NITROGEN NUTRITION AND RHIZOBIAL INOCULATION ON SOYBEAN (Glycine max (L.) Merrill)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Department of Agronomy

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D E C L A R A T I O N

I hereby declare that this thesis entitled "Nitrogen nutrition and rhizobial inoculation on soybean (Glycene max (L.) Merrill)" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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C E R T I F I C A T E

Certified that this thesis is a record of research work done independently by Sri. P. Geethakrishnan Nair under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to him.

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CERTIFICATE

We, the undersigned, members of the advisory committee of Sri. P. Geethakrishnan Nair, a candidate for the degree of Master of Science in Agriculture with major in Agronomy, agree that the thesis entitled "Nitrogen nutrition and rhizobial inoculation on soybean (Glycene max (L.) Merrill)" may be submitted by Sri. P. Geethakrishnan Nair in partial fulfilment of the requirement for the degree.

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INTRODUCTION

INTRODUCTION

Soybean (Glycene max (L.) Merrill) is cultivated in many parts of the world as a rich source of protein and oil. However, its cultivation in large areas in India is relatively recent.

Large scale experimentation of soybean in India was was started in the 1960's. By 1970 several varieties were released for cultivation especially in the northern States of the country. A world collection of over 800 varieties was screened for suitability in South India at IARI Sub Centre at Coimbatore. Based on this, about 25 varieties were selected as suitable for this region. These 25 varieties were again tested for their performance in red loam soils of Mannuthy, Trichur since 1976. The variety EC 39821 was found to perform well.

Being a legume, it is expected that the soybean plant will meet its own nitrogen requirement through symbiotic nitrogen fixation. However, as it is a new introduction, the soil may not be having the effective strains of nodulating bacteria, *Rhizobium japonicum.* If this is the case, most of nitrogen required for this crop should be met by soil supplies of mineral nitrogen. Again, if the native population of symbiotic nitrogen fixing organisms are not effective in nitrogen fixation, a drastic improvement of crop performance must be expected due to artificial inoculation with rhizobial cultures containing the appropriate strains.

In the presence of effective strains of symbiotic nitrogen fixers, an inverse relationship between nitrogen fixation and quantity of mineral nitrogen in soil is often observed. In many of the instances, a more or less effective balancing between nitrogen supply from soil and depression in symbiotic fixation have been reported. In such a situation no additional benefit due to nitrogen fertilisation may be expected in terms of growth, yield and nitrogen uptake in inoculated soybean. In the uninoculated crop, on the contrary, there must be favourable crop performance, yield and uptake of nitrogen with increasing levels of applied fertiliser nitrogen. If this is the trend of results, it must then be possible to arrive at the fertiliser equivalent of symbiotic nitrogen fixation due to culture inoculation by comparing the nitrogen uptake of inoculated unfertilised crop with the uptake of un-inoculated fertilised crop. It will thus be possible to work out the advantage due to rhizobial culture inoculation in quantitative terms.

It is recognised since long that the appropriate species of Rhizobium is different for different legumes. It was later established that there are strain differences within a species of Rhizobium and that the most effective strain was different for different legumes within a cross inoculation group. Recent work on this line indicates further that the most effective strain can be different for different soils. It is also known that in any soil, with the probable exception of soils in which a legume is continuously grown, there will not be an adequate rhizobial population initially. Population build up and effective nodulation occur gradually and the time lag varies with situations. Many an occasion, the time required is so long that the crop may initially suffer for want of nitrogen. In such situations also, artificial culture inoculation is recommended to ensure an adequate initial microbial population. Some advantage may thus be expected from rhizobial inoculation in the presence of native effective strains also.

The present study consisted of studying the performance of soybean (variety EC 39821) at graded levels of nitrogen with or without culture inoculation. The main objectives of the study were the following:

1. To assess the nitrogen requirement of the crop under inoculated and uninoculated conditions.
2. To arrive at the fertiliser equivalent of rhizobial inoculation.
3. To study the effect of rhizobial inoculation (Rhizobium japonicum) on the performance of soybean.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

A. Effect of applied nitrogen on soybean

A.1 Effect on growth and yield

Experimental results on the effect of nitrogen nutrition on the yield of soybean had been widely varying. Most of the experiments involving graded doses of nitrogen showed positive response upto a relatively low level of applied nitrogen only. There had been however, marked deviations from this general trend. In sons cases, there was no advantage due to nitrogen application and in some others significant response was obtained upto a relatively high level. A brief review of the work done on this aspect is given below, grouping the experiments under the above three categories.

Rao *et al* (1972) reported response in soybean yield upto 60 kg nitrogen per hectare during the *khariff* season. During the *rabi* season, on the contrary, there was significant response upto 180 kg nitrogen per hectare. Inoculation with rhizobial culture was done in both cases. Veeraswamy and Rethinaswamy (1974) observed that nitrogen application at 50 kg per hectare significantly increased grain yield over the control of no nitrogen supply and treatments with 25 kg nitrogen per hectare during the Monsoon season. During the other two seasons there was no yield increase due to application of nitrogen. Kesavan and Morachan (1974) observed that application of nitrogen resulted in a significant increase in protein content of grain, though a similar yield increase was not obtained. In a pot experiment by Nair and Tajuddin (1974) it was observed that there was a significant increase in plant height upto 200 ppm of applied nitrogen. Kang (1975) studied the effect of nitrogen fertilisers on soybean, with and without inoculation. Nitrogen application combined with inoculation was found to increase yield, dry matter, nitrogen uptake, pod number, seed weight and nitrogen content. It was concluded that 30 kg and 60 kg nitrogen per hectare were needed with and without inoculation respectively for maximum yield. Agarwal and Narang (1975) reported that maximum grain yield was obtained by the application of 20 kg nitrogen per hectare. Applied nitrogen was also found to increase the protein and oil content of grain appreciably. Sistachs (1976) based on field trials suggested that moderate yield of soybean can be obtained from inoculated currently available soybean cultivars with or without a small dressing of nitrogen. Sable and Khuspe (1976) reported significant linear increase in grain yield due to nitrogen upto 60 kg per hectare. Dry matter, pod and grain weight were also found to increase with increase in levels of nitrogen upto this level, but not beyond. Hundred grain weight also showed a similar trend. Bhangoo and Albritton (1976) found that seed yield from nodulating lines of soybean at low nitrogen level equals those from the non-nodulating lines at 112 kg and 224 kg applied nitrogen per hectare. At the higher nitrogen rates yield from both nodulating and non-nodulating lines were similar. Symbiotic nitrogen fixation was found to, practically, cease with 448 kg nitrogen per hectare while with 56 kg nitrogen per hectare there was no inhibition of symbiotic nitrogen fixation. Based on the experiments, it was recommended that for greatest utilization and efficiency of fixed nitrogen, applied nitrogen should be in the range 50 to 112 kg per hectare with allowance for residual soil nitrogen. Sable and Khuspe (1977), in an experiment found that there was increase in number of pods, plant height and grain and stover yield upto 30 kg nitrogen per hectare.

Andrews (1937) reported that ammonium sulphate increased the yield of soybeans significantly when applied in large amounts, but it had little influence on its nitrogen content. Takur and Hasan (1972) observed that the yield variations due to different levels of nitrogen were significant upto 190 kg per hectare. Though there was significant increase in yield due to *Rhizobium* inoculation, the deficiencies disappeared at the highest level of nitrogen viz 120 kg per hectare. The inhibiting effect of mineral nitrogen supplied through the fertilisers was attributed to be the reason for the lack of significant advantage due to inoculation at high nitrogen levels. In an experiment by Lawn *et al* (1974) supplemental nitrogen (448 kg per hectare) was applied as ammonium nitrate at the end of flowering. Seed yield and seed protein content increased by the application of nitrogen. Bhangoo and Albritton (1976) obtained yield increases in response to nitrogen (upto 220 kg per hectare) of 16 to 33 per cent more than check plots and 5 to 21 per cent more than PK plots during

1968 and 1969. Pal and Saxena (1976) conducted field experiments to determine the effect of varying levels of nitrogen fertilisation. They found that nitrogen fertilisation upto 300 kg per hectare had little effect on nitrogen concentration, amount of nitrogen in different plant parts or whole plants, nitrogen accumulation rate and seed yield per plant in inoculated nodulating lines. In non-nodulating iso-lines, the above attributes were significantly improved by application of nitrogen upto very high levels. The performance of non-nodulating lines reached parity with the nodulating lines at 200 to 300 kg nitrogen per hectare. Symbiotic nitrogen fixation was found to decrease practically to zero when nitrogen rate exceeded 224 kg per hectare. Fifty kg nitrogen had little effect on nitrogen fixation.

Andrews (1938) reported that inoculation produced significant increases in yield of soybeans where nitrate of soda or cyanamide was used and did not where urea or ammonium sulphate was used. He also found that inoculation did not increase the nitrogen content of soybean significantly where any sources of nitrogen were used. Lyons and Early (1952) in a two year field study observed that with adequate rainfall, moderate temperature and 30 to 40 days additional growing season, there was little or no response to added nitrogen. Welch (1974) found that neither nitrogen nor planting date affected yield. Kesavan and Morachan (1974) also reported that nitrogen application had no effect on seed yield or oil content. Carson and Shubeck (1974) in an experiment found that application of nitrogen upto 120 kg per hectare with 30 kg each of phosphorus and potassium did not increase soybean seed yield. Williamson and Diatloff (1975) conducted field experiments over four seasons. They observed that nitrogen upto 154 kg per hectare did not produce any response on nodulated soybeans. Olsen et al (1975) also observed a similar trend in Bragg soybean. Pal and Saxena (1975) reported that nodulating lines of soybean did not respond to nitrogen when they were either effectively inoculated or raised in a field which had been under inoculated soybean in the past. Ruiz et al (1976) found that nitrogen had no effect on yield of soybean upto 210 kg per hectare. Effect of concentration and source of added nitrogen ($NH4^{+}$ or $NO3^{-}$) on nodulation, nitrogen fixation and yield was studied in nutrient solution by Joseph (1977). Nodulation and nitrogen fixation were found to be severely suppressed by the addition of nitrogen either as NH_4^+ or NO_3^-.

A.2 Effect on nodulation

The reported relationship between levels of applied nitrogen and nodulation of soybean had been usually inverse, irrespective of the quantity of nitrogen applied. However, there are a very few instances in which there had been benefit on nodulation characteristics due to application of nitrogen in relatively small amounts.

Fellers (1918) reported that sodium nitrate inhibited nodule formation and consequent fixation of atmospheric nitrogen. Hels and Whiting (1928) found that normal nodulation of soybean was secured in non-fertilised soils of 25 to 40 per cent moisture content. Fertiliser application in amounts large enough to lower the percentage of germination also decreased nodulation. Application of either ammonium or nitrate salt in the above amounts decreased nodule formation. Doolas and George (1938) studied the effect of zonal distribution of nitrogen on nodulation of soybean. Application of nitrates to a portion of root zone resulted in a decrease in nodulation in this zone as compared to the nitrate free zone of the same plant. However, at very high levels of application of nitrates, the depressive effect of nitrate applied to a zone was also transmitted to the nitrate free zone. As compared to the decrease in nodulation due to the application of nitrates to the entire root zone, application to a single zone only resulted in a lower decrease in nodulation. In general, the depressive effects were more pronounced on the weight and development than in the number of nodules. Beard and Hoover (1971) reported that nodule number per plant was linearly and inversly related to the rate of nitrogen application. This inhibitory effect of fertilisers on nodulation was observed only when the fertilisers were applied at the time of planting but not when applied at flowering. Experiment conducted by Shakra and Bassiri (1972) indicated that nitrogen fertilisation upto 120 kg per hectare reduced the total number of nodules per plant. Rao and Reddy (1972) reported that highly significant reduction in nodule number was noticed due to nitrogen levels. The maximum number was at 0 level of nitrogen and lowest at 180 kg per hectare. Rao and Mader (1975) studied the nodulation process at different levels of nitrogen on the varieties EC 39824 and EC 14437. Nodulation started from the 22nd day and the maximum was attained on the 67th day. The nodule number declined then onwards through disintegration. Plants in control plot (no nitrogen) produced the largest number of nodules and the number decreased with increased nitrogen levels at each stage of observation, the decrease being significant 67 days after seeding. The number of nodules present at 60, 120 and 180 kg nitrogen per hectare levels worked out to 90.5, 79.4 and 68.4 per cent respectively. Ham et al (1975) found that nitrogen fertilisation increased seed yield, weight per seed and seed protein percentage in soybean. In nodulating plants, fertiliser nitrogen decreased nitrogen fixation, plant nodule weight, nodule number and weight per nodule. Hinson (1975) reported that nitrogen reduced nodule number. In an experiment Bhangoo and Albritton (1976) found that nodulation was decreased significantly by as much as 50 per cent with applied nitrogen.

Hatfield et al (1974) studied the effect of applied nitrogen on nodulation and early growth. They found that the number of nodules was increased by the addition of nitrogen for 2 weeks following seedling emergence in the variety tried.

B.1 <u>Effect of rhizobial inoculation on yield, nodulation and other characters</u>.

Results on the response to artificial inoculation with cultures containing <u>Rhizobium japonicum</u> had been contradicting, in some cases there being drastic increases in growth and yield of soybean and in some others very little or no response.

Lynch and Sears (1952) designed an experiment to evaluate the importance of inoculation of soybean when grown on land where well nodulated soybeans had been grown previously. They observed that neither soil treatment nor interval of time since the host plant had been grown had any influence upon crop response to inoculation. Even on plots which had not grown soybean since 1939 inoculation did not increase soybean yields. Dadarwal and Sen (1971) found that the survival of a large population of inoculated <u>Rhizobium</u> on the surface of legume seed is necessary for improving the chances of root-hair injection and thereby nodulation. According to Singh and Choubey (1971) the quantity of nitrogen fixed by the different strains of *Rhizobium* is in the range from 80 to 100 kg nitrogen per hectare in one planting season. Singh and Saxena (1972) conducted field experiments in 1968 and 1969 in silty clay loam soil rich in nitrogen and available phosphorus to evaluate the influence of nitrogen and inoculation on grain yield and yield contributing characters of Bragg soybean. They observed that inoculation increased grain yield considerably at all levels of nitrogen although the magnitude of differences was somewhat reduced at higher rates of fertilisation. An increase in nitrogen level from 0 to 80 kg per hectare in 1968 resulted in a linear increase in yield in the absence of inoculation because the nitrogen application recouped deficiency caused by lack of nodulations. But in the inoculated crop, there was a linear reduction in yield as a result of increase in the nitrogen level in the same range. It was concluded that the reduction in the nitrogen supply to the plants as a result of impaired symbiotic nitrogen fixation owing to the high rates of nitrogen application could not be counteracted by the increased mineral nitrogen supply from the fertiliser. The effect of inoculation and nitrogen upto 80 kg per hectare on the yield in 1969 was similar to those in 1968. In the experiment during 1969, nitrogen levels were raised upto 240 kg per hectare and the results showed similar performance of inoculated and uninoculated plants at 160 and 240 kg per hectare. Varma and Tiwari (1976) reported that oil percentage of seed decreased with increase in soybean yield and this decline was more marked following inoculation. In a small plot field trial conducted by Soos et al (1976) in Cuba, treatment with Rhizobium japonicum increased nodulation and yield of soybean. Peterson et al (1977) reported that high rates of inoculation at the rate of 10^4 to 10^8 rhizobia per seed at the time of planting increase soybean yields. Prokopenko and Vashchenko (1977) reported that seed inoculation increased plant height, nutrient uptake, plant dry matter, nitrogen content, seed yield and protein accumulation. The increase in seed yield was 17 per cent and increase in protein accumulation 2.34 per cent. Inoculation also tended to accelerate maturity.

Bajpal at al (1974) observed that rhizobial strains significantly increased yields of soybean. Pal and

Saxena (1975) reported a marked increase in nodulation of Clark and Harosay soybeans consequent to inoculation. Rao and Viswanatha (1974) found that inoculation increased number of nodules, dry weight of nodules and yield. Chesney Had *et al* (1973) and Tilak (1975) also reported increase in yield due to inoculation. Similar yield increases due to rhizobial inoculation on other legumes have been reported by Chahal *et al* (1976), Maheswari (1974), Sahu and Baheera (1972), Kadam *et al* (1977), Elango *et al* (1977) and Basdieek *et al* (1976).

Cardwell and Johnson (1971) found that soybean seed yields and seed protein percentages were not significantly increased by inoculating soybean seeds with *Rhizobium japonicum* at planting time. Miller (1972) reported that inoculation did not effect significantly the yield of soybean.

