# SESSION 3:

# GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Blake Cooper

# STUDIES ON BARLEY SPIKES TREATED WITH THE TRICHOTHECENE, DEOXYNIVALENOL: INSIGHT INTO BARLEY-*FUSARIUM GRAMINEARUM* INTERACTION.

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**ABSTRACT**

Fusarium head blight (FHB) of barley and wheat is a difficult disease to manage because of the complexity of the interactions. A serious problem associated with FHB is the accumulation of trichothecene mycotoxins such as deoxynivalenol (DON). Trichothecenes increase the virulence of the pathogen and reduce grain quality. A primary objective in our laboratory is to identify genes that reduce the impact of trichothecenes. Our laboratory has identified approximately 700 barley transcripts that respond to the invading pathogen and pathogen-derived trichothecenes. In an effort to further understand the barley-*F. graminearum* interaction, a subset of 54 genes encoding transcription factors, regulatory proteins, UDP-glucosyltransferases, cytochrome-P450s, and proteins participating in ubiquitination and cell death were selected and tested for their response to DON treatment compared to mock water inoculation at 1, 6 and 12 hours after inoculation (hai). Twenty-one transcripts showed a qualitative response and 28 transcripts showed quantitative response to DON treatment. Seven of the qualitatively responding genes responded by 1 hai, while 14 genes responded by 6 hai. All the quantitatively responding genes showed differential expression from 1 hai through 12 hai. To develop markers for mapping and other genetic studies, some of these genes were sequenced from barley mapping population parents and genotypes exhibiting FHB resistance and susceptibility. In separate experiments, the fate of DON *in planta* was tested. In barley spikes treated with DON, over 30.0% was converted to DON-3-O-glucoside. In addition, our preliminary experiments show a cell death-like phenotype on DON-treated barley leaves progressed in a distal manner, indicating that either DON or the signal transduction induced by DON traveled to the tip of the treated leaves.

# EXPRESSION OF A TRUNCATED FORM OF RIBOSOMAL PROTEIN L3 IN TRANSGENIC WHEAT CONFERS RESISTANCE TO DEOXYNIVALENOL AND FUSARIUM HEAD BLIGHT.

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**ABSTRACT**

DON belongs to the group of trichothecene toxins, which target ribosomal protein L3 at the peptidyltransferase site of eukaryotic ribosomes and inhibit protein synthesis. The goal of our work is to identify mutations in L3 that confer resistance to DON and to determine if FHB resistance can be engineered in transgenic wheat plants by expressing DON resistant L3 genes. In previous studies, we have demonstrated that overexpression of a truncated form of yeast ribosomal protein L3 (L3Δ) in transgenic tobacco plants confers resistance to deoxynivalenol (DON). To determine if expression of the yeast L3Δ in transgenic wheat plants would provide resistance to FHB, the susceptible spring wheat cultivar, Bobwhite was transformed with the yeast L3Δ under the control of the barley floret-specific *Lem1* or the maize constitutive *Ubi1* promoter. Three homozygous *Lem1*::yeast L3Δ lines (771, 772 and 773) and two homozygous *Ubi1*::yeast L3Δ lines (8133 and 8153) were evaluated for resistance to FHB in greenhouse tests. The disease severity was reduced by 48-56% in four different transgenic wheat lines compared to the untransformed Bobwhite plants. The reduction in disease severity correlated well with the level of expression of L3Δ mRNA. These results demonstrated that transgenic wheat plants expressing the yeast L3Δ showed improved resistance to FHB over the untransformed Bobwhite plants. To determine if resistance to FHB would result in a reduction in DON levels, the mature kernels above and below the inoculated spikelets were analyzed for DON levels. There was a 63-76% reduction in DON levels in the four different FHB resistant transgenic lines. The DON levels in one transgenic line were lower than the DON levels in the resistant line, Alsen. These results provided evidence that resistance to DON correlates with resistance to FHB and results in reduced accumulation of DON in transgenic wheat plants. We have identified four more homozygouns lines containing *Lem1*::yeast L3Δ, eight more homozygous lines containing *Ubi1*::yeast L3 and four new homozygous lines containing *Lem1*::yeast L3, which will be evaluated for resistance to FHB. The wheat *RPL3A1* and *RPL3B3* genes were cloned and wheat expression vectors were constructed with the L3Δ versions of these genes. Point mutations that confer a high degree of resistance to DON were introduced into the wheat *RPL3A1*. We have generated transgenic wheat plants containing the DON resistant forms of the wheat L3 genes to determine if their expression will lead to a higher level of resistance to FHB and a greater reduction in DON accumulation.

