinerea*, a necrotroph, initiates

an HR-like response on *Arabidopsis*; pathogenicity increases in response to the HR induced by inoculation with an avirulent biotrophic bacterium *Pseudomonas syringae* (Govrin and Levine, 2000). An *Arabidopsis* CC-NBS-LRR gene (a member of the most prevalent class of R-genes), *Lov1*, confers susceptibility to the necrotroph *Cochliobolus victoriae* and sensitivity to its victorin toxin (Lorang et al., 2007). Similarly, the *Pc* gene in sorghum is an NBS-LRR gene that conditions semi-dominant susceptibility to *Periconia circinata* peritoxin (Nagy et al., 2007; Nagy et al., 2008). The *Tsn1* gene in wheat, which conditions susceptibility to Ptr-Tox A from *Pyrenophora tritici-repentis* is also a member of the NBS-LRR family of genes (Faris et al., 2009).

While proteins with nucleotide binding and leucine rich repeat (LRR) domains are the most prevalent class of R-gene products, some contain additional domains such as coiled coil (CC) or toll interleukin-1 (TIR) domains. Additional classes of R-genes exist, such as cytoplasmic serine threonine kinases and proteins with transmembrane and extracellular LRR domains. One class contains proteins with all three of these domains (Ellis et al., 2000; Dangl and Jones, 2001). The three LRR-kinase candidate genes identified within the confidence interval of the major 3.04 QTL, GRMZM2G164024, GRMZM2G164030, and GRMZM2G010917, are homologous to a putative *Xa21* gene, an R-gene that imparts resistance to *Xanthomonas oryzae pv. oryzae*, a bacterial pathogen of rice (Song et al., 1995). The *Xa21* extracellular LRR domain provides race specificity and interacts with the pathogen effector; the intracellular kinase is responsible for signaling (Wang et al., 1998). One or more of these three maize LRR-kinase genes could impart dominant susceptibility to SLB by interacting with a fungal effector outside of the cell, activating a pro-cell death signaling

cascade via the kinase domain(s), and promoting growth of the necrotrophic fungus that causes SLB.

We plan to continue fine-mapping in this region to more precisely locate the causal gene or genes underlying this QTL. This will be achieved by increasing the number of near-isogenic $F_{2:3}$ families sampled, accurately phenotyping progeny resulting from genetic recombination in the QTL interval, and developing additional markers in the region between umc1772 and umc2000. Ultimately, cloning of this gene will aid our understanding of quantitative disease resistance to necrotrophs. Identification and characterization of genes conferring resistance to necrotrophic pathogens such as *C. heterostrophus* will enhance the utility of crop genomic sequences for resistance breeding.

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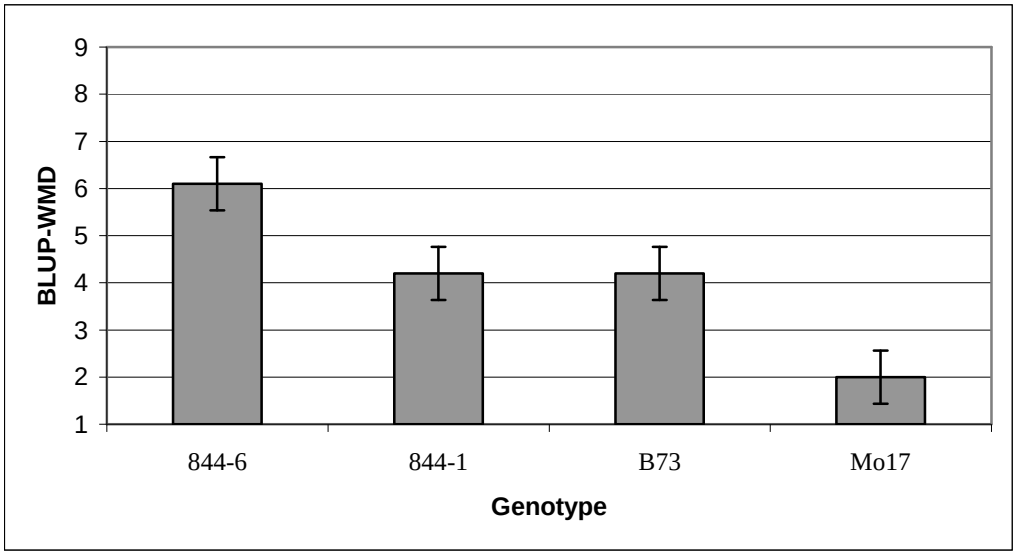

Figure 1. Best linear unbiased predictor weighted mean disease (BLUP-WMD) values for B73, Mo17, and Stuber NIL $F_{2:3}$ population parents, 844-1 and 844-6. Error bars reflect the standard error of the difference of means.

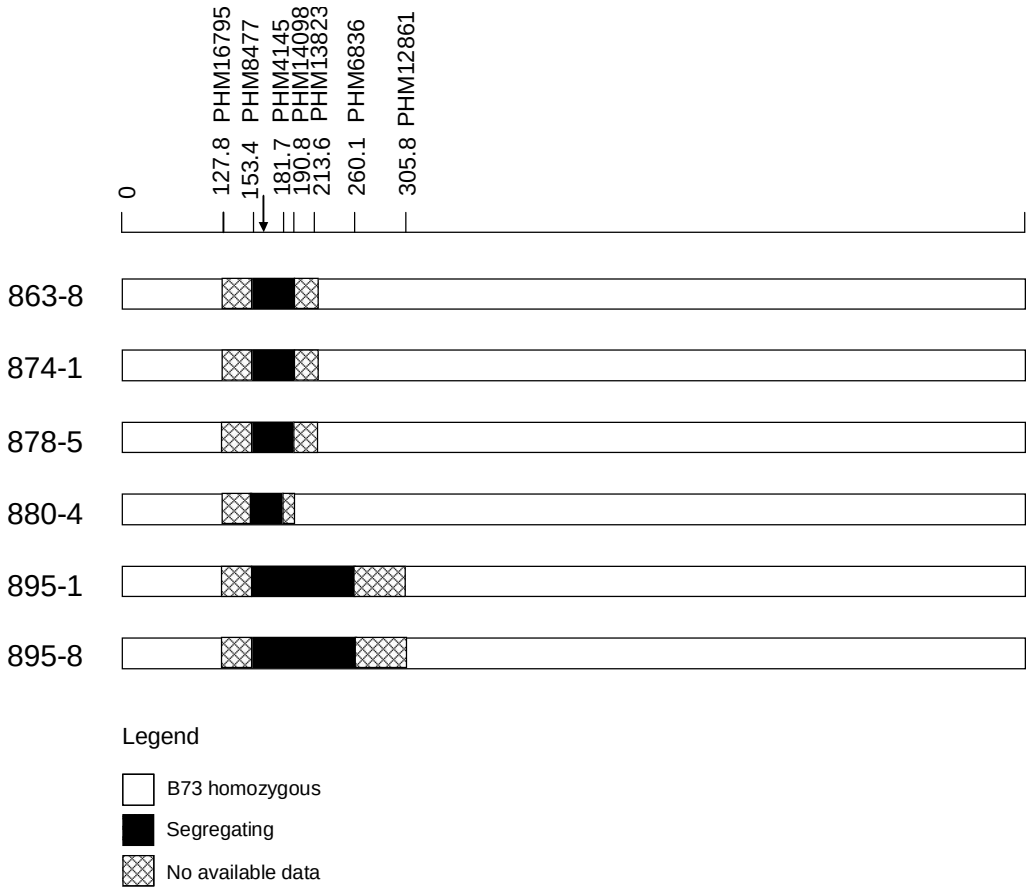

Figure 2. Chromosome 3 genotypes of the six B73 NIL $F_{2:3}$ subpopulations derived from the six BC_5F_2 lines listed. Map positions are in IBM2008 units (Schaeffer et al., 2008). Arrow indicates position of the bin 3.04 Southern leaf blight (SLB) resistance QTL.

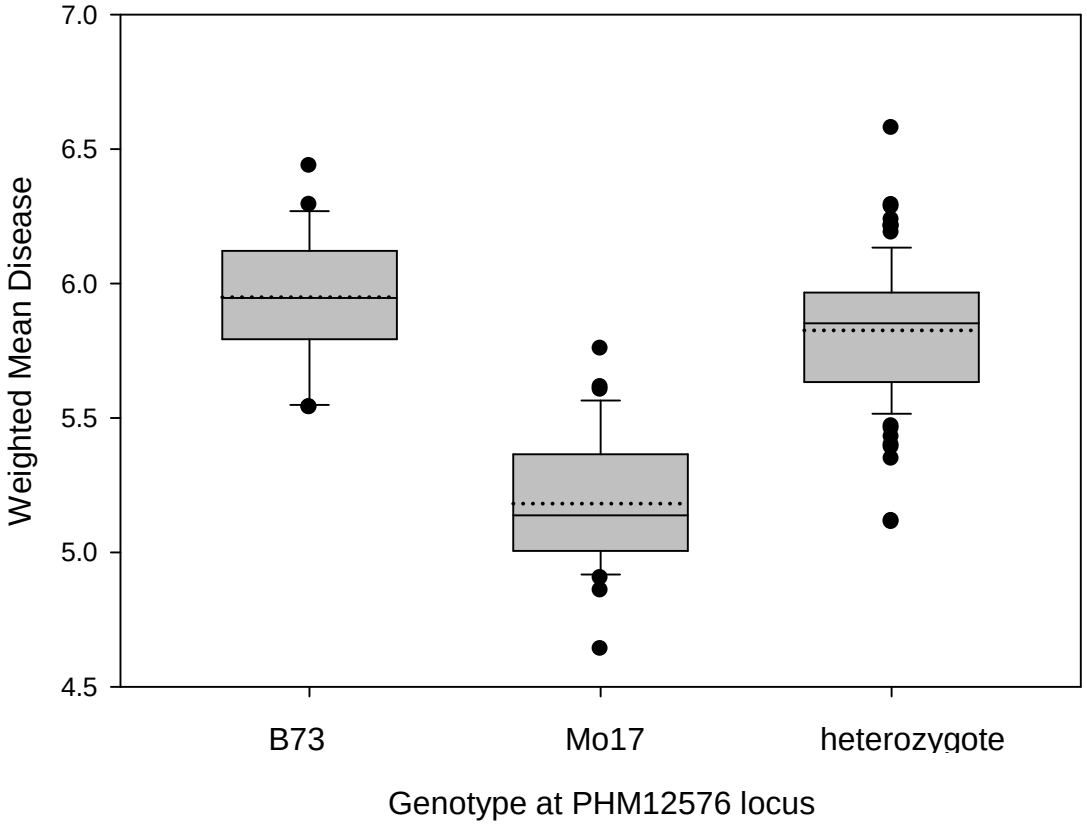

Figure 3. Boxplot of best linear unbiased predictor weighted mean disease (BLUP-WMD) scores for B73 NIL $F_{2:3}$ lines according to genotype at the PHM12576 locus. Boxes incorporate the 25^{th} to 75^{th} percentile of data, while dotted lines represent means and connected lines represent medians. Outliers are represented as circles.

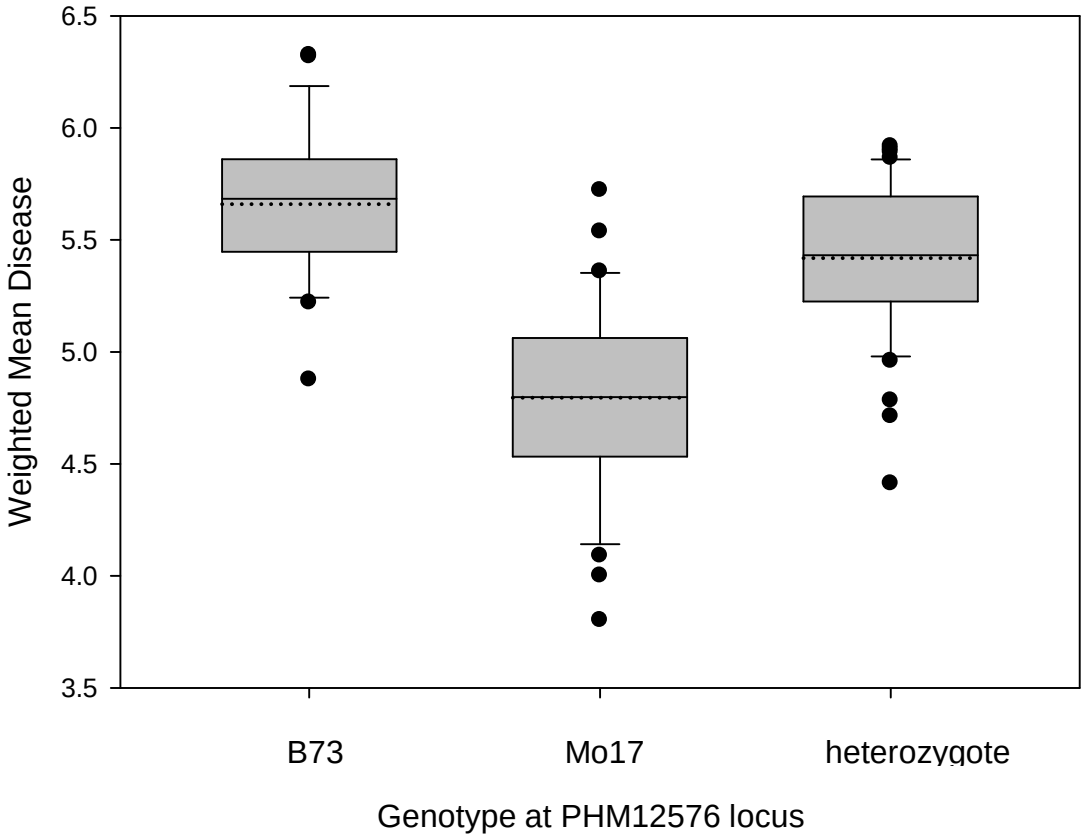

Figure 4. Boxplot of best linear unbiased predictor weighted mean disease (BLUP-WMD) scores for Stuber NIL $F_{2:3}$ lines according to genotype at the PHM12576 locus. Boxes incorporate the 25^{th} to 75^{th} percentile of data, while dotted lines represent means and connected lines represent medians. Outliers are represented as circles.

Table 1. Akaike Information Criterion (AIC) scores for regression of Southern leaf blight (SLB) scores on days to anthesis (DTA). Four models of SLB scores were considered in which linear, quadratic plus linear, cubic plus quadratic and linear, or quartic plus cubic, quadratic and linear DTA effects were fitted. The first column denotes the population considered and the rating time (first, second, third, or fourth week) that scores were taken. Shaded cells indicate the minimum values for that population and rating time.

Score by $F_{2:3}$ population and week	**DTA linear**	**DTA quadratic + linear**	**DTA cubic, quadratic +linear**	**DTA quartic, cubic, quadratic +linear**
B73 NIL- 1	1077.4	1069.1	1084.9	1104.6
B73 NIL- 2	1208.5	1192.8	1208.8	1225.8
B73 NIL- 3	1071.9	1062.4	1078.1	1096.3
B73 NIL- 4	988.6	989.7	1006.1	1024.2
Stuber NIL-1	914.1	820.4	736.4	753.8
Stuber NIL- 2	1487.5	1471.6	1480.1	1492.8
Stuber NIL- 3	1521.7	1516.3	1518.1	1526.9
Stuber NIL- 4	1004.6	1003.7	1009.9	1027.1

Table 2. Variance and covariance component estimates and ratios of variance or covariance components to their standard errors for the random sources of variation in the multivariate model of the Southern leaf blight (SLB) scores from weeks 1, 2, 3 and 4 taken on the B73 NIL $F_{2:3}$ population.

Source	**(Co)variance, Weeks**	**Component**	**Standard Error**
Residual	Variance week1	0.086	0.0063
Residual	Covariance weeks 1,2	0.049	0.0071
Residual	Variance week 2	0.20	0.014
Residual	Covariance weeks 1,3	0.0032	0.00071
Residual	Covariance weeks 2,3	0.11	0.011
Residual	Variance week 3	0.18	0.013
Residual	Covariance weeks 1,4	0.028	0.0075
Residual	Covariance weeks 2,4	0.11	0.012
Residual	Covariance weeks 3,4	0.14	0.013
Residual	Variance week 4	0.20	0.018
Rep	Variance week 1	0.0028	0.0037
Rep	Variance week 2	0.17	0.17
Rep	Variance week 3	0.024	0.026
Rep	Variance week 4	0.057	0.083
Block (Rep)	Variance week 1	0.0064	0.0028
Block (Rep)	Variance week 2	0.0091	0.0043
Block (Rep)	Variance week 3	0.010	0.0044
Block (Rep)	Variance week 4	0.0080	0.0047
Entry	Variance week1	0.12	0.015
Entry	Covariance weeks 1,2	0.19	0.023
Entry	Variance week 2	0.32	0.038
Entry	Covariance weeks 1,3	0.21	0.025
Entry	Covariance weeks 2,3	0.37	0.041
Entry	Variance week 3	0.43	0.048
Entry	Covariance weeks 1,4	0.23	0.027
Entry	Covariance weeks 2,4	0.40	0.045
Entry	Covariance weeks 3,4	0.46	0.051
Entry	Variance week 4	0.49	0.056

Table 3. Variance component estimates and ratios of variance components to standard errors for sources of variation in the multivariate model of the Southern leaf blight (SLB) scores from weeks 1,2,3 and 4 taken on the Stuber NIL $F_{2:3}$ population.

