SESSION 5:

GENE DISCOVERY AND ENGINEERING RESISTANCE

Chairperson: Nilgun Tumer

LIPID TRANSFER PROTEIN-MEDIATED RESISTANCE TO A TRICHOTHECENE MYCOTOXIN – NOVEL PLAYERS IN FHB RESISTANCE

Anwar Bin Umer[1], John McLaughlin[1], Susan McCormick[2] and Nilgun Tumer[1*]

[1]Department of Plant Biology and Pathology, S.E.B.S, Rutgers University, New Brunswick, NJ; and [2]Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS-NCAUR, Peoria, IL
[*]Corresponding Author: PH: 848-932-6359, Email: tumer@aesop.rutgers.edu

ABSTRACT

Lipid transfer proteins are a class of basic cysteine rich proteins characterized by an eight cysteine motif backbone with intrinsic antimicrobial activities against bacterial and fungal pathogens. Previously, we identified two type IV nonspecific lipid transfer protein (nsLTP) genes (LTP4.4 and LTP4.5) from screening 250,000 activation-tagged *A.thaliana* seeds. Overexpression of both genes enhanced resistance to trichothecin. We set up yeast as a model system to investigate the mechanism by which the LTP4.4 and LT4.5 mediate resistance to trichothecenes. LTP4.4 and LTP4.5 expression conferred resistance to 2µM and 3µM Tcin in yeast. LTP4.4 provided a greater level of resistance than LTP4.5. In contrast, expression of a different nsLTP (LTP 1.1) did not provide resistance to trichothecin. Moreover, expression of LTP4.4 and LTP4.5 did not provide any resistance to other translation inhibitors, such as cycloheximide, anisomycin or chloramphenicol. These results suggest that resistance to trichothecenes is not a general response, but a feature unique to LTP4.4 and LTP4.5.

Cell fractionation assays showed that while LTP4.4 remained largely in the cytosol, LTP4.5 was primarily associated with the membrane fractions, suggesting a difference in localization of the two nsLTPs. To explore the mechanism of nsLTP-mediated resistance to Tcin, we investigated the effects of Tcin on cytosolic and mitochondrial translation, two known targets of trichothecenes. Cytosolic translation was inhibited significantly (>65%), but mitochondrial translation was inhibited only minimally (<23%) by Tcin in cells overexpressing LTP4.4 and LTP4.5 relative to cells transformed with the vector alone. Reactive oxygen species (ROS) generation, an early time point event during trichothecene toxicity, was also alleviated in yeast overexpressing LTP4.4 and LTP4.5 with less than 2% of the cells generating any significant ROS at 2µM and 3µM Tcin. Taken together, these results suggest a likely role for mitochondria in nsLTP-mediated resistance to trichothecene toxicity.

2012 NORTH DAKOTA TRANSGENIC BARLEY FHB NURSERY REPORT

Lynn S. Dahleen[1*], Robert Brueggeman[2], Tilahun Abebe[3] and Ron Skadsen[4]

[1]USDA-ARS, Fargo, ND; [2]Dept. of Plant Pathology, North Dakota State University, Fargo, ND;
[3]Dept. of Biology, University of Northern Iowa, Cedar Falls, IA; and [4]USDA-ARS, Madison, WI
[*]Corresponding Author: PH: 701-239-1384, Email: lynn.dahleen@ars.usda.gov

ABSTRACT

The 2012 North Dakota transgenic field trials consisted of 23 barley lines, tested in three misted and three non-misted replicates. Plots were sown on May 9, 2012 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, the resistant checks Quest and CI4196, twelve primary transgenic lines derived from Conlon, and four transgenic-null pairs derived from crosses between primary Golden Promise transgenics and Conlon. 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 16% over all six replicates, 9% in the non-irrigated plots and 23% in the irrigated plots. Average DON contamination was 5.1ppm over all plots with 1.9ppm in the non-irrigated plots and 8.5ppm in the irrigated plots. Standard deviations for DON among the replicates were extremely high, reducing the power of comparisons. These lines will be analyzed again in the 2013 transgenic FHB nursery.

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PROTEOMIC DISSECTION OF NEAR ISOGENIC LINES FOR THE DISCOVERY OF SCAB RESPONSIVE GENES IN WHEAT

Moustafa Eldakak[1], Ansuman Roy[1], Yongbin Zhuang[2], Karl Glover[2], Shaukat Ali[2], Yang Yen[1] and Jai S. Rohila[1,2*]

[1]Department of Biology & Microbiology, and [2]Department of Plant Science, South Dakota State University, Brookings, SD 57007
[*]Corresponding Author: PH: (605) 688-4453, Email: jai.rohila@sdstate.edu

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 keep on maintaining the resistance levels in a resistant line of wheat, caused by scab are not well understood. Given the complexity of breeding for FHB resistance/susceptibility, a very first step is to get the fundamental and comprehensive knowledge for FHB responsive wheat functional proteins after *Fusarium* infection. This knowledge should accelerate the wheat breeding efforts to develop FHB resistant wheat cultivars. In the present investigation, we have evaluated the effect of scab infection on wheat heads of a resistant near isogenic line (NIL) and a susceptible NIL at cellular levels. The young heads of the two 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 proteins in wheat. A total of 80 protein spots were recorded displaying significantly differential expression on polyacrylamide gel. These protein spots were cut, trypsin digested and the protein was identified through MALDI-TOF mass spectrometry. Further, using the Gene Ontology (GO), functional pathways of these altered proteins discovered several up- and down- regulated biological processes and plant cellular components and organelles of wheat. Analysis of the various pathways affected in wheat plants by the *Fusarium* infection is done. This study shows that, there are significant functional differences in the regulation of host proteins that could be a cause and/or result of a successful or a defeated scab infection. In this study, combined use of a proteomic platform and GO analysis facilitated a better understanding of host-pathogen interactions at cellular levels.

