Form 2
Dissertation Abstract

Report no.	No.251 (Dissertation-based)	Name	FARHANA SHARMIN
Dissertation title	Ultrastructural studies on development and senescence of legume root nodules (マメ科植物根粒の発達と老化過程における微細構造の研究)		

Abstract

※The abstract should be in keeping with the structure of the dissertation (objective, statement of problem, investigation, conclusion) and should convey the substance of the dissertation.

The association of plants and bacteria results in the formation of nodules on the roots of leguminous plants. These root nodules fix nitrogen and after releasing it to the soil make it fertile without any hazardous impact on the soil. Soil and environment pollution is common in the world and soil is degrading day by day. Legume-bacteria association is an eco-friendly way of nitrogen fixation that plays a great role in agriculture and increases crop production. Legume-bacteria association is a type of symbiosis, characterized by the development of root nodules and nutrient exchange inside nodules. For many decades in research on legume nodulation, signal exchange between plant and bacteria has been studied. Studies of physiological and molecular aspects of nodule development were also carried out, but ultrastructural investigations related to legume root nodules are few. Nodule development and senescence are accompanied by many structural changes inside nodules. Not all ultrastructural changes have been identified, and many of their functions have not been described yet.

Our main objective in this study was to find out about the ultrastructural features of legume root nodules during development and senescence. Nodules fix nitrogen that is utilized by plants at different stages of their growth. It is very important to gain insight into the invasion of bacteria, the infection occurring inside nodules, and the interaction between them. After infection, bacteria are enclosed within organ-like structures known as symbiosomes which are currently the most interesting topic. The symbiosome is an important organ-like structure during development and senescence. Symbiosome membranes have the role to exchange nutrients with the upper portion of the host plant. Development of nodules at the early stage shows the development

of symbiosomes. At the time of symbiosome formation, different types of structural changes occur and new structures develop sometimes. Then these structures undergo senescence. Our main aim was to identify new features related to development and senescence at the ultrastructural level.

The structure of *Vicia faba* root nodules was investigated first. *Vicia faba* plants produce indeterminate type nodules, which retain meristematic activity at the tip of the nodules and newly produced cells get infected with infection threads containing bacteria. The released bacteria are instantly enclosed by membranes produced by plants and proliferate in the infected cells. Mature infected cells fix atmospheric nitrogen in the form of ammonia which is utilized by plants. The infected cells eventually senesce and become physiologically inactive. Mature *V. faba* root nodules retain an elongated shape and each area can be distinguished by color: the meristematic area is white; the nitrogen fixing area is pink because of leg hemoglobin to protect nitrogenase; the senescence area is green. During light microscopic observation of developing *V. faba* root nodules, I noticed peculiar massive structures observed near infection threads near the meristematic area. By transmission electron microscopic (TEM) observation, they were identified as para-crystalline membranous structures resembling pro lamellar bodies in etioplasts. Para-crystalline structures in the etioplasts are converted to massive thylakoid membranes upon illumination in leaf cells. Similarly, I suggest that the para-crystalline structures in the root nodules develop into peri-bacteroid membranes which will be required in mass during the development of the infected cells. The para-crystalline membranous inclusions in the root nodules were observed for the first time and we named them PSMB (pro-symbiosome membrane bodies).

Application of BODIPY TR Casein, which emits fluorescence when degraded by protease, to nodules at the beginning of senescence detected protease activities. By ultrastructural observation of the corresponding area, I learned that the bacteria tend to be degraded in the infected cells while plant cell structures remain intact. In the case of *V. faba* root nodules, symbiotic relationships end by degradation of the bacteria in the symbiosomes.

In the second stage of the research, development and senescence of Kudzu (*Pueraria montana*) root nodules were investigated. Kudzu is a fast growing perennial that belongs to the pea family Fabaceae. It originated in East Asia and, introduced to the USA and other countries, it eventually became an obnoxious weed because of its rapid growth and ability to climb up and cover other plants especially in hot climates. Root nodules of Kudzu were collected from the plants proliferating on a river bank of the Arakawa river. The shape of the nodules varied from globular to elongate and sometimes branched. Light microscopic investigation of the nodules revealed meristematic areas in several

directions in a nodule, which may be the reason for the branched irregular shape of mature nodules. From TEM observation, an abundance of pronounced peroxisomes and accumulation of ER, characteristics of ureide producing root nodules, were found in uninfected cells located among infected cells. Since ureides convey nitrogen more efficiently than amides under high temperature, this feature may explain the rapid growth of kudzu in a hot climate. Clusters of bacteria were sometimes observed between cells and some of the bacteria appear to be invading cells directly from colonies of bacteria between cells. In the infected cells bacteria vigorously proliferate and often appear to attack plant cell structures, including nuclei. Unlike *V. faba* root nodules in which bacteria degrade during early senescence, in Kudzu root nodules, plant cell structures disintegrate during senescence leaving bacteria intact. From winter to early spring, no intact nodules but only empty crusts were found on underground roots. The ultrastructural characteristics of kudzu root nodules seem to imply special adaptation tailored to provide support for rapid growth during hot summer.