

Genomic Evaluation of the Defense Response of Maize (*Zea mays* L.) against Herbivory by the Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

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The western corn rootworm (WCR) is the most important insect pest in the U.S. Corn Belt. In the past, breeding progress towards maize cultivars with improved native host plant resistance against root feeding by WCR larvae was minute due to the lack of resistant plant materials and detailed information about the defense responses of maize against WCR larvae root feeding and wounding. Recently maize germplasm with improved levels of native WCR resistance were developed by the USDA-ARS, Columbia, Missouri, and the University of Illinois, Urbana-Champaign, Illinois. These resistance sources combined with new genetic tools enabled us to investigate the genetic, genomic, and biochemical basis of the defense response in maize against WCR. In a companion project funded by the Illinois Missouri Biotechnology Alliance (IMBA, Project 2006-6), we evaluated gene expression patterns and metabolite profiles in one of the new resistance source associated with WCR larvae feeding.

The main goal of the present project was to investigate the genetic basis of WCR resistance in the newly developed maize cultivars with improved resistance levels. Employing the power of linkage disequilibrium mapping in segregating populations derived from crosses between resistant and susceptible maize cultivars will enable us to locate the genes involved in the improved resistance response and to estimate their effects. This information will be critical for improving breeding strategies in two ways: (1) Resistance genes tagged with molecular markers are the basis for marker-assisted selection strategies and (2) markers can be used to more efficiently screen germplasm banks for new resistance gene alleles.

Our first objective was to determine the genomic location and effect of major quantitative trait loci (QTL) involved in the inheritance of WCR resistance in maize using multiple mapping populations. The first mapping population consisted of 220 F_{2:3} families derived from the cross between four maize inbreds selected from population CRW3(C6) and LH51. CRW3(C6) derived inbreds showed improved levels of resistance to WCR, whereas LH51 is highly susceptible to WCR larvae feeding. The 220 F_{2:3} families and their parental inbreds along with susceptible and resistant (*Bt* hybrids) checks were evaluated in replicated field trials in four environments (Illinois, Missouri

[2], and South Dakota) in 2006. The largest mean root damage rating (RDR = 2.7) was observed in the Illinois location with a moderate repeatability (Fig. 1). All other locations showed less severe mean root feeding by WCR larvae. A detailed analysis confirmed that the response of the F_{2:3} families was highly effected by genotypic interactions of each family with the environment. Employing multivariate statistics, we identified a subset of environments that allowed us to significantly differentiate between resistant and susceptible genotypes (Fig. 2).

The exploitation of hybrid vigor ('heterosis') is the key concept in maize breeding. The performance of inbreds *per se* is only of secondary importance; therefore, the usefulness of a new inbred is determined by its performance in combination with other inbreds. In order to produce more relevant results from a breeder's perspective we crossed all F_{2:3} families to a common public inbred. These testcrosses were evaluated in 2007 in the same four environments (Illinois, Missouri (2), South Dakota) that were used in the previous year for the *per se* performance evaluation. Due to their increased vigor, the F_{2:3} testcrosses showed increased levels of root regrowth as a response to WCR larvae feeding, larger root sizes and substantially reduced root damage ratings (Fig. 3). As indicated by the increased heritability ($h^2 = 0.28$), we were also able to better estimate the resistance genotype of each F_{2:3} family using more vigorous testcrosses than inbred families *per se* ($h^2 = 0.20$). As expected for this highly quantitative resistance trait the association between *per se* and testcross performance was not significant. This result underscores the need of integrating testcross evaluations in the breeding process for improving WCR resistance.

