Plant Pathol. J. 15(1) : 1-7 (1999)

The Plant Pathology Journal

Heterodera glycines-Induced Syncytium Structures Related to the Nematode Growth and Reproduction in Susceptible Soybean Cultivars

Young Ho Kim*, Robert D. Riggs[1] and Kyung Soo Kim[1] 김영호

Division of Applied Biology and Chemistry, Seoul National University, Suwon 441-744, Korea
[1]Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, USA

(Received on January 8, 1999)

The reproduction of soybean cyst nematode (SCN), *Heterodera glycines*, including female formation and fecundity was much higher in SCN race 14 (R14) than in race 3 (R3) in susceptible soybean cultivars Bragg (intolerant), Lee74 (moderately tolerant), and PI 97100 (highly tolerant). The nematode body was also significantly larger in R14 than in R3 at 20 days after inoculation, but the further nematode growth appeared to be slower in R14 than in R3, resulting in no significant difference between the two races at 30 days after inoculation. Within each race, no significant difference was observed in the growth and reproduction among the soybean cultivars tested. Syncytial areas near the nematode lip regions (infection sites) were measured for each soybean cultivar-SCN race combination. R14 induced significantly larger syncytia than R3. Bragg had relatively larger syncytia than Lee74 and PI 97100, but the difference among the soybean cultivars was minimal or not significantly different. Syncytium occupation in the stelar region differed only between PI 97100 and the other two cultivars, which may be somewhat, but not exactly, related to tolerance levels. Syncytial cytoplasm was degenerated more with R14 and in Bragg than with R3 and in Lee74 and PI 97100, respectively. In light microscopy, degenerated syncytia were characterized by depleted and loose cytoplasm with less plastids than normal-looking (intact) syncytia which had dense syncytial cytoplasm. Electron microscopy revealed that degenerated syncytia contained highly vacuolated cytoplasm with degenerated plastids. The above results suggest that structural characteristics of syncytia may match the nematode growth and reproduction.

Keywords **:** *Heterodera glycines*, reproduction, soybean, syncytium, tolerance.

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is one of the most important pests of soybean, *Glycine max* (L.) Merr.

SCN induces syncytia in host root tissues (Endo, 1992; Endo and Veech, 1970; Jones, 1981). A syncytium induced by SCN is a group of cells with cytoplasmic continuity because of cell wall dissolution, which serves as a nutritional source for the infecting nematode. Syncytium development and persistence are related to the growth and reproduction of SCN (Kim et al., 1987; 1998). Kim et al. (1986) and Yum et al. (1992) reported that size and location of syncytium varied among susceptible plants with different tolerance levels. However, the syncytium characteristics have not been studied in relation to soybean cultivar and SCN race combinations. Therefore, in this study syncytium development in susceptible soybean cultivars with different tolerance levels was examined to evaluate the relationships between syncytium and nematode development and to investigate characteristics of syncytium that were related to tolerance.

Materials and Methods

Soybean cultivars and nematode inoculum. Susceptible soybean cultivars, Bragg, Lee74, and PI 97100 which were reported to be intolerant, moderately tolerant and highly tolerant, respectively (Anand and Koenning, 1986; Boerma and Hussey, 1984) were used in this experiment. Soybean seeds were germinated in vermiculite, and the seedlings were transplanted into 7.5-cm-diameter clay pots with sterilized river sand after 5 days.

Race 3 (R3) and race 14 (R14) of SCN, identified by using differential soybean cultivars and lines, and maintained on Lee74 and Pickett soybeans, respectively, were used as nematode inocula. Cysts extracted by rubbing the roots, suspending, and sieving, were crushed and placed on funnels, and second-stage juveniles (J2) were collected 3 days later. Two days after transplanting, nematode suspension (about 600 J2 per pot) was inoculated into the soybean rhizosphere in each pot, and the plants were transplanted to fresh soil 2 days after inoculation.

Growth and reproduction of SCN. Twenty and 30 days after inoculation, females on roots and in soil were collected by sieving through 850 μm (20 mesh) and 250 μm (60 mesh) sieves. The number of females and cysts was counted. Each cultivar and race combination of an infection stage had 4 replications. Twenty-day-old females and 30-day-old cysts were selected randomly and their widths of the largest body portions were measured individually under a stereomicroscope. For each soybean cultivar-SCN race combination, 50 females (cysts) were examined. Thirty days

*Corresponding author.
Phone) +82-331-290-2444, Fax) +82-331-294-5881
E-mail) yhokim@snu.ac.kr

after inoculation, cysts collected from each cultivar were crushed, and the number of eggs per cyst was examined under the stereomicroscope. Sixty cysts were examined from each soybean cultivar-SCN race combination.

