Effect of *Bradyrhizobium* Strains on the Yield of Accessions of Bambara groundnut (*Vigna subterranea (L) Verdc)*

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DECLARATION

I hereby certify and declare that the results contained in the Doctor of Philosophy in Biology thesis submitted to the North-West University, Faculty of Natural and Agricultural Science, Department of Microbiology, were all original research conducted by me, with the exception of the citations. I also certify that the results obtained from the research studies have not been submitted to or considered by any other university for the award of any degree.

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DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL ABSTRACT

A sizeable component of human diets consists of underutilised legumes. These legumes fix atmospheric nitrogen, giving smaller growers an economic advantage. Utilising the legumes in the appropriate way can help increase fertility of the soil and food security. A research was conducted to understand the growth, nutrient uptake, nitrogen fixation and yield of ten Bambara groundnut accessions in a screenhouse, field and laboratory at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria within 2018-2019 and 2019-2020 growing seasons. Ten Bambara groundnut accessions were randomly selected from IITA gene bank. *B. japonicum* strains (FA3, USDA110, IRJ2180A and RACA6) were coated separately to seeds of Bambara groundnut accessions before planting on the field and were applied as broth culture to seedlings of Bambara groundnut in the screenhouse. Nitrogen fertilizer was applied to seedlings of uninoculated Bambara groundnut accessions in both screenhouse and on the field, and an uninoculated control (no fertilizer and no inoculation). The screenhouse experiments were arranged in Completely Randomised Design (CRD), while the experiments on the field were arranged in Randomised Complete Block Design (RCBD).

The research identified traits such as plant height, number of leaves, terminal leaf length, terminal leaf width, seed per pod, number of seeds per plot, seed width, and seed length that enhanced vegetative and reproductive stages of Bambara groundnut accessions when *B. japonicum* strains were inoculated in both screenhouse and on the field. Comparing TVSu-1698 to other Bambara groundnut accessions inoculated with *B. japonicum* strains, the result showed that higher mean values were recorded in the yield per plot (80.92g), yield per ha (2205.5kg), and the number of pods per plot (91.13) in both locations and seasons.

The *B. japonicum* strains inoculated to the accessions of Bambara groundnut fixed nitrogen and produced root nodules. Three Bambara groundnut accessions (TVSu-1606, TVSu-365 and TVSu-1698) were identified as nitrogen-fixing accessions due to inoculation of *B. japonicum* strains. Inoculation of TVSu-1606 with RACA6 fixed nitrogen equivalent to 82.99 kg N ha^{-1} while inoculation of TVSu-365 with RACA6 fixed nitrogen equivalent to 81.10 kg N ha^{-1}, followed by the inoculation of TVSu-1698 with IRJ2180A which fixed 79.69 kg N ha^{-1}. *B. japonicum* strains inoculated to the accessions of Bambara groundnut, RACA6 and USDA110 strain, have stronger nitrogen-fixing capacity than other strains, with a mean value of 75.95 kg N ha^{-1} and 73.32 kg N ha^{-1} respectively. Nevertheless, the highest percentage of phosphorus uptake on the field was recorded in TVSu-787 at flowering, with a mean value of 0.21%, while the highest percentage of phosphorus at harvest was recorded in TVSu-378, with a mean value 0.15%. Also, the highest percentage of nitrogen uptake on the field was recorded in TVSu-1739 at flowering with a mean value of 2.09 %, and the highest percentage of nitrogen was recorded in TVSu-787 at harvest with a mean value of 1.75 %.

In the screenhouse experiment, *Bradyrhizobium* spp. (*B. diazoefficiens*, *B. japonicum*) and *Rhizobium spp* were isolated from Bambara groundnut roots, and characterisation of isolates was done using the 16S rRNA gene. However, the *nifH, Nod A*, and *Nod C* gene analyses showed that *Streptomyces bacillaris*, *Pseudomonas knackmussi*, *B. kanji*, *Sinorhizobium meliloti*, *Mesorhizobium* spp., *Bradyrhizobium* spp., and *Rhizobium* spp. were isolated on the fields. The amount of nitrogen (derived from the atmosphere (Ndfa) in Bambara groundnut shoots revealed significant differences in soil status: the non-sterile soil in the screenhouse had the highest percentage of nitrogen derived from the atmosphere value (38.25%), while the sterile soil had the lowest mean value (36.57%). Additionally, higher significant differences in the fresh and dry shoot weight at flowering and harvesting were recorded in the non-sterile soil than the sterile soil with a mean value of 4.44g and 1.23g (at flowering), 6.53g and 4.68g (at harvest) in the screenhouse. Significant differences between the *B. japonicum* strains and nitrogen fertilizer were equally observed. In comparison, the mean value recorded for nitrogen fertilizer applied to Bambara groundnut accessions at harvest were yield per plot (44.66g), and yield per ha (633.07kg ha^{-1}). Compared to other *B. japonicum* strains inoculated to Bambara groundnut accessions, RACA6 strain exhibited higher significant differences in the growth trait of Bambara groundnut accessions on the field at various weeks' intervals and yield component with a mean value of yield per plot (51.51g) and yield per ha (750. 72kg ha^{-1}). Findings of the study demonstrated the significance of applying inoculation of *B. japonicum* strains as well as the application of the inorganic nitrogen fertilizer (Urea) used. Finally, the study provided evidence that inoculation of *B. japonicum* strains on Bambara groundnut accessions can help improve the growth and yield traits, nutrient uptake, nitrogen-fixing potential, nodulation and diversity of the bacteria nodulating the roots of Bambara groundnut.

LIST OF PUBLICATIONS

Paper 1: Legumes and nutrient requirement

Authors: Bitire, T. D.; Abberton, M.T.; Oyatomi, O.A and Babalola, O.O. This paper has been formatted for Journal of Integrative Agriculture.

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Paper 3: Effect of *Bradyrhizobium japonicum* strains on the performance of accessions of Bambara groundnut (*Vigna subterranea (L) Verdc)*

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Paper 6: The influence of *Bradyrhizobium japonicum* on nodulation potential and nitrogen fixing efficiency of Bambara groundnut (*Vigna subterranea (L) Verdc)* accessions

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Candidate's contribution: Design of the study, literature search and developing the write up of the manuscript.

Paper 7: Effect of *Bradyrhizobium japonicum s*trains inoculation on growth and nutrient uptake of accessions of Bambara groundnut (*Vigna subterranea (L) Verdc)* in a sterile and nonsterile soil under screenhouse conditions

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Paper 8: Isolation and molecular characterisation of root nodules associated bacteria on inoculated Bambara groundnut (*Vigna subterranea (L) Verdc)* accessions

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List of Abbreviations

B…………………………………*Bradyrhizobium*

N………………………………… Nitrogen

Ib…………………………………..Ibadan

Ik…………………………………..Ikenne

Nig…………………………………Nigeria

P……………………………………Phosphorus

BNF………………………………..Biological nitrogen fixation

Spp…………………………………Species

WAP……………………………….Weeks after planting

RCBD………………………………Randomised complete block design

CRD………………………………...Completely Randomised design

MPN………………………………...Most Probable number

PGPM……………………………….Plant growth promoting microorganism

Ca……………………………………Calcium

Mg…………………………………..Magnesium

Fe……………………………………Iron

K…………………………………….Potassium

Na…………………………………...Sodium

OC…………………………………...Organic Carbon

CHAPTER ONE

General Introduction

1.1 Introduction

Bambara groundnut is a neglected member of the fabaceae family of legumes, which can grow in salt-stressed and poor-soil conditions. Moreover, it is drought-resilient (Nwadi *et al*. 2020). It was first cultivated in Asia, specifically in Malaysia, Thailand, and Indonesia, but its origins are precisely in West Africa where it is cultivated as a crop (Cheng *et al.* 2019). Bambara groundnut has a nutritional content of between 49% and 63.5% carbohydrates, 5.2% to 6.4% fibre, 15% to 25% proteins, 4.5% to 7.4% fat, 3.2% to 4.4% ash, and 2% minerals (Igwe and Afiukwa 2017). Micronutrient concentrations in it range from 95.5mg to 99.0 mg/100 g for calcium, 5.1 mg to 9.0 mg/100 g for iron, 11.45 mg to 14.36 mg/100 g for potassium, and 2.9 mg to 10.6 mg/100 g for sodium (Adeleke *et al*. 2018). For iron deficiency situations in human and livestock, Bambara groundnut is typically recommended (Muimba-Kankolongo *et al*. 2018).

Leucine, isoleucine, lysine, and methionine are abundant in Bambara groundnut (Adewumi *et al*. 2022). The legume also contains vitamins like vitamin A, vitamin C, and vitamin E (Harris *et al*. 2018). It provides a therapeutic function by assisting in the treatment of anaemia, diarrhoea, ulcer-infected wounds, internal injuries, heart illnesses, eye diseases, and colon cancer in both humans and animals (Harris *et al*. 2018). Despite its nutritional value and significance, Bambara groundnut is rarely consumed in most parts of the world, due to attracting difficulties in its meal preparation (Mubaiwa *et al*. 2018b).

Bambara groundnuts are among the least expensive proteins that can typically help to increase global food sustainability (Lin Tan *et al*. 2020). The nutritional investigation of Bambara groundnut revealed that it is a complete diet (Massawe *et al*. 2005). Unlike other leguminous crops like groundnut, soybean and cowpea which have 65%, 74%, and 64% of protein each, it contains a high level of crude protein, about 80% (Mubaiwa *et al*. 2018a). Additionally, it can be used to make locally produced milk and bread, both of which typically contain high antioxidant levels (Murevanhema and Jideani 2015) . Grounded wet paste of the seed can be used to produce okpa or moi-moi in various regions of Nigeria by steaming process (Ogunmuyiwa *et al*. 2017).

In west Africa, the flour obtained from Bambara groundnut can be used as composite supplements to maize or wheat flour to make bread (Muhammad *et al*. 2020). It can also be used as materials for pig and poultry feed (Bbebe 2019). When the host crop is inoculated with *Rhizobium* or *Bradyrhizobium* strains, this improves the host crop's ability to photosynthesise, thereby improving nodulation and enhancing the crop to fix the necessary amounts of atmospheric nitrogen for its nutritional utilisation (Gerrano *et al*. 2021). This results in the crop producing leaves for photosynthetic capacity improvement (Effa *et al*. 2016). To promote nodulation, nitrogen fixation, and optimal grain output, commercial inoculants are introduced and applied to leguminous crops as a way to enrich the crop's rhizosphere with rhizobia (Igiehon and Babalola 2018b). Adverse weather and harsh environmental conditions are a major barrier to the introduction of most exotic rhizobia strains, which frequently result in low yield, necessitating the introduction of indigenous rhizobia strains that have the potential to thrive in African soil, nodulate Bambara groundnuts, and promote nitrogen fixation in Africa (Puozaa *et al*. 2017b).

Nitrogen fixation in Bambara groundnut has received very little research attention, especially outside of the humid forest zone (Mohale *et al*. 2014b). The underutilised legume has the ability to fix up to 4 kg to 200 kg of nitrogen per hectare due to a symbiotic relationship with rhizobia, a type soil *bacterium* (Ibny *et al*. 2019a). A few studies demonstrated that it can be nodulated by the genus *Bradyrhizobium* spp, which was the only record of the biodiversity of the type of rhizobia nodulating the underutilised legume in tropical soils (Adjei *et al*. 2022; Ibny *et al*. 2019b; Puozaa *et al*. 2017a). Biological nitrogen fixation is the process of converting nitrogen in the atmosphere into a form the plant can use with the help of soil microorganisms, which typically results in the creation of root nodules (Bahati 2015). The plant in turn provides carbon to sustain the rhizobia bacteria's activity, and the bacteria fixes nitrogen for the leguminous crop (Taylor *et al*. 2020). In order for the nodule to form in the root and promote the nitrogen-fixing bacteria, the rhizobia often release a Nod factor termed lipochito-oligosaccharides which typically serves as a receptor to the leguminous plant (Garg *et al*. 2019).

With the help of rhizobia, legumes have the potential to convert and fix atmospheric nitrogen and the carbohydrates released from the legume crop typically helps the bacteria thrive (Mahmud *et al*. 2020). The microorganisms fix and convert atmospheric nitrogen and make it easily accessible for optimum plant growth (Garg *et al*. 2019). Nitrogen is a nutrient needed by plant in large quantities (macronutrient). It is required to obtain optimum growth of legumes and typically supports the production of amino acids, the protein's building block (Hasan *et al*. 2018b). The addition of nitrogen fertilizer to Bambara groundnut usually helps to enhanced agronomic factors like plant height and leaf count. The development of Bambara groundnut's root system and its potential to fix nitrogen are both aided by the rhizobia and the presence of nitrogen in the soil (Hasan *et al*. 2018b). Continuous application of nitrogen to legumes on tropical soil has frequently been harmful to human health and has led to the introduction of biofertilizer to increase the output of legumes and lessen the issue of food insecurity in the economy (Igiehon and Babalola 2018b).

1.2 Problem Statement

The most significant obstacle of farmers in the tropics is the high expense of obtaining inorganic nitrogen fertilizer, often used as a starter dose on legumes. Most farmers who can afford to buy the inorganic nitrogen fertilizers typically apply at rates below manufacturer's recommendation. The application of inorganic nitrogen fertilizers to legumes typically results in decrease in farmers' profit. The same equally contaminates and adversely affects soil health. Additionally, due to a number of agronomic constraints, smallholder farmers are increasingly losing interest in cultivating Bambara groundnut, which is on the verge of extinction. Bearing in mind soil and human health and safety, the investigation of how rhizobia bacteria strain can affect Bambara groundnut productivity is necessary. Therefore, this study was set up to improve the yield of Bambara groundnut accessions using *B. japonicum* strains (nitrogen fixing bacteria) and compare the yield response with the inorganic nitrogen fertilizer (Urea) treatment.

1.3 Justification

The goal of the research is to ascertain the potential of the *B. japonicum* strains and inorganic nitrogen fertilizer on yield response of Bambara groundnut accessions. The high cost of obtaining inorganic nitrogen fertilizer that is ravaging the world has led to sourcing for and using of affordable bacterial inoculant to coat the seeds of legumes as a starter dose to increase the yield and output of the farmers in the tropics.

1.4 The Aim and Objectives of the Research

The objectives of this research are:

1 to ascertain the impact of *B. japonicum* strains on the growth and yield of inoculated and non-inoculated Bambara groundnut accessions,

2 to compare the results of the inoculation of *B. japonicum* strains with inorganic nitrogen fertilizer (urea) on growth traits and nutrient uptake of Bambara groundnut accessions,

3 to identify variations in Bambara groundnut accessions capacity to fix nitrogen,

4 to examine the variability in the yield of inoculated Bambara groundnut accessions, and

5 to identify the diversity of root nodule associated bacteria recovered from inoculated Bambara groundnut accessions and to characterize, using molecular techniques.

1.5 Research Questions

1 What quantity of nitrogen will be fixed by the inoculated Bambara groundnut accessions?

2 What degree of variability will be recorded in the yield of inoculated Bambara groundnut accessions?

3 Nitrogen fertilizer application or *B. japonicum* strains inoculation: which of these treatment will promote the growth and development of Bambara groundnut accessions?

CHAPTER TWO

Legumes and Nutrient Requirement

Abstract

Small holder farmers have embraced the use of beneficial microorganism inoculants to improve growth and development of leguminous crops in order to decrease high production costs associated with the use of artificial nitrogen fertilizer to increase leguminous crop production. The significant importance of *B. japonicum* inoculation and nitrogen fertilizer treatment on various leguminous crops were examined thoroughly through a number of research. Following the evaluation of numerous studies, it was shown that inoculation of *B. japonicum* strains to leguminous crops outperformed the application of inorganic nitrogen fertilizers in terms of growth promotion, nitrogen fixation, yield, and yield components. Other studies in which *B. japonicum* inoculation and inorganic nitrogen fertilizer were combined recorded a very effective promotion of the yield and yield characteristics than when the treatments were separated.

Keywords- Residual, inoculation, fertilization, environmental contamination and legumes

2.1 Introduction

Agricultural sustainability is crucial to solving the need of the expanding population which has been predicted to reach 12 billion in the near future (Meena *et al*. 2017). Farmers are reportedly putting more effort to improve agricultural systems to increase the production of food that can sustain the expanding population in order to address food shortage (Ochieng *et al*. 2016). Legumes and nitrogen-fixing *bacterium* (rhizobia) symbiotic relationship has been in existence for many centuries because of its significance in sustainable agriculture such as lowering farmer costs, enhancing soil fertility and increasing plant tolerance to drought (Igiehon and Babalola 2018b).

An intense technology that might be utilised to decrease the usage of inorganic fertilizers for increased soil fertility and nutrient cycles is the inoculation of advantageous soil microbes to legumes in organic farming (N'cho *et al*. 2015). Rhizobia, a diazotrophic bacteria that colonises the host plant's rhizosphere and eventually forms nodules is the most advantageous microbe used as an inoculant. The microorganisms inside the nodules promote nitrogen fixation. Thus, the plant benefits from the nitrogen supply while the bacteria gain from the carbon molecules the plant provides (Andrews and Andrews 2017). The symbiotic relationship between legumes and rhizobia frequently results in biological nitrogen fixation, which provides the nitrogen needed by the plant for maximum development and production.

Furthermore, environmental factors, such as biotic and abiotic stress, reduced microbial survival rates, and exposure to adverse temperatures, limit the use of the beneficial inoculant in the northern regions in Africa (Ma 2019; Thilakarathna and Raizada 2017). By directly applying to plant tissues and rhizospheres or by inoculating the seeds before sowing, the plant growth promoting microbes increases legumes productivity.

2.2 *B. japonicum*

B. japonicum are key soil microbiota that contribute to soil fertility, plant nutrition, and growth. It plays a very important role in organic farming and can easily be procured by the farmers due to its affordability compared to the inorganic nitrogen fertilizers. *B. japonicum* colonises roots of legumes and develops symbiotic relationships to promote water and nutrient uptake to improve legumes growth and development (Mahanty *et al*. 2017; Srivastava *et al*. 2017). Biological nitrogen fixation by *B. japonicum* is ascertained (Gatabazi *et al*. 2021). Also, inoculation of legumes with *B. japonicum* is one of the main agronomic techniques aimed at enhancing symbiosis in sustainable agriculture. *B. japonicum* has a bipartite life cycle that differs from other nitrogen-fixing bacteria due to its symbiotic companionship inside the root nodules of legumes (Jaiswal and Dakora 2019a).

2.3 Nitrogen Fertilizer

The use of chemical nutrients, such as plant protection agents, and inorganic nitrogen fertilizers, leaves a severe damage on the soil, environment and water in agriculture (Nath *et al*. 2017). Furthermore, the incorrect use of chemical nutrients, pesticides and agrochemicals have a negative influence on biodiversity and soil qualities such as: organic matter, soil physical properties, soil fertility and water-holding capacity. Unfortunately, these inorganic inputs lead to the destruction of the environment (Abou-Shanab *et al*. 2017). Additionally, majority of smallholder farmers who lack access to resources find it impossible to afford artificial fertilizers (Majola *et al*. 2021). In contrast, organic farming focuses on the use of plant residue, off-farm organic wastes, crop rotation, animal wastes, mineral grade rock additions, biological systems of nutrient mobilization, solubilization and plant protection (Reganold and Wachter 2016). The primary aim of this review is to assess the impact of *B. japonicum* inoculation and nitrogen fertilizer application on the growth and development of diver's legumes.

2.4 Effects of *B. japonicum* on:

2.4.1 Agronomic Traits and Yield of Legumes

Evidence supporting the use of *B. japonicum* inoculation on Bambara groundnut productivity is rare compared to other leguminous crops. The impacts of soil and seed inoculation methods of *Bradyrhizobium* were studied to evaluate the growth and yield parameters of Bambara groundnut (Ikenganyia *et al*. 2017a). Findings revealed that both methods enhanced Bambara groundnut yield although soil inoculation method produced maximum yield compared to the seed inoculation. Gomoung *et al*. (2017) reported that the inoculation of Bambara groundnut with *rhizobium* strains resulted in growth traits improvement (plant biomass and plant height), and improvement of Bambara groundnut yield by 72.71% compared to 63.73% yield increase in groundnut. A recent study by Bitire *et al*. (2022a) revealed that *B. japonicum* strain, FA3, out-performed other *B. japonicum* strains and nitrogen fertilizer applied in the nutrient uptake, growth traits, and yield of Bambara groundnut accessions when inoculant were administered on the field. *Bradyrhizobium* inoculation considerably improved the growth parameters, nodulation potential, yield, and yield components of cowpea varieties, according to Yoseph *et al*. (2017). Furthermore, *Bradyrhizobium* inoculant was found to boost peanut growth and yield parameters when compared to the uninoculated control (Shang *et al*. 2021). According to Gitonga *et al*. (2021a), inoculating soybean with *B. japonicum* results in considerable improvements in the soybean nodule dry weight, shoot dry weight, and seed dry weight. Ayalew and Yoseph (2020) reported a substantial rise in the plant height and nodule number when cowpea cultivars were inoculated with *Bradyrhizobium* inoculants. Plant growth promoting bacteria and *B. japonicum* strains may consequently enhance the growth characteristics and yield of Bambara groundnut. *Bradyrhizobium* strains inoculation to early soybean cultivars increased growth, yield, protein content and nitrogen derived from the atmosphere when compared to the control (Zimmer *et al*. 2016). However, in a study conducted in Poland, Jarecki (2020) reported that inoculation of *B. japonicum* to seeds of soybean considerably increased the number of nodules, biomass dry weight, and pods per plant compared to the uninoculated control. Also, seed inoculation with *B. japonicum* increased protein and fat yield by 318 kg ha^{-1} and 101 kg ha^{-1} respectively, compared to the uninoculated control.

2.4.2 Nutritional Composition of Legumes

To increase the nutrition of leguminous crop and to promote the growth and development of legumes without contamination of the soil at a reduced cost, the use of biofertilizer must be embraced. *B. japonicum* inoculants must be introduced to legumes. *Bradyrhizobium* inoculation to Bambara groundnut increases the shoot concentration of Zn, Cu, and Mn but decreases that of Fe (Mbah and Dakora 2018a). Similarly, the application of *B. japonicum* inoculation to soybean enhanced the concentration of Fe, Mn, and Zn in shoot compared to inorganic nitrogen fertilizer and uninoculated control. Bitire *et al*. (2022b) also reported that inoculation of *B. japonicum* strains to accessions of Bambara groundnut in the screenhouse and on the field, showed significant influence on accessions in the uptake of nitrogen and phosphorus at flowering and at harvest compared to the uninoculated treatment.

Furthermore, Zoundji *et al*. (2022) concluded that inoculating Bambara groundnut with native rhizobia lead to greater nitrogen uptake than the uninoculated control. Puozaa *et al*. (2015) noted that *Bradyrhizobium* inoculation to Bambara groundnut landraces in two separate region resulted to variations in soil nitrogen uptake. According to Oyewole *et al*. (2018), inoculation with plant growth promoting microbe enhanced Bambara groundnut's ability to absorb available nutrients in the soil. *Bradyrhizobium* inoculation to soybean in double crop farming increased soil Nitrogen and Phosphorus uptake when compared with the uninoculated control (Namozov *et al*. 2022). Kyei-Boahen *et al*. (2017a) reported that the nitrogen content in the cowpea shoot inoculated with *Bradyrhizobium* was consistently higher, compared to the uninoculated control. Also, Neelipally (2020) concluded that *Bradyrhizobium* inoculation enhanced the biomass yield and nutrient uptake of peanuts compared to the uninoculated control.

2.4.3 Nitrogen Fixation by Legumes

The effectiveness of biological nitrogen fixation depends on the existence of favourable abiotic circumstances, including favourable climatic conditions, rhizobia populations and soil conditions (Soumare *et al*. 2020). In a study that involved inoculating Bambara groundnut with *Bradyrhizobium* strains, the result obtained revealed a great increase in symbiotic nitrogen fixation and grain yield (Gueye *et al*. 1997; Kishinevsky *et al*. 1996). Inoculation efficiency is not always at its best due to inadequately introduced symbiotic bacteria (Zoundji *et al*. 2022). Other studies reported that failure of exotic rhizobia or biological nitrogen fixation may occur due to incompatibility with agro-environmental conditions or due to competition with native rhizobia or other microorganisms presents in the soil (Mathu *et al*. 2012). The indigenous rhizobia population may also be scarce or ineffective in fixing nitrogen (Goyal *et al*. 2021). Inoculation of *B. japonicum* strain USDA110 on Bambara groundnut accession, in a study conducted in Kenya in 2015, resulted in the production of an average of over 49 nodules per plant, outperforming other Bradyrhizobia spp. that produced less than 25 nodules per plant (Benson and Fredrick).

The procedure of inoculating legumes with high inoculant strains typically results in increase in nitrogen fixation, nodulation, and optimal grain yield (Kyei-Boahen *et al*. 2017b). Additionally, Allito *et al*. (2020) revealed that inoculation of *rhizobium* strains greatly improved the nodulation, nitrogen fixation, nutrient absorption and soil nitrogen balance of faba bean (*Vicia faba L*.) in two locations. The maximum nitrogen fixation, nutrient absorption and soil nitrogen balance were produced by the inoculation with the NSFBR-12 and NSFBR-15 bacteria. The results also showed that faba bean nutrient uptake and soil nitrogen balance can be increased by inoculating the plant with competitive and efficient *rhizobium* strains.

When *Bradyrhizobium* strains were inoculated to mung bean and soybean, and regardless of the amount of nitrogen applied, significant improvements were recorded in plant growth, nitrogen fixation and nodulation potential, according to Htwe *et al*. (2019). Sibponkrung *et al*. (2020) also inoculated soybean with USDA110 strain, and the results showed that nodulation and nitrogen-fixing efficiency were improved with the development of bigger nodules compared to the uninoculated treatment.

2.5 Specific effects of N Fertilizers on:

2.5.1 Agronomic Traits and Grain Yield of Legumes

Legume growth and output are significantly boosted by the initial dose of inorganic nitrogen fertilizer applied. The application of nitrogen fertilizer in a pot experiment improved Bambara groundnut growth and yield parameters (Hasan *et al*. 2019a). The growth and yield parameters of Bambara groundnut recorded in the study were unaffected by nitrogen rates (20kg N ha^{-1}, 25kg N ha^{-1}, and 30kg N ha^{-1}) but nitrogen rates of 20 kg N ha^{-1} were recommended for Bambara groundnut cultivation in the tropics (Tyoakoso *et al*. 2019). Furthermore, a study conducted in Northern Nigeria revealed that fertilising Bambara groundnut seedlings with nitrogen increased plant height, number of leaves, and biomass yield (Sotayo and Donli 2021). Hasan *et al*. (2018a) observed improved performances in agronomic parameters (e.g. plant height and number of leaves) of Bambara groundnut when nitrogen fertiliser was used as an amendment. Ntambo *et al*. (2017a) showed that inoculating *rhizobium* with nitrogen fertilizer using soybean as a test crop resulted in maximum plant height of 50 kg N ha^{-1}. However, decrease in plant height was recorded for nitrogen fertiliser levels at 200 kg N ha^{-1}. Further results showed that increase in nodule number and nodule dry weight were recorded in the uninoculated control and application of 50 kg N ha^{-1} as compared to increased nitrogen application (100 kg N ha^{-1} and 200 kg N ha^{-1}). This indicates that increasing nitrogen levels reduced nodule size, number, and nodule dry weight per plant in the inoculated plants (Ntambo *et al*. 2017b).

2.5.2 Macro-nutrient absorption in Legumes

Application of nitrogen fertilizer to Bambara groundnut improved the crop's nutrient contents. According to a report by Hasan *et al*. (2019a), application of 30 kg N ha^{-1} increased the nutritional composition of Bambara groundnut. Additionally, due to the addition of nitrogen fertilizer to legumes, Morya *et al*. (2018) reported a substantial difference in soybean nitrogen and phosphorus uptake compared to the unfertilized area (control). When examined at 50% flowering, the uptake of nitrogen and phosphorus by legumes was improved by the initial dose of nitrogen fertilizer applied to leguminous crops. Bitire *et al*. (2022b) revealed that inoculation of *B. japonicum* strains increased nitrogen and phosphorus uptake of Bambara groundnut accessions in both seasons in two agro-ecological zones (Ibadan and Ikenne) in Nigeria. Nitrogen, phosphorus and potassium contents in the shoots and roots of chick pea (*Cicer arietinum L.*) were significantly ($p < 0.05$) higher in *rhizobium* inoculation treatment compared to uninoculated control (Abdiev *et al*. 2019). Singh *et al*. (2022) also reported increase in nutrient content (nitrogen, phosphorus and potassium) of chicken pea (*Cicer arietinum L.*) inoculated with *rhizobium* when compared with uninoculated control.

2.5.3 Nitrogen Fixation by Legumes

Amendments of Bambara groundnuts with inorganic nitrogen fertilizer aids both root development and nitrogen fixation (Hasan *et al*. 2018b). Yoseph *et al*. (2017) studied the impact of nitrogen fertilizer rate, *Bradyrhizobium* spp. inoculation, on common bean (*Phaseolus vulgaris L.*) in Ethiopia. The result showed that treatments with nitrogen fertilizer had the most increased nodule number and nodule dry weight. It also revealed that nitrogen promote nodulation when introduced in small amounts as a starter dose. The peak of biological nitrogen fixation was also decreased by the application of nitrogen fertilizer by up to 16% at the full flowering stage. In a different study conducted by Tamagno *et al*. (2018), designed to examine the relationship between nitrogen fertilizer application, biological nitrogen fixation and uninoculated control using soybeans as test crop, the result showed that the seed output fell to 13 kg ha^{-1} due to nitrogen fertilizer application when the expected grain yield area in Nigeria is 1.2 tons ha^{-1} and the worldwide average yield is 2.5 tons ha^{-1} (Iyorkaa *et al*. 2021).

Pampana *et al*. (2018) conducted an experiment in Italy to examine the grain yield response, nodulation capacity and biological nitrogen fixation in four common grain legumes grown in growth boxes: pea (*Pisum sativum L.*), white lupin (*Lupinus albus L.*), field bean (*Vicia faba L.*) and chick pea (*Cicer arietinum L.*). The soil was fertilized with five nitrogen fertilization rates (0, 40, 80, 120, and 160 kg ha^{-1}). It was noted that the amount of nitrogen fixed reduced with the increased supply of nitrogen fertilizer. The four grain legumes were distinct in how they responded to nitrogen fertilization. For example, white lupin and chickpea fixed more nitrogen per unit mass of nodules than other grain legumes and the amount of nitrogen derived via nitrogen fixation declined rapidly with increasing nitrogen supply. Although nodule fixation activity rose with increased nitrogen supply, the decline in nitrogen fixation in field bean and pea was only brought on by a drop in nodule biomass.

However, Mesfin *et al*. (2020) revealed that applying nitrogen fertilizer to various legumes faba bean (*Vicia faba L*.), field pea (*Vicia faba L.*) and dekoko (*Pisum sativum var. abyssinicum*) aided biological nitrogen fixation of the legumes. The amount of nitrogen fixed by each legume was 38, 49 and 97 kg ha^{-1} respectively. In contrast to the application of rhizobia, which affects growth traits and improves nitrogen fixation in common bean genotypes, the application of nitrogen fertilizer diminishes symbiotic nitrogen fixation in common bean genotypes (Reinprecht *et al*. 2020).

2.6 Effects of *B. japonicum* and N Fertilizer on:

2.6.1 Agronomic Traits and Yield of Legumes

Very few studies have been conducted to compare the effect of nitrogen-fixing bacteria (*B. japonicum*) with inorganic nitrogen fertilizer on agronomic and yield response of Bambara groundnut. Bitire *et al*. (2022b) found that inoculated Bambara groundnut accessions with *B. japonicum* strains promoted the yield and yield components of the crop in two locations (Ibadan and Ikenne) in Nigeria. The results obtained were significantly greater than the nitrogen fertilizer and uninoculated treatments. Similar to this, the results of a study conducted to ascertain the impact of nitrogen fertilizer application and *B. japonicum* inoculation on soybean seed showed that the two cultivars of soybean used in the study had increased seed yield following seed inoculation with *B. japonicum* strains HiStick, Nitragina and nitrogen fertilizer application at 30 kg N ha^{-1} and 60 kg N ha^{-1} than single application of nitrogen fertilizer and uninoculated control (Prusiński *et al*. 2020).

However, Mintah *et al*. (2020) reported the response of groundnut and cowpea to five rhizobia strains and nitrogen fertilizer application in Ghana. The results obtained revealed that the highest shoot biomass was recorded in cowpea and groundnut in three rhizobia strains but no significant difference was recorded among the nitrogen treatments and rhizobia inoculation combination in the nodule number when compared to uninoculated control. The nodule dry weight recorded in both groundnut and cowpea, however, showed a substantial difference. The quantity of pods, weight and size of cowpea and groundnut were increased by rhizobia inoculation. *Bradyrhizobium* spp. inoculation increased biomass accumulation in shoot, root, nodules and number of nodules (da Conceição Jesus *et al*. 2018). Furthermore, it was noted that inoculating soybean with *rhizobium* increased plant height compared to uninoculated plants (Alam *et al*. 2015; Janagard and Ebadi-Segherloo 2016). Another study indicated that velvet beans (*Mucuna pruriens*) inoculated with *rhizobium* and urea fertilizer (80 kg N ha^{-1}) resulted in accumulated biomass. A similar number of nodules and identical nodule dry weight were recorded in uninoculated velvet beans. Similar outcomes were recorded when urea fertilizer was applied in combination with *rhizobium* inoculation to velvet beans (Paudyal and Gupta 2018). The results of an experiment conducted by Szpunar-Krok *et al*. (2021) to examine the impact of *B. japonicum* inoculation and nitrogen fertilization application at 0, 30, and 60 kg ha^{-1} on the fatty acid profile of soybean seeds showed that nitrogen fertilizer application did not result in the anticipated changes. Furthermore, *B. japonicum* (HiStick, Soy and Nitragina) inoculation was recommended because it was observed to lower the levels of fatty acids in soybeans. In conclusion, a recent study utilising soybean as a test crop found that the combination of *Bradyrhizobium* strains inoculation and nitrogen fertilization significantly boosted soybean production, increased seed output by 42% and protein yield by roughly 28% (Księżak and Bojarszczuk (2022).

2.7 Conclusion and Prospect for the Future

High cost of procuring the inorganic nitrogen fertilizer is a major factor that contributed to the reduction of its use. Also, the continuous use of inorganic nitrogen fertilizers often causes detrimental effects on soil and human health which leads to the alternative by farmers to improve their grain yield. The use of bio-inoculant (Nitrogen fixing bacteria) was embraced and it has been widely accepted worldwide due to reduced cost and because it does not contaminate the soil or affect human health after use. The inoculation of the nitrogen-fixing bacteria (*B. japonicum*) increased production in legumes better than the application of nitrogen fertilization. The use of bacteria inoculant is easily accessible and affordable, improves nitrogen fixation, and provides a better yield than inorganic nitrogen fertilizer. The constraints to the use of bio-inoculant include unavailability in some designated places such as research institutes and farmers shops and limits in its use. The bio-inoculants need to be introduced to farmers and adequate training needs to be organized to present its importance to the end users, the farmers. It is crucial for smallholder farmers to adopt inoculation of bacteria to legumes to increase its production.

CHAPTER THREE

Identification of Bacteria from Nodules of Leguminous Crops

Abstract

Identifying bacteria species responsible for a particular legume's nodulation requires a great expertise. Even without the inoculation of exotic strains, a variety of native bacteria present in the soil have the potential to nodulate and fix atmospheric nitrogen to improve the growth and development of the legumes. In order to assess these native strains' potential, it is necessary to isolate, identify, and molecularly characterize them. An extensive review was conducted to examine the techniques used to isolate bacteria from legume nodules. The review revealed that majority of the isolates are highly fast growers, according to a reviewed study based on 16S rRNA gene, with high similarity index to *rhizobium* species.

Keywords: I6S rRNA, legumes, bacteria, isolation, *rhizobium*

3.1 Introduction

Nodulation is one of the most well-known processes for the symbiotic interaction of legumes with rhizobia. Host legumes use the fixed ammonia produced by rhizobia as a form of nitrogen and the carbon formed by the plants is used by the rhizobia. Consequently, root nodule symbiosis is crucial to agriculture and the nitrogen cycle since it is favourable to both parties (Duangkhet *et al*. 2018). In agriculturally polluted soils, almost all rhizobia type strains are said to encourage the generation of mineral uptake, phytohormones and a reduction in the effects of toxic metals, hence indirectly encouraging the growth and development of plant (Karthik *et al*. 2017). Mucilages, border cells, rhizodeposit nutrients and the exudates produced by plants all attract and provide food for the microorganisms found in the rhizosphere" (Igiehon and Babalola 2017). Studies have shown that there is the availability of increased diversity among rhizobial populations responsible for legume nodulation in African soils (Gyogluu *et al*. 2018). The list of nitrogen-fixing rhizobia responsible for the nodulation of legumes consist of over 100 species from 14 bacterial genera originating from diverse legumes and contrasting environments across different continents in the world *(Remigi et al*. 2016b).

