SESSION 4:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Michael Lawton

A GENOMICS APPROACH TO CHARACTERIZE TRICHOTHECENE MODE OF ACTION REVEALS A CELLULAR WIDE RESPONSE IN YEAST.

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ABSTRACT

Trichothecenes constitute a large family of low-molecular-weight sesquiterpenoid mycotoxins produced by various species of *Fusarium, Trichoderma*, *Cephalsporium*, and other fungi. Identified by their characteristic trichothecene ring, these toxins include deoxynivalenol (DON), diacetoxyscierpenol (DAS), T-2, and trichothecin (T-cin). Each toxin varies with regard to toxicity and as a group is known to have a wide array of effects in plants, animals, and humans. The plant pathogen *Fusarium graminearum* causes Fusarium head blight (FHB) in both wheat and barley resulting in reduced plant yield and contamination of cereal grains with trichothecenes, in particular DON. DON is an inhibitor of translation. However, the inhibitory effects of trichothecenes are often not limited to translation and information on other downstream targets of trichothecenes is lacking. To obtain a comprehensive picture of the pathways involved in trichothecene metabolism and resistance, we have used the yeast, *Saccharomyces cerevesiae*, as a model organism to study the impact of trichothecences on eukaryotic cells. We screened the yeast knockout (YKO) collection, composed of 4700 strains, to identify mutants that exhibit hypersensitivity to T-cin. We selected T-cin over DON to screen the library since yeast is sensitive to micromolar levels of T-cin compared to mM levels of DON. Bioinformatic analyses of the select sensitive mutants have revealed components of pathways that play a role in trichothecene resistance, such as MAP kinases, components of protein synthesis, vacuolar protein sorting, and ribotoxic stress pathways, suggesting a cellular-wide response. These genes represent new candidates for engineering resistance to DON and FHB in cereals. Further characterization of these genes will provide important new insights into the trichothecene metabolism.

ACKNOWLEDGEMENT AND DISCLAIMER

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2008 FHB ANALYSIS OF TRANSGENIC BARLEY LINES.

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ABSTRACT

Transgenic lines have been developed with the goal of reducing FHB and DON in barley. Replicated field trials for FHB reaction of 48 Conlon transgenic lines were conducted in 2008 in Langdon, ND and Rosemount, MN. The Langdon trials consisted of three replicates in hill plots in an inoculated misted nursery and three replicates in the adjacent un-inoculated un-misted nursery. The misted nursery was inoculated with a mixture of five *F. graminearum* isolates three times at two weekly intervals beginning 2 weeks prior to heading. Rosemount plots included 4 replicates of 8 foot rows which were spray-inoculated at heading and misted after inoculation. FHB severity (% infected kernels) and FHB incidence (% infected spikes) were measured at both locations. Disease severity was highest in the Langdon inoculated plots (31-55%) and lowest in the plots at Rosemount (2-10%). FHB incidence at Langdon was essentially 100%, with almost all spikes showing some FHB except the resistant checks. Data were ranked from low to high for each measurement and Spearman rank correlations were not significant between the locations, i.e. lines with the lowest measurements in either Langdon nursery showed moderate to high FHB at Rosemount. Lines did show significant correlations between measurements within a location. Two lines showed significant reductions in FHB (compared to Conlon) in the Langdon un-inoculated nursery and two different lines showed significant reductions in incidence and severity at Rosemount. All four lines contained rice genes for a chitinase and a thaumatin-like protein. All 48 lines will be tested again in 2009 to validate the reductions in FHB.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2008 FIELD NURSERY REPORT.

