GENE DISCOVERY AND ENGINEERING RESISTANCE

ANALYSIS OF THE SMALL INTERFERING RNA PROFILES OF RANDOMLY INSERTED PTRM-*TRI6* *FUSARIUM GRAMINEARUM* MUTANTS AND THEIR DON RELATED PHENOTYPES

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ABSTRACT

Deoxynivalenol (DON) production by *Fusarium graminearum* requires activation of the trichothecene pathway in which TRI5 catalyzes the first step of trichothecene synthesis and TRI6 is a transcription factor that activates the pathway. RNA interference (RNAi) has emerged as a useful fungal genetics tool for reducing the expression of specific genes such as *TRI6*. Reduced DON production and virulence on wheat has been demonstrated as a result of RNAi-induced reduction of *TRI6* expression via transformation of *F. graminearum* with the plasmid pTRM-TRI6. This plasmid contains the GDPA promoter driving *TRI6* linked to an inverted repeat of *TRI6* to generate a hairpin loop mRNA. Hairpin loop structures are known to be processed by the endoribonuclease dicer to produce a population of a specific type of non-coding small RNA approximately 20–30 bp long, called small interfering RNA (siRNA), that silence genes with homology to siRNAs. To verify and expand on the previous study, six additional fungal transformants containing pTRM-TRI6 were produced and evaluated by next generation sequencing for siRNA with homology to *TRI6* and for the genomic location of the pTRM-TRI6 insertions. The results support previous conclusions that expression of pTRM-TRI6 reduced DON production and virulence on wheat. Furthermore, experiments measuring the mycotoxin production capacity independent of host-pathogen interactions showed reduction in mycotoxin accumulation on rice cultures and lower expression of *TRI5* on toxin-inducing media (TBI) in association with the production of siRNAs with homology to *TRI6*. Understanding the resulting siRNA profiles from RNAi constructs is critical to optimizing RNAi applications. The results are being used to guide the development of vectors for barley transformation with the goal of elevating resistance to Fusarium head blight via host induced gene silencing, which is a promising application of RNAi.

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REACTIONS OF TRANSGENIC BARLEY LINES TO FHB INOCULATION IN 2015 NORTH DAKOTA FIELD TRIALS

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ABSTRACT

Transgenic lines were tested directly in the field in Langdon, ND. Lines and checks (Conlon, Quest, ND20448) were sown in hill plots in an augmented block design, with the checks repeated every 20 hills. Three replicates were planted in the inoculated, misted nursery and three replicates in the adjacent un-misted nursery. Ten seed were planted per hill with 30 cm hill spacing. Plots were bordered with wheat. The nursery was inoculated by grain spawn technique with a mixture of five *F. graminearum* isolates. From inoculation until grain ripening, the misted nursery was irrigated for 20 minutes early morning and again at late afternoon each day. FHB severity was evaluated at approximately three weeks after anthesis, by counting the total and infected number of seed on ten randomly selected spikes per hill. Samples from each plant were analyzed for DON content. For both misted and un-misted trials, there were significant differences for severity and DON content among lines. There were significant differences also among replicates for DON content. Pairwise comparisons ($p = 0.10$) between Quest, ND20448, and Conlon and their respective transgenic lines revealed few differences. In the misted nursery, ND20448-derived transgenic line ND2-6 had higher FHB severity and DON content than ND20448, and Quest-derived transgenic line 82Q3-6 had higher DON content than Quest. However, in the un-misted nursery, this line had lower severity. The other Quest-derived line, 82Q3-2 had lower DON content than Quest. 82DN2-6 again had higher severity and DON content than ND20448.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2015 FIELD NURSERY REPORT

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ABSTRACT

The 2015 field screening nursery consisted of 9 wheat and 12 barley entries evaluated in side by side experiments. Entries within each species experiment were arranged in a randomized complete block design with four replications in a field located at UMore Park, Rosemount MN. Trial entries and untransformed controls* were submitted by the University of North Texas (9 wheat lines + Bobwhite*) and the USDA (12 barley lines + Conlon* and ND20448*). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivar Alsen and the susceptible cultivars Roblin and Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivar Stander. Individual plots were 2.43 m long single rows. The trial was planted on June 2, 2015. All plots were inoculated twice with the exception of Wheaton and Stander non-inoculated controls. The first inoculation was applied at anthesis for wheat (July 13-July 22) and at head emergence (July 16-July 20) 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 40 *F. graminearum* isolates, applied 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 10 $ml.sec^{-1}$ at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on July 16 through August 6 to facilitate FHB development. FHB incidence and severity were assessed visually 18-24 d.a.i. for wheat and 17-21 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 hand harvested at maturity on August 17 (barley) and August 25 (wheat). Approximately sixty heads were harvested from each plot, threshed and the seed cleaned manually. The wheat grain was used to determine the percentage of visually scabby kernels (VSK) and then all samples (wheat and barley) were ground and submitted for deoxynivalenol (DON) analysis. In 2015, the disease severities were similar to the 2013 nursery and generally lower than the 2014 nursery. The mean FHB severity for the non-inoculated Wheaton control was 19%. Mean FHB severity for the untransformed wheat check Bobwhite was 31%. Mean FHB severities for the standard wheat checks, Alsen, Roblin and Wheaton were 9, 35 and 38%, respectively. The mean FHB severity for the non-inoculated Stander check was 17%, while the