B.2 Efficiency of rhizobial strains

Experiments on the comparison of efficiency of different strains of *Rhizobium* had been widely done. In almost all the experiments, considerable differences in the efficiency of strains had been observed. The results of experiments conducted to compare the performance of different rhizobial strains on different soybean varieties indicated specificity of strains on different varieties. The most effective strain was also found to be different in different seasons for the same variety in some cases. A few reports are available comparing the performance of Rhizobium japonicum isolates from other legumes on soybean. Only some of these strains were found to induce nodulation but even these strains were ineffective on soybean. Studies on the effect of competition by rhizosphere bacteria on Rhizobium japonicum in general indicated that though a significant competition is noticed in agar medium, such a competition is non-existant in culture solutions and sterile soils. Work on the residual effect of culture inoculation indicated a higher residual effect, when local strains were used as compared to introduced strain.

Harper and Murphy (1928) reported that considerable differences exist in the ability of various cultures of soybean bacteria to produce nodules. Ruf and Sarles (1937) studied the response of soybean inoculated with good strains and poor strains of Rhizobium japonicum. The relative effectiveness of the strains was studied. The effective strains of *Rhizobium japonicum* produced relatively few large nodules, the majority of which were located in the immediate vicinity of the tap root near the surface of the soil whereas ineffective strains produced very small nodules that were scattered over the entire root system. The ineffective strain produced a greater number, volume and weight of nodules on each plant than the effective strains. The effect of *Rhizobium japonicum* strains on several growth characteristic of Lee soybeans was evaluated in field experiments by Abel and Erdman (1964). In fields free of soybean rhizobia, some strains were more effective than others in increasing seed yield, protein percentage of seed, root nodule, green leaf colour and fresh plant weight and decreasing oil percentage of seed. One strain was effective on one year and ineffective in another. They found that single strains were not better than strain mixtures. Burton and Curby (1965) tested the effectiveness of 2 types of commercial inoculants commonly used in pre-inoculating soybeans in a field free of soybean *Rhizobium*. The peat inoculant was superior to the broth inoculant in all time periods. Highly significant increases over the non-inoculated plots were obtained where inoculant was applied as long as 21 days in advance of planting. They also found that effective nodulation of soybean under field conditions is dependant not only on the presence of large numbers of live rhizobia on the seed but also on conditions which are favourable to growth and development of rhizobia. Blair et al (1966), in a study found that inoculation with Rhizobium japonicum did not produce nodulation benefits in field work. But in the green house studies inoculation-nodulation responses were recorded but not of any great degree. Evidence is presented on strain differences of cultures of the nitrogen fixing bacteria and of the relationship of numbers of bacteria in the inoculum to nodulation. Caldwell (1966) found that certain strains of Rhizobium japonicum failed to produce effective nodules on soybean variety Hardee. Gerenko (1967) reported that inoculation of soybean seedlings with a strain of nitragin (Rhizobium) resulted in an increase of 24.5 per cent in yield whereas with another strain it was 16.2 per cent. There were corresponding increases in 1,000 seed weight and protein content. Rensburg et al (1969) observed that of six effective strains of Rhizobium japonicum present in an inoculum, three produced nodules on one or more of the three soybean varieties tested. These results indicated a selective competition for nodule sites among the three strains which produced nodules on the three varieties. Caldwell and Grant vest (1970) inoculated soybean varieties with 28 strains and 2 commercial preparations of Rhizobium japonicum. They found significant yield differences associated with certain strains. Visittipitakul (1970) observed that on sterile soil, inoculation with nitragin gave a higher dry matter yield at harvest and a greater number of nodules than inoculation with the locally produced inoculant. The local inoculant showed no beneficial effect compared with the control. Diatloff (1970) studied strains of Rhizobium japonicum including 4 alkali treatment strains in the field at two sites using

4 varieties of soybean. The strains differed in their infectivity both between sites and between varieties when assessed for percentage nodulation and nodule number per plant. Brown et al (1971) reported that five strains of Rhizobium japonicum showed effective nodulation on three varieties, but seven other strains did not improve plant growth significantly compared with the uninoculated control. Chonkar (1971) observed that when the variety 'Bragg' was inoculated with 10 strains of Rhizobium japonicum, some strains were not more efficient than others in increasing yield. Single strains were not better than multiple strain of commercial inoculum. Rensburg et al (1971) in an experiment found that none of the 49 slow growing rhizobial strains from a culture collection, isolated from 25 legume species was able to induce an effective response on soybeans, a ineffective nodules were incited by 25 of these strains. A few ineffective nodules were formed on soybean raised from uninoculated seed in soils obtained from each of 10 localities. Balasundaram and Iswaran (1972) found that 8 isolates of Rhizobium japonicum showed a differential performance in terms of nodulation and effect on yield in 6 varieties of soybean. Rollier and Obaton(1972)

reported that experimental results from glass house and field observations on nodulation in varieties inoculated with 17 strains of Rhizobium japonicum from various sources showed different degrees of effectiveness of the strains. Sharma and Tilak (1974) reported that inoculation with UPAU-2 culture resulted in higher bean yield followed by nitragin (USA), Kanpur culture and then by IARI culture. Rao and Patil (1974) tested peat based Rhizobium japonicum inoculant obtained from different sources on variety 'Davis' near Bangalore during khariff 1972. There were significant differences in nodulation and growth status of the crop as a result of seed inoculation with different strains. There was no relation between the extent of nodulation and the grain yield of soybean as a result of inoculation with different strains of Rhizobium japonicum. Bhargava et al (1974) conducted field experiments with different indigenous cultures of Rhizobium japonicum at Pantnagar. They found that inoculation with an effective strain of Rhizobium japonicum was imperative for successful cultivation of soybean. Boonkered (1974) reported that variations existed in the nitrogen fixing ability of different strains of Rhizobium and their effect on soybean seed yield. In field and glass house studies 10 out of

30 strains of *Rhizobium japonicum* tested had effective and efficient nitrogen fixing ability. Such strains were able to fix about 1.5 kg N/rai/season. (1 rai = 0.16 ha). Rao and Patil (1974) observed that there were significant differences in nodulation and growth status of the crop as a result of seed inoculation with different strains of *Rhizobium japonicum*. Though the increase in yield of beans as a result of seed inoculation was not significant, it ranged from 41.31 to 172.36 per cent compared to control. There was no relation between the extent of nodulation and the grain yield of soybean as a result of inoculation with different strains of *Rhizobium japonicum*. Subbarao (1976) found that inoculation with *Rhizobium japonicum* increased yield of soybean. Experiments conducted by Kumar *et al* (1976) at Pantnagar revealed that inoculation with effective culture of *Rhizobium japonicum* was imperative for successful cultivation of soybean. In an experiment by Sriramaraju and Samuel (1976) it was found that in black soils all the inoculants they tried except IARI culture gave very good nodulation and significantly increased yield which ranged from 63.8 to 134 per cent over control. IARI culture produced considerable number of nodules but did not

contribute in any way for the betterment of the crop and was on par with the uninoculated nitrogen control. Rao and Patil (1977) tested 5 commercial inoculants of *Rhizobium japonicum* obtained from different Agricultural Institutions under field conditions to study their effect on nodulation, nitrogen fixation and bean yield. There were significant differences in number and dry weight of nodules per plant and also dry weight of the plant top as a result of inoculation with different cultures. All the inoculants with the exception of one increased the bean yield significantly compared to uninoculated control. Patel *et al* (1977) reported that most of the Karnataka soils lacked in soybean rhizobia and several strains of *Rhizobium japonicum* obtained from Jabalpur were ineffective in the area.

Rensburg and Van (1969) found that rhizobial strain competed in the rhizosphere and varieties appeared to select a dominant strain. Caldwell (1969) reported that the 3 strains tested differed in their competitive ability. Performance of combinations of two strains also showed significant differences. Smith (1972) studied the interactions between *Rhizobium japonicum* and soybean rhizosphere bacteria on agar plates and in conjunction with the legume host in liquid culture, vermiculite and sterile soil. Seventy eight per cent of 18 bacterial isolates inhibited *Rhizobium japonicum* on agar. In liquid culture total nodule number was greater in 87 per cent of the treatments including a rhizosphere bacterium. But these effects were not noted in vermiculite or soil. Smith and Miller (1974) made a study to find out interactions of *Rhizobium japonicum* and soybean rhizosphere bacteria. Eight out of nine rhizosphere isolates inhibited *Rhizobium japonicum* in agar. Nodulation was not affected by rhizosphere bacteria when plants were grown in vermiculite or in sterile soil. Lahiri (1977) found that there was differential competition between strains of *Rhizobium* in nodulating soybean cultivars.

Rewari *et al* (1973) studied the varietal response of soybean to nodulation with 2 strains of *Rhizobium japonicum* of different origin and also with a mixture of these strains. Inoculation with mixture of strains of *Rhizobium japonicum* affected the growth and nitrogen uptake significantly in 4 varieties compared with the inoculation with the IARI isolate in 2 varieties at 6 weeks stage. In pot trials by Lagacherie *et al* (1977) soybeans were inoculated with nitrogen fixing strains of *Rhizobium japonicum* or mixtures of these strains with a non-fixing strain. The presence of non-fixing strain was found to decrease vigour and dry weight of aerial parts of the plants.

Diatloff and Brookwell (1976) reported that a particular strain of *Rhizobium japonicum* was ineffective in nitrogen fixation with the related cultivars, Hardee and Geduld, but was highly effective with the cultivar, Hampton. They also reported that there was no evidence that naturally occurring ineffective *Rhizobium japonicum* posed any threat to the nodulating ability of effective inoculant strains. Rewari *et al* (1973) in an experiment inoculated 6 soybean cultivars with 2 strains of *Rhizobium japonicum* to study the individual and combined effect of nitrogen uptake and yield of shoot, dry matter and seeds. All cultivars gave significant increases in dry matter yield and plant nitrogen content when inoculated.With the IARI strain, the yields of cultivars, Masterpiece, Clark-63, Bragg and Punjab-1, increased 11,5,2 and 2 fold respectively when compared with the uninoculated control. It was concluded that strain variations and cultivar specificity appear to control the efficiency of *Rhizobium*.

Kabi (1976) observed that in trials with soybeans, seed inoculation with local strains of Rhizobium japonicum showed the greatest residual effect on nodulation in the following years, while inoculation with the introduced strains showed the least residual effect. The experiment conducted by Patil et al (1977) revealed that inoculation with nitragin and IARI culture significantly increased nodule numbers and increased seed yield by 16 per cent. The grain yield of wheat Cv. Kalyan 227 sown in the same plots in winter increased and in plots previously supporting soybean inoculated with IARI culture, the yield increased by 29 per cent. The need is stressed for selection of rhizobial strains suitable for the legume cultivar, the environmental condition and of maximum benefit to the following non-leguminous crop.

Weaver (1974) reported that in glass house trials only a few, of the 21 rhizobial strains isolated from nodules proved to be effective on pea nuts. It was concluded that, ground nut grown in Texas would respond to inoculation with highly effective rhizobia and that the field test would be necessary to verify the potential benefit from inoculation.

MATERIALS AND METHODS

MATERIALS AND METHODS

A field experiment was conducted at the Research Station and Instructional Farm (Kerala Agricultural University), Mannuthy, during May-September, 1977 to study the response of soybean to different levels of nitrogen and rhizobial inoculation.

1. Materials

1.1 Site and soil

The Research Station and Instructional Farm is situated at 10°32" N latitude and 76°10" longitude at an altitude of 22.25 meters.

The soil was an acid sandy loam. Data on the mechanical and chemical analysis of soil are given below:

Mechanical composition of soil

(expressed as percentage on moisture free basis)

Coarse sand	--	44.00
Fine sand	--	18.50
Silt	--	13.00
Clay	--	22.00

Chemical analysis

Constituent	Content in soil	Method used
Total nitrogen (percentage)	0.095	Microkjeldahl method
Total phosphorus(percentage)	0.027	In HCl extract; as ammonium phospho-molybdate, Volumetric.

Total potassium (percentage)	0.025	In HCl extract; flamephotometric.
Available phosphorus (kg/ha)	17.00	In Bray I extract; Chlorostannus reduced molybdophosphoric blue colour method.
Available potassium (kg/ha)	18.55	In neutral ammonium acetate extract; flamephotometric.
P^H in water	5.2	1:2 soil; solution ratio using a p^H meter.

1.2 Climate

The details of meteorological observations for the cropping season are given in Appendix I, and Fig. I. The weekly average of daily maximum temperature ranged from 27.7°C to 31.6°C and the weekly averages of the daily minimum temperature from 22.8°C to 24.3°C during the crop season. The relative humidity ranged from 62 per cent to 86 per cent. There were 116 rainy days during the period of 153 days. The total rain fall received during the period was 1985.9 mm. The maximum rain fall was received during the first week of July. Number of hours of bright sun shine per week ranged from 1.1 to 14.9. It was relatively low during the period from 1st week of June to 3rd week of July.

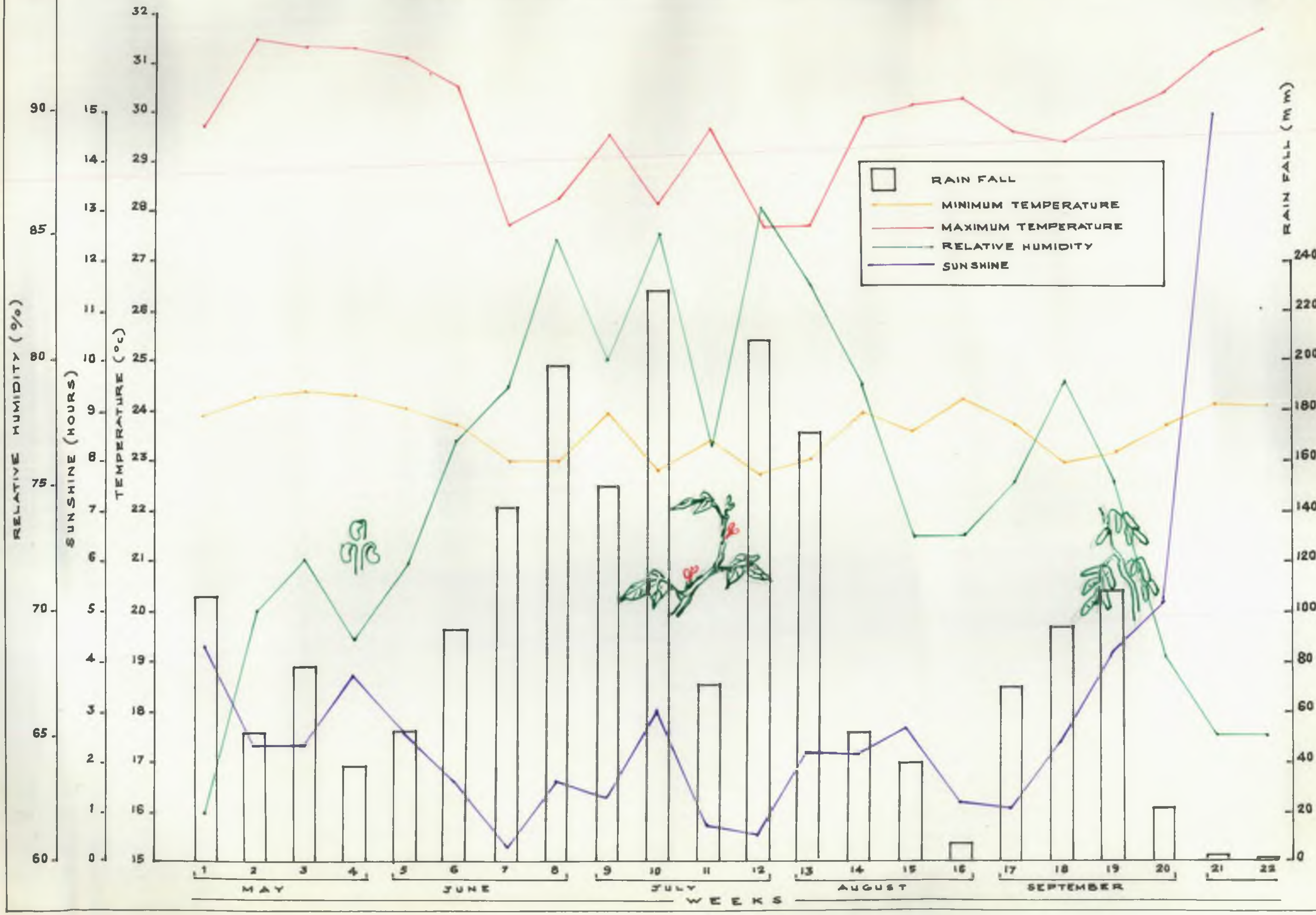


FIG. 1. WEATHER DATA FOR THE SEASON MAY - SEPTEMBER 1977.

