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OCCURRENCE OF PERSISTENT INFECTION THREADS IN THE ROOT NODULES OF *DALBERGIA SISSOO* ROXB.

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Abstract

Root nodules of *Dalbergia sissoo* Roxb., were globose and present in the axils of lateral roots. Histological studies showed that rhizobia entered the root *via* root hairs and formed infection threads some of which were branched.The nodule cortex showed a sclereid layer at its periphery. The bacteroid region showed infected and non infected (interstitial) cells intermingled with each other. Persistent infection threads were observed which are being reported for the first time.

Introduction

The Pakistani native flora contains a large number of leguminous trees growing along with their herbaceous relatives. A significant number of genera and species within both the subfamilies Papilionoideae and Mimosoideae are known to nodulate (Mahmood & Iqbal, 1994). The symbiotic association between indigenous legumes and their root nodule bacteria play an important role in the overall nitrogen increment of Pakistani soils (Mahmood, 1999). *Dalbergia sissoo* commonly forms a symbiotic relationship with native rhizobia (Javid & Fisher,1989). The process of nodule formation is intimately related with the infection of roots by appropriate rhizobia. In considering infection by rhizobia there are three alternatives: hairs, wounds (cracks) and entry through intact epidermis (Sprent *et al.*, 1989). Crack entry of *Rhizobium* into root tissue has been reported in *Dalbergia spp.*, (Sprent *et al.*, 1989; Sprent & Sprent, 1990; Sprent & Raven, 1992; Sutherland & Sprent, 1993). Faria *et al.*, (1987b) have surveyed the occurrence of persistent infection threads in the root nodule tissue of leguminous plants. Persistent infection threads were observed in several tree species belonging to the subfamily Caesalpinoideae and some species of Papilionoideae but none in the members of Mimosoideae (Quispel *et al.*, 1993). Although, persistent infection threads were present in some members of the tribe Dalbergieae but infection threads were never formed in *Dalbergia* (Sprent & Sprent, 1990; Sprent & Raven, 1992). In the present study infection process and structure of nodules in *D. sissoo* is being reported. There does not appear to be any previous report of the occurrence of persistent infection threads in the root nodules of *D. sissoo.*

Material and Methods

Nodules of *D. sissoo* were collected from plants growing at the Karachi University campus. Nodules and roots were washed in running tap water to remove adhering soil particles and prepared for structural and microbiological studies. Small lateral roots with nodules of different ages were prepared for structural studies following the histochemical methods described by Jensen (1962). The material was dehydrated with a tertiary butyl

alcohol (T.B.A.) series and embedded in paraffin wax (M.P. 56-58°C). Serial transverse and longitudinal sections (7-10 μm thick) were cut on a rotary microtome. The ribbons of sections were floated in 4% formaldehyde solution on glass slides coated with Haupt's adhesive and dried overnight at 40°C. Dried sections were passed through xylol-alcohol series for dewaxing and stained with Safranin-Harri's hematoxylin (Johansen, 1940) and toluidine blue (Faria *et al.*, 1986). Safranin-Harri's hematoxylin method was found most satisfactory as it stained vascular system, infection thread and infected tissues with equal clarity. Sections were stained in safranin for 18 h, washed with water and passed for 30 seconds through 50% alcohol slightly acidified with HCl and then washed with water. The sections were then stained with hematoxylin solution followed by washing with acidified water. The sections were dehydrated in absolute alcohol, then placed in 50% xylol-alcohol and finally in xylol (1 min., each), and mounted in Canada balsam (Johansen, 1940).

Preparation of material for light microscopy

After harvesting, the nodules and roots were fixed in F.A.A. for 18 h. Fixed pieces of 1-2 mm nodules were dehydrated in ethanol series and infiltrated with L.R. white resin at room temperature (two resin changes), and polymerised at 60°C for 24 hours (Faria *et al.*, 1986). Serial sections 0.5-2 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. The sections were examinated under Zeiss microscope.

