SESSION 3:

GENE DISCOVERY AND ENGINEERING RESISTANCE

TRICHOTHECENE EXPOSURE LEADS TO MITOCHONDRIAL ROS-MEDIATED CELL DEATH IN YEAST

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ABSTRACT

We had previously identified several yeast deletion mutants that conferred resistance to trichothecin (Tcin), a type B trichothecene and DON congener, which revealed a critical role for mitochondria in trichothecene-toxicity (1). Mitochondrial translation was directly inhibited prior to damage to mitochondrial membrane integrity and independent of cytosolic translation inhibition (2). To further explore the molecular mechanism of trichothecene toxicity we screened the yeast diploid knockout (YKO) library to identify deletion mutants that exhibited increased sensitivity to Tcin at concentrations that are sublethal to nonlethal (0.5-2μM) to the wild-type parental strain BY4743. We identified 121 deletion mutants which were disrupted in functions involved in cellular damage control from the toxic effects of trichothecenes, including DNA repair and response (15.7%), RNA degradation and stability (29.8%), ribosome biogenesis and protein degradation (10.7%). *Saccharomyces* Genome Database (SGD) phenotypic analysis revealed that a large fraction (42%) of the Tcin-sensitive mutants exhibited high sensitivity to oxidative stress. Oxidant-sensitive 2', 7'-dichlorofluorescin diacetate (DCFH-DA) staining of these mutants revealed that Tcin-induced ROS generation was up to 3-fold higher relative to BY4743. We observed a significant and dose-dependent increase in ROS levels (2-4 folds) in BY4743 treated with DON, T-2 and DAS and found a strong correlation between ROS generation and cell death. Moreover, treatment with antioxidants, such as ascorbic acid and vitamin E increased cell survival 3-4-fold in T-2 treated cells and 6-9-fold in Tcin treated cells, suggesting a direct role for ROS in trichothecene-mediated cell death. Trichothecenes failed to generate ROS in the petite strain lacking mitochondrial DNA (ρ^o) or when mitochondrial membrane potential (ψ_{mito}) was depolarized with the ionophore FCCP (carbonilcyanide p-triflouromethoxyphenylhydrazone), suggesting the mitochondrial origin of trichothecene-induced ROS.

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DOWN WITH DON: STRATEGIES FOR PRECISE TRANSGENE DELIVERY AND RNAI-BASED SUPPRESSION OF *FUSARIUM*

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ABSTRACT

Transgenic strategies can effectively supplement other methods for controlling Fusarium head blight (FHB). Impediments to deploying FHB-resistant transgenic barley include a long time-frame for creating and testing transgenes in barley, imprecise transgene insertions that lead to unstable gene expression, a poor understanding of exactly how to attack *Fusarium*, and negative public perceptions. Advances in *Fusarium* genetics have elucidated the genome sequence and genes critical to pathogenicity. Increasingly detailed knowledge of RNA interference (RNAi)-based gene regulation enables strategies to target specific *Fusarium* genes. The delivery of single copy transgenes using modified *Ds* transposable elements or by site-specific recombination (aka recombinase-mediated cassette exchange, RMCE) enables precise transgene insertion, stable and heritable transgene expression, and production of transgenic plants without bacterial genes or selectable markers. These characteristics should mitigate some of the public concern about transgenic crops. We will use *Ds*-mediated and RMCE for the delivery of transgenes encoding double-stranded RNA (dsRNA) capable of RNAi-based suppression of key *Fusarium* genes involved in virulence and mycotoxin production such as *Tri6*. These transgenes initially will be tested directly in *Fusarium*, to facilitate rapid efficacy assessments. Transgenes showing efficacy in *Fusarium* will be converted to plant transformation vectors and introduced into Conlon. Progress towards these objectives includes: 1) creation of near-isogenic Conlon lines expressing *Ac transposase*, for *Ds*-delivery; 2) design and progress in the construction of *Ds*-delivery backbone vectors; 3) design and construction of the TAG or recombination site platform vector for RMCE; and 4) the regeneration of fertile Conlon plants containing the TAG recombination site.

