

**PATHOGEN
BIOLOGY
AND
GENETICS**

SURVEY FOR *FUSARIUM GRAMINEARUM*
15-ADON, 3-ADON AND NIV CHEMOTYPES
IN WINTER WHEAT IN ONTARIO

Pragyan Burlakoti and Ljiljana Tamburic-Ilincic*

Ridgetown Campus, University of Guelph, 120 Main St. E. Ridgetown, Ontario, N0P2C0, Canada

*Corresponding Author: PH: 519-674-1500 x 63557; Email: ltamburi@uoguelph.ca

ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (FG) is a serious disease of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated wheat grain in Ontario, Canada. Grain samples from six winter wheat cultivars per year from 2013 and 2014 Ontario Performance Trial were included in the study. Grain samples were collected from Inwood, Elora, Woodslee, Palmerston and Ridgetown in 2013, and from Ottawa, Palmerston and Ridgetown in 2014 to assess the percentage of *Fusarium* infected kernels (FIK), the percentage of FG (identified as % of total *Fusarium* spp.) and the frequency of FG chemotypes (15-ADON, 3-ADON and Nivalenol-NIV). The cultivars were: highly susceptible to *Fusarium* head blight, moderately susceptible and moderately resistant. One hundred and fifty kernels of each cultivar were surface-sterilized in 0.16% NaOCl (dilute commercial bleach) for three minutes, air dried, and plated on acidified potato dextrose agar. The kernels were incubated for seven days under a 12:12 hr light: dark cycle at room temperature. Subsequently, single spore cultures of FG were recovered and identified morphologically. The FG isolates were also identified using molecular markers specific to *F. graminearum*. Genomic DNA was extracted from single spore isolates of FG. 15-ADON, 3-ADON and NIV chemotypes of the fungal strains were identified using *TRI3*- and *TRI12* based molecular markers. In 2014, the highest percentage of FG was observed in highly susceptible cv. in Ottawa (83.3%) and Palmerston (90.9%), and moderately susceptible cv. in Ridgetown (94.6%). Percentage of FG 15-ADON chemotype was 63.9%, 95.8 % and 97.9 % from Ottawa, Palmerston and Ridgetown, respectively. 2.1% of NIV was detected at Palmerston and Ridgetown, and NIV was not detected in Ottawa. 3-ADON was detected at 36.1% in Ottawa, only 2.1% at Palmerston and was not detected at Ridgetown. We concluded that the frequency of the FG 3-ADON chemotype in winter wheat in Ottawa was much higher in 2014 (36.1%) than recorded previously (2%-7%) from anywhere in Ontario. The results from 2013 season will be presented at the conference.

GENERATION OF *FUSARIUM GRAMINEARUM*
MUTANTS TO SCREEN CANDIDATE PATHOGEN-
ASSOCIATED MOLECULAR PATTERNS

A. Eranthodi^{1,4}, R. Subramaniam², C. Rampitsch³,
T. Ouellet², E.A. Schultz⁴ and N.A. Foroud^{1*}

¹Agriculture and Agri-Food Canada, Lethbridge, AB; ²Agriculture and Agri-Food Canada, Ottawa, ON; ³Agriculture and Agri-Food Canada, Morden, MB; and ⁴University of Lethbridge, Lethbridge, AB

*Corresponding Author: PH: (403) 317-2269; Email: nora.foroud@agr.gc.ca

ABSTRACT

Fusarium head blight (FHB) is a disease of wheat and other cereals caused predominantly by *F. graminearum* resulting in significant loss of grain yield and quality. Our study is aimed at screening candidate pathogen-associated molecular patterns (PAMPs) of *F. graminearum*. To this end, seven proteins (PAMP1-PAMP7) were selected from the *F. graminearum* secretome and knockout and overexpression mutants of *F. graminearum* were generated for the genes that encode these proteins. The selection of these proteins was based on their sequence homology to proteins having pathogenicity roles in other host-pathogen interactions. Mutants were generated by *Agrobacterium*-mediated transformation using plasmids designed for gene replacement with a hygromycin resistance gene for knockout (KO) mutants, and insertion of a hygromycin resistance gene adjacent to a fungal overexpression promoter for *in locus* overexpression (OX) of target genes. The mutants *PAMP1*-OX, *PAMP2*-KO, or *PAMP2*-OX were point-inoculated in Superb and GS-1-EM0040 ('CIMMYT 11'/'Superb'*2) that are moderately susceptible and moderately Type II resistant wheat lines respectively. *PAMP1*-KO appears to be a lethal mutant and hence was not evaluated here. The two wheat lines inoculated with the wild-type *F. graminearum* strain served as controls. Disease evaluations 18 days after inoculation showed that the number of infected spikelets was significantly lower for wheat lines inoculated with *PAMP1*-OX as compared to those inoculated with the wild-type *F. graminearum* strain. Similarly, *PAMP2*-OX inoculated wheat lines had fewer infected spikelets than wild-type inoculated wheat lines. Moreover, the number of diseased spikelets for *PAMP2*-OX inoculated wheat lines was significantly lower than those for *PAMP2*-KO inoculated wheat lines. From these preliminary results we hypothesize that PAMP1, and possibly PAMP2, activate wheat receptors triggering the basal immune response, known as PAMP-triggered immunity, in wheat. Generation of mutants for the remaining genes and their screening in additional wheat lines are currently underway.

