Pak. J. Bot., 9 (1): 39—46, 1977.

A CONTRIBUTION TO THE HISTOLOGY OF ROOT NODULES OF *SESBANIA SESBAN L.*

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Abstract

Rhizobia entered the root via root hairs and formed infection threads. The nodules were exogenous in origin. A mature nodule consisted of four regions, namely apical meristem, bacteroid region, vascular region and nodule cortex. The vascular supply of the nodule consisted of 1—2 strands which branched and encircled the bacteroid zone as a skeletal mantle. The vascular bundles were amphicribral. The cortex of 8 week old nodules showed a layer of sclereids with heavily lignified walls. Degeneration of the nodule occured due to the formation of a layer of suberized cells and appearance of tannin bodies at the base of the nodule.

Introduction

The importance of bacterial root nodules of herbaceous leguminous plants in agriculture is a well recognized fact and it is for this reason that major attention has been given in the past to the structure of nodules of these plants. The nodules of woody legumes on the other hand have received much less attention and there are only a few of them whose structure has been studied. They are *Acacia, Robiana* and *Sophora* species (Spratt, 1919) *Wistaria sinensis*, (Jimbo, 1927), *Cytisus capitatus* (Lechtova-Trunka, 1931) *Sesbania grandiflora* (Harris et al, 1949), *Aeschynomene indica* (Arora, 1954) and *Caragana arborescens* (Allen et al 1955).

In the present study some aspects of the histology of nodules of *Sesbania sesban* are described. This is a yellow flowering variety commonly cultivated in the plains of Sind and Punjab in Pakistan. It is a woody species and no published report is found on the histology of its nodules.

Materials and Methods

The strains of Sesbania rhizobia (*Rhizobium japonicum*) were obtained from nodules on young vigorous plants growing under natural conditions at the Karachi University Campus. Nodules were washed under running tap water and surface sterilized in 0.1% mercuric chloride solution for 2-4 minutes. Nodules were then rinsed in several changes of sterile distilled water and crushed in a tube containing 5-10 ml. of sterile water. Serial loop dilutions of nodule suspension were plated on yeast extract mannitol agar. After an incubation period of 1-2 days at 28°C the widely separated colonies typical of rhizobia were isolated in yeast extract mannitol broth. The broth cultures were sub-cultured several times till pure cultures were obtained.

The *Sesbania sesban* plants were raised from locally collected seeds. Surface sterilized seeds were grown in pots containing soil and manure. They were given a rhizobial dressing prepared in broth culture. Some seeds were treated before sowing and some after sowing. Nodules were formed on plants raised from seeds with both treatments.

For microtomy nodules were fixed in formalin-acetic-alcohol and usual techniques for dehydration in-filtration and embedding were followed. Serial sections of ten to fifteen microns thick were cut. Heidenhain's iron alum haematoxylin and safranin-light green stains proved useful for demonstrating nuclear and general anatomical details. A staining sequence described by Allen et al (1955) starting with 1 % alcholic safranin T for 16 hours, Gram's iodine 5 minutes, 1 % alcoholic crystal violet 1-2 minutes followed by clearing in clove oil was found satisfactory. This staining procedure differentiated with equal clarity the vascular system, bacteria and infection threads in the infected tissues.

Observations

The first nodule was observed on the primary root of the seedling close to the surface of soil on 7th day of its growth. After 15 days, nodules were found in clusters both on main tap root as well as on lateral roots (Fig. 1). The young nodules were spherical but the older ones were lobed.

