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INDIANA

PURDUE UNIVERSITY
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J.M. Anderson, S.E. Cambron, C. Crane, S.B. Goodwin, S. Scofield, B. Schemerhorn, R.H. Shukle, and C.E. Williams (USDA–ARS); H.W. Ohm and L. Kong (Department of Agronomy); G. Buechley, G. Shaner, and J.R. Xu (Department of Botany and Plant Pathology); and J. Stuart (Department of Entomology).

Wheat production.

According to the USDA National Agricultural Statistics Service, Indiana farmers harvested 150,000 hectares (370,000 acres) of wheat in 2007, down 18% from 2006. Wheat yields in Indiana averaged 3,830 kg/ha (57 bu/acre) in 2007, 15 bu less than the record high yield in 2005. Like most winters in Indiana since 1996, temperatures averaged above normal and winterkill due to low temperatures was limited. Unlike 2005 and 2006, growing conditions for winter wheat in 2007 were stressful; abnormally cool temperatures until late April, including a severe frost in mid-April that caused abandonment of some fields in southern Indiana. Beginning in early May, warm temperatures and increasingly dry soil with significant drought conditions developed by mid to late June, resulting in low grain yields and average to low test weight. Acreage prospects for 2007–08: preliminary reports are that 550,000 acres were seeded, and more acreage would have been seeded, but seed was limited. Wheat establishment was excellent and autumn growth was excellent prior to onset of winter.

Wheat disease summary.

Yellow dwarf, including BYDV and CYDV, were widespread and moderate to severe throughout the southern two-thirds of Indiana. Foliar diseases, including Fusarium head blight were present but not significant except in localized areas, likely due to unusually cool temperatures early in the spring growing season and dry conditions later in the growing season.

Performance of new cultivars.

Cultivar INW0731 yielded unusually well, ranking first or nearly first in multiple locations in Indiana and nearby regions, likely due to its demonstrated large root volume and moderate resistance to yellow dwarf. INW0731 has moderate resistance to Fusarium head blight from Freedom and Fundulea 201R, moderate resistance to leaf rust, resistance/tolerance to yellow dwarf, powdery mildew, Stagonospora nodorum blotch, Septoria leaf blotch, soil-borne wheat mosaic virus, and wheat spindle streak mosaic virus, and is susceptible to Hessian fly, stripe rust, and stem rust in Indiana.

INW0731 is adapted to southern Indiana and surrounding regions; it has survived winters very well in central and northern Indiana, but winters have been mild since 1996.

Cultivar INW0316, which has gene *Bdv3* introgressed from intermediate wheatgrass, a western U.S. range grass for grazing, continues to excel when yellow dwarf disease, caused by BYDV (PAV) and CYDV (RPV), is moderate to severe. INW0316 is especially well-suited to southern Indiana and adjacent areas because yellow dwarf is present many years, as in 2006 and 2007. In our multilocation performance tests in 2006 INW0316, Pioneer25R47, and Roane yielded, 103.2, 107.4, and 95.8 bu/a, respectively, $LSD_{0.05}$=7.7, averaged over four locations in mid to northern Indiana at which yellow dwarf was absent, and 89.0, 66.6, and 68.1 bu/a, respectively, $LSD_{0.05}$=6.5, at Evansville. Yellow dwarf severity on the three cultivars, respectively, at Evansville was 0, 3, and 4 (0=no symptoms to 9=severe leaf discoloration, plant stunting and little or no seed set). Infestation by viruliferous aphids, *Rhopalosiphum padi*, occurred in the sutumn of 2005. In 2007, INW0316, Pioneer25R47 and Roane yielded, 86.8, 86.2, and 78.2 bu/a, respectively, $LSD_{0.05}$=6.9, averaged over two locations in northern Indiana at which yellow dwarf was negligible. Grain yield averaged over three locations in central and southern Indiana at which yellow dwarf was moderately severe was, for the three cultivars 84.0, 78.6, and 73.8 bu/a, respectively, LSD=7.9. Yellow dwarf severity at these three locations averaged, respectively for the three cultivars, 0, 4 and 3.5, LSD=0.7. Infestation by viruliferous aphids occurred in autumn 2006 and in spring 2007 at Evansville.