ACKNOWLEDGEMENT

This material is based upon work supported by the U.S. Department of Agriculture. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

2013 NORTH DAKOTA TRANSGENIC BARLEY RESEARCH AND FHB NURSERY REPORT

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ABSTRACT

Research continues to develop and test new transgenic plants using genes provided by collaborators. As lines are developed in Golden Promise, they are crossed to Conlon for field testing. Transgenic lines developed in Conlon are being crossed to resistant lines developed by the breeding programs. Crosses between Conlon and five Golden Promise transgenic plants carrying different transgenes were begun in 2013. At least two of these lines will be ready for field tests in 2014. Crosses between Quest or ND20448 and two Conlon lines carrying either a tlp or a Tri12 transgene have continued, to determine whether the transgenes add to resistance from the breeding programs. Lines from this program should be ready for testing in 2014. The 2013 North Dakota transgenic field trials consisted of 15 barley lines, tested in three misted and three non-misted replicates. Plots were sown on May 29, 2013 in hill plots with 10 seed per hill spaced at 30 cm, and all plots were inoculated using the grain spawn method at heading. Lines included Conlon, Golden Promise, the resistant checks Quest and CI4196, four transgenic-null pairs derived from crosses between primary Golden Promise transgenics and Conlon, and one Golden Promise primary transgenic-null pair. FHB severity was evaluated approximately three weeks after anthesis, by counting the total and infected number of seed on ten randomly selected spikes per row. DON concentrations in the barley samples were determined by gas chromatography with electron capture detection using the method of Tacke and Casper. FHB and DON data were analyzed by SAS (SAS Institute, Cary, NC) with means adjusted for the nearest checks. Average FHB severity was 10% over all six replicates, 7% in the non-irrigated plots and 12% in the irrigated plots.

ACKNOWLEDGEMENTS

This material is based upon work supported by the US Department of Agriculture. This is a cooperative project with the US Wheat & Barley Scab Initiative. Thanks to Pat Gross, Megan Ramsett, and Cayley Steen for assistance with the field trials.

CHARACTERIZATION OF CEREAL GENES THAT ENHANCE DON/FHB RESISTANCE

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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. These studies have also highlighted a novel, evolutionary divergent protein involved in the wheat response to DON. Transient expression and microscopy studies showed this protein fused to a fluorescent tag localised within punctate areas of the nucleus of wheat cells. Yeast two hybrid and bifluorescent complementation experiments showed that this protein interacts with SnRK1 (SNF1-Related Kinase 1). We are currently characterizing the functional significance of this interaction. The association of a gene with DON resistance does not mean it plays a direct role in resistance or that it can be used to improve DON/FHB resistance. We use virus-induced gene silencing to determine if there is a direct relationship between transcript levels and toxin resistance. Gene silencing of the ABC transporter in wheat led to both enhanced DON tolerance and reduced grain number. Thus, from a breeding perspective, it warrants further investigation in terms of its potential for FHB resistance breeding and its effect on seed number.

DISCOVERY AND REVALIDATION OF SCAB RESPONSIVE GENES IN WHEAT BY 2D-DIGE AND Q-PCR

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ABSTRACT

Fusarium head blight (FHB) or scab, is a disease of economic importance affecting small grain crops every year causing loss of billions of dollars in grain quality and quantity. In wheat, FHB affects the developing heads directly and has been regarded as a severe threat to U.S. and global food security. The molecular mechanisms that underscore the complex disease etiology, leading to the suppression of innate resistance in a susceptible line or keeping on maintaining the resistance levels in a resistant line of wheat, are not well understood. Given the complexity of breeding for FHB resistance/susceptibility, identification of molecular functional markers and the discovery of FHB responsive genes associated with FHB resistance/susceptibility is valuable knowledge in accelerating the efforts to breed FHB resistant wheat cultivars in the coming years. Thus the focus of this project was to seek comprehensive and fundamental knowledge with the discovery of FHB responsive genes in wheat upon *Fusarium* infections. The young heads of near isogenic lines (NILs), resistant (260-2) and susceptible (260-4), were challenged with *Fusarium* and the infected heads were subjected to 2D-DIGE analysis for the identification of *Fusarium* responsive wheat proteins expressed in the wheat head. The selected 80 protein spots displaying significantly differential expression on the gel were cut, trypsin digested and identified through MALDI-TOF mass spectrometry. Further, we have evaluated the effect of scab infection on wheat heads of a resistant NIL and a susceptible NIL at the molecular level. We have analyzed the significantly altered wheat proteins due to scab infection in resistant and susceptible lines respectively. Functional analysis of these altered proteins using the Gene Ontology (GO) suggest unique multiple molecular function, biological process and cellular component involved in the resistant and susceptible near isogenic lines of wheat. This study shows that, there are significant functional differences in the host (wheat) proteins that respond to the pathogen (scab) infection. Selected genes were further validated by an independent experiment (quantitative RT-PCR or QPCR). In this study, combined use of a proteomic platform, QPCR, and GO facilitated a better understanding of wheat-*Fusarium* interactions at cellular levels.

