

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. 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 study diversity of the SCN and detect WSCN in Minnesota. Specifically, we phenotyped 182 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 study variations in SCN morphology and determine if WSCN occurs in Minnesota or not. The knowledge of SCN diversity and WSCN occurrence is highly useful for strategically breeding soybean cultivars resistant to the cyst nematode(s) with the most effective sources of resistance. In addition, we 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 182 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 180 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. Accomplishment

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

A total of 182 inbred lines of the soybean cyst nematodes were used in this study. Most of the lines were developed from SCN field populations that were ‘randomly’ collected across Minnesota soybean growing counties in 2013 and 2007-2008. 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 was 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 182 inbred lines may represent diversity of SCN populations in Minnesota.

We have finished tests of 182 SCN lines for their virulence on the six soybean lines (**Table 1**). Percentage of SCN lines, to which a soybean line is resistant ($FI < 10$), was 46.7% on Pickett, 50.5% on PI 88788, 76.9% on Peking, 77.5% on PI 567516C, 87.4% on PI 90763, and 87.4% on PI 834489B. A total of 8 races were found with 25.3% race 1, 4.9% race 2, 21.4% race 2, 2.2% race 4, 15.9% race 5, 14.3% race 6, 4.9% race 9, and 11.0% race 14 (**Table 2**).

The relative low number (50.5%) 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 SCN¹. Except for Pickett, which was derived from Peking and used for race determination, all other five lines are good alternative sources of resistance for breeding commercial SCN-resistant cultivars. PI 567516C is resistant to 80.0% of the SCN populations, to which PI 88788 is susceptible or moderately susceptible to ($FI > 30$) (data not shown). Therefore, PI 567516C is a good source of resistance alternative to Peking for rotation with PI 88788-derived cultivars. PI 567516C has been used in UMN breeding program as a new source of SCN resistance.

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

To detect white soybean cyst nematode (*Heterodera sojae*), DNA sequencing and morphological characterization will be carried out. Whole genomes of a total of 178 SCN lines have been sequenced and we are analyzing genome data to construct phylogenetic tree of the SCN inbred lines (see 2023-2024 project report). The lines that don’t fit in SCN based on their genome sequences will be further studied for their morphological traits and DNA sequences with specific primers (e.g., D2A, D3B, TW8, and AB28). Measurements of body size of SCN second-stage juveniles, and adult females and males of all SCN lines were taken. These measurements are being used for analyzing morphological diversity, and their association with genome.

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 that 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 3**). Nematode population densities were 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 September of 2020 and 2022 were determined on the sources of resistance PI 88788, Peking, and PI 437654 with Lee 74 or Williams 82 (2017 only) as susceptible soybean control (**Table 3**). Predicted female index (FI) of the soybean cyst nematode (SCN) on PI 88788 and Peking following different rotations of sources of resistance are illustrated in **Figure 1**. Based on the data, PI 88788 and PI 437654-derived cultivars increased FI on PI 88788. Peking and PI 437654-derived cultivars increased FI on Peking while planting the susceptible cultivar appeared to reduce FI on Peking in this field with original population of race 1.

VI. Milestones

- A total of 182 SCN lines were phenotyped for their virulence to eight soybean lines.
- The genomes of the SCN lines were sequenced, and the data are being analyzed.
- In Objective 3, all field samples taken have been processed for SCN soil egg population densities, and HG Type tests have been finished. A draft of manuscript have been written.

V. Deliverables

Abstracts and poster presentations:

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.

Docherty, L., Lorenz, A., and Chen, S. 2022. Long term storage of *Heterodera glycines* cysts. 2022 Soybean Nematode Conference.

Poster presentations:

Docherty, L., Lorenz, A., and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. Minnesota Ag Expo. January 19, 2023.

Docherty, L., Lorenz, A., and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. University of Minnesota College of Food, Agricultural, and Natural Resource Sciences Symposium. March 14, 2023.