# INHIBITION OF *FUSARIUM GRAMINEARUM* GERMLING DEVELOPMENT CAUSED BY COMBINATORIALLY SELECTED DEFENSE PEPTIDES.

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**ABSTRACT**

To address the problem of head blight in wheat, we are applying combinatorial peptide techniques to identify molecules that serve as antagonists to developing germlings of *Fusarium graminearum*. In this methodology, we mixed phage-display libraries that display 8-mer random peptides with *F. graminearum* germlings derived from macroconidia. Phage clones with binding affinity for germlings were recovered and amplified in *E. coli*. After additional rounds of incubation and amplification, we have recovered numerous peptides with affinity for surface molecules of germlings. We have sequenced an initial collection of selected peptides and are now evaluating their abilities to inhibit germling growth and development. At completion of these phenotype screens, we will test candidate peptides for inhibitory function when displayed on recently developed scaffold proteins.

# TRANSGENIC WHEAT EXPRESSING ANTIFUNGAL PLANT DEFENSIN MTDEF4 IS RESISTANT TO FUSARIUM HEAD BLIGHT (FHB).

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**ABSTRACT**

Plant defensin MtDef4 from *Medicago truncatula* is a potent inhibitor of *F. graminearum in vitr*o. Transgenic wheat lines expressing MtDef4 were generated using *Agrobacterium tumefaciens*-mediated transformation of spring wheat cultivar Bobwhite and a Chinese cultivar Xin Chun 9 (XC9). Single floret inoculation method was used to evaluate Type II resistance of these transgenics in the greenhouse. Of the two lines tested thus far, one Bobwhite transgenic line expressing MtDef4 has reduced FHB severity when compared to nontransgenic Bobwhite. The level of resistance in this line is similar to that of FHB resistant cultivar Alsen. Two more transgenic lines are being evaluated for Type II resistance in the greenhouse. The results of this study will be presented.

# REDUCING DON POTENTIAL IN VIRGINIA HULLESS BARLEY LINES THROUGH GENETIC ENGINEERING.

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**ABSTRACT**

Hulless barley (HLSB) is a new and emerging crop in Virginia, and may be an important source of biofuels in the future. Dried distillers grains with solubles (DDGS), a byproduct of ethanol fermentation, are rapidly becoming one of the main sources of feed for domestic animals. Traditional ethanol production may concentrate trichothecene mycotoxins such as deoxynivalenol (DON) in DDGS, posing a significant threat to domestic animal health. Our work aims to genetically engineer Virginia HLSB lines with reduced DON potential and thus provide a safe supply of DDGS for animal feed. In 2006 and 2007, we determined the DON potential of 20 Virginia HLSB lines; a number of these lines demonstrated low levels of DON in both years. We generated callus from 17 HLSB lines, and five of the lines were selected for further tissue culturing analyses and genetic transformation. We amplified *TRI101*, a gene encoding a 3-O-acetyltransferase responsible for the conversion of DON to 3-acetyl-DON, from four different species of *Fusarium*. Preliminary expression studies using the yeast expression vector pYES2.1 suggested that these genes differ in their relative ability to reduce DON *in vitro*. We are currently developing an *Agrobacterium* transformation vector to move *TRI101* into five selected HLSB lines, and we plan to monitor potential decreases in DON in both raw grain and DDGS following ethanol production using our genetically-engineered lines. We are currently exploring the function of additional genes that may detoxify DON (e.g., orthologs of *TRI101* in *Arabidopsis*), and we hope to harness the potential of these genes to enhance food safety and security in the eastern U.S.

# ENHANCING FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT BY MANIPULATING MECHANISMS CONTRIBUTING TO HOST RESISTANCE AND SUSCEPTIBILITY.

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## ABSTRACT

Fusarium head blight (scab) caused by the fungal pathogen, *Fusarium graminearum* is a serious menace in wheat and barley, severely limiting crop productivity and quality. Previously, we had demonstrated that ectopic expression of the *Arabidopsis thaliana* At*NPR1* gene, which is a key regulator of salicylic acid (SA) signaling, enhanced FHB resistance in the hexaploid wheat cv. Bobwhite (Makandar *et al*. 2006). Similarly, FHB resistance is enhanced in transgenic At*NPR1* expressing durum cvs. Ben, Maier and Belzer. Three field trials to monitor the impact of At*NPR1* on FHB have been completed. Results of these trials will be presented.

