SYNCYTIA DEVELOPMENT IN GERMLASM PEA ACCESSIONS INFECTED WITH HETERODERA GOETTINGIANA

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Summary. The ultrastructure of syncytial development in roots of pea accessions attacked by Heterodera goettingiana Liebscher is described 4 and 10 days after nematode invasion. Four days after inoculation a feeding plug was evident where the stylet had penetrated the wall of the initial syncytial cell. In the cell the fine structure was modified. Nonmembrane-bound materials, which appeared to be nematode secretions, were evident near the stylet terminus and within the syncytial cytoplasm. Syncytia continued to develop in susceptible pea accessions 10 days after nematode invasion. In the modified cells proliferation of actively synthetising cytoplasm indicate that the syncytial components function as secretory system. At the same time the content of syncytial cells of the resistant pea accessions is strongly disintegrated. The hypersensitive response in terms of physiological cell death is discussed.

Many investigations have been made on the ultrastructural changes induced in plant hosts by cyst nematodes. Second-stage juveniles enter the roots, moving intracellularly through the cortical cells and then usually fix their lip region on one endodermal cell from which they can stimulate a syncytium. The maturation of the nematode is directly related to the development and persistence of syncytia (Acedo et al., 1984). Many authors have given detailed descriptions of the ultrastructural features of syncytia induced in different susceptible and resistant hosts by Heterodera spp. (Hoopes et al., 1978; Kim et al., 1986; Wyss et al., 1984; Rice et al., 1985; Bleve-Zacheo and Zacheo, 1987) following the progressive changes in the root cells starting from 42 h after nematode inoculation (Riggs et al., 1973). In affected cells juveniles were observed to stimulate syncytial formation about two days after initial penetration of the roots and no differences were detected between susceptible and resistant roots during the following two days apart from more extended necrosis around the outer limits of syncytia in the resistant hosts. Seven days after penetration the syncytial components of the susceptible roots had expanded and contained dense and granular cytoplasm in contrast to those in resistant roots where the cytoplasmic contents were extremely vacuolated and reduced to a thin layer around the cell walls (Rice et al., 1985; Bleve-Zacheo et al., 1990; Melillo et al., 1990a).

Pea (Pisum sativum L.) is the principal host for the pea cyst nematode Heterodera goettingiana Liebscher. Little information is, however, available on the morphological response of pea roots to nematode invasion. In this paper we compare the ultrastructural changes induced by the pea cyst nematode in germplasm pea accessions. In previous studies they were selected as susceptible or resistant to H. goettingiana through the analysis of syncytial development and enzyme activities in roots (Melillo et al., 1990b; Zacheo et al., 1981, 1986).

The results reported here follow the course of structural changes at intervals after nematode invasion of this host by H. goettingiana with a view to elucidating the mechanism associated with resistance or susceptibility.

Materials and methods

Juveniles of H. goettingiana were obtained from cysts collected from infected pea plants that were grown in clay pots containing field soil infested with the pea cyst nematode. Seeds of six accessions of germplasm pea: MG 101877a and MG 101877b (Pisum arvense L., collected in Ethiopia), MG 101956a and MG 101956c (P. elatius Stev., obtained from Netherland collection), MG 101748 (P. arvense, collected in South Hungary) and MG 103738 (P. sativum sp. transcaucasicum, obtained from the Gatersleben collection), collected and multiplied at the Istituto of Germoplasm, Bari, were germinated under sterile conditions. When root initials appeared the seedlings were transplanted into clay pots containing 10 ml of sterilized sand and simultaneously a suspension of 50 second-stage juveniles of H. goettingiana was added to each pot. The inoculated plants were maintained at 17°C in growth chambers. At four and ten days after nematode inoculation, roots were removed and washed. Segments of infected roots were excised and fixed in 3% glutaraldehyde in 0.05
M sodium cacodylate buffer pH 7.2 for 4 h, rinsed in the same buffer, post-fixed in 2% osmium tetroxide for 4 h at 4°C, then stained in 0.5% uranyl acetate, dehydrated in Spurr’s medium (1969). Sections 2 µm thick were cut with an LKB ultratome IV, stained with 1% toluidine blue and observed under a light microscope to verify the syncytial location. Ultrathin sections were cut in that region and stained with uranyl acetate and lead citrate and examined under a Philips 400 T transmission electron microscope at 80 kV.

Results

Cells directly fed upon by the juveniles of *H. goettingiana* were easily identified because of a plug like deposit in the cell wall in both susceptible (MG 101956 c) (Fig. 1a) and resistant (MG 101748, 103738) pea roots (Figs. 1b, 1c). The electron dense deposits forming the plug close that part of the cell wall through which the nematode has inserted its stylet to establish its feeding site. In the same sections amphidial channels containing a matrix of dense material were detected, some of which appeared to be continuous with the plug material, suggesting that they may be related (Endo, 1978). In a 4-day infection of pea roots the feeding plug extended along the thickened wall of the initial cell of the syncytium. The odontostyle passed through the plug, pushing the plasma membrane deeply into the cell (Fig. 1a). Tubule-like material, which appeared as small vesicles when cut in transverse section, was aggregated around the stylet and was distributed throughout the cytoplasm (Figs. 1a, 1b). Similar material was observed around the feeding tube (Fig. 1b).

