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STRUCTURAL STUDIES ON ROOT NODULES OF *LEUCAENA LEUCOCEPHALA* WITH PARTICULAR REFERENCE TO THE INFECTION PROCESS

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Abstract

Rhizobial infection on roots of *Leucaena leucocephala* started with curling and deformation of root hairs. Infection threads were seen in the young infected cells travelling both inter and intracellularly. Bacteroid region of the nodule composed of both the infected parenchymatous cells and uninfected interestitial cells. Two types of infected cells viz., young vacuolated cell and older non vacuolated cells with deformed nuclei were observed in the developing nodules. Both determinate and indeterminate nodules were observed in *L. leucocephala*.

Introduction

Rhizobia have been reported to enter in majority of leguminous roots via root hairs (Baird *et al*., 1985). Infection through wound or cracks in the epidermis has been reported only in two genera viz., *Arachis* (Chandler, 1978) and *Stylosanthes* (Chandler *et al*., 1982) whereas penetration between intact epidermal cells occurs in *Mimosa scabrella* (Faria *et al*., 1988). In *Leucaena leucocephala* root infection via infection threads could not be observed by Dart (1977) and Mosse (1981) while infection threads were observed in the root hairs as well as inside the nodules of *Leucaena* by Chen & Rolfe (1988). The process of infection in *L. leucocephala* was therefore re-examined which is presented herein.

Materials and Methods

Locally collected seeds of *Leucaena leucocephala* were treated with concentrated sulphuric acid for 15 minutes to remove dormancy (Halliday & Billings, 1984). Seeds were washed with water and sown in the field (Naz & Mahmood, 1976). Six to eight week old seedlings were dug out and nodules and roots washed in running tap water to remove adhering soil particles. Small lateral roots with nodules were fixed in Formaline Aceto-Alcohol, dehydrated in Butyl alcohol and embedded in paraffin wax (Jensen, 1962). Sections of 8-12 m thickness were cut on a rotary microtome and stained with safranin and Harris hematoxylin (Johansen, 1940).

Preparation for Scanning Electron Microscopy (SEM)): Complete nodules and their free hand sections were dehydrated in acetone, dried using a polaron critical point drier (BIO-RAD), coated with platinum in coating unit (JFC-1100) and examined in a Jeol (JSM-T 200) scanning electron microscope (Faria et al., 1986).

Fig.1A. Infection thread (IT) seen inside the root hair (RH) of a lateral root of *Leucaena leucocephala*. The infection thread stains unevenly and its tip is broader than the rest. X4200. (phase contrast micrograph).

Fig.1B. L.S. of a root showing markedly curled root hair (RH) and infection thread (IT). X4200 (phase contrast micrograph).

Fig.2A. L.S. of root showing infection thread (IT) in the zone of dividing cells in the root cortex x2100.

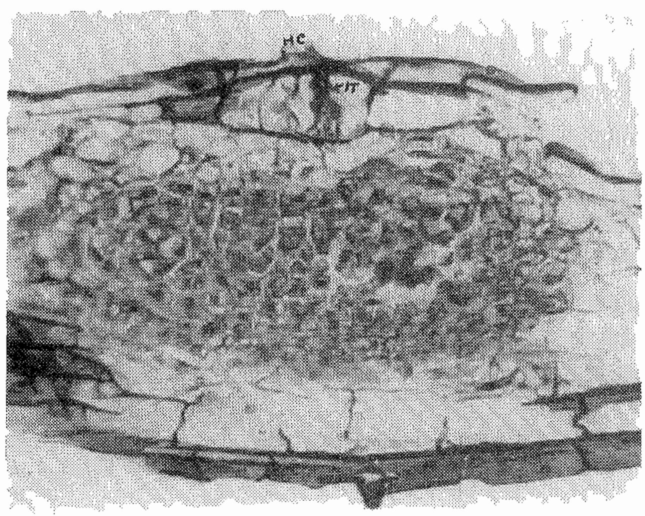

Fig.2B. A pre-emergent nodule composed of a mass of cells within the root cortex. Branched infection thread (IT) also visible subtending the root hair cell (HC). x1575.

