

HISTOLOGY OF THE ROOT NODULES OF *MELILOTUS ALBUS* AND *TRIFOLIUM ALEXANDRINUM*

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Abstract

The Rhizobia entered the root of *Melilotus albus* and *Trifolium alexandrinum* through the root hairs and formed infection threads. The infected root hairs were curled. The infection threads could be seen in the cortex of the root as well as in the bacteroid region of the nodules.

Origin of nodules was exogenous in both the plants. The mature nodule consisted of four regions, (1) the nodule meristem (2) the bacteroid region. The bacteroid region could be separated into 3 types of cells. (3) The vascular region and (4) The nodule cortex.

The vascular supply of the nodule consisted of two strands. The vascular bundles surrounding the central bacteroid region were amphicribal at the base and inversely collateral in the centre of the nodule.

Degeneration of the nodule progressed from the base upwards. It was characterized by the disintegration of root cell nuclei and their reducing affinities for staining.

Introduction

The controversy regarding the site of origin of the nodule on the parent root goes back into 19th century, when two opposite views were propounded. Wittmann (1968), has reviewed the literature on this subject and according to him Van Tiegham & Dulliot (1888), held that nodules initiated from the pericycle of the host root, whereas Prazmowski (1890) held the idea that nodules initiated from the cortex of the root. It is now known that both types of nodules exist in Angiosperms. Endogenous origin is more common in non-legumes whereas exogenous type is common in legumes. However, some legumes also show endogenous origin. Even contrasting histological sites for nodule initiation have been reported for the same species. The site of origin of nodule in *Phaseolus vulgaris* (McCoy, 1929), *Medicago sativa* (Thornton, 1930) and *Pisum sativum* (Bond, 1948; Torrey & Silder, 1969; Libbenga & Harkes, 1973) has been reported to be exogenous whereas Wittmann (1968) has described the origin of the nodules in these species and in *Trifolium pratense*, as endogenous. Histological studies on the structure of root nodules of two leguminous species namely *Melilotus albus* and *Trifolium alexandrinum* were therefor carried out. The anatomical criteria for differentiating between endogenous and exogenous nodules are discussed.

Materials and Methods

Trifolium alexandrinum L. and *Melilotus albus* Desr. were grown in the experimental field of Botany department University of Karachi. The nodules collected from these plants were washed in running tap water to remove the adhering soil particles and then rinsed with distilled water. The nodules were then fixed for 24 hours in

F.A.A. (formalin, acetic acid and alcohol or in Navashin's fluid). Infiltration was carried out in paraffin wax (M.P. 56-58°C). Serial median longitudinal sections were cut on a rotary microtome at 15-20 microns.

The staining combinations used were, safranin-light green, safranin—Harris Hematoxylin and Heidenhain's iron hematoxylin. The second combination was found most satisfactory, because it stained cell walls, nuclei and Rhizobia with equal clarity.

Observations

Mode of infection:

Rhizobia entered the root tissue through the root hairs by forming infection threads. Fig. 1 shows an infection thread in *Melilotus albus*. The infected root hairs underwent curling (Fig. 2). The infection threads could also be seen in the bacteroid region of the nodule (Fig. 3). Infection threads developed swellings adjacent to the cell wall of host cells and showed a marked affinity towards the nuclei of the host cells. In the early stages of infection they were found growing towards the nuclei of the host cells and in the later stages of infection either adhering to or encircling the nucleus.

Origin of the nodule:

The nodules originated from the cortical parenchyma of the root. The pericycle of the host plant was not involved in the nodule genesis, as it was found intact inside the endodermis, in front of the stele of the parent root, even after the emergence of the nodule (Figs. 4 & 5).

