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Identification of HG Types of Soybean Cyst Nematode *Heterodera glycines* and Resistance Screening on Soybean Genotypes in Northeast China

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Abstract

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is a serious soybean pathogen worldwide. HG Type 0 had been a predominant SCN in Heilongjiang province, the largest soybean (*Glycine max* L.) producing region in China. Recently, increased virulence on resistant cultivars originally developed for resistance to HG Type 0 was observed in fields. In order to identify new cultivars resistant to local SCN populations, two soil samples were collected from two counties (Anda and Wuchang) in which increased virulence on resistant cultivars occurred, and single-cyst cultures from each soil sample were maintained for more than five generations. Two single-cyst cultures from the Anda sample were identified as HG Type 1.2.3.5.6.7 and HG Type 1.3, and one single-cyst culture from Wuchang was identified as HG Type 2.5.7. Then 18 soybean genotypes, including 11 local cultivars originally developed for resistance to HG Type 0, were used to evaluate resistance response to the three identified SCN populations. Various levels of resistance or susceptibility to the three SCN populations were observed among 18 genotypes. Two tests produced similar results for the three SCN populations. Both 'Kangxian12' and 'Kangxian13' showed resistance or moderate resistance to HG Type 2.5.7, HG Type 1.2.3.5.6.7 and HG Type 1.3. The germplasm '09-138' was resistant to HG Type 1.3 and HG Type 1.2.3.5.6.7. Cultivars with 'Peking'-resistance were resistant or moderately resistant to HG Type 2.5.7 in both tests except for 'Kangxian8' in test 1. The identified resistant varieties would be valuable sources of breeding materials for resistance against multiple SCN populations.

Key words

Glycine max; *Heterodera glycines*; HG type; resistance screening; soybean; Soybean cyst nematodes.

Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most devastating pathogens of soybean (*Glycine max* L.) in the world, causing more than 1 billion dollars of yield losses each year in the USA (Wrather and Koenning, 2009). The nematode was first discovered in northeast China in 1899 (Ou et al., 2008; Li et al., 2011) and then was reported in most major soybean producing countries including the USA in 1954 (Winstead et al., 1955), Brazil in 1992 (Mendes and Dickson, 1993; Noel et al., 1994) and Argentina in 1997 (Dias et al., 1998). Once *H. glycines* is found in one location, nematodes can be quickly spread to more soybean-producing areas (Tylka and Marett, 2014). In China, *H. glycines* is present in almost every soybean producing area, which causes severe economic losses each year (Dong et al., 2008; Lu et al., 2006; Ou et al., 2008).

Genetic diversity among SCN populations was first reported in 1962 (Ross, 1962). A 16-race identification system was developed based on phenotypic response on five soybean lines ('Peking', 'Pickett', PI 88788, PI 90763 and 'Lee') (Golden, 1970; Riggs and Schmitt, 1988), and later an HG Type test was developed based on seven indicator lines (Niblack et al., 2002). Ten SCN races (1-7, 9, 13, and 14) and 9 HG Types (0, 7, 2.7, 5.7, 1.3.7, 2.5.7, 1.2.5.7, 1.3.4.7, and 1.2.3.5.7) have been reported in China. Two major soybean producing regions, in which SCN is an important disease, are Huang-Huai Valleys and northeast China. The predominant SCN populations in Huang-Huai Valleys and northeast China have been reported as HG Type 1.2.3- (designated as an incomplete HG Type denomination, Niblack et al., 2002) and HG Type 0-, respectively (Liu et al., 1985, 1995; Liu and Liu, 1989; Lu et al., 2006; Dong et al., 2008; Ou et al., 2008; Wang et al., 2014; Chen et al., 2015). Heilongjiang Province in northeast China is the province which produces the most soybean in China. SCN management mainly depends on crop rotation with non-host crops and host plant resistance. Host plant resistance is the most cost-effective and environmentally benign strategy against SCN. In China, major efforts were made to develop SCN cultivars resistant to HG Type 1.2.3- in Huang-Huai Valleys and to HG Type 0- in northeast China (Liu, 2005). For example, a series of varieties ('Kangxian1-13') resistant to HG Type 0- were developed by Heilongjiang Academy of Agricultural Sciences in northeast China (Li et al., 1998; Liu, 2005; Tian et al., 2007; Wu et al., 2011; Song, 2012; Wang, 2013; Yu et al., 2013). The major source of resistance in these varieties was soybean 'Franklin' containing 'Peking' resistance (Yuan et al., 2008; Li et al., 2014). However, these 'Peking'-derived SCN-resistant varieties have become less effective due to the emergence of new SCN populations with planting these varieties in the same field year after year (Tian et al., 2007; Chen et al., 2015; Yang et al., 2015). Increased virulence on resistant cultivars and the occurrence of virulent SCN populations were reported in the USA with continuous growing of PI88788-derived resistant varieties (Diers and Arelli, 1999; Mitchum et al., 2007; Hershman et al., 2008; Vuong et al., 2010; Tylka and Mullaney, 2015; Acharya et al., 2016). Selection pressure can make the SCN population shift to more virulent types which are able to adapt to the resistant varieties (Niblack, 2005). Although many soybean genotypes were screened for the soybean resistance breeding program against SCN, limited resistant varieties were available in northeast China (Kong et al., 2012; Cao et al., 2014; Li et al., 2014).

