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2021 National Fusarium Head Blight Forum

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Table of Contents

Food Safety & Toxicology 31

Gene Discovery & Engineering Resistance.. 36

Pathogen Biology & Genetics

2021 NFHB Forum Plenary Presentations

Resisting Susceptibility to FHB

Paul Nicholson, Benjamin Hales, Andrew Steed, John Haidoulis, Christopher Burt

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK
Corresponding Author: Paul Nicholson, paul.nicholson@jic.ac.uk

Abstract

Breeding for resistance to Fusarium head blight (FHB) is challenging because of the polygenic nature of resistance and interactions with environmental and host factors such as plant height and flowering time. While most research into Fusarium head blight (FHB) is understandably focused on increasing resistance through introducing beneficial genes it is becoming clear that removing deleterious ones may provide an alternative approach. Investigations of wheat, barley and *Brachypodium distachyon* have shown how various phytohormones affect susceptibility and resistance to FHB. The relationships between particular pathways and susceptibility are not always clear-cut, possibly because of the hemi-biotrophic nature of the interaction between *Fusarium graminearum* and wheat. It appears that *F. graminearum* may be exploiting certain pathways to prevent the plant from mounting an effective defense. This view is supported by the finding that isolates of *F. graminearum* are capable of producing some of the core phytohomones and these may be used to persuade the plant to maintain growth at the cost of defense. The negative association between plant height and FHB has long been recognized. Curiously, despite having similar effects on plant height, the semi-dwarfing gene/allele *Rht-D1b* has a more serious impact than *Rht-B1b* in winter wheat varieties. Our findings suggest that this is not a pleiotropic effect but is due to the introduction of a gene close to *Rht-D1* from the spring wheat donor. While the majority of wheat varieties lack the ability to prevent the spread of the fungus once it enters the spike, barley varieties have high levels of this so-called Type 2 resistance. We examined wheat barley chromosome addition/substitution lines to determine whether barley chromosomes could provide Type 2 resistance to wheat. Surprisingly, the most potent effect derived from the substitution of chromosome 4D with 4H suggesting that the susceptibility of wheat is due, in large part, to the presence of a susceptibility factor(s) on 4D rather than the absence of resistance factor(s). Work to identify the causal gene will be described. While resistance to FHB and DON mycotoxin accumulation in agronomically adapted varieties can undoubtedly be enhanced by the introduction of resistance from various sources it is also possible that resistance can be increased through the elimination of susceptibility factors. The challenge in both cases is to provide robust FHB resistance without compromising other important agronomic characteristics required by breeders and growers.

Challenges and Mitigations of Fusarium Impacts in Malting and Brewing

Xiang S Yin

Rahr Corporation. Shakopee, MN, USA
Corresponding Author: Xiang S Yin, xiangsyin@rahr.com

Abstract

In the malting and brewing industries, the impact of scab is far more than just management of food safety. Several key aspects of the impact lead to serious consequences to the processes and quality of the product. 1) Viable fungal hyphae on barley grains taken into the malthouse can proliferate further in the germination stage of the malting process causing uncontrolled out-of-specification increase of DON in the finished malt. 2) Certain polypeptides, also known as hydrophobins, secreted by some Fusarium species can survive the brewing and fermentation processes to the packaged beer and exhibit gushing propensity of beer upon opening the containers. 3) The action of antifungal response of barley when infected, can adversely produce high molecular weight substances impeding performance of yeast (also of fungal nature) in the brewery causing hung fermentation, known as the Premature Yeast Flocculation (PYF) phenomenon. The mechanisms may involve the multiple steps of interaction between pathogens and host plant, and the metabolites in turn affect the microorganisms on the plant or in later processes in the malting and fermentation media. Fusarium may also contribute to barley dormancy, malt flavor profile, and hydrolytic enzyme activities in some cases. After harvest and during storage, usually over the period of several months, the dominant form of microflora on the barley changes from the field fungi to storage fungi because of the shift in environmental conditions, such as temperature, kernel moisture, and storage time. With malting barley of average quality, the total plate count per gram (TPC/g) or colony-forming units per gram (CFU/g) of all viable microorganisms is in the order of millions. In most cases, bacteria were most prevalent, followed by yeasts, and then molds. The malting plant configuration, e.g., floor malting vs. tower malting, may have a strong influence on the types and proportions of microflora present due to the variation in moisture and temperature cycles employed, which in turn influence the flavor or quality of the finished malt. The change in microflora load on barley during malting could be up to a two-log increase (in CFU/g) during steeping but malt kilning under heat results in only limited reduction of the high CFU/g counts from germination. Options for managing the fusarium growth during germination are restricted as the germination capacity of the kernels should not be negatively affected, and the profile of flavor and taste of product should be preserved. Effects of selected physical and chemical treatments have been studied and recommendations are provided for practical applications.

FHB Management

Cost Effectiveness of Fungicides for FHB Control: Impacts on FDK and Test Weight

Adam Albright, Meghan Biggs, Daniel Sjarpe, Charles Victor, and Kaitlyn M. Bissonnette

University of Missouri, Division of Plant Science and Technology, Columbia, MO
Corresponding Author: Kaitlyn Bissonnette, bissonnettek@missouri.edu

Abstract

Since 1990, wheat and barley farmers in the United States have lost over 3 billion dollars in yield and quality lost due to FHB epidemics. FHB ultimately effects grain quality resulting in lower test weights and mycotoxin accumulation in grain which adversely affect livestock and consumers. Fungicides used for FHB control vary in cost and efficacy adding complexity to decision-making for producers when making management decisions. In this study the soft red winter wheat variety Pioneer 25R40 treated with Lumigen was planted at 2 different locations in Missouri. Small plots measuring 5 ft by 30 ft were planted and replicated four times. A non-treated control was included, and 5 fungicide treatments were applied: Prosaro® (6.5 oz per acre), Prosaro (8.2 oz per acre), Caramba®, and Miravis® Ace all applied at Feekes 10.5.1 (50% anthesis), and Miravis Ace applied at Feekes 10.3 (50% heading). All fungicide applications were applied with a 5 ft hand boom and pressurized backpack sprayer. To ensure uniform disease pressure, a spore suspension of 50,000 spores per mL of *Fusarium graminearum* inoculum was applied to each plot at Feekes 10.5.1 with a 5 ft hand boom and pressurized backpack sprayer. FHB ratings were conducted at 21- and 28- days post fungicide application, yield was collected at harvest, and percent Fusarium damaged kernels were calculated for each plot. After harvest, no statistical differences among treatments were observed for yield. Rather, yield was most impacted by the year-by-location interaction. While there was no statistical increase in yield for any treatment, there were statistical differences among treatments for both test weight and FDKs. The data indicated that Miravis Ace applied at Feekes 10.5.1 may not result in a yield increase but may result in a greater revenue due to less product discarded through dockage from low test weight or the presence of FDK at grain elevators. With the variability that occurs with FHB epidemics from year to year, it is important to consider the most effective products available to reduce quality losses from low test weights and high FDK percentages may not result in significant increases in yield.

Characterizing the Resistance of *Fusarium graminearum* to Quinone Outside Inhibitor Fungicides

Sheila M. P. Andrade [1], Guilherme R. Augusti [1], Gabriel F. Paiva [1], Heraldo R. Feksa [2], Dauri J. Tessmann [3], Franklin J. Machado [1], Eduardo S. G. Mizubuti [1], Emerson M. Del Ponte [1]

[1]Depto. de Fitopatologia, Universidade Federal de Viçosa, 36470-900 Viçosa, MG, Brazil
[2]Fundação Agrária de Pesquisa Agropecuária – FAPA, Entre Rios, 85139-400, Guarapuava, PR, Brazil
[3]Depto. de Agronomia, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil
Corresponding Author: Emerson Del Ponte, delponte@ufv.br

Abstract

Brazilian wheat farmers rely on fungicides to protect fields against several foliar and flowering diseases, including Fusarium head blight caused mainly by *Fusarium graminearum*. A range of active ingredients are used in isolation or dual premixes that include a demethyl (DMI) and a quinone outside inhibitor (QoI) fungicides. Comprehensive information on fungicide resistance of *F. graminearum* is available only for DMIs, while for QoI, data are scarce and results inconsistent. We studied 225 strains collected from two states in southern Brazil (RS and PR), in relation to their response to two QoIs. In vitro sensitivity of F. graminearum isolates was assessed through the conidia germination assay. The effective concentration leading to 50% inhibition (EC50) of conidial germination was obtained based on three-parameter Weibull function. Isolates, in which the EC50 was not previously determined, were screened using discriminatory doses (DD) for both fungicides. Molecular analysis of the cytochrome b (cytb) gene was performed, and nine isolates were selected and sequenced. Results showed that the median EC50 value for azoxystrobin (n = 25) was 2.20 µg mL^{-1} in the PR collection and 4.04 µg mL^{-1} in the RS collection. For pyraclostrobin (n =50), the median EC50 was 0.28 µg mL^{-1} in the PR collection and 0.24 µg mL^{-1} in the RS collection. A comparison between the two fungicides showed significant differences between them and pyraclostrobin was more fungitoxic than azoxystrobin. Evidence of cross resistance when correlating the EC50 values of the two fungicides could not be detected. Screening using DD for azoxystrobin, from PR (n = 75) and RS (n = 100) allowed the detection of 50% and 28% as less sensitive strains, respectively. Using DD for pyraclostrobin, 33% and 18.8% were classified as less sensitive in the PR and RS collections. In RS, the frequency of less sensitive isolates increased over five years (2007-2011). Sequence alignments showed no point mutation in any target spot (F129L, G137R, G143A) even in isolates with highly EC50. These results represent an important step towards monitoring the resistance to two most used QoIs in commercial premixes targeting FHB control in Brazil.

Acknowledgement and Disclaimer

Development of a Lightweight Quadruped for Real-Time FHB Phenotyping Under Variable Field Conditions

David Aviles[1], Ce Yang[2], An Min[2], Cory Hirsch[3], Brian J. Steffenson[3]

[1]University of Minnesota - Duluth, Department of Mechanical Engineering, Duluth, MN
[2]University of Minnesota - Twin Cities, Department of Bioproducts and Biosystems Engineering, Saint Paul, MN
[3]University of Minnesota, Department of Plant Pathology, Saint Paul, MN
Corresponding Author: Ce Yang, ceyang@umn.edu

Abstract

In Fusarium Head Blight (FHB) disease nurseries, overhead irrigation is typically used to maintain sufficient moisture on the spikes of wheat and barley plants to achieve optimal infection levels. However, these irrigation treatments can often lead to excessively wet and muddy field conditions, which may hinder the performance of many ground-based, high-throughput phenotyping platforms. A lightweight quadruped with walking capability was developed to handle phenotyping tasks under variable field conditions. The flexible quadruped prototype is controlled by a Raspberry Pi 4 microcontroller and contains 12 servo motors to achieve the needed degrees of freedom on the legs of the robotic dog. One more servo motor is used to control a Pi camera to capture images while traveling in the trial plots. The frame of the quadruped prototype was designed and printed by a 3D printer with durable polylactic acid plastic materials with 80% filling, and the shell of the quadruped was 3D printed with the same material with 100% filling. The size of the quadruped is 14-in in width, 21-in in length and 12-in tall when standing, which will accommodate with the spacing and plant height in the FHB disease nurseries and breeding plots. We have developed robust disease detection algorithms that work with images obtained from different distances and angles as illuminated by natural sunlight. By integrating the quadruped platform with improved lightweight edge-computing models that are currently under development in the AgRobotics Laboratory, we expect to achieve real-time FHB phenotyping under any type of weather and field conditions.

Effects of Environmental Conditions after Fusarium Head Blight Visual Symptom Expression on Deoxynivalenol-3-glucoside Accumulation in Wheat

Wanderson Bucker Moraes[1], Paul B. Schwarz[2], Byung-Kee Baik[3], Laurence V. Madden[1], and Pierce A. Paul[1]

[1]Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691
[2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108-6050
[3]USDA-ARS-CSWQRU, Soft Wheat Quality Laboratory, Wooster, OH 44691
Corresponding Author: Pierce Paul, paul.661@osu.edu

Abstract

Fusarium head blight (FHB) of wheat, caused by the fungus *Fusarium graminearum*, leads to grain contamination with mycotoxins such as deoxynivalenol (DON). Although FHB intensity is often positively correlated with DON, this relationship is quite variable and breaks down under certain conditions. One possible explanation for this could be the conversion of DON to DON-3-glucoside (D3G), a masked form of the toxin that is often missed by common DON testing methods. The objective of this study was to quantify the effects of temperature (20, 25, and 30°C), relative humidity (RH, 70, 80, 90, and 100%), and pre-harvest rainfall patterns (continuous [Rain1], intermittent, or no supplemental rainfall [check]) on DON, D3G, and their relationship in grain from wheat spikes with different levels of FHB index (IND). D3G levels were higher in grain from spikes exposed to 100% RH than to 70, 80, or 90% RH at 20 and 25°C across all tested IND levels. Mean D3G was highest at 20°C. There were significant positive linear relationships between DON and D3G. Rainfall resulted in significantly higher mean D3G than the rain-free check. All rainfall treatments induced pre-harvest sprouting, as indicated by low falling numbers (FN), with Rain1 having the lowest mean FN. There were significant positive relationships between the rate of increase in D3G per unit increase in DON (a measure of conversion) and sprouting. As FN decreased, the rate of D3G conversion increased, and this rate of conversion per unit decrease in FN was greater at low than at high mean DON levels. These results provide strong evidence that moisture after FHB visual symptom development influenced DON-to-D3G conversion. To our knowledge, this study was the first to: 1) associate cooler, humid conditions with high levels of D3G contamination of wheat grain, 2) quantify associations among rainfall after visual FHB symptom development, D3G, DON, DON-to-D3G conversion, and pre-harvest sprouting across different baseline IND levels, and 3) model D3G contamination of grain as a function of DON and FN. These constitute valuable new information for understanding this complex disease-toxin system.

Temperature, Moisture, Grain Development, and Harvesting Strategy Effects on Zearalenone Contamination of Grain Harvested from Fusarium Head Blight-affected Wheat Spikes

Wanderson Bucker Moraes[1], Paul B. Schwarz[2], Laurence V. Madden[1], and Pierce A. Paul[1]

[1]Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691
[2]Department of Plant Sciences, North Dakota State University, Fargo, ND 58108-6050
Corresponding Author: Pierce Paul, paul.661@osu.edu

Abstract

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum*, is associated with grain contamination with mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEA), a potent estrogenic secondary metabolite. Unlike DON, ZEA is rarely the focus to FHB-related research, and as such, less is known about factors affecting its production during FHB epidemics. The objective of this study was to quantify the contamination of wheat grain with ZEA as influenced by temperature (20, 25, and 30°C), relative humidity (RH, 70, 80, 90, and 100%), FHB index (IND), grain maturation, simulated last-season rainfall (0, 5, and 10 days of pre-harvest rainfall), and harvest timing. ZEA concentrations were low during early stages of grain development (25-31 days after anthesis [DAA]) but rapidly increased at latter stages (35 to 51 DAA) in field experiments, particularly under rainy conditions. Five or ten consecutive days with simulated rainfall shortly before harvest greatly increased ZEA contamination. Similarly, extremely high levels of ZEA were observed in grain from spikes exposed to 100% RH across all tested temperatures and mean IND levels under controlled conditions. Interestingly, at RH ≤ 90%, ZEA concentrations were very low at all tested temperatures, even at IND above 90%. Temperature affected ZEA contamination at 100% RH, with significantly higher mean levels of the toxin at 20 and 25°C than at 30°C. Grain harvested early and not exposed to simulated rainfall had lower mean ZEA than grain harvested late and subjected to pre-harvest rainfall. This study was the first to associate ZEA contamination of grain from FHB-affected wheat spikes with temperature and moisture and show through designed experiments that early harvest could be a useful strategy for reducing ZEA contamination. These results constitute new, valuable information for understanding this complex disease-toxin system and developing guidelines for managing FHB and minimizing grain yield and quality losses. In particular, finding from studies of conditions driving the production of ZEA will be helpful for predicting when this mycotoxin will likely be a concern and warrants more attention in terms of monitoring and testing.

Baseline Fungicide Sensitivity to Pydiflumetofen in *Fusarium graminearum* Isolated from Wheat Across 16 States

J.A. Cinderella[1], K. Anderson[2], G.C. Bergstrom[3], W.W. Bockus[2], C.A. Bradley[4], M. Breunig[5], E. Byamukama[6] M.I. Chilvers[5], C. Cowger[7], T.R. Faske[8], A.J. Friskop[9], J. Kelly[8], N.M. Kleczewski[10], S. Mideros[10], P.A. Paul[11], T. Price[12], N. Rawat[13], J. Rupp[2], S. Shim[14], J. Stevens[15], D. Telenko[14], A.M. Koehler[1]

[1]University of Delaware, Plant and Soil Sciences, Georgetown, Delaware
[2]Kansas State University, Department of Plant Pathology, Manhattan, Kansas
[3]Cornell, Plant Pathology and Plant Microbe Biology, Ithaca, New York
[4]University of Kentucky, Department of Plant Pathology, Lexington, Kentucky
[5]Michigan State University, Department on Plant, Soil and Microbial Sciences, East Lansing, Michigan
[6]South Dakota State University, Department of Agronomy, Horticulture and Plant Science, Brookings, South Dakota
[7]North Carolina State University, Department of Entomology and Plant Pathology, Raleigh, North Carolina
[8]University of Arkansas, Department of Entomology and Plant Pathology, Fayetteville, Arkansas
[9]North Dakota State University, Plant Pathology, Fargo, North Dakota
[10]Growmark Inc., Agronomy Services Plant Pathology, Entomology Specialist, Technical Agronomist East and Southern Illinois, Illinois
[11]Ohio State University, Department of Plant Pathology, Columbus, Ohio
[12]Louisiana State University, Department of Plant Pathology and Crop Physiology, Winnsboro, Louisiana
[13]University of Maryland, Plant Science & Landscape Architecture, College Park, Maryland
[14]Purdue University, College of Agriculture, West Lafayette, Indiana
[15]University of Nebraska-Lincoln, Department of Plant Pathology, Lincoln, Nebraska
Corresponding Author: Joseph Cinderella, jcinder@udel.edu

Abstract

In 2019, pydiflumetofen (Syngenta, Switzerland) was registered for use in the management of FHB in wheat, barley, rye, oats, and triticale. Pydiflumetofen is a new succinate dehydrogenase inhibitor (SDHI) and an in vitro screening of 65 isolates was conducted to establish baseline sensitivities in *F. graminearum* isolated from wheat. Isolates were submitted from 16 states representing numerous small grain production regions of the United States. The isolate set screened included 59 isolates collected in 2020-21 and 6 historic isolates collected from 1991-2013 that were never exposed to pydiflumetofen. The effective concentration to reduce mycelial growth by 50% (EC50) was determined for each isolate with a poison plate mycelial growth assay using fungicide-amended PDA plates at 0, 0.01, 0.05, 0.25, 1.0, and 5.0 µg/mL. Mycelial growth EC50 values averaged by state for 2020-21 isolates ranged from 0.09 to 0.52 µg/mL. The average EC50 value of post 2019 isolates was 0.25 µg/mL and the historic isolates 0.21 µg/mL. This study demonstrates the inhibition of mycelial growth by this new chemistry in vitro and establishes preliminary baseline sensitivity values of *F. graminearum* isolates from 16 states across the U.S.

Optimal Timing to Apply Fungicide to Winter Barley for FHB and DON Reduction

Christina Cowger[1, 2] and Logan Clark[1]

[1]USDA-ARS Plant Science Research Unit, Raleigh, NC
[2]North Carolina State University Department of Entomology & Plant Pathology, Raleigh, NC
Corresponding Author: Christina Cowger, Christina.Cowger@usda.gov

Abstract

In a Fusarium head blight (FHB) epidemic, the combination of cultivar resistance and effective fungicide application is expected to provide maximum reduction of FHB and DON in winter barley. Especially for malting end-use, there is a very low tolerance for *Fusarium* infections in barley grain. However, the best timing for fungicide application to manage FHB in barley is not well understood. An experiment on fungicide efficacy and timing was conducted at Raleigh in a misted, inoculated nursery with 4 trials, one per year from 2016 to 2020. Three winter barley cultivars were utilized that had different levels of FHB resistance: Violetta (MR), Thoroughbred (MR/MS), and Flavia (S). Violetta and Flavia were medium-late two-row malting cultivars, while Thoroughbred was a medium-maturing six-row feed type with acceptable malt quality. Inoculation was provided via *F. graminearum*-infected corn spawn, and the experiment was mist-irrigated. Fungicides were Miravis® Ace (adepidyn + pydiflumetofen), Prosaro® (prothioconazole + tebuconazole), and, starting in the second year, Caramba® (metconazole). The three timings for fungicide application were: 50% spike emergence (Feekes 10.3), 100% spike emergence (Feekes 10.5), and 100% emergence + 6 days. The experiment had a split-plot design with four replicate blocks in each year. Over the four years, two of the epidemics had high intensity and two had low intensity. Combining the data across years, cultivars, and fungicides, the latest timing was significantly superior at reducing DON, followed by the intermediate timing ($P < 0.05$). The early timing was least effective in reducing DON and was not significantly different from the untreated control. In an assay of percent kernels infected with *Fusarium*, the results were the same: across years, cultivars and fungicides, mean infected kernel percentages were significantly reduced by the late application, followed by the intermediate application, and the early application was not different from the untreated control ($P < 0.05$). The three fungicides performed similarly at the early timing except that Miravis Ace reduced visible symptoms more than did Caramba ($P < 0.05$). Taken together, these results indicate that the recommendation traditionally given for spring barley to apply an FHB-targeted fungicide at full spike emergence (Feekes 10.5) may not be best for winter barley. Waiting until 6 days after Feekes 10.5 to apply a fungicide appears preferable for reducing both DON and percentages of barley kernels infected with *Fusarium*.

Evaluation of Conventional and Organic Fungicide Applications Plus Cultivar Resistance to Reduce FHB and DON Infection of Barley in Vermont

Heather Darby and Hillary Emick

University of Vermont, Department of Plant and Soil Sciences, Burlington, VT
Corresponding Author: Heather Darby, heather.darby@uvm.edu

Abstract

Public interest in sourcing local foods has extended into beverages leading to a rapid expansion of the northeast malting industry. This has provided farmers with new market opportunities and many of these markets are interested in purchasing certified organic barley. However, all farmers are struggling to produce barley that is not infected with FHB and DON. Hence integrated management strategies are essential for managing yield and quality losses from FHB. The objective of this study was to evaluate the individual and interactive effects of moderately resistant cultivars and application timings of conventional fungicides and an organic copper fungicide on barley yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) in Vermont. The trial was conducted in Alburgh, Vermont in 2020 on a silt loam soil. The experiment was a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the fungicide treatments as subplots. The two spring barley varieties were 'Robust' (susceptible to FHB) and 'Genesis' (moderately resistant to FHB). The fungicide treatments included Caramba®, Miravis® Ace, Prosaro SC®, and Champ Ion (an organic fungicide) applied alone or in combination with each other. Fungicides were applied at two timings (Feekes growth stage, FGS 10.1 and/or four days after heading). Treatments were inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. An untreated control was sprayed with water.Yield, test weight, and FHB incidence and severity did not differ statistically by treatment. There were significant differences between treatments for DON concentrations. All treatments and timings, including the control and the Fusarium inoculated plots, had DON concentrations below 1 ppm. Eight treatments had DON concentrations less than that of the uninoculated control (0.19 ppm). These included Miravis Ace at heading, Miravis Ace followed by Caramba, Miravis Ace followed by Prosaro, Miravis Ace at Feekes 10.3, Miravis Ace post heading, Caramba, and Prosaro. The Fusarium inoculated plots had the highest DON concentrations as expected, and they were statistically similar to only the three ChampION treatments and the control.The DON concentrations in Genesis (0.08 ppm) were significantly lower than the DON concentration in Robust barley (0.22 ppm). When fungicide applications in this trial are compared, the results of this trial suggest that Miravis Ave applied at heading, whether combined with other products or not, was the most successful at reducing DON in comparison to an uninoculated control.

