Agric. Res. J. Kerala 1988.28 (2) 198 223

ULTRA STRUCTURES OF NODULES OF *CAJANUS CAJAN* (L) Millsp. INOCULATED WITH VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGUS

P. Sivaprasad[1], , V. Rai and S. S. Joshi

University of Agric. Sciences, GKVK, Bangalore 560 065, India

A mature nodule of legume 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 (Shah and Rao, 1982). The effectiveness and the extent of nitrogen fixation in legumes are related to the amount and persistence of bacteroid containing tissues in nodules (Bergersen, 1974). In the infected nodule cells, the bacteroids are enclosed in an intracytoplasmic membrane system and the number of bacteroid par envelop varies with legume species. Berger.sen and Briggs (1958) observed four to five bacteoids per membrane envelop in soybean nodule and there was only one in the case of clover (Dart and Mercer, 1963). No information is available on ultrastructures of Pigeon pea nodules. We report here some information on ultrastructures of pigeon pea nodule and the influence of vesicular-arbuscular mycorrhiza on fine structures.

Materials and Methods

Two sets of plants were raised in pots of 30 cm diameter filled with 10 kg of phosphorus deficient sterile Alfisol soil (pH 5.6, 0.3*M* NH_4F-HCl extractableP 2.4 mg/kg soil) with 50 per cent sand using selfed seeds of *Cajanus cajan* (L) Milisp. (variety: Pusa Agethi). One set was inoculated with *Rhizobium* strain IHP 100, and the other set was dually inoculated with surface sterilized spores (600 numbers/pot) of vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum* and *Rhizobium* strain IHP 100.

Healthy nodules of uniform size were collected from the collar region of 40 day old plants. Nodules were cleaned and cut into slices of approximately 0.5mm thickness and dropped immediately into 3 per cent glutaraldehyde for fixation, dehydration and infiltration process (Dixon, 1964). Potassium permanganate and uranylacetate were used in the fixation process for staining the specimen.

Polymerization/block making

Plastic capsules were filled with resin mix (with accelerator). One slice of nodule was transferred to each capsule and then allowed to polymerize at 70°C for 8 h.

Composition of resin mix used:

Resin.	Araldite	49 g
Hardener:	Dedecenylsuccinic anhydride	49 g
Plasticizer:	Dibutyl phthalate (DBT)	0.75 g
Accelerator:	Benzyldimethylamine	1.75 g

Present address: College of Agriculture, Vellayani 695 522, Trivandrum, India

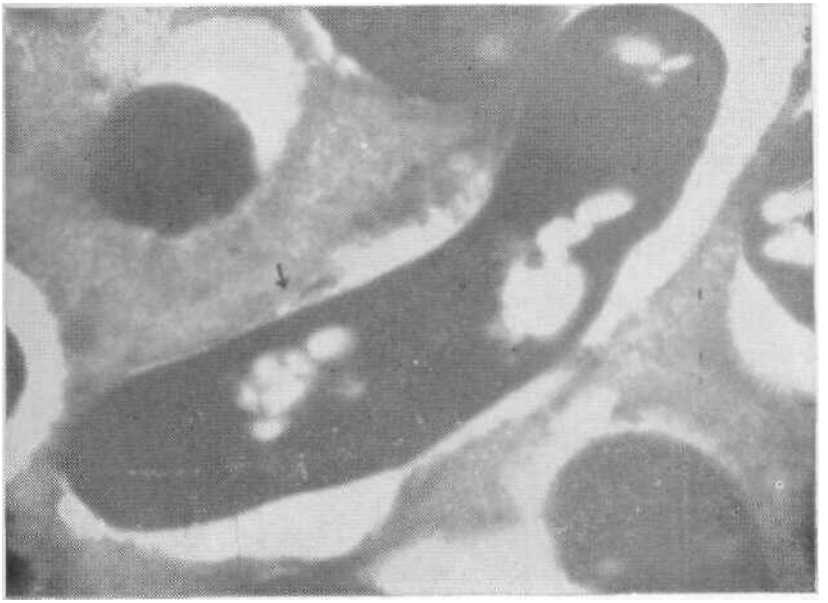

Plate 1 EM view of pigeon pea root nodules: Single bacteroid per membrane envelop (x 55COO)

