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GIANT CELL FORMATION IN PEA ROOTS INCITED BY *MELOIDOGYNE INCOGNITA* INFECTION

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Giant cells induced in roots of *Pisum sativum* by *Meloidogyne incognita* were studied by light microscopy. Three days after inoculation giant cell initials were observed near the head of nematode mostly in phloem parenchyma and rarely in pericycle and xylem parenechyama. They become multinucleate by synchronous mitosis without subsequent cytokinesis. Mature giant cells were bearing haustorial appendages which grew intrusively among the neighbouring tissue. Presence of wall in growths increased the internal surface area of giant cells resembling them with the transfer cells found in normal tissue.

Keywords : Giant cells; Pea; *Meloidogyne incognita*.

Introduction

Nematode infection causes marked morphological and physiological changes in plants. The infective stages of juveniles penetrate the roots and establish their feeding site in vascular parenchyma. In response to the feeding activity, some parenchyma cells become hypertrophied and multinucleate and are generally known as giant cells or syncytia. For the development of the root-knot disease and completion of life cycle of *Meloidogyne*, the formation of giant cells is an important prerequisite (Bird, 1961). Their obligate relationship with root-knot nematode and their peculiar morphological features have incited much curiosity among investigators since their discovery a century ago. The present investigation is directed towards their initiation, development and structure in pea infected by *Meloidogyne incognita*.

Material and Methods

Surface sterilized pea seeds of cultivar 'Bonneville' were sown in 15cm earthen pots containing steam sterilized soil (sand and farmyard manure in ratio of 4 : 1). The seeds were treated with the specific *Rhizobium* strain before sowing. When one week old, each seedling was inoculated with 1000 juveniles of *Meloidogyne incognita*. For a comprehensive study of giant cell formation and structure, a few plants were uprooted

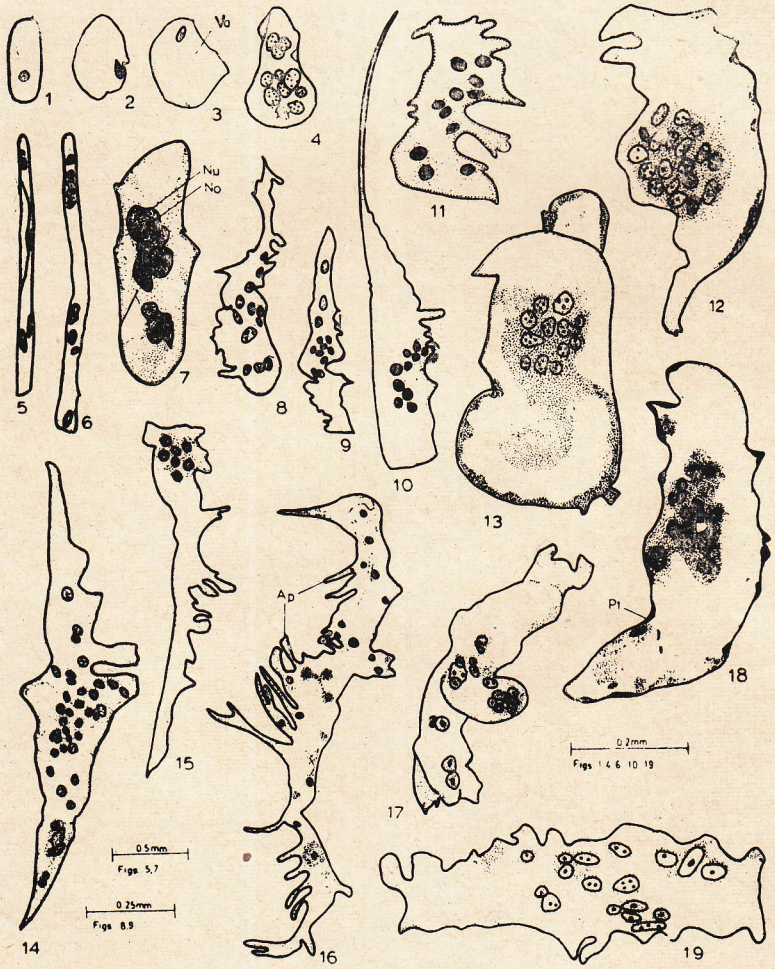
initially daily till 10 days, at three days interval afterwards, till 22 days and finally at one week interval till eight week after inoculation. Ninety days old infected plants were also uprooted. The roots were carefully washed and fixed in formalin–acetic–alcohol (FAA) for 24 hrs and stored in 70% ethanol. After a week, suitable portions of roots were dehydrated, embedded and microtomed at thickness ranging from 10μ to 15μ in transverse and longitudinal planes. The sections were stained in safranine-fastgreen combination and mounted in DPX–mountant for observations.

For isolation of gaint cells, galls of different ages were gently heated in 10% KOH for five minutes. They were washed in running water for 24 hrs and stained in Delafield's haematoxylin. A small portion of gall was macerated on the slide and mounted in 30% glycerine for observations.

