



**Full Length Article**

## **Distribution and Virulence Phenotypes of Soybean Cyst Nematode (*Heterodera glycines*) based upon Host Differentials in Jilin Province**

Xiujuan Yan<sup>1,2†</sup>, Jinwen Liu<sup>2†</sup>, Mingshu Li<sup>2</sup>, Qiang Qiu<sup>2</sup>, Xiaofeng Zhu<sup>1</sup> and Yuxi Duan<sup>1\*</sup>

<sup>1</sup>Nematology Institute of Northern China, Shenyang Agricultural University, Shenyang 110161, China

<sup>2</sup>Soybean Research Institute, Jilin Academy of Agricultural Sciences Changchun 130124, China

\*For correspondence: duanyx6407@163.com

†Contributed equally to this work and are co-first authors

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### **Abstract**

Jilin is the dominant soybean production province in China. Soybean cyst nematode [*Heterodera glycines*] (SCN) is one of the most important yield-limiting factors in soybean production. Information about the distribution and virulence phenotypes of SCN in soybean fields are essential for optimizing varieties choice in the region. The distribution and virulence phenotypes of SCN in 141 soil samples from 38 cities (cities, counties and towns) across Jilin province were investigated. One hundred and four (73.76%) of the samples from all 38 cities (counties, towns) tested positive for SCN and SCN population densities were more than 5 cysts/100 mL soil in 53 samples from 27 cities (counties, towns). In those 53 samples, we identified 7 races and 12 *Heterodera glycines* (HG) types, with Race 3 and HG Type 7 being the most dominant genotypes. Of all the genotypes identified, Race10 and HG Type 1.7 were found for the first time in China, and HG Type 3.4.5.7 was the first reported worldwide. Thirty-eight SCN populations (34.5%) were virulent on plant introduction (PI) 548316 (#7) and 15 of them had female indices (FI)  $\geq 10\%$  on Pickett. PI 548316 and Pickett were not recommended as parents of breeding against cyst nematode in Jilin province. Peking-type resistance sources were preferred to pi88788-type in Jilin province. In addition, it was found neither the race nor HG scheme is sufficient for differentiating SCN populations in Jilin province, nor the combination of the two methods is recommended for studying the genetic diversity of SCN in Jilin province. That is, Pickett which was removed in HG scheme should be included not as an indicator line but just to separate different races from the same HG type. © 2021 Friends Science Publishers

**Keywords:** Parasite; Virulence phenotypes; Cyst isolation

### **Introduction**

Soybean cyst nematode (SCN) is an obligate parasite, and its host specialization is obvious. The diversity of SCN is evaluated either by the race determination scheme (Golden *et al.* 1970; Riggs and Schmitt 1988) or HG Type classification scheme (Niblack *et al.* 2002).

The Race determination scheme was developed in 1970 (Golden *et al.* 1970); where four differential soybean lines including Pickett, Peking, PI 88788 and PI 90763 and the susceptible cultivar Lee 68 was used to characterize the heterogeneity of *H. glycines*. Based on this scheme, 16 potential races were proposed (Riggs and Schmitt 1988). Currently, it had become international scheme for classifying *H. glycines* virulence. Of the 16 races, race 11 and race 13 have not been found yet, and race 16 was reported only once. There are 13 races in the United States, including race 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 15 (Kim *et al.* 1997; Xu *et al.* 2010). Nine races 1, 2, 3, 4, 5, 6, 7, 9 and 14 had been found in China with 1, 3 and 4 being

the most widely distributed (Song *et al.* 2016). In 2017, a new SCN population (called race X12) was detected in Shanxi province, China (Lian *et al.* 2017). With more and more new SCN populations identified and reported, the race scheme became not sufficient to classify the virulence profile in SCN. Therefore, a new scheme for the virulence phenotype-HG Type classification was proposed (Niblack *et al.* 2002). HG Type testing enables more accurate management recommendations than the Race scheme does (Winter *et al.* 2006). Since the scheme was formally proposed at the national soybean cyst nematode conference in 2008, it has been widely accepted and used. Virulence phenotypes of *H. glycines* had been surveyed with HG Type in some U.S. states (Niblack *et al.* 2003; Zheng *et al.* 2006; Mitchum *et al.* 2007; Colgrove and Niblack 2008; Rzedkiewicz 2010; Acharya *et al.* 2016; Howland *et al.* 2018) and also in Korea (Kim *et al.* 2013). In China, the race scheme has always been used, and the application of HG scheme is relatively less (Wang *et al.* 2014; Chen *et al.* 2015; Cui *et al.* 2018).

