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ROOT NODULATION POTENTIALITIES OF LEUCAENA LEUCOCEPHALA IN KERALA

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ABSTRACT

Nodulation and growth of *Leucaena leucocephala* was found to be poor in soils with low pH (<5.5) and in degraded areas, especially in high ranges However, it nodulated well in places where other leguminous crops like Mimosa *pudica* and *Sesbania grandiflora* nodulated. Based upon the extent of nodulation and soil pH, *Rhizobium* was isolated from nodules of *Leucaena* growing in six localities of Kerala. Evaluation of these as well as seven strains obtained from abroad, showed that inoculation of *Leucaena* seeds with *Rhizobium* increased seedling biomass and fresh weight of nodules. *Rhizobium* isolates originating from Nilambur, Nandiyode (Palghat) and Trivandrum were equally good to the best exotic isolates Among the exotic isolates, RCR 3878, RCR 3817 and TAL 582 were promising. Low pH (<5.7) not only reduced the fresh weight of noduIes and seedling biomass but also affected root growth and seedling establishment. As the soil pH increased, improvement was noticed not only in the fresh weight of nodules and seedling biomass but also in the efficiency of nodules in increasing the biomass. The *Rhizobium* strain RCR 3817 was found suitable for soil with pH 5.7 and above; below 5.7 TAL 582 was suitable for seed inoculation. The isolate collected from Nilambur was as good as the above isolates at different pH levels though not superior.

INTRODUCTION

Leucaena leucocephala (Lam.) de Wit., commonly known as subabul, ipil ipil, etc, is a tropical evergreen tree belonging to the family Leguminosae. The plant reached Philippines from its native place in Central America through Spanish travellers who had business connections between their colonies in Central America and South-East Asia from sixteenth century onwards. Later the plant spread to other South Asian countries including India.

A lot of interest has been evinced on this plant, Various aspects of this crop was studied (Kushalappa, 1980: Kaul et al., 1981; Djo, 1983; IDRC, 1983; NAS, 1984) and results well documented in the journal *Leucaena Research Reports*. Under optimum growing conditions leucaena stands have yielded the highest annual amount of wood ever recorded. Leucaena coppices readily, Like most other members of the family Leguminosae, it forms symbiotic relationship with *Rhizobium*, the nitrogen fixing bacteria, now designated as *Rhizobium loti* (Halliday and Somasegaran, 1983). The *Rhizobium* penetrate young rootlets and multiply to form nodules. They absorb atmospheric nitrogen and transform it to ammonia which in turn is converted to other nitrogen containing organic and inorganic compounds. For average growth, leucaena requires no fertilisers and it can thrive in nitrogen poor soils that are inadequate to sustain many other crops. The nodules usually occur on rootlets in the aerated surface soil layers. In low land tropics, the plant grows luxuriantly in well-drained fertile soils (Pathak et al., 1982; Pathak and Patil, 1985). However, some authors dispute the superiority of leucaena (Chaturvedi, 1983; Patil and Prasunamma, 1986).

Leucaena's main uses: The wood yield is up to 40 to 50 m^3/ha/year. It has a higher density than other fast growing tree species like *Gmelina arborea* and *Anthocephalus chinensis*. The wood is used as furniture and other household materials, for paper pulp, poles and firewood. For coppicing, cutting cycle is 4 to 6 years.

When regularly mowed or clipped, large quantities of foliage (6 to 18 tonnes dry matter/ha/year), rich in proteins (25 to 30%) obtained. For ruminants such as cattle, goats and buffaloes, the forage is palatable and highly nutritious. Leucaena's use is slightly marred because of the presence of a harmful amino acid, mimosine in the foliage which causes epilation, weight loss and ill health in nonruminants like horses, pigs, rabbits and poultry, when fed at levels above

7.5% (dry matter) of the diet. However, ruminants in most of the South East Asian countries have stomach microorganisms that render mimosine harmless.

Genetics: There are more than 800 known varieties which are classified broadly into three types (NAS, 1984).

Common type (Hawaiian type): Short bushy variety, up to 5 m in height, flowers within 4 to 6 months, and round the year. Continuous and abundant flowering has made it an aggressive weed in several regions.

Giant type (Salvador type): Tall tree up to 20 m in height, flowers seasonally, usually twice a year. Some giant cultivars planted for timber, wood products and industrial fuel, are known as Hawaiian giants designated as K8, K28, K67, etc.

Peru type: Medium sized tree, up to 10 m in height but with extensive branching and high quantity of foliage.

Nursery techniques: Seeds are scarified after keeping them in hot water (about 80°C) for three minutes. Seeds are then soaked in cold water overnight, washed and pelleted (Vincent, 19/0) with appropriate *Rhizobium* strain and planted in soil.

Environmental tolerance: Leucaena grows successfully in a wide range of environments. It withstands large variation in rainfall, sunlight, salinity and terrain as well as periodic inundation, fire, wind storm, slight frost and drought. Good growth is restricted to the tropics and subtropics below 500 m elevation, At higher elevations the plant continues growing but without its low land vigour.

Soil: Leucaena's roots reach deep and far and wide for nutrients and water and allow the plant to tolerate a wide array of soil conditions. It thrives in soils with varying levels of rock, clay and coral. Leucaena grows well only in neutral to alkaline soil, growing best at pH 6.0 to 8.0. It often displays outstanding growth on coral or limestone outcroppings.

