STUDIES ON ISOLATION AND CHARACTERIZATION OF *Rhizobium* and NODULATION PATTERN IN HORSEGRAM (*Dolichos biflorus* L.)

BY

RAVINDRA TATYARAM GAIKWAD
REGD. NO. 93113

A THESIS SUBMITTED TO THE

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722. DIST. AHMEDNAGAR.
MAHARASHTRA STATE (INDIA)**

IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE

OF

MASTER OF SCIENCE (AGRICULTURE)

IN

AGRICULTURAL MICROBIOLOGY

DEPARTMENT OF PLANT PATHOLOGY
AND AGRICULTURAL MICROBIOLOGY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI-413 722. DIST. AHMEDNAGAR.
(MAHARASHTRA STATE) INDIA.

1997

STUDIES ON ISOLATION AND CHARACTERIZATION OF *Rhizobium* AND NODULATION PATTERN IN HORSEGRAM (*Dolichos biflorus* L.)

By

RAVINDRA TATYARAM GAIKWAD
Reg. No. 93113

[redacted]esis submitted to the

HATMA PHULE KRISHI VIDYAPEETH,
HURI-413 722, DIST. AHMEDNAGAR
MAHARASHTRA STATE (INDIA)

[redacted] fulfilment of the requirements for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

In

AGRICULTURAL MICROBIOLOGY

Approved by

(Dr. P.V. Wani)
Chairman and Research Guide

(Prof. D.V. Indi)
Committee Member

(Dr. K.R. Sonar)
Committee Member

(Dr. R.P. Aher)
Committee Member

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY,
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
POST GRADUATE INSTITUTE, RAHURI.
DIST. AHMEDNAGAR [MAHARASHTRA]

1997

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part thereof, has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma

Place : Rahuri. (Ravindra Tatyaram Gaikwad)

Date : 29 / 7 /1997

Dr. P.V. Wani
M.Sc. (Agri.), Ph.D.
Asstt. Professor of Agril. Microbiology,
Department of Plant Pathology and
Agricultural Microbiology,
Mahatma Phule Krishi Vidyapeeth,
Rahuri-413 722. Dist. Ahmednagar.
(Maharashtra State) India.

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON ISOLATION AND CHARACTERIZATION OF *Rhizobium* AND NODULATION PATTERN IN HORSEGRAM (*Dolichos biflorus* L.)", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (MS) India, for the award of the degree of MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL MICROBIOLOGY, embodies the results of the piece of *bona fide* research work carried out by Shri. RAVINDRA TATYARAM GAIKWAD, under my guidance and supervision and that no part of the thesis has been submitted for any other degree, diploma or publication in any other form.

The assistance and help received during the course of these investigation have been duly acknowledged.

(P.V. Wani)
Research Guide

Place : MPKV, Rahuri

Dated : 19/7/1997

Dr. V.M. Pawar.
Associate Dean,
Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri-413722. Dist. Ahmednagar.
Maharashtra (India)

CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON ISOLATION AND CHARACTERIZATION OF *Rhizobium* AND NODULATION PATTERN IN HORSEGRAM (*Dolichos biflorus* L.)", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (MS) India, for the award of the degree of MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL MICROBIOLOGY, embodies the results of the piece of *bona fide* research work carried out by Shri. RAVINDRA TATYARAM GAIKWAD, under the guidance and supervision of Dr. P.V. Wani, Asstt. Professor of Agricultural Microbiology, M.P.K.V., Rahuri and that no part of the thesis has been submitted for any other degree, diploma or publication in any other form.

Pawar

(V.M. Pawar)

Place : MPKV, Rahuri

Dated : 24/7/1997

ACKNOWLEDGEMENTS

Words are inadequate to express my feelings of gratitude towards my Research Guide Dr. P.V. Wani, Assistant Professor, Department of Plant Pathology and Agril. Microbiology for his scintillating, dynamic guidance, abounding cooperation, constant inspiration and finally withgoing through the draft manuscript of this investigation, he was to me the "Prime Mover" throughout the course of investigation.

I feel honoured to extend my grateful thanks to Prof. D.V. Indi, Prof. R.P. Aher and Dr. K.R. Sonar, the Members of my Advisory Committee for their valuable suggestions and kind cooperation in the conduct of the research work.

It gives me immense pleasure to acknowledge the able guidance of Dr. D.M. Sawant, Head, Department of Plant Pathology and Agril. Microbiology for his timely suggestions and helpful comments.

My special thanks and intellectual indebtedness are extended to Dr. R.V. Wuike who took keen interest in my research work and made constructive suggestions during the course of investigation.

I am thankful to all the staff members of this department S/Shri. Desai N.B., Kumbhar K.D., Kokare F.N., Narsale, Lahare, Pandhare, Pathare, Lagad and Sau. Tadamalbai

for their unstincted help rendered from time to time during the course of investigation.

I wish to express my sincere thanks to my colleagues and friends Nandu, Aju, Kailu, Piya, Prakash, Rajesatish, Kishor, Suresh, Ramu, Balu and other members of Foujdar loby for their valuable cooperation and help during the course this study.

Last but not least, words are small trophies to express my deep sense of love and affection to my loving parents Sau. Aai and Shri. Anna, brothers Rajesh and Nanda-kishor and my wife Sau. Bharati who are bright light for me in dark night and source of constant inspiration with which I am stepping up my career. Their contribution beyond acknowledge-ment.

I have no words to express my heartiest gratitude to my grandfather Bapusaheb and grandmother Aaisaheb and father-in-law Mamasaheb and mother-in-law Mami, brother-in-law Narendra and Devendra.

I am also grateful to Shri. Mukund S. Khopkar for his willing cooperation in neat and tidy typing of this manu-script.

I am deeply obliged to all the authors past and present whose contribution were a great help to undertake this

investigation.

During the completion of this course many hands pushed me forth, lips put elixin in my heart, learned heart put me on a right path, lightened by their knowledge and experience. I ever rest thankfully indebted to them all.

(Ravi)

CONTENTS

Contents Contd ....

Contents Contd ....

LIST OF TABLES

Contents Contd ....

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

cv.	= Cultivar
var.	= Variety
%	= Per cent
°C	= Degree centigrade
mg	= Milligram(s)
g	= Gram(s)
kg	= Kilogram(s)
ha	= Hectare(s)
viz.	= Namely
Fig.	= Figure
S.E.	= Standard Error
C.D.	= Critical Difference

ABSTRACT

STUDIES ON ISOLATION AND CHARACTERIZATION OF Rhizobium AND NODULATION PATTERN IN HORSEGRAM

By

RAVINDRA TATYARAM GAIKWAD

Post Graduate Institute,
Mahatma Phule Krishi Vidyapeeth,
Rahuri - 413 722

Research Guide : Dr. P.V. Wani
Department : Agricultural Microbiology

Six isolates of Rhizobium were obtained from horsegram varieties and these were studied for various morphological features. All the isolates were Gram negative, non-acid fast, non-spore formers and rod shaped measuring 2.30 to 3.24 µm in length and 0.63 to 0.96 µm in width and were encapsulated. Nodulation test for all the isolates using Leonard jar assembly recorded abundant nodulation in horsegram and confirmed that these isolates were of Rhizobium specific for horsegram. A pot culture experiment in sterile soil was conducted in a Factorial Completely Randomized Design with three replications. The inoculation treatments included rhizobial isolates viz., RS-1, RS-2, RS-3, RS-4, RS-5 and RS-6 and uninoculated control. The horsegram varieties viz., Sina, Man, TPK, HPK, Phule K-1 and KS-2 were used for the study. The observations on seed germination, nodulation (number, colour and dry weight) plant dry matter weight, N uptake and grain yield were recorded.

The results, in general, revealed that plants of horsegram varieties inoculated with different Rhizobium isolates recorded significant improvement in nodule number, nodule dry weight, plant dry matter weight, N uptake and grain yield over uninoculated control indicating that these isolates could effectively be used as inoculants for horsegram. There was an increase in number of total nodules at 30, 60 and upto 90 days after sowing, while at harvest the total number of pink and large sized nodules showed a decline. Rhizobium inoculants recorded grain yield of 2.89 (RS-1) to 3.41 g (RS-4) per plant as against 1.84 g per plant in uninoculated control. Amongst the Rhizobium isolates RS-4, RS-3 and RS-6 recorded significantly maximum improvement in production of large size and pink nodules, nodule dry weight, plant dry weight, N uptake and grain yield of horsegram varieties. Next to these isolates, RS-2, RS-5 and RS-1 also recorded significant improvement in nodulation, plant dry weight, N uptake and grain yield of horsegram varieties over uninoculated control. The results, in general, indicated that all the rhizobial isolates could effectively nodulate and gave significant increase in the yield of horsegram varieties over uninoculated control.

The results also indicated that the horsegram varieties responded differentially to inoculation with different isolates of Rhizobium. Differences in germination

percentage, production of large size and pink nodules, nodule dry weight, plant dry weight, N uptake by plant and grain yield were recorded amongst all six varieties of horsegram. All the varieties of horsegram viz., Sina, Man, TPK, HPK, Phule K-1 and KS-2 showed improvement in plant dry weight, N uptake and grain yield over uninoculated control. The response of varieties to inoculation with Rhizobium seemed to be genotype dependent.

The interactions of all the varieties with rhizobial inoculants recorded increased plant dry matter weight, N uptake and grain yield as compared to all the interaction of varieties with uninoculated control.

Considering all the traits studied in the present investigation it appears that all the rhizobial isolates could effectively nodulate and increase N uptake, dry matter weight and grain yield of horsegram varieties.

Introduction

and Madhya Pradesh. It occupies an area of about 2 million hectares and the annual production is about 8 lakh tonnes. It's mainly cultivated as feed and fodder for cattle. its consumption in North India is almost negligible. Kulthi can be cultivated on a wide range of soils except highly alkaline soils. It can be raised as a dry crop under low rainfall conditions. In Karnataka and Andhra Pradesh, it is sown in July and harvested in October. It is cultivated as a pure crop or mixed crop with jowar, bajra, arhar, niger etc.

In Maharashtra research on this crop is almost neglected. Inoculation of the seeds of pulses with an effective Rhizobium strain before sowing has become one of the important packages of practices in pulse crop production. Host-Rhizobium interaction is one of the most important factors in the success of symbiotic relationship. This aspect is receiving more and more attention now-a-days.

It has been proved beyond doubt that Rhizobium seed inoculation to seeds of leguminous crops enhance the yield by 15 to 20 per cent increase over the uninoculated control plants and also enhances the protein content of grain through the symbiotic nitrogen fixation by the rhizobia.

Thus the role of Rhizobium in improving the yield of leguminous crops and enriching the soil fertility has been well established. There is very little work carried out on

horsegram-*Rhizobium* symbiosis. The present investigation was, therefore, planned with the following objectives :

1. To isolate and characterize *Rhizobium* from different cultivars of horse gram.

2. To see the nodulation of different promising cultivars of horse gram using effective isolates *in vitro* and *in vivo*.

Review of Literature

2. REVIEW OF LITERATURE

Inoculation of Rhizobium to legume seeds is reported to increase nodulation, dry matter and grain yield and nitrogen uptake in various legume crops and now a days it is a general practice followed. The work on Rhizobium isolation and inoculation in horse gram is scanty. Therefore, in this chapter attempt is made to review the research work done on Rhizobium inoculation on growth, nodulation, nutrient uptake and yields of various legume crops.

2.1 Effect of Rhizobium inoculation on nodulation pattern and nodule dry weight

The importance of inoculation of legumes with cultures of root nodule bacteria was recognised in early 20^{th} century (Thornton, 1929) and since then the practice has been followed more extensively. It has become evident that all the strains or isolates of any species of the Rhizobium are not alike in their ability to benefit the host plant.

Stevans (1925) observed that the strains when used for inoculation of seeds of different varieties of same host varied in their efficiency.

Pohlman et al. (1929) tested 12 strains of Rhizobium japonicum of six varieties of soyabean and found that some strains gave good nodulation, some gave poor nodulation and one strain completely failed to nodulate any of the six varieties.

Allen and Allen (1939) observed variation in effectiveness of rhizobia on different plants belonging to cowpea group.

Linta (1963) reported that active strains of rhizobia not only produced greater number of nodules of larger size and fixed higher amount of nitrogen than less active strain did, but also proportionately enhanced the protein contents.

Jensen (1964) observed the host specificity of Rhizobium melioti, R. trifoli and R. leguminosarum. He suggested that ability of host to nodulate normal host plant may affect ecological factors in maintaining their survival in soil where, these host plant do not grow.

Visuttipitakul (1970) reported that inoculation produced a greater number of nodules (115 and 444 nodules per plant) at 60^{th} and 120^{th} days than inoculation with the locally produced inoculant (44.3% at the harvest, 20 to 29 nodules per plant at 60^{th} and 120^{th} days). The local inoculant showed no beneficial effect as compared with the control.

Chomchalow (1970) reported that Rhizobium inoculation significantly increased the dry matter yield, plant height, number of nodules and N uptake after 3, 6, 9 and 12 weeks of growth in pea nut.

Balasundaram et al. (1972) tested eight isolates of R. japonicum with six varieties of soyabean for their ability to nodulate and increase in the dry matter yield. They reported that the isolates differed in their performance with different varieties.

Prasad (1972) studied the biology of root nodule of Phaseolus mungo and Phaseolus aureus and described that the first sign of nodulation was observed on second day of seedling emergence. The maximum nodulation was observed at flowering stage and suggest best period of nitrogen fixation. The degeneration of root nodule were observed after flowering.

Morales and Graham (1973) reported that Phaseolus vulgaris, Stylosanthes guyanansis, Vigna sinensis and Leucaena leucocephala responded significantly to inoculation. Nodulation did not occur in plants grown from uninoculated seeds and their survival was very low which indicated that Leucaena needed inoculation.

Iswaran and Adwjar (1974) observed that the inoculation of Rhizobium leguminosarum to pea crop significantly increased nodule yield over uninoculated control.

Rao (1981) studied the effect of rhizobial inoculation on groundnut cultivar TMV-2 and reported that the seed inoculation of groundnut was an economic method of increasing nodulation and production.

Patil and Moniz (1974) studied the differential response of five varieties of gram viz., N-31, N-59, Chaffa, Early Gulab and Local against the efficient Rhizobium culture and reported that in general, inoculation of gram gave good nodulation and high dry matter production.

Chowdhary et al. (1974) observed that the root dry weight and number of nodules per plant of lentil increased due to inoculation of Rhizobium culture.

Dart et al. (1975) studied the effectiveness of fourteen Rhizobium isolates from cowpea under glass house conditions. After the eight week of growth, there were differences in nodulation and plant dry matter production between strains. There was good correlation between nodule tissue production and plant growth. There were differences in response of the different hosts (cowpea, sirato, pigeonpea and pueraric) to various strains. Thus, the strain very effective on cowpea may be only moderately effective with pigeonpea. Two or three strains performed well on all the hosts.

Kumar Rao and Dart (1975) tested 260 strains of Rhizobium of pigeonpea. Thirteen strains were very effective on pigeonpea. However, only four strains gave a significant response to inoculation in field trial for nodulation.

Singh (1977) reported that the inoculated plants of mung (Vigna radiata) nodulated profusely during normal period

of branching and flower primordial initiation. The yield contributing characters have been increased 51 per cent due to inoculation over control.

Bapat et al. (1977) reported the response of Bengal gram genotypes to Rhizobium inoculation and reported that they did not observe differences in nodule number and their weight. Nodulation study made at flowering stage gave significant correlation between nodule and shoot weight and also nodule number and shoot weight.

Gangawane and Datar (1977) inoculated seeds of lucerne, groundnut, cowpea, kulthi (Macrotyloma uniflorum) and bengal gram (C. arietinum) with mixture of 7 rhizobial strains or by individual strain including three lucerne strains and found that, inoculation increased nodulation, plant dry matter and nitrogen content in all treatments except composite inoculum on cowpea.

Pawar and Ghulghule (1977) reported that Rhizobium inoculation increased the nodulation, 'N' content of shoot and root, dry matter weight of root and shoot of mung.

Pawar et al. (1977) reported more dry weight of nodules in inoculated plants than in the uninoculated plants of two varieties of cowpea.

Bagyaraj and Hegde (1978) also reported that

inoculated of cowpea seeds with Rhizobium resulted in increased nodulation over control. No correlation between the number of nodules per plant and grain yield could be observed.

Khurana et al. (1978) observed direct correlation between nodule dry weigh and N content while, other correlations were non-significant. The increased nodulation ultimately helped in improving soil fertility by fixing more amount of atmospheric nitrogen in cluster bean.

Islam (1979) studied the differential response of two chickpea cultivars with eight Rhizobium strains and reported increased number of nodules per plant from 1 to 18 except the two strains UNR-1 and IC-13 which produced only three nodules per plant. inoculation of seed of cultivar Syrian Local with Rhizobium strain CC-1192 and cultivar NCE-2304 with strain IC-20 gave the highest nodule weight.

Kenjale (1979) found that inoculation of seeds with Rhizobium culture resulted into increased nodulation count, nodule dry matter weight over uninoculated control in 15 varieties of gram (C. arietinum L.).

Idris and Sandhu (1979) observed that significant increase in the number of nodules per plant and fresh nodule weight by inoculation of mung with Rhizobium over uninoculated control.

Koteswara Rao (1979) studied the effect of Rhizobium inoculation under graded levels of phosphorus application on nodulation in groundnut variety Asyria and observed significant increase in the nodule number on tap root at all the stages of crop growth except at 60^{th} days.

Reddy and Tanner (1980) reported that the rhizobial inoculant application increased nodulation in groundnut.

Balasubramanian et al. (1980) studied the field performance of the rhizobial inoculants viz., GG-2, GM-BS-1, BM-BS-P-47 and an equal mixture of these strains (multistrain inoculant) in green gram at Coimbatore and Kaveri Pattiwar under rainfed condition and Bhavanisagar under irrigated condition. The nodulation, yield and nitrogen assimilation responses of green gram were observed. At all three locations the multistrain rhizobial inoculants performed better with significance. They produce more number of nodules per plant than the uninoculated control and the single strain rhizobial inoculants.

Patil and Shinde (1980) reported that inoculation of gram with Rhizobium resulted in increased nodulation, more nodule dry weight in all the varieties over uninoculated control.


Vora and Desai (1980) conducted a field experiment by inoculating three Rhizobium cultures to four chickpea

cultivars at Dohad in Gujarat State and fond that inoculation with strain F-75 was the most effective in increasing nodulation in cultivar BG-203, Chaffa and Dohad yellow, followed inoculation with strain H-45 in cultivar N-208. Inoculation with strain H-45 was slightly superior to inoculation with strain F-75 for yield but not for nodulation.

Leonard (1980) reported that the effective strains produced very few but large nodules which were usually located on the upper part of root system whereas ineffective strains produced numerous small nodules widely distributed all over the root system.

Basak and Goyal (1980) reported that the inoculation of Vigna radiata and V. mungo seeds with Rhizobium strain from some tree legumes increased the nodulation and seed yield compared with inoculation with the specific Rhizobium strains and uninoculated control.

Bhore et al. (1980) reported that the nodulation of french bean was increased due to Rhizobium inoculation. The improved varieties "Contender" and "Pusa Parvati" responded better than local Waghya.

El-Said et al. (1981) reported that the inoculation with Rhizobium sp. increased the nodule number, dry weight of plant and "N" percentage in pea plants.

Ghag (1981) found that inoculation of seeds with Rhizobium culture resulted into increased nodulation count, nodule dry matter weight over uninoculated control in 5 promising varieties of gram (L. arietinum).

Singaravadivel and Prasad (1981) conducted pot trial and field experiment on groundnut inoculated with Rhizobium and observed that the nodule number and total N content were upto double that of the untreated control both in pot and field trial.

Khating and Ghonsikar (1981) studied the inoculation of gram with five Rhizobium strains and reported that nodulation was the greatest with strain Ca-7 while, seed yield and N-uptake were highest with strain PBN.

McNeil et al. (1981) reported that seed inoculation markedly increased the nodulation of plants of chickpea cultivar Burpee-5024 at 65 days after sowing in Hawai.

Singh and Misra (1981) conducted a field experiment by inoculating four Rhizobium strains with three varieties of gram and concluded that seed inoculation with strain F-75 significantly increased the number and weight of nodules per plant compared to inoculation with three other strains. The number and weight of nodule per plant of cultivar T-3 were higher than those of two other cultivars but the yield differences were not significant.

More et al. (1981) conducted a field experiment to study the effect of different commercial inoculants of Rhizobium under different levels of nitrogen on nodulation in groundnut. They observed a beneficial effect of seed inoculation with Rhizobium over the uninoculated control in respect of dry weight of nodules and nitrogen content in root and shoot.

Raut and Ghonsikar (1982) reported that seed inoculation with Rhizobium strain No. 24 increased the nodulation of four chickpea cultivars. Interaction between cultivar and inoculation was not significant. He also reported 30 per cent increase in nodule number and 65 per cent increased in fresh nodule weight and 22 per cent total N in grain due to Rhizobium inoculation.

Venkateswarulu et al. (1982) evaluated the symbiotic performance of four rhizobial strains on guar (FS-277, Durgapur Safed, B-19-1-55 and HFG-182) in sandy loam soil and observed increase in nodulation, dry matter production and nitrogenous activity. The number of nodules per plants varied between 30.4 and 32.9, nodule dry weight between 40.2 and 44.4 mg and dry weight of plant tops between 1.7 and 1.8 g.

Dahiya et al. (1983) reported that seed inoculation of pigeonpea with Rhizobium strain showed that the highest increase in nodule number, dry weight of nodule and nitrogen content of the plant.

Vaishya et al. (1983) reported that seed inoculation with Rhizobium strain M_1 significantly increased the number and weight of nodules in mung bean.

Vidaysurian et al. (1983) studied the response of mung bean (Vigna mungo L. Heppar) to fertilizer application and rhizobial inoculation and reported that nodulation was maximum and significantly higher with rhizobial inoculation and basal phosphate application.

Kremor and Peterson (1983) suggested that the grain-legume production and dinitrogen fixation was increased due to inoculation of Rhizobium, when the nodulation, plant growth and yield were determined. It showed that improved inoculant produce high number of nodules at flowering and increased yields and N-fixation of bean and cowpea.

Prasad and Ram (1984) reported in a field trial with three V. radiata cultivars and found that seed inoculation with Rhizobium phaseoli strains increased nodulation due to inoculation.

Raju and Verma (1984) studied the response of green grown (V. radiata) to rhizobial inoculation in relation to fertilizer nitrogen and reported that seed inoculation and or application of 15-60 kg N/ha significantly increased the nodulation in V. radiata.

Kulkarni *et al*. (1984) conducted field experiment during rainy season of 1981 was reported that inoculation of Robut-331 cultivar of groundnut with NC-92 *Rhizobium* resulted in an increase in nodulation.

 Sawashe and Patil (1984) inoculated chickpea cultivar Chaffa with 7 strains of *Rhizobium* and found that there was increase in nodule number and dry weight of nodule when inoculated with strain 5, 6 and 7.

 Mane (1985) found that inoculation of seed with *Rhizobium* culture resulted into increased nodule number, nodule dry weight over uninoculated control in all the varieties of gram (*C. arietinum* L.) under trial.

 Giller *et al*. (1985) conducted an experiment with ineffective *Rhizobium* strain IC-2094 and two chickpea varieties *viz*., K-850 and G-130. They observed that strain IC-2094 produced double the number of nodules on variety K-850 than the variety G-130.

Hungria and Neves (1986) conducted an experiment and studied the interaction of *Rhizobium phaseoli* and french bean (*P. vulgaris* L.). He reported that the dry weight and total N content of leaves, stem, pods and nodule efficiency was increased.

 Tellawi *et al*. (1986) inoculated chickpea genotypes with 3 different *Rhizobium* strains and observed significant increase in nodulation and N-uptake.

