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ULTRA-STRUCTURAL STUDIES ON ROOT NODULES OF *ALBIZIA LEBBECK* (L.) BENTH.

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Abstract

Albizia lebbeck (L.) Benth., commonly known as Siris is a mimosoid tree legume widely distributed in Pakistani soils. The roots of *A. lebbeck* establish symbiosis with root nodule bacteria and form nodules which develop singly as well as in clusters on the primary and secondary roots. Mature nodules are oblate, elongate, branched and coralloid. Rhizobia in root nodules from naturally nodulated plants showed intercellular movement. Both determinate and indeterminate type of nodules were observed having similar structure. Anatomically nodules could be differentiated into a nodule meristem, nodule cortex, containing the vascular bundles surrounding the infected tissues. Nodule meristem was multilayered, comprising of thin walled, tightly packed and actively dividing cells. Nodule cortex was mainly composed of parenchymatous tissue. Distinct periderm was present. Amphicribal vascular bundles were arranged around the bacteroid region which showed both the infected and un-infected interstitial cells with starch grains in their cytoplasm. Infected cells were non-vacuolated. Studies was carried out to examine the initiation, early development and ultrastructure of root nodules of *A. lebbeck*.

Introduction

Albizia lebbeck (L.) Bent (Leguminosae-Mimosoideae) is a multipurpose woody tree that is cultivated in many parts of Pakistan in farmlands, along roadsides, irrigated plantations and riveroin tracks (Ali, 1973). It provides shade and fuel wood. Its leaves contain 16.8-25.5% crude protein and is excellent source of fodder (Prinsen, 1986). *A. lebbeck* forms symbiotic relationship with *Rhizobium* and fix atmospheric nitrogen used for its growth and also for the enrichment of the rhizosphere. Although nitrogen fixation has been reported in tree legumes yet only few studies have been carried out on the nodule development and structure of tree legumes (Baird *et al*., 1985; Sprent, 2001; Gross *et al*., 2002). Studies on infection process and ultra structure of *P. dulce* nodules is lacking. The aim of the present study was to examine the initiation, early development and ultra-structural characteristics of the nodules.

Material and Methods

Nodules of *A. lebbeck* were collected from Karachi University campus. For light microscopy the nodules and roots were fixed in F.A.A. for 18 h. Fixed pieces of nodules 1-2 mm in size were dehydrated in ethanol series and infiltrated with L.R. white resin at room temperature (two resin changes), and polymerized at 60°C for 24 hours Serial sections 0.5-2 μm were cut with a glass knife on a J.B.-4 ultra microtome and transferred to glass slides in a large drop of water. The sections were dried on a hot plate at 40°C, stained with aqueous toluidine blue in 1.0% borax (pH 4.4) and mounted in D.P.X./ Canada balsam .The sections were examined using a Nikon HFX-11 Photomicroscope.

For Transmission Electron Microscopy (TEM) small pieces of nodules, 1-2 mm in size, were fixed in 2% gluteraldehyde in 0.1M phosphate buffer (pH 7) for 4h and then tissues were transferred in 1% aqueous Osmium tetroxide for 2-4 h at room temperature. The fixed material was dehydrated through ethanol-acetone series to absolute acetone, passed through acetone: propyleneoxide mixture in 3:1, 1:1 and 1:3 ratio and finally rinsed three times with 100% propylene oxide. Tissues were infiltrated in a gradually increasing concentration of Epon in propylene oxide and then transferred into fresh resin for 12-16 h before embedding and polymerised at 60°C for 24 h. Ultra-thin sections were cut with a glass knife on JB-4 Jeol ultra microtome, and stained in LKB ultra-stainer with 4% Uranyl acetate for 30 min., and with lead citrate for 5 min., (Callaham & Torry 1981). Specimens were examined in a Hitachi H-800 T.E.M. at 75-150 KV.

For scanning electron microscopy, complete nodules and free hand sections of nodules were fixed as for TEM. They were dehydrated in 100% ethanol followed by an ethanol: acetone series to 100% acetone. The specimens were then dried using a Polaron critical point drier (BIO-RAD), coated with gold in coating unit (JFC-1100) and examined with (Jeol T-20) scanning electron microscope.