EXPLORING THE FUNCTION OF GENES INVOLVED IN
DISEASE INITIATION BY *FUSARIUM GRAMINEARUM*
Lori Imboden and Frances Trail*

Department of Plant Biology, Michigan State University, East Lansing, MI

*Corresponding Author: PH: 517-432-2939; Email: trail@msu.edu

ABSTRACT

During a study to identify genes involved in early penetration of host plants by *Fusarium graminearum*, we investigated the role of the three aquaporin genes in this process. Aquaporins are membrane bound transport proteins, and are best known as water transporters in animal cells. Their function in the growth, development, and pathogenesis of microorganisms is poorly understood. Strains harboring knockouts of the individual genes can grow and develop functional perithecia under controlled laboratory conditions, but do so with deficiencies or delays in conidial germination, development of aerial hyphae, and development of perithecia relative to the wild-type fungus. Likewise, the mutants are capable of infecting excised barley florets under high humidity conditions. However, all three individual mutants have greatly reduced ability to spread beyond the site of inoculation in susceptible wheat. Generation and analysis of double aquaporin mutants is ongoing. Although the relationship between these aquaporins is unclear, they appear to be functioning in a similar manner and all are essential. These genes may be good targets for development of control of early infection of *F. graminearum*.

STAGE-SPECIFIC A-TO-I RNA EDITING IN THE WHEAT SCAB FUNGUS *FUSARIUM GRAMINEARUM*

Huiquan Liu¹ and Jin-Rong Xu^{2*}

¹Purdue-NWAFU Joint Research Center, State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China; and ²Dept. of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907. USA

*Corresponding Author: PH: 765-496-7496; Email: jinrong@purdue.edu

ABSTRACT

Yeasts and filamentous fungi do not have ADAR orthologs and are believed to lack A-to-I RNA editing, which is the most prevalent editing of mRNA in animals. However, during this study with the *PUK1* pseudo-kinase gene important for sexual reproduction in *Fusarium graminearum*, we found that two tandem stop codons UA¹⁸³¹G UA¹⁸³⁴G in its kinase domain were changed to UG¹⁸³¹G UG¹⁸³⁴G by RNA editing in perithecia. To confirm A-to-I editing of *PUK1* transcripts, strand-specific RNA-Seq data were generated with RNA isolated from conidia, hyphae, and perithecia. *PUK1* transcripts were almost specifically expressed in perithecia and 90% of them were edited to UG¹⁸³¹G UG¹⁸³⁴G. Genome-wide analysis identified 27,301 perithecium-specific A-to-I editing sites. Unlike those in animals, 70.5% of A-to-I editing sites in *F. graminearum* occur in coding regions and over two-thirds of them result in amino acid changes, including editing of 45 *PUK1*-like pseudogenes with stop codons in ORFs. Furthermore, *F. graminearum* differs from animals in the sequence-preference and structure-selectivity of A-to-I editing sites. Whereas As embedded in RNA stems are targeted by ADARs, RNA editing in *F. graminearum* preferentially targets As in hairpin loops, which is similar to the anticodon loop of tRNA targeted by ADATs, implying a potential evolutionary link between mRNA editing and ADATs in fungi. Overall, our results showed that A-to-I editing occurs specifically during sexual reproduction and mainly in the coding regions in filamentous ascomycetes, involving adenine deamination mechanisms distinct from metazoan ADARs.

A GENOME-WIDE VIEW OF POPULATION STRUCTURE AND GENETIC DIFFERENTIATION OF *FUSARIUM GRAMINEARUM* POPULATIONS FROM THE AMERICAS

Christopher Toomajian*, Wei Yue and John F. Leslie

Department of Plant Pathology, Kansas State University, Manhattan, KS

*Corresponding Author: PH: (785) 532-0879; Email: toomajia@ksu.edu

ABSTRACT

Population genomic studies of *Fusarium graminearum* (Fg) isolates provide an important complement to experimental studies that investigate the interaction of this important pathogen with its hosts and functional differences between different pathogen populations. Jointly, these studies can identify the genetic basis of functional differences between populations that can affect pathogen management and strategies for developing host resistance. Our broader aims include characterizing the variability along chromosomes of patterns such as variant density, genetic differentiation between populations, and genetic recombination to gain insight into evolutionary processes in this species. More specific goals include closer population genomics investigations of the fact that Fg isolates can be genetically clustered into groups that largely correspond to genotypes at the trichothecene gene cluster that determine whether 3-ADON or 15-ADON predominates, and observed population shifts in the prevalence of 3-ADON isolates in parts of North America.

