# Response of soybean genotypes to *Meloidogyne incognita* and *M. hapla* in Heilongjiang Province in China

**Chunjie Li[1], Cui Hua[1], Yanfeng Hu[1], Jia You[1], Yanzhi Mao[1], Jianying Li[2], Zhongyan Tian[2] and Congli Wang[1]**

**[1]Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, 138 Haping Rd., 150081, Harbin, China**
**[2]Daqing Branch of Heilongjiang Academy of Agricultural Sciences, Huoju New Street, 163000, Daqing, China**
**e-mail: wangcongli@iga.ac.cn**

Accepted for publication 9 July 2016

**Summary.** Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the economically important root parasites of soybean (*Glycine max*). RKN has been reported to infect soybean in central China, but no information existed about its presence in northern China, the major soybean production area. The northern RKN (*M. hapla*), however, was recently found in vegetable fields in Liaoning Province in northern China. The southern RKN (*M. incognita*) was reported under protected cultivation of vegetables in northern China, where the soybean cyst nematode (SCN) is the known damaging parasite on soybean. Although host-plant resistance is the best cost-effective strategy to control nematodes, no knowledge was available about the response of local soybean cultivars to RKN infection. To identify potential resistance sources of soybean to RKN, eleven local varieties originally bred for resistance to SCN race 3, five local cultivars susceptible to SCN race 3 and additional uncharacterised germplasms were inoculated with both *M. incognita* and *M. hapla* under controlled glasshouse conditions. The root galling index (GI) (0-10 scale), number of egg masses per gram of root (EM), eggs per gram of root (EGR) and nematode reproduction factor (Rf value) were used to evaluate how the different soybean genotypes reacted to RKN. The results indicated that out of 21 genotypes tested, 11 and 8 genotypes showed low GI (0-3) for *M. incognita* and for *M. hapla*, respectively. Low nematode reproduction ($Rf \leq 1$) was observed on 13 and 3 genotypes for *M. incognita* and for *M. hapla*, respectively, suggesting most local soybean cultivars are better hosts for *M. hapla* than *M. incognita*. The distribution of GI, EM, EGR and Rf for *M. incognita* and *M. hapla* suggested that they have different infection mechanisms. The great GI but with low number of nematode reproduction or low GI with high number of nematode reproduction on soybean suggested the genetic independence of resistance from root galling and/or nematode reproduction. Identification of resistant or tolerant varieties would be valuable resources for broad-based resistance breeding against RKN.

**Key words:** *Glycine max*, host-plant resistance, root-knot nematodes.

Root-knot nematodes (RKN, *Meloidogyne* spp.) are economically important pests worldwide with broad-host range including soybean (*Glycine max* L. Merr) (Nicol *et al.*, 2011). In particular, the southern RKN [*Meloidogyne incognita* (Kofoid & White) Chitwood] is one of the predominant pests on soybean and threatens soybean production in the USA and South Africa (Luzzi *et al.*, 1987; Wrather *et al.*, 1995; Tamulonis *et al.*, 1997a; Fourie *et al.*, 1999, 2013, 2015; Wrather & Koenning, 2006). *Meloidogyne arenaria* and/or *M. javanica* are also responsible for soybean yield losses in southern USA and South Africa (Luzzi *et al.*, 1987; Tamulonis *et al.*, 1997b, c; Fourie *et al.*, 2015). Information on the northern RKN, *M. hapla*, infection of soybeans, however, was not available.

In China, the northeast and central regions are major soybean producing areas. *Meloidogyne incognita* race 1 and *M. arenaria* race 2 were identified as the dominant species and races in soybean fields in the central regions with warm climate (Chen & Chen, 1989, 1990). Four species of RKN, *M. incognita*, *M. arenaria*, *M. javanica* and *M. bauruensis*, were found in Fujian Province of south China, with *M. arenaria* being the dominant species on soybean (Zhang, 1993). With green vegetables in huge demand throughout China, the increasing acreage of protected cultivation or glasshouses for vegetable production has enabled RKN to spread towards northeast China (including Liaoning, Jilin and Heilongjiang Provinces), where the winter climate is very harsh. Recently, *M. hapla*, that causes significant agricultural damage in cool climates (Walters & Barker, 1994), was found in vegetable fields in Liaoning Province (Zhao *et al.*, 2010), and *M. incognita*, with distribution typically in warmer climates, was identified in protected cultivation or glasshouses in both Liaoning Province (Zhao *et al.*, 2010) and Heilongjiang Province (Li *et al.*, 2016).

Crop rotation combined with deployment of different sources of resistant cultivars is a cost-effective and environmentally benign strategy to control pests and pathogens including nematodes. Screening of soybean for resistance against *M. incognita*, *M. javanica* and *M. arenaria* have been conducted in the USA and South Africa (Luzzi *et al.*, 1987, 1994a, b; Hussey *et al.*, 1991; Harris *et al.*, 2003; Fourie *et al.*, 2010, 2015; Yates *et al.*, 2010; Lee *et al.*, 2015); however, no such screens have been performed in China to evaluate local soybean cultivars. Since the soybean cyst nematode (SCN) is one of major pests on soybean in northeast China, major efforts have been dedicated for screening and breeding resistance against SCN (Li *et al.*, 1998; Tian *et al.*, 2007; Wu *et al.*, 2011; Yu *et al.*, 2013). However, little was known about the response of these local SCN-resistant and SCN-susceptible cultivars, as well as additional uncharacterised soybean germplasms to RKN. Therefore, the objective of this study was to evaluate these soybean resources with *M. incognita* and *M. hapla*.

## MATERIAL AND METHODS

**Plant materials.** Plant genotypes used in this study included 11 cultivars (Kangxian2, Kangxian4-13) originally developed for resistance to SCN race 3 (Li *et al.*, 1998; Tian *et al.*, 2007; Wu *et al.*, 2011; Yu *et al.*, 2013), five main local soybean cultivars (Hefeng25, Hefeng50, Suinong14, Dongsheng1 and Heinong35) susceptible to SCN races in Heilongjiang Province and additional uncharacterised soybean germplasm typically used for SCN-race identification (Pickett, Peking, PI88788, PI 90763 and Lee68).

**Nematode resistance screening.** A culture of *M. incognita* race 1, originally isolated from tomato plants growing in a glasshouse in Daqing city, Heilongjiang Province, China, was identified in the laboratory using host range and molecular markers (Li *et al.*, 2016). A culture of *M. hapla* was provided by Dr Yuxi Duan, Shenyang Agricultural University (Liaoning Province). China, and confirmed by molecular marker analysis (Li *et al.*, 2016). Nematodes were maintained and multiplied on tomato cv. Zhongshu 4 under controlled glasshouse conditions at 22-28° and 16 h daylight.

