Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala

By

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DECLARATION

I hereby declare that this thesis entitled **"Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala"** 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 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 **"Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala"** is a record of research work done independently by **Smt. Meena kumari. K.S.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS USED

µg	-	microgram
kg	-	kilogram
t	-	tonnes
q	-	quintals
ml	-	millilitre
g	-	gram
ppm	-	parts per million
m	-	meter
cm^2	-	centimeter square
%	-	percentage
YEMA	-	yeast extract mannitol agar
BTB	-	bromothymol blue
CRD	-	completely randomised design
POP	-	package of practices
FYM	-	farm yard manure
N	-	nitrogen
P	-	phosphorus
K	-	potassium
Ca	-	calcium
Mg	-	magnesium
Mo	-	molybdenum
Fe	-	iron
Al	-	aluminium
OD	-	optical density
CP	-	cowpea
BG	-	blackgram
GG	-	greengram
IARI	-	Indian Agricultural Research Institute
ha^{-1}	-	per hectare
NS	-	not significant

INTRODUCTION

INTRODUCTION

Nutrient imbalance is a major threat now a days to sustain soil fertility and crop productivity. The periodical increases in the market price of NPK fertilizers have further aggravated the situation. This problem can be met to a great extent by adopting an integrated nutrient management practice for the cultivation of different crops. Apart from chemical fertilizers, organic manures and biofertilizers are also important components of such a sustainable system. Among biofertilizers, the most important are those based on diazotrophic bacteria especially involved in legume - *Rhizobium* symbiosis. It is infact a gift of the nature to mankind. This can meet by itself nearly 60-70 per cent of the nitrogen requirement of most of the cultivated grain legumes. Besides, the cultivation of these crops will also result in enrichment of soil with fixed nitrogen. This phenomenon has been exploited from time immemorial to achieve nitrogen economy in legume-cereal crop rotation practices.

The success of legume - *Rhizobium* symbiosis, however, is dependent on several biotic as well as abiotic factors. The most important among them are the use of efficient strains of *Rhizobium*, *Bradyrhizobium* or *Azorhizobium* for seed treatment and the occurrence of ideal soil and environmental conditions favouring optimum root nodulation and plant growth. In India eventhough 23.9 million ha are under cultivation of different pulses, only 17,123 ha are used for this purpose in Kerala. This incidentally is not because of a lack of demand for grain and vegetable legume in Kerala, but it is mainly due to the non-availability of sufficient land area for large scale cultivation of pulses as a monocrop. Coupled with this problem, is the acidic nature of the soil with low organic matter content. The latter two factors are of greater importance because of their deleterious influence on legume -*Rhizobium* symbiosis.

In Kerala, the soil acidity is often in the range of 3.2 to 5.5. While soil acidity as such can inhibit growth and survival of rhizobia, the low availability of certain essential nutrient elements such as calcium and molybdenum besides the high aluminium content can adversely affect both nodulation process and nitrogenase activity by *Rhizobium*. These problems cannot be solved merely by identifying efficient native strains of *Rhizobium* or *Bradyrhizobium*. There is also a need for developing a comprehensive package of practices recommendations that can be easily adopted by the farmers for cultivation of legumes in acid soils. The present research project was taken up with these objectives with financial assistance from the Department of Biotechnology of Government of India.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Legumes are an excellent source of high quality protein. Cultivation of these crops can contribute significantly in building and conserving soil fertility. In India, nearly 23.9 million ha are under cultivation of different pulses with an annual production of about 13.2 million tonnes. In Kerala only 17,123 ha are under regular cultivation of these crops. Nearly 60 to 70 per cent of the nitrogen requirement of these crops can be met through legume -*Rhizobium* symbiosis.

The genus *Rhizobium* was first described in 1889 by Frank (Fred *et al.*, 1932) based on its ability to form root nodules in legumes. The three important genera, *Rhizobium, Bradyrhizobium* and *Azorhizobium* have been grouped along with *Agrobacterium* and *Phyllobacterium* into a single family, the Rhizobiaceae (Jordan, 1984). The use of modern methods of bacterial systematics such as numerical taxonomy, nucleic acid hybridisation and 16S rRNA analysis demonstrated the existence of marked genetic diversity within this family (Young *et al.*, 1991). Martinez (1994) confirmed that *Rhizobium* and *Bradyrhizobium* were only distantly related. Similarly Jarvis *et al.* (1986), Dreyfus *et al.* (1988) and Rhijn and Vanderleyden (1995) demonstrated that *Bradyrhizobium* and *Azorhizobium* species were genetically distinct from *Rhizobium* species.

Wood and Shephered (1987) reported that acid tolerant strains of *Rhizobium trifolii* often persisted in the acidic parts of the soil. The isolation of such strains have been reported from many other crops such as cowpea (Nair and Sivaprasad, 1982), *Psophocarpus tetragonolobus* (Lan-Gu and Cailongxiang, 1990) and subterranean clover (Rodrigues *et al.*, 1994).

2.1. Characterisation of *Rhizobium*

Allen and Allen (1958) reported that unlike *Agrobacterium* spp. none of the *Rhizobium* isolates grew well in an alkaline medium of pH 11.0. Kleczkowska *et al.* (1968) further reported that *Rhizobium* spp. also did not grow well in glucose peptone agar medium. Norris and Date (1976) observed that when different rhizobial strains were grown on yeast extract mannitol agar medium containing bromothymol blue, strains of *R. trifolii, R. meliloti, R. leguminosarum* and *R. phaseoli* produced an acidic reaction, while certain strains of *R. japonicum, R. lupini and Rhizobium* sp. (Cowpea group) produced alkalinity. But Bromfield and Rao (1983) observed that fast growing isolates from *Cajanus cajan* produced an acidic reaction in the same medium.

Norris (1964) developed a scheme for describing rhizobia based on the relative growth rate. He speculated that the fast growing acid producing strains of *Rhizobium* were evolved from the temperate regions while the slow growing alkali producing strains were evolved from the humid tropical regions. Jordan (1982) created a new genus, *Bradyrhizobium*, to include all slow growing species. However, a clear distinction between fast and slow growers was often not possible with the discovery of strains that had characters of both the groups (Broughton *et al.*, 1984).

2.2. Carbohydrate utilisation

Neal and Walker (1935) studied the utilisation of different carbon sources by *Rhizobium*. They found that while arabinose was superior to all other sugars, the utilisation pattern of glucose, galactose and xylose was almost identical. Graham and Parker (1964) reported that fast growing strains of rhizobia had better rate of growth with glucose, arabinose and xylose. The slow growers mainly used arabinose, xylose and galactose. Similar results were also reported by Zablotowicz and Focht (1981) and Bromfield and Rao (1983). Behari *et al.* (1994) made a detailed investigation on the pattern of carbohydrate utilisation by rhizobia. They found that maximum growth was with mannitol, glucose, sucrose and xylose. It was moderate with mannose, arabinose and maltose and least with fructose, lactose and galactose.

2.3. Pattern of antibiotic resistance

Antibiotics have been used to differentiate between *Rhizobium* sp. in selective media (Parttison and Skinner, 1974). Marques-Pinto *et* *al.* (1974) differentiated *R. meliloti* strain in nodulation from *Medicago sativa* L. grown under laboratory conditions by natural resistance to kanamycin and streptomycin. Resistance to streptomycin has been one of the most frequently used markers in genetic studies of rhizobia (Brockwell *et al.*, 1977). Cole and Elkan (1979) screened 48 strains of *R. japonicum* for their response to chloramphenicol, streptomycin, neomycin, polymyxin, penicillin, erythromycin and tetracyline. Over 60 per cent of the strains were resistant to chloramphenicol, polymyxin, erythromycin while 47 per cent were resistant to neomycin and penicillin.

Josey *et al.* (1979) observed certain variations in the intrinsic resistance pattern of rhizobia to low levels of antibiotics such as neomycin and kanamycin from 4.0 to 10.0 $\mu g\ ml^{-1}$. Beynon and Josey (1980) reported that *R. phaseoli* was resistant to high concentrations of streptomycin up to 200 $\mu g\ ml^{-1}$. Dakora (1985) found that slow growing rhizobia from *Vigna unguiculata* were generally more resistant to high concentrations of antibiotics. Dadarwal *et al.* (1987) observed that while slow growing rhizobia were resistant to tetracyline up to 50 $\mu g\ ml^{-1}$, fast growing strains showed better resistance to kanamycin, chloramphenicol and streptomycin up to 150 $\mu g\ ml^{-1}$. Borges *et al.* (1990) studied the antibiotic resistance of 50 numbers of *Bradyrhizobium japonicum* strains and found that a majority of them were resistant to ampicillin, kanamycin, streptomycin and tetracycline at concentrations below 100 $\mu g\ ml^{-1}$. Some of them were also resistant to more than 100 $\mu g\ ml^{-1}$ of chloramphenicol.

2.4. Serological characterisation

Charudathan and Hubbel (1973) using agar gel double diffusion technique compared the soluble antigens of three *Rhizobium* spp. with those of eight legumes representing compatible and non compatible hosts. Cross reaction was obtained between all the legume hosts and three rhizobia. Kishinevsky and Bar-Joseph (1978) used ELISA for serological identification of peanut rhizobia. Sheth (1979) investigated the antigenic relationship of inoculated strains of rhizobia for groundnut with native strains and found that inoculated strains differed from the native rhizobia.

The serological diversity of cowpea rhizobia was also examined by Ahmad *et al.* (1981) and Ortega (1989). Paterno (1987) conducted serological studies of indigenous rhizobia and obtained cross reaction with two of the test strains of TAL 209 and TAL 1000. Kishinevsky *et al.* (1993) conducted ELISA and antibody adsorption tests to determine the main somatic antigens constituent of 243 strains of *Bradyrhizobium*. He observed that the serological properties of indigenous *Bradyrhizobium* were not related to the cropping history of the cultivated fields from which they were isolated.

2.5. Soil acidity and legume cultivation

During the first half of this century, failure of legumes to achieve profitable yields was often attributed to the absence of symbiotically effective rhizobia in the soil (Cass-Smith and Pittman, 1938; Erdman, 1943; Vincent, 1954 and Baird, 1955). Environmental factors such as inconsistent rainfall, extreme temperature, acidic soils of low nutrient status and poor water holding capacity were also reported to adversely affect legume - *Rhizobium* symbiosis (Jenkins *et al.*, 1954; Loneragan *et al.*, 1955).

In acid soils, legume growth is considerably affected more commonly by factors related to acidity than by acidity itself (Arnon and Johnson, 1942; Yadav and Vyas, 1971). Vincent (1965) reported that hydrogen ion activity was a major factor restricting the survival and growth of rhizobia in acidic soils. In addition to the effect on root hair formation (Hecht - Buchholz *et al.*, 1990), low soil pH would also affect nodule initiation (de Carvalho *et al.*, 1982; Murphy *et al.*, 1984 and Brady *et al.*, 1990).

Raju (1977) reported that *Rhizobium* inoculation was essential for effective nodulation and better growth of cowpea under acidic soil conditions. Coventry and Evans (1989) found that the problems due to soil acidity were mainly because of the toxic effects of aluminium and manganese besides the limited availability of calcium and molybdenum. Such soils also had low levels of phosphorus (Kamprath, 1973; Pearson, 1975 and Franco and Munns, 1982). The growth and nodulation of many legumes were also found affected by aluminium toxicity (Andrew *et al.*, 1973 and Kim *et al.*, 1985). Delay in nodulation due to low calcium availability has been reported in *Vigna unguiculata* by Hohenberg and Munns (1984).

Date and Halliday (1977) and Keyser and Munns (1979) were the first to identify *Bradyrhizobium* strains capable of growth at low pH in the presence of soluble aluminium. They observed that cowpea rhizobia had more tolerance to aluminium than *R. japonicum*. Studies by Perkasem (1977) and de Carvalho *et al.* (1982) also showed that some rhizobia could survive high aluminium concentrations at low pH in soil and in solution media. Similar results were also reported by Wood and Cooper (1985, 1988) and Vargas and Graham (1988).

David *et al.* (1980) reported that the growth of three strains of blackgram rhizobia were maximum at pH 7.0. The growth was significantly reduced at pH below and above 7. Nair and Sivaprasad (1982) observed that cowpea rhizobia isolated from soil of pH 5.5 were more tolerant to still lower pH of 4.5 under *in vitro* conditions. Graham *et al.* (1982) found that acid tolerant strains of *R. leguminosarum* cv. *phaseoli* not only survived better in acid soils than acid sensitive strains but also gave better nodulation and yield in *Phaseolus vulgaris*. Thornton and Davey (1983) also reported that acid tolerant strains of *R. trifoli* could form better symbiotic association in *Trifolium* sp.

2.6. Benefits of *Rhizobium* inoculation

Sahu and Behera (1972) got significant increases in nodule number, nitrogen content of root and shoot in greengram due to *Rhizobium* inoculation. Similar results were also reported by Subba Rao and Balasundaram (1971) and Saxena and Tilak (1975) in soybean, Medhane and Patil (1974) and Rai *et al.* (1977) in bengalgram, Tripathi *et al.* (1975) in chickpea and Subba Rao (1972), Pahwa and Patil (1983), Shaktawat (1988) and Gandhi and Godbole (1990) in cowpea.

Chandramohan *et al.* (1980) studied the effect of *Rhizobium* inoculation in greengram and blackgram in Thanjavur delta. They obtained an increase of 33.5 and 23.6 per cent in yield in blackgram and greengram respectively. Nagre (1982) obtained significantly increased seed yield in blackgram and greengram by seed inoculation. Prasad and Ram (1982) found that inoculation of *Vigna radiata* with *Rhizobium* not only increased the yield but also the nitrogen content of nodules.

2.7. Influence of agronomic practices on legume - *Rhizobium* symbiosis

Araujo *et al.* (1982) studied the effect of FYM, phosphate fertilizer and *Rhizobium* inoculation on nitrogen fixation and yield in *Phaseolus vulgaris* for two seasons. The treatments were cattle manure (0 and 15 t ha^{-1}), soluble phosphate (0 and 100 kg P_2O_5 ha^{-1}), rock phosphate (0 and 300 kg P_2O_5 ha^{-1}) with and without inoculation. They found that in the wet season, cattle manure significantly increased nodulation, nitrogen fixation, dry matter yield and bean yield. In the dry season, there was a residual effect of cattle manure application on total nitrogen, dry matter yield and bean yield. Kadam and Desai (1983) got significantly increased yield due to FYM, molybdenum and *Rhizobium* application in groundnut. Kataoka and Haramaki (1985) also studied the effect of FYM application on nodulation in lucerne and found that application of 30-40 t FYM ha^{-1} increased the number of nodulated plants.

The influence of integrated nutrient management on yield, protein content and uptake of nutrients by pigeon pea was studied by Mathan *et al.* (1994). The application of urea, FYM, rockphosphate along with *Rhizobium* inoculation not only increased the seed yield but also the seed protein content and uptake of nitrogen, phosphorus and potassium. The available nutrient status of the soil was also increased significantly with these treatments. Lawson *et al.* (1995) also studied the effect of organic manure on growth, nodulation and nitrogen fixation of soybean. They found that the addition of organic manure increased shoot growth in soybean probably due to an improvement in soil moisture status and better nutrient uptake. Mathan *et al.* (1996) reported that the application of fertilizers, organic manures and *Rhizobium* inoculation enhanced the seed yield and nitrogen, phosphorus and potassium uptake by blackgram. The protein content was also increased by nearly 15 per cent over control treatment.

Rice (1975) demonstrated the importance of liming to decrease the soluble aluminium content of acid soils for alfalfa cultivation. Munns *et al.* (1977) reported that liming significantly improved nitrogen fixation in both tropical and temperate legumes. Increased nodulation and plant nitrogen content were obtained by the application of lime. Barthakur (1980) studied the effect of *Rhizobium* inoculation along with lime and molybdenum application on yield, dry weight and nodulation of soybean. He observed that inoculation and application of molybdenum and lime increased both plant dry weight and nodule number. Liming increased nodulation by 300 per cent. However, there was no correlation between plant dry weight and nodule number. The yield was increased by 27 and 30 per cent during the first and second year respectively.

Choe *et al.* (1980) reported that when lucerne was inoculated with an acid tolerant strain of *R. meliloti* and grown in an acid soil with 0,400 and 1020 kg lime ha^{-1} to give a soil pH of 5.4, 5.9 and 6.4 respectively, the nodule number, plant and nodule dry matter were significantly increased. Higgins and Olsen (1984) studied the effect of liming on soil acidity. They found that the addition of 5 and 10 t lime ha^{-1} was required to raise the pH to 5.6 and 6.3 respectively.

Barbo and Fabrico (1982) found that lime and phosphorus application increased not only calcium and magnesium content, but also reduced Al^{3+} content of soil. Lin *et al.* (1982) also observed that certain degree of liming was essential for growth of soybean in acidic soil. Mathew and Koshy (1982) reported that the application of lime and phosphorus along with *Rhizobium* inoculation improved both plant growth and yield of cowpea. The yield was increased by 24 per cent by applying 300 kg lime ha^{-1}. Kalia *et al.* (1984) studied the effect of nitrogen and phosphorus fertilization, with or without *Rhizobium* inoculation and liming on yield, quality and economics in soybean in clayloam acid soil of pH 5.6. The different treatments had a beneficial effect on seed yield and protein content. Hsin *et al.* (1985) also reported that use of lime and molybdenum increased nodule formation and yield in soybean. Chao and Young (1985) observed that liming not only increased soil pH to 7.0 but also decreased the percentage of soil aluminium content. This resulted in increased dry matter, yield, growth and nodulation by *Rhizobium* in *Leucaena leucocephala*. Koopman *et al.* (1995) obtained increased growth response in lucerne due to lime application.

Roughley (1970) reported that the use of lime pelleted seeds contributed to higher yield in alfalfa in moderately acidic soil. Choe *et al.* (1982) studied the effect of phosphorus fertilizer application, *Rhizobium* inoculation and lime pelleting on growth and yield of lucerne.

and phosphorus application in blackgram. Similarly Chanda *et al.* (1991) got better response to nodulation and grain yield in greengram due to *Rhizobium* inoculation along with fertilizer application. This increase was 57 per cent due to inoculation for nodule production, 77 per cent for dry matter yield and 64 per cent for grain yield. Hoque (1993) used *Bradyrhizobium* as a source of nitrogen for grain legumes in Bangladesh. He found that *Bradyrhizobium* inoculation was beneficial in that it increased the nodule number, nodule mass, shoot weight and yield of different grain legumes compared to uninoculated control and urea treated plants. In soybean, yield increase was 113 per cent over control and 49 per cent over urea treatments, whereas in groundnut, these were 36 and 11 per cent. In lentil and mung the yield increases were 30 and 13, 47 and 7 per cent respectively. Bagal and Jadhav (1995) also reported that *Rhizobium* inoculation along with 12.5 kg N ha^{-1} increased the yield and nutrient uptake by French bean.

Dube *et al.* (1995) studied the effect of *Rhizobium* inoculation and NPK fertilizer application on growth and yield of soybean. They observed that *Rhizobium* inoculation enhanced yield and net return up to 3.8 q ha^{-1}. The use of N-20 kg ha^{-1}, P-80 kg ha^{-1}, K-20 kg ha^{-1} gave the highest grain yield and net return up to 35.1 q ha^{-1}. Roy *et al.* (1995) reported that *Rhizobium* inoculation and nitrogen application increased nodulation, growth and seed yield of gram. Nitrogen alone or with inoculation increased leaf area index by 9 to 14 per cent and total dry matter by 24 to 68 per cent. Kaushik *et al.* (1995) also

The application of upto 300 kg P ha^{-1} increased fodder yield. Manquiat and Padilla (1982) reported that lime pelleting in *Albezzia* was beneficial in strongly acidic soils. Lu *et al.* (1983) obtained increased nodulation in chinese milk vetch raised from pelleted seeds. The fresh and dry weight of stem and leaves were also higher due to pelleting.

Hagedorn (1979) determined the extent of establishment of *Trifolium subterraneum* along with *Rhizobium* inoculation and fertilizer application at five field sites. Both plant dry weight and total nitrogen content showed an additive effect due to inoculation and fertilizer application. Nagre (1982) studied the effect of *Rhizobium* inoculation and nitrogen fertilizer application on yield of greengram and blackgram. He observed that seed inoculation or application of 10 or 20 Kg N ha^{-1} produced significant increases in seed yield of greengram and blackgram. Srivastava and Varma (1982) reported increased growth, nodulation and quality of greengram when 15 kg N ha^{-1} was applied along with seed inoculation with *Rhizobium*. Thakur and Negi (1985) found that that in blackgram seed yield was significantly increased by increasing nitrogen from 0-20 kg ha^{-1}, P_2O_5 from 0-60 kg ha^{-1} along with *Rhizobium* inoculation. Raju and Varma (1984) found that seed inoculation along with application of 15 kg N ha^{-1} significantly increased nodulation and seed yield of *Vigna radiata*.

Sudhakar *et al.* (1989) got increased leaf area index, leaf weight ratio, specific leaf weight, seed yield and harvest index by *Rhizobium* studied the effect of fertilizer nitrogen on nodulation, acetylene reducing activity and nitrogen uptake on pigeon pea (*Cajanus cajan*). They reported that these were significantly improved with 20 kg N ha^{-1}. However, at 40 and 60 kg N ha^{-1} the root system was poorly developed which affected nodule number, nodule dry weight, acetylene reducing activity, shoot weight and root and shoot nitrogen.

Arya and Singh (1996) reported that nitrogen and phosphorus application had a positive effect on plant height, number of leaves and leaf area $plant^{-1}$, dry matter $plant^{-1}$ and dry weight of nodules in horse gram. The grain and straw yields were increased with the application of upto 20 kg N ha^{-1} and phosphatic fertilizer upto 60 kg P_2O_5 ha^{-1}. Mathan *et al.* (1996) also studied the response of blackgram (*Phaseolus mungo*) to fertilization and *Rhizobium* inoculation. They found that the application of 25 kg N ha^{-1} as urea along with 50kg P ha^{-1} as single super phosphate and FYM recorded 75 per cent more yield. The available nitrogen, phosphorus and potassium in soil were also increased significantly by the above treatment combination. Rajput and Singh (1996) investigated the response of nitrogen and phosphorus with and without *Rhizobium* inoculation on fodder production in cowpea. Their study revealed that application of 50 kg N and 50 kg P_2O_5 ha^{-1} gave significantly higher fodder yield.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation on "Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala" was carried out as part of the Department of Biotechnology, Government of India, funded research project at the College of Agriculture, Vellayani, Trivandrum during 1993-96.

3.1. Isolation of native strains of *Bradyrhizobium*

The initial isolation of *Bradyrhizobium* was done from seven different locations in Kerala (Fig. 1). The first set of locations (Category A) namely Vellayani, Kayamkulam, Pattambi and Pilicode consisted of some of the major pulse growing regions in the state. Here different pulses were cultivated not only in garden land but also in rice fallows during summer season. Hence, the isolation of *Bradyrhizobium* was done under both these conditions. For this, cowpea (*Vigna unguiculata*), blackgram (*Vigna mungo*) and greengram (*Vigna radiata*) were raised in microplots of $1 m^2$ area for 45 days without resorting to any soil amendments such as liming and application of chemical fertilizers. The second set of locations (category B) namely Ambalapuzha, Kuttanad and Vyttila represented some of the problem zones for pulses cultivation

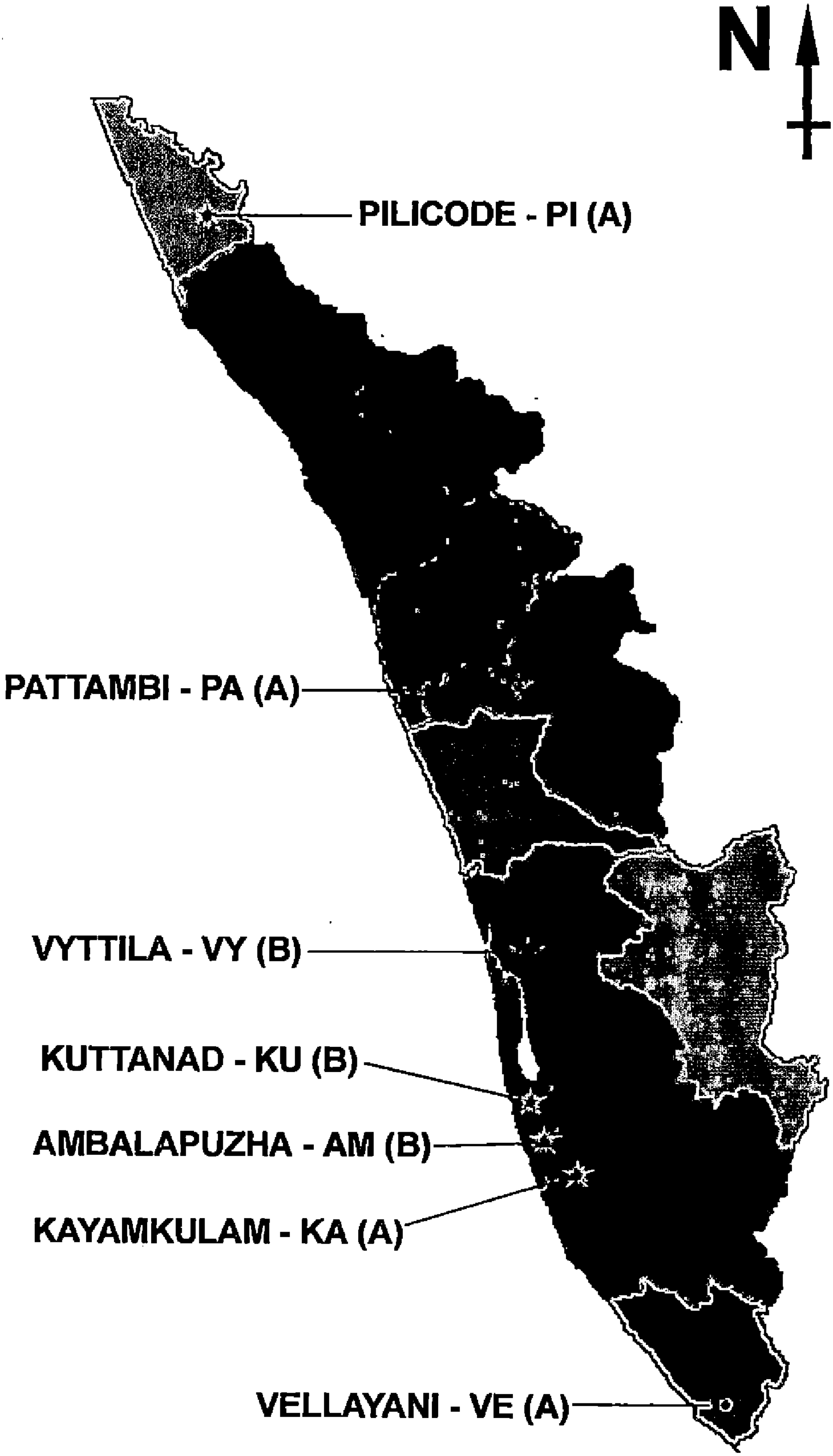


Fig. 1. Locations selected for the isolation of *Bradyrhizobium* in Kerala

in Kerala due to acid saline conditions or extreme soil acidity. These locations were also selected to find out the possible occurrence of *Bradyrhizobium* sp. under such adverse soil conditions. Here, the initial isolation of *Bradyrhizobium* was done only under pot culture conditions. In all these locations, the same crop varieties namely Kanakamani for cowpea, Shyama for blackgram and Co-2 for greengram were used.

The initial isolation of *Bradyrhizobium* was done by the method of Vincent (1970) using yeast extract mannitol agar medium (YEMA). Depending on the growth rate of different isolates, the plates were incubated for 3 - 5 days at 28 $\pm$ 1°C in an incubator and well isolated colonies showing the typical characters of *Bradyrhizobium* were selected. They were checked for purity by restreaking on YEMA and by Gram staining (Hucker, 1927). Pure cultures were then maintained on YEMA slants for further studies. All the isolates were given a specific code number indicating the location, whether isolated from gardenland (G) or rice fallows (F), crop (cowpea (C), blackgram (B) or greengram (G)) and a serial number. At the time of initial isolation, additional observations like number of nodules formed by native strains, nodule dry weight and plant dry weight were also recorded by standard procedures. The soil samples from each location (Appendix I) were also analysed for pH (Jackson, 1973) organic carbon (Walkley and Black, 1947), phosphorus (Jackson, 1973), potassium (Stanford and English, 1949), calcium and magnesium (Jackson, 1973) iron (Lindsay and Norvell, 1978) aluminium (Black, 1965) and molybdenum (Jackson, 1973).

3.2. Selection of efficient cultures of *Bradyrhizobium*

All the native isolates of *Bradyrhizobium* from cowpea, blackgram and greengram were screened for nodulation efficiency as per the Bureau of Indian Standard Specification for rhizobial inoculants (1S:8268/1976). The experiment was conducted under pot culture conditions using unamended and amended soils.

3.2.1. Screening in unamended soil

This experiment was conducted in completely randomised design with three replications for each isolate of *Bradyrhizobium*. Unsterilized soil of pH 4.9 without any amendment such as liming, application of FYM or chemical fertilizers were used for this purpose. Soil without *Bradyrhizobium* inoculation served as control. A similar treatment with the application of ammonium nitrate @ 100 kg N ha^{-1} (as per BIS requirement) was also maintained. The crop varieties used for initial isolation were used for this experiment also. Inoculation with *Bradyrhizobium* was done by using a thick aqueous suspension of respective isolates. The plants were grown for 45 days under regular irrigation and observations on nodule number, nodule dry weight and plant dry weight were recorded by standard procedures.

3.2.2. Screening in amended soil

The experiment under 3.2.1 was repeated by using sterilized soil amended with FYM @ 20 t ha^{-1} (as per the package of practices recommendations of Kerala Agricultural University for cowpea, blackgram and greengram). The pH of the amended soil was 6.9. Seed treatment with *Bradyrhizobium* was done only after surface sterilizing the required quantity of seed material with 0.1 per cent mercuric chloride followed by thorough washing with sterilised water. The plants were irrigated regularly with sterile water. The different treatments and observations recorded were similar to those under unamended soil.

The best isolates of *Bradyrhizobium* from both unamended as well as amended soils were selected as per the specification of Bureau of Indian Standards which stipulates atleast 50 per cent increase in plant dry weight in the inoculated series as compared to uninoculated and unfertilised control treatments. In all, six isolates, one each from unamended and amended soil for each crop were selected for further studies.

3.3. Characterisation of different isolates of *Bradyrhizobium*

The *Bradyrhizobium* isolates were characterised by recording its colony characteristics on YEMA with one per cent congored, acid production on YEMA with 0.5 per cent bromothymol blue, growth on lactose medium, glucose peptone agar and Hofer's alkaline medium of pH 11.0 (Vincent, 1970).

3.3.1. Utilisation of different carbon sources

The comparative growth rate of the six selected native isolates of *Bradyrhizobium* along with that of three exotic cultures for cowpea, blackgram and greengram from Indian Agricultural Research Institute, New Delhi, was studied under *in vitro* conditions. Normal YEM broth of pH 7.0 and modified yeast extract broth substituting mannitol with an equivalent quantity of either a pentose sugar such as xylose or arabinose and a hexose sugar such as glucose or galactose were used for this purpose. The growth rate was measured using a Baush and Lomb Spectronic-20 spectrophotometer.

The OD_{600} was determined after incubation at 28 $\pm$ 1°C once in 48 h for a maximum period of seven days. Each treatment was replicated twice. Data are presented as the mean OD_{600} for growth measurement for seven days.

3.3.2. pH sensitivity

The acid tolerance of the native as well as the exotic isolates of *Bradyrhizobium* was studied under *in vitro* conditions by using YEM broth of pH 4.5, 6.0 and 7.5 (control). The relative growth rate was measured by using a spectrophotometer. The OD_{600} was determined after incubation at 28 $\pm$ 1 ^{o} C once in 48 h for a maximum period of seven days. Each treatment was replicated twice. Data are presented as the mean OD_{600} for growth measurement for seven days.

3.3.3. Iron sensitivity

This was studied by using normal YEM broth supplemented with 50, 100 and 250 ppm ferric chloride. The growth of different native and exotic isolates were recorded by the method described earlier.

3.3.4. Aluminium sensitivity

The above experiment was repeated with 50, 100 and 250 ppm aluminium chloride. The growth of different native and exotic isolates were recorded by the method described earlier.

3.3.5. Antibiotic sensitivity

The presence of specific resistant markers against ampicillin (Ap), streptomycin (Sm) and kanamycin (Km) were studied by standard procedures. The concentration of different antibiotics used were 50-1000 ppm for ampicillin, 50-500 ppm for streptomycin and 50-100 ppm for kanamycin. The appropriate concentrations of each antibiotic were incorporated into YEMA medium at the time of plating. The presence or absence of growth at different concentrations of antibiotics was recorded after four days of incubation at 28 ± 1°C in an incubator.