Breeding/genetics. Combining multiple genes for resistance to foliar diseases, yellow dwarf, and Hessian fly in improved germ plasm and soft winter wheat cultivars adapted to Indiana.

Herb Ohm, Lingrang Kong, Xiaorong Shen, Judy Lindell, Stephen Baluch, Brett Ochs, and Kristen Rinehart.

Fusarium head blight. The chromosome segment 7EL from *Th. ponticum*, with resistance QTL, *Qfhs.pur-7EL*, was shortened to the distal one-third of T7DS·7DL7EL in wheat line P275-4 by crossing translocation line KS24-2 (T7DS·7EL) to the Chinese Spring wheat *Ph1b* deletion line. *Qfhs.pur-7EL* was mapped, by deletion bin mapping, to the distal portion of the introgressed 7EL segment. *Qfhs.pur-7EL* of P275-4 was combined with *Fhb1*, and in greenhouse tests using point inoculation (inoculation of a single floret at flowering with 500 *F. graminearum* macro spores in 10 μl dH_2O and placing a plastic bag over inoculated spikes for 3d) the disease severity averaged 0.75 diseased spikelets at 21 dai. We will carry out tests in the field under misted conditions in 2008.

Stem rust, yellow rust. We have identified and obtained germ plasm lines that have potentially new resistance to stem rust race TTKS (Ug99) and yellow rust. We have developed $F_{2:3}$ populations from crosses of the new resistant/susceptible lines. In collaboration with USDA–ARS laboratories at St Paul, MN; Pullman, WA; and Raleigh, NC; and at Purdue University for resistance to our local isolates of the causal fungal pathogens, the populations are being phenotyped for resistance. We will then screen the F_2 populations with SSR markers and map the resistance.

Marker-assisted selection. We have significantly expanded MAS as an integral part of the breeding program to combine a large number of desired QTL/genes for various important plant traits. MAS is a necessary technology to genotype parent lines for various desired traits and to plan parental combinations for efficiently combining a large number of desired plant traits.

Lab members. Lingrang Kong and Xiaorong Shen are Research Associates, Judy Lindell is a Research Molecular Biologist, and Stephen Baluch, Brett Ochs and Kristen Rinehart are doctoral students.

Interactions of wheat with virulent and avirulent Hessian fly larvae.

Christie Williams, Jill Nemacheck, Subhashree Subramanyam, Kurt Saltzmann, Marcelo Giovanini, and Stephen Baluch.

Wheat lectin deters insect feeding. During incompatible interactions, avirulent Hessian fly larvae are recognized by resistant wheat plants, resulting in the triggering a diverse set of plant defense responses. One component of this defense is the production of the HFR-1 protein. The ability of this protein to agglutinate red blood cells along with binding mannose-containing glycans demonstrates that this induced protein functions as a lectin. Because Hessian fly larvae are obligate parasites and cannot be grown in culture, feeding deterrent properties of HFR-1 were tested with *Drosophila*

melanogaster reared on artificial medium containing the protein. At low concentrations, the HFR-1 protein delayed larval development. At intermediate concentrations, larval development was arrested before pupation. At high concentrations, larvae crawled out of the medium and slowly starved to death on the side of the glass vial. These outcomes are consistent with HFR-1 lectin functioning as a feeding deterrent rather than an acute toxin.

Larvae manipulate wheat nutrient content. Virulent Hessian fly larvae manipulate their host plants to provide a good environment for their development. One plant component that is altered by these larvae is the production of certain amino acids. The increased production of methionine, histidine and phenylalanine is important because these essential amino acids must be obtained by the insect in its diet. Phenylalanine and tyrosine are necessary for the production of insect cuticle. Other amino acids that increase in abundance may contribute to energy production and other processes that benefit the larvae.