Here, we provide an update of our FY14-15 USWBSI population genomics project that uses genotyping by sequencing (GBS) for the genetic analysis of Fg isolates from the Americas. We have expanded our sample to nearly 400 isolates, focusing on 3-ADON and 15-ADON isolates from New York and the upper Midwest and also including isolates from Uruguay. We demonstrate that by scanning along chromosomes, we can identify loci with the highest levels of genetic differentiation between chemotypes, geographic regions, or sampling years, highlighting loci with potentially important adaptive roles. We also describe patterns of linkage disequilibrium between variants in the populations and other patterns consistent with footprints of natural selection.

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A SMALL PILOT GWAS FOR THE GENETIC BASIS
OF PATHOGENIC AND SAPROPHYTIC FITNESS
IN A SAMPLE OF NEW YORK *FUSARIUM*
GRAMINEARUM ISOLATES FROM WHEAT

Christopher Toomajian^{1*}, Wei Yue¹, Pierri Spolti²,
Gary C. Bergstrom² and John F. Leslie¹

¹Department of Plant Pathology, Kansas State University, Manhattan, KS; and ²Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY

*Corresponding Author: PH: (785) 532-0879; Email: toomajia@ksu.edu

ABSTRACT

The dominant mycotoxin of *Fusarium* head blight in the U.S. is the trichothecene deoxynivalenol (DON), a known virulence factor, and *Fusarium graminearum* (Fg) isolates can be categorized based on which of two acetylated DON forms, 3-ADON and 15-ADON, each isolate produces in greatest quantity. Surveys of genetic markers have found that Fg isolates can be genetically clustered into groups that correspond to genotypes at the trichothecene gene cluster that determine which acetylated form of DON predominates. Reports of the increase in prevalence of 3-ADON isolates led to a series of studies aimed at identifying factors that give 3-ADON isolates an advantage over 15-ADON isolates. While several studies that focused on pathogenic traits found evidence for a 3-ADON advantage, a study of 50 isolates from New York that considered traits both pathogenic and saprophytic fitness on a susceptible wheat cultivar found no detectable advantage of 3-ADON isolates. Several important questions remain unanswered: what factors help explain the increase in prevalence of 3-ADON isolates; what is the relationship between trichothecene genotypes and fitness traits; and what other genetic loci are associated with differences in saprophytic and pathogenic fitness found among Fg isolates?

Our FY14-15 USWBSI population genomics project uses genotyping by sequencing (GBS) markers to characterize the genome of hundreds of U.S. Fg isolates, including the 50 New York isolates measured for pathogenic and saprophytic fitness as described above. Here, we aim to address the latter two questions above by screening for statistical associations between the fitness traits and genetic variation throughout the Fg genome. We have performed a pilot genome-wide association study (GWAS) for the 14 fitness traits that takes into account the known population structure of Fg populations (in particular, the subdivision between the 3-ADON and 15-ADON chemotypes). Our GBS markers provided genotypes at thousands of SNPs densely distributed throughout the Fg genome. These SNPs were filtered for quality and allele frequency, and then we performed imputation to infer the allele state of the remaining missing genotypes. Due to our small sample size, our preliminary analysis has low power to detect associated genetic variants. Yet this pilot study has the potential to identify the strongest signals of association and helps to identify the challenges for future GWAS in Fg populations.

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USING ENZYMES AND MICROORGANISMS TO MODIFY THE MYCOTOXIN DEOXYNIVALENOL

Nina Wilson¹, Dash Gantulga¹, Nicole McMaster¹,
Ryan Senger², Susan McCormick³ and David Schmale^{1*}

¹Department of Plant Pathology, Physiology, and Weed Science, ²Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24061; and ³USDA-ARS, Mycotoxin Prevention and Applied Microbiology Peoria, IL 61604

*Corresponding Author: PH: (540) 231-0733; Email: nina09@vt.edu

ABSTRACT

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by the fungus *Fusarium graminearum* that contaminates staple crops such as wheat, barley, and maize when they are infected with this fungus. New strategies are needed to mitigate DON. We screened for microbes that could grow in the presence of 100 ppm DON and found two mixed cultures and two pure cultures that consistently detoxified DON in laboratory experiments. Sequencing analysis of the pure cultures indicated that they were *Pseudomonas* and *Achromobacter*. Nuclear magnetic resonance (NMR) analysis of one of the culture byproducts indicated that DON was converted to 3-keto-DON. In a second approach, we engineered yeast strains to be sensitive to 100 ppm DON and used them to screen library fragments generated from the mixed cultures and the *Pseudomonas* species and cDNA enzyme sequences created by Integrated DNA Technologies. Three library fragments and two cDNA enzyme sequences were identified that allowed the yeast to grow in the presence of 100 ppm DON. In future studies, microbes and enzymes that demonstrated DON detoxification will be tested on contaminated wheat and barley samples. Our research offers a unique approach to reduce DON in these grains, particularly in the context of ethanol co-products.

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