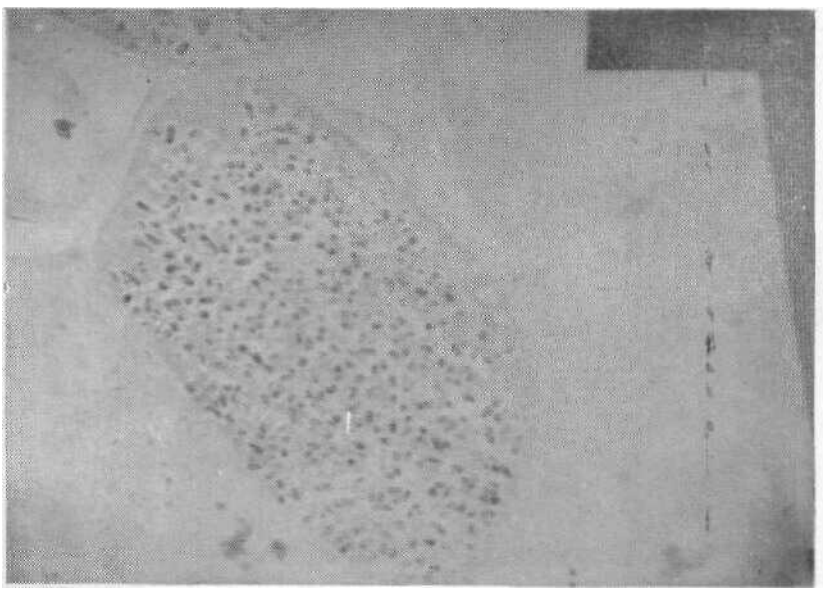

Plate 2 Typical infected nodule cell (transformed) of *Rhizobium* alone inoculated plants. Host cytoplasm is filled with *Rhizobium*/bacteroid (x 1600)

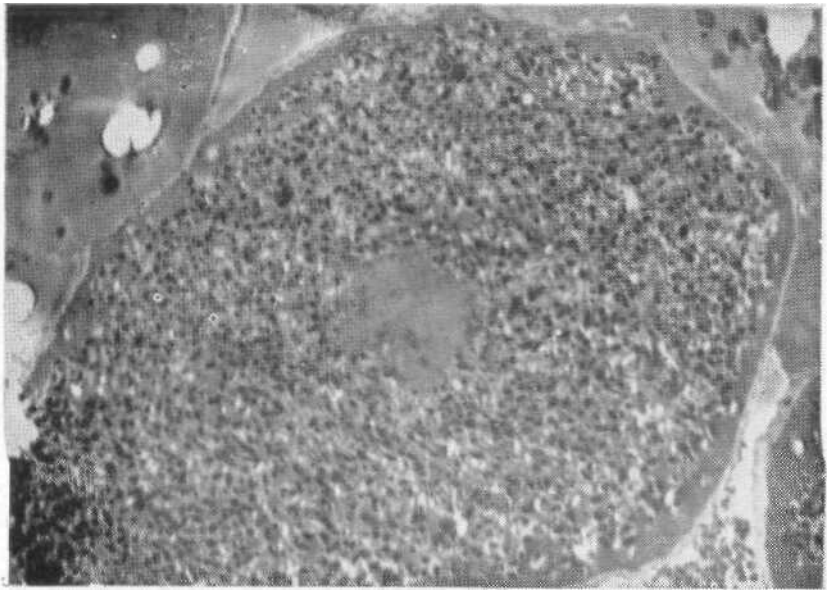

Plate 3 Typical infected nodule cell (transformed) of dually inoculated plants. Host cytoplasm is filled with bateroid/rhizobia (x 1600)

