



Uncovering Molecular Mechanisms of Quantitative Disease Resistance Using Nested Association Mapping

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Introduction

Northern Leaf Blight (NLB), caused by *Exserohilum turcicum*, is an endemic disease affecting maize production worldwide. The most economical and effective method for control of NLB is through the use of genetic resistance in the maize host. Quantitative resistance is important for resistance breeding to NLB and for crop species in general, as this type of resistance tends to be more durable and broad spectrum.

Previous work with NLB resistance has focused on two parameters of resistance: incubation period (IP; number of days until the appearance of disease symptoms) and disease severity (leaf area blighted). Although, IP and disease severity correspond to different stages in disease development, these studies have shown that IP and disease severity are well correlated, indicating a commonality in genetic control, and that IP can be used for selection early in the season.¹ Other studies have confirmed the polygenic nature of quantitative NLB resistance and shown moderate to high heritability.²

Previous work in our group has summarized the published literature for quantitative disease resistance in maize (blue bars in Fig. 2). The previous studies of NLB resistance have shown that resistance QTL are distributed throughout the genome without any apparent clustering.³

With the objective of uncovering molecular mechanisms of quantitative resistance, we have begun analyzing the nested association mapping (NAM) population for NLB resistance.⁴



NLB and GLS: two serious threats to maize.

Methods

We evaluated a sub-set of the NAM population during the summer of 2007 in Aurora, NY.

-We inoculated 120 lines from each of the 25 populations and evaluated two components of disease resistance:

1. Incubation Period (IP) – number of days after inoculation when 50% of the plants in a row showed disease symptoms.

2. Disease Severity – percentage of total leaf area blighted (3 time points).

-Area under the disease progress curve was calculated and standardized to 100.

Observed data for AUDPC was squared root transformed and SAS procedure GLMselect was used to select a linear model using marker intervals with marker effects nested within population. Flowering time has previously been associated with NLB resistance and was therefore included as a covariate during model selection. Permutations were conducted using the residual values from a population-only model. A LOD = 4 was found to correspond to an experimental alpha of 0.05 and was therefore used for model selection. Subsequently, the full model was fitted to each population and additional effects were selected on a single population basis with a selection criteria of LOD > 4.

Genome-wide analysis of marker association was conducted by testing single nucleotide polymorphisms against the LS means effect of the nearest identified QTL. Tests were considered significant for features within 10 Mb of the QTL peak at p-values < 0.001.

NLB – Northern Leaf Blight
IP – Incubation Period
AUDPC – Area Under the Disease Progress Curve
NAM – Nested Association Mapping
BAC – Bacteria Artificial Chromosome
QTL – Quantitative Trait Loci
LOD – Log of Odds

Results

As expected from previous studies with the maize diversity panel, the 25 parents of the NAM population showed a wide range of resistance for both IP and AUDPC (Fig. 1).

There was a highly significant (negative) correlation between IP and AUDPC for parental means ($R^2 = 0.76$), population means ($R^2 = 0.80$) and the individual lines ($R^2 = 0.53$).

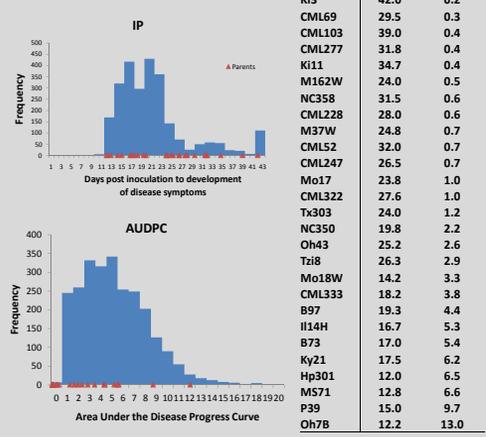
Initial model selection across all populations resulted in 4 QTL effects for IP and 11 QTL effects for AUDPC with LOD > 4 (Table 1). By analyzing populations separately, two additional QTLs were identified for IP and 4 additional QTLs were identified for AUDPC. These QTL were significant in a single population ($p < 0.0001$) and explained a substantial portion of that population variance ($R^2 > 0.09$) even though they were not detected across the full set of NAM populations.

