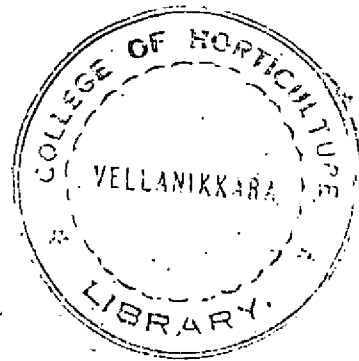


**VARIETAL RESPONSE TO *Rhizobium*
INOCULATION IN COWPEA UNDER
FIELD CONDITIONS**

BY
BEENA, S.



THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE
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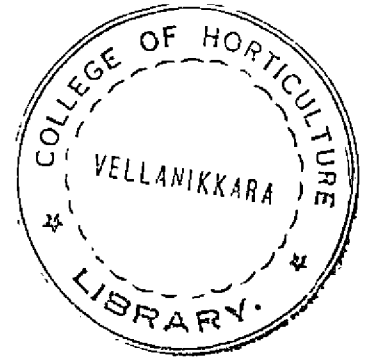
DECLARATION

I hereby declare that this thesis entitled " Varietal response to Rhizobium inoculation in cowpea under field conditions" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,

3- 3, 1983

Beena.S
(BEENA,S.)



CERTIFICATE

Certified that this thesis entitled " Varietal response to Rhizobium inoculation in cowpea under field conditions" is a record of research work done independently by Smt. Beena, S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

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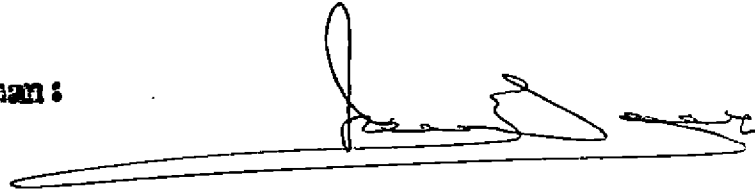
3. 3. 1983.

A handwritten signature in black ink, appearing to read "James Mathew". The signature is written over a horizontal line. To the right of the signature, the date "3. 3. 83" is written.

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INTRODUCTION

INTRODUCTION

More often than not, nitrogen in soil becomes a limiting factor for crop production. The green plants are incapable of utilizing the freely available nitrogen in the atmosphere, even though the atmosphere is rich enough in molecular nitrogen. The nitrogen requirement of crop plants is met by the addition of fertilizer nitrogen. But the dependence of fertilizer nitrogen production on fossil fuel resources and the diminished availability of this costly input for fertilizer production in the future has obviously brought the subject of biological nitrogen fixation in the fore front.

Biological nitrogen fixation is a unique process, characteristic to certain microorganisms and plant-microbe interactions capable of harnessing atmospheric nitrogen for the growth of plants. Biological nitrogen fixation is brought about by microorganisms in association with higher plants, particularly in root nodules. The best nodule forming plants are legumes, such as beans, clovers, grasses and peas. Legumes have been used in building and conserving soil fertility since the beginning of agriculture. The legumes are largely cultivated in tropical farming systems as pulses, palatable leafy vegetable and fodder crops.

As a rule, legumes are energy rich crops, grown more often than not under energy starved conditions. However, the yield

of the grain legumes, including cowpea which is of much importance in tropical farming system, is lower and more inconsistent when compared to cereals. The average yield of grain legume in Kerala is as low as 400 kg per hectare.

Cowpea (Vigna unguiculata) is a widely cultivated pulse crop in Kerala, both for use as a vegetable as well as a food grain. It is also grown in rotational sequence with paddy in summerfollows. Most often, it is felt that the maximum benefits of growing a legume crop is not obtained under these conditions, probably due to the lower rates of nitrogen contributed by them. This can be enhanced by properly exploiting the symbiotic association between the correct strain of pulses and Rhizobium. Such a situation can lead to greater nitrogen fixation as well as higher yield of pulses and can be achieved through the low cost technology of inoculating the legume crop with the specific strain of Rhizobium.

Keeping in view of these facts, the present study was undertaken with the following main objectives:

1. Varietal screening for nodulation efficiency using a known efficient strain of Rhizobium under field condition (10 different varieties of cowpea were used for this purpose).

2. Estimation of symbiotic efficiency in relation to host varietal specificity for nodulation. Efficiency were estimated in terms of comparative increase in number of nodule, nodule masses, plant dry weight, yield and total plant nitrogen.
3. Estimation of native nitrogen content of soil.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Biological nitrogen fixation is agriculturally the most important and significant process by which the fertility status of the soil is maintained. The renowned Dutch Microbiologist, Beijerinck (1888) was the first to recognise the bacterium causing the formation of root nodules in legumes and demonstrated that the purified bacteria formed nodules in plants grown from sterilized seeds. Inoculation of legume seeds with the appropriate cultures of Rhizobium was originally introduced as a means of ensuring the establishment of seedlings in nitrogen deficient soils which lacked adequate population of nodulating bacteria (Fred *et al.*, 1932). The ability of the Rhizobium to establish a symbiotic relationship with legume hosts is dependent on two important characteristics of the micro symbiont *vis.*, infectivity and effectiveness (Schwinghamer, 1964). It has been conceded that each of the partners, Rhizobium and legume is unable by itself to fix substantial amounts of nitrogen and the effective nitrogen fixation is the phenotypic expression of two associated genomes (Bergersen, 1971).

Nitrogen contribution by cowpea

Hartwell and Pember (1911) studying the nitrogen fixing phenomenon by legumes, found gains of nearly one tonne of soil

nitrogen per acre in pot culture experiments with cowpea and soybean. They also recorded an annual average gain of 180 lb of nitrogen per acre was credited by hairy vetch.

Hopkins as quoted by Wakeman (1952) stated that three tonnes of cowpea hay provided 26 lb of nitrogen per acre. Thompson (1952) reported that the amount of nitrogen fixed and that added by a legume to the soil depended upon its age, photosynthetic rate and presence of combined nitrogen in the soil. Mirchandani and Khan (1953) pointed out that the amount of nitrogen fixed by a legume depended upon its age, condition of growth, type of legume and the stage at which it is ploughed into. They found that the quantity of nitrogen added to the soil by a legume depend mainly on whether the legume was incorporated in the soil or not. Sen and Rao (1955) investigating the fixation of nitrogen under favourable conditions by cowpea and reported the quantity fixed symbiotically to be 251 lb per annum per acre. Alexander (1961) reported that nitrogen fixation by cowpea varied between 64 and 131 kg nitrogen per hectare per year and by soybean between 64 and 106 kg nitrogen per hectare per year. Hutman (1976) enlisted the amounts of nitrogen fixed by different legumes. He had pointed out that cowpea fixed an average of 198 kg nitrogen per hectare.

Rhizobium inoculation and crop response in cowpea

Crofts and Jenkins (1954) reported that inoculation of cowpea with a strain of Rhizobium resulted in good nodulation and growth.

Gargantini and Pimentel Wutke (1960) inoculated Rhizobium in sword bean (Cassia eschscholzii) and cowpea (Vigna sinensis) and reported that both plants fixed nitrogen well, sword bean fixing 48.5 kg nitrogen per hectare in the host treatment and cowpea 73 kg per hectare. Usedinna (1964) reported that in a split plot design experiment in green house, inoculation with isolates of cowpea Rhizobium increased the nitrogen content of cowpea plants. But symbiotic nitrogen fixation alone did not provide the plant with their optimal N requirement.

Nair et al. (1970 a) studied the effect of seed inoculation with Rhizobium on yield and nitrogen content of leguminous green manure crops and reported a fixation of 8 to 14 kg nitrogen per hectare and an increase in the dry matter production.

Sahu and Behara (1972) observed that inoculation increased the number of nodules and nitrogen content of shoot and root in cowpea, groundnut and green gram.

Sinha Rao (1972) reported an increased yield of 23 per cent over the uninoculated control in cowpea by the Rhizobium inoculation.

Summerfield et al. (1975) reported that seed inoculation of cowpea with Rhizobium increased the number of pods per plant from 63-92 and average seed weight and seed yield per plant from 56 to 100 g.

Sharma and Ghonsikar (1976) reported that application of micronutrients or addition of inorganic nitrogen did not alter symbiotic efficiency of Rhizobium spp. of cowpea. Summerfield et al. (1977) reported that effectively nodulated cowpea plants grown in pots without applied nitrogen were vegetatively equal to non-nodulated plants supplied with 60 ppm N throughout their growth period (83 days) and produced significantly greater seed yield.

Mughecho (1978) in a field experiment conducted on a highly weathered and leached sandy soil in Trinidad showed that cowpea (Vigna unguiculata) produced higher yield when inoculated with commercial Rhizobium strain. Uninoculated soybean produced better with indigenous strain present in the soil than with commercial strain.

Host varietal specificity of Rhizobium for nodulation in cowpea

Walker and Brown (1935) suggested that the root nodule bacteria of soybean and cowpea could be combined in one species

generally designated as Rhizobium japonicum (Kirchner). The basis for this suggestion was the finding that certain strains of soybean Rhizobium could produce nodules on cowpea roots (Walker and Brown, 1955) and vice-versa.

Doka (1969) found that cowpea nodulated well with rhizobia isolated from groundnut (Arachis hypogaea), bambara groundnut (Voandzeia subterranea) limbean (Phaseolus lunatus) and soybean (Glycine max). But cowpea Rhizobium nodulated only its host and limbean and vice-versa. Bart and Wilden (1970) in pot experiment with cowpea and purple vetch inoculated with different Rhizobium strains, it was found that nitrogen fixation by cowpea was markedly stimulated by combined nitrogen. Vincent (1974) explained that cowpea miscellany has no definite generalisations and a wide range of hosts has been grouped together for want of better definition as to their distinctions and relationships.

Stanford and Neotins (1976) inoculated three strains of rhizobia on to four cowpea cultivars and reported that three of the cultivars were more promiscuous in relation to the inoculated strains. They also found that nitrogen fixation was higher with strain isolated from the same species than with other two strains isolated from Centrosema pubescens.

Borgen (1977) evaluated the effectiveness of two Rhizobium strains on cowpea cv. Aranca in terms of their effect on stem

length, leaf area, shoot dry matter, water consumption and nitrogen content of roots and shoots. There was significant difference between the two strains and on the basis of the above parameters, one strain was selected. The result also indicated that the major proportion of atmospheric nitrogen fixed was in roots by the strain.

Pawar et al. (1977) found that inoculation of cowpea with Rhizobium increased seed yield from 0.52 t per hectare to 0.82 t per hectare depending upon the variety. There was no significant difference in the yield of cowpea inoculated with different strains of Rhizobium.

Raja (1977) conducted a study on the effect of inoculation with different strains of Rhizobium under different nutrient and soil conditions on cowpea variety New era. He reported that rhizobial inoculation of cowpea seeds resulted in significant increase in the number and dry weight of nodules, fresh weight of plants and dry weight of shoot and root.

Bagyaraj and Hegde (1978) reported that seed inoculation of cowpea with Rhizobium culture UASB 94 resulted in a significant increase in grain yield out of the four strains used. Increased nodulation and dry weight of plant tops occurred but was not statistically significant. No correlation between the number of nodules per plant and grain yield was observed. Symbiotic response of cowpea to the inoculated strain of Rhizobium was inferior.

Zary et al. (1978) studied the extent of intraspecific variability for nitrogen fixation among 100 southern pea (cowpea) genotypes and a screening technique was developed to identify cowpea plants with high nitrogen fixing efficiency. Significant difference in nitrogen fixation efficiency was found among host plant genotypes following application of a standard commercial mixed strain of Rhizobium inoculant. This variability was an evidence for genetic control of the trait and suggested the possibility of breeding cowpea for increased nitrogen fixation.

Bopalan and Rai (1979) reported that under acid conditions Rhizobium strain-12 produced greater dry weight of plant in cowpea.

Keyser et al. (1979) in a green house trials tested 21 strains of slow-growing rhizobia for symbiotic effectiveness and ability to nodulate three varieties of cowpea (Vigna unguiculata L.Walp). The result confirmed that cowpea rhizobia contain a large and perhaps continuous variation in symbiotic tolerance of soil acidity. Some strains combined acid tolerance with high effectiveness.

Ramachandran (1979) screened 20 rhizobial strains for efficiency in cowpea and found that strain S.17 and S.10 were

more effective than others. Fresh and dry weight of shoot, nodule number and dry weight of nodule were significantly increased with these strains.

In a comparative study with three cowpea varieties, Ifo Brown, Hala and Local Brown, Afkolabi (1980) found that the most important variables which contribute to yield potential were pod number per plant followed by seed number per pod. Seed size had a minor role in a seed yield.

Of the 20 isolates tried Prasad (1980) adjudged isolate 8 to be the most promising inoculant strain for cowpea (cv. Pusa Phalgani). Ramachandran *et al.* (1980) conducted a pot culture experiment on cowpea variety 'New Era' to determine the efficiency of mixed inoculation of strains of rhizobia in fixing atmospheric nitrogen. It was found that none of the combinations of strains of rhizobia was better than inoculation with single strain R5 isolated from *Crotalaria* gave higher dry weight of plants, number of nodules and nitrogen fixation per plant.