TRANSFER OF FUSARIUM HEAD BLIGHT RESISTANCE FROM *ELYMUS TSUKUSHIENSIS* TO WHEAT VIA A T1AL·1AS-1E^{TS}#1S TRANSLOCATION

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ABSTRACT

Elymus tsukushiensis Honda (2n=6x=42, S^{ts}S^{ts}H^{ts}H^{ts}Y^{ts}Y^{ts}, syn. *Roegneria kamoji* C. Koch) is a perennial cross-pollinating hexaploid species native to China, Korea, and Japan. *E. tsukushiensis* is a distantly related wild relative of bread wheat and a source for resistance to Fusarium head blight (FHB). Previously, we reported the production and characterization of wheat-*E. tsukusiensis* chromosome addition lines and showed that the disomic addition having a group-1 *E. tsukushiensis* chromosome, 1E^{ts}#1, or a wheat-E. *tsukushiensis* translocation chromosome TW·1E^{ts}#1S added to the wheat genome, conferred resistance to FHB.

We used *ph1b*-induced homoeologous recombinantion to produce wheat- *E. tsukushiensis* recombinants. After screening 488 progenies of plants homozygous for *ph1b* and heterozygous for TW·1E^{ts}#1S one interstitial and one distal recombinant were identified. Further analyses revealed that the interstitial recombinant is highly rearranged, of noncompensating type, and, thus, agronomically undesirable. FISH analysis identified the distal recombinant chromosome as T1AL·1AS-1E^{ts}#1S, consisting of the long arm of wheat chromosome 1A, part of the short arm of 1A, and a distal segment derived from 1E^{ts}#1S. T1AL·1AS-1E^{ts}#1S confers type-2 resistance to FHB after point inoculation in a greenhouse test and may be used in cultivar improvement.

RNA SEQUENCING ANALYSES OF TWO BARLEY NEAR-ISOGENIC LINE PAIRS IDENTIFY GENES ASSOCIATED WITH RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Previous studies have identified several quantitative trait loci (QTL) for barley FHB resistance. Two of these QTL are located on chromosome 2H bin8 and 6H bin7. Near-isogenic line (NIL) pairs carrying resistant or susceptible alleles for each of the QTL were developed. The 2H bin8 resistant NIL (Gen1-001) and 6H bin7 resistant NIL (Gen2-036) carry the Chevron allele in the susceptible parent M69 and Lacey backgrounds, respectively. Disease severity, deoxynivalenol concentration and fungal mass were evaluated for each NIL pair. Barley spike samples were collected at 48 and 96 hours after *Fusarium* inoculation and their transcriptomes were analyzed by RNA-Seq. Comparative analyses revealed differential expression profiles within each NIL pair and between the two QTL. A large set of differentially expressed genes (1,247) were identified for the 2H bin8 NILs. When comparing transcriptome profiles of the 2hb8 NILs after *Fusarium* inoculation, Gene Ontology (GO) analyses suggest enrichment in the resistant line of genes encoding proteins with oxidoreductase, glycosyltransferase, cellulose synthase, peptidase and enzyme inhibitor activities. Two hundred and forty-seven differentially accumulated transcripts were identified in the Gen2-036/Lacey NILs after *Fusarium* inoculation. Transcripts induced in Gen2-036 encode proteins functioning in cell wall, ethylene signaling, gibberellin signaling, pathogenesis, proteolysis, protease inhibition, transport and ubiquitination. A common set of transcripts were up-regulated in both Gen1-001 and Gen2-036 when compared with the respective susceptible genotypes, suggesting that certain components were employed by both FHB QTL to confer resistance.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2013 FIELD NURSERY REPORT