Objective

To evaluate the individual and interactive effects of moderately resistant cultivars and application timings of conventional fungicides and an organic copper fungicide on barley yield and the integrated management of Fusarium head blight (FHB) and deoxynivalenol (DON) in Vermont.

Introduction

Public interest in sourcing local foods has extended into beverages leading to a rapid expansion of the northeast malting industry. This has provided farmers with new market opportunities and many of these markets are interested in purchasing certified organic barley. However, all farmers are struggling to produce barley that is not infected with FHB and DON. Hence integrated management strategies are

essential for managing yield and quality losses from FHB. Most farmers in New England have experienced significant crop loss from FHB and some farmers have already stopped growing barley. At present, few farmers are specifically selecting varieties for resistance to FHB and even fewer are combining host resistance with fungicide applications. There has been little to no research conducted to evaluate organic approved fungicides. Other regions have shown that the use of a well-timed fungicide is an important management tool when suppressing FHB in barley production. In Vermont during 2020 we observed the disease and yield impact of cultivar susceptibility, inoculation with *Fusarium graminearum*, and treatment with fungicides (organic and conventional) at two timings.

Materials and Methods

The trial was conducted in Alburgh, VT in 2020. The soil type was a Benson silt loam soil. The plot size was 5 x 20 ft including seven rows with 7-in spacing. Planting occurred on 9-April 2020. Main plots were sown with barley at 125 lb ac^{-1} with a Great Plains grain drill (Salinas, KS). The experiment was set up as a completely randomized block design with a split-plot arrangement, with cultivar as the main plot and the fungicide treatments as subplots, randomized in four replicated blocks. The two spring barley varieties were 'Robust' (susceptible to FHB) and 'Genesis' (moderately resistant to FHB). Fungicide treatments are shown in Table 1. The first fungicide application (with surfactant at 0.125% V/V) was applied at heading (Feekes growth stage, FGS 10.1). After the fungicide had dried, plots were spray-inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ ml) to augment the development of FHB. The second fungicide application occurred four days after heading and inoculated with a conidial suspension of *F. graminearum* (40,000 conidia/ml) after the fungicide had dried. Fungicide and *F. graminearum* treatments were applied with a CO_2 backpack sprayer with paired TJ-60 8003vs nozzles mounted at an angle (30° from horizontal) forward and backward, 20-in. apart, pressurized at 30 psi, and calibrated to deliver 20 gal/A. Grain was harvested using an Almaco plot combine (Nevada, IA).. Grain moisture, plot yield, and test weight were recorded. Yield and test weight were adjusted to bushel ac^{-1} at 13.5% moisture. Analysis of DON content in grain was conducted at the University of Vermont Cereal Grain Testing Laboratory located in Burlington, VT. Treatment means were calculated, subjected to analysis of variance, and separated by Fisher's protected LSD test (P = 0.05).

Results and Discussion

Interactions

There were no variety by fungicide treatment interactions indicating that the varieties responded similarly to the fungicide treatments.

Impact of Fungicide and Timing

Harvest metrics are shown in Table 2 and DON concentrations and FHB severity are shown in Table 3. Harvest moisture, test weight, yield,100 kernel weights, and FHB incidence and severity did not differ statistically by treatment. There were significant differences between treatments for DON concentrations (Table 3).

All treatments and timings, including the control and the *Fusarium* inoculated plots, had DON concentrations below the 1 ppm threshold recommended by the FDA. It is important to note that DON results were below the detection minimum of 0.5, which means these results may not be precise. Eight treatments had DON concentrations less than that of the uninoculated control (0.19 ppm). These included Miravis Ace at heading, Miravis Ace followed by Caramba, Miravis Ace followed by Prosaro, Miravis Ace at Feekes 10.3, Miravis Ace post heading, Caramba, and Prosaro. The treatment with the lowest DON concentration was Miravis Ace at heading at 0.03 ppm, which was significantly lower than

all ChampION treatments, and the *Fusarium* inoculated plots. The *Fusarium* inoculated plots had the highest DON concentrations as expected, and they were statistically similar to only the three ChampION treatments and the control. All treatments were similar to the control, which is not surprising considering it was a hot and dry June and July, with poor conditions for DON.

There were no significant differences between treatments in the severity of FHB infection and incidence of infection. Caramba applied at heading had the lowest in average FHB severity (7.66%), and Prosaro applied at headed had the lowest FHB incidence (0.02%). The incidence of infected heads refers to the proportion of barley spikes showing any sign of FHB infection compared to the uninfected spikes in that treatment. The average infected head severity refers to the extent to which infected heads are affected by FHB symptoms. The trial average for FHB severity was 13.0% and the average incidence of FHB infection was 0.049%.

Impact of Variety
There were significant differences between varieties in harvest moisture, test weight, 100 kernel weights, yield, and DON concentrations (Table 4). There were no significant differences by variety in FHB severity and incidence of FHB infection.

Robust had a significantly lower harvest moisture and higher test weight than Genesis. Both varieties had to be dried down for storage. Genesis yielded 261 lbs ac^{-1} higher than Robust. The DON concentrations in Genesis (0.08 ppm) were significantly lower than the DON concentration in Robust barley (0.22 ppm), although both were well below the FDA threshold of 1 ppm. FHB severity and incidences were similar between the two varieties.

Higher levels of *Fusarium* infection and resulting DON vomitoxin concentrations in grain are associated with cool and damp weather conditions at the time of grain fill and heading. While early spring weather was slightly cooler than normal, precipitation was below the 30-year average during the entire growing season, and temperatures were warmer than average at grain fill in June and July. These conditions were not conductive for the development of the DON vomitoxin or other fungal pathogens. All fungicide applications reduced DON concentrations compared to the plots that were inoculated with *Fusarium* but not treated with fungicide. Some fungicide applications were statistically similar to the *Fusarium* inoculated plots, but that does not mean they would not be effective in a year with higher DON concentrations. These similarities can likely be attributed to the low DON concentrations overall due to the weather conditions. When fungicide applications in this trial are compared, the results of this trial suggest that Miravis Ave applied at heading, whether combined with other products or not, was the most successful at reducing DON in comparison to an uninoculated control. However, it is important to note that the DON test has a detection range of 0.5 to 5 ppm, and all DON results in this trial were lower than the recommended range for accuracy.

Table 1. Fungicide treatments, active ingredients and rates applied.

Fungicide treatments	Company	Fungicide active ingredient	Application rates
Control *Fusarium graminearum*			Water 40,000 spores/ml
Prosaro SC®	Bayer CropScience	Prothioconazole + tebuconazole	6.5 fl oz ac^{-1} + Induce at 0.125% V/V
Caramba®	BASF Ag Products	Metconazole	14 fl oz ac^{-1} + Induce at 0.125% V/V
Champ ION^{++}	NuFarm	Copper hydroxide	1.5 lbs ac^{-1}
Mirvais Ace®	Syngenta	Adepidyn/Pydiflumetofen) + Propiconazole	13.7 fl oz ac^{-1} Induce at 0.125% V/V

Table 2. Main effect of fungicide+timing on moisture, test weight, and grain yield at Alburgh, VT, 2020.

Fungicide + Timing Treatment	Harvest moisture	Test weight	Yield at 13.5% moisture	100 kernel weight
	%	lbs bu^{-1}	lbs ac^{-1}	g
Miravis Ace Post-Heading	14.20	45.7	3228	4.58
Miravis Ace Feekes 10.3	14.13	45.3	3406	4.51
Miravis Ace Heading	14.09	45.3	3369	4.49
Miravis Ace (Heading) & Caramba (Post)	15.13	45.8	3962	4.55
Miravis Ace (Heading) & Prosaro (Post)	14.50	45.1	3814	4.50
Caramba Heading	14.41	44.4	3314	4.46
ChampION Post-Heading	13.95	46.8	3746	4.53
ChampION Heading & Post-Heading	14.13	45.8	3946	4.54
ChampION Heading	13.96	45.3	3392	4.41
Inoculated *Fusarium* spores	14.40	46.2	3656	4.65
Prosaro Heading	13.76	45.6	3240	4.59
Non-sprayed, non-inoculated control	14.01	45.3	3281	4.40
LSD (p=0.10)[†]	NS[‡]	NS	NS	NS
Trial Mean	14.2	45.6	3530	4.52

[†] LSD- Least significant difference at p=0.10.
[‡]NS- Not significant.

Table 3. Main effect of fungicide+timing on deoxynivalenol (DON) contamination and FHB severity and incidence at Alburgh, VT, 2020.

Fungicide + Timing Treatment	DON	Average FHB severity	Incidence of FHB infected heads
	ppm	%	%
Miravis Ace Post-Heading	0.11[abc]	10.2	0.041
Miravis Ace Feekes 10.3	0.07[ab]	11.8	0.062
Miravis Ace Heading	0.03[a]	12.1	0.036
Miravis Ace (Heading) & Caramba (Post)	0.04[a]	15.5	0.070
Miravis Ace (Heading) & Prosaro (Post)	0.05[a]	11.0	0.058
Caramba Heading	0.14[abc]	7.66	0.026
ChampION Post-Heading	0.27[cd]	15.3	0.054
ChampION Heading & Post-Heading	0.26[cd]	10.9	0.073
ChampION Heading	0.22[bcd]	14.9	0.050
Inoculated *Fusarium* spores	0.33[d]	14.4	0.064
Prosaro Heading	0.14[abc]	14.9	0.020
Non-sprayed, non-inoculated control	0.19[abcd]	17.0	0.033
LSD (0.10) [‡]	0.153	NS[¥]	NS
Trial Mean	0.15	13.0	0.049

[†]Treatments within a column with the same letter are statistically similar.
[‡]LSD- Least significant difference.
[¥]NS- Not significant.

Table 4. Main effect of cultivar on deoxynivalenol (DON) concentration, grain yield, and test weight at Alburgh, VT, 2020.

Variety	Harvest moisture	Test weight	Yield @13.5% moisture	100 kernel weight	DON	Average FHB severity	Incidence of FHB infected heads
	%	lbs bu⁻¹	lbs ac⁻¹	g	ppm	%	%
Genesis	15.4	45.1	**3660**	**4.96**	**0.08**	13.4	0.056
Robust	**13.1**	**46.0**	3399	4.07	0.22	**12.6**	**0.040**
LSD (0.10) [†]	0.33	0.55	250	0.082	0.07	NS[‡]	NS
Trial Mean	14.2	45.6	3530	4.52	0.15	13.0	0.049

[†]LSD- Least significant difference.
[‡]NS- Not significant.

Fusarium Head Blight Resistance Exacerbates Nutritional Loss of Wheat Grain at Elevated CO$_2$

William Hay[1], James Anderson[2], Susan McCormick[1], Martha Vaughan[1]

[1]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Unit, 1815 N, University Street, Peoria, IL 61604
[2]Department of Agronomy & Plant Genetics, University of Minnesota, St. Paul, MN 55108
Corresponding Author: Martha Vaughan, martha.vaughan@usda.gov

Abstract

The nutritional quality of wheat is jeopardized by rising atmospheric carbon dioxide (CO$_2$) and the associated emergence and enhanced virulence of plant pathogens. To evaluate how disease resistance traits impact wheat nutritional content, 15 wheat cultivars with varying levels of resistance to Fusarium Head Blight (FHB) were grown at ambient and elevated CO$_2$. Although all wheat cultivars had increased yield when grown at elevated CO$_2$, on average the nutritional content of FHB moderately resistant (MR) cultivars was impacted more than susceptible cultivars. At elevated CO$_2$, the MR cultivars had more significant differences in plant growth and nutritional content. Furthermore, changes in protein, starch, phosphorus, and magnesium content were correlated with the cultivar FHB resistance rating, with more FHB moderately resistant cultivars having greater changes in nutrient content. This is the first report of a correlation between the degree of plant pathogen resistance and grain nutritional content loss in response to elevated CO$_2$. Our results demonstrate the importance of identifying wheat cultivars that can maintain nutritional integrity and FHB resistance in future atmospheric CO$_2$ conditions.

Utilizing a High-Throughput Field Based Rover for High Fidelity and High Temporal Resolution of FHB Phenotyping

Cory D. Hirsch[1], Julian Cooper[1], Joseph Wodarek[2], Rae Page[1], An Min[3], Jaafar Abdulridha[3], Ruth Dill-Macky[1], Kevin P. Smith[4], James A. Anderson[4], Ce Yang[3], Brian J. Steffenson[1]

[1]University of Minnesota, Department of Plant Pathology, Saint Paul, MN
[2]University of Minnesota, Northwest Research and Outreach Center, Crookston, MN
[3]University of Minnesota, Department of Bioproducts and Biosystems Engineering, Saint Paul, MN
[4]University of Minnesota, Department of Agronomy and Plant Genetics, Saint Paul, MN
Corresponding Author: Cory Hirsch, cdhirsch@umn.edu

Abstract

Fusarium head blight (FHB), a devastating disease of wheat and barley, can markedly reduce yield and grain quality. Current disease mitigation strategies including cultural practices, fungicide application, and planting resistant varieties rely on accurate and efficient phenotyping of FHB severity. Most projects that include field FHB screening utilize manual phenotyping methods, which do not provide adequate resolution for detecting small severity differences, is laborious, low throughput, and have rater bias and inter-rater variation. Increasing the throughput of FHB assessments in the field is necessary for continued improvement of varieties, efficacy of management practices, understanding disease development, and monitoring of FHB. We have deployed a sophisticated motorized phenotyping rover for high temporal and fidelity FHB detection in wheat and barley across resistance breeding and germplasm evaluation trials. This rover greatly increases the speed, accuracy, reliability, and automation of FHB severity assessment across experiments and scales. This past summer we used the rover to collect high-fidelity, field-based images over short time intervals (4 days) across hundreds of wheat and barley genotypes at two different field locations. After top and side images were acquired, machine learning algorithms are being used to identify spikes in each plot and develop highly accurate models for FHB detection and progression. Despite being at an early stage of development, these transformational, unique, and streamlined methods are markedly increasing the speed, accuracy, frequency of FHB phenotyping, and directly increasing the number of spikes evaluated per plot. The phenotyping pipeline and FHB assessment models generated from this work will be applicable to any researcher who could benefit from an easy to use, high throughput phenotyping rover for field FHB detection in wheat and barley. By combining high temporal and high-fidelity field-based phenotyping, we envision eliminating previous phenotyping limitations and foresee greatly increasing the phenotyping capabilities of programs with respect to the amount of germplasm screened for FHB resistance, the number of spikes evaluated per plot, and the precision of measurements.

Fungicide Sensitivity Towards the Predominant Pathogen Species of Fusarium Head Blight in Cereals from Manitoba, Western Canada

M. Nazrul Islam and Xiben Wang

Agriculture and Agri-Food Canada (AAFC), Morden Research and Development Centre, 101 Route 100, Morden, Manitoba, R6M 1Y5, Canada
Corresponding Author: M. Nazrul Islam, naz.islam@agr.gc.ca

Abstract

In recent years, *Fusarium* head blight (FHB) is the most aggressive fungal disease and growing threat to the economic sustainability of cereal crop production in Manitoba due to yield loss and downgrade grain quality as several mycotoxins contamination occurred in FHB infected grains. In our lab, *Fusarium poae* (*Fp*) and *F. graminearum* (*Fg*) were frequently isolated from FHB infected grains of barley and oats. Four fungicides, namely, caramba (metconazole), prosaro (prothioconazole+tebuconazole), proline (prothioconazole), folicur (tebuconazole) were tested against representative isolates of *Fp* (n=13) and *Fg* (n=13). These 26 isolates were selected from nearly 300 *Fp* and *Fg* strains isolated from commercial barley and oat fields of Manitoba, based on chemotypes diversity, virulence factors, mycelial growth performances and genotyping by sequencing (GBS). Fungicide sensitivity was quantified by measuring the radial growth of the cultures on PDA (potato dextrose agar) media amended at two concentrations of fungicides (active ingredient: 0.01 and 0.10 mg/L). Two-way analyses of variance (ANOVA) revealed on average, *Fp* isolates were statistically less sensitive to all four fungicides, compared to *Fg* regardless of doses. Specifically, the redial growth of *the Fp*-MRC585 strain cannot be restricted (24%, 5%, 2%, 3% inhibition) by the higher concentration of caramba, prosaro, proline and folicur amended plates, respectively. Similarly, the *Fg*-MRC572 strain also cannot be restricted (< 10% inhibition) to folicur, not the other three fungicides. This study confirmed the Caramba was consistent in controlling both *Fp* and *Fg* isolates tested. This *in-vitro* assay suggests that the selective pressure exerted by these commonly used fungicides may impact the population dynamics of FHB species, most likely the increasing insensitivity towards newly evolving/shifting *F. poae* communities in the regions due to different intrinsic characteristics and tolerance ability of these field isolates. The current results would assist Manitoba small grain producers to select effective fungicides in the integrated FHB management strategies.

Effect of Fungicide and Variety Resistance on the Suppression of Fusarium Head Blight and Deoxynivalenol in Dryland Hard Red Spring Wheat

LeAnn R. Lux[1], Jessica M. Halvorson[1], Bryan C. Hansen[1], Scott Meyer[1], Scott Fitterer[2], Dave Carruth[2], and Andrew Friskop[1]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58102 U.S.A
[2]BASF North Dakota Research Farm, Davenport, ND 58021 U.S.A
Corresponding Author: LeAnn Lux, leann.lux@ndsu.edu

Abstract

Fusarium head blight (FHB) continues to be one of most significant diseases affecting hard red spring wheat (HRSW) production in North Dakota. The implementation of integrated management practices such as the use of fungicides and host resistance, can drastically decrease yield and cause quality losses. However, as new fungicides and wheat varieties are released and disease risk fluctuates each growing season, the use of both management practices may not be necessary every year. The objective of this research was to evaluate fungicides and varietal resistance to suppress FHB and DON in HRSW in varying FHB risk dryland environments. From 2019 to 2021, seven integrated management field experiments were conducted at three locations in eastern North Dakota. The field experiments were conducted in a randomized complete block design, with a split plot arrangement. Two HRSW varieties, WB-Mayville (susceptible) and ND-VitPro (moderately resistant) served as the main plots and seven fungicide treatments including a non-treated control served as the sub-plots. Five single fungicide application treatments consisted of Prosaro® (prothioconazole+tebuconazole), Caramba® (metconazole), Sphaerex™ (metconazole+prothioconazole), Folicur® (tebuconazole), and Miravis® Ace (pydiflumetofen+propiconazole) and were applied at early anthesis (Feekes 10.5.1). One fungicide treatment of Sphaerex, was applied three to seven days after early anthesis. Individual plots were inoculated with Fusarium infected corn spawn at jointing (Feekes 6) to increase disease risk. DON, incidence, severity and Fusarium damaged kernel (FDK) data was used to create a DISK value. The DISK value was used to statistically categorize the seven location*years into four unique FHB management environments: high, moderate, low, and no risk. Additionally, yield and test weight data was collected to evaluate yield response from a fungicide application. In high and moderate environments, the moderately resistant variety significantly reduced DISK In the high environment, statistical differences were also observed among treatments. All fungicide treatments significantly reduced DON levels, except Folicur. In low FHB environments, Miravis Ace and both application timings of Sphaerex significantly reduced DON and had higher yield than the NTC. Additional analyses on the use of fungicides in varying FHB management environments will help refine decisions for hard red spring wheat producers.

Risk Prediction Models to Manage Fusarium Head Blight Epidemics in Canadian Prairie Cereal Production

Taurai T. Matengu[1], Paul Bullock[1], Manasah S. Mkhabela[1], Francis Zvomuya[1], Maria A. Henriquez[2], Timi Ojo[3], Réjean Picard[4], Raul Avila[5], Alireza Akhavan[5], and Michael Harding[6]

[1]Department of Soil Science, University of Manitoba, Winnipeg, MB, Canada
[2]Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB, Canada
[3]Soil and Ag Weather Surveillance, Manitoba Agriculture and Resource Development, Winnipeg, MB, Canada
[4]Ag Adaptation Specialist - Crops, Manitoba Agriculture and Resource Development, Morden, MB, Canada
[5]Ministry of Agriculture, Government of Saskatchewan, Regina, SK, Canada
[6]Plant and Bee Health Surveillance, Alberta Agriculture and Forestry, Brooks, AB, Canada
Corresponding Author: Paul Bullock, Paul.Bullock@umanitoba.ca

Abstract

Forecasting Fusarium head blight (FHB) epidemics based on visual disease observation in the field assists producers in preventing grain yield and quality loss by identifying where and when fungicide spray is required. However, visual symptoms do not always reflect the actual Fusarium-damaged kernels (FDK) and deoxynivalenol (DON) levels in the grain. Therefore, this study aims to develop weather-based risk models for predicting FHB index (FHBi), FDK, and DON in spring wheat, winter wheat, barley, and durum around the flowering period across three Canadian Prairie provinces. Disease data collected from 15 sites in western Canada in 2019 and 2020 were binary classified as either epidemic or non-epidemic using threshold values of 5% for FHBi (all crops), 1 ppm for DON (all crops) and 0.2, 0.3, 0.8, and 2% FDK for barley, spring wheat, winter wheat, and durum, respectively. Kendall correlation and stepwise logistic regression analysis identified combinations of temperature, relative humidity (RH), precipitation, and solar radiation at 4, 7, 10, 14 days pre-anthesis, and 3 days pre to 3 days post-anthesis that most accurately predicted the measures of FHB risk. RH was frequently the most highly correlated weather variable across crop types for FDK and DON. The prediction accuracy of the models ranged between 75 and 81, 77 and 85, 78 and 79% for FHBi, FDK, and DON, respectively. Dry and hot conditions during the 2019 and 2020 growing seasons likely suppressed FHB disease pressure in western Canada. These models, which will incorporate additional 2021 plot and field data, will be integrated into an online map viewer that will provide early warning of potential FHBi, FDK, and DON epidemics in cereal crops on the Canadian prairies.

Introduction

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum* in western Canada, is a significant threat to wheat and barley yield and quality, especially in warm and humid conditions (Tekauz et al., 2000). Producers mainly manage FHB through agronomic practices such as using resistant varieties and applying fungicides. Visual disease symptoms appear two to three weeks after flowering, posing a burden on producers who must decide whether to apply fungicides before knowing if or how much FHB infection will occur. Once symptoms appear, the damage has been done, and the application of fungicides is futile. Applying fungicides is costly and an unnecessary expense if applied when not needed, i.e., in years with low disease pressure. Considering the importance of fungicide application timing, growers require a risk advisory system to help them make informed management decisions. Several weather-based models have been developed to predict FHB levels in wheat (Moschini et al., 2001; Hooker et al., 2002; De Wolf et al., 2003; Rossi et al., 2003; Del Ponte et al., 2005; Shah et al.,

2013). Existing weather-based models utilized in western Canada that were developed in the USA many years ago may not represent the current FHB chemotypes. Additionally, these models predict FHB epidemics with a field severity or FHB index (FHBi) greater than 10%, which is a disease severity level strongly correlated with FHB yield losses and generally linked to high levels of deoxynivalenol (DON) in harvested grain (De Wolf et al., 2003). However, there are instances where disease symptoms in the field do not accurately reflect the amount of Fusarium damaged kernels (FDK) and DON, and recent research demonstrates complex relationships between disease symptoms and DON accumulation in the field (Miedaner et al., 2016). Therefore, the objective of this study was to develop weather-based models that predict FHBi, FDK, and DON in winter wheat, spring wheat, barley, and durum using data collected in western Canada.

