Project FY22-PB-002: Genetic Mapping of Genes Underlying Variation in Fusarium Head Blight Traits

---

1. **What are the major goals and objectives of the research project?**
   - **a.** In laboratory experiments, measure DON levels, fungicide sensitivity, ascospore discharge, and mycelial growth for 150 *Fg* isolates
   - **b.** Phenotype 150 *Fg* isolates for aggressiveness and DON production via greenhouse head inoculations on susceptible and resistant wheat
   - **c.** Perform genome-wide association (GWAS) to identify SNPs associated with variation in above traits

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 a): Before this period, all planned mycelial growth rate studies, perithecia formation/ascospore discharge experiments, DMI fungicide sensitivity experiments (using propiconazole and tebuconazole), and *in vitro* measurements of DON production had been completed. During this period, we conducted a small DMI fungicide sensitivity experiment in order to directly compare the sensitivity levels of isolates that come from different populations. For all traits, least squares means were estimated across the multiple replications and used subsequently in the GWAS analysis.

Goal b): Before this period, 2 seasons of greenhouse wheat head inoculations to measure aggressiveness and DON contamination of nearly 150 isolates on a susceptible and a moderately resistant wheat variety had been performed. During the current period, we attempted two additional replications (for a total of 4) of greenhouse wheat head inoculations of our nearly 150 isolates on both wheat varieties. Aggressiveness data were collected but not yet completely analyzed, and inoculated heads have not yet been sent to the USWBSI DON testing lab for *in planta* DON level data. From our first two replications, no isolate by cultivar interaction was observed.

Goal c): Previous to this period, we had performed preliminary GWAS with the available trait data. During the current period, we continued with our GWAS analyses for all traits, including aggressiveness and *in planta* DON production. We employed two software packages, mrMLM and GAPIT3, that each incorporate several GWAS models, in performing our analyses. We report SNPs identified as significant for any model after correcting for testing across the approximately 5000 SNPs used for each model and trait combination, and we highlight SNPs identified by multiple GWAS models.

During this period, we also took initial steps on one of our objectives on our FY24 USWBSI project – extracting DNA from over 150 *F. graminearum* isolates in preparation for the creation of whole genome sequencing libraries by K-State's Integrated Genomics Facility. The next step after these libraries are created will be sequencing them to an expected 30x depth of coverage in order to generate whole-genome genotypes of these isolates for further GWAS.

What were the significant results?

Goal a): The tebuconazole sensitivity experiment to compare 13 isolates with the NX-2 *TRI* genotype with 8 isolates from the NA1 population found no significant difference in

sensitivity between these two groups. Our earlier experiments had also demonstrated that in our assay tebuconazole was more effective at suppressing fungal growth, as the average EC50 was approximately half that of the EC50 for propiconazole.

Goal b): We were able to perform two replications of head inoculations on both of our wheat varieties during this season, however, for one replication on Wheaton, our FHB susceptible variety, the wheat heads suffered extensive damage from thrips. We do not anticipate that data from that variety-replication combination will be useable. This still leaves us with four total replications on Rollag (FHB moderately resistant) and three on Wheaton for the GWAS analysis that we will carry out in the coming months.

