FY23 Midterm Report NDSC

Project Title: **A tool for cheap and rapid tracking of soybean inoculant populations in field soil**

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a. Objectives of the Research:

Objective 1: Evaluate improvement of sensitivity with TaqMan probes and finalize technology platform (July 2022 – October 2022).

Objective 2: Establish reliability using different soil types and sampling procedures, and optimize as necessary (November 2022 – March 2022).

Objective 3: Test finalized assay using farmer's field soil, with a focus on inoculant survival in acidic soils from Western ND (April 2022 – June 2022).

b. Completed Work

Work thus far has focused on Objective 1, which involved improving the sensitivity of the assay and finalizing the technology platform. In the previous year, we set out to explore dPCR or qPCR as technology platforms for an assay to enumerate soybean rhizobia (symbiotic *Bradyrhizobium japonicum*) in farmer's soil. Work from FY22 culminated in a successful qPCR assay with a sensitivity limit of ~1000 rhizobia per gram (See FY22 report, Figure 1) (Assay Version 1.0). Development of an assay with a new technology platform, digital PCR (dPCR) which was advertised to have enhances specificity and sensitivity to qPCR, was also attempted. However, dPCR assays were unsuccessful due to high amounts of nonspecific signal in negative controls. Conversations with the manufacturer (QIAgen) indicated that incorporating TaqMan probes into the assay would overcome this issue in dPCR. TaqMan probes are a modification to amplicon-based molecular detection methods (qPCR/dPCR) that provide an added layer of specificity by binding to the amplicons and creating a detectable fluorescent signal when bound. Since TaqMan probes can also be utilized in qPCR and have the potential to increase sensitivity of our assay, we sought to evaluate incorporation of TaqMan probe technology into both qPCR and dPCR assays (Assay Version 2.0). Therefore, our first Objective of FY23 was to investigate the incorporation of TaqMan probes into qPCR and dPCR assays to both 1) finalize the selection of technology platform, and 2) evaluate improved sensitivity with their use.

We have now successfully designed an effective TaqMan probe and incorporated it into the previous assay, creating Assay Version 2.0. This has been tested on both dPCR and qPCR platforms. While the probe rendered the assay successful with dPCR, the sensitivity was far exceeded by qpCR (~10,000 rhizobia per gram with dPCR vs 1,000 rhizobia per gram with qPCR). These data have led us to finalize qPCR as the best platform for the assay. Furthermore, incorporation of the probe allowed an enhanced sensitivity in qPCR by allowing the assay to be

performed on concentrated DNA samples. This raised the assay sensitivity from ~1,000 cells per gram to ~100 cells per gram.
c. Preliminary Results

TaqMan probe evaluation

Two approaches to TaqMan probe design were explored.:

1) A custom TaqMan probe assay was designed by ThermoFisher Scientific and tested. This assay however was ineffective and showed significant amplification in non-rhizobia control samples indicating a lack of specificity (data not shown).

2) We manually designed a TaqMan probe to incorporate with our previously successful primer sets utilized in the Version 1.0 qPCR assay (Figure 1).

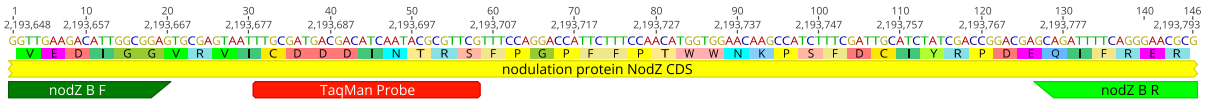

Figure 1. Design of TaqMan probe for incorporation into qPCR assay. Primer sequences for qPCR assay in green and the sequence of the TaqMan probe is in red.

To evaluate the accuracy of the new 2.0 TaqMan probe assay, we tested it using samples that were previously used to evaluate and calibrate the 1.0 qPCR assay. Overall, the 2.0 assay proved reliable and showed highly similar results to the qPCR assay with the same samples (Figure 2).

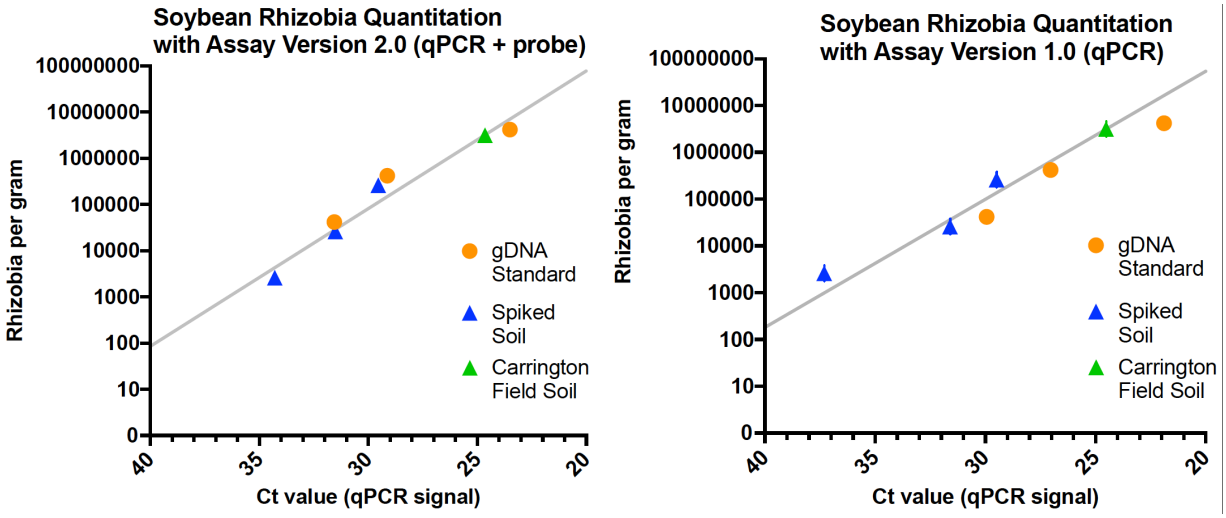

Figure 2. Assay Version 2.0 results using qPCR platform (left), Version 1.0 results (right)

