PERFORMANCE OF MUNGBEAN (*Vigna radiata* L.) TO DIFFERENT *BRADHYRHIZOBIUM* STRAINS

A THESIS

By

SHAZAL CHANDRA SHUTRADHAR

Registration No. 02180

Semester: July–December 2007

MASTER OF SCIENCE (M.S)

IN

SOIL SCIENCE

DEPARTMENT OF SOIL SCIENCE

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

SHER-E-BANGLA NAGAR, DHAKA-1207

MAY 2008

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Submitted to the Department of Soil Science,
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in partial fulfillment of requirement for the degree of

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CERTIFICATE

This is to certify that thesis entitled "PERFORMANCE OF MUNGBEAN (*Vigna radiata* L.) TO DIFFERENT *BRADHYRHIZOBIUM* STRAINS" submitted to the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka-1207 in partial fulfillment of the requirements for the degree of Master of Science (M.S) in Soil Science embodies the result of a piece of *bonafide* research work carried out by **Shazal Chandra Shutradhar**, Registration No. 02180 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged by him.

Dated: 10 May 2008
Place: Dhaka, Bangladesh

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Dedicated to

My

Beloved Parents and all of my teachers

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May 2008

The Author

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Abstract

A field experiment was conducted at the Soil Science Fields of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period from March to June 2007 to study the performance of mungbean (BARI Mung-5) to different *Bradyrhizobium* strains on growth, root nodulation, yield and yield contributing characters, nitrogen and protein content and protein yield in mungbean. There were six treatment combinations (BARI RVr-401, BARI RVr-402, BARI RVr-403, BARI RVr-404, mixed culture and uninoculated control) taking with single variety of mungban. The experiment was laid out in a randomized complete block design with four replications. The unit plot size was 4 m x 3 m. A basal dose of triple super phosphate (22 kg P ha^{-1}), muriate of potash (42 kg K ha^{-1}), gypsum (20 kg S ha^{-1}), zinc sulphate (5 kg Zn ha^{-1}) and 1 kg B ha^{-1} as boric acid. After 35 and 50 days of sowing, 10 plants were uprooted from each plot to study dry matter and nodulation. At maturity, yield and yield contributing characters were recorded. *Bradhyrhizobium* inoculation increased significantly the number of nodules, nodule weight, root and shoot length, seed and stover yield, and total protein content of mungbean compared to uninoculated control. Different responses were found among the cultures. Inoculation of mungbean seed with BARI RVr-404 strain performed the best in respect of nodulation, seed and stover yield, total protein yield and other characters studied. The performance of BARI RVr-404 strain was identical to other strains on nodulation which was superior to uninoculated control. Similarly, the number of seeds pod^{-1}, seed yield and nutrient content were influenced by the *Bradhyrhizobium* strain where BARI RVr-404 recorded the highest yield. The results indicate that the use of *Bradhyrhizobium* inoculants appears to be an effective method for successful mungbean production.

CHAPTER 1

INTRODUCTION

CHAPTER I

INTRODUCTION

Mungbean (*Vigna radiata L.* Wilzek) is one of the major pulse crops grown in Bangladesh. It is considered as the quality pulse in the country but the production per unit area is very low (763 kg ha^{-1}) as compared to other countries of the world (BBS, 2006). Among the pulses mungbean possess nutrient value having crude protein about 26.0%, crude fibre 5.2%, nitrogen free extract 62.9%, ether extract 1.1%, total ash 4.4%, Ca 0.2% and P 0.5% (Gowda and Kaul, 1982).

Mungbean is one of the widely grown pulse crops in Bangladesh for human consumption, animal fodder as well as soil fertility building purpose. But costly and environmentally risky chemical fertilizers cause serious and continuous problem for increasing mungbean production in developing countries including Bangladesh. These problems are likely to become serious in future. Biological nitrogen fixation (BNF) resulting from symbiosis between legume crops and root nodule bacterium *Bradyrhizobium* can ameliorate these problem by reducing the chemical N-fertilizer input required to ensure productivity.

The successful growing of mungbean is dependent on the availability of its microsymbiont bacteria in soil. *Bradyrhizobium* strains are present in all soils of Bangladesh but they may not be equally effective in nodulation and N-fixation. In this situation, inoculation can meet the challenge by providing superior strains in the soil, so that the most effective nodulation and nitrogen fixation are obtained. Thus it was thought that there is a scope for utilizing the effective bradyrhizobial strains for obtaining more yield of mungbean under field conditions which may play vital role in improving soil environment and agricultural sustainability.

Now a day a number of organisms like *Bradyrhizobium* has been identified to use as biological agent for fixing atmospheric nitrogen by processing with legume crops and make available to the plants. Bangladesh Agricultural Research Institute (BARI) isolated some *Rhizobium* strains for some pulse crops. It has already been selected some *Bradyrhizobium* strains especially for mungbean varieties. To reduce the production cost and to fulfill the demand, more pulse production could be achieved through seed inoculation with *Bradyrhizobium* strains which is known to increase biological nitrogen fixation. *Bradyrhizobium* inoculation increased mungbean seed yield from 4.3% to 16.2% (Vaishya *et al.*, 1983). In Bangladesh, inoculation with *Bradyrhizobium* increased 77% dry matter production, 64% grain yield and 40% hay yield over non-inoculated control (Chanda *et al.*, 1991). Maximum yields were obtained when fertilizers applied together with *Bradyrhizobium* inocula. Singha and Sarma (2001) reported that *Rhizobium* inoculation significantly increased the number (2.2%) and mass (9.5%) of root nodules plant^{-1} compared to the control. Meena Kumari and Nair (2001) reported that N content of fresh seed was 5.78% in the inoculated plants, while that in non-inoculated control was only 2.7%. Seed inoculation with *Bradyrhizobium* significantly increased seed yield (0.98 t ha^{-1} in 2001, 27.0% increase over control and 0.75 t ha^{-1} in 2002, 29.0% increase over control) and stover yield (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) of mungbean. *Bradyrhizobium* inoculation also significantly increased pods plant^{-1}, seeds pod^{-1} and 1000-seed weight (Bhuiyan and Mian, 2008).

In Bangladesh, few studies have been conducted on the effects of bio-fertilizers and chemical fertilizers on mungbean. Considering the above facts; present study has been undertaken with the following objectives:

1. To observe the effects of four *Bradyrhizobium* strains on nodulation, growth, yield and nitrogen uptake by mungbean.

2. To select most effective *Bradyrhizobium* strains for mungbean.-

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Biofertilizers are cultures of microorganisms which benefit the plants by providing nitrogen or phosphorus or rapid mineralization of organic materials. Of the biofertilizers, *Bradyrhizobium* is used in Bangladesh to some extent. However, only limited numbers of research works have so far been carried out on the use of different *Bradyrhizobium* strains on mungbean (*Vigna radiata* L.) and other pulse crops. Available information on the contribution of *Bradyrhizobium* inoculation on mungbean and other legumes has been reviewed in this chapter.

Effect of *Brayrhizobium* inoculation on different parameters of mungbean and other legumes have been presented below:

2.1 Effect of *Bradyrhizobium* inoculation on nodulation

Sattar and Ahmed (1995) carried out a field experiment on mungbean (*Vigna raidata* L.) to study the response of inoculation with *Bradyrhizobium* inoculants incorporating BINA 403, BINA 407, RCR 3824 and RCR 3825 strains as single and mixed culture. They observed that *Bradyrhizobium* inoculation increased the number of nodules and nodule weight significantly compared to non-inoculated treatments.

Behari *et al.* (1995) carried out an experiment with *Vigna radiata* cv. K851 and B165. Seeds were treated with 10 different *Bradyrhizobium* strains (applied alone or in pairs) and found that there was a significant interaction between different mixtures of strain and cultivars in terms of nodulation. Seed yields were generally improved by using multi-strain inoculants.

Patra and Bhattacharyya (1997) carried out a field trial with *Vigna radiata* cv. B-1, *Bradyrhizobium* and urea (25 kg ha^{-1}). They found that all treatments increased nodulation compared to control. They also reported that the highest nodules were obtained from *Bradyrhizobium* + urea treatment.

Das *et al*. (1997) conducted field trials where *Vigna radiata* cv. Noyagrah local seeds were inoculated with *Rhizobium* and/or VAM culture, applied at the rate of 15 kg ha^{-1}. They observed that number of nodules was increased with dual inoculation compared to non-inoculated control.

Goel *et al*. (1997) carried out an experiment with *Vigna radiata* cv. K851, where seed were inoculated with one or both of 2 bacteriocin-producing *Rhizobium* strains, VRF10 (poor nodulating capacity) and VRF57 (superior nodulating capacity), VRF57 fewer nodules, but nodule biomass was much greater in VRF57 treated plot than that of VRF10. When the two strains were inoculated together, VRF57 formed a higher proportion of nodules than that in VRF10 treatment, even when inoculated at a much lower ratio. This indicated that a factor other than bacteriocin affected its competitiveness.

Sharma and Khurana (1997) studied the effectiveness of single and multistrain inoculants in field experiments with summer mungbean variety SML 32. They found that number of nodules; nodule dry biomass and grain yield were better in multistrain inoculants. On an average, single strain and multistrain *Rhizobium* inoculants increased the seed yield by 10.4% and 19.3%, respectively, compared to the non-inoculated control.

Ghosh and Poi (1998) carried out a pot experiment where soybean, groundnut, mashkalai, mung and lentil were inoculated with *Bradyrhizobium*, *Bacillus polymixa* and *Glomus fasciculatum* in different combinations. They found that nodulation and population of microorganisms in the rhizosphere were highest from combined inoculation with all three microorganisms.

Gupta *et al.* (1998) reported that nodule occupancy of *Bradyrhizobium* strain S 24 was increased by 60% over the non-inoculated control.

Maldal and Ray (1999) conducted a field experiment where mungbean cv. B105, B1 and Hoogly local were untreated, seed inoculated with *Bradyrhizobium* and 20, 30, or 40 kg N ha^{-1} as urea were applied. The results revealed that nodulation was greatest with inoculation in B105 and Hoogly local while it was decreased by inoculation and N treatment in B105.

Sharma *et al.* (1999) conducted a field experiment in 1997/98 in Himachal Pradesh and *V. mungo* cv. Pant U-19 where seed inoculated with one of eleven *Rhizobium* strains or non-inoculated and applied with 0 or 20 kg N ha^{-1}. Seed yield was the highest on inoculation with a local strain (yield 1.30 t ha^{-1}). The application of 20 kg N produced higher seed yield (1.24 t ha^{-1}) than no N (1.14 t ha^{-1}). Application of nitrogen and inoculation increased nodulation and nodule dry weight $plant^{-1}$.

Kavathiya and Pandey (2000) conducted a pot experiment with *Bradyrhizobium* on seed inoculation of mungbean and found that nodule $plant^{-1}$ increased significantly over non-inoculated control.

Sharma *et al.* (2000) carried out a field experiment during *kharif* 1997 at Palampur, Himachal Pradesh, India where *V. mungo* seed was inoculated with 1 of 9 *Rhizobium* strains and planted with 0 or 20 kg N ha^{-1}. Growth, yield and dry matter accumulation increased with N application and *Rhizobium* inoculation, with the local strain giving the best results.

Tomar *et al.* (2001) conducted a field experiment at the G.B. Pant University Research Station, Ujhani, Uttar Pradesh, India, during *kharif* 1994-95 to study the effect of *Rhizobium*, vesicular arbuscular mycorrhiza (VAM, *Glomus caledonium*) and phosphate solubilizing bacteria (PSB, *Pseudomonas striata* strain P-27) inoculation, with and without P, on blackgram (*Vigna mungo*) seed yield. Phosphorus application in soil with medium P content (5.4 mg kg^{-1}) increased nodulation over no phosphorus control. Forty kilograms of P_2O_5 ha^{-1} recorded an increase of 20.6% in nodule dry weight, significant increases of 0.35 g kg^{-1} in N concentration and 1.28 g kg^{-1} in P concentration of plant over 20 kg P_2O_5 ha^{-1} treatment. *Rhizobium* gave the highest and 21.0% more nodule number, 34.7% more nodule dry mass, 0.73 g kg^{-1} more N in grain and 4.2% higher grain yield over PSB treatment.

A field experiment was conducted in Vamban, Tamil Nadu, India by Nagarajan and Balachandar (2002) during the *kharif* season of 1998 to study the effects of organic amendments on the nodulation and yield of blackgram cv. Vamban 1. The treatments consisted of *Rhizobium* (strains CRU 7 for blackgram and CRM 11 for greengram) seed inoculation, 15 t farmyard manure (FYM ha^{-1}), FYM + *Rhizobium*, 5 t compost ha^{-1} (prepared from leaves and twigs of *Sesbania sesban*, *S. grandiflora*, *Cassia fistula*, *Cassia auriculiformis*, and *Claricidia* (*Gliricidia*) along with cowdung and rock phosphate), compost + *Rhizobium*, 5 t biodigested slurry ha^{-1}, and biodigested slurry + *Rhizobium*. In general, inoculation of seed with *Rhizobium* and application of organic amendments enhanced root nodulation. Biodigested slurry at 5 t ha^{-1} + *Rhizobium* gave the greatest plant height (42.7 and 53.7 cm for blackgram and greengram, respectively), nodule number (23.3 and 24.0) and nodule weight (45.3 and 42.3 mg).

Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India, during the pre-*kharif* season to study the effect of *Bradyrhizobium* inoculation, P (at 0, 20 kg ha^{-1}) and Mo (at 0, 0.5 and 1 kg ha^{-1}) on the number of nodules plant^{-1} of summer greengram cv. T-44. *Bradyrhizobium* inoculation and application of P and Mo significantly influenced the number of nodules plant^{-1}.

Singha and Sarma (2001) conducted an experiment in India on blackgram cv. T-9 to study the effect of different levels of P fertilization and *Rhizobium* inoculation of seeds on yield and nutrient uptake. *Rhizobium* inoculation significantly increased the number (2.2%) and mass (9.5%) of root nodules plant^{-1} compared to the control indicating increased efficiency of the crop to fix the atmospheric N.

Meenakumari and Nair (2001) conducted a study in 7 different locations to evaluate root nodulation and plant growth characters of cowpea, blackgram and greengram and observed that root nodulation and plant growth characters of cowpea, blackgram and mungbean were uniformly better at category A locations.

The nodulation characteristics of 8 varieties of blackgram (*Vigna mungo*) were studied by Reddy and Mallaiah, 2001. *Rhizobium* sp. was isolated from the T-9 cultivar of the crop. The effect of three different methods of *Rhizobium* inoculation on the nodulation of blackgram was studied. The initiation of nodules was early and the numbers of nodules formed were more in the seed inoculation method than in soil inoculation or seedling inoculation methods. Three isolates of *Rhizobium*, viz. VM isolate, AH isolate and SG isolates, isolated, respectively from blackgram, *Arachis hypogaea* and *Sesbania grandiflora*, were used to study their effect on nodulation and nitrogen content of blackgram cultivar T-9. In plants inoculated with the VM isolate, nodules appeared 12 days after sowing, and a maximum of 84 nodules plant^{-1} were found during the reproductive stage of the crop. The nitrogen content of the nodules at the reproductive stage was 4.5%. The nitrogen content of the shoot was 1.9% at the vegetative stage (25-day-old plants), 3.0% at the reproductive stage

(45-day-old plants) and 1.0% at the harvesting stage. The nitrogen content of the fresh seeds was 5.78% in the inoculated plants, while that in non-inoculated controls was only 2.72%. The plants inoculated with the AH isolate showed better nodulation and nitrogen content compared to the plants inoculated with the VM isolate. However, the SG isolate completely failed to produce nodules on blackgram.

Sarkar *et al.* (2002) inoculated the seed of blackgram with strains of *Bradyrhizobium* viz. M-10, 129-USA, 480-M, and MK-5 before sowing in a field experiment conducted to determine the cultivars and *Bradyrhizobium* strain for suitable use in the locality. Cultivars M-16 produced longer roots and higher root volume plant^{-1}, number of nodules plant^{-1} and test weight compared to A-43. The interaction effects between cultivar A-43 and *Bradyrhizobium* strain MK-5 resulted in the highest root volume plant^{-1} (1.30), number of nodules plant^{-1} (7.03) and test weight (4.23 g), whereas the interaction effects between cultivar A-43 and *Bradyrhizobium* strain 480-M resulted in the longest roots (14.72 cm). Correlation coefficient studies showed high correlation between seed yield and dry weight, and root weight. Root length and root volume were inversely correlated with test weight.

Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and phosphate solubilizing bacteria (PSB) on nodulation and grain yield of mungbean cv. B-1 in field trial conducted in West Bengal. Seeds of mungbean were inoculated with strains of *Rhizobium*, i.e JCa-1 and M-10 strains, at a population of 28.20 x 10^6 and 32.66 x 10^6 cells mL^{-1}, respectively, phosphate solubilizing bacteria containing *Bacillus polymyxa* and *Pseudomonus striata* at a population of 7 x 10^8 cells mL^{-1} at the time of the sowing. The plants inoculated with *Bradyrhizobium* strains and PSB showed increased rate of nodulation and N content. The percentage increase in seed yield over control was observed to be highly significant in plants inoculated with *Bradyrhizobium* strains and PSB.

Sriramachandrasekharan and Vaiyapuri (2003) conducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on the nodulation, growth, and yield of blackgram cv. ADT 3. *Rhizobium*-inoculated blackgram showed better nodulation than the non-inoculated crop.

Osunde *et al.* (2003) tested the response of two mungbean cultivars (TGX1456-2E) and TGX1660-19F) to *Bradyrhizobium* inoculation in a two year trials in the farmers fields of Nigeria. The effect of cultivar on plant height and nodulation number was significant only in the first cropping season of the trial. Inoculation with *Bradyrhizobium* increased 40% seed yield in the first cropping season, while no such yield differences occurred in the second season. The proportion of nitrogen derived from nitrogen fixation ranged from 27% to 50% in the both cropping seasons and this was dependent on crop management on the farmer's field, rather than any cultivar or inoculation effect.

A study was conducted by Kumari and Nair (2003) to isolate efficient native strains of *Rhizobium* or *Bradyrhizobium* spp. and to develop suitable package of practices recommendations for their efficient use. The initial isolation of *Bradyrhizobium* spp. was done from seven different locations in Kerala, India, where the soil was generally acidic in nature. A total of 26 isolates (13 each from blackgram (*Vigna mungo*) and greengram (*V. radiata*) were collected and were screened for nodulation efficiency. The experiment was conducted in complete randomized block design with three replications for each isolate using unsterilized soil of pH 4.89 without any amendments such as applications of FYM or chemical fertilizers. The experiment also repeated under amended soil conditions. The selected isolates were further evaluated under field (Vellayani and Kayamkulam) conditions along with a package of practices recommendation (POP) developed by the Kerala Agricultural University. The extent of root nodulation, plant growth and yield were more in blackgram and greengram where *Bradyrhizobium* inoculation was done along with the POP recommendation. At Vellayani, the nodule number, plant dry weight and yield in blackgram were significantly high in the treatment combination of POP KA-F-B-6. At Kayamkulam, significant increases were obtained only in nodule number, nodule dry weight and yield. The results indicated that for acidic soils, the mere development of efficient native strains of *Rhizobium* or *Bradyrhizobium* alone was not sufficient but it should be along with a package of practices recommendation consisting of application of organic manure and liming to neutralize the soil pH.

An experiment was carried out by Bhuiyan *et al.* (2005) with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to find out the time of nodule initiation, nodulation pattern and their size distribution. Five mungbean varieties used were BARI Mung-2, BARI Mung-4, BARI Mung-5, BINA Mung-2 and Barisal local, and rhizobial inoculum (*Bradyrhizobium* strain BAUR-604). A few small nodules were first visible at 9 DAS. The number of nodules increased progressively with the increasing growth period and reached the peak at 42 DAS (i.e. at 50% flowering stage). Inoculated plants recorded higher number of nodules than non-inoculated plants at all the sampling dates. The results suggested that nodule initiation in the roots of mungbean varieties started at 9 days of sowing seeds (DAS), reached the peak at 42 DAS and thereafter started reducing in numbers until 70 DAS due to spontaneous degeneration.

Bhuiyan *et al.* (2006) carried out a field study with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe nodulation pattern and nodule dry matter production at different growth periods of mungbean. They found that inoculated plants produced significantly higher nodule number (17.51 plant^{-1} in 2001 and 17.61 plant^{-1} in 2002) at 42 DAS compared to non-inoculated plant (11.35 plant^{-1} in 2001 and 11.52 plant^{-1} in 2002). The lowest number of nodules (3.50 plant^{-1} in 2001 and 3.47 plant^{-1} in 2002) was produced at 14 DAS with non-inoculated plants.

Bhuiyan *et al.* (2007a) carried out an experiment with five mungbean varieties with or without *Bradyrhizobium* at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to find out the time of nodule initiation, nodulation pattern and their size distribution. The number of nodules increased progressively with the advancement of growth period and reached the peak at 42 DAS (i.e. at 50% flowering stage). The development of number of nodules of 2–4 mm size started to decline sharply after 42 DAS and in case of <2 mm size nodules, the declining was noticeable after 56 DAS, while the bigger nodules were increased up to 63 DAS. The results suggested that nodule initiation in the roots of mungbean varieties started at 9 days after sowing seeds (DAS), reached the peak at 42 DAS and thereafter started reducing in numbers until 70 DAS due to spontaneous degeneration. Higher number of nodules of different sizes (<2.0 mm, 2.1-4.0 mm and >4 mm) was observed in BARI mung-2 at different DAS in both the years. *Bradyrhizobium* inoculation produced 8.8 (<2.0 mm), 8.5-8.6 (2.1-4.0 mm) and 0.2-0.4 (>4 mm) nodules plant^{-1}, while non-inoculated plant produced 5.7 (<2.0 mm), 5.6 (2.1-4.0 mm) and 0.1-0.2 (>4 mm) nodules plant^{-1}.

Bhuiyan and Mian (2007) conducted experiments with or without *Bradyrhizobium* in five mungbean varieties at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe nodulation, biomass production and yield of mungbean. Five mungbean varieties viz. BARI Mung-2, BARI Mung-4, BARI Mung-5, BINA Mung-2 and Barisal local, and rhizobial inoculum (*Bradyrhizobium* strain BAUR-604) was used for the study. Application of *Bradyrhizobium* inoculant produced significant effect on nodulation, shoot dry weight, seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. Inoculated BARI Mung-2 produced the highest nodulation, dry matter production, seed and stover yields.

2.2 Effect of *Bradyrhizobium* inoculation on dry weight

Shukla and Dixit (1996a) laid out a field trial to study the response of summer mungbean to *Rhizobium* inoculation. *Rhizobium* inoculation delayed 50% flowering, whereas it increased number of branches $plant^{-1}$, plant height and the dry matter accumulation.

Kavathiya and Pandey (2000) conducted a pot experiment with *Rhizobium* on mungbean (*Vigna radiata* cv. K 851). They reported that maximum seed germination (96.6%), plant height (24.6 cm), fresh shoot weight (5.33 g), fresh root weight (4.42 g) and nodulation (69 healthy nodules $plant^{-1}$) was recorded in the *Rhizobium* inoculation treatment.

Chowdhury *et al.* (2000) carried out a pot experiment during *kharif* in 1995 with mungbean in Salna, Bangladesh where mungbean line NM-92 was inoculated with *Rhizobium* strain TAL 303. They found that dry matter production was increased by about 50% due to *Bradyrhizobium* inoculation.

Sharma *et al.* (2000) carried out a field experiment during *kharif* 1997 at Palampur, Himachal Pradesh, India where *V. mungo* was seed inoculated with 1 of 9 *Rhizobium* strains and applied with 0 or 20 kg N ha^{-1}. Growth, yield and dry matter accumulation increased with N application and *Rhizobium* inoculation, with the local strain giving the best results. They found that dry matter production was increased by about 50% due to *Bradyrhizobium* inoculation.

Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India, during the pre-*kharif* season of 1998 to study the effect of *Rhizobium* inoculation and reported that inoculation significantly influenced the number of nodules $plant^{-1}$, dry matter accumulation in the shoot, crop growth rate and plant height.

Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/ without *Bradyrhizobium* inoculation at the Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe shoot dry matter production and nitrogen uptake by mungbean at different growth stages. Significant improvement was observed on dry matter production and nitrogen uptake. *Bradyrhizobium* inoculant significantly increased dry matter production. The highest dry matter production $plant^{-1}$ at 77 DAS was recorded in *Bradyrhizobium* inoculated plots. Inoculated Barimung-2 produced the highest shoot weights.

Bhuiyan and Mian (2007) conducted experiments with or without *Bradyrhizobium* in five mungbean varieties at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe nodulation, biomass production and yield of mungbean. Five mungbean varieties viz. BARI Mung-2, BARI Mung-4, BARI Mung-5, BINA Mung-2 and Barisal local, and rhizobial inoculum (*Bradyrhizobium* strain BAUR-604) was used for the study. Each variety was tested with or without *Rhizobium* inoculation. Application of *Bradyrhizobium* inoculant produced significant effect on nodulation, shoot dry weight, seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. Inoculated BARI Mung-2 produced the highest nodulation, dry matter production, seed and stover yields.

2.3 Effect of *Bradyrhizobium* inoculation on plant height

Thakur and Panwar (1995) conducted a field trial where seeds of *Vigna radiata* cv. Pusa-105 and PS-16 were inoculated to observe the effect on plant height. They found that inoculation either singly or in combination increased plant height compared with no inoculation.

Das *et al.* (1997b) carried out a field trail where *Vigna radiata* cv. Nayagrah local seeds were inoculated with *Rhizobium* and/or VAM culture which was applied at 15 kg ha^{-1}. They found that shoot and root lengths were increased with dual interaction compared with uninoculated control.

Sharma (2001) conducted a field experiment in Palampur, Himachal Pradesh, India during the *kharif* season. Seeds of mungbean cv. Pusa Baisakhi were inoculated with three strains of *Bradyrhizobium* culture (Ludhiana, local isolate and IARI). The various physiological and yield parameters of mungbean were evaluated. Crop growth rate, relative growth rate, days to 50% flowering, days to maturity was at maximum when mungbean seeds were treated with the local isolate.

Bhattacharya and Pal (2001) conducted a field experiment in West Bengal, India during the pre-*kharif* season (February–May) to study the effect of *Rhizobium* inoculation, P and Mo on the growth of summer greengram. Crop growth rate was evaluated at 60 and 80 days. Inoculation of rhizobial inoculum and application of P and Mo influenced maximum crop growth and plant height compared with control. Maximum growth was obtained in *Rhizobium* treatments combined with P and Mo at 40 and 0.5 kg ha^{-1}, respectively.