The efficiency of nitrogen fixation must be determined for native isolates in order to maximise legume yield (Anglade *et al*. 2015). Additionally, resource-poor smallholder farmers may be able to produce crops utilising inoculants at a lower cost and with greater affordability (Singh *et al*. 2016). There are currently around 238 microorganisms that cause nodules formation on legumes (Shamseldin *et al*. 2017). The mutualistic interaction between legumes and rhizobia is host-specific, thus it is important to identify the rhizobia strains and diversity that are linked with particular kinds of legumes in order to maximise the potential for rhizobia biofertilizer benefits (Batista *et al*. 2015). Successful mutualistic relationship between the host and rhizobia relies on complex interactions of molecular signals (Roy *et al*. 2020). Under low-nitrogen conditions, the earliest signal exchange between the host and rhizobia occurs in the rhizosphere. Legume roots commonly release flavonoids into the soil, which attract rhizobia to the rhizosphere and induce these bacteria to secrete lipo-chitooligosaccharides called Nod factors (Yang *et al*. 2022).

Allorhizobium, Mesorhizobium, Bradyrhizobium, Methylobacterium, Sinorhizobium, Azorhizobium and *Rhizobium* are seven genera of Alpha-proteobacteria that make up around 40 rhizobia species that have been identified and named (Koskey *et al*. 2018). In addition, there are additional nitrogen-fixing bacteria that collaborate with legumes, which have recently been discovered in beta and gamma proteobacteria. Typically, each genus' rhizobia fix nitrogen with a particular legume through nodulation. *Rhizobium* strains, for example, are primarily linked to common beans (*Phaseolus vulgaris L.*) and chickpeas (*Cicer arietinum L.*) and whereas *Bradyrhizobium* strains are frequently observed to nodulate soybeans (*Glycine max L. Merrill*), Bambara groundnut (*Vigna subterranea L. Verdc*), cowpeas (*Vigna unguiculata L.*) and (*Vigna radiata L.*). The aim of this review is to identify the bacteria responsible for the nodulation of Bambara groundnut and other legumes.

3.2 Identification, Isolation, and Characterisation of Rhizobia from the root Nodules of Legumes

A field study conducted by Raissa *et al*. (2020) on the molecular investigation of 15 bacteria isolated from the root nodules of Bambara groundnut in the Republic of Côte d'Ivoire using 16S rRNA gene sequences indicated lineage linked to 80% *Rhizobium*, 13% *Bradyrhizobium*, and 7% *Densifier*. Isolates closely resemble *B. yuanmingense* and *Rhizobium* species. And in tests, it was found to have helped the host plant grow, stay healthy, and produce. Furthermore, in another study conducted in Ghana and South Africa, results revealed that Bambara groundnut nodulation was due to a group of *Bradyrhizobium* species precisely *Bradyrhizobium vignae*. Symbiotic transfer and several evolutionary trends were shown by the grouping of isolates based on *nifH* and *nod D* phylogenies (Puozaa *et al*. 2017a). In addition, in a study that was carried out in South Africa, Mali, and Ghana, *Bradyrhizobium* spp. were isolated from Bambara groundnut utilising 16S rRNA, *nifH* and *nod C* gene sequences. These lineages are closely related to *B. subterraneum, B. pachyrhiz, B. kavangense, B. elkanii* and *Bradyrhizobium vignae* through phylogenetic analysis (Ibny *et al*. 2019b). Eight rhizobia isolates from *Arachis hypogea* nodules were used in this study and their genotypes and phenotypes were characterised. The isolates were further tested for their tolerance to abiotic stress (temperature, salinity, and pH) (Ibny *et al*. 2019b).

The 16S rRNA genome analysis revealed that the isolates are fast-growing, phylogenetically related to *Rhizobium* species, tolerant to 3% naphthalene, able to survive between 20 and 37 °C, and are alkaline at pH values between 5 and 10. Three of the eight isolates have the ability to nodulate, increasing their potential for use as native groundnut inoculum and their ability to effectively use up as nitrogen fixers, *nifH* gene (Khalid *et al*. 2020). Furthermore, to characterise the rhizobia and non-rhizobia isolates obtained from the chickpea (*Cicer arietinum L.*) root nodules, Demissie *et al*. (2018) conducted another study in Ethiopia. The findings revealed that three chickpea cultivars were used to authenticate five bacteria found in chickpea root nodules, and the isolates were identified using morphological and phenotypic features. Bacteria nodulating chickpea (27 isolates) and bacteria not nodulating chickpea (23 isolates) are the two groups of tested isolates with 78% similarity levels to each other. Isolates may thrive in temperatures of 40 °C, salt concentrations of 2.5% and pH ranges of 5 to 9.5. However, a different investigation was designed to define the groundnut strains using the Sudanese soil's 16S rRNA gene sequence. Sequencing results showed that all three isolates have high similarity index of 98% to 99% to

Klebsiella spp. (Idris *et al*. 2015). More specifically, a study was conducted over the years to examine the genomic species distribution among 745 isolates on various leguminous hosts at different locations to determine the molecular variation of rhizobia occurring on legumes in south eastern Australia. These strains of rhizobia were isolated from nodule samples taken from 32 different legumes at 12 different locations. In the typically acidic soils where nodules were obtained, *Bradyrhizobium* spp. was the most prevalent and *Rhizobium tropici* predominated among the *Rhizobium* and *Mesorhizobium* isolates (Lafay and Burdon 1998).

A study conducted in India revealed the presence of thirty rhizobial strains from the root nodules of ten groundnut plant, of which twenty eight strains produced indole acetic acid, nine strains were able to solubilise phosphate, and twenty nine strains showed positive results for the production of siderophore and ammonia when the rhizobial strains were screened for growth promoting activities in plant. The results of the field tests showed that the *rhizobium* strains were more effective in terms of nodule number and dry weight, yield and yield components (Jain *et al*. 2020b).

The sequencing of the *nifH* and 16S rRNA genes amplified directly from the root nodules of mung bean revealed 32.05% *Bradyrhizobium* and 35.84% *Ensifer* of the retrieved 16S rRNA respectively. The coexistence of *Bradyrhizobium* with 39.21% and *Ensifer* with 59.23% strains were further supported by the *nifH* sequencing. The study came to the conclusion that the successful nodulation of mung bean by the isolates reflect that strains of both the general *Bradyrhizobium* and *Ensifer* can be used for the production of inoculum.

However, the *nifH* sequence of the genus *Rhizobium* was not present and those of the genus *Mesorhizobium* contain a minor fraction of the sequences retrieved from soil rhizosphere samples and from the nodules (Hakim *et al*. 2018). In another study conducted on rhizobial strains isolated from *Sesbania bispinosa* a leguminous crop grown in Bangladesh forty-four isolates were molecularly characterised and studied biochemically. It was found that the isolates were able to use carbohydrates and showed total resistance to cloxacillin and penicillin, which increased the population of the rhizobial in antibiotic-stressed conditions and isolates were able to nodulate. Nearly all of the isolates exhibited good results in the amplification of the *rhizobium* species' signature genes, *nod C* and *nifH*, and 16S rRNA was utilised to gauge the genetic distance between isolates. The research eventually assisted in the identification of certain *Sesbania bispinosa* strains which when introduced to the soil can be used as biofertilizers to increase agricultural yield (Nahar *et al*. 2017).

3.3 Conclusion and Prospect for the Future

According to the review, bacteria identification from the root nodules of various legumes differs base on the type of strains isolated, the habitats and the soil conditions where they are separated from. The 16S amplification of majority of the isolated genes showed that most isolates are *Rhizobium* spp. or *Bradyrhizobium* spp., with only a small percentage being slow growers. Many isolates from nodules of leguminous crop can live in high salt conditions, high temperatures and pH, according to the findings of this review. Nevertheless, *Bradyrhizobium* spp. and *Rhizobium* spp. isolates exhibit strong similarity index.

CHAPTER FOUR

Effects of *Bradyrhizobium japonicum* Strains on the Growth and Biomass Yield of Accessions of Bambara Groundnut (*Vigna subterranea (L) Verdc).*

Abstract

The use of inorganic nitrogen fertilizer often leads to environmental contamination and adverse effects on soil and human health. Also, the inability of farmers in the tropics to procure inorganic nitrogen fertilizer to amend legume as a starter dose resulted to the use of bacteria strains that are easily affordable and improved the growth and development of legumes. Pot experiments were carried out in the screenhouse at the International Institute of Tropical Agriculture (IITA), Ibadan, in 2018 and 2019, and field experiments in two geographical locations: Ibadan and Ikenne, within August to December in the 2019 and 2020 cropping seasons in Nigeria. Ten accessions of Bambara groundnut: TVSu-475, TVSu-1606, TVSu-305, TVSu-710, TVSu-787, TVSu-506, TVSu-1698, TVSu-1739, TVSu-365 and TVSu-378 were inoculated with broth culture of Bacteria strains (*B. japonicum* strains). RACA6, FA3, IRJ2180A, USDA110 and N (Urea, 20 kg ha^{-1}) were applied to uninoculated seedlings 2 weeks after planting (WAP) and an uninoculated control (no inoculation and no fertilizer application) in the screenhouse allowed. Seeds of Bambara groundnut accessions were coated with each of the *B. japonicum* strains and allowed to dry firmly with the seeds before planting on the fields. Data were collected on the biomass yield, growth response, and nutrient uptake in the screenhouse and on the field and were eventually subjected to analysis of variance, and means were separated using the Duncan multiple range test (DMRT) at ($P<0.05$) level of probability. The results obtained showed the importance of the inoculations of bacteria strains over the inorganic nitrogen fertilizer as a starter dose in improving the performance of Bambara groundnut accessions. Results obtained revealed that *B. japonicum* strains enhanced the growth of Bambara groundnut accessions in the screenhouse. FA3 strains was found to improve the growth traits and nutrients uptake significantly by 40% increase on the field as inoculant and was able to out-compete other strains and nitrogen fertilizer applied. Comparing the effect of treatments in both pot and field study, FA3 strains performed exceedingly and enhanced the growth, biomass yield, and nutrient uptake of Bambara groundnut accessions than other treatments used as amendments in the study.

Keywords: Underutilized legume, beneficial soil microorganism, inoculation, plant growth nutrition, fertilization

4.1 Introduction

Bradyrhizobium are beneficial symbionts that are highly present due to their availability as free-living bacteria in different habitats and in association with leguminous plants (Sprent *et al*. 2017a). Unlike some other N_2-fixing microsymbionts, they exhibit a life cycle (bipartite) which varies as a free-living state in soils and as a symbiotic partner inside root nodules of legumes (Jaiswal and Dakora 2019c). The inoculation of the *Bradyrhizobium* strains often leads to the supply of the nitrogen needed by plant through biological nitrogen fixation (BNF) without the addition of chemical nitrogen fertilizer which eventually leads to economic savings (Hungria and Mendes 2015b), decreases emission of greenhouse gases and reduce risk of contamination of surface and ground water with nitrate (de Moraes Sá *et al*. 2017; Hungria and Mendes 2015b).

Bradyrhizobium are major components of soil microbiota which enhance the improvement of plant growth nutrition, soil fertility, and also contribute an important role in organic agriculture which eventually lead to the reduced application of agrochemicals and inorganic fertilizers. They usually form symbiotic association and colonise roots of leguminous plants which eventually lead to utilisation of adequate nutrient uptake and water (Gitonga *et al*. 2021b). The use of beneficial soil microorganisms in organic farming, is an improving and promising smart-technology that could reduce the intensive use of inorganic fertilizers (Fasusi *et al*. 2021). The roles of the these bacteria have been marginalised in modern agriculture since the modification of the microbial communities in conventional farming systems due to high inputs of inorganic fertilizers, modern tillage (Kerry *et al*. 2018; Yadav *et al*. 2018) fertilizers, herbicides and pesticides usage (Malhotra *et al*. 2015; Prashar and Shah 2016).

Bambara groundnut (*Vigna subterranea* (L.) *Verdc*) is an underutilised and an indigenous African leguminous crop grown for human and animal consumption. It is ranked third in importance after other legumes like groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Africa (Mubaiwa *et al*. 2017). Bambara groundnut seed contains carbohydrate (58.3%), protein (23.7%) and fat (4.3%) with high amount of methionine content than other leguminous crops (Oyeyinka *et al*. 2018). It has the ability to produce high yields even in drought stress conditions and low nutrient soils (Ibny *et al*. (2019b).

Bambara groundnut has the potential to fix about 4 to 200 kg N ha^{-1} Mohale *et al*. (2014a) with a mutualistic relationship with soil bacteria called 'rhizobia' (Puozaa *et al*. (2017a). Because legumes vary between geographical locations, it is important to examine new geographic regions to identify rhizobia that are capable of effectively nodulating and promoting the growth of Bambara groundnut (Ibny *et al*. 2019b). Some of the established and promising strains of Bambara groundnut inoculation are: BAMKis strains (*Bradyrhizobium spp*.), BAMKbay8 and BAMsp3 (*Burkholderia spp.*). All these produce highly effective nodules and high plant biomass (Benson *et al*. 2015).

The deficiency of nitrogen in the soil often results to leaf senescence, lower yield production, and biomass in plants (Kant *et al*. 2011). High cost of procuring the inorganic nitrogen fertilizer by the tropical farmers is a major challenge and most farmers that can afford usually apply below the manufacturer's recommendation. Therefore, this study were conducted to evaluate the effects of inoculating different Bambara groundnut accessions with *B. japonicum* strains so as to understand its role in the growth and yield improvement of the legume.

4.2 Materials and Methods

4.2.1 Seed characteristic and phenotypic features

The seeds used were randomly selected from the early germination, maturing, seedling establishment and growth potential accessions at the International Institute of Tropical Agriculture Gene bank. The ten Bambara groundnut accessions include: TVSu-475, TVSu-1606, TVSu-305, TVSu-710, TVSu-787, TVSu-506, TVSu-1698, TVSu-1739, TVSu-365 and TVSu-378.

4.2.2 Seed scarification

The two ends of the Bambara groundnut seeds were scarified using scalpel before planting in the screenhouse. The seeds of different Bambara groundnut accessions were introduced into a beaker containing 3% w/v (Sodium hypochloride), the solution was decanted and rinsed with sterile water and 90% of ethanol was added for 30sec and was decanted and also rinsed after 1min with sterile water (Davis *et al*. 1991).

4.2.3 Authentication of the Strains

Seeds were sterilised using 3% w/v sodium hypochloride, planted in pots containing sterile soil and allowed to grow for 10days before inoculation of broth containing about 1×10^9 cfu/ml of rhizobia cell to seedlings using syringe. Plants were allowed to grow for about six to eight weeks to check for nodulation potential of rhizobia isolates. Most effective isolates were selected and used for pot experiments and field experiment (Vincent 1970b).

4.2.4 Broth Preparation

To prepare 1 litre of broth solution, mannitol 10 g, yeast extract powder 0.5 g, potassium phosphate 0.5 g, magnesium sulphate 0.2 g, sodium chloride 0.1 g, and agar powder 15 g were weighed and added to a beaker. The mixture was dissolved in a conical flask with 1000 ml of distilled water and stirred to homogenized the solution. The pH was adjusted to 6.8 and sterilized using the autoclave at 121^{o}C for 15 minutes at a pressure of 15 psi placed in the water bath to adjusted temperature of 47 $^{\circ}$C and was poured in sterile petri dishes.

4.2.5 Determination of Most Probable Number

The quantity of indigenous rhizobia present in the soil were determined using the most probable number. Soil sample were collected from the field in Ibadan and Ikenne and were carefully sieved, sterilized and placed in 2kg pots. Seeds of cowpea were planted in 2018 and seeds of Bambara groundnut were planted in 2019 and 2020 season. Rhizobia were isolated from nodules of the legumes on Congo red agar as reported in using spread plate method. Nodules samples were picked from the root of each legumes and were placed in sterile water for about 15 to 20 minutes, and were surface-sterilized using 3% sodium hypochlorite for few minutes. The nodules were carefully rinsed with sterile water and were further sterilized with 95% ethanol and then rinsed with six changes of sterile water (Woomer 1994). The nodules were then transferred into sterilized petri-dishes, crushed with flamed glass rod and mixed with a few drops of sterile water. Crushed nodules were streaked on Congo red agar and then incubated at 28^{o}C for 5-7 days, and isolates were purified and identified.

4.2.6 Nutrient Solution Preparation

To prepare 1 L of macronutrient, 100 g of calcium phosphate, 20 g of potassium phosphate, 20 g of hydrated magnesium sulphate, 20 g of sodium chloride and 10 g of iron (11) chloride were dissolved in 100 ml of distilled water and stirred till the solution became homogenous. To prepare 1 L of micronutrient solution, 2.86 g of orthoboric acids, 1.81 g of magnesium chloride, 0.22 g of zinc sulphate and 0.025 g of sodium molybdate were dissolved in 1000ml of distilled water and stirred (Steiner 1961).

4.2.7 Pre-Sowing Soil Analysis used in Pot and Field Experiments

The pH of the soils used in the screenhouse in both seasons were neutral. While pH of the soil in Ikenne were acidic in nature in both seasons, in Ibadan, pH were slightly acidic. The percentage organic carbon recorded for soils in both screenhouse and on the field were 0.25 to 0.40 which showed the soil used was moderate. The phosphorus in the soil used in the screenhouse was low. A higher percentage of phosphorus was recorded in the soil used in the field in both locations and seasons. The percent of potassium present in the soil used in both glasshouse and in the field in both seasons showed availability in moderate quantities.

The calcium in the soil used in the screenhouse were extremely high, while the calcium in the field in Ibadan were considered to be moderate (Table 4.1). The Mg present in the soils used for the screenhouse experiments were high in both sterile and non-sterile soil in both seasons. Higher Mg values were obtained in Ikenne in both seasons while lower quantities of Mg were obtained in Ibadan in both seasons. The particle size analysis were determined by the (hydrometer method). The soil textural class showed that both the soil used in the screenhouse and on the field were loamy (Table 4.1).

4.3 Pot Experiment

The soil used in the screenhouse was carefully sieved through 3 mm sieve and was sterilized at 121 °C for 1 hour using the autoclave. Ten accessions of Bambara groundnut, namely TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475 and TVSu-305, were randomly obtained from early maturing accessions from the International Institute of Tropical Agriculture (IITA) Gene bank and were inoculated with each *B. japonicum* strains: FA3, RACA6, USDA110 and IRJ2180A, containing 2.8×10^7, 7.2×10^6, 4.3×10^7, 1.4×10^7 cfu/ml respectively, nitrogen fertilizer (Urea, 20 kg/ha) and an uninoculated control. The choice of the *B. japonicum* strains depended on the availability and authenticity (ability to nodulate Bambara groundnut) and were inoculated to the seedlings of the ten accessions of Bambara groundnut in the screenhouse (Argaw 2014).

The experiment was laid out in a completely randomised design (CRD) with factorial arrangement using sterile and non-sterile soil and was replicated three time: 10 (Accession) * 6 (treatments)* 2 soil condition* 3 (replicate) =360 experimental unit. The seeds were surface sterilised and two seeds were sown into 10 kg pot containing the sterile and non-sterile soil in the screenhouse. Nitrogen-free nutrient solution and sterile water were added to each pot at regular intervals (twice a week) following the recommendation of Afzal *et al*. (2010). Data regarding growth traits and nutrient uptake were collected at 10 WAP while biomass yield was recorded at 50% flowering after drying at 72^{o}C for 48 h.

4.3.1 Data Collection

All data collected on growth parameters and biomass yield were taken based on the descriptor of Bambara groundnut Network (IPGRI). Vegetative traits recorded in this study from the Bambara groundnut descriptor include Peduncle length (cm), number of leaves, terminal leaflet length (cm), terminal leaflet width (cm), petiole length, plant spread (cm), plant height (cm) and numbers of stem and branches per plant.

4.4 Determination of Nitrogen in Plant.

4.4.1 Preparation of Digest: The 0.2 g of plant sample was weighed into a digestion tube, then 2.5 ml of acid mixture (3.5g of selenium powder was dissolved in 1 litre of conc H_2SO_4 and was placed on the hot plate at 300^oC till it turned colourless, 3.2g of salicylic acid was dissolved in 100ml of solution containing selenium powder and conc H_2SO_4), 3 ml of hydrogen peroxide was added to the plant samples and was stirred and placed on the digesting block at 150 °C for 30mins in the fume cabinet and temperature was increased to 330 °C. The sample was digested until extract turned colourless. Samples were removed from the fume cabinet and placed in the rack to cool and top up with 50 ml of distilled water (Anderson and Ingram 1989). All digest were diluted 1:9 (w/v) with distilled water and 0.2 ml of sample digested was taken with a micropipette and was placed in a clearly labelled test tube and 5.0 ml of the reagent N_1 and N_2 was added, vortexed and allowed to stay for 2 hours and absorbency was measured at 650 nm. The blue was stable for 10 hours and the concentration of nitrogen in the solution was measured. Nitrogen concentration in the sample materials expressed in nitrogen percentage was calculated using the formula by Lindner and Harley (1942).

$$\% N = \frac{(A-B) \times V \times K}{H^2 \times W \times al}$$

Where A = Concentration of N in the solution (n/mol), B = concentration of N in the blank (n/mol), V = total volume at the end of analysis procedure (ml), W = weight of dried samples (g), H= 1000, K = 100, and al = aliquot of the solution taken (ml).

N_1= 34g sodium salicylate, 25g sodium citrate and 25g sodium tartrate in 75ml water

N_2= 30g sodium hydroxide, 10ml sodium hypochlorite and make up to 1 litre

4.5 Determination of Phosphorus: 5 ml of the supernatant clear wet-ashed digest solution was pipetted into 50 ml volumetric flasks, then 20 ml of distilled water and 10 ml of ascorbic acid reducing agent were both added to each flask from the beginning of the standard. They were made to 50 ml with water and were shaken vigorously and allowed to stay for 1 hour to permit full colour development. Standard and sample absorbances (blue colouration) were measured at 880nm wavelength settings in a suitable colorimeter.

$$\% \text{ P in samples} = \frac{d \times t \times g}{v}$$

Where d = corrected concentration of P in the sample; t = volume of the digest; g = dilution factor; v = weight of the samples (Lindner and Harley 1942)

4.6 Field Experiments

Field experiments were carried out at two different geographical locations: Ibadan (7^o 38’N, 3^o 89’E) and Ikenne (6^o 86’N, 3^o 71’E), Nigeria in the 2019 and 2020 cropping seasons to determine the growth, nutrient uptake, and yield of inoculated accessions of Bambara groundnut. Each field was prepared using a tractor-driven plough and harrowed to remove plant debris. Two meter (2m) plot were made with a spacing of 25cm between plot and 1m between each replicate. The experiments were arranged in randomized complete block design (RCBD) on the field in both locations and cropping seasons and was replicated three (3) times.

Ten accessions of Bambara groundnut: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739 TVSu-710, TVSu-365, TVSu-475, and TVSu-305 used in the screenhouse were also used for field experiments. Six seeds from each accession were coated separately with each of the *B. japonicum* strains FA3, RACA6, USDA110 and IRJ2180A containing 2.8×10^7, 7.2×10^6, 4.3×10^7 and 1.4×10^7 Cfu/ml respectively, N fertilizer (Urea, 46%) and an uninoculated control. Seed of accessions were coated with the *B. japonicum* strains using Arabic gum and allowed to dry before planting on the field at both locations and cropping seasons. Regular weeding was done manually using hoe to remove weeds.

Data were collected at 12 weeks after planting (WAP) on growth trait and at 50% flowering on nutrient uptake. Data recorded on growth traits include peduncle length (cm), terminal leaflet length (cm), terminal leaflet width (cm), petiole length, plant spread(cm), and plant height (cm). Data were taken using a well calibrated metre rule, while the data on the number of stems, number of leaves, number of branches, and on flowering recorded were obtained by counting. Data on chlorophyll content of accessions of Bambara groundnut were obtained using the spad metre. Also, data on yield were obtained using the weighing balance.

4.6.1 Statistical Analysis

Data collected were subjected to four-way Analysis of Variance (ANOVA): pot (Accessions, treatments, soil status and season), field (Accessions, treatments, locations and seasons) statistical analysis system (SAS) package 9.4 and means were separated by using Ducan Multiple Range Test at $P \leq 0.05$

Figure 4.1: Accessions of Bambara groundnut used in the study.

Photo: T.D Bitire, 2018

4.7 Results

Table 4.1: Physiochemical and biological properties of soil used for the study in the screenhouse and on the field

Experiment	**Pot Experiment**				**Field Experiment**			
Year	2018	2018	2019	2019	2019	2020	2019	2020
Soil status/ Location	Sterile Soil	Non sterile Soil	Sterile soil	Nonsterile soil	Ikenne	Ikenne	Ibadan	Ibadan
pH (H_2O)	7.43	7.39	7.1	6.81	4.46±0.08	4.91±0.09	6.84 ± 0.1	7.13±0.11
N (%)	0.05	0.04	0.05	0.10	0.073±0.02	0.121±0.01	0.150±0.013	0.119±0.02
OC (%)	0.38	0.40	0.03	0.25	0.329±0.07	0.297±0.01	0.407 ± 0.10	0.336±0.06
Bray P (mg kg^{-1})	3.30	3.71	2.55	3.71	13.23±4.26	22.46±3.43	15.352±3.74	52.84±6.67
Sand (%)	81.00	83.00	77.0	81.00	76.00±1.16	76.00±1.16	83.00 ± 1.16	82.00±0.0
Clay (%)	13.00	11.00	19.00	11.00	16.00±1.16	20.00±2.00	10.00 ± 0.00	8.00±0.00
Silt (%)	6.00	6.00	4.00	8.00	6.00 ±1.15	3.00 ± 1.15	7.00 ± 1.15	10.00±0.0
Ca (Cmol/kg)	6.74	5.92	8.53	8.63	3.530±2.50	1.505±0.24	1.216 ± 0.10	1.101±0.30
Mg (Cmol/kg)	6.74	5.92	0.20	0.20	0.80 ±0.37	0.404±0.02	0.075 ± 0.01	0.158±0.05
K (Cmol/kg)	0.24	0.22	0.27	0.29	0.560±0.24	0.242±0.06	0.137 ± 0.02	0.201±0.02
Na (Cmol/kg)	0.06	0.06	0.08	0.08	0.076±0.01	0.082±0.00	0.055 ±0.02	0.057±0.01
ECEC (Cmol/kg)	13.79	12.12	9.08	9.21	4.96±1.56	2.23±0.57	1.29 ± 0.56	1.47±0.48
Zn (mg kg^{-1})	3.24	2.66	9.91	9.39	1.964±1.67	1.196±0.19	1.19 ± 0.10	0.66±0.04
Cu (mg kg^{-1})	0.95	0.49	0.64	0.70	1.167±0.62	2.045±0.19	0.86 ± 0.11	0.97±0.22
Mn (mg kg^{-1})	266.0	286.2	214.7	222.1	12.15±10.5	116.7±3.31	244.20±18.4	383.6±33.4
Fe (mg kg^{-1})	17.19	17.19	85.54	94.01	25.58±7.47	88.29±4.08	133.33±3.33	114.4±3.85
Glomus	-	159	-	145	106	117	135	152
acaulospora	-	211	-	200	192	185	202	208
enthrophospora	-	30	-	36	25	28	34	32
Spore/100gdwt	-	401	-	406	380	392	412	432
Soil textural class	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy sand	Loamy Sand	Loamy sand

Table 4.2: Rhizobia population of the soil used for both pot and field experiments

Experiment site	Year	Soil status/Location	Colony forming unit
Glasshouse	2018	Non sterile soil	1.28×10^{2}
	2019	Non sterile soil	1.53×10^{9}
Field	2019	Ibadan	1.83×10^{5}
	2019	Ikenne	2.03×10^{9}
	2020	Ibadan	3.05×10^{7}
	2020	Ikenne	2.47×10^{7}

Table 4.3: Analysis of variance (ANOVA) of accessions of Bambara groundnut inoculated with *B. japonicum* strains on the growth traits at 10WAP under screenhouse conditions in both seasons

Source of variation	DF	PLH (cm)	CS (cm)	NOS/plant	NOL/plant	CHPY
Accessions	9	407.97**	92.31**	1845.74**	17593.64**	417.89**
Treatments	5	12.017^{ns}	1.69^{ns}	274.76^{ns}	526.42^{ns}	95.11^{ns}
Soil Status	1	841.42**	159.41**	15797.87**	160776.32**	384.79^{ns}
Season	1	4969.64**	2134.50**	5037.88**	36155.86**	6896.92**
Rep	2	28.48^{ns}	17.25^{ns}	1673.01**	8792.17*	82.23^{ns}
Accessions*treatment	45	30.38^{ns}	8.90^{ns}	319.78^{ns}	2905.64^{ns}	232.18**
Accessions*soil status	9	26.95^{ns}	10.50^{ns}	293.01^{ns}	3224.99^{ns}	175.16^{ns}
Accessions*season	9	33.19^{ns}	5.79^{ns}	779.31**	6041.09*	148.13^{ns}
Treatment*soil Status	5	6.08^{ns}	4.47^{ns}	280.94^{ns}	3314.61^{ns}	240.49^{ns}
Treatment*seasons	5	52.34^{ns}	14.37^{ns}	620.32*	5978.13*	254.50^{ns}
Acce*treat*soil status	45	34.59^{ns}	8.83^{ns}	372.57*	8271.21**	161.86^{ns}
Treat*soil stat*season	5	38.53^{ns}	12.65^{ns}	384.43*	8041.32**	180.1^{ns}
Acct*treat*soil*seas	131	32.04^{ns}	7.51^{ns}	368.90**	8737.41**	150.86^{ns}

***=** Significant; **= Very significant; ns= Non-significant, DF= Degree of freedom; NOL= Number of leaves, CS= Canopy spread; PLH; Plant height; NOS= Number of stem; CHPY= Chlorophyll; WAP= Weeks after planting

Table 4.4: Analysis of variance (ANOVA) showing accession of Bambara groundnut inoculated with *B. japonicum* strains on the number of days to 50% flowering, %N and %P at flowering and harvest under screenhouse conditions in both seasons

Sources of variations	DF	Days to 50% Flw	P@Flw (%)	N@Flw (%)	N@Hvst (%)	P@Hvst (%)
Accessions	9	399.45**	0.012**	0.98**	0.67**	0.02**
Treatments	5	261.39ns	0.005ns	20.87**	16.07**	0.004ns
Soil Status	1	53.06ns	0.17**	0.28ns	3.01**	0.19**
Seasons	1	792.23**	1.06**	1.28ns	0.019ns	0.23**
Rep	2	196.78ns	0.012**	0.17ns	0.56ns	0.02**
Accession*treatment	45	282.45**	0.003ns	0.38ns	0.24ns	0.002ns
Access*soil Status	9	226.80ns	0.003ns	0.17ns	0.16ns	0.004ns
Accessions*season	9	226.80ns	0.01**	0.78**	0.25ns	0.003ns
Treatment*soil status	5	99.12ns	0.004ns	0.43ns	0.21ns	0.010ns
Treatment*season	4	189.71ns	0.003ns	0.48ns	0.85**	0.003ns
Acc*treat*soil status	45	198.53ns	0.002ns	0.38ns	0.23ns	0.004ns
Treat*soil stat*season	5	201.24ns	0.003ns	0.44ns	0.32ns	0.005ns
Acc*Strain*soil*season	131	163.25ns	0.002ns	0.29ns	0.003ns	0.003ns

*= Significant; **= Very significant; ns= Non-significant; DF= Degree of freedom; Flw= Flowering; Hvst= Harvesting; %P= Phosphorus; %N= Nitrogen; @= at, Acc-Accession

Table 4.5 Mean separation showing effect of treatments on growth traits at 10wap and nutrient uptake of Bambara groundnut accessions cultivated in two soil status under screenhouse conditions.

Soil Status	PLH (cm)	CS (cm)	NOS/plant	NOL/plant	CHPY	N@Flw (%)	N@Hvst (%)	P@Flw (%)	P@Hvst (%)	Days to 50% Flw
Sterile	17.03[b]	8.52[b]	30.58[b]	90.58[b]	35.49[a]	2.16[a]	2.0876[a]	0.086[b]	0.102[b]	46.294[a]
Nonsterile	19.34[a]	9.53[a]	41.48[a]	124.57[a]	37.15[a]	10.67[a]	1.9487[b]	0.118[a]	0.14[a]	45.903[a]

NOL= Number of leaves, CS= Canopy spread; PLH; Plant height; NOS= Number of stem; CHPY= Chlorophyll; WAP= Weeks after planting, Flw= Flowering; Hvst= Harvesting; %P= Phosphorus; %N= Nitrogen; @= at

Table 4.6 Mean separation showing effect of treatments on growth traits at 10WAP and nutrient uptake of Bambara groundnut accessions cultivated under screenhouse conditions in both seasons.

Season	PLH (cm)	CS (cm)	NOS/plant	NOL/plant	CHPY	N@Flw (%)	N@Hvst (%)	P@Flw (%)	P@Hvst (%)	Days to 50% Flw
1	21.37[a]	11.10[a]	39.44[a]	116.52[a]	39.98[a]	2.05[a]	1.99[a]	0.15[a]	0.14[a]	49.87[a]
2	15.55[b]	7.29[b]	33.22[b]	100.22[b]	33.28[b]	10.05[a]	2.04[a]	0.06[b]	0.10[b]	46.17[b]

NOS= number of leaves, NOL= Number of leaves, CS= Canopy spread; PLH; Plant height; NOS= Number of stem; CHPY= Chlorophyll; WAP= Weeks after planting, Flw= Flowering; Hvst= Harvesting; %P= Phosphorus; %N= Nitrogen; @= at

Table 4.7: Effect of the inoculation of *B. japonicum* strains on the growth traits of accessions of Bambara groundnut at 12WAP in Ibadan and Ikenne in both seasons.

Accessions	PLH (cm)	NS/ plant	NB/ plant	LA (cm^2)	NOL/ plant	TLL (cm)	TLW (cm)	PEL (cm)	CPHY
TVSu-506	24.01^{ab}	55.08^{d}	50.90^{e}	21.05^{a}	160.47^{f}	5.75^{b}	2.44^{b}	1.66^{c}	31.85^{d}
TVSu-1739	25.53^{a}	50.04^{e}	45.28^{f}	18.82^{b}	147.75^{g}	13.89^{a}	2.75^{a}	2.29^{a}	36.57^{b}
TVSu-305	20.42^{c}	69.64^{c}	62.76^{c}	14.89^{c}	209.36^{c}	5.75^{b}	2.64^{ab}	1.45^{d}	41.99^{a}
TVSu-787	21.76^{bc}	79.43^{b}	72.39^{b}	12.15^{d}	234.18^{b}	5.51^{bc}	2.00^{d}	1.78^{b}	33.13^{c}
TVSu-378	18.84^{d}	95.00^{a}	79.75^{a}	12.68^{d}	278.75^{a}	5.10^{cd}	2.20^{cd}	1.43^{d}	31.83^{d}
TVSu-1698	22.70^{b}	70.79^{c}	65.09^{c}	15.98^{c}	202.89^{d}	5.89^{b}	2.69^{a}	1.47^{d}	35.04^{b}
TVSu-710	18.77^{d}	39.40^{g}	34.94^{h}	12.76^{d}	121.68^{g}	5.35^{bcd}	2.22^{cd}	1.41^{d}	31.05^{d}
TVSu-475	21.29^{bc}	62.90^{d}	56.61^{d}	13.92^{d}	183.75^{e}	5.83^{b}	2.34^{c}	1.38^{e}	31.70^{d}
TVSu-365	18.85^{d}	78.62^{b}	65.27^{c}	10.20^{e}	210.12^{c}	4.45^{d}	1.74^{e}	1.39^{e}	27.84^{f}
TVSu-1606	17.23^{e}	47.01^{f}	39.71^{g}	12.61^{d}	133.51^{h}	4.89^{d}	2.08^{d}	1.22^{f}	29.38^{e}

Mean comparison is along each column, mean with the same letter are not significantly different at p<0.05 level of probability according to DMRT. PLH= Plant height; NS= Number of stem; NB= Number of branches; LA= Leaf area; NOL= Number of leaves; TLL= Terminal leaf length; TLW= Terminal leaf width; PEL= Petiole length; CHPY= Chlorophyl

Table 4.8: Effect of the inoculation of *B. japonicum* strains on the growth traits of accessions of Bambara groundnut at 12WAP in Ibadan and Ikenne in both seasons.

Strains	PLH (cm)	NB/plant	NS/plant	LA (cm^2)	NOL/plant	TLL (cm)	TLW (cm)	PEL (cm)	CS (cm)	CPHY units	PL (cm)
FA3	22.71^{a}	62.73^{a}	70.11^{a}	15.74^{ab}	209.25^{a}	6.00^{b}	2.56^{a}	1.73^{a}	12.21^{a}	34.53^{a}	12.69^{a}
USDA110	20.23^{b}	54.68^{bc}	60.83^{b}	12.93^{b}	177.48^{b}	5.44^{c}	2.22^{c}	1.49^{c}	10.37^{bc}	33.26^{ab}	9.89^{b}
N	18.99^{c}	48.20^{d}	53.59^{c}	13.02^{b}	156.83^{c}	5.18^{c}	2.21^{c}	1.47^{c}	9.97^{c}	31.48^{b}	9.03^{a}
RACA6	20.78^{b}	55.64^{b}	61.15^{b}	17.09^{a}	178.01^{b}	5.41^{c}	2.36^{b}	1.56^{bc}	10.99^{b}	33.35^{ab}	9.88^{b}
IRJ2180A	20.38^{b}	51.08^{cd}	58.84^{b}	13.45^{b}	167.07^{bc}	9.75^{a}	2.22^{c}	1.45^{c}	10.46^{bc}	30.96^{b}	9.46^{a}
Control	21.07^{b}	52.18^{bcd}	58.03^{bc}	14.83^{ab}	167.44^{bc}	5.90^{b}	2.46^{ab}	1.64^{ab}	11.06^{b}	34.09^{a}	9.48^{b}

Mean comparison is along each column, means with the same letter are not significantly different at $P<0.05$ level of probability according to DMRT. PLH= Plant height; NS= Number of stem; NB= Number of branches; LA= Leaf area; NOL= Number of leaves; TLL= Terminal leaf length; TLW= Terminal leaf width; PEL= Petiole length; CHPY= Chlorophyll; PL= Peduncle length

Table 4.9: Mean separation showing yield and nutrient uptake of accessions of Bambara groundnut Inoculated with *B. japonicum* strains at flowering and harvest in Ibadan and Ikenne in both seasons.

