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Pulse Microbiology  
Progress Report-3

# CHICKPEA MICROBIOLOGY

Report of work 1981-82

by  
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ICRISAT

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-- Authors

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## CHICKPEA MICROBIOLOGY

Staff, 1981-82

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List of approved projects

(1981-1983)

Sub-program Leader: J.A. Thompson

Project No.	Project Title	Project Scientist	Cooperating Scientist
CP-Micro-5 (81)	Ecology of chickpea rhizobia and selection of inoculant strains	O.P. Rupela	
CP-Micro-6 (81)	Nitrogen fixation by chickpea	O.P. Rupela	N.P. Saxena, J.R. Burford
CP-Micro-7 (81)	Selection of chickpea germ-plasm for N <sub>2</sub> -fixation	O.P. Rupela	Jagdish Kumar

**PROJECT: CP-MICRO-5 (81) (formerly CP-Micro-1 (76) and CP-Micro-2 (76)**

**ECOLOGY OF CHICKPEA RHIZOBIA AND SELECTION OF INOCULANT STRAIN**

**SUMMARY**

1. Indications are that the plant growth problems in test tubes were mainly due to high temperature inside the tubes. We did not face these problems after we started maintaining the room temperature  $20\pm 2^{\circ}\text{C}$ .
2. We could not satisfactorily nodulate chickpea plants in agar based media. More input is required to modify the system.
3. Tubed chickpea plants can be satisfactorily nodulated in lighted incubators besides plant growth units designed at ICRISAT.
4. Surveys for nodulation and native chickpea rhizobia in farmer's and research station fields in Rajasthan and Madhya Pradesh indicated poor correlation in root nodulation and the level of native chickpea rhizobia in soil. But the fields with  $<100$  rhizobia per g soil always had poorly nodulated plants. About 39 and 55% of the fields sampled in Madhya Pradesh and Rajasthan respectively had  $<100$  rhizobia per g soil. These figures include fields which did not grow chickpea at all at least for the past 5 years as per the information collected from the farmers.
5. Soil Rhizobium population in a soil profile of a sand dune in Rajasthan was very variable with depth as compared to an alluvial soil at Haryana Agricultural University.
6. Chickpea Rhizobium population measured in 73 fields indicated 28, 24 and 21 fields with  $<100$ , 100-1000 and  $>1000$  rhizobia per g soil respectively. Fields with  $<100$  rhizobia per g soil were either in unsprayed area at south end of the farm or Alfisols. Such fields are very useful in conducting some trials with specific objectives.
7. In the trial on Rhizobium screening involving 16 Rhizobium strains the noninoculated treatment receiving 150 kg N/ha produced maximum drymatter followed by strain IC-2002, CH-777 and IC-149. Noninoculated control, IC-2072 and IC-6 were among the lowest yielders. For grain yield, strain IC-2002

ranked first followed by CH-777, NIFTAL and IC-149. Again, noninoculated control, IC-2072 and IC-6 were significantly inferior to IC-2002. Nitrogen applied control ranked eleventh.

8. No interaction between three Rhizobium strains (IC-76, P-75 and H-45) and three cultivars (Annigeri, BDN 9-3 and K-850) was noticed for nodulation,  $N_2$ -fixation, N-uptake, drymatter production and grain yield in a trial conducted in a low Rhizobium field. Strain H-45 ranked first followed by P-75 and IC-76 though the differences between strains were not significant. All the three strains resulted in significant improvement in nodulation,  $N_2$ -fixation and grain yield over noninoculated control. Noninoculated control receiving 150 kg N/ha yielded more than Rhizobium strains.
9. Significant improvement in nodulation,  $N_2$ -fixation and plant growth in early stages was noticed, in a  $\pm$  Rhizobium inoculation trial in a Vertisol field having <10 rhizobia per g soil. About 10% increase in grain yield due to Rhizobium application was recorded in this trial which was not significant.
10. It was noticed that rhizobia applied as seed coat do not move lower down to the roots where they are required to infect the roots and form nodules. Such a possibility was recorded in an Alfisol field with <100 native chickpea rhizobia per g soil and tested in further field and pot trials.
11. Liquid method of Rhizobium application was found superior to traditional seed coat method.

#### Objectives

1. To quantitatively relate soil Rhizobium population to success of inoculation in a range of soils.
2. To identify and classify Rhizobium strains in field populations nodulating chickpea and study their variation between seasons and interaction with inoculant strains and environmental variables.
3. To determine whether the success of inoculant strains is dependent on inherent competitiveness.
4. To select effective competitive rhizobia as inoculants capable of forming nodules in both favourable and adverse soil conditions.

## Background

Work commenced on the two original projects in 1976. The major early achievement was to develop a technique of growing chickpea in test tubes thus making it possible to use the serial-dilution, plant-infection technique to estimate numbers of rhizobia in soils or any medium where other organisms were present. This was particularly important as no other host was available to be substituted for chickpea as a trap host.

The use of chickpea as a trap host not only provided the essential tool to count rhizobia but the nodules formed on the plants from the soil samples served as a source of Rhizobium for pure culture isolates for the Rhizobium collection. The collection also continued to build up from isolates obtained from nodules collected directly from farmer's fields during nodulation surveys and from overseas sources.

Previous work has provided a reasonably clear picture of the populations of chickpea rhizobia at ICRISAT. Normally cropped Vertisol fields generally have populations of  $10^6$  to  $10^8$  per g soil. Various experiments, with differing objectives, have included noninoculated controls, and significant yield responses to inoculation have been measured only in three out of 7 fields with low native Rhizobium populations i.e. <100 rhizobia per g soil (Table 1). No yield responses have so far been observed in fields with  $10^6$  or more rhizobia per g of soil. Also surprising has been the virtual absence of chickpea rhizobia from the Alfisols on ICRISAT, in spite of the close proximity of the differing soils, and the apparent opportunities for contamination in dust, on vehicles and farm equipment etc. Water of all ICRISAT lakes was also apparently free of chickpea rhizobia when checked in April 1982.

The work in this project in 1981-82 therefore concentrated on the relationship of soil populations and responses to Rhizobium inoculation.

### A. TESTS OF MODIFICATION OF COUNTING TECHNIQUE

For estimation of most probable number (MPN) of chickpea rhizobia the serial dilution plant infection technique uses chickpea plants grown aseptically in 200 x 25 mm test tubes. The plants are dwarfed by excision of cotyledons of germinating seedlings immediately before sowing. Tubes, plugged with cotton, contain 30 cc of coarse sand (2-4 mm diameter) supplied with 9 ml of nutrient

solution (Toomsan et al, 1984). Plants grown in plant growth units require at least one watering with 3-5 ml of nutrient solution per tube at about 20-25 day age. After 6 weeks the plants are harvested by washing the sand away for nodulation observations. We conducted some experiments in an effort to improve upon this method.

#### Experiment 1: Test of nutrient solutions and plant growth

In the summer of 1981 we noticed problems in tubed chickpea plants in the plant growth units. Leaflets showed yellow spots similar to K deficiency seen on white clover. The symptoms were evident on older leaves first and extended to growing leaves in about 2 weeks. About 40% of the plants were growing poorly and some died, while some nodulated and survived for 6 week growth period. In an experiment designed to test recovery of a rhizobia in suspension of known numbers using tubes plugged with sponge rather than cotton, only 50% of tubes formed nodules with either treatment, even when 15 rhizobia were added per tube. Samples from both normal and unhealthy plants were chemically analysed by Soil Chemistry section. These results were as follows:

	Elements							
	K%	Na%	Ca%	Mg%	Zn ppm	Cu ppm	Fe ppm	Mn ppm
Normal	2.10	0.94	1.41	0.26	47	25	286	120
Unhealthy	1.47	2.25	1.02	0.23	45	100	210	55

While the symptoms suggested K and Mn deficiency (Dr. N.P. Saxena personal communication) and this was supported by the data above, we could not explain why these elements should become deficient in some tubes and not in others while they are all supplied with same nutrient solution.

Toomsan, B., Rupela, O.P., Mittal, S., Dart, P.J., and Clark K.W. 1984. Counting Cicer Rhizobium using a plant infection technique. Soil Biology and Biochemistry, Accepted for publication.

Reading's N-free solution had been used since 1976. We set up a trial to compare recoveries of rhizobia when the host plants were grown with 4 different nutrient solutions (Table 2). Leaves had similar symptoms to those seen earlier. The results indicated that there were skips in nodulation with all four solutions. The plant growth problem was clearly not solved.

Examination of the pH of the sand and nutrient solution (Table 3) did not solve the problem although high pH levels of some of the sands were considered to be very marginal for nodulation.

However from 15th May 1981 we replaced Reading's N-free solution with Arnon's\* to provide a more repeatable solution using demineralized water + minor elements rather than relying on tap water to provide minor elements as is the case for Reading's solution.

We also examined the temperature levels in the plant growth units. The ambient temperature during the period April to August 1981 were usually above 22°C for most of the period during the day. Temperature charts of the same period in 1980, the previous year, showed temperatures mostly around 20°C and always below 25°C which is considered to be satisfactory and allows successful nodulation even when only one viable Rhizobium cell is present in the tube. A systematic set of temperature measurements was collected in April-May 1981 (Table 4). We concluded that with all lights 'ON' the temperature inside the tubes exceeded the air temperature by 3-7°C. With only one of each pair functioning the differential was 2-4°C. To achieve 25°C in the tubes therefore requires an air temperature of not more than 20°C and in the summer this can only be achieved by removing one of each pair of lights.

From 3rd August 1981 we maintained ambient air temperature at 20 ±2°C. The experiments conducted after this date did not give any problem of plant growth. We did not see any yellow spots on the leaves. We believe that our problem with growing chickpea plants was due to temperature rather than nutrients but this has not been conclusively shown.

\*Arnon, D.I. 1938. Micro elements in culture solution experiment with higher plants. American Journal of Botany 25:322-325

## 2: Replacing sand with agar for growing chickpea plants

The original decision to use sand instead of the more commonly used agar followed early experiments at ICRISAT in which nodulation of plants was more reliable with sand. However the chickpea plant tubes used for NPN estimates of chickpea Rhizobium require 1 to 2 waterings during the plant growth period. The amount and frequency of watering necessary is certainly based on a subjective decision and errors occur. Another problem associated with the use of sand is the need to wash and remove the sand at the end of experiment to see the nodules.

During 1981 the issue of agar vs. sand was again raised. Dr. R. Islam, Microbiologist at ICARDA claimed success in nodulation using agar and Dr. K. Lakshminarayana, Microbiologist at HAU showed plant tubes successfully nodulated in agar. Common to both situations was the use of Jensen's agar medium and the Gibson tube technique (roots enclosed and tops outside the tube). Dr. Lakshminarayana prepared his agar in such a way that the agar contained very many small air bubbles.

We examined plant growth assemblies by comparing the recovery of Rhizobium from a plate counted suspension. Following our normal practice the plants were grown wholly within the tube using pregerminated seeds with excised cotyledons. For preparation of "aerated agar", tubes were cooled to 55° C in a water bath and shaken vigorously on a "Vortex" shaker until the agar started to solidify. These were then cooled to prepare both "plugs" and "slopes".

Clearly agar based media were no more successful in our hands than it had been in the past (Table 5) as the numbers of positive tubes were much less than in sand where the expected number of positive tubes was obtained.

## Experiment 3: Alternative to plant growth unit

The plant growth units in use at ICRISAT are large and suitable for growing many plants. For an occasional user a smaller facility would be ideal particularly if it could be shifted easily. We therefore compared a lighted incubator (Percival, Boone, IOWA, USA model I 35 LL, with standard fittings) with ICRISAT-designed plant growth unit for suitability of growing and nodulating chickpea plants. The Percival incubator has 65 cm x 65 cm shelves fitted with two fluorescent tubes (60 cm, 20 W) per shelf. Height of a shelf can be adjusted to requirement. The results (Table 6) indicate that plants can be grown and nodulated in the Percival incubator with similar success to the plant growth



unit. It is preferable to provide lateral illumination light intensity was 13-31% less with top illumination because of the interference by the cotton plugs in the tubes.

## B. SURVEYS

### (a) Gwalior and Rajasthan

In January and February 1982 surveys were conducted in the Gwalior area of Madhya Pradesh and Rajasthan to examine the relationship of soil populations, soil characteristics and nodulation status. Soil samples were generally collected from the inter-row space in chickpea fields. In Gwalior area some samples were also collected from fields expected to grow chickpea in 1982-83, to help select a field with low chickpea Rhizobium population for a subsequent trial. The majority of samples came from farmers' fields and some from research stations. From each site, five separate samples, each to 15 cm depth (30 cm depth in case of Rajasthan area) were pooled, placed in a plastic bag sealed and stored in an insulated box. Samples were returned as soon as practicable to the laboratory and stored under refrigeration until all tests were completed within 15 days of collection.

In the Gwalior survey about 39% of farmer's fields had populations <100/g dry soil (Tables 7 and 8) - levels considered on ICRISAT experience to be likely to allow nodulation responses to inoculation (Table 1). In most of these sites nodulation of plants was poor with many plants not nodulated. However there was no significant correlation of populations with nodulation nor with other soil factors measured (Table 9) except that populations were negatively correlated with soil NO<sub>3</sub> level.

In the survey of Rajasthan, 29% of the fields were either free of rhizobia or contained very low numbers (< 20) (Tables 10 and 11). Although some plants were nodulated in fields from which no rhizobia were recovered, but which clearly must have contained the rhizobia, the plants in 2 fields were also free of nodules (Table 10). There was no obvious relationship between soil characteristics (pH, EC, soil moisture, nitrate nitrogen) and Rhizobium populations although there was some correlation of populations and nodulation with percentage soil moisture (Table 12).

The relationship of numbers of rhizobia to soil depth was also examined at 3 spots of a sand dune growing chickpea in Rajasthan (Table 13). Variation was great and rhizobia were found even below one metre, the only consistent factor

being that numbers in the 0-15 cm depth were low.

Modules formed at the highest dilution of each soil sample collected from Gwalior and Rajasthan were stored for isolation of rhizobia. These were to be used for testing of efficiency of fixation.

(b) Baryana Agricultural University, Bikaner

On 20 January 1982 a similar sampling was made on 5 spots in field 92/2 at the Baryana Agricultural University, Bikaner. Counts were made on all 35 samples from 5 spots with 7 depths per spot on 2 February (Table 14). Numbers were much less variable than at Rajasthan and similar to figures found at ICRI SAT in previous years.

(c) ICRI SAT Center

Rhizobium populations were also measured on pooled samples of 5 spots per field, collected from the top 15 cm in the pesticide-free fields at ICRI SAT (Table 15). The fields were examined mainly to identify fields devoid of rhizobia for experimental purposes. Of the 11 fields, 8 sub-fields were found to have <10 rhizobia/g.

Fields under chickpea in 1981-82, including Alfisols, were also sampled at ICRI SAT. Each sub-field was about 1 hectare. A total of 10 spots was sampled to 0-15 cm depth and the samples pooled. Samples were processed within 2 days of collection. Most of the Vertisol fields in Mamool basin and the precision fields had adequate populations while most of the Alfisol fields had <20 rhizobia per g of soil (Table 16).

Seventy four Rhizobium strains representing 18 ICRI SAT fields were isolated from nodules formed at the highest dilution used. These have been authenticated and freeze-dried for future use to determine effectiveness of native populations.

**C. SELECTION OF INOCULANT STRAINS**

Most of the strain collection has been made during nodulation surveys in farmer's fields, when nodules of interest have been collected and brought to the laboratory for isolation of rhizobia. Generally large sized, pink nodules are selected. After the isolation is made on yeast extract mannitol agar (YMA) with Congo Red the isolate is

used as an inoculum on a chickpea plant grown under aseptic conditions (authentication). If it forms nodules it qualifies as a chickpea Rhizobium strain. It is then necessary to determine both the effectiveness in N<sub>2</sub>-fixation and the competitive ability of the strain.

Authenticated strains are then tested in replicated trials in the glasshouse, in pots of sand for their relative efficiency in N<sub>2</sub>-fixation with one or more cultivars. Noninoculated and noninoculated N-fertilizer (KNO<sub>3</sub>) pots serve as controls. Nodule number, nodule weight, nitrogenase activity as measured by acetylene reduction, and shoot weight per plant are measured. The best performing strains, based on shoot weight, are tested in the field for their performance in fixing nitrogen and competitiveness. As techniques become available for measurement of competitive ability, as indicated by the proportion of nodules formed by the inoculant strain, the selection pressure can be increased. In the previous studies in glasshouse, the majority of the strains isolated from big pink nodules have turned out to be effective and did not differ significantly from each other even when collected from well separated sites.

In field conditions at ICRISAT only 2 out of 11 trials conducted since 1976 have shown significant increases in grain yield with any inoculant strains (Table 1). These 2 trials were conducted in fields with low native populations of chickpea rhizobia. However a total of 7 trials were conducted in fields with <100 rhizobia per g soil. Failure to respond to inoculation could have been due to i) soil N being high enough to meet crop demand, ii) strains not being competitive with native soil flora, iii) native strains being as effective as the inoculant strains, or iv) rhizobia not establishing in soil because of some soil factor(s). We need to review all our previous trials in detail.

In peninsular India chickpea is usually grown on Vertisols with residual moisture and generally have >1000 rhizobia per g soil. Such studies have not been rewarding in differentiating between strains, so in 1981-82 we departed from this practice to enable us to examine the performance of strains under low N conditions and with low native Rhizobium populations.

We conducted 4 field trials including our AICPIP commitment in field RCE-4. The field RCE-4 was chosen because of its low nitrogen status and low Rhizobium status (<10 rhizobia/gram). The soil nitrogen was depleted by growing a maize crop between July and October 1981 and the pooled soil sample of the whole field (also used for Experiments 2 and 3 below) had the following characteristics: NO<sub>3</sub> <2.5 ppm, pH 6.3, EC <0.15 m mhos/cm<sup>2</sup> (= 150 micro Siemens). Unfortunately the plant growth was not uniform, and, while the area subsequently sown to

Experiment 2 grew good maize, that sown to Experiment 1 and 3 was poor thus possibly failing to adequately reduce the available N in the soil.

#### Experiment 1: Screening of Rhizobium strains

This experiment was based on the AICPIP (All India Coordinated Pulse Improvement Project) screening trial which nominated 15 strains for inclusion. Because we had infective rhizobia from only six locations (one each), we substituted with our own strains.

The 15 strains used for this study comprised 5 effective strains (IC-53, IC-59, IC-76, IC-94 and IC-149) and one ineffective (IC-6) isolated at ICRISAT; 2 effective overseas strains (IC-2002 and IC-2072); 1 streptomycin resistant mutant of IC-2002 (CM-1); and 6 strains recommended by AICPIP from Indian research centres (B-1, Ca-181, CBH-32, CH-777, F-6 and KG-31). We also included NIFTAL inoculant which contained mixture of three strains (TAL-480, TAL-620 and TAL-1148). Noninoculated and urea applied (150 kg N/ha) controls were tested with the locally adopted cultivar Annigeri.

The soil was cultivated and formed into beds with centres 1.5 m apart. Each plot consisted of 6 m x 4 rows sown on one bed. A pre-sowing irrigation was given on 20 October 1981. The sowing was made on 1st November, 1981 in RCE-4 in a randomized block design. A subsequent irrigation schedule involved approximately 25 cm irrigations with 'perfo' pipes at 21, 40, 52, 64, 79 and 89 days. When urea was applied it was banded into shallow furrows at the side of the plant roots in equal doses of 75 kg N/ha at 21 and 39 days after sowing.

The inoculant used generally contained at least  $10^8$  rhizobia/g, with the exception of IC-6 (Table 17), and most seeds actually received  $>10^8$  rhizobia/seed. Nodulation was examined 20 and 45 days after sowing. On both days in spite of irrigation at 23 days and 40 days, the percentage of nodulated plants was low (Table 18). At 20 days none of the treatments nodulated better than the control. Although over half were superior at 45 days (Table 19) nodule numbers were low. The absence of significant differences in top weight between noninoculated controls and Rhizobium strains (Table 19) is only explicable in terms of the apparently poor growth of previous uniformity crop in this part of the field thus perhaps leaving more residual nitrogen for the chickpea.

At 113 days, total dry matter production and grain yield were measured (Table 20). Treatments in the first column have been ranked in order for dry matter production. With the notable exception of urea which clearly favoured dry matter production over grain yield, rankings of strains were not different. Strain IC 2002 (RCR 3889 ex-Rothamsted, -CC 1192 ex-Australia) ranked first in grain yield and dry matter IC-6, and IC-2072 ranked below the control, although IC-2072 was expected to be effective on the basis of our previous pot test in January 1980.

Because of the poor initial nodulation observed at 20 days, 10 plants were uprooted from each replication of each treatment on the following day. From the 40 plants the seed coats and cotyledons were separated, bulked and shaken in sterilized tap water from which aliquots of a serial dilution series were used to provide an MPN estimate of the rhizobia on cotyledons and seed coats. There were clearly still fairly large numbers of rhizobia adhering to the seed coat and cotyledons (Table 17) so that poor nodulation was not due to lack of inoculum.

A similar trial was conducted under AICPIP at 13 different locations in the country including ICRISAT Center. Inoculants for all the 15 proposed strains for all the locations were prepared at ICRISAT using gamma irradiated peat, from Australia, as a carrier. All the strains qualified all the requisite tests before being used as inoculant. Four (IC-59, IC-94, IC-2002 and IC-2072) of the 15 strains were contributed by ICRISAT. Besides these four, strains CM-1 and IC-2018 were also supplied on request. At ICRISAT, we included four more strains (Table 17) besides those agreed by AICPIP. Strains RG3 (Dholi), DG-34, DG-90 (Durgapura) and H-44 (Jabalpur) though agreed for inclusion by AICPIP were not used at ICRISAT because they did not nodulate chickpea during authentication tests though the inoculants for these strains were provided by the source.

Statistically analysed data were available only from 6 of the 13 locations where this trial was conducted. None of the 15 strains occurred in the top 5 positions at all the six locations. IC-94 and IC-2072 occurred in top 5 position at two of 6 locations and IC-59 and IC-2002 at one location each. On an average, strains IC-59, IC-94 resulted in 14.2%, 11.6% increase in grain yield. The data are summarised in appendix 1.

#### Experiment 2: Strain x cultivar interaction trial (AICPIP)

This experiment, with the same design and occasional modifications of strain and cultivar, has been carried out, at a number of ICAR centres in India for, at least last 6 years. At ICRISAT Center this has been conducted for 4

years on Vertisol soils and no significant yield or nodulation responses have been measured (Table 21). In 1981-82 the treatments sown in Alfisol field RCE-4 were as follows:

Annigeri		IC-76
BDN 9-3	x	H-45
K-850		F-75
		Controls: - noninoculated
		- noninoculated + urea (150 kg/ha)

In contrast to the normal AICPIP design of a split plot the experiment was sown as a randomized block. An Alfisol soil was (RCE-4) selected, as in experiment 1, to allow expression of the objective of the experiment viz., to see whether there was an interaction between strain and host. Clearly this is most likely to be expressed where other Rhizobium strains are absent and where soil nitrogen levels are low.

The experiment was sown on 1 November 1981. Inocula for the study were prepared at ICRISAT in sterilized peat carrier and forwarded to all collaborating centres in India. All inocula contained  $>10^9$  rhizobia/gram. The sowing was made on beds with centres at 1.5 m as in Experiment 1. However these plots of 4 rows were 10 metres long.

The following observations were made:

20 days	proportion of plants nodulated, nodulation
45 days	proportion of plants nodulated, nodulation
74 days	detailed measurements of nodule, nodule weight, nitrogenase activity and top dry weight of plants measured on 0.6 sq.m/plot which included 12-27 (mean 20) plants.
92 days	N-uptake on a 1.2 sq. m area
110 days	final harvest of grain and total dry matter.

## Results

At 20 days after sowing, when most nodules on chickpea grown on Vertisols are formed, nodulation was poor (Table 22 and 23). With the best strain, H-45, on the most prolifically nodulating cultivar, K-850, only 34% of plants carried nodules. The control plants were virtually free of nodules and nodulation with IC-76 was no better than the control (Table 23). At 22 days cotyledons from plants were removed and the number of surviving rhizobia determined (Table 24). As in the previous experiment there were still at least 900 rhizobia present per seed after 22 days although these had declined from the inoculum available at sowing. The nodules formed after irrigation were on epicotyl roots which were not evident at 20 days.

Without inoculation nodulation was never more than 5% even at 70 days (Table 22). At 20 days H-45 was the most successful strain and IC-76 the least, and this ranking continued throughout the 3 samplings. By 70 days some combinations were still <80% nodulated. Of the 3 cultivars BDN 9-3 was generally the most poorly nodulated but the magnitude of the differences was not great.

It is tempting to associate the superiority of H-45 with its faster growth in culture, and hence more rapid colonization, compared with the slower growing IC-76.

It was also concluded that the improved nodulation at 45 days was the result of an irrigation at 21 days but this needs to be further examined.

The data collected at 74 days have been examined on plant and area bases. The failure of controls to nodulate clearly indicates that the site was suitable for such an experiment. The most significant point is that while significant treatment effects were measured on all nodulation criteria on both plant and area bases and on specific activity there was never any significant interaction between host and Rhizobium (Tables 25, 26, 27 and 28). The cv K-850 carried more nodules (Table 25), which weighed more (Table 26) and gave higher nitrogenase activity (Table 28) than the other two cultivars when single plants were examined. Similar results were obtained on an area basis.

The superiority of H-45 and P-75 over IC-76 was consistent for nodule number and nodule weight on both plant and area basis but only H-45 was superior in  $N_2$ -fixation, and then only when examined on an area basis. Nevertheless, there was a consistent ranking of H-45, P-75 and IC-76 in all criteria. In contrast, the specific activity of IC-76 nodules was greater than the other two strains (Table 27) while there were differences between cultivars. This could

be due to the fact that nodules of IC-76 were younger as they could have formed later than H-45 and P-75. This view is strengthened by the fact that the grain yields of P-75 and IC-76 were similar. In dry matter yields at 74 days (Table 29) the effects of lack of nodulation and of nitrogen application were dramatic and significant. Under the given experimental conditions, H-45 was a significantly better strain than IC-76 on both individual plant and area bases.

Surprisingly the dry matter production of K-850 was no better than of the other cultivars, either as single plants or on an area basis (Table 29), in contrast to its nodulation performance (Tables 25, 26 and 27). Although the percentage N in the tops at 74 days was consistent between strains and cultivars (Table 30) the product of yield/ha x N% (ie N yield/ha) was Annigeri:BDN 9-3:K-850 :: 36:36:31.

At 92 days a small area was sampled for estimation of dry matter production and N-uptake. Statistical analyses were not possible (Table 31) because of heterogeneity of variances. Further, the apparent success of IC-76 and Annigeri even being superior to the urea treatment does not agree with observations, notes and photographs at other dates suggesting that some confusion has occurred in the sampling. Results of both dry matter and N-uptake of BDN 9-3 and K-850 confirm the poor ranking of IC-76 previously observed.