Sen and Weaver (1980) reported that acetylene reduction capacity and nitrogen accumulation in plant top per unit nodule mass were several fold higher in groundnut than cowpea and pinto when inoculated with strain 32 III. In a field study Sivaprasad and Shivappashetty (1980) noticed significant

increase in yield, plant top dry weight and leghaemoglobin content of nodule in cowpea cv. Pusa Phalguni when inoculated with strain IS-8 and IS-12. But increase in number of nodules and nitrogen content of roots were not significant. Correlation studies showed significant correlation between leghaemoglobin content of nodules, plant top dry weight and final grain yield. No significant correlation was observed between nodule number and nitrogen content of plant top and final grain yield.

Ahmad et al. (1981) conducted experiment with 400 genetically diverse lines of cowpea with and without applied nitrogen. They reported that many cultivars responded positively in shoot fresh weight at 5-6 week after sowing indicating inadequate infectivity or efficacy of indigenous rhizobia and a potential for improvement by use of inoculant.

Ninchin et al. (1981) reported that cowpea cv. (a) TVU 1469 and (b) TVU 2321 had greater growth and higher seed yields when inoculated with Rhizobium strain CB 1024 from Australia than with R-5008 from Nigeria. Seed yield of plant inoculated with R-5008 was decreased by application of 30 ppm nitrogen but nitrogen regime had no effect on the CB-1024 symbiosis.

Nair and Sivaprasad (1981) reported beneficial effects due to inoculation with different isolates of rhizobia in

cowpea. They could correlate symbiotic efficiency with increase in dry weight of cowpea plants.

Sharma et al. (1931) reported that seed inoculation with different rhizobial strains increased seed yield by 12-23 per cent in gram (chick pea), lentil, pea, cowpea, moth (Vigna acutifolia), Urd, (Vigna mungo), mung (Vigna radiata) and arhar (pigeon pea).

Zablatovic and Foelt (1931) inoculated cowpea plants with three slow growing strains and found that plants were nodulated by the fast growing Rhizobium spp. 176 A.28. They reported that the slow growing strains developed maximum acetylene reduction than fast growing strains.

Host-varietal specificity of Rhizobium for nodulation in other legumes

Albrecht (1943) showed that six single strain cultures of Rhizobium differed widely in their effectiveness on spanish pea nuts. The strains were assessed in terms of percentage of plant nodulated, dry matter yield per unit area, final rating of yield and mean dry weight of individually collected plants.

Krönan (1947) studied the strain variation and host specificity of Rhizobium trifolii on four species of Trifolium.

The result indicated the importance of the use of effective strain of Rhizobium trifolii as well as cultivation of efficient varieties within different Trifolium spp. for maximum nitrogen fixation and growth of plant.

Brakeš and Manil (1965) inoculated six dwarf bean varieties (Phaseolus vulgaris L.) with Rhizobium phaseoli and the effect of nodulation and yield were compared with uninoculated control with and without nitrogen application. They reported that inoculation caused a rapid and abundant nodulation and there was no significant difference among the varieties.

Saubert and Scheffler (1967) investigated the host specificity of Rhizobium trifolii on European clovers. Forty eight South African isolates were tested on five varieties of Trifolium subterraneum. In some cases there was considerable variation in the amount of nitrogen fixed by the various host inoculated with the same isolate.

Chhonker and Negi (1971) evaluated the response of soybean variety 'Bragg' to inoculation with different strains of Rhizobium japonicum in rhizobia free soil and reported that some strains were found to be more efficient than others in increasing yield.

Subba Rao and Balasundaram (1971) showed the practicability of inoculation of seeds of soybean with Rhizobium as an effective practice to maximise soybean yield.

Bhargava et al. (1974) reported that effective strains of Rhizobium japonicum produced better nodulation in soybean and indigenous strains were as effective as imported cultures.

Madhani and Patil (1974) investigated the comparative performance of different isolates of gram (Cicer arietinum L.) Rhizobium in pot and field experiment. They found that all the 10 isolates were superior to control. The increase in yield due to seed inoculation with Rhizobium strain was found to be in the range of 24-62 per cent.

Burton (1975) has shown that the use of effective strains of Rhizobium could make substantial increase in the yield of peanut. Suraj Bhan (1975) conducted an inoculation study with eight groundnut cultivars to evolve suitable plant type of the crop. He found that the variety AK-12-24 and Spanish Improved were the best with regard to nodulation and symbiotic efficiency.

Badarwal et al. (1976) showed that nodulation in chick pea was determined to a great extent by the host genotype. In a varietal and strain interaction test, it was found that

the isolate Ca 121 produced the maximum number of nodules. Among varieties, L 144 and DG-2 showed maximum nodulation. In symbiotic effectiveness, the most effective strains were Ca 121 and Ca 181 and showed an increase of more than 100 per cent in plant dry weight and nitrogen uptake with as many as seven genotypes. Among the varieties, H.551 and H.355 showed maximum increase in nitrogen uptake as well as in dry matter yield.

Lopes et al. (1976) conducted a study on natural nodulation in groundnut cultivars. Tatu1 and H.116 had the highest absolute value for dry weight of nodules on the main root of 21 varieties and lines observed. Difference between the varieties was not significant for nodule dry weight or for nodule weight or dry weight of the aerial parts.

Raju and Samuel (1976) studied the influence of different commercial inoculants on gram (Cicer arietinum). Except the I.A.R.I. culture all other inoculants gave very good results and produced high dry matter, high nitrogen uptake by the plant and translocation to seed.

Soos et al. (1976) investigated the effectiveness of Rhizobium inoculation on four soybean varieties grown in Cuba and found that the treatment with 'Rhizolegsoya' inoculum increased the nodulation and yield of all four varieties.

Gonzalez (1977) studied the behaviour of ten Rhizobium japonicum strains on soybean varieties. The result indicated that the relation between plant dry weight, nodule dry weight and nitrogen accumulation varied according to variety, strain and soil type.

Kumar Rao and Patil (1977) reported that soybean responded differently to different commercial inoculants of Rhizobium japonicum. The different cultures produced significant difference in number and dry weight of plant top. Except one inoculant, all others produced increase in yield as compared to uninoculated control.

Mishra and Srivastava (1978) conducted a pot culture experiment with four varieties of soybean namely Bragg, Punjab-1, UPSS-38 and Sepaya Black. It was found that the number of nodules did not differ significantly among the different varieties. Bragg variety appeared to have better nodulation capacity than the other varieties. Maximum nitrogen per cent was in UPSS-38 (3.38 per cent) followed by Bragg (3.22 per cent) and Punjab-1 (2.30 per cent). Variety Bragg appeared outstanding in fixing the maximum nitrogen which was about five times the value of nitrogen fixed by local Sepaya Black.

Saxena and Singh (1978) conducted experiments to study the response of gram varieties to Rhizobium cultures

isolated from different agroclimatic conditions. Their results indicated that varietal differences were significant and the interaction between culture and host variety was insignificant.

Caldwell and Vest (1979) reported the role of host genotype in nodulation and nitrogen fixation. They concluded that effective nodulation and nitrogen fixation were greatly influenced by genetic variation of both the symbionts.

Pareek (1979) studied the effectiveness of different strains of chick pea (Cicer arietinum) Rhizobium in field. There was difference in nitrogenase activity and dinitrogen fixation among the strains.

Ferrera (1980) tested three strains of Rhizobium phaseoli on seven species of Phaseolus under green house condition. It was found that three of the Phaseolus spp. showed a good level of nodulation and no nodules were observed on Phaseolus oligospermus. The other three species showed low levels of nodulation. Two species and to a lesser extent the wild form of P. vulgaris showed a good level of nitrogen fixation.

Haydock et al. (1980) reported that the most effective Rhizobium strain could be selected on the basis of dry matter yield of whole plant or plant top only.

Wynne et al. (1980) reported that specific host-strain combination can lead to increased biological nitrogen fixation in peanuts.

Peres and Vidor (1981) conducted a study on the selection of Rhizobium jannonicum strains and competitiveness for nodule site on soybean cultivars. They observed that there was a remarkable difference in nodule number and dry weight among cultivars in response to inoculation with individual strains. Strains 587 and 29 W nodulated all cultivars effectively and were very competitive for nodule sites, forming more than 70 per cent of the nodules.

Girija (1982) conducted a study on host varietal specificity for Rhizobium for nodulation in groundnut. She used seven varieties of groundnut and isolates of the root nodule bacterium from each of the seven varieties of groundnut and reported a favourable response for all plant characters studied in the variety due to inoculation with its respective homologous isolate of Rhizobium. The isolates R2 and R3 were found to be more compatible with different host varieties and the groundnut varieties USA-123 and Exotic-6 showed the most favourable response to inoculation with different isolates of rhizobia in terms of symbiotic efficiency.

Effect of symbiotic nitrogen fixation on the soil nitrogen status

Mirohandani and Khan (1953) reported that the quantity of nitrogen added to the soil by a legume depend mainly on whether the legume was incorporated into the soil or not.

Russell (1961) hold the view that the actual amount of nitrogen fixed by leguminous crops in the field was difficult to estimate because of the difficulty in determining accurately the nitrogen content of the soil on one hand and the amount of denitrification taking place during the growing season on the other. He expressed that probably all the nitrogen was transferred to the tops and seeds in view of the fact that the legumes increased the nitrogen content of the soil to a level not higher than that of the non-legume crop cultivated soils.

Abu-shakka and Bassiri (1972) found that the land upon which inoculated soybean had been grown the previous year produced more nodules and greater yield than did the land planted previously with non-inoculated seeds.

✓ Sahu and Bahara (1972) inoculated Rhizobium in cowpea , groundnut and green gram seeds and noticed a 29 per cent increase in soil nitrogen content as a result of culture application.

Sharma and Sillak (1974) compared the efficiency of different commercial inoculants of Rhizobium japonicum on field gram and soybeans. They found that inoculation with Nitragin culture increased the soil nitrogen to 43 per cent over initial level at the time of crop harvest. Other cultures (UPAU-2, Kappur and IARI culture) increased the soil nitrogen content only about to 20 per cent.

Nathan et al. (1979) reported that when a leguminous crop like cowpea was included in a crop rotation of Ganga 5 Maze, Co-7 ragi and Co-2 cowpea, the total nitrogen content in the soil was considerably increased even in the unfertilised plots. Considerable quantity of nitrogen fixed was observed to have been redistributed in the soil which depended on the fertilization pattern.

Kale and Patil, (1981) conducted a study in gram (Cicer arietinum L.) on the effect of nitrogen fixation and yield. They found that by the inoculation of gram seeds with an effective strain of Rhizobium leguminosarum along with Anotobacter chroococcum, the nitrogen and organic carbon content in soil was increased.

Alston and Graham (1982) evaluated the influence of soil nitrogen status and previous crop on nitrogen fixation

(Acetylene Reduction) in Barrel Medic, Medicago turneartula.

They reported that the rate of accretion of nitrogen in the soils from nitrogen fixation, was dependent of the total nitrogen content and was decreased when the soil mineral nitrogen was high. The results had implications for the maintenance of the nitrogen status of soils under cereal-pasture rotations.

MATERIALS AND METHODS

MATERIALS AND METHODS

The varietal response to Rhizobium Inoculation in cowpea was investigated in a statistically laid out field experiment. The experiment was conducted in rice fallow of the Rice Research station, Kayamkulam during the summer season of 1981-1982.

Materials

Varieties and seeds

The cowpea varieties selected were C-152, Ptb-1, Pusa-2, Hg-22, 522, 533, 534, 551, 779 and V-37.

All these varieties have about 90 days duration. The seeds required for the experiment were obtained from Rice Research Station, Pattambi.

Rhizobium culture was supplied by the Microbiology Unit of the Department of Plant Pathology, College of Agriculture, Vellayani. Cowpea Rhizobium strain 6050 was used as the inoculant in the present studies.

