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Screening and histopathological changes of Korean carrot lines for resistance to root-knot nematodes in different soil textures
A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Screening and histopathological changes of Korean carrot lines for resistance to root-knot nematodes in different soil textures

BY

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The Graduate School of Seoul National University

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ABSTRACT

Screening and histopathological changes of Korean carrot lines for resistance to root-knot nematodes in different soil textures

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Root-knot nematodes are major pathogens of carrot (*Daucus carota* var. *sativa* Dc.) in the crop-growing regions in the world, causing direct economic loss of the crop due to galling and forking of the carrot roots, rendering the infected carrots unmarketable. The most prevalent root-knot nematode species in Korea are *Meloidogyne incognita* and *M. hapla* in greenhouses with warm temperature and in open fields as in Jeju Province during the cool-temperature season, respectively. The use of resistant cultivars is a highly effective and environment-friendly method to control root-knot nematodes. In this study, 56 carrot lines were screened for resistance to both root-knot nematodes, among which 9 lines were resistant to both, one line was resistant only to *M. incognita*, and 12 lines were resistant only to *M. hapla*. Resistant carrot lines also showed apparently different responses from susceptible lines in light microscopy which were immediate and relatively slow responses of resistance to the infection of *M. incognita* and *M. hapla*, respectively, regardless of carrot lines examined. Furthermore, influence of soil textures on infectivity of root-knot nematode to carrot was examined by inspecting penetration rate and disease severity (gall formation and egg mass formation). For *M. incognita*, both penetration rate and disease severity were significantly increased in 100% sandy soil; however, for *M. hapla*, the disease severity was highly elevated in 100% sandy soil even though there was no significant difference in the penetration rate among different soil
textures. These results imply that the reason of severe damages reported in sandy soils by the root-knot nematodes may be due to the increased penetration rates of *M. incognita* in carrots probably by the increased porosity in sandy soil that enhances the mobility of the nematode, but due to the increased growth and reproduction after the penetration of *M. hapla* probably by the soil conditions favorable for root growths to provide better nutritional materials for the nematode. Screening of the carrot lines for resistance to the nematodes in this sandy soil texture may maximize the root-knot galling and the nematode reproduction rate, which is useful for the powerful selection of carrot lines with a strong resistance.

**Keywords:** *Meloidogyne incognita, Meloidogyne hapla, Daucus carota var. sativa* Dc., breeding, soil texture

*Student Number: 2015-21763*
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CHAPTER 1.

Screening and histopathological examination of Korean carrot lines for resistance to root-knot nematodes
ABSTRACT

In total, 56 carrot lines developed in Korea were screened for resistance to *Meloidogyne incognita* and *M. hapla* to develop nematode-resistant carrot cultivars. Approximately 1,000 second-stage juveniles of nematodes were inoculated for each carrot plant with 5 replications and 6 weeks after inoculation, carrot roots were carefully uprooted, washed free of soils and examined visually the gall formations for which the gall index (GI) was scored based on the root-knot scoring chart. For *M. incognita*, ten carrot lines were resistant (GI ≤ 1.0), while the other 46 were susceptible (GI > 1.0). For *M. hapla*, 21 carrot lines were resistant, while the other 35 were susceptible. Nine carrot lines were resistant to both *M. incognita* and *M. hapla*. The histopathological responses of various resistant and susceptible lines were examined after nematode infection. Carrot lines screened as the susceptible commonly showed well-developed giant cells in the stele around which xylem vessels were extensively formed regardless of nematode species. Histopathological responses of the carrot lines resistant to the nematodes differed apparently from those susceptible to the nematodes, showing the formation of modified, non-fully developed giant cells accompanying surrounding necrotic layers in *M. incognita*, while showing fully-developed but degenerated giant cells with severe vacuolations in *M. hapla*, indicating acute and retarded resistant responses for *M. incognita* and *M. hapla*, respectively. This suggests the resistance of the Korean carrot lines may be governed by the expression of different resistance genes for the resistance to *M. incognita* and *M. hapla*.
Keywords: root-knot nematode, carrot, resistant, breeding, gall formation, giant cell
INTRODUCTION

Carrot (*Daucus carota* var. *sativa* Dc.) is grown in temperate parts of the world and also in the subtropics and tropics. Besides being a popular vegetable, the roots are also a rich source of β-carotene, which is a precursor to vitamin A (Simon et al., 2008). One of the significant root diseases of carrot is root galling caused by root-knot nematodes. Symptoms of the disease are galling, stubbing, forking, and fasciculation of the roots (Roberts, 1987; Siroka and Fernandez, 1990). Root-knot nematodes, *Meloidogyne* species, are widespread pathogens worldwide. Among *Meloidogyne* species, *M. hapla*, *M. incognita*, *M. javanica*, and *M. arenaria* are reported as most common species globally and in Korea as well (Bridge and Starr, 2007; Korean Society of Plant Pathology, 2009). *M. hapla* is adapted to cool climates in northern United States and southern Canada in North America, in northern Europe and in northern Asia. *M. incognita*, *M. javanica*, and *M. arenaria* are most common in the tropic zone such as tropic Africa, Australia and southern Asia (Taylor and Sasser, 1978).

The most prevalent root-knot nematode species in Korea is *M. hapla*, considering that the major carrot growing areas are open fields in Jeju Province during the cool-temperature season (Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, 2012). However, the other three root-knot nematodes may be able to spread throughout carrot fields in the near future with increasing temperatures due to global climate change (Chakraborty et al., 2000; Harris et al., 2006; IPCC, 2007) and/or during
greenhouse cultivation at warmer temperatures, in which *M. incognita* and *M. arenaria* are most common (Kim, 2001; Kim et al., 2001b).