Goal c): Trait values were measured on a mixed sample from the NA1 and NA2 populations for growth rate and ascospore production, and on a sample from the NA1 population for the traits of propiconazole and tebuconazole sensitivity, *in vitro* DON production, and aggressiveness and *in planta* DON levels on wheat heads. The sensitivities to the two DMI fungicides were highly correlated (Pearson's coefficient = 0.9, **Table** 1). Also positively correlated, but not as strongly, were ascospore production and aggressiveness (0.54) and *in vitro* levels of 15ADON and DON (0.49). The strongest negative correlation was between growth and aggressiveness (-0.35). Significant SNPs were detected in GWAS for each trait. 13 distinct SNPs were significant for growth, with 8 significant in more than one model tested, and 2 SNPs significant at least 4 models (**Table 2**). 30 SNPs were significant for ascospore production, with 11 significant in more than one model tested (**Table 3**). This includes 2 SNPs significant in both the whole sample and the NA1 population subset. Consistent with the high correlation between tebuconazole and propiconazole sensitivity, 13 SNPs were found significant for both fungicides, while an additional 24 SNPs were significant for only tebuconazole and 35 SNPs were significant for only propiconazole (**Figure 1, Table 4**). None of the SNPs identified correspond to CYP51 genes, however one SNP common to both fungicides falls within the fusarin C gene cluster, and another SNP significant for tebuconazole only is in a multidrug resistant type ABC transporter. One SNP in a polyketide synthase cluster is significant for both DON and 15ADON levels, while two additional SNPs are significant for 15ADON levels only and seven additional SNPs are significant for DON levels only (**Figure 2, Table 5**). Finally, 2 SNPs were detected as significant for aggressiveness on wheat heads by four different models each, based on our first two replicates than have been analyzed (**Table 6**).

Table 1. Correlation between traits (Pearson's correlation coefficients)

TRAITS	growth	ascospore	propiconazole	tebuconazole	aggressiveness	DON *in vitro*
ascospore	0.112					
propiconazole	0.236	-0.151				
tebuconazole	0.249	-0.076	0.896			
aggressiveness	-0.347	0.537	0.176	0.096		
DON *in vitro*	-0.119	0.189	-0.173	-0.107	0.192	
15ADON *in vitro*	-0.189	0.318	-0.138	-0.113	0.214	0.488

Table 2. SNPs significantly associated with growth rate

Sample	Models	Chromosome	Position	Minor allele freq
NA1+NA2	1,2,3,4	1	7892085	0.16
NA1+NA2	1,4	1	9701935	0.06
NA2	6,7	2	400745	0.07
NA2	2	2	3635425	0.31
NA1+NA2	1	2	3764676	0.25
NA1	4	2	5712361	0.35
NA1	1	2	6320548	0.09
NA2	6,7	3	85478	0.07
NA1	1,4	3	523887	0.35
NA1	4	3	7346499	0.05
NA2	6,7	3	7509544	0.07
NA2	2,3,4,5,6,7	4	2946771	0.10
NA1	1,4	4	6548217	0.06

Model codes- 1: mrMLM, 2: FASTmrMLM, 3: FASTEMMA, 4: ISIS EM-BLASSO, 5: pKWmEB, 6: BLINK, 7: FarmCPU

Table 3. SNPs significantly associated with ascospore production

Sample	Models	Chromosome	Position	Minor allele freq
NA1	2,3,4	1	196392	0.16
NA1	1,6	1	540288	0.07
NA1+NA2	1,2,4,5,6	1	540304	0.08
NA1+NA2	4	1	677363	0.19
NA2	5	1	681329	0.24
NA2	4	1	6029759	0.34
NA1+NA2	2,6,7	1	10661762	0.46
NA1	4	1	10977911	0.41
NA1	4	1	11100468	0.24
NA1+NA2	1,3,4,5,6,7	2	160544	0.46
NA1+NA2	1,5	2	395059	0.11
NA1	4	2	455802	0.07
NA1+NA2	1,2	2	2271329	0.46
NA1+NA2	7	2	4489803	0.13
NA1	1,6,7	2	4489803	0.30
NA1+NA2	2	2	8649328	0.30
NA2	1	3	3451154	0.09
NA1+NA2	7	3	3453361	0.16
NA1+NA2	2	3	4181887	0.12
NA2	1	3	6652144	0.07
NA1	2,7	3	6679636	0.09
NA1	4	3	6694414	0.05
NA1	7	3	7079729	0.05
NA1	1	3	7230132	0.20
NA1+NA2	7	3	7239571	0.09
NA2	5	4	91835	0.33
NA1	2,3,4	4	290113	0.21
NA1+NA2	1,2,3,5	4	399628	0.21
NA1	2,3	4	399628	0.31