Accessions	N@Flw (%)	P@Flw (%)	N@Hvst (%)	P@Hvst (%)	Yield/plot (g)	Yield (kg ha^{-1})
TVSu-1739	2.09^{a}	0.19^{b}	1.67^{bc}	0.12^{b}	58.33^{ab}	784.3^{bcd}
TVSu-378	2.05^{ab}	0.21^{ab}	1.80^{a}	0.15^{a}	27.43^{de}	403.1^{def}
TVSu-305	2.03^{abc}	0.17^{d}	1.62^{cd}	0.12^{bc}	64.15^{ab}	825.2^{abc}
TVSu-710	$2.02^{a\text{-}d}$	0.17^{d}	1.68^{b}	0.12^{bcd}	30.06^{cde}	364.7^{ef}
TVSu-365	2.01^{bcd}	0.17^{d}	1.48^{f}	0.11^{d}	60.89^{ab}	812.7^{abc}
TVSu-1698	2.01^{bcd}	0.19^{bc}	1.53^{ef}	0.11^{d}	80.92^{a}	1205.5^{a}
TVSu-787	2.00^{bcd}	0.21^{a}	1.75^{a}	0.14^{a}	$39.99^{b\text{-}e}$	$669.6^{b\text{-}e}$
TVSu-475	1.99^{bcd}	0.17^{cd}	1.52^{ef}	0.11d	56.31^{b}	871.1^{ab}
TVSu-506	1.96^{cd}	0.19b	1.56^{ed}	0.12^{bcd}	42.42^{bcd}	$523.3^{c\text{-}f}$
TVSu-1606	1.95^{d}	0.21^{a}	1.53^{ef}	0.11^{cd}	51.08^{bc}	$733.5^{b\text{-}e}$

Means with the same letter are not significantly different at 5% level of probability using DMRT. N= Nitrogen; P= Phosphorus; Flw= Flowering; Hvst= Harvest; @= at

Table 4.10: Analysis of variance (ANOVA) showing growth traits of Bambara groundnut accessions inoculated with *B. japonicum* strains at 12WAP in Ibadan in both seasons

Source of Variation	Df	PLH (cm)	NB	NS	LA (cm^2)	NOL	TLL (cm)	TLW (cm)	PEL (cm)	CS (cm)	CPHY
Accessions	9	167.87**	5288.28**	6467.56**	643.31**	53917.39**	246.82**	2.446**	3.14**	39.67**	521.89**
Treatments	5	27.75**	1318.84**	1688.89**	297.70^{ns}	19014.32**	292.28**	0.269*	0.16^{ns}	8.26^{ns}	63.14^{ns}
Rep	2	41.91^{ns}	1771.18**	2580.95**	188.34^{ns}	23401.09**	1.263**	0.04^{ns}	0.67**	13.84^{ns}	350.17**
Seasons	1	25.71^{ns}	2543.77**	5813.20**	661.83^{ns}	41874.93**	353.42**	0.94**	0.15**	33.72**	548.64**
Accessions*treatments	45	11.66**	400.74**	577.39**	504.47**	5471.75**	187.47**	0.169**	0.30**	15.26**	116.71**
Accessions*seasons	9	16.29**	2543.77**	2643.39**	571.13**	26447.79**	202.12**	0.35**	0.76**	20.06**	202.46**
Treatments*seasons	5	46.38**	837.43**	796.00**	838.83**	9727.58**	307.08**	0.73**	0.65**	19.93**	62.82^{ns}
Accession*treatment*season	45	22.14**	386.32**	842.51**	632.14**	29112.64**	211.07**	0.32**	0.85**	26.61*	214.81*

Significant, ** very significant, ns- non significant, PLH- Plant height, NS-Number of stem, NB-Number of branches, LA-Leaf area, NOL-Number of leaves, TLL-Terminal leaf length, TLW- Terminal leaf width, PEL- Petiole length, CS- Plant spread, CHPY- Chlorophyll.

Table 4.11: Analysis of variance (ANOVA) showing growth traits of Bambara groundnut accessions inoculated with *B. japonicum* strains at 12WAP in Ikenne in both seasons

Source of Variation	DF	PLH (cm)	NS	NB	LA (cm^2)	NOL	TLL (cm)	TLW (cm)	PEL (cm)	CS (cm)	CPHY
Accession	9	262.54**	1708.93**	1762.92**	149.66**	15570.76**	15.31**	1.12**	5.18*	42.71**	241.56^{ns}
Treatment	5	13.81^{ns}	259.01*	255.10^{ns}	2.71^{ns}	2877.02**	0.29^{ns}	0.22^{ns}	0.15^{ns}	2.59^{ns}	150.36^{ns}
Rep	2	40.13*	1308.62**	1539.24**	5.50^{ns}	7235.08**	1.22^{ns}	0.139^{ns}	0.35^{ns}	1.23^{ns}	277.05^{ns}
Season	1	165.92**	6214.81**	7197.75**	434.97**	59359.90**	71.48**	0.84**	0.05^{ns}	78.75**	4276.27**
Accessions*treatment	45	10.81^{ns}	257.94**	249.84**	14.04^{ns}	2351.33**	1.21**	0.14^{ns}	0.18^{ns}	2.73^{ns}	104.42^{ns}
Accessions*seasons	9	82.73**	996.22**	1270.77**	70.05**	11984.91**	7.07**	0.74**	0.28^{ns}	13.76**	141.54^{ns}
Treatments*seasons	5	18.24^{ns}	147.23^{ns}	197.77^{ns}	38.81**	1176.80^{ns}	1.87^{ns}	0.39**	0.26^{ns}	7.06^{ns}	166.71^{ns}
Accession*treatments*seasons	45	25.60**	341.24**	645.81**	164.32**	2612.43**	10.24*	2.86*	0.54^{ns}	6.83*	121.33^{ns}

*Significant, ** very significant, ns- non significant, PLH- Plant height, NS-Number of stem, NB-Number of branches, LA-Leaf area, NOL-Number of leaves, TLL-Terminal leaf length, TLW- Terminal leaf width, PEL- Petiole length, CS- Canopy spread, CHPY- Chlorophyll, GH-Growth Habit, PL- Peduncle length

4.7.1 Rhizobial population of the soil used for both Pot and field experiments

The non-sterilized soils used in the screenhouse experiments, based on microbial analysis, indicated the presence of low rhizobia population during the 2018 season (1.28×10^2), and a high population in 2019 (1.53×10^9) season (Table 4.2). However, in the field used in Ikenne, rhizobia population were high i.e., 2.03×10^9 cfu/ml and 2.47×10^7 cfu/ml in 2019 and 2020 season, respectively. In Ibadan field, the rhizobial population were also high i.e., 1.83×10^5 cfu/mL and 3.05×10^7 cfu/mL in 2019 and 2020 season, respectively (Table 4.2).

4.7.2 Growth traits and nutrients uptake of inoculated accession under screenhouse conditions

At 10 weeks after planting (WAP), analysis of variance (ANOVA) indicated significant differences among accessions, soil status (sterile and non-sterile soil) and seasons in the growth traits recorded (Table 4.3). These significant differences among the accessions, soil status, and seasons were due to the inoculation of *B. japonicum* strains to Bambara groundnut accessions while non-significant differences were recorded among the treatments (strains) inoculated in all the growth traits under screenhouse conditions. The non-significant differences recorded among treatments inoculated under screenhouse conditions showed that the *B. japonicum* strains were nearly equal in performance. Furthermore, significant difference were not recorded for various interactions in Table 4.3 for most of the growth traits except chlorophyll content.

Analysis of variance (ANOVA) indicated that interaction effects of accessions and seasons had significant effects on the number of stem (NOS) and number of leaf (NOL)). Likewise, interaction of strains and seasons, accessions × strains × soil status × season had significant effects on NOS and NOL of Bambara groundnut (Table 4.3). ANOVA indicated that *B. japonicum* strains had significant effects on Bambara groundnut accessions on days to 50% flowering, and nutrient uptake percentage nitrogen and phosphorus at flowering and at harvest (Table 4.4). Moreover, non-significant differences were recorded among the strains inoculated in the number of days to 50% flowering, percentage phosphorus uptake at flowering, and at harvest under screenhouse conditions (Table 4.4). However, significant differences were recorded among the strains inoculated regarding nitrogen uptake at flowering and at harvest under screenhouse conditions due to the inoculation of *B. japonicum* strains (Table 4.4). Moreover, significant differences were also recorded in P_{flw} and P_{Hvst} in the soil status and season due to the inoculation of *B. japonicum* strains. Nevertheless, in the interactions of accessions with soil status, strains with soil status, and accessions with strains, soil status and season had non-significant effects on nutrients uptake recorded at 50% flowering under screenhouse conditions (Table 4.4). However, significant differences were also recorded regarding interaction of accessions with treatment on number of days to 50% flowering, accessions with season on the P uptake at flowering, and strains with season on nitrogen uptake at flowering and at harvest (Table 4.4). Table 4.5 revealed that inoculation of *Bradyrhizobium japonicum* strains to Bambara groundnut in the non-sterile soil significantly enhanced the growth traits and the nutrient uptake in flowering and at harvest stage than in the sterile soil. Also, the growth traits and the nutrients uptake of Bambara groundnut accessions were significantly enhanced in the first season than in the second season under screen conditions (Table 4.6).

4.7.3 Growth Traits, Nutrients Uptake and Yield of Inoculated Accessions on the Field

TVSu-1739 showed a higher significant difference in the plant height (25.53[a]) but not significantly different from TVSu-506 in plant height recorded. A higher significant difference was also recorded in TVSu-1739 in terminal leaf length (13.89[a]), terminal leaf width (2.75[a]), and petiole length (2.29[a]) compared to other inoculated Bambara groundnut accessions in both locations and seasons (Table 4.7). Likewise, TVSu-378 showed a higher significant difference in the number of branches, number of stems and number of leaves per plant in both locations and seasons compared to other inoculated Bambara groundnut accessions. Moreover, TVSu-506 and TVSu-305 showed higher significant difference in the leaf area and chlorophyll contents respectively (Table 4.7). The growth traits of inoculated Bambara groundnut accessions were best recorded in TVSu-1739, TVSu-378, TVSu-506 and TVSu-305 across locations in both seasons (Table 4.7).

Analysed data indicated that different *B. japonicum* strains inoculation had significant effects on the entire growth-related traits of Bambara groundnut on the field in both locations and seasons. Among different strains tested, FA3 had the highest plant height, number of branches and stems/plant, leaf area, terminal leaf length and width, number of leaves/plant and chlorophyll contents compared with all other strains and controls (Table 4.8).

Inoculation of different *B. japonicum* strains significantly improved the nutrients uptake and yield of inoculated accessions of Bambara groundnut. Higher nitrogen contents were recorded at flowering and harvest in TVSu-1739, TVSu-378, and TVSu-787 accessions respectively in Ibadan and Ikenne in both seasons and were significantly higher than other inoculated accessions of Bambara groundnut (Table 4.9). Similarly, high P uptake were recorded at flowering and at harvest in TVSu-787 and TVSu-1606, TVSu-378 and TVSu-787 respectively (Table 4.9). Among the inoculated accessions, higher yield and yield components were recorded by TVSu-1698 (1205.5

kg ha^{-1}) compared to other inoculated accessions at both locations and seasons due to the inoculation of *B. japonicum* strains that enhanced the yield components (Table 4.9). Significant differences were recorded among accessions in all the growth recorded in Ibadan in both seasons. Significant differences were also recorded in the treatment, replicate and season in some growth traits excluding the canopy spread, leaf area and petiole length which are not significantly different (Table 4.10). Furthermore, significant differences were recorded in the interaction of accessions with treatments, accessions with seasons and the interaction of treatments with seasons in the growth traits recorded in Ibadan in both seasons (Table 4.10). Also, significant differences were recorded among accessions and seasons in the growth traits in Ikenne in both seasons (Table 4.11). No significant differences were recorded in some growth traits in the treatments and replicates except in the number of stem and branches that showed significant differences in Ikenne in both seasons (Table 4.11). Significant differences were recorded in the growth traits in the interaction of accessions with treatment, accessions with seasons, but no significant differences were recorded in the interaction of treatments with seasons in Ikenne in both seasons (Table 4.11). Significant differences were also recorded in the interaction of accessions with treatments and seasons in the growth traits in Ibadan and also in Ikenne (Table 4.10 and 4.11).

4.8 Discussion

In general, the results obtained in this study revealed the relevance of *B. japonicum* strains to the improvement of the growth traits and nutrients uptake of accessions of Bambara groundnut under screenhouse (sterile and non-sterile soil) conditions. Also, inoculation of *B. japonicum* strains enhanced growth traits, nutrients uptake, and yield of accessions of Bambara groundnut in the field in both locations and seasons over the nitrogen fertilizer application.

Significant differences were recorded among the inoculated accessions in the growth traits and nutrients uptake in the screenhouse, and on the field on yield components in both locations and seasons. Among the *B. japonicum* strains inoculated to Bambara groundnut accessions, FA3 strains performed significantly better than other strains, nitrogen fertilizer applied, and uninoculated control which shows that significant differences exist among the *B. japonicum* strains. The results obtained in this study concurred with the result recorded by Yoseph *et al*. (2017). In their report, cowpea inoculated with *Bradyrhizobium* strains on the field significantly enhanced the growth traits, nodulation, and yield of cowpea when compared with the uninoculated control.

Furthermore, the nutritional benefit recorded in both studies was a result of easy mobilization of nutrients from the soil by the strains to a point where the root could intercept the nutrients for optimum utilization for growth and development. The result obtained on nutrition is similar to the findings of Biswas *et al*. (2000) which revealed that inoculation with rhizobia resulted to larger amount of nitrogen uptake when compared with the uninoculated control. A superior performance was recorded among inoculated accessions of Bambara groundnut, as the response to the *B. japonicum* strains varies probably due to genetic compositions of accessions that differ. The variability recorded among inoculated accessions of Bambara groundnut correlates with the findings of Nyoki and Ndakidemi (2014b) where varying responses were observed among varieties of soybean that were inoculated on the field.

It was apparent from the responses obtained in this study that inoculation of FA3 strains mostly supported the growth, nutrition, and yield of accessions of Bambara groundnut. FA3 strains performed better compared to other *B. japonicum* strains inoculated, nitrogen fertilizer applied, and uninoculated control in Ibadan and Ikenne in both seasons. The results obtained correlates with that of Kaschuk *et al*. (2016) in which neither basal nor topdressing of nitrogen application improved yields of determinate and indeterminate soybean cultivars, but the sole inoculation of *Bradyrhizobium* strains were enough to supply all nitrogen required by soybean plants. The results of this study also agrees with the research conducted by Hungria and Mendes (2015) that embraced rhizobia inoculation and zero nitrogen fertilizer application to enhanced legume production.

The findings in this study revealed that the inoculation of the *B. japonicum* strains can help to improved the growth, nutrient uptake and yield of Bambara groundnut with reduced cost as against the use of inorganic nitrogen fertilizers which is very expensive to procure and often contaminates the soil after use (Cordeiro and Echer (2019). Also, in another study conducted where five cowpea varieties with five *Bradyrhizobium* strains isolates were inoculated, the result revealed that *Bradyrhizobium* strains inoculation improved the growth, biomass and nodulation performance of the varieties of cowpea, according to Ayalew and Yoseph (2020).

4.9 Conclusions

Results of this study showed that inoculation of *B. japonicum* strains can help to improve the growth traits, nutrients uptake and yield of Bambara groundnut accessions under screenhouse and field conditions. Resultantly, it can reduce reliance on inorganic nitrogen fertilizer which most tropical farmers cannot afford and for those that can, apply at a low rate. Much effort is needed to introduce the inoculant to the farming community. It is, therefore, important to introduce and recommend the cheap and friendly technology to the poor farmers' communities of the nation. FA3 strain, therefore, can be introduced to the farmers to enhance optimum production of Bambara groundnut.

CHAPTER FIVE

Effects of *Bradyrhizobium japonicum* Strains and Inorganic Nitrogen Fertilizer on the Growth and Yield of Bambara Groundnut (*Vigna subterranea (L)* Verdc*)* Accessions.

Abstract

This study was carried out to compare the inoculation of *Bradyrhizobium japonicum* strains and the application of nitrogen fertilizers (urea with 46% nitrogen) on the growth and yield of Bambara groundnut accessions. The study results suggest the benefits of *Bradyrhizobium japonicum* (*B. japonicum*) strain inoculation. Field studies were conducted in two different geographical locations, Ibadan and Ikenne in Nigeria, during the rainy season between August and December in 2019 and 2020. The experiment was arranged in a randomized complete block design (RCBD) in both locations and seasons. It had a 10 × 6 factorial arrangement with one block holding the 10 accessions of Bambara groundnut inoculated with four *B. japonicum* strains. The second block had nitrogen fertilizer application and the third control block was without inoculation or fertilizer application. The 10 accessions of Bambara groundnut used in the study were: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475 and TVSu-305. Six seeds of each accession were coated with each of the four *B. japonicum* strains, namely FA3, USDA110, IRJ2180A and RACA6, before planting them in the field in both locations. In the next block, urea as nitrogen fertilizer (46% nitrogen) was applied to the uninoculated seedlings of accessions of Bambara groundnut 2 weeks after planting (WAP). The third block was the control with zero inoculation and zero fertilizer application. Data collected were subjected to an analysis of variance and mean were separated using Duncan's Multiple Range Test (DMRT) at a $p > 0.05$ level of probability. It was found that FA3 inoculation significantly enhanced the growth traits of the accessions than other strains and nitrogen fertilizer application.

In both locations and seasons, at 7 weeks after planting (WAP) and 12 WAP, plant height (19.54 and 22.71 cm), number of branches (33.63 and 62.77), number of leaves (116.54 and 209.25), terminal leaf length (5.62 and 6.00 cm) and width (2.09 and 2.56 cm) were recorded. The yield and yield components recorded at harvest were as follows: pod length (13.27 cm), pod width (9.08 mm), seed length (9.39 mm), seed width (6.92 mm), weight of 100 seeds (56.85 g), and yield/ha (750.72 kg). These values are recorded from the highest performing accessions. The yield and yield components were also significantly influenced by the inoculation of FA3 and RACA6 than other inoculated strains and nitrogen fertilizer application in both locations and seasons.

Keywords- Underutilised legume, inorganic fertilizer, inoculation, plant traits, bacteria strains.

5.1 Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is a leguminous crop usually cultivated in sub-Saharan Africa and some parts of Asia. It can thrive in all kinds of soil, especially in marginal soil (Mandizvo and Odindo 2019; Mayes *et al*. 2019; Soropa *et al*. 2019). *Bradyrhizobium* usually establishes mutual symbiosis with legumes by creating root nodules and fixing atmospheric nitrogen for the host plant and can be used as an inoculant for improving crop productivity (Jaiswal and Dakora 2019b). Also, inexpensive microbial inoculants help in improving plant growth, reducing heavy metal contaminations and controlling phyto-pathogens to enhance sustainable agriculture (Fasusi and Babalola 2021).

However, bacteria strains used in inoculation may not dominate during nodule formation due to competition with indigenous strains in the crop field (Suzuki *et al*. 2014). On the other hand, applying nitrogen fertilizers helps to improve the nutrition of the plant (Islam *et al*. 2018). It is also very essential for optimum growth and development and a constituent of various organic compounds such as protein, nucleic acid, and, hence, is part of protoplasm. It encourages rapid vegetative growth and regulates the utilization of phosphorus and potassium by the plant from the soil (Tyoakoso *et al*. 2019). Some agricultural practices which involved the use of agrochemicals are very harmful to human health due to their residue on food crops being consumed by humans (Enagbonma and Babalola 2019b).

The application of nitrogen fertilizer at the seedling stage usually increases the growth and yield of Bambara groundnut (Hasan *et al*. 2018c). However, its negative effect on the environment includes soil acidification and compaction (Cai *et al*. 2015), eutrophication of surface water (Zhang *et al*. 2011) and accelerating global warming by greenhouse gases emission (Battye *et al*. 2017).

Although urea is the most suitable source of nitrogen fertilizer recommended for Bambara groundnut (Hasan *et al*. 2019b), leguminous crops usually live in association with *Rhizobium* bacteria which helps to improve the soil status (Sarr *et al*. 2016).

Therefore, successful rhizobial inoculation in the field is usually measured by the extent of nodulation, nitrogen fixation (N_2), plant growth, and grain yield when compared with the uninoculated control (Kyei-Boahen *et al*. 2017b). They also play a vital role in the decomposition of organic matter, transformation of nutrients and regulation of soil productivity for plant growth and development (Oburger and Schmidt 2016). They also play a vital role in the decomposition of organic matter, transformation of nutrients, and regulation of soil productivity for plant growth and development (Ikenganyia *et al*. 2017b; Mahmood *et al*. 2016).

Farmers usually experience difficulty procuring inorganic nitrogen fertilizer due to its high cost and those who can afford it typically apply it below the recommended levels. Therefore, the aim of this study is to compare the effect of the inoculation of *B. japonicum* strains and nitrogen fertilizer application on the field performance of Bambara groundnut accessions.

5.2 Materials and Methods.

An experiment was carried out in 2019 and 2020 during the rainy season between August and December in two different geographical locations, Ibadan and Ikenne, in Nigeria. Ikenne can be characterized as a tropical savannah, with a latitude of 6° 51′ 56″ N and a longitude of 3° 42′ 54″ E while Ibadan can be characterized as sub-humid forest-savannah transition with a latitude of 7° 22' 30 N and a longitude of 3° 45' 54 E. The ten accessions of Bambara groundnut used in this study were randomly selected from the gene bank of the International Institute of Tropical Agriculture (IITA) while the *B. japonicum* strains were type strains readily available at the soil microbiology unit at IITA. The experiment was arranged in a randomised complete block design (RCBD) and was replicated three times in the field, with each block representing each replicate. The first block had accession seeds coated with the bacteria strains. The ten accessions were: TVSu-378, TVSu-506, TVSu-787, TVSu-1606, TVSu-1698, TVSu-1739, TVSu-710, TVSu-365, TVSu-475 and TVSu-305. Six sets of seeds of each accession were coated with each of the four *Bradyrhizobium japonicum* strains (FA3, RACA6, USDA110, and IRJ2180A) and with Arabic gum 20% (w/v) to attach the strain to the seed before planting.

The seeds were carefully dipped into the mixture of Arabic gum and *B. japonicum* strains, carefully stirred and allowed to stick firmly to the seeds, carefully spread and labelled to prevent mix up and allowed to dry properly before planting operation. The seeds coated with the bacteria strains were sown into a 2-m plot with 25 cm between each plot and 1 m between each replicate block.

In the second block, nitrogen fertilizer (20kg/ha) was applied to uninoculated seedlings at 2 WAP (Tyoakoso *et al.* 2019). The third block had the control which had no bacteria strain-coated seeds or seedlings administered with urea. Each block was identified with a plastic peg showing the accession number and bacteria strains and nitrogen fertilizer application. Data were collected on the percentage of germination after planting seeds at 2 WAP, on growth traits at 7WAP and 12 WAP in the flowering stage, and on yield and yield components at harvest in both locations and seasons.

5.2.1 Land Preparation: The fields were prepared mechanically using a tractor-driven plough in both locations carefully pulverised and harrowed to remove debris. Afterwards, plots of 2 m with spacing of 25 cm between the plot and 1 m between the blocks were prepared.

5.2.2 Arabic gum Preparation: A total of 20 g of Arabic gum was suspended in 100 ml of hot sterile water (22°C) to enable the Arabic gum to dissolve instantly. It was then mixed with 10 g of peat inoculant of *B. japonicum* strains, which was eventually used to inoculate 1 kg of the accessions of Bambara groundnut (Ogbuehi 2020).

5.2.3 Fertilizer Application

Urea was applied to seedlings at 2 WAP to compare the effect of the nitrogen-fixing bacteria, *B. japonicum* strains with the urea. Approximately 20 kg N ha^{-1} of urea was applied to each accession of the Bambara groundnut (Tyoakoso *et al*. 2019) and planted separately in the field without bacteria inoculation.

5.2.4 Weeding Operation

Weeding of the plot was carried out using chemical herbicides before their pre-emergence (lifeline 3 L/ha and metaforce 2 L/ha according to manufacturers' instructions) and manually using a hoe to weed at 6 WAP and 10 WAP to ensure optimum yield.

5.2.5 Insecticide Application

Spraying of insecticides was carried out as well as post-emergence using Karate and Termex, 1 L/ha in accordance to manufacturers' instructions in both locations and seasons to control whiteflies.

5.2.6 Data Collection

Data on percentage germination and growth parameters at 7 WAP and 12 WAP, which include plant height (cm), number of leaves, number of stems, plant spread (cm), number of branches, leaf area, terminal leaf length (cm), terminal leaf width (cm), chlorophyll content of leaf (mg/L), number of days to 1st flowering and to 50% flowering and fresh and dry shoot at 50% flowering and at harvest were collected in both locations. Additionally, the number of nodules, the weight of nodules (g) and the size and shape of nodules were also recorded. At harvest, the number of pods, the weight of pods (g), pod length (g), pod width (g), seed length (g), seed width (g), yield/plot (g), yield/ha (kg/ha) and weight of 100 seeds were further recorded.

5.2.7 Data Analysis

Data collected were subjected to a four-way analysis of variance (ANOVA) and Duncan Multiple Range Test at $p>0.05$ was used for treatments comparison.

5.2.8 Crop Establishment

Data were recorded for days to first flowering and days to 50% flowering. Data on canopy spread and days to pod formation were equally recorded.

Figure 5.1: Pods of Bambara groundnut after harvesting from the field.
Photo: T.D Bitire, 2020.

5.3 Results

Table 5.1: The influence of the six treatments on growth at 7WAP and floral parameters of the accessions of Bambara groundnut in Ibadan and Ikenne in 2019

Treatment	% GM	PLH (cm)	NOL	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	GH	D50% Flw	D1st Flw	PL (cm)	PEL (cm)	CS (cm)	LA (cm^2)
FA3	56.17^{d}	19.89^{a}	118.97^{ab}	35.41^{a}	39.83^{ab}	5.28^{a}	2.17^{a}	43.52^{a}	2.08^{a}	54.97^{bc}	41.63^{a}	7.74^{a}	1.77^{a}	10.44^{a}	11.79^{a}
USDA110	58.33^{cd}	19.66^{ab}	111.20^{b}	32.22^{b}	37.78^{b}	5.51^{a}	2.13^{a}	44.86^{a}	2.12^{a}	55.43^{ab}	42.54^{b}	7.91^{a}	1.67^{a}	10.70^{a}	12.38^{a}
N (fert)	63.75^{ab}	19.53^{ab}	129.36^{a}	37.89^{a}	43.52^{a}	5.37^{a}	2.13^{a}	44.85^{a}	2.14^{a}	54.71^{bc}	42.14^{b}	7.72^{a}	1.72^{a}	10.52^{a}	11.74^{a}
IRJ2180A	67.32^{a}	19.88^{a}	120.93^{ab}	33.18^{b}	39.46^{ab}	5.26^{a}	2.28^{a}	44.50^{a}	2.11^{a}	54.71^{bc}	41.82^{b}	7.61^{a}	1.82^{a}	10.48^{a}	12.24^{a}
RACA6	62.32^{bc}	19.69^{ab}	123.44^{ab}	36.14^{a}	42.04^{ab}	5.35^{a}	2.22^{a}	42.41^{a}	2.12^{a}	54.49^{c}	41.86^{b}	8.03^{a}	1.73^{a}	10.16^{a}	12.13^{a}
CONTROL	54.17^{d}	18.29^{b}	109.75^{b}	32.57^{b}	37.89^{b}	5.23^{a}	2.21^{a}	40.75^{a}	2.09^{a}	55.78^{a}	43.55^{a}	6.63^{b}	1.76^{a}	10.09^{a}	12.10^{a}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt). %GM –germination percentage, PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll, GH – growth habit, D50% Flw – days to 50% flowering, D1st Flw – days to first flowering, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting.

Table 5.2: The influence of the six treatments on the growth and biomass yield of the accessions of Bambara groundnut at 12WAP, in Ibadan and Ikenne, 2019

Treatments	PLH (cm)	CS (cm)	PEL (cm)	TLL	TLW	NOB	NOL	NOS	LA (cm^2)	CPYL (Mg/L)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.61^{a}	12.21^{a}	1.76^{a}	5.98^{b}	2.55^{a}	62.77^{a}	215.88^{a}	72.68^{a}	15.39^{b}	35.74^{a}	14.12^{a}	33.05^{a}	12.22^{a}	10.07^{a}	3.39^{a}
USDA110	21.68^{bc}	11.24^{b}	1.61^{a}	5.79^{b}	2.35^{b}	59.03^{a}	195.00^{a}	64.85^{b}	13.74^{b}	35.76^{a}	10.94^{b}	28.42^{a}	10.82^{a}	9.39^{a}	3.39^{a}
N (fert)	21.64^{bc}	11.39^{ab}	1.69^{ab}	5.90^{b}	2.46^{ab}	54.30^{b}	162.33^{b}	62.00^{b}	14.59^{b}	37.29^{a}	10.35^{b}	30.41^{a}	10.76^{a}	9.86^{a}	3.33^{a}
IRJ2180A	22.42^{ab}	11.53^{ab}	1.59^{b}	11.12^{a}	2.45^{ab}	56.59^{b}	190.77^{b}	63.59^{b}	14.89^{b}	36.40^{a}	10.73^{b}	31.59^{a}	11.71^{a}	9.69^{a}	3.48^{a}
RACA6	21.69^{bc}	11.62^{ab}	1.65^{ab}	5.69^{b}	2.47^{ab}	57.08^{b}	188.26^{b}	64.54^{b}	21.34^{a}	36.21^{a}	10.83^{b}	34.79^{a}	12.77^{a}	12.12^{a}	4.14^{a}
CONTROL	20.90^{c}	11.14^{b}	1.64^{ab}	5.84^{b}	2.44^{ab}	49.21^{c}	182.71^{b}	56.48^{c}	14.44^{b}	33.97^{a}	9.74^{b}	24.77^{b}	10.91^{a}	9.51^{a}	3.76^{a}

Means with the same letter are not significantly different at P<0.05 level of probability using the Duncan multiple range test (Dmrt). %GM –germination percentage, PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll, GH – growth habit, D50% Flw – days to 50% flowering, D1st Flw – days to first flowering, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting.

Table 5.3: The influence of the six treatments on growth and floral parameters of the accessions of Bambara groundnut at 7WAP in Ibadan and Ikenne in 2020

Treatment	% GM	PEL (cm)	PLH (cm)	NOL	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	D50% Flw	D1st Flw	PL (cm)	LA (cm^2)
FA3	78.17^{a}	1.43^{a}	19.62^{a}	116.10^{a}	38.45^{a}	38.92^{a}	6.06^{a}	2.06^{a}	42.37^{a}	54.93^{a}	45.83^{a}	7.99^{a}	12.56^{ab}
USDA110	80.38^{a}	1.42^{a}	19.63^{a}	118.32^{a}	39.13^{a}	39.68^{a}	5.99^{a}	2.08^{a}	43.73^{a}	49.03^{b}	44.18^{ab}	7.16^{a}	13.02^{a}
N (fert)	70.75^{a}	1.57^{a}	17.18^{b}	97.78^{b}	32.38^{b}	32.83^{b}	5.18^{b}	1.75^{b}	39.67^{a}	44.82^{c}	38.83^{c}	6.78^{a}	10.75^{b}
IRJ2180A	78.17^{a}	1.39^{a}	18.79^{ab}	114.88^{a}	37.04^{a}	38.30^{ab}	5.76^{ab}	2.04^{a}	43.78^{a}	52.95^{ab}	44.28^{ab}	6.72^{a}	12.24^{ab}
RACA6	77.00^{a}	1.41^{a}	18.01^{ab}	110.20^{ab}	36.30^{ab}	36.57^{ab}	5.48^{ab}	2.15^{a}	40.29^{a}	51.67^{ab}	41.63^{bc}	6.37^{a}	12.89^{a}
CONTROL	78.55^{a}	1.80^{a}	19.97^{a}	119.82^{a}	39.33^{a}	39.73^{a}	5.74^{ab}	2.13^{a}	43.80^{a}	54.87^{a}	43.27^{ab}	6.45^{a}	12.93^{a}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt). %GM – germination percentage, PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll content of leaf, GH – growth habit, D50% Flw – days to 50% flowering, D1st Flw – days to first flowering, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting.

Table 5.4: The influence of the six treatments on growth and biomass yield of the accessions of Bambara groundnut at 12WAP in Ibadan and Ikenne 2020

Treatment	PLH (cm)	PEL (cm)	TLL (cm)	TLW (cm)	NOB	NOL	NOS	LA (cm^2)	CPYL (mg/L)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.80^{a}	1.69^{a}	6.02^{b}	2.56^{a}	62.70^{a}	202.62^{a}	67.53^{a}	33.92^{a}	28.15^{a}	11.26^{a}	20.38^{a}	6.76^{ab}	6.21^{ab}	1.25^{ab}
USDA110	19.51^{c}	1.43^{bc}	5.29^{cd}	2.17^{c}	52.28^{b}	166.45^{b}	58.97^{a}	31.95^{a}	12.59^{d}	9.22^{ab}	19.42^{a}	6.73^{ab}	6.42^{ab}	1.16^{ab}
N (fert)	17.79^{d}	1.36^{c}	4.86^{d}	2.12^{c}	45.72^{c}	143.12^{c}	49.31^{b}	28.15^{b}	12.41^{d}	8.39^{b}	18.76^{a}	5.97^{ab}	6.08^{ab}	1.00^{ab}
IRJ2180A	21.71^{ab}	1.56^{ab}	10.06^{a}	2.37^{b}	54.05^{b}	171.98^{b}	63.63^{a}	30.99^{ab}	14.24^{bc}	9.81^{ab}	19.48^{a}	6.83^{ab}	5.98^{b}	1.28^{ab}
RACA6	20.95^{bc}	1.55^{ab}	5.42^{c}	2.37^{b}	57.05^{ab}	177.17^{b}	60.98^{a}	32.29^{a}	13.91^{c}	9.47^{ab}	23.19^{a}	7.55^{a}	8.08^{a}	1.37^{a}
CONTROL	21.25^{ab}	1.63^{a}	5.96^{a}	2.49^{ab}	55.15b	172.55^{b}	59.58^{a}	34.22^{a}	15.23^{a}	9.22^{ab}	17.34^{a}	5.13^{b}	6.66^{ab}	0.85^{b}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt). PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll content of leaf, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting. FSF – fresh shoot at flowering, DSF – dry shoot at flowering, FRF – fresh root at flowering, DRF – dry root at flowering.

Table 5.5: The influence of the six treatments on growth at 7WAP, and floral parameters of the accessions of Bambara groundnut Ibadan and Ikenne in both seasons

Treatment	%GM	PLH (cm)	NOL (cm)	NOB	NOS	TLL (cm)	TLW (cm)	CPYL (mg/L)	D1st Flw	D50% Flw	PL (cm)	PEL (cm)	CS (cm)	LA (cm^{2})
FA3	67.17^{a}	19.59^{a}	116.54^{a}	36.63^{a}	39.04^{a}	5.62^{ab}	2.09^{a}	42.22^{a}	43.38^{a}	54.95^{a}	7.80^{a}	1.58^{a}	10.74^{a}	12.08^{ab}
USDA110	69.36^{a}	19.47^{ab}	113.83^{a}	35.41^{a}	38.42^{a}	5.70^{a}	2.09^{a}	43.54^{a}	43.00^{ab}	51.31^{b}	7.47^{a}	1.53^{a}	10.65^{ab}	12.59^{a}
N (fert)	65.13^{a}	18.03^{b}	111.42^{a}	34.51^{a}	37.89^{a}	5.18^{b}	1.91^{b}	41.14^{a}	39.78^{c}	49.73^{c}	7.12^{ab}	1.62^{a}	9.72^{c}	11.05^{b}
IRJ2180A	70.50^{a}	18.84^{ab}	114.88^{a}	34.28^{a}	37.89^{a}	5.38^{ab}	2.10^{a}	43.03^{a}	42.00^{abc}	49.73^{b}	6.98^{ab}	1.56^{a}	10.37^{abc}	11.93^{ab}
RACA6	67.58^{a}	18.36^{ab}	113.73^{a}	35.32^{a}	38.25^{a}	5.28^{ab}	2.13^{a}	40.29^{a}	40.70^{bc}	51.72^{b}	6.99^{ab}	1.52^{a}	9.91^{bc}	12.20^{ab}
CONTROL	66.36^{a}	18.52^{ab}	111.13^{a}	34.87^{a}	37.55^{a}	5.31^{ab}	2.09^{a}	40.92^{a}	41.96^{abc}	55.33^{a}	6.32^{b}	1.72^{a}	10.05^{abc}	12.11^{ab}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt). %GM –germination percentage, PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll content of leaf, GH – growth habit, D50% Flw – days to 50% flowering, D1st Flw – days to first flowering, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting.

