COMPARATIVE STUDIES ON THE FIXATION OF NITROGEN BY CERTAIN COMMON LEGUMES.

By
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AGRICULTURAL COLLEGE AND RESEARCH INSTITUTE.
VELLAYANI.

This is to certify that the Thesis herewith submitted contains the results of bona fide research work carried out by Shri. H. L. ROSE under my supervision. No part of the work embodied in this Thesis has been submitted earlier for the award of any Degree.

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CONTENTS

CHAPTER I

INTRODUCTION

The process of symbiotic nitrogen fixation by legumes is of considerable importance in improving soils and their fertility.

Nitrogen fixation varies from legume to legume and can normally take place only if the crop is healthy and the nutrient supply adequate. The factors of particular importance to legumes are the presence of the proper strain of rhizobium, adequate quantities of nutrients, notably calcium, phosphorus, molybdenum and boron, an optimum pH and the physical capacity of the soil to hold and release water and oxygen to the plant.

Investigations reported reveal that the quantity of nitrogen fixed by different legumes varies not only among themselves but also from place to place. Hartwell and Pember (1911), Lohnis (1925), Sen and Rao (1953) and Mirchandani and Khan (1953) have studied several legumes and found varying quantities of nitrogen fixed by each. Mirchandani and Khan (1953) working in India reported that generally the quantity of nitrogen fixed by a legume varied between 20 to 120 pounds per acre. Russell (1961) holds the view that there is wide disparity in the nitrogen fixed by various legumes.

Hellreigal and Wilfarth (1886) and Beijerinck (1888) clearly demonstrated that nitrogen fixation occurred only in the presence of certain micro-organisms. Since then it has been shown that only particular strains of bacteria are associated with specific legumes in the process of nitrogen fixation.

Calcium is very important both for the nutrition of the legume as well as the rhizobia. Whyte _et al_. (1953) reported that under a low calcium regime the bacteria revert into a chromogenic form and fail to invade the plant. The calcium content of legumes as reported by McCalla (1937) is three to four times that of non-legume grasses on dry weight basis. Albrecht (1937) suggested that this perhaps may be the reason why rhizobia fail to invade non-legumes. Numerous workers like Mooers (1912), Lipman and Blair (1917), Fred and Graul (1918), Wilson (1917), Albrecht and Davies (1929) and Fred _et al_. (1932) have reported that the application of calcium to a legume enhanced its nitrogen fixation capacity as a result of promoting nodulation. Striking increases in the yield and dry matter content of legumes have also been recorded. The importance of calcium is recognised as a nutrient and also as instrumental in raising the pH of the soil to bring it to a condition optimum for the development of the soil microflora.

The effect of pH on symbiotic nitrogen fixation by legumes is governed by the availability of calcium in the soil. Work done by Albrecht and Smith (1952), Whyte et al. (1953) and Stout and Johnson (1957) indicate that irrespective of pH, if the calcium content of the soil was optimum, nitrogen fixation would continue normally. Certain investigators feel that a pH of between 5.5 and 6.5 is necessary for proper growth and functioning of the root nodule bacteria as evidenced by the work of Samuel and Landrau (1953) and McKee (1961).

Phosphorus plays a very important role in sustaining the soil microbial population at a high level. For rhizobia to migrate in the soil towards the root system of the plants, the cells must be in a flagellate state. Phosphorus has a stimulatory effect on the retention of this flagellate state by soil microflora. The rate of protein synthesis is found to be slow in plants grown in soils low in phosphorus. The classical experiments done by numerous workers like Trusdell (1917), Wilson (1917), Fellers (1918), McTaggart (1921) and Fred et al. (1932) have proved beyond doubt the dynamic role played by phosphorus in symbiotic nitrogen fixation by legumes.

Aeration and moisture have been shown to be very important in the formation of nodules and nitrogen fixation by legumes. Kellerman and Robinson (1906), Reynolds (1907), Prucha (1915) and Gangulee (1929) have substantiated this fact.

Thus it appears that the process of nitrogen fixation is influenced not only by the specificity of a legume, but also by the biological, chemical and physical condition of the soil. Consequently, comparative studies are imperative for selecting the right type of legume in a locality under a given set of conditions.

With reference to Kerala, loss of nitrogen in cultivated fields is very high due to high temperature and excessive rainfall prevalent in the State. Tapioca which is a popular crop of the State is grown continuously year after year without any rotation and often without proper manuring. Thus the exhausting tapioca crop leaves the soil depleted of its fertility. Further, vast stretches of land are available in coconut gardens which are generally intercropped to exhaustive crops like yams, bananas and tapioca. Growing legumes as a cheap source of maintaining the fertility status of soils is an established fact.

In Kerala no work appears to have been done comparing the efficiency of symbiotic nitrogen fixation through legumes. Hence it was felt that investigations which will lead to the determination of the rate of fixation of nitrogen by certain common legumes alone or in the presence of optimum doses of calcium and phosphorus were necessary.

CHAPTER II

REVIEW OF LITERATURE

Numerous aspects of the phenomenon of symbiotic nitrogen fixation through the agency of legumes and root nodule bacteria have been subjected to intensive study for many decades by several workers. The factors involved in this process are the specificity of the legumes themselves, their associated nodule organisms, soil and environmental conditions. The present investigation is primarily an attempt for ascertaining the relative rates at which free nitrogen could be fixed by certain common legumes, in a given soil.

Incidentally, the low calcium and phosphorus levels observed in the soil where this investigation was conducted led to the study of their influence on the legume also. The literature reviewed below is confined to the specific aspects studied.

I. Legumes and nitrogen fixation:

Though cultivation of legumes for improving and sustaining soil fertility was practised by Roman and Chinese agriculturists several thousands of years ago, it was not until 1813 that a suggestion was made by Sir Humphry Davy that legumes could obtain food directly from the atmosphere. Following this, Boussingault, (1837-'38) had demonstrated that when peas and beans were grown in pots, they acquired more nitrogen in their tissues than what was lost from the soil in the pot. He explained that those plants could assimilate nitrogen from the atmosphere whereas cereals like wheat and oats could not do so.

Hellreigal and Wilfarth (1886) showed that gains of nitrogen in peas took place only in the presence of certain specific soil micro-organisms. Ward (1887) also, from his investigations concluded that root nodules could be formed only in the presence of certain bacteria.

The chain of evidence was completed by Beijerinck (1888), who was able to isolate the bacterium concerned in symbiotic nitrogen fixation.

Hartwell and Pember (1911), studying the nitrogen fixing phenomenon by legumes for a period of five years in soybeans, cowpeas and hairy vetch, found gains of nearly one ton of soil nitrogen per acre in pots where cowpea and soybeans were grown. They reported that seven-tenths of the nitrogen was from the atmbsphere and only three-tenths from the soil. An annual average gain of 120 pounds of nitrogen per acre was credited to hairy vetch.

Lohnis (1925) reported that at least 80 to 120 pounds of nitrogen per acre were added to the soil by growing legumes.

Thornber (1920) found that clover grown in a Montana apple orchard continuously for eight years added 1,514 pounds of nitrogen per acre. He further reported that, when the hay was incorporated, the corresponding quantity of nitrogen added to the soil was of the order of 3,019 pounds.

Buckman and Brady (1960), estimating the quantity of nitrogen fixed in soil by a crop of red clover, reported the figure as 100 to 150 pounds per acre.

Hopkins, as quoted by Waksman (1952), stated that a three ton crop of cowpea hay added 86 pounds of nitrogen per acre; a four ton clover crop added 106 pounds of nitrogen per acre; and a four ton alfalfa crop added 132 pounds per acre.

Sen and Rao (1953), investigating the fixation of nitrogen under favourable conditions, reported the quantity fixed symbiotically was 251 pounds annually per acre.

Hayden <u>et al</u>. (1957) observed that four years of growing kudzu vine in Missisippi gave an average increase of 43% more of soil nitrogen compared to the fields where corn was cropped continuously for four years. They further held the view that cultivation of a legume preceding a cotton crop was tantamount to applying 24 pounds of commercial nitrogen to the cotton crop.

Virtanen *et al*. (1958), working on the relative quantities of fixation of nitrogen by pea, reported that 10 per cent of the nitrogen in the plant was from the soil and 90 per cent was accounted as having been assimilated from the atmosphere.

Thus, considerable variations are found in the assessment of the quantity of nitrogen fixed not only between legumes but even within the same species.

Russell (1961) holds the view that the actual amount of nitrogen fixed by leguminous crops in the field is difficult to estimate because of the difficulty of determining accurately the nitrogen content of a soil on the one hand and the amount of denitrification taking place during the growing season.

Some typical figures have been given by Lyon and Bizzell (1934) at Cornell. They found that two courses of a rotation consisting of one year clover cut hay and four year grain crops added 200 to 300 pounds of nitrogen per acre to the soil and 300 to 400 pounds to the harvested crop, compared with a similar rotation in which timothy grass replaced the clovers, indicating that each clover crop had fixed between 250 and 350 pounds of nitrogen per acre, which is comparable with the amount of nitrogen other workers have found a good crop of lucerne can fix in a year. Soybeans, field beans and peas harvested for grain on the other hand, depleted the soil of nitrogen as much as ordinary cereal crops; they fix between 100 and200 pounds of nitrogen, but it all appeared in the harvested material.

Little is known about the amount of nitrogen, tropical and sub-tropical crops fix. Russell (1961) expressed the view that probably all the nitrogen is transferred to the tops and seeds since the legumes increase the nitrogen contents of the soil no more than non-leguminous crops. The work of Vyas and Desai (1953) lends evidence to this view.

Thompson (1952) reported that the amount of nitrogen fixed and thus added by a legume to the soil depended upon its age, photosynthetic rate and presence or absence of combined nitrogen in the soil.

Mirchandani and Khan (1953) pointed out that the amount of nitrogen fixed by a legume depended upon its age and condition of growth, the type of legume and the stage at ploughing in. They suggested that the amount of nitrogen in a leguminous plant is the sum total of the nitrogen it had taken from the air and soil. They fixed it as two-thirds from the air and one-third from the soil.

