The Genetic Architecture of Southern Leaf Blight Resistance Revealed by Nested Association Mapping

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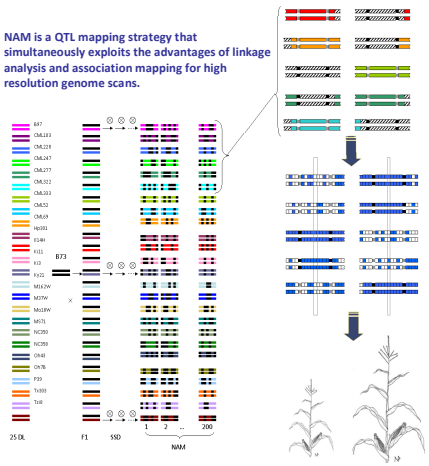
Introduction.

Southern leaf blight (SLB) is a fungal foliar pathogen of maize that occurs worldwide, but is more prevalent and destructive in warm temperate and tropical regions. SLB is caused by *Cochliobolus heterostrophus*, a necrotrophic member of the phylum Ascomycota.[1] Crop rotation and tilling under of debris can provide a moderate level of control through truncation of the infection cycle, but deployment of genetic resistance is the favored control method. Fortunately, many inbreds of tropical and warm temperate origin possess moderate to high levels of SLB resistance as severe infection can cause yield losses of 40%.[2] Several SLB resistance QTL have been mapped at low resolution (~2 – 15 cM) in various genomic regions in several segregating populations.[3,4,5,6,7] In order to fine-map the resistance genes and to elucidate the genetic architecture of this quantitative disease resistance, we evaluated the nested association mapping (NAM) population, developed as part of the NSF-funded Maize Diversity Project, for resistance to SLB. Exploiting advantages inherent in the NAM design, notably large population size, abundant marker data, and a broad sampling of maize germplasm, we hope to attain a high resolution, global QTL analysis of SLB resistance in maize.[8]

Figure 1. Overview of the NAM Strategy[8]

NAM is a QTL mapping strategy that simultaneously exploits the advantages of linkage analysis and association mapping for high resolution genome scans.



LOW resolution QTL-mapping

Detection of recombination break points within each population.

HIGH resolution LD-mapping

With rapid decay of LD, parental alleles at SNPs between mapped markers can be defined. Their inheritance is tracked in progeny using flanking mapped markers.

Association Detection

By searching for correlations between alleles and phenotypes, the causative SNPs for can be detected.

Genome reshuffling between 25 diverse inbred lines and the reference parent B73 in 5000 RILs. DL, diverse lines; ×, crossing; ⊗, selfing; SSD, single seed descent.

Figure 2. Rating Scale for Severity of Southern Leaf Blight

Each line was scored according to the following nine point rating scale, which measures the disease severity, or relative lesion area, of the ear leaf and the leaf directly above it. A rating of 1 would indicate death.

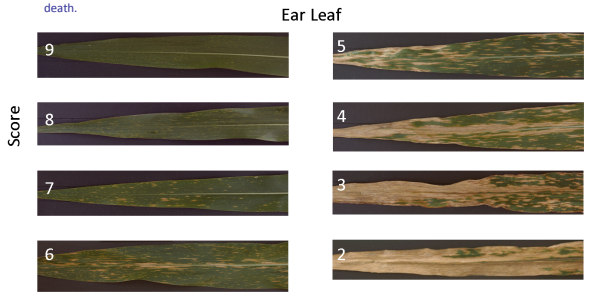
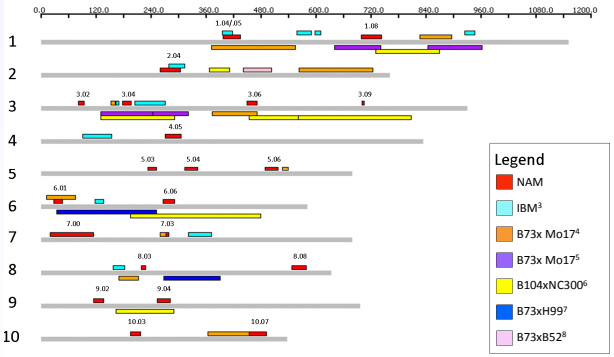


Figure 3. Comparative Map of QTL Detected in NAM and QTL reported in past publications



All QTL are mapped in IBM 2005 coordinates.

