

Project FY24-PB-004: Genome sequences and new annotated references for mapping Fusarium head blight traits

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

1. Perform additional years of greenhouse head inoculation experiments on susceptible and resistant wheat backgrounds, continuing the phenotyping of 150 Fg isolates from the NA1 population that we have used in USWBSI projects for aggressiveness and amount of DON produced, for the purpose of performing genome-wide association studies (GWAS) of these traits.
2. Vastly expand the set of genetic markers segregating in this sample of 150 Fg isolates and available for GWAS analysis by performing high-throughput whole genome sequencing for this sample using an inexpensive DNA library preparation protocol offered by K-State's Integrated Genomics Facility.

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

What were the major activities?

Goal 1): We performed one replication of the NA1 sample inoculation onto our wheat cultivar with partial FHB resistance, and two replications of inoculations onto the FHB susceptible cultivar. In each case, we took aggressiveness data by recording symptomatic spikelets at two time points post-inoculation. We have been processing all inoculated wheat heads from this reporting period (and some previous reporting periods), threshing and then grinding the grain from each set of heads inoculated with the same isolate in the same experiment. Some of these samples have been sent to the Virginia Tech USWBSI testing lab for DON levels, and we are awaiting the test results.

Goal 2): The most recent whole genome sequence (WGS) data produced (in spring 2025) were processed in our SNP calling pipeline, resulting in full WGS genotyping data for the entire sample. The WGS data from a portion of the isolates have also been used in a new pipeline, mapping these reads against a new pangenome graph produced by our group from high-quality genome assemblies that represent several of the major *Fusarium graminearum* populations. The variants called after mapping reads to the pangenome graph include those found in accessory genome regions (e.g., regions not found in the PH-1 reference genome assembly) as well as other structural variants.

What were the significant results?

Goal 1): Analyses of our early aggressiveness data found no significant isolate by cultivar interaction, so that the results could be combined across wheat varieties to provide estimates of relative aggressiveness suitable for GWAS.

Goal 2): Comparisons of variants detected by using a graph-based pangenome approach with those detected from an approach that compares genome assemblies individually against a single common reference genome indicated that the graph-based pangenome approach could detect many more large (>1 kb) insertion and deletion polymorphisms (Figure 1).