Basu et al. (1989) showed that seed inoculation of mung bean with Rhizobium strain JCa-1 and M-10, increased nodulation over uninoculated control.

Bhuiya et al. (1989) conducted a field trial with six rhizobial strains on T-9 variety of black gram and reported the significant beneficial effect on nodulation. The strain BAU-510 appeared to be the best in respect of all the parameters studied.

Namdeo et al. (1989) reported that inoculation of gram with Rhizobium strain increased the number and dry weight of nodules per plant.

Sairam et al. (1989) reported that phosphorus application at the rate of 90 kg/ha and inoculation with Rhizobium culture resulted in improvement in nodulation and physiological activity of nodules in cowpea as indicated by increase in leghaemoglobin content, nitrogen fixation and total nitrogen uptake plus increase in available soil nitrogen and total dry matter production.

Gandhi and Godbole (1990) studied the effect of 17 fast growing rhizobial strains (isolated from wild legumes) on a cultivated legumes V. unguiculata. The nitrogenase activity and the statistical analysis of pooled data of the experiment revealed that all fast growing rhizobial strains effectively nodulated V. unguiculata and gave significant increase in yield over uninoculated control.

Namdeo and Gupta (1990) conducted a field experiment with 8 authentic Rhizobium strains and JA-3 variety of pigeonpea. The inoculation with all the 8 Rhizobium strains resulted in a significantly higher nodule number per plant (2 to 3 fold) than the uninoculated control. The strain IC-3195 and CT-2014, however, performed better than others. All the eight Rhizobium strains produced a significantly higher nodule dry weight per plant. Nodule number and nodule dry weight exhibited a positive correlation with grain yield.

Nagre et al. (1991) studied the effect of Rhizobium inoculation with nitrogen application on nodulation of ground nut and soyabean. At every stage of recording, ground nut had significantly more number of nodules than soyabean. In both the crops nodules were more at 60 days than at 30 or 90 days. In groundnut nodules at 30 days were 80 nodules per plant, which increased to 294 per plant at 90 days. Similarly, in soyabean nodules at 30 days were 24 per plant which increased to 41 at 60 days and reduced to 35 per plant at 90 days. Like nodule number, nodule dry weight per plant in groundnut was significantly more than soyabean at 30, 60 and 90 days of recording.

Prabhakaran and Rangarajan (1992) studied the effect of rhizobial inoculation on nodulation, nitrogen fixation and yield of lablab. The rhizobial seed bacterization was found to enhance nodulation, nitrogen fixation and grain yield of

Phaseolus vulgaris cultivars over their controls. Among the cultivars of lablab CO-9 has recorded better nodulation and grain yield (556.9 per cent) followed by CO-8 cultivars. The interaction between lablab cultivars and rhizobial seed inoculation on nodulation, nitrogen fixation and grain yield at harvest were found significant. However, maximum nodulation was recorded by CO-9 cultivar with RvRp-2 inoculation which recorded 22 nodules per plant.

Tippannavar and Desai (1992) studied the effect of Rhizobium inoculation with cultural practices on Bengal gram production, the data indicated that the number of nodules were maximum in inoculated plants supplied with FYM followed by inoculation with green manure.

Prabhakaran and Srinivasan (1993) conducted a field experiment on performance of horse gram cultivars to rhizobial seed inoculation in acid soils. The cultivars Co-1 and Herbbal-1 were inoculated with three slow growing rhizobial strains VPR-1, VHG-1 and VBS-1 with and without N and they observed that the seed inoculation with rhizobial strain on the cultivar of horse gram recorded significantly an increased plant growth, nodulation and nodule dry weight at 30 and 45 DAS over uninoculated control.

Shaheen and Rahmatullah (1994) in laboratory experiment on groundnut cultivars Banki and ICGS-44, inoculated soil

with Rhizobium strain NC-92 or Ruddy Patric and they found that nodulation did not occur without inoculation. The Ruddy Patric strain significantly increased nodule number and weight compared with NC-92.

Wange and Patil (1996) conducted a field experiment to evolve best cultivar X rhizobial strain combination in respect of nodulation, vegetative growth and yield of tur crop over recommended dose of fertilizer nitrogen. Among the interactions V_2 X R_1 recorded significantly higher number of nodules (20 per plant) and dry weight of nodule (23 mg/plant). The nodule dry weight was significantly increased by strain R_1 (19.9 mg/plant) and cultivar PT-14 (15.5 kg/plant). The results clearly indicated that rhizobial strains R_1 and R_3 performed significantly well in improving nodulation over uninoculated control.

Surendragopal and Shivappashetty (1996) isolated the Rhizobium isolates from root nodules of Sesbania grandiflora (L.) grown in different soil samples. In Leonard jar experiment, the plants inoculated with the isolate SG-5 gave maximum dry weight (1 g/plant), number of nodule (47 per plant) and total nitrogen content (51.80 mg/plant). Similarly pot culture experiment also revealed that plant inoculated with SG-5 isolate gave maximum dry weight (1.51 g/plant) and total nitrogen content (43.34 mg/plant).

2.2 Effect of Rhizobium inoculation on dry matter weight and seed yield of plants

Hoffer (1949) reported 40 per cent enhanced yield of pea due to Rhizobium inoculation.

Yadav et al. (1967) observed that the per hectare production of green pods increased due to rhizobium inoculation over control in french bean. Yield was increased due to inoculation ranging from 2.00 to 32.2 per cent.

Hulmani et al. (1968) reported that the inoculation with Rhizobium culture increased the yield of garden pea (Pisum sativum L.) by 22.74 per cent over control.

Rewari et al. (1973) reported that the yield of soyabean crop was increased due to the inoculation of Rhizobium culture.

Richter (1974) conducted a pot culture trial to study the effect of nodulation in pea and reported that there was a highly significant positive correlation between the number of nodules and the total plant dry matter yield.

Patil and Moniz (1974) reported that the seed of five cultivar of groundnut (Arachis hypogene) and gram (Cicer arietinum) when inoculated with Rhizobium strains isolated from Cicer arietinum (cultivar N-59) produced profuse nodules in cultivar N-31 and N-59. They further stated that plant dry

matter weight and nitrogen recovery were greater in N-31 than in the other cultivars tested. He also studied the differential response of five varieties of gram against the efficient Rhizobium culture and reported 63.8 to 134 per cent increase in yield over control.

Bajpai et al. (1974) showed that the use of Rhizobium gave yield increase in crops of berseem, lobia (V. radiata) and groundnut of 74, 46 and 21 per cent, respectively.

Chowdhary et al. (1974) reported that Rhizobium strain significantly increased yield of gram, pea and soyabean.

Habish et al. (1974) reported that the inoculation of haricot bean with local strain of Rhizobium significantly increased seed yield ranging from 20-45 per cent.

Patil and Medhane (1974) recorded the inoculation with different strains of Rhizobium to gram increased the grain yield significantly.

Obliswami et al. (1976) reported that either individual or combination of strains gave better nodulation, dry matter and grain yield of black gram and green gram.

Agnihothrudu and Tripathi (1976) studied the effect of seed inoculation with Rhizobium on the yield of groundnut and bengal gram. Due to uninoculation, the increase in yield over the control was 10-57 per cent. All the treated plots

recorded higher yield than control plots. However, a variation was noted in yield from place to place which could be due to several factors like soil, climate and cultural practices.

Raju and Samuel (1976) reported that inoculation of gram except I.A.R.I culture gave good yield i.e. 63.8 to 134 per cent increase in yield over uninoculated control

Pawar and Ghulghule (1977) reported that inoculation increased the dry matter weight of root and shoot portion in mung.

Bapat et al. (1977) reported that the response of bengal gram genotypes to Rhizobium inoculation, in which significant increase in the seed yield was obtained. The yield responses to inoculation were ranging from negative to positive response upto 74 per cent.

Rai et al. (1977) reported that inoculation of C. arietinum seeds with 8 Rhizobium strains increased seed yields by 13.9 to 39.8 per cent. Strain GF-9 gave the highest yield following by strain GF-2, GF-6, GF-7 and GF-8.

Kenjale (1979) in a field experiment observed that inoculation of Rhizobium to gram increased the dry matter weight of shoot and root per plant in different varieties at different stages and the results were found to be statistically significant.

Idris and Sandhu (1979) reported that Rhizobium inoculation increased dry matter weight of shoot and root per plant of mung by 15.3 to 71.3 per cent and 13 to 42 per cent, respectively.

Islam (1979) studied the differential response of two chickpea cultivars with 8 Rhizobium strains and reported that seed yield increased from 1.25 to 1.51 t/ha in Syrin Local with inoculation with strain IC-26 and from 1.42 to 1.80 t/ha in NCF-2304 with strain Ca-7.

Rai and Singh (1979) studied the inoculation of chickpea cultivar C-235 with 9 strains of Rhizobium sp. and reported that there was no significant correlation between seed yield, number of nodules and dry weight of nodules, but yield was significantly correlated with leghaemoglobin content of nodules. Strain KG-38 gave significantly higher seed yield than the other strains.

Patil and Shinde (1980) in a field experiment observed that inoculation with Rhizobium to 6 chickpea cultivars increased the seed yield by 45 to 63 per cent, the response varied in different cultivars.

Vora and Desai (1980) conducted a field experiment by inoculating three Rhizobium cultures to four chickpea cultivars and found that inoculation with strain P-75 was the most effective in increasing the seed yield in cultivar

BG-203, chaffa and Dohad Yellow, followed by inoculation with strains H-45 in cultivar H-208. Inoculation with strain H-45 was slightly superior to inoculation with strain F-75 for yield but not for nodulation.

Barthakur (1980) reported 10 per cent increase in grain yield of soyabean with an application of Rhizobium culture.

McNeil et al. (1981) reported that seed inoculation markedly increased total dry weight of plants of chickpea cultivar Burpee-5024 at 65 days after sowing.

Bagchi et al. (1981) studied the effect of Rhizobium inoculation on a fodder variety of cowpea, T-2 and found that, there was significant increase in dry yield due to inoculation over control.

Khating and Ghonsikar (1981) reported that seed inoculation of gram seed with 5 Rhizobium strains, resulted in highest seed yield with strain PBN than the other strains.

Raut and Ghonsikar (1982) reported that seed inoculation with Rhizobium strain No. 24 increased the seed yield of four chickpea cultivars. Interaction between cultivar and inoculation was not significant.

Nagre (1982) reported that seed inoculation or application of 10 or 20 kg N per hectare produced similar

significant increase in seed yields of both green gram (Vigna radiata) and black gram (Vigna mungo). There was no significant differences between the two levels of N applied.

Raut and Ghonsikar (1983) reported that seed inoculation with Rhizobium gave significant increase over the control for grain yield per plant in pigeonpea. The interaction between cultivars and inoculation for grain yield was non-significant.

Natarajan and Prabhakaran (1983) conducted a field experiment with two cultivars of chickpea and three single strains of rhizobial inoculants to identify a best cultivar and Rhizobium strain combination. CO-1 cultivar showed better response to inoculation than the cultivar G-62-404. Inoculation with I.A.R.I. strain resulted in more nodulation in both the cultivars but its effect on dry weight and seed yield was not better than the other strains. Inoculation with TNAU-I strain gave higher yield than the other strains in CO-1 cultivar.

Vaishya et al. (1983) conducted an experiment to study the effect of Rhizobium inoculation with strain M_1 on nodulation and grain yield of 12 cultivars of mungbean. The statistical analysis of pooled data of the experiment revealed that inoculation had increased grain yield s The yield increased due to inoculation ranged f ent

with Pusa Baisakhi to 168.21 per cent with J-10 with a mean value of 48.28 per cent than the control. No significant interaction was observed with respect of grain yield.

Gupta and Bajpai (1984) observed that seed yield of pigeonpea was increased significantly by seed inoculation with Rhizobium strain KA-1.

Prasad and Ram (1984) reported increase in seed yield and plant dry weight due to rhizobial inoculation, in a field trial with 3 Vigna radiata cultivars and seed inoculation with Rhizobium phaseoli strain. Plant dry weight correlation with nodulation and nodule leghaemoglobin content.

Briner et al. (1985) conducted a pot trial with 5 strains of Rhizobium sp. and 4 chickpea cultivars and reported that seed inoculation with strains 395, 300 and 398 increased dry matter of stem, leaves, roots and nodules.

Thakur and Nagi (1985) reported during their field experiment that dry matter yield of black gram was increased due to rhizobial inoculation.

Pahuwa (1986) carried out studies on the response of cowpea to different levels of phosphate inoculation with Rhizobium and reported that inoculation alone increased dry matter yield of cowpea.

Churi and Nadar (1986) reported that Phaseolus vulgaris inoculated with Rhizobium strain No. 405 increased the dry weight of shoot, pod weight, total dry matter and grain yield.

Srivastava and Varme (1986) conducted field experiment at the Ranchi Agricultural College Farm during Kharif 1974-80 and 1980-81 and results revealed that Rhizobium inoculation alone or in combination with nitrogen significantly increased the grain yield.

Bhuiya et al. (1989) conducted a field trial with six rhizobial strains viz., BAU-501, BAU-502, BAU-504, BAU-5, BAU-511, BAU-512 on T-9 variety of black gram and reported the significant beneficial effect on total dry matter weight of plants and grain yield. The strain BAU-510 appeared to be the best in respect of all the parameters studied.

Joshi et al. (1989) found that Rhizobium inoculation significantly increased the yield of groundnut by 14.4 per cent and of soyabean by 10.69 per cent over uninoculated control. The increase in yield was due to favourable effect of Rhizobium on pod number and test weight.

Basu et al. (1989) showed that the seed inoculation of mungbean with Rhizobium strain JCA-1 and M-10 increased the seed yield and gave seed yield of 0.92 and 0.87 t/ha over uninoculated.

Namdeo *et al*. (1989) reported that inoculation of gram with *Rhizobium* strain, increased the seed yield by 22.4 to 37.8 per cent over uninoculated control.

Gandhi and Godbole (1990) studied the effect of 17 fast growing rhizobial strains (isolated from wild legumes) on a cultivated legume *Vigna unguiculata*. The nitrogenase activity and the statistical analysis of the pooled data of the experiment revealed that all fast growing rhizobial strains effectively gave significant increase in yield over uninoculated control.

Namdeo and Gupta (1990) conducted a field experiment with 8 authentic *Rhizobium* strains and seeds of pigeonpea variety JA-3. The seeds were treated with *Rhizobium* strains Inoculation resulted in a significant increase (19.20 to 32.08%) in grain yield. Strain (T-2014, IC-3195 and IC-3100) produced higher grain yield than the other but all the strains were at par among themselves. On an average the yield increased from 26.3 to 32.1 per cent due to inoculation with these strains.

Daterao *et al*. (1990) studied the effect of *Rhizobium* seed inoculation of green gram with and without molybdenum on grain yield and nitrogen status of soil. They observed that yield of greengram (variety Kopergaon) increased significantly with *Rhizobium* inoculation over uninoculated. The increase was 0.89 q/ha over the untreated control.

Tippannavar and Desai (1992) found that the dry matter production was maximum in rhizobial inoculation with nitrogen and phosphorus nutrition in bengal gram.

Prabhakaran and Rangarajan (1992) recorded that rhizobial seed bacterization was found to enhance grain yield of Phaseolus vulgaris cultivars over their controls. Among the cultivars of lablab CO-9 has recorded better grain yield (555.9%) followed by CO-8 cultivar. The interaction between lablab cultivars and rhizobial seed inoculation on grain yield at harvest were found significant.

Prabhakaran and Srinivasan (1993) conducted an experiment with horsegram cultivars viz., CO-1 and Hebbal-1 which were inoculated with three slow growing rhizobial strains VPR-1, VHG-1 and VBS-1 with and without nitrogen control. They observed that rhizobial seed inoculation increased the yield to an extent of 9.5 to 19.1 per cent in CO-1 whereas, in Hebbal-1 it ranged from 14.9 to 20.3 per cent over control. In case of Hebbal-1 the variable grain yield was found significant. In both cultivars, inoculation with VPR-1 gave highest seed yield which were greater than with nitrogen.

Kumpawat and Manohar (1994) conducted a field trial at Jobner, Rajasthan. Gram (C. arietinum) seeds were inoculated with Rhizobium or not inoculated and given 0 or 30 kg P_2O_5 and 20 kg each of zing sulphate or ferrous sulfate or 1 kg

ammonium molybdate. Seed yield was increased by seed inoculation (1.05 Vs. 0.85 t/ha) and P application (1.12 Vs. 0.79 t/ha).

Tomar and Raghu (1995) reported that seed inoculation with Rhizobium increased the seed yield of chickpea cultivar JG-315 compared with no inoculation (1.11 Vs. 0.86 t). This increase in yield was similar to uninoculated plants given 20 kg N/ha (1.15 t).

Prabhakaran et al. (1996) found that seed bacterization of Rhizobium with soil incorporation of amendents and their combinations in acid soil significantly enhanced the pod yield of red gram over control. Seed inoculation alone recorded 47.6 per cent enhanced grain yield over control.

Wange and Patil (1996) observed that inoculation with Rhizobium strain R_3 gave significantly more grain yield (1075 kg/ha) of tur. The interaction V_1 X R_3 recorded maximum grain yield (1277 kg/ha). The results clearly indicate that rhizobial strains R_1 and R_3 performed significantly well in improving the grain yield of tur crop in field condition over uninoculated control.

2.3 Effect of seed inoculation with Rhizobium on nitrogen content/N-uptake of plants

Sankaram et al. (1960) undertook studies on the selection of efficient strains of sun hemp, mung and found that all the strains employed were efficient in nitrogen fixation with their respective hosts.

Ramaswami and Nair (1965) isolated effective strains of Rhizobium from the nodules of red gram, green gram, cowpea, black gram and horse gram. The seeds were inoculated with the strains from respective plants and sown in the field. After 45 days, they showed that in case of red gram, cow pea and horse gram, there was a significant increase in the nitrogen content of the plants due to seed inoculation with Rhizobium.

Iswaran et al. (1969) reported that legumes respond to application of P and most often to inoculation with culture of respective Rhizobium species. In pot culture experiment, cowpea, urid and mung when treated with Rhizobium, induced the higher yield of dry matter and total uptake of phosphorus from the soil.

Chomchalow (1970) reported that Rhizobium inoculation significantly increased the dry matter yield, plant height, number of nodules and N-uptake after 3, 6, 9 and 12 weeks of growth in peanut.

Habish et al. (1974) reported the inoculating effect of Rhizobium on haricot bean. Local strain of Rhizobium significantly increased nitrogen content of plants.

Gangawane and Datar (1977) conducted an experiment to study the efficacy of individual and composite rhizobial inoculation on the nodulation of sown legume crop. They used

seven rhizobial strains and legume crops viz., lucerne, groundnut and cowpea. The Rhizobium inoculation indicate that both the individual and composite inoculants increased nitrogen content in all the legumes tested except in cowpea.

Pawar and Ghulghule (1977) reported that Rhizobium inoculation increased the N content of root and shoot in mung.

Idris and Sandhu (1979) reported that Rhizobium inoculation increased the N_2 fixing efficiency of mung plant by 31.6 to 141 per cent.

Shinde (1979) inoculated black gram with Rhizobium inoculant and found increased in nitrogen content in plant over uninoculated control.

Reddy and Tanner (1980) reported that the rhizobial inoculant application increased N fixation in groundnut.

More et al. (1981) conducted a field experiment to study the effect of different commercial inoculants of Rhizobium under different levels of nitrogen on nodulation in groundnut. They observed a beneficial effect of seed inoculation with Rhizobium over the uninoculated control in respect of nitrogen content in both root and shoot.

Singaravadivel and Prasad (1981) conducted a pot trial and field experiment on groundnut, inoculated with Rhizobium and observed that total N content were upto double that of the untreated control, both in pots and in the field.

El-Said et al. (1981) reported that inoculation with Rhizobium sp. increased the 'N' percentage in pea plants.

Ghag (1981) found that inoculation of seeds with Rhizobium culture resulted increased nitrogen content of plants over uninoculated control in all varieties of gram (C. arietinum L.).

Raut and Ghonsikar (1982) reported 22 per cent increase in total N in gram due to Rhizobium inoculation.

Mane (1985) found that the seed inoculation with Rhizobium enhanced the 'N' content of plants over uninoculated control in all the varieties of gram.

Kaliash and Gisela (1988) conducted an experiment on Rhizobium inoculation in alfalfa and showed that the 'N' accumulation increased after inoculation of Rhizobium strain.

Sairam et al. (1989) reported that phosphorus application at the rate of 90 kg/ha and inoculation with Rhizobium inoculant resulted in increase in nitrogen fixation and total nitrogen uptake plus increase in available soil nitrogen.

Bhuiya et al. (1989) conducted a field trial with six rhizobial strains on T-9 variety of black gram and reported that significant beneficial effect in N-uptake.

Beena *et al*. (1990) the studies were done in the fields of varietal response and host varietal specificity for nodulation by *Rhizobium*. Ten different varieties of cowpea and one *Rhizobium* strain was used for the experiment. It was found that *Rhizobium* inoculation resulted significantly increase in per cent nitrogen content by all the varieties.

Prabhakaran and Rangarajan (1992) conducted an experiment with two elite strains of *Rhizobium phaseoli viz*., PvRp-1 and PvRp-2 and four lablab cultivars *viz*., CO-6, CO-7, CO-8 and CO-9. The *Rhizobium* seed bacterization was found to enhance nitrogen fixation in *Phaseolus vulgaris* cultivars over their controls. The interaction between lablab cultivars and rhizobial seed inoculation on nitrogen fixation at harvest was found significant.

Shaheen and Rahmatullah (1994) conducted a laboratory experiment on groundnut cultivar Banki and ICGS-44 and soil inoculation with *Rhizobium* strains *viz*., NC-92 or Ruddy patric. They observed that nitrogen fixation of both cultivars were significantly increased by inoculation.

Material and Methods

3. MATERIAL AND METHODS

The present investigation was undertaken in the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722 (M.S.) India. The Glass House facilities of the Post Graduate Institute of the University were utilized to conduct pot culture experiment. The pot culture experiment was conducted during the Kharif season of 1995-96.

The details of material required and methods followed during the period of investigation are described briefly in the succeeding paragraphs.

3.1 Material

Material used for laboratory and pot culture experiments are as given below.

3.1.1 Collection of plant samples for isolation

Healthy, vigorously growing plant samples of Dolichos biflorus L. were collected from the Instructional Farm Area of the Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

3.1.2 Isolation of Rhizobia

Cango Red Yeast Extract Mannitol Agar medium (Vincent, 1970), sterilized water, petriplates, mercuric

bi-chloride ($HgCl_2$ 0.1 per cent), spirit lamp and inoculating needle were used for the isolation of Horse gram rhizobia.

3.1.3 Morphology and staining reaction

Different staining reactions were carried out by employing the standard methods described by Society of American Bacteriologist (1957).

3.1.4 Seeds

The seeds of horsegram cultivars viz., Sina, Man, TPK, HPK, Phule K-1 and KS-2 were obtained from the Pulse Breeder, All India Co-ordinated Pulses Improvement Project, M.P.K.V., Rahuri.

3.1.5 Soils

Soil used for conducting the pot culture experiment was obtained from the Instructional Farm Area of the Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri having pH 7.8, organic carbon 0.48%, available N 214 kg/ha and available P 9.16 kg/ha.

3.1.6 Earthen pots

The earthen pots of 22.5 cm diameter were used for conducting the pot culture experiment.

3.1.7 Disinfectant

Five per cent aqueous solution of copper sulphate ($CuSO_4$) was used for disinfecting the earthen pots.

3.1.8 *Rhizobium* culture

The *Rhizobium* cultures isolated from horsegram cultivars *viz*., Sina, Man, TPK, HPK, Phule K-1 and KS-2 were used.

