EVALUATION OF EFFECTIVENESS OF RHIZOBIA ISOLATES FROM RWANDAN SOILS ON COMMON BEAN (*Phaseolus vulgaris*)

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DECLARATION

This thesis is my original work and has not been presented for award of a degree/esearch in any other university.

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DEDICATION

This thesis is dedicated to my parents late Iyamuremye Theodomir and Mukabaziga Concessa, my wife Mukashema Umurerwa Chantal, my kids, my brothers, my sisters, their families, Buhiga family and all descendants from Gisazi Nkeramugaba.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA: Analysis of variance

Anthr: Anthracnose

Asc: Ascochytose

ALS: Angular leaf spot

BCMV: Bean common mosaic virus

Bact: Bacteria

CIAT: International Center for Tropical Agriculture

ISAR: Institut des Sciences Agronomiques du Rwanda (Rwanda Agronomic Sciences Research Institute)

ISAE: Institut Superieur d'Agriculture de l'Elevage (High Institute of Agriculture and Husbandry)

LSD: Least significant difference

Rlle: Rust (Fungal disease)

RAB: Rwanda Agriculture Board

MPN: Most probable number

NAR: N_2Africa in Rwanda (Rhizobia isolates code)

YMM: Yeast-mannitol medium

ABSTRACT

Greenhouse and field experiments were conducted to evaluate the effectiveness of rhizobia isolates from two Rwandan soils and their effectiveness on two types of common bean under different agro ecological zones. A preliminary greenhouse experiment using Leonard Jars in Rubona Station was conducted to evaluate the potential of 174 rhizobia isolates. A selection criterion was based on nodule number, color, size and nodule weight. After 21 days, the best 50 isolates were selected for further evaluation in pots. Highly effective isolates of rhizobia for Common bean were identified through a stepwise approach.. The effectiveness of rhizobia isolates on two types of beans (bush bean (RWR 1667) and climbing bean (Gasilida) was conducted in a pot experiment using CIAT 889, UMR 1597 as standard for comparison and and nitrogen nutrient as control. Both bush and climbing beans were inculated with appropriate rhizobia. tThe five best rhizobial isolates were (NAR 265, NAR 151, NAR 139, NAR75 and NAR 206) were evaluated for their effectiveness in two agro- ecological zones Ruhunde in the North and Rubona in the South. The design was a randomized completed block with nine treatments and three replicates. Before sowing, bean seeds were inoculated with test isolates, commercial strains (CIAT 889 and UMR 1597) while other bean plots were supplied with nitrogen and control plots were not inoculated.

The results showed that beans inoculated with Rwanda isolates had significantly higher number of nodules, plant dry fresh and weight at maturity. The nodules were also large and pink copared to where there no inoculation. These parameters compared well with beans inoculated with standard strains. The analysis of variance on biomass for N total % and P% showed that the difference was highly significant. The study concluded that five rhizobial isolates from Rwanda are highly effective on bush and climbing beans and compared well with rhizobia strains CIAT 899. The results further showed that these strains

were not only effective on beans but also able to reduce disease severity on beans thereby boosting bean production in Rwanda.

CHAPTER 1: GENERAL INTRODUCTION

Soil fertility degradation by nutrient depletion, caused by crop removal or by erosion is the greatest threat facing agricultural systems in Rwanda (Miniterre/Rwanda, 2003). Legumes are an important component of agricultural systems because of nitrogen fixation provided by their root nodule symbiosis with rhizobia (Maria et al, 2000). In many cases, inoculation with rhizobia serves to increase that nitrogen fixation (Giller, 1991). Rhizobium strains selected for use as inoculants must possess two important characteristics: show high nitrogen-fixing ability with their target host legume (Howison et al. 2000), but also the inoculant strains should be able to compete with indigenous rhizobia present in soils and capable of nodule formation on a plant host (Mårtensson 1989). Triplett (1990) indicated that a high competitiveness of inoculant strains in comparison with native rhizobia strains is as important as the effectiveness of symbiotic N_2 fixation itself. Rhizobium symbiosis with legumes species is of special importance, producing 50% of 175 million tons of annual biological nitrogen fixation worldwide (Sarioglu *et al.,* 1993).

Nitrogen deficiency can severely limit plant growth and productivity, particularly in legumes, where both plants and symbiotic bacteria are affected and this may have a definite effect on nodule formation, development and function (Miao *et al,*. 2007). Nitrogen is known to be an essential nutrient for plant growth and development. Intensive farming practices that achieve high yield require chemical fertilizers, which are costly but may also create environmental problems. The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been growing level of interest in environmentally friendly sustainable agricultural practices and organic farming system (Rigby and Caiceres, 2001; Lee and Song, 2007). Increasing and extending the role of biofertilizers such as legumes inoculants would reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementation of sustainable agriculture

techniques, biofertilization is of great importance in alleviating environmental pollution and the deterioration of nature (Elkoca et al., 2008).

In Rwanda, N depletion in most croplands is due to no application or addition of small quantities of fertilizers below the recommended rates and as a result, cereal, legumes and tubers yields are unsustainably low (<1 t ha $^{-1}$) as reported by ISAR (2000). Increased BNF by field legumes can reduce this ominous trend (Woomer et al., 1997).

1.1 Statement of the problem

Low productivity is a general problem facing most farming systems in sub-Saharan Africa (SSA). These low yields are pronounced in grain legumes and are often associated with declining soil fertility and reduced N2-fixation due to biological and environmental factors (Chianu *et al*., 2010*).* Beans often demonstrate reduced physiological potential for symbiotic nitrogen fixation, however, they are preferred for their quick maturity, tolerance to short-term drought, ease of harvesting, rapid cooking and favorable taste therefore many farmers are reluctant to consider other legumes (Woomer *et al.,* 1999). However, common beans are often considered as rather poor nitrogen fixers, although there are reports indicating high levels of fixation as well as the isolation of more efficient bean rhizobia (Aguilar *et al.,* 2001). Nitrogen replenishment particularly in smallholder agriculture remains a challenge as it is mainly fertilizer dependent. Nitrogen deficiency is one of the most widespread nutritional problems in most agricultural soils of Rwanda. Many soils are acidic and infertile representing N deficiency (ISAR, 2000). Yield responses of common bean to inoculation with a specific *Rhizobium* spp. are often variable and depend on environmental and agronomic factors (Tamimi, *2002*). This variability often limits the use of commercially available rhizobial inoculants and emphasizes the need to explore the potential of indigenous rhizobial strains for improving the symbiotic performance of *Phaseolus vulgaris.*

This study aims at evaluating the effectiveness of rhizobial isolates from Rwanda soils when the strains are used to inoculate the common bean.

1.2 Justification

Industrialization and green revolution have brought about an increase in productivity but have also resulted in massive environmental degradation**.** Extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been a growing level of interest in environmental friendly sustainable agricultural practices and organic farming systems (Rigby et al., 2001; Lee et al., 2007). Increasing and extending the role of biofertilizers such as legume inoculants decrease the need for chemical fertilizers and reduce adverse environmental effects. Development and implementation of sustainable agriculture techniques, such as biofertilizers is of major importance in alleviating environmental pollution and the deterioration of nature (Ogutcu et al., 2008). Rhizobia are a common soil bacteria and not toxic to humans, plants or animals. It is one of the most beneficial bacteria in agricultural practices. Some rhizobia are specific and nodulate only few hosts, while others may nodulate several legumes. Native rhizobia may be in sufficient numbers to nodulate both native and introduced legumes. In general, native *Rhizobium* are less effective than inoculant rhizobia, but are often much more numerous and competitive. Native rhizobia are adapted to their soil environments and responsive to environmental factors affecting their environmental niches (Somasegaran and Hoben, 1994).

Rhizobia entering into symbiosis with leguminous plants can produce nodules and fix nitrogen, which amounts to approximately 65% of the global biological nitrogen fixation, hence playing an important ecological role in nitrogen circulation on earth (Baoling*, et al.,* 2007*).* Although most farmers' think a response to inoculating their crops means yield increases, there are other important benefits such as improved protein content of seed or improved nodulation which means more BNF.

Effective rhizobia are essential to providing a beneficial symbiotic relationship with the host legume. In most parts of the world there is a broad range of rhizobial strains which vary in the degree of effectiveness and competitiveness. In some areas very effective and competitive strains may be the major constituents of the native rhizobial populations, but in other areas these strains may be: (1) lacking or (2) less effective and/or (3) less competitive. In the latter cases where there is no native rhizobial population or satisfactory strain, introduction of a superior strain must be made to create a greater potential for maximum yield (i.e. increase in nitrogen fixation). Many recent studies have been done which establish that inoculation with a superior strain is a method for increasing yields in legumes (Dube, 1976; Ham, Caldwelland Johnson, 1971; Subba Rao, 1975; Sundara-Rao et al, 1975; Sundara Rao, 1976). Some commercially prepared inoculants have also improved yield (Able and Erdman, 1964; Chhonkar and Negi, 1971; Dube, 1976; Dunphy, 1978, Dunphy et al., 1977). Before beginning any study on improving yield (enhancing nitrogen fixation) in legumes through Rhizobium strain selection, there must be an assessment of the need for inoculation.

1.3 Objectives

1.3.1 Broad Objective

To identify superior strains of native rhizobia associated with beans and establish their suitability for use as bean inoculants in Rwanda.

1.3.2 Specific Objectives

1. To identify elite rhizobia isolates from Rwandan soils.

2. To evaluate the effectiveness of isolated elite rhizobia strains from Rwanda and their effectiveness as innoculants for common beans in Rwanda.

3. Investigate the role of rhizobia isolates in reducing disease severity on beans

1.4 Working hypothesis

1. Rwanda soils have potential elite Rhizobia isolates, suitable to use in inoculants

2. Elite rhizobial isolates from Rwanda soils improve biomass and grain yields of beans.

3. Rwanda rhizobia isolates increase tolerance to diseases when used as inoculants on Common beans.

1.5 Outline of the thesis

This thesis is divided into six chapters addressing the evaluation of effectiveness of among rhizobial isolates from Rwanda soils on Common bean (*Phaseolus vulgaris*).

The first chapter provides the general introduction, the second presents the literature review and the third describes the materials and the methods. Chapter four documents the evaluation of the effectiveness of Rhizobia isolates from Rwanda soils on Common bean in the green house.

Chapter five discusses the performance of the best rhizobial isolates from Rwanda soils on Common bean in field and the last chapter concerns the general conclusions of the study and recommendations for using the rhizobial isolates selected in Rwanda.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin of bean

Common beans (*Phaseolus vulgaris L*.) originated from Latin America and have two primary centers of origin in the Mesoamerican and Andean regions that are easily distinguished by molecular means (Blair *et al*., 2006). Common bean, also referred to as dry bean, is an annual leguminous plant that belongs to the genus, *Phaseolus,* with pinnately compound trifoliate large leaves. It is largely a self-pollinated plant though cross-pollination is possible if the stigma make contact with pollen coated bee. Seeds are non-endospermic and vary greatly in size and color from the small black wild type to the large (7-16 mm long) white, brown, red, black or mottled seeds of cultivars (Katungi *et al*., 2009). Common bean shows variation in growth habits from determinate bush to indeterminate, aggressive climbing types. The bushy type bean is the most predominant type grown in Africa although climbers often greater yields (Buruchara, 2007).

2.2 Production and utilization of common beans

Common bean is used almost entirely for human consumption but beans require processing before they are eaten to degrade the toxic compound, lectin phyto-haemaglutinin, which would otherwise cause severe gastric upset (Ferris and Kaganzi, 2008). It is the most important food legume crop grown worldwide (Wortmann and Allen, 1994; Wortmann *et al*., 1998; Buruchara, 2006). Beans are considered by many to be the perfect food as they are nutrient dense with high contents of proteins, micronutrients, vitamins, dietary fiber, and also have a low glycemic index (Wortmann and Allen, 1994; Bennink, 2005; Widers, 2006). Common bean is grown extensively in five major continental areas: eastern Africa, North and Central America, South America, Eastern Asia, and Western and South-Eastern Europe (Adam, M.W. (1967).

Diverse forms of bean consumption including fresh or dry grains, green leaves and green pods (Kimani *et al.*, 2006) are common in Rwanda. World annual global production of dry beans is estimated at 19.5 million tons

with Brazil being the highest producer with an estimated annual production of 4 million tons (FAOSTAT, 2007). In Rwanda common beans play important roles in smallholder farmers' strategies for incomes, food security, nutrition, natural resource management and gender (Rusike, 2011). Rwanda has been among the countries which produce highest yields of beans, for example 9.151 Kg ha^{-1} (FAO, 2008). Deficient levels of nitrogen, results in poor yields therefore to improve bean yields in absence of effective rhizobia, it is recommended that nitrogen fertilizer should be applied. However, most of resource poor small scale farmers are unable to afford N fertilizers. The cheaper option, therefore, is to exploit biological nitrogen fixation through inoculation with rhizobia and use bean genotypes that respond well to inoculation (Waddington, 2003).

Common bean provide livestock feed and their crop residues offer benefit to soils through BNF that, in turn reduce the requirement for costly mineral fertilizers. A small-scale farming household that has incorporated legumes into enterprises is in better position to raise its wellbeing and to meet expectations in improved living standards (CIAT, 2009).

Legumes intensification was also found to increase subsequent cereal yield by approximately 40% with a net benefit increase of US $ 50 ha^{-1}(Snapp *et al*,2003).

2.3 Bean consumption

Beans are eaten as cooked dry or fresh grain, green leaves or pods by nearly all Rwandans, on a daily basis especially among the rural population. Beans contribute 84% of the pulse legume, and 65% of all plant and animal sources of proteins of Rwandan diets (Grisley, 1990). Beans are thus regarded as the *meat* for the poor (MINAGRI, 2000). Beans also contribute generously towards calories intake (32%) and the micronutrients: iron, zinc and vitamins A and B that enhance normal body and cognitive growth and development. Due to this diversified nutrients content, beans are regarded as a near-perfect food (CIAT, 1995).

However, there is a gap between consumption and production rates of about 20 to 30 kg per capita, making Rwanda a net importer of beans. This is mainly due to the fact that Rwanda is one of the highest consumers of beans in the world (50 – 60 kg per person) and its high population increase exacerbates consumption while constraining the scarce land resources; hence the overall decline in production potential (Ferris *et al*., 2002). Regular consumption of common bean and other pulses is now promoted by health organizations because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (Leterme, 2002, Leterme and Munoz, 2002). This is because common bean is low in fat and is cholesterol free. It is also an appetite suppressant because it digests slowly and causes a low sustained increase in blood sugar. Researchers have found that common bean can delay the reappearance of hunger for several hours, enhancing weight-loss programs.

2.4 Bean production

Common bean is an important component of the production systems and a major source of protein for the poor in Eastern and Southern Africa. Although largely grown for subsistence, and mainly by women, approximately 40 percent of production is marketed at a value of UDS 452 million (Wortmann et al., 1999, David *et al*., 2000). In recent years, the crop production trend has not kept pace with the annual growth rate (estimated above 2 percent) in population in some countries due to a number of biotic, abiotic and socio-economic constraints (Kambewa 1997; Chirwa *et al*., Forthcoming and Xavery *et al*., 2006).

In Africa, in terms of average production (tons) the four countries producers are Uganda, Kenya,Tanzanie and Rwanda respectively as shows by table 1

Table 1: List of Top 10 producers of common bean in terms of area in Africa in 2000-2007

Country	Average area (Ha)	Average production (Tons)
Kenya	910,478	412, 381
Uganda	794,75	478,625
Tanzania	373,125	285,414
Rwanda	340,055	231,882
Angola	290,391	92,786
Burundi	249,375	229,607
D.R.C	205,958	110,404
Malawi	197, 605	87,593
Ethiopia	188,000	143,414
Madagascar	820,96	77,273

Source: FAOstat at www.fao.org

2.5 Bean production constraints in Rwanda

Self sufficiency in bean production in Rwanda is severely constrained by field and storage losses due to damage caused by prevalent diseases and pests, (biotic factors) as well as soil and moisture related abiotic problems that are compounded by poor agronomic management practices (*Asareca*, 2013)

2.5.1 Biotic constraints

The important diseases of beans are angular leaf spot (*Phaeoisariopsis griseola*), and root rot caused by complex of soil pathogens, particularly *Pythium, Fusarium* and *Rhizoctonia* species, bean common mosaic virus (BCMV), and anthracnose (*Colletotricum lindemuthiunum*). Ascochyta blight (*Ascochyta phaseolorum*) and halo blight (*Psuedomonas syringae pv. phaseoli*) are important in higher and cooler altitudes (over 1700 m above sea level), while common bacterial blight and bean rust feature in the warmer lower altitudes zones (1000 –1400m asl). The fungal diseases (angular leaf spot, root rots, anthracnose, common blight and rust) alone cause grain yield loss of 219,575 tons per year, equivalent to 89 million USD in Rwanda (Buruchara *et al.*, 1996).

2.5.2 Abiotic constraints

Poor soil fertility (low N, P and K) and acidity are among the most important abiotic constraints. Drought is an important constraint in Eastern regions of Rwanda where the annual rainfall ranges from 800 – 1000 mm , but its erratic nature causes frequent spells of drought that limits bean yields. When beans are under drought stress they tend to flower very early prior to forming tiny and even one or two pods. At this stage whether the rain is resumed, the plants' growth circle would have been adversely affected.

The socio-economic factors that affect productivity include lack of varieties that combine market and consumer preferred seed-types and high yields that leads to slow or poor adoption. Besides their farmer preferred culinary attributes, the red-mottled, red, navy white and yellow seed market classes fetch premiums on urban markets in Rwanda (Spilsbury, *et al.*, 2004).