Results and Discussion

Nodules of *A. lebbeck* were woody and rough in texture, white in colour with pink apex and distributed on the main and lateral roots (Fig. 1A). The morphology of root nodules showed variation in different species of *Albizia* as described by various workers, for example nodules of *Albizia odianthifolia* were elongated and bifurcate, elongated and fan-shaped in *Albizia ferruginea* and palmate in *A. zygia* (Wester & Hogberg, 1989). Bifurcate or globose, Astragaloid type of nodules have been reported in *Albizia* sp., (Bergerson, 1982). Nodules were branched and Croterlaroid type in *Albizia polyphylla* (Faria *et al*., 1987a) and globose in *A. lebbeck* (Mahmood & Iqbal, 1994). In the present study globose, elongated, branched and coralloid nodules were observed.

An interesting feature was observed in the young nodules of *A. lebbeck* where bacteria entered through the root hair and multiplied within the epidermal cell. The root hairs were straight (Fig. 1B). From the epidermal cell they spread into the cortical region by intracellular movement by dissolving middle lamella (Fig. 2B). Movement of bacteria was also observed via pits (Fig. 3B). Both determinate and indeterminate type of nodules were observed (Fig. 4) which could be differentiated into nodule meristem, nodule cortex, vascular tissues and bacteroid region (Figs. 4A&B). A distinct periderm was present. Vascular bundles were amphicribal and distributed around the bacteroid region (Figs. 4A&B). The bacteroid region of the nodule showed both infected and non-infected cells mixed together. Infected cells of developing nodules as well as mature nodules were non-vacuolated (Figs. 5C, D, E & F). Nodule formation is initiated by the entry of rhizobia into root tissue. Rhizobia enter the root via root hairs in majority of legumes (Qadri & Mahmood 2004). Rhizobia were first found to colonize near the root surface (Fig. 3A) than entered the root via root hair and formed infection thread. Entry of *Rhizobium* into root cortex through root hairs was found prevalent mode of entry during the present studies. However, crack entry of *Rhizobium* through ruptured epidermis (Fig. 3A) was also observed. Crack entry of *Rhizobium* into root tissue has been reported in *Dalbergia* (Sprent & Sprent, 1990) and in *Arachis* (Chandler, 1978). The root hair infection process consists of several events which proceed to nodule formation in the *Rhizibium*-legume symbiosis. These steps include mutual host symbioint recognition, rhizobial adherence to root hair, root hair curling, root hair infection, nodulation and

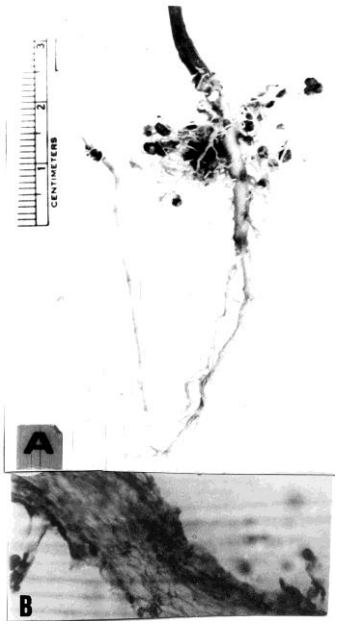

Fig. 1. A. Distribution of nodules on main and lateral roots of A. lebbeck
B. Infection thread (it) in the root hair (rh) of *A. lebbeck* ×150.

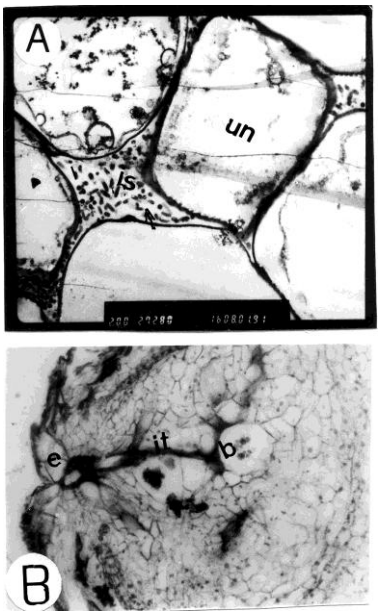

Fig. 2. A. Movement of rhizobia (b) through intercellular spaces (is) uninfected cell (un) in young nodule of *A. lebbeck*. (TEM electron micrograph) × 3000
B. Young nodule of *A. lebbeck* showing intercellular movement of infection thread (it) and bacteria (b). (Light micrograph) ×1700.