There were 20 treatment combinations as listed below:-

- | | | | |
|----------------------------------|-----------------------------------|-----------------------------------|------------------------------------|
| 1. V ₁ R ₀ | 6. V ₃ R ₁ | 11. V ₆ R ₀ | 16. V ₈ R ₁ |
| 2. V ₁ R ₁ | 7. V ₄ R ₀ | 12. V ₆ R ₁ | 17. V ₉ R ₀ |
| 3. V ₂ R ₀ | 8. V ₄ R ₁ | 13. V ₇ R ₀ | 18. V ₉ R ₁ |
| 4. V ₂ R ₁ | 9. V ₅ R ₀ | 14. V ₇ R ₁ | 19. V ₁₀ R ₀ |
| 5. V ₃ R ₀ | 10. V ₅ R ₁ | 15. V ₈ R ₀ | 20. V ₁₀ R ₁ |

Where:

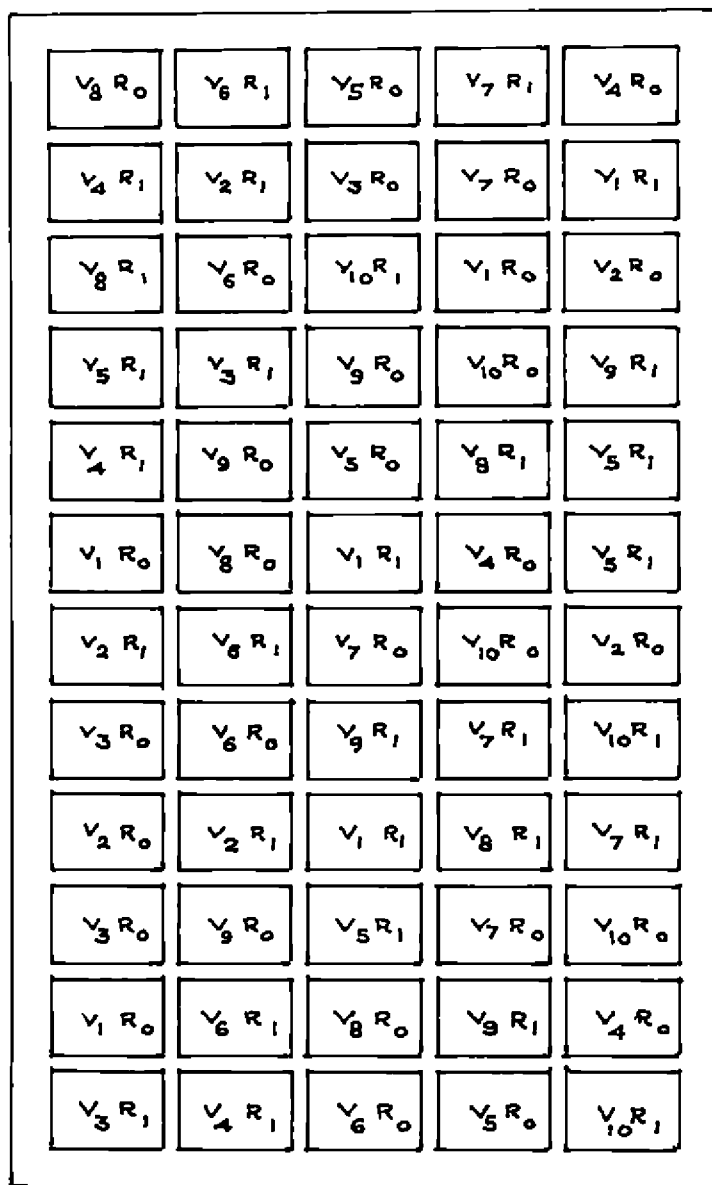
- R₀ - without Rhizobium (control)
- R₁ - with Rhizobium (cowpea Rhizobium strain 6050)
- V - represent variety
- V₁ - 534
- V₂ - V-37
- V₃ - 522
- V₄ - HG-22
- V₅ - C-152
- V₆ - 551
- V₇ - Pusa-2
- V₈ - 779
- V₉ - Ptb-1
- V₁₀ - 533

Design and layout

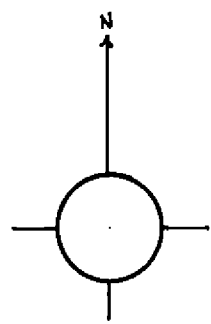
The experiment was laid out in factorial randomized block design with 3 replications. The treatment combinations were allotted to different plots randomly using the random number table.

Preparation of Rhizobium culture

The isolate of Rhizobium was tested for the purity by Gram staining (Hucker, 1927) and Ketolactose test (Bernsertz and de Ley, 1963). The isolate of Rhizobium was grown in yeast



REPLICATION. III.
 REPLICATION. II.
 REPLICATION. I.



V- VARIETIES R - *Rhizobium* CULTURE.
 TOTAL NUMBER OF PLOTS - 60
 GROSS PLOT SIZE - 4.40 x 3.90 Sq. m.
 NET PLOT SIZE - 4.00 x 3.60 Sq. m.
 SPACING - 20 x 15 cms.
 UNCULTIVATED BUFFER STRIPS WERE LEFT BETWEEN PLOTS

FIG: 1 LAY OUT PLAN: VARIETAL RESPONSE TO *Rhizobium* INOCULATION IN DIFFERENT VARIETIES OF COWPEA UNDER FIELD CONDITIONS

extract mannitol agar medium in petridishes and incubated in a B.O.D. incubator at $28 \pm 1^\circ$ for three days.

Yeast extract mannitol agar medium (Allen, 1953)

Mannitol.	10.0 g
K_2HPO_4	0.5 g
$Mg SO_4 \cdot 7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Yeast extract	1.0 g
Congo Red (1% aqueous solution)	2.5 ml
Agar	15.0 g
Distilled water	1000 ml
pH	7.0

Typical colonies of Rhizobium characterised by a white colour and gummy nature were selected and transferred to yeast extract mannitol agar slants.

The isolate of Rhizobium was also grown in yeast extract mannitol broth (Iswaran and Chhonkar, 1971). The conical flasks containing the broth with Rhizobium culture were incubated at $28 \pm 1^\circ C$ for 4-5 days in a B.O.D. incubator. The rhizobial growth in petridishes was transferred to the broth.

Dried well powdered and sterilized charcoal was used as carrier material for Rhizobium. Five hundred gram of charcoal was taken and mixed well with 750 ml of broth culture and was packed in polythene bags at the rate of 50 g each.

Field experiment

1. Preparation of field

The experimental area was tilled with a power tiller, clods were broken, weeds were removed, levelled and laid out into blocks and plots as given in the layout plan.

2. Collection of soil samples

After the layout, soil samples were collected from each plot for the analysis of per cent nitrogen in soil. Samples were taken by a spade to a depth of a plough furrow (6 inches) at several spots and then composited. The soil was mixed thoroughly and spread on a clean sheet of paper and divided into four equal parts. Two opposite quarters were rejected and samples from the other two quarters were taken and packed in plastic bags.

Soil samples were also collected after the harvest from each plots following the procedure given above.

3. Fertilizer application

Phosphorus at 30 kg per hectare as superphosphate and potassium at 10 kg per hectare as muriate of potash were

applied at the time of sowing as per the Package of Practice Recommendation of Kerala Agricultural University (Anon., 1981). Nitrogen fertilizer was not applied in this experiment.

4. Seeds and sowing

Sowing was done on 25th January 1982. The seeds were treated with Rhizobium culture. For that, 125 g of jaggery was dissolved in 500 ml of water and boiled for 30 minutes and then cooled. The carrier material with Rhizobium was mixed with the jaggery to form a slurry. Seeds were washed and dried under shade for half an hour before inoculation. The prepared slurry was well mixed with each variety of seeds separately so as to get an uniform coating of slurry on each seed. The seeds were then rolled over a bed of finely powdered calcium carbonate at the rate of 3.5 kg per 10 kg seeds to get an uniform coating. The seeds were allowed to dry under shade and culture treated seeds were sown immediately. Sowing was done by dibbling at a spacing of 20 x 15 cm. Thus in a plot there were about 440 plants. Light irrigation was also given after sowing and seedling emergence was completed in about 5-10 days time.

5. After care

One weeding was given 2 week after sowing. Incidence of pest attack was noticed for which Kkalux-25 B.C. at the

concentration of 0.1 per cent was sprayed twice at an interval of seven days. The stand of the crop was satisfactory throughout the period of growth.

6. Harvesting

From the net plot area, the dried pods were picked, sun dried and threshed plot wise. The seeds were packed in separate packets after recording the weight.

Observations recorded

1. Number of nodules per plant

The plants were uprooted carefully on the 35th day after sowing which corresponded to maximum flowering stage of the crop. The roots were washed free of soil particles. Nodules on the tap root and lateral roots were counted separately. Total number of nodules present in the root system of individual plant was recorded.

2. Plant height

Height of the plant from the soil level to the tip of the top most leaf was recorded. Plant height was recorded on 35th day of sowing and also before harvesting the crop.

3. Nodule dry weight

The nodules removed from the plants were packed plot wise and dried in the hot air oven at 65°C till the constant dry weights were obtained.

4. Plant dry weight

The uprooted sample plants were packed separately, partially dried in shade and then placed in the hot air oven at 65°C till the constant dry weights were obtained.

5. Yield

The dried pods from the net area were picked, sun dried and threshed plotwise and the weight of grains was recorded.

Plant analysis

The sample plants were oven dried at 65°C, powdered and used for the chemical analysis.

Nitrogen content in plant

The nitrogen content of the dried plant sample was determined by the modified micro-kjeldahl method (Jackson, 1967). Two hundred milligrams of the powdered plant sample along with 10 g of digestion mixture (potassium sulphate, cupric sulphate and selenium powder in the ratio of 10:1.0:0.1) was taken in a 100 ml kjeldahl digestion flask. Ten ml of concentrated sulphuric acid of specific gravity 1.84 was added slowly to the above digestion mixture and heated for 5 hours till the material was completely digested. The flasks were allowed to cool down to room temperature. Then added 25 ml of distilled water to each flask. On cooling, the contents were transferred to 100 ml volumetric flasks and the volume made up with distilled water. Ten ml aliquot of the sample from the volumetric flask was then added to the kjeldahl

flask along with 10 ml of 50 per cent sodium hydroxide solution and steam distilled till about 100 ml of the distillate was collected in the receiver flask containing initially 10 ml of 2 per cent boric acid solution with a drop of mixed indicator. The ammonical nitrogen content of the distillate was determined by a titration with 0.01 N hydrochloric acid. From the titre values the percentage N was determined by the following equation

$$= \frac{V \times N \times V_1 \times 0.014}{V_2 \times W}$$

Where V = Titre value - the blank

V₁ = Total volume of plant sample made up

V₂ = Volume of plant sample distilled

N = Normality of HCl

W = Weight of powdered sample used for digestion.

Soil analysis

The soil samples collected from the individual plots before sowing and after harvest were analysed for the total nitrogen content by modified micro-kjeldahl method (Jackson, 1967).

Statistical analysis

Data on various observations were analysed statistically by applying the technique of analysis of variance for

factorial randomized block design. The significance was tested by 'F' test (Snodgrass and Cochran, 1967).

RESULTS

RESULTS

Varietal response to Rhizobium inoculation in cowpea

The observations taken on thirtyfifth day after sowing on the number of nodules, nodule dry weight, plant height, plant dry weight and percentage of nitrogen content of shoot portion were statistically analysed.

The analysis of variance table for different observations is given in Appendix-I. Mean values of different observations, corresponding to the treatment are given in Table 1-5.

1. Number of root nodules produced

From the analysis of variance table given in Appendix-I, it was clear that the effects of Rhizobium, variety and the effect of interaction of variety and Rhizobium on nodule number were significant.

As seen from Table-1, Rhizobium inoculation resulted in significant increase in the total number of nodules produced. In the Rhizobium inoculated treatments the mean number of nodules recorded were 29.80 (R_1) as against 21.37 (R_0) in non-inoculated controls.

There was significant difference between varieties in the number of nodules produced. The maximum number of nodules (43.44) was observed in the variety 535, which was followed

Table-1

Module number - * Effect of Rhizobium inoculation and interaction with cowpea varieties

Cowpea varieties	Rhizobium culture	Control	Mean
	R ₁	R ₀	
V ₁ - 534	41.79	35.06	38.42
V ₂ - V-37	14.85	11.56	13.20
V ₃ - 522	23.29	12.10	17.69
V ₄ - Hg-22	19.62	14.22	16.92
V ₅ - C-152	27.33	21.50	24.41
V ₆ - 551	37.60	28.12	32.86
V ₇ - Pusa-2	29.81	21.43	25.62
V ₈ - 779	13.51	10.67	11.99
V ₉ - Ptb-1	41.58	20.95	31.27
V ₁₀ - 533	48.79	38.10	43.44
Mean	29.80	21.37	..

C.D.(5%) for comparison of levels of varieties - 3.37
 C.D.(5%) for comparison of levels of Rhizobium - 1.50
 C.D.(5%) for comparison of levels of variety X - 4.77
 Rhizobium interaction

* Mean of 3 replications

Ranking of varieties

V₁₀ V₁ $\overline{V_6 V_9}$ $\overline{V_7 V_5}$ $\overline{V_3 V_4}$ $\overline{V_2 V_8}$

Table - 2

Nodule dry weight (g)* - Effect of Rhizobium inoculation and interaction with cowpea varieties.

Cowpea varieties	Rhizobium culture	Control	Mean
	R ₁	R ₀	
V ₁ - 534	4.44	3.90	4.18
V ₂ - V-37	2.01	1.81	1.91
V ₃ - 522	3.31	2.99	3.16
V ₄ - Ng-22	2.44	2.24	2.34
V ₅ - C-152	2.69	2.24	2.46
V ₆ - 551	4.70	3.83	4.26
V ₇ - Pusa-2	4.45	3.93	4.18
V ₈ - 779	3.32	3.05	3.19
V ₉ - Ptb-1	2.13	1.41	1.77
V ₁₀ - 533	4.38	3.47	3.93
Mean	3.39	2.89	..