All five WCR resistant parental genotypes derived from CRW3(C6) and the susceptible parent LH51 were initially genotyped with 87 SSR markers for identifying polymorphic markers. Results of this marker analysis revealed the complex genetic relationship between the parental inbreds selected from population CRW3(C6) for QTL mapping. Given this complexity and the low to moderate levels of resistance of the parental inbreds, we decided to develop new and more informative mapping populations. To initiate this process a set of factorial crosses between six newly developed WCR resistant inbreds, *i.e.*, three inbreds selected from populations initially formed as part of the USDA "Germplasm Enhancement in Maize (GEM)" and three inbreds developed by the USDA-ARS (Columbia, Missouri), and five public and private inbreds (provided by AgReliant), were intensively evaluated for their level of WCR resistance and agronomic performance in multiple environments (Illinois, Missouri [2], South Dakota, Indiana) across multiple years. Based on results obtained from these experiments we selected four hybrids for double haploid (DH) induction employing AgReliant's proprietary DH system. All DH lines (DHL) were testcrossed in the winter nursery 2007 by AgReliant. DH populations derived from the crosses AG1×NGSDRW1 (250 DHL) and AG1×AR17056-16 (200 DHL) were planted in Illinois, Missouri, South Dakota using replicated field experiments. The DH populations will be evaluated for WCR resistance, root size, and root regrowth. All 450 DH lines will genotyped using Single Nucleotide Polymorphisms (SNPs) in the second half of 2008.

The screening of large mapping populations (>200 genotypes) for WCR resistance revealed that WCR resistance is characterized by large genotype-by-environment interactions. Therefore, this trait is a perfect candidate for marker-based selection procedures. In order to obtain reliable molecular marker-trait associations, we demonstrated the need to assess the level of resistance of F_{2:3} families or DHL *per se* and in testcross combinations. In addition, we increased the power of QTL detection through the development of mapping populations derived from crosses between the most WCR resistant genotypes available to date and elite maize inbreds known for their excellent agronomic performance.

In our effort to better understand the interactions between maize and WCR, we investigated the effect of the bacterium *Wolbachia* present in WCR on maize gene expression during larval feeding. *Wolbachia* plays an important role in insect speciation in some insects by permanently changing chromosome structure and gene expression patterns in its insect host. Preliminary evidence from microarray analysis of maize gene expression during WCR larval feeding indicates that chromosome structure and gene silencing are part of the maize defense response.

We performed a microarray experiment to determine whether *Wolbachia* plays a role in manipulating maize gene expression utilizing the maize oligoarray from University of Arizona (<http://www.maizearray.org/>). The treatments included feeding by WCR carrying a *Wolbachia* endosymbiont, feeding by WCR minus the *Wolbachia* endosymbiont, and mechanical wounding. Approximately 21,000 of the more than 50,000 maize genes displayed significant differences in gene expression when examining the effects of feeding by WCR with vs. without *Wolbachia*. Among the genes affected were members of all three major classes of plant host defense genes: the PR proteins, phytoalexins, and cell wall proteins. The data indicate that WCR carrying *Wolbachia* cause reduced expression of plant host defense genes in these classes as well as senescence genes. These findings suggest that *Wolbachia*, an obligate parasite of WCR, may have evolved the capacity to alter gene expression in the plant to prevent the death of its insect host. Chromatin remodeling and silencing genes also display significant differences in expression when *Wolbachia* are present during WCR feeding. Our results may help to explain the extraordinary ability of the WCR to overcome chemical and cultural control measures as due to mutation/selection within the bacterial genome either with or without parallel selection in the insect genome. Our findings suggest that insect vectored endosymbionts must be considered in the interpretation of future plant-insect interaction studies. *Wolbachia* is present in a large proportion of arthropods including many of agricultural significance and a number that easily adapt to various control measures. The question remains whether the ability of *Wolbachia* to alter plant gene expression is a general phenomenon within maize or within other plant species.

Publications

- Flint-Garcia A, Dashiell KE, Prischmann D, Bohn M, Hibbard B. 2008. Maize hybrid vigor is associated with resistance to western corn rootworm larval feeding. *Crop Science*. *Submitted*.
- Gray M, Bohn M, Moeser J, Sappinton T, Miller N. 2008. Adaptation and Invasiveness of Western Corn Rootworm: Intensifying Research on a Worsening Pest. *Annual Review of Entomology*. *Submitted*.
- Ivezić M, Raspudić E, Brmež M, Majić I, Brkić I, Tollefson JJ, Bohn M, Hibbard BE, Šimić D. 2008. A review of resistance breeding options targeting western corn rootworm (*Diabrotica virgifera virgifera* LeConte). *Agricultural and Forest Entomology*. *Submitted*.
- Bohn M, Bulhoses S, Rupassara I. 2008. Analysis of the root defense metabolome of maize cultivars in the presence and absence of the WCR larvae. *J. Economic Entomology*. *In preparation*.

