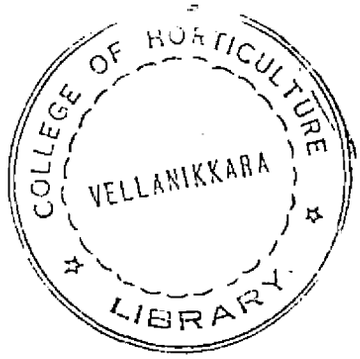
STUDIES ON HOST-VARIETAL SPECIFICITY FOR *Rhizobium* FOR NODULATION IN GROUNDNUT

By

V. K. GIRIJA



THESIS

Submitted in partial fulfilment of
the Requirement for the Degree
MASTER OF SCIENCE IN AGRICULTURE
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, TRIVANDRUM

1982

DECLARATION

I hereby declare that this thesis entitled "Studies on host-varietal specificity for Rhizobium for nodulation in groundnut" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,
18 -1-1982.

(V.K. GIRIJA)

CERTIFICATE

Certified that this thesis entitled "Studies on host-varietal specificity for Rhizobium for nodulation in groundnut" is a record of research work done independently by Kum. V.K. GIRIJA under my guidence and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship, or associateship to her.

(SASI KUMAR NAIR)
Chairman
Advisory Committee
Associate Professor of Microbiology

Vellayani,
18- 1-1982.

APPROVED BY:

Chairman:

Dr. SASI KUMAR NAIR 12.2.1982

Members:

1. Dr. M.CHANDRASEKHARAN NAIR 12.

2. Dr. JAMES MATHEW

3. Smt. ALICE ABRAHAM

ACKNOWLEDGEMENT

The author places on record her deep sense of indebtedness and gratitude to,

Dr. Sasi Kumar Nair, Associate Professor of Microbiology for his inspiring guidance, critical suggestions and constant encouragement during the course of investigation and also in the preparation of the thesis,

Dr. M. Chandrasekharan Nair, Professor of Plant Pathology, Dr. James Mathew, Associate Professor of Plant Pathology and Smt. Alice Abraham, Assistant Professor of Agricultural Chemistry, members of the Advisory Committee, for their constructive criticisms and valuable suggestions,

Dr. K.C. George, Associate Professor of Agricultural Statistics, College of Horticulture, Vellanikkara and Sri. Balakrishnan Asan and Sri. Yageen Thomas, Assistant Professors of Agricultural Statistics, Vellayani for their help in the statistical analysis of the data compiled during the investigation,

Dr. V.G. Malathy, Scientist, Central Tuber Crops Research Institute, Sreekariyam, Trivandrum for her help in centrifugation of rhizobial cultures,

Dean, College of Veterinary and Animal Sciences, Mannuthy, Trichur for supplying rabbits for serological studies,

Dr. A.D. Joy, Assistant Professor of Animal Husbandry for his help in serological work,

The Indian Council of Agricultural Research for awarding her the Junior Research Fellowship,

And to all the staff members and P.G. students of the Department of Plant Pathology for their help and co-operation.

V.K. GIRIJA.

Vellayani,

18-1-1982.

C O N T E N T S

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

Introduction

INTRODUCTION

The nitrogen requirement of crop plants is met either by the mineralisation of native soil nitrogen or by the addition of fertiliser nitrogen or by these two sources along with dinitrogen fixation as in the case of leguminous plants. Legumes have been used in building and conserving soil fertility since the beginning of agriculture. A review of the past work done in this field reveals the immense lot of research being carried out by various workers on this aspect. The root nodule bacterium, Rhizobium, in association with its host plant accounts for nearly 20 per cent of the dinitrogen fixed annually in the world (Dazzo and Hubbel, 1974). This indicates the importance of this process in restricting the use of fertiliser nitrogen for the cultivation of such leguminous crops. This is of much significance for our country which is at present facing an acute fuel crisis coupled with frequent shortage of nitrogenous fertilisers.

Groundnut (Arachis hypogaea L.) is an important oil seed crop in India occupying nearly 7.5 million hectares and producing about 6.5 million tons of pods annually. It is one of the main sources of edible oil contributing to more than 60 per cent of the total oil seeds production. In Kerala this crop is cultivated in an area of about 17,000 hectares with an average annual production of 23,000 tons.

Groundnut is cultivated as a pure crop in the Palghat district of the state. In other places, it is cultivated

either in rice fallows, as a catch crop especially in the Onattukara region or as an intercrop in coconut gardens. Recently, it has been recommended as an effective mixed crop with tapioca, by the Kerala Agricultural University and the Central Tuber Crops Research Institute of the Indian Council of Agricultural Research. All these indicate the enormous economic importance of this crop to our State.

Recent researches have led to the development of more and more groundnut varieties with high yield and oil content. However, one of the problems encountered with these new varieties is the lack of proper nodulation necessitating the use of high cost fertilizer nitrogen for their cultivation. The main reason for this appears to be the lack of compatible strains of rhizobia in the soil where these varieties are cultivated. Therefore, it has become essential to select appropriate strains of rhizobia for different varieties of groundnut in order to ensure adequate nodulation. This in turn can bring about much nitrogen economy in the cultivation of this crop in our country. Keeping in view of these facts, the present problem was undertaken with the following main objectives:

(1) Isolation of Rhizobium suitable for different varieties of groundnut.

(2) Testing for host-varietal specificity for nodulation by these isolates of rhizobia using selected varieties of groundnut.

(3) Physiological and serological characterisation of different isolates of rhizobia.

Review of literature

REVIEW OF LITERATURE

Rhizobium inoculation and crop response in groundnut

Groundnut is a crop that has great potentiality for nitrogen fixation under *Rhizobium* inoculated conditions. Dugger (1955) showed that *Rhizobium* inoculation to Spanish peanut resulted in an increase of the number and size of nodules and also the yield of nuts. Field trials with groundnut conducted by Seeger (1961) stressed the value of inoculation in increasing the yield of nuts. Rangaswamy and Oblisamy (1962) reported better response of groundnut crop to *Rhizobium* inoculation. However, Thronton (1963) found no significant yield increase due to *Rhizobium* inoculation in groundnut variety, Cambia bunch. He attributed this to the competition between the introduced strain of *Rhizobium* and the native strains already present in the soil.

Wetselaar (1967) made a nitrogen balance sheet over three years for four annual legumes, *Stylosanthes humilis*, *Vigna sinensis*, *Cyamopsis tetragonoloba* and *Arachis hypogaea*. He found that the groundnut crop alone recorded an average annual nitrogen fixation of 60 lbs per acre as compared to the adjacent fallow land. In the field trials conducted on the various tropical soils of Madagascar, Vuong and Andriamanatena (1970) reported increased nodulation in groundnut as a result of *Rhizobium* inoculation. Sahu and Behera (1972) found that the application of *Rhizobium* to

groundnut increased the grain yield as well as the nitrogen content of shoot, root and grains of the crop. Bajpai et al. (1974) reported a 21 per cent increase in the yield of unshelled nuts in groundnut due to inoculation.

Agnihotrudu and Tripathi (1976) conducted field trials with groundnut in Tamil Nadu, Karnataka and Andhra Pradesh, to study the effect of seed inoculation with Rhizobium on crop yield. The Rhizobium treated plots recorded 10 to 57 per cent increase in yield over the control. Study of the cost-benefit ratio of Rhizobium seed inoculation revealed a return of 9 to 39 rupees for every rupee spent in Rhizobium culture. Chitriv and Wangikar (1978) found that inoculated groundnut plants recorded 7.43 to 89.11 per cent increase in grain yield over the control under field conditions.

Effect of fertiliser application on nodulation in groundnut

In a field experiment on groundnut, cowpea and Sesbania spp. supplied with lime at 0 and 680 kg Ca $(OH)_2$ per hectare and P at 0 and 14 kg P_2O_5 per hectare, Rose (1963) found that lime alone or in combination with P significantly increased the height of plants at 30 days. At 60 days, lime applied along with P was superior to all other treatments in increasing plant height. Veeraraghavan (1964) reported that lime applied at the rate of 1,500 pounds per acre increased the number of nodules per plant under field conditions in groundnut in red loam of Kerala. Sreedharan and George (1965) working in the same soil got similar

results with the application of 2,000 kg of lime per hectare. In a field trial with groundnut, cowpea and greengram, Sahu and Behera (1972) showed that grain yields were significantly increased by the application of phosphorus at 22.4 kg phosphate per hectare combined with Rhizobium inoculation. The seed protein content was also increased. They also noticed a 29 per cent increase in soil nitrogen content as a result of culture application. Hall (1975) found that the combined application of phosphorus with Rhizobium inoculation resulted in better yield than with the separate application of either P or Rhizobium culture. Rani Perumal et al. (1978) reported that the application of phosphorus, magnesium and boron lead to increased yield and quality of groundnuts through the indirect way of beneficially influencing nodulation and nitrogen fixation processes.

Haque and Amara (1978) studied the effect of application of sodium molybdate and Rhizobium culture in groundnut grown on upland soil of Sierra Leone. They found that the dry matter yield increased from 74.5 g/m^2 in untreated control to 87.3 g/m^2 with inoculation and molybdate application. Dry matter yield decreased with inoculation alone.

Sharma and co-workers (1978) worked on the beneficial aspects of seed pelletting with calcium carbonate on nodulation in groundnut variety TMV-2. They obtained the highest number and weight of nodules in plants grown from seeds inoculated with Rhizobium and pelletted with $CaCO_3$.

Host-varietal specificity of *Rhizobium* for nodulation in groundnut

Rajagopalan (1938) studied the response of groundnut to two physiologically different *Rhizobium* isolates. One of them produced large number of small and medium sized nodules which were efficient and concentrated mainly on the tap root. The other strain, on the other hand, produced nodules of a smaller size mainly on the lateral roots. These nodules were incapable of fixing adequate quantity of nitrogen. Albrecht (1943) showed that six single strain cultures of *Rhizobium* differed widely in their effectiveness on Spanish peanuts. The strains were assessed in terms of percentage of plants nodulated, dry matter yield per unit area, visual rating of yield and mean dry weight of individually collected plants.

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

Muthusamy (1970) investigated the comparative efficiency of four isolates of *Rhizobium* for their nodulation and nitrogen fixation with TMV-2 groundnut plants. He found that there was some relationship between the groundnut variety TMV-2 and its

own rhizobial isolate. This isolate produced the maximum number of effective nodules. It also contributed to maximum length and fresh weight of plants than the other three isolates.

Dadarwal and associates (1974) investigated the nature of nodulation by rhizobia from six species of Arachis on the cultivated variety of A. hypogaea. The groundnut plants differed in the pattern of nodulation due to different Rhizobium isolates. Rhizobium from one species of Arachis viz., A. duranensis was most effective on A. hypogaea.

Rao (1974) tested the effectiveness of four different strains of Rhizobium (CA-2, 402-A, 402-B, 402-C) for their effectiveness against the groundnut variety TMV-2, Yield, nitrogen content and total nitrogen uptake by groundnut plants were taken as the main parameters for testing the comparative symbiotic efficiency of different isolates of Rhizobium. Strain 402B was found effective in pot culture as well as in field trials. Burton (1975) has shown that the use of effective strains of Rhizobium could increase the peanut yields, substantially. He advocated that an effective screening test for evaluation of rhizobial strains specific to groundnut cultivars should be conducted to enhance nut yield.

In a nodulation study conducted with eight groundnut cultivars to evolve suitable plant type of the crop for the arid zone, Suraj Bhan (1975) found that the varieties AK-12-24

and Spanish Improved were the best with regard to nodulation and symbiotic efficiency. Among the varieties tested, the procumbent ones had more and heavier root nodules with a higher nitrogen content than the erect varieties. But the latter ones had a higher dry matter yield and more efficient nitrogen use.

Date (1976) recommended that Rhizobium strains could be evaluated for their effectiveness on groundnut varieties in two phases. The strains should initially be screened under controlled environmental conditions for their efficiency of nitrogen fixation. Secondly, the selected efficient strains of Rhizobium should be evaluated under field conditions for its comparative ability, persistence and effectiveness in nitrogen fixation. Effectiveness of forty serologically distinct strains of Rhizobium, isolated from six wild species of Arachis on the cultivated variety of groundnut, A. hypogaea was studied in a pot culture trial by Singh et al. (1976). They noticed large variations in symbiotic efficiency between the strains. The strains GF-20 (isolated from Arachis hypogaea) and G-405 (isolated from A. glaberata) were found to be most effective. They also reported significant positive correlation between percentage nitrogen in nodules and weight and nitrogen content of seeds. However, they could not establish any significant correlation between the weight of nodules and nitrogen content. Likewise, no significant correlation was observed between any one of the physiological properties of different strains and their symbiotic efficiency.

Schneewis and associates (1978) and Singleton (1978) have emphasized the need for selection of bacterial strains and appropriate varieties for optimising nitrogen fixation potential in groundnut. The role of host genotype in nodulation and nitrogen fixation has been recently summarised by Caldwell and Vest (1979). The authors strongly conclude that effective nodulation and nitrogen fixation are greatly influenced by genetic variation of both the symbionts.

A total of seventy-eight isolates obtained from prominently nodulated groundnut plants from Karnataka and Gujarat were tested for their efficiency to fix atmospheric nitrogen in sand cultures under controlled conditions by Sheth (1979). Among them, 25 isolates were found to be superior to the standard inoculant strain CB 756. The results further established the potentiality of inoculating groundnut plants with suitable strains of rhizobia to get maximum benefit out of natural process of biological nitrogen fixation. An attempt was made by Alagawadi (1979) to evaluate the Rhizobium strains of groundnut isolated from Dharwar and Belgium for their symbiotic efficiency. Among the seventeen strains isolated, strains A_1, A_4 and RCP were used in a study for the comparative efficacy of nitrogen fixation with a standard strain TAL 309. The three selected isolates behaved in a varied manner in nodulation and nitrogen fixation in groundnut cultivars.

Ayalaba (1980) in his study on the evaluation of symbiotic nitrogen fixation by three peanut varieties inoculated with two Rhizobium strains, detected significant differences in

specific nitrogenase activity. However, these were not evident when the activity of the enzyme was expressed as total nitrogenase activity per plant. Sen and Weaver (1980) compared the relative efficiency of Rhizobium strain 32 H.I in nitrogen fixation in peanut and cowpea. Peanuts had 1.5 to 3.0 times higher acetylene reduction activity per unit mass than cowpea at different nodule ages. The nitrogen accumulated in the plant tops per unit weight of nodules on the roots was also greater in peanuts.

Sullia and Anasuya (1980) have observed the stimulatory effect of groundnut root exudates on the growth of three strains of Rhizobium spp. viz., CB-1024, CB-530 and BU-2, and the inhibitory effect on the strain BU-1. While the stimulatory effects of the root exudates was explained as a pre-requisite for the establishment of symbiotic relationship, the inhibitory effect of the strain BU-1 indicated incompatibility with the groundnut variety TMV-2.

Wynne (1980) reported that specific host-strain combination can lead to increased biological nitrogen fixation in peanuts. The authors also advocated that the most useful trait for measuring effectiveness of a rhizobial strain is the total nitrogen accumulated in the plant by their activity.

Host-varietal specificity for nodulation in other legumes

Erdman (1947) studied the strain variation and host specificity of Rhizobium trifolii on four species of Trifolium. His findings stressed the importance of the use of effective

cultures of Rhizobium trifolii as well as the cultivation of efficient varieties within different Trifolium spp for augmenting nitrogen fixation and crop growth.

Moniz and co-workers (1968) worked on the strain variation and efficiency of nitrogen fixation among eighteen alfalfa (Medicago sativa L.) and two berseem (Trifolium alexandrinum L.) strains of nodule bacteria. The strains were found to differ very markedly in their ability to fix nitrogen. The results of the cross-inoculation studies revealed that Rhizobium meliloti was specific to its own cross-inoculation group.

Trinick (1968) conducted an investigation on the specificity in Rhizobium symbiosis of Leucaena Leucocephala rhizobia isolated from a group of legumes including Leucaena leucocephala, Mimosa invisa, M. pudica, Acasia farnesiana and Sesbania spp. They were all found to be fast - growers capable of nodulating L. leucocephala effectively.

Weir (1969) investigated the interactions between three strains of Rhizobium meliloti and the host plant, Medicago sativa var. Hairy Peruvian. The strains showed no significant differences in the total number of nodules. But there was difference in the ratio of the effective to ineffective nodules produced by each strain.

Balasundaram et al.(1972) studied the interactions between soybean (Glycine max) genotypes and different isolates of Rhizobium japonicum. The different isolates varied in

their symbiotic efficiency with different varieties of soybeans.

Dadarwal et al. (1975) showed that nodulation in chickpea is also determined to a great extent by the host genotype. In tests carried out to determine the varietal and strain interactions, it was found that the isolate Ca 121 produced the maximum number of nodules followed by the isolates Ca 151, and Ca 191. On the other hand, among the varieties, L 144 and BG - 2 showed maximum response to nodulation. In the symbiotic effectiveness strains Ca 121 and Ca 181 were most effective and showed an increase of more than 100 per cent in plant dry weight and nitrogen uptake with as many as seven genotypes. Among the varieties, H 551 and H 355 showed maximum increase in nitrogen uptake as well as in the dry matter yield.

Raju and Samuel (1976) studied the influence of different commercial inoculants on gram (Cicer arietinum). All the inoculants, except the I.A.R.I. culture gave very good results as was noticed by the high dry matter production, high nitrogen uptake by the plant and translocation to the seed.

Borges (1977) evaluated the effectiveness of two Rhizobium strains on cowpea cv. Arauca in terms of their effect on stem length, leaf area, shoot dry matter, water consumption and nitrogen contents of roots and shoots. On the basis of such parameters, one strain was selected. The result also indicated that the major proportion of atmospheric nitrogen fixed was in roots by the strain.

González (1977) studied the behaviour of ten Rhizobium japonicum strains on soybean varieties. Their results brought to light the fact that the relation between plant dry weight, nodule dry weight, and nitrogen accumulation varied according to variety, strain and soil type.

Kumar Rao and Patil (1977) reported that soybean responded differently to different commercial inoculants of Rhizobium japonicum. The different cultures produced significant differences in number and dry weight of plant tops. With the exception of one inoculant, all the others produced increase in yield as compared to un-inoculated control.

Saxena and Singh (1978) conducted experiments to study the response of gram varieties to Rhizobium cultures isolated from different agro-climatic conditions. Their results indicated that varietal differences were significant and the interactions between culture and the host variety was insignificant.

Gaur and Sen (1979) studied the cross-inoculation group specificity in chickpea. They examined seventy-one strains of root-nodule bacteria of chickpea for nodulation in eighty-seven legume species, representative of all the legume cross-inoculation groups. In a reciprocal cross-inoculation study 287 strains of rhizobia from 52 to 87 species were examined on various chickpea isolates. Chickpea and its nodule bacteria showed no cross-inoculation specificity with any other legumes. The effectiveness of different strains of chickpea Rhizobium in field was evaluated by Pareek (1979). There was difference

in nitrogenase activity and dinitrogen fixation among the strains.

Dahiya (1979) conducted a study on the varietal interaction of pigeon pea with different strains of Rhizobium. Nodulation, shoot growth, total uptake of nitrogen and phosphorus were taken as measures of effective association. The results indicated a variety-strain specificity in case of variety T - 21 with strain A - 3. Another strain P - 4 was found to perform good with all the varieties.

pH tolerance and symbiotic efficiency of Rhizobium

Albrecht (1933) noted that nodulation in soybeans failed at pH values of 5.0 and less. Rajagopalan (1938) in his study of the root nodule bacteria of groundnut, found that out of the six rhizobial isolates tested, two showed good growth between pH 4.0 and 9.0 while others showed growth only between pH 5.5 and 8.5.

Muthusamy (1970) determined the pH tolerance of four isolates of Rhizobium of groundnut in yeast extract mannitol broth with varying pH ranging from 4.0 to 10.0. Among the four isolates, only one isolate showed good growth between pH 4.0 to 10.0. All the other isolates were able to grow only between pH 4.5 to 10.0.

Van Schreven (1972) studied the relation between tolerance to low pH and symbiotic effectiveness of Rhizobium trifolii. Three R. trifolii strains were repeatedly subcultured on

agar medium of pH values ranging from 3.7 to 7.0. The symbiotic effectiveness of the isolates after frequent subcultures on these pH was estimated by inoculation of aseptic seedlings of white clover in Jensen's medium. He found that the number of nodules formed, in the plants and the effectiveness of some strains seemed to be affected by growing Rhizobium for a long period at low pH.

Yadav and Vyas (1973) found that the optimum pH for growth of groundnut Rhizobium is in the range of 5.0 to 7.0. None of the Rhizobium strains were inhibited at pH 10.0, while low pH of 3.0 and 4.0 was lethal for all the strains tested.

A study involving five tropical legumes, five temperate legumes and Medicago sativa by Andrew (1976) revealed that nodulation and growth are strongly controlled by pH. Macroptilium lathyroides and Lotononis bainesii showed 100 per cent nodulation at pH 4.0, where as Medicago sativa, M. truncatula, M. scutellata and Glycine wightii achieved this level of nodulation only at pH 6.0 in the presence of high amount of calcium. In general, the legumes exhibited a marked decline in level of nodulation below pH 5.0.

Pandher and Kahlon (1978) reported the effect of pH on the growth of Rhizobium leguminosarum from pea (Pisum sativum L). None of the strains tested showed any growth at pH 3.0. All the strains grew though sparingly between pH 3.5 to 4.5. Good growth of the strains was noticed between pH 6.5 to 8.0.

The authors also reported decreased growth beyond pH 8.0, though it was not lethal to the bacterium.

Keyser et al.(1979) working on the acid tolerence of rhizobia in culture and in symbiosis with cowpea plants, found that cowpea rhizobia varied in its symbiotic tolerance of soil acidity.

Experiments were also conducted by Evans et al. (1980) to measure the effect of pH of the nutrient medium on symbiotic nitrogen fixation by Rhizobium leguminosarum and Pisum sativum. Both the bacterial growth and root development were adequate at all pH levels from 4.4 to 6.6. But efficient nodulation and nitrogen fixation did not occur at pH 4.8 and below.

Barber (1980) tested the growth of Rhizobium meliloti in media adjusted to pH values ranging from 4.5 to 6.5. The number of R. meliloti cells were greater with increase in pH of the nutrient medium.

In an in vitro study on the effect of different levels of pH on the growth of various isolates of cowpea rhizobia, Nair and Sivaprasad (1981) found that the native isolates were more tolerant to low pH such as 4.5 when compared to exotic isolates of rhizobia.