The plant is good for soil improvement, reforestation, reclaiming grass. lands, firebreaks, agroforestry, as a shade plant and nurse crop, living fences. and so on.

Root and nitrogen fixation: Leucaena develops a substantial tap root to reach water before the young plant is caught by drought. Seedlings often have a tap root longer than the plant. Small horizontal laterals that occur near the soil surface usually carry nitrogen fixing nodules of *Rhizobium,* 2 to 20 mm in diameter. They are occasionally single, astragalloid or coralloid with pink colouration inside.

Theannual nitrogen fixation by leucaena is intherange of 100 to 200 kg/ha (which is equivalent to 500 to 1000 kg ammonium sulphate (NAS, 1984). The nitrogen fixation in a four-year-old stand at a dry site was found to be 80 to 140 kg/ha annually. The nodules were seen at 10-30 cm soil depth (Hogberg and Kvarnstron, 1982).

Nitrogen fixation occurs only if the correct *Rhizobium* strain is present in the soil. Hence the plant is generally not nodulated in barren and uncultivated lands. Nodulation will be highly reduced if the natural *Rhizobium* population present in the soil is insufficient for effective nodulation or the soil is acidic. In addition to *Rhizobium,* fine roots and root hairs are also usually infected with a beneficial mycorrhizal fungus. Its vast network of hyphae aids the plant in obtaining and making more efficient use of mineral nutrients. This helps leucaena to grow in soils low in minerals such as phosphorus.

Although the plant survives and may outperform most of the fast growing trees, its exceptional yield occurs mainly in areas where soils are reasonably fertile and well-drained, and rainfall and temperature are adequate. However, there has been no attempt to raise leucaena in large scale plantations in Kerala. Nodulation is negligible or erratic and plant displays poor growth where it is grown in several parts of Kerala that have acidic, calcium poor oxisols with a below 5.0 and high levels of exchangeable aluminium. This has been found to be a big problem in raising large plantations of leucaena. So a study was undertaken to find out the extent of nodulation in leucaena in various parts of Kerala and its potentiality to nodulate when inoculated with different isolates of *Rhizobium* collected from various parts of Kerala and obtained from abroad.

MATERIALS AND METHODS

Collection of isolates: *Rhizobium* isolates used for the experiments were collected from the soils of various localities of Kerala and also obtained from different agencies abroad. Localities in Kerala where leucaena was found growing were visited during 1982-1983. Collection of nodules and isolation of Rhizobium were made based on the standard procedure of Vincent (1970). The isolates collected from the following six places in Kerala were used for the .evaluation of the efficiency of strains.

Isolates	Locality of isolation
TLY	Tellicherry (Cannanore Dist.)
NSC	Nilambur — KFRI sub centre (Malappuram Dist.)
NAR	Nilambur — Aruvakkode (Malappuram Dist.)
NDI	Nandiyode (Palghat Dist.)
PTD	Pattikkad (Trichur Dist.)
CDS	Centre for Development Studies (Trivandrum Dist.)

The following are the strains specific for leucaena obtained from abroad,.

Strain	Source and origin in parentheses
31A3	Nitragin Inc.
94A3 (CB 81)	Nitragin Inc. (Mexico)
94A5	TNAU (Mexico)
RCR 3817 (NGR 8)	Rothamsted (New Guineae)
RCR 3878 (CB 81)	Rothamsted (New Guineae)
TAL 82	University of Hawai
TAL 582	University of Hawai
TAL 1145	University of Hawai
X	Unknown

Evaluation of Isolates: Preliminary evaluation of isolates was done once in nursery bed (during rainy season) and twice in polythene bags (during. summer and rainy season).

In nursery bed: The following isolates were tried in nursery bed (10mx lm) in Nilambur. Leucaena seeds of variety K8 were scarified by keeping in hot water (80°C) for three minutes and kept in water overnight. The surface dried seeds were pelleted with peat based Rhizobium (procedure adopted from Vincent, 1970) and sown in rows of l0cm x l0cm spacing the same day. Each isolate was replicated in two beds. The following Rhizobium cultures were used for the field experiment

1. NSC
2. TAL 82
3. TAL 582
4. TAL 1145
5. TAL 1145+TAL 82+TAL 582
6. X
7, Control (pelleted excluding Rhizobiurn)

From each bed 20 seedlings were carefully removed, one from each alternate row (five border rows on either side rejected) and nodules collected carefully, gently removing the soil particles. The isolates were evaluated based on the number of nodules, shoot length, and oven dry weight of shoot and root of seedlings (biomass) after 6 weeks and biomass of above ground portion after 4 months.

In polytbene bags: The following isolates were used for the evaluation in polythene bags in KFRI campus, Peechi.

Local isolates	Obtained from abroad
1. TLY	1. 31A3
2. NSC	2. 94A3 (CB 81)
3. NAR	3. 94A5
4. NDI	4. RCR 3817 (NGR 8)
5. PTD	5. RCR3878 (CB 81)
6. CDS	6. TAL 582
	7. TAL 1145

The Rhizobium pelleted seeds were dibbled in polythene bags filled with gravel free garden soil. There were three types of control — seed only, seed+ lime and seed+lime+ peat. Though the experiment started with 1.5 replications, effective replications were between 10 and 15 because of non- viability of seed and mortality due to diseases. The experiment was done twice ie. during summer and rainy season. Seedlings were carefully removed and nodules collected after removing the soil floating the seedlings over water. The effectiveness of the isolates was evaluated based on the fresh weight of nodules and biomass after 15 weeks, using analysis of variance test.