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ABSTRACT

The 2013 field screening nursery consisted of 22 wheat and 15 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 Minnesota (16 wheat lines + Bobwhite[*] and CB037[*]), and the USDA (10 barley lines + Conlon[*] and Golden Promise[*]). Lines with known reactions to Fusarium head blight (FHB) were also included as checks. The wheat checks included were the moderately resistant cultivars Alsen, Rollag and Sumai 3 and the susceptible cultivar Wheaton. The barley checks were the moderately resistant cultivar Quest and the susceptible cultivars, Robust and Stander. Individual plots were 2.43 m long single rows. The trial was planted on June 3, 2013. All plots were inoculated twice. The first inoculation was applied at anthesis for wheat (July 16-Aug 2) and at head emergence (July 19-Aug 2) 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 30 *F. graminearum* isolates at a concentration of 100,000 macroconidia.ml^{-1} with Tween 20 (polysorbate) added at 2.5 ml.L^{-1} as a wetting agent. The inoculum was applied using a CO_2-powered backpack sprayer fitted with a SS8003 TeeJet spray nozzle with an output of 10 ml.sec^{-1} at a working pressure of 275 kPa. Mist-irrigation was applied from the first inoculation on July 16 through August 12 to facilitate FHB development. FHB incidence and severity were assessed visually 21 d.a.i. for wheat and around 18 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 27 (barley) and August 21 (wheat). Fifty heads where 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 2013 the disease severities were generally a little higher than in the 2012 nursery. Mean FHB severities for the untransformed wheat checks, Bobwhite and CB037 were 27 and 17 %, respectively. Mean FHB severities for the other standard wheat checks, Alsen, Wheaton, Rollag and Sumai 3, were 11, 33, 7 and 3%, respectively. For barley, the untransformed check variety Conlon had a mean FHB severity of 15%. The untransformed check Golden Promise and one transformant in the Golden Promise background, were very late heading and did not produce seed. The barley standard checks, Quest, Robust and Stander had mean FHB severities of 2, 16 and 24%, respectively. For the wheat entries in the Bobwhite background, the FHB severity data indicated that resistance was significantly expressed ($P<0.05$) in all transformed lines compared to the untransformed Bobwhite check,

with all transformed lines similar to Sumai 3 in response. For the entries with a CB037 background, resistance (FHB severity) was significantly ($P<0.05$) expressed in one transformed line compared to the untransformed background. The FHB severities of all the barley entries in the Conlon background were statistically similar to the untransformed Conlon check, which ranked between Quest and Stander.

ACKNOWLEDGEMENTS AND DISCLAIMER

We would like to acknowledge Beheshteh Zargaran and Julia Hagenguth for their assistance in completion of the work reported. We would also like to acknowledge Dr. Yanhong Dong for conducting the mycotoxin analysis.

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-069. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