Invited Presentations

- Bohn M. 2007. “Genomic Evaluation of The Defense Response of Maize Against The Western Corn Rootworm – How to Use This Information in Breeding Programs?” Workshop “Resistance Breeding in Maize Against Diabrotica”, European Union 6th Framework Programme Specific Support Action DIABR-ACT: Harmonize the strategies for fighting *Diabrotica virgifera virgifera*. Osijek, Croatia, April 11-13, 2007.
- Bohn M. 2007. “Synergism of Traditional and Molecular Approaches for Corn Rootworm Resistance and Susceptibility Research – How to Breed Corn Resistant Rootworms?” Annual Meeting of the Entomological Society of America, San Diego, CA, December 9-12, 2007.
- Bohn M, Davis G. 2006. Genomic evaluation of the defense response of maize against the western corn rootworm – How to use this information in breeding programs? 22nd IWGO Conference, 5 - 8 November 2006, Vienna, Austria.
- Davis G. 2007. “The Role of Wolbachia in Maize Response to Western Corn Rootworm Feeding” Annual Meeting of the Entomological Society of America, San Diego, CA, December 9-12, 2007.

Abstracts and Publications (non-peer reviewed)

- Barr K, Hearne L, Hibbard B, Davis G. 2007. A novel role for Wolbachia (Rickettsiales: Rickettsiaceae) in plant-insect interactions. Annual Meeting of the Entomological Society of America, San Diego, CA, December 9-12, 2007.
- Bulhoses S, Rupassara I, Bohn M, 2008. Analysis of the Root Defense Metabolome of Maize in the Presence and Absence of Western Corn Rootworm Larvae. 50th Maize Genetics Conference, Feb. 27-March 1, 2008. Marriott - Wardman Park, Washington, D.C.

- Bohn, M. 2007. Der Maiswurzelboher in den USA – Neue zuechterische Ansaetze zur Bekaempfung. Mais 2/2007:44-47. [A German Journal dedicated to all aspects of maize breeding.]
- Schaefer C, Kim JP, Barr K, Musket TA, Davis G. 2008. Evolution of maize defense gene expression altered by Wolbachia. 50th Maize Genetics Conference, Feb. 27-March 1, 2008. Marriott - Wardman Park, Washington, D.C.