Materials and Methods
Experimental site details, disease, and weather data collection
Small-plot trials at five sites per province (15 sites total) were conducted in Manitoba, Saskatchewan, and Alberta during the 2019 and 2020 growing seasons. The trials were established in areas where the fungal pathogen had been detected in the preceding two years and the soil already contained FHB inoculum. Sites were distributed geographically across western Canada to capture a range of weather conditions and FHB occurrences. FHB incidence and severity were assessed on the plots from 18 to 21 days after 50% anthesis (BBCH 65) and expressed as FHB index using the formula: FHBi = (FHB incidence x FHB severity)/100. One kilogram of grain from each plot was sent to a commercial laboratory for official grading, including FDK levels and DON analysis (Canadian Grain Commission, 2019). Portable weather stations were installed within 10 m of the plots to collect hourly growing-season weather data, including air temperature (°C), relative humidity (RH), precipitation (mm), solar radiation (W m^{-2}), and wind speed (m s^{-1}). In total, 84 weather predictor variables were calculated using hourly weather data from 4, 7, 10, and 14 days before 50% anthesis plus the period between 3 days before and 3 days after 50% anthesis.

Data analysis
SAS was used for all analyses. The Kendall Tau-b correlation coefficient was calculated using the PROC CORR procedure, and model fitting and validation were performed using the PROC LOGISTIC procedure.

Model fitting and validation
Multiple logistic regression with a stepwise selection was used to develop models that predict the occurrence (=1) and non-occurrence (=0) of FHB epidemics using selected weather predictor variables. FHBi was binary coded as 0 or 1 using FHBi ≥5% as the epidemic threshold. Observations of FDK were binary coded to 0 or 1 using 0.2, 0.3, 0.8, and 1.5% FDK thresholds for barley, spring wheat, winter wheat, and durum, respectively. These cut-off values reflect the maximum level permitted in the number one grade for each crop type under Canadian regulations and serve as a justification for the application of fungicides to avoid revenue loss due to downgrading (Canadian Grain Commission, 2019). A DON threshold of 1 ppm was used to differentiate epidemic from non-epidemic cases, and this value corresponds to a value that results in wheat being downgraded during marketing, as established by the Canadian Food Inspection Agency (CFIA, 2017). The models were selected based on the receiver operating characteristic (ROC) curve metrics of sensitivity (percentage of correctly classified epidemic cases) and specificity (percentage of correctly classified non-epidemic cases), accuracy (ability to correctly classify both epidemic and non-epidemic cases), and the Hosmer-Lemeshow goodness of fit test (Hosmer et al., 2013). Validation of the models was conducted using an independent dataset collected from producer fields during the same two growing seasons.

Results and Discussion

Disease status

Mean FHBi, FDK, and DON levels ranged from 2.9 to 11.9%, 0.03 to 2.98%, and 0.04 to 2.83 ppm, respectively, across crop type and FHB resistance ratings (Figure 1). The FDK and DON levels were lower in the moderately resistant varieties compared to the susceptible varieties. Durum had the highest disease levels while barley had the lowest levels. The occurrence of FHBi, FDK, and DON reflects weather conditions that occurred at the plot sites during the 2019 and 2020 growing seasons. Warm, dry weather was most likely unfavorable for FHB epidemics at most sites during the two growing seasons.

Variable selection

Eighteen variables were found to be independently or jointly associated with FHB epidemics across crop types and crop damage indicators through correlation analysis and stepwise logistic regression (Table 1). Compared to rainfall variables, RH was a more frequently used moisture variable in the models.

Logistic regression models

Initially, 5 FHBi models for each crop type; 6 and 7 FDK models for Spring wheat and durum, respectively; and 9 DON models for durum were identified with > 70% prediction accuracy (*data not shown*). However, some of these models had low sensitivity, while others had numerous predictor variables (complex), necessitating the application of the principle of parsimony. The list was narrowed to two models, those with high sensitivity, specificity, accuracy, and best fit for each crop type and crop damage indicator for further evaluation and validation (Table 2). Two winter wheat models (models WWFHB1 and WWFHB2) correctly classified 73 and 97% of the FHB epidemics, respectively (sensitivity), but correctly classified 76 and 61% of the FHB non-epidemics (Specificity). Spring wheat models (SWFHB1 and SWFHB2) had an equal prediction accuracy of 76%. The accuracy of the FHBi models for durum and barley ranged from 76 to 81%. The discrepancies in FHBi accuracy could be attributed to various factors, including late disease infection and differences in Fusarium species infecting the crop. This indicates that FHBi models may not accurately predict FDK and DON levels, and thus FDK and DON prediction models were developed.

Spring wheat and durum FDK models predicted epidemic cases more accurately than non-epidemic cases using RH as the sole predictor variable (Table 2). Weather conditions 10 days before mid-anthesis provided a more accurate FDK prediction than conditions at 4, 7, or 14 days before mid-anthesis. Shorter time periods may miss information about pathogen-environment interactions, whereas more extended periods may include unnecessary information (Shah et al., 2013; Giroux et al., 2016). The accuracy of the two Durum DON models was equal (78 and 79% for DONDU1 and DONDU2, respectively). However, these models predict more non-epidemic cases than epidemic cases. The FHBi, FDK, and DON models were validated using data from 199 producer fields collected over the same two growing seasons. The models exhibited accuracy ranges of 80 to 100 for FHBi, 54 to 89 for FDK, and 75 to 82% for DON (Table 2). However, sensitivity was infinite or low because there were no or few epidemic cases to predict.

Conclusion

Models for predicting FHBi, FDK, and DON had high prediction accuracy in both development and validation datasets. These models will be used to develop an online tool for assessing the risk of FHB and guiding the application of fungicides in the Canadian prairies. This risk assessment tool will allow producers to optimize fungicide application by avoiding unnecessary fungicide application and losses due to epidemics. The data used in this study were limited to two growing seasons and may not reflect

all possible disease-weather conditions that could favor FHB epidemics. Therefore, more data from the 2021 growing season will be added to refine and validate these models.
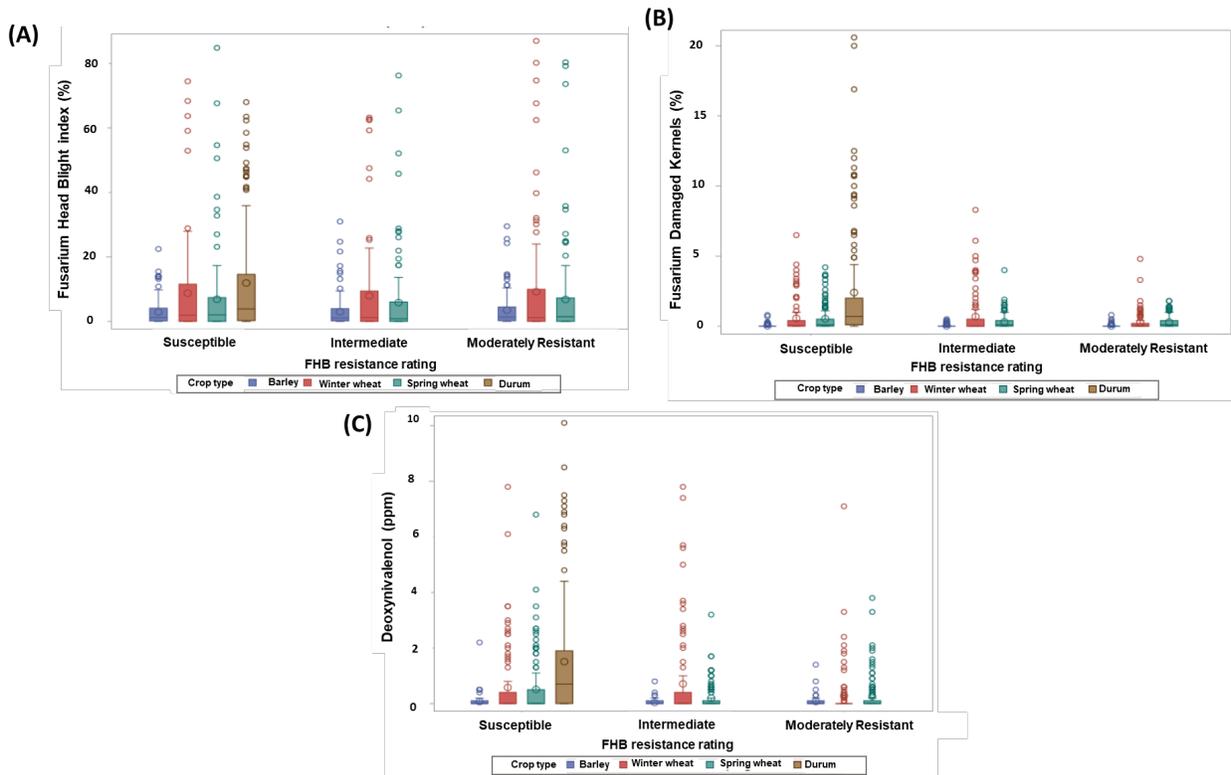
Figure 1. Fusarium head blight index (A) Fusarium damaged kernels (B) and Deoxynivalenol (C) occurrence in the 2019 and 2020 growing season in Manitoba, Saskatchewan, and Alberta.

Table 1. Description of selected weather predator variables

Variable	Variable Description	Days Prior to Mid-Anthesis
RH4MA	Mean daily relative humidity (%)	4
RH804MA	Duration (h) RH ≥ 80 %	4
T252804MA	Duration (h) air temperature 25 ≤ T ≤ 28°C	4
Tmin4MA	Mean daily minimum temperature (%)	4
TRH804MA	Duration (h) air temperature 15 ≤ T ≤ 30°C, and RH ≥ 80 %	4
TRH904MA	Duration (h) air temperature 15 ≤ T ≤ 30°C, and RH ≥ 90 %	4
R4MA	Mean daily rainfall (mm)	4
RH7MA	Mean daily relative humidity (%)	7
RH807MA	Duration (h) RH ≥ 80 %	7
T7MA	Mean daily temperature (°C)	7
Tmin7MA	Mean daily minimum temperature (%)	7
RH10MA	Mean daily relative humidity (%)	10
RH8010MA	Duration (h) RH ≥ 80 %	10
TRH8010MA	Duration (h) air temperature 15 ≤ T ≤ 30°C, and RH ≥ 80 %	10
RH8014MA	Duration (h) RH ≥ 80 %	14
T252814MA	Duration (h) air temperature 25 ≤ T ≤ 28°C	14
R14MA	Mean daily rainfall (mm)	14
RHmax14MA	Mean daily maximum relative humidity (%)	14

Table 2. Selected Fusarium head bight index, Fusarium damaged Kernel and Deoxynivalenol models for spring wheat, winter wheat, barley and durum.

Crop Type	Crop Damage Indicator	Model equation (p =1/1 +exp$^{- (a + bX +…)}$)[v]	Optimum predicted threshold[w]	Sensitivity[x]	Specificity[y]	Accuracy[z]
Winter Wheat	WWFHB1	-0.1188+0.0185RH807MA+0.7846Tmin7MA-0.6239T7MA	0.37	73.2	75.9	75
	WWFHB2	-5.1095+0.0312RH8014MA	0.17	96.9	61.3	79
Spring Wheat	SWFHB1	-6.1086+0.1267RH804MA+0.2461T252804MA-0.1414TRH904MA	0.25	82.4	68.9	76
	SWFHB2	-34.5786+0.3513RHmax14MA+0.0435T252814MA	0.39	79.8	72.7	76
Barley	BAFHB1	-6.4679+0.1560RH804MA+0.2981T252804MA-0.1137TRH804MA	0.42	74	86.6	80
	BAFHB2	-37.7241+0.2146R14MA+0.0495T252814MA	0.27	89.9	63.4	77
Durum	DUFHB1	-2.0665+0.0326TRH8010MA	0.39	91.5	70.2	81
	DUFHB2	-8.3268+0.5906Tmin4MA+0.2714R4MA	0.58	70.2	80.7	76
Spring Wheat	SWFDK1	-31.6372+ 0.40037RH10MA	0.37	86.4	83.4	85
	SWFDK2	-25.27+0.3167RH7MA	0.32	83.6	72.5	78
Durum	DUFDK1	-11.9932+0.0847RH8010MA	0.29	83.3	74.2	79
	DUFDK2	-17.9341+0.2185RH4MA	0.28	80	73	77
Durum	DUDON1	-20.7748+0.2646RH10MA	0.57	71.7	84.9	78
	DUDON2	-24.1039+0.3114RH14MA	0.51	69.6	89	79

[v]Logistic regression models were developed using 2019 and 2020 data collected in Manitoba, Saskatchewan, and Alberta. Variables are defined in Table 1. P= probability of an epidemic event (1), *a* and *b* are the model coefficients, and X is the predictor variable(s).

[w]The optimal predicted probability of an epidemic case, as determined by Youden index max (where sensitivity and specificity for the full range of *p* values, are high).

[x] Sensitivity is the percentage of correctly classified epidemics cases (epidemic = FHBi ≥ 5%).

[y] Specificity is the percentage of correctly classified non-epidemic cases.

[z] Accuracy is the percentage of correctly classified cases of epidemic and non-epidemic (true positive proportion + true negative proposition /2

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Pre-flowering Fungicide Applications for Fusarium Head Blight Management in Wheat

Pierce A. Paul

Department of Plant Pathology, The Ohio State University, OARDC, Wooster, OH 44691
Corresponding Author: Pierce Paul, paul.661@osu.edu

Abstract

Early anthesis (Feekes 10.5.1; Zadoks GS60-61), characterized in wheat by the extrusion of anthers from florets in the middle third of the spike, is recommended as the optimum growth stage for fungicide application for Fusarium head blight (FHB) and deoxynivalenol (DON) management. This is largely because wheat spikes are most susceptible to infection by *Fusarium graminearum*, the causal agent of FHB, during anthesis and early grain fill. Early anthesis at the plot or field level is not a fixed point in time but rather a window that varies in length from a few to several days, but since it is necessary to define a fixed time during the anthesis window for fungicide application, early anthesis is often considered to be reached when approximately 50% of the primary tillers are at Feekes 10.5.1. However, practical limitations often make it difficult for producers to treat fields at early anthesis. Therefore, a series of studies were conducted to evaluate the efficacy of pre- and post-anthesis fungicide programs for FHB index (IND) and DON management, including single applications of the demethylation inhibitor (DMI) fungicides metconazole (Caramba®) and prothioconazole + tebuconazole (Prosaro®) at Feekes 10.5 (Zadoks GS59; spike fully emerged from the leaf sheath of the flag leaf) and a new Succinate Dehydrogenase Inhibitor (adepidyn/pydiflumetofen) + DMI (propiconazole) premix fungicide (Miravis® Ace) at Feekes 10.3 (Zadoks GS55; spike half way out of the flag leaf sheath). Summary results from a quantitative synthesis of data from these studies will be presented showing that although pre-anthesis applications of Caramba, Prosaro, or Miravis Ace significantly reduced mean IND and DON relative to the nontreated check, in all cases, the overall mean efficacy in terms of percent control was substantially lower for the pre-anthesis timing compared to the early anthesis application. For Miravis Ace, a single pre-anthesis application was often just as effective as an early anthesis application against IND, but significantly less effective against DON. The economic consequences of pre-anthesis, relatively less effective, fungicide programs for FHB management in wheat will be discussed.

Introduction to Prosaro® Pro - a New Fungicide for Control of Leaf and Head Diseases in Wheat and Barley

Kevin B. Thorsness, Rory Cranston, and Randy A. Myers

Bayer Crop Science
Corresponding Author: Kevin Thorsness, kevin.thorsness@bayer.com

Abstract

Prosaro® PRO 400 SC Fungicide is a new fungicide from Bayer Crop Science for broad-spectrum control of leaf and head diseases in small grain cereals. Prosaro® PRO was registered in the 3rd quarter of 2021 in several states of the United States and will be available for commercial use in 2022. Prosaro® PRO is a mixture of prothioconazole plus tebuconazole plus fluopyram. These three active ingredients provide overlapping control of key head and foliar diseases in cereal small grains.   Prosaro® PRO provides increased control of Fusarium head blight and several important leaf diseases as well as suppression of ergot, compared to Prosaro®. Prosaro® PRO is registered for use in spring wheat, durum wheat, winter wheat, and barley. Prosaro® PRO is formulated as a soluble concentrate for ease of handling. Prosaro® PRO should be applied at 10.3 to 13.6 fl oz/ac with a non-ionic surfactant to wheat or barley, up to 30 and 32 days prior to harvest, respectively. For maximum performance of Prosaro® PRO in wheat, it is recommended to apply at flower initiation and up to 4-7 days later. For barley, it is recommended to apply Prosaro® PRO when barley heads are fully emerged on the main stems. Replicated trials were conducted at several locations throughout the United States from 2018 to 2021, with Prosaro® PRO at 10.3 fl oz/ac with a non-ionic surfactant at 0.125% v/v. Additionally, in 2018 to 2021, large demonstration trials were conducted by growers in commercial fields. The growers applied a tank mix treatment of Prosaro® plus Proline® plus Luna® Privilege at rates that delivered prothioconazole plus tebuconazole plus fluopyram equivalent to Prosaro® PRO at 10.3 fl oz/ac. These treatments were compared to either a non-treated check or Prosaro® at 6.5 to 8.2 fl oz/ac. The objective of these trials was to evaluate the influence of Prosaro® PRO on grain yield and grain quality in wheat and barley. Disease ratings were recorded where applicable. The trials were harvested, and grain yield and grain quality were determined.

The Impact of Row Spacing, Seeding Rate, and Fungicide Timing on Leaf Disease and Fusarium Damaged Kernel Severity, Deoxynivalenol, and Productivity of Spring Wheat

T.K. Turkington[1], H. Klein-Gebbinck[2], K. Xi[3], B. Beres[4], R. Aboukhaddour[4], Prabhath Lokuruge[5], A. Mulenga[5], G. Peng[6], W. May[7], R. Mohr[8], G. Telmosse[9], D. Pageau[9], A. Foster[10], B. Blackwell[11], H. Kubota[1], B. Tidemann[1], and G. Semach[2]

[1]Lacombe Research and Development Center, Agriculture and Agri-Food Canada, Lacombe, AB T4L 1W1, Canada
[2]Beaverlodge Research Farm, Agriculture and Agri-Food Canada, Beaverlodge, AB T0H 0C0, Canada
[3]Field Crop Development Centre, Olds College, Lacombe, AB T4L 1W8, Canada
[4]Lethbridge Research and Development Center, Agriculture and Agri-Food Canada, Lethbridge, AB T1J 4B1, Canada
[5]Scott Research Farm, Agriculture and Agri-Food Canada, Scott, SK S0K 4A0, Canada
[6]Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2, Canada
[7]Indian Head Research Farm, Agriculture and Agri-Food Canada, Indian Head, SK S0G 2K0, Canada
[8]Brandon Research and Development Center, Agriculture and Agri-Food Canada, Brandon, MB R7A 5Y3, Canada
[9]Normandin Experimental Farm, Agriculture and Agri-Food Canada, Normandin, QC G8M 4K3, Canada
[10]Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, Charlottetown, PEI C1A 4N6, Canada
[11]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON K1A 0C6, Canada
Corresponding Author: Thomas Turkington, kelly.turkington@agr.gc.ca

Abstract

The impact of row spacing, seeding rate, and fungicide timing were assessed at seven Canadian spring wheat sites in 2019. Narrow and wide row spacings (RS) were only set up at four sites. Seeding rates (SR) of 200 and 400 seeds m-2 were used, while fungicide (Prosaro® XTR) timings included: check no treatment; at the start of anthesis (early); a late application date 7-10 days after the start of anthesis (late); and a dual early and late application. Leaf spot levels were low at four sites, while low to moderate levels occurred at the remaining three sites. Overall, in 2019, RS and its interaction with other factors generally had limited impacts on disease, crop productivity and kernel quality. Seeding rate and fungicide tended to have the most frequent impacts on leaf disease and crop productivity. Higher seeding rates (SR) at two of seven sites increased leaf spot severity. Seeding rate also impacted yield, and thousand kernel weight (TKW) at four and three sites, respectively. Yield was increased with increased SR at three sites and decreased at one site. By increasing the seeding rate TKW was decreased at two sites but increased at the other. In 2019, fungicide timing impacted leaf disease and yield at three and two sites, respectively. Here the lowest leaf disease levels were similar for all fungicide treatments, while the highest yields occurred for early and dual applications. Fusarium damaged kernel severity was decreased by the increased seeding rate at one site only. Elevated deoxynivalenol (DON) levels only occurred at one site and were significantly impacted by RS, the interaction of RS and SR, and fungicide timing. At this one site DON was decreased with increased RS and SR, although there was an interaction whereby SR differences were only significant for the narrow RS. These observed impacts were likely due to more head emergence variability and perhaps a wider window for infection. Fungicide timing impacted fusarium damaged kernel severity at three sites and DON levels at one site. Overall, FDK and DON levels were generally lowest for the dual application treatment, intermediate for single early or late applications and highest for the check. Although dual post-head emergence applications did impact some parameters this treatment may be less economical, while also not being registered for use in Canada. The funding of the Canadian Agricultural Partnership Canadian National Wheat Cluster and Prairie producer/industry groups is graciously acknowledged.

Food Safety & Toxicology

Can Barley, High in Vomitoxin, Be Used to Grow Edible Mushrooms?

Aaron Gabriel

Cornell Cooperative Extension - Albany County, Voorheesville, NY
Corresponding Author: Aaron Gabriel, adg12@cornell.edu

Abstract

Barley (and other cereal grains) often become diseased in the field with *Fusarium graminearum* and contaminated with vomitoxin (DON), making them unsuitable for malting (1 ppm) and for animal feed (about 5 ppm). This experiment explored the use of barley with 3.1 ppm DON in substrate to grow edible mushrooms and determine if the mushrooms would contain any DON. *Pleurotis ostreatus* sawdust spawn (Poho oyster mushroom) and *Lentinula edodes* sawdust spawn (Shitake WR46[TM]) were used in two identical experiments. In polypropylene culture bags, a barley and hardwood sawdust mixture (4:1 ratio) was brought to 50% moisture and sterilized in a pressure cooker at 15 psi for one hour. Three replications were completed. Three flushes of mushrooms were harvested from each replication and dried at 125[O]F. Analysis was done by DairyOne (Ithaca, NY). No DON was detected in the oyster or shitake mushrooms. The spent oyster mushroom substrate plus mycelia was also tested and had a lower concentration of DON compared to the initial sterilized substrate. More rigorous and thorough testing is needed.