Two soybean seeds were planted into 11-cm-diam. × 9-cm-deep plastic pots filled with sandy soil (sand:soil = 2:1). After germination, seedlings were thinned keeping only one plant in each pot. Nematode inoculum was prepared by extracting eggs from tomato roots with NaOCl (Hussey & Barker, 1973). Seven-day-old seedlings were inoculated with approximately 5,000 RKN eggs. The hatch rate for each experiment was calculated by *in vitro* hatching tests at four days to be at about 25%. Five plants were used for each genotype and plants were randomly arranged in a complete block design on a bench in the glasshouse at 22-28°C. Plants were watered daily and fertilised every 2 weeks.

Plants were evaluated at 42 days and 45 days after inoculation for *M. incognita* and *M. hapla*, respectively. Since there were no known soybean varieties resistant or susceptible to *M. incognita* and *M. hapla* used in our screens, the evaluation standard for galling index was followed as described by Bridge & Page (1980) and Wang *et al.* (2006). The index for root galling (GI) was evaluated on a 0-10 scale: 0 = no symptom; 1 = few small galls; 2 = small galls with less than 10% of roots infected; 3 = 10% to 30% of roots infected; 4 = 31% to 40% of roots infected; 5 = 51% to 60% of roots infected, galling on parts of main roots; 6 = 61% to 70% of roots infected, galling on main roots; 7 = 71% to 80% of roots infected, majority of main roots galled; 8 = 81% to 100% of roots infected, all main roots galled; 9 = all roots severely galled and plant usually dying; 10 = all roots severely galled with diminished root system and plant usually dead (Bridge & Page, 1980; Wang *et al.*, 2006). Based on 0-10 scale, $GI \leq 3$ was classified as resistant, $3 < GI < 5$ as medium resistant, $\geq 5$ as susceptible. Host suitability for the two species of nematodes was also evaluated with the number of egg masses per gram of root (EM), which was counted after roots were stained with erioglaucine (Omwega *et al.*, 1988). After counting the egg masses, eggs from soybean roots were extracted by NaOCl (Hussey & Barker, 1973) and the number of eggs per gram root (EGR) was calculated. Nematode reproduction factor (Rf) values were determined by calculating the ratio of final egg number to initial egg number (5,000 eggs) for each plant (Fourie *et al.*, 1999). $Rf < 1$ was

**Table 1.** Response of soybean genotypes to *Meloidogyne incognita* and *M. hapla* in galling index (GI), egg masses g root$^{-1}$ (EM), eggs g root$^{-1}$ (EGR) and nematode reproduction factor (Rf) (mean ± S.E.).

| Genotype | *M. incognita* | | | | *M. hapla* | | | |
|---|---|---|---|---|---|---|---|---|
| | GI | EM | EGR | Rf | GI | EM | EGR | Rf |
| | Cultivars resistant to soybean cyst nematode (SCN) race 3 | | | | | | | |
| Kangxian2 | 5.4 ± 0.24 | 9.9 ± 3.74 | 22461 ± 4285.2 | 11.6 ± 2.16 | 5.8 ± 0.34 | 38.7 ± 8.68 | 21098 ± 5887.2 | 24.0 ± 7.45 |
| Kangxian4 | 4.2 ± 0.37 | 4.3 ± 3.04 | 14145 ± 3645.1 | 6.4 ± 1.60 | 6.0 ± 0.16 | 40.0 ± 13.07 | 23652 ± 5181.0 | 23.3 ± 4.42 |
| Kangxian5 | 0.2 ± 0.20 | 0 ± 0.00 | 2507 ± 2210.8 | 0.3 ± 0.22 | 3.6 ± 0.24 | 3.3 ± 1.37 | 1863 ± 427.5 | 2.0 ± 0.42 |
| Kangxian6 | 6.6 ± 0.24 | 3.1 ± 0.63 | 6535 ± 1510.3 | 2.1 ± 0.79 | 2.8 ± 0.20 | 2.1 ± 0.66 | 2126 ± 653.7 | 2.6 ± 0.83 |
| Kangxian7 | 2.2 ± 0.73 | 5.5 ± 3.70 | 3319 ± 1341.5 | 0.9 ± 0.46 | 5.7 ± 0.51 | 31.9 ± 11.78 | 16950 ± 6851.3 | 15.5 ± 6.26 |
| Kangxian8 | 0.6 ± 0.24 | 0.1 ± 0.10 | 1104 ± 398.3 | 0.5 ± 0.18 | 2.1 ± 0.64 | 5.2 ± 4.25 | 5647 ± 4101.2 | 5.3 ± 3.92 |
| Kangxian9 | 0.6 ± 0.40 | 0.1 ± 0.08 | 1291 ± 796.0 | 0.7 ± 0.43 | 4.8 ± 0.20 | 29.2 ± 2.20 | 13899 ± 1375.5 | 15.7 ± 1.58 |
| Kangxian10 | 0.6 ± 0.24 | 0.1 ± 0.08 | 746 ± 218.8 | 0.4 ± 0.11 | 5.0 ± 0.16 | 15.8 ± 3.96 | 8683 ± 1103.1 | 9.8 ± 1.52 |
| Kangxian11 | 3.6 ± 0.24 | 1.1 ± 0.77 | 4252 ± 590.6 | 1.9 ± 0.31 | 2.9 ± 0.24 | 4.3 ± 2.12 | 2898 ± 1067.4 | 2.6 ± 1.01 |
| Kangxian12 | 0.6 ± 0.24 | 0 ± 0.00 | 744 ± 354.3 | 0.4 ± 0.19 | 3.5 ± 0.39 | 1.8 ± 0.41 | 1552 ± 280.6 | 2.2 ± 0.40 |
| Kangxian13 | 0.6 ± 0.24 | 0.2 ± 0.12 | 629 ± 405.9 | 0.3 ± 0.14 | 3.2 ± 0.37 | 2.1 ± 0.26 | 1787 ± 365.3 | 2.1 ± 0.43 |
| | Cultivars susceptible to SCN race 3 | | | | | | | |
| Heinong35 | 3.8 ± 0.19 | 1.2 ± 0.95 | 9919 ± 2999.9 | 3.3 ± 1.13 | 5.3 ± 0.19 | 28.1 ± 2.29 | 13408 ± 252.0 | 11.4 ± 2.14 |
| Dongsheng1 | 3.6 ± 0.24 | 0.7 ± 0.40 | 6723 ± 1246.9 | 2.6 ± 0.45 | 5.4 ± 0.29 | 20.4 ± 9.64 | 13058 ± 2566.4 | 13.4 ± 2.67 |
| Hefeng25 | 0 ± 0.00 | 0 ± 0.00 | 1017 ± 808.4 | 0.2 ± 0.11 | 2.2 ± 0.20 | 3.3 ± 1.60 | 1718 ± 651.7 | 1.5 ± 0.56 |
| Hefeng50 | 0 ± 0.00 | 0 ± 0.00 | 83 ± 83.4 | 0 ± 0.02 | 4.2 ± 0.34 | 26.8 ± 12.10 | 9959 ± 2728.0 | 8.0 ± 2.48 |
| Suinong14 | 1.0 ± 0.32 | 0 ± 0.00 | 173 ± 108.1 | 0 ± 0.02 | 2.9 ± 0.37 | 4.4 ± 2.36 | 1252 ± 314.9 | 0.8 ± 0.23 |
| | Other soybean germplasms | | | | | | | |
| PI90763 | 7.6 ± 0.24 | 14.7 ± 5.21 | 25324 ± 14859.6 | 12.1 ± 7.03 | 6.2 ± 0.58 | 49.2 ± 10.65 | 12933 ± 3866.2 | 14.0 ± 5.73 |
| PI88788 | 0.2 ± 0.20 | 0 ± 0.00 | 1050 ± 414.6 | 0.3 ± 0.10 | 6.5 ± 0.50 | 42.8 ± 9.80 | 13569 ± 22.0 | 9.7 ± 3.88 |
| Picket | 6.4 ± 0.24 | 0.8 ± 0.48 | 364 ± 192.7 | 0.2 ± 0.12 | 2.4 ± 0.43 | 2.0 ± 0.28 | 937 ± 423.5 | 0.5 ± 0.11 |
| Peking | 6.4 ± 0.24 | 2.7 ± 1.01 | 9096.6 ± 1497.79 | 5.7 ± 1.02 | 2.0 ± 0.45 | 1.0 ± 0.34 | 1148 ± 306.0 | 1.5 ± 0.46 |
| Lee68 | 5.2 ± 0.20 | 0.3 ± 0.18 | 282.3 ± 149.51 | 0.2 ± 0.09 | 1.8 ± 0.49 | 1.3 ± 0.33 | 804 ± 217.8 | 1.0 ± 0.29 |

