PATHOGEN BIOLOGY AND GENETICS

ELUCIDATING THE ROLE OF SILENCING RNA *FGSIR34* IN FUSARIUM HEAD BLIGHT PATHOGENESIS IN WHEAT

Subha Dahal, Aravind Galla, Bimal Paudel, Anjun Ma, Andrea Zavadil and Yang Yen*

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

ABSTRACT

Fusarium head bligt (FHB) is a devastating disease of small grains. Mycotoxin deoxynivalenol (DON) produced by *Fusarium graminearum* is believed to be an important virulence factor. Our research has led to the hypothesis that silencing RNA *fgsiR34* of *F. graminearum* may play a key role in regulating DON production. In our experiments to test this hypothesis, *Dicer-like 2* (*Dcl2*) gene, which control the biogenesis of *fgsiR34*, was knocked down, and *fgsiR34* was overexpressed. The mutants have been studied for their impacts on expression of the genes that control DON biosynthesis. The FHB pathogenicity of the mutants has also been studied. Here we report the preliminary results.

OVER-EXPRESSION OF TRANSLATION ELONGATION FACTOR 1-ALPHA MODIFIES PATHOGENIC AND PHENOTYPIC TRAITS OF A *FUSARIUM GRAMINEARUM* STRAIN

Anas Eranthodi[1,2], Daria Ryabova[1], Ravinder K. Goyal[1] and Nora A. Foroud[1*]

[1]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5401 - 1st Avenue S, Lethbridge, AB T1J 4B1, Canada; and [2]Department of Biological Sciences, University of Lethbridge, 4401 University Drive W, Lethbridge, AB T1K 3M4
[*]Corresponding Author: PH: 403-317-2269; Email: nora.foroud@agr.gc.ca

ABSTRACT

Fusarium graminearum is the predominant causative agent in Fusarium head blight (FHB) of wheat and related cereals. We have generated a *F. graminearum* mutant (FgEF1a-OX) overexpressing a gene encoding for elongation factor 1-alpha (FgEF1a). Eukaryotic EF1a plays a vital role in protein synthesis, but has also been shown to involve in various other cellular activities including cytoskeletal organization, cell cycle and signalling. Compared to wild-type, the FgEF1a-OX mutant reduced the disease symptoms in susceptible wheat cultivar, Roblin by 77 and 61 % in spray and point inoculations, respectively. A reduction in visual symptoms was also observed in highly resistant cultivars, CM82036 and Tenacious. This apparent reduction in pathogenicity seems to be related to a loss of fitness in the FgEF1a-OX strain, which was observed through mycelial growth and spore germination assays. The germination of wild-type macroconidal spores was 60% at 6 h and increased to nearly 100% by 9 h incubation. In contrast, only 15 and 53 % of the FgEF1a-OX macroconidia were germinated at similar time points of incubation. The alteration in physiological levels of EF1a might have negatively impacted one or more aspects associated with cell biology/biochemistry. A report on EF1a overexpression in yeast leading to a similar loss in fitness due to interactions with actin suggests an altered cytoskeletal function in *F. graminearum*. Additional characterization of FgEF1a-OX is underway to identify changes in cell cycle and morphology to get in-sight into fitness loss associated with this strain.

LINKING HOST COMMUNITY TO *FUSARIUM GRAMINEARUM* DISTRIBUTION

Michael R. Fulcher and Gary C. Bergstrom*

School of Integrative Plant Science, Plant Pathology and Plant-Microbe Biology Section, Cornell University, Ithaca, NY 14850
*Corresponding Author: PH: 607-255-7849; Email: gcb3@cornell.edu

