

**THE PARTITIONING OF PHOTOSYNTHATE TO
NODULES AND NITROGEN FIXATION IN BEANS**
(Phaseolus vulgaris L)

By

Juma Kayeke Mohamed

**A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL
FULFILMENT OF THE REQUIREMENT OF THE AWARD OF DEGREE OF MASTER
OF SCIENCE IN AGRONOMY (CROP SCIENCE)**

THE UNIVERSITY OF ZAMBIA

**LUSAKA
256480
1998**

DECLARATION

I, JUMA KAYEKE MOHAMED, declare that this dissertation represents my own work and that it has not previously been submitted for a degree at this or another university.



Signature.....

Date..... 28th (May) 1998.....

DECLARATION

I, JUMA KAYEKE MOHAMED, declare that this dissertation represents my own work and that it has not previously been submitted for a degree at this or another university.



Signature.....

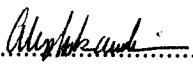
Date..... 28th (May) 1998.....

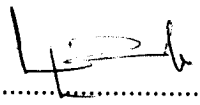
APPROVAL

This dissertation of Mr. Juma Kayeke Mohamed is approved as a fulfilling part of the requirements for the award of the degree of Master of Science in Agronomy (Crop Science) of the University of Zambia.

Examiner's signature:

Date:

1.  14/10/97

2.  4/8/98

3.  4/8/98

ABSTRACT

This study was designed to determine the influence of the maturity status on partitioning of photosynthate to the roots and nitrogen fixation in common beans (*Phaseolus vulgaris* L.). Two experiments (glasshouse at the University of Zambia and field at the Zambia Seed Company farm, Ngwerere, north of Lusaka) were conducted using a split- plot design in both cases. Three nitrogen treatments, i.e., N-fertilized (80 kgN/ha), inoculated with *Rhizobium* CIAT 899 (1ml of inoculum per plant in glasshouse and 2ml per hill in the field) and non-inoculated were the main plots. Six cultivars of beans each, two representing a maturing group; early (Chizi and Contender), medium (Kablengeti and CG 76-1) and late maturing (PVA 2280 and Carioca) were the subplots. Parameters measured were nodule number per plant, nodule dry weight per plant, carbohydrate content in roots and shoot nitrogen content. The data were collected at three growth stages, i.e., 50% flowering, pod initiation and mid-pod fill. It was found that the maturity status of bean cultivar had no influence on the partitioning photosynthates to the roots; amount of nitrogen in shoots was positively correlated with carbohydrate content in the roots. This confirms that the supply of photosynthates is very important in the roots.

DEDICATION

To my parents Moshi Kayeke (now the late) and Mwanamosi Mohamed, to my wife Sarah, my daughter Mosi and son Nkumuke for the love and patience for all the time I was away perusing my studies.

ACKNOWLEDGEMENT

I would like to extend my sincere thanks to Dr. D. Mbewe and Mr. R.C. Nyemba for their supervision, guidance, encouragement and useful criticisms which enable me to this work. I wish to express my thanks to Mr. M. Mwale , Mr. H. Tembo of Mount Makulu Research Station for their assistance especially with facilities which I used in this work. I also thank Mr. Tembo , the Zambia Seed Company Farm Manager for allocating me a plot on the farm.

I also thank all members of staff in Crop Science Department at the University of Zambia. The Soil Chemistry and Soil Microbiology sections at Mount Makulu Research Station are also acknowledged.

I am grateful to SACCAR/GTZ for awarding me the scholarship to undertake this Master degree and my employer, The Ministry of Agriculture, Tanzania , for granting me study leave.

Lastly, to my colleagues who made me enjoy my stay in Zambia.

TABLE OF CONTENTS

Declaration.....	i
Approval	ii
Abstract.....	iii
Dedication.....	iv
Acknowledgement.....	v
Table of contents.....	vi
List of Tables.....	x
1.0 Introduction.....	1
2.0 Literature Review.....	4
2.1 Climatic factors.....	4
2.2 Edaphic factors.....	5
2.2.1 Fertility.....	6
2.2.2.1 Major nutrients.....	6
2.2.2.2 Micronutrients.....	8
2.3 Biological factors.....	8
2.3.1 <i>Rhizobium</i> specie.....	8
2.3.2 The host plant.....	9
3.0 Materials and Methods.....	12
3.1 Glasshouse experiment.....	12
3.1.1 Experimental site.....	12
3.1.2 Design.....	12
3.1.3 Soil.....	13

3.1.4 Cultivars.....	13
3.1.5 Planting.....	13
3.1.6 Inoculation.....	14
3.1.7 Watering.....	14
3.1.8 Fertilizers.....	14
3.1.9 Sample collection.....	15
3.1.10 Data collection.....	15
3.1.11 Data analysis.....	16
3.2 Field experiment.....	17
3.2.1 Experimental site.....	17
3.2.2 Design.....	17
3.2.3.Cultivars.....	17
3.2.4 Planting.....	17
3.2.5 Inoculation.....	18
3.2.6 Fertilizers.....	18
3.2.7 Agronomic practices.....	18
3.2.8 Sample collection.....	19
3.2.9 Data collection.....	19
3.2.10 Data analysis.....	20
4.0 Results and Discussion.....	21
4.1 Results.....	21
4.1.1 Glasshouse experiment results.....	21
4.1.1.1 Nodule number per plant.....	21

4.1.1.2 Nodule dry weight per plant.....	24
4.1.1.3 Total carbohydrate in roots.....	24
4.1.1.4 Nitrogen harvest.....	28
4.1.1.5 Relationship between carbohydrate and shoot nitrogen	31
4.2 Discussion for glasshouse experiment.....	32
4.2.1 Nodule number	32
4.2.2 Nodule dry weight.....	33
4.2.3 Total carbohydrate in roots.....	33
4.2.4 Total nitrogen harvest.....	34
4.3 Field experiment results.....	36
4.3.1.Nodule number per plant.....	36
4.3.2 Nodule dry weight per plant.....	39
4.3.3 Total carbohydrate in roots.....	39
4.3.4 Nitrogen harvest.....	43
4.3.5 Relationship between carbohydrate and shoot nitrogen.....	47
4.4 Discussion for field experiment.....	48
4.4.1 Nodule number.....	48
4.4.2 Nodule dry weight.....	49
4.4.3 Total carbohydrate in roots.....	49
4.4.4 Total nitrogen harvest.....	50
5.0 Comparison of glasshouse and field experiment.....	51
5.1 Nodule number.....	51
5.2 Nodule dry weight.....	51

5.3 Carbohydrate in roots.....	52
5.4 Total nitrogen harvest.....	53
6.0 Conclusion.....	54
References.....	55
Appendices.....	59

LIST OF TABLES

Table 1a. Influence of cultivar on nodule number per plant at various harvesting stages.....	22
Table 1b. Effect of inoculation on nodule number per plant at various harvesting stages.....	23
Table 2a. Influence of cultivar on nodule dry weight at various harvesting stages.....	25
Table 2b. Effect of inoculation on nodule dry weight per plant at various harvesting stages.....	26
Table 3a. Influence of cultivar on total carbohydrate (%) in roots at various harvesting stages.....	27
Table 3b. Effect of inoculation on total carbohydrate (%) in roots at various harvesting stages.....	29
Table 4a. Influence of cultivar on nitrogen harvest (mg/pot) at various harvesting stages.....	30
Table 4b. Effect of inoculation on nitrogen harvest (mg/pot) at various harvesting stages.....	31
Table 5a. Influence of cultivar on nodule number per plant at various harvesting stages.....	37
Table 5b. Effect of inoculation on nodule number per plant at various harvesting stages.....	38
Table 6a. Influence of cultivar on nodule dry weight at various harvesting stages.....	40

Table 6b. Effect of inoculation on nodule dry weight per plant at various harvesting stages.....	41
Table 7a. Influence of cultivar on total carbohydrate (%) in roots at various harvesting stages.....	42
Table 7b. Effect of inoculation on total carbohydrate (%) in roots at various harvesting stages.....	44
Table 8a. Influence of cultivar on nitrogen harvest (mg/pot) at various harvesting stages.....	45
Table 8b. Effect of inoculation on nitrogen harvest (mg/pot) at various harvesting stages.....	46

CHAPTER ONE

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.), like other crops, requires nitrogen supply in order to express its yield potential (Karel *et al.*, 1981). The general deficiency of nitrogen in tropical soils is a major limiting factor to growth and productivity of beans (Wortmann and Zake, 1988). As a leguminous plant, the common bean plant has the ability to utilize atmospheric nitrogen fixed through symbiotic association with *Rhizobium* bacteria. The amount of nitrogen fixed by the common bean is a function of the interaction between the host plant (bean plant), the *Rhizobium* strain, and the environment (Salema, 1987). Based on the genetic potential, an important role in the interaction is played by the host plant (Graham, 1982; Nyemba *et al.*, 1989). When compared to other legumes, the common bean is inferior in fixing nitrogen (Piha and Munns, 1987; Salema, 1987; Nyemba *et al.*, 1989). Among bean cultivars, however, there are differences in their respective abilities to fix nitrogen as reported by Graham and Halliday (1977); Graham (1981); Rennie and Kemp (1981); Salema (1987). In most research conducted, it has been documented that the climbing and the late maturing cultivars are superior in fixing nitrogen to the bush type and early maturing cultivars (Sprent, 1979; Lynch and Piha, 1988). This inferiority is probably due to differences among the bean types in the duration of photosynthate supply to the roots and the nodules, since carbohydrate is the primary factor limiting nitrogen fixation in legumes (Graham, 1982).

Photosynthesis, as source of carbohydrate to the plant plays a major role in biological nitrogen fixation especially in symbiotic association. This is because the symbiotic bacteria in the nodules obtain the nutrient and energy required for the enzyme nitrogenase to function from the plant. Therefore, the supply of photosynthate to the roots and nodules is the most important aspect in symbiotic biological nitrogen fixation. Hardson (1990) reported that in the early vegetative phase, legumes tend to transport down the stem about 60% of the daily acquired photosynthate, out of which, about 40% is diverted to the roots and 30% to the nodules. On the other hand, approximately 50% of the carbon input to the nodule is returned to the shoot as fixation product and the rest is used for respiration and growth of nodules.

