ISRC Final Project Report

1) Tell us about your project

• Project Title: Characterization of iron deficiency and *Fusarium graminearum* interactive responses in soybean

• Lead PI: Silvina Arias, Plant Pathology, Entomology, and Microbiology. Collaboration with Jamie O'Rourke, Research Geneticist, Agronomy.

• Projects year(s): 2023-2024 (one-year project)

• Total amount of funding: $28,000

• Leveraged/Additional Funding, including federal or private organizations:

2) Project Summary

The objective of this study is to characterize soybean genes that are differentially regulated by the host during *F. graminearum* infection in an iron deficiency environment in order to identify new potential resistant mechanisms and candidate genes involved in the defense response.

Specific objectives:

1. Evaluate phenotypically IDC-resistant and susceptible soybean cultivars inoculated with *F. graminearum* under iron deficiency conditions in a hydroponic system.
2. To elucidate the comprehensive gene expression in response to the pathogen and iron deficiency simultaneously (RNA seq analysis).
3. Identify, compare, and analyze differentially expressed genes.

✓ **Report of deliverables**

- Assessment of the impact of low iron availability and *Fusarium* root rot (NRRL 13121 pathogenic *F. graminearum*) on two near-isogenic soybean lines.
- Understanding differential gene expression between roots and leaves within each genotype.
- Knowledge of the effect of *Fusarium* infection and an iron-deficient environment on soybean defense responses and other mechanisms to combat both stresses.

- ✓ **Non-technical summary (layman's terms)**

In the north central U.S., the calcareous soil favors the development of Iron deficiency chlorosis (IDC) in soybeans. An additional problem is that soybean plants with IDC symptoms often display *Fusarium* root rot (FRR) symptoms, caused by *Fusarium graminearum,* a major necrotrophic fungus and the most frequently recovered species of *Fusarium* in fields in Iowa. The combined impact of IDC and FRR results in yield loss, leading to substantial economic losses for soybean growers. Currently, the basis of the IDC-FRR association is not clear, and management becomes more complicated when they coexist. In general, research programs focus on identifying resistance to a particular stress and not multiple stress conditions. Consequently, improved varieties may respond unpredictably when grown in field conditions. To investigate soybean responses to both stresses, IDC and FRR, soybean lines Clark (iron efficient) and IsoClark (iron inefficient) were compared. *F. graminearum* inoculated and non-inoculated seeds were germinated on germination paper for 7 days. The seedlings were transferred to a hydroponic system and grown in iron-sufficient and iron-deficient nutrient solutions for 2 weeks. *F. graminearum* infestation and iron deficiency consistently impacted growth parameters, root morphological characteristics, and content of chlorophyll. Interestingly, the pathogen may strongly affect Clark (iron efficient cultivar) than IsoClark.

We also collected root and leaf tissue samples and we extracted the RNA for gene expression analysis (RNA seq analysis). Sequencing generated 1.9 billion reads, 1.7 billion of which (~84%) mapped uniquely to the soybean genome. The large quantity of sequence data generated by some of the samples meant the standard analysis pipeline failed at a critical juncture due to lack of computational power. Accordingly, we developed a novel method to handle the data that was much less computationally intensive and will be incredibly helpful in saving time and computational power for future experiments. Overall, we were able to determine that 33659 genes are expressed in leaves and 35028 genes are expressed in roots. We identified soybean genes in Clark and IsoClark responding to single stresses (iron deficiency and fungal infection) and combinatorial stresses (*Fusarium* infection + iron deficiency). The response to iron deficiency in Clark and IsoClark is well characterized by previous publications, and we will compare the genes identified in this study to previous studies to confirm that iron stress was induced. However, it is necessary to evaluate the impact of *F. graminearum* on IDC-tolerant/susceptible soybeans. We identified 9315 genes differentially expressed due to *Fusarium* infection in Clark leaves and 4765 genes in IsoClark leaves. Similarly, we identified 8195 genes differentially expressed due to the pathogen in Clark roots and 6407 genes differentially expressed in IsoClark roots.

