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PRESENCE OF RHIZOBIA IN THE XYLARY ELEMENTS OF ROOT NODULES IN *SAMANEA SAMAN* (JACQ.) MERR.

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Abstract

The paper reports the morphogenesis and anatomy of root nodules. The nodules were distributed singly as well as in clusters on the main and lateral roots of *Samanea saman* (Jacq.) Merr. Young nodules were globose whereas mature nodules were elongated, branched and coralloid. Rhizobia entered the root via root hair and formed infection thread. An interesting character was observed in the young nodules of *S. saman* where bacteria entered through the root hair and multiplied within the epidermal cell. From there they invaded into the cortical region by intracellular movement by dissolving middle lamella. Presence of bacteria inside, and their movement through xylary elements is being reported for the first time in a tree legume.

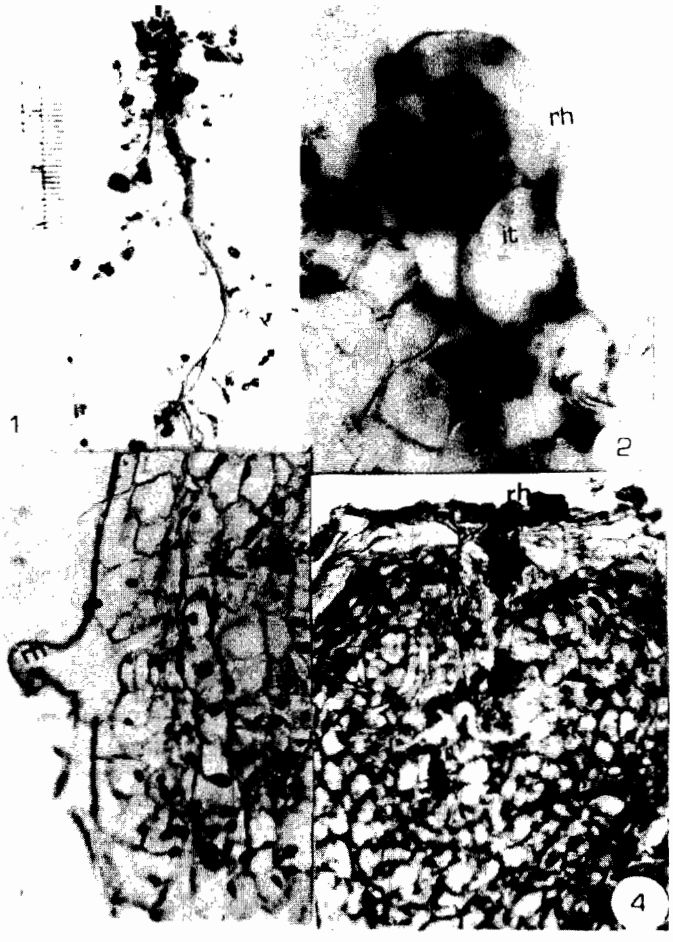
Introduction

Leguminosae is the third largest family of flowering plants (Allen and Allen, 1981). In Pakistan legumes form a considerable portion of the flora where Leguminosae ranks as the third largest family in the order of abundance (Ali & Qaiser, 1986). Nodulation in herbaceous and woody legumes of Pakistan in their natural habitat has been studied by Athar & Mahmood (1978, 1980, 1985, 1990, Mahmood & Athar (1985), Athar (1993, 1996) and Mahmood & Iqbal (1994). It is interesting to note that the bulk of the nodulated trees are distributed in Mimosoideae. The process of nodule formation is intimately related with the infection of roots by appropriate rhizobia; the infection may be through root hairs, wounds (cracks) or through intact epidermis (Sprent *et al.*, 1989). In majority of legumes, rhizobia enter the roots via root hairs (Callaham & Torrey, 1981; Chandler *et al.*, 1982; Baird *et al.*, 1985; Iqbal & Mahmood, 1992; Sprent & Raven, 1992; Qadri & Mahmood, 2002). We report here for the first time the presence and movement of rhizobia in the xylary elements of a tree legume *Samanea saman*.

