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Giant Cell Formation In Chilli Roots Incited By *Meloidogyne incognita* Infection

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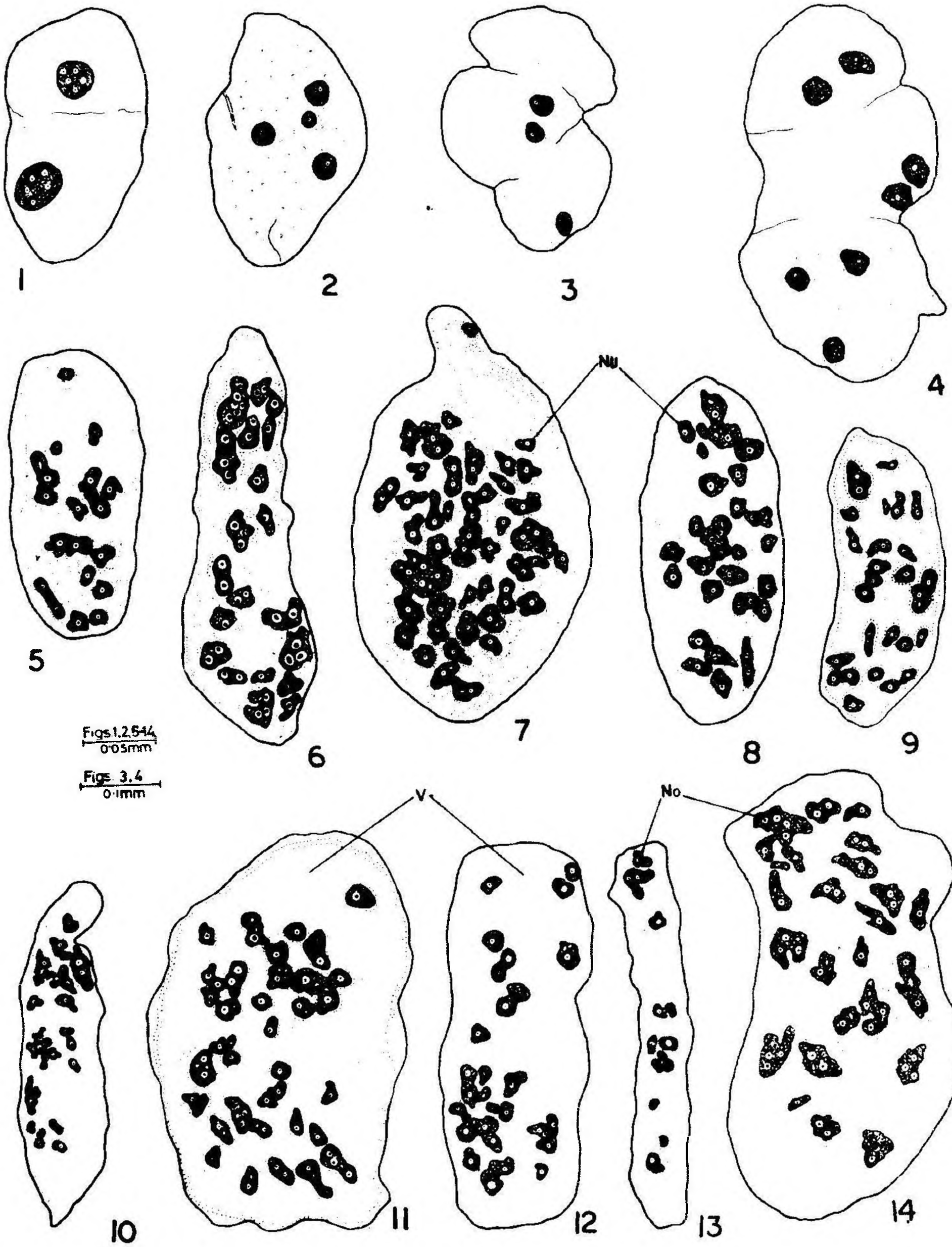
Giant cells induced in roots of *Capsicum annuum* by *Meloidogyne incognita* were examined by light microscopy. Giant cells were usually found in the pericycle, phloem, cambium or ray parenchyma of the xylem and rarely in cortical region. Giant cells developed by a combination of fusion of cells and synchronous mitosis without cytokinesis. Both nuclei and nucleoli were many times larger than those of normal cells. They were thick-walled and highly invaginated.