MOLECULAR PATHOGENICITY OF WHEAT – *FUSARIUM GRAMINEARUM* INTERACTION

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ABSTRACT

Fusarium graminearum is responsible for Fusarium head blight (FHB) disease in wheat which makes it's end-use quality unsuitable for end-use quality of wheat. A global gene expression study was undertaken to analyze and identify the wheat disease reaction mechanism. The study was conducted using three genotypes of wheat, Japanese landrace Nobeokabouzu-komugi (Highly resistant), cv. Sumai 3 (Resistant) and cv. Gamenya (Susceptible). Florets were inoculated with 10 µl (10^5 conidia / ml) of *Fusarium graminearum* "H-3" strain during the flowering stage. Microarray analysis was carried out by extracting RNA from 3 DAI (days after inoculation) and 7 DAI and using wheat custom array 4x38k. The expression of defense-related genes were more up-regulated in highly resistant landrace Nobeokabouzu-komugi followed by cultivar Sumai 3 and finally by susceptible cultivar Gamenya. Comparing the genotypes, in highly resistant Nobeokabouzu-komugi the expression of up-regulated genes at 3 DAI was more than 7 DAI, while in resistant cultivar Sumai 3 the expression pattern was the opposite, this could be due to a different resistant mechanism. Moreover in susceptible cultivar Gamenya expression was minimal at both time points. The most highly expressed genes in resistant wheats were UDP-glycosyltransferase and Multidrug resistance associated genes, both are genes which encode proteins involved in detoxification process.

TRANSGENIC WHEAT CARRYING A BARLEY UDP-GLUCOSYLTRANSFERASE EXHIBITS HIGH LEVELS OF FUSARIUM HEAD BLIGHT RESISTANCE BY DETOXIFYING TRICHOTHECENES

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ABSTRACT

Fusarium head blight (FHB) is a worldwide disease of wheat and barley, mainly caused by *Fusarium graminearum*. During infection, the fungal pathogen produces trichothecene mycotoxins, such as deoxynivalenol (DON) and nivalenol (NIV), which increase fungal virulence. Moreover, grains contaminated with trichothecenes threaten the health of humans and animals that consume them. Previous work had identified a barley UDP-glucosyltransferase (*HvUGT13248*) gene that exhibited resistance to DON via the conversion to DON-3-O-glucoside (D3G) in transgenic yeast and *Arabidopsis*. We developed transgenic wheat lines constitutively overexpressing the *HvUGT13248* gene in the background of cultivar Bobwhite and CB037. We performed point-inoculation tests in the greenhouse for three seasons (2011 spring, 2011 fall and 2012 spring) and found that transgenic wheat exhibited significantly higher type II resistance compared with the untransformed parental lines. Moreover, in two field tests (2012 and 2013 summer), *HvUGT13248*-overexpressing wheat lines also showed significantly less disease symptoms compared to the untransformed controls. To assess the mechanism of resistance, we treated plants with DON and examined the concentration of DON and D3G from 1-21 days after inoculation. *HvUGT13248*-overexpressing wheat plants converted DON to D3G more rapidly to a higher extent than untransformed plants. We also extended our exploration of the function of *HvUGT13248* gene toward NIV, and found that *HvUGT13248*-overexpressing wheat lines exhibits a high level of type II resistance to a NIV-producing *Fusarium graminearum* strain.

AN *ARABIDOPSIS* NON-SPECIFIC LIPID TRANSFER PROTEIN PROVIDES ENHANCED RESISTANCE TO A TRICHOTHECENE MYCOTOXIN BY REDUCING OXIDATIVE STRESS

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum*, is one of the most important diseases of wheat and barley. Trichothecene mycotoxins, such as deoxynivalenol (DON), are virulence factors of *F. graminearum* and accumulate in the grain causing a serious threat to human and animal health. Current methods for control of FHB have had limited success in reducing disease levels and toxin contamination of small grains. We screened an activation tagged *Arabidopsis* population for resistance to trichothecin (Tcin) and identified two closely linked nonspecific lipid transfer protein (*nsLTP*) genes, which were overexpressed in a resistant line. Treatment of wild type Col-0 with DON or Tcin induced reactive oxygen species (ROS) in leaves as measured by a quantitative Amplex Red assay. Confocal microscopy with 2',7'-dichlorofluorescin diacetate (H2DCF-DA) was used to examine the effect of Tcin on ROS generation. ROS generation was observed in the cell wall/apoplast region of the leaves and clearly colocalized with the chloroplast, suggesting that potential damage to the chloroplast is a source of Tcin-induced ROS in the cell. Treatment of *Arabidopsis* leaves with 2 mM vitamin C, PABA, or vitamin E protected against the toxic effects of Tcin in detached leaf assays, providing further evidence that ROS plays a role in Tcin mediated tissue damage (also see Mohamed Anwar Bin-Umer's abstract/poster). In addition, we found a significant effect of light on Tcin toxicity. Incubation of detached leaves in the dark with Tcin provided the greatest protection from chlorosis and tissue death relative to 16 h light/8 h dark treatments. Previously we have shown that Arabidopsis lines overexpressing two different *nsLTPs* showed reduction in chlorosis and cell death after Tcin treatment and were able to germinate and form roots on medium containing Tcin. Overexpression of *nsLTP*s in *Arabidopsis* and yeast reduced oxidative stress upon trichothecene exposure. *AtLTP* overexpressing lines had significantly attenuated ROS levels upon exposure to Tcin relative to the non-transgenic control. These results demonstrate that ROS production, a component of which is derived from the chloroplast, contributes to the toxicity of trichothecenes in plants and overexpression of an *nsLTP* enhanced trichothecene resistance, possibly by reducing oxidative stress.