Results

Origin and development—The development of multinucleate giant cells have been observed in relation to the developing nematodes. Infective juveniles (L_2S) penetrated within 24 hrs after inoculation, moved intercellularly in cortex and finally became sedentary in the stelar region. Three days after inoculation, giant cell initials were observed near the head of nematode mostly is phloem parenchyma and rarely in pericycle and xylem parenchyma. The initials became hypertrophied and their cytoplasm granular. A few nuclei immediately near the head of nematode appeared to be devoid of nuclear membrane but contained hypertrophied nucleoli (Figs. 20, 22). Later on, giant cell became muitinucleate by synchronous mitosis without subsequent cytokinesis (Figs. 4–6). In no case, evidence of wall dissolution during the development of giant cells was observed. However, sometimes, giant cells showed wall stubs on their inner walls Fig. 21). The syncytia elongated parallel to the long axis of root when they were intiated in phloem parenchyma (Fig. 25). When giant cells were induced in xylem parenchyma, they expanded radially, so much so, that they occupied almost the entire stele. Thin walled cells around the expanding giant cells of spike tail stage of nematode divided repeatedly and allowed the growth of giant cells and nematode without dislocation and disruption of tissues. However, the growth of giant cells was finally restricted by tracheary elements and reaction xylem (Fig. 23).

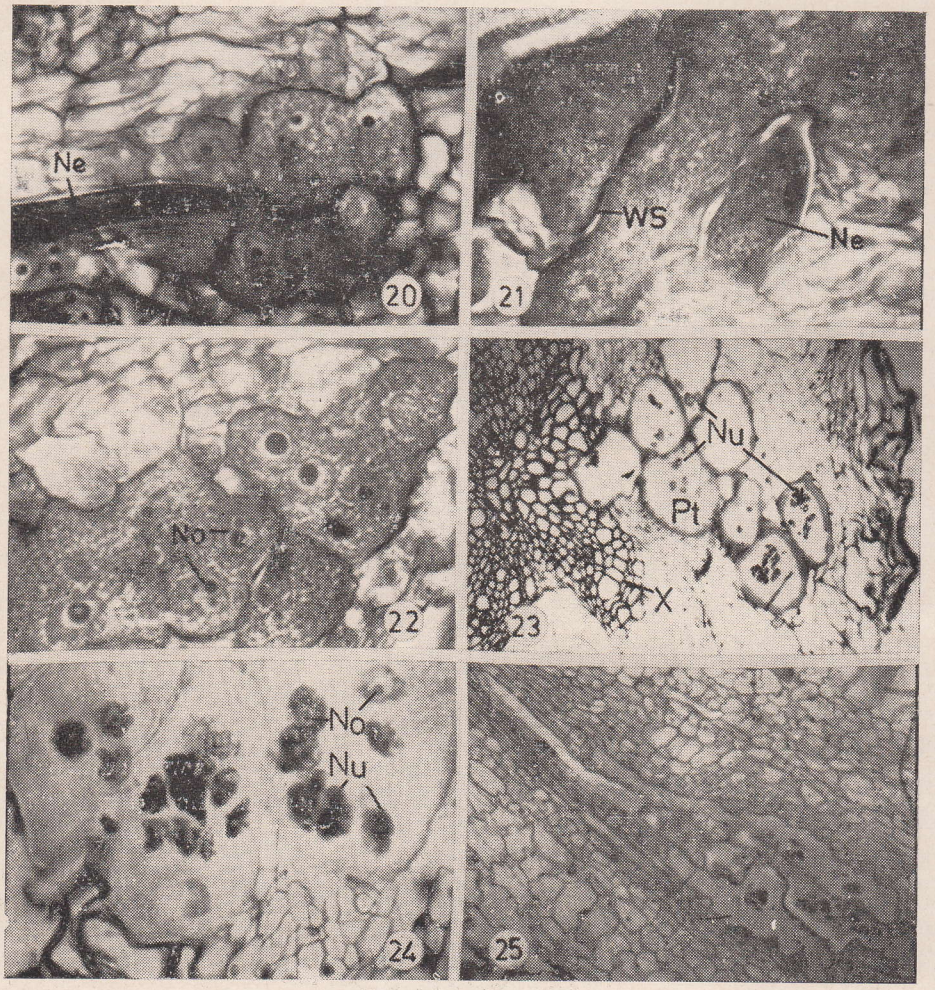
Giant cell appendages—Depending on the surrounding tissue, giant cells were bearing appendages which grew intrusively among the neighbouring cells (Figs. 8,15,16). These appendages were often blunt and club shaped and forming a closely fitting structure with the adjoining paren–



Figs. 1–19. *Meloidogyne incognita* on pea. Whole mounts of developing giant cells from macerated root tissue. 1–3 Prenchyma cells. 4–7 Young giant cells. 8–19 Mature giant cells of various shape and sizes.

Ap— Appendages; No–Nucleous; Nu–Nucleus;

Pt— Protuberance; Va–Vacuole.



