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# Ultra-structural Studies on Root Nodules of *Samanea saman* (Jacq.) Merr. (Leguminosae)

RAIHA QADRI[1], A. MAHMOOD[1] and MOHAMMAD ATHAR[2]*

[1] Department of Botany, University of Karachi, Karachi, Pakistan
[2] California Department of Food and Agriculture, Sacramento, CA, USA

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Abstract

Ultra-structural studies were conducted on root nodules of *Samanea saman* (Jacq.) Merr. collected from trees growing under natural conditions. Nodules were distributed singly as well as in clusters on the main and lateral roots. Mature nodules were elongated, branched and coralloid. Root hair curling was found but infection threads could not be observed. Rhizobia entered through the epidermis and moved intercellularly through the cortical region. Mature nodules of *S. saman* could be differentiated into meristem, cortex, vascular tissue and bacteroid tissue. The latter showed both infected and non-infected cells mixed together. Vascular bundles were inversely collateral and distributed around the bacteroid tissue. The bacteroids were enclosed in peribacteroid membrane in groups and showed prominent granules of polyhydroxybutyrate in their cytoplasm. Mycorrhizal hyphae were also observed along with rhizobia in the bacteroid tissue. *S. saman* with dual rhizobial and mycorrhizal infection is a potential tree for plantation in arid soils of Pakistan.

Key words: *Samanea saman*, bacteroids, mycorrhiza, rhizobia, root nodules ultra-structure, woody legume

## Introduction

*Samanea saman* is a fast growing woody mimosoid legume that is cultivated in many parts of Pakistan in farmlands and along roadsides. It provides shade and fuel wood. Its wood is also used for making bowls, trays, furniture *etc.* The tree forms nitrogen-fixing nodules with rhizobia (Allen and Allen, 1981). The indigenous woody legumes and their root nodule bacteria play an important role in the overall nitrogen increment of Pakistani soils (Mahmood, 1999). A diverse group of Gram-negative nodule forming bacteria namely *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Azorhizobium*, *Mesorhizobium, Sinorhizobium* and *Methylobacterium* have been recognized. They are the members of the α and β subgroup of the phylum *Proteobacteria* (Chen *et al.*, 2003), collectively known as rhizobia (Amarger, 2001; Vessey *et al.*, 2004). The fixed nitrogen is used by *S. saman* for its growth and enrichment of the rhizosphere. The process of nodule formation is closely related to the infection of roots by appropriate rhizobia. Rhizobia enter the root *via* root hairs in majority of legumes (Iqbal and Mahmood, 1992; Qadri and Mahmood 2003, 2004, 2005). Structural studies of tree legume nodules have been conducted on *Sesbania sesban* (Mahmood and Jamal, 1977), *Prosopis glandulosa* (Baird *et al.*, 1985), *Andira* sp. (Faria *et al.*, 1986), *Leucaena leucocephala* (Iqbal and Mahmood, 1992), *Anadenanthera peregrina* (Gross *et al.*, 2002), *Dalbergia sissoo* (Qadri and Mahmood, 2002, 2004), *Albizia lebbeck* (Qadri and Mahmood, 2005) and *Pithecellubium dulce* (unpublished). Although *S. saman* has been cultivated in Pakistan for a long time, studies on structure of its nodules are lacking. This paper describes mode of infection and development and structure of *S. saman* nodules.

## Experimental

### Materials and Methods

**Material collection and preparation for microscopy**. Nodules of *S. saman* were collected from roots of trees growing in the garden of the Department of Botany, University of Karachi. For light microscopy, the nodules and roots were fixed in F.A.A. (formaline-acetic acid-ethyl alcohol) in the ratio of 5:5:90 for

* Corresponding author: M. Athar, California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA 95832, USA; **e-mail: A.Tariq@cdfa.ca.gov**

18 hours. pieces of nodules (1–2 mm) were dehydrated in ethanol series and infiltrated with L.R. (London resin) white at room temperature and polymerized at 60°C for 24 hours. Serial sections (0.5–2 mm) were cut with a glass knife using a Sorrall 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 Canada balsam (Faria *et al.*, 1986). They were than examined under a Zeiss student microscope.