Lab members. Subhashree Subramanyam is a Purdue University postdoctoral researcher. Kurt Saltzmann is a USDA-ARS postdoctoral researcher. Jill Nemacheck is a research technician. Stephen Baluch is a joint Ph.D. student with Herb Ohm. Marcelo Giovanini, currently a corn breeder for Monsanto in his home country of Brazil, was a joint student with Herb Ohm.

Molecular interactions between Hessian fly and wheat.

Richard Shukle, Alisha Johnson, Kristin Saltzmann, Weilin Sun, and Jacob Shreve.

The objective of our research is to gain insight into the molecular interactions between Hessian fly and wheat. In this regard, previous research in our laboratory was directed toward transcriptional profiling of genes expressed in the larval Hessian fly during interactions with susceptible and resistant wheat. Results indicated that on susceptible wheat genes involved in establishing a feeding site, manipulation of host-plant cells, feeding and growth/development were up-regulated, while on resistant wheat genes involved in responding to stress and disruption of homeostasis (DAD–defender against apoptotic cell death, heat shock, detoxification, antioxidant defense, and excretion) were up-regulated. This supports the assumption that larvae on resistant plants encounter either toxic plant compounds, feeding deterrents, or cannot manipulate host-plant cells to develop a nutritive tissue to feed on. Of particular interest from this work was the transcriptional profile of one family of secreted salivary gland proteins (SSGPs). Results indicated the transcript levels for the SSGPs were equal in larvae at 6 hours on susceptible and resistant wheat. However, the transcript level in larvae on resistant wheat did not increase and showed a downward trend, whereas on susceptible wheat, the transcript level continued to increase peaking at 24 hours, suggesting that a rapid defense response in resistant wheat precludes the up-regulation of the genes encoding this family of SSGPs. Current research to further dissect Hessian fly/wheat interactions at the molecular level and identify novel approaches to genetically engineered resistance are focused toward (1) RNAi as a function genomics tool for genes expressed during Hessian fly/wheat interactions; (2) comparative salivary gland transcriptomics between divergent Hessian fly populations that differ in virulence to genes for resistance to reveal unique SSGPs involved in host-plant/tissue adaptation; and (3) electron microscopy studies of the larval midgut during compatible and incompatible interactions with wheat to reveal if the midgut is a target for toxic plant compounds in larvae on resistant plants and if so the possible mode of action.

Lab members. Alisha Johnson, USDA–ARS Research Technician and Ph.D. student; Kristin Saltzmann, USDA–ARS Research Technician; Weilin Sun, collaborating postdoctoral associate; and Jacob Shreve, undergraduate technician.

Population genetics of Hessian fly.

Brandon Schemerhorn, Yan M. Crane, Richard Smith, Philip Morton and Jennifer Sanders.

The objective of our research is to investigate the effects of genotype interaction between wheat and the Hessian fly on the genetic stability of the pest populations and risks to deployment of new resistance resources. In order to answer these questions, we have developed a microsatellite library and genetically mapped a set or markers to assess the population dynamics of the Hessian fly. We are currently using these markers to investigate geographic distance and biotype forms as barriers to gene flow. At this point, we have determined that in the southeastern United States, the barriers to gene flow between populations are not limited by distance, but rather geographical and climactic similarities in the

areas where populations were collected. Currently, this research has expanded into the Midwest, including Indiana, to determine if this trend will hold true. We currently are assessing E-chromosome makeup by AFLPs, the creation of a subtractive hybridization library and by in situ analysis of BAC clones from available libraries to investigate geographic distance and biotype forms as barriers to gene flow. We also have been working towards the elucidation of the processes of metabolic resistance to insecticides in the Hessian fly. The current data suggest that oxidative burst patterns are suggesting a complex cascade pathway whose function is yet to be determined.

Lab Members. Dr. Yan M. Crane, USDA–ARS Research Technician; Richard Smith, USDA–ARS Research Technician; Philip M. Morton, Ph.D. student; and Jennifer Sanders, undergraduate technician.