Nagarajan and Balachandar (2002) conducted a field experiment to observe seed inoculation of *Rhizobium* and application of organic amendments enhanced biomass, root nodulation and grain yield. Bio-digested slurry at 5 t ha^{-1} + *Rhizobium* produced the greatest plant height (44.7 and 53.7 cm for blackgram and greengram, respectively), nodule number (23.3 and 24.0), nodule weight (45.3 and 42.3 mg), and grain yield (758.3 and 732.0 kg ha^{-1}).

Malik *et al.* (2002) studied the effects of seed inoculation with *Rhizobium* and P application (at 0, 30, 50, 90 and 110 kg ha^{-1}) on the growth, seed yield and quality of mungbean cv. NM-98 in a field experiment conducted at Faisalabad in Pakistan during the autumn of 2000. Plant height at harvest was highest when inoculated with *Bradyrhizobium* (68.13 cm).

Srinivas and Shaik (2002) conducted a field experiment to find out the effects of N (0, 20, 40 and 60 kg ha^{-1}) and P (0, 25, 50 and 75 kg ha^{-1}) along with seed inoculation with *Rhizobium* culture on the growth and yield components of greengram. Plant height generally increased with increasing rates of P and with increasing rates of N up to 40 kg ha^{-1} followed by decrease with further increase in N.

Ashraf *et al.* (2003) conducted a field experiment to observe the effects of seed inoculation of a biofertilizer and NPK application on the performance of mungbean cv. NM-98 studied in Faisalabad, Pakistan. The treatments consisting of the seed inoculation of *Bradyrhizobium phaseoli* singly or in combination with 20:50:0, 40:50:0, 50:50:0, or 50:50:50 kg NPK ha^{-1} (urea), P (single superphosphate), and K (potassium sulphate) were applied during sowing. The tallest plants (69.93 cm) were obtained with seed inoculation + 50:50:0 kg NPK ha^{-1}.

2.4 Effect of *Bradyrhizobium* inoculation on yield and yield attributes

Bhalu *et al.* (1995) coducted a field experiment during the rainy season of 1990 at Junagadh, Gujarat with blackgram (*Vigna mungo*) and seed was inoculated with *Rhizobium* or not inoculated and fertilizer dose applied 10, 20 or 30 kg N and 20, 40 or 60 kg P_2O_5 ha^{-1}. Seed inoculation increased seed yield (471 vs. 434 kg ha^{-1}). Seed yield increased with up to 20 kg N (yield 464 kg) and 40 kg P_2O_5 (yield 475 kg). N and P uptakes and seed protein content increased with increasing N and P rates. Net return was highest with seed inoculation.

Satter and Ahmed (1995) carried out a field experiment at the farm of Rajbari, BARI, Dinajpur on mungbean inoculated with *Bradyrhizobium* and reported significant increase in hay and total protein yield.

Behari *et al.* (1995) carried out an experiment with *Vigna radiata* cv. K851 and B165. Seeds were treated with 10 different *Bradyrhizobium* strains (applied alone or in paris) before sowing and found that, there was a significant interaction between different mixtures of strain and cultivars in terms of nodulation. Seed yields were generally improved by using multi-strain inoculants.

Shukla and Dixit (1996) conducted a field experiment where greengram cv. Pusa Baisakhi seeds inoculated with *Rhizobium* or not inoculated were sown in rows, 20, 30 and 40 cm apart and applied with fertilizer 0-60 kg P_2O_5 ha^{-1}. They found that seed inoculation increased seed yield.

Sharma and Khurana (1997) studies the effectiveness of single and multi-strain inoculants in field experiment with summer mungbean variety SML-32 and found that grain yield was superior in multi-strain inoculants treatment. On an average, single strain and multi-strain *Rhizobium* inoculants increased the grain yield by 10.4% and 19.3% over non-inoculated control, respectively.

Paul (1999) conducted a pot experiment where mungbean cv. PS-16 seed was inoculated singly with 5 *Rhizobium* strains and cultivated under 3 water regimes. She found that seed yield was not increased by inoculation under excess water or normal irrigation conditions; rather seed yield was increased by inoculation.

Provorov *et al.* (1998) observed that seed inoculation of mungbean (*Vigna radiata*) strain CIAM 1901 augmented the herbage mass by 46.6%, seed mass by 39.2%, 1000-seed weight by 16%, seed N content by 58.3%, seed starch content by 30.0% and number of root nodules by 254%.

Patra and Bhattacharyya (1998) conducted a pot experiment to assess the effects of *Rhizobium* inoculation on seed of *Vigna radiata* cv. B1. They observed that plants grown from inoculated seeds exhibited significantly high root and shoot weights compared to the non-inoculated control plants. From a field trial, they also reported that plants from seeds inoculated with *Rhizobium* plus urea fertilizer dressing produced significantly high yield compared to the control.

Thakur (1999) conducted field experiments at Tendani, Chhindwara (Madhya Pradesh) during the rainy seasons of 1991 and 1992 to evaluate the effects of P, S and *Rhizobium* on growth and yield of blackgram. Inoculation of *Rhizobium* culture on the surface of dry seeds before sowing helped to improve the seed and straw yields. Significant increases in seed and straw yields were observed by application of up to 40 kg P_2O_5 and 20 kg S ha^{-1}, mainly due to improvement in plant height, branches $plant^{-1}$ and pods $plant^{-1}$.

Upadhyay *et al.* (1999) conducted a field experiment where green gram seed was inoculated with *Rhizobium* plus non-inoculated control and dressed with 0-60 kg P_2O_5 ha^{-1}. They observed that seed yield was higher in *Rhizobium* inoculation (2.02 vs. 1.87 t ha^{-1}) and the fertilizer up to 40 kg P_2O_5 (2.01 t ha^{-1}) treatments.

Sharma *et al.* (2000) carried out a field experiment during *kharif* 1997 at Palampur, Himachal Pradesh, India where *V. mungo* seed was inoculated with 1 of 9 *Rhizobium* strains and dressed with 0 or 20 kg N ha^{-1}. Growth, yield and dry matter accumulation were increased due to N application and *Rhizobium* inoculation, the local strain produced the best results.

Sharma (2001) conducted a field experiment in Palampur, Himachal Pradesh, India during the *kharif* season on mungbean cv. Pusa Baiosakhi inoculated with three strains of *Bradyrhizobium* culture. The various physiological and yield parameters of mungbean were evaluated. Crop growth rate, relative growth rate, days to 50% flowering, days to maturity and seed yield was at maximum when mungbean seeds were treated with local isolate of *Bradyrhizobium*.

Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India during February–May to study the effect of *Rhizobium* inoculation, P and Mo on the growth of summer greengram. Inoculation of *Rhizobium* influenced maximum seed yield in comparison to control.

El-Kramany (2001) conducted a pot trial to investigate the combined impact of biological (*Bradyrhizobium vigna* and *Azotobacter vinelandii*) and NPK fertilizers (25, 50 and 100%) on mungbean cultivars (Kawmy-1, VC-4, VC-9 and King) and found that the combined application of *B. vigna* and *A. vinelandii* increased seed yield, 100-seed weight and biological yield of mungbean significantly over non-inoculated control.

Nagarajan and Balachander (2002) conducted a field experiment during the *kharif* season to study the effects of *Bradyrhizobium* and organic amendments on nodulation and yield of blackgram cv. Vamban-1. The treatments consisted of *Bradyrhizobium* (strain CRU-

7) for blackgram and CRM 11 (for greengram) seed inoculation, 15 t FYM ha^{-1} and FYM + *Bradyrhizobium*, 5 t compost ha^{-1}. Seed inoculation with *Bradyrhizobium* and application of organic amendments enhanced biomass, root nodulation and grain yield.

Tomar *et al.* (2001) conducted a field experiment at the G.B. Pant University Research Station, Ujhani, Uttar Pradesh, India, during *kharif* 1994-95 to study the effect of *Rhizobium*, vesicular arbuscular mycorrhiza (VAM, *Glomus caledonium*) and phosphate solubilizing bacteria (PSB, *Pseudomonas striata* strain P-27) inoculation, with and without P, on blackgram (*Vigna mungo*) seed yield. Phosphorus application in soil with medium P content (5.4 mg kg^{-1}) increased nodulation, grain yield, N and P in plant and grain over no phosphorus control. Application of forty kilograms of P_2O_5 per hectare recorded an increase of 20.6% in nodule dry weight, significant increases of 0.35 g kg^{-1} in N concentration and 1.28 g kg^{-1} in P concentration of plant over 20 kg P_2O_5 ha^{-1} fertilizer dose. Similar significant increases of 0.59 g kg^{-1} in grain yield and 0.54 and 0.23 g kg^{-1} in N and P concentrations of the grain, respectively were also obtained with higher dose (over 20 kg P_2O_5 ha^{-1}). Inoculation of *Rhizobium* + VAM + PSB at all the stages of plant growth recorded maximum increases in all the parameters studied. Inoculation of *Rhizobium* resulted in the highest and 21.0% more nodule number, 34.7% more nodule dry mass, 0.73 g kg^{-1} more N in grain and 4.2% higher grain yield over PSB treatment.

Srinivas and Shaik (2002) studied the effects of N (0, 20, 40 and 60 kg ha^{-1}) and P (0, 25, 50 and 75 kg ha^{-1}) along with seed inoculation with *Rhizobium* culture on the growth and yields components of greengram. The field experiment was conducted during the kharif season. Number of seeds pod^{-1}, 1000-seed weight, seed and haulm yields were significantly increased. Seed inoculation with *Rhizobium* resulted in higher values for the parameters measured relative to the control. The interactions effects between N and P were not significant in the case of number of pods plant^{-1}, pod length and seed haulm yield.

Malik *et al.* (2002) reported the effects of seed inoculation with *Rhizobium* and P application (at 0, 30, 50, 90 and 110 kg ha^{-1}) on the growth, seed yield and quality of mungbean cv. NM-98 in a field experiment conducted in Pakistan. Seed inoculation with *Rhizobium* and application of 70 kg ha^{-1}) resulted in the highest number of pods plant^{-1} (22.47), number of seed pod^{-1} (12.06), 1000-seed weight (42.27 g) and seed yield (1158 kg ha^{-1}).

Perveen *et al.* (2002) conducted a field experiment to observe the effects of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiata*). The treatments were single, dual and combined inoculants of *Bradyrhizobium*, *Azotobacter chroococcum* and *Aspergillus*. The maximum root dry weight (0.37 g plant^{-1}) and seed yield (6.6 g plant^{-1}) were observed with single *Bradyrhizobium* sp.

Ashraf *et al.* (2003) conducted a field experiment to observe the effects of seed inoculation of a biofertilizer and NPK application on the performance of mungbean cv. NM-98 at Faisalabad in Pakistan. Seed inoculation + 50:50:0 or 50:50:50 kg ha^{-1} resulted in the highest number of pods plant^{-1} (28.97, 56.00, 63.90 and 32.56, respectively) and seed yield (1053, 1066, 1075 and 1072 kg ha^{-1}). Harvest index was the highest with seed inoculation in combination with NPK and 40:50:0 (25.23), 50:50:0 (24.70) or 50:50:50 (27.5). The effect of seed inoculation along with NPK at 30:50:0 kg ha^{-1}) was optimum for the production of high seed yield by mungbean cv. NM-98.

Sriramachandrasekharan and Vaiyapuri (2003) coducted a pot culture experiment to study the effect of carbofuran in association with *Rhizobium* on the nodulation, growth, and yield of blackgram cv. ADT 3. Plant height (40.3 cm), number of nodules plant^{-1} (36.4), effective number of nodules plant^{-1} (24.5), nodule dry weight (20.2 g), root length (23.5 cm), shoot weight (0.82 g), root weight (0.32 g), number of pods pot^{-1} (18.2), pod yield pot^{-1} (55.6 g), root weight (0.32 g), and stover yield pot^{-1} (90.1 g) was the highest in 2.50 ppm carbofuran applied treatment and decreased thereafter with further increase in carbofuran concentration. Irrespective of the levels of carbofuran, *Rhizobium*-inoculated blackgram showed better growth and higher pod yield (50.3 g) and stover yield pot^{-1} (81.1 g) than the uninoculated crop.

Bhuiyan and Mian (2007) conducted experiments with and without *Bradyrhizobium* in five mungbean varieties at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe nodulation, biomass production and yield of mungbean and reported that application of *Bradyrhizobium* inoculant produced significant effect on nodulation, shoot dry weight, seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. Inoculated BARI Mung-2 produced the highest nodulation, dry matter production, seed and stover yields.

Shil *et al.* (2007) reported that the highest seed yield, plant height, pod length, pods plant^{-1}, seeds pod^{-1}, 1000-seed weight were highest in full doses of fertilizers while control plants recorded the lowest yield and also other parameters.

Bhuiyan *et al.* (2008) carried out field studies with and without *Bradyrhizobium* with five mungbean varieties to observe the yield and yield attributes of mungbean. They observed that application of *Bradyrhizobium* inoculant produced significant effect on seed and stover yields. Seed inoculation significantly increased seed (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) and stover (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) yields of mungbean. *Bradyrhizobium* inoculation also significantly increased pods $plant^{-1}$, seeds pod^{-1} and 1000-seed weight. Inoculated BARI Mung-2 produced the highest seed and stover yields as well as other yield attributes such as pods $plant^{-1}$ and seed pod^{-1}.

