VARIETAL VARIATION FOR NODULATION BY *Rhizobium* IN GREENGRAM

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Varietal variation for nodulation by *Rhizobium* in greengram" 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.

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CERTIFICATE

Certified that this thesis entitled "Varietal variation for nodulation by Rhizobium in greengram" is a record of research work done independently by Sri.V. PADMANABHAN NAIR 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 him.

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CONTENTS

LIST OF TABLES

(contd.)

LIST OF TABLES (Contd.)

LIST OF PLATES

LIST OF FIGURES

Introduction

INTRODUCTION

Nitrogen is an essential nutrient for the normal growth and yield of crop plants. This requirement is usually met either by mineralisation of native soil nitrogen, application of nitrogenous fertilizers or by the unique process of biological nitrogen fixation. In most of the legumes, there is a symbiotic association between a soil bacterium and its root system resulting in the formation of root nodules. This root nodule bacterium, Rhizobium, in fact accounts for nearly 25% of all the dinitrogen fixed annually in the world (Dazze and Hubbel, 1974). Hence, a judicial exploitation of this natural phenomenon, a gift of the nature, can result in substantial saving of fertilizer nitrogen especially in the cultivation of different pulses. This will be of utmost significance for a country like India, which is even today not self sufficient in meeting all its fertilizer requirements. Any reduction in the use of nitrogenous fertilizer in the cultivation of pulses due to an effective Rhizobium symbiosis, will result in an additional availability of this high energy consuming and costly input for cereal production, where an effective biological nitrogen fixation system similar to that of legumes is yet to be established.

Besides, the cost of inoculation of pulses with an efficient Rhizobium culture in Kerala, comes to only about Rs.2 per hectare.

The use of bio-fertilizers, although has got many advantages to marginal and small scale farmers in terms of cost reduction in pulses production and improvement of native soil fertility, the adaptation of this technique is often affected by the use of inefficient and non-host specific Rhizobium inoculants often resulting in very poor crop response. At the same time, all our present knowledge on legume Rhizobium symbiosis indicate that it is better to use crop specific rather than cross inoculation specific Rhizobium inoculants for getting maximum benefits from its application. Further, it is also found that a sort of host varietal specificity is often exhibited by Rhizobium in forming effective root nodules within a particular pulse variety. It is under these circumstances, the present investigation was carried out with the main objective to develop an efficient and crop specific Rhizobium inoculant suitable for greengram.

Green gram (Vigna radiata L. Wilczek) is one of the important pulse crops cultivated in Kerala. It is mainly grown either as a pure crop in summer rice fallows or as an

intercrop in coconut plantations. However, the development of an efficient Rhizobium culture for this crop is yet to be achieved in Kerala. Therefore, this investigation was carried out with the following technical programme.

1. Isolation of native Rhizobium suitable for greengram from different locations in Alleppey, Quilon and Trivandrum District.

2. Preliminary screening of Rhizobium culture for nodulation efficiency in greengram under aseptic pot culture condition.

3. Selection of an efficient Rhizobium culture for field evaluation.

4. Multilocational field evaluation at Karamana and Pattom for nodulation efficiency and host varietal specificity by using five different varieties of greengram.

Review of Literature

REVIEW OF LITERATURE

The renowned Dutch Microbiologist Martinus Willem Beijerinck in 1888 first recognised the importance of Rhizobium in forming root nodules in legumes. However, under natural conditions, this type of nodulation may be insignificant especially in places where suitable Rhizobium for a particular leguminous crop is absent (Date, 1970 and Vincent, 1974). In fact, it was Beijerinck himself who first demonstrated that nodulation can be induced in such plants by using a pure culture of Rhizobium. This probably laid the foundation for the present day practice of using Rhizobium inoculant for large scale cultivation of pulses in many developing as well as developed countries.

Rhizobium inoculation and crop response in greengram (Vigna radiata (L) Wilczek)

Rajagopalan et al.(1965) got significant increases in nodule number, root weight, shoot length and shoot weight in greengram variety CO-1 due to inoculation with an efficient Rhizobium culture. Akinola et al. (1972) reported that green gram when inoculated with Rhizobium culture could fix about 324 kg N/ha as compared to only 63 kg N/ha fixed by uninoculated plants. Sahu and Behera (1972) also got significant increases in nodule number, nitrogen content

of root and shoot in greengram, as a result of Rhizobium inoculation.

Chahal et al.(1976) studied the effect of seed inoculation in moong bean in the presence and absence of phosphate fertilizer. They found that nodulation increased significantly in both the cases with an average increase in yield of about 13.5 per cent.

Oblisamy et al. (1976) tested the effect of composite culture of Rhizobium inoculant consisting of strains from greengram, blackgram and groundnut in two pulses, namely greengram and blackgram. They found that the composite culture performed better over single strains in greengram recording maximum grain yield. Sing (1977) observed that inoculated unfertilized moong bean nodulated profusely during the normal period of branching and flower primodial initiation. Such plants also gave 51 per cent higher yield than uninoculated control. The individual and combined effects of three rhizobial inoculants in greengram were also studied by Balasubramanian et al. (1980) at Coimbatore, Kaveripattinam and Bhavanisagar. In all these three locations the multistrain inoculants were found to perform better. They produced more number of nodules per plant, higher grain yield and more nitrogen assimilation

than uninoculated control and single strain inoculated plants. Chandramohan et al.(1980) studied the effect of peat based Rhizobium culture on greengram and blackgram in an experiment conducted in the Thanjavur delta. The treated plots were compared with the untreated plots and they obtained an increase in yield of 33.5 per cent in blackgram and 23.6 per cent in greengram.

Efficiency of different Rhizobium cultures namely MH-779 (Hisar), M-10 (IARI), KM-1 (Kanpur) GMVS-I (Coimbatore) and a composite culture were tested in moongbean varieties such as K-851, PS-16, MH-1 and T-44 by Dudeja and Khurana (1980). They found that all the strains tested increased the grain yield of different moongbean cultivars over the control. However, in a similar study, Rangarajan and Prabha karan (1980) reported that out of the seventeen Rhizobium strains, isolated from various agroclimatic regions of Tamil Nadu, only four strains namely VEP-1, MOV-1, KKRI-1 and GMBS-1 increased the growth, nodulation, nitrogenase activity and yield in greengram.

Studies conducted at the Kerala Agricultural University by Rajendran Pillai and Nair (1981) have shown a wide variation in the nodulation efficiency of different isolates of Rhizobium for greengram.

Ghai *et al.* (1982) in their study on the competitive ability of five different strains of rhizobia of cowpea group for nodulation in greengram variety ML-5, in loamy clay and loamy sand soils found that strains RM-5 and RM-6 were very effective for nodulation in loamy clay soil while the remaining strains such as MNH, M-20 and RM-4 were efficient only in loamy sand soil. Out of these, RM-6 and MNH predominated in nodule formation in loamy clay and loamy sand soil respectively. Rai and Prasad (1984) conducted an experiment in acid saline and alkaline media to study the growth and symbiotic nitrogen fixation in *Vigna radiata* (L) under stress conditions. They observed variations in nodulation and nitrogen fixation efficiency due to host variety while the strain characteristics were unaffected by soil stress conditions.

In an experiment conducted under field conditions using greengram variety Pusa baisakhi and various levels of nitrogen application, 15, 30, 45 and 60 kg N/ha with and without *Rhizobium* inoculation, Raju and Varma (1984) got maximum increase in dry weight, pod number, 100 grains weight and nitrogen uptake in treatment combinations consisting of *Rhizobium* inoculation and 15 kg N/ha.

Rhizobium inoculation and crop response in other legumes

Wetselsaar (1967) made a detailed study on nitrogen gain in four annual legumes such as Stylosanthes humilis, Vigna sinensis, Cyamopsis tetragonoloba and Archis hypogaea and reported that the cultivation of groundnut alone resulted in an average nitrogen fixation of about 60 lbs per acre as compared to the adjacent fallow land. Trinick (1968) conducted an investigation on the specificity of Rhizobium symbiosis in Leucaena leucocephala with Rhizobium isolated from a group of legumes like Leucaena leucocephala, Mimosa invisa, Mimosa pudica, Acasia farnesiana and Sesbania sp. and found that all are capable of nodulating Leucaena leucocephala effectively.

Muthusamy (1970) investigated the comparative efficiency of four isolates of Rhizobium for their nodulation and nitrogen fixation efficiency on TMV-2 variety of groundnut. He reported an apparent host varietal relationship between groundnut variety TMV-2 and its own rhizobial isolate. Inoculation with the homologous isolate also produced maximum number of nodules, plant length and fresh weight. Sahu and Behera (1972) found that application of Rhizobium to groundnut increased the grain yield and over all nitrogen content of root, shoot and grain of the crop. Dadarwal and associates

(1974) investigated the nature of nodulation of rhizobia from six species of Arachis in the cultivated variety of A. hypogaea. The plants differed in the pattern of nodulation due to different Rhizobium isolates. Rhizobium from one species of Archis viz. A.duranensis was found to be most effective on A. hypogaea.

Chhonkar and Negi (1971) evaluated the response of soybean variety "Bragg" to inoculation with different strains of Rhizobium japonicum. They found that indigenously produced peat based single strain inoculant was as effective as exogenous multistrain peat culture. Subba Rao and Bala sundaram (1971) also reported that seed inoculation of soybean with Rhizobium significantly increased the yield. Abu-Shakka and Bassiri (1972) observed that on a land upon which soybean was grown previously under Rhizobium inoculation, more number of nodules were formed in the succeeding crop when compared to uninoculated plants. An increase in yield to the extent of 165 to 226 per cent over control was obtained by Longari and Herrera (1972) in soybean grown in soils without any indigenous Rhizobium population. Rewari (1974) made an interesting observation that seeds of soybean and greengram carried rhizobia when the flowers were sprayed with homologous rhizobia. Such seeds when raised aseptically formed root nodules without any further Rhizobium inoculation.

Raut and Ghonsikar (1977) reported that the number of nodules formed in soybean could be increased significantly from 104 to 388.2 per plant due to Rhizobium inoculation. Okon et al. (1979) got 100 per cent increase in nodulation in soybean inoculated with an appropriate Rhizobium culture in Israel, where native population of this bacterium was completely absent. Rao and Sharma (1980) in their study on the effect of different levels of Rhizobium inoculation on yield in soybean found that maximum yield increase was obtained due to seed inoculation at the rate of 2×10^5 cells per seed.

Medhane and Patil (1974) laid out pot as well as field experiments to study the effect of Rhizobium inoculation in bengalgram. They got an increase in yield of 24 to 62 per cent due to inoculation with different isolates of rhizobia. Tripathi et al. (1975) also found that Rhizobium inoculation alone increased yield in chickpea by about 25 per cent over control. They further observed that although fertilizer application increased the yield upto 48 per cent, it resulted in considerable delay in flowering.

Chahal et al. (1976) inoculated seeds of chickpea variety G-130 and "Harechole" with Rhizobium cultures and got greater number of healthy and pinkish nodules, dark green

colour of foliage, and more number of pods in inoculated plants. Rai et al. (1977) studied the response of eight selected strains of Rhizobium in bengalgram and reported that the yield increase over the control was from 13.9 to 39.8 per cent due to inoculation. However, they could not get any correlation between yield and either the number of nodules formed or dry weight of nodule. Chahal and Joshi (1978) also conducted a field trial to find out the effect of inoculation with different strain of Rhizobium in chickpea. They observed that out of 17 strains tested, strain number 11 was very effective as it significantly increased the yield and nodule number per plant. Pareek (1979) has evaluated the effectiveness of various strains of chickpea Rhizobium in field for two consecutive years. Strains B-1 and 6042 recorded respectively about 26 and 141 per cent increase in nodule weight against control during the first two years. The response of nine different strains of Rhizobium on nodulation, grainyield, protein and aminoacid content of chickpea was studied by Rai and Singh (1979). However, they could not get any significant correlation between grain yield and nodule number.

An increase in yield of 23 per cent over uninoculated control in cowpea by Rhizobium inoculation was reported by Subba Rao (1972). Raju (1977) observed that Rhizobium

Ahmad *et al.* (1981) examined the potential for inoculant use in cowpea cultivation in West African soils and reported that many cultivars responded positively in shoot fresh weight at 3 to 6 weeks after sowing due to *Rhizobium* inoculations. Nair and Sivaprasad (1981) have also stressed the importance of *Rhizobium* inoculation in cowpea under Kerala conditions.

Graham and Rosas (1978) studied nodule development and nitrogen fixation in two climbing cultivars of *Phaseolus vulgaris* (L) grown in monoculture and associated with two maize varieties. They reported that the bean cultivar P-590 showed similar plant and pod fresh weight development in monoculture as well as in association with maize plants. Sekhon *et al.* (1978) found that phaseolus bean, inoculation with *Rhizobium* gave same yield as that of application of 25 kg/N/ha. Duque *et al.* (1985) made a comparative field evaluation on the effect of *Rhizobium* inoculation and nitrogen fertilization at the rate of 100 kg/N/ha on four cultivars of phaseolus bean and reported that nodulation of all cultivars in the uninoculated treatment was poor and that two of the cultivars nodulated well only when they were inoculated with *Rhizobium* culture.

