RHIZOBIUM REQUIREMENTS OF SESBANIA SESBAN (L). MERR.

A thesis submitted to the University of Queensland, Australia in partial fulfilment of the requirement of the degree of Master of Agricultural Studies, Department of Agriculture, Faculty of Agriculture.

BY

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DECLARATION OF ORIGINALITY:

This report describes the original work of the author, except as otherwise stated. It has not been submitted previously for a degree at any University.

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A B S T R A C T

Two experiments were conducted in a glasshouse at the University of Queensland, St.Lucia, Brisbane (Lat.27 30'S;153 E) between February 5th, 1990 and July 5th, 1990. The aim was to evaluate the effectiveness of strains of Rhizobium, isolated from various Sesbania species collected in Australia, Pakistan, Phillipines and Thailand, for nodulation and nitrogen fixation of Sesbania sesban var.nubica (CPI-30071). This constituted experiment-1 which was done using sand culture medium. The most promising Rhizobium strains from experiment-1 were used in experiment-2 on five lines of Sesbania sesban (9265, 10895, 15022, 15036 and CPI-30071) in loamy sand soil to evaluate strain by host interaction.

Results from experiment-1 showed that, Rhizobium strains PMA-295/2 and CB-3023 were the most effective on Sesbania sesban var nubica and were selected for use in experiment-2 the results of which showed that line CPI-30071 and strain PMA-295/2 produced the most significant results compared with all other interactions.

The conclusion was that, in the absence of a better strain, PMA-295/2 is a suitable strain for use with Sesbania sesban var.nubica. However it is not suitable for other lines nor is CB-3023. When introducing new lines of Sesbania sesban it is evident that each will need testing and perhaps a specific Rhizobium strain selected for each line.

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1. INTRODUCTION :

Nitrogen is one of the essential elements that drive biological systems. It is mainly a constituent of protein which is required by all animals and plants in a form they can utilize (Allen and Allen, 1981).

Rapid world population increase must be matched with increased food production, leading to greater use of inorganic fertilizers among other agricultural chemicals that are used to enhance food production. However there is a growing concern on enviromental degradation caused by inorganic agricultural chemicals. Continued use of inorganic nitrogen is being curtailed due to two major constraints. Firstly, unbalanced cost of fertilizer vis-a-vis prices of agricultural products and secondly the problem of nitrate pollution of ground water. Investigations into ways and means of sustaining production systems which are reasonably cheaper and less harmful to the environment in the long term are now actively being sought. Biological nitrogen fixation has a place in providing alternative and/or complementary source of nitrogen. Eighty percent of the world's atmosphere is nitrogen gas, and the significance of legumes in agricultural systems arises from their ability to form a symbiotic association with Rhizobium species and the consequent fixation of this vast atmospheric nitrogen in their nodules (Nurhayati et al.1988). Fixed nitrogen provides a significant input into the soil organic pool and is released to companion crops or subsequent cereal

crop (Peoples et al., 1989). The amount of nitrogen fixed is normally influenced by the legume species, climatic and edaphic variables and the management imposed on the system.

In the tropics and sub-tropics, tree legumes have a more dominant role in cropping and grazing systems than herbaceous legumes (Anon, 1973; Atta-krah, 1985; ILCA, 1985; Jones, 1979; and FAO, 1975) because of their relative ease of integration into the farming systems, and the advantage of remaining productive during the dry season when most grasses and herbaceous legumes out. However the introduction of new legumes and/or the extension of crop and pasture production into new locations and attempts to increase productivity of these systems require an evaluation of the effectiveness of symbiosis between legumes and Rhizobium species to ensure adequate nitrogen fixation. This is because many tropical legumes do not always nodulate effectively with strains of indigenous Rhizobium, despite their reputation for symbiotic promiscuity (Date, 1982). There is need for selection of suitable strains for tropical legumes.

Sesbania sesban has not been widely evaluated in Australia. At Mt. Cotton, South East Queensland, it has been grown under experimental conditions for three years on an infertile, seasonally water-logged podzolic soil. Yields of edible material (leaves and fine stems) from hedge rows 2m apart, and subjected to various cutting regimes, have ranged from 2-4 t/ha of dry matter over summer period (Nov-April) (Galang, 1989

unpublished data). Sesbania sesban was able to make some growth over cooler winter periods at Mt.Cotton, a feature which is not observed on most leguminous shrubs. It also shows very early growth.

In a preliminary feeding trial in which five browse legumes including Leucaena, were fed as supplements to low quality grass hay. Sesbania sesban gave the highest liveweight gains in goats over a 6-week period (Robertson, 1987 Unpublished data). The dry matter digestibility of Sesbania sesban leaves in the trial was greater than 90%. These preliminary trials have indicated that Sesbania sesban has potential for use on heavier, seasonally water-logged soils. It may also have a role in the reclamation of saline affected soils. However literature is silent on suitable strains of Rhizobium of this and other promising legumes, hence the present study was undertaken as an initial attempt to evaluate five lines of Sesbania sesban for Rhizobium requirement under tropical and sub-tropical conditions of Australia.

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2. L I T E R A T U R E R E V I E W :

2.1 THE LEGUME FAMILY:

Leguminosae is a large family with a world wide distribution currently estimated at 16000 - 19000 species in about 750 genera (Allen and Allen, 1981). The family is divided into three major sub-families based on floral differences as Caesalpinoideae, Mimosoideae and Papilionoideae. Hutchinson (1964) came up with a proposal to restructure the family by elevating sub-families to full families (Caesalpiniaceae, Mimosaceae and Papilionaceae or Fabaceae) and their original family became an order Leguminales. Corby et al., (1983), however pointed out that recent data from rhizobial associations as well as evidence from anatomy, chemistry chromosomes and pollen gives support to the older tradition of treating all legumes as one large family, the leguminosae. Allen and Allen (1981) similarly noted that whichever classification of legumes one uses, it is only a matter of choice, because the distinction between the sub-families is clear and universally accepted. For the purpose of this study, the word leguminosae has been retained to represent the large legume family, because most readers are more familiar with it.

2.2 CHARACTERISTICS OF LEGUMES:

Legumes are dicotyledonous plants, mostly trees, shrubs, woody vines or annual and perennial herbs. Leaves are usually alternate and compound, bipinnate, simply pinnate or palmate

and rarely simple. Flowers are variously racemes, panicles, spikes or heads. Fruits are pods (legumes) dehiscent or indehiscent (Allen and Allen, 1981). In this study, the major concern will be on the sub-family Papilionoideae, within which the genus Sesbania falls Gibson (1974) categorized legumes in three major groups according to their contribution to agricultural production as: those providing grains for human and livestock consumption, whose grain protein is rated between 20-45% ; those providing forage for animals, whose protein content is rated between 18-27% ; and those which raise nitrogen for non-legumes grown concurrently or subsequently, which are said to contribute about 40% of the world's 175-million tonnes of nitrogen fixed biologically each year.

2.2.1 CHARACTERISTICS OF SUB-FAMILY PAPILIONOIDEAE:

Sub-family Papilionoideae are trees, shrubs, herbs, both annual and perennials. Leaves are palmately-3-or more foliate, odd or evenly pinnate but never bipinnate. Flowers are very colourful (papilinous). Fruits are variously shaped pods, straight, curved winged or moniliform, usually 2-halved and dehiscent, some traversely jointed (loments), with ripe fruits separating. They are edible and highly nutritious crops for human and animal consumption (Allen and Allen, 1981).

2.3 TREE LEGUMES:

2.3.1 TAXONOMY AND DISTRIBUTION:

Most of the agriculturally important tree legumes belong to two

sub-families Mimosoideae and Papilionoideae, which includes: Acacia, Albizia, Calliandra, Gliricidia, Leucaena and Sesbania species among others. Table 2. 1 below, shows the origin and world distribution of some of the most important tree legumes which are generally found within the tropics.

TABLE 2.1: TAXONOMIC CLASSIFICATION AND DISTRIBUTION OF TREE LEGUMES:

SPECIES NAME	SUB-FAMILY	ORIGIN	DISTRIBUTION
1. Acacia albida	Mimosoideae	Africa	Africa, India, Middle East
2. Acacia aneura	"	Australia	Australia
3. Albizia lebbek	"	Africa, Asia	East Africa, South Africa
4. Calliandra calothyrus	"	C.America	C.America, Indonesia
5. Gliricidia sepium	Papilionoideae	Mexico, Costa Rica	Pan-tropical
6. Leucaena leucocephala	Mimosoideae	C/S.America	Pan-tropical
7. Sesbania sesban	Papilionoideae	Africa, Asia	Pan-tropical

Source: Skerman, 1977; NAS, 1979, 1983; Allen and Allen, 1981.

2.3.2 THE ROLE OF TREE LEGUMES IN AGRICULTURE:

Much work has been done in this area to establish the roles of tree legumes in agriculture throughout the world (Pittier, 1944; Takahashi and Ripperton, 1949; Dijkman, 1950; Gantt, 1953; Payne, 1954; Kinch and Ripperton, 1962; Charreau and

Vidal, 1965; Everist, 1969; Skerman, 1977; NAS, 1979; Atta-krah et al., 1985; Brewbaker, 1986; Evans and Rotar, 1987 among others). The most important roles of tree legumes are fodder production, soil reclamation, timber, wood fuel, shade, cover crop, pulp, gum, live fences, poles and posts, and human food production.

2.3.2.1 UTILIZATION AS ANIMAL FEED:

Tree legumes are utilized by livestock as supplementary feeds to grasses and other forages (Chadhokar, 1982). Young stems, leaves flowers and pods are palatable to both livestock and wildlife. Some tree legumes are more palatable than others, and some have been reported to have anti-nutritive factors such as saponins, tannins and alkaloids which are toxic to some animal species (Skerman, 1977). Feeding of tree legumes to domestic animals is mostly done by lopping of young shoots and branches, grazing of young trees and shrubs or their regrowths or by processing meals from leaves, pods and seeds. In Africa wildlife, cattle, sheep and goats utilize Acacia and Albizia leaves, flowers and pods fallen under the trees. Harvesting of leaves, flowers or pods is not feasible because of communal land ownership in many countries (FAO, 1975; and NAS, 1979). In Australia fallen leaves of Albizia lebbek are readily eaten by stock in Western Queensland and Cape York Peninsula (Everist, 1969). By contrast, in West Indies tree branches and pods are pollarded and fed to animals (Paterson, 1949). NAS (1979) reported the unusual phenological response of Acacia albida, in that it retains green leaves during the dry season

and sheds them at the beginning of the rain season. This has a number of economic benefits. High quality forage is made available throughout the dry season when other trees are leafless. At the end of the dry season when feed is often desparately scarce, the protein-rich pods are maturing and dropping off in large numbers. In addition during the hot months the tree's dense foliage provides cool shade for livestock. The leaf-drop and continuous presence of livestock near the tree greatly enriches the soil with their dung.

Mulga (Acacia aneura) is an outstanding Australian fodder tree. Pastoralists have high regard for Mulga and manage the trees as a reserve fodder source for use during drought. Leaves and branches are lopped or the trees are knocked down using tractors dragging heavy chains. A daily ration of 1.4kg of Mulga leaves can supply sheep with sufficient calcium and vitamin-A for growth (NAS, 1979). However Mulga has two major problems, firstly the of high tannin content which reduces the digestibility of the feed; and secondly it also contains very low levels of sulphur and phosphorus (NAS, 1979). Therefore sheep on Mulga diet have to be supplemented with sulphur and phosphorus, especially during the droughts when protein content is limiting.

Chadhokar (1982) reported that, contrary to a report by Mahadevan (1956) in India, that Gliricidia is not palatable to cattle. Other workers in Sri Lanka (Chadhokar and Kantharaju, 1980; Chadhokar and Lecamwasam, 1982) have shown that

Gliricidia is very palatable to both sheep and cattle. When Gliricidia was fed up to 75% as a supplement to Brachiaria miliformis, there was a significant effect on lambing weights, lambing rate and survival rate of both lambs and ewes (Chadhokar and Kantharaju, 1980).

Fresh Leucaena leaves have been reported in use in broiler feeds in Hawaii (Palafox, 1948) and Phillipines (Mollina, 1953). In Australia, trials on layers' rations have shown that, there is no advantage in using Leucaena in layers' rations because it caused delayed sexual maturity and lower body weight (Jones, 1979). Leucaena is normally grazed in paddocks in Australia where it is grown in wide hedge rows (5m apart). Jones (1979) reported that when Leucaena was used as a supplement for a diet of chopped sugar cane tops, so that the overall protein content was 9%, it gave an average daily live weight gain of 0.6kg/hd, identical with steers fed on sugar cane and meat meal with an overall crude protein of 10%. Leucaena use has also been reported in Columbia where it is used in layers' rations to improve yolk colour in eggs, and in Hawaii it is used for feeding dairy and beef cattle (Kinch and Ripperton, 1962).

2.3.2.2 FORAGE DRY MATTER PRODUCTION:

Work done on Leucaena species in different parts of the world show that Leucaena species at Samford, Queensland, Australia yielded 12.4 t/ha/yr (Hutton and Bonner, 1960), compared with Leucaena species in Hawaii which yielded 20-25

t/ha/yr (Kinch and Ripperton, 1962). Other species such as Gliricidia was reported by Catchpoole et al., (unpublished data) in Indonesia to have yielded 15-18 t/ha/8 months. Charreau and Vidal (1965) reported a yield of 400-600kg of pods/ha/yr from Acacia albida in the Sahelian region of Africa, while in Senegal (Africa) the yield from Acacia albida was only 40-50kg of pods/ha/yr, probably because there were fewer trees per hectare, compared with the other Sahelian countries.

2.3.2.3 NUTRITIVE VALUE OF TREE LEGUMES:

Table 2.2 shows the nutritive value of some tree legumes indicating edible parts and the level of available nutrients which could be utilized by animals when used as animal feedstuffs.

