

Project 2: Spherical Nucleic Acid Nanomaterials as Fungicide and FHB Resistance-promoting Agents

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

The primary goal of this project was to utilize spherical nucleic acid (SNA) nanomaterial-based technology to control *F. graminearum* and FHB. Two objectives were being pursued:

1. Identify candidate small interfering RNA (siRNA) sequences that effectively silence *F. graminearum* genes.
2. Synthesize lipophilic SNA nanoparticles and evaluate their efficacy in (i) delivering siRNA to the fungus, (ii) knocking down target gene expression, and (iii) limiting fungal growth and toxin accumulation, and (iv) enhancing FHB resistance in wheat.

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

What were the major activities?

- A variety of siRNA sequences and morpholinos that target different fungal genes, including house-keeping genes and virulence factors were designed for use in knocking down expression of the corresponding fungal gene.
- A variety of lipid-based SNA nanoparticles were made and used to study their efficacy in delivering siRNA into the fungus.
- The efficacy of delivery of siRNA by the SNA nanoparticles into the fungus and in knocking down fungal gene expression and limiting fungal growth was assessed in vitro.

What were the significant results?

- Lipid-based spherical nucleic acids (SNAs) that incorporate siRNA for gene knockdown application were generated. Confocal and epifluorescence micrographs confirmed that the nanomaterials were able to cross over the fungal cell wall, thus pointing to entry of the materials into the fungus
- Knockdown of fungal gene expression was observed. However, the efficacy was variable from experiment to experiment, when compared to the empty liposomes. This presumably is due to variations in the quality of different batches of SNA and uptake by the fungus among different experiments. Furthermore, the lipids in the SNA also had a fungicidal effect, thus adding further complexity in determining the true efficacy of the SNA-delivered siRNA in knocking down fungal gene expression.

List key outcomes or other achievements.

- Knockdown of fungal gene expression was observed with the SNA technology. However, this effect was highly variable from experiment to experiment. Furthermore, the lipids used in preparing the SNA themselves had a repressive effect on fungal growth, thus limiting the utility of using lipid-based nanoparticles in their current form as a means for delivering siRNA for fungal gene silencing.

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

Training: A postdoc who is spearheading this project was provided training in working with nanomaterials as a delivery system to target the knockdown of fungal genes. The postdoc also received training in working with *F. graminearum* and in molecular plant pathology. A new graduate student received training in molecular biology and plant pathology.

Professional Development: This project contributed to the professional development of the postdoc and the graduate student who participated in the weekly group meetings, weekly Department of Biological Sciences seminars, the BioDiscovery Institute research talks, the FHB forum and GDER/PBG virtual forum. The postdoc developed his presentation skills by preparing posters and oral presentations arising out of their work. The postdoc also received training on mentoring the graduate student. Co-PI Shah worked individually with the postdoc and graduate student, meeting with them biweekly to help them prepare towards their long-term professional goals.

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

The RNA silencing and SNA approach and their potential in controlling target gene expression, as well as their limitations when it comes to utilization on fungi was discussed in a graduate advanced molecular biology class and in an undergraduate Biology class taught by Co-PI Shah at the University of North Texas.

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

The outcomes of this project, will be submitted for submission as a short report.