Evaluation of isolates at different pH levels: The following six isolates, selected based on the three preliminary evaluations, were tested for effectiveness at various pH levels of soil.

1. NSC 2. NDI 3. CDS 4. RCR 3817 5. RCR 3878 6. TAL582.

Adjustment of soil pH: Soil from KFRI campus where leucaena was not raised earlier was collected, sieved using a 2 mm sieve and thoroughly mixed with appropriate quantity of lime and sulphur to get the desired pH level. In order to find out the quantity of lime required to increase the pH and sulphur to reduce the pH, initial trials were conducted in the laboratory.

Different quantities of lime was added to fixed quantity of sieved soil in plastic containers and initial pH was measured. The pH of the soil was measured once every month for three months. In the same way sulphur was added to another set of soil and pH measured every month. Quantity of sulphur or lime to be added to soil, to attain pH values from 3 to 8 was determined by correlating the weight of sulphur and lime added to the soil and the corresponding pH at the end of three months. After adding the required quantity of lime or sulphur, 2 kg. of soil was taken in polythene bags and incubated with water for three months to attain the required pH levels.

Seeds of *Leucaena leucocephala* var. K8 were inoculated with peat based Rhizobium (excluding lime) as per the standard procedure (Vincent, 1970) and dibbled in pH adjusted soil taken in polythene bags, Fourteen bags were used for each isolate at each level of pH. Seeds without any *Rhizobium* inoculation was used as control. The seedlings were harvested after 75 days, removing the soil by floating the bags in water over a sieve. Nodules were collected, size and shape noted and fresh weight determined. The height of seedlings measured, oven-dried and biomass determined.

RESULTS

Nodulation was found to be erratic in Kerala and growth of leucaena discouraging. Except for isolated trees in house compounds or in fertile soils, performance of the trees was not up to the expectations. Fertile soils with higher pH (> 6.0) produced good number of nodules in places where the plant was grown for the last several years and in places where some other leguminous plants nodulated. However, in remote areas irrespective of fertility of soil, grasslands of high ranges and degraded areas, the plant seldom nodulated. In fertile soil, plants devoid of nodules also performed well. But in non-fertile and highly degiaded areas with low pH. plants did not nodulate and invariably such plants were yellowish, unhealthy and stunted.

The performance of leucaena was better in places where it had nodulated. In such places, as the pH increased the number of nodules were more with large size and bright pink colour inside. Based upon the pH of soil and growth of leucaena in such soils, *Rhizobium* isolates from the following locations (Table l) were utilised for evaluation of performance in nursery bed at

seedling stage. Also, the table gives information on the growth of leucaena in those places.

Table 1. The locations of *Rhizobium* isolations made from Kerala and the status of leucaena in such places

Sl. No.	Location	Code of isolates	pH of soil	Nodulation	Status of tree growth	Remarks
1.	Tellicherry (Cannanore Dist.)	TLY	5.1	negligible	yellowing and stunting	degraded lateritic soil
2.	KFRI sub-centre, Nilambur (Malappuram Dist.)	NSC	6.2	good	good	fertile soil
3.	Aruvakkode, Nilambur	NAR	5.5	erratic	fairly good	forest soil
4.	Nandiyode (Palghat Dist.)	NDI	6.9	very good	good	farm land soil
5.	Pattikkad (Trichur Dist.)	PTD	5.6	fairly good	good	fertile soil
6.	Centre for Development studies, Trivandrum	CDS	7.7	very good	good	calcareous soil

It is apparent from the table that the higher the pH values and soil fertility better the nodulation and plant growth.

Table 2. Performance of leucaena with and without *Rhizobium* inoculation in nursery bed (mean of 40 seedlings)

Isolate	After 6 weeks			After 4 months
	Shoot length (cm)	No. of nodules	Biomass (g)	Biomass (g)
NSC	9.1 bcd*	1.8 a	0.9 a	2.1 b
TAL 82	9.6 abc	1.8 a	0.9 a	2.2 ab
TAL 582	10.1 a	2.1 a	0.9 a	2.3 a
TAL 1145	9.0 cd	1.8 a	0.9 b	1.8 b
TAL 582+ TAL 82+ TAL 1145	9.8 ab	1.8 a	0.9 a	2 2 ab
X	8.6 c	1.3 b	0.9 b	1.7 bc
Control		0.1 b	0.8 b	1.6 bc

*Any two means having a common letter are not significantly different at 5% level of significance.

Preliminary evaluation of isolates: Table 2 gives the performance of the isolates evaluated in nursery bed. Isolate X did not give appreciable number

of nodules and biomass. So it was eliminated from further experiments. TAL 82 was found contaminated and hence it was also rejected. The reason for the not so encouraging performance of TAL 1145 which was supposed to be a highly competitive strain (Halliday and Somasegaran, 1983) could not be assessed.

In polythene bags : Table 3 gives unidentical results in the performance of different isolates in rainy and summer seasons. The reason for the difference could not be ascertained. Generally seedlings from inoculated seeds gave higher fresh weight of nodules and biomass than uninoculated controls.