Antibiotic resistance and symbiotic efficiency in Rhizobium

In a study on the antibiotic resistance of thirty-three mutant clones from eleven normally effective strains of fast

growing rhizobia (Rhizobium trifolii, R. leguminosarum, R. Phaseoli and R. meliloti) Schwinghamer (1964) detected twenty-three mutant clones that were resistant to viomycin to be ineffective nitrogen fixers. Neomycin resistance gave a similar result and full cross-resistance was recorded between the two antibiotics. Acquisition of resistance to kanamycin was rarely associated with the loss of symbiotic effectiveness. No loss of effectiveness was also found associated with streptomycin resistant mutants.

The in vitro effect of streptomycin, aureomycin and terramycin was tested on ten rhizobial isolates of wal (Dolichos lablab) and fourteen isolates of methi (Trigonella foenum graecum) by Konde and Moniz (1968). Varying degrees of inhibition were produced by different antibiotics. All the rhizobial isolates were affected by streptomycin sulphate. Maximum inhibition was obtained, with streptomycin sulphate at 400 ppm.

Hendry and Jordan (1969) found that mutation to resistance to high level of viomycin in Rhizobium meliloti could involve selective reduction in permeability of the cells towards the antibiotic. They attributed this to the accumulation of phospholipid in the cell capable of forming complexes with the antibiotic, viomycin. Damery and Alexander (1969) noticed that in R. trifolii and R. meliloti kanamycin resistant mutants were in effective in nitrogen fixation, but could not detect any significant

physiological difference to account for the loss of symbiotic effectiveness.

Vincent (1971) stated that in Rhizobium simultaneous with the transfer of resistance to antibiotics there would also be the transfer of non-infectiveness property, since the genes associated with nitrogen fixation as well as antibiotic resistance are plasmid associated. Zelazna Kowalska (1971) reported that clones of Rhizobium trifolii resistant to streptomycin at levels varying from 10 to 100 ppm lost their nodulating capacity.

The response of selected strains of Rhizobium japonicum to fifty-five antibiotics was studied by Levin and Montgomery (1974). Their findings indicated no significant difference either in infectivity or effectiveness between the antibiotic sensitive strains and their resistant mutants. Abdel-wahab et al. (1976) also could not correlate resistance to chloramphenicol and penicillin with nitrogen fixation in Rhizobium trifolii mutants.

Brockwell and co-workers (1977) advocated the use of streptomycin resistant mutants for identification of rhizobial strains isolated from field environments. They also found that when streptomycin marked strains of Rhizobium trifolii were introduced into the field environment, a small proportion of the progeny of the inoculum sustained independant loss of streptomycin resistance in the field. Gollobin and Levin (1979) investigated the upper limit of resistance to the antibiotic, streptomycin of two strains of Rhizobium japonicum.

Only one grew well at 25,000 microgram per ml. Examination of the mutants showed that they all retained the symbiotic properties of parental strains. Josey et al. (1979) employed the variation in intrinsic resistance to low levels of eight antibiotics for identification of 26 Rhizobium leguminosarum strains. The wild type of R. leguminosarum strain used in the test were found to have unique patterns of resistance which remained stable and by which they could be identified.

Pain (1979) isolated a number of antibiotic-resistant mutants of Rhizobium leguminosarum and studied their symbiotic properties. Of the symbiotic mutants isolated, a minority of rifampicin resistant mutants were found to have an allied symbiotic phenotype which was ineffective.

Pankhurst (1979) evaluated the efficiency of two fast growing and two slow growing Rhizobium strains of Lotus pendunculatus. Resistance to streptomycin, spectinomycin, chloramphenicol and tetracycline was found associated with little or no loss of effectiveness with all the four strains tested. On the contrary, he noticed a significant loss of symbiotic effectiveness in 20 to 100 per cent of mutants in case of resistance to nalidixic acid, rifampicin, D-cycloserine, novobiocin and penicillin. Resistance to viomycin, neomycin, kanamycin and vibramycin was associated with loss of effectiveness with mutants of two fast-growing strains but not with those of the slow-growing ones.

Pankhurst and Craig (1979) found that one step mutants of Rhizobium strain 32 H1 resistant to D- cycloserine showed more than 90 per cent loss of asymbiotic nitrogenase activity under in vitro conditions and 25 to 30 per cent loss of symbiotic activity in the root nodules of the cowpea cultivar Caloona. Second and third step resistant mutants were found to exhibit a further decline or complete loss of symbiotic property.

Beynon and Josey (1980) demonstrated heterogeneity in a natural population of Rhizobium phaseoli using variation in antibiotic resistance. They recorded fifty-four different resistance patterns. Isolates having the same intrinsic resistance pattern were uniform in their reaction with angiserum raised against one of the inoculum strains and their colony morphology.

Om Prakash (1980) found that streptomycin resistant mutants were highly competitive but similar to parent strains in infectivity and efficiency. The chloramphenicol resistant mutants were of higher efficiency in nitrogen fixation but less competitive when compared to the parental strains.

Serological identification of rhizobial strains

Zipfel in 1912 for the first time showed the antigenic relationship between rhizobia forming nodules in Pisum sativum and Phaseolus vulgaris. Later, Klimmer and Kruger (1914) divided eighteen cultures from as many different legumes into 9 groups using agglutination tests and concluded

that the rhizobia isolated from different species of plants were serologically distinct but related at the same in other properties. Vincent (1941) distinguished two kinds of antigen namely, the heatlabile flagellar (H) antigens and the heatstable somatic (O) antigens by employing the agglutination test.

Koontz and Faber (1961) studied the somatic antigens of 25 strains of Rhizobium japonicum and classified thus into six groups. Graham (1963) classified rhizobia into three serologically distinct groups. (1) Rhizobium trifolii, R. leguminosarum and R. phaseoli; (2) R. lupini, R. japonicum and cowpea rhizobia and (3) R. meliloti. He also observed that the strains of R. meliloti showed serological affinities with Agrobacterium radiobacter and A. tumefaciens. All the groups showed wider flageller than somatic agglutination.

Johnson and Means (1964) serotyped large number of nodules obtained from soybean plants grown in different soils from Iowa, Maryland, Mississipii and South Carolina. The results indicated that the kind and abundance of serogroups of Rhizobium japonicum in nodules differed in each of the four states. Dudman (1964) employed the gel immune - diffusion technique for rhizobial strain identification. He suggested that several antigens could be recognised simultaneously by this technique, according to the position of precipitated bands. Employing a rapid agglutination test, Damirgi et al. (1967) distinguished serogroups of Rhizobium japonicum by using homogenised nodule suspensions as antigens.

Caldwell and Weber (1970) studied the distribution of Rhizobium japonicum serogroups in soybean nodules as affected by planting dates. The soybean variety 'Lee' was planted at different dates for three successive years in silt loam soil containing an established population of R. japonicum. Nodules were collected from each of the treatments and classified into appropriate serogroup by immuno fluroscent technique. Their work revealed that the occurrence of specific serological groups was influenced by planting date and the stage of plant growth, the reason being attributed to the influence of the environmentand climatic factors on the ecology of Rhizobium.

Using agar diffusion techniques and disrupted bacterial cells, Vincent and Humphrey (1970) found two internal antigens common to all the eighteen Rhizobium leguminosarum studied, but could not detect the antigens in nine slow-growing cultures of Rhizobium. Similarly, Vincent et al. (1970) found atleast one and generally two common antigens against the antisera from the soybean strains, but fifty-one fast growing rhizobia failed to give any reaction.

Studies conducted by Weber and Miller (1972) brought out the relationship between Rhizobium japonicum serogroups and soil temperature. The serogroups which were infrequently recovered at low temperature became prominent at 30°c, where as, the serogroups forming the majority of nodules at 10°c and 15°c formed fewer nodules at 30°c. Charudattan and Hubbel (1973) using agar-gel double diffusion technique compared

soluble antigens of three Rhizobium species with those of eight legumes representing compatible and non-compatible hosts. Cross reactive antigens were found between all the legume hosts and three rhizobia.

Dadarwal et al.(1974) studied the serology of forty-four isolates of Rhizobium from nodules of Arachis hypogaea, (cultivated species) A. duranensis, A. prostrata. A. villosa A. glaberata and A. marginata (wild species) by the tube agglutination and immuno-diffusion techniques. No cross-reaction was observed between isolates of Arachis hypogaea and antisera of isolates from A. marginata and A. glaberata. Likewise, antisera of isolates from A. hypogaea did not react with antisera of isolates from A. villosa and A. prostrata. They obtained diffusible precipitin bands in agar with all the cross-agglutinating strains at a titre value of 1/200 or above. Dadarwal and associates (1976) also found that rhizobia isolated from different varieties of chickpea (Cicer arietinum) grown in a locality were antigenically distinct. Strain specific precipitin reactions were observed only with Cicer rhizobia.

Kremer and Wagner (1978) advocated a new approach to the study of soil rhizobia by the use of direct diffusion of antigens from soils and development of specific bands. Using the technique of immuno-diffusion, they evaluated four strains of Rhizobium japonicum incubated in soil.

The technique of enzyme linked immuno-sorbent assay (ELISA) was described by Kishinevsky and Bar - Joseph (1978)

for serological identification of peanut Rhizobium strains both in cell suspensions of pure cultures and single root nodules of groundnut (Arachis hypogaea) plants. Antisera of three peanut Rhizobium strains were tested against eight different Rhizobium isolates. In this experiment, the workers found the ELISA to be four to six times more sensitive, than be agglutination and immuno-diffusion tests. The technique enabled detection of Rhizobium antigens in cell suspensions of 10^4 to 10^5 cells/ml. The reactions of the culture and nodule antigens were identical for all strains investigated. The minimum fresh weight of the nodule tissue necessary to perform the ELISA test was 0.4 microgram crushed in 1 ml of phosphate-buffered saline (PBS). This test was also successfully used for strain identification in mixed inoculated plants.

Sheth (1979) investigated the antigenic relationship of inoculated strain GB.755 of rhizobia for groundnut (Arachis hypogaea L.) with native rhizobia. The studies indicated that the inoculated strains differed from the native soil rhizobia. The study also indicated variation in the capacity of the rhizobial strains to establish in different soil types. The results indicated the possibilities of inoculating groundnut plants with suitable strains of rhizobia to get maximum benefit out of the natural process of biological nitrogen fixation.

Materials and methods

MATERIALS AND METHODS

An investigation to study the host-varietal specificity of *Rhizobium* in groundnut (*Arachis hypogaea* L.) was conducted at the College of Agriculture, Vellayani, Trivandrum. Fifteen varieties of groundnut of the following description were initially selected for this purpose.

Sl.No.	Variety	Source
1	CO-1	Germ plasm collection, Tamil Nadu Agricultural University, Coimbatore.
2	TMV-2	Government Seed Farm, Tindivanam, Tamil Nadu.
3	TMV-9	"
4	TMV-11	"
5	TMV-12	"
6	Ak-12-24	Germplasm collection, Department of Agricultural Botany, College of Agriculture, Vellayani.
7	Ah-32	"
8	Ah-4218	"
9	Ec-20187	"
10	Exotic-6	"
11	Red Spanish	"
12	Russia	"
13	Spanish Peanut	"
14	Uganda Local	"
15	USA-123	"

Preliminary screening for nodulation in different varieties of groundnut.

A pot culture experiment was conducted in completely randomised design with two replications each to study the nature of nodulation by native rhizobia in different varieties of groundnut. Earthern pots with an average diameter of 25 cm and depth of 30 cm were used for this purpose. These were filled with a potting mixture consisting of sand, garden soil and cowdung in the ratio of 1:1:2. Seeds of different varieties of groundnut were sown into these pots and irrigated daily. After germination and establishment, three healthy seedlings were retained in every pot. Observation on the average number of nodules formed per pot for each variety was taken on the 40th day of plant growth after carefully uprooting the plants with their intact root system. Data on the number of nodules formed was analysed statistically and based on this the different varieties were arranged into five closely related nodulation groups. Seven varieties, TMV-11, TMV-12, Ak-12-24, Ah-32, Exotic-6, Spanish Peanut and USA-123 representing these groups except group IV were thus selected to study the host-varietal specificity of Rhizobium for nodulation in groundnut. Variety belonging to group IV could not be included in the present investigation due to insufficient stock of seed material.

Isolation of Rhizobium from the above seven varieties was done by the method described by Vincent (1970). Healthy

nodules taken in test tubes were initially surface sterilized with 70 per cent ethanol for 30 seconds followed by 0.1 per cent mercuric chloride for two minutes. The nodules were then throughly washed in six changes of sterilized tap water and gently crushed with a sterilized glass rod. The residual milky fluid was streaked on sterilized yeast extract mannitol agar medium in petridishes and incubated in a B.O.D. incubator at 28 ± 1°C for three days.

Yeast extract mannitol agar medium (Allen, 1953).

Mannitol	-	10.0 g
K_2HPO_4	-	0.5 g
$MgSO_4.7H_2O$	-	0.2 g
NaCl	-	0.1 g
$CaCO_3$	-	3.0 g
Yeast extract	-	1.0 g
Congo Red (1% aqueous solution)	-	2.5 ml
Agar	-	15.0 g
Distilled water	-	1000 ml
pH	-	7.0

Typical colony of Rhizobium characterised by a white colour and gummy nature was selected and transferred to yeast extract mannitol agar slants. The various isolates were checked for their purity by repeated streaking on yeast extract mannitol agar medium. Single colony of Rhizobium thus selected for each variety was used as the stock culture.

Primary characterisation of different isolates of rhizobia.

a. Gram-staining (Hucker, 1927).

The staining was done to study the Gram reaction of different rhizobial isolates. Thin smear of 24 h. old broth culture of each isolate was prepared, heat fixed and stained with ammonium oxalate crystal violet for 1 minute. The slides were gently washed in tap water for not more than 2 seconds and treated with iodine solution for 1 minute. These were once again washed in tap water, blot dried and decolorized with 95 per cent ethyl alcohol for 30 seconds with gentle agitation. After drying the slides were counterstained for 10 seconds with safranin, washed in tap water, dried and examined under the oil-immersion objective of the microscope. The following were the compositions of different stains and reagents used for this purpose.

1) Ammonium oxalate crystal violet.

Solution A

Crystal violet (90%) - 2 g

Ethyl alcohol (95%) - 20 ml

Solution B

Ammonium oxalate - 0.8 g

Distilled water - 80 ml

Mixed together solutions A and B.

2) Gram's modification of Lugol's solution.

Iodine - 1 g

KI - 2 g

Distilled Water - 300 ml

3) Counter stain

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

Distilled water - 100 ml

b. Spore staining (Bartholomew and Mitwer, 1950)

Uniform smear of different isolates of rhizobia was heat fixed and stained with a saturated solution of malachite green (7.6% aqueous solution) for 10 minutes. The slides were then washed in tap water and counter stained with 0.25 per cent aqueous solution of safranin for 15 seconds, rinsed in tap water, blot dried and examined under the oil immersion objective for the occurrence of any endospore.

c. Growth on yeast extract mannitol agar medium with congo red.

All the seven isolates of rhizobia were streaked on yeast extract mannitol agar medium containing 2.5 ml of 1 per cent aqueous solution of congo red dye. The colour of the colonies as well as the number of days taken for the appearance of visible colonies were recorded.

d. Growth on glucose peptone agar medium.

Sterilised petri plates containing 25 ml medium of the following composition were streaked with the different isolates of rhizobia and incubated at 28 ± 1°C for 2 days. The extent of growth was recorded at the end of the incubation period.

Glucose peptone agar medium. (Vincent, 1970)

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

e. Ketolactolase test.

The solid medium of Bernaertz and de Ley, 1963 was used for this purpose.

Lactose	- 10.0 g
K_2HPO_4	- 0.5 g
$CaCl_2$	- 0.2 g
Yeast extract	- 0.5 g
$MgSO_4.7H_2O$	- 0.1 g
NaCl	- 0.2 g
$FeCl_3$	- 0.01 g
Distilled water	- 1000 ml
pH	- 6.8

The seven isolates of rhizobia were streaked on the above medium in sterile petridishes. After incubation for 7 days at 28 ± 1°C the different plates were flooded with Benedict's solution in order to test the production of lactonic acid from lactose. The change in colour of the reagent from blue to yellow around the colonies indicated the formation of lactonic acid and a positive reaction for the test.

Study of the host-varietal specificity of Rhizobium for nodulation in groundnut.

A pot-culture experiment was conducted in glass house under aseptic conditions to study the host-varietal specificity of different isolates of rhizobia. Seven groundnut varieties viz., TMV-11, TMV-12, Ak-12-24, Ah-32, Exotic-6, Spanish peanut and USA-123 selected after the preliminary screening for nodulation were used for this purpose. Homologous isolates of rhizobia from each variety was tested for compatibility and nodulation efficiency with all the seven varieties of groundnut. Appropriate control without Rhizobium inoculation was maintained in each case. An 8 x 7 factorial experiment in completely randomised design with 3 replications each was laid out with 8 levels of culture treatment and 7 varieties of groundnut (Fig.1.)

Earthern pots of 25 cm x 30 cm size and filled with a potting mixture consisting of sand, garden soil and codung in the ratio of 1:1:2 at the rate of 10 kg per pot were sterilised by autoclaving at 121°C and at a pressure of 15 p.s.i. for two hours. The various isolates of rhizobia were grown on yeast extract mannitol agar medium in sterilie petri dishes for three days at 28 ± 1°C in a B.O.D. incubator. Groundnut seeds from different varieties were initially shelled and surface sterilised with 0.1 per cent mercuric chloride for 1 minute. These were then washed throughly in three changes of sterilised tap water and treated with a thick suspension of the appropriate

Rhizobium ISOLATES / HOST VARIETY	V_1	V_2	V_3	V_4	V_5	V_6	V_7
R_1	$R_1 V_1$	$R_1 V_2$	$R_1 V_3$	$R_1 V_4$	$R_1 V_5$	$R_1 V_6$	$R_1 V_7$
R_2	$R_2 V_1$	$R_2 V_2$	$R_2 V_3$	$R_2 V_4$	$R_2 V_5$	$R_2 V_6$	$R_2 V_7$
R_3	$R_3 V_1$	$R_3 V_2$	$R_3 V_3$	$R_3 V_4$	$R_3 V_5$	$R_3 V_6$	$R_3 V_7$
R_4	$R_4 V_1$	$R_4 V_2$	$R_4 V_3$	$R_4 V_4$	$R_4 V_5$	$R_4 V_6$	$R_4 V_7$
R_5	$R_5 V_1$	$R_5 V_2$	$R_5 V_3$	$R_5 V_4$	$R_5 V_5$	$R_5 V_6$	$R_5 V_7$
R_6	$R_6 V_1$	$R_6 V_2$	$R_6 V_3$	$R_6 V_4$	$R_6 V_5$	$R_6 V_6$	$R_6 V_7$
R_7	$R_7 V_1$	$R_7 V_2$	$R_7 V_3$	$R_7 V_4$	$R_7 V_5$	$R_7 V_6$	$R_7 V_7$
R_0	$R_0 V_1$	$R_0 V_2$	$R_0 V_3$	$R_0 V_4$	$R_0 V_5$	$R_0 V_6$	$R_0 V_7$

HOMOLOGOUS *Rhizobium* ISOLATE - VARIETY INTERACTION

Treatment combinations

$R_1 - R_7$ REPRESENT THE *Rhizobium* ISOLATES FROM V_1 (TMV-12), V_2 (USA-123), V_3 (EXOTIC-6), V_4 (SPANISH PEANUT), V_5 (TMV-11), V_6 (AK 12-24) AND V_7 (Ah-32) RESPECTIVELY

R_0 - CONTROL (WITHOUT *Rhizobium* INOCULATION)

FIG: 1 HOST-VARIETAL SPECIFICITY OF *Rhizobium* FOR NODULATION IN DIFFERENT VARIETIES OF GROUNDNUT

Rhizobium culture prepared in 10 ml of sterilised distilled water. The seeds after culture treatment were sown immediately. Control treatments were maintained without any Rhizobium inoculation. P and K fertilisers were applied uniformly prior to sowing at the rate of 50 kg P_2O_5 and 40 kg K_2O per hectare. Observations on the average number of nodules formed, fresh and dry weight of nodules, fresh and dry weight of plants, plant height and percentage nitrogen content of the shoot portion were recorded for each treatment after 60 days of plant growth.

The number of nodules was counted after carefully removing each plant with its intact root system from the pot with the help of a mild jet of water. The separated nodules were weighed in a chemical balance to determine the fresh weight and then dried to a constant weight at 60°C in a drying over before taking the dry weight. The fresh weight of plants in each treatment was determined immediately after the harvest and the dry weight after drying the samples to a constant weight at 60°C. Plant height was measured from the base of the shoot portion to the growing tip of the longest branch.

The nitrogen content of the dried shoot portion was determined by the modified micro-kjeldahl method of Jackson, 1967. Hundred milligram of the powdered plant sample along with 10 g of the digestion mixture (Potassium sulphate, Cupric sulphate and Selenium metal powder in the ratio of 10:1.0:0.1) was taken in a 100 ml Kjeldahl digestion flask. Three ml of concentrated sulphuric acid of specific gravity 1.84 was added slowly to the abave digestion mixture and heated for 5 hours

till the material was completely digested. The flaks were allowed to cool down to room temperature before adding 25 ml of distilled water to each flask. After cooling the contents were transferred to 100 ml volumetric flasks and the volume made up with distilled water. Ten ml aliquot of the sample from the volumetric flask was then added to the kjeldahl flask along with 10 ml of 50 per cent sodium hydroxide solution and steam distilled till about 100 ml of the distillate was collected in the receiver flask containing initially 10 ml of 2 per cent boric acid solution with a drop of mixed indicator. The ammoniacal nitrogen content of the distillate was determined by titration with 0.05 N sulphuric acid. From the titre values, the percentage N was determined by the following equation.

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

where V = Titre value - the blank

V_1 = Total volume of plant sample made up

V_2 = Volume of plant sample distilled

N = Normality of H_2SO_4

W = Weight of powdered sample used for digestion.

Statistical analysis

Data on various observations was analysed statistically by the test of significance. The interactions between different isolates of rhizobia and host varieties was also studied for each character in order to evaluate the host varietal specificity of Rhizobium in groundnut.

Physiological and serological characteristics of different isolates of rhizobia.

