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/research in any other university.

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DEDICATION

This thesis is dedicated to my God, my parents, the late Iyamuremye Theodomir and Mukabaziga Concessa, my wife Mukashema Umurerwa Chantal, my kids Alain Rumongi Tresor, Aldo Muhizi, Agatho Nkeramugaba and Alida Mutoni Gloria, my brothers, my sisters, my cousins and their respective families, Buhiga family and all descendants from late Gisazi Nkeramugaba**.**

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA: Analysis of variance

Anthr: Anthracnose

Asc: Ascochyta

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 dôAgriculture de lôElevage (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)

YEMA: Yeast ExtractMannital Agar

General Abstract

The overall objective of this study was to identify superior strains of native rhizobia associated with beans and establish their suitability for use as bean inoculants. Greenhouse and field experiments were conducted to identify and evaluate the effectiveness of rhizobia isolates in Rubona/Huye and Ruhunde/Burera, both located in Rwanda. The first greenhouse experiment was conducted to evaluate the potential of 174 rhizobia isolates. Accordingly, 50 rhizobial isolates were found to be promising. The 50 rhizobial isolates were tested in a second greenhouse experiment and 5 isolates were identified as the most effective. The five isolates were: NAR 265, NAR 151, NAR 139, NAR75 and NAR 206 and they compared favorably with the standard commercial strains, CIAT 899 and UMR 1597. These five best strains were then evaluated in the field using the bush and climbing beans. A complete randomized block design with three replicates was employed. The findings showed that NAR 265 is the most effective elite native strain, followed by NAR 139. The other objective of this study was to investigate the effect of inoculation on susceptibility of the legume host to disease resistance. CIAT score technique was used to score the symptoms of diseases on common bean. The results showed that the severity and incidence on bean diseases were low for anthracnose, ascochyta, angular leaf spot, rusts, root rot and CBMV. A similar result was also shown on crop bean fertilized with nitrogen and crop bean inoculated with CIAT 899, NAR 265, NAR 139 and UMR 1597. The severity and incidence was however high for bean crop without inoculation or not fertilized on bush bean. The study concluded that the improved nodulation, both in the field and green house trials was influenced by the variety of bean used.

Key words: *Phaseolus vulgaris* root nodulation, commercial strains, biomass, Rhizobia isolates, disease incidence, severity and score.

CHAPTER 1: GENERAL INTRODUCTION

Soil fertility degradation caused by nutrient depletion, crop removal or 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 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. 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

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. 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 bio-fertilizers such as legume inoculants would reduce the need for chemical fertilizers and decrease adverse

environmental effects. Therefore, in the development and implementation of sustainable agriculture techniques, bio-fertilization is of great importance in alleviating environmental pollution and the deterioration of nature (Elkoca, 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 N_2-fixation due to biological and environmental factors (Chianu, 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 major 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 therefore aims at evaluating the effectiveness of rhizobial isolates from Rwandan soils on 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. Increasing and extending the role of bio-fertilizers such as legume inoculants decrease the need for chemical fertilizers and reduce adverse environmental effects. Development and implementation of sustainable agriculture techniques, such as bio-fertilizers 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, 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 H.P and Lipin F*,* 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 lacking or less effective and/or 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. Some commercially prepared inoculants have also improved yield (Dube *et al*, 1976). 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

i. To identify elite rhizobia isolates from Rwandan soils.

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

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

1.4 Working hypothesis

i. Rwandan soils have potential elite Rhizobia isolates suitable to use as inoculants.

ii. Elite rhizobial isolates from Rwandan soils improve biomass and grain yields of beans.

iii. Rwandan 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 rhizobial isolates from Rwandan 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 Rwandan 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, while chapter six discusses the investigation on the role of the rhizobia isolates in reducing diseases severity on bean crop. The thesis closes by 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 and 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. 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 (Wortman *et al*, 1998). 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 farmersô 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 and 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 provides 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 a better position to raise its wellbeing and to meet expectations in improved living standards.

Legumes intensification was also found to increase subsequent cereal yield by approximately 40% with a net benefit increase of US $ 50 ha^{-1}(Snapp, 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 levels 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 ï 60 kg per person) and its high population increase exacerbates consumption while constraining the scarce land resources, hence the overall decline in production potential. 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 *et al*, 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 USD 452 million (Wortmann *et al,* 2006). 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).

The world leader in production of dry bean is India, followed by Brazil and Myanmar. In Africa, the most important producers are Tanzania, Uganda and Kenya (FAO, 2012)

Table 1: List of best dry bean producers in World (Adapted from FAO, 2012)

Country	Production (Tons)
India	4,870,000
Brazil	3,202,150
Burma	3,029,800
People's Republic of China	1,538,693
United States	1,442,470
Mexico	1,156,250
Tanzania	950,000
Uganda	460,000
Kenya	390,598
Argentina	338,120
World	**23,230,000**

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. Others include 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 ï 1400m above sea level). 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, 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 ï 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 plantsô 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, 2004).

The low productivity is linked to non-use of 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, 2003). 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 such as the Nitrobacter species derive their energy by oxidation of their own food, rather than feeding on plants or other organisms (heterotrophic). The amount of autotrophic bacteria is small compared to heterotrophic bacteria, but are very important because almost every plant and organism requires nitrogen in some way, and would have no way of obtaining it if not for nitrogen-fixing bacteria.

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 (Burton, 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 shepherdôs 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 *et al,* 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. It is feasible that in Africa, BNF technologies can become extremely important in order to avoid the perpetual food shortages, elevation of standard of living and diminution of nutrition on the continent. Hence, BNF presents a great potential for increasing food production, through the application of bio-fertilizers and subsequent desirable effects on the N economy on the soils (H. Ssali and S.O Keya, 1985)

Dry bean seed is usually inoculated with a fungicide 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 (Hungria et *al, 2000*).

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 that those grain legumes are inefficient N_2-fixers, Hardy *et al,* (1975) showed that symbiotic nitrogen fixation may cost the legume only 12-15% in photosynthate.

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.,* 1996). 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 K.E, 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 farmerôs 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 laboratory made impressive progress towards collection of rhizobia and their preservation and use for legume production in Rwanda. 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.

During 2010, a team of soil microbiology laboratory staff at Rubona have contributed to this study by a bio-prospecting for rhizobia in all districts of Rwanda by collecting nodules from bean crop cultivated in farmerôs plots across the country.

After collection, the nodules were properly labeled and stored in cool conditions before returning to the laboratory. Nodules were placed in pre-sterilized plastic bottles and aseptic procedures observed to avoid cross contamination. In total 174 rhizobia isolates from nodules of bean crop were isolated and sterilized.

Similarly, the same team collected 100 rhizobia isolates nodules soya bean and after isolation and sterilization a study on evaluation of effectiveness of rhizobia isolates from Rwanda soils on soya bean was conducted in Rubona research station.

In Agronomy area; the main activities are emphasizing on different trials:

(1) Preliminary trials on soya bean for best lines selection on yield for advanced trials.

(2) Advanced trials on soya bean for performing lines selected on yield or comparatives multi-location trials.

(3) Multi-location trials on soya bean (on-station and on-farm) for best varieties selected for yield adaptability and acceptability.

(4) Adaptability and acceptability test for selecting best lines on date of maturity, size of grains, yield and oil content.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Bio-prospecting for rhizobia

Nodules were collected from bean crops planted in regularly cultivated farmerôs 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 isolated from bean grown in four provinces of Rwanda in different Districts (Fig. 1)

(i) Northern Province: Ruhengeri, Gakenke, Rulindo and Burera.

(ii) Southern Province: Ruhango, Gitarama, Gikongoro and Butaree.

(iii) Eastern Province: Cyangugu

(iv) Western Province: Rwamagana and Kibungo

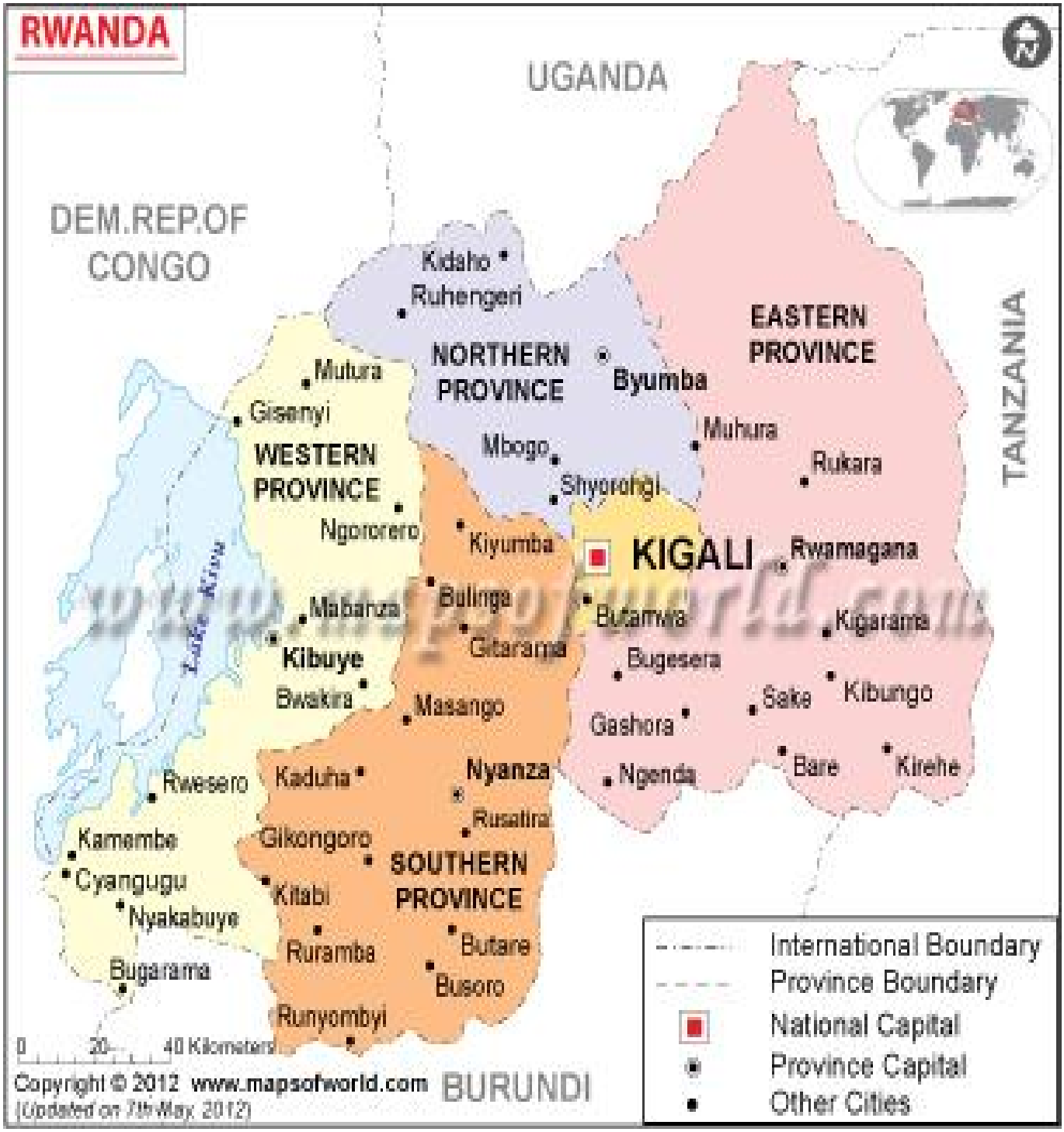

Figure 1: Rwanda Map and its Districts showing where nodules were sampled

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

3.2 Laboratory activities

3.2.1 Nodule sterilization

Nodules were surface sterilized following the procedures outlined by Somasegaran et al., (1994). The Rhizobia were then isolated 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 containing 96% alcohol to remove superficial microbes.

