RESEARCH ARTICLE

Different responses of soybean cyst nematode resistance between two RIL populations derived from Peking × 7605 under two ecological sites

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Abstract

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is a plant-parasitic nematode that feeds on the roots of soybean and most economically devastating pathogen of soybean (*Glycine max* (L.) Merr.) worldwide. Host plant resistance is the most effective control method. To understand SCN resistance in different environments, two recombinant-inbred lines (RILs) populations NJ(RN)P7 (217 $F_{2:8:11}$ lines) and JN(RN)P7 (248 $F_{2:7:9}$ lines) were developed from the cross of the cultivars Peking × 7605 in Nanjing and Jinan, respectively, and examined in this study. Peking is resistant to SCN race 1 (HG types 2.5.7), while 7605 is highly susceptible. Chi-square test of frequency distribution of families' female index (FI) showed that resistance to SCN was significantly different between NJ(RN)P7 and JN(RN)P7 populations. Three recessive genes conditioned the inheritance of resistance to SCN race 1 in both populations, but significant difference was detected for the mean of FI on two populations ($DM = -16.68$, $P < 0.01$). This indicated that natural selection may affect resistance to SCN. By analysing the variation of phenotype, the genetic structure of the two populations was determined to be different. The inheritance and variation of resistance were confirmed by simple sequence repeat (SSR) markers. For the two populations, 10 SSR markers showed polymorphism of resistant and susceptible DNA bulks. Some markers associated with the resistance of SCN races 1, 2, 3 and 5, and two markers, Satt163 and Satt309, reportedly related to *rgh1* were detected both in NJ(RN)P7 and JN(RN)P7 populations. The results support the view that a disease acts as a selective force on plant resistance characteristics, which may alter the relative fitness of resistance alleles.

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Introduction

Soybean is one of the most important crops worldwide accounting for about 30% of the vegetable oil and 60% of the vegetable protein in world production. However, the sustainability of soybean production has been challenged by intensified pest problems (Skorupska *et al.* 1994). Soybean cyst nematode (SCN, *Heterodera glycines* Ichinohe) is one of the most important pests of soybean (*Glycine max* (L.) Merr.) in the world. SCN lead to a large economic cost with annual losses of approximately $1.5 billion in USA (Wrather and Koenning 2006). The infection causes various symptoms that include chlorosis of the leaves and stems, root necrosis, loss in seed yield and suppression of root and shoot growth. It is also a significant problem in the soybean growing areas of China. While rotation with nonhost crops and nematode insecticide can partly reduce its loss, breeding resistance cultivars is the most economical and environmental friendly method to control SCN (Ma *et al.* 2006). However, the genetic complexity and the heterogeneity of SCN populations have limited our understanding of the nature of the resistance

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and the development of resistant cultivars (Faghihi *et al*. 1986a, b).

Soybean plant introductions (PIs) that are resistant to SCN suppress reproduction of the nematode, but cannot eliminate damage (Raoarelli and Anand 1988). During selection, SCN populations often develop the ability to overcome resistance (Riggs and Schmitt 1988). The value of diversity for disease control is well established experimentally. Intraspecific crop diversification provides an ecological approach to disease control that can be highly effective over a large area and contribute to the sustainability of crop production (Zhu *et al.* 2000). Experimental results indicate increased genetic diversity within a cultivar, for example, may stabilize a stand against pathogen invasion or spread (Burdon *et al.* 2006). SCN resistance is multigenic and quantitative, and the inheritance of resistance from Peking fits a three-recessive gene model, with the assigned symbols *rgh1*, *rgh2*, and *rgh3* (Mansur *et al.* 1993; Lu *et al.* 2006a). The fourth gene was reported as a dominant resistance gene and designated as *Rhg4* (Concibido *et al.* 2004). The genetic response of multiple alleles to SCN HG types consists of not only gene actions at single loci but also the inter-locus interactions and gene by environment interactions (Wu *et al.* 2009). It remains uncertain whether gene by environment interactions could condition SCN resistance in recombinant inbred line (RIL) populations derived from same soybean cross under two ecological sites.