In recent years, increased virulence on 'Peking'-derived SCN-resistant cultivars was observed without continuous cropping of resistant varieties in some fields in Heilongjiang Province. Initial nematode identification in different field locations indicated that HG Type 0- (Liu *et al.*, 1985, 1995) was not the predominant population anymore and multiple HG Types were detected in the same field but were not evenly distributed (unpubl. data). Since SCN virulence had increased in the field, it was unknown what the HG Types of the changed SCN populations were and how well these new SCN populations reproduced on the limited resistant varieties originally developed against HG Type 0- and current local varieties. The objectives of this study were to identify the HG types of the SCN populations and to evaluate the response of the local soybean cultivars to the identified SCN populations for determining resistant sources against multiple SCN populations in northeast China. This study would provide valuable information not only for SCN management but also for the SCN resistance breeding program.

Materials and methods

Soil sample collection and nematode culture

Soil sampling was conducted at soybean harvest time in two regions, Anda (N:46°24′, E:125°22′) and Wuchang (N:44°44′, E:127°14′), of Heilongjiang Province where SCN was present and increased virulence on resistant cultivars was known. Each soil sample contained more than 20 cores taken down to 20 cm depth with a 2.5-cm-diameter soil probe. The mixed soil samples were kept at 4°C and five cysts were isolated from each soil sample. Then each cyst was cultured on susceptible 'Dongsheng1' as the first generation (Hu et al., 2017) in 12-cm-diameter × 8-cm-deep plastic pots containing a 1:1 ratio of sand to soil under controlled greenhouse condition at 23°C to 28°C with 16-hr photoperiod. After 35 to 40 days, one single cyst was collected from the roots and the second generation was initiated. If there was no reproduction, the pot was discarded. To obtain a near-homogeneous population for producing more stable reactions on soybean plants, this process was continued for more than five generations.

Plant materials

Indicator lines PI 548402 ('Peking', indicator line #1), PI 88788 (#2), PI 90763 (#3), PI 437654 (#4), PI 209332 (#5), PI 89772 (#6), PI 548316 ('Cloud', #7) were utilized for HG Type determination (Niblack et al., 2002). 'Pickett' was added to identify the different SCN virulence profiles. Eighteen soybean cultivars/germplasms (Table 1) were utilized for resistance evaluation against SCN populations, including one wild type *G. soja* 'ZYD00006', three breeding lines, 11 cultivars developed primarily for resistance to HG Type 0- by Heilongjiang Academy of Agricultural Sciences in northeast China (Li et al., 1998; Liu, 2005; Tian et al., 2007; Wu et al., 2011; Song, 2012; Wang, 2013; Yu et al., 2013), and three main local commercial cultivars ('Hefeng25', 'Suinong14', 'Dongsheng1').