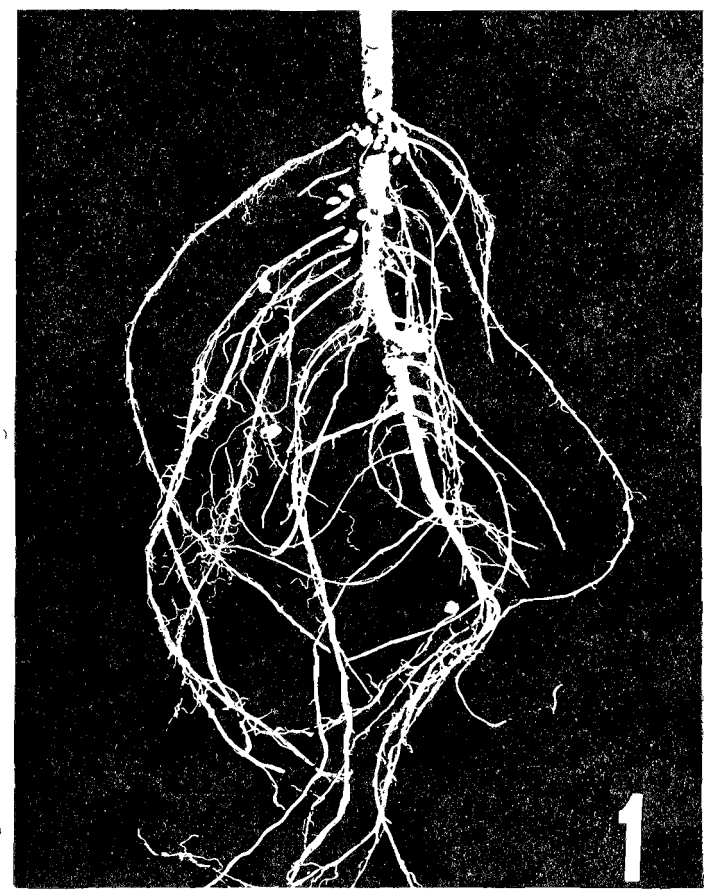


Fig 1. Roots showing nodules on 8 weeks old plant, some nodules are lobed.