Infectivity of soil populations of root-knot nematodes can be reduced by use of nematicides; by crop rotations, and sometimes by special methods such as flooding, or drying the soil by repeated plowings during dry seasons. Crop rotation can reduce nematode concentration, however, the practical applications are restricted due to the difficulties of controlling cultivation seasons and requirement of excessive workforce. Even though using nematicides are strong methods to control nematodes, their high toxicity and remaining residues contaminate underground water and non-selective action causes disruption of ecosystem. Therefore, breeding cultivars resistant to nematodes is a more efficient way of controlling the nematodes with great advantages of its environmental friendliness and no requirement of other costs for other control practices. Thus, this study aimed to screen Korean carrot lines for resistance to the root-knot nematodes (*M. incognita* and *M. hapla*) that can be used to breed nematode-resistant carrot lines.
MATERIALS AND METHODS

1. Screening of Korean carrot lines for resistance to root-knot nematodes

1-1. Carrot lines

A total of 56 carrot lines developed in a Carrot Breeding Institute, Korea, and a commercial carrot cultivar Shinheukjeon-5-chon (hereafter SHC) were used for this study.

1-2. Nematode preparation

The root-knot nematodes *M. incognita* and *M. hapla* used as in our previous studies (Park et al., 2014; Seo et al., 2014, 2015) were maintained as pure cultures respectively on chili pepper cv. Bugang and tomato cv. Rutgers at 20±5°C in a greenhouse, respectively. Egg masses of the nematodes were isolated with forceps by hand-picking. On Baermann funnels, the isolated egg masses were incubated for 3-5 days so that second-stage juveniles (J2) hatched out of the eggs were collected to be used for this study (Son et al., 2008; Southey, 1986).

1-3. Nematode inoculation

Seedlings of each carrot line were planted in tray filled with vermiculite and incubated in 25°C incubator about 3 weeks which is average duration for 3rd true-leaf starts to
grow. These carrot seedlings were transplanted to 9 cm (diameter) × 8 cm (depth) plastic pots filled with vermiculite and river sand in 1 to 1 ratio in weight. These J2 were diluted to about 200 J2/ml with sterile diluted water (SDW). Transplanted carrot seedlings were inoculated around rhizospheres with 5 ml of the nematode suspensions containing approximately 1,000 J2 for each, with 5 replications. Inoculated carrot lines were raised in a greenhouse, constantly maintaining the temperatures of 25±5°C and watered to field capacity three times a week until 6 weeks after inoculation.

1-4. Root-knot gall formation by *M. incognita* and *M. hapla*

Six weeks after inoculation, carrot roots were carefully uprooted and adhering soils were washed free with the tap water. Each root was visually scrutinized and scored gall index a measure of severity of root-knot galling based on the root-knot-scoring chart (Bridge and Page, 1980).

2. Structural changes of carrot root tissues infected with *M. incognita* and *M. hapla*

After the inspection of gall formation of carrot roots, those galls were cut and fixed with the modified Karnovsky’s fixative comprised of 2% paraformaldehyde and 2% glutaraldehyde in 0.05M sodium cacodylate buffer (pH 7.2) for 2-4 hours at 4°C in a vacuum. Then, roots fragments were washed in 0.05M sodium cacodylate buffer (pH 7.2) for 10 minutes at 4°C for three times and fixed again with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH7.2) at 4°C for 1 and a half hours. The fragments
were washed briefly two times at room temperature with sterile distilled water and en bloc stained in 0.5% uranyl acetate at 4°C for 30 minutes. A serial dehydration was processed from low concentration of ethanol to high one; 30%, 50%, 70%, 80%, 90%, and three times of 100% for 10 minutes each. The specimens were transited to 100% propylene oxide at room temperature and dehydrated twice for 10 minutes each and embedded in Spurr’s epoxy resin (Spurr, 1969), followed by polymerization at 70°C for 8 hours. With a glass knife mounted on a MT-X ultra-microtome (RMC, Tucson, AZ, USA), the embedded specimens were sectioned 600nm in thicknesses. The sections were then stained with 1% toluidine blue O in 2% sodium tetraborate and observed under a compound light microscope (Axiophot; Carl Zeiss, Oberkochen, Germany).
RESULTS

1. Screening of carrot lines for resistance to root-knot nematodes

When *M. incognita* was inoculated on 56 carrot lines and screened, average gall index was ranged from 0.4 to 4.0, for which 10 lines were resistant (GI ≤ 1.0) and the other 46 lines were susceptible (GI > 1.0) (Table 1). When *M. hapla* was inoculated on the 56 carrot lines and screened, average gall index was ranged from 0.2 to 4.2, for which 21 lines were resistant (GI ≤ 1.0) and the other 35 lines were susceptible (GI > 1.0) (Table 2). Of the 56 carrot lines, 9 lines were resistant to both *M. incognita* and *M. hapla*.