At 110 days dry matter production and grain yields showed similar trends as for nodulation and N<sub>2</sub>-fixation (Table 32). K-850 was ranked first among the cultivars although not significantly superior. Among Rhizobium strains H-45 ranked first and IC-76 last. The differences between strains were not significant but all strains exceeded the noninoculated control.

Correlations between N<sub>2</sub>-fixation and yield of inoculated treatments at 74 days were all of the order of 0.63 - 0.66 and the N-fixation parameters were significantly correlated with final grain yield at 0.57-0.61 (Table 33). Clearly, however, much of the variability is due to factors other than nodulation (N-uptake ?) and this is exemplified by the lack of growth response of K-850 at 74 days inspite of its superior nodulation.

Percent N in the grain was significantly different between cultivars and inoculation treatments, again without significant interaction (Table 34). The expression of these results in terms of protein (Table 35) illustrates very clearly that while cultivars can differ in protein percentage, the actual percentage can be markedly affected by the nitrogen regime. Thus selection for protein content is only practical by ranking of varieties tested under the same conditions.



The same trial with some changes in cultivars from one geographical zone to next was conducted at 14 locations including ICRISAT center. One of the strains in this trial was from ICRISAT - IC-76. Results were available from seven locations and interactions were significant only at two (Jabalpur, Varanasi) of these locations. Treatments differed significantly at six locations. With the best combination of cultivar the increase in grain yield due to IC-76 ranged from 0.5% to 42.9% at different locations with different cultivars. The results are summarized in appendix 2.

#### D. RHIZOBIUM INOCULATION STUDIES

##### Experiment 1: Test of response to inoculation

A number of the pesticide-free Vertisol fields at the south end of ICRISAT Center are known to have been free of chickpea for the 10 years of ICRISAT's presence. It was expected that Rhizobium numbers would be low and that inoculation responses may well be found. Such fields constitute a parallel with farmer's fields now being sown to chickpea for the first time.

Field BUS 11F was chosen after the population of rhizobia was found to be 48 per g soil. The experiment comprised 10 replicates of 2 treatments - inoculated and noninoculated. Seeds were inoculated with a peat culture of IC-76 and were hand sown into flat land in rows each 30 cm apart with 10 cm spacing on 2 November 1981. On each of following four occasions a strip of plants covering 9 rows near the end of the plot was removed for examination of nodulation and N<sub>2</sub>-fixation.

Days after sowing	No of plants examined
19	15
40	15
61	33-53
76	11-27

n a drymatter sampling at 74 days

7 rows x 1 m (= 2.1 m<sup>2</sup>) and thus provided an estimate of N-uptake.

The final harvest was made on 22nd January 1982 at 114 days from 7 rows of lengths between 5.2 to 5.7 m (area harvested =10.92 to 11.97 m<sup>2</sup>). Each sampling was separated from the next by at least one plant in the row.

#### ult

The periodic nodulation observations made between 19 and 76 days after sowing are presented in Tables 36 and those for shoot weight and N-uptake between 19 and 74 days in Table 37. Nodule number, nodule weight and nitrogenase activity clearly showed an inoculation response (Table 36). Plant growth followed an expected trend with a maximum at 74 days (Table 37 and 38), by which time nitrogenase activity had certainly declined (Table 36). N uptake per plant also increased to 74 days (Table 37). The decline in %N in the shoot after 40 days agreed with the evidence of decline in acetylene reduction (Table 36). Total dry matter production and N uptake at 74 days were significantly improved by inoculation (Table 38). Visual ratings at about 65 days, by officers not familiar with the plan, consistently ranked the growth of the inoculated plots above or not less than, those noninoculated but this was not reflected in any significant differences at final harvest (Table 38).

We manually picked Heliothis larvae from all the plots from 30 days until a week before harvest and removed about 17000 larvae from the trial. Despite this effort 33% pods were damaged - (31±1.0 and 34.2±2.4% pods were respectively damaged in noninoculated and inoculated plots). Taking the damage in account the grain yield was increased by 13% with inoculation but this was still not significantly superior to the noninoculated control (Table 39).

#### Experiment 2: Methods of Rhizobium application

In experiments 1 and 2 reported in Section C above, peat-based inoculant was applied to the seed by the traditional method using methyl cellulose as an adhesive. In the Alfisol soil of RCE-4, where soil moisture changes are so rapid, poor early nodulation was ascribed to the failure of rhizobia to migrate to the root although there was sufficient moisture for germination. A field experiment using strain IC-76 and cv. Annigeri was therefore designed to examine this hypothesis using the following treatments:

1. Traditional seed inoculation with adhesive (2.56 kg inoculant/ha i.e. approximately 15 times the recommended rate)

2. Traditional seed inoculation (2.56 kg inoculant/ha) followed by irrigation
3. Granular inoculant i.e. peat inoculant applied to sand as a carrier (4.8 g inoculant per kg 2-4 m diam sand applied to provide 3.17 kg inoculant/ha) and placed below the seed.
4. Liquid inoculant i.e. peat inoculant suspended in water and poured on seeds after sowing (1 g inoculant/litre water applied to provide 2.67 kg inoculant/ha. About 3 l inoculant suspension was used per plot of 7 m x 1.5 m)
5. Suitable controls viz., untreated seed, untreated seed + liquid and untreated seed + irrigation.

The estimated numbers of rhizobia applied per seed (based on a peat inoculant containing  $10^8$  rhizobia/g) were as follows:

1. Traditional	$9.5 \times 10^7$
2. Granular	$1.2 \times 10^8$
3. Liquid	$9.9 \times 10^7$

The experiment was sown on 27 November 1981 in RCE-4, the same field as for Experiments 1 and 2 on Rhizobium strain selection. The trial was replicated three times, in 4 row plots of 7 metres length, with 30 x 10 cm spacing. The experiment had to be irrigated 13 days after sowing because adjoining trials in the field used sprinkler irrigation which covered this experiment. This trial was also irrigated 25, 37, 51 and 61 days after sowing. Sampling for nodulation and  $N_2$ -fixation was made 60 days after sowing. Final harvest for dry matter and grain yield was done 87 days after sowing.

Though some plants nodulated without inoculation, inoculation significantly improved the proportion of nodulated plants, and the mean number of nodules per plant with all treatments (Table 40). In both criteria the presence of moisture as irrigation or as an inoculant carrier, gave better results, but nodule number, nodule mass and nitrogenase activity significantly exceeded the control with liquid inoculant only. As may reasonably be expected, specific activity was not very different between any of the treatments or the controls (Table 40). However the mean dry

weight of plant tops at 60 days was significantly increased by treatments receiving water as irrigation or as part of inoculum over relevant controls (Table 41), the only exception being the liquid inoculant. By the final harvest no treatment showed significant responses in either dry matter and grain, although the liquid and irrigated treatments were again ranked highest. Nodule number, nodule weight and N<sub>2</sub>-fixation were all correlated with grain yield (Table 42). The fairly consistent superiority of the liquid inoculant, and of the normal inoculant with irrigation lends some support to the hypothesis that colonization by the rhizobia is improved by the presence of liquid which presumably results in more rhizobia being available for infections away from the seed coat. While it is also possible of course that survival of the rhizobia is improved by the presence of added moisture, the low nodulation (56%) of plants nodulated by granular inoculant in spite of the highest inoculation rates, supports the former possibility.

The rates of inoculation in this experiment were of the order of about 15x normal rate, and provided about 10<sup>7</sup> rhizobia/seed, but no treatment achieved 100% nodulation.

### Experiment 3: Pot trial to examine methods of Rhizobium application

The results of the previous experiment suggested that poor nodulation in Alfisols could result from the inability of inocula to migrate in the absence of moisture. This experiment was designed to provide a range of moisture regimes expected to be adequate for seed germination but differentially affecting the migration of rhizobia from the seed.

The pot study was commenced in February 1982. Pots of 7" diameter were filled with Alfisol soil direct from field RCE-4, which was known to carry a low (48/g soil) population of chickpea rhizobia. Boiled deionized water was used for irrigation to about 19% moisture by weight (field capacity approximately 20%) by weight through saucers placed at the base of the pot.

At the start of the experiment, four replicate sets of pots were watered 7, 5, 3 and 1 day before sowing with cultivar Annigeri. Four seeds were sown per pot after inoculation by traditional seed inoculation, or by pouring 5 ml liquid (peat suspension) on each seed at sowing. Two inoculant strains IC-76 and H-45 were used separately. No watering was done for 15 days after sowing and the pots were subsequently watered 5 times during plant growth period via the saucers. Moisture percent by weight was measured frequently and is given in Figure 2. The initial watering was delayed to the point where plants were more severely

stressed than intended. All replicates of treatments (2 methods x 2 strains and 1 control) were completely randomized in blocks comprising one watering period. The experiment was harvested at 30 days when the proportion of plants nodulated, nodule number and fresh plant weights were measured. The use of delayed sowing provided the following initial moisture contents:

1 day	15.2%
3 days	11.8%
5 days	7.6%
7 days	5.4%

The experimental layout, using watering date as main plots and randomising the strain x method treatments, did not readily lend itself to a comparison of the separate strain or method treatments perhaps because of excessive drying before watering could be started and low moisture later on. However at 30 days the liquid treatment with both strains gave significantly greater proportion of plants nodulated (Table 43) and more nodules per plant (Table 44A) than normal seed inoculation. A delay in sowing affected the proportion of nodulated plants but did not consistently affect nodule number (Tables 43 and 44A). The absence of significant effects of delayed sowing on plant growth (Table 44B) was not surprising as plants were only 30 days old and they had encountered excessive moisture stress.

At 30 days after the original irrigation the proportion of plants nodulated was variable. H-45 seems to be consistently superior to IC-76. This result is comparable with that in Experiment 2. The expected superiority of liquid inoculant over seed inoculation is generally evident but not always at all moisture levels.

As in the previous field experiment, inoculation rates for seed were high. The liquid inoculum provided between  $3.4 \times 10^7$  (IC-76) and  $8.0 \times 10^7$  (H-45) per seed. The normally inoculated seeds in our experiments carry  $>10^7$ /seed (Table 17) and hence the treatments are quite comparable.

PROJECT: CP-MICRO-6 (81) (formerly CP-Micro-3 (76) )

### NITROGEN FIXATION BY CHICKPEA

#### SUMMARY

1. Cultivar K-850 was significantly superior to Annigeri for nodule number, nodule mass and nitrogen fixation per plant whether they were sown on flat beds, ridges or broad beds with and without mulch. For plant growth at different stages and drymatter at final harvest K-850 had an edge over Annigeri but the differences were not significant. Though Annigeri produced more grains than K-850 which might have suffered due to its medium maturity as it enters into unfavourable moisture and environmental conditions while still podding.
2. Nodule number, nodule mass and nitrogenase activity declined after 54 days sampling. Drop in nitrogenase activity was drastic might obviously be due to development of a more competitive sink - the flower and pods.
3. Upto 41 days no significant differences in nodule number, nodule mass due to different types of land preparations were noticed. After 59 days plants on mulched broad beds were better nodulated followed by flat, broad bed and ridges. Surprisingly, nitrogenase activity of K-850 was significantly inferior on flat beds as compared to other land treatments but was still superior to Annigeri which showed maximum activity when sown on flat beds.
4. In Vertisols, chickpeas sown at 10 cm depth had about 38% less nodule number and 52% less nodule mass than those sown at 5 cm depth. Plants from 10 cm sowings also formed epicotyl nodules. This work seems to have relevance in Vertisol soils where at least 90% of the nodules are formed in top 15 cm profile.
5. Nodule number, nodule mass and nitrogenase activity declined by about 26, 33 and 27% respectively in 30 day old plants exposed to 24 h day length for 16 days (from day 14 to day 29). At 50 days plants exposed to extended day treatment had 37, 63 and 87% less nodule number, nodule mass and nitrogenase activity respectively. By this stage they had been exposed to extra light for 30 days (from day 14 to day 43 after sowing).
6. Of the three cultivars, Annigeri, L-550 and G-130 under normal and extended day length, Annigeri had

significantly more nodule number, mass and nitrogenase activity at 30 days after sowing.

7. Decrease in Nodulation,  $N_2$ -fixation and plant growth was observed when the plants were exposed to four increasing soil temperature regimes for 40 days from day 7 to day 47 after sowing. At similar regimes significant effects were seen only on nitrogenase activity when the plants were exposed to differential soil temperatures for 6 days from day 46 to day 51 after sowing. At similar regimes significant effects were seen only on nitrogenase activity when the plants were exposed to differential soil temperatures for 6 days from day 46 to day 51 after sowing.
8. 48 Rhizobium strains were obtained from the nodules formed at various temperatures of which 16 are from pink green nodules formed at high and very high temperature regimes. We expect that these rhizobia will be effective in nitrogen fixation at higher temperatures which need to be tested.
9. Intercropping of chickpea with sorghum resulted in 59, 56 and 59% decrease in nodule number, nodule mass and nitrogenase activity per unit area basis respectively.
10. Cultivars K-850 and G-130 identified as high and low for nitrogen fixation by acetylene reduction technique were also high and low  $N_2$ -fixing by  $^{15}N$  technique. The percent fertilizer utilisation efficiency was <3% and this raises a concern on the usefulness of  $^{15}N$  technique for crops grown on residual moisture.  $^{15}N$  technique can certainly be used for ranking cultivars for nitrogen fixation.

#### Objectives

1. To measure nitrogen fixation by chickpea in the field
2. To determine the nutritional and environmental factors limiting nodulation and nitrogen fixation
3. To determine the amounts of fixed nitrogen made available to subsequent crops.

## **Background**

Most of the work on this project has used the acetylene reduction (AR) technique for measurement of nitrogenase activity. The technique is essentially as reported by Dart et al, 1972. Chickpea plants growing in a given area are carefully dug with maximum roots and nodules intact. Excised roots with nodules are placed in a container which is then carefully sealed and is injected with acetylene to achieve about 10% concentration inside the container. The container is incubated at about 25°C. A sample of the gas is then drawn after 30 minutes and stored in a pre-evacuated glass tube (vacutainer) until it is analysed on a gas chromatograph in the laboratory.

This technique has been of great advantage because it is quick and simple but its limitation is that it provides information on N<sub>2</sub>-fixation at a given point in time.

Our studies on this project started in 1976 with measurement on AR of five cultivars over time. The trial was repeated in four subsequent years with four cultivars. The questions addressed were on i) cultivar differences, ii) differences between years, iii) diurnal variability, iv) seasonal variability, v) effect of moisture, vi) effect of location. Salient features of these studies have been reported by Rupela and Dart (International Chickpea Workshop, 1980) and in ICRISAT Annual reports of 1977-78, 1978-79, 1979-80 and 1980-81.

## **Experiment 1: Land preparation and nodulation of chickpea**

This study was conducted in collaboration with Dr. K. L. Srivastava of Farming Systems and Dr. N. P. Saxena, Pulse Physiologist. Dr. Srivastava wished to study the effect of different land preparation treatments on the incidence of cracking of Vertisol soils and its effect on soil moisture status. Dr. N. P. Saxena (Pulse Physiology) wished to examine the effect of land treatments on plant growth. Our interest was to examine the effect of the treatments in N<sub>2</sub>-fixation. The treatments were as follows:

1. broad beds and furrows

Dart, P.J., Day, J.M., and Harvis, D., 1972. Assay of nitrogenase activity by acetylene reduction. Pages 88 to 100 In use of isotopes for study of Fertiliser Utilization by Legume Crops. IAEA /FAO, Vienna.



2. broad beds and furrows with straw mulch
3. ridges and furrows
4. flat

Two cultivars, Annigeri and at our request, K-850, were tested. The design was a split plot with land treatment as the main plot. Cultivars were sown in subplots 9 m x 7 m. However there were different numbers of rows between treatments. Within rows plant spacing was same in all treatments (10 cm). Broad beds with centers spaced at 150 cm each carried 4 rows. These rows were 30 cm apart but the space between the outer rows of each bed was 60 cm. Ridges were on 60 cm centres and one row was sown on each side of ridge so that all rows were consistently 30 cm apart. Flat sowings were also made with rows 30 cm apart. Thus the mean numbers of plants/m<sup>2</sup> were 27.16 for broadbeds and 33.33 for the other sowings.

The experiment in BP13 was dry sown with 2 seeds per hill on 2-3 November 1981, watered by "perfo" irrigation on 4 November 1981 and the plants thinned to one per hill after one week.

Nodulation and N<sub>2</sub>-fixation observations were made on at least 66 plants taken from a narrow strip across the end of each plot at 26, 42, 54, 70 and 84 days after sowing. Each sampling strip was separated from the next strip by at least one plant. The sampled strips were marked by two strings stretched across 8.1 m (22 rows) of the broad beds and 8.4 m (28 rows) of the ridge and flat sowings at row ends. The strings were 30 cm apart but it was evident from the sampling data that although the seeds had been very precisely sown on 10 cm spacing within the row (confirmed by subsequent counts of the remainder of each plot) the theoretically expected number of plants (e.g. broad beds 22 rows x 3 plants = 66) was greatly exceeded. There were clearly more than 3 plants removed from many rows so that the effective area was not for example 8.1 x 0.3 m. The calculations made on an area basis using the results from these strips were therefore derived from the single plant data x the theoretical number of plants/m<sup>2</sup>. The results are presented in Tables 45-53. The final harvest at 117 days for dry matter and grain yield (Table 54) was made on at least 6 m length of the 22 rows (broadbeds) or 28 rows (flat and ridges) in each plot and errors due to area measurement were minimal.

### Results

At 26 days there were more nodules and a greater nodule weight per plant (Table 45-48) on K-850 than Annigeri but no differences between cultural treatments. On an area basis

there were thus more nodules on the ridges and flat bed obviously due to more number of plants per unit area (Table 46, 48). Although Annigeri had a higher specific activity than K-850, the latter had more dry matter production per plant and per unit area than Annigeri (Table 52, 53).

At 41 days the superiority of K-850 was greater in terms of nodule number, nodule weight and fixation per plant and for unit area (Tables 45-51). The difference in specific activity shifted in favour of Annigeri (Table 49) but the previous differences between Annigeri and K-850 for plant size and yield/unit area (Table 52, 53) disappeared.

By 54 days significant differences in nodule number were no longer apparent (Table 45,46) but K-850 remained clearly superior in nodule weight in both single plant and unit area basis (Table 47,48). The greater population of plants on ridges and flat sowings had not maintained any greater quantity of nodule per unit area. Differences in specific activity again emerged (Table 49) in favour of K-850 so that fixation was clearly superior with K-850 (Table 50,51). This superiority was not evident in plant size or dry matter production per unit area. The greater number of plants on ridges and flat sowings perhaps resulted in greater plant yield per unit area (Table 52,53) on ridges and flat sowing than on broad beds.

At 69 days K-850 again had more nodules per plant (Table 45) and per unit area (Table 46), greater nodule weight (Table 47,48); retained its high specific activity (Table 49) so that fixation was greatly superior (Table 50,51). However, yields per plant and per unit area favoured Annigeri. This indicates that Annigeri is using the alternate source of N, the soil N pool. The treatments with more plants gave significantly greater top weight per unit area (Table 52).

The results at 82 days was essentially similar although nodule number and weights had declined markedly especially with Annigeri (Table 45-48) for which specific activity was also drastically reduced (Table 49).

The superiority of Annigeri in dry matter production on an area basis (Table 53) was also reflected in grain yield (Table 54). There is little doubt that K-850 fixed much more of its own nitrogen than did Annigeri but N was presumably not limiting and Annigeri was able to produce both more dry matter and higher yield.

At the final harvest (Table 54) broad beds with mulch resulted in significantly poor dry matter yield than all other treatments and ridges ranked best. Rankings of grain yield were similar. Cultivar K-850 produced significantly more dry matter than Annigeri but this trend was reversed for grain yield. Clearly we cannot ascribe the greater

yield of Annigeri to better nodulation and  $N_2$ -fixation as Annigeri always ranked lower than K-850 for nodulation and  $N_2$ -fixation. The total dry matter of Annigeri was lesser than K-850 while it ranked better than K-850 in all earlier samplings. This could be because of more leaf fall in Annigeri than in K-850. Also, Annigeri matures earlier than K-850 and might have shed leaves for larger period than K-850.

**Experiment 2: Nodulation and  $N_2$ -fixation with different sowing depths**

During a field visit to the Fruit Research Station of APAU at Sangareddy, chickpea planted at different depths were found to have different nodulation, with the deeper plantings having poorer nodulation. As about 90% of the chickpea nodules in Vertisols at ICRISAT Center are found in the 0-15 cm profile deeper planting may be expected to restrict the nodulation of chickpea.

To test this hypothesis a small trial with two cultivars Annigeri and K-850, was planted on 2 March 1982 at two depths, 5 and 10 cm. The trial was sown in RCB design with three replications and plot size of 4 m x 6 rows each 30 cm apart in field BP 13. Nodulation observations were made at 37, 47 and 59 days after sowing on 25-60 plants. Root nodules and epicotyl root nodules were recorded separately.

Irrespective of sowing depth all nodules occurred below 5 cm depth. (Fig.9) so that no epicotyl nodules formed on seeds sown at 5 cm. However they were formed when seeds were sown at 10 cm. The data in Tables 55-59 are on total treatment differences based on overall nodulation and  $N_2$ -fixation while the proportionate contribution of the epicotyl nodules to the overall nodule system is presented in Table 60.

Total nodule numbers per plant decreased with age (Table 55) with both cultivars although the changes were not significant and were not reflected in nodule weight per plant (Table 56). Cultivar K-850 sown at 5 cm carried significantly greater numbers and weight of nodules than 10 cm while with Annigeri, which was poorly nodulated as compared to K-850, depth had less effect. Similar trend for nitrogenase activity per plant (Table 58) was also observed but the activity declined with age of both cultivars although the decline was significantly more rapid with Annigeri. When activity was greatest (at 37 days) the shoot

weights of both cultivars were superior with 5 cm sowing (Table 59), all dates suggesting that activity may have commenced earlier in the shallower sowing. At other two samplings at 47 and 59 days also 5 cm sown plants were better grown than 10 cm sown plants.

The only significant feature of the data on the proportional contribution of the epicotyl nodules is that K-850 carried a greater proportion of its nodule numbers and weight in the epicotyl regions especially in the later samplings (Tables 60). The differences between the cultivars was even more marked in terms of nitrogenase activity. By 59 days only 1.3% of Annigeri's  $N_2$ -fixation was from epicotyl nodules while those of K-850 contributed 51.7% (Table 60). Similar trend was seen for specific activity also.

Quantitatively, the nitrogen fixation per plant in general was very poor for both cultivars particularly after 37 day sampling. This could be due to poor moisture status of soil and relatively warmer temperature than normal as the trial was sown at fag end of the proper chickpea season. Though the soil profile was recharged by watering with a tanker, before sowing, it might have depleted relatively faster than in a normal planted crop.

Two obvious questions arose from these data: (a) in the absence of sufficient moisture in the top 10 cm for formation of nodules does chickpea adequately compensate for the lack of epicotyl nodules by growing them in the hypocotyl region, (b) what is the significance of the cultivar difference evident in Table 60. Since the experiment was taken up at the fag end of chickpea growing season in relatively warmer temperatures the experiment should be repeated and also the answer to the questions raised be attempted.

### 3. Modulation and $N_2$ -fixation with extended day length

This experiment was conducted by Dr. N. P. Saxena, Pulse Physiologist. Treatments were normal and extended days with cultivars Annigeri, G-130, and L-550. They were sown in ridges and furrows in field BP 13 on 27 October 1981 under dry conditions and irrigated on 2 November 1981. Plot size was 4m, 13 rows with spacing of 30 x 10 cm in a split plot design with day length as the main plots and cultivar as subplots. The plots under different light regimes were separated by screens and the day length extended to 24 h by 100W tungsten lamps, suspended 0.9 m above ground and providing 20 lux at the plant surface. Lights were turned on between 6 pm to 6 am from 10 November 1981 until 11 December 1981 for 31 days. For plant growth and other details including final yield, please see Chickpea

Physiology Report of work, 1981-82. Nodulation examination was conducted on samples consisting of all plants from a 1.8 sq.m area at 30 and 50 day harvests.

At 30 days Annigeri had significantly superior nodule number, nodule mass and nitrogenase activity than both other cultivars, G-130 and L-550. However it also reacted least to extended day length although there was a general tendency with all three cultivars for all nodulation parameters except specific activity to drop with extended day length (Table 61,62). Shoot weight per plant or per unit area tended slightly in the opposite direction (Table 63).

At 50 days cultivars did not differ significantly in nodule number or weight but the treatment remained significantly superior in normal day temperature (Table 64). Specific activity was halved at normal daylength and reduced by about 80% with extended daylength so that nitrogenase activity per plant or per sq. m. was also reduced due to light treatment (Table 65). In contrast to the nodulation parameters, dry matter yields generally tended to be more with increased daylength (Table 66).

Also the significantly greater top growth of Annigeri at 30 days was not retained by 50 days when it was at least equalled by L-550 (Table 63,66). There seems little evidence of a controlling effect of plant growth by nodulation parameters.

#### Experiment 4: Nodulation and N<sub>2</sub>-fixation at different soil

At ICRI SAT Center N<sub>2</sub>-fixation in chickpea occurs only upto 40-50 days after sowing when the crop is grown under residual moisture conditions while at Hissar, in north India, it continues upto 150 days after sowing (ICRI SAT Annual Report, 1982). The levels of fixation are only 25-50% of those measured at Hissar. High soil temperature at Hyderabad could be one reason for the low levels of N<sub>2</sub>-fixation. Between sowings in mid-October and mid-December in 1980-81 soil temperature at 10 cm depth was above 30 C for about 6 hours per day (e.g. Figure 4). Dart *et al.*, 1975 found that 30 C was a limiting temperature for nodule formation and N<sub>2</sub>-fixation by chickpea under controlled environment conditions.

The present study was designed (a) to examine at the effect of temperature on nodulation N<sub>2</sub>-fixation and on Rhizobium populations in the soil and (b) to select Rhizobium strains which would fix nitrogen at the higher

temperatures. For this purpose we grew plants in mixture of soil from several fields mixed with small quantities of inoculants of chickpea rhizobia of several different origin in peat carrier. Plants were exposed to different soil temperature treatment after a period of 7 days till the plants emerged. This period of common temperature environment for all treatment in glass house was provided to facilitate infection process before the temperature treatment were introduced. It was hoped that the nodules thus formed and functioning at higher temperature may be from strains resistant at these temperatures. The strain, Ca-2 reported to be fixing nitrogen at above 30°C by Dart et al 1975 was planned to be used as check but could not be procured though all possible sources were tried.

We did not introduce the temperature treatment from sowing with the reason (a) even infection process may get affected at higher temperature and hence we may not get nodules functioning at these temperature which was one of the objective, (b) seed germination may get affected at different temperatures and plants may emerge at different times and hence confounding the studies with age of plants.

Soil from 8 different ICRISAT fields which were known to have >1000 rhizobia per g soil (Appendix 3) was collected, air dried, shredded and mixed thoroughly in equal proportions. Ten g each of the 35 different peat inoculants (Appendix 4) and 50-100 g soil from each soil sample from farmers fields in Madhya pradesh and Rajasthan which are listed in Table 7 and 10 were pooled and mixed with the main bulk of soil from 8 fields. The mixture was found to have >10<sup>8</sup> rhizobia per g soil, pH 8.3, and electrical conductivity of 0.24 mmhos/cm<sup>2</sup>. Each pot contained 16 kg mixed soil + inoculant.