Light and electron microscopy. Twenty days after inoculation, soybean root segments with female nematodes were processed for microscopy as follows: fixed in Karnovsky's fixative for 4 hr; post-fixed in 1% osmium tetroxide for 2 hr; dehydrated in an ethanol series; and embedded in Spurr's epoxy resin. The embedded samples were sectioned in 1 μm thickness in the vicinity of the nematode lip region with a glass knife on a Sorval ultramicrotome. The sections were stained with 1% toluidine blue and examined with a light microscope. Twenty to 30 sections were cut for each specimen for the light microscopy. For each cultivar and race combination, 20 specimens were examined and photographed. Syncytial and stelar areas in pictures were cut and the areas were measured with a Licor Area Meter (Model 3000).

For electron microscopy, embedded materials were sectioned in 80~90 nm thickness with a diamond knife. The sections were stained with uranyl acetate and lead citrate, and observed under a JEOL 100 CX transmission electron microscope.

Results

Growth and reproduction of SCN. There was no significant difference in female formation between 20 days and 30 days after inoculation. Also the number of females formed in each SCN race was not significantly different among the soybean cultivars tested, but R14 had more female formation rates than R3 approximately by 40% (Table 1). Fecundity was also higher in R14 than in R3 by 50%. R3 female

Table 1. Reproduction and growth of *Heterodera glycines* races in susceptible soybean cultivars 20 and 30 days after inoculation (DAI)

Race	Cultivar	Female formation (%)[a]		Fecundity[b] (No. eggs/cyst)	Body width ($\times10^{-2}$ mm)[c]	
		20 DAI	30 DAI		20 DAI	30 DAI
Race 3	Lee74	27.6±7.6	27.5±1.1	124.6±27.5	34.8±3.0	49.1±4.5
	Bragg	25.4±5.5	25.6±6.8	149.1±35.2	35.0±3.7	49.1±4.8
	PI 97100	23.2±4.2	20.7±2.5	136.5±36.3	33.7±4.8	49.8±4.8
	Average	25.4 X[d]	24.6 X	136.7 X	34.5 X	49.3 X
Race 14	Lee74	35.9±3.8	36.4±4.1	210.8±18.9	41.0±4.7	50.5±3.4
	Bragg	32.9±2.1	37.9±5.4	204.0±19.7	40.2±3.8	50.2±3.1
	PI 97100	38.1±7.2	28.6±3.9	199.7±31.4	37.8±4.0	49.1±4.5
	Average	35.6 Y	34.3 Y	204.8 Y	39.7 Y	49.9 X

[a] About 600 juveniles of *H. glycines* were inoculated into the rhizosphere of each soybean plant. Numbers are averages and standard deviations of 4 replications.
[b] For each cultivar and race combination, 60 cysts were collected and examined at 30 days after inoculation.
[c] Averages and standard deviations of 50 females or cysts.
[d] Same letters for averages within a column note no significant difference between *H. glycines* races at P=0.05 by the pooled standard deviations.

Table 2. Areas and characteristics of syncytia formed in root tissues of soybean cultivars infected with race 3 and race 14 of *Heterodera glycines*

Race	Cultivar	Syncytium area[a] ($\times10^{-2}$ mm^2)	Syncytium size[b]			% of degenerated syncytia[c]	Occupation of syncytium in stele (%)[d]
			Small	Medium	Large		
Race 3	Lee74	1.27±0.68	10	75	15	10	23.6±11.1
	Bragg	1.38±0.51	15	70	15	20	18.4± 9.9
	PI 97100	1.05±0.71	25	55	20	10	11.8±10.0
	Average	1.23 X[e]	16.7	66.6	16.7	13.3	17.9 X
Race 14	Lee74	1.72±0.65	0	70	30	20	30.7±14.4
	Bragg	2.14±1.05	0	55	45	45	25.0±12.1
	PI 97100	1.76±0.75	0	65	35	25	21.8±13.2
	Average	1.87 Y	0.0	63.3	36.7	30.0	25.8 Y

[a] Syncytium areas are averages and standard deviations of 20 syncytia.
[b] Number of syncytia out of 20 syncytia with areas smaller than 0.5×10^{-2} mm^2 (small), 0.5×10^{-2} mm^2-1.5×10^{-2} mm^2 (medium), and larger than 1.5×10^{-2} mm^2 (large) at the nematode infection sites.
[c] Degenerated syncytia were determined by the loose and vacuolated syncytial cytoplasm under the light microscope.
[d] Syncytium areas relative to stelar areas (%).
[e] Same letters for averages within a column note no significant difference between *H. glycines* races at P = 0.05 by the pooled standard deviations.

body widths were significantly smaller than those of R14 females 20 days after inoculation, but 30 days after inoculation, body widths were not significantly different between the two races (Table 1).