(iii) Nodules from bean crop were immersed in 90% alcohol for 10 seconds then washed in a second beaker containing sterile water.

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

(v) Finally nodules were washed by immersion again in sterile water then transferred into sterile Petri dishes using sterile forceps.

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

3.2.2 Rhizobia isolation

Nodules were crashed and washed with sterile water and the rhizobium was then isolated from the nodules. A loop full of crushed nodule was streaked across the Petri dishes containing yeast

manitol agar media and grown in an incubator maintained at optimum temperature of (28°C 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 basically entailed six steps as outlined below:

(i) Preparation of culture rhizobial isolates.

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

(iii) Pre-germination of seeds and thinning.

(iv) Inoculation of pre-germinated legume plant followed by watering.

(v) Observation of nodulation 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 experiments

One hundred and seventy four isolates (174) were collected. The isolates were tested in Leonard Jars 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 the plantsô aeration and for watering. The best 50 rhizobia isolates were selected on the basis of nodule numbers, nodules color, nodules size, nodule weight and biomass. The experiment was conducted in the greenhouse at ISAR Rubona. The experiment was laid out in a split plot design and replicated three times. The treatments were three: uninoculated control plus Nitrogen, uninoculated treatment minus Nitrogen and inoculated treatment. The greenhouse experiments were replicated three times resulting into 324 treatments. There were two controls and two commercial strains, CIAT 899 and UMR 1597. 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 plants per pot. Nitrogen-free nutrient solution (Broughton and Dillworth, 1970) plus N controls treatment, KNO_3 (0.05%) were 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. From pot experiment the evaluation was based on nodules number, nodules size, nodules color, dry weight nodules and biomass. The five best rhizobia isolates were NAR 265, NAR 151, NAR 139, NAR 75 and NAR 206. The number of rhizobia by type of evaluation is presented in figure 2.

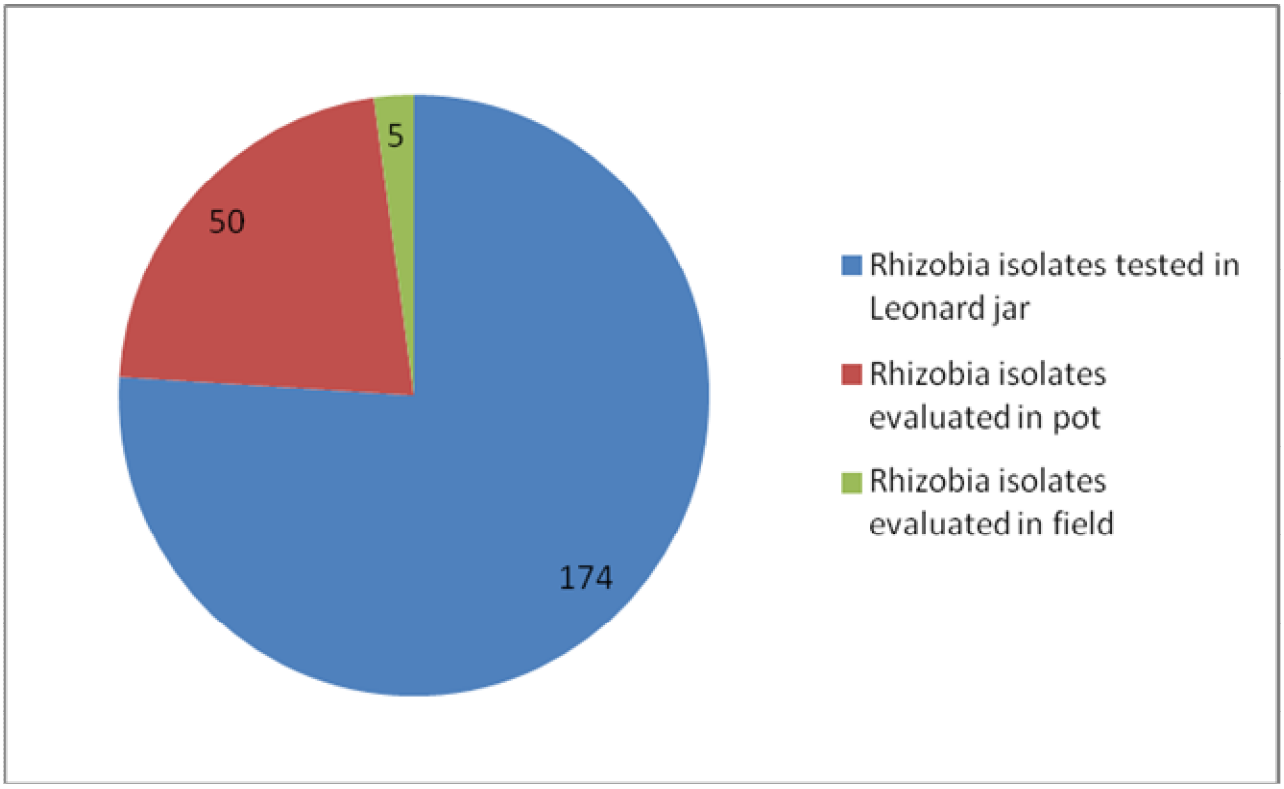

Figure 2: Number of rhizobia isolates by type of evaluation

Plate 2: Evaluation of nodulation in pot

Plate 3: Evaluation of nodulation on plant roots

3.4 Study site of field experiments

The field experiments were conducted in two different agro-ecological zones. The first site was Ruhunde, located in Burera District in Northern Rwanda at Longitude E 029 93ô38.5and Latitude 01S 55ô 83.5. The altitude ranges from 1800-2400 m above sea level and the mean annual temperature is 15 to 18^0 C. Rainfall is bimodal and the mean annual rainfall received ranges from 1800-2200 mm .The highest rainfall amount is received between February and May and the dry season is experienced between June and August. Ruhunde has a fertile volcanic soil with a high potential for agriculture (ISAR, 2000).

The second site was Rubona (Research Station) located in Huye District in Southern Rwanda. The altitude ranges from between 1600-1800 m above sea level with an annual mean temperature of 16^0C to 20^0C. Rainfall is bimodal and the mean annual amount received ranges from 1700-2000 mm. The highest amount of rainfall is received between February and May and the dry season occurs between June and August. Others physiochemical soil properties for the two sites are in table 2.

Table 2: Physiochemical soil properties of experimental sites

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

3.5 Soil sampling

Soil sampling was done from farmerôs 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 analyzed for pH, organic carbon, available phosphorus, exchangeable cations, total nitrogen and particle size. Procedures outlined in Okalebo *et al.,* (2007) were followed.

3.6 Soil chemical characterization

The composite sample was analyzed in the laboratory following the procedures outlined in Okalebo *et al.,* (2007).

3.6.1 Determination of soil pH

The pH by 1:2.5 ratios of water and calcium chloride was determined. 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 was added to the other set. The samples were shaken for 30 minutes in a reciprocating mechanical shaker, allowed to stand for 30 minutes and the pH reading was taken from the pH meter.

3.6.2 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 suited for acid soils of pH less than 6.5, soils with low CEC and soils with organic matter content of less than 5%. Available phosphorus was determined by weighing 5 g of air dried soil. The soil was mixed with 50 ml of Mehlich extracting solution (a double acid, containing 0.025N sulphuric acid and 0.05N hydrochloric acid) to produce a solution. 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.

3.6.3 Determination of 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 Walkley-Black (1934) oxidation method as (outlined by the Okalebo *et al.,* 2007). The method involved complete oxidation of soil organic carbon using concentrated sulphuric acid (H_2SO_4) and dichromate solution. The unused or residual $K_2Cr_2O_7$ was 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. This was followed by weighing 0.5g of air dried soil sieved through a 0.5 mm sieve .This was then transferred into a set of clean conical flasks. The next step involved addition of 10 ml of 1N $K_2Cr_2O_7$ into each conical flask and swirled gently followed by addition of20ml of 36N H_2SO_4 .This was then 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, and the color changes and end point were observed.

3.6.4 Determination of Cation Exchange Capacity

Cation exchange capacity (CEC) of the soil samples was determined using Metson method, (1961). The method uses 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 potassium ion salt solution was used to replace and leach out adsorbed NH_4^+ ions. The amount of NH_4^+ released gave the amount of CEC of a soil. The amounts of exchangeable Na, K, Ca and Mg in the extract were determined by flame photometry for Na and

K, and by atomic absorption spectrophotometer for Ca and Mg. Lanthanum (La) and strontium (SR) were added as a releasing agents to prevent formation of refractory compounds, which could interfere with the determination, of Phosphate.

3.6.5 Determination of total Nitrogen

In the determination of total nitrogen, the Kjeldahl, (1883) procedures as outlined by Okalebo *et al.,* (2007) were followed. This method entailed the conversion of nitrogen into $(NH_4)_2SO_4$ followed by distillation of NH_3 in an alkaline medium and titrating it with standard sodium hydroxide. One gram of a sample sieved through a 0.5 mm sieve was weighed and transferred into a clean digestion tube. A catalyst was then added followed by 8 ml of 36 N H_2SO_4. Samples were digested for 2 hours and were then titrated against 0.01N HCl. The volume of the titre used was then noted.

3.6.6 Isolation and codification of native rhizobia

Native rhizobia were isolated from nodules of legumes collected from farmersô fields. Isolation and preliminary characterization of the root nodule bacteria was done in the Rhizobiology Laboratory based at RAB Rubona Station. Each rhizobia isolate is known by a given code NAR (N2 Africa Rwanda) followed by a number.

3.6.7 Determination of Indigenous rhizobial populations

The most-probable-number (MPN) method outlined by 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 ensured for each soil by adding 9 ml of sterile water into 1 g of soil. This was mixed thoroughly by shaking on a rotary shaker for 20 minutes to disperse the soils. Serial dilutions were continued up to 10^{-6} for each of the soils.