Oral presentations:

Docherty, L. Diversity among inbred lines of soybean cyst nematode collected in Minnesota. October 24, 2022, University of Minnesota Department of Agronomy and Plant Genetics Seminar.

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, 2023, Columbus, Ohio.

Table 1. Female Index (FI) of the soybean cyst nematode (SCN) inbred lines on the SCN race differential lines and breeding soybean lines.

	Soybean lines					
	Pickett	Peking	PI 88788	PI 90763	PI 438489B	PI 567516C
Total populations	182	182	182	182	182	182
Number of populations with FI < 10	85	140	92	159	159	141
Number of populations with FI < 30	123	159	137	171	176	158
% of populations with FI < 10	46.7	76.9	50.5	87.4	87.4	77.5
% of population with FI < 30	67.6	87.4	75.3	94.0	96.7	86.8
Minimum FI	0.0	0.0	0.0	0.0	0.0	0.0
Maximum FI	107.8	128.1	105.8	71.7	108.7	117.9
Average FI	23.4	9.5	18.6	4.8	4.4	9.9
Median FI	10.6	0.2	9.2	0.0	0.1	0.5

Table 2. Resistance of the soybean cyst nematode race differential and breeding soybean lines to inbred SCN lines of different races. R is resistant to SCN at FI < 10, and MR is moderately resistant to SCN at 10 < FI < 30.

Race	Number of lines	Percent of total lines	Number lines of the race		Percentage lines of the race	
			MR	R	MR	R
Pickett						
1	46	25.3	0	46	0	100
2	9	4.9	2	0	22.2	0
3	39	21.4	0	39	0	100
4	4	2.2	0	0	0	0
5	29	15.9	16	0	55.2	0
6	26	14.3	15	0	57.7	0
9	9	4.9	2	0	22.2	0
14	20	11.0	3	0	15	0
Total	182	100	38	85	20.9	46.7
Peking						
1	46	25.3	0	46	0	100
2	9	4.9	7	0	77.8	0
3	39	21.4	0	39	0	100
4	4	2.2	0	0	0	0
5	29	15.9	0	29	0	100
6	26	14.3	0	26	0	100
9	9	4.9	4	0	44.4	0

14	20	11.0	7	1	35	5
Total	182	100	18	142	9.9	78.0

PI 88788						
1	46	25.3	24	0	52.2	0
2	9	4.9	4	0	44.4	0
3	39	21.4	0	39	0	100
4	4	2.2	4	0	100	0
5	29	15.9	12	0	41.4	0
6	26	14.3	0	26	0	100
9	9	4.9	0	9	0	100
14	20	11.0	1	16	5	80
Total	182	100	46	93	25.3	51.1

PI 90763						
1	46	25.3	0	46	0	100
2	9	4.9	0	9	0	100
3	39	21.4	0	38	0	100
4	4	2.2	0	0	0	0
5	29	15.9	0	29	0	100
6	26	14.3	0	26	0	100
9	9	4.9	0	9	0	100
14	20	11.0	13	0	65	0
Total	182	100	15	158	8.2	86.8

PI 438489B						
1	46	25.3	2	44	4.3	95.7
2	9	4.9	1	7	11.1	77.8
3	39	21.4	2	37	5.1	94.9
4	4	2.2	2	0	50.0	0.0
5	29	15.9	0	29	0.0	100.0
6	26	14.3	0	26	0.0	100.0
9	9	4.9	3	6	33.3	66.7
14	20	11.0	7	9	35.0	45.0
Total	182	100	19	159	10.4	87.4

PI 567516C						
1	46	25.3	2	37	4.3	80.4
2	9	4.9	3	4	33.3	44.4
3	39	21.4	4	32	10.3	82.1
4	4	2.2	1	2	25.0	50.0

5	29	15.9	2	25	6.9	86.2
6	26	14.3	2	24	7.7	92.3
9	9	4.9	1	3	11.1	33.3
14	20	11.0	1	13	5.0	65.0
Total	182	100	16	142	8.8	78.0

Table 3. Female Index on HG-Type indicator soybean lines Peking and PI 88788 of the soybean cyst nematode populations from plots treated with different cultivar sequences.