Next, we evaluated the new 2.0 TaqMan probe assay with dPCR using the same samples. While the assay was now successful using dPCR when the probes were incorporated, the sensitivity of the assay was vastly lower than qPCR (Figure 3). These data, combined with the higher reagent cost of dPCR lead us to finalize qPCR as the best platform for our assay.

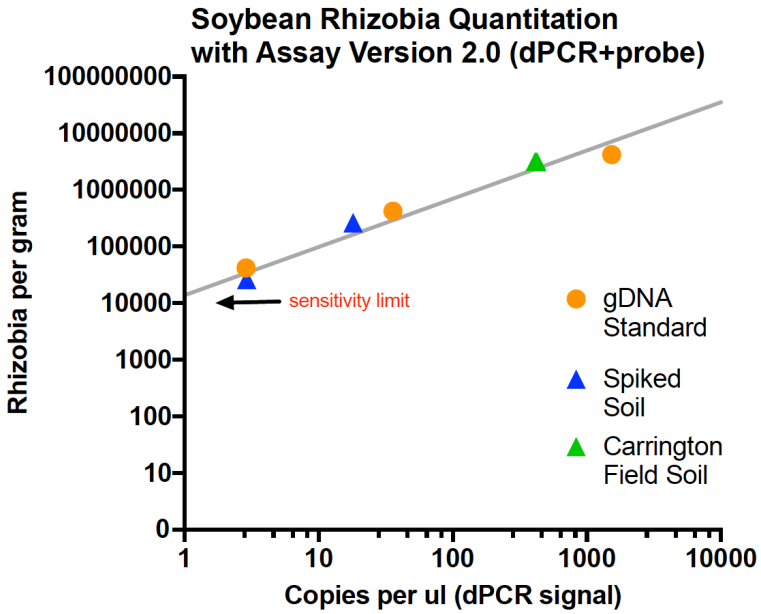

Figure 3. Assay Version 2.0 results using dPCR platform

Finally, we explored the capacity for increased sensitivity in qPCR using the new 2.0 TaqMan probe assay by using undiluted DNA samples from soil extractions. Previously, undiluted samples had too much background noise using the 1.0 assay and as a result samples needed to be diluted from ~100 ng/uL from the soil extraction to 10 ng/uL before the qPCR assay. Using the 2.0 TaqMan probe assay, undiluted samples were successfully detected without background noise and led to an enhanced sensitivity of the assay from ~1000 rhizobia per gram to ~100 rhizobia per gram.

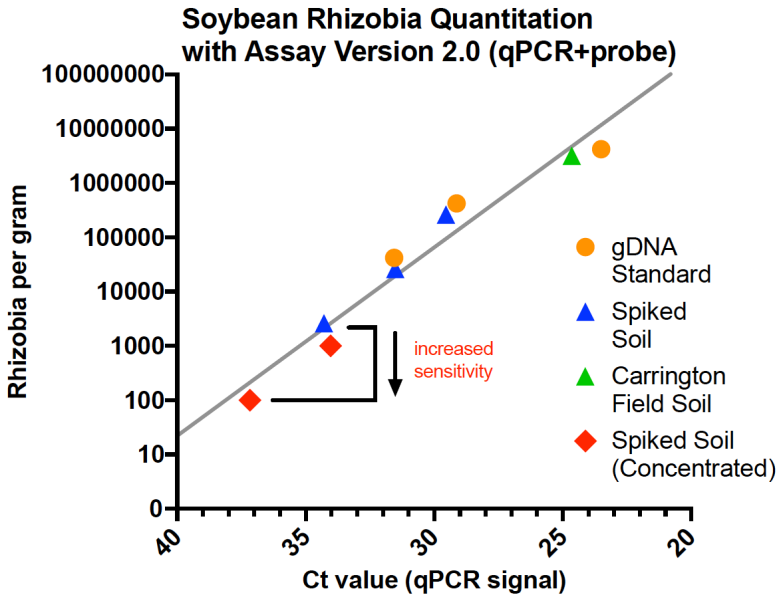

Figure 4. Assay Version 2.0 results using qPCR platform with concentrated (undiluted) DNA samples

d. Work to be Completed

Overall our results have led us to successfully finalize a technology platform and improve the sensitivity of our assay to enumerate soybean rhizobia from field soil, bringing us closer to a finalized molecular tool that could be used for agronomy research and provided as a service to farmers to guide inoculant decisions. Work on the remainder of the project will focus next on Objective 2 where we will evaluate the reliability of the assay with different soil types and sampling procedures, and then Objective 3 where we further test the assay with farmer's field soils from Western ND.

With a finalized assay in hand, work in future years will focus on the agronomy aspect of critical rhizobia thresholds to maximize yield. By establishing the number of rhizobia above which farmers can expect no benefit from inoculation, and below which farmers should see a yield boost in the context of the diverse soils and weather patterns across the state we will soon be able to deploy the tool as a valuable service for farmers.