RESPONSE OF COMMON BEAN GENOTYPES TO INOCULATION WITH *RHIZOBIA* AND EFFECTS OF P AND N ON BIOLOGICAL NITROGEN FIXATION

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ABSTRACT

The study on the response of common bean genotypes to *rhizobia* inoculation and effects of P and N on biological nitrogen fixation was carried out in field and Screen house experiments. Field experiments arranged in a split-split plot design were conducted to (a) Screen 16 common bean genotypes for capacity to fix nitrogen when inoculated with *Rhizobium* inoculants and (b) Assess genotype by environment interaction (G x E) on biological nitrogen fixation. The locations were the main-plot, two *rhizobia* inoculants Biostacked, Nitrosua and control as sub-plot, and 16 common bean genotypes as sub-sub plot factor. A Screen house experiment was carried out in factorial arrangement with rhizobia inoculant at three levels as one factor, and two levels of Nitrogen and Phosphorous (with and without) as second and third factor respectively and three common bean genotypes namely Kablanketi, Rojo and G51105-A as fourth factor. This resulted to 36 treatment combinations replicated three times. The results from the field experiments revealed high significant difference (P \leq 0.05) among genotypes and genotypes x environment interaction. Inoculation had significant effect in measured variables such as nodule number and dry weight per plant, seed percent nitrogen content, shoot ureides concentration. Biostacked inoculant was found to be superior compared to Nitrosua. Genotypes which showed promising results in nodulation included Lyamungu 85 closely followed by Pesa, Zawadi, Seliani 97, Rojo, Kablanketi and Carioka, indicating good adaptability for nodulation trait across the locations. Screen house experiment revealed that inoculation using either *rhizobia* in combination with application of Phosphorous increased nodule number, nodule dry weight, root dry weight, shoot

and seed nitrogen. Therefore, Commercial production of *rhizobia* inoculants should be explored in the country since use of inoculants has shown good results. Farmers should also be advised to use P-fertilizers as this will increase BNF and a consequence increase in yield.

DECLARATION

I Beata P. Khafa, do hereby declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently submitted for a degree award in any other institution.

Beata Paulo Khafa (MSc. Crop Science Candidate) Date

The above declaration is confirmed by;

Prof. Susan Nchimbi-Msolla

(Supervisor)

Date

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DEDICATION

This work is dedicated to my parents and friends for their love, endless support and encouragement.

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LIST OF ABBREVIATIONS AND SYMBOLS

- \$ Dollar sign
- % Percentage
- μM Micromole

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
ARA	Acetylene reduction assay
ARI	Agricultural Research Institute
ATP	Adenosine triphosphate
В	Boron
BNF	Biological nitrogen fixation
Bs	Biostacked(Becker nderwood)
C. mean	Combined mean
cm	Centimeter
C_2H_2	Acetylene
C_2H_4	Ethylene
CEC	Cation exchange capacity
CIAT	International centre for Tropical Agriculture
Cu	Copper
CV	Coefficient of variation
СV	Cultivar
DAP	Days after planting
DM	Dry matter
e	Electron
FAO	Food and Agriculture Organization
FAOSTAT	Food And Agriculture Organization Statistics
g	Gram
G x E	Genotype by environment interaction

H^+	Hydrogen ion
H_2	Hydrogen gas
К	Potassium
kg	kilogram
MAFCs	Ministry of Agriculture Food security and Cooperatives
masl	Metre above sea level
Mn	Manganese
Мо	Molybdenum
Ν	Nitrogen
N_2	Nitrogen gas
NH ₃	Ammonia
Nod dwt	Nodule dry weight
Nod No	Nodule number per plant
Ns	Nitrosua
°C	Degree Celsius
Р	Phosphorous
P ₂ 0 ₅	Phosphorus pentoxide
P ^H	Hydrogen ion concentration
Rdwt	Root dry weight
Sdwt	Shoot dry weight
SGM	Society for General Microbiology
SUA	Sokoine University of Agriculture
t/ha	Tonne per hactare
TSP	Triple super phosphate

xviii

Lymng 85

Lyamungu 85

Metre

m

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Common bean (*Phaseolus vulgaris* L.) is a member of the Fabaceae family originated from South America. It is a major source of protein for the poor in Tanzania. It has 38% of protein and caloric content of 12-16% (CIAT, 2008). The crop can be consumed in various forms like dry cooked seeds; green cooked seed or green leaves can be picked cooked and eaten as a green leafy vegetable (Katungi *et al.*, 2009). Apart from being source of protein, common beans like other legumes also supply carbohydrates, vitamins and minerals such as iron, phosphorus, magnesium, manganese, zinc, copper and calcium and complement cereals, roots and tubers that compose the bulk of diets in most developing countries (Beebe *et al.*, 1999; Broughton *et al.*, 2003; CIAT, 2009-2011). In Tanzania the crop is grown mainly for local consumption although a small portion of the produce finds its way to urban market (Wortman *et al.*, 1998). Bean yields are still low with an average of 729 kg/ha (FAO, 2005).

In Tanzania, crop production is constrained by a number of factors namely, unreliable weather conditions, lack of bean varieties adapted to soil related constraints, several diseases and insect pests, poor agronomic practices, also continue to be the major constraints of bean production. Small scale farmers in bean growing areas rarely use inorganic nitrogenous fertilizers which are very expensive, not easily accessible and potential pollutant to environment hence the crop is largely dependent on naturally biologically fixed nitrogen. The average production of the crop in East African countries are 0.453 t/ha, 0.603 t/ha, 0.682 t/ha, 0.765 t/ha and 0.921 t/ha for Kenya, Uganda, Rwanda, Tanzania and Burundi respectively (Appendix 1).

Common bean production areas in Tanzania are in the northern zone particularly in Arusha and Manyara regions, the great lakes region in the west particularly, Kigoma and in the Southern Highlands, in Mbeya, Rukwa and Iringa. Both local and improved varieties are grown but the most important ones are red, yellow medium sized, and grey spotted types (Wanda and Ferris, 2004). *Lyamungu 85* is the most common variety occupying about 38 percent of the area under beans in Northern and western Zone of the country (Katungi *et al.*, 2009). *Soya* and *Canadian wonder* type account for 22 percent of the area under the crop. In Southern highland of Tanzania, orange and yellow bean types, *Kablanketi* and *Uyole 96* are among the important bean types and also preferred in the neighbouring countries of DRC, Rwanda and Burundi (Katungi *et al.*, 2009).

1.2 Justification

There is a problem of low production of common bean in Tanzania an average of 0.75 t/ha from 2000 to 2008 (Katungi *et al.*, 2009, FAOSTAT, 2008) while under optimal management the yield of common bean ranges from 2- 3 t/ha (Kanyeka *et al.*, 2007). Low soil fertility has been observed to be one among the many causes of low production. Among nutrients, nitrogen is the critical limiting element for growth of most plants including common beans due to its unavailability (Vance, 2001 CIAT,

1989). Nitrogen (N) is a constituent of proteins, enzymes, chlorophyll, and growth regulators. Deficiency in N causes reduced growth, leaf yellowing, reduced branching and small trifoliate leaves in beans (CIAT, 1989). Small scale farmers in bean growing areas rarely use inorganic nitrogenous fertilizers which are very expensive and not easily accessible (FAO, 2008).

Plants acquire N from two principal sources which are the soil, (through commercial fertilizer, manure and/or mineralization of organic matter); and the atmosphere (through symbiotic N fixation) (Vance, 2001). Rodriguez (1993) and Urzua (2000b) revealed that, only 50 % to 60 % of the inorganic nitrogen fertilizer applied is used by the crop, the rest is lost by volatilization, denitrification or leaching of nitrate into the ground water.

Legumes including common beans have been recognized worldwide as an alternative means of improving soil fertility because of their ability to fix atmospheric nitrogen, enhancing soil organic matter and improving general soil structure. Legumes can meet most of their N needs and contribute to soil N through symbiotic nitrogen fixation (Maobe *et al.*, 1998). Estimates indicate that legumes can fix up to 200 kg N /ha/ year under optimal field conditions (Giller, 2001).

Inoculation with suitable *rhizobia* along with Phosphorus improves symbiotic nitrogen fixation and yield in common bean (Fatima, *et al.*, 2007). Inoculation of bean seed with appropriate *rhizobium* strains for enhanced nitrogen fixation provides an alternative to the application of nitrogenous fertilizers; however in

Tanzania studies towards identifying bean genotype/line with enhanced nitrogen fixation has not been conducted (Nchimbi-Msolla, S. personal communication, 2011). Therefore, it is important to evaluate various bean genotypes so as to identify the best genotype with higher response to inoculation with *rhizobium* strains in terms of biological nitrogen fixation.

1.3 Objectives

1.3.1 Overall objective

To improve yield of common bean (*Phaseolus vulgaris* L.) through enhanced nodulation and nitrogen fixation

1.3.2 Specific objectives

- To evaluate 16 common bean genotypes for capacity to fix nitrogen when inoculated with *rhizobia* inoculants in three locations in Tanzania and determine G x E interaction on BNF parameters and yield traits.
- ii. To determine the effect of different *rhizobia* inoculants in nodule formation and nitrogen fixation.
- iii. To determine the effect of phosphorous and nitrogen on nodulation and biological nitrogen fixation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nitrogen Fixation

Nitrogen fixation is the process by which atmospheric nitrogen gas (N_2) is converted into ammonia (NH_3) (Harrison, 2003; Postgate, 1998). Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called nitrogenase produced by bacteria particularly *Rhizobium* spp (Hubbell and Kidder, 2009). The process of biological nitrogen fixation is as illustrated in Fig.1

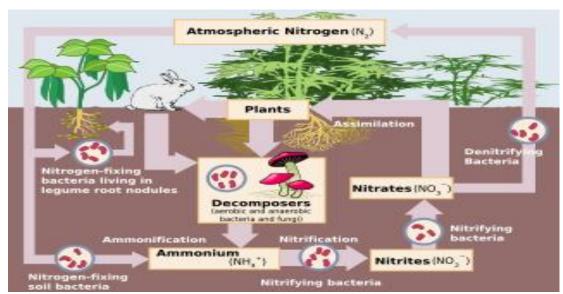


Figure 1: Illusrtation for process of Biological nitrogen fixation

Source: Harrison (2003)

2.2 Mechanism of Biological Nitrogen Fixation

Legumes release chemical compounds called flavonoids from their roots, which trigger the production of nod factors by the bacteria. When the nod factor is sensed by the root, a number of biochemical and morphological changes happen within the roots. Cell division is triggered in the root to create the nodule, and the root hair growth is redirected to fold around the bacteria multiple times until it fully encapsulates one or more bacteria. The bacteria encapsulated divide multiple times, forming a micro colony. From this micro colony, the bacteria enter the developing nodule through a structure called an infection thread, which grows through the root hair into the basal part of the epidermis cell, and onwards into the root cortex; they are then surrounded by a plant-derived membrane and differentiate into bacteroids that reduces nitrogen gas from the air into ammonium a form that can be used by its plant host to make proteins. In turn, the plant provides the *rhizobia* with products of photosynthesis: sugars and carbohydrates that can be used as fuel by the bacteria in the BNF process (Hubbell and Kidder, 2009; Barnish and Spinelli, 2011). The following equation explains the nitrogen-fixing reaction by which nitrogen is fixed from the atmosphere and thus become available for the plants (Urzua, 2005):

 $N_2 + 16 \text{ ATP} + 8e^2 + 8H + \frac{\text{nitrogenase}}{2NH_3} 2NH_3 + 8H_2 + 16 \text{ ADP} + 16 P_1....(1)$

2.3 Rhizobia

Rhizobia are microorganisms that are Gram-negative, motile, and non-sporulating and rods shaped (Burton, 1984). The organisms are capable of living symbiotically with plants forming nodules and fixing nitrogen into ammonia and supplying it to the host plants (that is, has nitrogen-fixing ability). The *rhizobia* are broadly classified as fast or slow-growing based on their growth on laboratory media and host plant. Further classification was according to their compatibility with particular legume (host range). Most of these bacterial species are in the *Rhizobiacae* family in the alpha-proteobacteria and are in the *Rhizobium*, *Bradyrhizobia*, *Azorhizobium*, *Sinorhizobium, Mesorhizobium,* and *Allorhizobium.* Recent research has shown that there are many other *rhizobial* species in addition to these. In some cases these new species have arisen through lateral gene transfer of symbiotic genes. (Burton, 1984; Giller, 2001; Weir, 2012).

2.4 Host Specificity

Legumes often require specific strains of rhizobia for maximum nitrogen fixation, some bacterial strains have a very narrow host range while others have broad host range (Burton, 1984). Host specificity in the rhizobium-legume symbiosis is controlled in the bacterium by host specific nodulation genes residing on its symbiotic plasmid. The plant signals, flavonoids exuded by the roots of the host plant, activate the expression of nodulation genes, resulting in the production of the rhizobial lipochitooligosaccharide signals (Nod factors), that under conditions of nitrogen limitation induces cells within the root cortex to divide and to develop into nodule primordial (Broughton et al., 2000). Pueppke and Broughton (1999) reported that Rhizobial spp NGR234, nodulates over 110 genera of legumes. The predominant rhizobial species in common bean nodules is Rhizobium etli, although many other species have been found such as R. leguminosarum bv.phaseoli, R. gallicum bv. phaseoli, R. giardinii bv.phaseoli, and R. tropici (Martinez, 2003). The large number of *rhizobia* species capable of nodulating the common bean supports the fact that this species is a promiscuous host and that a diversity of the bean-*rhizobia* interactions exists (Graham, 2008). Michiels et al. (1998) reported that common bean plant can form effective nodules with at least 20 species of *rhizobia*.

2.5 Rhzobia Effectiveness

An efficient *Rhizobium* strain is that which can compete in the field with other indigenous *rhizobia* for the colonization of the rhizosphere of its homologous legume partner, under various soil physical and chemical conditions. This efficient strain will form many large nitrogen-fixing nodules on the roots of the plant host. Effectiveness of the nodules can be determined to some degree by looking at the colour of the nodules. Numbers and size of nodules do not necessarily correlate to the effectiveness of the inoculant strain. The presence of plenty of bright red or pinkish colour (Plate 1) denotes the presence of leghaemoglobin (SGM, 2002). The presence of leghaemoglobin has been documented as a good indicator of active N fixation by the micro-symbiont (FAO, 1984). When nodules are young and not yet fixing nitrogen they are white or grey inside. Legume nodules that are no longer fixing nitrogen turn green and may be discarded by the plant. This may be the result of an inefficient *Rhizobium* strain or poor plant nutrition including P (SGM, 2002).



Plate 1: Effective nodules for fixing nitrogen of common bean (*Phaseolus vulgaris* L.)

2.6 Amount of Nitrogen Fixed

The amount of nitrogen fixed by legumes varies widely with host genotype, *Rhizobium* efficiency, soil and climatic conditions and, methodology used in assessing fixation (Unkovich *et al.*, 2008). Legume plants have ability to fix about 70 -300 kg N/ha/year; common bean can fix up to 40 - 70 kg N/ha/year (Table 1).

Plant	Scientific name	Nitrogen fixed(kgN/ha/yr)
Horse bean	Vicia faba	45–552
Pigeon pea	Cajanus cajan	168–280
Cowpea	Vigna unguiculata	73–354
Mung bean	Vigna mungo	63–342
Soybean	Glycine max	60–168
Chickpea	Cicer arietinum	103
Pea	Pisum sativum	55–77
Common bean	Phaseolus vulgaris	40–70
Source: FAO (1984)		

 Table 1: Estimated amounts of nitrogen fixed by various legume crops under field conditions

2.7 Methods of Determining Biological Nitrogen Fixation

One of the important reasons for the variation in symbiotic nitrogen fixation is the methods used in estimating biological nitrogen fixation. Generally, biological nitrogen fixation should lead to a measurable increase in the total nitrogen content of the system. In order to exploit the N fixing potential of different legumes under divergent agro climatic and management conditions, it is important to use suitable methodology that can distinguish between the contributions of N from fixation and other sources like soil organic matter and chemical fertilizers (Azam and Farooq, 2003). Several methods used includes.

2.7.1 Dry matter yield method

As biological N fixation is a major source of nitrogen for legumes, this biological activity is directly linked to dry matter (DM) yields in several legumes. These simple and inexpensive methods ideal in particular for field-based studies where other methods like the acetylene reduction technique are very variable. Harvested fresh

matter (shoots, roots, or pods) are dried in an oven at 70°C until it reaches a constant weight (approximately 48 h). dry matter yield of the legume plant should be positively correlated with the amount of N from fixation. This method is often used to screen large numbers of *rhizobial* strains and host plant lines. However, reliable quantitative estimates of the fixed N are difficult to be measured due to inherent differences in the cultivars for exploiting pre-existing soil nitrogen. Presence and absence of relevant *rhizobia* and the extent of effective nodulation will also have significant bearing on nitrogen fixation and consequent dry matter accumulation by different plant types (Azam and Farooq, 2003).

2.7.2 Nodule observations

Nodule number and weight have often been found to be positively correlated to the amount of N fixed. Such measurements (or visual scoring) can be useful when large numbers of *rhizobial* strains and plant germplasm are to be evaluated. However, the number and weight of nodules may not necessarily give a reliable clue to the amount of N fixed because of the changes of carbon compounds being made available at the time of sampling. Ineffective nodulation may be caused by failure of *rhizobia* to enter into the nodule, death of *rhizobia* within the nodule (Azam and Farooq, 2003).

2.7.3 Ureides determination

Ureides are nitrogenous compounds which includes allantoin and allantoic acid found in nitrogen fixing plants particularly legumes. Ureides can comprise up to 90% of the total nitrogen transported in the xylem of nitrogen-fixing tropical and sub tropical legumes like cowpea (*Vigna unguiculata*), soybean (*Glycine max*), and French bean (*Phaseolus vulgaris*) (Rifat Hayat *et al.*, 2008; Rainbird *et al.*, 1984). Pathways of assimilation of N derived from fixation and from soil are different, ammonia derived from symbiotic fixation is converted into the Ureides, allantoin and allantonic acid, in the nodule and then transported to the shoot in the chemical form in the transpiration stream. In contrast, N taken up from soil, which is primarily nitrate is transported either directly as nitrate or is assimilated into the amino acids asparagines or glutamine in the root prior to transport. Ureides are determined through xylem solute analysis (Murray *et al*, 1997; Unkovich *et al.*, 2008). Therefore, xylem sap composition changes from one dominated by ureides in fully symbiotic plants to one dominated by nitrate and amino acids in poorly nodulated plants utilizing soil N for growth (Rifata Hayat *et al.*, 2008).

2.7.4 Acetylene reduction assay

Acetylene reduction assay (ARA) is a simple indirect method to measure the activity of nitrogenase enzyme activity in nodules. When nodule nitrogenase is exposed to acetylene, the electron transfer to N_2 in the nodule is interrupted and the acetylene is converted to ethylene: Both gases can easily be quantified using gas chromatography. The equation below shows conversion of acetylene to ethylene.

 $C_2H_2+2e^{-}+2H^{+}->C_2H_4$(2)

The reaction with acetylene mimics the nitrogenase enzyme reaction with N_2 as shown below:

 $N_2 + 6e^{-} + 6H^{+} - -> 2NH_3$(3)

Scientists believed acetylene reduction would be useful to quantify BNF, the main benefits of ARA is that it is easy to use, sensitive and has a good reproducibility. There are, however, problems converting ethylene production measured in the assay into absolute amounts of N fixed by the crop over time. The most significant problem using the method to estimate BNF is the short incubation of nodules in acetylene (usually 30-90 minutes). Since nodule activity is very dependent upon current photosynthate availability and plant growth rate short term environmental factors that affect growth, such as reduced solar radiation during periodic cloud cover or temporary soil moisture deficit, immediately affects nodule activity. This fact makes it problematic to integrate short term nitrogenase activity estimates to quantify BNF over longer crop duration (Unkovich *et al.*, 2008).

2.7.5 Isotopic method

The ¹⁵N isotope dilution technique is considered to be one of the most reliable methods for estimation of nitrogen fixation by nodulated legumes in the field. The method depends upon differences in the isotopic composition between the sources of N to the plants, that is soil N, fertilizer N, and atmospheric N. A special advantage of the technique is that it assesses the integrated amount or proportion of nitrogen derived from atmosphere through N fixation in the field grown legume crops. The major limitation of this methods is that it is laborious and very expensive (Unkovich *et al.*, 2008).

2.8 Benefits Associated with Biological Nitrogen Fixation

2.8.1 Yield increase and higher protein content

When farmers purchase agricultural inputs, they expect to increase their yields. Legume inoculant is an input, and farmers expect to increase their legume yields when they inoculate with *rhizobia* strains. Yadegari and Rahmani (2010); Rifat hayat *et al.* (2008) reported increase in yield and yield components in common bean on inoculation with *rhizobium* strains Rb-133, and Rb-1360. Nitrogen fixation and shoot biomass yields of selected and check genotypes of *Phaseolus vulgaris* L. is shown in Appendix 3. Farmers are interested in yield increases, but these are not the only potential benefits from inoculation. There are other benefits from inoculation even though they are not easy to detect or measure. For example inoculation by *rhizobia* strains can increase the protein content of seed even if there is no increase in yield (Yadegari and Rahmani, 2010).

