PROFILES OF INFECTION THREADS IN ROOT NODULES AS REVEALED BY ELECTRON MICROSCOPY

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Various profiles of infection threads could be observed in the ultrastructural study of developing young root nodules of *Phaseolus mungo* L. The threads usually appear as hypha-like structures containing single rows of bacteria arranged end-to-end. The ramification of infection threads takes place in different directions soon after its initiation through the root hair cells. The thread walls are of cellulose origin and more or less uniform in cross sections. The tip regions of the thread, however, are thicker and intermittently show dilations prior to the bacterial release. Release of bacteria may be both terminal and subterminal. Branching of infection thread is very frequent with associated bacteria at the tip. Dissemination of bacteria occurs during its intercellular penetration and subsequently get distributed through cellular divisions.

INTRODUCTION

The information on the ultrastructure of infection threads following rhizobial invasion is meagre. It includes only a limited number of legume species. Moreover, these studies are confined to the gross morphology of the infection thread in relation to the formation of bacterial enveloping membranes (Bergersen and Briggs 1958; Mosse 1964; Jordan, Grinyer and Coulter 1963; Goodchild and Bergersen 1966 and Dixon 1964, 1967). There exist diverse opinions as regards the formation and behaviour of the infection threads. A broad spectrum of widely differing legume species should be attempted before making any generalisation.

The present study forms the part of a research project on the ultrastructure of developmental changes of root nodules of *Phaseolus mungo* L., and confines its scope only to the secondary ramification of the infection threads with various thread profiles. It reveals an important fact that the infection thread is a definite structure made up of cellulose and never are they formed from the plasmamembrane of the affected host cells. Branching of the infection threads and dissemination of bacteria in the cytoplasm are also described.

MATERIALS AND METHODS

The seedlings of black gram (*Phaseolus mungo* Var. T9) grown after inoculation with the specific rhizobial strain isolated in this laboratory and were raised on vermi-