2.5 Effect of *Bradyrhizobium* inoculation on nitrogen content and it's uptake

Bhalu *et al.* (1995) conducted a field experiment during the rainy season of 1990 at Junagadh, Gujarat with blackgram (*Vigna mungo*) using *Rhizobium* inoculation and 10, 20 or 30 kg N and 20, 40 or 60 kg P_2O_5 ha^{-1} fertilizer levels. Seed inoculation increased seed yield (471 vs. 434 kg ha^{-1}). Seed yield increased by applying with up to 20 kg N (464 kg) and 40 kg P_2O_5 (475 kg). N and P uptakes and seed protein content increased with increasing N and P rates. Net return was highest with seed inoculation.

Sharma *et al.* (1999) conducted a field experiment in 1997/98 in Himachal Pradesh with *V. mungo* cv. Pant U-19 and reported that though the result was not significant but a slight improvement in protein content of seed and straw were observed over control.

Srivastav and Poi (2000) conducted field experiments to determine the symbiotic efficiencies of greengram (*V. radiata*) and blackgram (*V. mungo*) after inoculation with a native *Bradyrhizobium* strain and the residual effects of 7 *Bradyrhizobium* strains (NG-13/1, M-10, Kuthi AR-1, Jea-1, Caj-3, NK-4 and Caj6/1) in neutral pH soil, in Mohanpur, West Bengal, India. Variations in symbiotic N fixation of greengram and blackgram were observed due to the effect of the host and inoculant strains. Inoculation with M-10 strain in greengram resulted in the highest dry matter production and nitrogen fixation, while inoculation with NK-4 into blackgram resulted in the highest nitrogen uptake and grain yield. The residual potentialities of the 7 strains were very low in subsequent seasons; however, strains M-10 and

NK-4 were better than the other strains. This was due to their higher adaptive nature and competitiveness over the native strains.

Tomar *et al.* (2001) conducted a field experiment at the G.B. Pant University Research Station, Ujhani, Uttar Pradesh, India, during kharif 1994-95 to study the effect of *Rhizobium*, vesicular arbuscular mycorrhiza (VAM, *Glomus caledonium*) and phosphate solubilizing bacteria (PSB, *Pseudomonas striata* strain P-27) inoculation, with and without P, on blackgram (*Vigna mungo*) seed yield. Phosphorus application in soil with medium P content (5.4 mg kg^{-1}) increased nodulation, grain yield, N and P content in plant and grain over no phosphorus control. Forty kilograms of P_2O_5 ha^{-1} recorded an increase of 20.6% in nodule dry weight, significant increases of 0.35 g kg^{-1} in N concentration and 1.28 g kg^{-1} in P concentration of plant over 20 kg P_2O_5 ha^{-1} fertilizer dose. Similar significant increases of 0.59 g kg^{-1} in grain yield and 0.54 and 0.23 g kg^{-1} in N and P concentrations of the grain, respectively, were also obtained with higher dose (over 20 kg P_2O_5 ha^{-1}). Inoculation of *Rhizobium* + VAM + PSB at all the stages of plant growth recorded maximum increases in all the parameters studied. Inoculation of *Rhizobium* resulted the highest and 21.0% more nodule number, 34.7% more nodule dry mass, 0.73 g kg^{-1} more N in grain and 4.2% higher grain yield over PSB treatment.

Perveen *et al.* (2002) conducted a field experiment to observe the effect of rhizospheric microorganisms on yield and N, P and K uptake of green gram (*Phaseolus radiata*). The treatments were single, dual and combined inoculants of *Bradyrhizobium*, *Azotobacer chroococcum* and *Aspergillus*. The effects of the rhizospheric microorganisms on the N, P and K uptake were also increased significantly.

Chatterjee and Bhattacharjee (2002) studied that plants inoculated with *Bradyrhizobium*, strains and PSB showed increased rate of nodulation and N content.

Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/ without *Bradyrhizobium* inoculation at the Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons to observe shoot dry matter production and nitrogen uptake by mungbean at different growth stages. They observed significant variation on dry matter production and nitrogen uptake among the mungbean varieties. Plants from seed inoculated with *Bradyrhizobium* significantly increased dry matter production and N uptake at 77 DAS. Inoculated BARI Mung-2 produced highest shoot weight and N uptake efficiency. The sequential analysis of plant samples of BARI Mung-2 on N concentration should the highest in most of the sampling dates and the N uptake efficiency was also the highest all through. Mungbean variety Barisal local ranked the lowest in respect of uptake of nitrogen from the atmosphere.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter deals with the experimental aspect of the work. The materials used and methods followed in performing this experiment has been presented in this chapter. This offers brief description of soil, experimental design, chemical fertilizer, intercultural operations and statistical analysis.

3.1 Site and soil

This experiment was conducted in the Soil Science Field Laboratory of the Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period from March to June 2007. The morphological characteristics of the land are presented in Table 3.1, and the physical and chemical characteristics of the soil are presented in Table 3.2.

Table 3.1. Morphological characteristics of the experiment field

Characters	BARI farm
Locality	BARI, Gazipur
Geographic position	24.09^{0} North Latitude 90.5^{o} Eeast Longitude 8.2 m height above the mean sea level
Agro-ecological zone (FAO and UNDP, 1988)	Madhupur Tract (AEZ-28)
General soil type	Shallow Grey Terrace Soil
Taxonomic soil classification:	
Order	Inceptisols
Sub-order	Aquept
Sub-group	Aeric Albaquept
Soil series	Chhiata
Parent material	Madhupur terrace
Topography	Fairly level
Drainage	Well drained
Flood level	Above Flood level

Table 3.2. Physical and chemical characteristics of the soils

Characteristics	BARI farm
Mechanical fractions:	
% Sand (0.2-0.02 mm)	27.4
% Silt (0.02-0.002 mm)	33.3
%Clay (< 0.002 mm)	39.3
Textural class	Clay loam
Colour	Grey
Consistency	Sticky and mud when wet
pH (1:2.5 Soil-Water)	6.2
CEC (cmol kg^{-1})	18.4
Exchangeable K (cmol kg^{-1})	0.21
Exchangeable Ca (cmol kg^{-1})	10.42
Exchangeable Mg (cmol kg^{-1})	7.34
Exchangeable Na (cmol kg^{-1})	0.16
Organic C (%)	0.93
Total N (%)	0.07
Available P (mg kg^{-1})	12.1
Available S (mg kg^{-1})	14.1
Available Zn (mg kg^{-1})	1.79
Available Cu (mg kg^{-1})	0.66
Available Fe (mg kg^{-1})	16.8
Available Mn (mg kg^{-1})	3.1

3.2 Collection of soil sample

Three composite soil samples were collected from a depth of 0-15 cm taking one from each block immediately before fertilizer application. Each composite sample was air dried and ground to pass through a 10 mesh sieve and stored in polythene bags for mechanical and chemical analysis.

3.3 Climate

The climatic condition of the experimental area was characterized by high temperature and heavy rainfall during *kharif* season (March-September) and low rainfall and moderately low temperature during *rabi* season (October-February).

3.4 Crop

Summer mungbean (*Vigna radiata* L.) variety BARI Mung-5 was used as the test crop. The seeds of mungbean were collected from Soil Science Division, BARI, Joydebpur, Gazipur. The seeds were healthy, pulpy, well matured and free from mixture of the other seeds, weed seeds and extraneous materials.

3.5 Variety

The salient characteristics of BARI Mung-5 are presented below:

BARI released BARI Mung-5 in 1997. Plant height of this variety ranges from 40 to 45 cm and seeds are deep green in colour. One thousand seed weight is about 40 to 42 g. The variety requires 55 to 60 days to mature, and average yield is 1200 kg ha^{-1}. It is resistant to *Cercospora* leaf spot and tolerant to yellow mosaic virus (BARI, 1998). One of the main characteristics of the variety is synchronization in pod ripening in the summer season.

3.6 Land preparation

The land was opened on 01 March 2007. Ploughing and cross-ploughing were done with disc ploughs and harrows, and then well prepared by ploughing and cross-ploughing with the country plough followed by laddering uniformly. Weeds and stubbles were collected and removed. Plots were made by raising bunds. Land preparation was completed on 07 March 2007 to make it ready for sowing.

3.7 Fertilizer application

After making the lay out of the experiment, the lands were fertilized on 06 March 2007 with 22, 42, 20, 5 and 1 kg ha^{-1} of P, K, S, Zn and B in the form of triple superphosphate (TSP), muriate of potash (MOP), gypsum, zinc sulphate and boric acid, respectively. No nitrogen as chemical fertilizer was applied in this experiment.

3.8 Treatment under investigation

The experiment consisted of the following six treatments. These were as follows:

Treatments: 6

i) T_1: Inoculated with *Bradyrhizobium* strain BARI RVr-401

ii) T_2: Inoculated with *Bradyrhizobium* strain BARI RVr-402

iii) T_3: Inoculated with *Bradyrhizobium* strain BARI RVr-403

iv) T_4: Inoculated with *Bradyrhizobium* strain BARI RVr-404

v) T_5: Mixed culture of strain BARI RVr-401, BARI RVr-402, BARI RVr-403 and BARI RVr-404

vi) T_6: Non-inoculated control

3.9 Experimental design

The experiment was laid out in randomized complete block design with 4 replications. Each replication was represented by a block, which was divided into 6 sub-plots. The treatments were randomly placed on the 6 sub-plots in each block. Thus, the total numbers of unit plots were 24 (6 treatments x 4 replications). The size of each unit plot was 4 m x 3 m and plot to plot distance was 1 m. Line to line distance was 30 cm and plant to plant distance was 10 cm.

3.10 Preparation of inoculants

3.10.1 Collection of strains

Strains were collected from Soil Microbiology Laboratory, BARI, Joydebpur, Gazipur. The mixed culture was prepared by mixing equal amount of fully grown individual culture broth in sterile conical flask.

3.10.2 Inoculation with peat media

From the ready broth, 20 mL were taken out with the sterile syringe and injected into polythene packet having sterile 50 g of peat in each packet. The inoculated packets were then incubated at 28^0C for two weeks and were ready for seed inoculation.

3.10.3 Viability count of *Bradyrhiozbium*

The viability count of *Bradyrhizobium* in peat based inoculum packets were made following plate count method before using (Vincent, 1970). BARI RVr-401, BARI RVr-402, BARI RVr-403, BARI RVr-404 and mixed culture containing 1.0×10^8, 1.3×10^8, 1.4×10^8, 4×10^8 and 1.2×10^8 cells g^{-1} of inoculant, respectively.

3.10.4 Seed coating with inoculants

Seeds of mungbean were taken in small polyethelene containing 42 g per packet. Out of 24 polythene packets 20 packets were made ready for mixing with 40% gum acacia @ per 1 kg seed. After gum coating the selected inoculum was mixed with the seeds (@ 50 g inoculum kg^{-1} seed) for each treatment by shaking the bag thoroughly. For each inoculant, separate polythene bag was used and mixed well with the seeds and care was taken to avoid contamination of the inoculants.

3.11 Sowing of seeds

Seeds were sown on the furrows on 08 March 2007 in the morning and the furrows were covered with soils soon after seeding. The line to line (furrow to furrow) distance was maintained at 30 cm with continuous distribution of seeds in the line.

3.12 Germination of seeds

Germination of seeds started from 3rd day of sowing. On the 4th day the percentage of germination was more than 80% and on the 5th day nearly all plants came out of the soil.

3.13 Intercultural operation

a) Weeding and thinning

Weeding and thinning were done on 20th day after sowing when the plants attained a height of about 8 to 10 cm. Plant to plant distance was maintained at 7.5 cm.

b) Drainage

During experimental period, once there was a heavy rainfall. At that time it was essential to drain the water from the field and the water was removed through the side drain.

c) General observation

The field was frequently observed to notice any change in plant characters as well as to check the attack of any pest and disease.

3.14 Plant sampling

Plant samples were collected from the field at 35 and 50 days after sowing and at harvest. From each plot, 10 randomly selected plants were carefully uprooted with the help of a spade so that no nodules were left in the soil. The roots were then washed out carefully in water. The nodules from main root and branch root of each were collected and counted. The shoot portion of each plant was then separated from the root. The length of roots and shoots of each plant were recorded. The oven dried weights of roots, shoots and nodules were recorded.

3.15 Harvest

The crop was harvested at physiological maturity on first week of June 2007 after 70 days after sowing. Data from 10 plants on number of pods and seeds plant^{-1} and 100-seed weight were recorded.

3.16 Threshing and processing

From each plot 10 randomly selected plants were collected and then plants of each of 3 square meters were harvested and tied with rope separately. The harvested plant materials of 3 square meters were allowed to dry in the sun for 3 days. After drying, threshing and processing were done plot wise carefully. The processed seed and straw were again dried in the sun for 3 days. Seed and straw yields were recorded plot wise, which were then converted into yield in kg ha^{-1}.

3.17 Collection of plant (stover and seed)

About 50 g each of dried seed and stover samples were collected from each plot. The sample were stored in polyethylene bags separately and tagged for chemical analysis.