Table. 2.2: Summary of Nutritive Value of Some Tree Legumes:

Species:	CP	EE	CF	NFE	ASH	Ca	P
A.albida (pods)	11.1	0.84	32.5	46.1	9.5	-	-
A.aneura (leaves)	11.7	2.7	29.0	49.6	6.9	1.29	0.07
A.lebbek (leaves)	29.2	-	25.3	43.8	7.5	1.8	0.2
G.sepium (twigs)	18.8	3.7	15.5	55.5	6.3	0.66	0.11
L.leucocephala							
(shoots)	19.8	5.1	16.1	49.3	9.9	2.2	0.08
(leaves)	20.0	6.5	14.1	48.3	1.2	-	-
(Meal)	24.4	6.4	9.6	-	10.6	-	-
S.sesban (leaves)	26.0	2.6	14.4	75.5	7.6	1.11	0.27
(pods)	7.8	0.5	10.0	49.4	6.2	1.4	0.4

Source: Skerman, 1977.

2.3.3 AGRONOMY OF TREE LEGUMES:

Tree legumes are well adapted to warm climates with or without high rainfall, from rich to poor soils, well drained to poorly drained soils in the tropics and sub-tropics (Cowie and Skerman, 1970). Species which are more widely distributed such as Leucaena leucocephala and Gliricidia sepium have a wider adaptation than those which are confined to a particular region such as Mulga, which is confined to the arid parts of Australia (Cowie and Skerman, 1970; Perry, 1970; Wilson and Bredon, 1963; Chadhokar, 1982; Evans and Rotar, 1987).

Establishment and persistence of tree legumes vary from one species to another. There are those that can establish from seed, with or without scarification such as Leucaena, Sesbania, Acacia and Calliandra, and those which establish better from stakes or stumps such as Gliricidia and Calliandra. Although the latter two species can establish from either seed or stake, it is common practice to use stakes because plants are lopped so frequently as fodder they do not get time to flower and seed. Most communities especially the Asian community prefer stakes because they can use them as live fences, support and shade for other crops soon after planting (Chadhokar, 1982).

Once established, most tree legumes can persist for many years under grazing or lopping with a few exceptions such as Leucaena leucocephala, which can not withstand heavy defoliation in the first year of establishment until it is mature. Others such as Sesbania sesban have shallow root systems and therefore short

lived (Takahashi and Ripperton, 1949; Gray, 1962; Wood and Larkens, 1987).

Tree legumes differ in their productivity depending on the management and mode of utilization accorded to them. Some species respond well to frequent defoliation either by lopping or grazing by producing more dry matter each time, such as Leucaena species (Hutton and Bonner, 1960; Takahashi and Ripperton, 1949; Kinch and Ripperton, 1962; and van Rensburg, 1968). Most other tree legumes respond well to lopping provided they are not lopped too low (0 - 10cm above ground) or too frequently (3 - 4 weeks intervals) during the establishment year (Brewbaker, 1986). Mulga is very sensitive to lopping, especially if all the lateral branches are lopped (Everist, 1969). It takes too long to regenerate. Under good environment, tree legumes have much higher food reserves stored in the roots and woody stems compared with herbaceous species. The reserves would help plants to resume growth and productivity very rapidly as conitions improve (Gray, 1970).

2.3.4 ANTI-NUTRITIVE FACTORS IN SOME TREE LEGUMES:

Chadhokar (1982) reported that when Gliricidia was fed as a complete diet to dairy cows it improved milk production but, the milk was tainted. Other workers have reported that Gliricidia has tannins and alkaloids (van der Walt and Steyn, 1943; Standley and Steyermark, 1946). There is no informationin the literature reviewed to show the levels of toxicity or which part of Gliricidia is more toxic than others.

A lot more work has been done on Leucaena toxicity because of its importance in agriculture in the tropics. Hergaty et al., (1976 and 1981) reported that the toxic principle to ruminants fed on Leucaena is DHP (3-hydroxy-4-1(H)-pyidone), the breakdown of mimosine (B-[N-(3-hydroxy-4-oxpyrdyl)]-L-Amino-propionic acid) in the rumen. DHP is a potent dipilatory agent, it affects reproductive processes in non-ruminants. Oakes (1963) reported that, mimosine concentrations vary between 3-8 % in leaves and 3-9 % in oven dry seeds, depending on Leucaena variety. Other compounds have also been isolated from Leucaena such as Troplone (2-hydroxy-2, 4, 6-cycloheptatrien-1-one), and 3-hydroxy-4-pyrone. The latter is mildly goitrogenic and carcinogenic. Recent developents have come up with a biological solution to these problems. It involves the introduction of rumen micro-flora from animals known to be resistant to mimosine toxicity into the rumen of susceptible animals. The resistant micro-flora is capable of breaking down DHP (Jones, 1980).

Hegarty et al., (1981) reported that Troplone is strongly anti-peroxidase and carcinogenic. The first clinical signs of mimosine toxicity are loss of hair especially from the tail and mane (Jones et al., 1976; Blunt and Jones, 1977; and Jones, 1980). The toxicity is cummulative in the sense that it depends on the amount of Leucaena consumed and the length of time the animal has been on that diet. The greater the amount of Leucaena consumed and the longer the animal has been

consuming it, the more toxic it becomes but, if the diet is dicontinued before it becomes lethal the toxic effects stop.

Other symptoms of Leucaena toxicity include loss of appetite, excessive salivation, incoordinated gait, enlarged thyroid glands and poor breeding performance. Calves born of cows grazing on Leucaena for more than 6 months have enlarged thyroid glands and some die a few hours after birth (Jones, 1979).

Unlike ruminants in Australia, which were affected by more than 30% of Leucaena in their diet (Jones, 1980), ruminants in Hawaii were not affected by Leucaena at any level. It was thus suggested that probably the Hawaii ruminants had rumen microflora which were capable of degrading DHP (Jones, 1980). This eventually led to the isolation of the bacteria that can be used to counter the mimosine problem. No limitations have been cited in the literature reviewed for the utilization of Acacia, Albizia and Calliandra species.

2.4 GENUS SESBANIA:

2.4.1 TAXONOMY AND DISTRIBUTION:

The genus Sesbania scopoli has 4 sub-genera and over 50 species which are widely distributed in the tropics. It belongs to the sub-family Papilionoideae, tribe Robinieae (Evans and Rotar, 1987).

Of the 4 sub-genera of the genus Sesbania, Agait and Sesbania are of agricultural importance. Sub-genus Agait is mostly found in Asia, with species members Sesbania grandiflora and Sesbania formosa. The other sub-genus Sesbania is pantropically distributed with 33 species in Africa (Gillet, 1963), 10 species in Australia (Burbridge, 1965), and 8 species in Hawaii (Evans and Rotar, 1987). Most of these species are used as green manure and forage especially Sesbania bispinosa and Sesbania sesban (Evans and Rotar, 1987). Table 2.3 shows the origin, description and potential uses of Sesbania species in the tropics. There is high potential for forage and green manure production where they are grown.

2.5 SESBANIA SESBAN:

2.5.1 GENERAL CHARACTERISTICS:

Sesbania sesban (L) Merr. (Sesbania aegyptiaca Poir.) is a small tree or shrub which grows to a height of 4-6m under good conditions (Andrews, 1952; Skerman, 1977). It is well branched, with abundant soft pinnate leaves, and regenerates rapidly after prunning or grazing. It has been reported to grow in a wide range of climatic and edaphic conditions (Burbridge, 1965; Gillet, 1963; Wood and Larkens, 1987). Its rainfall range is 350mm - 2000mm per annum, while it can also tolarate extremes of pH, periodic flooding, water-logging and soil salinity (up to 1.4% salt concentration).

Table 2.3 SEBANIA SPECIES WITH POTENTIAL AGRICULTURAL USES:

SPECIES NAME	ORIGIN	DESCRIPTION	USE
S.arborea	Hawaii	5m tree	Browse, Ornamental
S.bispinosa (S.aculeata)	Africa, Asia, Australia	4m tree	Browse, Pulp, Green manure, Wood fuel, gum
S.cannabina (S.roxburgii)	"	"	"
S.emerus	C/S.America	Annual shrub	"
S.exaltata	N.America	"	Green manure
S.grandiflora	Pan-tropical	15-20m tree	Fodder, Fence, Green manure, Ornamental, Gum and Pulp
S.macrantha	Africa	Perennial, Large seeded	Browse
S.pachycarpa	Africa	Annual shrub	Browse
S.rostrata	Africa	4m shrub, Noduled stem and leaves	Browse, Green manure
S.sericea	Africa, Caribbean	4m shrub	Green manure, Wood fuel, Pulp, Fodder, Gum pole
S.sesban	Africa, Asia	4-6m shrub	Browse, Gum, pulp, Green manure
S.speciosa	Africa	Annual shrub	Browse, Green manure
S.tetraptera	Africa	Annual shrub	Browse

SOURCE; Evans and Rotar, 1987

Wood and Larkens (1987) reported that Sesbania sesban is a shallow rooted perennial (mostly biennial). It establishes well from seeds, although its seeds require treatment for hardness before planting. Evans and Rotar (1987) reported the use of concentrated sulphuric acid for scarification and sterilization of Sesbania seeds for 30 minutes with much success. They also reported the remarkable growth rate of Sesbania sesban. They noted that it is a very good competitor with weeds at all stages. It is generally found in heavy textured , poorly drained soils, beside streams, road sides and sand banks. It can tolerate low pH acidity (below 5.0) and low temperatures (17 -20 C) unlike Leucaena leucocephala which can not thrive in soils of low pH or at low temperatures.

Gore and Joshi (1976) in India reported that Sesbania sesban yielded 142 t/ha/yr of dry matter when NPK fertilizer was applied in splits and when the harvesting frequency was stepped up from 7-8 week to 5-6 week intervals. In comparison, Evans and Rotar, (1987) in the Pacific region reported that Sesbania sesban yielded 51.5 t/ha/yr. No mention of fertilizer or the cutting frequency in this trial was made.

Ella et al., (1989) compared the response of Gliricidia. Calliandra, Leucaena and Sesbania to lopping. They found that all species except Sesbania sesban responded well to frequent lopping especially during the dry season. On the contrary, recent findings at the University of Queensland Research Farm, Mt. Cotton have shown that Sesbania sesban which was grown on

seasonally water-logged podzolic soil, responded well to frequent cutting (3-4 week intervals) (Galang, 1988 unpublished data).

2.5.2 THE ROLE OF SESBANIA IN AGRICULTURE:

2.5.2.1 FORAGE DRY MATTER PRODUCTION:

Sesbania species have a considerable potential as a source of fodder for animals, contrary to the suggestion by Briscoe and Andrews (1938) that, Sesbania has no forage value, since it is not palatable. There is strong evidence to show that Sesbania is very palatable. Gillet (1963) reported that, all Sesbania species including leaves of the toxic seed producing species are palatable to stock. The susceptibility of Sesbania sesban to browsing due to high palatability and hence the requirement for protection of areas under cultivation was noted as a limiting factor in wood fuel production from Sesbania sesban (NAS, 1983). Katiyar and Rhanjhan (1969) noted that Sesbania sesban and Sesbania cannabina are more palatable to stock if fed after a period of wilting than as green fodder. Sidahmed et al., (1985) reported that Sesbania hay was less palatable than fresh material when fed to pregnant goats. Dougall and Bogdan (1958) reported that Sesbania sesban is a useful browse in Kenya. Through research efforts in Kenya, Sesbania sesban and Sesbania macrantha have been incorporated in fodder production and stall feeding of small ruminants for small holder dairy industry in the country (Personal communication). In East Africa in general, Sesbania sesban is extensively browsed by elephants and cattle (Gillet, 1963). Allen and

Allen (1981) mentioned that, Sesbania sesban and Sesbania speciosa Taub. are excellent forage and fodder plants in India and Taiwan. Sesbania sesban is valuable on land which is suject to periodic flooding. It is cultivated profitably on irrigated pastures. Sesbania cineriscens and Sesbania sesban var. zambesiaca of the African species are valuable cattle feed on the flood plains of Chambeshi River in Zambia and Malawi in Southern Africa.

2.5.2.2 WOOD FUEL:

In developing countries where petroleum based fuel sources are expensive, wood fuel is gathered from shrubs and trees including Sesbania sesban and Sesbania grandiflora (NAS, 1979). Sesbania grandiflora produces the highest stem content and hence has a higher energy potential than Sesbania sesban which produces lighter wood (Wood and Larkens, 1987).

2.5.2.3 HUMAN FOOD:

In some Asian countries, India in particular, flowers and young pods of Sesbania grandiflora and Sesbania sesban are used as vegetables (Evans and Rotar, 1987).

2.5.2.4 GREEN MANURE:

Sesbania species have a potential for green manuring, especially of rice crops in South East Asia due to their ability to grow in heavy soils and withstand soil salinity, alkalinity and water logging (Evans and Rotar, 1987). Rice yield from Sesbania green manure depends on the line of

Sesbania (Ghai, Rao and Batra, 1985), the stage of growth of the green manure plant (Bhardwaj and Dev, 1985), the time of incorporation (Beri and Meelu, 1979), and the amount of the green manure incorporated (Singh and Sinha, 1964). In Tanzania, it is used for green manuring and shade for coffee plantations (Mengistu, 1987).

2.5.2.5 OTHER USES:

Various Sesbania species yield fibre suitable for rope making and fish nets. A Japanese patent specifies the stems of Sesbania aegyptiaca as a substitute for hemp (Matsuoka, 1920; cited by Allen and Allen, 1981). Dundee fibre or daincha is made from stems of Sesbania cannabina Roxb. and Sesbania aculeata Poir. in India as a substitute for jute and hemp (Evans and Rotar, 1987). The long stems of Sesbania aculeata are also used for making paper which is similar to rice paper (Allen and Allen, 1981). Sesbania sesban wood is light and suitable for making toys and small objects of art and gun powder charcoal (Allen and Allen, 1981).