Syncytium size, occupation and degeneration. Syncytia were always formed by SCN infection in all the soybean

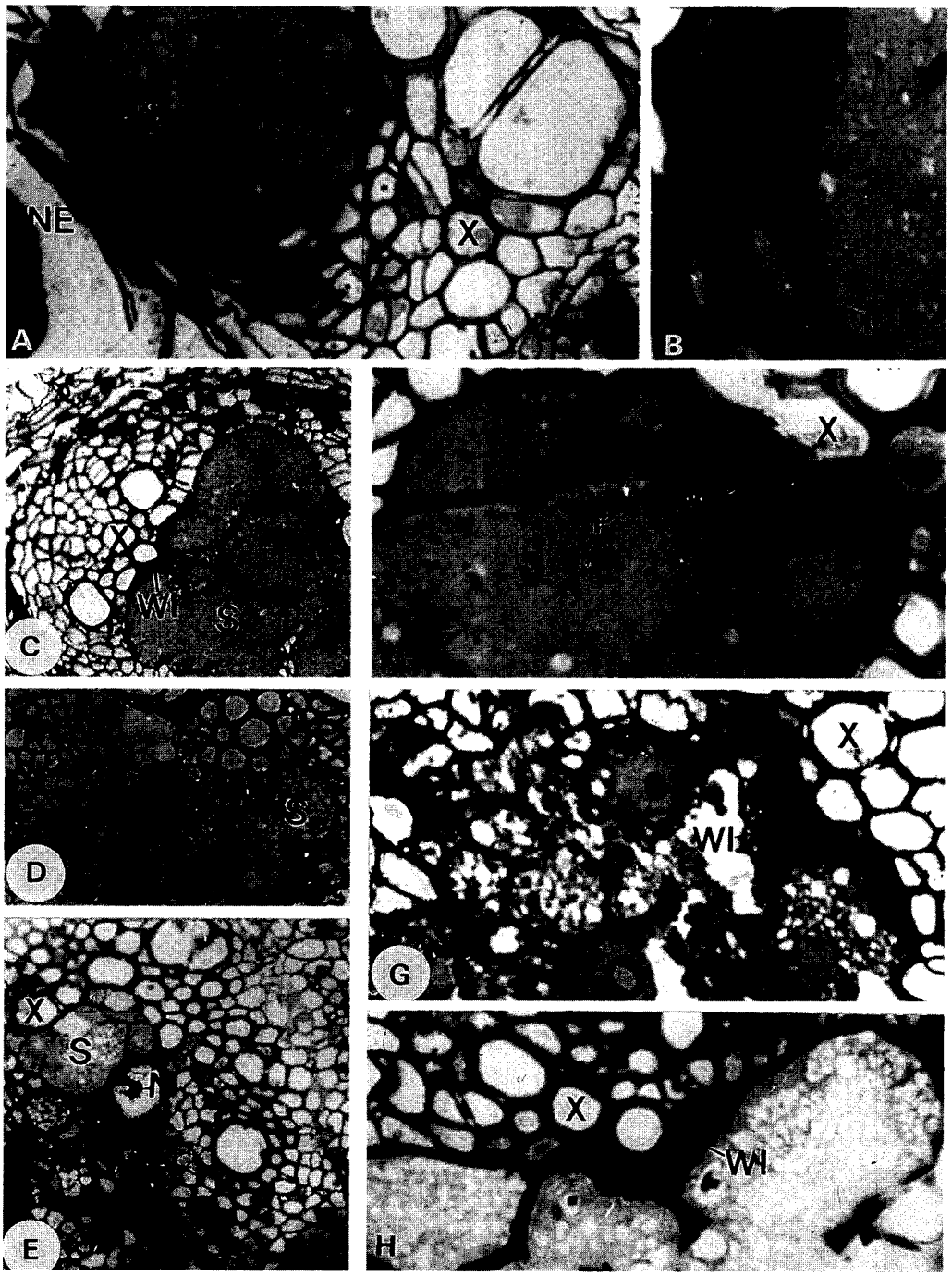

Fig. 1. Light micrographs of syncytia (S) formed in soybean root tissues 20 days after nematode inoculation. A: Nematode infection sites showing greatly thickened cell wall adjacent to the infecting nematode (NE) in Lee74 with R3. B: Higher magnification of (A), showing extremely thickened cell wall (W). C & D: Large and small syncytia formed in Lee74 with R14 and PI 97100 with R3, respectively. The large syncytium has prominent cell wall ingrowths (WI) adjacent to xylem vessels (X). The small syncytium has no or few cell wall ingrowths. E: Syncytium formed in the center of stele in Bragg with R14. F, G & H: Syncytia with intact (F) and degenerated (G & H) cytoplasm formed in Bragg with R3, PI 97100 with R4 and Bragg with R4, respectively. The intact syncytium has numerous plastids (P), while the degenerated syncytia have loose cytoplasm with few plastids. Magnifications: A=× 400, B=×1,200, C-E=×250, and F-H=×1,200.

cultivars used in this experiment. Syncytia varied little in size and shape within the 20~30 serial sections of a specimen near the infection site. However, syncytium size varied considerably even within the same cultivar and race combinations. Sizes of syncytium (areas of syncytium near the nematode infection) differed significantly between the nematode races; R14 syncytia were significantly larger than those produced by R3 by 52% in average (Table 2). The average syncytial area formed by SCN R3 was 1.23×10^{-2} mm^2, and that by R14 was 1.87×10^{-2} mm^2. Syncytia produced by either SCN race were not significantly different in size among the soybean cultivars, but Bragg had relatively larger syncytia for both SCN races. Small syncytia (not larger than 2.0×10^{-2} mm^2) were most fre-