One of the main reasons we grow legumes is for the protein content in the seed. Nitrogen is a key component of this protein. For example, soybean seed may have up to 6.5 % nitrogen (40.6 % protein) mungbean up to 3.8 % nitrogen (23.8 % protein) and common bean have up 2.56 - 5.28 nitrogen (16-33 % protein) (Osborn, 1988). The protein content of cereal crops is much lower. For example, maize seed may have only 2.2 % nitrogen. Legume plants produce as many seeds as they can. When available nitrogen is low, the plant reduces the protein content of each seed in order to produce the same number of seeds with a limited amount of nitrogen (Montanez, 2000).

2.8.2 Economics of biological nitrogen fixation

Biological nitrogen fixation reduces costs of production. Field trials have shown that N captured by crops due to the use of *rhizobia* inoculants costing \$ 3.00/ha is equal to fertilizer N costing \$ 87.00/ha (Silva and Uchida, 2000) which is equal to TSH 4 500 and 130 500 respectively. The primary energy source for the manufacture of nitrogen fertilizer is natural gas together with petroleum and coal. On the contrary, the energy requirements of BNF are met by renewable sources such as plant synthesized carbohydrates rather than from non-renewable fossil fuels. (Hubbell and Kidder, 2009). This makes biological nitrogen fixation less expensive in terms of energy use.

2.8.3 Environment and Sustainability

The use of inoculants as alternative to N fertilizer reduces problems of contamination of water resources from leaching and runoff of excess fertilizer. Utilizing BNF is part of responsible natural resource management (Montanez, 2000). Increased availability of inorganic nitrogen in aquatic ecosystems leads to water acidification; eutrophication of fresh and saltwater systems; and toxicity issues for animals, including humans.

Also transfer of nitrogen to the atmosphere and aquatic ecosystem has resulted into environmental problems; oxides of nitrogen and ammonia from inorganic nitrogen are greenhouse gases causing climate change and global warming (Vitousek *et al.*, 1997). Long-term sustainability of agricultural systems must rely on the use and effective management of internal resources. The process of BNF offers an economically attractive and ecologically sound means of reducing external nitrogen input and improving the quality and quantity of internal resources. The economic, environmental, and agronomic advantages of BNF make it a cornerstone of sustainable agricultural systems (Montanez, 2000).

2.9 Factors that affect Crop Response to Inoculation

2.9.1 Viability of inoculants

Inoculation failure is sometimes due to the loss of viability of *rhizobia* in the inoculant due to exposure to heat or prolonged storage. Others include improper handling, application, or planting methods that cause the *rhizobia* to die on the seed (Silvia, and Uchida, 2000). Many countries have no mandatory quality control standards for legume inoculant. The result can be products that are highly variable in quality as determined by symbiotic effectiveness, population density and shelf-life. The production of high-quality inoculant and proper quality control can still make a significant contribution in many countries (Keyser and Li, 1992).

2.9.2 Available soil N and native *rhizobia* in the soil

Legumes can get their N from BNF and from available N sources in the soil, often they use both sources (Vance, 2001). Legumes use these mineral sources of N because they require less energy for the plant to take up N. Therefore, availability of enough N in the soil will prevent bacteria from fixing atmospheric nitrogen, (Danso and Eskew, 1984). Some soils have high populations of native *rhizobia* that are compatible with the legume crop. If the crop's requirement for BNF can be met by these native *rhizobia*, legumes may not have good response to inoculation. Large native *rhizobial* populations often occur when legume crops are grown in the same field for many crop cycles or when crops have been previously inoculated and the *rhizobia* persist. Native *rhizobial* strains may not be as effective as the tested strains in inoculants, and inoculation assures the farmer that there will be sufficient numbers of superior *rhizobia* for the crop (Silvia and Uchida, 2000, Montanez, 2000).

2.9.3 Phosphorus availability

Phosphorus (P) is second only to N as the most limiting element for plant growth. Phosphorus deficiency limits the growth and development of crop (Vance, 2001). It has a major role in fat, carbohydrates and protein/amino acids metabolism. When no P is added there is little or no yield increase with inoculation. Adding P alone increases yields very little. Biological nitrogen fixation (BNF) is often limited by such soil constraints as low phosphorus availability in both tropical and Mediterranean regions of Africa and Latin America (Broughton *et al.*, 2003). Maximum benefits from N₂ fixation depend on soil P availability; plants dependent on symbiotic N₂ fixation have ATP requirements for nodule development and function and need additional P for signal transduction and membrane biosynthesis. Phosphorus concentrations in the nodule are often significantly higher than those in shoot or root tissue this suggest that bacteroids can be P limited even when plants have received otherwise adequate P levels (Graham and Vance, 2003). Additions of P plus inoculation result in large yield increases, and the response to inoculation increases as more phosphorus is applied. Hernandez *et al.* (2007) reported P deficiency in regions where the common bean is produced, and it is perhaps the factor that limits most nitrogen fixation. Analysis of soils from experimental sites indicates that P is moderately available (Table 2) suggesting application of Phosphate fertilizer to cover P deficit. Phosphorus recommendation in Tanzania in bean field is 60 kg P/ha (Kanyeka *et al.*, 2007).

2.9.4 High soil temperature and heat stress

Rhizobia populations can be reduced in hot, dry soil particularly at planting or may not be available to shallow-planted seed. For most *rhizobia*, the optimum temperature range for growth in culture is 28 to 31 °C, and many are unable to grow at 37 °C. High soil temperatures in tropical and subtropical areas are a major problem for biological nitrogen fixation of legume crops. High root temperatures strongly affect bacterial infection and N₂ fixation in several legume species, including soybean, guar peanut, cowpea, and beans. Critical temperatures for N₂ fixation are 30 °C for clover and pea and a range of 35 and 40 °C for soybean, guar, peanut, and cowpea. Nodule functioning in common beans (*Phaseolus* spp.) is optimal between 25 and 30 °C and is hampered by root temperatures between 30 and 33 °C. Nodulation and symbiotic nitrogen fixation depend on the nodulating strain in addition to the plant cultivar. Temperature affects root hair infection, bacteroid differentiation, nodule structure, and the functioning of the legume root nodule (Zahran, **1999**).

2.9.5 Soil acidity and alkalinity

Soil acidity is a significant problem facing agricultural production in many areas of the world and limits legume productivity. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation. Soil acidity constrains symbiotic N_2 fixation in both tropical and temperate soils, limiting *Rhizobium* survival and persistence in soils and reducing nodulation. Acidity affects several steps in the development of the symbiosis, including the exchange of molecular signals between the legume and the microsymbiont. *Rhizobia* and/or their effectiveness may be reduced in soils with a pH below 5.5 or above 8.0. Liming is effective in overcoming soil acidity and aluminum toxicity (Zahran; 1999).

2.9.6 Other soil micronutrients

Micronutrient deficiencies and toxicities are widespread south of the Sahara and deficiencies/responses in various crops and forages have been reported. Several microelements such as Mo, Cu, Mn, and B, had some degree of influence on nodule dry weight and nitrogen content of *Phaseolus vulgaris cv. Canellini* inoculated with *Rhizobium tropici* CIAT899 strain under green house hydroponic culture condition. Daza *et al.*, (2003) reported inhibition of biological nitrogen fixation when nutritive solution did not contain microelements. The study indicated that boron was the microelement that most affected the bean/*Rhizobium* symbiotic process (Daza *et al.*, 2003).

2.10 Ability of Common Bean (*P. vulgaris* L.) Fixing Atmospheric Nitrogen Compared to other Legumes

Some legumes are better at fixing nitrogen than others. Common beans are poor fixers and fix less than their nitrogen needs (Bliss, 1993). However, owing to genotypic variability for traits associated with N fixation potential selection has produced breeding lines able to fix high levels of N. In field experiments some bean lines have been shown to fix enough N to support seed yields of 1 000- 2 000 kg ha⁻¹, when effective *rhizobial* populations are present either naturally or from inoculation, and there are no other major yield limiting factors (Bliss, The selection of particular *rhizobial* strains with high nitrogen fixing 1993). potential is also important (Beaver and Osorno, 2009). Other grain legumes, such as peanuts, cowpeas, soybeans and faba beans are good nitrogen fixers and will fix all of their nitrogen needs other than that absorbed from the soil. These legumes may fix up to 250 kg N/ha/year and are not usually fertilized (Ralue and Petterson, 1981). Nitrogen fertilizer is normally or preferentially applied at planting to these legumes when grown on sandy or low organic matter soils to supply nitrogen to the plant before nitrogen fixation starts (Lindemann, 2003).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Experiment 1. Determination of the G x E interaction in Nitrogen Fixation

3.1.1 Experimental sites

In order to study the interaction of Genotype and environment in biological nitrogen fixation field experiments were carried out at Sokoine University of Agriculture (SUA), located in Morogoro (latitude 6°45° S, longitude 37°40' E and altitude 547 m above sea level (masl), Agriculture Research Institute (ARI) Uyole Mbeya (latitude 08°55' S, longitude 33°32' E and altitude 1 750 -1 850 masl) and ARI-Selian Arusha (latitude 03°14' S, longitude 37°15' E and altitude 1 268 masl). Site selection was based on differences in altitudes and areas preferred for growing common beans. Treatments were inoculants namely Biostacked (Becker Underwood) and Nitrosua inoculant which were directly applied to sixteen bean genotypes namely *Rojo, Kablanketi, Bilfa 4, Lyamungu 85, Carioka, Njano, Pesa, BAT 477, DOR 364, Seliani 97, Zawadi, Mshindi, Maini, SUA 90*, and two non nodulating varieties *G 4 445-A* and *G 5 1396-A* were also included in field experiment. *BAT 477* and *DOR 364* were used as positive checks for high biological nitrogen fixation. The experiment was laid out in a split split-plot design with three replications. Locations

were allocated to the main-plots, and the *rhizobia* inoculants were as sub-plots factor and genotypes as sub-sub factor.

Treatments: The treatments included; control (no inoculants) -T1, Biostacked inoculant (BS) - T2, and Nitrosua inoculant (NS) - T3. All these treatments were applied to 16 bean genotypes. Soil samples were collected from each site before planting and after harvesting for analysis. Before planting data collected included soil N, P, K, pH, CEC and soil type and physical characteristic (Soil particle analysis), after harvesting soil samples were for N content the results were presented in Table 2. Climatic data recorded include annual rainfall and temperature. Table 3 shows the temperature and rainfall levels of the sites during the experiments.

	Experimental sites										
Parameters analyzed before planting	Mbeya	Rating	Morogoro	Ratings	Arusha	Ratings					
pH in water (1:2.5)	5.92	Medium acidic	5.99	Medium acidic	5.99	Medium acidic					
Total N(%)	0.12	Low	0.16	Low	0.15	Low					
Bray-1-P(mg/kg)	18.52	Medium	7.78	Medium	15.21	Medium					
CEC (cmol/kg)	19.80	Medium	18.4	Medium	30.20	High					
Exchangeable K(cmol/kg) Particle size analysis	1.08	Medium	1.41	High	1.38	Very high					
Clay (%)	32		46		26						
Silt (%)	31		13		25						
Sand (%)	37		41		41						
Soli textural class	Clay		Clay		Sand						
	loam				clay						
					loam						
Parameters analyzed after harvesting Total N (%)											
Non inculated plots	0.13	Low	0.15	Low	0.12	Low					
Biostacked plots	0.13	Low	0.18	Low	0.12	Low					
Nitrosua plots	0.13	Low	0.15	Low	0.15	Low					

 Table 2: Physical and chemical properties of soil (0-30cm) used in the field experimental sites

			Loca	ations				
	Μ	beya	Mor	ogoro	Aru	Arusha		
Months	Mean	Total	Mean	Total	Mean	Total		
	Temp.(°C)	Rainfall	Temp.(°C)	Rainfall	Temp.(°C)	Rainfall		
		(mm)		(mm)		(mm)		
January	23.6	73.0	26.4	70.30	24.9	26.0		
February	24.5	129.7	27.2	71.70	25.7	76.2		
March	23.4	153.1	26.5	49.70	24.5	8.3		
April	22.5	26.8	25.2	124.90	23.4	201.4		
May	22.3	47.1	25.2	124.90	23.2	49.6		
June	22.0	-	24.0	22.90	23.1	-		
AverageTemp/T otal rainfal	23.1	429.7	25.5	464.4	24.1	361.5		

Table 3: Means of monthly temperature and total rainfall in three locations for
the whole crop growth period

3.1.2 Land preparation, Inoculation and Sowing

Experimental field was initially disc ploughed and levelling was done manually by hand hoe. Sub-sub- plot consisted of 5 rows separated by 50 cm apart and each row carries 10 hills separated by 20 cm apart. The sub-sub plot area was 2.5 m x 2 m giving 5 m². The sub- plot size was 80 m² separated by 1m. TSP Fertilizer ($46P_2O_5$) was applied uniformly in each plot at the rate of 40 kg P/ha. Seed lot of each genotype were inoculated separately and planted soon after seed inoculation, starting with the treatments without inoculants. The operators washed their hands prior to planting seed with a different *rhizobium* strain to avoid cross contamination. Inoculation was done using methods explained by Silvia and Uchida (2000). Inoculation of seeds was done under the shade to avoid heat stress to *rhizobium*.

Three seeds were sown per hill; the seedlings were thinned to two plants per hill after emergence. The outer rows in each plot were taken or treated as guard rows while the inner three rows were for data collection. To ensure good plant stands seed germination test was carried out before planting.

3.2 Experiment 2. Determination the Effect of P and N on Biological Nitrogen Fixation

The experiment was carried out at SUA in screen house as factorial experiment. Forest soil was collected and mixed with sand in the ratio of 50:50 and sterilized. Soils were put into 108 plastic containers of four litres each; four seeds were planted per pot and thinned out to three plants per pot after emergence. The experiment consisted of 3 bean genotypes which were (G1) *Kablanketi* (G2) *Rojo*, and (G3) non nodulating genotype *G51105-A*. Three levels of inoculants (NS) Nitrosua, (BS) biostacked and Non inoculation. Two levels of P and N (i.e. with and without), P in form of TSP at the rate of 40 kg P/ha was applied at planting and N in form of Urea at the rate of 30 kg N/ha was also applied. Application of urea took two phases, (1) Fertilizer was applied at a rate of 15 kg N/ha 14 days after planting. (2) Final application at the rate of 36 treatment combinations which were replicated three times; each variety consisted of twelve treatment combinations (Appendix 4).

3.3 Data Collection

Five plants from field experiment and one plant from screen house experiment were sampled from each plot and pot respectively 6 weeks after planting to count nodule number in each plant. Nodule counting was carried out manually after samples were separated into shoot, root and nodules then fresh weight were recorded. Samples were oven-dried at 70°C for 48 hours and their constant dry weight was also recorded using analytical weighing balance. Other variables included Days to 50 % flowering, days to 85 % maturity, at maturity five plants from field and two plants from screen house experiment were selected randomly from each plot/pot for yield assessments i.e. number of pods per plant, number of seeds per pod, weight of seeds per plant, 100 seed weight per plant were recorded and harvest index was determined by computation using the following formula, Harvest index = (Grain yield)/(Grain + Shoot + Root yield) (Fageria *et al.*, 2011). Plant tissue analysis (shoots and seeds) were done to determine total nitrogen content, Soil and plant tissue analysis for N were done using Kjeldahl method as describe by (Okalebo and Gathua 1993). Analysis of shoot ureides was done using method described by (Young and Conway, 1942).

3.4 Data Analysis

Analysis of variance (ANOVA) and Simple linear correlation analysis for nitrogen fixation parameters i.e. shoot fresh weight, nodule number, nodule fresh weight, nodule dry weight, shoot dry weight, root dry weight, number of pods per plant, number of seeds per pod, weight of seeds per plant, weight of 100 seeds, shoot N, seed N, ureides and yield, were analysed using GenStat software 14^{th} edition. Mean separation test was done using Student-Newman-Keuls test at P < 0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Field Experiment

4.1.1 Nodule number

There was significant interaction ($P \le 0.05$) among genotypes, locations and *rhizobia* in nodule number (Table 4). Locations wise higher number of nodules was recorded in Arusha followed by Mbeya and Morogoro (Table 5). Highest nodule number was recorded in inoculated treatments compared to non inoculated treatments (Appendices 7, 8, 9 and 13). Generally, Biostacked inoculants had higher mean nodules number compared to Nitrosua. The genotypes had variable performance with regard to nodule numbers in different locations. With regard to Biostacked inoculant, genotypes *Zawadi*, *Pesa*, *Seliani* 97 when grown at Selian-Arusha produced higher number of nodules (74), (70), (60) respectively compared to others, while at Mbeya Biostacked inoculants produced higher nodule number in genotypes *SUA* 90, followed by *Maini*, *Carioka* and *Lyamungu* 85. At Morogoro site higher nodule number were recorded in *Lyamungu* 85, *Pesa*, *Carioka* and *Maini* (Table 5).

Genotypes also had different performance using Nitrosua inoculants at different locations, in overall Arusha site had the highest nodule number with Selian 97 giving the highest number of nodules followed by *Lyamungo 85*, *Rojo* and *Zawadi*. At

Mbeya *Pesa* produced the highest nodule number followed by *Mshindi* and *Kablanketi*. In Morogoro Nitrosua produced the highest nodule number in *Zawadi* followed by *Bilfa 4*, *Rojo* and *Lyamungu 85* (Table 5). In all locations, in the non-inoculated treatment there were nodules formed in the plants, except for the non nodulating genotypes.

Regardless of inoculants used, the overall mean at Mbeya shows that Pesa produced highest number of nodules followed by SUA 90 and Kablanketi. On the other hand at Morogoro Lyamungu 85 produced highest nodules number followed by Zawadi and *Rojo*, while at Arusha the overall highest nodule number was observed in genotypes Zawadi (61), Pesa and Rojo having nodule number of 52 and 50 respectively (Table 5). (Plate 2) shows the nodulating ability of Zawadi. Combined analysis shows that across locations highest nodule number were obtained in Lyamungu 85, followed by Zawadi, Seliani 97 (Table 5 and Appendix Pesa, Rojo and 14).

		Sou	urce of variation				
Variables	Location	Inoculant	Genotypes	LxI	L x G	I x G	L x I x G
Shoot dwt g/plant Root dw g/plt	904.09*** 10.995***	3.834ns 0.006ns	2.49*** 0.205***	2.95ns 0.078ns	3.097*** 0.088***	1.01ns 0.017ns	0.934ns 0.024ns
Nod No/plt)	17397.3***	749.7***	3118.6***	62.09ns	581.1***	175.1***	249.8***
Nodw(g/plt) Days to 50% flw Days to 85% mat No of pods /plant	0.126*** 3840.55*** 2230.86** 3336.6***	0.009*** 16.41ns 6.13ns 55.03ns	0.0121*** 349.21*** 473.84*** 257.19***	0.0018* 5.64ns 7.06ns 24.10ns	0.0022*** 22.10*** 14.496*** 49.77***	0.001*** 5.33ns 6.84ns 7.699ns	0.001*** 4.214ns 4.24ns 7.34ns
No of seeds/pod	14.99**	1.57*	7.38***	0.611ns	1.64***	0.515ns	0.496ns
Wt seed/plant(g)	3143.1**	10.63ns	55.12***	3.65ns	24.36***	6.23ns	5.94ns
100seed wt(g) H.index Yield(t/ha)	2038.25*** 0.79** 110.99**	6.93ns 0.001ns 0.40ns	2193.68*** 0.026*** 2.06***	0.56ns 0.013ns 0.17ns	49.46*** 0.011*** 0.89***	6.80ns 0.004ns 0.23ns	5.93ns 0.003ns 0.23ns
Shoot ureides	106.83ns	72.97***	7.142***	3.371ns	4.687***	3.05***	2.893***
Seed %N	18.91***	0.58**	0.38***	0.22ns	0.153***	0.061***	0.091***
Shoot.%N	3.44*	0.099ns	0.536***	0.097ns	0.259***	0.072ns	0.047ns

 Table 4:
 Error mean square for combined analysis of variance for studied variables in field experiment

Key: L- Location, I- Inoculant, G- Genotype, ns- not significant at 0.05, *, **, and *** indicate significance at 0.05, 0.01 and 0.001 respectively



Plate 2: Nodulating common bean genotype (Zawadi)

Ns Mean	C.mea
49 61.0	3
0.0	
40 41.5	2
21 31.1	2
44 28.4	1
30.5	2
0.0	
57 49.6	3
29 27.4	2
28 26.8	2
39 37.4	2
29 34.3	2
52 50.3	3
45 52.9	3
57 42.9	3
39 44.5	2
Sa	
34.92c	24.0
9.756	6.9
5.85	4.2
16.8	17.
	Combined

 Table 5: Mean number of nodules of inoculated common bean genotypes at three locations

4.1.2 Mean nodule dry weight

Results for nodule dry weight were presented in Table 6. Inoculation had great influence on nodule dry weight. There was significant interaction ($P \le 0.05$) among genotypes, *rhizobia* and location in nodule dry weight (Table 4). Highest nodule dry weight was recorded when genotypes were grown in Arusha followed by Mbeya and Morogoro. Highest mean nodule dry weight was recorded in inoculated treatments compared to non inoculated control in all locations (Appendices 7, 8 9 and 13). In overall Biostacked inoculant had higher nodule dry weight compared to Nitrosua.

