Research Project Report

Project Title: Study of SCN diversity and detection of white soybean cyst nematode for strategically breeding resistant soybean

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I. Proposal Summary

The soybean cyst nematode (SCN, *Heterodera glycines*) is the most destructive pathogen of soybean and widely spread in Minnesota and most soybean-growing regions throughout the world. Recently, a new cyst nematode species, *Heterodera sojae,* also known as 'white soybean cyst nematode' (WSCN) as a common name, was found in Korea and China. Soybean cyst nematode has big variations in morphology and virulence phenotypes (ability of reproduction on different soybean germplasm lines). In this project, we propose to study diversity of the SCN and detect WSCN in Minnesota. Specifically, we will phenotype 184 inbred lines of cyst nematodes randomly selected from Minnesota soybean fields for their virulence phenotypes on the SCN-resistant source germplasm lines PI 88788, Peking, PI 437654, PI 567516C, PI 438489B, and a line that has novel SCN-resistance QTL/gene. We will study variations in SCN morphology and determine if WSCN occurs in Minnesota or not. The knowledge of SCN diversity and WSCN occurrence will be highly useful for strategically breeding soybean cultivars resistant to the cyst nematode(s) with the most effective sources of resistance. In addition, we will study effectiveness of rotation of different sources of resistance in managing SCN. This project will advance technology to manage the most destructive pests, the cyst nematodes, in soybean and maintain the crop productivity in Minnesota.

II. Goals and Objectives

Goal 1: Determine diversity of SCN virulence phenotypes in Minnesota
Objective 1. Characterization of virulence phenotypes of SCN inbred populations.

In this objective, we will characterize virulence phenotypes of 184 SCN inbred lines. Specifically, (1) the reproduction potential as measured with Female Index (FI) of the nematode lines will be determined on the SCN-resistance sources used in the University of Minnesota breeding program; (2) the data will be used to analyze the diversity of SCN in Minnesota.

Goal 2: Determine if white soybean cyst nematode occurs in Minnesota

Objective 2. Study of variations of SCN morphology and detection of white soybean cyst nematode.

In this objective, we will study the morphology and sequence DNA of the 184 inbred SCN populations to identify if all of these lines belong to SCN and if any of the lines is WSCN. In addition, we will analyze if any of the morphological parameters associated with virulence phenotype.

Goal 3: Predict the changes of SCN virulence phenotypes following the use of different sources of SCN-resistance

Objective 3. Determine the effect of sequences of SCN-resistance sources on SCN population densities and virulence phenotypes.

In this objective we will determine: (1) the effect of various sequences of three important sources of resistance PI 88788, Peking, and PI 437654 on the SCN population dynamics in a field initially infested by SCN race 1; (2) the effect of the sequences on change of SCN virulence phenotypes in the field.

III.

Progress and Accomplishment

Objective 1. Characterization of virulence phenotypes of SCN inbred populations.

A total of 184 inbred lines of the soybean cyst nematodes were selected from about 100 SCN field populations that were 'randomly' collected across Minnesota soybean growing counties in 2013. To develop an inbred line, a single cyst was transferred to an SCN-susceptible soybean plant. After 45 days, when the first generation of females (cysts) developed, a single cyst was transferred to a new soybean plant. Each of the cysts and females were developed from fertilization of the siblings within the same parent cyst. After a number of transfers (10 to 24 transfers), the SCN lines are relatively homogenous in genetics. The 184 inbred lines may represent diversity of SCN populations in Minnesota. So far, we have phenotyped 40 SCN lines for their virulence (Female Index) on eight soybean germplasm lines that have various genotypes of SCN resistance and additional 144 SCN lines on PI 88788 and Peking soybean lines.

Objective 2. Study of variations of SCN morphology and detection of white soybean cyst nematode.

To detect white soybean cyst nematode (*Heterodera sojae*), morphological characterization and DNA sequencing will be carried out. For morphological studies, SCN female, male, and juvenile specimen will be prepared. Key morphological traits will be measured and described. The morphological traits will be used to detect WSCN or confirm that these inbred lines are SCN. In addition, the morphological traits can also be used to study the morphological diversity of SCN. Specific DNA primers (e.g., D2A, D3B, TW8, and AB28) will be used to amplify DNA with PCR. The amplified DNA will be sequenced, and the data will be used to compare the published DNA sequence data of SCN, WSCN and other cyst nematodes, and determine any of the inbred lines is WSCN or confirm they are SCN. So far, we have extracted DNA of 175 lines.