Subba Rao et al. (1980) conducted root nodulation study in Aeschynomene aspera with fifteen isolates of nodule bacteria from root and stem nodules of Aeschynomene aspera itself. All these isolates together with other efficient strains of known rhizobia belonging to different cross inoculation groups were evaluated for their nodulation ability on Aeschynomene aspera, Cajanus cajan, chick pea, Trifolium, Medicago, Arachis and cowpea. They found that Rhizobium from Aeschynomene aspera could nodulate only its homologous host, but not in other legumes.

Garser (1977) studied the efficiency of Rhizobium meliloti strains and their effect on alfalfa cultivars. Five alfalfa cultivars were studied in green house in relation to their effective symbiotic relation with different strains of Rhizobium meliloti and reported a variety x Rhizobium strain interaction for yield in first cutting. Hagedorn (1979) determined the extent of establishment of Trifolium subterrenium along with Rhizobium inoculation and fertilization at five field sites in South West Oregon and found that both plant dry weight and total nitrogen content showed an additive effect due to inoculation and fertilization.

Tu and Mc-Donnell (1984) conducted experiments in green house as well as under field conditions to find out the effect of Rhizobium inoculation on nodulation, yield and winter survival of some alfalfa cultivars. They found that the number of nodules formed were more or less cultivar specific and that there were significant difference among alfalfa cultivars in their nodulation response.

Sharma and Singh (1986) reported that seed inoculation with and without nitrogen application improved significantly the number of nodule formed, root length, and root and shoot dry weight per plant in lentil. The application of phosphate fertilizer also promoted better root growth, nodulation and shoot per plant.

The effect of Rhizobium inoculation on nodulation and plant growth in winged bean was studied by Chakrabarti et al. (1987). They got significant increases in nodulation, pod number, pod fresh weight and pod dry weight, due to Rhizobium inoculation.

Varietal variation for nodulation by Rhizobium in different legumes.

Erdman (1947) studied the occurrence of any strain variation and host specificity for Rhizobium trifolii in four species of Trifolium and found that the use of appropriate strains of Rhizobium trifolii was essential for getting maximum nitrogen fixation and plant growth. Moniz et al. (1968) also reported marked variations for nitrogen fixation and nodulation by 18 alfalfa (Medicago sativa) and two berseem (Trifolium alexandrirum (L) strains of Rhizobium. They further observed that Rhizobium melliloti and Rhizobium trifolii were highly specific to their own cross inoculation groups.

Doku (1969) while studying the host specificity among five groups of cowpea cross inoculation groups found that Rhizobium from soybean, groundnut and bambara groundnut, nodulated cowpea and lima bean, while cowpea Rhizobium nodulated only its host, lima bean and vice-versa. Rhizobium from all species except cowpea and limabean nodulated groundnut and bambara groundnut.

An intraspecific variability for nitrogen fixation was also roported in the case of cowpea by Zary et al.(1978) in their detailed investigation involving nearly one hundred

host genotypes. They further reported that there was a genetic determinant for an effective symbiosis of Rhizobium in legumes.

Balasundaram (1971) studied the interaction between soybean genotypes and different isolates of Rhizobium japonicum and found that different isolates of Rhizobium varied in their symbiotic efficiency with different varieties of soybean. Gonzalez (1977) also reported that there was a relation between host variety and Rhizobium strain in terms of gain in plant dry weight, nodule dry weight and nitrogen content due to Rhizobium inoculation in soybean. Mishra and Srivastava (1978) conducted a pot culture experiment on four varieties of Soybean Bragg, Punjab-1, U.P.S. S-38 and Sepaya Black. It was noted that the number of nodules formed did not differ significantly among different varieties. The variety "Bragg" appeared outstanding in fixing maximum nitrogen.

The efficiency and specificity of Rhizobium cultures in bengalgram were investigated by Vaisbya and Saneria (1972). They reported that all the species of bengalgram exhibited a high degree of host specificity. Dadarwal et al. (1976) showed that nodulation in chickpea was determined to a greater extent by host genotypes. In the experiment conducted to determine the varietal and strain interaction, they found

that isolate Co-121 produced maximum number of nodules, while the varieties L-144 and B.G.-2 showed maximum response to Rhizobium inoculation. Variation in symbiotic effectiveness in bengalgram was also studied by Bapat et al. (1977). They examined nearly 50 genotypes of bengalgram and one effective Rhizobium strain. According to yield response to inoculation, they could group different genotypes into six different frequency classes.

Burton (1975) showed that an efficient strain of Rhizobium could increase substantially the yield in peanut. He further advocated an effective screening system for evaluation of Rhizobium strains to groundnut cultivars. Surajbhan (1975) in a nodulation study with eight groudnut cultivars to develop a suitable plant type for arid zone, found that the varieties A.K-12-24 and Spanish Improved were the best in relation to nodulation and symbiotic efficiency. Among the varieties tested, the procumbent ones had more and heavier root nodules with a higher nitrogen content than erect groundnut varieties.

Singh et al. (1976) tested the effectiveness of 40 genetically distinct strains of Rhizobium on six wild species of groundnut and cultivated varieties of Arachis hypogea under pot culture conditions and noticed large

variations in symbiotic efficiency between different strains, Strain GT-20 isolated from Arachis hypogea and G-405 isolated from Arachis glaberata were found to be most effective. Aylaba (1980) evaluated symbiotic nitrogen fixation by three peanut varieties inoculated with two Rhizobium strains and detected significant differences in specific nitrogenase activity. Wynne et al. (1980) conducted a green house evaluation of different strains of Rhizobium in peanuts and reported significant differences in nodulation. All the strains produced greener plants with higher nitrogen content per hectare than uninoculated control plants. Girija (1982) conducted a study on host varietal specificity of Rhizobium for nodulation in groundnut. The rhizobia were inoculated to the seven varieties of groundnut and reported a favourable response in all varieties mainly due to inoculation with homologous isolate of Rhizobium.

Singh et al. (1980) observed wide variations in symbiosis in 49 genotypes of moong bean inoculated with a cowpea Rhizobium strain M.O.J. Among the 49 genotypes tested, total amount of nitrogen fixed varied from 2 to 11 mg per plant and dry weights varied from 180 to 223 mg. There were also wide variations in the number of nodules formed in different genotypes.

Varietal variation and crop response to Rhizobium inoculation and zinc application in greengram was studied by Prasad and Ram (1982). They reported that treatment with Rhizobium and zinc exhibited variable response in different varieties of moongbean in significantly increasing nodulation, yield, protein and carbohydrate content of the seed.

Rajput and Varma (1982) conducted a field experiment with four dates of sowing and three levels of phosphate application with Rhizobium inoculation in three varieties of greengram during summer seasons of 1975, 1976 and 1977. They got maximum yield in Pusa baisakhi than in other varieties such as S-8 and Type-1. Vaishy and Gajendra gadkhar (1982) studied the effect of inoculation of Rhizobium strain, M1 on eight genotypes of Vigna mungo and found that various genotypes differed in nodulation and grain yield. The yield increase obtained ranged from 8.44 to 42.89 per cent. There was also a significant interaction between nodulation and grain yield in different genotypes.

Rai and Prasad (1983) conducted a study on the compatibility on N_2 fixation by high temperature adapted Rhizobium strain and Vigna radiata genotypes at two moisture levels in calcareous soil. They observed different types of interactions between strains and genotypes at

both moisture levels. Strain SY with greengram genotype S-8 gave maximum grain yield, nitrogenase activity, leghaemoglobin and ethanol soluble carbohydate of root nodules. Prasad and Ram (1984) studied the varietal response of Rhizobium strains for nodulation, chlorophyll and protein content in greengram and got different responses with different strains of Rhizobium in terms of above parameters which they tested for nodulation efficiency in greengram.

Materials and methods

MATERIALS AND METHODS

The present investigation on "Varietal variation for nodulation by Rhizobium in green gram" was conducted at College of Agriculture, Vellayani, Trivandrum. The field trial for host varietal specificity was done at the Cropping System Research Centre (C.S.R.C.), Karamana of Kerala Agricultural University and in a farmers' field at Pattom, Trivandrum.

1.1 Isolation of native Rhizobium specific for greengram

Soil samples from 14 different locations in Alleppey, Quilon and Trivandrum Districts were collected for this purpose.

Sl.No.	District	Location
1.	Alleppey	Kayamkulam
2.	Quilon	Anchal
3.	Quilon	Kottarakkara
4.	Quilon	Kulathupuzha
5.	Quilon	Pattathanum
6.	Trivandrum	Anad
7.	Trivandrum	Karamana
8.	Trivandrum	Kattakkada
9.	Trivandrum	Kazhakoottum
10.	Trivandrum	Kilimanoor
11.	Trivandrum	Neyyattinkara
12.	Trivandrum	Parassala
13.	Trivandrum	Pattom
14.	Trivandrum	Vellayani

A known variety of greengram, CO-3 obtained from Tamil Nadu Agricultural University, Coimbatore, was used for the isolation of native rhizobia under pot culture conditions. Three plants were maintained in each pot. They were grown for 45 days.

1.2 Isolation of Rhizobium

This was done by the method of Vincent (1970). Healthy root nodules collected from plants grown in different soil samples were initially transferred to clean test tubes, surface sterilized with 70 per cent ethanol for 30 seconds followed by 0.1 per cent mercuric chloride solution for two minutes. The nodules were then thoroughly washed in six changes of sterilized tap water and gently crushed with a sterilized glass rod. The resulting milky fluid was streaked on yeast extract mannitol agar medium in petridishes and incubated in a B.O.D. incubator at 28 $\pm$ 1°C for 3 days.

Yeast extract mannitol agar medium (Allen 1953)

Mannitol	- 10.0 g
K_2HPO_4	- 0.5 g
Mg SO_4 $7H_2O$	- 0.2 g
NaCl	- 0.1 g
$CaCO_3$	- 3.0 g

Yeast extract	- 1.0 g
Congo Red	- 2.5 ml (1% aqueous solution)
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 fresh slants of yeast extract mannitol agar medium for further studies. The various isolates were also checked for their purity by repeated streaking in the same medium. Single colony of Rhizobium thus selected from each location was used as the stock culture.

1.3 Primary characterisation of different isolates of Rhizobium

a. Gram staining

Thin smear of 24 h old broth culture of each isolate was prepared, heat fixed and stained with ammonium oxalate crystal violet for 1 minute. The slides were gently washed in tap water and treated with iodine solution for 1 minute. These were once again washed in tap water, blot dried and decolorised with 70 per cent ethyl alchohol for 30 seconds with gentle agitation. After washing, the slides were counterstained for ten seconds with safranin, rinsed in tap

water, dried and examined under the oil immersion objective of a microscope. The composition of different stains and reagents used are given below.

1. Ammonium oxalate crystal violet

Solution A -	Crystal violet (90%)	- 0.2 g
	Ethyl alcochol (95%)	- 20 ml
Solution B -	Ammonium oxalate	- 0.8 g
	Distilled water	- 80 ml

Solution A and B were mixed together.

2. Gram's modification of Lugol's solution

Iodine	- 1 g
KI	- 2 g
Distilled water	- 300 ml

3. Counter stain

Safranin (2.5% solution in 95% ethanol)	-10 ml
Distilled water	- 100 ml

b. Ketolactose test

Ketolactose medium (Bernaertz and de Ley, 1963)

Lactose	- 10.0 g
K_2HPO_4	- 0.5 g
$CaCl_2$	- 0.2 g
Yeast extract	- 0.5 g
$Mg\ SO_4(7H_2O)$	- 0.1 g
NaCl	- 0.2 g
$FeCl_3$	- 0.01 g
Distilled water	- 1000 ml
pH	- 6.8

c. Spore staining (Bartholomew and Mitwer, 1950)

A thin smear of different isolates of rhizobia was initially heat fixed and stained with a saturated solution of malachite green (7.0% aqueous solution) for 10 minutes. The slides were then washed in tap water and counter stained with 0.25 per cent aqueous solution of safranin for 15 seconds, rinsed in tap water, blot dried and examined under oil immersion objective for the occurrance of any endospore.

d. Growth on glucose peptone agar medium

Sterilized petri dishes containing 25 ml of glucose peptone agar medium of following composition were streaked with different isolates of rhizobia and incubated at 28 ± 1°C for seven days. The extent of growth was recorded at the end of the incubation, period.

Glucose Peptone agar medium (Vincent, 1970)

Glucose	- 5.0 g
Peptone	- 10.0 g
Agar	- 15.0 g
Bromocresol purple	- 10.0 ml (1% alcoholic solution)
Distilled water	- 1000 ml
pH	- 6.8

The different isolates of rhizobia were streaked on the above medium in sterile petri dishes. After incubation for 7 days at 28 ± 1°C, the plates were flooded with Benedict's solution in order to test the production of Ketolactose from lactose. A change in colour of the reagent from blue to yellow around the colonies indicated the formation of Ketolactose.