Figures

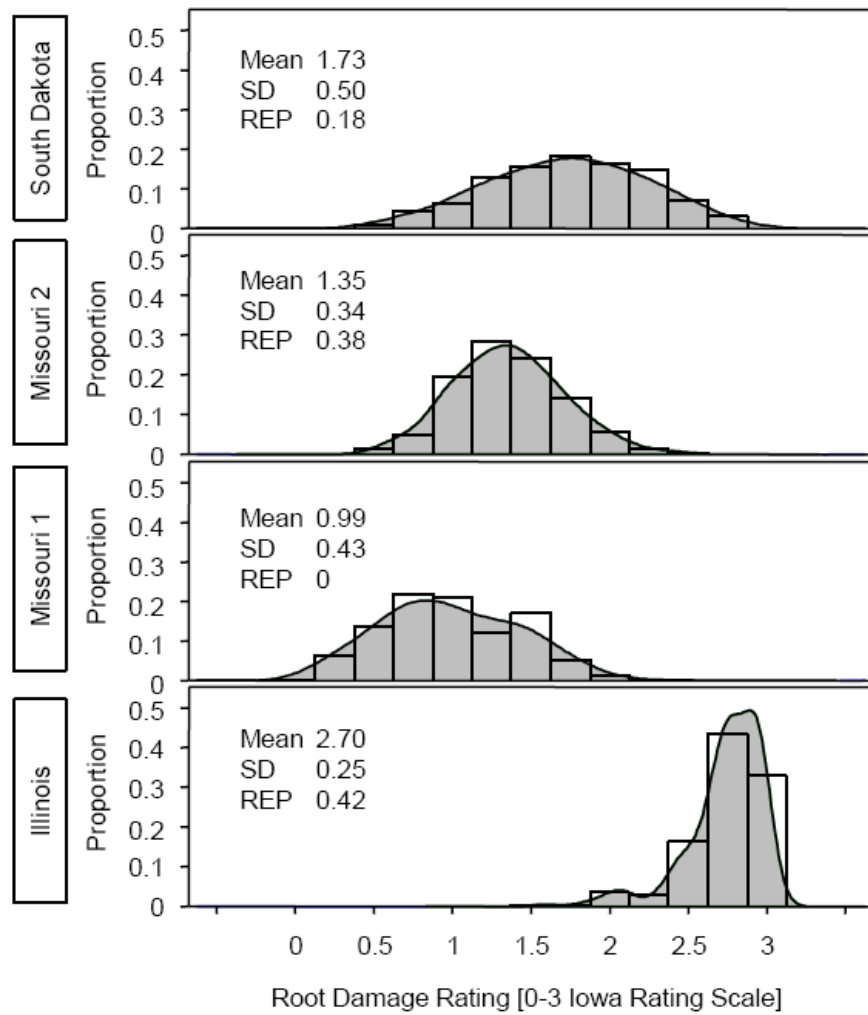


Fig. 1 Histograms for root damage ratings measured in four environments in 2006 for 220 $F_{2,3}$ families derived from cross CRW3×LH51. The evaluation was performed on families *per se*. (SD, standard deviation; REP, repeatability).