With regard to Biostacked inoculants highest nodule dry weight was observed in genotypes *Pesa* (0.14 g) followed by *Zawadi* (0.14 g), *Seliani* 97 (0.13 g), *Lyamungu* 85 (0.12 g) and *Rojo* (0.11 g) when genotypes were grown at Arusha. In Mbeya genotypes with highest nodule dry weight were *Carioka* (1.00 g), *Njano* (0.09 g) and *Pesa* (0.09 g). On the other hand genotypes *Lyamungu* 85 (0.07 g), *Maini* (0.06 g) and *Carioka* (0.06 g) had higher nodule dry weight when grown in Morogoro (Table 6).

Using Nitrosua inoculants genotypes performed differently in different locations, highest nodule dry weight was obtained in genotype *Rojo*, *Seliani 97* and *Njano* when grown in Arusha, while at Mbeya genotypes *Maini*, *Pesa* and *Kablanketi* had the highest nodule dry weight. In Morogoro genotypes *Rojo*, *Bilfa 4* and *Zawadi* performed better than others (Table 6). The overall means among locations indicate genotypes *Rojo*, *Lyamungu 85*, and *Pesa* had highest nodule dry weight closely followed by *Seliani 97* and *Zawadi* when grown in Arusha. On the other hand

genotypes Main, *Kablanketi, Pesa* and *Bilfa 4* had better performance compared to others when grown in Mbeya, while in Morogoro *Carioka*, followed by *Lyamungu 85, Maini* and *Bilfa 4* outcompeted other genotypes regardless of inoculant used (Table 6, Appendices 10, 11and 12). Across locations combined means show that highest nodule dry weight was recorded in *Carioka*, followed by *Lyamungu 85*, and *Pesa* (Table 6 and Appendix 14).

Table 6: Mean nodules dry weight (g) of inoculated common bean genotypes at three locations.	

						Locations	5						
	Ν	Ibeya (g)				Morogoi				Arush			
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	0.05	0.09	0.07	0.07	0.02	0.02	0.03	0.02	0.05	0.14	0.09	0.09	0.12
G4445A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Carioka	0.07	0.10	0.08	0.08	0.03	0.06	0.03	0.04	0.08	0.11	0.08	0.09	0.15
Maini	0.07	0.09	0.11	0.09	0.03	0.06	0.01	0.03	0.07	0.09	0.06	0.07	0.13
BAT 477	0.07	0.09	0.08	0.08	0.01	0.02	0.01	0.01	0.08	0.07	0.08	0.08	0.12
Mshindi	0.06	0.08	0.10	0.08	0.02	0.02	0.01	0.02	0.06	0.08	0.04	0.06	0.11
G51396A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Seliani 97	0.09	0.07	0.05	0.07	0.03	0.01	0.01	0.02	0.04	0.13	0.11	0.09	0.12
Bilfa 4	0.10	0.09	0.06	0.08	0.02	0.01	0.04	0.03	0.08	0.08	0.05	0.07	0.13
Njano	0.05	0.09	0.08	0.08	0.01	0.01	0.01	0.01	0.07	0.10	0.08	0.08	0.12
DOR 364	0.04	0.06	0.06	0.06	0.03	0.03	0.00	0.02	0.09	0.10	0.07	0.09	0.11
Sua90	0.07	0.06	0.07	0.07	0.00	0.01	0.00	0.00	0.07	0.05	0.06	0.06	0.09
Rojo	0.05	0.08	0.07	0.07	0.01	0.01	0.05	0.02	0.08	0.11	0.11	0.10	0.13
Pesa	0.06	0.09	0.11	0.09	0.02	0.03	0.01	0.02	0.07	0.14	0.07	0.10	0.14
Lymng 85	0.08	0.07	0.06	0.07	0.01	0.07	0.03	0.04	0.08	0.12	0.10	0.10	0.14
Kablanketi	0.08	0.09	0.10	0.09	0.01	0.01	0.01	0.01	0.07	0.09	0.07	0.08	0.12
Mean	0.059a	0.072b	0.069b		0.015b	0.023b	0.014a		0.059a	0.088b	0.0671a		
Grand mean				0.0668b				0.018a				0.0714b	0.11
LSD				0.0338				0.0148				0.0356	0.065
S.E				0.0202				0.0087				0.022	0.038
CV (%)				30.2				48.6				30.9	35
Key:	Bs-	Biostaked	(Be	eckerund	erwood),	Ns	-	Nitrosua,	(C.mean-	Con	nbined	mean

4.1.3 Shoot dry weight

There was a significant difference ($P \le 0.05$) among genotypes and among locations in shoot dry weight (Table 4). Inoculation did not have significant effect on shoot dry weight; however Location x genotypes interaction was significant. In general Biostacked inoculant had higher mean shoot dry weight compared to Nitrosua in Morogoro and Arusha except in Mbeya where Nitrosua had better performance (Table 7, Appendices 7, 8 9 and 13). Location wise, the highest shoot dry weight was recorded in Morogoro site followed by Mbeya while Arusha was the least. On averages Genotypes G 51 396-A and G 4 445-A produced the highest shoot dry weight (8.0 and 7.3 g respectively) followed by Selian 97 (6.8 g) and Carioka (6.4 g) when grown in Morogoro. Lowest shoot dry weight was observed in Maini (4.6 g) and Njano (4.7 g). The remaining genotypes had shoot dry weights ranging between 5.1 and 6.3 g (Table 7, and Appendix 11). In Mbeya genotypes with higher shoot dry weight included Seliani 97 (2.7 g), Rojo (2.6 g) and Lyamungu 85 (2.5 g). The least dry weight was obtained in non nodulating G 4 445-A (1.8 g). The remaining genotypes had shoot dry weights ranging between 2.0 and 2.4 g (Table 7, Appendix 10). When genotypes were grown in Arusha Lyamungu 85 and Carioka had the highest shoot dry weights each (1.5g) followed by Kablanketi (1.4g), and DOR 364 (1.4 g). Smaller dry weight was obtained in non nodulating G 4445-A (0.7 g) and Njano (0.85 g). The remaining genotypes had shoot dry weights between 0.9 and 1.4 g (Table 7, and Appendix 12). Across locations genotypes G 5 1396-A produced highest shoot dry weight followed by Seliani 97, Carioka and Rojo (Table7 and Appendix 14).

						Loca	tions							
		Mbey	/a (g)				Morogoro	(g)			Arusha	(g)		
Genotypes	С	ontrol	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi		2.4	2.2	2.3	2.3	6.0	6.9	5.3	6.1	0.9	1.3	1.0	1.1	3.13
G4445A		1.6	1.9	2.0	1.8	7.5	7.6	6.7	7.3	0.6	0.7	0.7	0.7	3.24
Carioka		2.3	2.3	2.1	2.2	5.0	8.1	6.2	6.4	1.4	1.6	1.3	1.5	3.37
Maini		2.5	2.2	2.3	2.3	4.3	4.6	4.9	4.6	1.2	1.5	1.2	1.3	2.75
BAT 477		2.0	2.3	2.5	2.3	4.9	5.7	7.1	5.9	1.0	1.4	0.6	1.0	3.05
Mshindi		2.2	2.5	2.4	2.4	4.1	6.2	5.0	5.1	1.0	1.3	1.1	1.1	2.86
G51396A		1.9	2.1	2.4	2.1	7.6	7.2	9.3	8.0	0.8	1.0	1.0	1.0	3.71
Seliani 97		2.9	2.3	3.0	2.7	6.4	5.5	8.7	6.8	0.9	1.2	1.2	1.1	3.56
Bilfa 4		1.9	2.2	2.0	2.0	5.1	5.6	5.5	5.4	1.4	1.6	1.0	1.4	2.93
Njano		2.0	2.0	2.2	2.1	5.1	4.9	4.0	4.7	0.7	0.8	0.8	0.8	2.50
DOR 364		2.6	2.1	2.3	2.4	4.4	6.9	4.2	5.2	1.3	1.5	1.4	1.4	2.98
Sua90		2.5	1.8	2.2	2.1	5.7	7.3	4.8	5.9	0.9	0.9	0.9	0.9	2.98
Rojo		2.6	2.3	2.8	2.6	5.8	7.5	4.9	6.1	1.3	1.3	1.3	1.3	3.31
Pesa		2.2	2.6	2.6	2.5	5.1	5.8	5.5	5.4	0.6	0.8	1.1	0.9	2.92
Lymng 85		2.6	2.8	2.1	2.5	5.5	6.0	5.5	5.7	1.4	1.8	1.4	1.5	3.24
Kablanketi		2.00	1.8	2.2	2.0	6.5	6.8	5.7	6.3	1.6	1.5	1.1	1.4	3.24
Mean		2.26a	2.20a	2.33a		5.56a	6.40b	5.82ab		1.08 a	1.26b	1.07 a		
Grand mean					2.26b				5.93c				1.14a	3.11
LSD					0.879				2.56				0.56	0.81
S.E					0.52				2.56				0.32	0.87
CV (%)					22.9				23.1				28.3	27.8
Key:	Bs-		Biostaked		(Beckeru	inderwood),	N	ls-	Nitrosua	, (C.mean-	Co	mbined	m

 Table 7: Mean shoots dry weight (g) of inoculated common bean genotypes at three locations

4.1.4 Root dry weight

The significant difference ($P \le 0.05$) among genotypes and among locations was observed on root dry weights. Interaction among locations x genotypes was also significant at $P \le 0.05$ (Table 4). Generally, highest root dry weight was recorded in Morogoro followed by Mbeya while Arusha was the lowest (Table 8). With regard to *rhizobia* inoculants, Biostacked had higher mean compared to Nitrosua in Morogoro site, however the means were more or less similar in Arusha, although Biostacked had slightly higher mean while in Mbeya Nitrosua performed the best (Table 8 and Appendices 7, 8, 9 and 13). With regard to Biostacked inoculant when grown at Arusha genotypes *Carioka*, and *Lyamungu 85* produced higher dry weights (0.41 g) and (0.30 g) respectively, followed by *Maini* (0.24 g) and *Bilfa 4* (0.23 g). With Nitrosua inoculant, the highest mean root dry weight was recorded in *Carioka* (0.28 g), followed by *Rojo* (0.27 g), *Lyamungu 85* (0.26 g) and *DOR 364* (0.26 g) (Table 8).

Genotypes showed significant difference ($P \le 0.05$) among locations on root dry weight where by genotypes *G* 4445-*A*, followed by *Carioka*, and G 51396-A, *Lyamungu* 85 and *Rojo* had higher root dry weights when grown at Morogoro (Table 8 and Appendix 11). At Mbeya genotypes *Carioka* followed by *Lyamungu* 85, *Rojo*, *BAT* 477 and *DOR* 364 had higher root dry weights (Table 8 and Appendix 10), while at Arusha genotypes with higher dry weights included *Carioka* followed by *Lyamungu* 85, *Rojo*, *DOR* 364, *Maini* and *Bilfa* 4 (Table 8 and Appendix 12). Across locations highest root dry weights were recorded in *Carioka*, *G* 4445-A, *Lyamungu* 85, G 51396-A, *Rojo*, *DOR* 364 and BAT 477 (Table 8 and Appendix 14).

						Locations							
	Mbe	eya (g)				Morogoro	o (g)			Arusha	(g)		
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	0.48	0.45	0.49	0.48	0.67	0.88	0.65	0.73	0.15	0.20	0.20	0.18	0.46
G4445A	0.53	0.48	0.66	0.56	1.08	1.28	0.93	1.09	0.14	0.17	0.20	0.17	0.61
Carioka	0.71	0.68	0.66	0.68	0.99	1.05	0.78	1.03	0.18	0.41	0.28	0.29	0.67
Maini	0.63	0.52	0.61	0.58	0.54	0.58	0.53	0.55	0.19	0.24	0.23	0.22	0.45
BAT 477	0.59	0.58	0.67	0.61	0.83	0.62	0.71	0.73	0.18	0.19	0.17	0.18	0.50
Mshindi	0.44	0.51	0.52	0.49	0.48	0.57	1.04	0.61	0.12	0.15	0.17	0.14	0.41
G51396A	0.53	0.46	0.56	0.52	1.03	1.12	0.88	1.01	0.18	0.18	0.22	0.19	0.57
Seliani 97	0.53	0.63	0.51	0.55	0.66	0.48	0.73	0.63	0.13	0.21	0.19	0.18	0.45
Bilfa 4	0.53	0.36	0.56	0.48	0.86	0.69	0.73	0.76	0.23	0.23	0.16	0.20	0.48
Njano	0.41	0.42	0.59	0.47	0.58	0.67	0.43	0.56	0.14	0.18	0.18	0.16	0.40
DOR 364	0.66	0.60	0.57	0.61	0.68	0.82	0.62	0.70	0.23	0.20	0.26	0.23	0.51
Sua90	0.55	0.40	0.60	0.52	0.63	0.96	0.68	0.75	0.13	0.12	0.21	0.15	0.47
Rojo	0.67	0.55	0.64	0.62	0.87	0.91	0.58	0.79	0.23	0.22	0.27	0.24	0.55
Pesa	0.53	0.32	0.53	0.46	0.62	0.67	0.81	0.70	0.17	0.18	0.19	0.18	0.45
Lymng 85	0.71	0.67	0.49	0.62	0.70	1.06	0.90	0.89	0.25	0.30	0.26	0.27	0.60
Kablanketi	0.43	0.37	0.38	0.39	0.46	0.31	0.44	0.40	0.14	0.20	0.18	0.17	0.32
Mean	0.56b	0.50a	0.57b		0.728a	0.79a	0.715a		0.17a	0.21b	0.21b		
Grand mean				0.54b				0.74c				0.20a	0.49
LSD				0.24				0.39				0.08	0.14
S.E				0.14				0.20				0.05	0.15
CV (%)				25.1				27.5				24	29.3
Key:	Bs-	Biosta	ked	(Becke	erunderwoo	d),	Ns-	Nitro	osua,	C.mean-	(Combined	1

 Table 8: Mean root dry weight (g) of inoculated common bean genotypes at three locations

4.1.5 Days to 50 % flowering

Days to 50 % flowering showed significant difference between genotypes and locations at $P \le 0.05$ (Table 4). Table 9 presents mean results of genotypes for days to 50 % flowering. Inoculation did not have any significant effects on days to 50 % flowering (Appendix 13). Location wise plants in Morogoro were the earliest to reach days to 50 % flowering followed by Arusha and Mbeya (Appendix 15). In Morogoro genotypes which had earlier 50% flowering were Zawadi (31), Pesa (31), Kablanketi (31), Mshindi (31) and Rojo (31). Late genotypes to reach 50 % flowering were DOR 364 (37), G 51396-A (37), Carioka (37), G 4445-A (37) and BAT 477 (36). The remaining genotypes reached 50% Flowering between 32 and 35 DAP (Table 9 and Appendix 11). When genotypes were grown in Mbeya Genotypes Zawadi, Kablanketi and Mshindi reached 50% flowering earlier at 37, 38, and 38 days after planting (DAP) respectively. The highest number of days to 50 % flowering was recorded in genotype Carioka (50) followed by G 4445-A (50), DOR 364 (49) and BAT 477 (49) (Table 9 and Appendix 10). The remaining genotypes reached 50 % flowering between 39 and 48 DAP. In Arusha, early 50 % flowering was observed in Kablanketi (36), Zawadi (36), Rojo (37) and Pesa (37). Genotypes with higher number of days to reach 50 % flowering were DOR 364 (47), BAT 477 (46), G 4445-A (46), Carioka (46), and Maini (45). Remaining genotypes had days to 50 % flowering varying between 38 and 41 (Table 9 and Appendix 12). Across all locations combined analysis showed that genotypes Zawadi, Kablanketi, Mshindi, Pesa, Rojo and Njano reached 50% flowering earlier, while DOR 364, G 4445-A, Carioka, BAT 477 and Maini took longer time to reach 50 % flowering (Table 9 and Appendix 14).

	Ι	Location		
Genotypes	Mbeya	Morogoro	Arusha	C. mean
Zawadi	37	31	36	35
G4445 A	50	37	46	44
Carioka	50	37	46	44
Maini	48	35	45	42
BAT 477	49	36	46	43
Mshindi	38	31	38	36
G51396 A	45	37	41	41
Seliani 97	45	33	41	40
Bilfa 4	48	34	42	41
Njano	41	32	37	37
DOR 364	49	37	47	44
SUA 90	43	33	40	39
Rojo	40	31	37	36
Pesa	39	31	37	36
Lyamungu 85	44	35	38	39
Kablanketi	38	31	36	35
Grand mean	44.03c	33.92a	40.80b	39.58
L.S.D	2.521	0.697	2.235	2.16
S.E	2.692	0.744	2.387	2.12
CV (%)	6.1	2.2	5.9	5.4

Table 9: Mean days to 50% flowering at three locations

Key: C.mean- Combined mean

4.1.6 Days to 85 % maturity

Inoculations did not have significant effects on days to 85 % Maturity, but the difference was significant among genotypes and among locations at $P \le 0.05$ (Table 4). Location x genotype interaction was also significant. Generally, Morogoro had the shortest days to maturity followed by Arusha and Mbeya (Table 10 and Appendix 13). When genotypes were grown in Morogoro *Mshindi* (67), *Zawadi* (67), *Kablanketi* (68), *Rojo* (68), and *Pesa* (68) were the earliest compared to *Carioka* (81), followed by *DOR 364* (80) and *BAT 477*(80) that took longest time to reach 85 %

maturity (81, 80, and 80 respectively). The remaining genotypes reached 85 % maturity between 72 and 78 DAP (Table 10 and Appendix 11). Genotypes *Zawadi* and *Kablanketi* had shortest days (75) while *DOR 364* (86) followed by *BAT 477* (86), *G 4445-A* (86) and *Carioka* (86) took longest time to reach 85 % maturity when grown in Mbeya (Table 10). In Arusha earliest 85 % maturity was observed in *Kablanketi* (73), *Zawadi* (73) and *Mshindi* (75), while genotypes *G 4445-A* (85), *Maini* (84), *BAT 477* (84) and *DOR 364* (84) had most days to reach (85 %) maturity. The remaining genotypes reached 85 % maturity between 77 and 84 DAP (Table10 and Appendix 12). Combined analysis also revealed that genotypes significantly differed ($P \le 0.05$). Across locations genotypes *Zawadi*, *Kablanketi*, *Mshindi*, *Pesa* and *Rojo* were the earliest while *Carioka*, *DOR 364*, *BAT 477* and *G 4445-A* was the latest genotypes (Table10 and Appendix 14).

	L	ocations		
Genotypes	Mbeya	Morogoro	Arusha	C.mean
Zawadi	75	67	73	72
G4445 A	86	78	84	83
Carioka	86	81	84	83
Maini	85	78	84	82
BAT 477	86	80	84	83
Mshindi	78	67	75	73
G51396 A	83	77	81	80
Seliani 97	84	77	83	81
Bilfa 4	84	76	82	80
Njano	82	76	80	79
DOR 364	86	80	84	83
SUA 90	83	72	79	78
Rojo	78	68	77	75
Pesa	78	68	77	74
Lyamungu 85	82	78	78	79
Kablanketi	75	68	73	72
Grand mean	81.90b	74.33a	79.97b	78.73
L.S.D	1.767	1.05	2.77	2.95
S.E	1.886	2.23	2.96	2.40
CV (%)	2.3	3.0	2.77	3.0

Table 10: Mean days to 85 % maturity at the three locations

Key: C.mean- Combined mean

4.1.7 Number of pods per plant

The difference among genotypes and among locations was significant, while the interaction between location x genotype was also significant ($P \le 0.05$) on number of pods per plants (Table 4). However, in overall performance of Biostacked inoculant was slightly higher than Nitrosua at two Locations i.e. Morogoro and Arusha (Table 11 and Appendices 7, 8 and 9). Genotypes alone differed significantly in number of pods per plant within Locations (Table 11 and Appendices 10, 11 and 12). Plants inoculated with Nitrosua generally had higher mean of number of pods per plant compared to those inoculated Biostacked and non inoculated control plants in Mbeya site (Table 11 and Appendix 7). With Nitrosua inoculation, genotypes with highest number of pods per plant were *DOR 364*, followed by *G 4445-A*, *BAT 477* and *Carioka*. Biostacked inoculant performed better in number of pods per plant with *DOR 364*, G 51396-A, *BAT 477* and *G 4445-A* (Table 11).

In overall, location, wise highest number of pods per plant was obtained in Morogoro followed by Mbeya and lastly Arusha. In Morogoro *DOR 364* and *G 4445-A* produced highest number of pods followed by G 51396-A, *BAT 477*, *Bilfa 4*, *SUA 90*, *Carioka* and *Maini*, while in Mbeya *DOR 364* was also the highest followed by *Maini*, *Carioka* and *G 4445-A*. In Arusha *BAT 477*, followed by *Bilfa 4* and *DOR 364* were the leading. Across locations genotype *DOR 364* had the highest number of pods per plant followed by *G 4445-A*, *BAT 477*, *G 51396-A* and *Carioka* (Table 11 and Appendices 10, 11 and 12).