QTL-ASSOCIATED ALTERNATIVE SPLICING AND MAPPING OF DIFFERENTIALLY EXPRESSED GENES TO THE WHEAT GENE-OME

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ABSTRACT

Transcriptomic analyses of wheat near-isogenic lines (NILs) segregating for prominent resistance QTL have not yet yielded the causal genes for either *Fhb1* or *Qfhs.ifa-5A*. A main disadvantage of such experiments in wheat is the still unavailable genome of wheat. To date the most complete assembly of the *T. aestivum* gene space is described by the released wheat low-copy-number genome (LCG) assembly, which provides partial sequences of an estimated 94 k genes. In addition, the transcriptome of the close relative barley (*Hordeum vulgare* L.) may be used. However these gene models are either incomplete and lack detailed annotations or do not differentiate between homeoalleles. The IWGSC (International Wheat Genome Sequencing Consortium) has advanced far in the generation of a whole gene-ome map of wheat. We have used these preliminary data as reference for a recent RNA-seq experiment that captured the response of NILs to *Fusarium graminearum* (Kugler et al. 2013). Mapping transcripts that show differences in the *Fusarium* response between lines harboring the resistant or susceptible alleles of either *Fhb1* or *Qfhs.ifa-5A* highlights several transcripts onto the respective regions of the QTL on chromosomes 3BS and 5A. These genes will be selected for further analyses.

With the gene models at hand, we also observed alternative splicing between the reference genome of Chinese Spring, a set of four NILs segregating for both QTL in different combinations and CM-82036, the resistant QTL-donor. Analyses are still ongoing, prelimilary data on *Fhb1* related alternative splicing events will be presented.

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DON INDUCES GENES THAT INCREASE WHEAT SUSCEPTIBILITY TO FUSARIUM HEAD BLIGHT IN WHEAT

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ABSTRACT

To characterize the effect of DON on the molecular defense response of wheat heads to *F. graminearum* infection, RNA profiling was performed using the Affymetrix GeneChip Wheat Genome Array, comparing the response of the FHB-susceptible wheat cultivar Roblin when inoculated with a wild type *F. graminearum* (DON+) strain and a related Tri5- knockout (DON-) strain. Several wheat genes that were specifically induced during infection with the DON+ *F. graminarum* strain were identified. This included two transcription factor genes, an ABC transporter, an AAA-type ATPase, aspartyl-tRNA synthase, gluthatione transferase, and two genes with unknown function. Additional experiments showed that most of those genes were induced directly by a treatment with DON. Further analysis of the DON-modulated genes across our expression profiles database showed a correlation between expression level of many of those genes and susceptibility of the genotypes to FHB. Silencing of one of the transcription factors, a homolog of AtNFXL1, and of the MRP-like ABC transporter in Roblin using a transient assay (VIGS) has showed reduction in infection spread and in head bleaching for both genes. These results support a contribution of DON to FHB-susceptibility in wheat via modulation of gene expression.

