Rhizobium INOCULATION AND NUTRIENT LEVELS ON NODULATION AND SEEDLING GROWTH IN TREE LEGUMES

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "*Rhizobium* inoculation and nutrient levels on nodulation and seedling growth in tree legumes" 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 University or Society.

Vellayani,
20th November, 1992

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CERTIFICATE

Certified that this thesis entitled "Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes" is a record of research work done independently by smt. Ragini,R. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her .

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LIST OF ABBREVIATIONS

cm - centimetre

m - metre

g - gram

ha - hectare

kg - kilogram

°C - degree celsius

DAS - days after sowing

% - per cent

Fig.- figure

con.- concentrated

No. - number

KAU - Kerala Agricultural University

INTRODUCTION

CHAPTER 1

INTRODUCTION

Agroforestry, the new multidisciplinary science encompasses growing of trees in farm lands with agricultural crops inorder to sustain productivity. There is a growing awareness, all over the world today on the scientific principles of agroforestry. This has generated enthusiasm among researchers, developmental experts and policy makers concerned with tropical land use system. Tree is an integral part of the agroforestry , and hence there is a renewed awareness on the productive and protective value of trees. Efforts are now being made, throughout the world to device scientific methods of integrating trees most efficiently in farm lands .

Of the variety of trees used, nitrogen fixing trees play a vital role in agroforestry systems. These trees have many useful attributes and provision for a number of end products and services. They have the capability of fast growth rate, tolerance to environmental extremes and have the potential for rapid genetic improvement.

Nitrogen fixing trees are also playing an important role as a major source of nitrogen that enters the ecosystem through nitrogen cycle. Since, these trees are generally subsistence crops and can fix nitrogen, their use will help to reduce the use of inorganic nitrogen fertilizers. Therefore, integration of fast growing multipurpose nitrogen fixing trees in farm lands has been considered as crucial and vital. However its operational aspects including management practices are yet to be studied.

Popularising trees in farm lands calls for finding out suitable species, both indigenous and exotic and also their rapid multiplication including nursery techniques. Hence, streamlining the nursery practices has been considered as an important area of research, which will help in large scale production of quality seedlings. Data available shows lack of sufficient informations on the germination behaviour of different tree legumes under Kerala conditions. It is generally observed that seeds of many trees possess hard and impermeable seed coats and this leads to dormancy and erratic germination behaviour of seeds. Under the circumstances faster multiplication of quality seedlings will be a difficult task. Hence investigations are to be initiated in these lines urgently.

Enhanced growth of plants is reported to be through improved mineral nutrition during the early stages of growth. Mineral nutrient deficiency is a major constraint limiting legume nitrogen fixation by legumes and their yield below maximum potential. It is also a fact that association of tree legumes with suitable strains of rhizobia could enhance the seedling vigour and the soil fertility status. It is, therefore, essential to understand the influence of nutrients on the legume-Rhizobium symbiosis. Scientific knowledge on the various strains of rhizobia and their interaction with nutrients in legume trees under our conditions are also lacking. So there is an urgent need to take up research programmes in the above aspects on a priority basis.

Keeping the above aspects in mind , the present investigation was carried out as a preliminary study to determine the effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes, under Kerala conditions and this investigation was carried out as three separate experiments with the following objectives.

(1) To find out the effect of different scarification methods on the germination of seeds of some commonly grown and newly introduced tree legumes .

(2) To screen out the best among the native and exotic rhizobial isolates along with different phosphorus levels in selected tree legumes.

(3) To study the effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth of tree legumes.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Literature available on the various aspects related to the present investigation on the germination of tree legumes in relation to different scarification methods and the effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes have been briefly reviewed here.

2.1 Germination studies on multipurpose tree species

Physical or seed coat dormancy in which hard seedness prevents germination is a general phenomenon in many leguminous trees, eg. Parkia clappretoniana (Etejere et al. 1982), Dialium quineeuse (Gill and Bamidele, 1981), Delonix regia (Gill et al 1981) and Cassia nodosa, Cassia sieberiana and Cassia spectablis (Gill et al. 1982). The strength of dormancy varied according to latitude, provenance and from year to year even in seeds from the same parent (Willian, 1985).

Impermeability to water and gases is also observed to be another most common form of seed coat dormancy and is characteristic to many legumes. (Krugman et al. 1984).

2.1.1 Pre-soaking of seeds with water

Various seed pretreatment methods have been found to be useful in breaking the impermeability of hard seed coat to water in many species (Alvarez _et al_. 1977; Heydecker and Coolbecur, 1977).

Favourable effect of presoaking of dormant seeds of trees have been reported by various workers (Motilal and Gilkar, 1967; Yeou-Der, 1968; Webb and Dunbroff, 1972; Kawecki, 1970). According to Ntumbula _et al_. (1990) there was 73 per cent germination for seeds of _Albizia lebbeck_ when soaked in water for 24 hours, compared to 9.5 per cent germination in control.

Germination could not be detected in seeds of _Cassia occidentalis_ soaked for 24 hours in water (Anitha kumari and Kohli, 1984).

2.1.2 Mechanical scarification

Moffett (1950) reported mechanical scarification as a suitable method to remove seed dormancy in _Acacia_ spp. Rubbing seed coat with a razor or sand paper was found to be effective in increasing germination in _Acacia_ seeds (Larsen, 1964).

Scarification of seeds with sand paper came out to be the most effective method of breaking dormancy in _Acacia farnesiana_, resulting in 98 per cent germination (Gill _et al_. 1986). In _Albizia lebbeck_, mechanical scarification resulted in 70 per cent germination compared to 9.5 per cent germination in control. Seeds of Cassia occidentalis which were surface scratched did not germinate (Anithakumari and Kohli, 1984).

Hawton and Drennan (1980) reported cent per cent germination in Crotalaria goreensis, when seeds were rubbed with glass paper. Mechanical scarification by rubbing the seeds of Leucaena leucocephala across a piece of sand paper enhanced germination (Visocky and Felker, 1989).

2.1.3 Germination in relation to time and hot water treatment

Variable response of boiling water treatments in relation to time in different leguminous species have been reported (Anon., 1948; Oseni, 1979; Gill et al. 1981).

Immersion in water at 90°C water for 30 seconds was recommended to enhance germination in Acacia mangium (Misra and Singh, 1981). According to Doran and Gunn (1986), germination of certain Australian acacias was enhanced by immersing the seeds for one to five minutes in boiling water. Boiling water treatment improved germination by 60 to 80 per cent in Acacia catechu (Babeley and Kandya, 1985; Doran and Guan, 1987). Bhatnagar et al. (1988) observed germination of 85 per cent for seeds of

Acacia maeonochieona and 80 per cent for Acacia ampliceps when treated with boiling water for one minute.

According to de Hoogh (1989), seeds of Acacia auriculiformis which were immersed in hot water for one minute and left to soak for 24 hours resulted in 25 per cent germination. Immersion in boiling water for one minute resulted in 75 per cent germination for Acacia wanyu(Gunn,1989).

When seeds of Acacia auriculiformis were treated with boiling water for one minute, there was 76 per cent germination and by the same method, there was 93 per cent germination for Acacia holosericea (Marunda, 1989).

Seeds of Acacia ampliceps, Acacia ligulata, Acacia salicina, Acacia saligna, Acacia victoriae, pretreated with boiling water for one minute, resulted in germination percentage of 90, 55, 50,65 and 35 respectively (Khajuria and Singh, 1990).

Seeds of Acacia farnesiana given boiling water treatment at 90°C for one and two minutes, resulted in poor germination percentage of 40 and 24 respectively (Gill et al. 1986). Seeds of Acacia murrayana when treated with boiling water for 60 seconds resulted in poor germination of seven per cent (Bhatnagar et al. 1988). Acacia xiphophylla and *Acacia coriacea* seeds showed less than 15 per cent germination (Gunn, 1989). There was only 30 per cent germination in seeds of *Cassia occidentalis* which were kept in water for one minute at 100°C (Anithakumari and Kohli, 1984).

Seeds of *Acacia auriculiformis* when treated with boiling water for one minute ,the germination percentage was 85, whereas in *Acacia holosericea* the germination was 93 per cent (Marunda, 1989). Seeds of *Acacia trachycarpa* responded favourably to immersion in boiling water for five minutes with a germination percentage of 93 [Gunn, 1989].

However, *Albizia lebbeck* seeds when soaked in hot water at 80°C for five minutes and 10 minutes showed poor germination of two and zero per cent respectively. Hot water treatment of seeds of *Dichrostachys cinerea* for 10 minutes resulted in poor germination percentage of 24 (Roy *et al*. 1984).

2.1.4 Scarification in relation to time and acids

The use of various acids for softening the hard seedcoat is already known in overcoming the dormancy in *Acacia albida* (Ford-Robertson, 1948), *Acacia cyanophylla* (Shaybany and Roughani, 1976), *Phaseolus mungo* (Subburamu and Sridhar, 1977), *Cuscuta campestris* (Hutchinson and

Ashton 1979), _Sesbania exaltata_ (Johnston _et al_. 1979) and _Acacia farnesiana_ (Gill _et al_. 1986). Sulphuric acid treatment has been reported to be effective in improving the germination of some Australian acacia seeds (Doran _et al_. 1983; Kariuki 1987, Acoba 1987).

Acacia farnesiana seeds, scarified with con. nitric acid for 10 minutes gave 65 per cent germination. But con. hydrochloric acid was found to be less effective in promoting germination of _Acacia farnesiana_, which gave only 33 per cent germination (Gill _et al_. 1986).

Seeds of _Dichrostachys cinerea_ treated with con. sulphuric acid for five minutes, resulted in 11.66 percent germination (Roy _et al_. 1984). Soaking of _Albizia lebbeck_ seeds for five minutes in con.sulphuric acid gave germination of 15 per cent as compared to boiling water treatment for three minutes and mechanical scarification (Ntumbula _et al_. 1990).

Gill and Bamidele (1981) reported 90 and 94 per cent seed germination in _Dialium guineeuse_ and _Parkia clappretoniana_ with con.sulphuric acid for 10 minutes. However, seeds of _Dichrostachys cinerea_ treated with sulphuric acid for 10 minutes resulted in germination percentage of 13.33 only (Roy _et al_. 1984).

Soaking seeds in con. sulphuric acid for 15 minutes produced 33.33 per cent germination in Dichrostachys cinerea (Roy et al. 1984), 50 per cent germination in Acacia auriculiformis and 30 per cent in Acacia holosericea (Marunda, 1989).

In Acacia farnesiana seed scarification with con.sulphuric acid for 20 minutes gave 66 per cent germination (Gill et al.1986). Sulphuric acid scarification of seeds for 20 minutes resulted in 95 per cent germination in Acacia holosericea (Sivasubramaniam et al. 1991).

Pretreatment of seeds of Cassia occidentalis for 20 minutes in con.sulphuric acid brought about 88 per cent germination (Anita kumari and Kohli, 1984). Seeds of Dichrostachys cinerea treated with con.sulphuric acid for 20 minutes gave 26.66 per cent germination (Roy et al. 1984). Natarajan and Vinayarai (1988) found that scarification with con.sulphuric acid for 20 minutes increased germination in Acacia planifrons. Seeds of Dichrostachys cinerea treated with con.sulphuric acid for 25 minutes gave 63.33 per cent germination (Roy et al. 1984).

Soaking seeds in sulphuric acid for 30 minutes produced 95 per cent germination in Acacia auriculiformis (Marunda, 1989). According to Dijk (1991) germination rate of Acacia auriculiformis could be increased from 25 per cent with hot water treatment to 58 per cent with sulphuric acid treatment for 30 minutes.

2.2 Effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth in legumes

Most of the research workers to this day are of the general opinion that legumes and nodules are always associated. Hutton and Coote (1972) have suggested that legume species may be selected for quicker and more efficient nodulation, although there is similar potential to select for more effective strains of Rhizobium (Norris and Date, 1976). Barnett (1986) showed the importance of inoculation with its specific Rhizobium on the growth of many indigenous legume tree species. However, the knowledge of symbiotic association with tree legumes is much less understood than with leguminous crops. According to O'Hara et al. (1988), mineral nutrient deficiencies limit nitrogen fixation by the legume - Rhizobium symbiosis in many agricultural soils and as a result seriously depress legume yields below their maximum potential. Research results in these lines are meagre. The literature available on the influence of Rhizobium inoculation and nitrogen and phosphorus levels on growth and nodulation of tree legumes and other related species pertaining to the nursery stage is reviewed here under.

2.2.1 Effect of *Rhizobium* inoculation on nodulation and seedling growth in tree legumes

2.2.1.1 Growth characters

Early growth of some nitrogen fixing trees have been reported to be slow (Bray *et al*. 1985). It was found that *Rhizobium* inoculation had a significant positive effect on their growth characters (Balaji and Rangarajan, 1987; Khajuria and Singh, 1990).

Barnett (1986) reported significantly increased nursery growth of indigenous legume tree species inoculated with specific *Rhizobium* strains.

In *Acacia manqium*, Umali-Garcia *et al*.(1988) observed an increase of 13.22 per cent in plant height over the conrol. They found increase in plant height from 16.48 cm in the control to 23.15 cm in inoculated seedlings in *Paraserianthes falcataria*.

However, inoculation, did not give significant increase in plant height in *Acacia cyanophylla* (Gardezi *et al*.1988).

There was an increase in seedling height from 31.29 to 34.64 cm by inoculation with local isolate and 31.27 cm to 36.36 cm by inoculation with exotic isolate in

Leucaena (Balasundaram and Mohammed Ali, 1988). Jiang et al. (1991) found an increase of 27.7 per cent in plant height by inoculation alone. In Gliricidia sepium, Ngulube (1989) observed an increase in plant height from 16.9 to 18.8 cm by inoculation.

Chang (1986) observed that Acacia seedlings inoculated with Rhizobium had greater shoot dry weight, compared to uninoculated plants. According to Balaji and Rangarajan (1987) there was 21.28 per cent increase in shoot dry weight of Acacia nilotica by inoculation. An increase in shoot dry weight from 89 g to 92 g in Albizia lebbeck in sterile soil was observed by inoculation (Ntumbula et al., 1990).

In Leucaena leucocephala (K-8) Mohammed (1988) noticed an increase in shoot dry weight from 1.41 to 3.88 g $plant^{-1}$ by inoculation.

However, Rhizobium inoculation, did not give significant response in shoot dry weight in Acacia cyanophylla (Gardezi et al. 1988) and in Gliricidia sepium (Ngulube, 1989).

Significant differences in root dry weight due to inoculation were not observed in Acacia mangium and Paraserianthes falcataria (Umali-Garcia et al. 1988) and in

Leucaena leucocephala (Ezenwa and Cobbina, 1991). In *Leucaena* an increase of 54.5 per cent over control with respect to root dry weight by inoculation was noticed (Jiang *et al*. 1991).

