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CRYSTAL-LIKE STRUCTURES IN PLASTIDS OF TOMATO ROOTS, INFESTED BY *MELOIDOGYNE INCOGNITA*

by
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Crystalline inclusions occur in the cytoplasm of healthy cells in many plant species and represent protein storage structures or the accumulation of fraction-I protein or enzymes. The inclusions are of various sizes and shapes and have been found in cell constituents such as spherosomes, mitochondria and plastids (Marinos, 1967; Martelli and Russo, 1977; Newcomb, 1967). Crystalloids have often been observed in the plastids of bean leaves treated with hypertonic solutions or poisonous gases (Shumway *et al.*, 1967; Gunning *et al.*, 1968; Wrischer, 1973).

There are cases in which ordinary crystalline cell contituents are much more abundant in diseased than in healthy cells. Esau (1975) related the higher number of the intraplastidial protein crystals, found in virus infected cells in spinach, as compared to healthy cells, to an effect of the causal agent on the metabolism and sugar translocation in leaves.

The results of cytological studies carried out to determine numerical and morphological differences between plastids in healthy and infested tomato roots, resistant to *Meloidogyne incognita* (Kofoid *et* White) Chitw., are discussed in this paper.

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Materials and Methods

Seedlings of tomato cv. Brech, resistant to *M. incognita*, were grown individually in 5 cm diameter plastic pots containing quartz sterilized sand. The pots were placed in a growth chamber (22°C 65% RH 3,000 lux) and each inoculated with one hundred juveniles of *M. incognita*. Six days after inoculation the roots were excised and fixed for 4 hours in 4% glutaraldehyde at 4°C, rinsed overnight in 0.05 M cacodylate buffer at pH 7.2 and postfixed for 4 hours in 2% osmium tetroxide. The samples were stained overnight at 4°C in 0.5 M uranyl acetate. They were than dehydrated in a graded ethanol series and embedded in Spurr's medium.

Healthy and infested root pieces were fixed in 3% glutaraldehyde at 4°C for 30 min. After several washes in cacodylate buffer, they were sectioned (60-100 µm thick) and incubated in DAB medium for one hour at 30°C. The medium contained 10 mg of 3-3' diaminobenzidine tetrahydrochloride (DAB Sigma), dissolved in 5 ml of 0.05 M 2 - methyl - 1,3 - propandiol buffer, pH 7.4. After incubation, the sections were rinsed repeatedly in buffer, postfixed in 2% osmium tetroxide and embedded in Spurr's resin.

Ultrathin sections, cut on an LKB ultratome IV, were stained in uranyl acetate and lead citrate (Reynolds, 1963), and examined at 60-80 Kv in a JEM 100 B electron microscope.

Areas of crystals were measured with a Hewlett-Packard 9874 A Digitizer connected to a 9835 A calculator system.

Results

The number of plastids per cell section was similar (Tab. I) in ultrathin sections obtained from both healthy and infested differentiated or undifferentiated tissues. Also the morphology of the stroma of the organules did not seem to differ in infested and healthy tissues. At the ultrastructural level differences were seen between healthy and diseased cells observed in the content of crystal-like bodies and in the accumulation of starch.

Crystalloids, characterized by a granular stroma and enveloped in a membrane, were present in the plastids of cells from both healthy and infested tissues. However, a larger number of plastids,

Table I - *Crystalline inclusions in roots of tomato cv. Brech, healthy and infested by Meloidogyne incognita.*

	Number of plastids/ section [1]	% of plastids with crystals	Number of crystals/ plastid	Area of crystals
Healthy roots	3.25	36	1.10	1.014
Infested roots	3.4	66	1.48	1.615

[1] Mean of 200 observations/plastids.

containing crystal-like inclusions, were detected in the cells from infested tissue (Figs 1 and 2).

An examination of 200 plastids showed crystalloids in 36% from healthy and 66% from infested tissues (Tab. I). The average number of crystalloids per plastid was 1.10 in healthy cells and 1.48 in cells from infested tissues. The crystalline core was larger (1.615 μm) in diseased cells, than in healthy ones (1.014 μm).

The crystalloids in the plastids of the healthy cells were spherical and of non-crystalline structure (Fig. 1), whereas in diseased cells the plastids contained crystalline bodies of a variety of shapes and structures, such as spherical and polyhedric (Figs 3 and 4), densely granular or rhomboid with crystal-like structure (Fig. 6).

The plastids treated with DAB were more intensively stained and electron dense than untreated (Fig. 3). The staining was more intense on the crystalline structures (Figs 3, 4 and 6).

