SESSION 3:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Nilgun Tumer

TRICHOTHECENE MYCOTOXINS INHIBIT MITOCHONDRIAL TRANSLATION- IMPLICATIONS FOR FHB RESISTANCE

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ABSTRACT

Trichothecenes are foodborne toxins produced by various fungi including the plant pathogen *Fusarium graminearum*, which causes head blight (FHB) or scab of wheat and barley, resulting in yield reduction and contamination of grains with trichothecene mycotoxins. Conventional approaches have not yet yielded effective resistance in the field. Identifying molecular mechanisms underlying trichothecene toxicity will therefore aid in understanding *Fusarium* pathology and engineering effective resistance against FHB. Trichothecenes first identified as inhibitors of translation are known to have multiple effects on eukaryotes, including inhibition of DNA, RNA synthesis, cell division, membrane structure and integrity and mitochondrial function. It is not clear if these are primary or secondary effects of inhibition of cytosolic translation. We previously showed that mitochondria play a critical role in the toxicity of a type B trichothecene, trichothecin (Tcin). In this study, we investigated the direct effects of type A and type B trichothecenes on mitochondrial translation and membrane integrity in *Saccharomyces cerevisiae*. Sensitivity to trichothecenes increased when yeast cells required functional mitochondria for growth, while cells devoid of mitochondria (ρ^0) showed increased tolerance. T-2, DAS and Tcin inhibited translation in isolated yeast mitochondria by 67% (T-2), 54% (DAS) and 70% (Tcin). Trichothecenes caused fragmentation of mitochondrial membrane when yeast cells were treated for 6 h with high doses at which total translation was inhibited by 44% (T-2), 33% (DAS) and 91% (Tcin). A corresponding drop in mitochondrial membrane fragmentation (ψ_{mito}) and ROS levels was also observed. Trichothecenes, at low doses, did not promote severe membrane fragmentation or affect mitochondrial membrane integrity. Mitochondrial translation was significantly inhibited by 48% (T-2), 42% (DAS), and 34% (Tcin) at the low doses, but not total translation. These results indicate that trichothecenes directly target mitochondrial translation. Inhibition of mitochondrial translation is a primary target of trichothecenes and is not secondary to the disruption of mitochondrial membranes or cytosolic translation inhibition.

FIELD TESTS OF TRANSGENIC BARLEY LINES IN NORTH DAKOTA

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ABSTRACT

Testing transgenic barley lines for FHB in the greenhouse does not necessarily give the same results as field tests. The objective of this project was to test 18 transgenic lines in replicated trials in an inoculated FHB nursery. Several programs have developed barley lines expressing anti-fungal and/or anti-toxin genes through partial funding from the USWBSI (Abebe, Dahleen, Skadsen). The Skadsen and Abebe labs inserted transgenes into the cultivar Golden Promise. These lines were backcrossed to Conlon for transgene expression in a cultivar that can be tested in North Dakota and Minnesota. In 2010, BC_1F_2 pooled seed of each line were tested. After another cross in 2010, the BC_2F_2 pooled seed were tested in 2011. In addition, 14 Conlon transgenic lines previously tested that showed reduced FHB and DON were evaluated again in 2011 along with wild type Conlon as the susceptible check and CI4196 as the resistant check. The mean FHB severity across all lines and replicates was 3.6%, mean FHB incidence was 48.5% and mean DON was 5.6 μg/g. Conlon and most of the transgenic lines showed significantly less DON than CI4196, but few other differences were detected in this single year trial. Comparison of seven lines tested in multiple years reveals that two transgenic lines (321-Tri12 and 823-tlp) consistently showed a 40% reduction in DON. Efforts to transfer these transgenes into resistant 6-rowed lines (Quest, ND20448) are underway to determine whether the transgene effect is additive to resistance being incorporated by the breeding programs.

