Project FY22-PB-001: Explore RNAi to Control FHB and Mycotoxin Contamination

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

The goal of this project is to develop an endophytic fungal RNAi delivery platform to reduce FHB and mycotoxin contamination.

The objectives of this proposal are:

- **Objective 1:** Design and generate *F. graminearum* RNAi mutants targeting genes that are essential for its pathogenesis, and trichothecene and zearalenone biosynthesis, and determine their effects on toxin production and FHB severity.
- **Objective 2**: Build, evaluate, and optimize the *S. zeae*-mediated RNAi delivery system. We will generate *S. zeae* GFP-RNAi strains, examine RNAi molecule production and the transferring of RNAi signals from *S. zeae* to plants and *F. graminearum*, and determine gene silencing efficacy.

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

What were the major activities?

Objective1

- We cocultured Fusarium strain and *S. zeae* TRI5 RNAi strain for toxin reduction assays.
- We performed wheat heads treatment with *S. zeae* TRI5 RNAi strain and examined disease and mycotoxin reduction efficacy.
- We performed wheat seeds treatment with *S. zeae* TRI5 RNAi strain at high concentration to increase RNAi efficacy.
- We developed a *S. zeae* strain carrying a GFP construct to investigate *S.* zeae colonization in wheat.

Objective 2:

- We conducted more liquid medium assays to confirm GFP signal reduction by *S. zeae* GFP RNAi strains.
- In collaboration with Dr. Inns from Indian University, isolate dsRNA and sRNA from cultures and media grown *S. zeae* GFP RNAi strain to examine RNAi signaling and transportation.

What were the significant results?

Objective 1

- We found *S. zeae* strain alone is able to suppress toxin production. *S. zeae* TRI5 RNAi strains further reduce toxin production in coculture.
- Seeds treatment with *S. zeae* TRI5 RNAi strain reduced FHB and toxin production.
- Heads treatment with *S. zeae* TRI5 RNAi strain did not reduce FHB. *S. zeae* TRI5 RNAi strain treatments alone appeared to induce necrosis symptoms on wheat heads under high humidity and high inoculation concentrations.
- Microscopic studies showed that *S. zeae* primarily colonized in wheat roots, but it did not move up to heads successfully.

Objective 2

- dsRNA and sRNA were isolated from *S. zeae* RNAi strains and media, and ready for Northern detection.

List key outcomes or other achievements.

An endophytic fungal RNAi delivery platform was developed successfully. Improvement of application efficiency and investigation of molecular mechanism are underway.

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

   One ORISE fellow, Nick Rhodes, has been trained in molecular biology, such as RNA and sRNA isolation, generation GFP-labelled fungal strain, confocal microscope to investigate fungal colonization. He was also trained in seed treatment, fungal inoculation and FHB virulence assays.

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

   None

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
   - Find methods to promote *S. zeae* colonization in wheat, and test if other *S. zeae* strains can move up to wheat heads.
   - Determine if RNA signaling can be transported to wheat heads without *S. zeae* colonization in heads.