Source	(Co)variance, Weeks	Component	Standard Error
Residual	Variance week1	0.27	0.024
Residual	Covariance weeks 1,2	0.22	0.024
Residual	Variance week 2	0.39	0.033
Residual	Covariance weeks 1,3	0.10	0.017
Residual	Covariance weeks 2,3	0.19	0.022
Residual	Variance week 3	0.26	0.023
Residual	Covariance weeks 1,4	0.082	0.015
Residual	Covariance weeks 2,4	0.12	0.017
Residual	Covariance weeks 3,4	0.14	0.015
Residual	Variance week 4	0.18	0.016
Rep	Variance week 1	0.037	0.041
Rep	Variance week 2	0.046	0.049
Rep	Variance week 3	0.016	0.018
Rep	Variance week 4	0.036	0.038
Block (Rep)	Variance week 1	0.030	0.011
Block (Rep)	Variance week 2	0.0071	0.007
Block (Rep)	Variance week 3	0.0064	0.005
Block (Rep)	Variance week 4	0.0056	0.005
Entry	Variance week1	0.31	0.048
Entry	Covariance weeks 1,2	0.37	0.056
Entry	Variance week 2	0.49	0.074
Entry	Covariance weeks 1,3	0.35	0.049
Entry	Covariance weeks 2,3	0.45	0.063
Entry	Variance week 3	0.41	0.059
Entry	Covariance weeks 1,4	0.29	0.043
Entry	Covariance weeks 2,4	0.39	0.055
Entry	Covariance weeks 3,4	0.36	0.051
Entry	Variance week 4	0.34	0.047

Table 4. Results from single marker regression analyses of adjusted Southern leaf blight phenotypic values on marker genotypes. Positions of markers are given in IBM cM units. For each marker tested, the r^2 value, additive effect (*a*), and dominance effect (*d*) are given. Negative additive effects indicate that the Mo17 allele increased resistance. Positive dominance effects indicate that heterozygotes have lower resistance than the midparent value. *p*-values associated with *a* and *d* are given in parentheses. Empty cells indicate unavailable marker information.

marker	**position**	**B73 NIL F2:3's**	**Stuber NIL F2:3's**	**IBM**	**Stuber RILs**	**all populations combined**
umc1772	163.5	r^2= 0.52 *a*= -0.89 (<0.0001) *d*= 1.36 (<0.0001)	r^2= 0.38 *a*= -0.78 (<0.0001) *d*= 0.60 (0.0487)	r^2= 0.19 *a*= -0.44 (<0.0001)	r^2= 0.060 *a*= -0.23 (0.0093) *d*= 0.61 (0.2268)	r^2= 0.21 *a*= -0.48 (<0.0001) *d*= 0.66 (<0.0001)
PHM12576	165	r^2= 0.58 *a*= -0.96 (<0.0001) *d*= 1.30 (<0.0001)	r^2= 0.47 *a*= -0.87 (<0.0001) *d*= 0.76 (0.0123)			r^2= 0.25 *a*= -0.54 (<0.0001) *d*= 0.74 (<0.0001)
umc1425	165	r^2= 0.55 *a*= -0.93 (<0.0001) *d*= 1.32 (<0.0001)	r^2= 0.41 *a*= -0.82 (<0.0001) *d*= 0.58 (0.0388)	r^2= 0.22 *a*= -0.49 (<0.0001)	r^2= 0.10 *a*= -0.32 (0.0003) *d*= 0.46 (0.0944)	r^2= 0.23 *a*= -0.53 (<0.0001) *d*= 0.70 (<0.0001)
umc2000	166	r^2= 0.55 *a*= -0.95 (<0.0001) *d*= 1.24 (<0.0001)	r^2= 0.37 *a*= -0.77 (<0.0001) *d*= 0.66 (0.0214)	r^2= 0.20 *a*= -0.44 (<0.0001)	r^2= 0.084 *a*= -0.28 (0.0021) *d*= 0.65 (0.1925)	r^2= 0.22 *a*= -0.50 (<0.0001) *d*= 0.70 (<0.0001)
umc2158	176.6	r^2= 0.50 *a*= -0.90(<0.0001) *d*= 1.12 (<0.0001)	r^2= 0.36 *a*= -0.76 (<0.0001) *d*= 0.72 (0.0144)	r^2= 0.083 *a*= -0.30 (<0.0001)	r^2= 0.047 *a*= -0.19 (0.0377) *d*= 0.90 (0.1242)	r^2= 0.16 *a*= -0.41 (<0.0001) *d*= 0.68 (<0.0001)

Table 4 Continued

marker	position	B73 NIL F2:3's	Stuber NIL F2:3's	IBM	Stuber RILs	all populations combined
umc1392	181.1	r^2= 0.50 *a*= -0.90 (<0.0001) *d*= 1.12 (<0.0001)	r^2= 0.35 *a*= -0.75 (<0.0001) *d*= 0.68 (0.0170)	r^2= 0.068 *a*= -0.26 (<0.0001)	r^2= 0.044 *a*= -0.19 (0.0385) *d*= 0.44 (0.1797)	r^2= 0.14 *a*= -0.39 (<0.0001) *d*= 0.68 (<0.0001)
PHM4145	181.7	r^2= 0.50 *a*= -0.93 (<0.0001) *d*= 1.10 (<0.0001)	r^2= 0.30 *a*= -0.67 (<0.0001) *d*= 0.70 (0.0223)			
PHM14098	190.8	r^2= 0.28 *a*= -0.67 (<0.0001) *d*= 1.32 (<0.0001)	r^2= 0.18 *a*= -0.59 (<0.0001) *d*= 0.14 (0.6704)			
PHM13823	213.6	r^2= 0.068 *a*= -0.37 (0.0032) *d*= 1.34 (0.0016)	r^2= 0.24 *a*= 0.76 (<0.0001) *d*= 0.26 (0.5821)			
PHM6836	260.1	r^2= 0.015 *a*= -0.16 (0.2081) *d*= 0.64 (0.1180)	r^2= 0.30 *a*= 0.83 (<0.0001) *d*= 0.08 (0.8649)			
PHM12861	305.8	r^2= 0.018 *a*= -0.20 (0.3361) *d*= -0.96 (0.3687)	r^2= 0.021 *a*= 0.02 (0.8664) *d*= -0.56 (0.1339)			

Table 5. LOD scores for both real and virtual markers in both combined and single population analyses. Virtual markers are located at the midpoints of adjacent real markers. Shading indicates marker positions that fall within the 2-LOD interval calculated for the population.

marker	position	all populations combined	B73 NIL F2:3's[1]	Stuber NIL F2:3's[1]	IBM	Stuber RILs[1]
umc1772	163.5	35.8	24.9	13.6	12.9	1.5
(umc1425+umc1772)/2	164.25	38.9	25.5	14.0	13.8	2.2
PHM12576	165	41.2[2]	25.5	14.1	14.6	2.8
(PHM12576 +umc1425)/2	165	41.0	25.5	14.2	15.1	3.3
umc1425	165	40.1	25.5	14.2[2]	15.3[2]	3.6[2]
(umc1425+umc2000)/2	165.95	38.9	25.7	13.0	14.6	3.3
umc2000	166.9	36.4	26.0[2]	11.4	12.9	2.6

[1] The 2-LOD interval in this population extends past the markers tested.
[2] Maximum LOD score for population

Table 6. Matrix of Pearson correlation coefficients between entry effect estimates for scores from weeks 1-4 in the B73 NIL $F_{2:3}$ population.

	Week 2	**Week 3**	**Week 4**
Week 1	.94	.92	.92
Week 2		.99	1.0
Week 3			1.0
Week 4			

Table 7. Matrix of Pearson correlation coefficients between entry effect estimates for scores from weeks 1-4 in the Stuber NIL $F_{2:3}$ population.

	Week 2	**Week 3**	**Week 4**
Week 1	0.96	0.99	0.90
Week 2		1.0	0.94
Week 3			0.95
Week 4			

Table 8. Matrix of Pearson correlation coefficients between residuals for scores from weeks 1-4 in the B73 NIL $F_{2:3}$ population.

	Week 2	**Week 3**	**Week 4**
Week 1	0.38	0.25	0.22
Week 2		0.57	0.57
Week 3			0.73
Week 4			

Table 9. Matrix of Pearson correlation coefficients between residuals for scores from weeks 1-4 in the Stuber NIL $F_{2:3}$ population.

	Week 2	**Week 3**	**Week 4**
Week 1	0.74	0.51	0.49
Week 2		0.69	0.58
Week 3			0.73
Week 4			

-CHAPTER III-

The Genetic Architecture of Quantitative Resistance to Southern Leaf Blight in Maize Revealed by Nested Association Mapping

by

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Abstract

The Nested Association Mapping (NAM) strategy offers an unprecedented ability to assess the genetic architecture of maize quantitative disease resistance through use of a combined linkage and association mapping approach. In this study, the NAM population, consisting of nearly 200 recombinant inbred lines (RILs) derived from each of 25 families created by crossing reference inbred B73 to 25 diverse founder inbred lines, was phenotyped for resistance to Southern leaf blight (SLB), a necrotrophic foliar disease of maize, in three environments. Phenotypic data and genotypic data from 1106 single nucleotide polymorphism markers on 4699 total progeny lines provided the most comprehensive sampling of genetic diversity for linkage analysis of a quantitative disease resistance trait in plants, with high statistical power to detect and estimate the effects of genome regions with minor effects on this complex phenotype. Through linkage analysis, 32 quantitative trait loci (QTL), each of which had relatively small effects on resistance to SLB, were identified. Additive effects relative to the B73 allele at most QTL varied widely among the 25 diverse parent alleles, but were skewed toward greater resistance, consistent with the relatively greater levels of resistance among those founders compared to B73. No significant epistatic interactions were detected among the 32 QTL; an additive model of 32 QTL explained 93% of the total genetic variation. Epistasis, as well as undetected QTL with smaller effects or segregating rarely, may contribute to the remaining 7% of genetic variation not explained by the additive model. Many of the QTL identified in this study colocalize with previously mapped QTL. Superior statistical power and diverse sampling of alleles allowed for the identification of 13 novel QTL. The genetic architecture of resistance to SLB in maize

involves many genes with relatively small effects, functional allelic variation for resistance at each QTL, dispersion of resistance alleles across diverse germplasm, and a limited contribution due to epistasis.

I. Introduction

Quantitative disease resistances (QDR) in plants provide a durable alternative to the often ephemeral major gene resistances deployed in many crops today (Landeo et al., 1995; Ayliffe et al., 2008). Whereas major "R–gene" resistance relies on an immune response triggered by the action of a single host gene product recognizing one corresponding avirulence protein in the pathogen, QDR generally relies on the combined smaller effects of many genes (Jones and Dangl, 2006; Bent and Mackey, 2007). Because QDR allows some level of pathogenesis and pathogen reproduction, it results in weaker selection intensity on the pathogen to overcome resistance compared to major gene resistance. Furthermore, since QDR is usually based on multiple loci with potentially diverse modes of action, multiple mutations in the pathogen would have to accrue to overcome this resistance. These factors likely explain why QDR is generally found to be more durable than major gene resistance (McDonald and Linde, 2002).

Unfortunately, the genetic basis of plant quantitative disease resistance is not well understood; very few genes underlying disease resistance quantitative trait loci (QTL) have been cloned, and those that have been represent a diverse array of gene functions (Broglie et al., 2006; Fukuoka et al., 2007; Fu et al., 2009; Manosalva et al., 2009; Poland et al., 2009; Krattinger et al., 2009). Even in those pathosystems for which a host gene imparting resistance has been cloned, little is known about the genetic architecture of host resistance, i.e., how many genes, for which variation is present, are involved in producing the phenotype, what effects these genes have singly or in combinations, and how they might

interact with different environmental factors. This knowledge is crucial to the success of fully exploiting quantitative resistance in breeding programs for crop improvement.

Traditional QTL mapping studies of biparental segregating populations offer limited answers to these questions. Only a subset of functional polymorphisms affecting the trait will be segregating in a given population, and typically only two alleles are sampled at these segregating loci (Xu, 1998). The sampled alleles may not be broadly representative of germplasm on the whole, such that the results obtained may not be applicable to breeding populations, limiting the practical exploitation through marker-assisted selection of many QTL identified (Holland, 2007).

The maize Nested Association Mapping (NAM) panel addresses some of these limitations by capitalizing on the benefits of both linkage and association mapping approaches. The complete NAM population will be a set of 5000 recombinant inbred lines (RIL) derived from crosses between 25 diverse maize inbred lines and B73, a common maize inbred. A genetic map comprised of 1106 single nucleotide polymorphism (SNP) markers was developed for this population (McMullen et al., 2009) and genotype scores are publicly available (www.maizegdb.org, verified 25 May 2009). The large population size offers superior statistical power (Yu et al., 2008) and the range of alleles sampled can directly capture information about genetic heterogeneity, with applicability to diverse breeding germplasm (Buckler et al., 2009).

Cochliobolus heterostrophus is an ascomycetous fungus that causes Southern leaf blight (SLB) on maize. Spores of this fungus germinate on and penetrate maize leaves, causing the appearance of tan elliptical lesions on the leaves of the plant. The disease is

prevalent in warm, humid, temperate and tropical maize growing regions, but is controlled mostly through host QDR. QDR is manifested through decreased lesion size and decreased lesion number (Hooker, 1972; Carson, 1999). Previous studies of the inheritance of resistance to SLB have highlighted the importance of several genes of mostly additive effect (Carson et al., 2004; Balint-Kurti and Carson, 2006; Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al., 2009). Mapping genome regions contributing to SLB resistance in the NAM population would complement these previous studies, significantly extending their scope of inference and providing a broader perspective on the genetic architecture of maize resistance to SLB. This study provides the most comprehensive analysis to date of the genetic architecture of QDR in plants by precisely localizing QTL affecting SLB resistance and estimating the effects of alleles representing a broad sampling of the maize gene pool.

II. Materials and Methods

Derivation of Lines

The NAM population is comprised of 5000 $F_{5:6}$ RILs that were derived from crosses between B73 and 25 diverse inbred lines. B73 was selected as the reference line for its ubiquity in maize inbred line development and its sequenced genome (Yu et al., 2008; Buckler et al., 2009). Other parents were chosen to maximize allelic diversity based on SSR marker data (Liu et al., 2003). B73 was crossed to each of the 25 inbred lines, and F_1 plants were selfed for six generations. Each RIL originally descended from a unique F_2 plant (Buckler et al., 2009). Two hundred RILs comprised each family.

Phenotyping

A set of 5000 NAM RILs, 200 RILs randomly sampled from the Intermated B73 x Mo17 (IBM) population (Lee et al., 2002; Sharopova et al., 2002), and 281 lines representing the global diversity of public maize inbreds and used as a standard association mapping population (Flint Garcia et al., 2005) were planted at three different environments: the summers of 2006 and 2007 in Clayton, NC and the winter of 2007 in Homestead, FL. Each location contained one complete replication of the experiment, except a partial second replication that included the B73×CML247, B73×CML277, B73×Ki3, B73×M162W, and B73×Tzi8 families, as well as the IBM population and the association mapping panel, was grown during the summer of 2006 in Clayton, NC. In the Clayton locations, plots were approximately 1.07 m in length with 0.97 m between rows and a 0.61-m alley between plots.

In Homestead, FL, plots were 0.91 m in length with 0.97 m between rows and a 0.61-m alley between plots. The 5481 entries were divided into 27 sets according to their family or subpopulation of origin (25 RIL families, the IBM lines, and the association mapping panel). Each set was subdivided into 10 incomplete blocks that were randomized as 20×10 alpha lattice designs across replications, where each environment contained one or two replications, as described above. Each incomplete block was augmented with the addition of two check lines, B73 and the other founder of the family, placed at random positions within the incomplete block. B73 and Mo17 were included as repeated checks for both the IBM population and association panel sets.

The lines were either exposed to SLB naturally (Clayton 2006) or inoculated artificially (Clayton 2007 and Florida 2007). *C. heterostrophus* inoculum of isolate 2-16Bm was prepared according to Carson et al. (2004). The Clayton 2007 experiment was inoculated 79 days after planting to enhance the infection provided by natural inoculum. The Florida 2007 experiment was inoculated 37 days after planting. Plants were inoculated by placing approximately 20 SLB-infected sorghum grains in the whorl of plants at the six to eight leaf stage (Clayton 2007 and Florida 2007). Because *C. heterostrophus* is endemic to North Carolina and Florida, all environments likely had a mixed isolate population. The rows were scored for severity of SLB symptoms twice, approximately 1 week apart in the North Carolina environments and 2 weeks apart in the Florida environment, using a nine-point scale, on which increasing score relates to greater SLB susceptibility (Balint-Kurti et al., 2006). Thus, asymptomatic rows would receive a score of 1, and rows completely killed by

SLB would receive a score of 9. Days to anthesis (DTA), the time between planting and 50% pollen shed, was also recorded on each plot.

Statistical Analysis of Phenotypic Data

To minimize the effects of environmental variation, Best Linear Unbiased Predictors (BLUPs) of each NAM line were used as phenotypic values for QTL mapping. To calculate a single average SLB prediction (BLUP) for each line, a combined multivariate mixed model analysis of the two SLB scores across all environments was performed with ASREML software (Gilmour et al., 2002). The model included random family and line within family genotypic effects, two levels of blocking effects (sets and incomplete blocks within sets), field range and row position effects, and family and within family genotype by environment effects. The IBM and association panels were coded as separate families. NAM RILs identified as having greater than 8% heterozygosity or containing non-parental alleles were coded as derived from a 28^{th} population to prevent them from affecting the estimates of family means or within-family genetic variances. Environment effects were fit as fixed factors to reduce computational demands, and unique variances and covariances for the two scores were fit within each environment for the residual, set, incomplete block, range, and row factors. The model also permitted unique genetic variances and covariances of the two scores within each family. Likelihood ratio tests were calculated and used to determine significant factors to retain in the final model (Holland et al., 2003). Genotypic BLUPs were computed for each of the two SLB scores; the two BLUP scores were then averaged for each line to obtain a single value for SLB resistance we refer to as the SLB index.

Because SLB is a late season disease that infects maize during the grain-filling period, special care was taken to avoid confounding resistance and maturity effects. Many later-maturing lines often exhibit delayed onset of the disease (Hooker, 1972). To minimize phenological effects, days to anthesis was treated as a quadratic plus linear covariate in the analysis of SLB. The linear plus quadratic relationship was determined through multivariate regression modeling with Proc Mixed, in SAS software v.9.1.3 (SAS Institute, Inc., 2004). The first or second SLB score from each environment was considered separately as a function of linear, quadratic, cubic, and quartic polynomials. The fit of different models was compared with Akaike Information Criterion (AIC) values (Lynch and Walsh, 1998). The quadratic plus linear model was selected because its AIC was lowest in the majority of data sets (combinations of score and environment, Table 1).

BLUPs were also calculated for days to anthesis in order to map and compare flowering QTL with SLB resistance QTL. DTA phenotypic values were treated similarly to SLB scores, although a univariate mixed model was employed. Random environment effects, family and within family genotypic effects, two levels of blocking effects, field range and field row positions were tested for inclusion in the model. Spatial correlations among residuals were modeled with heterogeneous anisotropic first-order autoregressive (AR1×AR1) residual structures within each environment (Gilmour et al., 1997). Likelihood ratio tests were calculated to determine which factors to retain in the final model.

The average variance of the BLUP of a family's number of days to anthesis was estimated according to the following formula

$$Var(BLUP_{family}) = \frac{\sigma^2_{family \times env}}{n_{env}} + \frac{\sigma^2_{entry(family)}}{n_{entry}} + \frac{\sigma^2_{entry(family) \times env}}{n_{entry \times env}} + \frac{\sigma^2_{error}}{n_{obs}}$$

where n_{env} is the harmonic mean of the number of environments in which a family was observed, n_{entry} is the harmonic mean of the number of entries per family, $n_{entry \text{ x } env}$ is the harmonic mean of the number of entry by environment combinations per family, and n_{obs} is the harmonic mean of the number of observations per family. Harmonic means were used to adjust for the effects of missing data on the contribution of different variance components to family mean variances (Nyquist, 1991; Holland et al. 2003). The average standard error of a difference between two family means was obtained as the square root of twice the variance of a family mean estimate.

To compare DTA between founder inbreds, the average variance of a founder inbred mean was calculated according to the following formula

$$Var(BLUP_{inbred}) = \frac{\sigma^2_{entry(family) \times env}}{n_{env}} + \frac{\sigma^2_{error}}{n_{obs}}$$

where n_{env} is the harmonic mean of the number of environments in which the inbred was scored and n_{obs} is the harmonic mean of the number of observations taken on the founder. The number of observations on diverse line parents varied little, so an average standard error for diverse line founders was estimated. The standard error of a difference in DTA between B73 and a diverse founder line was estimated as the square root of the sum of the variance of the DTA BLUPs for B73 and the average variance of DTA BLUPs for diverse line founders.