In Blackgram and horse gram, Sahu (1973) noticed an increase in root dry weight by inoculation.

2.2.1.2 Nodulation characteristics

According to Balaji and Rangarajan (1987) *Rhizobium* inoculated plants gave 2.2 nodules per plant in *Acacia nilotica*, as compared to no nodules in control. In *Albizia lebbeck*, inoculation increased the number of nodules per plant from zero to 13 (Ntumbula *et al*. 1990). Prabhakaran *et al*. (1991) observed an increase in nodule number from 2.4 to 9.4 $plant^{-1}$ by inoculation in *Acacia holosericea*.

Daroy *et al*. (1987) reported that nodule number of *Sesbania rostrata* was significantly increased by inoculation. . In *Leucaena leucocephala* Gunawardena and Pushpakumari (1988) reported that the number of nodules per plant increased from 29.2 to 45.8, and where as an increase in number of nodules from 5.30 to 26.20 was noticed by Mohammed (1988) by inoculation. Pahwa (1989) observed that in Leucaena, the number of nodules per plant increased from four in the control to 12 in inoculated plants. Gunawardena and Senanayake (1989) found that number of nodules per plant had increased from 0.30 to 4.08 in Leucaena.

In Acacia nilotica, nodule dry weight reached a maximum of 119.6 mg plant^{-1} in inoculated plants (Balaji and Rangarajan, 1987). Umali-Garcia et al. (1988) observed 50 times increase in nodule dry weight of inoculated plants in Paraserianthes falcataria. Prabhakaran et al. (1991) observed that nodule dry weight had increased from 15.3 to 42.3 mg plant^{-1} by inoculation in Acacia holosericea.

Mohammed (1988) observed that nodule dry weight in Leucaena increased from 0.003 to 0.145 g plant^{-1} and Gunawardena and Pushpakumari (1988) noted an increase in nodule dry weight from 0.29 to 0.66 g plant^{-1} by inoculation.

2.2.1.3 Nitrogen uptake

Nitrogen content of Acacia nilotica was increased from 7.84 per cent to 30.39 per cent due to rhizobial inoculation (Balaji and Rangarajan, 1987).

Mohammed (1988) noted an increase in nitrogen uptake from 27.72 to 96.23 mg plant^{-1} in Leucaena. Pahwa (1989) observed that nitrogen uptake in Leucaena was increased to 441.0mg plant^{-1} from 336.7mg plant^{-1}. Nitrogen uptake in pigeon pea was also increased by inoculation (de Lucena Costa and Paulino, 1989).

2.2.1.4 Phosphorus uptake

Mohammed (1988) noticed an increase in phosphorus uptake in Leucaena from 2.84mg per plant to 5.13mg plant^{-1} by inoculation. An increase in phosphorus uptake due to inoculation was observed in pigeon pea (de Lucena Costa and Paulino, 1989).

2.2.2 Effect of nitrogen and phosphorus on seedling growth and nodulation of tree legumes

Umali-Garcia *et al* (1988) observed that the average increase in plant height was highest in plants fertilised with 30kg N ha^{-1}.

In *Erythrina suberosa*, increase in plant height due to nitrogen fertiliser application was reported by Jones (1985) and Moloney *et al* (1986). Hussain *et al* (1989) found that in *Leucaena*, nitrogen application at the rate of 20kg N ha^{-1} resulted in an increase in height from 90cm 103cm. Sanginga *et al* (1988) and Cobbina (1991) reported significant response to applied nitrogen in *Leucaena* during early growth when the plant had not started nodulation and nitrogen fixation. According to Cobbina et al. (1990) nitrogen application at 20 Kg N ha^{-1} increased plant height at 84 days after planting of Leucaena and Gliricidia. Height of Leucaena was significantly higher in inoculated plants and without applied nitrogen (Ezenwa and Cobbina, 1991).

In Dalbergia sisso, Sheikh and Afzal (1985) observed 4.5 per cent increase in plant height over the control as a result of nitrogen addition. Hussain et al. (1990) noticed an increase in plant height upto 50 Kg N ha^{-1} six months after sowing in Dalbergia sisso.

On the contrary, plants fertilised with 100 Kg N ha^{-1}, exhibited stunted growth over plants receiving 0-60 Kg N ha^{-1} in Paraserianthes falcataria (Umali-Garcia et al. 1988).

As the level of nitrogen application increased above 50 Kg N ha^{-1}, a reduction in plant height was observed in Dalbergia sisso (Hussain et al. 1990).

Steward and Gwaze (1988) noticed that shoot dry weight of Acacia albida had significantly increased at nursery stage when nitrogen at the rate of 32 Kg ha^{-1} was applied. On the contrary, according to Umali-Garcia et al. (1988) shoot dry weight was not significantly affected by nitrogen application in Paraserianthes falcataria and Acacia *mangium*. According to Maasdorp and Gutteridge (1986), application of 100 Kg N ha^{-1} . increased shoot dry weight of *Sesbania* sp. Hussain *et al*. (1990) noticed an increase in shoot dry weight from 28.7 to 31.9 g $plant^{-1}$ by application of 20 Kg N ha^{-1} atSixmonths after planting in *Dalbergia sisso*.

Shoot dry weight of inoculated *Leucaena* plant, without applied nitrogen was significantly higher than the control (Gunawardena and Pushpakumari, 1988; Ezenwa and Cobbina, 1991).

Shoot dry weight of *Acacia albida* was found to be increased with application of phosphorus at the rate of 24 Kg ha^{-1} (Steward and Gwaze, 1988).

Shoot dry weight of pigeon pea increased by 32 per cent by application of phosphorus at the rate of 60 Kg ha^{-1} (Srivastava and Verma, 1986). Shoot dry weight of *Leucaena* was found to be significantly increased with application of phosphorus at the rate of 50 Kg ha^{-1} from 55.03 g to 87.75 g $plant^{-1}$ (Hussain *et al*. 1988). A three fold increase in shoot dry weight of *Leucaena leucocephala*, *Acacia albida*, *Tephrosia vogelii*, *Sesbania rostrata*, *Sesbania punctata* and *Sesbania grandiflora* was attained when plants grown in pots were applied with phosphorus at the rate of 250 mg super phosphate per pot (Sanginga, 1988).

According to Hussain et al. (1989) the shoot dry weight showed an increase from 28.7 to 31.9 g in the case of Erythrina suberosa due to 50 Kg ha^{-1} phosphorus application.

Root dry weight of plants was not significantly affected by application of nitrogen at the rate of 30,60 and 100 Kg N ha^{-1} in Paraserianthes falcataria (Umali-Garcia et al. 1988).

In Erythrina suberosa, nitrogen application at the rate of 20 Kg ha^{-1} increased the root dry weight from 5.70 g to 7.18 g $plant^{-1}$ (Hussain et al. 1989).

Increase in root dry weight from 26.88 g to 30.04 g $plant^{-1}$ by the application of phosphorus at the rate of 50 kg ha^{-1} was reported by Hussain et al.(1988) in the case of Leucaena.

According to Ezenwa and Cobbina (1991), root dry weight of Leucaena plants without applied phosphorus was significantly lower.

Adverse effect of nitrogen fertilization on nodulation of Albizia procera was reported by Hussain et al. (1986) and in Paraserianthes falcataria by Moloney et al. (1986). They observed that Paraserianthes falcataria have nodulated in both nitrogen fertilised and unfertilised pots, but effective and bigger nodules were observed in the later treatment. Umali-Garcia et al. (1988) noticed that nodulation was best in unfertilised treatments in Paraserianthes falcataria and nodulation in the absence of nitrogen exceeded those fertilised .

In Erythrina suberosa, the nodule number per plant was found to decrease from 13.60 to 9.33 due to application of nitrogen at the rate of 20 kg ha^{-1} (Hussain et al. 1989).

In Leucaena, suppression in nodulation by application of nitrogen fertiliser was noticed by Cobbina et al. (1990).

According to Hussain et al. (1990), in Dalbergia sisso, nodule number reduced to 42 from 88 with 25 kg N ha^{-1} after six months. Sundaram et al. (1979) reported reduced nodulation with increased levels of nitrogen in bengal gram. Application of 15 to 60 kg N ha^{-1} significantly increased nodulation of Vigna radiata (Raju and Verma, 1984). Similarly, nitrogen application at the rate of 20 kg N ha^{-1} was also found to increase nodulation of pea (Srivastava and Verma, 1985).

Manguiat et al. (1987) observed that best nodulation was obtained with application of phosphorus up to 30 kg ha^{-1}. In Leucaena, an increase in the number of nodules from Six in the control to 10 was obtained where phosphorus was applied at the rate of 50 kg ha^{-1} (Hussain *et al.* 1988). In *Erythrina suberosa*, the number of nodules per plant increased from 13.60 to 26.67 by phosphorus application 50 kg ha^{-1} (Hussain *et al.* 1989).

Srivastava and Verma (1985) reported that increasing the phosphorus rate upto 60 Kg ha^{-1} markedly increased the nodulation in pigeon pea. According to Singh and Faroda (1986) the number of nodules per plant increased significantly with phosphorus application upto 40 kg ha^{-1} in pigeon pea.

In *Acacia mangium*, Umali-Garcia *et al.* (1988) reported 15.2 per cent increase in nodule dry weight over control by nitrogen application at the rate of 15 kg N ha^{-1}. They also reported an increase of 97.17 per cent in nodulation by application of 30 kg N ha^{-1} and beyond that they could not find increase in nodule dry weight in *Paraserianthes falcataria*.

In *Erythrina suberosa*, nodule dry weight per plant decreased from 0.20 to 0.18 g by nitrogen application at the rate of 20 kg ha^{-1} (Hussain *et al.*, 1989). In *Dalbergia sisso*, application of nitrogen upto 25 kg ha^{-1} decreased the nodule dry weight per plant from 0.20 to 0.10 g and thereafter there was an increase in the dry weight of nodules with nitrogen upto 150 kg ha^{-1} (Hussain *et al.* 1990).

Gunawardena and Senanayake (1989) observed increased nodule dry weight per plant when phosphorus was applied at the rate of 15 kg ha^{-1} in Leucaena. In Erythrina suberosa the nodule dry weight per plant was increased from 0.20 g to 0.40 g by application of phosphorus at the rate of 50 kg ha^{-1}. (Hussain et al. 1989). Nodule dry weight per plant had increased significantly with 90 kg ha^{-1} application in Arachis hypogea (Sankar et al. 1984).

According to Singh and Faroda (1986), the nodule dry weight per plant was found to increase with application of phosphorus upto 40 kg ha^{-1}, in pigeon pea.

According to Umali-Garcia et al. (1988), nitrogen application did not give any significant difference with respect to plant nitrogen content in Acacia mangium. They also found a significant increase in plant nitrogen content from 1.97 to 2.12 per cent by nitrogen application.

In Erythrina suberosa, nitrogen content of plants increased from 1.17 to 1.21 per cent by the application of nitrogen at the rate of 20 kg ha^{-1}. According to Cobbina et al. (1990) application of nitrogen increased the nitrogen content of Gliricidia and Leucaena.

Oganwale and Olaniyi (1978) reported a significant increase of phosphorus content for pigeon pea as a result of phosphorus fertilization. In *Leucaena*, Hussain *et al.* (1988) noticed an increased in phosphorus uptake from 1.14 to 1.73g. An increase in the phosphorus content from 1.17 per cent to 1.28 percent was observed by the application of 50kg ha^{-1} (Hussain *et al.* 1989).

The role of woody perennial legumes in agro-forestry have been described by various authors (Brewbaker and Hu, 1981; Nair, *et al.* 1984). The existence of dormancy and erratic germination of seeds remains as a major constraint in large scale multiplication of seedlings for agroforestry programmes in a single growing season. The limited research work done on the germination behaviour of the different tree species reveals the necessity for further studies especially with respect to Kerala conditions.

Investigations on identification of specific micro-organisms along with selection of suitable tree species, for better and quicker nodulation under Kerala conditions are also less debated.

Mineral nutrient deficiencies are a major constraint limiting legume nitrogen fixation and yield below their maximum potential. The review of literature of the earlier studies undertaken on above aspects, showed that the attempts to understand the nature of limitations imposed by mineral nutrient deficiencies on the legume-*Rhizobium* symbiosis especially under Kerala conditions are meagre.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

An investigation was carried out with the objective of determining the effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes. This investigation was carried out as three separate experiments, the details of which are given below.

Experiment I

To find out the effect of different scarification methods on the germination of seeds of some commonly grown and newly introduced tree legumes.

Experiment II

To screen out the best among the native and exotic rhizobial isolates along with different levels of phosphorus in selected tree legumes.

Experiment III

To study the effect of Rhizobium inoculation, and nutrient levels on nodulation and seedling growth of tree legumes.

The experiments were carried out using the laboratory and greenhouse facilities available in the College of Agriculture, Vellayani.

3.1. Materials

3.1.1. Seeds

Seeds used for the investigation were procured from Pratap nursery, Dehradun and from the Department of Social Forestry, Government of Kerala. Nine species of tree legumes were considered for Experiment -I, namely, Acacia catechu, Acacia arabica, Albizia moluccana, Paraserianthes falcataria, Cassia fistula, Acacia mangium, Cassia javanica and Sesbania grandiflora. Out of this, the following tree species were used for experiment II and III.

Acacia. arabica is a small, thorny, irregular leguminous tree of the South Asian plains with a variety of human uses viz., fire wood, timber, tannin, gum, fodder and thorn hedges. It grows on sites with annual rainfall between 100 to 950 mm and five to 11 months dry period. The plant tolerate daily temperature upto 45^{o}C (Plate 1).

Acacia catechu is the most indigenous acacia in Nepal and is grown in plantations in terai region of Northern Gangetic plain, for its multipurpose products. It grows well in altitudes ranging from 50 m to 2000 m and also in a wide range of climatic conditions (Plate 2).