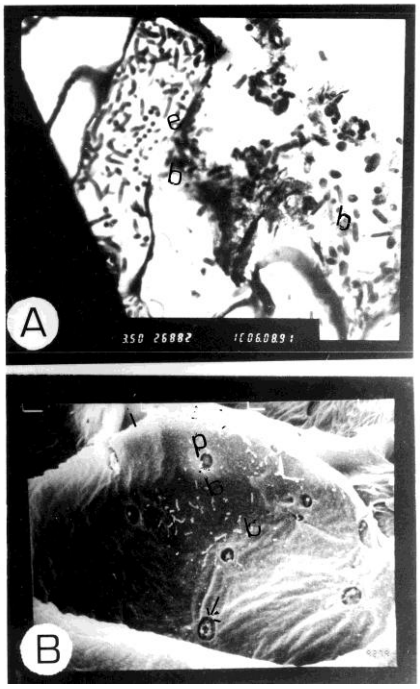

Fig. 3. A. Crack entry of rhizobia in *A. lebbeck* through epidermis. e= epidermis, b= bacteria, (TEM micrograph) ×4000
B. Movement of bacteria in the root of *Albizia lebbeck* through pits b= bacteria, p= pit, i= intercellular space (SEM micrograph) ×4000.

transformation of vegetative bacteria into enlarged pleomorphic bacteroids (Dart, 1977; Schmidt, 1979; Sprent & Sprent, 1990). The curling of root hairs seems to play an important role to facilitate the entry of rhizobia into the root cortex of host plant (Napoli & Hubbell, 1975). The infection thread is formed inside the root hair by multiplication of rhizobia which lie end to end within the thread. In *Phaseolus vulgaris* either straight or slightly curved infected hairs were observed (Baird & Webster, 1982). Similar type of infected hairs were found in *A. lebbeck* during the present studies (Fig. 1B). In addition to infection thread formation, accumulation of *Rhizobia* in the intercellular spaces of cortical tissue of nodule was also observed (Fig. 2A). After the infection thread reaches the root cortex, it ramifies and travels both inter- and intracellularly. Chen & Rolfe (1988) and Rubina & Mahmood (1992a) have described inter- and intracellular infection threads in *Leucaena*.

Persistent infection threads were observed in several species of trees of the subfamily Caesalpinoideae and some species of Papilionoideae but none in the members of Mimosoideae (Quispel *et al*., 1993). According to Faria *et al*., (1987b), species where persistent infection threads were observed, infection spreads in the developing nodules by movement of rhizobia intercellularly rather than by infection threads. In the present study intercellular movement of rhizobia was recorded (Fig. 2B) although persistent infection threads were absent.

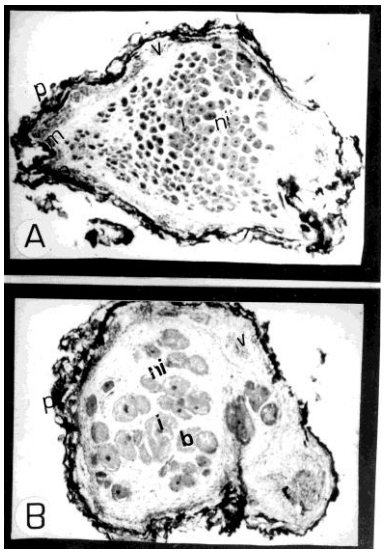

Fig. 4. Light microscopic photograph of *A. lebbeck.*
A. L.S. of indeterminate type root nodule of *A. lebbeck* showing periderm (p), vascular tissues (v), open meristem (m) and bacteroid region with infected (i) and uninfected (ni) cells ×100
B. L.S. of determinate type root nodule of *A. lebbeck* ×100.