The quantity of nitrogen added to the soil by a legume depends to a great extent on whether the legume is incorporated in the soil or not. Fellers (1918), after intensively studying the effect of growing alfalfa for 20 to 30 years, observed that growing alfalfa continuously for a long period depleted the soil nitrogen when compared to a soil that was left under sod. Greaves and Johnes (1950) corroborated the view on the strength of a 16 year study on alfalfa by concluding that harvesting and manuring the crop did not significantly increase the nitrogen status of the soil, whereas returning the crop to the soil significantly increased the soil nitrogen content.

From a similar investigation conducted by Waksman (1952) it was reported that if a legume crop was harvested and removed from the soil the amount of nitrogen added in the soil through the legume may be negligible owing to the amount assimilated from the atmosphere being generally found present in the tops.

Nair et al. (1957), while investigating the nitrogen balance in laterite soils of Pattambi by growing Sesbania speciosa , failed to get any significant increase in soil nitrogen.

Russell (1961) reported that legumes may not increase the soil nitrogen under all conditions. In case of legumes like peas, beans, soybeans and groundnut, even if their roots are often well nodulated, a large proportion of the nitrogen fixed

is removed in the seed, straw and other harvested portions of the crop.

II. (a) Calcium and nitrogen fixation:

Calcium is important in both the nutrition of the legume and rhizobia. The calcium content of the legumes is about three times that of grasses on the basis of dry weight. Consequently, a leguminous crop may respond to liming where a non-legume may not.

Application of lime to the soil tends to increase the nitrogen fixed by most legumes in the soil and their foliage. Mooers (1912), studying the effect of liming in crop production, revealed that the yield of cowpea hay was increased and its nitrogen content enhanced by application of lime.

Fred and Graul (1916) reported that on limed soils the nitrogen fixed through the agency of legumes varied. In three experiments conducted with alfalfa they reported that the nitrogen fixed in the tops of the crop was 51 pounds, 81 pounds and 269 pounds per acre, respectively. Thus, each experiment gave different results. Similar investigations by them on red clover indicated that the crop fixed 62 pounds of nitrogen in a silt loam and 145 pounds in a sandy soil.

Bear (1917), in his report onthe studies conducted on the application of calcium carbonate in soybeans, recorded an increase in the nitrogen content of both tops as well as the roots of the plant. He had selected nodulated plants for his investigation.

The above findings were corroborated by Lipman and Blair (1917). In their attempt for the assessment of the influence of lime on nitrogen fixation and yield of soybean on a loamy soil, they found that the nitrogen content in the tops grown on limed soils was 3.08 per cent as against 2.67 per cent for plants that did not receive lime. The figures for the roots in the same order of treatment were 1.4. per cent and 1.24 per cent respectively. On the above findings they concluded that liming had a positive influence on the nitrogen content of the plant material.

Klingebiel and Brown (1937) studied the nitrogen content of alfalfa through different methods of placement of lime and concluded that the increase of nitrogen content was not specifically influenced by any particular method of placement. However, the same workers conducting an investigation on the effect of lime on alfalfa in different types of soils having different lime requirements reported that fully limed soils produced highly significant yields as compared with those receiving inferior doses of calcium.

Russell (1961) contended that non-availability of calcium in acid soils appeared to restrict nitrogen fixation by

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Russell (1961) contended that non-availability of calcium in acid soils appeared to restrict nitrogen fixation by many legumes grown on soils having a low pH. He added that, if the pH was raised to over five or nitrogenous fertilizers were added, the nodules readily assimilated nitrogen.

Waksman (1952) also obtained data to show that in soils, low in nitrogen content and rich in lime, larger quantities of nitrogen were generally symbiotically fixed.

In trying to give an explanation to the positive effect of calcium on nitrogen fixation, Whyte *et al*. (1953) was of the view that, under a low calcium regime, the bacteria concerned failed to invade the root hairs of the legume.

McCalla (1937) has shown that the concentration of available calcium needs to be relatively high to sustain an active rhizobial population. Under a low calcium regime the bacteria change to an abnormal chromogenic form in which they are unable to invade the plant. Albrecht (1937) has even suggested that it is the low calcium content of non-legumes which determines their non-invasion.

(b) Effect of calcium on nodulation:

The earliest conceptions of the nature and functions of legume root nodules tended to regard them either as products of some pathological disorder or as storage organs. Following Ward's proof in 1887 that nodule formation results from bacterial infection, came the realisation that the nodule is a characteristic feature of some members of the Leguminosae and has nutritional significance.

Altogether, seventy-seven species of legumes, belonging to seventeen genera, do not form nodules under any known conditions; but, current records are imperfect. Published observations, according to Allen and Allen (1942), refer to only about 1,000 of the 10,000 or more species of Leguminosae as a whole for this character of nodulation.

Several workers have shown that application of calcium to legumes tend to increase their nodulation. Prucha (1915) stated that small amounts of calcium carbonate favoured nodulation in field bean.

Salfield Lingen, according to Wilson (1917) was one of the earliest to investigate the effect of calcium on nodulation. He observed that the addition of calcium in the form of oxide of calcium greatly influenced nodule formation in the pea plant. Continuing the study and comparing the effects of different forms of calcium, such as calcium oxide, calcium saccharate, calcium sulphate and calcium nitrate, he reported that calcium oxide gave the highest number of nodules.

Scanlon (1928) observed that, even though the application of calcium acetate to an acid soil brought about very little change in the soil acidity, the nodulation observed in soybeans grown in the same field was found to have increased by 1,000 per cent.

Studying the effect of inoculation, fertilizer treatment, certain minerals on yield, composition and nodule formation of soybeans in acid soils, Fellers (1918) concluded that liming increased nodule formation by as much as 1,500 per cent.

Pieters (1927) opined that lime and phosphorus application to soils have generally been found to increase the size and number of nodules present in the roots of legumes. He also reported the single exception that in seradella applications of lime was injurious both to the plant and its nodule formation.

Albrecht and Davies (1929), studying the problem in acid soils with pH 5.5, reported in favour of addition of limestone, it having been observed to have increased the number of nodules formed in just five weeks after germination by 336 per cent over the unlimed soils.

McKee (1961), studying the effect of liming on seedling growth and nodulation in bird'sfoot trefoil, reported that, though nodulation took place between pH 4.7 and 7.9, it was only at a pH above 6.2 that survival of seedlings and nodulation were observed to be satisfactory.

Nodulation in clover was found improved by the addition of lime in clover pastures as reported by McLachlan (1953).

Nair et al. (1957), in their studies on nodulation of *Sesbania speciosa* in laterite soils of Pattambi reported that oxide of calcium at 1,500 pounds per acre greatly increased the number of nodules formed.

Norris, as quoted by Russell (1961), has shown that, on a strongly leached acidic soil in Queensland, tropical legumes could take up adequate amounts of calcium for growth and nodulation, whereas temperate species of trifolium and medicago failed to grow properly unless they were given a dressing of ten hundredweights of carbonate of calcium.

(c) Effect of calcium on yield and dry matter content:

Recorded evidence from several investigations is available to show that calcium has a positive effect on the yield and dry matter content when applied to legumes.

Mooers (1912), studying the effect of liming on crop production, concluded that burnt lime increased yields of cowpea hay.

Lipman and Blair (1917) reported that application of ground limestone increased yield and dry matter content of soybeans. In a similar investigation in soybeans, Fellers (1918) observed that ground oyster shells and burnt lime were important and efficient in increasing the yield and total dry matter content of soybeans in acid soils. The increased yield ranged from 30 to 50 per cent for applications of 1,000 and 2,000 pounds of oyster shell and burnt lime per acre respectively.

In the same crop, Fred and Graul (1919) noted that application of lime to neutralise half the active acidity of a soil increased significantly the growth and yield of soybeans.

Klingebiel and Brown (1937), studying the effect of placement of lime in alfalfa, observed that applications of limestone significantly increased its yield and dry matter content irrespective of the method of placement. In the same study, they also reported that the soil receiving the entire requirement of lime produced greater yields and dry matter than those soil whose lime requirement was only partially met.

Jones and Edwards (1954), investigating the effect of liming in clover, concluded that on podzolic soils, increasing doses of liming material progressively increased forage and seed yields. Lawton and Davies (1956) indicated that the yield of beans was found increased subsequent to lime application on strongly acid peat soils.

Nair _et_ _al_. (1957), investigating the effect of calcium oxide on the yield and growth rate of _Sesbania_ _speciosa_ in the laterite soils of Pattambi, reported increased yield of both green and dry matter.

Studying the effect of calcium on groundnut, Piggot (1960) reported the beneficial effects of calcium in increasing the yield.

Investigations by many workers have shown that application of calcium to the soil tends to increase the height of many legumes.

Klingebiel and Brown (1937), studying the effect of lime at varying levels and stages of growth in alfalfa, observed positive response to lime. In their investigation, observations of growth at the 30th day of the crop did not show any significant difference in height between the treatments. However, the plants grown in treatments with lime requirement between 3.4 and 4.7 tons per acre, were taller at the fifty day stage and their heights were found significantly increased. The controls with no lime put forth the shortest plants. Nair et al. (1957), investigating on similar lines on sesbania crop, obtained corroborative results with regard to application of lime and height of the crop.

Birch (1960), studying the effect of calcium in combination with fertilizers, reported positive response for lime on the lucerne crop in increasing the height of plants.

III. (a) Phosphates and Nitrogen Fixation:

From the results of numerous scientific investigations conducted in various parts of the world, ample evidence has accumulated to show that phosphates stimulate nitrogen fixation in most of the legumes. Several workers have shown that not only increase in total nitrogen of the crop due to increased growth is obtained, but also the nitrogen percentage of the plant material is increased.

Truesdell (1917), working on alfalfa, concluded that fertilising the crop with phosphate brought about an increase in total nitrogen through increased yields. He also reported there was an increase in the nitrogen content of the plant associated with the addition of phosphate fertiliser.

