Chloroplast Differentiation in Tomato Root Galls Induced by the Root-knot Nematode Meloidogyne incognita[1]

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Abstract: Primary roots of tomato, *Lycopersicon esculentum* cv. Marglobe, were cultured aseptically on agar containing a standard nutrient formulation with or without kinetin. When secondary roots developed, cultures were inoculated with the root-knot nematode, *Meloidogyne incognita*. Following inoculation, the cultures were divided into two groups which were incubated either in total darkness or in 16-h light–8-h dark cycles. At 24 h, 1, 2, 3, and 4 wk after incubation, roots from all cultures were processed for transmission electron microscopy. Fine structural observation of the parenchyma tissue in galls from the inoculated cultures indicated that starch containing plastids or amyloplasts, which are usually present and remain undifferentiated in these root cells, developed into chloroplasts. These chloroplasts contained a membrane system indistinguishable from those found in leaves of intact plants. Although plastid development was not affected when uninoculated cultures were incubated in the light, differentiation of the amyloplast was induced when roots were cultured on the medium containing kinetin. These results suggest that plastid differentiation in the inoculated tissue may be influenced by an accumulation of kinetin in the gall, which is induced by the nematode and serves as the nutrient sink for its feeding.

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Cytological studies of the interaction between susceptible host plants and the root-knot nematode, *Meloidogyne incognita,* have concentrated on the changes that occur in the developing giant cells (1,3,4). However, enlarged cells in the cortical tissue of galls produced by *M. incognita acrita* have been reported (5). In addition, galled tissue, which developed in the light, turned green and was found to contain chlorophyll and a chloroplast-like organelle (8). Because chloroplast differentiation and green pigmentation do not generally occur in tomato roots, the authors concluded that the nematode influenced morphological properties in these cells.

The levels of constitutive cytokinins in the root have been correlated with nematode susceptibility of tomato plants (14) and increased levels of cytokinin have been reported in root homogenates following infection with *M. javanica* (2). Because cytokinins and light sensitive processes such as chlorophyll synthesis and the formation and maintenance of chloroplasts are closely associated (6), a transmission electron microscopic study was undertaken to determine if the development and ultrastructure of the plastids in galls from infected roots are similar to the changes that occur in uninoculated roots when exogenous cytokinin is added to the growth medium.

MATERIALS AND METHODS

Seeds of tomato, *Lycopersicon esculentum* cv. Marglobe, were surface sterilized for 15 min in 1% sodium hypochlorite, rinsed three times in sterile distilled water, and germinated at 25 C in petri plates containing 1% water agar. One-centimeter lengths of the primary root tips were excised and transferred to petri plates containing chemically defined media. The media consisted of either a standard formulation by Skoog, Tsui, and White (STW) (11) or STW plus 0.43 ppm kinetin (2μM) (STW + K). The roots were inoculated by placing egg masses of *Meloidogyne incognita,* obtained from monoxenic culture of this nematode, 1–2 cm from the root tips after the lateral roots had emerged. Treatments were replicated six or eight times. All cultures containing control or inoculated roots were incubated in the dark at 25 C.

After 48 h, half of the petri plates from each treatment were wrapped in aluminum foil to exclude the light. These and the unwrapped plates were transferred to a growth chamber and incubated at 25 C with a light-dark cycle of 16 and 8 hrs, respectively. The

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light period consisted of 100-foot candle illumination with Sylvania Gro-Lux fluorescent tubes.

Galls and root segments were processed for electron microscopic observation after 24 h, 1, 2, 3, and 4 wks incubation. Processing consisted of flooding the petri plates with 3% glutaraldehyde in 0.05 M phosphate buffer, ph 6.8 at 22 C. Segments (2–3 mm^3) of the infected galls or control roots were cut from the cultures and transferred to vials containing fresh fixative for 15 h. The root segments were then washed in six changes of buffer over a 1-h period, post-fixed in 2% buffered osmium tetroxide for 2 h, dehydrated in an acetone series, and infiltrated with a low viscosity resin (12). Silver-gray sections of selected root segments were cut with a diamond knife on a Sorvall MT-2 Ultramicrotome and mounted on uncoated 75 × 300 mesh copper grids. The sections were stained with 2% aqueous uranyl acetate for 10 min and with lead citrate for 5 min. Thin sections were viewed in a Hitachi H-500 transmission electron microscope operating at 75 kV.