1.3 Cropping history

The field was continuously under paddy during south-west and north-east Monsoon seasons. A crop of cowpea was raised in the preceding summer season.

1.4 Variety

Soybean variety EC 39821 obtained from the Botany Division of Tamil Nadu Agricultural University, Coimbatore, was used in the study. The variety was an introduction from Thailand (Kesavan 1970). The plant characters of this variety are furnished below:

Plant	—	Dwarf
Petiole	—	long leaflet - loosely arranged
Leaf shape	—	linear, lanceolate
Flower colour	—	purple
Days to flower	—	35
Days to maturity	—	90
Seed coat colour	—	yellow
Weight of 100 seeds	—	9.01 g

1.5 Fertilisers

Super phosphate and muriate of potash were used to supply phosphate and potash respectively at the rate of 80 kg per hectare each. Nitrogen in the form of urea was used in the study. Slaked line prepared on the spot was used at the rate of 1,000 kg per hectare.

2. <u>Methods</u>

2.1 <u>Lay out of experiment</u>

The experimental design was factorial in randomised block with 4 replications. Each replication consisted of 12 plots and each plot consisted of 4 beds of 4.5 m x 1m in size each. Each bed was seperated by furrows 0.3m wide and 20 cm deep. Randomisation of treatments was done with the help of random number table.

2.2 <u>Treatments</u>

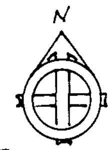
There were 12 treatment combinations of 6 levels of nitrogen and 2 levels of rhizobial inoculation.

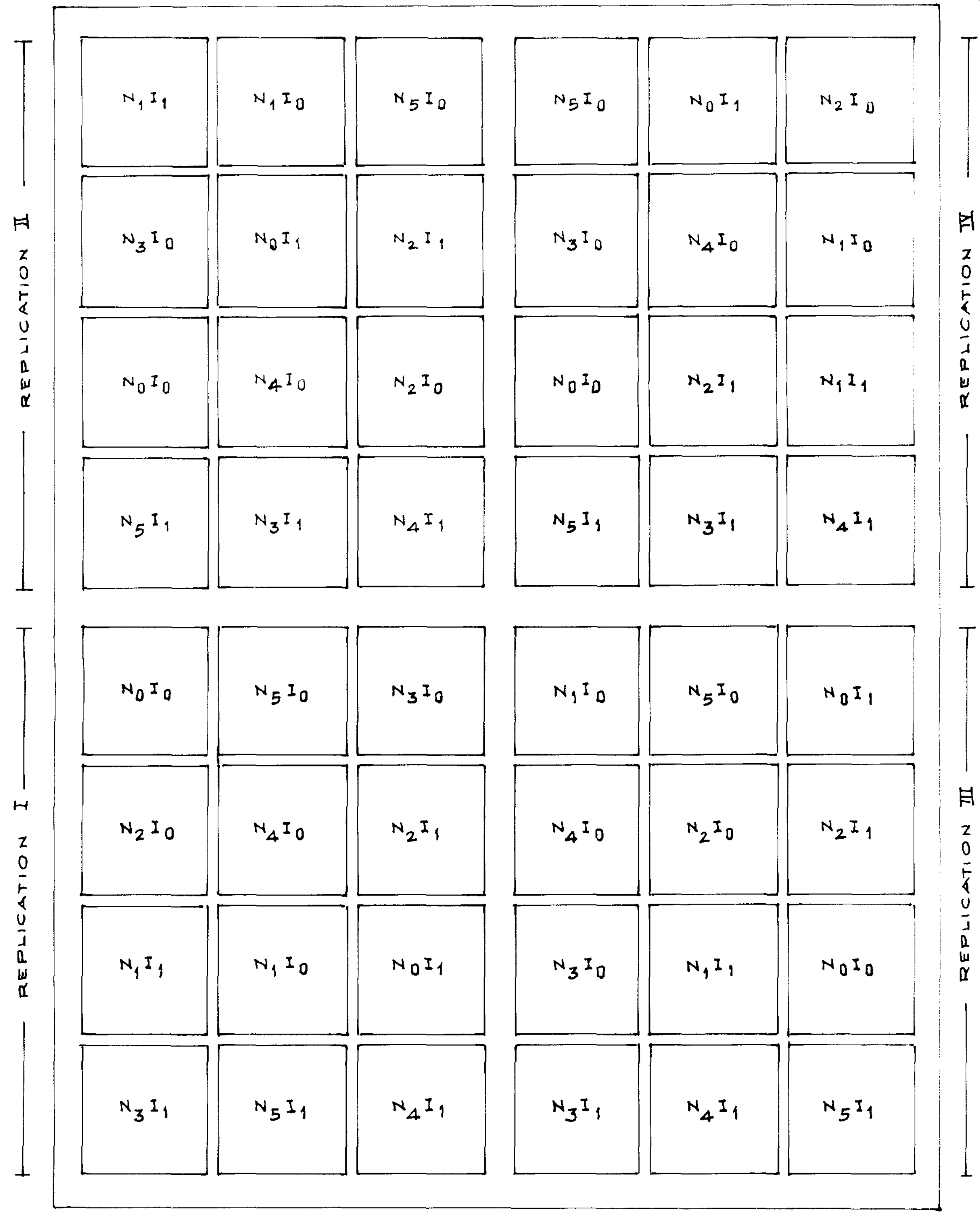
<u>Nitrogen</u>

N0	--	control, no nitrogen supplied
N1	--	50 kg per hectare
N2	--	100 kg per hectare
N3	--	150 kg per hectare
N4	--	200 Kg per hectare
N5	--	250 kg per hectare

FIG. 2.

LAY - OUT PLAN





N_0 - NO NITROGEN
N_1 - 50 kg N/ha.
N_2 - 100 kg N/ha.
N_3 - 150 kg N/ha.
N_4 - 200 kg N/ha.
N_5 - 250 kg N/ha.

I_0 - UNINOCULATED
I_1 - INOCULATED

GROSS PLOT SIZE - 4·5 m x 4 m.
NET PLOT SIZE - 3·90 m x 3·90 m.

Inoculation

I_0	—	Control
I_1	—	Inoculated

Treatment combinations were:

$N_0 I_0$	—	$N_0 I_1$
$N_1 I_0$	—	$N_1 I_1$
$N_2 I_0$	—	$N_2 I_1$
$N_3 I_0$	—	$N_3 I_1$
$N_4 I_0$	—	$N_4 I_1$
$N_5 I_0$	—	$N_5 I_1$

2.3 Field culture

2.3.1 Preparation of main field

The field was ploughed with tractor and later harrowed with a tiller to remove the weeds. Beds were taken and deep drainage trenches were provided along the boundary and also in between plots. Formation of trenches was necessary to provide drainage as the crop was raised in Monsoon season.

2.3.2 Liming and fertiliser application

Lime was broadcast on each bed and raked seven days prior to sowing. Fertilisers were applied as basal dressing. The three straight fertilisers were mixed and broadcast on beds and then mixed with the soil.

2.3.3 Seed treatment and sowing

For treating the seed with the inoculum, the procedure recommended by Tamil Nadu Agricultural University was followed. Jaggary syrup was prepared and cooled. The culture was added to the cooled syrup and mixed thoroughly. The seeds were mixed then with the culture and dried in shade.

Sowing was done on 21.5.1977. Sowing of inoculated seeds was done first. Thirty seeds were sown in each row of 1 m length and 45 cm space was given between rows. Though the stand per row was fixed as 20, excess seeds were sown to account for failure in germination. Seeds were sown in rows and a final population of 20 plants per row was retained to give an average spacing of 5 cm between plants in the row.

2.3.4 Thinning out

Germination was complete by 7th day. About 70 per cent germination was obtained in all the plots. Thinning out of seedlings was done to bring down the stand per row to 20.

2.3.5 Plant protection

Attack of leaf eating caterpillar was noticed when the plants were about 25 day old. Sevin was sprayed and the pest was brought under control.

2.3.6 Weeding and earthing up

Hand weeding and earthing up were done when the plants were 30 day old. Earthing up along with the deepening of drainage trenches was repeated after a month.

2.3.7 Harvesting

Harvesting was done when the plants were 106 day old. There was complete defoliation at the time of harvest.

3. Observations

Observations on the following characters were taken at various stages of growth.

3.1 Growth characters

1. Plant height
2. Number of branches
3. Leaf area index
4. Nodule count
5. Number of pods per plant
6. Dry weight of stem and petiole
7. Dry weight of pods
8. Seed weight
9. Shell weight

10. Number of seeds per pod

11. Net assimilation rate.

3.2 Observations at harvest

1. Grain yield
2. Stover yield
3. Moisture percentage
4. Test weight
5. Shelling percentage
6. Harvest index

3.3 Chemical studies

1. Per cent nitrogen in leaves
2. Per cent nitrogen in stem and petiole
3. Per cent nitrogen in seeds
4. Per cent nitrogen in shell

4. Sampling procedure

A bed in each plot was selected at random using randon numbers. One row of plants each was selected at randoa in these beds. Five plants were marked from the second plant in the row to take observations on plant characters. Similarly a set of five plants were selected on randomly selected rows for growth analysis. These plants were cut at the base at 40 days after sowing and from these samples, leaf samples were taken at random. Another set of five plants were cut for growth analysis at 70 days after sowing and a third set at harvest also.

5. Details of observation procedure

Details of observation procedure followed for each of the characters studied were as follows:

5.1 Plant height

Height of 5 plants selected was measured from the ground level to the terminal bud with a metre scale. The height was recorded in centimeters. Height was measured at 3 stages, 40 days after sowing, 70 days after sowing and harvest.

5.2 Number of branches

Number of branches was counted at two stages, 40 days after sowing and 70 days after sowing on the same plants on which plant height was measured. Number of branches was counted from the bottom to the top, leaving the terminal unopened bud.

5.3 Leaf area index

Five plants selected were cut at the base. Ten leaves were taken at random from the samples. Quality bond paper was weighed and the area per unit weight of the paper was determined. The paper was cut in the shape of 10 sample leaves and the weight of the 10 paper cuttings was determined. From this, the actual area of 10 leaves picked was calculated.

The leaves were dried in an oven at 70° to 80°C for 3 days and the leaf area per unit dry weight was calculated. The rest of the leaves was also picked seperately, dried in the oven, weighed and added to the weight of 10 leaves to get the total leaf weight. The leaf area of 5 plants was computed from the dry weight of total leaves and the area per unit dry weight. The leaf area index was then worked out by dividing the leaf area of 5 plants by the field area occupied by them.

5.4 Nodule count

Two sample plants from each plot were pulled out after lifting with a spade. The plants were transferred to a polythene sheet and the soil from the root was carefully removed. The total number of nodules was counted and the nodules per plant were worked out.

5.5 Number of pods

Number of pods on 5 plants was counted and pods per plant were worked out.

5.6 Dry weight of shoot

Dry weight of shoot was determined on the same plants on which leaf area studies were made. Immediately after all the leaves had been picked, these shoots were dried in an oven at 70° to 80°C for 3 days and then dry weight was taken. The dry weight of 10 leaf samples and of the rest of the leaves were added to get the total dry matter produced by 5 plants.

5.7 Net assimilation rate

The procedure given by Watson (1958) and Buttery (1970) was followed.

5.8 Dry weight of pods

Pods collected from 5 sample plants were dried in an oven at 70° to 80°C and weight was determined.

5.9 Dry weight of seeds and shell

Seeds and shells were separated from the pods which were used for determining the dry weight of pods. Weight of seeds and shell was determined separately.

5.10 Number of seeds per pod

Fifty pods were selected from 5 sample plants and the number of seeds per pod was counted and then the average worked out.

5.11 Grain yield

Net plots were harvested after excluding border plants. After threshing winnowing and cleaning, grain sample was drawn from each plot to determine the moisture percentage and test weight.

5.12 Stover yield

Stover from each plot was weighed separately after sun drying.

6. Chemical analysis

Total nitrogen was estimated by the Microkjeldahl procedure as given by Jackson (1958). Samples of shoot, leaves, seeds and shells were analysed seperately for total nitrogen.

7. Statistical analysis

Data on yield, yield attributes and growth characters were analysed statistically following the methods of Snedecor and Cochran (1967). 'F' test was carried out by analysis of variance method and significant results were compared by working out the critical differences.

RESULTS

EXPERIMENTAL RESULTS

The results of the experiment as influenced by the various treatments are summarised in the following text with the help of appropriate tables and suitable diagrams.

1. Yield

1.1 Grain yield

Data showing the grain yields are given in Table 1 and their analysis of variance in Appendix II.

There was no significant difference in yield between different levels of nitrogen. The yield obtained in control was almost equal to that obtained at 200 kg N/ha.

Inoculation has given a different trend. There was significant difference between treatments. Inoculation significantly depressed the yield.

The interaction effect was not significant.

1.2 Stover yield

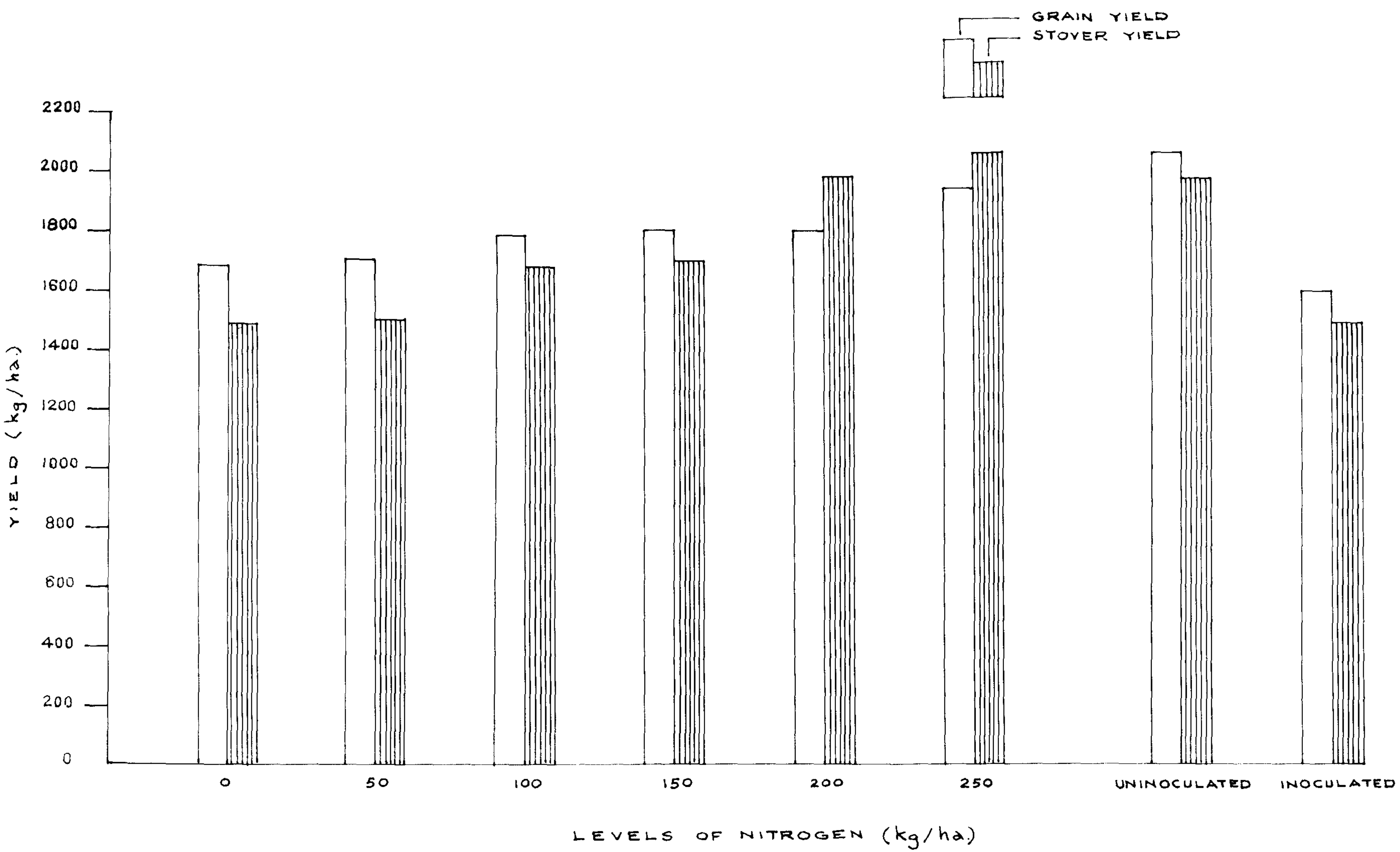
Data on stover yield are given in Table 1 and their analysis of variance in Appendix II.

