Initiation, development and structure of root nodules in some members of the tribe Trifolieae (Papilionaceae)

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Abstract. Initiation and development of root nodules are studied in 7 species and the structure in 4 species, belonging to 3 genera of the tribe Trifolieae. The shape of the mature nodules may be spherical, cylindrical, fan-like or coral-like. The bacterial threads enter the root through the intact epidermis and cause proliferation in cortex by liberating the bacteria. The origin of nodules in the investigated taxa is exogenous and they belong to the "apical" type in Kodama's classification. A mature nodule comprises of meristematic zone, cortex with vascular bundles and the bacteroid zone. The bacteroid zone is heterogeneous and is composed of infected and uninfected cells.

Keywords. Trifolieae ; root nodule ; exogenous origin ; bacterial thread ; proliferation of cortex ; bacteroid zone.

1. Introduction

The root nodules have been a subject of investigation because of nitrogen fixing function. Though extensive studies have been made on physiology, cytology and histology of root nodules in the leguminosae in general, only a few investigators have paid attention to the nodule anatomy in the tribe Trifolieae (Peirce 1902; Thornton 1930; Nutman 1948; Dart and Mercer 1963, 1964; Jordan et al 1963; Mosse 1964; Munns 1968; Tu 1977). The present investigation is a supplement to the existing data based on the study of initiation and development of nodules in Medicago orbicularis All., M. scutellata Mill., M. truncatula Gaertn., Melilotus officinalis Pallas, M. wolfiga Poir., Trigonella corniculata L. and T. foenumgraecum L. and structure of nodules in Medicago sativa L., Melilotus alba Med. M. indica All. and Trigonella foenum-graecum L.

2. Materials and methods

Seeds of Trigonella corniculata were obtained locally from a seedsman whereas fully developed nodules of Melilotus indica were collected from the plants growing wild in the University Campus. The rest of the species were raised from the seeds obtained from Berlin in the University Botanical Garden. Low viability
of the seeds precluded the investigation of nodular structure of some species. The roots and rootlets of seedlings and root nodules of different stages of development were fixed in FAA (Johansen 1940) and stored in 70% alcohol after 48 hrs. Longitudinal and transverse sections of roots and nodules (5-8 μm) were stained with safranin O and fast-green FCF (Berlyn and Miksche 1976) and made permanent in a customary way.

3. Observations

3.1. Morphological description

The developing nodules are spherical, but the fully developed ones are commonly cylindrical and variously lobed often becoming fan-like or coral-like and rarely spherical. They occur on primary, secondary and tertiary roots. There is no variation in size except that the smallest nodules are in *Melilotus alba* and the largest in *Trigonella foenum-graecum* (figures 1–4).

3.2. Infection, initiation and development

The entry of bacterial threads (bt) into the root cortex through the intact epidermis (e) is observed only in *Melilotus officinalis*, *Medicago truncatula* and *Trigonella foenum-graecum* (figure 5). The cortical cells through which the threads pass are relatively larger than the remaining cells (figure 6, at arrows). The threads are often found in close proximity or in contact with the host cell nucleus (N) while passing through the cells (figures 5, 7). Further, they develop bulbous or funnel-shaped swellings (s) adjacent to the cell wall in *Medicago truncatula*, *Melilotus officinalis* and *M. wolga* (figures 7, 8). The threads rupture and liberate the bacteria into the middle of the cortex. The bacterial infected cells contain dense cytoplasm and distinct nucleus, referred here as "proliferation cortical initials" (PCI) (figure 9). By repeated divisions these initials produce a mass of cells, each with dense cytoplasm and a nucleus (figure 10). Gradually, the divisions become restricted to the distal end as a result of which the mass of cells attains spherical shape (figure 11). The developing spherical nodule is now distinguishable into 2–3 layers of peripheral tangentially elongated cells (PE) around the inner mass of cells (MC) (figure 12). It is at this stage the inner mass