Thirty two pots were each sown on 29 January 1982 with 15 seeds of cultivar K-850 and thinned to 10 per pot 7 days after sowing. Four of these pots were placed in each of 4 temperature controlled water baths (Delta Cold Pty Ltd., Osborne Park, Western Australia) immediately after thinning and held for 40 days before harvest. The water level in baths was adjusted on alternate days to reach slightly above the level of soil in pots. The remaining 16 pots were grown in the glasshouse, beside the waterbaths under the same light and ambient temperature conditions (25 ±2°C) for 45 days before being split into group of four and placed in the waterbaths for 6 days (from day 46 to day 51).

Dart, P.J., Islam, R., and Eaglesham, A. 1975. The root nodule symbiosis of chickpea and pigeonpea. Pages 63-84 Proceedings of International Workshop on Grain Legumes, 13-16 January, 1975, Hyderabad, A.P., India.

A further four pots, without plants were prepared at the same time along with other pots and placed in the water baths at the same time as the long duration treatment for 114 days.

The water baths were adjusted in an attempt to provide 8 hour periods of 25, 30, 32 and 35°C temperatures between 0800 and 1600 hours each day. Figure 5 suggests that soil temperature could not be controlled precisely due a big lag period between water and the soil temperature. The periods necessary to reach the desired maximum were longer for the higher temperatures so that at the highest temperature settings the minimum temperature was also higher than the minimum of the lower temperature setting. This was later found to be due to the fact that soil temperature plotted in figure 5 were measured in the middle of the pot. Later studies indicated that there was a gradient of temperature decreasing from periphery to the middle of the pots. The difference in temperature from periphery to middle of the pot tended to get smaller with time. It appeared that water temperature should be taken as correct for treatment differences. The temperature measurements were done only for a limited period and the means are given below. The same timings for putting ON and OFF of the water baths were maintained throughout the experimental period. For practical purposes, four distinctly different temperature regimes could be established successfully.

	Mean		Minimum		Maximum	
	Soil	Water	Soil	Water	Soil	Water
Low	24.0	23.9	21.5	21.5	25.8	25.8
Medium	26.2	27.8	22.0	22.8	30.5	31.5
High	27.8	29.8	23.5	24.25	32.5	32.75
Very high	30.2	33.0	26.3	27.3	33.5	35.0
<b>Ambient</b>						
	Feb.1982		19.0		26.4	
	March 1982		19.4		27.1	

At harvest all plants were removed, examined and measured for nodulation and plant growth parameters.

Since few plants died in some pots which was not necessarily associated with temperature treatment we are presenting our results on per pot basis and not on per plant basis.

### Results

#### Soil Rhizobium population:

Rhizobium populations in soil samples at 53, 68, 83 and 114 days were not differentially affected by soil temperatures. A slight but significant decrease in population was observed over time. The interaction between duration of treatment and temperature treatment was not significant (Table 67).

#### Nodulation and $N_2$ -fixation:

After growing the plants in glasshouse for 45 days, various temperature treatment understandably did not affect nodule weights and numbers and plant growth (Table 68). However there was clearly a drastic effect on  $N_2$ -fixation with the higher temperatures. The specific activity of nodules and nitrogen fixation per plant were about 8-10 fold lower when soil temperature rose from medium to high. As expected the root over shoot weight ratio are not different at various temperatures.

The exposure of plants to differential soil temperatures for 40 days from day 7 to day 46 not only decreased the  $N_2$ -fixation and specific activity of nodules with increased temperature but also significantly decreased the number of nodules, nodule growth, shoot and root growth (Table 69). Even the ratio of root weight over shoot weight was also significantly affected which increased with increased temperature. This indicates that even the nutrient uptake might have been adversely affected at higher soil temperature such that plant had to produce more roots per unit shoot weight.

#### Isolation of rhizobia:

One of the major objectives of this trial was to obtain rhizobia from pink or pink green nodules formed at higher temperatures which were expected to be fixing nitrogen.



Some green, presumably ineffective nodules were also selected at these temperatures. A list of these strains (Appendix 5) will be tested in the future.

Out of the total of 83 isolates obtained from nodules only 48 (about 58%) were found to nodulate chickpea in authentication tests in test tube grown plants. Generally, more than 90% of our isolates turn out to be rhizobia. The poor success rate is surprising. Provided these isolates are rhizobia and not contaminants we wonder if they require a higher temperature to nodulate than the temperature in the plant growth room which was generally <25°C. All the non-nodulating isolates may be re-tested for nodulation at a range of temperatures.

**Experiment 5: Nodulation, nitrogen fixation in sole chickpea and sorghum/chickpea intercrop**

An experiment conducted by Farming Systems Research Program (Dr. M. Natarajan and Dr. Sardar Singh) studying moisture-use patterns of sole chickpea v/s intercropped chickpea with sorghum were examined for nodulation and N<sub>2</sub>-fixation at 32, 57 and 74 days after sowing. The experiment was planted on 29 October 1981 in field BW 3 with five treatments:-

1. chickpea with 30 cm between rows
2. chickpea with 60 cm between rows
3. sorghum with 30 cm between rows
4. sorghum with 60 cm between rows
5. chickpea/sorghum intercrop (alternate rows) 30 cm

Chickpea was not inoculated but soil contained about 10<sup>5</sup> rhizobia per g of soil. Chickpea plants were observed for nodulation and N<sub>2</sub>-fixation after sampling from 0.9 sq.m area in each plot except in intercrop when it was 0.45 sq.m. Chickpea occupies 50% area.

At 32 days nodule numbers per plant were similar in all treatments and the nodule number/sq.m was significantly higher at the 30 cm spacing (Table 70) obviously due to more number of plants per square meter area. However by 74 days the number/sq.m was similar with all treatments (Table 70)

due to a significant drop in the nodule number in 30 cm spacing while the drop in 60 cm spacings was not very pronounced. This drop in nodule number per plant may be due to degeneration of nodules with time, different sampling spots and significantly more sampling errors as by this time the soil gets very dry. This argument is strengthened by the nodule dry weights at 74 days.

Nodule weight per plant however was consistently and significantly greater with 60 cm spaced chickpea reflecting the trend towards larger individual plants with this treatment (Table 70). However, nodule weight per unit area was greater with 30 cm spaced chickpea until 53 days after which it declined and was no better than 60 cm spaced plants. Intercropped chickpea, presumably because of competition as evidenced by lower shoot weight per plant especially at 74 days (Table 72), carried less nodule weight per unit area and these reactions were reflected in lower nitrogenase activity per plant and per unit area (Table 71). With the drastic decline in specific activity after 32 days nitrogenase activity was virtually finished by 74 days in all treatments (Table 71).

By 74 days the dry matter yield of chickpea spaced at 60 cm was only 300 kg/ha lower than the more closely spaced treatment but intercropped chickpea only yielded half the dry matter of the 60 cm indicating the severity of the competition (Table 72). The sorghum in the intercrop in fact produced as much dry matter at 74 days as the sole chickpea.

There seems little doubt that intercropping adversely affects chickpea growth and N<sub>2</sub>-fixation presumably through reduced plant size rather than any special effect on particular nodulation parameters.

#### Experiment 6: Use of <sup>15</sup>N to measure N<sub>2</sub>-fixation by chickpea at ICRISAT

This was the first experiment where we used <sup>15</sup>N isotope dilution technique to measure N<sub>2</sub>-fixation and was conducted in collaboration with Rothamsted Experimental Station, U.K.

The objective of the experiment was to find out a <sup>15</sup>N based reliable field method to evaluate large number of cultivars for nitrogen fixation. Known high (cv.K-850) and low (cv. G-130) nitrogen fixing cultivars based on acetylene reduction were sown on 4 Dec. 1981 in field BP 13 on flat land in a systematic design such that all chickpea plots were surrounded from all sides by two safflower (Carthamus tinctorius L.). Safflower was seen growing slowly upto about first 60 days in water sheds and was felt to serve as a good non-fixing control. All the 18 chickpea plots were surrounded on all four sides by two rows of

safflower which was expected to provide an estimate of spacial variability in  $^{15}\text{N}$  uptake within a given control in an experiment.

Each of the 18 plots of chickpea were 2.1 m long and had 2 rows each of K-850 and G-130 such that 2 rows of a given cultivar did not occur as pairs (Figure 6) and were randomised within a plot. Spacing of 30 x 10 cm for safflower and 30 x 5 for chickpea was followed, whether the safflower rows were East-West or North-South. Some more details on lay out of the trial are given in figure 6.

After sowing the seeds in a dry piece of land, labelled nitrogen fertilizer was applied as ammonium sulphate at the rate of 10 kg N/ha over the complete experimental area of 13.8m x 9.9m (130.62 sq.m). Irrigation was immediately provided with 144 rose cans (=4.4 cm rain). The experiment was then covered with polythene for three days to prevent and conserve moisture. Germination and plant stand was good, except in one row of safflower which was missed during sowing. The missed row was filled with transplanting seedlings available at the time of thinning about 1 week after sowing. In another about 10 days some safflower seedlings died at 3-4 spots and resulted in gaps of about 30-50 cm. These gaps were also filled by transplanting plants from border row wherever excess. All the transplanted plants remained stunted till the harvest 67 days after sowing.

At harvest plants from 2.1m x 2 rows of safflower were brought to crop work area, chopped into pieces of about 1", approximately one fourth of the total amount was subsampled in oven at 80 C, weighed and ground in a cross beater mill with a sieve of 0.2 mm. There were 51 such plots of safflower numbered 1-51 in figure 6. Mean values for atom % excess of safflower for each plot were calculated from the enrichments of the four bordering rows of a given plot. Nitrogen fixed was then calculated in each chickpea cultivar using safflower as a non-fixing control plant and the relative nitrogen fixation in each chickpea cultivar compared by using the cultivar with highest enrichment as control for the other.

### Results

Growth of safflower plants in the rows which were completely transplanted was very poor, giving dry weights of only 700, 920 and 1210 kg/ha compared to a mean value of 4390 kg/ha in rows which had not been transplanted (Table 73). These plants had high enrichment of  $^{15}\text{N}$ , 0.076 - 0.090 compared to a mean of 0.027 atom %  $^{15}\text{N}$  excess, suggesting that they had taken up enriched nitrogen early but that growth and nitrogen uptake had then been markedly reduced.

Plants transplanted into gaps in other rows will have had some effect on the overall mean values for those rows for the same reasons. All data from these rows are excluded from further calculations. The total nitrogen content of G-130 was much lower than that of K-850, as was the total dry matter production (Table 74). Safflower had both higher total N content and dry matter than that produced by each chickpea cultivar. The percent fertilizer utilisation efficiency (% FUE) was very low in all the crops, the highest being found in safflower (<3%). The enrichment of the safflower and cultivar G-130 was similar while the enrichment of cultivar K-850 was quite consistently lower than G-130 in 16 of the 18 replicate plots.

The low uptake of fertilizer indicates that the fertilizer was unavailable for plant growth. It is likely that this was due to insufficient irrigation, resulting in the immobilisation of the fertilizer in the surface soil profile so that the labelled nitrogen could not be absorbed.

The  $^{15}\text{N}$ -enrichment of safflower in many cases approximately the same as that of G-130 indicating that negligible nitrogen fixation was taking place in G-130 (Table 75). However, in more than half of the plots there was more dilution of N in safflower than in G-130. This suggests that safflower was either absorbing nitrogen from different depths than G-130 where more  $^{15}\text{N}$  was available i.e. where N fertilizer had not been incorporated, or that safflower was taking up nitrogen later in the season than G-130 when the enrichment of the soil might have declined. As relatively rapid safflower growth in the seedling stage than G-130 was noticed in subsequent experiments, the former explanation is more likely.

The consistently lower enrichment in K-850 compared to G-130 indicates that the technique may be useful in providing a good comparative estimate of the nitrogen fixed by each cultivar over the growing season. Unfortunately as such a low amount of  $^{15}\text{N}$  was taken up by both cultivars the error due to heterogeneity in the amount of available soil nitrogen between plots and analytical precision was proportionately high.

If the estimates of nitrogen fixation by isotope dilution and the difference method are compared (Table 74), it can be seen that estimates of nitrogen fixed by each chickpea cultivar are similar. The difference method with safflower as a non-fixing control has not been used due to the higher total nitrogen content of safflower.

This experiment has confirmed that K-850 and G-130 are respectively high and low nitrogen fixers and has provided insight into experimental procedure which must be adopted to ensure a useful measurement of nitrogen fixation. Two recommendations for experimental procedure can be made; (a)

transplanting must be avoided as it can affect plant growth and nitrogen uptake considerably, (b) thorough irrigation is essential to ensure that fertilizer is washed into the soil to depths where it can be absorbed by the plants. This will help to reduce differences in fertilizer uptake due to contrasting rooting patterns of the legume and control plant. It is not clear whether the isotope dilution technique will be applicable for use under residual moisture conditions due to the problems of fertilizer incorporation into the soil and its subsequent unavailability.

Cultivar differences in nitrogen fixation were reasonably consistent and gave a comparative estimate of nitrogen fixation efficiency between the two chickpea cultivars similar to the difference method. The experiment suggests that the  $^{15}\text{N}$ -isotope dilution technique in the field will provide a useful method for cultivar screening and safflower can provide a factor on spacial variability for N-uptake.

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### SELECTION OF CHICKPEA GERMPLASM FOR N<sub>2</sub>-FIXATION

1. Due to problems of plant to plant variability within a cultivar experiments on this project were deferred.
2. Investigations in the glass house on a non-fixing line at F<sub>1</sub> stage reported from HAU found to have nodules at par and N<sub>2</sub>-fixation better than one of the parents. No yellowing symptoms as reported in the field studies at HAU were seen. Yellowing symptoms were seen at F<sub>1</sub> stage when the progeny was grown in field. All the plants died within 60 days of sowing.
3. Nine cultivars previously known for their nodulation were observed again and confirm for their high or low nodulation.
4. In the above trial we noticed better seedling emergence, plant stand, drymatter production and grain yield on the eastern side of a ridge than the western side. Nodulation and N<sub>2</sub>-fixation per plant did not differ significantly due to ridge placement though it was better on eastern side in some cultivars obviously due to differential plant population.

### Objectives

1. Characterise differences between chickpea lines in ability to nodulate and fix nitrogen
2. Monitor breeders material and germplasm for ability to nodulate and fix nitrogen
3. Select desirable material for use in breeding programmes
4. Determine heritability of nodulation

### studies

We screened about 200 crossing block lines grown in the field, over two seasons 1976-77, 1977-78 for variability in nodulation with the native soil Rhizobium populations.

Eighty lines were selected and further screened with and without inoculation at Hissar, Hyderabad and Parbhani in 1978-79. A further 30 of these lines were again screened at Hissar and Hyderabad in 1979-80. Some lines have performed consistently good or poor nodulating over locations and years, while there was an interaction with location and season with other lines. Crosses have been made in collaboration with chickpea breeders between consistently good and poor nodulation lines to study the inheritance of these traits.

A quick and reliable visual scoring technique for nodulation has been developed for screening large numbers of germplasm lines. This score correlates well with nodule number and nodule weight. Using this technique we screened about 500 germplasm lines at both Hissar and Hyderabad in 1980-81 using K-850 (a consistently good nodulating line across location) as a check. Two lines ICC-435 and ICC-685 nodulated better than K-850.

About 100 breeding lines (P<sub>1</sub> to P<sub>10</sub>) of crosses involving wilt resistant parents were screened for the occurrence of non-nodulating plants. Non-nodulating lines serve as a useful control in measurement of N<sub>2</sub>-fixation by nodulated plants. No non-nodulating plants were found although 10 plants had 2-6 nodules per plant which was less than a tenth of that formed by a well nodulating line.

Breeders elite material (117 lines) were screened for nodulation in 1980-81, 51 of these had poor nodulation, 61 average and only 5 were as good in terms of nodulation as the check line K-850. Though year to year variation in nodulation of check line K-850 has been seen but it has remained a consistently high nodulating line.

A method has been developed for estimating nitrogenase activity of intact plants, measuring their nodulation and transplanting to produce seeds with 98% survival of the transplants. Such methodology can be used in studies of inheritance of nodulation and N<sub>2</sub>-fixation.

Procedure for propagating chickpea vegetatively was developed for the first time in 1980-81 and had a 60% success rate. Mounded branches still attached to plants produce rootlets under glasshouse conditions. The branch can then be removed from the plant and grown on. This technique was published in International Chickpea Newsletter No.4, June 1981, pages 12-13. The technique allows examination of nodulation of wild chickpeas and breeding lines with limited seed availability and can be used for nodulation inheritance studies.

In a glasshouse study in November 1980 where P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> progenies of a cross (K-850 x I2-071-04144) were being studied for nodulation in pots growing single plants,

noticed large plant to plant variability for days to germination, nodulation and plant growth, not only in hybrid but also in the parents. This was the first time that we noticed large plant to plant variability within a given cultivar. Excess watering might have been one of the reason for such large variability. Plants were grown in 5" pots having sand and stood in half inch trays full of nutrient solution. We decided to review the situation carefully before undertaking further nodulation evaluation studies. Experiments undertaken in this year were (1) to examine a reported non-fixing chickpea line from Haryana Agricultural University using the watering system we successfully used for evaluation of Rhizobium strains (2) yield evaluation of cultivars known for their nodulation behaviour.

#### Experiment 1: Investigation of reported non-fixing chick line from HAU Hissar

In the December 1981 issue of International Chickpea Newsletter, B.S. Dahiya and A.L. Khurana reported a non-fixing F<sub>1</sub> progeny of a cross between NEC-721 and G-130. On request we received seeds of F<sub>1</sub> progeny and both the parents. 5-6 plants of each genotype were studied in a pot trial sown on 15 February 1982 for nodulation and N<sub>2</sub>-fixation with each of two types of inoculants, (a) mixture of 9 Rhizobium strains, (b) soil from field 92/2 of Haryana Agricultural University, Hissar from where the progeny was selected. The mixed inoculant comprised 5 strains from ICRISAT (IC-6, IC-13, IC-53, IC-59, and IC-76), 3 from NIFTAL (TAL-480, TAL-620, and TAL-1148) one John Innes (9036). The mixed inoculum was suspended in tap water, and applied to pots at sowing and provided 10<sup>8</sup> rhisobia per seed. Where HAU soil was used as inoculant, 5 g soil per pot was added with the seeds at sowing. Seeds of uniform size were sown two per pot in case of cultivars and one per pot in case of the F<sub>1</sub> progeny in 7 " plastic pots containing acid washed sand. The plants were grown in a glasshouse with day maximum temperature ranging 26-29 C and night minimum temperature ranging 18-20 C over the total growth period. Four days after emergence all plants were thinned to one per pot. Watering was done as required using Arnon solution containing 25 ppm N as KNO<sub>3</sub>.

#### Results

Observations on acetylene reduction, nodule weight and nodule number per plant were made 41 days after sowing (Table 76). After removal of nodules, plants were re-established for seed production. With the set of genotypes inoculated with soil, nodules formed near the cotyledons only, and the reason(s) for this are not clear. We did not observe the yellowing symptom described by Dahiya



and Khurana at any stage of plant growth.

The F<sub>1</sub> progeny had nodule numbers comparable to the poor nodulating parent G-130, nodule weight (with mixed inoculant) intermediate between the parents and N<sub>2</sub>-fixation at least as good as one of the parents but superior to the low N<sub>2</sub>-fixing check, Annigeri.

The transplanted plants experienced pot temperature periods when re-established for seed production on 29 March 1982. These plants produced 30 seeds, most of which were not fully developed. Only 8 of these germinated in field when planted in October 1982. In field these plants grew stunted and showed yellowing symptoms of the type described by Dahia and Khurana, 1981. All these plants died between 45-60 days after sowing. Though we have lost all the seed but the phenomena is quite interesting and deserves a fresh look.

**Experiment 2: Yield evaluation of nine chickpea cultivars known for their nodulation behaviour**

The main reason of conducting this trial was to multiply seeds of cultivars Rabat, E-100, P-310-1, P-319-1, ICC-435 and ICC-685 which we were running short. Because a good land piece was available we decided to take a trial with two additional objectives - yield evaluation and nodulation behaviour; besides the main objective of seed multiplication. Four replicate plots (5m, 8 rows on 60 cm ridges- 2 rows per ridge) in RCB design were sown on 2 November 1981 in field BP 13. We noticed differences in emergence and plant growth between rows on east and west sides of a ridge, while plant rows ran north-south. Therefore we decided to make all observations on east and west rows separately. Modulation and N<sub>2</sub>-fixation was observed at 45 days after sowing by uprooting plants from 0.9 sq.m area. Plants from East and West part of the 60 cm ridges of all rows were sampled separately, pooled and used for different measurements.

Cultivar K-850 had highest nodule mass and N<sub>2</sub>-fixation per plant. Modulation of other cultivar was as expected, cv P-310-1 had lower nodule number but was at par with other high nodulating lines for nodule mass. Cultivars differed significantly for all the nodulation parameters (Table 77). Cultivars G-130 and L-550 had lowest nodule weight and activity per plant. Ridge placement was not significant for all the parameters measured except for plant population. This may be due to differences in moisture conditions on east v/s west sides of a ridge. Modulating and N<sub>2</sub>-fixation

was observed at 45 days after sowing by uprooting plants, from 0.9 sq.m area. Plants from east and west part of the 60 cm ridges of all rows were sampled separately pooled and used for different measurements.

At the time of final harvest also east and west sides of the ridge were measured separately and the significant differences in dry matter and grain yield were seen not only for cultivars but also for ridge placement (Table 78). Cultivar ICC-435 produced maximum dry matter but for grain yields K-850 was at top followed by ICC-685 and P-319-1. Good nodulating cultivars in general yielded better than low nodulating cultivars. Significant correlation between grain yield and nodule weight,  $N_2$ -fixation were also seen (Table 79). Dry matter correlated poorly with nodule weight ( $r=0.262$ ) as compared to grain yield ( $r=0.454$ ).

We have seen differences in emergence and early plant growth in Vertisols in the past also. In 1981-82, we noticed that in field RCE-4 where ridges ran east-west, the north side of the ridge had poor plant population as compared to south side. The slope in this field was towards south-east. It seems that the plant stand and early growth on the side of a ridge opposite to slope gets affected adversely and could be due to the fact that this side allows water to stand for a more period. Such east-west differences are only seen if the crop is sown dry and irrigated for emergence and not when sown with dibblers, in an irrigated field of right moisture.

Table 1: Responses to *Rhizobium* inoculation in chickpea at ICRISAT, 1976 to 1981

Year	Field		Av. yield of control (kg/ha)	% Increase	Native <i>Rhizobium</i> population No/g soil
	old name	new name			
1976-77	Paddy field	p 6	1090	65 (P <.01)	2 <sup>a</sup>
	ST 2	RP 16A	1070	53 (unreplicated)	0 <sup>a</sup>
1977-78	B 9	BS 3	1190	10 (NS) <sup>b</sup>	14 <sup>a</sup>
	Manmool	RM 1C	1560	37 (P <.01)	44 <sup>a</sup>
1978-79	B. 8	BP 14C	930	4 (NS)	3090
	B 9	BS 3	900	14 (NS)	14
1979-80	BW 3	BW 3	1080	6 (NS)	19950
	M12	BM 16C	900	9 (NS)	40
	B 9	BS 3	410	19 (NS)	14
1980-81	BT 2	BP 10	1340	<1 (NS)	~10000
	BT 2	BP 10	1440	1 (NS)	~10000

<sup>a</sup>Measurements made in 1978 from adjoining parts of the respective fields.

<sup>b</sup>NS = Not significant.

Table 2: Comparison of recovery of Rhizobium strain IC-2002 by plate and by serial dilution plant infection technique (MPN) using four different nutrient solutions, July 1981.

Nutrient solution	Dilution level of <u>Rhizobium</u> suspension				Estimate of total numbers
	1:10 <sup>5</sup>	1:10 <sup>4</sup>	1:10 <sup>3</sup>	1:10 <sup>2</sup>	
	(68700)	(6870)	68.7±2.8	(6.87)	(0.687) 6.87 x 10 <sup>5</sup> <sup>a</sup>
	MPN count using 3 plants/level - expected positive tubes				
	3	3	3	3	1
	Numbers of positive tubes				
Reading (½ strength) <sup>b</sup>	2	2	0	1	2
Reading <sup>b</sup>	2	3	2	0	2
Arnon <sup>c</sup>	2	3	1	1	3
Jensen <sup>d</sup>	0	0	0	0	0
95% fiducial limit (p = .05) x or +					
					MPN
					3.8 x 10 <sup>6</sup>
					1.7 x 10 <sup>7</sup>
					3.7 x 10 <sup>7</sup>
					<1 x 10 <sup>8</sup>

<sup>a</sup> Estimate from count at 1:10<sup>5</sup> dilution

<sup>b</sup> Summerfield, R.J., Muxley, P.A., and Minchin, F.R. 1977. Plant husbandry and management techniques for growing legumes under simulated tropical conditions in controlled environments. Exp. Agric. 13:81-92

<sup>c</sup> Arnon, D.I. 1938. Micro elements in culture solution experiment with higher plants. Amer. J. Bot. 25: 322-325.

<sup>d</sup> Vincent, J.M. 1970. A manual for the practical study of the root nodule bacteria. IBP Handbook No.15. Blackwell Scientific Publications, Oxford.

Table 3: pH Measurements of sand, water and media used for plant tubes (sand:distilled water 1:2), 1981.

Treatment	pH range
Distilled water	6.0
Sand in test tubes where plants died (1:1 sand and water)	7.2-8.4
Sand in test tubes where plants with yellow spots but did not die	9.15-9.55
Sand from Glass house complex	
Unwashed	8.85-8.95
Washed (running water 5 days)	8.8
Acid washed (1% Hcl 24 hours)	8.3
Acid washed (6% Hcl 24 hours)	8.2
Sand from different sources	
Near Eucalyptus trees near SN gate	7.7
Near field RCE-21	8.1
Used by PPS for construction	8.4
Nutrient solutions (of pH 6.8) after autoclaving	
Reading's ½ strength	8.8
Arnon's ½ strength	8.9
Jensen's ½ strength	8.95
Reading's full strength	7.65
Arnon's full strength	7.10
Jensen's full strength	7.61

Use of deionised water or tap water for washing after acid treatment did not make any difference in pH of sand.