Genetic studies in *Arabidopsis thaliana* demonstrate that SA has an important role in plant resistance against *F. graminearum*. Pretreatment with SA enhances FHB resistance in wheat, also. Furthermore, SA accumulation in spikes correlates with FHB resistance in wheat. SA levels increase >200% in the fungus inoculated and distal spikelets of the resistant cv. Sumai-3, within 24 h of point inoculation with *F. graminearum* macroconidia. In contrast, a similar increase in SA content was not observed in the cv. Bobwhite, suggesting that SA accumulation can be targeted to enhance FHB resistance. Indeed, resistance against *F. graminearum* is enhanced in *Arabidopsis* plants that constitutively express the At*PAD4* gene, which modulates SA synthesis and signaling. We have initiated experiments to ectopically express At*PAD4* from the maize Ubi1 promoter in transgenic wheat. In addition, we have generated transgenic wheat plants that express a salicylate hydroxylase encoded by the bacterial *nahG* gene, to further test the involvement of SA in wheat defense against *F. graminearum*.

In contrast to SA, our experiments in wheat and *Arabidopsis* indicate that jasmonic acid (JA) accumulation and the activation of JA signaling inversely correlates with resistance to *F. graminearum*, suggesting that JA or a related oxidized lipid (oxylipin) may be a susceptibility factor. Indeed, in *Arabidopsis* a lipoxygenase involved in oxylipin synthesis contributes to susceptibility to *F. graminearum*. Thus, oxylipin synthesis could provide another target to control FHB. In *Arabidopsis*, JA antagonizes SA accumulation. Similarly, JA accumulation could result in the suppression of SA accumulation in the spikes of FHB susceptible wheat cultivars. Alternatively, as was recently shown in maize (Gao *et al*., 2007), JA or a related oxylipin may contribute to fungal development, thereby contributing to susceptibility.

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# ENGINEERING BARLEY WITH *GASTRODIANIN* FOR IMPROVED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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## OBJECTIVES

Develop transgenic barley lines expressing the anti-fungal gene *gastrodianin* for resistance to Fusarium head blight (FHB).

## INTRODUCTION

Control of Fusarium head blight (FHB) infection in barley remains difficult because of lack of genetic resistance. One strategy that has great potential to reduce FHB infection is introduction of anti-*Fusarium* genes into barley through genetic engineering. Unfortunately, engineering resistance has been slow since common pathogenesis-related (PR) proteins are not effective against *Fusarium graminearum*. Transgenic wheat over-expressing combinations of chitinases, glucanases, and thaumatin-like proteins (TLPs) had partial resistance to FHB in green house testing. However, the greenhouse results were not reproducible under field conditions and no resistance was observed (Anand *et al*. 2003). Apparently, genes known to specifically inhibit *F. graminearum* are required to give adequate protection from FHB. We have developed barley lines expressing anti-*Fusarium* gene *gastrodianin* for resistance to FHB.

*Gastrodianin* is an anti-fungal gene isolated from a traditional Chinese herb, *Gastrodia elata*. *G. elata* is devoid of chlorophyll and leads a parasitic life on the fungus *Armillaria mellea*. *A. mellea* hyphae usually infect the nutritive corms of *G. elata* but are digested in the cortical cells. The released nutrients are used by the host plant for growth and development. Expression of *gastrodianin* and other anti-fungal proteins protects the developing terminal corm from infection by *A. mellea* (Wang *et al*., 2007; Sa *et al*., 2003). *Gastrodianin* is a non-agglutinating, monomeric, mannose and chitin-binding lectin that belongs to the superfamily of monocot mannose-specific lectins (Liu *et al*., 2005; Wang *et al*., 2001). *Gastrodianin* effectively inhibits hyphal growth of pathogenic and saprophytic fungi including *Gibberella zeae*, *Armillaria mellea*, , *Rhizoctonia solani*, *Trichoderma viride* and *Valsa ambiens in vitro* (Wang *et al*., 2001). *In vivo* studies have also demonstrated the importance of *gastrodianin* in fighting pathogens. In transgenic tobacco, *gastrodianin* reduces root diseases caused by fungal pathogens *Rhizoctonia solani* and *Phytophthora nicotianae* (Cox *et al*., 2006). In cotton, field tests showed that transgenic plants expressing *gastrodianin* are resistant to another fungal pathogen *Verticillium* wilt (Wang *et al*., 2004). *Gastrodianin* maintains inhibitory properties at fluctuating temperatures (Wang *et al*., 2001, Xu *et al*., 1998). This stability and its inhibitory effects on *G. zeae* makes *gastrodianin* protein an attractive candidate for engineering resistance to fungal diseases.

