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Targeted Discovery and Characterization of Quantitative Trait Loci (QTL) for Resistance to Northern Leaf Blight and Other Foliar Fungal Diseases in Maize

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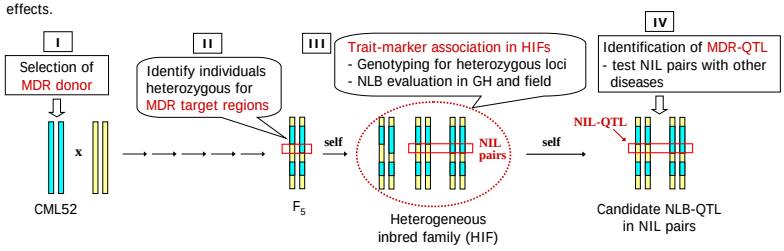
Introduction

In maize, quantitative resistance has been widely used to confer partial and durable resistance for disease control. A large number of quantitative trait loci (QTL) for disease resistance has been mapped in maize genome, and clusters of QTL for various diseases were identified in some chromosomal regions (Wisser *et al.*, 2006). This and other evidence suggests the existence of loci that condition **multiple disease resistance (MDR)**. We used the "heterogeneous inbred family" approach (HIF; Tuinstra *et al.*, 1997) to analyze disease QTL, focusing on regions of the genome associated with MDR. HIF analysis provides the flexibility of using intermediate materials from general breeding programs, to develop nearly-isogenic line (NIL) pairs that are isogenic at the majority of loci, but differ at a specific QTL. Our initial emphasis was on one of the most important diseases of maize in tropical and temperate environments, northern leaf blight (NLB, caused by *Setosphaeria turcica*, anamorph *Exserohilum turcicum*).

Strategy for identifying MDR-QTL by HIF analysis

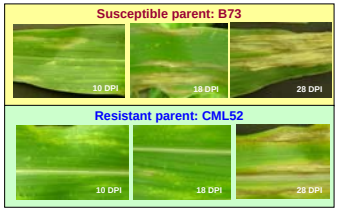
This study was initiated from the detection of heterozygous loci for markers of MDR interest in F_5 families derived during the development of a B73/CML52 recombinant inbred line (RIL) population. The F_6 progeny segregating for the target locus but isogenic at ~96 % of the genome are NIL pairs. Loci conditioning resistance can be identified if the NIL pairs show significant contrast for NLB resistance. The specific QTL in NIL pairs can be subsequently tested for their MDR effects.



I. Selection of MDR donor

The tropical maize line CML52 was chosen for analysis based on its superior resistance to NLB, GLS (gray leaf spot), SLB (southern leaf blight), and ear rot.

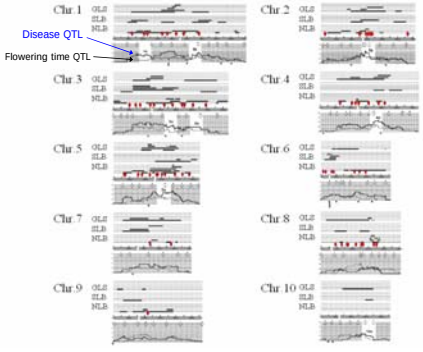
Evaluation of NLB resistance in parental genotypes



II. Identify individuals heterozygous for MDR target regions

Chromosomal regions associated with MDR

66 SSR markers covering 38 bins associated with MDR were selected based on a consensus map of disease QTL in maize (Wisser *et al.* 2006). Markers were chosen at smaller intervals for hot spots of QTL clusters.



♦ Polymorphic SSR markers

Maize disease QTL consensus map (Wisser *et al.*, 2006)

Population structure

Starting with F_5 derived from B73 x CML52, a sample set consisting of 94 individuals in 19 F_5 families allowed us to successfully identify heterozygous lines for almost every locus of interest.

Sample	Full set	Subset
Population Structure	94 individuals in 19 F_5	43 individuals in 13 F_5
No. marker	33	66
No. F_5 family het per locus	2.7	1.8
No. F_5 individual het per locus	5.8	3.2

III. Trait-marker association in HIFs

1. From 2005 to 2006, 15 F_6, 7 F_7, and 2 F_8 families (a total of 24 HIFs) were evaluated for NLB resistance in greenhouse and field (Aurora, NY). Significant phenotypic contrasts in near isogenic line (NIL) pairs were detected and verified in bins 1.06, 1.07-1.08, 2.10, 5.03, 6.05, and 8.02-8.03.
2. 34 F_6 families were also evaluated for NLB resistance at Aurora, NY in 2006. The across-family analysis largely confirmed the within-family HIF tests (data not shown).
3. 44 flanking SSR markers were used to estimate the start and end points of heterozygous regions in F_5 families.