ENGINEERING MICROBIAL ELICITORS OF DEFENSE TO PROMOTE RESISTANCE AGAINST *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat and barley. We had previously shown that *Arabidopsis thaliana* provides an excellent model system to study plant defense mechanisms against *Fusarium graminearum*, the principal causative agent of FHB in wheat and barley (Makander et al., 2006, 2010). *F. graminearum* is capable of infecting the leaves and flowers of *Arabidopsis*. In *Arabidopsis*, microbe-derived molecules can stimulate pathogen-triggered immunity (PTI), leading to enhancement of resistance against *F. graminearum*, thus suggesting that the PTI mechanism could be targeted for enhancing resistance against *F. graminearum*. We have engineered one of these defense elicitors for expression in *Arabidopsis* and find that these plants exhibit high level of resistance to leaf and floral inoculation with *F. graminearum*. Transgenic wheat plants expressing the defense elicitor have been generated and will be evaluated for resistance to FHB.

ACKNOWLEDGEMENT AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-3-003 and 59-0790-8-060. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. 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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UDP-GLUCOSYLTRANSFERASES: RESISTANCE TO *FUSARIUM* MYCOTOXINS AND FORMATION OF MASKED MYCOTOXINS

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ABSTRACT

Maximum tolerated levels for the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN) in food commodities have been enacted in the European Union. DON, a virulence factor of *F. graminearum*, is necessary for the spreading of the pathogen from the infection site to other spikelets in the wheat ear. The role of ZEN *in planta* is less clear, but both mycotoxins are inactivated *in planta* by formation of glucosides. We characterized candidate UDP-glucosyltransferases (UGTs) from barley, *Brachypodium*, Sorghum, rice and wheat by heterologous expression in yeast. Several genes encode enzymes with the ability to detoxify DON, and also NIV and other trichothecenes. Interestingly, one barley gene induced by DON had no DON-conjugating activity, but was able to convert ZEN to a mixture of the previously described ZEN-14-*O*-glucoside and the novel ZEN-16-*O*-glucoside. 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). Surprisingly, the detoxification capability and substrate specificity towards different toxins was quite different for products of genes with high sequence similarity. Presumably the diversification of trichothecene structures allows the fungus to escape detoxification by individual UGTs. In turn gene amplification and generation of novel UGT variants by mutation allow the plants to regain resistance. The gene family of *Brachypodium* UDP-glycosyltransferases consists of 178 predicted genes (IPR 002213), 159 of which encode potentially functional proteins. Analysis of the current draft wheat genome sequence indicates that there is even a further expansion of the UGT gene family with estimated 748 genes (many of which are pseudogenes). Due to the lack of conservation of copy number and changes in substrates specificity caused by point mutations it is difficult to predict the relevant orthologous genes responsible for mycotoxin inactivation in different plant species. Functional testing of candidate genes in yeast is warranted.

Since improved detoxification by glycosylation seems to be an option for biotechnological control and is (unintentionally) increased also by breeders by deploying *Fhb1*, the formation of masked mycotoxins may become a relevant issue. The glucosides escape routine detection methods, but they can at least partly be reactivated by glucosidases of intestinal bacteria.

Due to lacking toxicological data the masked forms are not yet considered in a maximum tolerated sum value of mycotoxins. To explore the fate of the masked mycotoxins in animals and to determine toxicity equivalence values, larger amounts of the mycotoxin glucosides are needed for toxicological studies. We expressed a recoded rice UGT gene with a solubility enhancing tag in *E. coli*. The availability of affinity purified active enzyme allows efficient enzymatic production of DON-3-*O*-glucoside, and also of nivalenol-3-*O*-glucoside. Standards for the glucosides of 15-ADON, fusarenone X and HT-2 toxin are in preparation. These compounds should become valuable reference substances to study metabolism of mycotoxins in various plants. They are also a starting point to investigate the fate of glucosides, which are further metabolized *in planta*.

Recently a novel detoxification mechanism for DON was demonstrated using stable isotope labeled DON, the formation of glutathione adducts and processing products derived from it (Kluger *et al.*, 2012). Methylthio-DON (MT-DON) - which was first reported as S-methyl-DON by Kushalappa et al. at the 2010 National Fusarium Head Blight Forum - is proposed to be generated *in planta* from the DON-glutathione conjugate processing product DON-cysteine by cysteine-S-conjugate-beta-lyase and subsequent methylation of DON-SH. Methylthio-DON was chemically synthesized and used to test its toxicity. The MT-DON concentration required for 50% growth inhibition of a sensitive yeast bioindicator strain was about 9-fold higher than for DON. Tests of the ability to inhibit protein synthesis of wheat ribosomes (using a wheat germ *in vitro* translation system) showed that MT-DON is at least 12-fold less toxic than DON. This strongly indicates that addition of the much larger cysteine and glutathione substituents should also lead to reduced toxicity due to steric hindrance preventing interaction of the toxin-conjugate with the ribosomal target.