Standard errors for differences between family and inbred mean SLB indices (average of first and second score BLUPs) were estimated in an analogous manner to those for DTA;

however, because the SLB index BLUP is a linear combination of two SLB score BLUPs, its variance was estimated as the variance of the linear combination (Lynch and Walsh, 1998). Specifically, the average variance of a population BLUP for SLB index was estimated as:

$$Var(BLUP_{family}) = \frac{0.5^2\sigma^2_{family\times env_1}}{n_{env1}} + \frac{0.5^2\sigma^2_{entry(family)_1}}{n_{entry1}} + \frac{0.5^2\sigma^2_{entry(family)\times env_1}}{n_{entry\times env1}} +$$
$$\frac{0.5^2\sigma^2_{error_1}}{n_{obs1}} + \frac{(2*0.5^2)\sigma_{family\times env_{1,2}}}{n_{env2}} + \frac{(2*0.5^2)\sigma_{entry(family)_{1,2}}}{n_{entry2}} +$$
$$\frac{(2*0.5^2)\sigma_{entry(family)\times env_{1,2}}}{n_{entry\times env2}} + \frac{(2*0.5^2)\sigma_{error_{1,2}}}{n_{obs2}} + \frac{0.5^2\sigma^2_{family\times env_2}}{n_{env2}} +$$
$$\frac{0.5^2\sigma^2_{entry(family)_2}}{n_{entry2}} + \frac{0.5^2\sigma^2_{entry(family)\times env_2}}{n_{entryxenv2}} + \frac{0.5^2\sigma^2_{error_2}}{n_{obs2}}$$

in which variance component subscripts indicate the variance component or harmonic mean's association with the *i*th scoring date. To compare parent checks, the average variance of the BLUP index of equally weighted SLB scores of a founder inbred was estimated according to the following formula:

$$Var(BLUP_{inbred}) = \frac{0.5^2\sigma^2_{entry(family)\times env_1}}{n_{env1}} + \frac{0.5^2\sigma^2_{error_1}}{n_{obs1}} + \frac{(2*0.5^2)\sigma_{entry(family)\times env_{1,2}}}{n_{env2}}$$
$$+ \frac{(2*0.5^2)\sigma_{error_{1,2}}}{n_{obs2}} + \frac{0.5^2\sigma^2_{entry(family)\times env_2}}{n_{env2}} + \frac{0.5^2\sigma^2_{error_2}}{n_{obs2}}$$

Approximate least significant differences for pairwise comparisons were calculated by multiplying the relevant standard error by the α=.05 critical value of a *t*-distribution with 52 degrees of freedom. The actual degrees of freedom for this variance are not easily computed, therefore, to be conservative, we used the minimum number of degrees of freedom among all

of the components of variation in the equation, which was 52 for family-by-environment interaction.

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Total genetic variance for a single SLB score or for DTA was estimated as the sum of the family variance component and the average entry within family variance component for that score. Phenotypic variance for individual genotypic BLUPs for a single score was estimated by summing the genetic variance, the average residual variance divided by the harmonic mean of number of observations on each entry, the family by environment variance divided by the number of environments, and the environment by entry within family variance divided by the harmonic mean of the number of environments per entry (Holland et al., 2003). Heritability of individual SLB scores or DTA was estimated as the ratio of the total genotypic to total phenotypic variances. Average within family heritability was also estimated for each trait by omitting the family and family by environment variances from the previously described variance calculations.

SLB index, the mean of the two SLB score BLUPs for each genotype, was used for QTL mapping. The heritability of SLB index values was estimated as:

$$h^2 = \mathbf{b'Gb} / \mathbf{b'Pb}$$

where **b** is the vector of index coefficients, and **G** and **P** are the genetic and phenotypic variance-covariance matrices (Lin and Allaire, 1977; Falconer and Mackay, 1996).

Genotyping and Mapping

Genotyping and map construction were performed by McMullen et al. (2009). 6825 RIL were genotyped at 1536 SNPs through use of the Illumina Golden Gate Assay System (Illumina, San Diego, CA). SNPs for mapping were chosen based on their ability to discriminate between B73 and diverse parent alleles, in addition to other criteria such as signal intensity and error rates. The genetic map was constructed based on genotyping of 4699 lines, approximately 200 per family, at 1106 SNPs. This number is lower than the starting 5000 lines because some lines were discarded due to high levels of heterozygosity or possible contamination (McMullen et al., 2009). These discarded lines were not used either in map construction, genetic variance component estimation, or QTL mapping.

QTL Detection and Mapping

Marker data were coded as "0" if the line possessed the B73 allele at that locus, and "2" if the line possessed the alternate parent allele at that locus. Heterozygotes were coded as "1". SNP markers were not informative in all families because some proportion of diverse line founders shared SNP alleles with B73 (McMullen et al., 2009). Therefore, missing marker data were imputed according to genotypes at flanking markers; imputed data are reasonable approximations of actual genotypes due to the density of available marker information (average marker density of 1.3 cM per marker; McMullen et al., 2009). If both flanking markers were of the same genotypic class, the missing marker was considered to be of the same genotype. If flanking markers were of different genotypes, genotype values at the flanking loci were averaged.

Proc GLMSelect (experimental), in SAS software v. 9.1.3 (SAS Institute, Inc., 2004) was used to scan the genome for SLB index or DTA QTL at each marker locus, using stepwise selection with a *p*-value threshold of 1×10^{-4} (Cohen, 2006; SAS Institute, Inc., 2004), a method that was previously demonstrated to provide reliable QTL results (Buckler et al., 2009). At each marker tested, unique allele effects for each diverse line founder were modeled.

After initial identification of loci, the model was optimized through an iterative process of sequentially dropping one marker in the model and separately refitting adjacent markers at up to eight consecutive positions on either side of that marker. Proc GLM, in SAS software v.9.1.3 (SAS Institute, Inc., 2004), was used to fit the regression model containing the effect of family and marker within family terms. Additional marker positions were tested after the seventeen consecutive positions if there was not a drop of at least two LOD points between the marker with the maximum LOD score and a marker to either side within the set of consecutive 17 markers tested for each QTL. LOD scores were calculated according to Haley et al. (1994):

$$LOD = \frac{n \ln(SSE_{reduced} / SSE_{full})}{2 \ln 10}$$

The error sums of squares of the full and reduced models are derived from models in which either all significant markers were fit or the marker in question was omitted, respectively. The QTL was considered to be located at the position of the maximum LOD score. Confidence intervals were considered as bracketed by the two markers on either side of the marker with the maximum LOD score for which the LOD score dropped at least two points

below the maximum (van Ooijen, 1992). This procedure was repeated for each of the markers in the model.

Allelic effects for each marker and family were estimated simultaneously from the final selected model through the solution option of Proc GLM in SAS software v.9.1.3 (SAS Institute, Inc., 2004). *t*-tests comparing the parental and B73 mean allelic effects were used to assess significance of specific allelic effects at α=0.05.

To determine if any loci pleiotropically affected both SLB resistance and DTA, Pearson correlation coefficients were calculated between allele effect estimates at SLB resistance and DTA QTL with overlapping confidence intervals. Because the Pearson correlation coefficient only characterizes the linear relationship between two variables, linear, quadratic, cubic, and quartic regression models of SLB on DTA were also tested with SAS Proc Mixed (SAS Institute, Inc., 2004).

To test for significant interactions among the 32 loci with additive contributions to the SLB resistance phenotype, all possible two-way combinations of these loci were tested for significance one at a time by inclusion of the interaction term in the final model. An interaction was considered significant if the *p*-value was less than 0.0001 in the context of the full additive model.

III. Results

Trait Variation and Heritability

Resistance to SLB varied widely among the twenty-five founder inbreds; the most resistant and susceptible founder lines differed by approximately three SLB index points, a difference that is easily observed visually (Figure 1). Mo17 was the most resistant inbred (SLB index of 1.96), while P39, a sweet corn, was the least resistant (SLB index of 4.85), compared to the reference parent B73 (SLB index of 4.44). P39, Hp301 (a popcorn), and Oh7B were not significantly different from B73 ($p > 0.05$, Figure 2). All other parents were more resistant ($p < 0.05$) than B73. Families of RILs also differed for mean resistance, with B73×CML52 producing the most resistant family of RILs (mean SLB index of 2.66), and B73×Hp301 producing the least resistant family of RILs (4.76, Figure 2). Family mean SLB index values were highly correlated with their diverse founder parent BLUPs ($r = 0.88$, $p < 0.0001$). DTA also varied by 28 days from the earliest (P39, 10 days prior to B73) to latest (CML52, flowering 18 days after B73) founder inbreds (Figure 3). B97 and NC358 were not significantly different from B73 for DTA.

Variance component estimates were generally greater for the second SLB score compared to the first (Table 2). Genetic variance components (both for families and RIL within families) were the most important components of variation for both scores, with heritabilities for the first and second SLB scores estimated to be 81 and 84%, respectively (Table 2). Although the second SLB score had both higher genetic variance and heritability than the first, it also had a higher proportion of missing data points (4.4 % vs. 41%, respectively), because earlier senescence of some genotypes prevented us from obtaining a

reliable second SLB score. The use of the multivariate mixed model permitted prediction of SLB index values even for those plots in which the second score was not available, based on the estimated covariances between first and second scores.

Heritability of SLB index across the entire NAM set was estimated to be 85.2%, slightly greater than both of the two individual score heritabilities. These estimates are consistent with previous estimates of SLB studies (Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al., 2009). Average within-family heritability was 78.1% and ranged from 58.8% (B73×MS71) to 86.6% (B73×Hp301). Heritability of DTA across all families was 89%; within family heritability of DTA was 81%, similar to results reported by Buckler et al. (2009) for this same population based on a set of eight summer environments, including the two North Carolina environments analyzed here.

QTL Identification

Thirty-two SLB resistance QTL were mapped jointly across all families (Table 3). The median length of 2-LOD support intervals for QTL position was 4.9 cM, and ranged from 0.6 cM to 21.8 cM. These 32 QTL jointly explained 79.6% of the phenotypic variation for SLB, and thus were associated with 93% of the observed genetic variation. No two-way epistatic interactions were detected among pairs of loci with additive effects.

All of the QTL had small effects on SLB resistance, with absolute values of significant additive SLB index effects averaging 0.13, and ranging from 0.0 to 0.38 among all QTL and allele combinations (Figure 4). The vast majority of allelic effects caused differences that would not be individually distinguishable on the 1-9 visual scale. For

example, the QTL with the largest effect estimate mapped to bin 3.04, a region which has previously been associated with SLB resistance (Bubeck, 1991; Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al., 2009). The additive effect of the alleles at this locus ranged from between -0.38 (Il14H) to +0.15 points (Hp301) relative to the B73 allele on the 1-9 scale, on which negative effect values reflect greater resistance to SLB than the B73 reference parent, and positive effect values reflect greater susceptibility than B73 (Figure 4). In comparison, the least significant difference for comparisons between SLB index values of founder lines replicated over three environments was 0.49.

At each detected QTL, only a subset of the 25 families segregated for significant functional allelic differences (as judged by the significance of allelic effects within families at $\alpha = 0.05$). The number of families that exhibited significant allelic segregation at a QTL ranged from three (bins 4.00/4.01 and 7.00) to 20 (8.03), with a median number of eight families (Figure 4). For three-quarters of the QTL, diverse parent founders contributed both positive and negative allele effects relative to the B73 allele. Diverse line founders contributed only negative significant effects (greater resistance) compared to B73 at five QTL (bins 1.05, 1.07, 7.00 8.03, and 9.03/.04, 7.00), and only positive effects (greater susceptibility) compared to B73 at three QTL (bins 3.06, 3.09, and 6.01; Figure 4). Approximately two-thirds of founder alleles with significant effects were negative (more resistant) relative to B73, as expected based on the greater resistance observed in most founders compared to the reference parent.

The predictive value of the QTL model was assessed by predicting SLB index values of founders based on the allelic effects estimated in their progeny RILs. Founder values were predicted by adding all 32 allelic effects and the corresponding family effect to the B73 SLB index. When predicted values for the founder inbreds were regressed on the observed SLB BLUP indices, the QTL model predicted 80% of the variation in resistance phenotypes of the parents.

Thirty QTL were mapped for DTA, explaining 85.2% of the variation for this trait (Table 4). Eight pairs of QTL for SLB and DTA had overlapping support intervals. To assess whether these loci may be affecting SLB and DTA pleiotropically, Pearson correlation coefficients were calculated for these eight colocalizing SLB and DTA QTL effect estimates in each of the 25 families. Only one pair of colocalizing SLB and DTA loci (in bin 1.05) had a significant correlation coefficient ($r = 0.55$, $p = 0.0047$), suggesting that only one of 32 SLB QTL is likely to be caused by a pleiotropic effect of later flowering. Regression analysis was also performed to assess importance of higher order relationships between colocalizing SLB resistance and DTA allelic effects, but none were significant.

In order to compare differences in the number of SLB resistance QTL detected between NAM and other mapping approaches, loci from eight different publications were projected onto the IBM2008 Neighbors map according to the positions of their closest flanking markers or positional confidence intervals (Figure 5). These previous publications represent germplasm from biparental populations derived from six different parents (Balint-Kurti and Carson, 2006; Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer, 2008; Zwonitzer et al., 2009). The median number of QTL detected in

previous studies was eight, four times fewer than detected in this study. Because most of the previous publications used different mapping strategies, marker density, and population sizes, precision of position estimates was difficult to compare. Positional comparisons revealed that 60% of loci detected with NAM overlap with previously identified QTL (Figure 5). Each of the 32 QTL for SLB resistance was assigned a rank, with the lowest rank corresponding to the QTL with the smallest *p*-value for inclusion in the regression model. The median rank of the QTL detected in NAM, but not previously was 19, slightly lower than the median rank of all QTL detected.

The most significant SLB resistance QTL previously identified in the IBM family (Balint-Kurti et al., 2007) mapped to the same genome region as the QTL with largest effect mapped in the NAM population in this study (Figure 5). To determine if the IBM and the NAM 3.04 QTL colocalize without confounding effects of genotype-by-environment interactions and differences among experimental procedures, we mapped QTL using phenotypic data taken by Balint-Kurti et al. (2007) and genotypes at the 1106 NAM SNP markers from a subset of 200 IBM lines. Seven of the 15 significant QTL (bins 1.07, 2.04, 3.04, 5.04, 7.03, 9.04, 10.03) detected with the Balint-Kurti et al. (2007) IBM data overlapped either exactly or very closely with NAM SLB resistance QTL confidence intervals, including the 3.04 QTL (Table 5).

IV. Discussion

We preferred to use the multivariate mixed model to simple averaging of scores or fixed effect multivariate modeling for two main reasons. The multivariate mixed model analysis of first and second SLB resistance scores allowed for recovery of information on plots for which senesced tissue made accurate scoring of the second rating impossible. The information on first SLB scores in such plots would not be used in traditional fixed effect multivariate analyses of variance; thus the multivariate mixed model uses available information more efficiently (Holland, 2006). The full mixed model approach also permitted fitting heterogeneous variance-covariance structures for residuals in different environments, which resulted in significantly greater likelihoods for these models, as residual variances were substantially higher for the Florida environment (Table 2).

Linkage analysis revealed that variation in resistance to SLB is controlled by at least 32 loci, each with relatively small additive effects. However, our results also indirectly suggest the existence of non-additive genetic variation for SLB resistance. The estimated correlation coefficient between diverse line founder inbred and family mean BLUPs was large, but not perfect (r = 0.88, Figure 2). Some important deviations from a perfect association were observed, such as a RIL family with only average level of resistance produced by the most resistant founder, Mo17 (Figure 2). Epistasis or selection within RIL families could account for deviations from a perfect relationship. Strong selection was detected at very few genome regions, and even in those cases, was restricted to a small number of families (McMullen et al., 2009). Similarly, the prediction of founder SLB index values based on the additive QTL model was high (r^2 = 0.80), but not perfect. Imperfect

prediction from the QTL model could be due to undetected QTL with very small effects, undetected QTL segregating in a small number of families, and epistatic interactions not included in the model. Epistatic effects among SLB QTL have been reported previously, but only between loci with significant additive effects (Balint-Kurti et al., 2006; Balint-Kurti et al., 2007; Balint-Kurti et al., 2008; Zwonitzer et al., 2009). In a meta-analysis of disease resistance QTL from a multitude of host and parasite species, Wilfert and Schmid-Hempel (2008) found that epistatic interactions between additive QTL were detected in 45.3% of studies, whereas 72.4% of epistatic loci reported did not have significant additive effects on disease resistance. Dominance effects that were not modeled but are expressed at low levels due to residual heterozygosity in the RILs (approximately 3.2 to 4.1% depending on distance from the centromere; McMullen et al., 2009) could also contribute to the discrepancy between predicted and observed founder variation as well.

The importance of additive over epistatic effects, the relatively high heritability, and the substantial phenotypic variation among inbred lines for resistance to SLB suggest that phenotypic selection for increased quantitative SLB resistance will be very effective. Examination of allele effect estimates across the different founder inbreds reveals that no single line offers the optimal combination of resistance alleles. The NAM results presented here provide, for the first time, identification of an optimal genotype for SLB resistance based on the evaluation of 27 alleles representing much of the global diversity for public maize inbreds at many loci. Development of this optimal genotype would require the intercrossing of 17 parental lines and selection for a single specific combination of SLB resistance alleles. This would be a technically formidable undertaking, but these results

provide the necessary framework needed to guide future efforts to mine the abundant natural genetic variation of maize for quantitative disease resistance alleles on a large scale.

Disease is a dynamic process in which plant and pathogen genomes interact at multiple regulatory levels. Due to selective pressures exerted on the plant genome by a multitude of pathogens, alleles at many loci responsible for disease responses may be conserved. If a mutation does occur, it may be present in only one inbred line. Of a set of 3641 SNPs that were sequenced in the 26 diverse founder inbred lines, approximately 40% were detected in only one or two lines (Buckler et al., 2009). This suggests that rare polymorphisms are a common phenomenon in maize inbreds; thus, it is likely that some alleles may not be represented in more than one inbred. In studying the inheritance of both resistance to SLB and DTA, no QTL were detected in only one or two families (Figure 6). Power to detect family-specific QTL may be lower in the NAM population than in individual biparental populations, assuming the biparental population chosen is segregating for the QTL of interest.

Sixty percent of the confidence intervals of NAM QTL colocalized with previously reported QTL for SLB resistance. Because many of the QTL that had not been detected in previous publications ranked high in terms of overall significance (or low in terms of *p-value*), it is likely not only enhanced statistical power, but also wider sampling of alleles allows for superior detection of QTL with the NAM strategy. The most significant QTL in the IBM (Balint-Kurti et al., 2007) and the QTL with largest allelic effect, explaining the second largest amount of variation among loci in the NAM are in bin 3.04. Comparison of the genetic positions of their flanking markers on the IBM2008 map suggests that they are

not identical. However, when the flanking marker positions are compared on the B73 physical map (http://www.maizesequence.org, verified 25 May 2009), the IBM QTL position lies within the NAM 3.04 QTL support interval. Several explanations may account for discrepancy. Microcollinearity is not necessarily preserved among maize lines because of high levels of physical rearrangements in the maize genome (Fu and Dooner, 2002), thus the NAM composite map may not offer accurate positions of the markers for all lines. Similarly, the IBM2008 map does not position markers across all of the genomes effectively. Because all positions are approximated, confidence intervals of QTL may not overlap even if they should. Since experiments were conducted in different environments, genotype by environment effects may contribute to differences in QTL detected between studies. Finally, however, it is also possible that the QTL segregating in the IBM is a rare functional variant physically distinct from, but genetically closely linked to, a QTL that segregates more commonly in the NAM.

The precision with which a QTL was mapped was largely dependent on the marker density within its specific region of the genome. Although the average distance between markers is 1.3 cM (McMullen et al., 2009), marker density does fluctuate along the map. The density of informative markers in each family also changes. Because certain families share alleles with B73, genotypes at these markers must be imputed. As distance between informative markers increases, these imputations become less accurate. Another complicating factor may be the difference in recombination rates between families at the QTL. Within intervals defined by adjacent markers, there is 2.9 fold difference, on average, between the highest and lowest recombination frequency per family. Within some intervals,

the recombination frequency differs by as much as a factor of 30 (McMullen et al., 2009). If, at a certain QTL, families with alleles of particularly large effect tend to recombine less, the precision of the QTL's position estimate will likely decrease. Although the recombination difference problem cannot be resolved easily, projection of parental sequence polymorphism data onto the 5000 NAM RILs should greatly enhance the precision of the positional estimates of SLB QTL and may even allow for gene level resolution without additional phenotyping (Yu et al., 2008).