A NOVEL SOURCE OF FUSARIUM HEAD BLIGHT RESISTANCE DERIVED FROM *ELYMUS TSUKUSHIENSIS*

B. Friebe[1*], J. Cainong[1], P. Chen[2], W.W. Bockus[3], and B.S. Gill[1]

[1]Wheat Genetic Resources Center and Department of Plant Pathology, Kansas State University; [2]The National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing Jiangsu, PR China; and [3]Department of Plant Pathology, Kansas State University
[*]Corresponding Author: PH: (785) 532-2364, Email: friebe@ksu.edu

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 recombination to produce wheat- *E. tsukushiensis* recombinants. The screening of 488 progenies of plants homozygous for *ph1b* and heterozygous for TW·$1E^{ts}$#1S identified one distal TWL·WS-$1E^{ts}$#1S and one interstitial TiWL·WS-$1E^{ts}$#1S-WS recombinant. Stocks homozygous for both recombinant chromosomes were recovered, conferred type-2 resistance to FHB after point inoculation in a greenhouse test, and may be used in cultivar improvement.

ETHYLENE-SIGNALING IS ESSENTIAL FOR BASAL RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

Megan E. Gillespie[1], Amanda S. Brandt[1] and Steven R. Scofield[1,2*]

[1]Department of Agronomy, Purdue University, 915 West State Street, West Lafayette, IN, 47907-2054; and [2]USDA-ARS, Crop Production and Pest Control Unit, 915 West State Street, West Lafayette, IN, 47907-2054
[*]Corresponding Author: PH: 765-494-3674, Email: scofield@purdue.edu

ABSTRACT

The role of ethylene (ET)-signaling in the mechanism of resistance to Fusarium head blight (FHB) has been controversial. Expression profiling analyses have identified induction of ET-signaling within the first 6-hours after challenge by *Fusarium graminearum* as a significant event correlated with resistance. However, another study employing an *Arabidopsis* model system and experiments in wheat concluded that ET-signaling was essential for FHB susceptibility. To further address this controversy we employed a virus-induced gene silencing (VIGS) system to silence genes required for ET-biosynthesis and signaling in an FHB resistant wheat genotype. These studies indicated that silencing genes encoding either S-adenosyl-methionine synthetase or an ET-responsive transcription factor, TaERF7-1, caused FHB resistant plants to become susceptible. These results were confirmed by methods independent of VIGS. Inhibition of ET-receptors by treatment with 1-methylcyclopropene caused FHB resistant plants to become susceptible and susceptible genotypes to develop significantly increased disease. Conversely, treatment of FHB susceptible plants with the chemical precursor of ET, 1-aminocyclopropane-1-carboxylic acid (ACC), at a concentration sufficient to induce the expression of TaERF7-1, resulted in increased resistance to FHB. The fact that these studies indicate manipulation of ET signaling affects FHB interactions of both resistant and susceptible genotypes indicates that ET signaling is controlling a component of basal defense. Taken together, these results strongly support ET-signaling as having an essential role in activating basal defense against FHB in wheat.

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

Anna Hofstad[1], Haiyan Jia[1], Benjamin P. Millett[1], Eduard Akhunov[2] and Gary J. Muehlbauer[1*]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108; and [2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506
[*]Corresponding Author: PH: 612-624-2755, Email: muehl003@umn.edu

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 found the lines to be 98% identical. Of the SNPs that could be mapped, 132 SNPs map to the 3BS region and 40% of the total SNP differences were found on chromosome 3BS. We established three experiments to examine gene expression in the NIL pair and to identify candidates for the *Fhb1* gene. We used next-generation sequencing of RNA from the following experiments to obtain the gene expression data: (1) point inoculation of spikelets with *Fusarium graminearum* and sampling the inoculated spikelets at 96 hours after inoculation; (2) point inoculation of deoxynivalenol (DON) or sterile water and sampling the inoculated spikelets at 12 hours after inoculation; and (3) point inoculation of *F. graminearum* and sampling of the rachis at 96 hours after inoculation. For all experiments at least 100 million sequencing reads were obtained for each genotype. For all analyses, differential expression is defined with at least a 2-fold change in expression and a q-value less than 0.05. For experiment #1, we identified 5,973 sequences that showed differential expression between the two genotypes. For experiment #2, we identified 2,210 sequences that showed differential expression between the two genotypes when DON inoculated and 866 sequences that showed differential expression when water inoculated. For experiment #3, we identified 4,771 sequences that showed differential expression between the two genotypes. We also mapped the sequencing reads from experiment #1 and experiment #3 to the *F. graminearum* transcripts to identify genes that are being expressed by the fungus during infection. For experiment #1, we identified 137 transcripts that showed differential expression between the two genotypes. For experiment #3, we identified 419 transcripts that showed differential expression between the two genotypes. Results of the differentially expressed genes will be presented.