3.7 Data analysis

Data were compiled into a spread sheet, inspected and were subjected to analysis of variance (ANOVA) using Genstat Discovery, 15th edition. 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 experiment was to identify the best elite rhizobia isolates from Rwandan soils based on their effectiveness compared to the commercial strains, CIAT 899 and UMR 1597. This experiment was conducted in the greenhouse. The 174 rhizobia isolates from Rwanda were used to innoculate common beans grown in Leonard jars and evaluated using bush and climbing bean varieties in the greenhouse. The rhizobia isolates formed effective nodules, red in color, large in size and showed vigorous growth. The measurement of dry weight of nodules indicated that 50 of the Rwanda rhizobia isolates were able to improve nodulation and biomass of both bush and climbing beans. The 50 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; number of nodules, nodule size, color of nodule and dry biomass. The 50 Rhizobia isolates from Rwanda showed a high significant difference on number of nodules, dry weight of nodules and dry weight biomass (P=0.005). However the size and the color of nodules did not show significant difference. Results further showed that 5 best rhizobia isolates compared favorably with the standard commercial strains and were proposed for further evaluation in field experiments**.**

Key words: *Phaseolus vulgaris*, root nodulation and 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, (Odame, 1997). Some of the options that are currently being pursued to address low soil fertility include integrated use of organic resources (e.g. crop residues, animal manures and agroforestry tree pruning) and inorganic resources (e.g. fertilizers and phosphate rocks), and use of rhizobia inoculants (Woomer, 2009). The use of crop residues usually conflicts with their other uses as fuel and fodder, while the use of animal manure is constrained by their difficulty in gathering, especially in a free grazing system and also by the labor required in their transportation to the intended fields due to their bulkiness. The use of manures is also constrained by their usually 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 bacteria fix atmospheric nitrogen (N_2) in leguminous plants through legume-rhizobium symbiosis and form nodules on the roots or stems of these plants. Auxin biosynthesis by rhizobia is increased many folds in supplementation with suitable precursor (Tryptophan) (Zahir *et al*., 2005).

4.2 Materials and methods

Rhizobia isolates derived through bio-prospecting in Rwanda were evaluated in the greenhouse using Leonard Jars and pots and their effects on the two types of common bean were assessed. The greenhouse was cleaned prior to the set-up of the experiment and Leonard Jars and pots were thoroughly sterilized by 95 % alcohol. The substrate was then put in the substrate. The bean seeds were sorted and 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. The seeds were then pre-germinated on sterile (autoclaved) vermiculite for 48 hours in an incubator at 28^0C, and regularly inspected to assure that the radical doesnôt 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 the 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 weights of host legumes were also considered. The numbers of nodules were examined at flowering time which was about 30 days after planting,

4.3 Results

4.3.1 Evaluating nodulation and effectiveness of rhizobia strains using Leonard jars.

Effectiveness Index was done based on plant biomass and means for internal nodule color and nodule number for authentication experiment in the greenhouse. From Leonard Jars experiment, nodule numbers and nodule biomass were found to be highly significant ($p<0.001$) for bush and climbing bean. The results indicated that nodulation was higher in bush bean than climbing bean across all strains. The average nodules numbers were 14 and 10 respectively for bush bean and climbing bean. CIAT 889 and UMR 1597, commercial strains yielded the highest numbers of nodules, 78.6 and 73.3 in bush bean and 75 and 69 in climbing bean 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 CIAT 899 giving 6.3 grams and 5.89 grams respectively for bush bean and

climbing (chart 1 and 2 below). In overall assessment, the treatments showed significant nodule weight differences ($p<0.001$)

However, when compared with 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) showed high nodule numbers and nodule weights that were statistically insignificant compared with commercial strains ($p=0.005$). 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 were divided in four groups: The first group of 5 rhizobia isolates (NAR 265, NAR 139, NAR 151, NAR 151 and NAR 206) was highly effective. The second group had 50 isolates and showed an intermediate effectiveness. The third cohort constituting 52 isolates were partially effective. The fourth group had 67 rhizobia isolates and was totally ineffective on bush bean (RWR 1668) with index 0.91 to 1.2; 0.81 to 0.9; 0.61 to 0.8; and 0.1 to 0.6 as illustrated in table 3 below.

Table 3: Effectiveness index by group description of rhizobia isolates and their underlying features

Index	**Group description**	**Underlying features**
0.91-1.2	Highly effective	Red nodule color, very big nodule size and very green plants.
0.81-0.9	Intermediate effective	Pink nodule, big nodule size and green plants
0.61-0.8	Partially effective	Yellow nodule, moderate nodule size and light green.
0.1-0.6	Non-effective	Brown nodule, small nodule size and yellowish plants.

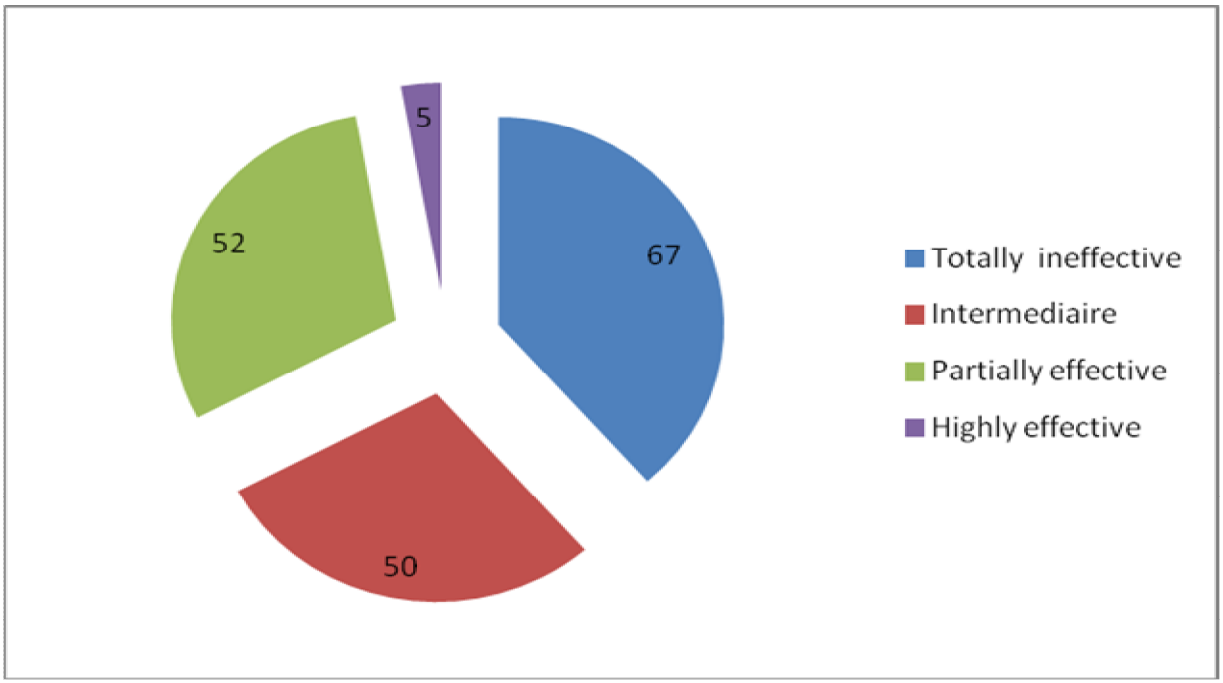

Figure 3: Group of rhizobia isolates on bush bean according their effectiveness index

In terms of effectiveness index on climbing bean, 4 rhizobia isolates (NAR 265, NAR 139, NAR 151 and NAR 75) were highly effective, 50 rhizobia isolates showed intermediate effectiveness; 50 rhizobia isolates were partially effective and 68 rhizobia isolates were totally ineffective on climbing bean (Gasilida) with index of 0.91 to 1.2; 0.81 to 0.9; 0.61 to 0.8 and 0.2 to 0.6 respectively as illustrated in figure 4 below.

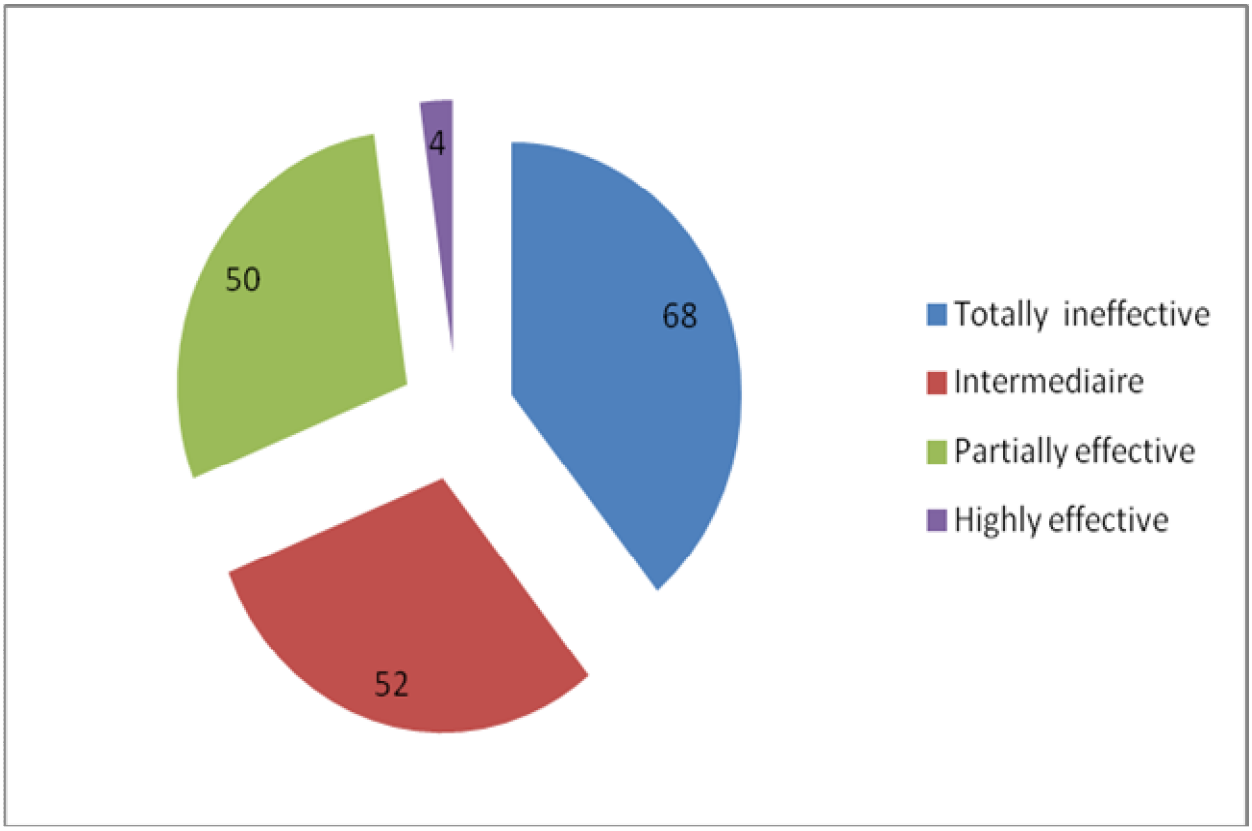

Figure 4: Group of rhizobia isolates on climbing bean according their effectiveness index

4.3.2 Pot experiment evaluation

i) Nodules number and dry weight

Evaluation of pot experiment showed that the nodule numbers and dry weight were highly significant ($p<0.001$), both for bush and climbing beans. However, bush bean generally showed higher nodule numbers across the strains (Figure 2 a & b).