Nematodes are widely distributed and cause considerable harm to crop husbandry. Nematode stimuli lead to marked morphological and physiological changes in plants. The most important criterion for a successful development of the nematode is the production of syncytia or giant cells or coenocyte or multinucleate transfer cells (Bird, 1961, 1979; Jones & Northcote, 1972; Pate & Gunning, 1972). This report deals with the giant cells induced by *M. incognita* in chilli roots.

MATERIALS & METHODS Surface sterilized chilli seeds were sown in 15 cm earthen pots containing steam sterilized soil (sand and farmyard manure, 4:1). One week old chilli seedlings were inoculated with 1000 juveniles of *Meloidogyne incognita* maintained and multiplied on tomato plants. Infected roots of chilli were collected and fixed in formalinacetic alcohol (FAA) after 10, 20, 30, 40, and 50 days of inoculation. Before processing of the material in tertiary butyl alcohol (TBA) series, it was washed in running water. Processed material was embedded in paraffin wax and sectioned in a microtome, at thickness ranging from 10 to 15 um in transverse and longitudinal planes. The sections were stained in safranin - fast-green stain. After staining, dehydration and clearing in alcohol-xylol series, the sections were mounted on slide in DPX.

In maceration study, the pieces of gall were gently heated in 10% potassium hydroxide, washed in running water and stained in Delafield's haematoxylin to stain the nuclei. A small stained piece was macerated, mounted in 25% glycerine, a coverslip was placed and giant cells were observed.

RESULTS **Infective juvenile (L_2S)** penetrated within 24 hrs after inoculation, moved intercellulary in cortex and finally resided in the stelar region. Giant cells were initiated in xylem parenchyma and phloem but rarely in cortex. The giant cells showed increase in their nuclei by mitosis without cytokinesis and coalescence with adjacent cells (Fig. 1-4). The cells adjacent the spike tail stage of nematode (L_3S) showed hyperplasia and hypertrophy to keep pace with is enlarging body. The syncytia associated with L_3S showed multinucleate thick-walled, and dense cytoplasm. While giant cells associated with young female had largest nuclei in volume, dense cytoplasm (Fig. 5-14), and developed prominent wall ingrowths adjacent another giant cell, xylem, phloem and nematode head but none on wall adjacent parenchyma (Fig.16,17). Male nematodes were



Figs. 1-14. *MELOIDOGYNE INCOGNITA* ON *CAPSICUM ANNUUM*.
Whole mounts of giant cells, obtained from root meceration.

Figs. 1-4. Giant cells, dissolution of separating walls, large nuclei.

Figs. 5-14. Giant cells (G), many large nuclei (NU) each with one to five nucleoli (NO), dense cytoplasam, thick-wall, vacuole (V).