				I	ocations								
	Mbey	/a			Moro	goro		Arusha					
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	8	8	8	8	14	12	12	13	5	7	5	6	9
G4445A	16	18	23	19	24	25	23	24	6	9	6	7	17
Carioka	19	17	20	19	16	21	17	18	8	11	8	9	15
Maini	12	13	13	19	16	17	20	18	7	9	7	8	13
BAT 477	19	19	20	10	19	21	21	20	9	10	10	10	16
Mshindi	10	10	11	17	13	13	17	14	6	7	9	7	11
G51396A	13	22	17	17	21	22	25	23	7	8	9	8	16
Seliani 97	10	10	14	11	14	15	14	14	6	5	6	6	10
Bilfa 4	15	14	16	15	18	20	17	18	8	9	9	9	14
Njano	14	14	16	15	16	19	15	17	7	7	7	7	13
DOR 364	21	23	29	24	24	25	23	24	9	9	9	9	19
Sua90	11	7	14	11	17	17	20	18	7	7	7	7	12
Rojo	11	9	11	11	13	13	11	12	7	7	7	7	10
Pesa	9	10	11	10	11	11	14	12	6	5	6	6	9
Lymng 85	11	13	11	12	16	17	15	16	8	9	8	8	12
Kablanketi	9	8	10	9	14	13	10	12	8	8	7	8	10
Mean	12.91a	13.35a	15.33b		16.49a	17.56a	17.14a		7.11 a	7.99b	7.7ab		
Grand mean				13.86b				17.06c				7.6a	12.84
LSD				3.89				6.33				2.48	2.52
S.E				2.35				3.68				1.49	2.67
CV (%)				16.9				21.6				19.7	20.8

 Table 11: Mean number of pods per plant of inoculated common bean genotypes at three locations

Key: Bs- Biostaked (Beckerunderwood), Ns- Nitrosua, C.mean- Combined mean

4.1.8 Number of seeds per pod

Number of seeds per pod among genotypes was not significantly affected by *rhizobia* inoculation (Appendix 13). Significant differences were observed among genotypes and among locations, the interaction between location and genotype was also significant at $P \le 0.05$ (Table 4). For locations, Morogoro performed better than Mbeya and Arusha (Appendix 15. At Morogoro, the highest number of seeds per pod were recorded in non nodulating genotype *G* 4445-*A* (6), and *G* 51396-*A* (6), closely followed by *DOR* 364 (5), *Carioka* (5) and *Bilfa* 4 (5), compared to *Lyamungu* 85 (4) and *Maini* (4) which had the lowest seeds per pod. The remaining genotypes had number of seeds per pod of between 4 and 5. (Table 12 and Appendices 11).

When grown in Mbeya genotypes *DOR 364* (6) had highest number of seeds per pod, followed by *BAT 477* (6), *G 51396-A* (6) and *Bilfa 4* (5) compared to *Lyamungu 85* (3) which had lowest number of seeds per pod. The remaining genotypes had number of seeds per pod of between 4 and 5 (Table 12 and Appendices 10). In Arusha genotype *BAT 477* (6), followed by *Carioka* (5) and *DOR 364* (5) had highest number of seeds per pod compared to *Njano* (3) and *Lyamungu 85* (4). The remaining genotypes had number of seeds per pod of between 4 and 5 (Table 12 and Appendices 12). Across locations highest number of seeds was recorded in genotypes *DOR 364*, *BAT 477*, *G 4445-A*, *Bilfa 4* and *Carioka* compared to *Lyamungu 85* and *Njano*.

		Locations	5	
	Mbeya	Morogoro	Arusha	
Genotypes	No seed/pod	No seed/pod	No seed/pod	C.mean
Zawadi	5	5	5	5
G4445 A	5	6	4	5
Carioka	5	5	5	5
Maini	4	4	5	4
BAT 477	6	5	6	5
Mshindi	4	5	4.	5
G51396 A	6	6	4	5
Seliani 97	4	5	4	4
Bilfa 4	5	5	5	5.
Njano	4	5	3	4
DOR 364	6	5	5	5
SUA 90	5	5	5	5
Rojo	4	5	4	5
Pesa	5	5	4	4
Lyamungu 85	3	4	4	4
Kablanketi	4	5	4	4
Grand mean	4.7b	5.0c	4.4a	4.7
L.S.D	0.39	0.75	0.70	0.63
S.E	0.41	0.80	0.75	0.68
CV (%)	8.8	16.1	17.1	14.4

Table 12: Mean number of se	eds per pod at three locations
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Key: C.mean- Combined mean

4.1.9 Weight of seeds per plant

The data for seeds weight per plant are presented in Table 13. The interaction between location and genotype was significant at $P \le 0.05$ (Table 4). Inoculations did not have significant effects on weight of seeds per plant across location (Appendix 130. There was also significant difference within locations and among genotypes on seeds weight per plant. In general, seeds weight per plant was found higher when genotypes were grown in Morogoro followed by Mbeya and Arusha (Table 13 and Appendix 15). In Morogoro Selian 97 (17 g) and *Bilfa 4* (17 g) performed the highest, followed by *DOR 364* (15 g) and *Carioka* (15 g). The lowest seed weight was observed in genotype *Rojo* (11 g) and *G 51396-A* (11 g). The remaining genotypes had weight of seed per plant of between 12 and 14 g (Table 13 and

Appendix 11). Genotypes *Bilfa* 4 (17 g), *DOR* 364 (16 g), *BAT* 477 (16 g) and *Njano* (16 g) had highest seed weight per plant contrary to non nodulating genotypes *G* 51396-A (9 g) and *G* 4445-A (9 g) with least seed weight in Mbeya. The remaining genotypes had weight of seeds per plant of between 9 and 15 g (Table 13 and appendix 10). At Arusha higher seed weight per plant were recorded in *Bilfa* 4 (7 g), *Rojo* (6 g), *Zawadi* (6 g), *SUA* 90 (6 g) and *Kablanketi* (6 g). Lower seed weight per plant was obtained in *G* 4445-A (3 g) and *Carioka* (4 g). The remaining genotypes had weight of seeds per plant of between 4 and 6 g (Table 13 and Appendix 12).

Results from the combined analysis of variance showed that genotypes significantly differ ($P \le 0.05$) across locations on seeds weight per plant, highest seeds weight per plant was recorded in *Bilfa 4* and *Seliani 97* followed by *DOR 364* and *BAT 477* while *G 4445-A*, *G 51396-A and Maini* which had the lowest weight of seeds per plant (Table 13 and Appendix 14).

		Locations		
	Mbeya Swt (g/P)	Morogoro Swt (g/P)	Arusha Swt (g/P)	C.mean
Zawadi	12	13	6	10
G4445 A	9	13	3	8
Carioka	10	15	4	10
Maini	9	13	4	9
BAT 477	16	14	5	12
Mshindi	12	14	5	11
G51396 A	9	11	5	8
Seliani 97	15	17	5	13
Bilfa 4	17	17	7	13
Njano	16	12	6	11
DOR 364	16	15	5	12
SUA 90	11	16	6	11
Rojo	12	11	6	10
Pesa	11	13	5	10
Lyamungu 85	15	13	6	11
Kablanketi	13	14	6	11
Grand mean	12.71b	13.84b	5.16a	10.57
L.S.D	2.87	2.71	0.71	3.73
S.E	3.06	2.89	0.76	2.47
CV (%)	24.1	21.1	14.7	23

Key: C.mean- Combined mean

4.1.10 Weight of 100 seeds (g)

Weight of 100 seeds was not significantly affected by *rhizobial* inoculation. The interaction between location and genotype was significant at $P \le 0.05$ (Table 4). Table 14 presents results for weight of 100 seeds. Location wise, genotypes planted in Morogoro gave heavier seeds followed by Mbeya and Arusha (Appendix 15). In Morogoro the highest weight of 100 seeds was recorded in genotype Lyamungu (56 g), followed by *Seliani 97* (48 g) and *Kablanketi* (47 g), genotypes *G 51396-A* (20 g), *G 4445-A* (20 g) and *BAT 477* (26 g) produced the lightest seeds. The remaining genotypes had 100 seed weights of between 26 and 45 g (Table 14 Appendix 11). When grown in Mbeya genotypes *Kablanketi* (43 g) had highest 100 seeds weight, followed by *Lyamungu 85* (41 g) and *Seliani 97* (41 g) compared to *G 51396-A* (16

g), *G* 4445-A (17g) and *BAT* 477 (18 g) which had lowest 100 seed weights. The remaining genotypes had 100 seed weight of between 19 and 39 g (Table 14 and Appendix 10). In Arusha genotype *Lyamungu* 85 (40 g), followed by *Kablanketi* (39 g) and *Seliani* 97 (38 g) had higher 100 seed weight compared to *G* 4445-A (18 g) and *G* 51396-A (20 g). The remaining genotypes had 100 seed weight of between 21 and 36 g (Table 14 and Appendix 12).

Combined means revealed that largest 100 seed weights were recorded in genotypes *Lyamungu 85* followed by *Seliani 97* and *Kablanketi* compared to *G 4445-A*, G 51396-A, *DOR 364* and *BAT 477* which had lowest 100 seed weight (Table 14 and Appendix 14).

		Locations		
	Mbeya	Morogoro	Arusha	
Genotypes	100 swt (g)	100 swt (g)	100 swt (g)	C.mean
Zawadi	32	35	31	33
G4445 A	17	20	18	18
Carioka	19	29	23	24
Maini	28	37	30	32
BAT 477	18	26	21	2
Mshindi	30	33	28	3
G51396 A	16	20	20	1
Seliani 97	41	48	38	4
Bilfa 4	26	33	27	2
Njano	39	45	33	3
DOR 364	19	26	22	2
SUA 90	27	31	26	2
Rojo	34	41	34	3
Pesa	38	44	36	4
Lyamungu 85	41	56	40	4
Kablanketi	43	47	39	4
Grand mean	29.31a	35.78b	29.22a	31.4
L.S.D	1.649	1.953	2.880	2.3
S.E	1.761	2.085	3.076	2.37
$\frac{\text{CV}(\%)}{\text{Kay: C mass } C}$	6.0	5.8	10.5	7.0

Table 14: Mean we	eight of 100 seeds at t	three locations
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Key: C.mean- Combined mean

4.1.12 Total yield (t/ha)

Inoculations did not have significant effects on total yield among locations. The interaction between location and genotype was significant at $P \le 0.05$ (Table 4).

Common bean yield was found to be higher when genotypes were grown in Morogoro followed by Mbeya and lastly Arusha (Table 15 and Appendix 15). In Morogoro Selian 97 performed the highest (3.3 t/ha) followed by Bilfa 4 (3.1 t/ha), DOR 364 (2.8 t/ha) and SUA 90 (2.7 t/ha). The lowest seed yield was observed in genotype Rojo (2.0 t/ha) followed by G 51396-A (2.2 t/ha) (Table 15 and Appendix 11). The remaining genotypes had yields of between 2.3 and 2.7 t/ha. Nodulating genotypes Bilfa 4 (3.2 t/ha), DOR 364 (3.1 t/ha), BAT 477 (3.0 t/ha) and Njano (3.0 t/ha) had highest seed yield compared to non nodulating genotypes G 51396-A (1.6 t/ha) and G 4445-A (1.7 t/ha), Maini (1.7 t/ha) and Carioka (1.8 t/ha) when genotypes were grown in Mbeya. The remaining genotypes had seed yields of between 2.0 and 2.9 t/ha (Table 15 and Appendix 10). In Arusha Bilfa 4 (1.3 t/ha) performed highest followed by Rojo (1.1 t/ha), Zawadi (1.1 t/ha), SUA 90 (1.1 t/ha), Kablanketi (1.1 t/ha), and Lyamungu 85 (1.1 t/ha). The lowest seed yield was observed in genotype G 4445-A (0.7 t/ha) followed by Carioka and Main which had similar yields (i.e. 0.8 t/ha). The remaining genotypes had yields of between 0.9 and 1.1 t/ha (Table 15 and Appendix 12)

Means from combined analysis of variance indicate that genotypes varied significantly ($P \le 0.05$) across locations. Highest yield was recorded from *Bilfa 4*, followed by *Seliani 97*, *DOR 364* and *BAT 477* whiles the non nodulating *G 4445-A*, *G 51396-A and Rojo* that had lowest seed yield (Table 15 and Appendix 14).

	Mbeya	Locations Morogoro	Arusha	
Genotypes	Seed yield (t/ha)	Seed yield (t/ha)	Seed yield (t/ha)	C.mean
Zawadi	2.3	2.5	1.1	2.0
G4445 A	1.7	2.4	0.7	1.6
Carioka	1.8	2.6	0.8	1.8
Maini	1.7	2.4	0.8	1.7
BAT 477	3.0	2.6	1.0	2.2
Mshindi	2.4	2.6	1.0	2.0
G51396 A	1.6	2.2	0.9	1.5
Seliani 97	2.9	3.3	1.0	2.4
Bilfa 4	3.2	3.1	1.3	2.5
Njano	3.0	2.3	1.1	2.1
DOR 364	3.1	2.8	0.9	2.3
SUA 90	2.0	2.7	1.1	2.1
Rojo	2.3	2.0	1.1	1.8
Pesa	2.1	2.5	1.1	1.9
Lyamungu 85	2.9	2.5	1.1	2.2
Kablanketi	2.4	2.6	1.1	2.0
Grand mean	2.41b	2.61b	0.98a	2.0
L.S.D	0.54	0.54	0.13	0.73
S.E	0.57	0.58	0.14	0.29
CV (%)	23.8	22.3	14.7	23.9

Table 15: Mean seed yield per hectare at three locations

Key: C.mean- Combined mean

4.1.13 Shoot nitrogen (%N)

The interaction between locations and genotypes was significant on shoot nitrogen at $P \le 0.05$ (Table 4). The difference was also significant among locations and among genotypes. Location wise Mbeya had the highest mean shoot N followed by Arusha and Morogoro was the lowest (Appendix 15). The difference was not significant

among inoculated and non inoculated (control) treatments with in locations (Table 16 and Appendix 13). In overall, Biostacked inoculant had highest mean compared to Nitrosua, with regards to Biostacked, genotype *Bilfa 4* (3.9), *Njano* (3.8) and DOR 477 (3.7) had highest shoot N while *Njano* followed by *Carioka, SUA 90* and *Maini* performed better when inoculated with Nitrosua inoculant (Table 16).

Genotypes also varied significantly (P \leq 0.05) within locations, whereby in Mbeya genotypes *Njano* performed better followed by *DOR 364* and *Bilfa 4* while non nodulating *G 51396-A* and *G 4445-A* were the least. In Arusha genotypes *Njano* (3.6), Bilfa (3.5) had highest shoot N, in contrast non nodulating *G 4445-A* (2.5) and *G 51396-A* (2.6) had lower shoot N. Further more in Morogoro, genotypes *Njano* (3.4) and DOR 477 (3.3) had highest shoot N compared to other genotypes. The remaining genotypes had shoot nitrogen (%N) of between 3.0 and 3.3 (Table 16 and Appendices 10, 11 and 12). Combined means revealed that across locations highest shoot N was recorded in *Njano*, *Bilfa 4*, *Carioka* and *DOR 364* as compared to non nodulating *G 4445-A* and *G 51396-A* that had lowest shoot N (Appendix 14).

					Loc	cations							
	Mbeya Morogoro Arusha												
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	3.5	3.5	3.4	3.5	3.2	3.2	2.8	3.1	3.5	3.3	3.4	3.4	3.3
G4445A	3.2	3.4	3.2	3.3	3.4	3.3	3.0	3.2	2.5	2.5	2.5	2.5	3.0
Carioka	3.5	3.5	3.6	3.5	3.4	3.5	3.0	3.3	3.4	3.4	3.5	3.4	3.4
Maini	3.5	3.4	3.6	3.5	3.3	3.1	3.3	3.2	3.4	3.2	3.3	3.3	3.3
BAT 477	3.5	3.6	3.4	3.5	3.3	3.2	3.4	3.3	3.0	3.3	3.4	3.2	3.3
Mshindi	3.2	3.6	3.5	3.5	3.1	3.4	3.2	3.3	3.4	3.2	3.1	3.2	3.3
G51396A	3.2	3.4	3.1	3.2	3.4	3.2	3.3	3.3	2.6	2.7	2.5	2.6	3.0
Seliani 97	3.4	3.4	3.4	3.4	3.4	3.2	3.3	3.3	3.4	3.4	3.1	3.3	3.3
Bilfa 4	3.4	3.9	3.5	3.6	3.3	3.3	2.9	3.2	3.4	3.6	3.6	3.5	3.4
Njano	3.7	3.8	4.0	3.8	3.3	3.4	3.4	3.4	3.5	3.8	3.5	3.6	3.6
DOR 364	3.6	3.7	3.5	3.6	3.2	3.5	3.3	3.3	3.2	3.4	3.4	3.3	3.4
Sua90	3.4	3.4	3.6	3.5	3.0	3.0	2.9	3.0	3.0	3.3	3.4	3.3	3.2
Rojo	3.3	3.5	3.6	3.4	3.1	3.0	3.0	3.0	3.1	3.3	3.3	3.2	3.2
Pesa	3.6	3.5	3.5	3.5	3.0	3.0	3.2	3.0	3.3	3.2	3.3	3.3	3.3
Lymng 85	3.4	3.3	3.5	3.4	3.3	2.9	3.2	3.0	3.4	3.2	3.3	3.3	3.3
Kablanketi	3.4	3.5	3.6	3.5	3.1	3.0	3.0	3.0	3.2	3.5	3.5	3.4	3.3
Mean	3.43a	3.52c	3.50b		3.22a	3.21a	3.142a		3.20a	3.271a	3.26a		
Grand mean	1			3.48b				3.20a				3.24a	3.31
LSD				0.09				0.47				0.45	0.25
S.E				0.05				0.28				0.27	0.23
CV (%)				1.5				8.8				8.2	6.8

Table 16: Mean shoots Nitrogen (%N) of inoculated common bean genotypes at the three locations

Key:Bs- Biostaked (Beckerunderwood), Ns- Nitrosua, C.mean- Combined mean

4.1.14 Seed Nitrogen (%N)

The interaction between location, genotype and *rhizobia* was significant at $P \le 0.05$ on seed nitrogen content among locations (Table 4). Inoculation had significant influence on seed nitrogen (%N) among locations and among genotypes (Appendices 14 and 15). Location wise, genotypes planted in Morogoro had higher nitrogen content in seeds followed by genotypes planted in Arusha and Mbeya (Table 17 and Appendix 15). Biostacked inoculant was found to have higher mean of percent seed N content compared to Nitrosua and Control in all locations. When genotypes were grown in Morogoro, the highest seed nitrogen content was obtained in Genotype *Maini* (4.5) followed by *DOR 364* (4.3) and Selian 97 (4.1) inoculated with Biostacked inoculant. In Arusha the highest seed N with Biostacked inoculant was observed in *Carioka, DOR 364, Rojo* and *SUA 90*, while in Mbeya genotypes *Njano* had best performance followed by *Pesa* and *Bilfa 4*.

Using Nitrosua inoculant in Morogoro site best performance was in genotype *Njano* (4.4) followed by *Maini* and *Carioka*. On the other hand in Arusha site highest seed N was recorded in *Maini*, *Carioka* and *BAT 477*, while at Mbeya genotypes *Bilfa 4*, followed by *Njano*, *Seliani 97*, and *Maini* had the highest performance on seed N.

Genotypes also differed significantly ($P \le 0.05$) among locations irrespective of the inoculant used, in Morogoro, genotypes *Njano* performed highest followed by *Maini* and *Seliani 97* (Appendix 11), while in Arusha highest performance was recorded in *Carioka* and *Maini* followed by *Seliani 97*, *DOR 364* and *Bilfa 4* (Appendix 12. In Mbeya genotypes *Njano* and *Bilfa 4* followed by *Pesa*, *Seliani 97*, *Maini* and *Zawadi* had the highest seed N content compared to other genotypes (Appendix 10). Overall means from combined analysis of variance indicate that genotypes varied significantly ($P \le 0.05$) across locations. Highest seed N was recorded from *Maini*, *Njano*, *Bilfa 4*, Selian 97 and *Carioka*.