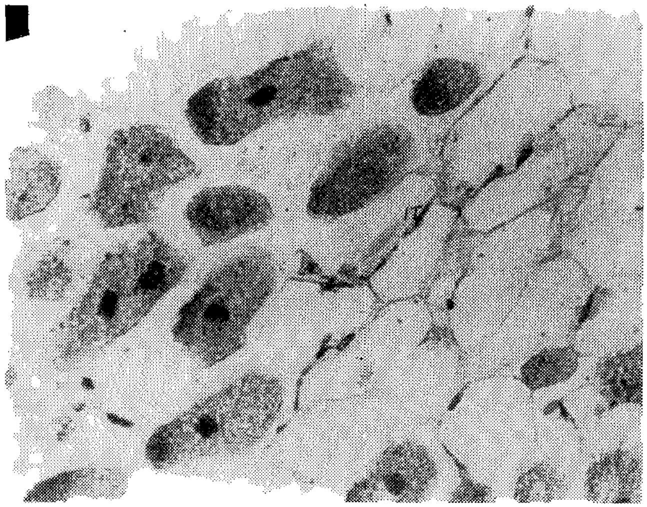

Fig.3A. L.S. of a portion of nodule showing infection threads (IT) travelling intercellularly, x2100.

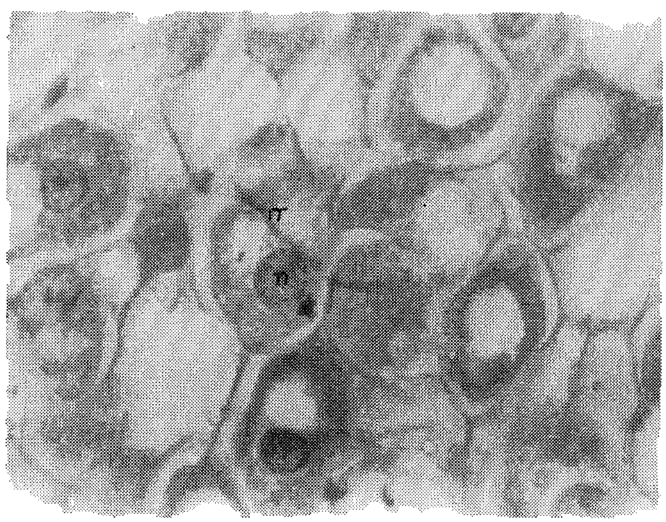

Fig.3B. The intracellular infection thread (IT) appearing broader when passing through the nodule cell wall. Infection thread adhering to the host cell nucleus (n). x4200.

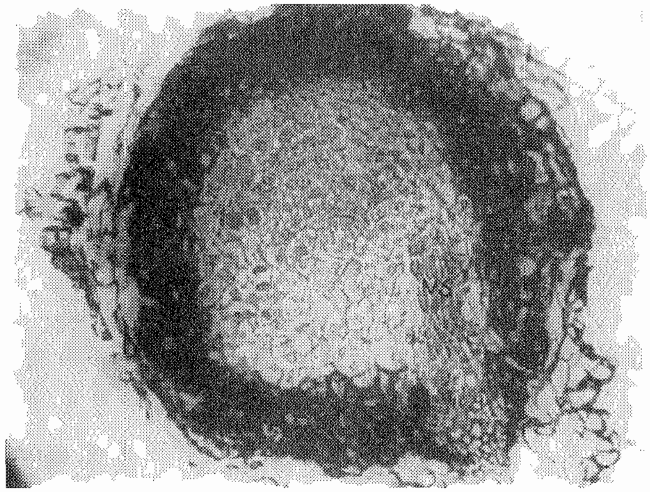

Fig.4A. L.S. of a young nodule composed of a mass of meristematic cells surrounded by darkly stained cells. Vascular strands (VS) initiating from the protoxylem point of the parent root stele, x1050.

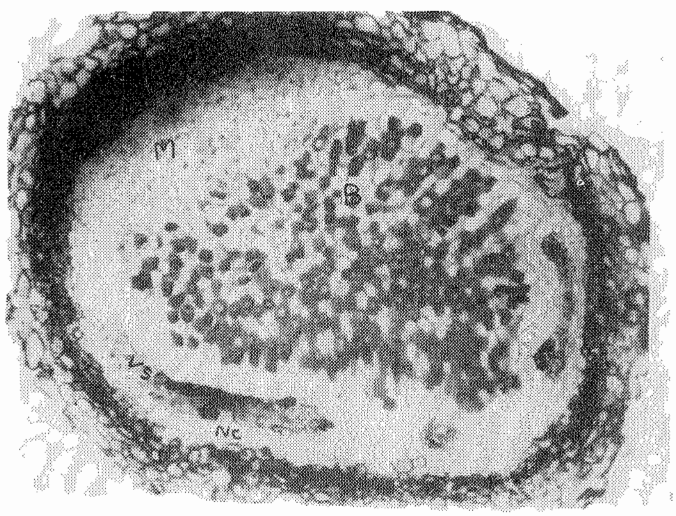

Fig.4B. L.S. of nodule showing three regions of nodule: nodule meristem (M), bacteriod region (B) and nodule cortex (NC). Nodule meristem is covered by darkly stained cells, x420.