Many Sesbania species produce bark and seed gum. The bark of Sesbania grandiflora, S. formosa and S. sesban (dark varieties) exude gum when cut (Evans and Rotar, 1987). The potential for S. grandiflora bark gum to substitute gum arabica was reported in NAS (1979). Sesbania sesban produces seed gum content of 31-32% (Hussain and Khan, 1962b).

2.5.3 FEEDING VALUE OF SESBANIA SESBAN:

A comparison of dry matter digestibility has shown that Sesbania species are superior to most of the tree legumes. Topark-Ngarm and Gutteridge (1985) compared the digestibility of 6 tropical grasses and 13 tree legumes. The digestibility values ranged from 38.5 - 47.5 % for grasses, and 52.7 - 66.9 % for tree legumes. The following digestibilities were observed for other tree legumes, 57.9 - 69.1 % for Sesbania species, 62.2 for Leucaena leucocephala, 65.2 % for Gliricidia maculata and 73.3 % for S. grandiflora (van Eyes et al., 1986), 74 % for S. aculeata (Katiyar and Rhanjhan, 1969), and 66.5 - 71.4 % for S. sesban (Singh, Kumar and Rekib, 1980).

Most of the studies in animal production with Sesbania were conducted on small ruminants and monogastrics. Evans and Rotar (1987), noted that Sesbania is a good protein supplement for ruminants feeding on poor quality roughage. Goats supplemented with S. grandiflora gained 20g/day, while control (fed on napier grass alone) lost 1g body weight per day (van Eyes et al., 1986). Goats fed on S. sesban forage alone gained 17.1g/day; but when S. sesban concentrated mixture replaced 20% of the diet, it increased the daily liveweight gain to 30.9g (Singh, Kumar and Rekib, 1980).

2.5.4 ANTI-NUTRITIVE FACTORS:

The incorporation of Sesbania leaf and seed in the diets of monogastrics appears to be less suitable. Sesbania grandiflora leaf meal progressively depressed chicken feed intake and body weight when incorporated at the rate of 0, 5, 10, and 15 % of

the diet (Prasad, Reddy and Reddy, 1970). Katosh and Chopra (1974b) suggested that the depression of the growth rate of chicks fed on S. bispinosa seed meal was attributed to trypsin inhibitors.

In a similar trial by Brown et al., (1987) Sesbania sesban var. nubica was used as a protein supplement for broilers. It was fed at two levels (15 and 30 %) of the diet against Lucarne fed at 15 % of a similar diet as a control. Results showed that, all broilers fed on 30 % S. sesban diet died during the first week and those fed on 15 %, only 4 birds died and the rest survived but were illthrift. Analysis showed that S. sesban var. nubica has at least one toxic principle which killed the birds and reduced the performance of the others.

Kingshorn and Smolenski (1978) also reported that genus Sesbania contains a long list of potentially toxic substances such as: saponins, tannins and alkaloids (eg. sesbanine). Kingsbury (1964) reported that some Sesbania species produce toxic seeds. He showed that animals were normally poisoned when forage on offer was inadequate in quantity. He observed that seeds of S. punicea, S. drummondii and S. vesicaria were lethal to poultry. Similar findings were also reported by Flory and Herbert (1984), who found that when S. drummondii seeds were fed to poultry at the rate of 2-3 seeds per day, they were found to be lethal. Allen and Allen, (1981) reported that in North America especially in Northern Texas and Florida, symptoms of poisoning among cattle, sheep and poultry, have

been attributed to the consumption of leaves and seeds of S. drummondii. S. exaltata. S. punicea and S. vesicaria. It was assumed that the toxic elements in Sesbania were saponins. Allen and Allen (1981) also noted that S. sesban seeds are used for medicinal purposes.

2.5.5 SYMBIOTIC RELATIONSHIP WITH RHIZOBIUM:

Harris et al., (1949) reported that young nodules on Sesbania species have hemispherical meristems which become interrupted and apical in old multilobular nodules. The nodules have been found to be long lived. This is attributed to the continuously active meristematic area, functional longevity of the infection thread, an elaborately developed vascular system, the prevalence of non-invaded cortical sclerenchyma and a gradual disintegration of the bacteriod area. They also found that, Sesbania seedlings were highly receptive to infection by their homologous rhizobia. However different Sesbania species have restricted susceptibility and Sesbania rhizobia have a restricted host range too. Briscoe and Andrews (1938) observed that Sesbania species and their symbionts constituted a separate rhizobia:plant group, contrary to the generalized grouping of inoculation groups (Peoples et al., 1989).

Johnson and Allen (1952a) observed that 39 strains of Rhizobium from 6 Sesbania species, ineffectively nodulated beans and cowpea plants but not the reverse. All Sesbania homologous rhizobia strains nodulated all Sesbania species but, the degree of effectiveness varied from high to no effect at all.

The Rhizobium associated with Sesbania species has distinctive characters, including the occurrence in saline and alkaline soils (Bhardwaj, 1972, 1974). The Rhizobium strain of S. rostrata (ORS-571), is capable of fixing nitrogen through stem nodules when the nitrogen content of the nutrient medium is high (Dreyfus, Elmerich and Dommergues, 1983). In 52 days S. rostrata can fix about 250kg N/ha (Tsien, Dreyfus and Schmidt, 1983). It can also fix nitrogen in pure culture.

2.6 THE GENUS RHIZOBIUM:

2.6.1 TAXONOMY:

The genus Rhizobium belongs to the family Rhizobiaceae (Vincent, 1977). In the 7th edition of Bergey's manual, genus Rhizobium was one of the three genera which made up the family Rhizobiaceae within the order Eubacteriales. The current revision reduced the number of genera to two-Rhizobium and Agrobacterium. Both Rhizobium and Agrobacterium can cause cortical hypertrophy on plants but, they can be distinguished on the basis of the nature of the hypertrophy caused. Rhizobium forms morphologically organized nodules in legumes, while Agrobacterium forms disorganized galls on many kinds of plants (Vincent, 1977).

Vincent (1977) reviewed the origin of the name Rhizobium and found that-Phytomxa schroeter. 1886 is the valid generic name of Rhizobium, but recently the Judicial Commission of International Committee on Nomenclature of bacteria validated

Rhizobium as the generic name.

2.6.2 GENERAL DESCRIPTION:

The genus Rhizobium includes those bacteria which are capable of forming morphologically defined nodules on the roots of a member of the family Leguminosae (Vincent, 1977).

They are gram-negative rods (0.5 - 0.9 X 1.2 - 3.0 um). They occur singly or in pairs, motile when young. They have peritrichous, polar or sub-polar flagella, with prominent poly-b-hydroxybutyrate granules, more often without endospores. They are aerobic chemorganotrophs. They grow best at 25-30 C on complex media such as yeast extracts and does poorly in peptone agar (glucose). Their growth on litmus milk is slow, resulting in acidic or alkaline reaction. They are capable of utilizing nitrate, ammonia and amino acids as a sole source of nitrogen. Atmospheric nitrogen is primarily utilized in symbiosis with a legume host. "Fast-growers" have a mean generation time of 2-4 hours, forming large (2-4mm D), gummy, colourless or white colonies in 3-5 days. "Slow-growers" have a mean generation time of 6-8 hours, forming small (about 1mm D) colourless , gumles, dense and sticky, white or creamy colonies after 7-10 days.

2.6.3 SPECIES OF THE GENUS RHIZOBIUM:

Rhizobium species are defined according to the prefered legume host plant (Vincent, 1977);

Rhizobium leguminosarum - for Pisum, Vicia and Lathyrus.

Rhizobium trifolii - for Trifolium.

Rhizobium phaseoli - for Phaseolus.

Rhizobium meliloti - for Medicago and Meliloti.

Rhizobium lupini - for Lupinus and Ornithopus.

Rhizobium japonicum - for Glycine.

The first four species of Rhizobium are "fast-growers" (Vincent, 1977) and the last two species are "slow growers". Strains of Rhizobium are formed when a rhizobial culture is not known to have common clonal history with another culture.

2.6.4 RHIZOBIUM-HOST INTERACTION:

Dart (1977) observed that Rhizobium-host interaction begins in the rhizosphere during the initial infection of the root, followed by entry of rhizobia into the host cells, their development and synthesis of nitrogenase. This interaction determines nodule number and their development. Environmental factors may influence the interaction positively or negatively.

Peoples et al., (1989) observed that there are five conditions which may render the soil devoid of Rhizobium to form an effective symbiosis with a legume, which may then warrant inoculation;

1. The absence of the same or related legume species in the past history of land use.
2. Poor nodulation of the previous crop.
3. When the legume follows a non-legume crop rotation.
4. In land reclamation.
5. When environmental conditions are adverse for Rhizobium survival.

2.6.5 NODULE DEVELOPMENT:

Allen and Allen (1936) found that nodule shape is a characteristic of the host plant rather than Rhizobium strain. It is determined by the pattern of meristematic activity. They may be broadly classified as; - round or oval, elongate to club-shaped, branched to coralloid and collar nodules. They observed that, nodule shape may differ within a genus, contrary to the observation made by Corby (1971) who suggested that nodule type within a tribe tended to be similar.

Pueppke (1986) studied nodule development and distribution on legume roots of plants grown in hydroponics and soil. He found that, the upper most nodules on the primary roots were clustered near the position occupied by the root tip at the time of inoculation. This is the region thought to be most susceptible to nodulation. A good number of nodules also developed down the length of the primary root. He found that soil culture reduced the extent of scattering of nodules alongthe primary root which shifted the nodule ratio to lateralroots. Soil also limited the interaction between the strains of Rhizobium and the host plant.

Dart (1977) reviewed the effect of delayed inoculation on nodule development. He observed that, when inoculation was delayed for 12-30 days after sowing of Trifolium pratense. there was an increase in nodule formation, which shortened the time between inoculation and nodulation. Similarly Nutman (1949) observed that even after 80-day delay, nodulation

occured successfully. Delaying inoculation for 5-25 days had a similar effect on nodulation of Medicago truncatula, Trifolium subterraneum, Pisum sativa and Glycine max (Nutman, 1967; Dart and Pate, 1959). As inoculation is delayed the older parts of the root become progressively resistant to infection (Dart, 1977) with the formation of nodules lower down the primary root. Delayed inoculation also causes nodules to form in clusters and many more nodules than those formed when inoculation was done at sowing.

2.6.6 MINERAL NUTRITION OF RHIZOBIUM:

Qualitative requirements for rhizobia growth have been demonstrated for Phosphorus, Potassium, Calcium, Magnessium, Cobalt, Molybdenum, Zinc, Manganese, Iron and recently Nickel (Robson, 1978). Norris (1965) observed that, if Rhizobium had a calcium requirement, it was very low and could be satisfied by the impurities contained in the culture media. Bergersen (1961) and Vincent (1962) on the other hand showed that there was a definite requirement for calcium by Rhizobium but, the response to calcium ceased at 1ppm, as agar is a calcium compound, hence there is no need to add calcium carbnonate to agar cultures because it has a deleterious effect on slow-growing Rhizobium. In the absence of calcium carbonate, Sesbania sesban strains of Rhizobium are acid producers (Norris, 1965), contrary to what Johnson and Allen (1952) reported earlier. They found that, all 39 strains of Rhizobium for S. sesban produced a slightly alkaline reaction on mannitol.

2.6.7 ENVIRONMENTAL FACTORS AFFECTING SAPROPHYTIC SURVIVAL OF RHIZOBIUM:

2.6.7.1 EFFECT OF COMBINED NITROGEN:

Graham and Chatel (1983); Harper (1974); and Mahon and Child (1979) observed that a small starter dose of combined nitrogen is beneficial to plant development and subsequent nodulation and function, especially when initial nodulation is retarded. However larger quantities of combined nitrogen reduced both nodulation and nitrogen fixation (Graham and Chatel, 1983; Raggio, Raggio and Torrey, 1965; Rigand, 1976; and Sprent, 1979).

Munn (1968) observed that combined nitrogen at the concentration of 0.1 uM in water culture considerably reduced root hair production and curling in Medicago sativa with Rhizobium melliloti When a concentration of 10-20 ug of nitrate and nitrite were applied, it stimulated nodulation but, when 40 ug was added, nodulation was delayed. On the other hand a concentration of 10ug of ammonium and urea had no effect but when 100ug was added, nodulation was delayed. Urea alone at 1mg N/plant prevented root infection.

Similarly, Graham and Chatel (1983) reported that the rate of seasonal nitrogen fixation in cultivars of Phaseolus vulgaris was reduced by 17.8 - 39.6 % when 15 kgN/ha were applied. This also affected various stages of nodulation and nitrogen fixation including, strain recognition, root hair production and curling, nitrogenase activity, leghaemoglobin synthesis,

energy supply to nodules and nodule life.

Yoshida (1979) observed that organic sources of combined nitrogen for example, farm yard manure are less inhibitory to nodulation than urea and ammonium and could be exploited much more in developing countries.

2.6.7.2 EFFECTS OF TEMPERATURE, LIGHT INTENSITY AND DAY LENGTH:

Elevated temperatures adversely affect the survival of rhizobia in the soil but, the rate of survival depends on the Rhizobium strain (Bowen and Kennedy, 1959; Danso and Alexander, 1974). Slow-growing strains were more tolerant of high temperatures than fast-growing strains (Marshall, 1964). Soil temperatures in the tropics often rise above 40 C, a temperature that would kill many rhizobia. Tolerance of such temperatures may be an important characteristic on which inoculum strains should be selected (Eaglesham et al., 1981).

Light intensity and day length affect nodule formation, distribution and structure because of the direct link between light intensity and photosynthesis, which are in turn affected by photoperiod. Low light intensity results in low rate of photosynthesis, hence low amount of assimilates being translocated to the roots. As a result, few or no nodules develop on seedlings in the dark, and those which develop, usually degenerate after two weeks (Dart, 1977).