untransformed barley check varieties Conlon and ND20448 had mean FHB severities of 25 and 15%, respectively. The barley standard checks, Quest and Stander, had mean FHB severities of 8 and 22%, respectively. The FHB severity data indicated that resistance was significantly ($P<0.05$) improved in some transformed barley lines compared to the untransformed checks. Preliminary analysis indicated that the FHB severities of several wheat entries may be better than the corresponding untransformed check, though the differences in FHB severities may not be statistically significant. The harvested grain is currently being analyzed for DON. The data are not yet available although they will be included in the poster presented at the forum.

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COARSE AND FINE MAPPING OF QUANTITATIVE TRAIT LOCI FOR FHB IN BARLEY

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ABSTRACT

Fusarium species cause Fusarium head blight (FHB) disease in wheat and barley. Resistance to the disease is controlled by quantitative trait loci (QTL) and previous genetic mapping studies have identified a number of resistant alleles from two- and six-rowed barley accessions. The aim of the current studies are to (1) identify additional QTL for resistance to initial infection (type I resistance); and (2) fine-map a QTL on chromosome 6H in barley. For the first study, a QTL mapping population comprising 93 $F_{6:7}$ recombinant inbred lines (RILs) derived from a cross between Rasmusson and PI383933 was developed. Rasmusson is a six-rowed malting barley cultivar released by University of Minnesota with moderately susceptible response to FHB. PI383933 is a six-rowed Japanese cultivar with susceptible response to FHB and often used as a susceptible check in FHB field screenings. The population was evaluated in FHB nurseries using a randomized complete block design with three replicates in St. Paul and two replicates in Crookston in 2015. Spray inoculation and grain spawn inoculation was applied at St. Paul and Crookston, respectively. Data for FHB severity, plant height, heading date and kernel density were recorded. The barley iSelect 9K SNP chip was used to genotype the RILs and a linkage map was generated with 1,394 SNPs and consisted of seven linkage groups. Composite interval mapping identified three QTL associated with FHB resistance on chromosomes 5H, 6H and 7H. The QTL on 5H and 6H were associated with PI383933 alleles and explained 6.2% and 5.5% of phenotypic variance, respectively. The 7H QTL was associated with a Rasmusson allele and explained 34.3% of phenotypic variance. Interestingly, the FHB QTL on 7H was coincident with a plant height QTL. To fine map the chromosome 6H QTL region, an F_2 population of 2,082 plants was derived from crossing lines carrying Chevron alleles in the 6H QTL region with the susceptible cv. Lacey. This population was genotyped with flanking SSR markers to identify 399 recombinants. These recombinants were further genotyped with 34 SNP markers covering the overlapping target region (2.8 cM), which resulted in the identification of 37 recombinants representing 13 recombinant classes. Selected recombinants will be tested for FHB response in 2016.

TRANSGENIC PLANTS EXPRESSING *HVUGT13248* EXHIBITS HIGH LEVELS OF RESISTANCE TO A WIDE SPECTRUM OF TYPE B TRICHOTHECENES

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ABSTRACT

Fusarium head blight (FHB) is a major disease problem in cereal crops. Trichothecenes produced by the main causal pathogen, *Fusarium graminearum*, play an important role in disease development and spread, and also pose threats to humans and animals that consume infected grains. However, research on FHB resistance has primarily focused on deoxynivalenol (DON), which is the major chemotype found in the United States. Other chemotypes such as nivalenol (NIV) are more prevalent in Asia, but have recently been identified in the US. Additional chemotypes include the 3-acetyldeoxynivalenol (3-ADON) and15-acetyldeoxynivalenol (15-ADON), and the newly identified NX-2. Previous research identified a barley UDP-glucosyltransferase gene, *HvUGT13248*, which provides high levels of type II resistance to FHB by converting DON to DON-3-O-glucoside (D3G) in transgenic wheat. Here, we report that this gene has a wide spectrum of resistance to different trichothecene chemotypes, including NIV, 3-ADON and NX-2. Transgenic wheat expressing *HvUGT13248* show high levels of type II resistance to a NIV-producing Fg strain in greenhouse point inoculation tests. The FHB severity of the transgenic events were reduced by up to 90% compared to the non-transgenic control, while NIV accumulation in the transgenic events were reduced up to 94% compared to the non-transgenic control. Transgenic wheat expressing *HvUGT13248* also exhibits type II resistance to Fg strains producing 3-ADON and NX-2. Using a root assay, we found that transgenic wheat and *Arabidopsis* expressing *HvUGT13248* show resistance to DON and 3,15-diA-NIV inhibited root growth. Taken together, *HvUGT13248* shows resistance to a wide range of type B trichothecenes.