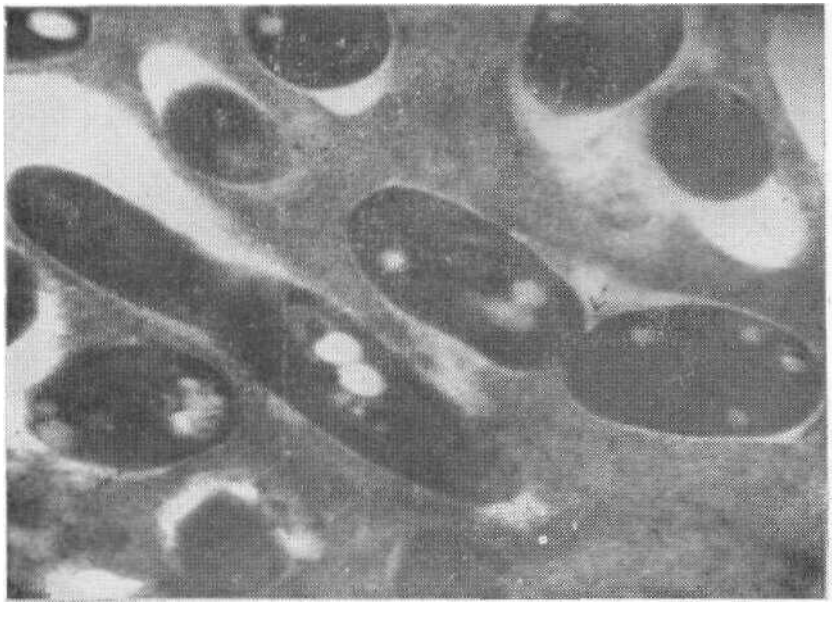

Plate 4 Dividing stage of bacteria in pigeon pea root nodule of *Rhizobium* alone inoculated plant (x 20000)

Sectioning and observation:

Ultra thin sections were made in an ultramicrotome. Sections were taken over copper grids and observed in a transmission electron microscope (Hitachi, HUIIF, 1972).

Result and Discussion

The electron microscopic observation made on nodule tissues revealed that the bacteroid present in a transformed cell of pigeon pea nodule is surrounded by a membrane envelop. Number of bacteroid present in an envelop was always one in both mycorrhizal aed non-mycorrhizal plant nodules (Plate 1). The transformed nodule cell size and the density of bacteroid/*Rhizobium* population in a unit area of transformed cell were higher in mycorrhizal plant nodule (Plate 2 and 3). Few dividing stages of bacterial cells were noticed in *Rhizobium* alone inoculated plant nodule but it was rarely observed in mycorrhizal plants (Plate 4). There was no striking cell size difference between *Rhizobium* and bacteroid. However, the shape of the bacteroid differed from bacterial cells (Plate 5 and 6).

It is well established that bacteroid in the nodule tissue is always enclosed by a membrane envelop (Bergersen, 1974) and the number of bacteriod per envelop varies, usually one or four to six. In the present study single bacteroid was observed per envelop irrespective of mycorrhizal inoculation. Hence, the mycorrhizal association did not influence the number of bacteroid formed per envelop.

Mycorrhiza induced higher transformed cell size is probably due to increased metabolic activity of the cell brought about by increased hormonal activity and better nutrient availability conferred by mycorrhizal association. Increased bacteroid/*Rhizobium* population observed in a unit area of transformed cell indicated that in mycorrhizal plant nodule there was a better bacterial multiplication and spread along with increase in transformed cell size.

In many legumes, compared to *Rhizobium,* a higher bacteroid size was recorded (Dixon, 1964). However, no such size difference between vegetative bacterial cell and bacteroid was noticed in cowpea (Bergerson, 1974). The present study with pigeon pea nodule also did not indicate any notable cell size difference.

Reduction in number of dividing stages (vegetative cells) observed in the nodule tissues of dually inoculated plant may be an indication of higher bacteroid number in dually inoculated plants than that of plants inoculated with *Rhizobium* alone.

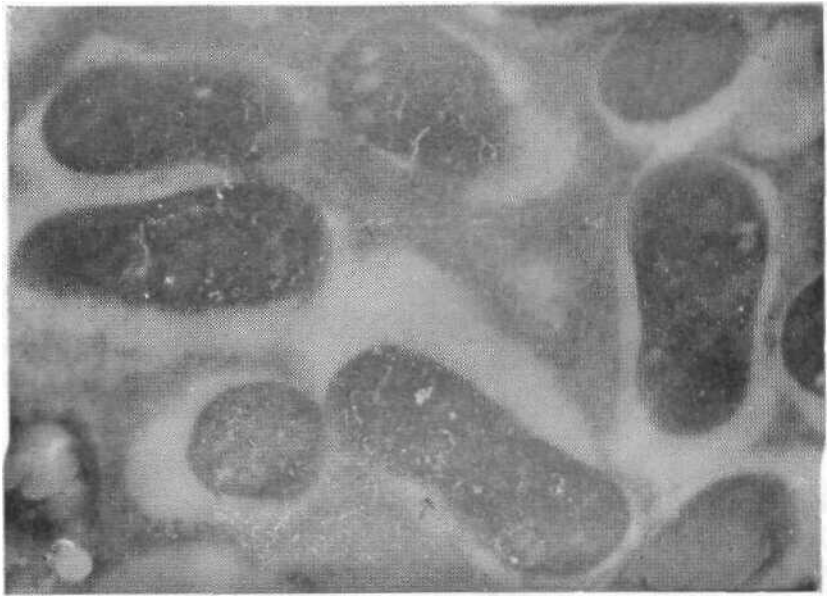

Plate 5 Shape of bacteroid (x 22000)