The low productivity is linked to none use of improved / certified seed whose current supply among farmers is estimated at only 3% necessitating farmers to plant saved seed of local varieties that are recycled over seasons. The yield loss associated with the use of poor seed quality progressively rises to about 86% and 75% of the

potential for the climbing and bush beans respectively. Small land area also disallows good husbandry practices such as rotations and fallows. Continuous cultivation exacerbates the cumulative effects and pressure of the diseases and pests on the bean crop and the depletion of soil nutrient (RADA, 2004).

The use of agro-inputs to replenish the nutrients or to control the pests is very low (the rate of fertilizer application is estimated at 1.3-3% of the recommendation (Kelly *et al*., 2002; Gahakwa, 2005). Lack of inexpressive staking options is a constraint that is peculiar to production of climbing beans, especially in deforested areas where agro-forestry is not well established.

2.6 Soil microorganisms

Plants thrive in a healthy soil environment. The mineral content of the soil and its physical structure are important for this well-being, but it is the life in the earth that powers its cycles and provides its fertility. Without the activities of soil organisms, organic materials would accumulate and litter the soil surface, and there would be no food for plants.

The nitrogen cycle in soils depends on the fixation of atmospheric nitrogen. One way this can occur is in the nodules on the roots of legumes hosts that contain symbiotic bacteria of the genera Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium, and Azorhizobium.

Bacteria are responsible for the process of nitrogen fixation, which is the conversion of atmospheric nitrogen into nitrogen which can be used by plants. Autotrophic bacteria derive their energy making their own food by oxidation, like the Nitrobacters species, rather than feeding on plants or other organisms. The bacteria that are heterotrophs, responsible for nitrogen fixation but the amount of autotrophic bacteria is small compared to heterotrophic bacteria (the opposite of autotrophic bacteria, acquire energy by consuming plants or other microorganisms), but are very important because almost every plant and organism require nitrogen in some way, and would have no way of obtaining it if not for nitrogen-fixing bacteria (Soltner, 2003).

2.7 Free- living Rhizobia in the soil

Rhizobia are facultative microsymbionts that live as normal components of the soil microbial population when not living symbiotically in the root nodules of the host legume. Outside the root nodule, rhizobial are mostly found on the root surface, soil around and close to the root surface, and to a lesser extent, non rhizosphere soil. The increase in numbers of rhizobia in rhizosphere is a response to excretion of nutrients by plants roots, especially the host legume (Broughton, 1981).

Rhizobia are somewhat unique among soil microorganisms in their ability to form N_2-fixing symbioses with legumes and occasionally, a non- legume (Parasponia). To enjoy the benefits of this partnership, any introduced rhizobia must not only exhibit saprophytic competence among other soil microorganisms, but they must out-compete other rhizobia for infection sites on legume roots. Therefore, potential for physiological versatility is an important trait contributing to their adaptation to the competitive and complex soil environment (Broughton, 1981).

2.8 Rhizobia as symbionts

The free-living rhizobia in the soil can enter the roots of the susceptible host legume by a complex series of interactions known collectively as the infection process. This begins with the adhesion of the specific rhizobia to the surface of the roots hair. Adhesion is followed by deformation, and curling of the root hair, which results in the characteristic shepherd's crook appearance. The enzyme nitrogenase is a complex of two enzymes, a Fe-containing protein and Fe-Mo protein. It is responsible for conversion (reduction) of atmospheric N into anion ammonium, and is synthesized in the cytosol on the bacteroids. The legumes utilize anion ammonium to convert certain precursor metabolites into amino acids, which in turn are synthesized into proteins (Somasegaran and Hoben, 1991).

2.9 Rhizobia in nitrogen fixation

While common beans have often been regarded as weak in their ability to fix nitrogen symbiotically, surprisingly large rates of N_2 fixation can be obtained under appropriate conditions (Vincent, 1974). The rates of N_2 fixation equivalent to 64-121 kg N per hectare per growth cycle (Ruschel, *et al.,* 1982) have been reported and give quite consistent values across dissimilar cultural and environmental regimes.

Many legumes have the ability to fix N from the air without the use of commercial fertilizers if inoculated with a nitrogen-fixing bacteria. The N-fixing bacteria for dry bean are called Rhizobium phaseoli and species nodulate with bean. Inoculant used for soybean or pea are different and will not infect dry bean. Unfortunately, the relationship between dry bean and its root nodules is not strong. Dry, hot weather, short periods of soil water saturation, and cold weather, will all result in sloughing off of nodules, so it may be difficult to achieve high dry bean yields consistently using inoculation for an N source (Vincent, 1974).

Dry bean seed is usually inoculated with a fungicides used to control bacterial blight. Until recently, many dry bean producers would not use an inoculation treatment because of the fear that the chemical would also kill the Rhizobium bacteria. It was recently shown that at least some newer strains or formulations resisted the seed treatment, and would produce greater nodule numbers when an inoculant was applied to seed immediately prior to planting. However, higher rates of soil N at planting decreased the number of nodules on the plant. Nitrogen fixation in leguminous plants involves a symbiotic relationship between nitrogen fixing bacteria and legume roots, and occurs within specialized root nodules. Low temperature stress is known to have an adverse effect on leguminous root nodule development(Hungaria et *al,*1982).

However, in several arctic legumes, the ability of the symbiotic nitrogen fixation process to function in a psychrophilic environment suggests a unique evolutionary adaptation and, also the strain of rhizobium

involved in a symbiotic association plays an important role in determining the efficiency of nitrogen fixation at low temperatures (Sarrantonio, 1991).

Despite claims those grain legumes are inefficient N2-fixers (Hardy and Havelka, 1975). Pate (1984) shows that symbiotic nitrogen fixation may cost the legume only 12-15% in photosynthate. Indeed, Gibson (1966) reported no significant difference in growth of clover plants fixing Nitrogen compared those utilizing Nitrate. Therefore legumes should have much the same potential for grain yield as wheat.

2.10 Impact of Rhizobium

In the quest to address declining soil fertility, grain legumes have often been proposed in Integrated Nutrients Management (INM) strategies due to their supply of nitrogen through Biological Nitrogen Fixation (BNF) processes (Sanchez *et al.,* 1997).

Although the magnitude of BNF is methodologically difficult to quantify, overall estimates are in the order of 25 to 100 kg N ha^{-1} per crop for grain legumes (Giller and Wilson, 1991). Besides nitrogen fixation, grain legumes also play an important role in human nutrition and market economies in rural and urban areas of Eastern Africa.

The integration of grain legumes, such as common bean (*Phaseolus vulgaris*) in INM strategies needs to be supported by well-structured research and extension services aimed at increasing capacity of farmers to be better learners and to rise to new challenges and dynamism in the farming environment (Hagmann *et al*., 1998). The development of soil fertility initiatives needs to take farmers perspectives and their indigenous technical knowledge into account if farmers have to adopt the developed technologies. In the past many soil fertility farm interventions have tended to ignore farmer's indigenous wisdom and to follow prescriptive methods of technology development and transfer on the assumption that farmers are ignorant and that they only needed to be told what to do. This has quite often led to selective adoption, modification, socially

discriminatory uptake, early abandonment or plain rejection of technologies on offer and even management methods associated with such technologies.

Grain legumes have been recognized worldwide as an alternative means of improving soil fertility through their ability to fix atmospheric nitrogen, increase soil organic matter and improve general soil structure (Musandu and Ogendo 2001). Besides having low nitrogen fixing ability under field conditions, the yield of beans has greatly declined due to pests and disease infections, mainly the bean-fly and bean root-rot. A sick plant cannot fix much nitrogen from the atmosphere.

2.11 Rhizobiology in Rwanda

The ISAR Microbiology Laboratory leads N2Africa rhizobiology activities in Rwanda and liaises with related actions in DR Congo and Rwanda. The team at ISAR is responsible for both Agronomy and Rhizobiology activities in Rwanda. The Microbiology Laboratory has cultured 80 isolates from bean and soya bean. Twenty-nine of these isolates were characterized and classified by Congo Red morphotype, BTB reaction and Gram Stain. To date, bio-prospecting has focused solely upon common bean (*Phaseolus vulgaris*) and soyabean (*Glycine max*), but 11 other genera and related species in Rwanda were sampled by the University of Nairobi MIRCEN team, reducing this possible additional shortcoming. Seven hundred (700) packets of bean inoculants containing 80g each were recently prepared (56 kg total) for use by project research and dissemination activities in the next growing season. The Soil Microbiology Laboratory of ISAR in Rubona had a strong presence in Rhizobiology in Africa backed by collaborative arrangements starting from the 1977 at the inception of the MIRCEN project hosted by the University of Nairobi, Kenya. The late Athanase Hakizimana was an active member of the MIRCEN core team and the ISA laboratory was a beneficiary of the FAO donated fermentor, autoclave, and laminar flow hood as basic support for pilot plant inoculants production. The laboratory made impressive progress towards collection of rhizobia and their preservation and use for legume production in Rwanda. The tragic events of the 1994 slowed scientific activities that eventually restored

following civil unrest in the country. The laboratory occupies a well designed building and has assembled a team of ambitious young scientists who must now demonstrate their ability to perform the full spectrum of microbiology skills.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Bio-prospection

Nodules were collected from bean crops planted in regularly cultivated farmer's plots at a time when nodulation was best. Generally the best time for nodule formation was when the plants were at the flowering stage. The nodules were placed in pre-sterilized plastic bottles and aseptic procedures observed to avoid cross contamination. The materials were properly labelled and stored in cool conditions before returning to the laboratory. The collection was undertaken at different provinces of Rwanda. Nodules were also collected from uncultivated legumes along an altitudinal transect between 1500 m and 2800 m of elevation. The 174 samples were taken in 12 districts located in four provinces:

(i) Musanze, Gakenke, Rulindo and Burera Districts in Northern Province(yellow)

(ii) Ruhango, Kamonyi, Nyamagabe and Huye Districts in Southern province (blue)

(iii) Nyamasheke and Rusizi Districts in Eastern province (red) and

(iv) Kayonza and Ngoma District in Western province (rose)

Nodules samples were collected aseptically using the sterilized forceps and gloves and reserved in a test tube containing silica gel.

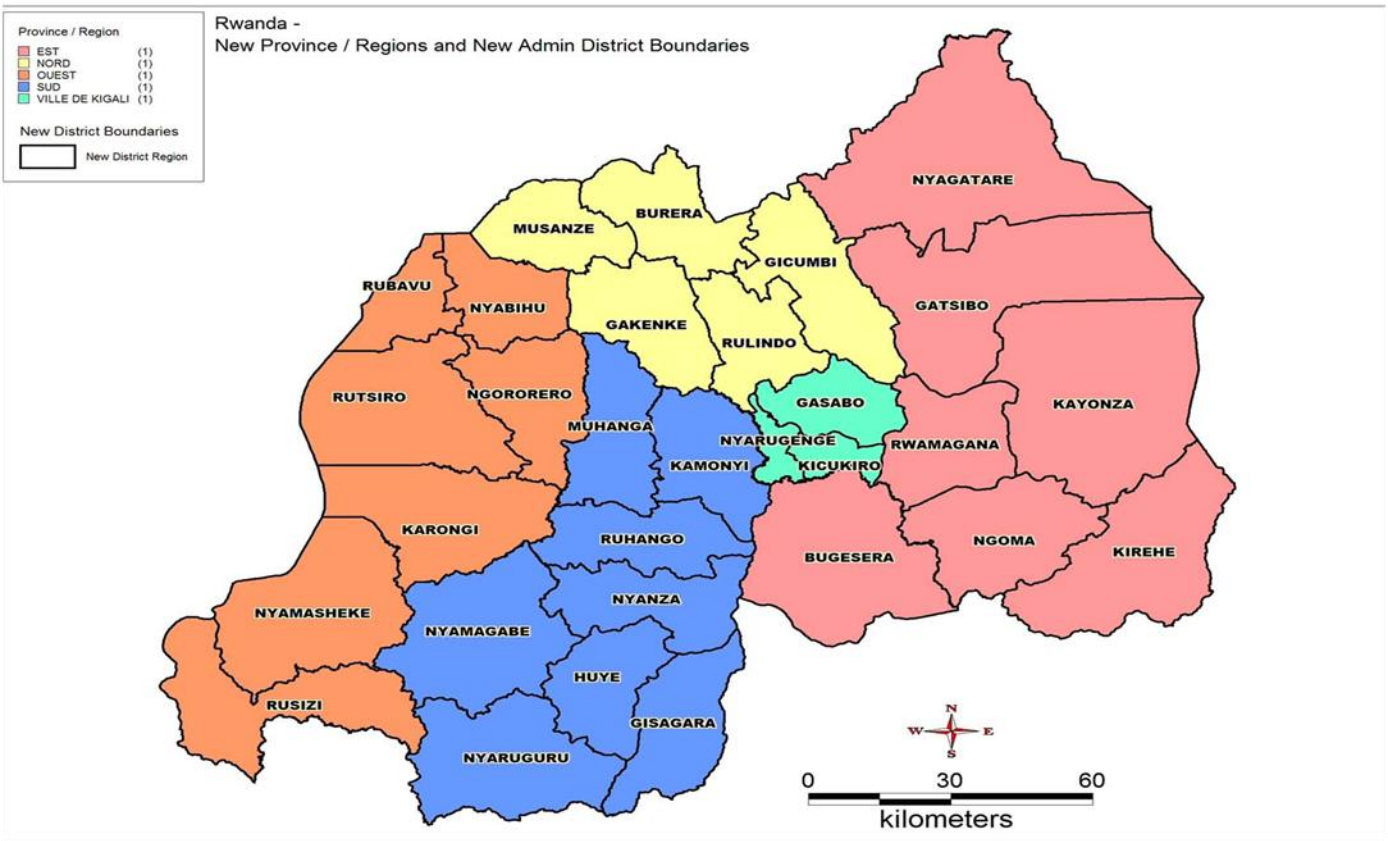


Figure 1: Rwanda Map and its Districts showing where nodules were sampled

3.2 Laboratory activities

3.2.1 Nodule sterilization

Nodules were surface sterilized according to Somasegaran (1994) method, before isolating the Rhizobium from the nodules. The process involved 5 important steps :

(i) Sterile water was poured in a beaker, where nodules were washed.

(ii) They were transferred in a second beaker of alcohol (96%) to remove superficial microbes.

(iii) Nodules from bean crop were immersed in 90% alcohol for one second then washed in a second beaker containing sterile water.

(iv) $HgCl_2$ was also used to remove contamination that might have been present and not removed by alcohol.

(v) Finally nodules were washed again in sterile water and put in sterile Petri dishes using sterile forceps.

A portion of the nodule sterilization process is shown in plate 1 below

3.2.2 Rhizobia isolation

Before isolating the rhizobium from the nodules, they were washed with sterile water.

Once surface sterilized, a loopful of crushed nodule was streaked across the Petri dishes containing yeast monitol agar media and grown in an incubator maintained at optimum temperature of (28° to 30°C) for 2 to 3 days.

The nitrogen fixation potential of the strains was compared by collecting plant growth data and analyzing the results. The process includes 6 steps:

(i) Preparation of culture rhizobial isolates

(ii) Preparation of seeding-agar plates and surface sterilize and germination of seeds

(iii) Pregermination of seeds and thinning

(iv) Inoculation of pregerminated plant followed by watering

(v) Observation of inoculation after 5 weeks

(vi) Collection of data and evaluation of results

Flask where nodules were washed

Plate 1: Assessing of Rhizobia isolates kept in the Rhizobiology lab of Rubona

3.3 Green house experiment

One hundred and seventy four isolates (174) were collected. The isolated were tested for their effectiveness on common beans using 3 liter pots and sterilized soil as media. Soil was covered with plate to minimize contamination from the surrounding, while two openings were developed for t the plants' aeration and a for

watering. A pot experiment was conducted in a greenhouse at ISAR Rubona to study the effect of rhizobia isolates on nodulation, growth and nitrogen fixation of climbing and bush beans. The plastic plate had two holes for the plants 6 cm in diameter and for the watering pipe 2 cm in diameter for The experiment was laid out in a split plot design and replicated three times. Treatments were; uninoculated control plus Nitrogen, uninoculated treatment minus Nitrogen and inoculated treatment. The greenhouse experiments were replicated three times and the treatments were 324; native (Rwanda) isolates while two were commercial inoculants (CIAT 899 and UMR 1597)\. Two controls and each treatment was replicated 3 times (54*3 replicates*2 type of common bean). Three sterilized and pre-germinated seeds were planted per pot and inoculated with 1 ml of log phase bacterial culture (10^8cfu/ml). After seven days, seedlings were thinned to two per pot.

The best five performing or effective isolates were selected for further testing in the field in two agro ecological zone of Rwanda using commonly grown bush and climbing bean varieties. Nitrogen-free nutrient solution (Broughton and Dillworth, 1970) plus N controls treatment, KNO_3 (0.05%) was added giving an N concentration of 70 ppm.Two healthy plants per pot were retained after the formation of first trifoliate leaf. Plants were harvested eight weeks after planting.

Nodules plot^{-1} evaluation **Nodules plant^{-1} evaluation**

Plate 2 a & b: Evaluation of bean nodulation in the green house

3.4 Study site of field experiments

The field experiment was conducted in two different agro-ecological zones. The first site was Ruhunde (Longitude: E 029 93'38.5''; Latitude 01S 55' 83.5''; Altitude 2293 m) located in Burera District in Northern Rwanda. Altitude ranges from 1800-2400 m above sea level with mean annual temperature of 15 to 18^0 C and bimodal rainfall ranging from 1800-2200 mm annually. This area receives the highest amount of rainfall that occurs between February and May and the dry season is experienced between June and August. The region has fertile volcanic soil and a high potential for agriculture (ISAR, 2000).

The second site was Rubona (Research Station) in Huye District located in Southern Rwanda. The altitude is between 1600-1800 m with an annual mean temperature of 16 to 20^0C and bimodal rainfall ranging from 1700-2000 mm annually.The highest amount of rainfall occurs between February and May and the dry season occurs between June and August. The region has acid soils (ISAR, 2004).