Table 4: Temperature measurements inside the plant tubes placed in Plant growth unit, 20-1-1981 to 26-5-1981

Place- ment	Wooden ON	Wooden OFF	Metal ON	Both units ON		Half lights ON		
				Metal OFF	Thermo- meters far end of AC	Thermo- meters near to AC	All <sup>a</sup> lights ON	Half lights ON
Wooden Unit								
Top shelf	E	26.8	23.1	20.8	27.3	21.8	21.1	18.7
	W	27.9	24.9	21.2	27.8	21.4	22.0	18.8
Middle shelf	E	25.2	23.9	20.1	26.1	22.4	23.0	19.5
	W	25.1	22.4	21.4	26.2	21.5	22.6	20.2
Bottom shelf	E	25.1	22.2	20.5	26.7	22.4	22.6	20.6
	W	22	21.7	21.1	25.0	23.3	23.5	20.9
		25.35	23.03	20.85	26.51	22.13	22.47	19.78
Metal Unit								
Top shelf	E	22.1	30.6	28.8	28.8	25.8	22.3	21.4
	W	21.9	28.5	29.8	28.1	24.1	21.3	20.9
Second shelf	E	22.9	N.D	26.4	28.7	25.0	21.3	21.3
	W	N.D	N.D	27.8	27.0	25.8	21.9	22.6
Third shelf	E	21.5	25.0	25.6	27.0	25.4	22.8	23.0
	W	21.4	24.9	N.D	27.6	25.8	22.6	23.4
Bottom shelf	E	20.7	26.1	25.1	26.2	21.8	22.1	21.7
	W	20.3	24.1	24.9	25.8	22.8	22.8	21.4
$\bar{x}$		21.54	26.53	26.91	27.4	24.6	22.14	21.96
Room tempe- rature								
		20.2	21.8	21.8	21.9	21.6	19.1	19.0

<sup>a</sup>It was difficult to keep plant tube temperature below 30°C as the room temperature approached 22°C. We could not run both 'light units' together 7 hours at a stretch with room temperature 22°C or less.

E = East )  
W = West ) while the units lie North-South.

Table 5: Comparison of recovery of Rhizobium strain IC-2002 by plate and by plant infection dilution (MPN) method using different plant support media, September 1981.

Medium	Dilution level of <u>Rhizobium</u> suspension.				Estimate of total numbers		
	1:10 <sup>0</sup>	1:10 <sup>1</sup>	1:10 <sup>2</sup>	1:10 <sup>3</sup>			
	(7600)	(760)	76±0.5	(7.6)	(0.76)	(0.076)	7.6 x 10 <sup>8</sup> <sup>a</sup>
	MPN count using 4 plants/level - expected positive tubes						
	4	4	4	4	1	0	
	Number of positive tubes						
Aerated agar (plug) 200 x 25 mm	4	2	3	2	1	0	1.7 x 10 <sup>8</sup>
Aerated agar (slope). 150 x 18 mm	1	1	3	1	1	1	1.7 x 10 <sup>7</sup>
Sand	4	4	4	4	3	0	1.0 x 10 <sup>11</sup>
95% fiducial limit (p = .05) x or +							3.8

<sup>a</sup>Estimates from counts at 1:10<sup>0</sup>.

Table 6: Comparison of recovery of Rhizobium strain IC-2002 by plate and plant infection dilution (MPN) method using Percival incubator and plant growth unit, November, 1981

Location - Percival	Dilution level of <u>Rhizobium</u> suspension				Estimate of total numbers
	1:10 <sup>0</sup>	1:10 <sup>1</sup>	1:10 <sup>2</sup>	1:10 <sup>3</sup>	
	Plate count - <u>Rhizobium</u> /ml				
	6000	600	60±2	(6)	(0.6) 6.0 x 10 <sup>3</sup> <sup>a</sup>
	MPN count using 3 plants/level - expected positive tubes				
	3	3	3	3	1-2
	Numbers of positive tubes				
					MPN <sup>b</sup>
top shelf, lateral lights	3	3	3	3	>10 <sup>3</sup>
mid shelf, top lights	3	2	1	3	1.8 x 10 <sup>3</sup>
bottom shelf, top lights	2	2	3	3	3.8 x 10 <sup>3</sup>
plant growth unit, single light lateral illum.	3	3	3	3	>10 <sup>3</sup>
95% fiducial limit x or +					

<sup>a</sup>Estimates extrapolated from counts at 1:10<sup>3</sup>

<sup>b</sup>Six dilution steps (1:10<sup>0</sup> - 1:10<sup>5</sup>) using 3 tubes per dilution.



Table 7: Chickpea *Rhizobium* populations at research stations and in farmers' fields around Gwalior - January 1982

S.No.	Location	Modulation (No/plant)	Rhizobia <sup>a</sup> (per g dry soil)	pH	EC (m mhos/ cm <sup>2</sup> )	Nitrate N ppm	Remarks
1.	Sonsa	5	1800	7.0	<0.15	9.5	
2.	Sonsa	- <sup>a</sup>	880	7.6	<0.15	1.0	<u>Eruca sativa</u> Mill.
3.	Sonsa	7	9160	7.5	<0.15	2.0	
4.	Bandha	29	4170	8.3	0.19	2.25	
5.	Bandha	7	20	7.6	0.21	10.0	Poor field, late sown
6.	Bandha	- <sup>a</sup>	90	8.4	<0.15	0.5	Pigeonpea
7.	Utilla	- <sup>a</sup>	1800	8.2	0.18	3.8	Sorghum-pigeonpea intercrop
8.	Utilla	9	8800	8.0	0.20	3.0	
9.	Utilla	- <sup>a</sup>	3930	7.0	<0.15	0.5	Sorghum-pigeonpea intercrop
10.	Dabka	3	880	6.7	<0.15	6.0	Poor field, browsing lower leaves
11.	Dabka	- <sup>a</sup>	1790	6.7	<0.15	0.5	Sorghum-pigeonpea intercrop
12.	Dhamal	- <sup>a</sup>	40	6.4	<0.15	6.0	<u>Sesamum indicum</u> L.
13.	Bandhapura	<1	<10	8.0	0.31	26.0	
14.	Bandhapura	- <sup>a</sup>	<10	7.8	<0.15	1.0	Millet-pigeonpea intercrop
15.	Karari Bhatari	11	47300	8.8	0.25	3.8	Tail end of waterway
16.	Badegaon	5	410	8.4	0.20	11.3	Field in waterway, at least sixth year of chickpea
17.	Badegaon	- <sup>a</sup>	<10	8.5	0.22	12.0	About 10 M left of s.no.16, 1 M elevated
18.	Badegaon	- <sup>a</sup>	<10	8.7	0.20	5.5	About 15 M left of s.no.16, 1 M elevated
<u>Agricultural Research Stn, Morena</u>							
19.	Field No.33	6	11500	6.9	<0.15	1.0	Irrigated crop
20.	Field No.16 (E)	<1	<10	8.0	0.19	7.5	
21.	Field No.16 (W)	<1	<10	7.9	0.19	4.5	
<u>ICRISAT, Gwalior</u>							
22.	Field No.305	<1	<10	7.8	0.19	11.8	
23.	Field No.321 (2 CVs in nearby plots)	24 (K-850) 8 (G-2)	1820	7.8	0.17	2.5	

-a = field under crops other than chickpea and likely to grow chickpea in 1982-83. MPN = most probable number

<sup>a</sup>Factor for 95% confidence limits: ± 4.68

Table 8: Rhizobium population in farmers' fields around Gwalior, January, 1982 - summary

<u>Rhizobium</u> <u>population</u>	% fields	Modules/ plant	pH Range	Range of EC (m mhos/cm <sup>2</sup> )
<100	38.9	1-7	6.4-8.8	<0.15-0.31
100 - 1000	16.7	3-5	6.7-8.7	<0.15-0.20
>1000	44.4	5-29	6.7-8.8	<0.15-0.25

**Table 9: Correlation between chickpea nodulation, Rhizobium population and soil properties of soil samples from fields around Gwalior, January 1982**

	Nodulation	pH	Electrical conductivity	Nitrate N	
<u>Rhizobium</u> population	0.466 <sup>NS</sup>	-0.216 <sup>NS</sup>	-0.278 <sup>NS</sup>	-0.512 <sup>**</sup>	(df 24)
Nodulation		0.257 <sup>NS</sup>	-0.117 <sup>NS</sup>	-0.459 <sup>NS</sup>	(df 14)
pH			0.573 <sup>**</sup>	0.197 <sup>NS</sup>	(df 24)
Electrical conductivity				0.756 <sup>**</sup>	(df 24)

df = degree of freedom

Table 10: Chickpea *Rhizobium* population at research stations and in farmers' fields of Rajasthan, February 1982

Observation spot no.	Modulation (No./plant)	<i>Rhizobium</i> <sup>e</sup> population (per g soil)	pH	E.C. (m mhos/cm <sup>2</sup> )	Percent moisture	Nitrate N
1	5	869	8.4	<.15	3.4	<0.5
2	14	398	9.0	0.20	7.8	0.5
3	10	44000	8.5	<.15	7.3	1.0
4 <sup>c,d</sup>	26	920	8.6	0.16	9.5	4.0
5 <sup>d</sup>	7	386	8.5	<.15	4.5	1.0
6	11	856	8.8	<.15	2.0	<0.5
31 <sup>c</sup>	7	43100	8.7	<.15	2.0	0.5
32	a	17	8.8	<.15	0.3	<0.5
33 <sup>b</sup>	1	18	8.7	<.15	3.6	<0.5
34 <sup>b</sup>	5	<10	8.8	<.15	1.9	<0.5
35	0	<10	8.6	<.15	2.4	<0.5
36 <sup>b</sup>	1	<10	8.4	<.15	1.0	<0.5
37 <sup>d</sup>	<1	<10	8.6	<.15	0.7	<0.5
38 <sup>d</sup>	12	>103000	8.6	<.15	3.8	<0.5
39 <sup>d</sup>	5	>104000	8.6	0.17	4.1	3.0
40 <sup>d</sup>	4	18400	8.7	<.15	2.3	<0.5
41 <sup>a</sup>	a	<10	8.6	<.15	2.1	<0.5
42 <sup>a</sup>	a	<10	8.7	<.15	1.0	<0.5
43 <sup>a</sup>	a	<10	8.4	<.15	1.8	<0.5
44 <sup>b</sup>	0	<10	8.4	<.15	1.1	<0.5
45	2	<10	8.4	<.15	1.0	<0.5
46	6	<10	8.6	<.15	0.4	<0.5
47	9	17	8.4	<.15	2.2	<0.5
48	3	173	8.6	<.15	1.9	<0.5
49	1	<10	8.5	<.15	2.3	0.7
50	10	<10	8.4	<.15	1.8	<0.5
51	6	<10	8.4	<.15	2.7	<0.5
52	7	3970	8.6	<.15	2.0	<0.5
53	18	177	8.9	<.15	4.6	<0.5
54	9	879	8.3	<.15	4.7	<0.5
55	19	1730	7.7	<.15	1.9	<0.5
56	13	1810	8.2	<.15	6.5	0.5

a = Barren land, b = poor crop, lower leaves reddening, c = irrigated at least once after sowing, d = research station, e = Factor for 95% confidence limits is  $x + 4.68$

**Table 11 Rhizobium population in farmers' fields in Rajasthan, February 1982 - summary**

Rhizobium population	Percent fields	Range of nodules/plant	Range of pH	Range of EC	Range of nitrate-N
<100	55.5	0-10	8.4-8.8	<.15	<0.5-0.7
100 - 1000	25.9	3-18	8.3-9.0	<.15-0.20	<0.5-1.0
>1000	18.5	7-19	7.7-8.7	<.15	<0.5-1.0

Table 12 Correlation between chickpea nodulation, Rhizobium population and soil properties of soil samples from field in Rajasthan, February, 1982.

	Modulation	pH	Electrical conductivity	Nitrate N	Percent moisture
<u>Rhizobium</u> <sup>a</sup> population	0.297 <sup>NS</sup>	0.033 <sup>NS</sup>	0.202 <sup>NS</sup>	0.328 <sup>NS</sup>	0.444 <sup>**</sup>
Modulation <sup>b</sup>		-0.208 <sup>NS</sup>	0.263 <sup>NS</sup>	0.422 <sup>*</sup>	0.654 <sup>***</sup>
pH			0.376 <sup>*</sup>	0.073 <sup>NS</sup>	0.028 <sup>NS</sup>
Electrical conductivity				0.303 <sup>NS</sup>	0.502 <sup>**</sup>
Nitrate N					0.552 <sup>**</sup>

<sup>a</sup>Correlations run on log numbers

<sup>b</sup>n = 28 while for other variables n = 32

Table 13 Rhizobium population over depth, on slope of a sand dune growing chickpea in Rajasthan, February, 1982.

Depth (cm)	Spot 1 (top of dune) <sup>a</sup>	Spot 2 (centre of dune)	Spot 3 (base of dune)
0-15	<10	17	172
15-30	<10	1760	18700
30-45	87	371	1780
45-60	179	10	177
60-75	19000	88	45000
75-90	393	386	4000
90-105	<10	874	903
105-120	<10	1750	403

<sup>a</sup>Top of sand dune, though sown, did not have any plants presumably due to shortage of water at sowing.

Table 14: Variation of soil characteristics over depth in field  
92/2 of HAU, January 1982

Depth (cm)	pH	Rhizobium <sup>†</sup> count/g of soil (log MPN)	Electrical conductivity (m mhos/cm <sup>2</sup> )	Per cent moisture
0-15	8.1	94 (1.87) <sup>d</sup>	0.25	6.0
15-30	8.2	9080 (3.85) <sup>ab</sup>	0.19	11.4
30-45	8.1	9330 (3.97) <sup>a</sup>	0.16	11.7
45-60	8.2	3800 (3.58) <sup>abc</sup>	0.15	12.5
60-75	8.1	1230 (3.09) <sup>abc</sup>	0.15	11.4
75-90	7.7	490 (2.69) <sup>cd</sup>	0.16	10.6
90-105	8.0	1050 (3.02) <sup>bc</sup>	0.16	9.8
SE±	0.12	0.32	0.012	0.39
CV %	3.4	23	15.6	8.2
F-test	NS	**	**	**

† Mean separated by Duncan's multiple range test at P < 0.05



Table 15: Rhizobium population in top 15 cm in Vertisols unsprayed (BUS) fields sampled in December, 1981

Field	Rhizobium population (log <sub>10</sub> MPN)	pH	EC	Percent moisture
BUS-1A	20 (1.31)	8.2	<0.15	19.4
BUS-1B	1000 (3.00)	8.1	<0.15	19.1
BUS-1C	436 (2.64)	8.1	<0.15	18.5
BUS-1D	2040 (3.31)	8.2	<0.15	20.9
BUS-2A	977 (2.99)	8.1	<0.15	18.9
BUS-3A	20 (1.31)	8.2	<0.15	20.4
BUS-3B	44 (1.64)	8.2	<0.15	17.6
BUS-3C	<10 (<1.00)	8.2	<0.15	16.6
BUS-3D	199 (2.30)	8.2	<0.15	18.3
BUS-3E-South	524 (2.72)	8.1	0.18	41.6
BUS-3E-North	<10 (<1.00)	8.2	<0.15	18.4
BUS-4D	4570 (3.66)	8.5	<0.15	19.8
BUS-5A	447 (2.65)	8.3	<0.15	21.3
BUS-5C	<12 (<1.07)	8.5	<0.15	17.6
BUS-5D	209 (2.32)	8.3	<0.15	21.8
BUS-6A	2090 (3.32)	8.3	<0.15	22.2
BUS-6B	10230 (4.01)	8.2	<0.15	19.2
BUS-7C	20 (1.30)	8.5	<0.15	17.2
BUS-8A	<12 (<1.07)	8.4	0.16	19.2
BUSBG-1	479 (2.68)	8.3	0.19	29.8
BUSBG-2	49 (2.34)	8.2	0.19	29.4
BUSBH	479 (2.68)	8.3	0.20	29.4
BUSBI	49 (2.34)	8.2	0.21	29.4
BUS-10C	199 (2.30)	8.1	0.16	19.2
BUS-10F	<12 (<1.07)	8.5	0.17	19.2
BUS-11A	44 (1.64)	8.4	0.17	18.3
BUS-11B	<10 (<1.00)	8.6	0.17	17.1
BUS-11C	<10 (<1.00)	8.3	0.19	30.0
BUS-11D	436 (2.64)	8.6	0.17	18.1
BUS-11E	436 (2.64)	8.5	<0.15	17.1
BUS-11F	48 (1.68)	8.2	0.19	30.0
BUS-12B	<12 (<1.07)	8.8	<0.15	17.7

Table 16: Rhizobium population within top 0-15 cm in ICRISAT fields, September, 1981. (proposed for chickpea in Pabi, 1981-82)

Field and position	Rhizobium population (log <sub>10</sub> MPN)	pH	E.C.	Percent moisture
RP4A-North <sup>a</sup>	19 (1.28)	7.8	<0.15	14.1
RP-4A South <sup>a</sup>	19 (1.28)	7.8	<0.15	12.6
RP15D-East	<10 (<1.00)	7.8	<0.15	15.8
RP15D-West	<10 (<1.00)	7.0	<0.15	21.8
RCE-4	<10 (<1.00)	6.3	<0.15	15.3
RCE14A-East	<10 (<1.00)	7.5	<0.15	11.1
RCE14A-West	<10 (<1.00)	7.6	<0.15	11.1
RL-9A	<10 (<1.00)	6.5	0.19	17.3
RL-9C	<10 (<1.00)	7.9	0.19	17.0
RL-9D	<10 (<1.00)	8.3	0.29	19.1
RM1A <sup>b</sup>	44 (1.64)	8.5	0.19	18.0
RM1B <sup>b</sup>	195 (2.29)	8.4	0.20	16.7
RM1C <sup>b</sup>	195 (2.29)	8.4	0.23	16.4
RP08-East	110 (2.04)	8.2	0.17	32.1
RP08-West	1100 (3.04)	8.4	0.20	32.4
RP0C-East	107 (2.03)	8.3	0.21	29.1
RP0C-West	2190 (3.34)	ND	ND	29.3
RP16A-East	2190 (3.34)	8.4	0.23	29.2
RP16A-West	219 (2.34)	8.2	0.25	31.0
RP16B-East	21 (1.33)	8.1	0.23	28.6
RP16B-West	724 (2.86)	8.3	0.23	30.2
RP16C-East	758 (2.88)	8.4	0.23	28.5

<sup>a</sup>Rhizobia introduced in RP4C in June 1978. Slope from RP4C to A

<sup>b</sup>Rhizobia (mixed inoculant) introduced in RM1C in October 1977. Slope from RM1C to RM1A.

ND = Not determined.

Table 17: Plant infection count ( $\log_{10}$  MPN) of rhizobia in inoculants, on seeds and on cotyledons after 22 days - Rhizobium screening trial in RCE-4, 1981-82

Strain	Log number Rhizobium/ g inoculant	Log number rhizobia per seed		MPN on cotyledons at 22 days
		Plate count	MPN <sup>b</sup>	
B-1	9.23	7.57	>6.00	>5.00
Ca-181	9.93	7.60	>6.00	>5.00
CBH-32	9.23	6.49	>6.00	>5.00
CH-777	8.93	7.06	>6.00	4.63
CM-1	8.93	7.48	>6.00	>5.00
F-6	8.93	7.63	>6.00	4.26
IC-6 <sup>a</sup>	6.23	7.57	-	1.92
IC-53 <sup>a</sup>	>9.00	7.76	>6.00	>5.00
IC-59	7.93	6.72	>6.00	>5.00
IC-94	9.93	>5.00	>6.00	>5.00
IC-149 <sup>a</sup>	10.93	7.21	5.63	>5.00
IC-2002	10.26	7.52	>6.00	4.26
IC-2072	8.93	5.91	4.23	3.58
KG-31	9.93	6.85	5.63	3.23
NIFTAL	8.23	-	5.63	>5.00

<sup>a</sup>MPN estimate of inoculant obtained by serial dilution infection technique on peat inoculant

<sup>b</sup>Seeds were processed within 3 days of treatment with peat inoculant after storage at ~ 4°C.

**Table 18: The percentage of chickpea plants nodulated in Rhizobium strain screening trial, RCE-4, 1981-82.**

Strain	At 20 days after sowing and before irrigation		At 45 days after sowing and after 2 irrigations
B-1	17.5	(18.1) <sup>a</sup>	57.5
Ca-181	0	(0)	27.5
CBH-32	7.5	(11.2)	17.5
CH-777	5.0	(9.2)	30.0
CM-1	7.5	(8.3)	85.0
F-6	10.0	(13.3)	47.5
IC-6	0	(0)	10.0
IC-53	20.0	(22.7)	87.5
IC-59	7.5	(11.2)	35.0
IC-76	17.5	(17.9)	62.5
IC-94	12.5	(17.5)	62.5
IC-149	2.5	(4.6)	42.5
IC-2002	10.0	(12.9)	70.0
IC-2072	10.0	(13.3)	92.5
KG-31	2.5	(4.6)	42.5
NIFTAL	12.5	(11.3)	87.5
Uninoculated	2.5	(2.4)	30.0
Uninoculated + N (150 kg N/ha)	17.5	(21.1)	25.0
SE		±(6.94)	±12.02

F. test:

NS

Values in parentheses are means after angular transformation

Table 19: Nodule number, mass and shoot weight of chickpea at 45 days in Rhizobium strain screening trial, RCE-4, 1981-82

Strain	Nodule No/ plant <sup>a</sup>	Nodule wt (mg/pl) <sup>c</sup>	Top wt (g/pl)
B-1	3 (1.79) <sup>b</sup>	27 (1.24) <sup>b</sup>	0.75
Ca-181	1 (1.13)	3 (0.53)	0.81
CBH-32	>1 (1.01)	5 (0.44)	0.57
CH-777	1 (1.22)	16 (0.96)	0.65
CM-1	7 (2.68)	20 (1.30)	0.72
F-6	2 (1.44)	16 (1.02)	0.63
IC-6	>1 (0.78)	>1 (0.08)	0.69
IC-53	8 (2.87)	17 (1.22)	0.67
IC-59	2 (1.71)	9 (0.93)	0.49
IC-76	3 (1.83)	17 (0.98)	0.60
IC-94	2 (1.45)	8 (0.80)	0.54
IC-149	2 (1.56)	7 (0.80)	0.67
IC-2002	5 (2.22)	9 (0.95)	0.93
IC-2072	5 (2.15)	15 (1.08)	0.60
KG-31	1 (1.19)	10 (0.81)	0.61
NIFTAL	7 (2.71)	25 (1.36)	0.76
Uninoculated	>1 (1.13)	11 (0.64)	0.70
Uninoculated + N (150 kg/ha)	1 (1.01)	9 (0.73)	1.11
SE	±(0.239)	±(0.205)	±(0.099)
CV%	±(29)	±(47)	±(29)
F.test	**	**	*

<sup>a</sup> Values in parentheses represent means after transformation

<sup>b</sup> Data analysed after  $\sqrt{x+0.5}$  transformation to bring independence of errors

<sup>c</sup> Data analysed after  $\text{Log}_e(x+1)$  transformation to bring activity and independence of errors

Table 20: Total dry matter production, grain yield and their rankings at final harvest in Rhizobium screening trial in RCE-4, 1981-82

Strain	Total dry matter <sup>**</sup> production (kg/ha)	Grain yield <sup>†</sup> (kg/ha)
Uninoculated + Urea (150 kg N/ha)	3180 (1) <sup>a</sup>	1450 (11) <sup>abc</sup>
IC-2002	3050 (2) <sup>ab</sup>	1870 (1) <sup>a</sup>
CH-777	2870 (3) <sup>abc</sup>	1720 (2) <sup>ab</sup>
IC-149	2820 (4) <sup>abc</sup>	1620 (4) <sup>abc</sup>
CH-1	2820 (4) <sup>abc</sup>	1540 (6) <sup>abc</sup>
NIFTAL	2730 (6) <sup>abc</sup>	1640 (3) <sup>abc</sup>
IC-76	2710 (7) <sup>abc</sup>	1550 (5) <sup>abc</sup>
IC-53	2680 (8) <sup>abc</sup>	1450 (11) <sup>abc</sup>
F-6	2580 (9) <sup>abc</sup>	1500 (7) <sup>abc</sup>
IC-94	2550 (10) <sup>abc</sup>	1480 (9) <sup>abc</sup>
Ca-181	2520 (11) <sup>abc</sup>	1490 (8) <sup>abc</sup>
KG-31	2510 (12) <sup>abc</sup>	1240 (17) <sup>cd</sup>
B-1	2510 (12) <sup>bc</sup>	1420 (13) <sup>bcd</sup>
IC-59	2470 (14) <sup>bc</sup>	1390 (14) <sup>bcd</sup>
CBH-32	2430 (15) <sup>bc</sup>	1480 (9) <sup>ab</sup>
Uninoculated control	2360 (16) <sup>c</sup>	1370 (15) <sup>bcd</sup>
IC-2072	2210 (17) <sup>cd</sup>	1270 (16) <sup>cd</sup>
IC-6	1770 (18) <sup>d</sup>	1040 (18) <sup>d</sup>
SE	±193	±126
CV %	15	17

F. test:

\*\*

\*

\*Figures in brackets indicate ranking

†Means were separated by Duncan's Multiple range test. Means with the same letter are not significantly different at P < 0.05

Table 21: Summary of results of host and Rhizobium interaction trials on chickpea at ICRISAT, 1977-1980.

Year	Cultivars	Strains <sup>a</sup>	% increase over control	Inter-action	Native rhizobia per g soil
1977-78	G-130, C-235, J6-62 Rabat, K-850	Ca-7, IC-2028 F-75, H-45, IC-2002	10 (NS)	NS	14
1978-79	BEG-482, H-208, J6-62, Rabat, K-850	9036, Ca-7, F-75, H-45, IC-59, IC-2002	2 (NS)	NS	3000
1979-80	Annigeri, H-208, CPS-1, K-850	F-75, H-45, IC-59, IC-2002, IC-76	12 (NS)	NS	<100 to >1000
1980-81	Annigeri, BDM 9-3, CPS-1, K-850	F-75, H-45, IC-59, IC-2002, IC-76	<1 (NS)	NS	1000 to 10000

<sup>a</sup>Control and N-control (150 kg N as urea or CAN) always included.

NS = Not statistically significant.

