RHIZOBIA INOCULANT EFFECTS ON BAMBARA GROUNDNUT GROWTH AND YIELD IN NAMIBIA

A

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN MICROBIOLOGY (BIOLOGICAL SCIENCES)

OF

THE UNIVERSITY OF NAMIBIA

BY

FELICITAS MANTJODI FWANYANGA

201608437

OCTOBER 2023

MAIN SUPERVISOR: DR. L.N. HORN (UNAM)

CO-SUPERVISOR(S): PROF. T. SIBANDA (UNAM)

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is the third most significant food legume in Africa after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*). It is normally grown in poor soils, is drought tolerant, and has a high potential for nitrogen fixation. Despite that, Bambara groundnut yields are on a gradual decline due to a combination of abiotic and biotic stresses such as erratic annual rainfall and climate-induced changes in soil microbial community compositions, which negatively impacting food security. Thus, the application of rhizobial inoculants at planting significantly improves yields in many leguminous crops. Moreover, symbiotic inoculants are mainly used in developed countries to improve nitrogen fixation and improve productivity of grain legumes. Sub-Saharan African countries like Namibia, however, still under-utilise the above practice. Three (3) studies were conducted, of which two were conducted in 2021, and in 2021/22 season. The first study was on farmer's appraisal, which was aimed to assess Bambara groundnut production systems in Kavango East Region. Bambara groundnut farming systems, farming inputs, and production constraints were explored in the two constituencies in Kavango East region. Subsequently, an experiment in a greenhouse of the ZERI at the University of Namibia using soil from the Kavango region to ascertain how well the various *Bradyrhizobium* strains promote nodulation and yield components among three varieties of Bambara groundnut was conducted. The study also evaluated the strains that are most competitive to reach the roots and promote nodulation in a given period. For the greenhouse experiment, the treatments included three different Bambara groundnut varieties that were inoculated with two types of inoculants (MIX inoculant with 7 *Bradyrhizobium* strains [1-7; 3B 4-1; 9-5, 36 1-1; 36 3-2; 55 1-1 and 60 2-1] and inoculant with *Bradyrhizobium vignae* 9-5 only), both of which were obtained from the University of Bremen in Germany the third treatment was an absolute control. The treatments were set up in a randomized complete block design with three replications. In comparison to the single strain 9-5, the mixed strains were found to be more productive and contagious (p=0.05). Moreover, the two inoculants outperformed the non-inoculated cultivars in terms of plant height, shoot dry weight. The brown variety, which was not inoculated, nevertheless, showed some nodules. Hence, in the next season (field experiment) the mixed inoculant strains of *Bradyrhizobium* were applied as single inoculants. The field experiment was conducted at Mashare Agricultural Development Institute (MADI) in Kavango East Region. The same three Bambara groundnut varieties which were used in the greenhouse were treated with chemical fertilizer (urea), 7 *Bradyrhizobium* strains, and negative control with no treatment. After 130 days of planting the cultivars were harvested and different yield parameters were assessed. The control Var03 (red) yielded the greatest grain at 1311.7 kg ha^{-1}, followed by Var01 (brown) treated with In1-7 at 1237.3kg ha^{-1}, while Var01 and Var02 (cream) inoculated with In36 3-2 yielded 1171.3kg ha^{-1} each. Based on the results, farmers are advised to consider inoculating Bambara groundnut seeds with *Bradyrhizobium* strains prior to planting, as this has been shown to have a positive impact on nodulation, grain yield, and biomass production compared to using urea fertilizer which is expensive and environmentally unfriendly. However, it is important to note that the control variety Var03 performed the best, likely due to its compatibility with native soil rhizobia. Therefore, it may be worthwhile for more studies to investigate and identify the unidentified strains used in the study as potential bio-inoculants. Additionally, it is recommended that farmers monitor the performance of different varieties in their specific soil conditions and choose the most suitable one for their crop management. Finally, it is important to adhere to best practices for inoculant application, such as using high-quality inoculants and ensuring proper handling and storage.