## MATERIALS AND METHODS

### *Expression vectors*

Plasmids used for transformation are shown in *Fig*. 1. There are four *gastrodianin* genes in *G. elata* differing only by 3 to 4 nucleotides (Wang *et al*., 1999). In this study the variant VGM was used (GeneBank Accession AJ277785). *Gastrodianin* has 513 nucleotides and encodes a polypeptide with 171 amino acids. The coding region was amplified by PCR from a binary vector generously provided by the Plant Biology Institute, University of Ghent, Belgium. The PCR fragment was digested with restriction enzymes (*Eco*RV and *Pst*I) and fused to a *Lem2* promoter (Abebe *et*

*al*., 2005). The resulting fragment was ligated to pLem2gfp (Abebe *et al*, 2005) to get pLem2VGM2 (Fig. 1). Plasmid pLem2VGM2 also contains *gfp* under the control of the *Lem2* promoter, making visual screening of transformed plants easier.

### *Transformation of barley*

Barley plants (*Hordeum vulgare* cv. Golden Promise) were transformed as described in Wang and Lemaux (1994) with minor modification. Immature kernels (approximately 14 days post-anthesis) were surface sterilized with 70% ethanol (v/v) and 20% chlorox (v/v). After three washes with sterile water, embryos were cut in half longitudinally and placed on callus induction medium (CIM), scutellum side down. After 3–5 days of incubation, embryos were bombarded with gold particles (0.6 µm) coated with an equimolar amounts of plasmids pLem2VGM2 and pAHC25 (contains the *bar*) using the He-driven PDS 1000 (Bio-Rad). The herbicide bialaphos was used to select transgenic calli and plantlets.

### *Characterization of transgenic plants*

Integration of *gastrodianin* into the genome of transgenic barley was verified by PCR. Genomic DNA was isolated from wild type and transgenic barley plants using CTAB (Murray and Thompson, 1980). PCR was performed using 100 ng of genomic DNA, along with upstream and downstream VGM primers.

## RESULTS AND DISCUSSION

We have recovered plants from 16 transformation events. Plants from four events were sterile, plants from two events were lost to fungal contamination and the remaining ten plants produced seeds. At least two plants were regenerated from each transformation event. Recovery of $T_0$ plants from tissue culture was significantly improved by visual screening of *gfp* expression. Both *gfp* and *gastrodianin* were placed in the same pLem2VGM2 plasmid (Fig. 1). By screening for *gfp* expression we were able to indirectly select plants incorporating *gasrodianin* in their genome. The *lem2* gene promoter directs tissue-specific expression (Abebe *et al.*, 2005). Visual screening of transgenic plants was performed by inspecting tissue-specific fluorescence of the GFP protein in the spike and auricle.

The sterile plants were bushy in appearance (had many tillers) and had thin stems and spikes (Fig. 2). We are screening plants for accumulation of the *gastrodianin* protein using western blotting and ELISA. It will be interesting to see if the phenotypes observed in sterile plants are due to high accumulation of the *gastrodianin* protein, disruption of spike-specific genes or somaclonal variations introduced during tissue culture.

To verify integration of *gastrodianin* in the genome, we screened some transgenic plants expressing *gfp* by PCR. This showed that all the plants expressing *gfp* also had the expected 0.5 kb *gastrodianin* insert (Fig. 3). In the next phase of the study, resistance of transgenic plants to *F. graminearum* will be tested under greenhouse conditions using $T_1$ and $T_2$ generations.