Materials and Methods

Nodules of *S. saman* were collected from plants growing at the Karachi University. For Light Microscopy, the nodules and roots were fixed in F.A.A. for 18 h pieces of nodules (1-2 mm) 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 h. Serial sections (0.5-2.0 µm) were cut with a glass knife in 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 (Qadri & Mahmood 2002).

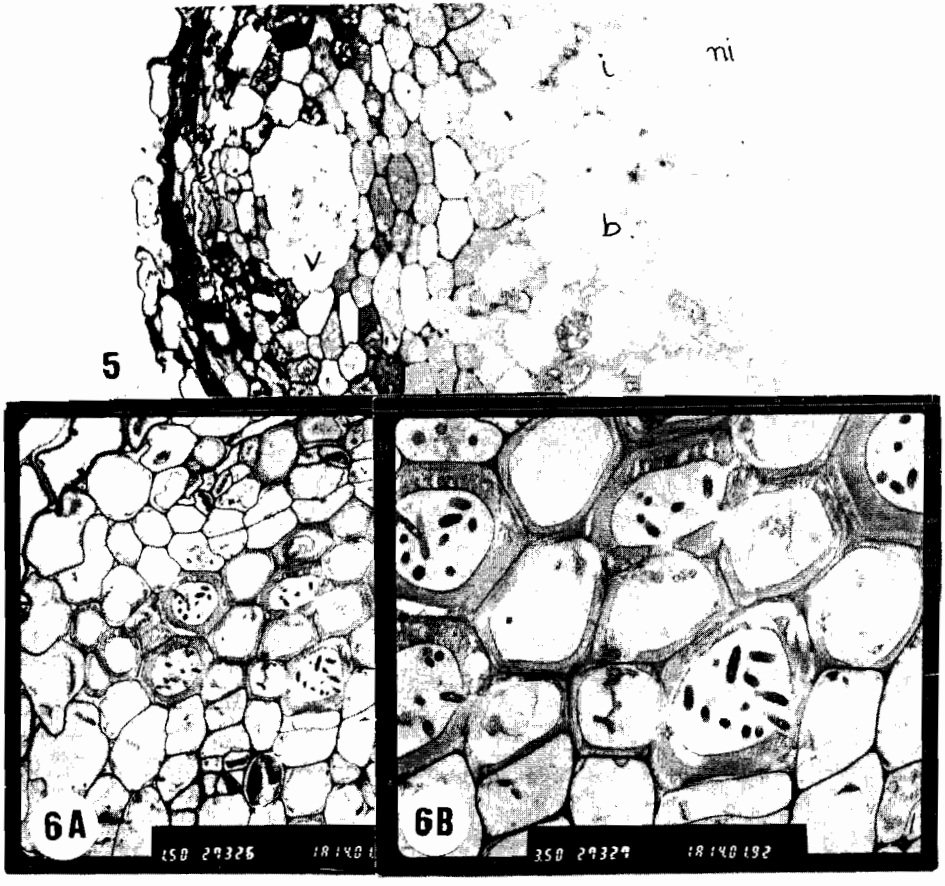
For Transmission Electron Microscopy, small pieces of nodules (1-2 mm) were fixed in 2 % gluteraldehyde in 0.1 M phosphate buffer (pH 7) for 4h. The tissues were then treated within 1 % aqueous osmium tetroxide for 2-4 h at room temperature (Faria *et al.*, 1986). The fixed material was processed for Transmission Electron Microscopy as described by Qadri & Mahmood (2002).