The accumulation of paracrystalline material in the stroma of plastids appeared to take place gradually and varied at different sites in the same plastid: one was observed with a crystalline core at an advanced stage and in another part small aggregates of particles were visibles within an electron light matrix indicating an early stage in the formation process (Fig. 3).

The membranes enveloping the crystals assumed different forms according to the stage of crystallization of the proteins. The membranes were shaped in angular patterns (Fig. 6) when crystallization was in a more advanced stage, but were rounded on one side and angular on the other at an intermediate stage of crystallization (Fig. 5).

Increased synthesis activity and the accumulation of plastids within the diseased cells, compared to the healthy ones, was corre-

lated with a remarkable accumulation of starch both in the number and the size of the granules (Figs 1 and 2). Also clusters of lipid droplets, more evident in the plastids treated with DAB, were present in the same plastids (Fig. 4).

Discussion

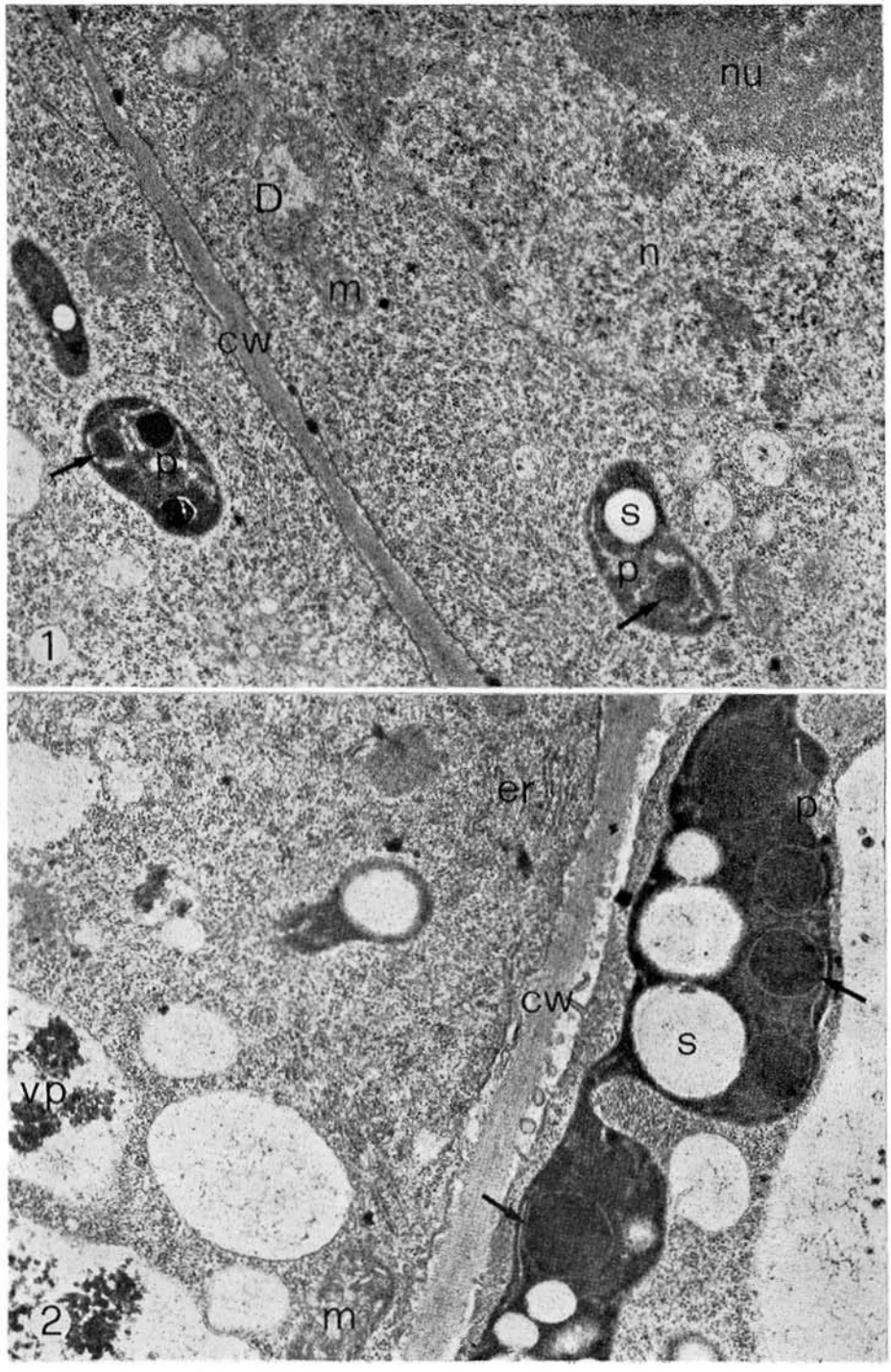
The process of penetration by *M. incognita* into roots of resistant tomato cv. Brech, induced an increase in the number and the size of crystalline structures in the plastids of the root cells. These structures possibly represent a response of the host to the nematode as it also occurs in tissues infected by other pathogens, such as fungi or viruses, where formation of crystals has been found in the microbodies or in the plastids respectively (Coffey *et al.*, 1972; Sargent *et al.*, 1973; Esau, 1975). The fact that the content of the crystals is markedly DAB-sensitive might then indicate that these are structures of proteic nature.

