



## Background

Quantitative resistance has been widely used to confer durable resistance for disease control. To gain insights into the nature and underlying genetics of quantitative resistance, our group has been using a range of complementary QTL approaches to identify, characterize and dissect loci conditioning disease resistance (disease QTLs) in maize. Two methods – chromosomal segment substitution lines (CSLSs; Szalma *et al.*, 2007) and heterogeneous inbred family strategy (HIF; Tuinstra *et al.*, 1997), were applied to simultaneously map QTLs and generate near-isogenic lines (NILs).

The primary emphasis of this study is to identify and characterize QTLs for resistance to northern leaf blight (NLB), one of the most important corn diseases in tropical and temperate environments. Second, the concept of multiple disease resistance (MDR) is being explored in order to help farmers reduce their loss to a range of diseases. A large number of disease QTLs have been mapped in the maize genome, and clusters of QTLs for various diseases were identified in some chromosomal regions (Wisser *et al.*, 2006). This and other evidence suggests the existence of loci that condition MDR. We used HIFs derived from MDR maize lines to analyze disease QTLs, focusing on regions of the genome associated with MDR.

## Outcomes

### I. QTLs identified using chromosomal segment substitution lines (CSLSs)

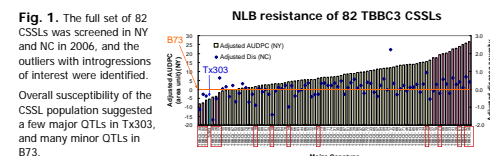
- Several NLB-QTLs were mapped in CSLSs derived from B73 x Tx303 (Fig. 1 and Table 1). Their phenotypic expression was similar at different plant developmental stages (Fig. 2).
- Two NLB-QTLs with larger effects, B73 allele at bin 1.02 (*qE11.02<sub>B73</sub>*) and Tx303 allele at bin 1.06 (*qE11.06<sub>Tx303</sub>*), have been further validated (Fig. 3). Their effectiveness on pathogenesis is under investigation.

### II. QTLs identified using heterogeneous inbred families (HIFs)

- A series of NIL pairs contrasting for chromosomal regions associated with MDR is developed (Table 2). By systematically characterizing the NIL pairs for resistance to multiple diseases, several disease QTLs were identified, most of them are effective for single diseases.
- The CML52 allele at bin 6.05 (*qE16.05<sub>CML52</sub>*) confers broad-spectrum resistance to three vascular diseases – NLB, ASR and Stewart's wilt (Fig. 4).
- The DK888 allele at bin 8.06 (*qE18.06<sub>DK888</sub>*) was found to condition quantitative race-specific resistance to NLB (Fig. 5). This locus, likely *Htn1*, has been fine-mapped to a region of ~ 5 - 7 Mb (Fig. 6).

### I. QTLs identified using chromosomal segment substitution lines (CSLSs)

The TBBC3 population (Szalma *et al.*, 2007) consists of 82 lines with an average 89% B73 constitution. Each line carries a different set of introgressions from Tx303. We screened this population for lines that differed significantly in disease resistance from the B73 recurrent parent line and from the rest of the population.



**Fig. 1.** The full set of 82 CSLSs was screened in NY and NC in 2006, and the outliers with introgressions of interest were identified.

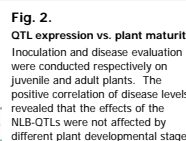
Overall susceptibility of the CSLS population suggested a few major QTLs in Tx303, and many minor QTLs in B73.

**Table 1.** Several NLB-QTLs were mapped by confirming the resistance effect in a subset of 15 selected CSLSs in NY and NC in 2007.