IDENTIFICATION AND CHARACTERIZATION OF BARLEY GENES THAT PROVIDE RESISTANCE TO TRICHOTHECENES

Yadong Huang[1], Sanghyun Shin[1], Benjamin P. Millett[1], Xin Li[1], Gerhard Adam[2], Susan McCormick[3], Kevin P. Smith[1], Brian J. Steffenson[4] and Gary J. Muehlbauer[1*]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA; [2]Center of Applied Genetics, BOKU-University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria; [3]Mycotoxin Research Unit, USDA-ARS-NCAUR, Peoria, IL 61604, USA; and [4]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
[*]Corresponding Author: PH: (612) 624-2755; E-mail: muehl003@umn.edu

ABSTRACT

Developing transgenic barley and wheat cultivars with increased trichothecene resistance is an important strategy to cope with Fusarium head blight (FHB). We have previously conducted several RNA microarray experiments using barley spikes inoculated with *Fusarium graminearum* or deoxynivalenol (DON). A number of up-regulated transcripts were identified and selected for further characterization. These genes are potentially involved in the metabolism or transport of trichothecenes, which include UDP-glucosyltransferases, glutathione-S-transferases, cytochrome P450s, ABC transporters and an epoxide hydrolase. We used a rapid *in planta* assay to test their efficacy against trichothecenes by overexpressing them in *Arabidopsis thaliana* and growing transgenic plants on media containing trichothecenes. The HvUGT13248 gene has been shown to confer resistance to DON in yeast, Arabidopsis and wheat. By re-sequencing and association analysis, a single nucleotide polymorphism (SNP) which causes a nonsynonymous mutation in the conserved substrate binding domain of HvUGT13248 was identified to be associated with FHB susceptibility. Transgenic Arabidopsis plants overexpressing this HvUGT13248 allele, as well as other aforementioned genes, are being tested on DON media and the results will be reported.

TRANSPOSON MUTAGENESIS FOR IDENTIFICATION OF STRESS RESPONSIVE GENES IN CEREALS

Ravneet Kaur, Surinder Singh and Jaswinder Singh*

Plant Science Department, McGill University, Ste Anne De Bellevue, QC, H9X3V9, Canada
*Corresponding Author: PH: (514) 398-7906, Email: Jaswinder.Singh@mcgill.ca

ABSTRACT

Wheat and Barley are two major food grain crops around the globe, which feed a large population of the world. Fusarium head blight (FHB) is an epidemic disease of wheat and barley causing heavy economic losses to farmers due to yield decreases. Mycotoxin produced by *Fusarium* makes these useless for flour and malt products. Varieties resistant to FHB are a matter of high priority in many areas where they are grown, but the complex nature of resistance makes this a highly challenging task. In barley, two major quantitative trait loci's (QTL) have been identified viz. QTL1 and QTL2 on chromosome 6H and 2H respectively which have a large effect on kernel discoloration. The resistant allele of QTL2 decreases the occurrence of head blight by nearly 50% in varieties in which it is present thus proving its importance. Efforts have been made to clone important QTL for better understanding of the mechanisms involved for FHB tolerance. Maize *Ac/Ds* system is one of the important tools that can be utilized for dissecting and saturating QTL through saturation mutagenesis. Previous and ongoing mapping studies in our lab indicate an added advantage of *Ds* transpositions, in gene rich linked positions; making this technique appropriate to dissect FHB QTL. Currently, our main focus is to saturate QTL2 region using maize *Ds* elements eventually facilitating identification and characterization of genes associated with FHB resistance. Plants with single *Ds* insertions (TNPs), mapping near QTL of interest are important vehicles for gene identification through re-activation and transposition of *Ds*. Recently we have reported that the frequency of Ds reactivation is higher using *in-vitro* transformation methods as compared to conventional breeding. Thus, *Ds* elements from TNP 41 (mapped near QTL2) will be re-activated by transforming the immature embryos with construct containing *Ac*TPase. The purpose is to identify phenotypes, morphology of which may be associated with FHB tolerance.

GENETIC AND GENOMIC APPROACHES FOR MANAGING *FUSARIUM* PATHOGENS CAUSING HEAD BLIGHT AND CROWN ROT IN WHEAT

Kemal Kazan*, Tim Fitzgerald, Chunji Liu, Mick Ayliffe and John Manners

CSIRO Plant Industry, Queensland Bioscience Precinct, St Lucia, Brisbane, 4067, Queensland, Australia
*Corresponding Author: PH: 61-7-3214-2678, Email: kemal.kazan@csiro.au

ABSTRACT

Fusarium head blight and crown rot are globally important fungal diseases of cereal crops such as wheat and barley. In Australia, these diseases are caused by two related *Fusarium* pathogens: *Fusarium graminearum* and *F. pseudograminearum*. We are employing genetic and genomic approaches to minimize crop losses by these pathogens through an improved understanding of pathogen biology and host plant resistance. In this talk, an overview of the work being conducted in CSIRO Plant Industry, Australia will be presented. Firstly, to better understand the weaponry used by the pathogen, we are comparatively analyzing the genomes of cereal infecting *Fusarium* spp. This topic will be covered in detail in *Pathogen Biology and Genetics* session of the Forum. Secondly, we are employing forward and reverse genetic approaches in wheat and Brachypodium to identify host factors that promote disease resistance or susceptibility. In particular, we are exploring the inactivation of susceptibility genes as a strategy to achieve novel resistance. Initially, wheat lines lacking selected putative susceptibility genes were identified within a mutant population and are currently being tested for disease resistance. Thirdly, quantitative trait loci (QTL) that confer *Fusarium* resistance in wheat are being identified and incorporated into elite germplasm. Efforts are also underway to clone and study the mode of action of these QTL. Finally, we have developed a high-throughput wheat transformation technique that allows testing the efficacy of large number of transgenes rapidly. The complementary approaches being explored should assist with development of wheat germplasm that is highly resistant to *Fusarium* spp.