2.1 Preliminary screening of Rhizobium for nodulation efficiency in greengram

A pot culture experiment was conducted under aseptic conditions to test the nodulation efficiency of different isolates of Rhizobium. This experiment was laid out in completely randomised design with three replications each using the greengram variety CO-3. Earthern pots of 25 x 30 cm size were filled with 10 kg of potting mixture consisting of sand, garden soil and cowdung in the ratio 1:1:2. The Rhizobium culture for seed treatment of greengram was initially grown in yeast extract mannitol agar medium in sterilized petri dishes for 3 days at 28 ± 1°C in a B.O. D. incubater. The seeds of greengram were first surface sterilized with 0.01 per cent mercuric chloride solution for one minute and washed thoroughly with 5 changes of sterilized tap water. These were then treated with a thick

suspension of appropriate Rhizobium culture prepared in 10 ml of sterilized distilled water and sown immediately. A treatment without Rhizobium inoculation served as the control. P and K fertilizers were applied uniformly to all pots before sowing at the rate of 30 kg P_2O_5 and 30 kg K_2O/ha. The plants were irrigated regularly with sterilised tap water and grown for 45 days. Observations on nodule number, fresh and dry weights of nodules, root length, fresh and dry weights of root, shoot height, fresh and dry weights of shoot were taken after carefully uprooting each plant with the help of a mild jet of water. Nodule number was counted after carefully separating them from the intact root system. They were weighed in a chemical balance to determine the fresh weight and then dried to a constant weight at 60°C in a drying oven for taking the dry weight. Root length and shoot height were measured from the base of the stem to the growing tip of the root and shoot respectively. The fresh weights of root and shoot in each treatment were determined immediately after harvest and the dry weights after drying the samples to a constant weight at 60°C.

2.2 Selection of Rhizobium for field evaluation

The nodulation and nitrogen fixation efficiency of different isolates of Rhizobium specific for greengram was

assessed as per the Indian Standard Institution specifications for Rhizobium inoculants (IS- 8268-1976)

3. Varietal variation for nodulation by Rhizobium in greengram.

A multi locational field trial in randomised block design was laid out at two different locations, Cropping System Research Centre, Karamana, Trivandrum of Agricultural University and in a farmer's field at Pattom, Trivandrum District. In all, there were 15 treatments consisting of 3 Rhizobium treatments and 5 different varieties of greengram (Vigna radiata (L) Wilczek), CO-3, CO-4, CV-2, KM-2 and PB (Pusa baisaki) obtained from Tamil Nadu Agricultural University Coimbatore. Three replications were maintained for each treatment in plots of 7.5 x 2.4 m in size. The details of the treatment combinations are given in Fig. I.

P and K fertilizers and lime were added uniformly to all plots prior to sowing at the rate of 30 kg P_2O_5, 30 kg K_2O and 500 kg lime/ha respectively. Seed treatment with appropriate Rhizobium culture was done as per package of practices recommendation, Kerala Agricultural University (1986). Irrigation was done only once at the time of sowing. The plants were grown for 85 days till harvest. Observations on nodule number, nodule fresh and dry weights shoot

Treatment combination

N

LOCATION KARAMANA

$R V_2$	$R_{II} V_3$	$R_0 V_3$	$R_0 V_4$	$R V_5$	$R_5 V_2$	$R_0 V_2$	$R_{II} V_4$	$R_0 V_5$	$R_5 V_3$	$R_5 V$	$R_0 V$	$R_5 V_5$	$R_5 V_4$	$R_1 V_1$
$R_5 V_3$	$P_1 V_4$	$R_0 V_5$	$R V_2$	$R_5 V_1$	$R_0 V_1$	$R_1 V$	$R_{II} V_5$	$R_0 V_3$	$R_5 V_4$	$R_5 V_5$	$R_0 V_4$	$R_5 V_2$	$R_1 V_3$	$R_0 V_2$
$R V_3$	$R_0 V_4$	$P_5 V_2$	$R_1 V_4$	$R_0 V_3$	$P_0 V_2$	$R_5 V_4$	$R_{II} V$	$R V_2$	$R_5 V_5$	$R_0 V_5$	$R_5 V_3$	$R_5 V$	$R_{II} V_5$	$R_0 V$

LOCATION PATTOM

$R_0 V_4$	$R_5 V$	$R_0 V_3$
$R V_3$	$R_5 V_3$	$R_0 V$
$R_5 V_5$	$R V_5$	$R_1 V_3$
$R V_4$	$R V_2$	$R_0 V_5$
$R_0 V_2$	$R_5 V_5$	$R V_2$
$R_0 V_5$	$R_0 V_4$	$P_{II} V_5$
$R_0 V$	$R V_4$	$R_0 V_2$
$R V$	$R_5 V_4$	$R_5 V_4$
$R_5 V_2$	$R V_2$	$R_0 V_4$
$R_0 V_3$	$R_0 V_5$	$R_5 V_5$
$R_5 V$	$R V_3$	$R V_1$
$R V_5$	$R V$	$R_5 V_3$
$R V_2$	$R_0 V$	$R_{II} V_4$
$R_5 V_4$	$R_5 V_2$	$R_5 V_2$
$R_5 V_3$	$R_0 V_3$	$R_5 V$

R_0 Without *Rhizobium*
R_5 Local isolate
R_1 Standard culture
V_1 - Green gram variety CO 3
V_2 CO 4
V_3 - CV 2
V_4 - KM 2
V_5 PB

PLOT SIZE - 18 m^2

Fig 1 Varietal variation for nodulation by *Rhizobium* in green gram

height, fresh and dry weights of shoot were taken on 45th day of plant growth. Additional observations on shoot height, fresh and dry weights of shoot and yield per plot were taken at the time of harvest.

4. Statistical Method of Analysis

The data on screening trial and selection of Rhizobium for field evaluation was analysed statistically using the analysis of variance technique suggested by Snedacor and Cochran (1967).

Results

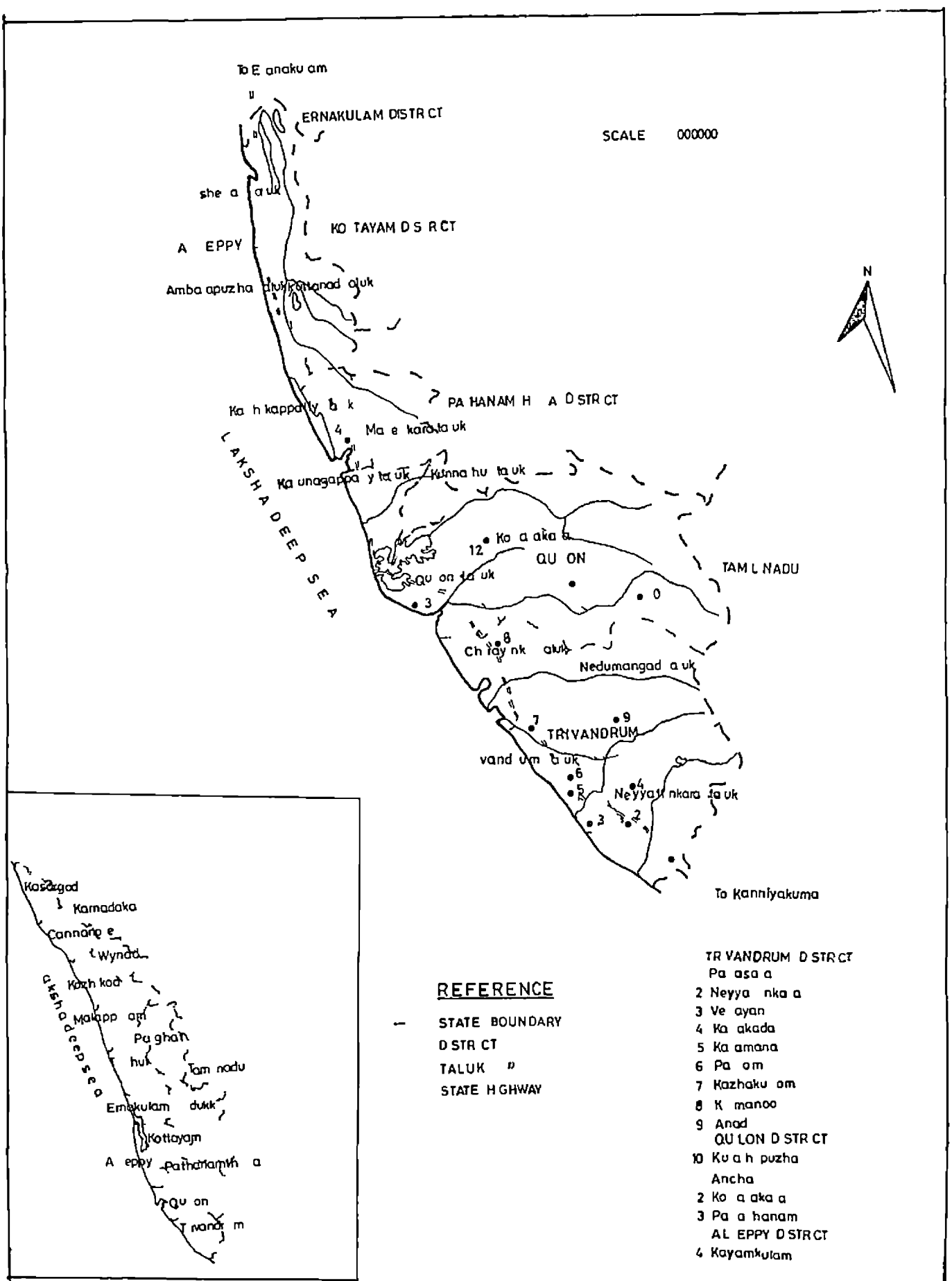
RESULTS

1. Isolation and characterisation of native Rhizobium specific for greengram.

Rhizobium capable of nodulation in greengram was isolated from 10 out of 14 different soil samples collected from Alleppey, Quilon and Trivandrum Districts (Fig. 2). All these isolates (R_1 - R_{10}) were gram-negative, Ketolactose negative, non sporulating and rod shaped (Table 1). They produced raised translucent gummy colonies on yeast extract mannitol agar medium after 72 h of incubation 28 ± 1°C. However, on glucose peptone agar the growth of these cultures were only minimum even after 7 days of incubation.

2. Preliminary screening of Rhizobium for nodulation efficiency in greengram.

The number of nodules formed due to seed inoculation with different isolates of rhizobia were significantly high in all treatments except in R_1 and R_8. This increase was maximum in R_5 treatment where 139.20 nodules were formed per plant when compared to only 8.33 in the uninoculated control (Table 2, Plate I and II). The number of nodules formed with isolate Nos. R_2, R_3, R_4, R_9 and R_{10} were also high and statistically on par with the above treatment. These were 86.66, 92.00, 96.66, 123.33 and 110.00, respectively.



F g 2 Locat on map of so l samples collected for the solat on of native *Rhizobium*

Table 1. Primary characters of different isolates of rhizobia

District	Location	Isolate No.	Gram reaction	Ketolactose	Sporulation	Growth on YEMA	Growth on GPA
Alleppey	Kayamakulam	R_1	-Ve	-	NS	F	±
Quilon	Kottarakkara	R_2	-Ve	-	NS	F	±
Quilon	Pattathanam	R_3	-Ve	-	NS	F	±
Trivandrum	Anad	R_4	-Ve	-	NS	F	±
Trivandrum	Karamana	R_5	-Ve	-	NS	F	±
Trivandrum	Kattakkada	R_6	-Ve	-	NS	F	±
Trivandrum	Kazhakkottam	R_7	-Ve	-	NS	F	±
Trivandrum	Parassala	R_8	-Ve	-	NS	F	±
Trivandrum	Pattom	R_9	-Ve	-	NS	F	±
Trivandrum	Vellayani	R_{10}	-Ve	-	NS	F	±

-Ve gram negative
F Fast rate of growth
- Ketolactose not produced
NS - Non-Spore forming
± Scanty growth

Table 2. Screening for nodulation efficiency of different isolates of Rhizobium in greengram.

Isolate number	Nodule number*	Nodule fresh weight* (mg)	Nodule dry weight* (mg)
R_1	20.66 (4.47)	36.66 (1.53)	7.00
R_2	86.66 (9.77)	115.00 (2.04)	23.66
R_3	92.00 (9.58)	70.00 (1.84)	16.66
R_4	96.66 (9.83)	98.33 (1.17)	16.66
R_5	138.30 (11.75)	455.00 (2.65)	118.33
R_6	55.00 (7.25)	70.00 (1.81)	15.00
R_7	90.00 (9.28)	86.60 (1.86)	21.00
R_8	11.66 (3.39)	17.00 (1.17)	2.33
R_9	123.33 (11.07)	243.33 (2.28)	61.66
R_{10}	110.00 (10.38)	170.00 (2.13)	44.33
R_0	8.33 (2.82)	5.66 (0.68)	1.66
C.D(0.05)	(2.17)	0.42	34.77

*Mean of 3 replications on 45th day.

