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# BRIEF ARTICLES

## NODULATION OF *CICER ARIETINUM* L. IN FIELD-SOIL

The common gram, *Cicer arietinum* L. is cultivated throughout the plains of India as a food crop and is of high commercial value among leguminous crops. Gram plants normally produce numerous nodules of two distinct types, simple ellipsoidal nodules and complex branched ones, in fields where they are cropped every winter from October to February.

Since 1949, attempts were made to collect nodulated roots of *Cicer* from areas in and about Calcutta for isolation of *Rhizobium*. Every year *Cicer* plants were raised from different varieties of seeds in manured and unmanured experimental plots of Bethune College, Presidency College, R. G. Kar Medical College and in the New Alipore area, Calcutta, but nodules were consistently absent on the roots. The range of *p*H of the soil in the experimental plots was 5.8 to 6.8. During 1953–55, three months prior to the growing season, experimental plots had a yearly dressing of farmyard manure and of fresh soil from fields in the vicinity of Calcutta. These yearly dressings had, however, no effect on the production of nodules on roots of *Cicer* plants. This was, probably, due to the absence of suitable strains of *Rhizobium* necessary to produce nodules on these plants growing in the soil of Calcutta, paritcularly in the experimental plots. Preliminary experiments were, therefore, set up to study the effects of seed inoculation with strains of *Rhizobium* isolated from nodules of *Cicer* growing in Murshidabad district of West Bengal, India.

The plants were raised from locally collected seeds. Only well formed ripe seeds with unbroken testa were selected for the purpose. *Rhizobium* was isolated from nodules of *Cicer* plants grown in Berhampore (Murshidabad). Cultures of *Rhizobium* were maintained in *yeast-water-agar* medium (Bhaduri, 1951). Seeds were superficially sterilized with 0.04% $HgCl_2$ solution for 1 minute and subsequently washed with sterile water under aseptic condition. These were, then, kept in the suspension of bacteria in sterile tapwater made from a 2 to 3-days-old culture of *Rhizobium*. After 18 hours, the suspension was drained off and the seeds were again treated with a freshly prepared suspension of the same bacteria for 1 hour. The seeds were then aseptically sown on sterile agar slants in test-tubes ($8'' \times 1\frac{1}{2}''$). Several pots containing sterilized washed sand were also sown with similarly inoculated seeds. Plants were daily watered with culture solution just to soak the sand without overflowing the pots. Duplicate experiments were, also, set up in pots with unsterilized washed sand. In sand pots, nitrogen deficient culture solution was added as a nutrient material for the plants. To this culture solution, 2% agar was added to prepare slants in test-tubes (Bhaduri, 1951). The culture solution, however, was not added to the field plots.

Plants grown in test-tubes with added suspension of *Rhizobium* became nodulated within 15 days, while the controls remained free from nodules until the end of the experiment.

Experimental plots (6×4 ft.) in the field were sown with inoculated seeds. In the control plots the seeds were sown without inoculation. All the plants were harvested after 24 days' growth. Bacteria isolated from nodules of plants in each experiment were tested by plant passage in controlled conditions as described by Nutman (1946).

Adequate controls without inoculum were kept under identical conditions in each of the experiments. These control plants remained without nodules even after 3 months' growth (Plate I. fig. 1).Nodules produced by Berhampore strain in plots were mostly complex (Plate I fig. 2) owing to the partial fused condition during growth of a number of elongated nodules from a few adjacent loci on a root, while those in sand pots were generally simple and spherical to ellipsoidal in shape (Plate I. fig. 3).

Table 1. *Number of nodules per plant and size of nodules*

| Experiment | Number of Replicates | Number of nodules per plant | Range of mean diameter of nodules in mm. | Remarks on elongated or ellipsoidal nodules |
|---|---|---|---|---|
| Pot (Sterilized sand) | 15 | 3.2±4.0 | 1 to 3 | 1 nodule, 6 mm. long |
| Pot (Unsterilized sand) | 18 | 4.6±0.2 | 1 to 3 | 3 nodules, 4 mm.–5.5 mm. long |
| Field Plot | 12 | 3.8±0.2 | 1 to 3 | 1 nodule, 5 mm. long |

Table 1 shows that mean number of nodules of plants in these experiments were not significantly different and generally the ranges of the mean size of nodules were not variable. Sometimes, however, one or more nodules of a significantly bigger size were produced, and these were elongated or ellipsoidal in shape.

It was, further, observed that there was no residual effect even after two consecutive years of seed inoculation with the given strain of bacteria in the same plot of the field. The result of the experiments indicated that the experimental fields were devoid of the strain *Rhizobium* suitable to produce nodules on roots of *Cicer*, and that a good strain of a different locality was able to produce nodules on roots of plants grown in the soil. There is, however, reason to believe that the introduced strain of *Rhizobium* could not remain active enough to produce nodules in the succeeding year. Since *Lens esculentum* L., *Phaseolus radiatus* var. *aurea* Prain. *Phaseolus mungo* L., *Lathyrus sativus* L. or *Trigonella foenum-graecum* L. plants growing in adjacent or in between two experimental plots produced abundant nodules on their roots, it might be assumed that the micro-environment of the population of *Rhizobium* in the soil had, at least, some


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general conditions suitable for the growth of these bacteria. It has been shown that genetic factors in the legume host is of importance in the production of nodules of plants (Nutman, 1955), but *Cicer* seeds obtained from the local market and other sources were from naturally cross-pollinated plants and were evidently heterozygous in so far as the genetic factors for nodulation were involved. Such factors were not known to have any adverse effect on nodule production. The question, therefore, of establishing a strain of *Rhizobium* producing nodules on roots of *Cicer*, will depend on various factors, the chief among them may be competition between micro-organisms including different strains of *Rhizobium* and the applied strain (Thornton, 1954), presence of *Rhizobiphage* in the soil (Allen and Allen, 1950) and the secretion of antibiotic substances produced by micro-organisms of the soil including other strains of *Rhizobium* capable of attacking of applied strain of *Rhizobium* (Allen and Allen, 1950). At present we know too little to be able to assess the importance of any one or of a combination of a few or all of these factors in the establishment of any strain of bacteria in the soil specially in connexion with the legume seed inoculation.

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