TESTING TRANSGENIC SPRING WHEAT AND BARLEY LINES FOR REACTION TO FUSARIUM HEAD BLIGHT: 2012 FIELD NURSERY REPORT

Koeritz, E.J.[1], Elakkad, A.M.[1], Dahleen, L.S.[2], Skadsen, R.[6], Abebe, T.[7], Shah, J.[3], Nalam, V.J.[3], Klossner, G.[3], Tumer, N.[4], Di, R.[4], Muehlbauer, G.J.[5], Li, X.[5], Shin, S.[5] and Dill-Macky, R.[1*]

[1]Department of Plant Pathology, University of Minnesota, St. Paul, MN; [2]USDA-ARS, RRVARC, Fargo, ND; [3]Department of Biological Sciences, University of North Texas, Denton, TX; [4]Department of Plant Biology and Plant Pathology, Rutgers University, New Brunswick, NJ; [5]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN; [6]USDA-ARS, Madison, WI; and [7]Department of Biology, University of Northern Iowa, Cedar Falls, IA

[*]Corresponding Author: PH: (612) 625-2227, Email: ruthdm@umn.edu

ABSTRACT

The 2012 field screening nursery consisted of 42 wheat and 24 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 (5+1 wheat), Rutgers University (7+1 wheat), the University of Minnesota (19+5 wheat), and the USDA (20+1 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, Robust and Stander. Individual plots were 2.4 m long single rows. The trial was planted on May 22, 2012. All plots, except a non-inoculated Wheaton check, were inoculated twice. The first inoculation, 6 July 2012, 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 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 CO2-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 before the first inoculation on July 5 through July 25 to facilitate FHB development. FHB incidence and severity were assessed visually 19 dai for wheat and 14 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 13 (barley) and August 22 (wheat). Fifty (barley) and 50 (wheat) heads where harvested from each plot, threshed and the seed cleaned manually. The wheat sub-samples were 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. The data indicated that resistance was expressed in some of the transformed lines.

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TRANSGENIC WHEAT CARRYING A BARLEY UDP-GLUCOSYLTRANSFERASE EXHIBIT HIGH LEVELS OF FUSARIUM HEAD BLIGHT RESISTANCE

Xin Li[1], Sanghyun Shin[1,7], Ruth Dill-Macky[3], Franz Berthiller[4], Thomas Clemente[5], Susan McCormick[6] and Gary Muehlbauer[1,2*]

[1]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA; [2]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108, USA; [3]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA; [4]Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna, 3430 Tulln, Austria; [5]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68588, USA; [6]USDA-ARS, Bacterial Foodborne Pathogen and Mycology Research Unit, Peoria, IL 61604, USA; and [7]Present address: National Institute of Crop Science (NICS), RDA, Suwon 441-857, Korea

[*]Corresponding Author: PH: (612) 624-2755, Email: muehl003@umn.edu

ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat and barley, mainly caused by *Fusarium graminearum*, leading to huge economic losses worldwide. During infection, the fungal pathogen produces trichothecene mycotoxins, such as deoxynivalenol (DON), that increase fungal virulence. Moreover, grain products contaminated with trichothecenes threatens 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 field tests HvUGT13248-overexpressing wheat lines also showed significantly less disease severity compared to the untransformed controls. To assess the mechanism of resistance, we inoculated 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 Bobwhite, indicating that the barley HvUGT13248 gene provides FHB type II resistance in transgenic wheat by converting DON to D3G.

ACTIVATION TAGGING IN *ARABIDOPSIS* IDENTIFIES TWO NOVEL NON-SPECIFIC LIPID TRANSFER PROTEINS WHICH PROVIDE ENHANCED RESISTANCE TO A TRICHOTHECENE MYCOTOXIN

John E. McLaughlin[1], Mohamed Anwar Bin-Umer[1], Thomas Widiez[1], Emily Salmon-Denikos[2], Debaleena Basu[1], Susan McCormick[3], Brian Gregory[2] and Nilgun E. Tumer[1*]

[1]Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University, New Brunswick, NJ; [2]Department of Biology, University of Pennsylvania, Philadelphia, PA; and [3]Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS-NCAUR, Peoria, IL
[*]Corresponding Author: PH: 848-932-6359, E-mail: tumer@aesop.rutgers.edu