Figures in parenthesis are after square root transformation for nodule number and log transformation for nodule fresh weight respectively.

Plate I Effect of inoculation with Rhizobium isolate R_5 on nodulation in greengram variety CO-3.

Plate II Effect of inoculation with Rhizobium isolate R_{10} on nodulation in greengram variety CO-3.

Plate I

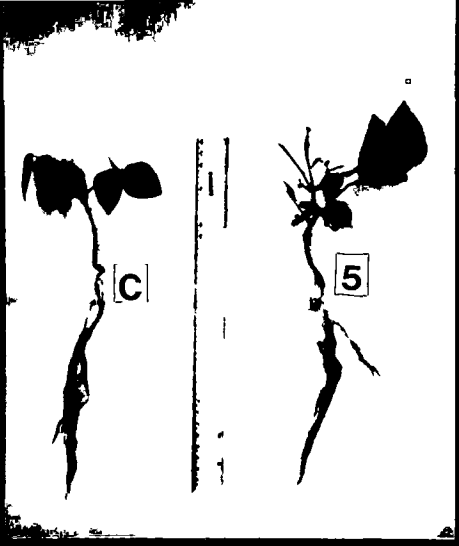


Plate II

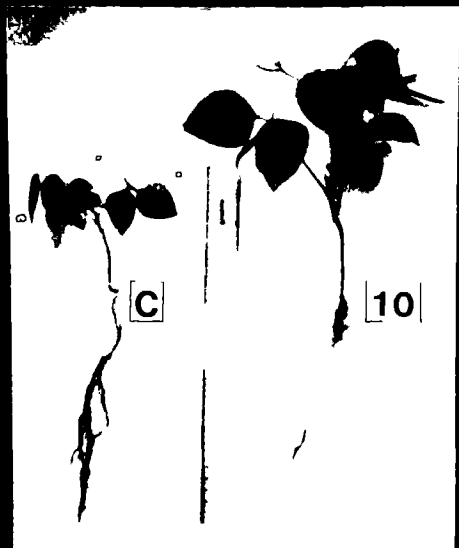


Table 3. Screening for nodulation efficiency of different isolates of Rhizobium in greengram - Effect of inoculation on other plant characters.

Isolate number	Root length* (cm)	Root fresh weight* (g)	Root dry weight* (g)	Shoot height* (cm)	Shoot fresh weight* (g)	Shoot dry weight* (g)
R_1	25.33	1.50	0.16	19.33	6.68	0.96
R_2	30.33	1.03	0.13	18.16	8.40	1.03
R_3	24.33	1.35	0.16	17.33	8.41	1.11
R_4	29.66	1.98	0.25	18.00	10.00	1.22
R_5	26.33	2.08	0.25	26.33	19.00	1.93
R_6	32.83	1.42	0.20	18.31	7.33	0.98
R_7	20.16	1.05	0.13	20.33	7.50	0.92
R_8	25.00	1.96	0.25	21.00	6.61	0.93
R_9	33.33	2.06	0.28	21.30	11.66	1.38
R_{10}	21.00	2.53	0.31	22.33	15.33	1.70
R_0	23.66	1.81	0.23	17.50	6.66	0.90
CD(0.05)	8.76	1.02	0.13	5.49	6.43	0.63

* Mean of 3 replications on 45th day.

The fresh and dry weight of nodules formed 455 and 118.33 mg respectively were maximum in the R_5 treatment (Table 2). The increase in fresh weight of nodules in the remaining treatments and dry weight in R_9 and R_{10} treatments were also significant when compared to that of control.

A significant increase in root length was obtained only in two of the above treatments, R_6 and R_9. These were 32.83 and 33.33 cm respectively (Table 3). However, there were no significant increase in fresh and dry weights of roots due to Rhizobium inoculation. The shoot height of 26.33 cm, its fresh and dry weights of 19.0 and 1.93 g respectively were maximum in the R_5 treatment (Table 3). These were significantly higher than rest of the treatments, except in R_{10}, where the fresh and dry weights of shoot recorded were statistically on par with the R_5 treatment.

2.1 Selection of an efficient local culture of Rhizobium for greengram.

The Rhizobium culture R_5, isolated from CSRC, Karamana, Trivandrum was found superior to all other local isolates of rhizobia. The number of nodules formed per plant, their fresh and dry weights, shoot height, fresh and dry weights of shoot were all maximum due to seed inoculation with this culture

Besides, the required minimum increase in dry weight of plants from Rhizobium inoculation, 50 per cent increase in dry mass over uninoculated control (as per the Indian Standard Institution specifications for Rhizobium inoculants- IS 8268-1976), was obtained only with this isolate of Rhizobium (Fig. 3). Therefore, this culture was selected for further studies along with a standard culture of Rhizobium obtained from Tamil Nadu Agricultural University, Coimbatore.

3. Varietal variation for nodulation by Rhizobium in greengram.

The multilocational field trial for studying the varietal variation for nodulation by Rhizobium was conducted at two different locations in Trivandrum District at Karamana and Pattom, using five varieties of greengram, CO-3, CO-4, CV-2, KM-2 and PB (Pusa baisakhi) and two cultures of rhizobia R_5 (local) and R_{11} (standard). The different biometric observations were taken on 45th day of plant growth and at the time of harvest.

3.A. Observations on 45th day of plant growth

1. Nodule number

The effect of Rhizobium inoculation in increasing the nodule number in different varieties of greengram was significant at both locations when compared to that of control

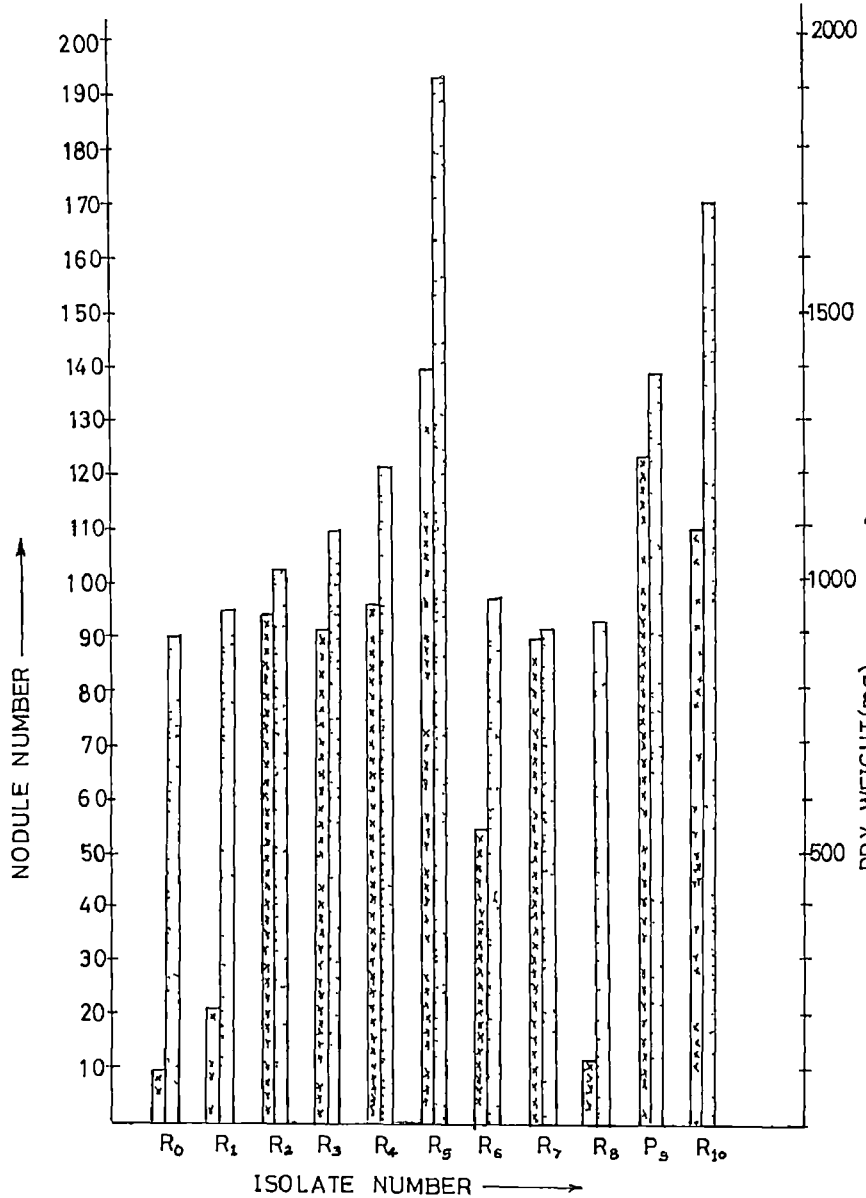


Fig 3 Relation between nodule number and dry weight of shoot in green gram

(Table 4). The mean number of nodules formed per plant, 220.59 at Karamana and 202.22 at Pattom were maximum due to seed inoculation with the standard culture of Rhizobium. The increase in number of nodules formed at both these locations with the local isolate of Rhizobium was also high and statistically on par with the above treatment.

Among different varieties of greengram, the maximum number of nodules were formed in CO-3 both at Karamana and Pattom. These were 199.33 and 194.02 respectively (Table 4). However, there was no significant differences between varieties in their mean nodule number. A significant interaction between Rhizobium inoculation and greengram varieties was also absent.

2. Fresh and dry weights of nodules

The fresh and dry weights of nodules were also significantly high due to Rhizobium inoculation at both locations when compared to that of control (Table 5 and 6). The mean fresh and dry weights of 1.42 g and 140.14 mg respectively at Karamana and 1.44 g and 146.0 mg respectively at Pattom were maximum in seed treatment with the local isolate of Rhizobium. The increase in fresh and dry weights of nodules due to seed inoculation with the standard culture of Rhizobium were also high and statistically on par with the above treat-

Table 4. Effect of Rhizobium inoculation on nodule number of different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Nodule number *					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	126.66 (11.25)	134.00 (11.57)	146.00 (12.11)	130.33 (11.41)	115.33 (10.73)	130.44 (11.42)
R_5	200.00 (14.14)	206.66 (14.37)	199.00 (14.10)	198.00 (14.07)	180.66 (13.41)	196.86 (14.01)
R_{11}	271.33 (16.47)	198.66 (14.09)	181.33 (13.46)	219.33 (14.80)	232.33 (15.24)	220.59 (14.81)
Mean	199.33 (13.94)	179.77 (13.34)	175.44 (13.22)	182.55 (13.42)	176.10 (13.12)	

C.D(0.05) for comparison between Rhizobium treatments = 1.08

Location 2. Pattom

Rhizobium treatment	Nodule number*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	136.66 (11.69)	121.66 (11.02)	140.00 (11.83)	125.00 (11.18)	125.00 (11.18)	129.66 (11.38)
R_5	219.60 (14.81)	171.33 (13.08)	195.33 (13.97)	199.33 (14.11)	192.00 (13.85)	195.51 (13.96)
R_{11}	225.80 (15.20)	188.00 (13.71)	197.33 (14.04)	209.66 (14.47)	190.33 (13.79)	202.22 (14.20)
Mean	194.02 (13.84)	160.33 (12.60)	177.55 (13.28)	177.99 (13.25)	169.11 (12.94)	

CD(0.05) for comparison between Rhizobium treatments = 0.86

* Mean of 3 replications on 45th day.
Figures in parenthesis are after square root transformation.

Table 5. Effect of Rhizobium inoculation on nodule fresh weight (g) of different varieties of greengram under field conditions.

Location. 1. Karamana.

Rhizobium treatment	Nodule fresh weight*					Mean
	CO-3	CO-4	CV-2	KM- 2	PB	
R_0	0.79	0.72	0.82	0.93	0.62	0.77
R_5	1.59	1.54	1.33	1.34	1.28	1.42
R_{11}	1.73	1.32	1.48	1.28	1.24	1.41
Mean	1.37	1.19	1.21	1.18	1.04	

CD(0.05) for comparison between Rhizobium treatments = 0.31
CD(0.05) for comparison between greengram varieties = 0.32

Location.2. Pattom

Rhizobium treatment	Nodule fresh weight*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	0.77	0.86	0.79	0.63	0.62	0.73
R_5	1.61	1.61	1.19	1.39	1.42	1.44
R_{11}	1.68	1.51	1.48	1.04	0.99	1.34
Mean	1.35	1.32	1.15	1.02	1.01	

CD(0.05) for comparison between Rhizobium treatments = 0.24
CD(0.05) for comparison between greengram varieties = 0.24
CD(0.05) for comparing Rhizobium x greengram variety interaction. = 0.53

* Mean of 3 replications on 45th day.

Table 6. Effect of Rhizobium inoculation on nodule dry weight(mg, of different varieties of greengram under field conditions.