RESULTS

Inoculated roots grown in the 16-h light–8-h dark cycles: After 24 h incubation the plastids in the cortical parenchyma cells surrounding the penetrating nematode did not exhibit any atypical or modified features. They were preferentially located in the cytoplasm near the cell wall (Fig. 1), were generally round or slightly oblate, measured 1.5–3.0 μm, and contained numerous starch granules 0.5 μm in diameter. Only an occasional internal membrane (thylakoid) was present. These starch-containing plastids (amyloplasts) were easily distinguished from mitochondria, organelles that appeared in about equal numbers, by their larger size, starch content, and electron dense stroma.

After 1 wk the amyloplasts became more oblate and generally measured more than

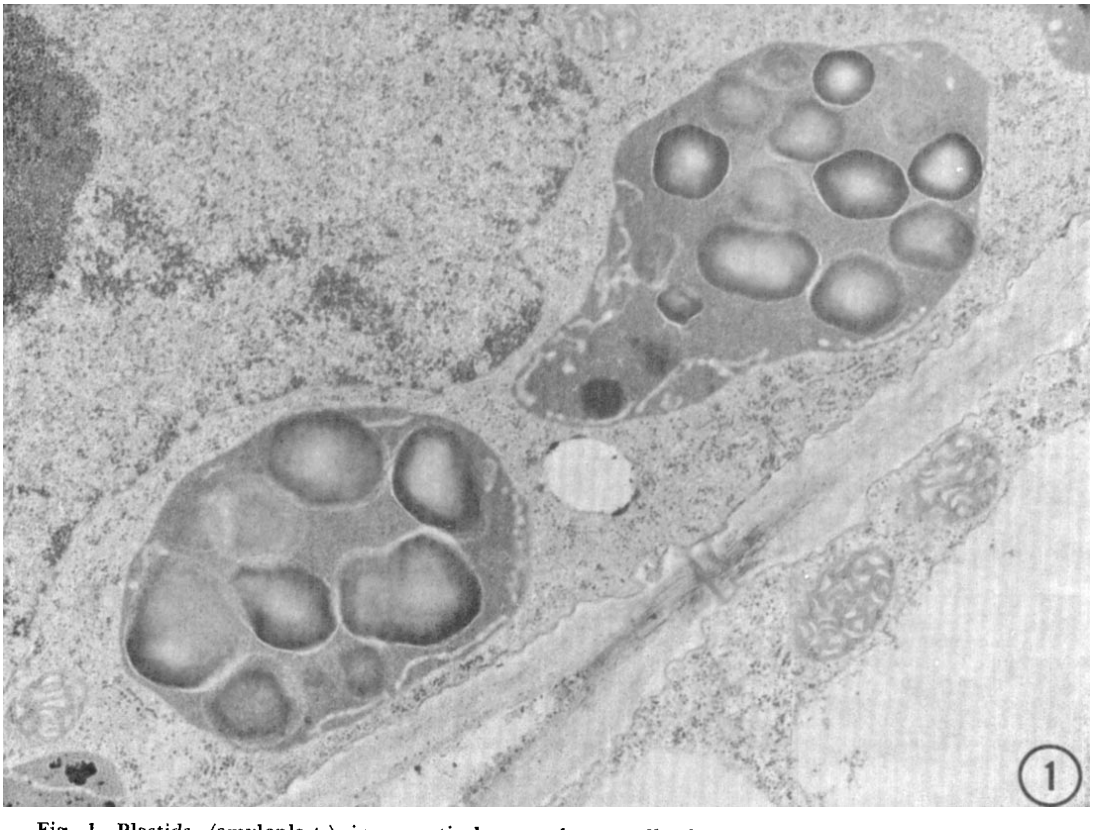

Fig. 1. Plastids (amyloplasts) in a cortical parenchyma cell of tomato root after inoculation with *Meloidogyne incognita* and incubation in 16 h light and 8 h dark. The amyloplasts contain numerous small starch granules but do not exhibit any atypical membrane structures. × 28,000.

