

Project 1: Characterization and Expression of Plant Transporters to Reduce FHB and Mycotoxins

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

The goal of this project is to identify plant transporters and utilize it to reduce FHB and mycotoxin contamination.

The objectives of this proposal are:

- Identification of transporters responsible for 3-ADON excretion using transgenic Arabidopsis expressing FgTri101.
- Generation of transgenic wheat expressing FgTri101 and determine if transgenic wheat expressing FgTri101 can excrete 3-ADON.
- Stacking FgTri101 and the Arabidopsis transporter in transgenic wheat and evaluate transgenic lines for DON detoxification and resistance to FHB.

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:

- We generated transgenic Arabidopsis expressing FgTri101 in Arabidopsis transporter mutant(Atdtx1) background.
- Performed root toxin resistance and seedling conversion assays.

Objective 2:

- Conducted FHB virulence assays on FgTri101 transgenic wheat.
- Compare disease and toxin level in FgTri101 transgenic wheat and controls.
- Optimized FgTri101 coding sequence with wheat codon, synthesized the optimized gene *TRI101* and sent to our collaborator Dr. Trick for wheat transformation.

What were the significant results?

Objective 1:

- We confirmed Arabidopsis DTX1 is involved in 3-ADON efflux. Our data suggest that at least one additional transporter is involved in 3-ADON excretion.

Objective 2:

- We showed that transgenic wheat expressing FgTRI101 increase FHB resistance and reduced DON contamination. The promising data led us to optimized FgTri101 coding sequence and introduce it to a moderately resistant wheat variety to enhance its effectiveness on FHB and mycotoxin control.

List key outcomes or other achievements.

First, we identified an Arabidopsis transporter involved in toxin efflux. Second, we increased FHB resistance and reduced DON contamination by expressing FgTri101 in wheat.

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

Two ORISE fellows (Gabdiel Yulfo-Soto and Nick Rhodes) have been trained in molecular biology including DNA isolation, RCR and RT-PCR, screening transgenic plants, root growth and DON to 3-ADON conversion assays, inoculation and scoring of FHB assays. Due to the training, Gabdiel obtained a permanent tech position in another USDA/ARS location.

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

Poster presentation at the 2023 National Fusarium Head Blight Forum, Dec. 7-11, 2023. Cincinnati, OH

Poster presentation at the 2024 Plant Biology 2024, June 22-2, 2024. Honolulu, HI

Paper "Reduction of Fusarium head blight and trichothecene contamination in transgenic wheat expressing *Fusarium graminearum* trichothecene 3-Oacetyltransferase" was published in Front. Plant Sci. 15:1389605. doi: 10.3389/fpls.2024.1389605

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

- Analyze data on Arabidopsis dtx1 mutant expressing FgTRI101 and prepare manuscript for publication.
- Obtain transgenic wheat expressing optimized FgTRI101, confirm gene integration by PCR and gene expression by RT-PCR.
- Propagate T2 seeds for toxin resistance assays and FHB virulence assays.