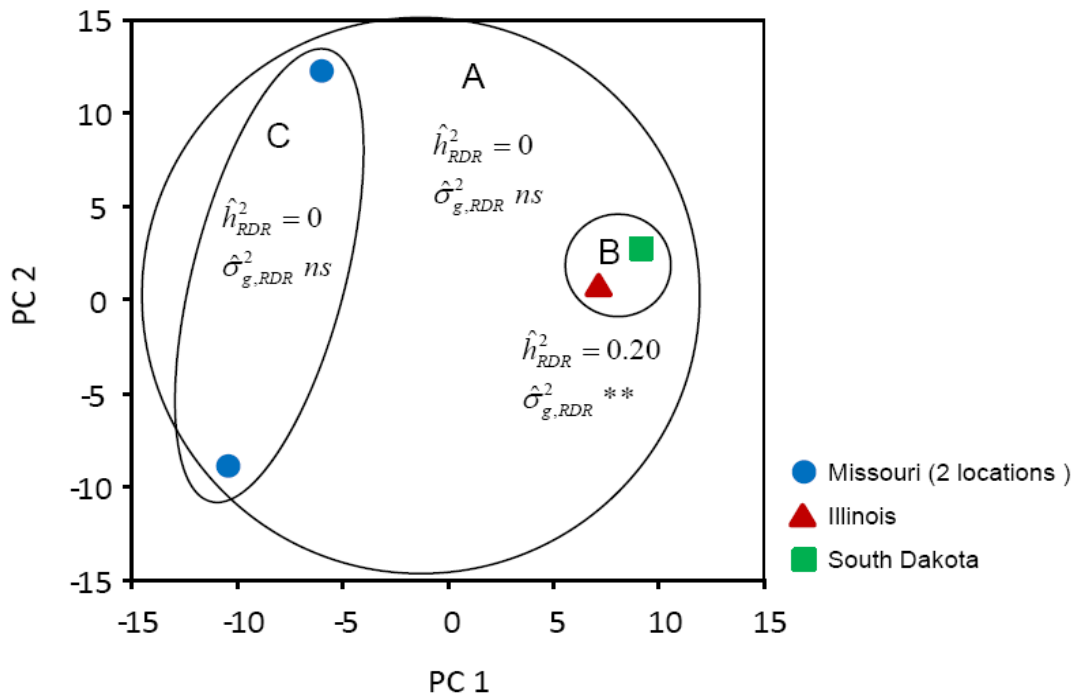


Fig. 2 After grouping the environments based on root damage ratings (RDR) observed after WCR feeding an analysis of variance was performed for each group (A = all environments; B = Illinois, South Dakota; C = two locations in Missouri). The above figure displays the grouping and presents heritability and genotypic variance estimates for RDR within groups A to C.

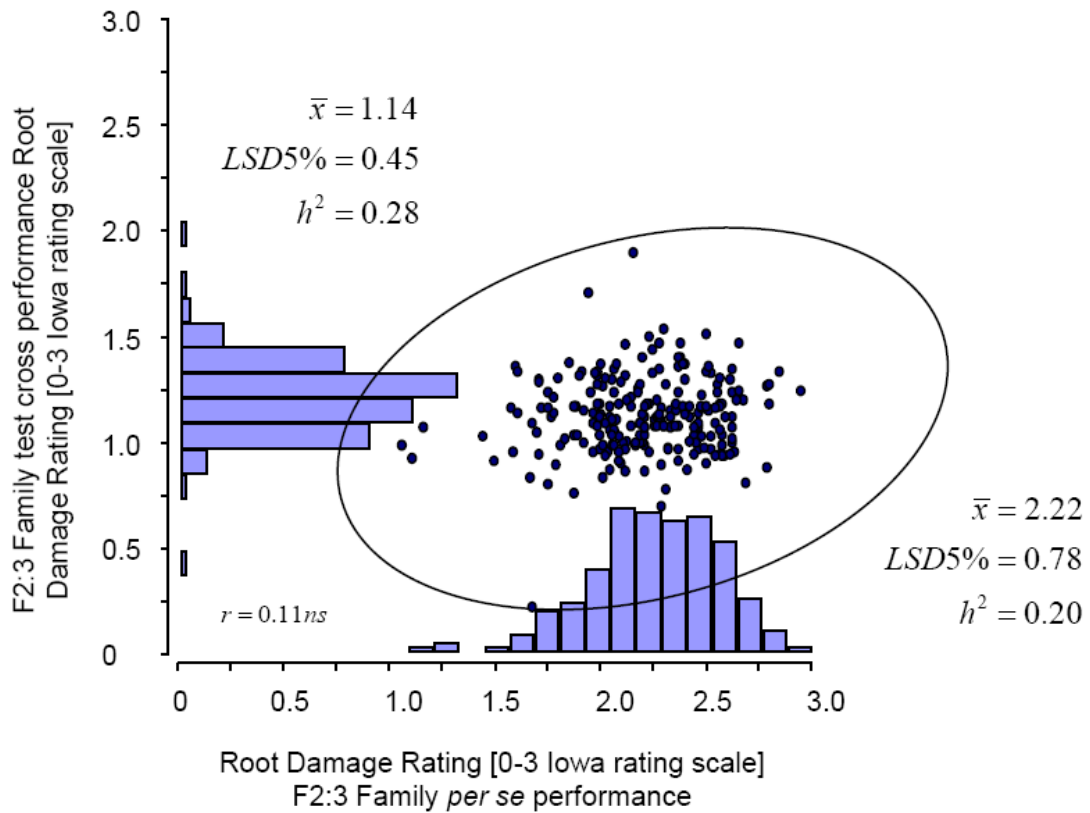


Fig. 3 Diagram for the relationship between inbred *per se* and testcross performance for WCR resistance evaluated in four environments in 2006 (*per se* performance) and 2007 (test cross performance) for 220 $F_{2:3}$ families derived from cross CRW3×LH51. Histograms for root damage ratings measured are also given. (\bar{x} , mean; $LSD5\%$, least significant difference at probability level 0.05, h^2 , heritability).