In China, most soybeans are produced in Jilin province and the SCN disease spreads widely in this area. The lack of distribution and phenotypic diversity surveys hinder the efficient management of SCN. The only study on the distribution of SCN in Jilin province was reported in 1988 (Liu and Wu 1988); In the past 30 years, there was no report about SCN distribution and genetic diversity of SCN population in Jilin Province, which seriously restricted the progress of soybean resistance breeding against cyst nematode. There are only a few resistant varieties bred from limited resistant sources available on the market, e.g., Bainong 8, Bainong 9 and Bainong 10 derived from Peking. Once SCN disease breaks out, only a few resistant varieties would not be enough.

The objective of this study is to determine the distribution, density, as well as the diversity of virulence phenotypes of *H. glycines* in Jilin province, thus to provide theoretical foundation for introducing and breeding suitable resistant varieties in the region.

## Materials and Methods

### Soil sample collection

Soil samples were collected from soybean fields in Jilin province. A total of 141 fields from 38 cities (counties, towns) were sampled after harvest (Table 1) via multi-point random sampling within the circumference of the soybean roots. The soil samples were divided into two parts, one for counting to determine the density of the cysts, the other were planted with the SCN-susceptible soybean Jiyu 86 to increase population densities for studying virulence phenotypes of SCN.

### SCN populations

Soil samples with population of more than 5 cysts/100 mL soil were further tested for distribution of virulence phenotypes by race scheme and HG Type scheme.

### Soybean differential lines

The four indicator lines Pickett, PI 548402(Peking), PI 88788 and PI 90763 and susceptible cultivar Lee 74 were from Soybean Research Institute, Jilin Academy of Agricultural Sciences. The other indicator lines PI 548402, PI 437654, PI 209332, PI 89772 and PI 548316, were from Nematology Institute of Northern China, Shenyang Agricultural University.

### Cyst isolation

The isolation of cyst was carried out as described previously (Liu 1995). In brief, 100 mL soil sample was mixed in 300 mL water in a 1000 mL measuring cup. Soil suspensions were mixed well by stirring. The floating particles and suspensions were filtered through 180- $\mu$ m-pore and 450-

$\mu$ m-pore sieves, 3 times. The residue on a 180- $\mu$ m-pore sieve was rinsed with a fine water flow into a beaker, filtered with a screen cloth, and dried. Each soil sample was prepared 3 times.

### Preparation of egg suspension

Under a stereomicroscope, full cysts were placed in a Petri dish and crushed, releasing eggs and juveniles (J2). The eggs and juveniles (J2) were transferred to a breaker and diluted to a final density of  $1 \times 10^6 \cdot L^{-1}$ .

### Inoculation

Plastic cups (6.4-cm diameter, 17-cm high) with bottom punched were filled with sterilized soil and sand (1: 3). For germination, surface-sterilized seeds of each soybean line were placed on filter paper under dark conditions for 48 h. One 2–3 cm long soybean seedling was then sown into each pre-irrigated plastic cup. After 3 days, a 2-mL egg suspension was injected into a 3-cm hole around the root of the seedling. There were 5 replicate cups for each line. The cups were placed in large plastic boxes according to sample origin and these boxes were placed in a greenhouse at 27–29°C. Plants were watered daily.

### Microscopic examination

After 30–35 days, the tops of the seedlings were removed. The soil and roots in the plastic cups were poured into a pot and then sprayed with a strong stream of water to dislodge the cysts. The cysts were isolated as described above and counted using a stereomicroscope.

### Identification

Virulence differentiation of SCN population was based on the difference in reproductive ability on indicator lines. Based on the average number of cysts formed on the indicator lines, Female Indices (FI) was calculated,  $FI \geq 10$  meant '+',  $FI < 10$  meant '-'. Virulence phenotypes were identified by the race scheme and HG Type scheme. The formula of the female index (FI):

$$FI = \frac{\text{Number of cysts and females per plant}}{\text{Average number of cysts and females on Lee74}} \times 100$$

### Statistical analysis

The correlation analysis among indicator lines was performed using IBM S.P.S.S. Statistics 21.

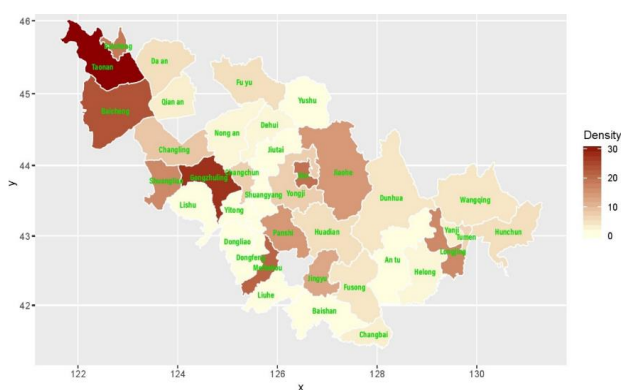
## Results

### Distribution and Density of SCN in Jilin province

Out of the 141 samples, 104 samples from all 38 cities (counties, town) were positive for SCN (73.76%) (Fig. 1).