Acknowledgements

Impact of Botanical Biofumigants on Grain Fungal Contaminants and Food Safety

William T. Hay[1], Susan P. McCormick[1], Mark A. Berhow[2], Steve F. Vaughn[2], Laurie Taylor[3], Martha M. Vaughan[1]

[1]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit, 1815 N. University, Peoria, IL 61604, USA
[2]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Functional Foods Research Unit, 1815 N. University, Peoria, IL 61604, USA
[3]Université de Poitiers, Poitiers, France
Corresponding Author: William Hay, William.Hay@usda.gov

Abstract

Mycotoxin accumulation during the malting of wheat and barley grain causes millions of dollars in annual losses for the U.S. malting and brewing industry. While the US malting industry has strict standards to avoid even minimally contaminated grain, the high moisture low temperature conditions during malting are ideal for residual *Fusarium graminearum* proliferation and production of deoxynivalenol (DON). This presentation discusses the potential use of biofumigant treatments from plant derived metabolites to reduce grain Fusarium contamination and mycotoxin production during malting. Defatted seed meals from three glucosinolate containing members of the Brassicaceae Family: *Brassica juncea*, *Brassica carinata* and *Thlaspi arvense* were utilized to fumigate contaminated wheat and barley. Upon wetting, myrosinase enzymes are activated in the seed meal and convert stable glucosinolate compounds into highly volatile isothiocyantes which can be used for fumigation. Furthermore, these naturally occurring plant defense compounds are US-FDA approved food additives known for their antimicrobial activity. We showed that the concentration of allyl isothiocyanate, the predominate compound emitted by wetted seed meals, caused complete inhibition of *Fusarium graminearum* growth without inhibiting wheat or barley germination. Fumigation of naturally contaminated wheat and barley under storage conditions significantly reduced the percent of infected kernels without affecting germination. Fumigation with *Brassica juncea* seed meal of contaminated barley during germination reduced DON contamination by 27% and increased barley germination success by 9%. This research provides producers and maltsters with an organic biofumigation treatment method for wheat and barley grain that can reduce grain Fusarium contamination and the likelihood of mycotoxin accumulation during the malting process.

Antifungal and Mycotoxin Inhibitory Activities of Nanoemulsified Hop Essential Oil Against *Fusarium graminearum* and Their Mechanism of Action

Haiyang Jiang and Jiajia Rao

North Dakota State University, Department of Plant Sciences, Fargo, ND,
Corresponding Author: Haiyang Jiang, haiyang.jiang@ndsu.edu

Abstract

Mycotoxins are ubiquitous on the basis of global surveillance of mycotoxin contamination in cereal-based food. Deoxynivalenol (DON), a mycotoxin produced by *Fusarium* spp. in the field, has been found with the greatest frequency in cereal-based food. The common way to control DON in food manufacture is to avoid the utilization of the mycotoxin contaminated grains, which oftentimes is not applicable. For example, previous studies have shown that DON levels can increase appreciably during the malting of so-called "clean grains" and that newly produced DON can be transferred to beer. The hops themselves, or the extracted oil, are extensively used in beer production for providing bitterness and characteristic aroma. Therefore, we explored the efficacy of hop oil nanoemulsion on *Fusarium graminearum* growth, mycotoxin production and understand their mechanisms of action on fungi membrane. The major chemical composition of hop essential oil was humulene, followed by beta-myrcene, caryophyllene, nerol and 3,3,6-Trimethyl-1,5-heptadiene. The 5 wt% of hop oil-in-water nanoemulsion could be fabricated by incorporating 70 wt% of hop oil with 30 wt% of medium chain triacylglycerol (MCT) in oil phase prior to homogenization. The hop oil-in water nanoemulsion with mean diameter < 145 nm showed highly stable against droplet growth during 30 days storage. The antifungal activities of hop nanoemulsion against mycelial growth and spore germination was dose dependent. The inhibition of *F. graminearum* growth was mainly attributed to increased lipid content of cell membrane, disputed cell wall composition and impaired cell viability as observed by three different fluorescent probes. Regarding mycotoxin inhibitory efficacies, 90% of mycotoxin production could be inhibited by applying 750 µg/g hop oil nanoemulsion in rice cultures. The results of this study have important implications for the design and utilization of hop oils as natural antifungal agents and mycotoxin inhibitors in the food industry.

Acknowledgement and Disclaimer

Essential Oil Based Nanoemulsions as Antifungal Agents in Food Processing

Jiajia Rao

North Dakota State University, Department of Plant Sciences, Fargo, North Dakota
Corresponding Author: Jiajia Rao, jiajia.rao@ndsu.edu

Abstract

Fusarium and mycotoxin contamination in cereal-based food is ubiquitous according to systematic review of the scientific documentation of worldwide mycotoxin contamination in cereal and their products between 2008 and 2018, thus representing food safety issue. Food processing is the last defense step to prevent mycotoxin contamination in foods for human consumption. Therefore, it is with great urgency to develop strategies to inhibit fungi growth and mycotoxin production during food processing. Recently, plant-based essential oils (EOs) have received considerable attentions in the food industry due to broad-spectrum of antifungal activities and inhibitory effect against mycotoxin biosynthesis. In this presentation, parameters that impact on the formation of EO-in-water nanoemulsions and functional properties including antifungal and mycotoxin inhibitory efficacy *in vitro* are discussed. Micro-malting process was selected to be as an example to testify the antifungal efficacy of proper-designed EO-in-water nanoemulsions. Results indicated that physically stable EO-in-water nanoemulsions can be fabricated by incorporating either ≥75 wt% of corn oil or ≥50 wt% of medium chain triacylglycerol (MCT) into EO before homogenization. In general, the mycotoxin inhibitory efficacy of EO was enhanced considerably in nanoemulsion form than bulk oil. Among all selected five EOs, thyme and clove oil-in-water nanoemulsions had the greatest antifungal and mycotoxin inhibitory activities. At last, clove oil-in-water nanoemulsions stabilized by three different emulsifiers (Tween 80, bovine serum albumin, quillaja saponins) were selected to apply in micro-malting process according to our germinative energy test of barley seeds. All clove oil-in-water nanoemulsions had the capability to inhibit fungal growth and DON production during the micro-malting process. Among the three emulsifiers, Tween 80-stablized clove oil nanoemulsion displayed largest reduction of mycotoxin and least flavor impact on the final malt. The overall project showed a great potential for utilization of EO-in-water nanoemulsion as antifungal agent and mycotoxin inhibitor in the food industry.

Gene Discovery & Engineering Resistance

Genetic Engineering to Improve Fusarium Head Blight Resistance in Barley

Abdullah Fahad Alhashel[1], Thomas Baldwin[1], Jack Rasmussen[1], Shaobin Zhong[1], Timothy Friesen[1,2], Shengming Yang[1,2,3]

[1]North Dakota State University, Department of Plant Pathology, Fargo, ND 58102
[2]USDA-ARS Cereals Research Unit, Edward T. Schafer Agriculture Research Center, Fargo, ND 58102
[3]North Dakota State University, Department of Plant Sciences, Fargo, ND 58102
Corresponding Author: Shengming Yang, Shengming.Yang@usda.gov

Abstract

Fusarium head blight (FHB) caused by the fungal pathogen *Fusarium graminearum* is one of the most devastating diseases in barley. However, effective resistance has not been identified in barley germplasm. We are using two strategies in this study to engineer the host with various genotypes, host-induced gene silencing (HIGS) and CRISPR-mediated gene-editing. The *FgGCN5* gene encodes a significant histone acetyltransferase in *F. graminearum* which is critical for pathogenicity, deoxynivalenol (DON) biosynthesis, and pathogen survival. Targeting the *FgGCN5* gene, we have generated transgenic plants to test if HIGS-mediated plant protection is gained in barley. The target for gene-editing is *HvHRC*, the barley ortholog of *FHB1* (*TaHRC*) in wheat. Since the dominant *TaHRC* allele is required for disease susceptibility, we expect that FHB-resistant barley can be obtained from *HvHRC* knockout mutants. Using CRISPR technology, we developed loss-of-function mutants of *HvHRC* for phenotype screening. All the derived transgenic plants and mutants will be assessed for FHB resistance in both greenhouse and field conditions.

Acknowledgment

The Barley UDP-Glycosyltransferase *UGT13248* is Required for Deoxynivalenol Conjugation and Type 2 Resistance to Fusarium Head Blight

Gerit Bethke[1], Yadong Huang[1], Goetz Hensel[2], Skylar Wyant[1], Xin Li[1] , Shane Heinen[1], Susan McCormick[3], Peter Morrell[1], Yanhong Dong[4], Jochen Kumlehn[2], Silvio Salvi[5], Franz Berthiller[6], Gary J. Muehlbauer[1]

[1]University of Minnesota, Department of Agronomy and Plant Genetics, Saint Paul, MN, USA
[2]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Plant Reproductive Biology, Gatersleben, Germany
[3]USDA-ARS NCAUR, Mycotoxin Prevention and Applied Microbiology Research, Peoria IL, USA
[4]University of Minnesota, Department of Plant Pathology, Saint Paul, MN, USA
[5]University of Bologna, Department of Agricultural and Food Sciences, Bologna, Italy
[6]University of Natural Resources and Life Sciences, Vienna (BOKU), Department of Agrobiotechnology, Tulln, Austria
Corresponding Author: Gary Muehlbauer, muehl003@umn.edu

Abstract

Fusarium head blight (FHB) of *Hordeum vulgare* (barley) can cause significant reduction in yield and grain quality by contamination with trichothecene mycotoxins including deoxynivalenol (DON). Glycosylation of DON to the less toxic DON-3-glucoside (D3G) is thought to be catalyzed by UDP-glucosyl transferases (UGTs). Barley UGT13248, was previously shown to convert DON to D3G in yeast, Arabidopsis and wheat. In wheat, expression of UGT13248 decreased FHB severity. To explore the natural genetic diversity of *UGT13248* in barley, we sequenced *UGT13248* from 28 barley accessions with varying degree of FHB resistance and identified six protein variants. A survey of the *UGT13248* sequence from exome capture sequencing data of 34 elite barley lines, 182 wild barley accessions and 317 barley landraces identified seven non-synonymous changes. Accessions carrying any of these UGT13248 protein variants did not show altered sensitivity to DON on seedling root growth assays. These results suggest that mutations in UGT13248 are rare and that UGT13248 is highly conserved. We next generated barley lines overexpressing UGT13248 and identified two independent TILLING lines carrying mutations in UGT13248 in close proximity to the UDP-sugar binding site, UGT13248 (T368I) and UGT13248 (H369Y). The UGT13248 (T368I) and (H369Y) mutants showed hypersensitivity to DON root growth inhibition in seedlings and strongly impaired conjugation of DON to D3G in barley spikes. Constitutively expressing *HvUGT13248* in a susceptible barley cultivar provided resistance to DON root growth inhibition and increased conjugation of DON to D3G in spikes. Field tests of TILLING mutants showed increased FHB disease severity, suggesting that DON to D3G conversion contributes to FHB resistance. Point inoculation experiments showed increased FHB disease severity and increased spread of FHB symptoms in the spikes of TILLING mutants as well as reduced disease severity in plants overexpressing UGT13248. The rachis of the UGT13248 (H369Y) mutant contained more *F. graminearum* and DON compared to wild-type plants. Further, spread of *F. graminearum* was detected in rachis nodes adjacent to inoculated spikelets of UGT13248 (H369Y) plants but not in wild-type plants. Taken together, our data suggest that UGT13248 is required for Type 2 resistance in barley.

Acknowledgement and Disclaimer

Nanoparticle-mediated Genome Editing System for FHB Resistance Improvement in Wheat

Hui Chen[1], Volodymyr Kavetskyi[1] and Guihua Bai[1, 2]

[1]Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA
[2]Hard Winter Wheat Genetics Research Unit, USDA-ARS, Manhattan, KS 66506, USA
Corresponding Author: Guihua Bai, guihua.bai@usda.gov

Abstract

Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide. Developing and growing FHB resistant wheat cultivars is one of the most practical and effective approaches to minimize FHB damage. Recently, manipulating susceptibility genes (*S-genes*) in crops has become one of the promising breeding strategies to create new sources of resistance. CRISPR/Cas genome editing technology provides a powerful tool to generate resistant mutants by knocking out *S-genes* precisely for functional validation of candidate genes and the creation of new resistant sources for breeding. However, this editing technology relies on gene transformation to deliver the CRISPR/Cas and gRNA into wheat plant cells and only a few wheat genotypes can be used for transformation because most wheat cultivars have very low rates of callus induction and tissue regeneration in the transformation process. Therefore, a novel efficient gene delivery system that bypasses tissue culture and regeneration is urgently needed for its application in wheat genomic research and breeding. Nanoparticle (NP)-mediated gene delivery system has been evaluated for potential application in crop improvement owing to its ability to reduce immune responses and cytotoxicity without host range restriction. In this study, we evaluated the feasibility of NP to deliver Cas9 and gRNA into wheat tissues using the floral dip method. We optimized the NP binding capacity, identified the best ratio of NPs with Cas9/gRNA editing reagents, and then directly dripped NPs-binding editing reagents into the immature spikes at preanthesis (Feekes 10.0). Sanger sequencing of the target gene from the progenies of the treated plants identified sequence mutations from the *Fhb1* susceptibility allele of Bobwhite and the resistance allele of Wangshuibai and Sumai3. These mutants will be phenotyped for FHB resistance to validate their functions on FHB resistance. This work demonstrated that NP directly delivered the editing reagents to target cells and generated inheritable mutations in the target gene regions, therefore the new NP-mediated genome-editing technology has high potential to be used to develop new FHB resistant wheat varieties.

Acknowledgement and Disclaimer

Discovery of a Susceptibility Factor for Fusarium Head Blight on Chromosome 7A of Wheat

Bhavit Chhabra[1], Vijay Tiwari[1], Bikram S. Gill[2], Yanhong Dong[3], and Nidhi Rawat[1]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742
[2]Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506
[3]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108
Corresponding Author: Nidhi Rawat, nidhirwt@umd.edu

Abstract

Fusarium head blight (FHB) disease of wheat caused by *Fusarium* spp. deteriorates both quantity and quality of the crop. Manipulation of susceptibility factors, the plant genes facilitating disease development, offers novel and alternative strategy for enhancing FHB resistance in plants. In this study, a major effect susceptibility gene for FHB was identified on the short arm of chromosome 7A (7AS). Nullisomic-tetrasomic lines for homoeologous group-7 of wheat revealed dosage effect of the gene, with tetrasomic 7A being more susceptible than control Chinese Spring wheat, qualifying it as a genuine susceptibility factor. The gene locus was found to be conserved in five chromosome 7A inter-varietal wheat substitution lines of diverse origin and a tetraploid *Triticum dicoccoides* genotype. The susceptibility factor was named as *Sf-Fhb-7AS* and mapped on chromosome 7AS to a 48.5-50.5 Mb peri-centromeric region between del7AS-3 and del7AS-8. Our results show that deletion of *Sf-Fhb-7AS* imparts 50-60% type-2 FHB resistance. Further work on mapping of *Sf-Fhb-7AS* is in progress. Identification and manipulation of *Sf-Fhb-7AS* will be useful for enhancing genetic resistance against FHB in wheat.

Acknowledgement and Disclaimer

Genetic Engineering of Barley to Improve Fusarium Head Blight Resistance

Alison Dineen, Michael A. Lawton, Rong Di

Rutgers, the State University of New Jersey, Plant Biology, New Brunswick, NJ
Corresponding Author: Rong Di, rongdi@sebs.rutgers.edu

Abstract

Fusarium head blight (FHB) caused by *Fusarium graminearum* (*Fg*) is an important disease of wheat and barley, resulting in significant yield loss and reduced grain quality due to mycotoxin contamination. Most commonly grown barley cultivars in the U.S. are susceptible to *Fg* infection. The mechanisms of FHB resistance in barley are not well defined. Genetic engineering of barley with transgenes including those that confer FHB resistance and the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-gene editing constructs can improve barley FHB resistance and aid our studies for the underlying molecular mechanisms of FHB resistance. To genetically engineer barley, we have developed an optimized plant tissue culture protocol to regenerate multiple barley plantlets from a single scutellum explant for cultivars of the two-rowed Conlon, Genesis and the six-rowed Morex. Many transgenic barley plants have been produced *via* both gene gun and *Agrobacterium*-mediated transformation. We have also developed our own CRISPR-gene editing platform to knock-out (KO) several host genes involved in conditioning *F. graminearum* susceptibility. Our studies with the model plant Arabidopsis have shown that CRISPR-mediated knock-outs of the *At2OGO* (*DMR6*) gene encoding a putative 2-oxoglutarate Fe(II)-dependent oxygenase and the *AtEIN2* (*ethylene insensitive 2*) gene result in an augmented *Fusarium* resistance. Another potential FHB susceptibility gene identified in Arabidopsis is *AtHSK* encoding homoserine kinase. We have constructed both transient and integrating CRISPR vectors to KO barley *Hv2OGO*, *HvEIN2* and *HvHSK*. We have also constructed CRISPR vectors to disrupt the promoter for barley *HvUGT* encoding the UDP gluconosyltransferase in order to study the dynamic role of *HvUGT* in FHB resistance in different barley cultivars. We have obtained CRISPR-edited *Hv2OGO* Conlon mutant plants and found that the mutations induced by our CRISPR-editing vector are inherited into the T_2 generation. Additionally, we have successfully applied the oligo-directed mutation method to enhance the CRISPR-gene editing efficiency and the online ICE (Inference of CRISPR Edits) analysis to efficiently screen for barley mutants.

Fine Mapping of FHB and DON Quantitative Trait Loci on Chromosome 2H in Barley

Yadong Huang[1], Shane Heinen[1], Brian Steffenson[2], Kevin P. Smith[1] and Gary J. Muehlbauer[1,3]

[1]Department of Agronomy and Plant Genetics
[2]Department of Plant Pathology
[3]Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN 55108
Corresponding Author: Gary Muehlbauer, muehl003@umn.edu

Abstract

Fusarium head blight (FHB) disease in wheat and barley is caused by *Fusarium* species. Genetic mapping has identified many quantitative trait loci (QTL) contributing to host resistance to FHB. Mapping studies utilizing 'Chevron', a six-rowed resistant landrace originated from Switzerland, have consistently detected QTL on chromosome 2H in barley, which co-localized with a heading date QTL. The aims of the present project are to (1) develop recombinant near-isogenic lines (rNILs) for the 2H QTL region and characterize their disease and correlated agronomic phenotypes; and (2) fine-map the QTL and identify candidate genes. To fine map the 2H QTL, an F_2 population of 2,038 plants was generated from a cross of 'Gen1-001' (resistant parent, 2H QTL region derived from Chevron introgressed into M69) to 'M69' (a susceptible breeding line) and was genotyped with SNP markers flanking the introgressed region (~26 cM). A total of 489 recombinants were identified which were further genotyped with 32 SNP markers spanning the introgression. This resulted in 17 recombinant classes and homozygous $F_{2:3}$ plants were identified. $F_{2:3}$ and $F_{2:4}$ plants were phenotyped for disease in field and greenhouse conditions from 2016-2021. Significant variation among rNILs and environments were detected. QTL for FHB resistance and DON accumulation were mapped in this population. Some lines exhibited lower disease severity than Gen1-001 and could be used as parents for breeding and further study.

Haplotype-Informed Prediction of FHB Resistance in US Wheat Breeding Programs

Katherine W. Jordan

USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
Corresponding Author: Katherine Jordan, katherine.jordan@usda.gov

Abstract

Effective imputation of missing genotypes in low coverage skim-sequencing or reduced-complexity sequencing projects is essential to identify potentially causal associations and to build robust genomic selection (GS) models. The newly developed Practical Haplotype Graph (PHG) tool presents this opportunity for wheat breeders to efficiently store sequence data and accurately impute genotypes across the wheat genome. A first-generation wheat PHG demonstrated over 92% imputation accuracy using very low (0.01x) exome sequencing coverage depth, and imputation accuracy of 89% using GBS sequencing technology. Our project builds on these results by creating a customized PHG database with wheat exome capture sequence of 200 FHB resistant and adapted wheat cultivars exhibiting different levels of resistance. These haplotypes will inform imputation of lower coverage sequenced breeding lines with high accuracy. By incorporating FHB resistant varieties in the database composition, FHB resistance-associated haplotypes are represented and will be used for developing a GS model for FHB resistance, identifying novel FHB resistant haplotypes/QTLs, and developing diagnostic markers for these QTLs. I will describe the progress of this new bioinformatics platform in wheat populations, and how it will be used to inform selection for FHB resistant germplasm in US breeding programs.

Using a New Genome Editing System to Validate the Functions of Wheat Candidate Genes of *FHB1* in Fusarium Head Blight Resistance

Volodymyr Kavetskyi[1], Hui Chen[1], Guihua Bai[1,2]

[1]Kansas State University
[2]USDA-ARS Hard Winter Wheat Genetics Research Unit, Manhattan, KS, 66506
Corresponding Author: Guihua Bai, Guihua.bai@usda.gov

Abstract

Fusarium head blight (FHB) infection significantly reduces wheat (*Triticum aestivum*) productivity and causes mycotoxin contamination in harvested grain. Growing resistant wheat cultivars is one of the most promising approaches to minimize FHB damage. *Fhb1* is the most stable quantitative trait locus (QTL) with a major effect on FHB resistance in wheat. Recently, three groups reported cloning of *Fhb1.* One group reported pore-forming toxin (*TaPFT*) as the candidate, and the other two reported histidine rich calcium binding protein (*TaHRC*) as the candidate, but they proposed different mechanisms of *TaHRC* in regulating *Fhb1* resistance. Therefore, the causal gene and mechanism for *Fhb1* remain to be determined*.* CRISPR/Cas9 genome editing technology can knock out a gene to determine its function. However, conventional gene editing is conducted using gene transformation, and most wheat cultivars have extremely low transformation rates. Wheat cultivars Bobwhite and Fielder is frequently used for wheat transformation, but none of them carries *Fhb1.* We developed an gene editing method that generates mutations in the targeted wheat genes without transformation. To edit a FHB resistant wheat accession Ning7840 carrying the *Fhb1* resistance allele*,* Cas9-overexpressed Bobwhite plants were crossed to Ning7840 and the leaf tissues of selected F_2 plants with both Cas9 and the target genes were inoculated with *Barley stripe mosaic virus* (BSMV) carrying the integrated guide RNA (gRNA). The progeny of BSMV infected plants were screened for the mutations using PCR cloning and sequencing. After sequencing 291 M_1 plants, we found two substitution mutations that alter amino acid sequences in *TaHRC*, and one deletion mutation and 1 substitution which caused a frameshift in *TaPFT*. Some of the homozygous M_2 mutant plants are being phenotyped for FHB resistance this fall to validate their functions on FHB resistance. Therefore, the new genome editing system successfully produced targeted mutations and can be used to validate gene functions in wheat.