Location.1. Karamana

Rhizobium treatment	Nodule dry weight*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	74.0	70.0	86.0	84.9	62.0	75.4
R_5	162.0	144.0	134.0	134.0	128.0	140.4
R_{11}	174.0	128.0	150.0	126.0	122.0	140.0
Mean	136.7	114.0	123.3	114.9	104.0	

C.D(0.05) for comparison between Rhizobium isolates = 30.4
CD(0.05) for comparison between greengram varieties = 32.4

Location.2. Pattom

Rhizobium treatment	Nodule dry weight*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	76.0	77.0	78.0	61.4	60.4	70.6
R_5	163.4	163.8	121.4	140.4	141.2	146.0
R_{11}	167.8	149.4	147.2	101.4	100.2	133.2
Mean	135.7	130.1	115.5	101.1	100.6	

C.D(0.05) for comparison between Rhizobium treatment = 25.4
C.D(0.05) for comparison between greengram varieties = 32.7
C.D(0.05) for comparing Rhizobium x grsengram variety interaction = 56.7

* Mean of 3 replications on 45th day.

ment. Significant differences between varieties were also observed in their mean fresh and dry weights of nodules at both locations. These were maximum in greengram variety CO-3.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed only at Pattom. This was most significant in the interaction between the standard culture of Rhizobium and greengram variety CO-3 (Table 5 and 6). The interaction between this culture and greengram varieties such as CO-4 and CV-2 and that of the local culture of Rhizobium with all varieties of greengram at this location were also significant and statistically on par with the R_{11} x CO-3 interaction.

3. Shoot height

The effect of Rhizobium inoculation in increasing the shoot height of different varieties of greengram was significant at both locations when compared to that of control (Table 7). The mean shoot height of 77.6 cm at Karamana and 87.2 cm at Pattom were maximum in seed treatment with the standard culture of Rhizobium. The increase in shoot height with the local isolate of Rhizobium was also significant when compared to that of control.

Table 7. Effect of Rhizobium inoculation on shoot height (cm) of different varieties of greengram under field conditions.

Location 1. Karamana.

Rhizobium treatment	Shoot height*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	79.0	69.0	75.0	62.0	63.0	69.6
R_5	78.0	60.0	65.0	80.0	78.0	72.2
R_{11}	80.0	85.0	80.0	74.0	69.0	77.6
Mean	79.0	71.3	73.3	72.0	70.0	

C.D(0.05) for comparison between Rhizobium isolates = 2.6
C.D.(0.05) for comparing Rhizobium x variety interaction = 5.7

Location 2. Pattom

Rhizobium treatment	Shoot height*					Mean
	CO-3	CO-3	CV-2	KM-2	PB	
R_0	85.0	75.0	81.7	69.0	80.0	78.1
R_5	79.0	70.0	75.0	95.0	92.0	82.2
R_{11}	105.0	83.0	93.0	80.0	75.0	87.2
Mean	89.7	76.0	83.2	81.3	82.3	

C.D(0.05) for comparison between Rhizobium treatment = 2.4
C.D.(0.05) for comparison between greengram varieties = 3.1
C.D.(0.05) for comparing Rhizobium x variety interaction = 5.6
* Mean of 3 replications on 45th day.

Significant differences between varieties in their mean shoot height were observed only at Pattom. The shoot height of 89.7 cm was maximum in greengram variety CO-3.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed at both locations. This was most significant in the interaction between the standard culture of Rhizobium and greengram variety CO-4 at Karamana and the variety CO-3 at Pattom (Table 7). The interaction of this culture with varieties such as CO-3 and CV-2 at Karamana and that of local isolate with variety KM-2 were also significant and statistically on par with the R_{11} x CO-4 interaction.

4. Fresh weight of shoot

The fresh weight of shoot was significantly high due to Rhizobium inoculation at both locations when compared to that of control (Table 8). The mean fresh weight of 150.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana and with the local isolate of Rhizobium at Pattom. The increase in fresh weight of shoot due to seed inoculations with the other Rhizobium culture at these locations was also significant and statistically on par with the treatments where maximum fresh weight of shoot was

Table 8. Effect of Rhizobium inoculation on fresh weight of shoot(g) in different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Fresh weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	114.0	122.0	84.0	132.0	128.0	116.0
R_5	162.0	150.0	158.0	138.0	107.0	143.0
R_{11}	160.0	167.0	151.0	137.0	136.0	130.2
Mean	145.3	146.3	131.0	135.7	123.7	

C.D.(0.05) for comparison between Rhizobium isolates = 25.5
C.D.(0.05) for comparing Rhizobium x variety interaction = 57.1

Location 2. Pattom

Rhizobium treatment	Fresh weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	108.0	116.0	102.0	142.0	136.0	120.8
R_5	160.0	162.0	165.0	142.0	122.0	150.2
R_{11}	170.0	154.0	144.0	126.0	142.0	147.2
Mean	146.0	144.0	137.0	136.6	133.3	

C.D(0.05) for comparison between Rhizobium isolates = 18.0
C.D(0.05) for comparing Rhizobium x variety interaction = 40.3
*Mean of 3 replications on 45th day.

obtained. However, there were no significant differences between varieties in their mean fresh weight of shoot at both locations.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed at both locations. This was most significant in the interaction between the standard culture of Rhizobium and the greengram variety CO-4 at Karamana and the variety of CO-3 at Pattom. The interaction of this culture with all the remaining varieties at theselocations except the variety KM-2 at Pattom and that of the local isolate with different varieties except Pusa baisakhi at Karamana and Pattom were also significant and statistically on par with the above interaction .

5. Dry weight of shoot

The dry weight of shoot was significantly high due to Rhizobium inoculation at both locations when compared to that of control (Table 9, Fig. 4 and 5). The mean dry weight o 25.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana.

The increase in dry weight of shoot due to seed inoculation with the local culture of Rhizobium at both locations were also significant and statistically on par with the above

Table 9. Effect of Rhizobium inoculation on dry weight of shoot(g) of different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Dry weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	18.0	20.8	14.0	22.0	23.0	19.6
R_5	27.0	24.6	24.0	24.0	18.0	23.5
R_{11}	26.6	28.8	25.0	23.2	22.6	25.2
Mean	23.9	24.7	21.0	23.1	21.2	

C.D(0.05) for comparison between Rhizobium isolates = 5.2

C.D(0.05) for comparing Rhizobium x variety interaction = 11.9

Location 2. Pattom

Rhizobium treatment	Dry weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	18.0	18.6	16.4	24.0	22.0	19.8
R_5	27.0	24.0	26.0	23.8	21.0	24.4
R_{11}	28.6	26.0	24.6	21.6	24.0	24.9
Mean	24.5	22.9	23.1	23.1	22.3	

C.D(0.05) for comparison between Rhizobium isolates = 3.3

C.D(0.05) for comparing Rhizobium x variety interaction = 7.4

*Mean of 3 replications on 45th day.

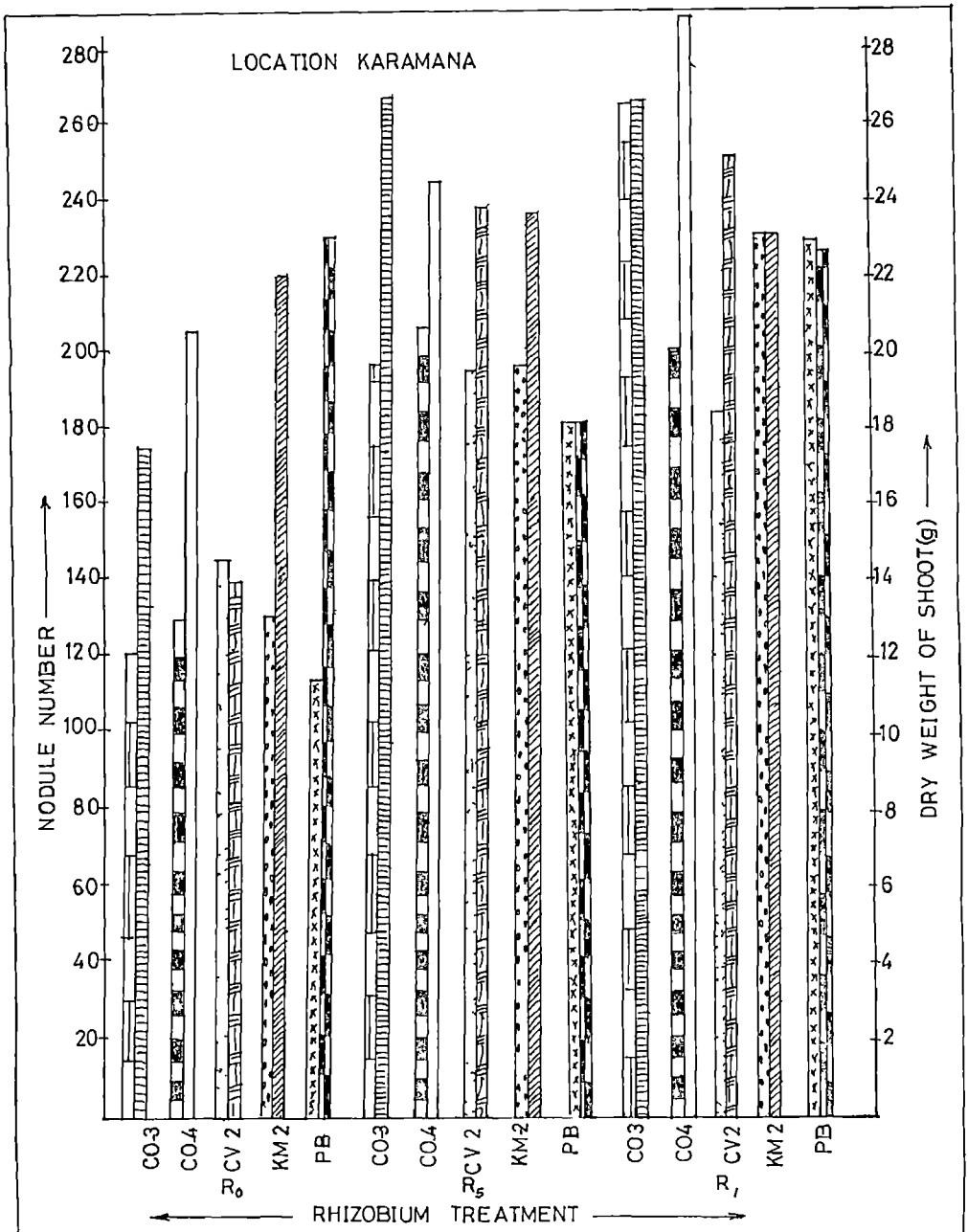


Fig 4 Relation between nodule number and dry weight of shoot in green gram under field condition

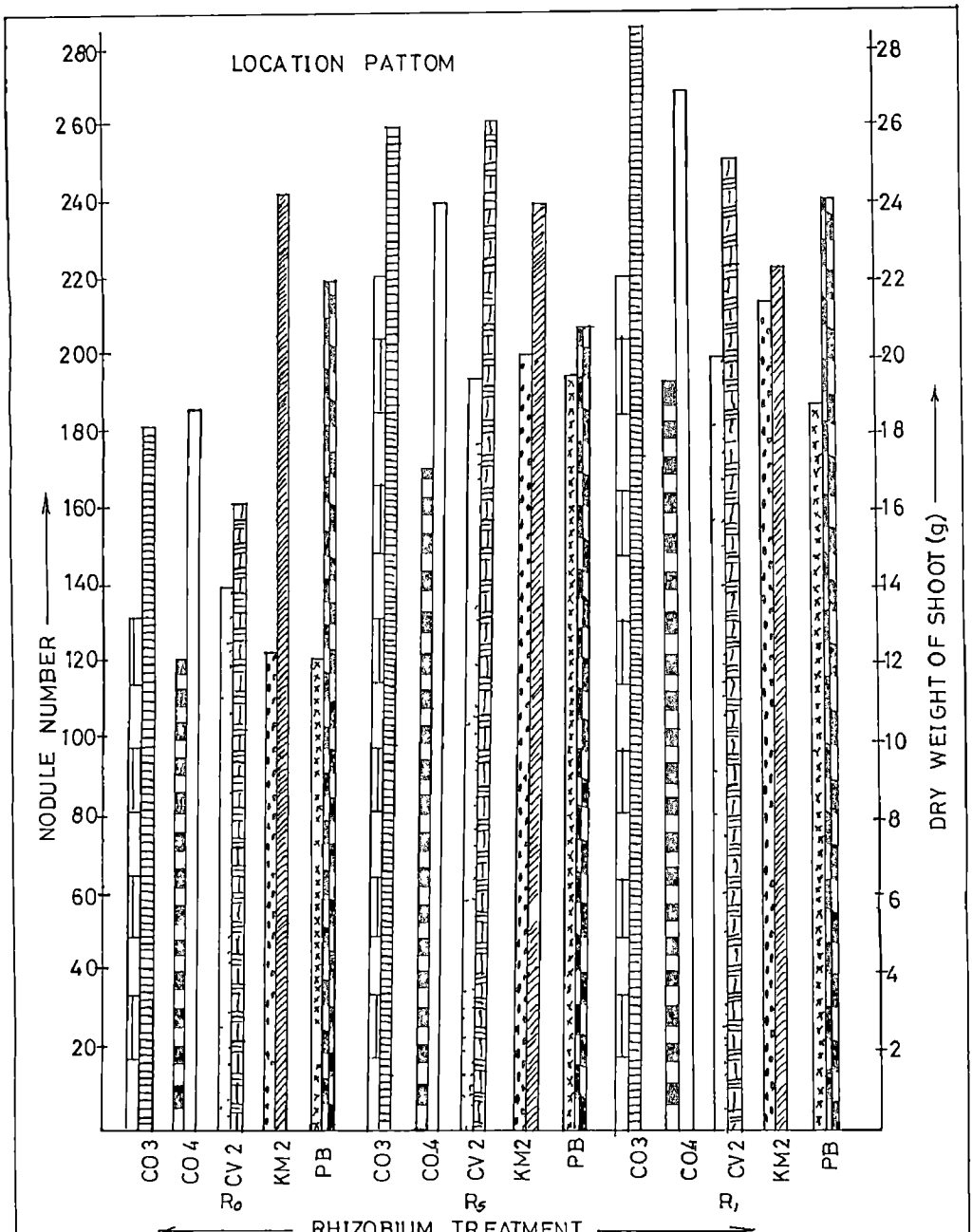


Fig 5 Relation between nodule number and dry weight of shoot in green gram under field condition

between varieties in their mean dry weight of shoot at both locations.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed at both locations. This was most significant in the interaction between the standard culture of Rhizobium and the greengram variety CO-4 at Karamana and the variety CO-3 at Pattom. The interaction of this culture with all the remaining varieties at both locations and that of the local isolate with different varieties except Pusa baisakhi at Pattom were also significant and statistically on par with the above interactions.