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IDENTIFICATION OF CANDIDATE GENES FOR HEAD BLIGHT AND DEOXYNIVALENOL RESISTANCE

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ABSTRACT

We are investigating the mechanisms underpinning host resistance to deoxynivalenol (DON) and Fusarium head blight disease of wheat and barley. We use a three-pronged approach to identify genes and pathways that enhance DON resistance – genes of interest have been identified based on studies of host resistance, biocontrol and hormonal signaling pathways. Using a double haploid wheat population segregating for QTL *Fhb1*, we identified several genes associated with DON resistance. These genes were up-regulated in response to DON and segregated at the RNA accumulation level with *Fhb1*. Ongoing characterisation of these genes includes analysis of the effect of gene knockout on disease and toxin resistance and cellular studies on protein -protein interactions. *Pseudomonas flourescens* strain MKB158 was indentified as a bacterium that reduces FHB levels, associated toxin accumulation and yield losses. Using a microarray, we identified wheat genes potentiated by this bacterium to respond to *Fusarium culmorum*. Serpins and lipoxygenase genes were highly potentiated to respond to the pathogen. Future studies will focus on the role of serpins in induced resistance to FHB. Studies on brassinsteroid signaling identified the receptor *Bri1* as a key regulator of FHB resistance and toxin build-up in grain. On the basis of microarray analysis, we identified genes and pathways differentially regulated in a *Bri1* mutant as compared to wild type barley. These pathways are currently being investigated. On the basis of all our studies, we can conclude that there is a biochemically diverse array of genes that can be targeted to control FHB, thus offering great scope for breeding genotypes with effective long-term resistance to FHB. However, in the context of a practical breeding programme, the effect of some such genes on other biotic and abiotic interactions must now be investigated.

ACKNOWLEDGEMENTS

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UTILIZING ALIEN SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT FOR WHEAT IMPROVEMENT

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ABSTRACT

We are currently working with *Fhb3* for resistance to Fusarium head blight (FHB) transferred from *Leymus racemosu*s and a second source of resistance derived from *Elymus tsukushiensis*. *Fhb3* is located in the short arm of the *L. racemosus* chromosome 7Lr#1 and was transferred to wheat in the form of a compensating T7AL·7Lr#1S Robertsonian translocation (RobT). Chromosome engineering was used to produce two distal (T7AL·7AS-7Lr#1S, rec 679 and rec 989) and one proximal (T7AL·7Lr#1S-7AS, rec124) recombinants in an Overley background. Our second source of FHB resistance is derived from a group-1 *E. tsukushiensis* chromosome 1Est#1. Chromosome engineering was used to produce distal (TWL·WS-1Est#1S) and interstitial (Ti1WL·1WS-1Est#1S-1WS) recombinants that are being evaluated for their resistance to FHB and DON accumulation. In addition, as one of our long-term objectives of conserving and utilizing wild germ plasm in wheat improvement, we are constructing chromosome introgression libraries of various alien species in Chinese Spring wheat. We are evaluating the following 23 RobTs for their resistance to FHB in the greenhouse using single-point inoculation. Promising lines will be crossed with Everest.

A PUTATIVE FUNGAL MIRNA THAT MIGHT PLAY A ROLE IN FUSARIUM HEAD BLIGHT PATHOGENESIS IN WHEAT

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ABSTRACT

miRNAs are endogenous, non-coding 21-23 nt RNA molecules that play important regulatory roles in eukaryotic gene expression by targeting mRNAs for cleavage or translational repression. Direct cloning has enabled the identification of many miRNAs; however, significant variation in their expression levels has made it difficult to clone low abundance miRNAs. No miRNAs has been reported so far by the traditional cloning method in Fungi. In our study of the role of miRNAs in the pathogenesis of Fusarium head blight in wheat, we identified a potential fungal miRNA which was specific to Fusarium inoculated wheat spikelets. Searching the genome of *Fusarium graminerum PH1* for its coding sequences revealed one where the putative transcript has the potential to form a hair pin loop secondary structure. Hence, the present study is taken up to prove it is indeed a fungal miRNA. The presence of this sequence will be verified in the genome of *Fusarium graminerum* isolate 4 by RT-PCR cloning of putative transcript. 5' and 3' RACE will be done to find the sequence of full length pre miRNA transcribed. A search against Rfam database that lists all the non-coding mRNAs will be useful to know if there is any protein being translated from this sequence. A target mRNA for the putative miRNA will be searched for in both *Fusarium* and wheat genomes which would be later verified by Northern or/and RT-PCR. RNAi studies will be conducted to better understand its function and relate its role in pathogenesis of Fusarium head blight in wheat.