3.3.6. Serological characterisation

The antiserum for the best isolate of cowpea (KA-F-C-7) was prepared by the method of Vincent (1970). The serological relationship of this isolate with other selected native isolates of cowpea, blackgram and greengram were studied by tube agglutination method.

3.4. Development of Package of Practices (POP) recommendations for acid tolerant strains of *Bradyrhizobium*

3.4.1. Pot experiments

3.4.1.1. Effect of FYM and liming

A pot culture experiment was conducted to study the effect of FYM and liming on root nodulation and plant growth in cowpea, blackgram and greengram. The experiment was laid out in CRD with three replications each using sterilised soil having an initial pH of 4.9. FYM (@20 t ha^{-1}) and lime (@250 kg ha^{-1}) were applied as per the POP recommendations of Kerala Agricultural University (1993).

3.4.1.2. Effect of FYM and pelleting

The above experiment was repeated and in treatments involving pelleting, liming was substituted by pelleting using finely powdered calcium carbonate of 300 mesh.

The best isolates of *Bradyrhizobium* selected initially from both unamended as well as amended soils were used for these experiments. Appropriate control treatments without FYM application, liming or pelleting were also maintained. The crop varieties which were used earlier namely Kanakamani for cowpea, Shyama for blackgram and Co-2 for greengram were used. Observations on nodule number, nodule dry weight and plant dry weight were recorded on 45th day of plant growth by standard procedures.

3.4.2. Field experiments

The field evaluation of selected isolates of *Bradyrhizobium* from both unamended and amended soils was done at two different locations namely College of Agriculture, Vellayani, Trivandrum and Rice Research Station of Kerala Agricultural University at Kayamkulam. The experiments were repeated during two identical seasons with the following treatments.

1. POP with seed treatment of *Bradyrhizobium* (POP^+BR^+)
2. Minus POP with seed treatment of *Bradyrhizobium* (POP^-BR^+)

3. POP without seed treatment of ***Bradyrhizobium*** ($POP^{+}BR^{-}$)
4. Minus POP without seed treatment of ***Bradyrhizobium*** ($POP^{-}BR^{-}$)

The treatments were laid out in Randomised Block Design with the crop varieties specified above with three replications each in plots of 5m x 2m. The plants were irrigated regularly till harvest. Observations on leaf area, nodule number, nodule dry weight, plant dry weight and NPK content of shoot were recorded on 45^{th} day after planting. Leaf area of cowpea, blackgram and greengram was however, taken only at College of Agriculture, Vellayani. This was due to the non-availability of a leaf area meter (LICOR-LI-3100) at RRS Kayamkulam. The grain yield was recorded as the average per plot yield of four picks for cowpea and three picks for blackgram and greengram respectively. The soil samples were also analysed for pH, organic carbon, P, K, Ca, Mg, Fe and Al content by the methods described earlier. The benefit cost ratio of all the treatments was also worked out.

3.5. Statistical analysis

The statistical analysis of the data was done by the methods described by Snedecor and Cochran (1967).

RESULTS

RESULTS

4.1. Isolation of native strains of *Bradyrhizobium*

4.1.1. Soil properties

4.1.1.1. pH

Soil sample collected from rice fallows at Pattambi had the maximum pH of 5.52 (Table 1) while the *Kari* soils of Ambalapuzha recorded the lowest pH of 3.20. The average soil pH (5.05) of category A locations was higher (Fig. 2 and 3) than category B (3.62) locations.

4.1.1.2. Organic carbon content

Marked variations in the organic carbon content were observed with different soil samples. The highest organic carbon content of 2.69 per cent was recorded in the *Kari* soil of Ambalapuzha (Table 1). It was considerably low (0.47 per cent) in the rice fallows of Kayamkulam. In the rest of the locations, the organic carbon content varied from 0.68 per cent (rice fallows of Vellayani) to 0.93 per cent (rice fallows of Pilicode) with a mean value of 0.76 and 1.79 per cent respectively for category A and B locations.

Table 1. Chemical properties of soil at different locations

Location	pH	Organic carbon %	Ca ppm	Mg ppm	Mo ppm	Fe ppm	Al ppm
Category A							
1. Vellayani							
Garden land	4.50	0.71	134.9	125.6	0.0145	94.3	17.6
Rice fallow	4.45	0.68	123.7	138.7	0.0175	86.5	11.2
2. Kayamkulam							
Garden land	5.10	0.77	146.7	119.3	0.0085	88.5	15.6
Rice fallow	4.42	0.47	136.7	176.3	0.0170	87.6	13.6
3. Pattambi							
Garden land	5.45	0.78	900.0	78.0	0.0050	82.1	90.0
Rice fallow	5.52	0.81	560.0	102.5	0.0105	80.3	61.5
4. Pilicode							
Garden land	5.51	0.90	990.0	155.6	0.0185	97.8	54.5
Rice fallow	5.45	0.93	810.0	54.5	0.0200	80.7	144.0
Mean of A	5.05	0.76	475.3	118.8	0.0139	87.2	51.0
Category B							
5. Ambalapuzha	3.20	2.69	405.0	52.6	0.0050	96.5	407.0
6. Kuttanad	3.55	1.95	930.0	231.5	0.0075	98.4	395.0
7. Vyttila	4.10	0.73	187.6	214.3	0.0150	85.2	35.3
Mean of B	3.62	1.79	507.5	166.1	0.0092	93.4	279.1

4.1.1.3. Calcium, magnesium and molybdenum content

Ca, Mg and Mo content of different soil samples also varied with locations. The Ca content of 990 ppm was higher in the garden soil of Pilicode. The Mg (231.50 ppm) and Mo (0.0200 ppm) contents were maximum in the *Kayal* soil of Kuttanad and rice fallows of Pilicode respectively (Table 1). In the other locations, presence of these elements varied from 187.6 ppm (*hydromorphic saline* soils of Vyttila) to 930 ppm (*Kayal* soil of Kuttanad) for Ca, 52.6 ppm (*Kari* soils of Ambalapuzha) to 214.3 ppm (*hydromorphic saline* soils of Vyttila) for Mg and from 0.0050 ppm (gardenland of Pattambi and *Kari* soils of Ambalapuzha) to 0.0185 ppm (gardenland of Pilicode) for Mo. The average content of these elements were 475.3, 118.8 and 0.0139 ppm for category A and 507.5, 166.1 and 0.0092 ppm for category B locations respectively.

4.1.1.4. Iron and aluminium content

The Fe and Al content of soil samples were uniformly higher in category B locations. These were 93.4 and 279.1 ppm in category B compared to only 87.2 and 51.0 ppm in category A locations respectively (Table 1, Fig. 4-7). The *Kayal* soil of Kuttanad and *Kari* soil of Ambalapuzha had the highest content of Fe (98.4 ppm) and Al (407.0 ppm).

4.1.2. Root nodulation and plant growth characteristics

Root nodulation and plant growth characteristics of cowpea, blackgram and greengram were uniformly better at category A locations. Here, the average nodule number and plant dry weight were 7.9, 8.7 and 8.2 and 9.7g, 7.6g and 7.2 g respectively for cowpea, blackgram and greengram (Table 2, Fig. 2-9). However, at category B locations, the corresponding figures were only 3.5, 3.2 and 5.3 and 3.7g, 2.7g and 1.7g respectively. A similar trend was also noticed in nodule dry weight of blackgram (14.0 mg) and greengram (13.8 mg). In cowpea this was higher for category B locations.

4.2. Selection of efficient cultures of *Bradyrhizobium*

Forty three cultures of *Bradyrhizobium* from cowpea (17), blackgram (13) and greengram (13) were isolated from different locations. Nodulation efficiency of these isolates were studied both in unamended and amended soils.

4.2.1. Cowpea

In unamended soil, the plant dry weight was maximum (17.3g) after seed treatment with Kayamkulam isolate KA-G-C-4. This culture was significantly superior to all the other isolates (Table 3, Plate 1). However, nodule number and nodule dry weight were more with another isolate (KA-G-C-5) from the same location. These were 28.1 and 46.5 mg respectively. The number of nodules formed with KA-G-C-4 was statistically on par with that of KA-G-C-5.

Table 2. Root nodulation and plant growth characteristics* of cowpea, blackgram and greengram at different locations

Location	Cowpea			Blackgram			Greengram		
	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
Category A									
1. Vellayani									
Garden land	12.3	13.8	20.5	8.9	32.9	16.1	9.7	54.3	9.7
Rice fallow	9.7	42.9	23.3	2.4	21.6	20.4	3.2	17.3	17.8
2. Kayamkulam									
Garden land	10.4	23.8	14.5	4.8	3.8	2.8	11.1	17.6	8.1
Rice fallow	2.9	5.7	13.8	3.2	27.0	19.0	2.7	4.2	17.7
3. Pattambi									
Garden land	7.3	12.5	0.7	11.6	6.9	0.4	10.3	5.2	0.5
Rice fallow	5.8	14.3	0.9	8.7	7.7	0.4	6.7	0.5	1.2
4. Pilicode									
Garden land	8.1	9.2	2.0	12.3	7.3	1.0	9.7	5.9	1.3
Rice fallow	6.3	11.8	1.7	17.8	4.4	0.7	11.9	5.5	1.2
Mean of A	7.9	16.8	9.7	8.7	14.0	7.6	8.2	13.8	7.2
Category B									
5. Ambalapuzha	2.1	3.1	0.5	2.2	1.3	0.2	7.6	4.4	0.3
6. Kuttanad	3.1	11.9	0.54	3.9	8.4	0.4	5.6	5.9	0.2
7. Vyttila	5.2	41.2	10.2	3.6	27.6	1.3	2.8	23.8	4.6
Mean of B	3.5	18.7	3.7	3.2	12.4	2.7	5.3	11.4	1.7

* Mean of 10 plants at each locations

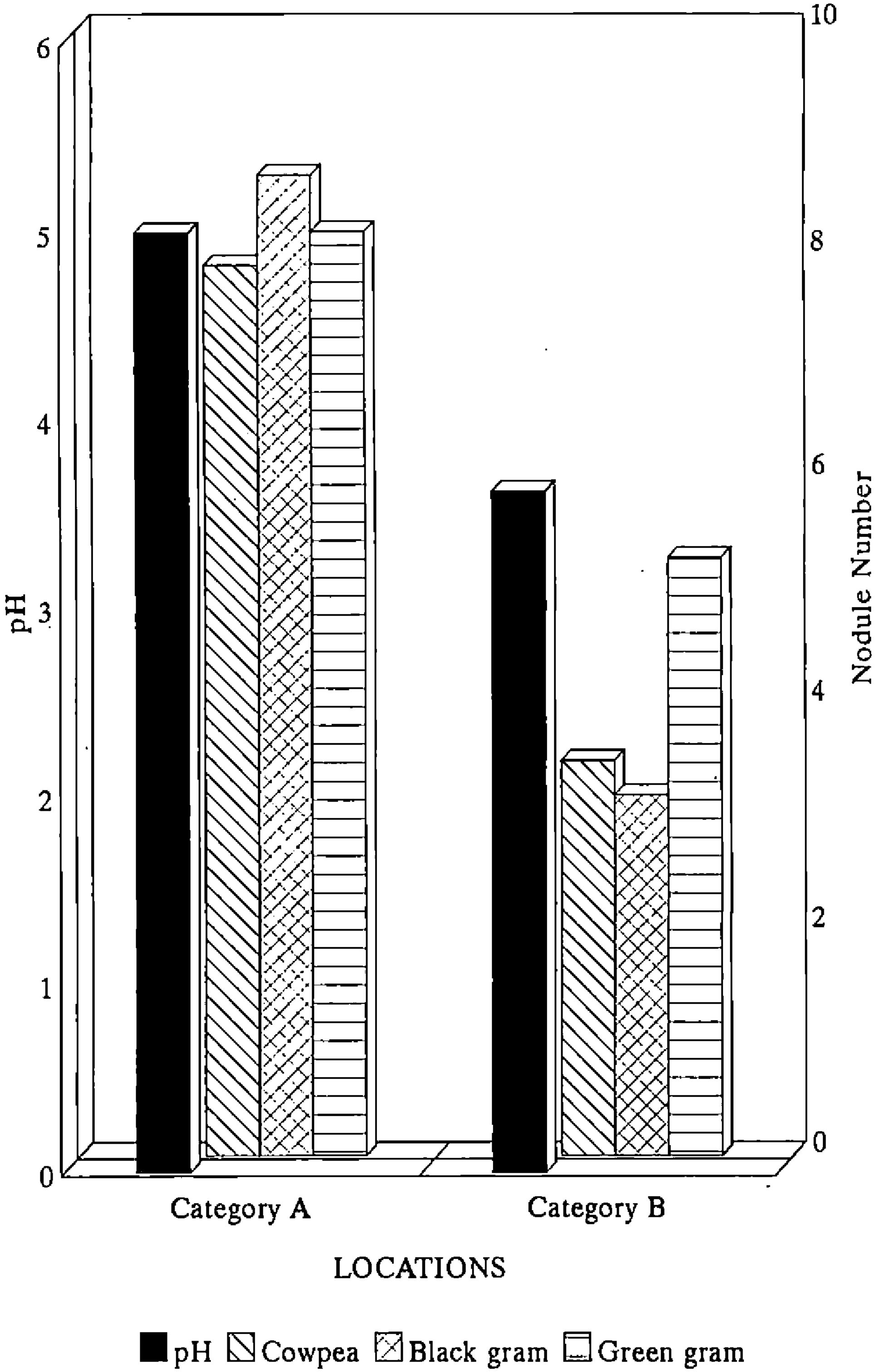


Fig. 2. Influence of Soil pH on root nodulation in cowpea, blackgram and greengram

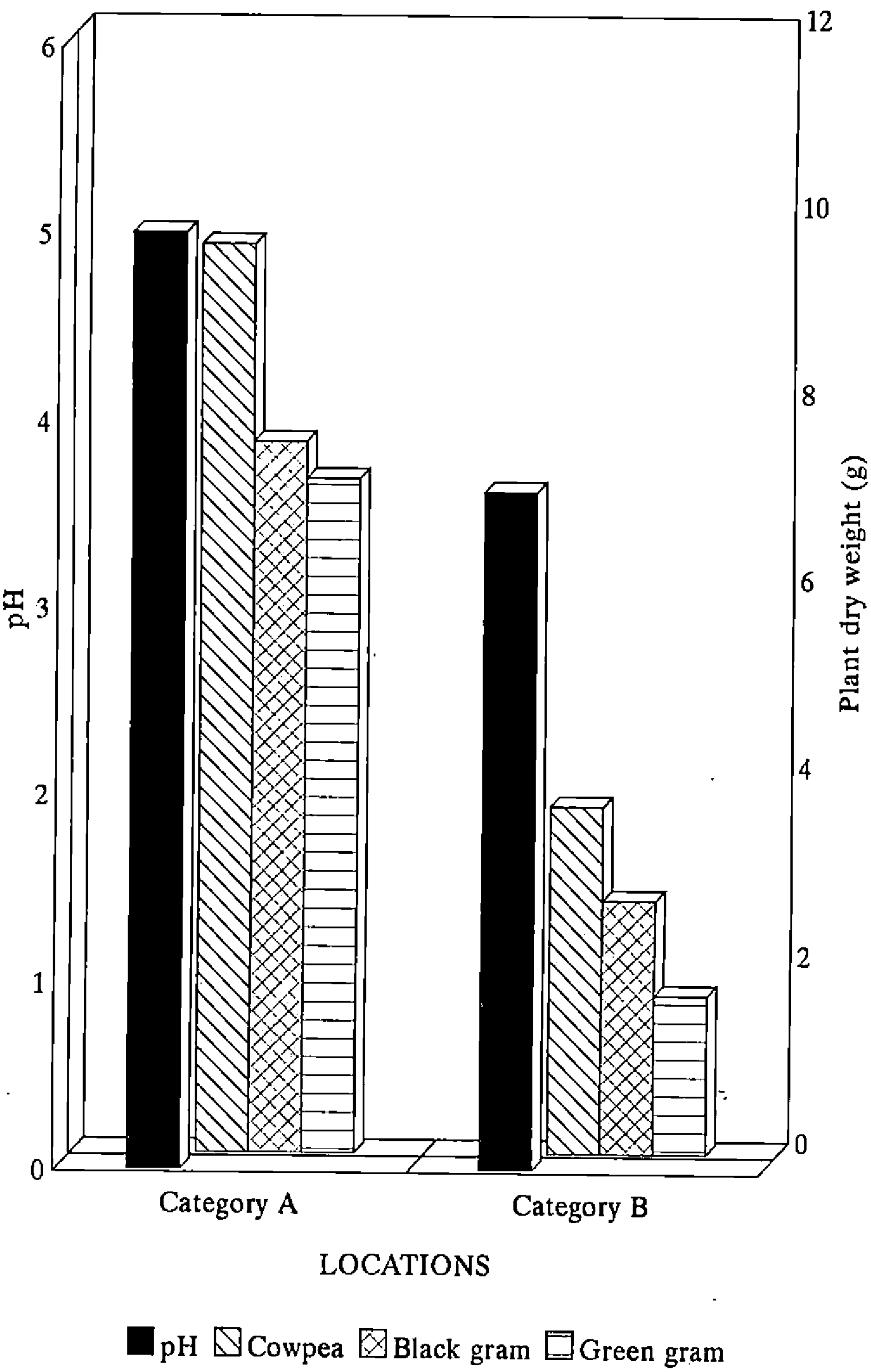


Fig. 3. Influence of Soil pH on plant dry weight in cowpea, blackgram and greengram

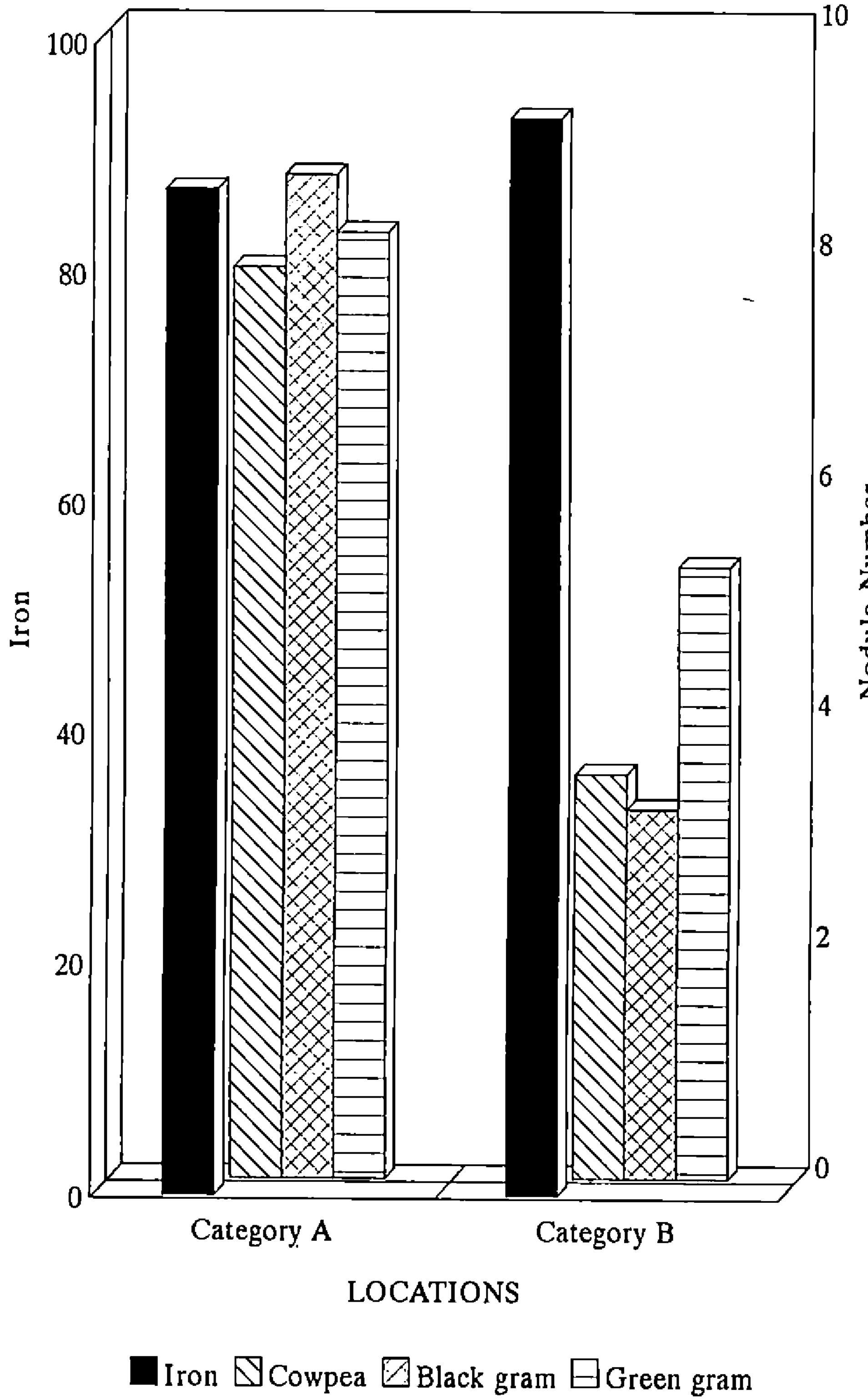


Fig. 4. Influence of iron on root nodulation in cowpea, blackgram and greengram

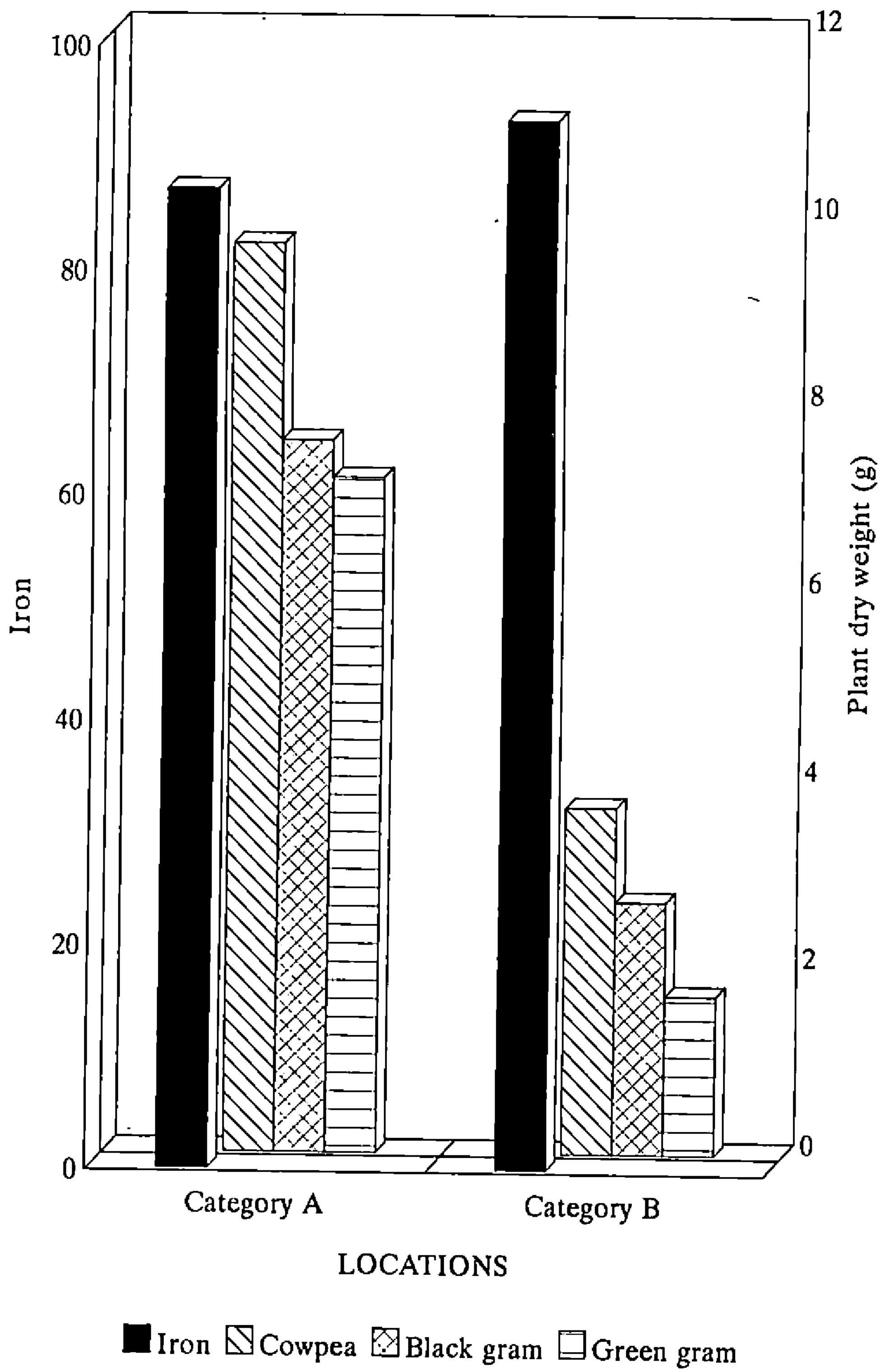


Fig. 5. Influence of iron on plant dry weight in cowpea, blackgram and greengram

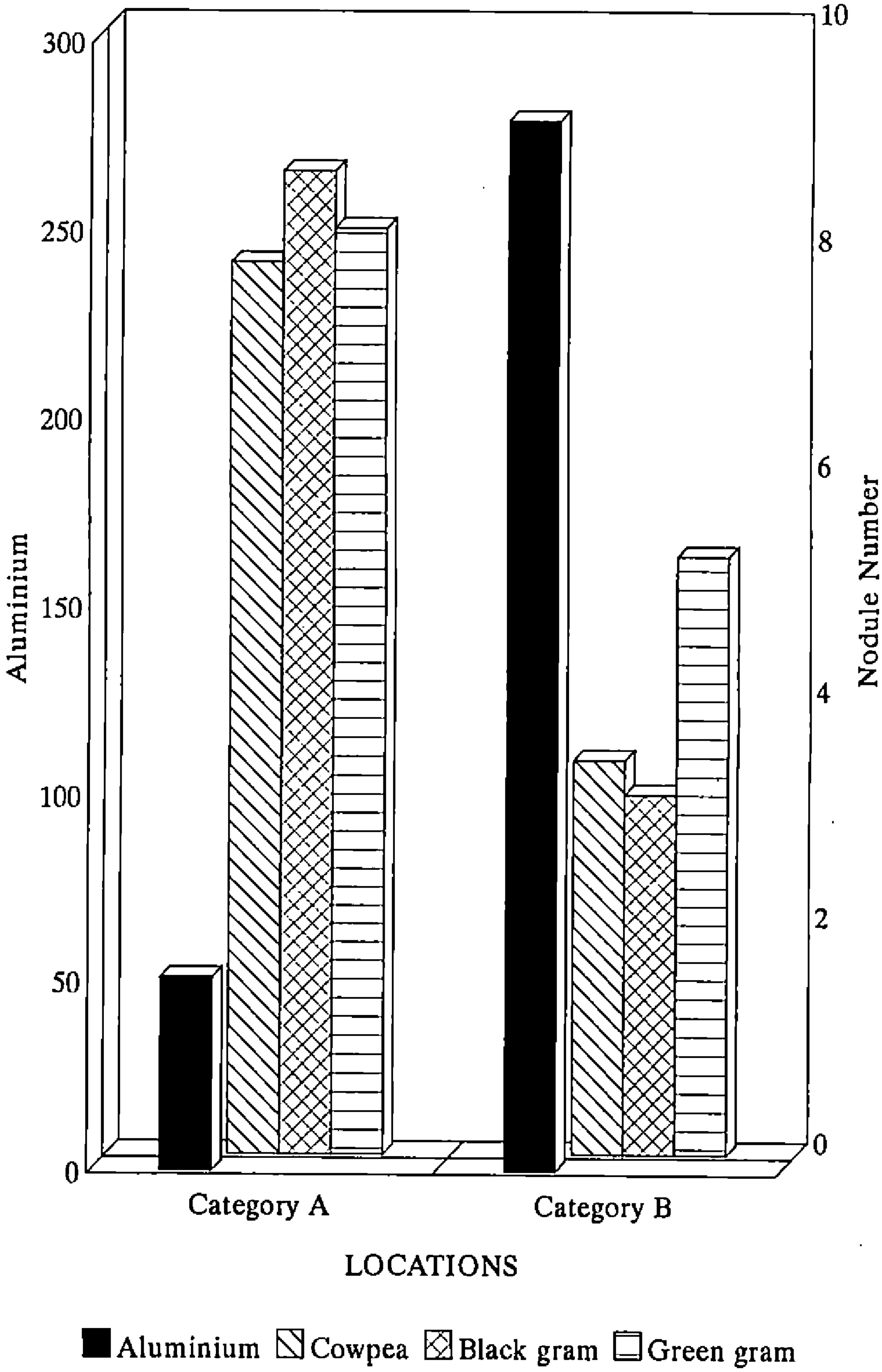


Fig. 6. Influence of aluminium on root nodulation in cowpea, blackgram and greengram

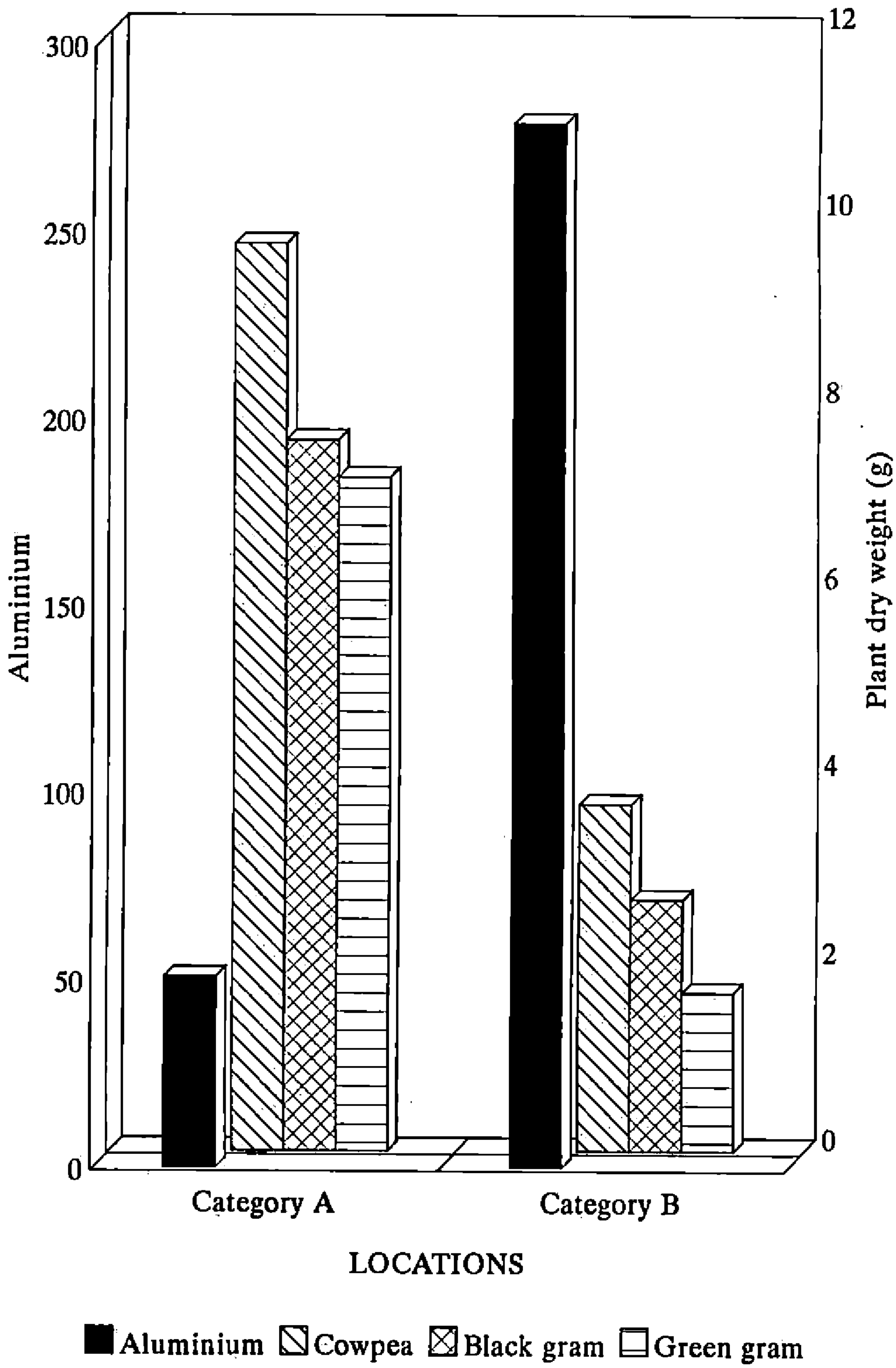


Fig. 7. Influence of aluminium on plant dry weight in cowpea, blackgram and greengram