Fellers (1918) studied the effect of inoculation, fertiliser treatment and certain minerals on the yield, composition and nodule formation of soybeans. Based on these investigations, he reported increased protein content in the seeds of plants receiving superphosphate. Conducting a similar investigation in Canada field beans, soybeans and alfalfa, McTaggart (1921) indicated that phosphates were responsible for increasing the total nitrogen in the soil and nitrogen percentage of the legume. James, *et al.* (1944) reported an uniform increase in the rate of growth as well as the quantity of nitrogen fixed in all the phosphate treatments. Vyas and

Desai (1953) studied the effect of phosphoric acid on nitrogen fixation in pea plants. Two graded doses of 60 pounds and 120 pounds of phosphoric acid were utilised for the trial. They reported a significant increase equal to 109.3 per cent and 51.7 per cent in the quantity of nitrogen fixed in the aerial parts of the plant by 120 and 60 pounds of phosphoric acid respectively. They further reported that the increase in quantity of nitrogen in the soil was not significant where the aerial parts were not incorporated as compared with pre-legume soil nitrogen values.

In a study on the effect of various indigenous phosphatic manures on the nitrogen fixation through pea plants, Vyas (1953) concluded that all the manures tried were efficient in increasing the nitrogen content of the pea plant. The respective increases obtained in nitrogen percentage over the control were 47.6 per cent, 18.4 per cent, 35.5 per cent and 24.8 per cent, the manures used being superphosphate bonemeal, Trichy nodule phosphate and singhhum phosphate respectively.

Investigations made by Sen and Bains (1955) on the effect of farm yard manure and superphosphate on the yield, nodulation and nitrogen fixation by berseem indicated that superphosphate alone or in combination with farm yard manure increased significantly the nitrogen content over that of the control. Further, the increase obtained in soil nitrogen was found to be directly proportional to the increase in application of phosphoric acid.

Sikka and Jain (1958), studying the effect of phosphate manuring on the physical and chemical properties of the soil, observed that when guar (Cymopsis tetragonoloba) was fertilised with phosphates, the nitrogen content of the soil was increased in comparison with the non-fertilised plots. Panos (1959) corroborated the above finding by reporting that protein content of certain winter legumes was found increased as a result of phosphate fertilisation.

Though there is sufficient experimental evidence to prove the capacity of phosphates to increase the nitrogen content of legumes, a few investigations have failed to observe the same effect.

Chandani and Oberoi (1956), working on the value of certain legumes as green manures, concluded that application of phosphates to legumes like sannhemp, berseem, senji, peas, lentil, cluster beans, soybeans, cowpea and daincha did not increase the nitrogen content.

Nair et al. (1957) failed to obtain an increase in nitrogen fixation in Sesbania speciosa on application of phosphoric acid singly at a level of 30 pounds per acre.

Sen and Bains (1957), pursuing studies on phosphate manuring of legumes, failed to get any positive response and reported that there was very little difference in soil nitrogen in the phosphate treated plots. This was in contradiction to the findings reported in 1955 by the same authors in a similar study.

(b) <u>Effect of Phosphate on Nodulation:</u>

Application of phosphates has been shown to have a marked influence on nodule formation in legumes, by several workers.

Notable among some of the earliest workers in this field who deserves mention is Prucha (1915) who studied the effect of phosphates on nodulation in legumes. He observed better nodulation in field beans when small quantities of potassium acid phosphate and calcium mono-basic phosphate were applied.

Helz and Whiting (1928) concluded that phosphate fertilisers increased nodulation in soybeans when applied in quantities which were not inhibitory to germination.

Sewell and Gainy (1930) demonstrated that in a soil, deficient in lime, application of superphosphate was more beneficial in inducing nodulation in a crop of alfalfa than application of calcium alone.

(c) Effect of Phosphates on Growth and Yield of Legumes:

Phosphates as reported by several investigators play an important role in increasing the yield and dry matter content of legumes.

Whyte et al. (1953) suggested that phosphorus is not only important for nutrition of legumes, but is also essential for sustaining the soil microflora at a high level.

Truesdell (1917), studying the effects of application of phosphates to alfalfa, observed that phosphates increased the dry weight of the material. Fellers (1917) obtained striking increases in the yield and dry matter content by application of phosphate.

Similar increase in the dry matter content was reported by McTaggart (1921) in Canada field beans, soybeans and alfalfa by application of phosphates.

James et al. (1944), Vyas (1953) and Vyas and Desai (1953) have corroborated the above findings.

Sen and Rao (1953), reviewing the work done in India on phosphorus nutrition of legumes, reported that in soils where the phosphoric acid status was low, as in Bihar state, there was a marked response to phosphorus in the growth of cowpea, sannhemp and daincha.

Rao *et al*. (1954), studying the effect of increasing levels of phosphate manuring on berseem, concluded that application of phosphoric acid at 66 pounds per acre increased yields considerably.

Winter and summer legumes, when manured with phosphatic fertilisers, were found to have increased in their dry matter content.

Chandani and Oberoi (1956) and Khan and Mathur (1957), in their studies on manuring sannhemp with superphosphate at 80 pounds per acre recorded an increase in yield of green matter to the extent of three and a half hundredweights per acre. On similar studies by Rao *et al*. (1957), the yield and dry matter content of sannhemp were found increased by superphosphate application.

Sikka and Jain (1958) concluded that application of phosphates in combination with boron and molybdenum increased yield of guar by 31 per cent. Panos (1959) has also reported increased yield in annual legumes as a result of superphosphate application.

In investigations carried out by Relwani and Ganguly (1959) on green manuring in conjunction with application of fertilisers, it was indicated that though daincha responded to the application of superphosphate by producing more yield, the difference in yield of green matter was not statistically significant.

Rao and Shanker (1960), conducting investigations on sannhemp manured with superphosphate, reported increased yield in the phosphate treated plots.

Ballal and Natu (1961) studied the response of groundnut and matki to phosphate manuring in the scarcity tracts of Maharashtra. They reported positive response in yield for doses from 10 pounds phosphoric acid per acre onwards. At Akola they obtained response to phosphoric acid treatment for doses varying from 30 to 40 pounds per acre.

CHAPTER III

MATERIALS AND METHODS

An investigation was undertaken to study the comparative fixation of nitrogen by certain common legumes, namely, a) Arachis hypogaea; b) Sesbania speciosa; c) Sesbania aculeata; and d) Vigna sinensis.

1. Seed Material:

The legumes taken for investigation were those frequently included in crop rotations in the State. The duration of the legumes tried were as follows:-

Arachis hypogaea (groundnut)	-	105 days
Sesbania speciosa (sesbania)	-	150 days
Sesbania aculeata (daincha)	-	150 days
Vigna sinensis (cowpea)	-	90 days

The varieties used were TMV-2 for groundnut and local for others.

Recommended quantities of seed at the following rates were utilised:-

Arachis hypogaea	-	35 kilograms of kernel per acre

Sesbania speciosa - 15 kilograms per acre

Sesbania aculeata - 10 kilograms per acre

Vigna sinensis - 15 kilograms per acre

Agronomic investigations regarding the fixation of nitrogen by these legumes under local conditions have not been reported. Hence, they were selected for this investigation.

2. Field:

The investigation was laid out at the Central Farm, Agricultural College and Research Institute, Vellayani.

The soil where the investigation was conducted was typical red loam characteristic of the tract. The soils were highly leached and low in calcium and available phosphorus. Soil analysis was conducted for the following constituents and for pH and values obtained are furnished in Table I. The procedure followed in soil analysis is that outlined by the A.O.A.C. (1955).

Total nitrogen

Available phosphoric acid

Exchangeable calcium

pH

3. Manures and fertilizers:

No basal dressing of farm yard manure was given as there is no such local practice for legumes.

Calcium at the rate of 680 kilograms per acre in the form of calcium hydroxide and phosphoric acid at the rate of 14 kilograms per acre in the form of superphosphate were applied as a basal dressing in the treated plots. Nair et al. (1957) have reported beneficial effects with calcium and phosphoric acid at the above rates in laterite soils and hence the levels were chosen.

4. Lay out:

Design	-	Split plot
Size of plot	-	Gross area = 22' x 10' Net area = 20' x 8'
Replications	-	four

Since the number of characters studied was two, namely, nitrogen fixation by legumes and the influence of calcium at 680 kilograms per acre of calcium hydroxide and phosphoric acid at 14 kilograms per acre in the form of superphosphate, the design shown above was selected as suggested for standard Agricultural experimental techniques by Panse and Sukhatme (1961).

5. Sowing:

Groundnut was dibbled in the prepared field at a spacing of 9" x 9". Decorticated seeds of TMV-2 were dibbled uniformly in the assigned plots during the month of May.

Similarly, uniform broadcasting of the other legumes was also finished by the same period. All the legumes were seeded on the same day.

The following were the 20 combinations of treatments included in the trial:-

Control:

1) $l_0 + m_0$ 2) $l_0 + m_1$ 3) $l_0 + m_2$ 4) $l_0 + m_3$

Arachis hypogaea:

1) $l_1 + m_0$ 2) $l_1 + m_1$ 3) $l_1 + m_2$ 4) $l_1 + m_3$

Sesbania speciosa:

1) $l_2 + m_0$ 2) $l_2 + m_1$ 3) $l_2 + m_2$ 4) $l_2 + m_3$

Sesbania aculeata:

1) $l_3 + m_0$ 2) $l_3 + m_1$ 3) $l_3 + m_2$ 4) $l_3 + m_3$

Vigna sinensis:

1) $l_4 + m_0$ 2) $l_4 + m_1$ 3) $l_4 + m_2$ 4) $l_4 + m_3$

The four legumes were assigned to the main plots and the manurial combination to the sub-plots. The legumes and treatments are denoted by the following notations:-

l_0	-	No legume
l_1	-	Arachis hypogaea
l_2	-	Sesbania speciosa
l_3	-	Sesbania aculeata
l_4	-	Vigna sinensis
m_0	-	No manure

m_1	-	Phosphoric acid @ 14 kilograms per acre
m_2	-	Lime @ 680 kilograms per acre
m_3	-	Phosphoric acid + lime @ 14 kilograms per acre and 680 kilograms per acre, respectively

6. Crop growth:

During the period of growth the meteorological conditions were normal and the general condition of crop was also normal.

There was no evidence of pests and diseases and hence no plant protection measures were taken.

7. Harvest:

The different legumes were harvested at the 60 day stage. Observations and recordings of characters selected for study were conducted at the assigned periods. The plants marked at random were separately studied, harvested and subjected to chemical analysis.

8. Characters studied:

20 plants per plot were selected at random, labelled and the following attributes were studied:-

All measurements were recorded in the metric units.