ETHYLENE-SIGNALING IN WHEAT IS ESSENTIAL FOR TYPE I AND II RESISTANCE TO F*USARIUM GRAMINEARUM* AND TOLERANCE TO DEOXYNIVALENOL

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ABSTRACT

Ethylene (ET) has 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 is a potential candidate 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. 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. S-adenosylmethionine synthetase (SAMs) and an Ethylene Response Factor (ERF) in particular demonstrated remarkable conversion to susceptibility upon silencing. The importance of ethylene signaling was also observed using the gaseous inhibitor of ethylene signaling 1-Methylcyclopropene (1-MCP). Ning plants exposed to this inhibitor became significantly more susceptible to *F. graminearum* than control plants. These plants also become substantially more sensitive to the toxin deoxynivalenol. Additionally, the susceptible variety 'Bobwhite' becomes significantly more susceptible when exposed to 1-MCP and challenged with *F. graminearum*. 1-aminocyclopropane-1-carboxylic-acid (ACC) is a precursor of ethylene and is converted by the enzyme ACC-oxidase into ethylene. Both varieties become significantly more resistant to spray (Type I resistance assay) and point (Type II resistance assay) inoculations when treated with ACC. Our findings contradict recently published work proposing that *F. graminearum* exploits ethylene signaling to create susceptibility in wheat.

IDENTIFYING FHB RESISTANCE GENES IN WHEAT USING A NEXT-GENERATION SEQUENCING APPROACH

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ABSTRACT

The *Fhb1* QTL on wheat chromosome 3BS confers type II resistance to Fusarium Head Blight (FHB). To gain a better genetic understanding of the *Fhb1* QTL locus, a near-isogenic line (NIL) pair carrying the resistant and susceptible alleles for *Fhb1* was subjected to SNP genotyping and gene expression analysis. We genotyped the NIL pair with 9,000 SNPs and defined the extent of the introgression and background variation. We established three experiments to examine gene expression in the NIL pair and to identify candidates for the *Fhb1* gene. These three experiments include: (1) point inoculation of spikelets with *Fusarium graminearum* and sampling the inoculated spikelets at 96 hours after inoculation; (2) deoxynivalenol (DON) inoculation and sampling at 12 hours after inoculation; and (3) point inoculation of *F. graminearum* and sampling the rachis at 48 and 96 hours after inoculation. We used next-generation sequencing of RNA to obtain the gene expression data. For experiment #1, approximately 250 million sequencing reads were obtained from each genotype. Overall, we identified approximately 425 genes that exhibited differential expression between the two genotypes. The expression of these genes provided the opportunity to better understand the resistant and susceptible interaction. In addition, we identified 31 candidate genes that showed high expression in the genotype containing the *Fhb1* resistant allele and no expression in the genotype with the *Fhb1* susceptible allele. Results of the differentially expressed genes will be presented.

SEQUENCE DIVERSITY AND HAPLOTYPE ANALYSIS OF FUSARIUM HEAD BLIGHT-RESPONSIVE GENES IN DIVERSE WILD AND CULTIVATED BARLEY

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ABSTRACT

Wild (*Hordeum vulgare* subsp. *spontaneum*) and cultivated barley (*Hordeum vulgare*) accessions possess various degrees of resistance to Fusarium head blight (FHB). Integration of resistance from diverse sources into elite lines has the potential to enhance resistance, ultimately helping barley producers manage FHB. To identify potential alleles for FHB resistance, a candidate gene-based approach was used to associate nucleotide polymorphisms with variation in disease resistance. Previous GeneChip experiments have identified hundreds of barley genes exhibiting significantly up-regulated transcript levels in response to treatment with *Fusarium graminearum* or deoxynivalenol (DON). Thirty-five of these genes, including those implicated in defense responses such as cytochrome P450s, ABC transporters, glutathione-S-transferases, and UDP-glucosyltransferases, and those with regulatory and signal transduction roles such as zinc finger proteins, transcription factors and protein kinases, have been successfully re-sequenced from 16-30 diverse barley genotypes and analyzed using an association-based approach. Ectopic expression of one of the candidate genes, *HvUGT13248*, conferred DON resistance in yeast and *Arabidopsis*. A 2.5 kb genomic region of the *HvUGT13248* gene was re-sequenced from 30 genotypes. *HvUGT13248* maps to chromosome 5H bin4, which does not correspond with any previously mapped FHB resistance QTL. Interestingly, a SNP was identified only in the two most susceptible genotypes, ICB111809 (two-rowed) and PI383933 (six-rowed) and caused an amino acid change from glycine to arginine, suggesting that it may have functional significance. Significant associations between SNP sites and disease resistance within a mitogen-activated protein kinase (MAPK) gene were also detected. The map location of the MAPK gene is on chromosome 1H bin12 which is coincident with an FHB resistance QTL identified from populations carrying Chevron or Zhedar2 alleles, indicating that this gene is a promising candidate for further analysis. Haplotype and diversity analysis of the other 33 genes will also be reported.