3.18 Analysis of soil and plant

3.18.1 Soil analysis

The initial soil sample was analyzed (texture, pH, CEC, organic matter, total nitrogen, available phosphorous, sulphur, potassium) using the following methodology.

i) Mechanical analysis

Mechanical analysis of the soil samples was carried out by hydrometer method (Black, 1965) and the textural class was determined by using Marshall's Triangular Diagram (1947).

ii) Soil pH

Soil pH was determined using a glass electrode pH meter, soil water ratio being 1:2.5 as described by Jackson (1973).

iii) Organic carbon

Soil organic carbon was determined by wet oxidation method as outlined by Jackson (1973). Organic matter content was calculated by multiplying the percent organic carbon with the "Van Bemmelen Factor of 1.723 (Piper, 1950).

iv) Total nitrogen

Total nitrogen in soil was determined by the Micro-Kjeldahl method. Digestion was made with H_2O_2, concentrated H_2SO_4 and catalyst mixture (K_2SO_4:$CuSO_4$, $5H_2O$:Se =

10:1:0.1). Nitrogen in the digest was estimated by distilling with 40% NaOH followed by titration of the distillate trapped in H_2BO_3 with 0.01 N H_2SO_4 (Page *et al.*, 1989).

v) Available phosphorus

Available phosphorus was extracted from the soil with 0.5 M $NaHCO_3$ at pH 8.5. The phosphorus in the extract was then determined by developing the blue colour by $SnCl_2$ reduction of phosphomolybdate complex and measuring the colour colorimetrically at 660 nm (Olsen *et al.*, 1982).

vi) Exchangeable potassium

Exchangeable potassium of the soil was determined from the 1N NH_4OAc extract of the soil using flame photometer as described by Page *et al.* (1989).

vii) Cation exchange capacity (CEC)

Cation exchange capacity of soil was determined by sodium saturation method as outlined by Page *et al.* (1989). The soil samples were saturated with 1N NaOAc solution followed by replacing the Na^+ from the saturated samples by 1N NH_4OAc at pH 7.0. The amount of Na^+ in the extract was then determined by flame photometer and putting the readings to the standard curve for Na^+. The results were expressed as me 100 g^{-1} soil.

3.18.2 Plant analysis (seed and stover)

The whole plant (seed and stover) sample of mungbean of all treatments were powdered to 60 mesh separately using a Wiley mill and stored in a desiccators for chemical analysis. For determination of nitrogen 0.1 g of oven dried ground sample was taken into a 100 ml digestion flask and then 1.1 g of catalyst mixture (K_2SO_4 : $CuSO_4$, $5H_2O$: Se = 100:10.01) 2 ml 30% H_2O_2 and 5 ml concentrated H_2SO_4 were added. The flask was swirled and allowed to stand for about 10 minutes then the flask was heated until became colorless. Digestion was carried out in digestion flask at 380^0C for 1.5 hour. After cooling the digest was transferred into a 100 mL volumetric flask and the volume was made up to the mark with distilled water. The distillate was titrated against 0.01 N H_2SO_4. A blank reagent was prepared by the same process. These digests were used to determine total N.

3.19 Data to be recorded

a) Before harvest (35 and 50 days after sowing)

i) Total nodule plant^{-1}

ii) Dry weight of nodules (mg plant^{-1})

iii) Root weight (g plant^{-1})

iv) Shoot weight (g plant^{-1})

v) Root length (cm)

vi) Shoot length (cm)

b) After harvest

i) Plant height (cm)

ii) Pod length (cm)

iii) Pods plant^{-1}

iv) Seeds pod^{-1}

v) Seed yield (kg ha^{-1})

vi) Stover yield (kg ha^{-1})

vii) 1000-seed weight (g)

viii) N content in seed

ix) N uptake by seed (kg ha^{-1})

x) N content in stover

xi) N uptake by stover (kg ha^{-1})

xii) P content in seed

xiv) Protein yield (kg ha^{-1})

3.20 Statistical analysis

Data of all the above characters have been analyzed statistically. Statistical analysis was done following RCBD design of the IRRISTAT package Programme. Duncan's Multiple Range Test was done as and when necessary.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

An experiment was conducted at the Soil Science Experimental Field of the Bangladesh Agricultural Research Institute (BARI) Farm, Joydebpur, Gazipur during 8th March to 1st July 2007 to evaluate the performance of four *Bradyrhizobium* strains on nodulation, dry matter weight, yield, yield attributes, protein content and N uptake by mungbean. The results are presented and discussed in this chapter.

4.1 Number of nodules plant^{-1}

Bradyrhizobium inoculants produced significantly higher number of nodules plant^{-1} compared to that found in uninoculated control (Figs. 4.1 and 4.2, and App. 4.1). The inoculant BARI RVr-404 produced the highest number of nodules plant^{-1} (8.21 nodules at 35 DAS i.e. days after sowing and 12.87 nodules at 50 DAS) and it was found that the nodulation efficiencies of the four strains viz. BARI RVr-401, BARI RVr-402, BARI RVr-403, BARI RVr-404 and their mixed culture were statistically identical at both 35 and 50 DAS (Figs. 4.1, 4.2 and App. 4.1). Nodule number plant^{-1}, 5.21 at 35 DAS and 7.25 at 50 DAS were found without any rhizobial inoculation in the control plot and 7.59 to 8.21 nodules plant^{-1} at 35 DAS and 10.10 to 12.87 nodules plant^{-1} at 50 DAS with *Bradyrhizobium* inoculation in the mungbean crop. In both at 35 and 50 DAS, BARI RVr-404 strain showed the highest number of nodules plant^{-1} compared to other strains including mixed culture. Nodule number was higher at 50 DAS than 35 DAS. It indicated that nodule formation at 50% flowering stage was higher than that at pre-flowering stage. The results agreed well with the findings of Chowdhury *et al.* (1997) who observed that nodule was higher in inoculated mungbean at flowering stage followed by pod filling and pre-flowering. Rao *et al.* (1991) found nodules in mungbean two weeks after sowing. Murakami *et al.* (1991) reported that in

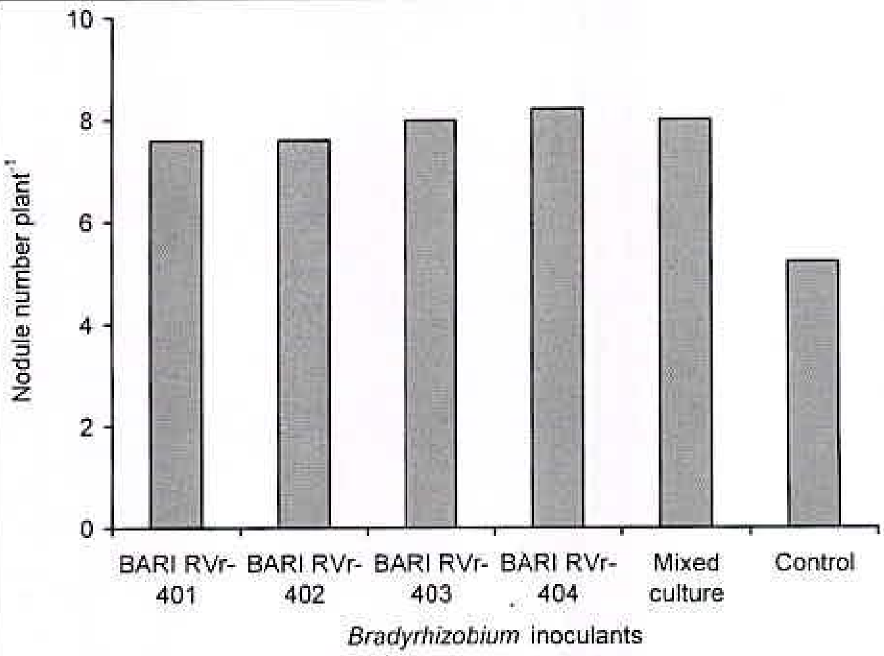

Fig. 4.1. Effect of bradyrhizobial inoculants on nodule number of mungbean at 35 DAS

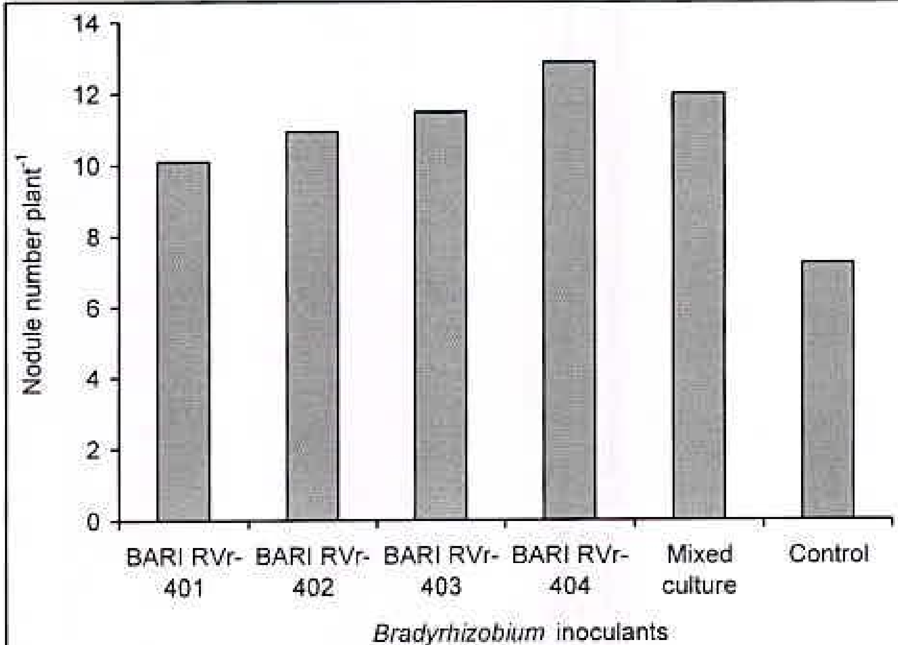

Fig. 4.2. Effect of bradyrhizobial inoculants on nodule number of mungbean at 50 DAS

mungbean, nodulation started at 12 DAS, increased rapidly at 34 DAS (flowering) reaching a peak at 45 DAS and then decreased until the plants died (83 DAS). Akhtaruzzaman (1998) also observed maximum nodulation at 40 DAS than that at 30 and 20 DAS in mungbean. With respect to time of sampling, greater nodule numbers were obtained at 50 DAS compared to all other sampling dates, which was supported by Datt and Bhardwaj (1995). They reported that the nodule number and nodule dry weight of cowpea increased significantly by *Rhizobium* inoculation at 45 DAS followed by 55, 30 and 15 DAS. This might be due to the high requirement of N at the flowering and pod-filling stage (Rennie and Kemp, 1984). Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and found that inoculation with *Bradyrhizobium* strains increased rate of nodulation and N content. Osunde *et al.* (2003) reported that inoculation increased nodule number in mungbean. Kumari and Nair (2003) found that the extent of improvement in root nodulation, plant growth and yield were more in blackgram and greengram inoculated with *Bradyrhizobium*. In another study, Bhuiyan *et al.* (2006) found that inoculated plants produced significantly higher nodule number (17.51 plant^{-1} in 2001 and 17.61 plant^{-1} in 2002) at 42 DAS compared to that in non-inoculated plant (11.35 plant^{-1} in 2001 and 11.52 plant^{-1} in 2002). The lowest number of nodules (3.50 plant^{-1} in 2001 and 3.47 plant^{-1} in 2002) was obtained at 14 DAS in non-inoculated plants. Bhuiyan *et al.* (2007a) found that the number of nodules increased progressively with the advancement of growth and reached the peak at 42 DAS (i.e. at 50% flowering stage). Bhuiyan and Mian (2007) also found that application of *Bradyrhizobium* inoculant induced significant effect on nodulation, shoot dry weight, seed and stover yields.

4.2 Nodule dry weight

Application of four *Bradyrhizobium* inoculants showed significant variation in the production of nodule dry weight (Figs. 4.3 and 4.4, and App. 4.1). The inoculant BARI RVr-404 produced the highest nodule dry weight which was statistically identical to that produced by BARI RVr-401, BARI RVr-402, BARI RVr-403 and mixed culture in both situations (35 DAS & 50 DAS). Non-inoculated control treatment produced the lowest nodule dry weight plant^{-1} (7.00 mg plant^{-1} at 35 DAS and 9.00 mg plant^{-1} at 50 DAS). At 35 DAS, the highest nodule weight (9.99 mg plant^{-1}) and at 50 DAS the highest nodule weight (16.35 mg plant^{-1}) were recorded when the plots were inoculated with the strain BARI RVr-404 which was identical with plot inoculated with BARI RVr-401, BARI RVr-402, BARI RVr-403 and mixed culture in both the sampling dates. Sattar and Ahmed (1995) carried out a field experiment on mungbean (*Vigna raidata* L.) to study the response of inoculation with *Bradyrhizobium* inoculants incorporating BINA 403, BINA 407, RCR 3824 and RCR 3825 strains as single and mixed culture. They observed that *Bradyrhizobium* inoculation increased the number of nodules and nodule weight significantly compared to non-inoculated treatment. Nagarajan and Balachandar (2002) reported that biodigested slurry at 5 t ha^{-1} + *Rhizobium* produced the highest nodule dry weight (42.3 mg).

4.3 Root dry weight

The effect of *Bradyrhizobium* did not show any significant influence on root weight of crop both at 35 and 50 DAS. The highest root weight (0.27 g plant^{-1} at 35 DAS and 0.34 g plant^{-1} at 50 DAS) was produced by BARI RVr-404 strain treated mungbean (Figs. 4.5 and 4.6, and App. 4.1). The root weight ranged from 0.22 g plant^{-1} recorded in non-inoculated crop to 0.27 g plant^{-1} due to inoculation at 35 DAS and at 50 DAS the range was from 0.24 g plant^{-1} to 0.34 g plant^{-1}. Kavathiya and Pandey (2000) conducted a pot experiment during the summer season of 1992-1993 in Gujrat, India and reported that fresh root weight (4.42 g) were recorded in the *Rhizobium* inoculation treatment. Perveen *et al.* (2002) conducted a field experiment to observe the effect of rhizospheric microorganisms on growth and yield of greengram (*Phaseolus radiata*) and reported that the maximum root dry weight (0.37 g plant^{-1}) was observed in inoculation with single *Bradyrhizobium* sp. only.