classified as poor host (Fourie *et al.*, 1999) or resistant (Windham & Williams, 1988). This experiment was repeated twice.

**Statistical analysis.** Data were subjected to one-way analysis of variance (one-way ANOVA) using SPSS software (SAS Institute, Cary, NC, USA). Results are reported as significant or non-significant in Tukey's Honestly Significant Difference (Tukey HSD) test ($P \leq 0.05$).

## RESULTS

**Response of soybean genotypes to *Meloidogyne incognita*.** Widely different responses ($P \leq 0.05$) to *M. incognita* were observed among the cultivars resistant and susceptible to soybean cyst nematodes and the uncharacterised soybean germplasms (Table 1). Among the 21 tested soybean genotypes, mean root GI ranged from 0-7.6, EM per gram of root ranged from 0-9.9 and EGR ranged from 83-22461, while RF ranged from 0-11.6 (Table 1). Similar results were obtained in the two replicated experiments and data from one experiment are presented.

**Cultivars resistant to SCN race 3.** Among the 11 soybean cultivars resistant to SCN race 3, GI ranged from 0.2-6.6, EM 0-9, EGR 629-22461 and Rf 0.3-11.6 (Table 1). The order of GI from greatest ($P \leq 0.05$) to smallest was Kangxian6 > Kangxian2 > Kangxian4 > Kangxian11 > Kangxian7 > Kangxian5, 8, 9, 10, 12, 13. Kangxian2 and Kangxian4 showed significantly greater EM, EGR and Rf ($P \leq 0.05$) than Kangxian5, 8, 9, 10, 12, 13 (Table 1). Seven soybean cultivars displayed low GI (≤ 3) and low Rf (< 1).