SUBCELLULAR TARGETING OF A PLANT DEFENSIN MTDEF4.1 PLAYS A MAJOR ROLE IN CONFERRING RESISTANCE TO HEMIBIOTROPHIC AND BIOTROPHIC PATHOGENS IN TRANSGENIC *ARABIDOPSIS*

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ABSTRACT

Plant defensin MtDef4.1 isolated from *Medicago truncatula* was shown to inhibit the hyphal growth of several *Fusarium* spp. *in vitro* at micromolar concentrations, including hemibiotroph *F. graminearum* which produces harmful mycotoxins. Transgenic *Arabidopsis* lines were generated in which MtDef4.1 was targeted to the extracellular space or to vacuoles or retained in the endoplasmic reticulum (ER), and tested for resistance to *F. graminearum* and to an obligate oomycete biotroph *Hyaloperonospora arabidopsidis* (*Hpa*). Using silique inoculations, we determined that transgenic *Arabidopsis* lines expressing MtDef4.1 targeted either extra- or intracellularly provided low level of resistance to this fungus. Interestingly, a couple of transgenic *Arabidopsis* lines expressing ER-retained and extracellular MtDef4.1 produced significantly reduced levels of mycotoxin deoxynivalenol. When evaluated for resistance to *Hpa*, transgenic lines expressing only extracellular MtDef4.1 displayed strong resistance to this pathogen, but the lines expressing intracellularly targeted MtDef4.1 did not. This is consistent with the lifestyle of this biotroph which grows intercellularly in the leaf. In contrast, since the lifecycle of *F. graminearum* consists of a brief biotrophic phase and a long necrotrophic phase, we postulate that co-expression of MtDef4.1 both extra- and intracellularly is required for effective control of this pathogen and mycotoxin accumulation in transgenic crop plants.

AN ACTIVATION TAGGING SCREEN TO IDENTIFY NOVEL GENES FOR FUSARIUM HEAD BLIGHT (FHB) RESISTANCE

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ABSTRACT

The goal of this project is to identify plant genes that confer resistance against FHB and reduced DON accumulation. The identification of such genes offers the possibility to more fully understand the mechanisms of *Fusarium* susceptibility and to design transgenic strategies to increase FHB resistance in barley and wheat. We are using activation tagging to identify genes which confer resistance to trichothecenes. Activation tagging uses a modified T-DNA vector which contains multiple copies of the cauliflower mosaic virus (CaMV) 35S gene enhancer arranged in tandem. In addition to knocking out genes, the modified T-DNA vector can also function as an enhancer when inserted either upstream or downstream of a gene to produce gain-of-function phenotypes. The genomic location of the tag is readily identifiable by thermal asymmetric interlaced (TAIL) PCR. Using this approach, we have screened ~250,000 activation tagged *Arabidopsis* seeds for resistance to trichothecin and identified 30 lines that showed resistance. These plants were able to form roots on 4 μM Tcin, a concentration which severely inhibits germination and prevents root formation of the Col-0 wild type. Characterization of two of these lines using RT-qPCR identified an activation genotype in one line, termed *Arabidopsis thaliana resistant root formation1* or *AtRRF1* and a knockout genotype in the other, termed *AtRRF5*. In *AtTRRF1*, two novel lipid transfer protein (LTP) genes, designated as LTP4 and LTP5, were overexpressed compared to the wild-type control. LTPs are small cysteine-rich proteins that transfer lipids between membranes *in vitro*. To verify resistance, both *LTP4* and *5* have been cloned into Gateway expression vectors and transformed into *Arabidopsis*. In *AtRRF5*, the activation tag was found in the second exon of TBR (Trichome Birefringence-Like), a gene that controls synthesis and deposition of secondary wall cellulose. Expression of *AtRRF5* was not detected in the activation tagged line while the wild-type control showed a detectable level of expression, confirming that the insertion created a knockout genotype. Resistance in *AtRRF5* was confirmed by testing two independent knockout lines which were obtained from the Arabidopsis Information Resource (TAIR) collection. This research has shown that activation tagging is a useful method to identify plant genes which play a role in trichothecene resistance. The next step will be to determine if the novel genes identified from the screen in *Arabidopsis* will confer resistance to DON and FHB in transgenic wheat and barley plants. In addition, we are exploring the use of activation tagging in both wheat and barley to directly identify genes for trichothecene resistance in these crop species.