A. Growth in yeast extract mannitol broth.

0.01 ml of seventy-two hour old broth cultures of all the seven isolates of rhizobia was added aseptically to 5 ml of freshly prepared sterile yeast extract mannitol broth taken in 10 cm x 1 cm capacity test-tubes. Three replications along with appropriate control without any Rhizobium inoculation were maintained in each case. The different tubes were incubated at 28 ± 1°C in a B.O.D. incubator for 72 hours. The growth rate of various isolates was determined colorimetrically at 640 nm using a Bausch and Lomb Spectronic - 20 colorimeter by measuring the optical density at 6 h. interval up to 72 h. of incubation.

b. pH tolerance

The ability of various isolates of rhizobia to grow in yeast extract mannitol broth adjusted to different pH ranging from 3.0 to 11.0 was determined colorimetrically. The pH of the broth was initially adjusted to 3.0, 5.0, 7.0, 9.0, and 11.0 by adding either 1 N hydrochloric acid or 1 N sodium hydroxide solution. Five ml aliquots of the above medium was dispensed in 10 cm x 1 cm test tubes and sterilized in an autoclave at 121°C for 20 minutes. The different tubes were thus inoculated in duplicate with 0.01 ml of 72 h. old broth culture of various isolates of rhizobia and incubated in a B.O.D. incubator at 28 ± 1°C for 72 hours. Growth at different levels of pH was determined colorimetrically at 640 nm using

a Bausch and Lomb Spectronic-20 colorimeter. Appropriate control without any Rhizobium inoculation was maintained in each case. Growth was measured in terms of optical density at the time of inoculation and at 24 h. intervals upto 72 h. of incubation. Data obtained for each culture was analysed statistically by the test of significance.

c. Antibiotic sensitivity

The seven isolates of rhizobia were tested for their sensitivity to different levels of streptomycin sulphate in yeast extract mannotol broth. Stock solution of streptomycin sulphate (Ambistryn - S, Sarabhai Chemicals, Baroda) prepared in sterilised distilled water was added to pre-sterilised yeast-extract mannitol broth taken in 10 cm x 1 cm test tubes in order toobtain a final concentration of 50, 125, 250 and 500 microgram per ml of the broth. These were thus inoculated in duplicate with 0.01 ml of 72 h. old broth culture of different isolates of rhizobia and incubated in a B.O.D. incubator at 28 ± 1°C for 72 hours. The antibiotic sensitivity of different isolates was determined by measuring the growth in terms of optical density using a Bausch and Lomb colorimeter at 640 nm. The optical density was measured at the time of inoculation and at 24 h. interval, upto 72 h. of incubation. Data thus obtained was analysed statistically by the test of significance.

d. Serological characteristics.

Preparation of antigen.

The antigens were prepared according to the method of Vincent (1970). Pure cultures of three isolates of rhizobia,

R_3, R_5 and R_6 were grown initially in yeast extract mannitol agar slants in Roux bottles at 28 ± 1°C for four days in a B.O.D. incubator. The bacterial cells were then harvested in 0.85 per cent NaCl solution and freed of large clumps by keeping in a rotary shaker for 1 hour after adding a pinch of Sodium EDTA. This suspension was then filtered through thin pads of cotton to remove the excess of gum and centrifuged at 15,000 rpm in a refrigerated high speed centrifuge for 30 minutes. The resulting pellet was washed three times by resuspending in 0.85 per cent sterilised NaCl solution and centrifuged at 15,000 rpm for 30 minutes. Finally, the washed pellet for each isolate of Rhizobium was suspended in 10 ml of sterile physiological saline and transferred to sterilized 20 ml screw-capped bottles for storing at 0°C in a deep freezer.

Preparation of antiserum

Healthy rabbits of unknown pedigree obtained from the Small Animal House, College of Veterinary and Animal Sciences, Mannuthy, Trichur were used for the preparation of antiserum. Emulsion of different antigens in Freund's (Difco) complete adjuvant was prepared by mixing equal quantity of both in a test tube using a syringe without needle. 2 ml of the above emulsion was then injected intramuscularly into the large thigh muscle of each rabbit. A similar injection was given one month followed by two more booster doses of 1 ml of each of the antigen without the adjuvant given intravenously through the large marginal ear vein of the rabbit at weekly intervals. In all

these cases, an antigen suspension containing approximately 10^9 cells/ml was used. Fifteen ml of blood was collected from each rabbit into a clean pre-sterilized beaker by making an incision on the large marginal ear vein. The blood was allowed to clot for 4 h. at 28 ± 1°C in a B.O.D. incubator followed by overnight incubation at 4°C in a refrigerator in order to allow the complete separation of blood serum. The separated serum was then centrifuged at 3000 rpm for 5 minutes in a laboratory centrifuge to sediment the residual blood cells. The clear supernatent after centrifugation was transferred to clean 5 ml screw capped bottles and stored at 0°C in a deep freezer.

Determination of serum titre

The titre value for different antisera was determined by the method of Vincent (1970). For each serum to be tested, two-fold serial dilution starting from 1:50 to an end dilution of 1:6, 400 was prepared according to the following schedule.

Tube	1	2	3	4	5	6	7	8
ml Saline	9.6	5	5	5	5	5	5	5
ml Serum	0.4	5(1)	5(2)	5(3)	5(4)	5(5)	5(6)	5(7)
Final dilution	50	100	200	400	800	1,600	3,200	6,400

One ml of the homologous antigen containing approximately 10^9 cells/ml was added to 1 ml of each of the above dilution of the antiserum. One ml of the antigen along with 1 ml of physiological saline served as the control. The different tubes were kept in a

water bath at 52°C for 4 hours before viewing the agglutination of various antigens in different dilutions of the homologuous antiserum.

d. Tube agglutination test for serological identification of different isolates.

Antiserum prepared for isolates R_3, R_5 and R_6 was used to test the serological relationship if any between these isolates and the remaining four isolates of rhizobia, R_1, R_2, R_4 and R_7 by the method described earlier. The antigens for the latter four isolates was prepared by harvesting the growth of these rhizobia from yeast extract mannitol agar slants in physiological saline and then concentrating it by centrifugation at 3000 rpm. One ml of these antigens containing approximately 10^9 cells/ml was then added to 1:800 dilution of each of the antiserum prepared for isolates R_3, R_5 and R_6. Serum tubes containing homologous antigens served as the control.

Results

RESULTS

Preliminary screening for nodulation in different varieties of groundnut (*Arachis hypogaea* L.)

A pot culture experiment was conducted to study the pattern nature of nodulation in 15 different varieties of groundnut. The number of nodules formed in each variety by the native rhizobia is given in Table 1. The analysis of variance for the same is presented in Appendix I.

There was significant difference in the number of nodules formed in different varieties of groundnut. Maximum number of 76.25 nodules was formed in variety TMV-12 followed by other varieties such as USA-123 (66.25), Exotic - 6 (65.25), KG-61-540 (59.25) and Spanish peanut (53.75). The number of nodules formed was less in Red Spanish (37.50), AK-12-24 (36.25) and Ah-32 (31.0) (Table 1).

Based on the significant difference between varieties in the number of nodules formed, they were serially arranged into the following five distinct nodulation groups (Table 1). In each of the group, the minimum number of nodules formed by a particular variety was always higher than the maximum number of nodules formed by the variety in the immediate succeeding group.

Table 1. Pattern of nodulation in different varieties of groundnut (*Arachis hypogaea* L.)

Sl.No.	Groundnut variety	Nodule number*	Nodulation group
1.	TMV-12	76.25	I
2	USA-123	66.25	
3	Exotic-6	65.25	II
4	KG-61-540	59.25	
5	Spanish peanut	58.75	
6	TMV-9	56.00	
7	Ec-20137	51.75	
8	TMV-11	49.00	III
9	Ah-4218	49.25	
10	Uganda local	48.50	
11	Co-1	46.25	
12	Russia	44.50	IV
13	Red Spanish	37.50	
14	Ak-12-24	36.25	V
15	Ah-32	31.00	
C.D (5%)		8.27	

* Mean of 2 replications.

Seven varieties of groundnut TMV-12, USA-123, Exotic-6, Spanish peanut, TMV-11, Ak-12-24 and Ah-32 were then selected to study the host varietal specificity of rhizobia isolated from them.

Characterisation of rhizobia from different varieties of groundnut

Pure cultures of rhizobia isolated from the above seven varieties of groundnut were maintained on yeast extract mannitol agar slants. The relation between the host variety and the Rhizobium isolate is given in Table 2.

Table 2. Relation between host-variety and Rhizobium isolate.

Sl.No.	Host-variety	Rhizobium isolate
1	V_1 - TMV-12	R_1
2	V_2 - USA-123	R_2
3	V_3 - Exotic-6	R_3
4	V_4 - Spanish peanut	R_4
5	V_5 - TMV-11	R_5
6	V_6 - Ak-12-24	R_6
7	V_7 - Ah-32	R_7

All the seven isolates of rhizobia were gram-negative, non-spore forming and rod-shaped. They produced raised, transluscent and gummy colonies on yeast extract mannitol agar

medium after 72 h. of incubation. On glucose peptone agar the growth of these isolates was minimum even after 7 days of incubation. Further, none of the isolates produced lactonic acid on ketolactose medium (Table 3).

Table 3. Primary characters of different isolates of rhizobia

Isolate No.	Gram reaction	Sporulation	Growth on yeast extract mannitol agar	Growth on yeast extract mannitol agar	Kelolactolase test
R_1	-ve	N.S	F	±	-
R_2	-ve	N.S	F	±	-
R_3	-ve	N.S	F	±	-
R_4	-ve	N.S	F	±	-
R_5	-ve	N.S	F	±	-
R_6	-ve	N.S	F	±	-
R_7	-ve	N.S	F	±	-

-ve - Gram negative; N.S. - non-sporeforming; F- Fast rate of growth

± - Scanty growth; - Lactonic acid not produced.

Host-varietal specificity of *Rhizobium* in groundnut

The biometric observations taken on the sixtieth day after planting viz., total number of nodules formed fresh and dry weight of nodules, fresh and dry weight of plants, plant height and percentage nitrogen content of the shoot portion were statistically analysed in order to determine the host-varietal

specificity of different isolates of rhizobia. The mean values for different observations are given in Tables 4 to 10 and their analysis of variance in Appendix II. Data for all characters is interpreted as the response of individual isolate of Rhizobium to different varieties of groundnut and also as the response of each variety of groundnut to various isolates of rhizobia. The comparitive effect of Rhizobium inoculation in different varieties of groundnuts is shown in plates 1 to 5.

Nodule number

a. Response of individual isolate of Rhizobium to different varieties of groundnut

All the isolates of rhizobia except R_5 produced the maximum number of nodules in their respective host varieties (Table 4). R_5 produced the maximum number of nodules, 65.77 in another variety, USA-123. The number of nodules formed by isolates R_3 and R_5 in variety TMV-12, R_3, R_4, R_6 and R_7 in USA-123, R_7 in Exotic-6, R_5, R_6 and R_7 in Spanish peanut, R_3 in TMV-11 and R_5 in Ah-32 were statistically on par with the number of nodules formed by these isolates in their respective host varieties.

b. Response of individual variety of groundnut to different isolates of rhizobia

The different varieties of groundnut exhibited a favourable response for nodulation with their homologous isolate of Rhizobium except in case of variety TMV-11 where significantly

Plate 1. Comparative effect of inoculation of Rhizobium isolate R_1 in varieties USA-123, IMV-12 and Ak-12-24. Control plants represent the homologous variety IMV-12.

Plate 2. Comparative effect of inoculation of Rhizobium isolate R_3 in varieties USA-123, Exotic-6 and Ak-12-24. Control plants represent the homologous variety Exotic-6.

Plate 3. Comparative effect of inoculation of Rhizobium isolate R_4 in varieties Exotic-6, Spanish peanut and Ak-12-24. Control plants represent the homologous variety Spanish peanut.

Plate 4. Comparative effect of inoculation of Rhizobium isolate R_5 in varieties USA-125, TMV-11 and Ak-12-24. Control plants represent the homologous variety TMV-11.

Plate 5. Comparative effect of inoculation of Rhizobium isolate R_7 in varieties Spanish peanut, Ah-32 and Ak-12-24. Control plants represent the homologous variety Ah-32.

higher number of nodules, 88.43 and 87.40 was produced by two other isolates R_2 and R_3, respectively (Table 4). The number of nodules formed in variety TMV-12 isolates R_2 and R_3, in Exotic-6, Spanish peanut and Ak-12-24 by R_2 was also higher than the number of nodules formed in these varieties by their homologous isolates. The response of Spanish peanut to isolates R_3 and R_7, TMV-11 to R_1, R_4 and R_7, Ak-12-24 to R_1, R_3 and R_5 and Ah-32 to R_2 was statistically on par with the response obtained with their homologous isolate of Rhizobium (Table 4).

In general, the average number of nodules produced by isolate R_2 in different varieties of groundnut was significantly higher than that produced by all the other isolates. Similarly, USA-123 was the best variety in terms of the average number of nodules formed by different isolates of rhizobia (Table 4).

Nodule fresh weight

a. Response of individual isolate of Rhizobium to different varieties of groundnut

Fresh weight of nodules was maximum with the homologous isolate of Rhizobium except with the isolates R_3 and R_5 which produced a higher fresh weight in varieties USA-123 and TMV-12, respectively (Table 5). The fresh weight of nodules due to isolates R_3, R_4, R_6 and R_7 in TMV-12, R_1, R_5, R_6, and R_7 in USA-123, R_2, R_5 and R_6 in Exotic-6 R_5 and R_6 in Spanish peanut, and all other isolates in Ah-32 was statistically on par with the fresh weight of nodules produced by these isolates in their respective host varieties.

Table 4. Nodule number* - Interaction between *Rhizobium* isolates and groundnut varieties.

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	81.42	54.40	48.00	60.67	58.50	44.26	54.17	57.35
R_2	92.67	128.03	99.43	80.33	88.43	53.97	71.33	87.72
R_3	85.77	79.56	89.76	69.67	87.40	46.00	64.10	76.39
R_4	59.70	79.50	59.87	79.50	59.70	30.00	36.86	58.08
R_5	61.55	65.77	47.17	59.07	61.55	46.87	54.67	57.04
R_6	30.00	47.00	37.97	46.20	31.00	53.47	35.10	40.00
R_7	61.58	68.93	68.93	68.20	52.83	35.40	76.77	61.70
Control	2.00	1.00	2.33	2.33	2.33	2.00	1.00	2.04
Mean	58.31	63.05	56.67	50.25	57.38	30.85	52.30	

C.D.(5%) for comparison of levels of varieties - 4.73

C.D.(5%) for comparison of levels of *Rhizobium* - 4.42

C.D.(5%) for comparison of levels of variety x *Rhizobium* interaction - 12.51

* Mean of 3 replications.

Table 5. Nodule fresh weight (mg)* - Interaction between *Rhizobium* isolates and groundnut varieties.

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	104.67	87.00	82.33	65.33	64.00	55.00	85.67	73.43
R_2	106.33	135.00	118.33	95.67	102.33	60.33	117.33	105.05
R_3	91.00	117.67	98.33	65.33	72.00	55.00	94.67	86.19
R_4	77.00	61.00	50.33	86.33	47.33	24.33	84.33	67.24
R_5	84.67	70.00	65.33	64.00	78.33	48.33	63.00	66.95
R_6	50.00	49.67	39.00	46.67	31.00	55.33	46.67	44.14
R_7	78.67	95.30	68.00	61.67	57.33	48.00	99.67	72.67
Control	3.00	3.33	2.66	2.33	3.33	1.67	2.00	2.62
Mean	77.25	70.29	65.54	62.71	57.00	42.25	77.29	

C.D. (5%) for comparision of levels of varieties - 7.55

C.D. (5%) for comparison of levels of *Rhizobium* - 8.07

C.D. (5%) for comparison of levels of variety x *Rhizobium* interaction - 21.35

*Mean of 3 replications.

b. Response of individual variety of groundnut to different isolates of rhizobia

A favourable response in terms of nodule fresh weight was obtained in all varieties with the homologous isolate of Rhizobium except in the case of TMV-11 where a significant increase in nodule fresh weight was produced by another isolate, R_2 (Table 5). The fresh weight of nodules was also higher in varieties TMV-12, Exotic-6, Spanish peanut, Ak-12-24 and Ah-32 due to isolate R_2. The response of variety TMV-12 to isolates R_3 and R_5, USA-123 to R_3, Exotic-6 to R_1, Spanish peanut to R_1 and R_3, TMV-11 to R_1, R_3 and R_7, Ak-12-24 to R_1, R_3, R_5 and R_7 and Ah-32 to R_1, R_3 and R_4 was statistically on par with that obtained with the homologous isolate of Rhizobium (Table 5).

In general, the average fresh weight of nodules produced by isolate R_2 in different varieties of groundnut was significantly higher than all the other isolates. Similarly, TMV-12 and USA-123 were the best varieties in terms of the fresh weight of nodules formed by different isolates of rhizobia (Table 5).

Nodule dry weight

a. Response of individual isolate of Rhizobium to different varieties of groundnut.

A uniform increase in nodule dry weight was produced in all varieties by the homologous isolate of Rhizobium except in the case of isolate R_1 which produced a significant increase in

nodule dry weight in varieties such as USA-123, Exotic-6 and TMV-11 when compared to TMV-12 (Table 6). The dry weight of nodules was also higher in Spanish peanut by this isolate. The dry weight of nodules due to isolate R_5 in variety TMV-12, R_3, R_5 and R_7 in USA-123, R_5 in Spanish peanut, R_3 in TMV-11, R_1 in Ak-12-24, and R_1, R_3 and R_5 in Ah-32 was statistically on par with the dry weight of nodules produced by the isolates in their respective host varieties (Table 6).

b. Response of individual variety of groundnut to different isolates of rhizobia

In variety TMV-12 and TMV-11 significant increase in dry weight of nodules was obtained with isolates R_2 and R_5, and R_2 R_3 respectively when compared to their homologous isolate of Rhizobium (Table 6). The dry weight of nodules in variety TMV-12 with isolate R_3, in Exotic-6 and Spanish peanut with R_2 was higher than with the homologous rhizobial isolates. The response of variety TMV-12 to isolates R_4 and R_7, Spanish peanut to R_3, TMV-11 to R_1 and R_7, Ak-12-24 to R_2 and R_7, Ak-12-24 to R_2 and R_7 and Ah-32 to R_2 and R_3 was statistically on par with that obtained with the homologous isolate of Rhizobium (Table 6).

In general, the average dry weight of nodules produced by isolate R_2 in different varieties of groundnut was significantly higher than in all the other isolates. Similarly, USA-123, TMV-11 and Ah-32 were the best varieties of groundnut in terms of dry weight of nodules formed by different isolates of rhizobia (Table 6).

Table 6. Nodule dry weight (mg)* - Interaction between *Rhizobium* isolates and groundnut varieties.

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	20.00	26.00	26.67	21.00	28.33	19.00	20.00	23.58
R_2	34.33	52.00	39.00	31.00	39.00	20.00	33.00	35.76
R_3	23.67	37.00	38.33	27.00	35.00	15.67	36.67	30.33
R_4	16.33	24.33	17.67	30.00	20.33	14.00	19.00	19.95
R_5	25.00	23.00	20.00	24.00	26.67	14.33	25.00	22.57
R_6	10.33	13.67	12.00	15.00	15.33	20.33	12.33	14.14
R_7	17.67	35.67	22.33	16.00	25.00	20.00	37.00	22.24
Control	1.64	1.76	1.66	1.34	1.31	1.00	1.00	1.39
Mean	18.66	24.71	21.54	20.79	24.66	14.79	24.42	

C.D. (5%) for comparison of levels of varieties - 2.41

C.D. (5%) for comparison of levels of *Rhizobium* - 2.57

C.D. (5%) for comparison of levels of variety x *Rhizobium* interaction - 4.40

* Mean of 3 replications.

Plant fresh weight

a. Response of individual isolate of Rhizobium to different varieties of groundnut

Fresh weight of plants was maximum with the homologous isolate of Rhizobium except with the isolates R_3, R_4 and R_5 which produced maximum fresh weight in the variety USA-123 (Table 7). The fresh weight of plants due to isolate R_4 was also higher in variety Exotic-6. Plant fresh weight due to isolates R_5 and R_7 in variety TMV-12, R_1 and R_7 in USA-123, R_2, R_5 and R_7 in Exotic-6, R_4, R_5 and R_6 in Ah-32 was statistically on par with fresh weight of plants by these isolates in their respective host varieties (Table 7).

b. Response of individual variety of groundnut to different isolates of rhizobia

A favourable response in fresh weight of plants was obtained with the homologous isolate of Rhizobium in all the varieties except in the case of variety TMV-11 where isolate R_2 produced significant increase in fresh weight. The fresh weight of plants in varieties TMV-12, Exotic-6 and Ah-32 with isolate R_2 was also higher than that obtained with their respective isolates of rhizobia (Table 7). The response of variety TMV-12 to isolate R_7, Exotic-6 to R_4, Spanish peanut to R_2 and R_3, TMV-11 to R_3 and R_7, Ah-32 to R_3 and R_4 and Ak-12-24 to all the other isolates was statistically on par with that obtained with the homologous isolate of Rhizobium (Table 7).

Table 7. Plant fresh weight (g)* - Interaction between *Rhizobium* isolates and groundnut varieties

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	7.95	7.85	4.54	4.55	5.44	4.55	5.92	5.69
R_2	8.20	10.23	9.28	6.68	8.37	4.95	7.84	7.94
R_3	5.60	8.60	8.58	7.23	6.09	5.33	7.21	6.95
R_4	5.30	7.52	7.63	7.35	5.70	4.90	6.90	6.47
R_5	5.85	6.95	5.92	5.60	6.80	5.05	5.89	5.90
R_6	4.03	3.02	3.98	4.00	4.09	5.82	5.00	4.37
R_7	7.15	7.60	6.72	6.12	5.82	5.43	7.75	6.54
Control	2.08	1.90	2.02	1.75	2.19	1.28	1.48	1.78
Mean	5.76	6.46	6.03	5.42	5.46	4.67	6.00	

C.D. (5%) for comparison of levels of varieties - 0.40

C.D. (5%) for comparison of levels of *Rhizobium* - 0.37

C.D. (5%) for comparison of levels of variety x *Rhizobium* interaction - 1.05

* Mean of 3 replications.

In general, the average fresh weight of plants due to isolate R_2 in different varieties of groundnut was significantly higher than all the other isolates. Similarly, USA-123 was the best variety in terms of fresh weight of plants due to inoculation with different isolates of rhizobia (Table 7).

Plant dry weight

a. Response of individual isolate of Rhizobium to different varieties of groundnut.