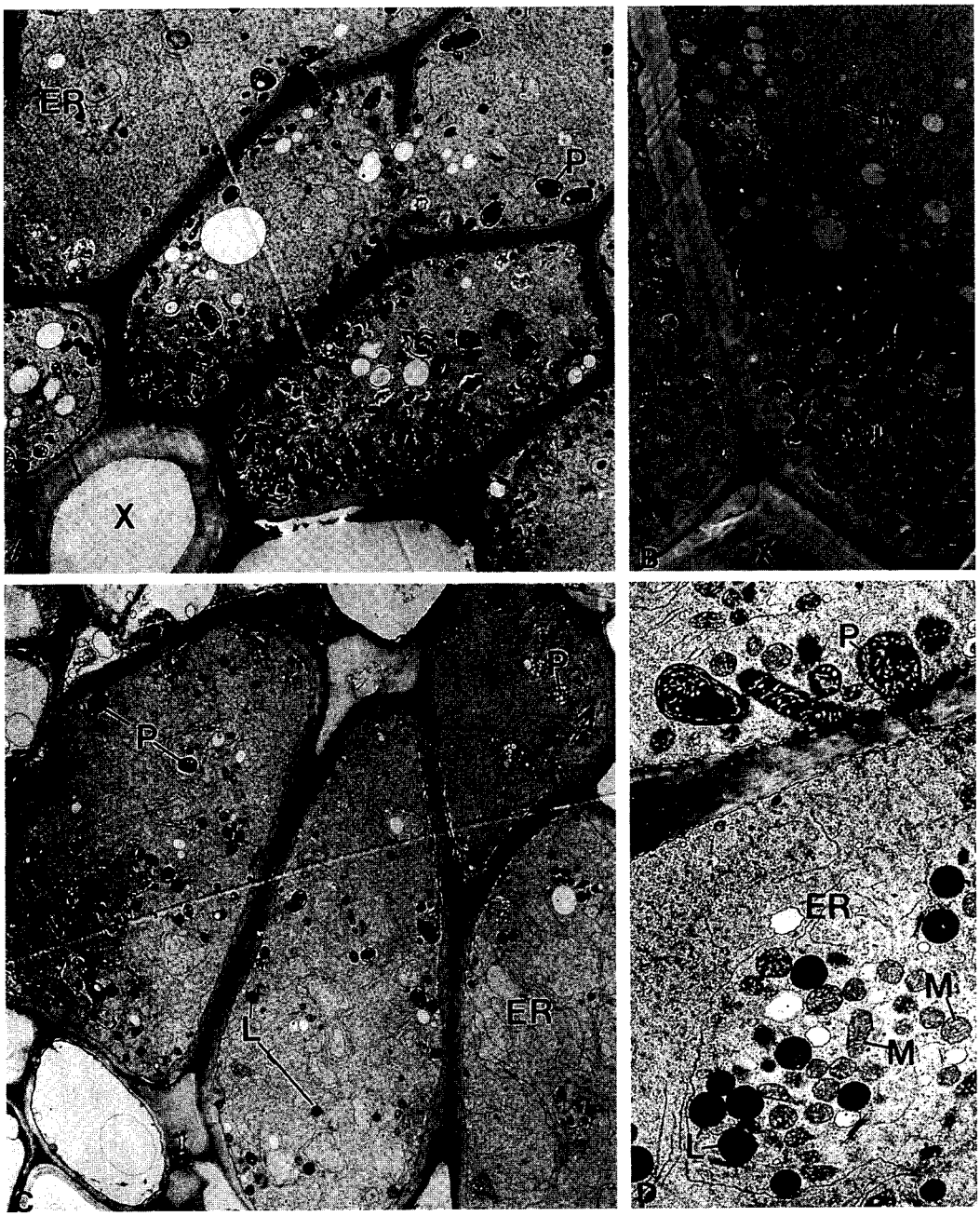

Fig. 2. Electron micrographs of intact syncytia formed in Lee74 with R3 (A) and R14 (B), and PI 97100 with R3 (C & D), showing dense syncytial cytoplasm with prominent endoplasmic reticulum (ER) and plastids (P). Large syncytia (A & B) contain prominent cell wall ingrowths (WI) adjacent to xylem vessels (X), while small syncytium (C) contains very few cell wall ingrowths (arrow), but more abundant lipid globules (L). D: Another portion of the small syncytium in (C), showing prominent lipid globules (L) and mitochondria (M). N: nucleus. Magnifications: A=×3,300, B=×5,000, C=×3,300, and D=×12,000.

quently found in PI 97100 infected with R3, while large syncytia (larger than 2.0×10^{-2} mm^2) were most abundant in Bragg infected with R14. None of such small syncytia were formed by R14.

Syncytium degeneration was examined based on the cytoplasmic features under the light microscope. Degenerated syncytia were observed more than two times in the R14 infections than in the R3 (Table 2). Also in the R14 infections, Bragg had the most numerous degenerated syncytia. Syncytial areas tended to increase with increase of stelar areas. Syncytial occupation of the stele (percentage of syncytium in stele) was higher in Lee74 and Bragg than in PI 97100 (Table 2).