**Table 1:** Densities of *Heterodera glycines* in Jilin province

Sampling point city (County)		Number of samples	Number of samples with SCN	Effective sample number ( $\geq 5$ cyst/100 mL soil)	Cyst density (cysts/100 mL soil)
City	County(town)				
Baishan	Baishan	5	2	0	0.4
	Jingyu	2	2	2	15
	Fusong	4	3	0	1.75
	Changbai Korean Autonomous County	4	3	1	2
Tonghua	Meihekou	3	2	2	12.67
	Liuhe	3	1	0	1
Liaoyuan	Dongliao	3	1	1	1.67
	Dongfeng	3	1	0	0.67
Baicheng	Baicheng	3	3	3	14.67
	Taonan	4	4	4	36.25
	Tongyu	3	3	3	18.33
	Daan	4	3	1	2.5
Siping	Gongzhuling	5	5	4	19.8
	Shuangliao	3	3	3	11.33
	Lishu	4	3	2	3.75
	Yitong	4	2	0	1.5
Songyuan	Fuyu	4	3	1	2.5
	Changling	4	3	1	3.25
	Qian'an	4	3	1	2
Jilin	Jilin	2	2	2	15.5
	Jiaohe	2	2	2	11.5
	Huadian	4	2	0	1.25
	Yongji	4	4	2	4
	Panshi	3	3	3	12.67
	Dunhua	6	5	2	3.33
Yanbian Korean Autonomous Prefecture	Wangqing	4	3	0	1.75
	Antu	4	3	2	3.75
	Helong	4	3	1	2.25
	Yanji	3	3	0	2
	Tumen	4	4	2	3.5
	Hunchun	5	3	0	1.2
	Longjing	3	3	3	13.67
	Yushu	4	2	1	2.25
	Changchun	4	4	2	4.75
	Dehui	4	3	0	1.75
Changchun	Nongan	4	3	1	2.75
	Jiutai	4	1	0	0.25
	Shuangyang	4	1	1	1.25
	Total/Average	141	104	53	6.33

**Fig. 1:** Distribution of soybean cyst nematode (*Heterodera glycines*) in Jilin province

100% of soil samples from Gongzhuling, Baishan, Yanji, Taonan, Tongyu and other 9 counties (cities) were positive for SCN.

The average cyst density of all soil samples was 6.33 cyst/100 mL. There were more than 10 cysts/100 mL soil in 24 soil samples from 11 cities (counties, towns), especially in samples from the cities of Taonan, Baicheng, Gongzhuling and Jilin, as well as Tongyu county. The cyst density in Taonan county was 28 cysts/100 mL soil. There were more than 5 cysts/100 mL soils in 53 soil samples from 27 cities (counties, towns).

### Race identification

Virulence phenotypes of 53 populations with more than 5 cysts /100 mL soil were tested by race scheme. Seven races were identified, *i.e.*, 1, 3, 5, 6, 9, 10 and 14 (Table 2). Race 3 was the most widely distributed, accounting for 62.26%, followed by race 1, accounting for 11.32%. Race 5 and 6 accounted for 9.43% respectively. The SCN in one soil sample was race 10, and one was race 14; of these, race 10 was first found in China.