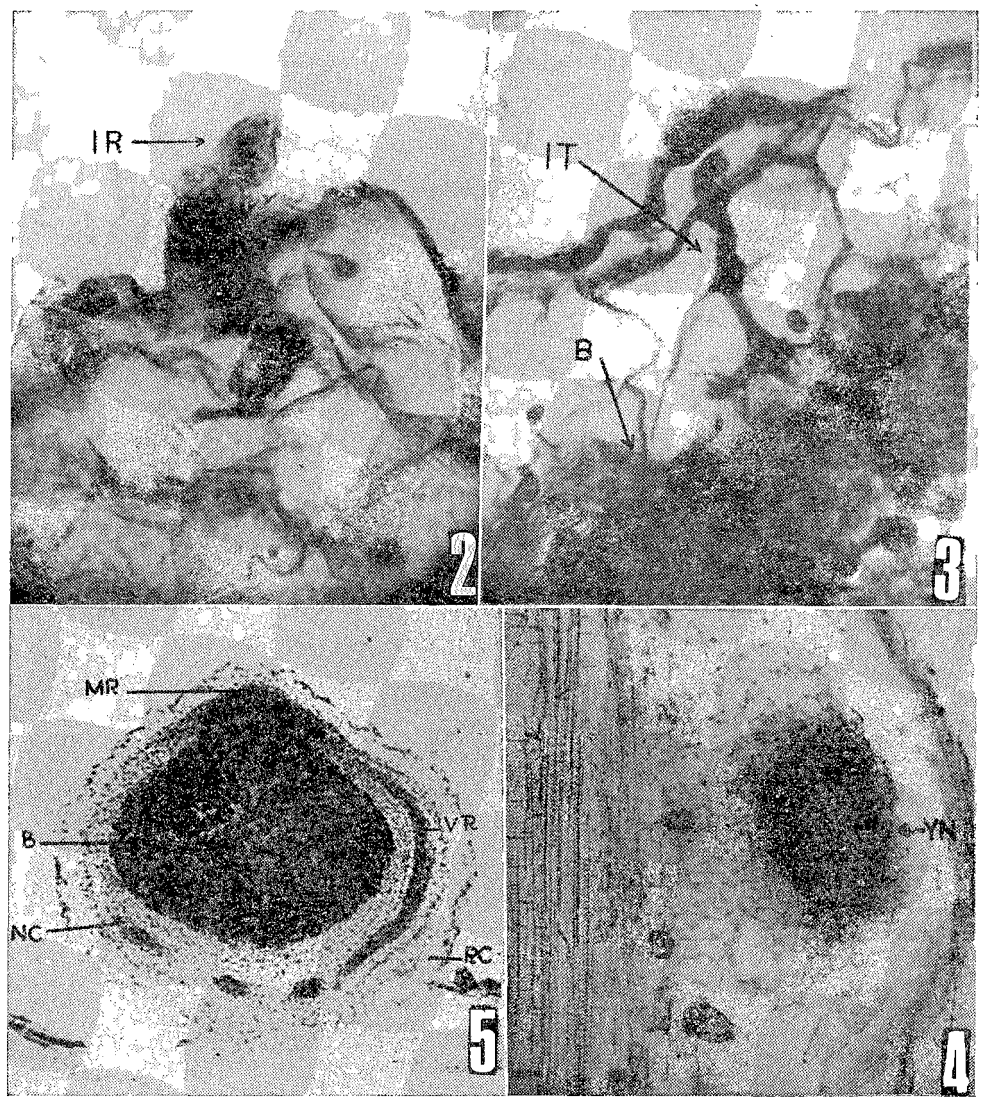


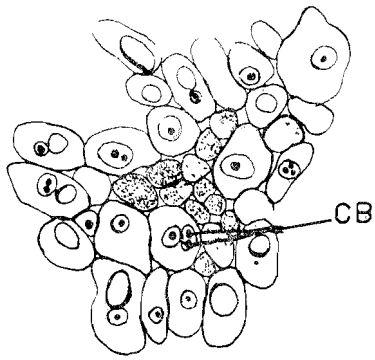
Fig. 2. Infection thread in the curled root hair, X 320.

Fig. 3. Infection thread with funnel shaped swelling passing through cortical parenchyma of root. The infection thread is ramified. X 80.

Fig. 4. Young nodular outgrowth in the root cortex, X 80.

Fig. 5. L. S. of the mature nodule showing four regions. X 80.

The root hairs were found to be the chief portals of entry of *Rhizobium* in the roots The rhizobia entered the curled root hair and formed an infection thread (Fig. 2). Infection thread usually passed through two or three cells of root cortex and then ramified (Fig. 3). At the juncture of the threads with the walls of the invaded cells the threads formed funnel shaped swellings (Fig. 3).



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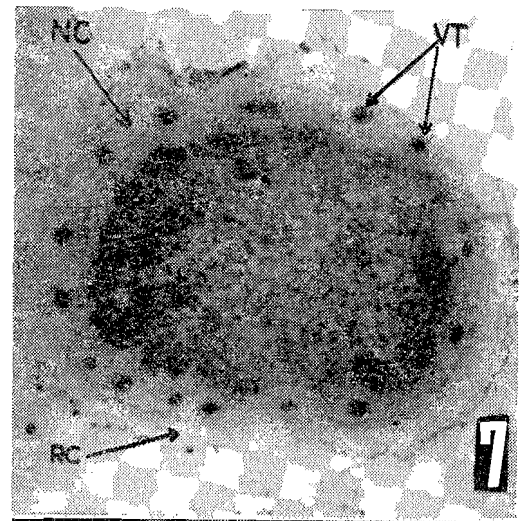

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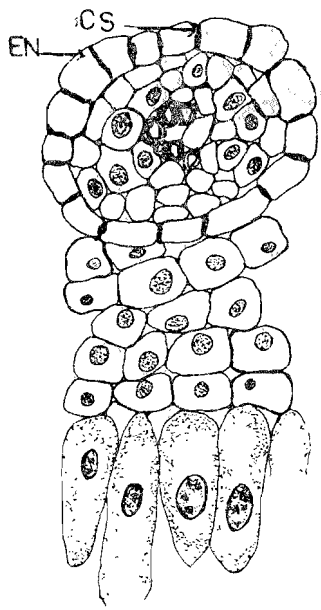