**Cultivars susceptible to SCN race 3.** Among the 5 local soybean cultivars susceptible to SCN mean

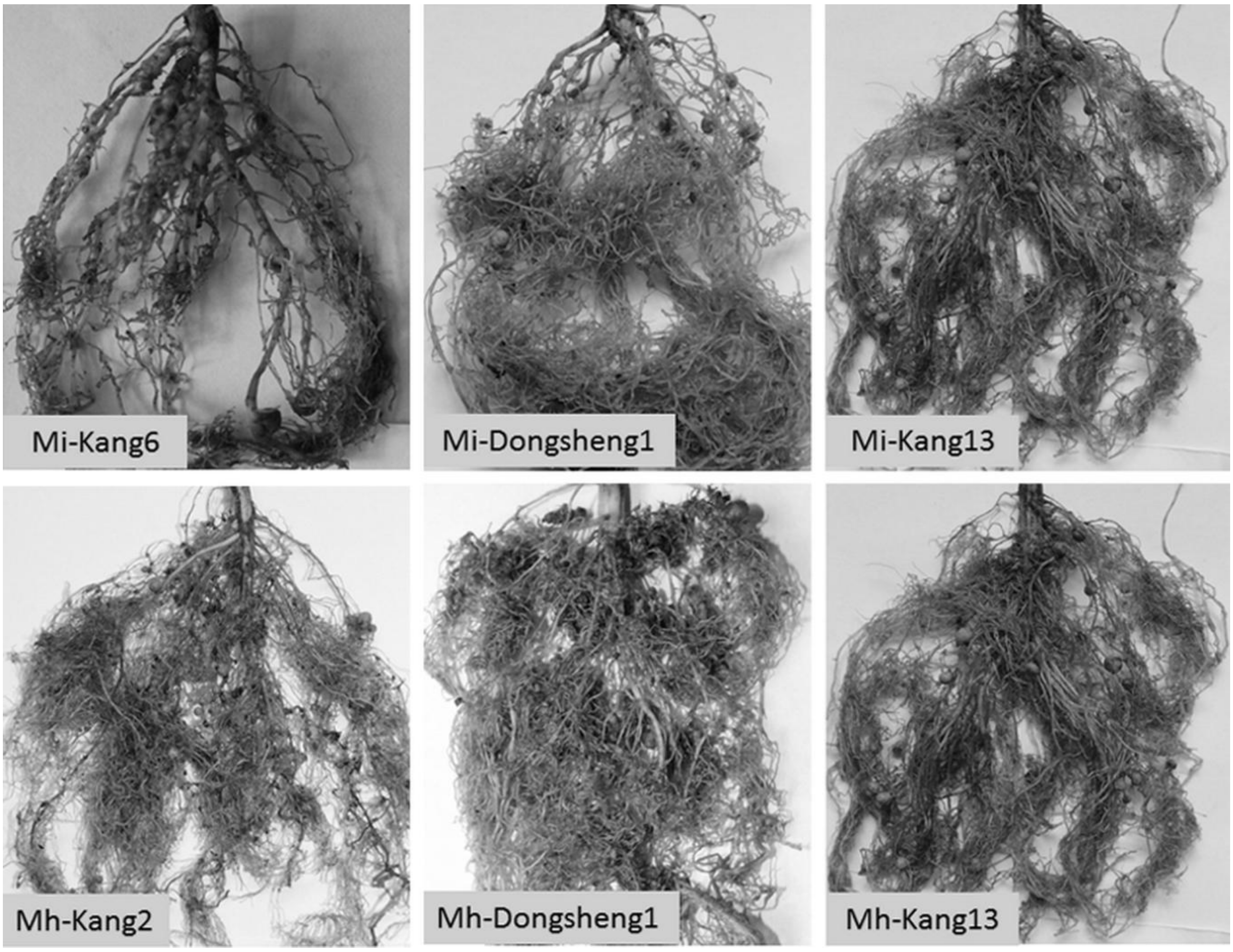


**Fig. 1.** Root-galling symptom and egg mass production of *Meloidogyne incognita* (Mi) and *M. hapla* (Mh) on different local soybean cultivars in Heilongjiang Province. Egg masses are stained blue with erioglaucine. Kang2, Kang6 and Kang13 mean Kangxian2, Kangxian6 and Kangxian13.

root GI ranged from 0-3.8, EM 0-1.2, EGR 83-9919 and Rf 0-3.3. The GI (range 0-1.2) observed for cvs Hefeng25, Hefeng50 and Suinong14 were significantly lower than those for Heinong35 and Dongsheng1 ($P \leq 0.05$) (Table 1). Heinong35 showed significantly greater number of EGR than cvs Hefeng25, Hefeng50 and Suinong14 but were not significantly different from cv. Dongsheng1 ($P \leq 0.05$) (Table 1). There was no significant difference ($P \leq 0.05$) in EM and Rf among the five local soybean varieties.

**Other soybean germplasms.** PI90763 was highly susceptible to *M. incognita* based on GI, EM, EGR and Rf (Table 1). Cultivars Lee68 and Picket were susceptible to *M. incognita* according to GI but had lower nematode reproduction as determined by EM, EGR and Rf, indicating that root galling response was not always positively correlated to nematode reproduction.

**Response of soybean genotype to *Meloidogyne hapla*.** Various ($P \leq 0.05$) responses to *M. hapla* were observed among the tested soybean genotypes (Table 1). Among the 21 soybean genotypes tested, GI ranged from 1.8-6.5, EM 1-40, EGR 803-23652, and Rf 0.5-24 (Table 1).

**Cultivars resistant to SCN race 3.** Among the 11 soybean cultivars resistant to SCN tested, mean root GI ranged from 2.1-6.0, EM 1.8-40, EGR 1552-23651, Rf 2.0-24 (Table 1). Cultivar Kangxian4 had no significant difference in GI, EM, EGR and Rf from cvs Kangxian2, 7, 9, 10, but with greater number in GI, EM, EGR and Rf than cvs Kangxian5, 6, 8, 11, 12, 13 ($P \leq 0.05$).

**Cultivar susceptible to SCN race 3.** Among the five local soybean cultivars susceptible to SCN, mean root GI ranged from 2.2-5.4, EM 3.3-28.1, EGR 1252-13408 and Rf 0.8-13.4 (Table 1). Cultivars Hefeng25 and Suinong14 showed significantly

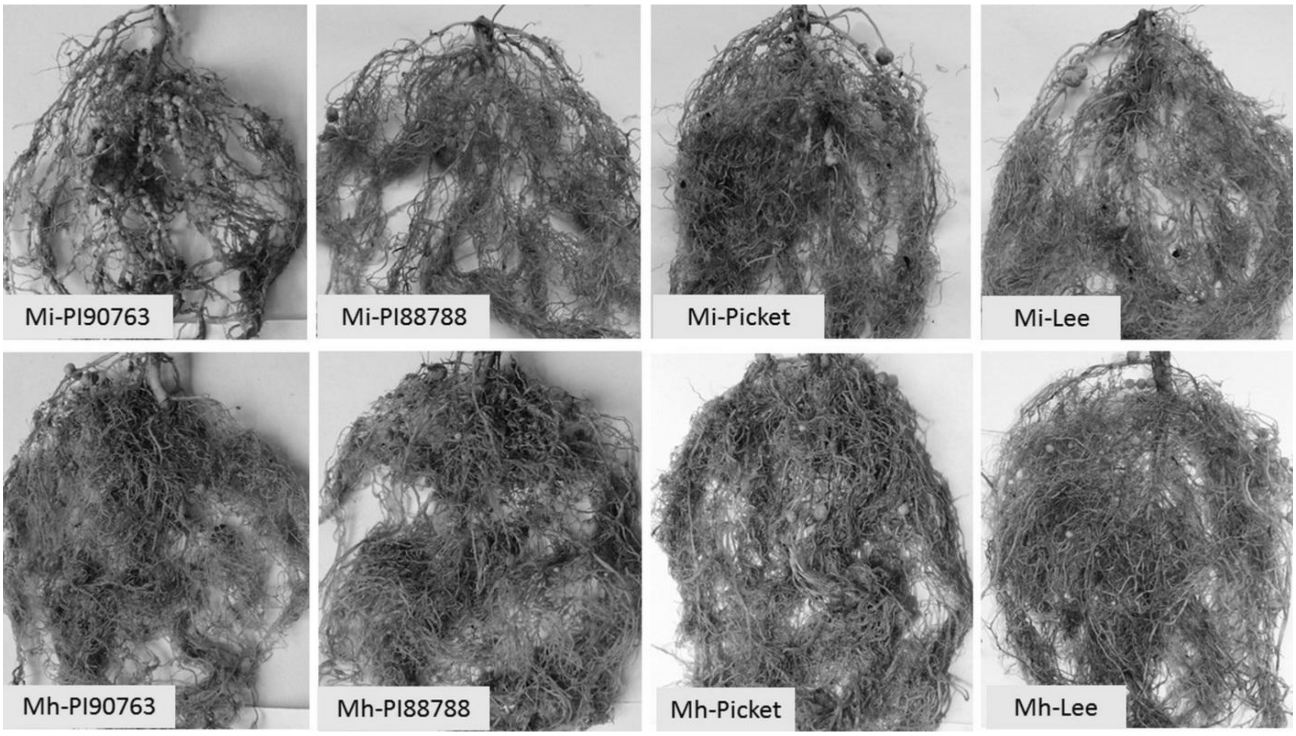


**Fig. 2.** Root-galling symptom and egg mass production of *Meloidogyne incognita* (Mi) and *M. hapla* (Mh) on other soybean germplasms. Egg masses are stained blue with erioglaucine.