						Loca	tions						
		Mbeya				Mor	ogoro			Arus	ha		
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	3.2	3.1	3.2	3.2	3.5	4.0	3.9	3.8	3.4	3.3	3.3	3.3	3.4
G4445A	2.8	2.8	2.8	2.8	3.6	4.1	3.7	3.8	3.6	3.6	3.4	3.5	3.4
Carioka	3.1	3.2	2.9	3.1	3.7	4.0	4.1	3.9	3.4	3.8	3.7	3.7	3.6
Maini	3.1	3.1	3.2	3.2	3.6	4.5	4.1	4.1	3.7	3.6	3.9	3.7	3.7
BAT 477	2.6	3.0	3.1	2.9	3.9	3.9	3.7	3.8	3.4	3.4	3.7	3.5	3.4
Mshindi	3.0	3.2	3.1	3.1	3.2	3.7	3.9	3.6	3.2	3.2	3.2	3.2	3.3
G 51396A	3.1	2.6	3.0	2.9	3.7	4.1	3.9	3.9	3.6	3.6	3.6	3.6	3.5
Seliani 97	3.2	3.3	3.2	3.2	3.9	4.1	3.9	4.0	3.6	3.6	3.6	3.6	3.6
Bilfa 4	3.4	3.4	3.3	3.4	3.8	4.0	4.1	4.0	3.7	3.4	3.6	3.6	3.6
Njano	3.3	3.6	3.2	3.4	3.9	4.0	4.4	4.1	3.4	3.5	3.5	3.5	3.6
DOR 364	3.0	3.1	3.1	3.0	3.7	4.3	3.8	3.9	3.6	3.8	3.3	3.6	3.5
Sua90	3.1	2.8	3.0	3.0	3.6	3.4	3.6	3.5	3.2	3.7	3.4	3.4	3.3
Rojo	3.0	3.2	3.1	3.1	3.6	3.8	3.6	3.7	3.6	3.7	3.4	3.5	3.4
Pesa	3.3	3.4	3.0	3.2	3.8	3.7	3.7	3.7	3.5	3.5	3.2	3.4	3.5
Lymng 85	3.0	3.2	3.0	3.0	3.8	4.1	3.5	3.8	3.3	3.2	3.7	3.4	3.4
Kablanketi	3.0	3.1	3.1	3.0	3.5	3.5	3.5	3.5	3.4	3.6	3.4	3.5	3.3
Mean	3.08a	3.12b	3.08a		3.67 a	3.95c	3.83b		3.48 a	3.54ab	3.49a		
Grand M				3.09a				3.82c				3.50b	3.47
LSD				0.15				0.38				0.22	0.27
S.E				0.09				0.22				0.12	0.15
CV (%)				2.9				5.7				3.5	4.4
Key:	Bs-	Biostak	ed	(Beckerı	Inderwood),	Ns-	Nitros	ua,	C.mean-	C	Combined	n

Table 17: Mean seed Nitrogen (%N) of inoculated common bean genotypes at the three locations.

4.1.15 Shoot Ureides (µM/g)

Results on mean of shoot ureides are presented in Table 18. The interaction between genotype, *rhizobia* and location was significant at $P \le 0.05$ on shoot ureides (Table 4). With regard to location, Morogoro was leading followed by Mbeya and the last was Arusha although the differences among locations were not significant statistically (Table 18 and Appendix 15). Inoculated treatments had higher shoot ureides concentration (μ M/g) compared to non inoculated treatments (Table 18).

Biostacked inoculant performed better in Morogoro and Arusha while Mbeya Nitrosua had higher mean of shoot ureides (Appendix 7, 8, 9 and 13). With regard to Biostacked inoculation at Morogoro, genotype which performed better than others were *Zawadi* (7.1 μ M/g), followed by *BAT* 477 (6.5 μ M/g), *Maini* (6.4 μ M/g), and *Bilfa* 4 (6.205 μ M/g). In Arusha the highest shoot ureides was observed in genotypes *Seliani* 97 followed by *Bilfa* 4 and *Pesa*, while at Mbeya *Seliani* 97 had highest shoot ureides followed by *Maini* and *Rojo*.

Using Nitrosua *Selian* 97 (6.6 μ M/g) had better performance followed by *Mshindi*, *Njano* and *DOR* 364 when grown in Morogoro, while in Arusha *Carioka* was the highest followed by *SUA* 90 and *Seliani* 97 and *Bilfa* 4 had better performance. On the other hand, genotypes *Seliani* 97, followed by *Zawadi* and *DOR* 364 had better performance when grown in Mbeya.

The genotypes also varied significantly ($P \le 0.05$) among locations regardless of the inoculant used. In Morogoro *Mshindi* was able to produce highest shoot ureides

followed by *Njano*, *Lyamungu 85*, *Seliani 97*, *Kablanketi* and *BAT 477* (Appendix 11). On the other hand in Arusha *Seliani 97* produced highest shoot ureides followed by *SUA 90* and *Carioka* (Appendix 12), while in Mbeya highest shoot ureides was recorded in *Seliani 97* followed by *Mshindi*, *Zawadi* and *Rojo*. Across locations highest shoot ureides was recorded in *Seliani 97*, *Mshindi* and *Lyamungu 85* (Appendix 10).

					Loc	ations							
		Mbeya				More	goro			Arus	sha		
Genotypes	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	Control	Bs	Ns	Mean	C.mean
Zawadi	2.9	3.8	4.8	3.9	4.7	7.1	2.5	4.8	1.4	3.3	2.9	2.6	3.7
G4445A	2.6	3.0	3.1	2.9	3.5	3.6	3.6	3.6	1.8	1.6	1.7	1.7	2.7
Carioka	1.3	3.3	3.1	2.6	3.0	3.6	3.7	3.4	1.9	3.4	6.7	4.0	3.3
Maini	1.1	4.6	4.0	3.2	3.2	6.4	3.5	4.4	2.1	3.2	3.1	2.8	3.4
BAT 477	1.7	3.6	3.2	2.8	3.6	6.5	4.9	5.0	3.7	3.5	2.9	3.4	3.7
Mshindi	4.0	4.2	3.8	4.0	5.1	6.1	6.1	5.8	2.2	3.0	2.8	2.7	4.1
G51396A	2.8	1.7	2.0	2.2	3.1	4.5	4.4	4.0	0.5	1.4	2.3	1.4	2.5
Selian97	2.5	5.2	5.0	4.2	3.5	5.4	6.6	5.2	3.0	8.6	3.2	4.9	4.8
Bilfa 4	1.5	3.4	3.4	2.8	4.4	6.2	3.5	4.7	2.3	5.7	3.2	3.7	3.8
Njano	1.7	2.7	3.5	2.6	5.4	5.4	6.0	5.6	1.6	3.3	2.7	2.5	3.6
JOR 364	2.7	3.8	4.4	3.7	4.3	5.0	5.5	4.9	1.5	3.1	2.2	2.3	3.6
Sua90	0.6	3.8	3.8	2.8	2.8	4.9	4.0	3.9	4.2	3.1	6.1	4.4	3.7
Rojo	2.8	4.4	4.1	3.8	2.8	3.4	5.1	3.8	2.3	3.1	2.6	2.7	3.4
Pesa	3.8	3.0	3.8	3.5	3.4	5.3	3.1	4.0	1.4	5.2	2.9	3.2	3.6
Lymng 85	2.1	4.1	2.8	3.0	6.0	5.7	4.3	5.3	3.2	4.7	2.9	3.6	4.0
, e Kablanketi	2.1	4.0	4.0	3.4	4.7	6.0	4.4	5.0	2.1	2.0	2.1	2.1	3.5
Mean	2.27a	3.66 b	3.69 b		3.97 a	5.33c	4.45b		2.20a	3.64c	3.14b		
Grand mean				3.20a				4.58ab				3.00a	3.59
LSD				0.65				1.89				1.29	1.40
S.E				0.42				0.95				0.77	0.75
CV (%)				13				20.8				25.8	20.8
Key:	Bs-	Biostak	ed	(Beckerı	underwood),	Ns-	Nitro	osua.	C.mear	1-	Combine	ed

Table 18: Mean shoots ureides $(\mu M\,/g)$ of inoculated common bean genotypes at the three locations.

4.1.16 Harvest index

The difference among genotypes was significant, and location x genotypes interaction was also significant $P \le 0.05$ (Table 4). Inoculation did not have significant effects on harvest index (Appendix 13). For location generally, Mbeya had the highest harvest index followed by Arusha and Morogoro (Table19 and Appendix 15). When genotypes were grown in Mbeya the higher harvest index were recorded in *Bilfa 4* (0.87), followed by *Njano* (0.86) and *DOR 364* (0.85) compared to *G 51396-A which* had lowest harvest index (Appendix 10). In Arusha, genotypes *Njano* had the highest harvest index (0.85) followed by *SUA 90* (0.84), *Pesa* (0.82) and *Zawadi* (0.82) compared to *Carioka*, *DOR 364* and *Maini* which had relatively low harvest index (Table 19 and Appendix 12). In Morogoro highest harvest index was observed in *Bilfa 4* (0.72) followed by *DOR 364* (0.71), *Maini* (0.71) and *Mshindi* (0.71), while genotypes G 51396-A, *G 4445-A* and *Rojo* had the lowest harvest indices (0.57), (0.60) and (0.61) respectively (Appendix 11).

Combined analysis also revealed that genotypes significantly differed (P \leq 0.05). Across locations genotypes *Bilfa 4* and *Njano* had the highest harvest indices followed by *Mshindi* as compared to G 51396-A, *Carioka* and *G 4445-A* which had the lowest harvest indices (Table19 and Appendix 14).

		Locations		
Genotypes	Mbeya	Morogoro	Arusha	C.mean
Zawadi	0.80	0.66	0.82	0.76
G4445 A	0.76	0.60	0.78	0.71
Carioka	0.75	0.66	0.71	0.71
Maini	0.73	0.71	0.75	0.73
BAT 477	0.84	0.67	0.81	0.77
Mshindi	0.81	0.71	0.81	0.78
G 51396 A	0.72	0.57	0.80	0.70
Seliani 97	0.82	0.70	0.79	0.77
Bilfa 4	0.87	0.72	0.81	0.80
Njano	0.86	0.70	0.85	0.80
DOR 364	0.85	0.71	0.74	0.77
SUA 90	0.80	0.68	0.84	0.77
Rojo	0.79	0.61	0.79	0.73
Pesa	0.78	0.67	0.82	0.76
Lyamungu 85	0.82	0.66	0.76	0.75
Kablanketi	0.84	0.68	0.79	0.77
Grand mean	0.80	0.67	0.79	0.76
L.S.D	0.06	0.06	0.05	0.07
S.E	0.06	0.06	0.06	0.06
CV (%)	7.6	9.3	7.10	7.9

Table 19: Mean harvest index at three locations

Key: C.mean- Combined mean

4.1.17 Simple correlations of studied variables at experimental sites

In Mbeya, simple correlation analysis showed seed yield in common bean genotypes significantly ($P \le 0.05$) correlated with 100 seed weight ($r = 0.2605^{**}$), shoot fresh weight (SFW. $r = 0.268^{***}$), number of pods per plant ($r = -0.2178^{**}$), shoot N ($r=0.2719^{***}$), Seed N ($r = 0.2627^{**}$), and nodule fresh weight (Nod fwt) per plant ($r = 0.1915^{*}$) (Table 20).

Simple correlation analysis between variables in Morogoro showed yield to be positive and significantly correlated with seed weight per plant (0.9851***), shoot fresh weight (0.3647***) and number of pods per plant (0.1989*) (Table 21). At Arusha, simple correlation analysis shows yield in common bean genotypes strong

and positively correlated ($P \le 0.05$) with 100 seed weight ($r = 0.3588^{***}$), shoot fresh weight ($r = 0.2142^{*}$), shoot dry weight ($r = 0.1637^{*}$), Nodule No per plant ($r = 0.3335^{*}$), nodule fresh weight (Nod fwt) per plant ($r = 0.3192^{***}$), nodule dry weight (NODDW) per plant ($r = 0.3271^{***}$) shoot N ($r = 0.2992^{***}$) and Seed N per plant ($r = -0.1696^{*}$) (Table 22).

	1	2	3	4	5	6	7	8	9	10	11	12
								0		10		
Yield(1)	-											
Wt of seeds per plant(2)	0.9985***	-										
No of seeds pod(3)	0.1132ns	0.1155ns	-									
No of pods plant(4)	0.2178**	0.2172**	0.5546***	-								
Shoot %N(5)	0.2719***	0.2637***	-0.136ns	0.068ns	-							
Shoot Ureides(6)	0.1008ns	0.1002ns	-0.132ns	-0.169*	0.121ns	-						
Sfwt per plant(7)	0.268***	0.263*	0.1823*	0.151ns	-0.100ns	0.021ns	-					
Seed %N(8)	0.2627**	0.2622**	-0.243**	-0.27***	0.358***	0.179*	0.037ns	-				
Sdwt per plant(9)	0.1273ns	0.1231ns	0.0225ns	-0.068ns	-0.047ns	0.110ns	0.790***	0.159ns	-			
Nodule No per plant(10)	0.0943ns	0.0856ns	-0.430***	-0.49***	0.343***	0.212**	0.004ns	0.383***	0.183*	-		
Nod fwt per plant(11)	0.1915*	0.1837*	-0.308***	-0.29***	0.471***	0.171*	-0.009ns	0.429***	0.156ns	0.700***	-	
Nod dwt per plant(12)	0.1277ns	0.1194ns	-0.307***	-0.33***	0.450***	0.206**	-0.009ns	0.451***	0.184*	0.733***	0.946***	-
100 seeds wt(13)	0.2605**	0.2551**	-0.676***	-0.62***	0.200*	0.251**	-0.052ns	0.435***	0.164*	0.577***	0.404***	0.404***

 Table 20: Simple Correlation of selected variables at Uyole-Mbeya

Key: * = significant at 0.05, ** =significant at 0.01 and *** =Significant at 0.001.

Table 21: Simple Correlation of selected variables at SUA-Morogoro

	1	2	3	4	5	6	7	8	9	10	11	12
Yield(1)	-											
Wt of seeds per plant(2)	0.985***	-										
No of seeds pod(3)	0.125ns	0.105ns	-									
No of pods plant(4)	0.199*	0.186*	0.267***	-								
shoot %N(5)	0.158ns	0.168*	0.072ns	0.173*	-							
Shoot Ureides(6)	0.022ns	0.016ns	-0.081ns	-0.06ns	-0.04ns	-						
Sfwt per plant(7)	0.365***	0.348***	0.189*	0.209**	0.132ns	0.024ns	-					
Seed %N(8)	-0.003ns	-0.019ns	-0.037ns	0.134ns	0.045ns	0.262**	0.030ns	-				
Sdwt per plant(9)	0.100ns	0.087ns	0.116ns	0.230**	0.052ns	0.07ns	0.695***	-0.05ns	-			
Nodule No per plant(10)	-0.005ns	-0.015ns	-0.225**	-0.27***	-0.251**	0.015ns	-0.03ns	0.112ns	-0.10ns	-		
Nod fwt per plant(11)	0.036ns	0.027ns	-0.143ns	-0.162*	-0.15ns	0.058ns	0.054ns	0.197*	-0.10ns	0.836***	-	
Nod dwt per plant(12)	0.045ns	0.033ns	-0.150ns	-0.16ns	-0.14ns	0.073ns	0.045ns	0.202*	-0.11ns	0.83***	0.995***	-
100 seeds wt(13)	0.1286ns	0.144ns	-0.443***	-0.51***	-0.12ns	0.213**	-0.101ns	-0.15ns	-0.15ns	0.431***	0.222**	0.226**

Key: * = significant at 0.05, ** =significant at 0.01 and *** =Significant at 0.001

 Table 22: Simple Correlation of selected variables at Seliani-Arusha

	1	2	3	4	5	6	7	8	9	10	11	12
Yield(1)	-											
Wt of seeds per plant(2)	0.990***	-										
No of seeds pod(3)	-0.033ns	-0.033ns	-									
No of pods plant(4)	-0.033ns	0.047ns	0.486***	-								
Shoot %N(5)	0.299***	0.299***	0.127ns	-0.036ns	-							
Shoot Ureides(6)	0.221ns	0.221*	0.192*	-0.0191ns	0.144ns	-						
Sfwt per plant(7)	0.214*	0.214*	0.232**	0.336***	0.172*	0.196*	-					
Seed %N(8)	-0.169*	-0.169*	-0.011ns	0.085ns	-0.131ns	-0.009ns	-0.095ns	-				
Sdwt per plant(9)	0.164*	0.163*	0.240**	0.348***	0.165*	0.167*	0.919***	-0.102ns	-			
Nodule No per plant(10)	0.334*	0.335***	0.077ns	-0.126ns	0.468***	0.275***	0.284***	-0.093ns	0.258**	-		
Nod fwt per plant(11)	0.319***	0.311***	0.130ns	-0.012ns	0.432***	0.404***	0.377***	-0.052ns	0.355***	0.778***	-	
NODDW per plant(12)	0.327***	0.321***	0.099ns	-0.031ns	0.379***	0.4436***	0.377***	-0.044ns	0.347***	0.778***	0.961***	-
100 seeds wt(13)	0.359***	0.358***	-0.325***	-0.270***	0.325***	0.142ns	0.217**	-0.154ns	0.191*	0.553***	0.450***	0.474**

Key: * = significant at 0.05, ** =significant at 0.01 and *** =Significant at 0.001

4.2 **Results for Screen House Experiment**

4.2.1 Nodule number

The interaction was significant $P \le 0.05$) among genotypes and fertilizer type on nodule number (Table 23). Results for nodule number per plant are presented in Table 24. Generally inoculation followed by application of P-fertilizer produced higher number of nodules compared to other treatments. The genotypes *Rojo* had higher number of nodule compared to *Kablanketi* and non-nodulating genotype. Higher number of nodules was recorded when the genotype *Rojo* (G2) was treated with *Rhizobium* Nitrosua inoculants and P-fertilizer (12.3), biostacked and P-fertilizer (11.9) followed by *Kablanketi* with biostacked and P-fertilizer (11.6) and Nitrosua and P-fertilizer (11.5) compared to non nodulating (G3) and non inoculated treatments

4.2.2 Nodule dry weight

The interaction was significant at ($P \le 0.05$) between genotypes and fertilizer type on nodule dry weight (Table 23). With regard to fertilizer type higher nodules dry weight was recorded in inoculated treatment in combination with P-fertilizer (Table 24). For interaction effect higher nodule dry weight were obtained when the genotypes *Kablanketi* and *Rojo* were treated with *Rhizobium* inoculant Nitrosua and P-fertilizer (0.015 g) and biostacked inoculant and P-fertilizer (0.012 g) respectively and *Kablanketi* inoculated with Nitrosua (0.012 g) and *Rojo* when treated with combination of Nitrogen (UREA), P- fertilizer (TSP) and biostacked inoculant (0.012 g).

4.2.3 Shoot dry weigh

The interaction was not significant at ($P \le 0.05$) between genotype and fertilizer types in shoot dry weigh. Genotypes also differed significantly (Appendix 17). On the other hand fertilizer types also differed significantly on shoot dry weight (Table 23 and Appendix 16). Generally, higher shoot dry weight was recorded in inoculated treatments with P-fertilizer for *Rojo* (G2) and *Kablanketi* (G1) and non nodulating genotype (*G51105-A*) applied with N and P fertilizers (Table 24).

	1	Sources of variation	
Variables	Fertilizer type	Genotype	Genotype x Fertilizer type
Shoot dwt g/plant	6.77***	2.08*	0.7864ns
Root dwt g/plt	0.005***	0.004**	0.0012**
NodNo/plt)	78.70***	338.5***	20.7***
Nodw(g/plt)	8.80***	4.14***	3.3***
Days to 50% flw	0.53ns	89.81***	0.29ns
Days to 85% mat	0.03ns	516***	0.12ns
No of pods /plant	6.29***	1.39ns	1.62ns
No of seeds/pod	0.36ns	0.77ns	0.17ns
Wt seed/plant(g)	72.33***	878.4***	12.71ns
100seed wt(g)	51.48ns	4486.2***	30.17ns
Harvest index	0.0145***	0.121***	0.004ns
Yield(t/ha)	2.34***	28.46***	0.41ns
Seed %N	0.296***	4.83***	0.217***
Shoot.%N	0.46***	2.11***	0.289***

Table 23: Error mean square for studied variables in screen house experiment

		Nod N	No/plant			Nod dwt g/j	plant			Sdwt g/p	lant	
Fertilizer type	G1	G2	G3	Mean	G1	G2	G3	Mean	G1	G2	G3	Mean
Control	0.0	0.0	0	0.0	0.000	0.000	0	0.000	2.1	2.1	1.8	2.0
Ns	7.3	8.8	0	5.4	0.012	0.007	0	0.006	3.5	2.0	2.8	2.8
Bs	5.9	9.0	0	5.0	0.006	0.010	0	0.005	2.8	1.7	2.7	2.4
N	0.0	0.0	0	0.0	0.000	0.000	0	0.000	1.5	2.1	1.9	1.9
Ns+N	4.0	5.5	0	3.2	0.008	0.005	0	0.004	2.0	0.6	2.2	1.6
Bs+N	4.3	7.0	0	3.8	0.009	0.008	0	0.006	1.2	1.2	2.0	1.4
Р	0.0	0.0	0	0.0	0.000	0.000	0	0.000	3.5	2.5	3.1	3.0
Ns+P	11.6	12.3	0	8.0	0.015	0.011	0	0.008	4.5	4.1	3.8	4.1
Bs+P	11.7	11.9	0	7.9	0.009	0.012	0	0.006	4.2	4.3	3.1	3.9
N+P	0.0	0.0	0	0.0	0.000	0.000	0	0.000	3.3	3.1	4.0	3.5
Ns+N+P	6.3	6.0	0	4.1	0.006	0.007	0	0.005	3.6	2.5	2.9	3.0
Bs+N+P	7.3	7.3	0	4.9	0.003	0.012	0	0.005	3.1	3.2	2.3	2.9
Mean	4.88	5.66	0		0.006	0.0061	0		2.94	2.46	2.73	
Grand mean				3.512				0.004				2.706
S.E				0.761				0.002				0.829
L.S.D				1.24				0.003				1.35
CV%				21.7				47.3				30.7
Key: G1=Kabla	anketi, G2= R	<i>Rojo</i> , G3= G	51105-A	, $Ns = Nitr$	osua, Bs =	Biostacked,	N = Ure	a, P = TSP,	Nod No =	Nodule nu	imber per	plant, No
wt =	N	odule	dry	V	weight,	Sdwt		=	shoot	d	ry	weigh

 Table 24: Mean comparison of the interaction effect of genotypes, *rhizobia*, P and N fertilizers on nodule number per plant, nodule dry weight and shoot dry weight

4.2.4 Root dry weight, Days to 50 % flowering and 85 % maturity

The interaction was significant at ($P \le 0.05$) between genotypes and fertilizer types on root dry weigh (Table 23). Generally, inoculation followed by application of Pfertilizer produced higher root dry weight compared to other treatments. For genotypes *Rojo* had highest root dry weight followed by non nodulating genotype (*G51105-A*) and *Kablanketi* (Appendix 17). Highest root dry weight was recorded in genotype *Rojo* treated with biostacked inoculant and P-fertilizer (0.18 g) followed by inoculation with strain Nitrosua and P- fertilizer (0.17 g) and with N-fertilizer and biostacked inoculant (0.15 g) respectively (Table 25). The lowest root dry weight was obtained in non inoculated control genotypes.