**Table 2:** Virulence phenotypes of *Heterodera glycines* in Jinlin province

No.	City	County(town)	Pickett	Peking(1)	PI88788(2)	PI90763(3)	PI437654(4)	PI209332(5)	PI89772(6)	PI548316(7)	Race	HG Type	
West	1	Songyuan	Fuyu	3.31	- 2.71	- 1.51	- 0.60	- 5.72	- 7.83	- 0.90	- 6.63	- 3	0
			Changling	3.55	- 1.66	- 7.58	- 4.98	- 1.90	- 7.82	- 2.61	- 19.67	+ 3	7
	Baicheng	Baicheng	Qian'an	7.47	- 2.67	- 6.93	- 3.47	- 0.80	- 7.73	- 4.00	- 25.87	+ 3	7
			Taonan	41.80	+ 3.28	- 2.62	- 25.25	+ 13.93	+ 11.48	+ 3.93	- 29.51	+ 10	3,4,5,7
			Taonan	18.22	+ 4.12	- 2.82	- 2.17	- 0.43	- 16.70	+ 4.34	- 37.96	+ 6	5,7
			Taonan	1.33	- 2.92	- 2.92	- 4.77	- 4.51	- 0.80	- 2.12	- 7.16	- 3	0
			Taonan	8.41	- 1.68	- 6.25	- 5.29	- 2.16	- 2.16	- 4.33	- 8.65	- 3	7
			Tongyu	3.77	- 2.08	- 0.19	- 2.26	- 5.66	- 4.91	- 5.09	- 23.58	+ 3	7
			Tongyu	0.98	- 3.58	- 5.21	- 4.56	- 4.56	- 2.28	- 5.86	- 6.51	- 3	0
			Tongyu	8.20	- 3.61	- 7.87	- 4.92	- 1.97	- 5.25	- 2.62	- 6.56	- 3	0
			Baicheng	4.61	- 1.05	- 1.05	- 1.26	- 1.05	- 3.14	- 0.84	- 1.68	- 3	5,7
			Baicheng	11.78	+ 11.36	+ 3.93	- 7.02	- 1.45	- 15.50	+ 2.48	- 30.37	+ 9	1,5,7
			Baicheng	4.86	- 2.13	- 3.04	- 2.74	- 3.95	- 9.12	- 2.43	- 27.66	+ 3	7
			Daan	14.60	+ 2.54	- 12.38	+ 1.59	- 0.63	- 13.33	+ 4.44	- 20.95	+ 5	2,5,7
East	15	Yanbian	Longjing	13.24	+ 11.35	+ 1.89	- 2.16	- 1.08	- 1.62	- 0.00	- 13.24	+ 9	1,7
			Longjing	3.27	- 0.30	- 5.36	- 2.38	- 4.76	- 3.27	- 2.68	- 23.21	+ 3	7
	17	Autonomous Prefecture	Longjing	2.32	- 0.70	- 3.25	- 2.55	- 2.32	- 2.32	- 2.55	- 3.02	- 3	0
			Duhua	8.82	- 1.26	- 1.76	- 8.82	- 4.03	- 2.02	- 8.06	- 8.56	- 3	0
	20	Helong	Duhua	7.21	- 4.08	- 0.31	- 0.94	- 0.00	- 3.45	- 7.21	- 15.05	+ 3	7
			Helong	12.70	+ 2.78	- 2.78	- 7.14	- 7.54	- 7.94	- 2.38	- 9.13	- 6	0
			Antu	11.18	+ 13.21	+ 4.47	- 12.60	+ 3.25	- 7.72	- 6.71	- 34.96	+ 14	1,3,7
			Antu	7.59	- 1.31	- 11.26	+ 2.36	- 4.97	- 2.36	- 0.79	- 32.46	+ 1	2,7
			Tumen	12.31	+ 2.24	- 19.78	+ 5.60	- 2.61	- 28.36	+ 4.10	- 16.42	+ 5	2,5,7
			Tumen	0.56	- 2.52	- 3.36	- 2.80	- 4.48	- 5.04	- 4.48	- 15.41	+ 3	7
			Jilin	36.04	+ 1.90	- 2.17	- 2.44	- 1.36	- 34.42	+ 2.17	- 27.37	+ 6	5,7
			Jilin	15.58	+ 3.90	- 13.96	+ 1.62	- 11.36	+ 6.17	- 1.30	- 20.45	+ 5	2,4,7
			Jiaohe	3.27	- 1.76	- 1.01	- 2.26	- 0.75	- 1.01	- 2.76	- 31.91	+ 3	7
			Jiaohe	4.82	- 1.29	- 11.58	+ 2.25	- 6.11	- 3.54	- 9.32	- 15.43	+ 1	2,7
Mid-region	29	Yongji	Yongji	7.51	- 3.18	- 2.31	- 4.34	- 2.60	- 8.96	- 2.02	- 6.07	- 3	0
			Yongji	5.65	- 4.32	- 5.98	- 4.32	- 0.00	- 3.65	- 7.64	- 8.31	- 3	0
	31	Panshi	Panshi	1.61	- 0.64	- 16.40	+ 1.29	- 0.64	- 4.82	- 2.89	- 19.61	+ 1	2
			Panshi	6.21	- 3.92	- 0.33	- 3.59	- 3.27	- 1.96	- 3.92	- 26.80	+ 3	7
	33	Panshi	Panshi	3.79	- 2.84	- 4.73	- 4.42	- 3.15	- 5.99	- 4.73	- 38.49	+ 3	7
			Gongzhuling	7.24	- 4.91	- 7.49	- 7.75	- 7.49	- 5.94	- 1.55	- 14.21	+ 3	7
	35	Siping	Gongzhuling	7.07	- 10.61	+ 5.14	- 5.47	- 7.07	- 9.32	- 7.72	- 28.94	+ 3	7
			Gongzhuling	3.97	- 3.44	- 4.76	- 3.44	- 4.23	- 6.88	- 7.94	- 9.79	- 3	0
	37	Gongzhuling	Gongzhuling	7.31	- 6.15	- 15.77	+ 3.46	- 5.77	- 6.92	- 4.23	- 25.00	+ 1	2,7
			Lishu	6.68	- 1.73	- 3.22	- 3.96	- 2.48	- 14.11	+ 3.22	- 21.04	+ 3	5,7
	39	Lishu	Lishu	2.25	- 2.46	- 2.66	- 3.89	- 2.25	- 0.41	- 3.69	- 30.12	+ 3	7
			Shuangliao	0.00	- 0.00	- 3.59	- 5.25	- 1.66	- 2.49	- 5.25	- 22.38	+ 3	7
	41	Shuangliao	Shuangliao	0.27	- 0.00	- 2.73	- 6.01	- 1.37	- 3.28	- 2.46	- 3.55	- 3	0
			Shuangliao	2.30	- 0.29	- 4.02	- 2.30	- 3.45	- 2.01	- 2.01	- 4.02	- 3	0
43	Changchun	Changchun	20.90	+ 6.35	- 11.64	+ 2.65	- 2.38	- 17.72	+ 6.35	- 4.50	- 5	2,5	
		Changchun	3.61	- 4.26	- 6.56	- 4.92	- 7.21	- 9.18	- 4.92	- 33.44	+ 3	7	
45	Shuangyang	Shuangyang	6.39	- 1.53	- 6.91	- 6.91	- 1.02	- 3.32	- 5.12	- 19.44	+ 3	7	
		Yushu	2.97	- 2.97	- 6.53	- 4.75	- 1.19	- 7.72	- 5.93	- 6.82	- 3	0	
47	Nong'an	Nong'an	12.15	+ 0.00	- 3.43	- 4.36	- 0.62	- 4.36	- 4.36	- 7.79	- 6	0	
		Jingyu	8.90	- 4.71	- 2.62	- 4.71	- 7.59	- 4.45	- 6.28	- 8.12	- 3	0	
49	Baishan	Jingyu	0.85	- 2.56	- 14.25	+ 0.57	- 0.57	- 15.67	+ 3.70	- 27.92	+ 1	2,5,7	
		Changbai Korean Autonomous	3.77	- 3.77	- 3.19	- 0.00	- 5.80	- 1.45	- 1.74	- 7.25	- 3	0	
51	Tonghua	Meihekou	13.33	+ 4.67	- 4.00	- 5.33	- 4.00	- 5.67	- 0.33	- 22.00	+ 6	7	
		Meihekou	17.59	+ 6.19	- 16.29	+ 0.98	- 4.89	- 3.26	- 0.33	- 33.55	+ 5	2,5,7	
53	Liaoyuan	Dongliao	2.67	- 0.00	- 13.07	+ 2.67	- 0.27	- 12.27	+ 4.27	- 6.40	- 1	2,5	