Table 2: Selected physiochemical soil properties of two sites (ISAE, 2013)

Properties	**Units**	**Site1: Huye/Rubona**	**Site 2: Burera/Ruhunde**
pH (H_2O)		4.9	5.4
Total N	%	0.16	0.45
P	Ppm	337	522
K	Me/100g	0.17	0.13
Mn	Ppm	128	218
Mg	Me/100g	0.035	0.038
CEC	Me/100g	26.8	27.0
Org C	%	5.13	6.93
Clay	%	60	62
Silt	%	15	18
Sand	%	25	20

3.4 Soil sampling

Soil sampling was done from farmer's fields at Burera and Kiruhura District where the field trial was conducted. The top 0-15 cm soil was dug randomly from the farms, mixed thoroughly, dried and stored in bags. A composite soil sample was taken and transported in a cool box to the laboratory and analysed for pH, organic carbon, available phosphorus, exchangeable cations, total nitrogen and particle size analysis as outlined in Okalebo *et al.* (2002).

3.5 Soil chemical characterization

The composite sample taken from the whole sample was used in laboratory for chemical analysis before setting up the experiments following the procedures outline in Okalebo *et al.* (2002). The soil pH and soil available P were repeated after the experiments in the greenhouse and field trials.

3.5.1 Determination of soil pH

The pH was determined by the 1:2.5 ratio of water and calcium chloride. The air dried sample was passed through a 2 mm sieve and used in determination of pH. Six grams of the sieved sample was weighed and put in two sets of clean plastic bottles. To one set, 15 ml of distilled water was added and 15 ml of calcium chloride to the other set. The samples were shaken for 30 minutes in a reciprocating mechanical shaker, allowed to stand for 30 minutes before reading the pH on a pH meter.

3.5.2 Determination of soil available Phosphorus

The Mehlich soil test for P also known as the dilute double acid as developed by Mehlich 1953 was used. This is a suitable method since it extracts P from aluminium, iron and calcium phosphates. The method is also suited for acid soils of pH less than 6.5, soils with low CEC and soils with organic matter content of less than 5%. P from 5 g of air dried and sieved (2 mm) soil was extracted using 50 ml of Mehlich extracting solution (double acid, containing 0.025 N sulphuric acid and 0.05 N hydrochloric acid). The solution was placed on a

reciprocating shaker and shaken for 30 minutes at 180 rpm at room temperature. The solution was filtered through a filter paper, The filtrate was thereafter analyzed for P colorimetrically using a blank and standards prepared in the Mehlich extracting solution and the absorbency read on a spectrophotometer at 882 nm wavelength.

3.5.3 Determination of Organic Carbon

The amount of organic matter in the soil indicated as percent organic carbon has an effect in determining the fertility status of a soil. High organic matter content indicates high base saturation as a source of nutrients for plant uptake. Organic carbon was determined using the Walkey-Black (1934) oxidation method. This method involves complete oxidation of soil organic carbon using concentrated sulphuric acid (H_2SO_4) and dichromate solution. The unused or residual $K_2Cr_2O_7$ is titrated against ferrous ammonium sulphate. The used $K_2Cr_2O_7$ which is the difference between added and residual $K_2Cr_2O_7$ gives a measure of organic carbon content of a particular soil. 0.5g of air dried soil sieved through a 0.5mm sieve is weighed into a set of clean conical flasks. 10ml of 1N $K_2Cr_2O_7$ was added to each and swirled gently. 20ml of 36N H_2SO_4 was rapidly added and allowed to stand. Distilled water was added followed by a drop of mixed indicator. The contents were thereafter titrated with 0.5N ammonium ferrous sulphate, observing the color changes and end point.

3.5.4 Determination of CEC

Cation exchange capacity of the soil samples was determined using Metson method (1961) which uses normal ammonium acetate as the exchange solution at pH 7. The exchange solution leaches out all the cations in a soil. Excess NH_4^+ ions were removed with an organic solvent alcohol. A K^+ salt solution was used to replace and leach out adsorbed NH_4^+ ions. The amount of NH_4^+ released gives the amount of CEC of a soil. The amounts of exchangeable Na, K, Ca and Mg in the extract was determined by flame photometry for Na and K, and by atomic absorption spectrophotometer for Ca and Mg. Lanthanum (La) or strontium (SR) was added as a

releasing agent to prevent formation of refractory compounds, which may interfere with the determination, for instance of phosphate.

3.5.5 Determination of total Nitrogen

In the determination of total nitrogen, the Kjeldahl (1883) method was used. This is basically the wet oxidation procedure. This method involves the conversion of nitrogen into $(NH_4)_2SO_4$ followed by distillation of NH_3 in an alkaline medium and titrating with standard sodium hydroxide. One gr of 0.5 mm sieved sample was weighed into a clean digestion tube and mixed catalyst added followed by 8 ml 36 N H_2SO_4. Samples were digested for 2 hours before titrating against 0.01N HCl and noting the volume used in titration.

3.5.6 Isolation and codification of native rhizobia

Native rhizobia were isolated from nodules of legumes collected from farmers' fields. Isolation and preliminary characterization of the root nodule bacteria was done at Laboratory of Rhizobiology based at RAB Rubona Station

3.5.7 Determination of Indigenous rhizobial populations

The most-probable-number (MPN) method (Woomer, 1994) was used to determine the number of viable and infective rhizobia in the soil. Gravimetric moisture content was determined by oven drying the soil samples at 105^0C for 24 hours. Ten grams of soil was wetted to 15% (w/v) moisture content and incubated at 28^0C for 7 days to simulate field conditions at the time of planting. A 10-fold dilution was done for each soil by adding 9 ml of sterile water into 1 g of soil. This was mixed thoroughly on a rotary shaker for 20 minutes to disperse the soils. Serial dilutions were continued up to 10^{-6} for each of the soils.

3.6 Data analysis

Data were compiled into a spread sheet, inspected and were subjected to analysis of variance (ANOVA) using Genstat Discovery edition 15th.· The treatment effects were tested for significance using F-test at 5%. Duncan Multiple Range Test (P=0.05) was used for mean separation. Analysis of correlation coefficients, at 5% level of significance, was done to determine the relationship between their yields and some other agronomic parameters (dry weight of biomass, pods and 100 seeds).

CHAPTER FOUR: PERFORMANCE OF RHIZOBIA ISOLATES IN GREEN HOUSE AT RUBONA RESEARCH STATION

ABSTRACT

The objective of this study was to identify to the indigenous rhizobial isolates and to test their effectiveness compared with commercial strains (CIAT 899 and UMR 1597). The 174 rhizobia isolates from Rwanda, were grown in Leonard jars and evaluated using bush and climbing bean varieties in the greenhouse. The rhizobia isolates formed effective nodules, red color, large in size caused vigorous growth of the respective legume host. The measurement of dry weight of nodules indicate that 14% of the Rwanda rhizobia isolates were able to improve nodulation and biomass of both bush and climbing beans. The effective isolates were subjected to futher evaluation in pots along with two commercial strains (CIAT 899 and UMR 1597) plus nitrogen control. To select the best rhizobial isolates, 6 parameters were used. These were: number of nodules, nodule size, color of nodule in and legume dry biomass. The results showed that the 50 Rhizobia isolates from Rwanda had a high significant difference on number of nodules, dry weight of nodules and dry weight biomass. However the size and the color of nodules didn't have significant difference. Results further showed that the 5 best rhizobia isolates compare favorably with the standard commercial strains and were proposed for further evaluation in field experiments**.**

Key words: Phaseolus vulgaris, root nodulation, commercial strains

4.1 Introduction

The major limitation to bean production in many smallholder farms is declining soil fertility as a result of continuous cropping with minimal inputs or rotation to replenish soil nutrients. Nitrogen, for example, is a limiting nutrient in crop production for 35 to 45 per cent of farmers in the highlands, one of the most productive areas of the country (Odame, 1997). Some of the options that are currently being pursued to address low soil fertility include integrated use of organic (e.g., crop residues, animal manures, agroforestry tree prunings) and inorganic (fertilizers, phosphate rocks) resources, and use of rhizobia inoculants (Okalebo *et al*., 2007). Uses of crop residues usually conflicts with their other uses as fuel and fodder, and while most farmers recognize the value of animal manures, most have only few animals so the manure produced is not enough. Manures are also bulky and usually of low and variable quality. Use of rhizobia inoculants in other countries has been successful, and is an option that has potential to increase legume production. Rhizobia are the bacteria which fix atmospheric nitrogen (N2) in leguminous plants through legume-rhizobium symbiosis by forming nodules on the roots/stems of these plants. Auxin biosynthesis by rhizobia is increased many folds in supplementation with suitable precursor (Tryptophan) (Zahir *et al*., 2005, 2010).

4.2 Materials and Methods

Rhizobia isolates from bio-prospecting across the country in Rwanda, were evaluated was sin the greenhouse using Leonard Jar and pots on two types of common bean. Before the experiment was set up, the greenhouse was cleaned and Leonard Jars and pots were thoroughly sterilized by 95 % alcohol before putting in the substrate. Before planting bean, the seeds were sorted, rinsed in 95% alcohol for 10 seconds to remove waxy material and trapped air. Sodium hypochlorite solution (2.5%) in sufficient volume to immerse the seeds completely was added for 3-5 minutes. Then seeds were rinsed with sterile water for 1 to 4 hours. Seeds were pre-germinated on sterile (autoclave) vermiculite for 48 hours in an incubator at 28^0C, and regularly inspected to assure that the radical doesn't become etiolated. The seeds were planted in Leonard Jars and in pots, and

then inoculated with appropriate rhizobia isolate, commercial strains, inoculated and non inoculated according to design. After germination, the plants were watered twice daily using rhizobium-free water. The evaluation of the Leonard Jars experiment considered 4 parameters, number, size, color and weight of nodules. However in pot experiments, fresh and dry weight of host legumes were also considered. The number of nodules was examined at flowering time which was close to 30 days after planting **.**

4.2.1 Leonard jars experiment

From Leonard Jars experiment, nodule numbers and nodule biomass were found to be highly significant ($p<0.001$) for bush and climbing bean as shown in table 3 and appendix 3

The results indicate that nodulation was higher in RWR 1668 than Gasilida across all strains. The average nodules population was 14 and 10 respectively for RWR 1688 and Gasilida. CIAT 889 and UMR 1597, commercial strains yielded the highest number of nodules, 78.6 and 73.3 in RWR 1688 and 75 and 69 in Gasilida respectively. These were followed by two rhizobia isolates NAR 256 and NAR 151 which produced 74 and 67 nodules on bush bean and 72 and 63 nodules on climbing bean respectively. The highest weight was observed with r CIAT 899 giving 6.3 grams and 5.89 grams respectively for RWR 1668 and Gasilida (table 3 above and figure 2 below). In overall assessment, the treatments showed significant ($p<0.001$) nodule weight differences. However, when compared to the commercial strains mentioned earlier, the following isolates (NAR 151, NAR 155, NAR 166, NAR 164, NAR 169, NAR 170, NAR 206, NAR 210, NAR 265, NAR 75 and NAR139) gave high nodule numbers and nodule weights that are statistically insignificant compared with commercial strains. The results of this experiment confirmed that the Rwanda rhizobia isolates are effective on both bush and climbing beans. There was negligible nodulation where N fertilizer was applied.

In terms of effectiveness index, 174 rhizobia isolates can be divided in four groups: The first group of 2.9% were highly effective. The second group comprising 28.7% of isolates showed an intermediate effectiveness. The third cohort constituting 30 % were partialy effective. Finally the fourth group comprising 38.8% were

totally ineffective on bush bean (RWR 1668) respectively with index 0.91 to 1.2; 0.81 to 0.9; 0.61 to 0.8; 0.41 to 0.6 and 0.2 to 0.4 as illustrated in figure 2 a& b below

Figure 2 a: Ineffective rhizobial isolates on bush bean

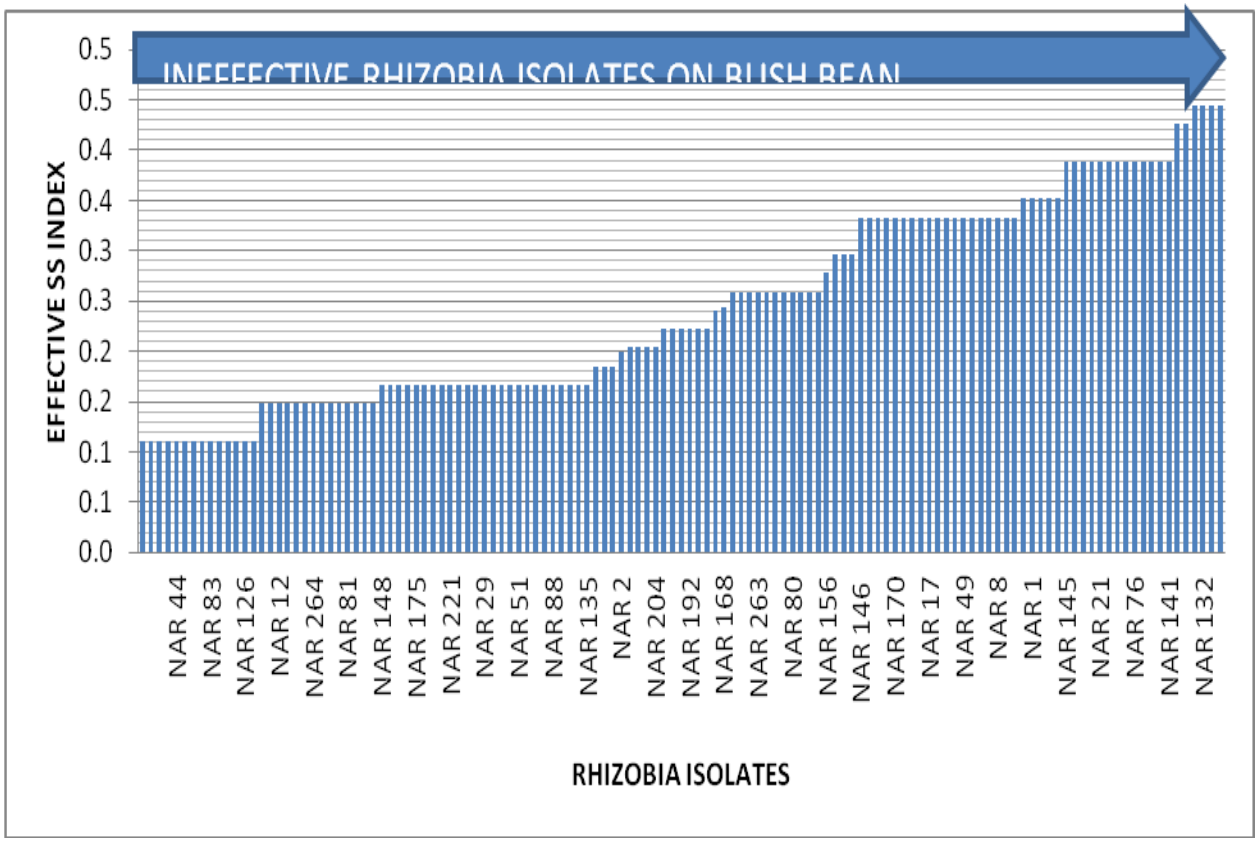
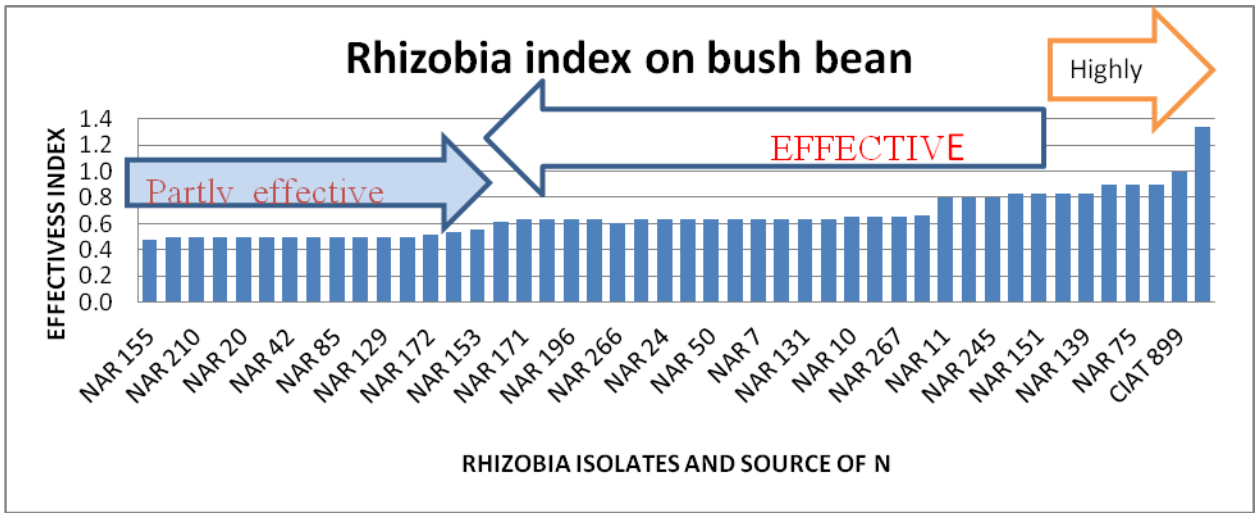


Figure 2 b: Partly effective, effective and highly effective on bush bean



In terms of effectiveness index on climbing bean, 2.5% were highly effective, 28.5% showed intermediate effectiveness; 30.0 were partially effective and 39% were totally ineffective on climbing bean (Gasilida) respectively with index 0.91 to 1.2; 0.81 to 0.9; 0.61 to 0.8; 0.41 to 0.6 and 0.2 to 0.4 as illustrated by figure 3 a & b.