The resistant carrot lines showed intact roots with no or slight gall formation (Fig. 1.B, E). On the other hand, the susceptible carrot lines showed noticeable gall formations and the size of galls were relatively larger in the carrot lines infected with *M. incognita* than those with *M. hapla* (Fig. 1.C, F).
Table 1. Resistance ratings (RR) of Korean carrot lines determined by gall index at 6 weeks after *Meloidogyne incognita* inoculation

<table>
<thead>
<tr>
<th>Lines</th>
<th>GI(^a)</th>
<th>RR(^b)</th>
<th>Lines</th>
<th>GI</th>
<th>RR</th>
<th>Lines</th>
<th>GI</th>
<th>RR</th>
</tr>
</thead>
<tbody>
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<td>2.4±1.1(^d)</td>
<td>S</td>
<td>1278-2</td>
<td>2.2±0.8</td>
<td>S</td>
<td>1234-1-2</td>
<td>0.4±0.5</td>
<td>R</td>
</tr>
<tr>
<td>1209♂</td>
<td>0.5±0.6</td>
<td>R</td>
<td>1278-3</td>
<td>3.0±0.0</td>
<td>S</td>
<td>1234-1-4</td>
<td>1.4±0.5</td>
<td>S</td>
</tr>
<tr>
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<td>1.7±0.6</td>
<td>S</td>
<td>1277-3</td>
<td>2.4±0.5</td>
<td>S</td>
<td>15KH-1</td>
<td>1.0±0.8</td>
<td>S</td>
</tr>
<tr>
<td>1219♂</td>
<td>1.0±0.0</td>
<td>R</td>
<td>1277-4</td>
<td>2.4±0.5</td>
<td>S</td>
<td>15KH-1♀</td>
<td>1.8±1.0</td>
<td>S</td>
</tr>
<tr>
<td>1219-1</td>
<td>0.6±0.5</td>
<td>R</td>
<td>1277-5</td>
<td>2.4±0.5</td>
<td>S</td>
<td>15KH-1♀</td>
<td>1.6±0.9</td>
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</tr>
<tr>
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<td>15KH-2</td>
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<td>1287-2</td>
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<td>15KH-2♀</td>
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<td>1281-4</td>
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<td>S</td>
<td>15KH-3</td>
<td>1.0±0.7</td>
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<td>15KH-3♀</td>
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</tr>
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<td>191</td>
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<td>15KH-4</td>
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<td>R</td>
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<td>24</td>
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<td>1054♀</td>
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<td>13-77♀</td>
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<td>2.2±0.8</td>
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<td>1123♀</td>
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<td>S</td>
<td>CV(^e)</td>
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<tr>
<td>1278♂</td>
<td>3.6±0.5</td>
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<td>1226-2-3</td>
<td>0.8±0.4</td>
<td>R</td>
<td></td>
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</tbody>
</table>

\(^a\) Gall index by Diagrammatic root-knot scoring chart (Bridge et al., 1980)

\(^b\) Host reaction of carrot lines determined by GI ≤ 0.1, highly resistant (HR); GI ≤ 1, resistant (R); GI > 1, susceptible (S) (modified from Sasser et al., 1984).

\(^c\) SHC: cv. Shinheukjeon 5 chon

\(^d\) Means ± standard deviations of five replications.

\(^e\) Average of coefficients of variation (CV) calculated by standard deviations/means of gall indices for the total carrot lines examined.
Table 2. Resistance ratings (RR) of Korean carrot lines determined by gall index at 6 weeks after *Meloidogyne hapla* inoculation

<table>
<thead>
<tr>
<th>Lines</th>
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<th>Lines</th>
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<td>1.0±0.0</td>
<td>R</td>
<td>1234-1-2</td>
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<td>1209♂</td>
<td>0.2±0.4</td>
<td>R</td>
<td>1278-3</td>
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<td>15KH-1♀</td>
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<td>1281-4</td>
<td>1.2±0.4</td>
<td>S</td>
<td>15KH-3</td>
<td>0.8±0.4</td>
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</tr>
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<td>1281-5</td>
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<td>14KH-191</td>
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</table>

a Gall index by Diagrammatic root-knot scoring chart (Bridge et al., 1980)
b Host reaction of carrot lines determined by GI ≤ 0.1, highly resistant (HR); GI ≤ 1, resistant (R); GI > 1, susceptible (S) (modified from Sasser et al., 1984).
c SHC: cv. Shinheukjeon 5 chon
d Means ± standard deviations of five replications.
e Average of coefficients of variation (CV) calculated by standard deviations/means of gall indices for the total carrot lines examined.
Figure 1. Gall formation in Korean carrot lines, Shinheukjeon-5-chon (A, D), 1219♂ (B, E), and 191 (C, F) at 6 weeks after inoculation with approximately 1,000 second-stage juveniles (J2) of *Meloidogyne incognita* (A, B, C) and *Meloidogyne hapla* (D, E, F)
2. Histopathological observation of carrot roots in nematode infection area

Carrot lines susceptible to the nematodes commonly showed well-developed giant cells in the stele, around which xylem vessels were also formed rather extensively, indicating a sophisticated system established for the development of the giant cells. However, cytoplasmic contents of the giant cells formed in the carrot root tissues susceptible to the nematodes were almost depleted probably because of the deprivation of nutritional contents by the infecting nematodes (Fig. 2 C, G, I, D, F, G). On the other hand, the carrot lines resistant to *M. incognita* showed the formation of relatively large modified cells, but not fully developed giant cells at the nematode infecting area, accompanying the formation of necrotic layers (Fig. 2 A, E). In the carrot lines resistant to *M. hapla*, the fully-developed giant cells were formed as in the susceptible lines; however, their cytoplasm appeared degenerated with severe cytoplasmic vacuolation, indicating the earlier degradation of giant cells before the consumption of nutritional substances for the full development of the female nematode (Fig. 2B, H).
Figure 2. Histopathological responses of Korean carrot lines 1234-1-2 (A, B), 15KH-3♀ (C, D), 15KH-4 (E, F), 15KH-7 (G, H), and Shinheukjeon-5-chon (I, J) at 6 weeks after inoculation of *M. incognita* (A, C, E, G, I) and *M. hapla* (B, D, F, H, J). G: giant cells, Ne: nematodes, St: stele, WI: cell wall ingrowth, X: xylem vessels, MC: modified cells, Nc: necrotic layers, Cr: cortex. Bars = 50 µm.
DISCUSSION