**Transmission electron microscopy assay**. For transmission electron microscopy (TEM), small pieces of nodules (1–2 mm) were fixed in 2% gluteraldehyde in 0.1 M phosphate buffer (pH 7) for 4 hours, washed with three changes of buffer solution during three hours and transferred to 1% aqueous osmium tetroxide for 2–4 hours at room temperature. The fixed material was processed for transmission electron microscopy as described by Qadri and Mahmood (2003).

**Scanning microscopy assay.** Complete nodules and free hand sections of nodules were fixed for scanning electron microscopy as for TEM. They were dehydrated in 100% ethanol followed by an ethanol/acetone mixtures up to 100% acetone (Faria *et al.*, 1986). The specimens were then dried using a Polaron critical point drier (BIO-RAD), coated with gold in coating unit (JFC-1100) and examined under (Jeol T-20) scanning electron microscope.

## Results and Discussion

Nodules of *S. saman* were distributed on the main as well as lateral roots and occurred singly and in clusters (Fig. 1A). Although root hair curling was observed, infection threads could not be seen (Fig. 1B). Faria *et al.* (1987a, b) have surveyed the occurrence of infection threads in the three sub-families of legumes namely *Caesalpinoideae*, *Mimosoideae* and *Papilionoideae*. According to their survey, infection in members of *Mimosoideae* occurs by the movement of rhizobia intercellularly rather than by infection threads. Similar observations have been reported by Dart (1977), Chandler *et al.* (1982) and Calvert *et al.* (1984). The bacteria entered the ruptured epidermis of the root, from where they spread intercellularly into the cortical region (Fig. 1C). Continuous proliferation of rhizobia in host cells resulted in the formation of well organized indeterminate nodules (Fig. 1D).

The general structure of the nodules of *S. saman* shared similarities with the majority of leguminous plants in having a nodule meristem (M), nodule cortex (NC), bacteroid region (B) and vascular supply (VS) (Fig. 1D). The nodule meristem was comprised of numerous small compact cells. This is the region of active nuclear division. Normally these cells contain neither infection threads nor rhizobia. The meristematic region persisted throughout nodule development. The nodule cortex was comprised of 4–10 layers of non-infected parenchyma, isodiametric in shape. Cortical cells are derived by division of cells of the meristematic zone. Tannins were found scattered throughout the cortical region as idioblasts (Fig. 1D). Bacteroid region occupied the central part of the nodule. The bacteroid tissues of the nodules showed both infected (IN) and uninfected (UN) cells mixed together (Fig. 1E). Similar observations have been made for *Sesbania grandiflora* (Harris *et al.*, 1949), *Cajanus indicus* (Arora, 1956), *Cyamopsis tetragonoloba* (Narayana, 1963), *Glycine max* (Bergersen and Goodchild, 1973), *Trifolium alexandrium* (Naz and Mahmood, 1976), *Albizia* spp. (Dart, 1977), *Sesbania sesban* (Mahmood and Jamal, 1977), *Parasponia andersonii* (Trinick, 1979), *Phaseolus vulgaris* (Baird and Webster, 1982), *Leucaena leucocephala* (Iqbal and Mahmood, 1992), *Dalbergia sissoo* (Qadri and Mahmood, 2002, 2004), *Albizia lebbeck* (Qadri and Mahmood, 2005) and *Pithecellobium dulce* (unpublished). A group of bacteria were enclosed in a common peribacteroid membrane (Fig. 2B). The bacteria contained prominent granules of polyhydroxybutyrate (PHB) (Fig. 2B). Both oval and rod shaped bacteria were observed (Fig. 2C). Enclosure of a group of bacteria in a common peribacteroid membrane is a distinctive feature of infected cells in leguminous root nodules as reported by a number of investigators (Newcomb, 1976; Lawrie, 1983; Chalifour and Benhamou, 1988; Qadri *et al.*, 2006). The peribacteroid membrane is derived initially from the host plasma membrane and is a plant product. The peribacteroid membrane become lost at certain points and bacteria are released into the cytoplasm of the cell from these sites (Fig. 2C). The liberated or free bacteria are always surrounded by a peribacteroid membrane which is derived from the bulges of the plasma membrane surrounding a group of bacteria (Fig. 2C) as described by Newcomb (1976).