Association Genetics of Fusarium Head Blight and Deoxynivalenol Resistance in *Aegilops tauschii*

Rizky Pasthika Kirana[1,6], Barbara Steiner[1], Kumar Gaurav[2], Sanu Arora[2], Gerlinde Wiesenberger[3], Maria Doppler[4], Rainer Schuhmacher[4], Sebastian Michel[1], Marc Lemmens[1], Gerhard Adam[3], Brande BH Wulff[2,5], Hermann Buerstmayr[1]

[1]Institute of Biotechnology in Plant Production, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Austria
[2]John Innes Centre, Norwich Research Park, Norwich, UK
[3]Institute of Microbial Genetics, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna (BOKU), Austria
[4]Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Austria
[5]Center for Desert Agriculture, Biological and Environmental Science and Engineering Division (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia
[6]Laboratory of Plant Breeding, Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia
Corresponding Author: Rizky Kirana, Pasthika pasthika@boku.ac.at

Abstract

Aegilops tauschii is the diploid progenitor of the wheat D subgenome and known as a precious resource of genetic diversity for wheat breeding. To evaluate the potential of *Ae. tauschii* for Fusarium head blight (FHB) resistance, 147 *Ae. tauschii* accessions were phenotyped for resistance against *Fusarium graminearum* and its major mycotoxin deoxynivalenol (DON). At anthesis, the accessions were treated either with *F. graminearum* spores or pure DON solution, and the spreading of the disease or the toxin-induced bleaching were recorded. A *k*-mer-based association mapping pipeline based on whole-genome sequence data was used to dissect the genetic basis of FHB and DON resistance and to identify candidate resistance genes.The accessions revealed broad variation for FHB spreading within the spike controlled by many minor-effect QTL. For DON resistance, less variation was detected, comprising no highly resistant accessions, but nine accessions showed severe bleaching symptoms after DON infiltration concomitant with lower conversion rates of DON into the conjugated non-toxic DON-3-O-glucoside. Association genetics for DON resistance identified a uridine diphosphate (UDP)-glucosyltransferase (UGT) on chromosome 5D in the nine susceptible accessions with lower DON detoxification rates.Only the full-length UGT, which is also polymorphic in hexaploid wheat, conferred resistance against DON, confirming its prominent role in DON detoxification and thus in the *Ae. tauschii*/wheat-*Fusarium*-interaction.*Aegilops tauschii*, Fusarium head blight, deoxynivalenol, UDP-glucosyltransferase.

Acknowledgment

Transfer *Fhb7* to Barley Through CRISPR-mediated Targeted Gene Insertion

Wanlong Li[1], Nicholas Santantonio[2], Mark E. Sorrells[3], Brian Steffenson[4], Steven S. Xu[5]

[1]Department of Biology and Microbiology, South Dakota State University, Brookings, SD
[2]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA
[3]School of Integrative Plant Science, Cornell University, Ithaca, NY
[4]Department of Plant Pathology, University of Minnesota, Saint Paul, MN
[5]Crop Improvement and Genetics Research, ARS-USDA, Albany, CA
Corresponding Author: Wanlong Li, wanlong.li@sdstate.edu

Abstract

Fusarium head blight (FHB; scab) is a devastating disease in barley and wheat caused by the same pathogen. While significant progress has been made in understanding and improving host resistance in wheat with molecular cloning of the major QTL *Fhb1* and *Fhb7*, similar research with barley has lagged behind due mainly to the lack of highly resistant genotypes, which makes it very difficult to effectively control FHB and DON contamination. Thus, there is an urgent need for a breakthrough in gene discovery and germplasm development to achieve higher levels of FHB resistance and a greater capacity to detoxify DON in barley using transformative approaches. The use of wheat genes to breed barley FHB resistance is the road not taken because of strong reproductive barriers. Considering that *Fhb7* detoxifies DON, we hypothesize that *Fhb7* can also contribute greatly to FHB resistance in barley. Taking the advantage of our ongoing work on *Fhb7* and CRISPR-based genome editing, we propose to continue our effort with an overall goal to transfer *Fhb7* to barley through CRISPR-mediated targeted gene insertion as a proof of concept. The proposed research includes three objectives: 1) Generate transgenic barley expressing both CRISPR/Cas9 and *Fhb7* donor, 2) Evaluate the *Fhb7* function in transgenic barley, and 3) Screen the transgenic plants for targeted *Fhb7* insertion events. The project is transformational because it 1) uses a wheat FHB-resistance gene to improve the FHB resistance of barley, which is unprecedented, 2) utilizes CRISPR-mediated target gene insertion to develop novel FHB-resistance germplasm, and 3) aligns with the multiple priorities of several USWBSI programs, including GDER, VDHR, and BAR-CP. This project serves our long-term goal to improve the FHB resistance of barley and wheat using CRISPR-based approaches Supported by the USWBSI-TRSC program, we are establishing a CRISPR-mediated targeted gene insertion system in barley. We have developed an all-in-one construct to express CRISPR/Cas9 and the *Fhb7* donor DNA. The construct has been used to transform barley cultivars Gold Promise (GP) and Quest, plantlets are regenerating from the transformed GP calluses. Results from the proposed research will have a positive impact on barley production and the (malting, feed, and food) industry, benefiting barley growers and end-users.

Deoxynivalenol Induces the Chloroplast Unfolded Protein Response (cpUPR) in Chlamydomonas

John E. McLaughlin[1], Friedrich Fauser[2], Mario Pinzas[3], Susan McCormick[4], Weronika Patena[5], Martin Jonikas[5], Nilgun E. Tumer[1]

[1]Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States of America
[2]Sangamo Therapeutics, Inc., Brisbane, CA, United States of America
[3]RUCDR Infinite Biologics, Piscataway, New Jersey
[4]Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS-NCAUR, Peoria, Illinois, United States of America
[5]Department of Molecular Biology, Princeton University, Princeton, NJ, United States of America
Corresponding Author: John McLaughlin, mclaughj@sebs.rutgers.edu

Abstract

We have discovered that deoxynivalenol (DON) induces the chloroplast unfolded protein response (cpUPR) in *Chlamydomonas* and that the cpUPR signaling mutant affected in chloroplast-to-nucleus retrograde signaling (MARS1) mutant is highly sensitive to this class of fungal toxins. This mutant, along with others, were identified by screening the indexed and mapped *Chlamydomonas reinhardtii* deletion library with 500 nM trichothecin (Tcin) by deep sequencing of mutant bar codes from DNA isolated from bulk flask cultures grown in duplicate. This *mars1* deletion mutant plus additional mutant alleles were screened on Tcin and DON and a high degree of sensitivity was confirmed for both. Previous work in the lab has documented chloroplast damage after exposure to DON via confocal microscopy in *Chlamydomonas*, *Arabidopsis*, and wheat. These new findings suggest a greater involvement of the chloroplast in the perception/response to DON than previously realized. This is critical to understand because the chloroplast can serve as a hub for ROS and programmed cell death (PCD) signaling impacting the plant response to the fungus and trichothecenes. A recent report indicates that cpUPR is critical for chloroplasts to mitigate photodamage. We also found that overexpression of the cpUPR gene MARS1 significantly enhances resistance to the virulence factor DON. Our results suggest that cpUPR is involved in increasing the tolerance to chloroplast stress due to DON exposure.

The *Chlamydomonas* knockout library, a "green yeast" mutant library for genome-wide screening, represents a unique and valuable resource to discover plant genes which are impacted by trichothecenes. Additional screening is likely to identify other mutants that play a role in how photosynthetic cells respond to DON. We will present our efforts to screen the B-Series CLiP Collection of ~13,000 high quality *Chlamydomonas* gene knockouts available from the *Chlamydomonas* Resource Center. Expanding these findings to targeted research in wheat and barley would enhance our understanding of the *Fusarium graminearum* pathosystem. Understanding how the chloroplast protein quality control system in higher plants is impacted by trichothecenes may provide unique methods to increase resistance to the mycotoxin and the fungus.

Acknowledgement

Exosome Mediated Protection against FHB

John E. McLaughlin[1], Götz Hensel[2], Jochen Kumlehn[3], Nilgun E. Tumer[1]

[1]Department of Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ, United States of America
[2]Institute of Plant Biochemistry, Heinrich Heine University, Düsseldorf, Germany
[3]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
Corresponding Author: John McLaughlin, mclaughj@sebs.rutgers.edu

Abstract

The discovery of sRNA uptake by fungal pathogens of barley and delivery of sRNAs and potentially proteins and other small molecules into fungal pathogens by exosomes provides a strong rationale to understand the contents of barley exosomes and the impact on *Fusarium graminearum*. Recent work in *Arabidopsis* has shown that sRNAs generated by the plant accumulate at infection sites and are taken up by fungal cells, host sRNAs that function to silence fungal genes that are critical for pathogenicity. Previously in our laboratory, we showed, using confocal microscopy, that infection of the barley variety Conlon with Ph1 spores induces multivesicular bodies (MVBs) accumulation. To determine if sRNAs and proteins are packaged into exosomes and delivered into *F.g.* during infection of barley, we isolated exosomes from barley apoplast fluid. Transgenic barley (Golden Promise) plants that accumulate the Arabidopsis (AtLTP4.4-GFP) and wheat (TaLTP3-GFP) nsLTP proteins in the apoplast were used in the experiment. We followed the Rutter and Innes exosome protocol to isolate and quantify plant extracellular vesicles. Proteins isolated from exosomes purified using a discontinuous iodixanol density gradient (OptiPrep) were run on a SDS-PAGE gel and analyzed by Western blotting using *Arabidopsis* TET8 antibodies. We found that the isolated barley exosome proteins contain an orthologous TET8 protein that will cross react with the *Arabidopsis* TET8 antibodies, indicating a promising marker for barley exosomes. Proteomic analysis via spectral scanning was performed between exosomes purified only by ultracentrifugation and exosomes purified by the OptiPrep gradient. An approximate 20% reduction in Rubisco was observed in the post-OptiPrep samples indicating enhanced purification. Gene Ontology (GO) term enrichment was identified for vesicle mediated transport, endomembrane system, GTPase activity, and several other classes of proteins. Specific proteins of potential interest identified in the enriched exosome fraction (http://microvesicles.org/) include small heat shock proteins, annexins, GAPDH enzymes, and GSH enzymes.

Acknowledgement and Disclaimer

Knockdown of *Lpx3* Function in Wheat Enhances FHB Resistance and Lowers DON Content

Isha Mittal[1], Syeda Alam[1], Bhavit Chhabra[2], Elena Shulaev[1], Vijee Mohan[1], Yanhong Dong[3], Steve Scofield[4], Nidhi Rawat[2], Jyoti Shah[1]

[1]University of North Texas, Department of Biological Sciences and BioDiscovery Institute, Denton, TX 76203
[2]University of Maryland, Department of Plant Science and Landscape Architecture, College Park, MD 20742
[3]University of Minnesota, Department of Plant Pathology, St. Paul, MN 55108
[4]USDA-Agricultural Research Service, West Lafayette, IN 47907
Corresponding Author: Jyoti Shah, Jyoti.Shah@unt.edu

Abstract

Fusarium head blight (FHB) caused by *Fusarium graminearum* is an economically important and serious disease of wheat and other small grain cereals that leads to reduced grain yield and mycotoxin contamination. 9-lipoxygenases catalyze the first step in the synthesis of oxylipins (oxidized lipids) that have multitude of roles in plants, including signaling associated with stress response. Oxylipins have also been reported to be involved in fungal development and inter-kingdom communication. In wheat, RNA-interference (RNAi)-mediated knockdown of the *Lpx3* locus resulted in enhanced resistance against *F. graminearum* in greenhouse studies. To identify non-GMO knockdown alleles of *Lpx3* that could provide new resistance-conferring genotypes with the potential for their integration into breeding programs, we have identified wheat TILLING lines with *Lpx3* variants containing mutations that are predicted to knockdown gene function. FHB disease severity and DON accumulation was significantly reduced in these *Lpx3* variants. Efforts are underway to develop lines with mutations at multiple *Lpx3* homeologs to evaluate the effect of the variant alleles on plant growth, development and grain yield, in addition to FHB. In parallel, physiological experiments are underway to identify the impact of *Lpx3* knockdown on oxylipin profile, and dual RNA-seq is being conducted to identify temporal changes in wheat and fungal gene expression that are influenced by the genotype at the *Lpx3* locus.

Development of Biocompatible siRNA Nanoparticles to Mitigate FHB in Wheat

Brandon Montoya[1], Isha Mittal[2,3], Jyoti Shah[2,3], and Brian Meckes[1,3]

[1]University of North Texas, Department of Biomedical Engineering, Denton, TX
[2]University of North Texas, Department of Biological Science, Denton, TX
[3]University of North Texas, BioDiscovery Institute, Denton, TX
Corresponding Authors: Brian Meckes, Brian.Meckes@unt.edu and Jyoti Shah, Jyoti.Shah@unt.edu

Abstract

Plants and fungi natively regulate expression of genes through the RNA interreference (RNAi) pathway, a mechanism where small interfering RNAs (19-21 base pairs in length) bind to target mRNAs leading to catalytic degradation of the mRNA. Degradation of the target mRNA prevents its translation into protein, effectively silencing the gene. Importantly, this process is highly specific, relying on perfect complementarity between the siRNA and the target mRNA, which significantly limits off-target effects. Plants have been engineered to produce siRNA that can be delivered into the invading fungi to interfere with gene function in the fungus, in a process known as host-induced silencing (HIGS). HIGS in wheat has shown ability to control *fusarium graminearum* growth, establishing RNAi as a viable means for controlling fungi growth. However, consumer opposition to genetically modified plants limits the widespread application of HIGS. Here, we will discuss approaches for developing non-toxic and biocompatible nanoparticles composed of natural products for the delivery of siRNA to plants. Specifically, we focus on the development of spherical nucleic acids, nanoparticle consisting of a spherical core with a dense and highly oriented nucleic acid shell, as potential fungicidal vehicles. We are currently developing liposome- and micelle- based structures for targeting susceptibility genes in wheat and virulence genes in *f graminearum* with the goal of mitigating FHB.

Acknowledgement and Disclaimer

A Wheat Practical Haplotype Graph to Facilitate FHB Resistance Mapping

Bikash Poudel[1], Katherine W. Jordan[2], Peter J. Bradbury[3], Jason D. Fiedler[1]

[1]USDA-ARS, Cereals Research Unit, Fargo, ND
[2]USD-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS
[3]USDA-ARS, Plant Soil and Nutrition Research Unit, Ithaca, NY
Corresponding Author: Jason Fiedler, jason.fiedler@usda.gov

Abstract

Wheat breeding for FHB resistance is a complex undertaking that relies heavily on the genetic variation among candidate germplasm. The quantitative nature of FHB resistance has made the task even more difficult. More than 500 QTL for FHB resistance have been detected in diverse wheat accessions worldwide, but characterization of the genes underlying these QTL has been limited. To improve the efficacy of the QTL in FHB resistance and to characterize the underlying genes, we created a wheat practical haplotype graph pangenome database using the Practical Haplotype Graph (PHG) tool. Whole exome-capture sequencing data from 95 spring wheat genotypes used in ND, SD, MN, and MT wheat breeding programs were used to build the PHG database. Based on the high confidence gene models from IWGSC Refseq v1.1, a set of 94,229 reference genome intervals were used to infer haplotypes from the wheat lines. In this ongoing study, we estimated the accuracy of PHG -imputed genotype calls in the wheat lines that were genotyped by exome capture, or whole-genome skim-sequencing approach. The PHG imputation accuracy varied between 97 - 98% for the exome capture data when compared against high confidence exome capture data concordant with 90K array sites. The accuracy of imputed skim-sequenced data with the PHG database ranged between 75-90%. The imputation accuracy for the sequencing data used in this study using other imputation methods was generally higher than the PHG but decreased as minor allele frequency increased. Further development of the PHG database is needed to evaluate its potential for large-scale imputation.

Meta-Analysis of the Genetics of Resistance to FHB and DON Accumulation Based on a New Barley Consensus Map

Ahmad H. Sallam[1], Matthew Haas[2], Yadong Huang[2], Yanhong Dong[1], Zenith Tandukar[2], Gary Muehlbauer[2, 3], Kevin P. Smith[2], and Brian J. Steffenson[1]

[1]University of Minnesota, Department of Plant Pathology, Saint Paul, MN, USA
[2]University of Minnesota, Department of Agronomy and Plant Genetics, Saint Paul, MN, USA
[3]University of Minnesota, Department of Plant and Microbial Biology, Saint Paul, MN, USA
Corresponding Author: Brian Steffenson, bsteffen@umn.edu

Abstract

Consensus maps are the product of integrating molecular marker information from several linkage maps and different molecular marker types. One of the goals of this study was the construction of a new barley consensus map that integrates SNP, SSR, RFLP, AFLP, and morphological markers with the purpose of developing a greater understanding of the genetic architecture of loci associated with Fusarium head blight (FHB) resistance and deoxynivalenol (DON) accumulation. Eight barley maps including six mapping populations and two previously developed consensus maps were used to construct the new consensus map. The linear programming theory implemented in the LPmerge package in R was used to integrate and order markers in these eight maps. A total of 4,788 markers on seven barley chromosomes covered about 1,299 cM. Marker positions on the consensus map were compared with the positions on other maps to confirm marker order. The average correlation of marker positions on the consensus map compared with the other eight maps is more than 0.95, indicating robust integration of these different marker types while preserving their relative order. The consensus map was used to consolidate mapping data for QTL associated with FHB resistance and DON accumulation from 13 bi- or tri-parental mapping populations and two genomewide association mapping panels. Based on this consensus map, we positioned 96 QTL associated with FHB resistance and 57 associated with DON accumulation across the barley genome. Most of the QTL explained a low percentage (<10%) of the variation for the traits and were often found significant in only one or a few environments in multi-year/multi-location field trials. Moreover, many of the major-effect FHB/DON QTL mapped to chromosomal positions coincident with various agro-morphological traits (e.g., heading date, height, spike density and spike angle). The large number of QTL with small effects that are not associated with agro-morphological traits suggests that genomic selection and rigorous phenotypic selection are appropriate approaches to improve disease resistance.

Towards Fine Mapping of a Native FHB Resistance QTL from Soft Red Winter Wheat Cultivar 'Jamestown'

Lovepreet Singh[1], Vijay Tiwari[1], Carl Griffey[2], Gina Brown-Guedira[3], Nidhi Rawat[1]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD 20742
[2]School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA 20460
[3]USDA-ARS, North Carolina State University, Raleigh, NC 27695
Corresponding Author: Nidhi Rawat, nidhirwt@umd.edu

Abstract

Fusarium Head Blight (FHB) is a devastating disease of wheat directly reducing yield and deteriorating quality due to mycotoxin contamination. Deploying genetic resistance is one of the most critical FHB management strategy. The majority of stable FHB resistance quantitative trait loci (QTL) have been derived from wild and non-adapted germplasm, which are not readily utilizable in the breeding programs due to the associated linkage drag. Therefore, it is crucial to identify and characterize native resistance derived from elite germplasm that could be available for immediate deployment in breeding programs. This study was conducted to fine map a native FHB resistance QTL on 1B chromosome of a high yielding moderately resistant soft red winter wheat cultivar Jamestown. Initial mapping of the QTL in a RIL population of 186 individuals identified two FHB QTL at long arm of 1B chromosome. In this study, genome-specific DNA markers were developed at 5 Mb intervals spanning the flanking region of 1B FHB QTL. The original RIL population was genotyped, and a critical set of recombinant RILs were identified. The critical RILs were robustly phenotyped for FHB severity and DON under greenhouse conditions for two years. Genotypic and phenotypic analysis narrowed the region to 25 Mb between 330-355 Mb region on 1B chromosome. High-resolution mapping population developed by crossing resistant and susceptible RIL and genotyped using genome specific Kompetitive allele specific polymerase chain reaction (KASP) assays marker revealed two recombinants. Subsequent phenotyping of F2 recombinants suggested a potential 15 Mb target region between 340-355 Mb. Genotyping and phenotyping of F3 families of recombinant and heterozygous F2 plants is underway. The fine mapping and KAPS markers developed in this study will enable precise selection for 1B FHB QTL and eventually facilitate cloning of the underlying candidate gene.

Phenylpropanoid-based Resistance to Fusarium Head Blight in Wheat

Shiv Singla[1], Zachary Duray[1,3], Ruth Dill-Macky[4], Patrick O'Neill[1,3], Lois Bernhardson[1,3], Satyanarayana Tatineni[1,3], Scott Sattler[2,3], Stephen Wegulo[1], and Deanna Funnell-Harris[1,3]

[1]University of Nebraska Lincoln, Departments of Plant Pathology, Lincoln, NE
[2]Department of Agronomy and Horticulture, Lincoln, NE
[3]USDA-ARS, Wheat, Sorghum, and Forage Research Unit, Lincoln, NE
[4]University of Minnesota, Department of Plant Pathology, St Paul, MN
Corresponding Author: Deanna Funnell-Harris, Deanna.Funnell-Harris@usda.gov

Abstract

Monolignol biosynthesis, part of the phenylpropanoid pathway, produces the lignin subunits and is associated with plant defense. We hypothesize that constitutive expression in wheat of sorghum genes involved in monolignol biosynthesis will increase resistance to FHB. The spring wheat variety CB037 (moderately susceptible) was transformed with constitutive expression constructs consisting of one of four sorghum genes: *SbMyb60*, encoding a transcriptional activator of monolignol biosynthesis, and, *SbC3'H* (*coumaroyl shikimate 3-hydroxylase*), *SbCCoAOMT* (*caffeoyl coenzyme A 3-O-methyl transferase*), or *Sb4CL* (*4 coumarate:CoA ligase*), that encode enzymes. In the field, the constitutive expression wheat lines were screened for Type-I resistance (initial infection) by determining disease index (DI), percent of Fusarium damaged kernels (FDK) and deoxynivalenol (DON) levels. In the greenhouse, two lines each constitutively expressing either *SbC3'H* or *SbCCoAOMT*, were tested for Type-I and Type-II resistance (spread of infection) by measuring FDK and the Area Under the Disease Progress Curve (AUDPC). In the field, a wheat line constitutively expressing *SbMyb60* had significantly less FDK than CB037 (the recipient check), but Sumai 3 (moderately resistant check) had lower FDK than all the lines. The DI and DON levels in transgenic lines were similar to or greater than CB037, but the DON level of one *SbC3'H* constitutive expression line was not significantly different from Sumai 3. In the greenhouse, for Type-I resistance, AUDPC and FDK in transgenic lines were similar to or greater than those of CB037. When screened for Type-II resistance, lines constitutively expressing *SbC3'H* and *SbCCoAOMT* had significantly less FDK and AUDPC than CB037. To identify genes and pathways involved in conferring Type-II resistance in *SbC3'H* and *SbCCoAOMT* constitutive expression lines, a global gene expression study is underway. Greenhouse-grown heads of these lines and CB037 were collected at 12 hours post-inoculation (hpi), 24 hpi, and 72 hpi. Total RNA will be extracted, followed by high-throughput RNA sequencing and analysis. We hypothesize that expression of genes involved in defense hormone production is increased in the constitutive expression lines. This study will improve understanding of the genes and mechanisms involved in FHB resistance.

Acknowledgement and Disclaimer

CRISPR-gene Editing to Engineer Plants for Disease Resistance

Bing Yang [1,2]

[1]University of Missouri, Bond Life Sciences Center, Columbia, Missouri
[2]Donald Danforth Plant Science Center, St. Louis, Missouri
Corresponding Author: Bing Yang, yangbi@missouri.edu

Abstract

Engineered CRISPR systems have been engineered into potent biotechnological tools for both basic and applied research. The most promising utilization of CRISPR/Cas9 is for targeted genome editing, leading to precise genetic alterations within any genome of interest, as demonstrated in a plethora of organisms including several important crop species. Bacterial blight (BB) is one of devastating rice diseases in Asia and Africa. The causal agent *Xanthomonas oryzae* pv. *oryzae* (Xoo) uses secreted TAL effectors (TALes) to ectopically activate the sucrose transporter SWEET genes of rice, conditioning a state of disease susceptibility. Xoo uses a limited set of TALes to target promoters of three SWEET genes (*SWEET11*, *13*, and *14*) in rice. Naturally occurring SWEET variants, with altered promoter TALe binding elements, act as recessive BB resistance genes by interfering with TALe functioning. We used CRISPR/Cas9 to engineer rice lines that have carried multiple mutations in three SWEET gene promoters. The SWEET promoter mutations were introduced into different rice varieties, and the disease evaluation showed that edited SWEET promoters generated robust, broad-spectrum BB resistance. We also developed rice lines that carried knockout mutations individually or in combination in three SWEET genes (*SWEET11*, *13*, and *14*). The knockout lines are useful diagnostic tools to determine SWEET-inducing TALes in Xoo isolates and guide the deployment of resistance genes derived from the naturally occurring or genome edited SWEET promoter mutations. Our results demonstrate a route to realize the promising potential of genome editing for crop improvement in agriculture.