Figure 3 a: Ineffective rhizobial isolates on climbing bean (Gasilida

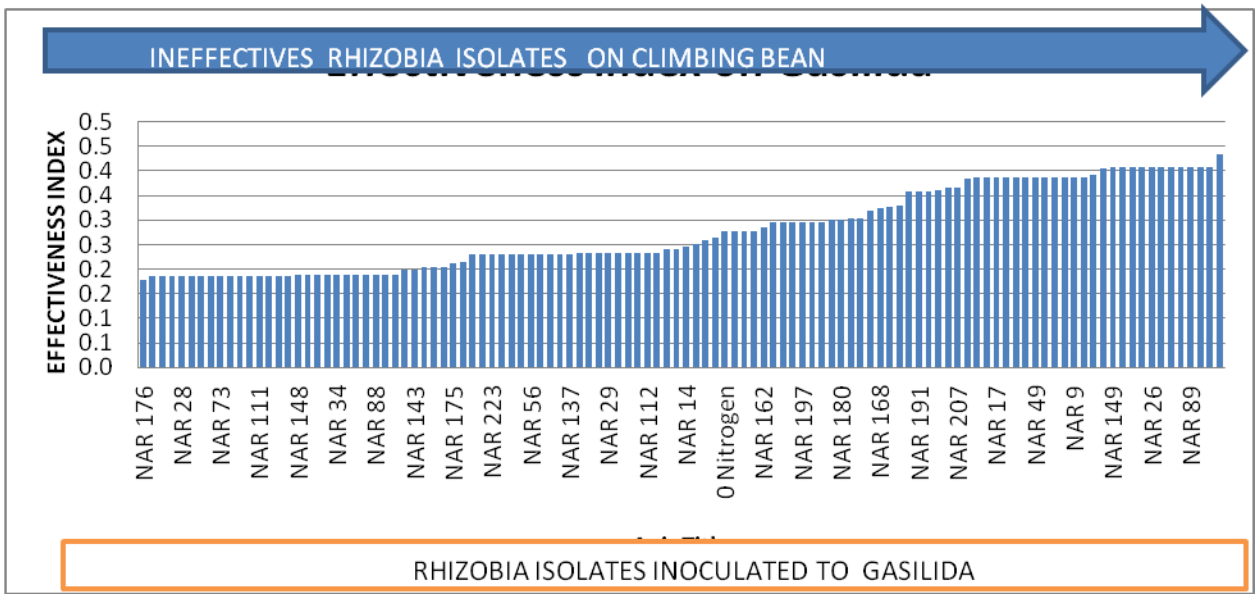
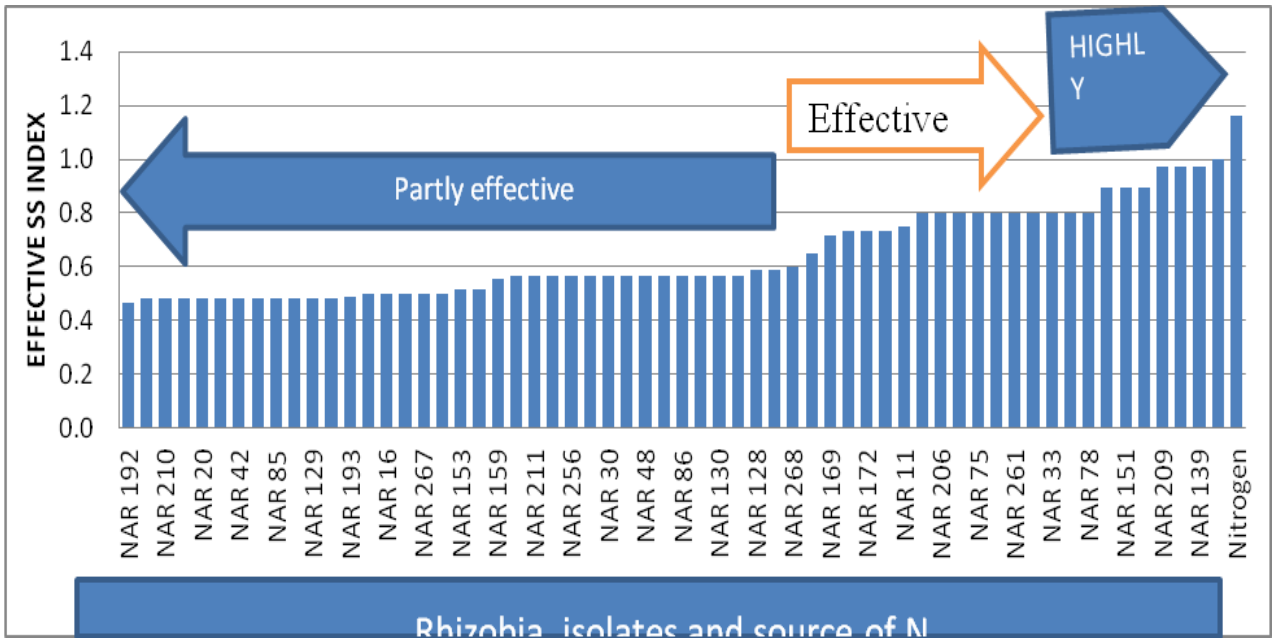


Figure 3 b: Partly effective, effective and highly effective



4.3.2 Pot experiment

i) Nodules number and dry weight

Evaluation of pot experiment shows that the nodule numbers and dry weight were highly significant ($p<0.001$) both for bush and climbing beans However, bush bean generally realized higher nodule numbers across the strains (Figure 4 a & b).

Figure 4 a: Nodule numbers from bush bean (RWR 1668)

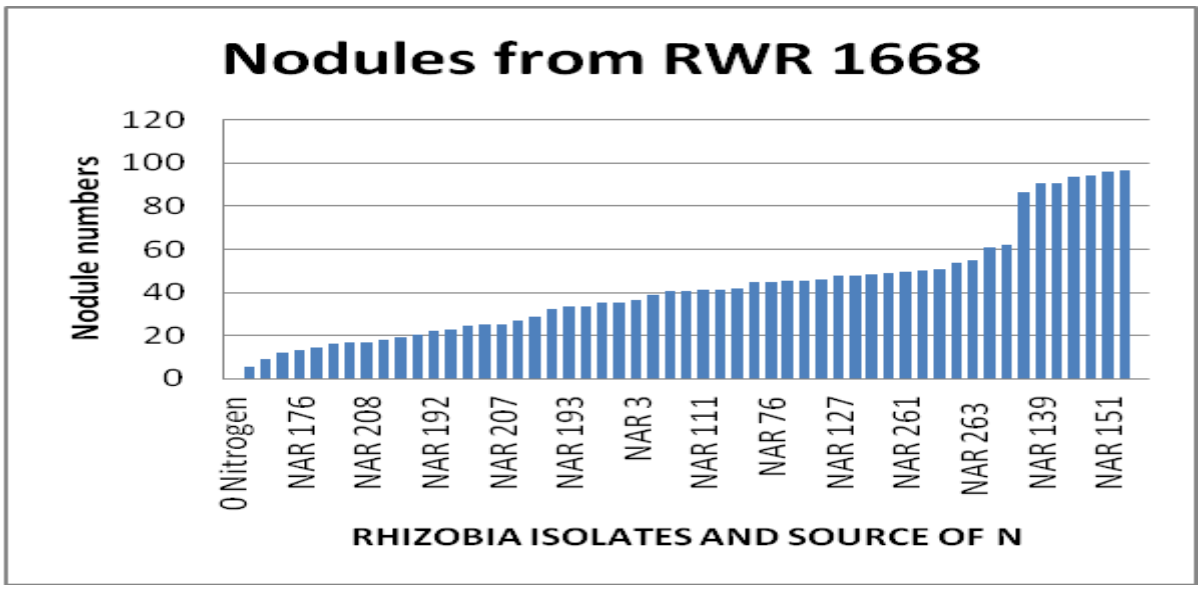
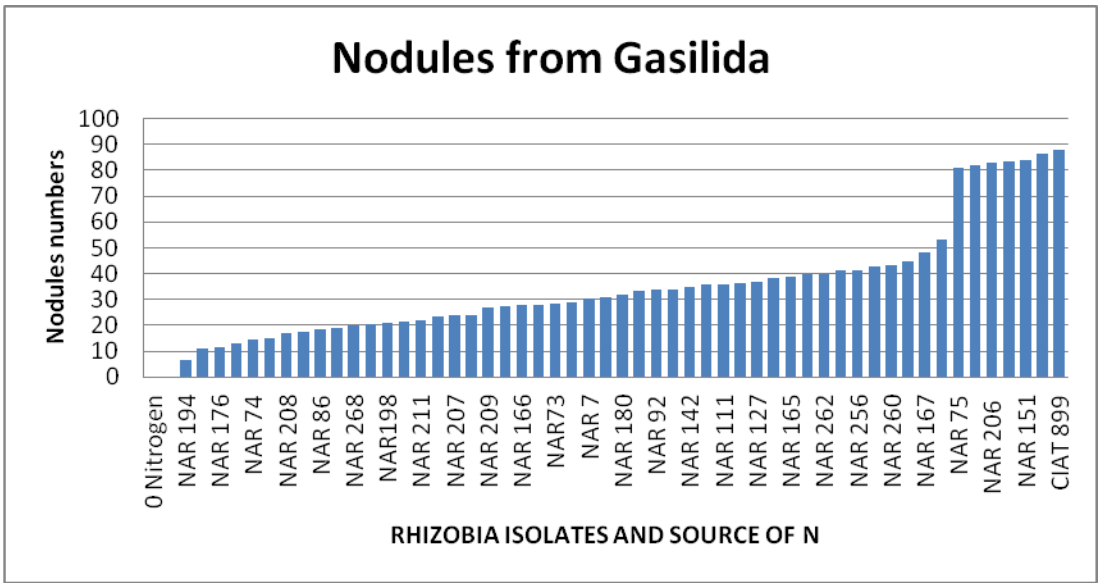


Figure 4 b: Nodule numbers from climbing bean (Gasilida) in pot experiment



CIAT 889 had the highest number of nodules, 96.7 and 88 in RWR 1688 and Gasilida respectively. However there was insignificant nodule population in both the beans among CIAT 899, NAR 265, NAR151, NAR139, NAR 206, UMR1597 and NAR 75 in climbing beans (with performance in that order) and bush beans. In bush

beans nodule numbers in NAR 75, NAR151 and NAR 206 was higher than with UMR 1597 but lower than with CIAT899. The performance of strains NAR139 and NAR 265 was lower than that of UMR 1597. Low nodulation was observed in plants where the nitrogen was applied, which is to be expected Biomass dry weight reflected magnitude of nodulation. the highest (dry weight) was realized under crop fertilized with nitrogen at 5.2 grams and 10.1 grams respectively for bush and climbing bean (figure 5 a & b) followed by CIAT 899 at 5 grams and 9.4 on bush and climbing bean.

Others strains also have the best dry weight biomass from RWR 1668, NAR 206 at 3.4 grams; NAR 265 at 3.4 grams; ; NAR 139 at 3.3 grams; NAR 151 at 3.3 grams and NAR 75 at 3.1 grams.

From climbing bean inoculated, NAR 139 occasions 8.2 grams; NAR 265 8.2 grams, NAR 206 7.3; NAR 151 7.2 grams and NAR 75 at 6.6 grams dry weight biomass plant^{-1}as illustrated in figure 5 a & b.

Figure 5 a: Dry weight biomass from RWR 1668 in pot experiment

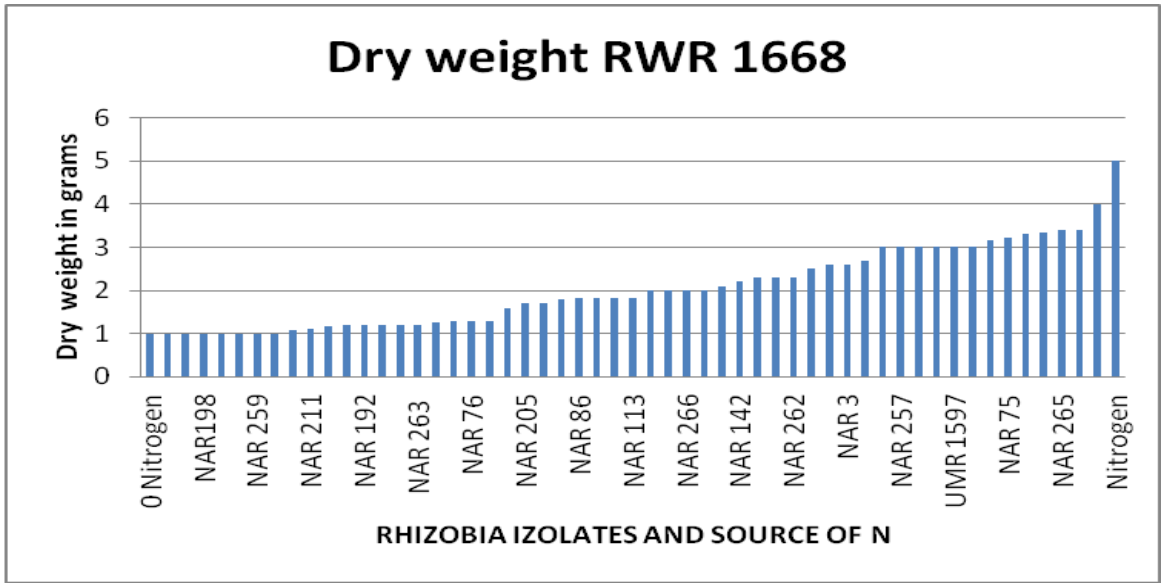
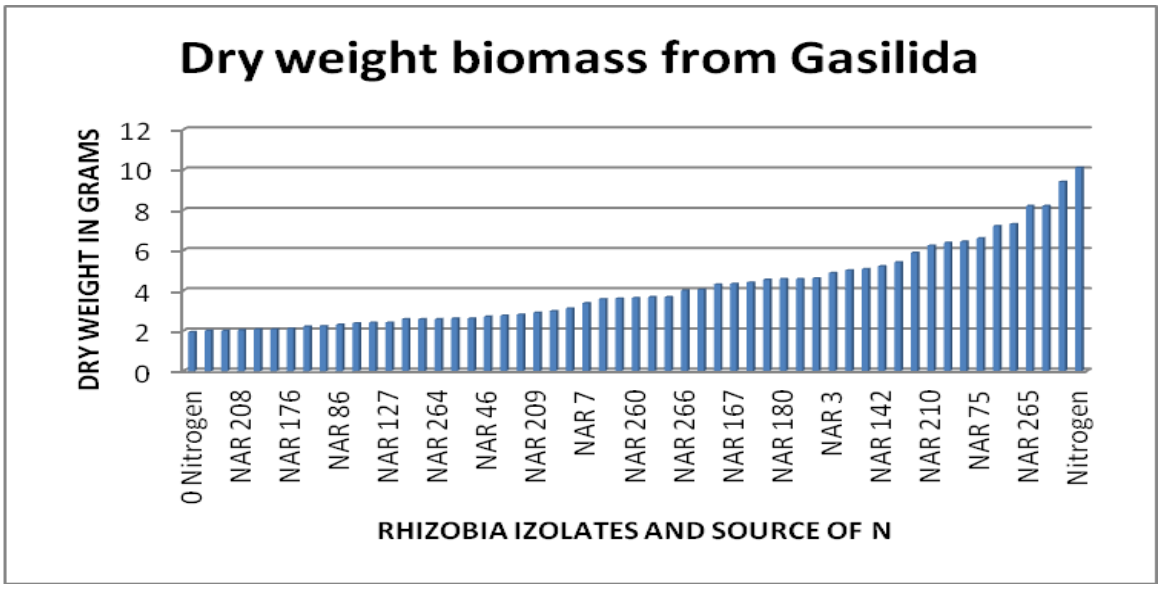


Figure 5 b: Dry weight biomass from Gasilida in pot experiment



4.4 Discussion

The analysis of variance on the results obtained showed that the strains had significant effect on agronomic performance in terms of dry weight biomass, weight biomass of (dry), nodule color, size of nodules and weight biomass(dry) as shown in figure 2 to 5. Most of the rhizobial isolates used in the experiment were effective on nodule population and biomass compared with the control pots (0 Nitrogen). The lowest values which were related to these parameters were obtained from this control treatment. The analysis of variance shows that the difference between inoculations was significant in terms of nodule population and their weight but not for size and color in Leonard jar experiment and in pot experiment.

Furthermore, inoculation with commercial strain CIAT899 and many natives rhizobium were more effective on nodule population and biomass on bean compared to the control. However, total nodule numbers in bean significantly increased ($P<0.05$) compared with the control, but few nodules were found in the control

treatments (Nitrogen and 0 Nitrogen treatments). The number of nodules differed significantly among native isolated strains. The number of nodules in the root hairs was found to be less than 85 except for native isolated strains No 108 NAR 151, No 96 NAR 139 and No 180 NAR 151 treatments. Nitrogen treatment was effective in inhibiting nodulation. Inoculation led to occurrence of significantly higher nodule number compared to the control. The highest nodule number was obtained from reference strain (CIAT899) and native isolates NAR 265, NAR 206, NAR 151, NAR139 and NAR 75. Those were selected and conducted in field experiment in two different agro ecological zones.

4.5 Conclusion

The Rwandan rhizobia isolates had positive effect on nodule numbers, nodules weight, plant fresh and plant dry weight of host legumes. However, a large number of rhizobial isolates were not effective and did not influence legume plant morphological properties. An explanation can be advanced that probably the condition for the rhizobium-legume symbiosis was unsuitable or unfavorable for matching between rhizobia and the legume host. It is also possible that nitrogenous fertilizers may have been used excessively on these soils. Further it could be argued that native rhizobium populations were many and outcompeted the introduced strains.

