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STRUCTURE OF BACTERIAL ROOT NODULE IN *INDIGOFERA GLANDULOSA* VAR SYKESII BAKER EX VIJAY KUMAR AND RAMAYYA : A HISTOCHEMICAL STUDY

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Mature nodule in *Indigofera glandulosa* var. *sykesii* has been described in terms of localization of major metabolites. The bacteriod zone is richest in all metabolites followed by vascular bundles of cortical zone. Starch deposition is seen only in internal cortical cells immediately encircling the bacteriod zone. All cells of bacteriodal zone are infected. Infection threads are absent.

Key words : Bacterial Root Nodules, Indigofera glandulosa Histochemistry.

Leguminous root nodules and their structures have been studied in a number of genera by several investigators (McCoy. 1929; Bieberdorf, 1938; Allen & Allen, 1954; Arora, 1954, 1956; Narayana, 1963 and Meijer, 1982). While various functional aspects of this association have formed subject matter of many investigations (Pate *et al.*, 1969 and Meijer, 1982), nodule structure from histochemical angle has been described for the first time in this communication in *I. glandulosa* var. *sykesii*.

MATERIALS AND METHODS

Root nodules of *I. glandulosa* var. *sykesii* fixed in F.A.A. and Carnoy's medium were dehydrated in ethanol and ethanol : xylol series and embedded in paraffin (m.p. 52 - 54°C). Sections were cut at 8 µm thickness with rotary microtome and mounted with Haupt's adhesive.

Total insoluble polysaccharides were localized by Periodic acid - Schiff's (PAS) reaction (Jensen, 1962), total insoluble proteins by mercuric bromophenol blue reagent (Maiza *et al.*, 1953) using trypsin extraction (Pearse, 1972) as control. Nucleic acids (DNA and RNA) were localised following methyl green pyronin method (Pearse, 1968). Control was run for RNA after ribonuclease extraction (Pearse, 1972).

The slides were then normally processed and mounted in DPX or euparal. All observations were made under light microscope.

OBSERVATIONS

Nodules in *Indigofera glandulosa* var. *sykesii* are distributed allover the root system and are diffused. They are of spherical type with corrugated surface, although some may be slighly lobed. They are distinctly pink, brown or pale.

Internally the nodule consists of a central bacteriodal zone encircled by the cortex (Fig. 1) traversed by a ring of vascular strands. The periderm formation is seen at places along the periphery of the cortical zone.

The cortex comprises of five to seven layers of polygonal parenchymatous cells (Fig. 1). Two to three layers of cells immediately surrounding the bacteriodal zone are smaller and are compactly arranged. Most of the cell lumen is occupied by a large vacuole within a thin layer of cytoplasm containing nucleus lining the vacuole. The nucleus is small and compact. Histochemically the cortical cells give a very faint reaction for total insoluble proteins (Fig. 2) and polysaccharides (Fig. 3) however their compact nuclei show intense reaction for these metabolites. The nucleic acids are solely restricted to the nucleus which show very intense reaction for both DNA and RNA. The nucleolus is intensely rich in polysaccharides, proteins and nucleic acids, especially RNA. Starch grains positive for I_2KI reaction are mainly restricted to the cells of the cortex immediately encircling the periphery of the bacteriodal zone. The cells surroun-ding the meristematic region of the bacteriodal zone are rich in starch grains.

The periderm has about six to eight layers of brick-shaped cells which lack cytoplasm and the nucleus. Histochemically these cells show reaction for polysaccharides mainly in their walls (Fig. 4).

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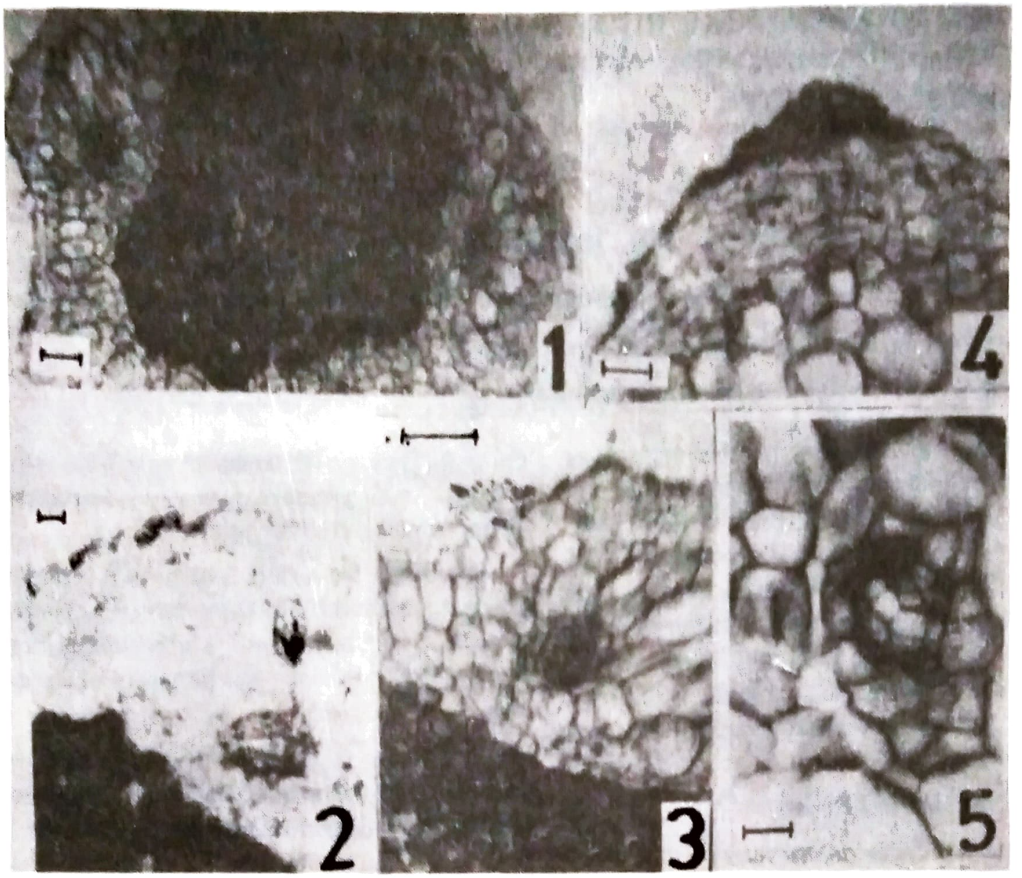