EXPRESSION OF A LIPID TRANSFER PROTEIN IN WHEAT TO ALLEVIATE OXIDATIVE STRESS INDUCED BY TRICHOTHECENES - A POSSIBLE MECHANISM TO INCREASE RESISTANCE TO FHB

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ABSTRACT

Trichothecene mycotoxins, such as deoxynivalenol (DON), are potent virulence factors of *Fusarium graminearum*, a causative agent of Fusarium head blight (FHB). Exposure to trichothecenes can trigger reactive oxygen species (ROS) production at toxic levels. Overexpression of a non-specific lipid transfer protein (AtLTP4.4) in *Arabidopsis* was found to enhance resistance to trichothecene exposure and led to significantly attenuated reactive oxygen species (ROS) compared to nontransgenic controls. In addition, overexpression of the cysteine-rich nsLTP was found to increase the total cellular glutathione (GSH) content of *Arabidopsis* leaf tissue. These results demonstrate that trichothecenes cause ROS accumulation and overexpression of AtLTP4.4 protects against trichothecene-induced oxidative stress by increasing the GSH-based antioxidant defense. We previously showed that exogenous addition of GSH and other antioxidants enhanced resistance to Tcin while the addition of buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, increased sensitivity to the toxin, providing further evidence of the link between oxidative stress and trichothecene sensitivity. To determine if expression of AtLTP4.4 confers resistance to trichothecenes in transgenic wheat, we constructed a monocot codon optimized version of AtLTP4.4 with HA and His tags, cloned the insert into the Ubi expression vector pAHC17, and generated transgenic Bobwhite, Rollag, Forefront and RB07 lines. We are analyzing these lines for RNA and protein expression and determining if the overexpression of nsLTP impacts the GSH content in wheat. Lines that show high expression and enhanced resistance to trichothecenes relative to the parental genotypes in the greenhouse will be tested in the field for FHB resistance during the 2016 season.

ENGINEERING RESISTANCE AGAINST *FUSARIUM GRAMINEARUM* IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is a damaging disease of wheat and barley. *Fusarium graminearum*, the principal causative agent of FHB, can also cause disease on leaves and flowers of *Arabidopsis thaliana*, a model plant for molecular-genetic studies. We have utilized the *Arabidopsis-F. graminearum* pathosystem to identify genes and mechanisms that can be targeted for promoting FHB resistance in wheat (Makandar et al. 2010, 2015, Nalam et al. 2015). Our results have demonstrated that defense regulatory genes as well as susceptibility factors can be engineered in wheat to enhance FHB resistance (Makandar et al. 2006, 2015; Nalam et al. 2015). Several of these transgenic wheat lines are undergoing field trials. Molecular-genetic studies have further demonstrated that the pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) mechanism can be targeted for enhancing resistance against *F. graminearum* in wheat. These results will be presented. In addition, we will also describe the utility of a lipase-encoding gene, which is expressed at elevated levels in floral tissues, in enhancing resistance against *F. graminearum*.

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HOW DOES THE *FHB1* LOCUS OF WHEAT AFFECT THE ABILITY TO DETOXIFY DON – A HYPOTHESIS

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ABSTRACT

The mycotoxin deoxynivalenol (DON) is a virulence factor of *Fusarium graminearum* required for spreading of the pathogen in the infected wheat head. The major spreading resistance QTL of wheat, the *Fhb1* locus, has been associated with increased ability to detoxify DON by formation of a glucose conjugate, leading to the hypothesis that *Fhb1* either encodes a UDP-glucosyltransferase (UGT) or a gene regulating its activity. Within the *Fhb1* interval of the sequenced susceptible cultivar Chinese Spring no UDP-glycosyltransferase (UGT), which might catalyze this detoxification-reaction, was found.

We have determined the K_m value (concentration of half-maximal enzyme activity) of a recombinant UGT capable of detoxifying DON into DON-3-O-glucoside for the UGT cosubstrate UDP-glucose. The observed K_m value of 2.2 mM [1] is about 10x higher than the concentration found in wheat heads at anthesis in *Fhb1* lines [2], suggesting that the availability of the co-substrate UDP-glucose is limiting the detoxification ability. Wheat lines containing *Fhb1* show about 2.53x higher levels of UDP-glucose than lines lacking it. A gene biochemically linked to UDP-glucose was found in the *Fhb1* interval of Chinese Spring, potentially encoding a truncated UDP-glucose dehydrogenase (UGDH), presumably a nonfunctional pseudogene. Both enzymes, UGT and UGDH, use UDP-glucose as a cosubstrate - the first to detoxify DON into DON-3-O-glucoside, the latter to produce UDPglucuronic acid, a precursor for activated sugar donors for cell wall biosynthesis. Coexpression of functional UGDH and UGT in yeast leads to a decrease of UDP-glucose levels and to sensitivity against DON compared to yeast expressing the UGT alone. We were therefore interested to learn what is encoded in the *Fhb1* interval of a resistant wheat cultivar. A comparison of the corresponding region between the *Fhb1* containing cultivar CM-82036 with Chinese Spring revealed that the pseudo-UGDH is absent in the *Fhb1* cultivar due to a 41.5 kb deletion.

The mechanism how the pseudogene lowers the UDP-glucose level is under investigation. A hypothesis how an absent pseudogene can confer a dominant resistance phenotype will be presented.

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