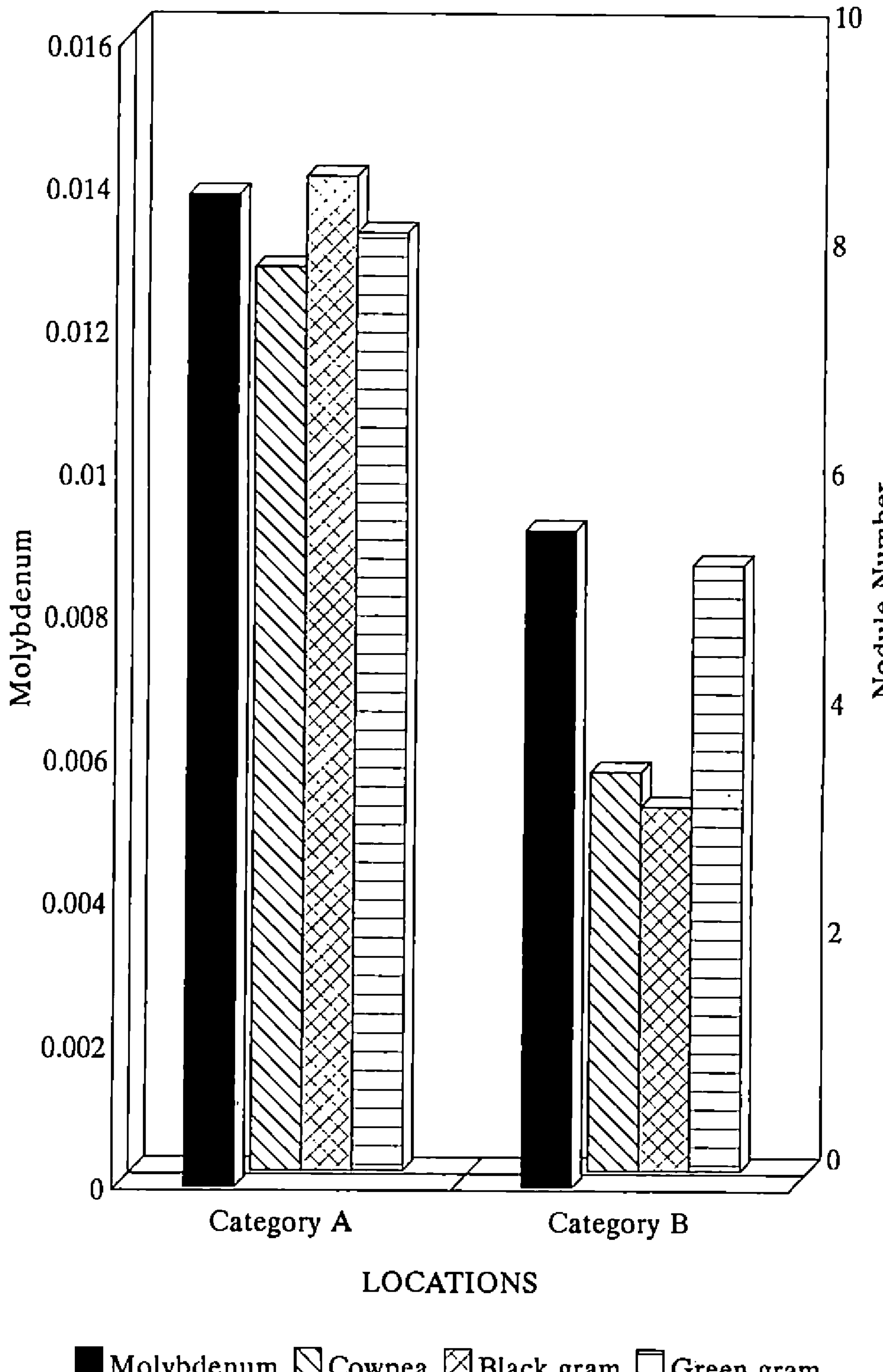


Fig. 8. Influence of molybdenum on root nodulation in cowpea, blackgram and greengram

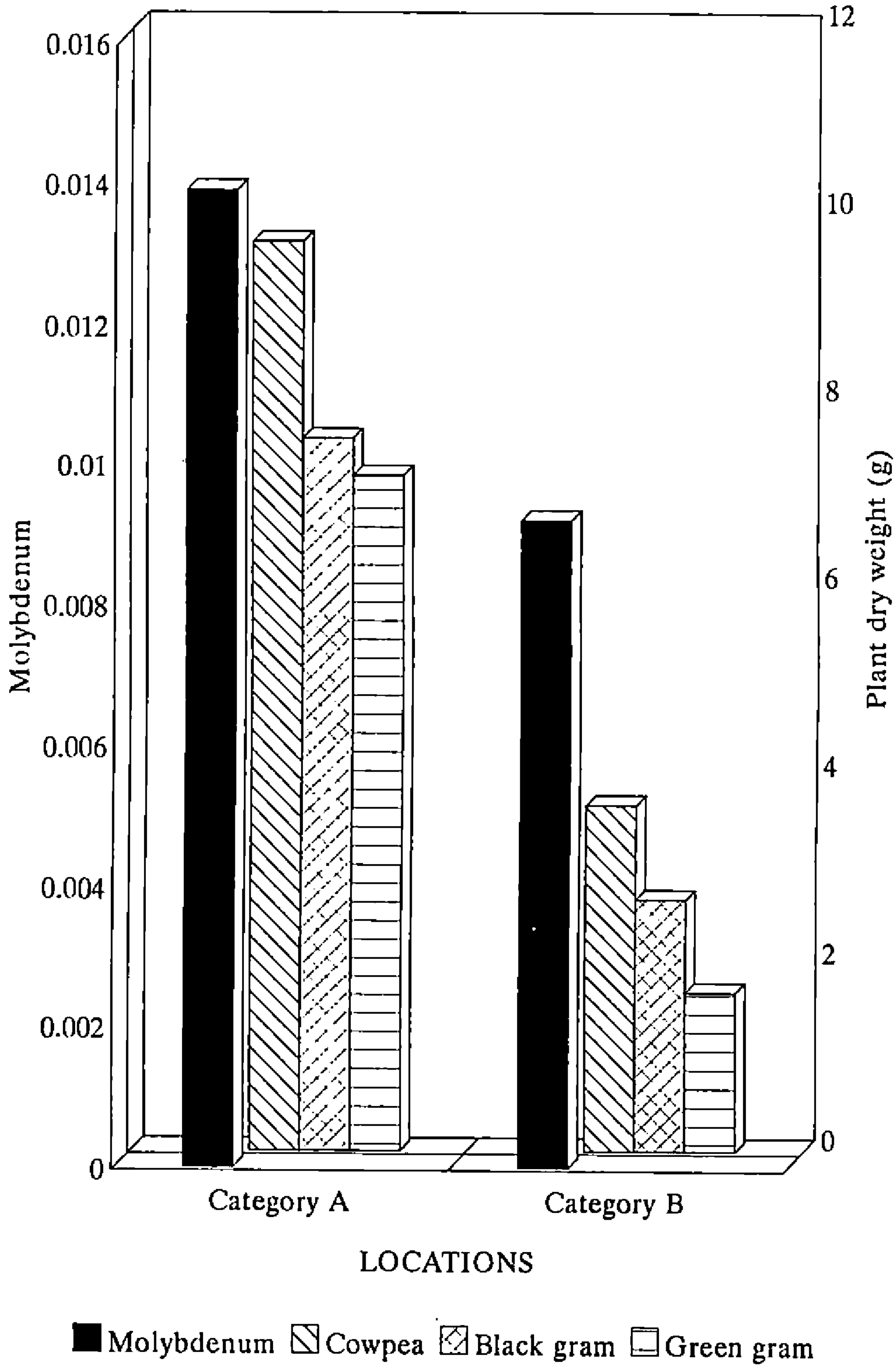


Fig. 9. Influence of molybdenum on plant dry weight in cowpea, blackgram and greengram

Table 3. Root nodulation efficiency of *Bradyrhizobium* isolates from cowpea in unamended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-C-1	10.6	30.1	9.4
VE-G-C-2	12.2	21.4	10.9
VE-F-C-3	11.3	24.9	9.3
KA-G-C-4	25.8	18.2	**17.3**
KA-G-C-5	28.1	46.5	10.4
KA-F-C-6	9.9	9.0	9.1
KA-F-C-7	14.1	10.5	13.3
PA-G-C-8	8.0	8.7	7.8
PA-F-C-9	9.4	8.3	8.6
PA-F-C-10	7.4	7.6	3.7
PA-F-C-11	12.7	23.7	10.0
PI-G-C-12	8.8	7.9	5.6
PI-G-C-13	6.0	4.1	4.1
PI-F-C-14	10.3	10.6	4.6
AM-K-C-15	5.1	5.8	6.5
KU-K-C-16	5.9	7.3	3.9
VY-S-C-17	11.1	9.2	5.8
Control B^-F^+	2.6	7.0	7.3
Control B^-F^-	1.8	5.3	4.7
CD(0.05)	2.9	7.2	2.6

B^- – without *Bradyrhizobium* inoculation

$F^{+/-}$ – with or without fertilizer application

1. Effect of inoculation with KA-G-C-4 isolate on plant growth of cowpea in unamended soil

   9 - inoculated with KA-G-C-4 isolate

   6 - control without *Bradyrhizobium* and fertilizer application

2. Effect of inoculation with KA-F-C-7 isolate on plant growth of cowpea in amended soil

   1 - inoculated with KA-F-C-7 isolate

   4 - control without *Bradyrhizobium* and fertilizer application

Plate 1

Plate 2

In amended soil also, the plant dry weight of 33.3 g was maximum with an isolate from Kayamkulam, KA-F-C-7 (Table 4, Plate 2). As in the above experiment, this culture was significantly superior to all other isolates. The nodule number of 26.2 and nodule dry weight of 81.1 mg were, however, more with two Vellayani isolates VE-F-C-3 and VE-G-C-1 respectively. The number of nodules formed (21.7) by VY-S-C-17 from Vyttila and nodule dry weight (71.9 mg) with VE-F-C-3 were statistically on par with the above treatments. A similar trend, particularly with respect to nodule number, was obtained with six other isolates from category A locations such as KA-G-C-4 (22.5), KA-G-C-5 (20.0), KA-F-C-6 (20.5), PI-G-C-12 (21.4), PI-G-C-13 (21.0) and PI-F-C-14 (23.9).

The best isolate from unamended, KA-G-C-4 and amended soil, KA-F-C-7 which produced maximum plant dry weight (Fig. 10) were used for further studies.

4.2.2. Blackgram

In unamended soil, the plant dry weight of 23.3 g was maximum after seed treatment with an isolate from Vellayani, VE-G-B-2 (Table 5, Plate 3). The increase in plant dry weight with three other isolates, VE-F-B-3 (17.8 g), KA-G-B-5 (18.4 g) and KA-F-B-6 (18.7 g) were statistically on par with the above treatment.

Table 4. Root nodulation efficiency of *Bradyrhizobium* isolates from cowpea in amended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-C-1	12.8	81.1	15.0
VE-G-C-2	9.3	1.7	12.5
VE-F-C-3	26.2	71.9	14.2
KA-G-C-4	22.5	44.3	10.8
KA-G-C-5	20.0	38.2	17.5
KA-F-C-6	20.5	42.6	15.0
KA-F-C-7	18.1	57.5	**33.3**
PA-G-C-8	10.6	17.1	14.4
PA-F-C-9	12.8	28.6	17.8
PA-F-C-10	11.3	13.2	11.1
PA-F-C-11	10.9	15.9	10.6
PI-G-C-12	21.4	15.0	12.2
PI-G-C-13	21.0	14.0	10.6
PI-F-C-14	23.9	19.6	17.3
AM-K-C-15	3.1	5.9	13.9
KU-K-C-16	4.3	8.1	7.2
VY-S-C-17	21.7	35.2	14.2
Control B^-F^+	2.0	1.0	6.1
Control B^-F^-	0.8	0.3	4.4
CD(0.05)	6.7	15.8	5.8

B^- – without *Bradyrhizobium* inoculation

$F^{+/-}$ – with or without fertilizer application

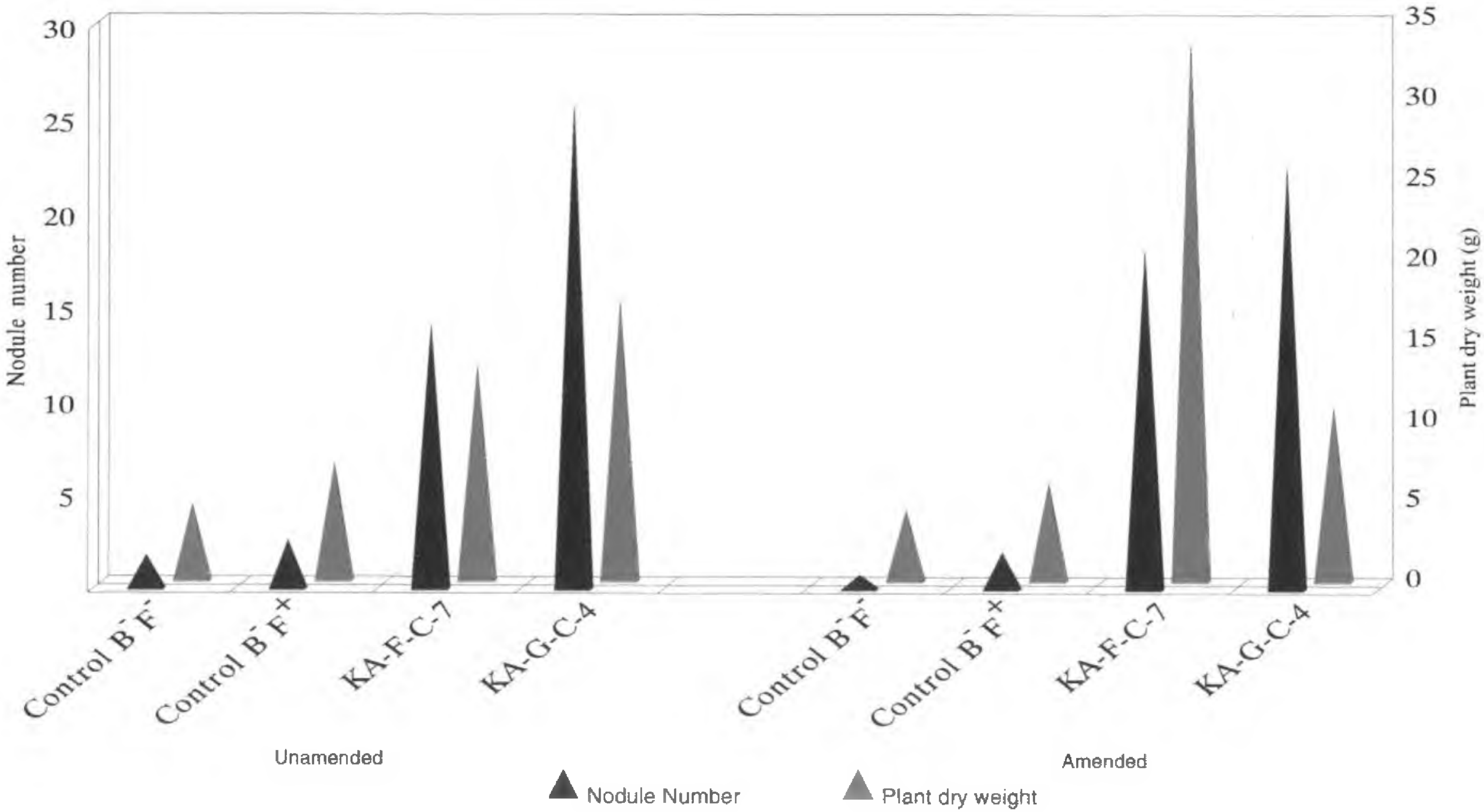


Fig. 10. Comparative performance of selected isolates of *Bradyrhizobium* suitable for cowpea

Table 5. Root nodulation efficiency of *Bradyrhizobium* isolates from blackgram in unamended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-B-1	24.4	20.0	16.7
VE-G-B-2	18.1	14.4	**23.3**
VE-F-B-3	13.4	11.9	17.8
VE-F-B-4	14.0	7.7	9.2
KA-G-B-5	11.7	8.3	18.4
KA-F-B-6	23.1	18.3	18.7
PA-G-B-7	12.4	8.2	8.1
PA-F-B-8	12.0	7.6	5.6
PI-G-B-9	14.3	9.1	14.4
PI-F-B-10	12.4	8.2	8.1
AM-K-B-11	7.0	3.3	4.5
KU-K-B-12	7.9	3.2	6.5
VY-S-B-13	16.1	19.6	12.5
Control B^-F^+	4.3	3.6	5.6
Control B^-F^-	3.1	4.1	6.2
CD(0.05)	6.0	7.0	5.6

B^- – without *Bradyrhizobium* inoculation

$F^{+/-}$ – with or without fertilizer application

3. Effect of inoculation with VE-G-B-2 isolate on plant growth of blackgram in unamended soil

2 - inoculated with VE-G-B-2 isolate

6 - control without *Bradyrhizobium* and fertilizer application

4. Effect of inoculation with KA-F-B-6 isolate on plant growth of blackgram in amended soil

1 - inoculated with KA-F-B-6 isolate

4 - control without *Bradyrhizobium* and fertilizer application

Plate 3

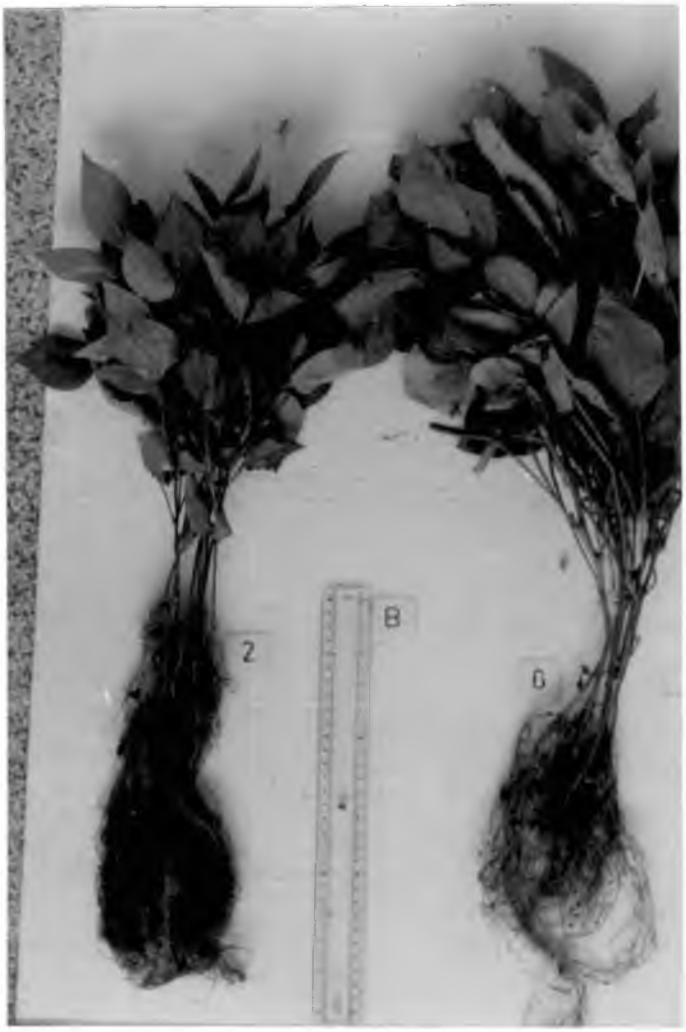
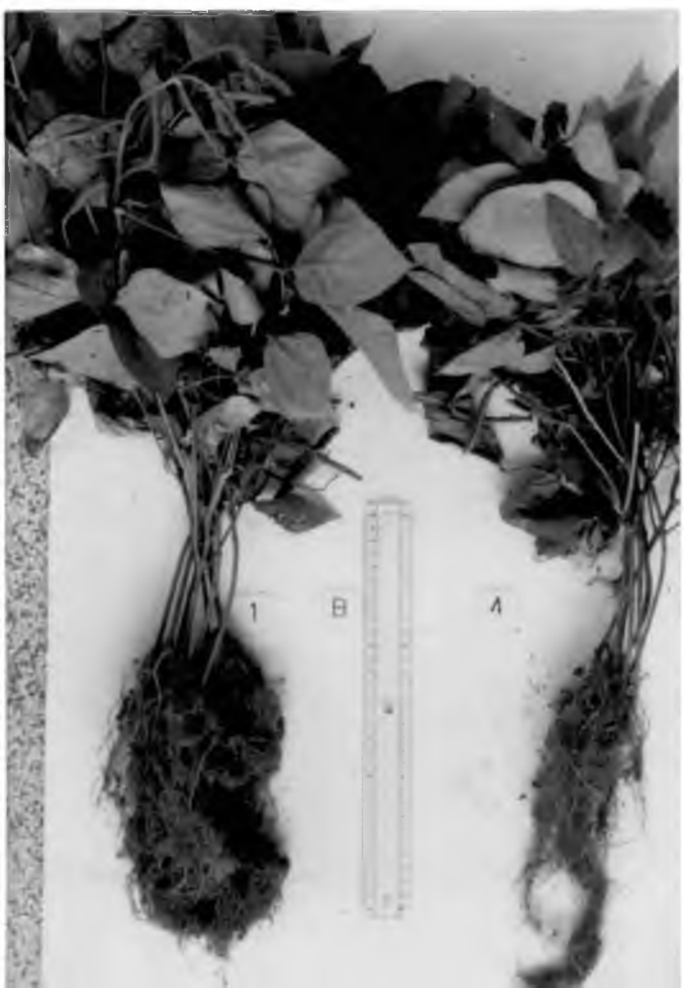


Plate 4



The nodule number of 24.4 and nodule dry weight of 20.0 mg were, however, more with VE-G-B-1 isolate. The increase in nodule number with KA-F-B-6 (23.1) and nodule dry weight with VE-G-B-2 (14.4 mg) and KA-F-B-6 (18.3 mg) isolates were statistically on par with that of VE-G-B-1 treatment. Such an effect particularly with respect to nodule dry weight was obtained with VY-S-B-13 isolate from Vyttila.

In amended soil, maximum plant dry weight of 18.9 g was obtained with Kayamkulam isolate KA-F-B-6 (Table 6, Plate 4). But the plant dry weight with the isolates PA-F-B-8 (18.3 g) and PI-F-B-10 (17.2 g) were statistically on par with the above treatment. The nodule number of 28.8 and nodule dry weight of 17.4 mg were, however, more with VE-F-B-3 and PA-G-B-7 isolates respectively. The increase in nodule number with VE-G-B-1 (26.8) and VE-F-B-4 (26.0) isolates were statistically on par with that of VE-F-B-3 treatment.

The best isolate from unamended, VE-G-B-2 and amended soil, KA-F-B-6 which produced maximum increase in plant dry weight (Fig. 11) were used for further studies.

4.2.3. Greengram

In unamended soil, plant dry weight of 9.4 g was maximum after seed treatment with Pattambi isolate, PA-G-G-5 (Table 7, Plate 5). The nodule number of 28.3 and nodule dry weight of 27.0 mg were, however, more with VE-F-G-2 and PA-F-G-7 isolates respectively.

Table 6. Root nodulation efficiency of *Bradyrhizobium* isolates from blackgram in amended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-B-1	26.8	6.4	7.5
VE-G-B-2	11.7	5.1	9.2
VE-F-B-3	28.8	1.9	8.3
VE-F-B-4	26.0	2.9	10.0
KA-G-B-5	4.0	1.6	11.7
KA-F-B-6	11.7	3.1	**18.9**
PA-G-B-7	19.6	17.4	12.5
PA-F-B-8	14.4	12.6	18.3
PI-G-B-9	19.1	11.2	10.5
PI-F-B-10	10.8	9.2	17.2
AM-K-B-11	6.0	4.4	7.8
KU-K-B-12	2.6	0.2	7.2
VY-S-B-13	22.6	1.1	10.8
Control B^-F^+	3.6	4.2	3.9
Control B^-F^-	2.9	0.3	6.7
CD(0.05)	6.3	2.8	6.2

B^- – without *Bradyrhizobium* inoculation

$F^{+/-}$ – with or without fertilizer application

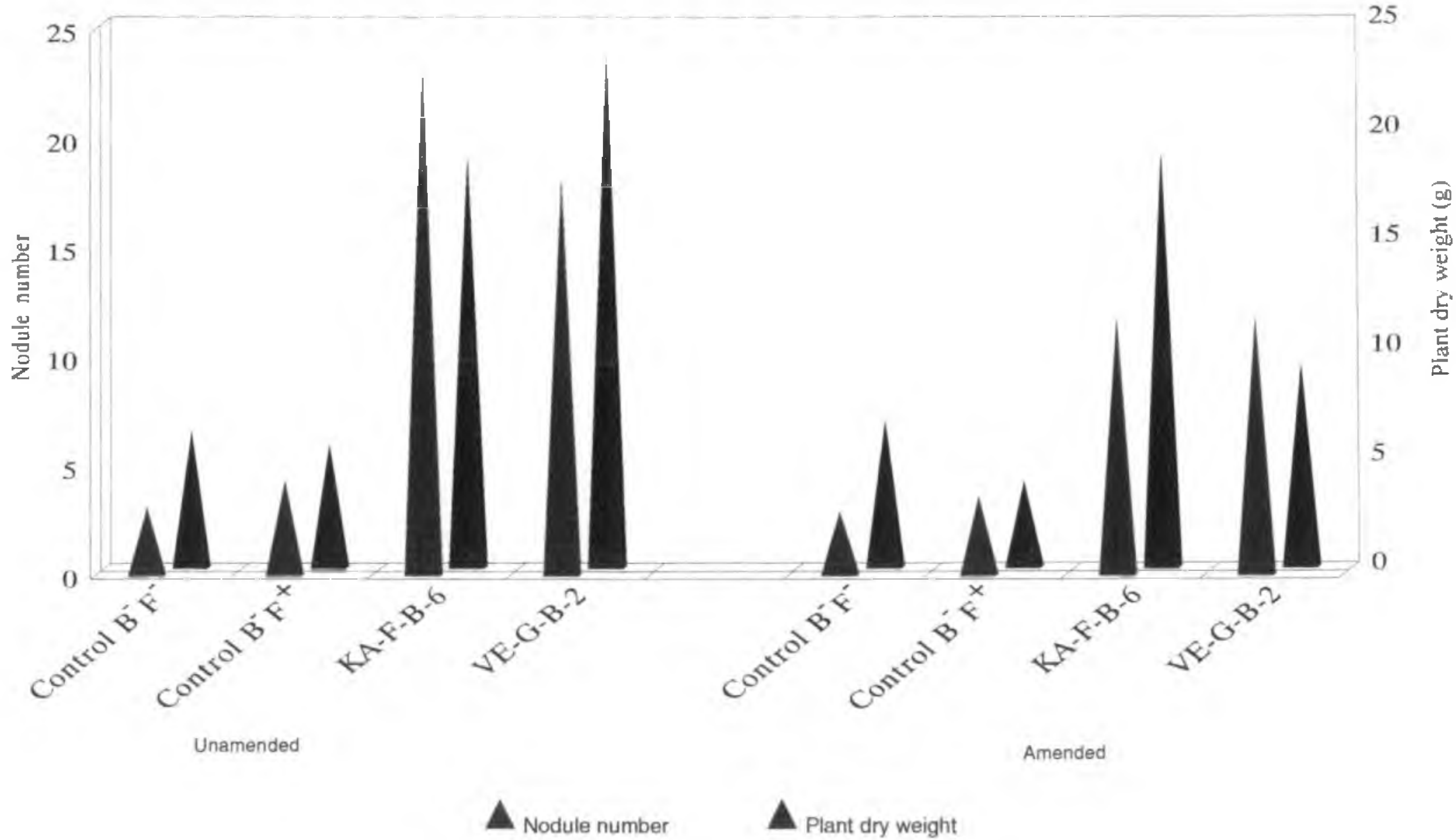


Fig. 11. Comparative performance of selected isolates of *Bradyrhizobium* suitable for blackgram

Table 7. Root nodulation efficiency of *Bradyrhizobium* isolates from greengram in unamended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-G-1	10.8	9.7	4.0
VE-F-G-2	28.3	17.1	5.5
KA-G-G-3	12.4	11.4	3.3
KA-F-G-4	16.8	10.0	3.6
PA-G-G-5	23.3	16.4	**9.4**
PA-F-G-6	21.9	8.5	5.8
PA-F-G-7	22.8	27.0	7.0
PI-G-G-8	17.3	12.9	3.9
PI-F-G-9	20.2	15.2	4.6
AM-K-G-10	6.2	4.1	3.0
KU-K-G-11	7.0	2.8	5.2
VY-S-G-12	24.4	16.8	3.0
VY-S-G-13	21.8	14.0	2.7
Control B^-F^+	16.7	8.5	3.9
Control B^-F^-	1.1	4.1	2.9
CD(0.05)	5.7	3.7	1.8

B^- - without *Bradyrhizobium* inoculation

$F^{+/-}$ - with or without fertilizer application

5. Effect of inoculation with PA-G-G-5 isolate on plant growth of greengram in unamended soil

2 - inoculated with PA-G-G-5 isolate

6 - control without *Bradyrhizobium* and fertilizer application

6. Effect of inoculation with PI-G-G-8 isolate on plant growth of greengram in amended soil

1 - inoculated with PI-G-G-8 isolate

4 - control without *Bradyrhizobium* and fertilizer application

Plate 5

Plate 6

The increase in nodule number with PA-G-G-5 (23.3) and PA-F-G-7 (22.8) were statistically on par with that of VE-F-G-2 isolate. A similar effect was also obtained with VY-S-G-12 isolate from Vyttila.

In amended soil, the maximum plant dry weight of 16.7 g was obtained with the isolate from Pilicode, PI-G-G-8 (Table 8, Plate 6). The increase in plant dry weight with KA-G-G-3 (15.8g) from Kayamkulam was statistically on par with the above treatment. The nodule number of 28.7 and nodule dry weight of 39.4 mg were, however, more with KA-F-G-4 and KA-G-G-3 isolates respectively. The increase in nodule number with PI-G-G-8 (24.0), PI-F-G-9 (21.3) isolates from Pilicode and VY-S-G-12 (24.7) isolate from Vyttila were statistically on par with that of KA-F-G-4 treatment. A similar response with respect to nodule dry weight was obtained with VE-F-G-2 (38.4 mg) isolate from Vellayani.

The best isolate from unamended, PA-G-G-5 and amended soil, PI-G-G-8 which produced maximum increase in plant dry weight (Fig. 12) were used for further studies.

4.3. Characterisation of different isolates of *Bradyrhizobium*

The six selected native isolates of *Bradyrhizobium* from cowpea, blackgram and greengram were fast growing, gram negative and did not produce 3-ketolactose on lactose medium. They produced, white translucent glistening and elevated colonies with entire margin on YEMA.

