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A COMPARATIVE STUDY OF THE ORIGIN AND THE CYTOLOGICAL DEVELOPMENT OF SOME LEGUME NODULES

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With the increased use of legumes as crop plants, there has been a growing interest in the root nodules or tubercles characteristic of legumes. From the literature it is apparent that there is considerable difference in the mode of development and structure of nodules in different legumes. These differences apparently are responsible for the lack of unity in interpretation of certain features of nodule development. Therefore, a comparative study of nodule development in a number of species of legumes was made.

However, although the nodule development in different species of legumes varies considerably, the mode of infection is very much the same in all plants that have been studied. The bacteria usually invade the host tissue through the root hairs, apparently dissolving the cell wall, and causing a characteristic curvature of the root hair. After passing through the cell membrane, the bacteria begin the formation of an infection strand and start migrating towards the center of the root. The cause of the migration is not known. Because of its directness, the course of the infection strand seems to be determined by an attraction exerted by the host cell upon the bacteria. The migration may be through or between the host cells. Once the infection strand has penetrated the cortex, bacteria are liberated directly into the cytoplasm. The distance which bacteria penetrate into the root and the exact point of origin of the nodule varies with different species of legumes.

A comparative histological and cytological study of the nodule development of the following leguminous plants was made: soybean (*Soja max* Piper), cowpea (*Vigna sinensis* Endl.), sweet clover (*Melilotus alba* Desr.), alfalfa (*Medicago* sativa L.), vetch (*Vicia villosa* Roth), and peanut *(Arachis hypogaea)*.

The nodules were obtained by growing the plants in:

(1) washed sand,
(2) Knop's water culture in bottles,
(3) a mixture of sand and loam soil.

The plants were grown in the greenhouse and in the laboratory under artificial light. Field grown nodules were also studied for comparison.

The bacteria used for inoculation were obtained as follows:

(1) By using soil already infested with bacteria.
(2) By grinding up mature nodules and preparing a water suspension,
(3) By using commercial "Nitragin,"
(4) By inoculation from agar slants

An abundance of material for study was obtained by the above method. Plants growing in Knop's water culture could be observed daily. Material was selected at various times of the day in order to increase the possibility of obtaining cells undergoing mitosis. Various killing and fixing solutions were used, depending upon the age of the material selected. The paraffin method of infiltration and imbedding proved most satisfactory. Free hand sections were used in the microchemical tests. Various staining methods were employed for the different kinds of tissues.

NODULE DEVELOPMENT OF SOYBEAN

As already mentioned, the bacteria causing nodule formation usually enter the host plant through the root hairs. A characteristic curving of the root hairs usually occurs at the point of contact between bacteria and root hair. In the soybean and cowpea, infection may take place without the formation of such a curvature of the root hairs. Root hairs were also found in which infection apparently took place simultaneously on opposite sides of the root hairs. From observations made, it was concluded that this curvature usually takes place when infection occurs during the elongation period of the root hairs. If infection occurs in the older root hairs, this curvature will not be exhibited. It is apparently possible for bacteria to enter the host through the epidermal cell. This was found to occur most frequently when seedlings were grown in a medium in which they obtained an abundance of water, and produced fewer root hairs.

It was noted that many seedlings would die or become severely stunted just at the stage at which they were breaking through the soil, before unfolding their cotyledons. This was especially found to occur when a medium was used which was highly infested with nodule-forming bacteria. Those seedlings which survived always showed a very heavy inoculation. Cross sections from the roots of these plants showed numerous infections that did not form nodules. From all observations, it was concluded that the stunting or death of these seedlings was a result of excessive infection. The bacteria apparently are parasitic during the early stages of infection.

Immediately after entering the cell, the bacteria begin the formation of the infection or zoogloeal strand which resembles a nonseptate fungus hypha. This strand is of a denser consistency than the surrounding cytoplasm. It requires approximately two days from the time that the bacteria come in contact with the root hair until they have reached the inner wall of the epidermal cell, a distance of 70 to 80 microns. The number of strands invading the root hairs was found to vary from one to three. The strands may remain single or branch. But when the cortex is penetrated, branching always occurs. The branching strands ramify the surrounding cortical parenchyma. Breaks in the strands liberate the bacteria into the cytoplasm. The infected cells, and those immediately surrounding them, have a rather dense cytoplasm and a very prominent nucleus. The infected cells enter into a period of active division. It is these cells which form the bacteroidal cells and give rise to the nodule. Because of the frequent cell division of infected cells of the soybean, the infection strand is broken up into small particles and its identity completely lost.

Nodules have been referred to as modified lateral roots arising in the pericycle. However, bacteria were never found penetrating the endodermis, nor was the pericycle found to be active.