ABSTRACT

Trichothecene mycotoxins are potent virulence factors produced by *Fusarium graminearum* during wheat and barley infection leading to the development and spread of Fusarium Head Blight (FHB). They inhibit cytosolic and mitochondrial protein synthesis in addition to having other complex cytotoxic effects. A genetic screen in *Arabidopsis* was undertaken to identify genes that provide resistance to trichothecenes. Approximately 250,000 activation tagged M2 generation *Arabidopsis* seeds were screened for resistance to trichothecin (Tcin), a type B trichothecene, and several lines from this population were identified that showed resistance. These plants were able to form roots on 4 μM Tcin, a concentration which severely restricts root initiation and elongation of the Col-0 wild type following germination. Characterization of one of these resistant lines using qRT-PCR identified an activation genotype, *Arabidopsis thaliana resistant root formation1* (*AtTRRF1)*. In *AtTRRF1*, two closely linked novel non-specific lipid transfer protein (nsLTP) genes, *LtpIV.4* and *LtpIV.5*, were found to be overexpressed compared to the wild-type control. Both proteins are classified as type IV nsLTPs, a largely uncharacterized class of nsLTPs with limited structural and functional information. Overexpression of both *LtpIV.4* and *LtpIV.5* independently in *Arabidopsis* confirmed resistance to trichothecin based on differences in the ability to form roots when grown on solid media containing 4 μM Tcin. Overexpression of *LtpIV.4* in *Arabidopsis* induced a high level (72.7% ± 10.5) of tolerance, as measured by the percentage seedlings that develop roots when grown on media containing 4 μM Tcin, as compared with control plants (5.6 ± 2.7). Overexpression of *LtpIV.5* induced a moderate level (58.6% ± 11.0) of tolerance. Expression of *LtpIV.4:GFP* and *LtpIV.5:GFP* was examined by transient expression in tobacco leaves and in the transgenic *Arabidopsis* lines by confocal microscopy. The GFP tagged *LtpIV.4* and *LtpIV.5* both localized near the cell wall, suggesting that they are likely targeted to the apoplast. In addition, coexpression analysis using an ER-mcherry marker indicates that *LtpIV.4* may also localize to the ER. This study identified two novel nsLTPs which function to provide enhanced resistance to a trichothecene mycotoxin in plants.

DEVELOPING FUSARIUM HEAD BLIGHT RESISTANT WHEAT

Muehlbauer, G.J.[1,2*], S. Shin[2], X. Li[2], J. Boddu[2], S. Heinen[2], J.A. Torres-Acosta[3], M.P.K. Paris[3], W. Schweiger[4], T. Clemente[5], R. Dill-Macky[6], S. McCormick[7], M. Lemmens[8], F. Berthiller[8] and G. Adam[3]

[1]Department of Plant Biology, University of Minnesota, St. Paul, MN 55108; [2]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108; [3]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, 3430 Tulln, Austria; [4]Department for Agrobiotechnology - IFA-Tulln, Institute for Biotechnology in Plant Production, University of Natural Resources, 3430 Tulln, Austria; [5]Department of Agronomy and Horticulture, University of Nebraska, Lincoln, NE 68588; [6]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; [7]USDA-ARS, Bacterial Foodborne Pathogens and Mycology Research Unit, Peoria, Illinois 61604, USA; and [8]Department for Agrobiotechnology - IFA-Tulln, Center for Analytical Chemistry (Christian Doppler Laboratory for Mycotoxin Metabolism), University of Natural Resources, 3430 Tulln, Austria

[*]Corresponding Author: PH: 612-624-2755, Email: muehl003@umn.edu

ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a major disease problem in wheat and barley around the world. During infection, *F. graminearum* produces a host of trichothecene mycotoxins, such as deoxynivalenol (DON), that act as virulence factors and cause a reduction in grain quality. Therefore, developing approaches to detoxify trichothecenes will serve the dual function of increasing disease resistance and improving grain quality. Numerous gene expression experiments were conducted to identify genes that are differentially expressed in barley and wheat after *F. graminearum* inoculation or treatment with DON. Previous work in *Arabidopsis thaliana* has shown that a UDP-glucosyltransferase can detoxify trichothecenes. We identified a set of barley UDP-glucosyltransferases that exhibited homology to the *Arabidopsis* gene. Examining these genes resulted in the identification of the barley UGT (*HvUGT13248*) gene that provided resistance to DON in yeast and *Arabidopsis*. Resistance to DON was shown to be via conjugation of DON with UDP glucose to form DON-3-O-glucoside (D3G). Transgenic wheat overexpressing *HvUGT13248* were developed in the FHB susceptible Bobwhite and CB037 backgrounds. These transgenics exhibited a statistically significant increase in type II resistance compared to the nontransgenic controls. In several of the lines the level of type II resistance was equivalent to that observed in the Sumai3 genotype. Field screening of the transgenic wheat showed a statistically significant decrease in disease severity compared to non transgenic controls. As in yeast and *Arabidopsis*, resistance in the transgenic wheat appears to be via conversion of DON to D3G. Our results show that developing wheat with the increased capacity to detoxify DON results in increased FHB resistance.

EXPRESSION-QTL MAPPING IN WHEAT TO IDENTIFY REGULATORY HOTSPOTS INVOLVED IN THE RESISTANCE TO *FUSARIUM GRAMINEARUM*

M. Samad-Zamini, W. Schweiger*, E. Sam, G. Siegwart, B. Steiner, M. Lemmens and H. Buerstmayr

BOKU-University of Natural Resources and Life Sciences Vienna, Department IFA-Tulln, Institute for Biotechnology in Plant Production, Konrad Lorenz Str. 20, A-3430 Tulln, Austria
*Corresponding Author: PH: 43-2272-66280-205, Email: wolfgang.schweiger@boku.ac.at

ABSTRACT

Expression QTL studies provide a technique to identify genetic markers (transcript derived marker, TDM), which can be used to generate a genetic map and subsequently identify eQTL based on differences in transcript abundance. This approach allows validating reported phenotypical QTL and simultaneously identifying candidate genes that are encoded or governed by these QTL.