Fusarium graminearum: Regulatory genes for DON.

Jin-Rong Xu.

The whole-genome microarray of *F. graminearum* and targeted deletion mutants are being used to identify regulatory genes controlling DON accumulation in infested grains.

Septoria tritici blotch.

Disease resistance (Stephen Goodwin, Jessica Cavaletto, Ian Thompson, Emily Helliwell, and Alisa Ponomarenko). Additional screening was done to find molecular markers closely linked to Septoria tritici blotch resistance gene *Stb2* on chromosome 3BS. Approximately 350 lines of an F_3 recombinant-inbred population, derived from a cross between the Swiss cultivar Arina and the doubled-haploid DH115, were screened for resistance to *M. graminicola*, and 20 SSR markers showing polymorphism between the parents were tested on the progeny. Linkage and QTL analyses identified five markers closely linked to *Stb2*, including two markers not previously identified as mapping to this region. Of these, marker *Xwmc754* was found to be closely linked to *Stb2*, and may be useful in future applications of marker-assisted selection.

Differences among susceptible and resistant interactions of *M. graminicola* on wheat and the non-host barley were compared to reciprocal interactions of the barley pathogen *Septoria passerinii* on barley and wheat. Trypan blue staining showed that *M. graminicola* germinates on barley leaves and enters via the stomata similarly to wheat, but fungal growth stagnates shortly after penetration. Staining with 3,3-diaminobenzidine showed an accumulation of H_2O_2 around stomatal cells and, later, epidermal cells, indicating a possible hypersensitive response. Quantitative real-time PCR showed differences in fungal biomass among the interactions. These data show that *M. graminicola* penetrates cells and triggers production of reactive oxygen species, providing further evidence for an active defense response of barley to this wheat pathogen.

Large-scale, cDNA-AFLP profiling previously identified numerous genes with increased expression during the resistance response of wheat to the Septoria tritici blotch fungus, *M. graminicola*. To test whether these genes were associated with resistance responses, their levels of expression were measured at 12 time points from 0 to 27 days after inoculation (DAI) in two resistant and two susceptible cultivars of wheat by real-time quantitative PCR. None of these genes was expressed constitutively in the resistant wheat cultivars. Instead, infection of wheat by *M. graminicola* induced changes in expression of each gene in both resistant and susceptible cultivars over time. Four genes were induced from about 10 to 60 fold only at early stages (3 h-1 DAI) during the incompatible interactions. Nine other genes had bimodal patterns with both early (1-3 DAI) and late (12-24 DAI) peaks of expression. The remaining gene had a trimodal pattern of expression in the resistant cultivar Tadinia. Therefore, the resistance response of wheat to *M. graminicola* is not completed during the first 24 hours after contact with the pathogen, as thought previously, but can extend into the period from 18 to 24 DAI when fungal biomass increases dramatically in susceptible interactions. Significant differential expression of the defense-related genes between the resistant and susceptible wheat cultivars and RILs after inoculation with *M. graminicola* suggests that these genes may play a major role in the resistance mechanisms of wheat.

Fungal genomics (Goodwin lab). The trigger for the switch from biotrophic to nectrotrophic growth of *M. graminicola* in wheat and the mechanisms of resistance in the host are not known. To better understand the biology of this pathosystem, the genome of the pathogen was sequenced completely at the Joint Genome Institute by filling in the gaps in an 8.9×

draft sequence. The essentially finished sequence contains 18 chromosomes from telomere to telomere, plus five fragments. Four of the five fragments contain telomeres so they presumably make up two additional chromosomes for a total of 20. A comparative bioinformatics analysis of *M. graminicola* with seven other sequenced fungal genomes revealed that *M. graminicola* possessed fewer enzymes than expected for degrading plant cell walls. Analyses of grass-infecting pathogens versus those from other hosts indicated that the suites of cell wall-degrading enzymes were tailored to break down the cell wall compositions of their particular hosts. The frequency of transposable elements in the genome of *M. graminicola* was intermediate between those of other sequenced fungi. Many long (> 10 kb) retrotransposons were identified in the finished genome compared to the draft sequence, indicating the need for finishing of other fungal genomes. Availability of the finished genome for *M. graminicola* should greatly aid research on this organism and will help to understand its interaction with wheat.