The vascular differentiation of the nodule is discernible within a week after nodule initiation. Literature on the subject has been reviewed by Bond (1948), Naz and Mahmood (1976) and Baird *et al.* (1985). The first indication of formation of conducting tissue becomes evident in the form of a few cortical cells that start dividing parallel to the radius of the root forming the procambial strands. Very soon these strands get connected with the protoxylem points of the vascular cylinder of the parent root. The vascular supply may consist of one to four vascular strands (Bond, 1948). In *S. saman* two vascular strands were seen making connection with the vascular supply of the main root (Fig. 1D). Two vascular strands have been

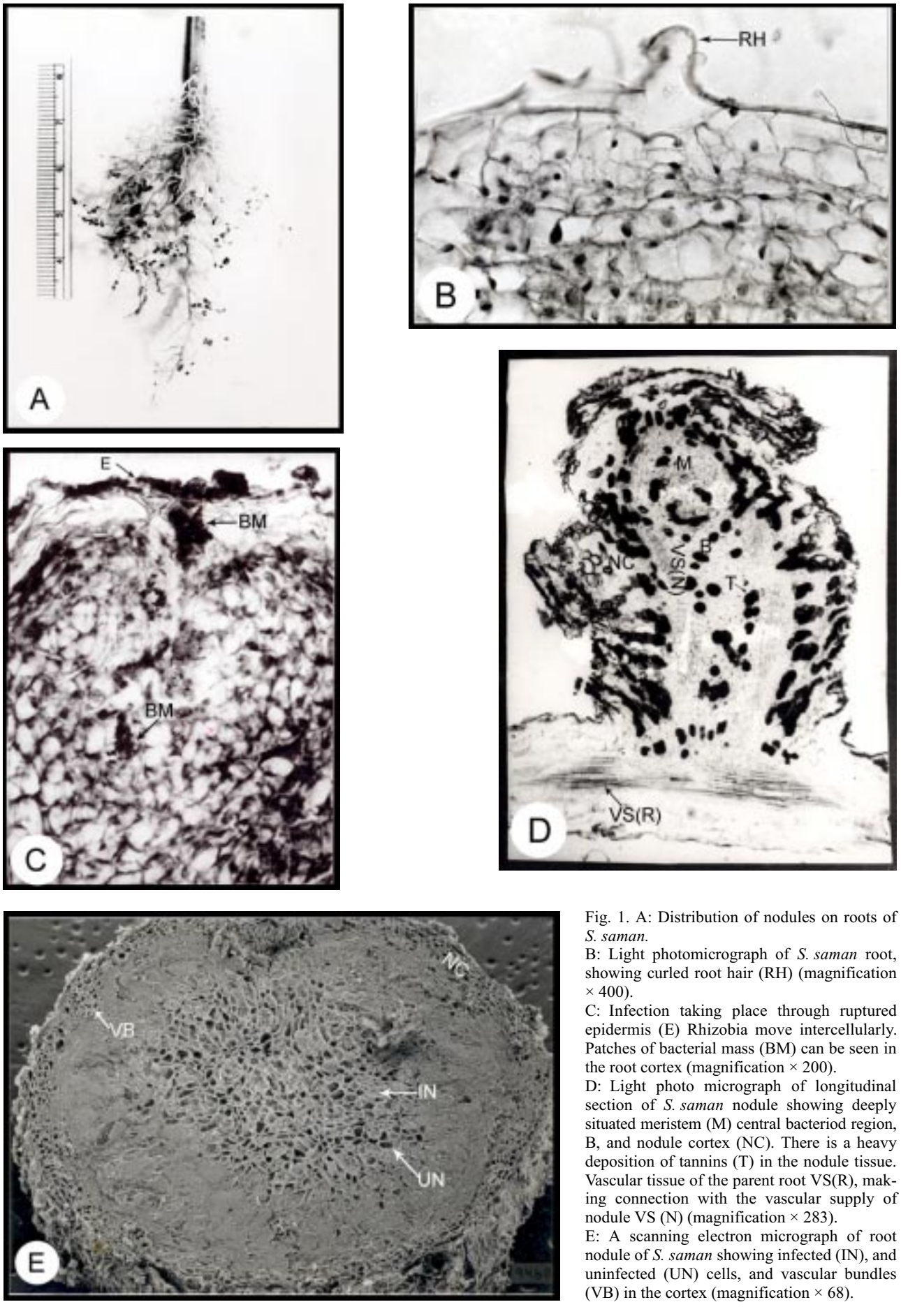
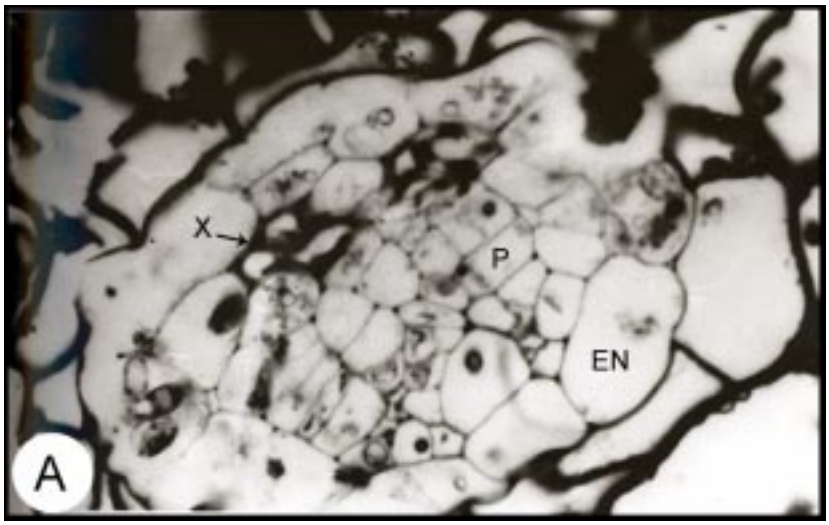
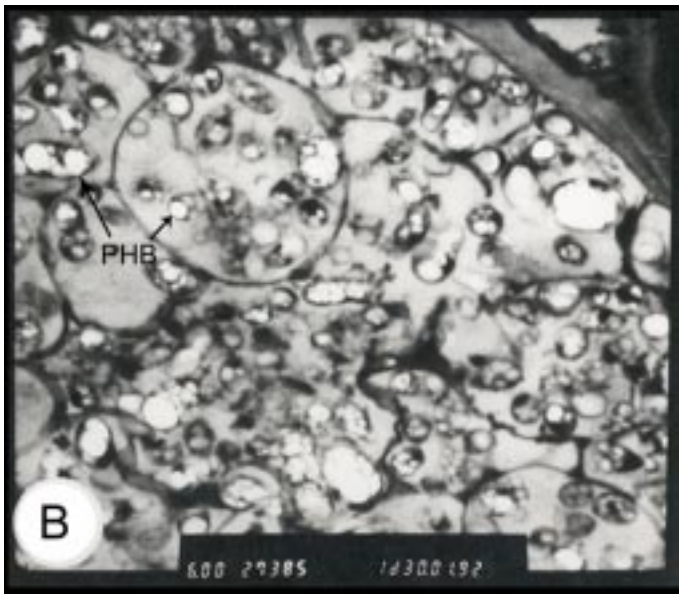
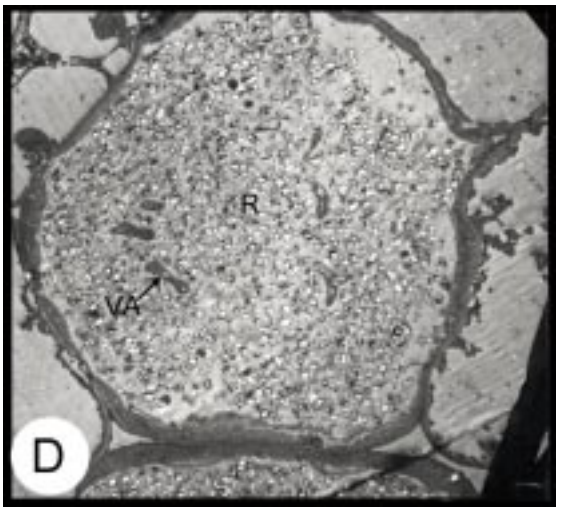
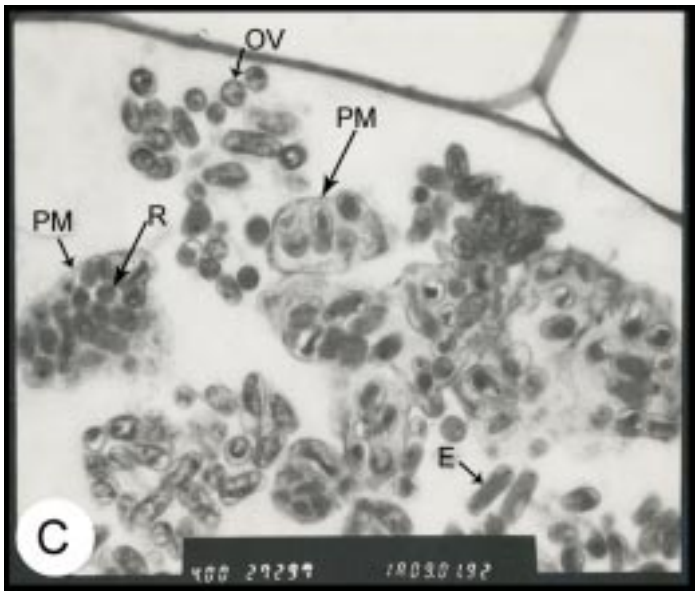