Conjugation with glutathione and glycosylation target different parts of DON, it should therefore be possible to combine increased detoxification capability of different cultivars by knowledge based plant breeding.

ACKNOWLEDGEMENTS AND DISCLAIMER

This work was funded by the Austrian Science fund (FWF) special research project “Fusarium” (F3706 and F3708), the Vienna Science and Technology Fund WWTF (LS12-021) and the graduate school program Applied Bioscience Technology of the Vienna University of Technology in cooperation with the BOKU Vienna. This material is also based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0206-9-073 (GM) and 59-0206-1-117 (IR). This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

TARGETING HOST DEFENSE AND SUSCEPTIBILITY MECHANISMS FOR ENGINEERING FHB RESISTANCE

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ABSTRACT

The interaction between *Arabidopsis thaliana* and *Fusarium graminearum* provides an excellent system to identify plant genes/mechanisms that govern resistance and susceptibility to *F. graminearum* (Makandar et al., 2006, 2010). We have targeted several of these genes/mechanisms for enhancing Fusarium head blight resistance in wheat. One of the defense mechanisms that has been successfully targeted for enhancing resistance against *F. graminearum* is systemic acquired resistance, which confers resistance against a broad-spectrum of pathogens. Host lipid metabolism genes involved in resistance and susceptibility have also been targeted for promoting disease resistance. A new approach under development involves utilizing a microbe-derived elicitor of plant defense for enhancing resistance against this fungus.

ACKNOWLEDGEMENTS AND DISCLAIMER

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0200-3-003 and 59-0790-8-060. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. 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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EXPRESSION QTL MAPPING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

To gain insights into the wheat response to Fusarium Head Blight (FHB) and uncover genes linked to known resistance QTL, we performed an expression quantitative trait loci (eQTL) study. eQTL studies allow for mapping of transcript abundances on the genetic map in order to infer genes that are involved in defense against FHB.

Our study captured expression profiles of 60k genes (Agilent wheat microarray) at two time points (30 and 50 hours) after inoculation with *Fusarium graminearum* spores from a population of 200 doubled haploid lines (CM-82036 x Remus). These segregate for the prominent resistance QTL *Fhb1* and *Qfhs. ifa-5A*. We found 400 genes at 30 hai and 5,000 genes at 50 hai differentially expressed (fold change >2, *adj.p*≤0.05) between the parental lines. We used a part of these to generate transcript derived markers (TDMs), which were used together with SSR and AFLP markers from a previous study (Buerstmayr et.al 2003) to improve the existing genetic map. Reanalysing the phenotypic data confirmed two major QTL (*Fhb1, Qfhs.ifa-5A*) and identified a novel QTL located on chromosome 6A. eQTL mapping for expression data revealed 14,994 and 13,116 significant eQTL at 30 and 50 hai, respectively. Distribution of these eQTL across the genetic map allowed us to identify eQTL that corresponded to phenotypic QTL (*Fhb1, Qfhs.ifa-5A* and 6A) and to hotspots. These 8 hotspots (which comprise between 350 to1900 genes) potentially encode for regulatory elements that govern the response to *F. graminearum*. To gain further insights into the activity of these hotspots and QTL we are currently working on GO enrichment analyses of the co-regulated genes. The results will be presented. Cis and trans-eQTL are defined based on distance of the eQTL from physical position of the respective gene. They are determined by plotting the physical position of eQTL against the genetic position of the TDMs. We mapped 1,500 eQTL, of which the majority (80 %) comprises trans-eQTL at either time points. Few cis-eQTL linked for instance to *Fhb1* are interesting candidates for further studies.

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