Paraserianthes falcataria (previously known as Albizia falcataria) is a valuable multipurpose leguminous

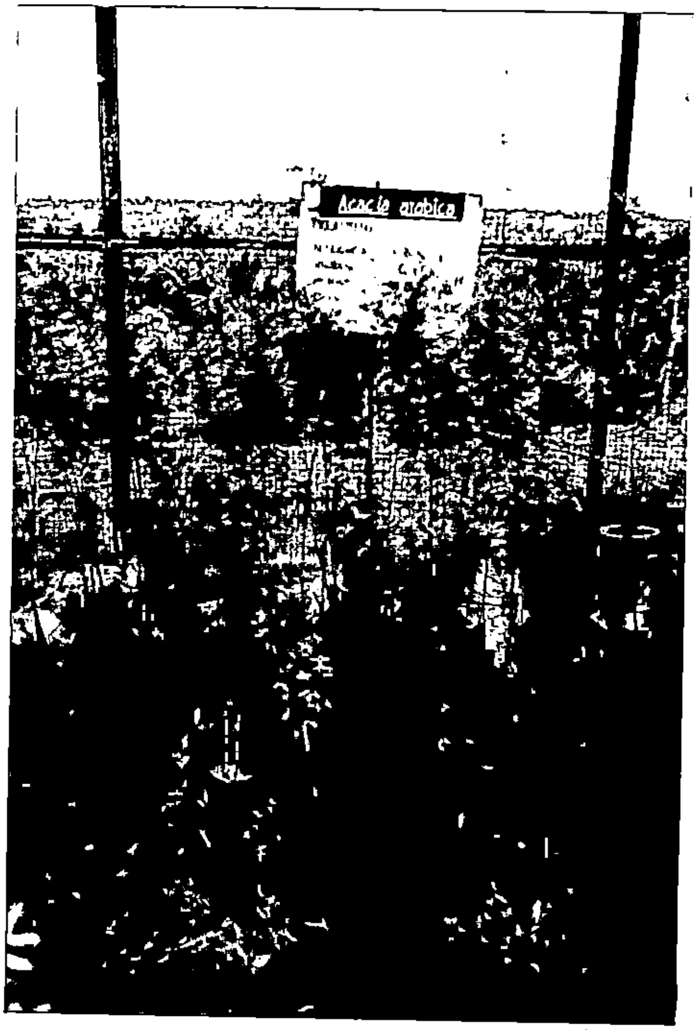

Plate 1: Acacia arabica at 180 DAS

Plate 2: Acacia catechu at 180 DAS

tree for the humid tropics. One of the fastest growing of all tree species, it is used for pulp and other wood products, fire wood, ornamental plantings and shade for coffee, tea and cattle. It naturally occurs in Indonesia, Papua New Guinea, and the Solomon islands from $10^{0}S$ to $30^{0}N$. It grows from sea level to 1200 m above MSL with an annual rainfall ranging from 2000 to 4000 mm, a dry season of less than two months, and a temperature range of $22^{0}C$ to $34^{0}C$ (plate 3).

3.1.2 Surface sterilizing agent

The seeds were surface sterilized by using 0.1 per cent mercuric chloride solution in Experiment I. Mercuric chloride and 70 per cent ethyl alcohol were used for isolation purposes in Experiment II.

3.1.3 Materials for scarifications

Analar quality con.HCl, con. HNO_3 and con. H_2SO_4 were used for acid scarification. Sand paper was used for mechanical scarification and water at $80^{0}C$ for hot water treatment. Cooled distilled water was used for presoaking the seeds in the control treatment.

3.1.4 Filter paper and petri dishes

Steam sterilized petridishes with moist filter paper inside were used for placing the scarified seeds.

Plate 3: <u>Paraserianthes falcataria</u> at 180 DAS

3.1.5 Potting mixture

The potting mixture used for the study consisted of soil, sand and dried cowdung in the ratio 1:1:1. Sterilized potting mixture was used for Experiment II.

3.1.6 Experimental site

Experiment I was carried out under laboratory conditions and the site chosen for Experiments II and III was a green house attached to the Department of Agronomy, in the College of Agriculture, Vellayani.

3.1.7 Season

Experiment I was carried out from August 1990 to September 1990. Experiment II was carried out from October 1990 to January 1991 and Experiment III from January to July 1991.

3.1.8 Weather conditions

The experiment site enjoys a tropical humid climate. Data on the maximum temperature, minimum temperature, rainfall and relative humidity during the experimental period were collected from the Meteorological Observatory at the College of Agriculture and is presented as monthly averages in Appendix I and Figure I.

On comparison, it was revealed that normal weather conditions prevailed during the experimental period.

3.1.9 Polybags

Black coloured polybags (500 guage) of size 30x20 cm were used for growing the seedlings.

3.1.10 Rhizobial inoculants

For Experiment II, native and exotic rhizobial isolates for Acacia sp. and Albizia sp. were used . For Experiment III, the best among the two isolates for each species were selected, based on Experiment II.

Native isolates were isolated from nodules of tree legumes, collected locally. Exotic isolates used for Acacia sp. was TAL 1868 and for Albizia sp. was TAL 45. Exotic isolates were obtained from NIFTAL Project, Hawaii (USA).

3.1.11 Fertilizers

Phosphorus as superphosphate analying 16 per cent P_2O_5 was used for Experiment II and III. In Experiment III urea analysing 46 per cent nitrogen was used.

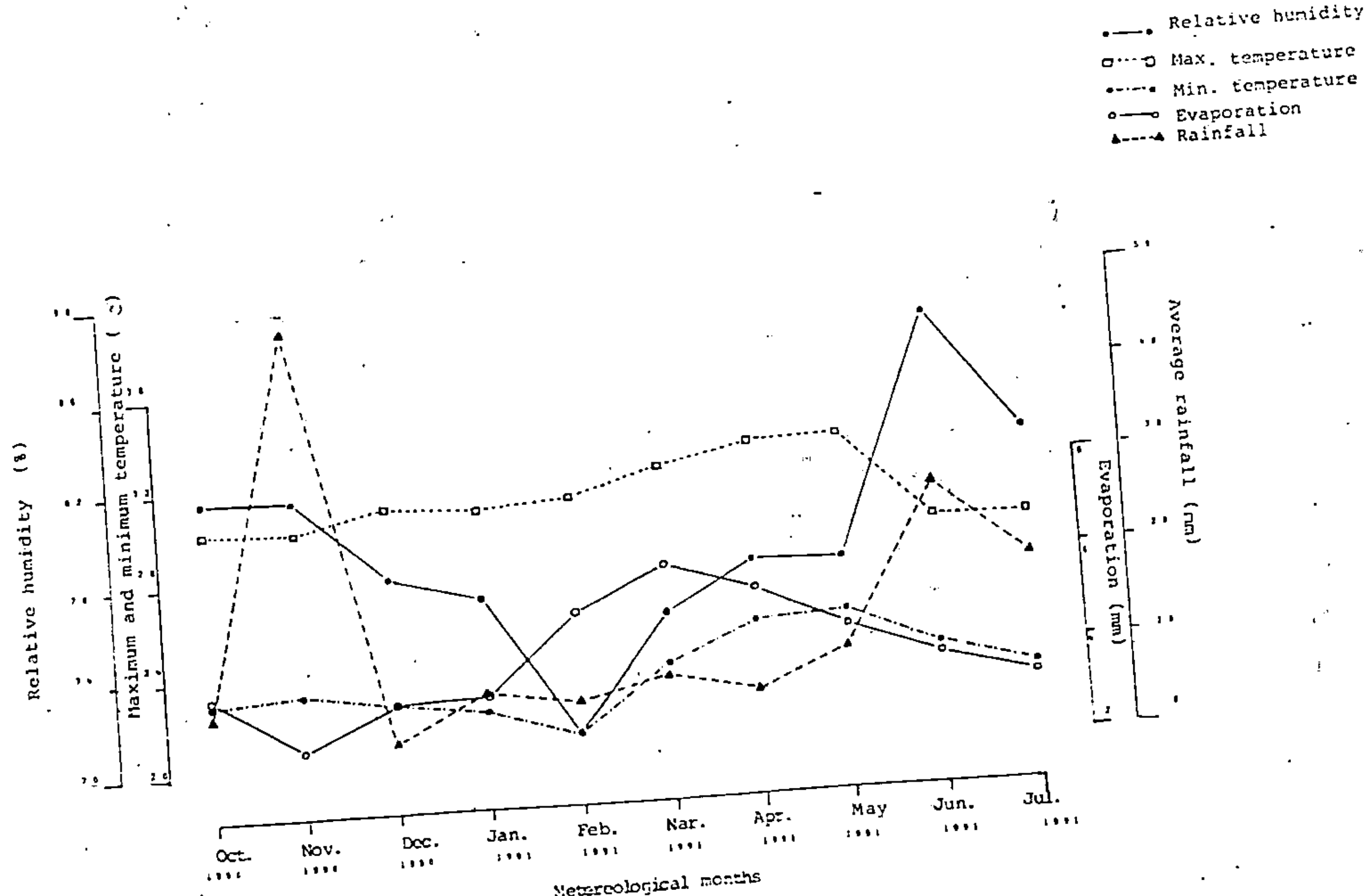

Fig. 1 Weather data during the cropping period.

3.2. Methods

3.2.1 Design and lay out

All the experiments were laid out in completely randomised design, Experiment I with five replications, Experiment II with three replications and Experiment III with four replications.

3.2.1.1 Experiment I

Design	- CRD
No. of treatments	- 15
Replications	- 5

Treatments

The treatments consisted of different scarification methods done in 9 species of tree legumes as given below.

Methods of scarification

s_1	con.HCl	1 minute
s_2	Do	5 minutes
s_3	Do	10 minutes
s_4	Do	15 minutes
s_5	con. HNO_3	1 minute
s_6	Do	5 minutes

S_7	Do	10 minutes
S_8	Do	15 minutes
S_9	con. H_2SO_4	1 minute
S_{10}	Do	5 minutes
S_{11}	Do	10 minutes
S_{12}	Do	15 minutes
S_{13}	Hot water treatment	3 minutes

S_{14} Mechanical scarification using sand paper

S_{15} Soaking in cold water 24 hrs. (Control)

Tree Species

Acacia arabica

Acacia catechu

Acacia mangium

Paraserianthes falcataria

Albizia lebbeck

Albizia moluccana

Cassia fistula

Cassia javanica

Sesbania grandiflora

3.2.1.2 Experiment II

Design - CRD

Number of treatments - 9

Number of replications - 3

Treatments

Treatments consisted of three levels of Rhizobium and three levels of phosphorus as detailed below.

Rhizobium Inoculation

r_0 – no inoculation

r_1 – inoculation with native Rhizobium isolate

r_2 – inoculation with exotic isolate.

Phosphorus levels

p_0 – 0 kg ha^{-1}

p_1 – 25 kg ha^{-1}

p_2 – 50 kg ha^{-1}

Treatment combinations

Treatment combinations are as follow:–

1 – r_0 p_0

2 – r_0 p_1

3 – r_0 p_2

4 – r_1 p_0

5 – r_1 p_1

6– r_1 p_2

7– r_2 p_0

8– r_2 p_1

9– r_2 p_2

Tree species

Acacia arabica

Acacia catechu

3.2.1.3. Experiment III

Design - CRD

Number of treatments - 18

Number of species - 3

Number of replications - 4

Treatments

Two levels of Rhizobium, three levels of nitrogen and three levels of phosphorus and their combinations were fixed as the treatments.

Inoculation	r_0	- no inoculation
	r_1	- inoculated with selected Rhizobium isolate.
Nitrogen	n_0	- 0 kg ha^{-1}
	n_1	- 20 kg ha^{-1}
	n_2	- 40 kg ha^{-1}
Phosphorus	p_0	- 0 kg ha^{-1}
	p_1	- 25 kg ha^{-1}
	p_2	- 50 kg ha^{-1}

Tree species

Acacia arabica

Acacia catechu

Paraserianthes falcataria

Treatment combinations

1	-	$r_0 n_0 p_0$	10	-	$r_{+} n_0 p_0$
2	-	$r_0 n_0 p_1$	11	-	$r_{+} n_0 p_1$
3	-	$r_0 n_0 p_2$	12	-	$r_{+} n_0 p_2$
4	-	$r_0 n_1 p_0$	13	-	$r_{+} n_1 p_0$
5	-	$r_0 n_1 p_1$	14	-	$r_{+} n_1 p_1$
6	-	$r_0 n_1 p_2$	15	-	$r_{+} n_1 p_2$
7	-	$r_0 n_2 p_0$	16	-	$r_{+} n_2 p_0$
8	-	$r_0 n_2 p_1$	17	-	$r_{+} n_2 p_1$
9	-	$r_0 n_2 p_2$	18	-	$r_{+} n_2 p_2$

Selection of seeds

Fully developed undamaged seeds were selected from seedlots for the experiments.

Cleaned seeds were surface sterilised with 0.1 per cent mercuric chloride for one minute and then rinsed with distilled water.

3.2.3 Methods of seed scarification

Acid scarification was done by soaking the seeds in acids, viz., con.HCl, con. HNO_3 and con. H_2SO_4 for one minute, five minutes, 10 minutes and 15 minutes respectively.

Mechanical scarification was done by rubbing the seeds against sand paper.

Hot water treatment was done by dipping the seeds in water at 80° C for one minute.

Soaking the seeds for 24 hours in distilled water served as control. The study was conducted at ambient temperature.

3.2.4 Preparation of Rhizobium isolates

For Experiment II, the native isolate of rhizobia was made by streak plate method of Vincent (1970) using yeast extract mannitol agar medium. Composition of yeast extract mannitol agar medium is given in Appendix II as given by Allen (1953).

Besides these, two exotic isolates of rhizobia were obtained from NIFTAL project, University of Hawaii. They were TAL 1868 for Acacia species and TAL 45 for Paraserianthes species.

The above isolates of rhizobia were tested for purity by gram staining (Vincent, 1970). Strains which were short to medium sized rods and gram negative were used for the study. Pure cultures of all these isolates were maintained on yeast extract mannitol agar slants at 4 ° C in a refrigerator.

3.2.5 Preparation of potting mixture

For Experiment II, the potting mixture was sterilised by autoclaving for two hr at 120° C.

Three-fourth of the polybags were filled with this sterilised mixture, each weighing 1.5 kg.

For Experiment III, the polybags were filled with non sterilised potting mixture, each weighing 3 kg.

3.2.6 Inoculation of *Rhizobium* isolates

For Experiment II, seeds of each species was inoculated with native isolates and exotic isolates and the best isolates selected for each species based on the performance of the Experiment II was used for Experiment III.

3.2.7 Fertilizer application

Phosphorus in the form of super phosphate was applied at the rate of 0,25 and 50 kg ha^{-1} in Experiments II and III.

In Experiment III, in addition to phosphorus, nitrogen was applied as urea at the rate of 0,20 and 40 kg ha^{-1}. The entire quantity of nitrogen and phosphorus was applied as basal dose at the time of sowing seeds.

3.2.8 Seeds and sowing

Bold seeds were selected for sowing. For Experiments II and III, the seeds were subjected to suitable pretreatment to enhance the germination based on the results of Experiment I. The soaked seeds of each species were inoculated with Rhizobium as per the treatments.

Two to three seeds were sown in all the bags. The bags were irrigated immediately after sowing.

3.2.9 Maintenance of the crop

In both the experiments, irrigation was given daily upto one week after sowing. Thereafter, irrigation was given on alternate days. Hand weeding was done for both the experiments as and when required.

3.2.10 Plant protection

No pests and diseases were observed in the polybag experiments and hence there was no necessity for plant protection operations during the experimental period.