Table 8. Root nodulation efficiency of *Bradyrhizobium* isolates from greengram in amended soil

Isolates	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)
VE-G-G-1	21.0	17.5	10.8
VE-F-G-2	21.2	38.4	3.3
KA-G-G-3	20.7	39.4	15.8
KA-F-G-4	28.7	19.2	6.7
PA-G-G-5	12.4	19.5	10.0
PA-F-G-6	9.1	10.4	9.4
PA-F-G-7	15.2	17.5	11.7
PI-G-G-8	24.0	30.7	**16.7**
PI-F-G-9	21.3	16.6	10.6
AM-K-G-10	10.8	8.5	9.4
KU-K-G-11	14.6	8.7	4.4
VY-S-G-12	24.7	28.4	9.2
VY-S-G-13	20.5	14.1	5.0
Control B^-F^+	1.9	5.2	5.0
Control B^-F^-	2.8	0.4	4.0
CD(0.05)	7.4	5.2	3.4

B^- – without *Bradyrhizobium* inoculation

$F^{+/-}$ – with or without fertilizer application

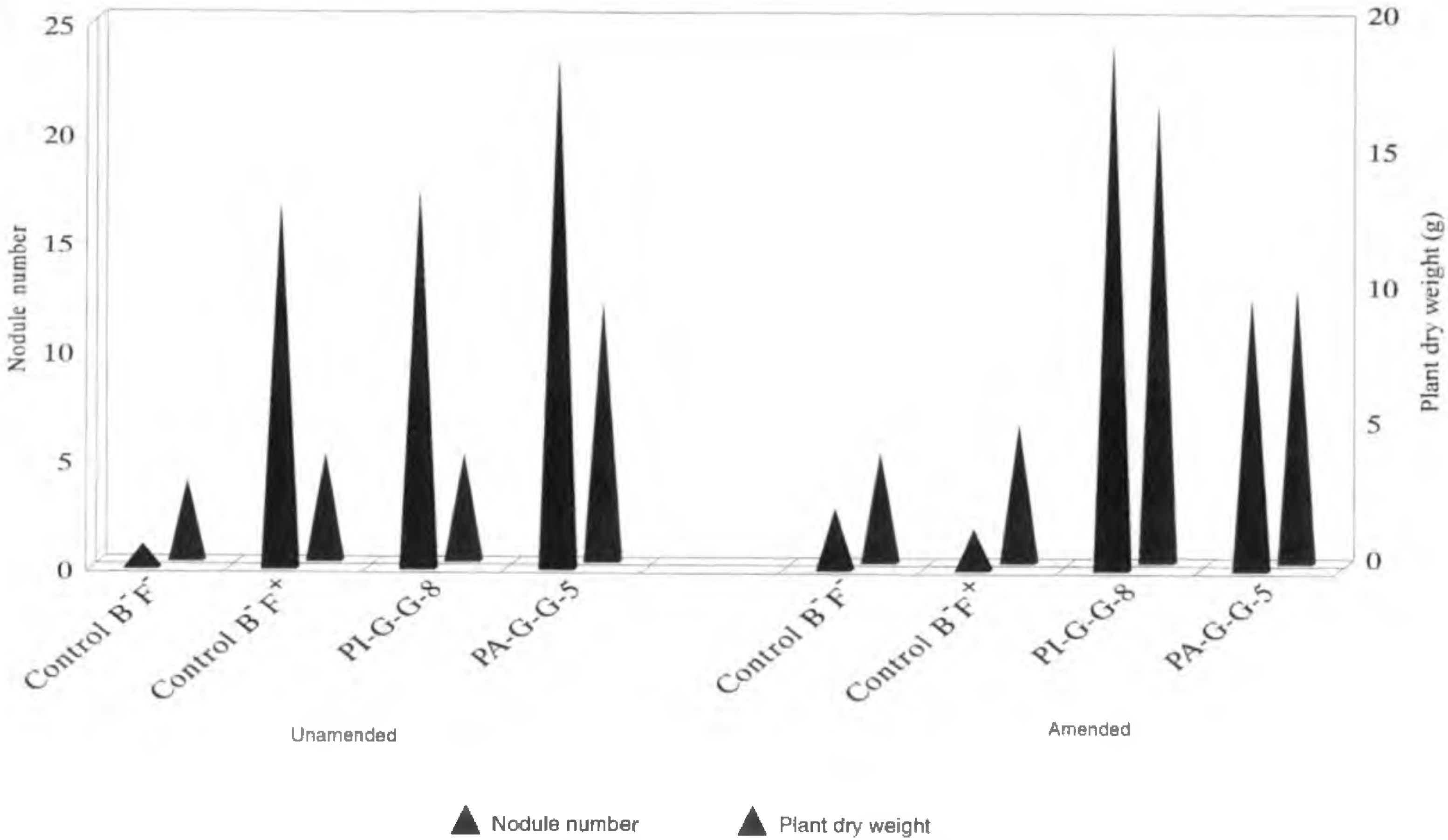


Fig. 12. Comparative performance of selected isolates of *Bradyrhizobium* suitable for greengram

An acidic reaction was observed on YEMA with bromothymol blue which resulted in change in colour of the medium to yellow with all the isolates except KA-F-C-7 (Table 9). The growth of all the isolates were poor on glucose peptone agar medium and totally absent in Hofer's alkaline medium of pH 11.0.

4.3.1. Utilisation of different carbon sources

The growth of the native isolates was maximum (mean OD_{600} = 0.216) in arabinose while for exotic isolates it was maximum in glucose (mean OD_{600} = 0.193) (Table 10, Fig. 13 and 14). The growth pattern was almost identical in mannitol (mean OD_{600} = 0.167 and 0.165) and xylose (mean OD_{600} = 0.159 and 0.155) for both the native as well as the exotic isolates. However, the growth of the native isolates was relatively poor in glucose and galactose with a mean OD_{600} of 0.167 and 0.160 respectively, compared to that of 0.193 and 0.179 for the exotic isolates. Among individual isolates, growth of VE-G-B-2 in mannitol (mean OD_{600} = 0.178), PA-G-G-5 in arabinose (mean OD_{600} = 0.227) and PI-G-G-8 in Xylose (mean OD_{600} = 0.169) was maximum. Similarly among exotic isolates growth of GG-IARI in glucose (mean OD_{600} = 0.202) and galactose (mean OD_{600} = 0.182) was maximum.

Table 9. Characteristics of selected native isolates of *Bradyrhizobium*

Bradyrhizobium isolates	Gram staining	YEMA + congored	YEMA + BTB	GPA	Hofer's alkaline medium	Ketolactose
KA-G-C-4		White translucent colonies		Little growth	No growth	Negative
KA-F-C-7		White translucent colonies		Little growth	No growth	Negative
VE-G-B-2		White translucent colonies		Little growth	No growth	Negative
KA-F-B-6		White translucent colonies		Little growth	No growth	Negative
PA-G-G-5		White translucent colonies		Little growth	No growth	Negative
PI-G-G-8		White translucent colonies		Little growth	No growth	Negative

Gram negative   Acid production   No acid production

Table 10. Carbohydrate utilisation pattern of *Bradyrhizobium* and *Rhizobium* isolates under *in vitro* conditions

Isolates	Carbon sources				
	Mannitol	Arabinose	Xylose	Glucose	Galactose
A. *Bradyrhizobium*					
KA-G-C-4	0.171	0.202	0.160	0.162	0.162
KA-F-C-7	0.161	0.211	0.155	0.157	0.154
VE-G-B-2	0.178	0.222	0.160	0.161	0.164
KA-F-B-6	0.163	0.219	0.153	0.167	0.159
PA-G-G-5	0.171	0.227	0.155	0.165	0.164
PI-G-G-8	0.157	0.212	0.169	0.189	0.155
Mean OD_{600}	0.167	0.216	0.159	0.167	0.160
B. *Rhizobium*					
CP-IARI	0.156	0.168	0.152	0.193	0.177
BG-IARI	0.169	0.158	0.163	0.183	0.179
GG-IARI	0.169	0.159	0.149	0.202	0.182
Mean OD_{600}	0.165	0.162	0.155	0.193	0.179
CD (0.05) : 0.007					

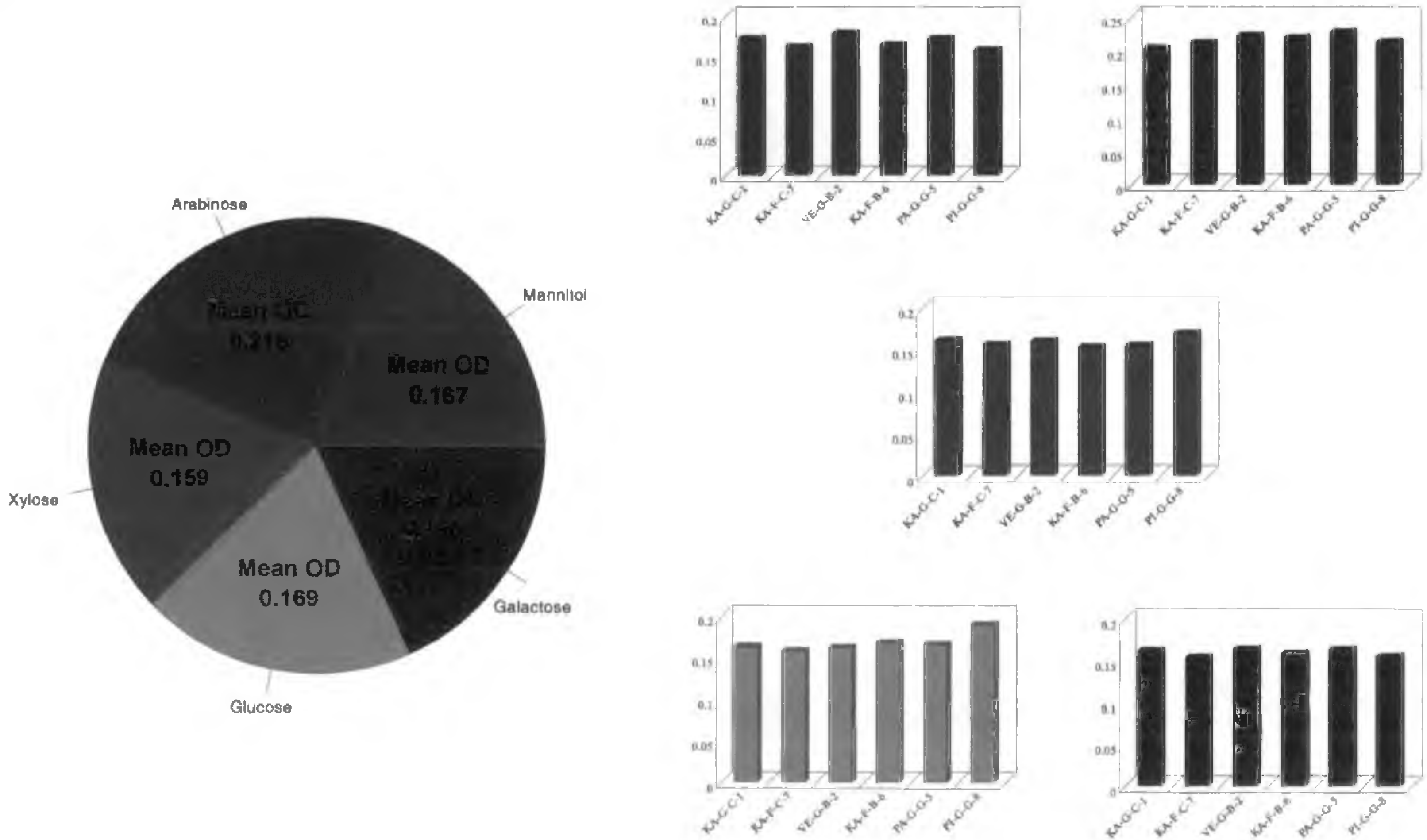


Fig. 13. Utilization of different carbon sources by native isolates of *Bradyrhizobium* under *in vitro* conditions

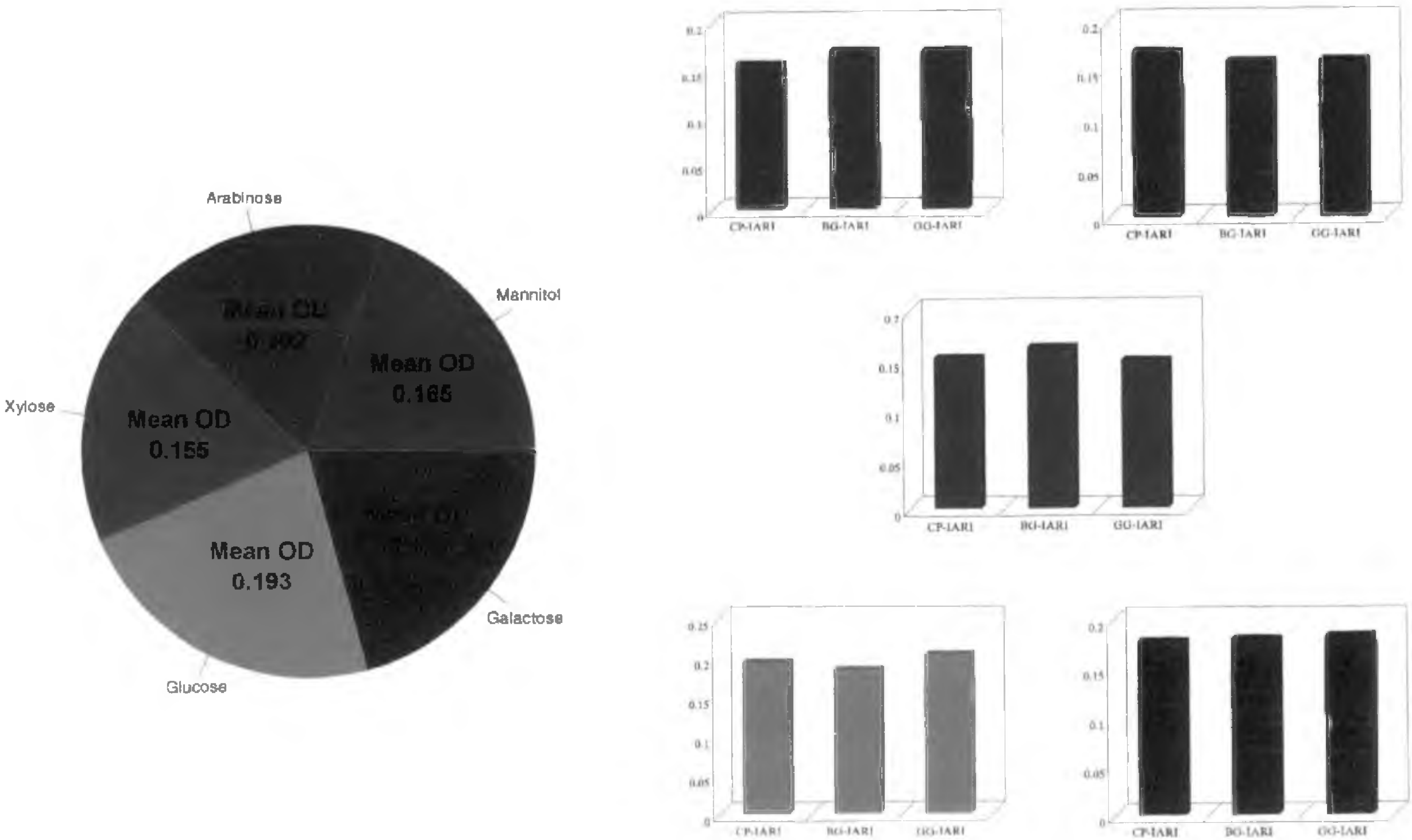


Fig. 14. Utilization of different carbon sources by exotic isolates of *Rhizobium* under *in vitro* conditions

4.3.2. pH sensitivity

The native isolates of *Bradyrhizobium* were more tolerant to low pH. The average OD_{600} at pH 4.5 and 6.0 for these isolates were 0.365 and 0.418 respectively when compared to that of 0.318 and 0.397 for the exotic isolates (Table 11, Fig. 15 and 16). Exotic isolates grew well (mean OD_{600} = 0.491) at pH 7.5.

Among individual isolates, exotic isolates GG-IARI (OD_{600} = 0.287) and BG-IARI (OD_{600} = 0.321) were more sensitive to highly acidic pH of 4.5. At this pH, growth of most of the native isolates except KA-G-C-4 and PA-G-G-5 was better. Under the moderately acidic pH of 6.0 also an almost similar trend was observed.

4.3.3. Iron sensitivity

The growth of both native and exotic isolates of *Bradyrhizobium* was progressively inhibited with increase in concentrations of ferric chloride from 50 to 250 ppm (Table 12, Fig. 17 and 18). The average OD_{600} varied from 0.377 to 0.133 for the native and from 0.373 to 0.160 for the exotic isolates. Some variations were also observed in the sensitivity pattern of individual isolates. At 50 ppm the growth was maximum (OD_{600} = 0.423) for the native isolate PI-G-G-8. At higher concentrations, the native isolate VE-G-B-2 and the exotic isolate BG-IARI were found more tolerant. The average OD_{600} for these isolates were 0.354 and 0.180 at 100 and 250 ppm respectively.

Table 11. pH sensitivity of *Bradyrhizobium* and *Rhizobium* isolates under *in vitro* conditions

Isolates	Levels of pH		
	4.5	6.0	7.5
A. *Bradyrhizobium*			
KA-G-C-4	0.334	0.435	0.481
KA-F-C-7	0.367	0.403	0.461
VE-G-B-2	0.405	0.434	0.509
KA-F-B-6	0.367	0.431	0.482
PA-G-G-5	0.343	0.380	0.498
PI-G-G-8	0.375	0.425	0.455
Mean CD_{600}	0.365	0.418	0.481
B. *Rhizobium*			
CP-IARI	0.345	0.407	0.490
BG-IARI	0.321	0.394	0.486
GG-IARI	0.287	0.391	0.497
Mean OD_{600}	0.318	0.397	0.491
CD(0.05) : 0.009			

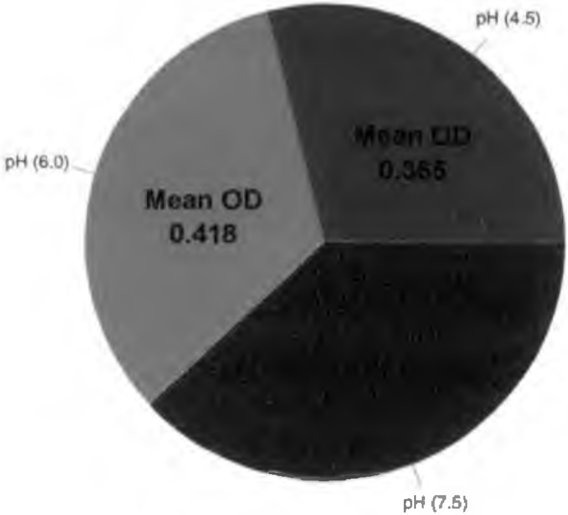
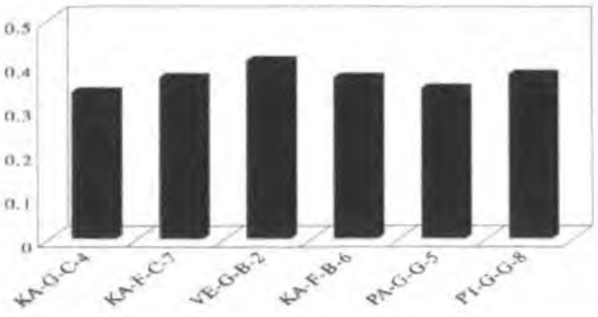
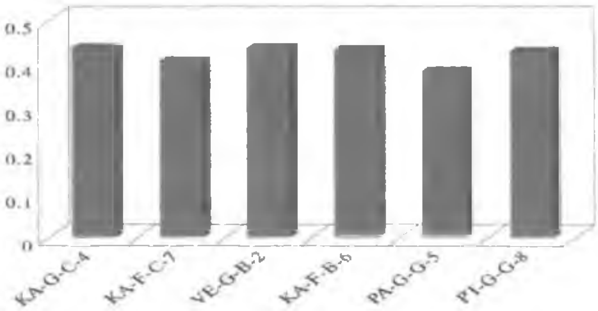
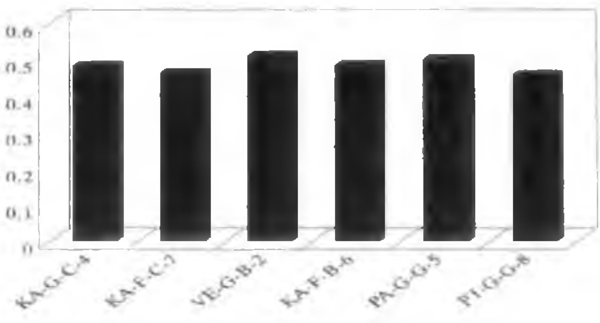


Fig. 15. pH sensitivity of native isolates of *Bradyrhizobium* under *in vitro* conditions


0.5
0.4
0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8


0.5
0.4
0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8


0.6
0.5
0.4
0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8

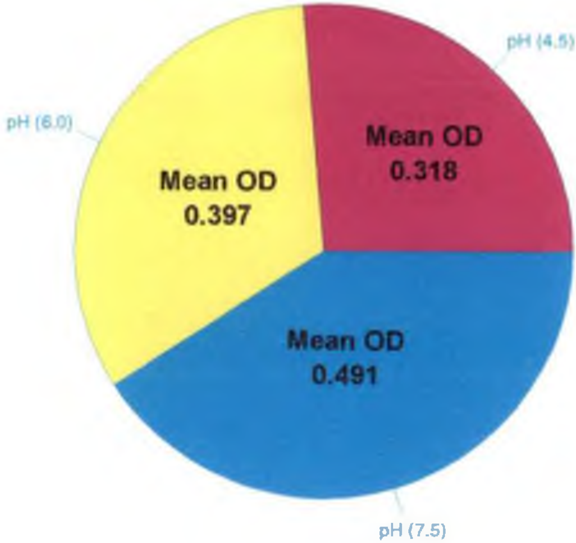
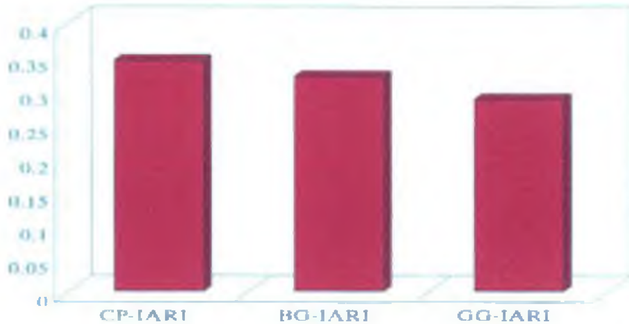
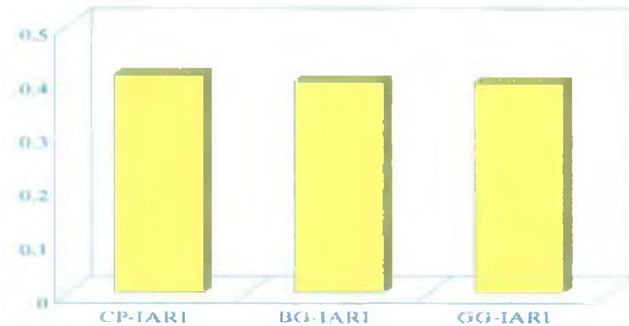


Fig. 16. pH sensitivity of exotic isolates of *Rhizobium* under *in vitro* conditions


0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CP-IARI
BG-IARI
GG-IARI


0.5
0.4
0.3
0.2
0.1
0
CP-IARI
BG-IARI
GG-IARI

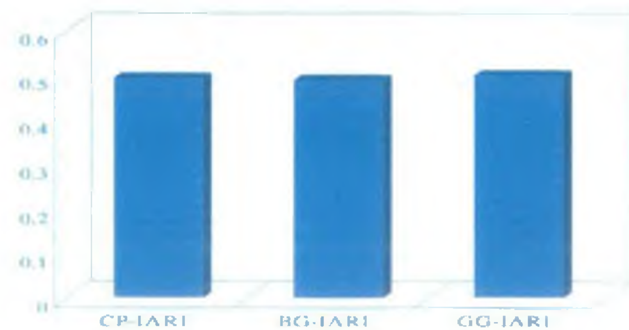

0.6
0.5
0.4
0.3
0.2
0.1
0
CP-IARI
BG-IARI
GG-IARI

Table 12. Iron sensitivity of *Bradyrhizobium* and *Rhizobium* isolates under *in vitro* conditions

Isolates	Concentration (ppm)		
	50	100	250
A. *Bradyrhizobium*			
KA-G-C-4	0.385	0.329	0.133
KA-F-C-7	0.366	0.294	0.095
VE-G-B-2	0.371	0.354	0.123
KA-F-B-6	0.396	0.262	0.133
PA-G-G-5	0.322	0.258	0.147
PI-G-G-8	0.423	0.290	0.165
Mean OD_{600}	0.377	0.298	0.133
B. *Rhizobium*			
CP-IARI	0.380	0.306	0.152
BG-IARI	0.384	0.296	0.180
GG-IARI	0.355	0.291	0.149
Mean OD_{600}	0.373	0.298	0.160
CD (0.05) : 0.028			

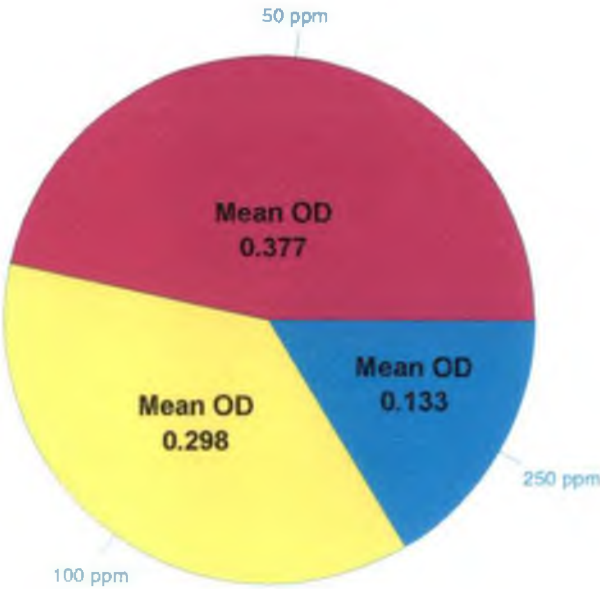
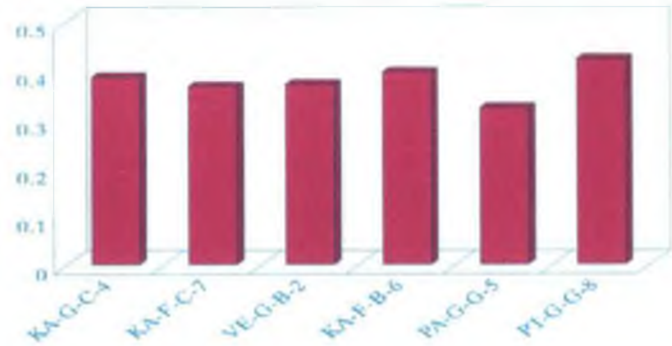


Fig. 17. Iron sensitivity of native isolates of *Bradyrhizobium* under *in vitro* conditions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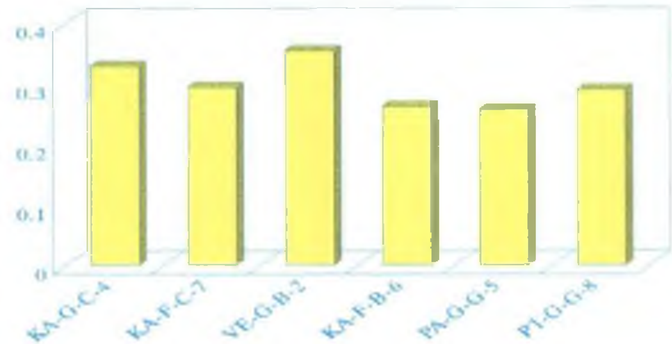
0.4
0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8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0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
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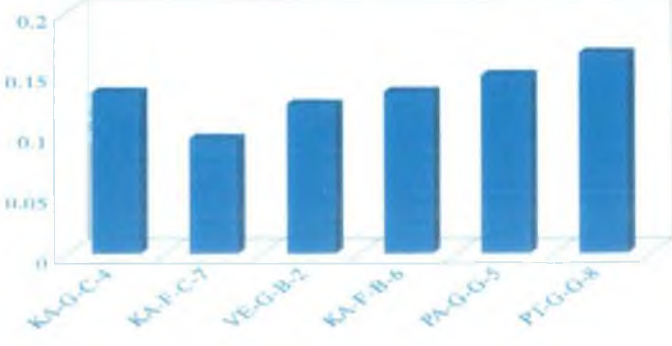
PI-G-G-8


0.2
0.15
0.1
0.05
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
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PI-G-G-8

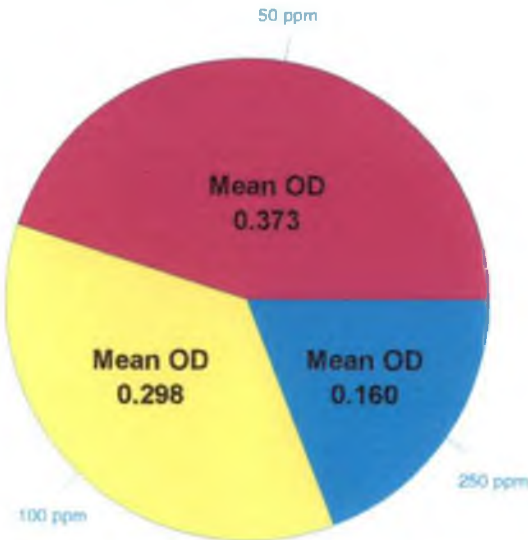
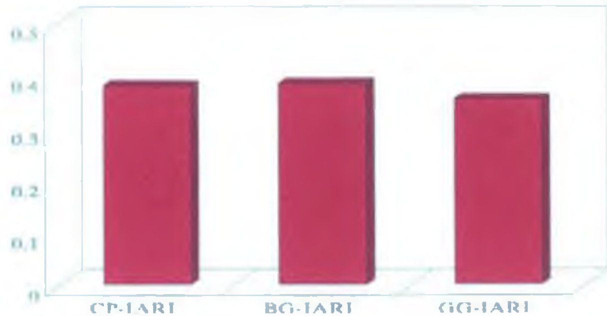
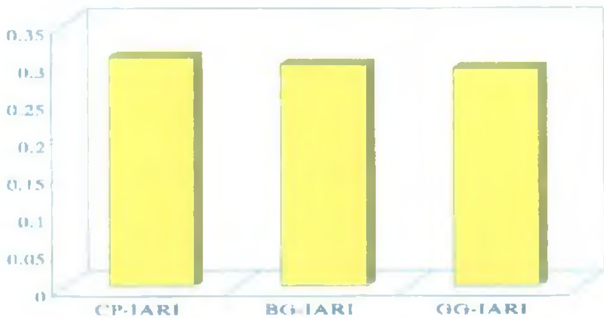
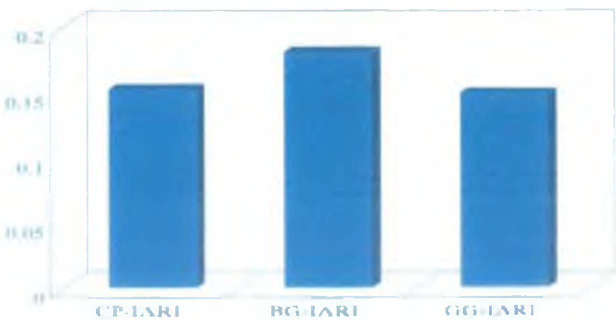


Fig. 18. Iron sensitivity of exotic isolates of *Rhizobium* under *in vitro* conditions


0.5
0.4
0.3
0.2
0.1
0
CP-IARI
BG-IARI
GG-IARI


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
CP-IARI
BG-IARI
GG-IARI


0.2
0.15
0.1
0.05
0
CP-IARI
BG-IARI
GG-IARI

4.3.4. Aluminium sensitivity

The growth of both native and exotic isolates of *Bradyrhizobium* were progressively inhibited with increase in concentrations of aluminium chloride from 50 to 250 ppm. The average OD_{600} value varied from 0.415 to 0.258 for the native isolates and from 0.449 to 0.180 for the exotic isolates (Table 13, Fig. 19 and 20). Some variations were also observed in the sensitivity pattern of individual isolates. The exotic isolates of *Bradyrhizobium* were more tolerant to lower concentrations of aluminium chloride. Thus at 50 and 100 ppm the mean OD_{600} for these isolates were 0.449 and 0.420 respectively. However, at 250 ppm the growth of the native isolate was more with a mean OD_{600} of 0.258 compared to that of only 0.180 for the exotic isolates. Among the individual isolates, the growth of the exotic isolate CP-IARI was more at 50 and 100 ppm. At 250 ppm, the growth of the native isolate KA-F-B-6 (OD_{600} = 0.307) was maximum.

4.3.5. Antibiotic sensitivity

Both the native as well as the exotic isolates of *Bradyrhizobium* had identical antibiotic resistant markers for ampicillin (Ap^{+++}), streptomycin (Sm^{++}) and kanamycin (Km^{+}). Growth of these isolates were not inhibited at concentrations up to 1000 ppm (Fig. 21) of ampicillin, 250 ppm of streptomycin and 50 ppm of kanamycin.