The *C. heterostrophus*-maize pathosystem serves as an excellent model for elucidation of quantitative disease resistance for several reasons: 1) the genetic resources available for mapping in maize are rapidly advancing with the recent completion of the draft sequence of the B73 genome (http://www.maizesequence.org), 2) resistance to SLB is a highly heritable trait in maize, 3) *C. heterostrophus* has a well-characterized, tractable mating system (Kolmer and Leonard, 1986; Yoder, 1988; Turgeon et al., 2008) and a completed genome sequence (http://genome.jgi-psf.org/CocheC5_1/CocheC5_1.home.html, verified 25 May 2009). Identification of the causal genetic components of maize quantitative resistance to SLB, which will be greatly enhanced by the NAM results presented here, will not only increase the efficiency of selection for increased disease resistance to SLB, but also serve as a rich reservoir of homologous candidate genes for disease traits that cannot be mapped with quite as high precision due to phenotyping difficulty or low heritability.

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Table 1. Akaike Information Criterion (AIC) scores for regression of Southern leaf blight (SLB) scores on days to anthesis (DTA). Four models of SLB scores were considered in which linear, quadratic plus linear, cubic plus quadratic and linear, or quartic plus cubic, quadratic and linear DTA effects were fitted separately for the data in each of the three environments (Clayton, NC 2006 and 2007, as well as Homestead, Florida). The first column denotes the environment considered and the rating time (first or second score). Shaded cells indicate the minimum values for that environment and rating time.

Environment and score	**DTA linear**	**DTA quadratic + linear**	**DTA cubic, quadratic +linear**	**DTA quartic, cubic, quadratic +linear**
NC06-1	18955.1	18916.7	18937.2	18952.5
NC07-1	13623.4	13638.3	13603.6	13609.6
FL-1	12727.1	12681.1	12698.5	12720.1
NC06-2	13393.3	13392.6	13410.0	13429.4
NC07-2	10564.0	10553.2	10572.2	10597.8
FL-2	9059.8	9071.4	9081.4	9100.1

Table 2. Variance component estimates (and their standard errors) for residual, family, family-by-environment, entry within family, and entry within family by environment terms in the multivariate model of the first Southern leaf blight (SLB) score, second SLB score, and days to anthesis (DTA) taken on the NAM population in the Clayton, NC 2006 and 2007 and Homestead, FL environments.

Source of Variation	**Variance of Score 1**	**Covariance of Scores 1 and 2**	**Variance of Score 2**	**Variance of DTA**
Residual- NC06	0.136 (0.004)	0.087 (0.005)	0.183 (0.008)	0.093 (0.003)
Residual-NC07	0.108 (0.005)	0.04 (0.006)	0.166 (0.01)	0.038 (0.002)
Residual-FL	0.494 (0.014)	0.297 (0.015)	0.517 (0.021)	0.211 (0.006)
Family	0.194 (0.055)	0.304 (0.087)	0.486 (0.141)	0.211 (0.059)
Family*Environment	0.005 (0.003)	0.012 (0.005)	0.04 (0.012)	0.013 (0.003)
Average Entry (Family)	0.338 (0.071)	.468 (.166)	0.689 (0.088)	0.22 (0.025)
Entry (Family)*Environment	0.049 (0.046)	0.038 (0.005)	0.067 (0.008)	0.026 (0.002)
Heritability	0.815	0.875	0.841	0.894
Average Within Family Heritability	0.736	0.812	0.764	0.808

Table 3. Bin number, left and right flanking markers, confidence interval (CI) position, partial R-square value, *p*-value, and number of families (out of 25) with significant effect estimates of each QTL mapped for Southern leaf blight resistance. The CI position denotes the position of the QTL in centiMorgans (cM) along the NAM map of the chromosome indicated. Partial R-square indicates the amount of variation explained by a single QTL after all other QTL were fit in the model. A QTL was considered detected in a family if its effect estimate was significantly different from B73 at the α=.05 level. Shaded cells indicate that the CI for this QTL overlaps with a QTL mapped for days to anthesis.

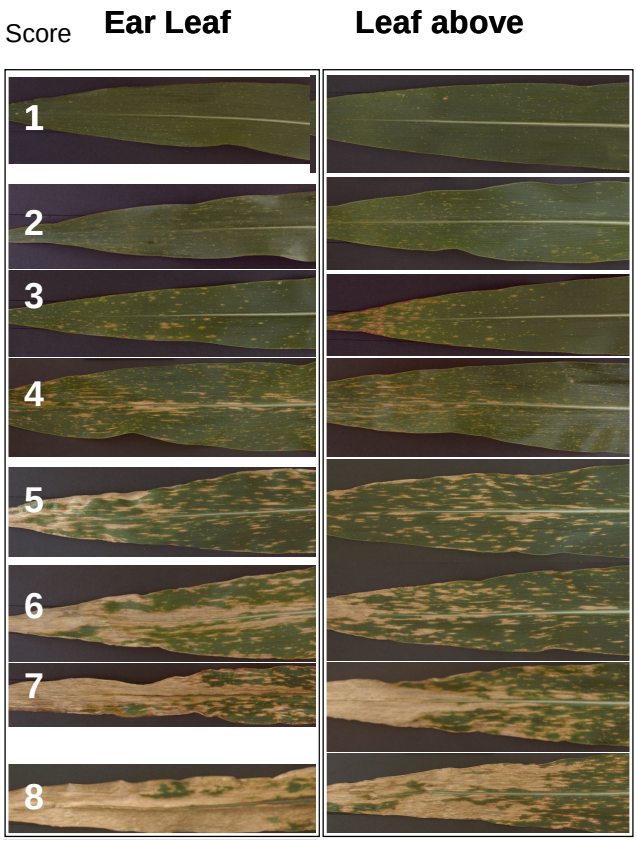
bin	left flanking marker	right flanking marker	CI map position	partial R-square	*p*-value	Number of families
1.03/1.04	PZA03243.2	PHM4913.18	60.8-64.9	0.0053	1.03E-10	6
1.05	PZA01135.1	PZB01235.4	82.4-86.6	0.0082	2.73E-20	10
1.07	PHM12706.14	PHM6043.19	123.7-127.6	0.0070	3.11E-16	8
1.09	PHM16605.19	PZA02269.3/4	146.6-149.7	0.0046	1.31E-08	8
2.02/2.03	PZA01753.1	PZA02417.2	40.6-47.7	0.0034	2.97E-05	4
2.04	PZA01993.7	PZB00183.4	64.2-69.4	0.0064	1.57E-14	12
2.05	PHM3626.3	PZA01232.1	77.4-81.7	0.0040	7.28E-07	5
3.03	PZA03527.1	PZB01944.1	22.5-28.9	0.0047	6.41E-09	8
3.04	PHM4204.69	PHM2343.25	38.3-53.4	0.0153	4.88E-45	15
3.05	PZA03198.3	PZD00016.4	60.5-62.4	0.0052	2.00E-10	11
3.06	PZA02654.3	PZA03733.1	90.1-92.6	0.0084	5.64E-21	8
3.09a	sh2.21	PZA01233.1	128.4-132.8	0.0088	2.69E-22	13
3.09b	PZA02182.1	PZA02423.1	146.8-155.5	0.0035	1.29E-05	5
4.00/4.01	PZA02509.15	PHM3301.28	0.1-19.4	0.0034	2.69E-05	3
4.05	PZA02457.1	PZA03385.1	49.4-53.4	0.0065	9.57E-15	9
4.09	PZA00694.6	PZA00513.1	116.1-118.9	0.0046	1.14E-08	5
5.03	PZA00517.7	PZA01563.1	49-57.6	0.0050	7.76E-10	8
5.04	PZA02164.16	PZB01017.1	70-74.5	0.0054	3.74E-11	8
5.06/5.07	PZA02667.1	PZA00963.3	110.7-127.2	0.0042	1.65E-07	6
6.01	PHM15961.13	PZA00158.2	0-5.1	0.0148	2.43E-43	17
6.06	PZA02436.1	PZA03027.12	61.8-75.1	0.0049	1.47E-09	8
7.00	PZA01426.1	PHM9241.13	0-21.8	0.0029	4.11E-04	3
7.01	PHM9241.13	PZA02872.1	21.8-39.9	0.0036	6.36E-06	7
7.03	PZA02352.1	PZA03583.1	64.9-69.8	0.0048	1.86E-09	9
8.03	PZA01209.1	PZA00379.2	52.4-53	0.0216	2.39E-67	20
8.06/8.07	PZA01316.1	PZA01964.29	99-106.9	0.0063	3.62E-14	6
9.02	PHM5181.10	PZA03416.7	26.8-30.4	0.0108	3.61E-29	12
9.03/9.04	PZA01791.2	PZA01281.2	46.1-49.1	0.0111	3.23E-30	12
9.04	PZA03671.1	PZA01096.1	65.5-69.4	0.0051	2.17E-10	8
9.07	PHM1911.173	PZA03573.1	107.5-114.8	0.0039	8.39E-07	7
10.03	PZA01597.1	PZA01877.2	36.5-38.6	0.0061	2.05E-13	10
10.07	PZA02969.9	PZA01001.2	81.3-91	0.0059	1.16E-12	9

Table 4. Bin number, left and right flanking markers, confidence interval (CI) position, partial R-square value, *p*-value, and number of families (out of 25) with significant effect estimates of each QTL mapped for days to anthesis. The CI position denotes the position of the QTL in centiMorgans (cM) along the NAM map of the chromosome indicated. Partial R-square indicates the amount of variation explained by a single QTL after all other QTL were fit in the model. A QTL was considered detected in a family if its effect estimate was significantly different from B73 at the α=.05 level. Shaded cells indicate that the CI for this QTL overlaps with a QTL mapped for Southern leaf blight resistance.

bin	left flanking marker	right flanking marker	CI map position	partial R-square	*p*-value	Number of families
1.01	PHM6238.36	PZA00528.1	13.4-15.6	0.00325	1.57E-08	9
1.03/1.04	PHM3726.129	umc13.1	59-62.1	0.004686	7.65E-15	9
1.05	PZA00752.1	PZB01235.4	81.4-86.6	0.003837	4.86E-11	9
1.06/1.07	PZA00619.3	PZA01963.15	108.4-116.4	0.003135	4.7E-08	10
1.11	PZA00894.7	PZA02044.1	180.9-188.2	0.004706	6.19E-15	8
2.04/2.05	PZA02168.1	PZA00485.2	72.1-77.1	0.007047	5.6E-26	10
2.07	PHM7953.11	PHM16125.47	106.3-107.5	0.002453	2.31E-05	13
2.08	PZA00804.1	PZA00527.10	123.9-129.8	0.005343	7.08E-18	7
3.04	PZA03070.9	PHM15474.5	56-57.1	0.008062	6.66E-31	6
3.05	PZA00828.2	PZA01396.1	70.6-75.9	0.003833	5.01E-11	8
3.07	PZA01035.1	PZA01501.1	103.2-106.3	0.003519	1.15E-09	11
3.08/3.09	PZA02616.1	PZA03146.4	120.7-127.7	0.005384	4.58E-18	10
3.09	PZA00402.1	PZA02182.1	140.7-146.8	0.002876	5.31E-07	6
4.04/4.05	PZA02457.1	PZA00218.1	49.4-56.4	0.00364	3.48E-10	7
4.08	PZA00344.10	PZA00193.2	101.3-105.4	0.003281	1.16E-08	5
4.09/4.10/4.11	PHM4125.11	PZA00282.19	129.3-141.1	0.002434	2.73E-05	9
5.00	PZA00191.5	PZA02316.22	3.5-9.1	0.004296	4.46E-13	6
5.04	PZA01693.1	PZA01365.1	68.7-71.3	0.006003	5.53E-21	9
5.06	PZA03024.16	PZA02068.1	106.4-115.1	0.002675	3.29E-06	8
6.05/6.06	PZA01144.1	PZA01462.1	59.5-72.3	0.002739	1.86E-06	6
6.07	PZA00889.2	PZA02141.1	94.1-101	0.005203	3.17E-17	5
7.02	PZA02018.1	PZA00616.13	50-63.2	0.00311	5.98E-08	7
8.02/8.03	PZA02454.2	PZA01196.2	42-50.6	0.003218	2.14E-08	9
8.05	PHM3993.28	PZA01038.1	64.2-67.1	0.012428	2.13E-52	5
8.07/8.08	PZA01964.29	PZA00189.23	106.9-119	0.002622	5.3E-06	17
9.03	PZA00693.3	PZA01791.2	44.5-46.1	0.00511	8.58E-17	5
9.04	PZA00840.1	PZA00213.19	62-62.9	0.006811	7.63E-25	7
9.06/9.07	PHM816.29	PZA02381.1	87.3-93.5	0.00244	2.6E-05	11
10.04	PZA01619.1	PZA00400.3	41.9-43	0.00847	6.76E-33	7
10.07	PZA01073.1	PZA01001.2	83.2-91	0.004235	8.38E-13	8

Table 5. Bin number, name of most significant marker and its map position, partial R-square (the amount of variation explained by a single QTL after all other QTL were fit in the model), *p*-value, effect estimate, and *p*-value of the *t*-test contrasting B73 and Mo17 allelic effects for each quantitative trait locus (QTL) mapped in the IBM population with data collected previously by Balint-Kurti et al., 2007. Shaded cells indicate that a QTL overlaps with one of the 32 QTL mapped in the combined analysis of the 25 recombinant inbred line families.

bin number	**marker name**	**map position**	**partial R -square**	***p*- value**	**effect estimate**	***p*-value t-test**
1.07	PZA02014.3	124.2	0.044	0.003	-0.17	<.0001
1.10	PZA02204.1	171.4	0.12	<.0001	-0.34	<.0001
2.04	PZA01993.7	64.2	0.035	0.0092	-0.16	0.0002
2.08	PZA02453.1	132.9	0.045	0.0036	-0.17	<.0001
3.04	PHM4145.18	50.2	0.054	0.014	-0.21	<.0001
3.04	PZA01114.2	55.5	0.023	0.0044	0.14	0.0022
5.01	PZA02753.1	27.5	0.042	0.0085	0.17	<.0001
5.04	PZA01796.1	75.6	0.037	0.0029	-0.17	0.0001
5.05	PZA03714.1	87.2	0.024	0.0089	0.14	0.0016
5.06	PHM532.23	101.9	0.036	0.0045	-0.17	0.0002
7.03	PHM9162.135	76.5	0.021	0.0063	0.11	0.0038
8.03	PHM1978.111	47.4	0.052	0.0088	-0.18	<.0001
9.06	PZA01096.1	69.4	0.064	0.0016	0.2	<.0001
10.03	PZA00409.17	36.5	0.044	<.0001	-0.11	0.0072
10.04	PZB01111.8	58.4	0.12	0.0021	-0.11	0.0046



1- No evidence of leaf blight
2- A few spots on the lower leaves
3-A few spots on the ear leaf
4-More spots on the ear leaf, but the lesions don't coalesce
5-Lesions on the ear leaf have grown together, particularly at the tip of the leaf to give quite large necrotic areas
6-Lesions on the leaf above the ear leaf have grown together too
7-Leaf above the ear leaf almost completely dead
8-Almost all tissue on the plant dead
9-Everything brown

Figure 1. Scale used for phenotyping Southern leaf blight

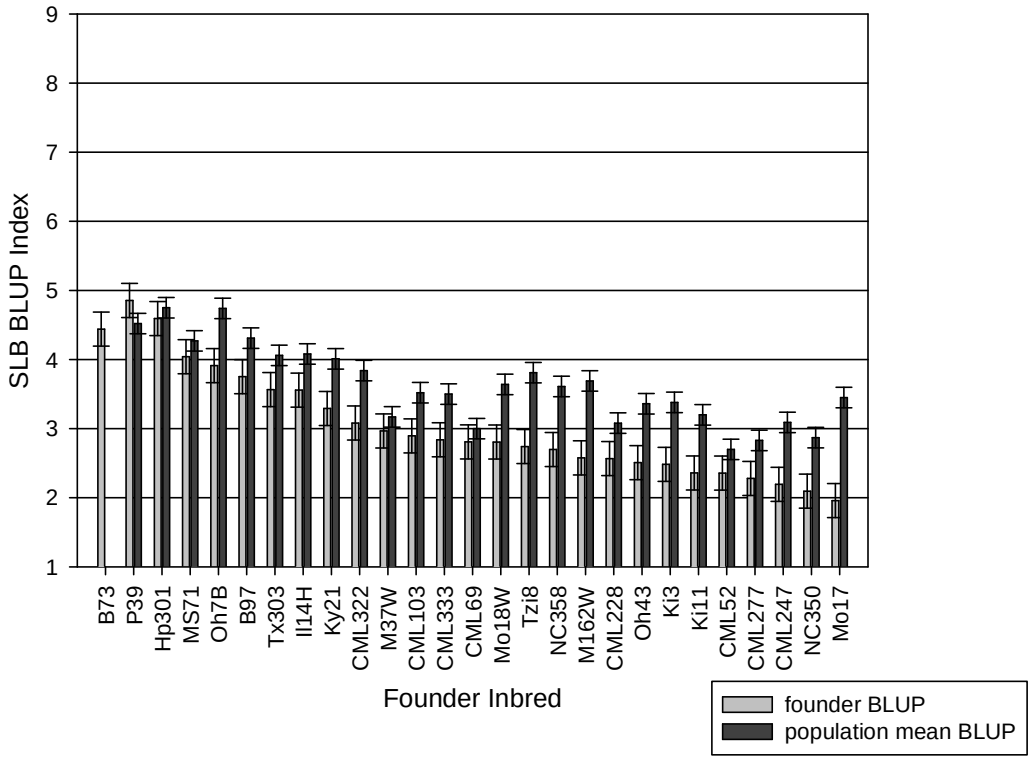


Figure 2. Graph of the equally weighted index of first and second Southern leaf blight (SLB) scores by family. The non-B73 Parent SLB BLUP index value is on the left and the average family index value is on the right. For the parent SLB BLUP indices, the error bars depict the average standard error of the difference between two parent checks. For family SLB BLUP indices, error bars depict the average standard error of the difference between families. The least significant difference between parents at the α=0.05 level is 0.49. The least significant difference between families is 0.30

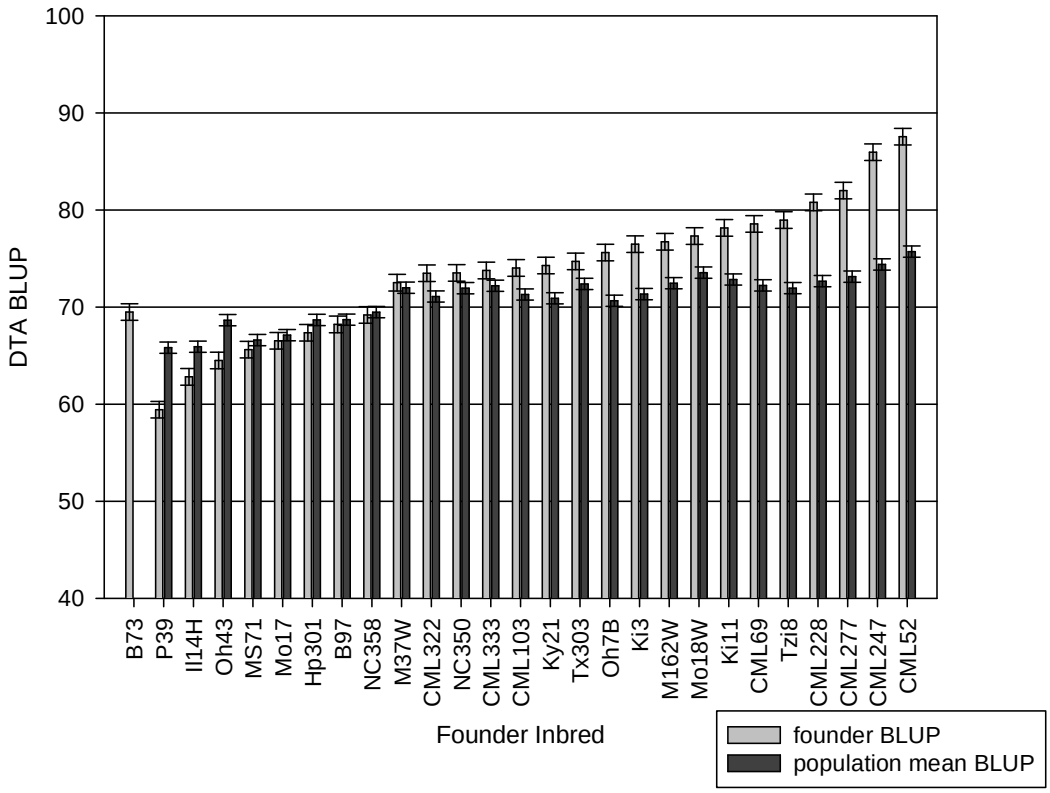


Figure 3. Graph of days to anthesis (DTA) values by family. Non-B73 parent DTA value is on the left and the average family DTA values is on the right. For the parent DTA values, the error bars depict the average standard error of the difference between two parent checks. For family DTA values, error bars depict the average standard error of the difference between families. The least significant difference between parents at the α=0.05 level is 1.72. The least significant difference between families is 1.16.