To investigate how soybeans respond to iron deficiency when previously inoculated with *F. graminearum,* we compared genes differentially expressed in plants inoculated with the fungus in sufficient and deficient iron conditions. These analyses identified 1425 genes in leaves and 1499 genes in roots of Clark and IsoClark combined. However, visualization of these genes showed that the majority of the genes were expressed in the same direction under both stresses, indicating a similar or possible additive response to combined stresses. We are particularly interested in genes with opposing

expression patterns under the two conditions. Initial investigations have identified 781 genes differentially expressed of interest in leaves and 190 in roots (depicted by * on the heat maps). Further investigation and characterization of these genes will continue as we prepare a manuscript for publication, i.e., to assign the biological roles to the identified gene clusters related to defense, iron homeostasis, cell cycle, gene silencing, and photosynthesis.

3) **Conclusions** (include observations, solutions, and/or is additional research warranted)

What new knowledge was developed/discovered in the research supported by the ISRC?

- We have developed a novel bioinformatics pipeline to manipulate the large file sizes generated in this experiment. This is going to greatly improve the speed at which future analysis takes place (~3 hrs for one step down to 1 min).
- We have identified genes differentially expressed due to *Fusarium* infection in Clark (9315 genes) and IsoClark (4765 genes). These genes can be mined to better understand the infection and plant response to *Fusarium* infection.
- We have identified 781 genes in leaves and 190 in roots with expression patterns that change direction under iron deficiency stress and *Fusarium* infection. These genes indicate an interaction between iron deficiency and *Fusarium* infection that has not been previously reported.

What are practical applications of the research? How long?

- The genes identified by this research immediately become high-priority genes to characterize and investigate in future iron and *Fusarium* studies. While tissue for this study was collected after extended stress, studies in iron deficiency have shown similar genes to be of high interest in understanding the soybean iron stress response.

How might this new knowledge/discovery affect the success of positive impact for industry and/or farmers?

- The knowledge provides the first evidence that there is an interaction between *Fusarium* infection and iron deficiency. Further, these results provide insight into how soybean responds to *Fusarium* infection. Understanding the soybean responses to these stresses is crucial for more effective management.
- In addition, these results may be useful in developing new methods of broadening the resistance of soybean to *F. graminearum* and iron deficiency.

4) Supporting attachments:

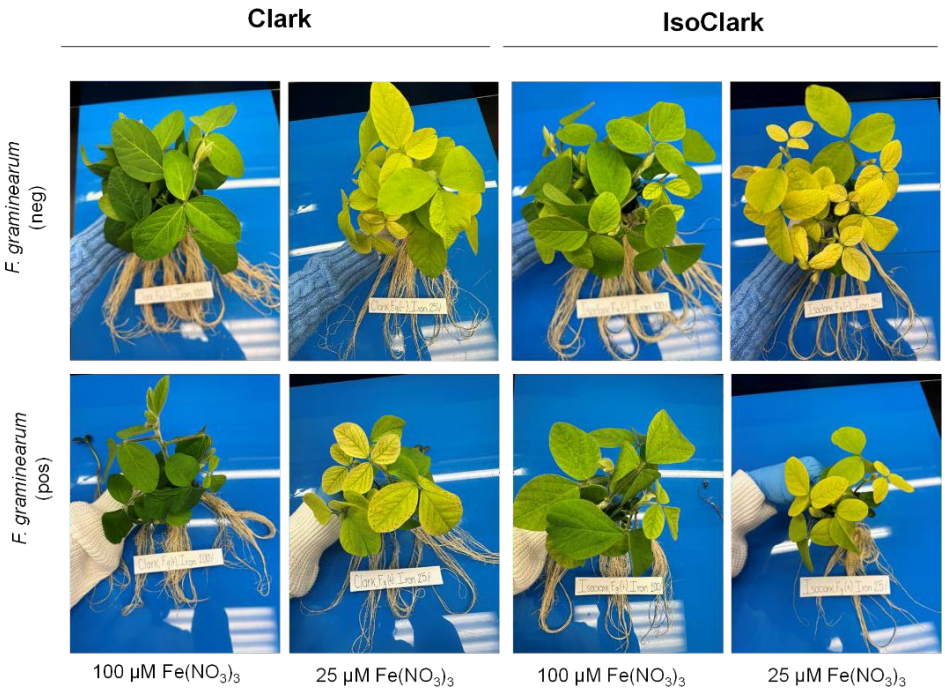

Figure 1. Chlorophyll content of Clark and Isoclark soybean seedlings grown under iron-deficiency treatment (25 µM Fe(NO3)3) and normal iron conditions (100 µM Fe(NO3)3); inoculated and non- inoculated with *Fusarium graminearum* (2.5 10^3 spores/ml).