NA1+NA2	7	4	7290074	0.13
NA1	7	4	7596245	0.34
NA1+NA2	7	4	7700303	0.13

Model codes- 1: mrMLM, 2: FASTmrMLM, 3: FASTEMMA, 4: ISIS EM-BLASSO, 5: pKWmEB, 6: BLINK, 7: FarmCPU

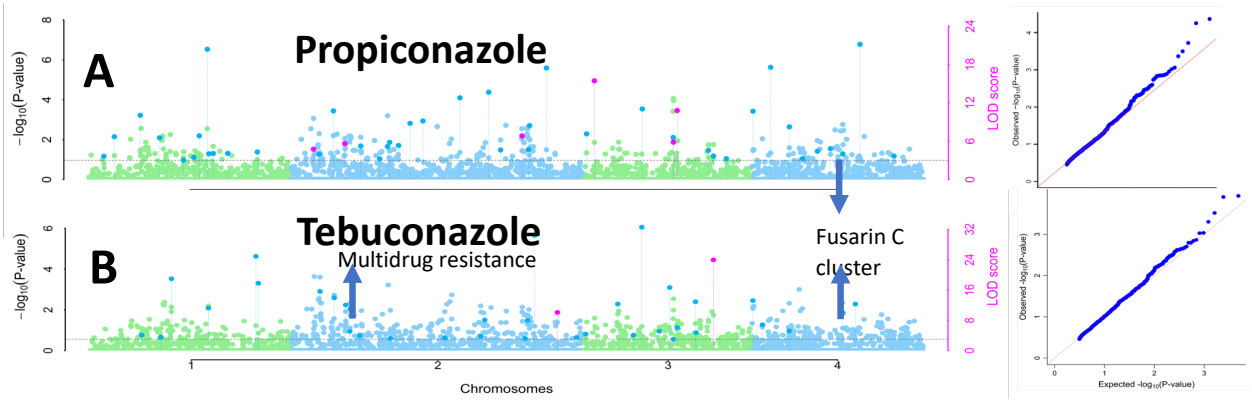

Figure 1: Manhattan and quantile-quantile plots for GWAS conducted on A) propiconazole and B) tebuconazole sensitivity.

Table 4. SNPs significantly associated with DMI fungicides propiconazole (pro) and tebuconazole (teb)

Trait	Models	Chromosome	Position	Minor allele freq
pro	5	1	237393	0.10
pro	4	1	489860	0.13
pro	5	1	3881005	0.34
teb	5	1	3885008	0.34
pro	4	1	**5731674**	0.09
teb	4	1	**5731674**	0.09
teb	5	1	5933539	0.47
pro	5	1	6829683	0.23
pro	4	1	7218760	0.38
pro	4	1	7356780	0.06
pro	4	1	**7467655**	0.05
teb	4	1	**7467655**	0.05
pro	4	1	7514533	0.29
pro	4	1	7585853	0.28
pro	4	1	9999192	0.31
teb	5	1	11258195	0.09
pro	5	1	**11270238**	0.22
teb	5	1	**11270238**	0.22
pro	1,4	2	459782	0.47
pro	5	2	**501355**	0.10
teb	5	2	**501355**	0.10
pro	4	2	**665406**	0.30
teb	4	2	**665406**	0.30
pro	2,4,5	2	**851037**	0.24