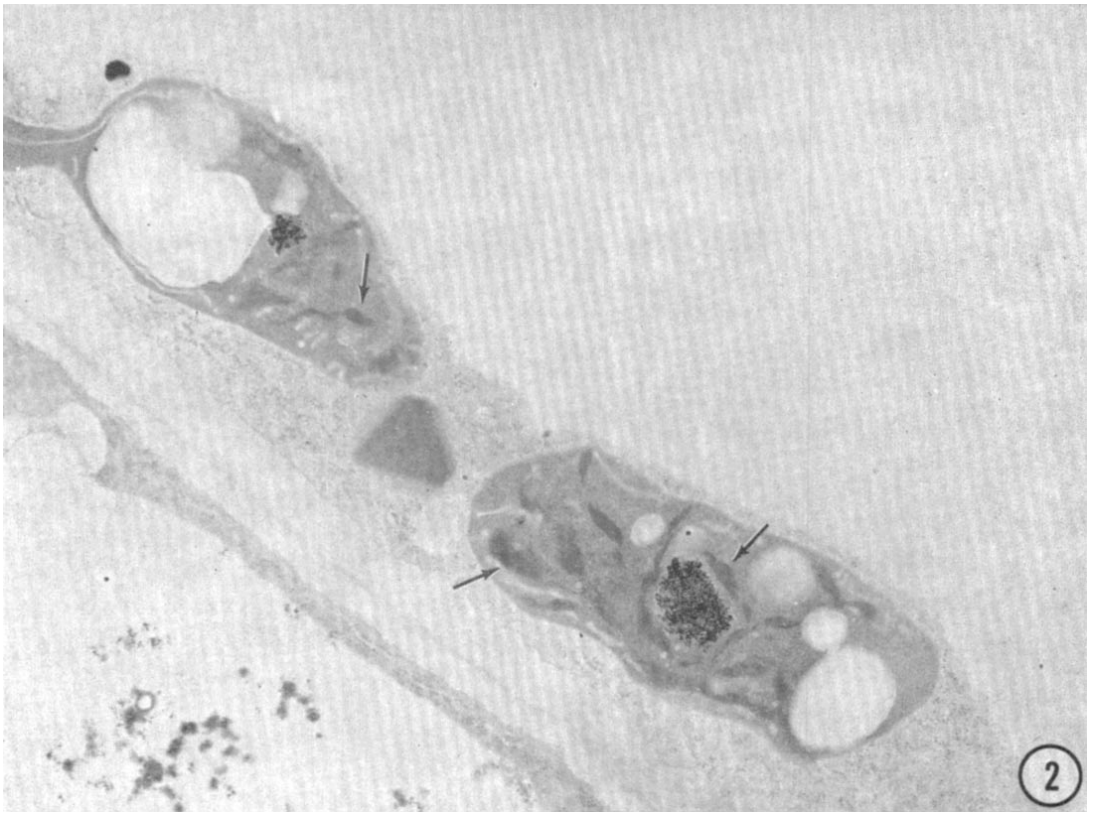

Fig. 2. Plastids from a tomato root parenchyma cell following inoculation with *Meloidogyne incognita* and incubation in 16-h light–8-h dark cycles for 1 wk. Stacked membranes (arrows) have begun to form grana in the stroma of the plastid. (Because the grana are randomly oriented at this stage, cross-sections do not reveal sharply delineated membranes). × 24,000.

3.0 μm in length (Fig. 2). Although starch granules were still present, they were less abundant than at 24 h. The most significant change at this time was the presence of vesiculated membranes around the periphery of the stroma and the appearance of groups of 2–4 stacked thylakoids which occurred randomly dispersed in the stroma of the plastid. In addition, crystal-containing microbodies, resembling the peroxisomes found in photosynthetic mesophyll tissue of leaves, were observed in the cytoplasm near the plastids.