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## DISCLAIMER

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

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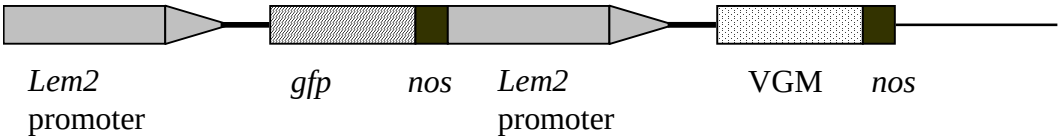
**pLem2VGM2 (7.9 kb)**




**pAHC25 (9.7 kb)**

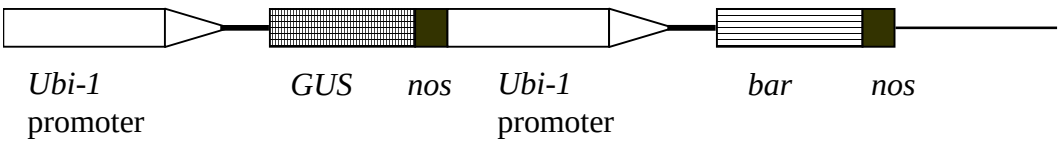



***Fig.1.*** Map of plasmids used for transformation. Plasmid pLem2VGM2 contains *gfp* and *gastrodianin* (VGM) driven by the Lem2 promoter. Plasmid pAHC25 contains the *bar* gene for selection. It also contains the *GUS* reporter gene.

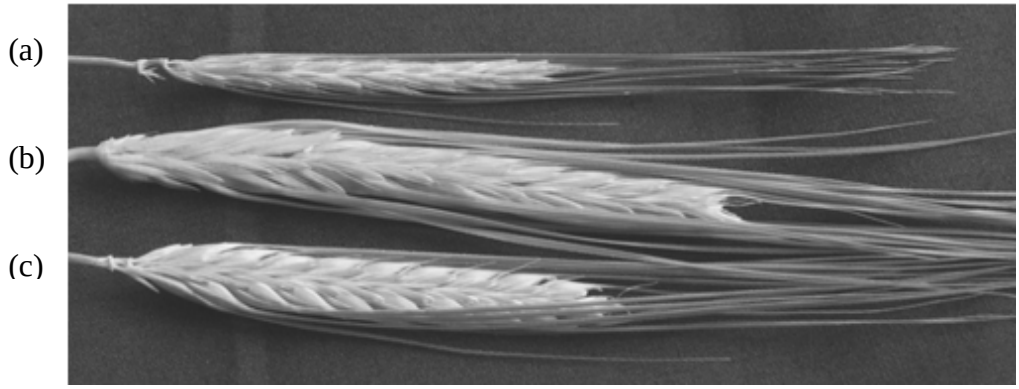



***Fig.2.*** Phenotype of $T_0$ plants. Sterile $T_0$ plants (a) have very thin spikes compared to fertile $T_0$ (b) and no-transgenic (c) Golden promise plants.

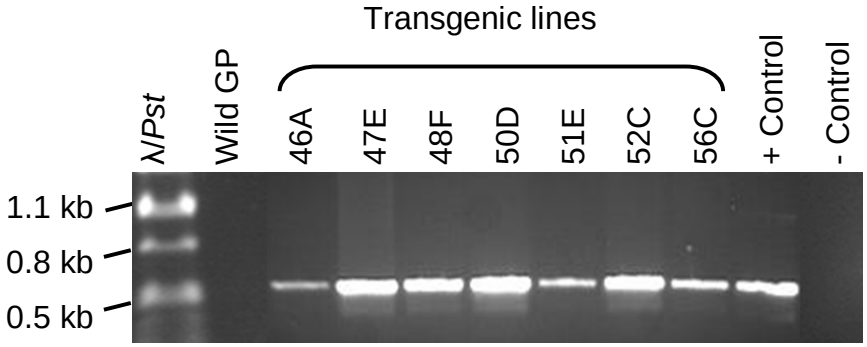



***Fig.3.*** PCR showing integration of the *gastrodianin* gene in the genome of Golden Promise (GP) barley. Positive control (+) for the PCR was plasmid pLem2VGM2 DNA and negative control (-) was water.