The modifications to the fine structure in the cells of 101956c consisted of numerous ribosomes, either as free particles in the cytoplasm or as polysomes, many mitochondria with dense matrices and enlarged cristae and endoplasmic reticulum in smooth and rough form (Fig. 1a). In MG 103738 roots the cytoplasmic ground substance appeared to be less dense (Fig. 1b) and in MG 101748 roots whorls of rough endoplasmic reticulum and small irregular vacuoles were present (Fig. 1c). Wall fragments inside the syncyta were thickened, and nuclei were amoeboid and the nucleolus highly vacuolated (Figs. 2a, 2b). The cytoplasm of cells incorporated into the syncytium in 101956 a had many small vacuoles, all containing protein storage. Some of them were fused to form an irregular larger vacuole (Fig. 2a). Numerous plastids were widely distributed within the syncytial cytoplasm of 101777 b. Their previous regular shape was distorted because of starch grains that they had accumulated (Fig. 2b).

The lytic process of syncytia in resistant roots started in the incorporated cells furthest from the nematode. In these cells there were vacuoles with indented tonoplast containing granular material or myelinic structures. Around each vacuole endoplasmic reticulum was present and a dark matrix appeared to be apposed to the tonoplast (Figs. 3a, 3b). This suggests that the ER, as the principal site of synthesis of digestive enzymes, could be involved in the deposition of vacuole contents. In the degenerating cytoplasm a tangle of membranous structures were associated with the lytic vacuoles (Fig. 3c).

Ten days after nematode inoculation the syncytia in the four resistant pea accessions had degenerated (Fig. 4). The cells appeared to be necrotic, with the typical hypersensitive response. Cytoplasmic contents were disintegrated and nuclei destroyed, a process referred to as karyolysis (Fig. 4b). The feeding tubes, which are stable structures, remained in the cytoplasm of the syncytial cells (Fig. 4c).

Ten days after nematode inoculation in susceptible pea accessions, the modified cells increased in number. Most of the cell walls, particularly in the initial cells of the structure, were digested and the communal cytoplasm was free to move within syncytium (Fig. 5a). The ground cytoplasm was granular with small vacuoles and occupied all the surface of the syncytium. Mitochondria were well preserved and similar to those in young syncytia. Endoplasmic reticulum, arranged in parallel arrays, was present in both smooth and rough forms (Fig. 5b). Nuclei were amoeboid in profile so that in section a single nucleus could appear as multinucleate. The amoeboid shape increases the capacity for nuclear-cytoplasm exchange. This features of nuclear content is typical of the reticulate nucleus reported in *Pisum* by Lafontaine (1974) (Fig. 6a). Accumulation of protein within rough endoplasmic reticulum profiles appears to be associated with Golgi cisternae as seen in Fig. 6b. Free polyribosomes and a large quantity of filamentous dark material (proteins?) were scattered in the cytoplasm (Fig. 6c). Wall ingrowths, typical of transfer cells, have been found adjacent to the xylem elements in both susceptible pea accessions (Fig. 6d).

Discussion

The results extend our previous observations (Melillo et al., 1990b) and confirm that there is a striking difference in reaction to *H. goettingiana* between the susceptible MG 101956 c and MG 101877 a and the resistant MG 101748, MG 103738, MG 101877 b, MG 101956 a, pea accessions. It appears to be a common feature that *Heterodera* spp. readily invade roots of resistant hosts and in-
Fig. 2 - Cross sections through syncytia of roots of resistant pea accessions (MG 101956 a and 101877 b) 4 days after nematode inoculation. Syncytial cells in MG 101956 a are filled with dense cytoplasm and numerous small vacuoles (va) containing protein bodies (a). Note that the syncytium has expanded right up to the vessels and wall fragments (cw) are evident inside it. The plastids (p) in the syncytium in 101877 b contain large starch grains (b). Nuclei (N) are amoeboid with dense vacuolated nucleolus (a x 2,100, b x 4,500).
Fig. 3 - (a) Sections through part of a syncytium in MG 101956 a 4 days after inoculation. Note the irregular profiles of the tonoplast of the vacuoles containing lytic material and myelin-like structures. Portions of endoplasmic reticulum enclose the vacuoles as rings (x 21,000). 
(b) Same features of the vacuoles in a syncytium induced in MG 103738 accession (x 7,600). 
(c) Section through a syncytial cell in MG 101748 accession, close to the xylem. Degeneration of syncytial cells starts furthest from the nematode head. Cytoplasmic content is transformed in a tangle of membranes (Me) occupying with the vacuoles, the most part of the degraded cytoplasm (x 10,400).
Fig. 4 - Sections through degenerated syncytia in resistant accessions MG 101877 b, MG 103738 and MG 101956a (a, b, c respectively) 10 days after inoculation. No cellular structures are evident and only fragments of preexisting cytoplasm and nuclear components remain. Note the well preserved feeding tubes (ft) (a x 1,180, b x 10,900, c x 6,600).
Fig. 5 - a) Section through a developed syncytium from the root of the susceptible accession (MG 101956 c) 10 days after nematode inoculation. A large number of cells are involved in syncytial formation. Nuclei and cytoplasm are well preserved and small vacuoles are present (× 1,250). b) Enlargement of a syncytium in MG 101877 b, same day old than in a. Plug, vesicle membranous material and feeding tubes present in the dense cytoplasm where profiles of endoplasmic reticulum arranged in parallel layers are detectable (× 5,870).
duce a syncytium, which disintegrated a few days later. The structures described in the resistant accessions are similar to those observed in other host cyst nematode interactions (Riggs et al., 1973; Wyss et al., 1984; Rice et al., 1985, 1987; Bleve-Zacheo et al., 1990). The changes occurring after the initiation of feeding sites can be directly implicated in the resistance mechanism. The development of a hypersensitive reaction enclosing the syncytium may result either from a component in the juvenile's saliva that directly induces the resistant reaction, or from a salivary component that initiates a series of biochemical reactions inducing the process. Rice et al. (1987) suggest that the second hypothesis is more likely because with the former an immediate response within the initial syncytial cells might be expected. We agree with this assumption because of the difference between necrotic cells (mechanically