Identification of SCN resistance is usually affected by environmental factors. Molecular markers can be used in the indirect selection of traits that are difficult to evaluate and/or largely affected by the environment (Paterson *et al.* 1988). The use of molecular markers is an efficient alternative to the tedious work of genotype evaluation for SCN resistance and allows for an efficient selection of polygenic resistance to SCN (Vierling *et al.* 1996). In this study, two RIL populations, NJ(RN)R7 and JN(RN)R7, which derived from Peking × 7605 under Nanjing (Jiangsu province) and Jinan (Shandong province), were used to estimate genetic effects and gene–environment interactions. SCN is a major pest of soybean in Jinan area, which belongs to the Huang-Huai valleys summer soybean production area in China. To our knowledge, the occurrence of SCN was not found in the Nanjing area in middle and lower Changjiang valley. We hypothesize that (i) resistance to SCN was significantly different between NJ(RN)P7 and JN(RN)P7 populations; and (ii) there is genetic heterogeneity for resistance to SCN between JN(RN)P7 and NJ(RN)P7. The differentiation of the genetic structure of the two RIL populations was identified by comparing their resistances to SCN, which were developed under two ecological sites derived from the cross of Peking × 7605. Further, we investigated the genetic relationships of SCN resistance originating from major SCN resistance genes in the two RIL lines by using SSR markers. Ecological strategy and theoretical foundation were provided to develop cultivars that are tolerant to SCN and RIL populations.

Materials and methods

Plant materials

Populations NJ(RN)P7 and JN(RN)P7 were developed from crossing the cultivars Peking (as female parent) and 7605 (as male parent) in Nanjing and Jinan, China, respectively, were used in this study. The predominant race of SCN in Huang-Huai valleys in China is race 1 (Lu *et al.* 2006b). Peking is resistant to SCN race 1, while 7605 is highly susceptible (table 1 in electronic supplementary material at www.ias.ac.in/jgenet). The importance of Peking in the development of resistant soybean cultivars and its use in the classification system of SCN races warrant its genomic characterization. The original germplasm of Peking was introduced in USA from China in 1906 (Skorupska *et al.* 1994). Peking can be characterized by the following agronomic traits: brown hilum, black seeds and purple flowers. The cultivar 7605 was bred by Shandong Academy of Agricultural Sciences and has the following characteristics: semidwarf, gray hilum, yellow seeds and white flowers. In 1995, Peking and 7605 were crossed in Jinan. The seeds of the F_2 generation were then divided into two in 1997. Generations $F_{2:3}$–$F_{2:7}$ were developed through adding-generation propagation from 1997 to 1999 in Nanjing and Jinan. Seeds were harvested by mixing plants within the family in every generation. Individual $F_{2:7}$ plants were randomly harvested, and $F_{2:7:8}$ generations were advanced. In 2000, two populations of $F_{2:7:8}$ and $F_{2:7:9}$ RILs were developed through adding-generation propagation, which was also applied in the two ecology sites. The population developed in Nanjing includes 217 $F_{2:8:11}$ RILs and designated as NJ(RN)P7; while the population developed in Jinan includes 248 $F_{2:7:9}$ RILs and designated as JN(RN)P7.

Two ecological sites of RILs populations developing and the nematode population level

The population NJ(RN)P7 was planted at experimental station of Nanjing Agricultural University in Nanjing, Jiangsu, located in the southeast China. Nanjing belongs to the north subtropical monsoon climate zone with four seasons, plenty of sunshine and rain. The average temperature of a year is 17.8°C and the amount of precipitation of a year is 1034 mm. The population JN(RN)P7 was grown at experimental field of Crop Institute of Shandong Academy of Agricultural Sciences in Jinan, Shandong, located in the north of China. It lies in the north-temperate zone and has a continental monsoon climate with four distinctive seasons. The annual average temperature is 14.3°C and average annual rainfall is 650–700 mm. Whether literature reports or in this study, the occurrence of SCN was not found in the Nanjing area in middle and lower Changjiang valley. Soybean and rice were rotated every summer season at Nanjing. The predominant race of SCN in the area of Jinan, in Huang-Huai valleys is race 1. At Jinan, field experiments were conducted at five plots numbered as plots 13–17, respectively. The summer crop were

planted as shown in table 2 in electronic supplementary material and the population level of SCN race 1 is described in figures 1 and 2 in electronic supplementary material.