# GENES THAT CONFER RESISTANCE TO *FUSARIUM*.

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## ABSTRACT

There is a pressing need for sources of germplasm or genes that are effective against *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) on wheat and barley. Since sources of resistance from wheat and barley are limited, we have developed a functional assay system to evaluate genes from other sources for their efficacy against FHB. The assay system is based on the plant *Physcomitrella patens*, which serves as a 'green yeast' for the rapid evaluation of novel genes. This plant, uniquely, allows the contribution of individual genes to be assessed through either the creation of targeted genes knockouts or through the introduction and overexpression of transgenes. Importantly, the wild type plant is fully susceptible to *F. graminearum* and highly sensitive to mycotoxins, including DON.

We have used this system to characterize genes that confer effective and robust resistance to FHB. The first set of genes acts through the plant programmed cell death pathway. Plants that contain knockouts for these genes are completely insensitive to DON and fully resistant to FHB. A similar level of FHB resistance can be conferred by overexpressing genes that suppress plant cell death. In these plants, FHB resistance is conferred by disabling a host susceptibility pathway (cell death) induced by mycotoxins.

A second set of genes confers FHB resistance through a pathway that is independent of cell death. These plants, which overexpress nuclease genes, are still sensitive to DON and other mycotoxins, yet display significant resistance to FHB. One explanation, indirectly supported by our studies, is that the overexpressed protein is itself directly antifungal. In these plants, FHB resistance is conferred by enhancing existing plant defense mechanisms.

A third set of genes that confer FHB resistance is associated with stress management, and in particular the response to reactive oxygen species (ROS), which are associated with the response to pathogen attack. Knockout and overexpressing lines for different genes associated with this response show enhanced resistance to both DON and to FHB but through a mechanism that acts downstream of the cell death pathway.

These results show that FHB resistance can be introduced by manipulating a variety of cellular targets. By combining these approaches it should be possible to introduce an enduring FHB resistance into wheat and barley plants. The efficacy of these FHB-resistance genes in wheat is currently being tested by the Scofield lab using a VIGS-based assay. This will provide an early indicator of likely performance in transgenic wheat.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# GENETIC STUDIES DEFINE DISTINCT PATHWAYS OF RESISTANCE TO FUSARIUM HEAD BLIGHT.

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## ABSTRACT

We have used the *Physcomitrella patens* rapid assay system to characterize a number of genes for their ability to confer resistance to *Fusarium graminearum*, the causal agent of Fusarium head Blight (FHB). Using this approach we have screened several dozen genes for their ability to confer resistance to fungal mycotoxins and FHB. These studies have revealed that resistance to FHB can be achieved by manipulating multiple cellular pathways, including those involved in the regulation of programmed host cell death, the production and elimination of reactive oxygen species, the production of lytic enzymes and the expression of host defense responses.

These mutant plants show distinct patterns of susceptibility to FHB and to various *Fusarium*-derived mycotoxins, compared to wild type plants, which are fully susceptible to FHB and highly sensitive to DON and T-2 toxin. Plants that are mutated in the cell death pathway are highly resistant to FHB, and insensitive to DON and T-2 toxin. In contrast, plants that are mutated in the regulation of reactive oxygen species are highly resistant to FHB, insensitive to DON but partially sensitive to T-2 toxin. A further contrast is provided by plants that overexpress nuclease genes. These plants are resistant to FHB but fully sensitive to DON and T2-toxin. These results illustrate that different FHB-derived toxins target different cellular pathways, and suggest that robust resistance to FHB in the field may require the concerted manipulation of more than one cellular pathway.

Several of the genes we have manipulated are induced during the response to FHB inoculation. We tested whether these genes form part of a natural defense response by pre-treating plants with the defense response elicitor chitosan. Plants exposed to chitosan are highly resistant to subsequent inoculation with FHB. This indicates that *Physcomitrella* plants possess a natural and highly effective mechanism of induced FHB resistance. Presumably this response is suppressed during the interaction with *F. graminearum*. We will present data on this induced FHB-resistance response and discuss other approaches we have used to suppress virulence and enhance resistance to FHB in this system.

## ACKNOWLEDGEMENT AND DISCLAIMER

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# RAPID FUNCTIONAL IDENTIFICATION OF GENES CONTRIBUTING TO FHB RESISTANCE.

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**ABSTRACT**

This presentation will describe a new method being employed 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. Examples of the utility of this important new method will be presented.