Table 13. Aluminum sensitivity of *Bradyrhizobium* and *Rhizobium* isolates under *in vitro* conditions

Isolates	Concentration of Al (ppm)		
	50	100	250
A. *Bradyrhizobium*			
KA-G-C-4	0.425	0.399	0.269
KA-F-C-7	0.403	0.349	0.209
VE-G-B-2	0.434	0.395	0.255
KA-F-B-6	0.410	0.352	0.307
PA-G-G-5	0.419	0.374	0.278
PI-G-G-8	0.401	0.340	0.229
Mean OD_{600}	0.415	0.368	0.258
B. *Rhizobium*			
CP-IARI	0.456	0.429	0.154
BG-IARI	0.444	0.409	0.187
GG-IARI	0.448	0.421	0.199
Mean OD_{600}	0.449	0.420	0.180
CD (0.05) : 0.006			

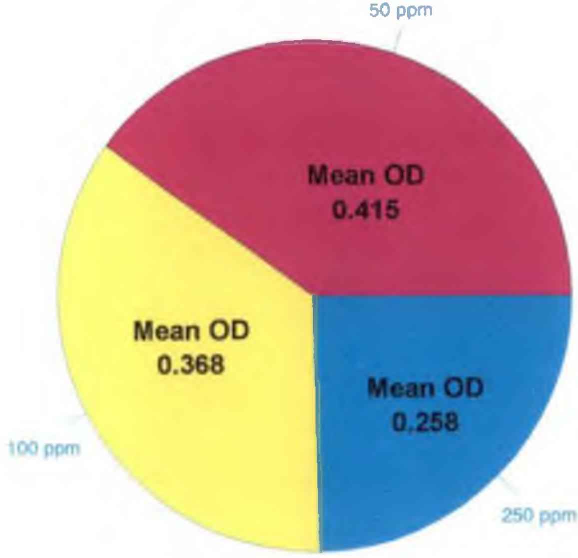
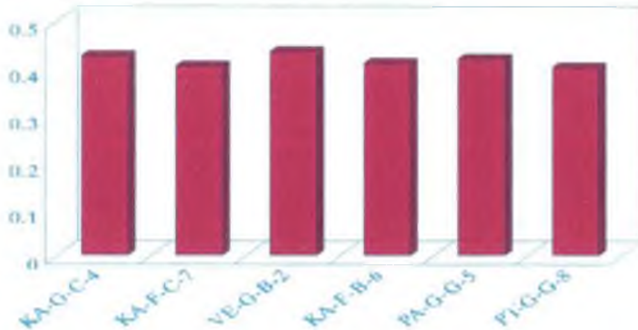


Fig. 19. Aluminium sensitivity of native isolates of *Bradyrhizobium* under *in vitro* conditions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0.4
0.3
0.2
0.1
0
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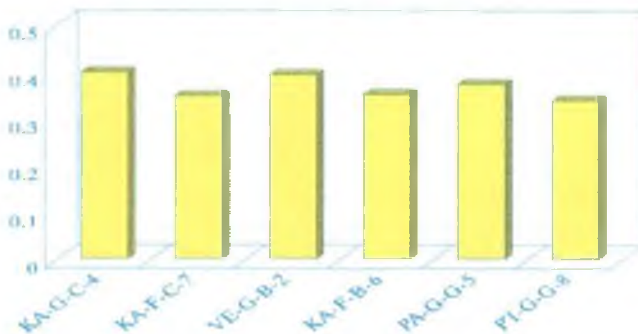
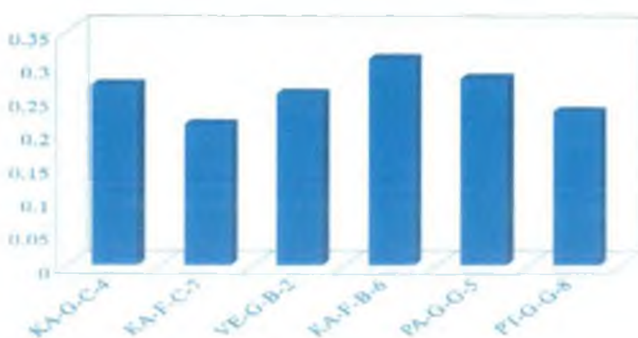
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8


0.5
0.4
0.3
0.2
0.1
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8


0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
KA-G-C-4
KA-F-C-7
VE-G-B-2
KA-F-B-6
PA-G-G-5
PI-G-G-8

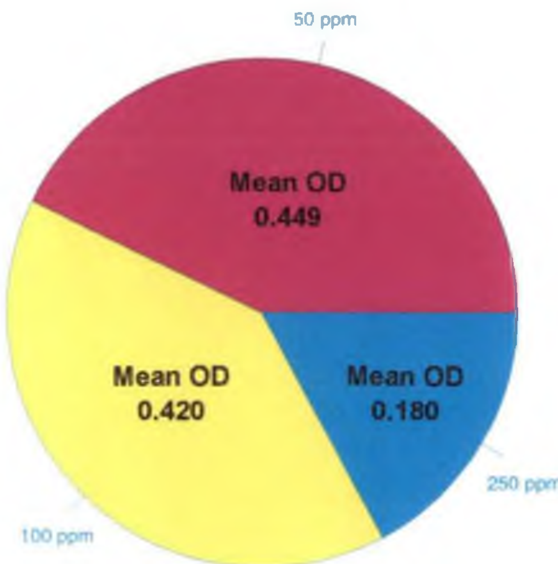
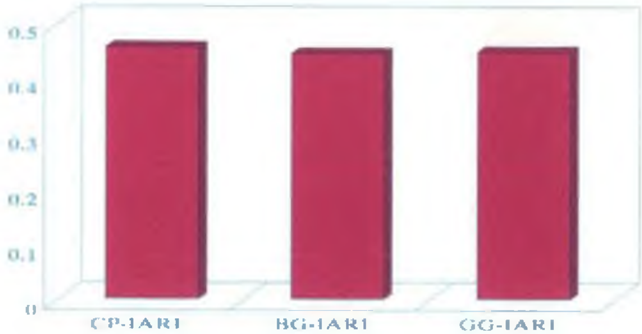
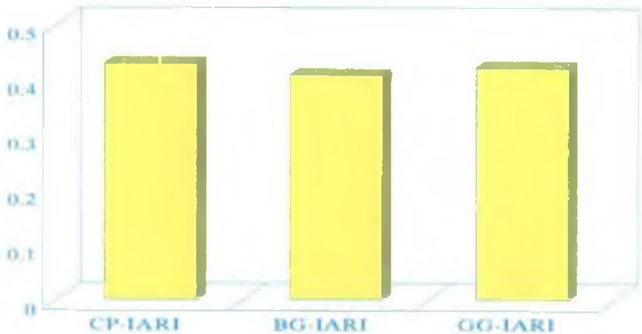


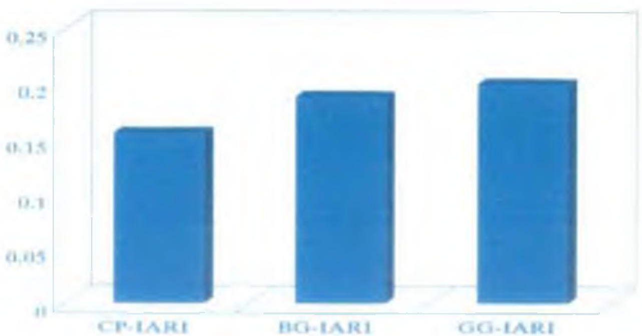
Fig. 20. Aluminium sensitivity of exotic isolates of *Rhizobium* under *in vitro* conditions



0.5
0.4
0.3
0.2
0.1
0
CP-IARI
BG-IARI
GG-IARI

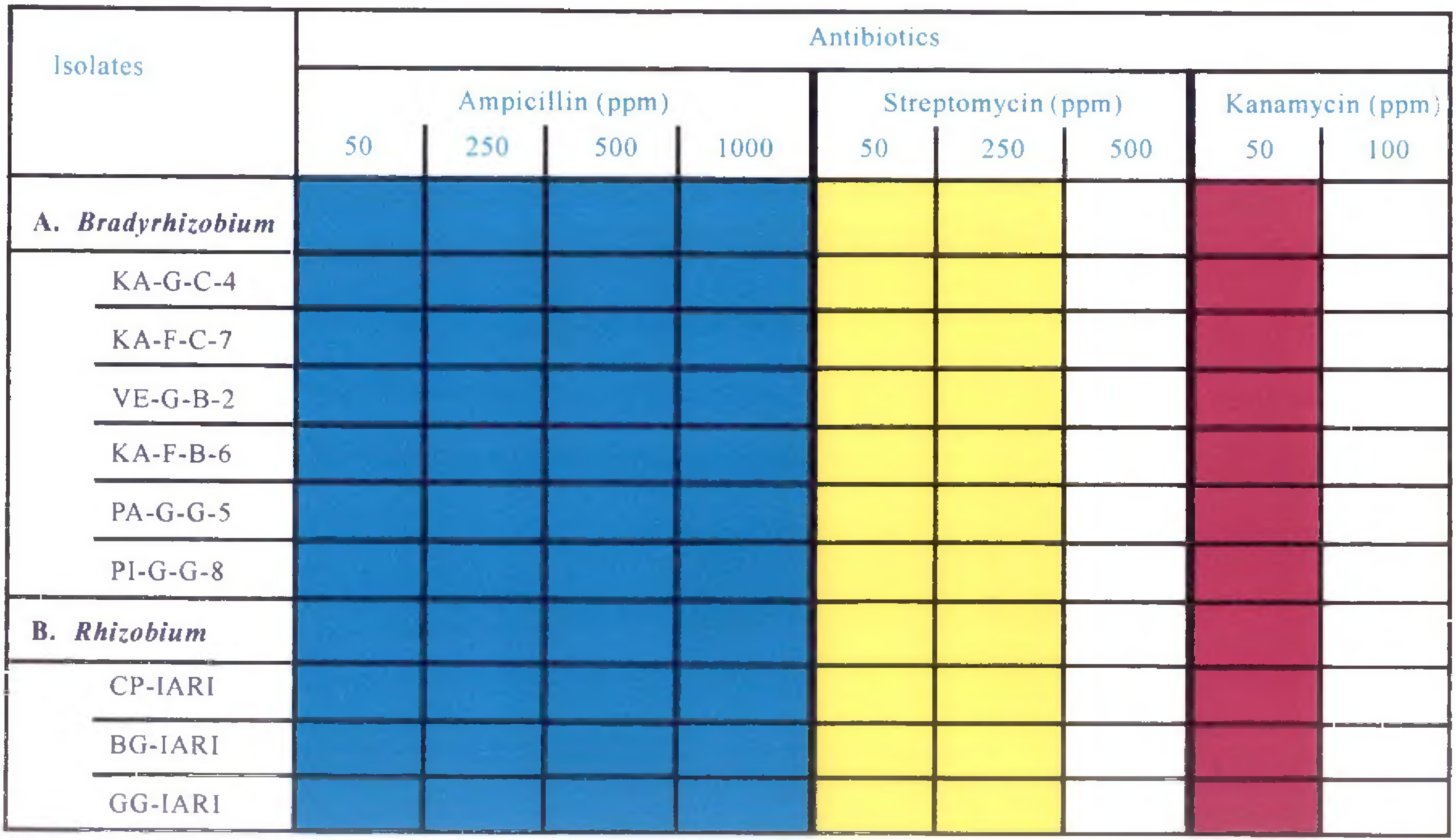


0.5
0.4
0.3
0.2
0.1
0
CP-IARI
BG-IARI
GG-IARI



0.25
0.2
0.15
0.1
0.05
0
CP-IARI
BG-IARI
GG-IARI

Fig. 21. Antibiotic sensitivity of native isolates of *Bradyrhizobium* and exotic isolates of *Rhizobium*



4.3.6. Serological characterisation

In the study on serological relationship between the best isolate of cowpea (KA-F-C-7) and that of blackgram (VE-G-B-2) and greengram (PI-G-G-8) by tube agglutination test, a positive agglutination was obtained only with the homologous isolate (KA-F-C-7) and an isolate from greengram, PI-G-G-8 (Plate 7). However, the interaction was negative with VE-G-B-2 antigen. A similar result was also obtained with the antigens of three isolates of *Bradyrhizobium* from the extremely acidic soil conditions (Plate 8) such as AM-K-C-15 for cowpea, AM-K-B-11 for blackgram and AM-K-G-10 for greengram.

4.4. Development of package of practices (POP) recommendations for acid tolerant strains of *Bradyrhizobium*

4.4.1. Pot experiments

4.4.1.1. Effect of FYM and liming

4.4.1.1.1. Cowpea

Significant increases in nodule number, nodule dry weight and plant dry weight were obtained in *Bradyrhizobium* treatments combined with FYM and lime application. The nodule number and nodule dry weight were maximum in FYM$^+$L$^+$KA-F-C-7 treatment. These were 72.7 and 196.9 mg respectively (Table 14, Plates 9-11).

7. Interaction between homologous and heterologous antigens of *Bradyrhizobium* from cowpea, blackgram and greengram

1 - Positive interaction of KA-F-C-7 x KA-F-C-7
2 - Negative interaction of KA-F-C-7 x VE-G-B-2
3 - Positive interaction of KA-F-C-7 x PI-G-G-8
4 - KA-F-C-7 antigen alone
5 - KA-F-C-7 antiserum alone
6 - Saline control

8. Interaction between homologous and heterologous antigens of *Bradyrhizobium* from cowpea, blackgram and greengram

1 - Negative interaction of KA-F-C-7 x AM-K-C-15
2 - Negative interaction of KA-F-C-7 x AM-K-B-11
3 - Negative interaction of KA-F-C-7 x AM-K-G-10
4 - KA-F-C-7 antigen alone
5 - Positive interaction of KA-F-C-7 x KA-F-C-7
6 - Saline control

Plate 7

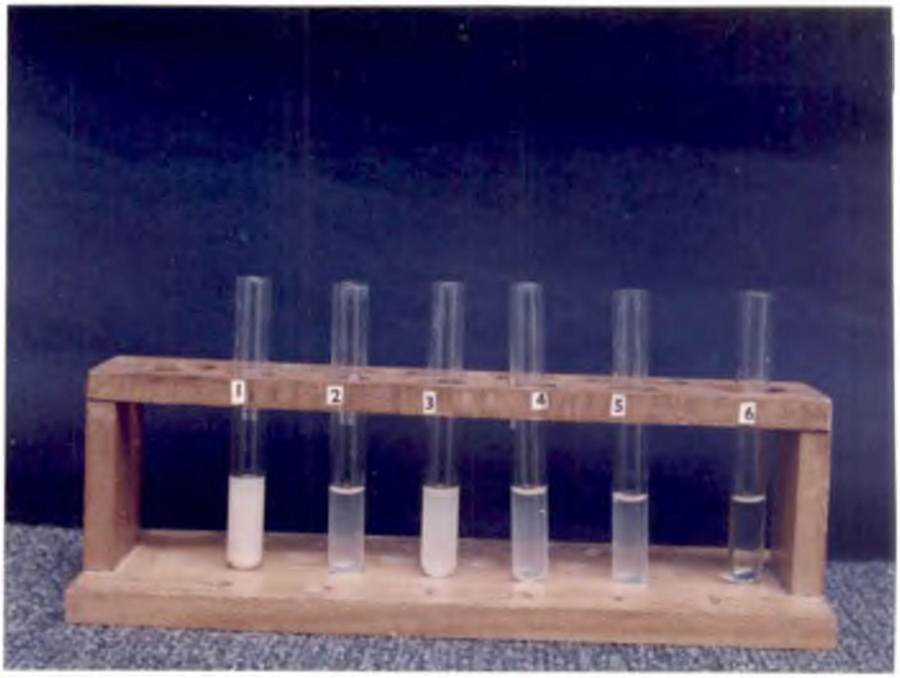


Plate 8

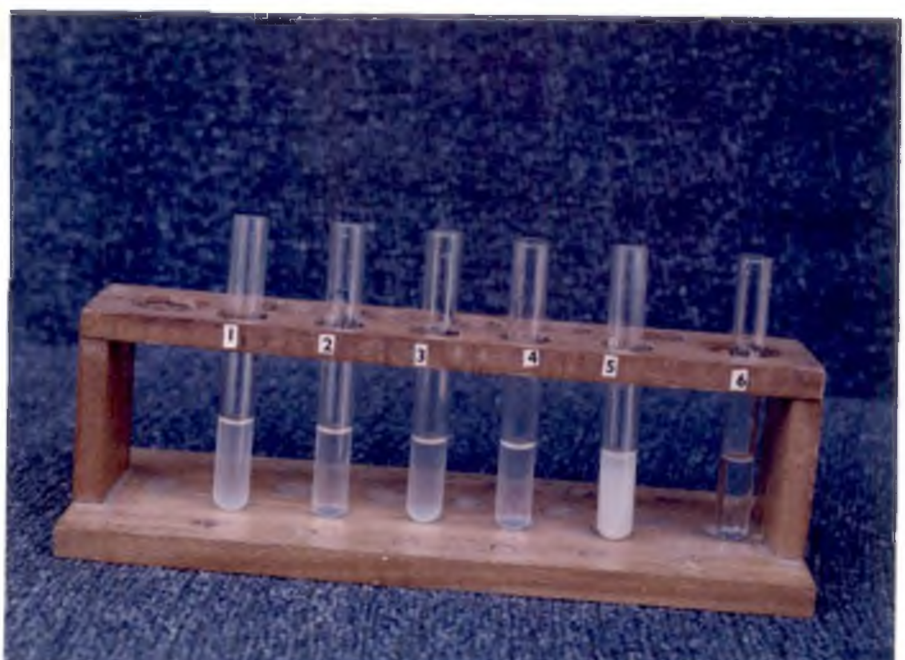


Table 14. Effect of FYM and liming on root nodulation and plant growth in cowpea

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
$FYM^{+}L^{+}$KA-G-C-4	65.0	108.9	**46.7**
$FYM^{+}L^{+}$KA-F-C-7	**72.7**	**196.9**	35.0
$FYM^{+}L^{-}$KA-G-C-4	30.3	75.1	35.0
FYM^{+}L-KA-F-C-7	50.7	114.2	41.7
$FYM^{-}L^{+}$KA-G-C-4	25.7	76.1	20.0
$FYM^{-}L^{+}$KA-F-C-7	22.3	89.9	18.3
$FYM^{-}L^{-}$KA-G-C-4	22.7	77.0	11.7
$FYM^{-}L^{-}$KA-F-C-7	15.7	98.0	13.3
CD (0.05)	16.3	19.2	12.5

$FYM^{+/-}$ with or without farmyard manure

$L^{+/-}$ with or without liming

9. Effect of FYM and liming on plant growth of cowpea

C - Control without FYM and liming

F^+L^+ - FYM + liming

10. Effect of FYM and liming on plant growth of cowpea

F - FYM alone

F^+L^+ - FYM + liming

Plate 9

Plate 10

11. Effect of FYM and liming on plant growth of cowpea

L - Liming alone

F^+L^+ - FYM + liming

Plate 11

The plant dry weight of 46.7g was, however, more in FYM$^+$L$^+$KA-G-C-4 treatment. The increase in nodule number in FYM$^+$L$^-$KA-F-C-7 and plant dry weight in FYM$^+$L$^-$KA-G-C-4 and FYM$^+$L$^-$KA-F-C-7 treatment with application of FYM alone were also statistically significant. These were 50.7 (nodule number) and 35.0g and 41.7g (plant dry weight) respectively. In the treatments without FYM, the nodule number, nodule dry weight and plant dry weight were significantly low even with the application of lime. Thus in the treatment combinations of FYM$^-$L$^+$KA-G-C-4 and FYM$^-$L$^+$KA-F-C-7, the nodule number, nodule dry weight and plant dry weight were only 25.7 and 22.3, 76.1 and 89.9 mg and 20.0 and 18.3 g respectively (Table 14). Such an effect was more pronounced in the control treatments (FYM$^-$L$^-$KA-G-C-4 and FYM$^-$L$^-$KA-F-C-7) where neither FYM nor lime was applied along with *Bradyrhizobium* inoculation.

4.4.1.1.2. Blackgram

Significant increases in nodule number (67.7), nodule dry weight (165.5 mg) and plant dry weight (40.0g) were obtained in the FYM$^+$L$^+$KA-F-B-6 treatment combination (Table 15, Plates 12-14). The plant dry weight of 41.7 g was however, maximum in the FYM$^+$L$^+$VE-G-B-2 treatment. The number of nodules formed in this treatment (60.3) was statistically on par with the best treatment combination of FYM$^+$L$^+$KA-F-B-6. The increase in nodule number with the application of FYM alone (FYM$^+$L$^-$VE-G-B-2 and FYM$^+$L$^-$KA-F-B-6) were also statistically on par with the corresponding treatment combination having lime application also.

Table 15. Effect of FYM and liming on root nodulation and plant growth in blackgram

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
$FYM^{+}L^{+}$VE-G-B-2	60.3	59.5	**41.7**
$FYM^{+}L^{+}$KA-F-B-6	**67.7**	**165.5**	40.0
$FYM^{+}L^{-}$VE-G-B-2	49.0	46.6	25.0
$FYM^{+}L^{-}$KA-F-B-6	45.7	48.4	30.0
$FYM^{-}L^{+}$VE-G-B-2	18.0	25.2	15.0
$FYM^{-}L^{+}$KA-F-B-6	28.7	25.8	20.0
$FYM^{-}L^{-}$VE-G-B-2	30.3	35.8	15.0
$FYM^{-}L^{-}$KA-F-B-6	25.3	36.6	13.3
CD (0.05)	35.2	22.8	12.5

$FYM^{+/-}$ with or without farmyard manure

$L^{+/-}$ with or without liming

12. Effect of FYM and liming on plant growth of blackgram

C - Control without FYM and liming

F^+L^+ - FYM + liming

13. Effect of FYM and liming on plant growth of blackgram

F - FYM alone

F^+L^+ - FYM + liming

Plate 12

Plate 13

14. Effect of FYM and liming on plant growth of blackgram

L - Liming alone

$F^{+}L^{+}$ - FYM + liming

Plate 14

These were 49.0 and 45.7 respectively. A similar response was obtained for nodule dry weight (46.6mg) in FYM^+L^-VE-G-B-2 treatment and for plant dry weight (30.0 g) in the FYM^+L^-KA-F-B-6 treatment. But in the treatments without FYM, the nodule number, nodule dry weight and plant dry weight were significantly low even with lime. Thus in the treatment combinations of FYM^-L^+VE-G-B-2 and FYM^-L^+KA-F-B-6, the nodule number, nodule dry weight and plant dry weight were only 18.0 and 28.7, 25.2 and 25.8 and 15.0 and 20.0 g respectively (Table 15). Such an effect was more pronounced in the control treatments (FYM^-L^-VE-G-B-2 and FYM^-L^-KA-F-B-6) where neither FYM nor lime was applied along with *Bradyrhizobium* inoculation.

4.4.1.1.3. Greengram

The number of nodules formed, nodule dry weight and plant dry weight in greengram were more in *Bradyrhizobium* treatments along with FYM and lime application. This increase was significant for nodule number (65.7), in FYM^+L^+PA-G-G-5, nodule dry weight (117.2 mg) and plant dry weight (71.7 g) in FYM^+L^+PI-G-G-8 (Table 16, Plates 15-17). The increase in nodule number (50.3 and 48.7) and nodule dry weight (103.8 and 98.0 mg) with the application of FYM alone (FYM^+L^-PA-G-G-5 and FYM^+L^-PI-G-G-8) were statistically on par with the corresponding treatment combination having lime application also. Such an increase in plant dry weight (38.3 and 46.7 g respectively) was statistically significant in both the above treatments.

Table 16. Effect of FYM and liming on root nodulation and plant growth in greengram

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
$FYM^{+}L^{+}$PA-G-G-5	**65.7**	115.7	63.3
$FYM^{+}L^{+}$PI-G-G-8	57.0	**117.2**	**71.7**
$FYM^{+}L^{-}$PA-G-G-5	50.3	103.8	38.3
FYM+L^{-}PI-G-G-8	48.7	98.0	46.7
$FYM^{-}L^{+}$PA-G-G-5	13.3	31.8	20.0
$FYM^{-}L^{+}$PI-G-G-8	17.7	31.6	18.3
$FYM^{-}L^{-}$PA-G-G-5	27.0	58.3	11.7
$FYM^{-}L^{-}$PI-G-G-8	25.3	57.4	15.0
CD(0.05)	35.1	58.3	18.1

$FYM^{+/-}$ with or without farmyard manure

$L^{+/-}$ with or without liming

15. Effect of FYM and liming on plant growth of greengram

C - Control without FYM and liming

$F^{+}L^{+}$ - FYM + liming

16. Effect of FYM and liming on plant growth of greengram

F - FYM alone

$F^{+}L^{+}$ - FYM + liming

Plate 15

Plate 16

17. Effect of FYM and liming on plant growth of greengram

L - Liming alone

$F^{+}L^{+}$ - FYM + liming

Plate 17

But in the treatments without FYM, the nodule number, nodule dry weight and plant dry weight were significantly low even with the application of lime. Thus, in the treatment combinations of FYM^-L^+PA-G-G-5 and FYM^-L^+PI-G-G-8, the nodule number, nodule dry weight and plant dry weight were only 13.3 and 17.7, 31.8 and 31.6 mg and 20.0 and 18.3 g respectively. However, unlike in cowpea and blackgram, a variable response was obtained in the control treatments.

4.4.1.2. Effect of FYM and pelleting

4.4.1.2.1. Cowpea

Significant increase in nodule number, nodule dry weight and plant dry weight was obtained in *Bradyrhizobium* treatments along with application of FYM and calcium carbonate pelleting. The number of nodules formed (62.0) and nodule dry weight (150.4 mg) were maximum in FYM^+P^+KA-G-C-4 and FYM^+P^+KA-F-C-7 treatments respectively (Table 17, Fig. 22, Plates 18-21). The plant dry weight was same in both these treatments. The increase in nodule number (50.7) and nodule dry weight (114.2 mg) with the application of FYM alone were significant in FYM^+P^-KA-F-C-7 treatment. Such an increase was also obtained in plant dry weight in FYM^+P^-KA-G-C-4 (35.0 g) and FYM^+P^-KA-F-C-7 (41.7 g) treatments.

Table 17. Effect of FYM and pelleting on root nodulation and plant growth in cowpea

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
FYM^+P^+KA-G-C-4	**62.0**	140.6	**46.7**
FYM^+P^+KA-F-C-7	51.7	**150.4**	**46.7**
FYM^+P^-KA-G-C-4	30.3	75.1	35.0
FYM^+P^-KA-F-C-7	50.7	114.2	41.7
FYM^-P^+KA-G-C-4	19.0	74.8	21.7
FYM^-P^+KA-F-C-7	22.3	80.8	23.3
FYM^-P^-KA-G-C-4	22.7	77.0	11.7
FYM^-P^-KA-F-C-7	15.7	98.0	13.3
CD (0.05)	19.4	19.0	7.7

$FYM^{+/-}$ with or without farmyard manure

$P^{+/-}$ with or without pelleting.

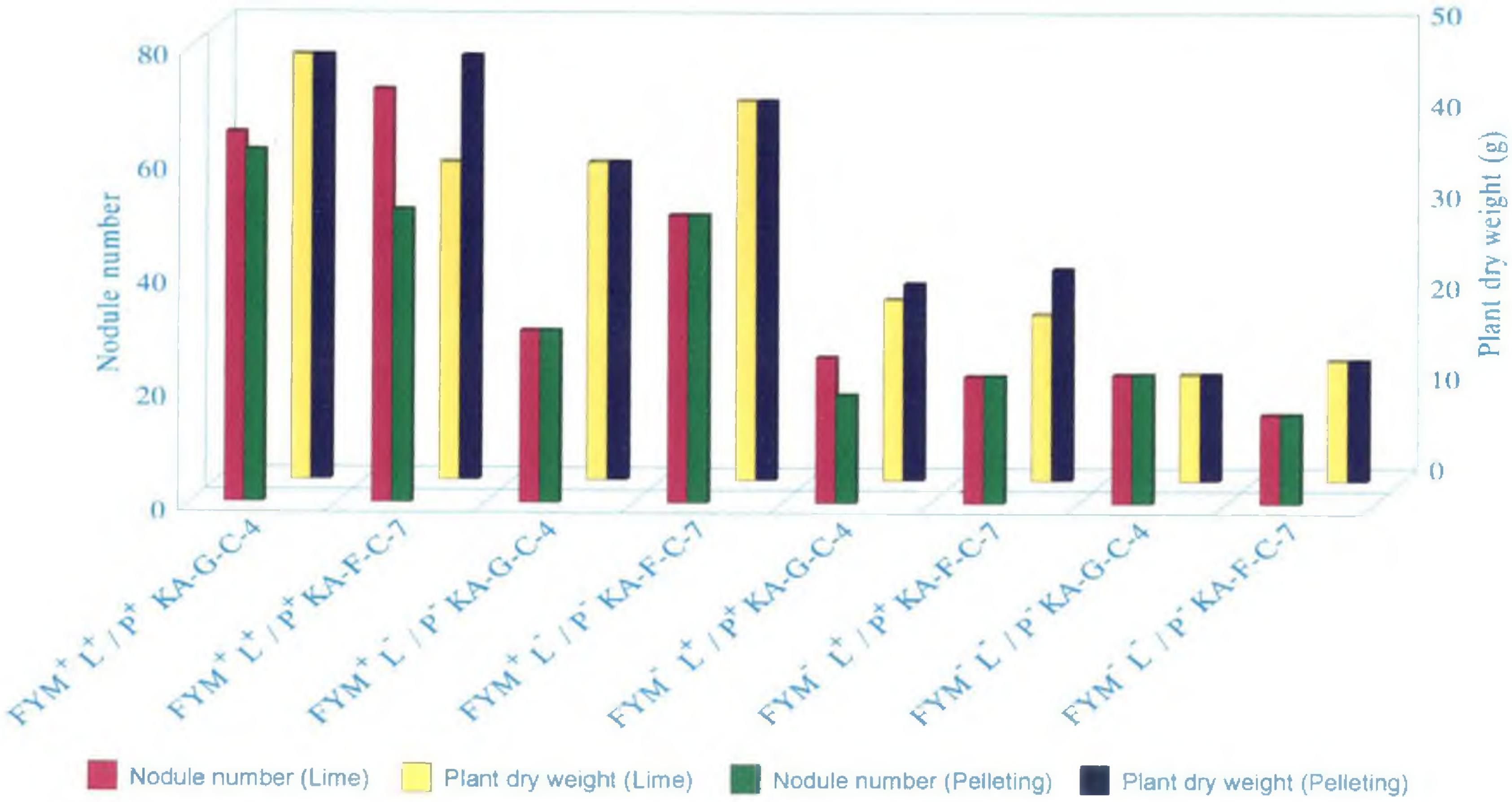


Fig. 22. Effect of FYM, lime application and pelleting on root nodulation and plant growth in cowpea

18. Effect of FYM and pelleting on plant growth of cowpea

C - Control without FYM and pelleting

F^+P^+ - FYM + pelleting

19. Effect of FYM and pelleting on plant growth of cowpea

F - FYM alone

F^+P^+ - FYM + pelleting

Plate 18

Plate 19

20. Effect of FYM and pelleting on plant growth of cowpea

P - Pelleting alone

F^+P^+ - FYM + pelleting

21. Effect of FYM and pelleting and FYM and liming on root nodulation of cowpea

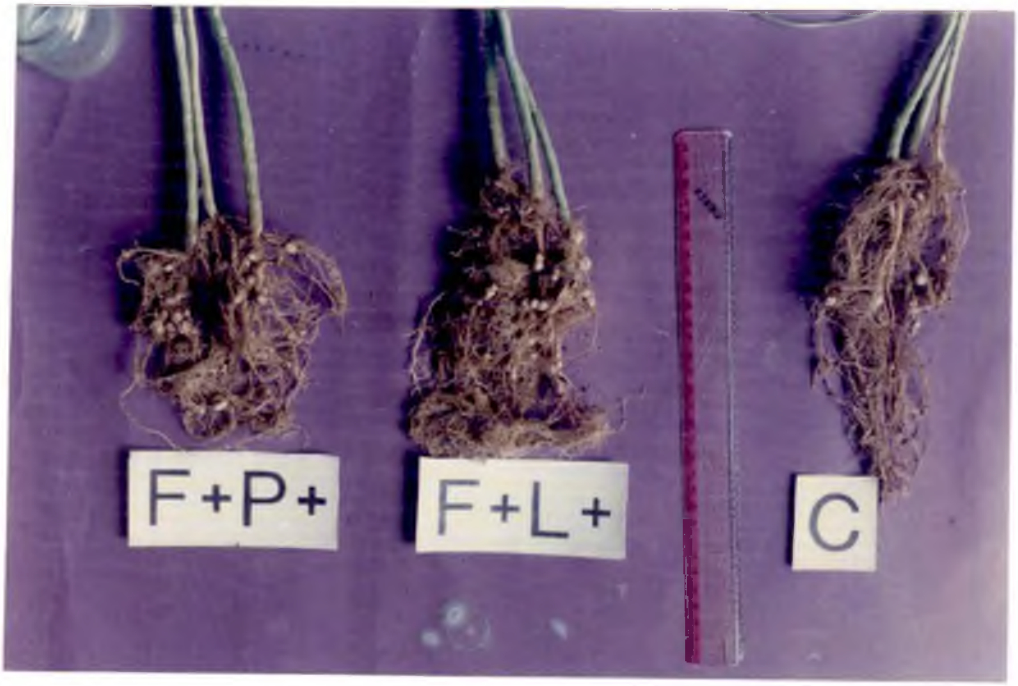
C - Control without FYM and pelleting or liming

F^+P^+ - FYM + pelleting

F^+L^+ - FYM + liming

Plate 20

Plate 21



In treatments without FYM (FYM^-P^+KA-G-C-4 and FYM^-P^+KA-F-C-7) eventhough the nodule number and nodule dry weight were significantly low, the plant dry weight (21.7 and 23.3 g respectively) were significantly increased by pelleting. However, in the control treatments without FYM and pelleting (FYM^-P^-KA-G-C-4 and FYM^-P^-KA-F-C-7) the nodule number, nodule dry weight and plant dry weight were significantly low.