2.6.7.3 EFFECT OF SOIL MOISTURE AND pH:

Both flooding and desiccation have negative effects. Flooding is known to reduce the supply of oxygen and may depress the pH of the soil, which may affect the survival of Rhizobium. On the other hand, desiccation affects the permeability of the membrane which causes the cell contents to leak out (Marshall, 1964; Bushby and Marshall, 1977a).

Sprent (1972) observed that a soil moisture potential close to field capacity is necessary for active nitrogen fixation. A reduction in nitrogen fixation is experienced when water potential reaches minus 2.5 bars and ceases totally at minus 15 to 20 bars (Patterson et al., 1979; Kuo and Boermaa, 1971).

Soil pH affects the Rhizobium species present in the soil. Slow-growing strains of Rhizobium are more tolerant of acid conditions. They can survive at pH 4.0 (Fred et al., 1932), whereas fast-growing strains prefer more alkaline conditions. Rhizobium trifolii prefers a pH greater than 4.8 - 5.0 while Rhizobium meliloti is more acid sensitive and will not grow below pH 5.5 - 6.0 (Vincent, 1981).

2.6.7.4 EFFECTS OF DISEASES AND PESTS:

Root-rot affects nodulation and nitrogen fixation (Graham and Chatel, 1983). Nodule infection by Bean Yellow Mosaic Virus (BYMV), nematodes and insect larvae, reduce nodule weight and therefore nitrogen fixation (Diatloff, 1965).

2.6.7.5 EFFECT OF HERBICIDES:

Graham and Chatel (1983) showed that, contrary to earlier report by Kapusta and Rouwenhorst (1973) Rhizobium is sensitive to herbicides. They observed that the sensitivity is only evident when abnormally high levels of herbicides are used. Jordan and Garcia (1969) also showed that a normal dose of 10 ug/ml of 2, 4-DB (2, 4-dichlorophenoxyl butyric acid) had no effect on Rhizobium. when it was sprayed on Lotus but, when a dose of 500ug per ml was sprayed, Rhizobium was adversely affected. The inhibition on nodulation at a higher dose was shown to be due to the effect of herbicide on the host plant. Dunigan et al., (1972) observed that, compensatory nodulation occurred after herbicide effects had worn off, by the production of new nodules or an increase in nodule size, and the final yield was not affected (Lesniuc, 1974; Kapusta and Rouwenhorst, 1973). Graham and Chatel (1983) concluded that herbicides are beneficial as long as recommended dosages and methods of application are used.

2.7 THE EVALUATION OF NITROGEN FIXATION:

2.7.1 WHY MEASURE NITROGEN FIXATION:

Nitrogen fixation is measured in an effort to understand the contribution of biological nitrogen fixation to the nitrogen cycle; in order to develop sustainable farming systems by using cultural methods; and to evaluate the symbiotic effectiveness of new legume introductions (Peoples et al., 1989).

2.7.2 METHODS OF EVALUATION:

Peoples et al., (1989) concluded that, there is no single correct method for measuring nitrogen fixation because there are many variations in legume plants and environmental conditions under which they are grown. They suggested that, each method has its advantages and limitations which have to be taken into account. Brockwell (1980) observed that regardless of the method used to measure nitrogen fixation, it is necessary to determine the amount of total plant nitrogen if inputs of nitrogen fixation are to be quantified in terms of kgN/ha. However the evaluation and interpretation of nitrogen fixation must take into account soil nitrogen status.

2.7.3 EVALUATION OF NODULATION:

Nodule score is judged according to the number of effective nodules in the crown zone (a region 5cm around the hypocotyl) and elsewhere on the root system (Brockwell, 1980). Nodule size and colour should also be taken into account when evaluating the effectiveness of nitrogen fixation qualitatively.

2.7.4 ANALYSIS OF TOTAL PLANT NITROGEN:

There are two methods on which all commonly used analyses are based (Bergersen, 1980). The first is the oxidative method, in which organic material is oxidized in the presence of copper oxide to prioduce Nitrogen dioxide gas, the volume of which is measured. This is based on the original Dumas technique, and is usually restricted now to commercially produced apparatus

such as the Coleman nitrogen analyser.

The other method which is commonly used is known as; The Kjeldahl Wet Digestion Method. In this method both organic and mineral nitrogen are reduced to ammonia in hot sulphuric acid in the presence of a catalyst. The ammonia is recovered by distilation or difussion and estimated by titration or calorimetrically (Bergersen, 1980) and calculated as below;

$$mgN = \frac{\text{Normality of HCl} \times 0.5 \times \text{Titre vol.} \times 1/0.03571.}{}$$

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3. MATERIALS AND METHODS :

3.1 LOCATION AND OBJECTIVES:

Two experiments were conducted in a glasshouse at the University of Queensland, St. Lucia, Brisbane (Lat.27 30'S;153 E) between Feb.5, 1990 and Jul.5, 1990, in glasshouse conditions (Temp.23 min.-27 C max.). The aim was to evaluate the effectiveness of strains of Rhizobium, isolated from various Sesbania species collected in Australia, Pakistan, Phillipines and Thailand, for nodulation and nitrogen fixation of Sesbania sesban var.nubica (CPI-30071). This constituted experiment-1, which was done using sand culture medium. The most promising Rhizobium strains from experiment-1, were used in experiment-2 on five lines of Sesbania sesban (9265, 10895, 15022, 15036 and CPI-30071) in loamy sand soil, to evaluate strain X host interaction.

3.2 CULTURE AND CULTURE MAINTENANCE:

Strains of Rhizobium used in this series of experiments are listed in Table 3.1. They were stored on either Tryptone Yeast Agar (TYA) of Yeast Mannitol Agar (YMA) at 5 C as indicated. The composition of the media is given in Table 3.3 and 3.5. Nine of the strains were Sesbania isolates held in the Department of Agriculture, University of Queensland; three were isolates made by the author from Sesbania sesban growing on California mix soil, and three were obtained as lyophilized cultures from CSIRO, Division of Tropical Crops and Pastures, Cunningham laboratories, St. Lucia.

Table 3.1 LIST OF STRAINS OF RHIZOBIUM:

CODE NAME	MEDIUM	ORIGINAL HOST	ORIGIN	FAST/SLOW
PMA-123	TY	S.rostrata	Thailand	F
" 124	"	S.speciosa	"	"
" 126	"	Sesbania spp	"	"
" 229/1	YMA	S.grandiflora	Phillipines	S
" 229/2	"	" "	"	"
" 252/1	"	S.aculeata	Pakistan	F
" 254/5	"	" "	"	"
" 256	"	" "	"	"
" 258/1	"	S.rostrata	"	"
" 295/1	"	S.sesban	Australia	"
" 295/2	"	" "	"	"
" 295/5	"	" "	"	"
CB- 2743	"	Sesbania spp	"	"
" 3023	"	" "	"	"
RAD-624/3	"	" "	"	"

*PMA = Strains held by the Department of Agriculture, Qld. Uni.

*CB and RAD = Strains held by CSIRO Laboratories, St. Lucia.

3.3 SESBANIA SEEDS:

Seeds from five lines of Sesbania sesban were used in the experiments as shown in Table 3.2 below;

Table 3.2 VARIETY OF SESBANIA SESBAN SEEDS:

Species Name	Code Name	Country of Origin
Sesbania sesban	CPI-30071	India
" "	-9265	ILCA-Ethiopia
" "	-10895	" "
" "	-15022	" "
" "	-15036	" "

3.4 EQUIPMENT AND FACILITIES:

3.4.1 CHEMICALS:

All chemicals used in these experiments were of analytical reagent grade.

3.4.2 LABORATORY GLASSWARE, EQUIPMENT AND FACILITIES:

All glassware used was of laboratory grade. Plastic pots and polythene liners were from Nally Plastics, Australia. All facilities for the experiments were provided by the Department of Agriculture, University of Queensland, St.Lucia. Routine sterilization of media and glassware was through the use of an autoclave run at 121 C for 20 minutes.

3.4.3 GLASSHOUSE FACILITIES:

Experiments were carried out under glasshouse conditions at a temperature range of 23 C min to 27 C max. Evaporative coolers

were used to maintain day temperatures below 30 C and night heaters were used to keep minimum temperature above 23 C. Sterilized deionized water was used for watering the experiments.

3.5 MEDIA:

Table 3.3 , 3.4 and 3.5 show the composition of media used for growth and storage of Strains of Rhizobium, after Vincent (1970).

Table 3.3 TRYPTONE YEAST AGAR (TYA):

Ingredient	Amount
Tryptone	5.0g
Yeast Extract	3.0g
CaCl2.2H2O	0.9g
Agar	15.0g
Deionize Water	1L

Table 3.4 TRYPTONE YEAST BROTH (TYB):

Ingredient	Amount
Tryptone	5.0g
Yeast Extract	3.0g
CaCl2.2H2O	0.9g
Deionized Water	1L

Table 3.5 YEAST MANNITOL AGAR (YMA):

Ingredient	Amount
Mannitol	10.0g
K2HPO4**	0.5g
MgSO4.7H2O**	0.8g
NaCl**	0.2g
Congo Red	0.025mg
Yeast Extract	0.4g
Agar	15.0g
Deionized Water	1L

**Added as 10ml of 50g/L stock solution

The composition of Yeast Mannitol Broth (YMB) is similar to that of YMA but Agar and Congo Red were excluded in YMB.

3.6 METHODS:

3.6.1 SUB-CULTURING OF STRAINS:

The strains used were previously listed in Table 3.1. The lyophilized cultures were reconstituted in 5ml YMB in capped test tubes and incubated at 28 C for 7 days before sub-culturing. In all cases, a loopful of culture containing strains was transfered into Petri dishes containing either YMA or TYA as the case required and streaked. Petri dishes were incubated at 28 C for 6-7 days and checked for purity by uniformity of colony type.

3.6.2 INOCULUM PREPARATION:

Previously prepared YMB and TYB in capped test tubes were used for the preparation of the inoculum. A loopful of each strain culture was transfered to the broth and shaken. Test tubes were incubated at 28 C for 6-10 days before the inoculum was used. During the incubation period test tubes were shaken daily.

3.6.3 SEED SCARIFICATION, STERILIZATION AND GERMINATION:

Seeds from five lines of Sesbania sesban were obtained, graded and dried well. Concentrated sulphuric acid [1M] was used for scarification and sterilization of seeds. Two hundred seeds were placed in a 50-ml flask with a rubber stopper. Twenty ml of acid was added and swirled to coat the inside walls of the flask for 15 minutes. The acid was decanted and 30ml of sterile water was added immediately and shaken vigorously to prevent localised heating of seeds by the acid when it comes in contact with water. Seeds were then subjected to 8 rinses with sterile water and the 8th rinse was retained for 2 hours in the flask to allow imbibition.

The water in the flask was decanted and the seeds were transfered using flamed forceps to Petri dishes containing 1 % water agar. About 100 seeds were transfered into each Petri dish, spread out evenly on the agar, and incubated for 24 hours before planting.

3.6.4 PLANTING, THINNING AND INOCULATION:

A sterile glass rod was used for making holes (1-2cm deep) in the culture (sand/soil).Flamed forceps were used to transfer germinated seeds from Petri dishes into the culture.Five seeds were placed in each jar/pot and covered well until the emergence of the cotyledons.

Seven days after planting,thinning was done by using a flamed forcep to pull out all but 2 healthy and uniform seedlings per jar/pot.

Inoculation with appropriate strains of Rhizobium (Table 3.1) was done by administering 2ml of inoculum per seedling at the base. The culture was then moistened with 30ml of deionized water to enable the bacteria to infiltrate the culture faster.

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4. E X P E R I M E N T - 1 :

THE EFFECTIVENESS OF 15 STRAINS OF RHIZOBIUM IN NODULATION AND NITROGEN FIXATION OF SESBANIA SESBAN var. NUBICA IN SAND:

4.1 EXPERIMENTAL DESIGN:

A randomized complete block design with 3 replicates was used.

Treatments: Fifteen (15) strains of Rhizobium as shown in Table 3.1, plus two controls (+N and -N) were used on Sesbania sesban var. nubica (CPI-30071).

4.2 METHODS:

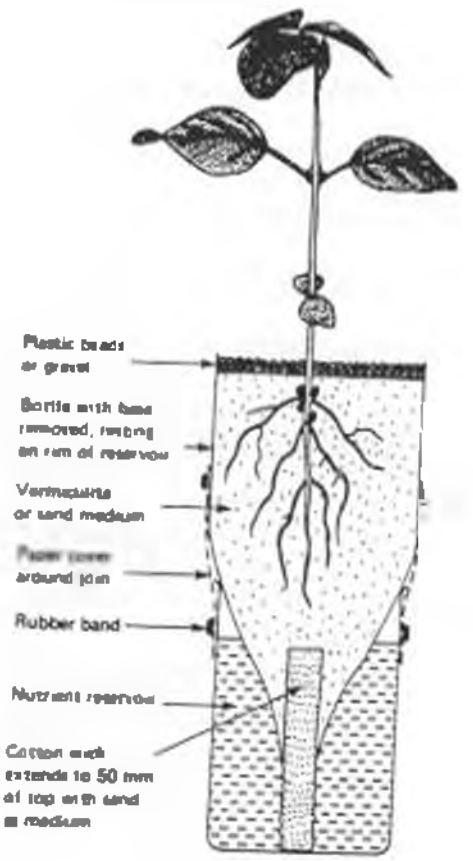
4.2.1 MODIFIED LEONARD JAR PREPARATION:

The modified Leonard jar assembly as described by Gibson (1980), consists of two parts: a wide-mouthed jar which serves as a reservoir, and an upper vessel mounted on it, made out of a 750-ml wine bottle with the bottom removed, containing sand, as shown in Figure 4.1.