Table 1. QTL effects selected for AUDPC and IP

EFFECT	CHR	cM	IBM2	LOD _{AUDPC}	R ² _{AUDPC}	LOD _{IP}	R ² _{IP}	QTL NAME
Pop	-	-	-	8.3	0.013	9.8	0.022	
Days to Silk	-	-	-	0.1	0.000	1.7	0.001	
i4(Pop)*	1	5.1	31.1	-	-	1.9	0.010	qEt1.01
i81(Pop)	1	92.3	508.19	10.6	0.015	-	-	qEt1.06
i108(Pop)	1	133	714.4	12.5	0.017	9.4	0.022	qEt1.07
i141(Pop)*	1	169.2	927.42	1.0	0.005	-	-	qEt1.10
i197(Pop)	2	27.6	94.4	2.5	0.007	-	-	qEt2.02
i217(Pop)*	2	60	251	0.8	0.005	-	-	qEt2.04
i266(Pop)	2	105.5	452.2	2.8	0.007	-	-	qEt2.07
i320(Pop)	3	52	189	3.5	0.008	-	-	qEt3.04
i372(Pop)*	3	75.5	377.5	-	-	5.3	0.016	qEt3.05
i383(Pop)	3	84.2	450.3	4.5	0.009	-	-	qEt3.06
i508(Pop)	4	98	443.22	7.2	0.012	-	-	qEt4.08.1
i515(Pop)	4	105.4	535.4	-	-	12.0	0.025	qEt4.08.2
i612(Pop)	5	65.7	286.7	6.6	0.011	-	-	qEt5.04
i720(Pop)	6	43.8	235.8	18.0	0.022	-	-	qEt6.04
i735(Pop)	6	54	302	-	-	21.9	0.039	qEt6.05.2
i746(Pop)	6	61.8	319.3	7.3	0.012	-	-	qEt6.05
i806(Pop)*	7	65.1	330.61	3.6	0.008	-	-	qEt7.04.1
i821(Pop)	7	77.1	381.5	5.8	0.011	-	-	qEt7.04.2
i921(Pop)	8	76.1	388.89	43.6	0.043	66.9	0.096	qEt8.06
i964(Pop)*	9	28.5	131.1	1.2	0.005	-	-	qEt9.02
i1096(Pop)	10	61.3	228.3	4.0	0.009	-	-	qEt10.04

* QTL effects identified in only one population (single population effects: LOD > 4; R² > 0.09)

Fig. 1 Phenotypic distribution of NAM and mean values of parents



Of the 21 QTL detected, only *qEt1.07* and *qEt8.06* (*qEt* for quantitative resistance to *Exserohilum turcicum*) were found to significantly contribute to both resistance parameters, IP and AUDPC. Most of the QTL identified in this study co-localized with previously reported disease resistance QTL for NLB, but novel QTL were also detected.

Preliminary analysis of ~236,000 genome-wide polymorphic features for association with the LS-means of QTL resulted in 35 significant tests ($p < 0.001$). Thus far a number of kinase like genes have been identified as potential candidates. Interestingly, none of the BACs examined for candidate genes have contained typical NL-LRR type genes.

Discussion

After a first year of evaluating the nested association mapping population for resistance to northern leaf blight, we have identified 6 QTL conditioning increased incubation period (delayed lesion formation) and 15 QTL conditioning decreased disease severity (AUDPC). Two of these QTL (*qEt1.07* and *qEt8.06*) contributed to both parameters of disease. Given that IP and disease severity are well correlated in this pathosystem, it is surprising that a larger overlap of QTL were not observed. However, the large effect of *qEt8.06* for both IP and AUDPC could explain a substantial portion this correlation.

This large-effect QTL was mapped to a <5 cM (7 Mb) region in maize bin 8.06. Concurrent work in our lab has co-localized the resistance gene *Htn1* to a 5 Mb region with 3.5 Mb overlap.* *Htn1* conditions race-specific resistance by prolonging IP rather than causing a typical hypersensitive response as do most major genes. Future work with the NAM populations will focus on dissection of this region and testing for race-specificity.

In addition to a second season of evaluation, our future work with NAM will also involve development of near-isogenic lines (NILs) using the heterogeneous inbred family (HIF) method to confirm and characterize these QTL. Using similar strategies, several of the QTL identified here (notably *qEt1.06* and *qEt6.05*) have already been confirmed in NILs and detailed characterization has been conducted.*

*see C. Chung et al. - Poster 197

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- ⁴see E. Buckler T8; M. McCullum T25; and J. Holland T26

Fig. 2. Position and relative effect of QTL for resistance to Northern Leaf Blight referenced against previously reported QTL.