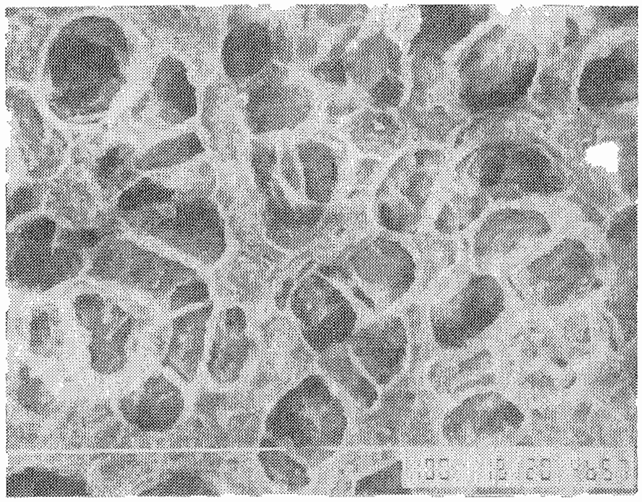

Fig.5A. T.S. of nodule showing noninfected cells intermingled with infected cells x500 (SE microphotograph)

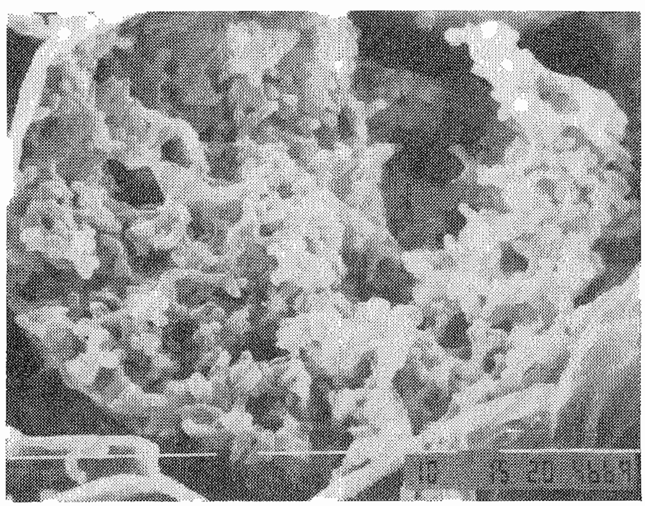

Fig.5B. A single infected cell at a higher magnification showing bacteroids x 3500 (SE microphotograph).

Results and Discussion

Infected roots showed curling and deformation of the root hairs with infection threads seen within them (Fig.1 A,B). Such similar reports have been made by Chen & Rolfe (1988). It may be mentioned that infection threads were not observed either in the root hairs or within the nodules of *L. leucocephala* by Dart (1977). Mosse (1981) not finding root hairs on the roots of *Leucaena*, described it as a legume highly dependent on mycorrhizae for rhizobial entry into the roots. The Infection thread upon reaching the root cortex ramified (Fig.2 A,B) and travelled both inter and intracellularly (Figs.3 A,B), as also found by Chen & Rolfe (1988). Infection threads were also seen in the young bacteroid region of mature indeterminate nodules (Figs.3 A,B). Lectova Trnka (1931) however failed to find infection threads in this region.

Young nodules were surrounded by deeply stained cells (Fig.4A) which stained dark blue green with toluidine blue suggesting the presence of polyphenolic compounds. These cells accompanied the nodules throughout their life and were more prominent at the apices of mature nodules (Fig.4B). Faria *et al.*, (1987) found similar deposits in nodules of most legume species. Such similar cells were observed in cortical region of *L. leucocephala* nodules by Pankhurst *et al.*, (1987) suggesting the presence of Flavolans in this region.