Development and Validation of Diagnostic Markers for the Wheat Fusarium Head Blight Resistance Gene *Fhb7*

Lanfei Zhao[1], Wenyang Ge[2], Zhongfan Lyu[2], Shoushen Xu[2], Yuzhou Xu[1], Amy Bernardo[3], Qijun Zhang[4], Steven S. Xu[5,4], Hongwei Wang[2], Lingrang Kong[2], and Guihua Bai[1,3]

[1]Kansas State University, Manhattan, KS 66506
[2]Shandong Agriculture University, Taian, Shandong 271018, China
[3]USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506
[4]North Dakota State University, Fargo, ND 58108
[5]USDA-ARS, Crop Improvement and Genetics Research Unit, Albany, CA 94710
Corresponding Author: Guihua Bai, guihua.bai@usda.gov

Abstract

Fusarium head blight (FHB), mainly incited by *Fusarium graminearum* Schwabe, has caused great losses in grain yield and quality of wheat and barley globally. *Fhb7*, a gene with a major effect on FHB resistance, was recently cloned and found to encode a glutathione S-transferase (GST). *Fhb7* originated from 7E chromosome of *Thinopyrum ponticum* and confers broad resistance to *Fusarium* species. However, the high-throughput diagnostic markers are not available for breeding selection, which hampers wide deployment of *Fhb7* in breeding programs. To develop such DNA markers for high-throughput screening of the *Fhb7*, we designed two kompetitive allele specific PCR (KASP) markers (KASP-GST1 and KASP-GST2) based on the promoter and coding sequences of *GST* homologs and both markers cosegregated with *Fhb7* FHB resistance in a mapping population. To validate their usefulness in marker-assisted selection (MAS) in breeding programs, the two codominant gene markers were validated to be diagnostic for *Fhb7* in another biparental mapping population and one natural panel. Analysis of 244 *Thinopyrum* accessions using the two diagnostic gene markers identified only six accessions with the *Fhb7* resistance allele, and three of them were cytologically confirmed to be *Th. Ponticum*, the species in which *Fhb7* was originally identified. In addition, sequencing *GST* homologs in selected samples from five *Thinopyrum* species identified three haplotypes of *GST*. The development of the diagnostic markers in this study will facilitate the deployment of *Fhb7* in wheat breeding programs to improve FHB resistance.

Pathogen Biology & Genetics

A Genome-Wide Association Study for the Genetic Basis of Saprophytic Fitness Traits in a Sample of Isolates of *Fusarium graminearum* from the Americas

Upasana Dhakal, John F. Leslie, and Christopher Toomajian

Kansas State University, Department of Plant Pathology, Manhattan, Kansas
Corresponding Author: Christopher Toomajian, toomajia@ksu.edu

Abstract

Our broader aims include identifying genes harboring functional variation that contributes to variation in critical saprophytic and pathogenic fitness traits within *Fusarium graminearum* (*Fg*) populations to provide targets for pathogen management and control, and toxin reduction. Specific goals include performing genome-wide association studies (GWAS) to find the genetic basis of these pathogen traits by scanning tag single nucleotide polymorphisms (SNPs) throughout the *Fg* genome generated by genotyping-by-sequencing (GBS). We are collecting trait data in laboratory experiments as well as more laborious greenhouse head inoculation experiments. We have genotyped nearly 600 *Fg* isolates from several geographical regions in the Americas, including the Upper Midwest, New York, and Louisiana in the U.S. as well as Uruguay. These samples include isolates from the 3-ADON, 15-ADON, NX-2, and NIV toxin chemotypes. To make this work feasible, we have focused on a subset of ~150 of our isolates for phenotyping and subsequent GWAS analyses. In this subset, we have attempted to preserve the diversity of the larger sample and avoid choosing genetically similar isolates. So far, these isolates have been phenotyped for the saprophytic traits of ascospore discharge rate and mycelial growth rate at three temperatures. We screened for statistical associations between the traits and SNPs, implementing a mixed linear model that accounts for the known population structure of *Fg* populations as well as cryptic relationships between isolates from the same populations. While our GBS markers provide genotypes at tens of thousands of SNPs densely distributed throughout the *Fg* genome, most SNPs are missing some genotype data. We performed imputation to infer the allele state at missing genotypes, then filtered the SNPs to exclude low frequency variants for which power to detect a robust association is low. This left us with ~6,000 SNPs for the GWAS scan. Due to the correction for strong population structure in our diverse sample, the significance of our top associations remains moderate. For ascospore discharge, we identify a handful of strong candidate loci where the model predicts about half will be true positives.

Linking the Effects of *Fusarium graminearum* Infection to Phenolic Acid Content in Malting Barley

Janice Fajardo[1], Franklin Apea-Bah[2], Trust Beta[2], and Matthew Bakker[1]

[1]Department of Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2 Canada
[2]Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB R3T 2N2 Canada
Corresponding Author: Matthew Bakker, Matthew.Bakker@umanitoba.ca

Abstract

Fusarium graminearum is a fungal plant pathogen, most known for causing Fusarium head blight (FHB) in wheat and barley. Globally, FHB is responsible for significant economic impacts on the grain industry arising through lowering of grain quality related to mycotoxin contamination and yield loss. In malting, the environmental conditions set to stimulate barley germination are also conducive to new fungal growth and can lead to production of mycotoxins such as deoxynivalenol (DON). One important aspect of *Fusarium* growth on barley in malting is the presence of defensive plant compounds such as phenolic acids. Functional and structural modifications to the grain that occur in malting, including modifications associated with the activities of *Fusarium*, may influence the composition and/or availability of the phenolic acids in barley, and may have implications for *Fusarium* growth and toxin production. In this study, we investigated the effects of *Fusarium* infection during steeping on the availability of phenolic acids. The concentrations of four phenolic acids (caffeic, *p*-coumaric, ferulic, and sinapic) in the soluble free and insoluble bound extracts from barley, green malt, and malt were determined using high performance liquid chromatography. The dominant phenolic acids that were detected were ferulic and *p*-coumaric acid. During the course of malting, the quantity of each phenolic acid decreased (barley > green malt > malt). However, the rate of decline in the quantity of extractable *p*-coumaric acid was slowed by the presence of *Fusarium*; in other words, more *p*-coumaric acid was extracted from green malt that was infested with *Fusarium*, compared to non-infested green malt. The antifungal activities of ferulic and *p*-coumaric acid were examined in-vitro. Ferulic acid demonstrated the most significant fungal inhibition, at over 70% growth reduction at the highest concentration (1.0 mM) tested 7 days post-inoculation. A dose-effect relationship was found, where higher concentrations of the phenolic acids resulted in greater growth reduction of fungal mycelia. Current work is focused on clarifying the influence of *Fusarium* degradative enzymes on the release of phenolic compounds during malting of barley. Outcomes from this study could provide a better understanding of the interactions between barley and *Fusarium* which may form the basis for practical approaches to improve malt quality.

Population Diversity of *Fusarium* Species Causing Fusarium Head Blight in Wheat and Greenhouse Pathogenicity Tests of *F. poae* Isolated from Georgia

Bikash Ghimire[1], Mohamed Mergoum[2,3], Anthony E. Glenn[4], Kira L. Bowen[5], Alfredo D. Martinez-Espinoza[1], and James W. Buck[1]

[1]Department of Plant Pathology, University of Georgia, Griffin Campus, Griffin, GA, USA
[2]Department of Crop and Soil Sciences, University of Georgia, Griffin Campus, Griffin, GA, USA
[3]Institute of Plant Breeding, Genetics, and Genomics, University of Georgia, Griffin Campus, Griffin, GA, USA
[4]USDA-ARS, Toxicology & Mycotoxin Research Unit, Athens, GA, USA
[5]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL, USA
Corresponding Author: James W. Buck, jwbuck@uga.edu

Abstract

Recent outbreaks of Fusarium head blight (FHB) in Georgia have negatively impacted soft red winter wheat (SRWW) production; however, information on pathogen population is lacking. To explore the distribution and diversity of *Fusarium* species, we collected 212 isolates from symptomatic wheat heads and corn stubble by sampling 46 wheat and 55 corn fields across 72 counties of Georgia in 2018 and 2019. Pure cultured and single hyphal tipped isolates were subjected to genomic DNA extraction. The sequencing date from translation elongation factor 1-alpha (*TEF1*) locus was used for species level identification by querying publicly available sequence databases in NCBI GeneBank and *Fusarium* MLST. Trichothecene chemotypes were determined using chemotype-specific primers designed from the *TRI3* and *TRI12* loci. We identified that the majority (nearly 84%) of isolates from wheat were *F. graminearum* of which 78.0% were 15ADON chemotype, 19% NIV chemotype, and few remaining isolates of 3ADON chemotype. *F. poae* was the second largest group of species recovered from wheat and were distributed across six counties of Georgia. Interestingly, two-thirds of the total isolates from corn were resolved under *Fusarium incarnatum-equisetum* species complex (FIESC) with a very few (5%) presence of *F. graminearum*. Additionally, several other species were identified including *F. armeniacum, F. proliferatum, F. verticillioides, F. fujikuroi, F. avenaceum, F. acuminatum,* and *F. chlamydosporum* belonging to different species complexes. Greenhouse pathogenicity tests were conducted on five isolates of *F. poae* and one isolate of *F. graminearum* in three hosts: SRWW, durum wheat, and six-rowed barley, each consisting of two to three susceptible and moderately resistant cultivars. Significant effects of isolates, cultivars, and their interaction were observed on FHB traits with *F. poae* isolates causing up to 40% FHB severity (SEV) and 75% *Fusarium*-damaged kernel (FDK) and producing type A trichothecene T-2/HT-2 as high as 45 ppb. On the other hand, *F. graminearum* isolate produced nearly 90% SEV and FDK and up to 40 ppm DON, while no disease was observed on the mock-inoculated plants. Overall, our findings on the widespread distribution of FHB pathogens and the ability of *F. poae* isolates to cause disease in the greenhouse demonstrate that FHB outbreaks will likely continue in Georgia whenever environmental conditions favor and thus growers should consider integrated disease management strategies, whenever applicable.

β-1,3-glucan, Laminarin, Triggered an Atypical Reactive Oxygen Response in Wheat and Barely

Guixia Hao, Helene Tiley, and Susan McCormick

USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention and Applied Microbiology Research Unit. 1815 N. University, Peoria, IL.
Corresponding Author: Guixia Hao, guixia.hao@usda.gov

Abstract

The fungal pathogen *Fusarium graminearum* causes Fusarium head blight (FHB) on wheat and barley and contaminates grain with trichothecene mycotoxins. In the presence of microbes, plants perceive microbe-associated molecular patterns (MAMPs). The production of reactive oxygen species (ROS) is a hallmark of plant defense responses during plant and pathogen interactions. Cell walls of filamentous fungi consist of two main components: chitin and β-glucans. Although the chitin-mediated immunity is well studied, less information is available about the role of β-glucans during plant and pathogen interactions. In this study, we investigated ROS production in wheat and barley tissues treated with laminarin, an essentially linear glucan composed of ca. 33 β-1,3-linked Glc residues. Using luminol-based chemiluminescent assays, we showed that laminarin did not induce ROS bursts in leaves from eight tested wheat varieties but induced a high and broad ROS burst in barley leaves. Our prior study found that chitin induced ROS bursts in wheat head tissues including rachis nodes. Therefore, we compared ROS production induced by laminarin and chitin in wheat head tissues. In lemmas and paleae, a ROS burst was barely induced by chitin, but highly induced by laminarin. In rachises and rachis nodes, a high and broad ROS peak was induced by laminarin. Overall, significantly higher ROS were induced in all tested tissues by laminarin compared to chitin. Furthermore, we determined that plant defense genes were upregulated in wheat heads treated with laminarin. Currently, we are investigating the effects of chitin or laminarin treatments on FHB severity and mycotoxin contamination.

Pathogenicity of *Fusarium graminearum* and *Fusarium poae* Causing Fusarium Head Blight in Barley Under Controlled Conditions

K. Hudson[1], L. J. Harris[1], D. P. Overy[1], A. Xue[1], A. Foster[2], and R. Khanal[1]

[1]Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada
[2]Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, Charlottetown, PE, Canada
Corresponding Author: Kerin Hudson, khuds071@uottawa.ca

Abstract

Fusarium head blight (FHB) is one of the most devastating diseases of barley. FHB is caused by a species complex of *Fusaria*, of which *Fusarium graminearum* is the species responsible for most FHB epidemics in Canada. Field surveys show that two or more *Fusarium* species often co-exist within the same field or grain sample and *F. poae* is reported as another dominant species in barley in eastern Canada. The aim of this study was to determine the pathogenicity of *F. graminearum*, *F. poae* and a co-inoculation of both species causing FHB in barley under controlled conditions. Two susceptible barley genotypes were spray-inoculated at 10 to 14 days after heading. Phenotypic disease severity was rated on a scale of 0-9 at 4, 7, 14, and 21 days after inoculation. There was a significant difference in FHB severity between *F. graminearum* and *F. poae,* where *F. graminearum* produced more severe disease ratings. *F. poae* was less pathogenic and not statistically different from the control treatment (inoculated with deionized water only). When heads were co-inoculated with both *Fusarium* species, the resulting FHB severity was lower than that caused by *F. graminearum* alone. This suggests that the presence of *F. poae* may reduce the pathogenicity of *F. graminearum* in causing FHB, however according to our preliminary data, this difference is not significant.

Objective

To assess the individual and interactive effects of *Fusarium graminearum* and *Fusarium poae* on Fusarium head blight symptom severity in barley under controlled conditions.

Introduction

Fusarium head blight (FHB) is a devastating fungal disease that causes massive losses in grain yield and quality in cereals and grasses. It is mostly distinguished by their shrivelled and/or discoloured 'tombstones'. Infected kernels are particularly dangerous to livestock and human health because of mycotoxins accumulated in the grain. When consumed beyond safe thresholds, these mycotoxins have adverse gastrointestinal and reproductive effects on consumer health.

FHB is caused by a complex of species, of which the predominant causal species in Canada is *Fusarium graminearum* (*Fg*). Results from a 2001-2017 survey in Ontario, Canada showed that *Fg* was most detected in grain samples in epidemic years. However, in non-epidemic years, a weaker pathogen, *F. poae* (*Fp*), was most detected, especially in barley. Furthermore, relative host species differences were observed where *Fg* was most dominant in wheat, *F. poae* was most dominant in oat, but in barley *Fg* and *Fp* were equally dominant. Seasonal and host differences prompted the question: what is the relationship between *Fg* and *Fp* in barley? To our knowledge, all published *Fusarium* interaction studies are performed *in vitro* or in wheat. The report below is the first observing *Fg* and *Fp* in barley.

Materials and Methods

Plant material

Two susceptible spring barley genotypes, Stander (six-row) and CDC Bold (two-row), were tested in growth cabinets at Agriculture and Agri-Food Canada's Ottawa Research and Development Centre in 2021. Seeds were germinated on soaked Whatman paper, and then five seeds per 7.5" pot were transferred to a growth cabinet at 20:17°C with a photoperiod of 16h light:8h dark and 70% relative humidity (RH). At two weeks after planting, plants were fertilized once a week with 20-20-20 until harvest. Two pots of each genotype were randomly assigned to each treatment and arranged in a completely random fashion in the cabinet.

Infection and disease severity rating

At 10-14 days after heading (i.e. base of spike has emerged from the flag leaf sheath collar), spikes were inoculated with one of four treatments: *F. graminearum* (*Fg*; DAOMC 180378, Ottawa, ON, Canada), *F. poae* (*Fp*; DAOMC 252242, Ottawa, ON, Canada), *Fg* + *Fp*, ddH$_2$O (control). Approximately 1x10^4 CFUs were sprayed onto each spike, and each entire pot was covered with a plastic bag. Following inoculation, pots were transferred to a growth cabinet with 25:20°C and 90% RH and remained in this cabinet until the end of the experiment.

After 72 hours, the bags were removed, and at 4-, 7-, 14-, and 21-days post-inoculation, a disease severity rating was assigned to each spike on a scale of 0-9 (see Figure 1). At 28 days post-inoculation, spikes were cut from the plant at the base of the head, individually wrapped, flash-frozen in liquid nitrogen, and stored in -80° C until ready for further molecular analysis.

Results and Discussion

Two-way ANOVA was used to analyze effects of genotype and treatment on visual FHB rating (see Table 1). There was a significant replication effect in these preliminary trials (p<0.01 at all timepoints) and research continues at the Ottawa Research and Development Centre. In the first two replicates, the plastic bags covered the entire pot and were removed after 72 hours. However, for the third replicate, the awns of each desired head were trimmed, the head was spray-inoculated and individually bagged, and was never removed for the remainder of the experiment, potentially explaining differences in replications. The decision to transition to individually bagging spikes stemmed from an effort to limit: 1) contamination between treatments, and 2) growth of foliar pathogens like mildews and molds.
The current data reflected a significant treatment effect at all stages of disease (p<0.0001), while the genotype effect in early stages of disease became not significant as disease progressed (p>0.5). Pairwise comparisons revealed *Fg* and co-inoculation were significantly different from the control (p<0.001, see Table 2). FHB ratings from *Fp* alone were significantly lower than FHB ratings in *Fg* alone. In early disease progression, *Fp* alone was not significantly different from the control treatment but was significant at 14 days post inoculation and beyond. However, when compared to the co-inoculation treatment, the opposite effect was observed where at 21 days post inoculation the *Fp* and *Fg+Fp* became significantly different.

Fg-infected heads has the highest visual FHB ratings, and *Fp*-infected heads had the lowest, corroborating other reports that *Fg* is more aggressive than *Fp*. Co-inoculation with *Fg+Fp* returned lower visual FHB ratings, suggesting that *Fp* may be competing with *Fg*, but from our pairwise comparisons of the current data, the difference observed is not significant.

To increase confidence in our findings, we will be repeating the indoor growth cabinet study, and then proceeding to a molecular analysis of the grain. From flour, fungal DNA will be extracted and ddPCR will

be used to identify the dominant species and contaminants in each treatment. Metabolomic analysis will be completed by High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) to observe differences in metabolite profiles between treatments. There is generally a positive correlation between visual disease symptoms and mycotoxin levels in the grain, but visual FHB symptoms are not required for the accumulation of mycotoxins. An *in vitro* interaction study of the two specific isolates has also been performed and data analysis is in progress.

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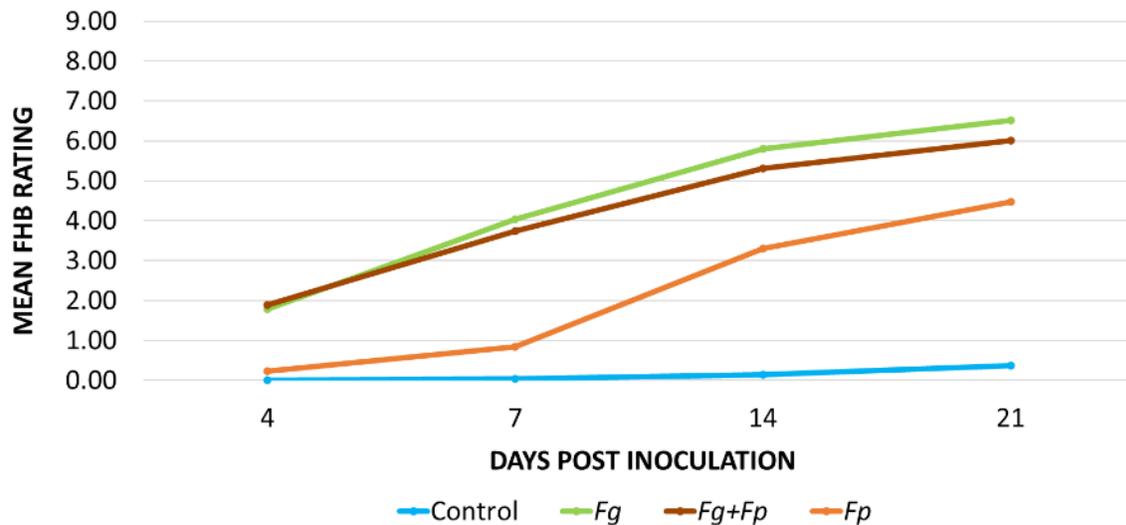
Figure 1 *- Mean disease progression per treatment, using visual disease rating scale of 0-9 (0 = no symptoms, 9 = severely diseased, spike dead); 0, no visible symptoms; 1, one diseased spikelet; 2, two diseased spikelets; 3, three diseased spikelets; 4, >3 diseased spikelets but 1/4 spike area with symptoms (sas); 5, <1/3 sas; 6, <1/2 sas; 7, <2/3 sas, slight peduncle discolouration; 8, <3/4 sas, restricted peduncle discolouration; 9, >3/4 sas, extended peduncle discolouration, spike dead. Each replication had 2 pots of each genotype for each treatment to give a total 16 pots per replication.*

Table 2 – *Two-way ANOVA for genotype and treatment effects*

		p-value			
Source	df	*4dpi*	*7dpi*	*14dpi*	*21dpi*
Replication	2	0.001	0.000	0.000	0.000
Genotype	1	0.003	0.003	0.115	0.628
Treatment	3	0.000	0.000	0.000	0.000
Genotype:Treatment	3	0.073	0.094	0.779	0.888

Table 1 – *Pairwise comparisons between single-species, multi-species and control treatments (dpi = days post inoculation)*

Treatment	4dpi		7dpi		14dpi		21dpi	
	diff	p adj	diff	p adj	diff	p adj	diff	p adj
Fg -Control	1.77	0.00	3.99	0.00	5.67	0.00	6.14	0.00
Fg+Fp -Control	1.87	0.00	3.70	0.00	5.17	0.00	5.63	0.00
Fp -Control	0.21	0.95	0.81	0.58	3.17	0.00	4.10	0.00
Fg+Fp-Fg	0.11	0.99	-0.28	0.97	-0.50	0.90	-0.50	0.89
Fp-Fg	-1.56	0.00	-3.18	0.00	-2.50	0.01	-2.03	0.03
Fp-Fg+Fp	-1.66	0.00	-2.90	0.00	-2.00	0.04	-1.53	0.16

Barley Resistance Ratings to Fusarium Head Blight Reflect *Fusarium graminearum* Growth and Deoxynivalenol Production During Malting

Anuradha Jayathissa[1], James Tucker[2,3], Ana Badea[3], Dilantha Fernando[2], Matthew Bakker[1]

[1]Department of Microbiology
[2]Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2 Canada
[3]Agriculture and Agri-Food Canada, Brandon Research and Development Centre, Brandon, MB R7A 5Y3 Canada
Corresponding Author: Matthew Bakker, Matthew.Bakker@umanitoba.ca

Abstract

Fusarium head blight (FHB) is a devastating disease of barley that leads to significant losses for the malting and brewing industry; however, there has been insufficient attention towards understanding the new fungal growth and the production of deoxynivalenol mycotoxin during the malting process. Field trials were carried out in 2019, 2020 and 2021 at Agriculture and Agri-Food Canada-Brandon Research and Development Centre, Brandon, MB. Barley varieties of different FHB resistant levels: Newdale (moderately resistant to moderately susceptible) and AAC Goldman (moderately resistant) were inoculated with single strain conidial suspensions of each of seven different *Fusarium graminearum* strains, plus a non-inoculated control. The severity of FHB (as a percentage of visibly symptomatic spikelets) was higher in Newdale than in AAC Goldman in all three years of the study (9% vs. 3%, respectively in 2019; 38% vs. 21% in 2020; 3.5% vs. 0.6% in 2021). FHB in non-inoculated controls was present at low levels compared to inoculated treatments each year. Cultivar differences were also evident and consistent across years when *Fusarium* density was assessed by quantitative PCR (*Fusarium*: barley gene abundance ratios averaged 74.3 in Newdale vs. 36.5 in AAC Goldman in 2019; 9.7 vs. 4.4 in 2020; 22.1 vs. 13.9 in 2021) and in non-inoculated controls, the *Fusarium* density was at very low levels. Deoxynivalenol content (determined via ELISA) in the harvested grain was also higher in Newdale than in AAC Goldman (11.6 vs. 5.0 ppm in 2019; 1.2 vs 0.7 ppm in 2020). We micro-malted this *Fusarium*-infested barley and found that cultivar differences in susceptibility factors persisted through malting; *Fusarium* density in finished malt remained higher in Newdale than in AAC Goldman (17.0 vs. 12.4 in 2019; 4.3 vs. 2.1 in 2020; 19.0 vs. 18.2 in 2021), as did deoxynivalenol content in the finished malt (3.8 ppm in Newdale vs. 1.4 ppm in AAC Goldman for 2019). Both *Fusarium* density and deoxynivalenol content were significantly correlated between barley and finished malt (P < 0.05). Differences among *Fusarium* strains were less frequent than expected, although in general deoxynivalenol content was higher for pathogen strains with a 3-acetyl-deoxynivalenol chemotype, compared to those with a 15-acetyl-deoxynivalenol chemotype. Future study objectives will be to understand the relationship between *Fusarium* trait variability and malt quality parameters which will suggest more effective criteria for grading barley grain or predicting final quality at early stages of malting.