ABSTRACT

The host range of *Fusarium graminearum* includes many non-cultivated grass species. Communities of these hosts may serve as sources of inoculum and as reservoirs of pathogen genetic diversity. In New York, there is a greater acreage of naturalized grassland than of wheat, barley, and corn combined. With funding from the United States Wheat and Barley Scab Initiative, the relationship between *F. graminearum* and host community was investigated at a cereal-natural grassland interface. In 2015, preliminary samples of wheat heads and wild grass inflorescences were taken from three wheat fields. In 2016, naturally occurring wild grass debris, wheat spikes, and wild grass inflorescences were collected from five winter wheat fields and one national wildlife refuge. In 2015, 112 *F. graminearum* isolates were captured from inflorescences of winter wheat, smooth brome grass (*Bromus inermis*), and timothy grass (*Phleum pratense*). Field conditions in 2016 were not conducive to scab development in wheat or to the infection of wild grasses. However, debris gathered from 181 individual sampling sites across the six locations yielded 370 *F. graminearum* isolates. Incidence and distribution were analyzed with reference to grass community composition, and models were produced for pathogen presence and abundance at individual sampling points. The recovery rate of *F. graminearum* from plant debris sampled at the grassland was equal to or greater than that of material sampled at wheat field margins. The grassland had a higher host richness and density than the wheat field margins, and these factors had significant effects in logistic regressions of both *F. graminearum* presence and the number of isolates found at individual sampling sites. Spatial autocorrelation was not detected for *F. graminearum* presence or abundance. These results indicate the potential for non-cultivated grass species, both on and off farms, to support sizeable *F. graminearum* populations. Ongoing work is examining the pathogen population structure in these locations and the extent to which specific host assemblages or other ecological factors impact *F. graminearum* populations.

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MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE AND DEOXYNIVALENOL ACCUMULATION IN KANSAS WHEAT

Cristiano Lemes da Silva[1*], Allan Fritz[1], Jesse Poland[1], Floyd Dowell[2] and Kamaranga Peiris[2]

[1]Kansas State University, Dept. of Agronomy, Manhattan, KS, 66502; and [2]USDA-ARS, Center for Grain and Animal Health Research Unit, Manhattan, KS, 66502
[*]Corresponding Author: PH: 785-477-7736; Email: cristianolemes@ksu.edu

ABSTRACT

Fusarium head blight (FHB) is a wheat disease caused by *Fusarium graminearum* that significantly reduces grain yield and produces mycotoxins that contaminate wheat grains and flour. Deoxynivalenol (DON) is the most prevalent mycotoxin and its advisory limit in U.S. is 1 ppm for wheat products. Thus, the objective of this study was to map quantitative trait loci (QTL) associated with FHB resistance (type II, III, and IV), and estimate the effect of stacking multiple QTL within breeding lines. A doubled haploid (DH) population with 202 lines was developed from a cross between Everest and WB-Cedar, which are moderately resistant and moderately susceptible to FHB, respectively. The experiment was conducted in the field at Rocky Ford FHB during 2014/2015 and 2015/2016 growing seasons in a randomized complete block design with 3 replications. Evaluations of percentage of symptomatic spikelets (PSS) started 21 days after heading and repeated every 3 days for a total of 5 evaluations. After harvest, a sample of 100 grains from each plot was collected to measure DON accumulation and *Fusarium* damaged kernel (FDK) using a single kernel near-infrared spectroscopy instrument. DH lines and parents were genotyped using genotyping-by-sequencing (GBS). A pipeline on TASSEL 4 was used to call and filter SNP markers. The final linkage map consisted of 3,005 SNP and 165 DH-lines. Phenotypic traits were analyzed in SAS with PROC GLM. Composite interval mapping and multiple QTL mapping were performed in Rstudio using Haley–Knott regression. Three QTL for type II resistance were found on chromosomes 3BS, 6AL and 6BL explaining 30.5% of PSS. Another three QTL located on 1B, 5AL, and 5DS from Everest together explained 29.2% of DON accumulation. The QTL on 3BL and 5DL were significant in 2015 and 2016 growing seasons. FDK and DON data from the second year of experiment are currently being analyzed. DH-lines containing all QTL for each trait were significantly more resistant than DH-lines with none or only one QTL. Everest is an elite source of FHB resistance with multiple QTL. GBS sequences flanking significant QTL for both years of experimentation will be later converted into diagnostic markers to assist breeding for FHB resistance.

