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A Mechanism of Density-Dependent Population Change in *Heterodera glycines*

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콩시스트線蟲의 密度變化 機作

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ABSTRACT

Penetration level, female development and histological changes in infected root tissues were investigated following inoculation with different inoculum levels (110, 440 and 1760 juveniles/plant) of *Heterodera glycines* (SCN) race 3 on susceptible 'Lee' and resistant 'Pickett' soybean cultivars. Penetration level was lower in Pickett at the higher inoculum levels but no differences were detected in Lee. However, the lower penetration level in the resistant soybean cultivar appeared not to be directly related to plant resistance (female maturation). The number of females recovered from Lee was lower at the highest inoculum level. The number of females maturing on Pickett was much less than that on Lee, showing that changes of SCN population is associated with the number of SCN maturing rather than nematode penetration. In Lee mono-infection sites (a single nematode per site) syncytia had dense cytoplasm and no central vacuole, while multi-infected sites (many overlapping nematodes per site) had syncytia with a large central vacuole and many small vacuoles. Resistant responses in mono- and multi-infected root tissues of Pickett were delayed and rapid necrosis, respectively. The differences in tissue response are suggested as a mechanism that controls density-dependent population changes in resistant and/or susceptible soybean cultivars.

Key words: soybean, soybean cyst nematode, cyst development, histopathology

要 約

콩시스트線虫 Race 3에 罹病性인 大豆品種 Lee와 抵抗性인 Pickett을 供試, 3가지 다른 接種水準(植物個體當 1,760, 440, 110 마리의 2期 幼虫)에 따른 線虫의 侵入과 시스트 形成을 조사하였고, 罹病 뿌리 組織의 形態的 變化를 觀察하였다. 線虫의 侵入率은 罹病性 大豆에서 接種水準間에 差異가 없었으나, 抵抗性 品種에서는 接種水準이 높아짐에 따라 감소하였다. 그러나, 接種水準에 따른 侵入率 差異의 程度는 시스트 形成, 즉 抵抗性과 直接的으로는 관련이 없는 것으로 나타났다. 罹病性 大豆品種에서 시스트 形成率

은 最高接種水準에서 多少 감소하였고, 抵抗性 品種에서는 罹病性 品種과 비교할 때 시스트 形成率이 현히 낮아, 大豆品種에서의 線虫의 密度變化는 線虫의 侵入보다는 線虫의 生長에 관계가 있는 것으로 나타다. 罹病組織의 形態的 特徵은 大豆品種間에 그리고 感染程度에 따라 差異가 있었다. 罹病性 品種의 뿌리 織에서는 syncytium이 形成되었으나, 여러 마리 線虫에 의해 感染된 部位에서는 syncytium이 接種 5 후에 퇴화하였다. 抵抗性 大豆에서도 線虫의 感染程度에 따라 罹病組織의 形態變化가 다르게 나타났다. 러한 組織病理學的인 差異는 罹病性 및 抵抗性 大豆에서의 콩시스트線虫의 密度變化에 영향을 주는 要인으로 사료된다.

INTRODUCTION

Heterodera glycines Inchinohe (SCN), is an important pest on soybean [*Glycine max* (L.) Merr.]. Changes in nematode population density are influenced by environmental conditions(3) and host suitability. Maturation rate of a nematode decreases with population density, which is associated with food resources(6). Miller(11) reported that on good hosts (soybean cultivars), the number of SCN females maturing was fewer at high inoculum levels than at low inoculum levels. In *Meloidogyne incognita*, multiplication rate decreased as initial population of the nematode in various crop fields increased(6). A population increases during continuous cultivation of susceptible plants, but does not exceed the ceiling level(9) and nematode populations decline in resistant crop fields.

SCN in roots of compatible hosts induce syncytia which are the nutritional sources for the nematode (8). Inhibition of syncytial formation and development is related to the mechanism of resistance (5,14,15). An understanding of the specific mechanisms of resistance (or susceptibility) related to inoculum level would aid in the determination of the host-parasite relationship and population dynamics. Therefore, this research was designed to investigate the reasons for differences in SCN penetration and development at different inoculum levels in susceptible and resistant soybean cultivars.