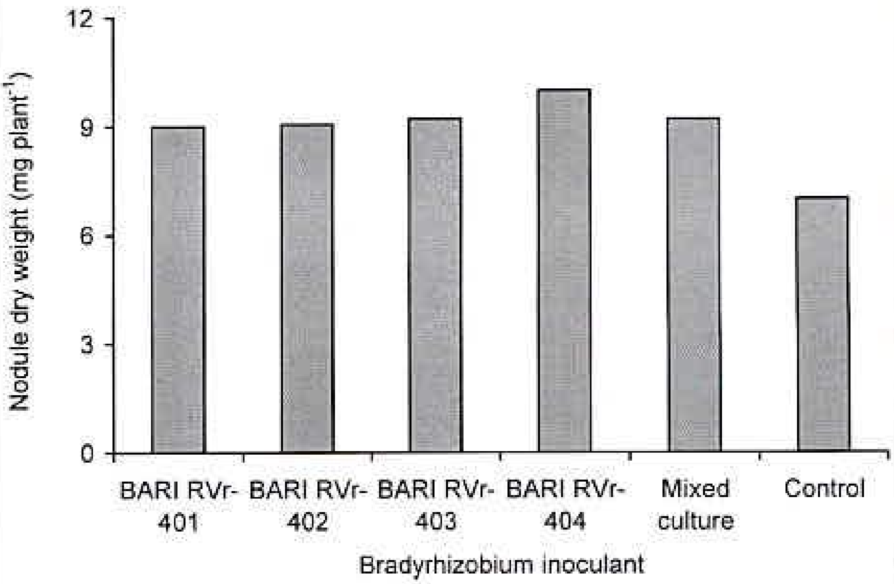

Fig. 4.3. Effect of bradyrhizobial inoculant on nodule dry weight of mungbean at 35 DAS

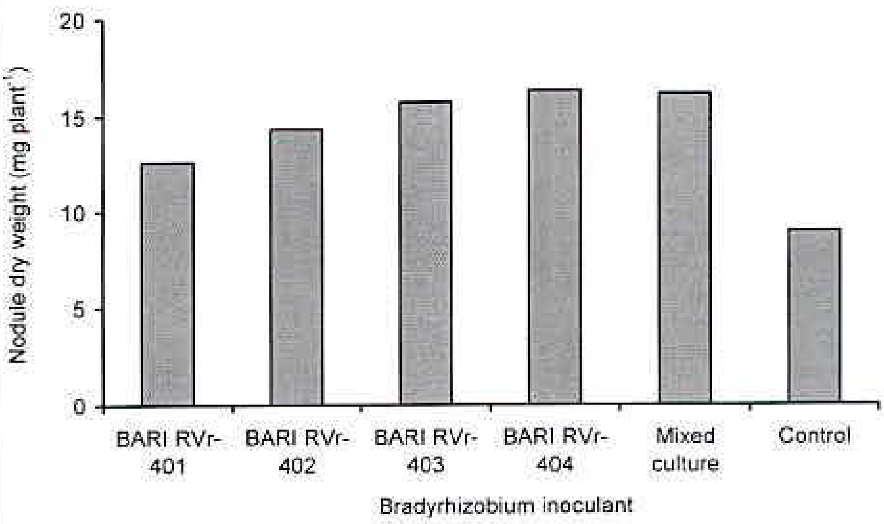

Fig. 4.4. Effect of bradyrhizobial inoculant on nodule dry weight of mungbean at 50 DAS

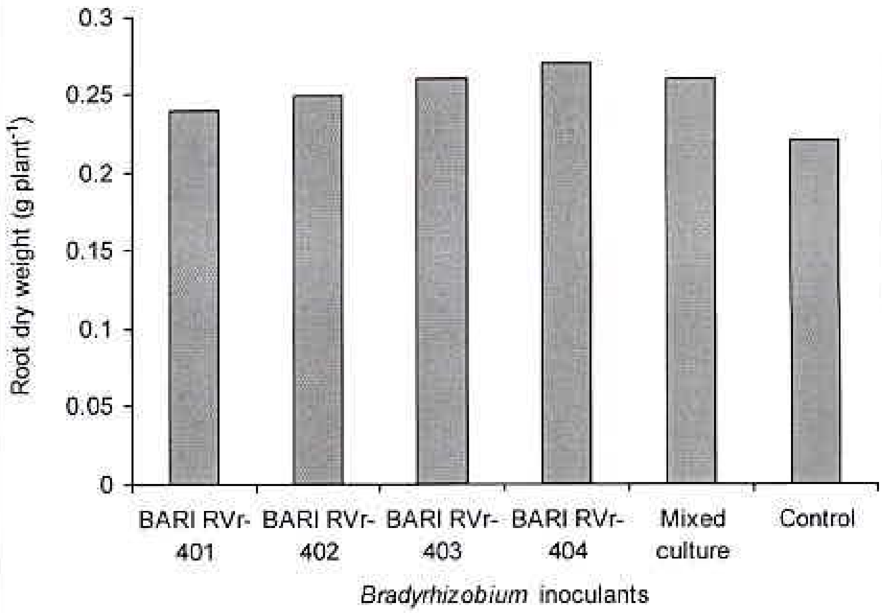

Fig. 4.5. Effect of bradyrhizobial inoculants on root dry weight of mungbean at 35 DAS

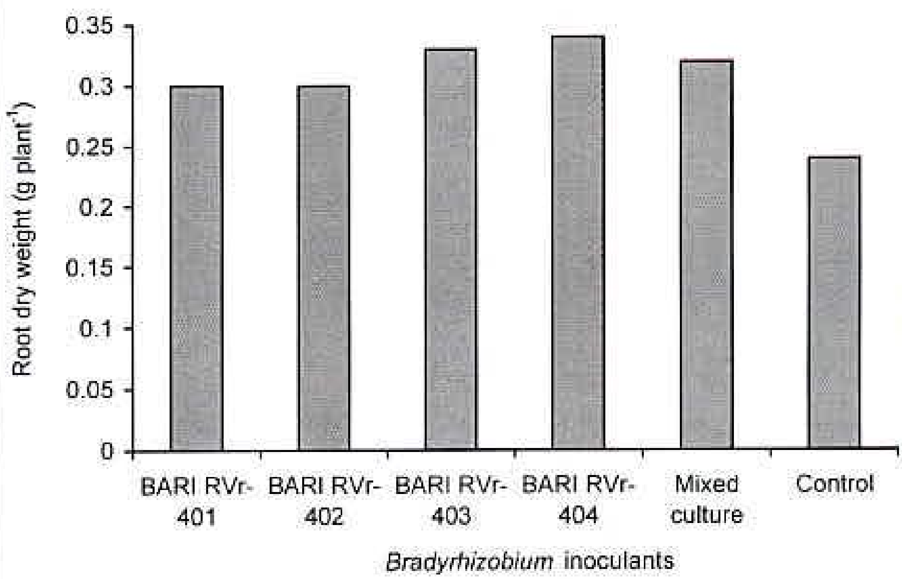

Fig. 4.6. Effect of bradyrhizobial inoculants on root dry weight of mungbean at 50 DAS

4.4 Shoot dry weight

The effect of inoculants on shoot weight was statistically significant (Figs. 4.7 and 4.8, and App. 4.1). The plants treated with the strain BARI RVr-404 recorded the highest shoot weight (1.20 g plant^{-1} at 35 DAS) which was superior to non-inoculated control but identical to other strains. At 50 DAS, the treatment containing same strain also recorded the highest shoot weight (2.11 g plant^{-1}) but it was identical to the effects of the rest three strains including non-inoculated control. The shoot weight was the lowest in non-inoculated control (0.91 g plant^{-1} at 35 DAS and 1.68 g plant^{-1} at 50 DAS). Kavathiya and Pandey (2000) reported that maximum fresh shoot weight (5.33 g) was recorded in the *Rhizobium* treated plot. Chowdhury *et al.* (2000) carried out a pot experiment during *kharif* season in 1995 with mungbean at IPSA, Salna, Gazipur, Bangladesh where mungbean line NM-92 was inoculated with *Rhizobium* strain TAL 303 and found that dry matter production was increased by about 50% due to *Bradyrhizobium* inoculation. Bhattacharyya and Pal (2001) conducted a field experiment in West Bengal, India, during the pre-*kharif* season of 1998 to study the effect of *Rhizobium* inoculation and reported that inoculation significantly influenced dry matter accumulation in the shoot. Bhuiyan *et al.* (2007b) carried out field studies with five mungbean varieties with/ without *Bradyrhizobium* inoculation at the Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 and observed that *Bradyrhizobium* inoculant significantly increased dry matter production. The highest dry matter production plant^{-1} at 77 DAS was recorded in *Bradyrhizobium* inoculated plots. Bhuiyan and Mian (2007) conducted experiments with or without *Bradyrhizobium* in five mungbean varieties at Bangladesh Agricultural University Farm during *kharif*-I 2001 and *kharif*-I 2002 seasons and observed that application of *Bradyrhizobium* inoculant recorded significant effect on shoot dry weight.

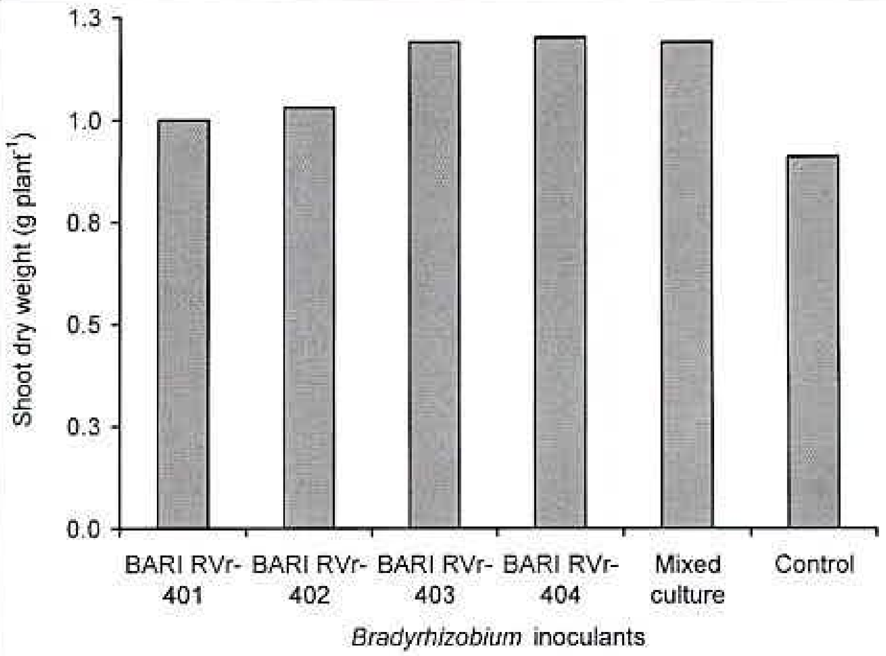

Fig. 4.7. Effect of bradyrhizobial inoculants on shoot dry weight of mungbean at 35 DAS

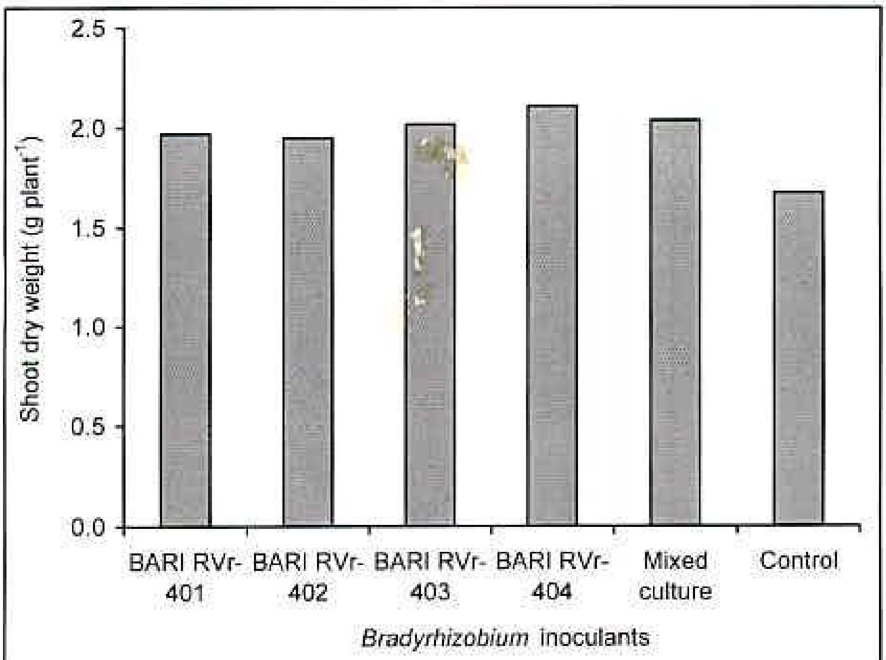

Fig. 4.8. Effect of bradyrhizobial inoculants on shoot dry weight of mungbean at 50 DAS

4.5 Shoot length

The effect of inoculants on shoot length was statistically insignificant (Figs. 4.9 and 4.10, and App. 4.1). The plants treated with the strain BARI RVr-404 recorded the highest shoot length (29.3 cm at 35 DAS and 39.4 cm at 50 DAS). The shoot length plant^{-1} was the lowest in the non-inoculated crop. Thakur and Panwar (1995) conducted a field trial where seeds of *Vigna radiata* cv. Pusa-105 and PS-16 were inoculated with inoculant. They found that inoculation either singly or combined increased plant height compared with non-inoculated control treatment. Bhattacharya and Pal (2001) reported that inoculation and application of rhizobial inoculum influenced plant height in comparison to that control. Nagarajan and Balachandar (2002) conducted a field experiment and observed that seeds treated with bio-digested slurry at 5 t ha^{-1} + *Rhizobium* produced the highest plant height (53.7 cm).