4.4.1.2.2. Blackgram

Significant increases in nodule number (122.3), nodule dry weight (96.1 mg) and plant dry weight (33.3g) were obtained in FYM^+P^+KA-F-B-6 treatments. A similar trend was observed in the other identical treatment combination of FYM^+P^+VE-G-B-2 for nodule number (69.3) and nodule dry weight (89.0 mg). The plant dry weight of 46.7 g was also maximum in this treatment. In the treatment with application of FYM alone (FYM^+P^-VE-G-B-2 and FYM^+P^-KA-F-B-6) a significant increase was obtained only in plant dry weight. These were 25.0 and 30.0 g respectively (Table 18, Fig. 23, Plates 22-24). In rest of the treatments, either without FYM or without FYM and pelleting, the nodule number, nodule dry weight and plant dry weight were significantly low when compared to that of $FYM^+P^+BR^+$ treatment.

Table 18. Effect of FYM and pelleting on root nodulation and plant growth in blackgram

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
$FYM^{+}P^{+}$VE-G-B-2	69.3	89.0	**46.7**
$FYM^{+}P^{+}$KA-F-B-6	**122.3**	**96.1**	33.3
$FYM^{+}P^{-}$VE-G-B-2	49.0	46.6	25.0
$FYM^{+}P^{-}$KA-F-B-6	45.7	48.4	30.0
$FYM^{-}P^{+}$VE-G-B-2	31.0	45.7	13.3
$FYM^{-}P^{+}$KA-F-B-6	36.0	46.8	18.3
$FYM^{-}P^{-}$VE-G-B-2	30.3	35.8	15.0
$FYM^{-}P^{-}$KA-F-B-6	25.3	36.6	13.3
CD(0.05)	41.9	12.6	9.0

$FYM^{+/-}$ with or without farmyard manure

$P^{+/-}$ with or without pelleting.

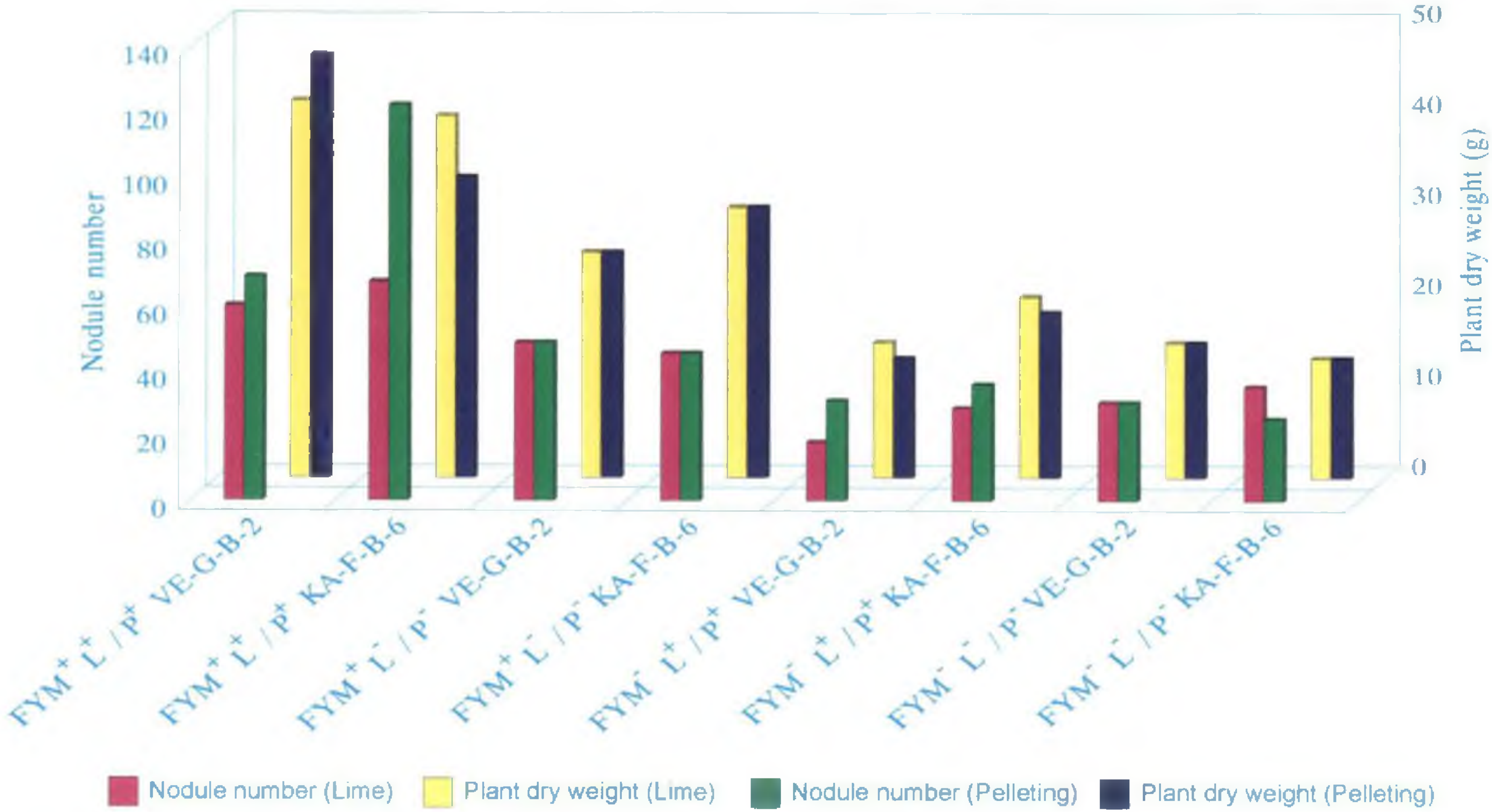


Fig. 23. Effect of FYM, lime application and pelleting on root nodulation and plant growth in blackgram

22. Effect of FYM and pelleting on plant growth of blackgram

C - Control without FYM and pelleting

F^+P^+ - FYM + pelleting

23. Effect of FYM and pelleting on plant growth of blackgram

F - FYM alone

F^+P^+ - FYM + pelleting

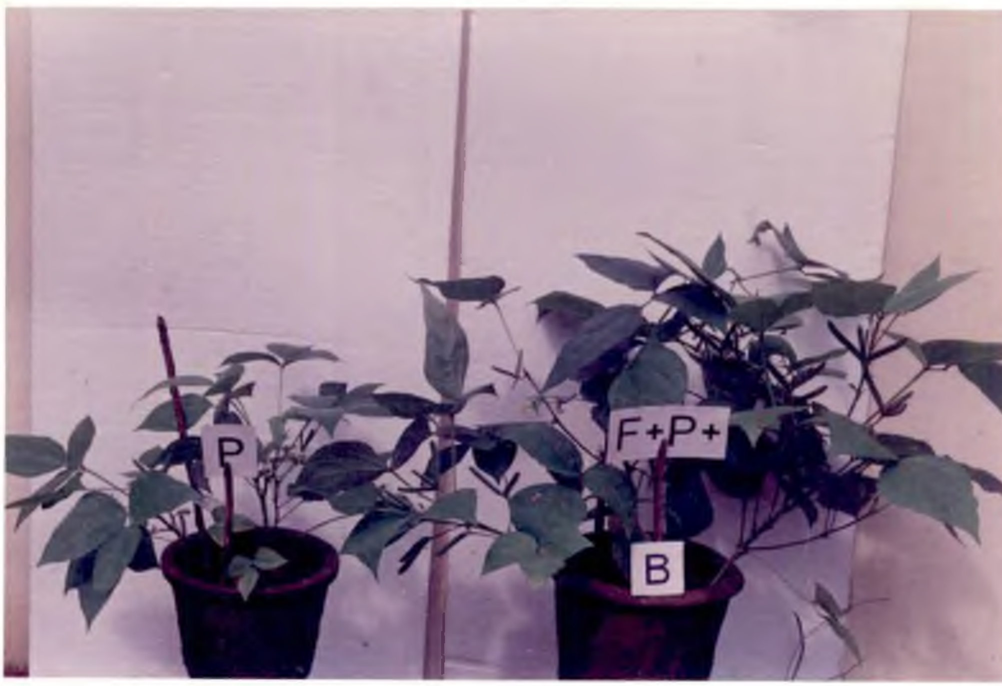
Plate 22

Plate 23

24. Effect of FYM and pelleting on plant growth of blackgram

P	-	Pelleting alone
$F^{+}P^{+}$	-	FYM + pelleting

Plate 24



4.4.1.2.3. Greengram

Significant increases in nodule number, nodule dry weight and plant dry weight were obtained in FYM^+P^+PI-G-G-8 treatments. These were 119.3, 145.7 mg and 51.7 g respectively (Table 19, Fig. 24, Plates 25-27). A similar trend was observed in the other identical treatment combination of FYM^+P^+PA-G-G-5 for nodule dry weight (109.8 mg) and plant dry weight (55.0 g). In the treatments with application of FYM alone (FYM^+P^-PA-G-G-5) and FYM^+P^-PI-G-G-8), a significant increase was obtained only in plant dry weight. These were 38.3 and 46.7 g respectively (Table 19). In rest of the treatments, either without FYM or without FYM and pelleting, the nodule number, nodule dry weight and plant dry weight were significantly low when compared to the $FYM^+P^+BR^+$ treatments.

4.4.2. Field experiments

4.4.2.1. Cowpea

4.4.2.1.1. Location - Vellayani

The nodule number, nodule dry weight, plant dry weight and yield were significantly increased due to *Bradyrhizobium* inoculation. The response was more in the treatment combinations of *Bradyrhizobium* inoculation along with the POP recommendation. The number of nodules formed, nodule dry weight, plant dry weight and yield were maximum in the POP^+KA-F-C-7 treatment.

Table 19. Effect of FYM and pelleting on root nodulation and plant growth in greengram

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)
$FYM^{+}P^{+}$PA-G-G-5	53.7	109.8	**55.0**
$FYM^{+}P^{+}$PI-G-G-8	**119.3**	**145.7**	51.7
$FYM^{+}P^{-}$PA-G-G-5	50.3	103.8	38.3
$FYM^{+}P^{-}$PI-G-G-8	48.7	98.0	46.7
$FYM^{-}P^{+}$PA-G-G-5	20.7	69.8	25.0
$FYM^{-}P^{+}$PI-G-G-8	24.3	62.5	21.7
$FYM^{-}P^{-}$PA-G-G-5	27.0	58.3	11.7
$FYM^{-}P^{-}$PI-G-G-8	25.3	57.4	15.0
CD(0.05)	53.4	46.9	18.8

$FYM^{+/-}$ with or without farmyard manure

$P^{+/-}$ with or without pelleting.

Fig. 24. Effect of FYM, lime application and pelleting on root nodulation and plant growth in greengram

25. Effect of FYM and pelleting on plant growth of greengram

C - Control without FYM and pelleting

F^+P^+ - FYM + pelleting

26. Effect of FYM and pelleting on plant growth of greengram

F - FYM alone

F^+P^+ - FYM + pelleting

Plate 25

Plate 26

27. Effect of FYM and pelleting on plant growth of greengram

P	-	Pelleting alone
F^+P^+	-	FYM + pelleting

Plate 27

These were 35.8, 109.6 mg, 25.1 and 556.3 g respectively compared to 16.1, 43.5 mg, 10.0 and 243.8 g respectively in the control (POP^-BR^-) treatment (Table 20). A similar response was also obtained in the POP^+KA-G-C-4 treatment. Here the nodule number, nodule dry weight, plant dry weight and yield were 28.6, 83.9 mg, 24.1 and 408.3 g respectively. In both the above treatments, the nodulation, plant growth and yield parameters were reduced when *Bradyrhizobium* inoculation was done without the POP recommendation. This reduction was significantly low for nodule number (22.4) and nodule dry weight (67.1 mg) in the POP^-KA-F-C-7 treatment and for yield in both POP^-KA-G-C-4 (297.2 g) and POP^- KA-F-C-7 (345.8 g) treatment. In the treatment without *Bradyrhizobium* inoculation, such a reduction was even more when compared to the best treatment combination of POP^+KA-F-C-7. Here (POP^+BR^-), the nodule number, nodule dry weight, plant dry weight and yield were only 18.4, 57.0 mg, 15.2 and 295.2 g respectively.

The total leaf area was also increased due to *Bradyrhizobium* inoculation along with the POP recommendation. This increase was significant (275.2 cm^2) in the POP^+KA-F-C-7 treatment when compared to the control (POP^-BR^-) treatment (Table 21). In the POP^+KA-G-C-4 treatment the total leaf area was 258.6 cm^2. In both the above treatments, the leaf area was reduced when *Bradyrhizobium* inoculation was done without the POP recommendation. These were only 221.9 and 219.0 cm^2 (Table 21). An increase in leaf area (256.0 cm^2) due to native flora was also observed in the POP^+BR^- treatment. But such a stimulation was not noticed in the absence of the POP recommendation.

Table 20. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of cowpea at Vellayani

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)	Yield per plot (g)	Percentage increase over control
POP^+KA-G-C-4	28.6	83.9	24.1	408.3	67.5
POP^-KA-G-C-4	24.5	68.6	19.7	297.2	21.8
POP^+KA-F-C-7	**35.8**	**109.6**	**25.1**	**556.3**	128.2
POP^-KA-F-C-7	22.4	67.1	21.4	345.8	41.8
POP^+BR^-	18.4	57.0	15.2	295.2	21.1
POP^-BR^-	16.1	43.5	10.0	243.8	
CD(0.05)	6.9	18.4	8.4	90.5	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

Table 21. Influence of POP recommendations and *Bradyrhizobium* inoculation on total leaf area* of cowpea at Vellayani

Treatments	Total leaf area (cm^2)
POP^+KA-G-C-4	258.6
POP^-KA-G-C-4	221.9
POP^+KA-F-C-7	275.2
POP^-KA-F-C-7	219.0
POP^+BR^-	256.0
POP^-BR^-	210.2
CD(0.05)	58.2

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

4.4.2.1.2. Location - Kayamkulam

At RRS, Kayamkulam, uniformly significant treatment effects were not obtained for nodule number, nodule dry weight and plant dry weight. However, the yield differences were significant. It was maximum (589.2 g) in the treatment combination of POP^{+}KA-F-C-7 followed by the POP^{+}KA-G-C-4 (528.8 g) treatment (Table 22, Fig. 25). The yield was significantly reduced in the corresponding treatment combinations (POP^{-}KA-G-C-4 and POP^{-}KA-F-C-7) without the POP recommendation. The yield in these treatments were only 459.2 and 433.3 g respectively. An increase in nodule number (26.7) and grain yield (468.3 g) due to native flora was also observed at RRS, Kayamkulam in the $POP^{+}BR^{-}$ treatment. But in the absence of the POP recommendation ($POP^{-}BR^{-}$) such a stimulation was absent.

4.4.2.2. Blackgram

4.4.2.2.1. Location - Vellayani

There were significant differences between treatments in nodule number, plant dry weight and yield. As in cowpea, the crop response was more in the treatment combinations where *Bradyrhizobium* inoculation was done along with the POP recommendation. Thus, the number of nodules formed, plant dry weight and yield were maximum in the POP^{+}KA-F-B-6 treatment.

Table 22. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of cowpea at Kayamkulam

Treatments	Nodule number	Nodule dry wt. (mg)	Plant dry wt. (g)	Yield per plot (g)	Percentage increase over control
POP^+KA-G-C-4	**28.7**	65.4	22.6	528.8	44.5
POP^-KA-G-C-4	24.7	69.8	**23.3**	459.2	25.5
POP^+KA-F-C-7	28.1	74.1	19.3	**589.2**	67.0
POP^-KA-F-C-7	27.5	**74.6**	19.0	433.3	18.4
POP^+BR^-	26.7	61.4	15.0	468.3	28.0
POP^-BR^-	18.8	52.8	12.6	366.0	
CD(0.05)	NS	NS	NS	14.0	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

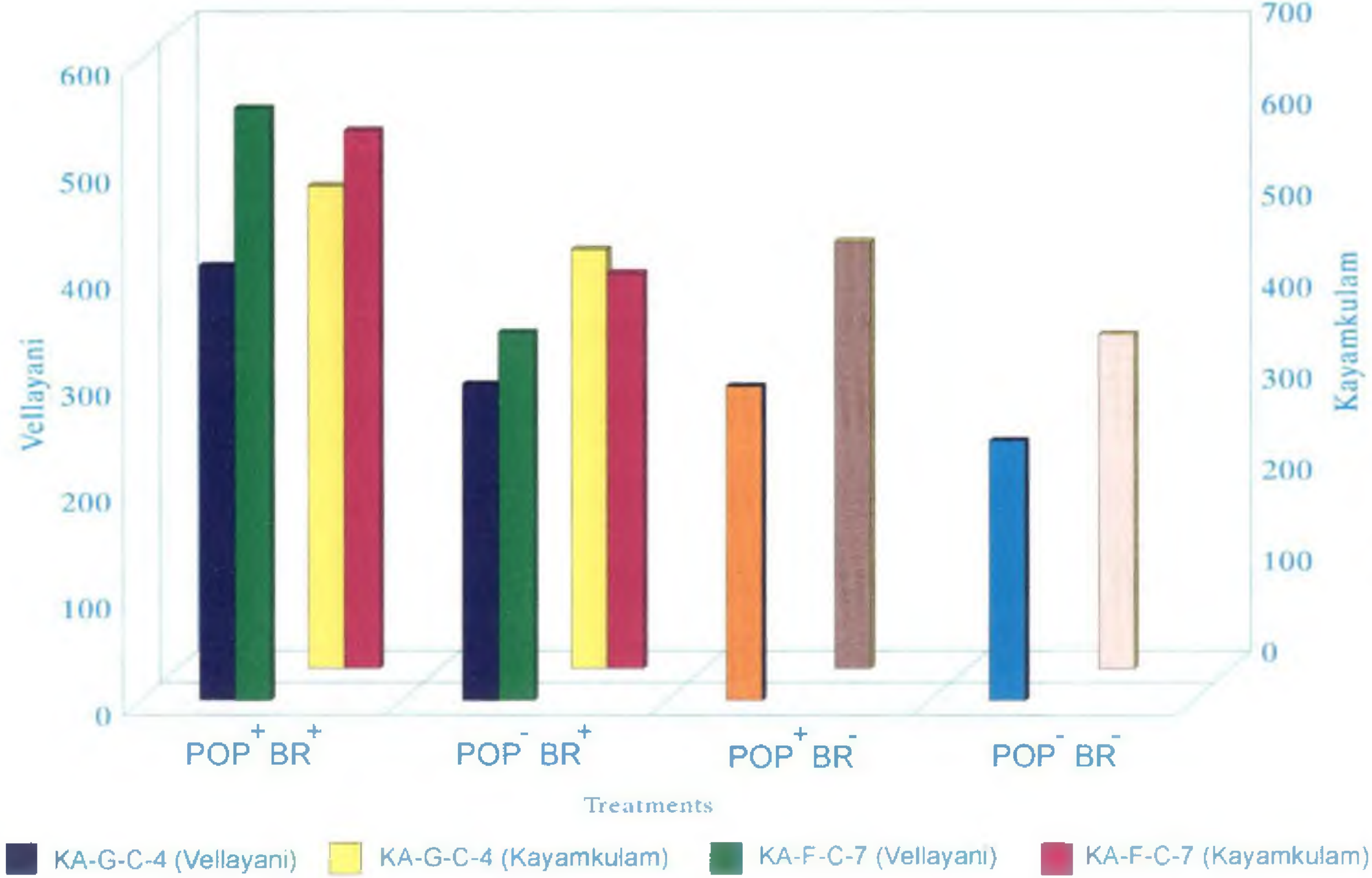


Fig. 25. Influence of POP recommendations and *Bradyrhizobium* inoculation on yield in cowpea

These were 33.5, 21.8 g and 322.5 g respectively when compared to that of 14.7, 11.8 g and 194.2 g in the control (POP⁻BR⁻) treatment (Table 23). A similar response was also obtained in the POP⁺VE-G-B-2 treatment. Here, the nodule number, plant dry weight and yield were 30.7, 18.3 g and 308.7 g respectively. There were no significant differences between treatments in nodule dry weight. The nodule number, nodule dry weight, plant dry weight and yield were reduced when *Bradyrhizobium* inoculation was done without the POP recommendations (Table 23). This reduction was significant for nodule number (25.3) in the POP⁻KA-F-B-6 treatment. In the treatment without *Bradyrhizobium* inoculation (POP⁺BR⁻) such a reduction was even more when compared to the best treatment combinations of POP⁺VE-G-B-2 and POP⁺KA-F-B-6. Here the nodule number, nodule dry weight, plant dry weight and yield were only 22.1, 50.4 mg, 15.0 and 214.0 g respectively.

A significant increase in leaf area (305.6 cm^2) was obtained in the POP⁺KA-F-B-6 treatment when compared to the control (POP⁻BR⁻) treatment (Table 24). But, in the absence of POP recommendation, the leaf area (241.8 cm^2) was significantly reduced. Such an effect was not observed in the POP⁻VE-G-B-2 treatment. As in cowpea, an increase in total leaf area (285.6 cm^2) due to the native flora was observed in the POP⁺BR⁻ treatment. However, such a stimulation was not there in the absence of the POP recommendation.

Table 23. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of blackgram at Vellayani

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)	Yield per plot (g)	Percentage increase over control
POP^{+}VE-G-B-2	30.7	**108.6**	18.3	308.7	59.0
POP^{-}VE-G-B-2	26.0	70.8	12.5	264.2	36.1
POP^{+}KA-F-B-6	**33.5**	70.1	**21.8**	**322.5**	66.1
POP^{-}KA-F-B-6	25.3	60.9	17.3	286.7	47.6
$POP^{+}BR^{-}$	22.1	50.4	15.0	214.0	10.2
$POP^{-}BR^{-}$	14.7	30.3	11.8	194.2	
CD (0.05)	8.2	NS	7.5	64.6	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 24. Influence* of POP recommendations and *Bradyrhizobium* inoculation on total leaf area of blackgram at Vellayani

Treatments	Total leaf area (cm^2)
POP^+VE-G-B-2	272.9
POP^-VE-G-B-2	272.6
POP^+KA-F-B-6	**305.6**
POP^-KA-F-B-6	241.8
POP^+BR^-	285.6
POP^-BR^-	256.0
CD(0.05)	40.4

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

4.4.2.2.2. Location - Kayamkulam

There were significant differences between treatments in nodule number, nodule dry weight and yield. The increase in nodule number due to *Bradyrhizobium* inoculation was significant when compared to the control (POP^-BR^-) treatment. These were maximum (27.0) in the POP^+VE-G-B-2 treatment. However the yield increase was significant (Table 25, Fig. 26) only in the POP^+VE-G-B-2 treatment combination. Here the per plot yield was 343.3 g (Table 25). Both the nodule number and yield were reduced where *Bradyrhizobium* inoculation was done without the POP recommendation. A variable response was however obtained in nodule dry weight. These were marginally higher in the POP^-VE-G-B-2 and POP^-KA-F-B-6 treatment. In the treatment without *Bradyrhizobium* inoculation (POP^+BR^-) such a reduction in nodule number, nodule dry weight, plant dry weight and yield were more when compared to the best treatment combinations of POP^+VE-G-B-2 and POP^+KA-F-B-6. These were only 18.5, 54.0 mg, 14.8 g and 223.3 g respectively (Table 25).

4.4.2.3. Greengram

4.4.2.3.1. Location - Vellayani

There were significant differences between treatments in nodule number, nodule dry weight and yield (Table 26). These were maximum in the POP^+PI-G-G-8 treatment for nodule number (30.4) and in the POP^+PA-G-G-5 treatment for nodule dry weight (65.9 mg).

Table 25. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of blackgram at Kayamkulam

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)	Yield per plot (g)	Percentage increase over control
POP^+VE-G-B-2	**27.0**	73.3	13.0	**343.3**	79.1
POP^-VE-G-B-2	23.3	**74.0**	14.6	274.2	43.0
POP^+KA-F-B-6	23.4	61.9	**17.0**	300.0	56.5
POP^-KA-F-B-6	22.9	62.5	11.5	272.5	42.2
POP^+BR^-	18.5	54.0	14.8	223.3	16.5
POP^-BR^-	9.8	44.8	11.3	191.7	
CD(0.05)	10.7	15.2	NS	117.1	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

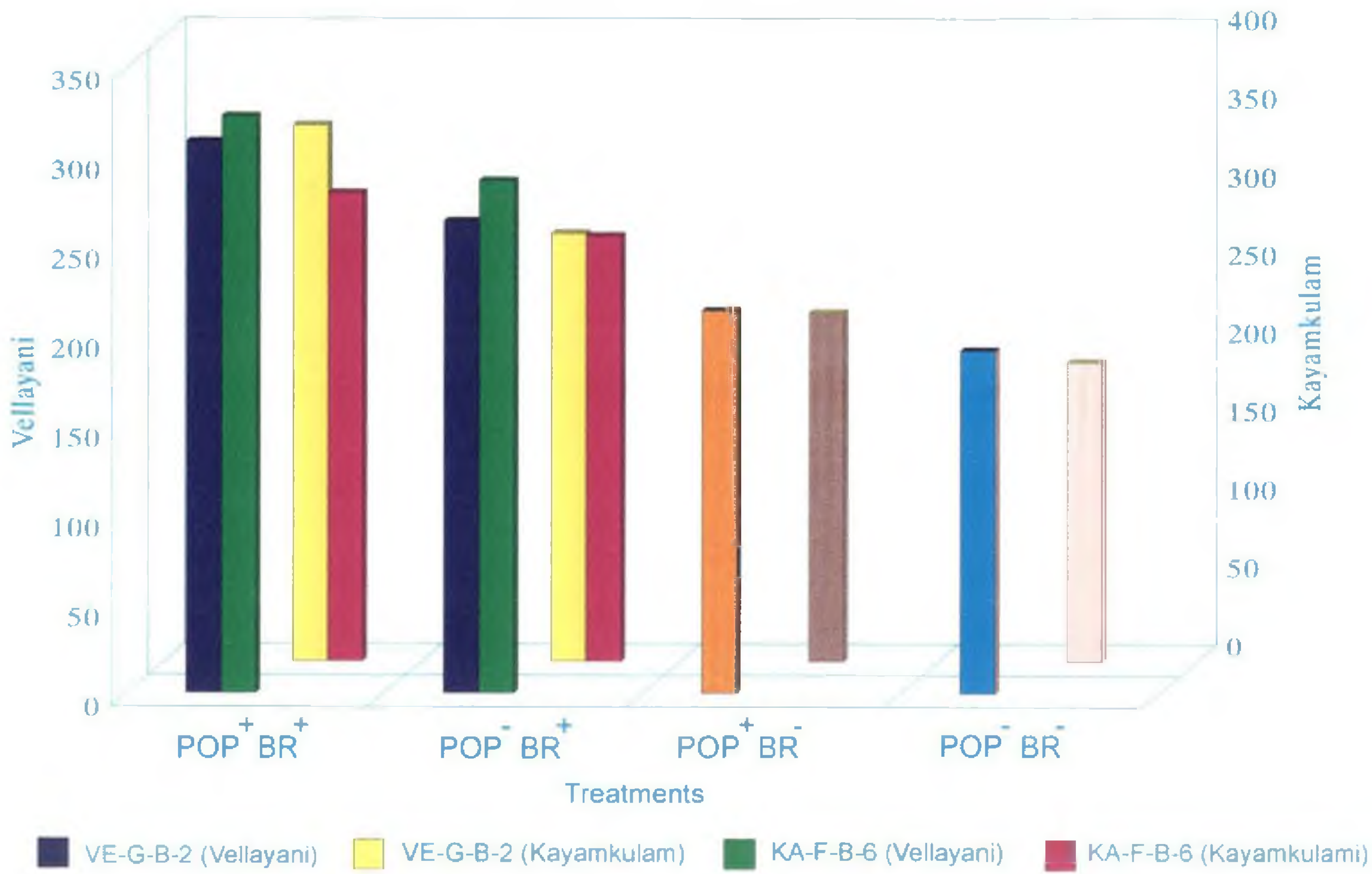


Fig. 26. Influence of POP recommendations and *Bradyrhizobium* inoculation on yield in blackgram

There were no significant differences between treatmentsin plant dry weight. As for the yield, the per plot yield of 355.8 g was maximum in the POP^{+}PI-G-G-8 treatment. A similar response (352.5 g) was also obtained in the POP^{+}PA-G-G-5 treatment. The nodulation, plant growth and yield parameters were reduced when *Bradyrhizobium* inoculation was done without the POP recommendation (Table 26). This reduction was significant for yield in the POP^{-}PA-G-G-5 (297.2g) and POP^{-}PI-G-G-8 (294.2 g) treatment. As in cowpea, at RRS, Kayamkulam, an increase in grain yield (250.8 g) was obtained in the $POP^{+}BR^{-}$ treatment. But in the absence of the POP recommendations such a stimulation by native flora was absent. Here in the ($POP^{-}BR^{-}$) treatment the yield was only 220.0 g.

In greengram also, the total leaf area was significantly increased when *Bradyrhizobium* inoculation was done along with the POP recommendation. These were 335.2 cm^2 and 329.4 cm^2 in the POP^{+}PA-G-G-5 and POP^{+}PI-G-G-8 treatments (Table 27). But in the absence of POP recommendation, the leaf area was significantly reduced in both the above treatments. These were only 304.9 and 305.0 cm^2 in the POP^{-}PA-G-G-5 and POP^{-}PI-G-G-8 treatments. As in cowpea and blackgram an increase in leaf area (322.0 cm^2) due to the native flora was observed in the $POP^{+}BR^{-}$ treatment. However, such a stimulation was not there in the absence of the POP recommendation.

Table 26. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of greengram at Vellayani

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)	Yield per plot (g)	Percentage increase over control
POP^{+}PA-G-G-5	29.8	**65.9**	12.2	352.5	60.2
POP^{-}PA-G-G-5	23.5	49.4	10.1	297.2	35.1
POP^{+}PI-G-G-8	**30.4**	65.5	**16.7**	**355.8**	61.7
POP^{-}PI-G-G-8	24.6	52.3	14.6	294.2	33.7
$POP^{+}BR^{-}$	19.9	41.9	10.0	250.8	14.0
$POP^{-}BR^{-}$	17.6	35.2	9.4	220.0	
CD (0.05)	9.0	18.3	NS	30.6	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 27. Influence* of POP recommendations and *Bradyrhizobium* inoculation on total leaf area of greengram at Vellayani

Treatments	Total leaf area (cm^2)
POP^+PA-G-G-5	**335.2**
POP^-PA-G-G-5	304.9
POP^+PI-G-G-8	329.4
POP^-PI-G-G-8	305.0
POP^+BR^-	322.0
POP^-BR^-	309.4
CD (0.05)	12.6

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

4.4.2.3.2. Location - Kayamkulam

There were significant differences between treatmentsin nodule dry weight and yield (Table 28, Fig. 27). These were maximum in the POP^{+}PI-G-G-8 treatment for nodule dry weight (67.4 mg) and in the POP^{+}PA-G-G-5 treatment for yield (289.2 g). A similar significant response was also obtained in the POP^{+}PA-G-G-5 treatment for nodule dry weight. However, there were no significant differences between treatments in nodule number and plant dry weight. The nodule dry weight, plant dry weight and yield were reduced when *Bradyrhizobium* inoculation was done without the POP recommendations (Table 28). These were only 60.3 and 60.7 mg, 15.9 and 20.6 g and 231.7 and 216.7 g respectively in the POP^{-}PA-G-G-5 and POP^{-}PI-G-G-8 treatments (Table 28). Such a reduction was even more in the $POP^{+}BR^{-}$ and $POP^{-}BR^{-}$ treatments. In both these treatments the yield were only 207.5 and 142.5 g respectively.

4.5. Influence of POP recommendations and *Bradyrhizobium* inoculation on NPK content of plants

4.5.1. Cowpea

4.5.1.1. Location - Vellayani

Significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. It was maximum (4.36 per cent) in the POP^{+}KA-F-C-7 treatment, when compared to that of 2.59 per cent in the control ($POP^{-}BR^{-}$) treatment (Table 29).