Preparation of material for Scanning Electron Microscopy (SEM)

Complete nodules and free hand sections were fixed in 2% gluteraldehyde in 0.1M phosphate buffer (pH 7) for 4h. The tissues were then treated with in 1% aqueous osmium tetroxide for 2-4 h at room temperature. The fixed material was dehydrated in 100% ethanol followed by an ethanol:acetone series to 100% acetone. The specimens were then dried using a Bio-Rad Polaron critical point drier, coated with gold in a JFC-1100 coating unit and examined with Jeol T-20 scanning electron microscope (Faria *et al.*, 1986).

Preparation of material for Transmission Electron Microscopy (TEM)

Small pieces of nodules (1-2 mm) were fixed as for SEM, dehydrated through ethanol-acetone series to absolute acetone, then passed through acetone:propylene oxide 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 then with lead citrate for 5 min., (Callaham & Torry 1981). Specimens were examined in a Hitachi H-800 TEM at 75-150 KV.

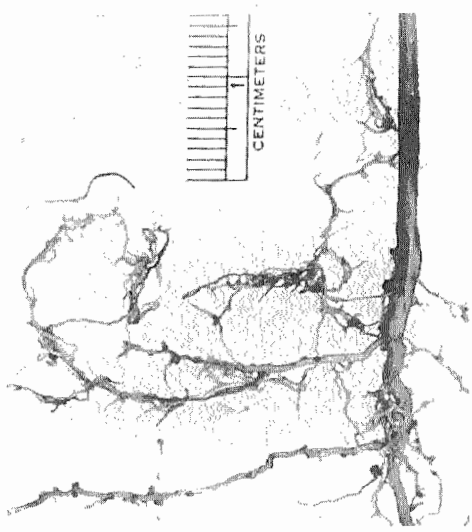

Fig.1. Distribution of nodules in the axil lateral roots.

Results and Discussion

Young as well as mature nodules of *D. sissoo* were globose and present in the axil of lateral roots (Fig. 1). Bacteria entered the root *via* root hairs and formed the infection threads (Fig. 2). General structure of nodules showed a cortex containing vascular bundles surrounding the infected tissues. There was a continuous layer of sclereids present in the periphery of the nodule cortex (Fig. 3). The bacteroid region consisted of infected and non infected (interstitial) cells intermingled with each other. Branched persistent infection threads were also found in the bacteroid region of nodules (Figs. 4 & 5). Faria *et al.*, (1984) observed similar type of nodules in *D. fructescens* and *D. glaucescens*. Aeschynomenoid type of globose-oblate nodules associated with lateral root have been reported in the tribe Dalbergieae (Chandler, 1978; Sprent *et al.*, 1989; Sprent & Raven, 1992; Sutherland & Sprent, 1993). However, Desmodioid nodules have been reported in Dalbergieae by Corby (1981). Interstitital cells were absent in *Dalbergia* but present in other members of the tribe Dalbergieae (Dart, 1977; Sprent & Sprent, 1990). Faria *et al.*, (1987b) have surveyed the occurence of persistent infection threads in the root nodule tissue of leguminous plants. 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). This feature had previously been observed only in *Parasponia spp.*, (Ulmaceae), in symbiosis with *Rhizobium* (Trinick & Galbraith, 1976; Trinick, 1979; Lancelle & Torry, 1984; Price *et. al.*, 1984). The tribe Dalbergieae and Millettieae (formerly Tephrosieae) both contain genera in which nodules have persistent infection threads (Faria *et al.*, 1987; Sprent *et al.*, 1987; Sprent & Raven, 1992). Although persistent infection threads have been reported in some members of the tribe Dalbergieae but not in *Dalbergia* (Sprent & Sprent, 1990; Sutherland & Sprent, 1993). Dart (1977) did not observe infection threads in the nodule of *D. sissoo*. In the present study, the occurrence of persistent infection threads in the bacteroid zone of *D. sissoo* are being reported for the first time.

