



Background

Northern Leaf Blight (NLB), caused by *Exserohilum turcicum*, is one of the most important diseases affecting maize production worldwide. Several qualitative loci (*Ht* genes) and a large number of quantitative trait loci (QTL) for NLB resistance have been identified and widely used in breeding programs for disease control. Qualitative race-specific resistance of *Ht* genes is characterized as inducing hypersensitive response and/or delaying lesion development, in a monogenic manner. However, the resistance phenotypes conferred by *Ht* genes can be difficult to distinguish in some environments and genetic backgrounds (1). Co-localization of major R genes and disease QTLs in some chromosomal regions of the maize genome (4) also suggests that the distinction between qualitative and quantitative resistance is ambiguous. Isolating and characterizing gene(s) underlying resistance loci is needed for resolving the question.

The sixth segment of maize chromosome 8 (bin 8.06) is known to be associated with resistance to NLB and several other diseases (4). Two qualitative resistance loci (*Ht2* and *Htn1*) and several QTLs for NLB resistance have been localized to this region. In response to a recurrent selection program for NLB resistance, significant changes in allele frequencies provided evidence of selection acting at several loci in bin 8.06. One of the putatively selected allele has been validated in F₂ families derived from the selection mapping population (5). To dissect the complex region, and to understand the relationship between qualitative and quantitative disease resistance in maize, a set of genetic stocks capturing a range of resistance alleles at bin 8.06 has been used for QTL mapping and characterization.

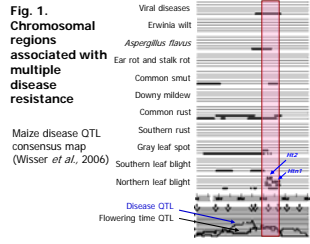


Fig. 1. Chromosomal regions associated with multiple disease resistance. Maize disease QTL consensus map (Wisser et al., 2006)

qEt8.06 is the largest-effect NLB-QTL identified in the nested association mapping (NAM) population

The nested association mapping (NAM) population is a large-scale mapping resource in maize, consisting of 5,000 recombinant inbred lines (RILs) developed from 25 diverse inbred lines crossed with a common inbred line B73. This resource is designed to combine the advantages of linkage mapping and association mapping, for high resolution QTL mapping with genome-wide coverage (7). Evaluating a subset of the NAM population for NLB for a first year led to mapping of 6 QTLs conditioning increased incubation period (IP) and 15 QTLs conditioning decreased disease severity (AUDPC) (Fig. 2). Of the 21 QTLs detected, *qEt8.06* (*qEt* for quantitative resistance to *Exserohilum turcicum*) was identified as the largest-effect QTL across all populations, and one of the two QTLs significantly contributing to both resistance parameters, IP and AUDPC (relative allele effects for decreasing AUDPC shown in Fig. 3). Most of the QTLs identified in this study co-localized with previously reported disease resistance QTLs for NLB, but novel QTLs were also detected. Evaluating a full set of the NAM population in summer 2008 largely confirmed the QTLs identified in the first year. Increase in sample size also provided better power in localizing and finding QTLs. With more recombination events captured in RILs, *qEt8.06* was found to consist of two closely-linked QTLs, suggesting the genetic complexity of this locus.

Fig. 3. Allele effects for *qEt8.06* from 25 NAM parents

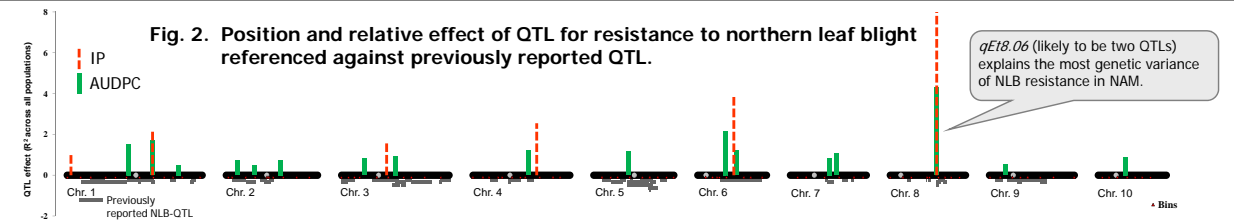
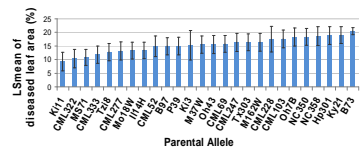


Fig. 2. Position and relative effect of QTL for resistance to northern leaf blight referenced against previously reported QTL.

qEt8.06 (likely to be two QTLs) explains the most genetic variance of NLB resistance in NAM.

Characterization of *qEt8.06* using near-isogenic line (NIL) pairs

For detailed analysis of *qEt8.06*, NIL pairs contrasting for the 8.06 region were developed using heterogeneous inbred family (HIF) strategy (2). In HIF analysis, intermediate materials from breeding programs are used to develop NIL pairs that are isogenic at the majority of loci, but differ at a specific QTL. In order to capture alleles contributing broad-spectrum resistance in NIL pairs, we started with F₂ families derived from DK888 x S11. DK888 is a tropical genotype with superior resistance to multiple diseases.

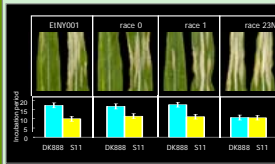
Resistance spectrum of *qEt8.06*

Although DK888 harbors multiple disease resistance, the DK888 allele at 8.06 (*qEt8.06_{DK888}*) is effective only for NLB resistance. Resistance spectra and effectiveness of diverse alleles at this locus will be characterized in NIL pairs being developed from the NAM population.