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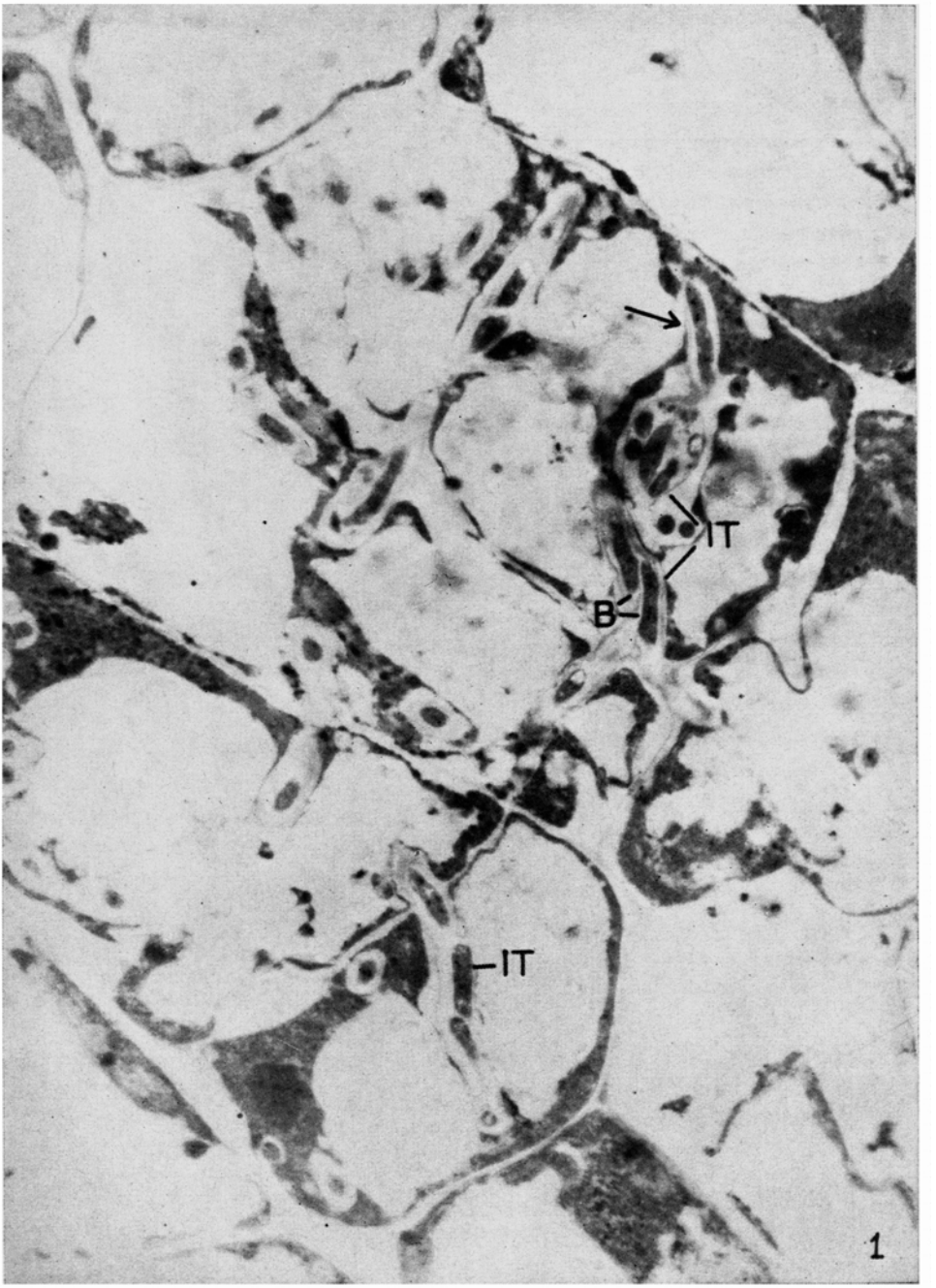
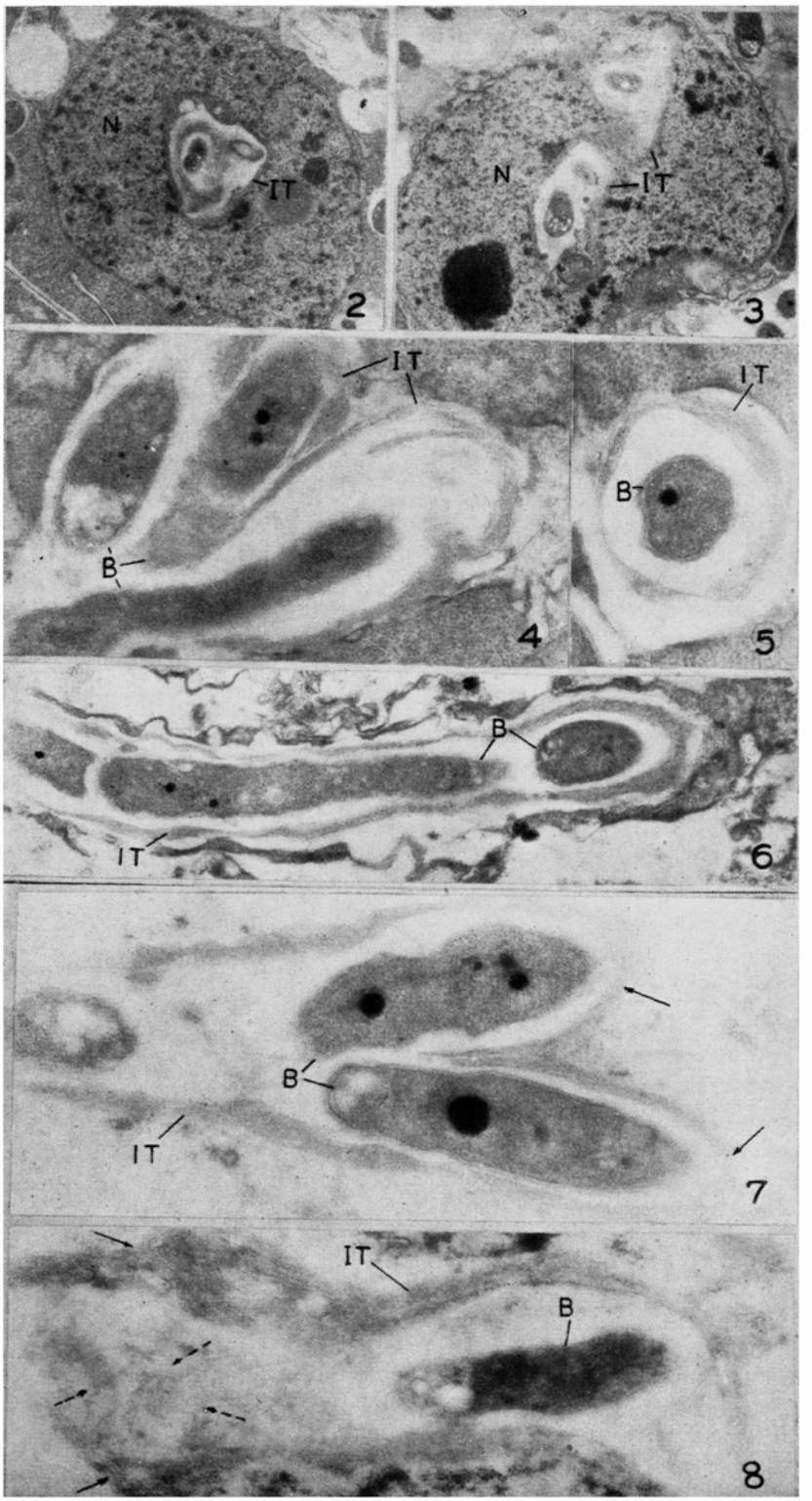