Nematode inoculum

Nematode inoculum was prepared from soybean plants infected for more than 35 days in the greenhouse. Cysts of *H. glycines* from roots and soil were extracted by hand over nested sieves. First, soil with roots was poured into 1-liter beaker containing water. After the mixture was vigorously stirred with a glass rod, it was decanted through an 800 µm pore sieve nested on a 150 µm pore sieve. After rinsing under a strong jet of water, the debris and materials on the 150 µm pore sieve were poured onto Whatman No. 4 filter paper which was supported by a 12.5-cm-diameter funnel. The filter paper was dried at room temperature. Most cysts were around the outer layer of the filter paper. The cysts were collected and were crushed over a 75 µm pore sieve nesting on a 25 µm pore sieve with hand-decanting to release eggs (Schmitt and Shannon, 1992). Eggs collected on the 25 µm sieve were incubated at 28°C for three or five days to obtain second-stage juveniles (J_2) as inoculum.

Identification of HG Types and resistance screening for soybean cyst nematodes

To determine SCN HG Types and to evaluate resistant sources, plants were grown in 12-cm-diameter × 8-cm-deep plastic pots containing a 1:1 ratio of sand to soil under controlled greenhouse condition at 23°C to 28°C with 16 hr of photoperiod. Two seeds were planted in each pot and thinned to one seedling after germination. Each treatment was replicated with five individual plants. Two holes around each seven-day-old plant were made with a 1 ml pipette tip and 0.5 ml water suspension containing 400 J_2 was injected into each hole (total 800 J_2 per plant). Then the holes were covered with soil. Plants were arranged on benches according to completely randomized design in the greenhouse. Twenty-one days after inoculation, white females were dislodged by washing plant roots individually under a strong jet of water over 800 µm and 150 µm aperture sieves. After females/cysts per plant were counted, eggs per plant were released over 75 µm and 25 µm aperture sieves as described above. Female index (FI) was calculated as follows: FI = (average number of females found on indicator lines or soybean genotypes/average number of

Table 1. Eighteen soybean germplasms tested in this study

Genotype category	Soybean germplasms
Glycine soja	ZYD00006
Breeding lines	Sien1-2, Sien1-3, 09-138
Peking-resistant cultivar to *Heterodera glycines*	Kangxian2, Kangxian4, Kangxian5, Kangxian6, Kangxian7, Kangxian8, Kangxian9, Kangxian10, Kangxian11, Kangxian12, Kangxian13
Local commercial cultivars	Dongsheng1, Suinong14, Hefeng25

females found on susceptible line Lee 68) × 100. Each experiment was repeated two times (Test 1 and Test 2).

For HG Type identification, an FI equal to or greater than 10 on any indicator line or soybean genotype was assigned as susceptible, and as resistant when FI < 10. HG Type determination was based on the schemes developed by Niblack et al. (2002). The response of soybean genotypes to SCN populations was evaluated based on the method developed by Schmitt and Shannon (1992). Plants were classified as resistant (FI < 10), moderately resistant (FI = 10-30), moderately susceptible (FI = 31-60) or susceptible (FI > 60).

Data analysis

Data were subjected to analysis of variance using SPSS One-Way ANOVA (IBM, Armonk, New York, USA). Tukey's HSD test ($P < 0.05$) was used to evaluate the influence of difference.

Results

HG Type of field SCN populations

After more than five generations of single-cyst culture, only two cultures from Anda soil sample (Anda1 and Anda2) and one from Wuchang were available to be used for HG Type identification. The other cultures with no reproduction were discarded. SCN reproduction from single-cyst cultures of three SCN populations on the indicator lines, 'Pickett' and the susceptible check 'Lee 68' are shown in Table 2. The Anda1 culture was identified as HG Type 1.2.3.5.6.7, HG Type 1.3 for Anda2, and HG Type 2.5.7 for Wuchang (Table 2).