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**Figures 1-10. 1-4. Mature root nodules. 1. Melilotus alba. 2. Medicago sativa. 3 and 4. Trigonella foenum-graecum. 5-10. T.S. of roots. 5. Melilotus officinalis showing the entry of bacterial thread into the root. 6. Medicago truncatula showing large cortical cells through which bacterial threads pass at arrows. 7. Melilotus wolga showing the contact of bacterial thread with host cell nucleus. 8. Melilotus officinalis showing the swelling of bacterial thread near cell wall, note the breaking of thread at arrow. 9. Melilotus wolga showing proliferation cortical initials. 10. Melilotus wolga showing mass of proliferated cells. 1-4 line indicates 1 mm; 5 × 260; 6 × 160; 7 × 650; 8 × 380; 9 × 430; 10 × 160.**

**Abbreviations:** BT, bacterial thread; N, host cell nucleus; E, epidermis; S, swelling; PCI, proliferation cortical initials.
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of cells transforms into the bacteroid zone (Bz) and the peripheral layers into nodule cortex (NC) with distinct apical meristematic zone (MZ) (figure 13). The ruptured threads even after liberating the bacteria remain in the developing nodule (figure 12 at arrow). The developing nodule now protrudes from the root with a protective covering of a few layers of the root cortical cells (figure 13).

3.3. Structure

A mature nodule consists of meristematic zone (MZ), nodule cortex (NC) with vascular bundles and the central bacteroid zone (Bz).

The meristematic zone is situated at the apex of the nodule, composed of multi-layered, thin walled, tangentially elongated cells with dense cytoplasm and prominent nuclei, arranged compactly in regular rows (figure 14).

The nodule cortex is homogeneous comprising of 3 to 6 layers of compact parenchymatous cells with vascular bundles (figures 15, 20). In *Medicago sativa*, *Melilotus alba* and *M. indica* 2 vascular bundles enter the base of the nodule (figures 16, 17 at arrows), but in *Trigonella foenum-graecum* there are 4 vascular traces two of which supply to each side of the nodule (figures 18, 19 at arrows). The vascular strands arise opposite to the protoxylem of the root stele (figure 17). The vascular strands during their upward course, branch repeatedly within the nodule cortex, but do not come in contact with the bacteroid zone (figure 20). The vascular bundles are “inversely collateral” and conjoint, surrounded by an endodermis (En) (figure 21).

The bacteroid zone is heterogeneous composed of approximately 75% infected cells (IC) and 25% uninfected cells (UC) (figure 22). The uninfected cells are packed with spherical starch grains and interspersed within the tissue of infected cells (figure 23 at arrow). The young infected cells are with distinct nucleus and contain bacteria (figure 24). In the maturing infected cells small vacuoles (V) appear (figure 25) and subsequently their fusion tend to form a large vacuole, pushing the contents and the nucleus towards periphery of the cell (figures 26, 27) and at this stage the mature infected cells are about four times larger than the uninfected ones (figure 22). At a later stage the disappearance of the nucleus

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11 x 532; 12 x 380; 13 x 43; 14 x 450; 15 x 32; 16 x 100; 17 x 48; 18 x 80; 19 x 38.

Abbreviations: PL, peripheral layers; MC, mass of cells; NC, nodule cortex; Bz, bacteroid zone; MZ, meristematic zone; VB, vascular bundle; RC, root cortex; RS, root stele.
and clumping of the bacteroids of the infected cells lead to the senescence of the nodule (figures 28, 29 at arrow).

4. Discussion

The mode of invasion of bacterial thread into the roots is considerably interesting. In most of the legumes the infection takes place through root hairs (Thornton 1930; Bond 1948; Harris et al 1949; Arora 1956b, c; Nutman 1959; Dart and Mercer 1963, 1964; Narayana 1963; Narayana and Gothwal 1964; Kapil and Kapil 1971), but in a few cases it is reported to enter through the intact root epidermal cells (McCoy 1929; Schaede 1940), broken epidermal cells (Bieberdorf 1938) and wounded and ruptured cortical cells during the emergence of lateral roots (Allen and Allen 1940; Arora 1954). Narayana and Gothwal (1964) reported the infection thread to enter through root hair in *Trigonella foenum-graecum*, but we have noted its entry through the intact root epidermis in *Trigonella foenum-graecum* and also in *Medicago sativa* and *Melilotus officinalis*.