teb	4	2	**851037**	0.24
teb	5	2	964956	0.33
teb	5	2	985340	0.17
teb	4	2	2645857	0.22
pro	5	2	2753268	0.11
pro	4	2	3804766	0.50
pro	4	2	3923133	0.08
pro	4	2	**3938384**	0.33
teb	4	2	**3938384**	0.33
pro	5	2	4138058	0.13
pro	4	2	4358147	0.05
pro	4	2	4679670	0.06
teb	4	2	5182922	0.10
pro	4	2	5664783	0.44
teb	4	2	6143802	0.15
teb	4	2	6173074	0.18
pro	5	2	6243290	0.31
pro	5	2	6487389	0.13
pro	4,5	2	8062916	0.39
teb	5	2	8129795	0.35
teb	4	2	8222086	0.22
pro	5	2	8232320	0.31
pro	4	2	8244606	0.47
teb	5	2	8322036	0.35
pro	5	2	8502325	0.14
teb	4,5	2	8566172	0.06
teb	5	2	8880263	0.18
teb	4	3	65770	0.45
pro	4	3	72709	0.14
pro	4,5	3	179032	0.30
teb	5	3	503820	0.34
teb	4	3	3183025	0.33
teb	5	3	3813789	0.07
pro	4	3	4095333	0.33
teb	4	3	6299555	0.09
teb	5	3	6562010	0.15
pro	4	3	**6640551**	0.05
teb	4	3	**6640551**	0.05
pro	1,4	3	6640556	0.05
pro	4,5	3	**6679615**	0.07
teb	4	3	**6679615**	0.07
teb	4	3	7066756	0.35
teb	4	3	7067865	0.43
pro	5	3	7294460	0.34
pro	4	3	**7346278**	0.20
teb	4,5	3	**7346278**	0.20

pro	5	3	7521430	0.09
pro	4	4	**25804**	0.25
teb	4	4	**25804**	0.25
teb	4	4	177976	0.08
pro	5	4	329152	0.05
pro	5	4	**2203719**	0.31
teb	4	4	**2203719**	0.31
pro	5	4	3862627	0.08
pro	4	4	4117472	0.13
pro	5	4	4362316	0.24
pro	4	4	**4545038**	0.11
teb	5	4	**4545038**	0.11
teb	5	4	5201402	0.05
pro	5	4	5602779	0.08
pro	4	4	7533605	0.09

Model codes- 1: mrMLM, 2: FASTmrMLM, 3: FASTEMMA, 4: ISIS EM-BLASSO, 5: pKWmEB

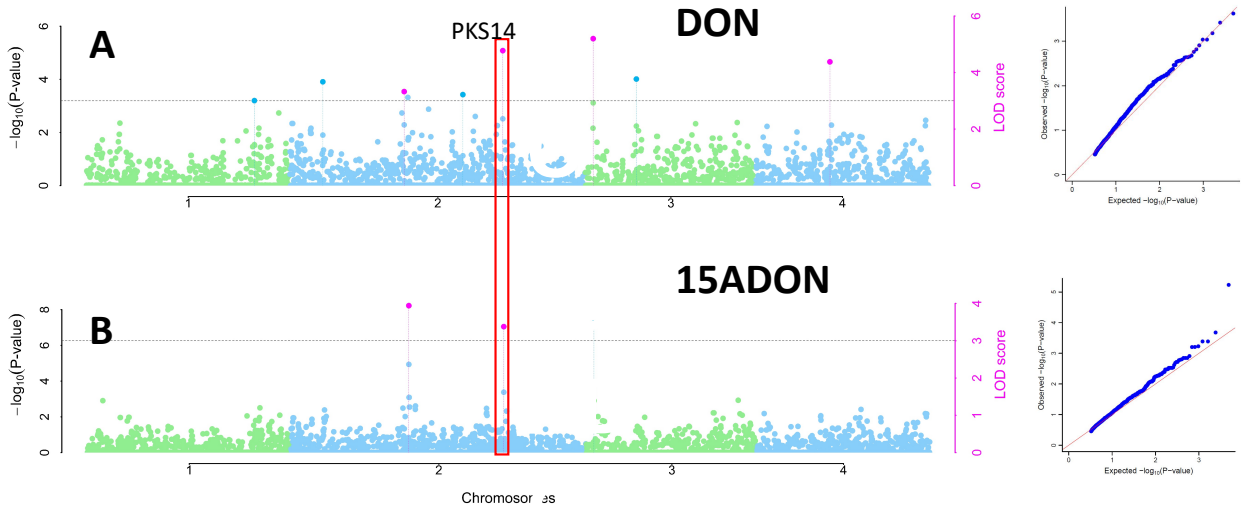

Figure 2: Manhattan and quantile-quantile plots for GWAS conducted on A) DON and B) its derivative 15ADON.