In wheat more than 200 QTL have been reported to contribute to resistance against Fusarium head blight. Most are minor contributors, although (prominently *Fhb1,* located on 3BS and *Qfhs.ifa-5A* on 5AS) explain up to 25 % of the observed resistance. To date, none of these QTL has been cloned and characterized. To characterize reported and newly identified QTL on the transcriptome level, we performed an eQTL study using a population of 200 doubled haploid (DH) lines segregating for *F. graminearum* resistance. This population derives from the resistant line CM82036 (derived from Sumai 3) and the susceptible European spring wheat cultivar Remus. Six central spikelets were inoculated with a Fusarium spore suspension at anthesis and samples were taken at two time points after inoculation. RNA was hybridized onto a custom-build microarray (Agilent 8x60k), comprising 44.000 wheat genes, several hundred wheat candidate genes, that have been reported responsive to *Fusarium* in literature and the entire transcriptome of *Fusarium graminearum* (ca. 14.000 genes). In total, we hybridized about 500 microarrays. We detected 2240 transcripts that show differential abundance between the parental lines. These transcripts and differential transcripts that are not differentially accumulating between the parental lines (due to transgressive segregation) are used as markers. At the time of abstract submission data analysis and QTL analysis as well as the identification of cis- and trans-regulated eQTL corresponding to each time point are still ongoing. These results, the identified regulative hotspots and related genes are presented.

FUNCTIONAL GENOMICS OF UDP-GLUCOSYLTRANSFERASES: HETEROLOGOUS EXPRESSION IN YEAST TO TEST FOR DEOXYNIVALENOL DETOXIFICATION CAPABILITY OF CANDIDATE GENES

W. Schweiger[1*], M.P. Kovalsky Paris [2], G. Wiesenberger [2], F. Berthiller [3], M. Lemmens[1], S. Shin[4], J.A. Torres Acosta[2], G.J. Muehlbauer[4] and G. Adam[3]

[1]Institute for Biotechnology in Plant Production, [2]Department of Applied Genetics and Cell Biology, [3]Center for Analytical Chemistry, Department of Agrobiotechnology, BOKU-University of Natural Resources and Life Sciences Vienna, IFA Tulln, 3430 Tulln, Austria; and [4]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

[*]Corresponding Author: PH: 43-2272-66280-205, Email: wolfgang.schweiger@boku.ac.at

ABSTRACT

The plant pathogenic fungus *Fusarium graminearum* produces the trichothecene toxin deoxynivalenol (DON). This protein biosynthesis inhibitor has been shown to be a virulence factor in wheat required for spread of infection. Resistance to the spreading of *Fusarium* is mediated by the ability of the plant to glucosylate DON into the non-toxic conjugate DON-3-O-glucoside by UDP-glucosyltransferases (UGT). The first gene encoding an enzyme with this activity from a monocotyledonous plant was recently described (HvUGT13248, Schweiger *et al.* 2010). Since the genomes of both wheat and barley have not yet been fully sequenced, we investigated the homologs of HvUGT13248 in the sequenced genome of the monocot model species *Brachypodium distachyon* (Bd) and the genomes of rice and sorghum. *Brachypodium* is closely related to wheat and barley and should therefore provide an insight into the architecture of wheat and barley UGTs. The gene family of Bd UGTs consists of 177 predicted genes. We characterized the cluster of six Bd UGTs with the highest amino acid sequence similarity to HvUGT13248 by expression in yeast. Only two of the candidate Bd UGTs were able to glucosylate DON to DON-3-O-glucoside. Also from the cluster of rice genes only one gene is capable of detoxifying DON. This is also the case for the single copy gene from *Sorghum*. Seemingly, the UGT genes undergo rapid evolution, and due to different copy numbers in gene clusters it is difficult to identify true orthologues.

Overexpression of a DON glucosylating UGT should increase DON resistance in plants. Yet, UGTs have also been shown to be capable of altering the activity of plant hormones, such as brassinosteroids, by glucosylation, which in turn leads to dwarfing. We tested HvUGT13248 and the two DON detoxifying Bd UGTs for their ability to glucosylate the brassinosteroid castasterone in a yeast assay. No castasterone-glucoside formation was detectable in yeast expressing the three candidate UGT genes. Overexpression of the barley HvUGT13248 gene in *Arabidopsis thaliana* led to increased DON resistance of seedlings without unwanted side effects such as dwarfing (Shin *et al*., 2012).

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TRANSCRIPTOMIC CHARACTERIZATION OF THE *FUSARIUM* RESISTANCE QTL *FHB1* AND *QFHS-IFA.5A*

W. Schweiger[1*], B. Steiner[1], C. Ametz[1], G. Siegwart[1], G. Wiesenberger[4], F. Berthiller[2], M. Lemmens[1], H. Jia[3], G. Adam[4], G.J. Muehlbauer[3], D.P. Kreil[5] and H. Buerstmayr[1]

[1]BOKU - University of Natural Resources and Life Sciences, Institute for Biotechnology in Plant Production and [2]Christian Doppler Laboratory for Mycotoxin Metabolism, IFA-Tulln, 3430 Tulln, Austria; [3]Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108 U.S.A.; [4]Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, 3430 Tulln, Austria; [5]Chair of Bioinformatics, Department of Biotechnology, University of Natural Resources and Life Sciences, 1190 Vienna, Austria and School of Life Sciences, University of Warwick, Coventry CV4 7AL, U.K.
[*]Corresponding Author: PH: 43-2272-66280-205, Email: wolfgang.schweiger@boku.ac.at

