Development of Molecular Soil Test for Soybean Cyst Nematode in Manitoba

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Introduction

Soybean Cyst Nematode (SCN), *Heterodera glycines,* is recognized as one of the major pests of soybean worldwide. In 2019, we reported the first occurrence of SCN in Manitoba. Precise identification and determination of SCN density in soil samples are essential for pest management decisions.

Objective

Developing a quantification method for the *H. glycines* targeting the CoxIII and SCAR genes correlated to the traditional microscopic counting of eggs in the soil.

Methods and Materials

• In July 2019, 20 fields with a range of SCN levels from Southern Ontario were sampled and used to optimize extractions and PCR reaction procedures (Fig 1),

• Cyst and egg extraction was performed using the wet-sieving method (Fig 3), and total genomic DNA was extracted from soil debris using the modified PowerSoil DNA Isolation Kit (Fig 5),

• A SYBR Green-based real-time PCR assay was optimized using the CoxIII (Madani et al. unpublished) and SCAR (Ou et al. 2008) primers. The melting profile further supported the specific detection of the SCN,

• Calibration curves were obtained by adding a different number of SCN eggs (10,100,500,1000) in three replicates to both suspension and soil debris in which the absence of *H.glycines* confirmed,

• The method was validated by quantifying the number of eggs in 10 composite soil samples collected from a naturally-infested soybean field in central Manitoba,

Results

• The relationship between Cq values and log of DNA concentration on the standard curve for SCAR and COXIII primer sets were linear with amplification efficiency of % 102.82 and % 100.3, respectively. Cq values increased proportionally with the dilution rate (Fig 7),

• The melt curves revealed the species-specificity of the assay by generating melt temperatures of 80±0.5 ◦C and 75±0.5 ◦C for DNA extracts positive for *H. glycines* when amplified with SCAR (Fig 8), and COXIII, respectively,

• There were highly significant negative correlations between the number of eggs added and Cq values in nematode suspension (Fig 9), as well as in soil debris (Fig 10) at all the inoculation levels for both Primer sets,

• There was a high correlation between the egg counts quantified by the qPCR and the conventional method for both primer sets (Fig 11),
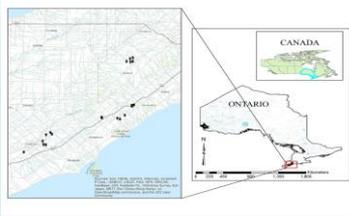

Figure 7. Real-time PCR standard curve of 10-fold serially diluted genomic DNA of *H. glycines* using SCAR primer set
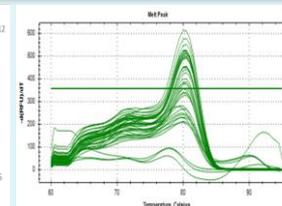
Figure 8. Melt curve generated in qPCR using SCAR primer set
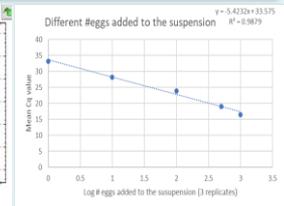
Figure 9. Relationship between the number of SCN eggs added to the suspension and Cq values
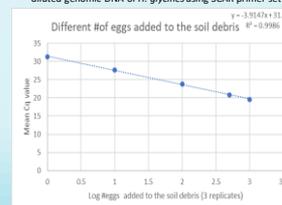
Figure 10. Relationship between the number of SCN eggs added to soil debris and Cq values
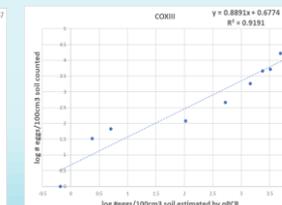
Figure 11. Correlation between the number of SCN eggs counted in 10 naturally-infested field soils under the microscope versus qPCR estimates of the same samples using the COXIII and SCAR primer sets. Each dot for qPCR represents the mean of three biological replicates (DNA extraction) and four technical replicates (qPCR run). Each dot for traditional counting represents three nematode extraction and three counting replicates.
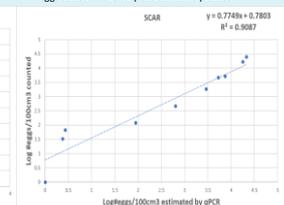
Conclusion

This study demonstrated a rapid and sensitive quantification method for soybean cyst nematode in soil.

Figure 1. Sampled fields in southern Ontario covering a range of SCN levels

Figure 2. sampling method

Figure 3. Wet- Sieving method to extract cysts and manually grinding cysts to release eggs

Figure 4. stained eggs to be counted under the microscope

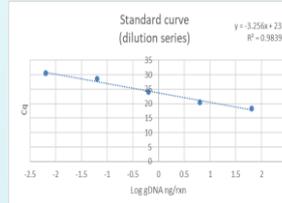**Figure 5.** Total genomic DNA extraction from nematode suspension and soil debris left on the sieve