Other authors have studied the nature of these crystals and identified their content as proteins on the basis of digestion with pepsin or positive reaction to mercuric bromophenol blue (Newcomb, 1967; Wrischer, 1967). Their appearance in form ranging from granular to crystalline is assumed to represent the intermediate steps to the accumulation of proteic material. However, the process of complete crystallization might be inhibited by the presence of a mixture of proteins or of extraneous material (Newcomb, 1967).

The reason for the accumulation of these proteins during infestation is still a matter of conjecture. The abundance of crystals observed in diseased cells, compared with the healthy ones, might suggest that the increase is the result of the effect of pathogens on the cellular metabolism. The same explanation could apply to the increased accumulation of starch in the plastids of infested plants (Kosuge, 1978).

Fig. 1. - Healthy cells of tomato root, showing plastids with crystalline inclusions (arrow) and starch grains; DNA filaments are visible in the mitochondria (x 18,000).

Fig. 2. - Very large plastids in cell of an infested root; five large crystalline bodies within membrane-bounded sacs and wide starch bodies in the stroma are recognizable (x 18,000).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Uritani (1976) reported that all injuries stimulate in plant cells the synthesis of essential components and secondary metabolites, including the hexoses pathway. There is no doubt, from these considerations, that the formation of crystalline structures is the consequence of an increased synthesis of proteins induced by the pathogen.

There is evidence that loss of water by osmosis induces the formation of crystals in the stroma of plastids (Perner, 1963) treated with hypertonic solutions; such crystallization should result from the concentration of soluble material within the stroma (Larsson *et al.*, 1973).

Wrischer (1967 and 1973) reported that formation of crystalloids within the stroma of bean chloroplasts was experimentally induced simply by placing the tissues in very low concentrations of sucrose. The formation of crystalline structures in the plastids was prevented by adding chloramphenicol, inhibitor of the proteic synthesis, to the solution.

It is therefore assumed that accumulation of proteins takes place in the plastids, during the invasion of resistant tomato roots by *M. incognita*, following water losses from tissues surrounding the nematodes. False plasmolysis triggered by attacks of pathogens and explained as a loss of the osmotic properties by infected and demaged tissues has been reported by various authors (Wheeler, 1975; Bleve-Zacchco *et al.*, 1982).

The penetration of the nematode into the root tissues causes the destruction of cells, due to lytic enzymes produced by the nematode

Fig. 3. - Plastid with two crystalline bodies, one completely filled and bounded by a membrane (arrow), and the other without crystalline material and incompletely filled with granular material (double arrow) (x 106,000).

