CHAPTER I.

INTRODUCTION

Contribution of nodule bearing legumes to the nitrogen economy of soils had been known since the discovery of symbiotic nitrogen fixation by the organisms harboured in the nodules and its recognition had long been the basis of rotation of crops. In most of the cultivated soils the stable equilibrium conditions which had been established through small but significant net annual gains in organic matter and nitrogen have long been disturbed and there are indications to believe that these constituents are in steady decline in many soils. For a good system of soil management, therefore, one has to look for conditions conducive to maximum fixation of nitrogen by a cultivated legume and also the factors which bring about such conditions.

Unlike other plants, the nutrition of a nodule bearing legume is modified to the advantage of the growing plant to a large extent by the presence in their root nodules of organisms capable of fixing atmospheric nitrogen. The organisms or rhizobia, as they are called, are ordinarily present in soils and at a certain stage of their life cycle they infect the plant roots. It is known that the activity of the organism is stimulated by certain substances and depressed by certain others present in the soil. They may form nodules but are unable to give rise to any fixation of nitrogen and are termed as inefficient strains. The differences among the rhizobial population are again further evident from differences in common characteristics and fixation of nitrogen, though they may all occur at the same time in the nodules of the same plant.
Oil and climatic conditions, agricultural practices like fertilization and irrigation which are known to affect generally the growth of crops, legumes included, must also be affecting growth development and activities of the rhizobial species occurring in soil. Beneficial effects of artificial inoculation of seeds of legumes with cultures of efficient strains of particular rhizobium at certain places had been reported as the cause of the spread of legume cultivation and this demonstrated the need to evaluate the influence of different conditions on the legume - rhizobium system.

During the present investigations studies have been made on growth and nitrogen fixation by two important legumes, gram (Cicer arietinum) and mung (Phaseolus vulgaris), the former a short term and the latter a long term crop, at different places in India, together with studies on the respective root nodule organisms and their characteristics. A study has also been made of the strain variation in Rhizobium japonicum, the root nodule organism of soybean (Glycine max) to evaluate the extent of variation that is likely to be exhibited in the efficiencies of a single species of rhizobium and its possible relationship with some well known characteristics of such group of organisms.

**Studies on gram (Cicer arietinum)** - Pure strains of gram root nodule organism were isolated from soils at Karnal, Hissar, Coimbatore and Delhi and the most efficient of the strains was used for inoculation of the crop grown at the four localities. Different methods of inoculation of the legume with the culture of the rhizobium and the effect of molybdenum on the fixation of nitrogen were also examined. It was expected that the results would present an overall picture on the influence of the rhizobium of different
soil and environmental conditions on the growth and yield of the crop at different places.

It was observed that the introduction of an efficient strain of the root nodule organism of gram from a different locality under almost identical climatic and soil conditions through inoculation of seeds gave rise to greater nitrogen fixation by the legume in a soil where the organism was already present. In a soil where the rhizobium was not detected, introduction of the same from an area where they occurred, nearest to the locality gave rise to large nodulation, nitrogen fixation and higher yields of the crop.

Among different methods of inoculation that of the seeds with a culture of an efficient strain of the root nodule organism had not been found to be very suitable for increasing nitrogen fixation and thereby for obtaining higher yields of gram. Inoculation of the soil with rhizobium had been found to be better instead. This might be achieved in two ways. One was, by broadcasting over the field, some soil from the field where gram was already well established. The other was, by broadcasting, over the field, a soil culture of the rhizobium with an adequate number of the organism per gram of the material. A count of eighty million live cells per gram was found to be a suitable titre for such purpose.

Application of molybdenum in the form of sodium molybdate up to a concentration of 5 p.p.m. gave rise to a higher number of nodules in the plant as compared to the number observed in the soil without any treatment of molybdenum. Application of 1 p.p.m. of molybdenum only had however, been found to result in definitely higher fixation of nitrogen by gram and greater yields of the same.
Studies on rahar (Calanucus cajan) - Pure strains of rahar root nodule organism were isolated from soil at different localities and the most efficient strain at each locality was used for inoculation of the crop. As the crop required several months to attain maturity and nodules were observed only for about a period of twelve weeks, it was probable that nitrogen fixed by the rhizobium could not supply all the nitrogen required by the crop and the extra nitrogen was supplied through the soil. Studies on the effect of fertilizers and molybdenum on the growth and the nitrogen fixation by the crop were therefore made. It was expected that differences in nitrogen fixing capacities, nodulation and yield due to treatments would be more clearly exhibited during the growth of the long term crop like rahar than in the case of short term legumes.

Environmental and soil conditions or treatments of the crop with nitrogenous, phosphatic and potash fertilizers alone or in combination with each other did not affect the period of active nodulation in Calanucus cajan which was observed to be limited to three weeks.

Significant differences were observed in the yields and nitrogen contents of inoculated and uninoculated rahar at the early stages of the growth of the plant. At the harvest stage, however, the differences in the yield and the nitrogen content of the inoculated and uninoculated crop were small.

The amount of nitrogen required by rahar for its full growth was supplied partly by the nitrogen fixed by the rhizobium in the nodule and partly by the soil. In an untreated soil, without any inoculation or any fertilizer treatment, these were almost equal.
Application of nitrogenous and phosphatic fertilizers reduced the amount contributed by the soil and increased nitrogen fixation by the crop from the atmosphere. The contribution from the soil might come from the applied fertilizer itself leaving the soil nitrogen undepleted and unused.

Application of molybdenum increased the yield and nitrogen content of radish and a concentration of 5 p.p.m. was found to be optimum for the crop in Delhi soil.

Studies on strain variation in *Rhizobium japonicum*: Twelve pure strains of *Rhizobium japonicum* were isolated from a single soybean (*Glycine max*) plant in Delhi soil from different nodules from different parts of the root system. Their morphological, cultural and biochemical characteristics were studied. It was believed that examination of the different strains of the same species of *Rhizobium*, isolated from different nodules of the same plant, would reveal the variations in the inherent character of the strains which had been subjected to the influence of similar environmental conditions.

The strains differed in their cultural characteristics and in action on milk and different sugars. They could be grouped in several classes according to their action on milk. They differed in their consumption of glucose in artificial culture. The differences between them were also reflected in their nitrogen fixing capacities as determined by sand culture experiments during which it was observed that larger number of healthy nodules were produced on the plant by inoculation with more efficient strains.

The strains with higher nitrogen fixing capacities generally did not exhibit any visible growth on potato and some growth occurred...
on potato in the case of the strains with low nitrogen fixing capacities. The strains having high nitrogen fixing capacities were also characterised by absence of a serum zone in milk and production of alkalinity.

The efficiency of a strain, as expressed by its nitrogen fixing capacity was found to be positively and significantly correlated with its capacity of consumption of glucose in artificial culture. The relationship between the efficiency of a strain and the amount of glucose consumed by the culture could be best expressed in the form of the equation

\[ E = 26.346G + 10.766, \]

where \( E \) was the nitrogen fixed in milligrams during growth of soybean plants inoculated with the strain for a period of eight weeks under conditions of the experiment and \( G \) was the amount of glucose in grams which disappeared during culture of the strain for six weeks.