Characterizing differential responses to *C. heterostrophus* in maize NILs differing for disease resistance QTL

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Introduction

Quantitative disease resistance, although the principal form of resistance used in maize, remains poorly understood (Carson *et al*, 2004) . We are working with the fungal foliar pathogen *Cochliobolus heterostrophus*, causal agent of southern leaf blight (SLB). The NC State corn breeding program developed two sister lines, **NC292** and **NC330**, that although near-isogenic to the standard line **B73, showed much higher SLB resistance (Fig. 1)**. **We have analyzed these lines to determine the genomic** regions which differentiate them from B73. We identified 11 introgressions and, using marker-assisted selection, we have created a series of near-isogenic lines (NILs) in a B73 background which carry between one and five of these introgressions. We are using this set of NILs to characterize the specific contributions of each introgression to SLB resistance.

Source of resistance

-The NILs share a B73 background , but differ by specific introgressions derived from the SLB-resistant sister lines NC292 and NC330.

-NC292 and NC330 were derived by recurrent phenotypic selection for quantitative resistance to southern leaf blight from an initial B73 (susceptible) x NC250 (resistant) cross and 4 to 5 subsequent backcrosses to B73.

-To create the NILS, NC292 and NC330 were (back)crossed to their parental line B73, and marker-assisted selection was used to identify NILs with specific NC250 introgressions.

-An NC250A x B73 $F_{2:3}$ mapping population was used to identify SLB resistance QTL. NC250A is a sister line of NC250 (~88% identical). These NC250A QTL were aligned with the NC250 introgressions identified in NC292 and NC330 **(Fig. 2)**.

-The NILs were assessed for SLB resistance in replicated field trials in the summer of 2007 ($BC_1F_{2:3}$ generation). For those lines with discrete NC250 introgressions verified by marker genotyping, disease levels were averaged across lines with each introgression or particular combination of introgressions **(Fig. 3)**.

-Recent genotyping with a larger array of markers has revealed a limited number of additional NC250 introgressions. In view of this data are being reanalyzed, though we expect the broad conclusion to remain unchanged.

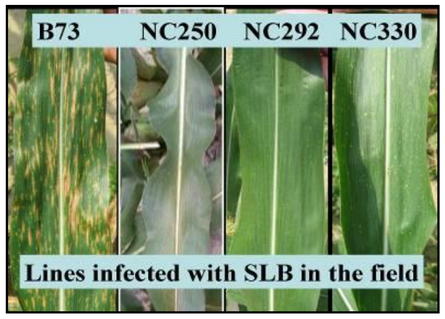

Fig. 1 Southern leaf blight (SLB) symptoms consist of small brown necrotic lesions on corn leaves, caused by the fungus *Cochliobolus heterostrophus.* NC292 and NC330 are the result of a B73 x NC250 cross with subsequent backcrossing to B73 and recurrent selection for SLB resistance.

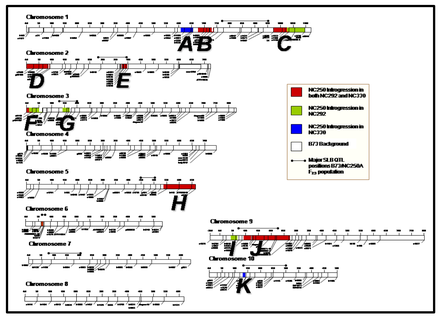

Fig. 2 A genome scan was used to determine regions of NC250 (SLB resistant) origin in the B73 (SLB susceptible) genomic background of sister lines NC292 and NC330. The resulting map was aligned with quantitative trait loci (QTL) for SLB resistance mapped in a related population.