Table 22: Percent plants nodulated before and after irrigation in strain x cultivar interaction trial, RCE-4, 1981-82

Treatment	Before irrigation				After irrigation							
	20 days after sowing		45 days after sowing		70 days after sowing							
	Anni-geri 9-3	BDM K-850 Mean	Anni-geri 9-3	BDM K-850 Mean	Anni-geri 9-3	BDM K-850 Mean	Anni-geri 9-3	BDM K-850 Mean				
H-45	31.3	11.3	28.5	23.7	91.3	75.0	83.8	83.3	96.7	94.4	98.9	96.7
F-75	12.5	7.5	13.8	11.3	71.3	57.5	68.8	65.9	86.7	88.0	91.6	88.8
IC-76	1.3	2.5	3.8	2.5	28.8	22.5	26.3	25.8	76.0	82.3	93.0	83.8
Control	1.3	0	0	0.4	3.7	0	1.2	1.7	4.0	10.5	1.3	5.3
Urea	0	0	0	0	1.2	0	0	0.4	1.9	0	5.0	2.3
SE		±2.87		±2.73		±6.49		±3.75		±3.93		±2.27
Mean	9.3	4.3	9.2		39.3	31.0	36.0		53.1	55.0	58.0	
SE		±2.11				±2.90				±3.93		
F. test: Interaction		*				NS				NS		
Treatment		**				**				**		
Cultivar		NS				NS				NS		



Table 23: Nodule number (nodule weight in mg) per plant at 20 (before irrigation) and 45 days (after irrigation) in strain x cultivar interaction trial, RCE-4, 1981-82

Treatment	at 20 days			At 45 days				
	Annigeri 9-3	BOM 9-3	K-850 Mean	Annigeri	BOM 9-3	K-850 Mean		
H-45	0.51	0.1	1.1	0.6	7.6 (31.4)	5.3 (12.6)	8.5 (35.4)	7.1 (26.5)
F-75	0.0	0.1	0.6	0.3	3.6 (11.1)	3.7 (8.3)	7.0 (21.1)	4.8 (13.5)
IC-76	0.0	0.0	0.1	0.1	0.7 (3.0)	0.3 (0.1)	0.6 (1.8)	0.6 (1.6)
Control	0.0	0.0	0.0	0.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
Urea	0.0	0.0	0.0	0.0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
SE	±0.15		±0.09		±1.39 (4.37)			±0.80 (2.52)
Mean	0.1	0.0	0.4		2.4 (9.4)	1.9 (4.2)	3.2 (11.9)	
SE	±0.07				±0.62 (1.95)			
F. test: Interaction	MS	MS	MS	MS	MS	MS	MS	MS
Treatment	**	**	**	**	**	**	**	**
Cultivar	**	**	**	**	**	**	**	**

**Table 24: Populations of chickpea rhizobia in inoculants and on Annigeri seeds at sowing and after 22 days**

Strain	Log <sub>10</sub> number rhizobia/ g inoculant at sowing		Log <sub>10</sub> number rhizobia/ seed at sowing		Log <sub>10</sub> number on cotyledons at 22 days
	Plate count	MPN estimate	Plate count	MPN estimate	MPN estimate
F-75	10.29	10.93	6.75	>6.00	2.92
H-45	9.73	10.23	6.74	>6.00	>5.00
IC-76	9.81	9.93	7.94	>6.00	4.26

Table 25: Modulation of chickpea at 74 days in Rhizobium strain and host interaction trial in RCE-4, 1981-82

Strain	Module No./plant <sup>b</sup>				Module No./sq. m <sup>b</sup>			
	Ami per/1	BM 9-3	K-850	Mean	Ami per/1	BM 9-3	K-850	Mean
H-45	22 (4.68)	20 (4.38)	33 (5.71)	25 (4.92)	820 (28.5)	681 (25.7)	1016 (31.5)	839 (28.6)
F-75	15 (3.59)	16 (3.68)	28 (5.15)	20 (4.21)	408 (19.3)	573 (23.4)	973 (30.4)	652 (24.4)
IC-76	5 (2.15)	5 (2.17)	13 (3.60)	8 (2.64)	153 (11.9)	125 (11.0)	391 (19.4)	223 (14.1)
SE		±(0.471)		±(0.272)		±(1.454)		±(1.417)
Mean	14 (3.47)	14 (3.47)	25 (4.82)		461 (19.9)	468 (20.0)	793 (27.1)	
SE		±(0.272)				±(1.417)		
Control <sup>a</sup>	1	1	0		3	12	0	
Urea <sup>a</sup>	0	0	1		2	0	5	

F-test:

Interaction	MS	MS
Cultivar	∞	∞
Strain	∞	∞

<sup>a</sup> Excluded for statistical analyses.

<sup>b</sup> Values in parentheses are means after  $\sqrt{x}$  transformation

Table 26: Modulation of chickpea at 74 days in Rhizobium strain and host interaction trial in RCI-4, 1981-82

Strain	Module wt. mg/plant <sup>b</sup>			Module wt. g/sq. m <sup>b</sup>				
	Ami- perl	BDM 9-3	K-850	Mean	Ami- perl	BDM 9-3	K-850	Mean
H-45	77 (8.43)	92 (9.47)	127 (11.18)	96 (9.69)	2.69 (1.60)	3.14 (1.76)	3.86 (1.95)	3.23 (1.77)
F-75	48 (6.57)	77 (8.72)	106 (10.2)	77 (8.50)	1.35 (1.13)	2.86 (1.68)	3.75 (1.91)	2.65 (1.57)
IC-76	40 (6.33)	28 (5.24)	69 (8.18)	46 (6.56)	1.22 (1.10)	0.73 (0.85)	2.02 (1.40)	1.32 (1.11)
SE		(±0.912)		(±0.526)		(±0.153)		(±0.088)
Mean	55 (7.11)	66 (7.81)	101 (9.86)		1.75 (1.28)	2.24 (1.43)	3.21 (1.75)	
SE		(±0.526)				(±0.088)		
Control <sup>a</sup>	2	6	0		0.06	0.25	0	
Urea <sup>a</sup>	0	0	2		0.01	0	0.07	

F. test:

Interaction	MS
Cultivar	ns
Strain	ns

<sup>a</sup> Excluded from statistical analyses.

<sup>b</sup> Values in parentheses are means after  $\sqrt{x}$  transformation.

Table 27: Specific activity of chickpea nodules at 74 days in Rhizobium strain and cultivar interaction trial in RCE-4 in 1981-82

Strain	$\mu\text{M C}_2\text{H}_4$ production/g.nod/hr			
	Annigeri	BDN 9-3	K-850	Mean
H-45	80	95	108	94
F-75	106	102	76	95
IC-76	142	114	151	136
SE		$\pm 12.9$		$\pm 7.5$
Mean	109	104	112	
SE		$\pm 7.5$		
Control <sup>a</sup>	20	16	106	
Urea <sup>a</sup>	9	0	5	

F.test:

Interaction	NS
Cultivar	NS
Strain	

24

<sup>a</sup> Excluded from statistical analyses

Table 28:  $N_2$ -ase activity in chickpeas at 74 days in Rhizobium strain and host interaction trial in RCE-4, 1981-82

Strain	$\mu M C_2H_4$ /plant/hr <sup>b</sup>		$\mu M C_2H_4$ /m <sup>2</sup> /hr <sup>b</sup>		Mean	SEM	K-850	K-850	K-850	Mean	SEM	K-850	K-850	Mean
	Amigerl	60M 9-3	Amigerl	60M 9-3										
H-45	7.0 (2.44)	9.0 (2.93)	14.1 (3.68)	10.0 (3.02)	245 (14.5)	313 (17.2)	428 (20.3)	329 (17.4)						
F-75	5.2 (2.15)	8.1 (2.79)	8.1 (2.81)	7.1 (2.58)	145 (11.6)	297 (16.7)	281 (16.5)	241 (15.0)						
IC-76	5.7 (2.38)	3.4 (1.74)	10.3 (3.13)	6.5 (2.42)	173 (13.1)	83 (8.8)	308 (17.0)	188 (13.0)						
SE		( $\pm 0.371$ )		( $\pm 0.214$ )										( $\pm 1.16$ )
Mean	6.0 (2.32)	6.8 (2.49)	10.8 (3.21)		188 (13.1)	231 (14.3)	339 (18.0)							
SE		( $\pm 0.214$ )												( $\pm 1.16$ )
Control <sup>a</sup>	0.1	0.3	0		3	14	1							
Urea <sup>a</sup>	0	0.1	0.1		0	3	2							

F-test:

Interaction	NS	NS
Cultivar	*	*
Strain	NS	**

<sup>a</sup> Excluded from statistical analyses

<sup>b</sup> Values in parentheses are means after  $\sqrt{x}$  transformation

Table 29: Dry matter production at 74 days in Rhizobium strain and host interaction trial in RCE-4 in 1981-82

Strain	Dry matter g/plant <sup>a</sup>			Dry matter kg/ha <sup>a</sup>				
	Annigeri	BOM 9-3	K-850	Mean	Annigeri	BOM 9-3	K-850	Mean
H-45	2.86 (1.68)	2.23 (1.48)	2.45 (1.56)	2.51 (1.58)	1060 (32.4)	780 (27.7)	760 (27.4)	870 (29.1)
F-75	2.40 (1.49)	1.92 (1.36)	2.08 (1.42)	2.13 (1.42)	730 (26.1)	700 (25.9)	710 (26.4)	720 (26.1)
IC-76	1.97 (1.39)	1.24 (1.11)	1.82 (1.33)	1.68 (1.28)	610 (24.4)	330 (18.1)	540 (22.7)	490 (21.7)
Control	1.34 (1.15)	1.67 (1.28)	1.41 (1.18)	1.47 (1.20)	410 (20.1)	590 (24.1)	400 (19.9)	470 (21.4)
Urea	3.72 (1.92)	6.30 (2.03)	3.97 (1.99)	4.00 (1.98)	1610 (39.7)	1580 (38.8)	1330 (36.4)	1510 (38.3)
SE		(±0.125)		(±0.072)		(±2.8)		(±1.4)
Mean	2.46 (1.53)	2.27 (1.45)	2.35 (1.50)		890 (28.5)	800 (28.9)	750 (26.6)	
SE		(±0.096)				(±1.1)		

F. test:

Interaction

Cultivar

Strain

MS

MS

MS

MS

MS

MS

<sup>a</sup> Data analysed after  $\sqrt{x}$  transformation.

**Table 30: Percent nitrogen in chickpea tops at 74 days  
in Rhizobium strain and host interaction trial  
in RCE-4 in 1981-82**

Strain	Cultivar			Mean
	Annigeri	BDN 9-3	K-850	
H-45	2.18	2.30	2.26	2.25
F-75	2.18	2.36	2.21	2.25
IC-76	2.46	2.27	2.22	2.32
Control	1.89	1.78	2.18	1.95
Urea	2.54	2.74	2.90	2.73
SE		±0.123		±0.071
Mean	2.25	2.29	2.36	
SE		±0.055		

F. test:

Interaction	NS
Cultivar	NS
Strain	



Table 31: Dry matter production and N-uptake at 92 days in<sup>a</sup>  
Rhizobium stain and host interaction trial in  
RCE-4 in 1981-82

Inoculation treatment	Dry matter (kg/ha)			N-uptake (kg/ha)		
	Annigeri	BDN 9-3	K-850	Annigeri	BDN 9-3	K-850
H-45	1880 <sup>b</sup>	1860	1870	40.5	42.5	42.0
F-75	1750	1600	2050	38.6	38.1	45.2
IC-76	2500 <sup>b</sup>	1140	1370	49.0 <sup>b</sup>	26.4	30.0
Control	1010	1030	1490	18.9	18.9	20.0
Urea (150 kg N/ha)	2090 <sup>b</sup>	3000	2820	68.2 <sup>b</sup>	82.0	80.9

<sup>a</sup> The data have not been statistically analysed as photographs and observations on other variables suggest that these measurements are in error.

Table 32: Dry matter production and grain yield at final harvest in Rhizobium strain and host interaction trial in RCE-4 in 1981-82

Inoculation Treatment	Dry matter (kg/ha)				Grain yield (kg/ha)			
	Cultivar		Mean	Anni-gerl	Cultivar			Mean
	Anni-gerl	BDM 9-3			K-850	BDM 9-3	K-850	
H-45	2290	1950	2350	2190	1310	1020	1410	1250
F-75	1710	1740	2100	1850	1040	970	1320	1110
IC-76	2190	1650	1850	1900	1240	900	1160	1100
Uninoculated	1190	1270	1370	1280	700	640	810	720
Urea (150 kg N/ha)	2520	2670	2830	2670	1380	1420	1690	1500
SE		±203		±117		±116		±67
Mean	1980	1860	2100		1130	990	1280	
SE		±91				±52		

F. test:

Interaction	NS
Cultivar	NS
Strain	**

Table 33: Correlations between N<sub>2</sub>-fixation parameters and yield of chickpea at 74 day harvest of host x Rhizobium strain interaction trial, 1981-82

	at 74 days			at final harvest	
	Module No.	Module wt.	N <sub>2</sub> -fixation	Shoot wt.	Dry matter
<u>At 74 days</u>					
Module wt.	.925**				
N <sub>2</sub> -fixation	.820**	.918**			
Shoot wt.	.668**	.639**	.663**		
<u>At final harvest</u>					
Dry matter	.555**	.545**	.549**	.477**	
Grain yield	.613**	.565**	.567**	.444**	.942**

\*\* = Significant at P < 0.01

n = 48

Table 34: Nitrogen percent in seeds of 3 chickpea varieties under different nitrogen regimes

Treatment	Cultivar			Mean
	Annigeri	BDN 9-3	K-850	
IC-76	2.84	3.26	2.90	3.00
H-45	2.75	3.21	2.96	2.98
F-75	2.79	3.07	2.85	2.91
Uninoculated	2.32	2.69	2.48	2.50
Nitrogen (150 kg N/ha)	3.62	4.10	3.74	3.82
SE		±0.090		±0.052
Mean	2.87	3.27	2.99	
SE		±0.040		

F. test:

Interaction	NS
Cultivar	**
Strain	**

**Table 35: Percent protein in seeds of 3 chickpea varieties under differing nitrogen regimes**

Treatment	Cultivar			Mean
	Annigeri	BDN 9-3	K-850	
H-45	17.2	20.1	18.5	18.6
F-75	17.5	19.2	17.8	18.2
IC-76	17.8	20.4	18.1	18.7
Uninoculated	14.5	16.8	15.5	15.6
Urea (150 kg N/ha)	22.6	25.7	23.4	23.9
SE		±0.56		±0.33
Mean	17.9	20.4	18.7	
SE		±0.25		

F. test:

Interaction	N.S.
Cultivar	
Strain	

Table 36: Nodulation and N<sub>2</sub>-fixation of chickpea in inoculation trial, BUS 11F, 1981-82

	Module number per plant				Module weight mg per plant				Specific activity $\mu\text{M C}_2\text{H}_4$ per g nod/hr				N <sub>2</sub> -ase activity $\mu\text{M C}_2\text{H}_4$ per pl/hr.			
	19	40	61	76	19	40	61	76	19	40	61	76	19	40	61	76
Inoculated	17	13	10	10	18	57	79	51	55.6	56.6	30.5	4.2	1.07	3.48	2.96	0.21
Control	2	3	2	2	3	22	24	24	30.0	38.0	18.3	2.2	0.08	0.93	0.48	0.06
SE	$\pm 0.8$	$\pm 0.7$	$\pm 0.6$	$\pm 0.5$	$\pm 1.3$	$\pm 2.7$	$\pm 8.6$	$\pm 3.7$	$\pm 3.14$	$\pm 3.47$	$\pm 4.22$	$\pm 0.66$	$\pm 0.118$	$\pm 0.407$	$\pm 0.613$	$\pm 0.032$
F-test	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Variances for each treatment were different after non-additivity of residuals was observed.

Table 17: Shoot weight, Nitrogen per cent and N uptake at different plant ages in inoculation trial, BUS 11F, 1981-82.

No. of days after sowing	Shoot wt. (g)/plant			Nitrogen percent in shoot			N uptake (mg)/plant					
	19	40	61	74 <sup>a</sup>	19	40	61	74	19	40	61	74
Inoculation	0.14	0.54	1.77	3.23	2.08	3.41	3.14	2.36	3.99	18.7	55.4	76.6
Control	0.14	0.54	1.56	2.05	2.96	3.29	2.97	2.13	4.13	18.0	48.7	61.1
SE ±	0.004	0.028	0.083	0.132	0.057	0.075	0.016	0.096	0.096	0.99	2.49	4.70
F. test	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	*	*

<sup>a</sup>Data from 74 day harvest based on larger sample (ca 70 plants) than at other dates. N uptake data were not collected on 76 day sampling.

Table 38: Dry matter production and N-uptake at 74 day harvest chickpea in inoculation trial, BUS 11F, 1981-82.

Treatment	Dry matter (kg/ha)	N-uptake (kg/ha)
Inoculated	1050	25.0
Control	900	19.3
SE	±44	±1.47
F. test	*	*



Table 39: Final harvest and grain yield in inoculation trial, BUS 11F, 1981-82

Treatment	Dry matter at final harvest (kg/ha)	Grain yield (kg/ha)	Potential <sup>a</sup> grain yield (kg/ha)
Inoculated	1490	470	710
Control	1370	430	630
SE	±41	NA <sup>b</sup>	±37
CV %	9	-	17
F. test:	NS	-	NS

<sup>a</sup> Yield calculated on basis of all seed being harvestable from Heliothis damaged pods.

<sup>b</sup> Data not analysed as they do not satisfy statistical assumptions.

Table 40: Effect of different methods of *Rhizobium* application on nodulation and nitrogenase activity of 60 days old chickpea plants grown in an alfisol field, RCE-4, 1981-82 (area harvested 0.6 sq. m)

Treatment	Percent plants nodulated	Module no. per plant	Module no. per sq. m	Module dry wt mg/pl	Module dry wt g/sq.m
Inoculated seed	76.7	6	230	26	1.02
Granular inoculum	56.7	5	272	45	1.75
Inoculated seed + irrigation	88.3	10	384	57	2.21
Liquid inoculum	80.0	16	744	108	5.16
Uninoculated controls:					
Seed	6.7	1	48	20	0.99
Seed + irrigation	8.3	2	55	44	1.48
Seed + liquid	15.0	1	43	38	1.32
SE	±10.25	±0.4	±52.1	±10.7	±0.466
F. test	**	**	**	**	**

Table 41: Effect of different methods of Rhizobium inoculum application on dry matter and grain yield (kg/ha) of chickpea at 60 days and at maturity in an alfisol field, RCE-4, 1981-82

Treatment	Dry matter yield at 60 days	Dry matter yield at final harvest	Grain yield
Inoculated seed	1100	1860	1150
Inoculated seed + irrigated	1300	2200	1320
Granular inoculum	1110	2020	1210
Liquid inoculum	1300	2240	1320
Uninoculated controls:			
Seed	750	1660	970
Seed + irrigation	760	1990	1040
Seed + liquid	690	2030	1040
SE	±127	±231	±143
F. test		NS	NS

Table 42: Correlation among N<sub>2</sub>-fixation parameters after 60 days of plant growth and yield parameters in method of inoculation trial, RCE-4, 1981-82

	N <sub>2</sub> -ase activity	Specific activity	Module number	Module weight	Shoot weight	Dry matter yield
Specific activity	0.653**					
Module No.	0.676**	0.133 <sup>MS</sup>				
Module wt.	0.901**	0.345 <sup>MS</sup>	0.803**			
Shoot wt.	0.393 <sup>MS</sup>	-0.036 <sup>MS</sup>	0.655**	0.492*		
Dry matter yield	0.460*	0.160 <sup>MS</sup>	0.5*	0.502*	0.655**	
Grain yield	0.449*	0.070 <sup>MS</sup>	0.576**	0.534*	0.783**	0.612**

MS = Not significant \* = Significant at 5% probability \*\* = Significant at 1% probability

**Table 43: Effect of delay in sowing after irrigation and method of inoculant application on percent plants nodulated at 30 days in glasshouse.**

Sowing day after irri- gation	Control		H-45		IC-76		Mean
	Seed coat	Liquid	Seed coat	Liquid	Seed coat	Liquid	
1	0	0	70.8	100.0	31.2	75.0	46.2
3	0	0	75.0	100.0	37.5	75.0	47.9
5	6.3	0	97.1	97.1	47.1	18.5	44.4
7	8.3	0	45.8	93.8	12.5	43.7	34.0
SE			±12.41 <sup>a</sup>				±4.20
Mean	3.6	0	72.2	97.7	32.1	53.1	
SE			±6.40				

F.test:      Sowing day      NS  
                  Treatment        \*\*  
                  Interaction      NS

<sup>a</sup> SE for comparison of treatment of treatment means within a sowing day.

Table 44A: Effect of delay in sowing after irrigation and method of inoculant application on nodule numbers per plant at 30 days, potted plants in glasshouse.

Sowing day after irri- gation	Control		H-45		IC-76		Mean
	Seed coat	Liquid	Seed coat	Liquid	Seed coat	Liquid	
1	0 (0)	0 (0)	3 (0.40)	8 (0.91)	<1 (0.14)	3 (0.62)	2 (0.36)
3	0 (0)	0 (0)	2 (0.46)	15 (1.17)	<1 (0.20)	3 (0.80)	4 (0.39)
5	<1 (0.04)	0 (0)	4 (0.66)	7 (0.87)	1 (0.24)	<1 (0.19)	2 (0.33)
7	<1 (0.03)	0 (0)	2 (0.44)	5 (0.76)	<1 (0.07)	2 (0.33)	2 (0.27)
SE			(±0.097) <sup>a</sup>				(±0.030)
Mean	<1 (0.02)	0 (0)	3 (0.62)	9 (0.93)	<1 (0.17)	2 (0.41)	
SE			(±0.044)				
F.test:	Sowing day	NS					
	Treatment	**					
	Interaction	NS					

<sup>a</sup> SE for comparison of treatment means within a sowing day

Table 44B: The effect of delay in sowing after irrigation and inoculation method on mean fresh weight per plant in pots at 30 days

Sowing day after irrigation	Control		H-45		IC-76		Mean
	Seed coat	Liquid	Seed coat	Liquid	Seed coat	Liquid	
1	940	920	980	1080	940	980	970
3	920	1020	1010	1070	1100	980	1020
5	820	790	1240	980	1000	870	940
7	1040	1000	1100	850	900	910	970
SE				±77 ±76 <sup>a</sup>			±32
Mean	930	930	1080	1000	990	920	
SE				±38			

F.test: Sowing day NS  
 Treatment \*  
 Interaction NS

<sup>a</sup>SE for comparison of treatment means within a sowing day.

Table 25: Module number per plant of chickpea at different times, under different types of<sup>a</sup> land preparations in a vertisol field, BP-13, 1981-82.

Treatment	Amalgari	K-950	Mean	Amalgari	K-950	Mean
	At 25 days			At 51 days		
Broad bed with furrow	18 (1.27)	26 (1.41)	23 (1.34)	21 (1.31)	43 (1.62)	32 (1.46)
Broad bed with mulch	18 (1.24)	25 (1.39)	21 (1.32)	19 (1.28)	39 (1.59)	29 (1.43)
Ridge of 60 cm	16 (1.19)	27 (1.42)	22 (1.31)	21 (1.32)	44 (1.63)	33 (1.48)
Flat bed	19 (1.28)	29 (1.46)	25 (1.37)	23 (1.35)	44 (1.65)	34 (1.50)
SE	$\pm(0.048)$	$\pm(0.052)$ <sup>b</sup>	$\pm(0.030)$	$\pm(0.038)$	$\pm(0.044)$ <sup>b</sup>	$\pm(0.021)$
Mean	18 (1.24)	27 (1.42)		21 (1.32)	43 (1.62)	
SE	$\pm(0.026)$			$\pm(0.022)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : ns			Cultivar : ns		
	Interaction: NS			Interaction: NS		
	At 34 days			At 62 days		
Broad bed with furrow	39 (1.51)	47 (1.53)	38 (1.52)	29 (1.27)	50 (1.58)	39 (1.43)
Broad bed with mulch	37 (1.54)	42 (1.49)	39 (1.54)	19 (1.28)	40 (1.60)	30 (1.44)
Ridge of 60 cm	40 (1.52)	43 (1.61)	42 (1.56)	21 (1.27)	33 (1.52)	27 (1.40)
Flat bed	41 (1.53)	35 (1.74)	39 (1.63)	21 (1.27)	42 (1.59)	31 (1.48)
SE	$\pm(0.109)$	$\pm(0.111)$ <sup>b</sup>	$\pm(0.076)$	$\pm(0.064)$	$\pm(0.068)$ <sup>b</sup>	$\pm(0.042)$
Mean	39 (1.52)	45 (1.61)		29 (1.27)	39 (1.57)	
SE	$\pm(0.058)$			$\pm(0.038)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : ns		
	Interaction: NS			Interaction: NS		
	At 82 days			At 100 days		
Broad bed with furrow	12 (1.07)	31 (1.48)	21 (1.27)			
Broad bed with mulch	14 (1.14)	40 (1.58)	27 (1.36)			
Ridge of 60 cm	18 (1.24)	29 (1.44)	23 (1.34)			
Flat bed	17 (1.23)	42 (1.60)	29 (1.41)			
SE	$\pm(0.069)$	$\pm(0.051)$ <sup>b</sup>	$\pm(0.034)$			
Mean	15(1.17)	35(1.53)				
SE	$\pm(0.023)$					
F-test:	Treatment : NS					
	Cultivar : ns					
	Interaction: NS					

<sup>a</sup> Data analyzed after log10 (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

Table 66: Module number per sq.m. of chickpea at different times, under different types of land preparations in a vertisol field, BP-13, 1981-82.

Treatment	Amnigeri	E-850	Mean	Amnigeri	E-850	Mean
	AS 28 Max			AS 31 Max		
Broad bed with furrow	518 (2.71)	718 (2.84)	618 (2.78)	560 (2.78)	1160 (3.85)	860 (2.90)
Broad bed with mulch	479 (2.67)	683 (2.83)	580 (2.75)	520 (2.72)	1050 (3.02)	790 (2.87)
Ridge of 60 cm	539 (2.71)	904 (2.94)	722 (2.83)	710 (2.85)	1470 (3.15)	1090 (3.00)
Flat bed	643 (2.80)	977 (2.99)	850 (2.89)	770 (2.88)	1480 (3.17)	1130 (3.02)
SE	$\pm(0.048)$	$\pm(0.048)$	$\pm(0.302)$	$\pm(0.030)$	$\pm(0.030)$	$\pm(0.021)$
Mean	545 (2.72)	825 (2.90)		640 (2.80)	1290 (3.10)	
SE	$\pm(0.026)$	$\pm(0.026)$		$\pm(0.022)$	$\pm(0.022)$	
F. test:	Treatment : NS	Treatment : NS		Treatment : NS	Treatment : NS	
	Cultivar : NS	Cultivar : NS		Cultivar : NS	Cultivar : NS	
	Interaction: NS	Interaction: NS		Interaction: NS	Interaction: NS	
	AS 28 Max			AS 31 Max		
Broad bed with furrow	1050 (2.94)	1060 (2.94)	1030 (2.95)	540 (2.72)	1000 (3.01)	810 (2.87)
Broad bed with mulch	1010 (2.97)	1140 (3.02)	1070 (3.00)	510 (2.72)	1000 (3.03)	800 (2.87)
Ridge of 60 cm	1340 (3.04)	1450 (3.13)	1390 (3.09)	690 (2.81)	1100 (3.04)	900 (2.93)
Flat bed	1300 (3.05)	1930 (3.26)	1650 (3.16)	690 (2.81)	1390 (3.12)	1040 (2.96)
SE	$\pm(0.109)$	$\pm(0.109)$	$\pm(0.076)$	$\pm(0.066)$	$\pm(0.066)$	$\pm(0.042)$
Mean	1190 (3.00)	1300 (3.09)		610 (2.77)	1160 (3.05)	
SE	$\pm(0.096)$	$\pm(0.096)$		$\pm(0.034)$	$\pm(0.034)$	
F. test:	Treatment : NS	Treatment : NS		Treatment : NS	Treatment : NS	
	Cultivar : NS	Cultivar : NS		Cultivar : NS	Cultivar : NS	
	Interaction: NS	Interaction: NS		Interaction: NS	Interaction: NS	
	AS 28 Max			AS 31 Max		
Broad bed with furrow	320 (2.50)	840 (2.91)	580 (2.71)			
Broad bed with mulch	380 (2.57)	1090 (3.02)	730 (2.79)			
Ridge of 60 cm	590 (2.77)	960 (2.96)	780 (2.86)			
Flat bed	570 (2.75)	1390 (3.12)	900 (2.94)			
SE	$\pm(0.049)$	$\pm(0.049)$	$\pm(0.038)$			
Mean	460 (2.65)	1070 (3.00)				
SE	$\pm(0.025)$	$\pm(0.025)$				
F. test:	Treatment : NS	Treatment : NS				
	Cultivar : NS	Cultivar : NS				
	Interaction: NS	Interaction: NS				

<sup>a</sup> Data analyzed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment



Table 47: Nodule dry wt (mg per plant) of chickpea at different times, under<sup>a</sup> different land preparations in a vertisol field, BP-13, 1981-82.