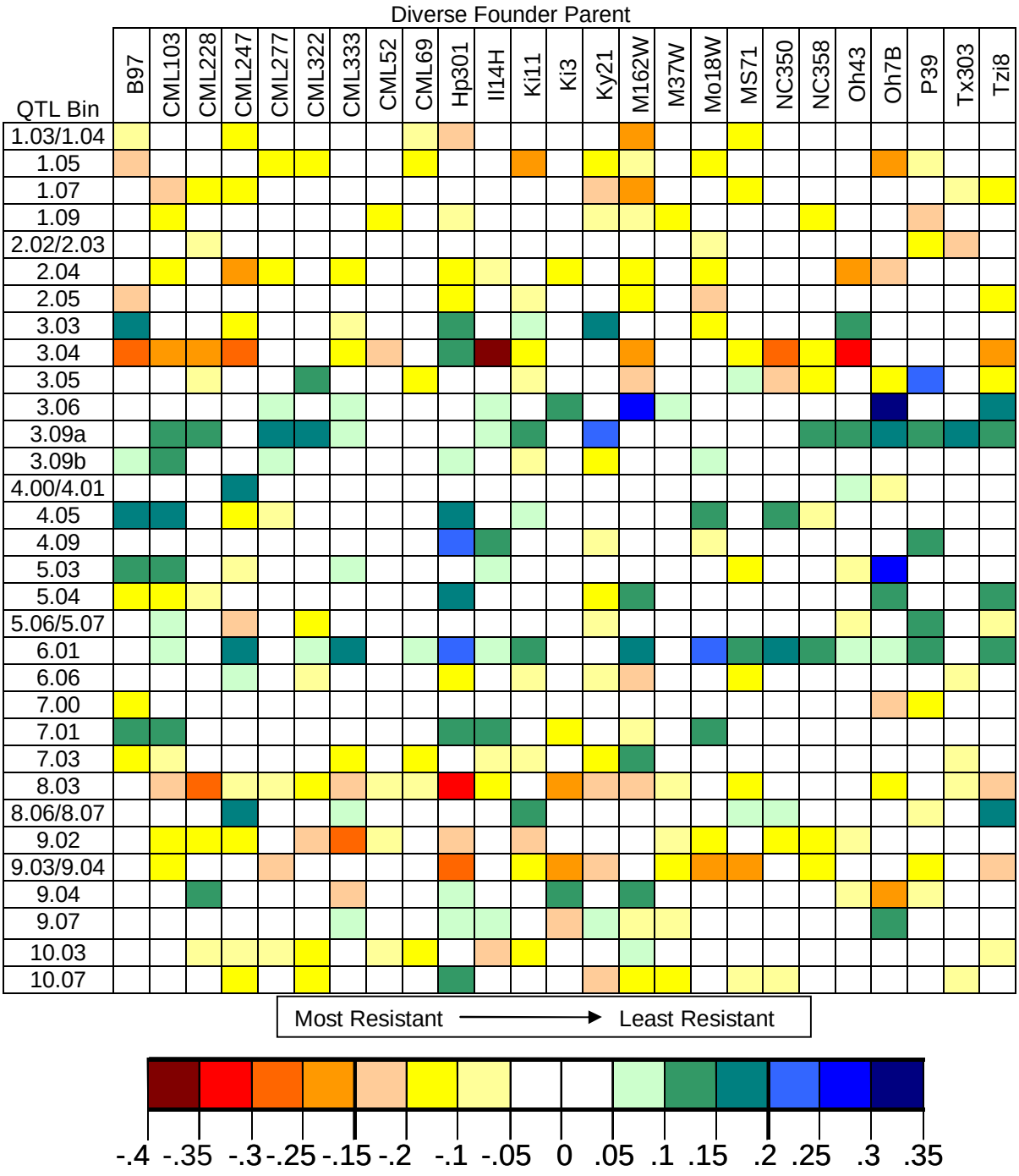
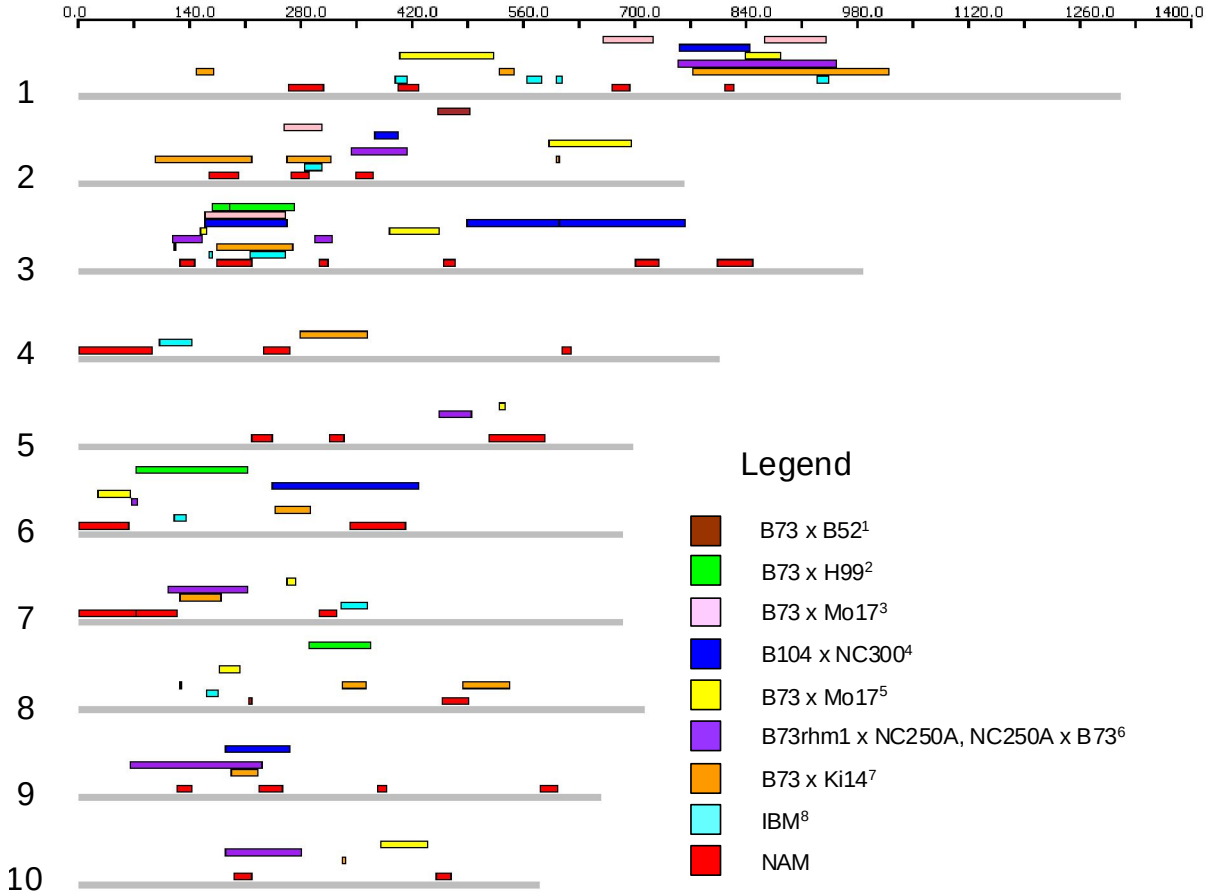


Figure 4. Heat map of significant additive effect estimates of the 25 founder inbred alleles at QTL for Southern leaf blight resistance (SLB) relative to B73 on the 1-9 scale. QTL are indicated by their chromosome and bin numbers (rows) and the allelic effect estimates for each founder allele (columns) are coded by color according to 0.05 score increments as shown in the legend.



[1,2] Balint-Kurti et al. 2008, [3,8] Balint-Kurti et al. 2007, [4] Balint-Kurti et al. 2006, [5] Balint-Kurti and Carson 2006, [6] Zwonitzer et al. 2009, [7] Zwonitzer 2008

Figure 5. Comparison of QTL identified in eight previous publications on the inheritance of Southern leaf blight resistance with QTL detected in this study. Map is presented in IBM2008 coordinates (Schaeffer et al., 2008).

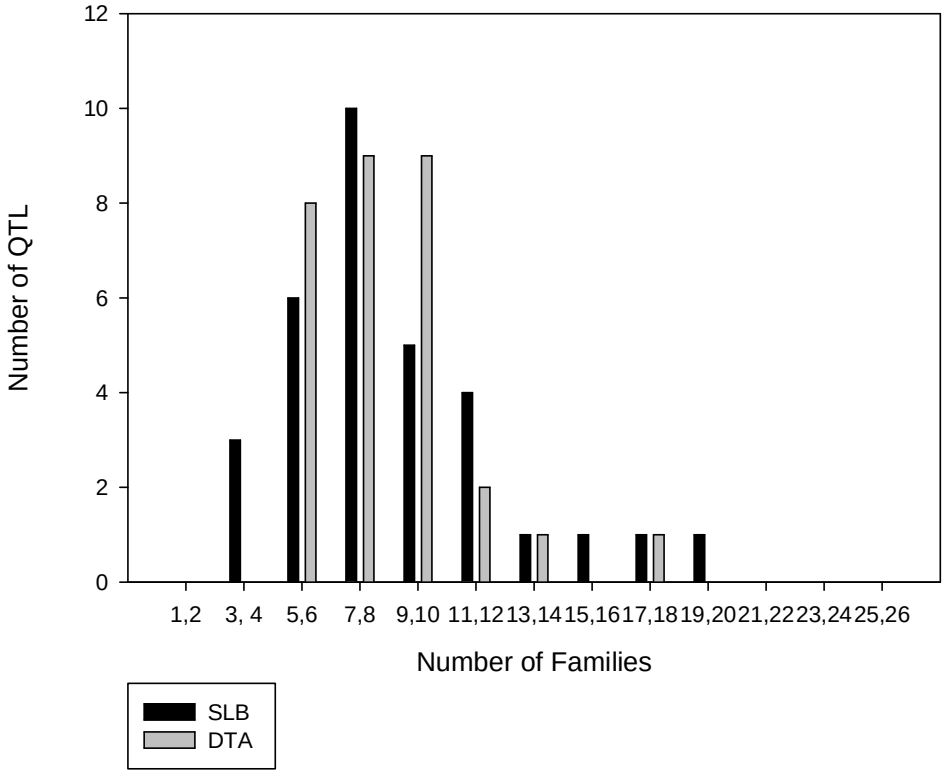


Figure 6. Graph of the number of families in which a Southern leaf blight resistance or days to anthesis QTL was detected. A QTL is considered to be detected in a family if its allele effect estimate is different from 0 at the α=0.05 level.

-APPENDICES-

Supplementary Material for Chapter 3

Table 1. Variance component estimates for random terms in the multivariate model of the first and second Southern leaf blight (SLB) scores. Standard errors of variance component estimates are given in parentheses.

Source of Variation	Variance of Score 1	Covariance of Scores 1 and 2	Variance of Score 2
Residual- NC06	0.136(0.004)	0.087(0.005)	0.183(0.008)
Residual-NC07	0.108(0.005)	0.04(0.006)	0.166(0.01)
Residual-FL	0.494(0.014)	0.297(0.015)	0.517(0.021)
Population	0.194(0.055)	0.304(0.087)	0.486(0.141)
Population*Environment	0.005(0.003)	0.012(0.005)	0.04(0.012)
Population-block(environment)-NC06	0.051(0.014)	0.022(0.007)	0.008(0.003)
Population-block(environment)-NC07	0.06(0.018)	0.02(0.022)	0.14(0.048)
Population-block(environment)-FL	0.169(0.053)	0.06(0.038)	0.058(0.033)
Block(population-block*environment)-NC06	0.019(0.003)	0.017(0.003)	0.022(0.004)
Block(population-block*environment)-FL	0.02(0.006)	0.017(0.006)	0.027(0.009)
Range(environment)-NC06	0.014(0.003)	0.022(0.004)	0.037(0.006)
Range(environment)-NC07	0.03(0.005)	0.042(0.007)	0.063(0.01)
Range(environment)-FL	0.011(0.006)	0(-0.004)	0(0.006)
Row(environment)-NC07	0.005(0.002)	0.005(0.002)	0.01(0.003)
Row(environment)-FL	0.013(0.004)	0.013(0.004)	0.015(0.005)
Entry(population B97)	0.178(0.027)	0.397(0.055)	0.935(0.14)
Entry(population CML103)	0.336(0.042)	0.508(0.058)	0.738(0.087)
Entry(population CML228)	0.273(0.035)	0.453(0.053)	0.706(0.084)
Entry(population CML247)	0.544(0.061)	0.723(0.08)	0.968(0.11)
Entry(population CML277)	0.144(0.021)	0.282(0.035)	0.494(0.061)
Entry(population CML322)	0.289(0.038)	0.363(0.044)	0.428(0.056)
Entry(population CML333)	0.477(0.056)	0.644(0.072)	0.847(0.098)
Entry(population CML52)	0.101(0.018)	0.203(0.027)	0.37(0.048)
Entry(population CML69)	0.18(0.026)	0.247(0.032)	0.338(0.045)
Entry(population Hp301)	0.459(0.056)	0.721(0.088)	1.363(0.165)
Entry(population IL14H)	0.337(0.043)	0.485(0.068)	0.85(0.133)
Entry(population Ki11)	0.323(0.04)	0.467(0.055)	0.665(0.081)
Entry(population Ki3)	0.288(0.046)	0.369(0.055)	0.422(0.07)
Entry(population Ky21)	0.388(0.048)	0.496(0.06)	0.606(0.083)
Entry(population M162W)	0.345(0.042)	0.551(0.069)	1.232(0.143)
Entry(population M37W)	0.215(0.03)	0.292(0.039)	0.415(0.059)
Entry(population IBM)	0.387(0.045)	0.502(0.058)	0.63(0.081)
Entry(population Mo18W)	0.27(0.036)	0.464(0.055)	0.765(0.092)
Entry(population MS71)	0.403(0.05)	0.261(0.075)	0.11(0.099)
Entry(population NC350)	0.328(0.042)	0.461(0.055)	0.635(0.079)
Entry(population NC358)	0.255(0.034)	0.319(0.041)	0.403(0.056)
Entry(population Oh43)	0.258(0.035)	0.375(0.047)	0.529(0.071)

Table 1. Continued

Source of Variation	Variance of Score 1	Covariance of Scores 1 and 2	Variance of Score 2
Entry(population P39)	0.427(0.057)	0.53(*)	0.77(*)
Entry(population Tx303)	0.133(0.022)	0.254(0.034)	0.436(0.058)
Entry(population Tzi8)	0.346(0.042)	0.484(0.055)	0.683(0.08)
Entry(population association)	0.79(1.162)	0.952(3.172)	1.226(0.13)
Entry(population)*environment	0.049(0.046)	0.038(0.005)	0.067(0.008)

* Standard error not estimated due to missing data.

Table 2. Variance component estimates for random terms in the mixed model for days to anthesis (DTA). Standard errors of variance component estimates are given in parentheses.

Source of Variation	Variance component (Standard error)
Residual Variance- NC06	0.093 (0.003)
Residual (AR1-range)-NC06	0.264 (0.018)
Residual (AR1-row)-NC06	0.153 (0.018)
Residual Variance- NC07	0.038 (0.002)
Residual (AR1-range)-NC07	0.222 (0.032)
Residual (AR1-row)-NC07	0.214 (0.033)
Residual Variance- FL	0.211 (0.006)
Residual (AR1-range)-FL	0.046 (0.019)
Residual (AR1-row)-FL	0.05 (0.02)
Environment	0.465 (0.47)
Population	0.211 (0.059)
Population*Environment	0.013 (0.003)
Population-block(environment)-NC07	0.007 (0.003)
Population-block(environment)-FL	0.055 (0.018)
Population-block(environment)-NC06	0.105 (0.028)
Block(Population-block*environment)-NC07	0.00008 (0.0004)
Block(Population-block*environment)-FL	0.002 (0.002)
Block(Population-block*environment)-NC06	0.024 (0.003)
Range(Environment)-NC06	0.001 (0.001)
Range(Environment)-NC07	0.002 (0.001)
Range(Environment)-FL	0.0003 (0.001)
Row(Environment)-NC06	0.004 (0.001)
Row(Environment)-NC07	0.004 (0.001)
Row(Environment)-FL	0.016 (0.004)
Entry(Population B97)	0.065 (0.01)
Entry(Population CML103)	0.061 (0.01)
Entry(Population CML228)	0.174 (0.021)
Entry(Population CML247)	0.265 (0.03)
Entry(Population CML277)	0.221 (0.026)
Entry(Population CML322)	0.114 (0.015)
Entry(Population CML333)	0.13 (0.017)
Entry(Population CML52)	0.181 (0.022)
Entry(Population CML69)	0.123 (0.016)
Entry(Population Hp301)	0.106 (0.015)
Entry(Population IL14H)	0.105 (0.015)
Entry(Population Ki11)	0.173 (0.021)
Entry(Population Ki3)	0.115 (0.018)
Entry(Population Ky21)	0.067 (0.011)
Entry(Population M162W)	0.124 (0.016)
Entry(Population M37W)	0.116 (0.015)
Entry(Population IBM)	0.139 (0.017)
Entry(Population Mo18W)	0.179 (0.022)
Entry(Population MS71)	0.041 (0.008)

Table 2. Continued

Source of Variation	Variance component (Standard error)
Entry(Population Oh43)	0.055 (0.01)
Entry(Population Oh7B)	0.114 (0.016)
Entry(Population P39)	0.18 (0.023)
Entry(Population Tx303)	0.179 (0.022)
Entry(Population Tzi8)	0.149 (0.018)
Entry(Population association)	2.366 (0.202)
Entry(Population)*Environment	0.026 (0.002)

Table 3. Additive effect estimates for QTL conditioning resistance to Southern leaf blight (SLB) in each family of the Nested Association Mapping (NAM) panel.