Out of 56 Korean carrot lines examined for resistance to the root-knot nematodes (*M. incognita* and *M. hapla*), 10 lines were resistant to *M. incognita* and 21 lines were resistant to *M. hapla*. Nine lines resistant to both nematodes can be developed as potential commercial cultivars that can be planted in both the greenhouse and open fields with major nematodes, *M. incognita* and *M. hapla*, distributed, respectively. Their resistance may not result from the disease escape as the GIs for both nematodes were less than 1.0 under the controlled environmental conditions which are most favorable for the development of the root-knot nematodes. Also the light microscopy of the root tissues infected with the nematodes showed the histopathological responses corresponding to the degrees of resistance, supporting further their resistance resulting from the plant-nematode interactions that exclude the possibility of the disease escape. However, the resistant responses differed between the nematode species. For *M. incognita* infection, no fully developed giant cells but modified cells were formed accompanying the formation of necrotic layers around the infecting nematodes. This implies resistant reaction occurs rapidly and immediately after penetration so that fundamentally hinders giant cell formation. For *M. hapla* infection, large giant cells similar to the susceptible responses were formed in the resistant carrot lines, whose differences from the susceptible ones were that the cytoplasm of giant cells in susceptible lines was almost vacant but were full of vacuoles in all resistant lines. If
nematode sufficiently uptake nutrients from the giant cells to become mature as female, giant cell structure is destroyed, leading to disappearance of cellular organelles (Paulson and Webster, 1970). The vacant giant cell cytoplasm indicates maturation of nematodes, which corresponded to the susceptible carrot lines. Formation and conservation of giant cells depend on a continuous stimulus from the nematodes and when this stimulus is interrupted, the cytoplasm of the giant cell becomes vacuolated (Bird, 1962). Vacuolated giant cells of resistant carrot lines infected with *M. hapla* may be derived from discontinuous access and stimulation from of the nematodes occurred after the formation of giant cells. All of these results suggest the resistance of the carrot lines result from the plant-nematode interactions, which is governed by the resistance genes but not by opportunistic escape of the nematode infection. Therefore, the Korean carrot lines resistant to the *M. incognita* and *M. hapla* infection may have true resistance so that they may be developed as carrot lines for the breeding of the carrots that can be commercialized as potential nematode-resistant carrot cultivars.
LITERATURE CITED


CHAPTER 2.

Effects of Soil Textures on Infectivity of Root-Knot Nematodes on Carrot
ABSTRACT

This study was conducted to examine infectivity (penetration and gall and egg-mass formations) of the root-knot nematodes, *Meloidogyne incognita* and *M. hapla*, on carrots grown in soil conditions of 5 different soil textures consisting of bed-soil (b) and sand (s) mixtures (b-s mixtures) at the ratios of 10:0, 7:3, 5:5, 3:7, and 0:10. For *M. incognita*, the nematode penetration rates in b-s of 0:10 (100% sand) were significantly higher than in the other b-s mixtures, more greatly at 2 and 5 days after inoculation (DAI) than at 10 DAI, while no significant differences in the penetration rates were mostly shown for *M. hapla* at the above DAI. However, for both nematodes, gall and egg-mass formations were remarkably increased in the b-s mixture of 0:10, compared to the other b-s mixtures, which is coincided with the general aspects of severe nematode infestations in sandy soils. This suggests the increased gall and egg-mass formations of *M. incognita* should be derived from the increased penetration rates in the sandy soil conditions, which provide a sufficient aeration due to coarse soil nature for the nematodes, leading to their mobility increased for the enhanced root penetration. For *M. hapla*, it is suggested that the sandy soil conditions affect positively on the healthy plant growth with little accumulation of the inhibitory materials and sufficient aeration, enhancing the nematode growth and feeding activities. All of these aspects provide information reliable for the development screening techniques efficient for the evaluation of the nematode resistance in the breeding programs.
Keywords: carrot, egg-mass, nematode penetration, root-knot galls, soil texture
INTRODUCTION

Agricultural economic losses caused by the plant-parasitic nematodes are estimated around 100 billion dollars (Chitwood, 2003; Oka et al., 2000; Sasser and Carter, 1985), among which the root-knot nematodes are the most important pathogens that attack a wide variety of host plants, causing serious economic losses, especially vegetable crops (Mai, 1985; Mitkowski and Abawi, 2003). The root-knot nematodes (*Meloidogyne* spp.) also cause one of the most serious damages to the carrot (*Daucus carota* var. *sativus* Dc.), an important root vegetable next to radish and potato in Korea, which comprises the total cultivation area of 2,849 ha and the total annual production amount of 93,694 tons in 2011 (Davis, 2004; Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, 2012). Four root-knot nematode species are recorded in the carrot worldwide and in Korea as well, including *Meloidogyne hapla*, *M. incognita*, *M. arenaria*, and *M. javanica* (Bridge and Starr, 2007; Korean Society of Plant Pathology, 2009). Especially, *M. hapla* and *M. incognita* are assumed to be most prevalent in Korea in open carrot cultivation fields and greenhouses, respectively, in which the environmental conditions are cool and warm that are favorable for the nematode growth and reproduction, respectively (Anwar and McKenry, 2010; Bridge and Starr, 2007; Kim, 2001; Kim et al., 2001; Sardanelli et al., 1983). Several management tactics are applied for controlling the root-knot nematodes, among which the use of resistant plants is a powerful tool for the nematode control in sustainable agriculture that is highly effective and economically
reliable with no or little additional cost for the nematode control (Kinloch and Hinson, 1972; Mitkowski and Abawi, 2003; Rhoades, 1976). Plant diseases develop under the conditions of virulent pathogen, susceptible host and favorable environment, all of which influence the occurrences and severities of diseases, minimizing disease escape that occurs when three factors in a disease triangle do not interact at the proper time and for sufficient duration (Agrios, 2004). Host-plant resistance to root-knot nematodes (*Meloidogyne* spp.) is determined by incompatible responses of the plants that vary qualitatively and quantitatively even among the cultivars of the same crops and is influenced by various factors such as temperature, planting time, plant age at the inoculation time, and origin of plants, all of which affect the survival and pathogenicity of the nematode pathogens (Mendosa and Jatala, 1985; Roberts, 1987). Especially, the plant-parasitic nematodes are soil-borne pathogens whose growths and pathogenicity are influenced by the soil conditions including soil texture, moisture, aeration and osmotic potential in field soils (Van Gundy, 1985). Among these soil conditions, soil texture, estimated by the relative amounts of sand, silt and clay particles in a soil, is an important component that determines soil compactness and thus availability of aeration and moisture, which is explored as a basis for management zones of plant-parasitic nematodes within a field (Moore and Lawrence, 2013). This implies the development and infestation of the plant-parasitic nematodes vary critically depending on the soil texture, which should be applied for the selection of plants with durable resistance in the breeding program of nematode-resistant crops. Thus, in this study, effects of soil textures on the infectivity of two root-knot nematodes, *M. incognita* and *M. hapla*, on the carrot
to select soil conditions suitable for the nematode disease development in the screening of the carrot plants for resistance to the plant-parasitic nematodes.
MATERIALS AND METHODS