With regard to maturity status, the late maturing cultivars are said to supply photosynthate to the roots and nodules for a longer period of time compared to short maturing cultivars. One of the reasons is that in late maturing varieties, there is a delay in the onset of competition for photosynthate between developing pods and nodules (Graham, 1982). Another reason is that late maturing cultivars tend to maintain their lower leaves which are important in producing photosynthates for the nodules. In addition, late maturing cultivars have longer leaf area duration (LAD), meaning that they maintain active assimilatory surface longer than the early maturing cultivars (Graham, 1982). Graham and Halliday (1977) reported that more than 30% of the carbohydrates produced by the late maturing cultivars are soluble while the early maturing cultivars have less than 30% soluble carbohydrates. The soluble carbohydrates are easily utilised in the nodules compared to less soluble carbohydrates. There is therefore, need

to confirm these physiological or phenotypic constraints to fixation of nitrogen by comparing the nitrogen fixing effectiveness of short and long maturing cultivars grown simultaneously under the same conditions.

The objective of this experiment was to determine the influence of maturity status of common bean (*Phaseolus vulgaris* L.) on partitioning of photosynthates to the roots and to examine the correlation between seasonal photosynthate supply and nitrogen fixed by the bean plant.

CHAPTER TWO

LITERATURE REVIEW

Beans (*Phaseolus vulgaris* L.) are an important crop for rural communities and poor urban dwellers of the tropics because it is the major source of protein. In order to have good yield it is recommended that beans be supplied with nitrogen at the rate of 25-30 kgN/ha as the starter dose (Masomo and Rweyemamu, 1989). Higher amounts above 30kg/ha of nitrogen can reduce nodulation (Rennie and Kemp, 1984; Salema, 1987) However beans are traditionally cultivated without application of chemical fertilizer. The traditionally cultivated bean therefore relies on naturally fixed nitrogen as a source of nitrogen for growth.

Symbiotic nitrogen fixation in common beans is affected by both environmental and biological factors (Rennie and Kemp, 1984; Buttery *et al*, 1987; Sprent, 1979). The environmental factors include climate and edaphic factors, while biological factors include the host plant (bean plant), *Rhizobium* bacteria and the interaction between the two. The interaction between plant and bacterium affects nodulation, nitrogen activity and accumulation of the vegetative and reproductive tissues (Hungria and Neves, 1987).

2.1 Climatic factors

Light, and temperature are very important for the growth and development of the bean plant. These factors affect the biochemical reactions of the plant such as production of photosynthetic material for the plant which is required in the active nodules (Sprent,

1979). Graham (1981) reported the optimum temperature for nodulation to be 25-30°C, while the highest temperature for nitrogenase activity is 20°C. Low temperatures below 12°C have been reported to adversely affect growth and nitrogen fixation in common beans by lowering the rate of biochemical reactions which result in reduced photosynthesis and hence reduced growth (Rennie and Kemp, 1984; Mehler *et al.*, 1979). Temperatures above 33°C have adverse effects on the respiration rate (metabolic activities), fixation rate, and nodulation. This is because many metabolic reactions are slowed down at temperatures above 35°C, and enzymes are denatured. According to Graham and Halliday (1977), fixation exceeded 20 micro mol acetylene produced per plant per hour at 29°C, while fixation was less than 10 micro mol acetylene per plant per hour at 35°C. The interaction of the *Rhizobium* and the bean plant is also variously affected by high and low temperatures due to differences in tolerance to those conditions by different strains of *Rhizobia*.

2.2 Edaphic factors

Munns (1977) observed that any nutritional disorder may in principle variously affect each separate phase of the legume *Rhizobium* symbiosis, such as rhizobia survival and growth in the rhizosphere, infection and nodule development, nodule function, and the growth of the host plant. Soil water status can affect plant growth and the nitrogen fixation processes, especially, nodule number, nodule size and specific nodule activity. The reduction in plant growth and nitrogen fixation is observed at a water deficit of only 2.5 bars, and at -15 up -20 bars nitrogen fixation is eliminated (Graham, 1983). The waterlogging condition has an adverse effect on the process because beans are very

poor in tolerating waterlogged soil conditions.

2.2.1 Fertility

The nutrition status of the soil can affect symbiotic nitrogen fixation directly by affecting initiation and development of nodules. Nutrition ultimately influences the efficiency of the legume - *Rhizobium* symbiosis, and plays an essential role in overall plant metabolism and growth. It has been reported by Franco (1977) that the toxicities of aluminium and manganese, soil pH, and levels of nitrogen, phosphorous and molybdenum are some of the nutritional factors which can affect the efficiency of the symbiosis in tropical soils. Franco and Munns (1982) reported that acidic conditions and aluminium toxicity in the soil will affect plant growth. Acidic conditions delay nodulation hence low fixation. Aluminium toxicity reduces the uptake and translocation of calcium and phosphorous in the plant (Mengel and Kirkby, 1987). The optimum pH for the growth of the bean plant and for fixation process is 5.5 - 6.7 (Graham, 1983).

2.2.1.1 Major nutrients

Phosphorous (P) plays a major role in symbiotic nitrogen fixation because it is a critical constituent in reactions involving energy transfer, especially ATP (Adenosine Tri-Phosphate) synthesis and activity of nitrogenase, the enzyme which catalyses reduction of dinitrogen to ammonia in the nodule. Therefore, the biological nitrogen fixing plants require more phosphorous than those dependent on combined nitrogen (Mengel and Kirkby, 1987; Franco, 1977; Salema, 1987; Graham and Rosas, 1979). Graham (1983) reported that nitrogen fixing plants require up to 50% P more than those supplied with

nitrogen, but at the same time the response differs within cultivars in their respective abilities to absorb phosphorus, and partitioning within the plants. In these nitrogen fixing plants, phosphorus increases the number of nodules per plant, encourages nodule development and longevity (Salema, 1987), as well as nodule dry weight and fixation activity (Graham and Rosas, 1979).

Sulphur (S) is part of nitrogenase molecule and is therefore important both for nitrogen fixation and metabolism in plants (Mengel and Kirkby, 1987).

Nitrogen (N) is required as a starter dose at the rate of 25 - 30 kgN /ha for plant growth, nodule formation and symbiosis establishment (Masomo and Rweyemamu, 1989; Salema 1987). However, when applied in excess it affects nitrogen fixation by inhibiting nodule formation, nodule development and nitrogenase activity. Park and BATTERY (1989) reported that as the level of nitrogen increases, there is a decrease in nodule weight and number. However, the amount of excess nitrogen required to suppress nodulation differs with cultivars.

In the plant, mineral nitrogen is reduced to ammonia, which inhibits nitrogenase synthesis and activity. Nitrite, the product of nitrate reduction, inhibits production of indole acetic acid (IAA) which is important for nodule initiation (Franco, 1977). Calcium is needed in the development of the nodule. Calcium is also important for rectifying soil pH by eliminating aluminium and manganese toxicity. Magnesium is necessary for bacterial growth and for ATP binding in nitrogenase activity (Franco, 1977).

2.2.2.2 Micronutrients

Molybdenum, iron and boron are important in symbiotic nitrogen fixation. Molybdenum and iron are components of nitrogenase and leghaemoglobin, the protein which participates in oxygen transport to the bacteroid in the nodule. An increase in molybdenum content reduces the number of nodules but increases the size of the remaining few and the nitrogen fixed per unit weight of nodule. Boron is important in the vascular tissue of the nodule (Franco and Munns 1982; Franco, 1977; Lynch and Piha, 1988).

2.3 Biological factors

2.3.1 *Rhizobium* species

The species of *Rhizobium* that associates with common beans is called *Rhizobium leguminosorum* bv. *phaseoli*. There are inherent variations among strains of this species in the ability to fix nitrogen. This strain variability may be related to differences in tolerance to environmental and edaphic factors (Franco and Munns, 1982; Nyiti *et al.*, 1987). In the soil, there are other differences associated with competition between the introduced (inoculated) *Rhizobium* and the naturally existing rhizobia. The naturally existing rhizobia compete for nutrients, and the site of infection (Danso, 1977). Competition for nodulating site is characterised by the presence of nodules in uninoculated plants, which are few, ineffective and sometimes the reverse (Graham, 1981). Sometimes these naturally existing rhizobia produce toxins which kill the inoculated *Rhizobium*. Nurhayat *et al* (1989) and Kucey (1989) reported that the *Rhizobium* have poor competitiveness against natural bacteria in the soil; sometimes

the *rhizobia* are completely inhibited. Competitiveness of the strain depends much on the biological factors like presence of bacterial phage. These factors may cause poor nodulation and hence poor nitrogen fixation activity by the plant.

2.3.2 The host plant

Common bean is poor in fixing nitrogen when compared to other legumes (Salema, 1987; Lynch and Piha, 1988; Nyemba *et al.*, 1989). But within bean cultivars, there is variation in the ability to fix nitrogen (Salema, 1987; Nyemba *et al.*, 1989). Three factors are said to contribute to the variability of nitrogen fixation in common beans; these are supply of carbohydrate to nodules, relative rates of nitrogen supply from soils, and duration to flowering time (Graham, 1981).

In the interaction between the host plant and the *Rhizobium*, the plant tops provide carbon source to its root system and the *Rhizobium* in the nodule by the supply of photosynthate. This provides energy which is required for nodule development and maintenance. It was reported by Graham (1982) that the common beans differ in their abilities to supply carbohydrates to the roots and nodules. Generally, carbohydrates in the roots are used for root growth, transport of products in the phloem and xylem, and to feed the nodules. The late maturing cultivars are generally more superior in fixing nitrogen compared to early maturing cultivars. Late maturing cultivars are said to supply more soluble carbohydrates to roots than the early maturing cultivars. Furthermore, in late maturing cultivars there is a delay of the onset of the competition for photosynthate between the developing pods and the nodules. Additionally, late maturing cultivars have

a long leaf area duration by shading their lower leaves late. Leaf area duration expresses how long a plant can maintain its active assimilatory surface.