Resistance response of soybean germplasms to soybean cyst nematode populations

Significant differences ($P < 0.05$) occurred in number of cysts among soybean lines with each individual population (Tables 3-5). Based on female indices and resistance classification (Schmitt and Shannon, 1992), none of the 18 tested soybean cultivars/germplasms showed resistance to any of the three populations. 'Kangxian12' and 'Kangxian13' showed resistance to HG Type 2.5.7 in both tests (Table 4) and moderate resistance to both HG Type 1.2.3.5.6.7 and HG Type 1.3 in one or two tests (Tables 3 and 5). One breeding line '09-138' showed resistance to HG Type 1.3 (Table 3) and susceptibility to HG Type 2.5.7 (Table 4) in both tests. Breeding line '09-138' showed resistance to HG Type 1.2.3.5.6.7 in test 1 but moderate susceptibility in test 2.

Out of eleven local cultivars originally resistant to HG Type 0-, at least nine cultivars were identified as resistant to HG Type 2.5.7 in both tests (Table 4). All other genotypes was moderately resistant, moderately susceptible or susceptible to the three HG Types (Tables 3-5). Within susceptible genotypes, wide variation of nematode reproduction was observed (Tables 3-5). For example, the range of female indices was 60 to 152 for HG Type 1.3, 71 to 146 for HG Type 2.5.7 and 66 to 213 for HG Type 1.2.3.5.6.7 (Tables 3-5).

Among all tested soybean genotypes, the correlation between egg number (data not shown) and cyst

Table 2. *Heterodera glycines* female indices[1] and HG Types associated with three different nematode populations

Location	1(PI 548402)	2(PI 88788)	3(PI 90763)	4(PI 437654)	5(PI 209332)	6 (PI 89772)	7(PI 548316)	Pickett	HG Type[2]
Anda1	71.93	24.03	25.21	0.00	33.45	21.57	66.72	78.38	1.2.3.5.6.7
Anda2	45.83	1.75	13.20	0.19	5.24	0.24	9.90	160.30	1.3
Wuchang	0.00	44.56	0.00	0.00	22.28	1.10	42.63	95.80	2.5.7

Notes: [1]Female index (FI) = (average number of females on indicator lines)/(average number of females on Lee 68) × 100 (n=10); [2]The average number of cysts on susceptible check 'Lee 68' was equal to or more than 100

Table 3. Response of germplasms to soybean cyst nematodes against HG Type 1.3

	Test 1			Test 2		
Genotype	Cyst/plant	FI[a]	R[b]	Cyst/plant	FI	R
Lee	92±25.63abcde			211±21.76defg		
ZYD00006	56±11.59abcde	60.43	S	131±6.39bc	61.90	S
09-138	0±0.2a	0.22	R	15±2.52a	7.01	R
Sein1-2	115±15.73cde	125.00	S	251±22.65fg	119.05	S
Sein1-3	92±23.33abcde	99.57	S	277±29.01g	131.18	S
Kangxian2	66±11.65abcde	71.52	S	127±8.45bc	60.09	S
Kangxian4	121±20.89de	131.52	S	197±8.17cdefg	93.36	S
Kangxian5	124±31.32de	134.78	S	251±21.24fg	118.96	S
Kangxian6	125±23.05de	135.87	S	253±15.28fg	120.09	S
Kangxian7	44±10.76abcd	47.39	MS	174±19.66cdef	82.65	S
Kangxian8	47±15.78abcd	50.87	MS	172±6.92cdef	81.71	S
Kangxian9	100±25.53bcde	108.70	S	219±14.02defg	103.60	S
Kangxian10	98±23.97bcde	106.96	S	147±16.31cd	69.76	S
Kangxian11	140±16.95e	152.17	S	167±7.80cde	79.34	S
Kangxian12	22±3.67ab	23.48	MR	64±7.52ab	30.24	MS
Kangxian13	27±6.24abc	29.13	MR	66±2.21ab	31.28	MS
Hefeng25	118±12.4cde	128.48	S	241±27.16efg	114.22	S
Dongsheng1	101bcde	110.00	S	152±25.63cd	71.85	S
Suinong14	80abcde	86.52	S	180±13.62cdef	85.12	S

