Pak. J. Bot., 40(2): 755-758, 2008.

EVIDENCE OF ENTRY OF RHIZOBIA INTO EPIDERMAL CELLS OF ROOTS OF *ALBIZIA LEBBECK* (L.) BENTH. (LEGUMINOSAE - MIMOSOIDEAE) THROUGH PITS

RAIHA QADRI[*], A. MAHMOOD[*] AND MOHAMMAD ATHAR[**]

[*]*Department of Botany, University of Karachi, Karachi-75270, Pakistan*
[**]*California Department of Food and Agriculture, 1220 N Street, Sacromena, CA 94814, USA.*

Root nodule initiation in leguminous plants is intimately related to the entry of rhizobia from rhizophere into cortical cells of the root, which then become, meristematic (Brewin ,2004). Three modes of entry of rhizobia into roots have been described. The first and most widely studied method is *via* root hairs, with the formation of a definite tube- like structure (the infection thread) which enclose the bacteria as they pass through cells towards the adjacent root cortex (Dart, 1977; Newcomb *et al*., 1981; Brewin, 2004). Second method is where bacteria enter through wounds at the place of lateral roots emergence (Chandler, 1978; Chandler *et al*., 1982; Mathesius *et al*., 2000; Uheda *et al*., 2001), without the formation of infection threads. In a third method where rhizobia colonize the surface of the primary root, penetrate through mucilage and primary layers of radial walls of epidermal cells. Further penetration may take place through primary wall layers and intercellular air spaces with occasional intracellular penetration of epidermal and cortical cells, always occurring from a boundary between the two neighbouring cells (Faria *et al*., 1988). In the fourth method rhizobia induce root hair deformations and penetrate root hair cell wall, where instead of forming infection thread, bacteria colonize the space between the cell wall and plasma membrane. There are no intracellular or transcellular infection threads and bacteria are released directly from infection droplets into the cytoplasm of cells in the root cortex, which then become meristematic (Lotocka *et al*., 2000).

Hilaire *et al*., (2001) while studying vascular defence responses in rice have shown beautiful scanning and transmission electronmicrographs of the rice bacterial blight pathogen *Xanthomonas oryzae* pv *oryzae* passing through the pits on the xylem vessels into adjacent parenchyma cells. Inspired by these pictures we scanned the external surface of the epidermal cells of the roots of *A. lebbeck* plants. *A. lebbeck* is a multipurpose woody legume that forms symbiotic relationship with *Rhizobium* and fix atmospheric nitrogen that is used for its growth and for the enrichment of the rhizophere (Qadri & Mahmood ,2005).

For scanning electron microscopy small pieces of roots (1-2mm) were cut longitudinally and fixed in 2% gluteraldehyde in 0.1M phosphate buffer (pH 7) for 4h and then transferred in 1% aqueous osmium tetroxide for 2-4 h at room temperature. Root segments were dehydrated in 100% ethanol followed by an ethanol: acetone series 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.

Fig. 1 shows pits on the epidermis of roots of *A. lebbeck* . Rhizobia are seen adhering the root surface. Both regular and pleomorphic forms are visible. Some of them can be seen inside the pits while a few are entering into the pits. In this method root hairs, wounds or direct entry through mucigel and primary walls are not involved. The bacteria can directly enter from the rhizosphere into the parenchyma cells of the root cortex. The entry of rhizobia into the epidermal cells of the root through pits does not appear to have been reported before.

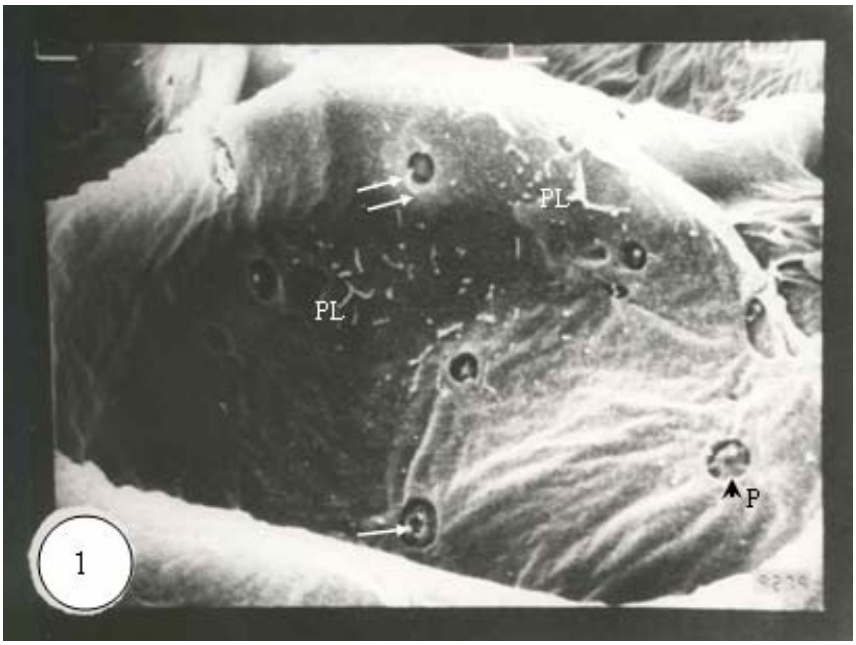

Fig. 1. Scanning electron micrograph of external surface of epidermal cell of root of *A. lebbeck* Note the movement of bacteria into the root through pits (P) (arrow head). Some of them can be seen inside the pits (↑) while a few are entering into the pits (↑↑) Both the normal and pleomorphic forms (PL) of rhizobia are visible × 3750.