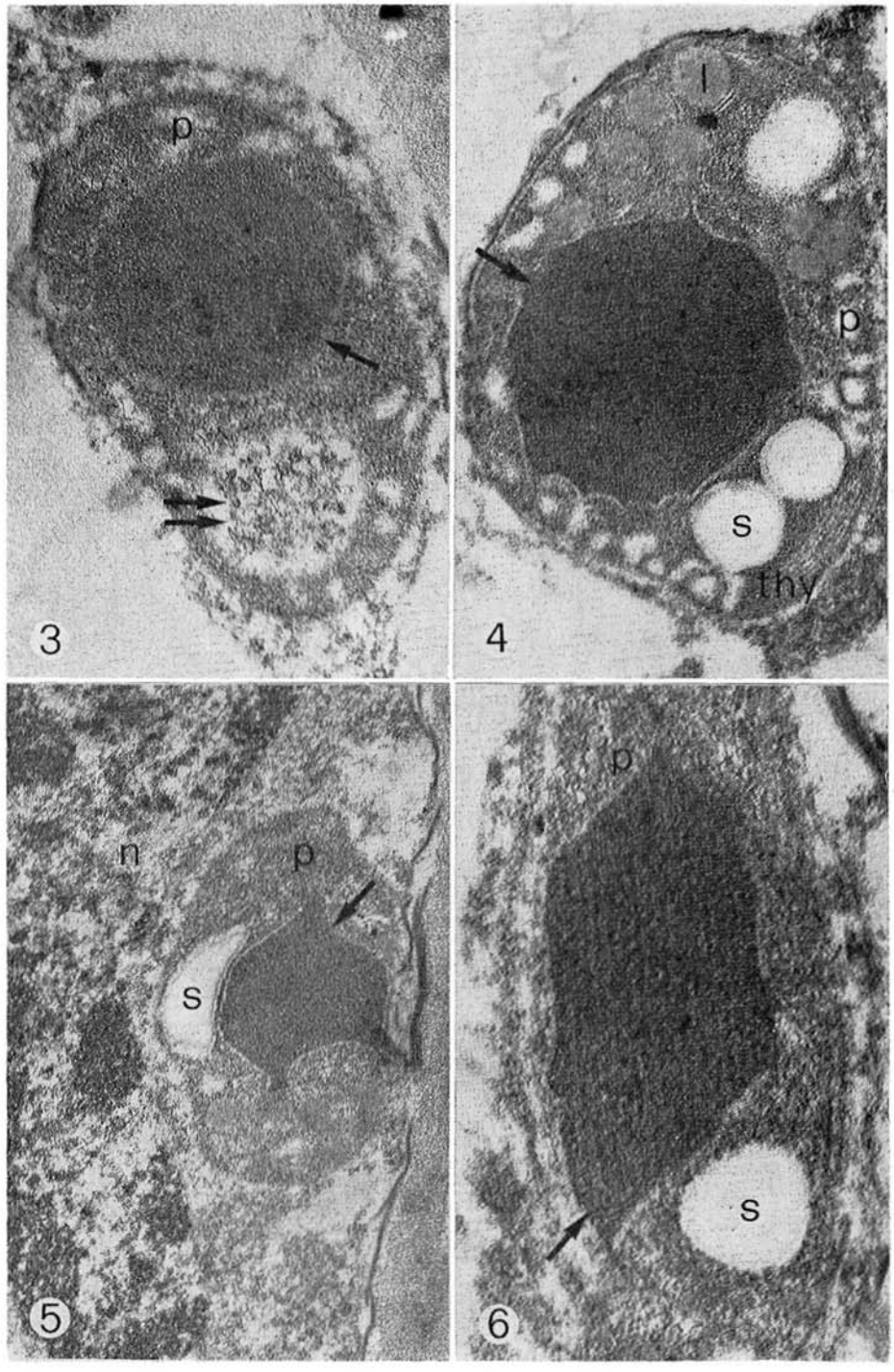
Fig. 4. - DAB stained with dense stroma; thylakoids, lipid droplets and starch grains are visible in the stroma; deeply stained large crystalline core occupies greater part of the plastid (x 120,000).

Fig. 5. - Portion of a cell with a plastid in whose stroma a crystalline body showing the bounding membrane, rounded in one region and angular in another is recognizable (x 92,000).

Fig. 6. - Portion of a plastid showing crystallized protein deposit and the bounding membrane conformed to the angles of the crystal (x 120,000).

LIST OF ABBREVIATIONS USED IN THE FIGURES

cw = cell wall; D = mitochondrial DNA; er = endoplasmic reticulum; l = lipid; m = mitochondrion; n = nucleus; nu = nucleolus; p = plastid; s = starch grain; thy = thylakoid; vp = vacuolar protein body.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(Deubert and Rohde, 1971; Hussey and Sasser, 1973; Bird, 1974). This leads to an osmotic imbalance, similar to that experimentally obtained by some authors (Perner, 1963; Wrischer, 1973), and to the accumulation of proteic material.

SUMMARY

Plastids from the roots of the tomato cv. Brech, resistant to *Meloidogyne incognita*, contained crystalline inclusions enclosed in membranes. Development of the inclusions involved the accumulation of granular material and the assembly of granules in the crystalline bodies. In nematode infested tissues the plastids contained more and larger crystalline cores than in plastids in healthy roots. It is assumed that these crystalline-like bodies are proteins and their formation is triggered by an osmotic imbalance in the cells caused by the nematode invasion of the roots.

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