CHAPTER III

THE SYNCHRONIZATION BETWEEN HOST PLANT DEVELOPMENT AND SYMBIOTIC DEVELOPMENT IN ARACHIS HYPOGAEA L.

The main features of nodule initiation and growth are known to vary from species to species (Nutman, 1958). While bacterial strains differ specifically in the number of nodules, they induce on the host (Chen, 1941), the hosts themselves differ in their readiness to form nodules. The controlling influence of the host is a genetic property and effects due to the host and bacterial strain are known to be quite independent (Nutman, 1948). Further, apparent differences in strain virulence appear to be related to the inherent capacity of the root to form lateral meristems as well as to the inhibition from meristems already present (Nutman, 1946 b, 1948, 1952 b, 1953, 1955). Besides, host physiology and the practice of inoculation profoundly affect nodule number, size and distribution. That there is an inverse relationship between nodule size and abundance and that it varies among species to the extent to which it is exhibited is well known (Nutman, 1949). For instance, in experiments on delayed inoculation Nutman (1949 b) showed that moderate delays augmented the hosts' capacity to form nodules.

Thus, infection by bacteria and development of nodules on legume hosts are to be integrated with host metabolism. In the present work the features of symbiotic development were studied in Arachi hypogaea in sand cultures using an effective and specific inoculant strain of Rhizobium (R_4).

The bunch variety of ground nut (Arachis hypogaea var. TMV 2) was grown in sterilized sand supplied with nitrogen-free nutrient salt solution (vide Materials and Methods). The methods of soil and seed sterilization, sowing, rhizobial inoculation and care of assemblies are described earlier (vide Materials and Methods). A total of 60 pots, 30 each for the inoculated and uninoculated controls, were maintained in the green house. The pots, each of which contained five plants, were randomized on the benches. During different periods of growth plants were analyzed (once in every seven days) for mean total nitrogen, root nodule haemoglobin, and mean nodule number, weight and volume. The mean values for each period are based on an analysis of five plants replicated three times. The plants were freed from sand at the time of each analysis, the roots washed free of sand, and nodules were detached carefully.

Dry Weight Determinations:

Shoots of 5 plants replicated three times were separately dried at 100°C to a constant weight.

Nitrogen determinations:

Dried plant material was ground and the quantity of nitrogen/plant determined by the Kjeldahl method (Humphries, 1956).

Root Nodule haemoglobin determinations:

Freshly removed nodules were washed, dried and weighed. One gram of nodules was used for each determination of haemoglobin. The nodule volume was determined by adding the nodules to water in a graduated cylinder and noting the displacement. The nodules were then ground thoroughly in a clean glass mortar and the extracts in water were centrifuged (3000 r.p.m. for ten minutes) and the supernatant so obtained was converted to pyridine haemochromogen as described earlier. The quantity of root nodule haemoglobin was calculated from the optical density values obtained with reference to the standard in the usual way (vide estimation of haematin).

A. NODULE FORMATION:

The development of nodules under the conditions of the experiment merits emphasis. In the bunch variety of *Arachis hypogaea* (TMV 2) nodules begin to be formed in 18-20 days following the sowing of seeds. Successive crops of nodules were formed at about 7 day intervals as the root system of the host plant enlarged. The mean nodule number, weight and volume are set forth in fig. 5.

B. THE MEAN NODULE NUMBER/PLANT:

The mean nodule number per plant gradually increased from about one week after their formation (25 days following sowing) to about 6 weeks (60 days following sowing). This period corresponds to the period of flowering of the host plant. After this period, the mean nodule number/plant dids not increase and remainsdstatic. Nodule degeneration dids not set in until late during pod formation (75-100 days from sowing) (fig.5).

C. THE MEAN NODULE FRESH WEIGHT AND VOLUME/PLANT:

Whilst the number of nodules formed gradually increased upto the period of maximum flowering (53-55 days), the mean fresh weight of nodules shows an abrupt increase from 3 weeks following nodulation (39 days from sowing) to 5 weeks of nodule age (53 days from sowing). The mean fresh weight thereafter declines gradually. A corresponding initial increase in nodule volume does not, however, follow the same trend after 5 weeks, thus showing that nodule degeneration does not set in until late during pod formation viz., 75-100 days from sowing (fig. 5).