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ABSTRACT

The 2008 field screening nursery, with 64 wheat and 208 barley plots was located at UMore Park, Rosemount MN. Trial entries were submitted by Rutgers University (5 wheat), University of North Texas (2 wheat) and USDA (48 barley). In addition to the submitted transgenic entries, untransformed controls were also submitted from each program. Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen and the susceptible cultivars Wheaton, Norm and Roblin while the barley checks were the moderately resistant line M122 and the susceptible cultivars Robust and Stander. The experimental design was a randomized block with four replicates. Plots were 2.4 m long single rows. The trial was planted on May 8, 2008. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation was applied at anthesis for wheat and at head emergence for barley. The second inoculation was applied three days after the initial inoculation (d.a.i.) for each plot. The inoculum was a composite of 41 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from June 26, two days prior to the first inoculation, till July 22 to facilitate FHB development. FHB incidence and severity were assessed visually 20-24 d.a.i. for wheat and 17-21 d.a.i. for barley on 20 arbitrarily selected spikes per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 spikes observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed in these 20 spikes. Plots were harvested at maturity on August 11. The harvested seed from each plot was split using a Boerner Divider to obtain a 50 g sub-sample, which was then cleaned by hand. These sub-samples were used to estimate the percentage of visually scabby kernels (VSK) for wheat and then all samples (wheat and barley) were analyzed for deoxynivalenol (DON). The data indicated that resistance was expressed in some of the transformed lines.

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DIFFERENTIAL TRANSCRIPTOMICS AND PROTEOMICS OF *FUSARIUM GRAMINEARUM*- AND TRICHOTHECENE-CHALLENGED WHEAT GENOTYPES.

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ABSTRACT

While a few fusarium head blight (FHB) host transcript- and protein-accumulation studies have emerged in the past few years, no global comparisons have been made between resistant and susceptible wheat genotypes, or between trichothecene- and *Fusarium*-induced defense responses. The current study explores the differential transcriptome and proteome of three wheat genotypes ('Superb', DH1 and DH2) in their response to isolated components of the FHB-wheat interaction (fungus, deoxynivalenol, and aggressive-factors) within the first 24h of interaction. 'Superb' is a FHB-susceptible Canadian wheat cultivar that shares 75% genetic identity with each of the double haploid lines, DH1 (CIMMYT11-derived type I resistance) and DH2 ('Sumai 3'-derived type II resistance). Uninfected spikelets of point-inoculated heads were harvested in order to identify changes in transcript and protein accumulation associated with induced systemic resistance. Differential transcription is elicited as early as 3hai (and up to 24hai) by the different components of the FHB-wheat interaction in the uninvaded tissues of all three wheat genotypes. Such an early induced response in distal spikelets suggests that a mobile alarm signal is produced in the infected tissue and transmitted to the uninvaded tissues, preparing the tissue for *Fusarium* invasion. On the other hand, few differences are elicited in the proteome of the aforementioned interactions at 3dai. It is possible that either (a) a change in the proteome of uninvaded tissues occurs later than 3dai, or (b) a change in proteome is induced at 3dai, but the assay method used is not sensitive enough to detect these changes. Fewest differences were observed in the uninvaded tissues of the type II resistant line, suggesting that resistance to disease spread is regulated at/near the site of infection.

DEOXYNIVALENOL-INDUCED GENE EXPRESSION IN BARLEY.

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ABSTRACT

Trichothecenes are a major group of mycotoxins that are produced by many types of phytopathogenic fungi including *Fusarium, Myrotherium, and Stachybotrys*. Trichothecenes inhibit protein synthesis in eukaryotic cells and function as phytotoxins that promote fungal disease. Therefore, the role of plant host response to trichothecene accumulation is an important aspect of plant defense to fungal disease overall. In FHB, the yield and quality of infected grain is severely reduced because of blighted kernels and the presence of the trichothecene deoxynivalenol (DON). Our objective was to examine the barley host response after DON application. A susceptible barley genotype (cv. *Morex*) inoculated with DON was shown to convert DON to DON-3-glucoside. In a subsequent experiment, a susceptible barley genotype (cv. *Morex*) was inoculated with the equivalent of 2.0 µg DON per floret or mock-inoculated with water. Microarray analysis was conducted with the Barley1GeneChip® to examine gene expression at 1, 12, 24, 48 hours after inoculation. A total of 255 transcripts exhibited increased accumulation, with fold changes of ≥ 2.0 in DON treated versus water treated plants. Eleven transcripts exhibited decreased accumulation (fold change ≤ 0.5 between DON and water treatment). Comparative analysis with previous barley-*Fusarium* studies shows that 135 of these genes may be trichothecene-specific. These genes comprised many functional classes; those groups that were of particular interest included putative trichothecene detoxification and transport, regulatory, signal transduction, and ubiquitination. We validated the expression of a subset of these genes in a near-isogenic line pair respectively containing resistant and susceptible alleles at an FHB resistance QTL on barley chromosome 2H-Bin8. Real-time PCR results validate the DON-specificity of the transcripts as observed from the microarray data. In addition, we found that some transcripts were differentially expressed between DON-treated resistant and susceptible near-isogenic lines, indicating that the transcripts encode genes that may be contributing to either resistance or susceptibility to FHB.