Keywords: Bambara groundnuts, biofertiliser, Bradyrhizobia, food security, legumes (Fabaceae), productivity

List of conference(s) proceedings

1. Fwanyanga FM, Horn LN, Sibanda T and Reinhold-Hurek B. Effects of different *Bradyrhizobium* inoculant strains on Bambara groundnut seed rhizospheric nitrogen levels, nodulation and apical plants growth in Namibia. Thesis presented at the mini-conference SusTec 2022, Held at the University of Bremen, Germany July 21st.

2. Fwanyanga FM, Horn LN, Sibanda T and Reinhold-Hurek B. Effects of different *Bradyrhizobium* inoculant strains on Bambara groundnut seed rhizospheric nitrogen levels, nodulation and apical plants growth in Namibia. Thesis presented at the Joint agricultural conference 2022, Held at the University of Namibia, Ogongo Campus, Omusati region, Namibia October 12th to October 14th.

3. Fwanyanga FM, Horn LN, Sibanda T and Reinhold-Hurek B. Effects of different *Bradyrhizobium* inoculant strains on Bambara groundnut seed rhizospheric nitrogen levels, nodulation and apical plants growth in Namibia. Chapter 3 presented at the National Student Research Symposium (NSRS) 2022, Held at NIPAM, Windhoek, Namibia November 22nd to November 23rd.

Publications pertaining to this thesis

Chapter 2

Fwanyanga FM, Horn LN, Sibanda T and Reinhold-Hurek B (2022) Prospects of rhizobial inoculant technology on Bambara groundnut crop production and growth. *Front. Agron.* 4:1004771. doi: 10.3389/fagro.2022.1004771

Table of content

List of tables

List of figures

Abbreviations and Acronyms

BNF	-Biological Nitrogen Fixation
GIZ	- Deutsche Gesellschaft für Internationale Zusammenarbeit
IRAD	-Institute of Agricultural Research for Development
ITS	- Internal Transcribed Spacer
N	-Nitrogen
SPSS	- Statistical Package for the Social Sciences
ZERI	- Zero Emissions Research Initiative

Acknowledgements

This significant research project's success hinges on the collaboration of a diverse group of individuals. Firstly, I extend my sincere gratitude to the Almighty God for enabling me, drawing strength from the saying, "I can do all things through HIM which strengthened me." I wish to acknowledge and thank the many individuals who have played a role in shaping this report. I am deeply appreciative of my supervisors, Dr. Lydia Horn and Prof. Timothy Sibanda, for their invaluable guidance, unwavering support, and the confidence they have shown in me throughout this study.

My heartfelt thanks extend to Prof. Barbara Reinhold-Hurek, Dr. Abhijit, and the entire team at the University of Bremen's Department of Microbe-Plant Interactions in Germany. Their extensive support and shared knowledge, both during and after my time at the University of Bremen, have been invaluable.

To my sisters and friends, I am grateful for your understanding and encouragement, which have been instrumental in so many ways. I also extend my appreciation to each respondent (farmers) who generously dedicated their time and interest to my research. The staff at Mashare Agricultural Developments Institute created a conducive environment during the field experiment, and I'm thankful for their assistance.

Furthermore, I wish to acknowledge the German-Namibian cooperation, which was made possible through grants from the Federal Ministry of Education and Research and the German Academic Exchange Service (DAAD). Their support has been pivotal.

Every individual involved has made a significant contribution to the final outcome of this project, and I want to express my deep gratitude to all. Thank you immensely

Dedication

This thesis is dedicated to my late grandmother, Roswitha Shirudi, who, despite lacking formal education for employment, tirelessly supported us as a smallholder farmer. To my caring mother, Katiku Stephania, your essential role has been instrumental in my achievements. I also extend a special dedication to the resilient under-resourced farmers who persistently ensure their families are nourished

Declaration

I Felicitas Mantjodi Fwanyanga (201608437)

Declare the following:

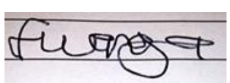
1. I understand what plagiarism entails and am aware of the University's policy in this regard.

2. I declare that this assignment is my own, original work. Where someone else's work was used (whether from a printed source, the Internet or any other source) due acknowledgment was given and reference was made according to departmental requirements.

3. I did not copy and paste any information directly from an electronic source (e.g. a web page, electronic journal article or CD ROM) into this document.