1. Plants, nematodes, soils and nematode inoculation
A commercial carrot cultivar Shinheukjeon-5-chon (hereafter SHC) and the maternal parent of a hybrid carrot line (13-77♀) currently developed in a Carrot Breeding Institute, Korea, were used for screening assays (nematode inoculation) in our experiments as susceptible host plants. The root-knot nematodes *M. incognita* Race 1 and *M. hapla* used in our previous studies (Park et al., 2014; Seo et al., 2014, 2015) were also used in this study. Soils used in our study were bed soil (b) (composed of 64.9% coco-peat, 15% peat-moss, 7% zeolite, 10% perlite, 2.6% dolomite, 0.03% wetting agent, and 0.47% N-P-K common fertilizer) and sand (s) at the ratios of 10:0, 7:3, 5:5, 3:7, and 0:10 (hereafter 10:0, 7:3, 5:5, 3:7, and 0:10 b-s mixtures, respectively) were used in this study. Seeds of SHC and 13-77♀ were planted in bed soil (sterilized at 15 psi, 121°C for 15 min) in a 50 cell-plug tray and grown around at 25°C for three weeks to become three-true leaf carrot seedlings, which were transplanted in 9 cm (diameter) × 8 cm (depth) plastic pots filled with b-s mixtures sterilized at 15 psi, 121°C for 15 min. The carrot seedlings were inoculated with the root-knot nematodes at three days after transplanting. Egg-masses of *M. incognita* and *M. hapla* were isolated by hand-picking with a forceps from the pure nematode cultures maintained on chili pepper cv. Bugang and tomato cv. Rutgers at 20 ± 5°C in a greenhouse, respectively. The egg-masses isolated were incubated on Baermann funnels for 3–5 days for egg hatching to second-stage juveniles (J2) of the
nematodes (Son et al., 2008; Southey, 1986), which were diluted to make nematode suspensions with the concentration of about 100 J2/ml in sterile distilled water. The nematodes were inoculated on the carrot seedlings by pouring 10 ml nematode suspensions (containing about 1,000 J2) around the plant rhizosphere with thirteen replications for each nematode on each carrot cultivar of line. The carrot seedlings inoculated with the nematodes were arranged in a split-plot design of the factorial experiment in greenhouse benches and grown at 20 ± 5°C in a greenhouse, watering to the field capacity three times per week throughout the experimental period.

2. Examination of the nematode penetration

Nematode penetration was examined microscopically. At 2, 5, and 10 days after inoculation (DAI), the carrot seedlings were carefully uprooted from the pots, and the root systems were washed free of adhering soil with tap water. All root systems were cut into 1–2 cm root segments with a razor blade and stained with red food coloring stain following the method described by Thies et al. (2002). The root segments stained were observed under a stereomicroscope to measure the number of nematodes in root tissues with three replications for each b-s mixture.

3. Formation of root-knot galls and egg-masses

Six weeks after nematode inoculation, plants were carefully uprooted from pots, and the root systems were gently washed with tap water to remove adhering soil. The roots were examined visually for root-knot gall formation on each root system and the severity of
root galling was graded using the gall index (GI) based on the root-knot scoring chart developed by Bridge and Page (1980); from no knots (GI = 0), few small knots difficult to find (GI = 1) ~50% of roots affected with some main roots knotting (GI = 5) through all roots severely knotted with concomitant plant death (GI = 10) with four replications for each b-s mixture. Also the number of egg-masses formed on each root system was examined by close looking into the root systems with four replications for each b-s mixture with naked eyes.