3.B. Observations at the time of harvest

1. Shoot height

There were no significant difference between treatments in the mean shoot height of plants with and without Rhizobium inoculation (Table 10). The plant height was maximum in the variety CO-4 at both locations due to seed inoculation with the standard culture of Rhizobium.

2. Fresh weight of shoot

The effect of Rhizobium inoculation in increasing the fresh weight of shoot was significant only at Pattom (Table 11

Table 10. Effect of Rhizobium inoculation on shoot height (cm) of different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Shoot height *					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	93.0	95.0	86.0	85.0	83.0	88.4
R_5	93.0	102.0	96.0	92.0	95.0	95.6
R_{11}	98.0	106.0	96.0	85.0	90.0	95.0
Mean	94.7	101.0	92.7	87.3	89.3	

Location 2. Pattom

Rhizobium treatment	Shoot height*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	92.0	91.0	89.0	78.0	90.0	88.0
R_5	89.0	93.0	92.0	87.0	90.0	90.2
R_{11}	92.0	107.0	89.0	91.3	95.0	94.9
Mean	91.0	97.0	90.0	85.4	91.7	

* Mean of 3 replications at harvest.

Table 11. Effect of Rhizobium inoculation on fresh weight of shoot (g) of different varieties of greengram under field conditions

Location 1. Karamana

Rhizobium treatment	Fresh weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	162.0	189.0	130.0	194.6	154.6	166.0
R_5	263.4	187.2	236.0	164.6	156.0	201.4
R_{11}	250.0	221.0	224.6	194.0	187.0	215.3
Mean	225.1	199.0	196.9	184.4	165.9	

C.D(C.05) for comparing Rhizobium x variety interaction = 114.2

Location 2. Pattom

Rhizobium treatment	Fresh weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	167.0	166.0	146.0	201.0	156.6	167.3
R_5	261.2	179.0	240.0	161.8	155.0	199.4
R_{11}	254.0	223.8	238.0	204.0	179.4	219.9
Mean	227.4	189.3	208.0	188.9	163.6	

C.D(0.05) for comparison between Rhizobium treatments = 37.7
C.D(0.05) for comparison between greengram varieties = 29.2
C.D.(0.05) for comparing Rhizobium x variety interaction= 65.4
* Mean of 3 replications at harvest.

The mean fresh weight of 219.9 g was maximum in seed treatment with the standard culture of Rhizobium. The increase in the fresh weight of shoot due to seed inoculation with the local isolate of Rhizobium at this location was also significant and statistically on par with the above treatment.

Significant differences between varieties in their mean fresh weight of shoot were observed only at Pattom. The mean fresh weight of 227.4 g was maximum in the variety CO-3. The increase in fresh weight of shoot in variety CV-2 was also statistically on par with the CO-3 variety.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed at both locations. This was most significant in the interaction between the local isolate of Rhizobium and the variety CO-3 both at Karamana and Pattom. The interaction of this culture with all the remaining varieties at Karamana and with the variety CV-2 at Pattom was also significant and statistically on par with the above interaction. A similar type of interaction was observed between the standard culture of Rhizobium and all varieties except with Pusa baisakhi at Pattom.

3. Dry weight of shoot

The effect of Rhizobium inoculation in increasing the dry weight of shoot was significant only at Pattom (Table 12). The mean dry weight of 41.8 g was maximum in seed treatment with the standard culture of Rhizobium. The increase in dry weight of shoot due to seed inoculation with the local isolate of Rhizobium at this location was also significant and statistically on par with the above treatment. Significant differences between varieties in their mean dry weight of shoot was also observed only at Pattom. The mean dry weight of 45.5 g was maximum in the variety CO-3. The increase in dry weight of shoot in variety CV-2 was also statistically on par with the CO-3 variety.

A significant interaction between Rhizobium inoculation and different varieties of greengram was observed at both locations. This was most significant in the interaction between the local isolate of Rhizobium and the variety CO-3 both at Karamana and Pattom. The interaction of this culture with all the remaining varieties at Karamana and with the variety CV-2 at Pattom were also significant and statistically on par with the above interaction. A similar type of interaction was observed between the standard culture of Rhizobium and all varieties except with varieties such as KM-2 and Pusa baisakhi at Pattom.

Table 12. Effect of *Rhizobium* inoculation on dry weight of shoot(g) of different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Dry weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	32.4	38.6	26.4	37.0	30.6	33.0
R_5	52.8	36.0	46.8	33.4	31.2	44.0
R_{11}	50.4	44.0	45.6	38.8	36.0	42.9
Mean	45.2	39.5	39.5	36.4	32.6	

C.D(0.05) for comparing *Rhizobium* x variety interaction = 33.2

Location 2. Pattom

Rhizobium treatment	Dry weight of shoot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	33.4	32.4	30.4	40.6	30.6	33.5
R_5	52.5	36.4	48.0	32.6	30.6	40.0
R_{11}	50.6	44.6	42.4	34.9	36.4	41.8
Mean	45.5	37.8	40.2	36.0	32.5	

C.D(0.05) for comparison between *Rhizobium* treatments = 8.1
C.D(0.05) for comparison between greengram varieties = 6.2
C.D(0.05) for comparing *Rhizobium* x variety interaction =14.0
* Mean of 3 replication at harvest.

4. Yield

The effect of Rhizobium inoculation in increasing the mean yield (g/plot) of different varieties of greengram was significant at both locations when compared to that of control (Table 13, Fig. 6 and 7). The mean yield of 1005.0 g at Karamana and 966.6 g at Pattom were maximum in seed treatment with the standard culture of Rhizobium. However, the increase in yield in different varieties of greengram due to seed inoculation with the local culture of Rhizobium was also significant when compared to that of control, and at one location, Pattom this increase was statistically on par with the standard cultures of Rhizobium.

Significant differences between varieties in their mean yield were observed at both locations. The mean yield of 1055.3 g at Karamana and 1015.6 g at Pattom were maximum in the variety CO-3 followed by varieties such as CO-4, CV-2, KM-2 and Pusa baisakhi. A significant interaction between Rhizobium inoculation and different varieties of greengram was however absent.

Table 13. Effect of Rhizobium inoculation on yield (g/plot 18 m^2) of different varieties of greengram under field conditions.

Location 1. Karamana

Rhizobium treatment	Yield per plot *					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	916.0	850.0	850.0	681.0	765.0	812.4
R_5	1050.0	1050.0	984.0	713.0	983.0	956.4
R_{11}	1200.0	1100.0	1002.0	903.0	820.0	1005.0
Mean	1055.3	1000.0	945.3	766.3	856.3	

CD(0.05) for comparison between Rhizobium treatments = 38.4

CD(0.05) for comparison between greengram varieties = 49.6

Location 2. Patton.

Rhizobium treatment	Yield per plot*					Mean
	CO-3	CO-4	CV-2	KM-2	PB	
R_0	907.0	834.0	834.0	670.0	736.0	786.2
R_5	970.0	970.0	960.0	949.0	890.0	949.0
R_{11}	1170.0	980.0	965.0	913.0	805.0	966.6
Mean	1015.6	930.0	919.6	827.3	810.3	

CD(0.05) for comparison between Rhizobium treatments = 36.4

CD(0.05) for comparison between green varieties = 47.0

*Mean of 3 replications.

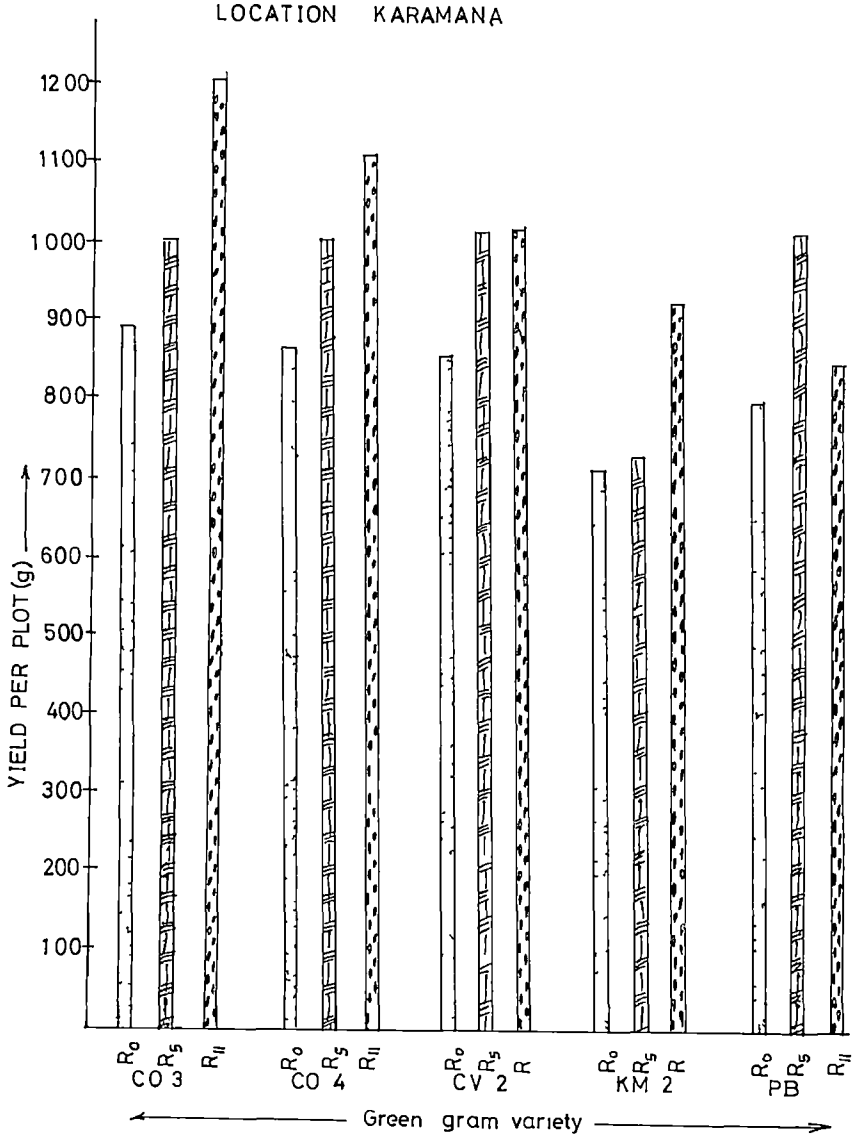


Fig 6 Comparative yield in different varieties of green gram with and without *Rhizobium* inoculation under field condition