Table 3. Performance of leucaena in polythene bags after 15 weeks

Rainy season				Summer season	
Isolate	Mean fresh weight of nodules (g)	Isolate	Mean biomass (g)	Isolate	Mean biomass (g)
CDS	0.28 a*	NSC	2.54 a	TAL 582	1.99 ab
RCR 3878	0.23 ab	CDS	2.16 ab	RCR 3817	1.85 ab
TLY	0.22 abc	RCR 3878	1.85 bc	RCR 3878	1.84 abc
NDI	0.21 abc	NAR	1.67 bcd	PTD	1.78 abcd
94A5	0.19 bcd	NDI	1.60 bcd	CDS	1.69 abcd
PTD	0.19 bcd	PTD	1.57 bcd	NAR	1.59 abcd
TAL 582	0.17 bcd	RCR 3817	1.50 cd	NSC	1.58 abcd
NSC	0.17 cd	31A3	1.49 cd	NDI	1.43 abcd
31A3	0.16 cd	94A3	1.42 cd	Peat+Lime	1.39 bcd
TAL 1145	0.15 cd	TAL 1145	1.37 cd	TLY	1.31 bcd
RCR 3817	0.14 cd	Lime+Seed	1.33 d	TAL 1145	1.19 bcd
NAR	0.14 cd	Seed	1.29 d	94A5	1.15 bcd
Lime+ Seed	0.13 cd	Peat+Lime+Seed	1.28 d	31A3	1.10 bcd
Seed	0.13 d	TAL 582	1.24 d	94A3	1.10 bcd
Peat+Lime+Seed	0.12 d	TLY	1.13 d	Seed	1.0 bcd
94A3	0.10 d	94A5	1.02 d	Peat+Lime+Seed	1.0 bcd

* Any two means having a common letter are not significantly different at 5% level of significance

There is no striking difference between local and exotic isolates in biomass production. During summer season the exotic isolates (TAL 582, RCR 3817 and RCR 3878) gave better performance in terms of seedling biomass while in rainy season local isolates (NSC and CDS) excelled. Hence based on the performance of the three preliminary evaluation trials, three local *Rhizobium* isolates (NSC, NDI and CDS) and three exotic isolates (RCR 3817, TAL 582 and RCR 3878) were considered promising for leucaena seed inoculation. These isolates were utilised for further evaluation at various soil pH levels.

Size and shape of nodules: The nodules were either single, astragalloid or coralloid (Fig. IA and B). In seedlings from uninoculated seeds, the nodules were generally single, close to the soil surface, at the collar region or away from it. Seedlings with astragalloid and coralloid nodules were healthier than with single nodules. Seedlings from inoculated seeds formed generally astragalloid and coralloid nodules, though single nodules were also formed. Generally they were found close to the tap root near the soil surface. But occasionally they were also found attached to the tap root through long narrow strand of roots.

Influence of soil pH on nodulation and plant growth: The pH values had more or less stabilised after 3 months of incubation of soil with the addition of sulphur or lime.

The final pH of different groups of the soils were 3.2. 3.7. 5.7. 7.0. and 7.8 The pH of the experimental soil without adjustment was 5.7. Since the gap in pH level between 3.7. and 5.7, was decisive, another experiment was carried out in the same way in summer season whereas the first experiment was conducted in rainy season. The pH levels in the second trials were 3. 7, 4. 1 and 5. 1. The performance of the various isolates at different pH levels was statistically analysed and the means compared. The results are given in Table 4 and 5.

First trial : *pH 3.2.* In the soil with pH 3.2, though seeds showed initial signs of germination, all of them dried within two weeks.

pH 3. 7. Most of the seeds germinated and the survival percentage was 80%. Seedlings were unhealthy, stunted and showed yellowing accompanied by heavy defoliation. Roots showed deformation, stunting and crowding at the collar region (Fig, 1C and D). There was no nodule formation. The seedling height and biomass were the lowest. There was no significant difference between the isolates for seedling height and biomass.