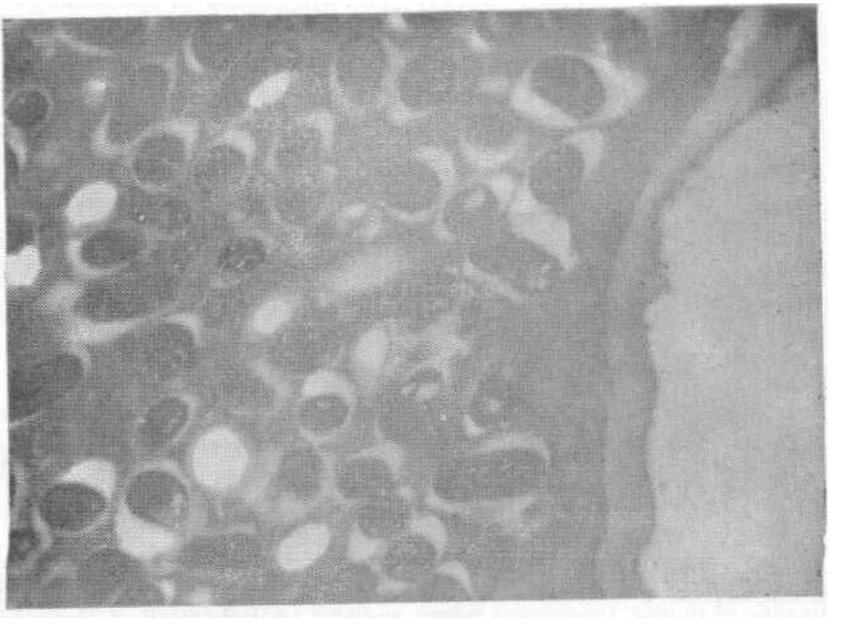

Plate 6 A general view of the transformed cell of pigeon pea nodule showing cell wall, membrane bacteroid/rhizobia etc. (x 4500)

Summary

Ultrastructural observations made on nodules of *Cajanus cajan* (L) Millsp. indicated that tha association of VA mycorrhizal fungus *Glomus fasciculatum* did not influence the numberof bacteroid formed per membrane envelop, which was always one. Both the transformed cell size and number of bacteroid/ *Rhizobium* present in transformed cell were increased due to mycorrhizal association. Bacteroidal shape differed from the *Rhizobium* However, no such difference was noticed in size.

Acknowledgement

The authors are grateful to Dr. R. V. Rammohan, Head of Department of Physics, UAS, Bangalore and Dr. Rammohan, Assistant Professor of Neuropathology NIMHANS, Bangalore for the help extended during the study.

References

Bergersen, F. J. 1974. Formation and function of bacteroid. *Biology of Nitrogen Fixation.* Ed. Quispei, A North Holland Publishing Company, Amsterdam, 473-498

Bergersen, F. J. and Briggs, M. J. 1958. Studies on the bacterial component of of soybean root nodules; Cytology and organisation in the host root tissue. *J, gen. Microbiol.* 19; 482-490

Dart, P. J. and Mercer, F. V. 1963. Development of bacteroid in root nodule of barrel medica *(Medicago tribuloides)* and subterraneum clover *(Trifoliumsubterraneum* L). *Arch. Microbiol.* 46: 382-401

Dixon, R. 0. D. 1964. Tha structure of infection thread and bacteria and bacteroid in pea and clover root nodules. *Arch. Microbiol.* 48: 166-178

Shah, G. L. and Rao, M. G. 1982. Initiation, development and structure of root nodules in some members of the tribe Trifolicae (Papilonaceae). *Proc. Indian Acad. Sci. (PI. Sci.)* 91: 309-318