Fig. 6 - a) Enlargement of an amoeboid nucleus within the syncytium of a susceptible root (MG 101956 c) 10 days after inoculation. The features of the nucleoplasm and nucleolus are typical of reticulate nuclei of unaffected roots (x 3,000). b) Profiles of endoplasmic reticulum aggregated in parallel cisternae, containing synthesized products, evident as electron dense material inside the cytoplasm of a syncytium as in a (x 23,700). c) Aggregates of filamentous material (proteins?) in the cytoplasm of a developed syncytium 10 days after inoculation in the susceptible MG 101877 b accession. These aggregates appear to be associated with polysomes free in the cytoplasm (pr) (x 17,900). d) Transverse section of a syncytium in MG 101877 b showing darkly-stained wall ingrowths near the xylem (x). The ingrowths are branched and anastomosed and related to mitochondria (m) (x 4,800).
damaged by nematode penetration) and physiological cell death (host response). There is no single mechanism to account for either the induction or the progression of cell death. Death may involve the activation of specific genes, with the synthesis of mRNA coding for novel protein transcripts. Such cell death in the hypersensitive reaction should clearly be regarded as an active process, induced by physiological factors to which particular cells are receptive. Physiological death of syncytial cells is at least, in its early stages, a controlled process and is strikingly different from the traumatic death of cortical cells, due to the mechanical damage by nematode penetration and in which a cell is suddenly challenged beyond its capacity to cope (Lockshin and Zakei-Milovanovic, 1984). Once the signal has been received, the cells initiate a sequence of biochemical events. Following minor cytoplasmic changes the syncytial cells usually show some condensation of chromatin, variable development of lysosomes and autophagic vacuoles, and changes in the protein-synthesis machinery. This suggests that the cells are destined to die because they lose the ability to respond to the specific needs indicated by the nematode. Thus, syncytial cell death must be invoked at the level of the chromosome, where the synthesis of new components capable of helping the cell adjust to its demand is blocked. This hypothesis envisages that the genes responsible for initiating physiological cell death are responsible for the synthesis of nascent degradative enzymes.

The accumulation of proteinaceous deposits in vacuoles could, for example, indicate the presence of a proteinase inhibitor. In detached tomato leaflets, storage proteins are induced in the vacuoles of mesophyll cells if illumination is provided. These protein bodies have been identified with a proteinase inhibitor (Ryan, 1973). This seems to be an unresponsive response to wounding since mechanical damage has the same effect of inducing the rapid accumulation of inhibitor protein in the wounded leaves. Green and Ryan (1973) suggest that the inhibitor protein impairs the digestion in the intestinal tract of insects attacking the plant and, therefore, represents a defense mechanism. In addition, several hydrolases increase their activity and particularly RNAse activity is reported during fungal infection (Pitt and Galpin, 1973) and it appears to be localized in the vacuoles. Hydrolases accomplish degradation of macromolecules with elimination of cytoplasmic organelles and disintegration of membranes. In contrast, increased metabolic activity of syncytial components elucidates the different manner in which the susceptible system works. The syncytial cells are induced to make proteins and the large proportion of ribosomes and polyribosomes lying free in the cytoplasm is similar to that in normal cells, where it is internal use. It can be speculated that they synthesize the many proteins that accumulate in the cytoplasm. Moreover, the accumulation of protein in the membrane of endoplasmic reticulum and presence of cell-wall ingrowths, responsible for transferring solutes into syncytia from surrounding tissues (Jones and Gunning, 1976), may indicate an enormous food reserve for the nematode.

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**Literature cited**