Figs. 1-5: Fig. 1. L. s. of nodule showing vascular bundles, cortex and bacteriod zone. Fig. 2 Nodule section stained for proteins. Fig. 3. Section stained for polysaccharides. Fig. 4 Periderm formation and Fig. 5. Section of vascular bundle showing bundle sheath, phloem and xylem.

Each nodule is supplied with a ring of about eight vascular strands traversing through the median region of the cortex (Fig. 1). The vascular strands are concentric and surrounded by well defined sheath of rectangular cells (Fig. 5). The sheath cells are thin walled without any special type of thickenings. The sheath cells and the cells of the phloem tissue show intense reactions for total insoluble proteins (Fig. 2) and polysaccharides (Fig. 3). The nuclei of phloem parenchyma and companion cells and those of sheath cells show intense reactions for total insoluble polysaccharides and proteins.

The central bacteriodal zone has enlarged polygonal to hexagonal cells (Fig. 3). Those towards the tip and the periphery are smaller and more or less squarish to rectangular.

The central cells of the bacteriodal zone have small central vacuole surrounded by a dense sheath of cytoplasm which almost entirely fills the cell cavity. Prominent enlarged nucleus with nucleolus is situated on one side of the central vacuole. The peripheral and the tip cells of the bacteriodal zone which form nodule meristem are densely cytoplasmic. The vacuoles are either absent or are small and are present throughout the cytoplasm. The nuclei often show division figures. The growth in length and diameter of the nodule is mainly brought about by divisions in these cells.

All the cells of the bacteriodal zone including those of bacteriod meristems are fairly rich in gram negative rod-shaped rhizobia. The cells of the bacteriodal zone are intensely rich for the reactions of

almost all the major metabolites (Fig. 2 and 3). Their cytoplasm is not only rich in polysaccharides and proteins but also in nucleic acids especially the RNA. However, their vacuolated nucleoli show less intense reaction as compared to those of cortical cells.

DISCUSSION

The nodular structure in *I. glandulosa* var. *sykesii* presents certain deviations from that described in other species which merit brief considerations. Several investigators (Allen and Allen, 1954; Arora, 1954, 1956 and Pate et al 1969) have described endodermis like thickenings on the walls of sheath cells of vascular bundles traversing the cortex. Allen and Allen (1954) have further reported endodermoid zone. Both these features are lacking in the species studied.

Almost all cell of bacteriodal zone appear to be infected in *I. glandulosa* var. *sykesii* as against in some legumes where uninfected cells with smaller nuclei and starch grains are found in the bacteriodal zone as well (Bieberdorf, 1938; Arora, 1956 and Narayana, 1963). No trace of infection threads could be recognised in the nodule though in several other species persistent remains of infection threads have been noted (Bieberdorf, 1938; Allen and Allen, 1954; Arora, 1956; Narayana, 1963 and Meijer, 1982).

For many of the features, nodular structure in this species resembles to that in *Arachis hypogaea* (Meijer, 1982) where the infection is direct without involving infection thread formation. According to Meijer (1982) such nodules are also found in species of *Aeschynomene* and *Stylosanthes* characterised by him as spherical nodules.

Starch grains are mainly localised in cortical cells immediately surrounding bacteriod zone. They are abundant in the cells surrounding the meriste-matic parts of the bacteriodal zone. In *Cicer arietinum* Narayana (1963) observed cortical cells to be rich in strach grains while in *Glycine max* (Bieberdorf, 1938) starch grains are reported in cells surrounding vacular bundlles.

Amongst different nodular tissues, the bacteriodal zone appears to be extermely rich in various primary metabolites localized during this study indicating high metabolic status of this zone. This is also borne out by the enlarged nuclei containing prominent vacuolar nucleoli in the cells of this zone. The richness of metabolites in conducting strands of cortical zone suggests their importance as pathways of transport between the bacteriod zone and other host tissues (Pate *et al* , 1969). The cortical zone appears merely to serve as a passive protective sheath around the active bacteriod zone.

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