Table 5.6: The influence of the six treatments on growth at 12WAP, and biomass yield of the accessions of Bambara groundnut in Ibadan and Ikenne in both seasons

Treatment	PLH (cm)	CS (cm)	PEL (cm)	TLL (cm)	TLW (cm)	NOB	NOL	NOS	LA (cm^2)	CPYL (SPAD)	PL (cm)	FSF (g)	DSF (g)	FRF (g)	DRF (g)
FA3	22.71^{a}	12.21^{a}	1.73^{a}	6.00^{b}	2.56^{a}	62.73^{a}	209.25^{a}	70.10^{a}	15.74^{ab}	34.53^{a}	12.69^{a}	21.68^{a}	6.31^{a}	8.38^{a}	1.90^{a}
USDA110	20.23^{b}	10.37^{bc}	1.49^{c}	5.44^{b}	2.22^{c}	54.68^{b}	177.48^{b}	60.83^{b}	12.94^{b}	33.26^{abc}	9.89^{b}	19.53^{a}	6.46^{a}	7.73^{a}	1.72^{a}
N (fert)	18.99^{c}	10.37^{c}	1.47^{c}	5.18^{b}	2.20^{c}	48.20^{c}	156.83^{c}	53.59^{c}	13.02^{b}	31.48^{bc}	9.025^{b}	18.29^{a}	5.94^{a}	7.54^{a}	1.80^{a}
IRJ2180A	20.38^{b}	10.46^{bc}	1.46^{c}	9.75^{a}	2.22^{c}	51.08^{bc}	167.07^{bc}	58.84^{bc}	13.45^{b}	30.96^{c}	9.46^{b}	19.55^{a}	6.41^{a}	7.78^{a}	1.83^{a}
RACA6	20.78^{b}	10.99^{b}	1.56^{bc}	5.42^{b}	2.36^{b}	55.64^{b}	178.01^{b}	61.15^{b}	17.09^{a}	33.35^{abc}	9.88^{b}	20.65^{a}	6.45^{a}	8.39^{a}	1.77^{a}
CONTROL	21.07^{b}	11.07^{b}	1.64^{ab}	5.90^{b}	2.46^{ab}	52.18^{bc}	167.44^{bc}	58.03^{bc}	14.83^{ab}	34.09^{ab}	9.48^{b}	19.74^{a}	5.86^{a}	8.15^{a}	1.68^{a}

Means with the same letter are not significantly different at P<0.05 level of probability using the Duncan multiple range test (Dmrt). PLH – plant height, NOL – number of leaves, NOS – number of stems, NOB – number of branches, TLL – terminal leaf length, TLW – terminal leaf width, CPYL – chlorophyll content of leaf, PL – peduncle length, PEL – petiole length, CS – canopy spread, LA – leaf area, WAP – weeks after planting. FSF – fresh shoot at flowering, DSF – dry shoot at flowering, FRF – fresh root at flowering, DRF – dry root at flowering.

Table 5.7: Effects of the six treatments on yield and yield components of accessions of Bambara groundnut at harvest in Ibadan and Ikenne in 2019

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield /Plot (g)	yield/ha (kg)	Pod /Plant	Pod/plot (g)	Wgt of pod/plot (g)	Seed/ plant	Seed/ plot	Seed wgt/plot (g)	Shelling %
FA3	17.12^{a}	11.49^{a}	12.64^{b}	9.23^{a}	55.79^{a}	70.25^{ab}	1401.8^{b}	29.39^{b}	100.25^{ab}	73.24^{ab}	27.23^{a}	93.89^{a}	41.61^{ab}	41.42^{ab}
USDA110	17.28^{a}	11.69^{a}	12.38^{b}	9.63^{a}	53.32^{a}	63.54^{b}	1367.7^{c}	27.65^{b}	95.20^{c}	67.62^{b}	27.01^{a}	96.13^{a}	38.14^{b}	44.49^{a}
N (fert)	17.85^{a}	12.02^{a}	13.59^{ab}	9.63^{a}	55.29^{a}	75.61^{a}	1394.9^{c}	31.26^{a}	109.39^{a}	73.71^{ab}	25.29^{a}	97.47^{a}	42.86^{ab}	42.86^{ab}
IRJ2180A	17.11^{a}	11.93^{a}	12.47^{b}	9.53^{a}	58.19^{a}	75.05^{a}	1459.9b	28.80^{b}	95.43^{c}	74.44^{ab}	27.61^{a}	88.68^{b}	42.38^{ab}	39.48^{b}
RACA6	16.89^{a}	11.78^{a}	13.39^{ab}	9.72^{a}	54.31^{a}	81.19^{ab}	1582.7^{a}	30.53^{a}	102.26^{ab}	86.08^{a}	27.74^{a}	96.72^{a}	44.66^{a}	45.38^{a}
CONTROL	17.34^{a}	11.76^{a}	14.78^{a}	9.85^{a}	55.19^{a}	64.41^{b}	1201.6^{d}	21.79^{c}	80.35^{d}	78.37^{ab}	28.47^{a}	70.82^{c}	38.99^{b}	41.89^{ab}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt).

Table 5.8: Effects of the six treatments on yield and yield component of accessions of Bambara groundnut at harvest in Ibadan and Ikenne in 2020

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield/plot (g)	yield/ha (kg)	Pod /Plant	Pod/plot (g)	Wgt of pod/plot (g)	Seed/ plant	Seed/ plot	Seed wgt/plot (g)	Shelling %
FA3	10.29^{a}	7.24^{a}	6.79^{a}	5.07^{a}	60.70^{a}	34.71^{ab}	125.04^{ab}	16.06^{a}	42.17^{ab}	32.02^{ab}	16.03^{a}	41.11^{a}	24.39^{ab}	26.79^{a}
USDA110	10.80^{a}	7.36^{ab}	6.66^{a}	5.01^{ab}	56.51^{ab}	43.85^{a}	130.59^{a}	12.88^{abc}	44.08^{a}	34.51^{a}	14.81^{ab}	42.91^{a}	27.10^{a}	23.20^{a}
N(fert)	9.74^{a}	6.30^{b}	5.78^{a}	4.23^{b}	45.05^{b}	25.04^{b}	118.58^{b}	9.26^{c}	28.65^{b}	22.23^{c}	10.97^{c}	27.48^{b}	16.01^{c}	24.51^{ab}
IRJ2180A	9.923^{a}	7.08^{b}	6.23^{a}	4.74^{ab}	54.84^{ab}	33.92^{ab}	106.56^{b}	12.15^{bc}	40.04^{ab}	28.29^{b}	12.99^{b}	39.26^{ab}	19.86^{bc}	23.24^{a}
RACA6	9.74^{a}	7.00^{b}	6.33^{a}	4.82^{ab}	56.46^{ab}	31.29^{b}	103.39^{b}	12.14^{bc}	37.12^{ab}	27.67^{b}	12.96^{b}	35.87^{ab}	20.49^{abc}	22.04^{b}
CONTROL	10.29^{a}	9.36^{a}	6.76^{a}	4.91^{ab}	42.09^{b}	26.69^{b}	80.47^{c}	14.19^{ab}	24.15^{c}	31.29^{ab}	6.50^{d}	20.62^{c}	23.61^{ab}	24.00^{ab}

Means with the same letter are not significantly different at P<0.05 level of probability using the Duncan multiple range test (Dmrt)

Table 5.9: Effects of the six treatments on yield and yield component of accessions of Bambara groundnut at harvest in Ibadan and Ikenne in both seasons

Treatment	Pod length (mm)	Pod width (mm)	Seed length (mm)	Seed width (mm)	100seed wgt (g)	Yield/plot (g)	yield/ha (kg)	Pod /plant	Pod/plot (g)	Wgt of pod/plot (g)	Seed/ plant	Seed/plot	Seed wgt/ Plot (g)	Shelling %
FA3	13.27^{a}	9.08^{ab}	9.39^{ab}	6.92^{ab}	56.85^{a}	50.72^{ab}	728.39^{ab}	21.99^{a}	68.70^{a}	50.79^{ab}	20.95^{a}	65.16^{a}	31.96^{a}	33.07^{b}
USDA110	13.18^{a}	8.94^{b}	8.90^{b}	6.84^{ab}	52.25^{ab}	50.52^{ab}	680.75^{b}	18.88^{ab}	64.88^{ab}	47.68^{ab}	19.56^{ab}	64.72^{a}	30.72^{ab}	31.62^{b}
N (fert)	12.01^{a}	8.26^{b}	8.67^{b}	6.21^{b}	46.03^{b}	44.66^{b}	633.07^{c}	17.91^{b}	63.73^{ab}	42.44^{b}	17.22^{b}	55.16^{b}	26.22^{b}	30.47^{b}
IRJ2180A	12.52^{a}	8.81^{b}	8.62^{b}	6.58^{ab}	53.12^{ab}	50.11^{ab}	698.06^{b}	18.79^{ab}	62.17^{b}	47.03^{ab}	17.67^{b}	58.79^{b}	28.65^{ab}	29.11^{c}
RACA6	12.33^{a}	8.70^{b}	9.07^{ab}	6.70^{ab}	52.22^{ab}	51.51^{a}	750.72^{a}	19.56^{a}	63.73^{ab}	51.86^{ab}	18.73^{ab}	60.65^{ab}	29.97^{ab}	31.06^{b}
CONTROL	13.39^{a}	10.27^{a}	10.40^{a}	7.135^{a}	47.26^{b}	48.44^{b}	533.82^{d}	12.20^{c}	54.97^{C}	32.87^{a}	11.78^{c}	46.78^{c}	25.07^{b}	41.90^{a}

Means with the same letter are not significantly different at $P<0.05$ level of probability using the Duncan multiple range test (Dmrt).

Table 5.10: Analysis of variance showing the sources of variations and degrees of freedom for seed and yield related traits of Bambara groundnut in both locations and seasons

Sources of variations	DF	Pod Length (mm)	Pod Width (mm)	Seed Length (mm)	Seed width (mm)	Wgt100 seed	Yield/plot (g)	yield/ha (kg/ha)	Pod/plot	Wgt pod/plot (g)	Seed/plot	Wgt seed/ Plot (g)
Accessions	9	157.89**	80.14**	66.29**	41.33**	11572**	19516.19**	4516451**	25275.49**	23518.6**	39987.39**	10103.8**
Treatments	5	37.89^{ns}	53.94**	52.68**	12.15*	1987.91*	2327.35*	565048.9*	3253.76**	1794.03*	2373.35*	1083.01*
season	1	4238.9**	1278.9*	5296.93*	2696.2^{ns}	6422.29^{ns}	201741.8**	2.6833**	505919.5**	272160.4**	414765.6**	52271.9**
Location	1	2966.4**	2231.0*	4875.5**	4066.9^{ns}	21817.9*	230306.6**	1.0923**	554858.6**	401441.3**	510664.9**	102848.9**
Rep	2	201.59**	43.00^{ns}	211.75**	43.02**	574.42^{ns}	693.39^{ns}	13.4^{ns}	360.86^{ns}	356.96^{ns}	7352.33^{ns}	160.69^{ns}
Accession*treatmt	45	26.61^{ns}	25.44^{ns}	21.94^{ns}	7.71^{ns}	759.33^{ns}	1665.43^{ns}	570363.7^{ns}	2968.66**	2138.72**	5237.34**	789.09^{ns}
Accesions*season	9	60.06*	35.15^{ns}	39.23^{ns}	17.50**	986.93^{ns}	6257.61**	3649903**	20459.03**	12296.21**	26870.41**	4297.76**
Accesion*location	9	47.89^{ns}	29.73^{ns}	41.97^{ns}	10.92^{ns}	510.95^{ns}	9438.24**	2705124**	15403.54**	10086.17**	28445.74**	4562.71**
Treatment*season	5	19.15^{ns}	17.53^{ns}	29.23^{ns}	2.77^{ns}	591.48^{ns}	3546.55^{ns}	473516.6^{ns}	2457.12^{ns}	1628.54^{ns}	1047.16^{ns}	900.31^{ns}
season*location	1	51.37^{ns}	1016.4*	125.96^{ns}	686.91*	8.01^{ns}	332521.6**	1.1322**	569157.2**	459783.1**	462964.6**	128785.2**
Ace*stra*sea*locat	131	20.41^{ns}	18.81^{ns}	18.43^{ns}	5.83^{ns}	445.15^{ns}	2296.82**	889695.4^{ns}	2822.56**	2493.77**	5679.69**	1005.93**

****P<0.01 Very significant, ***P<0.05 Significant, ns- not significant. Stra-strain, locat-location, sea-season.

5.3.1 Effects of Treatments Application on Growth Traits, Flowering Stages and Biomass Yield of Accessions of Bambara Groundnut.

Table 5.1 shows the comparison of the effects of *B. japonicum* strains and urea (46% N fertilizer) application on growth traits and flowering stages of inoculated accessions of Bambara groundnut at 7 WAP in Ibadan and Ikenne in the first season, 2019. As seen in Table 5.1, the IRJ2180A bacteria strain had a significantly higher germination percentage with a mean value of 67.32% compared to other inoculated strains, nitrogen fertilizers and control. Bacteria strains, FA3 and IRJ2180A, significantly enhanced plant height of the accessions with mean values of 19.88 and 19.89 cm, respectively, compared to all other treatments.

On the other hand, nitrogen fertilizer significantly enhanced the number of leaves and number of stems compared to other treatments. However, FA3 inoculated accessions and uninoculated control showed a significantly higher number of days to first flowering compared to other treatments. Significant differences were, however, observed in the number of branches among accessions in the treatments inoculated with FA3, RACA6 strains and with nitrogen fertilizer applied, compared to USDA110, IRJ2180A and the control.

5.3.2 Impacts of the Treatments on Growth Traits at 7WAP and 12WAP of Bambara Groundnut Accessions

The effects of *B. japonicum* strains and urea on the growth traits and biomass yield of Bambara groundnut accessions at 12 WAP revealed that the FA3 strain significantly enhanced plant height, canopy spread, petiole length, terminal leaf width, number of leaves, number of stems, and peduncle length compared to other treatments (Table 5.2). Similarly, the IRJ2180A strain significantly enhanced the terminal leaf length of the accessions of Bambara groundnut more than other bacteria strains, or nitrogen fertilizer applied and the control.

Furthermore, the number of branches was significantly enhanced by the inoculation with FA3, USDA110 and RACA6 compared to IRJ2180A inoculation, nitrogen fertilizer application and the control. Similarly, RACA6 significantly enhanced the leaf area compared to other strains and application of nitrogen fertilizer. However, there were no significant differences observed in the chlorophyll content of leaf, fresh and dry shoots and roots at flowering. The comparison of the effect of bacteria strains and urea application on growth traits at flowering at 7 WAP is shown in Table 5.3. At 7 WAP there were no significant differences in germination percentage, petiole length, chlorophyll content of leaf and peduncle length among the treatments considered in the study.

Similarly, there were no significant differences in the number of leaves, number of branches, and number of stems in all accessions barring those that were treated with RACA6 or urea. Plant height was significantly enhanced by FA3 and USDA110 strains compared to other bacteria strains. Accessions with nitrogen fertilizer application had the lowest mean value in plant height. Also, terminal leaf length was significantly enhanced by the inoculation with FA3 and USDA110 strains compared to IRJ2180A and RACA6 strains and the control. It was further noted that terminal leaf width and days to 50% flowering significantly decreased with nitrogen fertilizer application compared to the inoculation of bacteria strain and the uninoculated control.

5.3.3 Impacts of the Treatments on Growth Traits, Flowering, and Biomass Yield at 7 WAP and 12 WAP

Table 5.3 shows that FA3 significantly enhanced the number of days to the first flowering of accessions compared to other strains or control. Nitrogen fertilizer had the lowest mean value. Similarly, the growth traits and biomass yield at 12 WAP revealed that FA3 significantly enhanced plant height, petiole length, terminal leaf width, number of branches, number of leaves, chlorophyll content of leaf, and peduncle length more than other strains (Table 5.4). Nitrogen fertilizer recorded the lowest mean value on the growth traits. Also, RACA6 significantly enhanced biomass yield (dry shoot at flowering, and fresh and dry roots at flowering) compared to other strains, or when nitrogen fertilizer was applied or uninoculated control performed. The FA3 strain also significantly enhanced plant height while nitrogen fertilizer had the lowest mean value (Table 5.5).

Furthermore, USDA110 significantly enhanced terminal leaf length compared to other bacteria strains, nitrogen fertilizer applied and the uninoculated control. Table 5.5 further revealed that though there were no significant differences in terminal leaf length between inoculated accessions and uninoculated control in both seasons, there was a significant difference between the inoculated strains and nitrogen fertilizer-applied accessions. The peduncle length of the different accessions was significantly enhanced by FA3 and USDA110 inoculation in both seasons and the leaf area was significantly enhanced by USDA110 compared to nitrogen fertilizer. A comparison of the effects of the bacteria strains and nitrogen fertilizer on growth traits and biomass yield at 12 WAP across locations and seasons showed no significant differences. However, as seen in Table 5.6, FA3 significantly enhanced all growth traits at 12 WAP except for terminal leaf length and leaf areas, which were significantly enhanced by IRJ2180A and RACA6.

5.3.4 Impacts of Treatments on the Yield and Yield Components of Accessions of Bambara Groundnut

Significant differences were recorded in the seed length, yield/plot, yield/ha, pod/plant, pod/plot, weight of pod/plot, seed/plot, seed weight/plot, and shelling percentage at harvest among the different treatments in both locations in the first season (Table 5.7). The application of nitrogen fertilizer and inoculation with IRJ2180A were found to significantly enhance yield/plot at harvest. On the other hand, RACA6 strains significantly enhanced yield/ha, pod/plant, weight of pod/plot and seed weight/plot at harvest compared to other strains, nitrogen fertilizer application and the control in the first season.

Similarly, in both locations in the second season, significant differences were recorded in the pod width, seed width, 100 seed weight, yield/plot, yield/ha, pod/plant, pod/plot, weight of pod/plot, seed/plant, seed/plot, seed weight/plot and shelling percentage at harvest between accessions inoculated with bacteria strains, application of nitrogen fertilizer and uninoculated control. Inoculation with FA3 strain was found to significantly enhanced pod width, seed width, 100 seed weight, pod/plant and seed/plant at harvest compared to inoculations with other strains and application with nitrogen fertilizer in the second season. Yield/plot, yield/ha, pod/plot, weight of pod/plot and seed weight/plot at harvest were found to be significantly enhanced when inoculated with USDA110 strains (Table 5.8). Significant differences were also recorded in all other yield and yield components after harvest between accessions inoculated with bacteria strains, nitrogen fertilizer application, and the control across locations in both seasons. Interestingly, pod width, seed length, seed width, and weight of pod/plot at harvest were found to be significantly enhanced in the uninoculated control compared to inoculated accessions or those applied with nitrogen fertilizer (Table 5.9). The FA3 strain significantly enhanced 100 seed weight, seed/plant, and seed weight/plot of accessions at harvest in both seasons while the RACA6 strain significantly enhanced yield/plot and yield/ha. Among the bacteria strains, FA3, RACA6, and USDA110 significantly enhanced pod/plant and seed/plot of the various accessions. Table 5.10 provides a cumulative overview of the significant differences recorded among accessions inoculated with different *B. japonicum* strains in different seasons and locations.

Figure 5.2: Bambara groundnut establishment on the field in Ibadan

Photo: T.D Bitire, IITA 2019

5.4 Discussion

5.4.1 Response of Bambara groundnut accessions to inoculation and N fertilizer application

The differences in the impact recorded in the growth and yield of the Bambara groundnut accessions which were due to the inoculation of *B. japonicum* strains coated to the seeds before planting in both locations and seasons were similar to the result obtained by Sanginga *et al*. (1996b) and indicated that response to inoculation is likely to occur when the indigenous rhizobia population is less than 5 or 10 rhizobia cells g^{-1} soil.

Furthermore, significant differences observed in the biomass yield in this study show that *B. japonicum* strains enhanced the biomass yield more than the nitrogen fertilizer (Soe *et al*. 2010). This result agrees with the research conducted by Hungria and Mendes (2015a); (Hungria and Mendes 2015b) where, compared to nitrogen fertilizers, bacteria strains enhanced the growth traits and the biomass yield. The low yield recorded in the second season in both locations resulted from inadequate rainfall in both geographical locations—Ibadan and Ikenne. The study demonstrated that the growth traits, biomass yield, number of days to flowering and yield and yield components in Bambara groundnut accessions were significantly enhanced due to the inoculation of *B. japonicum* strains. This is in agreement with a study conducted by Solomon *et al*. (2012) which revealed that inoculation of *B. japonicum* enhanced nodulation, nitrogen fixation, and yield of soybean in Ethiopia. The results of our study will help farmers increase their profitability as the adoption of the use of bacteria strains will reduce the procurement of nitrogen fertilizers which are, according to Igiehon and Babalola (2018b), very expensive and lead to environmental contamination and adverse soil health.

5.4.2 Role of Bacteria Strains in Improving Legume Productivity

This study revealed the importance of the inoculation of bacteria strains (*B. japonicum*) over nitrogen fertilizers. The Bambara groundnut accessions in both locations inoculated with four bacteria strains in this study showed a significant difference in growth, yield and yield components compared to the nitrogen fertilizers applied. This indicates that the bacteria strains possessed a greater advantage over the nitrogen fertilizers. It is of paramount importance to introduce this technique to the end-users—the farmers—to improve their legume production and reduce the amount spent annually on inorganic fertilizers, which adversely affect the soil. The inoculation of bacteria strains to legumes is inexpensive and easily accessible.

5.4.3 Further Studies on Improving Legume Production by Means of Inoculation

In a research conducted in Chad and Cameroon, the inoculation of Bambara groundnut and groundnut in the field shows that inoculation contributed to the improvement of growth traits and biomass yield of the two legumes as well as their yield and yield components with a mean value of 63.73 kg/ha for groundnut and 72.71 kg/ha for Bambara groundnut (Gomoung *et al*. 2017), which is related to the results recorded in this study. In another study conducted in South Africa, inoculating *B. japonicum* and *Bacillus subtilis* strains on Bambara groundnut and cowpea revealed that co-inoculation of *B. japonicum* and *Bacillus subtilis* strains had the potential to improve the yield of both Bambara groundnut and cowpea (Nelwamondo 2020).

5.5 Conclusion

The bacteria strains inoculated in this study significantly influenced the growth traits, biomass yield, and yield traits of Bambara groundnut accessions during both seasons and both locations: Ibadan and Ikenne. The results revealed that it is important to promote the use of bio fertilizers over inorganic nitrogen fertilizers, which most farmers may not be able to afford due to their high cost, and those who can afford them usually apply nitrogen fertilizers below recommended levels. Rhizobia inoculants are readily available and farmers need to be informed about this new technique because the results of research such as this one tend to remain within the research community and do not reach the farmers. Therefore, more efforts are needed to disseminate new innovations to farmers in rural settlements and introduce this cheap and friendly technique to the poor farming community to improve the production of their legumes and increase their profit. From this study, the use of FA3, USDA110, and RACA6 can be recommended for Bambara groundnut increased production.

CHAPTER SIX

Variability in the Yield of Accessions of Bambara Groundnut (*Vigna subterranea (L) Verdc)* Inoculated with *Bradyrhizobium japonicum* Strains

Abstract

Field studies were conducted from August to December of the 2019 and 2020 agricultural seasons in two distinct geographic areas, Ibadan and Ikenne in Nigeria. Ten Bambara groundnut accessions: TVSu-378, TVSu-365, TVSu-1739, TVSu-475, TVSu-305, TVSu-506, TVSu-1606, TVSu-1698, TVSu-710 and TVSu-787 were obtained from the IITA gene bank. The following *B. japonicum* treatments: RACA6, FA3, IRJ2180A and USDA110 were applied to seeds from each Bambara groundnut accession before they were planted. Also, seedlings of Bambara groundnut accessions that did not receive *B. japonicum* strains at 2WAP were given nitrogen fertilizer (urea), as well as an uninoculated control (no inoculation and no fertilization). The experiment was a 10 x 6 factorial, containing 10 Bambara groundnut accessions and six (6) treatments (four B. *japonicum* inoculations. nitrogen fertilizer application and control).

The factorial arrangement was set up in Randomised Complete Block Design (RCBD) with three replication across locations and seasons. Bambara groundnut accessions, yield and yield components were significantly improved in both geographical locations and seasons. In particular, inoculation of *B. japonicum* strain RACA6 considerably increased the yield and yield component of TVSu-1698 with a value of 6234±86.70 kg ha^{-1} in both locations and seasons, compared to other Bambara groundnut accessions. In addition, the results also showed that FA3 and RACA6 strains considerably increased both the yield and yield traits of the inoculated accessions of Bambara groundnut when compared to other *B. japonicum* strains, nitrogen fertilizer treatment and uninoculated control. The study revealed that bacteria strains increased the grain yield of Bambara groundnut accessions.

Keywords- Inoculation, bacteria strains, underutilised legume, fertilizer, yield.

6.1 Introduction

Bambara groundnut is an underutilised and drought-tolerant legume (Olanrewaju *et al*. 2021). Compared to other leguminous crops, it can produce well and provide a good pod yield on marginal and water-stressed soil (Ajilogba *et al*. 2022). Bambara groundnut roots produce a beneficial effect when bacteria form symbiotic relationships with the roots because the bacteria absorb atmospheric nitrogen, trap it, convert it and make it available to the plants in the form that the plant can utilise and helps to raise the soil's nitrogen concentration (Babalola *et al*. 2017). Bambara groundnut roots are a source of nutrition for both people and animals, and they also aid in the treatment of nutritional disorders in both species (Olanrewaju *et al*. 2021).

Ecosystems and public health are at risk when chemical fertilizers are used to increase crop yields and improve soil fertility. Furthermore, some agricultural practices that use agrochemicals are particularly detrimental to human health because of the residue on food crops that people eat (Enagbonma and Babalola 2019b). Native rhizobial strains already present in the soil usually compete with exotic rhizobial strains introduced into the soil, so the survival rates of majority of the exotic rhizobial strains are frequently low to infect legume plants. Therefore, it is suggested that adequate inoculation may be necessary to achieve a better symbiosis (Thilakarathna and Raizada 2017). The use of inorganic nitrogen fertilizers on agricultural crops has been steadily rising throughout the last few decades and this is very detrimental. Although it was observed that applying nitrogen fertilizer at various rates increased the vegetative characteristics, nodulation, amino acid content and yield of Bambara groundnut root (Hasan *et al*. 2021) .

Additionally, the use of biofertilizer in the agricultural bio-economy can be optimized to ensure the sustainability of food production and can be incorporated into crop-breeding plans (Fasusi *et* *al*. 2021; Uzoh and Babalola 2018). However, the use of biofertilizer in agriculture is generally regarded as safe and environmentally beneficial and it can replace agrochemicals without having any adverse effects on the ecosystem (Fasusi *et al*. 2021; Glick 2020). As a result, several studies hypothesised that native rhizobial strains would be better suited to environmental stress conditions (low or high temperature) than exotic rhizobial strains. Therefore, the potential for local commercialization of native rhizobia may exist (Thilakarathna and Raizada 2017). Inoculating with *Bradyrhizobium* strain considerably improved groundnut pod yield in a study when compared to the uninoculated control, increasing it by 13% to 40% (Asante *et al*. 2020). In a different investigation on cowpea and peanuts, two rates of nitrogen fertilizer (20 kg N/ha and 40 kg N/ha), five rhizobia strains, and an uninoculated control were used in a different investigation. Results showed that rhizobial inoculation and nitrogen fertilizer application increased nodulation potential, biomass production, nodule dry weight, pod number, pod yield, pod weight and hundred seed weight of groundnut compared to the uninoculated control. In comparison to the uninoculated control, rhizobial inoculation generally boosted nodulation potential and pod yield by 63% and 67% respectively. In comparison to the uninoculated control, increase in nodulation and pod output were recorded in nitrogen fertilizer (20 kg N/ha) by 24% and 25%, respectively (Mintah *et al*. 2020).

In conclusion, the use of biofertilizers must be introduced to rural farmers and adequate training on how to apply them must be disseminated to the end users, as it has no residual effect on the soil. With easy affordability and increments of farm output, farmers can easily embrace the discovery. The aim of this study, therefore, is to examine the impact of *B. japonicum* strains on yield and yield components of Bambara groundnut accessions.

6.2 Materials and Methods

6.2.1 Field Experiments

Experiments were conducted on the field at two locations: Ibadan and Ikenne from August to December in the 2019 and 2020 planting seasons in Nigeria. Ikenne can be characterized as a tropical savanna, wet. Latitude (Lat) 6° 51' 56'' North and Longitude (Long) 3° 42' 54'' East with a temperature of 22.5°C-29.5°C. Ibadan fall in sub humid region, lat. 7° 30 North and long 3° 45 54 East with a temp of 21°C to 30.5°C. The fields were prepared using a tractor-driven plough and carefully harrowed to remove plant debris, and 2 m long plots were made (row) with 25 cm spacing between each plot and 1 m spacing between each rep. The fields were arranged in a random complete block design (RCBD) at both locations and seasons. Ten Bambara groundnut accessions were randomly selected from early maturing accessions at the IITA gene bank: TVSu-365, TVSu-787, TVSu-506, TVSu-1698, TVSu-710, TVSu-1606, TVSu-378, TVSu-475, TVSu-1739, and TVSu-305. Four *B. japonicum* strains inoculated in the study are: FA3, RACA6, USDA110, and IRJ2180A, containing $2.8x10^{7}$, $7.2x10^{6}$, $4.3x10^{7}$ and 1.4x107cfu/ml respectively. Two weeks after planting, nitrogen fertilizer 20 kg ha^{-1} was applied to the seedlings of accessions of Bambara groundnut that were not coated with *B. japonicum*, and an uninoculated control (no inoculation and no fertilizer application) was used. Each of the Bambara groundnut accessions had six rows in a block (4 rows for *B. japonicum* strains, 1 row for N fertilizer, and 1 row for uninoculated control), making a total of 60 rows in a block. This was replicated three times (180 rows).

The *B. japonicum* strains were grown on yeast extract mannitol agar (YEMA) (Bikrol *et al*. 2010), and were maintained on Congo red yeast extract mannitol agar (CRYMA) for 3 days at 28°C (Bikrol *et al*. 2010). The strains were selected based on authenticity, pot experiment in the growth room at 32°C to examine the potential of the selected strains which involves planting in 2kg pot containing sterile soil and Bambara groundnut seeds were sown into the pot in four replicates. After emergence of the seedlings, six different strains FA3, R25B, IRJ2180A, USDA110, RACA6 and IRJ2123 were inoculated as broth to the root of the seedlings. At flowering, plant samples were terminated to examine the nodulation potential of the strains and strains that were able to nodulate were selected for further studies in the glasshouse and on the fields in two different locations in Nigeria. Seeds of each Bambara groundnut accessions were coated with the strain using Arabic gum, carefully labelled to avoid mix-up and allowed to dry with the seeds according to Nodumax, IITA before planting on the field at both locations. Regular weeding was done manually. After harvesting, pods were preserved in the drying room and characterization was done on the seeds. Data were subjected to a four-way analysis of variance using the Statistical Analysis System (SAS) package and means of treatments were separated using the Ducan Multiple Range Test (DMRT) at $P \leq 0.05$. The fields used for this study (Ibadan and Ikenne) do not have any previous records of inoculation of *Bradyrhizobium* or previous cultivation of Bambara groundnut. The experiment were not repeated in the same spot in the second season in both locations but were sited few metres apart from the first season experiment to avoid residual contamination from previous inoculation from the experiment of the first season.

6.2.2 Data Collections on Yield

Data were collected from the harvested pod as described by the descriptor of Bambara groundnut, International Plant Genetic Resources Institute (IPGRI), Rome (Italy), IITA, and International Bambara groundnut Network (BAMNET).

6.2.3 Geographical Origin and Source of *B. japonicum* Strains Used in the Study

B. japonicum strains	Geographical origin	Source	Reference	Legumes
USDA110	Florida. USA	USDA	(Bai *et al*. 2003)	Soybean and Bambara groundnut
FA3	Cameroun	IRAT	(Brunel *et al*. 1988)	Soybean and Bambara groundnut
IRJ2180A	Nigeria	IITA	(Sanginga *et al*. 1996a)	Soybean and Bambara groundnut
RACA6	Nigeria	IITA	(Okogun and Sanginga 2003)	Soybean and Bambara groundnut

6.2.4 Method of Soil Collection

Soil samples were collected from both geographical locations and seasons with the aid of a soil auger in triplicate. After collection, soil samples were carefully labelled to prevent mix up, sieved using 2 mm sieve and air dried at 32°C before subjection to further analysis.

6.2.5 Method of Analysis

The percentage of organic carbon was analysed following the procedure of (Walkley and Black 1934), The percentage of nitrogen was determined using the Kjedahl method according (Mulvaney and Page 1982). Available phosphorus analysis was carried out as described in Bray's method (Carter and Gregorich 2007). The Exchangeable Sodium (Na), Calcium (Ca), and Potassium (K) was determined by ammonium acetate (Black *et al*. 1965).

6.2.6 Estimation of Yield Components

$$N = \frac{X}{Y} \times L$$

$$P = \frac{N}{Y}$$

$$H = \frac{W \times 10,000}{A}$$

$$S = \frac{T - G}{T} \times 100 \qquad \text{(Leonard 1980)}$$

Where:

N = yield/plot

X = number of seeds planted per plot

Y=number of harvested shoot per plot

L = weight of harvested seed per plot (g).

P = Yield/plant.

H = Yield/ha.

W = Seed weight/plot (kg).

A = Area of the plot (m^2)

S = Shelling %

T = Pod weight

G = seed weight.

1 hectare= 10,000m^2

Figure 6.1: Determining suitability of the *B. japonicum* strains, used in the study

Photo: T. D Bitire, 2018

6.3 Results

Table 6.1: Analysis of variance showing the yield and yield components of Bambara groundnut accessions inoculated with *B. japonicum* strains in Ikenne, 2019 and 2020

			Ikenne			**2019**							**2020**			
Source of variation	**Df**	**Pod lgt (mm)**	**Pod wdt (mm)**	**Seed lgt (mm)**	**Seed wdt (mm)**	**Wgt 100seeds (g)**	**Yield/ Pt (g)**	**Yield/ha (kg)**		**Pod lgt (mm)**	**Pod wdt (mm)**	**Seed lgt (mm)**	**Seed wdt (mm)**	**Wgt 100seeds (mm)**	**Yield/pt (g)**	**Yield/ha (kg)**
Accessions	9	76.09*	19.19ns	18.36ns	13.48ns	3006.94**	3402.00**	1214.7**		23.82ns	42.49ns	17.12ns	12.27*	3879.83*	2515.49**	2405.06*
Treatment	5	9.44ns	2.91ns	22.43ns	2.03ns	287.03ns	4662.52ns	1592.6ns		6.77ns	105.01ns	8.36ns	9.24ns	960.51ns	730.95ns	1251.9ns
Accessions*treats	45	30.64ns	13.01ns	18.44ns	10.39ns	301.20ns	4458.20ns	2324.8*		14.32ns	50.43ns	6.53ns	5.34ns	489.95ns	641.66ns	5440.1ns
Replicates	2	144.08*	29.02ns	51.41ns	14.85ns	122.25ns	534.19ns	1209.6ns		298.09**	47.15ns	124.41**	19.49*	356.59ns	1689.99*	7711.5ns

*****P<0.01 Significant, ******P<0.05 Highly Significant, ns- not significant. Lgt-Length, wdt- width, pt-plot, mm-millimetre, g- grams, kg-kilogram, Ha-hectare.

Table 6.2: Analysis of variance showing the yield and yield components of Bambara groundnut accessions inoculated with *B. japonicum* strains in Ibadan, 2019 and 2020

			Ibadan			**2019**							**2020**			
Source of variation	**Df**	**Pod lgt (mm)**	**Pod wdt (mm)**	**Seed Lgt (mm)**	**Seed wdt (mm)**	**Wgt 100 seeds (g)**	**Yield/pt (g)**	**Yield/ha (Kg)**		**Pod lgt (mm)**	**Pod wdt (mm)**	**Seed lgt (mm)**	**Seed wdt (mm)**	**Wgt 100Seed (mm)**	**Yield/pt (g)**	**Yield/ha (kg)**
Accessions	9	159.5**	98.7**	133.6*	50.3**	3687.4**	2496.2**	1268.7**		55.15**	28.23**	16.17*	4.59ns	2771.89*	2534.8*	3536.3**
Treatments	5	32.98ns	7.12ns	92.6ns	11.9ns	437.29ns	839.07ns	2628.4ns		38.06ns	6.52ns	7.35ns	4.04ns	1711.9ns	2770.5*	3253.5*
Accessions*treat	45	29.25ns	16.3ns	49.8ns	7.84ns	437.29ns	693.34ns	2942.3ns		21.44ns	6.27ns	3.94ns	1.99ns	1138.7ns	1112.1ns	1699.0*
Replicates	2	54.95ns	16.3ns	47.2ns	8.63ns	224.76ns	787.51ns	2435.9ns		44.70ns	55.47**	55.38**	42.45*	2819.9ns	101.5ns	3582.8ns

*****P<0.01 Significant, ******P<0.05 Highly Significant, ns- not significant. Lgt-Length, wdt- width, pt-plot, mm-millimetre, g- grams, kg-kilogram, Ha-hectare.