After 2 wk incubation a membrane system characteristic of that found in developing chloroplasts was present (Fig. 3); thylakoids were oriented parallel to one another and to the long axis of the plastid. Along the thylakoids were regions where membrane stacking had begun to form the grana that are characteristic of mature chloroplasts.

The formation of the photosynthetic membranes continued through the 3rd wk (Fig. 4) and by 4 wk (Fig. 5) structures very similar to those found in mature leaf tissues were observed. These organelles contained well-developed thylakoids consisting of the stacked membranes, which form the grana, and the single interconnecting stroma lamellae.

Control roots: Control treatments consisted of (i) inoculating the roots with nematodes and incubating the cultures in total darkness and (ii) exposing uninoculated cultures to the same light-dark cycles as those received by the inoculated roots described above. Four weeks after these treatments began, the ultrastructural features of the plastids from the parenchyma cells were similar in both treatments and resembled those shown in Fig. 1; i.e., the plastids were round, contained starch, and exhibited no unique membrane development.

Uninoculated roots grown with kinetin

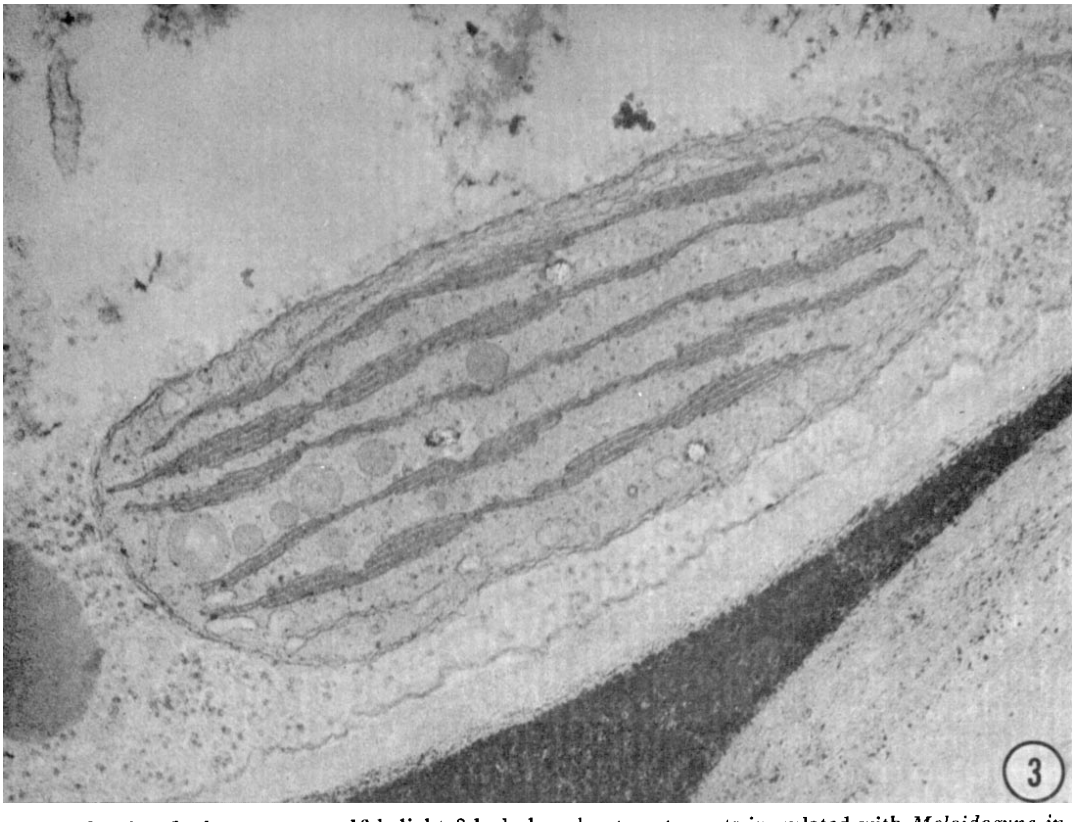

Fig. 3. After 2-wk exposure to 16-h light–8-h dark cycles, tomato roots inoculated with *Meloidogyne incognita* have cells that contain plastids whose ultrastructure resembles that of developing leaf chloroplasts. The internal membrane system consists of parallel arrays of single and stacked thylakoids. × 70,000.