SCN bioassay and data analysis

The SCN bioassay was performed to determine SCN reaction of families in NJ(RN)P7 and JN(RN)P7, this method established protocols by Arelli *et al.* (2000) with some modifications. For SCN bioassay, the soil was taken from plot 16 of Crop Institute of Shandong Academy of Agricultural Sciences (Jinan, Shandong, China) where two RILs populations were planted for investigating agronomic traits in previous year. Collection and culture methods of relative homogeneous SCN population used in this research have been reported in Diers *et al.* (1997). After the soil sample is mixed and dried in shade, cysts are floated and numbered in the diseased soil.

In 2009, SCN reaction was tested in two populations, at a maintained temperature above 26°C in a greenhouse at the Crop Research Institute of the Shandong Academy of Agricultural Sciences. The families of each population and their parents were sowed every 15 d, and two independent experiments were conducted for each population. Differentials or indicator lines included Pickett, Peking, PI88788, PI90763, PI437654, PI209332, PI89772, PI548316 and 7605 (susceptible control). The techniques involved growing plants in 200 mm × 25 mm plastic pots filled with fine sandy diseased soil. For each batch, five seeds of each indictor line and three single seeds per RIL were planted in pots. The pots were then covered with plastic film. Approximately 30 d after germination, nematode cysts were washed from the roots of each RIL and counted.

Female index (FI) was estimated to evaluate the response of resistant and susceptible individuals to each HG type of SCN based on the standard classification system with a specific number of SCN larvae and counting the number of white female cysts on roots (Riggs and Schmitt 1988) using the following formula.

FI = (number of female cyst nematodes on a given individual / average number of female nematodes on 7605) × 100%. FI ≤ 10% was considered a resistant (R) reaction whereas FI >10% was defined as a susceptible (S) reaction. FI was computed as a trait value in our study. Descriptive statistics and analysis of variance were processed by SPSS 16.0 (statistical product and service solutions) software.

Genomic DNA extraction and pooling for bulk segregant analysis

Genomic DNA was isolated from sampled leaves using a modified CTAB procedure (Saghaimaroof *et al.* 1984). Resistant and susceptible bulks for the bulk segregant analyses (BSA) were prepared from DNA samples of 10 homozygous-resistant (resistant bulk) and 10 homozygous-susceptible (susceptible bulk) RIL families of NJ(RN)P7 and JN(RN)P7, respectively (Michelmore *et al.* 1991). BSA was used to identify markers linked to SCN resistance loci in the RILs population.

SSR analysis

PCR amplifications with SSR primers were performed following the protocol of SoyBase (http://www.soybase.org/) with some modifications (Li *et al.* 2006). Each PCR reaction contained ~50 ng genomic DNA, 0.25 μmol/L of each primer, 1 U *Taq* DNA polymerase, 2 μL of 10× PCR buffer containing 15 mmol/L $MgCl_2$, 0.2 mmol/L of dNTPs in a total volume of 25 μL. PCR was performed in a Peltier thermal cycler (PTC-225), at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with final incubation at 72°C for 7 min before cooling to 4°C. PCR product was mixed with one-tenth of the volume of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 40% sucrose) and 3 μL were loaded for electrophoresis in vertical, nondenaturing 8% polyactylamide gels in 1× TBE at 25 W for 80 min, and then viewed by silver staining.

Results

Identification of SCN races and changes of pathogen at Jinan

To identify SCN races, soil samples were taken from Nanjing and Jinan. Neither nematodes nor cysts were found at the Nanjing site. The races of SCN were identified for soil samples from plots 14 and 15 in the Jinan site during 1988–1996 (table 3 in electronic supplementary material). The race status of race 1 did not change in these nine years, but the reproductive capability in Peking and Pickett (differential hosts) slightly increased 0.10 and 0.18 at plot 14 while 0.15 and 0.26 at plot 15, respectively. The reproductive capability in PI90763 showed little change, while the changes of PI88788 were negligent to the race status of race 1.