Dry weight of plants was maximum with the homologous isolate of Rhizobium except with the isolates R_4, R_5, R_6 and R_7 which produced maximum dry weight in the variety USA-123 (Table 8). The increase in dry weight due to isolate R_5 in variety TMV-12, Exotic-6 and Ah-32 was also higher when compared to its host variety TMV-11. The dry weight of plants due to isolate R_2, R_3 and R_6 in TMV-12, R_1 and R_3 in USA-123, R_1, R_2, R_4, R_5 and R_7 in Spanish peanut, R_1, R_4, R_6 and R_7 in TMV-11. R_1 and R_5 in Ak-12-24, R_1 and R_6 in Ah-32 was statistically on par with the dry weight of plants by these isolates in their respective host varieties (Table 8).

b. Response of individual variety of groundnut to different isolates of rhizobia

A favourable response in dry weight of plants was obtained with the homologous isolate of Rhizobium in all the varieties except in varieties TMV-12, TMV-11 and Ak-12-24. Increase in dry weight in TMV-12 due to isolates R_2 and R_3, in TMV-11 due

to R_1, R_2, R_3, R_4 and in Ak-12-24 due to R_5 was higher when compared to the dry weight of plants by their respective homologous isolates or rhizobia. The response of variety TMV-12 to isolates R_5 and R_7 Exotic-6 to R_2, Spanish peanut to R_2, R_3 and R_7, TMV-11 to R_6, Ah-32 to R_2, R_3, R_4 and R_5 and Ak-12-24 to all other isolates was statistically on par with the response of these varieties to their individual homologous isolate of Rhizobium (Table 8).

In general, the average dry weight of plants due to isolate R_2 in different varieties was significantly higher than all the other isolates. The increase in dry weight of plants due to isolate R_3 was also statistically on par with that of isolate of R_2. Similarly, USA-123 proved to be the best variety in terms of dry weight of plants due to inoculation with different isolates of rhizobia. The response of varieties such as Exotic-6, Spanish peanut and TMV-11 to Rhizobium inoculation was also equally good (Table 8).

Plant height

a. Response of individual isolate of Rhizobium to different varieties of groundnut

A general increase in plant height due to homologous isolate of Rhizobium was obtained only with isolates R_1 and R_7. Isolates R_2 and R_4 produced significant increase in plant height in varieties TMV-12 and Ah-32 respectively instead of in varieties USA-123 and Spanish peanut (Table 9). The isolate R_3 also produced a similar result in variety Ah-32. The increase in

Table 8. Plant dry weight (g)* - Interaction between *Rhizobium* isolates and groundnut varieties.

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	1.49	1.40	1.44	1.03	1.46	1.15	1.27	1.3
R_2	1.66	2.05	1.91	1.60	1.55	1.14	1.52	1.81
R_3	1.59	1.96	1.99	1.45	1.44	1.15	1.38	1.6
R_4	1.02	1.84	1.43	1.78	1.38	0.98	1.35	1.5
R_5	1.40	1.46	1.52	1.25	1.30	1.26	1.40	1.4
R_6	0.86	1.20	0.94	1.00	0.95	1.16	0.77	0.9
R_7	1.30	1.76	1.46	1.45	1.41	1.09	1.75	1.4
Control	0.63	0.42	0.40	0.29	0.55	0.27	0.36	0.4
Mean	1.26	1.43	1.34	1.29	1.31	1.03	1.19	

C.D.(5%) for comparison of levels of varieties - 0.14

C.D.(5%) for comparison of levels of *Rhizobium* - 0.16

C.D.(5%) for comparison of levels of variety x *Rhizobium* interaction - 0.42

* Mean of 3 replications.

plant height due to R_3, R_5 and R_6 in TMV-12, R_1, R_3, R_4 and R_6 in USA-123, R_1, R_2, R_4, R_5 and R_6 in Exotic-6, R_3, and R_6 in TMV-11, R_2, R_3 and R_4 in Ak-12-24, R_5 and R_6 in Ah-32 was statistically on par with the height of plants by these isolates in their respective host varieties (Table 9).

b. Response of individual groundnut varieties to different isolates of rhizobia

A favourable response in plant height was obtained with the homologous isolate of Rhizobium in all the varieties except in TMV-12 where the isolate R_2 produced significant increase in plant height (Table 9). The response of variety TMV-12 to isolate R_3, R_5 and R_7, USA-123 to R_1, R_3 and R_7, RMV-11 to R_1, R_2, R_3 and R_7, Ak-12-24 to R_1, R_2, R_3, R_4 and R_7, Ah-32 to R_2, R_3, R_4, R_5, and R_6 and varieties Exotic-6 and Spanish peanut to all the other isolates was statistically on par with that obtained with the homologous isolate of Rhizobium. The increase in plant height in control plants of varieties Spanish peanut and Ah-32 was also statistically on par with that of the homologous isolates of Rhizobium (Table 9).

In general, the average height of plants due to the isolate R_2 in different varieties of groundnut was significantly higher than that due to all the other isolates. Similarly, Ah-32 was the best variety in terms of plant height due to inoculation with different isolates of rhizobia.

Table 9. Plant height (cm)* - Interaction between *Rhizobium* isolates and groudnut varieties.

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	48.67	44.83	42.77	31.50	45.50	34.47	36.00	39.96
R_2	58.33	48.33	48.53	37.33	49.83	40.00	59.33	48.93
R_3	43.40	42.90	43.33	36.67	42.33	45.17	56.83	44.37
R_4	42.83	35.17	39.57	33.50	32.93	36.03	56.33	40.91
R_5	46.50	36.60	41.50	40.37	43.90	34.17	50.67	41.96
R_6	37.33	33.83	40.17	29.40	29.50	35.73	43.33	35.61
R_7	43.93	43.60	48.17	36.27	38.26	36.50	58.33	43.15
Control	30.07	32.40	31.67	29.50	30.53	29.83	35.83	32.83
Mean	43.94	38.46	43.21	35.57	37.48	37.24	50.88	

C.D. (5%) for comparison of levels of varieties - 3.19

C.D. (5%) for comparison of levels of *Rhizobium* - 3.41

C.D. (5%) for comparison of levels of variety x *Rhizobium* interaction - 9.03

* Mean of 3 replications.

Percentage nitrogen content of plants

a. Response of individual isolate of Rhizobium to different varieties of groundnut

The percentage nitrogen content of plants was maximum only with the homologous isolates R_2, R_4 and R_6 (Table 10). Isolate R_1 produced significantly higher nitrogen content in USA-123 when compared to TMV-12. The percentage nitrogen content of plants due to isolates R_3, R_5 and R_7 in USA-123, R_1, R_5 and R_7 in Exotic-6, R_7 in TMV-12 and Spanish peanut was higher than the nitrogen content of the homologous host variety. Increase in nitrogen content due to isolate R_5 and R_6 in TMV-12, R_2 in Exotic-6, R_1 and R_7 in TMV-11 and R_5 in Ah-32 was also statistically on par with the nitrogen content of plants by these isolates in their respective host varieties varieties (Table 10).

b. Response of individual variety of groundnut to different isolates of rhizobia

A favourable increase in nitrogen content of plants was obtained with the homologous isolate of Rhizobium in all the varieties except in the case of Exotic-6 and TMV-11 where significant increase in nitrogen content was obtained with another isolate R_2 (Table 10). The percentage nitrogen content in varieties TMV-12, Ak-12-24 and Ah-32 due to isolate R_2 and in Ah-32 due to isolate R_5 was higher than the nitrogen content obtained with the homologous isolate of rhizobia.

Table 10. Percentage nitrogen content* - Interaction between Rhizobium isolates and ground varieties

Rhizobium isolate	Groundnut variety							Mean
	TMV-12	USA-123	Exotic-6	Spanish peanut	TMV-11	Ak-12-24	Ah-32	
R_1	3.15	3.70	3.16	2.81	3.05	2.54	2.75	2.99
R_2	3.26	4.13	3.99	3.44	3.72	2.93	3.08	3.51
R_3	3.14	3.78	3.57	2.90	2.90	2.49	2.95	3.10
R_4	3.00	3.14	3.00	3.44	2.90	2.55	2.81	2.91
R_5	2.76	3.44	3.18	2.39	3.08	2.38	3.05	2.89
R_6	2.39	2.21	2.36	2.11	2.25	2.72	2.30	2.43
R_7	3.05	3.23	3.10	3.40	2.86	2.37	3.02	3.01
Control	1.41	1.48	1.25	1.48	1.34	1.46	1.54	1.43
Mean	2.74	3.08	2.95	2.74	2.76	2.43	2.78	

C.D. (5%) for comparison of levels of varieties - 0.15

C.D. (5%) for comparison of levels of Rhizobium - 0.14

C.D. (5%) for comparison of levels of variety x Rhizobium interaction - 0.39

* Mean of 3 replications.

The response of variety TMV-12 to isolates R_3, R_4 and R_7, USA-123 to R_3, Exotic-6 to R_5, Spanish peanut to R_2 and R_7, TMV-11 to R_3, R_4 and R_7, Ah-32 to R_1, R_3 and R_4 and Ak-12-24 to all other isolates was statistically on par with that obtained with homologous isolate of Rhizobium (Table 10).

In general, the average nitrogen content of plants due to isolate R_2 was significantly higher than all the other isolates. Similarly, USA-123 was the best variety in terms of percentage nitrogen content of plants due to inoculation with different isolates of rhizobia. The response of variety Exotic-6 to Rhizobium inoculation was also statistically on par with the variety USA-123 (Table 10).

Physiological and serological characteristics of different isolates of rhizobia

a. Growth in yeast extract mannitol broth

Rate of growth of all the seven isolates of rhizobia in yeast extract mannitol broth was determined colorimetrically. Mean values of O.D obtained at 6 h. intervals to 72 h. of incubation are presented in Table 11. All the isolates had an almost identical fast rate of growth (Fig.2). Isolates R_2 and R_3 reached the stationery phase in 42 h. while the remaining isolates R_1, R_4, R_5, R_6 and R_7 reached the same in 48 h. There was no growth for any of the isolates tested after 54 h. of incubation.

b. pH tolerance

Tolerance to a wide range of pH from 3.0 to 11.0 was tested for all the seven isolates of rhizobia in yeast extract mannitol

Table 11. Growth of different isolates of rhizobia in yeast extract mannitol broth.

Rhizobium isolate	O.D. values at 6 h. intervals*.											
	0	6	12	18	24	30	36	42	48	54	60	66	72
R_1	0.020	0.045	0.065	0.085	0.130	0.150	0.180	0.220	0.260	0.260	0.260	0.260	0.260
R_2	0.020	0.065	0.130	0.160	0.240	0.320	0.400	0.450	0.450	0.450	0.450	0.450	0.450
R_3	0.015	0.050	0.120	0.150	0.220	0.290	0.300	0.375	0.378	0.375	0.375	0.375	0.375
R_4	0.020	0.040	0.100	0.110	0.140	0.170	0.200	0.250	0.290	0.290	0.290	0.290	0.290
R_5	0.015	0.035	0.050	0.090	0.120	0.185	0.200	0.260	0.285	0.285	0.285	0.285	0.285
R_6	0.020	0.025	0.035	0.065	0.100	0.130	0.150	0.200	0.210	0.210	0.210	0.210	0.210
R_7	0.015	0.030	0.050	0.090	0.125	0.170	0.250	0.280	0.300	0.300	0.300	0.300	0.300

* Mean of 3 replications.

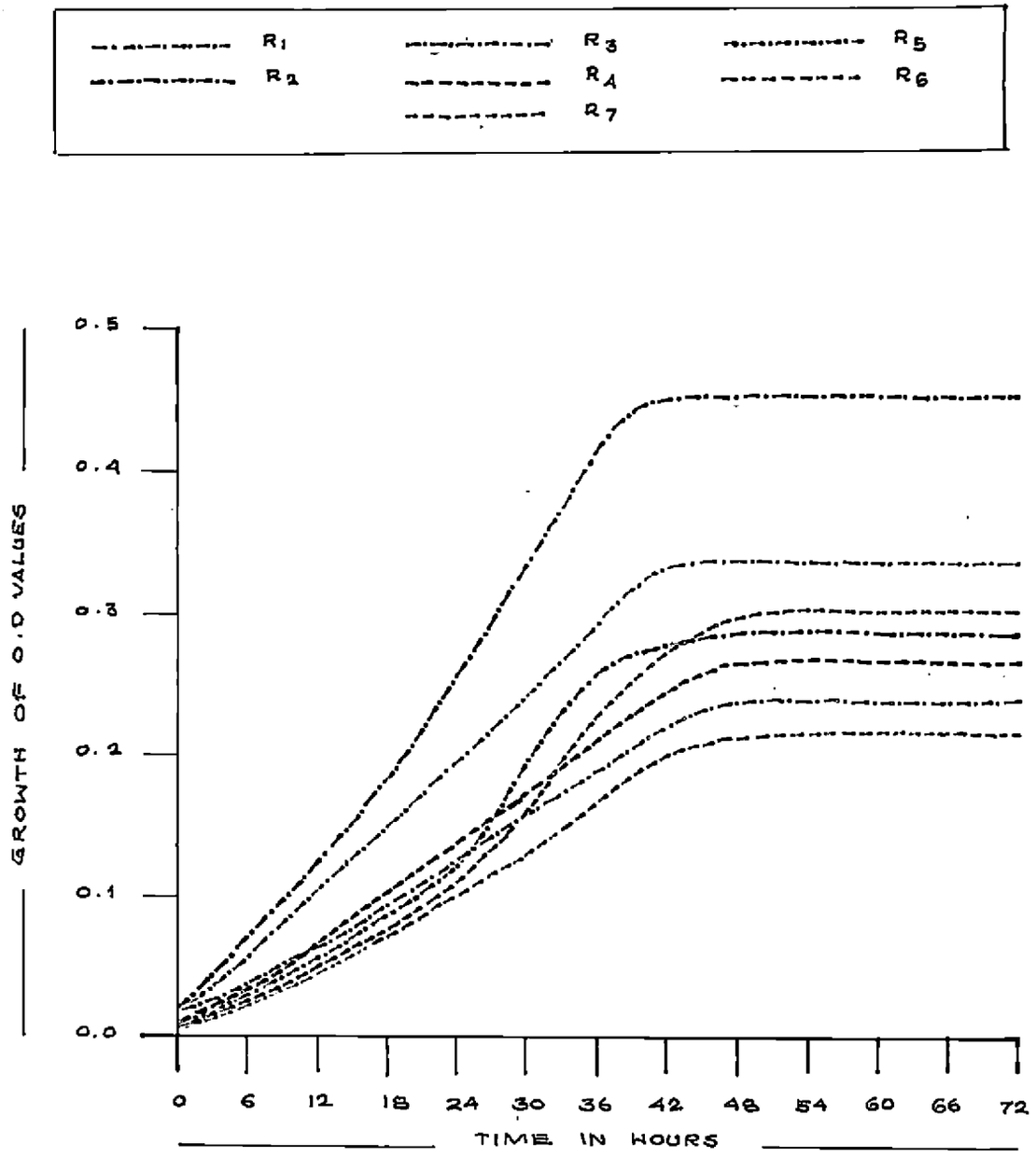


FIG: 2 GROWTH OF DIFFERENT ISOLATES OF RHIZOBIA IN YEAST EXTRACT MANNITOL BROTH

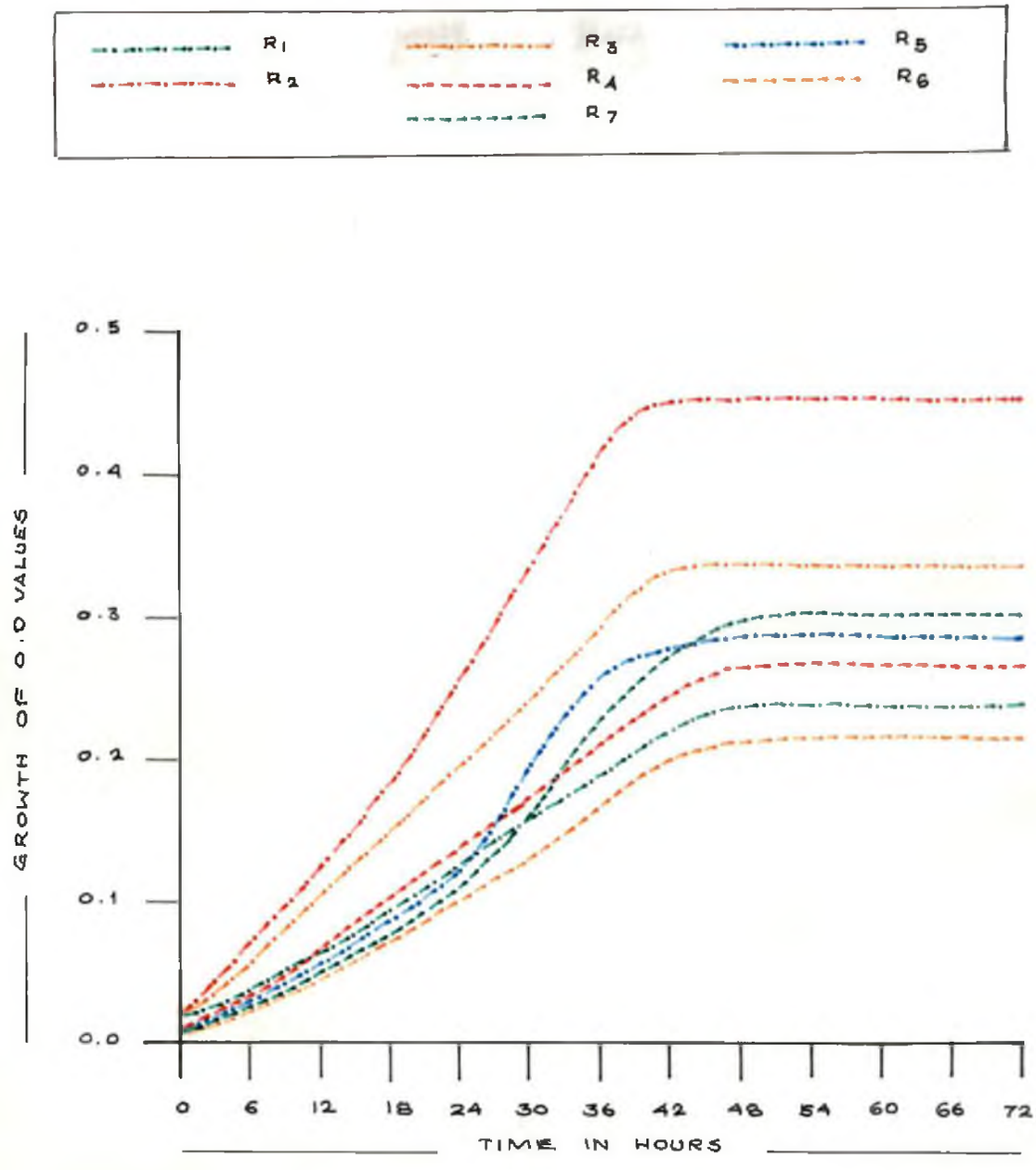


FIG: 2 GROWTH OF DIFFERENT ISOLATES OF RHIZOBIA IN YEAST EXTRACT MANNITOL BROTH

Table 12. pH tolerance of different isolates of Rhizobium

Isolate R_1

pH	O.D. values at 24 h. intervals*			
	0	24	48	72
3	0.025	0.025	0.025	0.025
5	0.020	0.050	0.095	0.095
7	0.015	0.205	0.280	0.280
9	0.010	0.070	0.115	0.115
11	0.020	0.020	0.020	0.020
C.D. (5%)		0.026	0.030	0.040

Isolate R_2

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.015	0.015	0.015	0.015
5	0.010	0.055	0.090	0.090
7	0.010	0.250	0.510	0.510
9	0.010	0.065	0.100	0.100
11	0.025	0.025	0.025	0.025
C.D. (5%)		0.025	0.070	0.070

Isolate R_3

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.015	0.015	0.015	0.015
5	0.010	0.095	0.155	0.155
7	0.010	0.220	0.370	0.370
9	0.015	0.110	0.195	0.195
11	0.020	0.020	0.020	0.020
C.D. (5%)		0.030	0.050	0.050

Isolate R_4

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.020	0.020	0.020	0.020
5	0.010	0.035	0.135	0.135
7	0.020	0.185	0.310	0.310
9	0.015	0.060	0.150	0.150
11	0.010	0.010	0.010	0.010
C.D. (5%)		0.031	0.190	0.190

Isolate R_5

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.010	0.010	0.010	0.010
5	0.020	0.040	0.090	0.090
7	0.010	0.190	0.280	0.280
9	0.025	0.065	0.165	0.165
11	0.025	0.025	0.025	0.025
C.D. (5%)		0.040	0.113	0.113

Isolate R_6

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.015	0.015	0.015	0.015
5	0.015	0.040	0.090	0.090
7	0.020	0.110	0.210	0.210
9	0.010	0.050	0.100	0.100
11	0.020	0.020	0.020	0.020
C.D. (5%)		0.030	0.090	0.090

Isolate R_7

pH	O.D. values at 24 h. intervals *			
	0	24	48	72
3	0.020	0.020	0.020	0.020
5	0.010	0.055	0.100	0.100
7	0.015	0.150	0.250	0.250
9	0.020	0.090	0.150	0.150
11	0.025	0.025	0.025	0.025
C.D. (5%)		0.038	0.051	0.051

* Mean of 2 replications.