in light: Uninoculated roots grown on a STW medium containing kinetin turned green when exposed to light but did not accumulate pigment when grown in the dark. Galls did not develop on these cultures; consequently, normal root segments exhibiting the green pigmentation were prepared for observation after a 4-wk incubation. Examination of the cortical parenchyma cells from these roots revealed enlarged plastids which contained a few starch grains and a well-developed internal membrane system consisting of single and stacked thylakoids (Fig. 6). The general appearance of these plastids was consistent with that found in the 4-wk-old inoculated, light grown roots (Fig. 5) and also with the typical chloroplast found in mature leaf tissue (Fig. 7).

DISCUSSION

Plastids are organelles ubiquitous in plant cells. In root tissues they normally occur as undifferentiated proplastids and differentiated amyloplasts, proteinoplasts, or oleioplasts which store starch, protein, or lipid, respectively (7). In some plants, such as carrot, plastids in root tissues may also differentiate into chloroplasts; however, light is required for this phenomenon. In our study the plastids in root tissues from uninoculated cultures failed to differentiate when exposed to light unless kinetin was present in the growth media. However, when the cultures were inoculated with *M. incognita,* which caused gall formation, chloroplast development occurred through a series of stages which resembled those that occur in the cotyledons of germinating seeds (15) and in leaf tissues (9,13). The resulting mature chloroplasts were similar to those described in a previous study (8), and they resembled the organelles normally found in mature leaf tissue.

Plastid differentiation failed to occur in root tissues from uninoculated cultures.