Light microscopy of syncytium structures. Syncytia

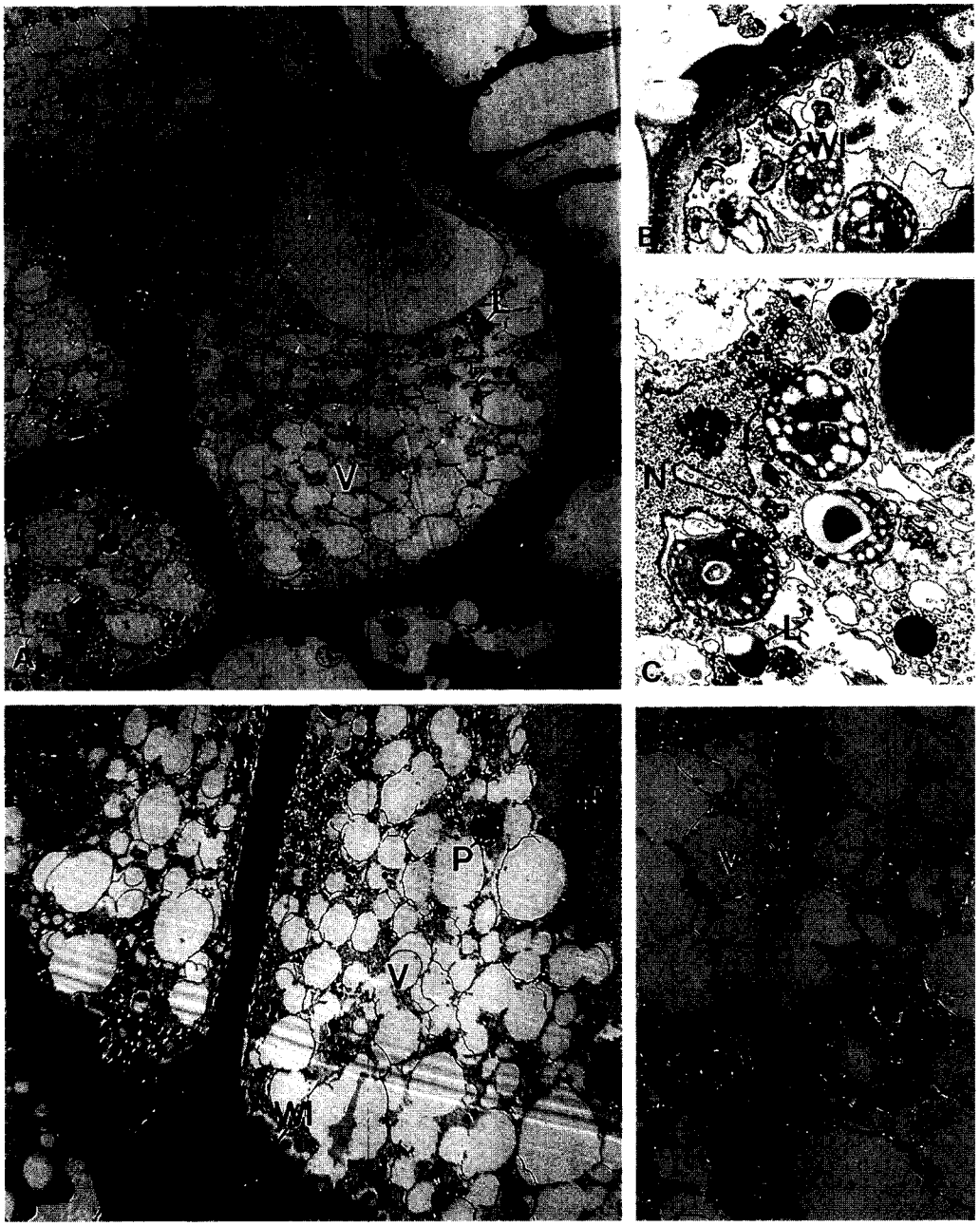

Fig. 3. Electron micrographs of degenerated syncytia formed in PI 97100 with R3 (A-C) and Bragg with R14 (D & E), showing that syncytial cytoplasm containing numerous small vacuoles (V) and degenerated plastids (P) and irregular-shaped mitochondria (M). A: Small syncytium in which no cell wall ingrowths are visible. B & C: Higher magnifications of the small syncytium showing few cell wall ingrowths (WI), degenerated plastids (P) and lipid globules (L). D: Large syncytium with prominent cell wall ingrowths (WI) adjacent to xylem vessel (X). E: Higher magnification in another portion of (D) with prominent irregular-shaped mitochondria. N=nucleus. Magnifications: A=×3,300, B & C=×12,000, D=×3,300, and E=×12,000.

examined in this study could be divided into two groups at 20 days after inoculation; syncytia with healthy-looking (intact) dense cytoplasm (Figs. 1A-1C, 1F) and degenerated cytoplasm (Figs. 1D-1F, 1G, 1H). Intact syncytia had numerous plastids (Fig. 1F). Cytoplasm of the degenerated syncytia was loose and sometimes depleted, and had few noticeable plastids under the light microscope (Figs. 1G, 1H). At the nematode infection site (near the nematode head), syncytial cell walls were greatly thickened, and identified as the infection site of the nematode (Figs. 1A, 1B). Infection sites of both SCN races were mostly located outside the stele in the cortex; however, R14 sometimes (about 10%) established the infection site in the center of the stele (Fig. 1E), in which stelar tissues were considerably damaged probably by nematode penetration, migration and growth. No such case was observed in R3 infections. Location of syncytium at the infection site varied, but syncytia produced by R14 females were located more in the center of stele than those by R3. In the highly tolerant soybean PI 97100 with R3, syncytia were mostly located outside of the stele (Fig. 1D).

The large and medium-sized syncytia had prominent cell wall ingrowths on the syncytial walls adjacent to xylem vessels (Figs. 1C, 1F-1H). However, no or few cell wall ingrowths were observed on the syncytial walls of the small syncytia (Figs. 1A, 1D, 1E).