MATERIALS AND METHODS

H. glycines race 3 was reared in a greenhouse on 'Kobe' lespedeza (*Lespedeza striata*) in an attempt to eliminate host bias toward soybean Cysts were crushed to release eggs, and they wer placed on Baermann funnels for 12 hours to allo second stage juveniles (J2) to hatch. Seeds o susceptible Lee or resistant Pickett soybean cultivar were germinated in vermiculite and transplanted i the cotyledon stage to sterilized fine sand in 10-cm d clay pots. At the true leaf stage, approximatel 110, 440 or 1760 J2 were added to the rhizospher of each plant (Pi) growing in a greenhouse at 25±2C

Two to five days after inoculation, soybean plant were selected for histological studies. Roots wer washed free of sand and selected root segments wer fixed with Karnovsky's fixative in 0.05 M cacody late buffer (pH 7.0) for 2 hours and postfixed i 1% osmium tetroxide in the same buffer for hours. These segments were dehydrated in a ethanol series and embedded in Spurr's epoxy resi (17). Darkly stained nematodes in root segment could be seen through the embedding resin, an mono- and multi-infection sites could be distingui shed. Silver-gold sections (ca. 80 nm thickness were cut with a glass knife on an ultramicrotome Sections were double stained with uranil acetat and lead citrate before examination using a JOE 100 x electron microscope. Thick sections (0.5 - 2μ stained with 1% toluidine blue in 50% ethanol wer examined using a phase-contrast light microscope

Five days after inoculation, root systems o soybean plants were fixed with FPA (formalin 5 ml; propionic acid, 5 ml; and 50% ethanol, 90 lm for 24 hours, cleared in 2% sodium hypochlorit for 10 minutes and stained with 0.07% bromo phenol blue in 50% ethanol for 8 hours, and rinse in 50% ethanol. The number of nematodes i mono-infection (a single nematode per site) o

multi-infection (two or more nematodes per site) sites in root tissues were counted using a stereomicroscope. After 30 days, mature females from soybean plants were collected on a 60-mesh sieve and counted using a stereomicroscope.

RESULTS

Penetration and Cyst Development. Most of the nematodes were located adjacent to the stele and had established feeding sites by 5 days after inoculation (Figs. 1 and 4). The number penetrating relative to the inoculum level was 21.0 - 25.8% on Lee and 14.4 - 24.8% on Pickett (Table 1). On Lee, the number penetrating increased as the Pi increased but not significantly. Penetration of Pickett was significantly less ($P = 0.05$) at Pi of 440 and 1760 compared to the Pi of 110 and those of Lee. The percentage penetration of Pickett compared to that of Lee was 106% at 110, 74% at 440, and 56% at 1760 inoculum levels. As Pi increased, the percentage of mono-infection sites decreased in both the Lee and Pickett soybeans (Table 1), while multi-infection sites increased.

Table 1. Percentage penetration and female development at different inoculum levels of *Heteroders glycines* race 3 applied to susceptible and resistant soybean cultivars

Cultivar	Inoculum level (J2/plant)	Percent penetration[a] by infection type[b]			J2 developing to females (%)[c]
		Mono	Multi	Total	
Lee (S)	110	19.7	1.3	21.0ab[d]	18.9a
	440	15.3	7.5	22.8ab	20.9a
	1760	14.0	11.8	25.8a	13.8b
Pickett (R)	110	23.7	1.1	24.8a	4.7c(25)[e]
	440	13.0	3.8	16.8bc	1.4d(4)
	1760	8.6	5.8	14.4c	0.4d(2)

[a] Nematodes found in root tissues as a percent of the number of juveniles in inoculum, average of 5 replicates.
[b] Mono-infection is one with a single nematode at an infection site.
Multi-infection is one with two or more nematodes at an infection site.
[c] Female development as a percent of the number of juveniles in inoculum, average of 5 replicates.
[d] Duncan's multiple range test; same letters indicate no significant differences ($P = 0.05$).
[e] The numbers in parentheses indicate mature female indices at the same inoculum level.

$$\text{Mature female index} = \frac{\text{average number of mature females on Pickett}}{\text{average number of mature females on Lee}} \times 100$$

Fewer ($P = 0.05$) females matured at Pi 1760 than at Pi of 110 and 440 on Lee (Table 1). Fewer females matured on Pickett than on Lee; however, 25% as many matured on Pickett as on Lee at the Pi 110, but only 4% at 440, and 2% at 1760 inoculum levels.