(a) Height of plants:

The height of plants was measured on the 30 day stage and the 60 day stage after germination. All measurements were taken from the base of the plant to the tip of the highest terminal leaf bud.

(b) Number of Nodules:

The number of nodules present on the root system of the legumes on the day of harvest was counted and recorded. A day prior to dislodging the plants, the field was put under irrigation. The plants were carefully lifted with the use of a spade to avoid as far as possible mechanical dislodging of nodules and injury to the root system. The root system was washed free of soil matter by gently cleaning in water. The nodule count was taken and recorded.

(c) Green matter content:

The entire harvested crop from each plot was separately harvested and bundled. The weight of the total plant material harvested from each plot was separately found out and recorded; after which the weight of the marked plants in each plot was also separately estimated.

(d) Dry matter content:

The marked plants were dried at 70° C. for 48 hours followed by drying at 107° C. in an air oven till two consecutive weighings agreed. The weight of the material was recorded and this gave the dry matter content of the legume.

Nitrogen was estimated by the Kjeldahl's method as described in A.O.A.C. (1955).

Before the seeds were sown a representative sample was drawn from the lot of seeds of each legume and its nitrogen content determined.

(e) Nitrogen content of plant material:

From the air-dried plant material of each treatment, representative samples were taken and nitrogen estimated. The nitrogen fixed in the plant material was reduced to acre basis.

The above data was subjected to statistical analysis and the findings are reported in the text.

The quantity of nitrogen fixed through the agency of legumes in association with symbiotic bacteria was computed on the following basis:-

Original soil nitrogen (+) Nitrogen applied through seed material	=	a
Post harvest soil nitrogen (+) Nitrogen contained in the plant material	=	b

Gain in nitrogen through legume) = (b - a)

CHAPTER IV

EXPERIMENTAL RESULTS

The observations made on the legumes and the data collected pertaining to this investigation were analysed statistically and are presented in the tables below:-

TABLE I

SOIL ANALYSIS VALUES

Total nitrogen	-	0.06 per cent
Available phosphoric acid	-	0.004 per cent
Exchangeable calcium	-	2.1 milli-equivalents per 100 grams of soil
Pre-legume soil pH	-	5.7
Post-legume soil pH in calcium treated plots	-	5.9

Analysis of variance table for each of the characters studied, namely, height of legume at 30 and 60 days, green matter yield, dry matter content, number of nodules, nitrogen content of plant material and difference between pre and post legume soil nitrogen content was worked out and is furnished in the appendix (I - VII).

TABLE II

MEAN HEIGHT OF LEGUMES IN CENTIMETRES
AT 30 DAYS

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_0)	8.72	10.60	11.10	15.65	11.52
Phosphoric acid (m_1)	9.40	12.40	11.25	16.10	12.28
Calcium (m_2)	10.82	15.22	14.15	18.10	14.57
Calcium and phosphoric acid (m_3)	10.92	14.95	14.25	18.47	14.65
Mean	9.97	13.29	12.68	17.08	14.26

C.D. (at five per cent) for legumes: 0.36 l_4 l_2 l_3 l_1

C.D. (at five per cent) for nutrients: 1.97 $\overline{m_3 \; m_2}$ $\overline{m_1 \; m_0}$

Table II clearly shows that the difference between the legumes was statistically significant.

Vigna sinensis recorded the maximum height at 30 days followed by Sesbania speciosa, Sesbania aculeata and Arachis hypogaea.

There was no significant difference between the treatments receiving calcium alone and the combination of calcium and phospherus. However, these treatments proved superior to phosphorus alone and the control which were on par.

TABLE III

MEAN HEIGHT OF LEGUMES IN CENTIMETRES AT 60 DAYS

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_0)	20.12	56.45	54.85	44.45	43.98
Phosphoric acid (m_1)	20.28	60.07	58.58	49.10	47.01
Calcium (m_2)	24.40	87.00	85.78	74.03	67.81
Calcium and phosphoric acid (m_3)	24.55	89.47	87.52	77.30	69.71
Mean	22.30	73.30	71.71	61.20	57.13

C.D. (at five per cent) for legumes: 4.03 $\overline{l_2 \; l_3} \; l_4 \; l_1$

C.D. (at five per cent) for nutrients: 2.19 $\overline{m_3 \; m_2} \; m_1 \; m_0$

At the 60 days stage Sesbania speciosa and Sesbania aculeata were superior to Vigna sinensis in height. There was no statistical significance between the heights of Sesbania aculeata and Sesbania speciosa. Arachis hypogaea recorded the least height at 60 days.

Application of lime and phosphoric acid in combination and lime alone resulted in taller plants, when compared with treatments receiving phosphorus only. The difference in heights between plants treated with a combination of calcium and phosphoric acid and calcium alone was not statistically significant. Phosphorus treated plants recorded heights statistically superior to those in the control plots.

TABLE IV

MEAN WEIGHT OF GREEN MATTER IN KILOGRAMS AT 60 DAYS

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_0)	7.48	30.93	32.25	33.85	26.12
Phosphoric acid (m_1)	8.58	33.05	31.43	38.90	27.99
Calcium (m_2)	13.28	56.08	55.88	51.20	44.11
Calcium and phosphoric acid (m_3)	13.45	61.05	57.10	52.10	45.93
Mean	10.69	45.28	44.17	44.01	36.04

C.D. (at five per cent) for legumes: 1.36 $\overline{l_2 l_3 l_4}$ l_1

C.D. (at five per cent) for nutrients: 1.52 m_3 m_2 m_1 m_0

The values in the table indicate that yield of green matter was not different between Sesbania speciosa, Sesbania aculeata and Vigna sinensis. But the green matter yield of these three legumes was superior to that of Arachis hypogaea.

The yield obtained from the lime and phosphorus treated plots was the highest. This was followed by the yields obtained from the plots receiving lime alone and phosphorus alone. The control recorded the least yield.

TABLE V

MEAN WEIGHT OF DRY MATTER IN KILOGRAMS

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_0)	1.22	5.52	6.97	6.47	5.05
Phosphoric acid (m_1)	1.32	5.90	6.82	7.40	5.36
Calcium (m_2)	2.12	10.27	12.25	9.95	8.62
Calcium and phosphoric acid (m_3)	2.15	11.02	12.72	10.52	9.11
Mean	1.70	8.17	9.69	8.58	7.04

C.D. (at five percent) for legumes: 0.62 l_3 $\overline{l_4 \quad l_2}$ l_1

C.D. (at five percent) for nutrients: 0.33 m_3 m_2 $\overline{m_1 \quad m_0}$

Regarding the dry matter content of the legumes, the dry matter content of Sesbania aculeata was superior to that of Sesbania speciosa and Vigna sinensis, there being no statistical significance between the latter two. However, the dry matter content obtained for these legumes was superior to that of Arachis hypogaea.

Calcium and phosphorus in combination influenced dry matter content more than only calcium treated plots. However, calcium treated plots in turn yielded more dry matter than both the phosphorus treated plots and the control which were on par.

TABLE VI

MEAN NUMBER OF NODULES PER PLANT AT 60 DAYS

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Means
No nutrients (m_1)	39.62	26.85	110.13	3.75	45.09
Phosphoric acid (m_2)	43.80	36.30	117.02	4.93	50.51
Calcium (m_3)	92.28	49.22	114.93	14.20	75.16
Calcium and phosphoric acid (m_4)	119.40	49.52	150.88	18.30	84.52
Mean	73.78	40.47	130.74	10.29	63.82

C.D. (at five per cent) for legumes: 8.72 $l_3 \; l_1 \; l_2 \; l_4$

C.D. (at five per cent) for nutrients: 5.58 $m_3 \; m_2 \; \overline{m_1 \; m_0}$

It is very clearly seen from the table that the number of nodules produced by <u>Sesbania aculeata</u> was greater than <u>Arachis hypogaea</u> followed by <u>Sesbania speciosa</u> and <u>Vigna sinensis</u>.

The average number of nodules produced in plants grown in plots receiving lime and phosphorus in combination was significantly greater than in plots receiving lime alone. Plants grown in phosphorus treated plots and the control recorded the least number of nodules, therebeing no statistical significance between them.

TABLE VII

NITROGEN CONTENT OF THE PLANT MATERIAL

	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_1)	1.33	1.53	2.30	1.89	1.86
Phosphoric acid (m_2)	1.30	1.53	2.59	1.83	1.81
Calcium (m_3)	1.43	1.63	2.95	1.93	1.99
Calcium and phosphoric acid (m_4)	1.53	1.70	3.03	2.00	2.07
Mean	1.41	1.69	2.72	1.91	1.91

C.D. (at five per cent) for legumes: 0.161 $l_3 \quad l_4 \quad l_2 \quad l_1$

C.D. (at five per cent) for nutrients: 0.77 $\overline{m_3 \quad m_2} \quad \overline{m_0 \quad m_1}$

The difference in nitrogen content between the four different legumes was statistically significant. The highest percentage of nitrogen was recorded in Sesbania aculeata followed by Vigna sinensis followed in turn by Sesbania speciosa and last of all Arachis hypogaea.

Plants receiving lime and phosphoric acid in combination and lime alone were statistically superior in nitrogen content to plants receiving phosphoric acid alone and no nutrients. There was no statistical difference between plants receiving a combination of lime and phosphoric acid and plants receiving lime alone. The difference between plants receiving phosphorus only and the control was statistically significant, but was inferior to the other treatments.

TABLE VIII

MEAN INCREASE IN SOIL NITROGEN AS A RESULT OF GROWING LEGUMES

	No legume (l_0)	Arachis hypogaea (l_1)	Sesbania speciosa (l_2)	Sesbania aculeata (l_3)	Vigna sinensis (l_4)	Mean
No nutrients (m_0)	0.002	0.004	0.001	0.008	0.009	0.006
Phosphoric acid (m_1)	0.004	0.003	0.009	0.017	0.016	0.012
Calcium (m_2)	0.003	0.004	0.006	0.014	0.011	0.009
Calcium and phosphoric acid (m_3)	0.002	0.007	0.004	0.017	0.005	0.009
Mean	0.003	0.004	0.005	0.014	0.010	0.009

C.D. (at 5 per cent) for legumes: 0.0061 $\overline{l_3 \quad l_4}$ $\underline{l_4 \quad l_2 \quad l_1 \quad l_0}$

From the data presented (Table VIII) it is apparent that Sesbania aculeata is superior to all the other legumes under study in increasing the nitrogen content of the soil. All the other legumes are on par and do not increase the soil nitrogen appreciably when compared to the soil in the control.