The distribution of nodules on the root was reported to influence nitrogen fixation (Danso and Bowen, 1989). Nodules on the lateral roots are said to supply a greater proportion of the fixed nitrogen as the plant grows. This is because the lateral roots develop late as well as their nodules so they stand a chance of fixing nitrogen later during plant growth than the tap root nodules which develop early and reach senescence before nodules on the lateral root. Tap root nodules are important in the early stages of plant growth while the lateral roots are important in the later stages of plant growth.

Differences in growth habit, that is determinate and indeterminate, which influence maturity status (determinate mature early, indeterminate mature late) are reported to affect nitrogen fixation with late maturing cultivars fixing more nitrogen than early maturing cultivars (Graham, 1982).

It was reported by Buttery *et al.* (1987), that rates of nitrogen fixation in common beans increase during vegetative growth period. Rates of fixation reach peak at flowering and early podding stage but start to fall during pod filling. This is related to the source/sink characteristic of bean plant during reproductive phase, whereby more photosynthate is concentrated to the reproductive parts and pod filling than that flowing to the roots (Salema, 1987). In other legumes such as soybeans and cowpeas, flowering occurs

later, thus giving a chance of having a longer nitrogen fixing period. This is regarded as the reason why the common bean is inferior in fixing nitrogen compared to other legumes. Duration of fixation period may therefore account for the influence of maturity status on total amount of fixed N at the end of the growth cycle.

In attempting to solve the problem of poor fixation in common bean, various suggestions have been made. These include suggestions by Rennie and Kemp (1984), who proposed selecting for beans with longer vegetative growth period. Buttery *et al*, (1987) suggested selecting for bean - rhizobia combination capable of maintaining high nitrogen fixation rates beyond the pod filling stage. Other proposals include selecting bean cultivars which nodulate early as suggested by Olivera and Graham (1990) and selecting bean cultivars which retain their lower leaves well into the reproductive cycle (Graham, 1982), in order to lengthen the period of photosynthate supply to the roots.

CHAPTER THREE

MATERIALS AND METHODS

The study was conducted in two sets of experiments; a glasshouse experiment and a field experiment. This was necessary in order to determine the performance of the different maturity status groups under controlled and under field conditions, respectively.

3.1 Glasshouse experiment

3.1.1 Experimental site

The glasshouse experiment was conducted at the University of Zambia. Soil was collected from the site of the field experiment at the Zambia Seed Company farm, Ngwerere, North of Lusaka. The area for soil collection was cleared using a hand hoe to remove grass, roots, and other plant materials. The soil was then dug to the depth of 15cm. After the soil was collected, clods were broken down and unwanted materials removed by using 2.5mm sieve. Soil analysis was done before conducting the experiment to determine inherent nitrogen, phosphorus, pH status, and soil texture. The results of the analysis showed the soil characteristics: nitrogen (0.16%), phosphorus (32.33mg/kg), pH (7.49); sand (73.2%), clay (14.4%) and silt (12.4%). The texture class was sandy loam ; Ferric Luvisol according to FAO classification.

3.1.2 Design

The experimental design was a split-plot, with *rhizobium* inoculated, non-inoculated and N-fertilizer as the main plots while varieties having different maturity status as the subplots. Treatments were six varieties of common beans (two each representing a

late, medium and early maturity group, respectively), and three sources of nitrogen (non inoculated, inoculated, and inorganic nitrogen fertilizer), resulting in eighteen treatment combinations. Three pots of each treatment combination were planted per replicate, and the experiment was replicated three times. The total number of planted pots was 162.

3.1.3 Soil

5kg fine soil was transferred into each pot, which had a surface area of 289cm².

3.1.4 Cultivars

The cultivars used in this experiment were Contender, Chizi, CG 76-1, Kablangeti, Carioca and PVA 2280. They were in three maturity groups, late (Carioca and PVA 2280), medium (CG 76-1 and Kablangeti) and early (Contender and Chizi). These maturity classes were established when the cultivars were grown for observation during the 1993/94 season at the Zambia Seed Company farm. Groupings were based on days to 50% flowering and days to physiological maturity.

3.1.5 Planting

Four seeds were planted per pot. Seven days after germination, thinning was done to reduce the number to two plants per pot.

3.1.6 Inoculation

The strain used in this experiment was CIAT 899, which was obtained from Mount Makulu Research Station, Chilanga, Zambia. The original cultures were purified by subculturing, and then reconstituted from slant cultures using yeast extract mannitol broth as described in Somesagaran and Hoben (1985). Inoculum was applied to the soil as a liquid broth seven days after germination to appropriate pots. Each plant received 1ml of inoculum, which supplied at least 10^9 *Rhizobium* cells per plant. Before inoculation the inoculum was kept in a flask containing ice cubes to avoid high temperatures which are detrimental to the *Rhizobium*.

Inoculation was done immediately after thinning on the seventh day. A glass rod was used to make a shallow hole in the soil about 2.5cm from the plant, then a dispensing bottle set at 1ml was used to transfer the inoculum in the hole after which the hole was covered with soil.

3.1.7 Watering

Watering was done daily to maintain moderate moisture in the pots. The amount of water supplied was 400 - 500ml per day

3.1.8 Fertilizers

Phosphorus (P) was applied in all pots at the equivalent rate of 40kg P/ha (0.02g P/kg soil). This fertilizer was incorporated in the dry soil soon after filling the pots.

Nitrogen (N) was applied at the equivalent rate of 80kg N/ha (0.04g N/kg soil) in the appropriate pots seven days after germination. All the pots were supplied with N - free nutrient solution, prepared and applied as described by Somesagaran and Hoben (1985). The application of N- free solution was done after every seven days where by 400 ml of the nutrient was applied per pot during the first four weeks after germination.

3.1.9 Sample collection

Two plants per pot were collected at each growing stage. Sampling was done at 28, 35, 42 days after germination (DAG) these stages were: 50% flowering, pod formation and mid pod filling, according to Fernandez *et al.* (1986)

The samples were cleaned carefully to remove soil without detaching nodules. Then the nodules were detached and counted separately. Roots from sampled plants were cut at the soil level and used for total carbohydrate determination. At each harvest, plant tops were obtained for nitrogen determination by Micro Kjeldahl analysis (Egan *et al.*,1981).

3.1.10 Data collection

Total carbohydrate content in the roots

The carbohydrate in roots was determined indirectly using the method of Shirlaw (1967). The separated roots were washed in water to remove soil and then nodules were detached. These root samples were dried at 70°C for 72 hours before grinding.

Nodule number per plant

This was done by washing the soil attached to the roots so as to remain with clean roots and nodules attached to them. The nodules then were detached and counted before drying.

Nodule dry weight

Nodule dry weight was determined by drying nodules in an oven at 70°C for 48 hours before weighing.

Nitrogen determination

Shoot nitrogen content was analyzed from shoot samples collected at all harvests by the Micro Kjeldahl method (Egan *et al.*, 1981). Plant shoot samples were dried at 70°C for 72 hours, then grinding was done by using Restch GMB type SM1 to make fine samples for N determination.

3.1.11 Data analysis

Analyses of variance were done by the MSTAT statistical procedure. Means were separated by the Duncan's Multiple Range Test (DMRT), (Gomez and Gomez, 1984). Correlation analysis (Gomez and Gomez, 1984) was done to determine the inter-relationships among the data collected.

3.2 Field experiment

3.2.1 Experimental site

The experiment was conducted at the Zambia Seed Company farm, Ngwerere, North of Lusaka during the 1994/95 rainy season. Soil samples were collected and analyzed as for the glasshouse experiment. After sampling the site was then ploughed and harrowed to prepare plots for planting.

3.2.2 Design

The same experimental design was used for glasshouse and field experiment. In the field the main plots were 16.8 x 4 m² while the subplots were 2.8 x 4 m². Each subplot had four lines of bean plants, consisting of two boarder rows and two middle rows which were used for data collection. Each row had 20 plants.

3.2.3 Cultivars

Cultivars used in this experiment were the same as those used in the glasshouse experiment.

3.2.4 Planting

Planting of seeds was done on small ridges which were 70cm apart, and the stations were 20cm apart. The non-inoculated seeds were planted and buried first followed by the inoculated ones in order to avoid contamination of inoculum to non inoculated seeds.

3.2.5 Inoculation

The strain used was CIAT 899 which was purified and prepared as explained for the glasshouse experiment. Inoculation was done by using a dispensing bottle, whereby 2ml was applied per station during planting. Inoculation was done in bands beneath the seed to avoid risk of toxic substances on the bean seed coat. The bacterial count was 10^9 *Rhizobium* cells per ml of liquid culture and this was done before inoculation.

3.2.6 Fertilizers

Phosphorus (P) at the rate of 40kg P/ha was applied by broadcasting and then incorporated in the soil before planting.

Nitrogen (N) at the rate of 80kg N/ha was applied to the appropriate plots 10 days after germination.

3.2.7 Agronomic practices

Thiodan (35ml per 15L water) was applied by sprayer to control bean stem maggot when plants were at the two leaf growth stage. Hand weeding using hand hoes was done eighteen days after germination to control weeds in the plots. Non-inoculated plots were weeded before inoculated plots to avoid contamination of *Rhizobium* from inoculated to non inoculated plots. Due to frequent dry periods during the cropping season, irrigation was carried out intermittently.

3.2.8 Sample collection

Four plants from each subplot were collected at 50% flowering, pod formation, and at mid-pod filling according to Fernandez *et al.* (1986). Four randomly selected plants were carefully dug up so as not to detach nodules and cleaned carefully to remove soil without detaching nodules. Nodule number per plant was determined by counting nodules of the four plants after which an average of the four plants was determined. Roots from sampled plants were cut at the level of soil surface and taken for total carbohydrate determination. At each harvest stage, plant tops were taken for nitrogen determination.