Light and Electron Microscopy. Structural changes of the susceptible and resistant soybean roots were different 2-5 days after inoculation. In Lee, syncytia were characterized by cell wall perforations and hypertrophied syncytial cells (Figs. 3 and 6). The characteristics of syncytia formed in mono-infection sites in Lee was dense cytoplasm with no central vacuoles (Figs. 2 and 3). Cytoplasm of syncytium component cells proliferated to replace the central vacuole, and endoplasmic reticulum tubules and mitochondria were numerous, indicating that they are metabolically active cells (Figs. 2 and 3). Multi-infection sites in Lee root tissues had syncytial cells with large central vacuoles (Fig. 5), many secondary vacuoles (Fig. 6), very sparse ground cytoplasm (Fig. 5), and mitochondria lacking inner membrane structures (cisternae) (Fig. 6). In mono-infected Pickett root tissues syncytia formed with cytoplasmic features similar to those of mono-infected Lee root tissues (Figs. 7, 8, 8A). However, necrotic layers (Fig. 7) surrounded the syncytial cells, and appeared to inhibit

the further syncytial development. In multi-infected Pickett root tissues, cellular changes included little or no hypertrophy (Fig. 9), no cell wall performations, and disorganized mitochondria and cellular appearance (Fig. 10).

DISCUSSION

The numbers of SCN J2 maturing to females in Lee root tissue appeared to be a function of density after penetration. Nematode maturation was associated with histopathological changes of infected root tissues. Syncytia with dense cytoplasm, small or no vacuoles, and abundant mitochondria and endoplasmic reticulum tubules resulted in a higher percentage of J2 maturing to adult females. The conditions which indicate a high metabolic activity would likely support more nematode development. Syncytia which were highly vacuolated with degenerated mitochondria and poorly developed endoplasmic reticulum probably would not be as metabolically active and would be less supportive of nematode development.

O'Brien *et al.*(12) reported that inoculation of resistant wheat cultivars with *Heterodera avenae* reduced the penetration level of inoculum applied later. The percentage of J2 penetrating was lower at higher inoculum levels in the resistant soybean. Syncytia in mono-infected resistant plants initally look very much like syncytia in susceptible plants. As the nematode develops, necrotic layers around the syncytium inhibit further syncytium development (10) and some nematodes die but a few are able to mature into females. In multi-infected resistant tissue, the necrotic reaction is more intense. Not only does the syncytium degenerate more quickly, resulting in the death of juveniles in the tissue, but the intense necrosis also may prevent penetration of J2 which hatch late. Resistance would be due to inhibition of penetration of nematodes into the resistant soybean cultivar and a lower percentage of juveniles that penetrate developing into females. This combination of lower penetration level and fewer penetrated J2 maturing probably is responsible for the rapid decrease in SCN popula tions in fields planted to resistant cultivars.

Various factors govern changes in nematod populations, such as environment(4), host suitabi lity(7,13,16) and inoculum density(2). Density dependent population changes may come fron plant damage which limits successful nematod development. Bonner and Schmitt(3) suggest tha a large initial nematode population in soil induc much root damage early in the season, limitin subsequent invasion and reproduction of *H. glycine* in susceptible soybeans. In the Lee soybean in ou experiment, there was no significant difference i percentage of J2 penetration among differen inculum levels, but the actual number penetratin was much higher at the higher inoculum levels. Th root damage appeared to be related to early degen eration of syncytia in heavily infected sites of Le sybean, possiblly because the penetrating J2 caus so much tissue damage. Root damage from J2 invad ing the tissue would not be nearly as much in ligh infections, and organized syncytial developmen would occur in root tissues infected by single nema todes. As inoculum level increases, multi-infectio sites increase, and reproductive rate may be lowe due to the syncytial degeneration.