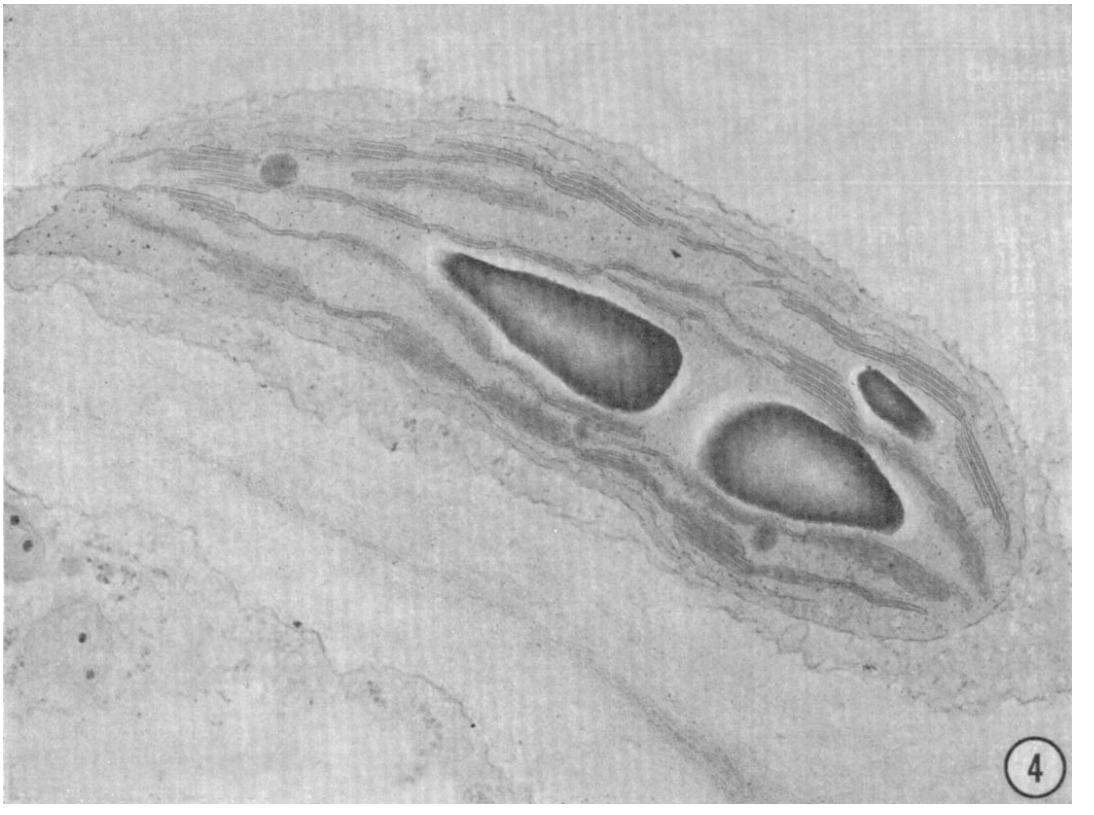

Fig. 4. After 3-wk exposure to the 16-h light–8-h dark cycles, plastids from the tomato roots inoculated with *Meloidogyne incognita* resemble the chloroplasts normally found in leaf parenchyma tissue. Generally, 4–5 stacked thylakoids form the grana which are interconnected by single membranes in the stroma. × 34,000.

However, when the medium was supplemented with kinetin, chloroplast differentiation proceeded. A similar response has been obtained on cultured cells of tobacco (10) where plastids remained devoid of thylakoid formation unless cytokinin was added to the medium.

Recent studies have shown that root tissues infected with nematodes contain elevated levels of cytokinin. Van Staden and Dimalla (14) concluded that roots from resistant and susceptible cultivars of tomato contained higher levels of cytokinin following infection. More recently Bird and Loveys (2) detected higher levels of cytokinin in root homogenates of tomato. In addition the authors concluded that newly hatched second-stage infective larvae of *M. javanica* were also capable of exuding cytokinin-like substances. Consequently, it appears that elevated levels of cytokinin, which apparently occur in the gall tissues from infected plants, may trigger the differentiation of root plastids. If this is the case, perhaps the nematode stimulates cytokinin synthesis in the gall. Alternatively, cytokinins could be transported from the root tip, where they are normally present in elevated concentrations, to the gall, which functions as a metabolic sink. The latter explanation would help to explain why nuclear division is stimulated in the gall, and why cell division and growth of the tips of the infected roots are slowed or arrested.

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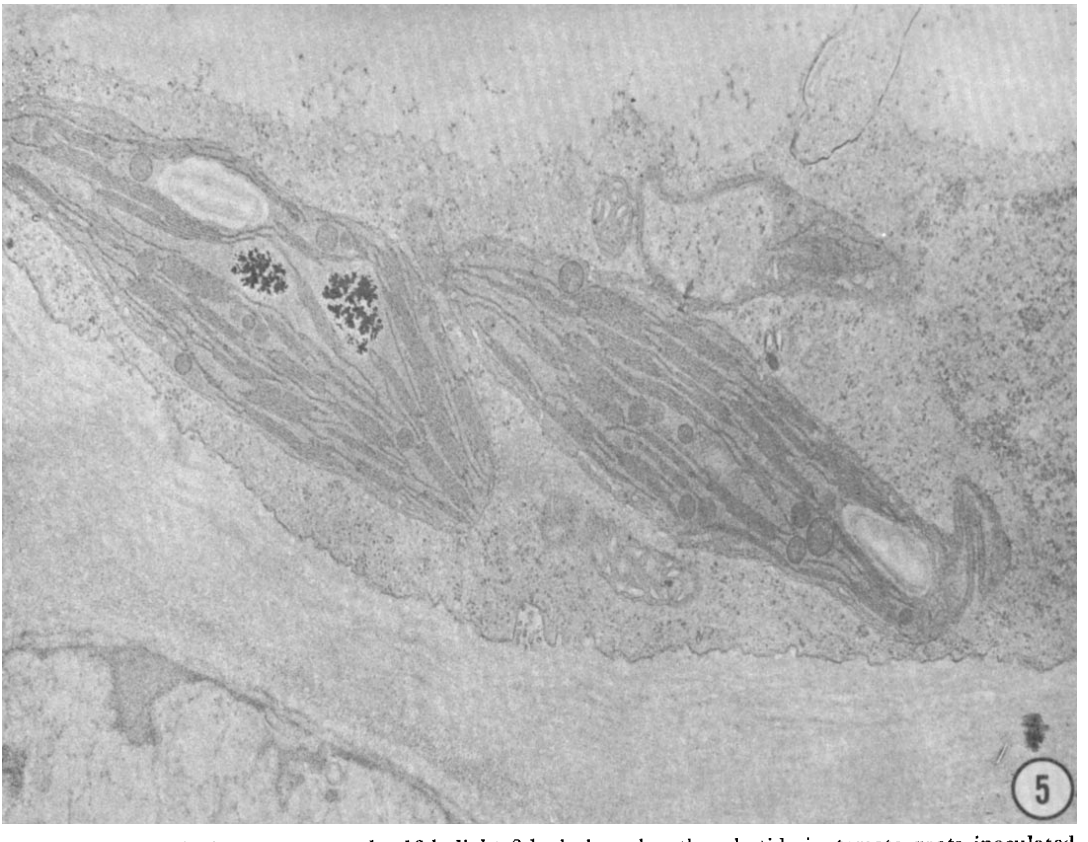