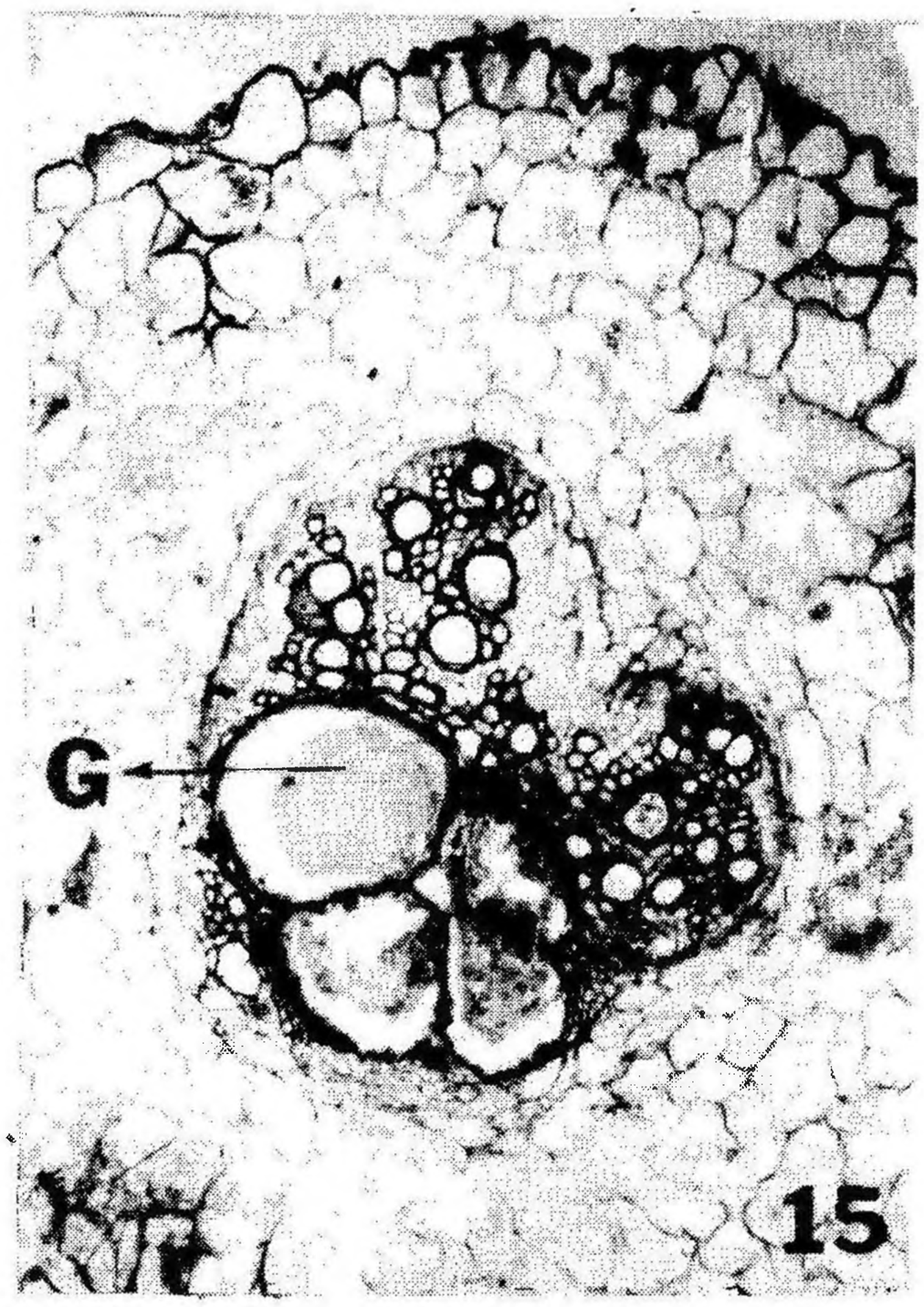


Fig. 15. T.S. a portion of root, giant cells in Primary medullary ray (PMR).

associated with thin-walled giant cells containing vacuolate cytoplasm with indistinct nuclei. They left the host upon maturity.

The anterior end of the nematode was in the pericycle, cambium, phloem or xylem parenchyma and giant cells were incited in some cells near its head (Fig. 16). Adjacent tissues showed hypertrophy and hyperplasia. Some of the parenchymatous cells, resulting from abnormal division might get transformed into reaction or abnormal xylem (Fig. 16). Giant cells were usually surrounded by the abnormal xylem elements. Formation of giant cells depended on four responses of the host: hypertrophy of the cells adjacent to the larvae; hyperplasia in the pericycle, phloem and xylem parenchyma (Fig.15,16); mitosis without cytokinesis and dissolution of cell walls between the cells taking part in the formation of giant cells (Fig. 1-4; 15,17).