In resistant wheat cultivars inoculated by *H avenae*, resistance was not expressed at a lov inoculum level (12). In our study of Pickett, cys formation was above the resistance level (10% o the Lee level) at the lowest inoculum level. Thi was probably due to a high percentage of mono infection sites for which the resistant response i delayed. Resistance is generally quickly expresse in heavy infections at higher inoculum levels

The differences in structural changes of roo tissues with mono- and multi-infections may b mechanisms of population regulation that lowe SCN multiplication in high population densities o susceptible soybean cultivars and that maximiz probability for survival in low populations. A susceptible cultivar growing in a soil with low SC numbers would have well-developed syncytia tha would provide a good food supply for a female t

produce a normal number of eggs with a minimum of root tissue damage. The roots would continue to grow and subsequent J2 generations would have adequate penetration sites for continued population increase. The same cultivar in a heavily infested soil would have more multi-infection sites, tissue destruction would be greater and syncytia unhealthy, which would result in an inadequate food supply for mature females. Thus, the females would likely produce fewer eggs. The heavily damaged root system would not produce new roots as readily, resulting in fewer sites for subsequent J2 generations to penetrate. In addition, the damaged root systems might not stimulate egg hatch as well as healthier root systems.

Resistant cultivars growing in soil with a low SCN population would react slowly to J2 penetration and syncytium formation, and some juveniles would mature into females; many of the juveniles destined to become mature males(15). This would provide means of survival when a resistant cultivar was planted. In a heavily infested field, the root tissue with many multi-infection sites would react more quickly, possibly because more nematodes would produce a greater stimulation, the necrotic layer would form more quickly, and syncytia would degenerate. The nematodes would then die without food source. The root system would produce new roots and a few J2 would remain to infect, probably establishing mono-infection sites, and a few females would be produced late in the season, again as a survival mechanism.

The effects would be as follows: 1) low initial population (Pi), susceptible cultivar, good reproduction, high final population (Pf); 2) high Pi, susceptible cultivar, poor reproduction, moderate Pf; 3) low Pi, resistant cultivar, poor reproduction, low Pf; 4) high Pi, resistant cultivar, very poor reproduction, low Pf.