Fig. 1. A: Distribution of nodules on roots of *S. saman.*
B: Light photomicrograph of *S. saman* root, showing curled root hair (RH) (magnification × 400).
C: Infection taking place through ruptured epidermis (E) Rhizobia move intercellularly. Patches of bacterial mass (BM) can be seen in the root cortex (magnification × 200).
D: Light photo micrograph of longitudinal section of *S. saman* nodule showing deeply situated meristem (M) central bacteriod region, B, and nodule cortex (NC). There is a heavy deposition of tannins (T) in the nodule tissue. Vascular tissue of the parent root VS(R), making connection with the vascular supply of nodule VS (N) (magnification × 283).
E: A scanning electron micrograph of root nodule of *S. saman* showing infected (IN), and uninfected (UN) cells, and vascular bundles (VB) in the cortex (magnification × 68).



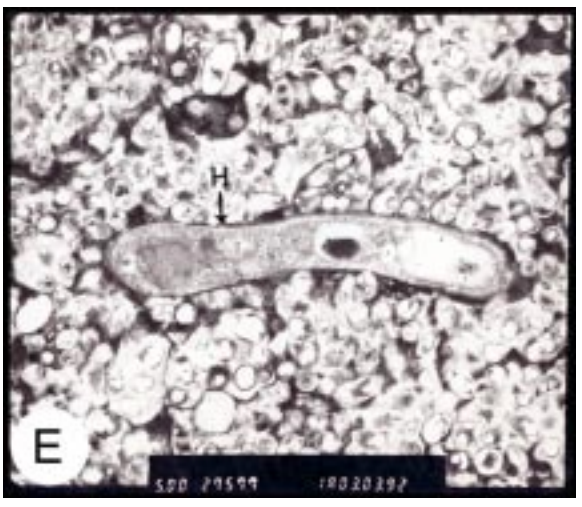




Fig. 2. A: An enlarged view of inversely collateral vascular bundle of *S. saman* showing xylem (X), phloem (P) and endodermis (EN) (magnification × 3000).
B: Transmission electron micrograph of a portion of bacteriod region (B) showing rhizobia enclosed in a common peribacteriod membrane (PM). They show a high content of polyhydroxybutyrate (PHB) granules (magnification × 6364).
C: Transmission electron micrograph of a portion of bacteroid region (B) of a root nodule cell. Note that rhizobia (R) are enclosed in a common peribacteroid membrane (PM) and some of them are coming out of the membrane at certain points. Both elongated (E) and oval (OV) forms are present (magnification × 15 600).
D: Transmission electron micrograph of a single bacteroid (B) cell of a root nodule showing rhizobia (R) and vesicular-arbuscular mycorrhizae – VAM (VA) (magnification × 2160).
E: Transmission electron micrograph of an enlarged portion of bacteroid cell in Fig. 2-D showing a single hypha (H) along with rhizobia (R) (magnification × 15000).

reported in *Vicia faba* (Bieberdorf, 1938), *Sesbania grandiflora* (Harris *et al.*, 1949), *Pisum sativum* (Bond, 1948), *Melilotus alba, Trifolium alexandrinum* (Naz and Mahmood, 1976) and *Pithecellobium dulce*. Once formed, the strands branched repeatedly encircling the central bacteroid region (Fig. 1E). The vascular strands never come in direct contact with the bacteroid tissue. A few layers of parenchyma always separate the vascular tissue from the bacteroid tissue (Fig. 1D). Vascular bundles were inversely collateral (Fig. 2A). The xylem elements faced away from while phloem elements faced towards the center. Inversely collateral bundles have been reported in pea nodules by Bond (1948). The vascular bundles were enclosed by an endodermis (Fig. 2A).

The infected cells of *S. saman* along with rhizobia also contained mycorrhizal hyphae (Figs. 2D and 2E). Vesicular-arbuscular mycorrhizae (VAM) have been reported in nodules of some leguminous trees such as *Sesbania grandflora* (Habte and Aziz, 1985), *Acacia mangium, Albizia falcata*, (Dela Cruz *et al.*, 1988) and *Leucaena leucocephala* (Young, 1990). The presence of mycorrhizae is known to enhance nodulation and nitrogen fixation by legumes (Amora-Lazecano *et al.*, 1998; Johansson *et al.*, 2004). Mycorrhizal fungi and nitrogen fixing bacteria often act synergistically on infection rate, mineral nutrition and plant growth (Rabie and Almadini, 2005). The beneficial effects of nitrogen-fixing bacteria in combination with mycorrhizal fungus on plant growth have been discussed by Patreze and Cordeiro (2004) and Domenech *et al.* (2004).

In conclusion it may be said that most of the mycorrhizal research with nitrogen fixing trees has revolved around only a few selected tree species (Aziz and Sylvia, 1992). Studies on VAM interactions with nitrogen-fixing tree species should be conducted on a large scale. Mahmood (1999) has analyzed the nitrogen-fixing potential of indigenous woody legumes and discussed their role in the improvement of denuded and derelict lands of Pakistan. *S. saman* with dual rhizobial and mycorrhizal infection is a potential tree for plantation in Pakistani soils in future afforestation schemes.

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## Literature

**Allen O.N. and E.K. Allen.** 1981. *The Leguminosae: a Source Book of Characteristics, Uses and Nodulation.* The University of Wisconsin Press, Madison.

**Amarger N.** 2001. Rhizobia in the field. *Adv. Agron.* 73: 109–168.

**Amora-Lazecano E., M.M. Vazquez and R. Azcon.** 1998. Response of nitrogen-transforming micro-organism to arbuscular mycorrhizal fungi. *Biol. Fertil. Soils* 27: 1–13.