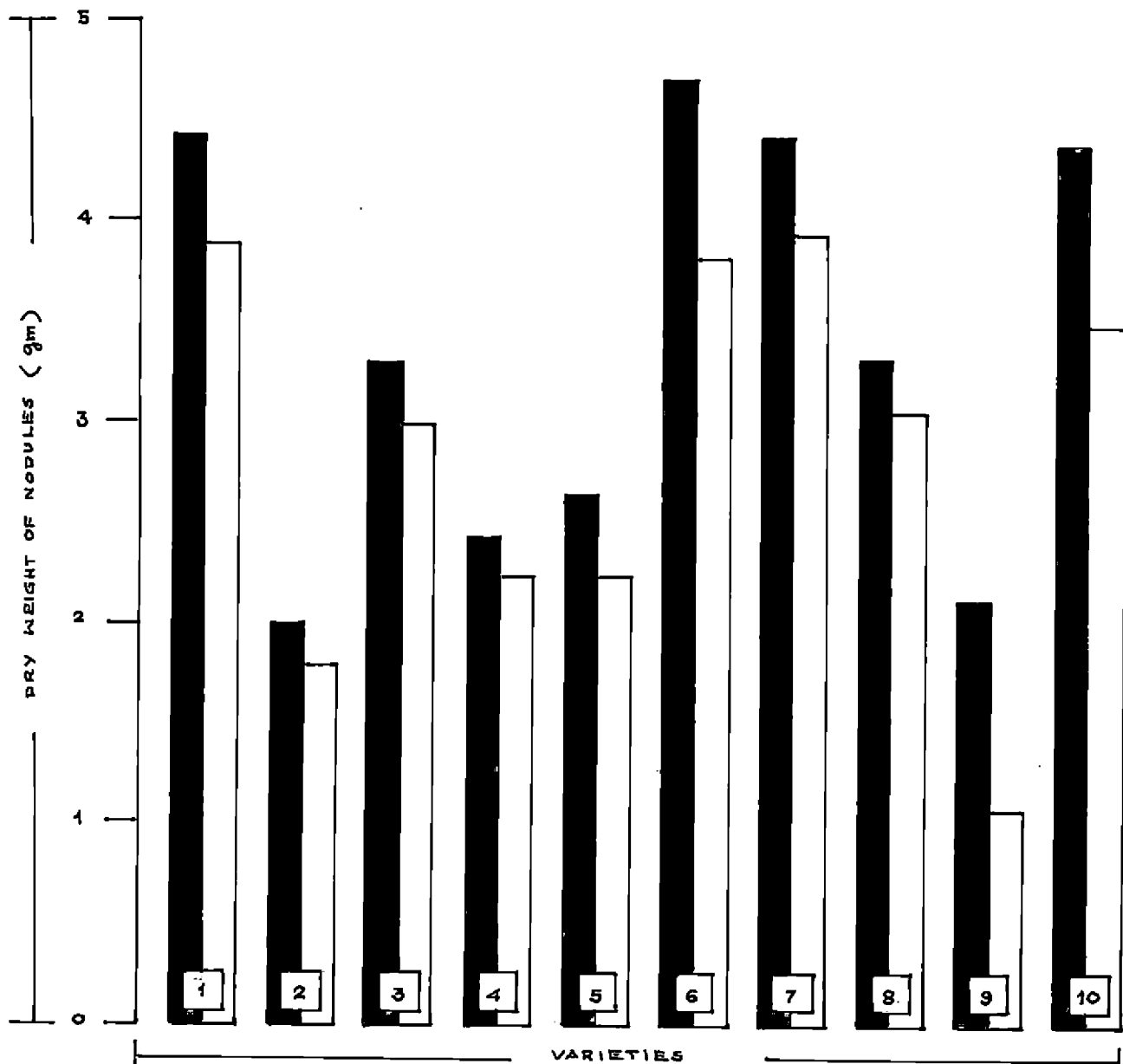
C.D.(5%) for comparison of levels of varieties - 0.77

C.D.(5%) for comparison of levels of Rhizobium - 0.34

* Mean of 3 replications

Ranking(varieties)

V₆ V₇ V₁ V₁₀ V₈ V₃ V₅ V₄ V₂ V₉



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (Ro)
3 - 522	6 - 551	9 - Ptb-1	□ WITHOUT <i>Rhizobium</i> (Ro)

FIG: 3. DRY WEIGHT OF NODULES, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

As seen from the Table-2, the Rhizobium inoculation had resulted in significant increase in nodule dry weight than control. Nodule dry weight obtained as a result of Rhizobium inoculation (R_1) was 3.39 g in contrast to the control (R_0) plants that produced only 2.89 g.

There was significant difference between varieties in nodule dry weight. With regard to the nodule dry weight the varieties were divided into three groups. The varieties 551, Pusa-2 and 534 were superior to other varieties, yielding a mean nodule dry weight of 4.26 g, 4.18 g and 4.18 g respectively. The second group included the varieties 533, 779 and 522, which were on par with each other yielding a mean nodule dry weight of 3.93 g, 3.19 g and 3.16 g respectively. The lowest nodule dry weight was observed by the third group of varieties consisting of G-152 (2.46 g), Hg-22 (2.34 g), V-37 (1.91 g) and Ptb-1 (1.77 g).

The varieties 551, Pusa-2 and 534 were the best varieties in terms of average nodule dry weight recorded by the inoculation of Rhizobium strain 6050.

5. Plant height

As seen from the analysis of variance table given in Appendix-I, it was clear that the effect of variety on the plant height was significant. The effect of Rhizobium and the

Table-3

Plant height (cm)* - Effect of Rhizobium inoculation and interaction with cowpea varieties

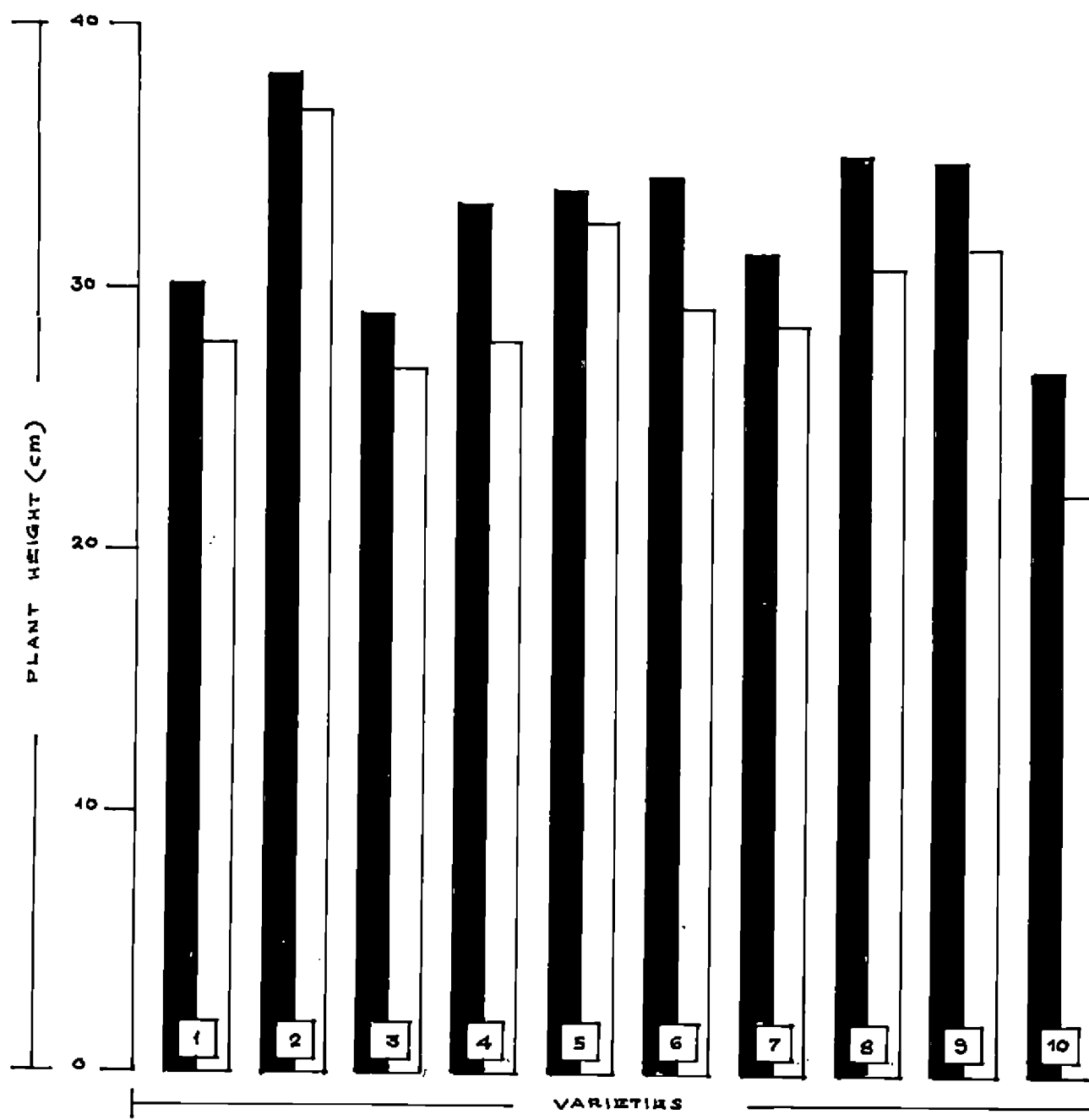
Cowpea varieties	Rhizobium culture	Control	Mean
	R ₁	R ₀	
V ₁ - 534	30.04	28.00	29.02
V ₂ - V-37	38.27	36.95	37.61
V ₃ - 522	29.16	27.00	23.08
V ₄ - Hg-22	33.27	27.95	30.61
V ₅ - G-152	33.90	32.33	33.11
V ₆ - 551	34.16	29.18	31.67
V ₇ - Pusa-2	31.25	28.54	29.89
V ₈ - 779	35.12	30.80	32.96
V ₉ - Ptb-1	34.68	31.53	33.11
V ₁₀ - 533	26.86	22.12	24.49
Mean	31.20	29.91	..

G.D.(5%) for comparison of levels of varieties = 5.45

* Mean of 3 replications.

Ranking of varieties

V₂ V₅ V₉ V₈ V₆ V₄ V₇ V₁ V₁₀ V₃



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R ₁)
3 - 522	6 - 551	9 - Pbb-1	□ WITHOUT <i>Rhizobium</i> (R ₀)

FIG: 4. PLANT HEIGHT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

effect of interaction of variety and Rhizobium were not significant.

From the Table-3 it was seen that the Rhizobium inoculation had resulted in higher plant height than control, but it was statistically not significant.

There was significant difference between the varieties in plant height. The maximum plant height was observed in the variety V-37 (37.61 cm). The rest of the varieties were divided into three groups. The first group included the varieties C-152 (35.11 cm), Ptb-1, (33.11 cm) and 779 (32.96 cm) and they were on par with each other. There was no significant difference between the varieties 551 (31.67 cm), Hg-22 (30.61 cm) and Pusa-2 (29.89 cm) and they formed the second group. The lowest plant height was observed in the third group of varieties 534 (29.02 cm), 533 (24.49 cm) and 522 (23.08 cm)..

4. Plant dry weight

From the analysis of variances table in Appendix-I, it was clear that the effect of variety and Rhizobium on plant dry weight were significant. The effect of interaction of variety and Rhizobium was not significant.

Table - 4
 Plant dry weight (g.)* - Effect of Rhizobium inoculation and interaction with cowpea varieties

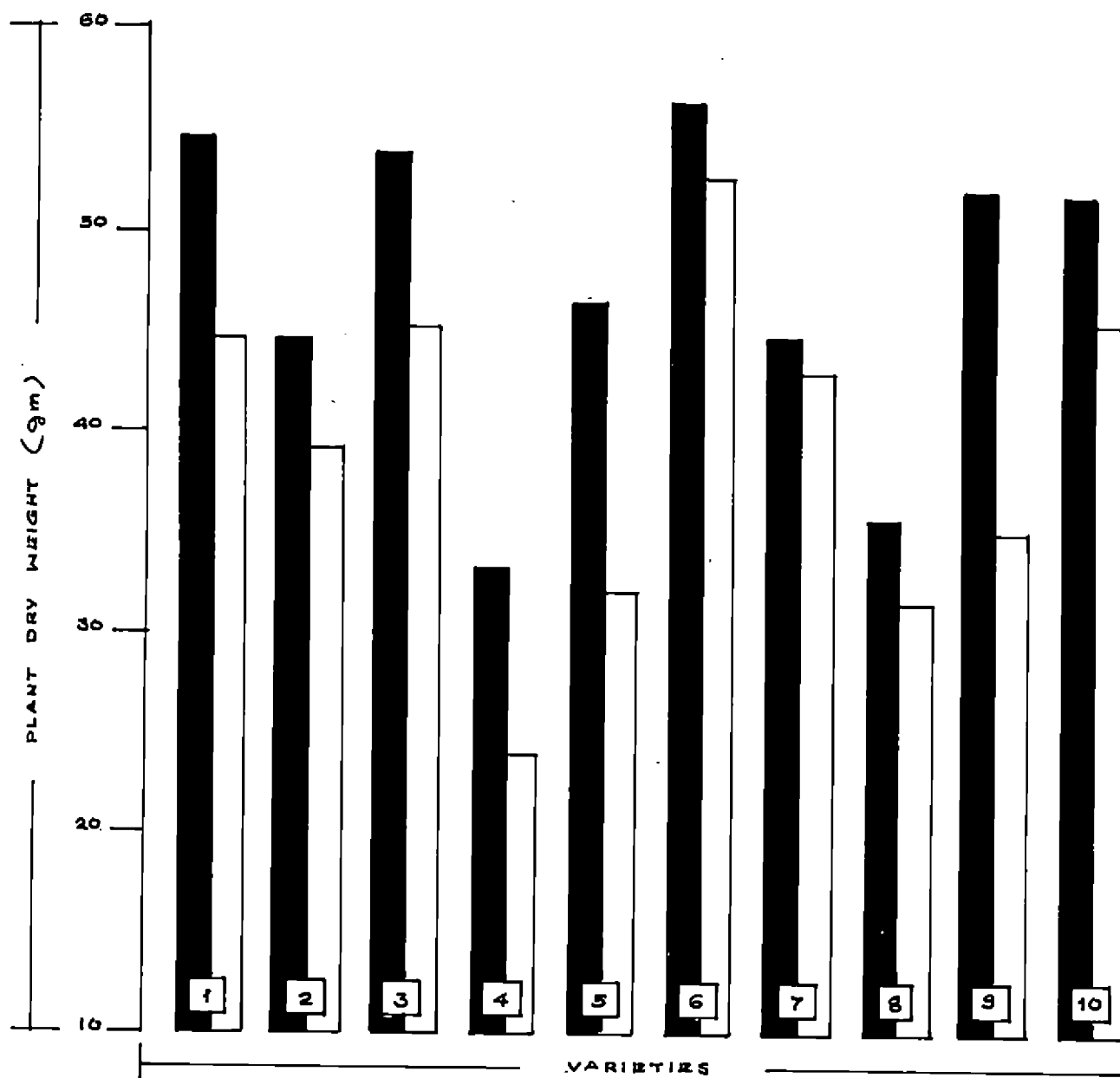
Cowpea varieties	Rhizobium culture	Control	Mean
	R ₁	R ₀	
V ₁ - 534	54.66	44.66	49.66
V ₂ - V-37	44.66	39.33	42.00
V ₃ - 522	54.00	45.33	49.66
V ₄ - Hg-22	30.33	24.00	27.16
V ₅ - C-152	40.66	32.00	36.33
V ₆ - 551	56.33	52.66	54.50
V ₇ - Pusa-2	44.66	40.33	42.50
V ₈ - 779	35.66	31.33	33.50
V ₉ - Ptb-1	52.00	35.00	43.50
V ₁₀ - 535	51.66	45.33	48.50
Mean	46.40	39.00	..