Table 5. SNPs significantly associated with DON or 15ADON

Trait	Models	Chromosome	Position	Minor allele freq
DON	4	1	11260778	0.18
DON	2	2	520988	0.19
DON	2,4	2	4175663	0.15
15ADON	1,2,3,4,5	2	4313942	0.11
DON	5	2	5687139	0.20
DON	4,5	2	**6526109**	0.21
15ADON	4,5	2	**6526109**	0.21
15ADON	4	3	98785	0.09
DON	4,5	3	102121	0.12
DON	1	4	3285338	0.12
DON	4,5	4	4261526	0.25

Table 6. SNPs significantly associated with aggressiveness

Models	Chromosome	Position	Minor allele freq
1,2,3,4	1	7371490	0.40
1,2,3,4	2	6675327	0.40

List key outcomes or other achievements.

Goal a): We have found our fungicide sensitivity assays in microtiter plates to be very efficient and repeatable. Work on other projects prevented us from extending these assays on our NA1 population beyond what we had originally planned. Yet our plans for the coming year include additional fungicide sensitivity experiments for both the NA1 population and other *F. graminearum* populations.

Goal b): As reported in Results above, we have three replications of aggressiveness data one the wheat variety Wheaton, and four replications on Rollag. These may prove sufficient to identify the major QTL for aggressiveness in our sample. However, the success of our FY24 USWBSI proposal includes the objective of additional replications of these experiments, to help us identify additional aggressiveness QTL that may have smaller effect sizes.

Goal c): We have evaluated several different GWAS methods and models, and found that multi-locus models and models that consider SNPs as random rather than fixed effects tend to identify the most significant SNPs. Our analyses have identified multiple significant SNPs for all traits analyzed, and while some SNPs fall in or near genes with known roles in the respective traits, in most cases the candidate genes identified are novel and provide new candidates for functional studies.

Outside of these main goals, we submitted a manuscript on structural variation across populations of *F. graminearum* that we reported on during the previous year's performance report. This manuscript was published in March 2024 in *G3*, and at the same time we released the four chromosome-level genome assemblies that were the focus of this publication through NCBI. The results of this manuscript on structural variation in *F. graminearum* and its relationship to repetitive elements and high recombination regions were also presented in an invited talk at the 2024 Fungal Genetics Conference.

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

The project has provided training for 3 PhD students: Upasana Dhakal, who graduated in December 2023, Sandhya Gopisetty, who started work on the project in May 2023, and Sumit Chowdhury, who began his work in August 2023. All have been trained on culturing and performing experiments with *Fusarium* in the lab, as well as greenhouse wheat head inoculations. Upasana has also been trained in data analysis and a range of bioinformatics and genomics analyses, while the other students will receive this training in the coming year. Sandhya and Sumit also benefitted from participating in the USWBSI GDER and PBG Joint Mid-year Virtual Meeting in April 2024. The project has provided training for two undergraduate hourly workers, Emily Gipson and Jamia Roberts. Both have helped to perform greenhouse inoculations and grain sample preparation for DON testing, and have learned additional techniques in cultivating *Fusarium* and harvesting both mycelia and macroconidia for experimental work. The project also provided training for two departmental interns during our greenhouse season, who helped with wheat head

inoculations. The project also helped to provide professional development and training to 26 participants in the 2024 Fusarium Laboratory Workshop. Finally, the PI and student Dhakal both had professional development opportunities through participation in the conferences listed in the next question (dissemination of results).

4. **How have the results been disseminated to communities of interest?**
   The results of this project were disseminated through presentations at the 2023 Plant Health (APS) conference, the December 2023 Scab Forum, the March 2024 Fungal Genetics Conference (including via an invited presentation), and through the publication of a Genome Report at the journal *G3* and the submission of another manuscript reporting on the population genetic analysis of *Fusarium graminearum* isolates to the journal *Fungal Genetics and Biology*. Results have also been included in the lab's Upasana Dhakal's PhD dissertation, which is freely available online. Further results of the project will be submitted for publication in peer review journals in the coming year. PI Toomajian participated in Kansas State University's 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?**
   Not applicable, this is the final report for the agreement.