Disease	Parameter	Unit	Allele(s) at <i>qEt8.06</i>	Student's t-test
Northern leaf blight (NLB)	Incubation period	days after inoculation	DK888 17.7 S11 10.0 ± 0.3	< 0.0001***
	Primary diseased leaf area	%	DK888 9.0 ± 4.1 S11 65.0 ± 6.2	< 0.0001***
Southern leaf blight (SLB)	Lesion length	mm	DK888 1.2 ± 0.05 S11 1.2 ± 0.06	0.719
	Primary diseased leaf area	%	DK888 29.5 ± 1.0 S11 30.0 ± 1.5	0.585
Anthracnose	Incubation period	days after inoculation	DK888 7.7 ± 0.2 S11 7.8 ± 0.4	0.698
	Latent period	days after inoculation	DK888 10.4 ± 0.7 S11 10.4 ± 0.7	1.000
Anthracnose stalk rot (ASR)	Primary diseased leaf area	%	DK888 39.1 ± 12.1 S11 38.6 ± 14.7	0.963
	Discolor internode tissue	Total % of internode	DK888 121.7 ± 12.9 S11 120.0 ± 23.0	0.901
Rust	First pustule appearance	days after inoculation	DK888 7.5 ± 0 S11 7.5 ± 0	1.000
	Number of pustules	# pustules	DK888 96.0 ± 64.0 S11 149.5 ± 37.7	0.706
Smut	Primary diseased leaf area	%	DK888 14.4 ± 3.1 S11 15.0 ± 2.7	0.790
	Volume of galls	cm ³	DK888 275.8 ± 157.4 S11 167.5 ± 99.1	0.258
Stewart's wilt	Weight of galls	grams	DK888 127.4 ± 68.5 S11 78.9 ± 46.1	0.247
	Primary diseased leaf area	%	DK888 72.5 S11 72.5	—

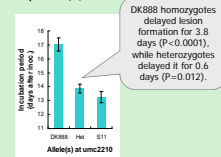
Race specificity of *qEt8.06*

qEt8.06_{DK888} conditions resistance to race 0, race 1, but not race23N of *E. turcicum*. Race specificity suggests that it may encompass the major genes *Ht2* and/or *Htn1*.



Gene action at *qEt8.06*

qEt8.06 identified in DK888 HIF showed **partially recessive resistance**, differing from the complete dominance of *Ht2* documented in previous reports (6).



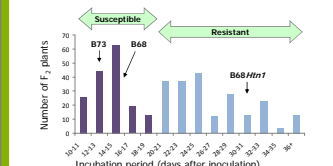
Genetic dissection of *Htn1*

Htn1 is a major gene that delays development of NLB lesions. The underlying mechanisms are of interest because unlike chlorotic lesions conferred by other *Ht* genes, *Htn1* does not confer a typical phenotype of hypersensitive response.

To fine-map *Htn1*, ~2600 F₂ plants derived from B68/B68 *Htn1* is being evaluated for marker-trait association. Preliminary X² analysis on 375 individual F₂ plants showed that the IP distribution fits a 9:7 distribution for resistance : susceptibility (X² = 0.01, P = 0.92, df = 1), indicating that two resistance genes are segregating in this population.



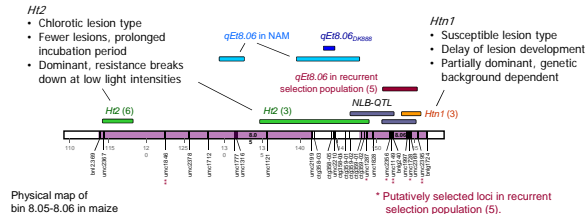
Distribution of NLB incubation period among B68/B68 *Htn1* F₂ plants



Conclusions

- Two linked QTLs (~5 cM apart) were detected at bin 8.06 in NAM. Consistent detection of the two *qEt8.06* in diverse mapping populations indicates that this locus accounts for a large proportion of NLB resistance in maize germplasm.
- Nested association mapping and break-point analysis using NIL pairs has localized *qEt8.06* to an overlapping region of ~2 Mb (142.9 – 144.5 Mb on physical map). The tightly linked SSR marker *umc2210* can be applied for marker-assisted selection in maize breeding.
- Race-specificity, map position and gene action of resistance suggested that *qEt8.06* can be *Ht2*, *Htn1* or a novel resistance locus. Concurrent work on fine-mapping of *Htn1* will resolve this question.

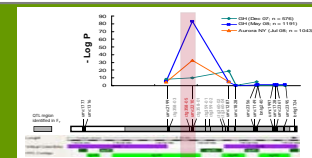
Evidence for NLB-QTLs in maize bin 8.05-8.06



- The enrichment of disease QTL in the 8.06 region and its genetic complexity implies the possibility that *qEt8.06* may consist of a cluster of resistance genes. Different levels and phenotypes of resistance can be due to various combinations of alleles for multiple genes, and their expression modified by genetic backgrounds and environmental conditions. The hypothesis will be further tested through map-based positional cloning.

Genetic dissection of *qEt8.06*

The QTL interval for *qEt8.06_{DK888}* in F₂ was ~20 Mb. Trait-marker association with ~2,800 individuals (F₂ or F₁₀) segregating for bin 8.06 has delimited the resistance locus to a region of <4 Mb tightly linked to the marker *umc2210*. Analysis of the NAM population also allowed mapping of *qEt8.06* to an overlapping region. Applying 7 newly-developed single nucleotide polymorphism markers (SNPs) surrounding *umc2210* on 40 representative individuals has narrowed the locus to a region of ~2 Mb. We are working to further saturate the region with SNPs to identify further recombinants for positional cloning.



References

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