ENGINEERING DEFENSE REGULATORY GENES AND HOST SUSCEPTIBILITY FACTORS FOR ENHANCING FHB RESISTANCE IN WHEAT

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ABSTRACT

Fusarium head blight (FHB)/scab is a destructive disease of wheat and barley. *Fusarium graminearum* is one of the major causative agents of FHB in the US. The interaction between *Arabidopsis thaliana* and *F. graminearum* has provided an excellent system to identify and characterize genes and mechanisms that are critical to host defense as well as genes that predispose plants to *F. graminearum* infection. These studies in *Arabidopsis*, and more recently in wheat have demonstrated that a complex interaction between salicylic acid (SA) and jasmonic acid (JA) signaling contribute to the overall basal resistance against *F. graminearum*. Previously, we showed that constitutive expression of the *Arabidopsis NPR1* gene, which is a key regulator of SA signaling, enhanced basal resistance against *F. graminearum* in *Arabidopsis* and wheat (Makandar et al. 2006, 2010). The *PAD4* and *WRKY18* genes are two other *Arabidopsis* genes that promote FHB resistance when constitutively expressed in wheat. *PAD4* encodes a putative lipase/hydrolase that regulates multiple defense mechanisms, including SA and phytoalexin accumulation, and *WRKY18* encodes a transcription factor involved in the activation of defense genes. In contrast to *NPR1*, *PAD4*, and *WRKY18*, which promotes defense against *F. graminearum*, a lipoxygenase (LOX) activity contributes to host susceptibility to this fungus. This LOX activity has been targeted for silencing in wheat to promote FHB resistance. In addition, the non-host resistance mechanism is also being targeted for enhancing FHB resistance in wheat.

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REFERENCES

Makandar, R., Essig, J. S., Schapaugh, M. A., Trick, H. N. and Shah, J. (2006). Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis NPR1*. Mol. Plant-Microbe Interact. 19:123-129.

Makandar, R., Nalam, V., Chaturvedi, R., Jeannotte, R., Sparks, A.A., and Shah, J. (2010). Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis* interaction with *Fusarium graminearum*. Mol. Plant-Microbe Interact. 23:861-870.

DEVELOPING A TRANSPOSON BASED GENOMIC APPROACH TO EXPLORE STRESS RESPONSIVE GENES IN WILD BARLEY

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ABSTRACT

Biotic and abiotic stresses pose major threats to crop species and result in heavy yield losses worldwide. The wild barley subspecies, *Hordeum spontaneum*, has shown high genetic variation in biotic and abiotic stress responses and represents an excellent candidate for improvement of cultivated barley. Although *H. spontaneum* and cultivated barley are inter-fertile, introgressions of complex traits from wild material are often unsuccessful due to linkage drag. Modern genetic and genomic tools, however, have the potential to provide new avenues for identification of unique genes from wild species and their introgression into cultivated barley and beyond to meet the challenges posed by biotic stresses such as tolerance to FHB. The transposon-based gene tagging system is an effective technique for identifying and determining gene functions in large genome crops. Maize Activator (*Ac*) and Dissociation (*Ds*) elements have been utilized for insertional mutagenesis in heterologous plant species by introducing *Ds* elements in one plant and then crossing with a transposase (*Ac*TPase) expressing plant. This transposon system is now well established in cultivated barley (*Hordeum vulgare* L.). The main objective of this study is to develop a transposon tagging system in wild barley (*H. spontaneum*) to explore genes related to biotic and abiotic stress. In the present study, two approaches are being applied to develop *Ds* insertion lines (TNPs) in wild barley population. Two wild barley accessions, Damon and Shechem of *H. spontaneum* were independently hybridized and back crossed with *Ds* containing lines from cultivated barley. In addition, a tissue culture system for this species has been explored for introduction of *Ac* and *Ds* elements through particle gun bombardment. After establishing *Ac*/*Ds* system in wild barley, we aim to perform functional genomics studies for identification of stress response genes for later improvement of small grain cereals.

CAN *BRACHYPODIUM* PROVIDE INSIGHT INTO FHB?