Treatment	Amalgari	K-050	Mean	Amalgari	K-050	Mean
	At 25 days			At 31 days		
Broad bed with furrow	18.1 (1.28)	23.3 (1.35)	20.7 (1.29)	42 (1.61)	142 (2.14)	92 (1.60)
Broad bed with mulch	15.7 (1.17)	23.1 (1.34)	19.4 (1.26)	39 (1.58)	122 (2.00)	80 (1.57)
Ridge of 60cm	15.5 (1.17)	20.3 (1.45)	22.4 (1.31)	43 (1.62)	126 (2.10)	84 (1.64)
Flat bed	21.5 (1.33)	26.5 (1.43)	25.0 (1.38)	62 (1.79)	131 (2.11)	97 (1.95)
SE	$\pm(0.055)$	$\pm(0.057)$ <sup>b</sup>	$\pm(0.038)$	$\pm(0.037)$	$\pm(0.038)$	$\pm(0.026)$
Mean	17.7 (1.23)	26.1 (1.39)		47 (1.65)	130 (2.11)	
SE	$\pm(0.029)$			$\pm(0.019)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : NS		
	Interaction: NS			Interaction: NS		
	At 33 days			At 43 days		
Broad bed with furrow	80 (1.89)	229 (2.37)	160 (2.13)	52 (1.68)	218 (2.32)	175 (2.00)
Broad bed with mulch	74 (1.83)	257 (2.18)	165 (2.12)	54 (1.72)	277 (2.42)	165 (2.07)
Ridge of 60 cm	58 (1.73)	234 (2.40)	157 (2.08)	38 (1.57)	144 (2.27)	115 (1.82)
Flat bed	90 (1.95)	275 (2.43)	182 (2.19)	58 (1.74)	239 (2.36)	148 (2.05)
SE	$\pm(0.047)$	$\pm(0.044)$ <sup>b</sup>	$\pm(0.034)$	$\pm(0.058)$	$\pm(0.073)$ <sup>b</sup>	$\pm(0.026)$
Mean	76 (1.86)	257 (2.40)		50 (1.68)	231 (2.34)	
SE	$\pm(0.023)$			$\pm(0.036)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : NS		
	Interaction: NS			Interaction: NS		
	At 42 days			At 48 days		
Broad bed with furrow	35 (1.51)	140 (2.13)	80 (1.82)			
Broad bed with mulch	31 (1.48)	179 (2.23)	105 (1.86)			
Ridge of 60 cm	26 (1.47)	175 (2.13)	81 (1.80)			
Flat bed	39 (1.57)	181 (2.24)	110 (1.90)			
SE	$\pm(0.058)$	$\pm(0.063)$ <sup>b</sup>	$\pm(0.037)$			
Mean	33 (1.51)	159 (2.18)				
SE	$\pm(0.032)$					
F-test:	Treatment : NS					
	Cultivar : NS					
	Interaction: NS					

<sup>a</sup> Data analyzed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

Table 48: Seedling dry wt (g per sq m) of chickpeas at different times, under different types of land preparation in a vertisol field, Dr-13, 1981-82.

Treatment	Amnigeri	E-950	Mean	Amnigeri	K-950	Mean
	At 26 days			At 31 days		
Broad bed with furrow	0.89 (2.87)	0.63 (2.78)	0.56 (2.73)	1.12 (3.04)	3.04 (3.58)	2.08 (3.31)
Broad bed with mulch	0.83 (2.61)	0.63 (2.77)	0.53 (2.69)	1.06 (3.01)	3.30 (3.52)	2.18 (3.27)
Ridge of 60 cm	0.52 (2.69)	0.98 (2.97)	0.75 (2.83)	1.44 (3.14)	4.19 (3.62)	2.81 (3.38)
Flat bed	0.72 (2.95)	0.95 (2.95)	0.83 (2.90)	2.08 (3.32)	4.36 (3.64)	3.22 (3.48)
SE	$\Delta(0.075)$	$\Delta(0.038)$	$\Delta(0.038)$	$\Delta(0.037)$	$\Delta(0.036)$	$\Delta(0.026)$
	At 62 days			At 68 days		
Broad bed with furrow	0.54 (2.70)	0.80 (2.87)		1.82 (3.13)	3.92 (3.59)	
Bred bed with mulch	0.93 (2.61)	0.63 (2.77)	0.53 (2.69)	1.06 (3.01)	3.30 (3.52)	2.18 (3.27)
Ridge of 60 cm	0.52 (2.69)	0.98 (2.97)	0.75 (2.83)	1.44 (3.14)	4.19 (3.62)	2.81 (3.38)
Flat bed	0.72 (2.95)	0.95 (2.95)	0.83 (2.90)	2.08 (3.32)	4.36 (3.64)	3.22 (3.48)
SE	$\Delta(0.075)$	$\Delta(0.038)$	$\Delta(0.038)$	$\Delta(0.037)$	$\Delta(0.036)$	$\Delta(0.026)$
	At 54 days			At 63 days		
Broad bed with furrow	2.18 (3.33)	6.48 (3.81)	4.33 (3.57)	1.81 (3.11)	5.92 (3.73)	3.64 (3.93)
Bred bed with mulch	2.01 (3.26)	6.97 (3.84)	4.49 (3.55)	1.85 (3.15)	7.33 (3.86)	4.49 (3.91)
Ridge of 60 cm	1.94 (3.26)	8.51 (3.92)	5.23 (3.60)	1.25 (3.09)	8.38 (3.79)	3.82 (3.80)
Flat bed	2.98 (3.47)	9.18 (3.96)	6.08 (3.71)	1.93 (3.26)	7.96 (3.88)	4.95 (3.58)
SE	$\Delta(0.047)$	$\Delta(0.034)$	$\Delta(0.034)$	$\Delta(0.048)$	$\Delta(0.038)$	$\Delta(0.026)$
	At 52 days			At 62 days		
Broad bed with furrow	0.94 (1.51)	3.81 (2.13)	2.38 (1.82)	1.51 (3.15)	6.95 (3.82)	
Bred bed with mulch	0.83 (1.48)	4.05 (2.23)	2.54 (1.86)	1.06 (3.01)	3.30 (3.52)	2.18 (3.27)
Ridge of 60 cm	0.94 (1.47)	4.53 (2.13)	2.73 (1.80)	1.44 (3.14)	4.19 (3.62)	2.81 (3.38)
Flat bed	1.30 (1.57)	6.03 (2.26)	3.67 (1.90)	2.08 (3.32)	4.36 (3.64)	3.22 (3.48)
SE	$\Delta(0.078)$	$\Delta(0.053)$	$\Delta(0.037)$	$\Delta(0.037)$	$\Delta(0.036)$	$\Delta(0.026)$
	At 62 days			At 68 days		
Broad bed with furrow	0.94 (1.51)	3.81 (2.13)	2.38 (1.82)	1.51 (3.15)	6.95 (3.82)	
Bred bed with mulch	0.83 (1.48)	4.05 (2.23)	2.54 (1.86)	1.06 (3.01)	3.30 (3.52)	2.18 (3.27)
Ridge of 60 cm	0.94 (1.47)	4.53 (2.13)	2.73 (1.80)	1.44 (3.14)	4.19 (3.62)	2.81 (3.38)
Flat bed	1.30 (1.57)	6.03 (2.26)	3.67 (1.90)	2.08 (3.32)	4.36 (3.64)	3.22 (3.48)
SE	$\Delta(0.078)$	$\Delta(0.053)$	$\Delta(0.037)$	$\Delta(0.037)$	$\Delta(0.036)$	$\Delta(0.026)$
	At 62 days			At 68 days		
Broad bed with furrow	0.94 (1.51)	3.81 (2.13)	2.38 (1.82)	1.51 (3.15)	6.95 (3.82)	
Bred bed with mulch	0.83 (1.48)	4.05 (2.23)	2.54 (1.86)	1.06 (3.01)	3.30 (3.52)	2.18 (3.27)
Ridge of 60 cm	0.94 (1.47)	4.53 (2.13)	2.73 (1.80)	1.44 (3.14)	4.19 (3.62)	2.81 (3.38)
Flat bed	1.30 (1.57)	6.03 (2.26)	3.67 (1.90)	2.08 (3.32)	4.36 (3.64)	3.22 (3.48)
SE	$\Delta(0.078)$	$\Delta(0.053)$	$\Delta(0.037)$	$\Delta(0.037)$	$\Delta(0.036)$	$\Delta(0.026)$

<sup>a</sup> Data analysed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

Table 29: Specific activity ( $\mu$ moles  $C_2H_4$  Production per g seed /h) of chickpea nodules at different times, under different types of land preparations in a vertisol field, MP-13, 1981-82.

Treatment	Amalgari	K-950	Mean	Amalgari	K-950	Mean
	AS 26 dms			AS 31 dms		
Broad bed with furrow	50.4 (1.69)	54.4 (1.71)	53.4 (1.70)	35.6 (1.55)	37.5 (1.57)	36.6 (1.56)
Broad bed with mulch	68.2 (1.81)	47.4 (1.67)	57.8 (1.74)	34.2 (1.52)	46.7 (1.67)	40.8 (1.60)
Ridge of 60 cm	61.6 (1.77)	49.6 (1.69)	55.6 (1.73)	36.0 (1.56)	40.3 (1.60)	38.2 (1.58)
Flat bed	81.0 (1.90)	58.5 (1.76)	69.8 (1.83)	32.2 (1.50)	48.6 (1.60)	40.4 (1.59)
SE	$\Delta(0.043)$	$\Delta(0.041)$ <sup>b</sup>	$\Delta(0.055)$	$\Delta(0.032)$	$\Delta(0.032)$ <sup>b</sup>	$\Delta(0.023)$
Mean	65.3 (1.79)	53.0 (1.71)		34.5 (1.53)	43.3 (1.63)	
SE	$\Delta(0.021)$	$\Delta(0.016)$		$\Delta(0.016)$	$\Delta(0.016)$	
F-test:	Treatment : NS	Treatment : NS		Treatment : NS	Treatment : NS	
	Cultivar : *	Cultivar : *		Cultivar : **	Cultivar : **	
	Interaction : NS	Interaction : NS		Interaction : NS	Interaction : NS	
	AS 28 dms			AS 29 dms		
Broad bed with furrow	24.4 (1.91)	47.6 (1.65)	37.0 (1.53)	2.1 (0.32)	9.7 (0.90)	5.9 (0.65)
Broad bed with mulch	25.6 (1.39)	47.1 (1.63)	36.4 (1.51)	2.6 (0.40)	11.7 (1.02)	7.1 (0.71)
Ridge of 60 cm	22.4 (1.32)	41.1 (1.59)	31.8 (1.46)	3.0 (0.34)	10.7 (1.16)	6.8 (0.60)
Flat bed	16.7 (1.21)	43.9 (1.63)	30.3 (1.42)	1.8 (0.23)	12.1 (1.07)	6.9 (0.65)
SE	$\Delta(0.040)$	$\Delta(0.042)$ <sup>b</sup>	$\Delta(0.020)$	$\Delta(0.024)$	$\Delta(0.024)$	$\Delta(0.040)$
Mean	22.8 (1.33)	44.9 (1.63)		2.4 (1.06)	12.1 (0.35)	
SE	$\Delta(0.031)$	$\Delta(0.030)$		$\Delta(0.030)$	$\Delta(0.030)$	
F-test:	Treatment : *	Treatment : NS		Treatment : NS	Treatment : NS	
	Cultivar : **	Cultivar : **		Cultivar : **	Cultivar : **	
	Interaction : NS	Interaction : NS		Interaction : NS	Interaction : NS	
	AS 22 dms <sup>c</sup>					
Broad bed with furrow	0.09	0.53	0.31			
Broad bed with mulch	0.13	0.07	0.50			
Ridge of 60 cm	0.05	0.81	0.73			
Flat bed	0.07	0.77	0.62			
Mean	0.24	0.75				

<sup>a</sup> Data analyzed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

<sup>c</sup> Data does not satisfy statistical assumptions and hence not analyzed

Table 50: Nitrogen activity (mass mole of C<sub>2</sub>H<sub>4</sub> production per pl / h) of chickpeas at different times,<sup>a</sup> under different types of land preparations in a vertisolic field, MP-13, 1991-92.

Treatment	Amigori	K-950	Mean	Amigori	K-950	Mean
	AS 24 dnx					
Breed bed with furrow	870 (2.92)	1930 (3.04)	1150 (2.99)	1790 (3.22)	4820 (3.67)	3300 (3.45)
Breed bed with mulch	1030 (2.98)	1060 (3.01)	1050 (2.99)	1390 (3.10)	5670 (3.75)	3530 (3.43)
Ridge of 60 cm	1030 (2.94)	1470 (3.10)	1250 (3.04)	1540 (3.17)	5030 (3.70)	3200 (3.24)
Flat bed	1740 (3.23)	1050 (3.19)	1690 (3.21)	1950 (3.20)	6630 (3.80)	4290 (3.54)
SE	$\pm(0.090)$	$\pm(0.065)$	$\pm(0.065)$	$\pm(0.060)$	$\pm(0.040)$	$\pm(0.042)$
	AS 51 dnx					
Mean	1170 (3.02)	1800 (3.1)		1670 (3.20)	5540 (3.73)	
SE	$\pm(0.045)$			$\pm(0.031)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : NS		
	Interaction : NS			Interaction : NS		
	AS 58 dnx					
Breed bed with furrow	2100 (3.30)	1070 (4.02)	680 (3.64)	110 (2.0)	2230 (3.29)	1170 (2.65)
Breed bed with mulch	1810 (3.22)	1220 (4.03)	700 (3.63)	280 (2.15)	3100 (3.42)	1090 (2.79)
Ridge of 60 cm	1210 (3.08)	1070 (3.99)	500 (3.54)	110 (2.01)	2790 (3.43)	1450 (2.72)
Flat bed	1530 (3.16)	1310 (4.04)	730 (3.62)	110 (1.97)	2000 (3.43)	1490 (2.70)
SE	$\pm(0.050)$	$\pm(0.029)$	$\pm(0.029)$	$\pm(0.007)$	$\pm(0.007)$	$\pm(0.053)$
	AS 69 dnx					
Mean	1660 (3.19)	1160 (4.03)		150 (2.03)	2750 (3.39)	
SE	$\pm(0.039)$			$\pm(0.049)$		
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : NS		
	Interaction : NS			Interaction : NS		
	AS 82 dnx <sup>c</sup>					
Breed bed with furrow	3	88	46			
Breed bed with mulch	4	177	90			
Ridge of 60 cm	67	122	94			
Flat bed	3	151	77			
Mean	19	136				

<sup>a</sup> Data analyzed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

<sup>c</sup> Data does not satisfy statistical assumptions and hence not analyzed

Table 51: Nitrogenase activity (µ moles C<sub>2</sub> H<sub>4</sub> production per sq m/h) of chickpea at different times<sup>a</sup> under different types of land preparations in a vertisol field, BP-13, 1981-82.

Treatment	Annigeri	K-850	Mean	Annigeri	K-850	Mean
AL 28 day						
Broad bed with furrow	23.6 (1.36)	38.7 (1.49)	31.1 (1.42)	49 (1.66)	131 (2.11)	90 (1.88)
Broad bed with mulch	27.9 (1.41)	28.8 (1.44)	28.4 (1.43)	38 (1.54)	154 (2.18)	96 (1.86)
Ridge of 60 cm	34.4 (1.46)	49.1 (1.67)	41.8 (1.56)	51 (1.70)	168 (2.22)	109 (1.96)
Flat bed	57.8 (1.75)	54.8 (1.72)	56.3 (1.73)	66 (1.81)	222 (2.32)	144 (2.06)
SE	s(0.090)	s(0.065)	s(0.065)	s(0.060)	s(0.060)	s(0.042)
Mean	35.9 (1.5)	42.9 (1.58)		51 (1.67)	169 (2.21)	
SE	s(0.045)	s(0.045)		s(0.031)	s(0.031)	
F-test:	Treatment : 0	Treatment : 0		Treatment : 0	Treatment : 0	
	Cultivar : NS	Cultivar : NS		Cultivar : NS	Cultivar : NS	
	Interaction : NS	Interaction : NS		Interaction : NS	Interaction : NS	
AL 53 day						
Broad bed with furrow	57 (1.74)	295 (2.46)	176 (2.10)	3.0 (0.43)	60.4 (1.73)	31.7 (1.08)
Broad bed with mulch	49 (1.66)	333 (2.87)	191 (2.06)	7.7 (0.58)	84.2 (1.95)	45.9 (1.22)
Ridge of 60 cm	40 (1.60)	336 (2.52)	188 (2.06)	3.7 (0.53)	92.9 (1.95)	48.3 (1.24)
Flat bed	51 (1.68)	340 (2.61)	245 (2.14)	3.6 (0.49)	95.9 (1.95)	49.8 (1.22)
SE	s(0.050)	s(0.029)	s(0.029)	s(0.047)	s(0.047)	s(0.053)
Mean	49 (1.67)	351 (2.51)		4.5 (0.51)	83.4 (1.87)	
SE	s(0.029)	s(0.029)		s(0.049)	s(0.049)	
F-test:	Treatment : NS	Treatment : NS		Treatment : NS	Treatment : NS	
	Cultivar : NS	Cultivar : NS		Cultivar : NS	Cultivar : NS	
	Interaction : NS	Interaction : NS		Interaction : NS	Interaction : NS	
AL 82 day						
Broad bed with furrow	0.09	2.39	1.24			
Broad bed with mulch	0.11	4.79	2.45			
Ridge of 60 cm	2.23	4.05	3.14			
Flat bed	0.10	5.05	2.58			
Mean	0.63	4.07				

<sup>a</sup> Data analyzed after log<sub>10</sub> (x) transformation

<sup>b</sup> SE for comparison of means within a treatment

<sup>c</sup> Data does not satisfy statistical assumptions and hence not analyzed

Table 52: Top wt (g per plant) of chickpea at different times, under different types of land preparations in a verticill field, BP-13, 1981-82.

Treatment	Annigeri	K-850	Mean	Annigeri	K-850	Mean
	At 26 days			At 31 days		
Broad bed with furrow	0.855	0.583	0.499	1.78	1.80	1.79
Broad bed with mulch	0.959	0.515	0.487	1.66	1.71	1.68
Ridge of 60 cm	0.809	0.521	0.505	1.74	1.74	1.74
Flat bed	0.533	0.541	0.537	2.09	1.91	2.00
SE	$\pm 0.031$		$\pm 0.023$			$\pm 0.075$
	At 26 days			At 31 days		
Mean	0.484	0.530		1.82	1.79	
SE	$\pm 0.015$					$\pm 0.056$
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : e			Cultivar : NS		
	Interaction : NS			Interaction : NS		
	At 58 days			At 63 days		
Broad bed with furrow	3.63	3.20	3.42	7.02	5.87	6.45
Broad bed with mulch	3.08	3.17	3.13	6.70	6.16	6.43
Ridge of 60 cm	3.27	3.60	3.44	7.10	6.37	6.74
Flat bed	3.59	3.22	3.40	6.92	6.08	6.50
SE	$\pm 0.224$		$\pm 0.129$			$\pm 0.302$
	At 58 days			At 63 days		
Mean	3.39	3.30		6.93	6.12	
SE	$\pm 0.130$					$\pm 0.183$
F-test:	Treatment : NS			Treatment : NS		
	Cultivar : NS			Cultivar : e		
	Interaction : NS			Interaction : NS		
	At 82 days			At 87 days		
Broad bed with furrow	10.8	9.6	10.2			
Broad bed with mulch	10.0	9.3	9.6			
Ridge of 60 cm	9.7	10.4	10.0			
Flat bed	10.4	9.4	9.9			
SE	$\pm 0.51$		$\pm 0.03$			
	At 82 days			At 87 days		
Mean	10.2	9.7				
SE	$\pm 0.19$					
F-test:	Treatment : NS					
	Cultivar : NS					
	Interaction : NS					

<sup>a</sup> SE for comparison of means within a treatment

Table 53: Dry wt. (g per sq m) of chickpea at different times, under different types of  
 land preparations in a vertisol field, BP-13, 1981-82.

Treatment	Amnigori	K-850	Mean	Amnigori	K-850	Mean
At 28 days						
Broad bed with furrow	12.4	14.7	13.6	48.3	48.9	48.6
Broad bed with mulch	12.5	14.0	13.2	45.1	46.4	45.7
Ridge of 60 cm	16.3	17.4	16.8	58.1	58.1	58.1
Flat bed	17.8	18.0	17.9	69.6	63.5	66.6
SE	±0.96		±0.69	±3.26		±2.10
		±0.94 a		±3.52a		
Mean	14.7	16.0		55.3	54.2	
SE	±0.87			±1.76		
F-test:	Treatment : ss			Treatment : ss		
	Cultivar : MS			Cultivar : MS		
	Interaction : MS			Interaction : MS		
At 53 days						
Broad bed with furrow	93	87	93	191	160	175
Broad bed with mulch	84	84	85	182	167	175
Ridge of 60 cm	109	120	115	237	213	225
Flat bed	120	107	114	231	203	217
SE	±6.8		±3.8	±10.6		±8.9
		±7.9a		±8.3a		
Mean	103	100		210	185	
SE	±4.0			±4.1		
F-test:	Treatment : ss			Treatment : ss		
	Cultivar : MS			Cultivar : ss		
	Interaction : MS			Interaction : MS		
At 82 days						
Broad bed with furrow	294	262	278			
Broad bed with mulch	272	252	262			
Ridge of 60 cm	323	346	334			
Flat bed	347	315	331			
SE	±14.9		±12.3			
		±11.9a				
Mean	309	294				
SE	±6.0					
F-test:	Treatment : ss					
	Cultivar : MS					
	Interaction : MS					

<sup>a</sup> SE for comparison of means within a treatment

Table 54: Total dry matter and grain yield of chickpea at 117 days harvest under different land preparations, field BP 13, (area harvested 27 sq. m). Data supplied by Dr. N.P. Saxena, Pulse Physiology.

Treatment	Total dry matter (kg/ha)			Grain yield (kg/ha)		
	Annigeri	K-850	Mean	Annigeri	K-850	Mean
Broad bed furrow	2980	3070	3030	1880	1720	1800
Broad bed + mulch	2720	2960	2840	1690	1650	1670
Ridge of 60 cm	3120	3160	3140	1930	1760	1850
Flat bed	2970	3110	3040	1810	1690	1750
SE	±59		±51	±40		±34
	±42 <sup>a</sup>			±28 <sup>a</sup>		
Mean	2950	3070		1830	1700	
SE		±21			±14	
CV %		3			4	
F. test: Treatment		**			*	
Cultivar		**			**	
Interaction		NS			NS	

<sup>a</sup> SE for comparison of cultivar mean within a treatment.



Table 55: Effect of depth of sowing on nodule number per plant of chickpea, BP 13, 1981-82

Cultivar	37 days after planting			47 days after planting			59 days after planting		
	5 cm	10 cm	Mean	5 cm	10 cm	Mean	5 cm	10 cm	Mean
Annigeri	10	7	8	8	6	7	7	6	6
K-850	20	11	15	16	10	13	15	8	11
SE	±0.7			±0.7			±0.7		
Mean	15	9		12	8		11	7	
SE	±2.3			±1.2			±1.7		

F.test: Cultivar      NS      NS  
 Depth                  NS      NS  
 Interaction          \*\*      \*

Table 56: Effect of depth of sowing on nodule weight (mg/plant) of chickpea, BP 13, 1981-82

Cultivar	37 days after planting		47 days after planting		59 days after planting	
	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
Annigeri	30	13	22	15	14	14
K-850	55	20	37	24	48	20
SE	±7.9		±5.6	±2.7	±7.5	±5.01
Mean	43	16		35	19	31
SE	±5.6			±7.5		±9.9
F.test: Cultivar	NS			NS		NS
Depth	*			NS		NS
Interaction	NS			*		*

Table 57: Effect of depth of sowing on nitrogenase activity ( $\mu$  moles  $C_2H_4$  per g nodule/hr) of chickpea. BP 13, 1981-82

Cultivar	37 days after planting		47 days after planting		59 days after planting	
	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
Annigeri	38.9	27.2	10.3	6.5	0.01	0.047
K-850	27.1	26.8	7.9	10.0	0.87	0.84
SE	$\pm 7.29$	$\pm 5.15$	$\pm 2.29$	$\pm 1.62$	$\pm 0.196$	$\pm 0.139$
Mean	33.0	27.0	9.1	8.2	0.44	0.66
SE	$\pm 5.15$		$\pm 1.62$		$\pm 0.139$	
F. test:	Cultivar	MS	MS	MS	MS	MS
	Depth	MS	MS	MS	MS	MS
	Interaction	MS	MS	MS	MS	MS

Table 58: Effect of depth of sowing in nitrogenase activity ( $\mu$ moles  $C_2H_4$  per plant/hr) of chickpea, BP 13, 1981-82

Cultivar	37 days after planting			47 days after planting			59 days after planting <sup>a</sup>		
	5 cm	10 cm	Mean	5 cm	10 cm	Mean	5 cm	10 cm	Mean
	Annigeri	1.26	0.38	0.82	0.21	0.10	0.15	0 (0.1)	.001 (0.04)
K-850	1.59	0.54	1.06	0.43	0.24	0.34	.024 (1.30)	.018 (1.19)	.021 (1.25)
SE		$\pm 0.047$	$\pm 0.331$		$\pm 0.06$	$\pm 0.042$		$\pm (0.162)$	$\pm (0.3)$
Mean	1.42	0.46		0.32	0.17		.012 (0.70)	.012 (1.02)	
SE		$\pm 0.331$			$\pm 0.042$			$\pm (0.3)$	
F.test: Cultivar			NS		*				NS
Depth			NS		*				NS
Interaction			NS		NS				*

<sup>a</sup> Data analysed after  $\log_{10} (x+1000)+1$  transformation

Table 59: Effect of depth of sowing on shoot weight of chickpea, BP 13, 1981-82

Cultivar	37 days after planting		47 days after planting		59 days after planting	
	5 cm	10 cm	5 cm	10 cm	5 cm	10 cm
Annigeri	1.96	1.64	3.07	2.71	5.57	4.50
K-850	1.79	1.79	3.78	3.22	5.12	4.57
SE	±0.102		±0.122		±0.455	
Mean	1.88	1.71	3.42	2.96	5.34	4.53
SE	±0.072		±0.086		±0.322	
F. test: Cultivar	NS		**		NS	
Depth	NS		*		NS	
Interaction	NS		NS		NS	

Table 60: Percentage contribution of nodules in epicotyl region in the trial studying effect of depth of sowing on various nodulation and  $N_2$ -fixation parameters, BP 13, 1981-82

Cultivar	Module number per plant			Module wt mg. per plant			$N_2$ -ase activity $\mu$ moles $C_2H_4$ per pl/hr		
	37	47	59	37	47	59	37	47	59
Annigeri	33.7	26.4	16.8	32.8	25.9	11.3	42	39.2	1.3
K-850	44.6	41.8	35.4	42.5	40.9	39.7	57	38.8	51.7
SE	$\pm 2.26$	$\pm 9.68$	$\pm 2.45$	$\pm 6.15$	$\pm 13.2$	$\pm 2.24$	$\pm 19$	$\pm 9.49$	$\pm 6.26$
F. test	NS	NS	*	NS	NS	*	NS	NS	*

Table 61: Modulation of chickpea at 30 days under normal and extended day length, BP 13, 1981-82.