✓ Consistent - 2 years  
✗ Inconsistent - 2 years

**NLB resistance of 15 selected TBBC3 lines**

Line	Introgressed Region (Chromosome)	Area (a.u.)	Area (a.u.)	Area (a.u.)
✓ TBBC3_1	1.02	0.4	0.4	0.4
✓ TBBC3_2	1.06	0.4	0.4	0.4
✓ TBBC3_3	1.02	0.4	0.4	0.4
✓ TBBC3_4	1.06	0.4	0.4	0.4
✓ TBBC3_5	1.02	0.4	0.4	0.4
✓ TBBC3_6	1.06	0.4	0.4	0.4
✓ TBBC3_7	1.02	0.4	0.4	0.4
✓ TBBC3_8	1.06	0.4	0.4	0.4
✓ TBBC3_9	1.02	0.4	0.4	0.4
✓ TBBC3_10	1.06	0.4	0.4	0.4
✓ TBBC3_11	1.02	0.4	0.4	0.4
✓ TBBC3_12	1.06	0.4	0.4	0.4
✓ TBBC3_13	1.02	0.4	0.4	0.4
✓ TBBC3_14	1.06	0.4	0.4	0.4
✓ TBBC3_15	1.02	0.4	0.4	0.4



**Fig. 3.** *qE11.02<sub>B73</sub>* and *qE11.06<sub>Tx303</sub>* are two NLB-QTLs with larger effects. They have been validated in F<sub>2</sub> populations and derived F<sub>3</sub> lines. Their effectiveness on pathogenesis is under investigation in derived NILs. Preliminary work suggested that *qE11.06<sub>Tx303</sub>* protects plants from fungal penetration, while *qE11.02<sub>B73</sub>* reduces the efficiency of hyphae growing into the vascular system (for details, see poster 192 by Walsh *et al.*).



### II. QTLs identified using heterogeneous inbred families (HIFs)

In HIF analysis, intermediate materials from breeding programs are used to develop nearly-isogenic line (NIL) pairs that are isogenic at the majority of loci, but differ at a specific QTL. 74 SSR markers covering 38 bins associated with MDR-QTL were targeted for NIL construction, based on a consensus map of disease QTL in maize (Wisser *et al.*, 2006). HIF-derived NILs were developed from B73 x CML52 and S11 x DK888. The tropical lines CML52 and DK888 were chosen based on their superior resistance to NLB, gray leaf spot (GLS), southern leaf blight (SLB), and other diseases. We hypothesized that: 1) maize genotypes showing MDR phenotypes harbor alleles contributing broad-spectrum resistance, and 2) chromosomal regions where disease QTLs co-localized are enriched with defense-related genes.

**Table 2.** The NIL pairs contrasting for different chromosomal regions were characterized for NLB, GLS, SLB, anthracnose leaf blight (ALB), anthracnose stalk rot (ASR), common rust, common smut and Stewart's wilt.

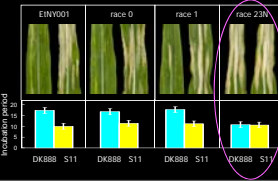
Cross of origin	Contrasting regions in available NIL pairs (bin)	Disease resistance (allele conferring resistance)								Differential in resistance, but causative QTL unknown
		GLS <sup>a</sup>	SLB <sup>a</sup>	ALB <sup>a</sup>	ASR <sup>a</sup>	Rust <sup>b</sup>	Smut <sup>c</sup>	Stewart's wilt <sup>d</sup>		
B73/	1.06	CML52 <sup>a</sup>	No	No	No	No	No	No	—	SLB, ALB
CML52	1.07/08	CML52 <sup>a</sup>	No	No	No	No	No	No	—	—
	2.04/06	No	No	No	No	No	No	No	—	SLB
	2.10, 5.03	CML52 or B73 (epistasis btw QTLs in bins 2, 10 and 5.03)	No	No	No	No	No	No	—	—
	3.06	No	No	No	No	No	No	CML52 <sup>a</sup>	—	NLB, ALB, rust
	6.05	CML52	No	No	No	CML52 <sup>b</sup>	No	No	CML52 <sup>a</sup>	SLB, ALB
	7.04	No	No	No	No	CML52 <sup>b</sup>	No	No	—	NLB, rust
	8.02/03	CML52 <sup>a</sup>	No	No	No	No	No	No	—	—
S11/	3.04	No	—	No	No	No	No	No	—	—
DK888	5.04	No	—	DK888 <sup>b</sup>	No	No	No	S11 <sup>a</sup>	—	—
	5.06	—	No	No	S11 <sup>a</sup>	No	No	—	—	—
	6.05	No	—	No	No	S11 <sup>a</sup>	No	No	—	—
	8.06	DK888	—	No	No	No	No	No	—	—

<sup>a</sup> The NLB-QTL was identified from corresponding heterogeneous inbred families in 2005 to 2006, but significant phenotypic contrast was not detected in selected NIL pairs at Aurora, 2007.  
<sup>b</sup> Data based on one-year field or GH trials, and need to be further validated. (Common rust and common smut are from natural infection.)