D. THE MEAN DRY WEIGHT OF INOCULATED AND UNINOCULATED PLANTS: (Fig.6):

The trends observed in nodule growth in terms of their mean number, weight and volume are reflected closely in the increases in the mean dry weight of inoculated plants. The pronounced growth rate in terms of mean plant yield increases from within a week after nodulation to about 8 weeks of nodule age (75 days from sowing).

E. THE MEAN INCREASE IN TOTAL NITROGEN/PLANT (Fig.7).

Under the symbiotic conditions of growth where a single *Rhizobium* strain was used, the total plant nitrogen of nodulated plants grown in sand cultures steadily increased at a constant rate from 2 weeks to 7 weeks after the commencement of nodulation. Nodules started decaying by 8 to 10 weeks of nodule age. From the time of germination the foliage of the nodulated plants were characteristically yellow resembling the uninoculated controls until 2 weeks after the beginning of nodulation when they started turning a bright green.

F. THE SYNTHESIS AND PATTERN OF DEVELOPMENT OF ROOT NODULE HAEMOGLOBIN:

Fig.8 shows that the synthesis of the pigment progressively increases during the first two weeks after nodule formation attaining a maximum at 3 weeks and subsequently decreasing during the 4th week. This decrease is followed by a steep increase, the pigment attaining a maximum concentration in nodules at 5 weeks of nodule age (53 days from sowing). The occurrence of the maximum concentration of the pigment in root nodules corresponds to the period of maximum flowering of the plant. Once again the pigment concentrations show a decrease and further rise during the 6th and 7th week of nodule age. Eight weeks after nodulation (75 days from sowing) with the cessation of nitrogen fixation, the nodules began to turn green and haemoglobin estimations were discontinued owing to possible interference effects of the green pigment.

G. PIGMENT TRANSFORMATIONS WITHIN THE NODULES IN RELATION TO NITROGEN FIXATION BY RHIZOBIUM: (Fig. 9).

Previous determinations have described symbiotic development in terms of nodule development, nitrogen fixation and haemoglobin formation during different stages of plant growth. Nodules were observed to form on roots by 18-20 days. The maximum development of nodules in terms of mean weight and volume and haemoglobin concentration were attained by about 53 days. Nodules began to turn green by 75 days from sowing.

A study of the absorption spectra of the pigment at these critical periods indicated that

1. on the 22nd day, the pigment haemoglobin was just forming in the root nodules,
2. on the 53rd day, the pigment in root nodules had attained maximum concentration with the characteristic absorption spectra comparable to mammation haemoglobin,

3. within 75 days the absorption maxima fade rapidly with the transformation of the red pigment into green one. This transformation appears to mark the onset of conditions which result in the cessation of N-fixation in the root nodules.

The points that emerge from the present investigation suggest the following conclusions:

Under symbiotic conditions of development where an effective rhizobial inoculant was used, the pattern of nodule development, nitrogen fixation and haemoglobin formation in root nodules could be construed in terms of a unified growth metabolism of the host plant in its vegetative, reproductive and seed setting periods within a time of three months.

The fluctuations of haemoglobin levels in the root nodules throughout the period of flowering are of interest since in the groundnut plant, unlike in other legumes, the period of flowering is extended and is punctuated by the appearance of successive crops of flowers throughout the 25-60 days following germination of the host plant.

The synthesis of haemoglobin in nodules becomes evident within a period of 2-5 days after nodulation. The absorption spectra of the pigment at the time it begins to form (22 days), at the time of its attaining a maximum concentration (53 days), and at the time of its gradual transformation into the green pigment (75 days) in the nodules, further show that the period of 22 days to 75 days following germination of the seedling represent the period of functional symbiosis in the host plant.

However, this synchronization between host development and symbiotic development is strongly influenced by the factors of the environment. These aspects are pursued in the following section.