Cultivar	Module no. per plant		Module no. per sq. m.		Module wt. mg. per plant		Module wt. g. per sq. m.			
	Normal	Extended	Normal	Extended	Normal	Extended	Normal	Extended		
Annigeri	26	20	995	736	67	45	56	2.52	1.66	2.09
G-130	12	10	449	355	58	39	49	2.17	1.38	1.78
L-550	18	12	673	428	46	29	37	1.72	1.07	1.40
SE	±1.0	±1.3	±46.3	±45.9	±4.2	±1.8	±0.156	±0.156	±0.116	±0.124
	±0.5 <sup>a</sup>		±32.4		±2.6					
Mean	19	14	706	506	57	38	.	2.14	1.37	
SE	±1.1		±37.5		±3.6			±0.082		
F-test: light	NS		NS		.			.		
Cultivar	*		*		*			*		
Interaction	**		**		**			**		NS

<sup>a</sup>SE for comparison of treatment means within a cultivar

Table 62: Nitrogenase activity of chickpea at 30 days under normal and extended day length, 09/13, 1981-82

Cultivar	μ moles C <sub>2</sub> H <sub>4</sub> per g nodule/hr		μ moles C <sub>2</sub> H <sub>4</sub> per plant/hr		μ moles C <sub>2</sub> H <sub>4</sub> per sq.m/hr			
	Normal	Extended	Normal	Extended	Normal	Extended		
Annigeri	53.5	64.0	58.8	2.88	3.23	134	107	121
G-130	49.2	50.1	49.7	1.95	2.41	107	70	88
L-550	53.0	56.1	54.6	1.62	2.02	91	59	75
SE			±1.78		±0.122			±5.0
			±2.6		±0.313			±118
			±2.52 <sup>a</sup>		±0.173			±7.0
Mean	51.9	56.7		2.96	2.15	111	79	
SE			±1.59		±0.279			±10.3
F-test: Light	NS			NS				NS
Cultivar	*			**				**
Interaction	NS			NS				NS

<sup>a</sup>SE for comparison of treatment means within a cultivar



Table 63: Dry matter yield of chickpea at 30 days under normal and extended day length, BP 13, 1981-82.

Cultivar	Dry matter (g) per plant		Dry matter yield (kg/ha)	
	Normal	Extended	Normal	Extended
Annigeri	0.93	1.01	350	371
G-130	0.63	0.63	234	234
L-550	0.74	0.85	279	313
SE	±0.04		±15.9	±10.6
	±0.04 <sup>a</sup>		±14.9	
Mean	0.77	0.85	288	306
SE		±0.023		±10.3
F.test: Light		NS		NS
Cultivar		**		**
Interaction		NS		NS

<sup>a</sup>SE for comparison of treatment means within a cultivar

Table 64: Modulation of chickpea at 50 days under normal and extended day length, BP 13, 1981-82.

Cultivar	Module no. per plant		Module no. per sq.m		Module wt. mg per plant		Module wt. g. per sq.m	
	Normal	Extended	Normal	Extended	Normal	Extended	Normal	Extended
Annigeri	15	10	455	341	84	31	2.84	1.07
G-130	15	8	468	291	82	40	2.63	1.39
L-550	17	11	580	350	93	25	3.16	0.80
SE	4.3	1.4	108.2	46.6	11.4	3.92	0.307	0.13
F. test	NS	NS	NS	NS	NS	NS	NS	*

Table 65: Nitrogenase activity of chickpea at 50 days under normal and extended day length, BP 13, 1981-82

Cultivar	$\mu$ moles $C_2H_4$ per g nodule/hr		$\mu$ moles $C_2H_4$ per plant/hr		$\mu$ moles $C_2H_4$ per sq. m/hr	
	Normal	Extended	Normal	Extended	Normal	Extended
Annigeri	28.8	10.4	2.42	0.328	81.3	11.2
G-130	25.4	8.0	2.13	0.333	67.1	11.6
L-550	29.9	10.8	2.74	0.280	93.1	8.8
SE	$\pm 2.92$	$\pm 1.38$	$\pm 0.441$	$\pm 0.071$	$\pm 13.56$	$\pm 2.32$
F. test	NS	NS	NS	NS	NS	NS

Table 66: Dry matter yield of chickpea at 50 days under normal and extended day length, BP 13, 1981-82.

Cultivar	Dry matter (g) per plant		Dry matter yield (kg/ha)	
	Normal	Extended	Normal	Extended
Annigeri	2.65	3.03	892	1020
G-130	1.98	2.47	634	850
L-550	2.13	3.54	725	1100
SE	±0.159	±0.075	±19.2	±44
F. test	NS	**	**	*

Table 67: Changes in Rhizobium population ( $\log_{10}$  MPN) over time at different temperatures in pots without plants, 1981-82

Incubation time (days)	Temperature				Mean
	Low	Medium	High	Very high	
0	5.00	5.00	5.00	5.00	5.00
53	5.00	5.00	5.00	5.00	5.00
68	4.5	4.68	5.1	4.84	4.78
83	4.49	4.84	4.24	4.41	4.50
114	4.24	4.41	3.99	4.34	4.20
SE		±0.206			±0.103
Mean	4.65	4.87	4.58	4.72	
SE		±0.092			
CV %			9		
F. test:	Time		**		
	Temperature		NS		
	Time x temperature		NS		

Table 68: Modulation,  $N_2$ -fixation and plant growth of chickpea plants exposed to different soil temperatures for 6 days from day 46 to day 51 after sowing, 1981-82.

Temperature	Module number/ pot	Module weight g/pot	Specific activity $\mu M C_2H_6$ / g.module/hr	$N_2$ -ase activity $\mu M C_2H_6$ / pot/hr	Shoot weight g/pot	Root weight g/pot	Root/ shoot ratio
Low	1810 $\pm$ 357	1.58 $\pm$ 0.330	20.2 $\pm$ 3.66	30.0 $\pm$ 6.61	29.4 $\pm$ 1.15	6.56 $\pm$ 0.515	0.22 $\pm$ 0.019
Medium	1670 $\pm$ 105	1.63 $\pm$ 0.113	22.6 $\pm$ 7.27	34.5 $\pm$ 8.64	26.7 $\pm$ 3.02	7.08 $\pm$ 0.185	0.28 $\pm$ 0.015
High	970 $\pm$ 233	0.89 $\pm$ 0.215	3.4 $\pm$ 0.60	3.3 $\pm$ 1.19	21.0 $\pm$ 4.25	5.30 $\pm$ 0.937	0.26 $\pm$ 0.030
Very high	1040 $\pm$ 166	1.04 $\pm$ 0.179	0.9 $\pm$ 0.34	0.9 $\pm$ 0.48	30.0 $\pm$ 0.58	5.93 $\pm$ 0.502	0.20 $\pm$ 0.009
SE	$\pm$ 235	$\pm$ 0.224	$\pm$ 4.08	$\pm$ 5.48	$\pm$ 2.68	$\pm$ 0.598	$\pm$ 0.020
CV %	34	35	70	64	20	19	17
F. test	NS	NS	**	**	NS	NS	NS

Table 69: Modulation,  $N_2$ -fixation and plant growth of chickpea plants exposed to different soil temperatures for 40 days from day 7 to day 47 after sowing, 1981-82.

Temperature	Module number/pot	Module weight g/pot	Specific activity $\mu M C_2H_4$ /production/g.module/hr	$N_2$ -ase activity $\mu M C_2H_4$ /production/pot/hr	Shoot weight g/pot	Root weight g/pot	Root/shoot ratio
Low	1480 $\pm$ 118	1.95 $\pm$ 0.077	11.8 $\pm$ 1.93	22.7 $\pm$ 3.25	21.9 $\pm$ 1.03	9.55 $\pm$ 0.611	0.44 $\pm$ 0.016
Medium	1580 $\pm$ 179	1.55 $\pm$ 0.223	7.1 $\pm$ 0.88	11.1 $\pm$ 2.05	16.9 $\pm$ 0.71	6.30 $\pm$ 0.533	0.37 $\pm$ 0.025
High	1490 $\pm$ 180	1.33 $\pm$ 0.196	3.7 $\pm$ 0.88	4.7 $\pm$ 1.22	10.4 $\pm$ 0.32	5.91 $\pm$ 0.183	0.57 $\pm$ 0.016
Very high	800 $\pm$ 173	0.83 $\pm$ 0.180	2.4 $\pm$ 0.38	2.2 $\pm$ 0.74	6.8 $\pm$ 0.47	4.86 $\pm$ 0.586	0.72 $\pm$ 0.070
SE	$\pm$ 165	$\pm$ 0.178	$\pm$ 1.16	$\pm$ 2.05	$\pm$ 0.69	$\pm$ 0.509	$\pm$ 0.039
CV %	25	25	37	40	10	15	15
F. test	*	**	**	**	**	**	**

Table 70: Modulation activity of intercropped chickpea in a deep vertisol field BM-3, 1981-82.

Treatment	Module number/plant			Module number/sq. m <sup>a</sup>			Module weight mg/plant <sup>b</sup>			Module weight g/sq.m		
	Days after sowing		32	Days after sowing		74	Days after sowing		53	Days after sowing		74
	32	53		74	53		74	32		53	74	
Chickpea at 30 cm	18	20	11	691 (26.1)	752 (27.2)	340 (18.1)	48 (1.68)	51 (1.70)	31 (1.48)	1.76	1.95	0.97
Chickpea at 60 cm	21	24	16	328 (18.0)	444 (21.0)	353 (18.2)	67 (1.80)	65 (1.80)	59 (1.74)	1.00	1.20	1.23
Chickpea + sorghum	16	16	14	280 (16.6)	280 (16.7)	257 (15.9)	44 (1.63)	40 (1.57)	36 (1.54)	0.78	0.71	0.67
SE	±2.3	±1.4	±1.5	±(1.36)	±(1.19)	±(1.91)	±(0.062)	±(0.065)	±(0.049)	±(0.148)	±(0.183)	±(0.194)
F. test	NS	**	NS	**	**	NS	NS	NS	**	**	**	NS

<sup>a</sup> Data analysed after  $\sqrt{x}$  transformation

<sup>b</sup> Data analysed after  $\log_{10}(x)$  transformation



Table 71: Nitrogenase activity of intercropped chickpea in a deep vertisol field BW-3, 1981-82.

Treatment	$\mu\text{M C}_2\text{H}_4$ production/g nodule/hr			$\mu\text{M C}_2\text{H}_4$ production/plant/hr <sup>a</sup>			$\mu\text{M C}_2\text{H}_4$ production/sq.m./hr <sup>b</sup>		
	Days after sowing			Days after sowing			Days after sowing		
	32	53	74	32	53	74	32	53	74
Chickpea at 30 cm	22.5	10.7	1.12	1.08 (2.99)	0.57 (2.71)	0.04 (1.47)	40.4 (6.19)	21.4 (4.48)	0.99 (0.95)
Chickpea at 60 cm	16.6	13.5	2.62	1.27 (3.01)	0.92 (2.88)	0.17 (2.12)	19.5 (4.36)	17.2 (3.96)	3.03 (1.69)
Chickpea + sorghum	20.8	5.7	1.35	0.94 (2.94)	0.26 (2.27)	0.05 (1.39)	16.6 (3.98)	4.6 (1.97)	0.94 (0.82)
SE	±2.55	±1.60	±0.539	±(0.107)	±(0.122)	±(0.208)	±(0.613)	±(0.494)	±(0.198)
F. test	NS	NS	NS	NS	*	NS	NS	*	*

<sup>a</sup> Data analysed after  $\log_{10}(x+1000)$  transformation

<sup>b</sup> Data analysed after  $\sqrt{x}$  transformation

Table 72: Dry matter yield of intercropped chickpea in a deep vertisol, BM-3, 1981-82.

Treatment	Shoot dry weight (g) of chickpea per plant		Dry matter yield (kg/ha) of chickpea per sq. m.		Dry matter yield (kg/ha) of sorghum + chickpea	
	Days after planting		Days after planting		Days after planting	
	32	53	74	32	53	74
Chickpea at 30 cm	0.63 (0.79)	2.51 (1.58)	8.5 (2.84)	230	920	2200
Chickpea at 60 cm	0.82 (0.89)	3.33 (1.81)	10.1 (3.10)	130	620	1900
Chickpea + sorghum	0.71 (0.84)	2.35 (1.52)	4.4 (2.09)	120	410	810
SE	±(0.035)	±(0.076)	±(0.286)	±27.4	±46.8	±119
F. test	NS	NS	NS	**	**	**

Table 73: Total dry matter harvest (kg/ha) of safflower from different plots (reference: figure 12)\*

Plot No.	Dry matter	Plot No.	Dry matter	Plot No.	Dry matter
1	3245	18	2987	35	3234
2	5439	19	6057	36	6297
3	4765	20	3214	37	3912
4	5558	21	5516	38	3937
5	3883	22	2765	39	2614
6	5480	23	4647	40	4736
7	5969	24	2331	41	3535
8	3101	25	3403	42	4075
9	1213	26	5943	43	5760
10	3760	27	5173	44	3597
11	917	28	4524	45	7187
12	4563	29	2868	46	3346
13	703	30	5755	47	5656
14	3664	31	3422	48	3589
15	3457	32	5267	49	6570
16	3819	33	4678	50	4586
17	3830	34	3826	51	5177

\*Calculated from net harvest area of 1.32 sq.m.

Table 74: Estimates of N<sub>2</sub>-fixation in chickpea using safflower as a non-fixing control, BP 13, 1981-82

	G-130	K-850	SE
Dry matter (kg ha <sup>-1</sup> )	1318	2052	±43.4
Total N (kg ha <sup>-1</sup> )	28.7	43.5	±0.69
Atom % <sup>15</sup> N excess	0.030	0.023	±.001
% N fixed	-	23	
N fixed (kg ha <sup>-1</sup> ) by isotope dilution		10	
N fixed (kg ha <sup>-1</sup> ) by difference		15	

Table 75: Estimates of nitrogen fixed by isotope dilution in chickpea in different plots of a <sup>15</sup>N trial, BP 13, 1981-82

Plot	K-850			K-850			6-130		
	Reference = G 130			Reference = Saff.			Reference = Saff.		
	X N fixed	N fixed (kg/ha)	% N fixed	X N fixed	N fixed (kg/ha)	% N fixed	X N fixed	N fixed (kg/ha)	% N fixed
A	36.4	13.0	37.8	37.8	13.5	2.2	2.2	0.5	0.5
B	28.2	11.1	14.8	14.8	5.8	†	†	†	†
C	48.4	22.4	0	0	0	†	†	†	†
D	†	†	†	†	†	0	0	0	0
E	7.4	3.7	10.7	10.7	5.3	3.6	3.6	1.0	1.0
F	†	†	†	†	†	4.3	4.3	6.2	6.2
G	47.3	11.2	42.4	42.4	14.8	†	†	†	†
H	21.4	9.1	18.5	18.5	7.9	†	†	†	†
I	16.7	7.7	16.7	16.7	7.7	0	0	0	0
J	22.3	9.7	8.7	8.7	3.8	†	†	†	†
K	44.8	22.2	38.5	38.5	19.1	†	†	†	†
L	29.2	12.6	29.2	29.2	12.6	0	0	0	0
M	27.6	11.4	4.5	4.5	1.9	†	†	†	†
N	25.9	11.0	31.0	31.0	13.2	6.9	6.9	2.0	2.0
O	50.0	23.5	53.8	53.8	25.2	7.7	7.7	3.0	3.0
P	3.3	1.3	†	†	†	†	†	†	†
Q	35.4	16.1	0	0	0	†	†	†	†
R	26.1	12.9	26.1	26.1	12.9	0	0	0	0
Means (excluding negative values)		12.4			10.6			1.6	

† = negative values.

N fixed by difference (Total N K-850) - (Total N G-130) = 14.8 kg/ha

Table 76: Modulation and  $N_2$ -fixation at 41 day harvest of parents,  $F_1$  and control cultivars with two different sources of inoculum, glass house, 1981-82

Genotype	Mixed inoculant				HAU soil		
	Module no. per plant	Module wt. per plant (g)	$N_2$ -fixation per plant ( $\mu M C_2H_4/pl/h$ )	Module no. per plant	Module no. per plant	$N_2$ -fixation per plant ( $\mu M C_2H_4/pl/h$ )	
NEC-721 (parent)	36 $\pm$ 12	0.23 $\pm$ 0.10	1.6 $\pm$ 0.7	28 $\pm$ 11.0	5.1 $\pm$ 1.5		
G-130 (parent)	17 $\pm$ 2	1.58 $\pm$ 0.40	10.8 $\pm$ 2.8	4 $\pm$ 3.5	7.3 $\pm$ 1.9		
NEC-721 x G-130 ( $F_1$ )	14 $\pm$ 4	1.01 $\pm$ 0.20	8.7 $\pm$ 1.1	6 $\pm$ 3.5	4.1 $\pm$ 1.8		
K-850 (check)	89 $\pm$ 18	1.47 $\pm$ 0.11	19.3 $\pm$ 0.7	15 $\pm$ 5.0	8.1 $\pm$ 1.4		
Annigeri (check)	79 $\pm$ 12	1.00 $\pm$ 0.33	3.4 $\pm$ 1.3	10 $\pm$ 4.0	1.6 $\pm$ 0.9		

Table 77: Plant growth and N<sub>2</sub>-fixation parameters at 45 days of nine chickpea cultivars, BP 13, 1981-82

	Nodule No/ plant			Nodule dry weight (mg/plant)			Specific activity μM C <sub>2</sub> H <sub>4</sub> /g dry nodule/hr			N <sub>2</sub> -fixation μM C <sub>2</sub> H <sub>4</sub> / plant/hr			Shoot dry weight (g/plant)			Plant population/ sq. m		
	East	West	Mean	East	West	Mean	East	West	Mean	East	West	Mean	East	West	Mean	East	West	Mean
E-100	15	10	12	60	34	47	82	84	83	4.8	2.8	3.8	2.1	2.5	2.3	20	15	18
G-130	7	7	7	60	72	66	51	49	50	2.9	3.4	3.1	1.9	1.9	1.9	29	23	26
L-550	9	8	8	53	50	52	47	30	39	2.5	1.4	2.0	2.7	2.6	2.6	21	16	19
Rabat	12	11	11	55	54	55	73	56	64	4.0	3.1	3.5	2.5	3.0	3.2	13	12	12
P-310-1	10	10	10	99	138	119	58	54	56	5.4	6.9	6.2	2.3	3.0	2.6	25	21	23
P-319-1	35	31	33	150	149	150	42	37	39	6.3	5.6	6.0	2.2	1.9	2.0	30	26	28
ICC-435	31	32	32	143	153	148	43	42	43	6.2	6.1	6.1	1.9	1.8	1.8	28	26	27
ICC-685	28	30	29	102	86	94	51	66	59	5.4	5.7	5.5	2.0	1.7	1.9	29	30	29
K-850	29	31	30	239	190	215	61	60	61	13.2	11.7	12.5	2.3	2.2	2.2	26	21	23
SE		±2.2	±1.6		±16.7	±11.8		±8.7	±6.2		±1.04	±0.73		±0.53	±0.38		±2.2	±1.5
Mean	20	19		107	103		56	53		5.6	5.2		2.2	2.4		25	21	
SE		±0.7			±5.6			±2.9			±0.35			±0.18			±0.7	
CV %		23			32			32			30			47			19	
F. test:																		
Cultivar		**			**			**			**			NS			**	
Ridge place- ment		NS			NS			NS			NS			NS			**	
Interaction		NS			NS			NS			NS			NS			NS	

Table 78: Grain yield and dry matter production of nine chickpea cultivars, BP 13, 1981-82.

Cultivar	Dry matter yield kg/ha			Grain yield kg/ha			Plant population/ sq. m.		
	East	West	Mean	East	West	Mean	East	West	Mean
E-100	3530	2110	2820	1190	710	950	18	12	15
G-130	3540	2780	3160	1680	1290	1490	29	23	26
L-550	4040	2390	3220	1980	1080	1530	27	22	24
Rabat	3350	2250	2800	1390	900	1140	19	15	17
P-310-1	3280	2430	2850	1400	1030	1220	24	20	22
P-319-1	4430	2700	3560	2190	1270	1730	30	26	28
ICC-435	3940	3120	3530	1820	1480	1650	30	27	28
ICC-685	3550	3160	3350	2060	1670	1860	31	31	31
K-850	4130	2590	3360	2430	1620	2030	30	23	26
SE	±266		±188	±139		±98	±1.3		±0.9
Mean	3760	2610		1790	1230		26	22	
SE	±89			±46			±0.4		
CV (%)	17			18			10		
F.test: Cultivar	*			**			**		
Ridge placement	**			**			**		
Interaction	NS			NS			NS		



Table 79: Correlations between  $N_2$ -fixation parameters and plant growth parameters of nine chickpea cultivars, BP 13, 1981-82

	$N_2$ -ase/ plant	Specific activity	Module number	Module dry wt (mg/pl)	Top weight (g)	Root weight (g/pl)	Dry matter yield (kg/ha)
Specific activity	0.285**						
Module number	0.618**	-0.024 <sup>NS</sup>					
Module dry weight	0.796**	-0.239**	0.653**				
Top weight	-0.086 <sup>NS</sup>	-0.027 <sup>NS</sup>	-0.291 <sup>NS</sup>	0.100 <sup>NS</sup>			
Root weight	0.148 <sup>NS</sup>	0.139 <sup>NS</sup>	-0.112 <sup>NS</sup>	0.016 <sup>NS</sup>	0.528**		
Dry matter yield kg/ha	0.199 <sup>NS</sup>	-0.103 <sup>NS</sup>	0.335**	0.262*	0.03 <sup>NS</sup>	0.174 <sup>NS</sup>	
Grain yield kg/ha	0.417**	-0.161 <sup>NS</sup>	0.506**	0.454**	-0.011 <sup>NS</sup>	0.095 <sup>NS</sup>	0.879**

\* = Significant at P < 0.05  
 \*\* = Significant at P < 0.01  
 NS = Not significant

n = 35

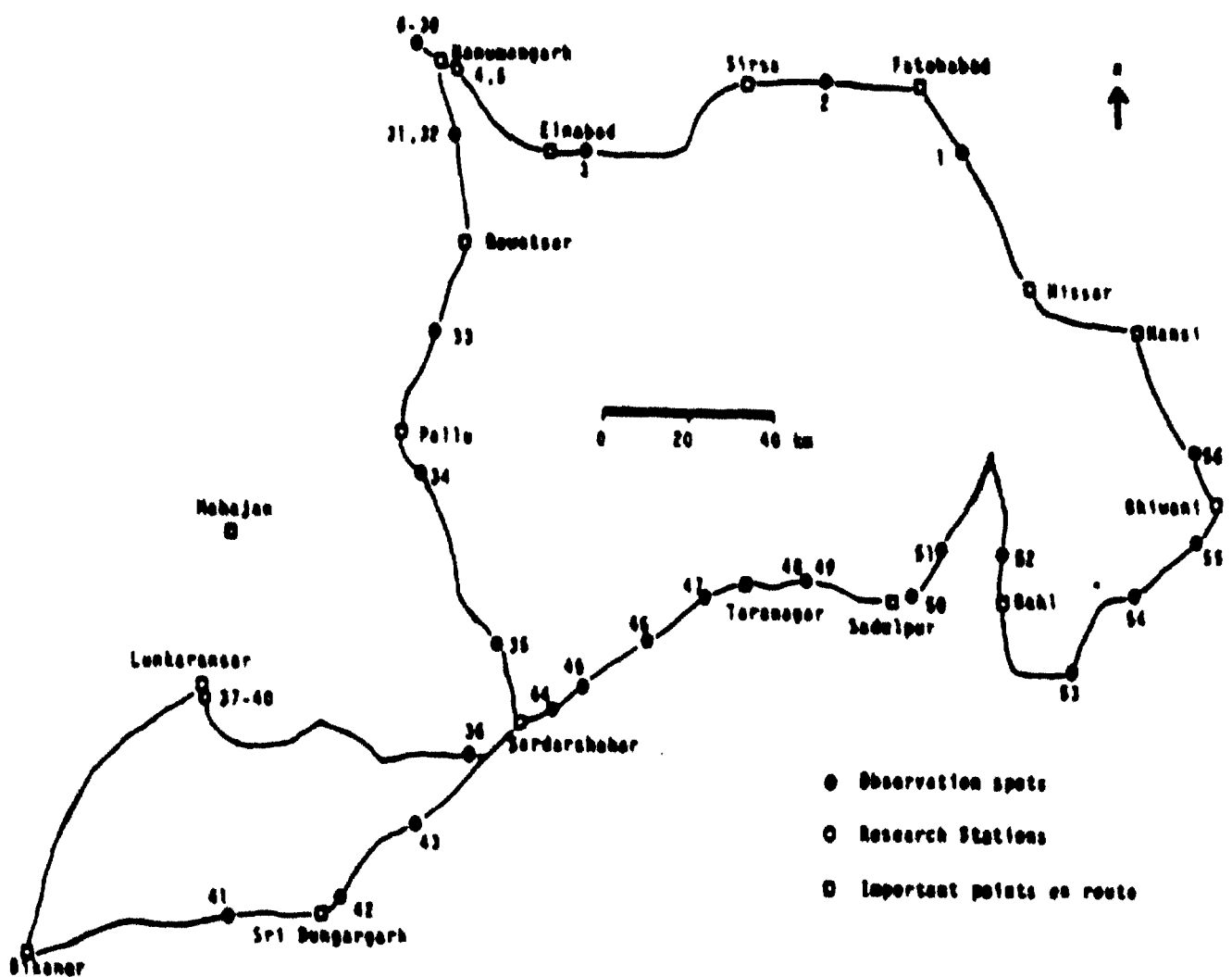


Figure 1: Touring route of Rajasthan, January 1982. Spot no. 33 to 51 fall in areas where chickpea is not preferred crop due to lack of moisture in the growing season. One may see an occasional field of chickpea on this route.