3.1.9 Fertilizers

Nitrogen and phosphorus were applied through urea and single super phosphate, respectively.

3.1.10 Insecticides

Endosulfan 35 EC and BHC 10 per cent dust were used for control insect pest of horsegram.

3.1.11 Glasswares

The Corning brand glasswares *viz*., beakers, pipettes, petriplates, test tubes, conical flask, volumetric flasks, measuring cylinders, microscopic glass slides, cover slips, funnels etc. were used whenever necessary.

3.1.12 Equipments

The laboratory equipments *viz*., digestion unit, distillation unit, autoclave, incubator, grinding machine,

weighing balance, microscope, filler micrometer, spectronic 20 etc. were used whenever necessary.

3.1.13 Miscellaneous material

Brown paper bags, weight box, labels, polythene paper bags, knife were used whenever required.

3.1.14 Material required for chemical analysis

3.1.14.1 Initial chemical analysis of soil

1. Soil reaction : pH of the soil was determined by using Elico digital pH meter.

2. Available nitrogen : Macro-Kjeldahl's distillation unit, 0.32 per cent $KMnO_4$, 2.5 per cent NaOH, 2 per cent boric acid, mixed indicator of bromocresol green and methyl red, ethyl alcohol, 0.02 N H_2SO_4, Devarda's alloy, MgO etc. were used.

3. Available phosphorus : Spectronic-20, 0.5 N $NaHCO_3$, Phosphorus free activated charcoal, ammonium molybdate, HCl, stannous chloride solution and KH_2PO_4 were used.

4. Organic carbon : Potassium dichromate solution (1 N), Ferrous sulphate solution (0.5 N), Ferroin indicator, conc. H_2SO_4 etc. were used.

3.1.14.2 Chemical analysis of plant samples

1. Total Nitrogen: Micro-Kjeldahl's digestion and distillation unit, conc. H_2SO_4, 30 per cent H_2O_2, NaOH 40 per cent solution,

2 per cent Boric acid indicator, mixed indicator of bromocresol green, methyl red (dissolved in 100 ml alcohol) and distilled water were used.

3.2 Methods

3.2.1 Isolation of Rhizobia

Isolation of Horsegram root nodule bacteria was done from six horsegram varieties viz., Sina, Man, TPK, HPK, Phule K-1 and KS-2.

For isolation, vigorously growing three months old plants with deep green foliage were selected from each of the above mentioned varieties and carefully uprooted with intact root system. The roots were washed thoroughly in running tap water and two to three large, hard and pinkish nodules located at tap root were selected and removed from each plant of horsegram cultivars. Isolation of the Rhizobium from these nodules was done by the method described by Vincent (1970) using Congored Yeast Extract Mannitol Agar medium. After solidification of the medium the plates were incubated at 30°C temperature for 5 days. After incubation, transfers from distinctly separate, raised, opaque, white well isolated colonies were made on YEMA slants and were coded as RS-1, RS-2, RS-3, RS-4, RS-5 and, RS-6 respectively.

3.2.2 Testing nodulation by Leonard jar assembly method

For testing nodulation ability of six isolates of horsegram rhizobia, Leonard jar assembly was used (Vincent, 1970). The seeds were then inoculated with rhizobial cultures and covered with gravel to prevent aerial contamination. One uninoculated control set was maintained for comparison. All these sets were kept for 21 days in glass house. The nutrient solution at the bottom of jar was changed by separating two halves of the assembly under aseptic condition.

3.2.3 Staining reactions and morphology

3.2.3.1 Staining reaction

In staining reaction viz., simple, Gram's, Capsule, Spore and Acid-fast staining were carried out for all isolates under study by employing the standard methods described by the Society of American Bacteriologist (1957).

3.2.3.2 Morphology

1. Shape : Smears of 48 hours old isolate grown on yeast extract mannitol agar medium were stained with Ziehl's Carbol Fuchsin and observed under microscope for shape.

2. Size : Measurement of cells were made with the help of filler micrometer, using smears stained with Carbol Fuchsin.

3.2.4 Pot culture experiment

3.2.4.1 Design of experiment

Factorial Completely Randomised Design (FCRD) was used for this experiment.

3.2.4.2 Experimental details

The experiment was conducted in a sterile soil with following two factors.

Factor-A :

a. Sina
b. Man
c. TPK
d. Phule K-1
e. KS-2

Factor-B :

a. RS-1 isolated from Sina
b. RS-2 isolated from Man
c. RS-3 isolated from TPK
d. RS-4 isolated from HPK
e. RS-5 isolated from Phule K-1
f. RS-6 isolated from KS-2
g. Uninoculated control

● REPLICATION - I

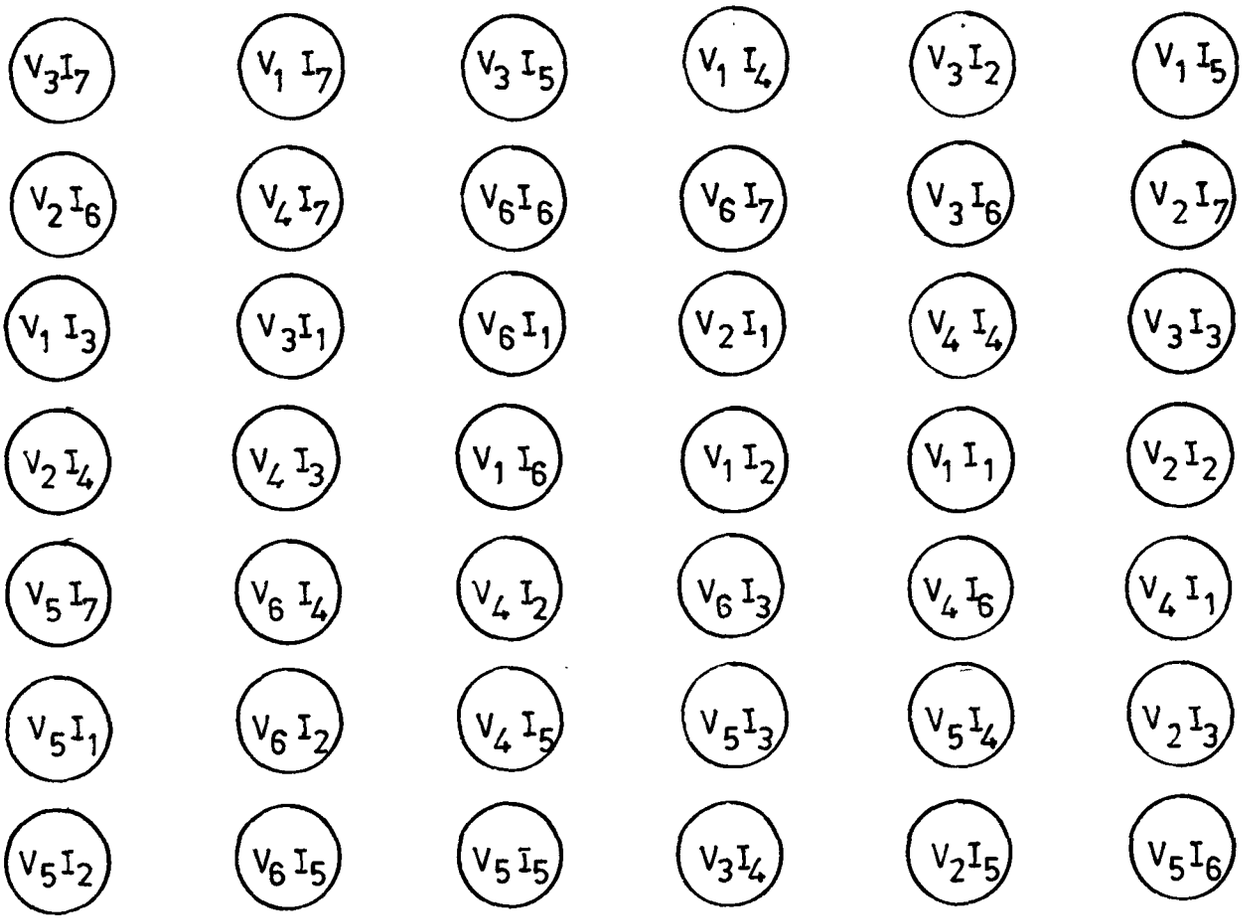
$V_1 I_4$	$V_6 I_7$	$V_1 I_1$	$V_1 I_3$	$V_6 I_2$	$V_1 I_2$
$V_2 I_3$	$V_1 I_6$	$V_2 I_4$	$V_5 I_1$	$V_3 I_7$	$V_1 I_5$
$V_2 I_5$	$V_3 I_5$	$V_4 I_6$	$V_5 I_4$	$V_2 I_1$	$V_6 I_2$
$V_3 I_1$	$V_2 I_6$	$V_6 I_1$	$V_2 I_7$	$V_3 I_4$	$V_5 I_2$
$V_4 I_2$	$V_3 I_6$	$V_4 I_1$	$V_3 I_2$	$V_4 I_3$	$V_4 I_5$
$V_5 I_7$	$V_3 I_3$	$V_5 I_6$	$V_2 I_2$	$V_4 I_7$	$V_5 I_3$
$V_6 I_6$	$V_1 I_7$	$V_6 I_5$	$V_4 I_4$	$V_5 I_5$	$V_6 I_4$

● REPLICATION - II

$V_6 I_7$	$V_4 I_7$	$V_2 I_2$	$V_2 I_8$	$V_3 I_5$	$V_6 I_1$
$V_1 I_4$	$V_2 I_6$	$V_4 I_3$	$V_3 I_7$	$V_5 I_4$	$V_6 I_2$
$V_6 I_6$	$V_4 I_5$	$V_1 I_7$	$V_2 I_4$	$V_3 I_3$	$V_1 I_5$
$V_3 I_2$	$V_4 I_1$	$V_5 I_1$	$V_3 I_4$	$V_5 I_6$	$V_2 I_3$
$V_1 I_6$	$V_3 I_6$	$V_4 I_6$	$V_5 I_7$	$V_2 I_7$	$V_4 I_4$
$V_6 I_3$	$V_5 I_3$	$V_1 I_2$	$V_2 I_1$	$V_5 I_5$	$V_3 I_1$
$V_4 I_2$	$V_1 I_1$	$V_5 I_2$	$V_6 I_5$	$V_1 I_3$	$V_2 I_5$

Contd ....

● REPLICATION-III



- Design : Factorial Completely Randomised Design (FCRD)
- Replication : Three
- Factors : Two

- Factor A : Horsegram varieties
  - V_1 - Sina
  - V_2 - Man
  - V_3 - TPK
  - V_4 - HPK
  - V_5 - Phule K-1
  - V_6 - KS-2
- Factor B : Rhizobium Isolates
  - I_1 - Isolated from Sina
  - I_2 - Isolated from Man
  - I_3 - Isolated from TPK
  - I_4 - Isolated from HPK
  - I_5 - Isolated from Phule K1
  - I_6 - Isolated from K S-2
  - I_7 - Uninoculated Control

Fig. 1. Plan of layout : Pot Culture Experiment

The experiment comprised of forty two treatment combinations which were replicated three times.

3.2.4.3 Pot filling

The soil used for pot culture experiment was passed through 2 X 3 mm sieve to remove stones and stubbles. The soil was sterilized in autoclave under pressure of 1.1 kg/cm^2 for three successive days. The earthen pots were sterilized with 5 per cent $CuSO_4$ solution and filled with sterile soil at the rate of 4.0 kg/pot.

3.2.4.4 Application of manures and fertilizer

The doses of manures and fertilizers per pot were worked out on the soil weight basis, keeping the base of their recommended dose *viz*., 20 kg N/ha and 40 kg P_2O_5/ha. The doses of nitrogen and phosphorus were applied through urea single super phosphate, respectively at the time of sowing as basal dose and uniformly covered with sub-surface soil.

3.2.4.5 Seed inoculation and sowing

For testing the nodulation ability and effectiveness in fixing nitrogen symbioticaly, all the isolates were tested in sterile soil in earthen pots. Eight to nine seeds of horsegram were sown in each pot varietywise. Prior to sowing the seeds were soaked for 12 hrs. in 7 days old culture suspension (10^8 cells/ml) of Rhizobium. Each treatment was

replicated three times including uninoculated control pots. Three such sets were prepared in order to record the observations at 30, 60 and 90 days after sowing and at harvesting stage.

Thinning was done after 15 days by keeping 5 healthy plants in each pot. All the pots were randomised to remove the positional effect. The plants were watered regularly whenever required.

3.2.4.6 Observation recorded

Following observations were recorded in the pot culture experiment.

1. Germination count (%) : Germination percentage of the seeds of each pot was recorded 10 days after sowing.

2. Nodulation count and dry weight : Nodulation count was recorded at 30, 60, 90 days after sowing and at harvest.

Nodulation count consisted of number of total nodules, number of large/medium/small nodules and also number of pink/white/black nodules.

A light irrigation was given on the previous day of observation, so as to uproot the plants with intact root system. One plant from each pot was selected randomly and plants were removed carefully by reversing the pot upside down

in water tought. The intact root system was washed with tap water and observation on nodulation were recorded and also dry weight of nodules/plant were recorded.

3. Dry matter yield of plant : The dry matter yield of plant was recorded at 30, 60, 90 days after sowing and at harvest of each treatment.

4. Yield/plant : Yield per plant was recorded for each treatment.

3.2.4.7 Chemical analysis of soil

1. Soil reaction : Soil reaction (pH) was determined before sowing by means of glass electrode using 1 : 2.5 soil : water suspension (Jackson, 1971).

2. Available Nitrogen : the available nitrogen from initial soil determined by modified alkaline permanganate method (Sahrawat and Burford, 1983).

3. Available Phosphorus : The available phosphorus of initial soil was estimated calorimetrically by using $NaHCO_3$ (0.5 N; 8.5 pH) as an extractant (Olsen et al., 1954).

4. Organic Carbon : Organic carbon of soil was estimated by modified method of Walkely and Black as described by Piper (1950).

3.2.4.8 Chemical analysis of plant

In order to study the effect of Rhizobium cultures on nitrogen content of whole plant of horsegram, the total nitrogen in plant was estimated by Micro-Kjeldahl's method (Jackson, 1971).

3.2.4.9 Statistical analysis

The data obtained on various parameters were subjected to statistical analysis by following the standard method of analysis of variance. The standard errors for the treatment means and critical differences were computed at 5 per cent level of significance (Panse and Sukhatme, 1957).

Experimental Results

4. EXPERIMENTAL RESULTS

4.1 Isolation of Rhizobium isolates from horsegram cultivars

The attempts were made to isolate of Rhizobium from the root nodules of different horsegram cultivars by using the method described by Vincent (1970) and the isolates were further coded as RS-1 to RS-6 as detailed below.

Isolated code	Isolate from horsegram variety
RS-1	Sina
RS-2	Man
RS-3	TPK
RS-4	HPK
RS-5	Phule K-1
RS-6	KS-2

4.2 Nodule forming ability of isolates

All the isolates under study proved to be horsegram Rhizobia, since they could nodulate the horsegram plants when tested in Leonard jar assembly.

4.3 Morphology and staining reaction

The results regarding the cell shape and size of the Rhizobium isolates are presented in Table 1. All the

isolates were invariably rod shaped. The size of cells varied from 2.30 to 3.24 µm in length and 0.63 to 0.96 µm in breadth.

Table 1. Morphological characters and staining reactions of Rhizobium isolates from horsegram

Isolate Number	Morphology			Staining reactions			
	Shape	Average size (µm)		Gram's	Spore	Capsule	Acid fast
		Length	Width				
RS-1	Rod	3.24	0.96	-	-	+	-
RS-2	Rod	3.17	0.91	-	-	+	-
RS-3	Rod	2.46	0.67	-	-	+	-
RS-4	Rod	2.30	0.63	-	-	+	-
RS-5	Rod	2.96	0.84	-	-	+	-
RS-6	Rod	2.80	0.79	-	-	+	-

- = Negative test

+ = Positive test

All the isolates were found to be Gram negative, non-spore forming, encapsulated and non-acid fast (Table 1). These results are in conformity with the morphological characters of Rhizobium spp. reported in the Bergey's Manual of Determinative Bacteriology (1974).

4.4 Seed germination (%)

The data represented in Table 2 in respect of germination count (%) were significant for inoculation treatments while non-significant for varieties and their interactions.

Table 2. Effect of inoculation with different isolates of *Rhizobium* on germination count (%) in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
RS-1	86.80	82.53	82.48	83.88	80.21	82.91	83.14
RS-2	83.20	85.75	78.02	80.84	82.67	82.57	82.17
RS-3	85.36	81.19	73.97	78.16	81.94	81.40	80.33
RS-4	87.96	85.46	85.38	84.63	83.82	84.27	85.25
RS-5	83.87	78.34	75.37	75.22	76.55	74.86	77.36
RS-6	84.51	84.28	78.41	82.02	82.16	81.52	82.15
U.C.	78.99	75.52	72.85	73.75	77.23	76.96	75.88
Mean	84.38	81.87	78.64	79.78	80.65	80.64	80.99

	S.E. ±	C.D. at 5%
Varieties (V)	0.14	N.S.
Inoculants (I)	0.15	0.45
Interaction (V X I)	0.39	N.S.

N.S. = Non-significant
U.C. = Uninoculated Control.

All the inoculation treatments recorded a significant improvement in germination count ranging from 77.36 to 85.25 per cent as compared to uninoculated control (75.88%). The inoculation with Rhizobium isolate, RS-4 recorded the highest germination count (85.25%) and it was significantly superior to the rest of the isolates. The rest of the inoculation treatments differed significantly except RS-2 and RS-6 which were at par with each other.

4.5 Number of nodules per plant

The results in respect of number of nodules per plant recorded at 30, 60 and 90 days after sowing and at harvest (Table 3, Fig. 1 & 2 and Plate 1 to 7) were significant for inoculation treatments, while non-significant for varieties and their interaction effects at all the stages.

At 30 days after sowing all the rhizobial isolates recorded significantly higher number of nodules per plant as compared to uninoculated control which recorded no nodules, as the experiment was carried out under sterile soil conditions. However, all the isolates were at par with each other. The number of nodules per plant ranged from 12.57 (RS-1) to 14.15 (RS-4). Although the results in respect of variety were non-significant, the variety Phule K-1 recorded the highest number of nodules per plant (12.51).

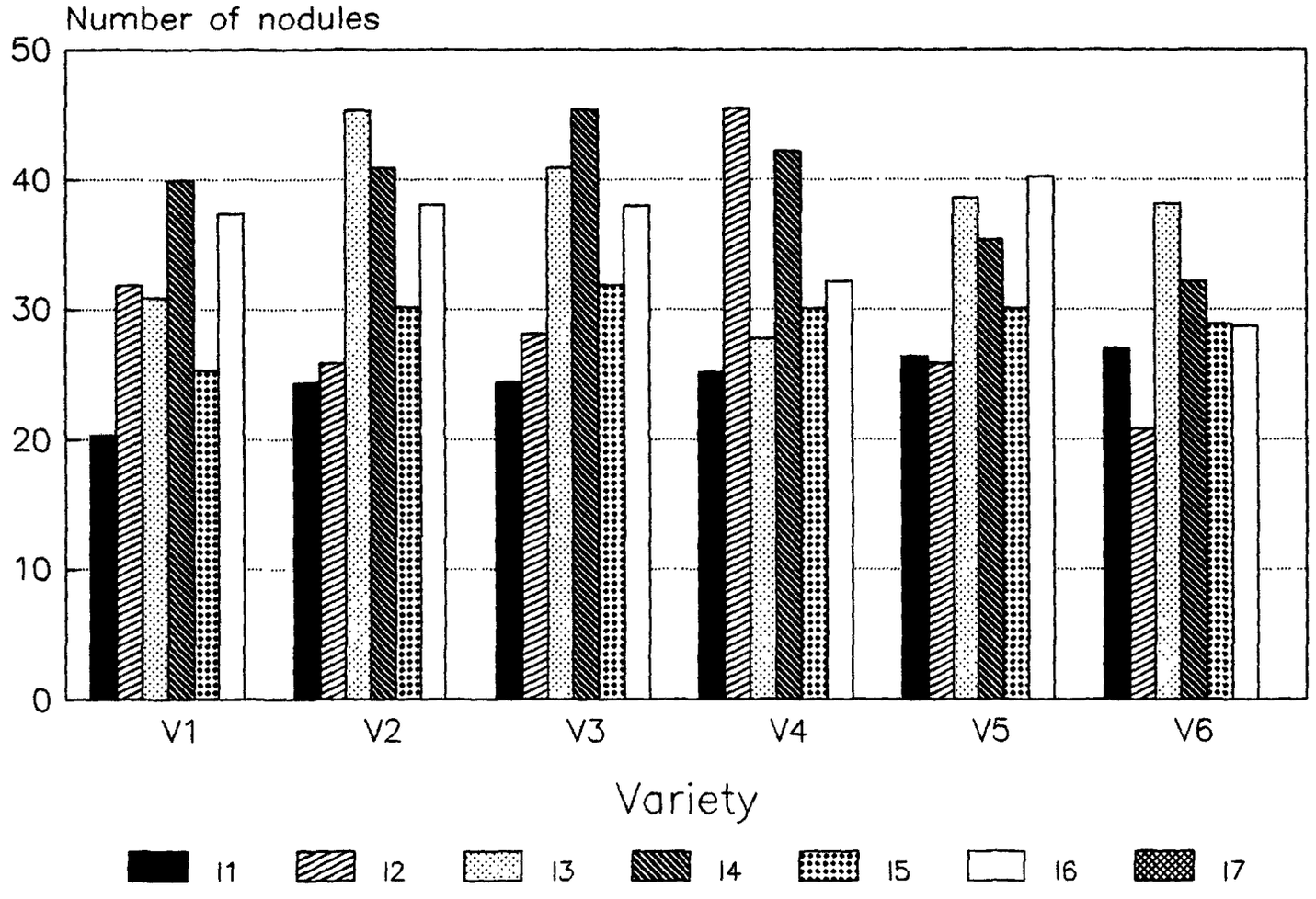
Table 3. Effect of inoculation with different isolates of Rhizobium on number of nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	14.87	10.96	12.06	12.78	15.15	9.60	12.57
RS-2	13.67	11.34	11.27	13.24	15.06	16.01	13.42
RS-3	14.00	9.76	11.36	18.44	14.15	12.50	13.53
RS-4	11.67	18.82	12.30	12.82	13.42	15.57	14.15
RS-5	14.00	10.30	13.25	12.22	14.00	13.37	12.85
RS-6	12.00	15.08	11.60	13.00	15.48	13.95	13.51
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	11.45	10.89	10.40	11.78	12.51	11.57	11.43
B] At 60 days							
RS-1	24.10	26.66	27.66	29.66	28.32	31.26	27.94
RS-2	32.57	30.40	38.00	34.80	27.00	30.00	32.12
RS-3	38.00	51.00	45.60	30.40	42.33	40.26	41.43
RS-4	48.00	50.00	52.00	45.65	38.00	35.20	44.82
RS-5	40.01	30.40	30.00	26.95	27.90	21.00	31.04
RS-6	45.30	44.33	42.20	34.60	42.20	36.26	40.81
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	32.56	33.25	33.63	30.29	29.53	27.72	31.16
C] At 90 days							
RS-1	20.40	24.36	24.42	25.16	26.34	26.96	24.59
RS-2	31.82	25.83	28.11	45.50	25.84	20.86	29.56
RS-3	30.88	45.33	40.92	27.75	38.51	38.12	36.91
RS-4	39.96	40.92	45.43	42.17	35.35	32.16	39.33
RS-5	25.34	30.12	31.86	30.06	30.06	28.86	29.38
RS-6	37.40	38.06	38.00	32.16	40.27	28.67	35.76
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	26.53	29.23	29.82	28.97	28.05	25.00	27.93
D] At harvest							
RS-1	15.02	20.26	22.42	23.51	22.65	26.34	21.70
RS-2	27.06	20.84	24.29	25.76	22.09	22.06	23.68
RS-3	26.02	39.22	38.16	25.91	31.70	32.75	32.29
RS-4	36.16	36.91	38.78	37.35	34.76	29.35	35.55
RS-5	24.03	26.86	29.78	27.83	20.11	22.33	25.15
RS-6	33.35	31.84	35.65	30.06	36.91	24.27	32.01
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	23.09	25.13	27.01	24.34	24.03	22.44	24.34

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.840	2.220	2.290	1.560	N.S.	N.S.	N.S.	N.S.
Inoculants (I)	0.910	2.400	2.480	1.680	2.570	6.760	6.98	4.74
Interaction (V X I)	2.230	5.880	6.080	4.130	N.S.	N.S.	N.S.	N.S.