The mouth of the bottle was plugged with cotton wool as shown in Figure 4.2, filled with washed sand of medium grade, (leaving 4cm at the top) and mounted on the jar. The sand was moistened with 200ml of plant nutrient solution shown in Table 4.1, and another 300ml of the same solution was poured into the jar. The whole assembly was then covered completely with a paper bag and sterilized in an autoclave at 121 C for 90 minutes. A total of 51 jars were prepared and transfered to a

FIGURE 4.1: LEONARD JAR ASSEMBLY:

(After Gibson, 1980)



glasshouse where the experiment was conducted on clean bench tops.

At planting, the paper bags were replaced with cylindrical laminated aluminium foil sheets which were used for blocking radiation from the rooting zone. All bottles were topped with sterile plastic beads after the emergence of the seedlings, to minimize chances of contamination. Deionized sterile water was added to the reservoir when necessary, generally after 4 weeks.

Inoculation with strains of Rhizobium was done 7 days after planting and thinning. Potassium nitrate fertilizer was also applied as a control in the appropriate jar to give 75ppm per jar in two splits. The first split was applied at inoculation and the second 4 weeks after planting.

Table 4.1 PLANT NUTRIENT MEDIUM : EXPERIMENT-1:

Ingredient	Amount/jar
KCl	0.001M
CaSO4.2H2O	0.002M
K2HPO4	0.001M
MgSO4.7H2O	0.001M
CuSO4.5H2O	0.157uM
ZnSO4.7H2O	0.382uM
MnSO4.4H2O	4.550uM
(Na)6Mo7O24.4H2O	0.004uM
H3BO3	11.560uM
C6H5O7.Fe.5H2O	5.400uM

Source: Norris and Date, 1976.

4.2.2 DATA COLLECTION:

4.2.2.1 PLANT HEIGHT MEASUREMENT:

Measurement of plant height was done every two weeks starting from week-2 to week-7 (harvest) as shown in Table 4.2 below;

Table 4.2 MEAN PLANT HEIGHT AT HARVEST AND GROSS RATE(GR):

Treatment	Mean Height(mm/jar)	GR(mm/jar/wk)
+N	362.5 d	7.25
CB-3023	85.8 c	1.72
PMA-295/2	84.5 c	1.69
RAD-624/3	48.3 b	0.97
PMA-123	36.8 ab	0.74
" -126	36.7 ab	0.73
-N	31.7 a	0.63
PMA-295/1	31.7 a	0.63
" -252/1	30.8 a	0.62
" -229/1	30.0 a	0.60
" -295/5	30.0 a	0.60
" -124	29.2 a	0.58
" -229/2	28.3 a	0.57
" -254/5	28.3 a	0.57
" -256	28.3 a	0.57
CB -2743	25.8 a	0.52
PMA-258/1	25.0 a	0.50

LSD (P = 0.05) =18.3 mm

** Means marked with a common letter are not significantly different at 5% level of significance.

4.2.2.2 HARVESTING:

Plants were harvested seven weeks after planting. Plants were harvested by cutting shoots at the root level and dried in separate labelled bags in a dessicator at 80 C for 48 hours. Plastic beads were tipped off and by applying high water pressure at the mouth of the bottle, all sand and roots were pushed out into a wash basin containing wire mesh for sand collection. All sand was washed away from the roots and observations of nodulation were made and recorded directly into a data collection sheet as shown in Appendix-B. Nodules were removed from the roots and dried in labelled tins. Roots were placed in paper bags and dried in the same way like roots.

4.3 RESULTS:

4.3.1 GENERAL OBSERVATIONS:

Plant growth rate was relatively slow for most of the treatments except, +N, CB-3023, PMA-295/2, and some plants in RAD-624/3, PMA-123 and PMA-126 as shown in Table 4.2. Apart from the above mentioned treatments, all others showed signs that may indicate nitrogen deficiency.

4.3.2 MEAN PLANT HEIGHT AT HARVEST:

Plants inoculated with strain PMA-295/2, CB-3023, RAD-624/3, PMA-123, PMA-126 and treatment +N, were significantly taller (P=0.05) than the control (-N) plants. The rest of the strains did not attain significantly different (P=0.05) height from the control plants.

4.3.3 NODULATION RATING:

At harvest most of the plants were yellow and small except five treatments (PMA-123, 295/2;CB-3023;RAD-624/3;and +N) which had green plants, conspicuously taller than the rest. However treatments, RAD-624/3 and PMA-123 had only 2 plants each which were green in one replicate and 4 yellow plants each in the other replicates, indicating a possibility of poor infection or contamination.

Most of the nodules were small, round, white and numerous, except for nodules on green plants which were few, large, round and pink. Nodule position was mostly crown and lateral on the same plant, except for a few plants which had only lateral nodules, and none of the plants had crown only nodules.

4.3.4 DRY WEIGHTS:

Table 4.3 shows mean dry weights of shoot, root, nodules and total dry matter yield in mg/treatment. Out of the 15 strains, only three (PMA-295/2;CB-3023 and RAD-624/3) produced significant dry weights above control treatment (-N).

Table 4.3 DRY MATTER YIELD: (mg/treat.):EXPT-1:

Treatment	Nodule wt.	Shoot wt.	Root wt.	Total wt.
+N	0.0	3113.2 f	790.8 c	3904.0 e
PMA-295/2	139.7 d	640.4 e	154.3 b	934.4 d
CB-3023	110.7 c	413.2 d	99.0 ab	622.8 c
RAD-624/3	47.7 b	163.6 c	61.4 ab	271.7 b
PMA-123	11.7 a	56.0 b	38.3 a	106.0 a
" -254/5	16.3 a	39.2 a	28.9 a	85.4 a
-N	0.0	45.2 b	36.2 a	81.4 a
PMA-126	12.7 a	37.2 a	29.9 a	79.8 a
" -295/5	4.3 a	41.6 ab	33.3 a	79.3 a
" -124	9.0 a	37.6 a	30.2 a	76.8 a
" -252/1	10.0 a	36.0 a	27.9 a	73.9 a
" -295/1	13.0 a	33.6 a	26.9 a	73.5 a
" -229/1	11.3 a	33.2 a	26.5 a	71.0 a
" -229/2	3.0 a	38.0 a	27.2 a	68.2 a
CB-2743	9.0 a	22.0 a	25.3 a	66.3 a
PMA-256	3.7 a	36.6 a	25.3 a	60.6 a
" -258/1	9.0 a	25.2 a	20.0 a	54.2 a
LSD(P=0.05)=	29.3mg	184.4mg	70.1mg	263.9mg

** Means marked with a common letter are not significantly different at 5% level of significance.

4.4 DISCUSSION:

The visual symptoms observed for most of the strains were similar to those described for nitrogen deficiency (Gonzales et al., 1980). The nitrogen deficiency may have been caused by the ineffectiveness of nodule Rhizobium as indicated by the size and colour of the nodules, the white colour being indicative of ineffective nitrogen fixation (Bergersen, 1980;

Dart, 1977). Thus the treatments that were infected with ineffective Rhizobium lacked enough nitrogen and therefore they did not make significant growth as shown in Table 4.2.

Possible causes of ineffectiveness may include, inhibition by environmental factors in the rhizosphere (Munns, 1968) and incompatibility between the host and strains of Rhizobium (Wilson, 1946; Date and Halliday, 1980).

Although treatments RAD-624/3, PMA-123 and PMA-126 gave mean height more than control plants, there is a possibility that they were contaminated or due to ineffective nodulation as shown in the results where some plants were tall and green while others were small and yellow. Thus these strains could not be selected as effective strains on Sesbania sesban var.nubica. Hence more work needs to be done on these strains to determine if the variable plant growth was due to genetically variable inoculum or due to cross contamination with other strains.

* * * * * *

5. E X P E R I M E N T - 2 :

THE EFFECTIVENESS OF TWO STRAINS OF *RHIZOBIUM* IN NODULATION AND NITROGEN FIXATION OF FIVE LINES OF *SESBANIA SESBAN* IN MOGILL LOAMY SAND:

5.1 EXPERIMENTAL DESIGN:

A randomized complete block design with 4 nitrogen treatments, 5 *Sesbania sesban* lines and 4 replicates were used. The 4 nitrogen treatments were:

(a) *Rhizobium* strain PMA-295/2

(b) *Rhizobium* strain CB-3023

(c) Combined nitrogen (+N) and

(d) Control (-N)

5.2 METHODS:

5.2.1 SOIL PREPARATIONS:

Mogill loamy sand, a podzolic type of soil was obtained, sun dried and sieved (5mm) to remove debris and large clods of soil. Tests were carried out to determine; the pH, bulk density and field moisture capacity as shown in Table 5.1 below.

Table 5.1 SOIL PROPERTIES:

pH in 1:5 water suspension	5.2
pH in 1:5 $CaCl_2.2H_2O$ (0.1M)	4.4
Bulk Density	1.5 g/cc
Field Moisture capacity	7.5 %

Eighty plastic pots (150mm D) were lined with polythene liners and filled with 1.5kg of soil. Basal fertilizer recommended for this type of soil as shown in Table 5.2 was weighed out for each pot and hand-mixed before planting. Pots were arranged on bench tops as shown in experimental layout appendix-A (2).

5.2.2 SEED PREPARATION:

Seeds were scarified, sterilized and germinated following standard procedures as described in section 3.6.3. Five lines of Sesbania sesban were used in this experiment as shown in Table 3.2.

5.2.3 WATERING:

Deionized water was used for watering the soil to field capacity when necessary.

5.2.4 PLANTING, THINNING AND INOCULATION:

Planting was done as described in section 3.6.4. Thinning and inoculation were done 7 days after planting. Two strains of Rhizobium (PMA-295/2 and CB-3023) selected on the basis of their outstanding effectiveness in nodulation in experiment-1, were used in experiment-2.

5.2.5 FERTILIZER APPLICATION:

Ammonium Nitrate fertilizer was applied at the rate of 125kg/ha (0.624g/pot) in two splits at 4 weeks intervals. The first split was applied one week after planting.

Table 5.2 BASAL FERTILIZER FOR POT CULTURE: EXPERIMENT-2:

Ingredient	Rate (kg/ha)	Amount/pot
$NaH_2PO_4.2H_2O$	503.0	0.890g
KCl	115.0	0.204g
Na_2SO_4	111.0	0.196g
$CaCl_2.2H_2O$	110.0	0.195g
$MgCl_2.2H_2O$	125.0	0.221g
$CuCl_2.2H_2O$	5.4	9.560mg
$ZnCl_2$	5.2	9.200mg
$MnCl_2.4H_2O$	16.2	28.670mg
$Na_2Mo_4.2H_2O$	0.5	8.850mg
$Na_2B_2O_7.10H_2O$	2.7	0.004mg
1 % Fe-EDTA	-	46.900mg

Source: Wallis et al., 1977.

5.2.6 DATA COLLECTION:

5.2.6.1 PLANT HEIGHT MEASUREMENTS:

Measurements of plant height were made weekly from the first week after planting (at inoculation) up to harvest as shown in Appendix-E.

5.2.6.2 HARVESTING:

Plants were harvested 9 weeks after planting, in a similar way as described in section 4.2.2.2.

5.2.6.3 ANALYSIS FOR PLANT NITROGEN:

The analysis for total plant nitrogen was done as described in section 2.7.4.

5.3 RESULTS:

5.3.1 GENERAL OBSERVATIONS:

5.3.1.1 GERMINATION:

After 24 hours of incubation at 27 C, line 9265, 10895, 15022 and 15036 attained above 95% germination with radicles 5-20mm long, while line CPI-30071 attained 75% germination with radicles 1-5mm for the same period of time.

5.3.1.2 EMERGENCE:

Four lines (9265, 10895, 15022, 15036) attained 100% emergence of seedlings in 48 hours, while line CPI-30071 attained 90% of emergence. All lines except CPI-30071, had very robust and uniform seedlings at thinning and inoculation time (1 week after planting. Some seedlings of this line had deformed small leaves on emergence.

5.3.1.3 SEEDLING PERFORMANCE:

Line 15022 and 15036 started branching on the 2nd week after planting from their first nodes, while the other 3 lines remained unbranched to harvesting. Lines 15022 and 15036 were leafier, with shorter internodes than the other 3 lines; and their stems were darker (brown), thicker ang angular with numerous small spines; while the other lines had pale green, thinner stems; longer internodes with numerous small spines.

5.3.1.4 DEFICIENCY SYMPTOMS:

At week-5, all plants under control treatment (-N) and some of the inoculated plants started showing signs of nitrogen deficiency. At week-7, the inoculated plants had improved, but control plants became progressively yellow and lost some of their leaves. At week-9, line CPI-30071 was at booting stage in two pots under +N treatment, while the rest of the plants remained vegetative. Sesbania sesban was susceptible to a number of pests which consumed leaves and sap, eg; caterpillars, aphids and red spiders. No pesticides were used but large pests were physically removed.

5.3.2 PLANT GROWTH RESPONSE:

Seedlings were relatively uniform at week 1, for all treatments attaining average height of 2.4cm for the inoculated, 2.3cm and 2.5cm for the +N and -N treatments respectively.

Figure 5.1-5.5 show that uniformity in growth was exihibited by all the Sesbania sesban lines, showing no treatment differences up to 5 weeks of age. The +N treatment picked up dramatically after the 5th week in all lines. However the response to Rhizobium depended on strain/host interaction. Strain PMA-295/2 was more uniform across the lines. Three lines (15036, 10895 and 15022) started showing improved growth at week-6 (Figure 5.2-5.4). The other two lines had delayed response, with line CPI-30071 responding at week-7 (Figure 5.5) and line 9265 not showing any appreciable response until week-8 (Figure 5.1).