There was no significant interaction between genotypes and fertilizer types on Days to 50 % flowering. The significant difference observed among genotypes at P<0.05 (Table 23). *Kablanketi* was the earliest (30) followed by *Rojo* (31) the longest time recorded was for genotype *G51105-A* (33) (Table 25 and Appendix 17).

There was no significant interaction ($P \le 0.05$) between genotype and fertilizer types on days to 85 % maturity. Significant differences were observed among genotypes at P<0.05 (Table 23 and Appendix 17). *Kablanketi* and *Rojo* were the earliest maturing genotypes (67) and (68) respectively, while genotype *G51105-A* took longest time to attain maturity (75) (Table 25 and Appendix 17).

		Rdwt	g/plant			50 % flo	owering			85 % r	naturity	
Fertilizer type	G1	G2	G3	Mean	G1	G2	G3	Mean	G1	G2	G3	Mean
Control	0.05	0.05	0.07	0.06	30	31	33	31	67	68	75	70
Ns	0.11	0.10	0.09	0.10	31	31	33	32	68	69	74	70
Bs	0.09	0.10	0.08	0.09	30	32	34	32	67	68	75	70
Ν	0.09	0.09	0.11	0.10	30	31	34	32	68	69	74	70
Ns+N	0.08	0.08	0.09	0.09	30	31	33	31	67	68	75	70
Bs+N	0.06	0.15	0.10	0.10	31	31	33	32	68	69	74	70
Р	0.10	0.08	0.13	0.11	30	32	33	32	67	68	75	70
Ns+P	0.12	0.17	0.13	0.14	30	31	34	31	68	69	74	70
Bs+P	0.11	0.18	0.12	0.14	31	31	33	32	67	68	75	70
N+P	0.08	0.12	0.09	0.10	30	31	33	31	68	69	74	70
Ns+N+P	0.10	0.10	0.08	0.09	31	32	34	32	67	68	75	70
Bs+N+P	0.08	0.08	0.09	0.08	30	31	34	32	68	69	74	70
Mean	0.089	0.110	0.099		30.3	31.2	33		67.5	68.5	75	
Grand mean				0.099				31.63				70.17
S.E				0.023				0.75				0.59
L.S.D				0.038				1.23				0.954
CV%				23.6				2.4				0.80

Table 25: Mean comparison of the interaction effect of genotypes, *rhizobia*, P and N fertilizers on root dry weight, days to 50 %flowering, and days to 85 %

*Key:G1= Kablanketi, G2= Rojo, G3= G 51105-A, Ns = Nitrosua, Bs = Biostacked, N = Urea, P =TSP, Rdwt = Root dry weight

4.2.5 Shoot nitrogen and seed nitrogen (%N)

The interaction between genotype and fertilizer type was significant at ($P \le 0.05$) on shoot nitrogen (Table 23). In general *Rojo* had higher shoot N compared to *Kablanketi* and *G51105-A* (Table 26 and Appendix 17). The highest nitrogen content was obtained when genotype *Rojo* was inoculated with *Rhizobium* biostacked inoculant and application of P-fertilizer (4.3) followed by when *Rojo* was inoculated with Nitrosua inoculant and application of P-fertilizer (4.3). The Lowest shoot nitrogen content was recorded when non nodulating genotype *G51105-A* was treated with P-fertilizer alone (2.8).

The interaction was significant ($P \le 0.05$) between genotypes and fertilizer types on seed N (Table 23). In general fertilizer types performed differently, inoculated treatment with N-fertilizer, followed by inoculation with P-fertilizer resulted to higher seed %N (Table 26 and Appendix 16). Genotypes also had different performance, *Rojo* had higher seed %N followed by *Kablanketi* and the least was *G51105-A* (Appendix 17). Higher seed nitrogen content was recorded when the genotype *Rojo* was treated with *Rhizobium* strain biostacked and P-fertilizer (4.3) followed by treatment with N-fertilizer and Nitrosua inoculants (4.2). Low seed nitrogen was obtained from non-inoculated control treatments and non nodulating genotype.

		Shoot N	N (%)		Se	ed N (%)		
Fertilizer type	G1	G2	G3	Mean	G1	G2	G3	Mean
Control	3.7	3.7	3.6	3.7	3.3	3.7	3.1	3.4
Ns	3.6	3.9	3.3	3.6	3.9	3.9	3.2	3.7
Bs	3.6	4.1	3.2	3.6	4.1	4.0	3.1	3.8
Ν	4.0	4.2	3.6	3.9	4.1	3.9	3.5	3.8
Ns+N	3.9	4.3	4.1	4.1	4.1	4.2	3.3	3.9
Bs+N	4.0	4.2	3.6	3.9	3.8	4.0	3.1	3.6
Р	3.3	3.5	2.8	3.2	3.5	3.7	2.9	3.4
Ns+P	4.0	4.3	2.9	3.7	4.1	4.1	2.8	3.7
Bs+P	4.1	4.3	3.0	3.8	4.2	4.3	3.0	3.9
N+P	3.6	3.5	3.9	3.7	3.6	4.0	3.7	3.8
Ns+N+P	3.6	3.4	3.7	3.6	4.0	3.9	4.0	3.9
Bs+N+P	3.8	3.9	3.8	3.8	3.7	3.9	3.7	3.8
Mean	3.77	3.93	3.45		3.87	3.97	3.29	
Grand mean				3.72				3.71
S.E				0.19				0.23
L.S.D				0.31				0.37
CV%				5.1				6.1

Table 26: Mean comparison of the interaction effect of genotypes, *rhizobia*, Pand N fertilizers on shoot and seed nitrogen (%N)

*Key:G1= *Kablanketi*, G2 = *Rojo*, G3 = G 51105-A, Ns = Nitrosua, Bs = Biostacked, N = Urea, P = TSP

Urea, P = 15P

4.2.6 Number of pods per plant and Number of seeds per pod

The interaction between genotype and fertlizer type was not significant at $P \le 0.05$, genotypes also did not differ significantly on number of pods per plant (Table 23). However, fertilizer types only differed significantly (Appendix 16). Generally, inoculated treatment with P-fertilizer had higher number of pods per plant compared to other treatments (Table 27).

The interaction between genotype and fertilizer types was not significant at $P \le 0.05$, on number of seeds per pod, genotypes and fertilizer types also did not differ significantly (Table 23). The highest number of seeds per pod was five while the lowest was four (Table 27).

4.2.7 Weight of seeds per plant (g)

The interaction between fertilizer type and genotype was not significant ($P \le 0.05$) on weight of seeds per plant but significant difference was observed among fertilizer types and among genotypes (Table 23). Generally, inoculated treatment followed by application with P-fertilizer had larger seed weight compared to non inoculated treatment. Genotype *Rojo* had a higher seed weight followed by *Kablanketi* and *G51105-A* was the lowest (Table 27 Appendix 17).

	No of	f pod/plant			No of	f seeds/pod			Swt g/p	lant		
Fertilizer types	G1	G2	G3	Mean	G1	G2	G3	Mean	G1	G2	G3	Mean
Control	9	9	9	9	4	4	4	4	13	11	6	10
Ns	10	9	10	10	4	4	4	4	14	17	8	13
Bs	10	10	10	10	4	4	5	4	15	16	10	14
Ν	11	10	10	10	4	4	4	4	18	16	8	14
Ns+N	10	11	10	10	4	4	4	4	15	17	10	14
Bs+N	11	11	11	11	4	4	4	4	19	24	12	18
Р	10	11	11	11	4	4	4	4	20	19	11	16
Ns+P	12	13	10	12	4	4	4	4	22	23	7	17
Bs+P	12	13	10	12	4	4	5	5	25	24	11	20
N+P	11	11	10	11	4	4	5	4	20	19	12	17
Ns+N+P	10	11	11	11	4	4	4	4	19	20	11	17
Bs+N+P	10	11	12	11	4	4	5	5	20	20	15	18
Mean	10.6	10.9	10.5		4.25	4.28	4.52		18.26	18.81	9.99	
Grand mean				10.7				4.35				15.7
S.E				1.09				0.61				3.8
L.S.D				1.79				0.98				6.22
CV%				10.3				13.90				24.3

Table 27: Mean comparison of the interaction effect of genotypes, *rhizobia*, P and N fertilizers on number of pods per plant,number of seeds per pod and weight of seed per plant

*Key:G1= Kablanketi, G2 = Rojo, G3 = G 51105-A, Ns = Nitrosua, Bs = Biostacked, N = Urea, P = TSP

4.2.8 Weight of 100 seeds, Harvest Index and yield (t/ha)

There was no significant interaction between genotype and fertilizer types at $P \le 0.05$ on weight of 100 seeds. The significant difference was observed among genotypes (Table 23). The largest 100 seed weight was recorded in genotypes *Kablanketi* and *Rojo*. Genotype *G51105-A* having smaller seeds produced the lowest 100 seed weight (Table 28 and Appendix 17).

There was no significant interaction ($P \le 0.05$) between genotype and fertilizer types on harvest index. Fertilizer types and genotypes only differed significantly on the studied variables (Table 23). With regard to fertlizer types harvest index was better in treatments with inoculants and N-fertilizer, followed by treatments with inoculants with combination of N and P-fertilizer. For the genotypes, *Rojo* had the highest harvest index followed by *Kablanketi* and lastly *G51105-A* (Table 28 and appendix 17).

There was no significant ($P \le 0.05$) interaction between fertilizer type and genotype, but there was a significant difference among fertilizer types and among genotypes at P<0.05 on seed yield (Table 23). For fertilizer type's highest yield was recorded in inoculated treatments with P-fertilizers followed by inoculated treatment with combination of N and P fertilizers (Appendix 16). With regard to the Genotypes, *Rojo* and *Kablanketi* had higher yields followed by *G51105-A* (Table 28 and Appendix 17)

	100	seed wt(g))		Har	vest index			Seed	d yield t/ha		
Fertilizer types	G1	G2	G3	Mean	G1	G2	G3	Mean	G1	G2	G3	Mean
Control	38	37	18	31	0.84	0.81	0.72	0.79	2.4	2.0	1.0	1.8
Ns	37	45	21	34	0.77	0.89	0.74	0.80	2.5	3.0	1.5	2.3
Bs	39	42	23	34	0.82	0.90	0.76	0.82	2.7	3.0	1.7	2.4
Ν	41	38	24	34	0.92	0.87	0.81	0.86	3.2	2.9	1.5	2.5
Ns+N	36	39	25	33	0.88	0.96	0.81	0.88	2.7	3.0	1.8	2.5
Bs+N	41	50	25	39	0.94	0.95	0.84	0.91	3.5	4.2	2.2	3.3
Р	47	42	23	37	0.84	0.88	0.76	0.83	3.5	3.3	1.9	2.9
Ns+P	45	42	19	35	0.82	0.84	0.63	0.76	4.0	4.1	1.3	3.1
Bs+P	48	44	21	38	0.85	0.83	0.76	0.82	4.4	4.4	1.9	3.6
N+P	43	42	27	37	0.87	0.85	0.74	0.82	3.6	3.4	2.2	3.1
Ns+N+P	46	42	23	37	0.83	0.88	0.78	0.83	3.3	3.6	2.0	3.0
Bs+N+P	47	43	27	39	0.86	0.86	0.86	0.86	3.6	3.7	2.7	3.3
Mean	42.18	42.16	22.83		0.855	0.88	0.77		3.29	3.39	1.8	
Grand mean				35.72				0.83				2.82
S.E				5.25				0.06				0.687
L.S.D				8.55				0.098				1.119
CV%				14.70				7.2				24.3

Table 28:Mean comparison of the interaction effect of genotypes, *rhizobia*, P and N fertilizers on 100 seed weight, harvest
index and yield per hectare

*Key:G1= Kablanketi, G2 = Rojo, G3 = G 51105, Ns = Nitrosua, Bs = B iostacked, N = Urea, P = TSP

4.2.9 Simple correlation of studied variables in screen house experiment

Simple correlation analysis showed that yield in common bean genotypes positively and significantly correlated ($P \le 0.05$) with 100 seed weight ($r = 0.83^{***}$), shoot dry weight (Sdwt) per plant ($r = 0.20^{*}$), Nodule No per plant ($r = 0.58^{***}$), nodule dry weight (Nod dwt) per plant ($r = 0.45^{***}$), shoot N ($r = 0.45^{***}$), seed N (0.58^{***}), number of pods per plant ($r = -0.56^{***}$) and number of seeds per pod ($r = 0.32^{***}$) (Table 29).

	1	2	3	4	5	6	7	8	9
Yield(1)	-								
Wt of seeds per plant(2)	0.9983***	-							
No of seeds pod(3)	0.32***	0.32***	-						
No of pods plant(4)	0.56***	0.56***	0.15ns	-					
shoot %N(5)	0.45***	0.45***	-0.03ns	0.19ns	-				
Seed %N(6)	0.58***	0.58***	-0.07ns	0.25ns	0.63***	-			
Nodule No per plant(7)	0.58***	0.58***	-0.07ns	0.34***	0.51***	0.60***	-		
Nod dwt per plant(8)	0.45***	0.45***	-0.14ns	0.24ns	0.45***	0.57***	0.88***	-	
100 seeds wt(9)	0.83***	0.83***	-0.07ns	0.17ns	0.45***	0.63***	0.57***	0.49***	-
Sdwt per plant(10)	0.20*	0.20*	0.32ns	0.56**	-0.11	0.07ns	0.24**	0.17ns	0.04ns

 Table 29: Simple Correlation of selected variables in screen house trial at SUA-Morogoro

Key: * = significant at 0.05, ** = significant at 0.01 and *** = significant at 0.001

CHAPTER FIVE

5.0 DISCUSSION

5.1 Field Experiment

5.1.1 Nodule number and nodule dry weight

Results from this study indicated large differences occurring among locations for nodule number and dry weight. Generally, higher nodule number and dry weights were observed among inoculated treatments when genotypes were grown in Arusha followed by Mbeya and Morogoro, this could be because soils in Arusha and Mbeya had slightly higher P content compared to soils in Morogoro site (Table 2). Phosphorus is needed for plant growth, nodule formation and development and ATP synthesis, each process being vital for nitrogen fixation (Dashora, 2011). In all Locations, in the non-inoculated treatment there were nodules formed in the plants, except for the non nodulating genotypes. This indicates that's there were native *rhizobia* in the soils where beans were planted.

Although symbiotic nitrogen fixing potential in common bean is considered to be low in comparison with other legumes, the present study also showed genotypic variability for traits associated with N fixation suggesting a prospect for improving nodulation by selecting and/or transforming legume genotypes for increased exudation of flavonoids and other signalling compounds.

These differences also reflect the sensitivity of the bean-*Rhizobium* symbiosis to many environmental factors that can have either an enhancing or reducing

effect on N fixation. Graham and Vance (2003) explained that numerous changes occur in host and bacterial gene expression during infection, nodule development, and function with approximately 100 host legume and *rhizobial* genes involved. Study by Chemining'wa *et al.* (2007) on effect of *rhizobia* inoculation and starter-N on nodulation and yield of grain legumes revealed that in most cases, common bean had significantly higher nodule numbers and nodule biomass than most of the other legumes. Otieno *et al.* (2007) also reported similar results that *rhizobial* inoculation significantly increases nodule number and dry weight in studied legume species compared to application of farmyard manure and N-fertilizer. Mehrpouyan (2011) also reported significant increase in nodule number and dry weight in common bean cultivars when inoculated with *Rhizobium leguminosarum* strain Rb117.

5.1.2 Shoot and root dry weight

Results for shoot and root dry weight had indicated no significant differences among inoculated and non-inoculated control although genotypes differed significantly on the studied variable. Higher shoot dry weigh were recorded in non nodulating genotypes *G* 51396-*A* and *G* 4445-*A*, closely followed by Selian 97 and *Carioka* grown in Morogoro. For Mbeya and Arusha genotypes *Seliani* 97 and *Rojo*, *Lyamungu* 85 and *Carioka* had the higher shoot dry weight respectively. This result indicates that the variations in biomass production among the common bean genotypes can be attributed to differences not only by BNF capacity but also in genetic potential, plant architecture and rooting pattern as were also reported by Mandel *et al.* (1990).

5.1.3 Shoot and Seed Nitrogen

Plant tissue analysis from this study also revealed that inoculation significantly increased total seed nitrogen (%N) but not shoot nitrogen. Genotypes varied significantly in their percent nitrogen content in the seeds across locations, with the genotypes grown in Morogoro having higher seed N content followed by genotypes grown in Arusha and Mbeya respectively. Increase in seed nitrogen content is attributed to increase in biological nitrogen fixation following inoculation with nitrogen fixing *rhizobia*, This finding confirms a similar phenomenon reported by Ahmed *et al.* (2008), that seed inoculation alone and in combination with soil inoculation increased significantly protein content as of Massor-93 cultivar compared to sole soil inoculation, whereas for Masoor-2002 the difference among the three methods remain non-significant though the seed inoculation and soil inoculation in combination increased seed protein contents against the control. Application of P during planting also may have contributed to accelerated plant N-uptake. Legumes requires sufficient amount of phosphorus for plant growth and nodules formation for nitrogen fixation (Neila *et al.*, 2012).

5.1.4 Shoot ureides

Variation was significant among genotypes across locations on shoot ureides Concentration. The ureides concentrations were higher in inoculated treatments compared to non-inoculated control and non nodulating genotypes. Presence of ureides content in shoot samples in nodulating and non nodulating genotypes indicated that nodules are not the only sites for ureides synthesis. However, the difference between the shoot ureide contents in nodulating and non-nodulating genotypes was large enough to permit use of the ureide technique for determination of biological nitrogen fixation. The presence of ureides in xylem sap, stem and leave extract of non-nodulated trees were also reported by Van kessel *et al.* (1998). In this study ureides concentration values was ranging between 2.99-4.58 μ M/g among locations and 2.5- 4.8 μ M/g among genotypes across locations. This indicates that there is variation in Biological nitrogen fixation (BNF) among genotypes and that there are environmental effects on BNF. Genotypes Selian 97, *Mshindi* and *Lyamungu 85* had the highest ureides content across all locations indicating their potentiality in fixing atmospheric nitrogen. Serraj and Sinclair (1998) reported the mean petioles ureides concentrations measured a range of between 2 and 10 μ M/g in two common bean varieties Roma and Kentucky Wonder respectively. The lower levels of ureides concentration in common bean is said to be associated with nitrogen fixation drought tolerance compared to other legumes like soybean where ureides levels reached up to100 μ M / g in petioles under drought (Serraj and Sinclair, 1997).

5.1.5 Days to 50 % flowering and 85 % maturity

Inoculation did not have significant effect on days to 50% flowering and 85% maturity. The results suggest that although genotypes vary on the measured variables, environments also had greater influence on the performance of the genotypes. Of these variables earlier flowering and maturity was obtained at Morogoro compared to Mbeya and Arusha. Climatic condition in Morogoro is warmer compared to Arusha and Mbeya sites which were relatively cooler. The wormer climate allows plants to grow faster and reach flowering earlier than when in the cool climate (Sinclair, (2004); Park *et al*, (1998). Temperature directly affects metabolic activities such as photosynthesis, respiration and transpiration which are the major processes in the growth and development of the crop plant. There was no relationship between BNF trait such as nodules and flowering and maturity of these genotypes.