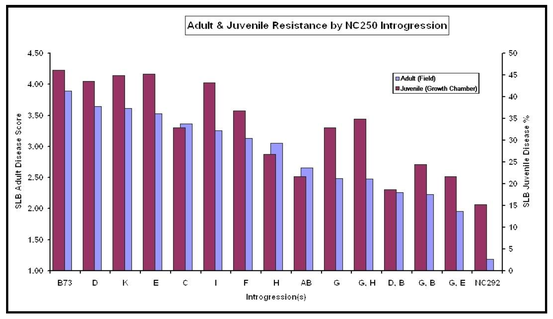

Fig. 3 Markers were used to identify those NILs containing only specific NC250 introgressions in the B73 genomic background. For the 3 parents each NC250 introgression (A through K – see figure at left) or specific combination of introgressions, the average SLB disease rating across all NILs with that particular genotype was calculated, both for adults in the field and juvenile plants in the growth chamber.

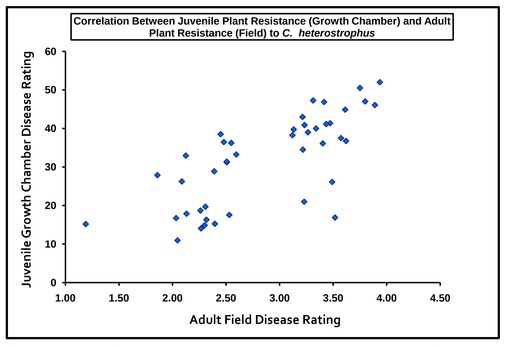

Fig. 4 Because of their different rating scales, direct correlation of juvenile and adult SLB disease scores would not meet the assumptions of that parametric comparison. However, combined with non-parametric correlation (see figure at left), a simple graphic comparison with juvenile ratings plotted against adult ratings indicates a clear correspondence of their responses to SLB.

Growth chamber results *are* applicable to the field

-Further characterization of the plant-pathogen interactions behind these different resistance responses will require growth chamber and laboratory work on juvenile plants. In the end, however, this work must be relevant to resistance in the field. Therefore, 42 of the 252 NILs were selected to compare juvenile resistance in the growth chamber with adult resistance in the field.

-Juveniles are generally more susceptible to SLB than adults, and may even express strikingly different resistance phenotypes (Simmons et al., 2001).

-Rating methods also differ. Adults rated on a 1 (no symptoms) – 9 (dead) 0.5-increment qualitative scale over the course of a field season. Juveniles are rated by the continuous scale % necrotic area of 2 inoculated leaves over the course of 4-5 days.

-These data do not meet the assumptions for standard correlation and regression statistics. However, non-parametric and qualitative comparisons highly suggest that juvenile response is an accurate relative assessment of adult resistance:

-Both adult and juvenile trials generated highly parallel effect estimates (based on least-square means and variances) **(Fig. 4)**.

-Based on Spearman's Rank Correlation Coefficient, adult and juvenile results were significantly correlated ($p<0.0001$, null hypothesis of no correlation, juvenile values from LSD estimates) **(Fig. 5)**. Mean values across all lines of a given set of introgressions were also highly correlated between adults and juveniles (Spearman's $r=0.73715$, $p<0.0001$) **(Fig. 3)**.

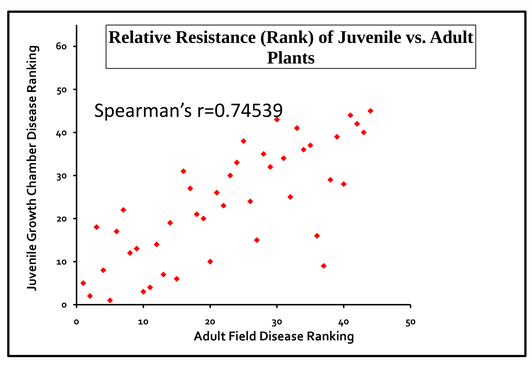

Fig. 5 Non-parametric statistical analysis of juvenile and adult plant response to SLB infection with Spearman's rank correlation coefficient demonstrated a statistically significant correlation between the juvenile and adult ratings for each line ($p<0.0001$, at alpha=0.05).