Young Giant Cells Young giant cells had thin walls and appeared to have been actively expanding (Fig. 15). The cells had dense cytoplasm. Mitotic division of nuclei was observed and the nuclei were scattered throughout the protoplast. Young giant cells were the primary site of nematode feeding. Young giant cells initially were hypertrophied cells with many enlarged nuclei and nucleoli, later on after

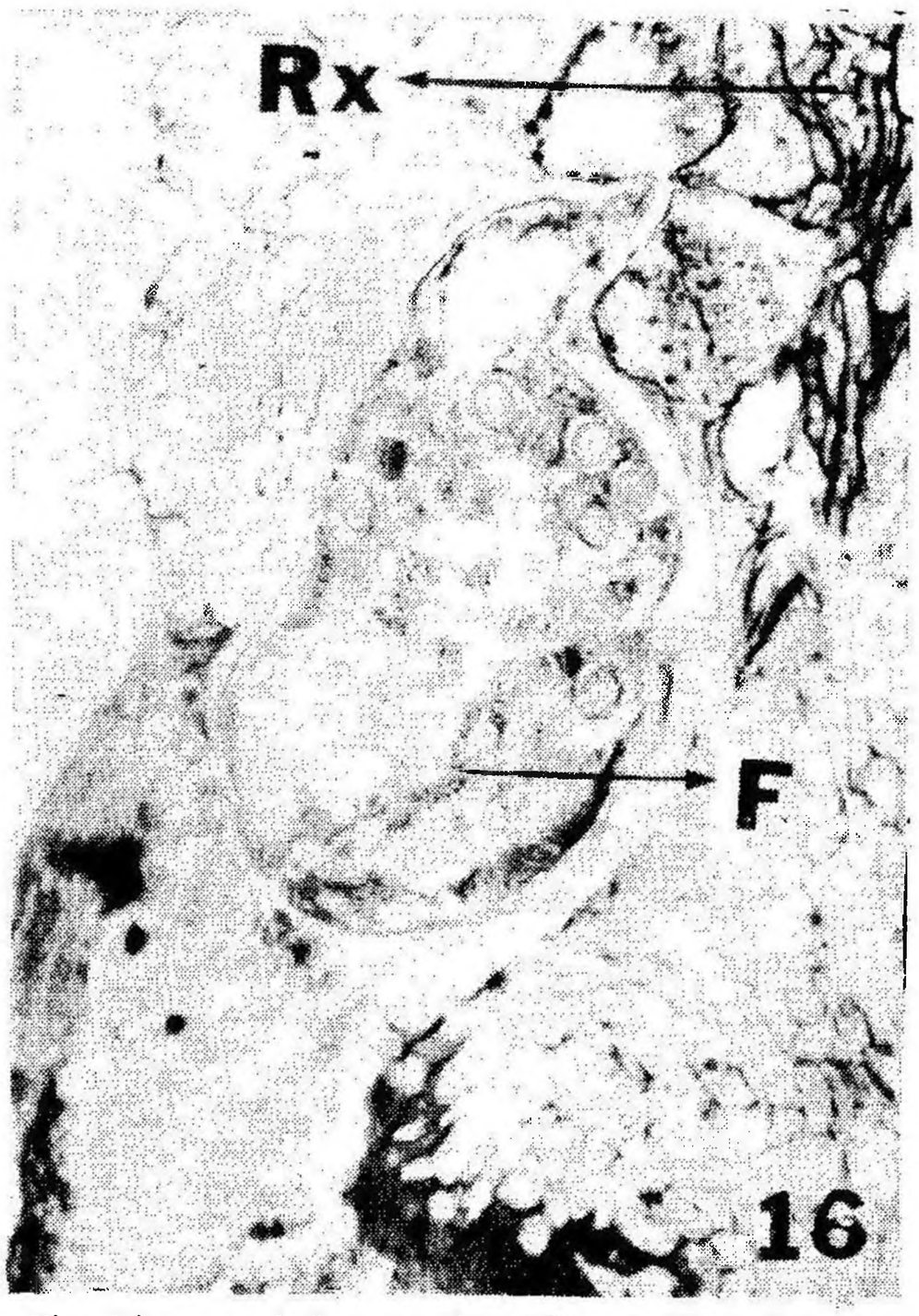


Fig. 16. L.S. a portion of root, Neck Female nematode surrounded by giant cells. Reaction xylem (RX).

dissolution of their adjacent walls became confluent and enlarged thick-walled (Fig. 1-4).