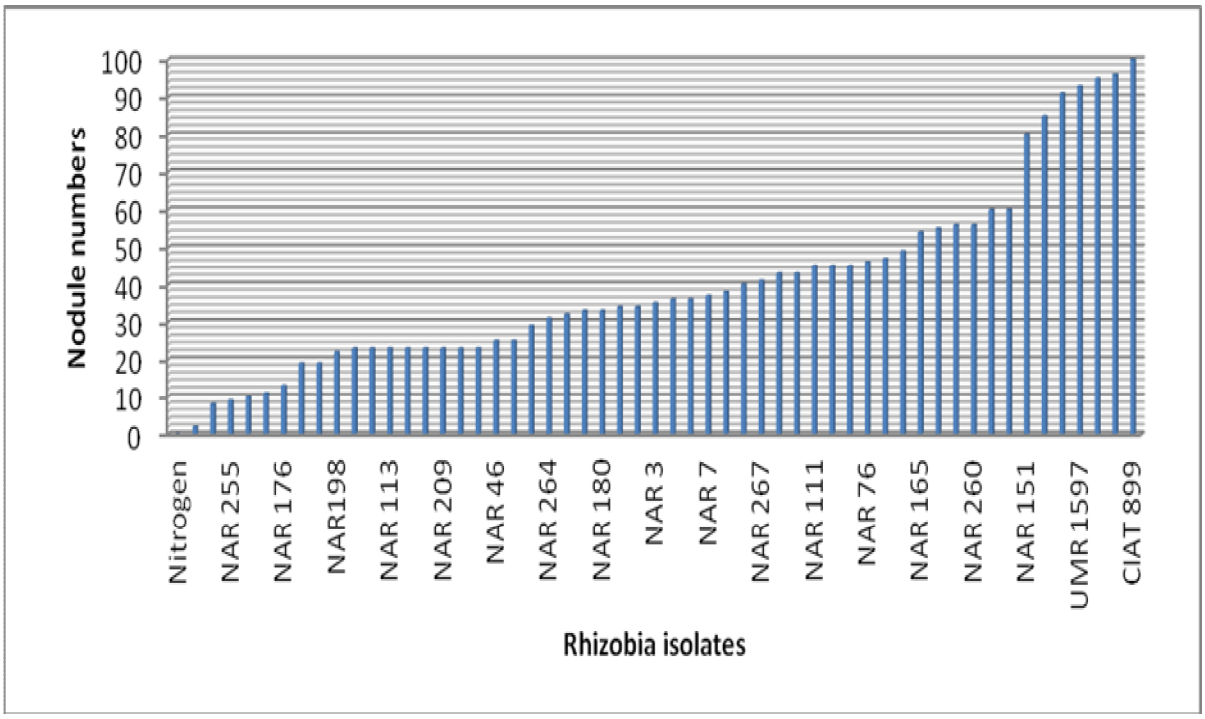

Figure 5a: Nodule numbers from bush bean

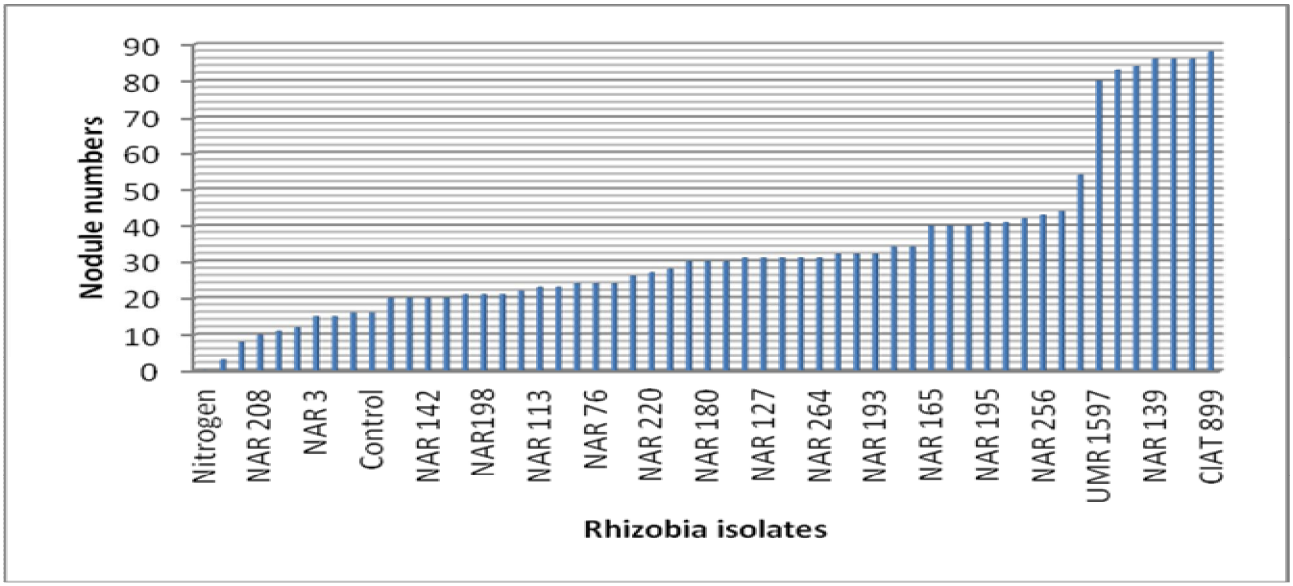

Figure 5b: Nodule numbers from climbing bean

CIAT 899 commercial strain showed the highest number of nodules, 96.7 and 88 in bush bean and climbing bean respectively. However, there was insignificant nodule population in both the

bush and climbing beans when CIAT 899, NAR 265, NAR151, NAR139, NAR 206, UMR1597 and NAR 75 strains were used, with performance in that order. In bush beans, nodule numbers with NAR 75, NAR 151 and NAR 206 was higher than with UMR 1597, but lower than with CIAT 899. The performance of strains, NAR 139 and NAR 265 was lower than that of UMR 1597. Low nodulation was observed in plants where the nitrogen was applied. Biomass dry weight reflected magnitude of nodulation. The highest dry weight was realized under crops fertilized with nitrogen at 5.2 grams and 10.1 grams for bush and climbing bean respectively (figure 5 a & b) followed by CIAT 899 at 5 grams and 9.4 for bush bean and climbing bean respectively.

Other strains also indicated high dry weight biomass; 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.

The climbing bean inoculated with NAR 139 recorded 8.2 grams dry weight per plant; NAR 265 recorded 8.2 grams, NAR 206, 7.3 grams; NAR 151 7.2 grams and NAR 75, 6.6 grams dry weight biomass per plant as illustrated in figures 5 a & b.

ii) Dry weight biomass

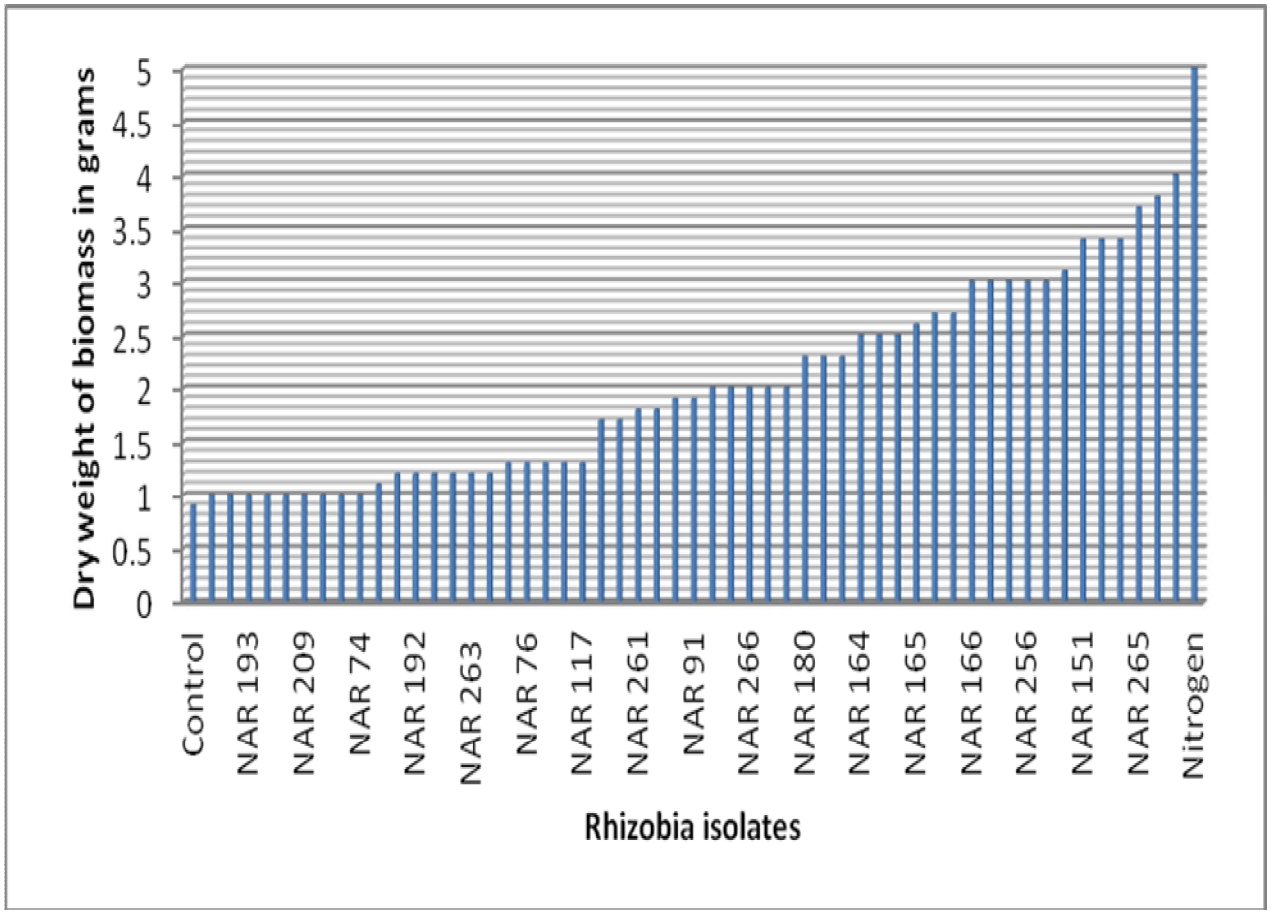

Figure 6a: Dry weight biomass of bush bean in pot.