There is no statistical difference in soil nitrogen due to any of the manure treatments.

CHAPTER V

DISCUSSION

An investigation was carried out at the Agricultural College and Research Institute, Vellayani, to compare the relative nitrogen fixing efficiencies of four common legumes, namely, *Arachis hypogaea*, *Sesbania speciosa*, *Sesbania aculeata* and *Vigna sinensis*.

Data in regard to the above attributes for the legumes under study are limited.

The experiment was laid out in split plot design. Lime and phosphoric acid were included in this investigation in the form of calcium hydroxide at the rate of 680 kilograms per acre and superphosphate at the rate of 14 kilograms phosphoric acid per acre.

The analysis of data for the various factors studied indicated that in the presence of lime and phosphoric acid, *Sesbania aculeata* was the most efficient in fixing nitrogen.

The function of lime and phosphoric acid on the various attributes studied was found varying.

The findings are discussed hereunder:-

1. Height of legumes:

The data presented in Tables II and III show the height of the four legumes under study at the 30th and the 60th day. It was observed that the trend of heights among the legumes differed at the first and second stages of observation.

At the first stage the order of superiority is *Vigna sinensis*, *Sesbania speciosa*, *Sesbania aculeata* followed by *Arachis hypogaea* and at the second stage as *Sesbania speciosa*, *Sesbania aculeata*, *Vigna sinensis* and *Arachis hypogaea*. At the first stage they were statistically superior in the order presented and in the second, *Sesbania speciosa* and *Sesbania aculeata* were superior to the other two, but were found on par as far as the height factor was concerned.

Since variations in height within the legumes is purely a function of the genetic make up under identical conditions of growth, the variation noted in the present study is to be attributed to the inherent character of the legume concerned. A comparison between the effects due to the application of lime and phosphoric acid, both singly and in combination, showed the following:-

Application of lime @ 680 kilograms per acre produced a significant increase in the height of the plants. This was also found statistically superior over phosphoric acid alone and the control. The soils in which the investigation was carried out had a very low calcium status (Table I). They were also highly leached.

The application of lime at rates utilised could have effectively raised the pH of the soil from the initial level of 5.7. Nair <u>et al</u>. (1957) have observed an increase in soil pH in lime applied plots at the above rates, under similar soil conditions. This raising of the pH to a range considered optimum for the activities of the soil microflora could have accounted for an increase in the available nitrogen. Since initial growth in plants is related to the available nitrogen status in soils, the differences in height of plants observed in the present study can be attributed to the increased availability of nitrogen, consequent on the application of lime.

Application of phosphoric acid at the rate of 14 kilograms per acre was observed to have failed to exert significant influence over the control for the factor studied. This can be accounted for, as follows:-

Phosphoric acid appears to have no profound direct influence on the initial vegetative growth rate of plants, other than promoting the growth of the root system. If at all phosphoric acid exerts a positive influence on the factor studied, the predominance of sesquioxides in the highly leached soils would have retarded the availability of the added mono-calcium phosphate.

However, for the character studied at the second stage in 60 days in contrast with the thirty days observation, phosphoric acid treatment has proved superior to the control. This could be due to the effect of phosphoric acid in increasing the total leaf area which consequently has a pronounced effect on photosynthetic efficiency of the plant, as proved by the classical experiments at Rothamstead and reported by Russell (1961). Visual observations of the crop made at the 60 day stage indicated an apparent increase in size of the leaf.

The application of lime and phosphoric acid in combination gave significantly different results. This treatment proved superior to all other treatments tried in this study. The fact that application of phosphoric acid alone failed to produce a significant difference in height of plants, proves that calcium was the limiting factor as far as growth was concerned. This becomes very clear from the fact that the height of plants in plots treated with phosphoric acid in combination with lime was on par with those treated with lime alone.

It was also observed that phosphoric acid in combination with lime was superior to the application of phosphoric acid alone. The superiority of phosphoric acid in the presence of lime can be explained as follows:-

When lime was added to the soil its pH was raised to a level where the availability of native as well as the added phosphoric acid was at a desirable maximum. This accounts for the superiority of phosphoric acid in combination with lime over phosphoric acid alone.

The same trend in the results was observed at the second stage also except for the fact that phosphoric acid exerted a significant superiority over the control. This obviously could be due to the legumes having reached the proper stage in growth, where phosphoric acid had started to exert all its magnifying functions, within the plant body.

2. Green matter:

A study of the comparative green matter output (Table IV) showed the legumes in the following order of superiority. *Sesbania speciosa* > *Sesbania aculeata* > *Vigna sinensis* > *Arachis hypogaea*. However, the yield of green matter for the first three legumes did not statistically differ among themselves. Since the green matter yield is mainly a function of the vegetative growth, by virtue of the inherent genetic makeup of each individual legume, the superiority of the three other legumes over *Arachis hypogaea* is explainable as a natural phenomenon.

The results presented in Table IV further reveal that maximum green matter output corresponded to the treatment involving a combination of lime and phosphoric acid. This finding is in common with the results presented for the height factor.

Legumes as a group are generally considered to be calcicoles. Also, the importance of phosphoric acid in the nutrition of legumes has been extensively observed. Therefore, it is reasonable to presume that the increased green matter output is the direct result of beneficial effects of lime and phosphoric acid, both individually and in combination. The increase in green matter yield could also be attributed to an increase in the available supply of nitrogen in presence of phosphoric acid and lime which was utilised by all the legumes. This view is in line with the conclusions arrived at by Mooers (1912). Curtis and Clark (1950) reported that growth is greatly retarded in the absence of calcium. This explains the superiority of treatments involving lime over phosphoric acid alone and the control. The importance of calcium, particularly in the nutrition of legumes under similar conditions, has been stressed by many other workers, notably Jones and Edwards (1954).

The response of legumes to added phosphoric acid appears to be conspicuous only under conditions of a very low level of available phosphoric acid. In the present study also the positive response observed in the green matter content under phosphoric acid treatments can be attributed to the poor status of available phosphoric acid (Table I). Relwani and Ganguly (1959) observed in daincha that the application of phosphoric acid to the soil failed to record any increase in green matter yield under a sufficiently high available phosphoric acid level. It appears that under conditions of adequate phosphoric acid, legumes may fail to respond to application of additional phosphoric acid. This may be due to the greater ability of legumes to utilise the native soil phosphoric acid.

3. <u>Dry matter:</u>

In the study of the dry matter production of the different legumes (Table V), it was observed that *Sesbania aculeata* was significantly superior to the rest. *Sesbania speciosa* and *Vigna sinensis* were among themselves, on par but superior to *Arachis hypogaea*. It is not very unreasonable to presume that under uniform conditions of growth the trend in the dry matter content should be more or less similar to that of green matter output. However, from the data for dry matter it was observed that statistically the trend is altered to some extent. This may be presumed to be due to variations in maturity of the plant tissue of the legumes at the second stage of harvest, viz., 60th day.

Regarding the effect of lime and phosphoric acid, it was observed that their combination was superiormost, followed by lime alone and phosphoric acid alone.

The positive role of lime in combination with phosphoric acid in the nutrition of legumes, as already discussed elsewhere, applies in this context as well. In addition, calcium, the active element in lime, is considered to be one of the few essential elements entering into the frame work of the plant. Curtis and Clark (1950) noted that no new cell walls were laid down when calcium became limiting.

Similarly, properties like increased cell division, fat and albumin formations and photosynthetic efficiency through an increased leaf area are attributed to phosphoric acid.

The positive response for phosphoric acid alone and in combination with lime, as shown in the tables, could be explained in the light of the above functions of calcium and phosphoric acid respectively.

4. <u>Nodules:</u>

From the figures presented in table VI the legumes in their capacity to produce nodules were in the following order of merit:-

Sesbania aculatea > *Arachis hypogaea* >

Sesbania speciosa > *Vigna sinensis*

The difference noted between the legumes in the character studied could be the cumulative result of the following factors:-

a) In the study undertaken, the legumes included are classified as belonging to different genera and species. Legumes are found to widely differ in their inherent capacity to form nodules in symbiosis with the respective organisms.

b) The organisms responsible for nodulation are specifically different for each legume, since no two legumes fall under the same cross inoculation group.

c) Even the specific strain of inoculum responsible for nodulation in a particular genera or species; may vary in their capacity to produce effective nodules among its different members.

In the data presented (Table VI) on the functions of lime and phosphoric acid on nodulation it was observed that the combined influence of lime and phosphoric acid was significantly superior to all other treatments. Lime supply was again observed to be superior to phosphoric acid alone and the control, the latter two of which, however, not differing between themselves.

From the trend of results it was observed that lime was the limiting factor for nodulation under conditions of of the present investigation. The direct role of calcium on the rhizobial activity and the subsequent nodulation process has been emphasised by many investigators. In the present investigation also, as a result of addition of lime, significant increase in nodulation has been recorded. This could be due to an increased maintenance of a rhizobial population in the soil followed by subsequent invasion of plant roots. McCalla (1937) reported that the concentration of available calcium needs to be relatively high to sustain an active rhizobial population, and under a low calcium regime the bacteria change to an abnormal chromogenic form in which they are unable to invade the plant. Albrecht (1937) has even suggested that it is the low calcium content of non-legumes that determine their non-invasion.

Although phosphoric acid treatment was observed to be on par with the control, when in combination with lime, it was observed to be the ideal combination for promoting nodulation. Since the combined applications proved superior to applications of lime alone, phosphoric acid could be presumed to have a positive influence on nodulation. Sufficient evidence is available in support of this view.

Phosphoric acid is an important element in influencing symbiotic nitrogen fixation. Phosphoric acid is also important in relation to the early infection stages of nodulation. In this case the effect is directly upon the bacteria and not the plant. For the rhizibia to migrate through the soil towards the root system of the legume they must be in a motile flagellate state on which phosphoric acid has a pronounced stimulatory effect.