Bin	B97	CML103	CML228	CML247	CML277	CML322	CML333	CML52	CML69	Hp301	Il14H	Ki11	Ki3
1.03/1.04	-0.099*	0.020	0.019	-0.138*	-0.031	-0.036	-0.047	0.005	-0.075*	-0.199*	-0.032	-0.021	0.038
1.05	-0.177*	-0.025	-0.037	-0.010	-0.132*	-0.101*	-0.046	-0.028	-0.103*	-0.016	-0.016	-0.224*	-0.019
1.07	-0.008	-0.154*	-0.110*	-0.149*	-0.078	-0.079	-0.069	-0.039	-0.059	-0.070	-0.084	-0.037	-0.096
1.09	0.052	-0.103*	0.012	0.069	-0.056	-0.069	0.006	-0.111*	0.005	-0.082*	0.016	-0.029	0.038
2.02/2.03	0.043	-0.046	-0.093*	-0.032	-0.023	-0.046	-0.038	-0.070	-0.034	0.068	0.008	-0.001	0.008
2.04	-0.029	-0.143*	-0.076	-0.240*	-0.113*	0.004	-0.128*	-0.076	-0.078	-0.143*	-0.099*	-0.093*	-0.134*
2.05	-0.195*	0.043	-0.058	0.007	-0.008	0.024	-0.031	0.021	0.038	-0.117*	-0.037	-0.074	0.021
3.03	0.156*	-0.011	-0.042	-0.101*	-0.044	-0.068	-0.085*	-0.064	-0.054	0.131*	0.031	0.076*	-0.011
3.04	-0.266*	-0.205*	-0.209*	-0.281*	-0.067	0.066	-0.141*	-0.160*	-0.065	0.147*	-0.380*	-0.130*	-0.090
3.05	-0.027	-0.068	-0.098*	-0.054	-0.023	0.105*	-0.034	-0.077	-0.069*	-0.050	-0.064	-0.093*	0.103
3.06	0.019	0.055	-0.023	0.058	0.074*	0.034	0.080*	0.056	0.029	0.012	0.088	-0.008	0.114*
3.09a	-0.035	0.122*	0.100*	0.038	0.154*	0.177*	0.076*	0.051	0.031	-0.007	0.058	0.102*	0.000
3.09b	-0.128	-0.072*	0.049	0.002	0.040	-0.1232	-0.058	-.103	-0.007	-0.077*	0.047	-0.132*	0.1458
4.00/4.01	0.036	0.055	0.048	0.175*	-0.033	0.018	0.002	0.019	0.065	0.051	0.043	0.057	-0.004
4.05	0.150*	0.165*	-0.013	-0.118*	-0.074*	-0.045	0.036	0.001	0.059	0.200*	-0.043	0.084*	0.091
4.09	-0.058	-0.006	0.025	-0.036	0.023	0.055	0.012	-0.008	0.037	0.224*	0.112*	-0.028	0.030
5.03	0.120*	0.134*	-0.032	-0.083*	0.069	0.035	0.077*	-0.050	-0.030	0.052	0.097*	-0.061	0.002
5.04	-0.135*	-0.121*	-0.082*	0.038	0.009	-0.072	-0.038	0.019	0.017	0.199*	-0.004	0.006	0.051
5.06/5.07	-0.006	0.092*	-0.033	-0.162*	-0.038	-0.107	-0.055	-0.008	-0.034	0.071	-0.069	-0.004	-0.063
6.01	-0.021	0.078*	0.050	0.211*	0.061	0.089*	0.173*	0.035	0.080*	0.202*	0.082*	0.102*	0.090
6.06	0.031	-0.039	0.020	0.093*	-0.067	-0.082*	0.052	-0.037	0.056	-0.133*	-0.067	-0.084*	0.015
7.00	-0.117*	-0.063	-0.047	0.008	-0.040	-0.043	0.010	0.022	-0.063	-0.049	-0.045	-0.056	-0.029
7.01	0.114*	0.109*	-0.033	0.070	0.058	0.032	-0.036	-0.039	0.027	0.137*	0.120*	0.070	-0.135*
7.03	-0.128*	-0.072*	0.049	0.002	-0.020	0.040	-0.123*	-0.058	-0.103*	-0.007	-0.069*	-0.077*	-0.063
8.03	-0.024	-0.198*	-0.263*	-0.089*	-0.073*	-0.108*	-0.192*	-0.073*	-0.099*	-0.345*	-0.130*	-0.037	-0.236*
8.06/8.07	0.070	0.034	0.056	0.201*	0.052	0.060	0.095*	-0.007	0.018	-0.048	0.068	0.129*	0.055
9.02	0.048	-0.135*	-0.147*	-0.135*	-0.061	-0.182*	-0.275*	-0.087*	-0.054	-0.163*	0.033	-0.187*	-0.008

* Significant at the α=.05 level.

Table 3. Continued

Bin	B97	CML103	CML228	CML247	CML277	CML322	CML333	CML52	CML69	Hp301	Il14H	Ki11	Ki3
9.03/9.04	0.020	-0.108*	-0.072	-0.011	-0.174*	0.005	-0.086	-0.078	-0.087	-0.251*	-0.081	-0.144*	-0.249*
9.04	0.074	0.077	0.138*	-0.052	-0.034	0.016	-0.153*	0.002	-0.023	0.086*	-0.069	0.058	0.116*
9.07	-0.052	0.047	0.005	-0.021	-0.010	0.027	0.059	-0.024	0.068	0.099*	0.088*	0.063	-0.158*
10.03	0.063	-0.043	-0.085*	-0.093*	-0.077*	-0.126*	0.028	-0.079*	-0.117*	-0.038	-0.184*	-0.108*	0.046
10.07	-0.048	-0.014	-0.022	-0.129*	0.030	-0.110*	-0.013	-0.018	-0.057	0.102*	-0.036	-0.036	-0.006

* Significant at the α=.05 level.

Table 3. Continued

Bin	Ky21	M162W	M37W	Mo18W	MS71	NC350	NC358	Oh43	Oh7B	P39	Tx303	Tzi8
1.03/1.04	0.020	-0.202*	0.072	0.017	-0.111*	-0.053	-0.052	-0.007	-0.031	-0.059	-0.023	-0.056
1.05	-0.109*	-0.088*	-0.073	-0.137*	0.002	-0.069	-0.055	-0.021	-0.246*	-0.096*	-0.041	0.003
1.07	-0.191*	-0.206*	-0.080	-0.047	-0.104*	-0.046	-0.026	-0.024	-0.052	-0.023	-0.088*	-0.104*
1.09	-0.080*	-0.099*	-0.145*	0.066	0.012	0.015	-0.115*	-0.028	-0.043	-0.161*	0.056	-0.065
2.02/2.03	0.050	-0.069	-0.049	-0.084*	-0.034	-0.032	-0.063	-0.047	-0.050	-0.134*	-0.160*	0.066
2.04	0.033	-0.125*	-0.034	-0.110*	0.039	-0.096	-0.091	-0.200*	-0.163*	0.071	-0.089	-0.098
2.05	0.038	-0.101*	-0.053	-0.182*	-0.060	0.047	-0.030	0.049	0.077	-0.053	0.052	-0.120*
3.03	0.170*	0.061	-0.031	-0.102*	0.035	-0.025	-0.073	0.123*	0.042	0.015	-0.006	-0.021
3.04	-0.094	-0.207*	-0.020	-0.035	-0.102*	-0.266*	-0.143*	-0.318*	-0.032	-0.076	0.023	-0.220*
3.05	0.066	-0.177*	-0.042	0.000	0.095*	-0.170*	-0.112*	0.009	-0.141*	0.200*	0.020	-0.105*
3.06	0.035	0.267*	0.089*	0.023	0.027	0.008	-0.002	0.070	0.311*	0.044	0.018	0.163*
3.09a	0.208*	0.045	0.060	-0.013	0.017	-0.001	0.150*	0.108*	0.164*	0.141*	0.163*	0.121*
3.09b	0.146*	0.075	-0.057	-0.079*	-0.017	-0.072	-0.055	0.018	-0.049	-0.060	-0.033	0.015
4.00/4.01	0.061	-0.013	0.034	-0.055	0.026	0.044	0.023	0.068*	-0.077*	0.030	0.039	0.050
4.05	-0.028	0.030	0.023	0.100*	-0.012	0.108*	-0.072*	-0.036	0.060	0.024	0.037	0.004
4.09	-0.092*	0.040	0.024	-0.087*	-0.024	-0.022	0.003	0.032	0.059	0.108*	0.016	-0.031
5.03	0.061	-0.022	0.047	-0.009	-0.124*	0.017	0.028	-0.084*	0.261*	0.040	0.046	-0.012
5.04	-0.134*	0.121*	0.013	0.000	0.057	0.087	0.067	0.003	0.125*	-0.074	-0.076	0.149*
5.06/5.07	-0.076*	-0.015	0.023	-0.046	-0.024	-0.021	-0.051	-0.070*	-0.020	0.123*	0.038	-0.089*
6.01	0.018	0.155*	0.036	0.214*	0.129*	0.158*	0.130*	0.081*	0.083*	0.121*	0.046	0.126*
6.06	-0.071*	-0.164*	-0.060	0.020	-0.108*	0.059	0.043	0.046	-0.017	-0.006	-0.076*	0.027
7.00	-0.035	-0.050	-0.014	-0.004	0.016	-0.008	0.005	0.001	-0.172*	-0.137*	0.014	-0.039
7.01	0.008	-0.093*	0.070	0.104*	0.037	0.026	-0.013	0.041	0.009	0.014	-0.019	0.048
7.03	-0.132*	0.146*	-0.003	-0.057	-0.004	0.046	-0.007	-0.009	-0.039	0.054	-0.090*	0.015
8.03	-0.183*	-0.172*	-0.088*	-0.022	-0.113*	-0.080*	0.011	-0.074	-0.142*	-0.040	-0.080*	-0.182*
8.06/8.07	0.044	0.066	0.032	0.032	0.074*	0.054	0.063	0.025	0.033	-0.072*	0.010	0.194*

* Significant at the α=.05 level.

Table 3. Continued

Bin	Ky21	M162W	M37W	Mo18W	MS71	NC350	NC358	Oh43	Oh7B	P39	Tx303	Tzi8
9.02	-0.004	-0.003	-0.090*	-0.136*	0.057	-0.140*	-0.109*	-0.074*	0.026	-0.039	0.004	0.043
9.03/9.04	-0.171*	-0.049	-0.139*	-0.229*	-0.235*	-0.025	-0.112*	0.003	-0.049	-0.116*	-0.063	-0.171*
9.04	0.054	0.104*	-0.001	0.073	-0.037	0.037	0.001	-0.095*	-0.238*	-0.087*	-0.037	-0.062
9.07	0.078*	-0.099*	-0.091*	0.063	0.056	-0.018	0.007	0.024	0.142*	-0.047	-0.003	0.035
10.03	0.007	0.076*	-0.055	-0.026	0.012	-0.067	-0.054	0.008	-0.056	-0.021	-0.037	-0.088*
10.07	-0.165*	-0.125*	-0.105*	-0.005	-0.073*	-0.096*	-0.066	-0.058	0.049	0.036	-0.093*	-0.011

* Significant at the α=.05 level

Table 4. Additive effect estimates for QTL conditioning days to anthesis (DTA) in each family of the Nested Association Mapping (NAM) panel.

bin	B97	CML103	CML228	CML247	CML277	CML322	CML333	CML52	CML69	Hp301	Il14H	Ki11	Ki3
1.01	-0.094	-0.029	0.317*	0.192	-0.559*	0.061	0.004	0.308*	-0.214*	0.055	0.241*	0.091	0.364*
1.03/1.04	0.009	-0.130	0.440*	0.135	0.280*	0.156	0.251*	0.222*	-0.206	0.316*	0.132	-0.159	0.103
1.05	0.297*	0.196	0.263*	-0.039	0.458*	0.411*	0.356*	0.415*	0.122	0.210	0.018	0.329*	0.033
1.06/1.07	-0.251*	0.198	-0.052	-0.443*	-0.118	-0.178	-0.222	-0.355*	-0.269*	0.323*	-0.413*	0.000	-0.202
1.11	-0.292*	0.014	-0.422*	-0.122	0.610*	-0.304*	0.092	0.013	-0.024	-0.158	-0.231*	-0.110	-0.627*
2.04/2.05	0.170	0.070	0.235*	0.573*	-0.043	-0.064	0.067	0.398*	-0.236*	-0.377*	0.195	0.129	-0.030
2.07	0.009	0.084	-0.059	0.490*	0.144	0.310*	-0.086	0.247*	0.572*	0.251*	0.375*	0.138	0.753*
2.08	0.268	0.036	0.506*	0.088	0.477*	0.240	0.080	0.787*	0.213	0.547*	-0.044	-0.039	0.702*
3.04	-0.373	-0.039	0.434*	0.744*	-0.214	0.252	0.626*	-0.289	-0.018	-0.064	-0.102	0.494*	-0.244
3.05	0.087	-0.239	-0.249	0.076	-0.207	-0.718*	-0.383*	0.420*	-0.102	0.012	-0.329*	-0.246	-0.063
3.07	0.239	0.227*	0.301*	0.070	0.434*	0.243*	0.340*	0.440*	0.273*	0.111	0.221	0.418*	0.064
3.08/3.09	0.107	0.068	0.666*	0.647*	0.146	0.293	0.064	-0.426*	0.144	0.087	0.066	0.068	0.477*
3.09	0.000	0.187	-0.571*	-0.181	0.876*	-0.001	0.101	0.617*	-0.116	0.119	0.057	-0.266*	-0.254
4.04/4.05	0.063	0.068	0.426*	0.478*	-0.043	0.099	0.263*	0.233*	0.364*	-0.139	-0.065	0.243*	-0.040
4.08	-0.229	0.141	-0.151	0.570*	0.095	-0.196	0.142	0.194	-0.009	0.030	0.175	-0.021	0.206
4.09/4.10/4.11	0.004	-0.142	0.019	-0.249*	-0.148	-0.008	-0.387*	-0.263*	-0.046	-0.394*	-0.031	-0.522*	0.039
5	-0.058	0.051	0.008	0.285*	0.092	0.005	-0.073	0.357*	0.519*	0.072	0.206	0.216	0.036
9.04	-0.149	-0.099	0.319*	-0.760*	-0.081	-0.185	-0.238	-0.205	-0.572*	-0.143	0.021	-0.175	-0.241
5.04	0.115	0.012	-0.533*	-0.433*	-0.161	-0.287*	-0.050	-0.341*	-0.021	-0.164	-0.074	-0.046	-0.012
5.06	-0.022	-0.108	0.045	0.579*	0.157	-0.333*	0.422*	0.532*	0.267*	-0.165	-0.125	0.025	0.612*
6.05/6.06	-0.207	0.086	0.051	-0.086	-0.001	-0.196	0.348*	-0.129	0.156	-0.269*	0.147	-0.066	-0.078
6.07	0.154	0.071	-0.195	-0.269*	-0.103	0.293*	0.051	0.144	-0.138	-0.229*	-0.076	0.028	-0.162
7.02	-0.034	0.017	-0.304*	0.916*	0.028	0.043	-0.107	0.072	0.280*	-0.078	0.264*	0.117	-0.216
8.02/8.03	0.115	0.062	0.235*	0.085	0.076	0.106	-0.246*	-0.088	0.279*	0.056	0.010	-0.422*	-0.230
8.05	0.165	-0.127	-0.639*	0.229	-0.151	-0.108	-0.438*	0.043	0.120	0.047	-0.117	-0.182	-0.162

* Significant at the α=.05 level.

Table 4. Continued

bin	B97	CML103	CML228	CML247	CML277	CML322	CML333	CML52	CML69	Hp301	Il14H	Ki11	Ki3
8.07/8.08	0.024	0.366*	0.788*	0.527*	0.268*	0.735*	0.661*	0.570*	0.412*	0.074	-0.733*	0.129	0.542*
9.03	0.138	0.061	0.035	0.374*	-0.028	0.021	-0.100	0.100	-0.032	0.115	0.051	-0.103	0.018
9.06/9.07	0.021	0.151	0.526*	0.572*	0.728*	0.568*	0.345*	0.899*	0.231	0.405*	-0.057	0.821*	0.384*
10.04	0.107	-0.057	0.540*	0.199	0.962*	-0.269*	-0.004	0.272*	0.239*	-0.071	0.109	0.897*	-0.209
10.07	-0.197	-0.171	-0.027	-0.367*	0.023	-0.018	-0.278*	-0.473*	-0.187	-0.367*	0.026	0.100	-0.192

* Significant at the α=.05 level.

Table 4. Continued

bin	Ky21	M162W	M37W	Mo18W	MS71	NC350	NC358	Oh43	Oh7B	P39	Tx303	Tzi8
1.01	-0.003	-0.017	-0.110	0.279*	0.068	-0.302*	-0.116	-0.163	0.227*	0.006	0.154	-0.075
1.03/1.04	0.223	0.419*	0.508*	0.487*	0.053	0.047	0.114	0.171	0.210	0.010	0.343*	0.125
1.05	0.061	0.238	0.046	0.195	0.065	0.297*	0.087	-0.036	0.568*	-0.003	0.148	0.192
1.06/1.07	-0.035	-0.283*	-0.242*	0.010	-0.118	-0.109	-0.079	-0.197	-0.190	-0.433*	0.257*	0.058
1.11	-0.007	0.016	-0.152	0.243*	-0.010	-0.177	-0.208	-0.129	-0.362*	-0.152	-0.133	0.023
2.04/2.05	0.017	0.794*	0.257*	0.167	0.005	0.033	0.159	-0.216*	0.661*	-0.112	-0.042	0.807*
2.07	0.294*	0.155	0.165	0.605*	0.161	0.225*	0.338*	0.071	0.112	-0.179	0.409*	0.333*
2.08	-0.112	0.395*	0.023	-0.257	0.015	-0.149	-0.095	0.130	0.204	-0.559*	0.027	0.256
3.04	0.058	0.321	0.294	0.409*	-0.029	0.341	0.138	0.222	-0.182	0.428*	-0.199	0.344
3.05	-0.211	-0.029	-0.284	-0.296	-0.093	-0.204	-0.078	-0.123	-0.470*	-0.620*	-0.432*	0.321*
3.07	0.121	-0.158	0.167	0.404*	0.079	0.381*	0.452*	0.059	-0.110	0.064	-0.101	0.065
3.08/3.09	0.017	0.169	-0.163	0.475*	0.069	0.369*	-0.070	0.357*	0.363*	0.341*	0.806*	0.004
3.09	-0.058	0.331*	0.531*	-0.154	0.000	0.043	0.144	-0.264	-0.056	-0.076	-0.094	0.001
4.04/4.05	0.039	0.158	0.210*	-0.065	0.015	0.068	0.091	-0.021	0.132	0.179	-0.009	0.184
4.08	0.010	0.056	0.466*	0.059	0.045	-0.118	-0.283*	-0.022	-0.040	0.497*	-0.106	-0.304*
4.09/4.10/4.11	-0.174	-0.384*	0.048	-0.018	-0.020	0.356*	0.079	-0.037	-0.104	-0.251*	0.161	-0.349*
5	0.217	0.132	-0.170	0.238*	-0.011	0.104	-0.204	0.072	-0.272*	-0.368*	-0.176	0.018
9.04	0.078	-0.474*	-0.184	-0.622*	-0.073	-0.558*	-0.322*	-0.103	0.209	0.104	-0.160	0.216
5.04	-0.271*	-0.311*	-0.067	-0.045	-0.096	-0.258*	-0.063	-0.038	-0.207	-0.255*	-0.028	-0.276*
5.06	0.130	0.081	0.068	0.179	0.073	0.047	-0.076	-0.025	0.013	0.384*	-0.066	0.602*
6.05/6.06	-0.072	-0.066	-0.089	0.402*	-0.064	-0.294*	-0.032	-0.115	-0.013	-0.441*	0.241*	0.211
6.07	0.081	0.219	0.128	0.037	0.196	0.566*	-0.184	0.009	0.155	-0.142	-0.012	-0.258*
7.02	0.181	-0.111	0.335*	0.152	0.001	0.347*	0.019	-0.064	-0.024	0.309*	-0.089	0.081
8.02/8.03	0.110	0.315*	-0.243*	0.329*	0.071	-0.132	-0.270*	-0.076	0.054	0.014	-0.202	-0.411*
8.05	-0.131	-0.158	-0.017	0.184	-0.097	-0.183	-0.139	-0.261*	-0.286	-0.448*	0.372*	-0.002

* Significant at the α=.05 level.