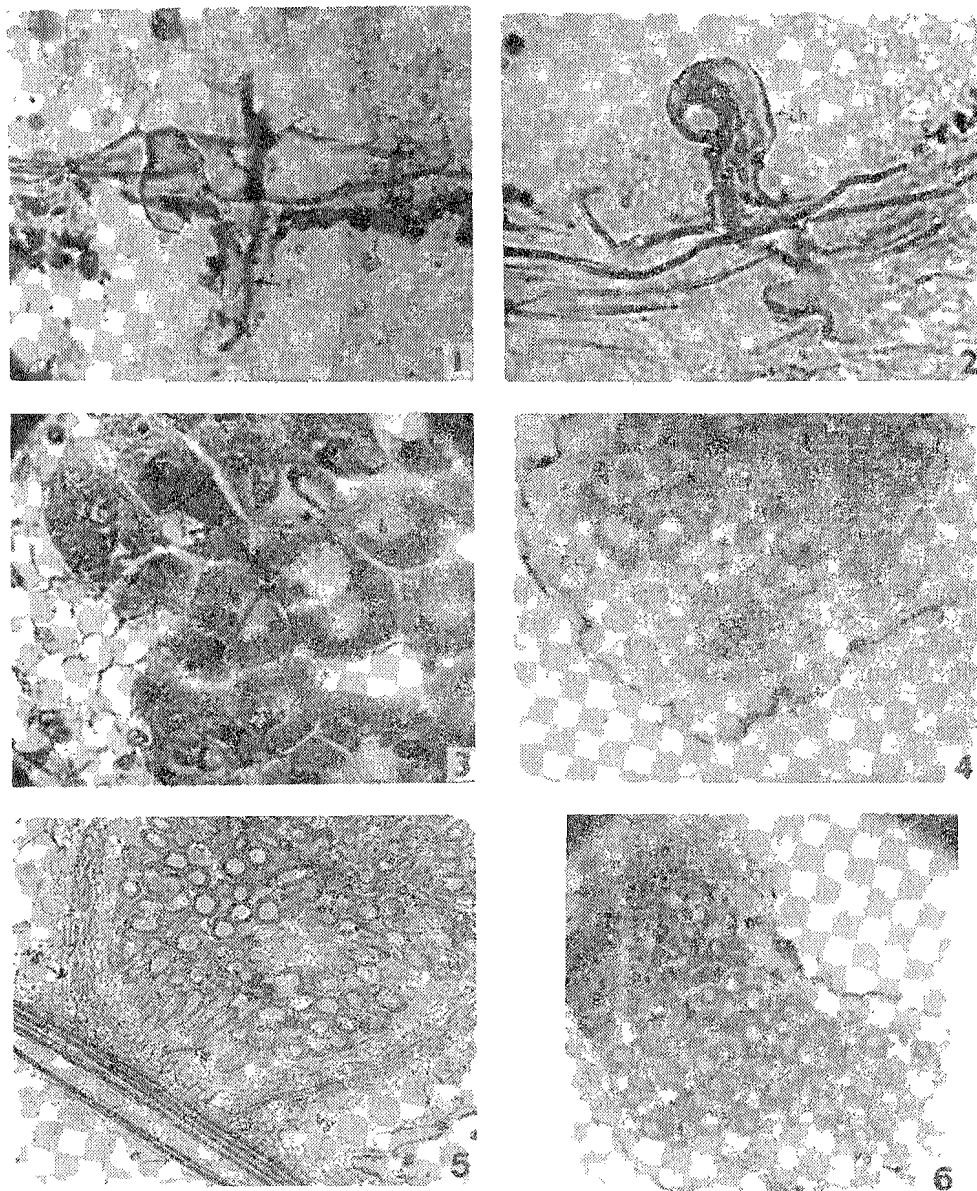
Histological zones of the fully developed nodule:

Median longitudinal sections of mature nodules of *Melilotus albus* and *Trifolium alexandrinum* showed the following four regions (Fig. 6) which are typically found in leguminous root nodules: —

- (1) Meristematic region, at the distal end of the nodule.
- (2) The bacteroid region occupying the centre of the nodule,
- (3) The vascular region enclosing the bacteroid region and
- (4) The nodule cortex.

Meristematic region consisted of multiple layers of thin walled, densely stained, actively dividing cells, with prominent nuclei. They were devoid of bacteria. The derivatives of these meristematic cells after their enlargement were invaded by Rhizobia.

The bacteroid region occupied the central part of the nodule. This region could be separated into three types of cells (Fig. 7) (a) cells of the bacteroid region just below the meristem, were small with somewhat prominent nuclei. (b) Below these cells was the region of non-vacuolated cells which were densely invaded by bacteria. These cells were large in size. Their nuclei were prominent and many chromatin



- Fig. 1. A Portion of the L.S. of root of *Melilotus albus*, showing an infected root hair with infection thread. X 1600.
- Fig. 2. A portion of the L.S. of the root of *Tritolium alexandrinum*, showing curled infected root hair. X 1600.
- Fig. 3. A part of T.S. of nodule of *Melilotus albus*. The infection thread is shown in bacteroid cells. X 1600.
- Fig. 4. T.S. of the mature nodule of *Melilotus albus* showing endodermis around the stele of the parent root. X 16000.
- Fig. 5. L.S. of the mature nodule of *Tritolium alexandrinum*, showing the endodermis between the two vascular strands of the nodule. X 320.
- Fig. 6. T.S. of mature nodule of *Melilotus albus* showing four regions of the nodules. X 700.