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ABSTRACT

Model species have the potential to provide significant insight into mechanisms involved in interactions between pathogens and crop hosts. We, along with others have used *Arabidopsis* as a model species to unravel aspects of the interactions between *Fusarium* and plant hosts. Our work revealed that *F. graminearum* exploits ethylene signalling to aid colonisation of *Arabidopsis* and wheat[1]. Although useful in many respects it is often difficult to undertake meaningful translation from a dicot model to crop species to identify candidate genes involved in FHB resistance. For this reason we turned to *Brachypodium distachyon* as an alternative. *Brachypodium* is a fully sequenced monocot species that has a small stature, rapid life cycle and non-demanding growth requirements. The high degree of genome conservation and synteny between *Brachypodium* and wheat makes this species even more attractive for providing information of direct relevance to cereal crop species. Furthermore, *Brachypodium* is being widely used in relation to bio-fuels and this is being accompanied by large-scale programmes to develop functional genomics resources in *Brachypodium*.

The ability of *F. graminearum* and *F. culmorum* to infect a range of *Brachypodium* tissues was examined in various bioassays. Histological studies were undertaken to investigate details of infection, colonisation and host response. Susceptibility to *Fusarium* and DON was assessed in two Brachypodium ecotypes.

Both *Fusarium* species infected all *Brachypodium* tissues examined and DON was produced in infected spike tissues. Hair cells were observed to be important sites of infection, supporting findings from other species. We also observed variation between ecotypes in resistance to both *F. graminearum* and DON.

Brachypodium exhibits characteristics of susceptibility highly similar to those of wheat, including susceptibility to spread of disease in the spikelets. This contrasts markedly with barley which exhibits almost complete resistance to spread between spikelets. We also found that DON appears to function as a virulence factor for infection of *Brachypodium* by *F. graminearum* as it does in wheat[2]. We concluded that *Brachypodium* potentially provides an excellent model for FHB in wheat.

More recently, we have been using T-DNA tagged lines (Brachy-TAG) to study the influence of a number of *Brachypodium* genes on resistance to *F. graminearum*. We will present some of our findings and discuss how they might influence attempts to improve FHB resistance in wheat and barley.

REFERENCES

[1]Chen et al (2009) *Molecular Plant Pathology* 2006, **7:**391-403.

[2]Peraldi et al (2011) *BMC Plant Biology* 11:100doi:10.1186/1471-2229-11-100.

IDENTIFICATION AND CHARACTERIZATION OF WHEAT GENES THAT ENHANCE PLANT RESISTANCE TO DON

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ABSTRACT

We focus on identifying biochemical pathways involved in the wheat response to the *Fusarium* virulence factor deoxynivalenol (DON). Using functional genomics techniques, DON-responsive transcripts were identified: these included transcripts encoding a basic leucine zipper transcription factor, a multidrug resistance protein ABC transporter, cytochrome P450s and novel proteins [1, 2]. Based on the results, we propose a model whereby the early oxidative stress response and a detoxification pathway contribute to DON tolerance. These studies have also highlighted that there are genes involved in the wheat response to DON that are not represented on the Affymetrix chip. For example, one DON-responsive wheat gene was identified via differential display analysis. It encodes a novel, evolutionary divergent protein. We are currently characterizing this gene at the molecular level.

REFERENCES

1. Ansari, K.I., et al., Retrotransposon and gene activation in wheat in response to mycotoxigenic and non-mycotoxigenic-associated *Fusarium* stress. Theor Appl Genet, 2007. 114(5): p. 927-37.

2. Walter, S., et al., Components of the gene network associated with genotype-dependent response of wheat to the *Fusarium* mycotoxin deoxynivalenol. Funct Integr Genomics, 2008. 8(4): p. 421-7.

THE UDP-GLYCOSYLTRANSFERASE (UGT) GENE FAMILY OF *BRACHYPODIUM DISTACHYON*: FUNCTIONAL CHARACTERIZATION OF A CLUSTER OF CANDIDATE UGTS INVOLVED IN DETOXIFICATION OF DON TO DON-3-O-GLUCOSIDE

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ABSTRACT

Deoxynivalenol production is a virulence factor supporting fungal spread in infected wheat ears. Resistance to *Fusarium* in wheat is at least partly determined by the ability to convert DON into the non-toxic conjugate DON-3-O-glucoside (Poppenberger *et al.* 2003, Lemmens et al. 2005). Also during infection of the model grass *Brachypodium distachyon* with *F. graminearum* the detoxification product DON-3-O-glucoside (D3G) is formed. The gene family of *Brachypodium* UDP-glycosyltransferases consists of 177 predicted genes. Frequently theses genes occur in clusters. We functionally characterized a cluster of six BdUGT genes displaying the highest sequence similarity with the DON resistance conferring barley *HvUGT13248* (Schweiger *et al.* 2010). The six BdUGT genes were cloned with an N-terminal cMYC-tag to monitor protein levels in yeast. Only two out of the six homologs conferred DON resistance in yeast.