4. I did not make use of another student's previous work and submitted it as my own.

5. I did not allow and will not allow anyone to copy my work with the intention of presenting it as his/her own work.

Signed:  Date: October 2023

Thesis Introduction

Background

Bambara groundnuts (*Vigna subterranea* (L.) Verdc) is the third most significant agricultural legume crop in Africa after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Puozaa et al., 2017). Bambara groundnut is a drought-tolerant but underutilized legume crop that subsistence farmers in Sub-Saharan Africa plant for its edible pulses (Egbe et al., 2013). This legume's edible grain contains a high protein content (20.6%), carbohydrate (56.5%), fat (6.6%), and fiber (6.3%), making it a well-balanced meal (Ibny et al., 2019).

Moreover, Bambara groundnut can form effective root nodules with suitable soil rhizobia that convert atmospheric nitrogen (N) to ammonia for bacterial and plant usage (Hillocks *et al.*, 2012; Zielinski and Reinhold-hurek, 2021). Despite its importance, Bambara groundnut production is hampered by poor crop establishment, low soil fertility, and a lack of adequate nodulation technology (Ikenganyia et al., 2018).

Farmers' efforts to increase soil N levels are limited by the fact that N fertilizers are expensive, insufficient, and may not be available on time. As a result, research into safer environmental control approaches has led to the usage of biofertilization techniques (Yakubu et al., 2010). After careful consideration, rhizobia inoculants were discovered to be useful and could be an alternate choice for crop growth improvement due to their low cost and environmental friendliness (Laurette et al., 2015). This technology has not yet been adopted in Namibia and there is limited information on the most competitive strain to colonize root nodules of Bambara groundnuts and its close relatives.

Overall research objectives

The overall objective of the study was to assess the effects of different rhizobia inoculant strains on Bambara groundnut growth in Namibia. This study will contribute to food security strategies of Namibia through improving yield and productivity of Bambara groundnut.

Specific objectives

The specific objectives of this study were to:

1. Assess farmers' perceived production constraints, preferred traits, and the farming system of Bambara groundnut for northern Namibia.
2. Evaluate how well the different strains of *Bradyrhizobium* promote nodulation, apical plant growth, yield and yield components among three varieties of Bambara groundnut in Namibia.
3. Determine which of the strains have occupied the nodules. That means, which strains is most competitive to reach the roots and promote nodulation in a specified period of time.

Research Hypothesis

This study was premised on the hypothesis that seed inoculation with different *Bradyrhizobium* strains has an effect on nodulation, apical growth, yield and yield components of Bambara groundnut crops grown in Namibia.

Significance of the study

The study used *Bradyrhizobium* spp because they were proven to fix nitrogen and increase the yield and yield components of Bambara groundnut (Zielinski and Reinhold-hurek, 2021), which is of major scientific interest and economic benefit to the country, particularly subsistence farmers.

References

1. Egbe, M. O., Alhassan, G. A., Ijoyah, M., & Onyilo, M. (2013). Nodulation , Nitrogen Yield and Fixation by Bambara Groundnut (Vigna Subterranea (L .) Verdc .) Landraces Intercropped with Cowpea and Maize in Southern Guinea Savanna of Nigeria. *Agricultural Science*, *1*(4), 15–28.

2. Hillocks, R. J., Bennett, C., Mponda, O. M., & Maritime, C. (2012). Bamnara nut: A review of utilisation, market potential and crop improvent. *African Crop Science Journal*, *20*(1), 1–16.

3. Ibny, F., Jaiswal, S., Mustapha, M., & Dakora, F. D. (2019). Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (Vigna subterranea L . Verdc .) in Africa and their relationship with phylogeny. *Scientific Reports*, *9*(1), 1–17. https://doi.org/10.1038/s41598-019-48944-1

4. Ikenganyia, E., Anikwe, M. A. N., & Ngwu, O. (2018). Impacts of Plant Growth Promoting Rhizobacteria Inoculation Configurations on Vegetative Growth , Nodulation and Yield of Bambara groundnut [Vigna subterranea (L .) Verdc .]. *International Journal of Horticulture, Agriculture and Food Science(IJHAF)*, *2*(3), 54–69. https://doi.org/10.22161/ijhaf.2.3.5