VIRUS-INDUCED GENE SILENCING IDENTIFIES A PUTATIVE ROLE FOR ETHYLENE SIGNALING IN TYPE II RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT.

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ABSTRACT

Ethylene (ET) and Jasmonic Acid (JA) have been shown to be important for resistance to necrotrophic pathogens in Arabidopsis. While it remains unclear as to whether *Fusarium graminearum* is a hemibiotroph or a necrotroph, its necrotrophic mode of growth is most damaging. Thus, ET and JA are potential candidates for disease resistance signaling. We have used a Virus-Induced Gene Silencing (VIGS) system to silence genes in both the ethylene biosynthesis pathway and the ethylene signaling pathway. Preliminary results indicate that a number of these genes may indeed be important for defense signaling against *Fusarium graminearum*. The genes were silenced in the resistant variety 'Ning' 7840. Upon application of the virus, containing a portion of a wheat gene, the plants were screened for conversion from resistance to susceptibility. The genes involved in ET signaling screened thus far include SAMs, ACS, ETO, CTR, EIN2, and an ERF.

FUSARIUM HEAD BLIGHT RESISTANT TRANSGENIC WHEAT EXPRESSING ANTIFUNGAL PLANT DEFENSIN FROM *MEDICAGO TRUNCATULA* (MTDEF4).

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ABSTRACT

Defensins belonging to a superfamily of similarly folded antimicrobial peptides comprise representatives in vertebrates, invertebrates and plants. Plant defensins are small cysteine-rich peptides with a net positive charge which have been isolated from both mono- and dicotyledonous plants. We have identified and isolated a plant defensin MtDef4 from *Medicago truncatula* which is a potent inhibitor of *Fusarium graminearum* (IC_{50} = 0.75-1.5 μM) during *in vitr*o antifungal activity assay. Transgenic wheat lines expressing monocot intron optimized MtDef4 were generated using *Agrobacterium tumefaciens*-mediated transformation of spring wheat cultivar Bobwhite (BW) and a Chinese cultivar Xin Chun 9 (XC9). A total of six and one events were generated in BW and XC9 background, respectively. Based on the segregation analysis of these 7 events, 3 in BW and single one in XC9 background segregated for single copy of the *MtDef4* gene. Homozygous plants from all four single-copy events were identified in the T2 generation, all of which were expressing MtDef4 protein based on ELISA. Type II resistance to FHB was evaluated in three homozygous lines using single floret inoculation method in the greenhouse. Of the three lines (independent events) tested, one transgenic line 431-1-3-1 showed improved resistance when compared to non-transformed Bobwhite both in the T3 and T4 generation, thereby showing the heritability of FHB resistance. Moreover, the level of resistance in this line was similar to that of FHB resistant cultivar Alsen. The results of this study show the potential of plant defensin *MtDef4* in conferring heritable resistance to FHB.

BIOPROSPECTING FOR *TRI101* IN *FUSARIUM*: SEARCHING FOR A BETTER ENZYME TO DETOXIFY DEOXYNIVALENOL (DON).