Application of superphosphate to the soil usually has the effect of markedly stimulating numbers of organisms of the soil bacterial flora, and rhizobia similarly respond. This has been reported by Whyte _et_ _al_. (1953).

5. _Nitrogen content of Plant material:_

From data presented in table VII it was observed that for the nitrogen content of the legumes they were in the following order:-

Sesbania aculeata _Vigna sinensis_ _Sesbania speciosa_ _Arachis hypogaea_

Sesbania aculeata was observed to have the highest nitrogen content among the four legumes studied. In the study of nodules (Table VI) the same legume showed the highest nodule count. From experiments on legumes, it is established that the tops are responsible for higher proportion of nitrogen.

Vigna sinensis was observed to be second in the order of merit for the character studied. This, however, has shown the least number of nodules. It follows that what little nodules were present should have been effective and that the balance in total nitrogen content increase could be attributed to the tops. This finding has been corroborated by Mirchandani and Khan (1953), who also put the above two legumes in the same order as in this study as far as nitrogen values were concerned.

From data observed as a result of application of lime and phosphoric acid, both singly and in combination, it was observed that the treatment where lime was applied in combination with phosphoric acid proved statistically superior to all other treatments. Second in order was the treatment where lime alone was applied and this was found to be on par with the former.

Control and treatments with only phosphoric acid between them were on par, but were found to be inferior to the first two treatments, This result can be explained as follows:-

The application of lime helped in the rapid mineralisation of organic nitrogen present in the soil. Plants could absorb greater amounts of available nitrogen and this in turn was metabolised into protein nitrogen in the presence of sufficient quantity of phosphoric acid. This explanation will hold true for the observed phenomenon of the superiority of lime and phosphoric acid over other treatments. Even the presence of active calcium in the lime alone might have helped the mineralisation of organic nitrogen, and the subsequent absorption of this nitrogen by the plants in the treated plots. This could possibly be the reason for the superiority of lime alone over the treatment involving phosphoric acid alone.

Role of phosphoric acid in nitrogen mineralisation does not appear to be so pronounced.

The positive role of calcium and phosphoric acid in nodule formation and subsequent fixation of gaseous nitrogen to the organic form is an established fact. In the present study also this finding has been upheld.

However, between calcium and phosphoric acid treatments under soil conditions investigated, calcium in the form of lime appeared to be more of a limiting factor than phosphoric acid. This explains the superiority of calcium alone. Nair _et al_. (1957) obtained similar results in a study involving the same treatments under similar conditions.

Regarding the effects of phosphoric acid, it was found that phosphoric acid in the form of superphosphate was not superior to no manure treatment. Under acidic conditions, superphosphate applied, in which the phosphoric acid is in the mono-calcic form, could possibly have rapidly combined with the sesquioxides present in the soil and got 'reverted' to unavailable forms. This could explain the lack of response for the applied superphosphate in the investigation.

6. Soil nitrogen:

From the study of the data presented in Table VIII, *Sesbania aculeata* seems to be more effective in enriching the soil nitrogen content than the other legumes under study. However, this capacity is equally shared by *Vigna sinensis* when statistically compared.

It is also seen that the nitrogen in the soil has not been increased by the three legumes, namely, *Vigna sinensis*, *Arachis hypogaea*, *Sesbania speciosa* as the soil analysis value obtained are on par with values of nitrogen of the soil in the control.

In the present investigation, *Sesbania aculeata* had larger number of root nodules compared with others. A good part of these nodules would have been returned in the organic form to the soil medium. A part of the nitrogen in *Sesbania aculeata* which recorded the highest values in nitrogen analysis of the plant material could have been excreted into the soil medium. Such a phenomenon has been observed and corroborated by Mirchandani and Misra (1957). Both these processes would account for the superiority of Sesbania aculeata in enriching the soil nitrogen.

However, Sesbania aculeata appears to be on par with Vigna sinensis. It is of interest to note that Vigna sinensis has recorded the least number of nodules, but the nitrogen content of the plant material was next only to Sesbania aculeata. It could be reasonably presumed that in the case of Vigna sinensis all the nodules were effective and the increase in soil nitrogen statistically equal to Sesbania aculeata could be exclusively due to the excretion of nitrogen into the soil medium.

In the case of Sesbania speciosa and Arachis hypogaea, the contribution towards enrichment of soil nitrogen either through the recognised phenomenon of nodule sloughings and excretion of fixed nitrogen do not appear to be as effective as compared with the control.

The results presented in tables for soil nitrogen analysis (Table VIII) clearly reveal that Sesbania speciosa and Arachis hypogaea though not as efficient as Sesbania aculeata and Vigna sinensis, have not depleted the soil nitrogen in spite of their standing in the field during the growth phase studied. In fact, they have exhibited a tendency to contribute slightly towards the increase in soil nitrogen content, when compared with the control. This proves that legumes are 'nitrogen savers' and exert the minimum draught on the soil nitrogen as reported by Buckman and Brady (1960). From the trend of the data on nitrogen status of the soil after the growth of the legume, the following facts also emerge:-

By growing a legume and its subsequent removal it does not necessarily contribute towards the enrichment of the soil nitrogen reserve. It is doubtful whether an increase, both in the total as well as available nitrogen, is effected by merely growing legumes.

In the present study the legumes were not incorporated in the soil and this could be the reason for the comparative inefficiency of *Sesbania speciosa* and *Arachis hypogaea* compared with the control.

However, inasmuch as there has been no depletion in soil nitrogen, as indicated by pre and post legume soil analysis data, it is obvious that legumes are to be preferred to non-legumes in regard to the sustained maintenance if not enrichment of soil nitrogen . They thus have their merited place in any proper cropping pattern.

CHAPTER VI

SUMMARY AND CONCLUSION

With a view to study the comparative nitrogen fixing capacities of the four legumes, namely, Arachis hypogaea, Sesbania speciosa, Sesbania aculeata and Vigna sinensis, an investigation was conducted at the Agricultural College and Research Institute, Vellayani, during 1962-'63 (May--July). Superphosphate at 14 kilograms phosphoric acid per acre and calcium in the form of calcium hydroxide at 680 kilograms per acre were the treatments included in the study.

Arachis hypogaea was dibbled 9" x 9" apart and the rest of the legumes were sown broadcast. Plant characters such as height, fresh weight, dry weight, number of nodules, nitrogen content of the plant material and pre-legume and post-legume soil nitrogen were estimated and recorded.

The studied characters were statistically analysed and the following conclusions are drawn:-

1. (a) Vigna sinensis recorded the maximum height at 30 days followed by Sesbania speciosa, Sesbania aculeata and Arachis hypogaea.

(b) There was no difference between heights of plants receiving calcium and phosphoric acid in combination and calcium alone; but these were superior to plants grown in plots receiving phosphoric acid alone and in the control.

2. (a) At 60 days stage there was no difference between heights of *Sesbania speciosa* and *Sesbania aculeata*. These proved superior to *Vigna sinensis* which in turn recorded greater height than *Arachis hypogaea*.

(b) Calcium and phosphoric acid in combination was superior to calcium alone in its influence in increasing the height of legumes. Plants receiving calcium alone were superior to those receiving phosphoric acid alone, which in turn was superior to the plants in the control for the height factor.

3. The green matter output on comparison between *Sesbania speciosa*, *Sesbania aculeata* and *Vigna sinensis* was found to be on par and *Arachis hypogaea* proved inferior with regard to green matter yield. Plants receiving a combination of calcium and phosphoric acid recorded a higher green matter out-turn than plants treated with calcium alone. Plants treated with phosphoric acid alone recorded still lower yields, but were superior to plants in the control.

4. (a) As regards the dry matter content, *Sesbania*

aculeata proved to be superior to the other three legumes. Vigna sinensis and Sesbania speciosa were on par, but superior to Arachis hypogaea.

(b) The influence of calcium and phosphoric acid in combination proved statistically superior to that of calcium alone in increasing the dry matter content. Plants grown in plots receiving phosphoric acid alone and control were on par and inferior to plants receiving lime alone.

5. (a) The nodule count made indicated that Sesbania aculeata was superior to Arachis hypogaea, which in turn was superior to Sesbania speciosa. Vigna sinensis recorded the least number of nodules.

(b) The effect on nodulation oflime and phosphoric acid in combination proved superior in increasing the number of nodules to that of lime alone. Plants treated with phosphoric acid and the control were on par and inferior to plants treated with calcium alone.

6. (a) The nitrogen content of the legumes on chemical analysis, was found highest in Sesbania aculeata followed by Vigna sinensis, Sesbania speciosa and Arachis hypogaea.

(b) Plants treated with calcium and phosphoric acid in combination and calcium alone proved to have the highest nitrogen percentage, followed by plants treated with phosphoric acid alone and in the control.

7. (a) Post-legume soil analysis proved that Sesbania aculeata was the most efficient in the capacity of enriching the soil nitrogen content. However, Vigna sinensis also was found to be on par with Sesbania aculeata. It was also observed that Vigna sinensis was again on par with the other two legumes, namely, Sesbania speciosa and Arachis hypogaea. In case of the latter two legumes, though not as efficient as Sesbania aculeata and Vigna sinensis, they have not depleted the soil nitrogen content despite their standing in the field during the growth phase studied.

(b) There was no statistical difference between the fertilizer treatments over that of the control.

(c) Thus the legumes under study were observed to be 'nitrogen savers' and exerted the minimum draught on the soil nitrogen.

In summarising, it is clear that under the laterite conditions of soil studied, Sesbania aculeata was the most efficient legume in fixing nitrogen. Its maximum efficiency was observed in the presence of lime and superphosphate at 680 kilograms of calcium hydroxide and 14 kilograms of phosphoric acid per acre respectively.

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* Original not seen.

APPENDIX I

Height in 30 days - Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	0.14	3	0.05	0.02
Legume	412.21	3	137.40	654.30*
$Error_1$	1.83	9	0.21	..
Nutrients	122.23	3	40.74	145.53*
Interaction	10.59	9	1.18	4.21*
$Error_2$	10.12	36	0.28	..

* Significant at 5 per cent level

APPENDIX II

Height in 60 days - Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	3.79	3	1.26	0.05
Legume	27181.26	3	9060.42	355.42*
$Error_1$	229.43	9	25.49	..
Nutrients	8766.03	3	2922.01	296.91*
Interaction	1961.24	9	217.91	22.94*
$Error_2$	341.36	36	9.48	..