To aid in comparative genomics, the sequence of the related banana pathogen *M. fijiensis* was obtained and released as a 7.1× draft during August of 2007. This genome was almost twice the size (73 Mb) of *M. graminicola* but had about the same number of genes. Much of the increased genome size seems to be due to higher numbers of families and higher copy numbers of retrotransposons in the genome of *M. fijiensis* compared to that of *M. graminicola*. The mitochondrial genomes of both species were obtained and also differed in size, with that of *M. fijiensis* about twice that of *M. graminicola*. Numbers of tRNA genes and unknown ORFs were higher in *M. fijiensis*. The set of structural genes within the two genomes were similar, but two genes in *M. fijiensis* contained introns that were absent from their homologs in *M. graminicola*. The reason for the increased genome size of *M. fijiensis* relative to that of *M. graminicola* is not known.

Lab members. Jessica Cavaletto and Dr. Ian Thompson are USDA–ARS Biological Science Research Technicians. Braham Dhillon is a Ph.D. student working on bioinformatics. Emily Helliwell completed her M.S. degree during the summer of 2007 and is now in a Ph.D. program at Pennsylvania State University. Alisa Ponomarenko also completed her M.S. during the summer of 2007 and is now in a Ph.D. program in bioinformatics at Purdue University.

Wheat viruses.

Epidemiology of wheat viruses (J.M. Anderson and B. Portwood). In this study, a multiplex reverse transcription polymerase chain reaction (M-RT-PCR) method was used to identify which viruses are present in field samples from 22 counties in Indiana. These samples were initially identified because they appeared to have viral disease symptoms. The multi-plex PCR can simultaneous detection and discrimination of eight viruses including five strains of barley/cereal yellow dwarf virus (B/CYDV), wheat spindle streak mosaic virus (wssmv), soil-borne wheat mosaic virus (SBWMV), and Wheat streak mosaic virus (WSMV). Analysis of these samples indicated that all 22 samples contained virus and all had mixed infections. BYDV-RMV was the least abundant virus as it was detected in five of the samples and generally appeared to present in very low levels. When the sample had B/CYDV typically they contained BYDV-PAV, -SGV, -MAV, and CYDV-RPV. WSSMV and SBWMV were present in 50% and 41% of the samples, respectively. Perhaps the most surprising result was the high percentage of samples (41%) that contained WSMV. This disease is transmitted by a mite that prefers a drier climate than that found in Indiana and, therefore, has not been considered to be a problem in Indiana or the Eastern U.S. These data suggest that this disease is more prevalent than previously thought. Although this is a very limited sample set collected just in the 2007 spring/summer these results demonstrate the utility of this method as a virus detection method and a tool for epidemiological studies.

Wheat-*Thinopyrum* mosaic chromosomes (K. Card and J.M. Anderson). A large number of *Thinopyrum*-wheat translocation recombinants in which the translocation chromosomes consist of an array of wheat and *Th. intermedium* chromatin segments were previously identified. These recombinants have been further characterized using additional DNA markers and are proving to be an excellent set of materials for identifying wheatgrass chromatin DNA markers.

Lab members. Brian Portwood is a USDA–ARS Biological Science Research Technician. Katie Card is currently a USDA–ARS Biological Science Research Technician at NCAUR in Peoria Illinois. Mahua Deb is currently a Research Associate with Chembiotek Research International, India.

Research personnel.

Paul Werner, an M.S. student with Herb Ohm, thesis research on characterizing and mapping yellow dwarf and crown rust resistance in oat, completed degree requirements in August 2007 and has joined his family's seed production business in Minnesota. Xiaorong Shen is the lab manager with a private pharmaceutical firm in Princeton, NJ.

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