ABSTRACT

Fusarium head blight caused by *Fusarium graminearum* is a devastating disease of wheat. We have developed near-isogenic lines (NILs) differing in the two major *F. graminearum* resistance quantitative trait loci (QTL), *Qfhs.ndsu-3BS* (also known *Fhb1*) and *Qfhs.ifa-5A*, which are located on the short arm of chromosome 3B and on 5A, respectively. These NILs show different levels of resistance and were used to identify transcripts that are significantly changed in a QTL-specific manner in reaction to the pathogen and between mock-inoculated samples. After inoculation with *F. graminearum* spores, 16 and 352 transcripts showed a significantly different response for the *Fhb1* and *Qfhs.ifa-5A* NIL pairs, respectively. Notably, we identified a lipid-transfer protein, corresponding to Ta.1282.4.S1_at that is 50-fold more abundant in plants carrying the *Qfhs.ifa-5A* resistant allele. In addition to the *Qfhs.ifa-5A*-associated candidate gene, we identified a UDP-glycosyltransferase, designated TaUGT12887, exhibiting a difference in response to the pathogen in lines harboring both QTL compared to lines carrying only the Qfhs.ifa-5A resistance allele, suggesting Fhb1 dependence of this transcript. Yet, this dependence was observed only in the NIL with higher basal resistance. The complete cDNA of TaUGT12887 was reconstituted from available wheat genomic sequences and expressed in a toxin sensitive strain of *Saccharomyces cerevisiae*. DON resistance, albeit weaker compared to the previously characterized barley HvUGT13248 was conferred. We will discuss possible interpretations of this result.

TARGETING DEFENSE REGULATORY GENES AND HOST SUSCEPTIBILITY FACTORS FOR ENHANCING FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

Jyoti Shah[1*], Vamsi Nalam[1], Guy Klossner[1], Syeda T. Alam[1], Sujon Sarowar[1], Hyeonju Lee[2], Dehlia McAfee[2] and Harold Trick[2]

[1]Department of Biological Sciences, University of North Texas, Denton, TX 76203; and
[2]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506
[*]Corresponding Author: PH: (940) 565-3535, Email: shah@unt.edu

ABSTRACT

Fusarium graminearum is one of the major causative agents of Fusarium head blight (FHB), a destructive disease of small grains. Genetic studies in *Arabidopsis thaliana* have provided insights into plant defense mechanisms that control severity of disease caused by *F. graminearum* (Makandar et al., 2006, 2010). Comparable mechanisms are also involved in controlling FHB severity in wheat (Makandar et al. 2006, 2012). In addition, host mechanisms that predispose plant tissue to fungal infection have also been identified. Several *Arabidopsis* genes that regulate defenses targeting *F. graminearum* have been constitutively expressed in wheat to enhance host plant resistance against FHB. Many of these have shown promise in greenhouse studies. In addition, wheat homologues of lipoxygenase genes that contribute to disease susceptibility have been targeted for silencing in wheat. Efforts are also underway to target genes involved in non-host resistance to promote FHB resistance in wheat.

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TRANSCRIPTOME SEQUENCING OF *FUSARIUM* CHALLENGED WHEAT NEAR ISOGENIC LINES: A COMPARISON OF METHODS

G. Siegwart, C. Ametz, M. Zamini, B. Steiner, W. Schweiger* and H. Buerstmayr

BOKU-University of Natural Resources and Life Sciences Vienna, Department IFA-Tulln, Institute for Biotechnology in Plant Production, A-3430 Tulln, Austria
*Corresponding Author: PH: 43-2272-66280-205, Email: wolfgang.schweiger@boku.ac.at

ABSTRACT

In an effort to identify differentially expressed transcripts in response to *Fusarium graminearum* in a quantitative trait loci (QTL) dependent manner, we performed transcriptome sequencing on NILs segregating for two prominent resistance QTL (*Qfhs.ndsu-3BS* and *Qfhs-ifa-5A*).

In order to identify the method best suitable for the entire set of samples, we compared two different sequencing approaches - full length RNA-seq and MACE (Massive Analysis of cDNA Ends), generating only one read/cDNA from the 3' end - on samples of the parental lines (susceptible cv. Remus and QTL-donor CM-82036). RNA from *F. graminearum* or mock inoculated floret tissue (50 hours after inoculation, hai) of both genotypes was provided to the sequencing facilities of 'GATC' (Konstanz, Germany) for RNA-seq and 'GenXPro' (Frankfurt/Main, Germany) for MACE. Both used one lane of an Illumina HighSeq2000 flow cell (yielding potentially 180 million reads). GATC used 8x multiplexing (22 M reads/sample) whereas GenXPro used 10x multiplexing, (18 M reads/ sample). Here higher multiplexing was used, because of the higher specificity of MACE, which should compensate for lower read numbers.

The generated number of reads for the respective methods differed significantly: For RNA-seq about 25 M reads/sample were produced, in contrast to 7 M reads/sample for MACE. After trimming, we were able to map 84% of the MACE reads and 72% of the RNA-seq reads to the publically available wheat flcDNA- (16k) and unigene-collections (65k). A pairwise comparison between *F. graminearum*/mock inoculated samples in both genotypes revealed about 19.500 and 9.500 differentially expressed genes between *F. graminearum* and mock-inoculated samples using RNA-Seq and MACE, respectively, with an overlap of 7.600 genes. Taken together, in our experiment the high amount of reads generated by RNA-seq outweighs the higher specificity and relatively higher number of mappable reads provided by MACE when given a limited budget. RNA-seq identifies most of the differentially expressed transcripts in response to *F. graminearum* and additionally, the high median number of reads per target allows for detection of polymorphisms and reconstruction of gene models. Consequently, the remaining samples were sequenced using conventional RNA-seq. Quality assessments of the sequenced samples attest good quality and confirms the choice of RNA-Seq over MACE.