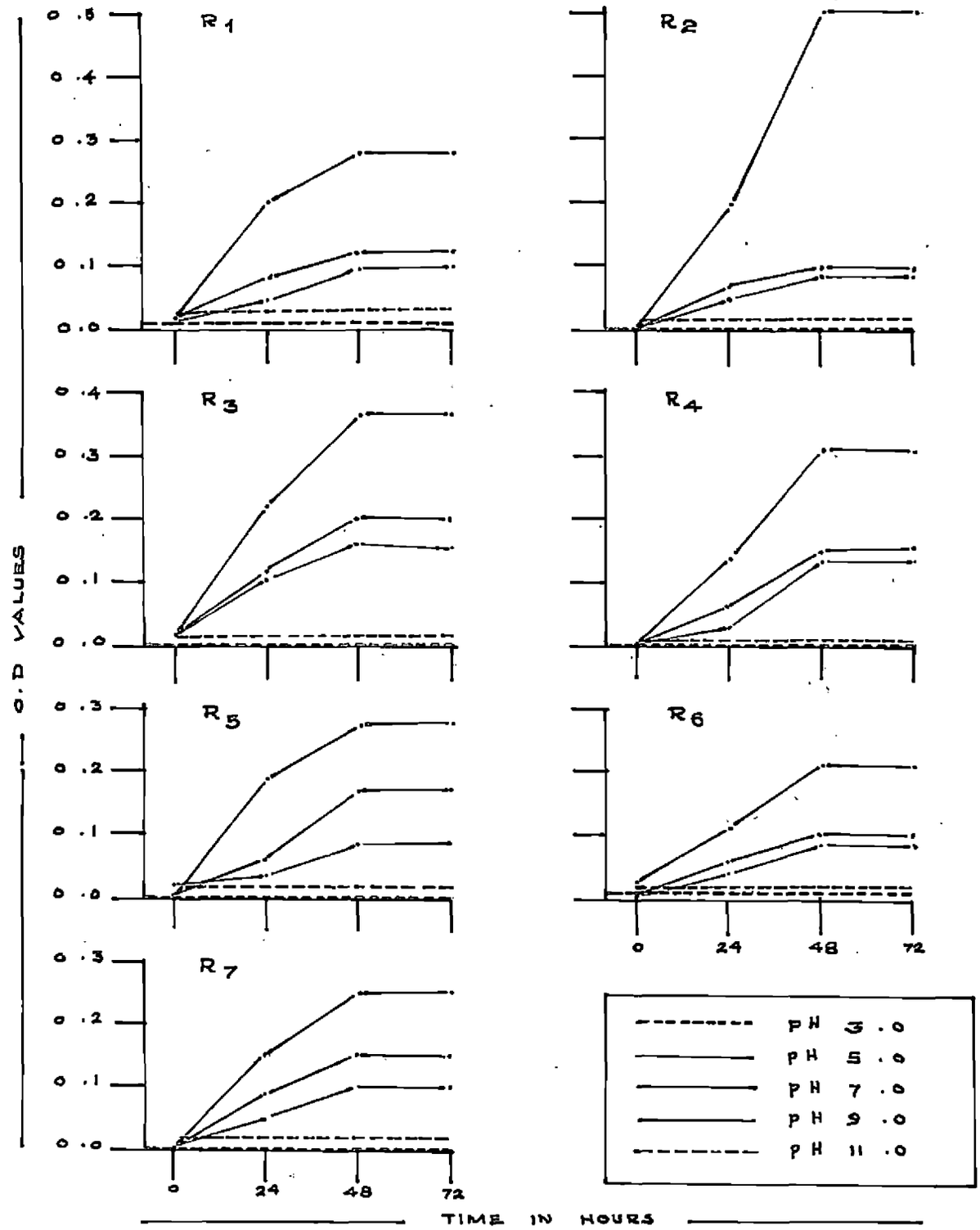


FIG: 3 EFFECT OF pH ON THE GROWTH OF DIFFERENT ISOLATES OF RHIZOBIA

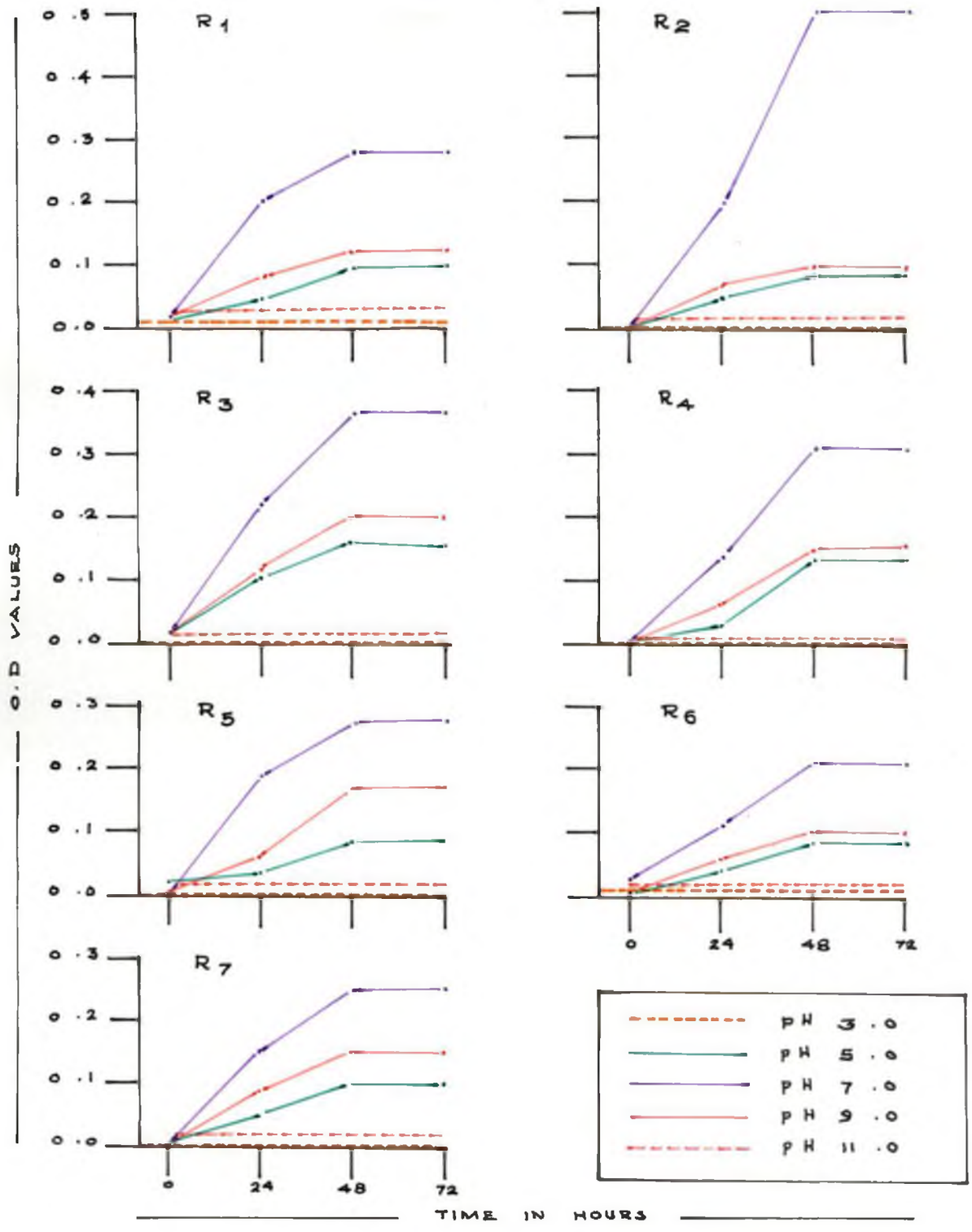


FIG: 3 EFFECT OF pH ON THE GROWTH OF DIFFERENT ISOLATES OF RHIZOBIA

broth. The extent of growth was measured in terms of O.D. The mean values for the same are presented in Table 12 and their analysis of variance in Appendix III.

The various isolates tested showed a similar pattern of growth at different levels of pH. Significant growth was obtained only at pH 7.0. Growth of all the isolated was minimum at pH 5.0 and 9.0 and absent at pH 3.0 and 11.0 (Table 12, Fig.3).

c. Antibiotic sensitivity

The seven isolates of rhizobia were tested for their sensitivity to the antibiotic, streptomycin at concentrations ranging from 50 to 500 microgram per ml. The growth in yeast extract mannitol broth containing the various concentrations of the antibiotic was measured in terms of O.D. Mean values of these observations are given in Table 13 and the analysis of variance in Appendix IV.

Growth of all the isolates of rhizobia except R_2 and R_3, was moderate at 125 microgram per ml and completely inhibited at 250 microgram per ml. Isolates R_2 and R_3 were able to grow to some extent at 250 microgram per ml. But there was no growth at 500 microgram per ml (Table 13, Fig.4).

d. Serological studies

Antiserum prepared for the three isolates of rhizobia, R_3, R_5 and R_6 was used to study the serological relationship of all the seven isolates of rhizobia, by the whole cell agglutination

Table 13. Sensitivity of different isolates of Rhizobium to Streptomycin sulphate.

Isolate R_1

Levels of antibiotic (µg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.015	0.215	0.215	0.215
125	0.020	0.095	0.190	0.190
250	0.020	0.020	0.020	0.020
500	0.015	0.015	0.015	0.015
C.D. (5%)		0.021	0.049	0.049

Isolate R_2

Levels of antibiotic (µg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.030	0.290	0.430	0.430
125	0.025	0.175	0.215	0.215
250	0.020	0.070	0.110	0.110
500	0.030	0.030	0.030	0.030
C.D. (5%)		0.024	0.081	0.081

Isolate R_3

Levels of antibiotic (µg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.030	0.200	0.340	0.340
125	0.010	0.140	0.210	0.210
250	0.040	0.065	0.090	0.090
500	0.045	0.045	0.045	0.045
C.D. (5%)		0.021	0.049	0.049

Isolate R_4

Levels of antibiotic (µg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.030	0.165	0.265	0.265
125	0.015	0.065	0.120	0.120
250	0.045	0.045	0.045	0.045
500	0.020	0.020	0.020	0.020
C.D. (5%)		0.037	0.063	0.063

Isolate R_5

Levels of antibiotic (μg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.010	0.190	0.285	0.285
125	0.020	0.145	0.175	0.175
250	0.025	0.025	0.025	0.025
500	0.020	0.020	0.020	0.020
C.D. (5%)		0.030	0.110	0.110

Isolate R_6

Levels of antibiotic (μg/ml)	O.D. values at 24 h. intervals*			
	0	24	48	72
50	0.015	0.155	0.225	0.225
125	0.015	0.100	0.165	0.165
250	0.020	0.020	0.020	0.020
500	0.010	0.010	0.010	0.010
C.D. (5%)		0.040	0.034	0.034

Isolate R_7

Levels of antibiotic (μg/ml)	C.D. values at 24 h. intervals*			
	0	24	48	72
50	0.030	0.145	0.260	0.260
125	0.030	0.090	0.165	0.165
250	0.020	0.020	0.020	0.020
500	0.025	0.025	0.025	0.025
C.D. (5%)		0.040	0.041	0.041

* Mean of 2 replications.

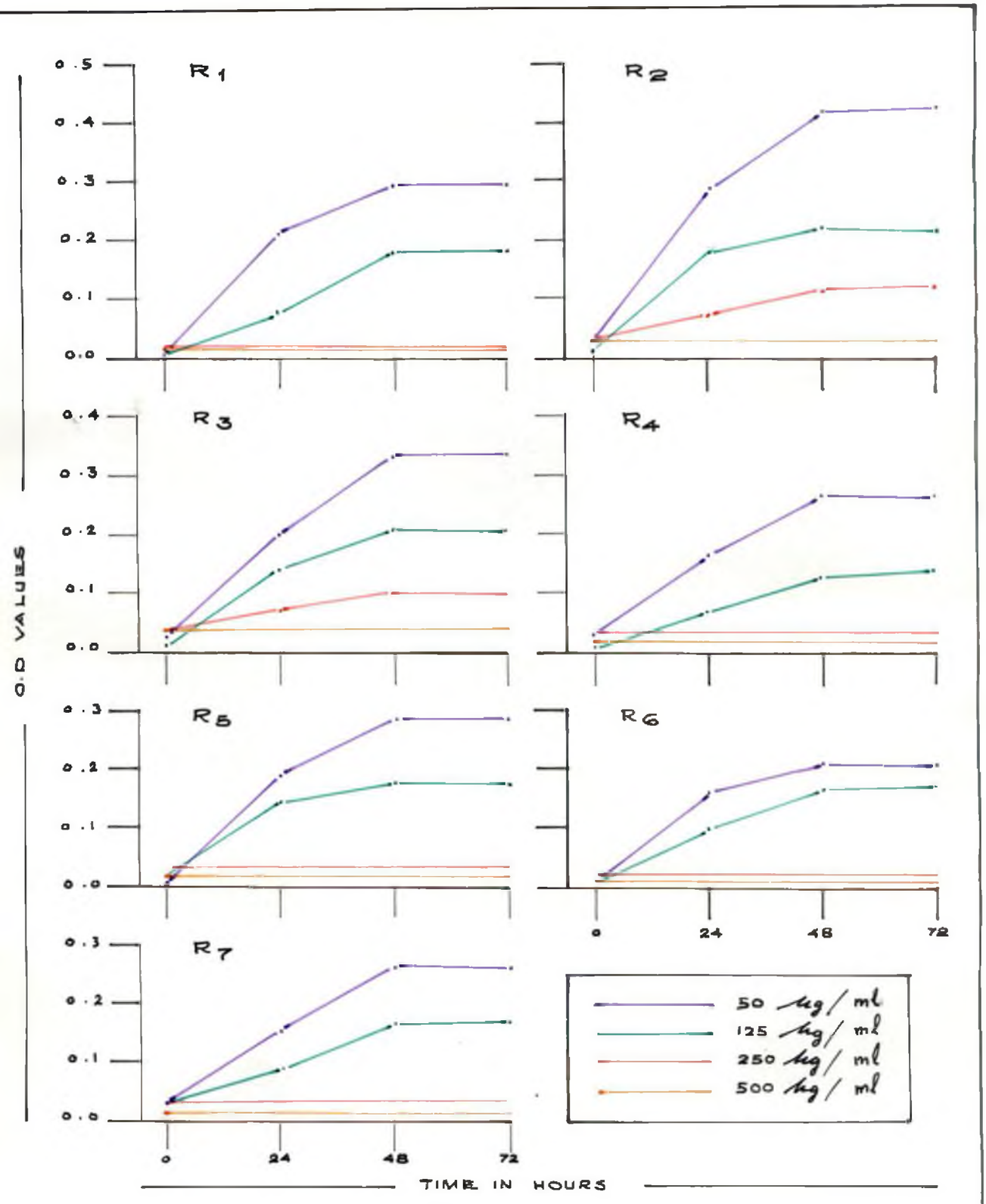


FIG: 4 TOLERANCE OF *Rhizobium* ISOLATES TO DIFFERENT LEVELS OF THE ANTIBIOTIC STREPTOMYCIN SULPHATE

test. The antiserum prepared for isolates R_3, R_5 and R_6 was initially tested for their titre values using homologous antigens. Agglutination of the homologous antigen was obtained with the 800 dilution of the respective antiserum (Table 14).

In the serological characterisation of different isolates, agglutination was obtained only with the homologous antigen except in the case of isolate R_1 which agglutinated with the antiserum for isolate R_5 (Table 15, Plates 6, 7 and 8).

Table 14. Titre value of antiserum by tube agglutination test

Antiserum for isolate of *Rhizobium*	Dilution of antiserum							
	50	100	200	400	800	1,600	3,200	6,400
R_3	+	+	+	+	+	-	-	-
R_5	+	+	+	+	+	-	-	-
R_6	+	+	+	+	+	-	-	-

\+ Positive reaction

\- Negative reaction

Table 15. Serological characterisation of different isolates of rhizobia

Rhizobium isolate	Antiserum for isolate		
	R_3	R_5	R_6
R_1	-	+	-
R_2	-	-	-
R_3	+	-	-
R_4	-	-	-
R_5	-	+	-
R_6	-	-	+
R_7	-	-	-

\+ Positive reaction

\- Negative reaction

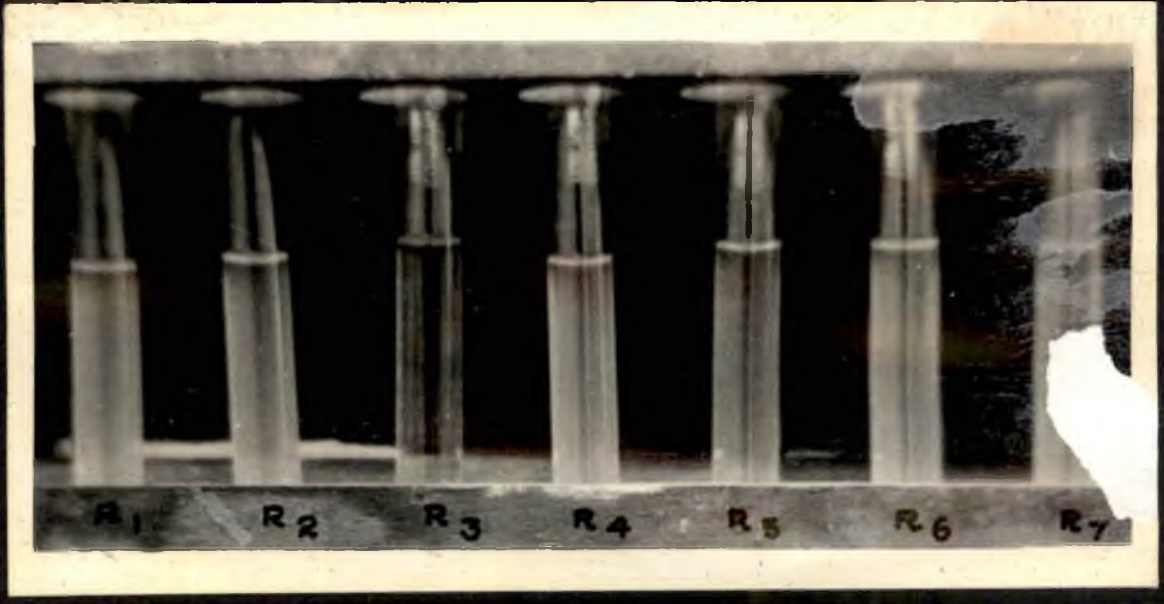


Plate 6. Tube agglutination test for serological characterisation of Rhizobium isolates using antiserum prepared against isolate R_3.

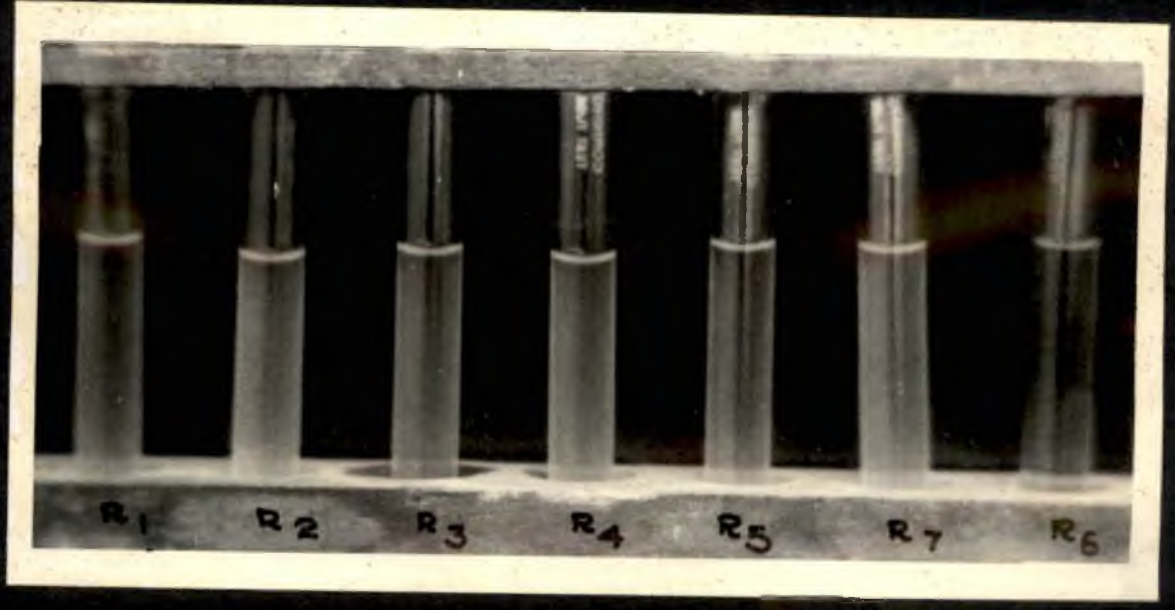


Plate 7. Tube agglutination test for serological characterisation of Rhizobium isolates using antiserum prepared against isolate R_6.

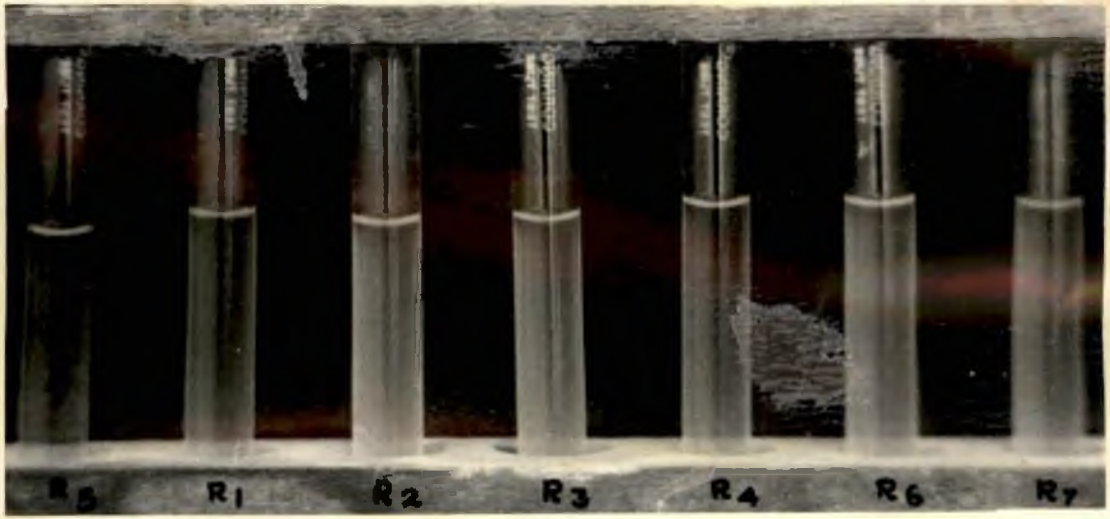


Plate 8. Tube agglutination test for serological characterisation of Rhizobium isolates using antiserum prepared against isolate R_5.

Discussion

DISCUSSION

The current interest in the use of plant proteins as a substitute for animal proteins in developing countries had led to the development of many new varieties in pulses with high yielding potential. However, one of the problems with these varieties is the lack of proper nodulation and the consequent necessity to use fertilizer nitrogen for their cultivation. This anomaly occurs either due to certain inherent physiological factors of the host variety acting against nodule formation or due to the lack of appropriate strains of rhizobia for nodulation in them. This has led to the intensive screening of different varieties of pulses for proper nodulation so that one can capitalise on the natural phenomenon of symbiotic nitrogen fixation for getting nitrogen economy and optimum yield. It is with this objective in mind that the present investigation was carried out. Can there be a host-varietal specificity for Rhizobium affecting nodulation in groundnut? An attempt is made to answer this question by studying the nature of nodulation and the resulting benefits in seven selected varieties of groundnut by rhizobia isolated from them in all possible combinations.

A pot culture experiment was done initially to study the pattern of nodulation in fifteen varieties of groundnut. The different varieties tested showed a clear difference in the number of nodules formed in them by native rhizobia already present in the soil. Based on the significant difference in

the number of nodules formed in these varieties they were arranged into five distinct nodulation groups. In each of this group, the minimum number of nodules formed in a particular variety was always higher than the maximum number of nodules formed in a variety in the immediate succeeding group. Having thus observed a certain degree of variation in the number of nodules formed, further study was conducted to see whether it was the same strain of Rhizobium that had formed nodules in all these varieties. This was tested by selecting seven varieties of groundnut representing each of the above mentioned nodulation group except group IV which could not be included due to reasons mentioned earlier.

Rhizobium was isolated from the following seven varieties of groundnut, TMV-12, USA-123, Exotic-6, Spanish peanut, TMV-11, Ak-12-24, and Ah-32. All these isolates were gram-negative, non-spore forming rods which produced the typical white, gummy and transluscent colonies on yeast extract mannitol agar medium after 72 h. of incubation at 28 ± 1°C. None of the isolates grew well on glucose peptone agar or produced acid on ketolactose medium confirming the basic characteristics of Rhizobium as described by Vincent (1970).