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Plate I

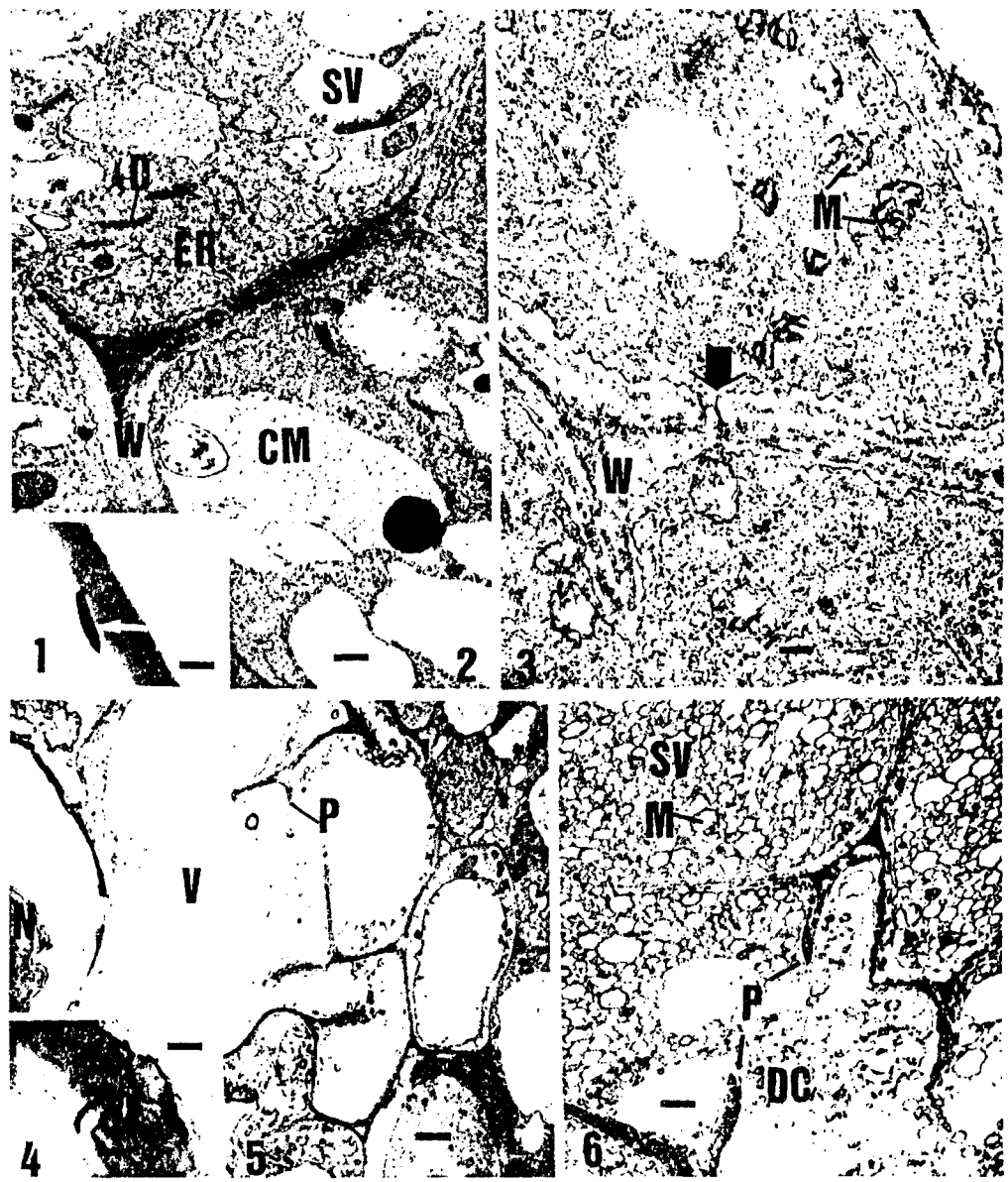

Fig. 1. Mono-infection site in Lee (susceptible) root tissue 5 days after inoculation (stained with bromophenol blue). Arrow: nematode. Bar: 100 μm.

Fig. 2. A portion of syncytium in mono-infection site in Lee root tissue 5 days after inoculation. Note numerous endoplasmic reticulum tubules (ER) and proliferated cytoplasmic material (CM) in a vacuole. D: dictyosome, SV: secondary vacuole, W: cell wall. Bar: 0.5 μm.

Fig. 3. Multi-infection site in Lee root tissue 5 days after inoculation (stained with bromophenol blue). Arrows: nematodes. Bar: 100 μm.

Fig. 4. A portion of syncytium induced in mono-infection site in Lee root tissue 2 days after inoculation. Note numerous mitochondria (M) and cell wall dissolution (arrow). Bar: 0.2 μm.

Fig. 5. A portion of syncytium in multi-infection site in Lee root tissue 5 days after inoculation. Note a large central vacuole (V) in two adjacent syncytial cells. P: cell wall perforation. Bar: 2 μ.

Fig. 6. Portion of syncytium showing proliferated secondary vacuoles (SV), mitochondria lacking cisternae (M), and degenerated cytoplasmic materials (DC). P: cell wall perforation. Bar: 1 μ.