Cultivar sequence† (2008 - 2022)		2008	2011	2013		2017		2020		2022		
Female Index on PI 88788												
1	S-S-S-S-S-S-S-S-S-C-S-S-S-S	29.1	37.1	ab	17.1	ab	15.8	ab	39.2	bc	29.8	bc
2	R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-C-R1-R1-R1	26.4	69.0	a	22.6	ab	28.1	a	79.6	a	64.5	a
3	R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-C-R2-R2-R2	17.5	54.4	ab	15.5	ab	8.1	b	26.7	c	17.6	c
4	R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-C-R3-R3-R3-S	33.2	51.8	ab	22.1	ab	17.5	ab	67.6	ab	46.4	ab
5	R1-S-R2-S-R1-S-R2-S-R1-S-C-R2-S-R1-S	18.2	25.2	b	31.1	ab	11.0	ab	49.5	abc	31.6	bc
6	R2-S-R3-S-R2-S-R3-S-R2-S-C-R3-S-R2-S	27.0	46.5	ab	12.9	b	12.5	ab	59.0	abc	43.9	ab
7	R3-S-R1-S-R3-S-R1-S-R3-S-C-R1-S-R3-S	23.5	51.5	ab	20.3	ab	11.1	ab	53.4	abc	32.7	bc
8	R1-R3-S-R1-R3-S-R1-R3-S-R1-C-R3-S-R1-S	28.6	44.0	ab	30.8	ab	9.0	b	46.4	abc	46.8	ab
9	R2-R1-S-R2-R1-S-R2-R1-S-R2-C-R1-S-R2-S	31.2	41.5	ab	32.5	ab	9.1	b	47.7	abc	40.5	abc
10	R3-R2-S-R3-R2-S-R3-R2-S-R3-C-R2-S-R3-S	26.2	39.7	ab	18.8	ab	16.8	ab	45.8	abc	36.8	bc
11	R1-R2-S-R3-R1-S-R2-R3-S-R1-C-R2-S-R3-S	18.7	44.8	ab	35.2	a	15.3	ab	55.8	abc	35.9	bc
Average Female Index		25.4	46.0		23.5		14.0		51.9		38.8	
ANOVA:												
F-value		1.3	2.02	P=0.06	3.35	**	3.14	**	3.68	**	5.49	***
Transformation		<i>x</i>	<i>ln x</i>		<i>x</i>		<i>x0.1</i>		<i>x</i>		<i>x</i>	
Female Index on Peking												
1	S-S-S-S-S-S-S-S-S-C-S-S-S-S		1.3	bc	1.1	c	1.9	bc d	3.7	def	2.3	c
2	R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-C-R1-R1-R1		5.8	bc	3	bc	2.7	bc d	8.7	cdef	5.5	bc
3	R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-C-R2-R2-R2		57.7	a	37.6	a	36.7	a	60.8	a	46.5	a
4	R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-C-R3-R3-R3-S		4.3	bc	5.6	bc	9.4	bc	37.1	ab	18.2	ab
5	R1-S-R2-S-R1-S-R2-S-R1-S-C-R2-S-R1-S		8.8	b	5.5	bc	2.7	bc d	13.7	cdef	7.6	bc
6	R2-S-R3-S-R2-S-R3-S-R2-S-C-R3-S-R2-S		7.6	bc	4.7	bc	11.2	ab	13.4	cdef	9.2	bc
7	R3-S-R1-S-R3-S-R1-S-R3-S-C-R1-S-R3-S		1.7	c	2.1	bc	1.3	cd	3.6	f	2.1	c
8	R1-R3-S-R1-R3-S-R1-R3-S-R1-C-R3-S-R1-S		3.4	bc	4.7	bc	1.5	d	5	ef	5	c

