

Research Project Report

Project Title: Characterizing and genetic mapping of virulence phenotypes in a unique collection of soybean cyst nematode inbred lines from Minnesota

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I. Proposal Summary

The soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pathogen of soybean and is widespread in Minnesota and most soybean-growing regions of the world. As a species, SCN contains significant variation in virulence (ability to reproduce on different soybean lines) and morphology. For this project we propose to study the phenotypic and genotypic diversity of SCN in Minnesota. Specifically, we phenotype 180 inbred lines of cyst nematodes randomly selected from Minnesota soybean fields for their virulence phenotypes on the SCN-resistant germplasm lines PI 88788, Peking (PI 548402), Pickett (PI 548988), PI 567516C, PI 438489B, PI 90763, and a line with a novel SCN resistance. These germplasm lines contain diverse SCN resistance genes. We are also studying variations in SCN morphology and conduct whole-genome sequencing on these inbred SCN lines. The knowledge of SCN phenotypic and genotypic diversity will be highly useful for strategically breeding soybean cultivars resistant to soybean cyst nematode with the most effective sources of resistance. This project will advance technology to manage the most destructive pest of soybean and maintain the crop's productivity in Minnesota.

II. Goals and Objectives

Goal 1: Determine diversity of SCN virulence phenotypes in Minnesota

Objective 1: Characterization of virulence phenotypes of inbred SCN lines.

In this objective, we will continue work to characterize the virulence phenotypes of 180 inbred SCN lines. Specifically, the reproduction potential as measured with the female index (FI) of the nematode lines will be determined on the SCN-resistance sources used or potentially used in public (e.g., UMN) and private soybean breeding programs. The data will be used to analyze the diversity of SCN in Minnesota.

Goal 2: Identify regions of the SCN genome controlling virulence

Objective 2: Whole genome sequencing and morphological analysis of inbred SCN lines.

To address this objective we will sequence the genomes of the 180 inbred SCN lines. We will compare this genomic data to virulence phenotype data to identify factors controlling virulence in the SCN genome. In addition, we will analyze morphological parameters and determine if they are associated with virulence phenotypes and regions of the genome. To our knowledge, nothing like this has ever been done. The unique collection of 180 SCN inbred lines at our disposal makes this possible.

III. Progress and Accomplishment

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

A total of 180 inbred lines of the soybean cyst nematodes were developed from 89 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 (8 to 24 transfers), the SCN lines are relatively homogenous genetically. The 180 inbred lines may represent diversity of SCN populations in Minnesota.

The virulence of the SCN inbred lines were/are being tested on SCN-resistant soybean lines that are used or could potentially be used for breeding SCN-resistance in the University of Minnesota breeding program as well as commercial programs. These include PI 88788, Peking (PI 548402), Pickett (PI 548988), PI 90763, PI 567516C, and PI 438489B.

Briefly, soybeans are planted in 100-mL cone-tainers in the greenhouse and inoculated with 3000 SCN eggs. Each soybean line is planted six times in six separate cone-tainers. After 35 days, the cysts are collected from each cone-tainer and counted. Female Index (FI) are calculated for each plant. $FI = \frac{\text{the number of cysts (females) on the SCN-resistant soybean line} \times 100}{\text{the number of cysts on the standard SCN-susceptible soybean line Williams 82}}$. The FI is used for evaluating the virulence of SCN on a soybean line.

So far, we have phenotyped 101 SCN lines for their virulence (Female Index) on eight soybean germplasm lines that have various genotypes of SCN resistance. In addition, the samples of the remaining 77 inbred lines on PI 88788 and Peking have been finished. The results of the soybean lines Pickett, Peking, PI 88788, PI 90763, PI 834489B, and PI 567516C are summarized in **Table 1**. Based the data of the 86 (by May 30) SCN lines, percentage of SCN lines, to which a soybean line is resistant (Female Index less than 10), was 47% on Pickett, 54% on PI 88788, 77% on Peking, 79% on PI 567516C, 86% on PI 90763, and 90% on PI 834489B (**Table 1**).

The relative low number of SCN lines with $FI < 10$ on PI 88788 indicates that PI 88788-derived soybean cultivars may have low level of resistance in many fields in Minnesota. Peking-derived cultivars are known to be good in rotation with PI 88788-derived cultivars for managing SCN. Except for Pickett, which was derived from Peking and used for race determination, all other three lines are good alternative sources of resistance for SCN management. For example, PI 567516C is resistant to 80% SCN populations, to which PI 88788 cultivars are susceptible or

moderately susceptible ($FI > 30$) (data not shown). PI 567516C has been used in UMN breeding program as a new source of SCN resistance.

Objective 2. Whole genome sequencing and morphological analysis of inbred SCN lines.

To prepare samples for sequencing, the inbred lines of SCN were cultured in sterilized soil. Cysts were extracted from soil and hand-picked to separate them from plant and soil debris. Cysts were crushed with a sterile glass crusher to release eggs and resulting eggs are relatively free from contaminating material. DNA extraction and sequencing were conducted in the University of Minnesota Genomics Center. The genomic DNA was sequenced using the Illumina® Nextera DNA Flex platform. A total of 177 SCN lines were successfully sequenced.

We are analyzing the genomes of the SCN lines and comparing them with reference SCN genome sequence to identify single nucleotide polymorphisms (SNPs). The SNPs will be used to study the SCN genetic diversity and their associations with the phenotypic traits including SCN virulence and morphometrics.

VI. Milestones

- A total of 101 SCN lines have been phenotyped for their virulence to eight soybean lines, and additional 77 SCN lines to PI 88788 and Peking.
- The genomes of 177 SCN lines were sequenced.