Stover yield showed a trend similar to that of grain. With increase in levels of nitrogen there was a more or less steady increase in yield upto 250 kg N/ha. The difference

Table 1. Effect of levels of nitrogen and inoculation on yield, number of pods per plant and number of seeds per pod.

Treatments	Yield (kg/ha)		Number of	
	Seed	Stover	pods per plant	seeds per pod
Nitrogen levels (kg/ha)				
0	1678.6	1488.8	24.0	2.1
50	1689.7	1495.7	22.7	2.2
100	1780.3	1674.0	19.6	2.1
150	1794.8	1695.1	26.9	2.2
200	1829.0	1980.0	23.6	2.2
250	1950.4	2058.6	26.9	2.1
F-Test	N.S	N.S	N.S	N.S
S. Em. ±	220.5	158.9	3.2	0.226
CD at 5%	—	—	—	—
Inoculation				
Uninoculated	2064.3	1975.4	25.1	2.2
Inoculated	1594.8	1488.8	22.8	2.2
F-Test	Sig	Sig	N.S	N.S
S. Em. ±	127.6	91.7	1.9	0.045
CD at 5%	367.5	263.8	—	—

FIG. 3. YIELD AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.



in yield between nitrogen levels was, however, statistically not significant.

There was significant difference between inoculated and uninoculated treatments. As in the case of grain yield there was a depression in yield in inoculated treatments.

The interaction effect was not significant.

2. Yield components

2.1 Number of pods per plant

Data on the number of pods per plant are given in Table 1 and their analysis of variance in Appendix II.

Nitrogen application did not increase the number of pods per plant significantly.

Though statistically not significant uninoculated treatment showed superiority over inoculated treatment.

2.2 Number of seeds per pod

Data on number of seeds per pod are given in Table 1 and their analysis of variance in Appendix II.

Neither different levels of nitrogen applied nor inoculation influenced the number of seeds per pod.

2.3 Test weight of seed (100 grain weight)

Data on 100 grain weight are given in Table 2 and their analysis of variance in Appendix III.

Levels of nitrogen and inoculation highly influenced the 100 grain weight. With increasing levels of nitrogen, the test weight also tended to increase upto 250 kg N/ha.

The levels of significance were as follows: 250 kg N/ha was significantly superior to all levels of nitrogen from control to 150 kg N/ha. 100 kg N/ha was superior to control.

Uninoculated treatment was significantly superior to inoculated treatment.

Interaction was not significant.

2.4 Shelling percentage

Data on shelling percentage are presented in Table 2 and the analysis of variance in Appendix III.

There was significant difference between levels of nitrogen. 100 kg N/ha and 50 kg N/ha were significantly superior to control and 250 kg N/ha. There was no significant difference between 100 kg N/ha, 50 kg N/ha, 150 kg N/ha and 200 kg N/ha. Also there was no significant difference between 200 kg N/ha and control and 250 kg N/ha and control.

The uninoculated treatment was superior to inoculated treatment.

Table 2. Effect of levels of nitrogen and inoculation on 100 seed weight, shelling percentage, harvest index and moisture percentage.

Treatments	100 seed weight (g)	Shelling percentage	Harvest index (%)	Moisture percentage
<u>Nitrogen levels</u>				
(kg/ha)				
0	12.7	60.3	53.9	1.9
50	12.8	64.9	52.6	1.2
100	13.2	66.7	53.7	1.6
150	13.3	64.3	50.0	1.9
200	13.5	62.4	49.1	2.0
250	14.0	60.0	47.9	1.9
F-Test	Sig	Sig	N.S	N.S
S. Em. ±	0.179	1.53	2.015	0.443
CD at 5%	0.449	4.40	—	—
<u>Inoculation</u>				
Uninoculated	13.590	64.5	51.3	2.1
Inoculated	13.016	61.7	51.1	1.4
F-Test	Sig	Sig	N.S	N.S
S. Em. ±	0.105	0.88	1.164	0.255
CD at 5%	0.302	2.53	—	—

Interaction effect was not significant.

2.5 Moisture percentage

Data on moisture percentage are given in Table 2 and their analysis of variance in Appendix III.

There was no significant difference either between different levels of nitrogen or between inoculated and uninoculated treatments.

2.6 Harvest index

Data on harvest index are presented in Table 2 and their analysis of variance in Appendix III.

Neither inoculation nor applied nitrogen had any significant influence on harvest index. However, increase in applied nitrogen beyond 100 kg /ha tended to lower the harvest index. The lowest values were noted at the highest level of nitrogen.

3. Growth characters

3.1 Number of branches

Data on number of branches recorded 40 and 70 days after sowing are given in Table 3 and their analysis of variance in Appendix IV.

There was no significant difference in the number of branches in the first stage either between different levels

FIG. 4. HEIGHT OF PLANTS AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.

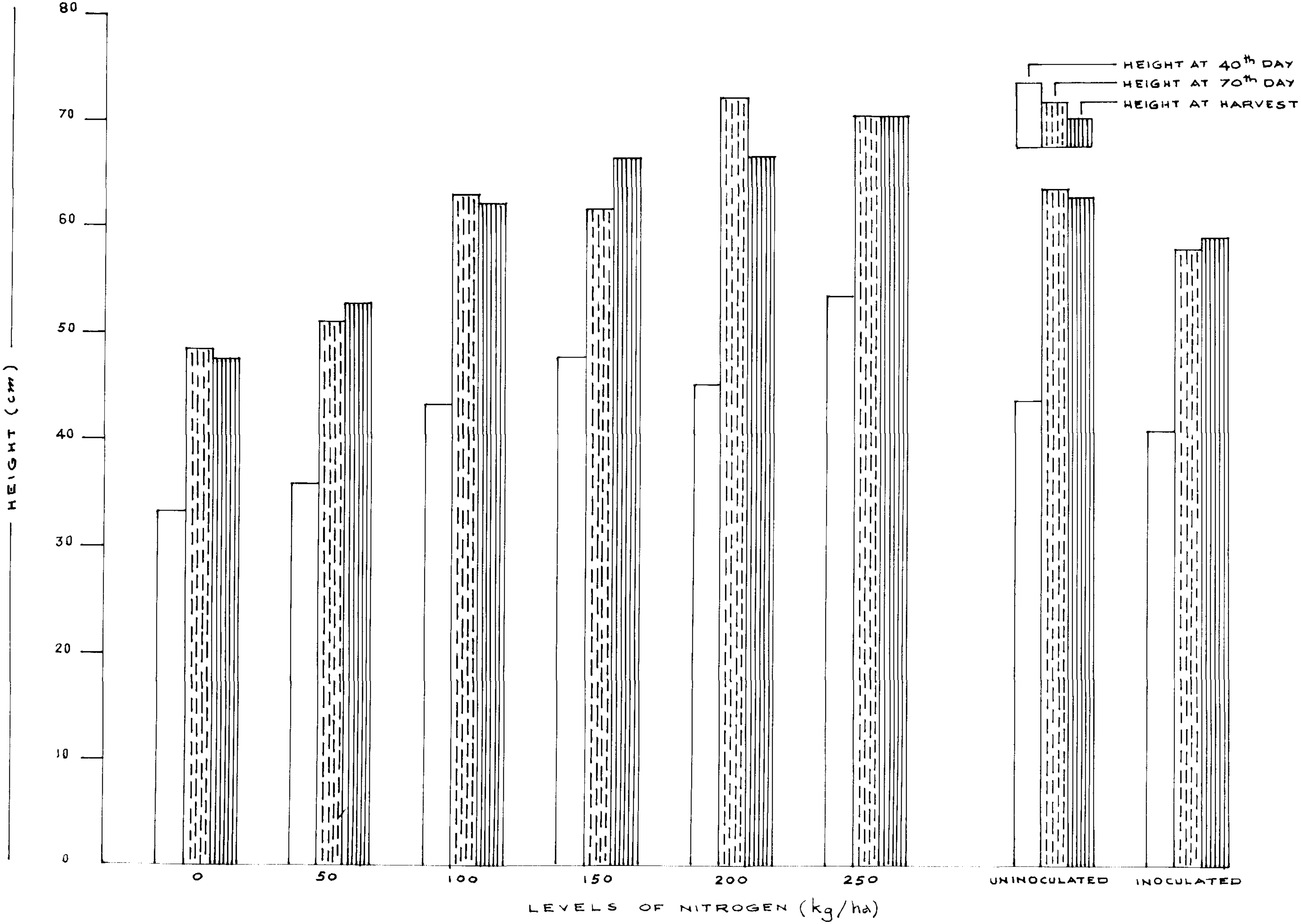
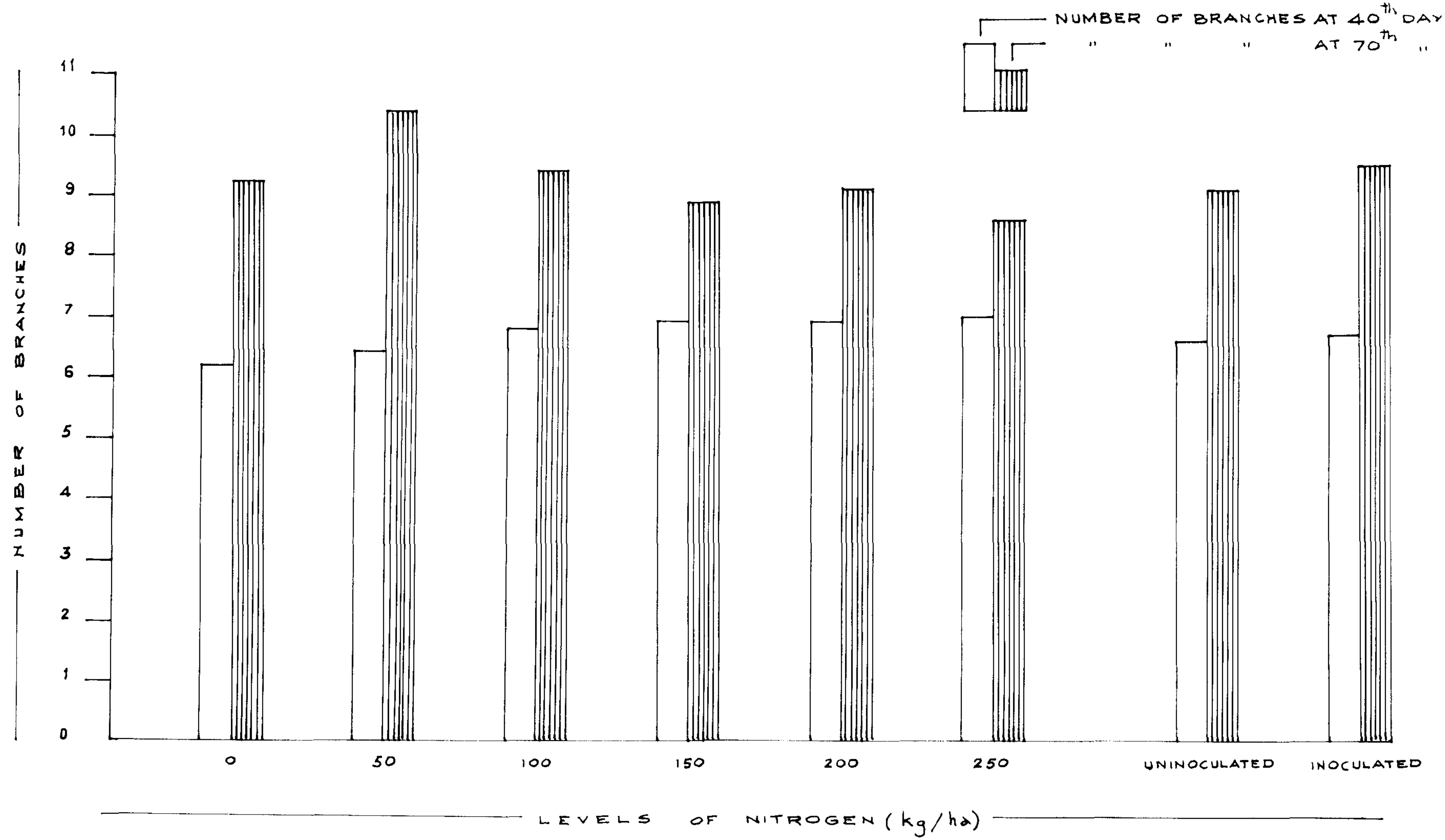


Table 3. Effect of levels of nitrogen and inoculation on number of branches and height.

Treatments	Number of branches		Height (cm)		
	40th day	70th day	40th day	70th day	Harvest
Nitrogen level (kg/ha)					
0	6.2	9.2	33.4	48.6	47.9
50	6.4	10.4	36.0	51.1	53.2
100	6.8	9.4	43.7	63.2	62.2
150	6.9	8.9	48.0	61.8	66.8
200	6.9	9.1	46.5	72.4	66.9
250	7.0	8.6	48.9	71.3	71.0
F-Test	N.S	Sig	Sig	Sig	Sig
S. Em. ±	0.22	0.37	2.17	11.8	3.05
CD at 5%	—	1.09	6.26	11.75	8.79
Inoculation					
Uninoculated	6.6	9.1	43.9	64.1	63.5
Inoculated	6.7	9.5	41.5	58.7	59.3
F-Test	N.S	N.S	N.S	N.S	N.S
S. Em. ±	0.12	0.21	1.25	2.35	1.76
CD at 5%	—	—	—	—	—

FIG. 5. NUMBER OF BRANCHES AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.



of nitrogen or between inoculated and uninoculated treatments. But levels of nitrogen influenced the number of branches in the second stage. 50 kg N/ha was superior to all other levels of nitrogen and control.

Though statistically not significant, number of branches showed slight increased mean value in the case of inoculated treatment in the second stage whereas in the first stage the difference was very negligible.

Interaction was not significant.

Number of branches increased from first stage to second stage.

3.2 Height of plants

Data on the height of plants are presented in Table 3 and their analysis of variance in Appendix IV.

The effect of levels of nitrogen was significant at all the three stages. With increase in levels of nitrogen, there was increase in plant height at all the three stages. At the first stage, 40 days after sowing, 250 kg N/ha, 150 kg N/ha and 100 kg N/ha were found to be superior to 50 kg N/ha and control.

At the second stage, 70 days after sowing, 250 kg N/ha,

Table 4. Effect of levels of nitrogen and inoculation on nodule number, leaf area index and net assimilation rate.

Treatment	Nodule number	Leaf area index		Net assimilation rate
		40th day	70th day	
Nitrogen levels (kg/ha)				
0	36.5	2.3	5.0	1.28
50	38.1	3.0	5.2	1.32
100	32.3	3.7	5.5	1.36
150	33.3	3.8	6.4	1.36
200	35.2	4.0	6.8	1.25
250	28.8	3.4	5.7	1.38
F-Test	N.S	N.S	N.S	N.S
S. Em. ±	6.75	0.41	0.83	0.302
CD at 5%	—	—	—	—
Inoculation				
Uninoculated	41.4	3.0	4.9	1.624
Inoculated	30.0	3.6	6.1	1.177
F-Test	Sig	N.S	N.S	N.S
S. Em. ±	3.9	0.24	0.48	0.173
CD at 5%	11.2	—	—	—

200 kg N/ha, 100 kg N/ha and 150 kg N/ha were found to be superior to 50 kg N/ha and control. 50 kg N/ha was significantly superior than control.

At harvest, the same trend as in the case of second stage was noticed.

Effect of interaction was not significant at any of these stages. However, the uninoculated treatments recorded higher mean values consistantly.

There was no significant difference between inoculated and uninoculated treatments. But, at all the stages, uninoculated treatments gave a higher mean value.

The height increased from first stage to second stage but there was not much difference between second stage and harvest.

3.3 Leaf area index

Data on leaf area index recorded 40 days and 70 days after sowing, are presented in Table 4 and their analysis of variance in Appendix V.

Neither levels of nitrogen nor inoculation caused significant difference in leaf area index at any of the stages.

FIG. 6. LEAF AREA INDEX AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.