The Effect of Wheat Resistance on the Aggressiveness of *Fusarium graminearum*

Mara Krone and Santiago Mideros

University of Illinois, Crop Sciences, Champaign-Urbana, Illinois
Corresponding Author: Mara Krone, mjkrone19@gmail.com

Abstract

Fusarium graminearum, the most common causal agent of Fusarium Head Blight on wheat, can cause significant yield loss and release mycotoxins that are known to cause diseases in both humans and animals. The level of aggressiveness in *F. graminearum* is both heritable and a highly variable trait. Genetic resistance in wheat is an important part of FHB management and could affect the populations of *F. graminearum.* I hypothesize that the aggressiveness of *F. graminearum* is affected by the resistance level of the wheat source. Isolates of *F. graminearum* were collected from naturally infected wheat lines with different levels of resistance. For this research 31 isolates from highly susceptible and 26 isolates from moderately resistant wheat lines were used. We conducted in vitro phenotyping of these isolates by recording the area of mycelial growth at 2, 4, 6, 8, and 10 days and sporulation at 10 days. To assess the aggressiveness of the isolates, 'Norm' spring wheat was grown in the greenhouse and inoculated at anthesis. Three disease ratings were taken weekly after inoculations and used to calculate an AUDPC value. Once harvested the percentage of *Fusarium* diseased kernels (FDK) per head was calculated. The harvested kernels will then be used for quantification of the mycotoxin deoxynivalenol (DON). Both the in vitro phenotyping and aggressiveness assay experiments were repeated twice. DNA was extracted from all the *F. graminearum* isolates and sequenced on an SP lane of a Novaseq6000. A linear mixed model was used to analyze the isolate's phenotypes and found that the isolate's source significantly affects the AUDPC, in vitro growth rate, and spore count. Isolates from the susceptible wheat lines were more aggressive than isolates from the resistant wheat lines. A strong significant correlation was found between AUDPC and FDK. There was also a significant correlation between the in vitro growth rate and FDK showing isolates that grow faster in vitro produce more diseased kernels in planta. The whole-genome sequences are currently being analyzed. Results from the data analyzed so far show resistant wheat lines are not selecting for more aggressive isolates. From these results, a better understanding of the factors affecting individual strain aggressiveness can be achieved and be useful for managing the deployment of disease-resistant wheat varieties.

Exploring the Genetics of Biofilm Development in *Fusarium graminearum*

Rebecca Shay[1] and Frances Trail[1,2]

[1]Michigan State University, Department of Plant Biology, East Lansing, MI
[2]Michigan State University, Department of Plant, Soil and Microbial Sciences, East Lansing, MI
Corresponding Author: Rebecca Shay, shayrebe@msu.edu

Abstract

Biofilms are known to play important roles in the disease cycles of bacterial pathogens of plants and animals, where the formations help protect cells from host defense responses and antimicrobial treatments. We have characterized the development of biofilms in *Fusarium graminearum in vitro*, and are now working towards elucidating the genetics behind the formation. The genetics of filamentous fungal biofilms have not been explored in depth, especially in plant pathogens. Through the use of RNA-sequencing, we have profiled the transcriptome of biofilm formation in *F. graminearum* over time, which was used to provide candidate genes for phenotypic analysis. The different stages of biofilm development were compared sequentially for differentially expressed transcripts. Stages selected were initial adhesion, initial development of the extracellular polymeric matrix, development of conidia within the biofilm, and mature biofilm formations. Additionally, we have selected a strain of the wild-type fungus which exhibits enhanced adhesion, an important biofilm phenotype. This strain was obtained by only eighteen rounds of selection for adhesive properties, indicating that adhesion is likely to be a property that can adapt quickly to new situations. Understanding the genetics behind the development of *F. graminearum* biofilms can provide insight into how these structures form and influencing disease development.

Unraveling the Mystery Behind Increased DON Level in the Malting Grains

Shyam Solanki[1], Zhao Jin[2], Gazala Ameen[1], Thomas Gross[3], Roshan Sharma Poudel[3], Pawel Borowicz[4], Robert S. Brueggeman[5], Paul Schwarz[2]

[1]South Dakota State University, Department of Agronomy, Horticulture and Plant Sciences, Brookings, South Dakota, 57007, USA.
[2]North Dakota State University, Department of Plant Sciences, Fargo, ND 58108, U.S.A.
[3]North Dakota State University, Department of Plant Pathology, Fargo, ND 58108, U.S.A.
[4]North Dakota State University, Department of Animal Sciences, Fargo, ND 58108, U.S.A.
[5]Washington State University, Department of Crop and Soil Sciences, Pullman, WA 99164, U.S.A.
Corresponding Author: Shyam Solanki, shyam.solanki@sdstate.edu

Abstract

Fusarium graminearum, the major species causing the Fusarium Head Blight (FHB) disease in cereals produces a variety of mycotoxins. Among these mycotoxins deoxynivalenol (DON) is a major challenge for quality of malting grains. An increase in DON following the malting of grain that had a relatively low initial level of these neurotoxins, and also had been stored for several months are seen as aberrant behavior. Maltsters have speculated that in barley, this may relate to internal vs external infection with *Fusarium* species. To track the presence and progression of *Fusarium* mycelia distribution during the malting of FHB infected small grains, we optimized and utilized a fluorescent staining method of cross sectioned grain samples using WGA-Alexa Fluor 488 fluorophore which preferentially binds with sialic acid and *N*-acetylglucosaminyl residues in fungal hyphae. Confocal laser scanning microscopy evaluation revealed that fungal hypha was mainly present in the husk (spongy parenchyma and cementing layer) of barley, but also can be found in pericarp, testa, aleurone layer, and even slightly into the interspace of starchy endosperm. Extensive growth of fungal hyphae was also observed in pericarp, testa, aleurone layer and the central endosperm of rye and triticale, which illustrated internal infection in rye, triticale and their malts. A sharp increase of DON and *Tri5* DNA levels were observed following the malting of these grain samples. To investigate the *Tri5* gene expression during infection, we are currently employing the RNAscope RNA *in situ* visualization. We conclude that the presence of internal infection and viable *Fusarium* hyphae provides a survival advantage to the pathogen during the harsh conditions of the malting process, thus, contributing to elevated DON level beyond the acceptable limits.

Impact of Microbial Associations on *Fusarium graminearum* Virulence and Disease

Briana Whitaker[1], Martha Vaughan[1], Susan McCormick[1], Matt Bakker[2]

[1]USDA, Agricultural Research Service, National Center for Agricultural Utilization Research, Mycotoxin Prevention & Applied Microbiology Unit, 1815 N University St., Peoria, IL 61604
[2]University of Manitoba, Department of Microbiology, Manitoba, Winnipeg, CA
Corresponding Author: Briana Whitaker, briana.whitaker@usda.gov

Abstract

Bacterial-fungal interactions can influence fungal phenotypes and shape the outcomes of plant-fungal interactions. Species of the fungus *Fusarium*, including *F. graminearum*, cause Fusarium head blight (FHB) of cereal crops and contaminate grain with harmful trichothecene mycotoxins. One approach to sustainably manage FHB includes harnessing the power of parasitic or antagonistic bacteria against *F. graminearum* in agroecosystems. In this talk, I will highlight two different approaches to managing FHB through use of isolated bacterial strains. First, through the study of existing *F. graminearum* genomes, a parasitic *Paenibacillus* species was identified and isolated. Scanning electron microscopy (SEM) imaging data indicate the association is at least partially ectopic, or ecto-hyphal, to the fungus. Experiments *in planta* (wheat) comparing wild type associations of the parasitic *Paenibacillus – F. graminearum* to the same *F. graminearum* strain cured of the bacteria, showed up to a 6-fold decrease in toxin (DON) when the parasitic bacteria were present. Additional tests to re-establish the association by simple mixing of the fungus and bacteria failed to reduce FHB spread and toxin accumulation *in planta*. In a second experiment, seven different species of bacteria were tested for their ability to reduce FHB *in vitro* and *in planta*. Results from a detached wheat head assay were promising, showing up to a 10-fold decrease in *F. graminearum* spread across several different environmental conditions tested. However, seed soak inoculations of the same bacteria in two different wheat varieties, varying in disease resistance, showed minimal changes in disease spread relative to the non-inoculated controls. Interestingly, the endophytic bacteria did have an impact on plant photosynthetic response, but not on metrics of disease. The results of these two studies highlight the complexities of harnessing bacterial-fungal associations to reduce FHB spread in living host plants and indicate more research is needed to understand the holistic response of small grain hosts to biocontrol applications.

Acknowledgements

Use of Mating-Type Gene Deletion Mutants for Genetic Analysis of *Fusarium graminearum*

Gabdiel Yulfo-Soto[1], Franklin Machado[1,2], Aline Vieira de Barros[1], David Van Sanford[3], Emerson Del Ponte[2], Frances Trail[4], Lisa Vaillancourt[1]

[1]University of Kentucky, Department of Plant Pathology, Lexington, Kentucky
[2]Universidade Federal de Vicosa, Departamento de Fitopatologia, Vicosa, MG, Brazil
[3]University of Kentucky, Department of Plant and Soil Sciences, Lexington, Kentucky
[4]Michigan State University, Department of Plant Biology, East Lansing, Michigan
Corresponding Author: Lisa Vaillancourt, vaillan@uky.edu

Abstract

Management of Fusarium Head Blight (FHB), caused by *Fusarium graminearum sensu stricto* and other members of the *F. graminearum* species complex (FGSC), is challenging because of its complex epidemiology and the strong influence of environmental factors on disease outcomes and toxin accumulation. There is a high degree of genotypic and phenotypic variation among pathogen species and strains. Current FHB models and treatments do not account for pathogen diversity, so it is difficult to predict what will happen if a new variant is introduced, or if changes in the environment favor one genotype over another. To improve management of FHB, we aim to (1) identify novel genetic markers associated with fitness and pathogenicity, and (2) incorporate these markers in multi-locus genotyping assays to monitor and predict population shifts. Although they are homothallic, outcrossing occurs between and within species. Controlled crosses are challenging because outcrossed perithecia must be differentiated from selfed ones. We produced deletion mutants of the MAT1-1-1 and MAT1-2-1 (MAT) genes and screened them to identify appropriate test maters with normal fitness- and pathogenicity-related phenotypes. The deletion strains engage only in heterothallic mating, solving the problem of identifying outcrossed perithecia. Many strains, especially the MAT1-2-1 deletions, were significantly reduced in pathogenicity or fitness compared with the wild type (WT). However, two highly female-fertile MAT1-1-1 strains did not differ from WT, and these were used in test matings with two MAT1-2-1 deletions varying in colony morphology and pathogenicity, and with WT strains including the PH-1 progenitor, another strain of *F. graminearum* (Gz3639), and *F. meridionale*. Antibiotic resistance, MAT alleles, and fertility had expected 1-1 segregation patterns in the crosses with *F. graminearum* WT strains, while segregation patterns related to colony morphology were complex and indicated absence of linkage to the MAT deletions. The interspecific cross resulted in non-Mendelian segregation patterns. Analyses involving additional molecular markers and pathogenicity-related phenotypes are continuing. However, results so far suggest that the MAT1-1-1 deletion strains will be suitable as test mates for crosses with WT FGSC strains, facilitating our efforts to identify molecular markers linked to fitness, pathogenicity, and toxigenicity-associated phenotypes.

Acknowledgements

Variety Development and Host Resistance

Quantitative Trait Loci Mapping for Fusarium Head Blight Resistance in a Wheat EMS Mutant from 'Jagger'

Ruolin Bian[1], Amy Bernardo[2], Paul St. Amand[2], Guihua Bai[2]

[1]Department of Agronomy, Kansas State University
[2]USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506
Corresponding Author: Guihua Bai, guihua.bai@ars.usda.gov

Abstract

Wheat Fusarium head blight (FHB) is one of the most destructive diseases of wheat and causes significant yield losses in wheat worldwide. Using resistant cultivars is one of the most effective approaches to control FHB. Although more than 600 quantitative trait loci (QTLs) have been reported, most of them showed minor effects and were not consistent across environments. To explore new sources of resistance, an EMS-induced mutant population derived from an FHB-susceptible hard winter wheat cultivar 'Jagger' was screened for FHB resistance and one mutant line (1076EMSMut) showed significantly higher FHB resistance than Jagger (< 0.01), with mean percentage of symptomatic spikelets in a spike (PSS) of 27% for 1076EMSMut and 80% for Jagger. A population of 164 recombinant inbred lines (RILs) was developed from 1076EMSMut x Jagger and phenotyped for FHB resistance and several agronomic traits, including plant height (PH), heading date (HD), spike length (SL), and spikelet number (SN) in four greenhouse experiments. A genetic map was constructed using 1,992 high-quality single nucleotide polymorphisms (SNPs) generated by the genotyping-by-sequencing (GBS). Analysis of quantitative trait loci (QTLs) identified ten QTLs for FHB resistance, only the QTL on 3A was significant for FHB resistance in multiple experiments and explained 9.19% - 12.01% of the phenotypic variation with the resistance allele from 1076EMSMut. Six QTLs were detected for SL, and only the 1B QTL was repeatable in two experiments and explained 11.44 and 17.06% of the phenotypic variation. Meanwhile, seven QTLs were identified for SN on 1B, 4A, 5A, 5B, 5D, 6A, and 7A. Among them, the 4A QTL was significant in two experiments and explained 10.78% and 11.51% of the phenotypic variation; the 7A QTL was significant in three experiments and increased SN by up to 13.66%. Repeatable QTL was not identified for PH and HD. The QTLs identified in this study should be useful for improvement of wheat FHB resistance and grain yield in hard winter wheat breeding programs.

DON Accumulation and Fusarium Head Blight Resistance in Winter Barley

Ben Eggers and Eric Stockinger

The Ohio State University, Department of Horticulture and Crop Science, Wooster, Ohio
Corresponding Author: Eric Stockinger, stockinger.4@osu.edu

Abstract

Increasing Fusarium Head Blight (FHB) disease on barley as a result of changes in climate and maize cultivation practices is placing the Nation's high-quality malt supply at risk of being inadequate. At the same time the Independent Craft Brewing Movement, which is undergoing exponential growth, is seeking locally produced barley. These trends are upending traditional systems. As a consequence, breeders across North America must develop portfolios of barley varieties, each resistant to FHB and adapted to its own unique environment. In autumn 2020 we planted the first FHB (scab) nursery for winter barley in Ohio. Included in the planting is a population of ~170 lines. This set consists of highly diverse two-row winter barley lines associated with our breeding program. We also planted a population of 82 recombinant lines from the 95SR316A × Charles cross, which we are referring to as the "Bregitzer population." Each line was planted in three reps. Also included were a set of six lines previously shown to differ in disease incidence and DON accumulation. Combined, we are testing ~260 different barley lines. Phenotypic data for the ~260 lines were obtained in the form of a visual fusarium incidence score. We are preparing samples for DON and related toxin level determinations at present. Each of the 170 lines were genotyped using a multiplexed sequencing (GMS) platform, and a subset using the Barley 50k iSelect SNP Array. The phenotypic data will then be tested for associations with regions of the barley genome, with the long-term goal being to utilize this information to select for resistances at the genotypic level. A subset of lines exhibiting low disease incidence have also been crossed to Ohio-adapted winter-hardy lines.

Serendipity and Strategy: Improving FHB Resistance in Hard Winter Wheat

Allan Fritz[1], Marshall Clinesmith[1], and Jessica Rupp[2]

[1]Kansas State University, Department of Agronomy, Manhattan, KS
[2]Kansas State University, Department of Plant Pathology, Manhattan, KS
Corresponding Author: Allan Fritz, akf@ksu.edu

Abstract

The Kansas State University wheat breeding program has been breeding for FHB resistance for more than 20 years. Identification and deployment of native resistance in the variety 'Everest' was a significant step toward improved management of disease but progress beyond the Everest level of resistance has been difficult. Phenotypic screening nurseries have facilitated the transfer of additional resistance from outside the hard winter wheat gene pool. Specifically, resistance derived from the soft wheat 'Truman' has been useful, but combining resistance with agronomic performance has been challenging. Recent results indicate a step change in the level of resistance with the best materials having 3 percent symptomatic spikelets compared to Everest at 20%. Materials are still in early generations, therefore agronomic performance has not be fully evaluated. However, visual phenotypes are superior to previous iterations. Additional sources of resistance are still needed. An effort to transfer resistance from *Aegilops tauschii* will be described. Future strategies for improvement of FHB resistance in the KSU wheat breeding program will also be discussed.

Evaluation of FHB Resistance in Hybrid Wheat

Pravin Gautam, Bill Berzonsky, Rima Thapa, Edward Souza, Ali ShaarbafToosi and Chelsea Puncochar

BASF Corporation
Corresponding Author: Pravin Gautam, Pravin.gautam@basf.com

Abstract

Hybrid wheat is considered as a key to yield boost, stability, climate resiliency, and enhanced biotic stress resistance. To understand the FHB resistance and heterosis in hybrid wheat, 1819 hybrid wheat along with 2167 inbred and parents and 53 commercial varieties were evaluated across three years (2019-2021) in corn spawn inoculated and misted FHB nursery in Nebraska. About 80% and 11% of the tested hybrids showed better resistance than susceptible and resistant check varieties, respectively. Similarly, 67% and 23% of inbred evaluated showed better resistance than susceptible and resistant check varieties, respectively. Mid-parent heterosis (MPH) for FHB index ranged from -74% to 154% with mean being -0.4%. Mean better parent heterosis (BPH) for FHB index was 11% ranging from -69% to 362%. MPH for FDK ranged from -47% to 66% with mean of 0.14%. Mean BPH was 9% ranging between -45%% and 101%. Anther exertion was negatively correlated with FHB index and significant in two of the three years tested. FDK was also negatively correlated with another exertion but was significant only in 2021. Anthesis date was positively correlated with FHB index and negatively correlated with FDK. Results suggests hybrid wheat can be alternative for increased FHB resistance in wheat.

Exploring Variation for FHB Resistance and Toxin Mitigation in Naked Barley

John Hawkins and Kevin Smith

University of Minnesota, Department of Agronomy and Plant Breeding, St. Paul, Minnesota
Corresponding Author: John Hawkins, hawki535@umn.edu

Abstract

Naked barley is receiving growing interest for malt, feed, and food applications. One potential advantage of naked barley over covered barley is lower levels of deoxynivalenol (DON) due to the loss of the hull. Studies on covered barley show hull removal can eliminate some amount of DON during processing, but the role of the hull as a DON sink in naked varieties hasn't been studied extensively. Variation for hull DON content could allow selection for stronger DON sink activity of hulls, reducing kernel DON content and the associated risks. The goals of this study are to evaluate a naked barley diversity panel for Fusarium head blight (FHB) resistance and favorable DON distribution and use that data to conduct genome-wide association studies (GWAS). The diversity panel, consisting of 242 naked spring barley lines along with one naked and five covered check varieties, was planted in irrigated FHB nurseries in St. Paul and Crookston in 2020. The St. Paul nursery was inoculated by spraying a solution containing macroconidia, while the Crookston nursery was inoculated with grain spawn. Data was collected on height, heading date and percent FHB infection in the field. Heads were harvested and separated into hull, kernel, and rachis subsamples for DON concentration analysis. Preliminary results show that the proportion of the DON mass localized in hulls, averaged across locations, ranged from 0.07 to 0.96, with mean of 0.36 and standard deviation of 0.15. This shows a high degree of variation in DON sink activity of the hull and potential for substantial toxin mitigation. On the other hand, there is also large variation among the lines for DON concentration in the kernel, suggesting variation for disease resistance per se. There is also a great deal of variation in the percent FHB infection, which ranges in St. Paul from 7.5% to 82.5%. Rachis subsamples showed comparable DON concentrations to other tissue types, with a mean of 21.9 ppm DON, which is surprising given that FHB in barley does not exhibit "spread in the head" as in wheat. Overall, naked barley shows potentially useful variation in FHB resistance and toxin distribution. A preliminary genome wide association study is in progress for this data. This experiment was repeated in 2021 and analysis of those samples is in progress.

Collaborative Doubled Haploid Breeding for Fusarium Head Blight Resistance in Barley

Patrick Hayes, Tanya Filichkin, and Laura Helgerson

Oregon State University, Corvallis, OR
Corresponding Author: Patrick Hayes, patrick.m.hayes@oregonstate.edu

Abstract

Breeding barley is a long-term process that requires multiple cycles of self-pollination to achieve complete homozygosity. Doubled haploid (DH) production leads to complete homozygosity in a single generation, thus bypassing the complications of field, greenhouse, or off-season generation advance. Completely homozygous material facilitates the phenotyping of complex traits and simplifies integration of phenotype with genotype for gene discovery and characterization. Here we present a summary of our USWBSI-supported DH production metrics.