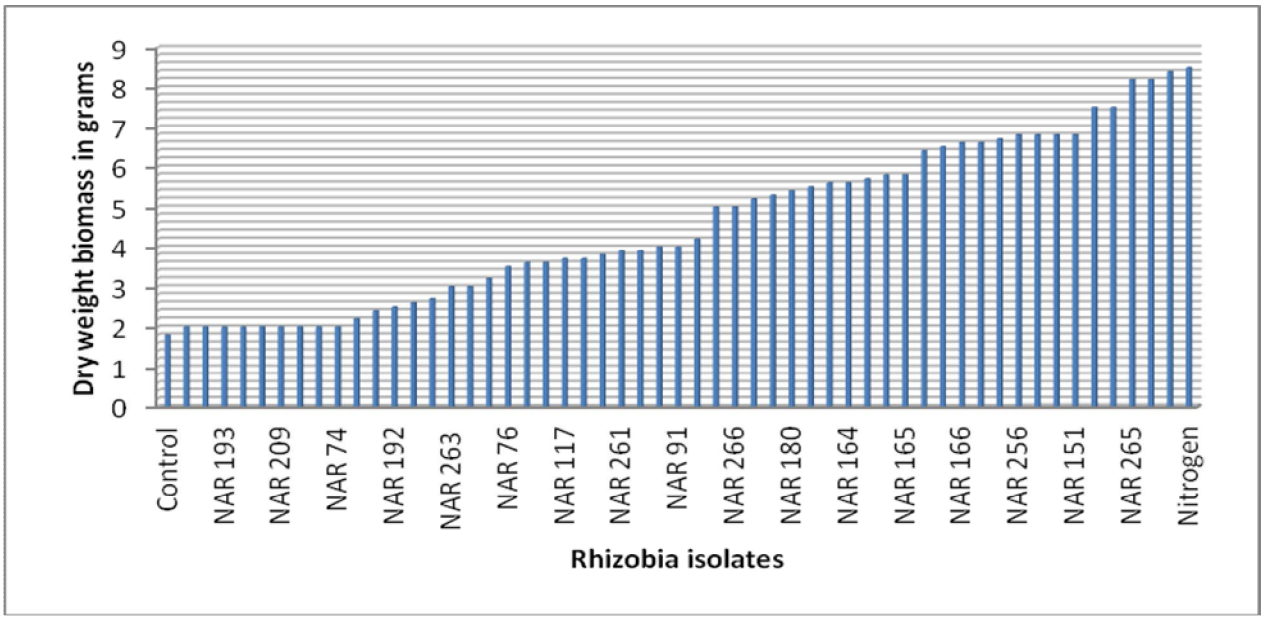

Figure 6b: Dry weight biomass of climbing bean

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, nodule color and size of nodules 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 the control treatment. The analysis of variance; both for the Leonard jar and pot experiments, showed that the difference between inoculations was significant in terms of nodule population and their weight, but not in terms of size and color.

Inoculations with commercial strain, CIAT 899 and most of native rhizobia were more effective on nodule population and on biomass compared to the control. However, total nodule numbers in bean significantly increased compared with the control ($P<0.05$), 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 (CIAT 899) and native isolates; NAR 265, NAR 206, NAR 151, NAR139 and NAR 75. These were selected and the experiment conducted in the field 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 might have been used excessively on these soils. Further it could be argued that native rhizobium populations were many and out-competed the introduced strains.

CHAPTER FIVE: EVALUATION OF EFFECTIVENESS OF RHIZOBIA ISOLATES IN FIELD EXPERIMENT, MPN AND MICROBIOLOGICAL TESTS

Abstract

The objective of this experiment was to evaluate the effectiveness of native rhizobia isolates in the field. The experiment was installed at Rubona and Ruhunde in Rwanda. A complete randomized block design with three replicates was employed in both cases. The findings indicated that nodule numbers were significantly different, both for the bush and the climbing beans. The bush beans inoculated with commercial strains, CIAT 899 and UMR 1597 recorded the highest mean nodule numbers of 82.1 and 73.1 respectively. Bush beans inoculated with, NAR 265, compared well with the commercial strains yielding 67.7 nodules, followed by NAR 139 that yielded 63.03 nodules. The lowest nodule numbers were observed where the controls were used; 8.47 nodules for N_2 and 13.7 for control (P=0.001). Dry weight biomass did not show any significant different (p=0.001) and the highest biomass weight was recorded by the CIAT 899 commercial strains (4.08 t ha^{-1}) while the lowest by control (2.38 t ha^{-1}). A similar trend was also shown by the strains inoculated with the climbing bean, whereby the highest nodule numbers were recorded when CIAT 899 was used (67.5 nodules), followed by UMR 1597 (61.83 nodules). NAR 265 and NAR 139, which are elite native strains significantly compared with the commercial strains at 61.8 and 58.8 nodules respectively (P=0.001). The lowest nodule populations were recorded with the controls whereby 9.4 and 11.1 nodules were recorded for N_2 and Control respectively. The findings showed that NAR 265 is the most effective native strain, followed by NAR 139.

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

5.1 Introduction

Rwanda is the largest producer of common bean (*Phaseolus vulgaris L.*) in East Africa and the grains represent the most important source of protein for the population. However, the production is still below the population demand (MINAGRI, 2002). This level of production has been mainly attributed to the use of inferior agricultural technology and cropping in soils low in nitrogen. Therefore an adequate supply of N through symbiosis with N_2-fixing rhizobia is necessary if the production has to be increased at a low cost. This approach will also protect water resources from pollution by excess mineral nitrogen which is normally washed by the runoff water. 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; Buttery *et al*.1987; Ramos and Boddey 1987; Hardarson *et al*., 1993). The failures in some trials have mainly been attributed to a high but inefficient population of indigenous rhizobia. 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 (Hungria and M.A. Vages, 2000).

The objective of this experiment was therefore to evaluate the effectiveness of native rhizobia isolates in the field.

5.2 Materials and methods

The five best rhizobia isolates (NAR 265, NAR 206, NAR 151, NAR139 and NAR 75) were selected after evaluation in field experiment. The experimental plots measuring 3m×3.5m were arranged in a complete randomized block design and a total of 9 different treatments were applied. Each treatment was replicated thrice in each plot giving a total of 27 plots which were prepared and sown with the two bean varieties; bush bean and climbing bean. The commercial

strains, (CIAT 899 and UMR 1597) and two controls (with nitrogen and without nitrogen) were applied. Plots within each block were separated by 1m apart and the distance between blocks was 3m. The native bean seeds were inoculated with filter mud-based inoculants of native rhizobia isolates. A solution of Gum Arabic (40%, w/v) was used as a sticker. Commercial rhizobia (CIAT 889 and UMR 1597) inoculants were also prepared. Based on the viable counts of inoculants (1ï 5×10^9 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, soil samples were taken from each site and viable microorganisms contained in g^{-1} solution was determined using MPN technique. Data were collected, recorded and analyzed using MPN technique and GenStat 15th edition**.**

5.3 Results

5.3.1 Nodulation

For the climbing bean, the highest numbers of nodules were observed in plants inoculated with Commercial strains; CIAT 899 which recorded mean nodule number of 67.50, and UMR 1597 which recorded 61.83 nodules. NAR 265, which is a native strain compared significantly with the commercial strains by yielding mean nodule number of 61.80. It was followed closely with NAR 139, which recorded 58.8 nodules and, NAR 206 (53.33 nodules), NAR 151 (43.8 nodules) and NAR 75(52.47 nodules) in that order (P=0.001). See table 4 below.

Table 4: Dry weight biomass and grains yield on bean varieties

	Bush beans			**Climbing beans**		
Rhizobia strains	Nodules number	Biomass (t ha^{-1})	Yield (t ha^{-1})	Nodule population	Biomass (t ha^{-1})	Yield (tha^{-1})
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
Nitrogen	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

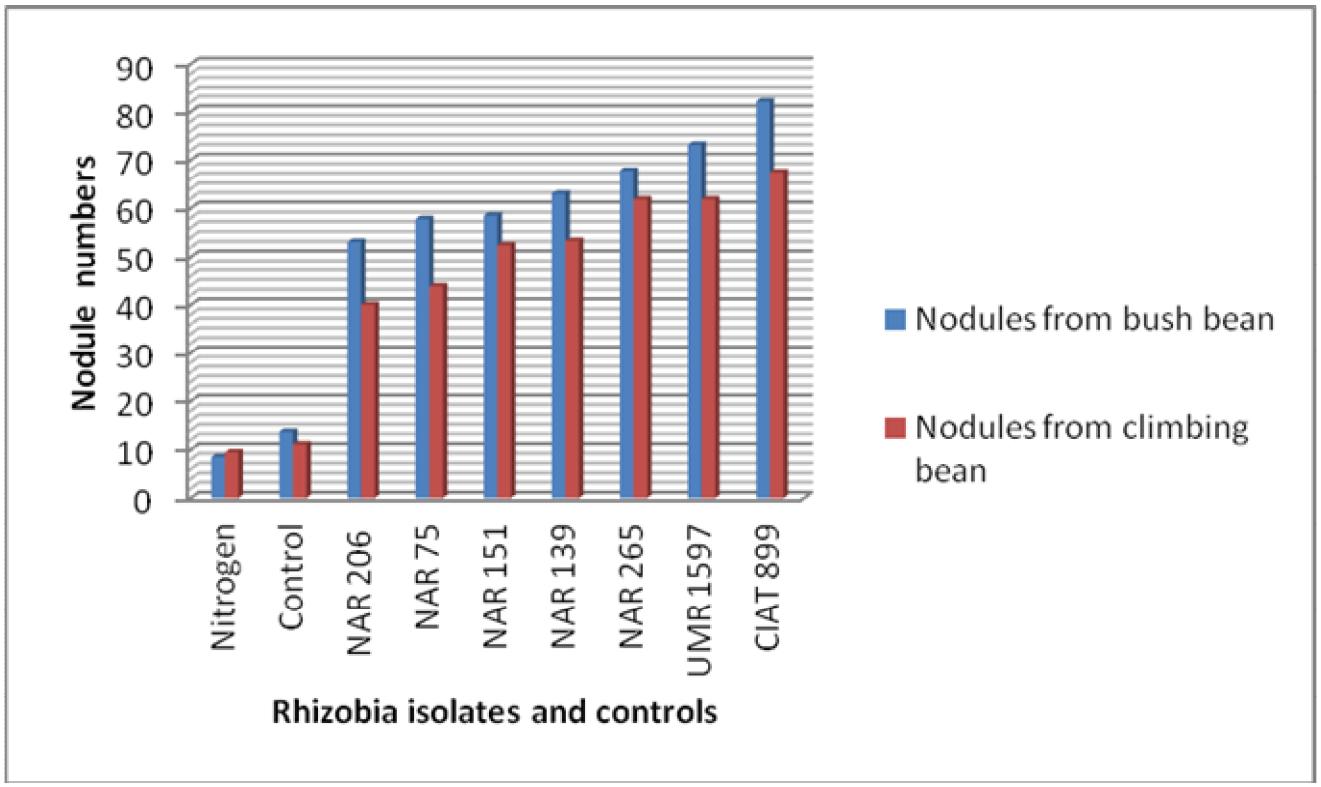

Figure 7: Nodule numbers obtained from bush bean and climbing bean

The climbing bean, just like the bush bean showed a similar trend. The highest number of nodules was observed where inoculation was done with the commercial strains; CIAT 899 which recorded mean nodule number of 82.1, and UMR 1597 which recorded 73 nodules. The native strains which significantly compared with these commercial strains were the NAR 265 which recorded 67.80 nodules and NAR 139 which recorded 63.0 nodules, NAR 151 yielded 58.5.4 nodules, while NAR 75 and NAR 206 recorded 57.8 and 53.1 mean nodule numbers respectively. The lowest mean nodule numbers were observed where common bean was fertilized with nitrogen and as low as 10 and 12 nodules plant^{-1} were recorded.