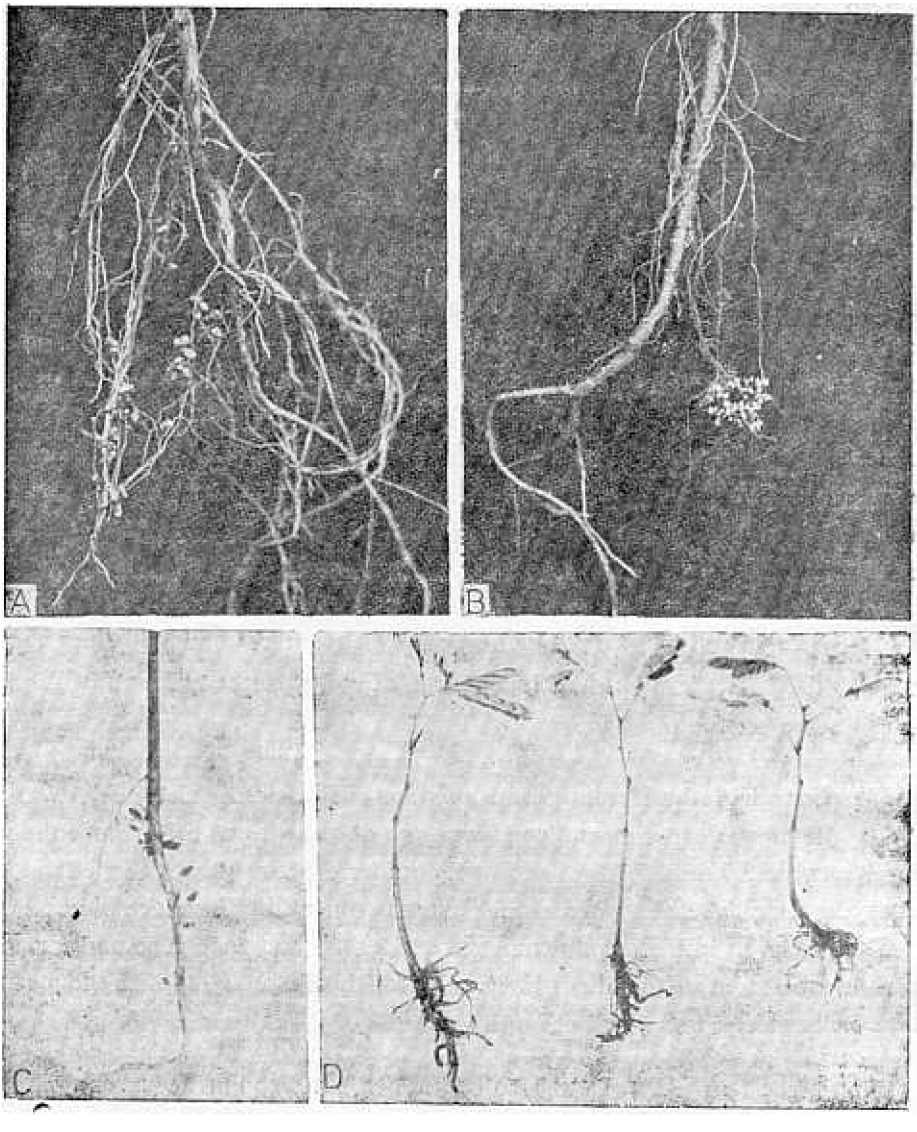

Fig. 1. A, single and astragalloid nodules formed by a seedling inoculated with isolate originating from Nilambur (NSC); B, One large coralloid nodule formed by a seedling inoculated with strain RCR 3817 at pH 7.0; C, The tap root of a seedling inoculated with the NSC isolate at pH 5.7; D, Seedling inoculated with isolate RCR 3878 at pH 3.7. Note the stunted growth of seedlings, malformed root system and absence of nodules.

pH 5.7. Germination and survival of the seedlings were normal. However, some of the seedlings showed yellowing and stunting. Nodules were present in all the groups including control. RCR 3817 gave the highest fresh weight of nodules, seedling height and biomass. The nodules formed in control treatments might be formed by the indigenous bacteria present in the soil.

pH 7.0. .TAL 582 gave the largest number of nodules. However, seedling height and biomass were highest in RCR 3817 inoculated plants.

pH 7.8. NSC gave the highest fresh weight of nodules. But seedling height and biomass were highest in RCR 3817 followed by NSC.

Second trial: *pH* 3, 7. Only 50 % of the seedlings survived. As in the case of first trial (pH 3. 7) all the seedlings showed stunting, yellowing and root deformation. Nodules were not formed, Seedlings from TAL 582 inoculated seeds gave the highest seedling height and biomass (Table 5).

pH 4.1 . Nodulation was negligible except in TAL 582. Also the same isolate gave the highest seedling height and biomass. As expected seedlings showed yellowing and stunting.

pH 5.1. Seedlings nodulated, but there was no significant difference between various *Rhizobium* isolates. Here also TAL 582 gave the highest seedling height and biomass. Partial yellowing and stunting were observed.

The higher seedling height and biomass values obtained generally in the second trial might be because of the favourable effect of summer season with copious sunlight and adequate water supply.

Correlation studies have shown that there was positive correlation between fresh weight of nodules and biomass (Table 6). Generally the correlation coefficient decreased in acid soils whereas it was higher in neutral and alkaline soils. It was interesting to note that there was least correlation in very acid soils when uninoculated seeds were used.

The study also indicated that the performaoce of leucaena is primarily determined not by the presence of suitable *Rhizobium,* but by the level of pH. In acid soils the roots are malformed and the growth of plant as a whole is very poor. Of course the survival of the *Rhizobium* and nodulation *of* the plant is also impaired in acid soils. The present experiment has shown that generally nodules will be progressively reduced as acidity of the soil increased.

Table 4. Mean fresh weight of nodules, seedling height and biomass at different pH levels (first trial)