* Significant at 5 per cent level

APPENDIX III

Weight of green matter - Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	6.59	3	2.16	0.73
Legume	13716.05	3	4572.02	1560.40*
$Error_1$	23.46	9	2.93	..
Nutrients	5214.84	3	1738.28	382.94*
Interaction	1205.84	9	123.98	27.30*
$Error_2$	163.31	36	4.54	..

* Significant at 5 per cent level

APPENDIX IV

Weight of dry matter - Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	0.31	3	0.10	0.02
Legume	627.81	3	209.27	387.54*
$Error_1$	0.49	9	0.54	..
Nutrients	218.17	3	72.72	346.29
Interaction	55.06	9	6.12	29.14*
$Error_2$	7.62	36	0.21	..

* Significant at 5 per cent level

APPENDIX V

Number of nodules - Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	309.82	3	103.27	0.85
Legume	127812.32	3	42604.11	356.87*
$Error_1$	1074.46	9	119.38	..
Nutrients	152706.71	3	50902.24	14.31*
Interaction	7516.13	9	835.13	0.23
$Error_2$	127969.83	36	3554.72	..

* Significant at 5 per cent level

APPENDIX VI

Nitrogen content of plant material
Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	0.13	3	0.04	1.00
Legumes	16.17	3	5.39	134.75*
$Error_1$	0.36	9	0.04	..
Nutrients	1.12	3	0.37	3.01*
Interaction	0.59	9	0.66	5.50*
$Error_2$	0.42	36	0.12	..

* Significant at 5 per cent level

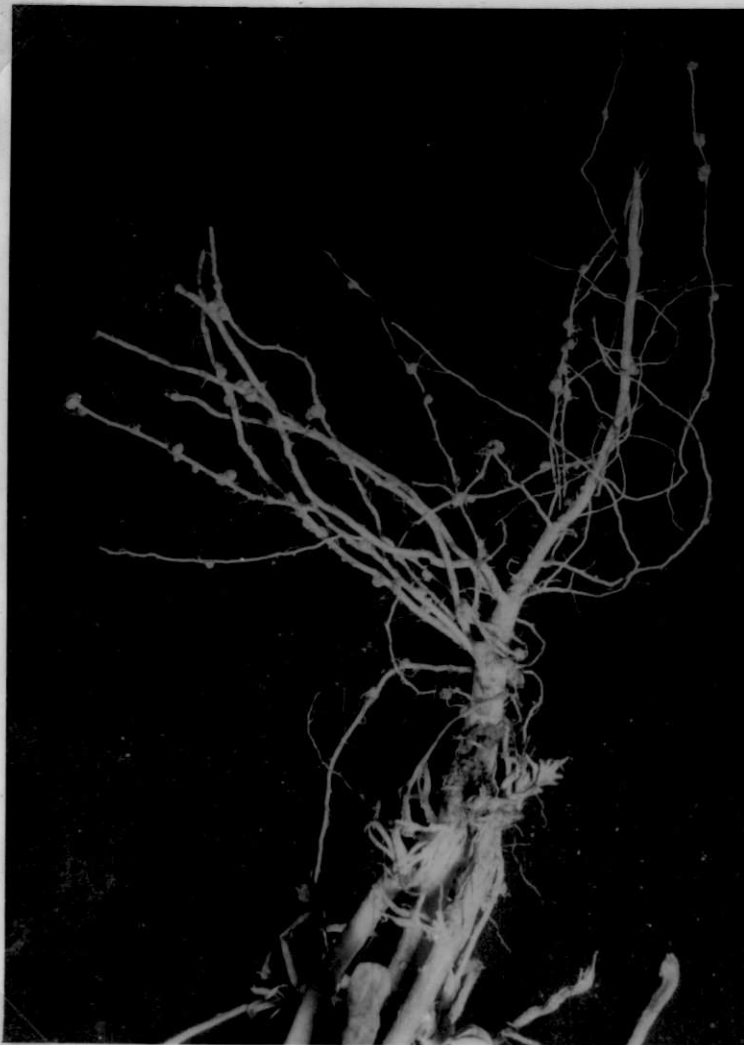
APPENDIX VII

Difference between pre and post legume soil nitrogen
Analysis of variance

Source	S.S.	D.F.	M.S.	'F'
Block	0.000098	3	0.000025	0.42
Legume	0.001315	4	0.000438	7.42*
$Error_1$	0.000710	12	0.000059	..
Nutrients	0.000194	3	0.000064	1.56
Interaction	0.001989	12	0.000165	4.00*
$Error_2$	0.001828	45	0.000041	..

* Significant at 5 per cent level

Nodules present in the root system of *Arachis hypogaea* in absence of both calcium and phosphorus.



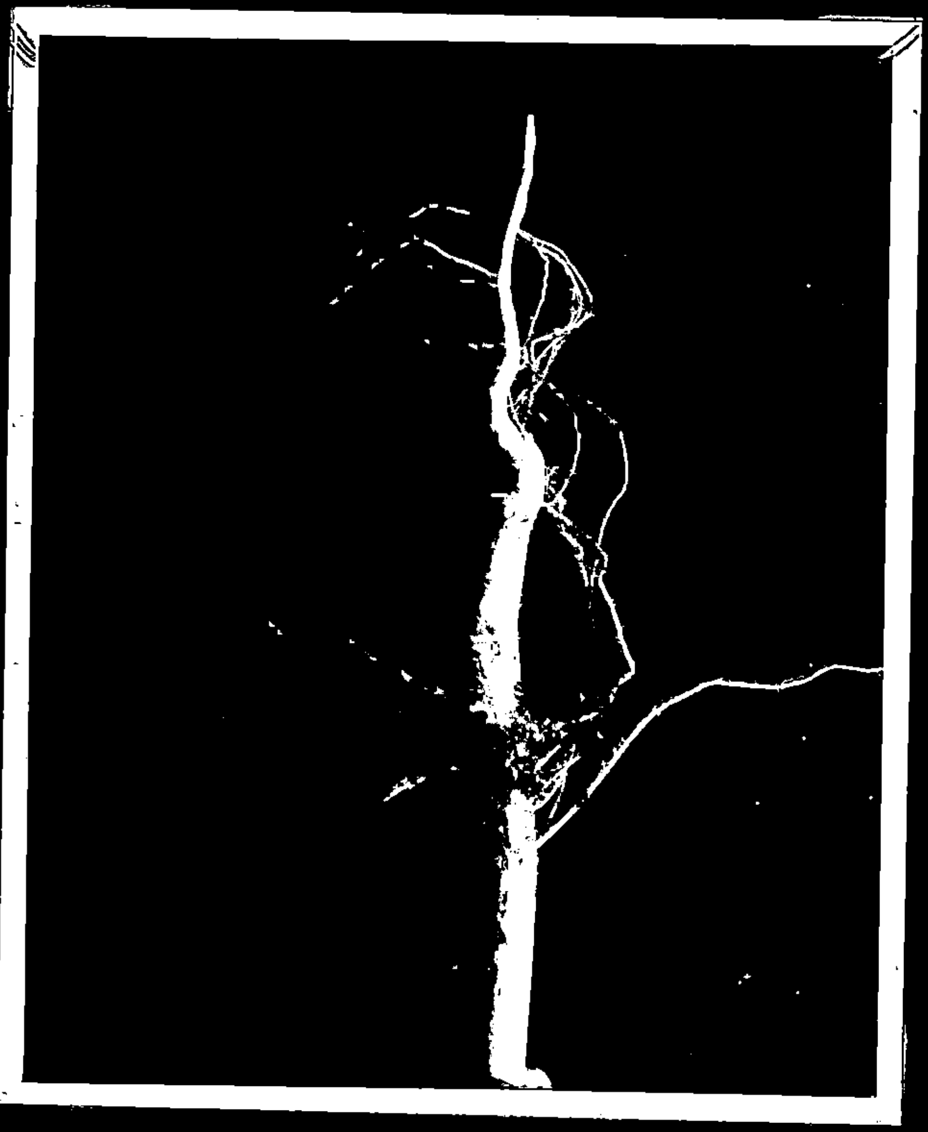
Nodules present in the root system of *Arachis hypogaea* in the presence of phosphorus.

Nodules present in the root system of *Arachis hypogaea* in the presence of calcium.

Nodules present in the root system of _Arachis hypogaea_ in the presence of phosphorus and calcium.

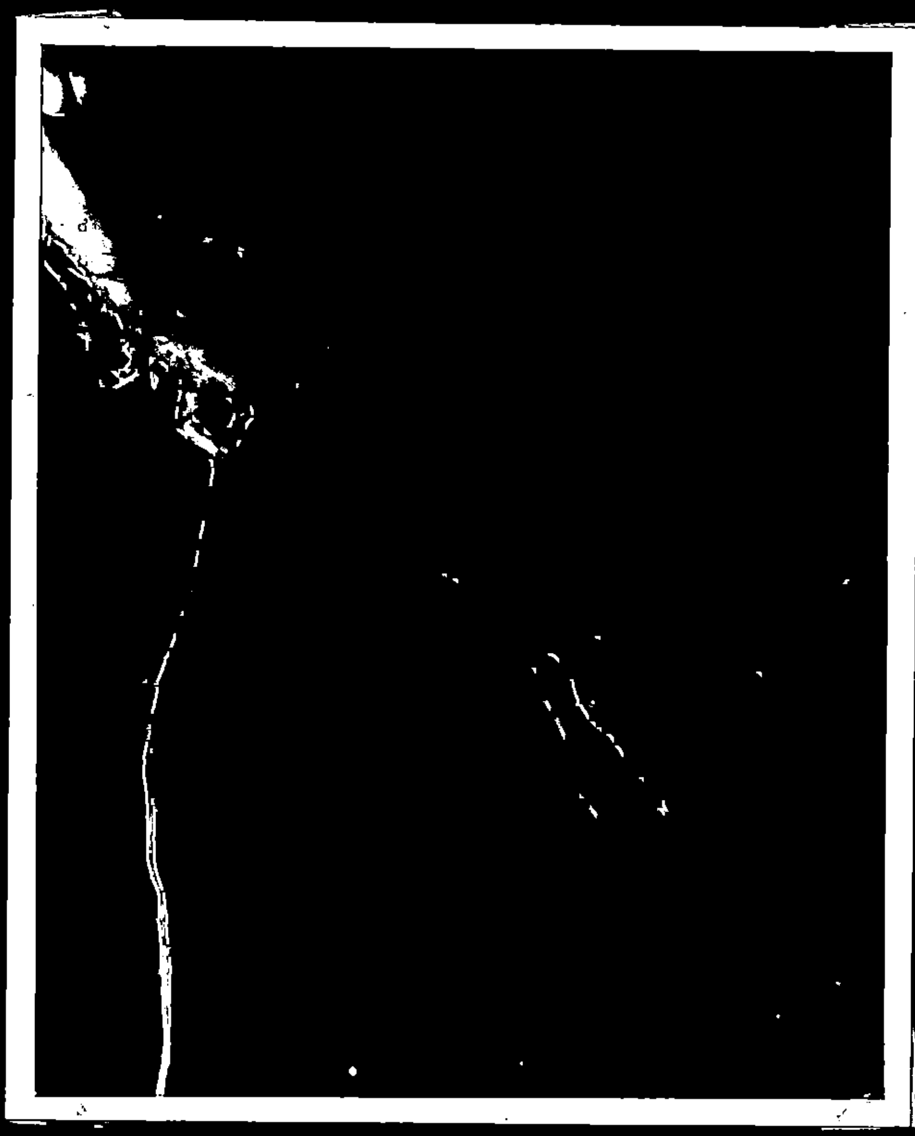
Nodules present in the root system of *Sesbania speciosa* in the absence of both calcium and phosphorus.