IDENTIFICATION AND CHARACTERIZATION OF *FUSARIUM GRAMINEARUM* PATHOGENESIS GENES

Melissa M. Salazar, Frederic L. Kolb and Santiago X. Mideros*

Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801
*Corresponding Author: PH: 217-265-6340; Email: smideros@illinois.edu

ABSTRACT

Fungal pathogens overcome plant host defenses by producing pathogenesis compounds. *Fusarium graminearum*, the causal agent of Fusarium Head Blight (FHB) of wheat, produces trichothecene secondary metabolites such as deoxynivalenol (DON). Apart from DON, little is known about other pathogenesis compounds used by *F. graminearum* to cause disease on wheat. To identify fungal pathogenesis genes that are essential for *F. graminearum*, a paired strategy of isolates and transcriptome characterization was implemented. This should allow not only to capture biological samples containing the pathogenesis genes but also to determine their role in pathogenesis. A field pathogenomics strategy to identify common transcription signals in naturally infected wheat lines with various levels of resistance was used. In order to collect a variety of *F. graminearum* strains, research sites were established in the following Illinois locations: Urbana, Savoy, Brownstown, St. Jacob, and Carmi. Within each site, five wheat lines were planted in a random block design using University of Illinois soft red winter wheat improvement program plots. Wheat lines included the following: a resistant line (IL11-28222), a moderately resistant line (IL07-19334), a moderately susceptible line (IL10-19464), and two susceptible lines (Kaskaskia and Pioneer 25R47). Ten naturally infected heads were identified for each line, and two spikelets were collected. One spikelet was kept on ice for isolation of the fungus and the other was immediately submerged in RNAlater. Close to 200 *Fusarium* single spore isolates have been recovered from over 90% of the samples. Selected strains will be used in a greenhouse assay to characterize the levels of aggressiveness. RNA extraction of selected infected samples yielded high-quality RNA and will soon be submitted for sequencing. RNASeq analysis will then be conducted to compare the transcriptomes of resistant, moderately susceptible, and susceptible interactions and to identify pathogenesis genes that are required for infection on wheat.

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IMPACT OF DROUGHT STRESS ON WHEAT ROOT AND STEM BASE INFECTIONS *(TRITICUM AESTIVUM* L.*)* WITH *FUSARIUM CULMORUM*

Sebastian Streit[1], Andreas von Tiedemann[1] and Mark Winter[1,2*]

[1]Georg-August-University Goettingen, D-37077 Goettingen, Germany;
and [2]University of Minnesota, St. Paul, MN 55108
[*]Corresponding Author: PH: 612-625-0234; Email: mark.winter@agr.uni-goettingen.de

ABSTRACT

Fusarium culmorum is the predominant causal agent of *Fusarium* foot and crown rot (FCR) of wheat in arid and semi-arid growing regions world-wide. Drought stress is considered to play a crucial role during severe *F. culmorum* root and stem base infections of wheat. However, there are apparently no published studies documenting the impact of induced drought stress under controlled conditions. Our aim was to quantify the infestation of wheat root and stem bases with *F. culmorum* under drought stress and to document the water stress status in planta in order to show whether drought stress increases the colonization rate of *F. culmorum*. Pre-germinated wheat seedlings were dip-inoculated with a spore suspension of *F. culmorum* (800.000 spores/mL) and planted into potting soil. During stem elongation (Feekes 5), drought stress was induced in half of the plants by reducing the field capacity in the potting soil to 45%. In the well-watered treatment, we kept the field capacity at 75%. Plant water status was assessed by four different drought stress parameters: Relative leaf water content (RWC) (Feekes 11.1), leaf turgor (throughout the experiment), leaf surface temperature by thermal imaging (Feekes 11.1) and proline content (Feekes 11.1). Colonization of roots and stem bases with *F. culmorum* was determined by quantifying the fungal DNA with quantitative PCR (qPCR) at late milk ripeness (Feekes 11.1) and maturity (Feekes 11.4.) and rating the disease symptoms on the respective organs. All four water stress parameters assessed indicated the presence of drought stress in wheat plants in 45% field capacity. In both organs, the fungal DNA content was significantly higher under drought stress than under well-watered conditions (stem bases 17 times and roots 3 times higher; $P \leq 0.05$). However, fungal biomass in roots always exceeded the stem base levels, regardless if drought stress was present or not. Proline and DNA content in roots and stem bases were correlated ($r^2 = 0.47$). Based on these results we conclude that severe drought stress leads to higher colonization rates of *F. culmorum* in roots and stem bases of wheat.