bodies could be seen in their nuclei. (c) The third type of cells were the older bacteroid cells. They were larger in size. These cells had a large central vacuole, with a deformed nucleus pressed to one side of the cell. The Rhizobia were located in the peripheral region of the cells. The cells of this region showed less affinity towards stain than the younger bacteroid cells. Differentiation of vascular tissue of the nodule started at the base of the nodule within the parenchymatous layer between the infected zone and the parent root stele. Soon after this, vascular strands were initiated from one or two protoxylem points of the parent root stele (Fig. 8). These strands progressed acropetally joining with the conducting tissues of the nodule and thereby provided vascular linkages between the nodule and the parent root (Fig. 9). There were two vascular strands found in these plants.

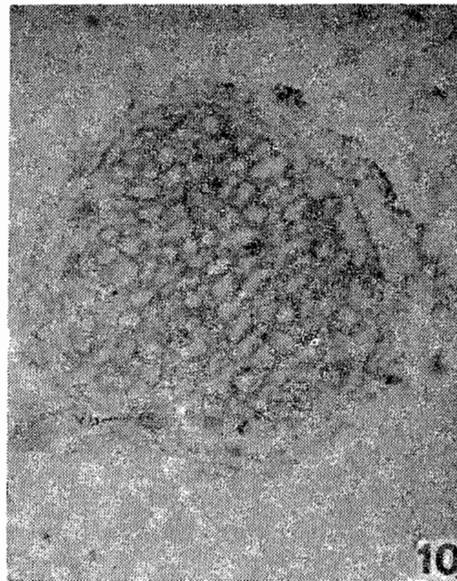
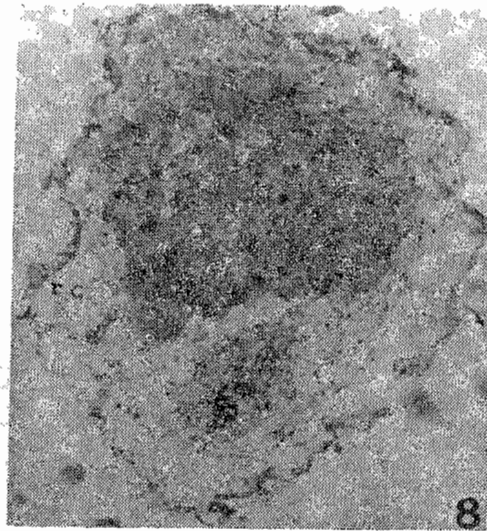
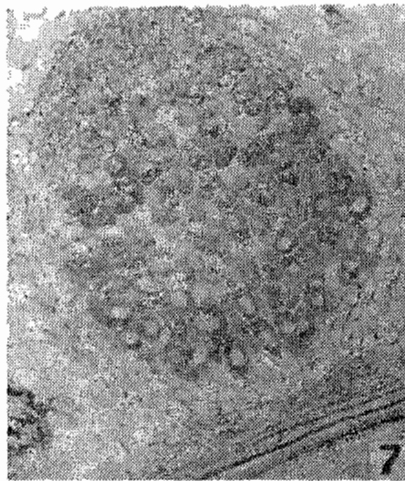
The vascular bundles were present around the bacteroid region (Fig. 10) except in the apical region of the nodules, where a well defined meristem persisted. The vascular bundles never came in direct contact with the bacteroid tissue owing to the interposition of 2-3 layers of non-infected parenchymatous cells, between the bacteroid region and vascular tissues. The vascular bundles were amphicribal at the base and inversely collateral towards the centre of the nodules. Each vascular bundle was encased within an endodermal sheath characterized by casparian strips.

The nodule cortex consisted of 2-4 layers of uninfected thin-walled parenchymatous cells which showed thickenings on their walls in the advance stages of degeneration of the nodule (Fig. 6). The degeneration of nodule was gradual from the base upwards. It was characterized by the disintegration of the nuclei of host cells and their reducing affinity for staining. (Fig. 9).