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ABSTRACT

The mycotoxin deoxynivalenol (DON) is a common contaminant of wheat and barley in the United States. New strategies to mitigate the threat of DON need to be developed and implemented. Previous research has shown the value of an enzyme (TRI101) to modify DON and reduce its toxicity. Recent work by Garvey et al. (2008) highlighted differences in the activity of TRI101 from two different species of *Fusarium* (*F. graminearum* and *F. sporotrichioides*), but little is known about the relative activity of TRI101 enzymes produced by other species of *Fusarium*. We cloned *TRI101* from four different species of *Fusarium*: *F. sporotrichioides*, *F. graminearum*, *F. oxysporum*, and *F. fujikuroi*. Pairwise comparisons of genetic identity between *TRI101* sequences ranged from 65% (*FgTRI101* and *FjTRI101*) to 85% (*FoTRI101* and *FjTRI101*). To increase the transfer of mycotoxin in and out of the yeast cells for our expression studies, we also cloned *TRI12* (a trichothecene efflux pump) from *F. sporotrichioides*. Both genes were cloned into the yeast expression vectors pYes2.1 (*TRI101*) and pESC-LEU (*TRI12*), and the resulting vectors were co-transformed into the yeast strain RW2802. Transformed strains of RW2802 expressing *TRI101* and/or *TRI12* were fed DON at a concentration of 10ppm for 4 days at 28C. Fungal secondary metabolites were extracted, and DON and 3-acetyl-deoxynivalenol (3-ADON) were quantified using GC/MS. All of the *TRI101* genes tested were able to acetylate DON *in vitro*, and the ratio of [3-ADON]/[DON] ranged from 0.77 (FoTRI101) to 10.44 (FsTRI101). Our results suggest that other species of *Fusarium* (even those that do not produce DON) may contain functional *TRI101* genes, some with the potential to 'outperform' those evaluated in the present study. We are currently developing an *Agrobacterium* transformation vector to move these *TRI101* genes into hulless barley lines. We plan to monitor potential decreases in DON in both raw grain and dried distiller's grains with solubles (a byproduct of ethanol fermentation and a significant source of feed for domestic animals) following ethanol production using our genetically-engineered lines. TRI101 has tremendous potential to enhance food safety in the United States in the near future.

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HR-LIKE LESION MIMIC CONTRIBUTES TO IMPROVED RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT.

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ABSTRACT

Lesion mimics (LM) that resemble plant disease symptoms have been reported to confer a broad spectrum resistance to diverse pathogens and to be involved in plant defense responses. A Chinese scab-resistant wheat line Ning7840 starts expression of LM around heading. To investigate whether LM in Ning7840 contributes to type II resistance to *Fusarium graminearum,* a recombinant inbred population from cross between Ning 7840 and Chokwang, a Korean moderately scab-resistant cultivars, was evaluated for LM and scab resistance. The gene responsible for LM in Ning 7840, designated as *lm,* also associated with type II resistance to scab. Lines with LM phenotype showed a significantly higher level of scab resistance than non-LM lines ($P < 0.05$). *lm* reduced the percentage of scabbed spikelets with or without presence of *Fhb1*, the major QTL responsible for type II resistance to scab on 3BS chromosome. The interaction between the two QTLs was not detected. Composite interval mapping consistently detected a minor QTL, *Qfhb.pser.1BL,* for type II scab resistance on 1BL across two experiments. *Qfhb.pser.1BL* was flanked by *lm* and SSR marker *Xbarc181*, and explained 5.0-8.0 % of phenotypic variation for scab resistance. *Qfhb.pser.1BL* is a new QTL that has not been reported before and may be due to a pleiotropic effect of *lm.*