Note: '+': FI ≥ 10; '-': FI < 10.

### HG Type

SCN virulence phenotypes in 53 soil samples with more than 5 cysts/100 mL soil were tested using HG Type scheme. We identified 12 HG Types 0, 2, 2.5, 2.7, 5.7, 7, 1.7, 1.3.7, 1.5.7, 2.4.7, 2.5.7, 3.4.5.7, (Table 2). HG Type 7 and 0 was predominant, accounting for 33.96% and 30.18%. Both HG Type 5.7 and HG Type 2.5.7 accounted for 7.55%. HG Type 2.5 and HG Type 2.7 accounted for 3.77% and 5.66%. The remaining types each accounted for 1.89%. HG Type 1.7 was first time recorded in China and HG Type 3.4.5.7 was the first found worldwide.

### Virulence of SCN groups on indicator lines

33 SCN populations in this study were virulent on PI548316: 62.26% had a FI ≥ 10 on the indicator line (#7), 26.42% FI ≥ 10 on Pickett, 20.75% FI ≥ 10 on PI88788 (#2) and 18.87% FI ≥ 10 on PI209332 (#5). PI89772 had the most resistance: 100% populations had a FI < 10 on the line (#6). 3.78% had a FI ≥ 10 on PI 90763 (#3), PI 437654 (#4) and 7.55% on Peking (#1), (Table 2).