5. Laurette, N. N., Maxémilienne, N. B., Henri, F., Souleymanou, A., & Kamdem, K. (2015). Isolation and Screening of Indigenous Bambara Groundnut (Vigna Subterranea) Nodulating Bacteria for their Tolerance to Some Environmental Stresses. *American Journal of Microbiological Research*, *3*(2), 65–75. https://doi.org/10.12691/ajmr-3-2-5

6. Puozaa, D. K., Jaiswal, S. K., & Dakora, F. D. (2017). African origin of Bradyrhizobium populations nodulating Bambara groundnut (Vigna subterranea

L . Verdc) in Ghanaian and South African soils. *PLOS ONE*, *12*(9), 1–23.

7. Yakubu, Kwari, & Ngala. (2010). N 2 Fixation by Grain Legume Varieties as Affected By Rhizobia Inoculation in The Sandy Loam Soil Of Sudano-Sahelian Zone of North Eastern Nigeria . *Nigerian Journal of Basic and Applied Science*, *18*(2), 229–236.

Chapter 1

Bambara groundnuts (*Vigna subterranea (L.)* Verdc) production systems in the Kavango East Region, Northern Namibia

Abstract

The productivity of Bambara groundnut (*Vigna subterranea* (L.) Verdc) is gradually decreasing in subsistence farming systems as a result of biotic and abiotic climatic factors as well as some socioeconomic limitations, amongst others. The objective of this study was to assess the factors behind the diminishing Bambara groundnut output in Namibia's Kavango East Region based on factors such as farming methods, preferred Bambara groundnut seed colour preference/choice, quantity of farming inputs, and production constraints. As a result, a participatory rural appraisal study was conducted in two constituencies of the Kavango East Region, namely Mukwe and Ndiyona, taking four villages per constituency where the crop is predominantly grown. Primary data was collected using structured interviews involving 70 households. Results showed that 77.8% of the farmers allocate less than a hectare for Bambara groundnut planting each year. About 66.7% of the farmers surveyed claimed their Bambara groundnut yields were low, with yield averages between 1 and 100 kg per season. On the economic side, 14.3% of farmers reported that they produced Bambara groundnut for income generation, while (85.7%) produce Bambara for home consumption. Farmers preferred cream seeded Bambara groundnuts which they said were early matures, high yielders, and for their ease of preparation. On the other hand, 8.7% of farmers in the Ndiyona constituencies indicated that they use artificial fertilizer while 91.3% did not use artificial fertilizer. In Mukwe constituencies, all farmers (100%) interviewed reported that they do not apply fertilizer in their Bambara groundnut fields due to the fact that they could not afford it and also due to lack of availability, while some believed that it was not necessary to apply fertlizers. Field pests, low yield, lack of improved varieties, expensive labour, and seed costs were cited as the main production barriers for Bambara groundnuts in these two constituencies. In some of the areas where the cost of artificial N fertilizer is very high, breeding for improved varieties as well as inoculant application are some of the approaches anticipated to boost the output of the Bambara groundnut crop. At the same time simultaneously add nitrogen to the soil medium for succeeding cropping.

Keywords: Bambara groundnut, subsistence farming, soil fertility

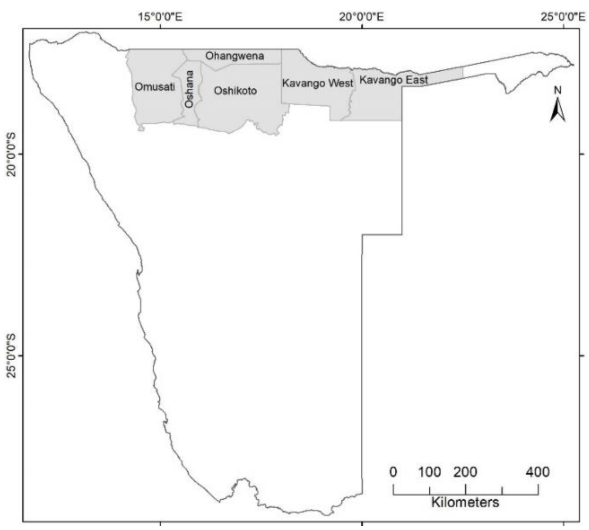
1.1. Introduction

The rate at which world population is growing relative to agricultural expansion suggests that, alongside population growth, attention should also be paid to the production of important crops and other previously neglected crops. The Bambara groundnut (*Vigna subterranean* (L) Verdc.), which thrived in Africa before the introduction of the peanut (*Arachis hypogea*), is one of the latter categories of crops (Aviara *et al.*, 2013; Khan *et al.*, 2021).