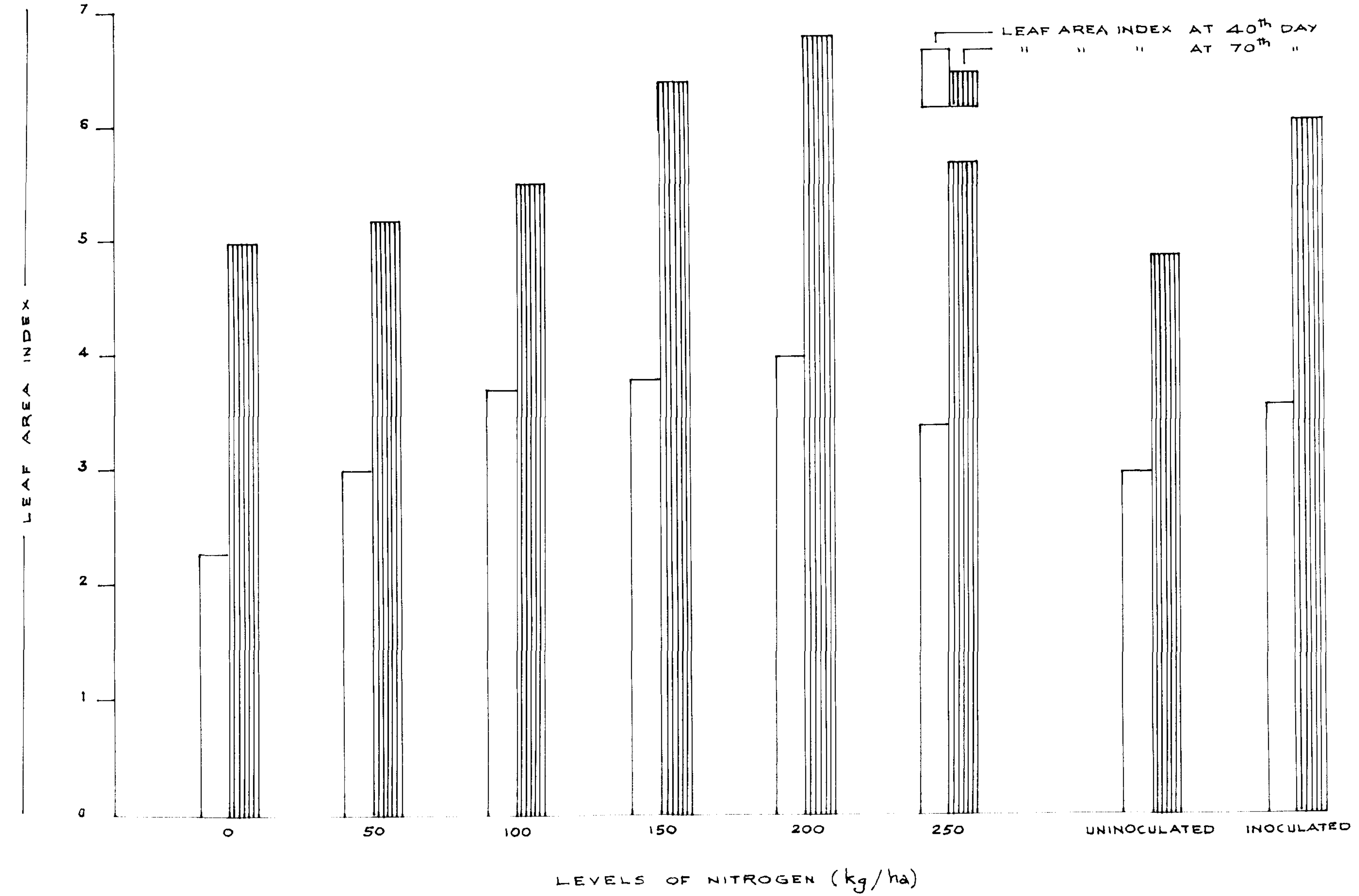


Table 5. Effect of levels of nitrogen and inoculation on dry weight of leaves and stem and petiole.

Treatments	Dry weight of leaves (g/5 plants)		Dry weight of stem and petiole (g/5 plants)		
	40th day	70th day	40th day	70th day	Harvest
Nitrogen levels (kg/ha)					
0	5.4	9.8	6.4	16.8	11.6
50	6.9	11.6	9.9	21.8	11.0
100	9.8	12.9	13.7	25.8	11.1
150	7.7	12.7	12.2	25.4	16.8
200	10.3	13.4	16.0	27.0	18.5
250	10.3	14.1	16.2	28.8	24.0
F-Test	N.S	N.S	Sig	N.S	Sig
S. Em. ±	1.360	1.482	1.905	3.160	2.304
CD at 5%	—	—	5.484	—	6.631
Inoculation					
Uninoculated	6.7	11.3	11.1	24.3	16.3
Inoculated	10.0	13.5	13.8	24.2	14.6
F-Test	Sig	N.S	N.S	N.S	N.S
S. Em. ±	0.785	0.856	1.100	1.824	1.330
CD at 5%	2.259	—	—	—	—

However, at the first stage, 40 days after sowing, control of no nitrogen supply recorded very low values as compared to treatments receiving fertiliser nitrogen.

In contrast to most of the other characters, inoculation resulted in higher leaf area index at both the stages.

Interaction was not significant in any of the stages. Leaf area index increased from first stage to second stage.

3.4 Net assimilation rate

Data on net assimilation rate between 40 days and 70 days after sowing are given in Table 4 and their analysis of varmiance in Appendix V.

Neither different levels of nitrogen nor inoculation influenced net assimilation rate significantly.

3.5 Dry weight of stem and petiole

Data on dry weight of stem and petiole at 40 days and 70 days after sowing and at harvest are given in Table 5 and their analysis of variance in Appendix VI.

Levels of nitrogen influenced the dry weight of stem and petiole in the first stage. There was no significant difference between 250 kg N/ha, 200 kg N/ha, 150 kg N/ha and 100 kg N/ha. There was no significant difference between

100 kg N/ha, 150 kg N/ha and 50 kg N/ha. Similarly, there was no significant difference between 150 kg N/ha, 50 kg N/ha and control. 250 kg N/ha and 200 kg N/ha were found to be superior to 50 kg N/ha and control. 100 kg N/ha and 150 kg N/ha were found to be superior to control.

However, in the second stage, dry weight was not influenced by any of the levels of nitrogen. But there was a slight linear increase in mean dry weight from control to 250 kg N/ha.

In the third stage, dry weight of stem and petiole was increased by different levels of nitrogen. 250 kg N/ha was significantly superior to all levels from control to 150 kg N/ha. 200 kg N/ha was superior to all levels including control but excluding 250 kg N/ha and 150 kg N/ha.

Inoculation had no influence on dry weight of stem and petiole at any of the stages. Dry weight of stem and petiole increased from first stage to second stage but then decreased.

3.6 Dry weight of leaves

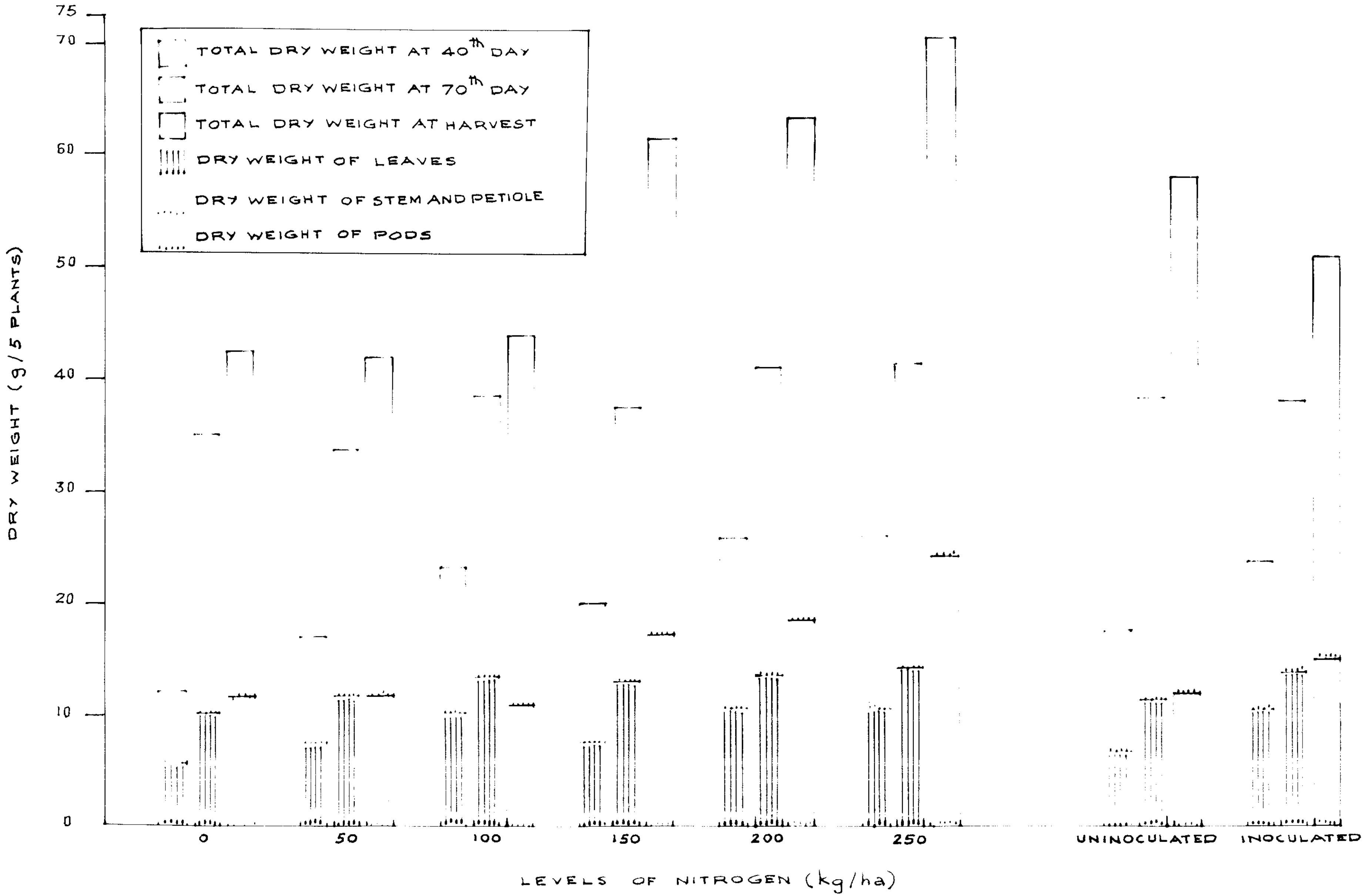
Data on dry weight of leaves at 40 days and 70 days after sowing are given in Table 6 and their analysis of variance in Appendix VI.

In both the stages, nitrogen levels did not influence

Table 6. Effect of levels of nitrogen and inoculation on dry weight of pods and total dry weight.

Treatments	Dry weight of pods (g/5 plants)	Total dry weight (g/5 plants)		
		40th day	70th day	Harvest
Nitrogen levels (kg/ha)				
0	30.8	11.9	33.3	42.4
50	32.8	16.8	33.5	42.3
100	32.9	23.1	38.7	44.0
150	44.9	19.9	37.6	61.7
200	44.9	25.9	40.5	63.7
250	41.7	26.3	42.9	70.5
F-Test	N.S	Sig	N.S	N.S
S. Em. ±	4.006	3.195	5.187	8.350
CD at 5%	—	9.197	—	—
Inoculation				
Uninoculated	40.9	17.4	37.8	58.2
Inoculated	36.1	23.8	37.7	51.0
F-Test	N.S	Sig	N.S	N.S
S. Em. ±	2.313	1.845	2.994	4.821
CD at 5%	—	5.311	—	—

FIG. 7. DRY WEIGHT OF STEM AND PETIOLE AND LEAVES AND TOTAL DRY WEIGHT AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.



dry weight of leaves. There was no significant difference between treatments. But though statistically not significant there was a linear increase in mean dry weight from control to 250 kg N/ha.

Inoculated treatment was superior to uninoculated treatment at the first stage and there was no significant difference in the second stage but at this stage also inoculated treatment gave a higher value.

Interaction effect was not significant.

Dry weight of leaves increased from first stage to second stage at levels of nitrogen and also at inoculation treatments.

3.7 Dry weight of pods

Data on dry weight of pods are presented in Table 6 and the analysis of variance in Appendix VII.

There was no significant difference either between different levels of nitrogen or between inoculated and uninoculated sets.

Interaction between levels of nitrogen and inoculation was highly significant.

3.8 Total dry weight

Data on total dry weight at 40 days and 70 days after sowing and at harvest are presented in Table 6 and their analysis of variance in Appendix VII.

Both levels of nitrogen and inoculation influenced the total dry weight at 40 days after sowing. 250 kg N/ha was superior to control and 50 kg N/ha. 200 kg N/ha was superior to control.

Inoculated sets were superior to uninoculated sets.

At 70 days after sowing or at harvest, neither levels of nitrogen nor inoculation influenced the total dry weight.

A similar trend as in the dry weight of stem and petiole was noticed in total dry weight, at different stages. The mean value was higher in inoculated sets at 40th day, it was almost equal at 70th day and was just reverse at harvest.

3.9 Number of nodules

Data on the number of nodules are presented in the Table 4 and their analysis of variance in Appendix V.

Levels of nitrogen did not influence nodule number.

There was significant difference between inoculated and uninoculated sets. Uninoculated sets were superior to inoculated sets.

Table 7. Effect of levels of nitrogen and inoculation on nitrogen content of stem and petiole.

Treatments	Nitrogen content (%) of stem and petiole		
	40th day	70th day	Harvest
Nitrogen levels (kg/ha)			
0	1.22	1.44	1.12
50	1.22	1.48	1.18
100	1.21	1.43	1.24
150	1.13	1.32	1.03
200	1.25	1.28	1.20
250	1.22	1.31	1.10
F-Test	N.S	N.S	N.S
S. Em. ±	0.122	0.138	0.126
CD at 5%	—	—	—
Inoculation			
Uninoculated	1.10	1.35	1.18
Inoculated	1.14	1.37	0.94
F-Test	N.S	N.S	Sig
S. Em. ±	0.071	0.077	0.02
CD at 5%	—	—	0.06

Interaction was not significant.

4. Chemical studies

4.1 Nitrogen content of stem and petiole

Data on nitrogen content of stem and petiole at three growth stages are given in Table 7 and their analysis of variance in Appendix VIII.

Different levels of nitrogen did not influence the nitrogen content of stem and petiole at any of the stages.

Inoculation had no effect at the first and second stages of observation. But uninoculated sets were found superior at harvest.

Interaction effect was significant at harvest, but not at the other two stages. The data showing the interaction effect are presented in Table 7a.

Nitrogen content increased from 40 days after sowing to 70 days after sowing at all levels of nitrogen applied and inoculation but then the values declined at harvest.

4.2 Nitrogen content of leaves

Data on the nitrogen content of leaves at two stages are given in Table 8 and their analysis of variance in Appendix VIII.

Neither applied nitrogen nor inoculation influenced the

Table 7a. Interaction effect of nitrogen and inoculation on nitrogen content of stem and petiole at harvest.

Nitrogen levels (kg/ha)	Inoculated	Uninoculated	Mean
0	1.40	0.84	2.24
50	1.63	0.74	2.37
100	1.33	1.13	2.46
150	0.96	1.10	2.06
200	0.91	0.50	1.41
250	0.89	1.31	2.20
Mean	7.12	5.62	12.74

CD for comparison between combinations.