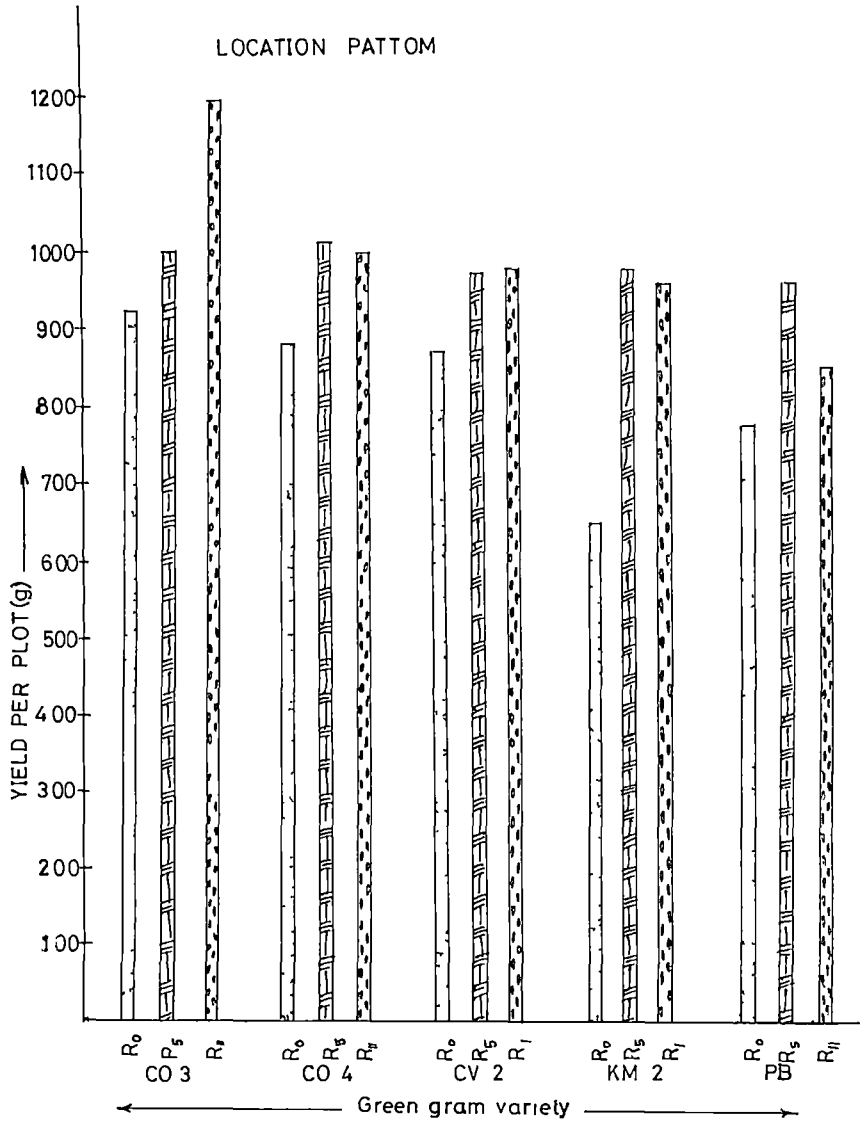


Fig 7 Comparative yield in different varieties of green gram with and without *Rhizobium* inoculation under field conditions

Discussion

DISCUSSION

The modern concept of biological nitrogen fixation is nearly hundred years old. Today we know that this unique phenomenon plays the most important role in the survival of plants and their dependant animal kingdom. In fact, one recent estimate of the quantum of nitrogen fixed by microorganism is to the extent of 150 to 300 million tons per annum on a global scale (Knowles, 1982) in comparison to this only 50 to 60 million tonsof nitrogenous fertilizer alone is produced through the industrial process. In nature there are three major groups of nitrogen fixing organisms, the symbiotic, associative and the free living diazotrophs. Among these organisms the most important are the bacteria belonging to the genera, Rhizobium and Bradyrhizobium which enter into a symbiotic association with appropriate leguminous and very rarely nonleguminous plants like parasponia andersonii (Trinick, 1973) and reduce the atmospheric nitrogen.

In an actively growing leguminous plant with an efficient nitrogen fixing system, at the time of its peak vegetative growth, nearly 32 per cent of the photosynthetate is diverted to root nodules (Atkins et al., 1978) (Pate and Herridge, 1978) (Layzell et al., 1979) and out of which, 10 per cent is returned to host plant in the form of fixed nitrogenous compounds either as amides or ureids (Pate et al., 1979) Once it became established that Rhizobium can meet a major

part of the nitrogen requirement of a pulse crop, the practice of seed treatment with appropriate Rhizobium culture in order to induce better nodulation became very popular throughout the world. Later within this system there gradually evolved the concept of using crop specific Rhizobium inoculants for better nodulation and nitrogen fixation (Muthuswamy, 1970; Burton, 1975, Raju, 1977, Subba Rao, 1980, Nair and Siva Prasad, 1982; Tu and Mc Donnell, 1984). This stress on host specific single or multistrain inoculants became important probably with the discovery of the mechanism of host recognition by Rhizobium involving a lectin-exopoly saccharide binding hypothesis (Bohlool et al. 1974).

In Kerala, host specific Rhizobium culture for greengram is yet to be developed. It is therefore with this objective, the present investigation was carried out. Soil samples from 14 different locations of Alleppey, Quilon and Trivandrum Districts were collected for the isolation of native Rhizobium suitable for greengram under pot culture conditions using the greengram variety CO-3. The plants were raised without any pretreatment or application of nitrogenous fertilizer. They were grown for 45 days and harvested for the isolation of native rhizobia. Rhizobium could be isolated from 10 out of 14 different locations survyed for this purpose (Table 1).

The inability to isolate Rhizobium capable of nodulating greengram from the remaining four locations, Anchal and Kulathupuzha of Quilon District, Kilimanoor and Neyyattinkara of Trivandrum District is not clear. It may be due to the absence of native rhizobia capable of nodulating the greengram variety CO-3 used for this preliminary isolation. Such lack of native Rhizobium suitable for a particular pulse crop has been reported earlier also in the case of soybean (Longari and Herrera, 1972 and Okon, 1979).

In the second part of this investigation, the above ten isolates of rhizobia (R_1-R_{10}) were screened for their nodulation efficiency under aseptic pot culture conditions. Different observations on nodule number, fresh and dry weights of nodules, root length, fresh and dry weights of roots, shoot height and fresh and dry weights of shoot were taken on 45th day. They were analysed statistically for selecting the most efficient native isolate of Rhizobium suitable for greengram. It was observed that out of the 10 different isolates of rhizobia screened for their nodulation efficiency, isolate No. R_5 from C.S.R.C. Karamana was superior to all other isolates both in terms of inducing better nodulation and plant growth. Thus the number of nodules formed by this isolate, 138.0 were maximum in comparison

to remaining treatments (Table 2). The fresh and dry weights of nodules were also significantly high due to seed inoculation with this culture of Rhizobium. This type of variation in the ability of different isolates of rhizobia in forming root nodules in a particular host variety has been observed earlier also by Chahal and Joshi (1978) in bengalgram. Ramachandran (1979) in cowpea, Balasubramanian et al. (1980) Rangarajan and Prabhakaran (1980) in greengram, Rajendran Pillai and Nair (1981) in blackgram and greengram.

The effect of efficient nodulation by the isolate R_5 was a significant increase in the fresh and dry weight of shoot (Table 3). In fact, an increase in dry weight of shoot over 50 per cent in comparison to the Rhizobium uninoculated control treatment was obtained only in seed treatment with this culture of Rhizobium (Fig.3). This also satisfied the mandatory requirement of ISI specification for an efficient Rhizobium inoculant in that Rhizobium inoculated plants should have a minimum 50 per cent increase in dry weight in relation to the uninoculated and unfertilized control plants (Indian Standard Institution specifications for Rhizobium inoculants (IS-8268-1976). Therefore the local isolate of Rhizobium, R_5 was selected for further studies on the 'Varietal variation for nodulation by Rhizobium in greengram under field conditions'. It may also be pointed out that the relative performance of another isolate R_{10}

from College of Agriculture, Vellayani was also good and statistically on par with the R_5 culture in terms of increase in nodule number, fresh and dry weight of shoot. However, this isolate was not used for subsequent studies as it did not satisfy fully the ISI standards for an efficient Rhizobium inoculant.

Once an efficient local isolate of Rhizobium for greengram was identified, it was used for a field evaluation trial along with a known standard culture obtained from Tamil Nadu Agricultural University, Coimbatore. Usually such experiments are laid out in one or more locations with a single host variety. In the present investigation the efficiency of the local isolate was tested in a multilocational field trial at C.S.R.C. Karamana of Kerala Agricultural University and in a farmer's field at Pattom, Trivandrum District. A second factor to find out the existence of any varietal variation for nodulation by these cultures was also introduced during this investigation. The concept of host varietal variation for nodulation by Rhizobium in legumes gained importance with the release of many high yielding or disease resistant varieties to increase the over all production of pulses in India. One thing that is usually observed in such group of new varieties available to farmers for cultivation, is the presence of a variety with varying degree of nodulation ability. This may

be due to the lack of certain factors contributed by the host itself favouring the enrichment of suitable Rhizobium in the plant rhizosphere, its subsequent root infection, nodulation and nitrogen fixation processes (Erdman, 1947, Vaishya and Saneria, 1972; Dadarwal, 1976; Bapat et al., 1977; Girija, 1982). Therefore, five different varieties of greengram CO-3, CO-4, CV-2, KM-2 and Pusa bisakhi were used for the present investigation.

The first set of observations were taken on 45th day when the vegetative growth of the plant was maximum and nitrogen fixation by Rhizobium was at its peak level (Sing, 1977). The second set of observations on height of shoot, fresh and dry weights of shoot and yield per plot were taken at the time of harvest.

In terms of nodulation, both the standard (R_{11}) and local isolate of Rhizobium (R_5) were statistically on par with one another in significantly enhancing the number of nodules formed in all varieties of greengram at both locations when compared to that of control (Table 4, Fig.4). This type of increase in nodulation in a pulse crop due to inoculation with an efficient Rhizobium culture is already reported in the case of greengram by Rajagopalan et al. (1965). The above results also indicated that both the local as well as

the standard culture of Rhizobium had a better competitive ability with the native rhizobia already present in the soil in forming root nodules in all varieties of greengram irrespective of their location of cultivation. Very often, one of the reasons for the failure of an introduced Rhizobium culture in a particular location is its poor competitive ability with the native flora, less efficient or inefficient, already present in the soil. In fact according to Alexander (1961), the extend of efficient Rhizobium normally present in any given soil sample is only 25 per cent. The rest of the population is made up of either with inefficient or less efficient rhizobia. This observation was substantiated in the present investigation also, because the number of nodules formed in the uninoculated control treatment exposed to native rhizobia was significantly low when compared to that of Rhizobium inoculated treatments (Table 4, Fig.4). A similar result was reported by Duqne et al. (1985) in Phaseolus beans. However, no particular host varietal specificity for nodulation was seen for either of the Rhizobium inoculants. This was due to the fact that there were no significant differences between varieties in their mean nodule number. This could also be a reason why a statistically significant interaction between Rhizobium inoculation and greengram varieties in terms of increase in nodule number was not observed during this investigation (Table 4).

The effects of better nodulation and nitrogen fixation by Rhizobium in a pulse crop are usually assessed in terms of increase in fresh and dry weights of shoot at maximum vegetative growth and also by estimating the final grain yield at the time of harvest (Rangarajan and Prabhakaran 1980, Raju and Varma 1984). Hence, these parameters were used along with the date on nodulation to evaluate the relative performance of the local and standard culture of Rhizobium and also to test the existence of any varietal variation for nodulation by these cultures in different varieties of greengram.

The analysis of the data on the increase in the fresh and dry weights of shoot showed that both the standard as well as the local isolate of Rhizobium were statistically on par with one another in significantly increasing the fresh and dry weights of shoot in all varieties of greengram at both locations when compared to that of control (Tables 8 and 9). This showed that, both the cultures of rhizobia were efficient in nitrogen fixation and this effect was translated into an increase in fresh and dry weights of shoot in all varieties of greengram irrespective of the locations of their cultivation. Similar results were reported by Rajagopalan (1965) in greengram and Ahmad (1981) in cowpea. As in the case of nodulation, significant

differences between varieties were not observed in the fresh and dry weights of shoot eventhough the interactions of the standard culture with variety CO-4 at Karamana (Tables 8 and 9) and CO-3 at Pattom were very significant. The interaction of the local isolate of Rhizobium at both these locations with different varieties except Pusa bisakhi at Pattom, were also significant and statistically on par with that of the standard culture.

The lack of significant differences between varieties in the fresh and dry weights of shoot may be due to the fact that all these varieties were more or less equally responsive to Rhizobium inoculation. A real significance for varietal variation for nodulation by Rhizobium in any puls crop probably arises only during the final stages of screening of a variety prior to its release for mass cultivation. The desirable traits in such a variety may be its better nodulation capacity, higher yield and increased resistance to disease and pest incidence. However, most often one of these aspects relating to nodulation is neglected resulting in the release of varieties that are poorly nodulating and thereby requiring a higher level of nitrogenous fertilization for optimum yield.

One may also point out here that the observed increase in dry weight of shoot in the above field experiment was not upto the extend of ISI specifications. This is mainly due to the reasons that ISI specifications are formulated under controlled and aseptic pot culture conditions. Since in a field experiment, the effects of different soil and environmental factors affecting plant growth will be varying with locations, the net effect of Rhizobium symbiosis as measured in terms of increase in dry weight of plants will not be always uniform. It is under these circumstances in a field evaluation trial, the estimate of the final grain yield of a pulse crop under Rhizobium inoculation becomes important.