FIG. 1. Electron micrograph of proliferating infection threads (*IT*) in the young nodule showing movement of bacteria (*B*) and their subsequent release at some regions. Dividing bacteria could also be noted (arrow). × 7,500.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culite and sand mixture. The samples were collected at 2, 5 and 10 days of emergence after carefully emptying the pots. The nodules so collected were washed thoroughly firstly with tap water and then with distilled water to remove any adhering soil particles.

The youngest visible nodules on two-day-old plants were carefully excised out through the help of a dissecting stereo-microscope. Larger nodules were cut into tiny pieces and immediately put in 2.5 per cent glutaraldehyde buffered in 0.05M cacodylate buffer and fixed for 2 hr at room temperature. Following fixation, the samples were washed with several changes in the same buffer solution (*p*H 7.1) for 12–14 hr at 4°C. After fixation, they were washed thoroughly with deionized water and kept in 0.5 per cent aqueous uranyl acetate in cold for 10 hr. Dehydration and embedding were carried out following the method of Mollenhauer (1964). Vacuum infiltration was used in each case for better penetration in the tissue.

Ultrathin sections were cut on an LKB 8800 Type III ultrotome using glass knives at a thickness of 400–600Å. They were collected on bare grids, post-stained with 0.5 per cent aqueous uranyl acetate for 10 min and lead citrate for 4 min (Reynolds 1963). Finally, they were washed with deionized water. Sections were examined in AEI EM-6 electron microscope operated at 75 kV and photographed on ORWO diapositive plates.

Results

The nodules on 2 to 5-day-old plants show the earliest presence of rhizobia disseminated through the infection threads. Due to the occurrence of a limited primary infections over large distance of the hypocotyl initiating from root hair cells, it has not been possible to observe the entire thread path under the electron microscope. After two days of emergence, ramification of the infection thread can be observed in a number of cortical cells.

Morphology of infection thread

The infection threads appear as a cellulose lined hypha-like structure running from one cell to another (Fig. 1).

The infection thread may pierce a cell and can branch profusely. The thread wall is smooth at the thicker regions and have little undulations at thinner regions. The thread diameter varies from 0.5 to 0.8 μ at different regions. The thread passes very close by the host nuclei and even right through the nuclei (Figs. 2 and 3). Blebbing of the outer membrane of the nuclei is commonly seen at this stage.

FIGS. 2–8. 2 and 3, Micrographs of cross section of invaded host nuclei (*N*) through infection thread (*IT*). × 12,000; 4, Oblique section through the ramifying infection thread (*IT*) with bacteria (*B*) within, during initial stage of release. × 40,000; 5, Cross section of an infection thread (*IT*) with a bacterium (*B*). Thread walls appear as parallel lamellar structures of cellulose. × 50,000; 6, L. S. of a part of an infection thread (*IT*) with a bacterium (*B*) at the tip. The tip region of thread has distinct thickened wall. × 20,000; 7, L. S. of part of the infection thread (*IT*) showing simple branching through a vacuole. The tip region is characteristically thickened (arrows). × 40,000; 8, Part of infection thread showing the site of penetration (arrows). Fibrillar net work of less electron dense matrix is also seen (broken arrows). × 38,000.

The tip of the thread is characterized by a thickened wall and appears more osmiophilic. Enlarged micrographs of the thread regions show parallel lamellae or fibrillar structure in their oblique and transverse sections (Figs. 4 and 5). The thickness at the tip region is usually wider, particularly during its advancing phase, measuring from 100–160 nm, as compared to the side walls which range from 30–50 nm. The tip of the infection threads invariably contain bacteria followed by a chain of newly divided ones.

Unbranched straight profiles of the advancing threads have been indicated in Fig. 6 with associated bacteria at the tip. Branching of the threads was commonly seen to be both terminal and subterminal (Fig. 7) and the threads advance in different directions following profuse branching.