Mature Giant Cells The giant cells developed after dissolution of adjacent walls of many young giant cells and existed in varying stages of confluence and development. They were found at the vicinity of the head of the parasite (Fig. 5-14; 16).

Nuclei in mature giant cells were uncountable in some cases due to their fusion or overlapping (Fig. 6, 7, 8, 11, 14). Nuclei in the giant cells were abundant in their processes as well as

in the centre or were restricted only to one side of the giant cell. Enlarged nuclei contained one or more than one nucleoli. Both nuclei and nucleoli were three or more times larger than the

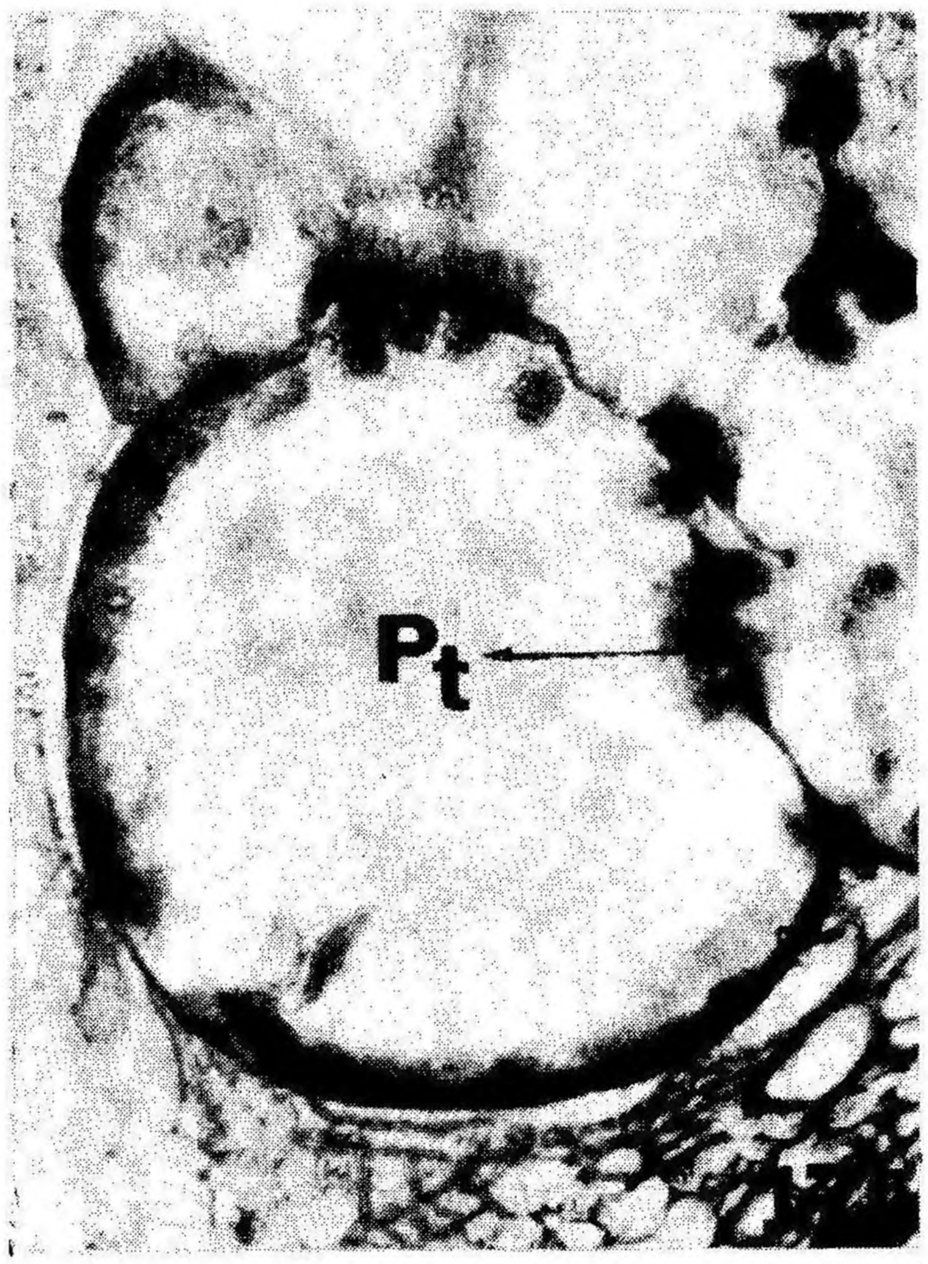


Fig. 17. T.S. magnified portion of root, protuberances (PT) on wall of giant cells.

normal cells (Fig. 1-14). Nuclei in mature giant cells often clumped together and their shapes were distorted. They were elongate, spherical, dumbbell shaped, sometimes possessing invaginations and exiginations (Fig. 5-14). The same giant cell contained both large and small nuclei (Fig. 5, 9, 10, 12, 13).

Old Giant Cells - Old giant cells contained fewer nuclei and nucleoli and vacuolate granular or reticulate consistency of cytoplasm. The cells showed the sign of degeneration and later on disintegrated. Walls of old giant cells were thick and they stained deeply. Their nuclei became deformed, nucleoli and nuclear membrane became indistinct and they stained darkly (Fig.7, 16).

Giant Cells Associated With Living and Dead Females - Giant cells associated with living nematodes had dense granular cytoplasm with prominent nuclei (Fig.16). Their nuclei and cytoplasm took a sharp staining. However, the giant cells associated with dead females possessed a vacuolated cytoplasm which stained weakly.

Giant Cells from Macerated Tissues - Giant cells of varying shapes and sizes were ob-