The different isolates of rhizobia were then used to study their host-varietal specificity, by conducting a pot-culture experiment under aseptic conditions in a glass house. The 8 x 7 factorial experiment had 8 levels of culture treatments and 7 levels of host varieties. In this experiment each of

the seven isolate of Rhizobium was used for inoculating its own homologous host variety as well as the remaining six heterologous host varieties.

The plants were grown for 60 days and observations on the number of nodules formed, fresh and dry weight of nodules and plants, plant height and percentage nitrogen content were taken. Data for these observations is interpreted in two possible ways as the response of individual isolate of Rhizobium to different varieties of groundnut and as the response of each variety to different isolates of rhizobia. Out of these, the former is mainly relied on to study the presence of host-varietal specificity of Rhizobium, if any, in groundnut.

In general, a favourable response for all the plant characters studied was obtained when an isolate of Rhizobium was used for inoculating its own homologous host variety. Muthusamy (1970), Caldwell and Vest (1979) and Wynne (1980) have also reported similar response for rhizobial strains in groundnut. The effect of inoculation with each of the seven isolates of Rhizobium on nodulation and other characters in both the homologous and heterologous host varieties is shown in Fig.5 to 11. Certain exceptions to the above general observation was also noticed. For example, isolate R_1 produced significant increase in nodule dry weight in varieties USA-123, Exotic-6 and TMV-11 when compared to its homologous host variety TMV-12. A significant increase in nitrogen content was also

Grading of plant characters for graphical representation.

Character	Grade		
	Low	Medium	High
Nodule number	30-59	60-89	90 and above.
Nodule fresh weight(mg)	20-59	60-109	110-150
Nodule dry weight (mg)	0-20	21-40	41-60
Plant fresh weight (g)	2.0-5.5	5.6-8.5	8.6-11.0
Plant dry weight (g)	0.45-1.05	1.06-1.70	1.71-2.30
Plant height (cm)	20-32	33-47	48-60
Percentage nitrogen content	2.0-2.74	2.75-3.49	3.50-4.25
Scale taken	0.5 cm	1 cm	2 cm
Value	1	2	3

Note: The figure within the circles in graphs represents the sum total of the values of grades for each character.

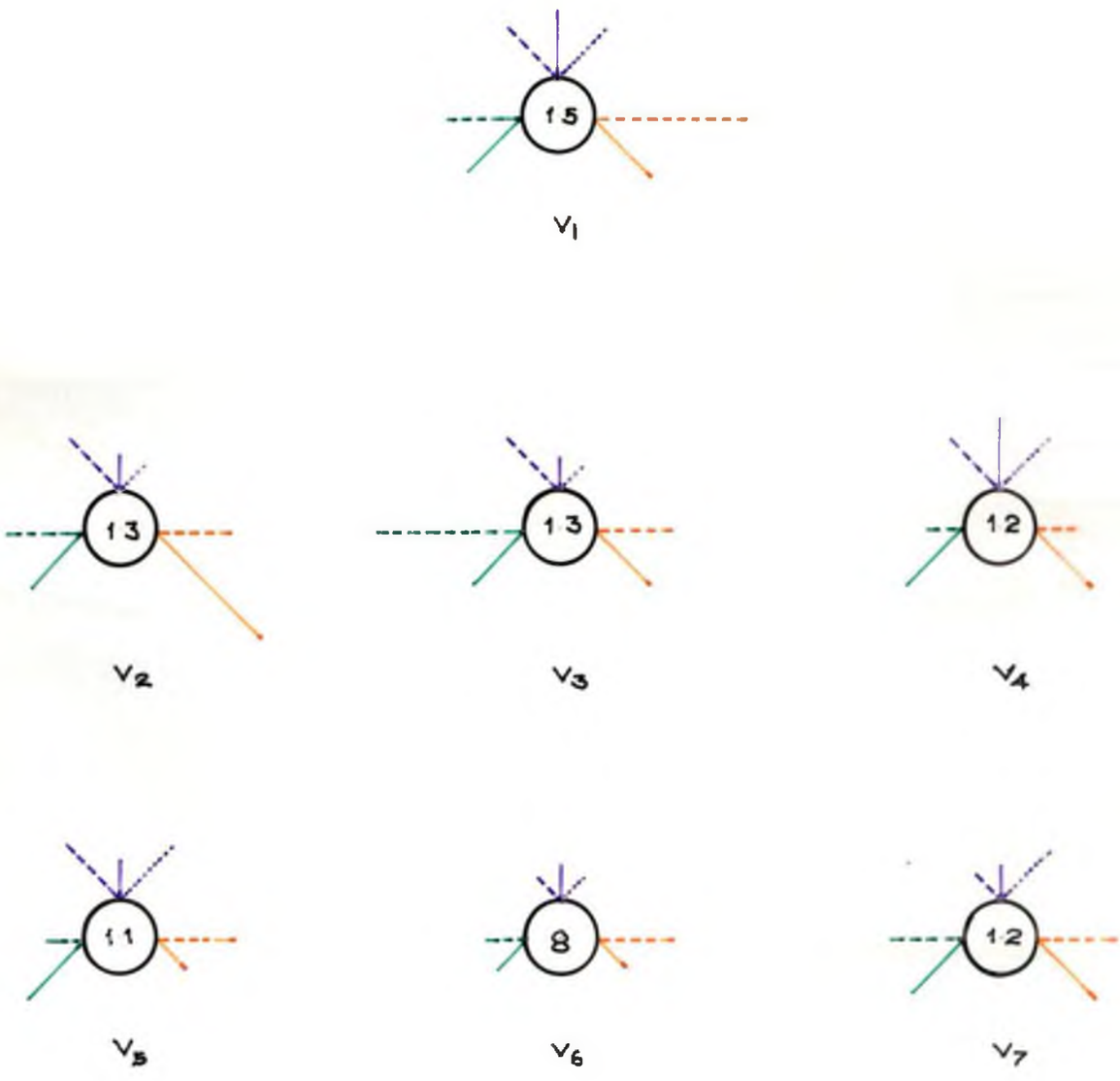


FIG: 5 EFFECT OF INOCULATION WITH ISOLATE R_1 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

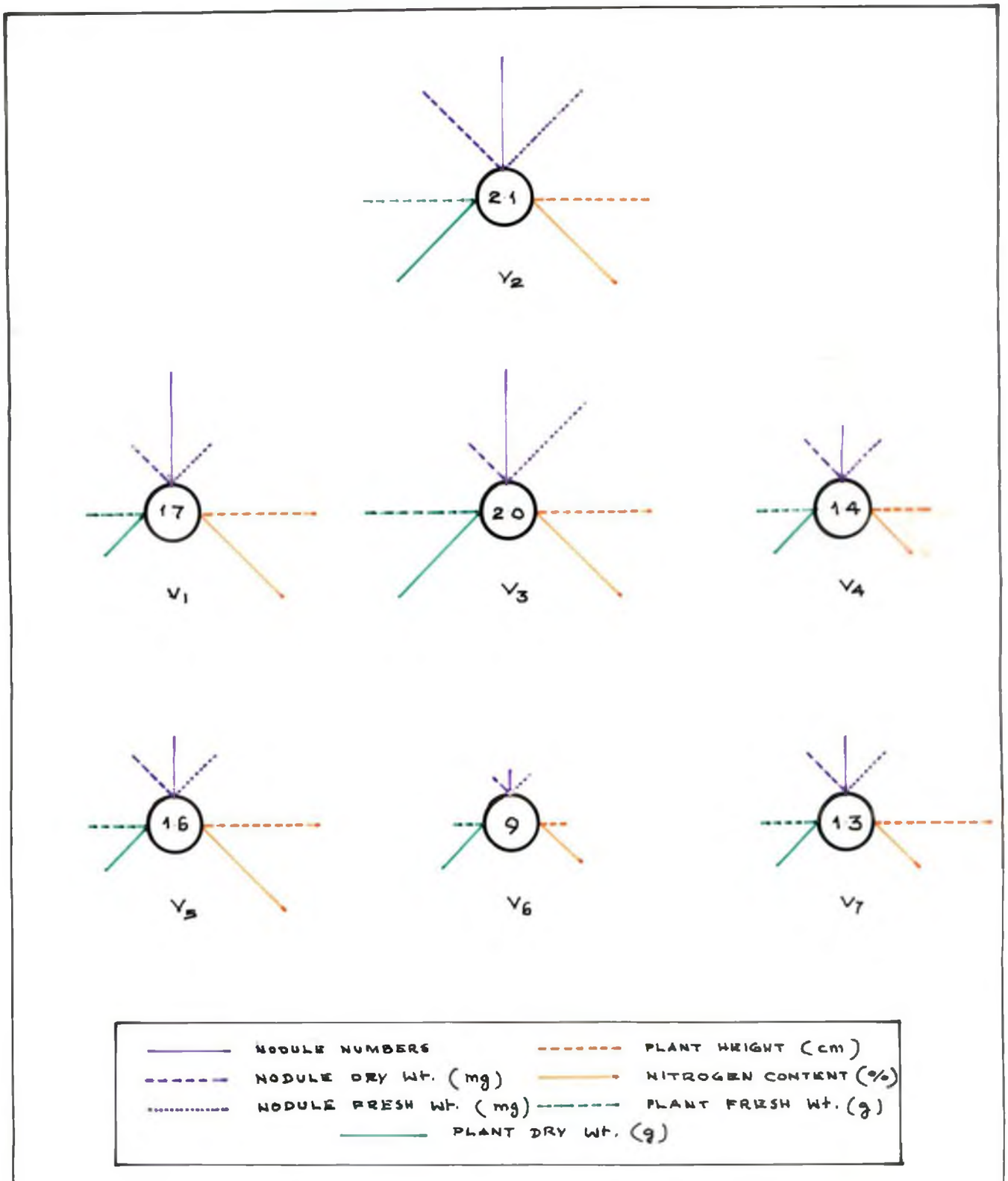


FIG: 6 EFFECT OF INOCULATION WITH ISOLATE R_2 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

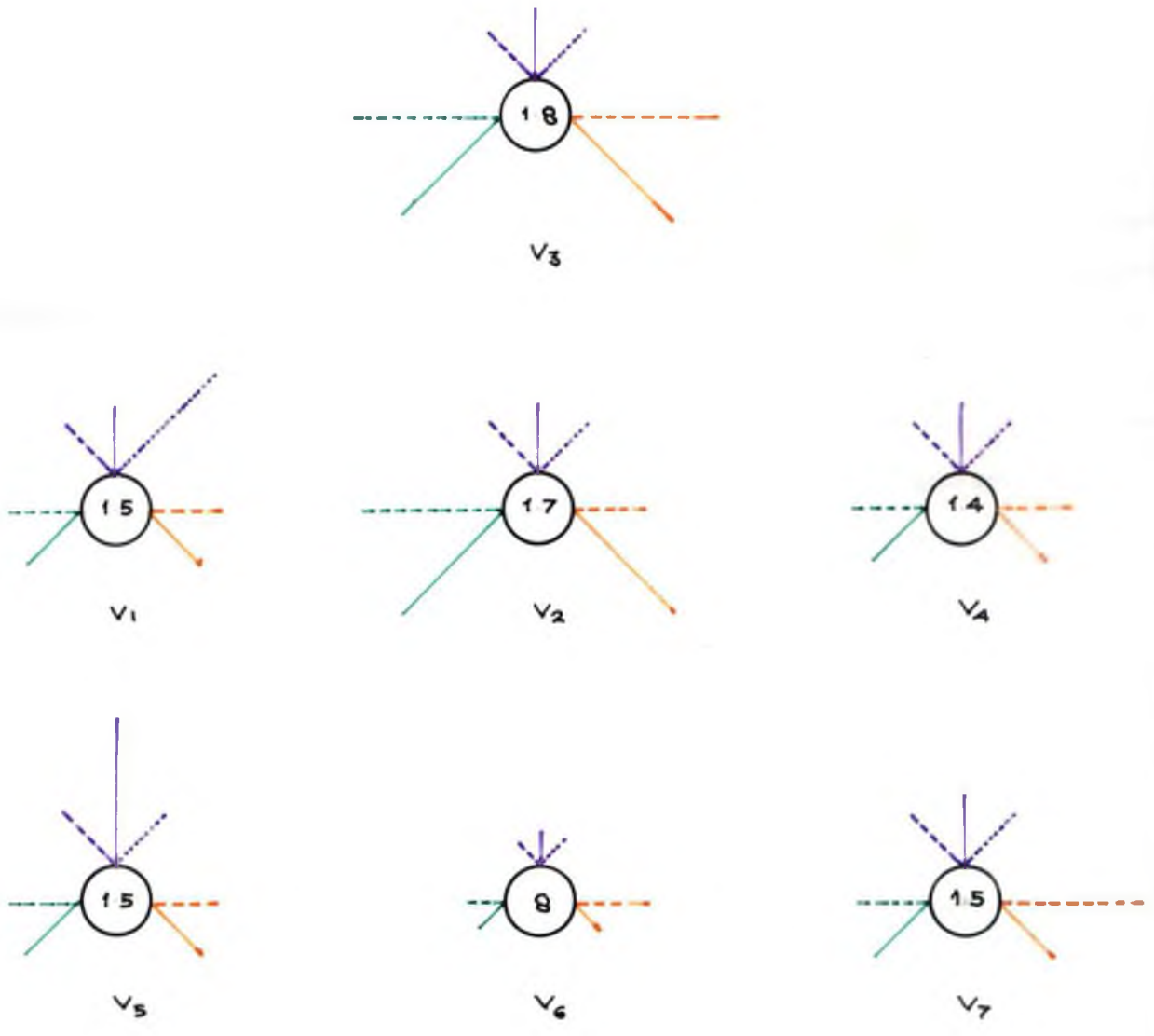


FIG: 7 EFFECT OF INOCULATION WITH ISOLATE R_3 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

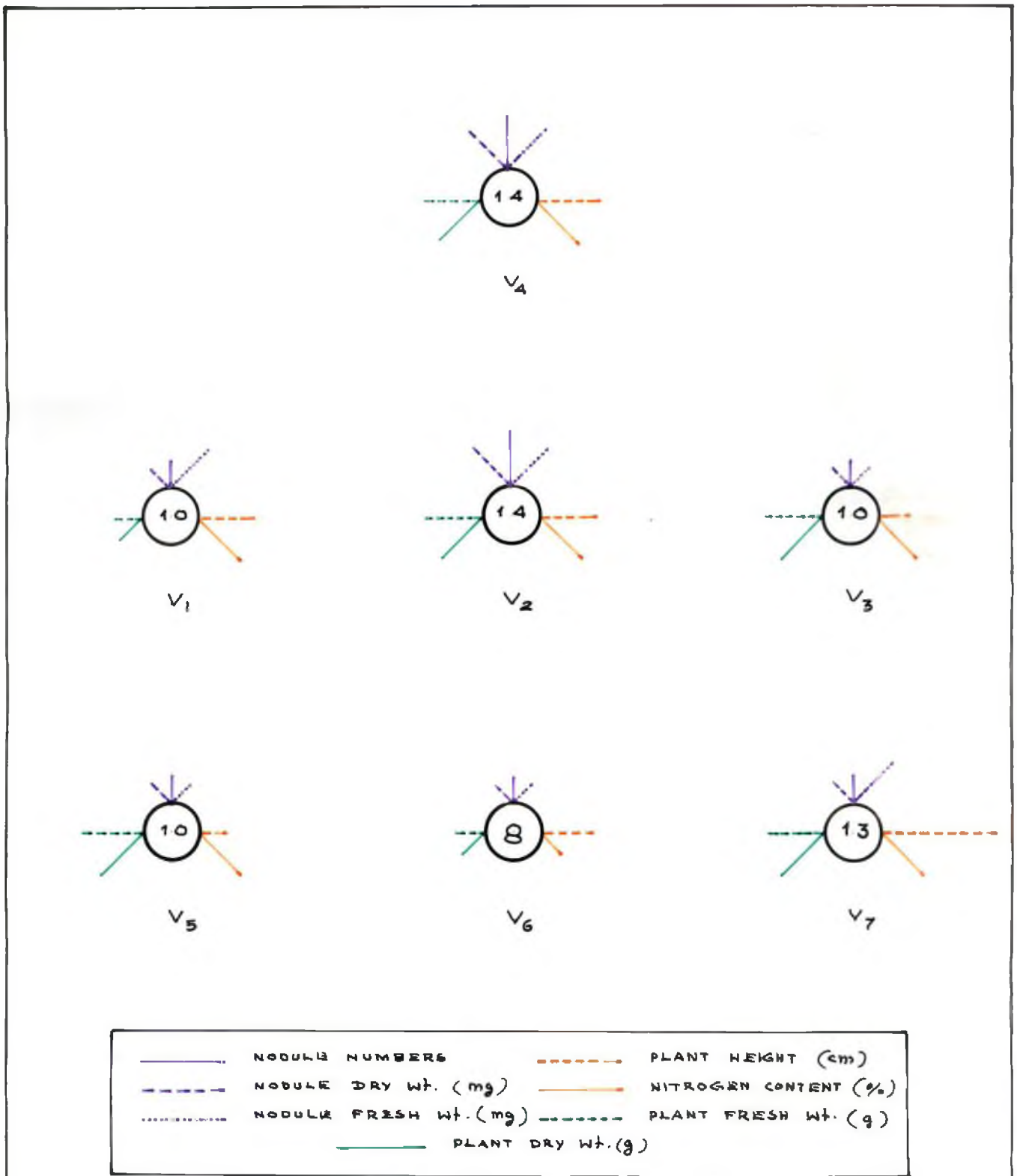


FIG: 8 EFFECT OF INOCULATION WITH ISOLATE R_A ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

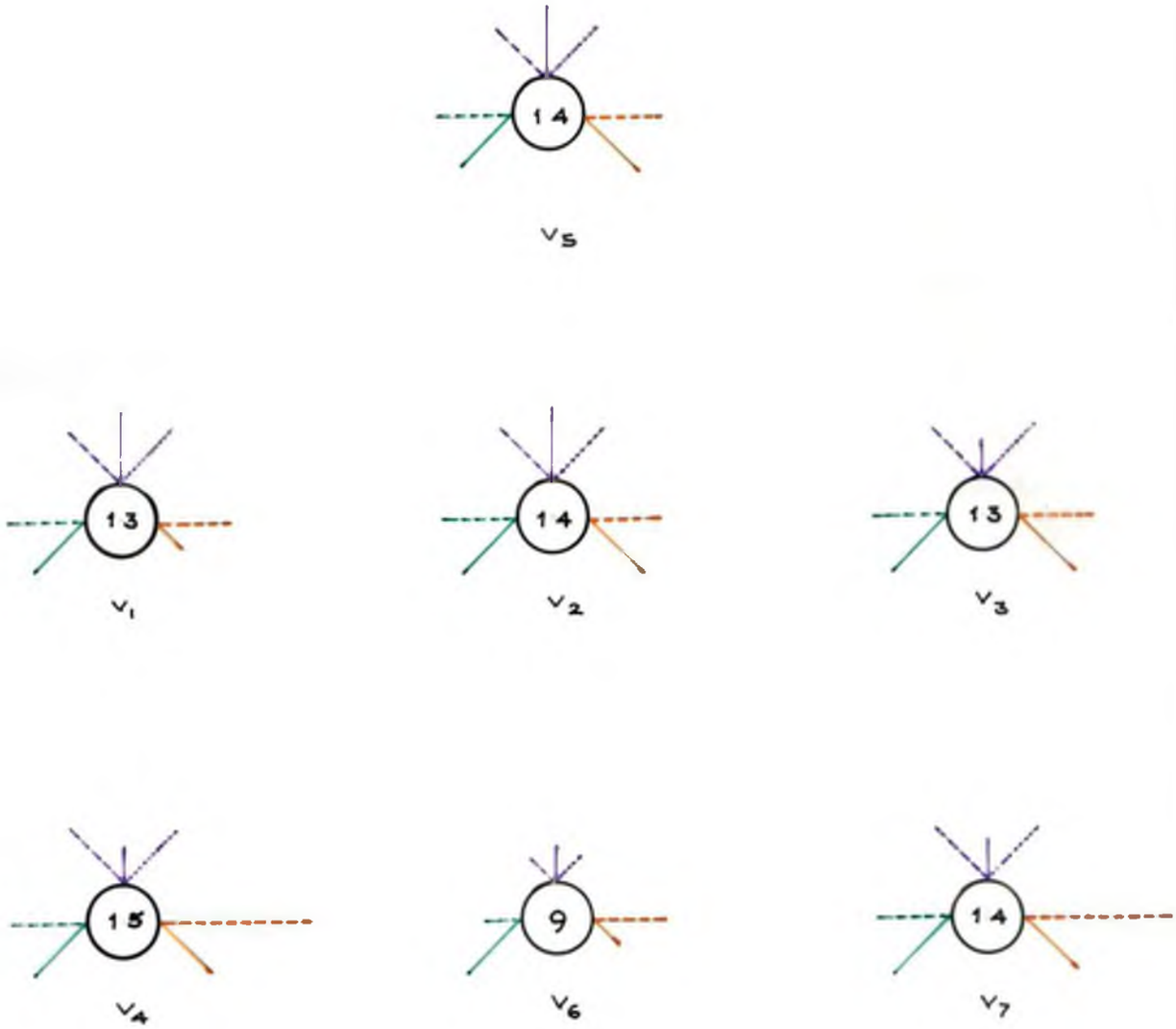


FIG: 9 EFFECT OF INOCULATION WITH ISOLATE R_5 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