Notes: Means (n = 5) followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's HSD test. The comparison was conducted within same column; [a] FI: Female Index (FI) = (average number of females on cultivars)/(average number of females on Lee 68) × 100; [b] R: Rating based on female index, where FI < 10 is resistant (R), FI = 10-30 is moderately resistant (MR), FI = 31-60 is moderately susceptible (MS), and FI > 60 is susceptible (S)

number per plant was 0.7247 (test 1) and 0.6560 (test 2) for HG Type1.3, 0.9474 (test 1) and 0.9774 (test 2) for HG Type2.5.7, and 0.6679 (test 1) and 0.7632 (test 2) for HG Type1.2.3.5.6.7.

Discussion

SCN populations in the field are usually a mixture of several populations with genetic heterogeneity (Dong et al., 1997; Niblack et al., 2008; Beeman et al., 2016). Beeman et al. (2016) found that HG Types varied in a small area of a field, confirming heterogeneity of SCN populations in the field. The two HG types identified from single-cyst cultures from Anda sample (Table 2) confirmed genetically heterogeneous SCN populations. We also detected multiple HG types present in the same field (unpubl. data), suggesting genetic heterogeneity. The genetic heterogeneity increases the difficulties for breeding programs. Cultivars with multiple resistance to SCN appear more important for SCN management.

To get cultures as homogeneous as we possibly can, single-cyst cultures with more than five generations each starting from a single cyst were obtained from Anda and Wuchang. These populations could be kept and utilized for resistance screening of soybean

Table 4. Response of germplasms to soybean cyst nematodes against HG Type 2.5.7

	Test 1			Test 2		
Genotype	Cyst/plant	FI[a]	R[b]	Cyst/plant	FI	R
Lee	199±35.09bc			166±20.92b		
ZYD00006	112±15.03ab	56.08	MS	132±2.91b	79.76	S
09-138	188±24.56bc	94.27	S	127±20.05b	76.63	S
Sein1-2	266±30.92c	133.57	S	142±13.89b	85.66	S
Sein1-3	290±53.11c	145.73	S	179±17.35b	108.07	S
Kangxian2	17±3.4a	8.64	R	26±23.46a	15.90	MR
Kangxian4	2±0.73a	1.11	R	1±0.58a	0.48	R
Kangxian5	8±1.74a	3.92	R	5±1.84a	3.01	R
Kangxian6	41±7.48a	20.60	MR	5±1.72a	3.25	R
Kangxian7	19±7.14a	9.75	R	14±6.62a	8.19	R
Kangxian8	68±12.7a	33.97	MS	16±3.35a	9.76	R
Kangxian9	6±0.97a	2.91	R	0±0a	0.00	R
Kangxian10	8±2.69a	4.22	R	2±1.17a	0.96	R
Kangxian11	12±4.42a	6.13	R	1±1.20a	0.72	R
Kangxian12	0±0.2a	0.10	R	0±0.20a	0.12	R
Kangxian13	3±0.86a	1.61	R	0±0a	0.00	R
Hefeng25	264±45.27c	132.46	S	137±22.92b	82.41	S
Dongsheng1	254±17.42c	127.64	S	118±14.55b	70.84	S
Suinong14	239±30.59c	120.00	S	147±20.95b	88.55	S

Notes: Means (n = 5) followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's HSD test. The comparison was conducted within same column; [a]FI: Female Index (FI) = (average number of females on cultivars)/(average number of females on Lee 68) × 100; [b]R: Rating based on female index, where FI < 10 is resistant (R), FI = 10-30 is moderately resistant (MR), FI = 31-60 is moderately susceptible (MS), and FI > 60 is susceptible (S)

cultivars or germplasms instead of heterogeneous soil samples. Furthermore, these SCN populations could also be utilized for other research purposes, such as genetic study of SCN through developing genetic population between different HG Types. Three different HG Types were identified in our research after at least 5 generations with only one cyst starting each generation. The results of these identified HG Types with single-cyst cultures confirmed the HG type shift which was reported in the same county by Yang et al. (2015).