Figs. 1-4. Nodules in *Samanea saman* roots: 1. Distribution of nodules on main and lateral roots. 2. Infection taking place through curled root hair (rh), infection thread (it) present in root hair (x 4034). 3. L.S. of root showing curled root hair (rh) with nodule primordium (x 400). 4. Young nodules showing intercellular movement of bacteria. e = epidermis, rh = root hair, b = bacteria (x 1520).

Results and Discussion

In *S. saman* nodules were distributed singly as well as in clusters on the main and lateral roots. Young nodules were globose, whereas mature nodules were elongated, branched and coralloid (Fig. 1). The bacteria entered the root via root hair and formed infection thread (Figs. 2 & 3). An interesting character was observed in the young nodules of *S. saman* where bacteria entered through the root hair and multiplied within the epidermal cell from where they spread into the cortical region through intracellular movement by dissolving middle lamella (Fig. 4). Bacteria were also observed in the xylary elements in *S. saman* (Fig. 6). Nodules could be differentiated into nodule meristems, cortex, vascular tissues and the bacteroid region. This region showed both



Figs. 5-6. Nodules in *Samanea saman* roots: 5. T.S. of root nodules showing vascular bundle (v) and bacteroid region (b) with infected (i) and non-infected cells (ni) x 98. 6a. Portion of vascular bundle showing bacteria in xylary elements (x 1800). 6b. Bacteria present in the xylary elements (showing at a high magnification) x 3845.

infected and non-infected cells mixed together (Fig. 5). Vascular bundles were amphicribal and distributed around the bacterioid region. Bacteria were also observed in the vascular region of the nodule (Fig. 6).

Nodule formation is initiated by the entry of rhizobia into the root tissue. Rhizobia enter the root via root hairs in majority of legumes (Mahmood & Jamal, 1977; Chandler, 1978; Newcomb *et al.*, 1979; Callaham & Torrey, 1981; Chandler *et al.*, 1982; Baird *et al.*, 1985; Iqbal & Mahmood, 1992; Sprent & Raven, 1992) and same was found during the present study. However, the curling of root hairs seems to play an important role to facilitate the entry of rhizobia into the root cortex of host plant (Nutman, 1956; 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 *Glycine max* and *Glycine soja* many infection threads were initiated at inner crook of short '?' shaped root hair (Pueppke, 1983). Similar observations were recorded in *S. saman* (Figs. 2 & 3). Accumulation of

Rhizobium in the intercellular spaces of cortical tissue of nodule was also observed. After infection, the thread reaches the root cortex, ramifies and travels both inter- and intracellularly. Iqbal & Mahmood (1992) have also found inter- and intracellular infection threads in *Leucaena*. Once the nodule has been formed it establishes connection with the vascular system of main root (Bond, 1948).

The vascular tissues of the nodule surrounded the central bacteroid region without coming in direct contact with it. Such observations have also been recorded by others (Wester & Hogberg, 1989; Sprent & Sprent, 1990; Iqbal & Mahmood, 1992). Amphicribal vascular bundles as found in *S. saman* have also been reported in *Phaseolus vulgaris* (Baird & Webster, 1982); *Sesbania sesban* (Mahmood & Jamal, 1977), *Leucaena leucocephala* (Iqbal & Mahmood, 1992). In *S. saman*, bacteria were also observed in the vascular region of nodules (Fig. 6). Presence and movement of diazotrophic bacteria inside the xylem elements of cereals and grasses is well documented. Schloter *et al.* (1993) reported that *A. brasilense* colonized the root xylem of wheat. Boddey (1995) has shown the presence of *Acetobacter diazotrophicus* within the xylem vessels of stem of sugarcane plantlets. Moreover protoxylem and metaxylem vessels of sugarcane leaves completely blocked with *Hebaspirillum rubrisubalbicans*. Boddey (1995) and Webster *et al.* (1996) have shown the presence of *Azorhizobium caulinodans* in the xylem vessels of *Sesbania rostrata* as well as in the wheat. Presence of rhizobia and their movement in xylary elements of *S. saman* is being reported here for the first time.

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