From the data presented regarding the depth of penetration of infection thread into the root cortex (Bieberdorf 1938; Bond 1948; Arora 1956c; Narayana 1963; Narayana and Gothwal 1964; Kapil and Kapil 1971) it appears that there is no correlation between the depth of penetration of the thread and the structure (thickness) of the root cortex. In the plants of present investigation also the penetration of the infection thread is up to middle region of the cortex in primary, secondary and tertiary roots.

The dissemination of the bacteria can be either by the invasion of the infection threads of the newly produced cells (Bond 1948; Harris et al 1949; Narayana 1963; Narayana and Gothwal 1964; Kapil and Kapil 1971) or by the division of the infected cells (McCoy 1929; Allen and Allen 1940; Arora 1954). In our plants it is by the second method.

Several viewpoints have been put forth to explain the formation of the funnel shaped swellings in the infection threads (McCoy 1929; Thornton 1930; Harris et al 1949; Arora 1956c; Narayana 1963; Narayana and Gothwal 1964; Dixon 1964). Such swellings are observed in the present investigation and may be due to emaciation of the bacterial mass caused by the stretching of the thread during the enlargement of the host cell harbouring it (Arora 1956c). The breaking of

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20 × 38; 21 × 768; 22 × 209; 23 × 380; 24 × 1040; 25 × 960; 26 × 640; 27 × 350; 28 × 1630; 29 × 560.

**Abbreviations.** NC, nodule cortex; EN, endodermis; XY, xylem; PH, phloem; UC, uninfected cell; IC, infected cell; N, nucleus; V, vacuole.
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the thread in the middle (Narayana 1963) further supports our conclusion (figure 8 at arrow).

The meristematic zone may be situated at the apex or at several places surrounding the nodule and accordingly they may be "apical" or "spherical" respectively (Kodama 1967). In the species of the present investigation the meristematic zone occurs at the apex and thereby the nodules confirm to "apical" type of Kodama (1967).

The structure of the nodule cortex is reported to be heterogeneous in some legumes (McCoy 1929; Harris et al. 1949; Arora 1954; Allen et al. 1955; Narayana and Gothwal 1964; Kapil and Kapil 1971), but it is homogeneous, comprising of 3-6 layers of compact parenchyma cells surrounded by 3-6 layers of root cortex in the species studied by us.

There is a great variation in the number and orientation of vascular strands connecting the root stele in different leguminous nodules (Bieberdorf 1938; Bond 1948; Harris et al. 1949; Arora 1954, 1956a-c; Narayana 1963; Kapil and Kapil 1971). We have observed four vascular connections in *Trigonella foenum-graecum* (see also Narayana and Gothwal 1964) and two in *Medicago sativa, Melilotus alba* and *M. indica*.

The arrangement of phloem and xylem in the vascular bundles in different leguminous nodules is variable (Allen and Allen 1940; Allen et al. 1955; Kapil and Kapil 1971). It is inversely collateral in the nodules of the investigated species (see Kapil and Kapil 1971).

All the cells in the bacteroid zone contain bacteria (Allen and Allen 1940; Arora 1954, 1956a) or some of them may be infected by bacteria and others may not (Harris et al. 1949; Arora 1956b, c; Narayana 1963; Kapil and Kapil 1971). The latter situation is observed in our plants and the percentage of infected and uninfected cells is 75% and 25% respectively.

We are in agreement with the observations of Allen and Allen (1958) who report that the earliest indication of senescence is the change in colour of the bacteroid zone from red to green and a change in the nodule surface from smooth to wrinkled. Clumping of bacteria is quite common during the nodule senescence.

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