CHAPTER FIVE: EVALUATION OF EFFECTIVENESS OF RHIZOBIA ISOLATES IN FIELD EXPERIMENT

ABSTRACT

A field experiment was conducted in order to evaluate the performance of the best rhizobial isolates selected in pot experiments carried out in the greenhouse of rhizobiology based at Rubona research station. The field experiment was installed in two sites, Rubona/ Huye in South of Rwanda where experimental plots had been sowed with bush bean (RWR 1668) inoculated and Ruhunde/ Burera site in North of Rwanda where experimental plots were sowed with Gasalida climbing bean variety also inoculated. Twenty seven plots were prepared in each targeted site and fertilized with farm yard manure. A randomized block design with three replicates was employed Nodule number were significantly different, dry weight biomass was also significant ($p<0.005$). Pods weight, haulms weight, 100 bean seeds (grams) and yield differed significantly.

Key words: Rhizobia isolates, strains, control, root nodule, biomass and haulms

5.1 Introduction

Today Rwanda is the largest producer of common bean (*Phaseolus vulgaris L.*) in East African Community and the grains represent the most important source of protein for the population. The

demand is higher than the production (MINAGRI, 2002). Poor use of technology and cropping in low fertility soils, especially with low N content, contribute greatly to this scenario. Therefore an adequate supply of N through symbiosis with N2-fixing rhizobia should increase yield at a low cost as well as protect water resources from pollution by leached mineral nitrogen. Poor nodulation and lack of responses to inoculation in field experiments have been frequently reported worldwide, raising doubts about the efficiency of bean inoculation, (Graham 1981; Pereira *et al.* 1984; Buttery *et al*.1987; Ramos and Boddey 1987; Hardarson 1993). The explanation for the failure in some trials mainly attributed to a high but inefficient population of indigenous rhizobia (Graham 1981; Thies *et al*. 1991). Furthermore, the common bean-rhizobia symbiosis is quite sensitive to environmental stresses, such as high temperatures and soil dryness, leading to low N_2 fixation efficiency (Graham 1981; Hungria et al. 1997; Hungria and Vargas 2000). Nodulation is improved when the number of viable rhizobial isolated per seeds increases as accomplished by having greater numbers of viable rhizobia in the inoculants and delivering larger doses (Catroux et al, 2001).

5.2 Materials and Methods

Five best rhizobia isolates from pots experiment on two types of common bean were evaluated in field experiments. The experimental plots were arranged nn a randomized complete block design with three replicates. In total 27 plots were prepared and sowed with the two bean varieties in with 9 treatments. Five best rhizobia isolates selected from among those evaluated in pot experiment consisted of strains(NAR 265, NAR 206, NAR 151, NAR139 and NAR 75. Two commercial strains (CIAT 899 and UMR 1597) and two controls (with nitrogen and without nitrogen)were also applied**.** Each plot (3m×3.5m) had seven rows, spaced 0.5m apart. Plots, within each block, were separated by 1m apart and the distance between blocks was 3m.

Common bean seeds were inoculated with filter mud-based inoculants of native rhizobia isolates using a solution of Gum Arabic (40%, w/v) as a sticker. Commercial rhizobia (CIAT 889 and UMR 1597) inoculants were also prepared. Based on the viable counts of inoculants ($1–5\times10^{9}$ rhizobia g^{-1}) and on the average weight of the individual seeds, seeds lots was inoculated to give a population of 10^{6} rhizobia/seed**.** Before planting and after harvesting, soils for MPN test were taken in each site for determination of viable microorganisms contained in $gram^{-1}$ solution.

Data were collected, recorded and analyzed using MPN technique and GenStat 15^{th} edition**.**

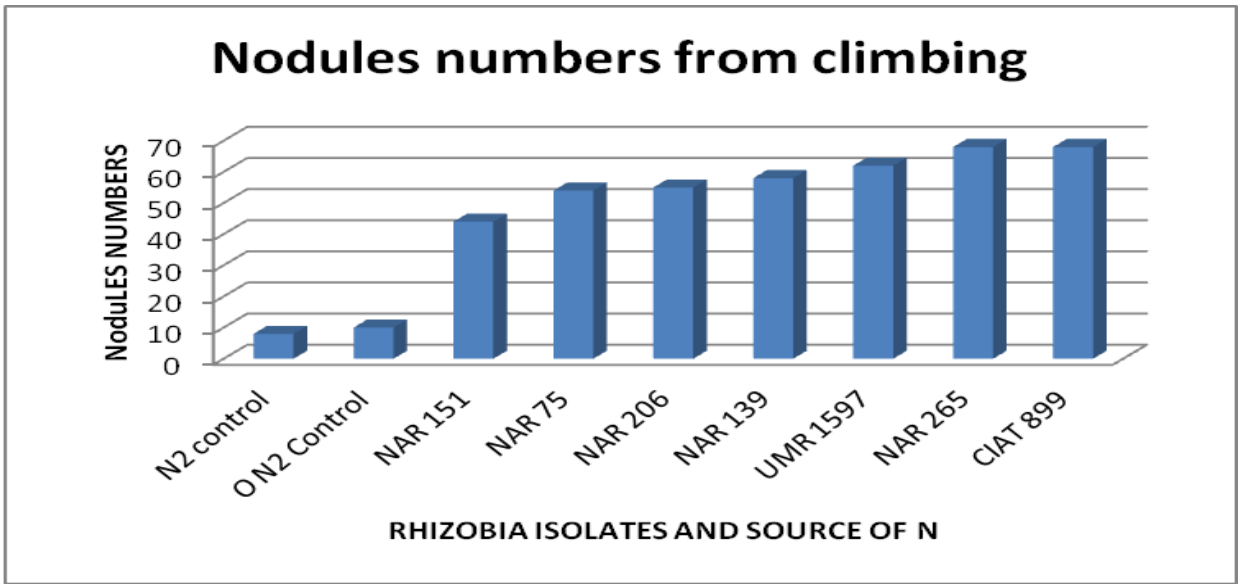
5.3 Results

5.3.1 Nodulation

The statistical analysis showed a significant difference on nodules population (p value=0.001) due to rhizobiainoculant treatment. From climbing bean(Gasilida) the highest number of nodules were observed in plants inoculated with CIAT 89 (67.50), UMR 1597 (61.83) which are commercial strains followed by NAR 265(61.80), NAR 139(58.8), NAR 206 (53.33), NAR 151 (43.8) and NAR 75(52.47) in that order.

Figure 6 a below shows the results obtained in non-inoculated and inoculated plots in Ruhunde/Burera where the rhizobial population increased slightly with the presence of the common bean plant, but a further increase was obtained by inoculation with rhizobial isolates and commercial strains. There was a significant difference (p=0.001) in nodules population observed in both the beans.

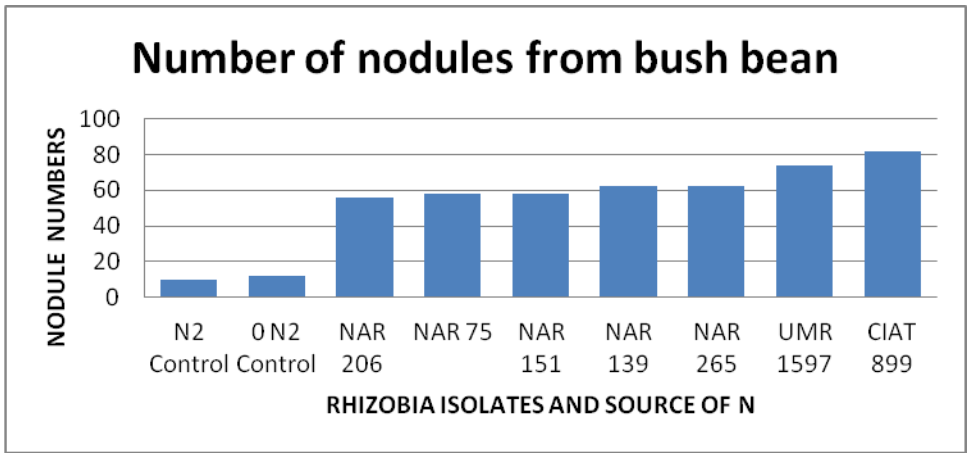
Figure 6 a: Nodule numbers obtained with climbing bean



The highest number of nodules were observed in bush bean inoculated with CIAT 89 (82.1), UMR 1597(73) as commercial strains followed by NAR 265(67.80), NAR 139(63.0), and NAR 151 (58.5.4), NAR 75(57.8) and NAR 206(53.1) The lowest nodule numbers were observed where common bean was fertilized with nitrogen. In this situation as low as 10 and 12 nodules plant^{-1} were found.

Figure 6 b below shows the results obtained in non-inoculated and inoculated plots in Rubona where the rhizobial population increased slightly with the presence of the common bean plant. However, a further increase was obtained by inoculation with rhizobia isolates and commercial strains. There was a significant difference (p=0.001) in nodules population observed in both climbing and bush beans.

Figure 6 b: Nodule numbers from bush bean



The indigenous rhizobial population before the first sowing was estimated at 15 and 3,594 cells g^{-1} soil in Rubona and in Ruhunda, respectively. However, despite the high population of rhizobia, inoculation allowed an increase in rhizobial population resulting in an increased nodules population, biomass weight and the yield of climbing and bush beans. At harvesting, in June 2012, MPN test was used to determine the population of microorganisms and subsequently calculated at 3,594 cells g-1. In October 2012, the population of microorganisms in Ruhunde was estimated at 15,926 cells g^{-1} soil as shown in the appendix 1.

5.3.2 Yield components and seed quality

The results shows a significant effect of rhizobial treatment in yield components pods (p=0.01), seed quality (p=0.01), haulms(p=0.08) for bush beans, while for climbing beans, all the yield components gave a significant response (p=0.010 to treatments (Table 3).

Table 3: Weight of pods and 100 seeds weight of bean varieties

	Climbing beans		**Bush beans**	
Treitments	**Pods (t ha^{-1})**	**100 seeds(g)**	**Pods (t ha^{-1})**	**100 seeds(gr)**
0 N_2 Control	3.31	45.6	1.88	44.77
CIAT 899	5.59	57.03	2.33	55.83
N_2 Control	5.7	58.7	2.35	56.67
NAR 151	4.89	51.9	2.15	51.17
NAR 206	4.37	54.33	2.29	51.67
NAR 265	5.3	55.63	2.3	54.97
NAR 75	5.14	52.93	2.2	51.83
NAR 139	5.19	54.67	2.22	54.8
UMR 1597	5.26	56.77	2.3	55.77
P value	0.01	0.01	0.01	0.01
LSD 0.005	0.17	4.81	0.072	2.37

Climbing beans had a better performance compared to bush beans in all the yield parameters assessed. Maximum yield for pods was realized in plots fertilized with nitrogen for both climbing (5.7 t/ha) and bush (2.35 t ha^{-1}) respectively (table 6). This was followed closely by commercial strains CIAT 899 (5.59 t ha^{-1}) and UMR1597 (5.26 t ha^{-1}) respectively. However isolates performance was better in plots that were inoculated with strains NAR 265, NAR75 and NAR 139 outperforming the commercial strain UMR1597. In bush beans, nitrogen fertilized plots produced the highest pods weight (2.35t ha^{-1}), followed by CIAT899 (2.33 t ha^{-1}), NAR 206 (2.29t ha^{-1}), NAR139 (2.22 t ha^{-1}). Plots without fertilizer application and not inoculated with any rhizobia gave lowest yield (Figure 7 a).

Figure 7 a: Pods yield (t ha^{-1}) for climbing bean

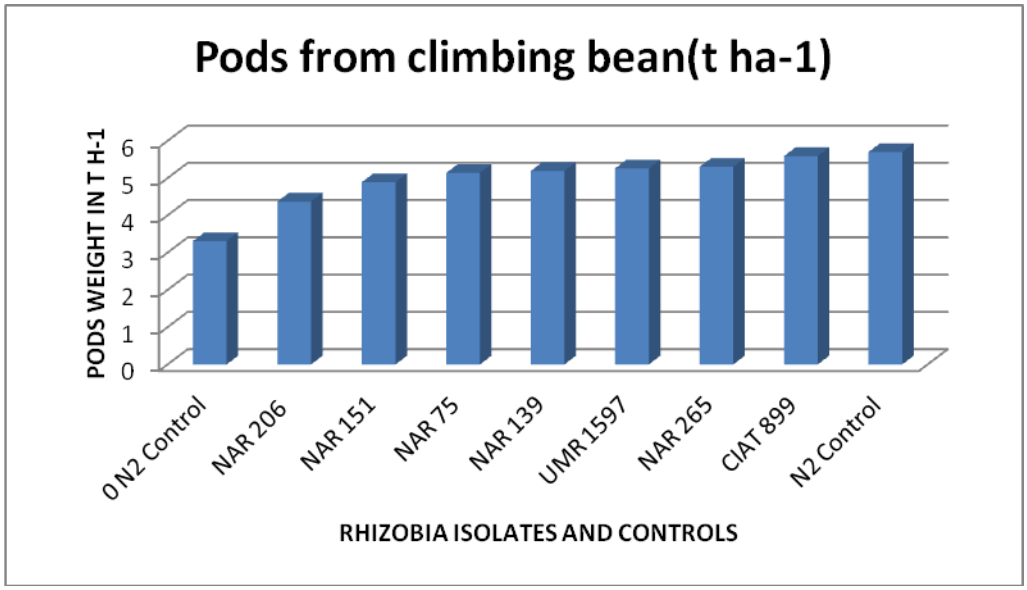
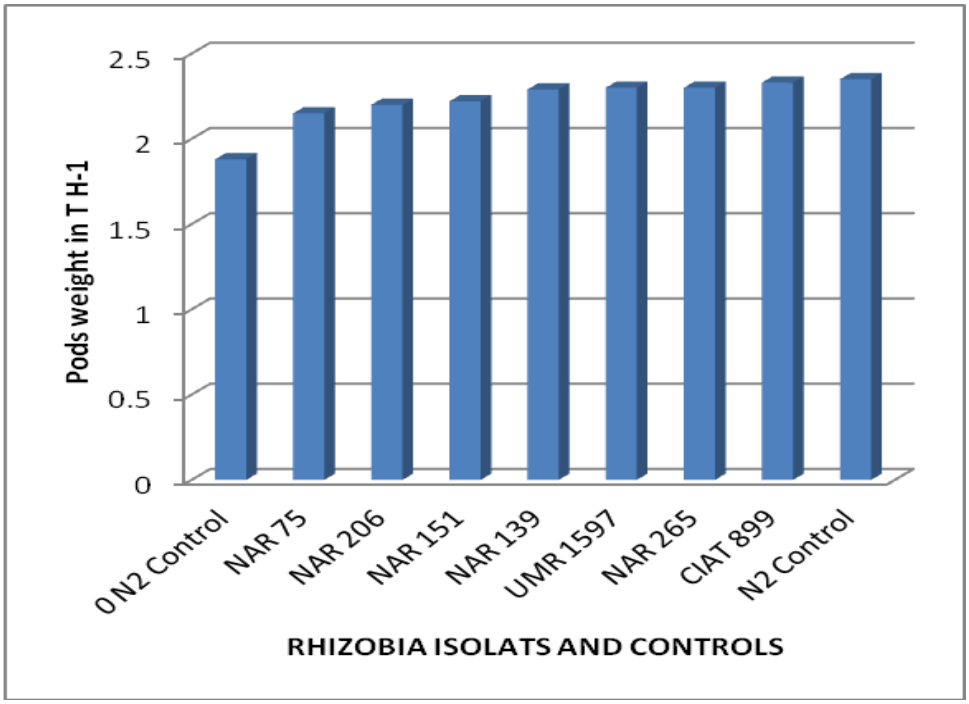


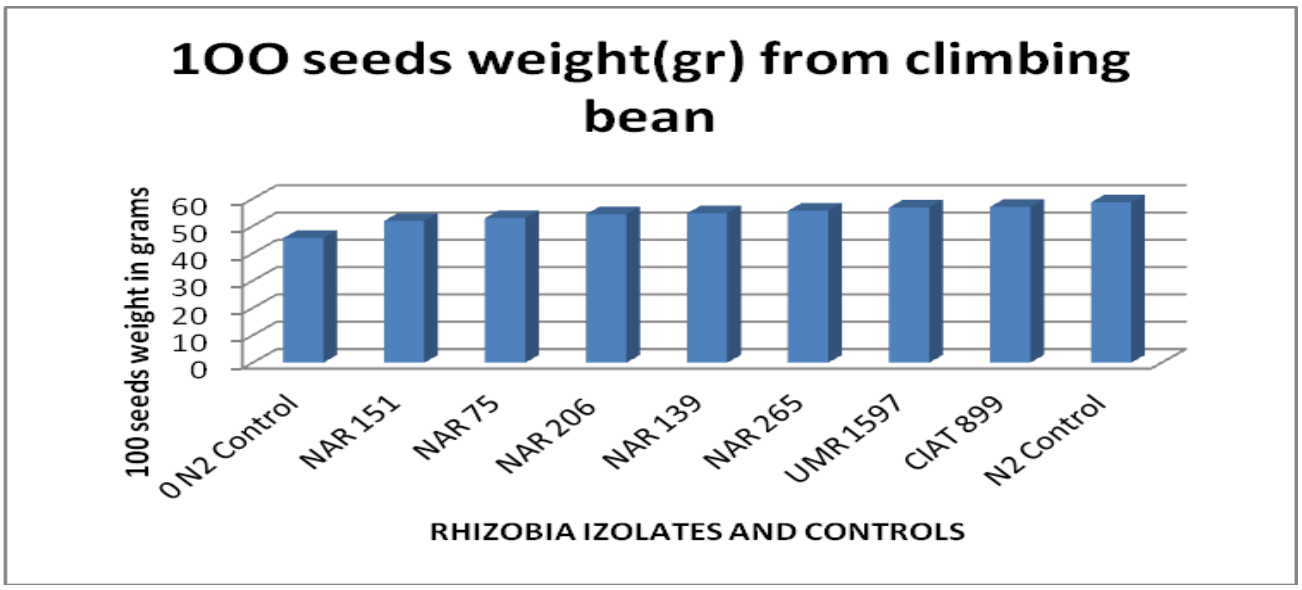
Table 7a shows that climbing beans inoculated or fertilized with nitrogen had a better performance compared to bush beans in all the yield parameters assessed. But the data also reveal the ability of best strains specifically being able to to increase the yield of pods as demonstrated in in figure 7 b. It was equally observed that there was no statistical difference in yield at (p=0.01).