3.2.11 Harvest

The plants were harvested at 90 DAS and at 180 DAS for Experiment II and III respectively.

3.3. Observations

In both the experiments, observations on growth characters of all the plants were taken from all the replications.

3.3.1 Growth characters

3.3.1.1 Height of plants

The height of the plants were measured from the base to the growing tip at 90 DAS for Experiment II and at 180 DAS for Experiment III and expressed as cm.

3.3.1.2 Number of root nodules per plant

The plants were carefully uprooted and the roots were washed carefully with water. The root nodule number was counted and expressed as mean value.

3.3.1.3 Dry weight of nodules per plant

The dry weight of nodules per plant was determined after drying the samples to a constant weight at 70°C in a hot air oven and expressed as g plant $^{-1}$.

3.3.1.4 Dry weight of roots

Dry weight of the below ground portion of each plant was taken after drying the samples to a constant weight at 70°C in a hot air oven and expressed as g plant^{-1}.

3.3.1.5 Dry weight of shoots

Each plant was dried separately and dry weight of the above ground portion of each plant was recorded and expressed as g plant $^{-1}$.

3.3.2 Chemical analysis

The plant samples collected from each polybag at 180 DAS in Experiment III were dried to constant weight in a hot air oven at 70° C, ground and passed through a 0.5 mm mesh in a Willey mill. The required quantity of samples were then weighed out accurately in an electronic balance, subjected to triacid extraction as given by Jackson (1973) and the nutrient contents were determined and expressed as percentage on dry weight basis.

3.3.2.1 Total nitrogen content of plant

Total nitrogen content in plant was estimated by modified microkjeldahl method as given by Jackson (1973).

3.3.2.2 Total phosphorus content of plant

Phosphorus content was estimated colorimetrically by Vanado-molybdo- phosphoric yellow color method (Jackson, 1973) and read in Klett Summerson photoelectric colorimeter.

3.3.3 Statistical analysis

In Experiment I, the germination studies were conducted twice and the average of the two observations were taken for statistical analysis. The data were analysed using angular transformation employing F test.

For Experiment II and III, the data relating to each character were analysed using the analysis of variance technique, as applied to completely randomised design. Whereever the effects were found to be significant, critical differences were calculated for effecting comparison among the mean.

RESULTS

CHAPTER 4

RESULTS

4.1 Experiment I

The results of the investigation on the effect of different scarification methods on the germination of seeds of various multipurpose leguminous tree species are given below.

4.1.1 Germination percentage

The data on the mean germination percentage are presented in Table 1 to 3.

The data revealed that highest germination was obtained with hot water treatment (s_{13}) in the case of <u>Acacia mangium</u> and lowest with treatments s_{14} and s_{18}. In the case of <u>Acacia arabica</u>, treatment with con.H_2SO_4 for five minutes (s_{10}) recorded high per cent germination compared to other treatments. However, <u>Acacia catechu</u> showed comparatively high per cent germination with all the treatments. The highest germination was obtained with con.H_2SO_4 treatment for five minutes (s_{10}) which was at par with hot water treatment.

The germination of <u>Albizia lebbeck</u> was maximum with treatment s_7. Treatments s_1 and s_2 also recorded high per cent germination. <u>Acacia moluccana</u> seeds had significantly higher germination with treatment s_9. The per

Table-1. Effect of different scarification methods on seed germination (%)

Treatments		*Acacia mangium*	*Acacia arabica*	*Acacia catech*
s_1 con. HCl	1 min.	21.39	29.96	91.23
s_2 ' '	5 min.	15.77	46.39	87.42
s_3 ' '	10 min.	13.79	37.99	89.40
s_4 ' '	15 min.	09.38	39.58	88.40
s_5 con. HNO_3	1 min.	15.77	45.99	89.83
s_6 ' '	5 min.	05.30	49.59	78.02
s_7 ' '	10 min.	10.35	58.41	75.61
s_8 ' '	15 min.	12.07	49.99	69.61
s_9 con. H_2SO_4	1 min.	39.19	42.15	91.12
s_{10} ' '	5 min.	35.19	69.84	99.41
s_{11} ' '	10 min.	29.99	17.94	80.01
s_{12} ' '	15 min.	11.18	11.15	00.00
s_{13} hot water treatment (3 min.)		74.38	42.73	98.99
s_{14} mechanical scarification		00.00	34.08	70.94
s_{15} soaking 24 hours in water		00.00	00.00	59.82
CD		07.47	04.52	03.61

CD at 5 % level.

Table-2 Effect of different scarification methods on seed germination(%)

Treatment		*Albizia lebbeck*	*Albizia moluccana*	*Paraserianthes falcataria*
S_1 con. HCl	1 min.	93.34	34.79	22.59
S_2 ' '	5 min.	90.02	26.77	24.34
S_3 ' '	10 min.	82.03	25.58	27.59
S_4 ' '	15 min.	87.24	45.99	15.16
S_5 con. HNO_3	1 min.	87.63	35.59	19.98
S_6 ' '	5 min.	82.04	19.57	29.98
S_7 ' '	20 min.	98.27	27.58	19.53
S_8 ' '	15 min.	93.23	33.57	23.97
S_9 con. H_2SO_4	1 min.	19.55	80.44	19.05
S_{10} ' '	5 min	34.96	77.57	14.68
S_{11} ' '	10 min.	08.86	70.21	19.39
S_{12} ' '	15 min.	08.68	05.77	10.40
S_{13} hot water treatment (3 min.)		09.54	72.33	74.61
S_{14} mechanical scarification		29.05	29.76	15.93
S_{15} soaking 24 hours in water		04.85	29.39	11.35
CD		04.38	02.79	03.46

CD at 5% level.

Table-3. Effect of different scarification methods on seed germination (%)

Treatment		*Cassia fistula*	*Cassia javanica*	*Sesbania grandiflo*
S_1 con. HCl	1 min.	70.03	26.18	33.59
S_2 ' '	5 min.	65.80	26.19	12.97
S_3 ' '	10 min.	47.59	21.98	19.98
S_4 ' '	15 min.	60.20	21.79	27.61
S_5 con. HNO_3	1 min.	31.18	27.99	29.17
S_6 ' '	5 min.	40.99	37.99	29.39
S_7 ' '	10 min.	43.99	27.56	31.19
S_8 ' '	15 min.	49.80	21.78	41.39
S_9 con. H_2SO_4	1 min.	35.47	36.41	25.99
S_{10} ' '	5 min.	19.57	70.14	45.41
S_{11} ' '	10 min.	22.99	32.39	30.59
S_{12} ' '	15 min.	27.39	28.99	16.99
S_{13} hot water treatment (3 min.)		09.76	00.00	68.55
S_{14} mehcanical scarification		00.00	33.96	44.92
S_{15} soaking in water	24 hour	00.00	00.00	31.78
CD		03.37	02.26	03.03

CD at 5% level.

cent germination under con. H_2SO_4 treated and hot water treated seeds were on par. In the case of <u>Paraserianthes falcataria</u>, high per cent germination was recorded with hot water treatment (s_{13}).

High per cent germination was observed in <u>Cassia fistula</u> with con.HCl treatment for one minute (s_1) and other treatments recorded comparatively lower values. The lowest value was recorded for mechanical scarification (s_{14}) and soaking for 24 hours (S_{18}). In the case of <u>Cassia javanica</u>, the different treatments gave lower values of germination. Among these, the highest value was recorded with treatment s_{10}, which was at par with treatment s_{14}. As compared to other treatments, in the case of <u>Sesbania grandiflora</u>, better germination was obtained with hot water treatment .

4.2 Experiment II

The results of the investigations to screen out the best among the native and exotic rhizobial isolates along with different levels of phosphorus in selected tree legumes are given below.

4.2.1 Height of plants

The data on mean plant height at 180 days after sowing are presented in Table 4.

Table-4. Effect of *Rhizobium* inoculation and phosphorus levels on plant height (cm)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	31.81	11.84	08.84
r_1	32.17	16.83	07.60
r_2	33.50	18.96	10.60
CD	NS	01.95	01.15
p_0	34.60	15.96	08.31
p_1	32.18	16.46	08.68
p_2	30.70	15.22	10.06
CD	NS	NS	01.15

CD at 5% level NS - Not significant

Inoculation with different isolates of *Rhizobium* had significant effect on plant height in all the tree species studied except *A. arabica*. In *A. catechu* and *P. falcataria* the exotic isolate (r_2) produced significant increase in plant height.

In *A. catechu*, r_0 produced significantly lower plant height compared to r_1 and r_2.

Phosphorus application had significant effect on plant height in *P. falcataria* only and highest plant height was resulted at p_2 level.

4.2.2 Dry weight of shoots

The data on the mean shoot dry weight of plants as affected by the treatments are presented in Table 5.

Rhizobium inoculation had significant influence on shoot dry weight of *A. catechu* and *P. falcataria*.

In *Acacia catechu*, r_2 produced significantly higher shoot dry weight followed by r_1 and r_0 which were on par.

Inoculation levels had no significant influence on shoot dry weight in *A. arabica* and *P. falcataria*.

Among the three species studied, phosphorus application increased shoot dry weight in the case of *A.*

Table-5. Effect of *Rhizobium* inoculation and phosphorus levels on shoot dry weight (g)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	0.41	0.12	0.28
r_1	0.37	0.18	0.23
r_2	0.43	0.27	0.41
CD	NS	0.06	NS
p_0	0.39	0.18	0.34
p_1	0.33	0.19	0.31
p_2	0.49	0.19	0.27
CD	0.09	NS	NS

CD at 5% level. NS - Not significant

arabica only. Here p_2 level recorded highest value, whereas effects of p_1 and p_0 were on par.

4.2.3 Dry weight of roots

The mean data on the root dry weight are presented in Table 6.

Inoculation levels had significant influence on the root dry weight in *A. arabica*, *A. catechu* and *P. falcataria*.

In *A. arabica*, r_2 produced highest shoot dry weight and was at par with r_1. Treatment r_0 recorded lowest root dry weight which was on par with r_1. *A. catechu* exhibited same pattern with the exception that r_0 recorded significantly lower value. But in *P. falcataria*, r_2 was found to be superior with r_1 which was on par with r_0.

Phosphorus levels had significant influence on the root dry weight of *P. falcataria*. Highest root dry weight was recorded by p_1 level in *P. falcataria* which was on par with p_2 and the effects of p_2 and p_0 were at par.

4.2.4 Number of root nodules per plant

The mean data on nodule number are presented in Table 7.

			falcataria
r_0	0.08	0.05	0.07
r_1	0.12	0.11	0.08
r_2	0.16	0.14	0.15
CD	0.05	0.04	0.03
p_0	0.11	0.11	0.09
p_1	0.12	0.08	0.11
p_2	0.13	0.11	0.10
CD	NS	NS	0.01

CD at 5% level. NS - Not significant

Table-7. Effect of *Rhizobium* inoculation and phosphorus levels on nodule number per plant.

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	0.00	1.00	1.44
r_1	1.22	1.33	2.78
r_2	2.00	2.11	15.00
CD	0.40	0.55	1.85
p_0	0.67	1.33	8.11
p_1	1.22	1.67	7.89
p_2	1.33	1.44	3.22
CD	0.40	NS	1.85

CD at 5% level.

NS - Not significant

Inoculation levels produced significant influence on nodule number in all the species. In *A.arabica*, r_2 increased nodule number followed by r_1 and r_0. In *A.catechu* and *P. falcataria*, treatment r_2 was found to be significantly superior compared to r_1 and r_0.

Significant influence on nodule number was exerted by phosphorus application in *A. arabica* and *P. falcataria*. In *A. arabica*, p_2 produced highest nodule number and was on par with p_1. A reduction in the nodule number with increasing levels of phosphorus was recorded in *P. falcataria*.

4.2.5 Dry weight of nodules per plant

The data on mean nodule dry weight of plants are presented in Table 8.

Nodule dry weight of plants was significantly influenced by levels of inoculation. In all the species, treatment r_2 was significantly superior to all others and r_0 recorded the lowest value. In *A. arabica* and *P. falcataria*, treatments r_1 and r_0 on par, whereas in *A. catechu* r_0 was significantly inferior to r_1.

Levels of phosphorus did not influenced the nodule dry weight of plants in all the three species.

Table-8. Effect of *Rhizobium* inoculation and phosphorus levels on nodule dry weight (g)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	0.000	0.001	0.000
r_1	0.000	0.018	0.000
r_2	0.014	0.037	0.040
CD	0.007	0.004	0.010
p_0	0.001	0.020	0.010
p_1	0.003	0.017	0.020
p_2	0.009	0.180	0.010
CD	NS	NS	NS

CD at 5% level. NS - Not significant

4.3 Experiment III

The results on the effect of Rhizobium inoculation, nitrogen and phosphorus application on seedling growth and nodulation in A. arabica , A. catechu and P. falcataria are given below.

4.3.1 Growth characters

4.3.1.1 Plant height

The data on the mean height of plants as affected by the treatments are presented in Table 9.

Rhizobium inoculation had significant effect on plant height for all the species except, A. arabica.

Nitrogen application had significant effect in plant height in A. arabica. Significantly higher plant height was recorded by n_1 and the effects of n_0 and n_2 were on par. In A. catechu, n_1 had significantly increased plant height followed by n_2 and n_0. Nitrogen application did not have significant effect with respect to this parameter in the case of P. falcataria. Application of different levels of phosphorus also had influenced plant height significantly in A.catechu. Treatment p_1 recorded highest value for plant height and it was at par with p_2 and treatment p_0 recorded the lowest value.

Table-9. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on plant height (cm).

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	129.63	119.95	69.72
r_+	132.28	125.44	82.32
CD	NS	003.25	10.61
n_0	121.88	109.75	75.25
n_1	154.88	131.94	79.96
n_2	126.13	126.42	72.85
CD	011.49	003.99	NS
p_0	138.75	117.68	72.08
p_1	125.50	126.75	72.55
p_2	132.67	123.67	83.44
CD	NS	003.99	NS

CD at 5% level   NS - Not significant

4.3.1.2 Dry weight of shoots

The mean table on shoot dry weight is given in Table 10 and Fig.2.

Inoculation did not have significant effect with respect to this parameter in all the three species.

Nitrogen application had significantly influenced the shoot dry weight of plants. In _A. arabica_, a reduction in shoot dry weight was noticed with increasing levels of nitrogen application, maximum with n_0 level and minimum with n_2 level. In the case of _P. falcataria_, n_1 level had produced maximum shoot dry weight, which was on par with n_0 level and n_2 recorded the lowest shoot dry weight.

Phosphorus application had exerted significant effect in shoot dry weight in the case of _A. catechu_. However, the pattern of influence was not similar. In _A. catechu_, p_2 level produced maximum shoot dry weight which was on par with p_1.