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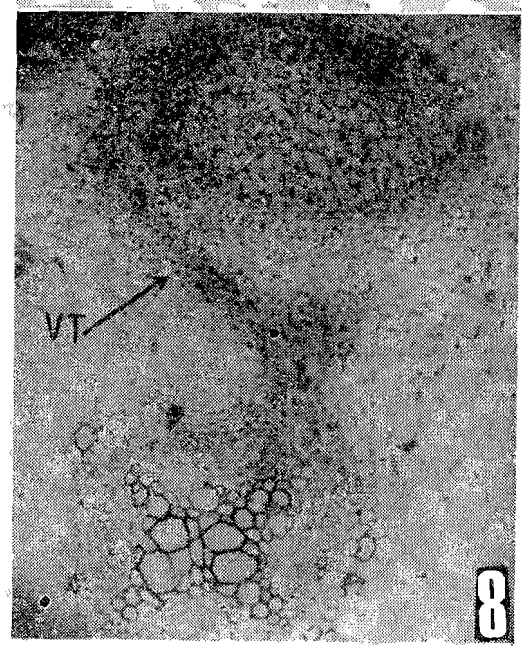

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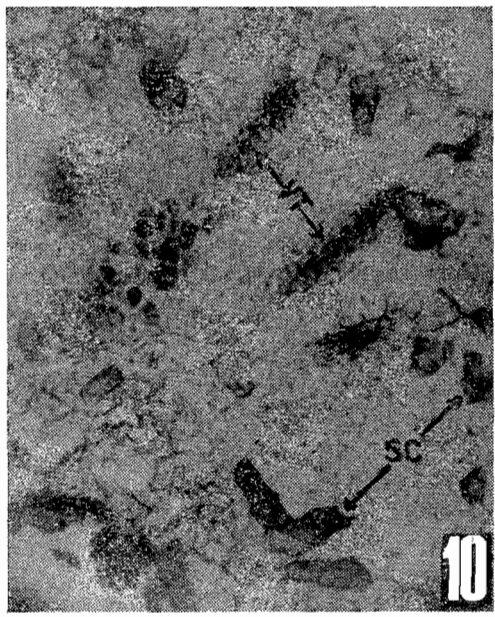
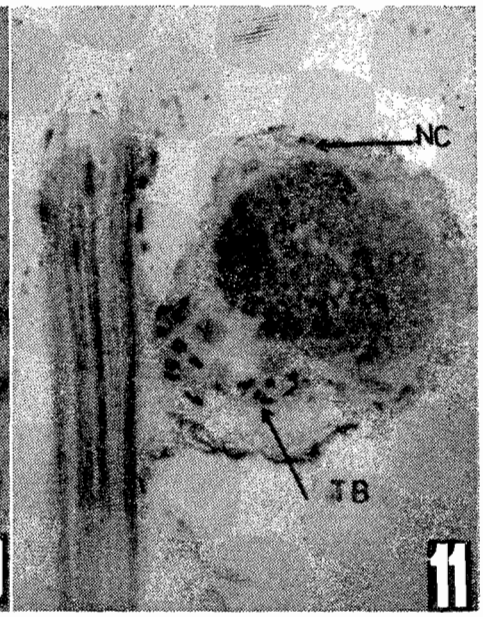
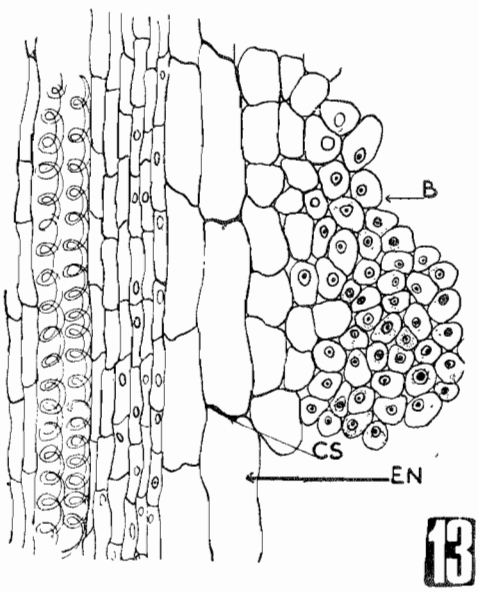
Fig. 6. Mature bacteroid cells showing central vacuole and more than one chromatin bodies. X 250.
Fig. 7. T. S. through nodule showing a ring of vascular bundles in the nodule cortex. X20.
Fig. 8. Initiation of vascular strand of nodule from one protoxylem point, X 80.
Fig. 9. A single amphircribral vascular bundle from nodule cortex, X 60.