Currently, the HG Type test (Niblack et al., 2002) is more commonly used for SCN population identification (Tylka, 2016). Eight HG Types were identified by Wang et al. (2014) which was the first report of HG Types in China. Chen et al. (2015) investigated the virulence of SCN populations under continuous cropping in Daqing and Anda counties in Heilongjiang province, and identified them as HG Types 7 and 1.3.4.7, respectively. In this study, we identified two new HG Types (HG Type 1.2.3.5.6.7 and HG Type 1.3) in Anda county.

It is very common for *H. glycines* virulence to occur when the same resistant sources are continuously planted in the field (Niblack, 2005; Tylka and Mullaney, 2015; Acharya et al., 2016). Since PI 88788 is the major SCN resistance source in the USA, the FI on PI 88788 as an aggressiveness index provides very important

Table 5. Response of germplasms to soybean cyst nematodes against HG Type 1.2.3.5.6.7

	Test 1			Test 2		
Genotype	Cyst/plant	FI[a]	R[b]	Cyst/plant	FI	R
Lee	56±4.39bcde			57±16.47abcde		
ZYD00006	64±14.27cdef	113.93	S	78±6.25bcde	137.19	S
09-138	4±1.45a	7.14	R	22±14.45ab	38.95	MS
Sein1-2	103±12.48ef	183.21	S	86±25.77bcde	150.53	S
Sein1-3	71±14.37cdef	126.07	S	70±8.77abcde	122.11	S
Kangxian2	106±11.5f	188.93	S	33±8.58abc	58.60	MS
Kangxian4	86±6.5cdef	154.29	S	73±8.62abcde	128.42	S
Kangxian5	45±12.46abc	80.00	S	30±10.89abc	51.93	MS
Kangxian6	61±7.04cdef	108.57	S	53±6.39abcd	93.33	S
Kangxian7	55±11.51bcd	98.21	S	38±3.44abc	65.96	S
Kangxian8	63±13.66cdef	111.79	S	75±19.11abcde	131.58	S
Kangxian9	41±6.83abc	72.86	S	19±6.04ab	33.33	MS
Kangxian10	56±6.69bcde	99.29	S	68±1.63abcde	118.60	S
Kangxian11	78±4.5cdef	138.93	S	43±13.66abc	75.09	S
Kangxian12	13±1.56ab	23.57	MR	41±16.71abc	71.49	S
Kangxian13	13±2.12ab	23.21	MR	9±3.51a	15.44	MR
Hefeng25	101±10.23def	180.71	S	114±9.96de	200.70	S
Dongsheng1	64±5.42cdef	113.93	S	122±22.11e	213.33	S
Suinong14	83±9.05cdef	148.57	S	97±15.21cde	169.12	S

Notes: Means (n = 5) followed by the same letter are not significantly different ($P < 0.05$) according to Tukey's HSD test. The comparison was conducted within same column; [a] FI: Female Index (FI) = (average number of females on cultivars)/(average number of females on Lee 68) × 100; [b] R: Rating based on female index, where FI < 10 is resistant (R), FI = 10-30 is moderately resistant (MR), FI = 31-60 is moderately susceptible (MS), and FI > 60 is susceptible (S)

information to indicate how well the *H. glycines* virulent population reproduces on 'PI 88788'-derived SCN-resistant varieties (Beeman et al., 2016; Tylka, 2016). Similarly, the FI on 'Peking' can also be thought of as an aggressiveness index to indicate how well the virulent *H. glycines* population reproduces on 'Peking'-derived SCN-resistant varieties. For example, 'Peking'-derived SCN-resistant 'Kangxian' cultivars were originally developed for resistance to HG Type 0- in northern China. When the FI on 'Peking' (PI 548402) increased (Table 2), resistance of these 'Kangxian' cultivars decreased (Tables 3 and 5), indicating more aggressive SCN population(s) had appeared. In this study, both HG Type 1.2.3.5.6.7 and HG Type1.3 reproduced well on 'Peking', confirming SCN virulence change.