Predicting Fusarium Head Blight Resistance for Advanced Trials in a Soft Red Winter Wheat Breeding Program with Genomic Selection

Dylan L. Larkin[1], R. Esten Mason[2], David E. Moon[3], Amanda L. Holder[4], Brian P. Ward[5] and Gina Brown-Guedira[6]

[1]Aardevo North America, Boise, ID, USA
[2]Colorado State University, Department of Soil and Crop Sciences, Fort Collins, CO, USA
[3]University of Arkansas, Crop Soil and Environmental Sciences, Fayetteville, AR, USA
[4]Agriculture Department, Crowder College, Joplin, MO, USA
[5]Ohio State University, Department of Horticulture and Crop Science, Wooster, OH, USA
[6]USDA-ARS SEA, Plant Science Research, Raleigh, NC, USA
Corresponding Author: Esten Mason, esten.mason@colostate.edu

Abstract

Many studies have evaluated the effectiveness of genomic selection (GS) using cross-validation within training populations; however, few have looked at its performance for forward prediction within a breeding program. The objectives for this study were to compare the performance of naïve GS (NGS) models without covariates and multi-trait GS (MTGS) models by predicting two years of $F_{4:7}$ advanced breeding lines for three Fusarium head blight (FHB) resistance traits, deoxynivalenol (DON) accumulation, Fusarium damaged kernels (FDK), and severity (SEV) in soft red winter wheat and comparing predictions with phenotypic performance over two years of selection based on selection accuracy and response to selection. On average, for DON, the NGS model correctly selected 69.2% of elite genotypes, while the MTGS model correctly selected 70.1% of elite genotypes compared with 33.0% based on phenotypic selection from the advanced generation. During the 2018 breeding cycle, GS models had the greatest response to selection for DON, FDK, and SEV compared with phenotypic selection. The MTGS model performed better than NGS during the 2019 breeding cycle for all three traits, whereas NGS outperformed MTGS during the 2018 breeding cycle for all traits except for SEV. Overall, GS models were comparable, if not better than phenotypic selection for FHB resistance traits. This is particularly helpful when adverse environmental conditions prohibit accurate phenotyping. This study also shows that MTGS models can be effective for forward prediction when there are strong correlations between traits of interest and covariates in both training and validation populations.

Multi-trait Genomic Selection and it's Potential to Streamline Scab Resistance Phenotyping

Jessica Rutkoski[1], Rupesh Gaire[2], Marcio Arruda[3], Mohsen Mohammadi[4], Gina Brown-Guedira[5], Frederic Kolb[1]

[1]University of Illinois, Department of Crop Science, Urbana IL
[2]Bayer Crop Science, St. Louis, MO
[3]HM Clause, Davis, CA
[4]Purdue University, Agronomy, West Lafayette, IN
[5]USDA-ARS Plant Science Research, Raleigh, NC
Corresponding Author: Jessica Rutkoski, jrut@illinois.edu

Abstract

Breeding programs that select for scab resistance aim to reduce Deoxynivalenol (DON), the primary trait. Because evaluating DON is costly, the secondary scab resistance traits: incidence, severity and Fusarium damaged kernels (FDK) are typically evaluated on large numbers of lines while DON is measured on fewer lines at later stages of selection. Genomic selection (GS), selection based genomic estimated breeding value, can improve plant breeding efficiency in two ways. GS can accelerate rates of genetic gain by enabling parent selection prior to phenotyping. At later stages of selection, GS can improve phenotypic selection accuracy or achieve the same level of selection accuracy with less phenotypic data by tapping into information from relatives, and from correlated traits in the case of multi-trait GS. Using datasets from Purdue University and the University of Illinois, we tested whether multi-trait GS models could be used to reduce phenotyping costs for scab resistance without sacrificing selection accuracy. We considered a scenario where selection for scab resistance among breeding candidates is done based on traits other than DON. We compared multi-trait GS models for DON which included DON phenotypes on the training set and other scab resistance traits on both the training set and the selection candidates. Multi-trait GS models which included FDK on the selection candidates were the most predictive of DON. Furthermore, we discovered that once FDK was included in the model, adding data on severity and incidence did not improve accuracy. These results indicate that once breeding programs begin using GS, phenotyping severity and incidence may no longer be necessary. Regardless of the selection method, phenotyping FDK and DON will remain critical.

Breeding for FHB Resistance in Hard Winter Wheat

Sunish K. Sehgal[1], Jinfeng Zhang[1], Harsimardeep S. Gill[1] Navreet K Brar[1], Jyotirmoy Halder[1], Shaukat Ali[1], Xiaotian Liu[2], Amy Bernardo[3], Paul St Amand[4], Guihua Bai[4], Brent Turnipseed[1]

[1]Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD, USA.
[2]School of Computing, Queen's University, Kingston, ON, Canada
[3]Department of Plant Pathology, Kansas State University, Manhattan, KS, USA
[4]USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS, USA.
Corresponding Author: Sunish Sehgal, Sunish.Sehgal@sdstate.edu

Abstract

South Dakota winter wheat breeding program routinely develops winter wheat varieties under a 100% regenerative management system working closely with producers through on-farm trials. The main goal of the breeding program is to deliver hard winter wheat varieties with stable yield potential, good end-use quality, and resistance against disease and pests for the producers of South Dakota and the northern Great Plains. Fusarium head blight (FHB) followed by rust and Bacterial Leaf Streak are the major threats to sustainable wheat production in South Dakota. Breeding efforts for FHB resistance have successfully exploited native resistance. Over the years, SD winter wheat breeding program has released several winter wheat varieties like Lyman, Oahe, Winner, and Draper with above-average FHB resistance through conventional breeding focusing on phenotypic selection. Recently, we evaluated the potential of genomic selection in predicting FHB disease index (DIS) and Fusarium damaged kernels (FDK) in the early generation breeding lines using advanced breeding lines as training populations. We observed moderate prediction accuracy of up to 0.59 for DIS and 0.54 for FDK, demonstrating the promise in genomic prediction for FHB resistance in earlier generations using advanced lines. Further to increase the frequency of *Fhb1+Sr2, Fhb6, and Fhb1+2DL* in SD breeding materials, we are coupling marker-enrichment with rapid generation advancement.

Evaluation of Winter Rye (*Secale cereale* L.) Resistance to Fusarium Head Blight in Kentucky

Elzbieta Szuleta, Tim Phillips, and David Van Sanford

University of Kentucky, Department of Plant and Soil Sciences, Lexington, KY 40546
Corresponding Author: David Van Sanford, dvs@uky.edu

Abstract

Fusarium head blight (FHB) is a fungal disease that causes yield and quality loss in cereal crops. In Europe, the crop most susceptible to Fusarium damage and DON accumulation is durum wheat, followed by bread wheat, triticale, and winter rye with the least susceptibility. Because FHB in winter rye is not a major problem, its resistance to Fusarium has not received as much attention as has winter wheat resistance. While trying to bring back winter rye production in Kentucky, farmers face Fusarium kernel damage and difficulties in protecting rye from this fungal disease. A big problem is to determine right fungicide application date, because of rye's open-pollinating nature and non-uniform heading date in population varieties. In 2020 and 2021 we tested 24 commercially available winter rye varieties for FHB resistance in the Fusarium inoculated nursery in Lexington, Kentucky. Traits measured included heading date, incidence, severity, and FHB index on a 0-9 scale. Post-harvest analysis included FDK rate measured with light seeds vacuum sorter. In 2021 we measured heading date, FHB index and DON content. Overall, the most FHB - resistant varieties were Daniello and Kentucky line KYSC1710 (with the FHB index 0.5), followed by hybrids Bono and Serafino and population variety Wheeler (FHB index 0.7). Over two years of observation, the most susceptible varieties were Wrens Abruzzi, Rymin and Kentucky line KYSC1704. FHB index means were statistically higher in 2020 comparing to 2021 season. 2020 data analysis showed a weak positive correlation (0.27) between the FHB index and FDK rate. We found a strong negative correlation (-0.57) between earliness and FDK, a moderate correlation (0.45) between earliness and incidence, and a negative correlation (-0.47) between earliness and FHB index. The next step will be to identify the best sources of winter rye FHB resistance and to begin incorporating the resistance into the breeding program.

Exploring the Genetic Diversity of Fusarium Head Blight Resistance in a Diverse Triticale Collection

Sydney Wallace[1], Bhavit Chhabra[1], Yanhong Dong[2], Xuefeng Ma[3], Gary Coleman[1], Vijay Tiwari[1], and Nidhi Rawat[1]

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland 20742, USA
[2]Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55108, USA
[3]Noble Research Institute, Ardmore, Oklahoma 73401, USA
Corresponding Author: Nidhi Rawat, nidhirwt@umd.edu

Abstract

Fusarium Head Blight is a destructive disease affecting the grain yield and quality of wheat, barley, rye and triticale. Developing varieties with genetic resistance is integral to successfully managing FHB. Triticale is an important forage and cover crop with steadily increasing acreage throughout the USA. Triticale's high protein content and favorable composition of amino acids makes it a desirable livestock feed, whereas high biomass and nitrogen use efficiency make it a highly recommended cover crop. However, a significant knowledge gap exists in the genetic variation for FHB resistance in triticale. This information is critical for breeding new varieties of triticale as its production continues to increase. In the present study, a set of 298 winter-type Triticale accessions from a worldwide collection were screened for their type-2 FHB resistance in a misted nursery using high levels of FHB inoculum. Most of the Triticale accessions were susceptible to FHB, and only 8% of accessions showed resistance in the nursery screening. The resistant accessions identified in the nursery screening were selected and further screened for three years in greenhouse conditions. Seven accessions were found to show robust FHB resistance over the three years of greenhouse testing. Thirteen accessions showed significantly lower levels of DON accumulation when compared to a susceptible Triticale control. The accessions identified in the study will be useful in Triticale breeding programs for enhancing FHB resistance and reducing DON accumulation.

Breeding for Fusarium Head Blight Resistance of Wheat (*Triticum aestivum*) by Marker-Assisted Selection and Genomic Selection

Fang Wang[1], P. Stephen Baenziger[1], Stephen Wegulo[2], Julie Stevens[2], Vikas Belamkar[3], Katherine Frels[1]

[1]University of Nebraska-Lincoln, Department of Agronomy and Horticulture, Lincoln, NE
[2]University of Nebraska-Lincoln, Department of Plant Pathology, Lincoln, NE
[3]Corteva Science, Moorhead, MN
Corresponding Author: Katherine Frels, kfrels2@unl.edu

Abstract

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a devastating disease of wheat (*Triticum aestivum* L.). Improvement of host resistance is an effective strategy to mitigate yield and quality losses. In this research, we used marker-assisted selection (MAS) and genomic selection (GS) to develop adapted wheat cultivars and make accurate selections to advance breeding for resistance to FHB. Overland_Fhb10 is a moderately resistant hard winter wheat cultivar with *Fhb1*. Backcross populations were developed between Overland_Fhb10 as a donor parent and six elite domestic breeding lines as recurrent parents which are NE14696 (very good native tolerance to FHB), NE14421, NE16562, NE15624, NE14434 and NE10478-1 (very good native tolerance to FHB and marketed as LCS Valiant) to introgress *Fhb1* into advanced or modern adapted germplasm for potential release or use as parents. The diagnostic KASP marker conferring *Fhb1* was used in the genotypic identification from BC_1 to BC_3 generations. Disease resistance was significantly improved for each genotype in each backcross population. NE14696 carrying *Fhb1* showed lowest severity (SEV) in three backcross generations. In addition to improving NE germplasm with marker-assisted selection, genomic selection was considered as a feasible solution to save field screening effort and data loss under unsuitable environments. To evaluate the prediction accuracy of genomic selection, SEV and incidence (INC) were evaluated for 1199 winter wheat lines planted in 2015 to 2019. The phenotypes were evaluated in replicated and misted disease nurseries located at Mead or Lincoln, Nebraska. In total, 62,478 SNPs were obtained by genotyping-by-sequencing and fitted in the statistical model. The heritability (H^2) of SEV and INC varied through years with an overall H^2 at 0.53 for SEV and 0.82 for INC. The cross-validation accuracy of the population ranged from 0.22 to 0.42 for SEV and from 0.39 to 0.67 for INC. MAS and GS results indicate the potential to improve the breeding program to advance FHB resistant lines along with good agronomic traits.

Acknowledgement and Disclaimer

Recurrent Selection to Develop Fusarium Head Blight Resistance Germplasm for Durum Wheat

Runhao Wang[1], Jason Axtman[1], Evan Salsman[1], Justin Hegstad[1], Jason Fiedler[2], Steven Xu[2], Shaobin Zhong[3], Elias Elias[1], and Xuehui Li[1]

[1]North Dakota State University, Department of Plant Sciences, Fargo, ND
[2]USDA-ARS, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND
[3]North Dakota State University, Department of Plant Pathology, Fargo, ND
Corresponding Author: Xuehui Li, xuehui.li@ndsu.edu

Abstract

Fusarium Head Blight (FHB) is a major disease that can cause severe loss of grain yield and quality of durum wheat in the northern Great Plain of the US. FHB resistance in wheat is a complex trait controlled by many genes. Recurrent selection is an effective way to increase frequencies of favorable resistant alleles and to develop improved germplasm. In this study, three cycles of recurrent phenotypic selection were conducted for reducing FHB severity from 2019 to 2021 in a durum wheat population derived from intercrossing of 15 elite cultivars and breeding lines. The FHB severity was reduced 13.8% from Cycle 0 to Cycle 1 population, and 25.9% from Cycle 1 to Cycle 2 population. Significant negative correlations were found between FHB severity and plant height and between FHB severity and days to flowering in Cycle 0 population, but not observed in Cycle 1 and Cycle 2 populations. Genomic selection (GS) can speed up selection and increase genetic gain in terms of time and cost. A total of 161 S1 families in Cycle 2 population were genotyped using 90K SNP array and obtained 4,614 SNP markers. Using ridge regression best linear unbiased prediction (rrBLUP) model, the prediction accuracy for FHB severity was 0.39 with cross-validation. Our results indicate that recurrent phenotypic selection can improve FHB resistance in durum wheat. Implementing GS in the recurrent selection is possible to further accelerate genetic improvement.

Acknowledgement and Disclaimer

Imputation of Fusarium Head Blight Resistance QTL Through Molecular Markers, Genotyping-by-Sequencing, and Machine Learning

Zachary J. Winn[1], Jeanette Lyerly[1], Gina Brown-Guedira[1, 2], and J. Paul Murphy[1]

[1]North Carolina State University, Department of Crop and Soil Sciences, Raleigh, NC
[2]United States Department of Agriculture - Agricultural Research Service, Raleigh, NC
Corresponding Author: Zachary Winn, zjwinn@ncsu.edu

Abstract

Breeders screen germplasm with molecular markers to identify and select individuals that have desirable alleles. In the SunGrains collaborative breeding group in the southern United States, genotyping-by-sequencing (GBS) is conducted annually in the $F_{5:7}$ generation to identify single nucleotide polymorphisms (SNPs) for use in genomic selection. Subsequently, a reduced number of $F_{5:9}$ generation lines are screened with markers for 60 QTL via Kompetitive allele specific PCR (KASP). The objective of this research was to investigate if major effect QTL can be accurately called in $F_{5:7}$ generation breeding lines by using the SNPs derived by GBS. In 2020 and 2021, 2376 and 3423 SunGrains lines submitted for GBS were genotyped via KASP for the *Fusarium* head blight QTL: *Fhb1* from 'Sumai 3', *Qfhb.vt-1B* from 'Jamestown', and *Qfhb.nc-1A* and *Qfhb.nc-4A* from 'NC-Neuse'. In parallel, data was compiled from the 2011-2020 Southern Uniform Winter Wheat Scab Nursery (UFHBN), which had been screened for the same QTL via KASP, sequenced via GBS, and phenotyped for: severity (SEV), percent *Fusarium* damaged kernels (FDK), deoxynivalenol content (DON), plant height, and heading date. Three machine learning models were evaluated: random forest, k-nearest neighbors, and gradient boosting machine. The SunGrains data was randomly partitioned into training-testing splits. The QTL call and 100 most correlated GBS SNPs on the chromosome containing the QTL were used for training and k-fold cross validation tuning for each model. The cross-validated machine learning models were used to predict QTL calls in the testing partition of the SunGrains lines and the UFHBN. Phenotypic data and observed QTL calls were compared to predictive QTL calls in the UFHBN. Random subsetting of training and testing partitions in the SunGrains material, prediction of QTL calls in the SunGrains testing partitions and UFHBN, and estimation of QTL call effects were repeated 20 times and results were averaged. The average predictive accuracies for *Fhb1* calls in the 2020 SunGrains testing partitions ranged from 97.2 - 98.9%. The observed *Fhb1* call estimated effects for SEV, FDK, DON, plant height, and heading date in the UFHBN were not significantly different from any of the predicted *Fhb1* call effects. Similar results were observed in the 2021 SunGrains and UFHBN populations. This indicates that machine learning may be utilized in breeding programs to accurately estimate QTL calls in earlier generation germplasm via a GBS and KASP genotyped training population.

Characterization of Quantitative Trait Loci for Resistance to Fusarium Head Blight in a Winter Wheat Population

Yuzhou Xu[1], Ruolin Bian[1], Yanhong Dong[2], Lanfei Zhao[1], Yunfeng Xu[1], Amy Bernardo[3], Paul St. Amand[3], Jessica Rupp[1], Myron Bruce[1], and Guihua Bai[3, 1]

[1]Kansas State University, Manhattan, KS 66506
[2]University of Minnesota, St. Paul, MN 55108
[3]USDA-ARS-Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506
Corresponding Author: Guihua Bai, guihua.bai@usda.gov

Abstract

Fusarium head blight (FHB) is a devastating disease that threatens wheat production worldwide, and using genetic resistance is one of the most effective approaches to combat FHB. Plant heading date (HD) has often been associated with FHB resistance. To identify quantitative trait loci (QTLs) for the FHB resistance and HD, an F_8 recombinant inbred line (RIL) population derived from the cross G97252W x G97380A was evaluated for the two traits in two field trials and genotyped by genotyping-by-sequencing (GBS) markers. Both parents are hard winter wheat, but G97252W showed higher FHB resistance (moderate FHB resistance) and later heading date than G97380A (FHB susceptible). Single nucleotide polymorphism (SNP) markers generated by GBS in the same map positions were binned to identify unique SNPs for map construction and the resulting genetic linkage map consists of 38 linkage groups. The map was constructed with 586 SNP bin markers covering 2,131 cM genetic map with a marker density of 3.6 markers per cM. The RIL population was evaluated for percentage of symptomatic spikelet in a spike (PSS), Fusarium damaged kernel (FDK) and deoxynivalenol (DON) in the two field experiments. QTL mapping using the SNP bin map and FHB trait data identified a cluster of QTLs between 86.5 to 97.5 cM on chromosome 2D that explained 17.7%, 38.9%, 45.1% of the phenotypic variation for PSS, FDK and DON, respectively. Three QTLs for HD were detected on chromosomes 2A, 2D and 7D and explained 4.7%, 63.9% and 6.5% of the phenotypic variation, respectively. The 2D QTL for HD completely overlapped with the QTLs for the three FHB traits, suggesting these QTLs may be tightly linked or have pleiotropic effects on the four traits. Significantly positive correlations were also observed among the three FHB traits and HD ($P < 0.01$). These results implied that the 2DS QTL region may play a significant role in both FHB resistance and HD in the population.

Acknowledgement and Disclaimer

A Diploid Tall Wheatgrass-Derived *Fhb7* Allele Integrated into Wheat B Genome Conditions FHB Resistance in Wheat

Wei Zhang[1], Tatiana Danilova[1], Mingyi Zhang[1], Shuangfeng Ren[1], Xianwen Zhu[1], Qijun Zhang[1], Shaobin Zhong[2], Linda Dykes[3], Jason Fiedler[3], Steven Xu[3], Jeffrey Boehm Jr.[4], and Xiwen Cai[4]

[1]Departments of Plant Sciences, North Dakota State University, Fargo, ND 58108
[2]Department of Plant Pathology, North Dakota State University, Fargo, ND 58108
[3]USDA-ARS, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND 58102
[4]USDA-ARS, Wheat, Sorghum and Forage Research Unit, Lincoln, NE 68583
Corresponding Author: Xiwen Cai, xiwen.cai@usda.gov

Abstract

The tall wheatgrass-derived Fusarium head blight (FHB) resistance gene, *Fhb7*, was cloned from the decaploid species *Thinopyrum ponticum* and found to detoxify *Fusarium*-produced trichothecenes, such as deoxynivalenol (DON), in addition to conditioning Type II FHB resistance in wheat.
Several *Fhb7* resistance alleles have been integrated into the wheat D genome through 7D-7E translocations from diploid (*Th. elongatum*) and decaploid (*Th. ponticum*) tall wheatgrass that share the E genome containing the *Fhb7* locus. Additionally, a *Th. ponticum*-derived *Fhb7* resistance allele was transferred to the wheat A genome through 7A-7E translocation. The present study aimed to transfer the *Th. elongatum*-derived *Fhb7* allele, designated *Fhb7^{The}*, to the wheat B genome by inducing meiotic homoeologous recombination between wheat chromosome 7B and *Th. elongatum* chromosome 7E, thereby making this FHB resistance allele *Fhb7^{The}* usable in common wheat as well as durum wheat breeding. In addition, *Fhb7^{The}* was characterized for its genomic structure and resistance to FHB in wheat. A small 7EL segment containing *Fhb7^{The}* was incorporated into wheat chromosome 7B by *ph1b* mutant-induced 7B-7E translocation. FHB evaluation of the 7B-7E translocation line and its wheat parental line 'Chinese Spring' indicated that *Fhb7^{The}* conditioned significant Type II resistance in the wheat background. Also, we found this translocation line does not contain the gene for yellow flour pigment, which is closely linked with the *Fhb7* alleles from other sources. Thus, *Fhb7^{The}* can be utilized immediately in wheat breeding without obvious linkage drag. DNA sequence analysis identified 10 SNPs between *Fhb7^{The}* and the cloned *Fhb7* allele, leading to seven amino acid differences between these two *Fhb7* alleles. A user-friendly STS marker was developed specifically for *Fhb7^{The}*. Thereby, *Fhb7^{The}* is a new *Fhb7* allele that conditions FHB resistance in wheat and can be deployed in both common and durum wheat varieties.

Acknowledgement and Disclaimer

Identification of QTL for Type I Resistance to Fusarium Head Blight in Two Spring Wheat Mapping Populations

Shaobin Zhong[1], Subidhya Shrestha[1], Bikash Powdel[1], Anil Karmacharya[1], Mingxia Zhao[1], Yueqiang Leng[1], Joe Mullins[1], Chenggen Chu[2], Steven Xu[2]

[1]North Dakota State University, Fargo, North Dakota
[2]USDA-ARS, Fargo, North Dakota
Corresponding Author: Shaobin Zhong, shaobin.zhong@ndsu.edu

Abstract

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is an important disease of wheat and other small grains in North America. Use of resistance is one of the most important components in management of the disease. Although different types of FHB resistance are recognized, only type II resistance (resistance to disease spread) have extensively been studied. This study was focused on mapping QTL associated with Type I resistance to FHB (resistance to initial infection) in spring wheat using 130 doubled haploid (DH) lines from the cross Grandin × PI277012 (GP) and 237 recombinant inbred lines (RILs) from the cross Bobwhite × ND2710 (BN). The GP population was previously genotyped with SSR markers and the 9K SNP chips while the BN population was genotyped with the 90K SNP chips. The two populations were evaluated for type I resistance by spay inoculation in field and greenhouse experiments. For the GP population, QTL analysis using composite interval mapping (CIM) identified three QTL on chromosomes 1A, 4B and 6B, respectively, under field environments, and two QTL on chromosomes 2B and 5B, respectively, under greenhouse conditions. These QTL explained 10.7-19% of the total phenotypic variation. For the BN population tested under field conditions, three QTL were detected on chromosomes 2A, 5A and 6B, respectively, whereas one QTL was detected on chromosome 5A under greenhouse conditions. These QTL explained 6.2-13.7% of the total phenotypic variation. The QTL identified in this study mapped to genomic regions with previously reported QTL for FHB resistance, except for one QTL (*Qfhb.ndwp-5A.2*) on chromosome 5A which explained 6.6% of the phenotypic variation. The markers associated with the QTL for type I resistance will be useful for selection and pyramiding of different types of FHB resistance in breeding programs.