4.6 Plant height

Mungbean seeds inoculated with *Bradyrhizobium* sp. produced significantly increased in plant height (Table 4.1). The plants in the BARI RVr-404 treated plots were the tallest (44.1 cm) and it was statistically identical to that found in BARI RVr-403 and mixed culture treatments. The shortest plants (35 cm) were found in non-inoculated control plots. Thakur and Panwar (1995) found that inoculation either singly or combined increased plant height compared with no inoculation. Bhattacharya and Pal (2001) reported that application of rhizobial inoculum influenced plant height comparing with control. Nagarajan and Balachandar (2002) found that *Rhizobium* inoculation increased induced the higher plant height (53.7 cm).

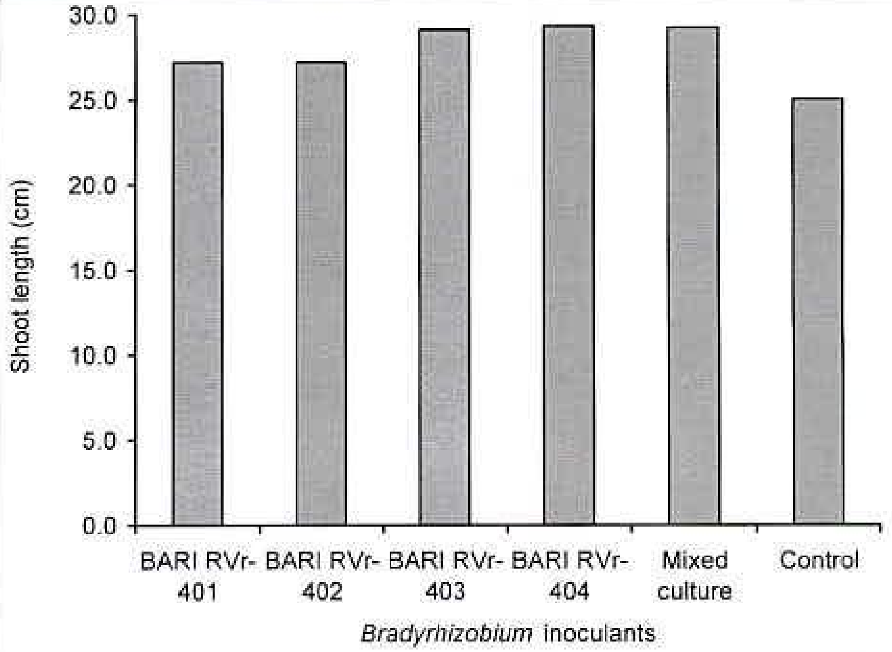

Fig. 4.9. Effect of bradyrhizobial inoculants on shoot length of mungbean at 35 DAS

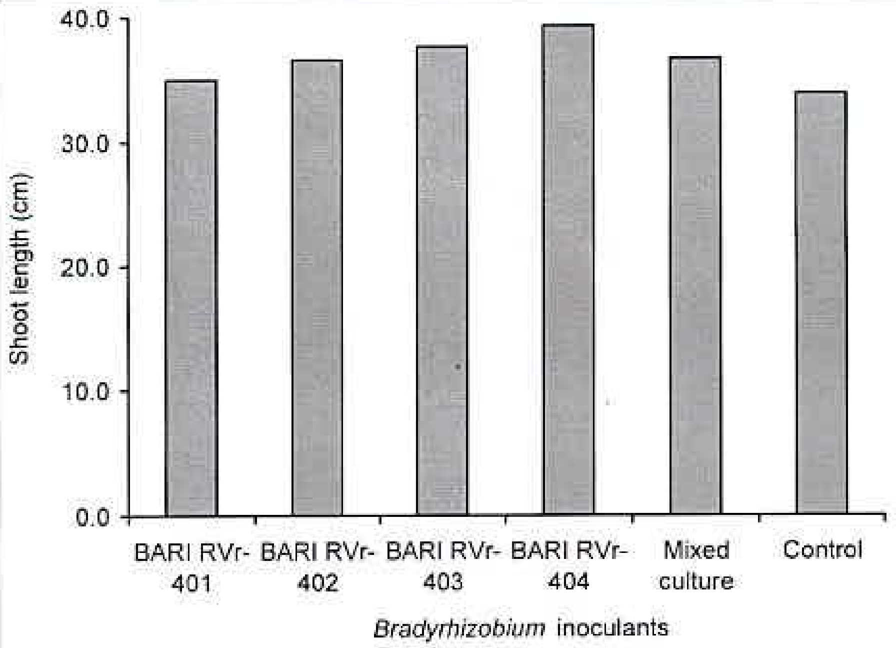

Fig. 4.10. Effect of bradyrhizobial inoculants on shoot length of mungbean at 50 DAS

Table 4.1. Effects of *Bradyrhizobium* inoculant on plant height and yield attributes of mungbean

Bradyrhizobium strains	Plant height (cm)	Pod length (cm)	Pods plant^{-1}	Seeds pod^{-1}	1000-seed weight (g)
BARI RVr-401	39.9b	7.80	16.5a	11.4	44.0
BARI RVr-402	40.0b	765	17.5a	11.5	43.0
BARI RVr-403	43.2ab	7.50	17.8a	11.4	43.1
BARI RVr-404	44.1a	8.00	19.3a	12.4	43.6
Mixed culture	43.9a	7.95	17.3a	11.9	44.0
Control	35.0c	7.35	12.5b	10.0	43.0
LSD (0.05)	3.71	NS	3.6	NS	NS
CV (%)	6.0	8.5	14.1	15.5	2.4

NS = Not Significant
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

4.7 Pod length

The effects of inoculants viz. BARI RVr-401, BARI RVr-402, BARI RVr-403, BARI RVr-404 and mixed culture were not statistically significant on the pod length of mungbean (Table 4.1). The inoculant BARI RVr-404 induced plots produced the longest pod (8.00 cm) which was statistically similar to that of all other strains and mixed culture treatments. The shortest pod (7.35 cm) was found in non-inoculated control plots. Shil *et al.* (2007) reported that the highest pod length was the highest in full doses of fertilizers while control plants recorded the lowest pod length. Srinivas and Shaik (2002) observed that seed inoculation with *Rhizobium* culture enhanced number of seeds pods^{-1}, 1000-seed weight, seed and haulm yields. Seed inoculation with *Rhizobium* resulted in higher values for the parameters measured relative to the control.

4.8 Pods plant^{-1}

Application of different *Bradyrhizobium* inoculants in mungbean showed significant variation in number of pods plant^{-1} (Table 4.1). The number of pods plant^{-1} was the highest (19.3 plant^{-1}) in inoculation with BARI RVr-404 strain, it was statistically identical to that of other inoculants treated plots. The minimum number of pods plant^{-1} was appeared in non-inoculated control plants. Malik *et al.* (2002) reported that seed inoculation with *Rhizobium* resulted in the highest number of pods plant^{-1} (22.47). Ashraf *et al.* (2003) observed that seed inoculation + 50:50:0 or 50:50:50 kg N: P: K ha^{-1} resulted in the highest number of pods plant^{-1} (28.97, 56.00, 63.90 and 32.56, respectively). Bhuiyan *et al.* (2008) reported that *Bradyrhizobium* inoculation in mungbean plots also significantly increased pods plant^{-1}.

4.9 Seeds pod^{-1}

The effect of inoculant on number of seeds pod^{-1} was not statistically significant (Table 4.1). The highest number of seeds pod^{-1} (12.4) was found in BARI RVr-404 inoculated plants. The lowest number of seeds pod^{-1} was seen in non-inoculated control (10.0). Bhuiyan *et al.* (2008) observed that *Bradyrhizobium* inoculation also significantly increased seeds pod^{-1}. Shil *et al.* (2007) reported that seeds pod^{-1} was the highest in full doses of fertilizers while control plants recorded the lowest seeds pod^{-1}. Srinivas and Shaik (2002) conducted trial on seed inoculation in *Rhizobium* culture and reported that *Rhizobium* inoculation in mungbean increased the number of seeds pod^{-1}.

4.10 1000-seed weight

No significant variation in 1000-seed weight of mungbean was observed due to seed inoculation with *Bradyrhizobium* inoculants. The 1000-seed weight ranged from 43.0 g to 44.0 g. The result showed the insignificant effect of different *Bradyrhizobium* inoculation on 1000-seed weight of mungbean. Srinivas and Shaik (2002) reported that 1000-seed weight generally increased due to rhizobial inoculation. Shil *et al.* (2007) reported that 1000-seed weight was the highest in full doses of fertilizers while control plants recorded the lowest in 1000-seed weight. Bhuiyan *et al.* (2008) opined that *Bradyrhizobium* inoculation also significantly increased 1000-seed weight.

4.11 Seed yield

Seed yield of mungbean varied significantly due to *Bradyrhizobium* inoculation (Fig. 4.11 and App. 4.2). The inoculant BARI RVr-404 induced plot showed the highest seed yield (1.43 t ha^{-1}) which was superior to BARI RVr-401, BARI RVr-402 and uninoculated control plot but identical to BARI RVr-403 and mixed culture. The higher yield recorded in inoculated plants might be due to higher nodulation which fixed more atmospheric nitrogen. Sharma and Khurana (1997) studied the effectiveness of single and multi-strain inoculants in field experiment with summer mungbean variety SML-32 and found that grain yield was higher in multi-strain inoculant treatments. On an average, single strain and multi-strain *Rhizobium* inoculants increased the grain yield by 10.4% and 19.3% over uninoculated control, respectively. Bhattacharya and Pal (2001) conducted a field experiment and reported that inoculation of *Rhizobium* influenced maximum seed yield comparing with control. Perveen *et al.* (2002) observed the maximum seed yield (6.6 g $plant^{-1}$) with single *Bradyrhizobium* sp. inoculation. Bhuiyan and Mian (2007) reported that application of *Bradyrhizobium* inoculant produced significant effect on seed yields in case of crop. Seed inoculation significantly increased seed yield (0.98 t ha^{-1} in 2001, 27% increase over control and 0.75 t ha^{-1} in 2002, 29% increase over control) of mungbean.

4.12 Stover yield

The *Bradyrhozibium* inoculants influenced significantly in stover yield of mungbean over non-inoculated control (Fig. 4.12 and App. 4.2). The highest stover yield of 2.31 t ha^{-1} was obtained due to application of BARI RVr-404 inoculant and it was statistically identical to that of BARI RV-401, BARI RV-402, BARI RV-403 strains and mixed culture. Therefore, it was found that mungbean crop inoculated with *Bradhyrhizobium* produced higher stover yield also. Bhuiyan and Mian (2007) reported that application of *Bradyrhizobium* inoculant produced significant effect on stover yields (2.31 t ha^{-1} in 2001 and 2.04 t ha^{-1} in 2002) of mungbean.

4.13 Nitrogen content in seed and stover

The effect of *Bradyrhizobium* inoculants on mungbean did not show any significant influence on N content in seed and stover. The result showed (Table 4.2) that the highest N content in seed and stover was obtained in BARI RVr-404 inoculant treated crop. The lowest N content in seed and stover was recorded in non-inoculated control plots. Chatterjee and Bhattacharjee (2002) studied the effects of inoculation with *Bradyrhizobium* and reported that the plants inoculated with *Bradyrhizobium* strains and PSB showed increased rate of N content.

4.14 Protein content

Bradyrhizobium strains had no significant effect on protein content of mungbean when *Bradyrhizobium* inoculants were used (Table 4.2). The highest protein content (21.3%) and yield (301 kg ha^{-1}) was observed in BARI RVr-404 inoculant treated crop and the lowest protein content and yield was noted in non-inoculated control. Bhuiyan *et al.* (2007b) found that inoculation significantly increased the protein yield in mungbean.