The indigenous rhizobial population before the first sowing was estimated at 15 and 3,594 cells g$^{ï 1}$ 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 $g^{ï\,1}$ soil as shown in the appendix 1.

5.3.2 Yield components and seed quality

For the bush bean, the results showed a significant effect of rhizobial on yield component: pods (p=0.01), seed quality (p=0.01), haulms (p=0.08), while for climbing beans, all the yield components showed a significant response (p=0.010 to treatments (Table 5).

Table 5: Weight of pods and 100 seeds of two bean varieties

	Climbing beans		**Bush beans**	
Treatments	**Pods(t ha^{1})**	**100 seeds(g)**	**Pods (t ha^{-1})**	**100 seeds(g)**
Control	3.31	45.6	1.88	44.77
CIAT 899	5.59	57.03	2.33	55.83
Nitrogen	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	0.89	0.072	0.37

Climbing beans showed a better performance compared to bush beans in all the yield parameters assessed. Maximum pod yield was realized in plots fertilized with nitrogen for both climbing (5.7 t/ha) and bush (2.35 t ha^{-1}) beans (table 6). This was followed closely by commercial strains CIAT 899 (5.59 t ha^{-1}) and UMR1597 (5.26 t ha^{-1}). However, isolates performance was better in plots that were inoculated with strains NAR 265, NAR75 and NAR 139, which performed better than the commercial strain, UMR1597. In bush beans, plots applied with nitrogen fertilizer produced the highest pods weight (2.35t ha^{-1}), followed by CIAT 899 (2.33 t ha^{-1}), NAR 206 (2.29t ha^{-1}), NAR139 (2.22 t ha^{-1}). Plots not applied with rhizobia inoculants showed the lowest yield.

Figure 5a indicates that climbing beans inoculated or fertilized with nitrogen showed a better performance compared to bush beans in all the yield parameters assessed. The data also showed the ability of best strains to increase the yield of pods as demonstrated in figure 6b. It was equally observed that there was no statistical difference in yield (p=0.01).

The weight of seeds ranged between 58 grams to 44 grams with an average of 54.4 grams and 53.5 grams for bush and climbing beans. Seed weights in grams for climbing bean were 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. There was no significant response on 100 seed weight for climbing bean (p=0.01).

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 5b. Statistically, the weight for all treatments were not significant (p=0.01).

Plate 4: An evaluation of crop performance during harvesting

Evaluation of diseases before the maturity(climbing bean0

Evaluation of grains yield and biomass at the maturity(climbing bean)

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

Yields for both grain and above ground biomass was significant (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 (Fig 8)).

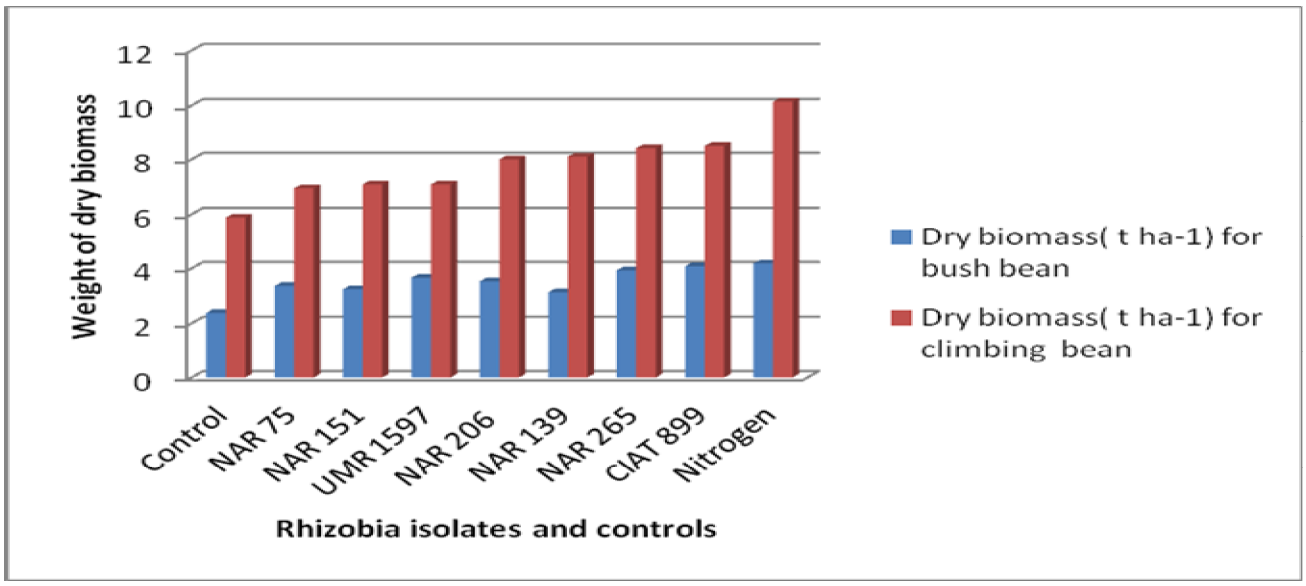

Figure 8: Biomass yield (t ha-1) of bean inoculated or non-inoculated at Rubona and Ruhunde, Rwanda.

Biomass weight was highest under N fertilized control plots for both bean varieties, followed by CIAT 899, NAR 265 and NAR 139. Climbing beans realized higher biomass, doubling those of bush beans across all the strains. On average, plots with no fertilizer and without rhizobia inoculation applied recorded the lowest biomass yield.

Inoculation showed 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 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 lowest grain yield (2.16 t ha^{-1}) was obtained in the control (Figure 8).

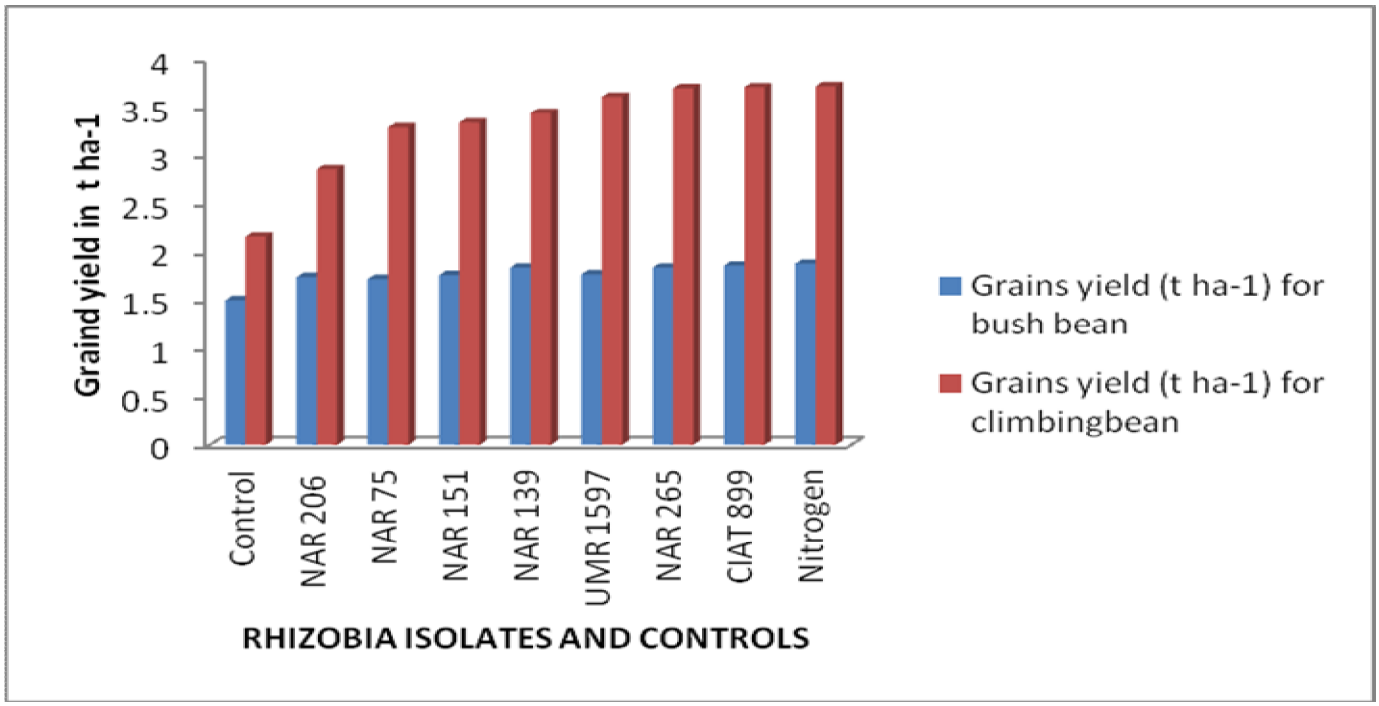

Figure9: Effect of rhizobia isolates on grain yield of bush and climbing beans

Bush beans recorded the highest grain yield with an average of 1.88 t ha^{-1} while the lowest yield of 1.5 t ha^{-1} was recorded in plots fertilized with nitrogen and without any input. Plots where there was no inoculation and no fertilizer yielded the lowest grain of 1.50 t ha^{-1}.

The isolates evaluated showed the ability to increase the yield according to their performance comparatively with 0 N (Control) and the improvement in grains yield on bush bean (Table 6).

Table 6: Yield increase of beans due to inoculation by elite rhizobia isolates.

Isolates and N source	Grains yield (t ha^{-1}) of bush bean	Grains yield increased %of bush bean	Grains yield (t ha^{-1}) of climbing bean	Grains yield increased %
Control	1.5	0	2.16	0
NAR 206	1.74	0.16	2.86	0.32
NAR 75	1.72	0.14	3.29	0.52
NAR 151	1.76	0.17	3.34	0.54
NAR 139	1.84	0.22	3.44	0.59
UMR 1597	1.77	0.18	3.61	0.67
NAR 265	1.84	0.22	3.7	0.71
CIAT 899	1.86	0.24	3.71	0.71
Nitrogen	1.88	0.25	3.72	0.72

The grain yield increase varied from 16% to 25% on bush bean and 32% to 72 % on climbing bean. The best performing rhizobia isolate was NAR 265 followed by NAR 139, NAR 151, NAR 75 and the least performing rhizobia isolate was NAR 206.