TOWARD POSITIONAL CLONING OF *FHB1*, A MAJOR QTL FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

DNA markers near *Fhb1*, a major QTL for Fusarium head blight resistance on chromosome 3BS in wheat, were used to screen the Chinese Spring chromosome 3B BAC library, and a BAC contig spanning the *Fhb1* region was constructed. The genic regions of two overlapping BAC clones were sequenced. Based on new DNA markers developed from the BAC sequences, *Fhb1* was narrowed down to a 261 kb region with seven putative genes. The expression of the candidate genes was examined by RT-PCR. Four out of the seven genes are expressed in wheat spikes. But, there is no clear expression difference between water-inoculated and Fusarium-inoculated wheat spikes. Five cosmid clones containing all seven candidate genes were isolated from a cosmid library of Sumai 3. The cosmid clones were used to transform FHB-susceptible cultivar Bobwhite and transgenic plants were obtained for all cosmid clones. Transgenic plants for four out the five cosmid clones have been tested for Type II resistance to FHB, and none of them are resistant. FHB evaluation of the transgenic plants of the fifth cosmid clone is in progress. A highly diagnostic, codominant marker, UMN10, was developed and used for MAS for gene *Fhb1*.

A GENOME-WIDE SCREEN IN YEAST TO IDENTIFY POTENTIAL TARGETS OF TRICHOTHECENE MYCOTOXINS.

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ABSTRACT

Fusarium graminearum infection contaminates wheat and barley grain with the potent trichothecene mycotoxin deoxynivalenol (DON). Trichothecene mycotoxins are known to target cytosolic ribosomes and can cause cell death by permanently disrupting translation. In addition to the inhibition of protein synthesis, these toxins have been reported to influence a diverse set of biochemical processes in the eukaryotic cell. However, the molecular mechanisms that control sensitivity of wheat and barley to trichothecenes are not well understood and cellular factors that influence how the toxins are taken up, processed, and transported before inhibiting translation have not been identified. The goal of our research is to develop a better understanding of the genetic basis of eukaryotic cell susceptibility to trichothecene mycotoxins. Yeast, *Saccharomyces cerevisiae*, is sensitive to a wide variety of trichothecene mycotoxins and thus provides an ideal model organism to identify the cellular targets of these toxins. The availability of several complete sets of deletion libraries provides a powerful approach to identify genes critical for conferring sensitivity to trichothecenes on a genome-wide scale. We have carried out a genome-wide screen of the non-essential yeast knockout library (YKO) to identify the genes that confer resistance to trichothecenes when deleted. We screened 4720 homozygous diploid YKO strains and identified 122 strains that showed resistance to 4 μM trichothecin (T-cin), 27 of these strains also showed resistance to 6 μM T-cin and 14 strains showed resistance to 8 μM T-cin. The broad categories of genes identified in the resistance screen include genes that influence translation, trafficking, signal transduction, protein folding/degradation, biosynthesis/metabolism, the cell cycle, membranes, gene regulation, and mitochondria. The majority of identified genes were associated with mitochondria, implicating mitochondria in the toxin mechanism of action. These genes represent potential targets for engineering resistance to FHB and for developing effective approaches to prevent mycotoxin contamination of cereals.

IDENTIFYING PLANT GENES AND MECHANISMS THAT CONTRIBUTE TO DEFENSE AND SUSCEPTIBILITY TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Fusarium head blight (FHB)/scab caused by the fungus *Fusarium graminearum* is a destructive disease of wheat and barley. Previously, we had demonstrated that ectopic expression of the *Arabidopsis thaliana* At*NPR1* gene, which is a key regulator of the salicylic acid-dependent systemic acquired resistance (SAR) and the jasmonic acid-dependent induced systemic resistance (ISR) mechanisms, enhanced FHB resistance in the hexaploid wheat cv. Bobwhite and in Arabidopsis (Makandar *et al*. 2006). Subsequent studies have indicated that both SA and JA contribute to wheat and Arabidopsis resistance to *F. graminearum* during different stages of the infection. The Arabidopsis-*F. graminearum* host-pathogen system has been utilized to identify additional plant genes and mechanisms that modulate host defense or susceptibility against *F. graminearum*. For example, mutations in the *PAD4* and *WRKY18* genes enhanced Arabidopsis susceptibility to *F. graminearum*. In contrast, overexpression of *PAD4* and *WRKY18* enhanced disease resistance. PAD4 is an important modulator of camalexin and salicylic acid synthesis and is also required for phloem-based defenses against sap sucking insects. WRKY18 on the other hand is a transcription factor that regulates expression of defense associated genes. Transgenic wheat containing a *Ubi*:At*PAD4* chimera, in which *PAD4* expression is driven from the maize *Ubi* gene promoter have been generated and efforts are underway to transform a *Ubi*:At*WRKY18* chimera into the wheat cv Bobwhite. Other mechanisms that contribute to controlling *F. graminearum* growth in Arabidopsis include a flagellin-inducible non-host defense mechanism that enhances FHB resistance and a 9-lipoxygenase-dependent mechanism that enhances susceptibility to the fungus. Efforts are underway to determine if these processes also impact FHB severity in wheat.