3.2.9 Data collection

Total carbohydrate content in the roots

The total amount of carbohydrate content in roots was determined by indirect method as described by Shirlaw (1967). After detaching the nodules, plant roots were separated from the shoot system at the ground level. Then these root samples were dried at 70°C for 72 hours before grinding and carbohydrate determination. Equipment used were Muffle furnace for ashing, Soxhlet apparatus for ether extraction and Micro kjedahl apparatus for crude protein.

Nodule number per plant

This was done by washing the soil attached to the roots so as to remain with clean roots and nodules attached to them. Then the nodules were detached and counted.

Nodule dry weight

Nodule dry weight was determined by drying nodules separately in an oven at 70°C for 48 hours before weighing.

Nitrogen determination

Shoot nitrogen was determined for samples collected at all harvests by the Micro Kjeldahl method (Egan *et al.*, 1981). Plant shoot samples were dried at 70°C for 72 hours, then grinding was done to make the sample fine for N determination. Nitrogen was determined by Micro - kjedahl apparatus

3.2.10 Data analysis

Analysis of variance were done by using the MSTAT statistical procedure. Means were separated by the Duncan's Multiple Range Test (DMRT), (Gomez and Gomez, 1984). Correlation analysis (Gomez and Gomez, 1984) was done to establish inter-relationships among the data collected.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Glasshouse experiment

4.1.1.1 Nodule number per plant

Cultivars differed in nodule number as shown in Table 1a. Contender formed the highest number of nodules at all growth stages. At flowering (28 DAG) PVA 2280 had the lowest number, while at pod-initiation (35 DAG) and mid-pod filling (42 DAG). Carioca had the least number of nodules. The number of nodules was increasing from one growth stage to another irrespective of the maturity status of the cultivar except for PVA 2280 which showed a decline from 35 DAG to 42 DAG. No noticeable relationship between maturity status and number of nodules was observed. There was, however, a tendency by early maturing cultivars to produce more nodules than late maturing ones. The late maturing cultivars PVA 2280 and Carioca, had the least numbers of nodules at all sampling stages while Contender, the earliest maturing had the greatest number at all stages.

With regard to the effect of inoculation (Table 1b), it was observed that at 50% flowering (28 DAG), the inoculated pots had the highest number of nodules followed by non inoculated and the N-fertilized pots, respectively. At pod initiation (35 DAG) the highest number was obtained in the non-inoculated plants followed by inoculated and N-fertilized. During mid pod-filling stage (42 DAG) the N-fertilized plants had the highest number followed by the non inoculated and the inoculated treatments, respectively.

Table 1a. Influence of cultivar on nodule number per plant at various sampling stages

Cultivars	28 DAG	35 DAG	42 DAG	Mean
Contender	43.67 a	80.33 a	91.89 a	71.96
Chizi	30.89 b	58.00 c	57.33 d	48.78
Kablangeti	25.89 c	64.44 b	79.67 b	56.67
CG 76-1	31.00 b	54.22 c	67.22 c	50.81
PVA 2280	20.00 e	53.89 c	46.11 e	40.00
Carioca	22.67 d	48.89 d	66.56 c	46.06
Mean	29.02	59.96	68.13	
LSD	1.651	4.769	5.467	
CV %	5.91	8.17	8.34	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

N.B. Cultivars are arranged according to maturity status early (Contender, Chizi), medium (Kablangeti and CG 76-1) and late (PVA2280, Carioca). The same applies for Table 1a to 5b.

Table 1b. Effect of inoculation on nodule number per plant at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non-inoculated	27.67 b	81.39 a	62.28 b	57.11
N fertilized	20.83 c	39.33 c	84.06 a	48.08
Inoculated	38.56 a	61.17 b	58.06 b	52.60
Mean	29.02	60.63	68.13	
LSD	1.368	3.406	5.915	
CV %	5.91	8.17	8.34	

Means in the same column followed by a common letter are not significantly different from each other at according to Duncan's Multiple Range Test.

DAG = Days after germination

The numbers of nodules generally increased with time although a decrease was recorded in the case of non inoculated and inoculated treatments, respectively at 42 DAG compared to the numbers at 35 DAG

4.1.1.2 Nodule dry weight (g)

Table 2a shows that Contender had the highest nodule dry weight at 50% flowering (28 DAG) while at pod initiation (35 DAG) and at mid pod-fill (42 DAG) Carioca had the highest nodule dry weight. Across the sampling stages, Contender, Carioca, Chizi, PVA 2280 and Kablangeti showed an increase in nodule dry weights, while for CG 76-1 the nodule dry weight decreased at 42 DAG. The increase and decrease of the nodule dry weight did not follow the maturity status of the bean cultivar.

In Table 2b, the inoculated plants had higher nodule weight per plant at 50% flowering (28 DAG) compared to the non-inoculated and N-fertilized plants. At pod initiation (35 DAG) and at mid pod fill (42 DAG) the non-inoculated plants had the highest nodule dry weight followed by the inoculated and N fertilized treatments respectively. Across the sampling stages there was an increase in the nodule weight from first harvest to the second harvest in all treatments.

4.1.1.3 Total carbohydrate in roots (%)

The highest percentage of carbohydrate in the root was recorded from Kablangeti at the first and second harvests (Table 3a). At the third harvest the highest carbohydrate content was recorded in PVA 2280, but not different from Kablangeti. Across the growth stages, cultivars differed in the carbohydrate contents in the roots. Contender, PVA

Table 2a. Influence of cultivar on nodule dry weight(g) per plant at various sampling stages

Cultivars	28 DAG	35 DAG	42 DAG	Mean
Contender	0.2 a	0.22 c	0.25 b	0.22
Chizi	0.15 b	0.21 c	0.20 cd	0.18
Kablangeti	0.12 bc	0.21 c	0.22 c	0.18
CG76-1	0.14 b	0.26 b	0.18 d	0.19
PVA 2280	0.09 c	0.22 c	0.18 d	0.16
Carioca	0.09 c	0.47 a	0.52 a	0.36
Mean	0.13	0.26	0.26	
LSD	0.036	0.052	0.03	
CV %	6.04	13.42	7.90	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 2b. Effect of inoculation on nodule dry weight (g) per plant at various sampling stages

N -source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	0.02 c	0.40 a	0.33 a	0.25
N fertilized	0.08 b	0.16 c	0.21 b	0.15
Inoculated	0.17 a	0.23 b	0.24 b	0.21
Mean	0.09	0.26	0.26	
LSD	0.02	0.101	0.071	
CV %	6.04	13.42	7.90	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 3a. Influence of cultivar on total carbohydrate in roots(%) at various sampling stages

Cultivar	28 DAG	35 DAG	2 DAG	Mean
Contender	24.42 e	27.84 b	27.9 b	26.72
Chizi	27.33 ab	27.98 b	26.48 c	27.26
Kablangeti	28.04 a	29.19 a	28.92 a	28.72
CG 76-1	26.80 bc	26.92 c	23.60 d	25.77
PVA 2280	25.10 de	26.41 d	29.31 a	26.94
Carioca	25.79 cd	24.13 e	6.71 e	22.21
Mean	26.25	27.08	25.49	
LSD	1.177	0.147	0.879	
CV %	4.66	3.63	3.37	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

2280 and Kablangeti showed overall increases in the carbohydrate percent with time while Carioca, Chizi and CG 76-1 had overall decreases in the carbohydrate percent as the plant growth progressed. This increase and decrease in root carbohydrate content had no systematic relationship with maturity group of the cultivar

Inoculated roots contained the highest carbohydrate percent when arranged over the three growth stages. Across the growth stages, inoculated and N fertilized plants showed a slight increase in the carbohydrate percent with time while the non inoculated pots showed a slight decline (Table 3b).

4.1.1.4 Nitrogen harvest (mg/pot)

Cultivars differ significantly in terms of N harvest showing no defined trend to maturity group (Table 4a). At first harvest, the cultivar Chizi had the highest plant nitrogen content while Kablangeti had the lowest. At the second harvest, CG 76-1, gave the highest nitrogen harvest and Kablangeti had the least, but at the last harvest PVA 2280 showed the highest nitrogen harvest and Carioca became the least. When examining the performance across the growth stages, there was a substantial increase in N harvest among all cultivars independent of their maturity classes (Table 4a).

The inoculated plants differed from non-inoculated and N-fertilized at the first harvest in terms of nitrogen harvest. At the second harvest, no significance difference between N-fertilized and inoculated were noted. During the third harvest, N-fertilized plants had higher nitrogen harvest than inoculated ones and non-inoculated (Table 4b)

Table 3b. Effect of inoculation on total carbohydrate (%) in the root at various sampling stages

N- source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	26.09 a	26.14 c	25.12 b	25.78
N fertilized	25.71 a	27.22 b	27.91 a	26.95
Inoculated	26.94 a	27.88 a	28.17 a	27.66
Mean	26.25	27.08	27.07	
LSD	1.261	0.453	0.53	
CV %	4.66	3.63	3.37	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 4a. Influence of cultivar on N harvest (mg/pot) at various sampling stages

Cultivars	28 DAG	35 DAG	42 DAG	Mean
Contender	11.23 d	16.54 a	16.66 c	14.81
Chizi	12.93 a	14.93 b	15.90 d	14.59
Kablangeti	9.74 e	14.45 c	18.35 b	14.18
CG 76-1	12.52 b	16.74 a	18.63 b	15.96
PVA 2280	11.05 d	14.56 c	27.76 a	17.79
Carioca	12.22 c	14.98 b	14.41 e	13.87
Mean	11.62	15.35	18.62	
LSD	0.292	0.332	0.424	
CV %	2.61	2.25	2.48	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 4b. Effect of inoculation on N harvest (mg/pot) at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	10.42 c	14.09 b	15.28 c	13.26
N fertilized	11.44 b	16.12 a	19.58 a	15.71
Inoculated	12.98 a	15.89 a	18.57 b	15.81
Mean	11.61	15.37	17.81	
LSD	0.447	0.24	0.354	
CV %	2.61	2.25	2.48	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

4.1.5 Relationship between carbohydrate and shoot nitrogen.

Correlation analysis shows a positive but low correlation between carbohydrates in roots and N harvest ($r = 0.26^*$, $r = 0.37^*$, $r = 0.34^*$) at 50% flowering, pod initiation and mid pod -fill, respectively.