9	R2-R1-S-R2-R1-S-R2-R1-S-R2-C-R1-S-R2-S	6.2	bc	8.7	ab	3.7	bc d	12.9	bcd e	17.2	ab
10	R3-R2-S-R3-R2-S-R3-R2-S-R3-C-R2-S-R3-S	5.1	bc	4.4	bc	5.5	bc d	17	bcd	7.6	bc
11	R1-R2-S-R3-R1-S-R2-R3-S-R1-C-R2-S-R3-S	4.2	bc	5.4	bc	4.4	bc d	15.6	bc	8.6	bc
Average Female Index		9.6		7.5		7.4		17.4		11.8	
ANOVA:											
<i>F-value</i>		11.73	*****	6.94	**** *	8.54	** **	13.32	**** *	10.8 0	*** *
Transformation		$\ln x$		$\ln x$		$x0.1$		$\ln x$		$\ln x$	
Female Index on PI 437654											
1	S-S-S-S-S-S-S-S-C-S-S-S-S	0		0.04		0.94		0.03		0.09	
2	R1-R1-R1-R1-R1-R1-R1-R1-R1-R1-C-R1-R1-R1	0		0		0.27		0.05		0.07	
3	R2-R2-R2-R2-R2-R2-R2-R2-R2-R2-C-R2-R2-R2	0		0		0.08		0.05		0.20	
4	R3-R3-R3-R3-R3-R3-R3-R3-R3-R3-C-R3-R3-R3	0		0		0.20		0.08		0.04	
5	R1-S-R2-S-R1-S-R2-S-R1-S-C-R2-S-R1-S	0		0		0.21		0		0.07	
6	R2-S-R3-S-R2-S-R3-S-R2-S-C-R3-S-R2-S	0		0		0.20		0		0.14	
7	R3-S-R1-S-R3-S-R1-S-R3-S-C-R1-S-R3-S	0		0		0.37		0		0.03	
8	R1-R3-S-R1-R3-S-R1-R3-S-R1-C-R3-S-R1-S	0.32		0.08		0.26		0.03		0.05	
9	R2-R1-S-R2-R1-S-R2-R1-S-R2-C-R1-S-R2-S	0		0		0.22		0.05		0.03	
10	R3-R2-S-R3-R2-S-R3-R2-S-R3-C-R2-S-R3-S	0		0		0.62		0.03		0.17	
11	R1-R2-S-R3-R1-S-R2-R3-S-R1-C-R2-S-R3-S	0		0.22		0.29		0.05		0.27	
Average Female on Lee 74		96		188		422		263		312	

† S = SCN-susceptible ‘Pioneer 92B13’; S1 = SCN-susceptible ‘Pioneer 92B38’; R1 = SCN-resistant ‘Latham EX547 RR N’ with PI 88788 source of resistance; R2 = SCN-resistant ‘91M90’ with Peking source of resistance; R3 = SCN-resistant ‘AR5084’ with PI347654 source of resistance. All plots were planted to corn in 2018.

‡ The values followed by the same letter(s) in the same column are not different according to least significant difference test at $P > 0.05$.