Figure 7 b: Pods yield (t ha^{-1}) for bush bean



The weight of seeds ranged between 58 grams to 44 grams with an average of 54.4 grams and 53.5 for bush and climbing. Seed weight in grams was 57.03, 56.77, 55.63, 54.67, 54.33 and 52.93 for CIAT 899; UMR 1597; NAR 265; NAR 139; NAR 206 and NAR 75 respectively on climbing. There was no significant response on 100 seed weight for climbing bean at (p=0.01).

Figure 8 a: 100 seeds weight (gr) of climbing bean



For bush bean seeds weight in grams was 55.83, 55.77, 54.97, 54.80, 51.83 and 51.67 for CIAT 899; UMR 1597; NAR 265; NAR 139; NAR 75 and NAR 206 respectively as shown in figure 9 b but statistically the weight for all treatments were not significant (p=0.01).

Figure 8 b: 100 seeds weight (gr) of bush bean

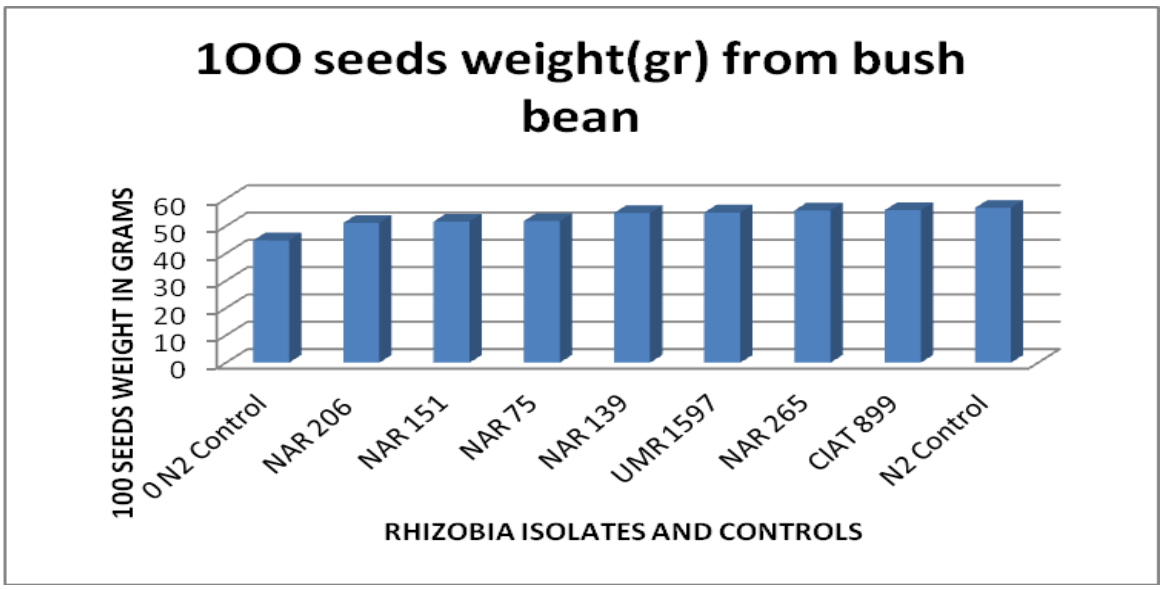


Plate 3 below shows an evaluation of crop performance during harvesting

Evaluation of diseases near the maturity on** climbing **bean

Evaluation of grains yield and biomass nodulation before at the maturity on climbing bean

Plate 3*:* Evaluation of rhizobial isolates effect on climbing bean before harvesting at Ruhund*e*

5.3.3 Biomass and grains yield (t ha^{-1})

Yields for both grain and total above ground biomass was significant at (p=0.01 and p=0.001) respectively when rhizobia inoculation treatment was applied. Biomass yields ranged from 2.38 t ha^{-1} in plots where fertilizer was applied (Table 4).

Table 4: Biomass and grains yield on bean varieties

	Bush beans v			Climbing beans		
Rhizobia strains	Nodules number	Biomass (t ha^{-1})	Yield (t ha^{-1})	Nodule population	Biomass (t ha^{-1})	Yield (tha^{-1})
0 N_2 Control	13.7	2.38	1.50	11.07	5.88	2.16
CIAT 899	82.13	4.08	1.86	67.5	8.49	3.71
N_2 Control	8.47	4.18	1.88	9.47	10.14	3.72
NAR 151	58.5	3.24	1.72	43.83	7.09	3.29
NAR 206	53.13	3.53	1.74	53.33	8	2.86
NAR 265	67.77	3.93	1.84	61.8	8.41	3.70
NAR 75	57.77	3.36	1.76	52.47	6.95	3.34
NAR 139	63.03	3.13	1.77	58.8	8.1	3.61
UMR 1597	73.17	3.66	1.84	61.83	7.09	3.44
P value	0.001	0.001	0.01	0.001	0.003	0.001
LSD 0.05	23.78	0.308	0.56	5.98	1.84	0.55

Biomass was highest under N fertilized control plots for both bean varieties, followed by CIAT99, NAR 265 and NAR139. Climbing beans realized higher biomass doubling those of bush beans across all the strains. On average, plots with no fertilizer and without rhizobia inoculantion applied recorded the lowest biomass production (Figure 9).

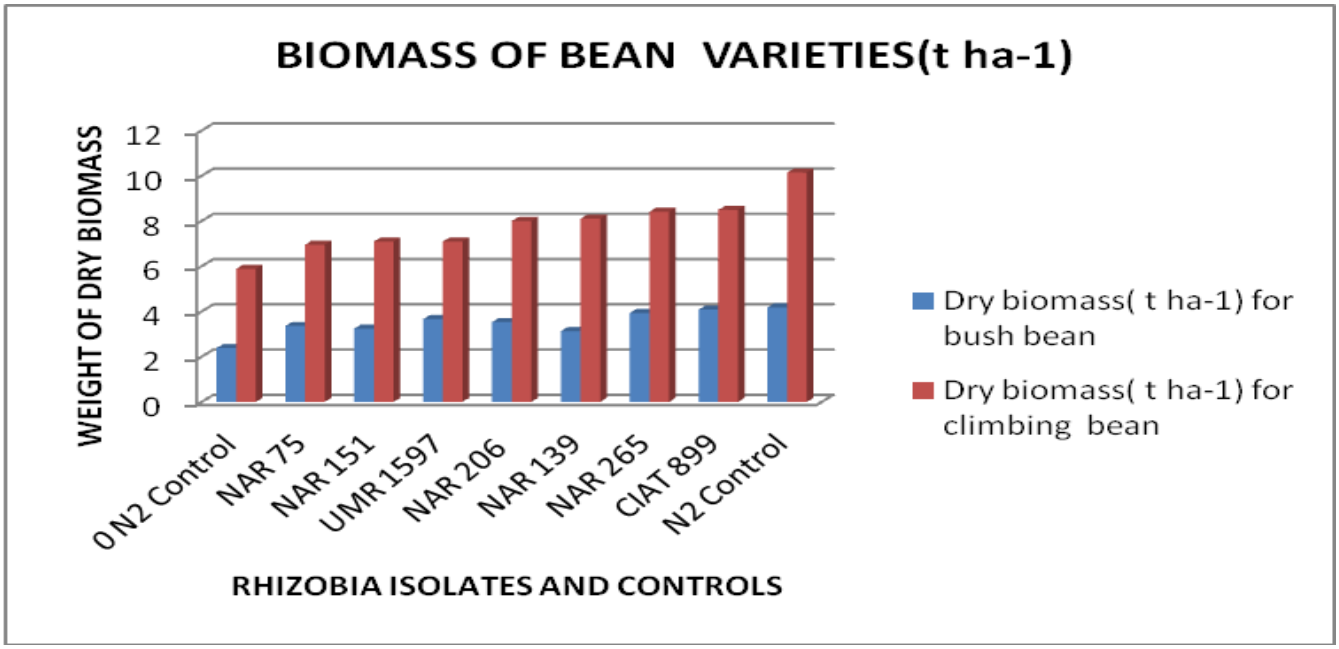


Figure 9: Biomass yield (t ha^{-1}) of bean inoculated or non-inoculated at Rubona and Ruhande

Inoculation had a significant effect (p= 0.001) on grains yield for both the bean varieties. The highest grain yield of 3.72 t ha^{-1} was recorded in nitrogen fertilizer plots for climbing beans followed by CIAT 899 (3.71 t ha^{-1}) inoculated beans. Grain yields observed, declined in this order NAR 265 (3.70 t ha^{-1}), NAR 139 (3.62 t ha^{-1}), UMR 1597 (3.44 t ha^{-1}), NAR 75 (3.34 t ha^{-1}), NAR 151 (3.23 t ha^{-1}) and NAR 206 (2.86 t ha^{-1}). The least grain yield (2.16 t ha^{-1}) was obtained in the control (Figure 10).

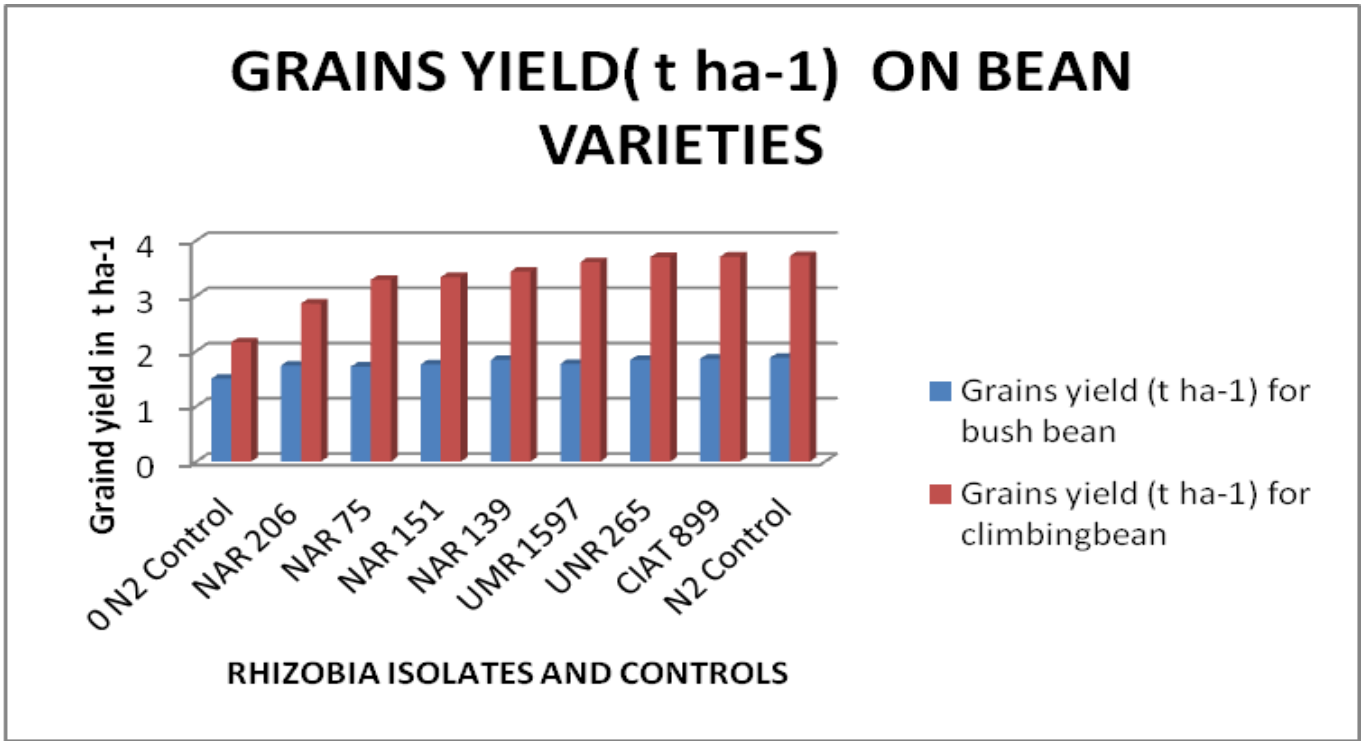


Figure 10: Effect of rhizobia treatment on grains yield of bush and climbing beans

The results demonstrate that bush beans realized the a relatively highest grain yield with an average of 1.88 t ha^{-1} and lowest of (1.5 t ha^{-1}) recorded in plots fertilized with nitrogen and without any input. Plots where there was no inoculation and no fertilizer yielded the least grain 1.50 t $ha^{-1)}$.

The isolates evaluated show the ability to increase the yield according their performance comparatively with 0 N (Control) and the improvement in grains yield on bush bean (Table 5).

Table 5: Yield increased (%) according the performance of 5 rhizobial isolates

Isolates and N source	Grains yield (t ha^{-1}) of bush bean	Grains yield increased %	Grains yield (t ha^{-1}) of climbing bean	Grains yield increased %
0 N_2 Control	1.5	100.0	2.16	100.0
NAR 206	1.74	116.0	2.86	132.4
NAR 75	1.72	114.7	3.29	152.3
NAR 151	1.76	117.3	3.34	154.6
NAR 139	1.84	122.7	3.44	159.3
UMR 1597	1.77	118.0	3.61	167.1
NAR 265	1.84	122.7	3.7	171.3
CIAT 899	1.86	124.0	3.71	171.8
N_2 control	1.88	125.3	3.72	172.2

The best grains yield increased varied from 16% to 22.7% and 32.4% to 71.3 % respectively on bush bean and bean climbing. The best performing rhizobia isolate was NAR 265 followed by NAR 139, NAR 151(17.3% and 54.6%), NAR 75 (14.7 % and 15.3%) and the least performing rhizobia isolate was NAR 206.

5.3.4 Crop tissue nutrient content

Tissue nutrient content Phosphorous (P) and Nitrogen (N) was significantly affected ($p=0.001$ and $p<0.001$) by rhizobia treatments as observed in Gasilida and RWR 1668 beans respectively. Table 8 below shows that the highest P and N was obtained where beans were fertilized by Nitrogen (0.96 %, P on Gasilida; 0.90% P from RWR 1668; 6.32 N Total from Gasilida and 6.10 total N on bush beam respectively) followed by where the beans were inoculated by the commercial strains CIAT 899 (0.86 P%, 5.99 N Total from Gasilida; 0.82 %P and 5.87 N Total from RWR 1668) and the second commercial strains such us UMR 1597 (0.82% P and 5.82 N Total from Gasilida; 0.80% P and 5.44 N Total from RWR 1668) in table 6.

Table 6: Tissue nutrient content on bean varieties

	P%		Total N %	
Rhizobia isolates/strains	**Gasilida**	**RWR 1668**	**Gasilida**	**RWR 1668**
0 N_2 Control	0.20	0.15	2.82	2.28
CIAT 899	0.86	0.82	5.99	5.87
NAR151	0.62	0.59	4.17	3.94
NAR 206	0.61	0.60	4.15	4.02
NAR 265	0.82	0.69	4.90	4.58
NAR 75	0.73	0.60	4.23	4.10
NAR 139	0.80	0.62	4.28	4.14
N_2 control	0.96	0.90	6.32	6.10
UMR 1597	0.82	0.80	5.82	5.44
LSD 0.05	0.14	0.42	1.00	0.90
				11.60
p value	0.001	0.001	<0.001	<0.001

It was observed that beans treated with the commercial strains had higher nutrient P and N contents relative to the isolates. According to Haynes *et al.*, (1986) and Mengel and Kirkby, (1987), N concentration in plant tissues range between 1 and 6 % depending on plant species, age, plant organ and environment and the results here fits within these limits.

5.3.5 Investigation of the role of rhizobia isolates in reducing disease severity on beans

The third objective of the thesis was to investigate whether inoculation had effect on susceptibility of the legume host to disease resistance.

Beans are generally characterized by their instable yield resulting from biological, climatic and edaphic factors which affect plant grow and productivity.

Rhizabia isolates have ability to induce diseases tolerance by nodulation and fixation of nitrogen to common bean crop.

To evaluate the effect on legume hote to disease, a score used by CIAT was used to cote the symptoms of several diseases on common bean.

CIAT gives a standard system for the evaluation of bean germoplasm under field conditions

The more commonly used scale goes from 1 to 9 grouped to 1-3, 4-6 and 6-9 as ululated in table 7.

Table 7: CIAT scare for diseases evaluation on bean crop

No	SCORE(Group)	Symptoms	Yield loss estimated
1.	1-3	Negligible	Less than 20%
2.	4-6	Intermediates	Moderate less than 50%
3.	7-9	Very susceptible	Highly to totally , 60% to 100%

It is important to note that when evaluation bean crop particulary to their reaction to pathogen or insects it is highly desirable and useful to have check cultivars or source of resistance. This helps diseases level assessment as well as in evaluation pathogens or insect distribution thought the nursery.

According the results diseases evaluation showed that bean diseases of significance were Anthracnose, Ascochyta, angular leaf pot, rusts and root rot for Rubona station. While in Ruhunde, anthracnose, ascochyta, angular leaf spot and halo blight were the most important (Tables 8 and 9).