4.2. Discussion for glasshouse experiment

4.2.1 Nodule number

It was observed that inoculated plants had higher number of nodules at the first harvest compared to non-inoculated, but at second and third harvests, the numbers in non inoculated and N- fertilized plants exceed those in inoculated pots. Perhaps the activity of the naturally existing rhizobia was activated by the favourable moisture level in the soil and a better supply of micronutrients. The decrease of nodule number observed in inoculated plants was probably due to earlier senescence of nodules. Nodules in the non- inoculated treatments developed much later compared to inoculated plants and therefore may have lasted longer as the plant developed. The same results were observed by Danso and Bowen (1989). The fact that nodules developed even where nitrogen was applied may indicate that the amount applied was not sufficient to inhibit natural nodulation.

The differences in nodule numbers among cultivars were unrelated to maturity status of the cultivar. The variations were apparently caused by varietal factors implying that there were cultivar related differences in development and sustaining of nodules as was also observed by Rennie and Kemp (1984).

4.2.2 Nodule dry weight

The nodule dry weights in Table 2b showed that the naturally existing *Rhizobium spp* had higher nodulating ability under controlled conditions than in the field. In the presence of chemical nitrogen fertilizer, nodule weight was reduced.

Cultivars manifested different abilities in maintaining nodule weight regardless of the maturity group but generally there was an increase in weight towards the third harvest. The implication of this observation is that the fixation process continued up to the last sampling stage.

4.2.3 Carbohydrates in roots

The carbohydrate content found in inoculated roots was higher than that found in non-inoculated roots. This is probably due to high demand for carbohydrate by nodulated roots (active sink effect). Herridge and Pate (1977) reported that higher amount of carbohydrate was partitioned to nodulated roots compared to non nodulated (less active sink) . The other reason for the higher carbohydrate content in inoculated plants could be enhanced photosynthetic capacity and carbohydrate partitioning as a result of improved plant nitrogen nutrition through fixation.

Hungria and Neves (1987) concluded that cultivars differed in the amounts of carbohydrate in their roots due to differences in the partitioning of carbohydrates to the roots, in N fixing capacity, and in utilization of fixed nitrogen and rates of exudate.

The positive correlation between carbohydrates in roots and N harvest ($r = 0.26^*$, $r = 0.37^*$, $r = 0.34^*$) at 50% flowering, pod initiation and mid pod -fill, respectively implies that the increase in carbohydrate in roots resulted in the increase of shoot nitrogen. These findings agree with those by Vasilas and Nelson (1992) who found that biological nitrogen fixation was affected by carbohydrate production. Higher carbohydrate supplied to the roots gives higher nitrogen in the leaves.

4.2.4 Total Nitrogen Harvest

There was greater amount of nitrogen in leaves in the inoculated pots compared to non-inoculated and N-fertilized plants for the two harvests (28 DAG and 35 DAG). At the third harvest (42 DAG), nitrogen fertilized plants had the highest amount of nitrogen in leaves. This could be the result of the mineral nitrogen ultimately supplying much more nitrogen to the bean plant than that was made available through biological fixation.

The differences among cultivars in total nitrogen in leaves can be explained by genotypic differences in dry matter production and utilization of N derived from fixation as observed by Vasilas and Nelson (1992), and variations in N fixing ability (Graham and Halliday, 1977).

The amount of nitrogen in leaves of inoculated and non-inoculated plants was increasing at all growth stages indicating that biological nitrogen fixation continued into the reproductive phase. This was also observed by Fernandez and Miller (1986), Israel (1981) and Imsande (1989). The building up of N in leaves depends on the interaction

between plant cultivar and the *Rhizobium* strain, dry matter production, partitioning of nitrogen to the leaves and nodule efficiency (Hungria and Neves, 1987; Israel, 1981).

4.3 Field Experiment Results

4.3.1 Nodule number per plant

Cultivars differed significantly in the number of nodules at the first harvest (Table 5a). Kablangeti had the highest mean nodule number amounting to 44.0 per plant, while Contender had the lowest mean number of 23.6. There was no defined relationship between maturity group and number of nodules. The cultivars significantly differed in nodule number within and between maturity groups.

At the second harvest, the highest number was once again recorded from Kablangeti, while the lowest number was obtained from CG76-1. At the third harvest, Kablangeti, CG76-1 and Chizi showed a decrease in nodule number compared to the previous sampling, while PVA 2280, Carioca and Contender on the other hand, showed an increase in the number of nodules. This increase and decrease was irrespective of the maturity group. The number of nodules generally increased with the growth stage when averaged over all cultivars (Table 5a).

Inoculation produced significantly higher number of nodules at the second and the third harvest, respectively, when compared to non- inoculated and N- fertilized plants (Table 5 b). At the third harvest, non- inoculated and N- fertilized plots showed an increase in nodule number compared to the second harvest while inoculated plots showed a decrease in nodule number. On the average, the mean number of nodules increased with sampling stage when averaged over inoculation treatment (Table 5b).

Table 5 a. Influence of cultivar on number of nodules per plant at various sampling stages

Cultivar	28 DAG	35 DAG	42 DAG	Mean
Contender	23.63 c	39.89 b	40.33 c	34.61
Chizi	40.89 b	55.78 a	36.67 c	44.44
Kablangeti	59.78 a	59.89 a	53.89 b	57.85
CG 76-1	34.56 bc	37.22 b	32.11 c	34.63
PVA 2280	49.89 ab	38.67 b	71.56 a	53.37
Carioca	44.00 b	55.56 a	66.89 a	55.48
Mean	42.13	47.84	50.24	
LSD	4.95	8.17	11.48	
CV %	35.29	17.95	23.74	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

N.B. Cultivars are arranged according to maturity status (early - Contender, Chizi), medium Kablangeti and CG 76-1) and late (PVA 2280, Carioca). The same applies for Table 5a to 8b).

Table 5 b. Effect of inoculation nodule number per plant at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	38.11a	30.17b	44.00b	37.43
N fertilized	46.28a	26.06b	33.17c	35.17
Inoculated	42.00a	82.78a	73.17a	65.98
Mean	42.13	46.34	50.11	
LSD	14.09	7.62	4.83	
CV %	35.29	17.95	23.74	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

4.3.2 Nodule dry weight per plant

There were significant differences in nodule dry weight among varieties (Table 6a) at all three stages of growth. At the first harvest, the highest weight of nodules was recorded from Contender and the lowest from CG 76-1. At the first and second harvests, nodule dry weights varied irrespective of the maturity group of the cultivar. However, in the third harvest there was a defined trend in relation to maturity group. The late and medium maturing cultivars showed a continued increase in the nodule weight while the early maturing cultivars showed a reduction in nodule weight compared to the second harvest.

Inoculation had an influence on nodule weight (Table 6b). At all harvest times, inoculated plants had higher mean nodule weights per plant followed by non-inoculated and N-fertilized. In all cases nodule weights from non-inoculated and N fertilized were not significantly different.

4.3.3 Total carbohydrate in the roots (%)

PVA 2280 had the highest percent carbohydrate content at all the three sampling times (Table 7a). CG76-1 had the lowest percentage at first and the second harvests. There was a general increase in the carbohydrate percent content with time among all the cultivars, the biggest increase being in PVA 2280. This degree of increase in carbohydrate content was irrespective of maturity group of the cultivar.

Table 6a. Influence of cultivar nodule dry weight (g) per plant at various sampling stages

Cultivar	28 DAG	35 DAG	42 DAG	Mean
Contender	0.31 a	0.15 b	0.13 de	0.2
Chizi	0.17 b	0.20 a	0.11 e	0.16
Kablangeti	0.17 b	0.19 a	0.20 c	0.19
CG 76-1	0.13 b	0.15 b	0.17 cd	0.15
PVA 2280	0.15 b	0.19 a	0.48 b	0.27
Carioca	0.14 b	0.14 b	0.78 a	0.35
Mean	0.18	0.17	0.31	
LSD	0.052	0.03	0.043	
CV %	0.61	19.85	15.47	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 6b. Effect of inoculation on nodule dry weight (g) per plant at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	0.11 b	0.12 b	0.15 b	0.13
N fertilized	0.09 b	0.11 b	0.12 b	0.11
Inoculated	0.25 a	0.28 a	0.67 a	0.40
Mean	0.15	0.17	0.31	
LSD	0.07	0.07	0.14	
CV %	10.61	19.85	15.47	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 7a. Influence of cultivar on total carbohydrate (%) in roots at various sampling stages

Cultivar	28 DAG	35 DAG	42 DAG	Mean
Contender	18.43 e	19.50 e	22.06 e	20.00
Chizi	20.31 d	21.52 d	25.70 c	22.51
Kablangeti	23.25 c	24.45 c	24.90 cd	24.20
CG 76-1	17.43 f	18.77 e	24.53 d	20.24
PVA 2280	26.55 a	30.37 a	36.01 a	30.98
Carioca	25.39 b	27.18 b	31.89 b	28.15
Mean	21.89	23.63	27.52	
LSD	0.49	1.34	0.88	
CV %	2.35	5.90	3.32	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Inoculation had a significant effect on the carbohydrate content in the roots as shown in Table 7b. All roots from inoculated plants had significantly lower total carbohydrate content than roots from non-inoculated or N-fertilized plants at each harvesting time. The respective differences between non-inoculated and N fertilized were not significant. All treatments resulted in increased carbohydrate content with time during the period of investigation.

4.3.4 Nitrogen harvest

There was an increase in the shoot nitrogen harvest at the second and third harvest periods compared to the first harvest among all cultivars (Table 8a), but the level of increase did not show any defined relationship to the maturity group of the cultivar. There was, however, a tendency for later maturing cultivars to have more shoot nitrogen content than earlier maturing ones.

With regard to source of N, there were significant differences within harvests between the inoculated, non-inoculated, and N-fertilized treatments, respectively. At the first harvest, the inoculated plants had highest nitrogen content. However, at the second harvest, there was no significant difference between inoculated and N-fertilized plants. During the third harvest, the N fertilized plants had the highest N harvest (Table 8b), followed by inoculated and non-inoculated. Across the growth stages, the shoot nitrogen harvest increased in all inoculation treatments.