5.3.4 Bean 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 climbing bean and bush beans respectively. Table 7 below shows that the highest P and N was obtained where beans were fertilized by Nitrogen (0.96 %, P on climbing bean; 0.90% P from bush bean; 6.32 total N for climbing bean and 6.10 total N for bush bean) followed by where the beans were inoculated by the commercial strains, CIAT 899 (0.86 P%, 5.99 total N for Climbing bean; 0.82 %P and 5.87 N Total for bush bean) and UMR 1597 (0.82% P and 5.82 total N for climbing bean 0.80% P and 5.44 total N for bush bean)-table 7.

Table 7: Bean tissue nutrient content

	P%		**Total N %**	
Rhizobia isolates/strains	**Climbing bean**	**Bush bean**	**Climbing**	**Bush bean**
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
Nitrogen	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 P and N contents relative to the isolates. N concentration in plant tissues ranged between 1 and 6 % depending on plant species, age, plant organ and the environment. The results here fit within these limits.

5.3.5 Most Probable Number (MPN) test

The estimation of native rhizobia nodulation on common bean was done before and after seeding of bean**.** The plant infection count, also known as most-probable number (MPN) was used to determine the number of viable and infective rhizobia following the procedures outlined by Somaseragan 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 inoculate the common bean seedling grown in acid treated and sterilized sand using plastic cups in four replicates. Nodule observation was performed 21 days after inoculation. Positive and negative nodulation were recorded for all dilutions and converted into number of rhizobia g^{-1} using MPN table.

Table 8: Number of viable rhizobia isolates in some Rwanda

	Site	**Period**	**Host Plant**	**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 rhizobia isolates before seeding were very few (15) but after harvesting bush bean that were inoculated the rhizobial count showed an increase (rhizobia g^{-1}sol by 40.9 %), an increase by 599 viable bacteria. However in Ruhunde the rhizobia g^{-1} were important but after harvesting climbing bean the increase rates were estimated at 4.4%.

5.3.6 Microbiological test

After several evaluations on the best 5 rhizobial isolates, the microbiological test confirmed the growth rate, characteristics on YEM-broth absorption, reaction on bromothymol blue and growth at different temperatures. The characteristics of NAR 265 and NAR 151 are similar to CIAT 899 not significantly different from the other strains.

Table 9: Characteristics of the best rhizobia isolates

Strains	Host plant	Growth rate	Colony Characteristics YEMA	Reaction on Bromothymolblue	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	Fast (3 days)	Center absorbent	Yellow	30
NAR 206	Bean	Fast (4 days)	Partly absorbent	Yellow	32
NAR 265	Bean	Fast (3days)	Fully absorbent	Yellow	30
CIAT 899	Bean	Fast (3 days)	Fully absorbent	Yellow	30
UMR 1597	Bean	Fast (3 days)	Fully absorbent	Yellow	30

Table 10: Growth rate of Rhizobia on 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 the rhizobium isolates evaluated in the field, induced nodulations in bush bean and climbing bean varieties. The study showed that there were significant differences among the inoculated strains on some properties of dry bean such as dry nodules numbers and weights, fresh and dry biomass, weight of pods, husk, 100 seeds weight and yield of bean grains. This study is in agreement with a study by (Chaverra and Graham, 1992) on native inoculation of *Rhizobium spp.* on dry bean have which showed that the isolated strains used significantly increased nodulation and other morphological parameters ($P<0.05$). In terms of the number and weight of dry nodules, only three of the isolated strains (NAR 265, NAR 139 and NAR 206) showed a significant symbiotic efficiency. The differences among isolated strains could be attributed to the differences in soils since soil properties have important influence in such microorganisms as

reported by other related studies. The soils from where these rhizobia were isolated and on which dry bean was grown had alkaline pH, clay loam texture, high amounts of $CaCO_3$ and low organic matter. The results showed 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 revealed that the native strains had significant effect on the plant biomass and grain yields. Besides, the same strains had significant effects on number of nodule and nodule weight ($P<0.05$).

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.

5.5 Conclusion

The most effective isolates of rhizobium were confirmed to be (NAR 265 and NAR 139). These isolates have the ability to fix nitrogen and thus have a commercial potential. They compared favorably with the commercial strains, (CIAT 899 and UMR 1597). However, rhizobium strains need to be genetically identified before they are recommended for use as commercial products. This reality comes from the results obtained from in Leonard Jars test, in pot and field experiments.

CHAPTER SIX: INVESTIGATION OF THE ROLE OF RHIZOBIA ISOLATES IN REDUCING DISEASE SEVERITY ON BEAN CROPS IN THE FIELD

Abstract

The third objective of this study was to investigate the effect of inoculation on susceptibility of the legume host to disease resistance. The field experiment was conducted in Rubona/ Huye and Ruhunde/Burera in Rwanda. Plants were evaluated in twenty seven plots, each 6 m^2. A Complete randomized block design with nine treatments (5 rhizobia isolates, 2 commercial isolates and 2 controls) was employed. Each treatment was replicated three times. CIAT score technique was used to score the symptoms of several diseases on common bean from just before the flowering until the maturity period. The diseases were scored according to their severity and incidence on leaves, stems and pods.

The results showed that the score for severity and incidence on bean diseases were low for anthracnose, ascochyta, angular leaf spot, rusts, root rot and Common Bean Mossaic Virus (CBMV). A similar result was shown on crop bean fertilized with nitrogen and crop bean inoculated with CIAT 899, NAR 265, NAR 139, and UMR 1597. The severity and incidence was however high for bean crop without inoculation or not fertilized on bush bean in Rubona research station. The study concluded that inoculation has effect on susceptibility of the legume host to disease resistance.

Key words: Rhizobia isolates, diseases, incidence, severity and score.

6.1 Introduction

Rhizabia isolates have the ability to induce disease tolerance by nodulation and fixation of nitrogen to common bean crop. Beans are generally characterized by their variable yield resulting from biological, climatic and edaphic factors which affect plant growth and productivity (H.Ssali and Mokwunye, 1986).

Most bean plant diseases are caused by fungi (anthracnose, angular leaf spot, ascochyta and rust). Others are caused by bacteria (halo blight, common bacteria) and viruses (bean common mosaic virus). Although the term disease is usually used only for the destruction of live plants, the action of dry rots and the rotting of harvested crops in storage or transport is similar to the rots of growing plants; both are caused by bacteria and fungi (Thusten,H.D., 1998). Any environmental factor that favors the growth of parasites or disease transmitters or that is unfavorable to the growth of the plants will lead to increase in the likelihood of infection and the amount of destruction caused by parasitic disease. Parasitic diseases are spread by dissemination of the agent itself (bacteria and viruses) or of the reproductive structures (Murray *et al.*, 1998). Wind, rain, insects, humans, and other animals may provide the means for dissemination (Andy Kirmayer, 2012)

6.2 Materials and Methods

To evaluate the effect of inoculation on legume host to disease, a score standardized by CIAT was used to score the symptoms of several diseases on common bean from before the flowering period until the maturity period. Plants were evaluated in twenty seven plots and each plot had 6 m^2. A complete randomized block design with nine treatments (5 rhizobia isolates, 2 commercial isolates and 2 controls) with three replicates was employed. CIAT score technique was used to

score the symptoms of several diseases on common bean from before the flowering period until the maturity period. The diseases were scored according the severity and incidence on leaves, stems and pods.

The parameters measured were:

(1) The incidence of the disease, i.e., the number or proportion of plant units that are diseased (i.e., the number or proportion of plants, leaves, stems, and fruit that showed any symptoms) in relation to the total number of units examined;

(2) The severity of the disease, i.e., the proportion of area or amount of plant tissue that is diseased; and

(3) The yield loss caused by the disease, i.e., the proportion of the yield that the grower would not be able to harvest because the disease destroyed it directly or prevented the plants from producing (the yield loss is the difference between attainable yield and actual yield).

Table 11: CIAT score 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%

During the disease evaluation, the score of each plot for bean crop inoculated or not inoculated was noted and for each treatment the highest score for each disease was considered.

6.3 Results

The results on diseases evaluation showed that bean diseases were significant for 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 12 and 13).

Table 12: Disease evaluation on bush beans inoculated or none inoculated in RUBONA field

	Scoring for severity of diseases							
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
Nitrogen	3	2	3	2	1	1	1	2
Control	6	5	5	3	1	4	1	5

Table 13: Diseases evaluation on climbing bean at Ruhunde field experiment

	Diseases scoring							
Treatments	**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
Nitrogen	3	2	2	2	1	2	1	1
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 blight also had a score of 3 in Ruhunde but was negligible in Rubona site but root rot (3) was more prominent. The following treatments had the lowest score of (3): nitrogen, CIAT 899, UMR 899, NAR 265 and NAR 139.

6.4 Discussion

Diseases scores were higher where fertilizer was not applied or where bean plants were not inoculated. This was shown in both the two sites and could be attributed to the enhanced plant vigor and imparted disease tolerance brought about by the increased nitrogen from the inoculant and the fertilizer. Disease score of between 4-6 caused serious crop damage and affected crop growth and ultimately reduced yields. A score of 1-3 resulted in the crop damage that did not

affect the bean yield, but the score of between 6-9 resulted in crop damage that led to the yield loss of more than 60%. The latter damage was severe due to the high susceptibility of beans to diseases at this score.

6.5 Conclusions and Recommendations

Nutrient application had a much greater effect on reducing disease when the plants were at deficiency levels. In cases where the addition of a nutrient has exacerbated the disease it is possibly because of toxicity rather than deficiency; or in other cases, the addition of a nutrient can aggravate the primary deficiency. In sustainable agriculture, balanced nutrition is an essential component of any integrative crop protection program because it is more cost-effective and also environmentally friendly to control plant diseases with the adequate amount of nutrients and with no pesticides. Nutrients can reduce disease to acceptable levels, or at least to a level at which further control by other cultural practices or conventional organic biocides are more successful and less expensive.

This study showed that effective rhizobia isolates can improve tolerance to diseases when nodulated legume host, in this case the common beans are inoculated. Further studies are recommended to evaluate the effectiveness of these inoculants on other viral, bacterial and fungal diseases. The rationale for the disease resistance was not provided and could also be explored by subsequent studies. This study however, pointed out that when legumes are effectively nodulated, they are vigorous and have higher probability of taking up nutrients and further able to resist diseases.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

CONCLUSION

The improved nodulation observed in both field and green house trials through inoculation with rhizobia was influenced by the variety of bean used. Some of the isolated strains (NAR 265 and NAR 139) performed better than the commercial strain (UMR 1597) in terms of nodulation, crop growth and yields underscoring the huge potential. They have the ability to induce nodule formation and N_2 fixation on bean variety. They bring about effective N_2 fixation in association with a wide range of several types of bean varieties.

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 also 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.