4.3.1.3 Dry weight of roots

The mean data on root dry weight are given in Table 11. The effect of inoculation was found to be significant except in _A. catechu_. In the other two species, inoculation (r_+) recorded higher root dry weight. Nitrogen application

Table-10. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on shoot dry weight (g)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	37.25	28.06	19.56
r_+	37.68	28.82	22.74
CD	NS	NS	NS
n_0	41.15	27.49	22.58
n_1	38.95	31.63	24.75
n_2	32.29	26.19	16.10
CD	02.64	01.42	04.25
p_0	37.92	26.35	21.69
p_1	36.28	29.08	21.10
p_2	38.19	29.88	20.65
CD	NS	01.42	NS

CD at 5% level. NS - Not significant

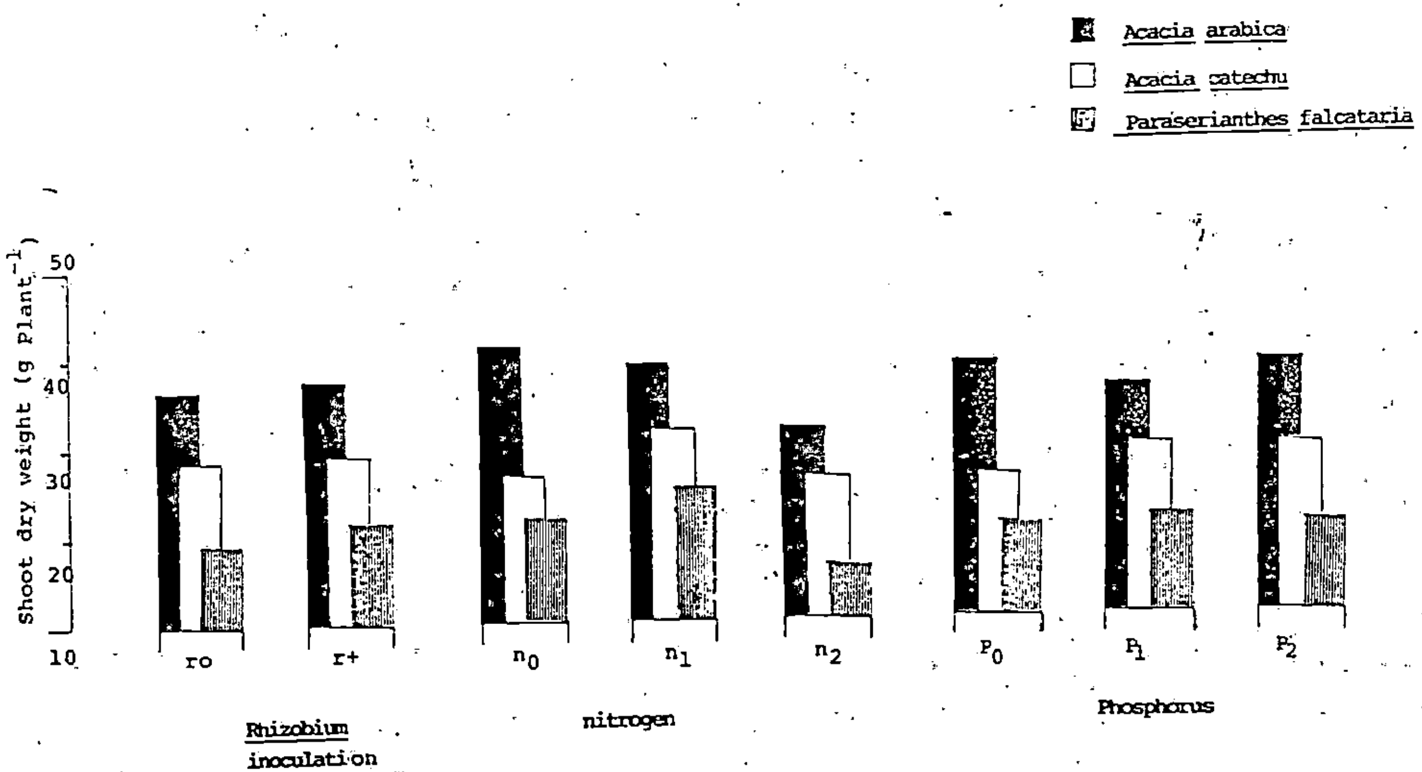

Fig.2 Effect of Rhizobium inoculation,nitrogen and phosphorus levels on shoot dry weight.

Table-11. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on root dry weight (g)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	5.15	19.06	11.05
r_+	6.73	19.78	12.91
CD	0.71	NS	01.57
n_0	5.60	18.04	12.01
n_1	6.08	20.89	13.75
n_2	6.14	19.33	10.16
CD	NS	01.37	01.92
p_0	4.75	18.15	12.29
p_1	7.18	21.31	12.17
p_2	5.89	18.81	12.47
CD	0.88	01.37	NS

CD at 5% level. NS - Not significant

had significant influence on root dry weight in all species except *A. arabica*. Maximum root dry weight was recorded at n_1 level, in *A. catechu* and *P. falcataria*.

Phosphorus application had significant effect on root dry weight on all the three species. Highest value was recorded by p_1 level in *A. arabica* followed by p_2 and p_0 which were on par. In *A. catechu*, the level p_0 resulted in lowest root dry weight with p_1 producing significantly higher value.

4.3.2 Nodule characters

4.3.2.1 Number of nodules per plant

The mean data on the nodule number per plant are presented in Table 12 and Fig.3.

Data revealed that inoculation had significantly influenced the nodule number per plant.

Nitrogen application had not influenced this parameter in all the three species, maximum number of nodules being produced at the lowest nitrogen level, which was significantly higher than n_1 and n_2 level.

In *A.arabica* and *A.catechu*, n_0 was significantly superior to n_1 and n_2. In *P. falcataria* n_0 and n_1 were on par and n_2 recorded the lowest value.

Table-12. **Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on nodule number per plant**

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	06.75	02.64	186.69
r_+	15.91	08.78	244.64
CD	02.45	00.91	25.92
n_0	16.25	11.42	253.33
n_1	10.91	09.46	241.13
n_2	06.87	03.25	152.54
CD	03.00	01.12	31.75
p_0	08.92	02.79	233.96
p_1	12.83	09.08	221.79
p_2	12.29	07.25	191.25
CD	03.00	01.12	31.75

CD at 5% level. NS - Not significant

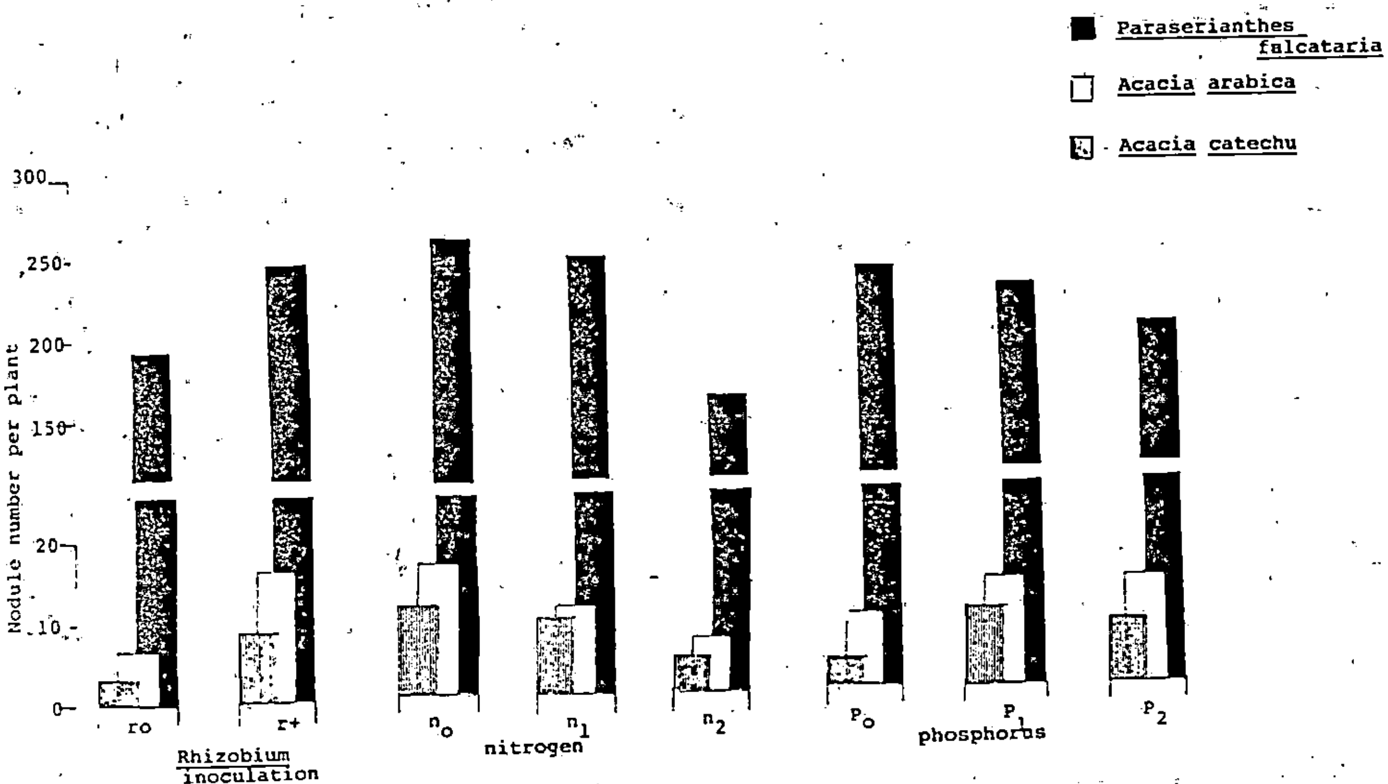

Fig. 3 Effect of Rhizobium inoculation, nitrogen and phosphorus levels on nodule number.

The number of nodules per plant varied significantly in all the species with phosphorus application. In *A. arabica*, p_1 recorded highest value which was on par with p_2. In *A. catechu*, p_1 level produced significantly higher number of nodules per plant, followed by p_2 and p_0. In *P. falcataria* number of nodules per plant decreased significantly with increase in phosphorus levels, p_0 level recording maximum value which was at par with p_1.

4.3.2.2 Dry weight of nodules per plant

The data on the mean nodule dry weight per plant are presented in Table 13 .

With respect to *Rhizobium* inoculation, significant increase in nodule dry weight was noticed due to inoculation in all the species.

Nitrogen application had significant effect on nodule dry weight in all the species. In *A. arabica*, n_1 recorded highest nodule dry weight which was on par with n_0, followed by n_2. In *A. catechu*, effect of n_1 was significantly superior with respect to this parameter followed by n_0 and n_2. Nitrogen levels did not exert any influence on nodule dry weight. In *P. falcataria* , n_1 level recorded maximum dry weight which was on par with n_2.

Table-13. Effect of <u>Rhizobium</u> inoculation, nitrogen and phosphorus levels on nodule dry weight (g)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	0.29	0.01	07.72
r_+	0.36	0.26	10.69
CD	0.05	0.08	01.20
n_0	0.39	0.12	08.09
n_1	0.40	0.28	09.92
n_2	0.16	0.01	09.61
CD	0.06	0.01	01.47
p_0	0.40	0.01	08.08
p_1	0.25	0.26	10.65
p_2	0.31	0.13	08.89
CD	0.06	0.01	01.47

CD at 5% level.

Phosphorus application had exerted profound influence on nodule dry weight. In *A. arabica*, p_1 recorded significantly superior value with respect to this parameter. Treatments p_0 and p_2 were found to be on par. Highest nodule dry weight was recorded at p_1 level in *A.catechu* and p_0 recorded lowest value. It was also found that in *P.falcataria*, p_1 level produced highest nodule dry weight and effects of p_2 and p_0 were on par.

4.3.3 Analysis of plant samples

4.3.3.1 Total nitrogen content of plant

The data on total nitrogen content of plants are given in Table 14.

Inoculation was found to have a significant influence on this parameter, with r_+ recording higher value.

Nitrogen application had significant influence on the total nitrogen content of plants. In *A. arabica* and *A. catechu*, significant increase in nitrogen content was seen at n_1 level. In *P. falcataria*, highest value was recorded at n_1 level which was on par with n_0.

Phosphorus application had significantly influenced the nitrogen content, with p_1 recording significantly higher value.

Table-14. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on plant nitrogen content (%)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	1.47	0.86	1.18
r_1	1.62	1.08	1.69
CD	0.08	0.09	0.06
n_0	1.33	0.93	1.45
n_1	1.48	1.13	1.51
n_2	1.23	0.86	1.35
CD	0.10	0.12	0.07
p_0	1.22	0.89	1.24
p_1	1.42	1.03	1.80
p_2	1.33	1.01	1.27
CD	0.10	0.12	0.07

CD at 5% level.

4.3.3.2 Total phosphorus content of plant

The mean data on the total phosphorus content of the plants are presented in Table 15.

Different levels of inoculation did not influence the P content in all the species under study.

Nitrogen application significantly influenced the total phosphorus content of the different species. In *A. arabica*, as the nitrogen level increased from n_0 to n_1, the total phosphorus content of the plant also increased . Effects of n_1 and n_2 were on par. In *P. falcataria*, n_1 recorded highest value which was on par with n_0. In *A. catechu*, effect of nitrogen application with respect to this parameter was not statistically significant.

Phosphorus application exerted significant influence on the total phosphorus content of plants. In *A. arabica* and *A. catechu*, p_2 recorded significantly higher values. In *A. arabica* effects of p_0 and p_1 were at par. In *A. catechu* effects of p_1 and p_0 were on par. In *P. falcataria*, phosphorus application did not have significant effect on phosphorus content .

Table-15. Effect of *Rhizobium* inoculation, nitrogen and phosphorus levels on plant phosphorus content (%)

Treatments	*Acacia arabica*	*Acacia catechu*	*Paraserianthes falcataria*
r_0	0.161	0.153	0.146
r_+	0.160	0.157	0.147
CD	NS	NS	NS
n_0	0.154	0.149	0.148
n_1	0.167	0.152	0.149
n_2	0.161	0.156	0.141
CD	0.006	NS	0.005
p_0	0.153	0.157	0.146
p_1	0.152	0.161	0.148
p_2	0.178	0.169	0.145
CD	0.006	0.006	NS

CD at 5% level. NS - Not significant

DISCUSSION

CHAPTER 5

DISCUSSION

5.1. EXPERIMENT I

An experiment was undertaken to determine the effect of different scarification methods on the germination of various tree legumes. The results obtained are discussed below.

5.1.1. Germination percentage

The data depicted in Table 1, 2 and 3 in general revealed significant increase in germination percentage by different scarification methods.

In *Acacia mangium*, high per cent germination (74.38) was recorded with hot water treatment for three minutes. However, germination was zero with mechanical scarification and soaking for 24 hours in water. Acid scarification was also found to be less effective in this species, with values ranging from 5.30 per cent for treatment with con. HNO_3 for five minutes to 39.19 per cent for treatment with con. H_2SO_4 for one minute.