Table 7b. Effect of inoculation on total carbohydrate (%) in roots at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	22.52 a	24.61 a	27.97 a	25.03
N fertilized	22.84 a	24.64 a	27.87 a	25.12
Inoculated	20.45 b	21.82 b	26.71 b	22.99
Mean	21.94	23.69	27.52	
LSD	0.60	1.22	0.64	
CV %	2.35	5.90	3.32	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 8a. Influence of cultivar in N harvest (kg/ha) at various sampling stages

Cultivar	28 DAG	35 DAG	42 DAG	Mean
Contender	11.9 bc	14.07 b	5.77 d	13.91
Chizi	12.36 a	13.23 c	13.83 f	13.14
Kablangeti	11.68 cd	13.20 c	16.17 c	13.68
CG 76-1	11.42 d	14.79 a	17.46 b	14.56
PVA 2280	12.30 ab	14.65 a	20.32 a	15.76
Carioca	11.08 e	13.35 c	14.79 e	13.07
Mean	11.79	13.88	16.39	
LSD	0.337	0.392	0.347	
CV %	2.97	2.94	2.2	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

Table 8b. Effect of inoculation on total N harvest (kg/ha) at various sampling stages

N - source	28 DAG	35 DAG	42 DAG	Mean
Non inoculated	11.33 b	12.38 c	13.55 c	12.42
N fertilized	11.69 b	14.15 a	19.03 a	14.96
Inoculated	12.40 a	15.11 a	16.60 b	14.70
Mean	11.81	13.88	16.39	
LSD	0.655	0.581	0.258	
CV %	2.97	2.94	2.2	

Means in the same column followed by a common letter are not significantly different from each other according to Duncan's Multiple Range Test.

DAG = Days after germination

4.3.5 Relationship between carbohydrate and shoot nitrogen.

Correlation analysis show that there was no correlation between carbohydrates in roots and shoot nitrogen at flowering, pod initiation and mid pod -fill respectively.

4.4 Discussion for Field experiment

4.4.1 Nodule number

The number of nodules was highly affected by inoculation. Inoculated plants had significantly higher number of nodules compared to non-inoculated and N-fertilized except at the first sampling. This can be probably attributed to slow colonization of the CIAT 899 *Rhizobium* strain. Presence of nodules on non-inoculated and N-fertilized roots was due to the presence of naturally existing *Rhizobia* which were not inhibited by nitrogen fertilizer. These findings agree with Rennie and Kemp (1984), who reported no apparent suppression effect on mean nodulation across sites when N was applied at the rate of 80 - 100 kg/ha. Graham (1981) also reported that number of nodules reached 145 - 278 per plant for non-inoculated plots. At the third harvest, the number of nodules was lower in N-fertilized plants compared to inoculated plants, possibly due to earlier senescence of nodules.

Differences among the cultivars in nodule numbers, were probably an expression of the interactions between *Rhizobium* strain and the host plant. Rennie and Kemp (1984) and Graham (1982) reported cultivar differences in nodulation probably caused by the presence of toxic substances in seed coats, or differences in root exudate. In this experiment, some cultivars showed an increase in the number of nodules towards the third harvest, while others showed a decrease, a result probably due to the cultivars having different abilities to maintain the number of nodules, and differences in nodulation time. Generally, there was a tendency for increased nodulation with time among later maturing cultivars while the number of nodules decreased among the

earlier maturing ones, when the first and third harvest were compared.

4.4.2 Nodule dry weight

At all growth stages, the inoculated plants had the highest nodule dry weight which differed significantly from non-inoculated and N-fertilized plots. The early and medium maturing cultivars showed that the nodule weight was falling at the later sampling stages while the nodule weight was increasing across the sampling stages for the late maturing cultivars. This probably signifies that the later maturing cultivars had greater capacity to sustain nodule function with time under field condition.

4.4.3 Total carbohydrate in roots

Carbohydrates are required in the root system together with other nutrients to enable the plant roots to function. For nodulating, roots carbohydrates are more important because nodules also need energy for the fixation process. Graham (1981) reported that supply of carbohydrate to the root is one of the factors that contributes to variability in nitrogen fixation in *Phaseolus vulgaris*.

In the present study, there were significant differences among cultivars with respect to carbohydrate percent in plant roots showing that cultivars had different abilities in partitioning carbohydrates to their root systems. Although there was an increase in root carbohydrate content from first harvest to the third harvest it was not related to cultivar maturity groups as cultivars even in the same maturity group varied in the amount of carbohydrate partitioned to the roots.

With inoculation, it was found that the non-inoculated and N- fertilized roots had higher levels of carbohydrates in the root system than the inoculated roots, probably implying that these were less active sinks. The lack of correlation at all stages of growth between carbohydrate and shoot nitrogen harvest meant that plants did not respond to the partitioned carbohydrate due to moisture stress conditions which prevailed during the course of the experiment.

4.4.4 Total nitrogen harvest

Cultivars showed significant differences in the amounts of nitrogen in the plant shoots which were unrelated to the maturity group of the cultivar. According to Graham and Halliday (1977) and Graham and Rosas (1979), nitrogen fixation effectiveness is a variety specific characteristic. In this experiment it was observed that the later maturing cultivars CG 76-1, PVA 2280 and Kablangeti had accumulated more nitrogen than the shorter maturing cultivars Chizi and Contender at the last harvest.

The inoculated plants had accumulated highest amount of nitrogen at the second sampling stage. In the third harvest the N-fertilized plants had the highest amount of nitrogen in leaves. This showed the limited amount of nitrogen that can be obtained by the plant through fixation as compared to nitrogen supplied by fertilizer. In the case of inoculated plants nitrogen mobilization and accumulation in plant tops depends on interaction with the *Rhizobium*, while for the N-fertilized plants the nitrogen content increase with the increase in nitrogen level in the soil (Park and Buttery, 1989).

CHAPTER FIVE

COMPARISON OF GLASSHOUSE AND FIELD EXPERIMENT

5.1 Nodule number

In the glasshouse experiment, cultivars had more nodules than in the field experiment, except at the first harvest (28 DAG) where plants in the field experiment had more nodules than plants in the glasshouse experiment. This was probably because in the field, inoculation was done during planting which enabled earlier initiation of nodules compared to the glasshouse experiment where inoculation was done after germination.

When comparing inoculated, non-inoculated and N - fertilized plants in the pot experiment, at 28 DAG, inoculated pots had more nodules than non inoculated and N - fertilized, at 35 DAG non-inoculated had more nodules than inoculated and N - fertilized; and at 42 DAG, N-fertilized had more nodules than others. Perhaps at 28 DAG the strain used (CIAT 899) colonized the rhizosphere faster than the naturally existing rhizobia which picked up at 35 DAG, and at 42 DAG. In the field, inoculated plants had high numbers of nodules at all sampling stages. The reason for the difference is not clear perhaps this observation can be investigated further.

5.2 Nodule dry weight

Inoculated plants maintained high nodule dry weights throughout the sampling stages in the field most likely because enhanced infection caused by CIAT 899 compared to natural rhizobia. The situation was different in the glasshouse experiment where inoculated plants had higher nodule weight than non-inoculated and N - fertilized plants

at 28 DAG. At 35 DAG and 42 DAG, the non-inoculated plants had higher weight than inoculated and N - fertilized. These results indicate that the conducive environment in the glasshouse probably activated the natural rhizobia more than the inoculum strain. This observation also requires further investigation.

5.3 Carbohydrate (%) in roots

In the glasshouse experiment, carbohydrate content found in inoculated roots was higher than that found in non-inoculated roots. This is probably due to the high demand for carbohydrate by nodulated roots (active sink effect). In addition, since harvesting was normally done in the morning, low night temperatures would have affected the partitioning of carbohydrates as was reported by Sprent (1979). The other reason for the higher carbohydrate content in inoculated plants could be enhanced photosynthetic capacity and carbohydrate partitioning as a result of improved plant nitrogen nutrition through fixation.

In the field the N - fertilized and non-inoculated roots showed more carbohydrate because of being less active sinks for carbohydrates compared to inoculated roots.

Due to lack of sufficient nodule materials for carbohydrate determination in these experiments the percentage of carbohydrate in roots was measured to reflect the percentage of carbohydrates available to nodules basing on the observations made by Heridge and Pate, 1977. In their work they found that in annual legumes the amount of photosynthate partitioned and consumed by nodule from the nodulated roots it is in the

ratio of about 4:1 to fix 1g of nitrogen. This means four parts of photosynthate are required by nodulated root and one part by nodules in order to fix 1g of nitrogen.

5.4 Total nitrogen harvest

In both field and glasshouse experiment, the amount of nitrogen in leaves in the inoculated plants was higher than non-inoculated and N fertilized plants at the first harvest (28 DAG). At 35 DAG, there was no significant difference between inoculated and N-fertilized plants in both experiments. At the third harvest (42 DAG) nitrogen fertilized plants had the highest amount of nitrogen in leaves. This could be the result of the mineral nitrogen ultimately supplying much more nitrogen to bean than what was available through biological fixation.

The amount of nitrogen in leaves was increasing with growth stage indicating that biological nitrogen fixation continued up to the last sampling stage. With regards to the individual cultivars, shoot nitrogen content was higher in the glasshouse experiment than the field experiment.

CHAPTER SIX

CONCLUSION

In the glasshouse experiment results showed a positive correlation between carbohydrates in roots and nitrogen in leaves. It can be concluded that carbohydrate partitioning to the roots affected biological nitrogen fixation in the sense that the higher the carbohydrate in roots, the greater the amount translocated to the nodules and hence, the higher the nitrogen fixed. The partitioning of carbohydrates did not necessarily follow the maturity status of common bean plants. This study has shown that within the same maturity group bean plants had different abilities to supply photosynthate to the roots and different abilities to fix nitrogen. Therefore, in selection of the breeding materials for biological nitrogen fixation, the cultivar with good ability to sustain biological nitrogen fixation by sustaining carbohydrates to nodules even during podding can be chosen regardless of the maturity status.