Table 28. Influence* of POP recommendations and *Bradyrhizobium* inoculation on nodulation, plant growth and yield of greengram at Kayamkulam

Treatments	Nodule number	Nodule dry weight (mg)	Plant dry weight (g)	Yield per plot (g)	Percentage increase over control
POP^{+}PA-G-G-5	25.4	64.1	16.3	**289.2**	103.0
POP^{-}PA-G-G-5	**28.2**	60.3	15.9	231.7	62.6
POP^{+}PI-G-G-8	27.0	**67.4**	**21.0**	274.2	92.4
POP^{-}PI-G-G-8	26.4	60.7	20.6	216.7	52.0
$POP^{+}BR^{-}$	23.4	55.4	12.7	207.5	45.6
$POP^{-}BR^{-}$	15.9	41.7	11.3	142.5	
CD (0.05)	NS	14.6	NS	13.67	

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

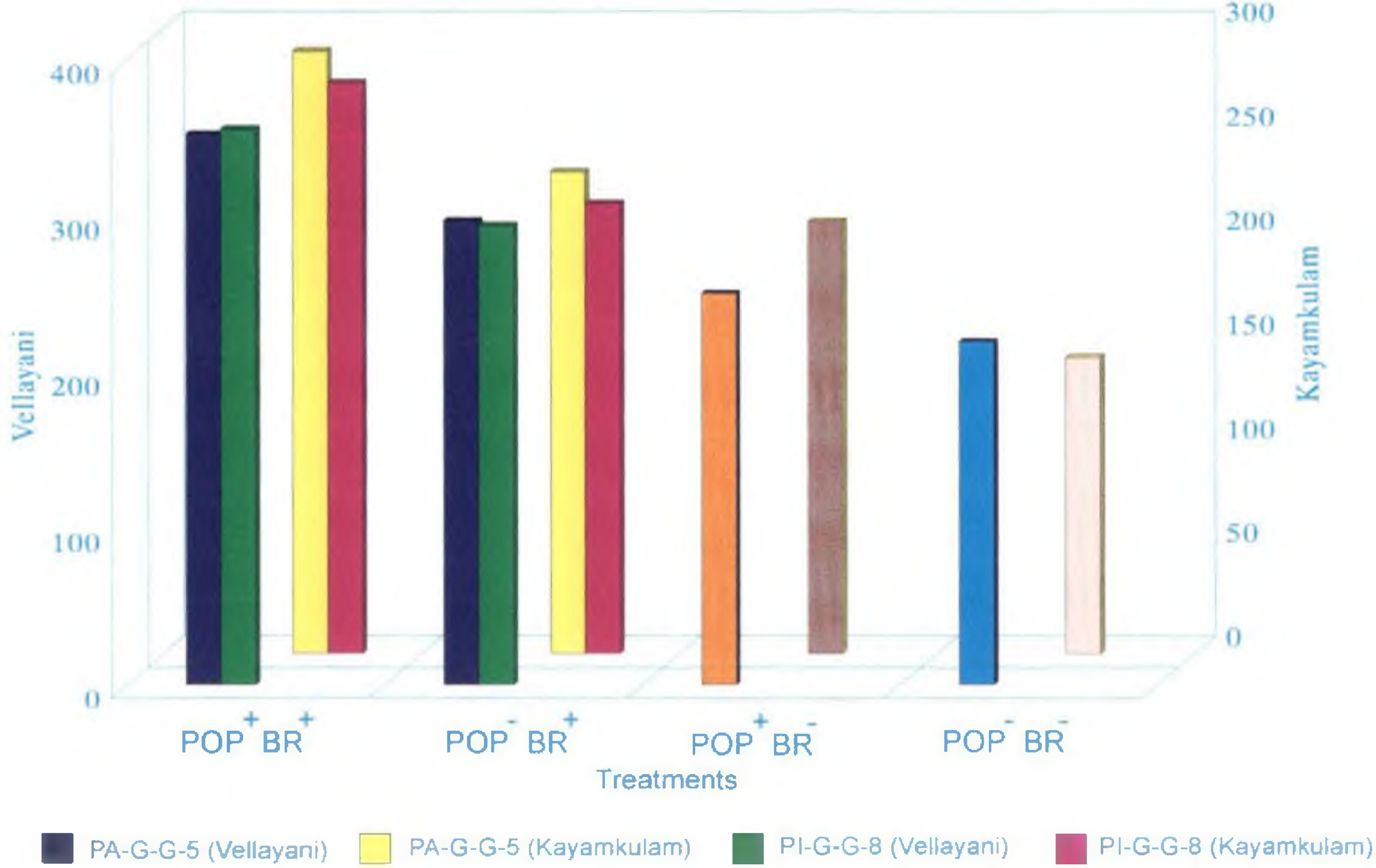


Fig. 27. Influence of POP recommendations and *Bradyrhizobium* inoculation on yield in greengram

Table 29. Influence of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of cowpea at Vellayani

Treatments	N	P	K
POP^{+}KA-G-C-4	3.93	1.35	1.47
POP^{-}KA-G-C-4	3.65	1.10	1.44
POP^{+}KA-F-C-7	4.36	1.33	1.37
POP^{-}KA-F-C-7	3.50	1.01	1.35
$POP^{+}BR^{-}$	2.61	1.21	1.47
$POP^{-}BR^{-}$	2.59	1.30	1.44
CD(0.05)	0.65	0.17	0.10

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

A similar response (3.93 per cent) was also observed in the POP^{+}KA-G-C-4 treatment. In both the above treatments N content was reduced when *Bradyrhizobium* inoculation was done without POP recommendation. Such a reduction (3.50 per cent) was significant in the POP^{-}KA-F-C-7 treatment (Table 29). The P content was also increased in treatments with POP recommendation. This was maximum (1.35 per cent) in the POP^{+}KA-G-C-4 treatment. However, the P content of plants was significantly reduced in treatments without POP recommendation. These were only 1.10 and 1.01 per cent respectively in the POP^{-}KA-G-C-4 and POP^{-}KA-F-C-7 treatments (Table 29). There were some variations in the K content of plants. It was maximum (1.47 per cent) in the POP^{+}KA-G-C-4 and $POP^{+}BR^{-}$ treatments.

4.5.1.2. Location - Kayamkulam

Significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. It was however, maximum (4.16 per cent) in the POP^{-}KA-G-C-4 treatment when compared to that of 2.39 per cent in the control ($POP^{-}BR^{-}$) treatment (Table 30). There were no significant differences between treatments in the P and K content.

Table 30. Influence of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of cowpea at Kayamkulam

Treatments	N	P	K
POP^{+}KA-G-C-4	4.00	0.74	1.35
POP^{-}KA-G-C-4	4.16	0.62	1.26
POP^{+}KA-F-C-2	3.64	0.71	1.24
POP^{-}KA-F-C-2	3.46	0.62	1.27
$POP^{+}BR^{-}$	2.70	0.71	1.31
$POP^{-}BR^{-}$	2.39	0.74	1.40
CD(0.05)	0.71	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.5.2. Blackgram

4.5.2.1. Location - Vellayani

In blackgram also, significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. It was maximum (3.87 per cent) in the POP^{+}VE-G-B-2 treatment when compared to that of 2.41 per cent in the control ($POP^{-}BR^{-}$) treatment (Table 31). Unlike in cowpea, a somewhat variable response in N content was observed when *Bradyrhizobium* inoculation was done without the POP recommendation. There were no significant differences between treatments in the P and K content.

4.5.2.2. Location - Kayamkulam

Significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. It was maximum (4.0 per cent) in the POP^{+}KA-F-B-6 treatment when compared to that of 2.47 per cent in the control ($POP^{-}BR^{-}$) treatment (Table 32). A similar response (3.59 per cent) was obtained in the POP^{+}VE-G-B-2 treatment. In both the above treatments, the N content was reduced when *Bradyrhizobium* inoculation was done without POP recommendation. These were only 3.81 and 3.45 per cent respectively in the POP^{-}KA-F-B-6 and POP^{-}VE-G-B-2 treatments. There were no significant differences between treatments in the P and K content of plants.

Table 31. Influence of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of blackgram at Vellayani

Treatments	N	P	K
POP^{+}VE-G-B-2	3.87	0.76	0.65
POP^{-}VE-G-B-2	3.85	0.85	0.62
POP^{+}KA-F-B-6	3.71	0.79	0.55
POP^{-}KA-F-B-6	3.72	0.57	0.62
$POP^{+}BR^{-}$	2.67	0.67	0.56
$POP^{-}BR^{-}$	2.41	0.73	0.52
CD (0.05)	0.84	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 32. Influence of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of blackgram at Kayamkulam

Treatments	N	P	K
POP^{+}VE-G-B-2	3.59	0.31	0.47
POP^{-}VE-G-B-2	3.45	0.32	0.48
POP^{+}KA-F-B-6	4.00	0.34	0.38
POP^{-}KA-F-B-6	3.81	0.35	0.43
$POP^{+}BR^{-}$	2.65	0.36	0.36
$POP^{-}BR^{-}$	2.47	0.40	0.47
CD (0.05)	0.79	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.5.3. Greengram

4.5.3.1. Location - Vellayani

There were no significant differences between treatments in the NPK content of plants at Vellayani (Table 33). In general, the N and P contents were higher in the treatment combination of POP$^+$ BR$^+$ inoculation. These were maximum (3.81 and 0.92 per cent respectively) in the POP$^+$PI-G-G-8 treatment. A similar response was not obtained in the K content of plants.

4.5.3.2. Location - Kayamkulam

Significant increase in N content of plants were obtained due to *Bradyrhizobium* inoculation. It was maximum (3.87 per cent) in the POP$^+$PA-G-G-5 treatment when compared to that of 2.59 per cent in the control (POP$^-$BR$^-$) treatment (Table 34). A similar response (3.77 per cent) was also obtained in the POP$^+$PI-G-G-8 treatment. In both the above treatments, the N content was reduced when *Bradyrhizobium* inoculation was done without the POP recommendation. Thus in the POP$^-$PA-G-G-5 and POP$^-$PI-G-G-8 treatments, the N content was only 3.41 and 3.68 per cent respectively. A variable response was obtained in the P content of plants. There were no significant differences between treatments in the K content.

Table 33. Influence* of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of greengram at Vellayani

Treatments	N	P	K
POP^{+}PA-G-G-5	3.45	0.85	0.39
POP^{-}PA-G-G-5	3.42	0.84	0.38
POP^{+}PI-G-G-8	3.81	0.92	0.48
POP^{-}PI-G-G-8	3.21	0.88	0.44
$POP^{+}BR^{-}$	2.50	0.70	0.38
$POP^{-}BR^{-}$	2.29	0.61	0.50
CD (0.05)	NS	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 34. Influence* of POP recommendations and *Bradyrhizobium* inoculation on (%) NPK content of greengram at Kayamkulam

Treatments	N	P	K
POP^{+}PA-G-G-5	3.87	0.22	0.36
POP^{-}PA-G-G-5	3.41	0.30	0.40
POP^{+}PI-G-G-8	3.77	0.48	0.32
POP^{-}PI-G-G-8	3.68	0.34	0.42
$POP^{+}BR^{-}$	2.48	0.33	0.40
$POP^{-}BR^{-}$	2.59	0.30	0.42
CD (0.05)	0.59	0.20	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.6. Influence of POP recommendations and *Bradyrhizobium* inoculation on soil nutrient status

4.6.1. Cowpea

4.6.1.1. Location - Vellayani

In general, the availability of P, K, Ca and Mg were significantly increased in treatments involving *Bradyrhizobium* inoculation along with the POP recommendation. These were maximum in the treatment combination of POP^+KA-G-C-4 where the soil P, K, Ca and Mg contents were 15.87, 47.97, 146.02 and 22.34 ppm respectively (Table 35). In the control treatment (POP^-BR^-) these were only 13.77, 41.67, 121.37 and 18.72 ppm respectively. The increase in P (15.60 ppm), Ca and Mg (140.5 and 22.06 ppm) in the POP^+KA-F-C-7 treatment was also significant. Such an increase in K and Mg (47.77 and 21.46 ppm) was also obtained in the POP^+BR^- treatment. However, in *Bradyrhizobium* treatments without POP recommendation, there was a significant reduction in the availability of P, K, Ca and Mg. These were only 14.00, 38.82, 126.23 and 19.45 ppm respectively in the POP^-KA-G-C-4 treatment and 13.98, 38.82, 121.65 and 18.98 ppm respectively in the POP^-KA-F-C-7 treatment (Table 35). There were no significant differences between treatments in soil pH, organic carbon, Fe and Al content.

Table 35. Influence* of POP recommendations and *Bradyrhizobium* inoculation in cowpea on soil nutrient status at Vellayani

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^{+}KA-G-C-4	15.87	47.97	1.36	4.65	146.02	22.34	127.95	17.22
POP^{-}KA-G-C-4	14.00	38.82	0.76	4.64	126.23	19.45	129.3	14.82
POP^{+}KA-F-C-7	15.60	46.63	0.73	4.61	140.50	22.06	125.1	14.60
POP^{-}KA-F-C-7	13.98	38.82	0.70	4.53	121.65	18.98	125.38	18.35
$POP^{+}BR^{-}$	14.62	47.77	0.99	4.73	133.07	21.46	126.13	14.73
$POP^{-}BR^{-}$	13.77	41.67	0.91	4.79	121.37	18.72	125.4	16.31
CD (0.05)	0.98	6.10	NS	NS	18.30	2.31	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.6.1.2 Location - Kayamkulam

The availability of P, K, Ca and Mg were significantly increased in treatments involving *Bradyrhizobium* inoculation along with the POP recommendation. These were maximum in the treatment combination of POP^+KA-G-C-4 for P (13.90 ppm), K (13.48 ppm) and Ca (40.15 ppm) (Table 36). The Mg content of 26.69 ppm was, however, higher in the POP^+KA-F-C-7 treatment. In the control treatment (POP^-BR^-), these were only 10.48, 11.70, 27.82 and 21.44 ppm respectively. A significant increase in P (13.28 ppm) and Mg (26.33 ppm) was also obtained in the POP^+BR^- treatment. As in the previous experiment, the availability of these elements particularly that of P, K and Mg were significantly reduced in *Bradyrhizobium* treatments without POP recommendation. The soil P, K and Mg content in the POP^-KA-G-C-4 and POP^-KA-F-C-7 treatments were only 11.57 and 11.08, 12.02 and 12.0, 21.35 and 20.71 ppm respectively. However, such a reduction was not observed in Ca content. There were no significant differences between treatments in the soil pH, organic carbon, Fe and Al content.

4.6.2. Blackgram

4.6.2.1. Location - Vellayani

There were no significant differences between treatments in P, K, organic carbon, pH, Fe and Al content. A significant variation was observed only in POP^-KA-F-B-6 treatment.

Table 36. Influence* of POP recommendations and *Bradyrhizobium* inoculation in cowpea on soil nutrient status at Kayamkulam

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^+KA-G-C-4	13.90	13.48	0.57	5.16	40.15	24.71	120.42	12.37
POP^-KA-G-C-4	11.57	12.02	0.64	5.21	39.47	21.35	120.80	11.55
POP^+KA-F-C-7	13.08	13.30	0.50	5.22	37.40	26.69	117.05	11.83
POP^-KA-F-C-7	11.08	12.00	0.51	5.17	36.43	20.71	115.93	12.11
POP^+BR^-	13.28	12.93	0.53	5.21	33.53	26.33	117.12	12.95
POP^-BR^-	10.48	11.70	0.46	5.24	27.82	21.44	119.47	13.35
CD (0.05)	1.99	1.25	NS	NS	6.80	1.78	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^- without *Bradyrhizobium* inoculation

As in cowpea, the availability of Ca and Mg was significantly increased in treatments where *Bradyrhizobium* inoculation was done along with POP recommendation. These were 150.85 and 151.88 ppm and 22.27 and 23.07 ppm respectively (Table 37). In the control treatment, (POP^-BR^-) Ca and Mg contents were only 130.08 and 18.38 ppm respectively. However, in the absence of POP recommendation, availability of these elements were reduced. Such a reduction was significant for Ca in the POP^-KA-F-B-6 treatment and for Mg in both the POP^-VE-G-B-2 and POP^-KA-F-B-6 treatments.

4.6.2.2. Location - Kayamkulam

There were no significant differences between treatments in soil P, K, organic carbon, pH, Fe and Al content. The availability of Ca and Mg was significantly increased in the treatment combinations of POP^+VE-G-B-2 and POP^+KA-F-B-6. These were 37.47 and 40.13 ppm and 26.91 and 25.90 ppm respectively when compared to that of 26.38 and 21.85 ppm respectively in the control treatment (Table 38). However, the Ca and Mg contents were significantly reduced in treatments without POP recommendation. These were only 22.75 ppm for Mg in the POP^-VE-G-B-2 treatment and 29.97 and 21.90 ppm for both Ca and Mg in the POP^-KA-F-B-6 treatment.

Table 37. Influence* of POP recommendations and *Bradyrhizobium* inoculation in blackgram on soil nutrient status at Vellayani

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^{+}VE-G-B-2	15.87	47.65	0.64	4.68	150.85	22.27	126.2	17.18
POP^{-}VE-G-B-2	14.42	44.27	0.69	4.62	143.12	19.55	125.1	17.48
POP^{+}KA-F-B-6	15.67	46.12	0.68	4.63	151.88	23.07	123.7	17.85
POP^{-}KA-F-B-6	14.78	43.82	0.66	4.72	133.37	18.87	123.6	16.13
$POP^{+}BR^{-}$	16.02	46.65	0.63	4.66	141.20	21.54	124.4	14.93
$POP^{-}BR^{-}$	14.47	42.78	0.69	4.61	130.08	18.38	122.4	17.05
CD(0.05)	NS	NS	NS	NS	13.30	2.21	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 38. Influence* of POP recommendations and *Bradyrhizobium* inoculation in blackgram on soil nutrient status at Kayamkulam

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^{+}VE-G-B-2	14.50	14.20	0.54	5.20	37.47	26.91	120.45	14.63
POP^{-}VE-G-B-2	13.68	11.65	0.54	5.19	33.70	22.75	115.5	14.27
POP^{+}KA-F-B-6	14.17	13.78	0.58	5.20	40.13	25.90	118.77	14.15
POP^{-}KA-F-B-6	12.95	12.05	0.57	5.20	29.97	21.90	119.67	13.78
$POP^{+}BR^{-}$	16.20	13.27	0.56	5.16	35.65	23.83	121.15	13.00
$POP^{-}BR^{-}$	13.80	11.08	0.51	5.18	26.38	21.85	115.12	12.37
CD (0.05)	NS	NS	NS	NS	9.59	3.10	NS	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.6.3. Greengram

4.6.3.1. Location - Vellayani

In general, the availability of P, K, Ca and Mg were significantly increased in treatments involving *Bradyrhizobium* inoculation along with the POP recommendation. These were maximum in the treatment combinations of POP^{+}PA-G-G-5 for P (16.52 ppm) and K (49.02 ppm) and in the POP^{+}PI-G-G-8 for Ca (147.98 ppm) and Mg (23.86 ppm). In the control treatment ($POP^{-}BR^{-}$) the P, K, Ca and Mg content were only 14.53, 41.05, 128.10 and 17.85 ppm respectively (Table 39). A significant increase in K (47.22 ppm) and Mg (21.12 ppm) was also obtained in the $POP^{+}BR^{-}$ treatment. However, the availability of these elements were reduced in treatments without POP^{-} recommendation. This reduction was significant in the POP^{-}PA-G-G-5 for P (14.93 ppm), K (43.82 ppm) and Mg (19.02 ppm) and in the POP^{-}PI-G-G-8 treatment for K (42.97 ppm) and Mg (18.26 ppm). But there were no significant differences between treatments in soil pH, organic carbon, Fe and Al content.

4.6.3.2. Location - Kayamkulam

There were significant differences between treatments in the P, K, organic carbon, Ca, Mg and Fe content of the soil. However, much variations were observed in the availability of these elements with and without the POP recommendations (Table 40). Further, there were no significant differences between treatments in soil pH and Al content.

Table 39. Influence* of POP recommendations and *Bradyrhizobium* inoculation in greengram on soil nutrient status at Vellayani

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^{+}PA-G-G-5	16.52	49.02	0.89	4.58	147.35	22.98	124.97	14.07
POP^{-}PA-G-G-5	14.93	43.82	0.82	4.59	133.65	19.02	123.22	15.67
POP^{+}PI-G-G-8	16.30	48.05	0.71	4.61	147.98	23.86	126.60	14.88
POP^{-}PI-G-G-8	14.95	42.97	0.69	4.59	123.23	18.26	126.33	14.42
POP^{+}BR^{-}	15.87	47.22	0.80	4.55	141.02	21.12	126.93	19.81
POP^{-}BR	14.53	41.05	0.80	4.57	128.10	17.85	125.97	19.72
CD (0.05)	1.48	4.53	NS	NS	20.41	3.26	NS	NS

* Mean of 2 seasons

POP$^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

Table 40. Influence* of POP recommendations and *Bradyrhizobium* inoculation in greengram on soil nutrient status at Kayamkulam

Treatments	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
POP^{+}PA-G-G-5	13.62	14.41	0.58	5.17	36.97	27.43	120.32	16.35
POP^{-}PA-G-G-5	15.97	13.35	0.58	5.14	37.3	27.02	113.95	15.11
POP^{+}PI-G-G-8	12.12	11.14	0.49	5.24	33.22	20.72	13.28	16.81
POP^{-}PI-G-G-8	12.10	10.57	0.59	5.21	25.18	21.25	111.87	16.22
$POP^{+}BR^{-}$	15.37	11.77	0.55	5.16	31.18	24.81	112.57	16.87
$POP^{-}BR^{-}$	12.60	10.28	0.53	5.14	23.45	21.99	110.28	15.21
CD (0.05)	3.63	1.76	0.08	NS	9.04	4.97	6.44	NS

* Mean of 2 seasons

$POP^{+/-}$ with or without package of practices recommendations

BR^{-} without *Bradyrhizobium* inoculation

4.7. Influence of POP recommendations and *Bradyrhizobium* inoculation on the economics of cowpea, blackgram and greengram cultivation

4.7.1. Cowpea

The maximum net return of Rs. 11456 was obtained from the best treatment combination of POP^{+}KA-F-C-7 (Table 41). This was followed by the treatment combination of POP^{+}KA-G-C-4 (Rs. 9372). The benefit cost ratio for these treatments were 1.82 and 1.49 respectively when compared to 1.28 for the control ($POP^{-}BR^{-}$) treatment.

4.7.2. Blackgram

The maximum net return of Rs. 9780 was obtained from the best treatment combination of POP^{+}VE-G-B-2 (Table 42). This was followed by the treatment combination of POP^{+}KA-F-B-6 (Rs. 9339). The benefit cost ratio for these treatments were 1.49 and 1.42 respectively when compared to 1.03 for the control ($POP^{-}BR^{-}$) treatment.

4.7.3. Greengram

The maximum net return of Rs. 9627 was obtained from the best treatment combination of POP^{+}PA-G-G-5 (Table 43). This was followed by the treatment combination of POP^{+}PI-G-G-8 (Rs. 9450).

Table 41. Influence of POP recommendations and *Bradyrhizobium* inoculation on the economics* of cowpea cultivation

Treatment	Cost (Rs.)	Yield/ha (kg)	Net return (Rs.)	Benefit cost ratio
POP^{+}KA-G-C-4	6294	468.6	9372	1.49
POP^{-}KA-G-C-4	4800	378.2	7564	1.58
POP^{+}KA-F-C-7	6294	572.8	11456	1.82
POP^{-}KA-F-C-7	4800	389.6	7792	1.62
$POP^{+}BR^{-}$	6194	381.8	7636	1.23
$POP^{-}BR^{-}$	4750	304.9	6098	1.28

* Based on pooled data for two locations

Table 42. Influence of POP recommendations and *Bradyrhizobium* inoculation on the economics* of blackgram cultivation

Treatment	Cost (Rs.)	Yield/ha (kg)	Net return (Rs.)	Benefit cost ratio
POP^{+}VE-G-B-2	6575	326.0	9780	1.49
POP^{-}VE-G-B-2	5675	269.2	8076	1.42
POP^{+}KA-F-B-6	6575	311.3	9339	1.42
POP^{-}KA-F-B-6	5675	279.6	8388	1.48
$POP^{+}BR^{-}$	6500	218.7	6561	1.01
$POP^{-}BR^{-}$	5600	193.0	5790	1.03

* Based on pooled data for two locations

Table 43. Influence of POP recommendations and *Bradyrhizobium* inoculation on the economics* of greengram cultivation

Treatment	Cost (Rs.)	Yield/ha (kg)	Net return (Rs.)	Benefit cost ratio
POP^{+}PA-G-G-5	6812	320.9	9627	1.41
POP^{-}PA-G-G-5	5912	264.5	7935	1.34
POP^{+}PI-G-G-8	6812	315.0	9450	1.39
POP^{-}PI-G-G-8	5912	255.5	7665	1.30
$POP^{+}BR^{-}$	6737	229.2	6876	1.02
$POP^{-}BR^{-}$	5837	181.3	5439	0.93

* Based on pooled data for two locations

The benefit cost ratio for these treatments were 1.41 and 1.39 respectively when compared to 0.93 for the control ($POP^{-}BR^{-}$) treatment.

DISCUSSION

DISCUSSION

Among all diazotrophic systems, legume - *Rhizobium* symbiosis is probably the most beneficial to crop plants. The importance of this symbiotic association in improving and sustaining soil fertility was known to mankind right from the days of early agriculture. Later investigations by several workers have helped to understand the true nature of legume - *Rhizobium* symbiosis. These studies clearly demonstrated the occurrence of several *Rhizobium* species in the soil capable of nodulating one or more specific leguminous host plants with occasional incidence of symbiotic promiscuity. Today, the existence of three different genera of rhizobia such as *Rhizobium, Bradyrhizobium* and *Azorhizobium* comprising nearly 17 species have been recognised (Jordan, 1984).

The important soil and other environmental factors influencing the efficiency of legume - *Rhizobium* symbiosis under actual field conditions were also investigated by many scientists (Jenkins *et al.*, 1954; de Carvalho *et al.*, 1982 and Brady *et al.*, 1990). Such studies, infact, enabled to derive maximum benefits from this symbiotic association to a standing crop or to a subsequent non-leguminous crop involved in a crop rotation practice.

In Kerala, the cultivation of pulses is on a small scale when compared to that in other states in India. Among the different pulses cultivated in the state, cowpea is the most important one followed by blackgram and greengram. The non-availability of sufficient land area and the marketing facility for the end produce is often reported to be responsible for this situation. It is further aggravated by the existence of unfavourable soil conditions such as acidity and poor organic matter content. The soil acidity, depending on the locations, vary from pH 3.20 to 5.52, a range often found to be quite unfavourable for the growth and nitrogen fixation of rhizobia. These problems can be solved only by selecting suitable acid tolerant strains of either *Rhizobium* or *Bradyrhizobium* for large scale seed treatment. There is also a need for evolving appropriate agronomic practices for optimum field performance of such introduced inoculum in acid soils. It was with these objectives, the Department of Biotechnology, Government of India, sponsored a research project entitled "*Rhizobium* - Pulses Association - Development of efficient strains of *Rhizobium* suitable for acid soil conditions" for investigation by the Kerala Agricultural University in 1993. The present research project was taken up as part of this DBT project.

The initial isolation of *Bradyrhizobium* was done from seven different locations in the state. They were broadly grouped into category A and B locations. In the former locations such as Vellayani, Kayamkulam, Pattambi and Pilicode, pulses especially cowpea, blackgram and greengram were cultivated regularly under both gardenland and in rice fallows during the summer season. The isolation was done by raising uniformly the same crop varieties namely Kanakamani for cowpea, Shyama for blackgram and Co-2 for greengram at different locations without resorting to any soil amendments. The average pH, organic carbon, Ca, Mg, Mo, Fe and Al content of soil samples collected from these sites were 5.05, 0.76 per cent, 475.3, 118.8, 0.0139, 87.2 and 51.0 ppm respectively (Table 1, Fig. 2-9). On the contarary, in the second set of locations such as Ambalapuzha, Kuttanad and Vytilla, where either extreme soil acidity or saline acidic conditions were the limiting factors for regular cultivation of pulses, the average soil pH, organic carbon, Ca, Mg, Mo, Fe and Al content were 3.62, 1.79 per cent, 507.5, 166.1, 0.0092, 93.4 and 279.1 ppm respectively (Table 1, Fig. 2-9). The isolation of *Bradyrhizobium* was attempted from these locations mainly to find out the occurrence, if any, of extreme acid tolerant strains of *Bradyrhizobium* suitable for cowpea, blackgram and greengram.

Observations on the native root nodulation and plant growth characters of cowpea, blackgram and greengram were taken for both category A and B locations. It was observed that in general, plant growth was better in plants raised under category A locations. Here, the average nodule number, nodule dry weight and plant dry weight were 7.9, 16.8 mg and 9.7 g in cowpea, 8.7, 14.0 mg and 7.6 g in blackgram and 8.2, 13.8 mg and 7.2 g in greengram respectively. At the same time, in category B locations these were only 3.5, 18.7 mg and 3.7 g in cowpea, 3.2, 12.4 mg and 2.7 g in blackgram and 5.3, 11.4 mg and 1.7 g in greengram respectively (Table 2, Fig. 2-9). The negative influence of soil factors such as extreme acidity (pH 3.62), high Fe (93.4 ppm) and Al (279.1 ppm) content may be responsible for this. Besides the Mo content of the soil was also very low. It was only 0.0092 ppm when compared to that of 0.0139 ppm for category A locations. The deleterious effects of high Fe and Al content particularly that of Al on root nodulation have been reported earlier also by several workers (Andrew *et al.*, 1973; Kim *et al.*, 1985; Wood and Cooper, 1988c and Coventry and Evans, 1989).

In all, 43 native isolates of *Bradyrhizobium* including 17 from cowpea, 13 each from blackgram and greengram were obtained during this investigation. They were initially characterised based on their typical colony characteristics on YEMA medium. The screening for nodulation efficiency was done as per the Bureau of Indian Standard Specification (IS:8268/1976) which stipulates that "a given culture of *Rhizobium* can be considered of required quality if seed treatment with the culture will result in significant with atleast 50 per cent increase in dry weight of inoculated plants when compared to the uninoculated and unfertilised control treatment". Eventhough the main screening trial was done by using sterile soil amended with FYM (@ 20 t ha^{-1}), a parallel pot trial was also conducted using unsterilised soil without the addition of FYM. The analysis of the data from both these trials showed that the performance of individual isolates of *Bradyrhizobium* in amended and unamended soils were not identical for any of the nodulation and plant growth parameters studied (Table 3-8, Fig. 10-12, Plates 1-6). Thus while in amended soil, the isolates KA-F-C-7, KA-F-B-6 and PI-G-G-8 from cowpea, blackgram and greengram produced maximum increase in plant dry weight (33.3g, 18.9g and 16.7 g respectively), in unamended soil, the isolates KA-G-C-4, VE-G-B-2 and PA-G-G-5 produced maximum increase in plant dry weight (17.3g, 23.3g and 9.4 g respectively). The best isolates from both amended as well as unamended soils were selected for further studies. This type of procedure had been adopted earlier also by Heydock *et al.* (1980). In these studies it was observed that there was no direct correlation between nodule number and plant dry weight. Such an observation has been made earlier also by Barthakur (1980). In fact, what is actually important in a nodulated legume is not the total number of nodules formed but its nitrogen fixation ability.

The plant dry weight in the above experiments, especially cowpea and greengram were more when grown in the amended soil. This gave an indication that the native isolates of *Bradyrhizobium* were amenable to soil amendments such as the addition of FYM which resulted in better growth response in both cowpea and greengram. The influence of soil amendments on root nodulation and plant growth have been reported earlier also by Kadam and Desai, 1983; Kataoka and Haramaki, 1985 and Mathan *et al.* 1994. It is likely that the addition of FYM might have resulted in changes in soil pH and availability of nutrients favouring better root nodulation and plant growth by *Bradyrhizobium.*

The six selected isolates of *Bradyrhizobium* based on plant dry weight were further studied in detail for their growth characters on YEMA with congored, YEMA with BTB, glucose peptone agar, lactose agar and Hofer's alkaline medium of pH 11.0. The colony characteristics as studied earlier during initial isolation were typical of *Bradyrhizobium* forming white translucent glistening and elevated colonies with entire margin on YEMA with congored. All the six isolates were gram negative and fast growers attaining satisfactory growth on YEMA within three days (Table 9). This observation was contradictory to some of the earlier reports that *Bradyrhizobium* isolates were generally slow growers (Jordan, 1982). However, the occurence of fast growing strains of *Bradyrhizobium* have been reported by many workers such as Bromfield and Rao (1983) and Broughton *et al.* (1984). Hence, growth rate need not be an important factor for differentiating *Rhizobium* and *Bradyrhizobium* species. Similarly, it was also found that most of these isolates were capable of acid production on YEMA with BTB. The only exception was the isolate KA-F-C-7. This observation was also not in agreement with some of the earlier reports indicating an alkaline reaction of isolates from acidic soils (Bromfield and Rao, 1983). Eventhough the growth rate and acid production reactions of the native isolates were not in agreement with some of the earlier reports, they had a negative growth response on lactose agar and glucose peptone agar and Hofer's alkaline medium. These tests are carried out to differentiate *Rhizobium* from *Agrobacterium* sp. which are classified under the same family Rhizobiaceae (Allen and Allen, 1958).