Fig. 5. After 4-wk exposure to the 16-h light–8-h dark cycles, the plastids in tomato roots inoculated with *Meloidogyne incognita* are fully differentiated. Their size and ultrastructure cannot be readily distinguished from those of the leaf chloroplasts. × 16,000.

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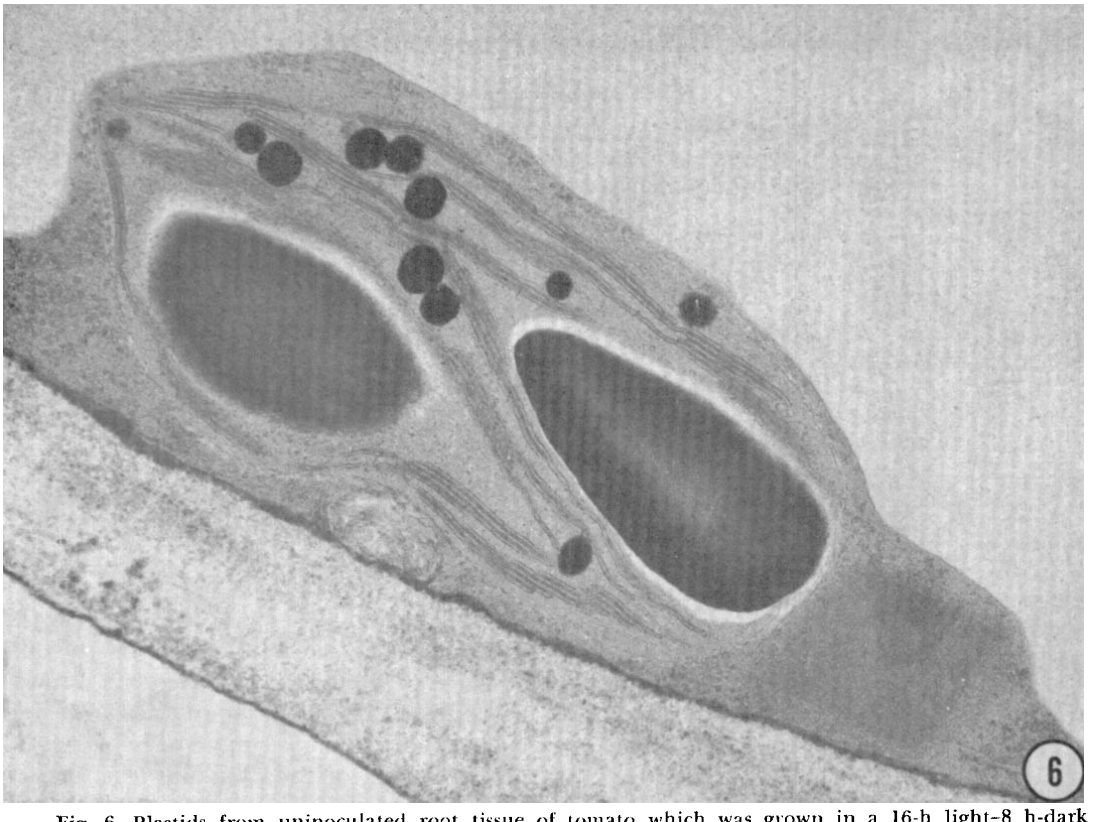