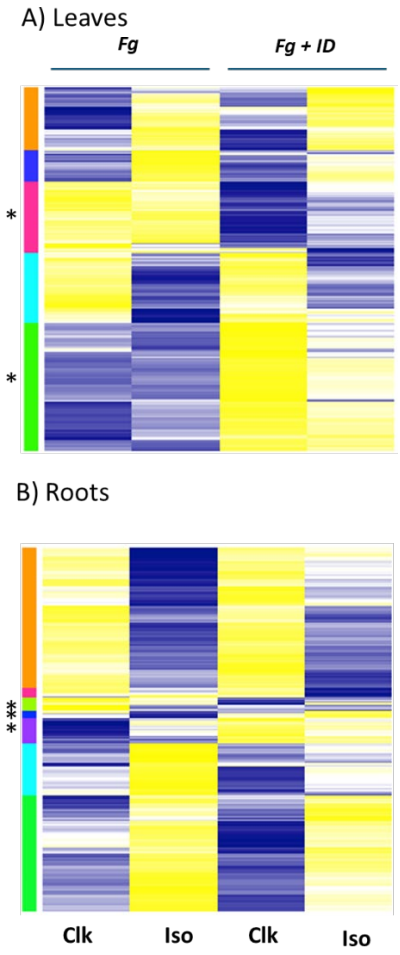

Figure 2. Heat map illustrating the expression patterns of genes differentially expressed (DEGs) in soybean A) leaves and B) roots due *Fusarium graminearum (Fg)* infected plants and to *Fg* + iron deficiency conditions (ID) in Clark (Clk) and IsoClark (Iso). Analysis of leaf samples (A) identified 1425 DEGs under iron deficiency and *Fg* inoculation, while 1499 genes were identified in root samples (B).

Hierarchical clustering was used to generate heat maps to identify genes with conserved expression patterns of interest. Clusters are represented by the colored bars to the left of each heat map. Clusters with conserved expression patterns of interest are denoted by an asterisk to the left of the cluster bar. These clusters of interest represent 781 genes in leaves and 190 in roots.

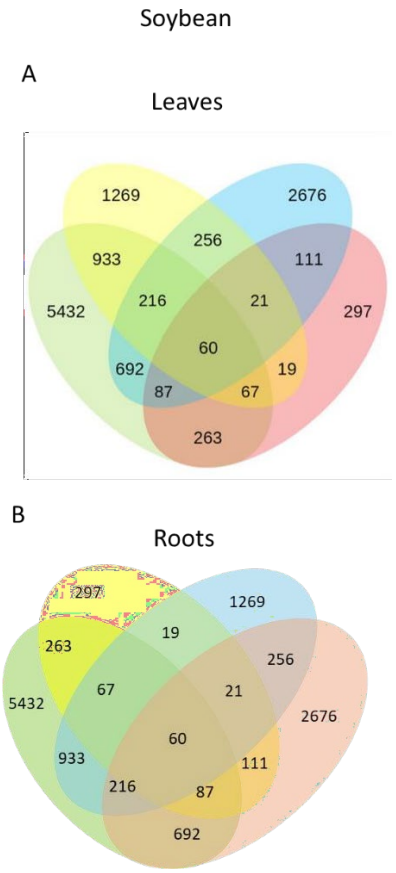

Figure 3. Venn Diagram of soybean illustrating the distribution of differentially expressed genes (DEGs) in leaves (A) and roots (B) due to *Fusarium* infection in Clark under iron sufficient (green) and deficient conditions (yellow) and IsoClark under iron sufficient (blue) and deficient conditions (red).

5) List conferences, publications, etc. in which this research was shared

- Presented our research as a poster at the American Phytopathological Society meeting in July 2024, in Memphis, Tennessee.
- Presented our research at the ISU Research Day hosted by ISRC in 2024.
- I will present as a poster at the Soy 2025: 19th Biennial Conference on Molecular and Cellular Biology in Soybean in July 2025 in Madison, Wisconsin.