Table 6.3: Mean separation showing the effects of treatments on yield and yield components of Bambara groundnut accessions in Ibadan in both cropping seasons

Accessions	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt/100 seeds (g)	Yield/pt (g)	Yield/ha (Kg)	Pod/pt	Pod wgt/pt (g)	Seed/plot	Seed wgt/pt (g)	Shelling %
TVSu-305	15.35^{a}	10.11^{ab}	10.11^{ab}	7.43^{ab}	69.52^{a}	64.15^{ab}	825.2^{abc}	60.18^{de}	60.69^{ab}	59.82^{bcd}	37.75^{b}	31.55^{abc}
TVSu-475	14.49^{ab}	10.35^{a}	9.99^{ab}	7.38^{abc}	53.72^{bc}	56.31^{b}	871.1^{abc}	79.35^{abcd}	65.14^{a}	78.63^{abc}	37.01^{b}	37.42^{a}
TVSu-1739	14.41^{abc}	10.66^{a}	10.86^{a}	8.07^{a}	73.81^{a}	58.33^{ab}	784.3^{bcd}	66.56^{bcde}	63.03^{a}	60.79^{bcd}	40.01^{ab}	35.03^{ab}
TVSu-365	13.59^{abcd}	8.27^{abc}	8.57^{ab}	6.48^{abc}	48.71^{bcde}	60.89^{ab}	812.7^{abc}	93.42^{a}	64.76^{a}	90.26^{ab}	40.96^{ab}	29.79^{abc}
TVSu-1698	13.39^{abcd}	9.43^{abc}	9.90^{ab}	7.28^{abc}	62.91^{ab}	80.92^{a}	1205.5^{a}	91.13^{ab}	77.77^{a}	103.62^{a}	51.88^{a}	23.83^{bc}
TVSu-710	11.94^{abcd}	7.84^{abc}	8.49^{ab}	6.11^{bc}	42.14^{cde}	30.06^{cde}	364.7^{ef}	60.02^{de}	31.49^{c}	36.35^{cde}	20.00^{c}	30.59^{abc}
TVSu-1606	10.91^{cd}	7.87^{abc}	7.75^{b}	5.81^{bc}	53.97^{bc}	51.08^{b}	733.5^{bcde}	31.38^{f}	28.03^{c}	53.31^{cde}	18.72^{c}	26.76^{abc}
TVSu-378	11.59^{bcd}	8.97^{abc}	8.11^{ab}	6.17^{bc}	38.82^{cde}	27.43^{de}	403.1^{def}	65.68^{bcde}	30.51^{c}	46.46^{de}	17.39^{c}	33.09^{ab}
TVSu-506	11.77^{bcd}	9.37^{abc}	8.79^{ab}	6.66^{abc}	51.99^{bcd}	42.42^{bcd}	523.3^{cde}	43.17^{ef}	32.50^{c}	36.35^{de}	20.18^{c}	32.56^{ab}
TVSu-787	10.79^{d}	7.57^{abc}	9.27^{ab}	6.06^{bc}	36.53^{de}	39.99^{bcde}	669.6^{bcde}	72.95^{abcd}	40.43^{bc}	59.68^{bcd}	23.88^{c}	33.38^{ab}

Mean with the same letter are not significantly different at 5% level of probability using the (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g-grams, kg-kilogram, Ha-hectare, wgt-weight, %-percentage

Table 6.4: Mean separation showing the effects of treatments on yield and yield components of Bambara groundnut accessions in Ikenne in both cropping seasons

Accessions	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt 100 seeds (g)	Yield/pt (g)	Yield/ha (Kg)	Pod/pt	Pod wgt/pt (g)	Seed/plot	Seed wgt/pt (g)	Shelling %
TVSu-305	13.31^{a}	7.93^{abc}	7.41^{ab}	5.05^{ab}	63.35^{ab}	43.01^{ab}	749.1^{abc}	33.00^{cde}	26.81^{abcd}	30.23^{bc}	19.71^{abc}	30.16^{a}
TVSu-475	12.49^{ab}	8.36^{ab}	6.79^{abc}	4.67^{abc}	47.72^{abcd}	42.69^{ab}	860.4^{ab}	61.20^{a}	42.73^{a}	57.49^{a}	26.89^{ab}	33.85^{a}
TVSu-1739	13.51^{a}	9.59^{a}	9.07^{a}	6.31^{a}	68.65^{a}	37.35^{ab}	790.5^{abc}	38.62^{bcde}	37.69^{ab}	37.11^{abc}	28.31^{a}	30.77^{a}
TVSu-365	11.33^{abc}	6.46^{abc}	5.76^{abc}	4.56^{abc}	46.98^{abcd}	25.56^{abc}	289.3^{bc}	34.83^{cde}	20.67^{bcde}	31.28^{bc}	15.70^{abc}	30.91^{a}
TVSu-1698	11.47^{abc}	7.84^{abc}	7.37^{ab}	5.04^{ab}	59.59^{abc}	47.37^{a}	881.2^{a}	47.19^{abc}	34.90^{abc}	46.32^{ab}	26.52^{ab}	21.78^{ab}
TVSu-710	8.81^{abcd}	5.33^{bcd}	5.32^{abc}	3.47^{bcd}	36.01^{cde}	18.25^{bc}	116.9^{c}	24.72^{cde}	12.56^{de}	20.86^{bc}	9.14^{c}	27.59^{a}
TVSu-1606	7.62^{bcd}	4.82^{ad}	3.94^{bc}	2.76^{cd}	41.76^{bcde}	27.93^{abc}	245.6^{bc}	21.44^{de}	16.43^{cde}	21.44^{bc}	12.74^{bc}	17.07^{ab}
TVSu-378	8.43^{abcd}	5.50^{bcd}	4.87^{abc}	3.86^{bcd}	35.62^{cde}	23.99^{abc}	262.7^{bc}	39.53^{bcde}	16.06^{cde}	35.26^{abc}	12.48^{bc}	27.90^{a}
TVSu-506	9.24^{abc}	6.75^{abc}	5.63^{abc}	4.13^{abc}	46.39^{abcd}	37.77^{ab}	783.3^{abc}	32.39^{cde}	24.156^{bcde}	30.21^{bc}	18.51^{abc}	26.53^{a}
TVSu-787	9.45^{abc}	5.73^{bc}	8.18^{ab}	3.94^{bc}	33.68^{de}	30.37^{abc}	783.3^{abc}	50.26^{ab}	21.29^{bcde}	46.08^{ab}	16.13^{abc}	28.98^{a}

Mean with the same letter are not significantly different at 5% level of probability using the (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g-grams, kg-kilogram, Ha-hectare, wgt-weight, %-percentage

Table 6.5: Yield response of accessions of Bambara groundnut inoculated with *B. japonicum* strains in Ibadan and Ikenne in the 2019 cropping season

Accession	Pod Lgt(mm)	Pod wdt(mm)	Seed lgt (mm)	Seed wdt(mm)	Wgt100 seeds (g)	yield/pt (g)	Yield/ha (kg)	Pod/pt	Pod wgt/pt (g)	Seed/plot	Seed wgt/pt (g)	Shelling %
TVSu-305	19.94^{a}	12.71^{ab}	13.99^{a}	10.14^{ab}	72.46^{a}	89.32^{bc}	1641.7^{bc}	83.42^{d}	90.37^{bc}	85.33^{bcd}	50.59^{bc}	42.05^{ab}
TVSu-475	18.31^{ab}	12.94^{ab}	13.45^{a}	9.98^{abc}	55.51^{cd}	89.32^{bcd}	1635.7^{bc}	117.06^{abc}	98.11^{b}	117.72^{b}	52.54^{bc}	42.81^{ab}
TVSu-1739	18.18^{ab}	13.43^{a}	14.41^{a}	10.75^{a}	76.16^{a}	76.12^{bcd}	1445.2^{bcd}	95.09^{cd}	86.31^{bc}	82.86^{bcd}	48.71^{bc}	42.67^{ab}
TVSu-365	17.62^{bc}	10.27^{e}	11.93^{a}	8.72^{d}	45.77^{e}	90.42^{bc}	1670.8^{bc}	142.76^{a}	94.02^{bc}	151.62^{a}	57.00^{b}	39.53^{bc}
TVSu-1698	17.61^{bc}	11.93^{bc}	13.29^{a}	9.97^{abc}	62.59^{b}	118.75^{a}	2374.9^{a}	135.47^{a}	121.09^{a}	162.38^{a}	75.88^{a}	31.36^{c}
TVSu-710	16.93^{bc}	10.63^{de}	12.69^{a}	8.71^{d}	44.03^{e}	39.68^{ef}	774.6^{e}	94.80^{cd}	49.24^{d}	80.50^{cd}	28.08^{d}	45.83^{ab}
TVSu-1606	16.83^{bc}	11.80b^{cd}	12.87^{a}	9.70^{bcd}	61.18^{bc}	94.32^{b}	1885.7^{b}	43.38^{e}	37.06^{d}	36.12^{e}	22.209^{d}	44.32^{ab}
TVSu-378	16.42^{bc}	10.82^{cde}	12.11^{a}	8.92^{d}	40.60^{e}	35.68^{f}	776.9^{e}	104.82^{bcd}	47.07^{d}	64.79^{de}	22.69^{d}	51.04^{a}
TVSu-506	15.94^{dc}	12.16^{b}	13.00^{a}	10.02^{ab}	54.24^{d}	58.29^{de}	1130.6^{de}	55.80^{e}	42.10^{d}	43.87^{e}	23.18^{d}	44.42^{ab}
TVSu-787	14.49^{d}	10.87^{cde}	14.19^{a}	8.99^{dc}	40.29^{e}	67.27^{f}	1355.3^{cd}	129.37^{ab}	72.17^{c}	102.29^{bc}	40.41^{c}	42.89^{ab}

Mean with the same letter are not significantly different at 5% level of probability using the (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g-grams, kg-kilogram, Ha-hectare, wgt-weight, %-percentage.

Table 6.6: Yield response of accessions of Bambara groundnut inoculated with *B. japonicum* strains in Ibadan and Ikenne in the 2020 cropping season

Accession	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt100 seeds (g)	yield/pt (g)	Yield/ha (kg)	Pod/pt	Pod wgt/pt(g)	Seed/pt	Seed wgt/pt(g)	Shelling %
TVSu-305	12.43^{a}	8.57^{a}	7.40^{ab}	5.56^{ab}	72.63^{a}	46.41^{ab}	145.56^{ab}	43.89^{bc}	38.55^{a}	41.42^{b}	29.11^{ab}	24.55^{ab}
TVSu-475	10.67^{ab}	7.77^{a}	6.54^{abcd}	4.77^{abc}	51.93^{abc}	34.7^{5abcd}	106.56^{abcd}	41.65^{bc}	32.18^{abc}	39.54^{ab}	21.49^{abcd}	32.04^{a}
TVSu-1739	11.14^{ab}	8.26^{a}	7.70^{a}	5.69^{a}	73.57^{a}	42.65^{abc}	163.47^{a}	40.68^{bc}	42.15^{a}	41.03^{ab}	32.66^{a}	28.57^{a}
TVSu-365	10.69^{ab}	6.75^{a}	6.12^{abcd}	4.53^{abc}	54.6^{abc}	38.52^{abcd}	116.26^{abcd}	46.70^{bc}	31.15^{abc}	44.49^{ab}	23.91^{abc}	20.46^{ab}
TVSu-1698	10.16^{ab}	7.59^{a}	7.26^{abc}	5.14^{abc}	66.72^{ab}	49.69^{a}	167.94^{a}	54.31^{a}	41.17^{a}	53.88^{a}	32.10^{a}	17.88^{ab}
TVSu-710	9.77^{ab}	6.81^{a}	6.40^{abcd}	4.97^{abc}	47.58^{abcd}	27.07^{abcd}	84.02^{abcd}	41.04^{bc}	21.94^{abcd}	39.53^{ab}	16.60^{abcd}	22.98^{ab}
TVSu-1606	9.66^{ab}	7.22^{a}	6.20^{abcd}	4.62^{abc}	63.75^{ab}	34.04^{abcd}	105.03^{abcd}	31.42^{bc}	29.29^{abc}	32.47^{ab}	21.40^{abcd}	21.51^{ab}
TVSu-378	7.873^{c}	7.79^{a}	4.89^{cd}	4.03^{bc}	39.57^{bcd}	20.86^{bcd}	64.84^{bcd}	32.15^{bc}	16.81^{bcd}	31.32^{ab}	13.27^{bcd}	18.54^{ab}
TVSu-506	10.26^{ab}	8.62^{a}	6.75^{abcd}	4.98^{abc}	58.79^{abc}	36.26^{abcd}	104.55^{abcd}	39.83^{bc}	29.93^{abc}	36.14^{ab}	21.04^{abcd}	28.11^{a}
TVSu-787	7.91^{bc}	4.87^{a}	5.13^{bcd}	3.63^{c}	35.01^{cd}	16.44^{cd}	59.16^{cd}	23.72^{c}	12.71^{cd}	22.75^{b}	9.59^{cd}	26.24^{ab}

Mean with the same letter are not significantly different at 5% level of probability using the (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g-grams, kg-kilogram, Ha-hectare, wgt-weight, %-percentage

Table 6.7: Yield response of accessions of Bambara groundnut inoculated with *B. japonicum* strains in Ibadan and Ikenne in both cropping seasons

Accession	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt100 seeds (g)	yield/pt (g)	Yield/ha (kg)	Pod/pt	Pod wgt/ pt (g)	Seed/pt	Seed wgt/ pt (g)	Shelling %
TVSu-305	15.35^{a}	10.11^{ab}	10.11^{ab}	7.43^{ab}	69.52^{a}	64.15^{ab}	825.2^{abc}	60.18d^{e}	60.69^{ab}	59.82^{bcd}	37.745^{b}	31.55^{abc}
TVSu-475	14.49^{ab}	10.35^{a}	9.99^{ab}	7.38^{abc}	53.72^{bc}	56.31^{b}	871.1^{ab}	79.35^{abcd}	65.14^{a}	78.63^{abc}	37.01^{b}	37.42^{a}
TVSu1739	14.41^{abc}	10.66^{a}	10.86^{a}	8.073^{a}	73.81^{a}	58.33^{ab}	784.3^{bcd}	66.56^{bcde}	63.03^{a}	60.79^{bcd}	40.01^{ab}	35.03^{ab}
TVSu-365	13.58^{abcd}	8.27^{abc}	8.58^{ab}	6.48^{abc}	48.71^{bcde}	60.89^{ab}	812.7^{abc}	87.65^{ab}	64.76^{a}	100.33^{a}	40.96^{ab}	29.79^{abc}
TVSu1698	13.39^{abcd}	9.43^{abc}	9.90^{ab}	7.28^{abc}	62.91^{ab}	80.92^{a}	2205.5^{a}	91.13^{a}	77.76^{a}	103.62^{a}	51.88^{a}	23.83^{bc}
TVSu-710	11.94^{abcd}	7.84^{abc}	8.49^{ab}	6.11^{bc}	42.14^{cde}	30.06^{cde}	364.7^{ef}	60.02^{de}	31.49^{c}	53.31^{cde}	20.00c	30.59^{abc}
TVSu1606	10.91^{cd}	7.87^{abc}	7.75^{b}	5.81^{bc}	53.97^{bc}	51.08^{bc}	733.5^{bcde}	31.38^{f}	28.03^{c}	29.28^{de}	18.72c	26.76^{abc}
TVSu-378	11.59^{bcd}	8.97^{abc}	8.11^{ab}	6.17^{bc}	38.82^{cde}	27.43^{de}	403.1^{def}	65.68^{bcde}	30.51^{c}	46.46^{de}	17.39c	33.09^{ab}
TVSu-506	11.77^{bcd}	9.37^{abc}	8.79^{ab}	6.66^{abc}	51.99^{bcd}	42.42^{bcd}	523.3^{cdef}	43.17^{ef}	32.50^{c}	36.35^{de}	20.18c	32.56^{ab}
TVSu-787	10.79^{d}	7.57^{abc}	9.27^{ab}	6.06^{bc}	36.53^{de}	39.99^{bcde}	669.6^{bcde}	72.95^{abcd}	40.43^{bc}	59.68^{bcd}	23.88c	33.38^{ab}

Mean with the same letter are not significantly different at 5% level of probability using the (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g-grams, kg-kilogram, Ha-hectare, wgt-weight, %- percentage

Table 6.8: Effects of N fertilizer and *B. japonicum* strains on yield and yield component of Bambara groundnut accessions at harvest in Ibadan and Ikenne in both seasons

Treatment	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt 100seeds (g)	Yield/pt (g)	yield/ha (kg)	Pod /plt	Pod/pt (g)	Wgt of pod/plt (g)	Seed/ plt	Seed/pt	Seed wgt/ plt (g)	Shelling %
FA3	13.27^{a}	9.08^{ab}	9.39^{ab}	6.92^{ab}	56.85^{a}	50.72^{ab}	728.39^{ab}	21.99^{a}	68.70^{a}	50.79^{a}	20.95^{a}	65.16^{a}	31.96^{a}	33.07^{b}
USDA110	13.18^{a}	8.94^{b}	8.90^{b}	6.84^{ab}	52.25^{ab}	50.52^{ab}	680.75^{b}	18.88^{ab}	64.88^{ab}	47.68^{ab}	19.56^{ab}	64.72^{a}	30.72^{ab}	31.62^{b}
N	12.01^{a}	8.26^{b}	8.67^{b}	6.21^{b}	46.03^{b}	44.66^{b}	633.07^{c}	17.91^{b}	63.73^{ab}	42.44^{b}	17.22^{b}	55.16^{b}	26.22^{b}	30.47^{b}
IRJ2180A	12.52^{a}	8.81^{b}	8.62^{b}	6.58^{ab}	53.12^{ab}	50.11^{ab}	698.06^{b}	18.79^{ab}	62.17^{b}	47.03^{ab}	17.67^{b}	58.79^{b}	28.65^{ab}	29.11^{c}
RACA6	12.33^{a}	8.70^{b}	9.07^{ab}	6.70^{ab}	52.22^{ab}	51.51^{a}	750.72^{a}	19.56^{a}	63.73^{ab}	51.86^{a}	18.73^{ab}	60.65^{ab}	29.97^{ab}	31.06^{b}
CONTROL	13.39^{a}	10.27^{a}	10.40^{a}	7.135^{a}	47.26^{b}	48.44^{b}	533.82^{d}	12.20^{c}	54.97^{c}	32.87^{c}	11.78^{c}	46.78^{c}	25.07^{b}	41.90^{a}

Means with the same letter are not significantly different at P<0.05 level of probability using the Duncan multiple range test (Dmrt). Lgt-Length, wdt- width, pt-plot, mm-millimetre, g- grams, kg-kilogram, Ha-hectare, wgt-weight, %- percentage, plt-plant

Table 6.9: Mean and standard deviation of yield and yield components of Bambara groundnut accessions' six treatments in Ibadan and Ikenne in both cropping seasons

Accessions	Treatments	Pod Lgt (mm)	Pod wdt (mm)	Seed Lgt (mm)	Seed wdt (mm)	Wgt100 seeds(g)	Yield/plt (g)	Yield/ha (kg/ha)	Wgt pod/plt (g)	Wgt of seed/plt (g)
TVSu-365	**FA3**	20.1±2.37^{a}	11.8±2.57^{a}	15.8±0.73^{a}	10.1±2.57^{a}	57.8±19.9^{a}	96.4±38.7^{d}	1928.3±774.0^{c}	147.8±93.4^{bc}	59.8±26.4^{c}
	RACA6	21.6±0.86^{a}	8.43±2.84^{b}	15.3±1.76^{a}	6.84±2.58^{bc}	49.8±2.86^{bc}	176.9±24.5^{b}	3536±492.7^{b}	226.2±77.3^{a}	138.8±33.7^{a}
	USDA110	20±5.250^{a}	11.1±0.96^{a}	12.8±3.23^{b}	9.8±1.57^{a}	47.1±6.29^{bc}	195.3±39.7^{a}	3906±793.8^{ab}	174.8±35.3^{b}	118.6±28.1^{b}
	IRJ2180A	15.4±13.6b	8.21±7.31^{b}	10.6±9.22^{bc}	7.22±6.45^{bc}	32.7±28.3^{d}	97.2±98.3^{e}	1943.7±1965.3^{c}	86.5±75.7^{c}	48.3±42.4^{d}
	N	18.9±5.50^{ab}	9.89±1.93^{ab}	13.9±2.57^{ab}	8.68±1.94^{ab}	46.0±4.36^{c}	145.4±85.6^{c}	1703.6±614.4^{d}	153.5±29.8^{bc}	87.9±29.6^{bc}
	CONTROL	21.9±2.94^{a}	11.2±1.92^{a}	14.23±2.53^{a}	9.29±0.88^{a}	52.9±2.42^{b}	111.8±43.4^{d}	4236.3±856.3^{a}	201.6±79.1^{ab}	136.8±58.1^{a}
TVSu-305	**FA3**	17±14.70^{b}	9.04±7.86^{b}	9.66±9.38^{c}	6.86±6.19^{c}	50.2±43.5^{c}	74.4±78.6^{d}	1487.3±1571.9^{d}	97.5±115.2^{e}	63.3±82.6^{d}
	RACA6	23.3±3.85^{ab}	15.1±0.17^{a}	18.4±0.67^{a}	14.7±2.19^{a}	87.8±8.85^{a}	150.1±95.2^{a}	3002.6±1904.9^{b}	218.8±36.39^{a}	89.7±44.9^{b}
	USDA110	22.7±3.91^{ab}	14.36±3.64^{a}	14.4±2.19^{b}	11.1±2.44^{ab}	75.9±9.43^{b}	139.4±66.3^{b}	3500±2721.4^{a}	168.4±72.3^{b}	110.2±59.3^{a}
	IRJ2180A	26.7±2.78^{a}	12.22±3.69^{ab}	14.4±4.19^{b}	9.85±3.29^{ab}	88.5±6.67^{a}	153.2±36.0^{a}	3064.7±720.0^{b}	103±35.3^{d}	27.3±12.3^{e}
	N	24.6±6.73^{a}	15.5±0.52^{a}	20.18±3.21^{a}	13.39±1.39^{a}	84.1±5.98^{ab}	129.5±48.5^{bc}	2590.7±969.9^{c}	126.4±64.00^{c}	70.1±43.8^{c}
	CONTROL	14.7±13.6^{c}	9.15±7.89^{b}	12.21±10.7^{bc}	8.44±7.40^{b}	61.3±53.8^{bc}	107.6±94.4^{c}	1089±1591.3^{e}	91.9±79.8^{e}	53.8±47.2^{d}
TVSu-378	**FA3**	18±3.550^{ab}	11.5±0.13^{a}	14.2±0.15^{ab}	10.2±0.08^{a}	34.4±3.96^{c}	20.21±6.92^{d}	404.2±138.3^{f}	38.8±16.1^{d}	12.18±6.39^{d}
	RACA6	18.2±2.86^{ab}	12.5±1.22^{a}	14.9±2.53^{ab}	11.00±0.50^{a}	44±11.79^{a}	45.2±22.1^{b}	904.5±441.1^{c}	72.17±22.04^{b}	31.79±13.16^{b}
	USDA110	20.1±2.10^{ab}	12.4±1.64^{a}	14.18±1.32^{ab}	11.04±1.59^{a}	37.9±9.22^{b}	43.07±26.03^{b}	1786.3±1142.1^{a}	83.8±64.8a	35.03±23.04^{a}
	IRJ2180A	14.9±2.66^{c}	9.70±0.54^{b}	10.3±1.74^{b}	8.25±1.26^{ab}	40.3±4.10^{ab}	66.3±19.9^{a}	1326±395.5^{b}	83.3±52.2^{a}	37.5±19.32^{a}
	N	18.5±4.10^{ab}	11.8±1.11^{a}	15.49±3.00^{ab}	9.96±0.92^{ab}	44.5±3.59^{a}	30.76±7.97^{c}	615.3±159.5^{e}	75.37±17.8^{b}	25.64±6.65^{c}
	CONTROL	23±11.40^{a}	10.8±0.44^{ab}	19.5±7.66^{a}	9.45±1.88^{ab}	34.2±7.93^{c}	43.3±34.4^{b}	866.6±687.9^{d}	66.04±12.48^{bc}	36.11±28.66^{a}
TVSu-475	**FA3**	20.9±3.21^{b}	13.21±0.31^{a}	17.8±2.34^{a}	11.5±0.89^{a}	63.3±11.7^{a}	111.6±26.9^{b}	2232.3±540.3^{b}	157.9±92.4^{b}	72.9±42.8^{b}
	RACA6	20.5±5.23^{b}	13.6±0.72^{a}	17.6±1.80^{a}	12.6±1.04^{a}	58.2±15.4^{b}	103.9±56.3^{b}	2078.6±1126.7^{c}	155.7±90.3^{b}	74.9±50.2^{b}
	USDA110	20.2±3.81^{b}	13.3±0.73^{a}	14.7±2.54^{b}	11.2±2.74^{a}	60.1±6.71^{b}	101.5±15.9^{b}	2031.6±319.5^{c}	132.4±35.9^{c}	67.8±19.2^{c}
	IRJ2180A	15.5±3.34^{c}	12.9±1.64^{ab}	12.4±2.19^{b}	10.3±1.16^{a}	59.8±10.8^{b}	125.5±29.9^{a}	2510±597.9^{a}	176.3±46.9^{a}	91.9±31.1^{a}
	N	23±2.260^{a}	14.5±0.77^{a}	18.5±0.63^{a}	12.8±1.87^{a}	61.1±4.54^{ab}	98.2±50.2^{c}	1964.3±1003.3^{d}	153.9±68.8^{b}	81.8±41.8^{ab}
	CONTROL	19.9±3.32^{b}	13.2±1.26^{a}	17.08±0.35^{a}	11.6±1.00^{a}	56.2±1.04^{c}	122.4±51.3^{a}	2448.6±1026.1^{ab}	126.4±17.9^{cd}	65.1±19.9^{c}
TVSu-506	**FA3**	18.1±0.86^{a}	13.1±1.79^{a}	16.7±0.57^{a}	11.6±1.44^{b}	58.7±9.90^{b}	65.8±43.0^{c}	1233.7±1002.1^{c}	68.1±20.1^{a}	27.5±17.6^{b}
	RACA6	13.5±11.8^{c}	9.19±7.98^{c}	11.7±10.15^{c}	8.47±7.34^{bc}	39.8±34.8^{c}	71.8±84.3^{b}	1436.7±1685.5^{b}	33.03±28.7^{c}	17.6±15.3^{d}
	USDA110	12.8±12.3^{c}	7.59±6.73^{cd}	10.69±9.74^{c}	6.25±5.57^{c}	37.2±34.1^{c}	64.8±89.2^{c}	1296.7±1783.7^{c}	46.1±60.1^{b}	23.2±28.9^{c}
	IRJ2180A	17.6±2.47^{ab}	13.1±1.16^{a}	15.3±0.89^{b}	12.1±2.55^{a}	62.5±4.09^{ab}	82.8±20.3^{a}	1656.7±405.3^{a}	67.0±43.1^{a}	38.28±22.9^{a}
	N	15.6±0.51^{b}	12.22±1.23^{ab}	14.7±0.61^{b}	10.9±1.07^{b}	58.5±8.49^{b}	70.3±19.5^{b}	1406.7±390.6^{b}	42.1±4.51^{b}	26.2±2.02^{b}
	CONTROL	14.1±3.34^{b}	12.5±16.8^{ab}	11.6±2.97^{c}	9.51±1.14^{b}	69.3±7.37^{a}	20.3±11.6^{d}	75.10±33.10^{d}	21.1±17.3^{d}	15.3±6.4^{d}

TVSu-710	**FA3**	19.3±4.12^{a}	11.7±0.89^{a}	14.6±1.49^{a}	10.2±1.49^{a}	48.6±1.05^{a}	81.6±41.1^{a}	1632±822.3^{a}	81.3±16.9^{c}	55.2±35.5^{a}
	RACA6	18.4±3.46^{a}	11.8±0.96^{a}	14.5±1.99^{a}	9.76±1.29^{b}	49.5±11.03^{a}	30.7±36.1^{cd}	613.3±724.3^{d}	41.5±41.4^{e}	20.8±23.7^{e}
	USDA110	13.2±11.4^{c}	6.96±6.18^{b}	10.4±9.09^{b}	7.18±6.75^{c}	30.4±27.1^{c}	56.2±56.9^{b}	1123.9±1192.1^{cd}	65.8±68.2^{d}	41.3±51.4^{c}
	IRJ2180A	18.9±5.90^{a}	10.5±0.50^{a}	13.5±2.18^{a}	8.87±0.77^{c}	42.8±3.56^{ab}	79.8±41.5^{a}	1595±829.5^{ab}	91.4±53.1^{b}	51.1±34.1^{ab}
	N	18.4±6.10^{a}	10.3±1.04^{a}	14.4±3.61^{a}	8.64±2.06^{c}	40±5.700^{b}	62.3±12.2^{b}	1245±244.6^{c}	107.2±18.0^{a}	56.0±17.0^{a}
	CONTROL	17.1±3.31^{ab}	10.0±0.24^{a}	14.3±2.20^{a}	9.08±0.89^{b}	41.7±4.93^{b}	37.5±23.1^{c}	560.3±623.6^{e}	66.5±4.9^{d}	29.5±13.6^{d}
TVSu-787	**FA3**	16.9±0.67^{a}	12.7±0.94^{a}	15.1±0.86^{a}	11.2±0.55^{a}	47.7±4.01^{a}	132.5±32.4^{a}	2649.7±646.5^{a}	151.6±29.8^{b}	87.7±29.6^{ab}
	RACA6	14.8±1.24^{ab}	11.2±1.65^{a}	12.7±1.76^{ab}	9.92±1.96^{ab}	40.7±6.73^{b}	58.4±9.35^{c}	1168.8±186.9^{e}	91.7±33.2^{c}	38.9±6.20^{c}
	USDA110	9.82±8.51^{b}	7.69±6.66^{c}	8.34±7.24^{c}	6.86±5.94^{c}	25.2±21.8^{c}	44.1±43.6^{d}	888.1±872.2^{f}	59.1±57.5^{d}	22.4±21.8^{d}
	IRJ2180A	16.2±2.43^{a}	12.9±2.32^{a}	14.2±3.44^{a}	10.82±1.00^{a}	47.1±8.60^{a}	106.1±41.9^{b}	2123.3±839.4^{cd}	91.8±18.6^{c}	49.4±10.94^{b}
	N	15.4±1.14^{a}	9.46±2.92^{b}	12.4±0.87^{ab}	8.5±2.630^{b}	47.7±10.7^{a}	98.5±46.2^{bc}	1698.2±1394.3^{c}	98.2±48.7^{c}	49.7±32.7^{b}
	CONTROL	15.6±1.21^{a}	12.2±0.68^{a}	14.5±1.69^{a}	11.7±1.46^{a}	46.4±4.92^{a}	94.4±68.8^{bc}	2273.3±739.0^{b}	175.1±41.9^{a}	98.7±24.1^{a}
TVSu-1606	**FA3**	18.3±5.18^{ab}	12.5±1.41^{b}	13.7±2.58^{ab}	10.1±1.09^{b}	74.1±6.75^{a}	125.1±78.5^{c}	2502.7±1570.1^{c}	42.6±26.4^{c}	20.9±13.1^{c}
	RACA6	11±9.950^{c}	8.02±6.96^{c}	9.47±8.57^{c}	6.80±5.90^{c}	43.8±38.7^{c}	140.9±122.0^{b}	2817.3±2440.1^{b}	57.1±54.3^{b}	35.4±35.8^{b}
	USDA110	15.1±4.05^{b}	12.5±2.86^{b}	13.7±3.96^{ab}	10.2±2.99^{b}	50.8±12.9^{bc}	49.5±77.50^{e}	989.9±1549.4^{e}	23.7±15.5^{e}	8.87±12.8^{d}
	IRJ2180A	20.6±4.63^{a}	14±1.990^{a}	16.42±2.14^{a}	12.21±3.21^{a}	74.9±2.93^{a}	158.8±49.1^{ab}	3169.7±983.6^{a}	75.3±14.1^{a}	41.3±9.03^{ab}
	N	15.1±13.1^{b}	8.52±7.45^{c}	11.01±9.57^{b}	6.98±6.04^{c}	49.1±43.3^{c}	61.8±58.8^{d}	1235±1175.4^{d}	33.4±39.4^{d}	16.8±20.1^{cd}
	CONTROL	16.4±2.13^{b}	12.8±1.90^{b}	15.04±3.14^{a}	11.7±1.96^{a}	57.7±13.5^{b}	163.3±83.3^{a}	3271.3±166.3^{a}	35.8±19.2^{d}	46.1±36.9^{a}
TVSu-1698	**FA3**	17.4±3.11^{b}	12.3±0.33^{a}	14.9±2.84^{b}	10.3±0.89^{b}	62.8±5.35^{c}	220.5±43.92^{b}	4410±878.6^{b}	59.7±28.22^{e}	878.6±59.7^{a}
	RACA6	19.8±1.98^{ab}	13.2±1.05^{a}	15.4±2.40^{b}	10.9±1.25^{b}	61.7±2.69^{c}	311.7±53.3^{a}	6234±86.70^{a}	327.2±205.5^{a}	155.8±26.7^{c}
	USDA110	19.9±3.82^{ab}	12.7±0.65^{a}	16.3±2.50^{ab}	12.2±1.14^{a}	61.0±2.17^{c}	119.3±51.3^{d}	2386±1025.3^{d}	119±32.4^{d}	73.9±18.3^{e}
	IRJ2180A	11±9.820^{c}	7.75±6.81^{b}	9.50±8.42^{b}	6.99±6.15^{c}	45.6±39.3^{d}	83.7±72.50^{e}	1674±1450^{e}	101.8±88.3^{d}	69.9±60.6^{e}
	N	22.4±0.57^{a}	12.9±0.50^{a}	17.1±1.57^{a}	12.7±3.14^{a}	66.9±10.14^{b}	163.2±47.2^{c}	3264±943.2^{c}	153.9±74.9^{c}	105.8±65.4^{d}
	CONTROL	22.1±1.55^{a}	12.6±0.31^{a}	14.2±2.88^{b}	10.55±0.91^{b}	69.1±8.64^{a}	212.3±9.96^{b}	4245.7±198.9^{b}	245.1±67.6^{b}	165.8±45.8^{b}
TVSu-1739	**FA3**	20.6±2.02^{a}	12.4±3.75^{ab}	14.5±2.47^{b}	9.17±1.84^{c}	83.8±11.6^{a}	130.4±152.0^{b}	3176.7±2519.7^{a}	173±58.3^{a}	104.3±46.2^{a}
	RACA6	17.3±1.14^{b}	12.8±1.60^{ab}	16.1±1.16^{a}	10.9±1.72^{b}	80.5±98.4^{ab}	97.7±53.1^{d}	1307.6±1074.9^{e}	115.7±34.1^{d}	57.9±30.9^{d}
	USDA110	18.9±3.91^{b}	14±1.690^{a}	16.5±2.75^{a}	12.6±1.35^{a}	72.8±11.9^{b}	104.5±49.1^{cd}	1519.0±1577.3^{d}	177.3±72.1^{a}	68.4±33.3^{c}
	IRJ2180A	20.9±4.95^{a}	13.5±0.88^{a}	15.2±2.99^{a}	11.34±0.97^{b}	82.9±8.49^{a}	112.8±7.09^{c}	2125.3±207.6^{b}	145.1±46.9^{bc}	70.01±31.8^{c}
	N	12.6±11.0^{d}	9.11±7.92^{b}	12.4±10.81^{c}	8.35±7.39^{c}	52.5±45.51^{c}	98.2±85.10^{d}	1965.7±1702.3^{c}	82.2±71.21^{e}	49.1±42.6^{d}
	CONTROL	16.6±1.8.0^{c}	12.9±0.88^{ab}	14.0±1.420^{b}	11.5±0.65^{b}	83.7±7.60^{a}	161.1±100.1^{a}	3222.7±2002.1^{a}	156.2±36.3^{b}	107.3±66.8^{b}
CV (%)		33.1	29.4	32.0	32.0	32.7	55.6	56.9	51.8	54.9

Means with the same letter are not significantly different at P<0.05 level of probability using the Duncan multiple range test (Dmrt). Lgt-Length, wdt- width, plt-plot, mm-millimetre, g- grams, kg-kilogram, Ha-hectare, wgt-weight.

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6.3.1 Rainfall Pattern during Bambara Groundnut Production in Both Locations

Adequate rainfall was recorded in Ibadan and Ikenne throughout the production of the Bambara groundnut in the first season but a slight break in rainfall occurred in the second season in both locations for a one month period and alternative means of irrigation were introduced in both locations. Water from the irrigation was not sufficient for the legume compared to the natural rain, which adversely affected the yield of the underutilized legume. In the second season, the lack of rain significantly reduced pod formation in both locations. This eventually resulted in low yield and yield components recorded in the second season in both locations.

6.3.2 Most Probable Number of Soil Used for the Study

The number of indigenous rhizobia present in the soil was determined using the most probable number (MPN), in Ikenne the result obtained were 2.03×10^9 cfu/ml and 2.47×10^7 cfu/ml in 2019 and 2020 respectively, while in Ibadan, the value obtained were 1.83×10^5 cfu/ml and 1.83×10^5 cfu/ml in 2019 and 2020 respectively. The available rhizobial count in the soil was moderately abundant in both seasons and locations, except for the soil in Ikenne in the 2019 (1.53×10^9 cfu/ml) season which seemed to be relatively abundant.