For SCN bioassay of the population, the parents and differential hosts were used to perform SCN bioassay in the

Table 1. Hosts' reactions to diseased soil during SCN bioassay of NJ(RN)P7 and JN(RN)P7 (Jinan site, 2009).

		Peking		PI88788		PI90763		Pickett			
Population	Batch	AFN	FI	AFN	FI	AFN	FI	AFN	FI	Race	HG type
NJ(RN)P7	First	0.7	R	59.0	S	2.4	R	5.3	R	1	HG 2,5,7
	Second	1.1	R	42.4	S	0.0	R	1.9	R	1	HG 2,5,7
JN(RN)P7	First	0.4	R	66.8	S	0.9	R	4.8	R	1	HG 2,5,7
	Second	4.6	R	42.4	S	0.0	R	5.9	R	1	HG 2,5,7

FI, female index; AFN, average female number; S (susceptible) FI >10%; R (resistant) FI ≤ 10%.

Table 2. Statistical description of FI of the populations NJ(RN)P7 and JN(RN)P7.

Population	Minimum	Maximum	Range	Mean	SD	Variance	CV (%)	Skew	Kurt
NJ(RN)P7	0.68	105.60	104.92	34.06	21.86	477.98	64.19	0.72	0.17
JN(RN)P7	0.00	136.51	136.51	50.73	28.01	784.39	55.20	−0.12	−0.27

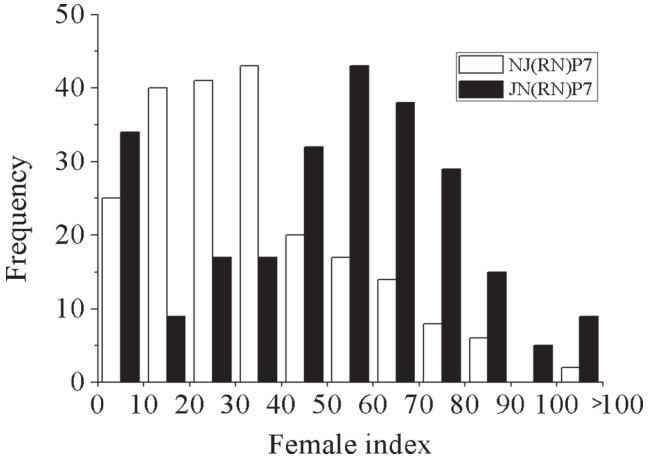

Figure 1. Frequency distribution of the FI for the populations NJ(RN)P7 and JN(RN)P7.

soil sample from Jinan. There were differences in the average female number and the mean of FI at the roots of differential hosts in every batch (table 1). However, based on the Riggs mode, SCN reactions of differential hosts were unique to the diseased soil, indicating that the dominant population in the diseased soil was SCN race 1. Further, the results showed that the independent phenotypes of each population were consistent with the response of the genotypes to HG types 2.5.7.

SCN reactions of the two populations

The FI of the families of the two populations is described statistically in table 2. The range and mean of FI for JN(RN)P7 (136.51 and 50.73, respectively) were greater than those for NJ(RN)R7 (104.92 and 34.06, respectively). The coefficient of variation (CV) of the former (55.20%) was smaller than that of the latter (64.19%). Generally, the symptoms of the population developed in Jinan were more severe than those experienced by the population developed in Nanjing. The resistant variation of the population developed in Nanjing was richer than that of the population developed in Jinan. The Skewness and Kurtosis of the distribution of FI across the entire RIL populations deviated slightly from zero (table 2). This indicates that the frequency distributions of RILs for FI were not normally distributed for race 1 (HG types 2.5.7).

Significant difference was observed in the frequency distributions of FI of the families for the two populations (figure 1). The SCN bioassay indicated that the genetic structure of the population derived from the cross between Peking and 7605 was changed because of the effects of natural selection under the two ecological sites. The frequency of the resistant gene of the population was most likely conditioned due to disease pressure. As a result, the resistance performance of families was significantly different ($\chi^2 = 90.75$, $P < 0.01$).