This is the first study showing that water deficit in wheat plants followed by limited water supply significantly increases FCR severity in wheat caused by *F. culmorum*. Therefore, we conclude that FCR might be a threat to wheat production in areas with low precipitation with increasing importance.

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COMPARATIVE POPULATION GENOMICS OF *FUSARIUM GRAMINEARUM* REVEALS ADAPTIVE DIVERGENCE AMONG CEREAL HEAD BLIGHT PATHOGENS

Todd J. Ward* and Amy Kelly

United States Department of Agriculture, Agricultural Research Service, Peoria, IL, USA
*Corresponding Author: PH: 309-681-6394, Email: todd.ward@ars.usda.gov

ABSTRACT

During the last decade, a combination of molecular surveillance and population genetic analyses have significantly altered our understanding of *Fusarium graminearum*, the major FHB pathogen in North America. In addition to the native NA1 population (largely 15ADON toxin type) and the invasive NA2 population (largely 3ADON toxin type), which has rapidly increased in frequency in some areas, isolates with a novel trichothecene toxin type (NX-2) were recently found to cause FHB in the northern U.S. and southern Canada. In this study, we sequenced the genomes of 60 *F. graminearum* isolates to understand how NX-2 isolates relate to the previously characterized NA1 and NA2 populations; and to identify potential adaptations that distinguish the various populations of *F. graminearum* responsible for FHB in the U.S. and Canada. Genome-wide patterns of SNP diversity revealed that most isolates with the NX-2 toxin type represent a novel genetic population (termed NA3), although genetic exchange among populations was documented. The three genetic populations were found to differ in gene content, with 122 genes showing population-specific patterns of gene conservation. An additional 16 loci, varying in size from 10-40 kb exhibited patterns of adaptive divergence between pathogen populations. Functional annotation of these population-differentiating genes and genomic regions indicated that *F. graminearum* populations in North America harbor unique sets of adaptations that contribute to differences in how these pathogens exploit the agricultural landscape.

DON MODIFICATION IN NATURALLY-ONTAMINATED WHEAT SAMPLES USING MICROORGANISMS ISOLATED FROM THE ENVIRONMENT

Nina Wilson[1*], Dash Gantulga[1], Nicole McMaster[1], Ken Knott[1], Susan McCormick[3], Ryan Senger[2] and David Schmale[1]

[1]Department of Plant Pathology, Physiology, and Weed Science, [2]Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061; and [3]USDA-ARS, Mycotoxin Prevention and Applied Microbiology, Peoria, IL 61604
[*]Corresponding Author: PH: 540-231-0733; Email: nina09@vt.edu

ABSTRACT

The fungus *Fusarium graminearum* produces the toxic compound deoxynivalenol (DON) that contaminates wheat, barley, and maize. New strategies are needed to mitigate DON in the United States. Microbes were isolated from different soil types, and cultured in a mineral salt media using 100 ppm DON as the sole carbon source. We identified two mixed cultures, Soil 1 and Soil 2, which consistently modified DON. Nuclear magnetic resonance (NMR) was used to determine the structure of the culture byproducts of Soil 1 and Soil 2. Sequencing of the mixed cultures showed that Soil 1 contained mostly members of the genera *Acinetobacter* and *Enterobacter*, and Soil 2 contained mostly members of the genera *Pseudomonas* and *Comamonas*. Soil 1 and Soil 2 were incubated in two naturally contaminated wheat samples containing two different concentrations of DON (7 ppm and 41 ppm). Gas chromatography mass spectrometry (GC/MS) analysis showed nearly complete DON reduction in two samples (7 ppm DON) using the Soil 1 culture. GC/MS analysis of these two samples showed that DON was converted to another metabolite, 3-epi-DON. This research highlights the various ways DON can be modified under certain conditions and offers a platform to detoxify DON in naturally contaminated samples.