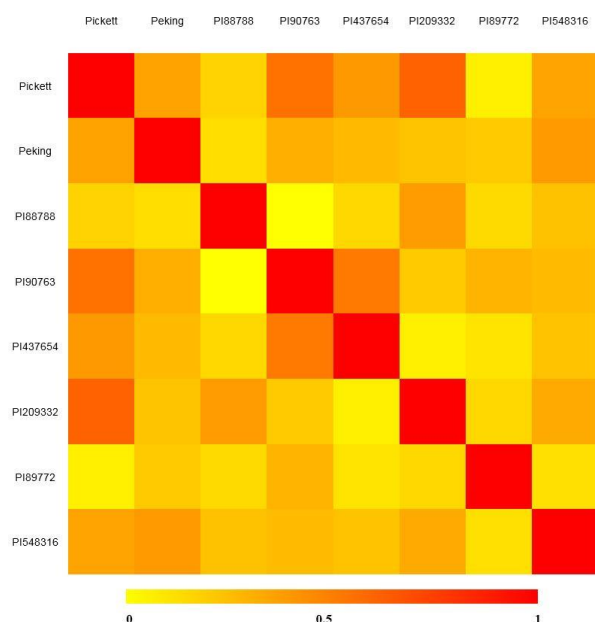
A highly significant positive correlation ( $P < 0.01$ ) was found between the FI on PI 90763 (#3) and PI 437654 (#4), as were correlations among Pickett, PI 90763 (#3) and

**Table 3:** Correlation coefficients among soybean differential lines with resistance to *Heterodera glycines* based on female indices (FIs) from 53 populations in Jilin province

Correlation coefficients	Pickett	Peking	PI88788	PI90763	PI437654	PI209332	PI89772	PI548316
Pickett	1	.254	.038	.479**	.301*	.555**	-.099	.253
Peking		1	-.014	.202	.147	.106	.074	.295*
PI88788			1	-.168	.013	.282*	.002	.114
PI90763				1	.442**	.071	.179	.142
PI437654					1	-.098	-.041	.110
PI209332						1	.015	.224
PI89772							1	-.024
PI548316								1

\*  $P < 0.05$ , \*\*  $P < 0.01$ **Table 4:** Correspondence between race and HG Type

Race	HG Type															
1	2	2.4	2.5	2.6	2.7	2.4.5	2.4.6	2.4.7	2.5.6	2.5.7	2.6.7	2.4.5.6	2.4.5.7	2.5.6.7	2.4.5.6.7	
2	1.2	1.2.4	1.2.5	1.2.6	1.2.7	1.2.4.5	1.2.4.6	1.2.4.7	1.2.5.6	1.2.5.7	1.2.6.7	1.2.4.5.6	1.2.4.5.7	1.2.5.6.7	1.2.4.5.6.7	
3	0	4	5	6	7	4.5	4.6	4.7	5.6	5.7	6.7	4.5.6	4.5.7	5.6.7	4.5.6.7	
4	1.2.3	1.2.3.4	1.2.3.5	1.2.3.6	1.2.3.7	1.2.3.4.5	1.2.3.4.6	1.2.3.4.7	1.2.3.5.6	1.2.3.5.7	1.2.3.6.7	1.2.3.4.5.6	1.2.3.4.5.7	1.2.3.5.6.7	1.2.3.4.5.6.7	
5	2	2.4	2.5	2.6	2.7	2.4.5	2.4.6	2.4.7	2.5.6	2.5.7	2.6.7	2.4.5.6	2.4.5.7	2.5.6.7	2.4.5.6.7	
6	0	4	5	6	7	4.5	4.6	4.7	5.6	5.7	6.7	4.5.6	4.5.7	5.6.7	4.5.6.7	
7	2.3	2.3.4	2.3.5	2.3.6	2.3.7	2.3.4.5	2.3.4.6	2.3.4.7	2.3.5.6	2.3.5.7	2.3.6.7	2.3.4.5.6	2.3.4.5.7	2.3.5.6.7	2.3.4.5.6.7	
8	3	3.4	3.5	3.6	3.7	3.4.5	3.4.6	3.4.7	3.5.6	3.5.7	3.6.7	3.4.5.6	3.4.5.7	3.5.6.7	3.4.5.6.7	
9	1	1.4	1.5	1.6	1.7	1.4.5	1.4.6	1.4.7	1.5.6	1.5.7	1.6.7	1.4.5.6	1.4.5.7	1.5.6.7	1.4.5.6.7	
10	3	3.4	3.5	3.6	3.7	3.4.5	3.4.6	3.4.7	3.5.6	3.5.7	3.6.7	3.4.5.6	3.4.5.7	3.5.6.7	3.4.5.6.7	
11	1.2	1.2.4	1.2.5	1.2.6	1.2.7	1.2.4.5	1.2.4.6	1.2.4.7	1.2.5.6	1.2.5.7	1.2.6.7	1.2.4.5.6	1.2.4.5.7	1.2.5.6.7	1.2.4.5.6.7	
12	1.3	1.3.4	1.3.5	1.3.6	1.3.7	1.3.4.5	1.3.4.6	1.3.4.7	1.3.5.6	1.3.5.7	1.3.6.7	1.3.4.5.6	1.3.4.5.7	1.3.5.6.7	1.3.4.5.6.7	
13	1	1.4	1.5	1.6	1.7	1.4.5	1.4.6	1.4.7	1.5.6	1.5.7	1.6.7	1.4.5.6	1.4.5.7	1.5.6.7	1.4.5.6.7	
14	1.3	1.3.4	1.3.5	1.3.6	1.3.7	1.3.4.5	1.3.4.6	1.3.4.7	1.3.5.6	1.3.5.7	1.3.6.7	1.3.4.5.6	1.3.4.5.7	1.3.5.6.7	1.3.4.5.6.7	
15	2.3	2.3.4	2.3.5	2.3.6	2.3.7	2.3.4.5	2.3.4.6	2.3.4.7	2.3.5.6	2.3.5.7	2.3.6.7	2.3.4.5.6	2.3.4.5.7	2.3.5.6.7	2.3.4.5.6.7	
16	1.2.3	1.2.3.4	1.2.3.5	1.2.3.6	1.2.3.7	1.2.3.4.5	1.2.3.4.6	1.2.3.4.7	1.2.3.5.6	1.2.3.5.7	1.2.3.6.7	1.2.3.4.5.6	1.2.3.4.5.7	1.2.3.5.6.7	1.2.3.4.5.6.7	