Bacteroidal region of *L. leucocephala* nodules contained approximately 43% of uninfected cells intermingled with infected cells (Table 1; Fig.5A,6). Uninfected cells were not observed in this region by Dart (1977) whereas Durvasula (1984) reported that 50% cells were uninfected. Lack of infection in these cells may be failure of infection to reach in these cells or due to unfavourable conditions within the cells which did not permit the entry of rhizobia (Bergersen & Goodchild, 1973). Uninfected cells were slightly larger in size (Table 1) and contained huge deposits of starch (Fig.6). Such deposits have also been observed in uninfected cells in nodules of *Phaseolus vulgaris* (McCoy, 1929); *Cicer arietinum* (Arora, 1956), *Trifolium alexandrinum* (Naz & Mah-

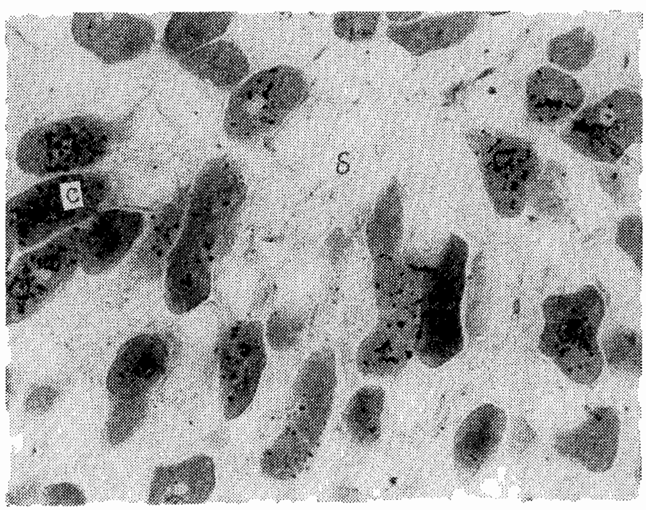

Fig.6. A portion of central bacteroid zone showing uninfected cells containing huge deposits of starch (S). Infected cells showing some contaminants (C). x 1050.

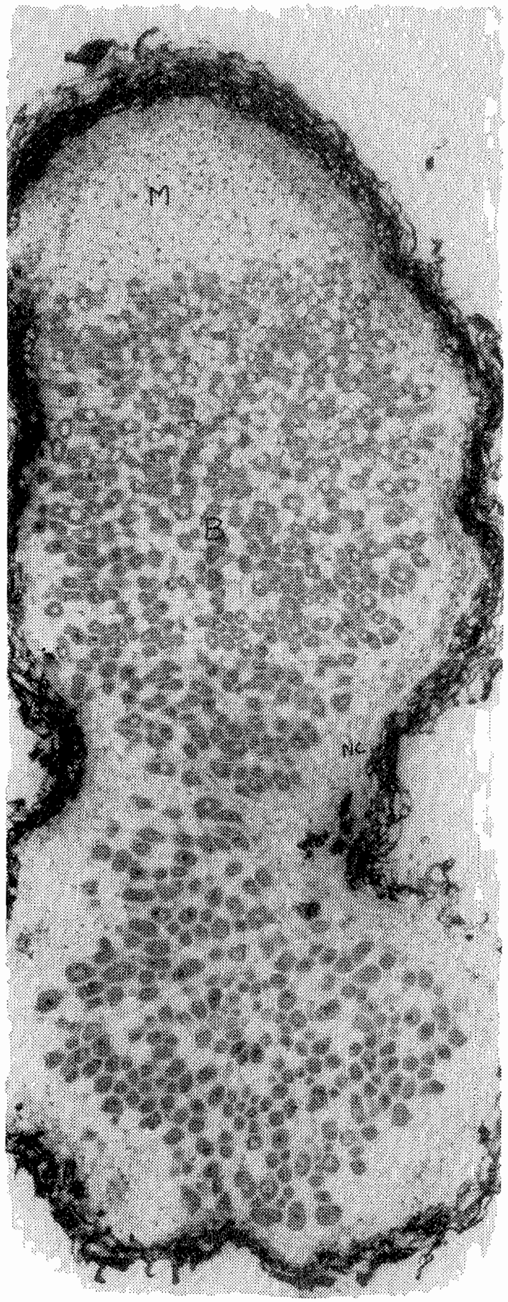

Fig.7. L.S. of a mature nodule showing three regions: nodule meristem (M), cortical region (NC), and central bacteroid region (B) showing two types of infected cells, i.e. young vacuolated cells and older non vacuolated cells, x 420.