UGT genes highly similar to *HvUGT13248* are also present in the genomes of *Sorghum bicolor* and rice. The cluster of the Bd genes on chromosome 5 consists of 4 genes in a row and another 2 separated by 6 and 52 unrelated ORFs. The two separated genes were identified as functional DON detoxification genes, while the gene with the highest blast score (Bradi5g03390, 1.3e-190) was inactive. In *Sorghum* the gene with the highest similarity to *HvUGT13248* is a single copy gene on chromosome 6 (BlastP score 6.5e-185). Yet, two complex clusters of also highly similar genes (5 genes in mixed orientation from Sb02g030020 to Sb02g030060 with scores from 2.6e-126 to 8.5e-74) are present on chromosomes 2, and on chromosome 1 (two genes Sb01g031560 and Sb01g031540, scores 5.9e-168 and 7.4e-161), respectively. Also in rice the situation is complex, with a cluster of 3 homologous genes on chromosome 4 (Os04g0206500 to Os04g0206700 with scores of 1.4e-180 and 1.8e-166). UGT gene clusters seem to evolve rapidly and with changes in copy number. We therefore expect that it will be difficult to identify the true orthologs of UGT genes in different crop plants. Validation of the presumed function of candidate genes by heterologous expression and functional testing in yeast seems therefore warranted.

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IDENTIFYING AND CHARACTERIZING BARLEY GENES THAT PROTECT AGAINST TRICHOTHECENES

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ABSTRACT

Our overall goal is to identify genes that play a role in resistance to Fusarium Head Blight (FHB) and to develop and test transgenic wheat carrying these genes. In particular, we are interested in identifying genes that protect barley and wheat from the effects of trichothecenes. Previously, we conducted a large set of RNA profiling experiments during *Fusarium graminearum* infection of barley and inoculation with the trichothecene deoxynivalenol (DON). We identified a set of potential resistance genes that respond to trichothecene accumulation that encode a cysteine synthase, ABC transporters, UDP-glucosyltransferases (UGTs), cytochrome P450s, and glutathione-S-transferases (GST). In parallel, we developed an *Arabidopsis* assay to functionally test genes for their efficacy against trichothecenes. We then generated transgenic *Arabidopsis* over expressing one of the barley UGTs and five GSTs and tested these plants for their ability to grow on media containing trichothecenes. Transgenic *Arabidopsis* overexpressing the barley UGT and the GST genes exhibited high and moderate levels of tolerance to DON, respectively. Resistance to DON via UGT activity is considered an important component of resistance against FHB and is related to the ability to detoxify DON into DON-3-O-glucoside (D3G). DON feeding studies on the transgenic *Arabidopsis* carrying the barley UGT showed that DON was converted to D3G. More recently, we developed 18 events of transgenic wheat overexpressing the barley UGT and tested the transgenic lines for resistance to FHB. We identified five transgenic lines that expressed the UGT transgene and exhibited high type II FHB resistance.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2011 FIELD NURSERY REPORT

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ABSTRACT

The 2011 field screening nursery, with 56 wheat and 88 barley plots was located at UMore Park, Rosemount MN. Trial entries and untransformed controls were submitted by the University of North Texas (9+1 wheat), and USDA (17+2 barley). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks used were the moderately resistant Alsen, the susceptible cultivars Wheaton and Roblin, and a non-inoculated Wheaton check. The barley checks were the moderately resistant Quest and the susceptible cultivars Conlon (2-rowed), 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 18, 2011. 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 (dai) for each plot. The inoculum was a composite of 50 *F. graminearum* isolates at a concentration of 100,000 (barley) or 200,000 (wheat) 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 the first inoculation on July 7 till July 25 to facilitate FHB development. FHB incidence and severity were assessed visually 17 d.a.i. for wheat and 13 d.a.i. for barley on 20 arbitrarily selected heads per plot. FHB incidence was determined by the percentage of spikes with visually symptomatic spikelets of the 20 heads observed. FHB severity was determined as the percentage symptomatic spikelets of the total of all spikelets observed. Plots were harvested at maturity on August 5 (barley) and 11 (wheat). Fifty (barley) and 30 (wheat) heads where harvested from each plot, threshed and the seed cleaned manually. The wheat sub-samples were used to determine the percentage of *Fusarium* damaged kernels (FDK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis. The data indicated that resistance was expressed in some of the transformed lines.