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RAPID GENE ASSAY IN *PHYSCOMITRELLA PATENS* REVEALS MULTIPLE MECHANISMS AND APPROACHES FOR CONTROLLING FUSARIUM HEAD BLIGHT.

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ABSTRACT

There is a pressing need for novel sources of resistance against *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) and mycotoxin contamination in wheat and barley. Our ability to perform functional screens for novel genes that can confer FHB resistance has been limited by the relatively inefficiency of transformation in wheat and barley. We have developed the recombinogenic plant *Physcomitrella patens* as a rapid assay system for genes that confer resistance to FHB and Fusarium-derived mycotoxins such as deoxynivalenol (DON).

We have used this system to define a collection of genes (conserved between *Physcomitrella* and wheat) that condition FHB resistance and DON sensitivity. Our studies have revealed that a number of distinct mechanisms can contribute to resistance and susceptibility to FHB. These include: (i) the suppression of host programmed cell death (PCD) through the overexpression of anti-PCD genes; (ii) the suppression of host PCD through the disruption of genes required for PCD; (iii) the overexpression of mutant versions of natural genes that are known targets of *Fusarium* mycotoxins; (iv) the disruption of genes that affect recognition at the plant cell surface: (v) the alteration of cell wall properties; (vi) the overexpression of genes involved in induced immunity.

These reverse genetic approaches define useful genes whose efficacy in crops can be evaluated through the use of VIGS and transgenic plants. In addition, we have also found *Physcomitrella* to be a useful system for chemical genetic studies to define compounds that phenocopy specific mutants and protect plants against infection with *Fusarium*.

USING VIRUS-INDUCED GENE SILENCING (VIGS) TO IDENTIFY GENES MAKING ESSENTIAL CONTRIBUTIONS TO FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT.

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ABSTRACT

This presentation will describe a new method we have developed to rapidly identify genes that function in the Fusarium head blight (FHB) resistance mechanism of wheat. In this method, called virus-induced gene silencing (VIGS), genes thought to function in FHB resistance are switched-off, or silenced, and their role in FHB resistance is inferred if silencing results in resistant wheat plants becoming susceptible to FHB. This method utilizes the RNA virus, Barley stripe mosaic virus (BSMV), to activate RNA-mediated gene silencing in wheat. RNA-mediated gene silencing is an evolutionarily conserved defense mechanism in plants and animals that targets viral RNAs for sequence-specific degradation. In VIGS, the plant's RNA-based defense response is exploited to cause plant genes selected by the experimenter to be silenced by inserting a piece of the chosen plant gene into the viral RNA. In this way, the messenger RNA from the chosen plant gene is targeted for degradation, thus silencing the expression of the gene, as the plant defense mechanism works to degrade all the viral RNA. This approach has several important advantages: 1) As it is homology-dependent, it can simultaneously silence multiple copies of genes, which are almost always present in hexaploid wheat. Without this capability, the expression of any closely related genes would prevent observation of the effects of silencing. 2) It is rapid; an experiment can be accomplished in as little as 2 months from identification of a candidate gene to observing the effect of its silencing. We are using VIGS to test if candidate genes make essential contributions to FHB resistance by silencing the target gene in an FHB resistant wheat genotype and assessing whether or not the silenced plant remains resistant to FHB.

GENES AND MECHANISMS ASSOCIATED WITH PLANT INTERACTION WITH *F. GRAMINEARUM*.