C.D.(5%) for comparison of levels of varieties- 11.57
 C.D.(5%) for comparison of levels of Rhizobium - 5.17

* Mean of 3 replications

Ranking of varieties

V₆ V₁ V₃ V₁₀ V₉ V₇ V₂ V₅ V₈ V₄



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - Y-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R _i)
3 - 522	6 - 551	9 - Pcb-1	□ WITHOUT <i>Rhizobium</i> (R _o)

FIG: 5 PLANT DRY WEIGHT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

As seen in the Table-4, the Rhizobium inoculation resulted in significant increase in plant dry weight than control. By the inoculation of Rhizobium the average plant dry weight was 46.40 g compared to the control plants weighing only 39.00 g.

There was also significant difference in plant dry weight, between the varieties. On the basis of plant dry weight, the varieties were divided into three groups. The highest plant dry weight was observed in the varieties 551 (54.50 g), 534 (49.66 g), 522 (49.66 g) and 533 (48.50 g). The second group consisted of the varieties Ptb-1 (43.50 g), Pusa-2 (42.50 g) and V-37 (42.00 g). The lowest dry weight was observed in the third group of varieties C-152 (36.33 g), 779 (33.50 g) and Hg-22 (27.16 g).

The best varieties in terms of mean plant dry weight were 551, 534, 522 and 533 recorded by the inoculation of Rhizobium strain 6050.

5. Percentage of nitrogen content in plant

As seen in the analysis of variance table in Appendix-I it was clear that the effect of Rhizobium, variety and the effect of interaction of variety and Rhizobium on percentage nitrogen content were significant.

From the Table-5, it was seen that the Rhizobium inoculation had resulted in significant increase in percentage

Table - 5
 Percentage nitrogen content* - Effect of Rhizobium
 inoculation and interaction with cowpea varieties

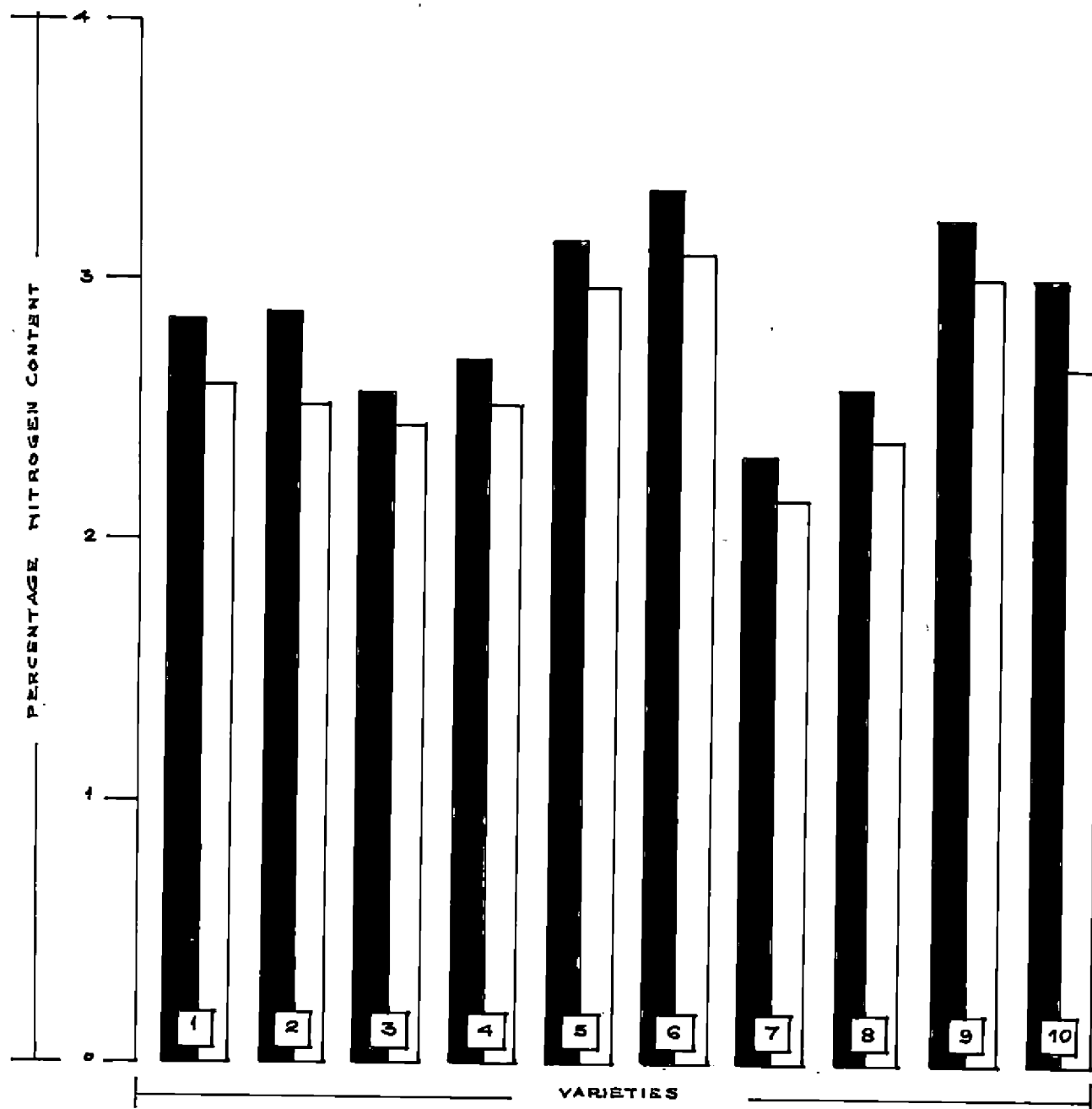
Cowpea varieties	Rhizobium culture R ₁	Control R ₀	Mean
V ₁ - 534	2.85	2.61	2.73
V ₂ - V-37	2.89	2.53	2.71
V ₃ - 522	2.58	2.45	2.52
V ₄ - Hg-22	2.70	2.52	2.61
V ₅ - C-152	3.16	2.97	3.07
V ₆ - 551	3.35	3.10	3.23
V ₇ - Pusa-2	2.32	2.15	2.24
V ₈ - 779	2.58	2.38	2.48
V ₉ - Ptb-1	3.23	3.02	3.12
V ₁₀ - 533	3.02	2.66	2.84
Mean	2.87	2.64	..

C.D. (5%) for comparison of levels of varieties - 0.072
 C.D. (5%) for comparison of levels of Rhizobium - 0.032
 C.D. (5%) for comparison of levels of varieties X Rhizobium interaction.

* Mean of 3 replications

Ranking of varieties

V₆ V₉ V₅ V₁₀ V₁ V₂ V₄ V₃ V₈ V₇



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R _i)
3 - 522	6 - 551	9 - PEb-1	□ WITHOUT <i>Rhizobium</i> (R _o)

FIG. 6 PERCENTAGE NITROGEN CONTENT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

of nitrogen content of plant compared to control, Rhizobium inoculation had resulted in an increase in average nitrogen content of 2.87 per cent compared to the control which was 2.64 per cent.

Varieties also showed significant difference in percentage of nitrogen content of plant. The highest nitrogen content was recorded by the variety 551 (3.23 per cent). It was closely followed by two varieties Ptb-1 and C-152, recording an average nitrogen content of 3.12 per cent and 3.07 per cent respectively eventhough the first one was significant differ from the latter. There was no significant difference in per cent nitrogen content between the varieties 534 (2.73 per cent) and V-37 (2.71 per cent), and 522 (2.52 per cent) and 779 (2.48 per cent). The lowest percentage of nitrogen content was observed in the variety Pusa-2 (2.24 per cent).

By the interaction of Variety and Rhizobium, the highest nitrogen content was recorded in the combination of 551 and Rhizobium (V_6R_1) which resulted in 3.35 per cent. The lowest nitrogen content recorded by the combination of Pusa-2 and Rhizobium (V_7R_4) was 2.32 per cent. The variety 551 was the best variety in terms of percentage nitrogen content obtained by the inoculation of Rhizobium strain 6050.

The observations taken at the time of harvest were plant height, plant dry weight, yield per plot and percentage of nitrogen content of plant. The analysis of variance table for different observations given in Appendix-II. Mean values of different observations are given in Table 6-9.

6. Plant height

From the analysis of variance table in Appendix-II, the effect of Rhizobium, variety and the effect of interaction of variety and Rhizobium on plant height were significant.

As seen in the Table-6, the Rhizobium inoculation resulted in significant increase in plant height at the time of harvest. An average plant height of 65.29 cm was observed by the Rhizobium inoculation in contrast to the control which was 50.85 cm.

The maximum plant height (144.14 cm) was recorded in the variety V-37 and was superior to all other varieties. It was followed by Ptb-1 having a plant height of 101.44 cm. There was no significant difference in plant height between the varieties Pusa-2 (60.51 cm) and C-152 (58.04 cm). The varieties 522 (45.13 cm) and Hg-22 (41.35 cm) were also on par with each other. The lowest plant height was observed in the varieties 551 (35.78 cm), 554 (32.37 cm), 779 (32.28 cm) and 553 (29.67 cm) and which were on par with each other.