Table 8: Disease evaluation on bush beans inoculated or none inoculated in RUBONA field

	Diseases evaluation							
Treatments	**Anthracnose**	**Asco**	**ALS**	**Rust**	**Bact**	**HB**	**BCMV**	**Root rot**
NAR 139	3	3	4	2	1	1	1	3
NAR 265	3	4	3	1	1	1	1	2
NAR 206	4	4	4	1	1	1	1	3
NAR 75	3	3	3	1	1	1	1	3
NAR 151	4	3	3	1	1	1	1	3
CIAT 899	3	3	2	1	1	1	1	2
UMR 1597	3	3	3	1	1	1	1	2
N_2 Control	3	2	3	2	1	1	1	2
0 N_2 Control	6	5	5	3	1	4	1	5

Diseases scores were higher where N was not applied in both sites with the highest score of 6 being recorded for anthracnose and halo blight (Table 8 & 9) in Rubona and Ruhunde respectively. Disease score of between 4-6 has a potential to cause serious crop damage and may affect crop growth and ultimately yields, 1-3 the crop damage don't affect the bean yield but the score between 6-9 the damage affect the loss yield more than 60%.

Table 9 Diseases evaluation on climbing bean at Ruhunde field

	Diseases evaluation							
Traitments	**Anthr**	**Asco**	**ALS**	**Rust**	**Bact**	**HB**	**BCMV**	**Root rot**
NAR 139	3	3	3	2	1	2	1	1
NAR 265	3	4	2	1	1	2	1	1
NAR 206	4	4	2	1	1	3	1	1
NAR 75	4	4	2	1	1	4	1	1
NAR 151	4	3	3	1	1	4	1	1
CIAT 899	3	3	2	1	1	2	1	1
UMR 1597	3	3	3	1	1	2	1	1
N_2 Control	3	2	2	2	1	2	1	1
0 N_2 Control	5	5	4	3	1	6	1	2

Anthracnose had the highest average score of 4 in both sites followed by Ascochyta (3) and angular leaf spot (3). Halo bligh also had a score of 3 in Ruhunde but was negligible in Rubona site but root rot (3) was more prominent.

5.3.6 MPN Test

The estimation of native rhizobia nodulating on common bean was done before and after seeding of bean**.** The plant infection count, also known as most-probable number (MPN) counts was used to determine the number of viable and infective rhizobia following the procedure stated by Somasegaran and Hoben (1994). Ten grams of soil sample was diluted in aseptic condition in 90 mL sterilized distilled water. Then 1 mL from first dilution was transferred into 9 mL sterilized distilled water up to 10^{-10} and was used to inoc ulate a common bean seedling adequately grown in acid treated and sterilized sand using plastic cups in four replication. Nodule observations were made 21 days after inoculation. Positive and negative nodulation of growth unit were recorded for all dilutions and converted into number of rhizobia g^{-1} using MPN table. The results are noted in two sites are noted in table 10.

Table 10: MPN results on two sites

	Site	Period	Plant hot	Number of viable rhizobia
1.	Rubona	Before seeding	-	15
2.	Rubona	After harvesting	Bush bean	614
3.	Ruhunde	Before seeding	-	3,594
4.	Ruhunde	After harvesting	Climbing bean	15,924

In Rubona site, the viable rhizobial isolates before seeding were very few (15) but after harvesting bush bean inoculated the increasing on rhizobia g^{-1}sol is estimated at 4093.3 %. However in Ruhunde the rhizobia g^{-1} were important but after harvesting climbing bean the rate were estimated at 443.07%.

5.3.7 Microbiology test

After several evaluations on the best 5 rhizobial isolates the microbiology test confirms their growth rate, their characteristic on YM-broth absorption, their reaction on bromothio blue and their growth on different temperatures. The characteristics of NAR 265 and NAR 151 are similar to CIAT899 not fare to others news 3 strains. Their performance is noted in tableau 9.

Tableau 11: Microbiology test on the 5 best rhizobia isolates

Strains	Host plant	Growth rate	Colony Characteristics on YMM	Reaction on Bromothio blue	Growth Optimum Temperature
NAR 75	Bean	Intermediate (5 days)	Partly absorbent	Yellow	34
NAR 139	Bean	Fast (3 days)	Partly absorbent	Yellow	32
NAR 151	Bean	Very fast (3 days)	Center absorbent	Yellow	30
NAR 206	Bean	Fast (4 days)	Partly absorbent	Yellow	32
NAR 265	Bean	Very fast (3days)	Fully absorbent	Yellow	30
CIAT 899	Bean	Very fast (3 days)	Fully absorbent	Yellow	30
UMR 1597	Bean	Very fast (3 days)	Fully absorbent	Yellow	30

Growth rate Yema/Congo red

Rhizobia/Strains	**Growth rate (days)**
NAR 75	5
NAR 139	3
NAR 151	3
NAR 206	4
CIAT 899	3
NAR 265	3
UMR 1597	3

5.4 Discussion

All of the rhizobium isolates evaluated in the field induced nodulations in both RWR 1668 and Gasilida bean varieties. In greenhouse and in the field reaffirming the assumed promiscuity of beans as a host to rhizobia (Michiels *et al.*, 1998, Kellman 2008). According to this study, the increases in the number and weight of nodules, dry biomass, weight of pods, haulms, 100 seeds weight and yield of bean grains were investigated after inoculation. Factors such as salinity, temperature, water supply, pH, mineral nutrition and combined nitrogen have a great importance in the symbiosis process (Elsheikh and Elzidany, 1998). A favorable rhizosphere environment is highly important for the interaction between root hairs and rhizobia as it does not only encourage the growth and multiplication of the micronutrient, but also ensures the healthy development of root hairs. Any environmental stress that affects these processes is also likely to also affect infection and nodulation (Alexander, 1984; Cordovilla, et al., 1999). Also the present study showed that there are significant differences among the inoculated strains in some properties of dry bean such as dry nodule weight in the number and weight of dry nodules, fresh and dry biomass, weight of pods, haulms, husk, 100 seeds weight and yield of bean grains. Moreover, other studies with native inoculation of *Rhizobium sp.* and dry bean (Cebel, 1988; Chaverra and Graham, 1992; Özdemir, 2002; Slattery, et al., 2004) have shown that isolated strains used, significantly ($P<0.05$) increased nodulation and other morphological parameters. Significant differences existed in the symbiotic potential of the isolates examined. In terms of the number and weight of dry nodules, dry biomass only three of the isolated strain (NAR 265, NAR 139 and NAR 206) possessed as promising symbiotic efficiency. In this study, the differences among isolated strains were also found and such distinction could be explained by environmental condition in the experimental soil. That is to say that the soil properties have the main importance in such microorganism-related studies. The match between rhizobia and the legume host is particularly important. The soils (Rhizobium was isolated from and on which dry bean was grown) had alkaline pH, clay loam texture, high amounts of $CaCO_3$ and low organic matter. The results show that the inoculations were significantly different from each other with respect to

plant agronomic properties. Moreover, the results of agronomic and symbiotic efficiency indicated that rhizobium strains which were isolated from soils grown with dry bean can be in harmony with *P. vulgaris L*. The results of the present studies reveal that the native strains had significant effect on the plant biomass and grain yields. Besides, the same strains had significant ($P<0.05$) effects on number of nodule and nodule weight. In addition, the isolated strains had positive effect on root weight, total dry matter, total nitrogen, total symbiotic efficiency and efficiency rate. However, the rhizobial isolates NAR 75 and NAR 151 did not improve the performance of dry bean.

This study showed also the ability of effective rhizobia isolates to improve the tolerance to diseases when nodulated legume host in this case the common beans are inoculated. That inoculation with various rhizobia conferred resitance to a number of diseases is a preliminary but very interestingfindings worth following up in the future. The rationale for the disease resistance was not provided and could also be explored by subsequent students. For now it can be speculated that when legumes areeffectively nodulated, they are vigorous and have higher probability of taking up nutrients and further able to resist diseases and shwn by this study. Colonization of therhizosphere by compatible rhizobia could also have modified the root zone where most of these disease invade t plant.

5.5 Conclusion

We consider that the best five isolates of rhizobium have an ability to fix nitrogen and thus have a commercial potential among them two (NAR 265 and NAR 139) are offered as elites strains comparing favorable with the commercial strains (CIAT 899 and UMR 1597). However, rhizobium strains are to be genetically identified before they are recommended for use commercial products. This reality come from the results obtained from in Leonad Jars test, in pot experiment and in field experiment

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CHAPTER SIX: GENERAL RECOMMENDATION AND CONCLUSION

6.1 RECOMMENDATIONS

- Inoculation boosts nodulation, growth and yields of common bean in Rwanda and can be employed by small scale farmers and lower their consumption of N fertilizer and thus should be promoted as a green alternative.
- *Rhizobium* isolates evaluation should be espouse greenhouse work and field trials before recommendations on the effectiveness of isolates are made.
- This being the first extensive rhizobia isolation and selection work carried in Rwanda to the best of authors knowledge, there is need to carry further trials (covering more and distended geographical areas) to ascertain the behavior observed in this report.
- Isolates NAR 265, NAR 139, NAR 75 and NAR151 gave promising results in terms of nodulation, yield components and seed quality, biomass and grain yield and it is necessary to carry out further evaluation.

- To effectively and quickly identification and characterization of rhizobia, molecular methods such as rDNA analysis should be juxtaposed to other methodology. There is also need to carry out genetic mapping of the isolates.

6.1 CONCLUSION

The improved nodulation observed in both field and green house trials through inoculation with rhizobia was by and large influenced by the variety of bean used.

Some of the isolated strains (NAR 265, NAR 139 and NAR 151) performed better than the second commercial strain (UMR 1597) in terms of nodulation, crop growth and yields underscoring the huge potential. They have ability to induce nodules formation and N^2 fixation on bean variety.

They bring about effective N^2 fixation in association with a wide range of several type of bean variety.

Common beans showed promiscuity as a rhizobia host because provide evidence. However, it could be advisable to carry out rhizobia identification and cultivar selection in the future to boost symbiosis process.

Inoculation improved below ground microbial activity thus promoting healthy soils at the same time lowering carbon foot print in small holder farming systems.

Inoculation on the bean varieties showed a significant increase on yield biomass, yield bean grains, nutrient content on N and P and on the tolerance to diseases.

This should open an opportunity for further and more identification native rhizobia in Rwanda.

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Appendices

Appendix 1 : MPN test

No 1: SITE: RUBONA

Sampling date: 14th February 2012

Sowing date: 20th February 2012

REPETITION	REP I	REP II	REP III	REP IV
DILUTION				
10^{-1}	+	+	+	-
10^{-2}	-	+	-	-
10^{-3}	-	-	-	-
10^{-4}	-	-	-	-
10^{-5}	-	-	-	
10^{-6}	-	-	-	-
10^{-7}	-	-	-	-
10^{-8}	-	-	-	-
10^{-9}				
10^{-10}				

Dilution: 3-1-0-0

Population estimated: 15 microorganisms gram $^{-1}$soil

After harvesting

SITE: RUBONA

REPETITION	REP I	REP II	REP III	REP IV
DILUTION				
10^{-1}	+	+	+	+
10^{-2}	+	+	+	+
10^{-3}	+	+	-	-
10^{-4}	-	-	-	-
10^{-5}	-	-	-	-
10^{-6}	-	-	-	-
10^{-7}	-	-	-	-
10^{-8}	-	-	-	-
10^{-9}				
10^{-10}				

Number of dilution =10; results on 4 repetitions=4-4-2-0-0-0; population estimated: 614 microorganisms gram $^{-1}$soil

SITE 2: RUHUNDE

SOWING DATE 15th March/2012

SAMPLING DATE: 9 th February/2012

REPETITION	REP I	REP II	REP III	REP IV
DILUTION				
10^{-1}	+	+	+	+
10^{-2}	+	+	+	+
10^{-3}	+	+	+	+
10^{-4}	-	+	-	-
10^{-5}	-	-	-	
10^{-6}	-	-	-	-
10^{-7}	-	-	-	-
10^{-8}	-	-	-	
10^{-9}	-	-	-	-
10^{-10}	-	-	-	-

Results after 10 dilutions on 4 repetitions: 4 -4-4-1-0-0

Population estimated= 3,594 microorganisms gram $^{-1}$soil

After harvesting

REPETITION	REP I	REP II	REP III	REP IV
DILUTION				
10^{-1}	+	+	+	+
10^{-2}	+	+	+	+
10^{-3}	+	+	+	+
10^{-4}	+	+	+	_
10^{-5}	+	-	-	
10^{-6}	-	-	-	-
10^{-7}	-	-	-	-
10^{-8}	-	-	-	
10^{-9}	-	-	-	-
10^{-10}	-	-	-	-

Results after 10 dilutions on 4 repetitions: 4 -4-4-3-1-0

Population estimated= 15,926 microorganisms gram $^{-1}$soil

Appendix 2: Localization of rhizobia isolates screened

Code		Origin					Host
No	NAR	Country	Contributor	Altitude	Longitude	Latitude	Sub-family
1	1	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
2	2	Rwanda	RAB	1717m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
3	3	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
4	4	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
5	5	Rwanda	RAB	1685m	E 029°50’47.6’’	S 02°02’16.8’’	Bean
6	6	Rwanda	RAB	1684m	E 029°50’47.6’’	S 02°02’16.8’’	Bean
7	7	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
8	8	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
9	9	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
10	10	Rwanda	RAB	1684m	E 029°50’47.6’’	S 02°02’16.8’’	Bean
11	11	Rwanda	RAB	1691m	E 029^0 50’ 46.2’’	S 02^0 00’ 07.0’’	Bean
12	12	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
13	13	Rwanda	RAB	1500m	E 030º 27' 08.2"	S 01º 49' 13.8"	Bean
14	14	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
15	15	Rwanda	RAB	1730m	E 029^0 50’ 57.9’’	S 02^0 01’ 52.0’’	Bean
16	16	Rwanda	RAB	1730m	E 029^0 50’ 57.9’’	S 02^0 01’ 52.0’’	Bean
17	17	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
18	18	Rwanda	RAB	1730m	E 029^0 50’ 57.9’’	S 02^0 01’ 52.0’’	Bean
20	20	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
21	21	Rwanda	RAB	1500m	E 030º 27' 08.2"	S 01º 49' 13.8"	Bean
23	23	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
24	24	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean
25	25	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
26	26	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
27	27	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
28	28	Rwanda	RAB	1500m	E 030º 27' 08.2"	S 01º 49' 13.8"	Bean
29	29	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
30	30	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
31	31	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
32	32	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean
33	33	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
34	34	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
35	35	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Soybea
36	36	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean
37	37	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean

38	38	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
39	39	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
40	40	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
41	41	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
42	42	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
43	43	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
44	44	Rwanda	RAB	1730m	E 029^0 50’ 57.9’’	S 02^0 01’ 52.0’’	Bean
45	45	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
46	46	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
48	48	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean
49	49	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
50	50	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
51	51	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
52	52	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
2	55	Rwanda	RAB	1783m	E 029°48’35,6’’	S 02°05’55.1’’	Bean
54	72	Rwanda	RAB	1906m	E 029°53’42.9	S 01°35’50.3’’	Bean
55	73	Rwanda	RAB	1906m	E 029°53’42.10	S 01°35’50.3’’	Bean
56	74	Rwanda	RAB	1906m	E 029°53’42.11	S 01°35’50.3’’	Bean
57	75	Rwanda	RAB	1906m	E 029°53’42.12	S 01°35’50.3’’	Bean
58	76	Rwanda	RAB	1906m	E 029°53’42.13	S 01°35’50.3’’	Bean
59	77	Rwanda	RAB	1906m	E 029°53’42.14	S 01°35’50.3’’	Bean
60	78	Rwanda	RAB	1906m	E 029°53’42.15	S 01°35’50.3’’	Bean
61	79	Rwanda	RAB	1906m	E 029°53’42.16	S 01°35’50.3’’	Bean
62	80	Rwanda	RAB	1906m	E 029°53’42.17	S 01°35’50.3’’	Bean
63	81	Rwanda	RAB	1906m	E 029°53’42.18	S 01°35’50.3’’	Bean
64	82	Rwanda	RAB	1906m	E 029°53’42.19	S 01°35’50.3’’	Bean
65	83	Rwanda	RAB	1906m	E 029°53’42.20	S 01°35’50.3’’	Bean
66	84	Rwanda	RAB	1906m	E 029°53’42.21	S 01°35’50.3’’	Bean
67	85	Rwanda	RAB	1906m	E 029°53’42.22	S 01°35’50.3’’	Bean
68	86	Rwanda	RAB	1906m	E 029°53’42.23	S 01°35’50.3’’	Bean
69	87	Rwanda	RAB	1906m	E 029°53’42.24	S 01°35’50.3’’	Bean
70	88	Rwanda	RAB	1906m	E 029°53’42.25	S 01°35’50.3’’	Bean
71	89	Rwanda	RAB	1906m	E 029°53’42.26	S 01°35’50.3’’	Bean
72	90	Rwanda	RAB	1991m	E 029º 44' 03.4"	S 01º 25' 51.1"	Bean
73	91	Rwanda	RAB	1991m	E 029º 44' 03.4"	S 01º 25' 51.1"	Bean
74	92	Rwanda	RAB	1991m	E 029º 44' 03.4"	S 01º 25' 51.1"	Bean
75	111	Rwanda	RAB	1691m	E 029^0 50’ 46.2’’	S 02^0 00’ 07.0’’	Bean
76	112	Rwanda	RAB	1691m	E 029^0 50’ 46.2’’	S 02^0 00’ 07.0’’	Bean
77	113	Rwanda	RAB	1691m	E 029^0 50’ 46.2’’	S 02^0 00’ 07.0’’	Bean