At least nine out of 11 local cultivars in two tests originally resistant to HG Type 0- showed <10 FI to SCN HG Type 2.5.7, confirming the 'Peking' resistant source. The resistance source of 'Kangxiang2-11' was derived from 'Franklin' ('Peking'-derived resistance) or its generations crossed with various local varieties (Li et al., 1998; Liu, 2005; Tian et al., 2007; Wu et al., 2011; Yu et al., 2013; Li et al., 2014).

'Kangxian12' and 'Kangxian13' (also called 'Qingdou13') were derived from the generations of the cross between 'Heikang002-24' (resistant to *H. glycines*) and 'Nongda5129' and its resistance was selected in the field in the presence of HG Type 0- (Song, 2012; Wang, 2013). 'Kangxian12' was reported to display resistance to HG Type 0- as well to HG Type 2 and HG Type 1.3- (Song, 2012). The parental line 'Heikang 002-24' has SCN-resistance from 'Peking' (Z. Tian, DBHAAS, pers. comm.). Therefore, it was not surprising that these cultivars displayed resistance to HG type 2.5.7 population. Furthermore, 'Kangxian12' and 'Kangxian13' were selected in the field more recently than other resistant varieties in Anda county. The breeding line '09-138', which was also selected in the field of Anda county, was supposed to contain 'Hartwig'- and 'Peking'-resistance sources (unpubl. data). 'Hartwig'-resistance was derived from PI 437654 (indicator line #4) (Anand, 1992). Surprisingly, it was first reported in northeast China that the breeding line '09-138' showed resistance to HG Type 1.2.3.5.6.7 and HG Type 1.3 but was susceptible to HG Type 2.5.7. Further study of genetic resistance for '09-138' might expand the application of the SCN resistant breeding program.

Wide variation in numbers of cysts on the tested germplasms in this study indicated that the infectivity of each SCN population differs on same cultivar. The similar result was also reported previously (Epps and Hartwig, 1972; Young, 1990; Riggs et al., 1991; Davis et al., 1996; Arelli et al., 1997, 2000, 2015). In addition, with the same level of inoculum among three HG Types, the average number of cysts formed on the susceptible check Lee 68 for HG Type 1.2.3.5.6.7 in both tests (Table 5) was low, indicating low disease pressure. Even so, most soybean genotypes were good hosts for HG Type 1.2.3.5.6.7 in both tests (Table 5). The reason for low disease pressure was not clear and high disease pressure will be required for future study. However, HG Type 1.2.3.5.6.7 produced more than 100 cysts on susceptible Lee 68 and soybean '09-138' and 'Kangxian13' consistently displayed resistance or moderate resistance to HG Type 1.2.3.5.6.7 in other tests (data not shown) as reported in this study.

The high correlation of egg number with cyst number among all tested soybean germplasms suggests non-differential genetic characters between nematode reproduction and cysts. However, the negative correlation between egg number (nematode reproduction) and egg masses or egg-laying females (EFL) was reported in root-knot nematode-plant interaction (Windham and Williams, 1987, 1988; Fourie et al., 1999; Li et al., 2016).

In conclusion, our study showed that the mixed SCN populations were presented in Anda county. At least nine out of 11 local cultivars in the two tests originally resistant to HG Type 0- also displayed resistance to HG Type 2.5.7. No genotype showed multiple resistance to all the three SCN populations. 'Kangxian12' and 'Kangxian13' showed resistance or moderate resistance to HG Type 2.5.7, HG Type 1.2.3.5.6.7 and HG Type 1.3. The breeding line '09-138' was resistant to both HG Type 1.2.3.5.6.7 and HG Type 1.3. The identified resistant varieties would be valuable sources of breeding materials for resistance against multiple SCN populations.

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