V. Deliverables

Abstracts and poster presentations:

1. Docherty, L., Lorenz, A., and Chen, S. 2023. Virulence diversity of soybean cyst nematode in Minnesota. Society of Nematologists Annual Meeting, July 9-14, Columbus, Ohio.

Oral presentation:

1. Docherty, L. (*presenter*), and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. North Central Nematology Research Committee Annual Report Meeting, July 14-15, Columbus, Ohio.

Table 1. Resistance of soybean to inbred lines of different SCN races.

SCN Race	# of lines	% of total lines	# lines of the race		% lines of the race		Female index (FI)				
			MR	R	MR	R	Min	Max	Median	Average	
Pickett											
1	17	19.8	0	17	0.0	100.0	0.0	5.9	0.4	1.4	
2	5	5.8	1	0	20.0	0.0	24.0	74.6	70.3	35.3	
3	24	27.9	0	24	0.0	100.0	0.0	9.8	0.2	1.7	
4	4	4.7	0	0	0.0	0.0	33.1	93.7	64.5	63.9	
5	14	16.3	7	0	46.7	0.0	10.3	66.7	30.7	34.1	
6	11	12.8	5	0	45.5	0.0	13.9	53.6	33.8	34.6	
9	4	4.7	1	0	25.0	0.0	13.2	86.8	60.6	55.3	
14	7	8.1	0	0	0.0	0.0	34.8	104.7	69.9	70.4	
Total	86	100.0	14	41	16.1	47.1	0.0	188.2	35.6	48.3	
Peking											
1	17	19.8	0	17	0	100	0.0	1.0	0.0	0.1	
2	5	5.8	4	0	80	0	10.9	36.9	11.9	19.9	
3	24	27.9	0	24	0	100	0.0	4.2	0.0	0.3	
4	4	4.7	0	0	0	0	41.1	54.7	51.5	49.7	
5	14	16.3	0	14	0	100	0.0	7.9	0.4	1.5	
6	11	12.8	0	11	0	100	0.0	8.4	0.7	1.8	
9	4	4.7	2	0	50	0	11.7	80.4	31.5	38.8	
14	7	8.1	2	0	28.6	0.0	10.7	113.5	62.6	60.9	
Total	86	100.0	8	66	9.3	78	0.0	188.2	35.6	48.3	
PI 88788											
1	17	19.8	9	0	52.9	0.0	10.2	105.8	27.4	39.3	
2	5	5.8	2	0	40.0	0.0	11.1	100.7	35.0	48.5	
3	24	27.9	0	24	0.0	100.0	0.0	8.6	1.3	2.2	
4	4	4.7	4	0	100.0	0.0	13.6	21.0	15.1	16.2	
5	14	16.3	4	0	28.6	0.0	10.5	98.2	36.3	48.7	
6	11	12.8	0	11	0.0	100.0	0.0	9.0	1.6	3.0	
9	4	4.7	0	4	0.0	100.0	1.1	4.5	3.2	3.0	
14	7	8.1	0	7	0.0	100.0	0.2	7.8	1.2	2.6	
Total	86	100.0	19	46	22.1	53.5	0.0	188.2	35.6	48.3	
PI 90763											
1	17	19.8	0	17	0	100	0.0	1.5	0.0	0.1	
2	5	5.8	0	5	0	100	0.0	6.7	0.1	1.4	
3	24	27.9	0	24	0	100	0.0	0.8	0.0	0.0	
4	4	4.7	0	0	0	0	35.3	58.6	45.5	46.2	

5	14	16.3	0	14	0	100	0.0	1.2	0.0	0.1
6	11	12.8	0	11	0	100	0.0	1.1	0.0	0.1
9	4	4.7	0	4	0	100	4.3	9.4	6.5	6.7
14	7	8.1	4	0	57.1	0	12.0	71.7	20.6	35.1
Total	86	100.0	4	74	4.7	86.0	0.0	188.2	35.6	48.3

PI 438489B

1	17	19.8	0	17	0	100	0.0	1.1	0.0	0.2
2	5	5.8	1	4	20	80	0.0	17.3	0.2	4.4
3	24	27.9	0	24	0	100	0.0	4.0	0.0	0.3
4	4	4.7	2	0	50	0	12.6	37.0	27.2	26.0
5	14	16.3	0	14	0	100	0.0	0.4	0.0	0.1
6	11	12.8	0	11	0	100	0.0	2.4	0.0	0.3
9	4	4.7	0	4	0	100	0.2	4.5	2.1	2.2
14	7	8.1	2	3	28.6	42.9	1.3	103.2	12.6	27.2
Total	86	100.0	5	77	5.8	89.5	0.0	188.2	35.6	48.3

PI567516C

1	17	19.8	0	15	0	88.2	0.0	48.2	0.5	6.3
2	5	5.8	1	3	20	60.0	0.0	34.7	1.6	13.0
3	24	27.9	2	21	8.3	87.5	0.0	47.5	0.0	3.7
4	4	4.7	2	1	50	25.0	7.0	37.0	17.0	19.5
5	14	16.3	0	12	0	85.7	0.0	66.0	0.2	9.3
6	11	12.8	0	11	0	100.0	0.0	2.0	0.0	0.3
9	4	4.7	1	1	25	25.0	0.4	117.9	45.6	52.4
14	7	8.1	0	4	0	57.1	0.1	91.8	3.1	32.2
Total	86	100.0	6	68	7.0	79.1	0.0	188.2	35.6	48.3
