Project FY22-BA-007: Molecular Genetics Approaches to Developing Scab Resistant Barley

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1. What are the major goals and objectives of the research project?

The major goal of this project is to develop genetic tools for increasing FHB resistance in barley. There are three major objectives that will be addressed including: (1) characterize the role of trichothecenes on infection and host responses; (2) fine map and characterize the chromosome 2H bin8 FHB resistant QTL; and (3) identify DON and FHB resistant mutants.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address these three items below.)*

What were the major activities?

Objective 1. Characterize the role of trichothecenes on infection and host responses. We characterized the function of *HvUGT13248*, a barley gene that encodes a UDP-glucosyltransferase. We used two *HvUGT13248* mutations and wildtype controls to examine tricothecene detoxification, type II resistance, and movement of the fungus during infection. We collected RNA-seq data, and corresponding DON and ergosterol data from the *HvUGT13248* mutant and wildtype plants after *F. graminearum* and identified families of fungal genes that we are beginning to characterize. To characterize the ability of *HvUGT13248* to detoxify a broad set of trichothecenes (NIV, 3-ADON, 15-ADON and NX-2), we conducted three replicates of an experiment to characterize a set of barley sister genotypes (transgenic Genesis UGT+ and UGT-, transgenic Rasmussen UGT+ and UGT-, and a Morex UGT13248 mutant and Morex control) inoculated with *F. graminearum* 3-ADON, 15-ADON, NIV and NX-2 producing strains. The plants were scored for FHB severity and spike tissue was sampled 21 days after inoculation. When compared to wildtype plants, mutant plants exhibited increased disease severity after inoculation with each of the four chemotypes; however, we did not detect a statistical difference between transgenic and non-transgenic plants. Preliminary measurements of a small set of samples from the first replicate demonstrate that HvUGT13248 glucosylates DON derived from 15-ADON and 3-ADON to make DON-3-Glucoside (D3G), NIV to make NIV3G, and NX2 to make NX3G. We also detected 15-ADON-Glc in the 15-ADON producer. The complete set of sampled tissues from all three replicates were ground and extracted and sent to Franz Berthiller (University of Natural Resources and Life Sciences, Vienna, Austria) for the trichothecenes and trichothecene-glucoside conjugates.

We tested *HvUGT13248* for type I resistance using floral dip inoculation by examining *HvUGT13248* mutant and wildtype plants and assessing disease progression. While disease severity (number of infected spikelets) was higher in *HvUGT13248* mutant plants at 6, 9, 14 and 21 days, there was no difference in disease severity at 3 days after inoculation between the wildtype sister line and the UGT13248 mutant line (H369Y). The number of infection points, defined as an area where infected spikelets are directly adjacent to each other, was the same between wildtype and H369Y mutant plants. We conclude that *HvUGT13248* is required for type II, but not type I resistance in barley.

Objective 2. Fine map and characterize the chromosome 2H bin8 FHB resistant QTL. We fine mapped the chromosome 2H bin8 region using 2,000 F2 individuals and phenotyped the recombinants in 2016 and 2018-2023 for FHB severity, DON accumulation, heading date and height.

Objective 3. Identify DON and FHB resistant mutants. In 2022-2024, we screened 1,250 M_3 lines for FHB severity in a Conlon mutagenized population. An additional 1,000 M_3 lines are growing in Crookston, MN and will be screened later this summer.

Progress on related activities
We worked with Brian Steffenson to assemble a meta-analysis of the barley QTL that are associated with FHB resistance and agro-morphological traits.

What were the significant results?
We showed that the rachis node is the site important for resistance and that HvUGT13248 conjugated DON, NIV, 3-ADON, 15-ADON and NX2 to glycoside conjugates. We also showed that *HvUGT13248* is the primary gene conferring type II resistance against a broad set of chemotypes but does not play a role in type I resistance. From our screening of a Conlon mutagenized population, we identified 58 lines with increased susceptibility and 16 lines that exhibit decreased susceptibility. We have also shown that the 2H QTL is a complex of QTL for DON and FHB resistance. Meta analysis demonstrated that most of the QTL associated with FHB resistance are associated with an agromorphological trait.

List key outcomes or other achievements.
We showed that the rachis node is the site important for resistance and that HvUGT13248 conjugated DON, NIV, 3-ADON, 15-ADON and NX2 to glycoside conjugates. We also showed that *HvUGT13248* is the primary gene conferring type II resistance against a broad set of trichothecenes but does not play a role in type I resistance. The barley mutants in the Conlon background may provide insight into the barley-*F. graminearum* interaction and provide genetic stocks for developing resistant barley cultivars. We have also shown that the 2H QTL is a complex of QTL for DON and FHB resistance.

3. What opportunities for training and professional development has the project provided?
Three postdocs have worked on this project. One postdoc has taken a position at a start-up company called Exreprotein LLC. Each of the postdocs meet with me regularly and attend and present results in weekly lab meetings. One postdoc presented a poster at the 2023 National Fusarium Head Blight Forum.

4. How have the results been disseminated to communities of interest?
A paper describing the role of *HvUGT13248* in FHB resistance was published in *Plant Physiology*. A paper was published in *Plant Breeding* describing a meta-analysis of the barley QTL associated with FHB resistance, DON accumulation and agro-morphological traits. A poster was presented at the 2023 National Scab Forum.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives? We plan to complete a comprehensive characterization of *HvUGT13248* with regards to glucoside conjugation of a broad set of trichothecene mycotoxins, and more closely examine the infection process at the node and internode regions in the spike. We also plan to complete our analysis of the RNA-seq data derived from inoculated mutant and wild type plants. We also plan to rescreen all barley mutants that exhibit susceptibility and resistance and initiate genetic characterization of promising lines.