The bacteria always appear to move towards one of the protoxylem points. Soon after bacteria have penetrated the cortex, a direct vascular connection is formed between the infected area and one of the protoxylem points. It appears that the materials utilized by the bacteria and the dividing cells of the young nodule are obtained through a vascular system connected to the protoxylem points. As the nodule begins to enlarge, the bacteroidal cells cease their meristematic activity, and the nodule increases in size merely by the enlargement of the bacteroidal cells. Uninfected parenchyma cells of normal size are found scattered throughout bacteroidal tissue, which indicates that when the identity of the infection strand is lost, no more cells are infected.

A rather elaborate conductive system is developed connecting the nodule with the xylem and phloem of the root. This conductive system becomes evident very early in the development of the nodule. The first conductive tissue formed connects the infected area with one of the protoxylem points. The parenchyma cells between these two regions are differentiated into conductive cells. The conductive system branches, surrounding the bacteroidal area, and uniting again at the opposite side. A layer of parenchyma cells is found between the bacteroidal area and the conductive tissue. The conductive bundle is composed of xylem and phloem-like cells. The bundles have no definite arrangement except that the xylem is surrounded by phloem-like parenchyma.

When the nodule is about three-quarters matured, a very conspicuous layer of sclerenchyma cells is formed in the cortex. This layer of thick walled cells originates in the outer layer of the cortical parenchyma, completely surrounding the bacteroidal and vascular tissues. This layer of sclerenchyma cells develops very rapidly and when liginification has been completed, little if any growth occurs in the nodule. The size of the nodule is limited by this layer.

NODULE DEVELOPMENT OF THE COWPEA

Nodules of the cowpea *(Vigna sinensis)* are spherical and develop to about the size of a small pea. Cowpea nodules, like soybean, arise in the cortical parenchyma of the root, the bacteria usually entering through the root hairs. The essential differences between soybean and cowpea nodules are that the layer of cambium surrounding the bacteroidal tissue is more conspicuous in the cowpea nodule, and that it does not form a layer of thick walled cells in the cortex.

STARCH GRAINS

The presence of starch grains in nodules has been reported by many investigators. In the soybean and cowpea, starch grains are found most abundantly in the normal cells scattered throughout the bacteriodal tissue, in cells surrounding the vascular bundles, and in the cortical parenchyma bordering the infected tissue. Many of the starch grains appear to be exceedingly large and show a typical tetrad formation.

NODULE DEVELOPMENT OF VETCH AND SWEET CLOVER

Nodules on vetch *(Vicia villosa)* and sweet clover *(Melilotus alba)* are found prevalent even on those plants having escaped cultivation. The nodules of vetch, sweet clover and alfalfa are very similar in origin, form and structure and for that reason only one, the vetch, will be described. The nodules of vetch arise from the cortical parenchyma. Bacteria gain entrance to the host tissue in the same manner as already described for the soybean. There being comparatively little cortical parenchyma in the roots of vetch, the bacteria may penetrate the cortex until the endodermis is reached. Therefore, it may appear as if the nodule arises in the pericycle, like a lateral root. However, bacteria were not found penetrating the endodermis.

The nodules of vetch are club or pear shaped, sometimes forming large clusters. The bacterial infection strand does not lose its identity on entering the cortex, but can be found in the old or mature nodule. The strands shrink somewhat and the bacteria apparently are all liberated from the strands into the surrounding cytoplasm. In the bacteroidal tissue of vetch nodules, cell division is not as obvious as in the soybean. The bacteroidal area is increased mainly by the continual invasion of the infection strands of new tissue laid down by a peripheral meristem instead of by division of infected cells. As new tissue is laid down, the cells are invaded by the numerous infection strands that run parallel toward the meristem. The infection strands have a tendency to branch and spread out, thus causing the club-shaped nodule.

If during growth, some of the cambial cells in the periphery function at a different rate or some of the cells fail to function, the result will be branching of the nodule. Mitotic figures were not found in the bacteroidal cells of vetch. The cells begin to enlarge soon after infection occurs, and vacuoles are formed. The vacuoles increase in size very rapidly, often occupying half the volume of the cell. The nucleus and bacteria are seen closely crowded together. In the older cells, the vacuoles often disappear. The nucleus becomes flattened, and then broken into small fragments.

A vascular system is formed connecting the xylem and phloem with the nodule. The vascular bundle is formed from procambium strands. The branches of the bundle surrounding the bacteroidal area are laid down by the peripheral meristem. The branching of the nodule and the growth from one meristematic region prevents the vascular bundle branches from uniting at the apex. The nodules continue their growth and development throughout the growing period of the plant. A corky layer is formed around the nodule, but it is not as highly diffentiated as in soybean nodules. Cell division is not obvious in the uninfected cells and apparently their function is storage.

FUNGI

Filamentous fungi were found within the nodule of the soybean and cowpea. The fungus hyphae somewhat resembled an infection strand. The fungus hyphae were found penetrating the nodule through the corky layer and cortical parenchyma, until they reached the bacterial area. From all indications, these fungi are able to penetrate the nodule anywhere, and for entrance do not depend upon a break in the epidermal layer. These fungi may cause a premature disintegration of the nodules.