6.3.3 Response of Bambara Groundnut Accessions to *B. japonicum* Strains Inoculation

Results obtained revealed that no notable differences were recorded between Bambara groundnut accessions in terms of yield traits in Ikenne in both seasons (Table 6.1). Precisely in Ibadan, there were notable variations in the yield traits among Bambara groundnut accessions in both seasons (Table 6.2). Additionally, no discernible differences were recorded in yield traits among the *B. japonicum* strains inoculated to the Bambara groundnut accessions (Table 6.2).

The interaction of Bambara groundnut accessions with *B. japonicum* strains on yield traits recorded in Ibadan and Ikenne in both seasons were not significantly different (Table 6.1 and Table 6.2). However, considerable variations in some yield traits of Bambara groundnut accessions were found in replicates in Ibadan and Ikenne in both seasons (Table 6.1 and Table 6.2). Higher significant differences were recorded in TVSu-1739 compared to other inoculated Bambara groundnut accessions in the pod length, width, seed length, width and weight of one hundred seeds in Ibadan (Table 6.3). Also, TVSu-1698 recorded a significant difference in Ibadan compared to other inoculated Bambara groundnut accessions in the yield/plot, yield/ha, number of pod/plot, number of seed/plot, pod weight/pot and seed weight per pod (Table 6.3). The highest shelling percentage were recorded in TVSu-475, which is significantly different from other inoculated Bambara groundnut accessions (Table 6.3). Furthermore, higher significant differences were also recorded in TVSu-1739 compared to other inoculated Bambara groundnut accessions in the pod length, width, seed length, width and weight of hundred seeds in Ikenne (Table 6.4). TVSu-1698 recorded a significant difference in Ikenne compared to other inoculated Bambara groundnut accessions in the yield/plot and yield/ha (Table 6.4). TVSu-475 significantly enhanced the number of pod/plot, number of seed/plot, pod weight/pot, seed weight per pod and highest shelling percentage in Ikenne (Table 6.4). These findings showed that TVSu-1739, TVSu-1698 and TVSu-475 are super accessions compared to other Bambara groundnut accession inoculated in Ibadan and Ikenne. The inoculated Bambara groundnut accessions TVSu-305, TVSu-475 and TVSu-1739 did not significantly differ in pod length, width, or seed width according to the results of the first experiment conducted in the 2019 crop season in both geographical locations (Ibadan and Ikenne) but the values obtained were significantly higher than those of other inoculated Bambara groundnut accessions (Table 6.5). Inoculated Bambara groundnut accessions did not significantly differ in terms of seed length (Table 6.5). Furthermore, significant differences were recorded between TVSu-305, TVSu-365, TVSu-1739 and TVSu-1698 than other inoculated Bambara groundnut accessions in both locations in the first season. Likewise, TVSu-1698 in both locations recorded the highest mean value for the yield and yield component during the first season (Table 6.5).

Except for TVSu-378 and TVSu-787 at both locations, there were no discernible changes in the second season between the inoculated Bambara groundnut accessions in terms of pod length, width, seed length, or width (Table 6.6). Also, in the second season, TVSu-1739 and TVSu-1698 showed significant differences in the yield traits recorded compared to other genotype. However, other genotypes with exception of TVSu-378 and TVSu-787 were not significantly different in the yield traits recorded (Table 6.6). Also, no discernible differences were recorded in the number of pod per plot and seed per plot except TVSu-1698 that showed a significant difference compared to other accessions of Bambara groundnut (Table 6.6). No significant difference was recorded among Bambara groundnut accessions in the seed length and width in either location (Table 6.6). With a higher yield trait than other Bambara groundnut accessions in the second season in both locations, a significant difference was recorded in the inoculated Bambara groundnut accession, TVSu-1698 (Table 6.6).

TVSu-305 and TVSu-1698 responded to inoculation with higher yield traits recorded than other genotypes, which were significantly not different from yield traits recorded in the first, second, and combine seasons (Table 6.7).

Figure 6.2: Bambara groundnut establishment in Ikenne field

Photo: T.D. Bitire, IITA (2019).

6.3.4 Interactions between Accessions and Treatments.

FA3 strains significantly differed from other *B. japonicum* strains inoculated to Bambara groundnut accessions, inorganic nitrogen fertilizer application, and uninoculated control in both sites and seasons in the weight of 100 seeds and seed weight per plot (Table 6.8).

These findings showed that FA3 and RACA6 strains have better potentials than other *B. japonicum* strains, inorganic nitrogen fertilizer treatment and uninoculated controls (Table 6.8). The yield response of Bambara groundnut accessions and the *B. japonicum* strains were recorded (Table 6.8). The interactions of Bambara groundnut accessions with *B. japonicum* strains on yield traits revealed significant differences.

Different variability were noted in the yield traits of inoculated Bambara groundnut accessions (Table 6.9). The combination of TVSu-305 with IRJ2018A produced the greatest mean values for 100 seeds, pod length and weight (88±56.67g, 26.7±2.78mm, and 12.22±3.68mm respectively) (Table 6.9). TVSu-365 and USDA110 had the highest yield/plot. Moreover, the interaction between TVSu-305 with RACA6 was found to produce the longest pod width and seed width, with mean values of 15.1±0.17mm and 14.7±2.19mm respectively (Table 6.9). The interaction of TVSu-1698 with RACA6 produced the highest weight of pod per plot and yield per hectare, with mean values of 327.2±205.5g and 6234±86.70 kg ha^{-1}, respectively. The inoculation of Bambara groundnut accessions with *B. japonicum* strains leads to differences observed in the yield traits of inoculated Bambara groundnut accessions (Table 6.9).

6.5 Discussion

Due to the second season's low rainfall and break in rainfall which caused a decline in yield, comparing the yield recorded in both season, the yield and yield components of the inoculated Bambara groundnut accession differed from the first season in both geographical areas. Most notably, the soil cultivated on, helped boost the yield and the yield components that were measured. The above indicates that the result of soil sample revealed that the macronutrient, micronutrient and biological nutrient needed for crop sustainability were accessible in sufficient amounts in the soils which buttress the inoculation of the *B. japonicum* strains to improve Bambara groundnut accessions' optimum yield and yield components.

The importance of soil quality cannot be overstated because it has a direct impact on the yield response recorded in the inoculated accessions of Bambara groundnut in both locations and seasons. The key nutrients the crop needed for maximum growth are readily available in the soils. Additionally, the soil's pH in both locations is a factor in determining the success of the production of the legume since poor yields were also observed in the acidic soil (Ikenne) compared to the site with neutral soil pH conditions in Ibadan. The study recorded that, in comparison to the locations where the study was conducted, the inoculation of the strains improved the yield traits.

The examination of the native rhizobia found in the soils in both locations, however, revealed that the nitrogen fertilizer could not boost output above the uninoculated control. Results indicate that native rhizobia, which are present in the soils in modest quantity, outperform nitrogen fertilizer in terms of yield and the components of yield that were measured. The underutilised legume responded to the inoculation in both seasons, as shown in the result obtained which showed a great difference among Bambara groundnut accessions. It is possible to link the genotype in connection to the environments and seasons to the response shown in the yield and yield component of

Bambara groundnut accessions. The results obtained are related to that published in Allito *et al*. (2021) which found that rhizobia inoculation increases the nodulation of bean compared to the uninoculated control. Bambara groundnut accessions, in both cropping seasons, showed superior performance, including TVSu-1698 and TVSu-1739.

However, differences in yield and yield components were recorded among inoculated Bambara groundnut accessions with the exception of TVSu-1698, which exhibits a higher response to the inoculation with more seeds per area compared to other Bambara groundnut accessions. The results showed that inoculating legumes with *B. japonicum* strains increases yield. This is related to the findings of Santos *et al*. (2019), who discovered that inoculating rhizobia to legumes sparked a lot of interest among researchers in the 1970s. The outcome of the interactions also demonstrated the applicability and inoculation of bacteria strains to Bambara groundnut accessions, demonstrating different variability in the yield traits measured.

Significant differences in yield traits of the accessions of Bambara groundnut were also recorded among the *B. japonicum* strains inoculated. The present result revealed that genotype, the environmental circumstances, the crop management and the type of *B. japonicum* strain all play a major role in the production and yield of Bambara groundnut (Abdullahi and Abubakar 2022). Nevertheless, significant variations in pod length, width, seed length, weight of 100 seeds, yield/plot, pod weight/plot, seed weight/plot and yield/ha were observed in both seasons and locations. This is consistent with the findings of a study carried out at Minna, northern Nigeria which was done using an early maturing promiscuous cultivar soybean (TGX 1485), inoculated with one of the four *B. japonicum* strains (IRc291, IRj2180A, IRc461 and R25B). All parameters, including grain yields were increased by inoculating with the four rhizobia inoculants (Abdullahi *et al*. 2020). The yield traits of the inoculated accessions of Bambara groundnut showed varying levels of variability, with TVSu-1698 demonstrating a greater response to the inoculation in both sites in the yield traits examined than other accessions of Bambara groundnut. The fruits (pod), seed size, shape, and shelling % of TVSu-1698 were precisely and significantly different from those of other Bambara groundnut accessions. It was evident that the *B. japonicum* strains' inoculation greatly increased the yield traits of accessions of Bambara groundnut compared to the inorganic nitrogen fertilizer applied and the uninoculated control.

In this study, nitrogen fertilizer application also had a significant impact on some traits of Bambara groundnut accessions. This highlights the value of inorganic nitrogen fertilizer application and indigenous rhizobia than the uninoculated control. Native rhizobia in the soil form nodules in the uninoculated plant and in the plant that has received nitrogen fertilization. It was found that several Bambara groundnut accessions had improved yield and yield components due to the two native strains (RACA6 and IRJ2180A) and the two non-native strains (FA3 and USDA110). In contrast to the USDA110 strain, the FA3 strain increased the yield of the following Bambara groundnut accessions: TVSu-710, TVSu-787 and TVSu-1739. The improvement in production recorded in TVSu-365, TVSu-1606 and TVSu-1739 without strain inoculation indicates that the native rhizobia strains proved effective to enhanced grain yield.

6.6 Conclusion

Farmers can use *B. japonicum* strains as an alternate source to inoculate legumes as a starter dosage, which farmers can readily afford, compared to using nitrogen fertilizers to increase yield. Among the other bacteria strains, FA3 and RACA6 strains improved the yield traits of Bambara groundnut accessions.

In conclusion, farmers in the tropics should adopt the use of bacteria strains (FA3 and RACA6) in order to increase productivity as opposed to applying inorganic nitrogen fertilizer, which most farmers in the tropics cannot afford. In the future, it could be required to repeat this research on Bambara groundnut accessions using various inorganic nitrogen fertilizer rates and *B. japonicum* strains or to determine the combine effectiveness of the inorganic nitrogen fertilizer and *B. japonicum* strains on accessions of Bambara groundnut.

CHAPTER SEVEN

The Influence of *Bradyrhizobium japonicum* on Nodulation Potential and Nitrogen Fixing Efficiency in Bambara Groundnut (*Vigna subterranea (L) Verdc)* Accessions.

Abstract

Nitrogen fixation is essential for sustaining growth and development in legumes. This study was designed to understand the efficiency of nitrogen-fixing bacteria (*B. japonicum)* over the inorganic nitrogen fertilizer in different Bambara groundnut accessions. Pot experiments were conducted in the screenhouse from August to December of the 2018 and 2019 cropping seasons in Ibadan, Nigeria. Field experiments were also performed in Ibadan and Ikenne from August to December of the 2019 and 2020 cropping seasons to evaluate the nitrogen-fixing potentials of ten Bambara groundnut accessions: TVSu-305, TVSu-1739, TVSu-475, TVSu-1606, TVSu-710, TVSu-506, TVSu-1698, TVSu-365, TVSu-787, and TVSu-378. Seeds of each Bambara groundnut accessions were carefully coated with gum Arabic and nitrogen-fixing bacteria: FA3, USDA110, IRJ2180A and RACA6. The coated seeds were sown on the field in both locations and seasons. Inorganic nitrogen fertilizer (urea, 20kg/ha) was applied to seedlings of Bambara groundnut accessions that were not inoculated with the strains, and an uninoculated control was adopted (no inoculation and no fertilizer application). The experiment was a 10×6 factorial arrangement in a randomized complete block design. The results obtained revealed that higher nodule numbers and weight were recorded in TVSu-1739 and TVSu-475 in both locations and seasons, compared to other accessions. The highest (62-67 kg ha^{-1}) N fixed was recorded in TVSu-1698 in both locations and seasons. Additionally, *B. japonicum* strains—RACA6 and USDA110—significantly enhanced the amount of N fixed (73.32kg ha^{-1} and 75.95kg ha^{-1} respectively) than other *B. japonicum* strains inoculated and inorganic N fertilizer (urea) applied.

Keywords: Bacteria strains, inoculation, nitrogen fixation, nodule formation, underutilised legume.

7.1 Introduction

The most abundant gas in the atmosphere is N. But, only the reactive types of N, that is, the oxidized form can be absorbed by the plants (Burén and Rubio 2018). Nucleic acids (DNA and RNA), urea, adenosine triphosphate (ATP), amino acids (proteins) and nicotinamide adenine dinucleotide (NAD) are components of nitrogen in living cells (Burén and Rubio 2018). Nitrogen gas (N_2) conversion to the biologically acceptable form of nitrogen (NH_3) by plant could be performed either by Biological Nitrogen Fixation (BNF) by certain bacteria or via the industrial Haber-Bosch process. Different types of plant-beneficial microrganisms live in the soil and usually interact with plants and other types of microorganisms (Igiehon and Babalola 2018a).

The interaction between rhizobia and legume type is usually determined by nodulation from rhizobia and structure of Nod factor in the host (Oldroyd *et al*. 2011).This is usually initiated by the host plant which exudes from the root flavonoid molecules which are recognised by their rhizobia partners (Ferguson *et al*. 2010; Sprent *et al*. 2017b). These flavonoids attract the bacteria to the roots and help them to produce nod factor molecules in the host plants, leading to the development of root organs called nodules (Ferguson *et al*. 2010). These nodules therefore provide a conducive environment for BNF which the rhizobia release for their host plant for carbohydrates exchange. (Ferguson *et al*. 2019).

Soil structure and fertility, diversity of the beneficial microorganisms and water-holding capacity have also been affected as a result of excessive use of agrochemicals (Enagbonma and Babalola 2019a). *Rhizobium* was the first genus of bacteria which resulted to the use of the name for the nitrogen-fixing bacteria of legumes (Basile and Lepek 2021). There are about 112 species of *rhizobium* thus making rhizobia the largest root-nodulating genus (Lindström and Mousavi 2020). The *nod*, *nif*, and *fix* genes usually control the symbiotic nitrogen fixation in rhizobia. The bacteria accessory genes are enclosed in the *nod* and *nif* proteins and are placed in genetic elements like chromids, plasmids and symbiotic islands. These can be transferred in high frequencies within species of a bacteria genus and infrequently between genera (Remigi *et al*. 2016a). Bambara groundnut is a leguminous crop that is underutilized and under-researched in Africa. Usually, it can fix nitrogen through a mutual relationship with bacteria in the soil called 'rhizobia' (Puozaa *et al*. 2017b).

There is little information on the type of rhizobia that nodulate Bambara groundnut in tropical soils. Nevertheless, some research studies have reported that Bambara groundnut can be nodulated by *Bradyrhizobium* (Grönemeyer *et al*. 2014). Legumes can be nodulated by different rhizobia. Also, the inoculation of stress-tolerant strains of *rhizobium* may enhance nodulation and nitrogen-fixation ability of Bambara groundnut under stress conditions. Furthermore, very little research has been done on nitrogen fixation in Bambara groundnut (Mohale *et al*. 2014b). Based on a research conducted by Yakubu *et al*. (2010), Bambara groundnut has a high N fixing potential provided an effective and appropriate *rhizobium* strain is used as an inoculant for the underutilized legume. Adegboyega *et al*. (2021a) reported that the amount of nitrogen fixed (kg ha^{-1}) in the shoot of wing bean varies among accessions. Therefore, this study aims to evaluate the effect of *B. japonicum* strains on different accessions of Bambara groundnut which differs in genetic composition and nitrogen-fixing ability to improve the yield of the legume and reduce the problem of food insecurity in the global economy.

7.2 Materials and Methods

Pot experiments were conducted in a screenhouse from August to December in the 2018 and 2019 cropping season in Ibadan, Nigeria. Field studies were also carried out from August to December in the 2019 and 2020 cropping season in two locations: Ibadan and Ikenne, Nigeria. The pot experiments were arranged in a completely randomized design (CRD) in triplicates. The field experiments were arranged in Randomized complete block design (RCBD). Soil samples for these experiments were sieved through 3mm sieve, sterilized at 121°C for 1 hour using the autoclave before planting according to (Davies and Owen 1951). The pot and field experiment were laid out in 10 x 6 factorial arrangement. Ten accessions of Bambara groundnut, namely: TVSu-305, TVSu-365, TVSu-787, TVSu-1698, TVSu-710, TVSu-506, TVSu-1606, TVSu-475, TVSu-1739 and TVSu-378 were randomly selected at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Strains of *Bradyrhizobium japonicum*, USDA110, IRJ2180A, RACA6 and FA3, an un-inoculated control and N fertilizer (Urea-46% N). The strains were obtained at Soil Microbiology Laboratory, IITA, Ibadan, Nigeria.

The seeds of accessions of Bambara groundnut were carefully surface sterilized and two seeds were sown in 10kg pot containing sterile and non-sterile soil in the screenhouse. Ten (10) days after emergence of the seedlings, 3ml of broth form of the *B. japonicum* strains were inoculated to some seedlings and N fertilizer 20kg/ha in form of urea (46%) were also applied to seedlings that were not inoculated. The uninoculated plants served as control. N free nutrient solution and sterile water were added to each pot at a regular interval (Shamseldin 2013). Ten Bambara groundnut accessions used in the pot experiments were also used in the field in both locations and seasons. Six seeds from each accessions were coated with gum Arabic and *B. japonicum* strains USDA110, IRJ2180A, RACA6, and FA3 separately before planting in the both locations and seasons.

Nitrogen fertilizer 20kg/ha in form of urea (46%) was applied to the seedlings that were not coated two weeks after emergence. Seeds neither coated with bacteria strains nor supplied with nitrogen fertilizer served as control.

At 50% flowering, plant samples were collected from pots and fields (Ibadan and Ikenne) in both seasons, 2018/2019 and 2019/2020, respectively. The samples were taken to Soil Microbiology Laboratory, IITA for further analysis. Plant roots were carefully rinsed to recover the nodules. The nodules were then counted and carefully placed on the Mettler balance to determine the fresh weight. Fresh weight of the shoots were determined before samples were oven-dried for the dried weight determination. The dried samples were used to determine the total nitrogen available by the Macro kjeldahl oxidation method which entails digestion and distillation. The estimation of nitrogen fixation on the field was determined by ureide method (Giller *et al*. 2016). Nitrogen fixation in the screenhouse was determined by nitrogen difference method, as described by Yakubu *et al*. (2010).

7.2.1 Pot Experiments

% Nitrogen derived from atmosphere (Ndfa) was calculated using the formula:

$$X = \frac{D \times N}{V}$$

$$Y = L - R$$

$$K = \frac{L - R \times 100}{L}$$

Where,

X= Total Nitrogen in plants

D = Dry matter weight

N= % Nitrogen in plants

V= 100

Y= Nitrogen fixed

L= Total Nitrogen in legume

R= Total Nitrogen in reference crop (maize)

K= % (Ndfa).

7.2.2 Field Experiments

Field studies were also carried out from August to December of the 2019/2020 cropping season in Ibadan and Ikenne, Nigeria. Ten Bambara groundnut accessions that were used in the pot experiments were also used on the field. Ikenne belongs to Tropical savanna, Latitude (Lat) 7° 51' 56" North and Longitude (Long) 3° 42' 54" East while Ibadan belongs to Sub humid, Lat 7° 22´ 30 N and long 3° 45´ 54 E.

Seeds from each accessions were coated with Arabic gum and nitrogen-fixing bacteria—FA3, USDA110, IRJ2180A, and RACA6—and allow to dry firmly to the seeds before sowing in both sites and seasons. Nitrogen fertilizer 20kg/ha in form of urea (46%) was applied to the seedlings that were not coated with *B. japonicum* strains two weeks after emergence. Seeds that were not coated served as control (zero inoculation and zero fertilizer applications) and maize was planted as a reference crop also in triplicate to determine the nitrogen-fixing potential of the legume, using the nitrogen difference method. Weeding was done manually at regular intervals.

At 50% flowering, plant samples were collected from the fields (Ibadan and Ikenne) in both seasons, 2018/2019 and 2019/2020, respectively at the same time. Plant roots were carefully rinsed to separate nodules from roots. The nodules were carefully counted and then placed on a mettler balance to determine the fresh weight. Fresh weight of the shoots were determined before samples were oven-dried at 65°C for 48 hour. Analysis was carried out in the Soil Microbiology Laboratory of IITA. The grinded samples were used to determine the estimation of nitrogen fixation on the field using the ureide method (Giller et al. 2016).

The relative ureide in stem extract can be calculated as

$$\text{Relative Ureide N} = \frac{K \times a}{(L\,a + b)}$$

Where N= N %

K= 400

a = Molar concentration of ureides

b = molar concentration of nitrates

L = 4 (Herridge and Peoples 1990)

7.2.3 Ureide Determination

7.2.3.1 Extraction

Zero point five gram (0.5 g) of dried and grinded stems were transferred into 100 ml beaker and 25 ml of distilled water was introduced to each subsample and boiled for 1-2minutes. The samples were carefully filtered into a volumetric flask (50 ml) using filter paper (15 cm) and distilled water was added to make it up to 50ml. Eluent were kept and stored in vials in a freezer (-18°C) until analysis of N solutes (Shamseldin 2013).

7.2.3.2 Reagent Preparation

Two gram (2 g) of sodium hydroxide (NaOH) was added to 100ml beaker with further addition of 6.5ml concentrated hydrogen Chloride (Hcl). Also, 0.33g of phenylhydrazine and 1.67g of Potassium ferricyanide were added to 100ml distilled water. Furthermore, 400ml concentrated Hcl was decanted from the bottle and 39.53mg allantoin was added to 250ml of distilled water (Shamseldin 2013)

7.2.3.3 Procedure

Two point five millilitres (2.5 ml) each of the 5 concentrations was added to duplicate test tube and 0.5 ml of 0.5 Normal sodium hydroxide. The tubes were preserved in a boiling water bath for 10minutes (incubated). Then mixture of 1.0ml of 0.65 Normal HCl and phenylhydrazine inside the tubes were placed in boiling water for exactly 2 minutes. The tubes were later incubated in ice for 15minutes, after which 2.5ml of HCl/KFeCn was added, thoroughly mixed, and allowed to stay for 10 minutes for the development of colour and accurate results. The samples were measured at 525 nm (absorbance) using a spectrophotometer (Peoples *et al*. 1989).

7.2.4 Nitrate Determination

7.2.4.1 Reagent Preparation

Half of a grams (5 g) of salicylic acid was added to 100 ml H_2SO_4 in a beaker. Also, 40 g of NaOH was added to the 500 ml of distilled water and 632 mg KNO_3 was added to 250 ml of distilled water.

7.2.4.2 Procedure

Point zero five millimetre (0.05 ml) of each of the concentration was pipetted into duplicate test tubes and 0.02 ml of salicylic acid was pipetted into the tubes and allowed to mix and leave on the bench for 20 minutes. The solution was allowed to clear and left on the bench for 10minutes and was later read at O.D (optical density) at 410nm on a spectrophotometer.

7.2.4.3 Data Analysis

Data were subjected to a four-way ANOVA: pot (Accessions, treatments, soil status and season), field (Accessions, treatments, locations and seasons) using SAS package 9.4 and means obtained were therefore separated using Ducan Multiple Range Test (Dmrt) at $P \leq 0.05$ (Zatybekov *et al*. 2017).

7.3 Results

Table 7.1: Analysis of variance showing the sources of variation for nodule numbers, weight and fixed nitrogen on the field

Source of variation	DF	Nodule number	Nodule weight(g)	Nitrogen fixed(kg/ha)
Accessions	9	629.24**	19.55**	217.84*
Treatments	5	97.38**	3.59ns	15336.58**
season	1	204.68*	797.11**	5548.10**
location	1	22.63ns	25.07**	823.69**
Accessions*treatments	45	40.51ns	1.89ns	126.58ns
Accessions*season	9	168.21**	18.14**	287.71**
Accessions*location	9	72.31ns	3.42ns	151.19ns
Treatments*season	5	52.73ns	2.51**	348.06**
season*location	1	1393.73**	79.67**	6.19ns
Treatments*location	5	66.09ns	1.66ns	156.95ns
Accessions*treatment*location	45	25.60ns	3.53ns	89.17ns
Treat*location*season	5	72.14ns	3.02ns	136.21ns
Access*treatm*location*season	131	32.38ns	1.94ns	111.66ns
Rep	2	47.83ns	29.57**	116.09ns

Key: DF-Degree of freedom, ** (p<0.01)-Very significant, * (p< 0.05)-significant, ns- not significant.

Table 7.2: Influence of *B. japonicum* strains on nodule number, weight and N fixation of Bambara groundnut in both locations and seasons

Strains	Nodule number	Nodule weight (g)	Nitrogen fixed (kg/ha)
FA3	7.80[a]	1.16[a]	69.95[b]
IRJ2180A	7.49[a]	1.17[a]	72.02[b]
RACA6	7.32[ab]	1.47[a]	75.95[a]
USDA110	6.92[ab]	1.26[a]	73.32[ab]
N	5.35[b]	1.06[a]	42.41[c]
control	7.07[ab]	1.42[a]	44.07[c]

Mean followed by the same letter are not significantly different at 5% level of probability using the (DMRT), and mean comparison for each trait is along each column

Table 7.3: Accessions by treatment interaction for nodule number, weight and N fixation in both locations and seasons

Accession	Strains	Nodule number	Nodule weight (g)	Nitrogen fixed (kg/ha)
TVSu-305	FA3	4.70 ± 5.40	1.04 ± 1.93	68.22 ± 17.81
	IRJ2180A	4.62 ± 7.24	1.24 ± 2.56	73.57 ± 13.01
	N	2.32 ± 4.22	0.50 ± 1.05	40.91 ± 5.80
	RACA6	5.92 ± 6.76	0.85 ± 1.70	72.52 ± 13.83
	USDA110	5.64 ± 7.49	0.99 ± 1.59	62.63 ± 11.87
	control	4.06 ± 6.38	0.73 ± 1.41	42.29 ± 4.14
TVSu-365	FA3	10.41 ± 9.3	1.26 ± 1.48	70.86 ± 12.29
	IRJ2180A	6.83 ± 5.81	1.13 ± 1.58	73.11 ± 9.63
	N	5.86 ±5.55	1.89 ±2.20	43.64 ± 6.47
	RACA6	4.17 ± 4.51	1.14 ± 1.75	81.10 ± 9.52
	USDA110	5.48 ± 5.55	1.35 ± 1.75	73.26 ± 14.22
	control	8.15 ± 6.16	1.85 ± 2.39	42.29 ± 4.14
TVSu-378	FA3	8.26 ± 5.85	1.08 ± 1.68	71.09 ± 9.89
	IRJ2180A	5.99 ± 6.56	0.69 ± 1.39	65.31 ± 15.92
	N	5.09 ± 4.72	0.82 ± 1.16	37.07 ± 8.14
	RACA6	6.69 ± 6.53	2.05 ± 2.99	72.64 ± 8.49
	USDA110	10.96 ± 6.9	1.73 ± 1.83	73.42 ± 10.96
	control	6.73 ± 6.88	1.53 ± 2.52	41.27 ± 4.96
TVSu-506	FA3	4.52 ± 7.41	0.63 ± 1.18	67.14 ± 5.39
	IRJ2180A	2.37 ± 5.52	0.69 ±1.61	66.37 ± 11.70
	N	1.80 ± 3.08	0.49 ± 1.14	38.89 ± 4.90
	RACA6	3.13 ± 5.95	0.68 ± 1.43	73.86 ±13.59
	USDA110	2.83 ± 4.48	0.70 ± 1.53	68.10 ± 6.45
	control	4.06 ± 4.39	0.91 ± 1.31	41.82 ± 4.94
TVSu-710	FA3	2.15 ± 2.71	0.33 ± 0.71	76.51 ± 12.83
	IRJ2180A	5.51 ± 5.56	0.81 ± 1.29	76.84 ± 9.96
	N	2.31 ± 2.72	0.94 ± 2.38	40.45 ± 10.57
	RACA6	4.29 ± 7.78	0.43 ± 0.75	72.19 ± 17.97
	USDA110	6.11 ± 8.12	0.78 ± 1.10	75.54 ± 16.80
	control	4.65 ± 5.57	0.61 ± 0.92	41.82 ± 8.94

TVSu-787	FA3	8.42 ± 9.22	1.60 ± 2.23	73.06 ± 9.94
	IRJ2180A	7.30 ± 4.96	1.59 ± 2.05	71.21 ± 12.53
	N	5.38 ± 4.93	0.96 ± 1.52	46.74 ± 11.13
	RACA6	11.30 ± 12.6	2.27 ± 2.88	76.88 ± 5.83
	USDA110	6.95 ± 5.23	1.27 ± 1.59	76.30 ± 9.23
	control	7.28 ± 6.69	1.67 ± 2.28	50.57 ± 18.03

TVSu-1698	FA3	10.76 ± 10.8	1.57 ± 1.81	64.87 ± 22.51
	IRJ2180A	8.49 ± 9.89	1.16 ± 1.56	79.69 ± 10.21
	N	4.59 ± 7.18	0.84 ± 1.49	48.92 ± 13.55
	RACA6	7.80 ± 6.00	2.16 ± 2.62	78.32 ± 13.38
	USDA110	7.21 ± 5.64	1.36 ± 1.61	75.92 ± 13.23
	control	11.7 ± 12.7	3.08 ± 4.38	45.08 ± 12.30
TVSu-1739	FA3	12.9 ± 12.0	1.29 ± 1.46	75.08 ± 10.79
	IRJ2180A	14.6 ± 14.5	1.61 ±2.29	71.01 ± 17.69
	N	10.1 ± 10.4	0.99 ±1.29	40.96 ± 6.03
	RACA6	14.5 ± 10.9	1.84 ± 1.92	70.86 ± 15.33
	USDA110	13.4 ± 5.96	1.66 ± 1.64	74.43 ± 12.56
	control	9.49 ± 7.95	1.18 ± 1.19	42.59 ± 5.60
TVSu-475	FA3	10.6 ± 12.9	1.89 ± 2.66	61.09 ± 12.43
	IRJ2180A	12.3 ± 9.65	1.98 ± 2.49	66.99 ± 12.87
	N	13.6 ± 9.60	2.94 ± 3.38	43.77 ± 5.93
	RACA6	10.2 ± 5.43	2.49 ± 2.69	77.33 ± 11.13
	USDA110	6.79 ± 8.88	2.01 ± 4.43	77.37 ± 4.32
	control	11.7 ± 9.68	2.24 ± 2.35	44.54 ± 5.03
TVSu-1606	FA3	3.36 ± 4.48	0.53 ±1.20	75.08 ± 9.07
	IRJ2180A	6.98 ± 10.7	0.77 ± 1.55	77.87 ± 12.02
	N	2.33 ± 4.52	0.25 ± 0.83	40.56 ± 3.59
	RACA6	5.17 ± 7.23	0.81 ±1.52	82.99 ± 4.99
	USDA110	3.88 ± 5.62	0.74 ± 1.53	73.09 ± 18.27
	control	2.92 ± 3.30	0.38 ± 0.97	40.32 ± 5.51
CV (%)		106.6	117.6	17.1

Mean ± Standard error

Table 7.4: Analysis of variance showing the sources of variation for four traits under screenhouse conditions in both seasons

Source of Variation	DF	Nodule number	Nodule weight (g)	Nitrogen fixed (kg/ha)	% Ndfa
Accessions	9	72.26*	0.41^{ns}	25.1^{ns}	1709.2*
Treatments	5	975.45**	3.05**	0.55*	532.2^{ns}
Soil status	1	376.65*	0.70ns	28.7**	303.8^{ns}
Season	1	3249.73**	146.45**	55.6^{ns}	520.1^{ns}
Accessions*treatments	45	28.40^{ns}	0.09ns	24.6^{ns}	532.3^{ns}
Accessions*soil status	9	27.73^{ns}	0.71*	26.6^{ns}	361.2^{ns}
Acessions*season	9	22.12^{ns}	0.30ns	36.0^{ns}	491.3^{ns}
Treatments*soil status	5	67.50*	1.33**	53.5^{ns}	540.9^{ns}
Treatments*season	5	365.46**	3.51**	23.5^{ns}	1044.6^{ns}
Accessions*treatment*season	45	38.25ns	0.164^{ns}	0.32^{ns}	852.00^{ns}
Treatment* soil status*seasn	5	325.21*	4.21*	34.24^{ns}	731.21^{ns}
Aces*treat*soil*season	45	35.91*	0.19ns	30.3^{ns}	801.0^{ns}
Rep	2	433.08**	7.06**	26.9^{ns}	3608.1*

** (P<0.01)-Very significant, * P (<0.05)-significant, ns- not significant.

Table 7.5: Response of Bambara groundnut accessions to treatments on nodule number and the % N derived from the atmosphere under screen House conditions

Accessions	Nodule Number	%Ndfa
TVSu-787	12.02^{a}	31.03^{b}
TVSu1739	11.35^{ab}	33.48^{b}
TVSu-305	10.96^{abc}	36.73^{b}
TVSu1606	10.62^{abcd}	38.59^{ab}
TVSu-378	10.08^{abcd}	35.79^{b}
TVSu-475	9.76^{bcd}	47.93^{a}
TVSu-506	9.58^{bcd}	32.52^{b}
TVSu1698	9.35^{bcd}	40.59^{ab}
TVSu-365	9.11^{dc}	37.36^{ab}
TVSu-710	8.64^{d}	39.89^{ab}

Mean followed by the same letter are not significantly different at 5% level of probability using the (DMRT), and mean comparison for each trait is along each column

Table 7.6: Influence of treatments on nodule number, weight, and N fixation of Bambara groundnut under screenhouse conditions in both seasons

Strains	Nodule number	Nodule weight(g)	Nitrogen fixed (kg/ha)
FA3	11.10[a]	0.62[b]	29.8[a]
USDA110	11.12[a]	0.57[b]	29.3[a]
IRJ2180A	12.34[a]	0.66[b]	29.6[a]
RACA6	11.36[a]	0.57[b]	28.4[a]
N	10.75[a]	1.00[a]	28.2[a]
CONTROL	4.55[b]	0.22[c]	15.1[b]

Mean followed by the same letter are not significantly different at 5% level of probability using the (DMRT), and mean comparison for each trait is along Each column

Table 7.7: **Soil analysis before and after growing Bambara groundnut of nodulating legume in locations**

Year	2019		2020		2019		2020	
Soil properties	After	Before	After	Before	After	Before	After	Before
pH(H_2O) 1:1	4.86	4.46	4.85	4.91	6.65	6.84	6.61	7.13
N (%)	0.09	0.07	0.09	0.07	0.08	0.15	0.07	0.12
OC (%)	0.44	0.33	0.49	0.29	0.45	0.41	0.59	0.34
Bray P (ppm)	23.17	13.23	24.22	22.46	12.69	15.35	18.02	52.84
Sand (%)	76.00	76.00	78.00	76.00	82.00	83.00	83.00	82.00
Clay (%)	18.00	16.00	16.00	20.00	10.00	10.00	11.00	8.00
Silt (%)	6.00	6.00	6.00	3.00	8.00	7.00	6.00	10.00
Ca (Cmol/kg)	1.32	3.53	1.42	1.51	0.845	1.22	0.64	1.10
Mg (Cmol/kg)	0.42	0.80	0.38	0.40	0.039	0.08	0.04	0.16
K (Cmol/kg)	0.27	0.56	0.38	0.24	0.163	1.38	0.04	0.20
Na (Cmol/kg)	0.07	0.08	0.07	0.08	0.067	0.06	0.08	0.06
Zn (ppm)	1.19	1.96	1.62	2.23	1.376	1.19	1.64	0.66
Cu (ppm)	2.26	1.17	0.95	1.19	0.222	0.86	0.39	0.97
Mn (ppm)	15.29	12.15	11.88	2.05	136.74	244.2	133.39	383.6
Fe (ppm)	28.38	25.58	20.92	116.7	133.33	133.3	128.67	114.4

7.3.1 Response of Accessions to Inoculation

Significant differences were recorded among the accessions, treatments, location, season, interactions of accessions with season, interactions of treatments with season and season with location in nodule number, weight and nitrogen fixation in both locations and seasons (Table 7.1). No significant differences were recorded in the interactions of accessions with treatments, accessions with locations, treatments with location and accessions with treatment with season with location in the nodule number, weight and nitrogen fixed in both locations and seasons (Table 7.1). No significant differences were recorded in the nodule number, weight and nitrogen fixed in the interaction of accessions with treatments and location and interaction of treatments with soil status and season (Table 7.1). No significant differences were recorded among the treatments and uninoculated control in the nodule number and weight in both locations and seasons but were significantly higher than the nitrogen fertilizer applied in the nodule number (Table 7.2). RACA6 and USDA110 strains are not different in the amount of nitrogen fixed but are significantly higher than FA3 and IRJ2180A strains which are significantly higher than the nitrogen fertilizer applied and the uninoculated control in both locations and seasons (Table 7.2). The *B. japonicum* strains and nitrogen fertilizer applied to the Bambara groundnut accessions enhanced the nodule number, weight and nitrogen fixed of all the accessions in both locations and seasons compared to the uninoculated control (Table 7.3).