On critical examination of the known mechanisms of rhizobial entry into host tissue two features appear to be common. Firstly the host cells undergo specific modifications at the site of bacterial entry to facilitate infection. This is formation of an infection thread during root hair infections as observed in majority of legumes (Dart, 1977; Brewin, 2004), presence of large basal cells at the base of root hairs in *Arachis hypogea* L. (Chandler, 1978), direct invasion of rhizobia through space between epidermal cells (wound infection) coupled with early collapse of invaded cells in *Stylosanthes* species (Chandler *et al*., 1982) and entry of rhizobia between the epidermal cells of root in *Mimosa scabrella* Benth. (Faria *et al*., 1988). Secondly during the passage of bacteria from epidermal or cortical cells of the root , they often penetrate a rigid cell wall made up of cellulose fibrils that is normally completely impermeable to microorganisms. Bacterial penetration involves remodeling and restructuring of cell wall (Nutman, 1956; Napoli & Hubbell, 1975; Vessey *et al*., 2004; Brewin, 2004). Bacteria were never seen to enter the secondary layer (Foster, 1986; Faria *et al*., 1988). It may be stated that in the case of pits, secondary walls are completely absent from pit field area (Esau, 1965). Thus bacteria entering through pits will have to cross the pit membrane in order to land in the neighboring cell. Structural alterations of the primary wall at these sites may occur similar to other modes of infection described earlier in the text.

Acknowledgements

The first author expresses her gratitude to the British Council for providing technical assistance under the ODA linkage programme to work at the University of Reading, England. We are thankful to Dr. J. Barnett for providing research facilities at the School of Plant Sciences , University of Reading ,England.

References

Brewin, N.J. 2004. Plant cell wall remodeling in the *Rhizobium* - legume symbiosis. *Critical Reviews in Plant sciences*, 23(4): 293-316.

Chandler, M.R. 1978. Some observations on infection of *Arachis hypogaea* L., by *Rhizobium*. *J. Exper. Bot*., 29(110): 749-755.

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(132): 47-57.

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

De Faria, S.M., H.T. Hay and J.I. Sprent. 1988. Entry of rhizobia into roots of *Mimosa scabrella* Bentham., occurs between epidermal cells. *J. Gen. Microbiol*., 134: 2291-2296.

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

Esau, K. 1965. *Plant anatomy*. John Wiley & sons, Inc; New York, pp 1-767.

Foster, R.C. 1986. The ultrastructure of the rhizoplane and rhizosphere. *Annual Review of phytopathology*, 24: 211- 234.

Hilaire, E., S.A. Young, L.H. Willard, J.D. McGee, T. Sweet, J.M. Chittoor, J.A. Guuikema and J.E. Leach. 2001. Vascular defense responses in rice: Peroxidase accumulation in xylem parenchyma cells and xylem wall thickening. *MPML*., 14(12): 1411-1419.

Lotocka, B., J. Kopcinska, M. Gorecka and W. Golinowski. 2000. Formation and abortion of root nodule primordia in *Lupinus luteus* L. *Acta Biologica cracoviensia series Botanica*, 42: 87-102.

Mathesius, U., J.J. Weinman, B.G. Rolfe and M.A. Djordjevic. 2000. Rhizobia can induce nodules in white clover by hijacking mature cortical cells activated during lateral root development. *Mol. Plant Microbe Interact*, 13: 170-182.

Napoli, C.A. and D.H. Hubbell. 1975. Ultrastructure of *Rhizobium*-induced infection threads in clover root hairs. *Appl. Microbiol*., 30: 1003-1009.

Newcomb, W., S. Creighton and L. Latta. 1981. A reinvestigation of the origin of the peribacteroid membrane in root nodules of *Vicia faba*. *Can. J. Bot*., 59: 1547-1552.

Nutman, P.S. 1956. The influence of the legume in root- nodule symbiosis.A comparative study of host determinants and functions. *Biol. Rev*., 31: 109-151.

Qadri, R. and A. Mahmood. 2005. Ultra-structural studies on root nodules of *Albizzia lebbeck* (L.) Benth. *Pak .J. Bot*., 37(4): 815-821.

Uheda, E., H. Daimon and F. Yoshizako. 2001. Colonization and invasion of peanut (*Arachis hypogea* L.) roots by gus A- marked *Bradyrhizobium* sp. *Can. J. Bot*., 79: 733-738.

Vessey, J.K., K. Pawlowski and B. Bergman. 2004. Root-based N2–fixing symbiosis: Legumes, actinorhizal plants, *Parasponia sp.* and Cycads. *Plant and Soil*, 266: 205-230.

(Received for publication 10 April 2007)