REFERENCES

1. Buttery, B.R., S.J. Park, W.I. Findlay (1987). Growth and yield of white beans (*Phaseolus vulgaris* L.) in response to nitrogen, phosphorus and potassium fertilizers and to inoculation with rhizobia. *Can. J. Plant Sc.* 66: 825 - 835. ✓
2. Danso, S.K.A. (1977). The ecology of *Rhizobium* and recent advances in the study of the ecology of *Rhizobium*: In A. Ayanaba and P.J. Dart (Eds.) Symposium on Nitrogen Fixation in Farming Systems of the Tropics, Ibadan 1975. International Institute of Tropical Agriculture, Ibadan Nigeria pp 115 - 125. ✓
3. Danso, S.K.A. and G.D. Bowen (1989). Methods of inoculation and how they influence nodulation pattern and nitrogen fixation using the contrasting strains of *Bradyrhizobium japonicum*. *Soil Biol. Biochem* 21(81): 1053 - 1058. ✓
4. Egan, H., R.S. Kirk and R. Sawy (1981). Pearson's chemical analysis of foods (Eighth edition) Longman Burnt Mill Harlow England. pp 15-20. ✓
5. Fernandez F., P. Gepts, M. Lopez, O. Arregoces, R. Hidalgo, and A. Correa, (1986). Stages of development of common bean plant; CIAT Series 04EB - 09 03 Cali, Colombia, 32p. ✓
6. Fernandez, C.J. and C. J. Miller (1986). Relation between rhizobial inoculation and fertilizer nitrogen in five Cowpea cultivars. *Crop Sc.* 21 (6):1345-1348. ✓
7. Franco, A.A. and D.N. Munns, (1982). Acid and aluminium restraints on nodulation, nitrogen fixation and growth of *Phaseolus vulgaris* L. in solution culture. *Soil Sc. Soc. Amer. J.* 4 :296 - 30 . ✓
8. Franco, A.A. (1977). Nutritional restraint for tropical grain legume symbiosis: In J.M. Vincent ,A.S. Whitney and J. Bose (Eds.) Exploiting Legume - Rhizobium Symbiosis in Tropical Agriculture. University of Hawaii Coll. Trop. Agrc. Misc. Publ. 145 pp 313 - 334. ✓
9. Gomez, K.A. and A.A. Gomez (1984). Statical Procedure for Agricultural Resaearch (second edition), John Wiley \$ sons. New York . pp. 207-215. ✓
10. Graham, P.H. and J. Halliday (1977). Inoculation and nitrogen fixation in the genus *Phaseolus* :In J.M. Vincent ,A.S. Whitney and J. Bose (Eds.) Exploiting Legume - *Rhizobium* symbiosis in Tropical Agriculture. University of Hawaii Coll. Trop. Agric. Mis. Publ. 313 - 334. ✓
11. Graham, P.H. and J.C. Rosas (1979). Phosphorus fertilization and symbiotic nitrogen fixation in common beans (*Phaseolus vulgaris* L.) *Agr. J.* 71: 925 - 927. ✓

12. Graham, P.H. (1981). Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L. a review Field Crops Res. 4: 93 - 112. ✓
13. Graham, P.H. (1982). Plant factors affecting symbiotic nitrogen fixation in legumes: In Graham P.H. and C.S. Harris (Eds.). Biological Nitrogen Fixation Technology for Tropical Agriculture. CIAT Series No 03E - 5 (82) pp 27 - 37. ✓
14. Graham P.H. (1983). Plant factors affecting nodulation and symbiotic nitrogen fixation in Legumes: In Biological Nitrogen Fixation and its Ecological Basis. Alexander (Ed.) pp 1 - 31. ✓
15. Hardson, G (1990). Use of nuclear technique in studies of soil - plant relationship. International Atomic Energy Agency, Vienna, Austria pp.161- 182. ✓
16. Herridge, D.F. and J.S. Pate (1977). Utilization of net photosynthate for nitrogen fixation and protein production in an annual legume. Plant Physiol. 60: 759 - 764. ✓
17. Hungria M. and C.P. Neves (1987). Cultivar and *Rhizobium* strain effect on nitrogen fixation and transport in *Phaseolus vulgaris* L.:Plant and Soil 103, 111-121. ✓
18. Imsande, J. (1989). Rapid dinitrogen fixation during soybean pod-fill enhances net photosynthetic output and seed yield: A new perspective. Agr. J. 81 (4) 549 - 556. ✓
19. Israel,D.W.(1981). Cultivar and *Rhizobium* strain effect on nitrogen fixation and remobilization by soybean. Agr.J. 73:509 - 516. ✓
20. Karel, A.K., B.G.Ndunguru, M.Price, S.H.Semuguruka and B.B.Singh (1981). Bean production in Tanzania: In Proceedings of Regional Workshop on Potential for Field Beans in East Africa, Lilongwe ,Malawi, 1980. CIAT series 03EB 1 Cali Colombia pp. 112 - 154. ✓
21. Kucey, R.M.N. (1989). Response of field bean (*Phaseolus vulgaris* L.) to level of *Rhizobium leguminosorum* bv. *phaseoli* population. Can. J. Plant Sc. 69: 419 - 426. ✓
22. Lynch, J. and M. Piha (1988). Adaptation of beans to infertile soils. Proceeding of a Workshop in Soil Fertility Research for Bean Cropping Systems in Africa, Addis Ababa, Ethiopia, 5 - 9 September 1988. ✓

23. Masomo, S.M.S. and C.L. Rweyemamu (1989). Evaluation of the effect of cattle and poultry manure in combination with inorganic fertilizers in seed yield and seed quality of common beans (*Phaseolus vulgaris* L.) grown different stand per hill: In E.E. Maeda and S.F. Nchimbi (Eds.) Proceedings of the Eighth Bean Research Workshop held at Sokoine University of Agriculture, Morogoro, Tanzania. September 27 - 29th 1989
24. Mehler, R.L., D.F. Bezdicek and R.E. Witters (1979). Influence of slope position on nitrogen fixation and yield of dry peas. Morogoro, Tanzania Agr. J. 71: 348 - 351.
25. Mengel, K. and E.A. Kirkby (1987). Principles of plant nutrition. International Potash Institute. Bern, Switzerland. pp. 335-359.
26. Munns, D.N. (1977). Soil acidity and related problems: In J.M. Vincent, A.S. Whitney and J. Bose (Eds.) Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture. University of Hawaii Coll. Trop. Agr. Misc. Publ. 145: 211-236.
27. Nurhayat, D.P., D.A. Ivory and W.W. Stur (1989). The effectiveness and competitiveness of some Indonesia *Rhizobium* strains on tropical legumes grown in four soil types of Java. Plant and Soil 117: 146 - 150.
28. Nyemba, R.C., K. Munyinda, H. Tembo, M. Mwale, and G. Sakala, (1989). Evaluating biological nitrogen fixation in bean in Zambia: In Smithson J.B. (Eds.) Proceeding of First SADC Region Bean Research Workshop. Mbabane, Swaziland. 4 - 7th October 1989. CIAT Series No 6 pp 7 - 14.
29. Nyiti, E.J., M.P. Salema, M.S. Chowdhury (1987). Effect of rhizobial inoculation, phosphorus, molybdenum fertilizer on nodulation and nitrogen fixation in common beans grown on acidic soils: In M.P. Salema and A.N. Minjas (Eds) Proceeding of the Fifth Bean Research Workshop, held at Sokoine University of Agriculture, Morogoro Tanzania. September 9 - 12th 1986
30. Oliviera, L. A. de and P.H. Graham (1990). Evaluation of strain competitiveness in *Rhizobium leguminosarum* bv. phaseoli using nod fix natural mutant. Archives of Microbiology 153: 305 - 310.
31. Park S.J. and B.R. Buttery (1989). Identification and characterization of common bean (*Phaseolus vulgaris* L.) well nodulated in presence of high nitrate. Plant and Soil 119: 237 - 244.
32. Piha, M.I. and D.M. Munns, (1987). Nitrogen fixing capacity of field grown beans compared to other legumes. Agr. J. 79: 690 - 696.

33. Rennie, R.J. and G.A. Kemp, (1981). Dinitrogen fixation of pea bean (*Phaseolus vulgaris* L.) as affected by growth stage and temperature regime. Can. J. Bot. 59: 1181 - 1188. ✓
34. Rennie, R.J. and G.A. Kemp (1984) ¹⁵N determined time course for N₂ Fixation in two cultivars of field beans. Agr. J. 76: 146 - 154. ✓
35. Salema, M.P. (1987). Variation in nodulation, nitrogen fixation and yield in various bean (*Phaseolus vulgaris* L.) genotypes: In Salema M.P. and A.N. Minjas (Eds.). Proceeding of Sixth Bean Research Workshop held at Sokoine University of Agriculture, Morogoro, Tanzania 1st - 3rd October 1987. ✓
36. Shirlaw D.W.G. (1967). A practical course in Agricultural Chemistry. Pergamon press, London. p.165. ✓
37. Somasegaran, P and Hoben (1985). Methods in Legume - *Rhizobium* Technology. University of Hawaii NifTAL Project. Department of Agronomy and Soil Science. University of Hawaii. p. 365. ✓
38. Sprent, J.T. (1979). The biology of nitrogen fixing organisms. McGraw Hill Book company UK., p196 ✓
39. Vasilas, B.L. and R.L. Nelson (1992). N fixation and dry matter accumulation in soy bean lines with different seed filling periods. Can. J. Plant Sc. 72: 1067-1074. ✓
40. Wortmann, C.S. and J.Y.K. Zake (1988). Review of bean response to applied fertilizer in Africa. Proceeding of a Workshop in Soil Fertility Research for Bean Cropping Systems in Africa, Addis Ababa, Ethiopia, 5 - 9 September 1988. ✓

Appendix 1 (Field experiment)

Interactions of cultivar and source of nitrogen on nodule number and nodule dry weight per plant at various sampling stages.