After the initial characterisation of native isolates of *Bradyrhizobium* was done, the pentose and hexose sugar utilisation pattern, tolerance to different levels of acidic pH, iron and aluminium chloride were studied under *in vitro* conditions. The data were compared with that of three exotic isolates of *Rhizobium* for cowpea, blackgram and greengram obtained from the Indian Agricultural Research Institute, New Delhi. The carbohydrate utilisation pattern showed that arabinose, a pentose sugar, was the most preferred carbon source for the native isolates. The mean OD_{600} for these cultures was 0.216 when compared to that of only 0.162 for the exotic isolates (Table 10, Fig. 13 and 14). At the same time, the growth of the exotic isolates were maximum in glucose. The average OD_{600} for the exotic isolates was 0.193 as compared to 0.167 for the native isolates. An almost similar pattern was observed in the utilisation pattern of another hexose sugar, the galactose. The preferential utilisation of pentoses by *Bradyrhizobium* sp. and hexoses by *Rhizobium* have been reported earlier by Graham and Parker (1964) and Stanier *et al.* (1989). But, there were not much difference between these isolates in the utilisation pattern of either mannitol or xylose.

The ability to tolerate low levels of acidic pH up to 4.5 was more for the native isolates (Table 11). At this extreme acidic pH, the mean OD_{600} for these isolates was 0.365 when compared to that of 0.318 for the exotic isolates (Fig 15 and 16). The growth of both these isolates gradually improved and attained a peak level at pH 7.5. The mean OD_{600} at this pH for the native and exotic isolates were 0.481 and 0.491 respectively. The relatively better tolerance of native isolates of *Bradyrhizobium* to low pH might be due to the fact that they were initially isolated from acidic soils of pH ranging from 5.52 to 4.42. Such observations have been made earlier also by Graham *et al.* (1982) and Thornton and Davey (1983a). This character should be an essential criterion in the selection of *Rhizobium* or *Bradyrhizobium* isolates for seed treatment of crops meant for cultivation in acid soils. This will enable better survival of the introduced inoculum in competition with the already existing acid tolerant strains in the soil.

The *in vitro* tolerance to Fe and Al were almost identical for both the native and exotic isolates. In general, the growth was progressively inhibited with an increase in the concentration of these elements from 50 to 250 ppm (Table 12 and 13, Fig. 17-20). However, at 50 and 100 ppm concentrations of aluminium chloride, the growth of the native isolates were more suppressed than the *Rhizobium* isolates. The OD_{600} at these levels of aluminium chloride were 0.415 and 0.449 for the exotic isolates and 0.368 and 0.420 ppm respectively for the native isolates (Table 13, Fig. 19 and 20). This showed that the native isolates of *Bradyrhizobium* were more sensitive to Al. Similar observations have been made earlier by Andrew *et al.* (1973), de Carvalho *et al.* (1982) and Kim *et al.* (1985).

The antibiotic resistant markers for ampicillin, streptomycin and kanamycin were same for both the native and exotic isolates (Fig. 21). The occurrence of similar types of antibiotic markers have been reported earlier also by Josey *et al.* (1979), Beynon and Josey (1980), Dakora (1985) and Borges *et al.* (1990). In the serological characterisation it was observed that one of the heterologous antigens (PI-G-G-8) from greengram showed positive agglutination with that of the cowpea isolate, KA-F-C-7 (Plate 7). This indicated that these two isolates were either the same or very closely related to each other in their antigenic properties. This could be so because, *Rhizobium* sp. grouped earlier under the cowpea miscellany (Fred *et al.*, 1932) were capable of nodulating many different host genera like cowpea, blackgram and greengram. Another important observation was the lack of any serological relationship between the cowpea isolate (KA-F-C-7) and the isolates of cowpea, blackgram and greengram from the extreme acidic soil (Plate 8). This indicated that these strains were probably distinct from those present in "normally acidic" soils.

The earlier observation that the performance of even acid tolerant strains of *Bradyrhizobium* could be improved by soil amendments prompted the lay out of two separate pot trials to find out the relative benefits of the same on root nodulation and plant growth in cowpea, blackgram and greengram. It was found that out of the three variable factors used for this purpose, application of FYM, liming or pelleting either alone or in combination (FYM^+L^+/FYM^+P^+), the use of FYM was more critical for inducing better root nodulation and plant growth in all the three crops (Table 14-19, Fig. 22-24, Plates 9-27). But such an effect was not seen when liming or pelleting was practised alone.

Significant increases were obtained in nodule number, nodule dry weight and plant dry weight in the treatment combination of *Bradyrhizobium* inoculation along with FYM and lime application in cowpea, blackgram and greengram. These effects were more apparent in cowpea with the isolate KA-G-C-4 for plant dry weight (46.7 g). Similarly in blackgram the response was more with KA-F-B-6 isolate for nodule number (67.7) and nodule dry weight (165.5 mg) and with the isolate VE-G-B-2 for plant dry weight (41.7 g). In greengram such an effect was obtained with PA-G-G-5 and PI-G-G-8 isolates. In all these crops, in the absence of FYM application, the root nodulation and plant growth were greatly reduced even with seed treatment with appropriate *Bradyrhizobium* culture.An almost similar result was also obtained when liming was substituted with calcium carbonate pelleting. However, here variations in plant growth response were observed particularly in cowpea. These were not in conformity with that of lime application. Here a positive response in plant dry weight was obtained even by pelleting alone. The increase in plant dry weight with the isolates, KA-G-C-4 and KA-F-C-7 were significant when compared to the control treatment. Thus in acidic soils with poor organic matter content, liming or pelleting alone will not be sufficient to induce proper root nodulation and plant growth. There is always a need for application of FYM. The need for liming or pelleting to improve root nodulation have been reported earlier also by many workers (Roughley, 1970; Rice, 1975; Choe *et al.*, 1980; Barbo and Fabrico, 1982; Manquiat and Padilla, 1982 and Lu *et al.*, 1983). When the relative benefits of liming and pelleting were studied by comparing the data of best treatment combinations such as $FYM^+L^+BR^+$ and $FYM^+P^+BR^+$, a definite trend was not observed. The only probable exception was with the isolate PA-G-G-5 of greengram where the application of lime along with FYM uniformly increased nodule number (65.7), nodule dry weight (115.7 mg) and plant dry weight (63.3 g) when compared to the corresponding pelleting treatment.

The pot trial experiments conducted above clearly demonstrated the importance of soil amendments in enhancing root nodulation and plant growth in cowpea, blackgram and greengram in acidic soils. The efficacy of the same was further tested under field conditions as part of an existing POP recommendation of Kerala Agricultural University for the cultivation of cowpea, blackgram and greengram. The salient aspects of this POP recommendations were, the application of FYM (@ 20 t ha^{-1}), lime (@ 250 kg ha^{-1}) and NPK (@ 20:30:10 for cowpea and

20:30:30 kg ha^{-1} for blackgram and greengram). The experiments were laid out at two different locations namely College of Agriculture, Vellayani and RRS, Kayamkulam during identical cropping season for two years. The seed treatment effects of the two selected *Bradyrhizobium* isolates for cowpea (KA-G-C-4 and KA-F-C-7), blackgram (VE-G-B-2 and KA-F-B-6) and greengram (PA-G-G-5 and PI-G-G-8) were evaluated with and without POP recommendations along with appropriate control treatments. In general, the root nodulation, plant growth and yield were significantly improved in all the three crops where *Bradyrhizobium* inoculation was practised along with the POP recommendation for each crop. Thus at Vellayani, the nodule number (35.8), nodule dry weight (109.6 mg), leaf area (275.2 cm^2), plant dry weight (25.1 g) and yield (556.3 g) in cowpea were maximum in the treatment combination of POP^+KA-F-C-7 (Table 20 and 21, Fig. 25). A similar response was also obtained with POP^+KA-G-C-4 isolate. At RRS, Kayamkulam also, the yield increase were maximum in the treatment combinations of POP^+KA-F-C-7 and POP^+KA-G-C-4. But, here, there were no significant differences between treatments in nodule number, nodule dry weight and plant dry weight. This was inspite of the fact that the best isolates of cowpea for all the locations taken together (KA-F-C7) was initially isolated from Kayamkulam itself. The lack of a significant response may be due to a stimulatory effect on the native flora present in the soil. But it was also observed that in the absence of the POP recommendations, such a stimulation was absent.

If the overall treatment effect of *Bradyrhizobium* inoculation was assessed in terms of increase in grain yield, it was observed that in cowpea, due to the best treatment combination of POP^{+}KA-F-C-7 and POP^{+}KA-G-C-4, the yield increases were 128.2 and 67.5 per cent respectively (Table 22, Fig. 25). In the corresponding treatments without the POP recommendation, the yield increases were only 41.8 and 21.8 per cent respectively. The yield increase was further reduced in the absence of *Bradyrhizobium* inoculation. Here, the yield increase was only 21.1 per cent. Similarly at Kayamkulam also, maximum increase in yield was obtained in the treatment combination of POP^{+}KA-F-C-7 and POP^{+}KA-G-C-4. These were 67.0 and 44.5 per cent respectively. Here also, the yield increase was considerably low, 18.4 and 25.5 per cent in the corresponding treatment combinations without the POP recommendation.

In blackgram and greengram also the treatment effects were more or less similar to that of cowpea (Table 23-28, Fig. 26 and 27). The yield increase of 66.1 per cent in blackgram was maximum in POP^{+}KA-F-B-6 treatment combination followed by 59.0 per cent in POP^{+}VE-G-B-2 treatment. At Kayamkulam, however, increase was maximum (79.1 per cent) in the treatment combination of POP^{+}VE-G-B-2. Similarly, in greengram also, the yield increases of 61.7 and 60.2 per cent at Vellayani were maximum in the treatment combinations of POP^{+}PI-G-G-8 and POP^{+}PA-G-G-5. At Kayamkulam, such a response was even more. Here an yield increase of 103.0 and 92.4 per cent respectively were obtained in POP^+PA-G-G-5 and POP^+PI-G-G-8 treatment combinations. As in cowpea, both in blackgram and greengram, the yield was reduced when *Bradyrhizobium* inoculation was done without the POP recommendation. These were only 36.1 and 47.6 per cent respectively for VE-G-B-2 and KA-F-B-6 isolates at Vellayani and 43.0 and 42.2 per cent at RRS, Kayamkulam. In the absence of *Bradyrhizobium* inoculation POP^+BR^-, the yield increase in blackgram was only 10.2 and 16.5 per cent respectively at these locations. In greengram also the yield was considerably reduced when *Bradyrhizobium* inoculation was done without POP recommendation. Thus at Vellayani, in the treatment combination of POP^-PA-G-G-5 and POP^-PI-G-G-8, the yield increases were only 35.1 and 33.7 per cent respectively. In the treatment without *Bradyrhizobium* inoculation, the yield increase was further reduced to the extent of 14.0 per cent. However, at RRS, Kayamkulam, the yield reduction in these treatments were not to the extent at Vellayani. These observations, infact, confirmed the results obtained earlier in the pot trial and once again demonstrated the importance of adopting suitable POP recommendation for the use of *Bradyrhizobium* in acidic soils with poor organic matter content. The improvement in root nodulation, plant growth and yield by *Rhizobium* or *Bradyrhizobium* inoculation have been reported earlier by several other workers in crops such as groundnut (Kadam and Desai, 1983), blackgram (Sudhakar *et al.*, 1989 and Mathan *et al.*, 1996), greengram (Chanda *et al.*, 1991) pigeon pea (Mathan *et al.*, 1994), soybean (Lawson *et al.*, 1995), gram (Roy *et al.*, 1995) and horse gram (Arya and Singh, 1996).

In the last part of the present investigation, the influence of the POP recommendation on plant and soil nutrient status was studied. The NPK content of plant samples were estimated on 45th day of plant growth which approximately corresponded with the peak nitrogen fixation and active growth phase in all the three crops. The soil factors including the positively influencing (organic carbon, P, K, Ca and Mg content) and negatively influencing (pH, Fe and Al content) factors were studied in the samples collected immediately after harvest of the crop. The data obtained were more or less in agreement with that of the observations made earlier on nodulation, plant growth and yield characters in cowpea, blackgram and greengram.

The increase in N content of cowpea plants at Vellayani was maximum (4.36 per cent) in the best treatment combination of POP$^+$KA-F-C-7 followed by that of 3.93 per cent in POP$^+$KA-G-C-4 treatment (Table 29). A similar treatment effect was also obtained in both blackgram and greengram (Table 31-34). The only exception was in greengram at Vellayani where there were no significant differences between treatments. The increase in plant N content is an indicator for the extent of N economy achieved through legume - *Rhizobium* symbiosis. Since in the present investigation, the data was compared with normal cultivation practices involving fertiliser application (@20kg

N ha^{-1}), any increase in N content of plants in the treatment combination of POP^+BR^+ should be due to *Bradyrhizobium* inoculation. These observations indicated that the cultures used for field evaluation had also better competitive ability with the native flora in forming efficient root nodules. The increase in plant N content due to *Rhizobium* inoculation have been reported earlier also (Bagal and Jadhav, 1995; Hagedorn, 1979 and Kaushik *et al.*, 1995).

Unlike the N content, a uniform treatment effect was not observed in the P and K content of plants (Tables 29-34). However, at two locations, there was significant differences between treatments in P content. These were in cowpea at Vellayani and greengram at Kayamkulam. In the former location, the P content was maximum (1.35 per cent) in the treatment combination of POP^+KA-G-C-4. At this location, it was also observed that the P content of plants was reduced in the absence of POP recommendation. However, such an effect was not observed in greengram. Much variations were also observed in the K content of plants.

In the studies on soil nutrient status, it was observed that, in general, the availability of P, K, Ca and Mg were increased in the treatment combination where the POP recommendations were practised along with *Bradyrhizobium* inoculation. Thus at Vellayani, in the soil samples collected after harvest of cowpea, the P, K, Ca and Mg content of soil in the POP^+KA-G-C-4 treatment were 15.87, 47.97, 146.02 and

22.34 ppm respectively. However, when the POP recommendation were not practised, availability of these elements were greatly reduced (Table 35). An almost similar trend was observed at RRS, Kayamkulam. In blackgram, both at Vellayani and Kayamkulam, a positive response was obtained only for Ca and Mg content of the soils. Here also the availability of these elements were significantly increased when POP recommendations were practised along with *Bradyrhizobium* inoculation. In greengram, significant differences between treatments were also obtained in the P and K content of soils. Such enhanced availability of nutrients like N, P and K in soils have been reported earlier by Mathan *et al.* (1996).

Thus the results of the field trial clearly showed that by adopting suitable package of practices for the cultivation of cowpea, blackgram and greengram in acid soils, the benefits of *Bradyrhizobium* inoculation can be significantly improved by enhancing root nodulation, plant growth and yield. This will also lead to certain favourable changes in the availability of some of the essential nutrient elements such as Ca, Mg and probably Mo which are critical for nodule initiation and subsequent nitrogenase activity of bacteroids. However, application of present POP recommendations for cowpea, blackgram and greengram did not result in any significant differences in soil pH, organic carbon, Fe and Al content of different soil samples. Further studies will have to be taken up on these aspects to arrive at some definite conclusions.

When the economics of cowpea, blackgram and greengram cultivation in acidic soils was worked out, it was found that maximum net return could be obtained when *Bradyrhizobium* inoculation was practised along with a set of POP recommendations. Thus the net return of Rs. 11456, Rs. 9780 and Rs. 9627 were maximum in the treatment combinations of POP^{+}PA-F-C-7 for cowpea, POP^{+}VE-G-B-2 for blackgram and POP^{+}PA-G-G-5 for greengram (Table 41-43). The benefit cost ratio for these treatments were 1.82, 1.49 and 1.41 respectively when compared to 1.28 for cowpea, 1.03 for blackgram and 0.93 for greengram respectively in the control ($POP^{-}BR^{-}$) treatments.

SUMMARY

SUMMARY

The present investigation on "Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala" was conducted at College of Agriculture, Vellayani, Trivandrum during 1993-96.

The isolation of native strains of *Bradyrhizobium* was done from seven different locations of Kerala. These locations were grouped into category A and B. Root nodulation and plant growth characteristics of cowpea, blackgram and greengram were uniformly better in category A locations. Here, the average nodule number and plant dry weight were 7.9, 8.7 and 8.2 and 9.7 g, 7.6g and 7.2 g respectively for cowpea, blackgram and greengram. However, at category B locations the corresponding figures were only 3.5, 3.2 and 5.3 and 3.7 g, 2.7 g and 1.7 g respectively. The average pH, organic carbon, Ca, Mg, Mo, Fe and Al content of soil samples collected from the category A locations were 5.05, 0.76 per cent, 475.3, 118.8, 0.0139, 87.2 ad 51.0 ppm respectively. On the contarary, in the second set of locations such as Ambalapuzha, Kuttanad and Vytilla where either extreme soil acidity or saline acidic conditions were the limiting factors for regular cultivation of pulses, the average soil pH, organic carbon, Ca, Mg, Mo,

Fe and Al content were 3.62, 1.79 per cent, 507.5, 166.1, 0.0092, 93.4 and 279.1 ppm respectively.

Fortythree native isolates of *Bradyrhizobium* including 17 from cowpea, 13 each from blackgram and greengram were obtained during this investigation. The screening for nodulation efficiency was done as per the Bureau of Indian Standard specification for rhizobial inoculants using unamended and amended soils. In unamended soil, the isolates KA-G-C-4, VE-G-B-2 and PA-G-G-5 from cowpea, blackgram and greengram produced maximum increase in plant dry weight of 17.3 g, 23.3 g and 9.4g respectively. In amended soil, the isolates KA-F-C-7, KA-F-B-6 and PI-G-G-8 from cowpea, blackgram and greengram produced maximum increase in plant dry weight of 33.3g, 18.9 and 16.7 g respectively. The best isolates from both unamended and amended soils were selected for further studies.

The colony characteristics of all the six selected isolates were typical of *Bradyrhizobium* forming white translucent glistening and elevated colonies with entire margin on YEMA with congored. All the six isolates were gram negative and fast growers attaining satisfactory growth on YEMA within three days. An acidic reaction was observed on YEMA with bromothymol blue which resulted in change of colour of the medium to yellow with all the isolates except KA-F-C-7. The growth of all the isolates were poor on glucose peptone agar medium and totally absent in Hofer's alkaline medium of pH 11.0. The carbohydrate utilisation pattern showed that arabinose, a pentose sugar, was the most preferred carbon source for the native isolates. The mean OD_{600} for these cultures was 0.216 when compared to that of only 0.162 for the exotic isolates. At the same time, the growth of the exotic isolates was maximum in glucose. The ability to tolerate low levels of acidic pH up to 4.5 was more for the native isolates. At this extreme acidic pH, the mean OD_{600} for these isolates was 0.365 when compared to that of 0.318 for the exotic isolates. The growth of both these isolates gradually improved and attained a peak level at pH 7.5. The *in vitro* tolerance to Fe and Al were almost identical for both the native and exotic isolates. In general, the growth was progressively inhibited with an increase in concentration of these elements from 50 to 250 ppm. However, at 50 and 100 ppm, concentration of aluminium chloride, the growth of the native isolates were more suppressed than the *Rhizobium* isolates.

The antibiotic resistant markers for ampicillin, streptomycin and kanamycin were same for both the native and exotic isolates. The growth of these isolates were not inhibited at concentrations up to 1000 ppm of amipicillin, 250 ppm of streptomycin and 50 ppm of kanamycin. In the serological characterisation it was observed that one of the heterologous antigens (PI-G-G-8) from greengram showed positive agglutination with that of the cowpea isolate, KA-F-C-7.

Two seperate pot trials were conducted to study the effect of FYM and liming and FYM and pelleting in cowpea, blackgram and greengram. Significant increases were obtained in nodule number, nodule dry weight and plant dry weight in the treatment combination of *Bradyrhizobium* inoculation along with FYM and lime application in all the three crops. These effects were more apparent in cowpea with the isolate KA-G-C-4 for plant dry weight (46.7 g). Similarly in blackgram the response was more with KA-F-B-6 isolate for nodule number (67.7) and nodule dry weight (165.5mg) and with the isolates VE-G-B-2 for plant dry weight (41.7 g). In greengram such an effect was obtained with PA-G-G-5 and PI-G-G-8 isolates. An almost similar result was also obtained when liming was substituted with calcium carbonate pelleting. Significant increases in nodule number, nodule dry weight and plant dry weight were obtained in *Bradyrhizobium* treatments along with application of FYM and calcium carbonate pelleting.

The pot trials demonstrated the importance of soil amendments in enhancing root nodulation and plant growth in cowpea, blackgram and greengram in acidic soils. The efficacy of the same was further tested under field conditions. In general, the root nodulation, plant growth and yield were significantly improved in all the three crops where *Bradyrhizobium* inoculation was practised along with the POP recommendation for each crop. Thus at Vellayani, the nodule number (35.8), nodule dry weight (109.6 mg), leaf area (275.2 cm^2), plant dry weight (25.1 g) and yield (556.3 g) in cowpea were maximum in the treatment combination of $POP^{+}KA$-F-C-7. At RRS, Kayamkulam also, the yield increase were maximum in the treatment combinations of $POP^{+}KA$-F-C-7 and $POP^{+}KA$-G-C-4. But here, there were no significant differences between treatments in nodule number, nodule dry weight and plant dry weight. It was observed that in cowpea, due to the best treatment combination of $POP^{+}KA$-F-C-7 and $POP^{+}KA$-G-C-4, the yield increases were 128.2 and 67.5 per cent respectively.

In blackgram and greengram also the treatment effects were more or less similar to that of cowpea. In blackgram at Vellayani, the number of nodules formed, plant dry weight, total leaf area and yield were maximum in the $POP^{+}KA$-F-B-6 treatment. These were 33.5, 21.8 g, 305.6 cm^2 and 322.5 g respectively. At RRS, Kayamkulam, significant increases were obtained only in nodule number, nodule dry weight and yield due to *Bradyrhizobium* inoculation. But there were no significant difference between treatments in plant dry weight. The nodule number (27.0) and yield (343.3 g) were maximum in the $POP^{+}VE$-G-B-2 treatment. The yield increase of 66.1 per cent in blackgram was maximum in $POP^{+}KA$-F-B-6 treatment followed by 59.0 per cent in VE-G-B-2 treatment. At Kayamkulam such an increase was maximum (79.1 per cent) in the $POP^{+}VE$-G-B-2.

In greengram, at Vellayani, significant increase in nodule number, nodule dry weight, total leaf area and yield were obtained only in treatments where *Bradyrhizobium* inoculation was done along with POP recommendation. These were maximum in the POP+PI-G-G-8 for nodule number (30.4) and with POP+PA-G-G-5 for nodule dry weight (65.9 mg). There were no significant differences between treatments in plant dry weight. The per plot yield of 355.8 g was maximum in the POP+PI-G-G-8 treatment. The total leaf area was maximum (335.2 cm^2) in the POP+PA-G-G-5 treatment. At RRS, Kayamkulam, significant increase due to *Bradyrhizobium* inoculation was obtained only in nodule dry weight and yield. These were maximum (67.4 mg and 289.2 g) in the POP+PI-G-G-8 and POP+PA-G-G-5 treatment respectively. The yield increases of 61.7 and 60.2 per cent at Vellayani were maximum in the treatment combination of POP+PI-G-G-8 and POP+PA-G-G-5 respectively. At RRS, Kayamkulam, the yield increase of 103.0 and 92.4 per cent respectively were obtained in the POP+PA-G-G-5 and POP+PI-G-G-8 treatment combination.

The increase in N content of cowpea plants at Vellayani was maximum (4.36 per cent) in the best treatment combination of POP+KA-F-C-7. At RRS, Kayamkulam also significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. In blackgram also significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. It was maximum (3.87 per cent) in the POP+VE-G-B-2 treatment. At RRS, Kayamkulam also significant increase in N content of plants was obtained due to *Bradyrhizobium* inoculation. In greengram, at Vellayani, there were no significant differences between treatments in the NPK content of plants. At RRS, Kayamkulam, significant increases in N content of plants were obtained due to *Bradyrhizobium* inoculation. It was maximum (3.87 per cent) in the POP+PA-G-G-5 treatment. Unlike the N content a uniform treatment effect was not observed in the P and K content of plants.

In general, the availability of P, K, Ca and Mg increased in the treatment combinations where POP recommendation was practised along with *Bradyrhizobium* inoculation. Thus at Vellayani, in the soil samples collected after harvest of cowpea, the P, K, Ca and Mg content of soil in the POP+KA-G-C-4 treatment were 15.87, 47.97, 146.02 and 22.34 ppm respectively. However, when the POP recommendation was not practised, the availability of these elements were reduced. Similar trend was observed at RRS, Kayamkulam. In blackgram, both at Vellayani and Kayamkulam a positive response was obtained only for Ca and Mg content of the soils. Here also the availability of these elements were significantly increased when POP recommendation was practised along with *Bradyrhizobium* inoculation. In greengram at Vellayani, the availability of P, K, Ca and Mg were significantly increased in treatments involving *Bradyrhizobium* inoculation along with POP recommendation. These were maximum in the treatment combinations of POP+PA-G-G-5 for P (16.52 ppm) and K (49.02 ppm) and in the POP+PI-G-G-8 for Ca (147.98 ppm) and Mg (23.86 ppm). There were no significant differences between treatments in soil pH, organic carbon, Fe and Al content. At RRS, Kayamkulam, there were significant differences between treatments in the P, K, organic carbon, Ca, Mg and Fe content of the soil. However, much variations were observed in the availability of these elements with and without the POP recommendations. There were no significant differences between treatments in soil pH and Al content.

The benefit cost ratio of the different treatment combinations was worked out for cowpea, blackgram and greengram. In cowpea the maximum net return of Rs. 11,456/- was obtained from the best treatment combination of POP^{+}KA-F-C-7. The benefit cost ratio for this treatment was 1.82. In blackgram, the maximum net return of Rs. 9780/- was obtained from the best treatment combination of POP^{+}VE-G-B-2. The benefit cost ratio for this treatment was 1.49. In greengram, the maximum net return of Rs. 9627/- was obtained from the best treatment combination of POP^{+}PA-G-G-5. The benefit cost ratio for this treatment was 1.41.

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* Originals not seen

APPENDICES

APPENDIX I

Locations selected for the isolation of *Bradyrhizobium* sp. suitable for cowpea, blackgram and greengram

Sl. No.	District	Location	Location code	Soil type
Category - A				
1.	Trivandrum	Vellayani	VE	Redloam
2.	Alappuzha	Kayamkulam	KA	Sandy soil
3.	Palghat	Pattambi	PA	Laterite
4.	Kasargod	Pilicode	PI	Lateritic alluvium
Category - B				
5.	Alappuzha	Ambalapuzha	AM	Kari soil
6.	Alappuzha	Kuttanad	KU	Kayal soil
7.	Ernakulam	Vyttila	VY	Hydromorphic saline

APPENDIX II

pH and soil nutrient status of soil at Vellayani and Kayamkulam

Location	P ppm	K ppm	Organic carbon %	pH	Ca ppm	Mg ppm	Fe ppm	Al ppm
Vellayani	13.21	38.24	0.71	4.70	120.91	18.99	126.09	17.27
Kayamkulam	10.18	11.80	0.42	5.23	26.09	20.52	120.82	13.23

APPENDIX III

Media composition

1. Yeast extract mannitol agar medium (YEMA)

K_2HPO_4	-	0.5 g
$MgSO_4 7H_2O$	-	0.2 g
NaCl	-	0.1 g
Mannitol	-	10.0 g
Yeast extract	-	1.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
Congored (1%)	-	2.5 ml
pH	-	7.0

2. Glucose peptone agar

Glucose	-	5.0 g
Peptone	-	10.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0

3. Hofer's alkaline medium

K_2HPO_4	-	0.5 g
$MgSO_4$ $7H_2O$	-	0.2 g
NaCl	-	0.1 g
$CaCO_3$	-	0.05 g
Yeast extract	-	1.0 g
Mannitol	-	10.0 g
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	11.0

Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala

By

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ABSTRACT

The present investigation on "Development of acid tolerant strains of *Bradyrhizobium* sp. suitable for certain pulse crops of Kerala" was conducted at College of Agriculture, Vellayani, Trivandrum during 1993-96.

The initial isolation of acid tolerant strains of *Bradyrhizobium* suitable for cowpea, blackgram and greengram was done from seven different locations in Kerala. These locations were broadly grouped into category A and B. The root nodulation and plant dry weight were uniformly higher in category A locations compared to category B locations in all the three crops. In all, 43 native isolates of *Bradyrhizobium* were obtained including 17 from cowpea, 13 each from blackgram and greengram. The native isolates of *Bradyrhizobium* obtained were screened as per the Bureau of Indian Standard specification. The screening trial was done both under unamended and amended soil conditions. Thus in unamended soil, the isolates KA-G-C-4, VE-G-B-2 and PA-G-G-5 for cowpea, blackgram and greengram respectively were selected based on plant dry weight and in amended soil, the isolates KA-F-C-7, KA-F-B-6 and PI-G-G-8 for cowpea, blackgram and greengram respectively were selected for further studies.

The colony characteristics of the selected isolates were typical of *Bradyrhizobium* forming white translucent glistening and elevated colonies with entire margin on YEMA with congored. All the six isolates were gram negative and fast growers attaining satisfactory growth on YEMA within three days. The carbohydrate utilization pattern showed that arabinose, a pentose sugar was the most preferred carbon source for the native isolates. At the sametime, the growth of the exotic isolates were maximum in glucose. The ability to tolerate low levels of acidic pH upto 4.5 was more for the native isolates. The growth of both these isolates gradually improved and attained a peak level at pH 7.5. The *in vitro* tolerance to Fe and Al were almost identical for both the native and exotic isolates. In general, the growth was progressively inhibited with an increase in the concentration of these elements from 50 to 250 ppm. The antibiotic resistant markers for ampicillin, streptomycin and kanamycin were same for both the native and exotic isolates. The growth of these isolates were not inhibited at concentrations upto 1000 ppm of ampicillin, 250 ppm of streptomycin and 50 ppm of kanamycin. In the serological characterisation it was observed that one of the heterologous antigens (PI-G-G-8) for greengram showed positive agglutination with that of the cowpea isolate, KA-F-C-7.

The effect of application of FYM, liming or pelleting on root nodulation and plant growth characters in cowpea, blackgram and greengram was studied. Significant increases in nodule number, nodule dry weight and plant dry weight were obtained in the treatment combination of *Bradyrhizobium* inoculation along with FYM and lime application in all the three crops. An almost similar result was also obtained when liming was substituted with calcium carbonate pelleting. The efficacy of the selected isolates of cowpea, blackgram and greengram were tested under field conditions at two locations namely College of Agriculture, Vellayani and Rice Research Station, Kayamkulam during identical cropping season for two years. The seed treatment effects of the two selected *Bradyrhizobium* isolates for cowpea (KA-G-C-4 and KA-F-C-7), blackgram (VE-G-B-2 and KA-F-B-6), greengram (PA-G-G-5 and PI-G-G-8) were evaluated with and without POP recommendations along with appropriate control treatments. In general, the root nodulation, plant growth and yield were significantly improved in all the three crops where *Bradyrhizobium* inoculation was practised along with the POP recommendation for each crop. Thus at Vellayani, the nodule number, nodule dry weight, leaf area, plant dry weight and yield in cowpea were maximum in the treatment combination of POP+KA-F-C-7. A similar response was also obtained with POP+KA-G-C-4 treatment. At Rice Research Station, Kayamkulam also, the yield increase was maximum in the treatment combinations of POP+KA-F-C-7 and POP+KA-G-C-4. In blackgram and greengram also the treatment effects were more or less similar to that of cowpea.

In the last part of the present investigation, the influence of the POP recommendations on plant and soil nutrient status was studied.

The nitrogen content of plants was significantly higher in *Bradyrhizobium* inoculated plants in cowpea and blackgram at both the locations. In greengram, at Vellayani there were no significant differences between treatments in the NPK content of plants. At Rice Research Station, Kayamkulam, also significant increases in nitrogen content of plants were obtained due to *Bradyrhizobium* inoculation. Unlike the nitrogen content a uniform treatment effect was not observed in the P and K content of plants.

In the studies on soil nutrient status, it was observed that in general the availability of P, K, Ca and Mg were increased in the treatment combinations where POP recommendation was practised along with *Bradyrhizobium* inoculation. However, there was no significant difference between treatments in soil pH, organic carbon, Fe and Al content of different soil samples.