pH	Isolate	Fresh weight of nodules (g)	Isolate	Seedling height (cm)	Isolate	Seedling biomass (g)
7.8	NSC	0.19 ab*	RCR 3817	36.36 a	RCR 3817	1.60 a
	TAL 582	0.14 abc	NSC	34.64 ab	NSC	1.60 a
	RCR 3817	0.13 bcd	NDI	33 23 abc	NDI	1.51 abc
	NDI	0.12 bcdef	CDS	32.50 cd	TAL 582	1.35 abc
	Seed	0.10 bcdef	TAL 582	31.64 bcde	Seed	1.34 abc
	CDS	0.09 bcdefg	Seed	31.29 bcde	CDS	1.23 bcdef
	RCR 3878	0.08 cdefghi	RCR 3878	27.57 efghi	RCR 3878	0.94 efg
7.0	TAL 582	0.14 ab	RCR 3817	34.85 ab	RCR 3817	1.53 ab
	NSC	0.13 bcde	RCR 3878	32.57 abcd	NSC	1.29 abcd
	RCR 3817	0.12 bcdf	TAL 582	31.92 abcde	CDS	1.29 bcd
	NDI	0.11 bcdefg	CDS	31.21 bcdef	Seed	1.28 bcde
	RCR 3878	0 08 bcdefgh	NSC	30.50 bcdefg	TAL 582	1.27 bcde
	Seed	0.07 efghi	NDI	29.79 cdefg	NDI	1.26 bcde
	CDS	0.03 i	Seed	27.79 defghi	RCR 3878	1.20 cdef
5.7	RCR 3817	0.10 bcdefgh	RCR 3817	28.85 cdefgh	RCR 3817	1.00 defg
	TAL 582	0.07 defghi	RCR 3878	26.27 fghi	TAL 582	0.92 fgh
	NDI	0.07 defghi	TAL 582	26.77 fghi	RCR3878	0.90 fgh
	Seed	0 07 efghi	CDS	26.14 ghi	NSC	0.89 fgh
	RCR 3878	0 07 fghi	NSC	24.57 hi	NDI	0.88 gh
	NSC	0.08 ghi	Seed	24.00 i	Seed	0.85 gh
	CDS	0.05 hi	NDI	12.95 j	CDS	0.85 gh
3 7	Nodules not formed		NSC	11.22 j	NSC	0.29 hi
			CDS	10.82 j	CDS	0.28
			Seed	10 45 j	Seed	0.23 i
			RCR 3878	9 82 j	NDI	0.21 i
			NDI	9.17 j	TAL 582	0.17 i
			TAL 582	8.64 j	RCR 3878	0.17 i
			RCR 3817	8 08 j	RCR 3817	0.15 i

*Any two means having a common letter are not significantly different at 5% level of significance.

Table 5. Mean seedling height and biomass at different pH levels (Second trial)

pH	Isolate	Seedling height (cm)	Isolate	Seedling biomass (g)
5.1	TAL 583	54.62 a*	TAL 582	2.36 a
	RCR 3878	44.86 ab	NDI	1.89 ab
	CDS	43.93 bc	CDS	1 83 b
	NDI	41.21 bcd	RCR 3878	1.76 bc
	NSC	39.69 bcde	NSC	1.72 bc
	RCR 3817	36.20 cdef	RCR 3817	1.19 def
	Seed	84 64 defg	Seed	1.13 defg
4.1	TAL 582	36.62 cde	TAL 582	1.40 bcd
	NDI	35 85 cde	NSC	1.28 cde
	NSC	35.77 cde	NDI	1.13 defg
	RCR 3878	35.64 de	RCR 3878	1.04 defg
	CDS	34 92 def	RCR 3817	0.88 defg
	RCR 3817	31.17 efgh	CDS	0.87 defg
	Seed	25 82 ghi	Seed	0.66 efgh
3.7	TAL 582	26.00 fghi	TAL 582	0 75 defgh
	RCR 3817	21.40 hi	NDI	0.49 efgh
	NSC	21.25 hi	RCR 3817	0.41 fgh
	WDI	20.14 hi	NSC	0.37 gh
	RCR 3878	18.83i	CDS	0.30 h
	CDS	17.29 i	RCR 3878	0.28 h
	Seed	11.83 i	Seed	0.10 h

*Any two means having a common letter are not significantly different at 5% level of signi

Table 6. Correlation coefficient between fresh weight of nodules and biomass of leucaena seedlings inoculated with different *Rhizobium* isolates at various pH levels

pH	Isolates						
	RCR 3817	RCR 3878	NDI	CDS	NSC	TAL 582	Control
5.1	0.68*	0.30	0.40	0.54*	0.68	0.44	0.01
5.7	0.56*	0.25	0.52	0.72*	0.68*	0.23	0.02
7.0	0.62*	0.62*	0.42	0.60*	0.72*	0.94*	0.58*
7.8	0.74*	0.80	0.58*	0.70*	0.72*	0.63*	0.76*

* Significant at 5% level

Table 7. Intercept, slope and R^2 of regression equation of fresh weights of nodules, height of seedlings and biomass on soil pH level in respect of various *Rhizobium* isolates

Isolate	Fresh weight of nodules			Height of seedlings			Biomass		
	R^2	Intercept	Slope	R^2	Intercept	Slope	R^2	Intercept	Slope
RCR 3817	0.92*	-0.11 (0.04)	0.03 (0.001)	0.91*	-27.80 (12.52)	8.77 (2.00)	0.98*	-1.16 (0.25)	0.37 (0.04)
NSC	0.94*	-0.18 (0.05)	0.05 (0.008)	0.99*	-9.16 (2.25)	5.68 (0.36)	0.99*	-0.89 (0.06)	0.31 (0.01)
NDI	0.97*	-0.10 (0.02)	0.03 (0.004)	0.88*	-16.73 (10.27)	6.28 (1.64)	0.99*	-0.94 (0.05)	0.31 (0.008)
RCR3878	0.92*	-0.07 (0.03)	0.92 (0.004)	0.77	-5.25 (11.70)	4.86 (1.87)	0.76	-0.50 (0.53)	0.22 (0.08)
CDS	0.65	-0.07 (0.05)	0.02 (0.009)	0.95*	-7.53 (5.75)	5.41 (0.92)	0.94*	-0.62 (0.28)	0.25 (0.04)
TAL582	0.96*	-0.13 (0.03)	0.04 (0.005)	0.90	-10.52 (8.54)	5.83 (1.37)	0.97*	-0.86 (0.23)	0.30 (0.04)
Seed	0.90	-0.07 (0.03)	0.02 (0.005)	0.97*	-6.95 (3.99)	5.01 (0.64)	0.98*	-0.79 (0.17)	0.28 (0.03)