FIG: 5.1: GROWTH RESPONSE OF LINE 9265 TO TREATMENTS

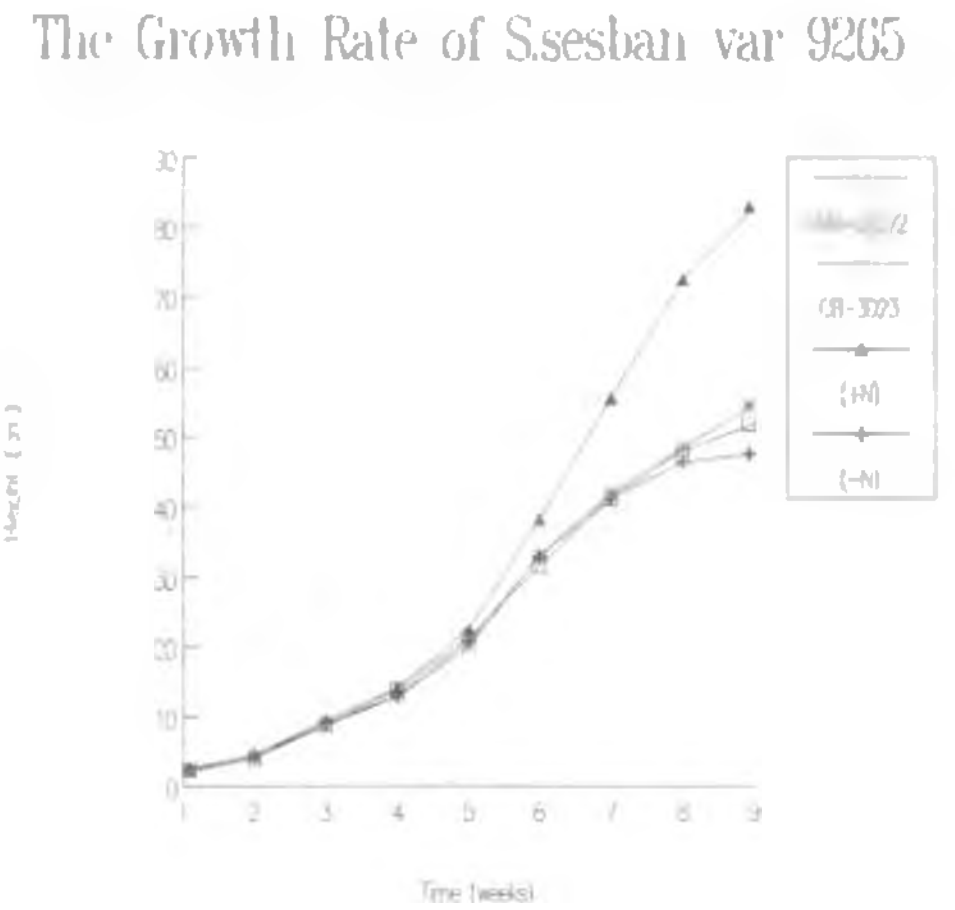


FIG. 5.2 : GROWTH RESPONSE OF LINE 15036 TO TREATMENTS

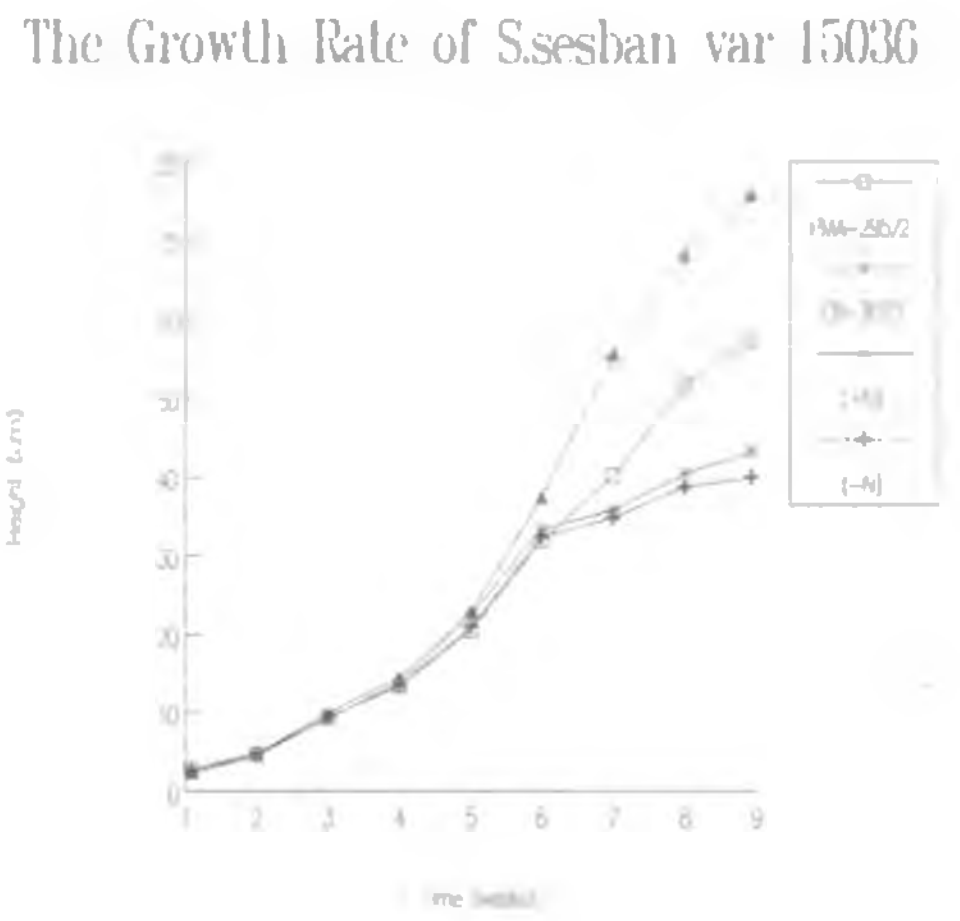


FIG: 5.3: GROWTH RESPONSE OF LINE 10895 TO TREATMENTS:

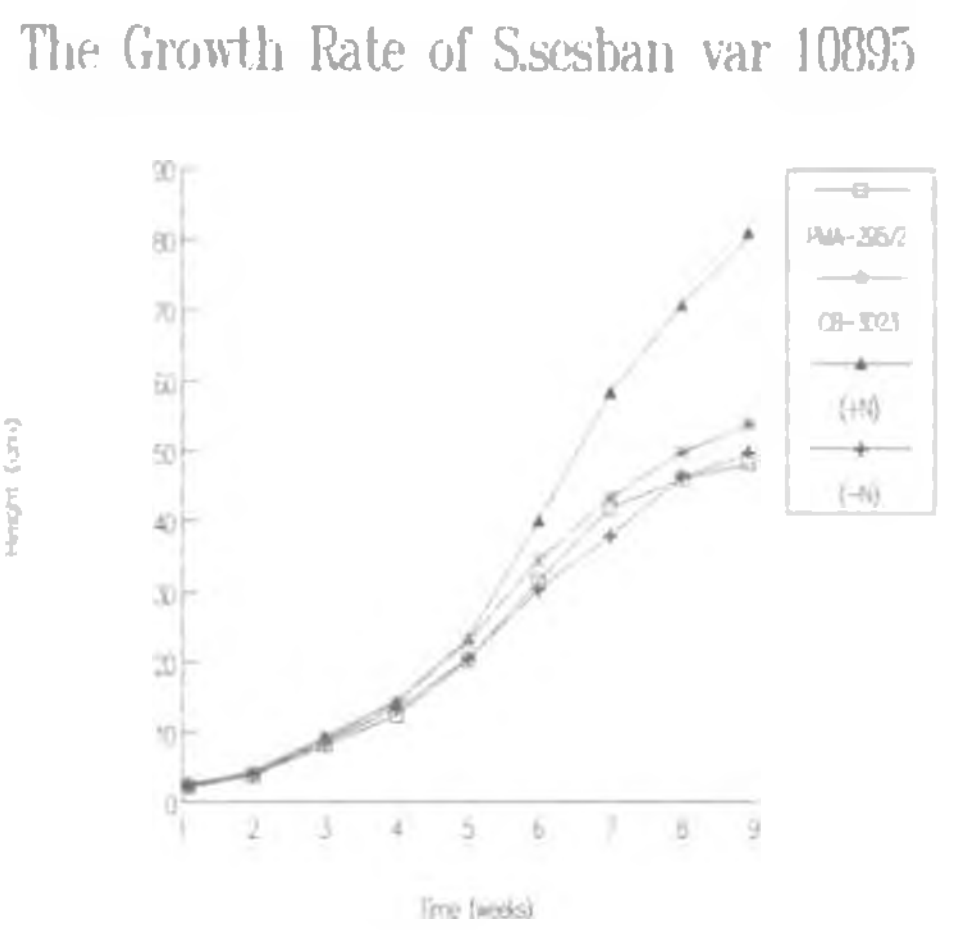


FIG: 5.4: GROWTH RESPONSE OF LINE 15022 TO TREATMENTS

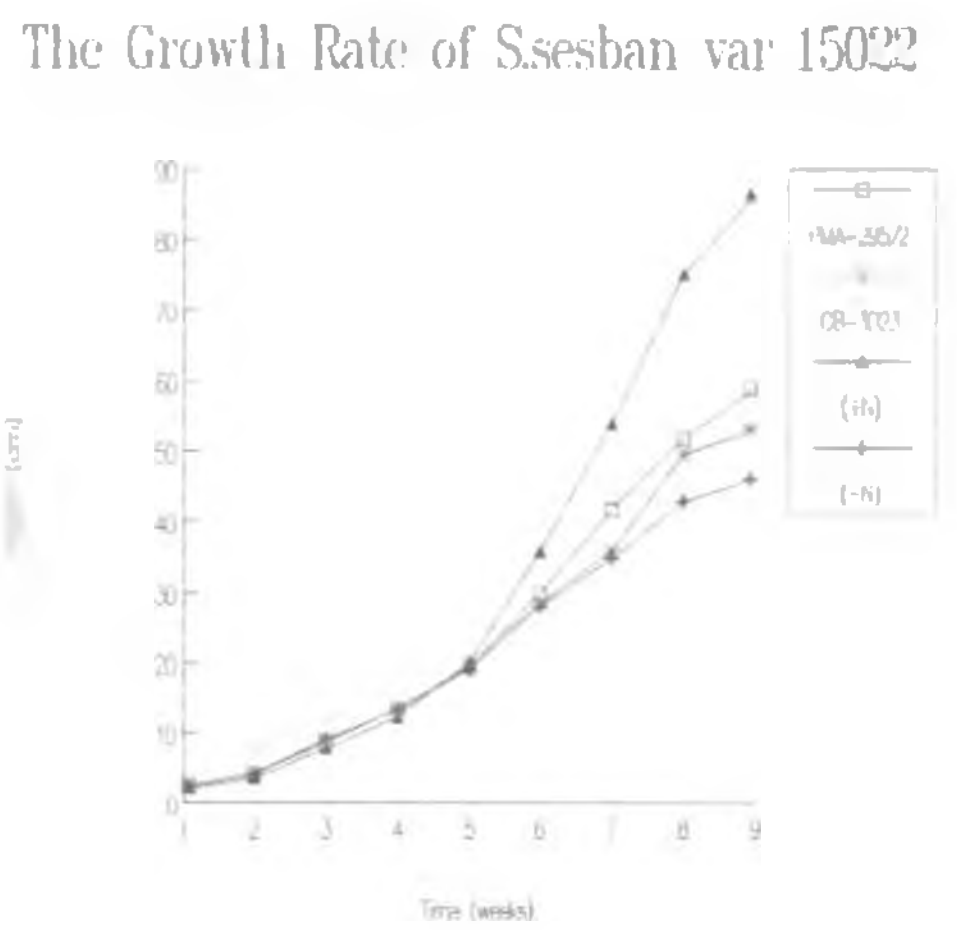


FIG: 5.9: GROWTH RESPONSE OF LINE 30071 TO TREATMENTS

Strain CB-3023 was more varied in its effect. Line 10895 responded as early as week-4 (Figure 5.3) with line CPI-30071 and 15022 responding at week 6 and 7 respectively (Figure 5.4 and 5.5). Two lines (15036 and 9265) did not respond to this particular strain until the 8th week (Figure 5.1 and 5.2). Table 5.3 illustrates the mean height achieved by the five lines of Sesbania sesban under two strains of Rhizobium and two nitrogen controls (-N and +N).

Both strains of Rhizobium enabled plants to attain a mean height which was significantly ($P<0.05$) greater than that of -N control treatment. Strain PMA-295/2 however gave a significantly ($P<0.05$) larger response (mean height 56.4cm) than strain CB-3023 (mean 50.9cm). Plants which received +N treatment, attained the greatest mean height (81.5cm) which was significantly greater than any other treatment.

Among five lines of Sesbania sesban, line CPI-30071 responded best to strain PMA-295/2 attaining a mean height of 65.8cm during the experimental period, although it was not significantly ($P<0.05$) taller than line 15036 which attained a mean height of 58.9cm. Line 15022 did not respond at all to this particular strain, achieving a height of 48.3cm, which was practically the same as that of the -N treatment (cf 49.8cm). Although strain CB-3023 produced plants which were taller than the -N treatment, the height difference was not significant. However among the five lines, line 9265 responded best to this strain attaining a height of 54.6cm (Table 5.3).

Table 5.3 MEAN PLANT HEIGHT AT HARVEST (cm); EXPERIMENT-2:

	T R E A T M E N T				
LINE	PMA-295/2	CB-3023	+N	-N	MEAN
9265	51.9	54.6	82.8	47.5	59.2
10895	57.3	43.3	76.0	40.1	54.2
15022	48.3	53.9	81.1	49.8	58.3
15036	58.9	53.0	86.3	46.1	61.1
30071	65.8	49.8	81.4	46.3	60.8
MEAN	56.4	50.9	81.5	46.0	

LSD (P=0.05) = (a). Treat. = 3.3cm
(b). Lines = 3.7cm
(c). L x T = 7.3cm
(d). Block = 3.3cm

5.3.3 NODULATION OBSERVATIONS:

Sesbania sesban nodules are round or lobed, single or clustered, firmly attached on the roots. Effective nodules were pink/purple to bright red, while ineffective ones were white.