Cytology of syncytia. Electron microscopic studies were focused on cytoplasmic features of intact and degenerated syncytia with small and large sizes to search for any significant relationships between syncytial characteristics and nematode growth. Both large and small intact syncytia had dense cytoplasm with few small vacuoles (Fig. 2). In large intact syncytia, prominent cell wall ingrowths were formed on syncytial walls adjacent to xylem vessels, and numerous plastids were formed and usually arranged along the syncytial cell walls (Figs. 2A, 2B). On the other hand, no or few cell wall ingrowths were formed in the small syncytia (Fig. 2C). Lipid globules were abundant in the small syncytia, and often accumulated together with mitochondria (Fig. 2D).

Degenerated syncytia, large or small, contained highly vacuolated cytoplasm usually with few plastids (Figs. 3A, 3D). As in the intact syncytia, prominent cell wall ingrowths were formed in large degenerated syncytia (Fig. 3D), but not in small syncytia (Fig. 3A). As in intact syncytia, lipid globules were more abundant in small syncytia than in large ones (Fig. 3C). Plastids appeared to be degenerated in the small syncytia, as indicated by low electron-density and depletion of the contents (Figs. 3B, 3C). In large degenerated syncytia, mitochondria were irregular-shaped (Fig. 3E).

Discussion

Kim and Riggs (1987) reported that SCN race 4 (believed to be R14 or a similar race by the complete race scheme for SCN (Riggs and Schmidt, 1988)) was more reproductive than R3 in susceptible soybean cultivars. In this study also, nematode reproduction including female formation and fecundity was higher in SCN R14 than in R3 on all the soybean cultivars tested. The syncytia induced by R14 were larger than those by R3. As the syncytium development is required for the nematode to mature and reproduce, the reproduction and growth results in this experiment may be corresponding to the syncytium sizes. The fact that degenerated syncytia were more frequent in R14 infections than the in R3 also indicates that R14 nematodes required more nutrients for their growth and reproduction. The more syncytial depletion and degeneration of syncytia might hinder the further nematode growth of R14 from 20 days through 30 days after inoculation, so that no difference in female size was observed between the two races at 30 days after inoculation. R14 penetrated and established the infection site more deeply, and gave more damages to the host plants by forming larger syncytia. In these respects, R14 seems to be more aggressive than R3.

Gipson et al. (1971) described that degradation of syncytial cytoplasm is accompanied with loss of plastids and swelling of cisternae of the endoplasmic reticulum (ER). Syncytia have numerous plastids at the late stage of infection, often containing starch granules, regardless of host species (Kim et al., 1986; 1989). Degenerated syncytia were highly vacuolated and less abundant with plastids than intact syncytia in this study. Loss of plastids and vacuolation of cytoplasm may result from depletion of cytoplasmic contents and digestion of stored food in plastids for feeding the infecting nematodes.

Cell wall ingrowth forms mostly adjacent to xylem vessels or sometimes sieve tubes, which functions to increase the absorption of water and nutritional materials from the adjacent cells, and is a typical structure feature at late stages of infection (Endo, 1992; Jones, 1981; Kim et al., 1987). Large syncytia formed prominent cell wall ingrowths, whereas small syncytia usually contained very few of them. Instead, the small syncytia were abundant with lipid globules, which may serve as another food source for the infecting nematodes.

Plant tolerance, which differs from resistance, does not suppresses nematode development and reproduction (Anand and Koenning, 1988), and is race independent (Boerma and Hussey, 1984; Boerma et al., 1986). In this study also, female formation and fecundity within the same races were not significantly different among the soybean cultivars tested. Tolerance of a plant to the nematode is a matter of population densities of SCN (Baker and Olthof, 1976), for the amount of plant damage differs with the number of

nematodes infecting root tissues. In field microplot tests, shoot and root growth of both cultivars were stimulated at low initial population densities of SCN but were suppressed at high densities (Boerma et al., 1986), suggesting that compensatory root growth (tolerance) may be dependent upon degree of root damages caused by nematode infection. Boerma and Hussey (1984) reported that cultivars tolerant to SCN also exhibited tolerance to *Hoplolaimus columbus*; however, tolerance limits of the two nematode species differed. Young (1992) indicated that some of the differences in tolerance limits may be due to the predominance of different races in fields. In this study, SCN R14 formed larger syncytia and damaged root tissue more than R3, suggesting that the tolerance limit for R14 may be lower than that for R3. Tolerance is likely to be less effective to R14 than R3 at high SCN population densities.