Table - 6
 Plant height (cm)* - Effect of Rhizobium inoculation
 and interaction with cowpea varieties

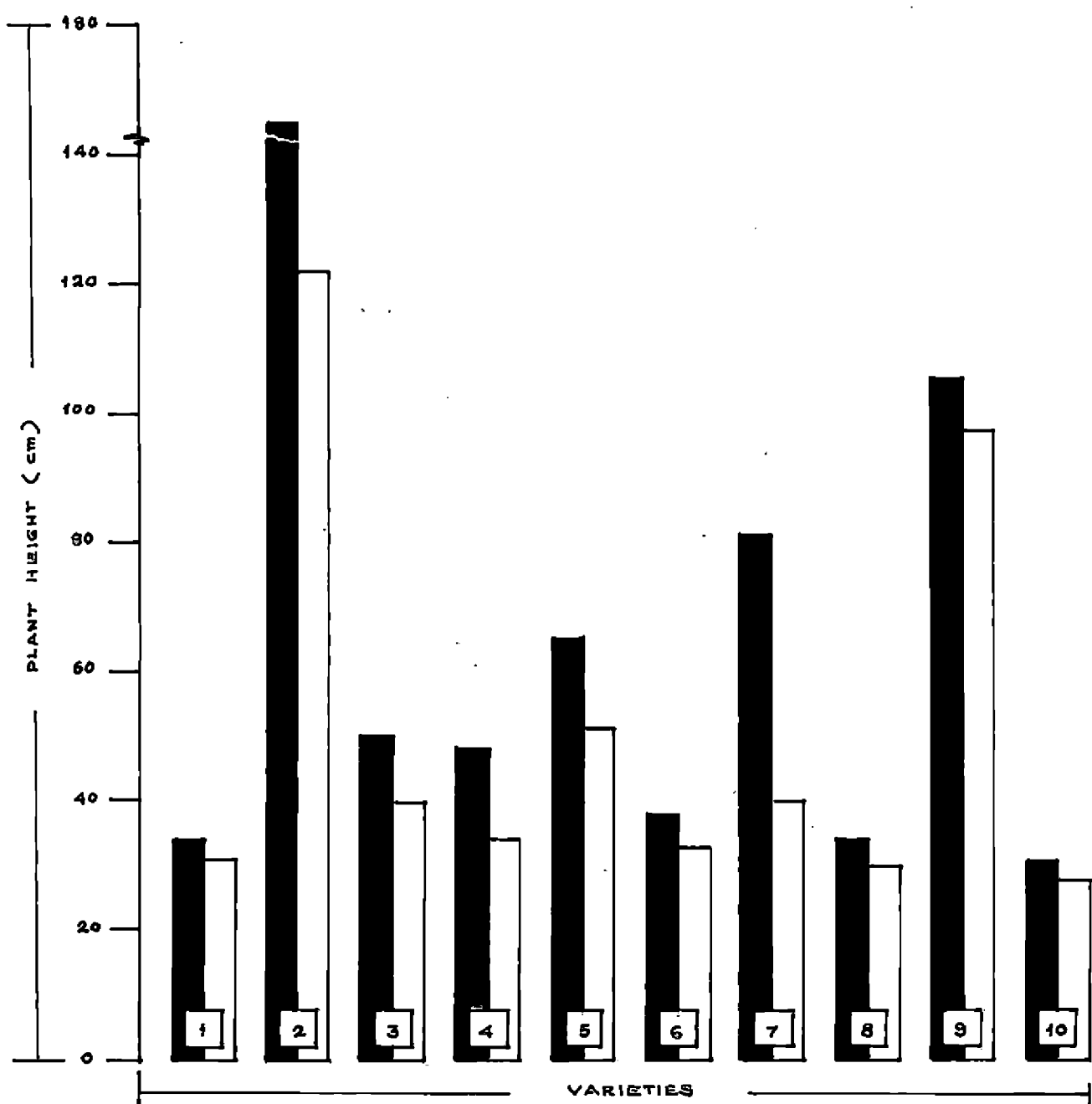
Cowpea varieties	Rhizobium culture		Mean
	R ₁	Control R ₀	
V ₁ - 534	34.08	30.66	32.37
V ₂ - V-37	165.89	122.39	144.14
V ₃ - 522	50.54	39.72	45.13
V ₄ - Hg-22	48.02	34.68	41.35
V ₅ - C-152	64.54	51.54	58.04
V ₆ - 551	38.52	33.04	35.78
V ₇ - Pusa-2	80.97	40.04	60.51
V ₈ - 779	34.00	30.56	32.28
V ₉ - Ptb-1	105.56	97.33	101.44
V ₁₀ 553	30.63	28.52	29.67
Mean	65.29	50.85	..

C.D. (5%) for comparison of levels of varieties - 6.32
 C.D. (5%) for comparison of levels of Rhizobium - 2.82
 C.D. (5%) for comparison of levels of variety X - 8.94
 Rhizobium interaction

* Mean of 5 replications

Ranking of varieties

V₂ V₉ V₇ V₅ V₃ V₄ V₆ V₁ V₈ V₁₀



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R ₁)
3 - 522	6 - 551	9 - Pcb-1	□ WITHOUT <i>Rhizobium</i> (R ₀)

FIG: 7. PLANT HEIGHT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

The variety V-37 was the best variety in terms of plant height by the inoculation of Rhizobium strain 6050.

7. Plant dry weight

As seen in the analysis of variance table given in Appendix-II, it was clear that the effects of Rhizobium and variety were significant. The effect of interaction of variety and Rhizobium was not significant.

From the Table-7, it was seen that the Rhizobium inoculation resulted in significant increase in plant dry weight than control. By the Rhizobium inoculation an average plant dry weight of 87.63 g was observed in contrast to the control which was 71.80 g.

The variety V-37 produced the maximum plant dry weight (113.83 g) and it was superior to all other varieties. There was no significant difference between the varieties 779 (87.83 g), 551 (85.16 g), 533 (80.00 g), Hg-22 (79.33 g), Ptb-1 (75.33 g), 534 (75.00 g) and Pusa-2 (71.33 g) in plant dry weight. The lowest plant dry weight was recorded by the varieties 522 (69.66 g) and C-152 (59.66 g).

V-37 was the best variety in terms of plant dry weight at the time of harvest, obtained by the inoculation of Rhizobium strain 6050.

Table - 7
 Plant dry weight (g.)* - Effect of Rhizobium
 inoculation and interaction with cowpea varieties

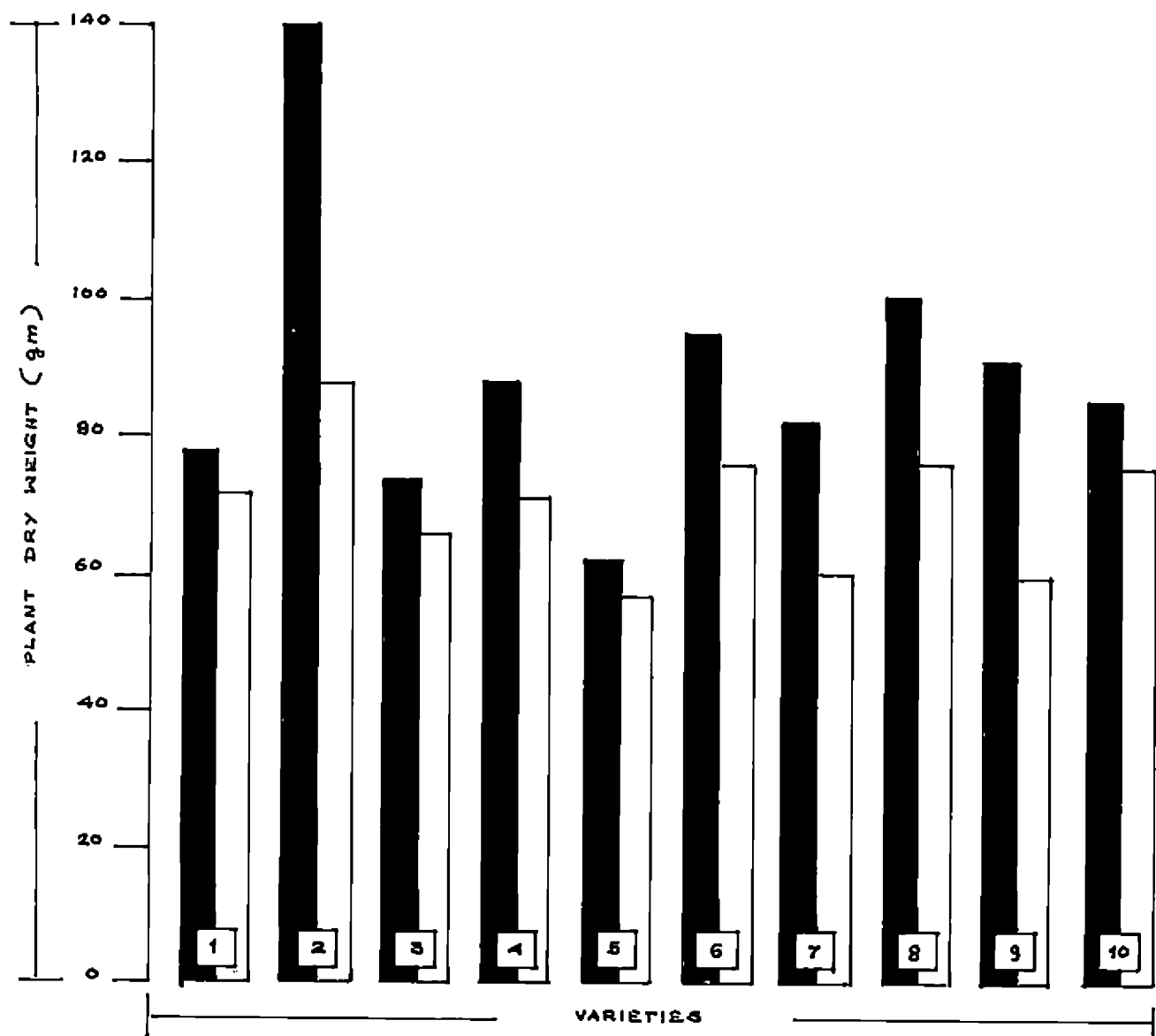
Cowpea varieties	Rhizobium culture R ₁	Control R ₀	Mean
V ₁ - 554	77.66	72.33	75.00
V ₂ - V-37	139.66	88.00	113.83
V ₃ - 522	73.66	65.66	69.66
V ₄ - HG-22	88.00	70.66	79.33
V ₅ - C-152	62.33	57.00	59.66
V ₆ - 551	94.66	75.66	85.16
V ₇ - Pusa-2	82.33	60.33	71.33
V ₈ - 779	99.66	76.00	87.83
V ₉ - PBL-1	91.33	59.33	75.33
V ₁₀ - 533	85.00	75.00	80.00
Mean	87.63	71.80	..

C.D. (5%) for comparison of levels of varieties - 26.00
 C.D. (5%) for comparison of levels of Rhizobium - 11.63

* Mean of 3 replications

Ranking of varieties

V₂ V₈ V₆ V₁₀ V₄ V₉ V₁ V₇ V₃ V₅



1 - 534	4 - Hg 22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (Ri)
3 - 522	6 - 551	9 - Pbb-1	□ WITHOUT <i>Rhizobium</i> (Ro)

FIG: 8 PLANT DRY WEIGHT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

8. Yield per plot

As seen in the analysis of variance table given in Appendix-II, it was clear that the effects of Rhizobium and variety were significant. The effect of interaction of variety and Rhizobium was not significant.

As seen in Table-8, the Rhizobium inoculation resulted in significant increase in yield than the control. By the Rhizobium inoculation an average yield of 1.000 kg was observed compared to the control yielding only 0.816 kg.

The varieties were divided into four groups. The variety 522 (1.310 kg) and 534 (1.142 kg) were the superior varieties in terms of average yield. The second group included the varieties 779 (1.036 kg), V-37 (1.028kg) and 551 (0.862 kg) and the third group included the varieties Hg-22 (0.825 kg), Ptb-1 (0.805 kg) and C-152 (0.762 kg). The lowest yield producing group was Pusa-2 (0.720 kg) and 533 (0.593 kg).

The best varieties were 522 and 533 in terms of average yield production by the inoculation of Rhizobium strain 6050.

9. Percentage of nitrogen content in plant

From the analysis of variance table in the Appendix-II it was clear that the effect of Rhizobium, variety and the effect of interaction of variety and Rhizobium were significant.

Table - 8
Yield per plot (kg.)* - Effect of Rhizobium inoculation and interaction with cowpea varieties

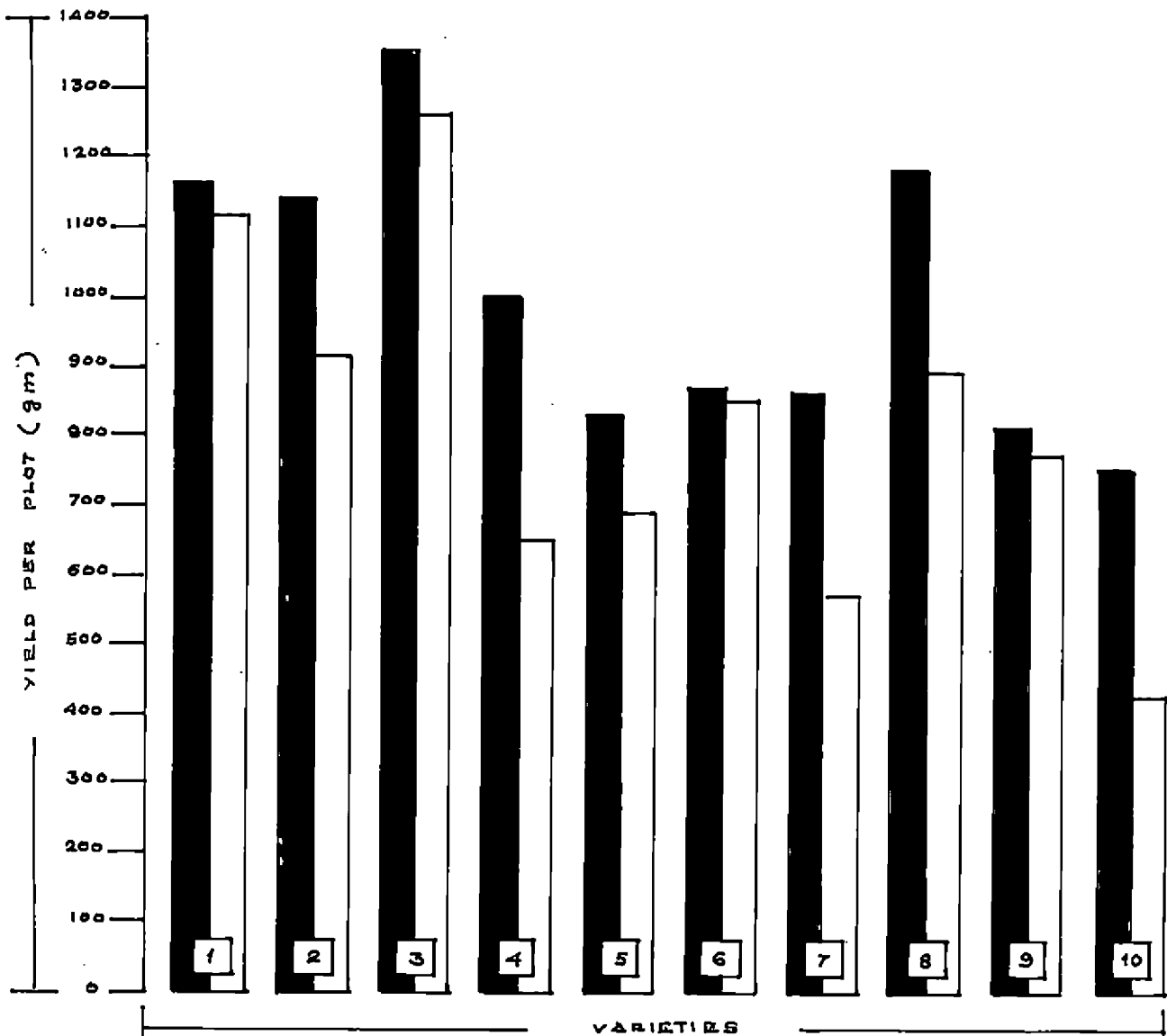
Cowpea varieties	Rhizobium culture R ₁	Control R ₀	Mean
V ₁ - 534	1.163	1.115	1.142
V ₂ - V-37	1.140	0.916	1.028
V ₃ - 522	1.353	1.266	1.310
V ₄ - Hg-22	1.000	0.650	0.825
V ₅ - C-152	0.853	0.695	0.762
V ₆ - 551	0.875	0.850	0.862
V ₇ - Pusa-2	0.866	0.573	0.720
V ₈ - 779	1.180	0.893	1.036
V ₉ - Ptb-1	0.813	0.773	0.805
V ₁₀ - 533	0.755	0.431	0.593
Mean	1.000	0.816	..