N.S. = Non-significant

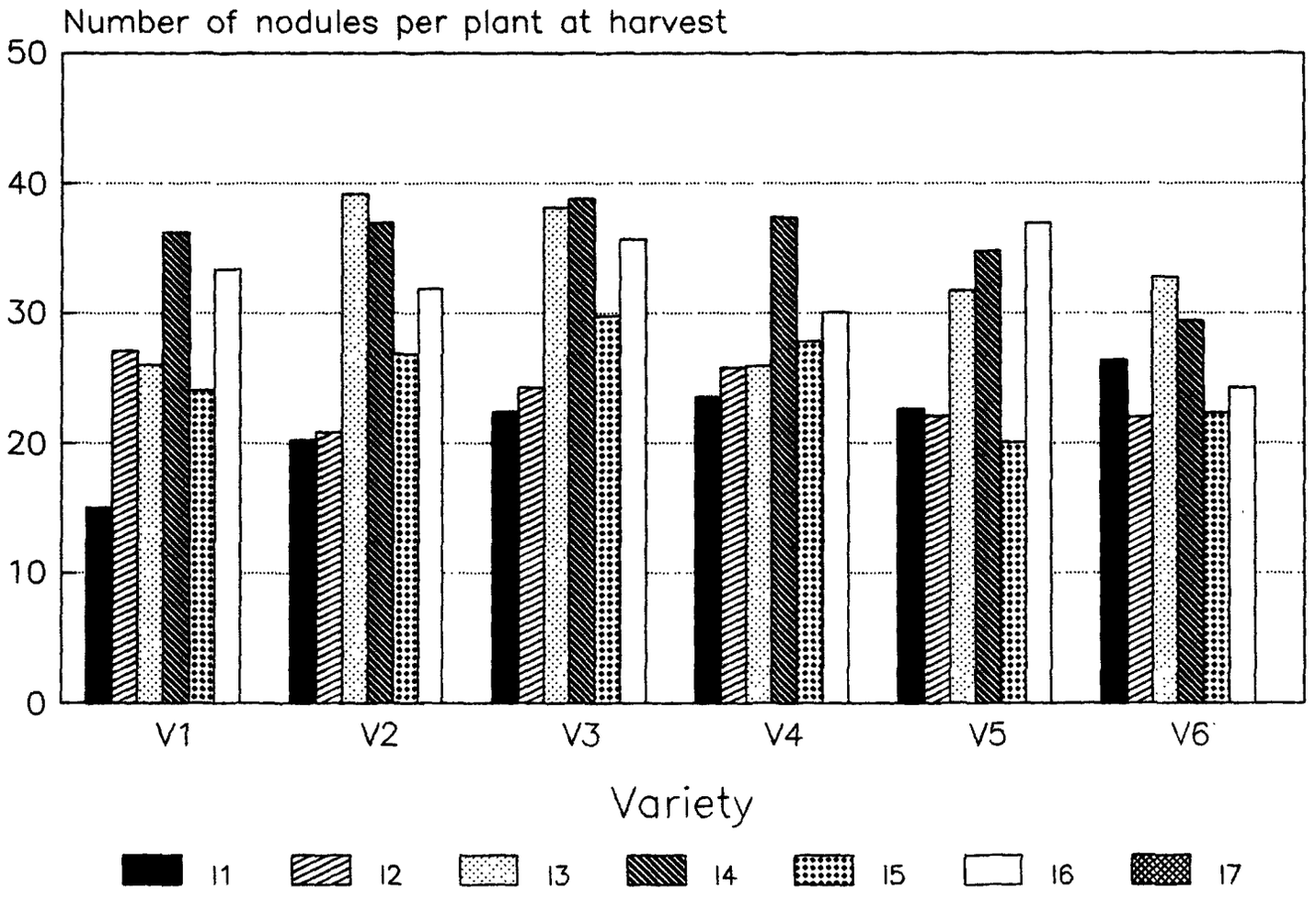
T-3859



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 1. Number of nodules per plant of horsegram varieties at 90 days as influenced by rhizobial inoculants



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 2. Number of nodules per plant of horsegram varieties at harvest as influenced by rhizobial inoculants

Plate No. 1. Maximum nodules produced in variety TPK by inoculation with rhizobial isolate No. 4

Plate No. 2. Effect of different rhizobial isolates on nodulation in var. Sina (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

Plate No. 3. Effect of different rhizobial isolates on nodulation in var. Man (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

Plate No. 4. Effect of different rhizobial isolates on nodulation in var. TPK (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

Plate No. 5. Effect of different rhizobial isolates on nodulation in var. HPK (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

Plate No. 6. Effect of different rhizobial isolates on nodulation in var. Phule K-1 (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

Plate No. 7. Effect of different rhizobial isolates on nodulation in var. KS-2 (1 to 6 indicates Rhizobium isolate numbers and 7 being control)

At 60 days after sowing, all the isolates had significantly improved the number of nodules per plant as compared to uninoculated control which recorded no nodules. Among the six isolates, the isolate RS-4 recorded the highest number of nodules per plant (44.82) and was significantly superior to the other isolates except RS-3 (41.43) and RS-6 (40.81).

At 90 days after sowing, all the isolates recorded significantly higher number of nodules per plant as compared to uninoculated control which did not record any nodules. The number of nodules ranged from 24.59 (RS-1) to 39.33 (RS-4). The inoculation treatment RS-4 recorded the highest number of nodules (39.33) per plant and was significantly superior to the other isolates except RS-3 (36.91 nodules) and RS-6 (35.76 nodules). The variety TPK recorded the highest number of nodules (29.82).

At harvesting stage, all the inoculation treatments recorded significantly higher number of nodules per plant as compared to uninoculated control which recorded no nodules. The inoculation treatment RS-4 recorded the highest number of nodules per plant (35.55) and was significantly superior to other isolates except RS-3 (32.29 nodules) and RS-6 (32.01 nodules). Although the results in respect of variety were non-significant, the variety TPK recorded maximum number of nodules per plant (27.01).

4.6 Number of white nodules

The results in respect of white nodules per plant (Table 4) were significant for inoculation treatments at all the stages. The results were non-significant for varieties and interactions at 30 days, however, they were significant for varieties and interactions at 60 days, 90 days and at harvest.

At 30 days stage, all the isolates recorded significantly higher number of white nodules per plant as compared to uninoculated control which recorded no nodules. However, all the isolates were at par with each other. The isolate RS-4 recorded higher number of white nodules per plant (9.02).

At 60 days all the inoculation treatments differed significantly from each other and recorded higher number of nodules. The number of white nodules per plant ranged from 3.48 (RS-6) to 5.91 (RS-4). Among the varieties all the varieties differ significantly from each other. The number of white nodules per plant in different varieties ranged from 2.90 (Man) to 4.47 (Phule K-1). Among the interactions RS-2 X Phule K-1 recorded significantly highest number of white nodules (7.45/plant) while, RS-5 X HPK and RS-6 X KS-2 recorded least number of white nodules (2.00/plant).

At 90 days, all the inoculation treatments differed significantly from each other. All the inoculation treatments recorded higher number of white nodules per plant as compared

Table 4. Effect of inoculation with different isolates of Rhizobium on white colour nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	10.33	6.76	7.66	8.00	9.66	5.60	8.00
RS-2	9.66	7.00	7.74	7.66	9.33	10.66	8.67
RS-3	9.00	6.37	7.00	13.00	9.33	8.00	8.78
RS-4	8.33	13.40	7.40	8.00	8.00	9.00	9.02
RS-5	9.00	6.80	8.76	8.33	9.66	8.00	8.42
RS-6	8.00	9.33	7.73	8.66	8.66	9.34	8.62
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	7.76	7.09	6.61	7.95	7.80	7.22	7.35
B] At 60 days							
RS-1	3.25	2.25	3.33	3.06	6.07	5.11	3.84
RS-2	3.02	2.10	4.00	4.15	7.45	7.00	4.62
RS-3	4.00	4.00	4.15	4.20	2.33	4.26	3.82
RS-4	6.75	6.00	7.07	5.65	6.00	4.00	5.91
RS-5	5.01	3.00	6.50	2.00	6.35	2.50	4.22
RS-6	5.30	3.00	3.00	4.50	3.10	2.00	3.48
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	3.90	2.90	4.00	3.36	4.47	3.55	3.69
C] At 90 days							
RS-1	2.22	2.20	6.22	2.12	6.00	5.05	3.96
RS-2	4.80	2.33	6.20	6.36	5.30	3.00	4.66
RS-3	5.44	3.80	8.35	3.92	2.20	4.10	4.63
RS-4	4.36	5.32	4.15	5.10	4.00	3.90	4.47
RS-5	4.10	6.06	6.40	4.00	6.80	6.10	5.57
RS-6	4.10	2.60	5.16	4.15	4.10	2.17	3.71
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.00
Mean	3.57	3.18	5.21	3.66	4.05	3.47	3.85
D] At harvest							
RS-1	1.80	2.12	4.40	2.02	4.22	3.02	2.93
RS-2	2.22	3.15	4.15	2.20	4.30	2.05	3.01
RS-3	3.02	3.85	6.35	3.65	2.15	3.10	3.68
RS-4	3.10	4.30	3.16	4.15	3.90	3.04	3.60
RS-5	4.05	3.06	3.40	2.08	4.31	4.00	3.48
RS-6	2.35	2.35	4.20	4.00	3.65	2.00	3.09
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	2.36	2.69	3.66	2.58	3.21	2.45	2.82

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.610	0.004	0.004	0.003	N.S.	0.010	0.010	0.007
Inoculants (I)	0.660	0.004	0.004	0.002	1.880	0.011	0.011	0.007
Interaction (V X I)	1.630	0.009	0.009	0.006	N.S.	0.027	0.027	0.019

N.S. : Non-significant

to uninoculated control which recorded no nodules. The number of white nodules per plant ranged from 3.71 (RS-5) to 5.57 (RS-5). All the varieties differed significantly from each other. The number of white nodules per plant in the varieties ranged from 3.18 (Man) to 5.21 (TPK). Among the interactions the interaction RS-5 X Phule K-1 recorded significantly highest number of white nodules per plant (6.80), while RS-1 X HPK recorded least number of white nodules (2.12).

At harvesting stage, all the inoculation treatments differed significantly from each other and recorded higher number of white nodules per plant as compared to uninoculated control which recorded no nodules. The number of white nodules per plant ranged from 2.93 (RS-1) to 3.68 (RS-3). All the varieties differed significantly from each other. The number of white nodules per plant recorded by different varieties ranged from 2.36 (Man) to 3.66 (TPK). Among the interactions, the interaction RS-3 X TPK recorded significantly highest number of white nodules per plant (6.35), while RS-1 X Sina recorded least number of white nodules per plant (1.80).

4.7 Number of pink nodules

The results on the number of pink nodules per plant (Table 5) were significant for varieties, inoculation treatments and their interactions at 60 days, 90 days and at harvesting stage, whereas they were significant only for inoculation treatments at 30 days.

Table 5. Effect of inoculation with different isolates of *Rhizobium* on pink colour nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	4.54	4.20	4.39	4.78	5.48	4.00	4.56
RS-2	4.00	4.34	3.53	5.57	5.72	5.35	4.75
RS-3	5.00	3.38	4.36	5.44	4.81	4.50	4.58
RS-4	3.33	5.42	4.90	4.82	5.42	6.57	5.07
RS-5	5.00	3.50	4.48	3.88	4.33	5.37	4.42
RS-6	4.00	5.74	3.86	4.33	6.81	4.61	4.89
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	3.69	3.79	3.64	4.11	4.65	4.34	4.18
B] At 60 days							
RS-1	18.20	19.00	18.30	21.25	19.00	22.00	19.62
RS-2	25.20	20.00	31.00	21.20	17.00	18.00	22.06
RS-3	31.00	40.00	33.05	21.30	38.00	26.00	31.55
RS-4	37.25	39.00	39.93	31.00	28.00	27.00	33.69
RS-5	27.00	22.00	18.50	20.00	18.00	16.50	20.33
RS-6	35.00	35.23	35.10	22.10	35.00	30.00	32.07
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	24.80	25.03	25.12	19.55	22.14	19.92	22.76
C] At 90 days							
RS-1	12.08	15.06	7.20	15.00	10.40	13.85	12.26
RS-2	13.00	13.00	13.00	24.80	13.30	9.46	14.42
RS-3	10.22	25.43	20.27	11.42	21.15	18.00	17.74
RS-4	20.30	17.30	27.02	21.07	17.35	15.12	19.69
RS-5	17.67	14.02	15.36	16.06	13.10	12.36	14.76
RS-6	17.05	25.35	17.42	14.01	14.17	14.20	17.03
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	12.90	15.73	14.32	14.62	12.78	11.85	13.70
D] At harvest							
RS-1	4.35	5.47	4.44	7.40	2.80	11.08	5.92
RS-2	5.64	8.09	6.14	5.56	2.49	5.01	5.48
RS-3	6.00	12.20	6.91	6.20	8.60	10.40	8.38
RS-4	13.06	18.50	13.20	13.05	10.20	8.26	12.71
RS-5	8.20	7.60	7.30	8.50	2.80	6.30	6.78
RS-6	14.50	10.30	7.40	8.06	12.20	6.20	9.77
U.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mean	7.39	8.88	6.48	6.96	5.58	6.75	7.00

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.350	0.950	0.010	0.520	N.S.	2.690	0.029	1.470
Inoculants (I)	0.380	1.030	0.011	0.560	1.070	2.910	0.031	1.590
Interaction (V X I)	0.930	2.530	0.027	1.380	N.S.	7.120	0.078	3.900

N.S. = Non-significant

At 30 days all the inoculation treatments recorded significantly higher number of pink nodules per plant as compared to uninoculated control which recorded no nodules. However, all the inoculation treatments were at par with each other. The number of pink nodules per plant due to different isolates ranged from 4.42 (RS-5) to 5.07 (RS-4).

At 60 days stage, all the inoculation treatments recorded significantly higher number of pink nodules per plant as compared to uninoculated control which recorded no nodules. The inoculation treatment RS-4 recorded the highest number of pink nodules (33.69) per plant and was significantly superior to the rest of the isolates except RS-3 (32.55) and RS-6 (32.07). Among the varieties, the variety Sina (24.80), Man (25.03) and TPK (25.12) were significantly superior to the others but were at par with each other. Among the interactions, the interaction RS-3 X Man recorded the highest number of pink nodules per plant (40.00) while RS-5 X KS-2 recorded the least number of pink nodules per plant (16.50).

At 90 days stage, all the inoculation treatments recorded significantly higher number of pink nodules per plant as compared to uninoculated control which recorded no pink nodules. All the inoculation treatments differed significantly from each other. The number of pink nodules per plant ranged from 12.26 (RS-1) to 19.69 (RS-4). All the varieties differed significantly from each other in respect of number of pink

nodules per plant. The variety Man recorded the highest number of pink nodules (15.73) whereas KS-2 recorded the least (11.85). Among the interactions, the interaction RS-4 X TPK recorded significantly higher number pink nodules per plant (27.02), while RS-1 X TPK recorded the least number of pink nodules per plant (7.20).

At harvesting stage all the inoculation treatments recorded significantly higher number of pink nodules per plant over the uninoculated control which recorded no pink nodule. The inoculation treatments RS-4 recorded the highest number of pink nodules per plant (12.71) and was significantly superior to rest of the isolates. The number of pink nodules per plant ranged from 5.48 (RS-1) to 12.71 (RS-4). Among the varieties, the variety Man recorded significantly highest number of pink nodules per plant (8.88). The number of pink nodules per plant in the varieties ranged from 5.58 (Phule K-1) to 8.88 (Man).

Among the interactions, the interaction RS-4 X Man recorded significantly higher number of pink nodules per plant (18.50) while RS-2 X Phule K-1 recorded the least number of pink nodules per plant (2.49)

4.8 Number of black nodules

The results in respect of number of black nodules per plant (Table 6) were significantly influenced by the varieties (except at 90 days). Inoculation treatments and their interactions as well.

Table 6. Effect of inoculation with different isolates of Rhizobium on black colour nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 60 days							
RS-1	2.55	5.40	6.03	5.35	3.25	4.15	4.45
RS-2	4.35	8.30	3.00	9.45	2.55	5.00	5.44
RS-3	3.00	7.00	8.40	4.90	5.00	10.00	6.38
RS-4	4.00	5.00	5.00	9.00	4.00	4.30	5.21
RS-5	8.00	5.40	5.00	4.95	3.55	2.00	4.81
RS-6	5.00	6.10	4.10	8.00	4.10	4.26	5.26
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	3.84	5.31	4.50	5.95	3.20	4.24	4.50
B] At 90 days							
RS-1	6.10	7.24	11.00	8.04	10.00	8.06	8.40
RS-2	7.54	10.90	8.91	14.34	7.24	8.40	9.55
RS-3	15.22	16.10	12.30	12.41	15.16	16.02	14.53
RS-4	15.30	18.30	14.03	16.00	14.00	13.14	15.12
RS-5	10.05	10.04	10.10	10.00	6.16	10.40	9.45
RS-6	16.25	10.11	15.42	14.00	18.00	12.30	14.34
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	10.06	10.38	10.25	10.68	10.08	10.19	10.19
C] At harvest							
RS-1	8.87	12.00	12.25	14.09	11.40	12.24	11.80
RS-2	19.20	10.16	14.00	18.00	15.00	15.00	15.22
RS-3	17.00	23.07	25.00	16.06	21.03	19.25	20.23
RS-4	20.00	20.21	22.42	20.15	20.66	18.05	20.24
RS-5	12.05	16.20	19.08	17.25	13.00	12.03	14.93
RS-6	16.50	19.19	24.05	18.00	21.16	16.07	19.16
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	13.37	14.16	16.68	14.79	14.60	13.23	14.51

	S.E. ±			C.D. at 5%		
	A	B	C	A	B	C
Varieties (V)	0.400	0.590	0.190	1.140	N.S.	0.540
Inoculants (I)	0.430	0.640	0.200	1.230	1.820	0.590
Interaction (V X I)	1.070	1.580	0.510	3.020	4.460	1.440

N.S. = Non-significant

At 60 days after sowing, all the inoculation treatments recorded significantly higher number of black nodules per plant as compared to uninoculated control which recorded no black nodules. The inoculation treatment RS-3 recorded the highest number of black nodules (6.38). However, it was on par with RS-2, RS-4 and RS-6. Among the varieties, the variety HPK recorded the highest number of black nodules (5.95) per plant and was significantly superior to the other except Man (5.31). Among the interactions, the interaction RS-3 X KS-2 recorded the highest number of black nodules per plant (10.00), while RS-5 X KS-2 recorded the least number of black nodules per plant (2.00).

At 90 days after sowing all the inoculation treatments had significantly higher number of black nodules per plant as compared to control which recorded no black nodules. The isolate RS-4 recorded the highest number of black nodules (15.12) per plant. However it was at par with RS-3 (14.53) and RS-6 (14.34). Although, the results in respect of varieties were non-significant, the variety HPK recorded the highest number of black nodules per plant (10.68). Among the interactions, the interaction RS-4 X Man recorded the highest number of black nodules per plant (18.30), while RS-1 X Sina recorded the least number of black nodules per plant (6.10).

At harvesting stage all the inoculation treatments recorded significantly higher number of black nodules per

plant over the uninoculated control which recorded no black nodules. The inoculation treatment RS-4 recorded the highest number of black nodules per plant (20.24) and was significantly superior to the other isolates except RS-3 (20.23). Among the varieties TPK recorded significantly highest number of black nodules (16.68). The varieties Man (14.16), HPK (14.79) and Phule K-1 (14.68) were next effective and were at par with each other. Among the interactions, the interaction RS-3 X TPK recorded the highest number of black nodules per plant (25.00).

4.9 Number of small size nodules

The results (Table 7) in respect of number of small nodules per plant were significant for varieties and inoculation treatments at 30, 60 and 90 days after sowing and at harvesting. The interaction effects were significant at all the stages except at 60 days.

At 30 days after sowing, all the inoculation treatments differed significantly and recorded higher number of small nodules per plant as compared to the uninoculated control which did not record any nodules. The number of small nodules per plant ranged from 8.70 (RS-5) to 9.86 (RS-4). All the varieties differed significantly from each other. The number small nodules per plant ranged from 7.00 (TPK) to 8.82 (Phule K-1). Among the interactions, the interaction RS-4 X Man recorded significantly higher number of small nodules per

Table 7. Effect of inoculation with different isolates of Rhizobium on small size nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	10.00	6.68	9.00	9.00	10.15	7.05	8.64
RS-2	10.00	8.00	8.00	8.00	10.60	12.10	9.45
RS-3	9.10	7.00	7.00	10.00	10.00	9.05	8.69
RS-4	8.00	14.00	9.00	8.00	9.00	11.20	9.86
RS-5	9.00	7.00	8.00	9.00	10.00	9.20	8.70
RS-6	9.60	11.90	8.00	8.00	12.00	9.20	9.78
U.C.	0.00	0.00	0.00	0.00	0.0	0.00	0.00
Mean	5.10	7.94	7.00	7.42	8.82	7.97	7.73
B] At 60 days							
RS-1	3.10	5.25	4.82	4.32	8.60	8.45	5.75
RS-2	4.02	3.28	5.76	5.09	7.14	6.01	5.21
RS-3	4.10	4.30	5.14	5.01	9.12	8.10	5.96
RS-4	5.75	4.50	8.09	5.40	7.12	7.10	6.32
RS-5	5.00	4.48	7.20	4.46	8.16	7.48	6.13
RS-6	4.25	3.10	5.27	4.75	6.20	9.36	5.48
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	3.74	3.55	5.18	4.14	5.70	6.64	4.97
C] At 90 days							
RS-1	3.78	3.72	3.26	3.21	4.60	4.80	3.89
RS-2	4.10	5.48	4.37	3.07	5.28	5.83	4.68
RS-3	3.25	5.10	3.14	4.14	6.01	4.01	4.27
RS-4	4.65	3.58	6.38	4.22	5.26	5.09	4.86
RS-5	4.07	4.27	3.79	3.72	4.14	3.02	3.83
RS-6	3.40	3.02	4.00	3.10	5.10	6.74	4.22
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	3.32	3.59	3.56	3.06	4.34	4.21	3.67
D] At harvest							
RS-1	2.19	3.20	2.63	3.10	4.20	3.64	3.16
RS-2	4.00	4.36	3.47	3.01	4.82	3.21	3.81
RS-3	4.19	4.09	2.14	3.41	4.89	3.02	3.62
RS-4	4.28	2.40	4.30	3.29	4.62	4.80	3.94
RS-5	3.04	4.10	2.97	2.27	3.41	2.02	2.96
RS-6	6.32	3.05	3.02	3.01	4.10	5.63	4.18
U.C.	0.00	0.00	0.00	0.00	0.0	0.00	0.00
Mean	3.43	3.02	2.64	2.58	3.72	3.18	3.09

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.007	0.420	0.005	0.260	0.020	1.190	0.014	0.740
Inoculants (I)	0.008	0.450	0.005	0.280	0.022	1.280	0.015	0.800
Interaction (V X I)	0.019	1.120	0.001	0.700	0.055	N.S.	0.039	1.970

N.S. = Non-significant

plant (14.00), while RS-3 X Man and RS-3 X TPK recorded the least number of small nodules per plant (7.00). At 60 days all the inoculation treatments were at par with each other. The number of small nodules per plant ranged from 5.21 (RS-2) to 6.32 (RS-4). Among the varieties, Phule K-1 (5.70) and KS-2 (6.64) were significantly superior to the others.