7.3.2 Interplay Between the *B. japonicum* and Nitrogen Fertilizer

No significant differences were recorded among the Bambara groundnut accessions inoculated in the screenhouse in the nodule weight and nitrogen fixed, but significant differences were recorded among the Bambara groundnut in the nodule number and the amount of nitrogen derived from the atmosphere in the screenhouse (Table 7.4). Significant difference were also recorded among the treatments in the nodule number, weight and nitrogen fixed in the screenhouse in both seasons but no significant differences were recorded among the treatments in the amount of nitrogen derived in the atmosphere in the screenhouse in both seasons (Table 7.4).

Furthermore, significant differences were recorded among the treatments in the nodule number, nitrogen fixed and amount of nitrogen derived in the atmosphere in the screenhouse in both seasons but no significant differences were recorded among the treatments in the nodule weight in the screenhouse in both season. No significant differences were recorded in the interaction of accession with treatments, accessions with soil status and accession with season in nodule number, nodule weight, nitrogen fixed and the amount of nitrogen derived from the atmosphere in the screenhouse in both seasons (Table 7.4). No significant differences were recorded in the interaction of treatments with season, treatments with soil status and accessions with treatments with soil status and season in the nitrogen fixed and the amount of nitrogen derived in the atmosphere in the screenhouse but significant differences were recorded among the interactions in the nodule number and weight (Table 7.4). Significant differences were recorded in the nodule number and weight but no significant differences were recorded in the nitrogen fixed and nitrogen derived from the atmosphere in the interaction of treatments with soil status with season (Table 7.4). No significant difference were recorded in the nodule number, weight, nitrogen fixed and nitrogen derived from the atmosphere in the interaction of accession with treatments with season (Table 7.4).

No significant difference were recorded among TVSu-787. TVSu-1739, TVSu-305, TVSu-1606 and TVSu-378 in the nodule number recorded in the screenhouse in both seasons but they were significantly higher than TVSu-475, TVSu-506 and TVSu-1698 which are not significantly different in the number of nodules recorded in the screen house in both seasons. These were, however, significantly higher than TVSu-365 and TVSu-710. Furthermore, no significant differences were recorded among TVSu-475, TVSu-1698, TVSu-365, TVSu-710 and TVSu-1606 in the amount of nitrogen derived from the atmosphere in the screenhouse in both seasons but they were significantly higher than TVSu-787, TVSu-1739, TVSu-305, TVSu-378 and TVSu-506 which were not significantly different (Table 7.5)

No significant differences were recorded among the *B. japonicum* strains and the nitrogen fertilizer applied to the Bambara groundnut accessions in the number of nodules and nitrogen fixed recorded in the screenhouse in both seasons but both were significantly higher than the uninoculated control (Table 7.6). The nitrogen fertilizer applied was significantly higher than the *B. japonicum* strains and uninoculated control in the weight of nodules recorded in the screenhouse in both seasons but no significant differences were recorded among the *B. japonicum* strains in the nodule weight recorded in the screenhouse in both seasons which were, however, significantly higher than the uninoculated control (Table 7.6).

The soil analysis results before and after growing Bambara groundnut, eventually revealed that the inoculation of bacteria strains have a significant effect on the soil even after uptake of essential nutrients needed for growth and developments. After harvesting the legume, high nitrogen content, organic carbon, and other soil properties were available in the soil in moderate quantities, which showed that inoculation of bacteria strains helped to improve soil nutrients unlike nitrogen fertilizers that contaminate the soil and affect human health after use (Table 7.7).

7.4 Discussion

The inoculation of *B. japonicum* strains to accessions showed diverse responses in nodulation and nitrogen-fixing potentials in relation to locations and years. Significant differences were observed among accessions, interaction of accessions and strains in nodulation and Nitrogen fixation in Ibadan and Ikenne in 2019 and 2020. These findings are in accordance with Htwe *et al*. (2018) in which it was revealed that *B. japonicum* inoculation singly promote nodulation potential and nitrogen fixation of soybean. Also, significant differences were observed among strains inoculated to accessions during nodulation in Ibadan in 2019 but there were no significant differences recorded among strains inoculated during nodulation in Ibadan in 2020, Ikenne in 2019 and Ikenne in 2020 respectively. These observations correlate with the findings of Egamberdieva *et al*. (2018) in which the lowest nodule number was recorded on soybean roots grown under a low Phosphorus supply level regardless of the nitrogen supply.

Notably, inoculation of different strains improved nodulation of accessions in both locations and years, though significant differences were not recorded among the strains inoculated. This is in accordance with the findings of Leggett *et al*. (2017) where inoculation of bacteria strain enhanced nodulation potential of soybean in United states and Argentina in different years. Conversely, inoculation of strains also enhanced the nitrogen-fixation of accessions in Ibadan and Ikenne in both years, which correlates with a study of Zimmer *et al*. (2016) in which *B. japonicum* inoculation enhanced nitrogen fixation of soybean. Mbah and Dakora (2018b) observed that *B. japonicum* inoculation enhanced nodulation potential and nitrogen fixation in kersting groundnut and Bambara groundnut.

Over 20% increase was recorded among accessions in Ibadan and Ikenne in nodule number, nodule weight and nitrogen fixation. Moreover, the inoculation of FA3 strains enhanced nodule number, weight and nitrogen fixed of TVSu-1739 in both Ibadan and Ikenne in both years than other inoculated accessions. Inoculated strains also enhanced nodulation potential and derived nitrogen from the atmosphere of accessions when compared to the uninoculated control in the sterile and non-sterile soil in the screenhouse which were related to a report of a research conducted by Adegboyega *et al*. (2021b) that concluded that nitrogen fixation and derived nitrogen from the atmosphere were obtained in the shoot and root of accessions of wing bean. Inoculated strains significantly enhanced nodule number and derived nitrogen from the atmosphere in TVSu-787 and TVSu-475 respectively than other inoculated accessions.

B. japonicum strains inoculation enhanced the nodule number and nitrogen fixation respectively in Ibadan and Ikenne in both years compared to the uninoculated control. This study eventually revealed the response of inoculated accessions in both locations and seasons and in the screenhouse. Greater responses were recorded among accessions in the nodule weight and number, nitrogen fixation and derived nitrogen from the atmosphere in relation to inoculated strains. The inoculation of *B. japonicum* strains could help improve the nitrogen-fixing potentials of accessions, thereby improving productivity of the Bambara groundnut and reducing the problem of food shortage in the economy.

7.5 Conclusions

The inoculation of bacteria strains improved the nodulation potential and nitrogen fixation of Bambara groundnut accessions than the inorganic nitrogen fertilizer. When seed are coated with nitrogen-fixing bacteria (*B. japonicum*) before planting, the bacteria develop a mutual relationship with the root of the legume and develop inside the nodule structure which converts the atmospheric nitrogen to a form utilizable to the Bambara groundnut accessions for optimum fixation.

Precisely, in this study, FA3 and RACA6 strains significantly improved nodulation potential and nitrogen fixation of inoculated Bambara groundnut accessions than other bacteria strains, inorganic nitrogen fertilizer application, and uninoculated control. Therefore, the use of bacteria strains to replace the use of inorganic nitrogen fertilizer can help to promote the nodulation potential and nitrogen fixation of accessions of Bambara groundnut. Also the use of bacteria strains give no residual effect on the soil after planting unlike inorganic nitrogen fertilizer.

CHAPTER EIGHT

Effects of *Bradyrhizobium japonicum* Strains on Growth and Nutrient Uptake of Bambara Groundnut (*Vigna subterranea (L) Verdc)* Accessions in Sterile and Non-sterile Soil under Screenhouse Conditions

Abstract

An experiment was carried out in the screenhouse from August to December, 2018 and 2019 using a sterile and non-sterile soil. The study used ten accessions of Bambara groundnut: TVSu-475, TVSu-1698, TVSu-787, TVSu-1606, TVSu-506, TVSu-710, TVSu-365, TVSu-378, TVSu-1739 and TVSu-305. *B. japonicum* strains coated to seeds of Bambara groundnut accessions were: USDA110, RACA6, FA3, IRJ2180A and inorganic nitrogen fertilizer was applied to the seedlings of uninoculated plants at two weeks after planting, and an uninoculated control was used. The experiments were arranged in a completely randomized design (CRD) and were replicated three times. Data were collected on growth parameters, fresh and dry biomass yield, nutrients uptake at flowering and at harvest, nodulation, and N fixing potential. Data collected were subjected to analysis of variance and means were separated using the Ducan multiple range test (DMRT) at 5% level of probability. The study revealed that inoculation of *B. japonicum* strains in non-sterile soil significantly enhanced the growth parameters at 5WAP and 10WAP. Also, fresh and dry biomass yield, nutrient uptake at flowering and at harvest, and nodulation potential were significantly enhanced in non-sterile soil than inoculation in the sterile soil in both seasons. N fixed in the sterile soil reflect significant differences over the non-sterile soil in both seasons.

Keywords: Inoculation, indigenous rhizobia, underutilized legume, soil condition, fixation

8.1 Introduction

The harmful effect of inorganic nitrogen fertilizer increases due to its continuous use, and these are harmful to human health and ecological systems (Enagbonma and Babalola 2019a). However, smallholder farmers usually have difficulty in procuring inorganic nitrogen fertilizers to amend their soil because of the expensive rate (Ngetich *et al*. 2012). Transformation of carbon-based organic materials is the beneficial role that rhizobacteria can play to enhanced plant health and promote sustainable agriculture (Olanrewaju *et al*. 2017). Nitrogen-fixing bacteria are affordable and suitable alternative to inorganic N fertilizers for small-scale farmers in Africa.

Nodulating legumes are also well known to support and contribute substantial amount of symbiotic Nitrogen to cropping systems (Mohale *et al*. 2014b). These bacteria are helpful in different ways of mechanisms which include stimulation of root growth crucial for nutrient acquisition, recycling of essential elements, solubilization of mineral nutrients, producing numerous plant growth regulators, decomposition of organic matter, soil structure formation, degrading organic pollutants, bio-control of soil and seed borne plant pathogens, and in promoting changes in vegetation (Sivasakthi *et al*. 2014).

Plant growth promoting bacteria can reduce the use of inorganic nitrogen fertilizers and it is economically and environmentally beneficial due to the reduced cost of procuring the bacteria. It is suitable for soil fertility restoration and sustainable crop production (Maheshwari 2012). The application of beneficial microorganisms by coating the seeds or directly putting them into the soil are processes for introducing the microbial inoculants into the soil where they will be well positioned to effect seedling roots. However, based on the history of inoculation of legume seeds with rhizobia species and laboratory operation of the ability of a wide range of other beneficial microorganisms to improve crop performance, there are still very few commercially available microbial seeds or soil inoculants made available to small scale farmers because most research on microbial seeds are limited to researchers only and do not get to small scale farmers. Leguminous plants often require high amount of Nitrogen for optimum grain yield, which is achievable through Biological Nitrogen Fixing (Hungria and Kaschuk 2014). Most low income farmers usually plant legumes without adding fertilizers to the legume thereby resulting to low grain yields. Under such conditions, legumes depend primarily on indigenous Biological Nitrogen Fixation through mutual symbiosis with rhizobia to partially or fully meet the amount of Nitrogen required (Hungria and Kaschuk 2014). Such attribute of native rhizobia is among the several major factors limiting legume production. In Ghana, the use of bacteria strains to inoculate legume has just been introduced and is still new to small-scale farmers. The beneficial effect and importance of rhizobia inoculant on soybean were also demonstrated in Kenya (Thuita *et al*. 2012). *Rhizobium* inoculation is classified as an option for increasing yield of leguminous crop in N deficient soils. The objective of this study is, therefore, to access the effect of inoculation of *B. japonicum* strains on Bambara groundnut accessions in two different soil conditions (sterile and non-sterile soil).

Figure 8.1: Determining the quantity of indigenous rhizobia in the soil used in 2018 and 2019
Photo: T. D Bitire

8.2 Materials and Methods

An experiment was carried out in the screenhouse from August to December, 2018/2019 at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The experiment was arranged in a completely randomized design (CRD). Ten accessions of Bambara groundnut were used--TVSu-475, TVSu-1698, TVSu-787, TVSu-1606, TVSu-506, TVSu-710, TVSu-365, TVSu-378, TVSu-1739 and TVSu-305—as well as four strains of *B. japonicum*—namely FA3, RACA6, USDA110, and IRJ2180A, ($2.8x10^7$,$7.2x10^6$,$4.3x10^7$ and $1.4x10^7$cfu/ml). N fertilizer (urea- 46%) application was applied two weeks after planting and an uninoculated control was used. 10 kg of sterile and non-sterile soil each were filled into each pot and two seeds of each accessions were sown into pots containing sterile and non-sterile soil.

The experiment was replicated three times. Two weeks after germination, broth solution containing each of the strains was applied separately to the seedlings in the glasshouse and regular application of sterile water and Nitrogen-free nutrients solution (micronutrient composition) was applied into both sterile and non-sterile pots. Adequate weeding and spraying were done (termex) to control insecticides from destroying the vegetative stage of the Bambara groundnut accessions. Data collected were growth traits which includes number of leaves, number of branches, plant height, chlorophyll, leaf area, plant spread and number of stem.

At 50% flowering, and at harvest, weight of fresh and dry shoot as well as fresh and dry root of samples were also recorded. Data obtained were subjected to analysis of variance using the two-way ANOVA, in SAS version 9.4 and mean was separated using the Duncan multiple range test (DMRT) at ($P<0.05$). The presence of native rhizobia in the pots was estimated using most probable number (MPN). The counting method was estimated from six folds dilution series replicated four times (Somasegaran and Hoben (1985) and the diluent used for the MPN determination containing salt found in yeast-extract mannitol broth Vincent (1970a). The amount of indigenous rhizobia was determined using a sterile soil in the growth room, with temperature of 28^{o}C, of the Soil Microbiology Unit, IITA, Ibadan, Nigeria (Figure 8.1).

8.3 Results

Table 8.1 Analysis of variance showing growth traits of accessions of Bambara groundnut with treatments under screenhouse conditions at 5WAP in both seasons

Source of Variation	DF	NOB	NOL	CS(cm)	PLHT(cm)	NOS	CLPHY
Accessions	9	181.75**	1967.60**	89.75**	295.48**	169.58**	1205.09**
Treatments	5	52.48*	441.15*	2.91*	2.57**	52.63**	61.80**
Soil Status	1	52.48**	32108.95**	3.17**	644.54**	3991.85**	196.51^{ns}
Seasons	1	1409.18**	27037.16**	319.02**	2398.63**	3148.98**	21210.33**
Accessions*treatment	45	14.90^{ns}	141.69^{ns}	5.85^{ns}	11.68^{ns}	14.68^{ns}	81.18^{ns}
Accessions*soil Status	9	39.97*	367.74^{ns}	4.28^{ns}	13.81^{ns}	28.83^{ns}	404.39**
Accessions*season	9	67.73**	651.12**	5.81^{ns}	14.04^{ns}	72.35**	330.46**
Treatments*soil Status	5	25.78^{ns}	182.53^{ns}	1.74^{ns}	1.45^{ns}	28.02^{ns}	97.03^{ns}
Treatment*season	5	12.07^{ns}	108.15^{ns}	3.99^{ns}	20.17^{ns}	14.76^{ns}	85.26^{ns}
Acc*treatment*soil status	45	14.24^{ns}	127.07^{ns}	3.41^{ns}	13.21^{ns}	16.97^{ns}	121.26^{ns}
Rep	2	4.94^{ns}	271.4^{ns}	6.43^{ns}	17.77^{ns}	29.97^{ns}	262.11^{ns}
Aces*treat*soil*season	45	16.11^{ns}	176.25^{ns}	4.90^{ns}	11.76^{ns}	29.97^{ns}	145.42^{ns}
CV (%)		31.1	31.9	18.4	17.7	33.5	31.5

* (P<0.05) significant, ** (P<0.01) Very significant, ns-not significant. NOB-number of Branches, NOL-number of leaves, CS- canopy spread, PLHT-plant height, NOS-number of stem, CLPHY-chlorophyll content

Table 8.2: Analysis of variance showing biomass yield of Bambara groundnut accessions under screenhouse conditions at flowering and harvest in both seasons

Source of variation	DF	FSF(g)	DSF(g)	FSH(g)	DSH(g)	FRH(g)	DRH(g)	%N Flw	%P Flw	%N Hvst	%P Hvst
Accessions	9	30.50**	2.13**	102.85**	32.31**	59.19**	6.90**	0.98^{ns}	0.01**	0.69**	0.02**
Treatments	5	5.98**	0.33**	34.47*	33.73**	11.08**	4.22*	20.87^{ns}	0.005*	16.07**	0.004*
Soil Status	1	550.91**	19.05**	548.64**	427.99**	11.91^{ns}	22.31**	0.28^{ns}	0.16^{ns}	3.01**	0.19**
Season	1	2786.50**	221.94**	628.86**	4.56^{ns}	1562.75^{ns}	7.57*	1.28**	1.06^{ns}	0.019^{ns}	0.23**
Accessions*treat	45	10.15^{ns}	0.69^{ns}	13.15^{ns}	4.14^{ns}	13.54^{ns}	1.86^{ns}	0.38^{ns}	0.003^{ns}	0.24^{ns}	0.002^{ns}
Accessions*soil stat	9	4.59^{ns}	0.25^{ns}	18.20^{ns}	7.25^{ns}	18.93^{ns}	2.68^{ns}	0.17^{ns}	0.003^{ns}	0.16^{ns}	0.004^{ns}
Accessions*season	9	13.95^{ns}	1.30*	54.62^{ns}	9.54*	44.17**	6.49**	0.78^{ns}	0.011**	0.25^{ns}	0.004^{ns}
Treatments*soil stat	5	7.39^{ns}	0.33^{ns}	15.86^{ns}	5.23^{ns}	18.20^{ns}	2.27^{ns}	0.43^{ns}	0.004^{ns}	0.21^{ns}	0.006^{ns}
Treatment*season	5	9.87^{ns}	0.35^{ns}	47.84**	39.96**	1.88^{ns}	0.99^{ns}	0.48^{ns}	0.004^{ns}	0.85^{ns}	0.003^{ns}
Acc*treat*soil status	45	6.51^{ns}	0.50^{ns}	19.56*	5.43^{ns}	17.35^{ns}	1.53^{ns}	0.85^{ns}	0.002^{ns}	0.13^{ns}	0.003^{ns}
Rep	2	3.25^{ns}	1.14^{ns}	27.53^{ns}	10.59^{ns}	27.19^{ns}	1.32^{ns}	0.17^{ns}	0.012^{ns}	0.56^{ns}	0.013^{ns}
Aces* treat*soil*sea	45	8.93^{ns}	0.53^{ns}	18.03*	5.92*	13.44^{ns}	1.64^{ns}	0.29^{ns}	0.003^{ns}	0.20^{ns}	0.003^{ns}
CV (%)		84.6	77.8	66.7	54.2	52.1	62.7	17.2	51.9	24.4	47.6

* ($P<0.05$) highly significant, ** ($P<0.01$) Very significant, ns-not significant FSF- Fresh shoot at flowering, DSF-Dry shoot at flowering, DSH-Dry shoot at harvest, FRH-Fresh root at harvest, DRH-Dry root at harvest, N-Nitrogen, Flw-Flowering, P-Phosphorus, %-percentage, Host- Harvest.

Table 8.3: Biomass yield, nutrients uptake and N fixation of Bambara groundnut accessions in sterile and non-sterile soils under screenhouse conditions in both seasons

Soil status	FSF(g)	DSF(g)	FSH(g)	DSH(g)	FRH(g)	DRH(g)	%N Flw	%P Flw	%N Hvst	%P Hvst	Nod No	Nod wgt	N uptake	N fixed (Kg/ha)	Ndfa
Sterile	2.59[b]	0.89[b]	4.76[b]	3.06[b]	3.56[a]	1.29[b]	2.16[b]	0.09[b]	2.09[a]	0.10[b]	9.32[b]	0.640[a]	0.065[b]	28.12[a]	36.57[a]
Non Sterile	4.44[a]	1.23[a]	6.53[a]	4.68[a]	3.69[a]	1.63[a]	10.67[a]	0.12[a]	1.95[b]	0.14[a]	10.97[a]	0.540[a]	0.098[a]	15.49[a]	38.25[a]

Mean with same letter is not significantly different at 5% level of probability using (Dmrt). FSF-fresh shoot at flowering, Dry shoot at flowering, FSH-fresh shoot at flowering, DSH-dry shoot at harvest, FRH-fresh root at harvest, Flw-flowering, Hvst-harvest, Nod-nodule, No-number, wgt-weight, dfa-derived from atmosphere

Table 8.4 Mean separation showing effect of treatments on growth traits of Bambara groundnut accessions of Bambara groundnut at 5WAP in both seasons under screenhouse conditions

Accessions	NOB	NOL	CS(cm)	PLTH	NOS	CLPHY
TVSu-365	17.47^{a}	54.56^{a}	9.58^{e}	15.54^{f}	18.05^{a}	28.99^{e}
TVSu-305	15.88^{b}	50.17^{ab}	11.93^{bc}	18.78^{bc}	16.45^{ab}	39.54^{ab}
TVSu-710	15.24^{b}	47.39^{b}	10.39^{d}	16.44^{ef}	15.94^{bc}	31.77^{de}
TVSu-787	14.83^{bc}	46.02^{bc}	10.39^{bc}	19.15^{bc}	15.55^{bc}	36.41^{bc}
TVSu-1698	14.38^{bcd}	45.53^{bcd}	11.73^{c}	19.94^{b}	15.03^{bc}	33.01^{cde}
TVSu-1739	13.54^{cde}	37.36^{e}	14.08^{a}	23.44^{a}	12.924^{e}	42.08^{a}
TVSu-475	13.54^{cde}	41.93^{cde}	11.77^{c}	18.96^{bc}	14.79^{bcde}	30.15^{e}
TVSu-506	13.03^{de}	40.44d^{e}	12.67^{b}	19.38^{bc}	13.59d^{e}	42.07^{a}
TVSu-378	12.98^{de}	40.85^{cde}	11.63^{c}	17.44^{de}	14.14^{cde}	35.13^{dc}
TVSu-1606	12.69^{e}	39.68^{e}	11.76^{c}	18.17^{cd}	13.27^{de}	35.88^{bc}

Mean followed by the same letter are not significantly different at 5% level of probability using the (DMRT), and mean comparison for each trait is along each column. NOB-number of Branches, NOL-number of leaves, CS-canopy spread, PLHT-plant height, NOS-number of stem, CLPHY-chlorophy content

Table 8.5 Mean separation showing the effects of treatments on biomass yield and nutrients uptake in both seasons under screenhouse conditions

Accessions	FSF (g)	DSF (g)	FSH (g)	DSH (g)	FRH (g)	DRH (g)	%N Flw	%P Flw	%N Hvst	%P Hvst
TVSu-365	2.53^{d}	0.79^{c}	4.04^{c}	3.18^{cd}	2.12^{d}	1.08^{de}	1.93^{a}	0.11^{abc}	1.95^{bc}	0.11^{bc}
TVSu-305	4.25^{ab}	1.27^{a}	6.40^{ab}	4.36^{ab}	4.87^{a}	1.77^{ab}	2.31^{a}	0.09^{bcd}	2.06^{ab}	0.11^{cd}
TVSu-710	3.22^{bcd}	1.00^{bc}	3.93^{c}	2.62^{d}	2.21^{d}	0.815^{e}	2.11^{a}	0.08^{d}	1.85^{c}	0.09^{d}
TVSu-787	3.88^{abc}	1.08^{bc}	6.91^{a}	4.68^{a}	4.22^{abc}	1.61^{abc}	2.08^{a}	0.11^{ab}	2.02^{ab}	0.13^{ab}
TVSu-1698	3.56^{bcd}	1.09^{bc}	4.83^{c}	3.81^{bc}	2.83^{cd}	1.21^{cde}	2.23^{a}	0.10^{bc}	2.05^{ab}	0.11^{bc}
TVSu-1739	4.82^{a}	1.47^{a}	7.78^{a}	4.86^{a}	4.63^{a}	1.88^{a}	2.34^{a}	0.12^{a}	2.09^{ab}	0.14^{a}
TVSu-475	3.03^{cd}	0.98^{bc}	5.34^{bc}	3.81^{bc}	3.77^{abc}	1.55^{abcd}	2.13^{a}	0.10^{bc}	1.94^{bc}	0.10^{cd}
TVSu-506	3.80^{abc}	1.04^{bc}	5.17^{bc}	3.48^{c}	4.08^{abc}	1.70^{abc}	2.18^{a}	0.09^{bcd}	1.92^{bc}	0.12^{bc}
TVSu-378	2.91^{cd}	0.89^{c}	7.09^{a}	4.41^{ab}	4.38^{ab}	1.68^{abc}	1.87^{a}	0.11^{ab}	2.16^{a}	0.14^{a}
TVSu-1606	3.17^{bcd}	0.98^{bc}	4.94^{c}	3.48^{c}	3.18^{bcd}	1.27^{bcde}	2.09^{a}	0.08^{cd}	2.14^{a}	0.12^{bc}

Mean with same letter is not significantly different at 5% level of probability using (Dmrt). FSF-fresh shoot at flowering, Dry shoot at flowering, FSH-fresh shoot at flowering, DSH-dry shoot at harvest, FRH-fresh root at harvest, Flw-flowering, Hvst-harvest.

Table 8.6 Mean separation showing the effects of treatments on growth traits of Bambara groundout accessions at 5WAP in both season under screenhouse conditions

Treatments	NOB	NOL	CS(cm)	PLTH	NOS	CLPHY
FA3	14.26^{ab}	44.38^{ab}	11.86^{a}	18.77^{a}	15.43^{ab}	34.12^{b}
IRJ2180A	15.50^{a}	48.19^{a}	11.54^{ab}	19.17^{a}	16.00^{a}	36.02^{b}
RACA6	13.96^{b}	44.35^{ab}	11.79^{ab}	18.84^{a}	14.66^{abc}	34.19^{b}
N	13.35^{b}	39.92^{c}	11.21^{b}	16.88^{b}	13.82c	40.17^{a}
USDA110	14.37^{ab}	44.70^{ab}	11.94^{a}	18.96^{a}	15.04^{abc}	35.09^{b}
CONTROL	13.61^{b}	42.58^{bc}	11.96^{a}	18.79^{a}	14.31^{bc}	34.79^{b}

Mean followed by the same letter is not significantly different at 5% level of probability using the (DMRT), and mean comparison for each trait is along each column. NOB-number of Branches, NOL-number of leaves, CS-canopy spread, PLHT-plant height, NOS-number of stem, CLPHY-chlorophyll content

Table 8.7 Mean separation showing the effects of treatments on biomass yield and nutrients uptake of Bambara groundnut accessions in both seasons under screenhouse conditions

Treatments	FSF (g)	DSF (g)	FSH (g)	DSH (g)	FRH (g)	DRH (g)	% P Flw	% N Flw	% N Hvst	% P Hvst
FA3	3.15[b]	0.96[b]	5.75[b]	3.99[ab]	3.44[b]	1.45[a]	0.09[a]	2.97[a]	2.18[a]	0.11[ab]
IRJ2180A	3.45[b]	1.05[b]	5.56[bc]	4.09[ab]	3.53[b]	1.58[a]	0.09[a]	2.23[a]	2.10[a]	0.12[a]
RACA6	3.08[b]	0.94[b]	5.70[b]	3.89[b]	3.69[b]	1.58[a]	0.08[a]	2.36[a]	2.19[a]	0.12[a]
N	5.18[a]	1.55[a]	7.06[a]	4.62[a]	5.52[a]	1.68[a]	0.08[a]	2.32[a]	2.19[a]	0.10[b]
USDA110	3.63[a]	1.05[b]	5.98[b]	4.12[ab]	3.59[b]	1.49[a]	0.09[b]	2.34[a]	2.25[a]	0.13[a]
CONTROL	3.45[b]	1.05[b]	4.51[c]	2.89[c]	2.92[b]	1.08[b]	0.06[b]	1.29[a]	1.28[b]	0.11[ab]

Mean with same letter is not significantly different at 5% level of probability using (Dmrt). FSF-fresh shoot at flowering, Dry shoot at flowering, FSH-fresh shoot at flowering, DSH-dry shoot at harvest, FRH-fresh root at harvest, Flw-flowering, Hvst-harvest.

8.3.1 Response of Accessions of Bambara Groundnut Inoculation under Screenhouse Conditions

The result indicated that significant differences were recorded in the growth traits at 5WAP (Table 8.1), biomass yield and nutrient uptake of Bambara groundnut accessions inoculated with treatments. Also, differences were observed in the soil status (sterile and nonsterile soil) and growth traits of inoculated accessions which was as a result of the presence of native rhizobia present in the non-sterile soil (Table 8.1 and 8.2).

The population of the indigenous rhizobia in the soil and adaptability to the environment with inoculated *B. japonicum* strains promoted the growth traits of the accessions in the non-sterile soil than the exotic strains inoculated in the sterile soil. There were no significant differences recorded among the strains inoculated in some growth traits biomass yield and nutrient uptake of accessions inoculated in both seasons (Table 8.1 and 8.2). No significant differences were recorded in the interaction of accessions with strains, strains with soil status, interaction of accessions with soil status for biomass yield and nitrogen and phosphorus uptake at flowering and at harvest (Table 8.2).

Significant differences were recorded in dry shoot at flowering, and at harvest, fresh and dry root at harvest and percentage phosphorus at flowering in the interaction of accessions and seasons. Also, significant differences were recorded in fresh and dry shoot at harvest in the interaction of strains with season, accessions with strains and soil status with the seasons. Similarly, a significant difference was recorded in the non-sterile soil than in the sterile soil for biomass yield, nutrient uptake and the derived nitrogen from the atmosphere (Table 8.3).

In all, the results recorded in this study shows that inoculation of the *B. japonicum* strains in the non-sterile soil enhanced the growth parameters, nutrient uptake, flowering, and biomass yield in the inoculated Bambara groundnut accessions compared to the result obtained in the sterile soil which shows that the activities of the plant growth promoting bacteria (PGPB) and rhizobia in the non-sterile soil is significantly better compared to the sterilized soil for the different Bambara groundnut accessions. (Table 8.3). TVSu-1739 and TVSu-365 showed higher significant differences in the growth traits, biomass yield and nutrient uptake of inoculated Bambara groundnut accessions, compared to other accessions (Table 8.4 and Table 8.5). The treatments used in the study and control enhanced the growth traits of Bambara groundnut accessions. The difference recorded in the control was due to the presence of indigenous rhizobia present in the soil (Table 8.6). The nitrogen fertilizer applied significantly enhanced the biomass yield and nutrient uptake in the screenhouse compared to other treatments used in the study (Table 8.7)

Figure 8.2: Accessions of Bambara groundnut in a sterile and non-sterile soil.

Photo: T.D. Bitire, IITA (2019)

8.4 Discussion

The result of the analysis of variance revealed variability in the growth traits among accessions of Bambara groundnut inoculated with *B. japonicum* strains in a sterile and non-sterile soil at 5WAP showing that inoculation significantly enhanced the plant height, number of branches, chlorophyll content, number of stem, and number of leaf in the non-sterilized soil over the sterilized soil. The significant differences which were observed in the non-sterilized soil over the sterilized soil on growth trait and chlorophyll content were due to the availability of plant growth promoting bacteria (PGPB) and the native rhizobia in the non-sterilised soil. This correlates with a study conducted by Stajkovic *et al*. (2011) that reported that inoculation of bacteria strains in the screenhouse significantly enhanced growth and biomass yield of common bean in non-sterilized soil over the sterilized soil.

Also, no significant difference was observed in the number of days to 50% flowering among the accessions inoculated with the *B. japonicum* strains in the sterile soil and non-sterile soil (Table 8.1). The biomass yield of accessions recorded at 50% flowering, and at harvest—fresh and dry shoot at flowering, fresh and dry shoot at harvest, and fresh root and dry root at harvest—were significantly enhanced by the inoculation in the non-sterile soil over the sterile soil in both seasons which is as a result of the plant growth promoting bacteria and native rhizobia in the non-sterilized soil and this correlates with a research reported by Ulzen *et al*. (2016) which disclosed that inoculation of *B. japonicum* inoculant enhanced the biomass yield and yield of soybean.

Furthermore, the % N and % P recorded at flowering and at harvest in the shoot of the accessions in the non-sterile soil were significantly different from the sterile soil and this reflects that the accessions of Bambara groundnut were able to utilize the nutrient in the non-sterile soil than the sterile soil. This was supported by the plant growth promoting bacteria and the indigenous rhizobia with the *B. japonicum* strains inoculated which correlates with a trial conducted by Nyoki and Ndakidemi (2014a). Also, the number of nodules recorded at 50% flowering in both seasons were significantly enhanced by the non-sterilized soil than the sterilized soil, but no significant difference was recorded between the sterilized and non-sterilized soil in the weight of the nodule of accessions inoculated with the *B. japonicum* strains.

Similarly, inoculation of Bambara groundnut accessions with *B. japonicum* improved nitrogen uptake although no significant differences were recorded between the inoculated accessions in the sterile and non-sterile soil in the amount of nitrogen derived from the atmosphere.

8.5 Conclusion

The result of the trial concluded that inoculation of the *B. japonicum* strains to the accessions in the non-sterile soil significantly supported the growth, nutrient uptake, and biomass yield of the accessions over the sterile soil. This seems to reflect that plant growth promoting bacteria in the non-sterilized soil and the native rhizobia with the inoculated *B. japonicum* enhanced the growth and development of the legume better than the sterile soil. It is equally clear from this study that indigenous rhizobia also enhanced the success recorded in the non-sterile soil. However, the Bambara groundnut refused to produce pod in the screenhouse in both seasons which limited its yield evaluation except TVSu-710 that was able to produce in both the sterile and the non-sterile soil out of the ten accessions used in the study. The refusal of Bambara groundnut to pod formation in the screenhouse may be as a result of the quantity of soil in the pot. This therefore limited the research in the screenhouse to only the growth parameters, nutrient uptake, and biomass yield.

CHAPTER NINE

Isolation and Molecular Characterization of Root Nodules Associated Bacteria on Inoculated Bambara groundnut (*Vigna subterranea (L) Verdc)* Accessions.

Abstract

Soils in the tropics have often shown diverse reduction in the population of bacteria due to the pressure on the agricultural system with the nature of the climatic conditions, which eventually affect soil health. High priority has been placed on rhizobia inoculants over the inorganic fertilizer by farmers and this has led to the onset of great research on tropical rhizobia strains. In this study, bacteria isolates from root nodules of inoculated Bambara groundnut were characterized using 16S rRNA, *nifH*, *nod A* and *nod* C gene analysis. The result of the 16S rRNA revealed that the root of Bambara groundnut are mostly associated with *Bradyrhizobium* spp. (*B.* spp.), which include: *B.* diazoefficiens, *B. japonicum* and *Rhizobium* spp. in the screenhouse. The *nifH*, *nod A* and *nod* C gene analysis revealed that *Streptomyces bacillaris, Pseudomonas knackmussi, B. ekanii, Sinorhizobium meliloti, Mesorhizobium spp., Bradyrhizobium* spp., and *Rhizobium* spp were isolated from the field in two locations. The rhizobia isolates have been previously confirmed to be playing an important role in nodulation and nitrogen fixation of Bambara groundnut. The result of the study, therefore, revealed the isolation of bacteria species from the root nodules of Bambara groundnut accessions using molecular techniques.

Keywords: Bacteria, underutilised legume, 16S rRNA, *nifH*, nod A and nod C.

9.1 Introduction

Nitrogen fixation and nodulation of rhizobia are most important factors in understanding the symbiotic relationship with legumes. The microbial communities in the roots are usually recruited from large and diverse pool of microbes in bulk soils through root exudate chemical signaling (Adedeji and Babalola 2020; Hartman and Tringe 2019). The rhizosphere, which is the medium between plants and soil, has been labelled a hotspot for new genes and biomolecules *(Babalola et al*. 2020).

Several genes are present in the nodulation process; the early nodulation genes, which include *nod* ABC, are responsible for the important structure of the *Nod* factors (Etesami 2022). The terminology 'biovar' has been frequently used in bacteria taxonomy to group and categorize strains of the same species with different physiological characters (Teresa *et al*. 2021). In rhizobia research, it refers to strains symbiotic with the same host species (Jain *et al*. 2020a). The characterization and relationship of peanut *Bradyrhizobium* spp. applying a molecular approach with the evaluation of housekeeping and symbiotic gene sequences was recently carried out in China (Chen *et al*. 2016a) and South Africa (Puozaa *et al*. 2017b) showing a large diversity of bacteria isolates. Puozaa *et a*l. (2015) postulated that the bacteria isolates studied from two different regions of South Africa were classified as *B. kavangense, B. stylosanthis*, *B. elkanii* and *B. manauense* along with four bacteria isolates which are close to *B. diazoefficiens*. Chen *et al*. (2016b) showed that among 36 bacteria isolates, 11 of them were closely related to *B. japonicum* and other 21 to *B. guangxiense*, in addition to other four bacteria related to *B. iriomotense.* The evaluation of symbiotic gene sequences is important to indicate the range of hosts nodulated by the bradyrhizobia isolates due to the determination of their "symbiovar" affiliation (Hungria and Mendes (2015a).