C.D.(5%) for comparison of levels of varieties = 0.280
C.D.(5%) for comparison of levels of Rhizobium = 0.125

* Mean of 3 replications

Ranking of varieties

V₃ V₁ V₈ V₂ V₆ V₄ V₉ V₅ V₇ V₁₀



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R ₁)
3 - 522	6 - 551	9 - Pbb-1	□ WITHOUT <i>Rhizobium</i> (R ₀)

FIG: 9 YIELD PER PLOT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

As seen in the Table-9, the Rhizobium inoculation resulted in significant increase in nitrogen content. Rhizobium inoculation resulted in an average nitrogen content of 2.24 per cent in contrast to the control (2.09 per cent).

The highest nitrogen content was recorded by the variety 551 (2.62 per cent). It was followed by the varieties C-152 (2.49 per cent), Ptb-1 (2.38 per cent) and 535 (2.20 per cent). There was no significant difference in per cent nitrogen content between the varieties 534 (2.11 per cent) and Hg-22 (2.09 per cent), 779 (2.00 per cent) and V-37 (1.99 per cent). The lowest nitrogen content was recorded by the variety Pusa-2 (1.85 per cent).

The highest plant nitrogen content recorded by the combination of variety 551 and Rhizobium (V_6R_1) was 2.63 per cent and the lowest recorded by the combination of Pusa-2 and Rhizobium (V_7R_1) was 1.91 per cent.

The variety 551 was the best variety in terms of percentage of nitrogen content in plant by the inoculation of Rhizobium strain 6050.

Table - 9

Percentage nitrogen content * - Effect of Rhizobium inoculation and interaction with cowpea varieties

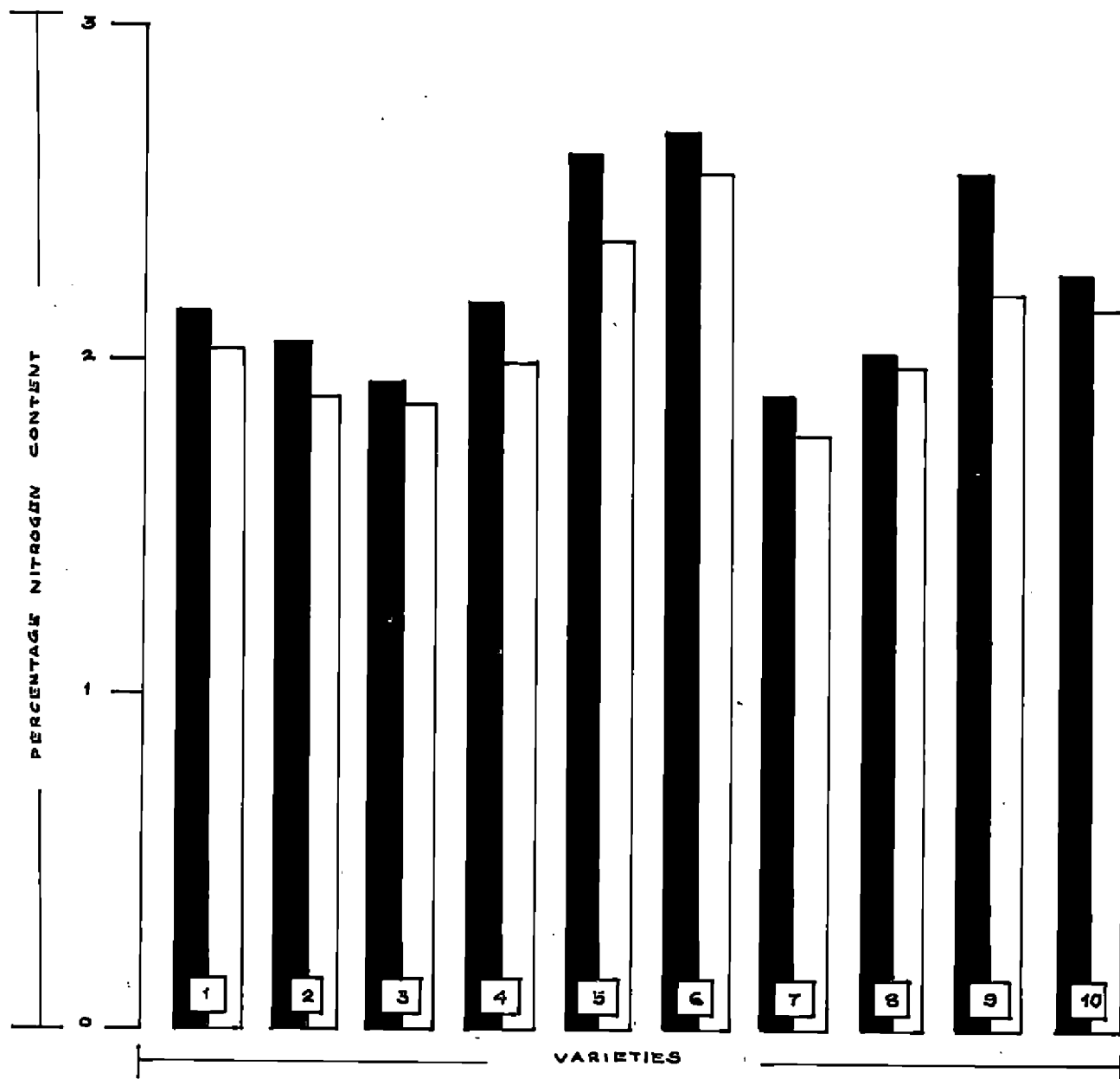
Cowpea varieties	Rhizobium culture	Control	Mean
	R ₁	R ₀	
V ₁ - 534	2.17	2.05	2.11
V ₂ - V-37	2.07	1.91	1.99
V ₃ - 522	1.96	1.89	1.93
V ₄ - HG-22	2.17	2.00	2.09
V ₅ - G-152	2.62	2.36	2.49
V ₆ - 551	2.68	2.56	2.62
V ₇ - Pusa-2	1.91	1.79	1.85
V ₈ - 779	2.03	1.98	2.00
V ₉ - Ptb-1	2.56	2.19	2.38
V ₁₀ - 533	2.26	2.15	2.20
Mean	2.24	2.09	..

C.D.(5%) for comparison of levels of varieties - 0.058
 C.D.(5%) for comparison of levels of Rhizobium - 0.026
 C.D.(5%) for comparison of levels of varieties X Rhizobium interaction. 0.083

* Mean of 3 replications

Ranking of varieties

V₆ V₅ V₉ V₁₀ V₁ V₄ V₈ V₂ V₃ V₇



1 - 534	4 - Hg-22	7 - PUSA-2	10 - 533
2 - V-37	5 - C-152	8 - 779	■ INOCULATED WITH <i>Rhizobium</i> (R)
3 - 522	6 - 551	9 - Pbb-1	□ WITHOUT <i>Rhizobium</i> (Ro)

FIG: 10 PERCENTAGE NITROGEN CONTENT, EFFECT OF *Rhizobium* INOCULATION AND INTERACTION WITH COWPEA VARIETIES

Effect of cowpea cultivation on native soil nitrogen content

The nitrogen content of soil before sowing was determined. But it was found that there was no significant difference between the plots in the native nitrogen content. The Rhizobium inoculation resulted in significant increase in residual nitrogen content of soil. Increase in per cent nitrogen content ranged from 1.7 to 6.3 per cent (Table-10). Correlation studies showed that there was slight increase in native nitrogen content in soil in the control plots also and it ranged from 0.4 to 3.8 per cent (Table-10). But it was found that the nitrogen fixed in the Rhizobium inoculated plots was significantly higher than that fixed in the control plots.

Table-10

Comparison of increase in soil nitrogen content by inoculated and non-inoculated cowpea plants

Cowpea varieties	Percent increase in soil nitrogen	
	in control plots	in treated plots
534	0.4	1.7
V-37	2.1	3.1
522	0.4	2.1
Hg-22	1.2	3.4
C-152	2.1	3.8
551	3.8	6.3
Pusa-2	2.1	3.2
779	1.4	2.6
Ptb-1	1.2	3.8
533	2.1	2.5

DISCUSSION

DISCUSSION

An important problem in the pulse cultivation is the lack of proper nodulation that will lead to the extensive use of fertilizer nitrogen for their cultivation. This has led to the intensive screening of different varieties of pulse crops for proper nodulation. With this objective, the present investigation was carried out to study whether there is any varietal response among the cowpea varieties to Rhizobium inoculation on nodulation and other plant characters. In this attempt 10 varieties of cowpea were used for selecting the best variety for effective symbiotic nitrogen fixation. Cowpea Rhizobium strain 6050 was used to study the host varietal specificity.

Plants were maintained till the harvest and observations on number of nodules, nodule dry weight, plant height, plant dry weight and percentage of nitrogen content were taken on 35th day after sowing. At the time of harvest, plant height, plant dry weight, percentage of nitrogen content and yield were noted. The symbiotic efficiency of the Rhizobium strain on different varieties of cowpea was observed on the basis of the above plant characters.

In the present study on the response of different cowpea varieties to Rhizobium inoculation, it was found that the inoculation resulted in significant increase in total number of nodules produced. The cowpea variety 553 recorded the maximum

number of nodules. It was closely followed by the variety 534. The variety 551, Ptb-1, Pusa-2, C-152 were recorded a significantly lower nodule number than the above mentioned varieties. The lowest nodule number was observed in the varieties V-37 and 779. In the case of nodule dry weight, the maximum dry weight was recorded by the varieties 551, Pusa-2 and 534. The lowest nodule dry weight was observed in the group of varieties including C-152, Hg-22, V-37 and Ptb-1. In the case of nodule dry weight, the variety 534 was there among the superior varieties. 534 was also selected as best variety for the nodule number production, eventhough 553 recorded the maximum number of nodules. With regard to the yield, the varieties were divided into four groups. The first and superior yield producing group included the varieties 522 and 534. Eventhough 522 recorded a higher yield than 534, there was no significant difference between them in mean yield. So in the case of highest yield production also the variety 534 was considered as best. The lowest yield was recorded by the varieties C-152, Pusa-2 and 553. So with regard to the nodule number, nodule dry weight and yield, the variety 534 was considered as the best variety.

In the case of plant dry weight, the variety V-37 was superior to all other varieties at the time of harvest. But at

the flowering time, the maximum plant dry weight was recorded by the varieties 551, 534, 522 and 533. The variety Ptb-1, Pusa-2 and C-152 recorded significantly lower plant dry weight.

With regard to the percentage of nitrogen content, the variety 551 recorded the maximum value. It was followed by C-152 and Ptb-1. The other varieties recorded significantly less nitrogen content than the above mentioned varieties. The lowest nitrogen content was observed in Pusa-2.

The observations therefore indicate that the host varietal specificity of rhizobia as seen here is not absolute. There is no consistency among the varieties in all characters recorded. This type of varying association is more of a host determined phenomenon. Such a role for the host genotype has been put forward by other workers also. Saubert and Scheffler (1967) reported that there was considerable variation in the amount of nitrogen fixed by various host varieties of *Trifolium* inoculated with the same isolate of Rhizobium. Dadarwal et al. (1976) showed that nodulation in chickpea was determined to a great extent by the host genotype. They reported that the varieties L-144 and BG-2 recording the maximum nodulation, while H-551 and H-355 :

recording the maximum nitrogen uptake and dry matter yield. Caldwell and Vest (1979) reported that effective nodulation and nitrogen fixation were greatly influenced by genetic variation of both the symbionts.