Objective 3. Determine the effect of sequences of SCN-resistance sources on SCN population densities and virulence phenotypes.

In previous studies, we have demonstrated that the use of the SCN-resistant cultivars resulted in SCN populations that are able to break the resistance of existing cultivars. The SCN resistance in most current commercial cultivars is from PI 88788, only a few from Peking. The selection pressure of SCN-resistance on SCN populations may differ in different sources of resistance. We have initiated long-term field experiments to determine how the cultivars from the three sources of resistance PI 88788, Peking, and PI 437654 affect the reproductive ability of SCN over time. Based on the data of HG Type analysis of the populations collected in 2007, 2008, 2009, 2010, 2012, and 2014 from a field experiment in Waseca, MN, where the initial SCN population was race 3 (HG Type 0), SCN populations selected by the cultivar with PI 88788 source of resistance can only overcome the resistance of PI 88788 not the other two, and Peking-derived cultivar selected SCN populations can only overcome the resistance in Peking. In contrast, PI437654-derived cultivar

selected SCN populations that could overcome both Peking and PI 88788 sources of resistance (Chen, 2020: https://doi.org/10.1094/PDIS-09-19-1916-RE).

In this project, experiment was initiated in 2008 to study how the rotations of different resistance sources affect the dynamics of SCN population densities and their virulence phenotype in a field with initial population race 1 (HG Type 2.5.7), which is virulent to cultivars carrying the PI 88788 resistance. The main aim of the experiment is to determine whether any cultivar sequence can change the population from virulent to avirulent or change to other HG Types so that the PI 88788-source and/or Peking-source cultivars can be used.

The treatments include different combinations of the four cultivars Pioneer 92B13 (susceptible), Latham EX547 RR N (PI 88788 resistance), Pioneer 91M90 (Peking resistance), and Latham AR5084 (PI 437654 resistance). This is a long-term study (Table 1). Nematode population densities were/will be determined at planting and harvest every year. The virulence phenotypes (HG Types) of the populations collected from different crop sequences from the site in the spring of 2008, 2011, 2013, and 2017, and 2020 have been determined on the sources of resistance PI 887888, Peking, and PI 437654 with Lee 74 or Williams 82 as susceptible soybean control. In 2022, in addition to soil samples taken at planting and at harvest for determining SCN population densities. These samples are yet to be processed. Another set of soil samples were collected in September for testing virulence phenotypes. Nematode numbers were increased in greenhouse pot culture. HG Type bioassay has been set up in the greenhouse. Soybean yield was recorded.

VI. Milestones

- A total of 40 SCN lines have been phenotyped for their virulence to eight soybean lines, and additional 144 SCN lines to PI 88788 and Peking.
- In Objective 2, DNA has been extracted for most nematode lines.
- In Objective 3, all field samples have been taken. HG Type tests have been set up.

V. Deliverables

No deliverables to report.

Table 1. Cultivar sequences at Lamberton site.

Sequence	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
1	S	S	S	S	S	S	S	S	S	S	Corn	S	S	S	S
2	R1	R1	R1	R1	R1	R1	R1	R1	R1	R1	Corn	R1	R1	R1	R1
3	R2	R2	R2	R2	R2	R2	R2	R2	R2	R2	Corn	R2	R2	R2	R2
4	R3	R3	R3	R3	R3	R3	R3	R3	R3	R3	Corn	**S**	R3	R3	**S**
5	R1	S	R2	S	R1	S	R2	S	R1	S	Corn	R2	S	R1	S
6	R2	S	R3	S	R2	S	R3	S	R2	S	Corn	R3	S	R2	S
7	R3	S	R1	S	R3	S	R1	S	R3	S	Corn	R1	S	R3	S
8	R1	R3	S	R1	R3	S	R1	R3	S	R1	Corn	R3	S	R1	S
9	R2	R1	S	R2	R1	S	R2	R1	S	R2	Corn	R1	S	R2	S
10	R3	R2	S	R3	R2	S	R3	R2	S	R3	Corn	R2	S	R3	S
11	R1	R2	S	R3	R1	S	R2	R3	S	R1	Corn	R2	S	R3	S

S = Pioneer 92B13, susceptible to SCN; R1 = Latham EX547 RR N (PI 88788 resistance); R2 = Pioneer 91M90 (Peking resistance); R3 = Latham AR5084 (PI 437654 resistance)