5.1.6 Number of pods per plant and number of seeds per pod

Findings from this study reveal that interaction was significant between location and genotypes on number of pods per plant and number of seeds per plant. Inoculation did not have significant effect on number of pods per plant except number of seeds per plant. Variation in the number of pods per plant and number of seeds per pod among genotypes was most likely due to differences in genotypes and the G x E interaction observed was due differences in adaptability and stability to the environment in which genotypes were grown. The environments were different in climatic conditions and soil characteristics. Despite the fact that the number of pods per plant and seeds per pod is also genotype specific, differences in moisture around the time of pod setting and grain filling also led to differences in number of pods per plant and seeds per pods. The overall performance of the genotypes for various traits was better at SUA and Mbeya than at Arusha, Such traits included seed weight, number of pods per plant and seed yield, this means that the environment at SUA and Mbeya appear to allow easy discrimination of the best genotypes for expression of their maximum potential and could thus prove to be good breeding and production sites for common bean.

5.1.7 Weight of 100 seeds, seeds weight of per plant and yield

Statistically inoculation had no significant effect on 100 seeds weight (seed size), of seeds weight per plant and overall yield per ha, however genotypes and genotype x environment interaction showed significant effects on these studied variables. This indicates that increased N supply did not increase seed filling phase of reproductive growth. Seed size is highly heritable therefore it is not very much affected by environment. Also the significant difference among genotypes tested implies genetic potential to direct assimilates efficiently towards sinks. Ahmed *et al.* (2008) reported insignificant increase of 100 seed weight in two Lentil varieties upon inoculation.

Low yield which occurred at Arusha and Mbeya sites compared to Morogoro may be attributed to insufficient rainfall during pod-filling stage (Table 3), Because at this stage crop demands ample amount of water. Yield also has been considered a quantitative character, i.e., influenced by many genes with the effects of individual genes normally unidentified, its expression depends upon interaction of many edaphic and climatic variables (Wallace *et al.*, 1972). Similar results were also reported by Rifat hayat *et al.* (2008), that yield of Mash and Mung bean did not increase irrespective of inoculation with *rhizobia* species.

5.1.8 Correlation among traits

Simple correlation analysis showed that positive correlation existed in yield and weight of seeds per plant, 100 seed weight, shoot fresh weight, shoot nitrogen, pod per plant, and seeds per pod. This suggests that breeding for one trait may lead to significant progress in the other. Similar results were reported by Fivawo and Nchimbi-Msolla (2012). Yield also was positively correlated with nodule number, nodule dry weight and shoot ureides, implying that number of nodules, nodules dry weight and shoot ureides are a reliable indicator of yield per plant during the early stages of the crop growth, and it could be a helpful tool in plant breeding programs if a selection in the early stages can be achieved. Similar results were reported by Stajkovic *et al.* (2011), Rifat hayat *et al.* (2008) and Fernandez *et al.* (2012). There was no significant correlation between seed yield and seed N, indicating when available nitrogen is low; the plant reduces the protein content of each seed in order to produce the same number of seeds with a limited amount of nitrogen (Montanez, 2000).

5.2 Screen House Experiment

5.2.1 Nodule number and nodule dry weight

For screen house experiment higher nodule number and dry weight were obtained when genotypes *Rojo* and *Kablanketi* were inoculated with *rhizobia* strains Biostacked and Nitrosua and application of P-fertilizer. When genotypes were inoculated followed by application of N-fertilizer nodule number were reduced, the range was between 4-7. This indicates the preference of host plant to utilize available N present in the soil which requires less energy than fixing N from the atmosphere as explained also by Silvia and Uchida (2000). Nodule counts were zero for non inoculated control and application of N and P-fertilizers treatments alone. The result of this study was in accordance with earlier finding by Thair *et al.* (2009) who reported increase of nodule number and nodule dry weight from 73 to 125 and 1.36 to 1.53 g respectively in soybean by inoculation alone, where addition of P-fertilizer

resulted to more increase in nodule number and dry weight. Cheema and Ahmad (2000) also reported that application of nitrogenous fertilizer resulted in reduction of nodules number and rate of nitrogen fixation in soybean.

5.2.2 Shoot and root dry weight

Inoculation in combination with fertilizer P resulted significantly in increased shoot and root dry weight compared to other treatments. The results indicate that phosphorus is important for normal plant growth and functioning, also it may have improved the uptake of other nutrient like nitrogen. This result confirms finding by Rifat hayat *et al*, (2008b), who found that shoot dry matter yield of mung bean and mash bean on an average was 2.99 and 2.78 t/ha with phosphorus fertilization and 2.79 and 2.72 t/ha without phosphorus application, respectively. He further reported that Phosphorus fertilizer produced 7 and 2 % higher biomass yield of mung bean and mash bean respectively.

Phosphorus is important for plant growth and its deficiency limits legume production in most agriculture soils (Vance, 2001). It is also required for normal functioning in N-fixing bacteria and for effective nodulation on the root system of leguminous crops (Brady and Weil, 2004)

5.2.3 Shoot and seed Nitrogen

Results from this study showed that, inoculation in addition to P-fertilizer increased both shoots and seed nitrogen as for other traits. Tahir *et al.* (2009) reported similar results whereby *Rhizobium* inoculation and P increase nitrogenease activity, nodule mass that ultimately increased plant N content and uptake while addition of reduced level of N (starter fertilizer) fulfils the immediate need of N to plants and these combinations leading to higher N content and N uptake in plant. As an important nutrient, P deficiency constraint is considered the principal limiting factor for legume growth, strongly reduced nitrogen fixation and resulted in decreased nitrogen availability for subsequent crops (Neila *et al.*, 2012).

5.2.4 Days to 50 % flowering and 85 % maturity

Days to 50 % flowering and 85 % maturity was not significantly affected by treatment used. Significant difference was observed among genotypes only. Genotypes *Kablanketi* and *Rojo* were found to be the earliest in flowering and maturity compared to G 51105-A which took long time to produce flower and to mature. Results indicated that early flowering and maturity is mostly genotypes specific although environmental conditions such as high temperature in lower altitude hastened earlier flowering and maturity in most of the cultivated crops.

5.2.5 Number of pods per plant and number of seeds per pod

Number of pods per plant was not significantly different among genotypes. Interaction between cultivar and fertilizer type was also not significant. However significant difference was observed among fertilizer type only whereby inoculation in combination with P-fertilizer resulted to increased number of pods in nodulating genotypes, while combination of N and P had better performance in number of pods per plant in non nodulating genotype. This study also reported that increase in nodulation increased nitrogen fixation efficiency which influenced formation of more pods per plant. On the other hand number of seeds per pod was not significantly affected by treatments used. It is obvious that the number of seeds per pod is one of the most constant grain yield components, that they are determined mainly by the genotypes, that genotypes with smaller seed size usually have more number of seeds per pod compared to genotypes with larger seed size which has few seeds per pod. The results indicated that as number of seeds per pod is a genetic trait, it is not affected by environmental effects.

5.2.6 Weight of 100 seeds, weight of seeds per plant and yield

The 100 seed weight is a measure of seed size, it is the weight in grams of 100 seeds. Weight of 100 seeds can vary from one crop to another, between varieties of the same crop and even from year to year or from field to field of the same variety. Results from this study reveal that a significant difference ($P \le 0.05$) was observed among genotypes for 100 seed weight. This result suggests that genotypes have different genetic potential in producing different seeds size. Similar findings were also reported by Safapour *et al.* (2011).

On the other hand inoculation in addition with P- fertilizers application significantly increased seeds weight per plant and yield of tested genotypes, variation was also observed among genotypes. The results suggest that increase in nodulation and root and shoot biomass due to inoculation and application of P may have resulted to slight increase in final yield. The significant difference among genotypes tested implies genetic potential to direct assimilate efficiently towards sinks.

5.2.7 Correlation among traits

Positive and significant correlation between seed yield and other traits such as nodule number and dry weight, seed nitrogen, number of pods per plant and number of seeds per pod suggest the possibility of selecting desired traits simultaneously in successful breeding program. These results are in agreement with the earlier findings reported by Fivawo and Nchimbi-Msolla (2012).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Common bean has usually been considered a poor N fixing grain legume; however there is a large genotypic variability for N fixation potential which offers hope for significantly improving this trait in bean through appropriate breeding programs. In this study combined analysis of variance showed that genotypes which had good nodulation included *Lyamungu 85* by having higher mean nodule number per plant closely followed by *Pesa*, *Zawadi*, *Seliani 97*, *Rojo*, *Kablanketi* and *Carioka*, indicating good adaptability of these genotypes for nodulation trait across those three locations, also possibility of having genes for nodulation therefore genetic improvement of other genotypes. Positive correlation between yield and nodule number and dry weight have demonstrated that it is possible to breed common bean genotypes that are highly nodulating and give high yields in Tanzania. Genotype *Bilfa 4* nodulates moderately and had higher yield across all three locations, therefore it can be recommended to be grown in all three location or utilized as source of good breeding material for yield improvement.

In this study G x E interaction was highly significant on traits determining biological nitrogen fixation such as nodule number, nodule dry weight, shoot and root dry weight, shoot Ureides, shoot and seed N. The variation observed in nodulation capacity of tested genotypes in different environment indicate importance of testing genotypes in various locations to improve selection of genotypes for required trait they have been tested for.

Rhizobia inoculation of common bean seeds had enhanced nodulation and hence nodule dry weight in genotypes tested across locations. The effect of inoculation was also significant on shoot ureides and seed N. In this study Biostacked inoculant was found to be superior compared to Nitrosua.

Application of N-fertilizer alone or in combination with *rhizobial* inoculants was found to reduce formation of nodules while combination of *rhizobial* inoculants and P-fertilizers gave better results in tested common bean genotypes.

6.2 Recommendations

- i. More research has to be done to test these genotypes with other commercial strains of *rhizobia* to maximize use of biological nitrogen fixation for sustainable agriculture.
- ii. As far as phenolic compounds (i.e Isoflavonoids) are involved in the signaling pathway in the *rhizobia*-legume symbiosis, quantity of these compounds produced by the genotypes/host legume and their quality should also be analysed to improve host-*rhizobia* symbiosis.
- iii. Almost all of the genotypes tested had nodulated in control plots. Therefore survey should be done to observe and isolate native *rhizobia* from the soils and root nodules of growing plants in all legume/bean growing areas so as to identify effective native strain to make effective use of our native *rhizobia*.
- iv. Extension program should be facilitated by Ministry of Agriculture Food security and Cooperatives (MAFCs) to disseminate information to

farmers on use of micro-symbiont (*Rhizobia*) as an alternative source of nitrogenous fertilizers for improving legume production in a sustainable manner.

- v. Commercial production of *rhizobia* inoculant should be explored in the county since use of inoculants has shown good results.
- vi. Farmers should be advised to continue growing genotypes that showed to have good nodulation and potentially good biological nitrogen fixers.
- vii. In legume fields farmers should be advised to use P-fertilizers as this will increase BNF and a consequence increase yield.

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APPENDICES

Appendix 1: Common bean production in East Africa in terms of area in 2000-2007

Country	Average area(ha)	Average production (ton)	Production(ton/ha)
Kenya	910 478	412 381	0.453
Uganda	794 375	478 625	0.603
Tanzania	373 125	285 414	0.765
Rwanda	340 055	231 882	0.682
Burundi	249 375	229 607	0.921

Source: FAOstat at <u>www.fao.org</u> in Katungi et al. (2009)

Genotypes	enotypes Source S		Seed size	Growth habit
Rojo	SUA	Released variety	Large	Determinate upright
Kablanketi Bilfa 4	SUA Uyole	Local variety Released variety	Medium Medium	Indeterminate upright Determinate bush
<i>Lyamungu 85</i> Carioca	Seliani CIAT	Released variety	Released variety Large Small	
Njano	SUA	Local	Large	Indeterminate semi climber
Pesa	SUA	Released variety	Medium	Determinate upright
BAT 477	CIAT	Improved line	Small	Indeterminate upright
DOR 364	CIAT	Improved line	Small	Indeterminate upright
Selian 97	Seliani	Released	Large	Determinate bush
Zawadi	SUA	Improved line	Medium	Determinate upright
SUA 90	SUA	Released variety	Medium	Determinate upright
Mshindi	SUA	Released variety	Medium	Determinate upright
Maini	SUA	Local	Medium	Indeterminate semi

Appendix 2: Genotypes used for trials at Uyole, SUA, and Selia
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			climber
G 4445-A	Check	Small	Indeterminate upright
G 51396-A	Check	Small	Indeterminate upright

Appendix 3: Nitrogen fixation and shoot biomass yields of selected and check genotypes of *Phaseolus vulgaris*

Genotype	N_2 fixation (% relative ureid-N)			Shoot dry matter (t/ha			
	1995 ^A	1996 ^A	1997 ^A	1995 ^B	1996 ^B	1997 ^B	
	Higl	n nitrogen-fixin	ig genotypes				
ICA20667	74	46	56	2.0	5.1	2.7	
ICA21573	53	38	55	2.1	4.6	3.0	
High shoot bion	nass genotyp	e					
RIZ53	27	26	36	2.5	5.4	2.6	
	Che	ck cultivars					
Rainbird	55	33	43	1.3	4.3	2.7	
Spearfelt	63	30	47	1.1	4.2	2.5	
Gallaroy	38	n.d.	50	1.3	n.d.	2.8	

A= Mean of 2 samplings (flowering and pod-fill), B= Pod-fill sampling only, and n.d.= not determined.

Source: Herridge et al., 2001

Appendix 4: Treatments combination for screen house experiment

Treatment	Description
T1	Control (genotypes 1-3)
T2	Genotypes 1-3 + NS
Т3	Genotypes 1-3 + BS
T4	Genotypes $1-3 + N$
Τ5	Genotypes $1-3 + N + NS$
Τ6	Genotypes $1-3 + N + BS$
Τ7	Genotypes 1-3 + P
Τ8	Genotypes $1-3 + P + NS$

T9				Genotypes $1-3 + P + BS$
T10				Genotypes $1-3 + N-P$
T11				Genotypes 1-3 + N-P +NS
T12				Genotypes 1-3 + N-P +BS
Kev:	NS-Nitrosua	BS-Biostacked	P-Phosphatic f	fertilizer and N nitrogenous

Key: NS-Nitrosua, BS-Biostacked, P-Phosphatic fertilizer and N nitrogenous fertilizer

Appendix 5: Statistical models: for factorial and split-split- plot respectively

 $Y_{ijkl} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + \mathcal{E}_{lijk} \qquad (4)$ $X_{ijk} = \mu + M_i + B_j + d_{ij} + S_k + (MS)_{ik} + f_{ikj} + T_1 + (MT)_{il} + (ST)_{kl} + (MST)_{ikl} + \mathcal{E}_{ijk} \qquad (5)$

X _{ijk}/Y_{ijkl} =an observation or response, μ = the experiment mean, M _i= the main plot treatment effect, B _j = the block effect, d _{ij} = the main plot error (error a), S _k = the subplot treatment effect (MS) _{ik} = the treatment interaction effect, f _{ikj}= the subplot error (error b), T₁ = the sub subplot treatment effect, (MT)_{il} = the treatment interaction effect, (ST)_{kl} = the treatment interaction effect, (MST) _{ikl} =the treatment interaction effect, e _{ijk} = the sub subplot error (error c), i, k, l = a particular treatment, j = a particular block.

Appendix 6: Ranks of Yield across three locations

LocationsMbeyaMorogoroArusha								
Genotypes	Yield	Rank	Rank Genotypes Yield Ran				Yield	Rank
Bilfa 4	3.2	1	Seliani 97	3.3	1	Bilfa 4	1.3	1
DOR 364	3.1	2	Bilfa 4	3.1	2	Zawadi	1.1	2
BAT 477	3.0	3	DOR 364	2.8	3	Njano	1.1	3
Njano	3.0	4	Sua90	2.7	4	Sua90	1.1	4
Seliani 97	2.9	5	Carioka	2.6	5	Rojo	1.1	5
Lymng 85	2.9	6	BAT 477	2.6	6	Pesa	1.1	6

Mshindi	2.4	7	Mshindi	2.6	7	Lymng 85	1.1	7
Kablanket	2.4	8	Kablanket	2.6	8	Kablanket	1.1	8
Zawadi	2.3	9	Zawadi	2.5	9	BAT 477	1.0	9
Rojo	2.3	10	Pesa	2.5	10	Mshindi	1.0	10
Pesa	2.1	11	Lymng 85	2.5	11	Seliani 97	1.0	11
Sua90	2.0	12	G4445A	2.4	12	G51396A	0.9	12
Carioka	1.8	13	Maini	2.4	13	DOR 364	0.9	13
G4445A	1.7	14	Njano	2.3	14	Carioka	0.8	14
Maini	1.7	15	G51396A	2.2	15	Maini	0.8	15
G51396A	1.6	16	Rojo	2.0	16	G4445A	0.7	16

	١	ARIABLE	ES					
Inoculants	Nod No/pla nt	Nod dwt(g/p lant)	Sdwt (g/plant	Rdwt (g/pla nt)	Days to 50%f	Days to 85%m	No of pods/pl ant	No of seeds /pod
Contol	23	0.06	2.26	0.56	44	82	13	5
Bs	26	0.07	2.20	0.50	44	82	13	5
Ns	24	0.07	2.33	0.57	44	82	15	5
Grand mean	24.29	0.07	2.26	0.54	44.03	81.90	13.86	4.71
L.S.D(0.05)	1.12	0.01	0.22	0.06	1.07	0.75	0.96	0.17
SE	2.77	0.02	0.53	0.14	2.64	1.86	2.38	0.42
CV %	11.40	30.80	23.60	26.50	6.00	2.30	17.20	8.80

Appendix 7 continues,

VARIABLES											
Inoculants	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot. % N	Seed %N	Ureides (µM/g)				
Contol	12.16	29.18	0.79	2.29	3.43	3.08	2.27				
Bs	13.32	29.48	0.82	2.531	3.52	3.12	3.66				
Ns	12.65	29.26	0.79	2.403	3.50	3.08	3.69				
Grand mean	12.71	29.31	0.80	2.41	3.48	3.09	3.21				
L.S.D(0.05)	1.23	0.71	0.03	0.23	0.02	0.04	0.16				
SE	3.03	1.75	0.06	0.57	0.05	0.09	0.41				
CV %	23.90	6.00	7.80	23.50	1.50	3.00	12.70				

			VA	RIABLE	S			
Inoculants	Nod No/pla nt	Nod dwt(g/p lant)	Sdwt (g/plant	Rdwt (g/pla nt)	Days to 50%f	Days to 85%m	No of pods/pl ant	No of seeds/pod
Contol	10	0.02	5.56	0.73	34	75	16	5
Bs	15	0.02	6.40	0.79	34	74	18	5
Ns	14	0.02	5.82	0.72	34	74	17	5
Grand mean	12.94	0.02	5.93	0.74	33.91	74.33	17.06	5.02
L.S.D(0.05)	1.53	0.00	0.61	0.09	0.31	0.90	1.55	0.32
SE	3.77	0.01	1.50	0.23	0.76	2.22	3.82	0.79
CV %	29.10	49.90	25.30	30.30	2.20	3.00	22.40	15.70

Appendix 8: Mean effects of inoculants for the studied variables at Morog

Appendix 8: continues,

			VARIABLE	ES			
Inoculalants	wt of seeds/pl ant	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	Seed %N	Ureides (µM/g)
Contol	13.57	35.51	0.68	2.54	3.22	3.67	3.97
Bs	13.81	35.98	0.65	2.59	3.21	3.95	5.33
Ns	13.74	35.85	0.68	2.61	3.14	3.83	4.45
Grand mean	13.71	35.78	0.67	2.58	3.20	3.82	4.58
L.S.D(0.05)	1.42	0.98	0.03	0.28	0.12	0.09	0.44
SE	3.49	2.41	0.06	0.69	0.29	0.23	1.08
CV %	25.50	6.70	9.40	26.90	9.00	6.00	23.60

Appendix 9: Mean effect of inoculants for the studied variables at Arusl	or the studied variables at Arusha
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			V	ARIABLES	;			
Inoculants	Nod No/pl ant	Nod dwt(g/p lant)	Sdwt (g/pla nt	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/pl ant	No of seeds/p od
Contol	32	0.06	1.08	0.17	40	80	7	4
Bs	39	0.09	1.26	0.21	41	80	8	5
Ns	34	0.07	1.07	0.21	41	80	8	4
G.Mean	34.92	0.07	1.14	0.20	40.80	79.97	7.60	4.37
L.S.D(0.05)	2.42	0.01	0.14	0.02	0.97	1.18	0.62	0.31
SE	5.96	0.02	0.34	0.05	2.39	2.91	1.52	0.76
CV %	17.10	30.80	29.70	25.00	5.90	3.60	20.00	17.30

Appendix 9: o	continues,
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		V	ARIABI	ES			
Inoculalants	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	Seed %N	Ureides (µM/g)
Contol	5.11	29.03	0.80	0.97	3.20	3.48	2.20
Bs	5.32	29.55	0.79	1.01	3.27	3.54	3.64
Ns	5.06	29.08	0.79	0.96	3.26	3.49	3.14
Grand mean	5.16	29.22	0.79	0.98	3.24	3.50	3.00
L.S.D(0.05)	0.30	1.25	0.02	0.06	0.11	0.05	0.32
SE	0.75	3.09	0.06	0.14	0.27	0.13	0.79
CV %	14.60	10.60	7.40	14.60	8.50	3.80	26.30