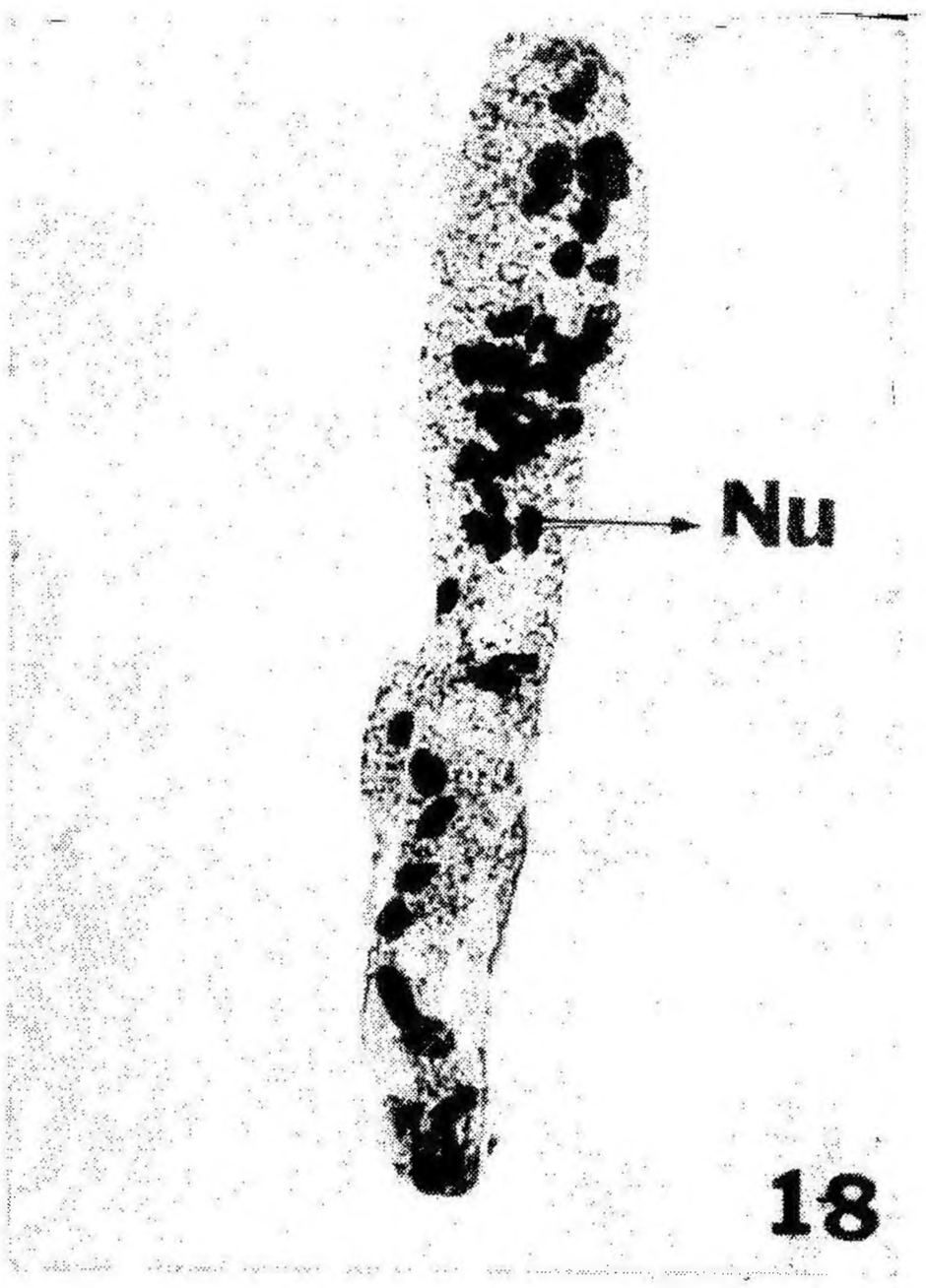


Fig. 18. Whole mount of giant cell, Densely protoplasmic with large nuclei (NU).

tained by macerating galled tissue. Young cells had densely cytoplasmic contents and finely granular cytoplasm. Sometimes giant cells possessed appendages but mostly they were rounded or elongated, without any appendage. These giant cells showed large number of enlarged polyploid nuclei with dense cytoplasm (Fig.5-14, 18).

Cell Wall - The cell wall in young giant

cells, was sufficiently thick which stained deeply. Each cell wall had two portions, a comparatively thin external layer and a thicker internal layer. From the internal layer many thick walled protuberances emerged, projecting inside the lumen of the cell. These protuberances increased the internal surface area of giant cell (Fig. 17).

Cytoplasm - Giant cell cytoplasm near the head region of the nematode was more dense than the rest of the giant cell (Fig.16). In newly formed giant cells, only a few vacuoles were present, but in older one, vacuoles were large and numerous (Fig. 11, 12).

Nuclei and Nucleoli - Nuclei in young giant cells enlarged and stained deeply. Afterwards, the old giant cells nuclei increased in diameter with irregular nuclear membranes. Nuclear enlargement may be due to the fusion of many nuclei, or due to ploidy of their chromosome complements (Fig.1 -4).

In old giant cells disintegration of nuclei occurred, only dark fibrilar mass appeared inside the nucleus.

DISCUSSION Giant cells are highly specialized cellular adaptations induced and maintained by the nematode. Its cells showed high metabolic activity as long as in association with nematode and degenerate when abandoned by it (Bird, 1962). Giant cell formation in cortex had been reported (Ogbuji, 1976; Trivedi *et al.*, 1979). Giant cells were initiated 96 h after inoculation in chilli but in okra, it occurred within 48 h (Littrell, 1966), 4th day in tomato (Bird, 1961) and 5th day in wheat (Siddiqui & Taylor, 1970) infested by *Meloidogyne* sp.