4. Statistical analysis

Data obtained from the experiments were subjected to analyses of variance (ANOVA) in splitplot design of 2 (nematode species) × 2 (plant cultivar/ line) × 5 (b-s mixtures) factorial experiments using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Fisher’s least significant difference was employed to test for significant difference among the factors examined using critical values from the t-distribution table at $P \leq 0.05$ and $P \leq 0.01$. 
RESULTS

1. Penetration of root-knot nematodes in different soil conditions.

In microscopic examination, thread-like nematodes were mostly found in the proximity of apical meristem tissues at 2 DAI, in which neither definite root swelling nor giant cell formation was noted, regardless of the b-s mixtures, nematode species and carrot cultivar/line (Fig. 1). At 5 DAI, the root tissues of the nematode infection sites were somewhat swollen and giant cell-looking hypertrophied tissues were intermittently found in the stelar parenchyma adjacent to vascular tissues around the nematode infection sites (Fig. 2). However, the nematode morphology was mostly thread-like at this stage of infection, indicating the nematode growth should be minimal, regardless of the root-knot nematode species. At 10 DAI, the root tissues of the infection sites were more swollen than those at 5 DAI, some of which were definitely hypertrophied to form galls, in which the infecting nematodes were mostly located in the stelar parenchyma around the vascular tissues with mostly thickened shapes like sausages (Fig. 3).
Figure 1. Penetration of *Meloidogyne incognita* (A–D) and *M. hapla* (E–H) in carrot cultivar Shinheukjeon-5-chon (A, B, E, F) and a crossing parent line 13-77♀ (C, D, G, H) at 2 days after inoculation, showing the nematode juveniles with no swelling (arrows) located in the proximity of the apical meristem. Scale bars = 1.0 mm.
Figure 2. Penetration of *Meloidogyne incognita* (A–D) and *M. hapla* (E–H) in carrot cultivar Shinheukjeon-5-chon (A, B, E, F) and a crossing parent line 13-77♀ (C, D, G, H) at 5 days after inoculation, showing the nematode juveniles with little swelling (arrows) located inside the stele of upper differentiated root tissues. Note the formation of giant cells (,Object) and somewhat swelled cortical tissues (†), indicating the initial stage of gall formation. Scale bars = 1.0 mm.
Figure 3. Penetration of *Meloidogyne incognita* (A–D) and *M. hapla* (E–H) in carrot cultivar Shinheukjeon-5-chon (A, B, E, F) and a crossing parent line 13-77♀ (C, D, G, H) at 10 days after inoculation, showing the swollen nematode juveniles (arrows) located inside the galled root tissues (asterisks) containing the giant cells (ⓡ). Scale bars = 1.0 mm.
2. The nematode penetration rates at 2, 5, and 10 DAI

For *M. incognita*, the penetration rates were significantly higher in 0:10 b-s mixture (100% sand) than in the other b-s mixtures with high degrees at 2 and 5 DAI, but with a lowered degree at 10 DAI due to the increased penetration rates in the b-s mixtures other than 0:10 b-s mixture (Table 1). There were no significant differences in the penetration rates of *M. incognita* between the carrot cultivar SHC and the hybrid line 13-77♀. On the other hand, the penetration rates of *M. hapla* were not significantly different among all b-s mixtures except for SHC at 2 DAI (Table 1). Statistical comparisons of the penetration rates between the two nematode species showed significantly (P ≤ 0.05) higher penetration rates for *M. hapla* than *M. incognita* at 2 and 5 DAI, but not at 10 DAI (Table 1).
Table 1. Penetration rates of MI and MH in carrot cultivar SHC and a crossing parent line (13-77♀) in pots containing b-s mixtures at the ratios of 10:0, 7:3, 5:5, 3:7, and 0:10 at 2, 5, and 10 DAI

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</tbody>
</table>

Significance (LSD)  

- MI, *Meloidogyne incognita*; MH, *Meloidogyne hapla*; SHC, Shinheukjeon-5-chon; b-s mixtures, bed soil-sand mixtures; DAI, days after inoculation; LSD, least significant difference.

- Averages followed by the same letters among b-s mixtures in a column for the same nematode are not significantly different at $P \leq 0.05$ by LSD.

Values are presented as average ± standard deviation of three replications.

MI, *Meloidogyne incognita*; MH, *Meloidogyne hapla*; SHC, Shinheukjeon-5-chon; b-s mixtures, bed soil-sand mixtures; DAI, days after inoculation; LSD, least significant difference.

*aAverages followed by the same letters among b-s mixtures in a column for the same nematode are not significantly different at $P \leq 0.05$ by LSD.*
Averages followed by the same letters between SHC and 13-77♀ in a row are not significantly different at $P \leq 0.05$ by LSD.

All average is the averages of the nematode penetration rates for both carrot plants in all b-s mixtures at the same DAI.

LSD test for all averages between MI and MH (**, significantly different at $P \leq 0.01$; NS, not significantly different at $P \leq 0.05$).
3. Formation of root-knot galls and egg-masses in different b-s mixtures

At 6 weeks after inoculation, root-knot nematode disease severities assessed by the gall formation were significantly higher in 0:10 b-s mixture (100% sand) than the others regardless of the nematode species and carrot cultivar/line, which revealed no significant differences between the carrot cultivar and line as well as between the nematode species (Table 2, Fig. 4). Also egg mass formation was all higher in 0:10 b-s mixture (100% sand) than the other b-s mixtures examined, more greatly in *M. hapla* compared to *M. incognita* that even showed no significant difference among b-s mixtures in SHC (Table 2).
Table 2. Formation of root-knot galls and egg-masses of MI and MH on carrot cultivar SHC and a crossing parental line (13-77♀) in pots containing b-s mixtures at the ratios of 10:0, 7:3, 5:5, 3:7, and 0:10 at 6 weeks after inoculation