Table. NLB-QTL identified in CML52 x B73 HIFs

dQTL (Bin)	Position [a] (cM)	Donor	Allele effect [b,c] (Mean $_{CML52}$ – Mean $_{B73}$)						Genetic background of NIL pairs	Co-localized with published NLB-QTL (mapping population)
			IP	LN	DLA1	DLA2	DLA3	AUDPC		
1.06	529.0	CML52	0.8*	-9.1**	-7.2*	-8.7*	-11.4**	-20.0**	Susceptible	D32 x D145
1.07/08	693.6 – 737.5	CML52	2.2	-9.2*	-2.5*	-0.7	-6.8***	-5.4*	Intermediate resistant	D32 x D145 IL731a x W6786
2.10	712.1	CML52	2.4*	-16.1	-3.6*	–	–	–	Intermediate	–
		B73	-1.7*	5.3*	1.0	3.3*	4.0*	5.7*		
5.03	189.8 – 217.4	B73	-0.3*	6.2*	4.1*	2.8**	4.5**	5.0**	Intermediate	B52 x Mo17 D32 x D145
6.05	271.5 – 373.8	CML52	2.1*	-12.3**	-6.5**	-6.3**	-6.8**	-13.2***	Highly susceptible	D32 x D145
8.02/03	132.4 – 239.8	CML52	6.0*	-5.7*	–	–	–	–	Highly resistant	D32 x D145
Resistant parental genotype CML52			*4.6****	*-26.0****	*-11.1****	*-16.7****	*-37.1****	*-40.8****		

[a] The map position was based on genetic map of the intermated B73 x Mo17 population (IBM 2005 neighbors).

[b] Resistance traits included: IP (incubation period, number of days after inoculation when a plant starts showing wilt lesions), LN (lesion number), DLA (diseased leaf area, the percentage of infected leaf area of the entire plant disregarding decayed bottom leaves), and AUDPC (area under the disease progress curve).

[c] Phenotypic differences between NIL pairs in each HIF were analyzed by: (1) nonparametric Kaplan-Meier estimate of survival function for IP; and (2) two-sided student's t test for LN, DLA, and AUDPC. (* 0.01 < P < 0.05 ; ** 0.001 < P < 0.01 ; *** P < 0.001)

IV. Identification of MDR-QTL

To test for MDR-QTL in the CML52 x B73 population, the NIL pairs contrasting for each identified NLB-QTL will be evaluated for resistance to GLS and SLB. Dissection and fine-mapping of the MDR-QTL will be performed if genetic correlation of resistance to NLB and other fungal diseases can be found.

Conclusions:

1. By investigating 24 HIFs from B73 x CML52, six NLB-QTL have been identified. The candidate loci will be subsequently characterized in derived NIL pairs for multiple disease resistance.
2. There is preliminary evidence that the effectiveness of candidate NLB-QTL changes over different disease developmental stages as well as plant maturity stages (eg. QTL in bin 1.07/08 was effective for LN and DLA, but not IP). The developed NIL pairs will be used for further understanding the defense mechanisms underlying quantitative resistance.
3. Implications of using HIF approach for QTL analysis:
   - HIF analysis was found to be an effective shortcut for targeted QTL analysis and NIL development.
   - Each derived NIL pair is in unique genetic background. Susceptible backgrounds are more suitable for further characterization and fine-mapping, while dQTL detected in highly resistant backgrounds still need to be backcrossed. Small scale, across-family pre-testing can provide useful information on the resistant levels and phenotypic variations of different families, which can help in eliminating the highly resistant ones.
   - To increase efficiency, the HIF approach can be applied through a more genotype-driven way: identify NIL pairs for target loci in F_5 or F_6 first, and evaluate the $F_{5:7}$ or $F_{5:8}$ progeny with traits of interest later.

References:

Tuinstra *et al.* (1997) Theor. Appl. Genet. 95: 1005-1011.

Wisser *et al.* (2006) Phytopathology 96(2): 120-129.

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