Various mechanisms for tolerance of plants to nematode infection have been suggested in terms of enhanced and compensational root growths (Cook and Evans, 1987; Miltner et al., 1997; Wallace, 1987). Another mechanism was suggested to be related to syncytium location and size (Kim et al., 1986; Yum et al., 1992). In this study, the tolerant cultivar PI 97100 had smaller syncytia and less syncytial occupation than the less tolerant cultivars Bragg and Lee74. However, the syncytium size and occupation were not clearly differentiated between the intolerant cultivar Bragg and the moderately tolerant cultivar Lee74. Also size and location of a syncytium are affected by soil environments such as water potential (Johnson et al., 1993). Because the longitudinal transport of water and nutrients occurs through the stelar region, obviously the occupation of the stele by syncytia is related to plant function and thus to plant tolerance. However, use of this method in screening for tolerance may be tentative because of variations of syncytium structures caused by various unknown factors.

References

Anand, S. C. and Koenning, S. R. 1986. Tolerance of soybean to *Heterodera glycines*. *J. Nematol.* 18:195-199.

Barker, K. R. and Olthof, T. H. A. 1976. Relationships between nematode population densities and crop responses. *Annu. Rev. Phytopathol.* 14:327-353.

Boerma, H. R. and Hussey, R. S. 1984. Tolerance to *Heterodera glycines* in soybean. *J. Nematol.* 16:289-296.

Boerma, H. R., Hussey, R. S. and Reese, Jr. P. F. 1986. Tolerance to soybean cyst nematode. Proceedings of the Soybean Seed Research Conference, XVIII, pp.53-58. Am. Seed Trade Assoc., Washington, DC.

Cook, R. and Evans, K. 1987. Resistance and tolerance. In: *Principles and Practice of Nematode Control in Crops*, ed. by R. H. Brown and B. R. Kerry, pp.179-231. Academic Press, Orlando, Florida.

Endo, B. Y. and Veech, J. A. 1970. Morphology and histochemistry of soybean roots infected with *Heterodera glycines*. *Phytopathology* 60:1493-1498.

Endo, B. Y. 1992. Cellular responses to infection. In: *Biology and Management of the Soybean Cyst Nematode*, ed. by R. D. Riggs and J. A. Wrather, pp.37-49. APS Press, St. Paul, Minn.

Gipson, I., Kim, K. S. and Riggs, R. D. 1971. An ultrastructural study of syncytium development in soybean roots infected with *Heterodera glycines*. *Phytopathology* 61:347-353.

Johnson, A. B., Kim, K. S., Riggs, R. D. and Scott, H. D. 1993. Location of *Heterodera glycines*-induced syncytia in soybean as affected by soil water regimes. *J. Nematol.* 25:422-426.

Jones, M. G. K. 1981. The development and function of plant cells modified by endoparasitic nematodes. In: *Plant Parasitic Nematodes,* Vol. 3, ed. by B. M. Zuckerman and R. A. Rohde, pp.255-279. Academic Press, New York.

Kim, Y. H., Kim, K. S. and Riggs, R. D. 1986. Morphological characteristics of syncytia in susceptible hosts infected by the soybean cyst nematode. *Phytopathology* 76:913-917.

Kim, Y. H. and Riggs, R. D. 1987. Reproductivity of mixtures of race 3 and race 4 of *Heterodera glycines* on soybean cultivars. *Korean J. Plant Pathol.* 2: 199-206.

Kim, Y. H., Riggs, R. D. and Kim, K. S. 1987. Structural changes associated with resistance of soybean to *Heterodera glycines*. *J. Nematol.* 19:177-187.

Kim, Y. H., Riggs, R. D. and Kim, K. S. 1998. Effect of acetylsalicylic acid on the reproduction of soybean cyst nematode in susceptible soybean. *Korean J. Plant Pathol.* 14: 368-392.

Miltner, E. D., Karnok, K. J. and Hussey, R. S. 1991. Root responses of soybean tolerant to soybean cyst nematode. *Agron. J.* 83:571-576.

Riggs, R. D. and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. *J. Nematol.* 20: 392-395.

Wallace, H. R. 1987. A perception of tolerance. *Nematologica* 33:419-432.

Young, L. D. 1992. Epiphytology and life cycle. In: *Biology and Management of the Soybean Cyst Nematode*, ed. by R. D. Riggs and J. A. Wrather, pp.27-36. APS Press, St. Paul, Minn.

Yum, K. J., Park, E. W. and Kim, Y. H. 1992. Morphological characteristics of syncytia related to tolerance of soybean to *Heterodera glycines*. *Korean J. Plant Pathol.* 8:286-292.