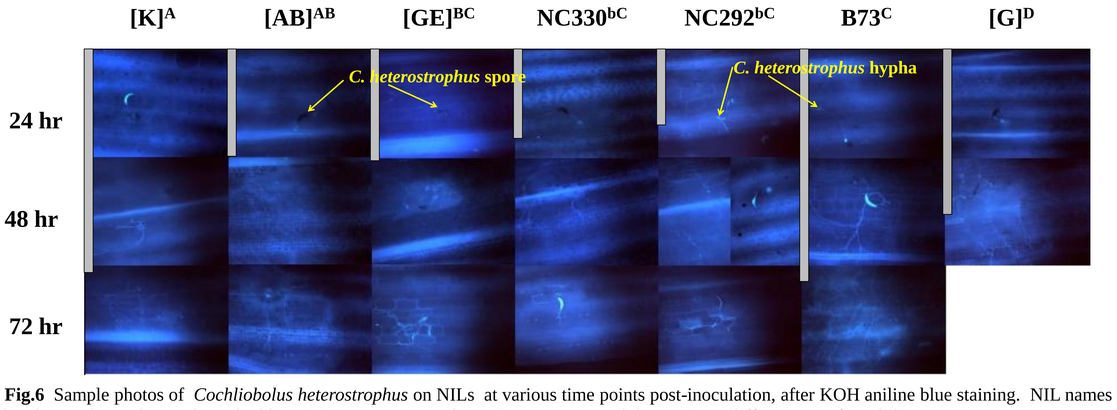

Fig.6 Sample photos of *Cochliobolus heterostrophus* on NILs at various time points post-inoculation, after KOH aniline blue staining. NIL names in brackets indicate the single or double NC250 introgression that NIL contains. At alpha=0.05, no differences in fungal diameter or necrotic tissue diameter were detected at 24 hr. Significant differences in fungal diameter at 48 hr post-inoculation (alpha=0.05) are indicated by super-scripted group letters. A lower-case group letter for a line designates inclusion of the line in that group at the alpha=0.05 level of significance, but not at the alpha=0.10 level. Lines are arranged in order of smallest (left) to largest (right) fungal diameter at 48 hr post-inoculation. Gray bars indicate average % infected leaf area for that line or introgression set in the juvenile plant growth chamber experiments. The disparity between resistance in the growth chamber study and fungal infection diameter at 48 hrs post-inoculation is interesting, but not conclusive. These statistics must be compared with data from further time points, alternate measures of disease and fungal growth and subsequent replications of this experiment. Moreover, as hypha farther below the leaf surface are not always visible, it is unclear whether smaller fungal diameter indicates lower fungal growth or faster fungal penetration.

Current efforts

-The correspondence of our growth chamber results with those in the field has allowed us to begin a more detailed exploration of host-parasite interactions.

-To date, preliminary work has consisted of evaluation for consistent, low-variance inoculation methods and microscopic examination of whole mounts from the first 5 days post-inoculation using a GFP-transformed fungal strain and KOH aniline blue staining **(Fig. 6)**.

-In general, resistant lines are not distinguishable from susceptible B73 plants until around 48 hours post-inoculation **(Fig. 6)**.

-Images are being analyzed for trends in lesion diameter, fungal growth vs. lesion expansion, hyphal branching, penetration depth and lesion variance.

-GFP-transformed strain (gift of Charlotte Bronson) will be used to assess infection germination and infection efficiency of spores.

-Conserved defense-related genes (e.g., PR-1) will be examined for induced expression in the NILs (Basse, 2005).

In summary

Dissecting the genetic interaction of resistance QTL in the field is in and of itself of value to understanding quantitative resistance. We also would like to characterize the biology of these interactions at the microscopic and molecular levels. The highly parallel results with juvenile plants in the growth chamber when compared with adults under field conditions enables us to confidently begin these characterizations with juveniles in the lab.

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References

Basse. (2005) Plant Physiology, 138(3), 1774. Carson *et al*. (2004). Phytopathology, 94, 862-867. Simmons *et al*. (2001). MPMI, 14(8; 8), 947-954.