<table>
<thead>
<tr>
<th>Nematode</th>
<th>b-s mixture</th>
<th>Gall formation (gall index)</th>
<th>Egg mass formation (No. of egg masses/root)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SHC</td>
<td>13-77♀</td>
</tr>
<tr>
<td>MI</td>
<td>10:0</td>
<td>2.25±0.50X</td>
<td>1.00±0.00X</td>
</tr>
<tr>
<td></td>
<td>7:3</td>
<td>2.50±0.58X</td>
<td>2.00±0.00Y</td>
</tr>
<tr>
<td></td>
<td>5:5</td>
<td>2.25±0.50X</td>
<td>1.33±0.58XY</td>
</tr>
<tr>
<td></td>
<td>3:7</td>
<td>2.25±0.50X</td>
<td>2.00±0.82Y</td>
</tr>
<tr>
<td></td>
<td>0:10</td>
<td>4.25±0.50Y</td>
<td>4.25±0.96Z</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>2.70±0.52A</td>
<td>2.12±0.47A</td>
</tr>
<tr>
<td></td>
<td>All avg.</td>
<td>2.41±0.50</td>
<td>4.04±3.31</td>
</tr>
<tr>
<td>MH</td>
<td>0</td>
<td>1.75±0.50X</td>
<td>1.00±0.00X</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.00±0.00X</td>
<td>2.25±0.50Y</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>1.75±0.50X</td>
<td>1.50±0.58XY</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>2.75±0.50Y</td>
<td>1.00±0.00X</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.25±0.50Z</td>
<td>4.00±0.00Z</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>2.50±0.40A</td>
<td>1.95±0.22A</td>
</tr>
<tr>
<td></td>
<td>All avg.</td>
<td>2.23±0.31</td>
<td>5.46±2.94</td>
</tr>
</tbody>
</table>

Values are presented as average ± standard deviation of four replications.


Averages followed by the same letters among b-s mixtures in a column of the same nematode are not significantly different at $P \leq 0.05$ by LSD.

Averages followed by the same letters between SHC and 13-77♀ in a row are not significantly different at $P \leq 0.05$ by LSD.
All average is the averages of the nematode penetration rates for both carrot plants in all b-s mixtures at the same days after inoculation (DAI).

LSD test for all averages between MI and MH (NS, not significantly different at $P \leq 0.05$).
Figure 4. Gall formation of *Meloidogyne incognita* (A–D) and *M. hapla* (E–H) in carrot cultivar Shinheukjeon-5-chon (SHC) (A, B, E, F) and a crossing parent line 13-77♀ (C, D, G, H) in 0:10 bed soil-sand (b-s) mixture (100% sand) (A, C, E, G) and the other b-s mixtures (B, D, F, H) at 6 weeks after inoculation. Note the different sizes of galls (yellow circles) formed by *M. incognita* (large) in SHC (A) and 13-77♀ (C) and *M. hapla* (small) in SHC (E) and 13-77♀ (G).
DISCUSSION

A variety of abiotic and biotic factors affect the establishment of nematode populations in soil. Soil nematodes including plant-parasitic nematodes are mostly aerobic and originally aquatic animals, requiring proper moisture contents and aeration from their immediate surrounding soil water films, preferring most to moisture levels 40–60% of filed capacity (Dropkin, 1980; Kim, 2015; Van Gundy, 1985). Among soil conditions, soil texture, a mixture of solids (sand, silt and clay particles and organic matters) determines soil compactness and porosity (thereby availability of moisture and aeration for the nematodes) is one of the most important soil characteristics related to nematode infestations in crop fields (Moore and Lawrence, 2013; Stolzy and Van Gundy, 1968). Generally light sandy soils are more favorable to large populations of nematodes than heavy clay soils owing to more adequate aeration provided in soils consisting of coarse particles, but cause more nematode damages on plants that suffer from water stress due to easy drainage of water in coarse particulate sandy soils (Dropkin, 1980). *M. incognita* and *Hoplolaimus columbus* prefer to soils with high sand content (Koenning et al., 1996; Lewis and Smith, 1976). Soil water regimes, related with soil textures and water contents affect the penetration of the soybean cyst nematode (SCN) and the tolerance of susceptible soybean cultivars to SCN (Johnson et al., 1993b, 1994). However, little study has been made on the reasons of heavy infestation and sever damages of *Meloidogyne* spp. in the carrot fields in sandy soils until now.
In this study, gall and egg-mass formations of the root-knot nematodes were remarkably increased in 100% sandy soils. These results agree with the general aspects that the agricultural importance of the root-knot nematodes is associated with sandy soils and that crop damages associated with root-knot nematode infections are highly reflective of sandy soils and sandy patches within fields (Van Gundy, 1985). Infestations of *M. incognita* and *M. hapla* occur more frequently in sandy loam soils than in clay soils (Sasser, 1954).

For *M. incognita* in our study, the increased gall and egg-mass formations were due to the significantly increased penetration rates of the nematode J2 in the sandy soil compared to other soil textures (Table 1). This is consistent with another study that the mobility and root penetration of the nematode juveniles decreases as the clay and silt fractions in the soil increase (Prot and Van Gundy, 1981).

On the other hand, the root penetration rates of *M. hapla* juveniles were not much increased in 100% sandy soils as compared to *M. incognita* in our study, although its gall and especially egg-mass formations were significantly increased in the sandy soil over the other soil textures (Table 1, 2). This suggests that the increased nematode damage (the root-knot gall formation) and reproduction (egg-mass formation) should not be related with the nematode penetration, but with nematode growth and development after infection in the carrot root tissues, which are dependent on the giant cell formation, leading to the enhanced reproduction and root-knot galling (Seo et al., 2015). This may be supported in our study by the results that showed similar penetration rates among b-s mixtures but differentially increased gall and egg-mass formations in 100% sandy soil.
compared to the other b-s mixtures (Table 1, 2).