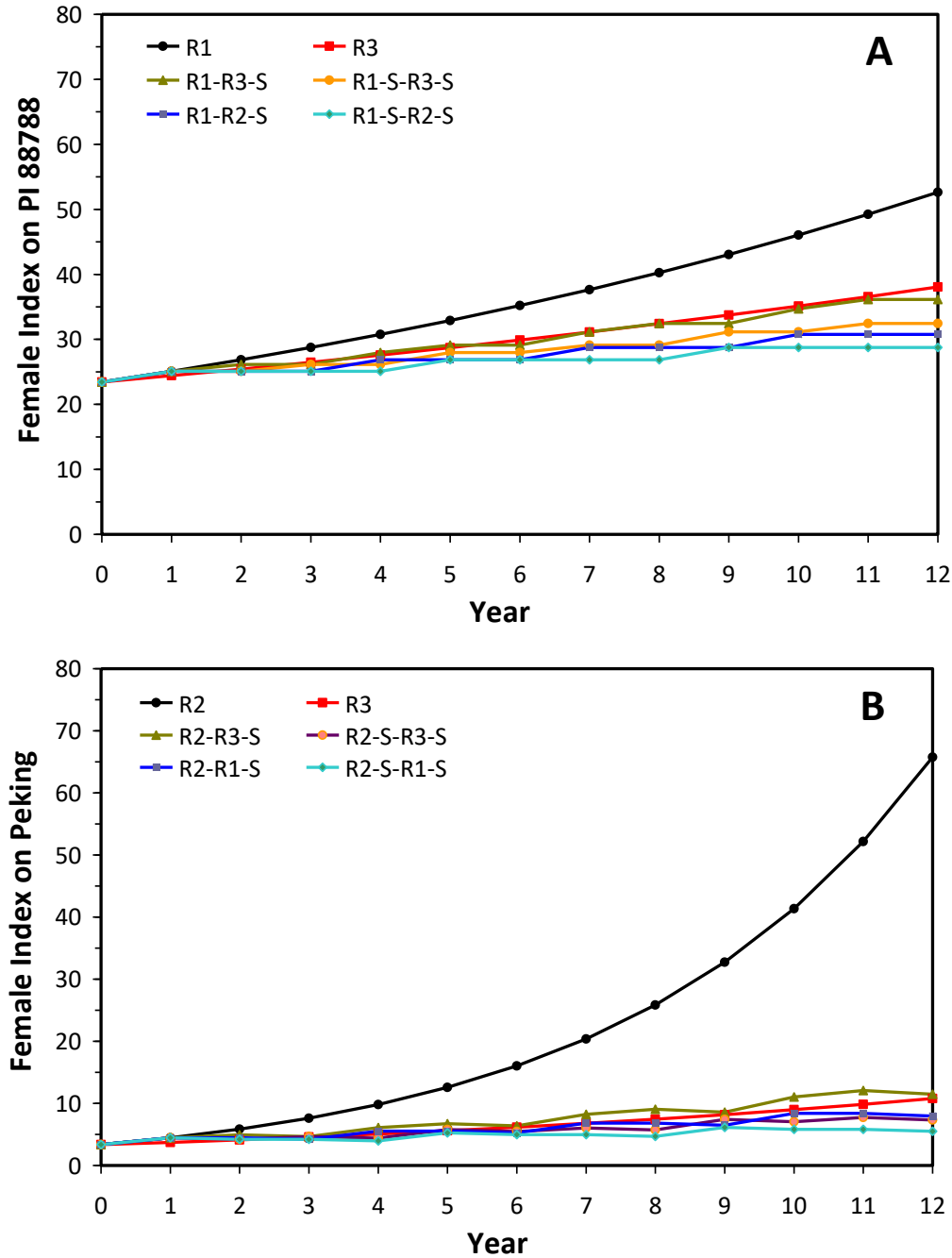


Figure 1. Predicted female index (FI) of the soybean cyst nematode (SCN) in different rotations of sources of resistance. **A)** The model for FI on cultivar PI 88788 after a cultivar sequence is $FI = 34.44e^{(0.06549R1 + 0.03911R3)} - 1$ ($r^2 = 0.08$, $p < 0.001$). **B)** The model for FI on cultivar Peking is $FI = 3.35e^{(0.22758R2 + 0.08305R3 - 0.04689S)} - 1$ ($r^2 = 0.4541$, $p < 0.0001$). S is SCN-susceptible ‘Pioneer 92B13’; R1 is SCN-resistant ‘Latham EX547 RR N’ derived from PI 88788; R2 is SCN-resistant ‘Pioneer 91M90’ derived from Peking; R3 is SCN-resistant ‘Latham AR5084’ derived from PI 437654. In the models, R1, R2, R3, and S are number of years of the soybean cultivars in a crop sequence.