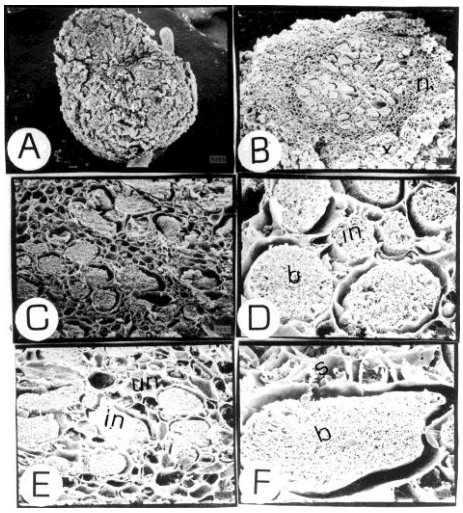

Fig. 5. Scanning Electromicrographs (SEM) of *A. lebbeck* nodules.
A. Woody semi globose determinate type of nodule of *A. lebbeck* with rough texture ×50
B. T.S. of nodule, with central bacteroid (b), nodule cortex (n) and vascular tissues (v) outside the bacteroid region. (SEM micrograph) ×100
C. Portion of bacteroid region of root nodule showing infected and uninfected cells. × 300
D. Infected (in) cells of bacteroid zone filled with bacteria (b) ×1000
E. Infected cells (in) intermingled with interstitial cell (un) ×500
F. An infected cell (in) without vacuole filled with bacteria (b) and interstitial cells (un) with starch (s) ×1300.

Once the nodule has been formed it establishes connection with the vascular system of main root (Bond, 1948). The vascular system of the nodule of *A. lebbeck* surrounded the central bacteroid region without coming in direct contact with it. Such observations has been recorded by Mahmood & Jamal, (1977); Wester & Hogberg, (1989); Sprent & Sprent, (1990); Rubina & Mahmood, (1992a). The vascular bundles found in the nodules of *A. lebbeck,* were amphicribal. Amphicribal vascular bundles have been reported in a number of nodules of leguminous plants (Qadri & Mahmood, 2004). Bacteroid region occupies the central part of the legume nodule. Composition of this region may vary in different legumes. In the present study bacteroid region consisted of infected and uninfected (interstitial) cells intermingled with each other. Similar observations have been made IN a number of nodules (Qadri & Mahmood, 2004). The bacteroid cells contained large central vacuole in *Arachis hypogaea* (Allen & Allen, 1940) and *Crotalaria juncea* (Arora, 1956a). Vacuoles in the infected cells of indeterminate nodules of tribe vicieae were reported by Sprent (1981), Which could act as temporary stores for fixed nitrogen. During the present investigation vacuolated infected cells were not observed in the infected zone of *A. lebbeck*. Faria *et al*., (1987b) have surveyed this feature in tribes of subfamilies Caesalpinoideae, Papilionoideae and Mimosoideae and found that prominent central vacuole was found only in the genera *Cyclolobium* and *Poecilanthe* of the tribe Tephrosieae. Non-infected cells found in the bacteroid and cortical regions of *A. lebbeck* contained starch. It has been reported that nodules of several legume species store carbohydrates in the form of starch (Rubina & Mahmood, 1992a; Gross *et al*., 2002). Hostak *et al*., (1987) found that uninfected cells of the nodules may prove an important source of carbohydrates for nodular tissues because legume nodules require large amount of carbohydrates in order to generate adequate supply of energy needed in nitrogen fixing process.

The general structure of nodules of *A. lebbeck* showed similarities with majority of leguminous plants, in having a nodule meristem, nodule cortex, vascular tissues surrounding the bacteriod region. However two distinct features observed were the presence of straight infected root hairs and the presence of bacteria on the root surface. Bacteria could be seen around the pits and some of them reaching inside the pits (Fig 2B). Further studies will be needed to establish the entry of bacteria in the root cortex through the pits and their role in the initiation of the process of infection.

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