With a proper moisture content, aeration of the soil enhances stem growth and root elongation, the rate of transpiration and the intensity of the respiratory activity of the shoot, leading to healthy plant growth with a full function of nutritional absorption by root hairs and reduced accumulation of potentially inhibitory soil products (Drew and Sisworo, 1979; Hunter and Rich, 1925). Endoparasitic sedentary nematodes such as *Meloidogyne* and *Heterodera* species induce the formation of specialized nursing cells, giant cells and syncytia, respectively, from which they acquire nutrients for their growth and development (Jones, 1981; Kim et al., 1999). Soil environments such as soil water regimes and inhibitory materials influence on the location of nematode-induced syncytia by altering nematode feeding behavior and the development of giant cells and syncytia, which affects the nematode growth and development (Johnson et al., 1993a; Kim et al., 1986, 1998; Moon et al., 2010; Orion et al., 1980; Stender et al., 1986). All of these aspects suggest the increased reproduction of *M. hapla* in the sandy soil may derived from the healthy plant growth with little accumulation of the inhibitory materials in the soil conditions with sufficient aeration available for the nematodes.

Minimizing the crop losses of the root-knot nematodes through the use of nematicides is expensive and provides environmental and human health problems, which makes the breeding for the nematode-resistance an attractive alternative (Fassuliotis, 1985; Oka et al., 2000; Osman and Viglierchio, 1981). Screening techniques efficient for the evaluation of the nematode resistance should be developed, for which the screening should be done under conditions that are conductive for good plant growth and that no
plants escape contact with infective nematode juveniles (Fassuliotis, 1985). In our study, in b-s mixtures other than 100% sandy soil, the plant roots were not in full contact with the infective juveniles of *M. incognita*, resulting in low penetration rates, and not in such healthy growth status as sufficiently supporting the growth and reproduction of both root-knot nematodes, resulting in the decreased root-knot gall and egg-mass formations. This is not the true plant resistance to the nematodes, but disease escape. Therefore, we suggest the screening of the carrot for resistance to the root-knot nematodes should be done under the soil conditions of 100% sandy soil texture with proper moisture contents and fertilizers to support full contact of the infective juveniles with the plant roots and healthy plant growth to provide the soil conditions favorable for the growth and reproduction of the infecting nematodes to minimize disease escape.
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다양한 토질에서의 한국 당근 계통의 뿌리혹선충

저항성 검정과 조직병리학적 변화

김은지

초록

뿌리혹선충은 전세계적으로 재배되는 당근(Daucus carota var. sativa Dc.)의 주요 병원체이며 당근 뿌리의 혹을 유발하고 갈라지게 하는 등의 피해를 주어 당근의 상품성을 잃게 한다. 국내의 당근 재배지 중 비교적 따뜻한 온도의 온실에 가장 많이 존재하는 뿌리혹선충은 Meloidogyne incognita이며 서원한 계절에 재배하는 제주도의 노지에서는 Meloidogyne hapla가 가장 많이 분포한다. 저항성 종자의 사용은 이러한 뿌리혹선충을 방제하기에 매우 효과적이고 친환경적이다. 본 연구에서는 두 가지 선충에 대하여 56 개의 당근계통의 저항성을여부를 조사하였으며 그 결과 9계통은 두 선충에 모두 저항성이었고, 1 계통은 M. incognita에만 저항성이었으며, 12 계통은 M. hapla에만 저항성이었다. 광학현미경을 통해 침입부위를 확인하였을 때에도 저항성과 감수성 계통은 분명한 차이를 보였다. 또한 당근계통과는 무관하게 선충의 종류에 따라서 저항성 반응이 일어나는 시점이 상이했는데, 상대적으로 M. incognita의 침입 시에는 빠른 조직병리학적 저항성 반응의 특징(피사의 동반한 거대세포로 완전히 분화되지 않은 비대화된 세포 형성)이 나타났고, M. hapla침입 시에는 느린 저항성 반응의 특징(거대세포 형성 후 세포질의 심한 액포화로 인한 세포의
퇴화)가 나타났다. 더 나아가 뿌리혹선충의 감염력에 토질이 미치는 영향을 파악하기 위해 각 토질별 선충 침입률과 병의 심도(혹 형성과 알집형성)를 조사하였는데, *M. incognita*는 100% 모래 토양에서 침입률과 병의 심도가 모두 증가하였는데, *M. hapla*는 100% 모래 토양에서 병의 심도가 크게 증가하였음에도 불구하고 침입률은 다른 토질과 비교하여 유의적인 차이가 없었다. 이러한 결과는 모래 토양에서 뿌리혹선충의 피해가 심해지는 것은 *M. incognita*의 경우 모래 토양의 높은 공극률로 인하여 선충의 운동성이 증가하여 침입률 상승으로 이어져 선충의 감염력이 증가하였고, *M. hapla*의 경우엔 모래 토양에서 통기성이 제고되어 이로 인한 뿌리의 생육이 조장되어 뿌리 내 침입한 선충의 발달에 도움을 준 것이 선충의 감염력 증가의 원인임을 의미한다. 이러한 토양 조건은 뿌리혹선충에 저항성을 당근을 검정할 때 선충의 감염력을 극대화시켜 강력한 선충 저항성 당근 계통을 선발하기 위한 유용한 검정시스템을 제공할 수 있을 것이다. 이러한 모든 결과는 한국 당근의 뿌리혹선충에 대한 저항성은 선충의 감염을 우발적으로 피한 결과(병회피)가 아닌 당근과 선충의 상호작용의 결과에서 나타난 진정저항성으로 선충저항성 당근 계통들은 뿌리혹선충 저항성 당근 품종의 개발과 상용화가 가능할 것으로 생각된다.

중요어 : Meloidogyne incognita, Meloidogyne hapla, Daucus carota var. sativa Dc, 육종, 토질

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