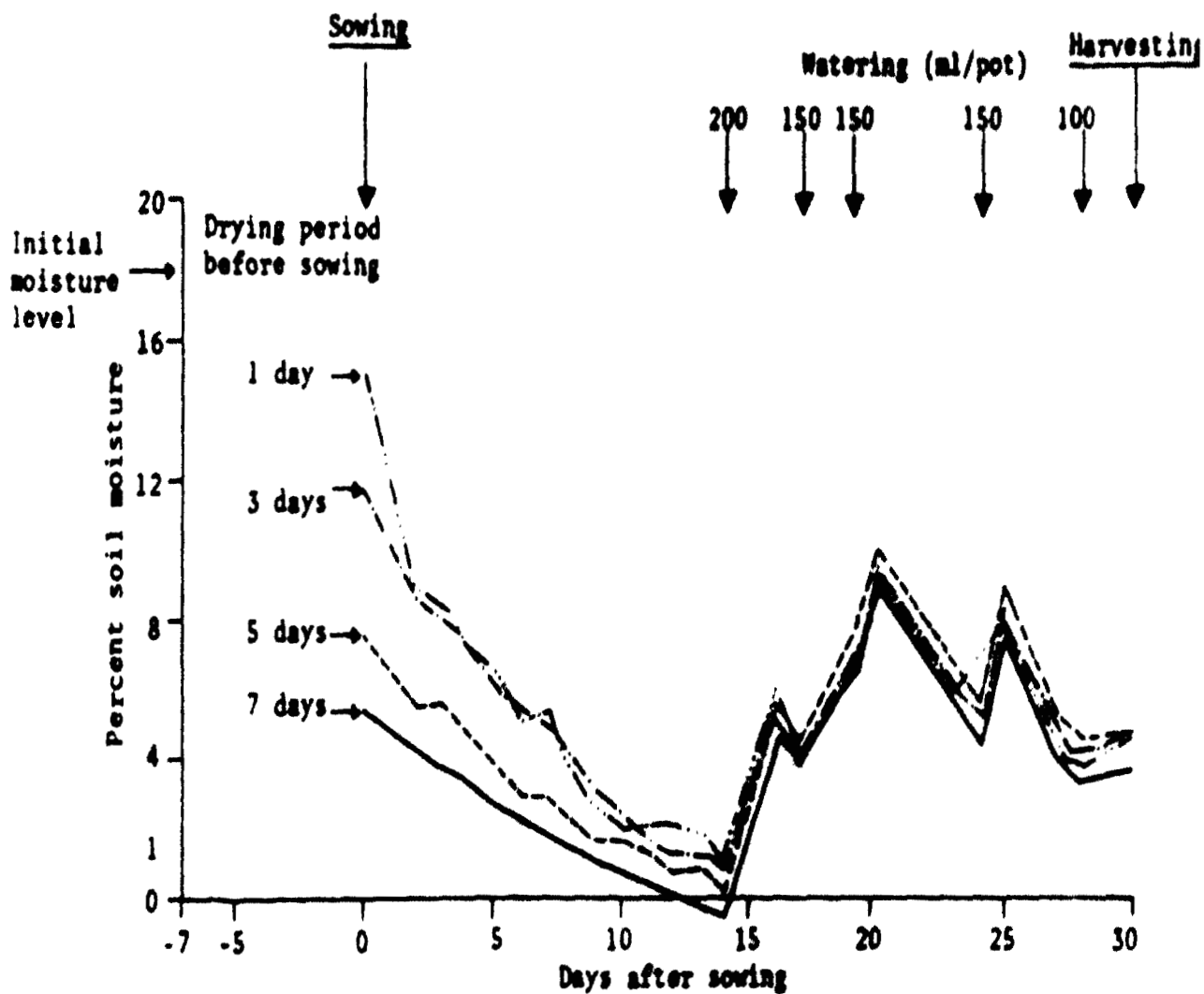


Figure 2. Moisture content of soil used in study of delayed sowing and method of inoculant application, Glass house, 1981-82.

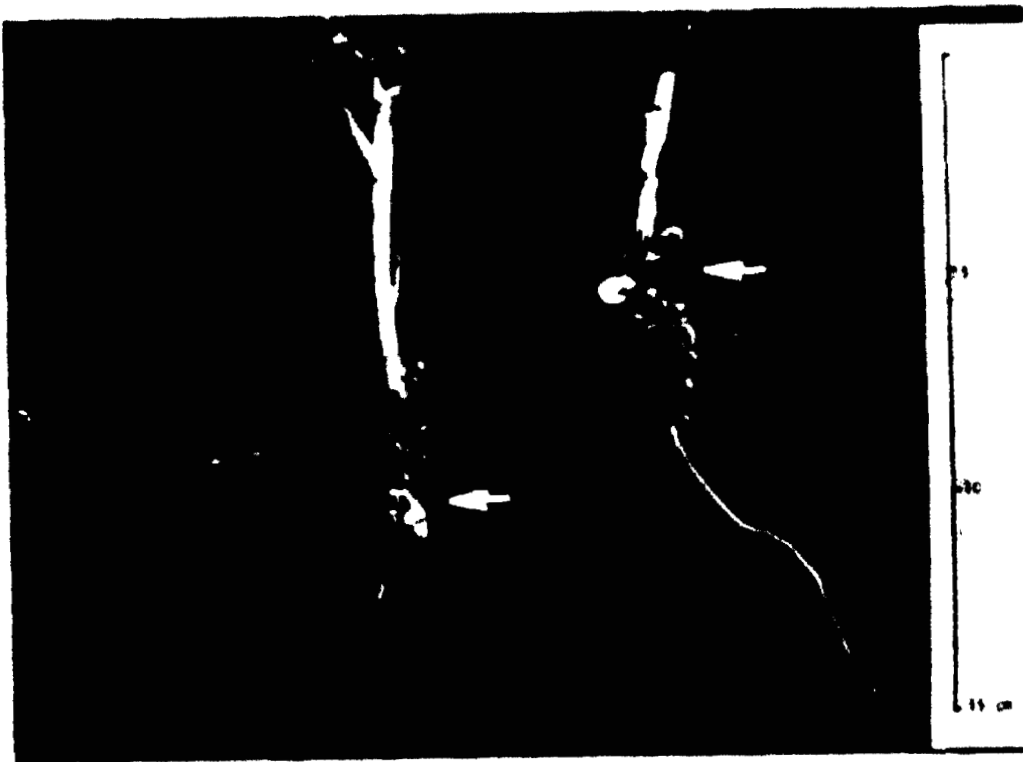


Figure Effect of depth of sowing on nodulation pattern of chickpea, 1981-82. Arrows indicate position of cotyledons.

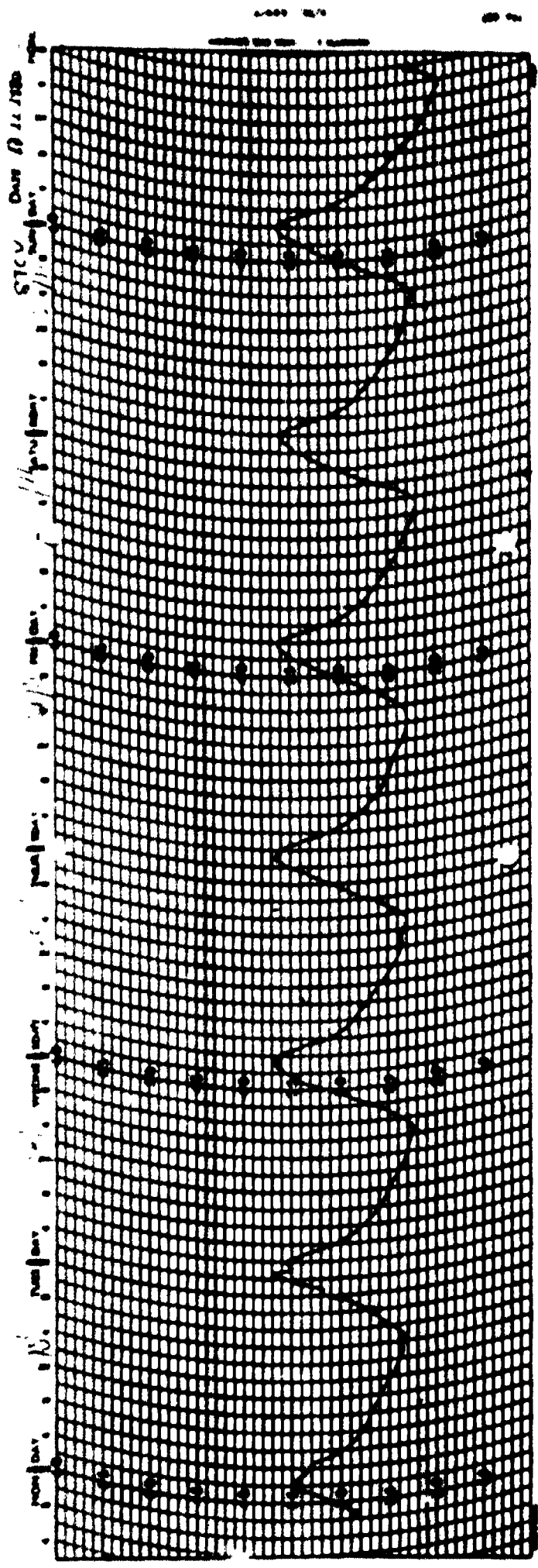


Figure 4: Soil temperature at 10 cm depth, between 17 and 24 November 1980  
 i.e. between 26 and 33 days after sowing chickpea in field 9T2,  
 1980-81.

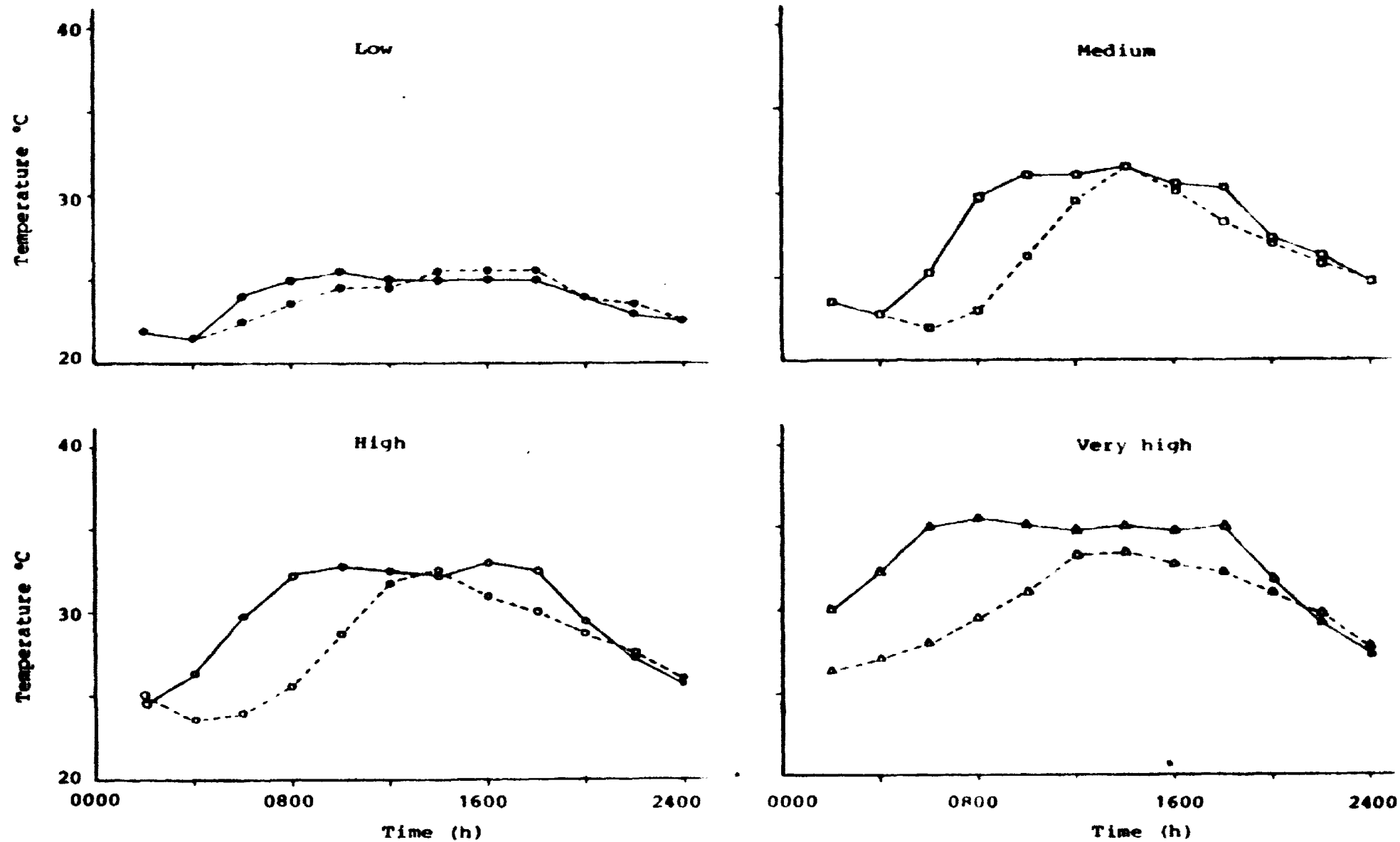
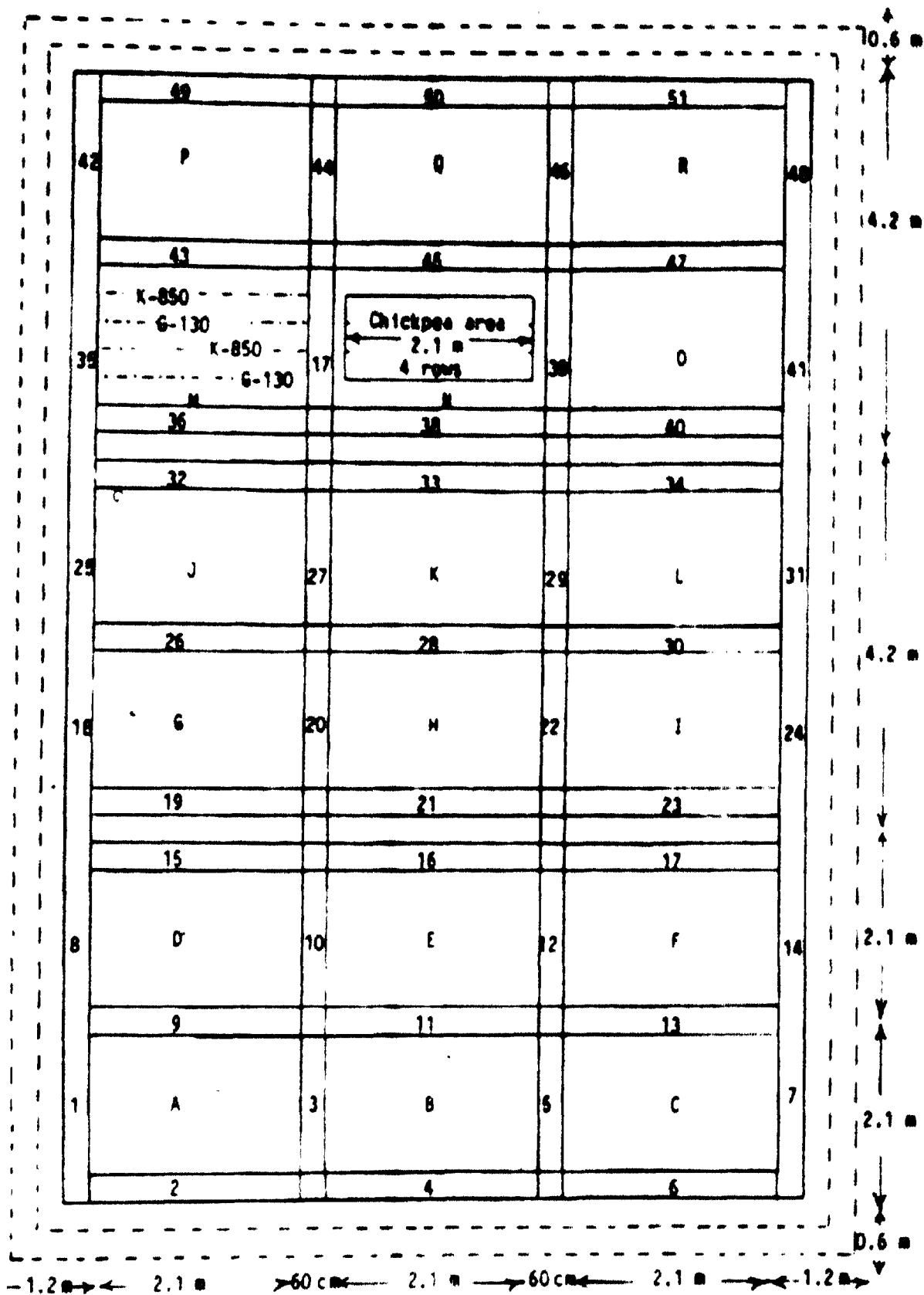


Figure 5: Temperature recordings in water (—) in the water baths and soil (---) in pots placed in four different water baths. Each figure represents a different temperature regime called low, medium, high and very high.

Figure 6: Lay out map of <sup>18</sup>m trial, BP 13, 1981-82

N-



Total area including borders i.e. dotted line on all four sides = 13.8 x 9.9 m

Net area from where harvest made = 12.6 x 7.5

Chickpea plot size (total 18 plots, A to R) = 2.1 m, 4 rows

Safflower plot size (total 51 plots, 1 to 51) = 2.1 m, 2 rows

Appendix 1: Performance of ICRISAT *Cicer Rhizobium* strains in multilocation trial of AICPIP, 1981-82

Reporting Location	Strain	Test cultivar	Yield of uninoculated control (kg/ha)	Change in grain yield (%) over control	Statistical significance	Strain rank/total no. of treatments	Top 5 treatments	Remarks
Burgapura (Rajasthan)	IC-59	RSG-2	1180	12.7	NS	7/16	IC-94, B-1, DG-90, IC-2072, DG-34	
	IC-94		1180	51.7	*	1/16		
	IC-2002		1180	-8.5	NS	13/16		
	IC-2072		1180	30.5	NS	4/16		
ICRISAT (Andhra Pradesh)	IC-59	Annigeri	1370	1.5	NS	14/18	IC-2002, CH-777, NIFTAL, IC-149, IC-76	Trial conducted on alfisol having <10 rhizobia per g of soil. NIFTAL is a mixture of 3 strains with IC-2002 as one of the three.
	IC-94			8.0	NS	9/18		
	IC-2002			36.5	*	1/18		
	IC-2072			-7.3	NS	16/18		
IARI (New Delhi)	IC-59	NA	1690	15.4	NS	9/16	Ca-181, H-44, CH-777, KG-31, B-1	H-44 did <10 rhizobia per g peat when tested at ICRISAT
	IC-94			18.3	*	6/16		
	IC-2002			3.6	NS	12/16		
	IC-2072			17.2	NS	7/16		
Jabalpur (Madhya Pradesh)	IC-59	NA	1580	12.7	NS	6/17	H-44, DG-90, IC-94, CBH-32, B-1	H-44 and DG-90 had respectively <10 and 10 <sup>6</sup> rhizobia per g inoculant when tested at ICRISAT
	IC-94			17.1	*	3/17		
	IC-2002			14.6	NS	17/17		
	IC-2072			12.0		7/17		
Kanpur (Uttar Pradesh)	IC-59	T-3	1610	28.6	NS	1/18	IC-59, NIFTAL, F-6, Ca-181, H-44	H-44 had <10 rhizobia per g of peat when tested at ICRISAT
	IC-94			-0.6	NS	17/18		
	IC-2002			20.5	NS	6/18		
	IC-2072			12.4	NS	13/18		
	IC-2018			19.3	NS	11/18		
Varanasi (Uttar Pradesh)	IC-94	T-3	770	-24.7	NS	14/16	DG-34, NIFTAL, IC-2072, DG-90, Ca-181/CH-777	DG-34 and DG-90 had <10 rhizobia per g of peat when tested at ICRISAT CV % = 21
	IC-2002			6.5	NS	9/16		
	IC-2072			29.2	NS	3/16		

NA = Data/information not available, \* = Significant, NS = Not significant.



Appendix 2: Performance of IC-76 in *Rhizobium* strain and host interaction trial of AICPIP 1981-82 on the basis of grain yield

Location	Mean yield (kg/ha) of un inoculated control	Mean yield (kg/ha) of IC-76	F-test		Response of IC-76	Interaction	Highest yielding cultivar/strain combination (% change in yield over respective uninoc. control)	Best yielding cultivar with IC-76 and (% change over respective uninoc. control)	Cultivars used in the trial	Rank of IC-76/total no. of treatments	Remarks
			Treatment	Cultivar							
Badnagar (Maharashtra)	1780	1950	NS	*	NS	NS	Annigeri/F-75 (10.7)	Annigeri (15.3)	A,B,R	4/5	
Dhoni (Bihar)	3830	4950	NA	NA	NA	NA	Part G-114/IC-76 (27.0)	Part G-114 (27.0)	O,P,C	3/5	Station report not available. Information from PIR report of 1980-81
Durgapura (Rajasthan)	1630	1830	NS	NS	NS	NS	Part G-114/M-85 (25.3)	Part G-114 (8.1)	O,P,R	4/5	
ICRISAT (Andhra Pradesh)	720	1100	*	*	*	NS	K-650/M-85 (79.1)	Annigeri (77.1)	A,B,E	4/5	Trial conducted in red soil with 42 ppm of MB, 1000 rhizobia per g soil from (150 kg N/ha) used as one of the treatment
ZARI (Muz Belhi)	1660	2110	*	*	*	NS	86-231/F-75 (41.3)	86-209 (42.9)	O,P,R	2/5	
Jabalpur (M.P.)	1700	1840	NS	*	*	*	JG-125B (24.4)	JG-125B (19.3)	O,E,F	4/5	Recommended cultivar Part G-114 not used
Kanpur (Uttar Pradesh)	1550	1650	NS	*	*	NS	86-209/F-75 (12.2)	Part G-114 (0.5) 86-209 (0.5)	O,P,K,R	4/5	
Baramasi (Uttar Pradesh)	1910	1980	NS	*	*	*	Part G-114/M-85 (38.4)	86-209 (26.6)	O,P,T	3/5	

\* = Significant, NS = Not significant, A = Annigeri, B = BDM 9-3, H = H-59, D = 86-209, P = Part G-114, R = Radway, C = C-238, K = K-650, E = JG-125B, F = JG-315, T = T-3; NA = Data/information not available, PIR = Principal Investigator, Microbiology.

Appendix 3: List of fields from where soil samples were taken  
for Experiment 4, Project CP-Micro-6, 1981-82

Field	Soil pH	EC m mhos/cm	MPN count ( $\log_{10}$ rhizobia/g soil) in top 15 cm profile
BIL 7B	8.1	0.18	3.57
BM 16C	8.5	0.23	3.84
BM 17	8.3	0.22	3.73
BP 2C	-	-	-
BP 8	8.4	0.20	4.05
BP 10 (BT-2)	8.3	0.20	3.24
BP 14 (BP)	8.0	0.21	3.46
BW 3	7.93	0.19	4.30

Appendix 4: List of strains used in Experiment 4, Project CP-Micro-6,  
1981-82.

S.No.	Strain	Source	Growth rate
1.	IC-8	ICRISAT Center	Medium
2.	IC-11	-do-	Slow
3.	IC-13	-do-	Medium
4.	IC-20	-do-	Slow
5.	IC-21	-do-	Medium
6.	IC-23	-do-	Slow
7.	IC-24	-do-	Slow
8.	IC-25	-do-	Slow
9.	IC-30	-do-	Slow
10.	IC-35	-do-	Slow
11.	IC-44	-do-	Slow
12.	IC-52	-do-	Medium
13.	IC-53	-do-	Slow
14.	IC-59	Hissar, Haryana	Slow
15.	IC-66	-do-	Slow
16.	IC-76	-do-	Medium
17.	IC-97	-do-	Medium
18.	IC-128	Rajasthan	Medium
19.	IC-143	ICRISAT Center	Medium
20.	IC-144	-do-	Medium
21.	IC-145	-do-	Medium
22.	IC-149	-do-	Medium
23.	IC-2001	Ex Rothamsted 3827	Medium
24.	IC-2002	Ex Rothamsted 3889	Medium
25.	IC-2027	Ex HAU CH-827	Fast
26.	IC-2028	Ex HAU CH-777	Medium
27.	IC-2046	Ex HAU CA-2	Medium
28.	IC-2048	Ex HAU CA-7	Medium
29.	IC-2058	Ex HAU CA-181	Medium
30.	IC-2091	Ex John Innes 9036	Medium
31.	B-1	IARI, New Delhi	Medium
32.	F-6	-do-	Medium
33.	F-75	-do-	Medium
34.	H-45	JNKVV, Jabalpur	Fast
35.	KG-31	CSAUAT, Kanpur	Medium
36.	TAL-480	Ex Bangalore UAS B57	Medium
37.	TAL-620	Ex ICRISAT IC-2002	Medium
38.	TAL-1148	Ex USA 27 A2	Medium

Fast = About 2 mm size in 3-4 days  
 Medium = About 2 mm size in 7 days  
 Slow = About 2 mm size in 7-10 days.

Appendix 5: List of rhizobia isolated from nodules formed in Experiment 4

Strain	Module size	Module colour	Temp. at which plant was growing
CM-120	S	Pg	35
CM-121	B	Green	35
CM-122	B	Green	35
CM-123	S	Pink green	35
CM-124	B	Pink green	35
CM-125	B	Pink green	35
CM-126	B	Pink green	35
CM-127	B	Pink green	35
CM-128	S	Green	35
CM-129	S	Green	35
CM-130	S	Green	30
CM-131	S	Green	30
CM-132	S	Green	30
CM-107	S	Green	35
CM-108	S	Green	35
CM-109	S	Green	35
CM-110	S	Green	35
CM-111	S	Pg	35
CM-112	B	Pg	35
CM-113	B	Pg	35
CM-114	B	Pg	35
CM-115	B	G	35
CM-116	B	G	30
CM-117	B	G	30
CM-118	B	G	30
CM-119	S	G	35
CM-105	S	G	35
CM-106	S	G	35
CM-133	B	Pg	32
CM-134	B	Pg	35

Strain	Module size	Module colour	Temp. at which plant was growing
CM-135	S	G	35
CM-158	S	G	25
CM-163	S	G	30
CM-164	S	Pg	32
CM-165	S	Pg	25
CM-212	S	G	35
CM-213	S	G	35
CM-214	S	Pg	35
CM-215	S	Pg	35
CM-216	S	Pg	35
CM-217	S	Pg	35
CM-218	B	NR	30
CM-219	B	NR	30
CM-220	S	G	32
CM-221	B	Pg	25
CM-222	B	Pg	25
CM-223	B	G	25
CM-251	S	G	32

S = small, 3-5%; B = big, 5-15%; G = green; Pg = pink; NR = not recorded.

3/15/88  
C