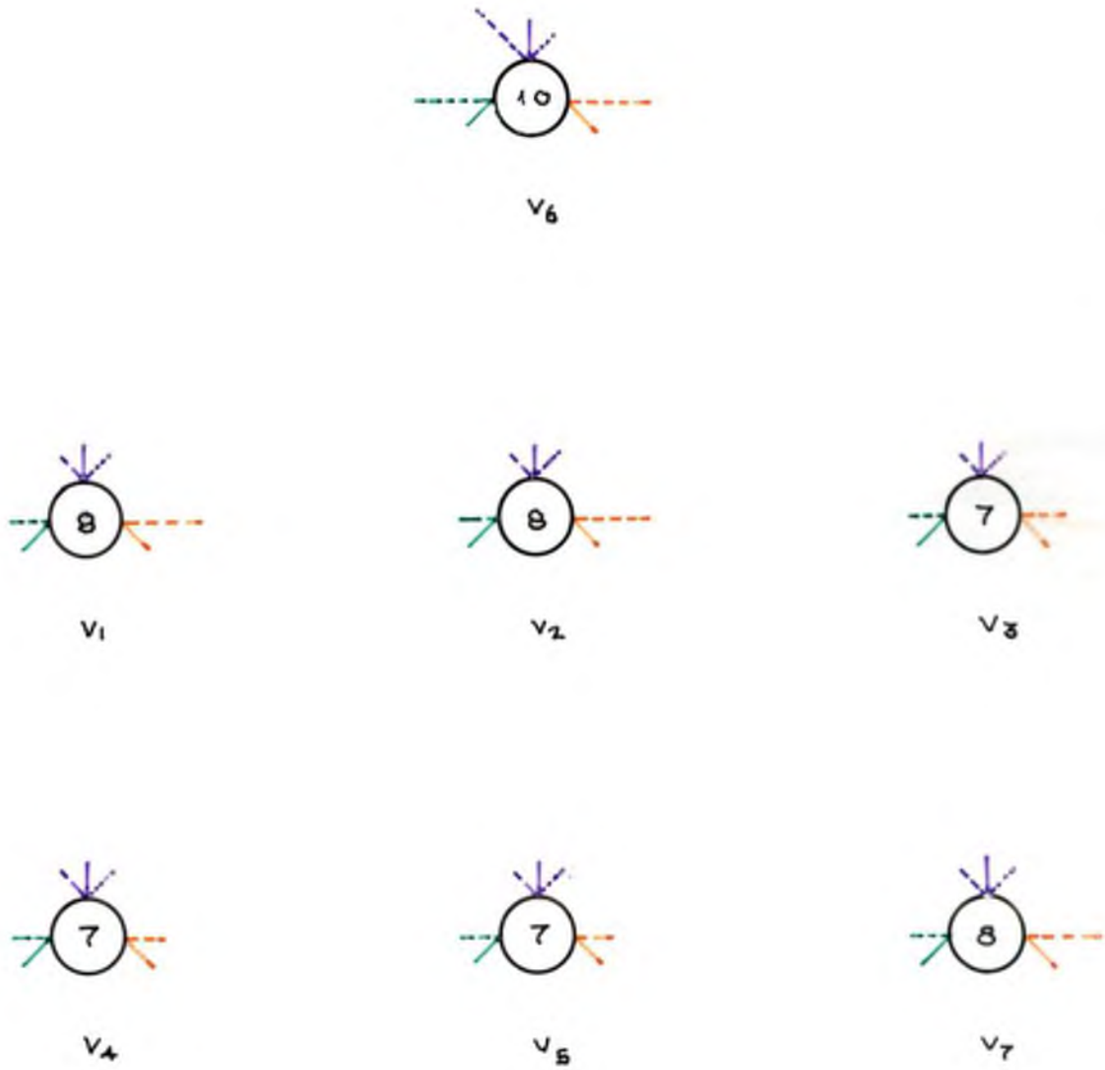


FIG: 10 EFFECT OF INOCULATION WITH ISOLATE R_6 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

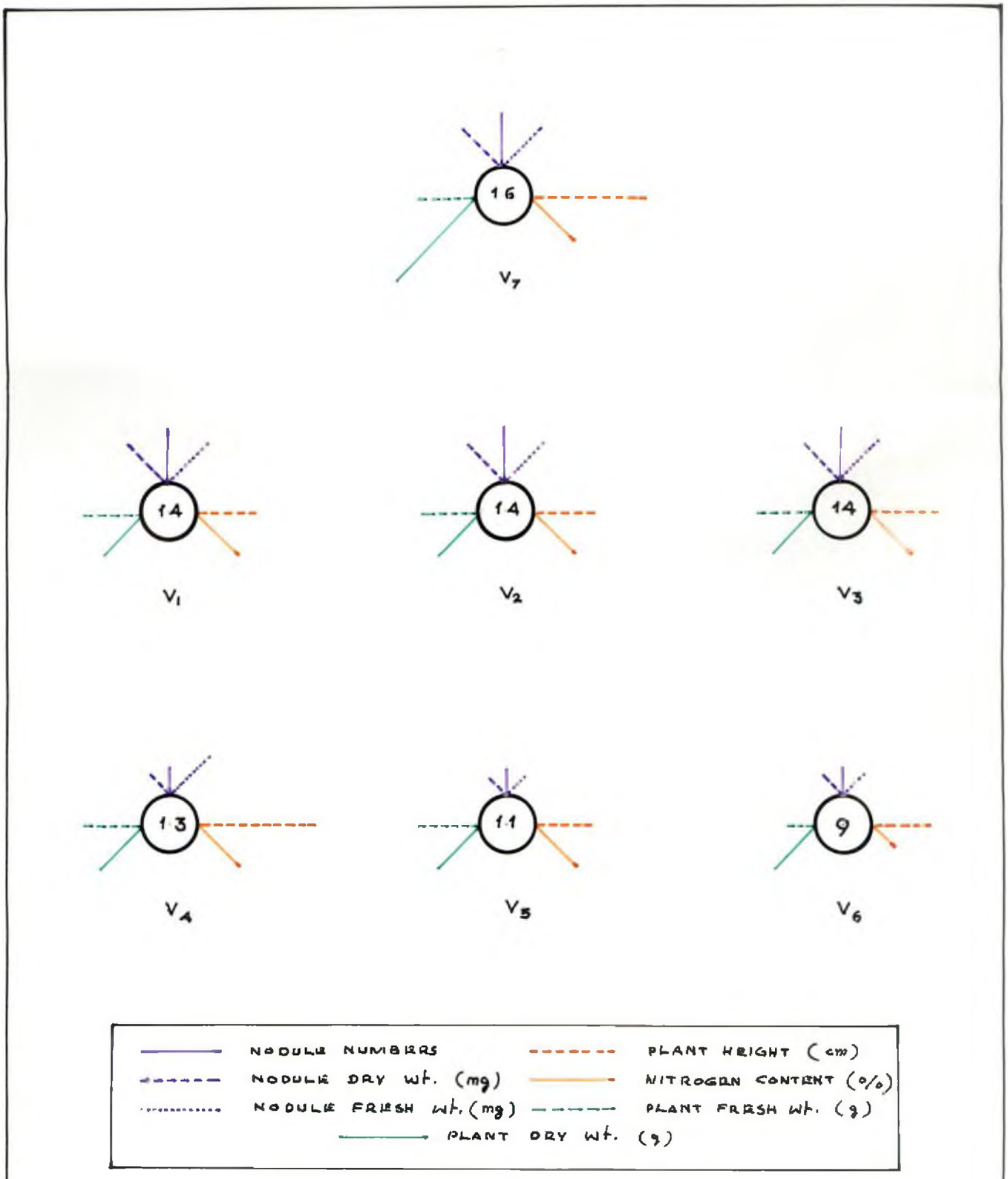


FIG: 11 EFFECT OF INOCULATION WITH ISOLATE R_7 ON VARIOUS PLANT CHARACTERS IN DIFFERENT VARIETIES OF GROUNDNUT

produced by this isolate in variety USA-123. Similarly, isolates R_2, R_3 and R_4 produced significant increase in plant height in varieties TMV-12 and Ah-32. Such variations in the response of rhizobial strains in groundnut is observed earlier also by Singh et al. (1976) when they found that the Rhizobium isolate from groundnut variety TMV-8 was ineffective in this variety when compared to two other isolates of rhizobia obtained from a wild variety, Arachis duranensis.

Although during the present investigation, observations on seven different plant characters were taken to study the host-varietal specificity of Rhizobium, it may be pointed out that the efficiency of a legume-Rhizobium symbiotic system is primarily reflected in the number of nodules formed and the resulting nitrogen content of the plants. The former character indicates the competitive and invasive ability of a rhizobial strain and the latter its nitrogen fixing ability. A brief review of early work done in this field supports this concept. Albrecht (1943) assessed the effectiveness of six single strains of cultures of rhizobia with spanish peanuts in terms of the number of nodules formed and nitrogen content of plants. Erdman (1947) found that the total nitrogen content and nodule number are highly correlated and used them to study the strain variation and host specificity of Rhizobium trifolii in four species of Trifolium. Vincent (1970) reported that the most direct basis for evaluation of symbiotic efficiency is to measure the nitrogen content of the plants. Similar assessment of symbiotic system is also made by Schneewis et al (1978), Alagawadi (1979), Sheth

(1979) and Wynne et al. (1980) in groundnut; Varela and Munéwar (1978) and Pugashetti and Wagner in soybean; Dadarwal et al.(1975) and Raju and Samuel (1976) in chikpea, and Singh et al. (1979) in pigeon pea. Hence, in the present study also an attempt is made to interpret the host-varietal specificity in terms of the number of nodules formed and nitrogen content of the plants.

In the study on the response of individual isolates of Rhizobium to different varieties of groundnut, it was found that all the isolates except R_5 produced maximum number of nodules in their respective host varieties. However, a corresponding high nitrogen content of plants was obtained only with three of the isolates, R_2, R_4 and R_6. This indicates a beneficial association as well as an apparent host-varietal specificity of these isolates. This observation is further supported by the fact that the association of these rhizobia with certain other host varieties resulted in significant reduction in nodule number and nitrogen content when compared to their respective homologous host-varieties. The isolate R_2 produced significantly less number of nodules in all host varieties other than USA-123. A corresponding significant decrease in nitrogen content of plants was also observed except in the case of variety Exotic-6. Isolate R_4 produced significantly less number of nodules and nitrogen content in all varieties except, USA-123. Similarly, isolate R_6 produced significantly less number of nodules and nitrogen in varieties such as TMV-11 and Ah-32.

However, it would be seen that the varietal specificity shown by these isolates was not an absolute phenomenon. Evidence for this comes from the fact that these isolates could produce equally good results in some other varieties of groundnut. Thus, the nitrogen content of variety Exotic-6 due to isolate R_2 was statistically par with the nitrogen content of variety USA-123. The number of nodules formed and nitrogen content of variety USA-123 , by isolate R_4 was also statistically on par with that of Spanish peanut, the homologous host-variety of this isolate. Similarly, the number of nodules produced by isolate R_5 in USA-12 and Spanish peanut, and nitrogen content of plants in varieties TMV-12 and Exotic-6 was statistically on par with that of AK-12-24. These observations therefore indicate that the host-varietal specificity of rhizobia as seen here is not absolute. Further, it appears that the benefits resulting from this type of varying association is more of a host determined phenomenon. Such a role for the host genotype has been put forward by other workers also (Caldwell and Vest, 1979, and Wynne et al., 1980). The behaviour of other isolates of rhizobia also supports this hypothesis. The nitrogen content of plant varieties USA-123, Exotic-6, Spanish peanut and TMV-11 was either significantly higher or statistically on par with TMV-12 despite the fact that isolate R_1 did not produce any significant increase in nodule number in these varieties. Similarly, the number of nodules produced by isolate R_3 in TMV-12 and TMV-11 although was statistically on par with its host variety Exotic-6, the nitrogen

content of these plants was significantly less. Such variations can also be seen in the case of isolates R_5 and R_7. Thus a given strain of Rhizobium eventhough competitive and efficient in nodule formation and nitrogen fixation in one variety may not produce the same result in another host variety which may have certain inherent physiological limitations to favour this symbiotic phenomenon. It is worthwhile to recall here the existence of non-nodulation varieties in soybeans. This will, in fact, be a serious problem which breeders of new varieties of pulses will have to take into consideration, for, the lack of effective nodulation in pulses will lead to the higher use of fertilizer nitrogen for their cultivation.

Another aspect of the present investigation was to see whether one can select a rhizobial strain which has got an efficient compatibility with more than one groundnut variety and whether one can select a variety which can show a favourable response to inoculation with more than one rhizobial strain. From the mean values presented in tables 4 and 10 for nodule number and nitrogen content, one can see that isolates R_2 and R_3 are more compatible with different host varieties, when compared to all other isolates of rhizobia in terms of the symbiotic efficiency measured by the average number of nodules formed and the nitrogen content of plant varieties. Similarly, groundnut varieties USA-123 and Exotic-6 showed the most favourable response to inoculation with different isolates of rhizobia. Similar response of certain varieties of groundnut to Rhizobium inoculation is

reported by Wynne et al. (1978). Further, Wynne et al. (1980) have suggested that by selection of specific host-strain combinations or by selecting strains and/or varieties with adaptation to a broader range of host varieties/Rhizobium isolates, increased symbiotic benefits can be obtained in groundnut crop.

The last part of this study was mainly concerned with the possible strain variations, if any, between the seven isolates of rhizobia. It was observed that all the isolates had an indential fast rate of growth and tolerance to different levels of pH between 5.0 and 9.0 Studies conducted by Rajagopalan (1933), Habish and Khairi (1965) and Yadav and Vyas (1970) support this observation. In the case of antibiotic sensitivity, some difference between the seven isolates of rhizobia were noticed. The isolates R_2 and R_3 were more resistant to streptomycin sulphate upto 250 μg/ml while the remaining isolates were all sensitive to this concentration of the antibiotic. Use of resistance to streptomycin sulphate has been recommended for strain identification by Brockwell et al. (1979). But the more interesting observation here was that isolates R_2 and R_3 were the most efficient and compatible strains when compared to all other isolates of rhizobia indicating a certain degree of correlation between resistance to this antibiotic and symbiotic efficiency. The more competitive nature of such rhizobial strains is reported earlier by Om Prakash (1980).

In the serological characterisation, complete identification of various isolates of rhizobia was not done since serum for only three isolates R_3, R_5 and R_6 could be prepared. Tube agglutination studies showed that these isolates were distinct, from one another and also from the remaining isolates of rhizobia except R_1 which showed a positive agglutination with the antiserum for isolate R_5. Regarding the agglutination of isolate R_1 with the antiserum for R_5, one may conclude that these isolates are one and the same strains. But the apparent difference in plant characters due to inoculation with these isolates in varieties TMV-12 and TMV-11 indicate that they are related but not identical strains. A more conclusive result would have been obtained by a precipitin reaction which could not be done during the present investigation due to the relatively low titre value of various antisera. However, it is interesting to note that host varieties TMV-11 and TMV-12 are probably the most related of all the different varieties of groundnut used for the present investigation. Can there be then such subtle variations in rhizobial strains also to indicate such close relation not amounting to the complete identify of both the isolates as one and the same strain? However, much more work in this direction would be necessary to arrive at defenitive conclusions.

Summary

SUMMARY

An investigation was carried out at the College of Agriculture, Vellayani, Kerala to study the possibility of host-varietal specificity of Rhizobium for nodulation in groundnut.

A pot culture experiment was done initially to study the pattern of nodulation in fifteen varieties of groundnut. Based on the significant difference in the number of nodules formed in these varieties they were arranged into five distinct nodulation groups. In each of this group, the minimum number of nodule formed in a particular variety was always higher than the maximum number of nodules formed in a variety in the immediate succeeding group. Seven varieties of groundnut TMV-12, USA-123, Exotic-6, Spanish peanut, TMV-11, Ak-12-24 and Ah-32 were selected from among these groups for further studies.

Rhizobium was isolated from the above seven varieties and pure cultures were maintained. All these isolates were gram-negative, non-spore forming rods which produced the typical, white, gummy and transluscent colonies on yeast extract mannitol agar medium after 72 h. of incubation at 28 ± 1°C. None of the isolates grew well on glucose peptone agar or produced acid on ketolactose medium, confirming the basic characteristics of Rhizobium as described by Vincent (1970).

A pot culture experiment was then conducted under aseptic conditions in a glass house to study the host-varietal specificity of different isolates of rhizobia. The 8 x 7 factorial experiment had 8 levels of treatments and 7 levels of host varieties. One representative rhizobial isolate taken from each variety was thus used for inoculating its homologous host variety as well as the remaining six heterologous host varieties.

The plants were grown for 60 days when the observations such as nodule number, fresh and dry weight of nodules and plants, plant height and percentage nitrogen content was taken. Data for these observations is interpreted in two possible ways as the response of individual isolate of Rhizobium to different varieties of groundnut and as the response of each variety to different isolates of rhizobia. Out of these, the former was mainly relied on to study the presence of host varietal specificity of Rhizobium in groundnut.

In general, a favourable response for all the plant characters studied was obtained when an isolate of Rhizobium was used for inoculating its own homologous host variety. However, certain exceptions were also observed. For example, isolate R_1 produced significant increase in nodule dry weight in varieties such as USA-123, Exotic-6 and TMV-11 when compared to its homologous host variety TMV-12. A significant increase in nitrogen content was also produced by this isolate in USA-123. Similarly, isolates R_2, R_3 and R_4 produced significant increase in plant height in varieties TMV-12 and Ah-32.

Although during the present investigation, observations on seven different plant characters were taken to study the host-varietal specificity of Rhizobium, it may be pointed out that the efficiency of a legume-Rhizobium symbiotic system is primarily reflected in the number of nodules formed and the resulting nitrogen content of the plants. Hence, for the present study also attempt is made to interpret the host-varietal specificity in terms of these two characters.

In the study on the response of individual isolate of Rhizobium to different varieties of groundnut, it was found that all the isolates except R_5 produced maximum number of nodules in their respective host varieties. However, a corresponding high nitrogen content of plants was obtained only with three of the isolates, R_2, R_4 and R_6. This indicates a beneficial association as well as an apparent host-varietal specificity of these isolates. This observation is further supported by the fact that the association of these rhizobia with certain other host varieties resulted in significant reduction in nodule number and nitrogen content when compared to their respective homologous host varieties. Thus, isolate R_2 produced significantly less number of nodules in all host varieties other than USA-123. A corresponding decrease in nitrogen content of plants was also observed except in the case of variety Exotic-6. Isolate R_4 produced significantly less number of nodules and nitrogen content in all varieties

except, USA-123. Similarly isolate R_6 produced significantly less number of nodules and nitrogen content in varieties TMV-11 and Ah-32.

However, certain isolates were also found to produce equally good results in some other varieties of groundnut. Thus, the nitrogen content of variety Exotic-6 due to isolate R_2 was statistically on par with the nitrogen content of variety USA-123. The number of nodules formed and nitrogen content of variety USA-123 was also statistically on par with that of Spanish peanut, the homologous host-variety of this isolate. Similarly, the number of nodules produced by isolate R_6 in USA-123 and Spanish peanut, and nitrogen content of plants in varieties TMV-12 and Exotic-6 was statistically on par with that of Ah-12-24. These observations therefore indicate that the host-varietal specificity of rhizobia as seen here is not absolute. Further, it appears that the benefits resulting from this type of varying association is more a host determined phenomenon. The behaviour of other isolates of rhizobia also supports this hypothesis. The nitrogen content of plant varieties USA-123, Exotic-6, Spanish peanut and TMV-11 was either significantly higher or statistically on par with TMV-12 despite the fact that isolate R_1 did not produce any significant increase in nodules number in these varieties. Similarly, the number of of nodule produced by isolate R_3 in TMV-12 and TMV-11 although was statistically on par with its host variety Exotic-6, the

nitrogen content of these plants was significantly less. Such variations can also be seen in the case of isolates R_5 and R_7. Thus a given strain of Rhizobium eventhough competitive and efficient in nodule formation and nitrogen fixation in one variety may not produce the same result in another host variety which may have certain inherent physiological limitations to favour the symbiotic phenomenon. This will be a serious problem which breeders of new varieties of pulses will have to take into consideration.

Another aspect of the present investigation was to see whether one can select a rhizobial strain which has an efficient compatibility with more than one groundnut variety and whether one can select a variety which can show a favourable response to inoculation with more than one rhizobial strain. The isolates R_2 and R_3 were found to be more compatible with different host varieties, when compared to all other isolates of rhizobia in terms of symbiotic efficiency. Similarly, groundnut varieties USA-123 and Exotic-6 showed the most favourable response to inoculation with different isolates of rhizobia.

The last part of this study was mainly concerned with the possible strain variations, if any, between the seven isolates of rhizobia. It was observed that all the isolates had an identical fast rate of growth and tolerance to different levels of pH between 5.0 and 9.0. In the case of antibiotic sensitivity some difference between the seven isolates of rhizobia was

noticed. The isolates R_2 and R_3 were more resistant to streptomycin sulphate upto 250 μg/ml while the remaining isolates were all sensitive to this concentration of the antibiotic. It is also interesting to note that the isolates R_2 and R_3 were also the most efficient and compatible strains when compared to all other isolates of rhizobia indicating a certain degree of correlation between resistance to this antibiotic and symbiotic efficiency.

In the serological characterization, complete identification of various isolates of rhizobia was not done since serum for only three isolates R_3, R_5 and R_6 could be prepared. Tube agglutination studies showed that these isolates were distinct from one another and also from the remaining isolates of rhizobia except R_1 which showed a positive agglutination with the antiserum for isolate R_5. Regarding the agglutination of isolate R_1 with antiserum for R_5, one may conclude that these isolates are one and the same strains. But the apparent difference in plant characters due to inoculation with these isolates in varieties TMV-12 and TMV-11 indicate that they are related but not identical strains.

References

REFERENCES

Abdel - Wahab, S.M., Rifaat, D.M., Ahmed, K.A. and Hamdi, Y.A. (1976). Resistance to antibiotic in Rhizobium trifoli and its relation to nitrogen fixation. Zbl.Bakt. Abt II, 131 : 170-178.

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

Alagawadi, A.R. (1979). Evaluation of suitable strains of Rhizobium for groundnut (Arachis hypogaea L.) M.Sc. (Ag) thesis University of Agricultural Sciences, Dharwar.

Albrecht, W.A. (1933). Inoculation of legumes as related to soil acidity. J. Amer. Soc. Agron., 25 : 512-522.

Albrecht, W.A. (1943). Factors influencing the effect of inoculation of peanuts on new peanut lands. Soil Sci. Soc. Amer. Proc., 8 : 217-220.

Allen, O.N. (1953). Experiments in soil bacteriology. Burgees Publication Co., Minneapolis Minn. USA,,I Ed. pp. 69-70.

Andrew, C.S. (1976). Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate pasture legumes on nodulation and growth. Aust. J. Agric. Res., 29 : 611-623.

Ayalaba, L.B. (1980). Symbiotic nitrogen fixation by three peanut genotypes inoculated with two Rhizobium spp. strains. Agronomia Tropical, 27 : 343-344.

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. Ind. Nat. Sci. Acad., 40 : 571-575.

Balasunderam, V.R. (1971). Composite cultures of Rhizobium a better means of increasing yield of soybeans. Curr. Sci., 40 : 441-442.

Barber, L.E. (1980). Enumeration effectiveness and pH resistance of Rhizobium meliloti population in Oregon soils. Soil Sci. Soc. Amer. J., 44 : 3-4.

Bartholomew, J.W. and Mitwer, T. (1950). A simplified bacterial spore stain. Stain Technol., 25 : 153-156.

Bernaertz, M.J. and de Ley, J. (1963). A biochemical test for crown gall bacteria. Nature, 197 : 406-407.