In this experiment, the variety 534 recorded the maximum number of nodules. But at the same time high nitrogen content of plant was obtained in the variety 551 at the flowering and harvesting time. So it was found that association of this Rhizobium resulted in maximum nodule number in the variety 534, but it resulted in significant reduction in nitrogen content than 551. The highest nitrogen content was observed in the variety 551 by the association of Rhizobium and less nodule number than in the variety 534. Among the varieties of cowpea, there were considerable differences in nodule number and nitrogen content, so it was not possible to correlate the nodule number and nitrogen content. The correlation study proved that there was no significant correlation between these two characters. Studies conducted by Sivaprasad and Shivappashetty (1980) support this observation. They showed that there was no significant correlation between nodule number and nitrogen content of plant top.

Results of the present investigation showed that the highest number of nodule was produced by the variety 534.

The same variety also recorded the highest yield. But such a relationship was not seen in other varieties. The highest nodule number was also recorded by the variety 555 but at the same time the yield was the lowest. The correlation study proved that there was no significant correlation between the nodule number and yield. Previous reports by some workers also support this result. Bagyaraj and Hegde (1976) reported that the seed inoculation of cowpea with Rhizobium culture UASB 94 resulted in increase in nodule number and grain yield. But the correlation study showed that there was no correlation between the number of nodules per plant and grain yield. Sivaprasad and Shivappashetty (1980) in a field study of the inoculation of cowpea cv. Pusa Phalguni, with strain IS-8 and IS-12 observed that there was no significant correlation between nodule number and final grain yield.

The last part of this study was to investigate whether there was any significant effect on native nitrogen content of soil by Rhizobium inoculation. The increase in percentage nitrogen content ranged from 1.7 to 6.5 per cent among the plots where the different cowpea varieties were cultivated. The variety 551 was the most efficient one that increased the soil nitrogen content to 6.5 per cent as a result of

Rhizobium inoculation. The variety 534 recorded the minimum increase in nitrogen amounting to 1.7 per cent only. Various reports from previous workers also proved that there was significant increase in native soil nitrogen by Rhizobium inoculation (Sahu and Bahara, 1972; Sharma and Sillak, 1974; Nathan et al. 1979 and Kale and Patil, 1981). The present investigation showed that there was slight increase in soil nitrogen content in the control plots also and it ranged from 0.4 - 3.8 per cent. But the statistical analysis showed that the nitrogen fixed in the Rhizobium inoculated plots was significantly higher than that fixed in the control plots. The increase in soil nitrogen in control plots may be due to the presence of rhizobia originally present in the soil.

In general a favourable response for all the plant characters was obtained by the inoculation of Rhizobia strain on all of the cowpea varieties. Stanford and Hectane (1976), Raju (1977), Bagyaraj and Hegde (1978), Ramachandran (1979), Sivaprasad and Shivappaobetty (1980) and Hair and Sivaprasad (1981) have also reported similar response by the inoculation of Rhizobium strain in cowpea. The effect of Rhizobium inoculation in cowpea varieties on nodulation and other plant characters and interaction between Rhizobium and varieties were shown in Fig. 2-10. Certain exceptions to

the above general observations was also noticed. Rhizobium inoculation resulted in higher plant height at the time of flowering, but statistically it was not significant. Such results were reported by Bagyaraj and Hegde (1978) that in cowpea increased nodulation and dry weight of plant top occurred, but was not statistically significant. Sivaprasad and Shivappasetty (1980) also reported that there was significant increase in nodule number and nitrogen content in cowpea inoculated with strain IS-3 and IS-12. But the increase in number of nodules and nitrogen content of roots were not found to be significant. In the case of interaction between varieties and Rhizobium, there was no significant effect in nodule dry weight, plant height, and plant dry weight at flowering time and plant dry weight and yield at the time of harvest, even though the values were higher than the control. Similar result was reported by Saxena and Singh (1978) that by Rhizobium inoculation in gram varieties the varietal differences were significant, but the interaction between culture and host variety was not significant.

A more conclusive result would have been obtained by including some more observations like plant fresh weight, nodule fresh weight, leghaemoglobin content of nodules, and root and seed nitrogen content which could not be taken

during the present investigation. Some important varieties like Ptb-1, Pusa-2 and C-152 did not perform well in this experiment. This may be due to the difference in the soil reaction or climatic conditions. Sometimes the soil environment affect the survival and persistence of rhizobia, or there may be competition with native rhizobia. So complete absence of native population of rhizobia should be necessary and that would be assured by selecting the field which was previously under non-legume crops, at least in the known recent past. This study was only of a preliminary nature. Detailed studies are necessary to understand the performance of different varieties under varied agro-climatic conditions before arriving at definite conclusions on varietal response of cowpea to Rhizobium. The most responsive genotype, can be identified and used by breeders for developing varieties for improved nodulation and nitrogen fixation preserving all agronomic characters and yield.

SUMMARY

SUMMARY

An investigation was undertaken at the Rice Research Station, Kayankulam during the summer season of 1981-1982 to study the varietal response to Rhizobium inoculation in cowpea under field conditions. A field experiment was laid out as a 2 x 10 factorial randomized block design with three replications.

The plants were maintained till the harvest and observations on nodule number, nodule dry weight, plant height, plant dry weight and percentage of nitrogen content were taken on 35th day after sowing. At the time of harvest, plant height, plant dry weight, percentage of nitrogen content and yield were taken. The symbiotic efficiency of the Rhizobium strain on different varieties was observed on the basis of the above characters.

In the present study on the response of different cowpea varieties to Rhizobium inoculation, it was found that the Rhizobium inoculation resulted in significant increase in total number of nodules produced. The variety 534 was considered as the best variety in terms of mean nodule number production. The lowest nodule number was recorded by the varieties V-37 and 779. In the case of nodule dry weight 534 was considered as best variety. The lowest nodule dry weight was observed by the group of varieties including C-152, Ng-22,

V-37 and Ptb-1. With regard to the yield also the variety 534 was the best one. In this case the lowest yield was recorded by the varieties C-152, Pusa-2 and 533. So with regard to nodule number, nodule dry weight and yield, 534 was the best variety.

In the case of plant dry weight, the varieties 551, 534, 522 and 533 recorded the maximum dry weight at the flowering time. But at the time of harvest V-37 recorded the maximum plant dry weight. The lowest plant dry weight was observed in the variety C-152.

With regard to percentage of nitrogen content, the variety 551 recorded the maximum nitrogen content. The lowest nitrogen content was recorded by Pusa-2. These observations, therefore, indicate that the host varietal specificity of rhizobia as seen here is not absolute. This type of varying association is more of a host determined phenomenon.

In the present experiment, the variety 534 recorded the maximum number of nodules. But at the same time high nitrogen content of the plant was obtained in the variety 551. The association of this Rhizobium resulted in maximum nodule number in the variety 534, but it resulted in significant reduction in nitrogen content. The high nitrogen content was resulted in the variety 551 and less nodule number than the variety

534. The correlation study proved that there was no significant correlation between nodule number and nitrogen content.

Results of the present investigation showed that the highest nodule number and yield were recorded by the variety 534. But such a relationship was not seen in other varieties. The statistical analysis showed that there was no correlation between the nodule number and grain yield.

The last part of this study was to investigate whether there was significant effect on native nitrogen content of soil by Rhizobium inoculation. The result showed that there was significant increase in nitrogen content of soil and it ranged from 1.7 to 6.3 per cent. The result also showed that there was slight increase in nitrogen content in the control plots and it ranged from 0.4 to 3.8 per cent. But the statistical analysis concluded that the nitrogen fixed in the Rhizobium inoculated plots was significantly higher than that fixed in the control plots.

In general a favourable response for all the plant characters was obtained by the inoculation of Rhizobium strain on all of the cowpea varieties. However, certain exceptions were also observed. Rhizobium inoculation resulted in higher plant height at the flowering time, but statistically it was not

significant. In the case of interaction between varieties and Rhizobium, it was found that, statistically there was no significant effect in nodule dry weight, plant height and plant dry weight at flowering time and plant dry weight and yield at the time of harvest, eventhough the values were higher than that of the control plots.

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* Original not seen

APPENDICES

Appendix-I

Effect of rhizobial inoculation in cowpea (Vigna unguiculata)
on nodulation and other plant characters at the flowering time.

Analysis of variance table

Source	df	Mean square				
		Total number of nodules per plant	Dry weight of nodules per plant	Height of plant	Total dry weight per plant	Nitrogen content per plant
			g.	cm.	g.	%
Total	59
Replication	2	45.94**	5.02**	307.60**	650.06**	0.004
Variety	9	697.24**	5.67**	110.25**	425.04**	0.591**
Treatment	1	1064.50**	3.73**	24.59	836.26**	0.793**
Variety X Treatment	9	40.22**	1.02	27.45	23.56	0.009
Error	38	8.33	0.44	21.78	97.94	0.004

** Significant at 1 per cent level of significance

Appendix-II

Effect of rhizobial inoculation in cowpea (*Vigna unguiculata*) on nodulation and other plant characters at harvesting time.

Analysis of variance table

Source	df	Mean square			
		Height of plant cm.	Total dry weight per plant g.	Yield per plot. kg.	Nitrogen content per plant %
Total	59
Replication	2	54.99	532.26	0.135	0.0013
Variety	9	8247.74**	1249.66**	0.232**	0.3850**
Treatment	1	3130.23**	3760.41**	0.507**	0.5430**
Variety X Treatment	9	345.26**	614.82	0.023	0.0014**
Error	38	29.27	494.86	0.057	0.0025

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Appendix-III

Increase in soil nitrogen by inoculated
cowpea plants

Cowpea varieties	Nitrogen content of soil before cultivation	Nitrogen content of soil after cultivation	Per cent increase
534	0.0460	0.0468	1.7
V-37	0.0420	0.0435	3.1
522	0.0460	0.0470	2.1
Hg-22	0.0460	0.0476	3.4
C-152	0.0350	0.0371	3.8
551	0.0340	0.0352	6.3
Pusa-2	0.0340	0.0351	3.2
779	0.0460	0.0472	2.6
Ptb-1	0.0390	0.0413	3.8
533	0.0350	0.0363	2.5

Appendix - IV

Increase in soil nitrogen by non-inoculated cowpea plants

Cowpea varieties	Nitrogen content of soil		Per cent increase
	Before cultivation	After Cultivation	
534	0.0380	0.0382	0.4
V-37	0.0380	0.0388	2.1
522	0.0420	0.0422	0.4
Hg-22	0.0430	0.0435	1.2
C-152	0.0550	0.0562	2.1
551	0.0580	0.0594	3.8
Pusa-2	0.0290	0.0296	2.1
779	0.0420	0.0426	1.4
Ptb-1	0.0550	0.0557	1.2
535	0.0420	0.0429	2.1

**VARIETAL RESPONSE TO *Rhizobium*
INOCULATION IN COWPEA UNDER
FIELD CONDITIONS**

BY
BEENA, S.

**ABSTRACT OF A THESIS
SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENT FOR THE DEGREE
MASTER OF SCIENCE IN AGRICULTURE
KERALA AGRICULTURAL UNIVERSITY**

**DEPARTMENT OF PLANT PATHOLOGY
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VELLAYANI, TRIVANDRUM**

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ABSTRACT

An investigation was undertaken at the Rice Research Station, Kayankulam during the summer season of 1981-1982 to study the varietal response to Rhizobium inoculation in cowpea under field conditions. Ten varieties of cowpea, C-152, Ptb-1, Pusa-2, Hg-22, 522, 533, 534, 551, 779 and V-37 were used in this experiment. The Rhizobium strain 6050 was used for the inoculation.

A field experiment was laid out as a 2 x 10 factorial randomized block design with 2 levels of treatments and 10 levels of host varieties. Nodule number, nodule dry weight, plant height, plant dry weight, percentage nitrogen content were taken at the time of flowering. Plant height, plant dry weight, percentage nitrogen content and yield were taken at the time of harvest.

The variety 534 was found to be the best variety in terms of nodule number, nodule dry weight and yield. In terms of plant dry weight, the varieties 551, 534, 522 and 533 were found to be superior at the flowering time. At the time of harvest, the variety V-37 recorded the maximum plant dry weight. The variety 551 recorded the maximum nitrogen content of the plant.

The correlation studies showed that there was no significant correlation between the nodule number and percentage of

nitrogen content of plant and also between nodule number and grain yield.

The study on the effect of Rhizobium inoculation on the native nitrogen content of soil revealed that there was significant increase in the nitrogen content and it ranged from 1.7 to 6.5 per cent. There was slight increase in nitrogen content in the control plots also. But the nitrogen fixed in Rhizobium inoculated plots was significantly higher than that fixed in control plots.

In general a favourable response for all the plant characters was obtained by the inoculation of Rhizobium strain on all the cowpea varieties. However, certain exceptions were also observed. Rhizobium inoculation resulted in higher plant height at the flowering time, but statistically it was not significant. In the case of interaction between varieties and Rhizobium, it was found that, statistically there was no significant effect in nodule dry weight, plant height and plant dry weight at flowering time and plant dry weight and yield at the time of harvest, even though the values were higher than that of the control plots.