At 90 days after sowing all the inoculation treatments differed significantly from each other. The number of small nodules per plant ranged from 3.83 (RS-5) to 4.86 (RS-4). All the varieties differed significantly from each other. The number of small nodules per plant ranged from 3.06 (HPK) to 4.34 (Phule K-1). Among the interactions, the interaction RS-6 X KS-2 recorded significantly higher number of small nodules per plant (6.74), while RS-5 X KS-2 and RS-6 X Man recorded the least number of small nodules per plant (3.02).

At harvesting stage, all the inoculation treatments were at par with each other and recorded significantly higher number of small nodules per plant as compared to uninoculated control which recorded no nodules. The number of small nodules per plant ranged from 2.96 (RS-5) to 4.18 (RS-6). All the varieties were at par with each other. The number of small nodules per plant ranged from 2.58 (HPK) to 3.72 (Phule K-1). Among the interactions, the interaction RS-6 X Sina recorded significantly higher number of small nodules per plant (6.32), while RS-5 X KS-2 recorded the least number of small nodules per plant (2.02).

4.10 Number of medium size nodules

The data presented (Table 8) in respect of number of medium size nodules per plant were significant for inoculation treatments and interaction effects. The results were non-significant for varieties at 90 days after sowing and significant at 30, 60 days after sowing and at harvest.

At 30 days after sowing all the inoculation treatments recorded significantly higher number of medium size nodules per plant as compared to uninoculated control which recorded no nodules. Similarly, all the inoculation treatments differed significantly from each other. The number of medium size nodules per plant ranged from 2.88 (RS-1) to 3.60 (RS-3). All the varieties differed significantly from each other. The number of medium size nodules per plant ranged from 2.16 (Man) to 3.57 (HPK). Among the interactions, the interaction RS-3 X HPK recorded significantly higher number of medium size nodules per plant (7.00), while RS-6 X Sina, RS-3 X Man, RS-5 X Man, RS-2 X TPK, RS-1 X TPK and RS-3 X Phule K-1 recorded the least number of medium nodules per plant (2.00).

At 60 days after sowing all the inoculation treatments recorded significantly higher number of medium size nodules per plant as compared to the uninoculated control which recorded no nodules. The isolate RS-4 recorded significantly highest number of medium sized nodules per plant (28.61). The number of medium size nodules per plant ranged

Table 8. Effect of inoculation with different isolates of Rhizobium on medium size nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	3.07	3.00	2.00	3.00	4.20	2.04	2.88
RS-2	3.00	2.10	2.00	4.00	3.34	3.06	2.91
RS-3	4.00	2.00	4.00	7.00	2.00	2.60	3.60
RS-4	3.00	4.00	2.20	4.00	3.67	3.51	3.39
RS-5	4.00	2.00	4.00	3.00	2.80	3.48	3.21
RS-6	2.00	2.08	3.00	4.00	2.80	3.80	2.94
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	2.72	2.16	2.45	3.57	2.68	2.64	2.70
B] At 60 days							
RS-1	14.20	12.01	14.26	16.71	12.09	20.00	14.87
RS-2	20.10	11.30	17.43	17.09	11.58	14.03	15.25
RS-3	26.05	36.25	30.05	17.18	30.25	24.05	27.30
RS-4	30.25	32.10	34.70	26.52	22.06	26.03	28.61
RS-5	25.10	18.65	12.35	25.43	12.36	12.50	17.73
RS-6	30.05	30.23	30.80	19.02	29.52	22.03	26.94
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	20.85	20.07	19.94	17.42	16.83	16.94	18.65
C] At 90 days							
RS-1	6.54	7.55	10.82	9.63	10.69	13.33	9.76
RS-2	13.67	9.29	10.39	18.67	8.55	4.82	10.89
RS-3	11.48	20.38	21.52	11.58	22.41	16.02	17.23
RS-4	16.21	19.09	14.32	17.79	14.07	14.78	16.04
RS-5	6.05	13.20	16.33	11.24	9.92	10.52	11.21
RS-6	17.80	18.00	12.64	12.97	22.78	11.01	15.86
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	10.25	12.50	12.28	11.69	12.63	10.68	11.57
D] At harvest							
RS-1	2.28	9.26	9.03	9.11	7.25	9.27	7.70
RS-2	12.97	10.22	12.05	10.11	9.10	8.16	10.43
RS-3	8.53	12.25	12.25	11.05	10.09	11.63	10.96
RS-4	17.87	13.07	16.20	16.04	12.41	10.35	14.32
RS-5	3.78	10.20	12.80	10.19	7.70	9.69	9.06
RS-6	16.01	12.17	18.30	13.63	12.60	7.68	13.39
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	8.76	9.59	11.51	10.01	8.45	8.11	9.48

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.002	0.025	0.750	0.010	0.007	0.072	N.S.	0.029
Inoculants (I)	0.002	0.027	0.810	0.011	0.007	0.078	2.280	0.031
Interaction (V X I)	0.006	0.068	1.990	0.027	0.019	0.191	5.600	0.078

N.S. = Non-significant

from 14.87 (RS-1) to 28.61 (RS-4). Among the varieties, the variety Sina recorded the highest number of medium sized nodules per plant (20.85) and was significantly superior to rest of the varieties. The number of medium size nodules per plant ranged from 16.83 (Phule K-1) to 20.85 (Sina). The interaction RS-4 X TPK recorded significantly higher number of medium sized nodules per plant (34.70), while RS-2 X Man recorded the least number of medium size nodules per plant (11.30).

At 90 days after sowing the inoculation treatments RS-6 (15.86), RS-3 (17.23) and RS-4 (16.04) were significantly superior to the others. The number of medium size nodules per plant ranged from 9.76 (RS-1) to 17.23 (RS-3). Among the interactions, the interaction RS-6 X Phule K-1 recorded the highest number of medium size nodules per plant (22.78), while RS-2 X KS-2 recorded the least number of medium size nodules per plant (4.82).

At harvesting stage all the inoculation treatments differed significantly from each other. They recorded significantly higher number of medium size nodules per plant as compared to the uninoculated control which recorded no nodules per plant. The number of medium size nodules per plant ranged from 7.70 (RS-1) to 14.32 (RS-4). All the varieties differed significantly from each other. The number of medium size nodules per plant ranged from 8.11 (KS-2) to 11.51 (TPK).

Among the interactions, the interaction RS-6 X TPK recorded the highest number of medium size nodules per plant (18.30), while RS-1 X Sina recorded the least number of medium size nodules per plant (2.28).

4.11 Number of large size nodules

The data on number of large size nodules recorded at 30, 60 and 90 days after sowing and at harvesting stage (Table 9) were significant for the inoculation treatments and interactions, whereas the varieties were found to be non-significant at harvesting, while significant at all the remaining stages i.e. 30, 60 and 90 days after sowing.

At 30 days after sowing, all inoculation treatments recorded significantly higher number of large nodules per plant as compared to the uninoculated control which recorded no nodules. The number of large nodules per plant ranged from 0.78 (RS-6) to 1.11 (RS-5). The isolate RS-5 recorded the highest number of large size nodules per plant (1.11). However, it was on par with RS-3 (1.07) and RS-2 (1.06). Among the varieties, the variety Man (0.92), TPK (0.94), HPK (0.92) and Phule K-1 (0.86) were significantly superior to KS-2 and Sina. Among the interactions, the interaction RS-3 X HPK recorded the highest number of large size nodules per plant (1.44), while RS-6 X Sina recorded the least number of large nodules per plant (0.40).

Table 9. Effect of inoculation with different isolates of Rhizobium on large size nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	0.80	1.28	1.06	0.78	0.80	0.51	0.87
RS-2	0.67	1.24	1.24	1.24	1.12	0.85	1.06
RS-3	0.90	0.76	1.37	1.44	1.15	0.85	1.07
RS-4	0.67	0.82	1.10	0.82	1.08	0.86	0.89
RS-5	1.00	1.30	1.25	1.22	1.20	0.69	1.11
RS-6	0.40	1.10	0.60	1.00	0.68	0.95	0.78
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.63	0.92	0.94	0.92	0.86	0.58	0.82
B] At 60 days							
RS-1	6.80	9.40	8.58	8.63	7.63	2.81	7.30
RS-2	8.45	10.82	4.81	12.62	8.28	9.96	9.15
RS-3	7.85	10.45	10.40	8.21	3.96	8.11	8.16
RS-4	12.00	13.40	9.21	13.73	8.82	2.17	9.88
RS-5	9.91	7.27	10.45	7.06	7.38	1.02	7.18
RS-6	11.00	11.00	6.13	10.83	6.48	4.87	8.38
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	8.00	8.90	7.08	8.72	6.07	4.13	7.15
C] At 90 days							
RS-1	10.02	13.09	10.32	12.32	11.05	9.03	10.97
RS-2	14.05	11.06	13.35	23.76	12.01	10.21	14.07
RS-3	16.15	19.85	16.26	12.06	10.09	18.09	15.41
RS-4	19.10	21.05	24.73	20.16	16.01	12.29	18.89
RS-5	15.22	12.65	11.74	15.10	16.00	15.32	14.33
RS-6	16.20	17.04	21.36	16.09	12.39	10.92	15.66
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	12.96	13.53	13.96	14.21	11.07	10.83	12.76
D] At harvest							
RS-1	10.55	7.80	10.76	11.30	11.20	13.43	10.75
RS-2	10.09	6.26	8.77	12.64	8.17	10.69	9.43
RS-3	13.30	22.88	23.77	12.45	16.72	18.10	17.87
RS-4	14.01	21.44	18.28	18.02	17.73	14.20	17.28
RS-5	17.21	12.56	14.01	15.37	9.00	10.62	13.12
RS-6	11.02	16.61	14.33	13.42	20.21	10.96	14.42
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	10.81	12.50	12.87	11.88	11.86	11.14	11.83

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.060	0.012	0.960	0.950	0.169	0.036	2.700	N.S.
Inoculants (I)	0.064	0.013	1.030	1.020	0.182	0.039	2.920	2.880
Interaction (V X I)	0.159	0.034	2.540	2.510	0.447	0.959	7.160	7.070

N.S. = Non-significant

At 60 days, all the inoculation treatments differed significantly from each other. The number of large nodules per plant ranged from 7.18 (RS-5) to 9.88 (RS-4). All the varieties differed significantly from each other. The variety Man recorded the highest number of large size nodules (8.90). Among the interactions, the interaction RS-4 X HPK recorded the highest number of large size nodules per plant (13.73), while RS-5 X KS-2 recorded the least number of large size nodules per plant (1.02).

At 90 days after sowing, all the inoculation treatments recorded significantly higher number of large size nodules per plant as compared to the uninoculated control which recorded no nodules. The isolate RS-4 recorded significantly highest number of large size nodules per plant (18.89). The number of large size nodules ranged from 10.97 (RS-1) to 18.89 (RS-4). The varieties Sina (12.96), Man (13.53), TPK (13.96) and HPK (14.21) were significantly superior to Phule K-1 and KS-2.

At harvesting stage, all the inoculation treatments recorded significantly higher number of large size nodules per plant as compared to uninoculated control which recorded no nodules per plant. The inoculation treatments RS-3 (17.87) and RS-4 (17.28) were significantly superior to the others. Among the interactions, the interaction RS-3 X TPK recorded the highest number of large size nodules per plant (23.77), while

RS-2 X Man recorded the least number of large nodules per plant (6.26).

4.12 Nodule dry matter (mg)

The results in respect of dry matter weight of nodules per plant at 30, 60 and 90 days after sowing and at harvest (Table 10, Fig. 3 & 4) were significant for inoculation treatments, while non-significant for varieties and their interactions at all the stages.

At 30 days after sowing, all the inoculation treatments recorded significantly higher dry matter weight of nodules per plant as compared to the uninoculated control. However, all the inoculation treatments were at par with each other. The dry matter weight of nodules per plant ranged from 3.79 mg to 4.32 mg.

At 60 days after sowing, all the inoculation treatments recorded significantly higher dry matter weight of nodules compared to the uninoculated control. The dry matter weight of nodules per plant ranged from 15.88 (RS-1) to 28.25 (RS-4). The isolate RS-4 recorded significantly highest dry matter weight in the nodules (28.25 mg).

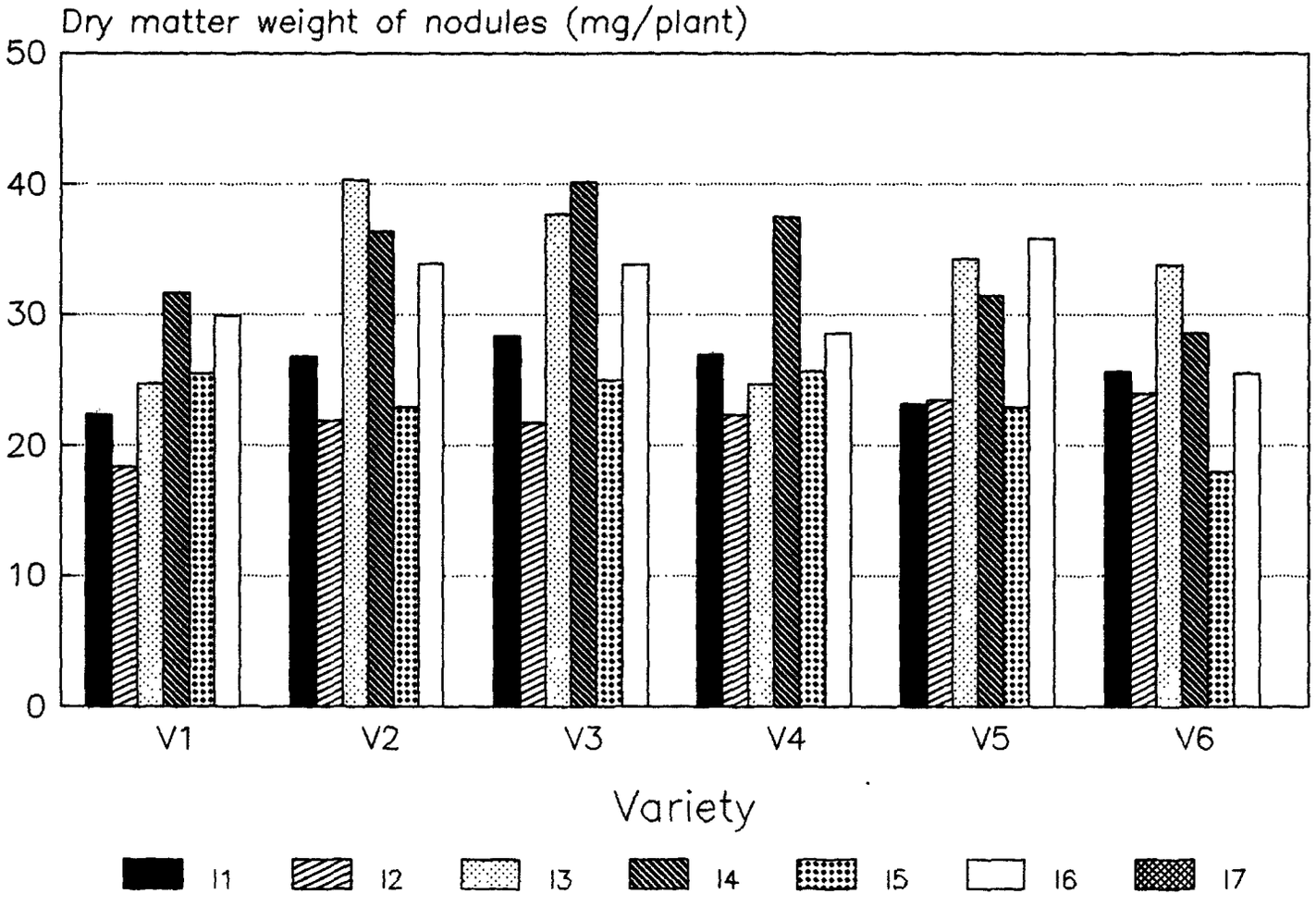
At 90 days after sowing, all the inoculation treatments recorded significantly higher dry matter weight of nodules per plant as compared to the uninoculated control. The

Table 10. Effect of inoculation with different isolates of Rhizobium on dry matter weight (mg/plant) of nodules per plant in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	4.35	3.35	3.69	3.91	4.63	2.86	3.79
RS-2	4.01	3.47	3.44	4.05	4.61	4.69	4.04
RS-3	3.67	4.66	3.55	3.97	4.73	4.27	4.14
RS-4	4.72	5.76	3.76	3.92	4.01	4.76	4.32
RS-5	4.27	3.15	4.05	3.74	4.28	4.01	3.91
RS-6	4.22	2.98	3.48	5.64	4.33	3.82	4.07
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	3.46	3.33	3.13	3.60	3.79	3.48	3.47
B] At 60 days							
RS-1	13.67	15.18	15.78	16.85	16.13	17.68	15.88
RS-2	18.55	17.31	21.64	19.82	15.38	17.09	18.29
RS-3	21.64	29.05	25.97	17.31	24.68	22.93	23.59
RS-4	27.34	28.48	46.48	25.66	21.64	20.11	28.25
RS-5	27.79	17.31	17.09	21.05	15.89	11.96	17.68
RS-6	25.68	25.25	24.04	19.71	24.04	20.65	23.22
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	18.52	18.94	21.54	17.20	16.82	15.77	18.13
C] At 90 days							
RS-1	22.32	26.77	28.32	26.89	23.16	25.65	25.51
RS-2	18.32	21.86	21.70	22.36	23.46	23.96	21.94
RS-3	24.70	40.29	37.70	24.66	34.23	33.88	32.57
RS-4	31.61	36.37	40.17	37.48	31.42	28.58	34.27
RS-5	25.45	22.96	24.98	25.63	22.96	18.00	23.33
RS-6	29.92	33.83	33.77	28.58	35.79	35.48	31.22
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	21.76	26.01	26.66	23.65	24.43	22.22	24.12
D] At harvest							
RS-1	26.03	29.11	32.28	32.36	28.93	24.36	28.84
RS-2	16.47	23.96	22.86	26.64	27.70	28.80	24.40
RS-3	36.15	34.51	38.64	32.79	40.26	29.47	35.40
RS-4	39.19	40.01	42.03	40.74	38.58	32.01	38.76
RS-5	25.72	23.59	26.33	28.10	26.09	24.06	25.64
RS-6	28.40	40.40	41.47	28.26	34.66	31.05	34.04
U.C.	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	24.56	27.36	29.08	26.98	28.03	24.25	26.71

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.290	1.520	1.490	1.650	N.S.	N.S.	N.S.	N.S.
Inoculants (I)	0.310	1.640	1.610	1.780	0.890	4.620	4.540	5.010
Interaction (V X I)	0.770	4.020	3.950	4.360	N.S.	N.S.	N.S.	N.S.

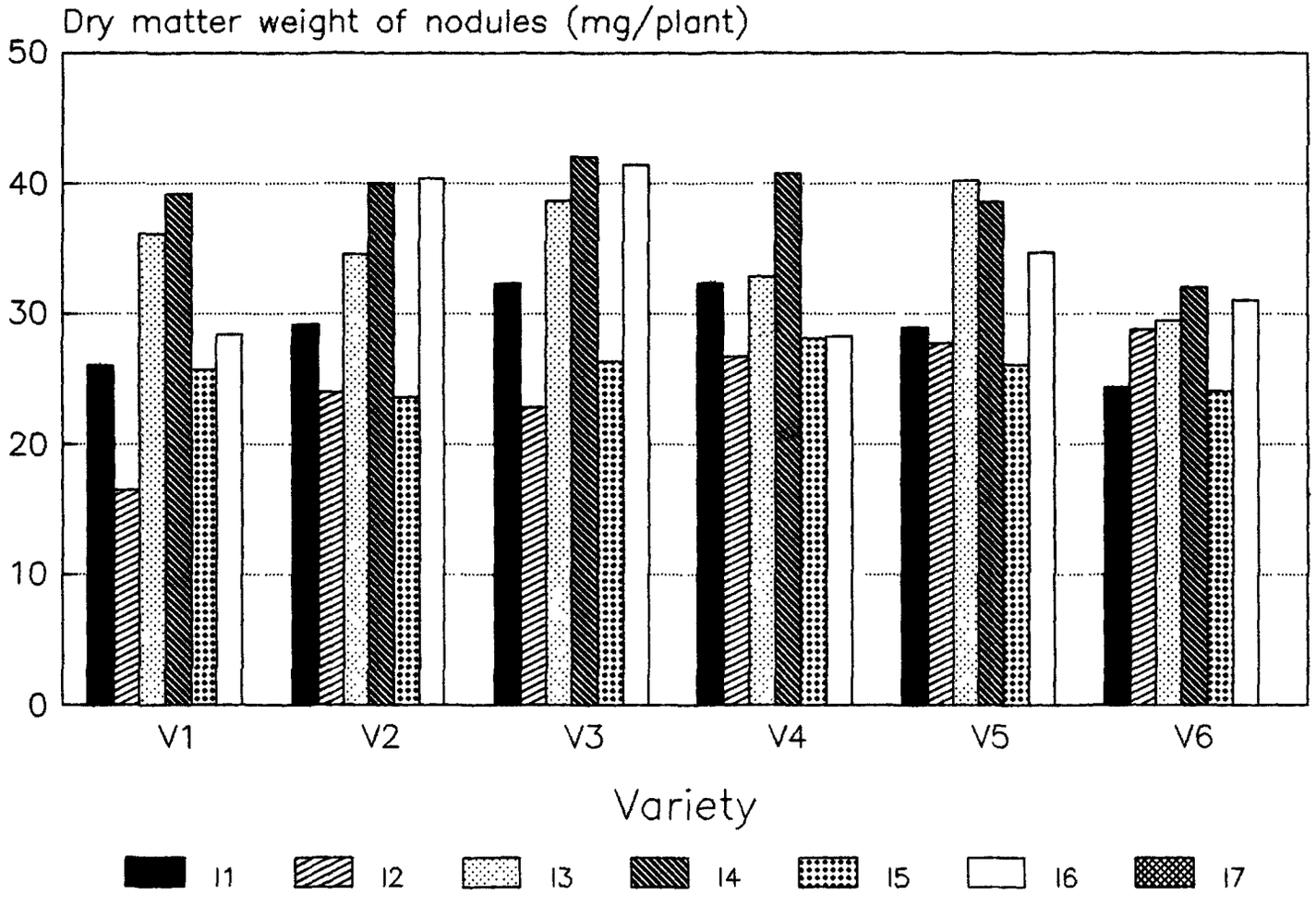
N.S. = Non-significant



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 3. Dry matter weight of nodules per plant (mg/plant) of horsegram varieties at 90 days as influenced by rhizobial inoculants



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 4. Dry matter weight of nodules per plant (mg/plant) of horsegram varieties at harvest as influenced by rhizobial inoculants

dry matter weight of nodules per plant ranged from 21.94 mg (RS-2) to 34.27 mg (RS-4). The isolate RS-4 recorded the highest nodule dry matter per plant (34.27 mg) and was significantly superior to the other isolates except RS-3 (32.57 mg) and RS-6 (31.22 mg).