Appendix 10: Mean effects of genotypes for the studied variables at Mbeya

Genotypes	Nod No/pla nt	Nod dwt(g/pl ant)	Sdwt (g/plant	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/ plant	No of seeds/ pod
Zawadi	22	0.07	2.28	0.48	37	75	8	5
G4445A	0	0.00	1.81	0.56	50	86	19	5
Carioka	29	0.08	2.21	0.68	50	86	19	5
Maini	33	0.09	2.34	0.58	48	85	12	4
BAT 477	18	0.08	2.27	0.61	49	86	19	6
Mshindi	31	0.08	2.35	0.49	38	78	10	4
G51396A	0	0.00	2.14	0.52	45	83	17	6
Selian97	32	0.07	2.74	0.55	45	84	11	4
Bilfa 4	24	0.08	2.03	0.48	48	84	15	5
Njano	23	0.08	2.06	0.47	41	82	15	4
DOR 364	19	0.06	2.36	0.61	49	86	24	6
Sua90	34	0.07	2.14	0.52	43	83	11	5
Rojo	22	0.07	2.57	0.62	40	78	11	4

VARIABLES

Pesa	37	0.09	2.46	0.46	39	78	10	5
Lymng 85	32	0.07	2.49	0.62	44	82	12	3
Kablanket	32	0.09	1.99	0.39	38	75	9	4
Grand mean	24.29	0.07	2.26	0.54	44.03	81.90	13.86	4.71
L.S.D(0.05)	2.59	0.02	0.50	0.13	2.47	1.74	2.23	0.39
SE	2.77	0.02	0.53	0.14	2.64	1.86	2.38	0.42
CV %	11.40	30.80	23.60	26.50	6.00	2.30	17.20	8.80

Appendix 10: continues,

		V	ARIAB	LES			
Genotyeps	wt of seeds/pla nt	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	seed.% N	Ureides (µM/g)
Zawadi	11.86	32.03	0.80	2.25	3.47	3.15	3.87
G4445A	9.05	17.11	0.76	1.72	3.28	2.80	2.90
Carioka	9.72	19.44	0.75	1.85	3.55	3.06	2.56
Maini	9.17	28.26	0.73	1.74	3.51	3.15	3.23
BAT 477	15.97	18.41	0.84	3.03	3.51	2.91	2.83
Mshindi	12.40	30.04	0.81	2.36	3.46	3.11	3.99
G51396A	8.72	16.10	0.72	1.60	3.22	2.88	2.19
Selian97	15.38	40.79	0.82	2.92	3.42	3.23	4.22

Bilfa 4	16.76	25.74	0.87	3.18	3.58	3.36	2.79
Njano	15.88	39.19	0.86	3.02	3.82	3.36	2.63
DOR 364	16.47	18.91	0.85	3.13	3.60	3.04	3.66
Sua90	10.60	27.10	0.80	2.01	3.48	2.97	2.76
Rojo	12.31	33.81	0.79	2.34	3.45	3.13	3.78
Pesa	11.27	37.98	0.78	2.14	3.54	3.23	3.55
Lymng 85	15.00	41.23	0.82	2.85	3.38	3.05	3.01
Kablanket	12.78	42.76	0.84	2.43	3.48	3.04	3.35
Grand mean	12.71	29.31	0.80	2.41	3.48	3.09	3.21
L.S.D(0.05)	2.84	1.64	0.06	0.53	0.05	0.09	0.38
SE	3.03	1.75	0.06	0.57	0.05	0.09	0.41
CV %	23.90	6.00	7.80	23.50	1.50	3.00	12.70

Appendix 11: Mean effects of genotypes for the studied variables at Morogoro

			VA	RIABLES				
Genotypes	Nod No/p lant	Nod dwt(g/p lant)	Sdwt (g/plant	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/ plant	No of seeds/p od
Zawadi	20	0.02	6.06	0.73	31	67	13	5
G4445A	0	0.00	7.25	1.09	37	78	24	6
Carioka	18	0.04	6.44	1.03	37	81	18	5
Maini	14	0.03	4.60	0.55	35	78	18	4
BAT 477	8	0.01	5.87	0.72	36	80	20	5
Mshindi	10	0.02	5.12	0.61	31	67	14	5
G51396A	0	0.00	8.03	1.01	37	77	23	6
Selian97	11	0.02	6.83	0.63	33	77	14	5

Bilfa 4	19	0.03	5.40	0.76	34	76	18	5
Njano	10	0.01	4.67	0.56	32	76	17	5
DOR 364	12	0.02	5.18	0.70	37	80	24	5
Sua90	6	0.00	5.91	0.75	33	72	18	5
Rojo	19	0.02	6.07	0.79	31	68	12	5
Pesa	17	0.02	5.44	0.70	31	68	12	5
Lymng 85	34	0.04	5.68	0.89	35	78	16	4
Kablanket	9	0.01	6.30	0.40	31	68	12	5
Grand mean	12.9 4	0.02	5.93	0.74	33.92	74.33	17.06	5.02
L.S.D(0.05)	3.53	0.01	1.40	0.21	0.71	2.08	3.58	0.74
SE	3.77	0.01	1.50	0.23	0.76	2.22	3.82	0.79
CV %	29.1 0	49.90	25.30	30.30	2.20	3.00	22.40	15.70

Appendix 11: continues,

		VA	RIABLI	ES			
Genotypes	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot.% N	seed. %N	Ureides (µM/g)
Zawadi	13.32	35.48	0.66	2.53	3.06	3.80	4.78
G4445A	12.89	19.72	0.60	2.45	3.24	3.78	3.56
Carioka	14.50	28.72	0.66	2.75	3.30	3.93	3.42
Maini	13.35	36.90	0.71	2.43	3.23	4.07	4.36
BAT 477	13.64	25.84	0.67	2.59	3.28	3.82	5.02
Mshindi	14.10	32.81	0.71	2.62	3.25	3.60	5.77
G51396A	11.44	19.67	0.57	2.17	3.32	3.88	4.01
Selian97	17.16	48.38	0.70	3.26	3.31	4.00	5.17

Bilfa 4	16.52	33.14	0.72	3.14	3.17	3.97	4.73
Njano	12.11	45.00	0.69	2.26	3.38	4.08	5.59
DOR 364	14.80	26.19	0.71	2.81	3.32	3.93	4.93
Sua90	14.26	31.34	0.68	2.65	2.98	3.53	3.89
Rojo	10.55	40.92	0.61	1.96	3.04	3.66	3.79
Pesa	12.98	44.46	0.67	2.47	3.09	3.71	3.95
Lymng 85	13.31	56.41	0.66	2.53	3.09	3.78	5.32
Kablanket	14.40	47.49	0.68	2.63	3.05	3.50	5.03
Grand mean	13.71	35.78	0.67	2.58	3.20	3.82	4.58
L.S.D(0.05)	3.27	2.25	0.06	0.65	0.27	0.21	1.01
SE	3.49	2.41	0.06	0.69	0.29	0.23	1.08
CV %	25.50	6.70	9.40	26.90	9.00	6.00	23.60

Appendix 12: Mean effects of genotypes for the studied variables at Arusha

		VARIAB	SLES					
Genotypes	Nod No/pl ant	Nod dwt(g/ plant)	Sdwt (g/plant	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/pl ant	No of seeds/ pod
Zawadi	61	0.09	1.06	0.18	36	73	6	5
G4445A	0	0.00	0.66	0.17	46	85	7	4
Carioka	41	0.09	1.46	0.29	46	84	9	5
Maini	31	0.07	1.30	0.22	45	84	8	5
BAT 477	28	0.08	1.00	0.18	46	84	10	6
Mshindi	31	0.06	1.12	0.14	38	75	7	4
G51396A	0	0.00	0.94	0.19	41	81	8	4

Selian97	50	0.09	1.10	0.18	41	83	6	4
Bilfa 4	27	0.07	1.36	0.20	42	82	9	5
Njano	27	0.08	0.78	0.16	37	80	7	3
DOR 364	37	0.09	1.39	0.23	47	84	9	5
Sua90	34	0.06	0.90	0.15	40	79	7	5
Rojo	50	0.10	1.29	0.24	37	77	7	4
Pesa	53	0.10	0.87	0.18	37	77	6	4
Lymng 85	43	0.10	1.54	0.27	38	78	8	4
Kablanket	45	0.08	1.42	0.17	36	73	8	4
Grand mean	34.92	0.07	1.14	0.20	40.80	79.97	7.60	4.37
L.S.D(0.05)	5.58	0.02	0.32	0.05	2.24	2.73	1.42	0.71
SE	5.96	0.02	0.34	0.05	2.40	2.91	1.52	0.76
CV %	17.10	30.80	29.70	25.00	5.90	3.60	20.00	17.30

Appendix 12: continues,

	VARIABLES										
Genotypes	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	seed. %N	Ureides (µM/g)				
Zawadi	5.75	31.31	0.82	1.09	3.40	3.32	2.56				
G4445A	3.46	17.96	0.78	0.66	2.52	3.54	1.69				
Carioka	4.30	23.26	0.71	0.82	3.41	3.67	3.98				

Maini	4.41	30.31	0.75	0.84	3.29	3.73	2.81
BAT 477	5.00	20.67	0.81	0.95	3.23	3.52	3.35
Mshindi	5.31	28.14	0.81	1.01	3.24	3.19	2.67
G51396A	4.55	19.79	0.80	0.86	2.60	3.61	1.41
Selian97	5.02	38.38	0.79	0.95	3.30	3.63	4.95
Bilfa 4	6.71	27.02	0.81	1.28	3.53	3.55	3.74
Njano	5.60	32.63	0.85	1.07	3.61	3.47	2.51
DOR 364	4.50	21.63	0.74	0.86	3.33	3.59	2.30
Sua90	5.70	26.31	0.84	1.08	3.27	3.40	4.44
Rojo	5.87	34.36	0.79	1.12	3.21	3.54	2.68
Pesa	5.09	36.22	0.83	0.97	3.25	3.41	3.17
Lymng 85	5.66	40.19	0.76	1.08	3.30	3.40	3.59
Kablanket	5.68	39.36	0.79	1.08	3.40	3.46	2.09
Grand mean	5.16	29.22	0.79	0.98	3.24	3.50	3.00
L.S.D(0.05)	0.70	2.89	0.06	0.13	0.26	0.12	0.74
SE	0.75	3.09	0.06	0.14	0.27	0.13	0.79
CV %	14.60	10.60	7.40	14.60	8.50	3.80	26.30

			VA	RIABLES				
Inoculants	Nod No/p lant	Nod dwt(g/ plant)	Sdwt (g/plant	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/ plant	No of seeds/pod
Contol	22	0.04	2.97	0.49	39	79	12	5
Bs	26	0.06	5.93	0.50	40	79	13	5
Ns	24	0.05	1.14	0.50	40	79	13	5
Grand mean	24.0 5	0.05	3.11	0.49	39.58	78.73	12.84	4.70
L.S.D(0.05)	1.58	0.01	0.50	0.08	0.44	0.42	1.06	0.16
SE	1.54	0.01	0.49	0.08	0.43	0.41	1.04	0.15
CV %	6.40	10.40	15.70	16.70	1.10	0.50	8.10	3.30

Appendix 13: Mean effect of inoculants for the studied variables across locations

Appendix 13: Continues,

			VARIAI	BLES			
Inoculants	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot.% N	seed. % N	Ureides (µM/g)
Control	10.00	21.24	0.74	1.04	2.20	2 41	2.02
Contol	10.28	31.24	0.76	1.94	3.29	3.41	2.82
Bs	10.82	31.67	0.67	2.04	3.34	3.53	4.21
Ns	10.48	31.40	0.79	1.99	3.30	3.47	3.76
Grand mean	10.53	31.44	0.75	1.99	3.31	3.47	3.60
L.S.D(0.05)	1.51	1.07	0.02	0.30	0.08	0.07	0.42
SE	1.47	1.04	0.02	0.29	0.08	0.07	0.41
CV %	14.00	3.30	3.10	14.60	2.50	2.00	11.50

			1	VARIAB	LES			
Genotypes	Nod No/pl ant	Nod dwt(g/plant)	Sdwt (g/plant	Rdwt (g/pla nt)		85%	No of	No of seeds /pod
Zawadi	35	0.06	3.13	0.46	35	72	9	4
G4445A	0	0.00	3.24	0.61	44	83	17	5
Carioka	29	0.07	3.37	0.67	44	83	15	4
Maini	26	0.06	2.75	0.45	42	82	13	4
BAT 477	18	0.05	3.05	0.50	44	83	16	5
Mshindi	24	0.05	2.86	0.41	36	73	11	5
G51396A	0	0.00	3.71	0.57	41	80	16	4
Selian97	31	0.06	3.56	0.45	40	81	10	2
Bilfa 4	23	0.06	2.93	0.48	41	81	14	5
Njano	20	0.06	2.50	0.40	37	79	13	2
DOR 364	23	0.05	2.98	0.51	44	83	19	4
Sua90	25	0.04	2.98	0.47	39	78	12	4
Rojo	31	0.06	3.31	0.55	36	75	10	4
Pesa	35	0.07	2.92	0.45	36	74	9	4
Lymng 85	36	0.07	3.24	0.59	39	79	12	4
Kablanket	29	0.06	3.24	0.32	35	72	10	2
Grand mean	24.05	0.05	3.11	0.49	39.58	78.73	12.84	4.70
L.S.D(0.05)	2.29	0.01	0.46	0.08	1.14	1.29	1.43	0.36
SE	4.28	0.02	0.87	0.14	2.12	2.40	2.67	0.68
CV %	17.80	34.50	27.80	29.30	5.40	3.00	20.80	14.40

Appendix 14: Mean effect of genotypes for the studied variables across locations

Appendix 14:	Continues,
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		V	ARIAB	SLES			
Genotypes	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot.% N	seed.%N	Ureides (µM/g)
Zawadi	10.31	32.94	0.76	1.96	3.31	3.43	3.73
G4445A	8.46	18.26	0.71	1.61	3.01	3.37	2.71
Carioka	9.51	23.81	0.71	1.81	3.42	3.55	3.32
Maini	8.98	31.82	0.73	1.67	3.34	3.65	3.47
BAT 477	11.53	21.64	0.77	2.19	3.34	3.42	3.74
Mshindi	10.61	30.33	0.78	1.99	3.32	3.30	4.15
G51396A	8.24	18.52	0.70	1.55	3.05	3.46	2.53
Selian97	12.52	42.51	0.77	2.38	3.34	3.62	4.78
Bilfa 4	13.33	28.64	0.80	2.53	3.43	3.63	3.75
Njano	11.20	38.94	0.80	2.11	3.60	3.64	3.58
DOR 364	11.92	22.24	0.77	2.27	3.42	3.52	3.63
Sua90	10.19	28.25	0.77	1.92	3.24	3.30	3.69
Rojo	9.58	36.36	0.73	1.81	3.23	3.44	3.42
Pesa	9.78	39.55	0.76	1.86	3.30	3.45	3.56
Lymng 85	11.32	45.94	0.75	2.15	3.26	3.41	3.98
Kablanket	10.96	43.20	0.77	2.05	3.31	3.33	3.49
Grand mean	10.53	31.44	0.75	1.99	3.31	3.47	3.60
L.S.D(0.05)	1.32	1.27	0.03	0.26	0.12	0.08	0.40
SE	2.47	2.37	0.06	0.48	0.23	0.15	0.75
CV %	23.50	7.60	7.90	23.90	6.80	4.40	20.80

	Nod No/plant	Nod dwt(g/ plant)	Sdwt (g/pla nt	Rdwt (g/pla nt)	Days to 50%f	Days to 85%m	No of pods/ plant	No of seeds/pod
Locations								
Mbeya	24	0.07	2.26	0.54	44	82	14	5
Morogoro	13	0.02	5.93	0.74	34	74	17	5
Arusha	35	0.07	1.14	0.20	41	80	8	4
Grand mean	24.05	0.05	3.11	0.49	39.58	78.73	12.84	4.70
L.S.D(0.05)	0.92	0.01	0.34	0.07	1.36	2.53	1.09	0.24
SE	0.41	0.01	0.15	0.03	0.60	1.12	0.48	0.11
CV %	1.70	12.00	4.80	6.20	1.50	1.40	3.80	2.30

Appendix 15: Mean effect of locations for the studied variables

Appendix 15: continues,

Locations	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	seed. %N	Ureides (µM/g)
2.4	10.71	20.21	0.00	0.41	2 49	2.00	2.21
Mbeya	12.71	29.31	0.80	2.41	3.48	3.09	3.21
Morogoro	13.71	35.78	0.67	2.57	3.20	3.82	4.58
Arusha	5.16	29.22	0.79	2.58	3.24	3.50	3.00
Grand mean	10.53	31.44	0.75	1.99	3.31	3.47	3.60
L.S.D(0.05)	3.59	1.61	0.06	0.71	0.19	0.12	1.4
SE	1.58	0.71	0.03	0.31	0.09	0.05	0.61
CV %	15.00	2.30	3.50	15.70	2.60	1.50	17.00

VARIABLES									
Fertilizer type	Nod No/pl ant	Nod dwt(g/pla nt)	Sdwt (g/pla nt	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/plant	No of seeds/ pod	
Control	0	0.000	2.01	0.06	31	70	9	4	
Ns	5	0.006	2.77	0.10	32	70	10	4	
Bs	5	0.005	2.42	0.09	32	70	10	4	
Ν	0	0.000	1.87	0.10	32	70	10	4	
Ns+N	3	0.004	1.60	0.09	31	70	10	4	
Bs+N	4	0.006	1.44	0.10	32	70	11	4	
Р	0	0.000	3.02	0.11	32	70	11	4	
Ns+P	8	0.009	4.12	0.14	31	70	12	4	
Bs+P	8	0.007	3.89	0.14	32	70	12	5	
N+P	0	0.000	3.48	0.10	31	70	11	4	
Ns+N+P	4	0.005	2.99	0.09	32	70	11	4	
Bs+N+P	5	0.005	2.87	0.08	32	70	11	5	
G.Mean	3.51	0.004	2.71	0.10	31.63	70.17	10.68	4.35	
L.S.D(0.05)	0.72	0.002	0.78	0.02	0.71	0.55	1.03	0.57	
SE	0.76	0.002	0.83	0.02	0.75	0.59	1.10	0.61	
CV %	21.70	47.30	30.70	23.60	2.40	0.80	10.30	13.90	

Appendix 16: Mean effect of fertilizer types for the studied variables in screen house experiment

Appendix 16: Continues,

VARIABLES								
	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot.% N	seed.% N		
tilizer type								
Control	9.95	30.94	0.31	1.79	3.65	3.35		
Ns	13.00	34.24	0.38	2.34	3.61	3.67		
Bs	13.57	34.48	0.39	2.44	3.64	3.75		

Ν	14.05	34.17	0.40	2.53	3.94	3.82	
Ns+N	13.76	33.28	0.42	2.48	4.07	3.86	
Bs+N	18.28	38.69	0.47	3.29	3.93	3.63	
Р	16.28	37.28	0.44	2.93	3.20	3.38	
Ns+P	17.41	35.00	0.47	3.13	3.72	3.68	
Bs+P	19.84	37.60	0.52	3.57	3.81	3.85	
N+P	17.08	37.41	0.46	3.07	3.68	3.77	
Ns+N+P	16.64	36.92	0.45	2.99	3.55	3.94	
Bs+N+P	18.37	38.63	0.49	3.31	3.84	3.79	
Grand mean	15.68	35.72	0.43	2.82	3.72	3.71	
L.S.D(0.05)	3.59	4.94	0.08	0.65	0.18	0.21	
SE	3.82	5.25	0.08	0.69	0.19	0.23	
CV %	24.30	14.70	19.30	24.30	5.10	6.10	

Appendix 17: Mean effect of inoculants for the studied variables in screen house experiment

	VARIABLES										
Genotypes	Nod No/pl ant	Nod dwt(g/p lant)	Sdwt (g/plant	Rdwt (g/plant)	Days to 50%f	Days to 85%m	No of pods/pl ant	No of seeds/p od			
Kablanketi	5	0.006	2.94	0.09	30	68	11	4			
Rojo	6	0.006	2.46	0.11	31	69	11	4			
G 51105-A	0	0.000	2.73	0.10	33	75	11	5			
Grand mean	3.51	0.004	2.71	0.10	32	70	11	4			
L.S.D(0.05)	0.36	0.001	0.39	0.01	0	0	1	0			
SE	0.76	0.002	0.83	0.02	1	1	1	1			
CV %	21.70	47.30	30.70	23.60	2	1	10	14			

Appendix 17: Continues.

	VARIABLES								
Genotypes	wt of seeds/plant	100 seeds wght	H. Index	Yield kg/ha	shoot. %N	seed. %N			
Kablanketi	18.26	42.18	0.43	3.29	3.77	3.87			
Rojo	18.81	42.16	0.44	3.39	3.93	3.97			
G 51105-A	9.99	22.83	0.43	1.80	3.45	3.29			
Grand mean	15.68	35.72	0.43	2.82	3.72	3.71			
L.S.D(0.05)	1.79	2.47	0.04	0.32	0.09	0.11			
SE	3.82	5.25	0.08	0.69	0.19	0.23			
CV %	24.30	14.70	19.30	24.30	5.10	6.10			