Table 3. Variance analysis of FI in the populations NJ(RN)P7 and JN(RN)P7.

Source of variation	DF	MS	F
Population	1	64224.30	66.99**
Sowing date	1	24776.04	25.84**
Population × sowing date	1	55120.03	57.49**
Error	924	958.76	

DF, degree of freedom; MS, mean square; F, Fisher's test;
**significant difference at $P<0.01$.

Phenotypic variation in resistance to SCN

The variance analysis of the FI of families in the two populations revealed that resistance performance between NJ(RN)P7 and JN(RN)R7 was significantly different (table 3). This is because the population genetic structure varied between the two populations derived from the crossing Peking and 7605. There was significant variation observed in the FI of the sowing date between the two populations, suggesting that the resistance of families to SCN is significantly affected by the sowing date. Further, the difference of performance in various sowing dates cannot be eliminated by converting female number to FI. There also exists a significant interaction variation between populations and sowing dates.

Genetic analysis of soybean resistance to SCN

In this study, χ^2 tests were performed to test the goodness of fit between the observed values and the expected ratios of the two populations. It is not possible for temperature, moisture and soil water content to be completely consistent between two batches of each population during the growth stage; therefore, two batches of each population were used for the genetic analysis through replication after converting female number to FI. Based on the segregation ratio of the observed and expected values, a three-gene model is fitful to resistance inheritance of the populations (table 4), indicating that resistance is controlled by three pairs of genes.

Test for association between SCN resistance and SSR markers

Thirty-three markers associating with SCN resistance and the other 15 markers on linkage group A2 and G were selected

Table 4. Genetic model analysis of resistance to SCN in the populations NJ(RN)P7 and JN(RN)P7.

Population	Families number	R:S observe	R:S expect	Hypothesized resistance genes	Expect ratio	χ^2	*P*
NJ(RN)P7	216	25:191	13.5:202.5	*rhg1*, *rhg2*, *rhg3*, *Rhg4*	1:15	9.56**	
			27:189	*rhg1*, *rhg2*, *rhg3*	1:7	0.10	0.90–0.75
JN(RN)P7	248	34:214	15.5:232.5	*rhg1*, *rhg2*, *rhg3*, *Rhg4*	1:15	22.30**	
			31:217	*rhg1*, *rhg2*, *rhg3*	1:7	0.23	0.75–0.50

df=1, $\chi^2_{0.05} = 3.84$, $\chi^2_{0.01} = 6.63$. **Significant difference at $P<0.01$.

Table 5. Results of polymorphism between resistant and susceptible DNA bulks for NJ(RN)P7.

Marker	LG	Position	Interval	Locus	Race	Cross	Reference
Satt333	A2	119.59			1	Peking × 7605	
Satt453	B1	123.96	Satt453–Satt359		2,5	PI90763× Hamilton	Guo *et al.* (2005)
Satt359	B1	102.56					
Satt168	B2	55.20	Satt168–A329	unknown	1,3	Hamilton × PI438489B	Yue *et al.* (2001a)
Satt163	G	0	Satt038–Satt163	*rgh1*	3	Y23 × Hartwig	Cervigni *et al.* (2004)
			Satt163–Satt688	*rgh1*	2,3,5	PI90763 × Hamilton	Guo *et al.* (2005)
Satt309	G	4.53	B053–Satt309	*rgh1*	1,2,3,5	Hamilton × PI89772	Cregan *et al.* (1999)

Table 6. Results of polymorphism between resistant and susceptible DNA bulks for JN(RN)P7.