Discussion

Nodules were found to be exogenous in both the plants. This conclusion is mainly based on three observations. (1) The endodermis and pericycle of parent root were encircling the vascular elements, even after the emergence of the nodule (Fig. 4). This is a strong evidence to suggest that pericycle was not involved in the genesis of nodule in these plants. If the nodules were endogenous in origin then the nodular tissue would have been enclosed within the stretched endodermis of the parent root. (2) Bond (1948), studying the anatomy of Pea nodules, had observed that the endodermis of the parent root was intact in front of the stele of the parent root, between the two vascular connections, which the nodule established with the main root. Allen and Allen (1950) have regarded this observation of Bond as a strong evidence for the recognition of exogenous origin. It can be seen from Fig. 5, that the endodermis of the parent root is intact in front of the stele of the parent root, between the two vascular connections which the nodule established with the main root. (3) Allen & Allen (1940) studying the nodules of Peanut have discussed that nodules of exogenous origin differ from those of endogenous origin in the sense that they always remain enclosed within a layer of parent root cortex. In the present study the root cortex can be seen extending around the nodules (Figs. 4 & 8).

The central part of the nodule comprising the bacteroid region shows variation in the composition of cells in both the plants. In *Melilotus albus* the bacteroid area consisted of a dense mass of cells full of bacteria, a situation reported by Arora (1954) in *Aeschynomene indica* while in *Trifolium alexandrinum* cells of bacteroid region showed a large central vacuole as reported by Allen & Allen (1940) in *Arachis hypogaea* and by Arora (1956 a) in *Crotalaria juncea*.



- Fig. 7. I.S. of mature nodule of *Melilotus albus* showing three types of bacteroid cells, X 360.
- Fig. 8. T.S. of the nodule of *Melilotus albus* showing the vascular strand, (indicated by arrow) establishing connection with the protoxylem point of parent root stele, X 400.
- Fig. 9. T.S. of the mature nodule of *Trifolium alexandrinum*, showing the vascular strand connecting with the protoxylem point of parent root stele, X 320.
- Fig. 10. T.S. of the mature nodule of *Trifolium alexandrinum*, showing vascular bundles, surrounding the bacteroid region, X 190.

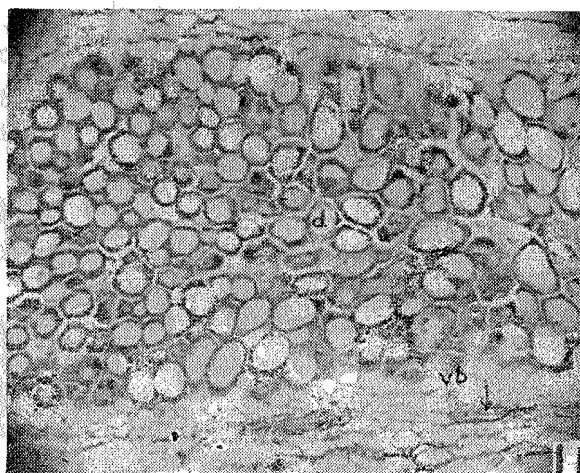


Fig. 11. A portion of the L.S. of older nodule of *Melilotus albus*, showing the degenerated cells. X 1408.

Abbreviations Used

b=	bacteroid tissues.	d=	degenerated cells.
nc=	nodule cortex.	e=	endodermis of parent root.
vb=	Vascular bundle of nodule.	cs=	casparian strips.
vs=	Vascular strand.	ih=	Infected root hair.
m =	nodule meristem.	i =	Infection thread.
t=	central cylinder of parent root.	rc=	root cortex.

The mode of differentiation of conducting elements in the early stage of nodule development, initiation of vascular strands in the parent root and acropetal extension of these strands to join with the conducting elements of the nodule, as reported in the present study, have also been reported in *Pisum sativum* (Bond, 1948), *Caragana arborescens* (Allen et al, 1955) and *Cicer arietinum* (Arora, 1956 c). However Bieberdorf (1938) observed that in soya bean, the provascular strands arose near the base of the nodule and progressed forward towards the apex and also backwards, towards the root stele.

Two vascular strands were found in these plants, however, number of vascular strands that supply the nodule show great variation in different legumes. There is one strand in *Glycine max* (Bieberdorf, 1938), *Sesbania grandiflora* (Harris et al, 1949), *Aeschynomene indica* (Arora, 1954) and *Cyamopsis tetragonoloba* (Narayana, 1963). Two vascular strands have been reported in *Vicia faba* (Bieberdorf, 1938), *Pisum*

sativum (Bond, 1948) and *Cajanus indicus* (Arora, 1956 b). Three to five vascular strands have been reported in *Cicer arietinum* (Arora, 1956 c).

The mode of degeneration of older nodules in both *Melilotus albus* and *Trifolium alexandrinum* was gradual from the base upwards. It was characterized by the disintegration of host cells nuclei and their reducing affinity for staining. This situation is similar to that reported by Harris et al (1949), Arora (1954), Allen et al (1955) and Narayana (1963).

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