The increase in yield due to seed treatment with the standard culture of Rhizobium was more in different varieties of greengram (Table 13). The effect of inoculation with the local isolate of Rhizobium R_5 was equally good in that, the increase in yield obtained was significantly high when compared to that of the uninoculated control and at one location, Pattom, it was even statistically on par with the standard culture. Therefore, it may be pointed out that the local culture of Rhizobium isolated from CSRC, Karamana was comparable in its performance with the standard

culture of Rhizobium obtained from Tamil Nadu Agricultural University, Coimbatore. The fact that the inoculation with an efficient Rhizobium culture will increase the yield of pulses has been reported earlier also by many workers in diverse pulse crops such as greengram (Chahal et al. (1978), groundnut (Sahu and Behera, 1972), Soybean (Subba Rao and Balasundaram, 1971, Rao and Sharma, 1980), bengalgram (Madhane and Patil, 1974; Tripathi, 1975 and Rai, 1977).

Significant differences between varieties were also observed in their average grain yield (Table 13). However, this may not be due to a direct consequence of Rhizobium inoculation as it can also depend on the inherent yield potential of a particular greengram variety. If one accepts this hypothesis, then it will be possible to conclude that no particular host varietal specificity was shown by either of the Rhizobium cultures used during this investigation in enhancing the final grain yield of different varieties of greengram. This was further supported by the observation that the yield increase in different varieties of greengram followed a similar pattern both at Karamana and Pattom. The yield was maximum in greengram variety CO-3 followed by CO-4, CV-2, KM-2, Pusa bisakhi. Such variations in yield in

different varieties/ genotypes of greengram under Rhizobium inoculation have been reported earlier also by Prasad and Ram (1982), Rajput and Varma (1982) and Vlashya and Gajendra Gadkar (1982).

What is the reason for the better performance of the standard culture over the local isolate of Rhizobium? If a Rhizobium culture is isolated from a region, where a particular pulse crop is cultivated regularly and extensively, there is always a possibility of natural selection of better strain of Rhizobium having higher nodulation and nitrogen fixing efficiency. Therefore, the standard culture of Rhizobium obtained from Tamil Nadu Agricultural University, Coimbatore, where greengram is usually cultivated on an extensive scale was found superior to the local isolate, eventhough this isolate was comparable to the standard culture in many of the parameters tested for nodulation efficiency under field conditions. This means that if greengram cultivation is taken up on a large scale in Kerala, thus there is the possibility for isolating a better strain of native Rhizobium specific for this pulse crop from within the State itself. Finally we should also take into consideration, the relative economic benefits of seed treatment with Rhizobium in order to popularise this practice among farmers of Kerala. It was found during this investigation that on an averag

one can get an yield increase of about 21.3 per cent in greengram due to Rhizobium inoculation (pooled data for both the locations) under field conditions when compared to that of the uninoculated control treatment.

Summary

SUMMARY

The present investigation on varietal variation for nodulation by Rhizobium in greengram was conducted at College of Agriculture, Vellayani, Trivandrum. The field trial for host varietal specificity was done at C.S.R.C. Karamana of Kerala Agricultural University and in a farmer's plot at Pattom, Trivandrum.

Rhizobium capable of nodulation in greengram was isolated from 10 out of 14 different soil samples collected from Alleppey, Quilon and Trivandrum districts. All these isolates (R_1- R_{10}) were gram negative, Ketolactose negative, non sporulating and rod shaped. They produced raised translucent gummy colonies on yeast extract mannitol agar medium after 72 h of incubation at 28 $\pm$ 1°C. However, on glucose peptone agar, the growth on these cultures were only minimum even after 7 days of incubation. The ten isolates of Rhizobium obtained intially were screened for their nodulation efficiency under aseptic pot culture conditions by using the greengram variety CO-3. In this screening it was found that the increase was maximum in R_5 treatment where, 138.30 nodules were formed per plant when compared to only 8.33 in the uninoculated control The number of nodules formed with isolate numbers R_2, R_3, R_4, R_9 and R_{10} were also high and statistically on par with the above

treatment. The fresh and dry weight of nodules formed 455 and 118.33 mg respectively were also maximum in the R_5 treatment. A significant increase in root length was obtained only in two of the above treatments, R_6 and R_9. The shoot height of 26.33 cm its fresh and dry weights of 19.0 and 1.93 g respectively were maximum in the R_5 treatment. These were significantly higher than rest of the treatments except in R_{10}, where the fresh and dry weights of shoot recorded were statistically on par with the R_5 treatment.

The Rhizobium culture R_5 isolated from C.S.R.C. Karamana, Trivandrum was found superior to all other local isolates of rhizobia. The number of nodules formed per plant, their fresh and dry weight, shoot height, fresh and dry weight of shoot were all maximum due to seed inoculation with this culture. Besides, the required minimum increase in dry weights of plants from Rhizobium inoculation, 50 per cent increase in dry mass over uninoculated control (as per the Indian Standard Institution Specifications for Rhizobium inoculants IS 8268-1976) was obtained only with this isolate of Rhizobium. Therefore, this culture was selected for further studies along with a standard culture of Rhizobium obtained from Tamil Nadu Agricultural University, Coimbatore.

The multilocational field trial for studying the varietal variation for nodulation by Rhizobium was conducted at two different locations in Trivandrum district at Karamana and Pattom using five varieties of greengram and two cultures of rhizobia. The different biometric observations were taken on 45th day of plant growth and at the time of harvest. The effect of Rhizobium inoculation in increasing the nodule number in different varieties of greengram was significant at both locations when compared to that of control. The mean number of nodules formed per plant, 220.59 at Karamana and 202.22 at Pattom were maximum due to seed inoculation with the standard culture of Rhizobium. Among different varieties of greengram the maximum number of nodules were formed in CO-3 both at Karamana and Pattom. However, there were no significant difference between varieties in their mean nodule number. The fresh and dry weights of nodules were also significantly high due to Rhizobium inoculation at both locations. The mean fresh and dry weights of 1.42 g and 140.14 mg respectively at Karamana and 1.44 g and 146.0 mg respectively at Pattom were maximum in seed treatment with the local isolate of Rhizobium.

The mean shoot height of 77.6 cm at Karamana and 87.2 cm at Pattom were maximum in seed treatment with the

standard culture of Rhizobium. The increase in shoot height with the local isolate of Rhizobium was also significant when compared to that of control. The fresh weight of shoot was significantly high due to Rhizobium inoculation at both locations when compared to that of control. The mean fresh weight of 150.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana and with the local culture of Rhizobium at Pattom. It was also observed that the dry weight of shoot was significantly high due to Rhizobium inoculation at both locations. The mean dry weight of 25.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana. The increase in dry weight of shoot due to seed inoculation with the local culture of Rhizobium at both locations were also significant and statistically on par with the above treatment. There were no significant difference between varieties in their mean dry weight of shoot at both locations.

There was no significant difference between treatments in the mean shoot height of plants with and without Rhizobium inoculation at the time of harvest. The increase in fresh weight of shoot was significant only at Pattom. The mean fresh weight of 219.9 g was maximum in seed treatment with the standard culture of Rhizobium. The increase

in the fresh weight of shoot due to seed inoculation with the local isolate of Rhizobium at this location was also significant and statistically on par with the above treatment. As in the case of fresh weight of shoot, the increase in the dry weight of shoot was significant only at Pattom. The mean dry weight of 41.8 g was maximum in seed treatment with the standard culture of Rhizobium. The increase in dry weight of shoot due to seed inoculation with the local isolate of Rhizobium at this location was also significant and statistically on par with the above treatments.

The increase in the mean yield (g/plot) of different varieties of greengram due to Rhizobium inoculation was significant at both locations when compared to that of control. The mean yield of 1005.0 g at Karamana and 966.6 g at Pattom were maximum in seed treatment with the standard culture of Rhizobium. However, the increase in yield in different varieties of greengram due to seed inoculation with the local culture of Rhizobium was also significant when compared to that of control, and at one location, Pattom, this increase was even statistically on par with the standard culture of Rhizobium. Significant differences between varieties in their mean yield were observed at both locations. The mean yield of 1055.3 g

at Karamana and 1015.6 g at Pattom were maximum in the variety CO-3 followed by varieties such as CO-4, CV-2, KM-2 and Pusa baisakhi. A significant interaction between Rhizobium inoculation and different varieties of greengram was however absent.

Thus the important findings in the present investigation can be summarised as follows:

1. It was possible to isolate native rhizobia capable for nodulating greengram from ten out of fourteen different locations survyed during this investigation from Alleppey, Quilon and Trivandrum Districts of Kerala.

2. In the screening trial for nodulation efficiency in greengram with the above ten isolates of rhizobia, it was possible to select a local culture that could satisfy the minimum requirements as per I.S.I. specifications for Rhizobium inoculants (IS-8268-1976). This culture was isolated from Cropping System Research Centre, Karamana of the Kerala Agricultural University.

3. In the multilocational field evaluation study using five different varieties of greengram it was found that no particular host varietal specificity was shown by either of Rhizobium culture, local as well as standard

culture in increasing the number of nodules formed, fresh and dry weight of shoot and the final grain yield of different varieties of greengram. The differences in yield in different varieties of greengram followed a similar pattern both at Karamana and Pattom indicating that such differences may be due to the variation in the yield potency of the different varieties of greengram rather than due to a differential response to Rhizobium inoculation.

4. It was found during this investigation that on an average one can get an yield increase of about 21.3 per cent in greengram due to Rhizobium inoculation (pooled data for both the locations) under field conditions when compared to that of the uninoculated control treatment.

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* Originals not seen.

VARIETAL VARIATION FOR NODULATION BY *Rhizobium* IN GREENGRAM

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ABSTRACT OF A THESIS

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ABSTRACT

The present investigation on varietal variation for nodulation by Rhizobium in greengram was conducted at College of Agriculture, Vellayani, Trivandrum. The field trial for host varietal specificity was done at C.S.R.C. Karamana of Kerala Agricultural University and in a farmer's plot at Pattom, Trivandrum.

Rhizobium capable of nodulation in greengram was isolated from 10 out of 14 different soil samples collected from Alleppey, Quilon and Trivandrum districts. The ten isolates of Rhizobium obtained initially were screened for their nodulation efficiency under aseptic pot culture conditions by using the greengram variety CO-3. In this screening it was found that the increase was maximum in R_5 treatment where 198.30 nodules were formed per plant when compared to only 8.33 in the uninoculated control. The fresh and dry weights of nodules formed 455 and 118.33 mg respectively were also maximum in the R_5 treatment. A significant increase in root length was obtained only in two of the above treatments R_6 and R_9. The Rhizobium culture R_5 isolated from C.S.R.C. Karamana, Trivandrum was found superior to all other local isolates of rhizobia. Besides, the required minimum increase in dry weight of plants from Rhizobium inoculation, 50 per cent increase

in dry mass over uninoculated control (as per the Indian Standard Institution Specifications for Rhizobium inoculants IS 8268-1976) was obtained only with this isolate of Rhizobium.

The multilocational field trial for studying the varietal variation for nodulation by Rhizobium was conducted at two different locations in Trivandrum district at Karamana and Pattom using five varieties of greengram and two cultures of rhizobia. The effect of Rhizobium inoculation in increasing the nodule number in different varieties of greengram was significant at both locations when compared to that of control. The mean number of nodules formed per plant, 220.59 at Karamana and 202.22 at Pattom were maximum due to seed inoculation with the standard culture of Rhizobium. Among different varieties of greengram the maximum number of nodules were formed in CO-3 both at Karamana and Pattom. However, there was no significant differences between varieties in their mean nodule number. The fresh and dry weights of nodules were also significantly high due to Rhizobium inoculation at both locations. The mean fresh and dry weights of 1.42 g and 140.14 mg respectively at Karamana and 1.44 and 145.0 mg respectively at Pattom were maximum in seed treatment with the local isolate of Rhizobium. The fresh weight of shoot was significantly

high due to Rhizobium inoculant at both locations when compared to that of control. The mean fresh weight of 150.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana and with the local culture of Rhizobium at Pattom. It was also observed that the dry weight of shoot was significantly high due to Rhizobium inoculation at both locations. The mean dry weight of 25.2 g was maximum in seed treatment with the standard culture of Rhizobium at Karamana. The increase in dry weight of shoot due to seed inoculation with the local culture of Rhizobium at both locations were also significant and statistically on par with the above treatment. There were no significant difference between varieties in their mean dry weight of shoot at both locations.

The increase in mean yield (g/plot) of different varieties of greengram due to Rhizobium inoculation was significant at both locations when compared to that of control. The mean yield of 1005.0 g at Karamana and 966.6 g at Pattom were maximum in seed treatment with the standard culture of Rhizobium. However, the increase in yield in different varieties of greengram due to seed inoculation with the local culture of Rhizobium was also significant when compared to that of control and at one

location, Pattom, this increase was even statistically on par with the standard culture or Rhizobium. Significant differences between varieties in their mean yield were observed at both locations. The mean yield of 1055.3 g at Karamana and 1015.6 g at Pattom were maximum in the variety CO-3 followed by varieties such as CO-4, CV-2, KM-2 and Pusa barsakhi. A significant interaction between Rhizobium inoculation and different varieties of greengram was however absent.