There are three views regarding the ontogeny of giant cell : (i) that multinucleate giant cell is derived from a single uninucleate initial cell solely by mitosis without cytokinesis. (Huang & Maggenti, 1969 a, b; Paulson & Webster, 1970; Siddiqui & Taylor, 1970; Jones & Northcote, 1972; Swamy & Krishnamurthy, 1971; Siddiqui *et al.*, 1974; Jones & Payne, 1978). (ii) The uninucleate initial cells first coalesce by the dissolution of cell walls. Thus, a multinucleate cavity results in which the nuclei divide mitotically but without cytokinesis and further enlargement continues by fusion with adjacent parenchymatous cells (Christie, 1936; Dropkin & Nelson, 1960; Owens & Specht, 1964; Rao & Kumar, 1973). (iii) Involved enlargement of parenchyma, multinucleation as a result of mitosis without cytokinesis and coalescence with adjacent cells. We support the third view and confirm observation of Swarup & Pillai (1963), Littrell (1966), Dalal & Thakur (1971), Bird (1974), Shah & Raju (1977) & Bhatnagar *et al.* (1978).

Recently however, Jones & Payne (1978) examined early stages of giant cell formation in *Impatiens* by *M. javanica* and *M. incognita* by light and electron microscopy. They emphatically supported the view held by Huang & Maggenti (1969a). However, they did not totally rule out the possibility that cell wall breakdown may occur 3 to 6 days after induction but they felt that if it did occur, it was a rare phenomenon.

The present investigation on chilli is supported by Bird's (1961) observations that initially the development of giant cell involved repeated mitosis without cytokinesis but as the giant cell enlarged and grew intrusively between cells, the cell walls of such adjacent cells, wherever thin broke and intermingling of cytoplasm resulted. Cell wall dissolution was seen by Owens & Specht (1964).

Diminishing central vacuole in giant cell associated with second stage larva by Owens & Specht (1964) and Jones & Payne (1978) and nuclear clumping were seen in chilli.

The giant cell associated with spike-tail stage of larva showed reticulate cytoplasm in which uni-to multinucleolate nuclei of various shapes and sizes were present. The nuclei in

chilli reached a maximum size at this stage as confirmed by the observations made by Owens & Specht (1964) and Rubinstein & Owens (1964). Bird (1972) reported that in 2-3 weeks after inoculation, the size of nuclei in the syncytia and their DNA content were high and just before the start of egg-laying, they declined. Our data support these observation.

As the nematode moulted into a female in chilli roots, the syncytial nuclei became fewer and smaller but remained multinucleoate. The cytoplasm became dense and granular while the wall thickened and prominent protuberances developed. This stage represented high metabolic activity corresponding to nematode feeding. Since feeding appeared to be carried on in one syncytium at a time, the syncytia of same complex represented different conditions of nuclei and cytoplasm, representing different physiological states (Owens & Specht 1964).

The second stage larvae hatching out from embedded egg masses never seem to feed from already existing giant cell induced by the mother female. In tomato galls Paulson & Webster (1970) obtained similar data.

Giant cell walls become thicker and they possess irregular rounded protuberance, attached to their inner walls. Huang & Maggenti (1969a) noted that the cytoplasmic boundary membrane of the giant cell develops vesicular and tubular invaginations in the cytoplasm which gradually get filled as the secondary-wall materials are deposited in them. Gunning & Pate (1969) and Jones & Gunning (1976) use the term "transfer cells" to those cells which possess ingrowths of wall material and hence having protoplasts with an unusually high ratio of surface to volume. Jones & Northcote (1972) noted extensive protuberances on walls of giant cells, adjacent to xylem vessels.

The nuclei of giant cells show varying degree of overlapping and fusion between them. Often large nuclei were observed in some giant cells. The present work supports Huang & Maggenti (1969b) but is in disagreement with Tischler (1902,quoted in Endo,1971) who reported amitotic divisions and fragmentation of giant cell nuclei.

A number of giant cells showed large nuclei with irregular nuclear membrane and many enlarged nucleoli, confirming the published observations (Paulson & Webster, 1970). Old giant cells had fewer nuclei and disintegration of their nuclei occurred.

Young giant cells were densely protoplasmic, almost without vacuoles. However,in older ones, many large vacuoles were observed. According to Owens & Specht (1964) newly formed syncytia contained large vacuoles.

Khan & Tiagi (1977) reported haustorial appendages of giant cells in root galls of tomato. Giant cells do not bear any conspicuous appendages. Irregularly shaped giant cell outline and their appendages may be due to coalescence of several irregular cells, that extent into several planes.

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