In addition, the concomitant evaluation of the sequences of housekeeping and symbiotic genes lead to a better understanding of the taxonomic position and phylogenetic relationships of the bacteria isolates (Delamuta *et al*. 2012). Therefore, the aim of this study is to isolate, identify, and characterize using molecular technique to isolate from the root nodules of inoculated Bambara groundnut accessions in the screenhouse and in two different geographical locations (Ibadan and Ikenne) in Nigeria.

9.2 Materials and Methods

The experiment was carried out at the Bioscience Centre at the International Institute of Tropical Agriculture (IITA) headquarters, Ibadan, Nigeria. The nodule extracted from the experiment in the screenhouse in the 2018 and 2019 cropping seasons and also from the field in two locations namely Ibadan and Ikenne in the 2019 season were prepared into broth solution in the Soil Microbiology Laboratory of IITA and taken to the Bioscience Centre for further analysis such as DNA Extraction, PCR and Sequencing. The 16S primers was used during the PCR of the nodules extracted from the screenhouse while the *nifh*, *nod* A, and *nod* C primers were used to determine the nodules extracted from the field.

9.2.1 Isolation of Bacteria from Root Nodules of Bambara Groundnut Accessions

Recovering of nodules from the root of Bambara groundnut accessions involves the removal of outside contamination by thorough washing of the root and nodules in water. Samples were collected at 50% flowering in the screenhouse and on the field with water. Nodules were washed in Gooch crucible/ glass vial (desiccators). After washing, the crucible containing the nodules was dipped into a petri dish containing mercury chloride solution (Conc. 1:1000). Floating nodules were held beneath surface of the liquid about two to five minutes. Large nodules were held in the solution much longer without injury to the nodule content. The crucible containing the nodules was removed from the mercury chloride solution and immersed into successive dishes of sterilized water (washing all mercury chloride from nodules). It was placed in a sterile petri dish with sterile forceps. Nodules were then open with sterile knife while the inoculating needle was used to carry one drop of sterile water into the centre of the broken surface and rotated slightly to secure the bacteria. The material was transferred into a drop sterilized water in a petri dish and was stirred to distribute the organism. Sterilized needle was used to transfer the loop-full of the culture to a second petri dish and it was stirred and transferred to the third petri dish. The rhizobia isolates which would be considered as representative strains were selected for further molecular studies.

9.2.2 Culturization

Yeast mannitol broth (mannitol-10 g, magnesium sulphate 0.1 g, sodium chloride-0.05 g, potassium phosphate- 0.25 g and yeast extract- 0.25 g) was prepared in conical flasks and sterilized at 121°C for 15 minutes. Isolates from the nodules of the accessions of Bambara groundnut and indigenous rhizobia in the soil (nodules from uninoculated control) were placed in a rotator shaker for 7 days at 28°C with a revolution of 100 rev/ min.

9.2.3 DNA Extraction

Twenty-six and seventy-one pure isolates were characterised using the 16S rRNA gene approach in the glass house in the first and the second season respectively while seventy pure isolates were obtained from two locations (Ibadan and Ikenne) on the field in the first and second season, using the *nifh*, *Nod* A and *Nod* C gene. Genomic DNA was extracted from pure bacteria isolates, using ZR Bacterial DNA miniprep™ Kit according to the recommendation by Zymo Research Corp South Africa—the manufacturer. The concentration and the purity of the DNA were estimated using the namedrops lite spectrophotometer, (Thermo Scientific Inc, USA) at 260-280 nm and by the horizontal gel electrophoresis (Thistle Scientific Ltd, USA) on a 0.8% (w/v) agarose gel at 100 V for 30min and was visualised under UV after staining with GelRed TM (Thermo Scientific Inc, USA). The PCR cocktail mix consist of 2.5µl of10x PCR buffer, 1µl of 25Mm MgC12, 1 µl each of forward and Reverse primers for the 16S rRNA gene (27F:AGAGTTTGATCMTGGCTCAG), (1525R:AAGGAGGTGWTCCARCCA), *nifH* gene (F: AAGTGCGTGGAGTCCTCCGGTGG) (R:GTTCGGCAAGCATCTGCTCG),NodC(F:AYGTHGTYGAYGACGGTTC)(R:CGYGACA GCCANTCKCTATTG),NodA(F:TTTGAGCCCGACCCCCGA)(R:CCGTTTCGGTCGCTGAT GGC) 1 µl of DMSO, 2 µL OF 2.5mMDNTPs, 0.1 µl of 5 µl/ul Taq DNA polymerase, and 3µl of 10ng/µl DNA. The total reaction Volume was made up to 25 µl using 13.4 of nuclease-free water. PCR was determined in gene Amp PCR System 9700 thermo cycler (Applied Biosystems), with an initial denaturation at 94°C for 5min, followed by 36 cycles of denaturation at 94°C for 30sec, annealing temp at 56°C for 30sec and elongation at 72°C for 45 sec. A final elongation procedure at 72°C For 7min and a hold temperature at 10°C and an amplified fragment was visualized on a safe view-stained 1.5agarose gel electrophoresis. Products were purified using ethanol and the EDTA precipitation protocol developed in IITA and Bioscience Centre. The purified PCR products were sequenced at the Iqaba, South Africa, using ABI3500 genetic Analyser (Applied Biosystems, Thermo Fisher Scientific Inc, USA). The sequence that were generated were analyzed using the NCBI BLAST portal.

9.2.4 Data Analysis

Sequence was submitted to the NCBI BLAST portal (www.ncbi.blast.Inm.nih.gov) for the sequence similarity search and the sequence that were greater than 75% similarity were retrieved for phylogenetic analysis.

9.3 Results

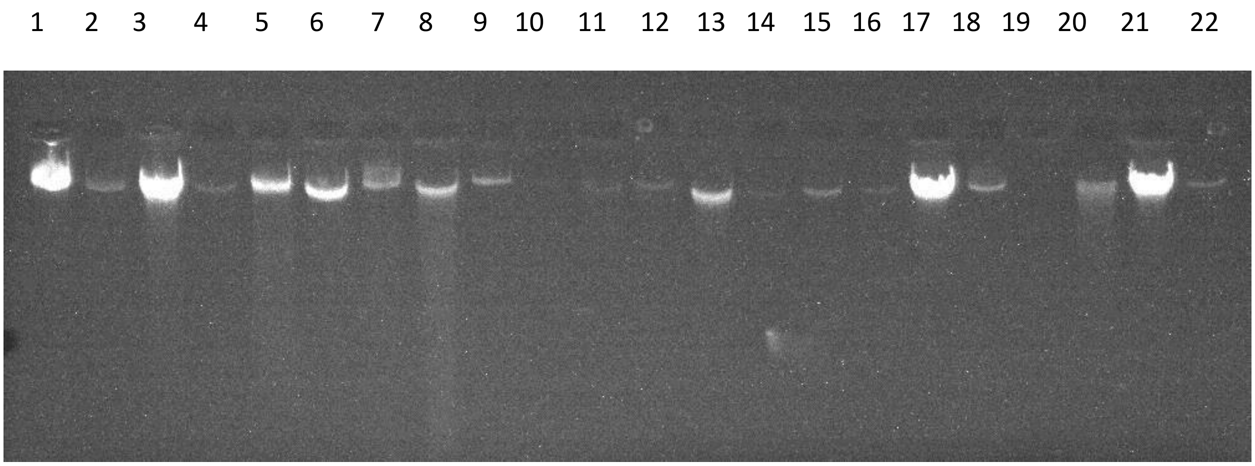
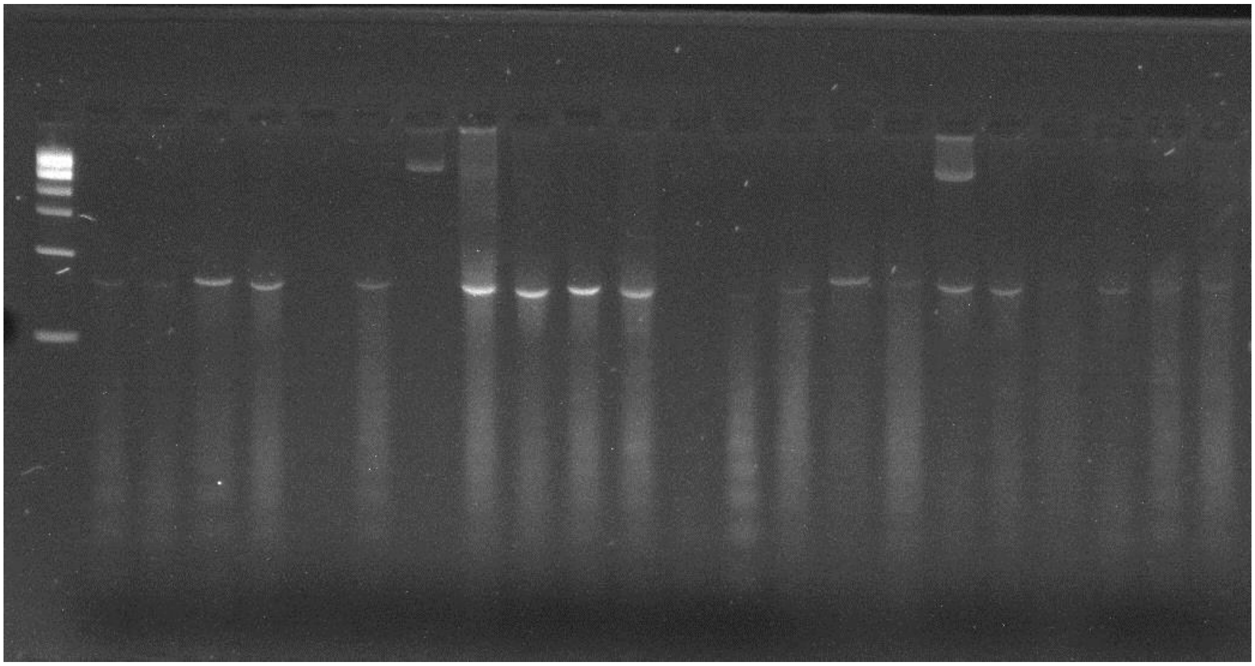


Figure 9.1: DNA Samples of Bambara groundnut bacterial isolated under screenhouse conditions



M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

Figure 9.2: PCR gel picture of Bambara groundnut bacteria isolated under screenhouse conditions

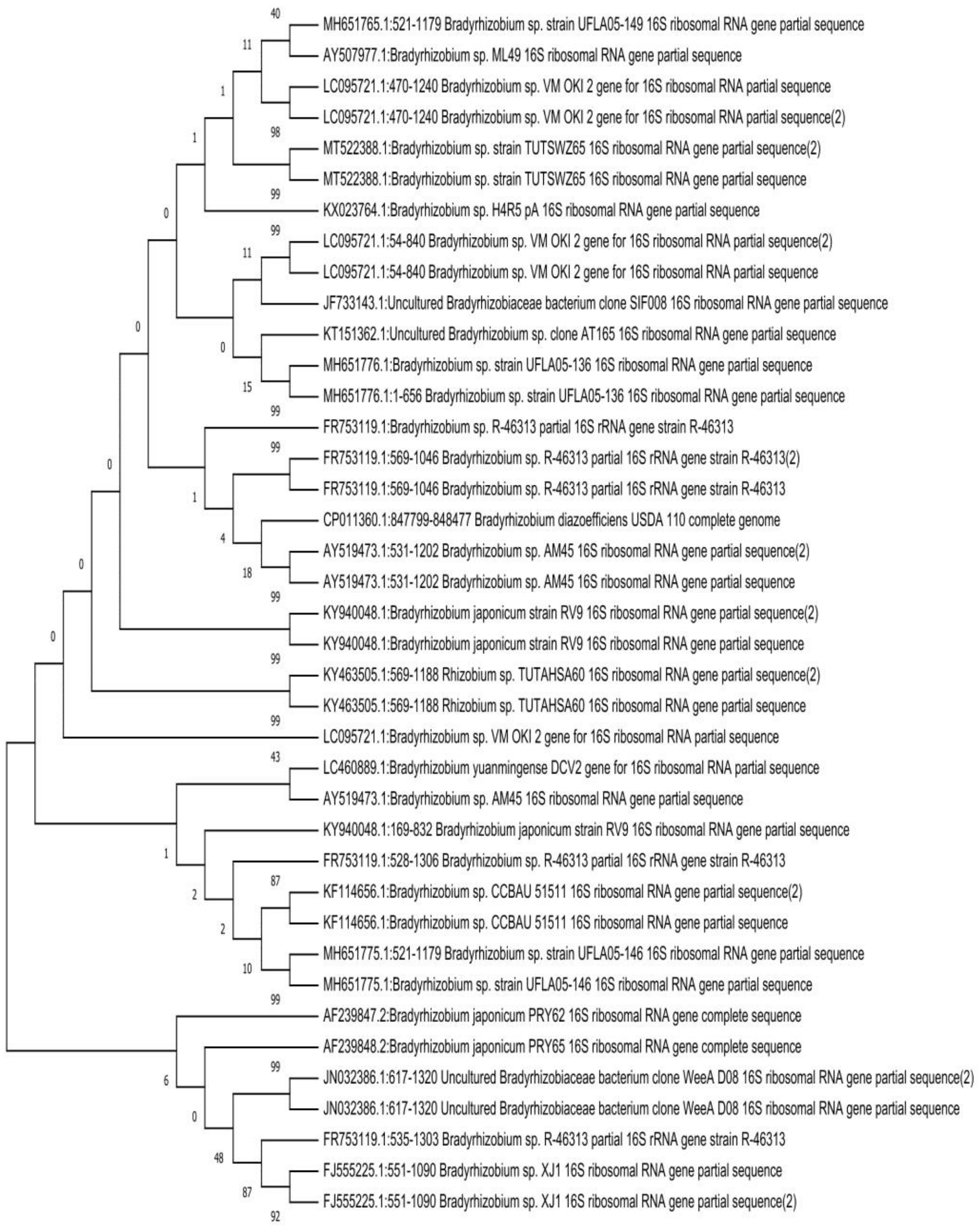


Figure 9.3: Phylogenetic tree of Bambara groundnut bacteria under screenhouse conditions

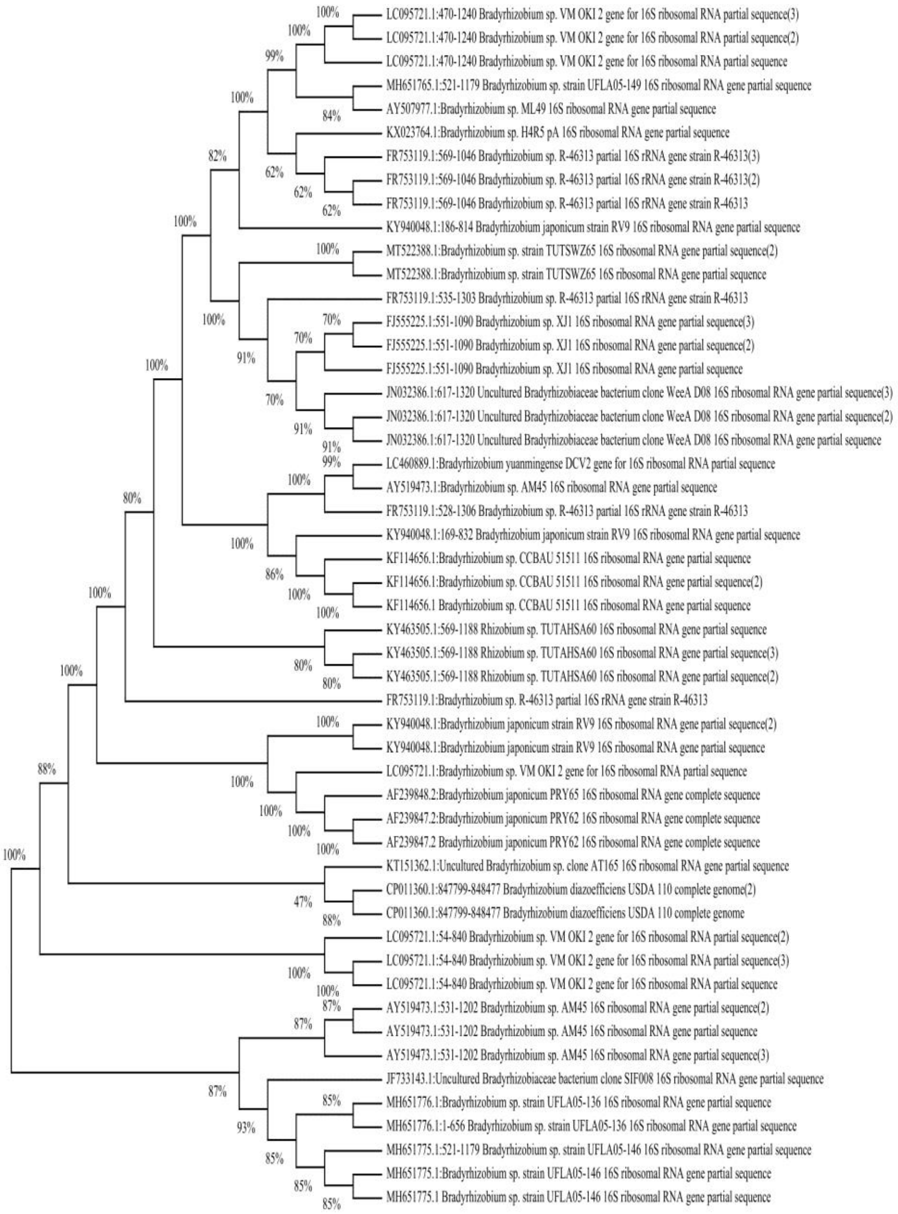
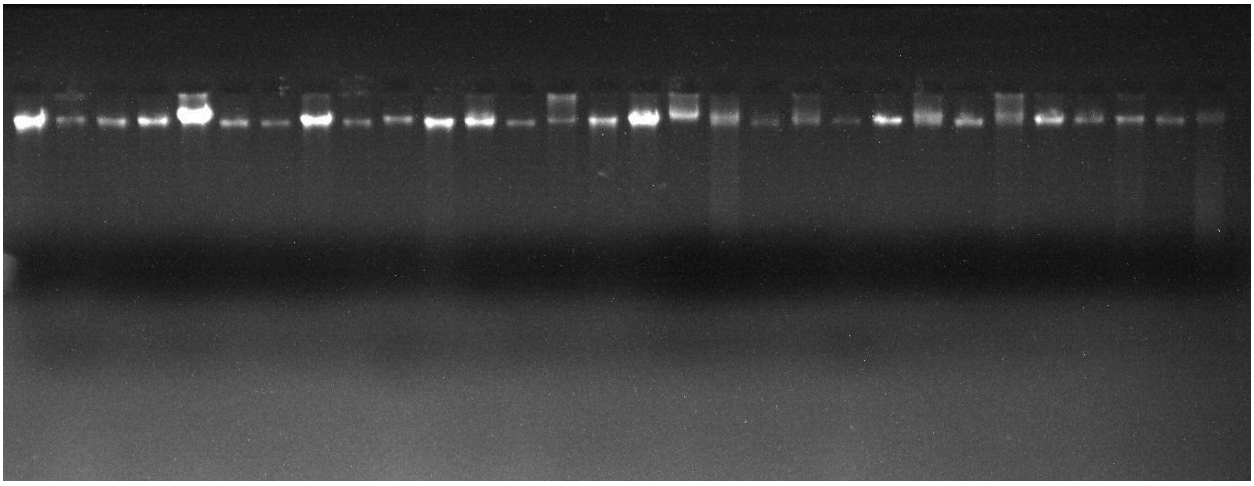
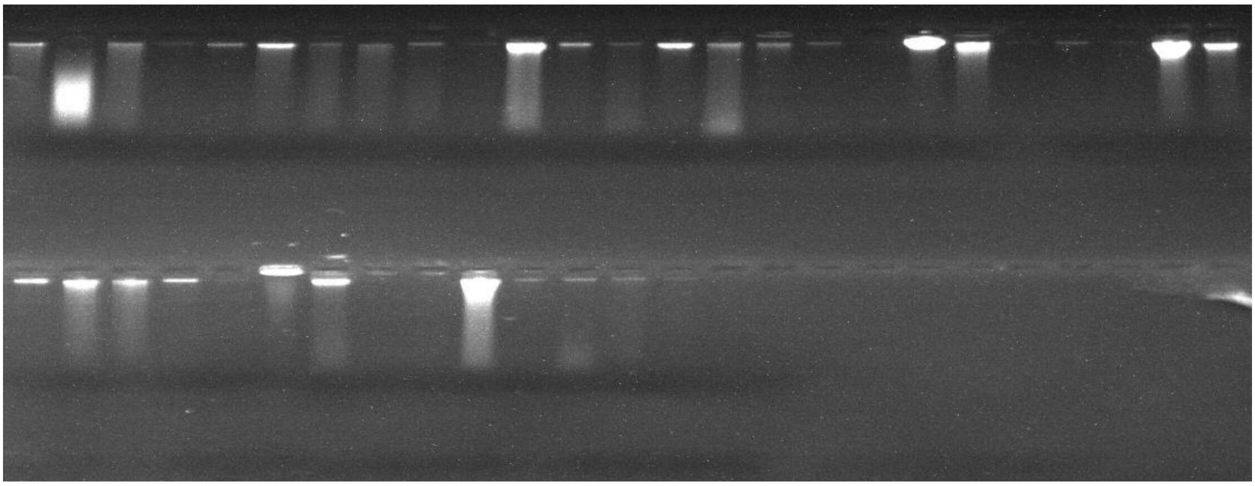


Figure 9.4: Phylogenetic tree of Bambara groundnut bacteria isolated under screenhouse conditions in both seasons



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29

31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53



54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69

Figure 9.5: DNA Samples of bacteria isolated from the field of Bambara groundnut (Ibadan and Ikenne**)**

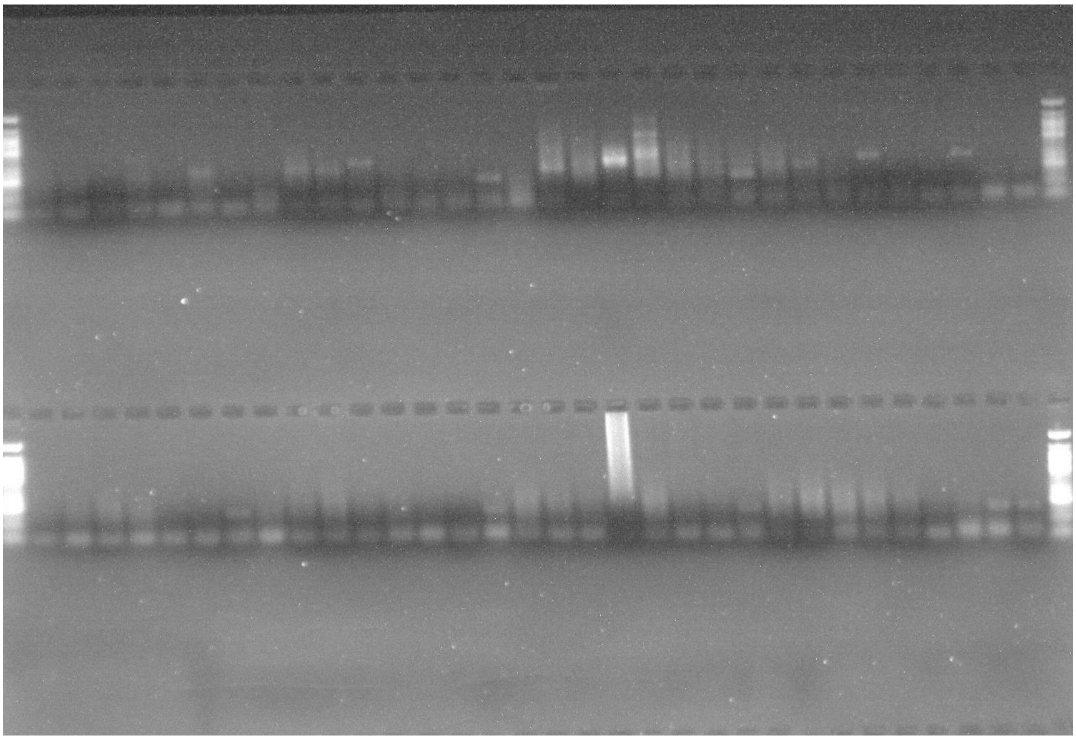


Figure 9.6: PCR NOD A gene from Bambara groundnut isolates (Ibadan and Ikenne).

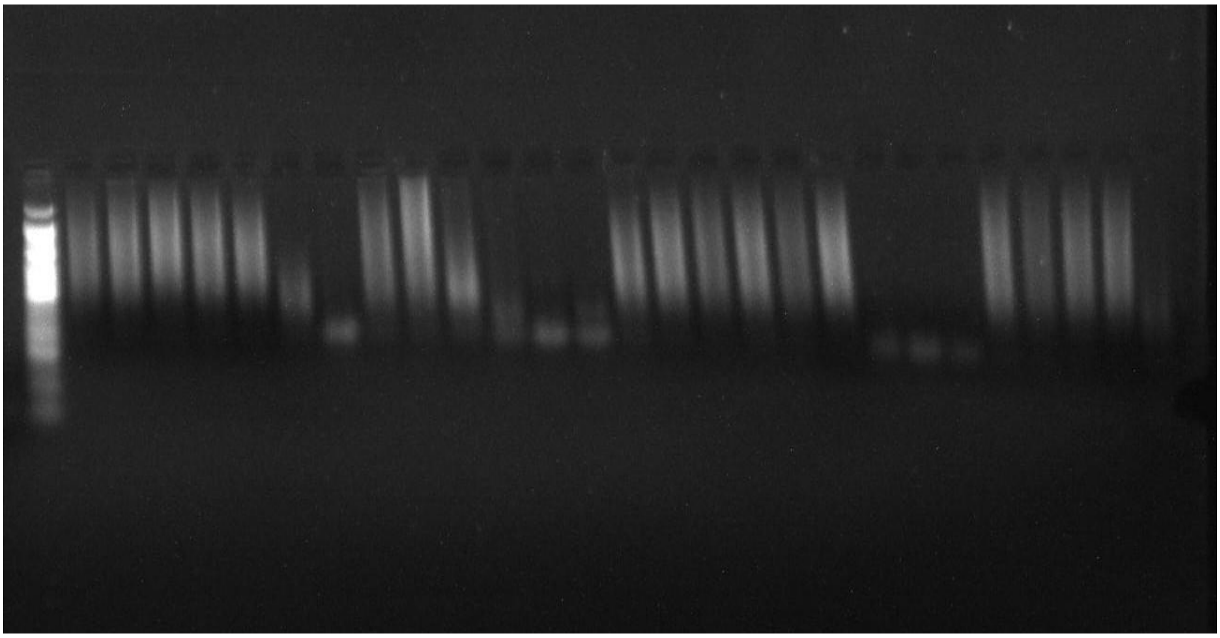


Figure 9.7: PCR NOD C gene from Bambara groundnut isolates (Ibadan and Ikenne).

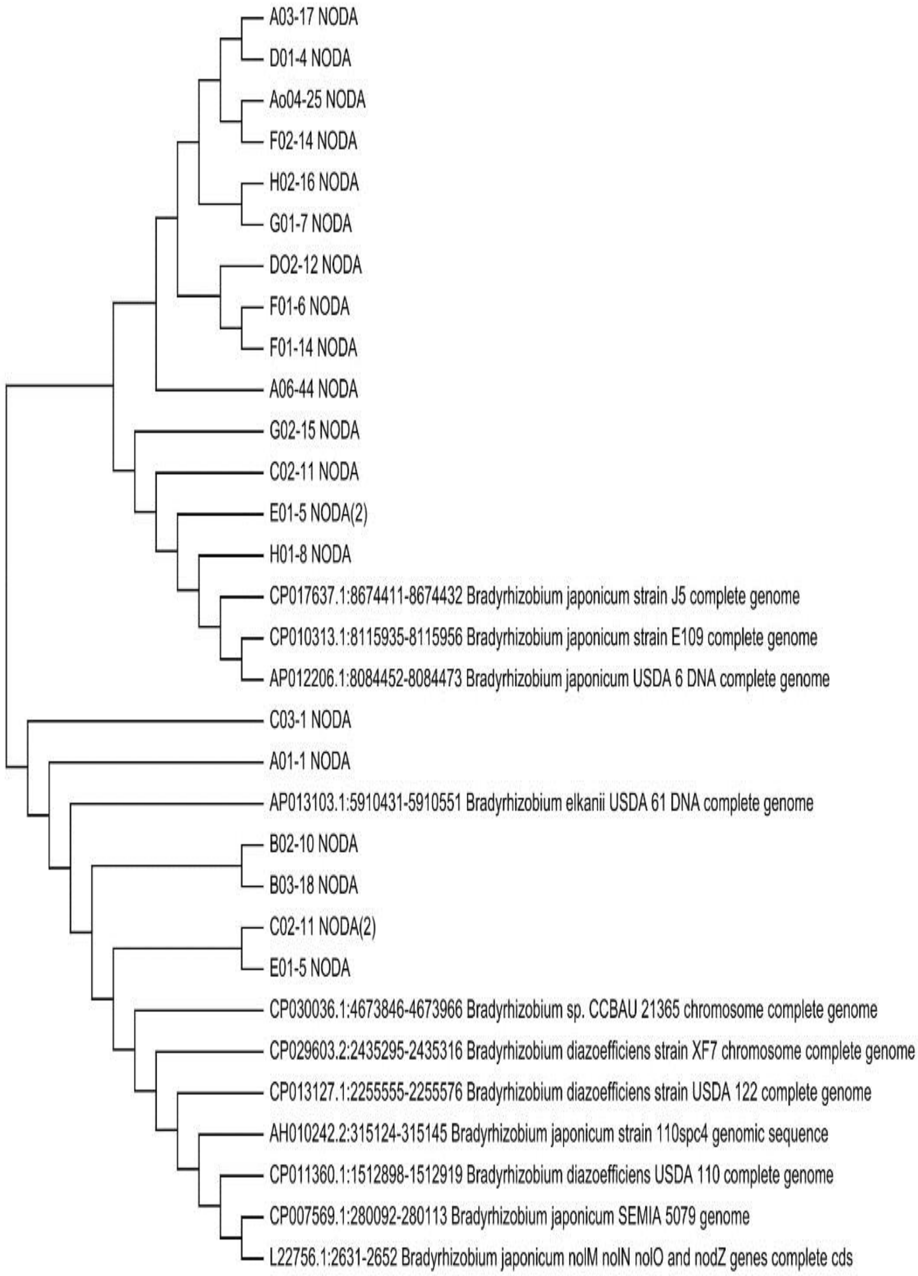


Figure 9.8: Phylogenetic tree using NOD A gene sequences obtained from root nodules of bacteria isolated from Bambara groundnut (Ibadan and Ikenne).

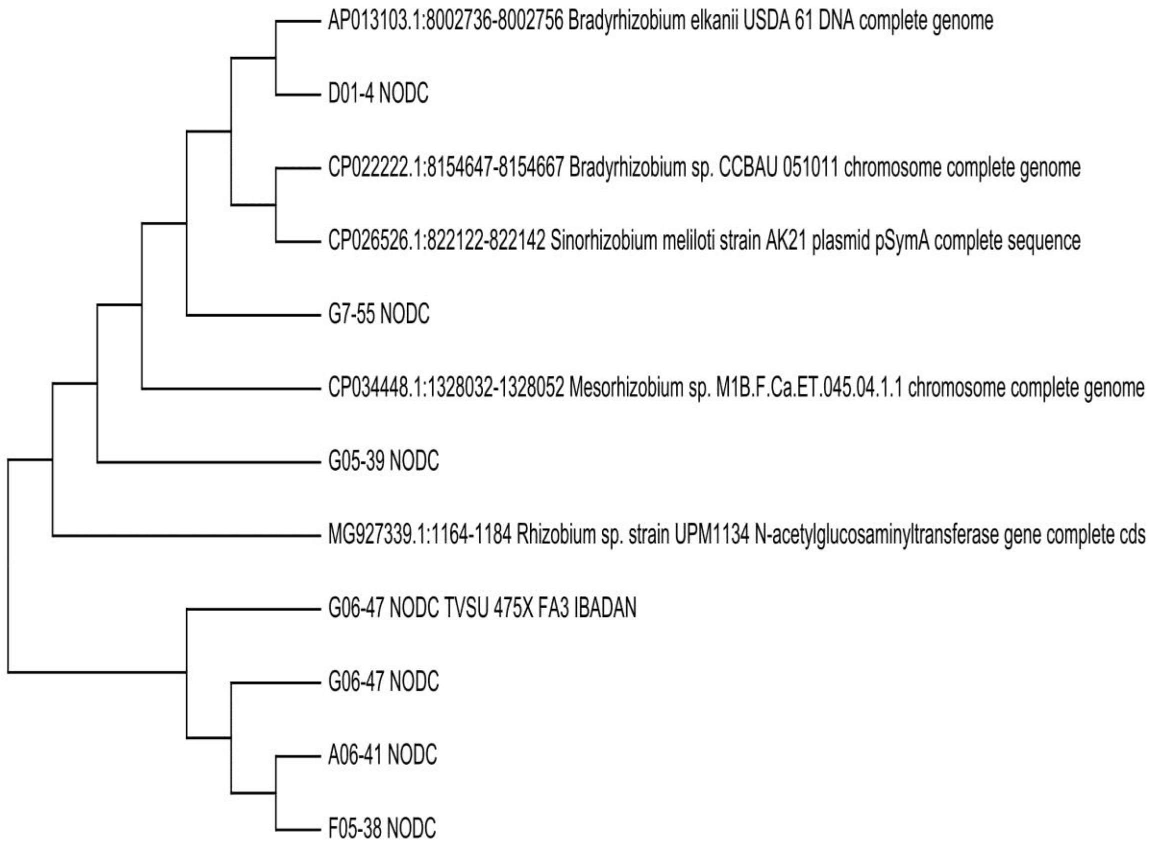


Figure 9.9: Phylogenetic tree using NOD C gene sequences obtained from root nodule bacteria isolated from Bambara groundnut (Ibadan and Ikenne).

9.4 DNA, PCR and Sequencing

DNA and PCR of Bambara groundnut accessions in the screenhouse and on the field revealed that bacteria are present in the rhizosphere of Bambara groundnut. This was revealed from the images recorded which shows that the bacteria inoculated are responsible for nodulation and fixation (Fig 9.1, 9.2, 9.5, and 9.6). This also shows that the bacteria inoculated were responsible for most of the activities in the root of Bambara groundnut accessions and not the indigenous rhizobia in the soil.

Furthermore, the phylogenetic tree also shows that nearly all the bacteria isolated from the roots of Bambara groundnut accessions in the glasshouse were *Bradyrhizobium* spp. (Fig 9.3, 9.4, 9.8, and 9.9). However, very few isolated bacteria from the root of Bambara groundnut accessions have close resemblance to *Rhizobium* spp., *mesorhizobium* spp., *sinorhizobium* spp, *pseudomonas* spp., and *Streptomyces* spp. With the result recorded, it was revealed that the *B. japonicum* inoculated to the Bambara groundnut accessions in the screenhouse and fields were responsible for the nodulation and fixation potential of the Bambara groundnut accessions.

9.5 Discussion

Twenty-six and Seventy-one pure isolates were respectively characterized using the 16S rRNA gene approach in the screenhouse for the first and the second seasons. The difference in percentage recovery of rhizobia in the two seasons may be probably due to differences in seasons. The indigenous strains recovered alongside the *B. japonicum* strains used to inoculate Bambara groundnut accessions were mostly *Bradyrhizobium* spp. but also included the *Rhizobium* spp. This was similar to the findings of Ibny *et al*. (2019b); Kanu and Dakora (2012) and Mohale *et al*. (2014a) where the biodiversity of the rhizobia nodulating Bambara groundnut is diverse including microorganisms that belong to both α and β rhizobia spp. but they were mostly the *Bradyrhizobium* spp.

Furthermore, seventy pure isolates obtained from the field of two locations (Ibadan and Ikenne) were used for detection of nodulation genes *nifH*, Nod A and Nod C gene. The amplified genes had about

50bp in the three genes which was different from the findings of Haukka *et al*. (1998) which showed that the amplification of the *nifh* resulted in a single band at 601bp. While Nod A had a faint band at 666bp, the report in AJAYI (2020) held that Nod A had a single band at 100bp. It is evident that these genes are present in rhizobia-nodulating Bambara groundnut accessions, playing an important role but may vary in size from strain to strain probably due to difference in genetic arrangements.

The results of the study in both the screenhouse and on the field revealed the presence of *Bradyrhizobium* spp. and *Rhizobium* spp. in the root of Bambara groundnut accessions which is in accordance with the results obtained by Bumunang *et al*. (2015) in which sequence analysis revealed that uncultured bacteria, *Actinobacterium, Bradyrhizobium* spp. and *Sphingomonas* spp. were common in the samples where maize plants were cultivated.

9.6 Conclusion

A great diversity of rhizobia is able to nodulate Bambara groundnut accessions but the most common rhizobia nodulating Bambara groundnut are *Bradyrhizobium* spp. In addition, *Bradyrhizobium japonicum* can effectively nodulate the plant. Nevertheless, further studies may need to be carried out to also study potential of indigenous rhizobia in the soils. The nodulation genes Nod A, Nod C and nitrogen fixing gene *nifh* were present in all the strains and are important for the process of nodulation and nitrogen fixation of Bambara groundnut accessions.

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Appendix 1: *Bradyrhizobium japonicum* strains

Appendix 2: Urea fertilizer