At harvesting, all the inoculation treatments recorded significantly higher dry matter weight of nodules per plant compared to the uninoculated control. The dry matter weight of nodules per plant ranged from 24.40 mg (RS-2) to 38.76 mg (RS-4). The isolates viz., RS-4, RS-3 and RS-6 were significantly superior to the others.

4.13 Plant dry matter (g)

The results (Table 11, Fig. 5 & 6) in respect of dry matter weight of plant were significant for inoculation treatments at all the stages. The varieties had a non-significant influence on plant dry matter at all the stages. Similarly, the interactions had significant effect on plant dry matter only at harvest stage.

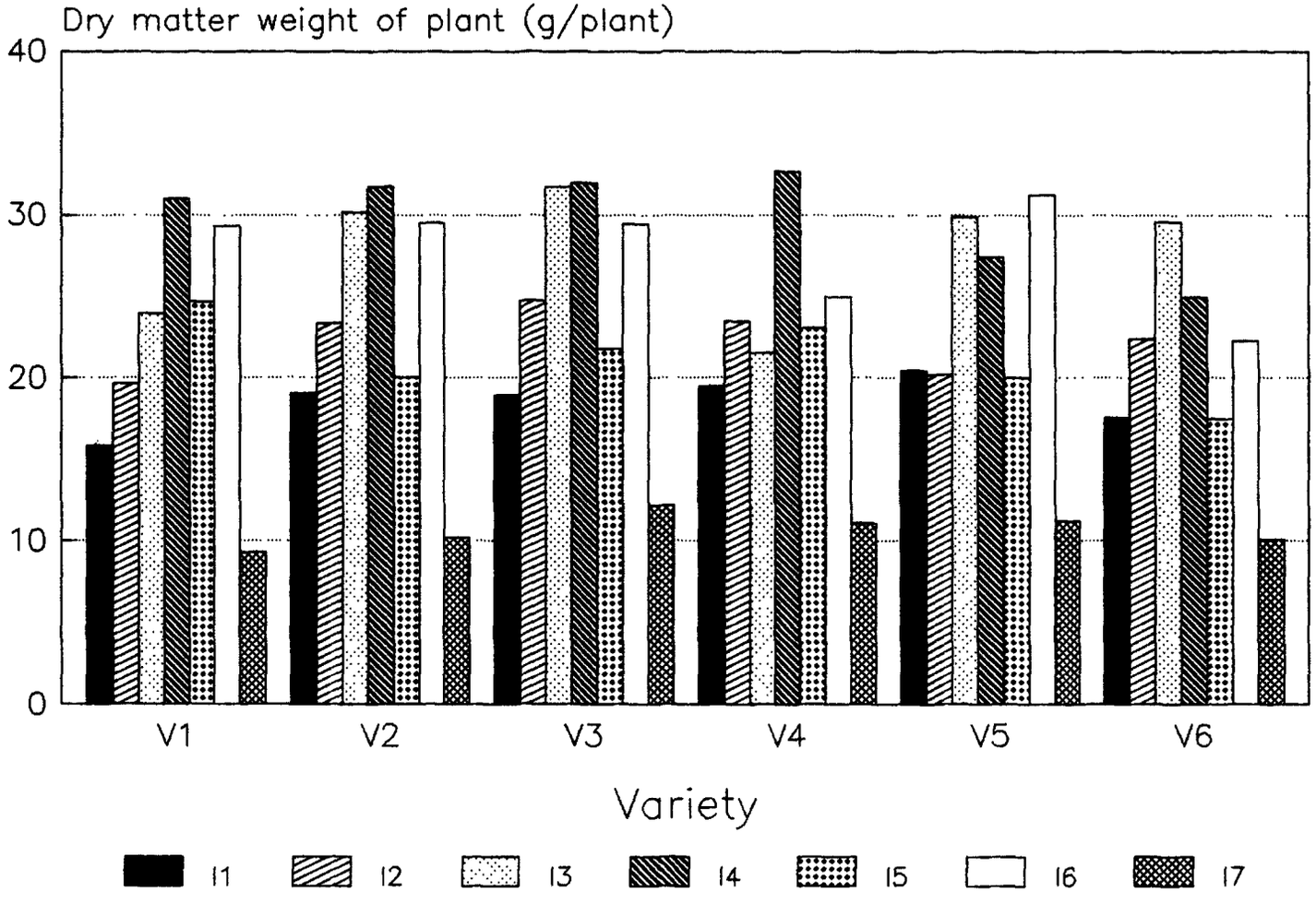
At 30 days after sowing, all the inoculation treatments recorded significantly higher plant dry matter over the uninoculated control (0.67 g) per plant. However, all the inoculation treatments were at par with each other. The dry matter weight of plant due to different isolates ranged from 0.92 g (RS-1) to 1.02 (RS-4).

Table 11. Effect of inoculation with different isolates of Rhizobium on dry matter weight of plant (g/plant) in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	1.05	0.80	0.88	0.89	1.11	0.80	0.92
RS-2	1.02	0.71	0.83	1.35	1.03	0.91	0.97
RS-3	0.88	1.11	0.85	0.95	1.13	1.02	0.99
RS-4	0.85	1.34	0.90	0.94	0.98	1.14	1.02
RS-5	1.02	0.75	0.97	0.89	1.02	0.98	0.93
RS-6	1.00	0.83	0.82	0.97	1.10	1.17	0.98
U.C.	0.71	0.70	0.66	0.68	0.66	0.61	0.67
Mean	0.93	0.89	0.84	0.95	1.00	0.94	0.92
B] At 60 days							
RS-1	8.20	8.99	8.29	9.70	8.49	9.90	8.92
RS-2	12.00	9.12	10.00	11.08	8.37	9.50	10.01
RS-3	11.40	15.30	14.34	12.12	12.99	12.07	13.03
RS-4	14.40	15.00	15.40	13.69	11.40	10.59	13.41
RS-5	9.77	10.12	11.40	10.44	8.10	9.45	9.88
RS-6	13.59	13.29	13.66	10.38	12.66	10.87	12.40
U.C.	7.68	7.0	6.44	7.48	6.20	7.42	7.05
Mean	11.00	11.27	11.36	10.69	9.74	9.97	10.67
C] At 90 days							
RS-1	15.81	19.06	18.92	19.49	20.46	17.55	18.54
RS-2	19.68	23.34	24.69	23.45	20.19	22.36	22.28
RS-3	23.93	30.13	31.71	25.50	29.84	29.54	27.77
RS-4	30.96	31.71	31.93	32.68	27.39	24.92	29.93
RS-5	24.66	20.01	21.78	23.01	20.02	17.49	21.16
RS-6	29.21	29.49	29.45	24.92	31.20	22.21	27.76
U.C.	9.28	10.22	12.15	11.10	11.24	10.05	10.67
Mean	21.94	23.42	24.67	22.30	22.90	20.59	22.59
D] At harvest							
RS-1	35.59	36.50	28.00	30.95	35.65	29.50	32.69
RS-2	33.28	38.08	32.86	33.06	36.73	36.60	35.10
RS-3	39.93	35.50	41.00	33.05	34.85	45.00	38.22
RS-4	38.10	36.88	40.68	41.00	36.60	38.78	38.67
RS-5	34.65	34.00	36.73	41.08	33.95	28.00	34.73
RS-6	31.96	30.85	45.00	43.90	42.00	32.84	37.75
U.C.	20.42	22.46	22.48	25.25	28.85	26.02	24.24
Mean	33.41	33.46	35.25	35.47	35.51	33.82	34.49

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.063	0.560	1.270	1.210	N.S.	N.S.	N.S.	N.S.
Inoculants (I)	0.068	0.600	1.370	1.310	0.190	1.700	3.880	3.680
Interaction (V X I)	0.160	1.480	3.370	3.210	N.S.	N.S.	N.S.	9.030

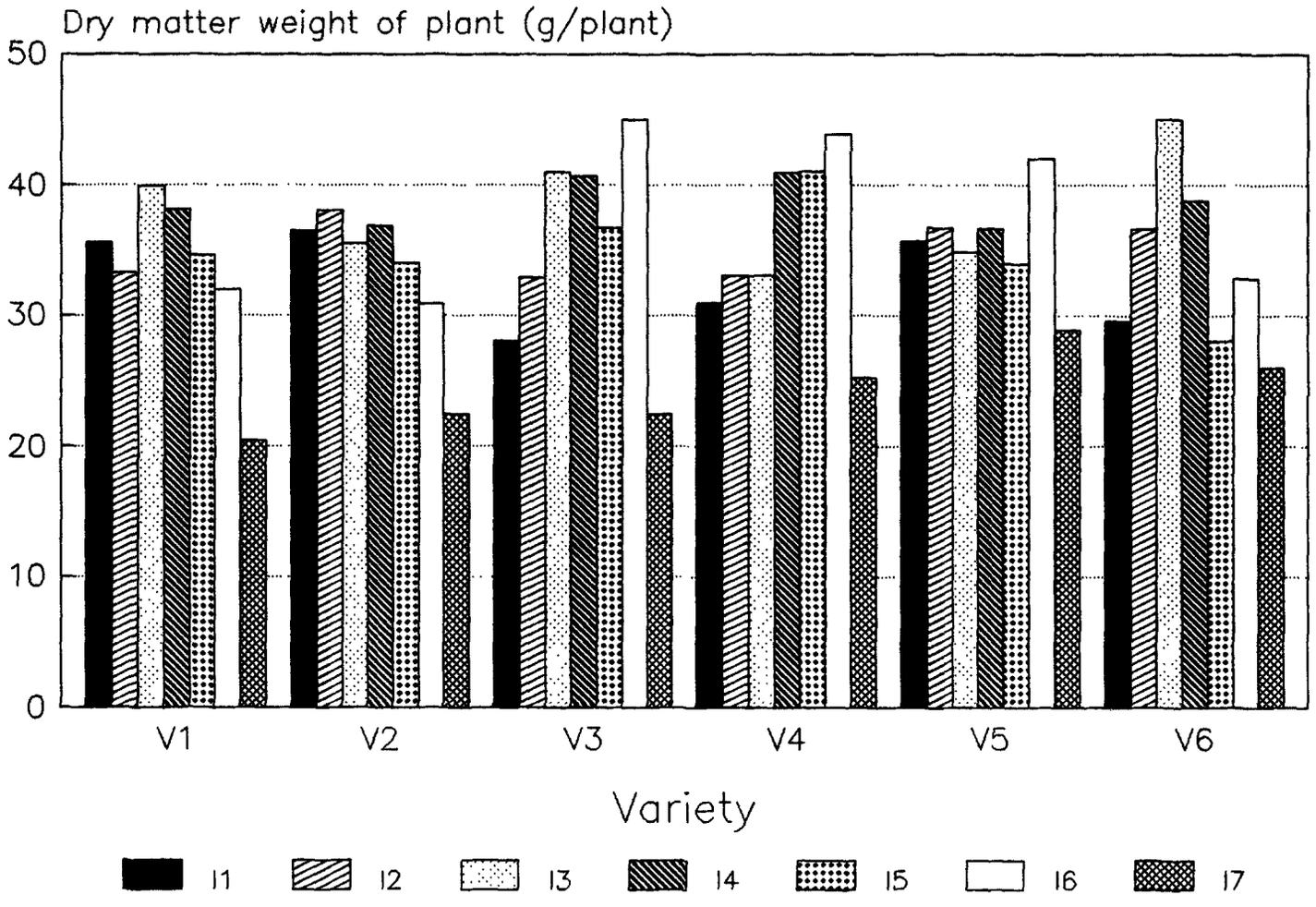
N.S. = Non-significant



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 5. Dry matter weight of plant (g/plant) of horsegram varieties at 90 days as influenced by rhizobial inoculants



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 6. Dry matter weight of plant (g/plant) of horsegram varieties at harvest as influenced by rhizobial inoculants

At 60 days after sowing all the inoculation treatments had significantly higher plant dry matter as compared to uninoculated control (7.05 g/plant). The dry matter weight of plant ranged from 8.92 g (RS-1) to 13.41 g (RS-4). The inoculation treatments RS-4, RS-3 and RS-6 were significantly superior to the others.

At 90 days after sowing, all the inoculation treatments recorded significantly higher plant dry matter as compared to the uninoculated control (10.67 g/plant). The plant dry matter ranged from 18.54 g (RS-1) to 29.93 g (RS-4). The isolates viz., RS-4, RS-3 and RS-6 were significantly superior to the others.

At harvesting stage, all the inoculation treatments recorded significantly higher dry matter weight of plant as compared to the uninoculated control (24.24 g/plant). The dry matter weight of plant ranged from 32.69 g per plant (RS-1) to 38.67 g per plant (RS-4). The isolate RS-4 recorded the highest plant dry matter (38.64 g). However, it was on par with RS-3 (38.22 g), RS-6 (37.75 g) and RS-2 (32.10 g).

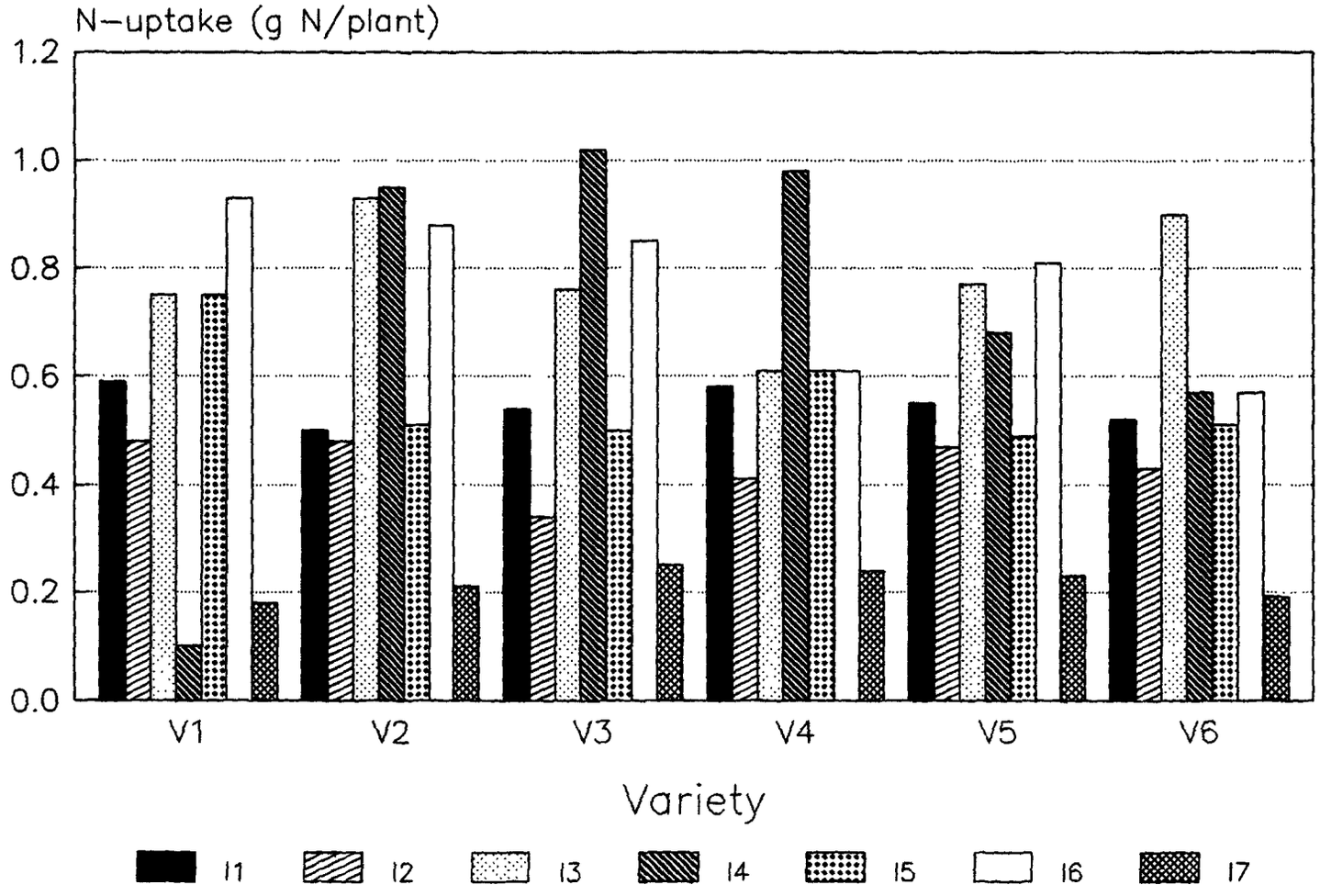
4.14 N-uptake by plant

The results (Table 12, Fig. 7 & 8) in respect of the N-uptake by plant at 30, 60 and 90 days after sowing and at harvest were significant for inoculation treatments and non-significant for their interaction effect. The varieties

Table 12. Effect of inoculation with different isolates of *Rhizobium* on N-uptake/plant (g N/plant) in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	0.034	0.026	0.027	0.030	0.035	0.026	0.030
RS-2	0.036	0.027	0.031	0.029	0.032	0.032	0.031
RS-3	0.031	0.044	0.028	0.031	0.037	0.034	0.034
RS-4	0.034	0.053	0.029	0.033	0.032	0.037	0.036
RS-5	0.035	0.024	0.027	0.045	0.034	0.030	0.032
RS-6	0.035	0.029	0.027	0.030	0.037	0.038	0.033
U.C.	0.022	0.020	0.020	0.020	0.016	0.019	0.019
Mean	0.033	0.032	0.027	0.031	0.032	0.031	0.031
B] At 60 days							
RS-1	0.317	0.237	0.300	0.277	0.223	0.270	0.271
RS-2	0.273	0.303	0.197	0.233	0.207	0.257	0.245
RS-3	0.443	0.440	0.450	0.357	0.343	0.333	0.394
RS-4	0.563	0.503	0.503	0.420	0.273	0.350	0.436
RS-5	0.463	0.253	0.267	0.320	0.233	0.250	0.298
RS-6	0.517	0.317	0.410	0.327	0.297	0.317	0.364
U.C.	0.230	0.167	0.143	0.170	0.127	0.183	0.170
Mean	0.401	0.317	0.324	0.300	0.243	0.283	0.311
C] At 90 days							
RS-1	0.593	0.503	0.547	0.580	0.557	0.520	0.550
RS-2	0.480	0.487	0.347	0.410	0.473	0.430	0.338
RS-3	0.753	0.930	0.767	0.610	0.773	0.907	0.790
RS-4	0.010	0.950	1.027	0.983	0.683	0.573	0.871
RS-5	0.750	0.510	0.503	0.617	0.490	0.517	0.564
RS-6	0.933	0.883	0.857	0.617	0.813	0.570	0.779
U.C.	0.187	0.210	0.253	0.240	0.237	0.297	0.221
Mean	0.672	0.639	0.614	0.580	0.575	0.521	0.602
D] At harvest							
RS-1	0.737	0.903	0.807	0.783	0.910	0.847	0.831
RS-2	0.963	0.820	0.430	0.720	0.743	0.667	0.724
RS-3	0.830	0.723	1.063	1.103	0.947	0.823	0.915
RS-4	0.980	0.887	1.170	1.070	0.810	0.970	0.981
RS-5	0.947	0.840	0.853	0.803	0.757	1.033	0.872
RS-6	0.817	0.613	1.027	1.087	1.140	0.677	0.893
U.C.	0.337	0.407	0.370	0.480	0.490	0.383	0.411
Mean	0.801	0.742	0.817	0.864	0.828	0.771	0.804

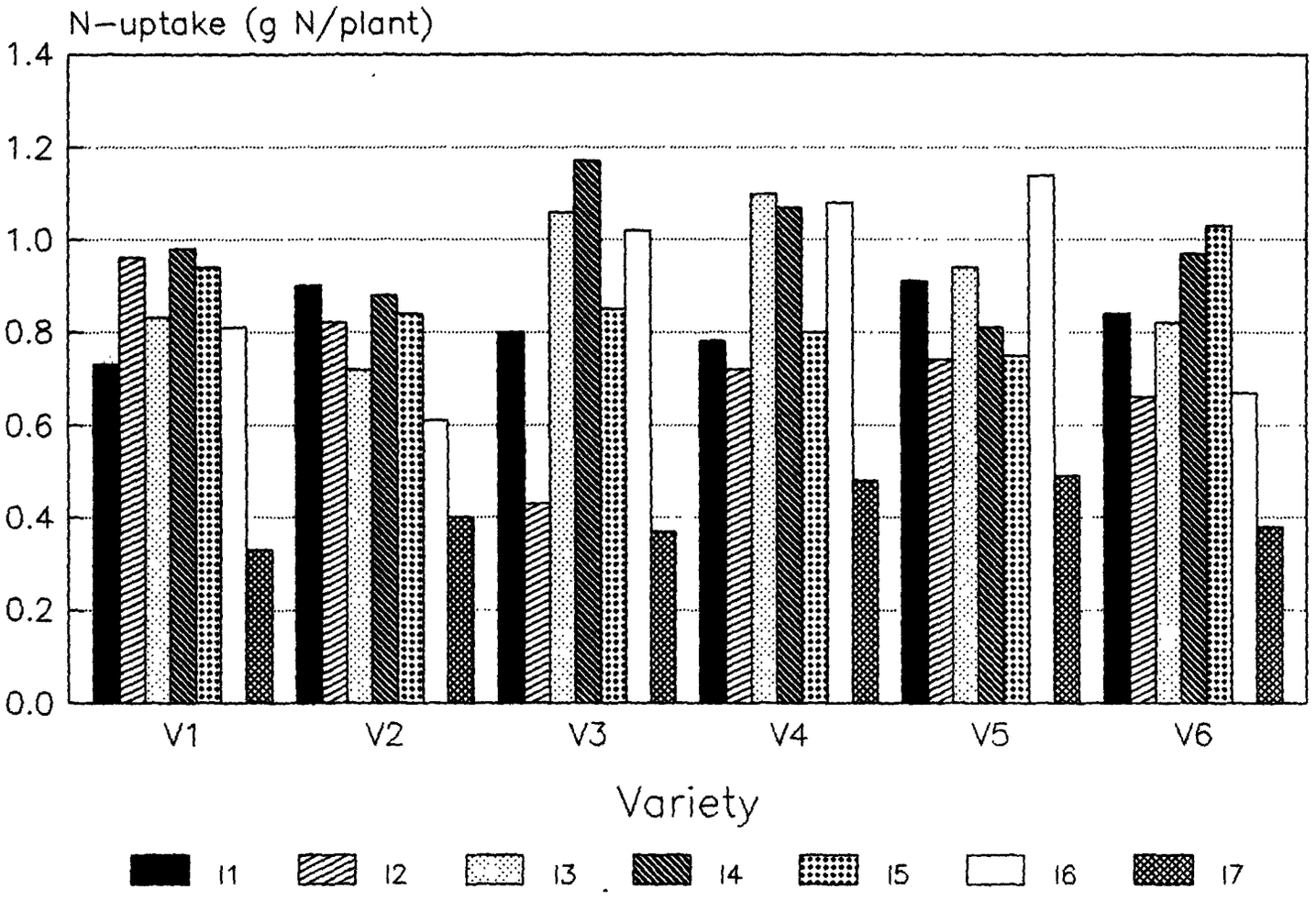
	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.003	0.020	0.047	0.058	N.S.	0.058	N.S.	N.S.
Inoculants (I)	0.003	0.020	0.050	0.063	0.009	0.063	0.140	0.170
Interaction (V X I)	0.007	0.050	0.124	0.154	N.S.	N.S.	N.S.	N.S.



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 7. N-uptake per plant (g N/plant) of horsegram varieties at 90 days as influenced by rhizobial inoculants



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 8. N-uptake per plant (g N/plant) of horsegram varieties at harvest as influenced by rhizobial inoculants

had a significant effect on N-uptake only at 60 days after sowing.