All plants inoculated with strain CB-3023 nodulated as shown in Appendix-B (2). Fifteen percent of the treatment had crown nodules only; 5% lateral only; and 80% both crown and lateral nodules.

Sixty percent of the plants inoculated with strain PMA-295/2, nodulated both crown and lateral. Twenty-five percent of the

+N treatment nodulated across 3 host lines with nodules on lateral roots only.

Sixty percent of -N treatment plants were nodulated. Two thirds were lateral and one third were both crown and lateral nodules. Line 10895 failed to nodulate, indicating that no native rhizobia were present for this particular host.

5.3.4 DRY WEIGHTS:

5.3.4.1 NODULE DRY WEIGHT:

Line CPI-30071 produced the highest mean nodule dry weight followed by line 15036, 15022, 9265 and 10895 as shown by Appendix-E.Strain PMA-295/2 produced the highest nodule weight followed by strain CB-3023, -N and +N (Appendix-E).

5.3.4.2 SHOOT DRY WEIGHT:

Line 9265 produced the highest shoot dry weight, followed by line CPI-30071, 10895, 15022 and 15036 (Appendix-E). Combined nitrogen (+N) produced the highest shoot dry weight, followed by strains PMA-295/2, CB-3023 and control (-N) (Appendix-E).

5.3.4.3 ROOT DRY WEIGHT:

Line CPI-30071 produced the highest root dry weight, followed by line 15036, 15022, 9265 and 10895 (Appendix-E). Combined nitrogen produced the highest root dry weight, followed by strains PMA-295/2, CB-3023 and control.

5.3.4.4 TOTAL DRY MATTER:

Table 5.4 gives the total dry matter yield of nine week old plants. Strain PMA-295/2 gave a mean yield of 9.7g which was significantly (P<0.05) higher than strain CB-3023 and control (-N) treatments, although the effect was much lower than the +N treatment which enabled mean yield of 19.0g. Among the lines used line CPI-30071 again responded best (giving yield of 14.0g) and line 15036 least (with 7.0g), to strain PMA-295/2. Nevertheless the response of the Sesbania sesban lines to strain CB-3023 apparently favoured line 15022, which was the only one that significantly outyielded the -N treatment. The rest of the lines did not respond to strains significantly.

Table 5.4 TOTAL DRY MATTER YIELD AT HARVEST(g): EXPERIMENT-2:

	T R E A T M E N T				
LINE	PMA-295/2	CB-3023	+N	-N	MEAN
9265	8.6	8.8	19.9	7.4	11.2
10895	9.7	6.4	19.2	5.6	10.2
15022	7.0	8.4	18.6	6.6	10.2
15036	9.2	7.4	18.9	6.7	10.6
30071	14.0	6.8	18.5	6.5	11.5
MEAN	9.7	7.6	19.0	6.6	

LSD (P=0.05) = (a). Treat. = 1.0g
(b). Lines = 1.1g
(c). L x T = 2.1g
(d). Block = 1.0g

5.3.8 TOTAL PLANT NITROGEN (%):

At harvest time the inoculated plants had significantly ($P<0.05$) higher mean % plant nitrogen content (2.5% for strain PMA-295/2 and 2.0% for strain CB-3023), than the two controls (+N and -N) which had 1.4% and 1.2% respectively (Table 5.5). However, of the two strains of *Rhizobium*, the effect of strain PMA-295/2 was slightly better than strain CB-3023. The -N treatment gave the least % nitrogen content. Among the five lines of *Sesbania sesban*, strain PMA-295/2 was not significantly ($P<0.05$) different, and the least effect was on line 10895. Strain CB-3023 had the highest effect on line 10895, with nitrogen content of 2.5% followed by line CPI-30071 with 2.3%.

Table 5.5 TOTAL PLANT NITROGEN (%): EXPERIMENT-2:

	T R E A T M E N T				
LINE	PMA-295/2	CB-3023	+N	-N	MEAN
9265	2.0	1.9	1.2	1.1	1.6
10895	1.6	2.5	1.3	0.5	1.5
15022	3.1	1.8	1.3	1.4	1.9
15036	2.4	1.7	1.5	1.7	1.8
30071	3.6	2.3	1.9	1.3	2.3
MEAN	2.5	2.0	1.4	1.2	

LSD (P=0.05) = (a) Treat. = 0.7 %
(b) Lines = 0.7 %
(c) L x T = 1.5 %
(d) Block = 0.7 %

5.4 DISCUSSION:

There was no difference in growth between the control (-N) and the other treatments up to five weeks of age (Figure 5.1-5.5). This may indicate the fact that the soil nitrogen content was adequate for five weeks requirement. Thus there was no noticeable differences between treatments within this period. The differences in growth rate that showed after the 5th week indicate that there were significant ($P<0.05$) line/treatment interactions (Appendix-C-6). The lag in growth rate for the first three weeks may have been partly due to low winter temperatures at night before heaters were repaired. Although Ammonium Nitrate fertilizer was applied to the +N treatment at week-1, lack of response by the seedlings may have been due to the low nitrogen level applied (125 kg/ha in two splits of 62.5 kg/ha each) which may have been inadequate. It was not until the soil nitrogen was depleted that the effect of the strains of Rhizobium were noticed. The level of soil nitrogen may have been high enough to exhibit effective nitrogen fixation (Graham and Chatel, 1983; Sprent, 1979; Raggio, Raggio and Torrey, 1975; and Munns, 1968).

Although strain CB-3023 nodulated all the Sesbania sesban lines, unlike strain PMA-295/2 (Appendix-B-2). The poor performance of plants inoculated with strain CB-3023 may be attributed to less effective nitrogen fixation since its nodules were small in size and weighed less than the nodules from strain PMA-295/2 (Appendix-E-7). Strain PMA-295/2 was more specific in its nodulation. This phenomenon has been observed by Wilson, 1946; Date and Halliday, 1980; and Evans and Rotar, 1987).

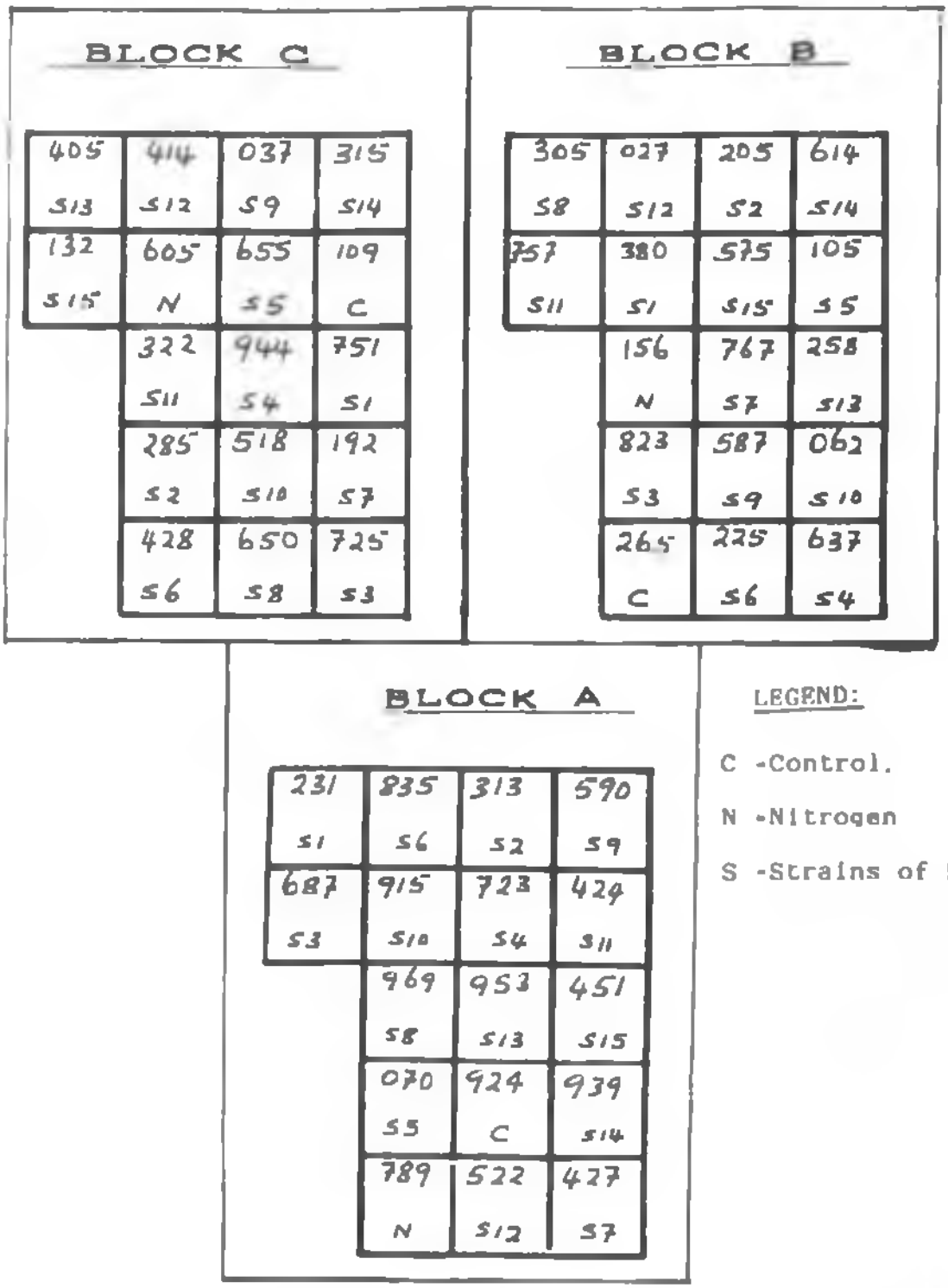
The effectiveness of strain PMA-295/2 and its specificity is further exhibited in plant height gained (Table 5.3) and dry matter production (Table 5.4). The specificity of PMA-295/2 was such that it interacted most significantly with line CPI-30071.

* * * * * * * * * * *

A P P E N D I X - A :

EXPERIMENTAL LAYOUTS:

EXPERIMENT-I. DESIGN LAYOUT :



LEGEND:

C -Control.

N -Nitrogen

S -Strains of Rhizobi

EXPERIMENT-2. DESIGN LAYOUT :

LEGEND: C =Control, 1-5 = Lines of S.sesban.
N -Nitrogen
S11 -Strain PMA-295/2
S14 - Strain CB-3023

BLOCK-D

3 S14	2 N	4 C	5 S11
1 S11	4 S14	3 C	5 S14
1 C	4 N	2 S11	2 C
5 N	1 S14	2 S14	1 N
4 S11	5 C	3 S11	3 N

BLOCK-A

4 N	3 N	3 S11	1 S11
1 S14	2 S11	4 C	4 S11
1 C	4 S14	2 C	3 C
5 S14	2 S14	1 N	5 C
5 S11	5 N	3 S14	2 N

BLOCK-C

5 S11	3 N	5 N	4 C
1 C	3 C	4 N	2 C
1 S14	2 S14	2 N	3 S11
5 S14	1 S11	5 C	4 S14
1 N	3 S14	2 S11	4 S11

BLOCK-B

2 S14	5 C	5 S14	5 S11
3 C	2 S11	1 S11	4 S11
5 N	4 C	1 C	2 N
3 S14	1 S14	2 C	3 S11
4 N	1 N	4 S14	3 N

A P P E N D I X - B :

NODULATION RATING:

DATA COLLECTION SHEET:

Treatment	Pl/jar	Pl-colour	Pl-height 1.	2.	mean	Nodule P	No	C	Dry wts. St	Rt	Nd	Tdm
PMA-123-I	2	yellow	30	30	30	1	10	w	35	38	14	87
II	2	yellow	50	50	50	c	15	w	76	71	7	154
III	2	yellow	30	40	35	1	5	w	29	51	14	94
MEAN												
PMA-124-I												
II												
III												
MEAN												
PMA-126-I												
II												
III												
MEAN												

** P = Nodule Position
No = Nodule Number
C = Nodule Colour
St = Shoot dry weight
Rt = Root dry weight
Nd = Nodule dry weight
Tdm = Total dry matter

NODULATION RECORD: EXPERIMENT-2:

SESBANIA LINE	BLOCK	CB-3023	PMA-295/2	+N	-N
9265	A	C,L	NIL	NIL	NIL
"	B	C,L	NIL	NIL	NIL
"	C	C	C,L	NIL	NIL
"	D	C,L	NIL	NIL	NIL
15036	A	C,L	C,L	L	L
"	B	C,L	C,L	NIL	C,L
"	C	C,L	NIL	L	NIL
"	D	C,L	C,L	NIL	C,L
10895	A	C	NIL	NIL	NIL
"	B	C,L	NIL	NIL	NIL
"	C	C,L	NIL	NIL	NIL
"	D	C	NIL	NIL	NIL
15022	A	L	C,L	L	L
"	B	C,L	C,L	L	C,L
"	C	C,L	C,L	NIL	L
"	D	C,L	C,L	NIL	L
30071	A	C,L	C,L	NIL	NIL
"	B	C,L	C,L	NIL	C,L
"	C	C,L	C,L	L	L
"	D	C,L	C,L	NIL	L

** C = CROWN NODULATION
L = LATERAL NODULATION

SUMMARY OF DATA:

STRAIN CB-3023:

NODULATION	= 100 %
CROWN NODULES	= 15 %
LATERAL NODULES	= 5 %
C AND L NODULES	= 80 %

CONTROL TREATMENT:

NODULATION	=60 %
CROWN	= 0 %
LATERAL	=67 %
C,LATERAL	=33 %

STRAIN PMA-295/2:

NODULATION	= 60 %
CROWN NODULES	= 0 %
LATERAL NODULES	= 0 %
C,L NODULES	= 80 %

COMBINED NITROGEN (+N):

NODULATION	= 25 %
CROWN NODULES	= 0 %
LATERAL NODULES	= 100 %
C,L NODULES	= 0 %

A P P E N D I X - C:

ANALYSIS OF VARIANCE TABLES:

TABLE 1. THE EFFECT OF TREATMENTS ON PLANT GROWTH: EXPT-1.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT.	16	313535.2	19596.0	18.3	0.0000	**
BLOCK	2	241.8	120.9	10.3	0.3780	NS
ERROR	32	3857.6	120.6			

** = HIGHLY SIGNIFICANT.
NS = NOT SIGNIFICANT.

TABLE 2. THE EFFECT OF TREATMENT ON SHOOT DRY WEIGHT: EXPT-1.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT.	16	26874190	1679637	184.4	0.0000	**
BLOCK	2	24251.7	12125.8	77.5	1.0000	NS
ERROR	32	393106.4	12284.6			

TABLE 3. THE EFFECT OF TREATMENT ON ROOT DRY WEIGHT: EXPT-1.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	16	1635011.7	102188.2	70.07	.0000	**
BLOCK	2	3255.3	1627.6	29.4	1.0000	NS
ERROR	32	1695018.4	33900.4			

TABLE 4. THE EFFECT OF TREATMENT ON NODULE DRY WEIGHT: EXPT-1.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	16	75865.3	4741.6	29.3	.0000	**
BLOCK	2	362.2	181.1	12.3	1.0000	NS
ERROR	32	9907.8	309.6			

TABLE 5.THE EFFECT OF TREATMENT ON TOTAL PLANT DRY WEIGHT:EXPT1

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	16	42000658	2625041	263.9	.0000	**
BLOCK	2	56552.6	28276.3	110.9	.3374	NS
ERROR	32	804915.2	25153.6			

TABLE 6. MEAN PLANT HEIGHT: EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	3	14970.6	4990.2	3.2	.0000	**
LINE	4	496.0	124.0	3.7	.0027	**
T x L	12	998.3	83.2	7.4	.0020	**
BLOCK	3	687.8	229.3	3.3	.0001	**
ERROR	57	1534.0	26.9			

TABLE 7. NODULE DRY WEIGHT: EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	3	3149470	1049823	149.5	.0000	**
LINE	4	1124042.5	281010.6	167.1	.0015	**
T x L	12	2040617.5	1700514.6	334.3	.0022	**
BLOCK	3	116230.0	38743.3	149.5	1.0000	NS
ERROR	57	3175020.0	557021.0			

TABLE 8. SHOOT DRY WEIGHT: EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F
TREAT	3	1381.9	460.6	6.0	.0034 **
LINE	4	233.6	58.4	6.7	1.0000 NS
T x L	12	1130.9	94.2	13.5	0.4247 NS
BLOCK	3	163.5	55.8	6.0	1.0000 NS
ERROR	57	5154.4	90.4		

TABLE 9. ROOT DRY WEIGHT: EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F
TREAT	3	77.9	26.0	0.21	.0000 **
LINE	4	4.1	1.0	0.23	.0000 **
T x L	12	4.2	0.4	0.47	.0015 **
BLOCK	3	0.6	0.2	0.21	.1638 NS
ERROR	57	6.2	0.1		

TABLE 10. TOTAL DRY MATTER YIELD: EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F
TREAT	3	1925.2	641.7	0.95	.0000 **
LINE	4	20.3	5.1	1.1	.0723 NS
T x L	12	117.1	9.7	2.1	.0001 **
BLOCK	3	19.8	6.6	0.95	.0404 **
ERROR	57	127.4	2.2		

TABLE 11. TOTAL PLANT NITROGEN (%): EXPERIMENT 2.

SOURCE	DF	S.SQ	M.S.SQ	LSD 5%	F	
TREAT	3	21.5	7.1	0.66	.0000	**
LINE	4	6.1	1.5	0.74	.2358	NS
T x L	12	9.7	0.8	1.47	1.0000	NS
BLOCK	03	0.4	0.1	0.66	1.0000	NS
ERROR	57	61.4	1.1			

A P P E N D I X D :

NITROGEN ANALYSIS:

TOTAL PLANT NITROGEN:(mg):EXPERIMENT-2.

LINE	BLOCK	PMA-295/2	CB-3023	+N	-N
9265	A	69.89	97.07	16.84	51.78
	B	26.99	57.34	67.10	35.03
	C	117.05	56.48	15.55	28.19
	D	72.65	47.33	73.32	36.63
15036	A	97.88	37.49	63.39	61.61
	B	75.82	141.82	80.68	68.48
	C	37.24	31.09	44.24	67.73
	D	105.41	39.88	30.54	36.52
10895	A	40.43	91.99	32.93	0.00
	B	69.23	57.21	24.75	11.90
	C	64.24	102.36	70.40	43.52
	D	41.50	93.06	63.30	17.93
15022	A	36.77	99.38	25.67	87.96
	B	149.30	66.97	10.99	40.39
	C	85.21	42.78	44.26	47.16
	D	174.99	48.76	98.29	19.99
30071	A	88.62	128.59	142.97	78.19
	B	99.52	54.77	52.14	29.21
	C	227.48	69.60	39.75	6.36
	D	88.51	42.61	31.54	79.18

TOTAL PLANT NITROGEN: (%):EXPERIMENT-2.

LINE	BLOCK	PMA-295/2	CB-3023	+N	-N
9265	A	2.00	2.62	0.47	1.38
	B	0.78	1.77	1.91	0.99
	C	3.15	1.64	0.49	0.77
	D	1.94	1.41	1.99	1.16
15036	A	2.92	1.10	1.78	1.74
	B	2.41	3.78	2.29	2.07
	C	1.03	0.92	1.19	1.81
	D	3.18	1.08	0.85	1.02
10895	A	1.28	2.78	0.93	0.00
	B	2.07	1.53	0.73	0.33
	C	2.05	2.80	1.83	1.17
	D	1.19	2.76	1.78	0.48
15022	A	1.06	2.77	0.66	2.63
	B	3.97	1.93	0.28	1.15
	C	2.30	1.13	1.29	1.26
	D	4.96	1.31	2.78	0.57
30071	A	2.02	4.21	3.92	2.06
	B	3.03	1.55	1.43	0.86
	C	6.45	2.01	1.24	0.16
	D	2.70	1.27	0.99	2.13

A P P E N D I X - E

EXTRA DATA: 1. MEAN PLANT HEIGHT - EXPT-1.
2. MEAN PLANT HEIGHT - EXPT-2.
3. DRY MATTER YIELD - EXPT-2.

1. MEAN PLANT HEIGHT - EXPERIMENT-1.

TABLE 1. MEAN PLANT HEIGHT (WEEK-3)(mm/jar):

Treatment	Rep-1	Rep-2	Rep-3
PMA-123	20	40	20
" 124	35	20	20
" 126	30	30	20
" 229/1	30	20	25
" 229/2	25	20	30
" 252/1	20	20	20
" 254/5	25	20	20
" 256	15	15	20
" 258/1	20	20	15
" 295/1	35	15	25
" 295/2	25	25	20
" 295/5	15	15	25
CB -2743	15	20	30
" 3023	25	25	25
RAD-624/3	35	10	25
+N	50	60	55
-N	25	20	20

TABLE 2. MEAN PLANT HEIGHT (WEEK-5)(mm/jar):

Treatment	Rep-1	Rep-2	Rep-3
PMA-123	30	50	35
" 124	35	30	20
" 126	30	40	20
" 229/1	30	30	25
" 229/2	25	20	35
" 252/1	32.5	20	20
" 254/5	30	30	25
" 256	20	25	20
" 258/1	20	30	20
" 295/1	35	25	25
" 295/2	70	70	50
" 295/5	25	25	25
CB-2743	20	25	30
" 3023	45	30	45
RAD-624/3	35	30	30
+N	190	205	220
-N	30	30	30

TABLE 3. MEAN PLANT HEIGHT (WEEK-7)(mm/jar):

Treatment	Rep-1	Rep-2	Rep-3
PMA-123	30	50	35
" 124	32.5	30	25
" 126	40	40	30
" 229/1	30	30	30
" 229/2	25	20	30
" 252/1	32.5	30	30
" 254/5	30	30	25
" 256	25	30	30
" 258/1	20	30	25
" 295/1	35	30	30
" 295/2	137.5	150	92.5
" 295/5	25	30	35
CB-2743	20	25	32.5
" -3023	112.5	70	35
RAD-624/3	40	70	35
+N	342.5	380	365
-N	30	30	40

2. MEAN PLANT HEIGHT - EXPERIMENT - 2.

TABLE 1. MEAN PLANT HEIGHT - RHIZOBIUM STRAIN - PMA-295/2.
(cm)

	SESBANIA SESBAN LINES				
WEEK	9265	10895	15022	15036	30071
1	2.2	2.8	2.2	2.5	1.9
2	4.1	4.7	3.9	4.2	3.7
3	8.8	9.4	8.2	8.8	7.7
4	14.0	13.4	12.5	12.5	12.5
5	21.4	20.6	20.3	19.4	21.9
6	31.5	32.0	31.5	29.8	31.0
7	41.1	40.3	41.9	41.8	42.9
8	48.0	51.8	46.0	51.8	55.8
9	51.9	57.3	48.3	58.9	65.8

TABLE 2. MEAN PLANT HEIGHT - RHIZOBIUM STRAIN - CB-3023.
(cm)

	SESBANIA SESBAN LINES				
WEEK	9265	10895	15022	15036	30071
1	2.7	2.4	2.3	2.3	2.2
2	4.3	4.5	4.2	4.3	3.9
3	8.7	9.3	9.1	8.9	8.6
4	12.9	13.8	14.4	13.3	13.9
5	20.1	22.4	22.9	19.6	20.5
6	33.0	33.5	34.4	28.4	32.6
7	41.9	35.9	43.5	35.9	41.5
8	48.5	40.5	49.8	49.6	45.9
9	54.6	43.3	53.9	53.0	49.8

TABLE 3. MEAN PLANT HEIGHT - COMBINED NITROGEN (+N).
(cm)

	SESBANIA SESBAN LINES				
WEEK	9265	10895	15022	15036	30071
1	2.5	2.4	2.4	2.0	2.2
2	4.5	4.7	4.5	3.6	3.5
3	9.5	10.0	9.3	7.9	7.7
4	14.1	14.4	14.6	12.3	12.4
5	22.5	22.9	23.4	20.1	20.9
6	38.1	37.4	40.1	35.8	36.4
7	55.5	55.6	58.4	53.8	55.5
8	72.5	68.3	70.9	75.0	71.0
9	82.8	76.0	81.1	86.3	81.4

TABLE 4. MEAN PLANT HEIGHT - CONTROL (-N).
(cm)

WEEK	SESBANIA SESBAN LINES 9265	10895	15022	15036	30071
1	2.4	2.8	2.6	2.5	2.2
2	4.5	4.8	4.1	4.2	3.3
3	9.1	9.4	8.6	9.1	7.5
4	13.0	13.4	13.3	13.4	11.5
5	20.9	21.0	20.5	19.0	18.9
6	32.9	32.5	30.3	28.0	30.6
7	41.2	34.9	37.9	34.9	39.3
8	46.4	38.9	46.3	42.9	44.0
9	47.5	40.1	49.8	46.1	46.3

TABLE 5. BLOCK MEAN PLANT HEIGHT - (cm).

LINE	PMA-295/2	CB-3023	+N	-N
9265	51.9	54.6	82.8	47.5
10895	57.3	43.3	76.0	40.1
15022	48.3	53.9	81.1	49.8
15036	58.9	53.0	86.3	46.1
30071	65.8	49.8	81.4	46.3

3. DRY MATTER YIELD - EXPERIMENT - 2.

TABLE 1. NODULE DRY MATTER - (mg).

TREATMENT	SESBANIA SESBAN LINES 9265	15036	10895	15022	30071
PMA-295/2	312.5	710.0	0.0	600.0	1132.5
CB-3023	350.0	310.0	247.5	287.5	300.0
+N	0.0	110.0	0.0	90.0	2.5
-N	7.5	145.0	0.0	265.0	130.0

LSD (P=0.05) =334.3 mg

TABLE 2. SHOOT DRY MATTER - (g).

TREATMENT	SESBANIA SESBAN LINES 9265	10895	15022	15036	30071
PMA-295/2	6.7	5.6	6.8	7.0	10.5
CB-3023	7.0	6.6	5.5	4.5	5.0
+N	16.5	15.5	14.5	14.7	14.2
-N	6.0	5.3	4.8	4.0	4.9

LSD (P=0.05) = 13.47g

TABLE 3. ROOT DRY MATTER - (g).

TREATMENT	SESBANIA SESBAN LINES 9265	15036	10895	15022	30071
PMA-295/2	1.6	2.0	1.4	1.8	2.4
CB-3023	1.4	1.6	1.6	1.6	1.5
+N	3.4	4.4	3.1	4.3	4.3
-N	1.4	1.5	1.3	1.6	1.5

LSD (P=0.05) = 0.47 g

TABLE 4. TOTAL DRY MATTER -(g).

TREATMENT	SESBANIA SESBAN LINES 9265	15036	10895	15022	30071
PMA-295/2	8.6	9.7	7.0	9.2	14.0
CB-3023	8.8	6.4	8.4	7.4	6.8
+N	19.9	19.2	18.6	18.9	18.5
-N	7.4	5.6	6.6	6.7	6.5

LSD (P=0.05) = 2.12 g