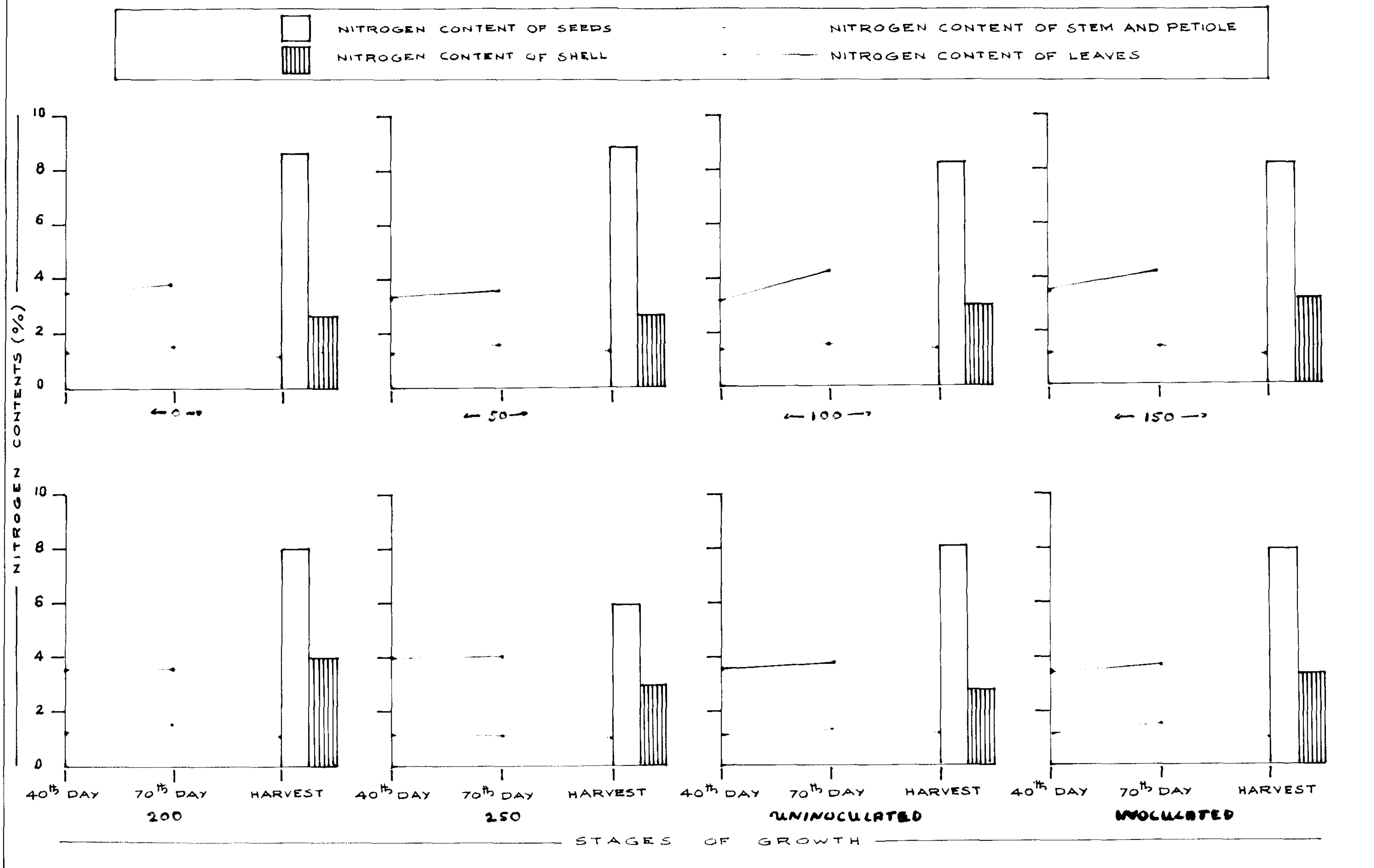
S. Em. ± = 0.090

CD at 5% = 0.259

Table 8. Effect of levels of nitrogen and inoculation on nitrogen content of leaves, seeds and shell.

Treatments	Nitrogen content of leaves (%)		Nitrogen content (%) of	
	40th day	70th day	Seeds	Shell
Nitrogen levels (kg/ha)				
0	3.38	3.64	8.64	2.47
50	3.18	3.51	8.75	2.62
100	3.08	3.89	8.16	2.91
150	3.35	3.98	8.12	3.20
200	3.62	3.61	7.59	4.01
250	4.16	3.87	6.22	3.07
F-Test	N.S	N.S	Sig	Sig
S. Em. ±	0.342	0.295	0.307	0.35
CD at 5%	—	—	0.884	1.007
Inoculation				
Uninoculated	3.50	3.74	8.14	2.82
Inoculated	3.42	3.62	7.85	3.28
F-Test	N.S	N.S	N.S	N.S
S. Em. ±	0.197	0.170	0.176	0.197
CD at 5%	—	—	—	—

FIG. 8. NITROGEN CONTENTS OF LEAVES, STEM AND PETIOLE, SEEDS AND SHELL AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.



leaf nitrogen content at both the stages. Though statistically not significant, uninoculated sets gave higher mean values. Nitrogen content increased from first stage to second stage at all levels of nitrogen applied except 250 kg N/ha. Inoculation also gave same trend.

4.3 Nitrogen content of shell

Data on nitrogen content of shell are presented in Table 8 and their analysis of variance in Appendix VIII.

Applied nitrogen influenced the nitrogen content of the shell. 250 kg N/ha, 200 kg N/ha and 150 kg N/ha were significantly superior to 100 kg N/ha, 50 kg N/ha and control.

Though statistically not significant inoculated treatment gave higher mean value.

4.4 Nitrogen content of seeds

Data on nitrogen content of seeds are given in Table 8 and their analysis of variance in Appendix VIII.

Applied nitrogen had a marked influence on the nitrogen content of seeds. Increasing levels of nitrogen tended to decrease nitrogen content. All levels of nitrogen from control to 200 kg N/ha were superior to 250 kg N/ha.

Table 9. Effect of levels of nitrogen and inoculation on nitrogen uptake by leaves and stem and petiole (kg/ha).

Treatments	Nitrogen uptake by leaves.		Nitrogen uptake by stem and petiole.		
	40th day	70th day	40th day	70th day	Harvest
Nitrogen levels (kg/ha)					
0	16.8	27.5	8.2	22.6	11.4
50	19.8	37.8	10.8	29.6	12.9
100	26.6	43.3	10.8	32.4	12.5
150	21.1	44.7	12.3	32.9	15.3
200	30.8	38.1	15.2	27.6	12.1
250	38.2	46.0	13.7	33.6	22.6
F-Test	Sig	N.S	N.S	N.S	N.S
S. Em. ±	5.011	6.7	2.2	5.1	3.146
CD at 5%	14.424	—	—	—	—
Inoculation					
Uninoculated	21.8	35.6	11.9	30.2	15.6
Inoculated	32.0	42.5	14.0	29.4	13.3
F-Test	Sig	N.S	N.S	N.S	N.S
S. Em. ±	3.034	3.9	1.7	2.9	1.817
CD at 5%	8.733	—	—	—	—

50 kg N/ha and control were superior to 200 kg N/ha also.

Inoculation did not influence nitrogen content of seeds. But the mean value was higher in uninoculated sets.

4.5 Nitrogen uptake by stem and petiole

Data on the nitrogen uptake by stem and petiole are presented in Table 9 and the analysis of variance in Appendix IX.

At all the three stages, 40 and 70 days after sowing and at harvest neither levels of nitrogen nor inoculation influenced the nitrogen uptake by stem and petiole significantly. However, there was linear increase in mean value from control to 250 kg N/ha though statistically not significant.

Inoculation gave a slightly lower value at the second and third stages. At all levels of applied nitrogen and inoculation, nitrogen uptake by stem and petiole increased from first stage to second stage and then decreased.

4.6 Nitrogen uptake by leaves

Data on the nitrogen uptake by leaves at 40 days and 70 days after sowing are given in Table 9 and their analysis of variance in Appendix IX.

Nitrogen uptake by leaves at 40 days after sowing was influenced by levels of nitrogen applied. 250 kg N/ha was significantly superior to 150 kg N/ha, 50 kg N/ha and control. Inoculated treatment was significantly superior to un-inoculated treatment.

The trend was different in the second stage. Neither levels of applied nitrogen nor inoculation influenced the nitrogen uptake by leaves.

With increasing age, there was increasing nitrogen uptake by leaves. The increase from 40th day to 70th day ranged from 20 per cent at 250 kg N/ha to 111 per cent at 150 kg N/ha.

4.7 Nitrogen uptake by seeds

Data on nitrogen uptake by seeds are given in Table 10 and the analysis of variance in Appendix X.

Both levels of nitrogen and inoculation did not influence the nitrogen uptake by seeds.

4.8 Nitrogen uptake by shell

Data on nitrogen uptake by shell are presented in Table 10 and their analysis of variance in Appendix X.

Neither levels of nitrogen nor inoculation did influence

Table 10. Effect of levels of nitrogen and inoculation on nitrogen uptake (kg/ha) by seed and shell and total nitrogen uptake.

Treatments	Nitrogen uptake by		Total nitrogen uptake at		
	Seeds	Shell	40th day	70th day	Harvest
Nitrogen levels (kg/ha)					
0	163.3	34.0	25.1	50.2	209.3
50	152.4	25.0	30.6	67.5	190.4
100	152.1	29.4	37.4	77.8	194.0
150	196.5	47.4	33.5	77.7	259.3
200	162.7	51.3	57.1	70.8	226.2
250	157.6	46.1	52.0	79.7	226.4
F-Test	N.S	N.S	Sig	N.S	N.S
S. Em. ±	23.7	7.9	6.9	8.6	31.1
CD at 5%	—	—	20.0	—	—
Inoculation					
Uninoculated	174.4	37.0	32.5	65.8	227.1
Inoculated	153.7	40.9	46.1	72.0	208.0
F-Test	N.S	N.S	Sig	N.S	N.S
S. Em. ±	13.7	4.6	4.0	5.0	17.9
CD at 5%	—	—	11.5	—	—

the nitrogen uptake by shell.

4.9 Total nitrogen uptake

Data on total nitrogen uptake are presented in Table 10 and their analysis of variance in Appendix X.

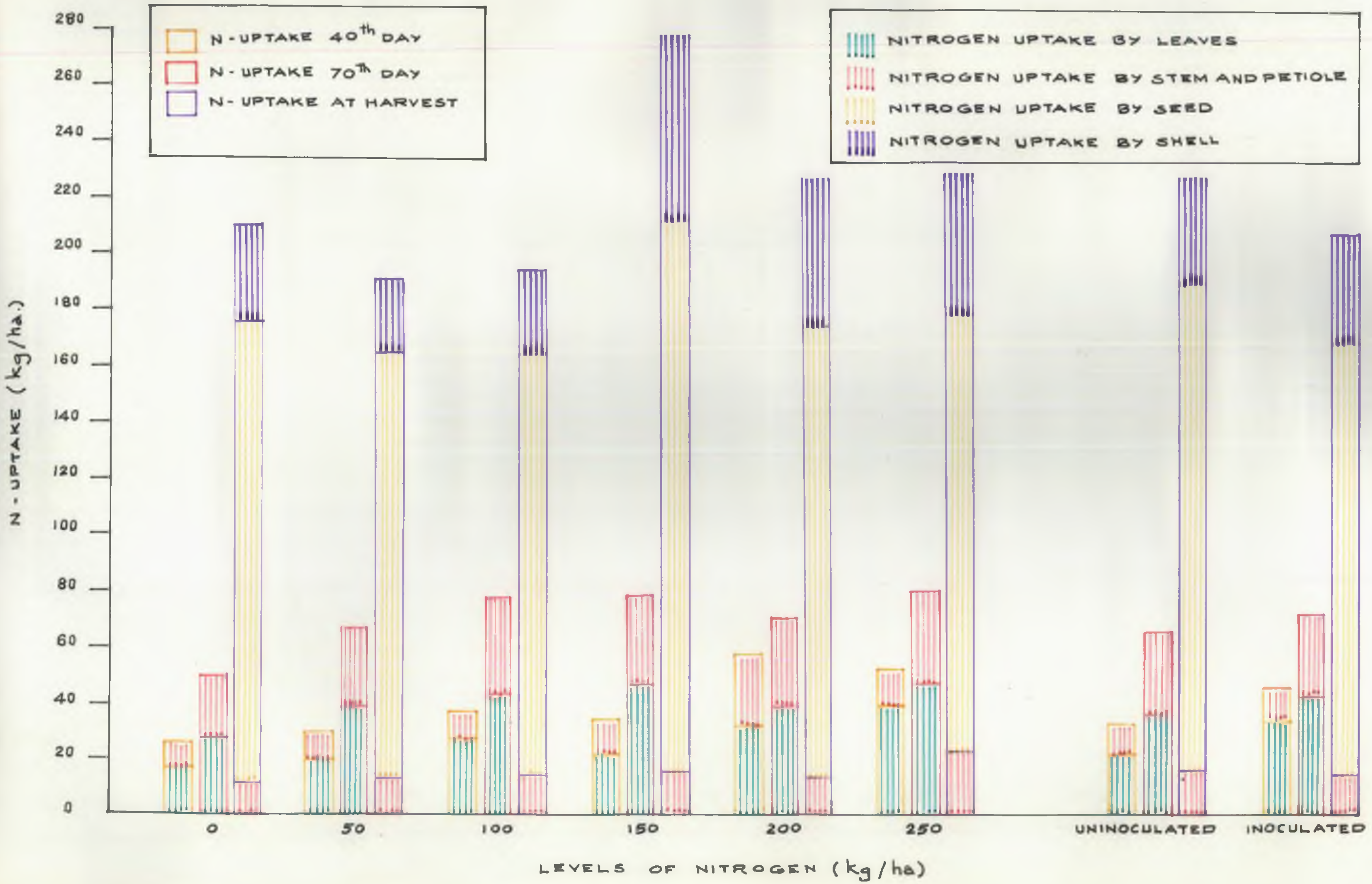
Levels of nitrogen had marked significant influence on total nitrogen uptake at 40th day. 250 kg N/ha was superior to all other levels of nitrogen except 200 kg N/ha. 200 kg N/ha was superior to all other levels of nitrogen.

Inoculated sets showed superiority over uninoculated sets.

At the second stage, 70 days after sowing, the difference in nitrogen uptake between levels of nitrogen narrowed down. Though the differences were not significant there was still a trend towards increase in uptake with increasing levels of nitrogen. The lowest mean uptake of 50.2 kg N/ha was observed for control as against 67.5 kg for nitrogen application at 50 kg N/ha and 79.7 kg for the highest level of 250 kg N/ha.

The inoculated sets recorded higher mean nitrogen uptake at this stage also, the difference were however not significant.

FIG. 9. N-UPTAKE OF LEAVES, STEM AND PETIOLE, SEEDS, SHELL AND TOTAL UPTAKE AS AFFECTED BY DIFFERENT LEVELS OF NITROGEN AND INOCULATION.



At harvest the trend of increasing uptake with increase in rate of application almost disappeared. The differences were not significant.

Unlike the previous two stages, the uninoculated treatment recorded higher mean nitrogen uptake at harvest. The differences as compared to treatment receiving inoculation was not significant.

Interaction was not significant at any of the above three stages.

Over the stages, there was an increase in nitrogen uptake with advancing crop growth in all the treatments. However, the uptake between the 70th day and the harvest was much more than the uptake between 40th and 70th day after sowing. The interval between the three stages of observation were more or less the same, 30 days between the first and second stages and 34 days between second and third stages.

DISCUSSION

DISCUSSION

The present investivation was taken up with a view to study the effect of different levels of nitrogen and rhizobial culture inoculation on growth and yield of soybean variety EC 39821. The soil of the experimental field was an acid sandy loam which contains 0.095 per cent nitrogen, 0.027 per cent phosphorus, and 0.062 per cent potassium.

The effect of nitrogen and inoculation on the plant characters contributing directly or indirectly to yield, growth and quality was studied to ascertain the magnitude of response to each character under observation.

From the result of the present investigation, it can be seen that the difference between treatments in respect of most of the characters studied are not statistically significant, though there is a trend in favour of certain treatments.

Grain yield

Different levels of nitrogen did not influence grain yield. Yield of control plot was almost on par with that of the highest level of nitrogen applied. This shows that nitrogen fixed symbiotically was adequate for crop growth.

The lack of significant response even upto the highest level of 250 kg N/ha also indicates that with increasing levels of applied nitrogen, there was depression in symbiotic nitrogen fixation. At all the varying levels of applied nitrogen, quantities of applied nitrogen were more or less balanced by the decrease in symbiotic fixation. This will be further evidenced by the data on nitrogen uptake which showed practically little difference between levels of nitrogen application. Such a depression in nitrogen fixation consequent to mineral nitrogen supply has been widely observed (Bhangoo and Albritton (1976), Fellers (1918), Ham et al (1975) and Hat field (1974)). Similar observations on lack of significant improvement in yield of soybean in field experiments were reported earlier by Lynch and Sears (1952), Sriramaraju and Samuel (1976).

The following discussion on the effect of fertiliser nitrogen on growth characters and yield components will further support the above conclusions. Among the various growth measurements, only the following characters showed significant increase due to nitrogen application. These are number of branches at the second stage of observation

40 days after sowing, plant height at all the three stages, total dry weight at the first stage and dry weight of stem petiole at the first and third stages. Among these characters, the variation in number of branches was inconsistent and progressive increase was not observed with increasing levels of nitrogen. In the case of total dry weight at the first stage there was a steady increase with increasing levels of nitrogen. This trend however did not persist and at the subsequent two stages, there was no significant difference between levels of nitrogen. Dry weight of stem and petiole on the contrary was consistently higher at higher levels of nitrogen at all the three stages though the differences fell short of statistical significance at the second stage. Plant height increase with increasing levels of nitrogen was consistent and was statistical significant at all the three stages. None of the other characters was significantly affected by nitrogen levels. The results thus show that in at least a few growth characters, there was significant improvement upto the first stage of observation. This may be mainly because applied nitrogen contributed substantially to nitrogen requirement of the crop upto the stage of initial nodule formation and

effective symbiotic nitrogen fixation. According to Norman (1963) nodulation starts 9 days after sowing in soybean and effective nitrogen fixation starts after about 22 days. When symbiosis became effective and nitrogen fixation could take care of the nitrogen requirement of the crop, the advantage due to mineral nitrogen supply disappeared. This explanation, however will not hold true in the case of two plant characters viz. plant height and dry weight of stem and petiole which showed significant differences upto the stage of harvest. The probable reasons for this deviation will be discussed separately.

