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 continued to characterize the function of *HvUGT13248*, a barley gene that encodes a UDP-glucosyltransferase. Previously, we showed that HvUGT13248 functions to glycosylate trichothecene mycotoxins and provide type II resistance in barley. To examine if *HvUGT13248* played a role in type I resistance, we conducted floral dip inoculation of the *HvUGT13248* mutant and wildtype plants and assessed disease progression. While disease severity (number of infected spikelets) was higher in *HvUGT13248* mutant plants at 6, 9, 14 and 21 days after inoculation, there was no difference in disease severity at 3 days after inoculation between the wildtype sister line and the *HvUGT13248* mutant line. The number of infection points, defined as an area where infected spikelets are directly adjacent to each other, was the same between wildtype and *HvUGT13248* mutant plants. We conclude that *HvUGT13248* is required for type II, but not type I resistance in barley. Furthermore, we point inoculated the *HvUGT13248* mutant and wildtype plants with either wildtype *F. graminearum* capable of producing trichothecenes or a mutant strain (Δtri5) which does not produce trichothecenes. WT Morex was able to resist spread of FHB symptoms when inoculated with either *F. graminearum* genotype. The *HvUGT13248* mutant was unable to contain the spread of the wildtype *F. graminearum* strain. However, the resistance exhibited by the *HvUGT13248* mutant and wildtype plants was similar when point inoculated with Δtri5. These findings indicate that *HvUGT13248* is directly responsible for detoxifying the trichothecenes, restricting the spread of disease severity, and is not required for Type II resistance in the absence of trichothecene production.

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. In collaboration with Franz Berthiller (University of Natural Resources and Life Sciences, Vienna, Austria), we also showed 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.

To examine host response during infection, we examined transcript levels in the *HvUGT13248* mutant and wildtype plants after *F. graminearum* inoculation. We also collected corresponding DON and ergosterol data from *HvUGT13248* mutant and wildtype plants after *F. graminearum*

inoculation. Our data showed that DON and ergosterol were higher in the mutant than wildtype plants. Analysis of the RNA-seq dataset showed the *HvUGT13248* mutant upregulates defense response genes 2-3 days faster than wildtype plants. When looking at gene sets which are related to defense or altering metabolites (UGTs, GSTs, and transcription factors), there was no difference in the total number of up-regulated genes between the two genotypes, but the *HvUGT13248* mutant genotype exhibits down-regulation of 2-3 times as many genes. This may be due to the earlier response to infection and subsequent tamping down of defense-related genes as the spike tissues senesce. A weighted gene co-expression network analysis was created from the RNA-seq dataset and indicated a single module out of nine which was enriched in defense-related genes. This module shows significant differential gene expression during infection, but almost no differential gene expression during mock inoculation. Furthermore, genes highly associated with this module were not shared with any of the other eight modules of the network, indicating they may be solely differentially expressed during infection.

We also used the RNA-seq data to examine the accompanying transcript accumulation data for *F. graminearum* during infection of the *HvUGT13248* mutant and wildtype plants. When comparing differential gene expression between WT and the *HvUGT13248* mutant plants inoculated with *F. graminearum*, we observed that most of the TRI gene transcripts involved in trichothecene biosynthesis were significantly increased in WT barley tissue. This result is consistent with our other results that show wildtype trichothecene-producing fungal strains exhibit high disease spread on *HvUGT13248* mutant plants, but little disease spread on wildtype plants. In addition, there was little disease spread when *HvUGT13248* mutants and wildtype plants were inoculated with the Δtri5 non-trichothecene producing strain of the fungus. Taken together, the RNA-seq and phenotyping results highlight the interaction between HvUGT13248, trichothecene production and disease severity. We are also investigating whether any of the tested and predicted effector genes in *F. graminearum* are differentially expressed between the two barley genotypes, but to date no significant patterns have emerged.

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. We showed that the barley 2H QTL is a complex locus composed of DON and FHB resistance. However, there is not a clear result that warrants publication or moving forward.

Objective 3. Identify DON and FHB resistant mutants.

In 2022-2024, we screened 2,250 M_3 lines for FHB severity in a Conlon mutagenized population. For the summer 2025 growing season in Crookston, MN, we planted 127 lines (82 susceptible lines and 45 resistant lines) of mutagenized Conlon which had previously shown either FHB resistance or susceptibility relative to the average disease for their given growing season. Another 776 lines which have not been previously screened were planted at the same time. We are planning to score for FHB symptoms in early August 2025.

Other related activities

Fusarium graminearum species produce non-trichothecene mycotoxins including zearalenone, an estrogenic compound. Previous work in collaboration with Professor Gerhard Adam (University of Natural Resources and Life Sciences, Vienna, Austria) showed that the barley UGT14077 was found to glucosylate zearalenone *in vitro* (Michlmayr *et al*., 2017). We have identified four non-synonymous mutations in the *HvUGT14077* gene in the barley cv. 'Sebastian' (Szurman-Zubrzycka *et al.*, 2018), three of those are located within the PSPG box, the predicted UDP-glucose binding region. We are currently identifying homozygous mutants to test if they affect *HvUGT14077* function with the goal to test if HvUGT14077-dependent glucosylation of zearalenone affects FHB disease severity in barley.

Michlmayr *et al.*, Toxins, 2017, https://doi.org/10.3390/toxins9020058.
Szurman-Zubrzycka *et al.*, Front. Plant Sci., 2018, https://doi.org/10.3389/fpls.2018.00216.

What were the significant results?

We showed that HvUGT13248 conjugates DON, NIV, 3-ADON, 15-ADON and NX2 with a glucoside group, resulting in detoxified 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. We also showed that the *HvUGT13248* mutant upregulates defense response genes 2-3 days faster than wildtype plants. A weighted gene co-expression network analysis was created on the RNA-seq dataset and indicated a single module out of nine which is enriched in defense response genes. This module shows significant differential gene expression during infection, but almost no differential gene expression after mock inoculation. We also observed increased expression of the *F. graminearum* trichothecene biosynthetic genes when wildtype plants were inoculated compared to *HvUGT13248* mutant plants. We also showed that the barley 2H QTL is a complex locus composed of DON and FHB resistance. However, there is not a clear result that warrants publication or moving forward. From our screening of a Conlon mutagenized population, we identified 82 lines with increased susceptibility and 45 lines with decreased susceptibility. We identified mutations in the *HvUGT14077* gene for further characterization of the role of *HvUGT14077* in FHB resistance.

List key outcomes or other achievements.

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3. What opportunities for training and professional development has the project provided?

Two postdoctoral research associates have worked on this project. They meet with me weekly and attend my weekly lab meeting where recent research results are presented and current journal articles discussed. Both postdocs attended the National Scab Forum in December 2024 and presented a poster.

4. How have the results been disseminated to communities of interest?

Two posters were presented at the 2024 National Scab Forum, and a talk was presented at the American Society of Plant Biologists Mid Atlantic meeting in College Park, Maryland. A manuscript is in preparation describing the role of *HvUGT13248* in resistance, trichothecene glucoside conjugation, and host and pathogen responses during infection.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

We plan to assemble and submit a paper on *HvUGT13248* with regards to glucoside conjugation of a broad set of trichothecene mycotoxins, the RNA-seq data derived from inoculated mutant and wild type plants, and the role of *HvUGT13248* in type I resistance. We also plan to rescreen all barley mutants that exhibit susceptibility and resistance and initiate genetic characterization of promising lines through developing populations that can be used for genetic mapping. We will continue to characterize the *HvUGT10477* mutants for their role in FHB resistance.