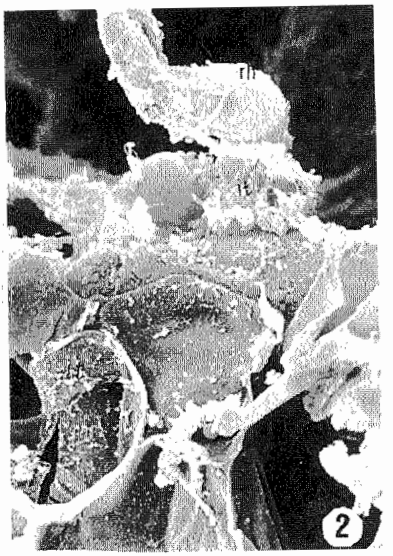

Fig. 2. Portion of root showing curled root hair (rh) with infection thread (it) and bacteria. ×8759 (S.E.M. Photograph).

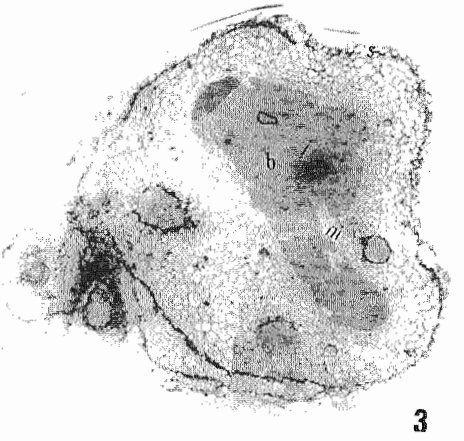

Fig.3. L.S. of lobed root nodule of *D. sissoo* with root showing lobed, central bacteroid region (b) with loosely arranged infected cells (i) intermingled with interstitial cells (ni) and a layer of sclereids in the cortex (s). ×80.

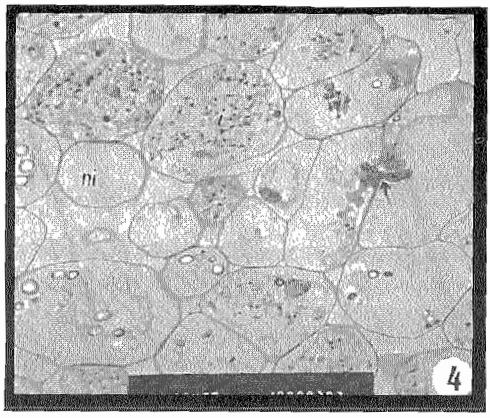

Fig. 4. Bacteroid region of nodule in *D. sissoo* showing infected cell (i) and interstitial (ni) and penetration of persistent infection thread through cell wall (→). ×1772 (Electron micrograph).

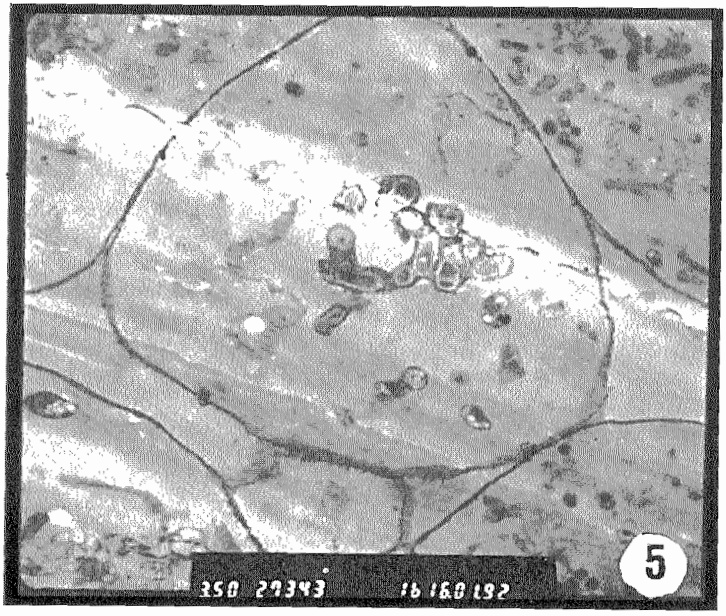

Fig. 5. Infected cells of root nodule in *D. sissoo* with branched persistent infection thread. x 1611 (Electron micrograph).

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