Of the yield components, test weight and shelling percentage were significantly affected by levels of applied nitrogen. The variation in shelling percentage with increasing levels of nitrogen was inconsistent. In the case of test weight, however, there was progressive increase with increasing levels of nitrogen. As evidenced by the lack of significant increase in yield, this improvement in this yield component alone could not affect the final yield appreciably.

Artificial inoculation with rhizobial culture resulted in a significant depression in yield. This shows in all probability that the introduced strain of Rhizobium japonicum was less effective in nitrogen fixation on soybean than the strains already present in the soil. Differences in effectiveness of various strains of Rhizobium japonicum on soybean have been reported by several workers {Sharma and Tilak (1974), Rao and Patil (1977), Rensburg H.J. Van (1969)}. According to Norman (1963) there can be different strains of bacteria in different nodules of a legume though in general, a single nodule will contain only a single strain. By artificial culture inoculation a certain percentage of nodules would have been formed with the introduced strain. If this introduced strain was less effective than the strains already available in the soil, it would then explain why the yield of inoculated set was lower than the uninoculated series.

A similar trend of superiority of uninoculated series over the inoculated was noted also in several growth characters, nodulation and yield of stover. Among these, the depressive effect of inoculation was significant in the case of nodule number and yield of stover.

A perusal of the data on nitrogen uptake will further substantiate the effect of nodulation. At the first stage of observation, total nitrogen uptake was significantly higher in the inoculated treatment. At the next two stages, differences were not significant. Though not significant, even at the second stage, the inoculated set recorded a higher mean nitrogen uptake. At harvest, the reverse was the trend and inoculation led to a decrease in total uptake. As indicated by a significantly higher nitrogen uptake in the inoculated set at the first two stages, inoculation was beneficial initially. This may be due to the supply through culture inoculation of a large number of nitrogen fixing organisms, though less effective than the native population. This trend also indicates that the soil initially did not have a sufficiently large rhizobial population of the required strain. At later stages, this setback was more than compensated by multiplication of the native, more effective strains. The over all effect was thus a superiority of the native strains in terms of nitrogen uptake, nodulation and yield. Data on nodulation are however not adequate to explain such a larger initial nodulation due to culture inoculation. Only one observation on nodule count was taken and that was at a relatively later stage - 80 days after sowing.

Yield of stover

With increasing levels of nitrogen, there was a trend towards increase in stover yield. The differences, however were not significant.

As in the case of grain yield, inoculation with the culture led to a significant decrease in stover yield also. The reasons for the lack of response of the crop to applied nitrogen and the significant superiority of uninoculated treatment have been discussed already.

Yield components

Data on the yield components indicate that among the observations recorded, viz. number of pods per plant, seeds per pod, test weight and shelling percentage, only the last two showed significant differences between levels of nitrogen. Among these two characters the trend in variation of shelling percentage was inconsistent. In the case of test weight, on the contrary, there was significant increase with increasing levels of nitrogen. Such an improvement in this growth character alone indicates that enhanced soil nitrogen supply was beneficial towards the later stages of crop growth. The other two characters viz. pod number and seeds per pod are decided much earlier. According to Norman (1963) symbiotic nitrogen fixation ceases 50 days prior to maturity. It is probable that residual applied nitrogen contributed substantially towards crop requirement at this stage when symbiotic nitrogen fixation stopped. At the earlier stages when pod number and seed number per pod could be effected by nitrogen supply through fertiliser, symbiotically fixed nitrogen could, probably take care of nitrogen requirement completely. Continued nitrogen supply either in itself or through the continued maintenance of photosynthetically active leaves for a longer period might have contributed towards the higher test weight in treatments of nitrogen supply. Again, data are not adequate to substantiate such a continued activity of photosynthetic organ for a longer period. It may, however, be recalled in this context that the improvement in only one of the yield components was not adequate enough to significantly increase final grain yield of soybean.

Harvest index was worked out taking into account the dry weight of aerial parts only, excluding the root portion. As may be deduced from the lack of significant variation in grain and stover yield, harvest index also did not significantly differ between treatments.

Growth characters

1. Number of branches

The variation in number of branches with increasing levels of nitrogen was significant at the second stage of observation only. Even at this stage, the variation was not steady and consistent. Inoculation had no effect on this character.

2. Plant height

There was significant increase in plant height at all the three stages. This is in contrast to most of the other growth characters, yield components and yield. A close study of the data over the different stages will explain this deviation. As had been mentioned elsewhere, effective symbiotic nitrogen fixation in soybean starts by about 22 days after sowing. The requirement of nitrogen upto this stage would have to be met from soil nitrogen supplies. Applied fertiliser nitrogen apparently must have had a decisive role in deciding growth upto this stage. In the case of soybean a substantial part of increase in plant height is achieved in the early stages. The mean plant height 40 days after sowing was around 42.5 cm as against 61.4 cm. 70 days after sowing and at harvest. Calculated as percentage of the height at harvest, the height 40 days after sowing was about 69 per cent. It is this pattern of plant height increase in early stages when symbiotic nitrogen fixation could not make any substantial contribution towards nitrogen supply that might have resulted in a significant effect of applied nitrogen on this character.

Inoculation did not apparently have any significant effect on plant height, though there was a tendency for a higher mean value for the uninoculated set.

3. Leaf area index (LAI)

Neither levels of nitrogen nor inoculation had any significant effect on leaf area index. The average for the first stage, 40 days after sowing was 3.3 as against 5.5 for the second stage. According to Evans (1975) the optimum LAI for soybean at bloom is between 5 and 8.

The mean values observed in this experiment are not much deviating from this optimum value reported. In all probability, there was practically no marked mutual shading due to excessive foliage development in any of the treatments.

The reasons for lack of growth response to applied nitrogen and inoculation have been discussed already.

4. Net assimilation rate (NAR)

As in the case of leaf area index, net assimilation rate also did not differ significantly between nitrogen levels and also due to culture inoculation. This lack of significant difference indicates that there was no appreciable difference in the efficiency of leaves for photosynthesis in any of the treatments. This may also support the conclusion that there was little mutual sheding in any of the treatments. Inoculation also did not appear to effect NAR markedly.

5. Number of nodules

Levels of nitrogen did not influence the number of nodules. As a discussion on yield of grain and nitrogen uptake will reveal, there was evidently inhibition of symbiotic nitrogen fixation due to application of nitrogen fertilisers. Such an inhibition could express itself either through a reduced nodule number or through lowering of effectiveness of nodules. The lack of significant differences in nodule number in this experiment indicate that the contributing factor was mainly the subdued effectiveness of nodule bacteria.

Inoculation, on the contrary, led to a significantly lower nodule number. It was earlier mentioned that inoculation might have probably resulted in a larger initial nodulation due to culture inoculation. The observations recorded at a relatively late stage, 80 days after sowing, however indicate a reverse trend. Such a reversal in the nodulation pattern is possible because several batches of nodules form during the growth period of soybean. When the native bacteria could multiply and build up the required more effective population at a later stage, they were probably more effective than the introduced strains in bringing about nodulation. According to Rao and Mader (1975) several batches of nodules form on soybean during its life cycle and the average life span of a nodule is about 45 days.

6. Dry weight

Total dry weight of aerial parts differed significantly due to levels of nitrogen at the first stage of observation only. The trend of increase in dry weight with increasing levels of nitrogen, however, continued till harvest but the differences fell short of statistical significance at the last two stages. As had been mentioned elsehwere, the reason for a significant treatment difference at the first stage and lack of significant variation later lies in the time lag between sowing and symbiotic nitrogen fixation. In soybean, effective nitrogen fixation starts by about 22 days after sowing. Applied nitrogen had its impact on dry matter accumulation during this period when symbiosis could not take effect . At later stages when nitrogen fixation became effective, the differences between levels of nitrogen tended to narrow down.

Among the plant parts that contributed to total dry weight the variation in dry weight of stem and petiole followed an exactly identical trend as that of total dry weight. The dry weight increase of stem and petiole with increasing levels of nitrogen also was significant at the first stage. The differences in dry weight of leaves at the first two stages and those of pods at harvest were not significant.

The differences in total dry weight due to culture inoculation was significant at the first stage only when inoculation led to a significant superiority. The reasons for the initial advantage due to culture inoculation have been discussed already. At the next stage, treatment differences tended to equalise and at harvest, uninoculated treatment recorded a higher mean total dry weight. Among the components contributing to total dry weight, dry weight of leaves at the first stage gave significantly higher mean figures due to culture inoculation. Differences in leaf dry weight at the next stage and those of stem and petiole and pods at all the stages were not significant.

Over the stages, there was a more or less steady increase in total dry weight upto the harvest stage. Among the components, leaf dry weight increased upto 70 days after sowing. Observations at harvest could not be taken as there was more or less complete defoliation prior to harvest. In the case of stem and petiole there was increase upto the 70th day after which there was a drastic decrease at harvest. Loss of petiole by defoliation might have been the dominant factor for this loss in dry weight. However there might also have been translocation of some carbohydrates from these parts to the developing pods leading thus to a decrease in dry weight of stem and petiole.

Among the components contributing to total dry weight, stem and petiole had a dominant role at the first two stages, the percentage contributions being 60.4 and 64.3 respectively. At harvest on the contrary, pods contributed about 70.5 per cent of the total dry weight.

Nitrogen content

Percentages of nitrogen in plant parts excepting seeds and shell did not differ significantly between levels of nitrogen. In the case of seeds, nitrogen content decreased with increasing levels of nitrogen, the decrease being conspicuous especially at very high levels. In the case of shell, on the contrary, the trend was reverse and nitrogen percentages increased with increasing levels of nitrogen applied. Such a marked difference in the nitrogen accumulation pattern between seeds and shell is possible if the rate at which nitrogen is translocated from other parts towards seeds differs from the rate at which this nutrient moves to the shell. It may however, be noted that the decrease in nitrogen content of seeds assumes statistical significance only at and beyond 200 kg N/ha.

The difference in nitrogen content due to culture inoculation was significant only in the case of stem and petiole at harvest. At the earlier two stages the differences were not significant. The occurrence of a significantly lower nitrogen content at harvest alone might be probable because of a higher rate of translocation of nitrogen from stem and petiole in the inoculated series. This is possible especially as the inoculated plants were less effective in nitrogen fixation and because there might have been a greater strain for nitrogen at the grain filling stage in the plants that were less efficient nitrogen fixers.

Over the stages, there was an increase in nitrogen content of stem and petiole and leaves upto the 70th day. At harvest on the other hand, there was a marked decrease in content of nitrogen in stem and petiole. Such a trend of increase in nitrogen content of tissues with growth is not common in plants, excepting probably in legumes. In soybean the increase in nitrogen content upto 70th day is probably a consequence of continued effectiveness of symbiotic nitrogen fixation upto this period. It may also be noted that even by an application of fertiliser nitrogen at 250 kg/ha at the time of planting, this trend was not reversed in the case of stem and petiole. However, in the case of nitrogen content of leaves, the trend of increasing nitrogen content with advancing crop growth did not persist beyond 150 kg N/ha. At harvest, the nitrogen content of stem and petiole dropped down considerably indicating thereby a fair degree of translocation of nitrogen from these vegetative parts to the developing reproductive organs. There might have been such a similar translocation from leaves also.

Nitrogen uptake

Total nitrogen uptake was significantly different between levels of applied nitrogen at the first stage of observation 40 days after sowing. With increasing levels of nitrogen, this trend of increase in uptake continued upto 200 kg N/ha. At the

the subsequent two stages significant treatment difference disappeared. The reasons for such an observation have been discussed already. An exactly identical trend was noted in the case of nitrogen uptake by leaves also which significantly increased with increasing levels of nitrogen at the first stage of observation but not later. In the case of stem and petiole, there was no significant difference between nitrogen levels at any of the three stages of observation.

Culture inoculation led to a significant improvement in nitrogen uptake at the first stage of observation. The same trend continued at the subsequent stage also without there being statistically significant differences. At harvest the reverse was the trend and the treatment with culture inoculation gave a lower mean value. The reasons for enhanced nitrogen uptake at the early stages and the discontinuance of this trend at later stages have been discussed in detail earlier.

Between the stages, there was an increase in total nitrogen uptake upto harvest. The rates of uptake between the stages were, however, widely different. The mean uptake on the 40th day and was 39.3 kg /ha as against 68.9 kg on the 70th day and 217.5 kg at harvest, 110 days after sowing. Calculated as uptake per day, the mean value for the first 40 days was

0.98 kg/day and that between 40th day and 70th day, was also 0.98. The corresponding figure for the period from 70th day till harvest was 3.71 kg/day. This means, in other words, that the average rate of uptake was more than thrice during the last phase. A significant part of uptake and for that reason symbiotic fixation should have thus continued during this period of 70-110 days . According to Norman (1963) nitrogen fixation in soybean stops 50 days prior to maturity. The uptake pattern observed in this experiment indicates that atleast in the variety under trial, nitrogen fixation should have continued for a substantially longer period.

A similar trend of increasing nitrogen uptake was noted in the case of leaves and also stem and petiole upto the second stage, 70 days after sowing. However the increase over the stages was more or less steady and in terms of quantity meagre as compared to total uptake. The mean uptake by leaves on the 40th day was 26.9 kg/ha and that on the 70th day, 39.0 kg/ha, the percentage increase being 45. The corresponding figures for uptake by stem and ptiole were 12.9 kg, 29.8 kg and 130 per cent respectively. In terms of quantity, thus uptake by leaves was more than petiole but the rate of increase in uptake by petiole was more conspicuous.

At harvest, uptake by stem and petiole dropped substantially, the overall mean percentage drop in uptake being 52. The mean quantity of decrease in nitrogen uptake is 15.4 kg. This loss in uptake between 70th day and harvest might have been partially because of loss of some part of petiole at the time of harvest and partly because of translocation of nitrogen from these vegetative parts to the developing seeds.

A comparison of the relative contribution by the different parts towards total uptake will reveal that at the first stage, 40 days after sowing, leaves contributed more towards uptake, its percentage being 67. The remaining 33 per cent was by stem and petiole. With advancing growth, the relative contribution by stem and petiole increased to 43 per cent and that by leaves decreased to 57 per cent on the 70th day after sowing. At harvest stage, the major contributing component was seeds, the percentage share by this part being 75, shell and stem and petiole contributing to 18 and 7 per cent respectively. The discussion on percentage contribution by plant parts towards total uptake may be summarised as follows: In the early stages, most of the nitrogen in the plant is concentrated in the leaves. With advancing growth, more of nitrogen accumulated in stems and petiole. At harvest, the major part of nitrogen occurs in seeds followed by a relatively small quantity in shells. Negligibly small quantities occur in the remaining plant parts at harvest. It may also be worthwhile emphasising that about 75 per cent of the nitrogen accumulated by the soybean plant, be it through nitrogen fixation or by other means, is getting concentrated in the harvestable produce.

One of the primary objectives of conducting the experiment was to arrive at the fertiliser equivalent of rhizobial inoculation. The results indicate a non-significant variation in total nitrogen uptake due to culture inoculation. The fertiliser equivalent of culture inoculation or the quantity of applied nitrogen that is equivalent in effect to culture inoculation in this experiment therefore works out to zero.

It may also be worthwhile exploring the possibility of arriving at an approximate value of the quantity of nitrogen symbiotically fixed by soybean or the fertiliser equivalent of it from the uptake data. The lack of significant increase in nitrogen uptake consequent to application of graded doses of nitrogen upto 250 kg/ha indicates that even these heavy rates of applied nitrogen are not equivalent in effectiveness to the quantity of nitrogen symbiotically fixed. It may thus be concluded that under the conditions of experimentation, the quantity of nitrogen fixed by the soybean plant exceeds the fertiliser equivalent of 250 kg N/ha applied basally.

SUMMARY

SUMMARY

An experiment was conducted at the Research Station and Instructional Farm, Mannuthy on the nitrogen nutrition and rhizobial inoculation on soybean.