Data analysis including test for differential expression in response to *F. graminearum* between NILs differing in either or both QTL is currently ongoing and will be presented as a poster.

STRUCTURAL AND FUNCTIONAL ANALYSIS OF A STRESS RESPONSIVE GENE FROM BARLEY

Kashmir Singh, Ravneet Kaur and Jaswinder Singh*

Plant Science Department, McGill University, Ste Anne De Bellevue, QC, H9X3V9, Canada
*Corresponding Author: PH: (514) 398-7906, Email: Jaswinder.Singh@mcgill.ca

ABSTRACT

Biotic and abiotic stresses pose major threats to crop species, causing heavy yield losses worldwide. Barley being an excellent model grain crop used as food for humans and animal feed stock; it is beneficial to study stress tolerance mechanism in this plant. Insertional mutagenesis has been widely employed to characterize stress-responsive genes in plants such as *Arabidopsis* and rice. We are currently employing this tool in barley to identify important traits that can be used to enhance plant's performance. We have identified a Wall–associated receptor-like kinase 1 (WAK1) by analyzing flanking sequence of *Ds* transposon insertion site in barley cv Golden Promise. Wall–associated receptor-like kinases (WAKs) are candidates for directly linking the extracellular matrix with intracellular compartments and are involved in developmental processes and studies have shown that this gene is involved in defense response against pathogen attack. For example, Induction of *WAK1* expression by salicylic acid (SA) is required by arabidopsis plants to survive infection by the bacterial pathogen, *Pseudomonas syringae*. SA is a signaling molecule that accumulates in plants in response to pathogen attack and is required for the establishment of systemic acquired resistance (SAR). Rice *WAK1* is induced by infection of an incompatible race P131 of *Magnaporthe oryza* which provides evidence that WAK take part in plant fungal disease resistance. For characterization of *WAK1* gene from barley, three BAC clones corresponding to WAK fragment were sequenced and full length *WAK1* gene was identified. The gene has 3 exons and two short introns with a coding region of 2178 bp encoding a protein of 725 amino acids. Regulatory region was also identified and analyzed in -1000 bp sequence upstream to start codon. Using CDD and SMART, various conserved domains such as GUB WAK Bind, EGF_CA and PKc including other regions like signal peptides, active sites and transmembrane domains were identified. The gene organization of *WAK1* was compared with that of wheat and arabidopsis, which was found to be similar, thus, suggesting that *WAK1* gene remained unchanged during the evolution. Nonetheless, WAK1 shared very low similarity with protein sequences available from barley cultivar Haruna Nijo, rice, wheat, *Arabidopsis*, maize and was found to be 50%, 46%, 21%, 25% and 19% respectively. This divergence may have helped the plants to adapt themselves according the surrounding environment, as WAKs are main proteins helping in exchange between the cytoplasm and outer environment through the cell wall. Semi-quantitative RT-PCR based expression analysis indicates its expression is specific to roots.

IDENTIFICATION OF CANDIDATE GENES OF MAJOR FHB-RESISTANT QTL IN WHEAT CULTIVAR SUMAI 3

Yongbin Zhuang, Aravind Gala and Yang Yen*

Department of Biology and Microbiology, South Dakota State University, Brookings, SD 57007
*Corresponding Author: PH: (605) 688-4567, Email: yen.yang@sdstate.edu

ABSTRACT

Major FHB resistant QTL have been identified in Sumai 3. To identify the genic components of these QTL, 406 FHB-related wheat ESTs revealed by microarray assay were investigated in two sets of material: two bulked resistant/susceptible pools constructed from 10 $F_{2:8}$Sumai3 / Y1193 RILs per pool and two near-isogenic lines (NILs) differed only in the *Fhb1* containing region on chromosome arm 3BS. The plants grown in greenhouse were investigated for FHB severity by calculating the rate of FHB-diseased kernels and for expression of the FHB-related genes by qPCR assay. Genes showed significant differential expression (≥2 fold) either between the two bulks or the two NILs in both years were subjected to eQTL mapping for their association with FHB-resistance QTL. The identified candidate genes were then physically mapped to their carrier chromosomes with the Chinese Spring nulli-tetra deficiency set. One gene, designated *WFhb1_c1* (Wheat*Fhb1* candidate gene 1), was both functionally associated with and physically located within *Fhb1*, and was found to be weakly similar (*E* = 5e+0) to a gene encoding pectin methyl esterase inhibitor. Two other genes, designated as *WFI_6BL1* and *WFI_6BL2* (Wheat-*Fusarium* interaction gene 6BL1/6BL2), were functionally associated with *Fhb_6BL*, but physically mapped on chromosomes 7D and 5A, respectively. *WFI_6BL1* was annotated as a 13-lipoxygenase gene and *WFI_6BL2* might encode a PR-4 like protein. Study of the dynamic expression of the three genes in the early stage of FHB pathogenesis suggested that: 1) *Fhb1* seems to contribute to FHB resistance by reducing susceptibility in the first 60 hours; 2) *Fhb_6BL* made its contribution to FHB resistance via the JA-mediated pathways; and 3) wheat seemed to activate its defense mechanism in the biotrophic phase of FHB pathogenesis.