Marker	LG	Position	Interval	Locus	Race	Cross	Reference
Sat_406	A2	25.90	Satt632–Sat_406		1	PI404198A× Magellan	Guo *et al.* (2006)
Satt368	D1a	43.84	Satt342–Satt368		5	Hamilton × PI89772	Yue *et al.* (2001b)
Satt163	G	0	Satt038–Satt163	*rgh1*	3	Y23 × Hartwig	Cervigni *et al.* (2004)
			Satt163–Satt688	*rgh1*	2,3,5	PI90763 × Hamilton	Guo *et al.* (2005)
Sat_210	G	3.70	Sat_210–Sat-168	*rhgR1-1*	1	(Essex × ZDD2315) × ZDD2315	Lu *et al.* (2006c)
Satt309	G	4.53	B053–Satt309	*rgh1*	1,2,3,5	Hamilton × PI89772	Cregan *et al.* (1999)
Sat_141	G	9.18	Sat_168–Sat_141	*rhgR4-2*	1	(Essex × ZDD2315) × ZDD2315	Lu *et al.* (2006c)

as candidate markers (table 4 in electronic supplementary material). Forty-eight candidate markers were screened between resistant and susceptible DNA bulks for NJ(RN)P7, and six markers (Satt333, Satt453, Satt359, Satt168, Satt163 and Satt309) were performed for polymorphism between resistant and susceptible bulks (table 5). Marker Satt168 was related to race 1 and race 3, while markers Satt163 and Satt309 were related to *rgh1*, which is a major resistant gene for SCN. Marker interval Satt453–Satt359 was revealed for polymorphism and associated with resistance to races 2 and 5. Meanwhile, marker Satt333 was tested for polymorphism between resistant and susceptible bulks, which can be seen on linkage group A2, and had not been reported previously with relation to SCN.

For JN(RN)P7, six markers (Sat_406, Satt368, Satt163, Sat_210, Satt309 and Sat_141) showed polymorphism (table 6). Four SSR markers (Sat_406, Sat_210, Satt309 and Sat_141) were associated with resistance to SCN race 1. Moreover, marker Satt368 was tested for polymorphism, and had been associated to race 5. From the results, polymorphism markers Satt163 and Satt309 were detected in the two populations, and the two were related to *rgh1*.

Discussion

We have hypothesized that resistance to SCN was significantly different between NJ(RN)P7 and JN(RN)P7 populations. In this study, χ^2 test showed that the severity of disease in the two populations was different. In comparison, the SCN symptom of JN(RN)P7 that developed at the Jinan site was more serious. From the results of the variance analysis, the disease severity of JN(RN)P7 was proved to be significantly different from NJ(RN)P7. The populations of NJ(RN)P7 and JN(RN)P7 were derived from the same soybean cross under two ecological sites. The vital difference between the two ecological sites is that SCN is a major pest of soybean in Jinan, whereas the occurrence of SCN was not found in the Nanjing area, with the exception of different climatic conditions. Natural genetic heterogeneity may lead

to this variation, which is the result of selection pressures that are created by environmental conditions. Therefore, SCN most likely acts as a selective force on soybean resistance characteristics for the two RIL populations.

Previous research suggested that the resistance difference of soybean varieties can be demonstrated if the number of cysts is more than 20 per 100 g soil (Wu *et al.* 1984). Our research showed that a significant regression between the mean of females on 7605 roots (y) and the mean of eggs and second-stage juveniles (J2s) per 100 g of soil (x) was fitted as [Ln(y+1) = 0.6628Ln(x+1) – 1.3994, r = 0.9742**]. According to the concept of significant regression, the lowest limit of high susceptibility could be speculated for 7605. The mean of the sum of eggs and J2s per 100 g soil were 1475.2 when the average female number per plant on 7605 was 30.1. The sum of eggs and J2s per 100 g of soil went up to 6998.9 when the lower confidence interval of 7605 was an average 30.1 of female nematodes per plant. Therefore, difference of soybean resistance to SCN could be demonstrated for SCN bioassay when the sum of eggs and J2s was more than 6998.9. The average number of female nematodes per plant on 7605 was 162.4 from 1988 to 1996, indicating that the number of pathogens was sufficient to act as a selective force on plant resistance characteristics (table 3). Findings of the present study support our first hypothesis and imply that the resistant difference is caused partly by genetic heterogeneity of the two populations.