The growth characters, yield attributes and yield as influenced by various treatments were statistically analysed. The percentages of plant nitrogen on different plant parts was also analysed. The results of the investigation are summarised below:

1. Different levels of nitrogen did not influence grain yield. This shows that nitrogen fixed symbiotically was adequate for crop growth. The lack of significant response even upto highest level of 250 kg N/ha indicated that with increasing levels of applied nitrogen, there was a depression in symbiotic nitrogen fixation. The affect of fertiliser nitrogen on growth characterestics and yield components supported the above conclusions. Artificial inoculation with rhizobial culture, resulted in a significant depression in yield. This indicates that the introduced strain of *Rhizobium japonicum* was probably

less effective in nitrogen fixation on soybean than the strains already present in the soil.

2. With increasing levels of nitrogen, there was a trend towards increase in stover yield. The differences, however, were not significant. As in the case of grain yield, inoculation with the culture led to a significant decrease in stover yield also.

3. Among the observations recorded on the yield components, test weight andshelling percentage only showed significant difference between levels of nitrogen. The trend in shelling percentage was inconsistent. But there was significant increase with increasing levels of nitrogen in the case of test weight. This indicate that enhanced soil nitrogen supply was beneficial towards the later stages of crop growth. The other two characters viz. pod number and seeds per pod showed no response to applied nitrogen. Harvest index also did not differ significantly between treatments.

4. The variation in the number of branches with increasing levels of nitrogen was significant at

the second stage of observation only. The variation was not consistent. Inoculation had no effect.

5. Plant height increased with increasing levels of nitrogen. The plant height increase in the early stages when symbiotic nitrogen fixation could not make any substantial contribution towards nitrogen supply might have resulted in a significant effect of nitrogen on this character.

6. Neither nitrogen nor inoculation influenced the leaf area index. The mean values in this experiment are not much deviating from the optimum values reported.

7. Net assimilation rate also did not differ significantly between nitrogen levels and also due to culture inoculation. This indicated that there was no appreciable difference in the efficiency of leaves for photosynthesis in any of the treatments.

8. Levels of nitrogen did not influence the number of nodules. The lack of significant differences in nodule number in this experiment indicated that

the contributing factor was mainly the subdued effectiveness of nodule bacteria. Inoculation reduced the nodule number.

9. Total dry weight of aerial parts differed significantly due to the levels of nitrogen at the first stage only, 40 days after sowing. The reason for this and significant variation only in the early stages lies in the tine lag between sowing and symbiotic nitrogen fixation. The dry weight of stem and petiole followed the same trend. Inoculation influenced the total dry weight at the first stage only. At harvest, uninoculated treatment recorded a higher value. Over the stages, there was a more or less steady increase in total dry weight upto the harvest stage. Dry weight of stem and petiole increased upto 70th day and then decreased.

10. Percentage of nitrogen in plant parts excepting seeds and shell did not differ significantly between levels of nitrogen. In the case of seeds nitrogen content decreased with increasing levels of nitrogen. In the case of shell, the trend was reverse. This may be due to the difference between

the rates at which nitrogen is translocated from other parts towards seeds and shell. The difference in nitrogen content due to culture inoculation was significant only in the case of stem and petiole at harvest. Over the stages there was an increase in nitrogen content of stem and petiole and leaves upto 70th day.

11. The total nitrogen uptake was significantly different between levels of applied nitrogen at the first stage of observation, 40 days after sowing. At the subsequent two stages significant treatment differences disappeared. Similar trend was noticed in the nitrogen uptake by leaves also. In the case of stem and petiole there was no significant response to levels of nitrogen. Culture inoculation led to a significant improvement in nitrogen uptake at the first stage of observation. At harvest, the reverse was the trend. Between the stages, there was an increase in total nitrogen uptake up to harvest. Similar trend was noticed upto second stage in nitrogen uptake by stem and petiole and leaves but later declined.

12. The quantity of nitrogen fixed by the soybean plant appears to exceed the fertiliser equivalent of 250 kg N/ha applied basally.

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* Original not referred.

APPENDIX I

Weather data (weekly averages) from 1st May to 30th September, 1977

Date	Weeks	Temp °C Maximum	Temp °C Minimum	Relative humidity	Total rainfall (mm)	No. of rainy days	Sun shine (hours)
1	2	3	4	5	6	7	8
May 1 – May 6	1	29.7	23.9	62	106.6	4	4.3
May 7 – May 13	2	31.4	24.3	70	51.8	5	2.4
May 14 – May 20	3	31.3	24.4	72	78.2	5	2.5
May 21 – May 27	4	31.3	24.3	69	39.0	5	3.7
May 28 – June 3	5	31.1	24.0	72	52.6	5	2.6
June 4 – June 10	6	30.5	23.7	77	92.8	6	1.6
June 11 – June 17	7	27.7	23.0	79	140.8	7	0.3
June 18 – June 24	8	28.2	23.0	85	198.3	7	1.7
June 25 – July 1	9	29.5	23.9	80	150.5	7	1.3
July 2 – July 8	10	28.1	22.8	85	227.7	7	3.1
July 9 – July 15	11	29.7	23.4	77	69.6	6	0.7

1		2	3	4	5	6	7	8
July 16	– July 22	12	27.7	22.8	86	207.7	7	0.5
July 23	– July 29	13	27.8	23.0	83	173.8	7	2.1
July 30	– August 5	14	29.9	23.9	79	52.5	5	2.2
August 6	– August 12	15	30.1	23.6	73	40.5	5	2.6
August 13	– August 19	16	30.3	24.2	73	7.3	2	1.2
August 20	– August 26	17	29.6	23.7	75	69.6	7	1.1
August 27	– September 2	18	29.4	22.9	79	93.9	5	2.4
September 3	– September 9	19	29.9	23.1	75	107.5	7	4.2
September 10	– September 16	20	30.3	23.7	68	22.1	5	5.2
September 17	– September 23	21	31.2	24.0	65	2.1	1	14.9
September 24	– September 30	22	31.6	24.0	65	1.0	1	

APPENDIX II

Analysis of variance for yield, number of pods and seed per pod.

Source	DF	MEAN SQUARES			
		Yield (Kg/ha)		Number of	
		Grain	Stover	pods per plant	seeds per pod
Block	3	2.84	1.68	418.14	0.02
Treatment	11	0.91	1.75	60.54	0.01
Nitrogen (N)	5	0.25	1.42	59.78	0.01
Inoculation (I)	1	8.15*	8.76	60.75	0.00
N x I	5	0.13	0.68	61.25	0.01
Error	33	1.19	0.62	84.11	0.40

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX III

Analysis of variance for 100 seed weight, shelling percentage, harvest index and moisture percentage.

Source	DF	MEAN SQUARES			
		100 seed weight	shelling percentage	Harvest index	Moisture percentage
Block	3	2.37	33.80	3.01	5.30
Treatment	11	1.45	48.06	35.63	1.38
Nitrogen (N)	5	1.89**	57.22*	42.86	0.85
Inoculation (I)	1	3.95**	94.64*	0.33	4.75
N x I	5	0.51	29.59	35.43	1.22
Error	33	0.26	18.70	32.49	1.57

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX IV

Analysis of variance for hight and number of branches

Source	DF	MEAN SQUARES				
		Number of branches		Height (cm)		
		40th day	70th day	40th day	70th day	Harvest
Block	3	2.57	2.30	304.78	55.97	33.07
Treatment	11	0.59	2.05	173.95	460.81	318.47
Nitrogen (N)	5	0.83	3.06*	339.09**	786.63**	644.86**
Inoculation (I)	1	0.09	2.04	68.16	358.61	212.52
N x I	5	0.45	1.03	29.98	155.43	13.28
Error	33	0.39	1.15	37.92	133.31	74.60

* - Significant at 5 per cent level.

** - Significant at 1 par cent levol.

APPENDIX V

Analysis of variance for nodule number, leaf, area index and net assimilation rate.

Source	DF	MEAN SQUARES			
		Nodule number	Leaf area index		Net assimilation rate
			40th day	70th day	
Block	3	44.55	1.92	17.09	1.26
Treatment	11	444.72	2.69	4.75	0.44
Nitrogen (N)	5	257.20	2.99	3.15	0.99
Inoculation (I)	1	1541.33	4.06	17.91	2.39
N x I	5	412.93	2.12	3.72	0.39
Error	33	365.43	1.40	5.54	0.72

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX VI

Analysis of variance for dry weight of leaves and stem and petiole.

Source	DF	MEAN SQUARES				
		Dry weight of leaves		Dry weight of stem and petiole.		
		40th day	70th day	40th day	70th day	Harvest
Block	3	33.10	22.99	54.20	161.72	121.18
Treatment	11	37.12	19.09	78.83	84.82	115.58
Nitrogen (N)	5	31.99	18.92	113.53**	150.33	221.90**
Inoculation (I)	1	127.49	58.24	87.98	0.24	34.40
N x I	5	24.11	11.26	42.31	36.22	25.50
Error	33	14.81	17.55	29.01	79.87	42.46

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX VII

Analysis of variance for dry weight of pods and total dry weight

Source	DF	MEAN SQUARES			
		Dry weight of pods	Total dry weight		
			40th day	70th day	Harvest
Block	3	588.28	170.90	145.56	1308.50
Treatment	11	637.73	219.56	88.33	768.74
Nitrogen (N)	5	304.39	252.86*	116.60	1205.70
Inoculation (I)	1	271.78	497.90*	0.2	613.50
N x I	5	1044.27	130.60	1.80	362.84
Error	33	128.432	81.66	215.2	557.758

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX VIII

Analysis of variance for N content (%) of leaves, stem and petiole, seeds and shell

Source	DF	MEAN SQUARES						
		Nitrogen content of leaves		Nitrogen content of stem and petiole			Nitrogen content of	
		40th day	70th day	40th day	70th day	Harvest	seeds	shell
Block	3	0.97	0.73	0.01	0.23	0.42	0.47	0.46
Treatment	11	0.80	0.91	0.11	0.33	0.40	3.56	1.43
Nitrogen (N)	5	1.23	0.32	0.17	0.10	0.28	6.86*	2.39*
Inoculation (I)	1	0.15	0.19	0.02	0.01	0.72*	0.18	2.58
N x I	5	0.51	1.63	0.08	0.63	0.45	0.94	0.24
Error	33	0.93	0.69	0.12	0.15	0.13	0.75	0.94

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX IX

Analysis of variance for nitrogen uptake (Kg/ha) by leaves and stem and petiole

Source	DF	M E A N S Q U A R E S				
		Nitrogen uptake by leaves.		Nitrogen uptake by stem and petiole.		
		40th day	70th day	40th day	70th day	Harvest
Block	3	290.43	524.70	44.10	231.08	107.20
Treatment	11	668.50	496.96	78.35	309.58	159.02
Nitrogen (N)	5	729.60*	463.90	94.06	190.53	142.18
Inoculation (I)	1	1259.7**	574.20	131.85	6.50	56.50
N x I	5	489.12	514.58	41.93	539.24	196.36
Error	33	220.90	365.80	41.02	211.22	79.18

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

APPENDIX X

Analysis of variance for nitrogen uptake (Kg/ha) by seed, shell and total uptake

Source	DF	MEAN SQUARES				
		Nitrogen uptake by		Total nitrogen uptake		
		seeds	shell	40th day	70th day	Harvest
Block	3	15694.56	1344.56	552.08	1122.71	28229.98
Treatment	11	2080.08	493.20	1157.04	1292.34	4307.09
Nitrogen (N)	5	2203.25	934.55	1270.03*	1107.07	5207.16
Inoculation (I)	1	5154.88	180.92	2161.998	458.53	4363.71
N x I	5	1341.95	114.31	893.061	1644.37	3393.71
Error	33	4506.71	510.72	387.508	604.45	7763.69

* - Significant at 5 per cent level.

** - Significant at 1 per cent level.

NITROGEN NUTRITION AND RHIZOBIAL INOCULATION ON SOYBEAN (*Glycine max* (L.) Merrill)

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the

requirement for the degree

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

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COLLEGE OF HORTICULTURE, VELLANIKKARA-TRICHUR

1978

ABSTRACT

An experiment was conducted at the Research Station and Instructional Farm, Mannuthy during 1976 on the nitrogen nutrition and rhizobial inoculation on soybean (Glycene max (L.) Merrill).

The investigation was undertaken to assess the nitrogen requirement of the crop under inoculated and uninoculated conditions. Also the study was aimed to arrive at the fertiliser equivalent of rhizobial inoculation.

The experiment was laid out in factorial in randomised block with 12 treatments and 4 replications.

The study revealed that nitrogen fixed symbiotically, was adequate for the crop. Application of nitrogen depressed symbiotic nitrogen fixation and with increasing levels of nitrogen, there was depression in symbiotic nitrogen fixation. The study also revealed that introduced strain of Rhizobium japonicum was less effective in nitrogen fixation on soybean than the strains already present in the soil.

Application of nitrogen did not influence the stover yield. Inoculation of an ineffective strain tended to

depress the stover yield.

Application of nitrogen increased the plant height in the early stages when symbiotic nitrogen fixation could not make any substantial contribution towards nitrogen supply.

Significant increase in test weight with increasing levels of nitrogen indicated that enhanced soil nitrogen supply was beneficial towards the later stages of crop growth.

The quantity of nitrogen fixed by the soybean plant appears to exceed the fertiliser equivalent of 250 kg N/ha applied basally.

I. A general view of the experimental plot, 90 days after sowing.

II. No nitrogen, inoculated

vs

100 kg nitrogen, uninoculated.


N
CONTROL
INOCULATED

III. No nitrogen, inoculated

vs

150 kg nitrogen per hectare, uninoculated.

IV. No nitrogen, uninoculated

vs

200 kg nitrogen per hectare, uninoculated.


CONTROL
INOCULATED


CONTROL

V. No nitrogen, inoculated
vs
250 kg nitrogen per hectare, uninoculated.

VI. No nitrogen, uninoculated
vs
250 kg nitrogen per hectare, uninoculated.


CONTROL
INOCULATED
N250 kg/ha


CONTROL
N250 kg/ha

VII. General view of experimental plot,
90 days after sowing.

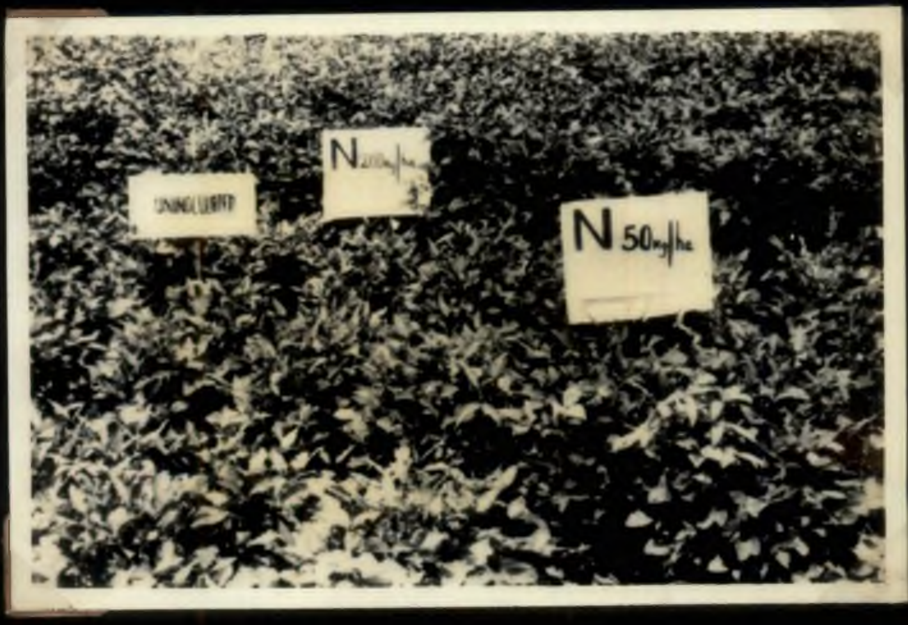
VIII. 50 kg nitrogen per hectare, inoculated
vs
150 kg nitrogen per hectare, uninoculated.

N 50
INOCULATED
N

IX. 100 kg nitrogen per hectare, uninoculated
vs
200 kg nitrogen per hectare, uninoculated.

X. 200 kg nitrogen per hectare, uninoculated
vs
50 kg nitrogen per hectare, uninoculated.


N100kg/ha


N 50kg/ha

XI. No nitrogen, inoculated
vs
250 kg nitrogen per hectare, uninoculated.

XII. 50 kg nitrogen per hectare, inoculated
vs
150 kg nitrogen per hectare, uninoculated.


CONTROL
INOCULATED
N250 kg/ha


N 50 kg/ha
INOCULATED