lower GI, EM, EGR and Rf than the other three genotypes ($P \leq 0.05$).

**Other soybean germplasms.** Cultivars Picket, Peking and Lee68 showed lower GI, EM, EGR and Rf compared to PI90763 and PI88788 ($P \leq 0.05$).

**Comparison of root galling index and nematode reproduction for *Meloidogyne incognita* and *M. hapla* on 21 soybean genotypes.** Large sized galls were developed on soybean roots (cvs Kangxian6 and Dongsheng1) infected with *M. incognita*. However, the large size galls were typically associated with lower numbers of EM and EGR to *M. incognita* than the root response to *M. hapla* (Table 1; Figs 1 & 2). For example, cv. Kangxian6 showed big galls on roots when infected with *M. incognita* but lower EM (3.1), EGR (6535) and Rf (2.1) values. By contrast, galls on soybean cv. Kangxian2 infected with *M. hapla* were typically small in size with higher EM (38.7), EGR (21098) and Rf (24.0) values (Table 1; Figs 1 & 2). Clearly distinct root symptoms were observed on the roots of the uncharacterised soybean germplasms infected with *M. incognita* and *M. hapla* (Fig. 2). Big galls by *M. incognita* and small galls with higher EM, EGR and Rf values by *M. hapla* appeared on PI90763 (Table 1; Figs 1 & 2). PI88788 showed lower GI (0.2), EM (0), EGR (1050) and Rf (0.3) values to *M. incognita* but greater values for the four measured indices (GI 6.5; EM 42.8; EGR 13569 and Rf 9.7) to *M. hapla*. Soybean cv. Peking showed the opposite phenotype, greater value for GI 6.4; EM 2.7; EGR 9097 and Rf 5.7 to *M. incognita* and lower value for GI 2.0; EM 1; EGR 1148 and Rf 1.5 to *M. hapla*. Based on GI, cvs Picket and Lee68 showed susceptibility to *M. incognita* but resistance to *M. hapla* (Fig. 2). By contrast, based on EM, EGR and RF indices, cvs Picket and Lee68 were resistant to both *M. incognita* and *M. hapla* (Table 1).

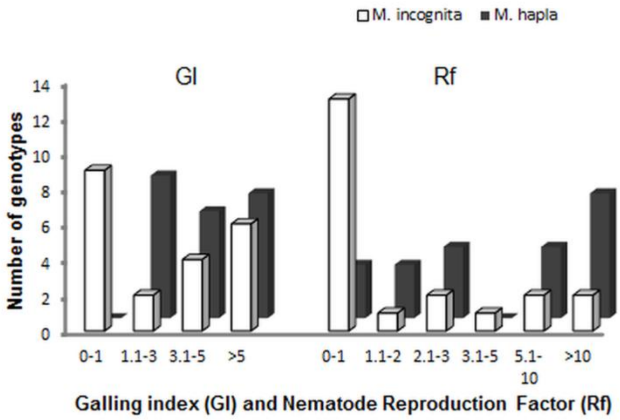


**Fig. 3.** Distribution of galling index (GI) and nematode reproduction factor (Rf) for *Meloidogyne incognita* and *M. hapla* among 21 soybean genotypes.

A comparison of root response and reproduction for *M. incognita* and *M. hapla* among the 21 soybean genotypes tested appears in Fig. 3. Eleven and eight genotypes showed low GI (0-3) for *M. incognita* and *M. hapla*, respectively (Fig. 3). Low *M. incognita* reproduction (Rf ≤ 1) occurred on 13 genotypes and three for *M. hapla*. Moreover, four genotypes showed Rf > 5 for *M. incognita* and 11 showed Rf > 5 for *M. hapla* (Fig. 3). Combined these results indicated that most of the local SCN varieties were better hosts for *M. hapla* compared to *M. incognita*.

## DISCUSSION

This is the first study to evaluate the response of local SCN-resistant/susceptible soybean genotypes to RKN in northern China. Six SCN-resistant (Kangxian5, 8, 9, 10, 12 and 13) and three SCN-susceptible cultivars (Hefeng25, Hefeng50 and Suinong14) showed poor host suitability for *M. incognita* with very low GI and Rf (both less than 1) (Table 1). Three SCN-resistant cultivars (Kangxian6, 8, and 11) displayed low GI (2.1-2.9) but supported high nematode reproduction (Rf = 2.6-5.3) by *M. hapla* and only one SCN-susceptible cultivar (Suinong14) displayed a low Rf value (< 1) and low GI (2.9). All SCN-resistant cultivars showed Rf greater than 2 indicating that these local soybean cultivars are good hosts for *M. hapla*, which is adapted to a cold climate compared to *M. incognita*. The potential of an outbreak by *M. hapla* in Heilongjiang Province is high. If this happens, no highly resistant soybean cultivars to both SCN and RKN are available for cultivation in this region. Therefore, additional screens of soybean cultivars and germplasms to *M. hapla* are necessary to identify resistance sources for regions of northern China. Furthermore, these soybean screens with both *M. incognita* and *M. hapla* could provide valuable information for breeding resistance against RKN in combination with resistance to SCN. Breeding for combined resistance for SCN and RKN is necessary as our results suggest that different resistance mechanisms exist against SCN and RKN species.