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ABSTRACT

Genes and signaling mechanisms associated with plant interaction with *F. graminearum* were identified in *Arabidopsis thaliana* and their involvement in wheat interaction with *F. graminearum* validated. These studies in wheat and Arabidopsis have indicated an important role for salicylic acid (SA) and jasmonic acid (JA) signaling in plant defense against *F. graminearum*. SA-dependent defenses were most effective during the early stages of infection, inhibiting germination of fungal spores. In contrast, JA-dependent defenses were most effective during later stages of infection. However, during the early stages of infection, JA attenuated the activation of SA signaling, thereby contributing to susceptibility. The induction of SA signaling can be expedited in susceptible hexaploid wheat and durum cultivars by constitutive overexpression of Arabidopsis *NPR1*, resulting in enhanced FHB resistance. Similarly, overexpression of two other genes associated with SA signaling, enhanced resistance to *F. graminearum* in Arabidopsis. Resistance could also be enhanced by knockdown of lipoxygenase expression in Arabidopsis, suggesting that plant oxylipins function as susceptibility factors. Experiments are underway to test the role of these and additional genes/mechanisms in wheat interaction with *F. graminearum* and to determine if their manipulation provides a viable strategy for enhancing FHB resistance in wheat.

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RAPIDLY IDENTIFY AND TEST SCAB RESISTANCE GENES.

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ABSTRACT

Our overall goal is to identify genes that play role in resistance to Fusarium Head Blight (FHB) and to develop and test transgenic wheat carrying these genes. Previously, we conducted a large array of RNA profiling experiments during *Fusarium graminearum* infection of barley. We identified a set of regulatory genes that respond to trichothecene accumulation and a set of genes encoding UDP-glucosyltransferases that may detoxify trichothecenes. The regulatory genes encode a WRKY transcription factor, a Myb transcription factor, a Spl7 cell death regulatory protein, a Cys2/His2 zinc-finger protein, an F-box domain containing protein, and a NF-X1 zinc finger protein. We used a virus-induced gene silencing (VIGS) assay to functionally test the regulatory genes for their role in FHB resistance/susceptibility in wheat. The NF-X1 gene functions as a negative regulator of trichothecene-induced defense response in Arabidopsis. Wheaton and Bobwhite inoculated with VIGS-NF-X1 constructs exhibited statistically significant reduction in disease severity during the early stages of disease development compared to the empty vector VIGS control lines ($P<0.05$). In addition, we have established collaboration with Dr. Gerhard Adam (Universität für Bodenkultur Wien, Austria) and to study barley UDP-glucosyltransferases in yeast.

ARABIDOPSIS THALIANA AS A MODEL PLANT TO TEST ANTIFUNGAL GENES FOR RESISTANCE TO *FUSARIUM GRAMINEARUM*.

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ABSTRACT

Recent studies have shown that *Arabidopsis thaliana*, a model host plant is susceptible to *F. graminearum*. Taking advantage of the foliar *Fusarium-Arabidopsis* pathosystem, we tested antifungal defensins, MsDef1 and MtDef4, from *Medicago* spp., for their ability to confer resistance to this pathogen. We generated chimeric defensin gene constructs that resulted in overexpression of MsDef1 or MtDef4 either extracellularly or intracellularly (*i. e.,* vacuole or endoplasmic reticulum) in transgenic *A. thaliana* ecotype Columbia). Here, we demonstrate that constitutive overexpression of MsDef1 and MtDef4 confers strong resistance to *F. graminearum*. Transgenic *A. thaliana* lines overexpressing MsDef1 or MtDef4 either extracellularly or intracellularly showed up to 68% reduction in disease severity (DS) index as compared to that of the wild type plants (100%) and supported significantly less fungal growth as evaluated by trypan blue staining. Transgenic inoculated plants also bolted normally like the mock inoculated wild-type plants, whereas the inoculated wild-type plants showed much delayed bolting. Our results indicate that *A. thaliana* is a useful model plant to test antifungal genes for their ability to confer resistance to *F. graminearum.*