78	114	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
79	115	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
80	116	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
81	117	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
82	118	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
83	119	Rwanda	RAB	1658m	E 029º 51' 10.7"	S 02º 00' 55.1"	Bean
84	125	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
85	126	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
86	127	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
87	128	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
88	129	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
89	130	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
90	131	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
91	132	Rwanda	RAB	1730m	E 029^0 50' 57.9''	S 02^0 01' 52.0''	Bean
92	135	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
93	136	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
94	137	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
95	138	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
96	139	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
97	140	Rwanda	RAB	1787m	E 029^0 41' 02.6''	S 01^0 32' 32.7''	Bean
98	141	Rwanda	RAB	1787m	E 029^0 41' 02.6''	S 01^0 32' 32.7''	Bean
99	142	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
100	143	Rwanda	RAB	1732m	E 029°43'08.5	S 01°34'32.3''	Bean
101	144	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
102	145	Rwanda	RAB	1732m	E 029°43'08.5	S 01°34'32.3''	Bean
103	146	Rwanda	RAB	1732m	E 029°43'08.6	S 01°34'32.3''	Bean
104	147	Rwanda	RAB	2050m	E 029°44'00.1''	S 01°25'46.2''	Bean
105	148	Rwanda	RAB	2050m	E 029°44'00.1''	S 01°25'46.2''	Bean
106	149	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
107	150	Rwanda	RAB	1732m	E 029°43'08.5	S 01°34'32.3''	Bean
108	151	Rwanda	RAB	2050m	E 029°44'00.1''	S 01°25'46.2''	Bean
109	152	Rwanda	RAB	2050m	E 029°44'00.1''	S 01°25'46.2''	Bean
110	153	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
111	154	Rwanda	RAB	1732m	E 029°43'08.5	S 01°34'32.3''	Bean
112	155	Rwanda	RAB	1732m	E 029°43'08.6	S 01°34'32.3''	Bean
113	156	Rwanda	RAB	1732m	E 029°43'08.4	S 01°34'32.3''	Bean
114	157	Rwanda	RAB	1732m	E 029°43'08.5	S 01°34'32.3''	Bean
115	158	Rwanda	RAB	1732m	E 029°43'08.6	S 01°34'32.3''	Bean

116	159	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
117	160	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
118	161	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
119	162	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
120	163	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
121	164	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
122	165	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
123	166	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
124	167	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
125	168	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
126	169	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
127	170	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
128	171	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
129	172	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
130	173	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
131	174	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
132	175	Rwanda	RAB	1539m	E 030°27’00.4’’	S 01°48’46.3’’	Bean
133	176	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
134	177	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
135	178	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
136	179	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
137	180	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
138	181	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
139	182	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
140	183	Rwanda	RAB	1906m	E 029°53’42.26	S 01°35’50.3’’	Bean
141	189	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
142	190	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
143	191	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
144	192	Rwanda	RAB	1539m	E 030°27’00.4’’	S 01°48’46.3’’	Bean
145	193	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
146	194	Rwanda	RAB	1539m	E 030°27’00.4’’	S 01°48’46.3’’	Bean
147	195	Rwanda	RAB	2082m	E 029º 44' 13.2"	S 01º 26' 07.3"	Bean
148	196	Rwanda	RAB	1787m	E 029^0 41’ 02.6’’	S 01^0 32’ 32.7’’	Bean
149	197	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
151	204	Rwanda	RAB	1906m	E 029°53’42.26	S 01°35’50.3’’	Bean
152	205	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
153	206	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
154	207	Rwanda	RAB	1684m	E 029°50’47.6’’	S 02°02’16.8’’	Bean

155	208	Rwanda	RAB	2050m	E 029°44’00.1’’	S 01°25’46.2’’	Bean
156	209	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
157	210	Rwanda	RAB	1732m	E 029°43’08.4	S 01°34’32.3’’	Bean
158	211	Rwanda	RAB	1745m	E 029^0 41’ 21.0’’	S 01^0 32’ 31.1’’	Bean
159	220	Rwanda	RAB	1906m	E 029°53’42.26	S 01°35’50.3’’	Bean
160	221	Rwanda	RAB	1906m	E 029°53’42.26	S 01°35’50.3’’	Bean
161	222	Rwanda	RAB	1783m	E 029°48’35,6’’	S 02°05’55.1’’	Bean
162	223	Rwanda	RAB	1783m	E 029°48’35,6’’	S 02°05’55.1’’	Bean
164	253	Rwanda	RAB	1783m	E 029°48’35,6’’	S 02°05’55.1’’	Bean
163	254	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
173	255	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
174	256	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
166	257	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
167	258	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
168	259	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
175	260	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
176	261	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
177	262	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
178	263	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
179	264	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
180	265	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
170	266	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
171	267	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean
172	268	Rwanda	RAB	1716m	E 029º 51' 01.2"	S 02º 01' 50.3"	Bean

RAB, N2Africa (2011): Bioprospection in Rwanda

Appendix 3: Nodules population and weight in bean variety from Leonard Jars test

	Nodules (population plant^{-1})		Weight (grams)
Treatments	**RWR 1668**	**Gasilida**	**RWR 1668**
NAR 128	**0.00**	**0.00**	**0.00**
0 Nitrogen	**0.00**	**0.00**	**0.00**
CIAT 899	**78.67**	**73.33**	**6.30**
NAR 171	**0.00**	**0.00**	**0.00**
NAR 32	**0.00**	**0.00**	**0.00**
NAR 41	**0.00**	**0.00**	**0.00**
NAR 114	**0.00**	**0.00**	**1.33**
NAR 132	**8.00**	**0.00**	**0.61**
Nitrogen	**0.00**	**0.00**	**0.00**
NAR 1	**0.00**	**0.00**	**0.00**
NAR 10	**6.67**	**0.00**	**0.48**
NAR 143	**0.00**	**0.00**	**0.00**
NAR 144	**0.00**	**0.00**	**0.00**
NAR 145	**0.00**	**0.00**	**0.00**
NAR 146	**0.00**	**0.00**	**0.00**
NAR 147	**0.00**	**0.00**	**0.00**
NAR 148	**0.00**	**0.00**	**0.00**
NAR 149	**0.00**	**0.00**	**0.00**
NAR 150	**0.00**	**0.00**	**0.00**
NAR 151	**72.00**	**63.67**	**5.71**
NAR 152	**0.00**	**0.00**	**0.00**
NAR 11	**0.00**	**0.00**	**0.00**
NAR 153	**0.00**	**0.00**	**0.00**
NAR 154	**0.00**	**0.00**	**0.00**
NAR 155	**60.67**	**46.33**	**4.88**
NAR 156	**14.33**	**0.00**	**1.11**
NAR 157	**0.00**	**0.00**	**0.00**
NAR 158	**0.00**	**0.00**	**0.00**
NAR 159	**0.00**	**0.00**	**0.00**
NAR 160	**0.00**	**0.00**	**0.00**
NAR 161	**0.00**	**0.00**	**0.00**
NAR 162	**0.00**	**0.00**	**0.00**
NAR 12	**0.00**	**0.00**	**0.00**
NAR 163	**21.00**	**16.00**	**1.42**
NAR 164	**53.67**	**47.33**	**4.62**
NAR 165	**68.67**	**47.67**	**5.50**
NAR 166	**66.33**	**48.00**	**5.28**
NAR 167	**67.00**	**50.00**	**4.68**

NAR 168	**0.00**	**0.00**	**1.90**
NAR 169	**66.00**	**50.67**	**5.23**
NAR 170	**63.00**	**50.67**	**4.95**
NAR 172	**0.00**	**0.00**	**0.00**
NAR 13	**0.00**	**0.00**	**0.00**
NAR 173	**0.00**	**0.00**	**0.00**
NAR 174	**0.00**	**0.00**	**0.00**
NAR 175	**0.00**	**0.00**	**0.00**
NAR 176	**16.00**	**12.00**	**1.23**
NAR 177	**0.00**	**2.67**	**0.43**
NAR 178	**0.00**	**0.00**	**0.00**
NAR 179	**0.00**	**0.00**	**0.00**
NAR 180	**49.00**	**40.00**	**2.63**
NAR 181	**0.00**	**0.00**	**0.00**
NAR 182	**0.00**	**0.00**	**0.00**
NAR 14	**0.00**	**0.00**	**0.00**
NAR 183	**0.00**	**0.00**	**0.00**
NAR 189	**0.00**	**0.00**	**0.00**
NAR 190	**0.00**	**0.00**	**0.00**
NAR 191	**0.00**	**0.00**	**0.00**
NAR 192	**0.00**	**0.00**	**0.00**
NAR 193	**48.33**	**40.33**	**3.77**
NAR 194	**45.00**	**0.00**	**3.53**
NAR 195	**39.00**	**48.00**	**3.53**
NAR 196	**0.00**	**0.00**	**0.00**
NAR 197	**0.00**	**0.00**	**0.00**
NAR 15	**0.00**	**0.00**	**0.00**
NAR 204	**0.00**	**0.00**	**0.00**
NAR 205	**58.67**	**45.00**	**4.60**
NAR 206	**73.00**	**64.33**	**5.81**
NAR 207	**39.67**	**25.00**	**3.10**
NAR 208	**0.00**	**0.00**	**0.00**
NAR 209	**53.67**	**52.00**	**4.26**
NAR 210	**53.67**	**42.33**	**4.23**
NAR 211	**0.00**	**0.00**	**0.00**
NAR 220	**36.00**	**27.00**	**2.82**
NAR 16	**0.00**	**0.00**	**0.00**
NAR 221	**0.00**	**0.00**	**0.00**
NAR 222	**0.00**	**0.00**	**0.00**
NAR 223	**0.00**	**0.00**	**0.00**
NAR 253	**0.00**	**0.00**	**0.00**
NAR 254	**0.00**	**0.00**	**0.00**
NAR 257	**0.00**	**0.00**	**0.00**

NAR 258	**0.00**	**0.00**	**0.00**
NAR 259	**0.00**	**0.00**	**0.00**
NAR 17	**7.00**	**0.00**	**0.55**
NAR 266	**42.67**	**44.00**	**3.35**
NAR 267	**40.67**	**45.00**	**3.18**
NAR 268	**25.00**	**11.00**	**1.89**
NAR 255	**19.33**	**8.00**	**1.46**
NAR 256	**69.67**	**44.00**	**5.56**
NAR 260	**0.00**	**0.00**	**0.00**
NAR 261	**0.00**	**0.00**	**0.00**
NAR 262	**50.33**	**34.00**	**3.97**
NAR 263	**49.67**	**44.33**	**3.97**
NAR 264	**50.33**	**36.67**	**3.68**
NAR 18	**0.00**	**0.00**	**0.00**
NAR 265	**74.00**	**66.33**	**5.30**
NAR 2	**2.67**	**0.00**	**0.20**
NAR 20	**0.00**	**0.00**	**0.00**
NAR 21	**0.00**	**0.00**	**0.00**
NAR 23	**0.00**	**0.00**	**0.00**
NAR 24	**0.00**	**0.00**	**0.00**
NAR 25	**0.00**	**0.00**	**0.00**
NAR 26	**0.00**	**0.00**	**0.00**
NAR 27	**0.00**	**0.00**	**0.00**
NAR 28	**0.00**	**0.00**	**0.00**
NAR 29	**0.00**	**0.00**	**0.00**
NAR 3	**63.00**	**50.00**	**4.93**
NAR 30	**0.00**	**0.00**	**0.00**
NAR 31	**0.00**	**0.00**	**0.00**
NAR 33	**8.00**	**0.00**	**0.59**
NAR 34	**0.00**	**0.00**	**0.00**
NAR36	**0.00**	**0.00**	**0.00**
NAR 37	**0.00**	**0.00**	**0.00**
NAR 38	**0.00**	**0.00**	**0.00**
NAR 39	**0.00**	**0.00**	**0.00**
NAR 40	**18.67**	**0.00**	**1.36**
NAR 42	**0.00**	**0.00**	**0.00**
NAR 43	**0.49**	**0.00**	**0.04**
NAR 44	**0.00**	**0.00**	**0.00**
NAR 45	**0.00**	**0.00**	**0.00**
NAR 46	**28.00**	**10.67**	**2.50**
NAR 48	**0.00**	**0.00**	**0.00**
NAR 49	**0.00**	**0.00**	**0.00**
NAR 50	**0.00**	**0.00**	**0.00**

NAR 51	**0.00**	**0.00**	**0.00**
NAR 52	**0.00**	**0.00**	**0.00**
NAR 56	**18.67**	**9.00**	**1.44**
NAR 72	**0.00**	**0.00**	**0.00**
NAR 73	**45.00**	**25.00**	**3.47**
NAR 74	**20.00**	**8.00**	**1.56**
NAR 75	**73.33**	**52.67**	**5.83**
NAR 76	**37.33**	**28.33**	**2.93**
NAR 77	**0.00**	**0.00**	**0.00**
NAR 78	**0.00**	**0.00**	**0.00**
NAR 79	**0.00**	**5.67**	**0.00**
NAR 80	**0.00**	**0.00**	**0.00**
NAR 81	**5.00**	**0.00**	**0.37**
NAR 82	**0.00**	**0.00**	**0.00**
NAR 83	**0.00**	**0.00**	**0.00**
NAR 84	**0.00**	**0.00**	**0.00**
NAR 85	**0.00**	**0.00**	**0.00**
NAR 86	**35.00**	**15.00**	**2.70**
NAR 87	**0.00**	**0.00**	**0.00**
NAR 7	**45.00**	**26.67**	**3.65**
NAR 88	**0.00**	**0.00**	**0.00**
NAR 89	**0.00**	**0.00**	**0.00**
NAR 90	**0.00**	**0.00**	**0.00**
NAR 91	**48.00**	**29.67**	**3.80**
NAR 94	**53.33**	**37.67**	**4.21**
NAR 111	**50.33**	**35.00**	**3.99**
NAR 112	**0.00**	**0.00**	**0.00**
NAR 113	**53.33**	**45.33**	**4.21**
NAR 115	**0.00**	**0.00**	**0.00**
NAR 8	**0.00**	**0.00**	**0.00**
NAR 116	**0.00**	**0.00**	**0.00**
NAR 117	**16.67**	**10.00**	**1.27**
NAR 118	**0.00**	**0.00**	**0.00**
NAR 119	**0.00**	**0.00**	**0.00**
NAR 125	**0.00**	**0.00**	**0.00**
NAR 126	**0.00**	**0.00**	**0.00**
NAR 127	**54.67**	**38.00**	**4.37**
NAR 129	**0.00**	**0.00**	**0.00**
NAR 130	**0.00**	**0.00**	**0.00**
NAR 9	**0.00**	**0.00**	**0.00**
NAR 131	**0.00**	**0.00**	**0.00**
NAR 135	**0.00**	**0.00**	**0.00**
NAR 136	**0.00**	**0.00**	**0.00**

NAR 137	**16.67**	**0.00**	**1.27**
NAR 138	**0.00**	**0.00**	**0.00**
NAR 139	**68.67**	**60.00**	**5.45**
NAR 140	**20.00**	**12.00**	**1.55**
NAR 141	**0.00**	**0.00**	**1.17**
NAR 142	**48.33**	**48.67**	**3.83**
UMR 1597	**75.00**	**68.67**	**6.00**
Mean	**14.38**	**10.68**	**1.15**
P value	**<0.001**	**<0.001**	**<0.001**
Lsd (5%)	**6.67**	**9.00**	**0.89**
CV %	**11.40**	**13.00**	**19.60**

Appendices 4: Nodule numbers, dry biomass weight on bean varieties

	Nodules (population plant^{-1})		Biomass dry (grams)	
Strains	**Gasilida**	**RWR 1668**	**Gasilida**	**RWR 1668**
0 Nitrogen	0	0	1.93	1
CIAT 899	88	96.67	9.4	4
NAR 151	84	94	7.2	3.3
NAR 265	86.33	93.33	6.73	3.4
Nitrogen	0	0	10.1	5
NAR 163	21.67	22.67	4.6	2.3
NAR 164	38.33	50.67	3.67	2.1
NAR 165	39	49.67	5.4	2.6
NAR 166	27.67	48	6.37	3
NAR 167	48.33	50	4.33	2
NAR 169	34	36.67	10	1.2
NAR 176	11.67	12	2.1	1
NAR 180	32	33.67	4.57	2.3
NAR 192	20.33	20.33	2.57	1.2
NAR 193	31	32	2.07	1
NAR 194	6.33	5.67	2.6	1.2
NAR 195	36.33	45.67	5.07	2.7
NAR198	21	22	2	1
NAR 205	41.33	55	3.1	1.7
NAR 206	83	90.33	7.3	3.4
NAR 207	24	25	2.97	2
NAR 208	17	17	2.03	1
NAR 209	26.67	41.33	2.9	1
NAR 210	28.67	62.33	6.23	4

NAR 211	22	18	2.8	1.1
NAR 220	28	27	2.6	1.2
NAR 257	24	24.33	2.2	3
NAR 259	17.33	17	2.07	1
NAR 266	42.67	48	4	2
NAR 267	44.67	45	5.87	3
NAR 268	20	35.33	4.4	3.17
NAR 255	11	9	4.05	2
NAR 256	41.33	48.33	4.3	3
NAR 260	43.33	60.67	3.63	1.7
NAR 261	39.67	49	3.6	1.8
NAR 262	40	41.67	4.57	2.3
NAR 263	53	53.67	2.57	1.2
NAR 264	35.67	38.67	2.57	1.3
NAR 3	19	35.33	4.87	2.6
NAR 46	15	16	2.7	1.27
NAR73	28.33	29	2.37	1.07
NAR 74	14.33	14.33	2	1
NAR 75	81	96	6.6	3.23
NAR 76	23.67	44.67	2.4	1.3
NAR 86	18.33	19.33	2.3	1.83
NAR 7	30.33	33.33	3.37	1.83
NAR 91	33.33	40.33	3.57	1.83
NAR 92	33.67	41.33	4.53	2.5
NAR 111	36	40.67	2.75	1.3
NAR 113	27.33	25.33	3.67	1.83
NAR 117	13	13.33	2.23	1.17
NAR 127	36.67	46	2.4	1.57
NAR 139	83.33	86.33	8.2	4.17
NAR 142	34.67	45.33	5.2	2.2
UMR 1597	82	90.33	6.43	3
Mean	35.11	40.81	4.18	2.11
Max	88	96.67	10.1	5
Min	0	0	2	1
p value	<0.001	<0.001	<0.001	<0.001
LSD (5%)	10.22	11.47	0.84	0.3
CV	18	17.3	12.7	8.6