**Fig. 2:** Heat map for correlation between indicator lines

PI 209332 (#5). Pickett and PI 437654 (#4) were positively correlated ( $P < 0.05$ ). PI 88788 (#2) and PI209332 (#5) were also positively correlated (Table 3). Additional correlation was found between Peking (#1) and PI548316 (#7) (Fig. 2).

## Discussion

In a previous survey conducted in 1988, SCN was distributed over 83% of Jilin province, especially in the counties of Zhenlai, Yushu, Tongyu and Jingyu and in Baicheng city (Liu and Wu 1988). A total of 38 (100%) cities (counties, towns) were infested with SCN in this study. Taonan, Baicheng, Gongzhuling, Jilin city and Tongyu county are the hotspots for SCN distribution (Fig. 1), the same as previous survey, except Jilin city. Significantly, SCN was not detected in Jilin and Longjing cities in the survey conducted in 1988 and cyst density of sample from Gongzhuling increased, possibly due to the long-term cultivation of soybeans and the lack of disease resistant varieties in these regions. As shown in Fig. 1, the density of SCN was the highest and widely distributed in the western of Jilin Province, which may be due to the most of saline-alkali land, followed by the middle part, the lowest in the east.

According to similarities of cellular resistance response in tests, soybean resistance to SCN was classified into two main types: the PI 88788-type, including PI 209332 and PI 548316; and the Peking-type, including PI 90763, PI 89772 and perhaps, PI 437654 (Endo 1965; Kim *et al.* 1987; Halbrendt *et al.* 1992; Mahalingham and Skorupska 1996). The results of this study are similar to and different from those of previous studies. The resistance of PI 209332 was similar to that of PI 88788 and PI 548316 (Anand 1992). *H. glycines* reproduction on these three differentials was

significantly positively correlated (Zheng *et al.* 2006; Colgrove and Niblack 2008; Acharya *et al.* 2016). In this study, PI 209332 (#5) and PI88788 (#2) had positive correlation on FI ( $P < 0.05$ ), but not PI 548316 (#7) (Fig. 2). What's interesting was that significant correction was found between PI 548316 and Peking in this study. PI 548316, originally assigned to PI88788-type, now was classified into the Peking-type. Differentials PI 90763 (#3) and PI 437654 (#4) exhibited significant positive correlations on FI ( $P < 0.01$ ), but not Peking. PI 90763 and PI 437654 were not classified into the Peking-type now. An observation of a stage-related effect of resistance on juvenile development supports that the resistance response may be different between the types (Halbrendt *et al.* 1992). In addition, Pickett is considered to be the progeny of Peking and may carry similar resistance genes. However, in this study, Pickett and PI 90763 (#3), PI 437654 (#4), PI 209332 (#5) were found to have significant positive correlation but not Peking. This result may be due to the high genetic diversity of the field population. Pickett should be used to distinguish the virulence phenotypes of SCN field populations in Jilin province.