# ENGINEERING RESISTANCE TO *FUSARIUM GRAMINEARUM* USING ANTIFUNGAL PLANT DEFENSINS.

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**ABSTRACT**

Small cysteine-rich plants defensins have potential as antifungal agents in transgenic crops. Two such defensins, MsDef1 and MtDef4, from *Medicago* spp., share only 41% amino acid sequence identity, but potently inhibit the growth of *Fusarium graminearum in vitro*. These two defensins exhibit different modes of antifungal action. Using the *Fusarium graminearum-Arabidopsis thaliana* pathosystem, we have found that over-expression of either MsDef1 or MtDef4 extracellularly or intracellularly (in the vacuole or endoplasmic reticulum) conferred strong resistance to this pathogen in transgenic *A. thaliana* plants. Transgenic plants exhibited reduced foliar symptoms and growth of fungal hyphae. Moreover, growth of the pathogen-challenged transgenic plants was similar to that of non-inoculated wild-type plants. Since *F. graminearum* colonizes host tissue by both inter- and intra-cellular growth, we will develop and test transgenic *A. thaliana* lines co-expressing extra- and intracellular defensins for more robust resistance to this pathogen. In parallel experiments, we have generated seven transgenic wheat lines over-expressing extracellular MtDef4. Of the two lines tested thus far, one line displayed improved FHB resistance when compared to non-transgenic Bobwhite. Furthermore, the level of resistance in this line was comparable to that of the disease resistant check, Alsen. No pleiotropic effects resulting from over-expression of defensins were observed in transgenic *A. thaliana* or wheat.

# ENGINEERING SCAB RESISTANCE IN WHEAT WITH PLANT DEFENSE SIGNALING GENES.

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## ABSTRACT

*Fusarium graminearum* is the principal causative agent of Fusarium Head Blight (FHB)/scab, a devastating disease of wheat and barley that severely limits crop productivity and grain quality. Our approach has been to utilize plant genes that regulate defense responses for enhancing FHB resistance in wheat. A plant-pathogen system consisting of *Arabidopsis thaliana* and *Fusarium graminearum* has been developed to identify genes involved in regulating plant defense against *F. graminearum*. The *Arabidopsis NPR1* (At*NPR1*) gene was one of the promising genes identified in this screen. NPR1 is a key regulator of salicylic acid (SA) signaling in plant defense. Our studies in *Arabidopsis* and wheat have indicated that SA is also an important regulator of defense against *F. graminearum*. Expression of At*NPR1* gene (At*NPR1*) was successfully engineered in the hexaploid wheat cultivar Bobwhite. In green house and growth chamber studies, At*NPR1* expression resulted in heightened FHB resistance in transgenic wheat. Furthermore, DON content was lower in the transgenic seeds. SA-regulated defense responses were turned on faster and to higher levels in the At*NPR1* expressing plants. Three field trials have been completed with At*NPR1* expressing transgenic Bobwhite plants. At*NPR1* expression has also been successfully engineered into the durum varieties Ben, Maier and Belzer. FHB evaluations of these transgenic plants are ongoing. Results on other promising genes identified in our *Arabidopsis-F. graminearum* screen and the status for engineering their expression in wheat will also be discussed.

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# TRANSGENIC WHEAT WITH ENHANCED RESISTANCE TO FUSARIUM HEAD BLIGHT.

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**ABSTRACT**

We are developing and testing transgenic wheat for resistance to Fusarium Head Blight (FHB). We developed transgenic wheat carrying genes encoding chitinase, thaumatin-like protein 1 (tlp-1), ribosome-inactivating protein (RIP), lipid transfer protein (LTP), glutathione-S-transferase (GST), jasmonic acid inducible Myb transcription factor (JaMyb), germin-like protein1 (GLP1), and pathogenesis-related protein1 (PR1). Transgenic lines over-expressing these genes were generated using micro-projectile bombardment of the wheat cultivar 'Bobwhite'. Both single and combinations of transgenes were generated. We developed 4, 4, 2, 2, 1, and 4 lines carrying LTP, RIP, RIP/tlp-1, TRI101/tlp-1, TRI101/ß-1,3-glucanase, and tlp-1/ß-1,3-glucanase, respectively. In multiple greenhouse screens of these lines, we identified five lines (one RIP, two TRI 101/tlp-1, and two tlp-1/ß-1,3-glucanase) that exhibited statistically significant reductions in FHB severity compared to the non-transgenic controls ($p<0.05$). Combined with our previous greenhouse screens, we identified and evaluated 24 lines (seven chitinase, two RIP, two chitinase/tlp-1, one chitinase/RIP, six RIP/tlp-1, two TRI 101/tlp-1, two tlp-1/ß-1,3-glucanase, and two LTP) in field trials in 2005 and/or 2007. Three lines (two chitinase and one RIP) exhibited statistically significant reductions in FHB severity and very scabby kernels (VSK) compared to the non-transgenic control ($P<0.05$) in 2005 and 2007. In 2007, four lines (one TRI 101/tlp-1, two tlp-1/ß-1,3-glucanase, and one RIP) showed reduced FHB severity and five (two TRI 101/tlp-1, one tlp-1/ß-1,3-glucanase, two LTP) showed reduced VSK ($p<0.05$). We also crossed three transgenic wheat lines (two chitinase and one RIP), that exhibited statistically significant reductions in FHB severity in the field, to the type II resistant cv. Alsen. In addition, we developed 13, 10, 10, and 6 transgenic lines carrying GST, JaMyb, PR1, and GLP genes, respectively. Six lines (one GST, two JaMyb, and three GLP1) exhibited statistically significant reductions in FHB severity in compared to the non-transgenic Bobwhite in greenhouse screens ($p<0.05$).