At 30 days after sowing, all the inoculation treatments recorded significantly higher N-uptake by plant as compared to the uninoculated treatment (0.019 g/plant). However, all the inoculation treatments were at par with each other. The N-uptake by plant due to different isolates ranged from 0.030 g per plant (RS-1) to 0.036 g per plant (RS-4).

At 60 days after sowing, all the inoculation treatments recorded significantly higher N-uptake by plant as compared to the uninoculated control (0.170 g/plant). The N-uptake by plant due to different isolates ranged from 0.245 g per plant (RS-2) to 0.436 g per plant (RS-4). The isolate RS-4 recorded the highest N-uptake (0.436 g/plant) and was significantly superior to rest of the isolates except RS-3 (0.394 g N/plant). Among the varieties, the variety Sina recorded significantly highest N-uptake (0.401 g/plant).

At 90 days after sowing all the inoculation treatments recorded significantly higher N-uptake by plant as compared to uninoculated control (0.221 g/plant). The N-uptake by plant due to different isolates ranged from 0.338 g per plant to 0.871 g per plant. The inoculation treatments RS-3 (0.790 g N/plant), RS-4 (0.871 g N/plant) and RS-6 (0.779 g N/plant) were at par with each other but significantly superior to the other isolates.

At harvesting stage, all the inoculation treatments recorded significantly higher N-uptake by plant as compared to the uninoculated control (0.411 g N/plant). The N-uptake by plant due to different isolates ranged from 0.724 g per plant (RS-2) to 0.981 g per plant (RS-4). All the other isolates were significantly superior to RS-2 in respect of N-uptake by plant.

4.15 Grain yield

The results (Table 13, Fig. 9) in respect of grain yield per plant were significant for inoculation treatments, while non-significant for varieties and interaction effects.

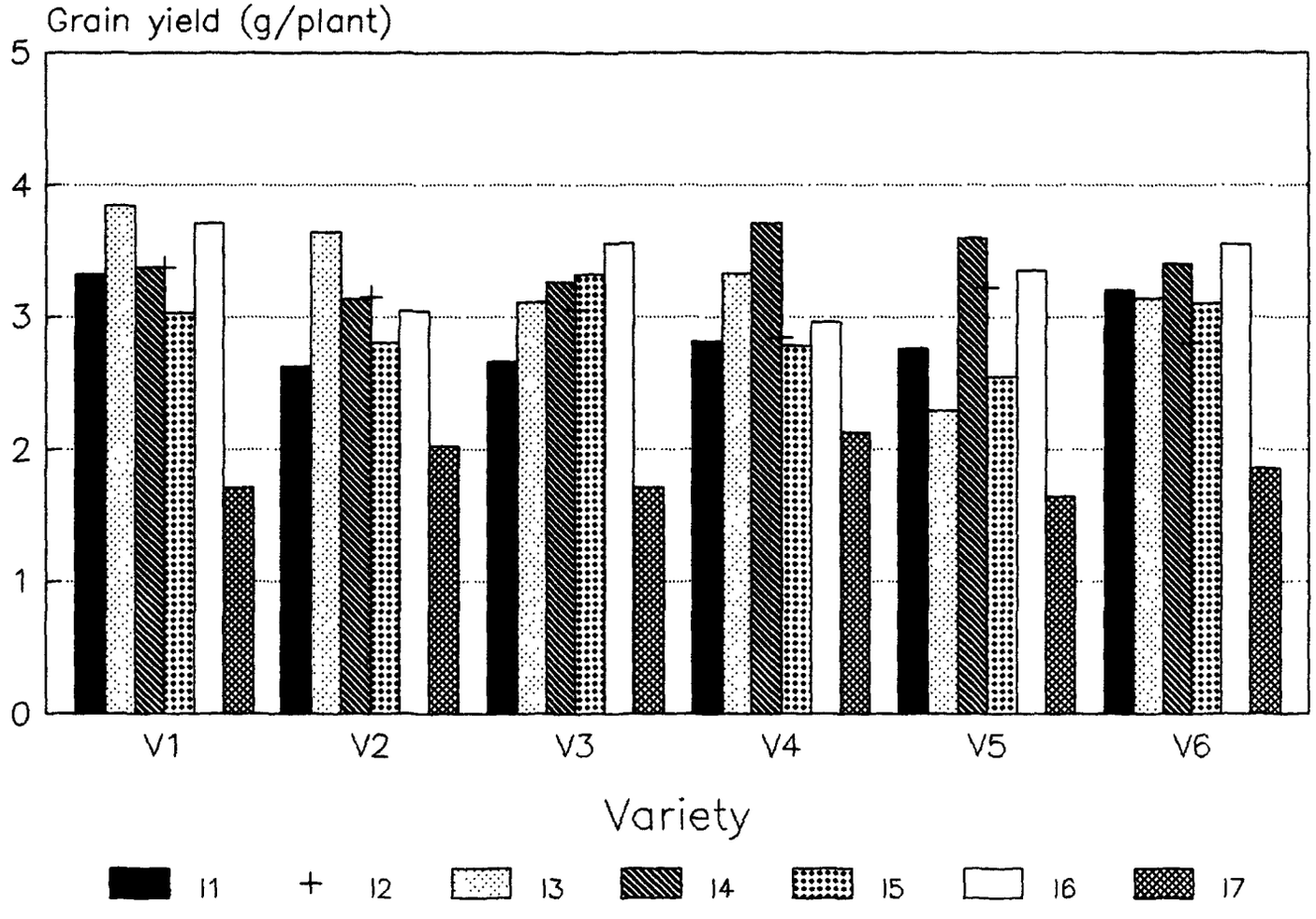
All the inoculation treatments recorded significantly higher grain yield per plant compared to the uninoculated control which recorded grain yield of only 1.84 g per plant. The grain yield per plant due to different isolates ranged from 2.89 g per plant (RS-1) to 3.41 g per plant (RS-4). The isolates viz., RS-4 (3.41 g/plant), RS-3 (3.39 g/plant), RS-6 (3.36 g/plant) and RS-2 (3.07 g/plant) were significantly superior to RS-1 and RS-5 but were at par with each other.

Table 13. Effect of inoculation with different isolates of *Rhizobium* on grain yield (g/plant) in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
RS-1	3.32	2.62	2.66	2.81	2.76	3.20	2.89
RS-2	3.37	3.15	3.05	2.84	3.22	2.80	3.07
RS-3	3.84	3.64	3.11	3.33	3.29	3.14	3.39
RS-4	3.37	3.13	3.26	3.71	3.60	3.40	3.41
RS-5	3.03	2.80	3.32	2.78	2.55	3.10	2.93
RS-6	3.71	3.04	3.56	2.96	3.35	3.55	3.36
U.C.	1.71	2.02	1.71	2.13	1.64	1.86	1.84
Mean	3.19	2.91	2.95	2.94	2.91	3.01	2.99

	S.E. ±	C.D. at 5%
Varieties (V)	0.14	N.S.
Inoculants (I)	0.15	0.42
Interaction (V X I)	0.37	N.S.

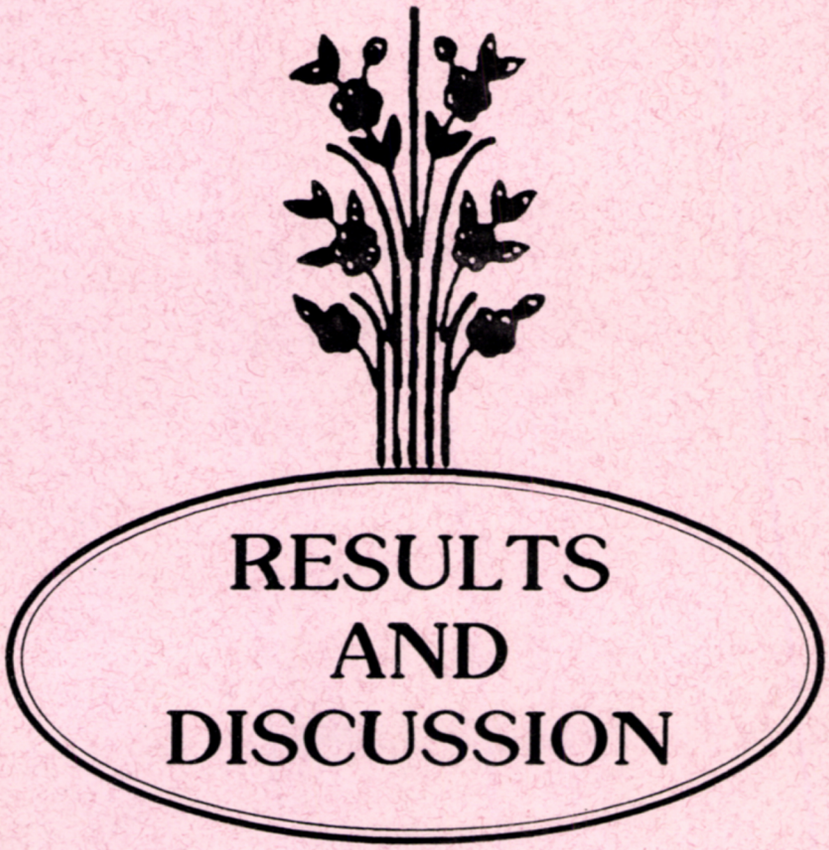
N.S. = Non-significant



V_1 = Sina V_2 = Man V_3 = TPK V_4 = HPK V_5 = Phule K-1 V_6 = KS-2

I_1 = RS-1 I_2 = RS-2 I_3 = RS-3 I_4 = RS-4 I_5 = RS-5 I_6 = RS-6

Fig. 9. Grain yield per plant (g /plant) of horsegram varieties as influenced by rhizobial inoculants



RESULTS AND DISCUSSION

5. DISCUSSION

5.1 Isolation and characterization of rhizobial isolates

The samples of horsegram varieties collected for rhizobial isolation showed large, pink and abundant nodules which indicated the presence of homologous rhizobia in the soil. All the isolates were non-spore formers, gram negative, non acid fast, rod shaped, encapsulated showing translucent milky white colonies on congo red yeast extract mannitol agar. These characters were identical as described in recent edition of Bergey's Manual (1984). Nodulation testes conducted for all the isolates using Leonard jars confirmed them as rhizobia specific for horsegram. Further, all the isolates when used as seed inoculant for six varieties of horsegram in a pot culture experiment using sterile soil showed production of abundant nodulation and thus confirmed as rhizobial isolates specific for horsegram.

5.2 Seed germination

The results in general indicated that inoculation with different strains of Rhizobium increased the germination percentage of all the varieties of horsegram. This shows effective symbiotic association of all the rhizobial strains with horsegram genotypes. Obviously there are no reports on effect of rhizobial inoculation on seed germination of crops.

5.3 Observations on nodulation

The results on nodulation in general indicated that all the rhizobial inoculants significantly increased the number of nodules per plant, pink nodules per plant and large size nodules per plant. Although, the varieties of horsegram showed non-significant response, a variations and increase in total number of pink and large size nodules were observed due to inoculation with Rhizobium. Interactions of all the varieties with rhizobial inoculants recorded from 20.34 to 45.50 nodules per plant at 90 days (maximum flowering period) after sowing, while uninoculated control combinations did not record any of the nodules as soil used for pot culture experiment was sterile. There was an increase in number of total, pink and large size nodules from 30 to 90 days after sowing, while at harvest there was a decline. This could be due to more diversions of plant nutrients for grain formation and decrease in nutrient supply to microsymbiont (Rhizobium). All the isolates could produce large and pink nodules indicating their effectiveness in symbiosis with horsegram.

The present results of increase in nodulation due to Rhizobium inoculation in horsegram varieties are similar to those of Bagyaraj and Hegde (1978) and Sairam et al (1989) in cowpea; Gangawane and Datar (1977) and Rice and Olsen (1983) in lucernae and Prasad and Ram (1984) and Basu et al. (1989) in green gram. Differential response of gram cultivars to

interactions of varieties with uninoculated control. The increased dry matter weight in plants inoculated with Rhizobium could be attributed to the increase in nutrient uptake especially N as evidenced from the data on N uptake. It is proved beyond no doubts that Rhizobium acts as symbiont with legume host, fixed atmospheric nitrogen and ehances its uptake by legume host which helps in accumulation of dry matter in inoculated host.

The inoculation with Rhizobium has been reported to increase dry matter weight of various crops viz., lucerne (Gangawane and Datar, 1977); clover (Nuti et al., 1981); gram (Patil and Medhane, 1974; Raju and Samuel, 1976; Gangawane and Datar, 1977 and Sing et al., 1978); cowpea (Bagchi et al., 1981) and green gram (Idris and Sandhu, 1979); Prasad and Ram, 1984 and Basu et al, 1989). The present results are therefore, in conformity with these earlier reports. Gasser et al. (1972) in alfalfa and Pawar et al. (1977) in cowpea cultivars reported variations in dry matter production due to Rhizobium inoculation. Similarly, Marevckova and Brozeva (1984) and Hegaje et al. (1989) reported varied increase in dry matter weight of lucerne genotypes due to Rhizobium inoculation. The present results of variations in increased dry matter weight of horsegram cultivars were in close agreement with the above reports.

5.5 Nitrogen uptake by horsegram varieties

The results on nitrogen uptake in general indicated that all the rhizobial isolates increased N uptake by horsegram varieties over their corresponding uninoculated treatments. All the varieties differed greatly in N uptake. As already stated, main function of Rhizobium in symbiosis with legume crop is to fix atmospheric nitrogen and increase its uptake by inoculated crop and this is the reason to note the increased uptake of N is all inoculated treatment combinations as compared to uninoculated treatment combinations. The enhanced N uptake due to inoculation with Rhizobium has been reported in peanut (Chomchalow, 1970), mung (Nagi and Thakur, 1985); black gram (Bhuiya et al., 1989) and in cowpea. Patil and Moniz (1974) in gram cultivars reported varied increase in N uptake due to inoculation with Rhizobium. The present results were therefore, in conformity with these earlier reports.

5.6 Grain yield of horsegram varieties

The results on grain yield of horsegram varieties as influenced by Rhizobium inoculants in general indicated that Rhizobium inoculation increased grain yields from 2.89 to 3.41 g per plant as against 1.84 g per plant in uninoculated control. The varieties showed differences in grain yield which could be mainly attributed to the inherent yielding capacity of the horsegram varieties. The rhizobial inoculation is known

for its nitrogen fixation and increasing nitrogen uptake of legume host which increase growth, accumulates dry matter and simultaneously increases yield. The increase in grain yield due to inoculation with Rhizobium has been reported in green gram (Idris and Sandhu, 1979; Balasubramanian et al., 1980, Vidayasurian et al., 1983; Prasad and Ram, 1984 and Basu et al., 1989), cowpea (Bagyaraj and Hegde, 1978) and groundnut (More et al., 1981; Singaravadivel and Prasad, 1981) with which the present results are in close agreement. The inoculation of Rhizobium has been reported to be crop genotype dependent. The variation in grain yields of horsegram varieties due to inoculation with Rhizobium could be attributed to the fact that effectiveness of Rhizobium inoculation is crop genotype dependent as reported by Pawar et al. (1977), Patil and Shinde (1980) and Jadhav et al. (1990).

Considering all the traits studied in the present investigation appears that all the rhizobial isolates could effectively nodulate, increase N uptake, dry matter weight and grain yield of horse gram varieties.

Summary and Conclusions

6. SUMMARY AND CONCLUSIONS

The present investigation was carried out with the objectives (1) to isolate and characterise Rhizobium from six varieties of horsegram and (ii) to see the effect of inoculation with different isolates of Rhizobium on nodulation, N uptake and yield of six genotypes of horsegram.

Six isolates of Rhizobium were obtained from horsegram varieties and these were studied for various morphological features. All the isolates were rod shaped, Gram negative, non-acid fast, non-spore formers, rods measuring 2.30 to 3.24 µm in length and 0.63 to 0.96 µm in width and all the isolates were encapsulated. Nodulation test for all isolates using Leonard jar assembly, recorded abundant nodulation in horsegram and confirmed that these isolates were of Rhizobium specific for horsegram. A pot culture experiment in sterile soil was conducted in a Factorial Completely Randomized Design with replications. The treatments of inoculation included inoculation with rhizobial isolates viz., RS-1, RS-2, RS-3, RS-4, RS-5 and RS-6 and uninoculated control. The horsegram varieties included were Sina, Man, TPK, HPK, Phule K-1 and K-2. The observations on seed germination (%), nodulation (number of varying sized and coloured nodules and dry weight of nodules per plant) plant dry matter weight, N uptake and grain yield of horsegram varieties were recorded.

The results of pot culture experiment in general revealed that plants of horsegram varieties inoculated with different Rhizobium isolates recorded significant improvement in nodule number, nodule dry weight, plant dry matter weight, N uptake and grain yield over uninoculated control indicating that these isolates could effectively be used as inoculants for horsegram. There was an increase in number of total nodules at 30, 60 and upto 90 days after sowing, while at harvest the number of total pink and large sized nodules showed a decline. Rhizobium inoculants increased grain yield from 2.89 to 3.41 g per plant as against 1.84 g per plant in uninoculated control. Amongst the inoculation treatments, Rhizobium isolates RS-4, RS-3 and RS-6 recorded significantly maximum improvement in production of large size and pink nodules, nodule dry weight, plant dry weight, N uptake and grain yield of horsegram varieties. Next to these isolates, RS-2, RS-5 and RS-1 also recorded significant improvement in nodulation, plant dry weight, N uptake and grain yield of horsegram varieties over uninoculated control. The results in general indicated that all the rhizobial isolates effectively nodulate and gave significant increase in the yield of horsegram varieties over uninoculated control.

The results also indicated that the horsegram varieties responded differentially to inoculation with different isolates of Rhizobium. Differences in germination

percentage, production of large size and pink nodules, nodule dry weight, plant dry weight, N uptake by plant and grain yield were recorded amongst all six varieties of horsegram. All the varieties of horsegram viz., Sina, Man, TPK, HPK, Phule K-1 and KS-2 showed improvement in plant dry weight, N uptake and grain yield over uninoculated control. The response of varieties to inoculation with Rhizobium seemed to be genotype dependent.

The interactions of all the varieties with rhizobial inoculants recorded increased plant dry matter weight, N uptake and grain yield as compared to all the interaction of varieties with uninoculated control.

Considering all the traits studied in the present investigation it appears that all the rhizobial isolates could effectively nodulate, increase N uptake, dry matter weight and grain yield of horsegram varieties.

Literature Cited

9. LITERATURE CITED

Agnihothrudu, V. and Tripathi, S.C. (1976). Effect of seed inoculation with Rhizobium on the yield of groundnut and bengal gram. Madras agric. J., 63(11-12) : 655-656.

Allen, O.N. and Allen, E.K. (1939). Cross-inoculation test within cowpea group. Soil Sci., 471 : 63-76.

Bagchi, D.K.; Chanda, S. and Banerjee, A. (1981). Effect of inoculation and phosphorus on dry yield and utilization of nitrogen by cowpea. Indian J. agric. Chem., 14 : 163-166.

Bagyaraj, D.T. and Hegde, S.V. (1978). Response of cowpea to Rhizobium seed inoculation. Curr. Sci., 47(5) : 548-549.

Bajpai, P.D.; Lehri, L.K. and Pathak, A.N. (1974). Effect of seed inoculation with Rhizobium strains on the yield of leguminous crops. Proc. Indian Natl. Sci. Acad., 40(13) : 571-575.

Balasubramanian, A.; Prabhakaran, J. and Sundaram, S.P. (1980). Influence of single and multi-strain rhizobial inoculants on nodulation, yield and nitrogen assimilation by green gram. (Phaseolus aureus Roxb.). Madras agric. J., 67(2) : 90-93.

Balasundaram, V.R.; Iswaran, V. and Sundara Rao, W.V.B. (1972). Interactions between soyabean (Glycine max (L.) Merril.) genotypes and different isolates of Rhizobium japonicum. Indian J. agric. Sci., 42(5) : 387-389.

Ball, S.T.; Coynne, J.C.; Guerrant, S.M. Schneeweis, T.T. (1981). Response of peanut to irrigation and inoculation. Proc. Ame. Peanut Res. and Edn. Soc., 13(1) : 82.

Bapat, P.N.; Singh, L., Vaishya, U.K. and Dubey, J.N. (1977). Variation in symbiotic effectiveness in bengal gram (Cicer arietinum L.) Indian J. Microbiol., 17(4) : 163-166.

Barthakur, B.C. (1980). Effect of Rhizobium inoculation, application of molybdenum, lime and nitrogen on soyabean (Glycine max (L.) Meri.). J. Res. Assam. agric. Univ., 1(1) : 19-23.

Basak, M.K. and Goyal, S.K. (1980). Studies on tree legume further addition to the list of tree legumes. Plant and Soil, 56(1) : 33.

Basu, T.K.; Barai, A.K. and Bandyopadhyay, S. (1989). Nodulation, growth and seed yield of mung in response to Rhizobium inoculation and nitrogen application. Indian J. Mycol. Res., 27(2) : 153-157.

Beena, S.; Nair, S.K. and Mathew James. (1990). Varietal response and host-varietal specificity for nodulation by Rhizobium in cowpea. Legume Res., 13(3) : 136-139.

Bhore, D.P.; Patil, S.D. and Patil, P.L. (1980). Response of frenchbean varieties to Rhizobium phaseoli. Rhizobium Newsletter, 25(2) : 156.

Bhuiya, Z.H.; Hoque, M.S.; Islam, M.R. and Hoque, S.A. (1989). Effectiveness of some Rhizobium strains on black gram. Bangladesh J. Agril. Sci., 16(1) : 56-62.

*Briner, G.C.; Fernandez, P.M. nd Cafati, K.C. (1985). Selection of strans of Rhizobium sp. for distinct cultivars of chickpea (Cicer arietinum L.). Simiente, 55(1/2) : 19. Estaction Experimental L. Platina, INIA, Santiago, Chile (cited from Field Crops Abst., Sept. 1986, 39(9) : 784.)

←

*Chomchalow, S. (1970). The effectiveness of introduced Rhizobium strain on "Rayong" peanut. Report on Res. Project No. 44/3, Res. Programme No. 44. Appli. Scientific Res. Corn., Thailand. (2) : 10 (cited from Field Crop Abstract, 28(4) : 1975).

Chowdhary, S.L.; Ram, S. and Giri, G. (1974). Effect of inoculation, nitrogen and phosphorus on root nodulation

and yield of lentil varieties. Indian J. Agron., 19 : 274-276.

Churi, J.N. and Nadar, H.M. (1986). Evaluation of effects of Rhizobium phaseoli strain on nodulation, dry matter and grain yield of two bean (Phaseolus aureus) varieties. Soils and Ferti., 49(8) : 995.

Dahiya, J.S.; Khurana, A.L. and Dudeja, S.S. (1983). Evaluation of Cajanus cajan (L.) rhizobia. Int. Workshop of pigeonpea. 2(15-19) : 373-379.

Dart, P.J.; Islam, R. and Egleman (1975). The root nodules symbiosis of chickpea and pigeonpea. An International Workshop on Grain Legumes. ICRISAT, Hyderabad, India. Jan. 1975. 63-68.

Daterao, S.H.; Lakhdive, B.A. and Hanwante, P.R. (1990). Effect of Rhizobium seed inoculation of Green gram with and without molybdenum on grain yield and nitrogen status of soil. P.K.V. Res. J., 14(1) : 75-77.

El-Said, H.M.; Salam, S.H.; Yehia, A.H. and El-Dech, A.A. (1981). Interaction between root rot pathogen of peanut. Rhizoctoria solani (Corticum solani) and Sclerotium rolfsi and inoculation with Rhizobium sp. on dry weight of plants and nodulation. Microb. Abst., 16(9-12) : 185.

Gandhi, M.B. and Godbole, S.H. (1990). Effect of fast growing rhizobia from wild legumes on V. unguiculata L. Walp. Indian J. Expt. Biol., 28 : 438-440.