Virulence phenotypes of *H. glycines* populations were evaluated by race scheme and HG Type scheme. A total of 7 races and 12 HG Types were identified, and race 10 and HG Type 1.7 were first recorded in China. In 1988, race 10 was first discovered in Arkansas (Riggs and Schmitt 1988). In 1996, the race was also identified in Ohio (Willson *et al.* 1996). HG Type 3.4.5.7 was first found worldwide.

If Jilin Province is divided into eastern, western and central regions, race 1, 3, 5, 6 are distributed in all the three regions in common. Race 1 is dominantly distributed in the mid-region, while the other three races are common. Race 9 was found sporadically in the east and west, but not in the central Jilin Province. Race 10 and 14 distributed in one sampling point in the West and East respectively.

A population's HG Type name can clearly show invalid indicator plants. Any cultivar that has a similar FI  $\geq 10$  should not be used in a field that has an *H. glycines* population that is compatible with that indicator line. In this student (Table 2), due to FI  $\geq 10$  on PI 548316 and Pickett in most of populations, the use of PI 548316 and Pickett as resistant parents should be minimized in Jilin province. The use of PI 88788 (#2) should be also reduced in the west. PI 89772, PI 437654 and PI 90763 are better sources for resistance to SCN. Recently, multi resistant varieties, such as Kangxian 3 (Cao *et al.* 2014), Dongnong L-10 (Wu *et al.* 2016), ZDD24656, a variety derived from PI 437654 and ZDD2315 (Lian *et al.* 2017), Andou 162, a descendant of Franklin, Bedford and Hartwig (Wang *et al.* 2019), Zhonghuang 26 and Zhonghuang 54, varieties derived from PI 437654 (Wang and Wang 2016) can be used as source of resistance.

When comparing the two schemes, the same race corresponded to multiple HG types, *e.g.*, race 3 corresponded to HG Type 7 and HG Type 5.7 in this study.

Additionally, the same HG type may also correspond to multiple races, *e.g.*, HG Type 7 corresponded to race 3 and race 6, and HG Type 5.7 also corresponded to race 3 and race 6 in this study. In a previous study (Wang 2015), HG Type 2.7 corresponded to race 1 and race 5. We considered the SCN population with HG Type 2.7 (race 1) in this study was different from the population with HG Type 2.7 (race 5) in the previous study (Wang 2015). Possibly, that was the same as the population with HG Type 2.7 (race 1) in the previous study. In this way, neither scheme could completely distinguish the virulence phenotypes of SCN populations. HG Type testing is internationally accepted because it is relatively accurate and easily expandable as new soybean germplasm is released and deployed (Winter *et al.* 2006). The resistant line PI 438489B (Yue *et al.* 2001) was added to the set of differentials used in the HG Type test in 2007 (Mitchum *et al.* 2007). However, as the number of identified hosts increases, HG type testing will become more complex. Currently, HG Type + Race are used in SCN research fields (Han *et al.* 2015; Lin *et al.* 2016).

Although there is no one-to-one correspondence between race and HG type, there are some corresponding relationships (Table 4). Theoretically, 128 HG types are possible with HG Type testing. Each race corresponded to 16 HG types, and each two races corresponded to the same HG type, *e.g.*, race 3 and race 6 corresponded to the same 16 HG Types. Accordingly, in theory, 256 different SCN virulence types are possible with the combination of the two methods, but the lack of characterization of them. More trials are needed for further validation. According to this study, the combination of the two methods was suitable for the study of genetic diversity of SCN in Jilin province. Pickett which was removed in HG scheme should be included not as an indicator line but just to separate different races from the same HG type in Jilin province.

## Conclusion

In this study, 104 soil samples from 38 cites (cities, counties and towns) were positive for SCN across Jilin province 7 races and 12 HG types were identified, with Race 3 and HG Type 7 being the most dominant genotypes. PI 90763, PI 437654 and PI89772 were recommended as excellent sources against cyst nematode. It suggests that Peking-type resistance sources were preferred to pi88788-type in Jilin province. The combination of the race and HG scheme is recommended for studying the genetic diversity of SCN in Jilin province. That is, Pickett should be included not as an indicator line but just to separate different races from the same HG type.

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## Author Contributions

Xiujuan Yan and Yuxi Duan conceived and designed the experiments; Qiang Qiu and Xiaofeng Zhu collected soil samples; Xiujuan Yan, Jinwen Liu and Mingshu Li performed the experiments; Jinwen Liu analyzed the data; Xiujuan Yan and Jinwen Liu wrote the paper.

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