In the early stages of development of the nodule the entire mass of cells comprising the young nodules was meristematic (Fig. 4) but as nodule matured it could be differentiated into four regions, namely meristematic region bacteroid region, vascular region and nodule cortex, as characterized by most legumes (Fig. 5).







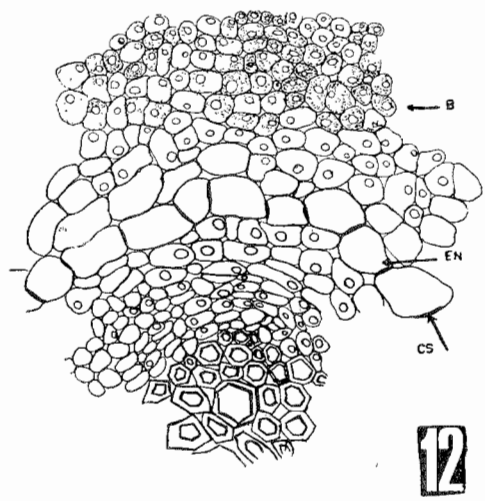


Fig. 10. Vascular strands and sclereids scattered in nodule cortex, X 80.

Fig. 11. Accumulation of tannin bodies at the base of 8 weeks old nodule, X 20.

Fig. 12. T. S. of root along with the nodule showing intact endodermis around the parent root stele, X 250.

Fig. 13. L. S. of the root along with nodule showing nodular tissue outside the parent root stele endodermis, X 250.

The meristematic region was located at the tip of nodule and consisted of multiple layers of thin walled deeply stained actively dividing cells with prominent nuclei. The bacteroid region occupied the central part of the nodule. Towards the apex just below the meristematic zone bacteroid cells were small in size. These cells showed a large central nucleus with one or two prominent nucleoli. The older bacteroid cells were found below the young bacteroid cells and were elongated. They contained a large central vacuole with a deformed nucleus. Many chromatin bodies were observed in their nuclei (Fig. 6).

The conducting system of the nodule showed dichotomous branching. As a result of this the bacteroid area was surrounded by numerous vascular strands. These strands did not come in contact at any time with the bacteroid tissue (Fig. 7). Provascular strand was initiated from one protoxylem point within the root stele (Fig. 8).

Vascular bundles were conspicuous in the nodule cortex and could be seen in all the cross sections. They were amphicribral. The bundles were enclosed by a typical endodermis (Fig. 9). In median longitudinal sections of 6 weeks old nodules approximately 16 vascular bundles were found in the nodule cortex, 2-3 layers outside the bacteroid area (Fig. 7). The nodule cortex consisted of 3-5 layers of non-infected parenchymatous cells. These cells are readily distinguished from cells of root cortex by their compactness and smaller size (Fig. 5 & 7). In these older nodules sclerenchymatous tissue was present in the region of the nodule cortex where normally nodular endodermis is found. This tissue consisted of sclereids characterized by heavily lignified walls which showed pits. In 8 week old nodules small clusters of sclereids were found scattered in the cortex external to the fibrovascular system (Fig. 10). In young nodules sclereids were not observed.

The degeneration of the nodules was accompained by appearance of tannin bodies in the cortical cells at the base of the nodule (Fig. 11). The nuclei and cytoplasmic contents of these cells disintegrated and they showed a large central vacuole.