**Arora N.** 1956. Morphological study of root nodules on *Cajanus indicus*. *Proc. 43rd Indian Sci. Congr.* (*Agr.*): 224–245.

**Aziz T. and D.M. Sylvia.** 1992. Utilization of vesicular-arbuscular mycorrhizal fungi in the establishment of nitrogen-fixing trees. pp. 167–194. In: N.S. Suba Rao and G. Rodriguez Barrueco (eds). *Symbiosis in Nitrogen Fixing Tree.* Oxford & IBH Publishing Co. (Pvt.) Ltd., New Delhi.

**Baird L.M. and B.D. Webster.** 1982. Morphogenesis of effective and ineffective root nodules in *Phaseolus vulgaris* L. *Bot. Gaz.* 143: 41–51.

**Baird L.M., R.A. Virginia and B.D. Webster.** 1985. Development of root nodules in a woody legume, *Prosopis glandulosa* Torr. *Bot. Gaz*. 146: 39–43.

**Bergersen F.J. and D.J. Goodchild.** 1973. Aeration pathway in soybean root nodules. *Australian J. Biol. Sci.* 26: 729–740.

**Bieberdorf F.W.** 1938. The cytology and histology of the root nodules of some Leguminosae. *J. Amer. Soc. Agron*. 30: 375–389.

**Bond L.** 1948. Origin and developmental morphology of root nodules of *Pisum sativum* L. *Bot. Gaz.* 109: 411–434.

**Calvert H.E., M.K. Pence, M. Pierce, N.S.A. Malik and W.D. Bauer.** 1984. Anatomical analysis of the development and distribution of *Rhizobium* infection in soybean roots. *Can. J. Bot.* 62: 2375–2384.

**Chalifour F.P. and N. Benhamou.** 1989. Indirect evidence for cellulase production by *Rhizobium* in pea root nodules during bacteriod differentiation: cytochemical aspects of cellulose breakdown in rhizobial droplets. *Can. J. Microbiol.* 35: 821–829.

**Chandler M.R., R.A. Date and R.J. Roughley.** 1982. Infection and root nodule development in *Stylosanthes* species by *Rhizobium*. *J. Exp. Bot.* 33: 47–57.

**Chen W.M., L. Moulin, C. Bontemps, P. Vandamme, G. Bena and C. Boivin-Masson.** 2003. Legume symbiotic nitrogen fixation by $\beta$-*proteobacteria* is widespread in nature. *J. Bacteriol.* 185: 7266–7272.

**Dart P.J.** 1977. Infection and development of leguminous nodules. pp. 367–472. In: R.W.F. Hardy and W.S. Silver (eds). *A Treatise on Dinitrogen Fixation. Section III. Biology.* John Wiley and Sons, New York.

**Dela Cruz R.E., M.Q. Manalo, N.S. Aggangan and J.D. Tambalo.** 1988. Growth of three legume trees inoculated with VA mycorrhizal fungi and *Rhizobium*. *Plant Soil* 108: 111–115.

**de Faria S.M., H.C. de Lima, A.A. Franco, E.S.F. Mucci and J.I. Sprent.** 1987a. Nodulation of legume trees from South East Brazil. *Plant Soil* 99: 347–356.

**de Faria S.M., S.G. McInroy and J.I. Sprent.** 1987b. The occurrence of infected cells, with persistent infection threads in legume root nodules. *Can. J. Bot.* 65: 553–558.

**de Faria S.M., J.M. Sutherland and J.I. Sprent.** 1986. A new type of infected cells in root nodules of *Andira* spp. (Leguminosae). *Plant Sci.* 45: 143–147.

**Domenech J., B. Ramos-Solano, A. Probanza, J.A. Lucas-Garcia, J.J. Colon and F.J. Gutierrez-Manero.** 2004. *Bacillus* spp. and *Pisolithus tinctorius* effects on *Quercus ilex* ssp. *ballota*: a study of tree growth, rhizosphere community structure and mycorrhizal infection. *Forest Ecol. Manage*. 19: 293–303.