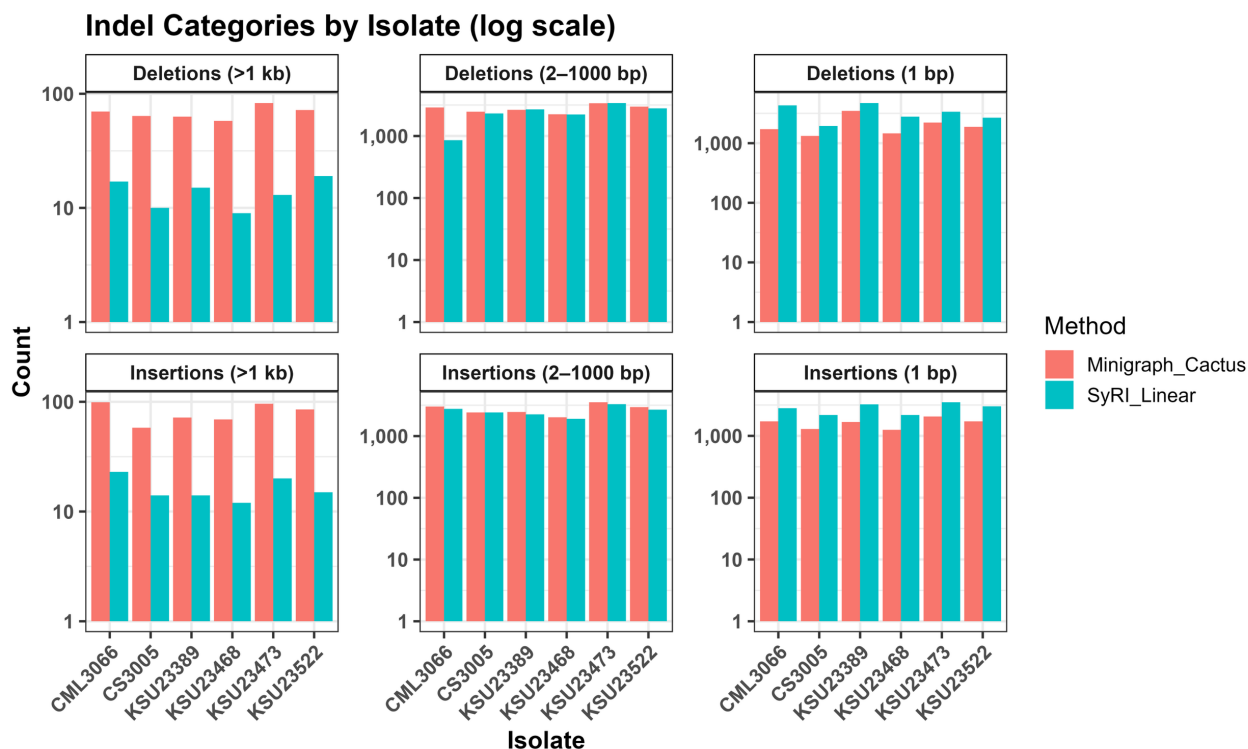


Figure 1: Graph-based pangenome analysis recovers more large indels (> 1kb) than the approach of comparing to a single reference genome

List key outcomes or other achievements.

Goal 1): Including greenhouse inoculations performed in the previous USWBSI project, we now have completed five replications on each of the two wheat varieties.

Goal 2): The availability of new methods for variant calling WGS data from samples of isolates allows for more structural variants from *F. graminearum*'s accessory genome to be used in GWAS. This increases the likelihood that causal variants that influence pathogen traits can be mapped in these analyses.

Outside the main goals of the current project, but related to goals from past USWBSI-funded projects, we have published our first paper on GWAS of DON levels in *F. graminearum* isolates in the journal *Toxins*, as an open-access publication. We have also submitted, and subsequently revised and resubmitted another *F. graminearum* GWAS paper, this on sensitivity to two DMI fungicides, propiconazole and tebuconazole. These publications provide dozens of associated SNPs and highlight the nearby candidate genes which may influence the fungal traits, such as the role of transmembrane transporters in self-protection from DON.

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

The project provided training opportunities for PhD students Sandhya Gopisetty and Sumit Chowdhury, including performing genetic crosses with *F. graminearum* strains and carrying out data analysis for GWAS. They have had opportunities to present at several national and international conferences, including Sandhya at the 2025 Plant Health Conference and both at the 2025 National FHB Forum and both at the 33rd Fungal Genetics Conference in March

2026. The project has provided training for two undergraduate workers, Brianna Goebel and Mark Andres, the former not only working in the lab providing research support during the semesters, but also participating in the summer of 2026 in a USDA-funded REU experience performing independent research in the lab. The project provided professional development and training to 45 participants in the 2026 Fusarium Laboratory Workshop. The PI had professional development opportunities by attending the 33rd Fungal Genetics Conference and attending and presenting at the 2025 FHB Forum, the 2026 USWBSI GDER and PBG Joint Midyear Online Meeting, and the joint NC1183 and Annual Corn Dry Milling Conference in May 2026.

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

Project results have been disseminated through presentations at Plant Health 2025, the 2025 FHB Forum, at the 33rd Fungal Genetics Conference, at the 2026 USWBSI GDER and PBG Joint Midyear Online Meeting, and at the joint NC1183 and Annual Corn Dry Milling Conference. Manuscripts from project results have also been published or are being reviewed in peer-review journals (*Toxins* and *Microbiology*). Additional peer-reviewed manuscripts will be submitted during the final year of this project. PI Toomajian participated in the Kansas State University annual open house for the purpose of outreach to enhance public understanding of plant pathology and increase interest in careers in this field.

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

Goal 1): In the lab, we will process the remaining inoculated wheat head samples, threshing the grain and grinding it, before sending the samples to the Virginia Tech DON testing lab. Once we receive the results reporting the DON and 15ADON levels, we will compare these trichothecene levels with those that these same isolates produce on a rice culture medium. We will also perform GWAS for the *in planta* DON levels from both wheat varieties, using the WGS data obtained in goal 2. We will also perform a comprehensive analysis of isolate aggressiveness across the two wheat varieties and all five experimental replications that have been performed. Aggressiveness values will be compared with the DON levels for the same sets of inoculated wheat heads, and GWAS will be performed to identify variants associated with variability in aggressiveness across our sample.

Goal 2): With the full WGS data from the 150 isolates used for many trait measurements, we will repeat GWAS for these traits. This includes GWAS for radial growth rate at three different temperatures and ascospore production in a set of *F. graminearum* isolates, preliminary results of which have never been published. Even in the case of the traits where preliminary GWAS has been published after GBS genotyping, the WGS data and repeat of GWAS is critical to determining the causal changes that affect pathogen traits. GWAS results using GBS genotypes only identify markers associated with the trait of interest, but are less likely to include the actual causal variants nearby associated markers. The WGS data provide information on all the variants within candidate genes identified in the preliminary GWAS, and after predicting the effect of each variant on gene function, we can strategically prioritize the individual variants and candidate genes for functional validation.