Fig. 6. Plastids from uninoculated root tissue of tomato which was grown in a 16-h light–8 h-dark cycle for 4 wk on a medium containing kinetin. Under these conditions, the plastids develop an internal membrane system very similar to that shown in Fig. 4; i.e., grana consisting of 2–5 thylakoids are interconnected by the single stroma membranes. × 32,000.

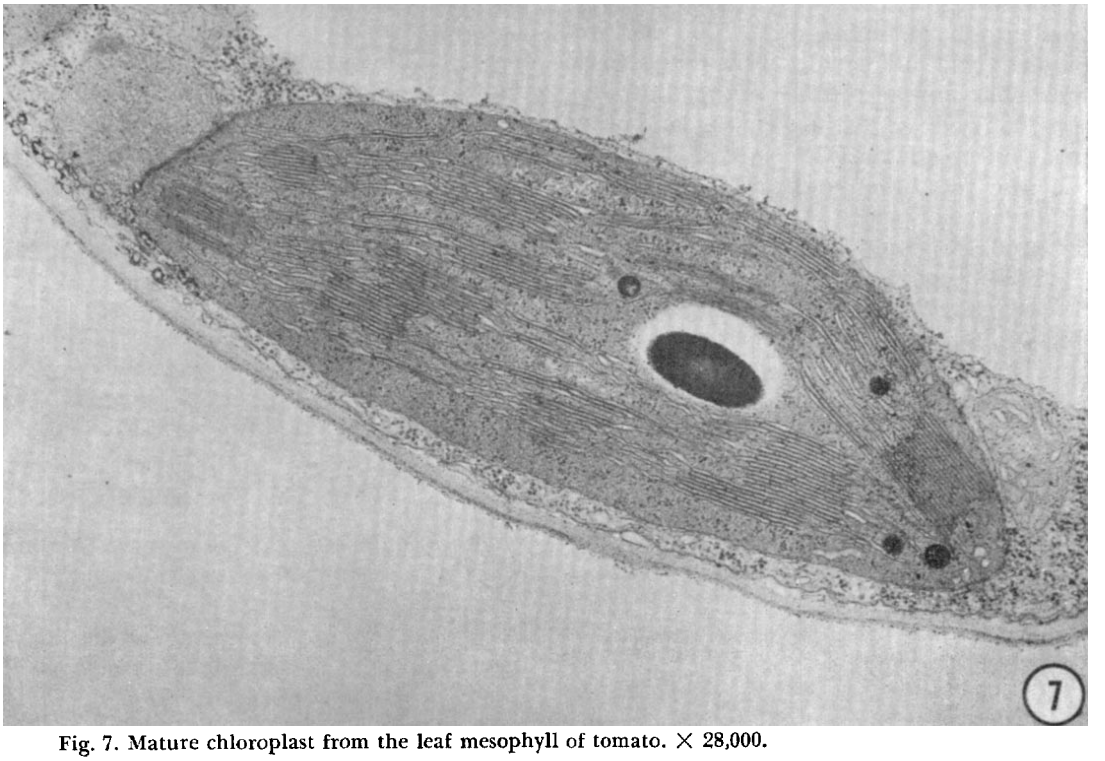

Fig. 7. Mature chloroplast from the leaf mesophyll of tomato. × 28,000.