**Gross E., L. Cordeiro and F.H. Caetano.** 2002. Nodule ultrastructure and initial growth of *Anadenanthera peregrina* (L.) Speg. var. *falcata* (Benth.) Altschul plants infected with rhizobia. *Ann. Bot.* 90: 175–183.

**Habte M. and T. Aziz.** 1985. Response of *Sesbania grandiflora* to inoculation of soil with vesicular-arbuscular mycorrhizal endophytes in an oxisol. *Biol. Fertil. Soils* 7: 164–167.

**Harris J.D., E.K. Allen and O.N. Allen.** 1949. Morphological development of nodules on *Sesbania grandiflora* Poir. with reference to the origin of nodule rootlets. *Amer. J. Bot.* 36: 651–661.

**Iqbal R. and A. Mahmood.** 1992. Structural studies on root nodules of *Leucaena leucocephala* with particular reference to the infection process*. Pak. J. Bot*. 24: 142–152.

**Johansson J.F., L.R. Paul and R.D. Finlay.** 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* 48: 1–13.

**Lawrie A.C.** 1983. Infection and nodule development in *Aotus ericoides* (Vent.) G. Don., a woody native Australian legumes. *J. Exp. Bot.* 34: 1168–1180.

**Mahmood A.** 1999. A comparison of nitrogen concentration between wild and cultivated legumes of Sindh. *Pak. J. Bot.* 31: 183–192.

**Mahmood A. and S. Jamal**. 1977. A contribution to the histology of root nodules of *Sesbania sesban* L. *Pak. J. Bot.* 9: 39–46.

**Narayana H.S.** 1963. A contribution to the structure of root nodules in *Cyamopsis tetragonoloba* Taub. *J. Ind. Bot. Soc.* 42: 273–279.

**Naz S. and A. Mahmood.** 1976. Histology of the root nodules of *Melilotus albus* and *Trifolium alexandrinum. Pak. J. Bot.* 8: 95–101.

**Newcomb W.** 1976. A correlated light and electron microscopic study of symbiotic growth and differentiation in *Pisum sativum* root nodules. *Can. J. Bot.* 54: 2163–2186.

**Patreze C.M. and L. Cordeiro.** 2004. Nitrogen-fixing and vesicular-arbuscular mycorrhizal symbioses in some tropical legume trees of tribe Mimoseae. *Forest Ecol. Manage.* 196: 275–285.

**Qadri R. and A. Mahmood**. 2002. Occurrence of persistent infection threads in the root nodules of *Dalbergia sissoo* Roxb. *Pak. J. Bot.* 34: 397–403.

**Qadri R. and A. Mahmood.** 2003. Presence of rhizobia in the xylary elements of root nodules in *Samanea saman* (Jacq.) Merr. *Pak. J. Bot.* 35: 819–823.

**Qadri R. and A. Mahmood.** 2004. Structural study of the root nodules of *Dalbergia sissoo* Roxb. *Int. J. Biol. Biotech.* 1: 535–538.

**Qadri R. and A. Mahmood.** 2005. Ultrastructural studies on the root nodules of *Albizia lebbeck* (Roxb.) Benth. *Pak. J. Bot.* 37: 815–821.

**Rabie G.H. and A.M. Alamdini. 2005**. Role of bioinoculants in development of salt tolerance *Vicia faba* plants under salinity stress. *Afr. J. Biotech.* 41: 210–222.

**Trinick M.J.** 1979. Structure of nitrogen-fixing nodules formed by *Rhizobium* on roots of *Parasponia andersonii* Planch. *Can. J. Microbiol.* 25: 565–578.

**Vessey J.K., K. Pawlowski and B. Bergman.** 2004. Root-based $N_2$-fixing symbiosis: legumes, actinorhizal plants, *Parasponia* sp. and Cycads. *Plant Soil* 266: 205–230.

**Young C.C.** 1990. Effects of phosphorus solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the growth of tree species in subtropical soils. *Soil Sci. Plant Nutr.* 36: 225–231.