Project FY22-PB-007: Signal Recognition by GPCRs During Plant Infection in Fusarium graminearum

---

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

The wheat head blight fungus *Fusarium graminearum* has 105 G-protein coupled receptor (GPCR) genes, including six GIV (GPCR important for virulence) genes important for plant infection. This study aims to further characterize the roles of these six *GIV* GPCRs in fungal pathogenesis and develop approaches to identify plant compounds (ligands) recognized by them. It consists of three objectives. Objective 1 is to characterize the functional relationships among these *GIV* GPCR genes. Objective 2 is to screen for anther compounds stimulatory to Gpmk1 activation via Giv1. Objective 3 is to develop a yeast reporter system to screen for anther compounds recognized by Giv1 and other GIV GPCRs.

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. The *giv1 giv2 giv3* and *giv4 giv5 giv6* triple mutants are further characterized. They have no other defects but reduced virulence in infection assays. The *giv1 giv2 giv3* triple mutant was similar to the *giv1 giv2* double mutant, indicating that *GIV2* likely functions upstream from *GIV3*. *GIV1* and *GIV2* functions independently because the *giv1 giv2* double mutant has more severe defects in plant infection than the *giv1* and *giv2* single mutants. In repeated assays, the *giv4 giv5 giv6* mutant is only slightly more reduced in virulence than the *giv4* and *giv5 giv6* mutants, indicating that *GIV4* has overlapping functions with *GIV5* and *GIV6*. We also have generated the *giv1 giv4* double mutant and the *giv1 giv2 giv4* and *giv1 giv2 giv5* triple mutants. Preliminary data showed that the *giv1 giv2 giv4* and *giv1 giv2 giv5* trouble mutants are slightly more reduced in virulence than the *giv1 giv2* double mutants. The *giv1 giv4* double mutant has no significant difference with the *giv1* mutant in virulence. These results indicate that *GIV1* and *GIV2* plays more critical roles than other GIV genes during initial infection and infectious growth in the rachis, respectively.

Objective 2. We have generated transformants expressing the *FST12*-GFP fusion construct under the control of its native promoter and strong, constitutive RP30 promoter. The P_{FST12}-*FST12*-GFP transformants have no detectable GFP signals in all the cell types examined. In the P_{RP30}-*FST12*-GFP transformants, GFP signals were observed in the vacuoles. Because FST12 encodes a transcription factor that should localize to the nucleus, localization to the vacuoles is likely an overexpression effect. We also expressed GFP fusion constructs of MST12, an ortholog of FST12 in the rice blast fungus and obtained similar results. Therefore, it is likely that the FST12 transcription factor is only transiently localized to the nucleus and *FST12*-GFP is not a suitable reporter for screening wheat compounds recognized by Giv1. One alternative approach is to identify genes regulated by *FST12* during plant infection by RNA-seq analysis and use one of these genes as a reporter.

Objective 3. We have generated the GIV-Ste2 chimeric constructs for all six *GIV* genes and transformed them into yeast cells expressing the PFuz1-GFP construct. Preliminary screening showed that treatments with yeast alpha pheromone and crude extracts of flowering wheat heads had similar effects due to spontaneous fluorescence signals in yeast cells and weak GFP signals from the PFuz1-GFP construct. These GIV-Ste2 chimeric constructs with extracellular domains of GIV genes and intracellular and transmembrane helices of Ste2 will be transformed into yeast cells expressing the PFuz1-Mel1 and PFuz1-LacZ constructs. The resulting transformants will be screened for the inducive effects of crude extracts of flowering wheat heads.

What were the significant results?

Objective 1. Our results showed that *GIV1* and *GIV2* functions independently in regulating the initial infection processes and infectious growth in rachis tissues but *GIV2* function upstream from *GIV3* for regulating infectious growth in the rachis. *GIV4* has overlapping functions with *GIV5* and *GIV6*.

Preliminary analysis with the *giv1 giv4, giv1 giv2 giv4,* and *giv1 giv2 giv5* mutants suggest that *GIV1* and *GIV2* play more important roles in regulating earlier infection processes than *GIV4* and *GIV5*.

Objective 2. Our results indicate that the *FST12* transcription factor is expressed at a relatively low level and only transiently localized to the nucleus in *F. graminearum*. Therefore, *FST12*-GFP is not a suitable reporter for screening wheat compounds recognized by Giv1. Alternative approaches are being pursued to identify more suitable report genes for the Giv1 GPCR.

Objective 3. All six GIV-Ste2 chimeric constructs have been generated and transformed into yeast cells expressing the PFuz1-GFP reporter construct. These GIV-Ste2 chimeric constructs will be transformed into yeast cells expressing the PFuz1-Mel1 and PFuz1-LacZ constructs. The resulting transformants will be screened for the inducive effects of crude extracts of flowering wheat heads.

List key outcomes or other achievements.

Objective 1

- Generated and phenotypically characterized double and triple mutants of different *GIV* genes.
- Attempted to generate *giv1 giv2 giv3 giv4* mutants by CRISPR in the wild type and *giv5 giv6* double mutant.

Objective 2

- Generated and characterized transformants expressing *FST12*-GFP fusion constructs under the control of its native promoter and the RP30 promoter.
- Examined the expression and localization of MST12-GFP.

Objective 3

- Generated yeast transformants expressing the P_{Fuz1}-GFP reporter and *GIV*-Ste2 chimeric constructs.
- Conducted a preliminary screening with crude extracts of flowering wheat heads.

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

Two PhD students are involved in conducting all the experiments related to project. They were trained in basic molecular techniques and fungal genetics, including yeast vector modifications, fungal transformation, qRT-PCR, and RNA-seq data analysis. Both of them attended the 2024 Fungal Genetics Conference and presented posters and an oral presentation.

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

Results from this study were included in talks presented by the PI at the PBP and GDER mid-year symposium, Golden conference on Cellular and Molecular Mycology, research seminars at two universities. The PI also included yeast report strains in the invited review on fungal GPCRs submitted to Current Opinion in Plant Biology.

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

Most of planned experiments for objective 1 have been accomplished. Next step is to finishing some assays and prepare a manuscript for publication. For objective 2, we reached the conclusion that although FST12 is a major transcription factor, it is not suitable for the planned experiment. Alternative approaches to develop a suitable reporter for screening wheat head compounds recognized by Giv1 will be tried. Objective is a pilot experiment and we will keep on trying different reporter genes and GIV-Ste2 chimeric constructs for screening wheat compounds recognized by Giv1-Giv6.