Table 4. Continued

bin	Ky21	M162W	M37W	Mo18W	MS71	NC350	NC358	Oh43	Oh7B	P39	Tx303	Tzi8
8.07/8.08	0.287*	0.273*	0.166	0.701*	-0.215	0.303*	0.348*	0.074	0.292	-0.587*	0.295*	0.205
9.03	0.182	-0.020	-0.123	0.359*	0.076	0.108	0.158	-0.244*	0.187	-0.362*	0.002	-0.355*
9.06/9.07	0.181	0.164	0.153	0.552*	0.025	0.094	-0.107	0.056	0.141	0.217	0.136	0.321*
10.04	0.157	0.121	-0.071	0.124	0.008	-0.161	0.044	-0.084	0.144	0.022	-0.342*	0.035
10.07	-0.062	-0.247*	-0.234*	-0.427*	-0.112	-0.024	0.109	-0.060	0.086	-0.406*	0.114	0.038

* Significant at the α=.05 level.

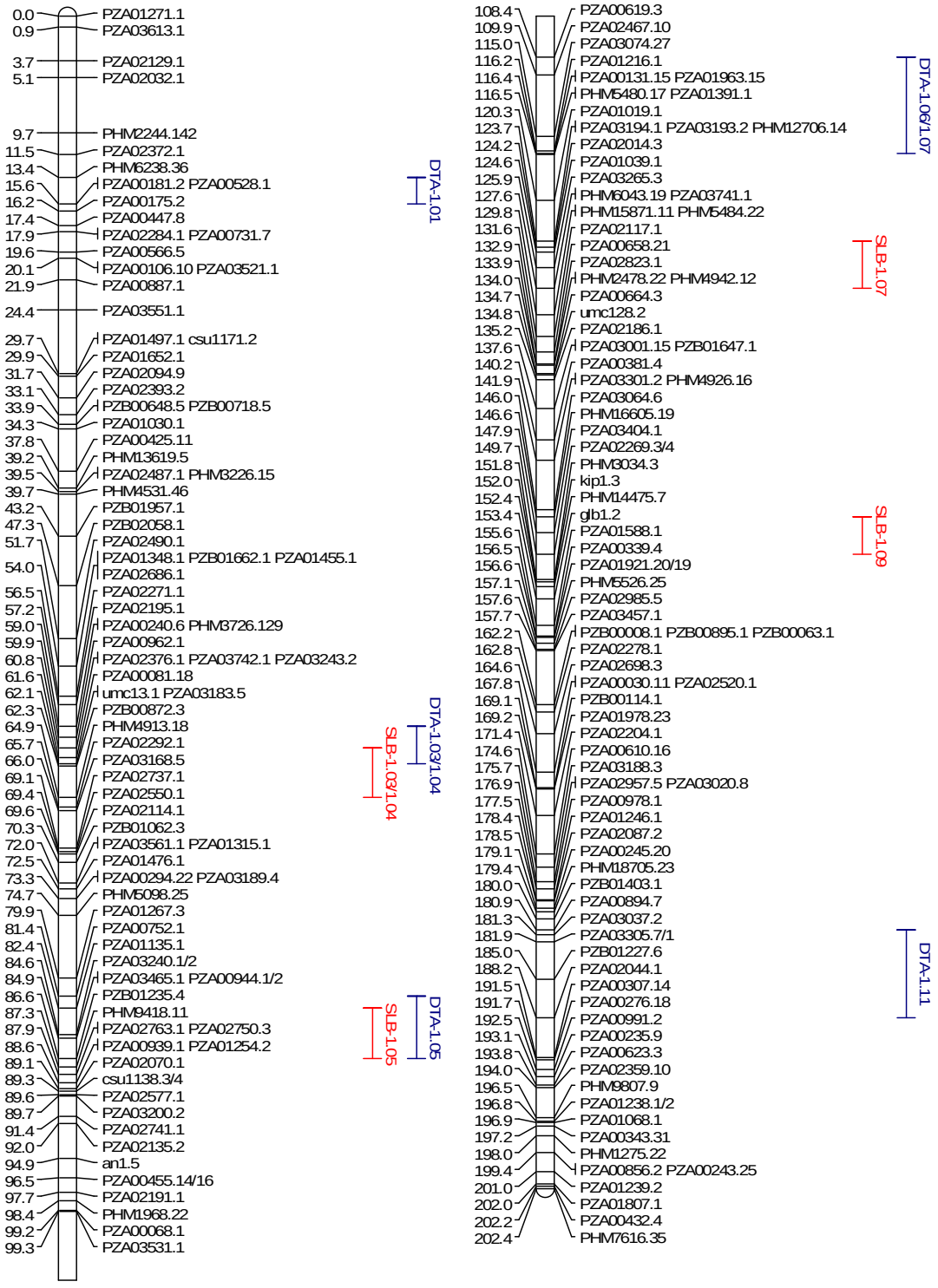


Figure 1. Linkage map of chromosome 1. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

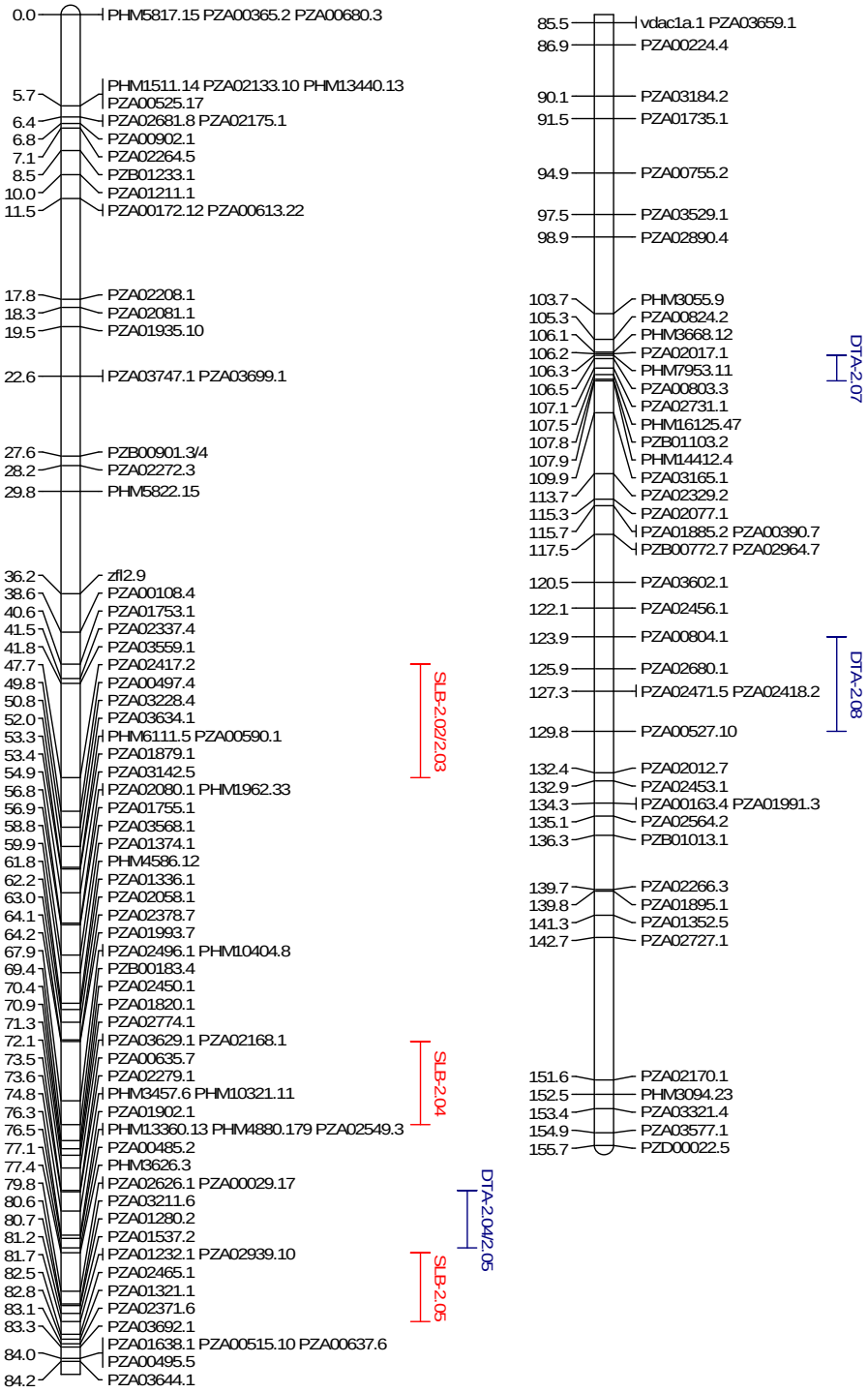


Figure 2. Linkage map of chromosome 2. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

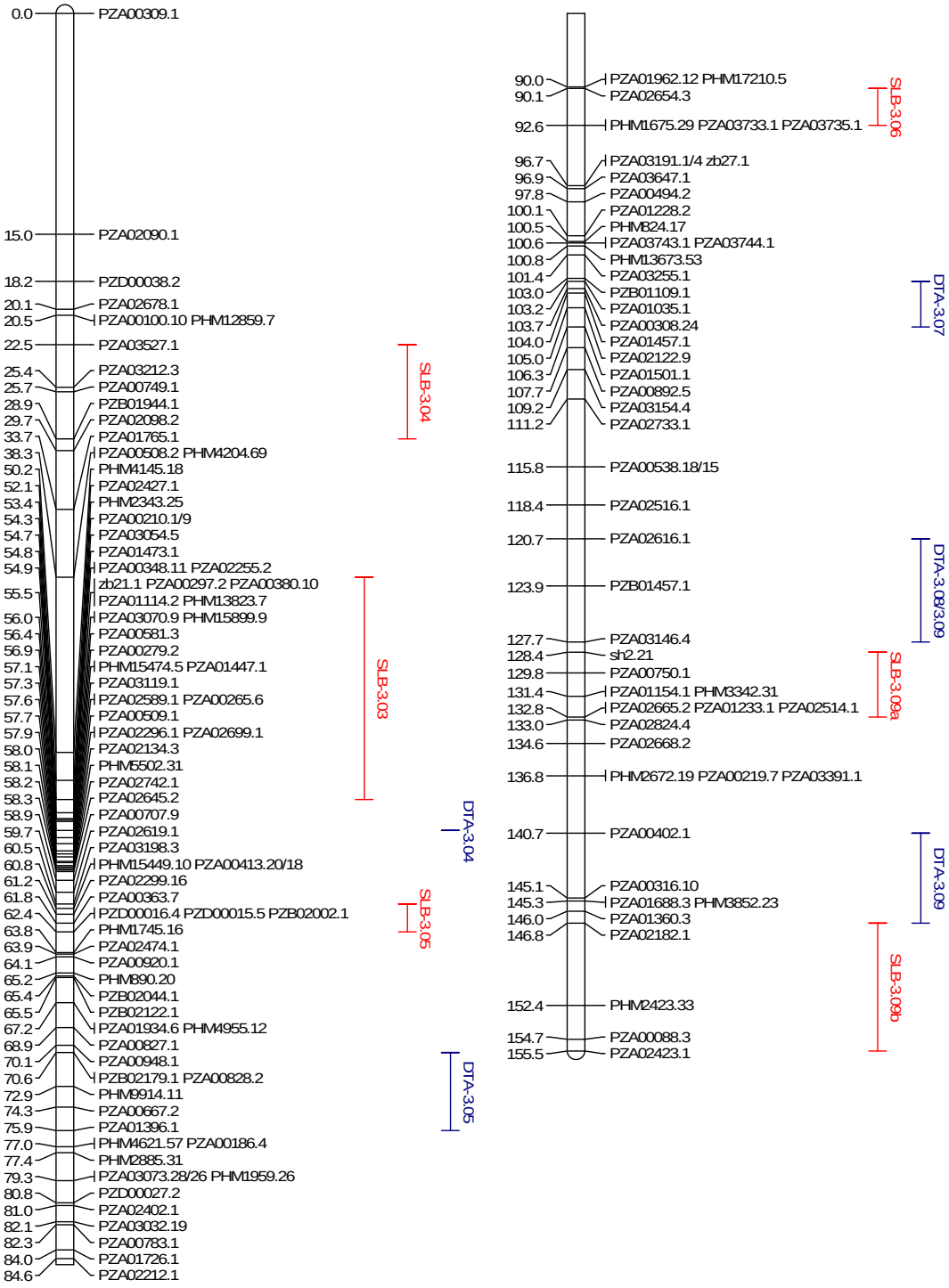


Figure 3. Linkage map of chromosome 3. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

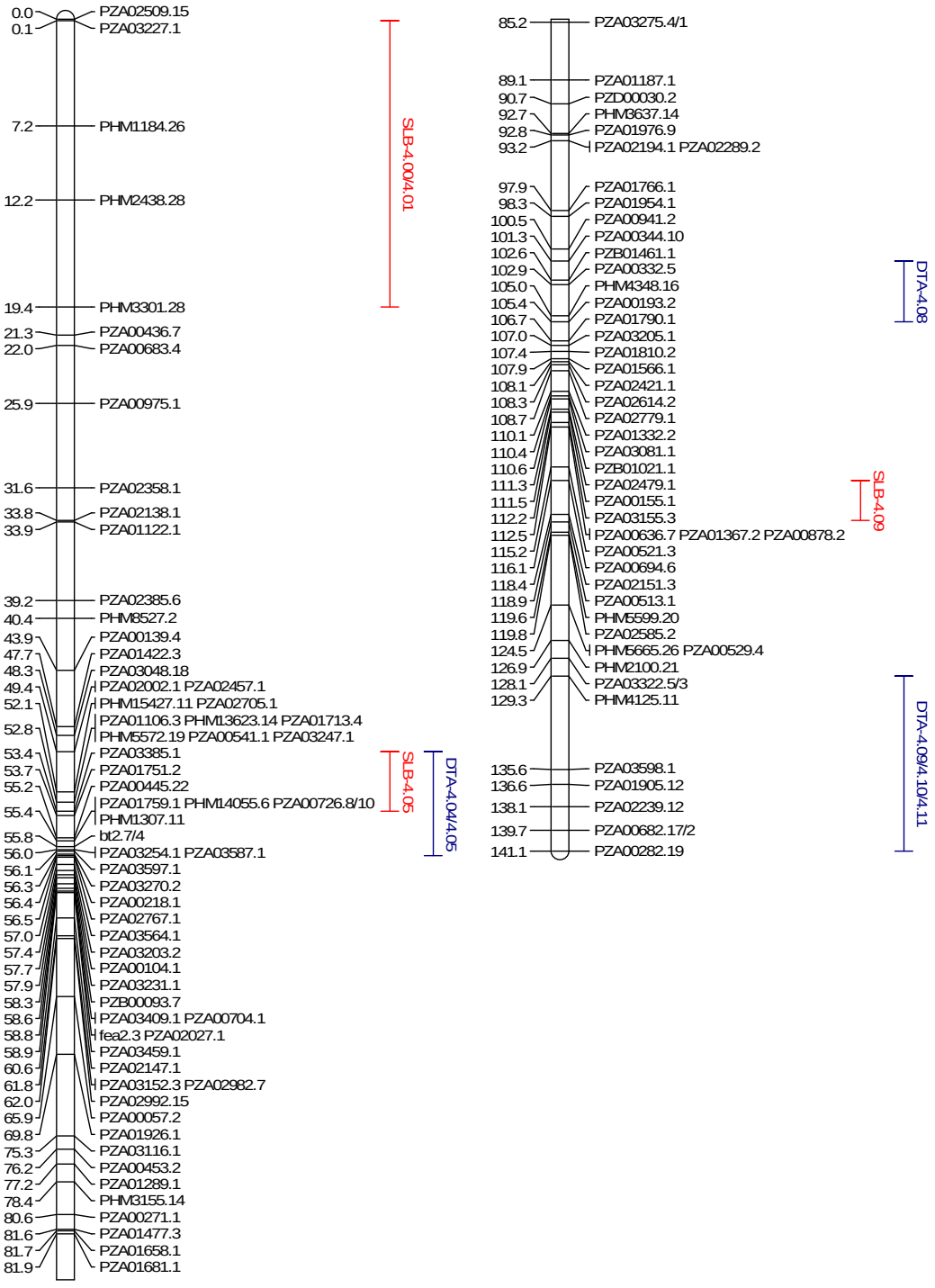


Figure 4. Linkage map of chromosome 4. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

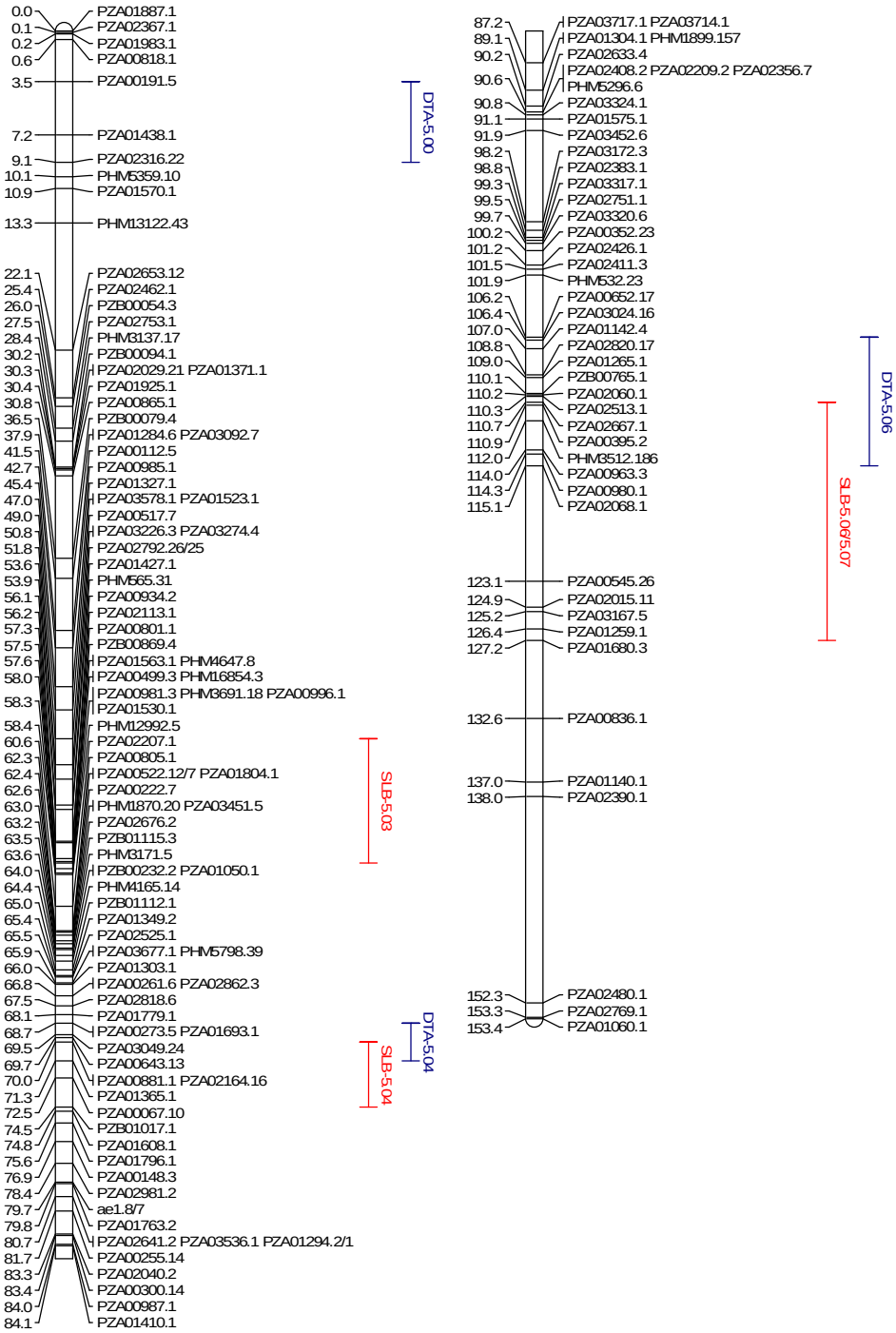


Figure 5. Linkage map of chromosome 5. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