The genetic variation of resistance could be analysed in terms of pathogens and host plants. SCN is a population phenotype while the genotype of the pathogen could be distinguished by the mode 'HG type' (Niblack *et al.* 2002). As both phenotype and genotype pathogens were consistent for the two independent SCN bioassays of each population in this study, the SCN population was regarded as relatively homogeneous. The genotype of pathogen could not be compared with the phenotype results of our previous research (from 1988 to 1996). In addition, it was impossible for the prevalent population of SCN in 2009 to be completely consistent with the initial period when the crossing of Peking and 7605 was conducted in 1995. However, the impact on the results, which caused by the artificial cut-off point in resistance and susceptibility cannot be eliminated in the genetic analysis. In this study, the continuing variation in data was treated as a quality trait and χ^2 test was performed to determine the inheritance model. However, the genetic system of the quantitative traits of plants was not used for the analysis because a number of major genes were limited (Wang and Gai 2001). From the perspective of the genetic analysis of resistance to SCN, there seemed to be little difference in the resistance between NJ(RN)P7 and JN(RN)P7. Nevertheless, the real difference was evident on the resistance performance of the frequency distribution of the FI. In addition, coevolution may also exist between the pathogens and host plants. One of the main reasons of genetic difference in resistance was the difference in the genetic structure between the two populations due to natural selection.

A total of 684 microsatellite markers that spans 20 chromosomes of the soybean genome (Song *et al.* 2004) were used to analysis in this study, and 210 markers showed polymorphism between Peking and 7605. According to the previous researches (Concibido *et al.* 2004; Glover *et al.* 2004), 33 SSR markers were associated with SCN resistance. The resistance genes on linkage groups G and A2 of the soybean genetic map are especially important (Webb *et al.* 1995; Concibido *et al.* 1996). Based on the marker polymorphism, 10 unique markers were screened against resistant or susceptible DNA bulks and two markers were detected in the both populations. At the same time, marker Sat_141 associated with *Rhg4*, a major gene related to SCN resistance, were not tested for polymorphism in NJ(RN)P7. However, a possible explanation for this is the case of candidate markers having no complete genome coverage, which may have caused some markers associated with resistance to be missed (Concibido *et al.* 2004). Nevertheless, the results of our study indicate that there is genetic heterogeneity for resistance to SCN between JN(RN)P7 and NJ(RN)P7, supporting our second hypothesis. Both theory and observation indicate that genetic heterogeneity provides greater disease suppression (Garrett and Mundt 1999; Zhu *et al.* 2000). The success of modern agriculture is partly due to the biological diversity available in nature. This variation provides the resources that we can make selections for the genetic improvement of crops. The resistance performances of the two populations and the tolerance of soybean to SCN are significantly different due to genetic heterogeneity.

Quantitative trait loci (QTL) mapping is a powerful tool to identify genomic regions responsible for important agronomic traits. Molecular markers, such as SSR, SNP, that are tightly linked to the desired QTL can be applied in marker-assisted selection breeding to improve and shorten the process of developing resistant cultivars. To date, many QTL conferring resistance to SCN in soybean have been identified on the chromosomes (Concibido *et al.* 2004; Yamasaki *et al.* 2005; Ellegren and Sheldon 2008; Wu *et al.* 2009; Jiao *et al.* 2015). Among these, two QTL, *rhg1* on linkage group (LG) G (chr. 18) and *Rhg4* LG A2 (chr. 8) (Concibido *et al.* 2004), were mapped in various sources of resistance. The soybean *rhg1* (resistance to *H. glycines*) consistently contributes much more effective SCN resistance than any other known loci (Concibido *et al.* 2004). The SCN resistance conferred by *rhg1* in PI88788 was found to be controlled by three genes (Cook *et al.* 2012). *Rhg4* in Forrest encodes a serine hydroxymethyltransferase that is essential for cellular one-carbon metabolism (Liu *et al.* 2012). A number of major and minor QTLs associated with SCN resistance to different races have been mapped, but only a few loci, including *rhg1* and *Rhg4*, are extensively used in marker-assisted selection. This is due to the limited coverage of linkage maps for QTL analysis, inadequate statistical procedures, small mapping populations, and the fact that the epistatic effects among resistance loci have been ignored (Wu *et al.* 2009). Therefore, the results of this study demonstrate that

an ecological strategy to disease control could be used to effectively develop cultivars that are tolerant to SCN and RIL populations.

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