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AN OVERVIEW OF WHEAT TRANSFORMATION AT K-STATE

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ABSTRACT

Genetic engineering of wheat holds great potential for trait development and plays a critical role in gene validation. Many wheat breeders see this technology as a valuable component of their wheat breeding toolbox. The U.S. wheat industry has also recognized the benefits and value of biotech wheat and is willing support the commercialization of transgenic wheat. More than half of the research projects at Kansas State University's Plant Transformation Facility are now focused on transgenic wheat. Over the past decade, the facility has collaborated on a number of projects both applied and basic, including projects funded through USWBSI. This presentation will give an overview of wheat transformation at K-State: the overall transformation process; past and present transformation capacities of the facility and; examples of the collaborative projects using transgenic wheat.

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A NEW SOURCE OF RESISTANCE TO FUSARIUM HEAD BLIGHT FROM WHEAT- *ELYMUS REPENS* INTROGRESSIONS

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ABSTRACT

Elymus repens (L.) Gould (2n = 6x = 42, **StStStStHH**) is a hexaploid wild grass species, distantly related to bread wheat (*Triticum aestivum* L. em Thell; 2n = 6x =42, **AABBDD**). It has a high level of resistance to Fusarium head blight (FHB). The objective of this study was to transfer genes for resistance to FHB from *E. repens* to common bread wheat. The cross Crocus/*E. repens* was made in Crop Development Center experimental field, Department of Plant Sciences, University of Saskatchewan. The F_1 plants were back-crossed to Crocus, then seeds from the BC_1F_1 plants were bulked and advanced to the BC_1F_7 generation. Sixteen lines were selected and evaluated for FHB reaction in the nursery in Ottawa in 2007 and 2008. Two lines, P1142 and P1131 (F8), were re-selected based on agronomic traits and FHB resistance performance. The results showed that the line P1142 was still segregating, with chromosome numbers ranging from 42 to 56, while the line P1131 with 56 chromosomes was stable morphologically. Cytological study and *in situ* hybridization analyses indicated that we obtained several wheat-*E. repens* addition and translocation lines, and two partial amphiploids. The results of greenhouse FHB evaluation by point inoculation showed that all the lines had a high level of resistance to FHB with only one spikelet infected (6%), compared to the check Roblin (100%) and the parent Crocus (85%). This new resistance source will be useful for the improvement of FHB resistance in wheat.

IDENTIFYING AND CHARACTERIZING CANDIDATE GENES ASSOCIATED WITH FHB RESISTANT QTL *QFHB1*

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ABSTRACT

The goal of this research is to identify and characterize genes associated with *Qfhb1*, a major FHB resistance QTL that has been mapped on wheat chromosome 3BS. Our previous study of FHB molecular mechanism has identified 281 resistance-related and 79 susceptibility-related genes. We screened these 360 genes for their differential expression with qPCR between a pair of near-isogenic lines 260-2 (R) and 260-4 (S) developed by Anderson's group at University of Minnesota and potential candidates were confirmed by screening their differential expression between a resistant and a susceptible pools, which consist 10 most resistant or susceptible lines from a $F_{2:8}$ recombinant inbred population between Sumai 3 and Y1193-6. Plants were grown in green house under a light cycle of 16 h light and 8 h dark. The first flowering floret of randomly chosen plants was either challenged with *Fusarium graminearum* macroconidia diluted in water (70,000~ (100,000 conidia /mL) or with water as the control. The inoculated spikes were immediately covered with plastic bags to maintain the humidity. RNA samples were isolated from spikelets collected from 10 plants in 24, 48 or 72 h after inoculation, respectively, for each treatment, and used for qPCR assay. Each qPCR assay was composed of three biologic and three technic repeats. Statistic analysis was done to identify significant differential expressions at $p \leq 0.05$. Of the genes screened, 16 potential candidates have been identified. Further validation and gene-disease association are in progress.