RECOMMENDATIONS

1. 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.
2. The findings of this study showed that the best two isolates of rhizobium (NAR 265 and NAR 139) have the ability to fix nitrogen and to improve the tolerance to diseases and also improve the yield when used as inoculants for a nodulated legume host. However, rhizobium strains need to be genetically identified before they are recommended for use as commercial products.
3. There is need to carry out further trials, covering more and extended geographical areas in Rwanda to ascertain the performance of these rhizobia isolates.
4. To effectively identify and characterize 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.
5. It is advisable that rhizobia identification and cultivar selection be carried out in the future to boost symbiosis process.

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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

a.Before sowing

SOWING DATE 15th March/2012

SAMPLING DATE: 9 th February/2012

REPLICATION	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

b.After harvesting

REPLICATION	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 plant
No	NAR	Country	Contributor	Altitude	Longitude	Latitude	Sub-family
1	1	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
2	2	Rwanda	RAB	1717m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
3	3	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
4	4	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
5	5	Rwanda	RAB	1685m	E 029°50ô47.6ôô	S 02°02ô16.8ôô	Bean
6	6	Rwanda	RAB	1684m	E 029°50ô47.6ôô	S 02°02ô16.8ôô	Bean
7	7	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
8	8	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
9	9	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
10	10	Rwanda	RAB	1684m	E 029°50ô47.6ôô	S 02°02ô16.8ôô	Bean
11	11	Rwanda	RAB	1691m	E 029^0 50ô46.2ôô	S 02^0 00ô 07.0ôô	Bean
12	12	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
13	13	Rwanda	RAB	1500m	E 030^0 27' 08.2"	S 01^0 49' 13.8"	Bean
14	14	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
15	15	Rwanda	RAB	1730m	E 029^0 50ô57.9ôô	S 02^0 01ô 52.0ôô	Bean
16	16	Rwanda	RAB	1730m	E 029^0 50ô57.9ôô	S 02^0 01ô	Bean

						52.0ôô	
17	17	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
18	18	Rwanda	RAB	1730m	E 029^{0} 50ô57.9ôô	S 02^{0} 01ô 52.0ôô	Bean
20	20	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
21	21	Rwanda	RAB	1500m	E 030^{0} 27' 08.2"	S 01^{0} 49' 13.8"	Bean
23	23	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
24	24	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
25	25	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
26	26	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
27	27	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
28	28	Rwanda	RAB	1500m	E 030^{0} 27' 08.2"	S 01^{0} 49' 13.8"	Bean
29	29	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
30	30	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
31	31	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
32	32	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
33	33	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
34	34	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
35	35	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Soybean
36	36	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
37	37	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
38	38	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
39	39	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean

40	40	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
41	41	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
42	42	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
43	43	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
44	44	Rwanda	RAB	1730m	E 029^0 50ô57.9ôô	S 02^0 01ô 52.0ôô	Bean
45	45	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3ôô	Bean
46	46	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
48	48	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
49	49	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
50	50	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
51	51	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
52	52	Rwanda	RAB	2050m	E 029°44ô00.1ôô	S 01°25ô46.2ôô	Bean
2	55	Rwanda	RAB	1783m	E 029°48ô35,6ôô	S 02°05ô55.1ôô	Bean
54	72	Rwanda	RAB	1906m	E 029°53ô42.9	S 01°35ô50.3ôô	Bean
55	73	Rwanda	RAB	1906m	E 029°53ô42.10	S 01°35ô50.3ôô	Bean
56	74	Rwanda	RAB	1906m	E 029°53ô42.11	S 01°35ô50.3ôô	Bean
57	75	Rwanda	RAB	1906m	E 029°53ô42.12	S 01°35ô50.3ôô	Bean
58	76	Rwanda	RAB	1906m	E 029°53ô42.13	S 01°35ô50.3ôô	Bean
59	77	Rwanda	RAB	1906m	E 029°53ô42.14	S 01°35ô50.3ôô	Bean
60	78	Rwanda	RAB	1906m	E 029°53ô42.15	S 01°35ô50.3ôô	Bean
61	79	Rwanda	RAB	1906m	E 029°53ô42.16	S 01°35ô50.3ôô	Bean
62	80	Rwanda	RAB	1906m	E 029°53ô42.17	S 01°35ô50.3ôô	Bean

63	81	Rwanda	RAB	1906m	E 029°53ô42.18	S 01°35ô50.3ôô	Bean
64	82	Rwanda	RAB	1906m	E 029°53ô42.19	S 01°35ô50.3ôô	Bean
65	83	Rwanda	RAB	1906m	E 029°53ô42.20	S 01°35ô50.3ôô	Bean
66	84	Rwanda	RAB	1906m	E 029°53ô42.21	S 01°35ô50.3ôô	Bean
67	85	Rwanda	RAB	1906m	E 029°53ô42.22	S 01°35ô50.3ôô	Bean
68	86	Rwanda	RAB	1906m	E 029°53ô42.23	S 01°35ô50.3ôô	Bean
69	87	Rwanda	RAB	1906m	E 029°53ô42.24	S 01°35ô50.3ôô	Bean
70	88	Rwanda	RAB	1906m	E 029°53ô42.25	S 01°35ô50.3ôô	Bean
71	89	Rwanda	RAB	1906m	E 029°53ô42.26	S 01°35ô50.3ôô	Bean
72	90	Rwanda	RAB	1991m	E 029^0 44' 03.4"	S 01^0 25' 51.1"	Bean
73	91	Rwanda	RAB	1991m	E 029^0 44' 03.4"	S 01^0 25' 51.1"	Bean
74	92	Rwanda	RAB	1991m	E 029^0 44' 03.4"	S 01^0 25' 51.1"	Bean
75	111	Rwanda	RAB	1691m	E 029^0 50ô46.2ôô	S 02^0 00ô 07.0ôô	Bean
76	112	Rwanda	RAB	1691m	E 029^0 50ô46.2ôô	S 02^0 00ô 07.0ôô	Bean
77	113	Rwanda	RAB	1691m	E 029^0 50ô46.2ôô	S 02^0 00ô 07.0ôô	Bean
78	114	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean
79	115	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean
80	116	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean
81	117	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean

82	118	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean
83	119	Rwanda	RAB	1658m	E 029^0 51' 10.7"	S 02^0 00' 55.1"	Bean
84	125	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
85	126	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
86	127	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
87	128	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
88	129	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
89	130	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
90	131	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
91	132	Rwanda	RAB	1730m	E 029^0 50ô 57.9öö	S 02^0 01ô 52.0öö	Bean
92	135	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ö32.3öö	Bean
93	136	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ö32.3öö	Bean
94	137	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ö32.3öö	Bean
95	138	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ö32.3öö	Bean
96	139	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ö32.3öö	Bean

97	140	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
98	141	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
99	142	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
100	143	Rwanda	RAB	1732m	E 029°43ô08.5	S 01°34ô32.3öö	Bean
101	144	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
102	145	Rwanda	RAB	1732m	E 029°43ô08.5	S 01°34ô32.3öö	Bean
103	146	Rwanda	RAB	1732m	E 029°43ô08.6	S 01°34ô32.3öö	Bean
104	147	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
105	148	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
106	149	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
107	150	Rwanda	RAB	1732m	E 029°43ô08.5	S 01°34ô32.3öö	Bean
108	151	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
109	152	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
110	153	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
111	154	Rwanda	RAB	1732m	E 029°43ô08.5	S 01°34ô32.3öö	Bean
112	155	Rwanda	RAB	1732m	E 029°43ô08.6	S 01°34ô32.3öö	Bean
113	156	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
114	157	Rwanda	RAB	1732m	E 029°43ô08.5	S 01°34ô32.3öö	Bean
115	158	Rwanda	RAB	1732m	E 029°43ô08.6	S 01°34ô32.3öö	Bean
116	159	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
117	160	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean

118	161	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
119	162	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
120	163	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
121	164	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
122	165	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
123	166	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
124	167	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
125	168	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
126	169	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
127	170	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
128	171	Rwanda	RAB	1787m	E 029^0 41ô02.6öö	S 01^0 32ô 32.7öö	Bean
129	172	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
130	173	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
131	174	Rwanda	RAB	2082m	E 029^0 44' 13.2"	S 01^0 26' 07.3"	Bean

132	175	Rwanda	RAB	1539m	E 030°27ô00.4öö	S 01°48ô46.3öö	Bean
133	176	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
134	177	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
135	178	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
136	179	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
137	180	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
138	181	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
139	182	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
140	183	Rwanda	RAB	1906m	E 029°53ô42.26	S 01°35ô50.3öö	Bean
141	189	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
142	190	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
143	191	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
144	192	Rwanda	RAB	1539m	E 030°27ô00.4öö	S 01°48ô46.3öö	Bean
145	193	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
146	194	Rwanda	RAB	1539m	E 030°27ô00.4öö	S 01°48ô46.3öö	Bean
147	195	Rwanda	RAB	2082m	E 029^{0} 44' 13.2"	S 01^{0} 26' 07.3"	Bean
148	196	Rwanda	RAB	1787m	E 029^{0} 41ô02.6öö	S 01^{0} 32ô 32.7öö	Bean
149	197	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
151	204	Rwanda	RAB	1906m	E 029°53ô42.26	S 01°35ô50.3öö	Bean
152	205	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
153	206	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean

154	207	Rwanda	RAB	1684m	E 029°50ô47.6öö	S 02°02ô16.8öö	Bean
155	208	Rwanda	RAB	2050m	E 029°44ô00.1öö	S 01°25ô46.2öö	Bean
156	209	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
157	210	Rwanda	RAB	1732m	E 029°43ô08.4	S 01°34ô32.3öö	Bean
158	211	Rwanda	RAB	1745m	E 029^{0} 41ô21.0öö	S 01^{0} 32ô 31.1öö	Bean
159	220	Rwanda	RAB	1906m	E 029°53ô42.26	S 01°35ô50.3öö	Bean
160	221	Rwanda	RAB	1906m	E 029°53ô42.26	S 01°35ô50.3öö	Bean
161	222	Rwanda	RAB	1783m	E 029°48ô35,6öö	S 02°05ô55.1öö	Bean
162	223	Rwanda	RAB	1783m	E 029°48ô35,6öö	S 02°05ô55.1öö	Bean
164	253	Rwanda	RAB	1783m	E 029°48ô35,6öö	S 02°05ô55.1öö	Bean
163	254	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
173	255	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
174	256	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
166	257	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
167	258	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
168	259	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
175	260	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
176	261	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
177	262	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
178	263	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean
179	264	Rwanda	RAB	1716m	E 029^{0} 51' 01.2"	S 02^{0} 01' 50.3"	Bean

180	265	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
170	266	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
171	267	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean
172	268	Rwanda	RAB	1716m	E 029^0 51' 01.2"	S 02^0 01' 50.3"	Bean

RAB, N2Africa (2011): Bioprospection in Rwanda

Appendix 3: Nodule numbers and dry biomass weight on bean varieties

	Nodules (population plant^{-1})		Biomass dry (grams)	
Strains	**Climbing bean**	**Bush bean**	**Climbing bean**	**Bush bean**
Control	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 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