In the case of *Acacia arabica*, treatment with con. H_2SO_4 for five minutes recorded high per cent germination of 69.84 compared to other treatments. The lowest values of zero and 11.15 per cent were obtained with soaking for 24 hours and treatment with con.H_2SO_4 for 15 minutes respectively.

Scarification with con.H_2SO_4 for five minutes and hot water treatment for three minutes recorded high per cent germination in Acacia catechu with values of 99.4 per cent and 98.99 per cent respectively. The lowest value of zero was obtained with con.H_2SO_4 treatment for 15 minutes.

In Albizia lebbeck, scarification with con.HNO_3 for 10 minutes recorded maximum germination per cent of 98.27. Treatments with con.H_2SO_4 for 10 minutes and five minutes recorded only 8.86 per cent and 8.68 per cent respectively. The lowest value of 4.85 per cent was obtained with soaking the seeds in water for 24 hours.

Scarification with con.H_2SO_4 for one minute recorded 80.44 per cent germination in Albizia moluccana and soaking for 15 minutes recorded lowest germination percentage of 5.77 . Hot water treatment for three minutes recorded 72.33 per cent germination.

In the case of Paraserianthes falcataria, the germination per cent was highest (74.61) with hot water treatment and lowest (10.40) with con.H_2SO_4 treatment for 15 minutes.

In *Cassia fistula*, the highest germination per cent of 70.03 was recorded for scarification with con.HCl for one minute. Germination percentage was zero with mechanical scarification and soaking for 24 hours.

In *Cassia javanica*, the germination per cent was highest (70.14) with con.H_2SO_4 for one minute. Zero value was obtained for hot water treatment and soaking for 24 hours.

In *Sesbania grandiflora*, the germination per cent was highest (68.55) with hot water treatment for three minutes and lowest (12.97) with con.HCl scarification for five minutes.

Among the nine species studied, the highest germination percentage of 99.41 and 98.27 were obtained for *A. catechu* and *A. lebbeck* respectively. The highest germination percentage of *A. mangium*, *A. arabica*, *A. moluccana*, *P. falcataria*, *C. fistula*, *C. javanica* and *S. grandiflora* were 74.38, 69.84, 80.44, 74.61, 70.03, 70.14 and 68.55 respectively.

Among the different scarification methods employed, hot water treatment for three minutes proved to be effective in bringing more than 50 per cent germination in all the species except *A. arabica*, *A. lebbeck*, *C. fistula* and C. javanica. Acid scarification with con.H_2SO_4 for varying durations were found to be effective in A. arabica, A. catechu, A. moluccana, and C. javanica. Acid scarification with con.HCl significantly improved the germination percentage in C. fistula only. Further scarification with con.HNO_3 improved germination percentage from 4.85 to 98.27 in A. lebbeck. More than 80 per cent germination could be achieved by acid scarification with con.HCl in A. lebbeck and C. fistula for varying durations.

Scarification with mechanical means such as sand paper and soaking the seeds in water for 24 hours promoted more than 50 per cent germinationin A. catechu .

It is presumed that like many of the legumes the tree species under the present study also are found to invariably possess hard and impermeable seed coats which act as a mechanical barrier to entry of water and gaseous exchange and thus do not permit the protrusion of the radicle out of the seedcoat, if at all imbibition occurs.

Promotion of germination beyond 50 per cent in A. mangium, A. catechu, A. moluccana, P. falcataria and S. grandiflora may probably be due to softening and dissolving of waxes that may be present on the seed coat. It may be expected that the waxy material could be dissolved with hot water treatment . Similar results of increased germination with hot water treatment were also reported in C. occidentalis (Anitakumari and Kohli, 1984); in Acacia maeonochieona and Acacia ampliceps (Bhatnagar et al. 1988); Acacia coriaceae. (Gunn, 1989); Acacia holosericea (Marunda, 1989); and in Acacia spp. (Khajuria and Singh, 1990).

The fact that pretreatment of seeds with con.H_2SO_4 for one minute resulted in above 80 per cent germination in A. moluccana and above 65 per cent with five minutes in A. arabica, A. catechu and C. javanica, indicated the predominant effect of HCl treatment to dissolve the waxy coating of the seeds and thereby reducing the seed coat resistance and enabling the penetration of water through the testa. The use of H_2SO_4 for softening the hard seed coat and also as an efficient chemical for breaking the seed dormancy had already been reported in Acacia cyanophylla (Shaybany and Roughani, 1976); Sesbania exaltata (Johnston et al. 1979); Acacia farnesiana (Gill et al. 1986); Acacia planifrons (Natarajan and Vinaya rai, 1988); and in Acacia holosericea (Sivasubramaniam et al. 1991). Decrease in germination percentage with an increase in the duration of soaking in acids observed in the present study may probably be due to the damaging of the tissues by contact and direct action of the chemical with seed coat and acid. Similar results of reduced germination percentage of leguminous tree species had also be been reported by Some *et al* (1989).

Similarly, germination percentage of 70 was recorded, where the seeds were treated with con.HNO_3 for 10 minutes in the case of *A. lebbeck* and with con.HCl treatment for one minute in the case of *C. fistula* . Similar results of improved germination of seeds with con.HNO_3 were also reported in *Acacia farnesiana* (Gill *et al*. 1986).

Mechanical scarification and soaking for 24 hours enchanced germination above 50 per cent in *A. catechu*. This indicated that the hard seed coat in *A. catechu* will not permit water uptake and gaseous exchange which could be overcome by mechanical means such as softening by surface scratching or presoaking method. The result is in conformity with the findings of Anitakumari and Kohli, (1984) and Gill *et al* (1986).

5.2 EXPERIMENT II

The results of the experiment conducted to screen out the best among the native and exotic rhizobial isolates along with different levels of phosphorus in selected tree legumes are discussed below.

5.2.1 Growth characters

5.2.1.1 Plant height, shoot dry weight and root dry weight

An appraisal of the data depicted in Table 4 revealed significant variations in growth parameters by inoculation with different strains of Rhizobium. However, the influence of phosphorus on growth characters was much less as compared to different strains of Rhizobium.

Types of Rhizobium had significant influence on plant height in all the species except Acacia arabica. In Acacia catechu and Paraserianthes falcataria, inoculation with exotic isolates TAL 1868 and TAL 45 produced highest plant heights of 18.96 cm and 10.60 cm respectively.

Different phosphorus levels had significant effect only in P. falcataria, in which plant height showed an increasing trend with increasing levels of phosphorus.

Shoot dry weight was not much influenced by different treatments as evident from Table 5.

Exotic Rhizobium isolate TAL 1868 produced maximum shoot dry weight in A. catechu, which increased shoot dry weight from 0.12 to 0.27 g $plant^{-1}$. Phosphorus levels had significant effect only in A. arabica, wherein application of phosphorus upto 50 kg ha^{-1} increased the shoot dry weight from 0.39 to 0.49 g $plant^{-1}$.

Results of the study showed that exotic isolate produced maximum root dry weight as revealed from Table 6. Lowest root dry weight has been recorded in all the species with no inoculation. It could be seen that the increase in root dry weight was from 0.08 to 0.16 g plant^{-1} in A. arabica and from 0.05 to 0.14 g plant^{-1} in A. catechu. Exotic isolate increased the root dry weight from 0.07 to 0.15 g plant^{-1} in P. falcataria.

Phosphorus treatments exhibited significant variation with respect to this parameter in P. falcataria. The resultant root dry weight by phosphorus application upto 50 Kg ha^{-1} (0.10 g plant $^{-1}$) and upto 25 kg ha^{-1} (0.11 g plant^{-1}) were on par.

5.2.2 Nodule characters

The data on the nodule number are depicted in Table 7.

An appraisal of the data indicated that the exotic isolate was effective in producing higher nodule number per plant in all the species. Lowest nodule number per plant was observed where there was no inoculation.

Phosphorus application had a significant positive influence on nodule number per plant in A. arabica. With increase in phosphorus levels upto 50 kg ha^{-1} the nodule number per plant increased from 0.67 to 1.33 in A. arabica.

With increasing levels of phosphorus, a decrease in nodule number per plant, ie., from 8.11 to 3.22 was observed in P. falcataria. Phosphorus application had not significantly affected nodule number per plant in A. catechu.

Scrutiny of Table 8 revealed significant increase on nodule dry weight by inoculation with exotic isolate in all the species. In A. arabica, the increase was from zero to 0.01 g plant $^{-1}$. Nodule dry weight per plant increased from zero to 0.04 g plant^{-1} in A. catechu and from zero to 0.04 g plant $^{-1}$ in P. falcataria. In all species, lowest nodule dry weight was observed in the control . Phosphorus had no significant effect in all the species with respect to this parameter.

Selection of indigenous and exotic isolates was done to compare the relative efficiency of both types of strains for nodulation and other beneficial characters in the tree legumes studied. In general, the results suggest that inoculation with suitable specific Rhizobium strain is to be adopted for better and effective performance of these species with respect to growth and nodulation.

It could be further noted that growth and nodulation was increased by inoculation with exotic isolates in A. arabica, A. catechu and P. falcataria as compared to

Acacia arabica

1 cm 1 cm

Fig.4 Rooting pattern and nodulation behaviour of Acacia arabica at 180 DAS.

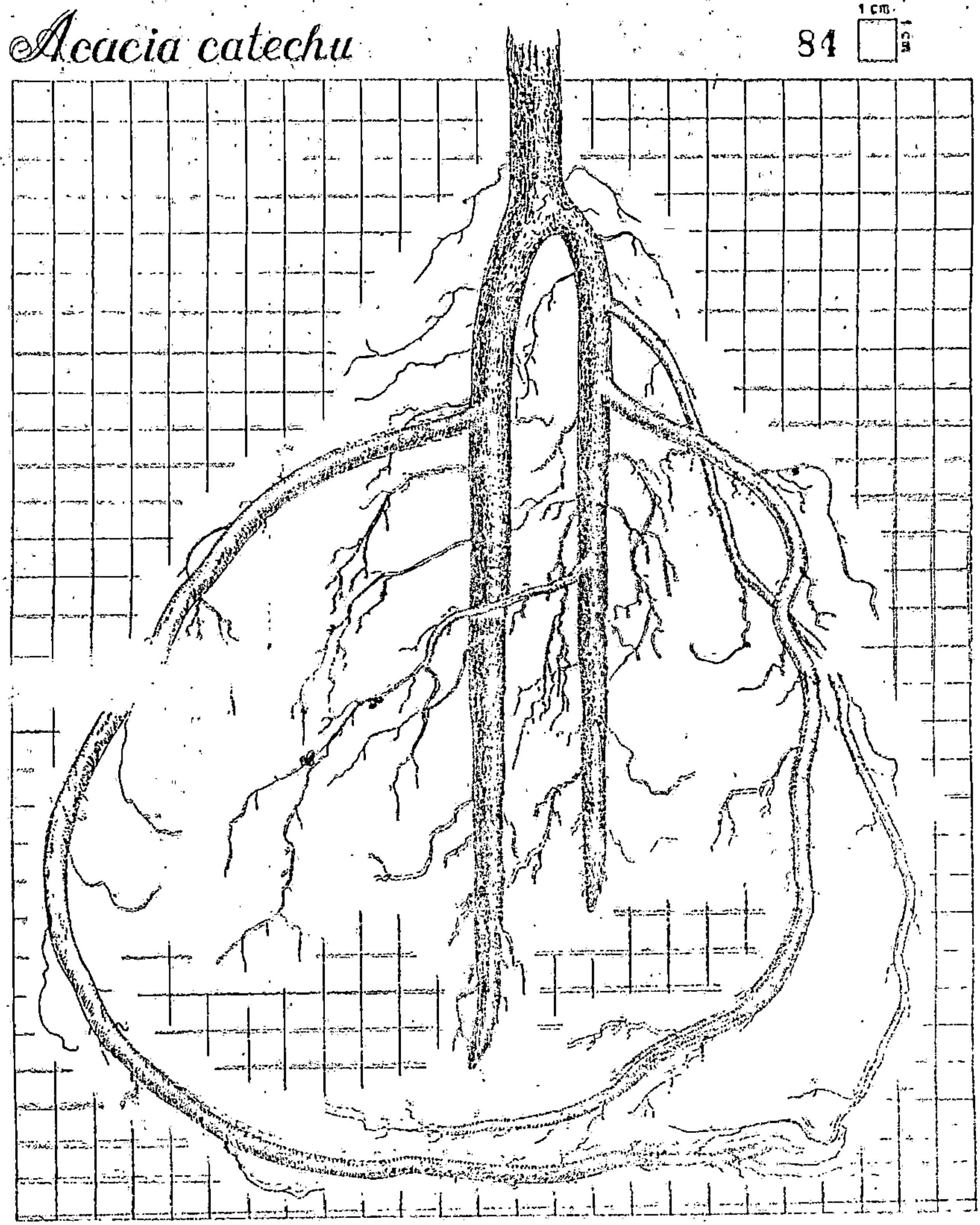

Fig.5 Rooting pattern and nodulation behaviour of Acacia catechu at 180 DAS.