Discussion

Although there are reports of entry of rhizobia through the broken epidermal cells (McCoy, 1929; Bieberdorf, 1938) through the ruptured cortex in the vicinity of rootlet emergence (Allen & Allen, 1940; Arora, 1954), through epidermal cells alone (Schaede, 1940), but entry of rhizobia through root hairs is more prevalent mode of infection in majority of plants investigated (Thornton, 1930; Fred, Baldwin & McCoy, 1932; Bieberdorf, 1938; Wipf & Cooper, 1940; Bond, 1948a; Harris *et al*, 1949; Allen *et al*, 1955; Arora, 1956b & c; Narayana, 1963; and Wittmann 1968). Root hairs were the principal avenue of entry of rhizobia in the root system of *Sesbania sesban* and the infection thread was single and branched.

The origin of nodules of *Sesbania sesban* is cortical. This conclusion is based on two observations.

1. Endomermis of the parent root can be seen encircling the stele of the root although the development of the nodule has been completed (Figs 12 & 13).

List of Abbreviations

B—Bacteroid area, CB—Chromatin bodies, CS—Casparian strips, EN—Endodermis, IR—Infected root hair, IT—Infection thread, MR—Meristematic region, NC—Nodule cortex, RC—Root cortex, SC—Sclereids, TB—Tannin bodies, VR—Vascular region, VT—Vascular tissue, YN—Young nodule.

2. Nodules of exogenous type remain enclosed within a layer of root cortex (Allen & Allen 1940). This is shown in (Figs. 5 & 7).

Differenitation of vascular strands in *Sesbania sesban* is intiatied in cortical tissue of the nodule and these elements are not out growths from the mother root stele. Similar observations were made by (Bieberdorf, 1938; Frazer, 1942; Bond, 1948a). The bundles were amphicribral. Similar type of vascular bundles have been reported in *Phaseolus vulgaris* (McCoy, 1929), *Arachis hypogea* (Allen & Allen 1940) and *Sesbania grandiflora* (Harris *et al*, 1949).

The constitution of bacteroid tissue varies in different plants. In *Sesbania sesban*, the bacteroid tissue shows unifected cells intermingled wth the infected ones. Such a condition has been reported in *Sesbania grandiflora* (Harris et al, 1949), *Cajanus indicus* (Arora, 1956b) *Cicer arietinum* (Arora 1956c), and *Cyampopsis tetragonoloba* (Narayana, 1963). The bacteroid tissue consists of cells packed with bacteria in *Aeschynomene indica* (Arora, 1954), and infected cells possess large central vocuole in *Arachis hypogoea* (Allen & Allen 1940) and *Crotalaria juncea* (Arora, 1956a).

Considerable attention has been given in the past to the layers of cells which seperate the nodule cortex from the nodular vascular system. The cells of this layer may show casparian strips or they may be simply suberized. Nodule endodermis as typified by casparian strips was not observed in *Sesbania sesban* nodules, a situation also reported in *Wistaria sinensis* (Jimbo, 1927), *Sojaman* (Bieberdorf, 1938), *Sesbania grandiflora* (Harris et al 1949), and *Caragana arborescens* (Allen et al, 1955). However sclerenchymatous tissue was seen in the nodules of 8 weeks old *Sesbania sesban* plants in the area where nodule endodermis is usually present. This tissue consisted of sclereids characterized by heavily lignified cell walls showing pits. Similar type of sclereid tissue has been reported by Spratt (1919) in *Acacia* and *Robiana* species and in *Sesbania grandiflora* by (Harris et al, 1949). Older *Sesbania sesban* nodules undergo radial increase in diameter. As a consequence of this increase the outer cortical layers of the nodule get ruptured. The sclereidal layer in such a situation performs the function of a secondary cortex or periderm as suggested by Harris et al (1949) for *Sesbania grandiflora*.

Degeneration of nodules occurs in several ways. Our observations corroborate with the findings of Allen & Allen (1940) & Arora (1954) that the degeneration of nodules occurs due to the formation of suberized thickenings in parenchymatous cells at the base of the nodule.

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