Gangawane, L.V. and Datar, V.V. (1977). Efficacy of individual and composite rhizobial inoculations on the nodulation of some legume crops. Fert. Tech., 41(1-2) : 134-135.

Gasser, H.; Gux, P.; Obaton, M. and Sikora, I. 1972. Efficiency of Rhizobium meliloti strains and their effect on alfalfa cultivars. Can. J. Pl. Sci., 52 : 441-448.

Ghag, M.S. (1981). Studies on nodulation pattern and its relationship with morpho-physiological characters and yield of gram (Cicer arietinum L.). M.Sc. (Agri.) thesis submitted to M.P.A.U., Rahuri (Maharashtra).

*Gill, M.A.; Nirmal Ali and Nayyar, M.M. (1985). Relative effect of phosphorus combined with potash and Rhizobium phaseoli on the yield of Vigna aureus. Mung. J. agric. Res. Pakistan. 23(4) : 279-282 (cited from Field Crop Abstract, 1987). (40-91).

Gupta, B.R. and Bajpai, P.D. (1984). Studies on response of early maturing varieties of pigeonpea to rhizobia inoculation. Agril. Sci. Dig., 1(4) : 207-209.

Habish, H.A. and Ishag, H.M. (1974). Nodulation in legumes in Sudan. Expt. Agric., 10(1) : 45.

Hegaje, K.S.; Wani, P.V.; Konde, B.K. and Indi, D.V. (1989). Genotypical response of lucerne to inoculation with Rhizobium meliloti and Glomus fasciculatum. Paper presented during the national seminar on Biofertilizer, Technology Transfer held at Marathwada Univ., Aurangabad (M.S.) India, Oct., 14-16. Abst. pp. 16.

Hoffer, A.W. (1949). Effect of inoculation on yield of canning peas in New York. Soil Sci., 46 : 117-126.

Hulmani, N.C.; Sulikeri, G.S. and Kologi, S.D. (1968). Effect of Rhizobium inoculation and molybdenum treatment on nodule and yield of gardenpea (Pisum sativum). Mysore J. agric. Sci., 6(2) : 55.

*Hungria, M. and Neves, M.C.P. (1986). Interaction between Phaseolus vulgaris cultivars and Rhizobium strains in nitrogen fixation and transport. Pesquisa Agropecuria Brasileria, 21(2) : 127-140 (cited from Field Crop Abstract, 1988. 41(4)).

Idris, M. and Sandhu, G.R. (1979). Rhizobium inoculation as an aid in the mung bean (Phaseolus aureus / Vigna radiata) incubation. Pakistan J. Sci. Res., 31(3/4) : 165-173.

Islam, R. (1979). Research on ICARDA on improving nitrogen in chickpea. International Chickpea Newsletter, 1 : 11-12.

Iswaran, V. and Adwajar, C.V. (1974). Comparative efficiency of slurry inoculation and slurry containing molybdenum compound on nodulation, yield and nitrogen uptake by pea crop. Curr. Res., (3) : 141.

Iswaran, V.; Karimadan, S.K. and Kamath, M.B. (1969). Response of inoculated and uninoculated legumes to application of phosphate. Curr. Sci., 38(10) : 251-252.

Jackson, M.L. (1971). Soil chemical analysis. Pentice Hall of India (Ltd.), New Delhi. pp. 159-161.

Jadhav, V.T.; Kore, S.S. and Mayee, C.D. (1990). Effect of Rhizobium inoculation with Nitrogen on Nitrogen fixation and yield of Peanut. Paper presented at VIIIth Southern Regional Conference, held at College of Agriculture, Pune (M.S.). India on 5-6th Feb., 1990. Abst. pp. 11-12.

* Jensen, H.L. (1964). The relationship between host plant and root nodule bacteria in certain leguminous plants. Tidsskr. Planteavl., 68 : 1-22 (cited from Soil and Fertilizer, 1965. 28(28) : 294).

Joshi, S.S.; Thorve, P.V. and Nagre, K.T. (1989). Effect of Rhizobium and nitrogen on the yield and quality of groundnut and soyabean. P.K.V. Journal, 13(2) : 152-155.

*Kaliash, J. and Gisela, H. (1988). Effect of alfalfa inoculation with Rhizobium on shoot and root growth and available inorganic nitrogen content of the soil. ARCH ACKER PILANEZE NBAU BODEND. 32(3) : 187-197 (cited from Biol. Abst., 1988. 86(37)).

Kenjale, L.D. (1979). I. Effect of ground leaf rust on nodulation and yield. II. Studies on nodulation pattern in gram (C. arietinum L.). M.Sc. (Agri.) thesis submitted to M.P.A.U., Rahuri (Maharashtra).

Khating, E.A. and Ghonsikar, C.P. (1981). Effect of different Rhizobium strains on nodulation, N uptake and grain yield of chickpea. International Chickpea Newsletter, 5 : 14-16.

Khurana, A.L.; Saini, M.L.; Jhorar, B.S. and Dudeja, S.S. (1978 b). Nodulation studies in cluster bean. Forage Res., 4(2) : 195-198.

Koteswara Rao, S. (1979). Effect of Rhizobium inoculation and P_2O_5 fertilization on nodulation in groundnut. Andhra agric. J., 26 :

Kremer, R.J. and Paterson, H.L. (1983). Field evaluation of selected Rhizobium in an improved legume inoculant. Agron. J., 75(1) : 139-143.

Kulkarni, J.H.; Joshi, P.K. and Sajitra, V.K. (1984). Response of groundnut varieties to inoculation with rhizobial strains. Indian J. Agric. Sci., 54(7) : 586-589.

Kumar Rao, J.D.V.K. and Dart, P.J. (1975). Biology of nodulation of pigeonpea (Cajanus cajan (L.) Millsp.). ICRISAT, Patancheru, P., 503 : 325.

Kumpawat, B.S. and Manohar, S.S. (1994). Response of gram to bacterial inoculation, phosphorus and micronutrients. Madras Agric. J., 81(7) : 396-397.

Leonard, L.T. (1980). A failure of Australian winter pea opportunity due to nodule bacteria. Amm. Soc. Agron., 22 : 297-279.

Linta, L. (1963). The nitrogen fixing capacity of bacteria in the nodules of pea and vetc and dynamics of nitrogen accumulation in plants. Agrokem. Talajt., 12 : 647-660.

Mane, P.B. (1985). Nodulation studies in new promising varieties of gram (Cicer arietinum L.). M.Sc. (Agri.) thesis submitted to MPAU, Rahuri, Dist. Ahmednagar (M.S.).

Marevckova, H. and Brozeva, O. (1984). Symbiotic nitrogen fixation in lucerne. Rostlinna Vyroba, 30(11) : 1169.

McNeil, D.L.; Croft, L.K. and Sandhu, T.S. (1981). Response of chickpea to inoculation with Rhizobium in Hawai. International Chickpea Newsletter, 4 : 25-26.

*Morales, V.M. and Graham, P.H. (1973). Influence of inoculation method and liming of the soil at Carmgua (E. Lianos Colombia) on nodulation of legumes. Turrialba., 23(1) : 52-55 (cited from Herbage Abst. 1975. 45(9) : 352).

More, B.B.; Patil, S.L. and Konde, B.K. (1981). Effect of Rhizobium inoculant under various levels of nitrogen on nodulation and yield of peanut (Arachis hypogea L.). J. Maharashtra agric. Univ., 6(1) : 13-16.

*Nagi, S.C. and Thakur, R.C. (1985). Effect of nitrogen and phosphorus and Rhizobium inoculation on mash (viana mungo) yield and N and P uptake. Himachal J. of Agric. Res., 11(1) : 96-99 (cited from Field Crop Abst., 1987. 40 : 9).

Nagre, K.T. (1982). Effect of Rhizobium and nitrogen on yield of green gram and blackgram. Agril. Sci. Digest., 2(1) : 27-26.

Nagre, K.T.; Joshi, S.S. and Thorve, P.V. (1991). Effect of Rhizobium inoculation and nitrogen application on root nodulation of groundnut and soyabean. P.K.V. Res. J., 15(2) : 157-159.

Namdeo, S.L. and Gupta, S.C. (1990). Studies on competiveness of Rhizobium strains with pigeonpea var. JA-3. Indian J. Pulses Res., 3(1) : 181-182.

Namdeo, S.L.; Gupta, S.C.; Jain, R.C. and Kakran, M.S. (1989). Response of chickpea genotype to inoculation with Rhizobium strains under rainfed conditions. Legume Res., 12(2) : 96-100.

Natarajan, T. and Prabhakaran, J. (1983). Influence of rhizobial inoculants on nodulation and yield of chickpea. Sixth Southern Regional Conference on Microbial Inoculants, held at University of Agricultural Sci., Banglore. 560 065 on Sept., 22-23. Abst. pp. 6.

Nuti, M.P.; Casella, S., Filippi, C.; Lepidi, A.A. and Galluzzi, P. (1981). Rhizobia as inoculants for field trials in marginal soil of middle and northen Italy. In : Current perspectives in nitrogen fixation (Gibson, A.H. and Newton, W.E. (Editors), Australian Acad. Sci., Elsevier/North Holland, Biomedical Press, Amsterdam, Netherland, 1981. pp. 514.

Obliswami, G.; Balaraman, K. and Natarajan, T. (1976). Effect of composite cultures of Rhizobium on two pulse crops. Madras Agril. J., 63(11-12) : 587-589.

Olsen, S.R.; Cole, C.V.; Watanabe, P.S. and Dean, L.A. (1954). Estimation of available phosphorus in soil dry extraction with sodium bicarbonate. U.S.D.A. Circ. 939. pp. 1035-1040.

Pahuwa, M.R. (1986). Response of forage cowpea to phosphate and Rhizobium inoculation. Agric. Sci. Digest. 6(2) : 96-97.

Panse, V.G. and Sukhatme, P.V. (1957). Statistical methods for Agricultural Workers, ICAR Publ., New Delhi.

Patil, B.D. and Moniz, L. (1974). Differential response of gram varieties to efficient isolate of rhizobia from gram (Cicer arietinum L.). Res. J. M.P.A. Univ., 5 : 42-46.

Patil, P.L. and Medhane, N.S. (1974). Seed inoculation studies in gram (Cicer arietinum L.) with different strains of Rhizobium sp. Pl. Soil., 40(1) : 221-223.

Patil, R.N. and Shinde, P.A. (1980). Studies on nodulation pattern in gram. J. Maharashtra agric. Univ., 5(3) : 211-213.

Prabhakaran, J. and Rangarajan, M. (1992). Effect of rhizobial inoculation on the nodulation, nitrogen fixation and yield of lablab (Phareolus vulgaris L.). Madras Agric. J., 79(3) : 172-174.

Prabhakaran, J. and Srinivasan, K. (1993). Performance of horsegram cultivars to rhizobial seed inoculation in Acid-Soils. Madras Agric. J., 88(11) : 654-655.

Prasad, D.N. (1972). Biology of root nodules of Phaseolus spp. with reference to their anatomical structure. Proc. Ind. Nat. Sci. Acad., B. 38 : 1-7.

Prasad, J. and Ram, H. (1984). Varietal response to rhizobial strain for nodule characters, chlorophyll and protein content in green gram (V. radiata). J. agric. Sci., 102(1) : 245-246.

Rai, R. and Singh, S.N. (1979). Interaction between chickpea (Cicer arietinum Linn.) genotypes and strains of Rhizobium sp.) J. of Agril. Sci., 92(2) : 437-441 (cited from Trop. Grain Legume Bulletin. 15, 1979. pp. 51).

Rai, R.; Singh, S.N. and Murtuza, M. (1977). Differential response of Rhizobium strains of bengal graim (Cicer arietinum L.). Curr. Sci., 46(16) : 572-573.

Raju, K.S. and Samuel, A.V. (1976). Response of gram (Cicer arietinum L.) to different rhizobial inoculants. Madras Agric. J., 63(11-12) : 582-586.

Raju, M.S. and Verma, S.C. (1984). Response of green gram (Vigna radiata) to rhizobial inoculation in relation to fertilizer nitrogen. Legume Res., 7(2) : 73-76.

Ramaswmi, P.P. and Nair, K.S. (1965). Effect of seed inoculation with Rhizobim on yield and nitrogen content of pulses under field conditions. Indian J. Microbiology, 5(2) : 95-96.

Rao, N.S.S. (1981). Contribution of biofertilizers in supplementing the nitrogen requirement. Indian Farm., 31(7) : 13-15.

Raut, R.S. and Ghonsikar, C.P. (1983). Response of two pigeonpea cultivars to inoculation with Rhizobium at Parbhani, Maharashtra, International Pigeonpea Newsletter, 2 : 70.

Raut, R.S. and Ghonsikar, C.P. (1982). Response of chickpea cultivars to inoculation with Rhizobium. International Chickpea Newsletter, 6 : 25-26.

Reddy, V.M. and Tanner, J.W. (1980). The effect of irrigation, inoculants and fertilizer N on peanut nitrogen fixation. Peanut Sci., 7(2) : 114-119.

Rewari, R.B.; Jain, M.K. and Bhatnagar, R.S. (1973). Varietal response of soyabean (Glycine max (L.) Merr.) to different strains of Rhizobium japonicum. Indian J. Agric. Sci., 43(8) : 801-804.

Rai, W.A. and Olsen, P.E. (1983). Inoculation of alfalfa seed for increased yield on moderately acid soil. Can. J. Soil. Sci., 63(3) : 541-545.

Richter, E. (1974). Nodulation, yield and protein content of Pisum sativum seeds. Land Wirtschafldiche. Forchung, 27(3/4) : 330-342 (cited from Field Crop Abst., 28(7) : 3652, 1975).

Sarhawat, K.L. and Burford, J.R. (1982). Modification of alkaline permanganate method for assessing the availability of soil nitrogen in upland soils. Soil Sci. 113(1) : 53.

Sairam, R.K.; Tomer, P.S.; Harika, A.S. and Ganguly, T.K. (1989). Effect of phosphorus levels and inoculation with Rhizobium on nodulation, leghaemoglobin content and nitrogen uptake in fodder cowpea. Legume Res., 12(1) : 27-30.

Sankaram, A.R.P.V.L.; Narayan Sinha and Kaisaiah, S. (1960). Studies on selection of efficient strains of root nodule bacteria. Indian J. Agric. Sci., 4(3) : 140-143.

Sawashe, S.G. and Patil, P.L. (1984). A study on nodulation by different strains of Rhizobium sp. in gram. J. Maharashtra agric. Univ., 9(1) : 75-77.

*Shaheen, A. and Rahmatullah. (1994). Differential growth, nodulation and N fixation efficiency of groundnut cultivars inoculated with different strains of Rhizobium. J. of Agronomy and Crop Sci., 173(5) : 289-392 (cited from Field Crop Abst. 1995. 48(7) : 640).

Shinde, D.B. (1979). Studies on nodulation pattern in black-gram (V. mungo L. Wilzek) and interaction between root infecting fungi and Rhizobium sp. M.Sc. (Agri.) thesis submitted to MPKV, Rahuri (Maharashtra).

Singaravadivel, K. and Prasad, N.N. (1981). Saprophytic competence of inoculated strains of groundnut Rhizobium. Madras agric. J., 68(6) : 403-405.

Singh, R.K. and Misra, G.C. (1981). Interaction between Rhizobium strains and cultivars of gram (Cicer aeritinum L.) for increasing nitrogen fixation ability. Pulse Crop Newsletter, 1(3) : 62.

Singh, S.D. (1977). Effect of rhizobia inoculation on nodulation and yield of mung (Vigna radiata). Ann. Arid Zone, 16(1) : 79-84.

Singh, D.V.; Chauhan, R.P.S.; Srig, K. and Pal, B. (1978). Nitrogen and phosphorus needs of gram (Cicer arietinum L.) alongwith bacterial fertilization. Madras Agric. J., 65(1) : 22-24.

Society of American Bacteriologist. (1957). Manual of Microbiological Methods. McGraw Hill Book Co., New York.

Srivastava, G.P. and Varma, U.K. (1986). Response of pigeonpea to phosphorus and Rhizobium inoculation. Indian J. Agron., 30(2) : 131-134.

Stevens, J.W. (1925). The value of litmus bromocresol purple and Jenus-green milk in study of nodule organisms of leguminasac. J. Agric. Res. (U.S.). 31 : 997-1000.

Surendragopal, K. and Shivappa Shetty, K. (1996). Gram manuring for rice with Rhizobium inoculated Sesbania grandiflora. Madras agric. J., 83(4) : 249-250.

*Tellawi, A.; Haddad, N. and Hattaz, B. (1986). Effect of several Rhizobium strains on nodulation, nitrogen uptake and yield of chickpea (S. arietinum L.). Zeitschrift Fijr pflanzenerna bruna and Bodenkunde, 149(3) : 314-322. (Cited from Soils and Fertilizer, 1987. Vol. 50, No. 1. pp : 98.).

Thakur, R.C. and Nagi, S.C. (1985). Effect of fertilizers and Rhizobium inoculation in blackgram. Indian J. Agron., 30(4) : 501-504.

Thornton, H.C. (1929). Legume bacterial population of soil. J. Agric. Sci., 19 : 48-70.

Tippannavar, C.M. and Desai, S.A. (1992). Effect of Rhizobium with cultural practices on Bangalgram production. J. Maharashtra agric. Univ., 17(2) : 326-327.

*Tomar, R.K.S. and Raghu, J.S. (1995). Response of chickpea to phosphorus and Rhizobium inoculation under rainfed (cited from Field Crop Abst. 1995. 48(10)).

Vaishya, U.K.; Gajendragadkar, G.R. and Pandey, R.L. (1983). Effect of Rhizobium inoculation on nodulation and grain yield of mung bean (Vigna radiata). Indian J. Microb., 23(4) : 228-230.

Venkateswarulu, B.; Rao, A.V. and Lahiri, A.N. (1982). Symbiotic performance of rhizobial strains on Gaur grown in desertic soil. Egyptian J. Microbiol., 18 : 9-14.

Vincent, J.M. (1970). A manual for the practical study of root nodule bacteria. Blackwell Sci. Publ., Oxford and Edinburg. pp. 164.

*Visuttipitakul, S. (1970). Preliminary study on the effect of Rhizobium inoculants on the yield of peanut. Report on Res. Project No. 4413. Res. Programme No. 44, Appl. Scientific Res. Corporation, Thailand. 1 : 12 (cited from Field Crops Abst. 1975. 28(2) : 3018).

Vora, M.S. and Desai, B.G. (1980). Interaction of chickpea cultivars with Rhizobium strains at Dohad in Gujarat State. International Chickpea Newsletter, 2 : 19-20.

Wange, S.S. and Patil, P.L. (1996). Effect of Rhizobium strains on pigeonpea cultivar. Madras agric. J., 83(6) : 401-402.

Yadav, E.D.; Bhore, D.P.; Patil, A.V. and Patil, V.L. (1967). Response of different frenchbean (Phaseolus vulgaris L.) varieties to Rhizobium strains. Rhizobium Newsletter, 21(1) : 56.

Vidyasurian, V.; Sahul, M. and Sivaprasad, P. (1983). Response of mungbean to fertilizer application and rhizobial inoculation. Sixth Southern Regional Conference on Microbial Inoculants, held at Univ. of Agril. Sci. Banglore on Sept., 22-23. Abst. pp. 9.

* Original not seen.

Appendix

8. APPENDIX : Effect of inoculation with different isolates of *Rhizobium* on N-content of plant (N%) in horsegram

Inoculant	Variety						Mean
	Sina	Man	TPK	HPK	Phule K-1	KS-2	
A] At 30 days							
RS-1	3.52	3.47	3.44	3.36	3.37	3.40	3.42
RS-2	3.51	3.45	3.42	3.39	3.36	3.32	3.40
RS-3	3.44	3.49	3.48	3.37	3.35	3.39	3.42
RS-4	3.46	3.42	3.32	3.35	3.30	3.37	3.37
RS-5	3.45	3.47	3.37	3.38	3.33	3.32	3.38
RS-6	3.48	3.51	3.39	3.34	3.36	3.35	3.40
U.C.	3.11	3.02	3.05	3.09	2.43	3.02	2.95
Mean	3.42	3.40	3.35	3.32	3.21	3.21	3.31
B] At 60 days							
RS-1	3.90	3.42	3.30	3.08	2.37	3.39	3.24
RS-2	3.80	2.39	3.00	3.22	2.40	2.93	2.95
RS-3	3.92	2.93	3.11	3.00	2.70	2.83	3.08
RS-4	3.48	2.70	2.75	2.92	2.76	2.75	2.96
RS-5	3.30	2.40	2.68	2.70	2.75	2.92	2.79
RS-6	3.39	3.45	2.42	2.46	2.39	2.61	2.78
U.C.	3.18	2.18	2.32	2.20	2.15	2.30	2.38
Mean	3.63	2.78	2.79	2.79	2.50	2.82	2.88
C] At 90 days							
RS-1	3.27	3.02	3.20	3.02	2.48	2.29	2.88
RS-2	3.20	3.01	2.90	2.50	2.60	2.48	2.78
RS-3	3.18	3.05	2.45	2.92	2.63	3.05	2.81
RS-4	3.06	2.50	2.20	2.82	2.77	3.02	2.72
RS-5	3.05	2.09	2.17	2.42	2.70	2.27	2.45
RS-6	3.04	2.48	2.20	2.17	2.40	2.18	2.41
U.C.	3.06	2.10	1.90	2.12	2.14	2.00	2.22
Mean	3.98	2.60	2.43	2.56	2.48	2.47	2.75
D] At harvest							
RS-1	2.43	2.16	2.88	2.75	2.82	1.83	2.47
RS-2	2.57	2.00	2.30	2.45	2.70	2.01	2.33
RS-3	2.58	2.43	2.89	2.65	2.20	2.48	2.53
RS-4	2.35	2.45	2.09	2.67	2.17	2.28	2.33
RS-5	2.25	2.37	2.48	2.40	2.48	2.30	2.38
RS-6	2.74	2.22	1.56	2.35	2.09	2.35	2.21
U.C.	1.75	1.49	1.75	1.89	1.72	1.57	1.69
Mean	2.38	2.16	2.28	2.45	2.31	2.11	2.28

	S.E. ±				C.D. at 5%			
	A	B	C	D	A	B	C	D
Varieties (V)	0.20	0.10	0.12	0.14	N.S.	0.30	0.35	N.S.
Inoculants (I)	0.21	0.11	0.13	0.15	N.S.	0.32	0.38	0.45
Interaction (V X I)	0.53	0.28	0.33	0.30	N.S.	N.S.	N.S.	N.S.

N.S. = Non-significant

Vita

9. VITA

RAVINDRA TATYARAM GAIKWAD

A candidate for the degree

of

MASTER OF SCIENCE (AGRICULTURE)

Title of thesis	:	Studies on isolation and characterization of Rhizobium and nodulation pattern in horsegram (Dolichus biflorus L.)
Major field	:	Agricultural Microbiology
Biographical information	:	Born at Borgaon, Tal. Barshi, Dist. Solapur on June 1, 1970. Son of Sau. Suman and Shri. Tatyaram Namdeo Gaikwad. Having two brothers.
Educational	:	Attended Secondary School at Savitribai Phule Madhyamik Vidyalaya, Rahuri. Higher Secondary at Vidyamandir Prashala, Rahuri. Received Bachelor of Science (Agri.) degree from College of Agril., Dhule of Mahatma Phule Krishi Vidyapeeth, Rahuri in June 93.
Extra Curricular Activities	:	1. Selected for the "Inter-University Tournament", held at Jodhpur (Rajasthan) in Hockey in 1992-93. 2. Participated in Inter-collegiate sports events viz., Kho-Kho, Football, Cricket, Hockey etc. 3. Working as a Junior Research Assistant at Regional Wheat Rust Research Station, Mahabaleshwar under M.P.K.V., Rahuri.