Treatment	Nodule number			Nodule dry wt (g)		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
1. Contender	15.67	28.67	53.67	0.12	0.12	0.15
1. Chizi	17.00	9.00	47.00	0.11	0.09	0.11
1. Kablangeti	12.33	55.00	40.00	0.10	0.17	0.12
1. CG 76-1	18.00	23.67	33.67	0.11	0.11	0.13
1. PVA 2280	17.33	14.33	33.67	0.11	0.07	0.15
1. Carioca	21.67	40.33	53.33	0.12	0.13	0.22
2. Contender	10.33	34.33	32.33	0.07	0.14	0.12
2. Chizi	18.00	49.33	19.33	0.11	0.15	0.10
2. Kablangeti	20.33	21.00	25.67	0.11	0.08	0.11
2. CG 76-1	42.67	47.00	30.33	0.83	0.15	0.10
2. PVA 2280	17.33	11.00	54.67	0.10	0.07	0.19
2. Carioca	10.33	11.67	36.67	0.07	0.08	0.12
3. Contender	45.00	56.67	35.00	0.21	0.20	0.12
3. Chizi	87.67	99.00	43.67	0.29	0.35	0.13
3. Kablangeti	146.67	94.67	96.00	0.30	0.32	0.373
3. CG 76-1	43.00	41.00	32.33	0.20	0.19	0.27
3. PVA 2280	115.00	90.67	123.33	0.25	0.43	1.10
3. Carioca	100.00	114.6	108.67	0.23	0.20	2.00
CV	35.29	17.95	23.74	10.61	19.85	15.47

Key:

1. = Non inoculated

2. = N fertilized

3. = Inoculated

DAG = Days after germination

Appendix 2 (Field experiment)

Interactions of cultivar and source of nitrogen on carbohydrate in roots and nitrogen harvest in leaves at various sampling stages

Treatment	Total CHO(%)			Nitrogen harvest (mg/pot)		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
1. Contender	18.77	20.03	18.63	12.50	15.65	18.37
1. Chizi	19.99	22.43	26.11	10.10	9.67	10.47
1. Kablangeti	27.72	28.01	28.50	12.11	10.16	10.37
1. CG 76-1	18.03	19.16	24.75	10.48	14.43	15.22
1. PVA 2280	25.73	30.08	37.62	11.24	13.25	14.58
1. Carioca	24.88	27.97	32.23	11.53	11.10	12.27
2. Contender	20.28	22.34	23.93	11.15	13.14	18.27
2. Chizi	22.38	22.16	26.02	13.42	15.54	14.99
2. Kablangeti	24.97	27.06	24.54	10.50	14.98	20.72
2. GC 76-1	16.41	19.05	23.49	13.31	15.39	20.49
2. PVA 2280	28.01	30.87	38.49	10.30	10.37	24.38
2. Carioca	24.99	25.28	30.72	11.48	15.45	15.35
3. Contender	16.24	16.13	23.63	12.33	13.40	10.67
3. Chizi	18.57	19.99	24.97	13.56	14.43	16.04
3. Kablangeti	17.87	18.29	21.65	12.45	14.46	17.44
3. CG 76-1	17.84	18.09	25.36	10.48	14.54	16.67
3. PVA 2280	25.90	30.15	31.93	5.37	20.34	22.01
3. Carioca	26.30	28.28	32.72	10.23	13.50	16.75
CV	2.35	5.90	3.32	2.97	2.94	2.2

Key:

1. = Non inoculated

2. = N fertilized

3. = Inoculated

DAG = Days after germination

Appendix 3 (Glasshouse experiment)

Interactions of cultivar and source of nitrogen on nodule number and nodule dry weight per plant at various sampling stages

	Nodule number/plant			Nodule dry wt/plant		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
Treatment						
1. Contender	43.00	86.00	74.67	0.23	0.23	0.20
1. Chizi	28.33	95.67	43.00	0.18	0.32	0.11
1. Kablangeti	21.67	59.67	86.33	0.11	0.12	0.22
1. CG 76-1	22.33	94.00	52.67	0.15	0.38	0.12
1. PVA 2280	24.00	63.00	32.67	0.11	0.24	0.14
1. Carioca	26.67	90.00	84.33	0.13	1.09	1.17
2. Contender	56.00	57.33	115.00	0.21	0.18	0.22
2. Chizi	18.00	39.00	87.67	0.06	0.10	0.24
2. Kablangeti	11.33	47.00	92.67	0.05	0.20	0.23
2. CG 76-1	13.67	20.33	95.33	0.05	0.19	0.22
2. PVA 2280	10.67	60.00	57.67	0.05	0.20	0.21
2. Carioca	15.33	12.33	56.00	0.05	0.10	0.17
3. Contender	32.00	97.67	86.00	0.16	0.21	0.34
3. Chizi	46.33	39.33	41.33	0.10	0.22	0.24
3. Kablangeti	44.67	98.67	60.00	0.20	0.30	0.21
3. CG 76-1	157.00	48.33	53.67	0.02	0.20	0.22
3. PVA 2280	25.33	38.67	48.00	0.11	0.22	0.21
3. Carioca	26.00	44.33	59.33	0.16	0.22	0.21
CV	5.91	8.17	8.34	6.04	13.42	7.90

Key:

1. = Non inoculated

2. = N fertilized

3. = Inoculated

DAG = Days after germination

Appendix 4 (Glasshouse experiment)

Interactions of cultivar and source of nitrogen on carbohydrate in roots and nitrogen harvest in leaves at various sampling stages

Treatment	Total CHO (%)			Nitrogen harvest (mg/pot)		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
1. Contender	28.06	28.09	27.13	12.10	15.45	19.12
1. Chizi	27.50	26.85	23.93	10.62	10.76	12.11
1. Kablangeti	26.36	28.59	26.97	7.67	12.23	12.05
1. CG 76-1	26.04	26.58	18.14	8.31	16.85	16.19
1. PVA 2280	23.35	23.37	28.93	9.51	14.46	18.32
1. Carioca	25.24	23.39	25.64	14.30	14.77	13.86
2. Contender	16.69	26.14	27.66	9.44	16.42	19.30
2. Chizi	27.27	27.85	31.25	11.61	16.21	16.64
2. Kablangeti	28.86	28.98	30.23	7.06	15.50	21.52
2. CG 76-1	27.56	27.86	24.36	17.42	18.55	21.54
2. PVA 2280	26.02	26.54	27.97	11.05	11.96	26.24
2. Carioca	27.75	26.00	25.97	12.08	18.09	1.84
3. Contender	28.52	29.27	27.39	12.15	17.75	11.56
3. Chizi	27.21	29.25	24.25	16.55	17.80	18.95
3. Kablangeti	28.92	30.04	29.55	14.50	15.62	21.47
3. CG 76-1	26.70	26.33	28.31	11.82	14.83	18.17
3. PVA 2280	25.92	23.37	31.03	12.59	17.42	23.72
3. Carioca	24.38	23.03	28.51	10.28	12.08	17.54
CV	4.66	3.63	3.37	2.61	2.25	2.48

Key:

1. = Non inoculated

2. = N fertilized

3. = Inoculated

DAG = Days after germination

Appendix 5

Correlation between nitrogen in leaves and nodule number, nodule dry weight and carbohydrate in the root

Glasshouse experiment

	Nodule number	Nodule dry wt	Carbohydrate(%)
28 DAG	0.198*	0.123ns	0.263*
35 DAG	-0.135*	-0.112ns	0.369*
42 DAG	-0.048ns	-0.211*	0.336*

Field Experiment

28 DAG	0.416*	0.34 *	-0.131ns
35 DAG	0.314*	0.504*	-0.136ns
45 DAG	0.261*	0.202*	0.119ns

Appendix 6

SUMMARY OF ANOVA TABLES

6a Glasshouse Experiment

Source	Nodule number			Nodule dry wt		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
N-source	*	*	*	*	*	*
Cultivars	*	*	*	*	*	*
N*C	*	*	*	*	*	*
CV	5.91	8.17	8.34	6.04	13.42	7.90

Appendix 6a continued

Source	Carbohydrate in roots			Nitrogen harvest		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
N-source	*	*	*	*	*	*
Cultivars	*	*	*	*	*	*
N*C	*	*	*	*	*	*
CV	4.66	3.63	3.37	2.61	2.25	2.48

* = Indicate significance difference according to Duncan's Multiple Range Test

DAG= Days after germination

Appendix 6 continued

SUMMARY OF ANOVA TABLES

6b Field Experiment

Source	Nodule number			Nodule dry wt		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
N-source	*	*	*	*	*	*
Cultivars	*	*	*	*	*	*
N*C	*	*	*	*	*	*
CV	35.29	17.95	23.74	10.61	19.85	15.47

Appendix 6b continued

Source	Carbohydrate in roots			Nitrogen harvest		
	28DAG	35DAG	42DAG	28DAG	35DAG	42DAG
N-source	*	*	*	*	*	*
Cultivars	*	*	*	*	*	*
N*C	*	*	*	*	*	*
CV	2.35	5.90	3.32	2.61	2.25	2.48

* = Indicate significance difference according to Duncan's Multiple Range Test

DAG= Days after germination

Appendix 7

Climatic data for 1994/95 cropping season at Lusaka International Airport

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Rain (mm)	43.5	99.5	41.8	105.4	23.9	0	0	0
Max.temp monthly°C)	32.3	28.7	28.0	26.9	27.9	28.7	26.6	23.8
Min.temp monthly°C)	20.3	19.6	17.4	16.3	15.8	16.7	13.4	10.9

Source: Meteorological Department, Lusaka, Zambia 1995.

Appendix 8

Stages of growth of cultivars during harvesting (for Field and Glasshouse)

Cultivar	28DAG	35DAG	42DAG
Contender	50% flowering	Pod initiation	Mid pod-fill
Chizi	50% flowering	Pod initiation	Mid pod-fill
Ablangeti	Budding	35% flowering	Pod initiation
CG 76-1	Budding	35% flowering	Pod initiation
GA 2280	-	Budding	20% flowering
Parioca	-	Budding	20% flowering

DAG = Days after germination