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DEOXYNIVALENOL (DON) AND NIVALENOL (NIV) PLAY A ROLE AS VIRULENCE FACTORS FOR WHEAT ROOT AND STEM BASE INFECTION BY *FUSARIUM CULMORUM* AND *F. GRAMINEARUM*

Mark Winter*, Peter L. Samuels, Yanhong Dong and Ruth Dill-Macky

University of Minnesota, St Paul, MN 55108
*Corresponding Author: PH: 612-625-0234; Email: mark.winter@agr.uni-goettingen.de

ABSTRACT

Fusarium culmorum and *F. graminearum,* along with *F. pseudograminearum*, are the predominant causal agents of *Fusarium* foot and root rot in wheat. Populations of these two species vary broadly in their production of a variety of mycotoxins, including deoxynivalenol (DON) which is considered a virulence factor for wheat head and stem base infections. It is likely that DON is also a virulence factor in root infections incited by *F. culmorum* and *F. graminearum*, but there are apparently no published studies documenting infection under controlled conditions, or whether trichothecenes including DON are virulence factors in that process.

In this study, we screened a collection of 21 *F. culmorum* and six *F. graminearum* isolates for their ability to produce the mycotoxins DON, 3 acetyl-deoxynivalenol (3ADON), 15 acetyl-deoxynivalenol (15ADON), nivalenol (NIV), and zearelanone (ZEA) in rice culture. We selected two isolates of *F. culmorum* that demonstrated high and low production, respectively, of DON and 3ADON, and a third isolate for which ZEA was the only detected mycotoxin produced. Of the two *F. graminearum* isolates selected, one isolate only produced NIV and the other produced high levels of DON and 3ADON. Wheat plants were inoculated by planting pre-germinated seedlings (2 seedlings per pot) into potting soil enriched with *Fusarium*-colonized wheat straw (72 g straw per 10 kg soil). For each treatment, 16 plants were inoculated with the respective isolate. A non-inoculated treatment served as a control. For analysis, 4 plants were merged to one sample (n = 4). At the late milky ripe stage, root and above-ground biomass were determined. The roots and stem bases were excised and assessed visually for disease symptoms, and the DNA of *Fusarium* spp. and mycotoxin content were determined. Inoculation with the high DON/3ADON-producing isolate of *F. culmorum* resulted in the highest disease rating and a significant reduction in the biomass of roots and above-ground plant material. Additionally, levels of *Fusarium* DNA and DON in root and stem base tissues were significantly higher compared to the other isolates tested. *Fusarium* DNA and DON readings were significantly correlated ($r^2 = 0.67$ for roots, $r^2 = 0.92$ for stem bases). For *F. graminearum*, the NIV- and the DON/3ADON-producing isolates both caused higher disease symptoms on roots and stem bases, compared to the non-inoculated control, which was only significant for stem bases in plants inoculated with the NIV-producing isolate. Elevated DNA levels were seen in roots in both treatments and both isolates significantly reduced above-ground plant material compared to the control. Interestingly, only the DON/3ADON isolate caused a significant reduction of root biomass, and only the NIV-producing isolate colonized the stem base at significantly higher levels compared to the control plants, based on *Fusarium* DNA. NIV and DNA content were correlated in root and stem tissue ($r^2 = 0.66$ for roots, $r^2 = 0.59$ for stem bases). Based on these results it appears that the trichothecenes DON and NIV are virulence factors for root and stem base infection of wheat by *F. culmorum* and *F. graminearum*.

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