# COMPARATIVE ANALYSIS OF FHB QTLS IN THE MINI MANO/ FRONTANA AND FRONTANA/REMUS DH POPULATIONS.

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**ABSTRACT**

Fusarium head blight (FHB) is one of the most important diseases in the aspects of food safety and yield quality also. The most effective strategy for controlling FHB in wheat is through the development of resistant cultivars. This can be reached by analyzing QTLs, and using them in a marker-assisted selection.

Frontana is a Brazilian spring wheat cultivar that has small and medium effective QTLs. These types of QTLs are sensitive for the environmental factors and for the problems of heterogeneity.

220 DH lines from Frontana/Remus (IFA Tulln) /2005-2006/ and 110 DH lined of Mini Mano/Frontana (CRC Szeged) /2006-2007/ were inoculated with four *Fusarium* isolates of *F. graminearum* and *F. culmorum*. The Frontana/Remus population was developed traditionally, with about up to two weeks difference in flowering time and 60-70 cm differences in plant height. MM/Frontana was created by us so that too early and late DH lines were discarded and the remaining lines flowered within five days, so one inoculation time was enough to cover all genotypes and plant height differences were kept within 20-30 cm depending on season. The rest of the lines were discarded.

In the Frontana/Remus population QTLs were identified on the chromosomes 2D, 3A, 5A, 5B, 3B, 6B, 7A/ 7D. The most consequent markers were found on 5A and 5B chromosomes (BARC197 and GWM156), the others gave positive signal seldom and the LOD values were around 2 and 2,8. In the MM/Frontana population 2B, 3B, 5A, 5B and 7B gave positive signal. The LOD values on 5B chromosome were the highest (BARC115), between 2,76 and 5,32. Even so in both populations Frontana was the resistant parent not the same markers gave positive signal. It seems that the more homogeneous population increases the accuracy of the QTL analysis. An increased morphologic homogeneity is necessary to decrease „noise" in QTL analyses and increase preciosity. Until now no QTL were found that gave positive signs for all epidemic situations. Therefore, the conditions to perform consequent MAS to identify superior genotypes in Frontana descendants are not yet in sight.

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# EXTRA- AND INTRACELLULAR TARGETING OF ANTIFUNGAL PLANT DEFENSINS IN TRANSGENIC *ARABIDOPSIS* 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 this 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 will result in over-expression of MsDef1 or MtDef4 either extra-cellularly or intra-cellularly (i.e., vacuole or endoplasmic reticulum) in transgenic *A. thaliana* ecotype Columbia (Col-0). Here, we demonstrate that constitutive overexpression of MsDef1 and MtDef4 confers strong resistance to *F.graminearum*. Transgenic Arabidopsis lines overexpressing MsDef1 or MtDef4 either extra-cellularly or intra-cellularly showed 59-68 % 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. Since *F. graminearum* has both biotrophic and necrotrophic life-cycles, we hypothesize that MsDef1 and MtDef4 co-expressed extra- and intra-cellularly will confer much higher level of resistance to FHB. Hence, transgenic *A. thaliana* lines co-expressing extra- and intracellular defensins will be tested for increased resistance to this pathogen.