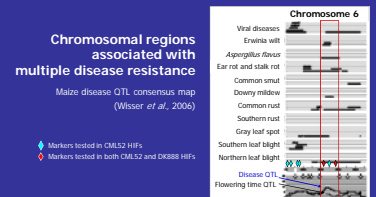
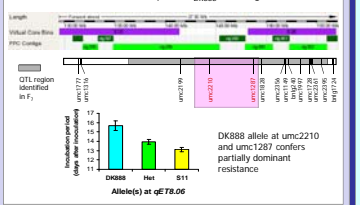
### QTL for race-specific resistance to NLB

Bin 8.06 in the maize genome is known associated with resistance to NLB and several other important diseases. Two qualitative resistance loci and several QTLs for NLB resistance were localized to this chromosomal region, and the effect has also been identified by us in the nested association mapping (NAM) population (poster 220 by Poland *et al.*) and recurrent selection mapping population (Wisser *et al.*, submitted). To elucidate this complex locus, we captured the resistance allele from DK888 (*qE18.06<sub>DK888</sub>*), and are dissecting this region for map-based cloning.

**Fig. 5.** Race specificity and map position of *qE18.06<sub>DK888</sub>* suggest that it encompasses *Htn1*, a major gene that delays lesion development.



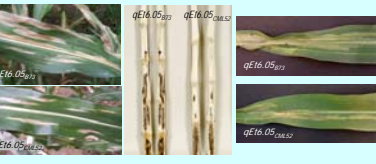
**Fig. 6.** Trait-marker association in a population of ~200 F<sub>2</sub> recombinants has localized *qE18.06<sub>DK888</sub>* to a region of 5-7 Mb.



**Fig. 4.** QTL for multiple disease resistance

*qE16.05<sub>CML52</sub>* conditions resistance to NLB, ASR, and Stewart's wilt. Development of the three diseases all involves biotrophic interaction with newly infected cells, and subsequent colonization and destruction of the vascular tissue. We hypothesize that resistance is expressed in the xylem, and thus not against pathogens attacking other tissues. Genetic dissection of this MDR-QTL (~24 Mb) is undergoing, in order to determine whether the associated resistance is due to pleiotropy or linkage, and to shed light on the underlying defense mechanisms.

### CML52 NILs contrasting for bin 6.05



*qE16.05<sub>CML52</sub>* is the only MDR-QTL found in this study. The lack of MDR-QTL can be due to:

- CML52 and DK888 have distinct QTLs for resistance to different diseases.
- Background effect: Each derived NIL pair is in a unique genetic background. A susceptible background is needed for accurate detection of disease QTLs, especially minor QTLs. However, it is rather difficult to create a genetic background susceptible to many diseases, considering the diverse requirements of different pathogens and their different interaction patterns with host plant.

## Conclusions

1. The use of CSLSs and HIFs was found to be effective for QTL analysis and NIL development.
2. The disease QTL consensus map served as a reference for targeted QTL mapping. By targeting 25 out of 41 bins that were previously reported to be associated with NLB resistance, we have successfully identified seven NLB-QTLs in bins 1.06, 1.07-1.08, 5.03, 6.05, 8.02-8.03, and 8.06. With the derived NILs, we are working towards map-based cloning for two QTLs of interest – *qE16.05<sub>CML52</sub>* and *qE18.06<sub>DK888</sub>*.
3. The value of the disease QTL consensus map to MDR-QTL prediction is still unclear. The limitation comes from low precision and accuracy of QTL mapping, and disease QTLs initially identified in diverse mapping populations. Our work on HIFs from only two resistance donors is not sufficient to clarify the question.

## References

Tuinstra *et al.* (1997) Theor. Appl. Genet. 95: 1005-1011.  
Szalma *et al.* (2007) Theor. Appl. Genet. 114: 1211-1228.  
Wisser *et al.* (2006) Phytopathology 96: 120-129.  
Wisser *et al.* submitted.

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