In most cases of nematode-host plant interactions, major disease resistance genes suppress both root galling and nematode reproduction (Roberts, 1995). In some of the soybean genotypes we tested, we were able to identify such characteristics. However, contrasting combination of GI response and nematode reproduction were observed for *M. incognita*. High GI and low reproduction were observed for some genotypes [*e.g.*, cv. Picket by *M. incognita* (GI = 6.4; Rf = 0.2) and cv. Lee68 (GI = 5.2; Rf = 0.2)] (Table 1; Fig. 2), while low GI and high reproduction were observed for others [cv. Kangxian8 by *M. hapla* (GI = 2.1; Rf = 5.3)]. These results revealed the genetic independence of the resistance from root galling response and nematode reproduction. The independence of root galling and nematode reproduction has been reported in various crops including soybean (Harris *et al.*, 2003; Fourie *et al.*, 2008), common bean (Fassuliotis *et al.*, 1970), lima bean (Roberts *et al.*, 2008), cotton (Shepherd, 1979; Gutiérrez *et al.*, 2010; Wang *et al.*, 2012; He *et al.*, 2014) and potato (Thomas & Williamson, 2013). Moreover, the EM values were not always positively correlated with the EGR or Rf values on some of the soybean genotypes, indicating differential genetic characters between egg-laying females (ELF) and nematode egg production. This phenomenon has also been reported previously (Windham & Williams, 1987, 1988; Fourie *et al.*, 1999).

So far, there are no standard phenotypic indices for screening RKN resistance or susceptibility in soybean. Hartman & Sasser (1985) classified plants with ELF index greater than 2 (*i.e.*, egg masses > 10 per root system) as susceptible, whilst Windham & Williams (1988) classified plants with Rf value more than 1 as susceptible. The same authors found Rf value as a more reliable value to evaluate crop resistance to *Meloidogyne* spp. than root GI and EM indices (Windham & Williams, 1987, 1988). Genetic analysis of the genes contributing to GI, EM and EGR indices will shed more light to our understanding of the complex interactions between nematodes and plants. The known soybean germplasms (PI90763, PI88788, Picket, Peking and Lee68) for SCN-race identification had different characteristics in GI, EM, EGR and Rf for both *M. incognita* and *M. hapla* which could be utilised as susceptible or resistant controls in genetic studies contributing to both root responses and RKN reproduction.

Most previously reported RKN-resistant gene mapping was based on GI on soybean. One major QTL on chromosome (Chr) 10 was identified to contribute to resistance to GI by *M. incognita* in various soybean genotypes (Tamulonis *et al.*, 1997a; Li *et al.*, 2001; Ha *et al.*, 2004, 2007; Pham *et al.*, 2013; Xu *et al.*, 2013; Jiao *et al.*, 2015). Other minor QTL associated with resistance to *M. incognita* based on GI index were mapped to Chr 8, 13 , 17, 18 (Li *et al.*, 2001; Ha *et al.*, 2007; Xu *et al.*, 2013; Jiao *et al.*, 2015). One QTL, linked to egg production (total number of eggs per plant) with *M. incognita* infection, was found on Chr 6 (Shearin *et* *al.*, 2009). Fourie *et al.* (2008) identified one major QTL on Chr 7 accounting for 80% of the phenotypic variation in eggs and juveniles and 62.4% for GI by *M. incognita* race 2 on the resistant soybean cultivar LS5995. Genetic markers associated with soybean resistance based on GI index were also reported on Chr 1, 13 to *M. javanica* and on Chr 13, 15 by *M. arenaria* (Tamulonis *et al.*, 1997b, c). These data suggested that multiple genes were associated with RKN resistance on soybean. Our data also indicates a complex interaction between the resistance indices and the RKN species used. Resistance based on GI, EM (or ELF) and EGR depends on both *Meloidogyne* species and the genetic background of the soybean genotypes. Therefore, broadly to utilize these resistance sources, it is necessary to combine all resistance indices including GI, EM, EGR, and Rf to evaluate nematode resistance and to understand how they contribute to soybean yield damage threshold.

## ACKNOWLEDGEMENTS

This study was supported by ‘One Hundred Talent Program Grant’ from Chinese Academy of Sciences to Congli Wang and by the Chinese National Scientific Funding (project no. 31471749). We thank Dr Qingshan Chen (Northeast Agricultural University, China) for providing Suinong14 and Hefeng25 soybean cultivars and Dr Isgouhi Kaloshian (University of California, Riverside, USA) for helpful comments on the manuscript.

## REFERENCES

BRIDGE, J. & PAGE, S.L.J. 1980. Estimation of root-knot nematode infection levels on roots using a rating chart. *Tropical Pest Management* 26: 296-298.

CHEN, P. & CHEN, S. 1989. [Identification and regional distribution of root-knot nematodes (*Meloidogyne incognita, M. arenaria, M. hapla*) on soybean China]. *Soybean Science* – Da Dou Ke Xue 8: 167-176 (in Chinese).

CHEN, S. & CHEN, P. 1990. [Observation on biological characteristics of soybean root-knot nematodes (*Meloidogyne incognita*, *M. arenaria*)]. *Acta Phytopathologica Sinica* – Zhi Wu Bing Li Xue Bao 20: 253-257 (in Chinese).

FASSULIOTIS, G., DEAKIN, J.R. & HOFFMAN, J.C. 1970. Root-knot nematode resistance in snap beans: breeding and nature of resistance. *Journal of the American Society for Horticultural Science* 95: 640-645.

FOURIE, H., MC DONALD, A.H. & LOOTS, G.C. 1999. Host suitability of South African commercial soybean cultivars to two root-knot nematode species. *African Plant Protection* 2: 119-124.

FOURIE, H., MIENIE, C.M.S., MC DONALD, A.H. & DE WAELE, D. 2008. Identification and validation of genetic markers associated with *Meloidogyne incognita* race 2 resistance in soybean, *Glycine max* (L.) Merr. *Nematology* 10: 651-661.

FOURIE, H., MC DONALD, A.H. & DE WAELE, D. 2010. Relationships between initial population densities of *Meloidogyne incognita* race 2 and nematode population development in terms of variable soybean resistance. *Journal of Nematology* 42: 55-61.

FOURIE, H., MC DONALD, A.H. & DE WAELE, D. 2013. Host and yield responses of soybean genotypes resistant or susceptible to *Meloidogyne incognita in vivo*. *International Journal of Pest Management* 59: 111-121.

FOURIE, H., DE WAELE, D., MC DONALD, A.H., MIENIE, C. & MARAIS, M. 2015. Nematode pests threatening soybean production in South Africa, with reference to *Meloidogyne*. *South African Journal of Science* 111: 1-9.

GUTIÉRREZ, O.A., JENKINS, J.N., MCCARTY, J.C., WUBBEN, M.J., HAYES, R.W. & CALLAHAN, F.E. 2010. SSR markers closely associated with genes for resistance to root-knot nematode on chromosomes 11 and 14 of Upland cotton. *Theoretical & Applied Genetics* 121: 1323-1337.