Plate II

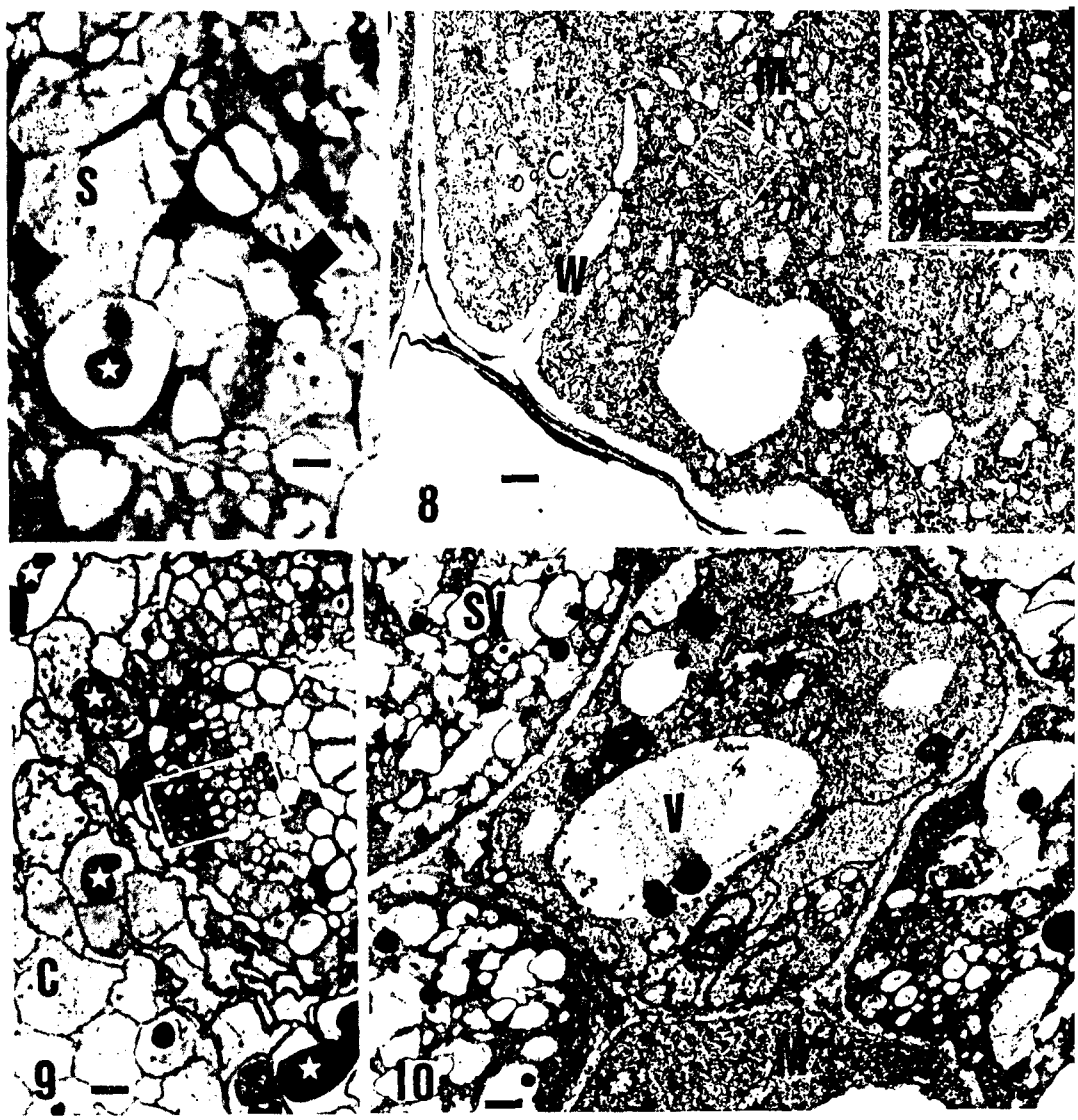

Fig. 7. Syncytium (S) induced in mono-infection site in Pickett (resistant) root tissue 5 days after inoculation (stained with toluidine blue). Note necrotic layers (arrow) around syncytial cells. Star: nematode. Bar: 10 μm.

Fig. 8. A portion of syncytium shown in Fig. 7, showing dissolution of cell wall (W), numerous mitochondria (M), and increased endoplasmic reticulum (rectangle). Bar: 0.5 μm.

Fig. 8A. Higher magnification of the portion in Fig. 8 (rectangle), showing numerous rough endoplasmic reticulum. Bar: 0.2 μm.

Fig. 9. Multi-infection site in Pickett root tissue 5 days after inoculation. Note numerous nematodes (stars) and affected cells (rectangle) in the stele. The affected cells are not hypertrophied. C: cortex Bar: 20 μm.

Fig. 10. A detailed structural feature of affected cells of Fig. 9 (a portion in the rectangle). Note mitochondria lacking cisternae (M) and cell wall thickening (arrow), and numerous secondary vacuoles (SV). Bar: 0.5 μm.