Nodules present in the root system of <u>Sesbania speciosa</u> in the presence of phosphorus.



Nodules present in the root system of *Sesbania speciosa* in the presence of calcium.

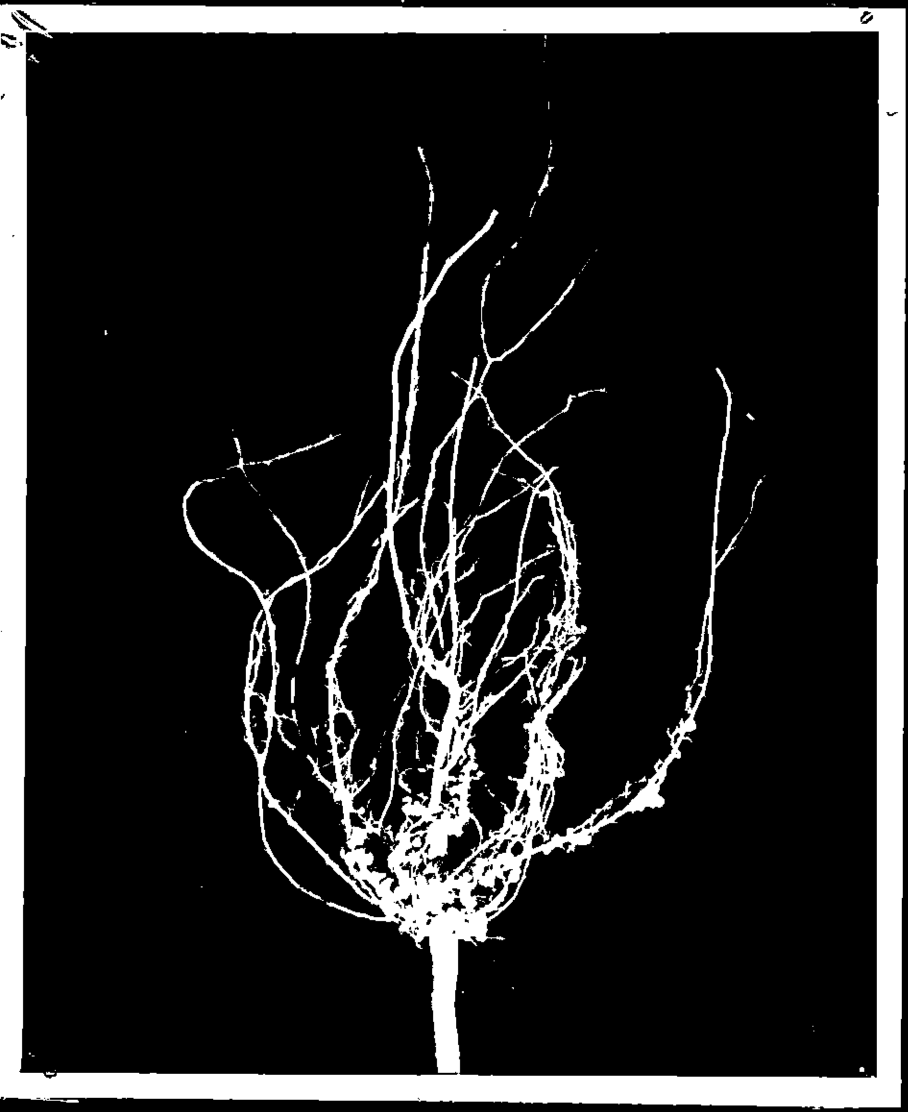
Nodules present in the root system of <u>Sesbania</u> <u>speciosa</u> in the presence of phospherus and calcium.



Nodules present in the root system of _Sesbania aculeata_ in the absence of both calcium and phosphorus.

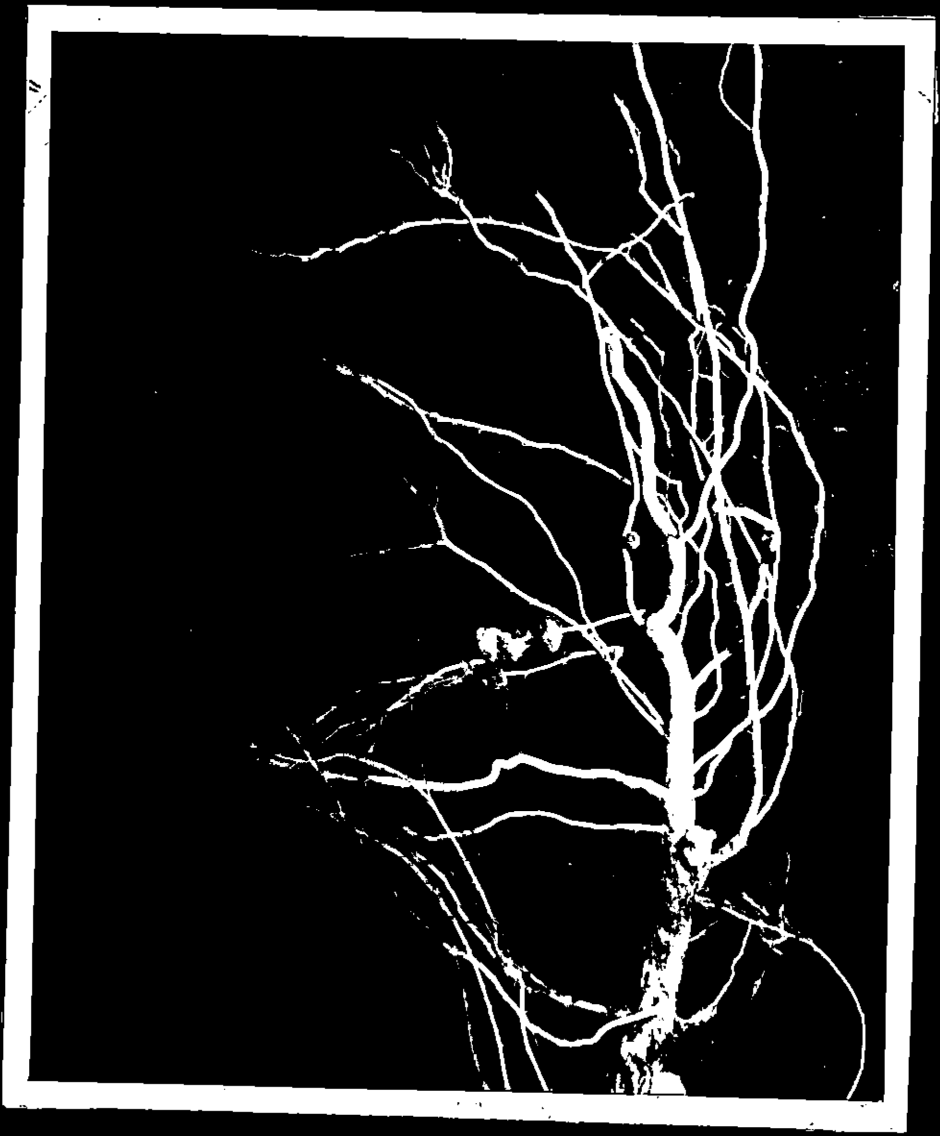
Nodules present in the root system of _Sesbania_ _aculeata_ in the presence of phospherus.

Nodules present in the root system of *Sesbania aculeata* in the presence of calcium.



Nodules present in the root system of *Sesbania aculeata* in the presence of phosphorus and calcium.

Nodules present in the root system of Vigna sinensis in the absence of both calcium and phosphorus.



Nodules present in the root system of _Vigna sinensis_ in the presence of phosphorus.

Nodules present in the root system of _Vigna sinensis_ in the presence of calcium.

Nodules present in the root system of *Vigna sinensis* in the presence of phosphorus and calcium.

is removed in the seed, straw and other harvested portions of the crop.

II. (a) Calcium and nitrogen fixation:

Calcium is important in both the nutrition of the legum and rhizobia. The calcium content of the legumes is about three times that of grasses on the basis of dry weight. Consequently, a leguminous crop may respond to liming where a non-legume may not.

Application of lime to the soil tends to increase the nitrogen fixed by most legumes in the soil and their foliage. Mooers (1912), studying the effect of liming in crop production, revealed that the yield of cowpea hay was increased and its nitrogen content enhanced by application of lime.

Fred and Graul (1916) reported that on limed soils the nitrogen fixed through the agency of legumes varied. In three experiments conducted with alfalfa they reported that the nitrogen fixed in the tops of the crop was 51 pounds, 81 pounds and 269 pounds per acre, respectively. Thus, each experiment gave different results. Similar investigations by them on red clover indicated that the crop fixed 62 pounds of nitrogen in a silt loam and 145 pounds in a sandy soil.

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