HA, B.-K., BENNETT, J.B., HUSSEY, R.S., FINNERTY, S.L. & BOERMA, H.R. 2004. Pedigree analysis of a major QTL conditioning soybean resistance to southern root-knot nematode. *Crop Science* 44: 758-763.

HA, B.-K., HUSSEY, R.S. & BOERMA, H.R. 2007. Development of SNP assays for marker-assisted selection of two southern root-knot nematode resistance QTL in soybean. *Crop Science* 47: S73-S82.

HARRIS, D.K., BOERMA, H.R., HUSSEY, R.S. & FINNERTY, S.L. 2003. Additional sources of soybean germ-plasm resistant to two species of root-knot nematode. *Crop Science* 43: 1848-1851.

HARTMAN, K., & SASSER, J.N. 1985. Identification of *Meloidogyne* species on the basis of differential hosts test and perineal pattern morphology. In: *An Advanced Treatise on Meloidogyne, Volume II: Methodology* (K.R. Barker, C.C. Carter & J.N. Sasser Eds). pp. 69-77. Raleigh, USA, North Carolina State University Graphics.

HE, Y., KUMAR, P., SHEN, X., DAVIS, R.F., VAN BECELAERE, G., MAY, O.L., NICHOLS, R. & CHEE, P. 2014. Re-evaluation of the inheritance for root-knot nematode resistance in the Upland cotton germplasm line M-120 RNR revealed two epistatic QTLs conferring resistance. *Theoretical & Applied Genetics* 127: 1343-1351.

HUSSEY, R.S. & BARKER, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Report* 57: 1025-1028.

HUSSEY, R.S., BOERMA, H.R., RAYMER, P.L. & LUZZI, B.M. 1991. Resistance in soybean cultivars from maturity groups V-VIII to soybean cyst and root-knot nematodes. *Journal of Nematology* 23: 576-583.

JIAO, Y., VUONG, T.D., LIU, Y., LI, Z., NOE, J., ROBBINS, R.T., JOSHI, T., XU, D., SHANNON, J.G & NGUYEN, H.T. 2015. Identification of quantitative trait loci underlying resistance to southern root-knot and reniform nematodes in soybean accession PI 567516C. *Molecular Breeding* 35: doi: 10.1007/s11032-015-0330-5.

LEE, J., KIM, H., ROBBINS, R.T., WRATHER, J.A., BOND, J., NGUYEN, H.T. & SHANNON, J.G. 2015. Reaction of soybean cyst nematode resistant plant introductions to root-knot and reniform nematodes. *Plant Breeding & Biotechnology* 3: 346-354.

LI, C., HU, Y., HUA, C. & WANG, C. 2016. [Identification of species and races of root-knot nematodes in greenhouse from Daqing city in Heilongjiang Province]. *Soil & Crop* – Tu Rang Yu Zuo Wu 5: 105-109 (in Chinese).

LI, Y., LI, X., TIAN, Z. & CHEN, P. 1998. [A new soybean variety: Kangxian2]. *Bulletin of Agricultural Science and Technology* – Nong Ye Ke Ji Tong Xun 12: 28 (in Chinese).

LI, Z., JAKKULA, L., HUSSEY, R., TAMULONIS, J. & BOERMA, H. 2001. SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. *Theoretical & Applied Genetics* 103: 1167-1173.

LUZZI, B.M., BOERMA, H.R. & HUSSEY, R.S. 1987. Resistance to three species of root-knot nematode in soybean. *Crop Science* 27: 258-262.

LUZZI, B.M., BOERMA, H.R. & HUSSEY, R.S. 1994a. A gene for resistance to the southern root-knot nematode in soybean. *Journal of Heredity* 85: 484-486.

LUZZI, B.M., BOERMA, H.R. & HUSSEY, R.S. 1994b. Inheritance of resistance to the southern root-knot nematode in soybean. *Crop Science* 34: 1240-1243.

NICOL, J.M., TURNER, S.J., COYNE, D.L., DEN NIJS, L., HOCKLAND, S. & TAHNA MAAFI, Z. 2011. Current nematode threats to world agriculture. In: *Genomics and Molecular Genetics of Plant-Nematode Interactions* (J. Jones, G. Gheysen & C. Fenoll Eds). pp. 21-43. Dordrecht, The Netherlands, Springer Science + Business Media B.V.

OMWEGA, C.O., THOMASON, I.J. & ROBERT, P.A. 1988. A nondestructive technique for screening bean germ plasm for resistance to *Meloidogyne incognita*. *Plant Disease* 72: 970-972.

PHAM, A.T., MCNALLY, K., ABDEL-HALEEM, H., ROGER BOERMA, H. & LI, Z. 2013. Fine mapping and identification of candidate genes controlling the resistance to southern root-knot nematode in PI 96354. *Theoretical & Applied Genetics* 126: 1825-1838.

ROBERTS, P.A. 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annual Review of Phytopathology* 33: 199-221.

ROBERTS, P.A., MATTHEWS, W.C., EHLERS, J.D. & HELMS, D. 2008. Genetic determinants of differential resistance to root-knot nematode reproduction and galling in Lima Bean. *Crop Science* 48: 553-561.

SHEARIN, Z.P., FINNERTY, S.L., WOOD, E.D., HUSSEY, R.S. & BOERMA, H.R. 2009. A southern root-knot nematode resistance QTL linked to the *T*-locus in soybean. *Crop Science* 49: 467-472.

SHEPHERD, R.L. 1979. A quantitative technique for evaluation cotton for root-knot nematode resistance. *Phytopathology* 69: 427-430.

TAMULONIS, J.P., LUZZI, B.M., HUSSEY, R.S., PARROTT, W.A. & BOERMA, H.R. 1997a. RFLP mapping of resistance to southern root-knot nematode in soybean. *Crop Science* 37: 1903-1909.

TAMULONIS, J.P., LUZZI, B.M., HUSSEY, R.S., PARROTT, W.A. & BOERMA, H.R. 1997b. DNA marker analysis of loci conferring resistance to peanut root-knot nematode in soybean. *Theoretical & Applied Genetics* 95: 664-670.

TAMULONIS, J.P., LUZZI, B.M., HUSSEY, R.S., PARROTT, W.A. & BOERMA, H.R. 1997c. DNA markers associated with resistance to Javanese root-knot nematode in soybean. *Crop Science* 37: 783-788.