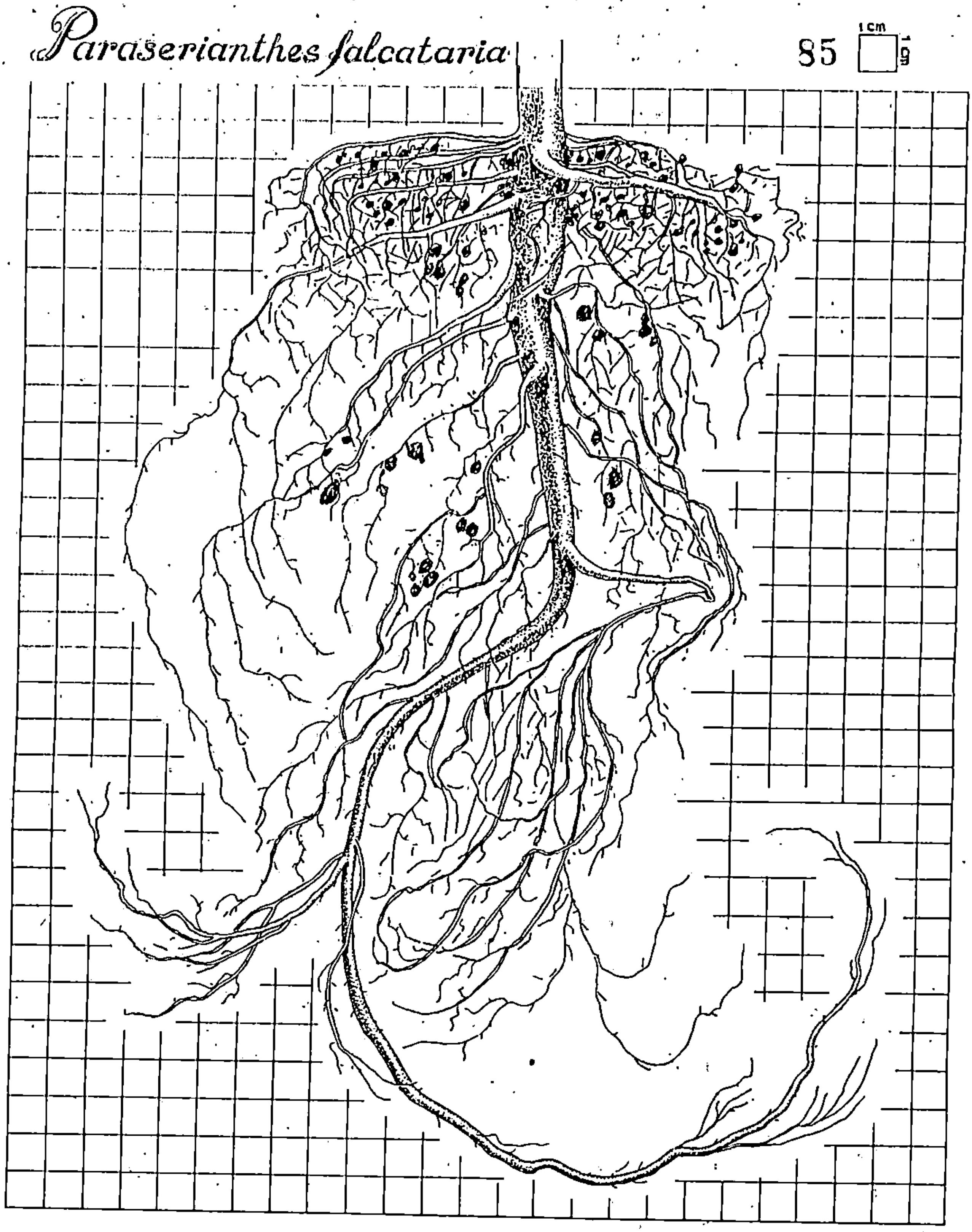

Fig.6 Rooting pattern and nodulation behaviour of <u>Paraserianthes falcataria</u> at 180 DAS.

native isolates. An increase in growth and nodulation with treatment of exotic strains of *Rhizobium* was reported in *Leucaena* (Gunawardena and Pushpakumari, 1988), in *Acacia nilotica*. (Balaji and Rengarajan, 1987) and in *Acacia mangium* and *P. falcataria* (Umali-Garcia *et al*. 1988). Similar results were also reported in trials undertaken with different isolates of rhizobia by Subba Rao *et al*.(1974), Ramachandran (1979) and Dean *et al*. (1980).

Phosphorus is considered to be essential for increased root growth which in turn helps in better absorption of other nutrients by plants. This leads to better plant growth (Gauch, 1957; Ohlrogge, 1962 and Tisdale *et al*. 1985). Increase in root dry weight and plant height in the different species observed in the present study indicates the beneficial effects of phosphorus application on plant growth.

However, recent studies indicate that rhizobia should be able to grow well at the phosphorus concentrations commonly found in soil solutions (Cassman *et al*. 1981 Robson and Pitman 1983; Smart *et al*. 1985) . According to them additional supply of phosphorus may also enhance the rhizobial activity and general growth of plants.

In this connection, it is also worth mentioning here that work carried out in Soybean revealed that phosphorus requirements for its nodulation and maximum nodule activity were much higher than plant growth (Edwards, 1975). For enhancing rhizobial activity and nodulation higher levels of phosphorus application was essential (de Mooy and Pesek, 1966). This was also supported by the finding of Luse et al. (1975) in which higher levels of phosphorus application was found to increase nodule number, nodule activity and seed yield in cowpea.

Strains of rhizobia explore in differing ways to obtain mineral nutrients from the environment for their growth and development (O'Hara et al. 1988). The absorption of nutrients from soil by plants also depends upon the inherent capacity of the plants to respond to varying levels of nutrients and the form in which it is available to the plants. The observed variation in nodulation characters in each species with respect to phosphorus application in the present study could be attributed to the above facts. This is further established by difference in rooting pattern exhibited by the species of plants (Fig 4,5 and 6).

5.3 EXPERIMENT III

This experiment was undertaken to determine the effect of Rhizobium inoculation, nitrogen and phosphorus levels on nodulation and seedling growth in tree legumes. The results of the study are discussed here under.

5.3.1 Growth characters

An appraisal of the data in Table 9 indicates that Rhizobium inoculation did not have significant influence on plant height in A. arabica. In all other species, inoculation had a positive influence on plant height.

However, nitrogen application had significant influence in enhancing plant height. Maximum height of 154.88 cm was recorded with nitrogen at 20 kg ha^{-1} and as the nitrogen level increased to 40 kg ha^{-1} the plant height was 126.13 cm in A. arabica. An increase in plant height from 109.75 cm to 131.94 cm was recorded when nitrogen application increased from 0 - 20 kg ha^{-1} in the case of A. catechu. Nitrogen application did not significantly influence this parameter in P. falcataria. This lack of response may be due to genetic character of the species to respond to higher doses of nitrogen even though the rate of production of nodules are higher (Table 12).

Phosphorus application did not have much influence with respect to this parameter in A. arabica and P. falcataria. Phosphorus application upto 25 kg ha^{-1} resulted in highest plant height of 126.75 cm in A. catechu.

The data presented in Table 10 and Fig. 1 revealed that <u>Rhizobium</u> inoculation had not influenced the shoot dry weight of all the three species. However, a marginal increase in shoot dry weight was observed.

Nitrogen application had a significant influence with respect to this parameter for all the species. In <u>A. arabica</u>, the influence of zero nitrogen and nitrogen application at 20 kg ha^{-1} were on par with respect to this parameter. Application of nitrogen upto 20 kg ha^{-1} resulted in highest shoot dry weight in <u>A. catechu</u> (31.63 g) and <u>P. falcataria</u> (24.75 g).

Phosphorus application had significant influence on shoot dry weight of <u>A. catechu</u> and phosphorus application upto 50 kg ha^{-1} increased the shoot dry weight from 26.35 to 29.88 g $plant^{-1}$.

The data from Table 11 revealed that <u>Rhizobium</u> inoculation significantly increased root dry weight in <u>A. arabica</u> from 5.15 to 6.73 g $plant^{-1}$. Inoculation had significant influence on root dry weight in <u>P. falcataria</u> and it increased from 11.05 to 12.91 g $plant^{-1}$. However, in the case of <u>A. catechu</u> inoculation had not influenced this parameter.

Nitrogen application did not significantly increase root dry weight in A. arabica. The increase in root dry weight was from 18.04 to 20.89 g plant $^{-1}$ in A. catechu and 12.01 to 13.75 g plant $^{-1}$ in P. falcataria when nitrogen level was increased from 0 to 20 kg ha^{-1}. Nitrogen application upto 40 kg ha $^{-1}$ decreased this parameter in both the species.

With increasing levels of phosphorus application upto 25 kg ha $^{-1}$, there was an increase in root dry weight in A. arabica from 4.75 to 7.18 g plant $^{-1}$, whereas the root dry weight per plant increased in A. catechu from 18.15 to 21.31 g. However, phosphorus application had not significantly influenced the root dry weight in P. falcataria.

According to Barnett (1986) the nursery growth of legume tree species can be significantly increased by Rhizobium inoculation.It may be presumed that in the present study selection of suitable rhizobial strains, its inoculation combined with nutrients would have helped to activate the bacterial strains and subsequently nodulation and this would have resulted in better performance of the tree species . Reports of similar increase of growth with Rhizobium inoculation visa-a-vis increased growth of plants were reported in other species like Acacia nilotica (Balaji and Rangarajan, 1987), Acacia mangium and P. falcataria (Umali -Garcia et al. 1988) and Leucaena leucocephala (Mohammed, 1988; Jiang et al. 1991).

Nitrogen application was observed to increase the growth characters favourably in the present study in the case of A. catechu and A. arabica. In this connection, it is worth mentioning here the role of nitrogen, as an essential element, necessary for plant growth and development.Small amounts of nitrogen added during the nitrogen hunger period probably help leguminous plants for steady growth and better development in the initial stages (Olvera et al. 1982; Gunawardena and Senanayake, 1989). Significant response to applied nitrogen during early growth of Leucaena when the plant had not started nodulating and fixing nitrogen was reported (Sanginga et al. 1988 and Cobbina, 1991). The response to nitrogen application upto 20 kg ha^{-1} by an increase in the growth parameters of the two species in the present study could be attributed to the above facts . Similar results were also reported in A. mangium and P. falcataria (Umali- Garcia et al.1989) and Dalbergia sisso (Hussain et al. 1990). It may be furthur stated that after a certain period of time, with growth and development of roots, the plants become pot bound and thereafter the chances of response to increased doses of fertilizers is very little. The lack of response of fertilizer nitrogen beyond 20 kg ha $^{-1}$ for P. falcataria in this study may probably be due to this reason. Results of reduction in growth with higher doses of fertiliser nitrogen was also reported by Belen, (1987), Powell and Webb (1972) and Umali-Garcia et al. (1988).

Like nitrogen, phosphorus is also an essential element necessary for the growth of plants especially for root growth. In A. catechu, phosphorus application resulted in an increase in growth as evident from the increased growth parameters. The same trend was also reported in Gliricidia (Manguiat et al. 1987). However, response to phosphorus application in improving growth characters was not observed in A. arabica and P. falcataria. It may be due to the differences among the species and their own inherent capacity to absorb phosphorus from the medium. Phosphorus would have helped better absorption of nitrogen and its utilisation which might have resulted in increasing the growth characters by increasing the protoplasmic activity.

In this context, in the present investigation the rooting pattern studies undertaken is worth mentioning. It can be seen from Fig.4,5 and 6 that the density of root hairs and root spread is maximum in the peripheral layers of the soil in the case of P. falcataria. The roots in the plough layer would be active and in constant touch with the rich nutrient medium. This would facilitate faster absorption of nutrients and translocation to growing parts of the plants. The plant also would have utilised more minerals from the soil solution than in the applied fertilizers. Naturally a lower fertilizer response could be expected . Both A. catechu and A. arabica are deep rooted, with more number of nodules being located deeper in the soil. Thus the variation in rooting pattern and nodulation in the tree species might also be the reasons of poor response to added nitrogen fertilizers.

5.3.2 Nodule characteristics

5.3.2.1 Number of root nodules per plant

An appraisal of the data in Table 12 and Fig.3 revealed that Rhizobium inoculation significantly increased nodule number per plant from 6.75 to 15.91 in A. arabica, 2.64 to 8.78 in A. catechu and 186.69 to 244.64 in P. falcataria.

It may be furthur seen that there was an adverse effect of nitrogen application on nodulation in all the three species. Highest number of nodules were produced at zero level of nitrogen. When nitrogen level increased from zero to 20 kg ha^{-1}, the nodule number reduced from 16.25 to

10.91 per plant in *A. arabica*, 11.42 to 9.46 per plant in *A. catechu* and 253.33 to 241.13 in *P. falcataria*.

While considering the growth pattern and nodulation in relation to different levels of nitrogen in the case of *P. falcataria*, it may be noted that the growth characters were not significantly influenced by nitrogen application. However, the nodule number per plant in this species is much higher and it recorded almost twenty to thirty fold increase. This is an indication of the better establishment of the species, its suitability and adaptability in the agro-ecological conditions of this region. As compared to other species, *P. falcataria* performed well. Because of higher number of nodules present, the rate of fixation of biological nitrogen in this tree will be high and hence it can be safely recommended as a suitable agroforestry species.

Phosphorus application had significant influence on the nodule number per plant. The effect of phosphorus was positive and the number of nodules increased as the level of phosphorus increased and the order of increase was 8.92 to 12.83 in *A. arabica* and 2.79 to 9.08 in *A. catechu*, with application of phosphorus from 0 kg ha^{-1} to 25 kg ha^{-1}. However, beyond 25 kg ha^{-1}, a decline in nodule number in both the species were noticed.

Many of the leguminous plants are generally considered to have specific strain requirements for nodulation. In the present study also the responses to inoculation were highly variable. The probable reason for the variation in the response and nodule number might be due to the soil effect, preferably acidity of the soil and also the resultant nitrogen - nodulation interaction. Investigations on plant root - soil - nutrient interaction are to be considered and taken up in future and research programmes are to be initiated to evaluate the performance of these species, especially when adaptability trials are undertaken.

5.3.2.2 Dry weight of nodules per plant

Data from Table 13 revealed that inoculation had a positive influence on the dry weight of root nodules per plant in all the species.

Nitrogen application had significant effect on nodule dry weight per plant in all the species studied.

In A. arabica nodule dry weight per plant increased from 0.39 to 0.40 g when the level of nitrogen increased from zero to 20 kg ha^{-1} and they were on par. There was decline in the value (0.16) when the level of nitrogen was increased to 40 kg ha^{-1}.

An increase in nodule dry weight (from 0.12 to 0.28 g plant^{-1}) was recorded in A. catechu, when the nitrogen level increased from zero to 20 kg ha^{-1}. However a sharp decline in the nodule dry weight was recorded when the nitrogen level was raised to 40 kg ha^{-1}.

In P. falcataria nodule dry weight per plant increased from 8.09 to 9.92 g as the nitrogen level increased from zero to 20 kg ha^{-1} and then decreased to 9.61 g with increase in nitrogen level to 40 kg ha^{-1}.

Phosphorus application also influenced this parameter. An appraisal of the data indicated that as the level of phosphorus increased from zero to 25 kg ha^{-1}, the nodule dry weight was also increased in A. arabica, A. catechu and P. falcataria. The increase was from 0.25 to 0.40 per plant in A. arabica, 0.01 to 0.26 g per plant in A. catechu and 8.08 to 10.65 g per plant in P. falcataria.

The direct role of inoculation on nodule formation is widely accepted . Increased nodulation and nodule dry weight due to Rhizobium inoculation of legumes has been reported by workers like Vincent (1958), Gunawardena and Pushpakumari (1988) and Pahwa (1989).

Mineral nutrient defeciencies could specifically limit nodulation at root infection stage of the nodule bacteria in legumes (O'Hara *et al*. 1988). The depressive influence of nitrogen on nodulation and nitrogen fixation using lucerne as the test plant has been shown by Subba Rao *et al*. (1974). Lower levels of nitrogen application was found to increase the nodulation and nodule dry weight and decrease with higher levels was reported in *Glycine max* (Kotoch *et al*. 1983), *A. mangium* and *P. falcataria* (Umali - Garcia *et al*. 1988). Once the *Rhizobium* have filled a proliferating cell in roots , they change their form into a bacteroid for which it requires optimal amount of nitrogen and other nutrients and subsequently increasing the nodule size (Russell, 1977). Increase in nodule dry weight at 20 kg ha^{-1} nitrogen level may be attributed to this reason. However, the process of infection and nodule formation can be disturbed if the nitrate or ammonium concentration around the plant roots is too high (Russell, 1977).