Figures in parentheses are standard errors

* Significant at 5% level

A simple linear regression analysis was carried out to examine the response of the Rhizobium isolates to varying soil pH. The intercept, slope and coefficient of determination (R^2) are given in Table 7. The significance of R^2 values shows that variation in soil pH affected the fresh weight of nodules, seedling height and biomass. It is also observed that increased slope in respect of RCR 381 7 for height of seedlings and biomass is an indication of its response to increasing soil

DISCUSSION

Seed inoculation with *Rhizobium* is normally not adopted in areas where leucaena is found growing luxuriantly because in such places good nodulation is generally expected. Leucaena rhizobia will nodulate other tropical legumes, usually nodulated by fast-growing rhizobia and will also nodulate some species normally noduated by slow growers (Halliday and Somasegaran, 1983). Trinnick (1980) has also described the ability of leucaena *Rhizobium* to cross-inoculate with some of the few other tropical legumes that have fast growing rhizobia, viz., *Mimosa invisia, M. pudica, Acacia farnesiana, Sesbania grandiflora* and *Calliandra rallothyrsus.* In agglutination tests, isolates from each host shared antigens with one or more of five *Rhizobiurn* strains of leucaena. Probably this cross-compatibility may be the reason for the nodulation of leucaena even in remote areas where the plant had not grown earlier, but some of the above species nodulated and grew well. A study by Basavaraju and Hegde (1983) indicated the presence of effective native leucaena rhizobia in soils of Karnataka. But they were not effective for seratro, leucerne, soybean, common bean, pea, groundnut and cowpea. Contrary to the general belief, eroded soils support leucaena poorly. Nodulation also is found negligible. Studies adopting simulated erosion by removing top soil in a silt loam clay soil inoculated with *Rhizobium* strain TAL 1145 showed significantly reduced nodulation and growth (Habte and El-Swaify, 1985). Such a situation reduces the scope of leucaena in degraded soil especially if the pH also is low. The critical pH level reported for establishment of *Leucuena Ieucocephala* was between 4.45 and 4.70 below which species cannot be established satisfactorily(Ahmad and Ng, 1981) In our study also the percentage of survival was reduced considerably (20 to 50 %) at pH 3.7. AtpH 3.2 none of the seedlings survived. Though the seedlings survived at pH levels 4.1 and 5.1 the growth was very poor indicating harmful effect; at pH 5. 7 also yellowing was noted. A direct relationship also exists between root formation and soil pH. Low pH in turn reduces the biomass.

The poor nodulation and performance of leucaena in soils of low pH (< 5.5) observed in the present study is expected in the light of experiences by several authors. The survival and multiplication of *Rhizobium* and nodulation in acid soils is a matter of debate. Lowendorf et al. (1981) found that the minimum pH values of strains of *R. meliloti* in liquid medium ranged from 5.3 to 5.9. It appears that leucaena Rhizobia are able to multiply at a pH in which leucaena itself cannot grow satisfactorily. In our experiment, effective nodulation was found at pH 4. 1 in TAL 582 inoculated seedlings and at pH 5. 1 in seedlings inoculated with all the tested isolates. However, yellowing and stunting of seedlings as well as root damage and reduced growth were observed. It seemed that had the root development were more, there could have been more chance for increased production of nodules.

Hence root damage and reduction in root biomass reduce the infection sites on the roots. Such situations have been reported earlier also (Aquiahuatl and Munoz, 1983). In leucaena, nodulation does not involve root hairs or infection thread (Dart, 1977). Such plants are certainly affected by soil acidity. The results obtained in the present study is in agreement with the opinion of Halliday and Somasegaran (1983) that leucaena's intolerance of soil acidity can be attributed to the leucaena-plant genome rather than to its rhizobial partner, some of which have been shown to be acid competent. Generally legume roots appear less sensitive to soil acidity than nodulation (Munns and Mosse, 1980), but in the case of leucaena this is not found true.

The improved growth of seedlings over uninoculated ones are significant in lower as well as higher pH levels. Dutt and Pathania (1983) reported improved growth of leucaena over uninoculated plants of 24 to 30 months in Jammu when CB81, TAL 82, and 94A5 were used as Rhizobium strains for inoculation. The capability of TAL 582 to produce nodules even at pH 4.1I and its increased efficiency in nodule formation and biomass production at 5.1 and 5.7 are noteworthy. This indicates that TAL 582 is more suitable than other isolates in acid soils. The superiority of RCR 3817 in nodulation as well as for increased biomass at pH 5.7, 7.0 and 7.8 is indicative of its suitability in neutral and alkaline soils. Dutt et al. (1982) also found that among RCR 3878 (CB 81), TAL 82, 94A5 and RCR 3817 (NGR 8), RCR 3817 was the most effective inoculum in increasing biomass, fresh weight of nodules and percentage of N in nodules and leaves in 60-day-old seedlings. In another trial (Chandrasekharan, 1981) it was found that RCR 3817 caused the maximum nodulation on the 60th day whereas it was 94A5 which resulted in maximum nodulation on 30th day. Maximum nitrogenase activity at 60th- day was given by local isolate whereas NGR 8 exhibited maximum activity at 90th day.