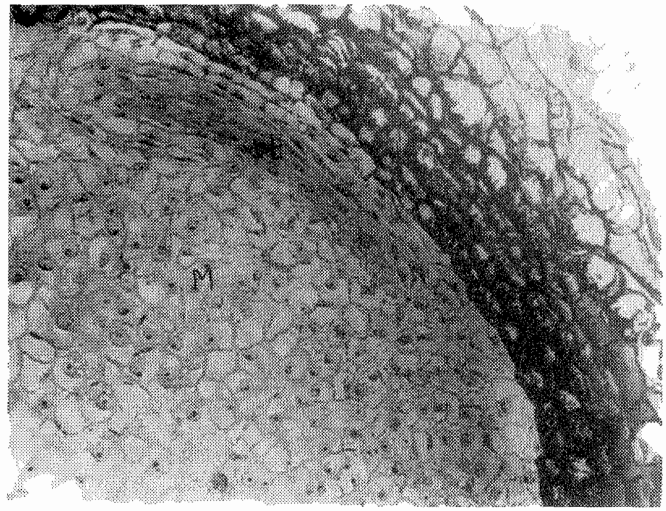

Fig.8A. L.S. of apical portion of a determinate nodule showing spherical meristem (M) and traces of vasculatures (Vt.) x 1050.

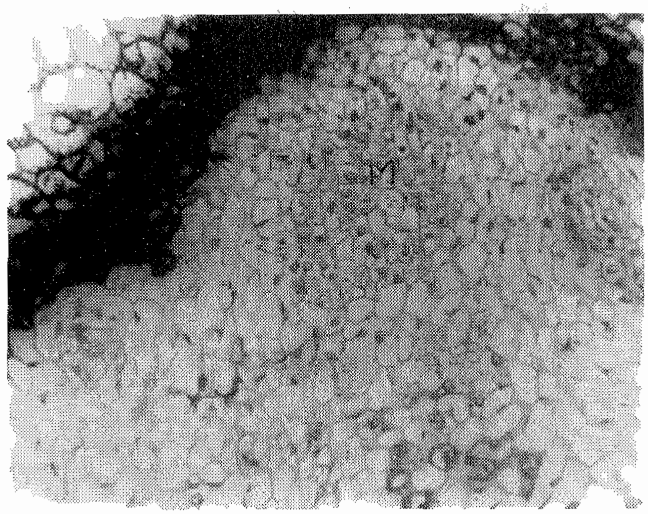

Fig.8B. L.S. of apical portion of an indeterminate nodule showing apical meristem (M). x 1050.

Table 1. Comparison of cell area and cell number of infected and noninfected cells in the central bacteroid region of *Leucaena leucocephala*.

Cell type	% of cells against total No. of cells in the bacteroid region.	Area of one cell in the bacteroid region (Mean o 00 cells) in um^2
Infected cells	57.2	417.34
Non infected cells	42.8	516.98

mood, 1976) and *Medicago sativa* (Hostak *et al.*, 1987). The presence of starch may be an important source of carbohydrate in the nodule tissue (Hostak *et al.*, 1987).

Two types of infected cells were observed in a developing nodule, young newly infected, vacuolated cells and older nonvacu olated cells with deformed nuclei (Fig.7). Numerous vacuoles of different sizes were also observed in the cytoplasm of infected cells of *Cyamopsis tetragonoloba* which disappeared with the increasing number of rhizobia within the nodules (Narayana, 1963). Prominent central vacuole was found only in the genera of the tribe Tephrosieae (Faria *et al.*, 1987).

Infected cells contained some black coloured contaminants (Fig.6) as also observed in isolations made from nodules by (Fred *et al.*, 1932) but proof that these organisms were actually present inside the nodule was lacking.

In the present study the mature nodules of *L. leucocephala* showed close vascular system characteristic of determinate nodules (Fig.8A) and an open vascular system

Fig.9. Section showing vascular bundle (VB) of a nodule. Xylem (X) is surrounded by Phloem (P). x 2100.

characteristic of indeterminate nodules (Fig.8B). Baird *et al.*, (1985) also observed elongated indeterminate and spherical determinate nodules in *Prosopis glandulosa*. Vascular bundles were amphicribral where xylem was surrounded by phloem (Fig.9). Amphicribral and bicollateral vascular bundles have also been reported in *Leucaena* by Durvasula (1984). Amphicribral vascular bundles are reported in majority of legumes (Naz & Mahmood, 1976; Mahmood & Jamal, 1977).

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