Phosphorus is necessary for enhancing nodule number and nodule activity in many legumes as demonstrated by de Mooy and Pesek (1966) on soybeans and Luse *et al*. (1975) on cowpea. Positive influence of phosphorus on nodule number and nodule dry weight was reported by Srivastava and Verma (1985) in pigeon pea, Manguiat *et al*. (1987) in *Gliricidia*, Hussain *et al*. (1988) in *Leucaena* and Hussain *et al*. (1989) in *Erythrina suberosa*. Results of this experiment are also in conformity with the findings of the above workers.

5.3.3. Total nitrogen content of plant

It can be seen from Table 14 that inoculation had resulted in an increase in nitrogen content in all the species.

Nitrogen application upto 20 kg ha^{-1} resulted in an increase in nitrogen content and then a reduction in the parameter beyond application of 20 kg ha^{-1} .

The nitrogen application in excess of 20 kg ha^{-1} might have been utilised for the growth and cell division of plants as the seedlings were activily growing. Naturally a reduction in the nutrient content could be expected.

Phosphorus application upto 25 kg ha^{-1} generally resulted in an increase in nitrogen content of plants and thereafter a slight reduction beyond 25 kg ha^{-1} . Phosphorus application thus, would have synergistic effect on the absorption of nitrogen in plants. This might have resulted in favourable effects on absorption of nitrogen in plants. The increased nitrogen content with application of phosphorus was reported earlier (Tisdale and Nelson, 1985; Russell, 1977).

5.3.4 Total phosphorus content of plant

An appraisal of the data given in Table 15 showed that inoculation had no significant influence with respect to this parameter.

Nitrogen application had a significant influence on phosphorus content of plant in A. arabica and P. falcataria . Nitrogen application at the rate of 20 kg ha^{-1} gave the highest phosphorus content in A. arabica (0.167 and P. falcataria (0.149).

Phosphorus application at 50 Kg ha^{-1} gave highest phosphorus content in Acacia arabica (0.178) and in A. catechu (0.169). In P. falcataria , phosphorus application had no significant influence with respect to this parameter. This might be due to the higher number of nodules produced in this species. Phosphorus is highly essential for nodulation and increase in the nodulation, in turn will reduce the plant phosphorus content and this would have happend in this case also.

Rhizobium inoculation was resulted in increase of nitrogen content in Acacia nilotica (Balaji and Rangarajan, 1987), Leucaena (Mohammed, 1988; Pahwa, 1989). Rhizobium inoculation increased nitrogen uptake in Leucaena (Mohammed, 1988) and pigeonpea de Lucena Costa and Paulino (1989). Results of the present study are also in conformity with the above findings.

According to Tanaka et al.(1964), the nutrient content in plants is generally controlled by factors like nutrient availability in soil, nutrient absorption power of roots and rate of increase of dry matter production. Hence, the nutrient content of plants at any stage of growth is mainly related to dry matter production. Nitrogen and phosphorus application resulted in an increase in growth parameter showing an increase in dry matter production. Similar results were reported by workers like Dreyfus et al. (1985), Manguiat et al. (1987) and Umali-Garcia et al. (1988) in different legumes.

SUMMARY

CHAPTER 6

SUMMARY

An investigation was carried out at College of Agriculture, Vellayani during August 1990 to July 1991 to determine the effect of Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes. This investigation was carried out as three separate experiments. The main objective of Experiment I was to find out the effect of different scarification methods on the germination of seeds of some commonly grown and newly introduced tree legumes. The aim of Experiment II was to screen out the best among the native and exotic rhizobial isolates along with different levels of phosphorus in selected tree legumes. The objective of Experiment III was to determine the effect of Rhizobium inoculation, nitrogen and phosphorus levels on nodulation and seedling growth. The experiment(s) were carried out in a completely randomised design with five, three and four replications each for experiment I,II and III respectively. The results of the experiment(s) are summarised below.

(1) Hot water treatment for three minutes enhanced germination per cent to 74.38 in Acacia mangium.

(2) Con.H_2SO_4 treatment for five minutes recorded 69.84 per cent germination in Acacia arabica.

(3) In *Acacia catechu*, 99.41 per cent germination was recorded by treatment with con. H_2SO_4 for five minutes and 98.99 per cent germination with hot water treatment.

(4) Scarification with con. HNO_3 for ten minutes resulted in 98.27 per cent germination in *Albizia lebbeck*.

(5) Treatment with con. H_2SO_4 for one minute gave 80.44 per cent germination in *Albizia moluccana*.

(6) Hot water treatment for three minutes gave 74.61 per cent germination in *Paraserianthes falcataria*.

(7) Scarification with con. HCl for one minute resulted 70.03 per cent germination in *Cassia fistula* .

(8) Con. H_2SO_4 treatment for five minutes gave 70.14 per cent germination in in *Cassia javanica*.

(9) Hot water treatment for three minutes resulted 68.55 per cent germination in *Sesbania grandiflora*.

EXPERIMENT II

(1) Type of *Rhizobium* had significant influence on plant height. Inoculation with exotic isolates TAL 1868 and TAL 45 produced highest plant heights of 18.96 cm and 10.60 cm in *A. catechu* and *P. falcataria* respectively.

(2) Shoot dry weight was significantly influenced by inoculation in A. catechu.

(3) Inoculation of Rhizobium with TAL 1868 produced root dry weight 0.16 g and 0.14 g per plant in A. arabica and A. catechu respectively. Inoculation with TAL 45 produced root dry weight of 0.15 g per plant in P. falcataria.

(4) Plant height was significantly influenced by phosphorus application in P. falcataria. Maximum plant height was recorded by 50 kg ha^{-1} phosphorus application.

(5) Shoot dry weight of A. arabica was significantly increased by phosphorus application upto 50 kg ha^{-1}.

(6) Root dry weight of P. falcataria reached maximum with 25 kg ha^{-1} phosphorus application.

(7) Inoculation with exotic isolate TAL 1868 produced the maxinum number of nodules per plant in A. arabica and A. catechu. In P. falcataria inoculation with exotic isolate TAL 45 produced 15 nodules per plant.

(8) Exotic isolate TAL 1868 produced maximum nodule dry weight per plant in A. arabica and A. catechu. Inoculation with exotic isolate TAL 45 produced maximum nodule dry weight per plant in P. falcataria .

(9) The nodule number per plant increased from 0.67 to 1.33 in A. arabica by application of phosphorus upto 50 kg ha^{-1}.

Results of screening for suitable rhizobial isolate clearly indicated that growth and nodulation of all the species under the study were improved by inoculation with exotic isolates in combination with phosphorus application.

EXPERIMENT III

(1) Inoculation with Rhizobium had significant influence on plant height. Plant height reached maximum in A. catechu and P. falcataria by inoculation with exotic isolates of Rhizobium.

(2) Inoculation resulted in highest root dry weight per plant of 6.73 g in A. arabica and 12.91 g in P. falcataria.

(3) Nitrogen application had significantly influenced plant height. Maximum plant height was recorded in A. arabica and A. catechu due to application of nitrogen upto 20 kg ha^{-1}.

(4) Application of nitrogen upto 20 kg ha^{-1} resulted in highest shoot dry weight per plant in A. catechu and

in *P. falcataria*, where as in the case of *A. arabica*, nitrogen application did not influence this character.

(5) As the level of nitrogen increased upto 20 kg ha^{-1}, root dry weight per plant had increased in *A. catechu* and *P. falcataria* with 20.89 g and 13.75 g per plant respectively.

(6) Levels of phosphorus application had significantly influenced plant height in *A. catechu*, maximum value being recorded with 25 kg ha^{-1} .

(7) Application of phosphorus upto 50 kg ha^{-1} produced maximum shoot dry weight of 29.88 g in *A. catechu*.

(8) Application of phosphorus at 25 kg ha^{-1} resulted in maximum root dry weight in *A. arabica* and in *A. catechu* .

(9) Inoculation with *Rhizobium* increased nodule number per plant in *A. arabica*, in *A. catechu* and in *P. falcataria*.

(10) There was significant increase in nodule dry weight by inoculation in all the species.

(11) There was no increase in the number of nodules per plant with nitrogen application in all the species.

(12) Nodule dry weight per plant increased with application of nitrogen upto 20 kg ha^{-1} in all species.

(13) Phosphorus application upto 25 kg ha^{-1} resulted in maximum number of nodules per plant in A. arabica and A. catechu . Whereas, in P. falcataria , nodulation was not affected by nitrogen application.

(14) Nodule dry weight per plant was not affected by phosphorus application in A. arabica. On the contrary, application of phosphorus at the rate of 25 kg ha^{-1} resulted in highest nodule dry weight per plant in A. catechu and P. falcataria.

(15) Rhizobium inoculation had influenced plant nitrogen content in all the species. Rhizobial inoculation resulted in increase nitrogen content.

(16) Rhizobium inoculation did not influence plant phosphorus content.

(17) Application of nitrogen upto 20 kg ha^{-1} recorded maximum plant nitrogen content .

(18) Similarly, plant phosphorus content reached maximum values in A. arabica and P.falcataria. with 20 kg ha^{-1} nitrogen application.

(19) With phosphorus application at 25 kg ha^{-1}, plant nitrogen content increased to 1.42 per cent in *A. arabica*, 1.03 per cent in *A. catechu* and 1.80 per cent in *P. falcataria* respectively. Application of phosphorus (50 kg ha^{-1}) recorded 0.178 per cent and 0.169 per cent phosphorus in *A. arabica* and *A. catechu* respectively.

The investigation clearly revealed that application of nitrogen and phosphorus along with inoculation using specific strains of *Rhizobium* is essential for better growth and nodulation of leguminous tree species .

It may be worthwhile to take up future studies on the interaction of soil/fertilizer/ species under different agroclimatic conditions for confirmatory results.

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APPENDIX

APPENDIX I

Weather data during the cropping period

(October 90 to July 1991)

Month	Average rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)	Relative humidity (%)
Oct. 90	6.11	30.34	23.23	81.62
Nov. 90	47.74	30.14	23.27	81.58
Dec. 90	2.86	31.01	22.78	78.10
Jan. 91	7.14	30.80	22.30	77.80
Feb. 91	6.63	31.10	21.30	71.20
Mar. 91	8.00	32.30	23.98	76.10
Apr. 91	6.24	33.40	25.40	78.70
May. 91	10.93	33.20	25.75	78.66
Jun. 91	27.88	29.50	24.00	88.20
Jul. 91	19.43	29.40	23.47	83.50

APPENDIX II

Composition of Yeast Extract Mannitol Agar Medium (Allen,1953)

1	Mannitol	10.0 g
2	Dipotassium hydrogen phosphate	0.5 g
3	Magnesium sulphate	0.2 g
4	Sodium chloride	0.1 g
5	Calcium carbonate	3.0 g
6	Yeast extract	1.0 g
7	1 % Aqueous solution of Congo red	2.5 ml
8	Agar	15.0 g
9	Distilled water	1000 ml
	p^H	7.0

RHIZOBIUM INOCULATION AND NUTRIENT LEVELS ON NODULATION AND SEEDLING GROWTH IN TREE LEGUMES

By

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ABSTRACT OF A THESIS

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ABSTRACT

The present investigation entitled Rhizobium inoculation and nutrient levels on nodulation and seedling growth in tree legumes was carried out at College of Agriculture, Vellayani during the period August 1990 to July 1991. The investigation was carried out as three separate experiments. The main objective of Experiment I was to determine the effect of different scarification methods on the germination of some commonly grown and newly introduced legumes, the objective of Experiment II was to screen out the best among the native and exotic isolates of Rhizobium with different levels of phosphorus on the nodulation and seedling growth and the objective of Experiment III was to study the effect of Rhizobium inoculation, and nutrient levels on nodulation and seedling growth of tree legumes. The experiment(s) were carried out in a completely randomised design with five, three and four replications each for Experiments I, II and III respectively. An abstract of the results is given below.

Experiment I

Hot water treatment for three minutes was found to be effective for enhancing the germination in *Acacia mangium*, *Paraserianthes falcataria* and *Sesbania grandiflora*. Treatment with Con.Hcl for one minute recorded highest

germination per cent in Cassia fistula. Germination percentage of 98.27 was recorded with con.HNO_3 treatment for 10 inutes. In Acacia arabica, Acacia catechu and Cassia javanica maximum germination was obtained with con.H_2SO_4 treatment for five minutes, while that for one minute resulted in highest germination in Albizia moluccana.

Experiment II

Exotic isolates, TAL 1868 and TAL 45 were proved to be the best among the rhizobial isolates for Acacia and Albizia sp. respectively with respect to growth and nodulation characteristics as compared to the local strains. Plant height, shoot dry weight and root dry weight were significantly influenced by phosphorus application. Nodule number per plant had increased from 0.67 to 1.33 in Acacia arabica.

Experiment III

Nitrogen application along with inoculation significantly influenced growth characters such as plant height, shoot dry weight and root dry weight. Maximum plant height was recorded due to application of nitrogen upto 20 kg ha^{-1} in Acacia arabica and Acacia catechu. Nitrogen application upto 20 kg ha^{-1} resulted in highest shoot dry weight and root dry weight in both Acacia catechu and Paraserianthes falcataria. Application of nitrogen did not increase nodule number per plant in all the tree species. However, nodule dry weight per plant increased with application of nitrogen upto 20 kg ha^{-1}. Phosphorus application upto 50 kg ha^{-1} produced maximum shoot and root dry weights in Acacia catechu. Application of phosphorus at 25 kg ha^{-1} resulted in maximum root dry weight in Acacia arabica and Acacia catechu. Phosphorus application upto 25 kg ha^{-1} resulted in maximum number of nodules per plant in Acacia arabica and Acacia catechu. Highest nodule dry weight per plant was recorded with phosphorus application upto 25 kg ha^{-1} in Acacia catechu and Paraserianthes falcataria. Nitrogen application upto 20 kg ha^{-1} resulted in an increase in plant nitrogen content and reduction thereafter. Phosphorus application upto 25 kg ha^{-1} resulted in an increase in nitrogen content of plants and a reduction was noticed beyond 25 kg ha^{-1}. Nitrogen application had significant influence on phosphorus content of plants in A arabica and P. falcataria. Phosphorus application at 50 kg ha^{-1} gave highest phosphorus content in A. arabica and A. catechu.

On the basis of the present study it can be concluded that nitrogen and phosphorus application along with Rhizobium inoculation using specific strains is essential for better growth and nodulation of leguminous tree species.