Figs. 20-25. *Meloidogyne incognita* on pea forming giant cells(GC). 20 L_2S feeding on young GC (x400). 21 GC with wall stubs (x400). 22 GC with hypertrophied nucleoli (x400). 23 GC with wall protuberances (x100). 24 GC with fragmented nucleoli (x400). 25 Developing GC with hyperplastic phloem parenchyma (x100).

Ne–Nematode, No–Nucleolus; Nu–Nucleus;

Pt–Protuberance; WS– Wall Stub; X-Xylem.

chyma (Fig. 25). These appendages contained granular to dense cytoplasm but generally nuelei were not visible in them due to high density of the cytoplasm.

Giant cells from Macerated galls—Giant cells of varying shapes and sizes were obtained by macerated galled tissues of different ages (Figs. 4–19). Young giant cells had dense cytoplasm containing large number of nuclei of almost equal size (Figs.4-6). They were globular (Fig. 4) or elongated with blut ends (Figs. 5–6). Mature giant cells possessed a large number of appendages of varying lengths and their cytoplasm became more vacuolated and nuclei of various sizes and shapes tended to coalesce in the centre (Figs. 12, 18).

Syncytial wall—The newly formed giant cells had very thin walls but as they matured their walls became unevenly thickened. This wall consisted of two layers, a comparatively thin external and thick internal layer. From the internal layer many knob-tike protuberances emerged projecting inside the lumen of the syncytium (Figs. 18, 23). These ingrowths increased the internal surface area of syncytium.

Syncytial cytoplasm—Syncytial cytoplasm near the head region of nematode appeared to be denser than in rest of the giant cell. The amount and nature of the cytoplasm varled in different giant cells of the same complex. In newly formed giant cells vacuoles were few and small (Figs. 4, 8) whereas in older ones they were larger and numerous (Figs. 18, 19). As the syncytium matured the cytoplasm became denser and granular or reticulate.

Syncytial nuclei and nucleoli—The number of nuelei reached upto 50 in weil developed giant cell. The number of nucleoli varied within a giant cell suggesting the fusion of some of nucleoli (Fig. 7). In young giant cells nucleoli were rounded and fewer in number, however, in giant cells associated with mature and egg laying females they lost their rounded shape and fragmented. These fragments, which stained like nucleoli, were either scattered throughout the nucleus or they were arranged at the periphery of the nucleus (Fig. 24)

Discussion

The highly specialized giant cells were induced, maintained and completely dependent on a continuous stimulus from the nematode, the removal of which led to their atrophy (Bird, 1962). The nematode repressed a part of cell's genetic coding and activated other part so that a special type of cell was produced (Bird, 1974). Giant cells were initiated in pea three days after the inoculation by the nematode. Other

workers, dealing with different hosts, have found this period ranging from a few hours to upto fifth day. The period depended on the environmental conditions and host species involved.

The multinucleate condition of giant cells was achieved by synchronous mitosis without subsequent cytokinesis and hence the present work supported the view regarding the ontogency of giant cells held by Huang and Maggenti, 1969; Paulson and Webster, 1970; Jones and Payne, 1978 ahd Finley, 1981. It appeared that the presence of wall stubs on the inner walls of giant cells might erroneously led to the conclusion that giant cells were formed by cell wall dissolution. This might have been the reason why many investigators like Christie; 1936; Krusberg and Nielsen, 1958; Bird, 1968; Birchfield, 1964 thought that giant cell formation involved cell wall dissolution and subsequent cytoplasmic fusion between the neighbouring cells. The observation of wall dissolution was difficult to ascertain with paraffin wax sections because wax was not firm enough to exclude the possibility that industinguishable atifects might have been formed during the sectioning. Furthermore, the generally thick wall of giant cells have thin areas that are close to the limit of LM resolution (Huang and Ma genti, 1969; Jone and Payne, 1978) thus giving an impression of 'gaps' in sections in some planes.

The most appropriate functlon of giant cell appendages appeared to be the absorption of nourishment from the phloem and other adjoining tissue. Khan and Tiagi (1977) had also assigned a haustorial function to them in *Lageneria leucantha.* However, Trivedi and Tiagi (1987) did not odserve any conspicuous appendages in chilli roots.

Wall ingrowths or protuberances formation in syncytia led to their being considered as a form of transfer cells, similar to function to those found in normal tissue such as companion cells and xylem parenehyma cells (Jones and Northcote, 1972; Pate and Gunning, 1972). The ingrowths greatly increased the surface area of the plasmalemma of the giant cells. Jones (1976), on the basis of scanning electron micrographs estimated ten-fold increase in the surface area of wall of giant cells over the wall without ingrowths.

As the nematode moulted into a female in pea roots the cytoplasm became denser and granular. Bird (1961) from ultrastructural studies had showed that the increased granulation was due to the presence of mitochondria, plastids and other cell organelles, thus closely resembling the meristematic cells at interphase. The amount and nature of cytoplasm

varied in the different giant cells of the same copmlex as also observed by Owens and Specht (1964) and Trivedi and Tiagi (1987). This suggested differential feeding by the nematode and consequently differential absorption of nutrients and development of giant cells in the same complex.

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