Though RCR 3878 (CB 81) is considered a very effective strain in acid soil, it did not give the expected result: in fact some of the local strains were found to perform better than RCR 3878. Halliday (1981) also reported that many strains of Rhizobium were more efficient nitrogen fixers than RCR 3817 (NGR 8) and RCR 3878 (CB 81).

The liming of the soil was found to have tremendous influence in determining the fresh weight of nodules and the biomass of seedlings. The fresh weight of nodules, seedling height and biomass steadily increased as pH also increased. Halliday (1981) also reported effective nodulation of leucaena insoils of pH 4.5 to 5.0 by inoculating and pelleting seed with lime. But he also found that even though seedlings were effectively nodulated production of leucaena in acid soils was greatly retarded, but at the same time yield increased 15 fold by liming to a pH of 6.0.

The generally low values of correlation coefficient between fresh weight of nodules and biomass of leucaena seedlings at lower pH levels indicates that in low pH soils, proliferation of nodules is not an indication of its efficiency in nitrogen fixation. In other words there need not be a direct relationship between biomass and fresh weight of nodules. But in higher pH there is direct relationship between pH and fresh weight of nodules. This shows that leucaena is sensitive to pH in different ways. In a Hawaiian trial, though leucaena nodulated fairly abundantly at low pH it responded to lime, evidently because the nodule function was markedly sensitive to variation in pH (Munns and Fox, 1977).

Several others have studied the effect of liming on nodulation and growth of leucaena (Dutt and Pathania, 1985; Chu-Chung Young and Chen-Ching Chao, 1983). Calcium influences the legumes in different ways. Initiation of infection (of Rhizobium for nodulation) is the most Ca demanding. Root extension, root hair production and nodulation is affected by low calcium concentration or low pH (Munns, 1970) Aluminium toxicity also is responsible for poor growth of the plant as well as growth of slow growing rhizobia with significant variation between strains (Munns, 1978). Aquiahuatl and Munoz (1983) also found that increased levels of soluble A1 inhibited growth of rhizobia associated with leucaena in culture medium.

Hence the scope of leucaena cultivation, intended only for biomass production is limited in soils of low pH and due to the inherent difficulty in promoting nodulation in such soil. However, it is reported that improved plant growth can be attained by addition of lime. Surface liming of soil allows plant root system to develop through acid soil layers as thick as 90 cm (Olvera et al., 1982).

Munns et a1 (1977) concluded that for most legume species improved nitrogen fixation was the cause of growth improvement associated with liming. Leucaena will grow very well in deep fertile soil with adequate irrigation. Whatever drawbacks the plant has, its utility for other purposes (mentioned under introduction) cannot be underestimated.

In Kerala, soils are predominantly acidic except in isolated pockets (ISSS, 1976: Koshy and Varghese, 1972). Hence there is every likelihood that leucaena may not give the expected good performance even if inoculated with efficient Rhizobium strain. The long term presence of nitrogen fixing symbiosis will depend on post-transplanting reinfection by soil rhizobia if the original inoculant is an ineffective competitor. The average life span of a leucaena nodule is 8-12 weeks. Studies with TAL 582 showed that the original inoculant was lost after sometime of transplantation (South, 1982). Such a chance cannot be ruled out in Kerala soils also. Though exact life span is not determined, it is generally observed that nodules disintegrate once soil moisture is depleted after the North-East monsoon. Fresh nodule formation takes place after the pre-monsoon showers only. A study in a prairie soil inoculated with Rhizobium strain CB 81 showed that nodule representation of CB 81 decreased from 100% 3 months after sowing to between 12 and 16% after two years (Bushby, 1982). Sinceleucaena is a perennial crop, seed inoculation with a particular strain at the time of sowing should give persistence, multiplication and infection of leucaena roots at least till its rotation period. This necessitates the need for a better competitor than the indigenous rhizobia strains for improved nitrogen fixatoins. TAL 1145 has been found to be a better competitor (Halliday and Somasegaran, 1983). But the performance of this strain was not found good in the present study.

Unlike other tropical legumes, leucaena is poorly adapted to acid soils. Though leucaena Rhizobium is fast growing its inability to produce alkali is singled out as a reason for lack of acid tolerance. A search for base-exuding rhizobia is seen as a key to alleaviate acid soil stress on leucaena-Rhizobium symbiosis (Halliday, 1981)

Association of vesicular arbuscular mycorrhiza (VAM) with roots of leucaena which helps in the uptake of P is a promising area of study. A few reports have already appeared indicating encouraging results. Munns and Mosse (1980) reported that when *Glomus fasciculatus,* a VAM was inoculated, the dry weight of plants increased three-fold and the number of nodules-several fold. The beneficial effect of VAM in increasing biomass and nodule numbers are reported by several authors (Sivaprasad et al., 1983: Manjunath et al., 1984: Kun-Piao Chang et al., 1986). VAM enhances plant growth in unfertile soils with adverse pH levels (Huang et al., 1984). Hence studies on this aspect seem to be rewarding.

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