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The Infection Process in the Formation of Nitrogen-Fixing Stem Nodules of *Sesbania rostrata*

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USAID Grant DPE-5542-G-SS-3012-00 was awarded for the period 21 June 1983 through 19 June 1984. The grant was awarded to support research on the nature of the process whereby Sesbania rostrata interacts with a Rhizobium bacterium to form nitrogen-fixing nodules located on the plant stem.

The annual tropical legume Sesbania rostrata was found in 1980 to bear nodules profusely along the stem as well as on the roots. The stem nodules are caused by specific, fast-growing rhizobia and are capable of nitrogen fixation. The goal of this research program was to determine how the infection process is initiated at the stem nodule site and how it leads to the development of the nitrogen-fixing nodule. The specific objectives of the research were to resolve the following aspects of nodule formation at stem sites in S. rostrata.

1. What is the site of localization of the Rhizobium partner prior to infection?
2. What are the earliest observable morphological changes in the infection site following inoculation?
3. What is the changing pattern of distribution of rhizobia during early infection as host cells begin to differentiate into nodule structures?
4. Is an infection thread or some analagous mechanism involved in the entry of Rhizobium into cortical cells?
5. How do the infection centers seen in a preliminary study progress to the mature nodule stage?

Resolution of the above questions has determined that the early events in the formation of stem nodules in S. rostrata may be summarized as follows: 1) Colonization of the nodulation site by specific Rhizobium, 2) Intercellular penetration of rhizobia into the meristematic zone, 3) Establishment by rhizobia of localized infection sites (infective centers) within the

meristematic zone, 4) Invagination of intercellular infection threads into cells of infective center and intercellular release of rhizobia, 5) Intracellular growth of rhizobia and bacteroid formation, 6) Progressive enlargement of infective centers with continued cortical cell division and continued infection thread expansion and invagination. The more unusual aspects of nodule formation in this system include the nature of the nodulation site, the mode of invasion of the rhizobia, and the proliferation of cortical tissues surrounding the incipient adventitious root primordium wherein rhizobial infection begins and within which it expands and spreads.

The stem nodulation site is a beautifully evolved structure capable of initiating either a root system or a nitrogen fixing system depending on the environment. Prior to its activation as a nodulation site, it appears as a small, sub-epidermal swelling, one of hundreds in vertical lines on the stem. Centered beneath each swollen area is the tip of an adventitious root. The conditions that trigger activation of the site are not known, but in view of the natural occurrence of stem nodules only under water-logged soil conditions, it is reasonable to expect that moisture is an important factor. The site becomes a nodulation site with the emergence of the tip of the adventitious root through the slightly swollen cortical tissues, leaving a narrow circular cavity of fissure surrounding the root primordium. This fissure provides an excellent microenvironment for appropriate inoculant rhizobia. Just how this microenvironment selects for an appropriate Rhizobium strain when the nodule sites are exposed to natural windborne inoculation is an interesting ecological question. In any case, the specific Rhizobium establish and begin to divide at widely scattered sites on the V-shaped bottom of the fissure. The plant cells exposed at the base and inner face of the fissure are mature, highly vaculated, non-dividing cells with wide intercellular spaces. The rhizobia colonize the exposed open spaces and crevices between the cells.

Rhizobia penetrate laterally from the intercellular bridgeheads established in the fissure in the direction of the base of the incipient root primordium to enter a zone of small, dense, cytoplasm-rich cells. The invasion route quickly narrows down from the large intercellular spaces of the fissure-associated cells to small tubular channels a few rhizobia-wide between the more active underlying cortical cells. Since the channels invaginate into adjacent cells and are clearly enclosed by plant cell wall as they penetrate the cells, we designated the channels as infection threads. Within the infection thread the bacteria appear variable in shape and somewhat deformed, suspended in a moderately electron dense matrix having the appearance of mucopolysaccharide. Once released into the cytoplasm, individual rhizobia are promptly surrounded by a peribacteroid membrane presumed to be of plant origin, and assume a regular rod shape.

The mode of penetration of rhizobia in the S. rostrata stem nodule system is generally similar to that of the peanut, A. hypogaea, root nodule system. Unlike most root nodules systems where the rhizobia enter cortical cells through curled root hair tips by forming an infection thread, infection on the peanut root starts by entry into intercellular spaces at the base of the root hairs on emerging lateral roots. The S. rostrata stem nodule formation differs from the peanut, however, in that the Sesbania nodulation site is without root hairs, and the intracellular penetration in Sesbania is via an infection thread surrounded by a cell wall-like structure. In A. hypogaea, penetration into the host cortical cell apparently does not involve a cell wall-bound infection thread.

The target area for rhizobia penetrating the Sesbania root primordium from the fissure is a relatively thin zone of small, dense, cytoplasm-filled cells. This tissue occurs surrounding the base of the root primordium, and underlying the base of the fissure by a distance of a few cortical cells. These cells

appear to be cortex-derived and highly meristematic. Infection spreads within the meristematic zone by extension of intercellular infection threads and into cells in contact with the infection thread.

As in other Rhizobium-legume symbioses, the Sesbania stem rhizobia grow profusely within host cells which become densly packed with bacteroids. The infective centers within the meristematic zone clearly expand and merge as nodules mature at stages later than those examined in this study. It is not clear from our data how the infective zones proliferate, but if analagous to nodule morphogenesis in root nodules, it may be expected that cell division in the meristematic zone is stimulated by the presence of the rhizobia.