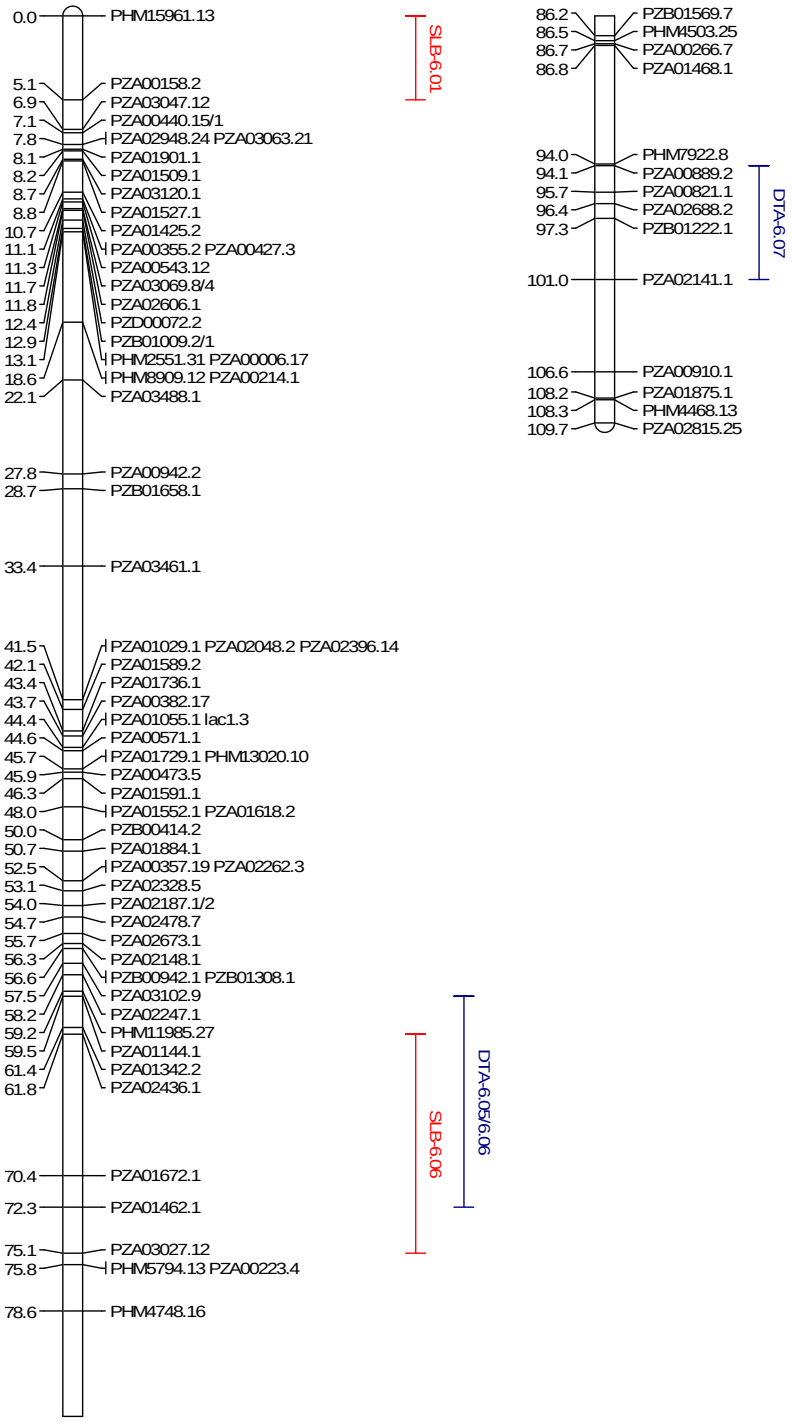


Figure 6. Linkage map of chromosome 6. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

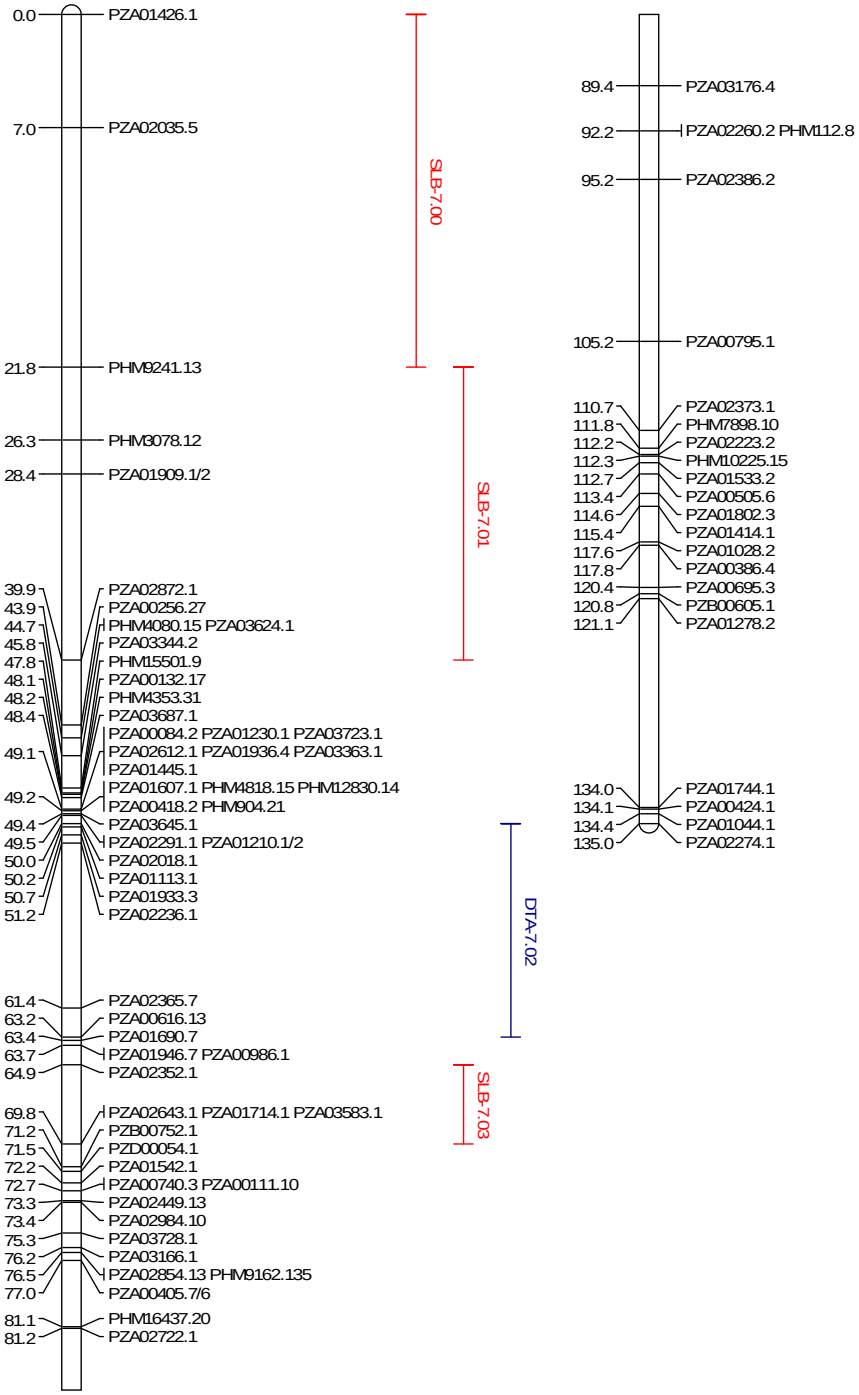


Figure 7. Linkage map of chromosome 7. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

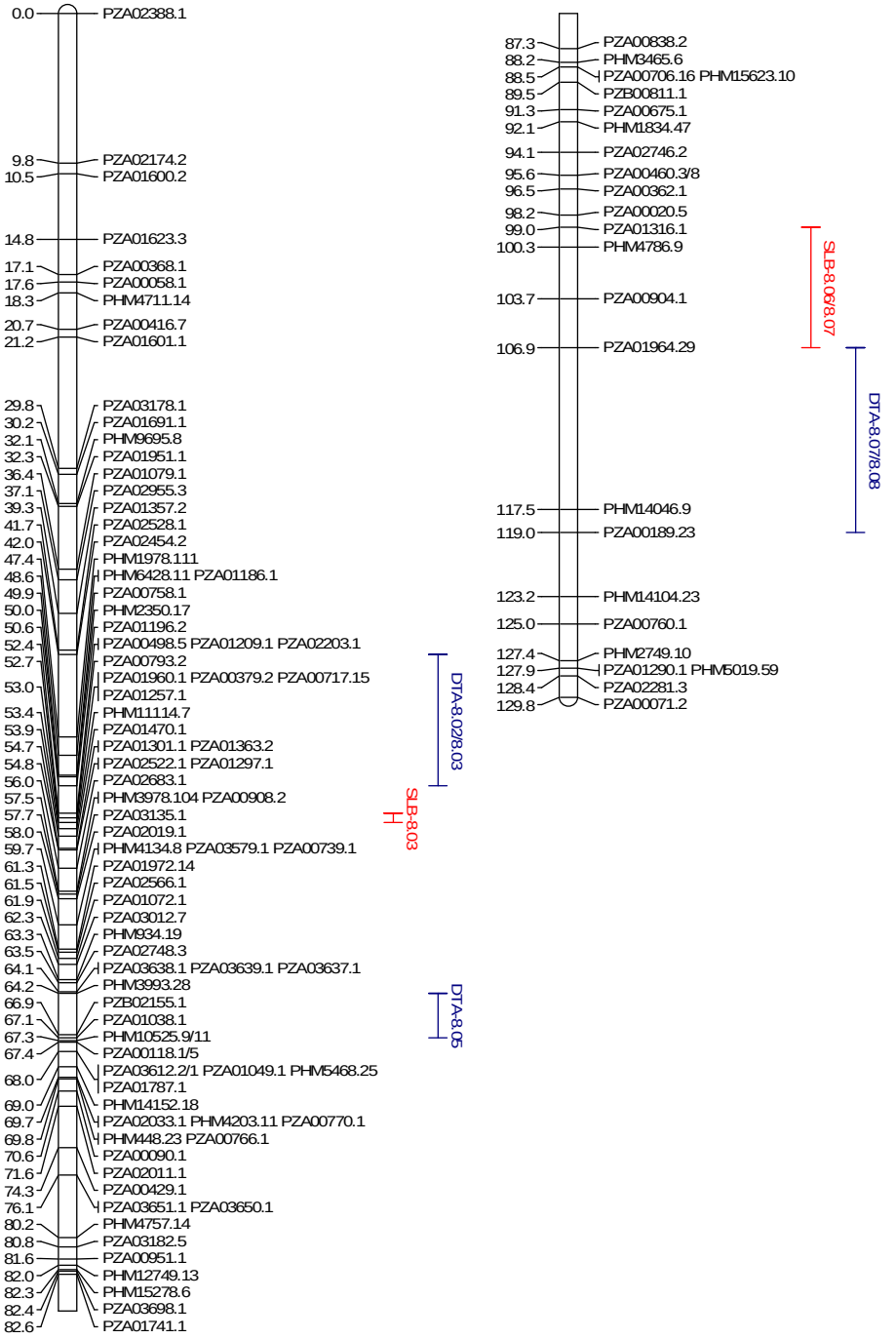


Figure 8. Linkage map of chromosome 8. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

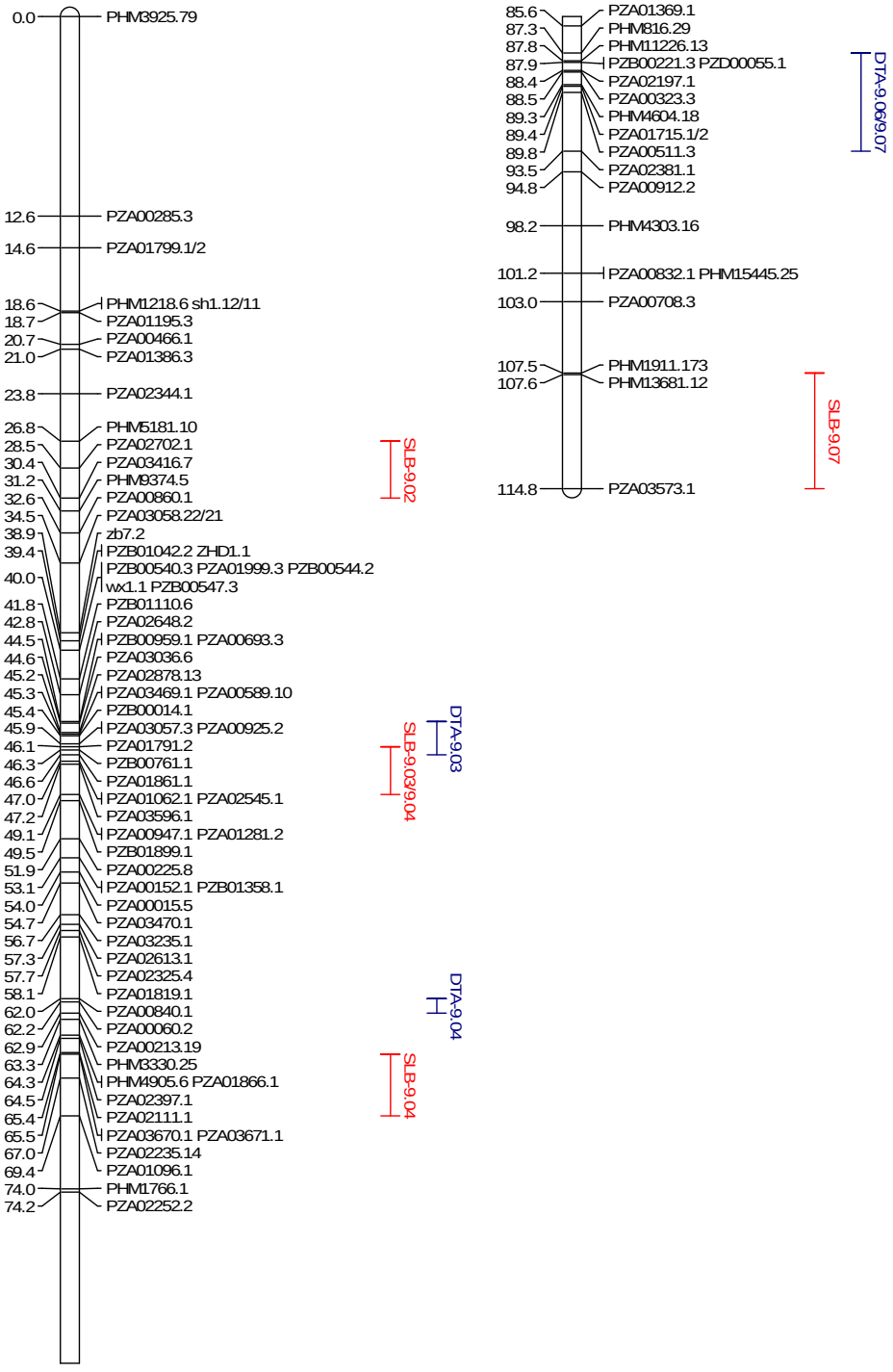


Figure 9. Linkage map of chromosome 9. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.

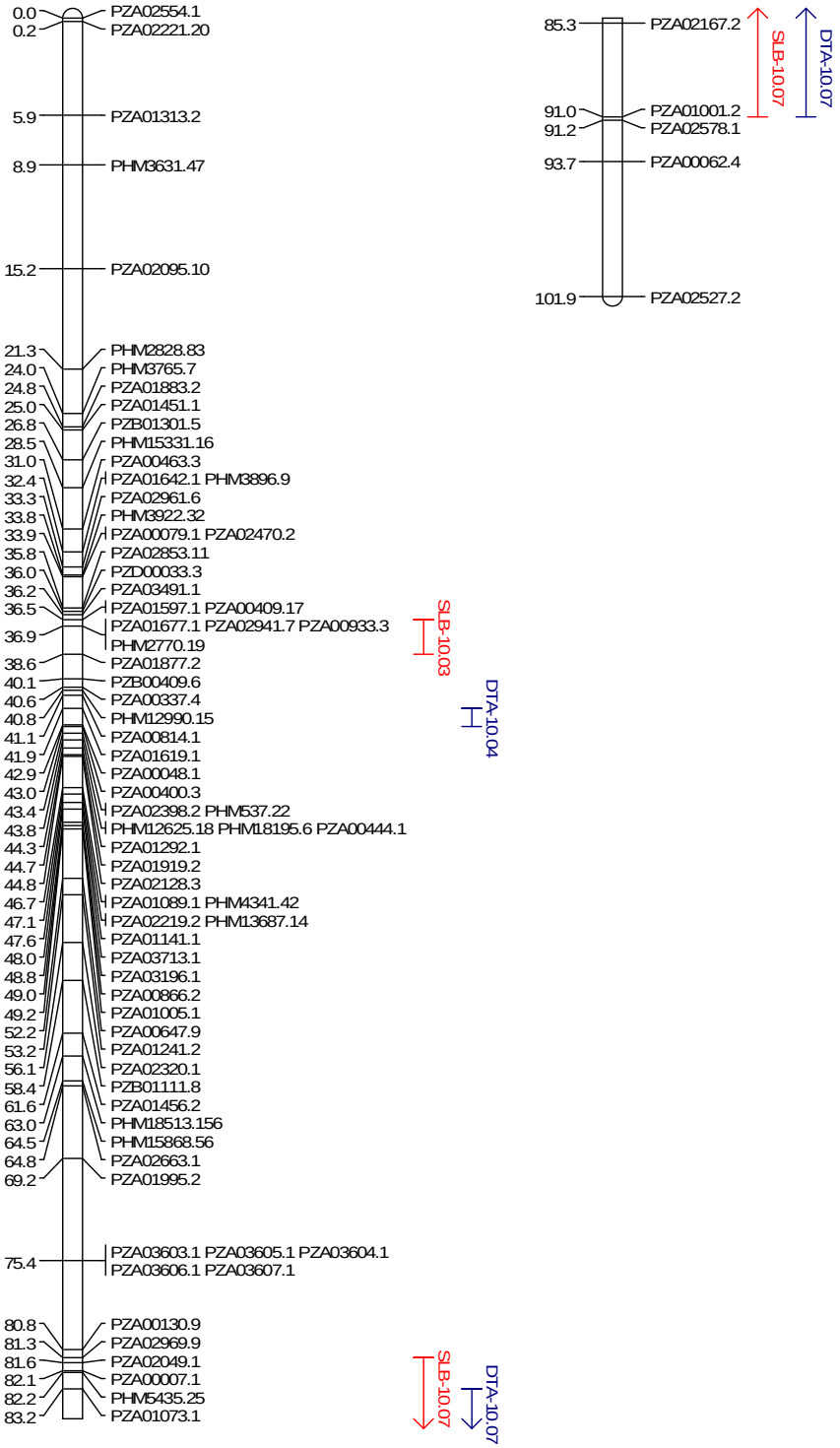


Figure 10. Linkage map of chromosome 10. SLB Resistance (in red) and DTA QTL (in blue) are identifed by their respective bins.