Project FY24-PB-006: Characterize the regulatory mechanism of DON biosynthesis in infected wheat heads

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1. **What are the major goals and objectives of the research project?**
   The goal of this study is to characterize the mechanism for regulating DON biosynthesis by *TRI14* in infected plant tissues and determine its functional relationship with *TRI6* and *TRI10*. Specific objectives are to: 1) Characterize the Tri14-Tri6 interaction and regulation of *TRI* gene expression; 2) Determine the functional relationship of Tri14 with Tri10 in DON biosynthesis; 3) Comparative analysis of genes regulated by *TRI6, TRI10,* and *TRI14* during plant infection.

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

   What were the major activities?
   For objective 1, we have finished the characterization of the interaction of Tri6 with Tri14 in yeast two-hybrid assays. We have generated the *tri14*/*TRI14*$^{\Delta T6B1}$ transformant that was defective in TRI14 expression but normal in DON production in in vitro DON production media. For objective 2, we fail to detect the direction interaction between Tri14 and Tri10 in yeast two-hybrid and co-immunoprecipitation assays, indicating that these two proteins do not interact. For objective 3, RNA samples have been prepared with wheat kernels inoculated with the *tri6, tri10,* and *tri14* mutants. RNA-seq analysis to identify genes differentially expressed in these mutants is in progress.

   What were the significant results?
   We have confirmed the regulation of *TRI14* expression by Tri6 and dispensability of *TRI14* in DON biosynthesis in axenic cultures under inducible conditions. The interaction of Tri14 with Tri6 in yeast two-hybrid assays is weak and will be verified by in vivo assays. For Tri10, our data showed that there is no direct interaction between Tri10 and Tri14. For objective 3, we have isolated RNA samples of tri6, tri10, and tri14 mutant cultures for RNA-seq analysis.

   List key outcomes or other achievements.
   Confirmed the regulation of TRI14 expression by Tri6 and Tri10.
   Ruled out the direct interaction of Tri10 with Tri14.
   During this study, we found that some mycoviruses that infect *Fusarium graminearum* are subjected to RNA editing in fungal hyphae.

3. **What opportunities for training and professional development has the project provided?**
   One new PhD student was hired and trained to conduct molecular genetic studies with *Fusarium graminearum*.

4. **How have the results been disseminated to communities of interest?**
   Not yet

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
   -Characterize the Tri14-Tri6 interaction in vivo and regulation of *TRI14* by *TRI6*
   -Comparative analysis of genes regulated by *TRI6/TRI10/TRI14* and verification
   -EMSA assays of the effects of Tri14 and Tri10 on target DNA binding of Tri6.