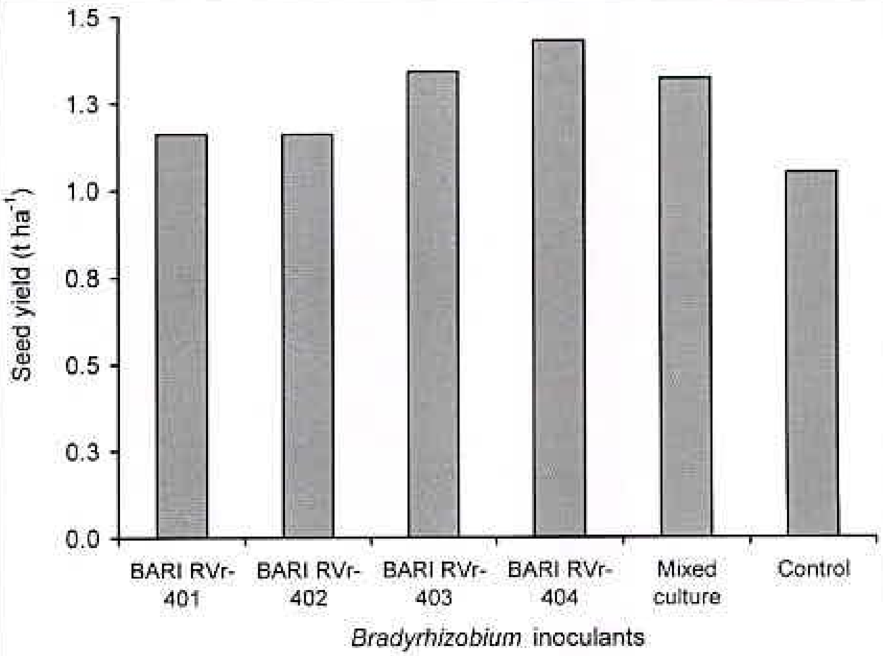

Fig. 4.11. Effect of bradyrhizobial inoculants on seed yield of mungbean

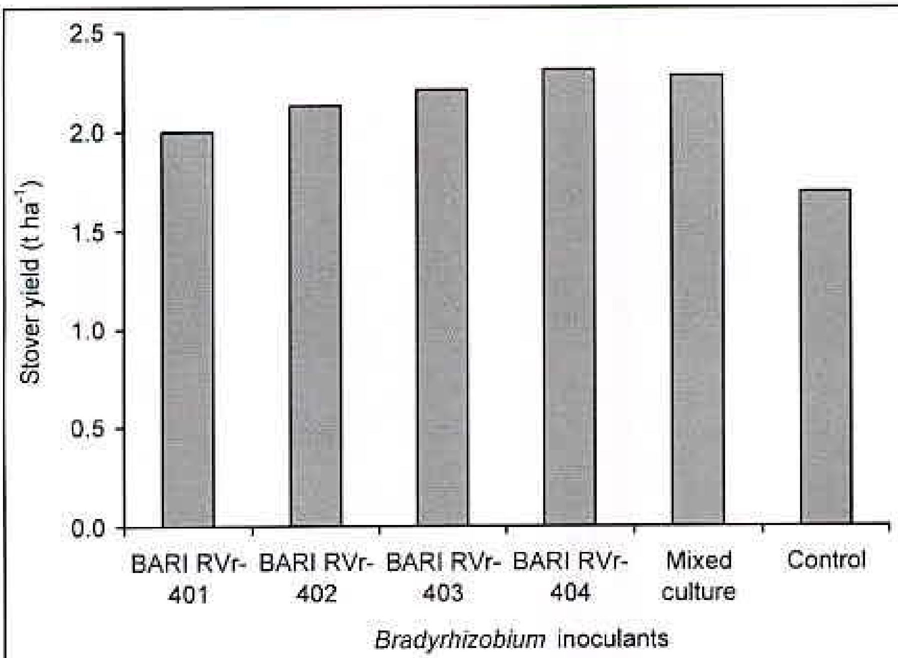

Fig. 4.12. Effect of bradyrhizobial inoculants on stover yield of mungbean

Table 4.2. Effects of rhizobial inoculant on N content in seed and stover, and protein content in seed of mungbean

Rhizobium strains	N content in seed (%)	N content in stover (%)	Protein content in seed (%)
BARI RVr-401	3.35	1.53	20.9
BARI RVr-402	3.34	1.54	20.9
BARI RVr-403	3.36	1.53	21.0
BARI RVr-404	3.36	1.55	21.3
Mixed culture	3.35	1.54	20.9
Control	3.30	1.51	20.7
LSD (0.05)	NS	NS	NS
CV (%)	2.8	4.4	2.7

NS = Not Significant

4.15 Correlation

Correlation matrix among the plant characters of mungbean has been shown in Table 4.3. Most of the plant characters were strongly correlated among themselves. In the present study, nodule number had positive and significant correlation with nodule weight, root weight, shoot weight, plant height, seed yield, stover yield, N uptake by seed and stover, and protein content. Nodule weight had also positive and significant correlation with root weight, shoot weight, plant height, seed yield, stover yield, N uptake by seed and stover, and protein content; root weight with shoot weight, plant height, seed yield, stover yield, N uptake and protein content; shoot weight with plant height, seed yield, N uptake and protein content; plant height with seed yield, N uptake and protein content; seed yield with stover yield, N uptake and protein content; stover yield with N uptake and protein content; N uptake with protein content. Sarker *et al.* (2002) reported that correlation coefficient studies showed high correlation between seed yield and test weight, shoot length and shoot fresh weight. Root length and root volume were inversely correlated with test weight. These results confirmed the findings of Khanam (2002). They observed positive and significant correlation of nodule number with nodule weight, root weight, and shoot weight of inoculated chickpea and soybean. Solaiman (1999) found positive correlation among mungbean growth, N uptake and yield parameters.

Table 4.3. Correlation matrix of different parameters of mungbean

Parameters	Correlation coefficient (r value)								
	Nodule weight	Root weight	Shoot weight	Plant height	Seed yield	Stover yield	N content in seed	N content in stover	Protein content in seed
Nodule number	0.899**	0.910**	0.891**	0.834**	0.883**	0.908**	0.894**	0.779**	0.772**
Nodule weight	-	0.901**	0893**	0.950**	0.910**	0.952**	0.919**	0.835**	0.773**
Root weight	-	-	0.899**	0.892**	0.865**	0.891**	0.897**	0.761**	0.777**
Shoot weight	-	-	-	0.842**	0.850**	0.846**	0.873**	0.745**	0.847**
Plant height	-	-	-	-	0.887**	0.942**	0.878**	0.883**	0.749**
Seed yield	-	-	-	-	-	0.912**	0.993**	0.851**	0.861**
Stover yield	-	-	-	-	-	-	0.904**	0.905**	0.720**
N content in seed	-	-	-	-	-	-	-	0.846**	0.868**
N content in stover	-	-	-	-	-	-	-	-	0.704**

** Significant at 1% level

CHAPTER 5

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

A field experiment was conducted at Soil Science Field of Bangladesh Agricultural Research Institute, Joydebpur, Gazipur using Madhupur Tract (Chiatta series) during the period of March to June 2007 to evaluate the performance of four *Bradyhizobium* inoculants as regard to nodulation, growth, nitrogen uptake and yield of BARI Mung-5 following RCBD technique with four replication. The unit plot size was 4 m x 3 m. The total number of unit plot was 24. In the present study the bacterial fertilizers *Bradyrhizobium* strains (BARI RVr-401, BARI RVr-402, BARI RVr-403, BARI RVr-404, mixed culture) and one non-inoculated control were used to compare their effects on mungbean. Triple super phosphate (22 kg P ha^{-1}), muriate of potash (42 kg K ha^{-1}), gypsum (20 kg S ha^{-1}), zinc sulphate (5 kg Zn ha^{-1}) and boric acid (1 kg B ha^{-1}) were applied as basal dose at the time of land preparation. Mungbean seeds were inoculated with respective inoculants and were sown on 8 March, 2007. The spacing was maintained 30 cm x 10 cm and seeds were sown in furrows. Necessary moisture was maintained to the plot for proper growth.

Data for nodule number, nodule weight, root dry weight, shoot dry weight and shoot length were recorded at two times of growth viz. 35 and 50 DAS, and for seed and stover and other yield attributing data characters were taken at harvest. Nitrogen content and protein content data were taken after chemical analysis of plant and seed samples.

Abridged account of the different plant characters were analyzed statistically and the significant mean differences were adjusted by DMRT.

Inoculation of mungbean with *Bradyrhizobium* strains produced higher number of nodules both at 35 and 50 DAS. The maximum number of nodules was produced by BARI RVr-404 strain both at 35 DAS (8.01 nodule plant^{-1}) and 50 DAS (16.11 nodule plant^{-1}). The effect of inoculation with *Bradyrhizobium* cultures were also significantly superior to non-inoculated control and gave significant results with BARI RVr-404.

Effect of *Bradyrhizobium* inoculation on dry weight of nodule was found significant both at 35 and 50 DAS. The highest nodule dry weight (9.99 mg plant^{-1}) was produced by BARI RVr-404 treatment which was 29.9% higher over non-inoculated control at 35 DAS and the highest nodule dry weight (16.35 mg plant^{-1}) at 50 DAS which was 44.5% higher volume over control. Other cultures were also found significantly superior to non-inoculated control.

Bradyrhizobium inoculation had no significant effect on shoot length. Although maximum shoot length (29.3 cm at 35 DAS) and (39.4 cm at 50 DAS) was recorded in BARI RVr-404 treatment but the result was not statistically significant.

There was no statistically significant effect of *Bradyrhizobium* inoculation on root dry weight. But maximum root weight was observed in BARI RVr-404 treatment both at 35 and 50 DAS.

The effect of *Bradyrhizobium* inoculation on shoot weight was statistically significant at 35 DAS but non-significant at 50 DAS stage. It was fact that the highest shoot weight (1.20 g plant^{-1} at 35 DAS and 2.11 g plant^{-1} at 50 DAS) of mungbean was observed in inoculant treatment.

Inoculation of seeds of mungbean with *Bradyrhizobium* inoculation had also significant effect on stover yield of mungbean. The maximum stover yield (2.31 t ha^{-1}) was recorded by BARI RVr-404 culture treatment which was statistically identical to rest of strains. BARI RVr-404 inoculated treatment produced 2.31 t ha^{-1} stover yield which was 26.4% higher over non-inoculated control.

The effect of *Bradyrhizobium* inouclation on N content in seed and stover was non-significant but the highest N content and N uptake were recorded in BARI RVr-404 inoculant treatment which was not statistically significant.

Inoculation of *Bradyrhizobium* had no significant effect on protein content in seed but the highest protein content in seed was recorded in BARI RVr-404 inoculant treatment.

Bradyrhizobium inoculation had significant effect on N uptake by seed. The highest N uptake (48.1 kg ha^{-1}) was observed in BARI RVR-404 strain inoculated mungbean crop which was statistically different from non-inoculated control.

The effect of inoculation of *Bradyrhizobium* with mungbean had significant effect on N uptake by stover. The highest N uptake (35.9 kg ha^{-1}) was recorded in the inoculation of BARI RVr-404 strain and it was statistically identical to BARI RVr-403 and mixed culture treatments but significantly different from all other treatments.

The analysis of variance showed that *Bradyrhizobium* inoculation had significant effect on total N uptake by seed and stover. The highest N uptake by seed and stover was recorded (83.9 kg ha^{-1}) in the inoculation of BARI RVr-404 strain in mungbean seed which was statistically identical to rest of strains and mixed culture.

Bradyrhizobium inoculation had no significant effect on protein content of munbean seeds though the highest protein content was recorded in the inoculation of BARI RVr-404 strain in mungbean seed and the lowest was in non-inoculated control.

From the above findings, it can be concluded that all the four *Bradyrhizobium* strians and their mixture were effective in terms of nodulation, dry matter production, seed and stover yield compared to non-inoculated control. Among the strains, the performance BARI RVr-404 was better than the other strains under the study. However, the results obtained need to be substantiated through trials in various regions in the country, and the strain may be recommended for use in the farmers' field.

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App. Table 4.1. Effects of bradyrhizobial inoculants on nodulation and dry matter production of mungbean

Bradyrhizobium strains	35 DAS					50 DAS				
	Nodule number $plant^{-1}$	Nodule weight (mg $plant^{-1}$)	Root weight (g $plant^{-1}$)	Shoot weight (g $plant^{-1}$)	Shoot length (cm)	Nodule number $plant^{-1}$	Nodule weight (mg $plant^{-1}$)	Root weight (g $plant^{-1}$)	Shoot weight (g $plant^{-1}$)	Shoot length (cm)
BARI RVr-401	7.59a	9.00a	0.24	1.00ab	27.2	10.10ab	12.59ab	0.30	1.97	35.0
BARI RVr-402	7.60a	9.05a	0.25	1.03ab	27.2	10.93a	14.31a	0.30	1.95	36.6
BARI RVr-403	7.99a	9.21a	0.26	1.19a	29.1	11.49a	15.78a	0.33	2.02	37.7
BARI RVr-404	8.21a	9.99a	0.27	1.20a	29.3	12.87a	16.35a	0.34	2.11	39.4
Mixed culture	8.00a	9.20a	0.26	1.19a	29.2	12.00a	16.21a	0.32	2.04	36.8
Control	5.21b	7.00b	0.22	0.91b	25.0	7.25b	9.00b	0.24	1.68	34.0
LSD (0.05)	1.44	1.77	NS	0.20	NS	3.32	3.76	NS	NS	NS
CV (%)	12.8	13.2	13.1	12.2	8.70	20.5	17.8	14.40	12.6	7.50

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
NS = Not Significant.

App. Table 4.2. Effects of bradyrhizobial inoculants on seed and stover yield of mungbean

Bradyrhizobium strains	Seed yield (t ha^{-1})	Stover yield (t ha^{-1})
BARI RVr-401	1.16bc	2.00a
BARI RVr-402	1.16bc	2.13a
ARI RVr-403	1.34ab	2.21a
RI RVr-404	1.43a	2.31a
ixed culture	1.32ab	2.28a
ntrol	1.05c	1.70b
LSD (0.05)	0.20	0.29
CV (%)	10.9	9.0

lumn, means followed by a common letter are not significantly different at the 5%
RT