Growth of infection thread, penetration and bacterial dissemination

The bacteria initially present from the primary entry through root hair cells undergo a rapid division concomitant with the growth of the infection threads. Dividing bacteria could be seen frequently within the threads. The infection thread appears to be filled up with a mucillaginous 'slime' mass where bacteria grow and move. Occasionally, they present a less electron dense fibrillar net work (Fig. 8, broken arrows).

The intercellular penetration of these threads appear to be rather indirect and associated with a little narrowing of the impeding threads at the site of entry. The wall so affected remains more or less undisturbed.

The bacteria are released intermittently during their intercellular penetration in the cortex. Sometimes these threads do not penetrate much deeper into the tissue and release its entire bacterial content right in one cell. The mode of bacterial release from the infection threads seem to be very specific through the tip-formed spear structures. During this, the thread tip dilates to such an extent that the bacteria easily move into the cytoplasm and remain free-floating. No membranous structures have been seen to be associated with these disseminated bacteria.

Other descriptions—The ultrastructure of host cells containing the invading infection threads reveals prominent nuclei and other cytoplasmic bodies. In any case, there is no prevalence of endoplasmic reticulum or golgi bodies around the growing threads but at times heavy accumulation of ribosomal particles could be noticed. The bacteria within the threads form the largest in size during its entire developmental cycle in the host tissue. Individual bacterium invariably shows prominent fine cytoplasmic and nuclear structures.

Discussion

Most of the workers on electron microscopy of root nodules have failed to study the ultrastructure of primary infection thread passing from root hair cells to the cortex. The available literature on infection thread deals chiefly with the secondary ramification and subsequent growth.

Infection threads can be observed in nodules as late as 7 days after infection. Although a direct evidence is not clear, it is considered that the thread is outlined by a cellulose wall. In some cases as reported by Mosse (1964), Goodchild and

Bergersen (1966) and Dixon (1967), the plasmamembrane is found to surround the infection thread. The presence of such a plasmamembrane is rather ill-defined in the electron micrographs of the present study. In *P. mungo*, at the early stage, the infection thread is found to traverse right through the cells as has been reported by Goodchild and Bergersen (1966) in soybean. The threads have never been observed in the intercellular spaces but isolated bacteria are sometimes observed in the present finding. Similar observations were made by Jordan, Grinyer and Coulter (1963). Mosse (1964) reported a close association between the host nucleus and the extending infection threads; sometimes even causing nuclear indentation. The release of bacteria near the nucleus has also been recorded by him and Goodchild and Bergersen (1966). In the present investigation also, the infection thread has been found to pass very close by the nucleus and occasionally, nuclei appear to have penetrated by the invading threads. Whether this is by chance or by a definite design cannot be ascertained.

In all the published electron micrographs of infection threads as well as in the present case, the bacteria are seen to be separated from the thread wall and between themselves by an electron transparent mass—the 'slime' layer. Slime layer is a mucilaginous matrix of complex chemical composition. In fact, it is not yet clearly known whether the bacteria start synthesizing nitrogenous compounds right after their entry within the infection threads or only at a later stage of nodule growth in well defined enveloping membrane system.

Normally, these infection threads have limited space just enough to accommodate only a single row of bacteria. Goodchild and Bergersen (1966) reported that sometimes two bacteria can lie side by side. Capsulation of bacteria within infection threads was not recorded any time as reported by Dixon (1964).

Generally, the release of bacteria from the infection threads takes place through the well-defined tip of either the main thread or its subterminal branches. Mosse (1964) reported that there is no wall around a growing infection thread. However, the present study and that by Goodchild and Bergersen (1966) show that there is indeed wall around the growing tip. Moreover, in soybean, the tip bulges out with the intact wall and enclose a group of bacterial cells. In the present study, it has been indicated that the thread wall swells and finally dilates to the extent that bacteria are easily released. The individual bacterium in soybean is reported to be released within so called ingestion vacuoles, however, in *P. mungo,* the release appears to take place either to a vacuole or near it in the cytoplasm. Thus, the bacteria become chiefly free-floating inside the pre-existing vacuole or in the cytoplasm and become surrounded by the nuclear-derived membranes (Prasad and De 1971*a*). In certain cases, however, the primary infection threads branch profusely before release, releasing a number of rhizobia at the same time for efficient dissemination. During this, the rhizobia invariably show higher cellular activity and present distinct ultrastructural organization (Prasad and De 1971*b*).

From the evidences available as regards the formation of the infection thread, it appears that there are a number of ways through which it is accomplished. The lack of any specific cytoplasmic organelle like Golgi bodies near or around the growing infection thread suggests a *de novo* synthesis of the thread membrane. It

is also likely that some reaction is definitely offered by the host tissue as a result of defence mechanism following rhizobial invasion.

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