Figure 5. SLB Score BLUPs for parents and their derived populations

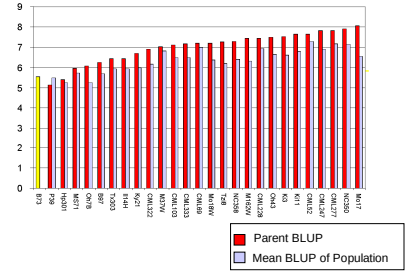
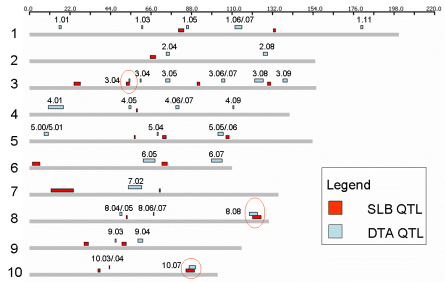


Figure 6. Comparison and Colocalization of QTL for SLB Resistance and Days to Anthesis



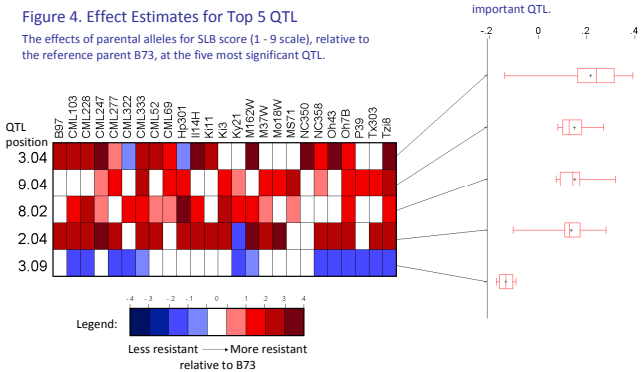
Materials and Methods.

- The NAM population is comprised of 5000 recombinant inbred lines (RILs) derived from crosses between B73 and 25 diverse inbred parents (Figure 1). The full set of RILs was planted in an augmented design and evaluated for resistance to SLB in three different location-year combinations: the summers of 2006 and 2007 in Clayton, NC and the winter of 2007 in Homestead, FL. Adequate disease pressure occurred during the summer of 2006 despite exposure to natural inoculum only. In contrast, the summer 2007 experiment was inoculated post-anthesis to increase disease pressure; the winter 2007 experiment was inoculated at the 6-8 leaf stage to ensure adequate disease. Each line was scored on a nine-point scale according to the severity of disease symptoms (Figure 2). In each environment, every line was scored at two different times. The number of days between planting and anthesis was also recorded for each line.
- We employed a combined multivariate mixed model analysis of the two SLB scores across all environments to calculate a single average SLB prediction (BLUP) for each line using ASREML. Significant variation in flowering time was observed in the NAM populations and later-maturing lines often exhibited higher resistance to SLB. For this reason, days to anthesis (DTA) was included as a covariate to minimize phenological effects. BLUPs were also calculated for DTA in order to map and compare flowering associated QTL with SLB resistance QTL.
- Each line was genotyped with 1106 B73-specific SNP markers. SAS Proc GLMSelect was used to scan the genome for SLB or DTA QTL across each interval defined by adjacent markers, using stepwise selection with a p-value threshold of 1x 10^{-6}. At each interval tested, unique allele effects for each diverse line founder were modeled.

Figure 4. Effect Estimates for Top 5 QTL

The effects of parental alleles for SLB score (1 - 9 scale), relative to the reference parent B73, at the five most significant QTL.

Box plot of founder allele effects relative to B73 at five most important QTL.



Results.

Significant genetic variation in response to SLB was observed; parental BLUPs ranged from 5.15 (P39) to 8.04 (Mo17), while average population BLUPs ranged from 5.25 (Hp301) to 7.3 (CML52) (Figure 5). Mean SLB score was highly heritable (85.2%). Twenty-one QTL for resistance to SLB were detected. Together, these QTL account for 75% of the genetic variation present among these lines. This, as well as rather low effect estimates for most of the QTL (Figure 4), indicate that many genes of small effect contribute to SLB resistance. QTL were mapped on every chromosome, with the five most significant in bins 3.04, 9.04, 8.03, 2.04 and 3.09. Of the twenty-one QTL mapped, many colocalize with QTL detected in other populations (Figure 3). Thirty QTL were mapped for DTA. Three QTL in bins 3.04, 8.08, and 10.07 colocalize with SLB resistance QTL (Figure 6). If alleles for later maturity pleiotropically caused increased SLB resistance, we would expect positive correlations between the SLB and DTA allele effect estimates at these QTL. The correlation was positive at bin 10.07 (r = 0.62), but only weakly positive at bin 3.04 (r = 0.21) and was negative at bin 8.08 (r = -0 .62). Thus, only one of the 21 SLB QTL was likely to be a maturity gene that pleiotropically affects resistance. At this point, QTL are localized to intervals of about 5 cM. Refinement of crossovers within the most important QTL with additional marker information will improve resolution of QTL positions. When a higher level of precision is achieved, candidate gene alleles in the intervals will be sequenced in the founders, and founder allele information will be projected on progeny RILs to conduct nested association mapping aimed at uncovering the genes responsible for SLB resistance.

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