THOMAS, V.P. & WILLIAMSON, V.M. 2013. Segregation and mapping in the root-knot nematode *Meloidogyne hapla* of quantitatively inherited traits affecting parasitism. *Phytopathology* 103: 935-940.

TIAN, Z., ZHOU, C., WU, Y., LI, J. & GAO, G. 2007. [The breeding of one new soybean variety: Kangxian6]. *Crop Research* – Zuo Wu Yan Jiu 3: 373 (in Chinese).

WALTERS, S.A. & BARKER, K.R. 1994. Current distribution of five major *Meloidogyne* species in the United States. *Plant Disease* 78: 772-774.

WANG, C., ULLOA, M. & ROBERTS, P.A. 2006. Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (*rkn1*) in Acala NemX cotton (*Gossypium hirsutum* L.). *Theoretical & Applied Genetics* 112: 770-777.

WANG, C., ULLOA, M., MULLENS, T.R., YU, J.Z. & ROBERTS, P.A. 2012. QTL Analysis for transgressive resistance to root-knot nematode in interspecific cotton (*Gossypium* spp.) progeny derived from susceptible parents. *PLoS One* 7: e34874.

WINDHAM, G.L. & WILLIAMS, W.P. 1987. Host suitability of commercial corn hybrids to

*Meloidogyne arenaria* and *M. incognita*. *Journal of Nematology* 1: 13-16.

WINDHAM, G.L. & WILLIAMS, W.P. 1988. Reproduction of *Meloidogyne javanica* on corn hybrids and inbreds. *Annals of Applied Nematology* 2: 25-28.

WRATHER, J.A., CHAMBERS, A.Y., FOX, J.A., MOORE, W.F. & SCIUMBATO, G.L. 1995. Soybean disease loss estimates for the southern United States, 1974-1994. *Plant Disease* 79: 1076-1079.

WRATHER, J.A. & KOENNING, S.R. 2006. Estimates of disease effects on soybean yields in the United States 2003 to 2005. *Journal of Nematology* 38: 173-180.

WU, Y., TIAN, Z., ZHOU, C., LI, J., YANG, L., DU, Z. & ZHU, C. 2011. [The breeding of one new soybean variety: Kangxian9 resistant to soybean cyst nematodes]. *Heilongjiang Agricultural Sciences* – Hei Long Jiang Nong Ye Ke Xue 11: 160-161 (in Chinese).

XU, X., ZENG, L., TAO, Y., VUONG, T., WAN, J., BOERMA, R., NOE, J., LI, Z., FINNERTY, S., PATHAN, S. M., SHANNON, J.G. & NGUYEN, H.T. 2013. Pinpointing genes underlying the quantitative trait loci for root-knot nematode resistance in palaeopolyploid soybean by whole genome resequencing. *Proceedings of the National Academy of Sciences of the United States of America* 110: 13469-13474.

YATES, J.L., HUSSEY, R.S., FINNERTY, S.L. & BOERMA, H.R. 2010. Three soybean plant introductions possess unique resistance to peanut root-knot nematode. *Crop Science* 50: 118-122.

YU, J., CHEN, J., MA, L., LIU, B., ZHANG, B. & LI, X. 2013. [The breeding of Kangxian11 with high yield and resistance to nematodes]. *Heilongjiang Agricultural Sciences* – Hei Long Jiang Nong Ye Ke Xue 2: 147-148 (in Chinese).

ZHANG, S. 1993. [Occurrence and pathogen identification of soybean root-knot nematodes in Fujian Province]. *Plant Protection* – Zhi Wu Bao Hu 19: 14-15 (in Chinese).

ZHAO, L., DUAN, Y., BAI, C., CHEN, L., ZHU, X. & SUN, X. 2010. [Identification and distribution of root-knot nematodes in Liaoning Province]. *Hubei Agricultural Sciences* – Hu Bei Nong Ye Ke Xue 49: 865-868 (in Chinese).

**Chunjie Li, Cui Hua, Yanfeng Hu, Jia You, Yanzhi Mao, Jianying Li, Zhongyan Tian and Congli Wang.** Организменный ответ различных генотипов сои на заражение *Meloidogyne incognita* и *M. hapla* в провинции Хэйлунцзян Китая.

**Резюме.** Цистообразующие нематоды (ЦН, *Meloidogyne* spp.) относятся к числу наиболее важных в экономическом отношении вредителей сои *Glycine max*. ЦН были отмечены на сое в Центральном Китае, но данных об их присутствии в северных областях Китая нет, хотя именно эти регионы являются основными производителями сои. Недавно северная ЦН *M. hapla* была обнаружена на овощных полях провинции Ляонин в Северном Китае. Южная ЦН *M. incognita* была отмечена здесь в защищенном грунте, где также отмечался ущерб от соевой ЦН. Хотя выращивание устойчивых сортов представляет одну из наиболее эффективных стратегий контроля ЦН, отсутствует информация об устойчивости местных сортов сои к поражению ЦН. Для определения потенциальных генетических источников устойчивости сои против расы 3 соевой ЦН исследовали в условиях теплиц особенности заражения ЦН *M. incognita* и *M. hapla* одиннадцати местных устойчивых сортов, полученных в результате селекции, пяти местных сортов, чувствительных к поражению соевой ЦН, а также нескольких местных сортов, для которых степень устойчивости не была определена. Определяли индекс галлобразования (GI) по шкале от 0 до 10, число комков яиц на грамм корней (EM), число яиц на грамм корней (EGR) и фактор размножения нематод (величина Rf) для оценки реакции отдельных генотипов сои на поражение ЦН. Как показали эксперименты, из 21 исследованного генотипа 11 и 8 генотипов показали низкое значение GI (0-3) при поражении *M. incognita* и *M. hapla,* соответственно. Низкие показатели размножения нематод ($Rf \leq 1$) отмечены для 13 и 3 генотипов *M. incognita* и *M. hapla,* соответственно, что позволяет рассматривать местные сорта сои в качестве более подходящих растений-хозяев для *M. hapla,* чем для *M. incognita*. Сравнение таких показателей зараженности, как GI, EM, EGR и Rf для *M. incognita* и *M. hapla* указывает на существенные различия в биологических особенностях паразитирования этих видов. Высокий индекс GI при низких показателях размножения нематод или низкие значения GI при высоких показателях размножения говорят в пользу существования у сои различных генетических источников подавления формирования галлов и нарастания численности при поражении двумя этими видами нематод. Выявление устойчивых сортов – наиболее перспективное направление поиска в селекции новых сортов сои.