Beynon, J.L. and Josey, D.P. (1980). Demonstration of heterogeneity in a natural population of Rhizobium phaseoli using variation in intrinsic antibiotic resistance. J. Gen. Microbiol., 118 : 437-442.

*Borges, de T.D. (1977). Isolation, identification and effectiveness of two Rhizobium strains from cowpea (Vigna sinensis L. Savi) Revista de la facultad de agronomia, 9 : 5-13..

Brockwell, J., Schwinghamer, E.A. and Gault, R.R. (1977) Ecological studies of root-nodule bacteria introduced intro field environment II. A critical examination of the suitability of antigenic and streptomycin markers for identifications of strains of Rhizobium trifolii. Soil Biol. Biochem., 9 : 19-24.

Burton, J.C. (1975). Pragmatic aspects of Rhizobium-leguminous plant association. In Proceedings of the First International Symposium on Nitrogen fixation. Washington State University Press. Pullman. (Eds) W.E. Newton and C.J. Nyman. Vol. II pp. 429-446.

Caldwell, B.E. and Vest, H.G. (1979). Genetic aspects of nodulation and dinitrogen fixation by legumes - the macrosymiont. In A Treatise on Dinitrogen Fixation, Section III Biology (Eds) R.W.F. Hardy and W.S. Silver. John Wiley and Sons, Inc., pp 328.

Caldwell, B.E. and Weber, O.F. (1970). Distribution of Rhizobium japonicum serogroups in soybean nodule as affected by planting dates. Agron. J., 62 : 12-14.

Chahal, V.P.S., Joshi, P.K. Chahal, D.S. and Rewari, R.B. (1973). Studies on serotyping and competitiveness of different isolates of gram-Rhizobium. Indian J. Microbiol., 18 : 148-150.

Charudathan, B. and Hubbel, D.H. (1973). The presence and possible significance of cross-reactive antigens in Rhizobium legume associations. Antonie van Leewenhock, 39 : 619-627.

Chitriv, A.J. and Wangikar, P.D. (1973). Note on the performance of legume nodulation with Rhizobium in Dry farming. Food, Farming and Agriculture, 10 : 165-166.

Dadarwal, K.R. Singh, C.S. and Subha Rao, N.S. (1974). Nodulation and serological studies of rhizobia from six species of Arachis. Pl. Soil, 40 : 535-544.

Dadarwal. K.R. Shashi Prabha and Tauro, P. (1976). Varietal differences with regard to Rhizobium compatibility and efficiency in nitrogen fixation of chick pea (Cicer arietinum. L). Proceedings of National Symposium on Nitrogen Assimilation and Crop Productivity pp. 235-243.

Damery, J.T. and Alexander, M. (1969). Physiological differences between effective and ineffective strains of Rhizobium, Soil Sci., 108 : 209-211.

Damirigi, S.M., Frederick, L.R. and Anderson, J.C. (1967). Serogroups of Rhizobium japonicum in soybean nodules as affected by soil types. Agron. J., 59 : 10-12.

Date, R.A. (1976). Principles of Rhizobium strain selection. In Symbiotic Nitrogen Fixation in Plants (Ed) P.S. Nutman. IBP-7. Cambridge University Press. pp. 157-180.

Dazzo, W.F. and Hubbel, U.D. (1974). Nitrogen Fixation Research, a key to world food. In Proceedings of the Final Inputs Review, meeting, Honolulu, Hawaii (Eds) D.R. Bouldin, S. Mughogho., D.J. Lathwell, and T.W. Scott, pp. 20-24.

De, N.K. and Chatterjee, B.N. (1976). Effect of trace elements on the growth and yield of groundnut in leached sandy loam soil. Indian J. agron., 21 : 209-216.

Dudman, W.F. (1964). Immuno-diffusion analysis of extra cellular soluble antigens of two strains of Rhizobium meliloti. J. Bact., 88 : 732-944.

Duggar, J.F. (1955). The effect of inoculation and fertilization of Spanish peanuts in root-nodule numbers. J. Amer. Soc. Agron., 27 : 128-135.

Erdman, L.W. (1947). Strain variation and host-specificity of Rhizobium trifolii on different species of Trifolium. Soil Sci. Soc. Amer. Proc., 11 : 255-259.

Evans, L.S., Levin, K.F. and Vella, F.A. (1980). Effect of nutrient medium pH on symbiotic nitrogen fixation by Rhizobium leguminosarum and Pisum sativum. Pl. Soil, 56 : 71-80.

Gaur, Y.D. and Sen, A.D. (1979). Cross-inoculation group-specificity in Cicer Rhizobium symbiosis. New Phytologist, 83 : 745-754.

Gollobin, G.S. and Levin, R.A. (1974). Streptomycin resistance in Rhizobium japonicum. Arch. Microbiol., 101 : 83-90.

*González, R.A. (1977). Behaviour of some Rhizobium japonicum strains in three varieties of soybean (Glycine max L.) Merr. Thesis, Universidad Nacionals, Bogota, Colombia. Taken from Revista Institute Colombiano Agropecuario, 12 : 588-590.

Habish, H.A. and Khairi, S.K.M. (1968). Nodulation of legumes in Sudan: Cross-inoculation groups and the associated Rhizobium strains. Exp. Agric., 4 : 227-234.

Hall, A.M. (1975). Effect of P, K and calcium on peanuts at Mauke, N. Z. J. Exp. Agric., 3 : 117-120.

Haque, I. and Amara, D.S. (1978). Effect of inoculation and sodium molybdate on groundnuts. Trop. grain Leg. Bull., 11-12 : 35-37.

*Hendry, G.S. and Jordan, D.C. 1969. Cation content and cation exchange capacity of intact cells and cell envelopes of viomycin - sensitive and - resistant strains of Rhizobium japonicum. Can. J. Microbiol., 15 : 671-675.

Hucker, G.J. (1927). Further studies on methods of Gram-staining. N.Y. Agr. Expt. Sta. Tech. Bull., 128-130.

Jackson, M.L. (1967). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd. New Delhi.

Johnson, H.W. and Means U.M. (1964). Selection of competitive strains of soybean nodulating bacteria. Agron. J., 56 : 60-62.

Josey, D.P., Beynon, J.L., Johnston, W.B. and Beringer, J.E. (1979). Strain identification using intrinsic antibiotic resistance. J.Appl. Bacteriol., 46: 343-350.

Keyser, H.H.,Munns, D.N. and Hohenberg, J.S. (1979). Acid tolerance of rhizobia in culture and in symbiosis with cowpea. Soil Sci. Soc. Amer. J., 43 : 719-722.

Kishinevsky, B. and Bar - Joseph, M. (1978). Rhizobium strain identification in Arachis hypogaea nodules by enzyme - linked inmunosorbent assay (ELISA). Can. J. Microbiol., 24 : 1537-1543.

*Klimmer, M. and Kruger, R.(1914). Sind debeider verschiednen Legumi nosen gef unden knollehem-backlarien ant verschieden. Centbl. Backt.Abt., 240: 256-265.

Konde, B.K. and Moniz, L. (1968). Effect of antibiotics on Rhizobium strain of wal (Dolichos lablab L.) and methi (Trigonella foenum-graecum L.) Indian J. Microbiol., 8 : 199-202.

Kremer, R.J. and Wagner, G.H, (1978). Detection of soluble Rhizobium japonicum antigens in soil by immuno-diffusion. Soil Biol. Biochem., 10 : 247-250.

Kumar Rao, J.V.D.K. and Patil, R.B. (1977). Symbiotic response of Glycine max (L.) Merril. to inoculation with different commercial inoculants of Rhizobium japonicum from agricultural institutions. Mysore J. agric. Sci. 11 : 342-244.

Levin, R.A. and Montgomery, M.P.,(1974). Symbiotic effectivenss of antibiotic - resistant mutants of Rhizobium japonicum. Pl. Soil, 41 : 669-676.

Moniz, L., Shete, G.S. and Konde, B.K. (1968). Strain variation and effeciency of nitrogen fixation among strains of Rhizobium melliloti Dangegard and Rhizobium trifolii Dangegard. Indian J. Microbiol., 8 : 159-164.

Muthusamy, S. (1970). Studies of Rhizobium of groundnut. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.

Nair, S.K. and Sivaprasad, P. (1981). Preliminary study on nodulation in cowpea in acid soil. Agric. Res. J. Kerala, In Press.

*Om Prakash. (1980). Antibiotic resistance in Cicer rhizobia and its relation to efficiency. M.Sc. thesis, Haryana, Agricultural University, Hissar.

Pandher, M.S. and Kahlon, S.S. (1978). pH and salt tolerance of Rhizobium leguminosarum isolates from pea (Pisum sativum). Indian J. Microbiol., 18 : 81-84.

Pain, A.N. (1979). Symbiotic properties of antibiotic resistant and auxotrophic mutants of Rhizobium leguminosarum. J. appl. Bact., 47 : 53-54.

Pankhurst, C.E. (1977). Symbiotic effeciency of antibiotic-resistant muntants of fast-and slow-growing strains of Rhizobium nodulating Lotus species. Can. J. Microbiol., 23 : 1026-1033.

Pankhurst, C.E. and Craig, A.S.(1979). Morphology and nitrogenase activity of agar cultures and root nodule formed by D - cyclo serine resistant mutants of Rhizobium sp. strain 32 H 1. J. Gen. Microbiol., 110 : 177-184.

Pareek, R.P. (1979). Studies on the effectiveness of different strains of chickpea (Cicer arietinum L.) Rhizobium in field. Indian J. Microbiol., 19 : 123-129.

Rajagopalan, T. (1938). Studies on groundnut root nodule organism. I. Isolation of strain and study of culture. Indian J. agric. Sci. 8 : 331-348.

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

Rangaswami, G. and Oblisami, G. (1962). Quantitative studies on nitrogen fixation by five legume nodule bacteria. J. Annamalai Univ., 23 : 119-121.

Rani Perumal, Venketaramanan, C.R., Krishnamoorthy, K.K. and Ramaswamy, P.P. (1978). Influence of mode of Rhizobium incorporation with phosphorous, magnesium and boron on yield and quality of groundnut (Arachis hypogaea L.). Foodd, Farming and Agriculture, 9 : 256-258.

Rao, H.S.N. (1974). Response of groundnut variety TMV-2 to rhizobial inoculation. Proc. Ind. Nat. Sci. Acad. B. Biol. Sci., 40 : 650-655.

Robinson, H.L. (1975). Soil fertility studies with peanut in Louisiana. La Agric., 19 : 4-5.

Rose, H.L. (1963). Comparative studies on the fixation of nitrogen in certain common legumes, M.Sc. (Ag) thesis, University of Kerala.

Sahu, S.K. and Behera, B. (1972). Note on the effect of Rhizobium inoculation on cowpea, groundnut and green gram. Indian J. Agron., 17 : 359-360.

Saxena, M.C. and Singh, H.P. (1978). Response of gram varieties to rhizobial culture. Annual Report of the Research Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, A - 166.

Schneewis, T.J., Elkan, G.H., Ligon, J.M., Wynne, J.H. and Isleeb, T.G. (1978). Isolation and evaluation of Rhizobium from Arachis nodules collected in South America. American Peanut Research and Education Association Proceedings, 10 : 16-20.

Schwinghamer, E.A. (1954). Effectiveness of Rhizobium as modified by mutation for resistance to antibiotics. Antonie van Leewenhoek, 33 : 121-136.

*Seeger, J.R. (1961). Effects of nitrogen manuring on nodulation and yield of groundnut. Bull. Inst. agron. gembl., 29 : 197-218.

Sen. D. and Weaver, R.W. (1980). Nitrogen fixing activity of rhizobial strain 32 H 1 in peanut (Arachis hypogaea) cultivar Starr. and cowpea (Vigna unguicultata) cultivar California Buckaye nodules. Plant Sci. Lett., 18 : 315-318.

Sharma, A., Sen, A.N. and Rao, N.S.S. (1978). Beneficial effects of seed pelletting with calcium carbonate on nodulation of groundnut. Sci. Cult., 44 : 172-173.

Sheth, R.D. (1979). Studies on the development of Rhizobium inoculants for groundnut (Arachis hypogaea L.) Ph.D. Thesis, University of Agricultural Sciences, Bangalore.

Singh, C.S., Dadarwal, K.R, and Rao, N.S.S. (1976). Effectiveness of rhizobia from wild species of Arachis on the cultivated species of Arachis hypogaea and their physiological characteristics. Zbl. Bakt. Abt. III, 131 : 72-78.

Singh, H.D., Baghel, S.S., Singhania, R.A. and Pareek, R.P. (1979). Variety-strain interaction between pigeon pea (Cajanus cajan L.) and Rhizobium spp. Indian J. Microbiol., 19 : 170-172.

*Singleton, P.T., Sanford, W.G. and Stockinger, K.R. (1978). Green house studies on the symbiotic effectiveness of eleven tropical peanut cultivars. Agron. Abstr., 85 : 12.

Sreedharan, C. and George, C.M. (1965). Effect of calcium, phosphorous and magnesium on growth, yield and shelling percentage of groundnut in red loam soils of Kerala. Agric. Res. J. Kerala, 6 : 74-78.

Sullia, S.B. and Anasuya, D. (1980). Effect of groundnut root exudates on different strains of Rhizobium sp. Curr. Sci. 49 : 489-490.

Suraj Bhan. (1975). Suitable plant type for arid zone of groundnut (Arachis hypogaea L.) 4. Nodulation studies. Oilseeds Journal, 5 : 5-7.

Thronton, H.G. (1963). Nodule bacteria and their host-legume: some problems that they still present. Sci. Prog., 42 : 185-204.

Trinick, M.J. (1968). Nodulation of tropical legumes 1. Specificity in the Rhizobium symbiosis of Leucaena leucocephala, Exp. Agric., 4 : 243-253.

Van Schreven, D.A. (1972). On the resistance of effectiveness of Rhizobium trifolii to a low pH. Pl. Soil, 37 : 49-55.

*Varela, G.R. and Munevar, M.F. (1978). Performance of strains of Rhizobium japonicum associated with cultivars of soybean (Glycine max (L.) Merr.) selected for Tolima Department. Revista Institute Colombiano Agropecuario, 13 (2) : 249-255.

Veeraraghavan, P.G. (1954). Effect of lime and potash on the yield and quality of groundnut (Arachis hypogaea L.) in red loam soils of Kerala. M.Sc. (Ag) thesis submitted to University of Kerala.

Vincent, J.M. (1941). Serological studies of the root-nodule bacteria I. strains of Rhizobium melliloti. Proc. Linn. Soc. (N.S.W.)., 66 : 145-154.

Vincent, J.M. (1970). A Manual for the Practical Study of the Root-nodule Bacteria. IBP - Handbook No. 15. Blackwell Scientific Publications, Oxford. pp. 196.

Vincent, J.M. (1971). Rhizobium : General Microbiology Taken from Biological Nitrogen Fixation in Natural and Agricultural Habitats (Eds). T.A.Lie and E.G. Mulder Martinus Nijhoff. The Hague.

Vincent, J.M. and Humphrey, B.A. (1970). Taxonomically significent group of entigens in Rhizobium. J.Gen. Microbiol., 63 : 78-82.

*Vuong, H.H. and Andriamanatena, S. (1970). Legume inoculation. Results of 1968 experiments. Agron. Trop., 6-7 : 596-603.

Weber, O.F. and Miller, J.P. (1970). Serogroups of Rhizobium japonicum as influenced by soil temperature. Agron. J., 62 : 10-12.

Weir, J.B. (1959). Investigations on adaptation between strains of root nodule bacterium. Rhizobium meliloti and the host plant. Medicago sativa L., Phyton, 13 : 165-169.

Wetselsaar, R. (1967). Estimation of nitrogen fixation of four legumes in a dry monsoonal area of north western Australia. Aust. J. Exp. Agric. Husb., 7 : 578-522.

Wynne, J.C., Elkan, G.H., Schneewis, T.J., Isleab, T.G., Preston C.M. and Meisher, C.A. (1978). Increasing nitrogen fixation in peanuts. Proceedings of American Peanut Research and Education Association, 10 : 22-29.

Wynne, J.C., Elkan, G.H., Meisner, C.A., Schneewis, T.J. and Ligon, J.M. (1980). Greenhouse evaluation of strains of Rhizobium for peanuts. Agron. J., 72 : 645-659.

Yadav, N.K. and Vyas, S.R. (1973). Effect of salt and pH on the growth of Rhizobium strains. Indian J. Microbiol., 11 : 97-102.

*Zelazna - Kowalska, I. (1971). Correlation between streptomycin resistance and symbiotic properties of Rhizobium. I. Conversion of spheroplasting, effective Rhizobium trifolii strain B_1 to avirulent rods with changed phage and antibiotic patterns after mutation to a high level of streptomycin resistance. Acta Microbiol. Polon., 26 : 233-241.

*Zipfel, H., (1912). Beilrage Bumorphologie and Biologieder knollehen bakterein deleguminosen. Zent-11. Bakt. Parasit. Ked., Abt.11. 32 : 97-137.

* Original not seen

Appendices

APPENDIX I

Pattern of nodulation in different varieties of groundnut (*Arachis hypogaea* L.)

Analysis of variance table

Source	df	Mean Square
Total	105	..
Treatment	14	11023.3 *
Error	91	43.81

* Significant at 5% per cent level of significance

APPENDIX II

Effect of different rhizobial isolates on the nodulation, plant characters and nitrogen content of groundnut varieties.

Analysis of variance table

Source	df	Mean Square						
		Nodule number per plant	Nodule fresh weight mg	Nodule dry weight mg	Plant fresh weight g	Plant dry weight g	Plant height cm	Nitrogen content %
Total	167	..	..	..	..	..	..	..
Variety	6	13824.53*	3781.08*	363.49*	8.13*	0.393*	694.58*	0.97*
Rhizobium	7	1326.60*	19552.57*	2247.97*	75.85*	3.39*	529.99*	8.14*
Variety x Rhizobium	42	455.72*	752.79*	91.11*	2.99*	0.107*	54.10*	0.29*
Error	112	59.79	172.79	17.56	0.42	0.065	30.87	0.006

* Significant at 5 per cent level of significance.

APPENDIX III

pH tolerance of different isolates of *Rhizobium*

Analysis of variance table

Isolate R_1

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.000225	0.0096*	0.0250*	0.0250*
Error	5	0.00014	0.00031	0.00195	0.00195

Isolate R_2

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.00009	0.0119*	0.015*	0.015*
Error	5	0.00005	0.00011	0.00019	0.00019

Isolate R_3

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.00010	0.0046*	0.000675*	0.000675*
Error	5	0.00034	0.00039	0.00018	0.00018

Isolate R_4

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.00004	0.0060*	0.[illegible]069*	0.069*
Error	5	0.00005	0.0001	0.00086	0.00086

Isolate R_5

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.00005	0.005*	0.031*	0.031*
Error	5	0.00004	0.0002	0.0004	0.0004

Isolate R_6

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.000016	0.0078*	0.0316*	0.0316*
Error	5	0.00025	0.00015	0.00006	0.00006

Isolate R_7

Source	df	Mean Square			
		0	24	48	72
Total	10	..	..	..	..
Treatment	4	0.0003	0.005*	0.0097*	0.0097*
Error	5	0.0002	0.00018	0.000015	0.000015

* Significant at 5 per cent level of significance.

APPENDIX IV

Sensitivity of different isolates of rhizobia to the antibiotic, streptomycin sulphate

Analysis of variance table

Isolate R_1

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.0002	0.096*	0.139*	0.139*
Error	4	0.00014	0.0031	0.002	0.002

Isolate R_2

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00004	0.006*	0.0152*	0.0152*
Error	4	0.00005	0.00006	0.00032	0.00032

Isolate R_2

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00036	0.0138*	0.0277*	0.0277*
Error	4	0.00045	0.002	0.0010	0.0010

Isolate R_4

Source	df	Mean Sqaure			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00044	0.0238*	0.0411*	0.0411*
Error	4	0.00036	0.00018	0.00052	0.00052

Isolate R_5

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00009	0.0331*	0.020*	0.020*
Error	4	0.00005	0.00018	0.00017	0.00017

Isolate R_6

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00005	0.020*	0.059*	0.059*
Error	4	0.00002	0.0002	0.0009	0.0009

Isolate R_7

Source	df	Mean Square			
		0	24	48	72
Total	7	..	..	..	..
Treatment	3	0.00079	0.007*	0.028*	0.028*
Error	4	0.0003	0.0002	0.0002	0.0002

* Significant at 5 per cent level of significance.

STUDIES ON HOST-VARIETAL SPECIFICITY FOR *Rhizobium* FOR NODULATION IN GROUNDNUT

By

V. K. GIRIJA

ABSTRACT OF A THESIS
Submitted in partial fulfilment of
the Requirement for the Degree
MASTER OF SCIENCE IN AGRICULTURE
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, TRIVANDRUM

1982

ABSTRACT

An investigation was carried out at the College of Agriculture, Vellayani, Kerala to study the host-varietal specificity, if any, for Rhizobium for nodulation in groundnut. A total of fifteen groundnut varieties were subjected to a preliminary screening on the basis of the number of nodules formed and arranged into five distinct nodulation groups. Seven varieties of groundnut TMV-12, USA-123, Exotic-6, Spanish Peanut, TMV-11, Ak-12-24 and Ah-32 were selected from among these groups for use in further studies.

Isolates of the root nodule bacterium from each of the seven varieties of groundnut were identified as Rhizobium by morphological and cultural characteristics. These rhizobial isolates along with their host varieties were used in a pot culture experiment to study their host-varietal specificity. Nodule number, fresh and dry weight of nodules and plants, plant height and percentage nitrogen content were taken as the important parameters for studying the efficiency. In general, a favourable response for all plant characters studied was obtained in a variety due to inoculation with its respective homologous isolate of Rhizobium. Exceptions to this were also noticed. The isolates R_2 and R_3 were found to be more compatible with different host varieties in terms of symbiotic efficiency. Similarly, the groundnut varieties USA-123 and Exotic-6 showed the most favourable response to inoculation with different isolates of rhizobia.

The physiological studies with the rhizobial isolates revealed that all of them had an identical fast rate of growth and tolerance to different levels of pH between 5.0 and 9.0. Regarding the sensitivity of isolates to antibiotic, isolates R_2 and R_3 were more resistant to streptomycin upto a concentrat of 250 μg/ml while the remaining isolates were all sensitive to this concentration of the antibiotic.

Serological studies on the antigenic relationship of the isolates, revealed that the isolates R_3, R_5 and R_6 for which antisera were prepared were antigenically distinct from one another and also from the remaining isolates except R_1. The isolate R_1 showed a positive agglutination with the antiser for R_5. However, the isolates R_1 and R_5 produced differential responds in varieties TMV-12 and TMV-11, indicating that they are related but not identical strains.