The Bambara groundnut has advantages over more favoured species in terms of nutritional value and tolerance to adverse environmental conditions (Temegne *et al.*, 2018). In Namibia, which is a semi-arid country in southwest Africa, 82% of subsistence farming households grow Bambara groundnuts (Fleissner, 2006). The crop, which is the second most significant legume after cowpea, is mostly farmed in the northern regions (Figure 1.1) of Omusati, Oshana, Oshikoto, Ohangwena, Kavango West, and Kavango East (Valombola *et al.,* 2021). Depending on the language, the Bambara groundnut is known by a variety of vernacular names in Namibia, including ngomene (Gciriku), nyimu (Thimbukushu), eefukwa (Oshiwambo) among others.

According to Mukakalisa (2010), the crop is commonly used in Namibia as both a source of income and for domestic consumption. Farmers usually continue using saved seeds from previous harvests since there is no commercial seed production or sale for this crop in Namibia. Luchen *et al.* (2018) claims that most soils in Namibia's northern areas have poor nutrition as well as poor water retention. Despite the low potential of the poor soils to produce crops, subsistence farming remains the primary source of livelihood and food security for the local population. The yield level on farms is currently unclear and is anticipated to be substantially lower making it necessary for an actions to investigate strategies for enhanced Bambara groundnut production and improve the soil fertility (Grönemeyer and Reinhold-Hurek, 2018).

Figure 1.1. Bambara groundnut plant at early flowering (left); Map of Namibia showing the Northern regions where Bambara groundnut occurs (right)



Understanding Bambara groundnut farming systems, variety preferences, choice and quantities of farming inputs, and production constraints is critical for the development and widespread acceptance of production technology (Horn *et al*., 2015). This study was therefore conducted to assess the Bambara groundnut seed preferences by farmers, as well as choice and quantity of farming inputs, as well as the production constraints in the Kavango East Region of Namibia. The Kavango East Region was selected for the study due to a widely held perception that it could serve as Namibia's "Breadbasket" if the local farms were productive (Luchen *et al.*, 2018). Hence, this study focuses on Bambara groundnut farmers in Kavango East region.

1.2. Materials and methods

This study was conducted among two constituencies in the Kavango East Region, namely Ndiyona constituency and Mukwe constituency during- August 2021. The study was conducted at the time when farmers were off season preparing for the new cultivation period. These constituencies were selected because they are known for their production of various crops including Bambara groundnut. Kavango East is located in the northeast of Namibia, with an average annual rainfall between 450–600 mm. The average minimum and maximum temperatures is 23–39 °C (Valombola *et al.*, 2021). In order to identify Bambara groundnut producers in the Kavango East Region, a systematic sampling approach was used. Data was collected from four villages from the Ndiyona constituency (Dumushi, Kakekete, Dosa, Rucara, and Shikoro) and four villages from the Mukwe constituency (Mbapuka, Tjova, Katenture, and Shamangorwa). The sampling constituted 80 households in total, with 10 Bambara farming homes (samples) in each village. Only 70 households were contacted, as some farmers were not available during the interviews. A structured questionnaire was used to conduct interviews and gather the data. Demographic data, Bambara groundnut farming systems (farm size, area planted with Bambara groundnut, average yield per/ha per year, main uses and seed preference). Bambara groundnut farming inputs and production constraints made up four sections of the questionnaire. The interview was contacted in the local languages of Rugciriku and Thimbukushu while the information was gathered and subsequently translated into English. The accrued data was analysed using descriptive statistics in the form of cross-tabulation, frequencies, and graphical display using Statistical Package for Social Sciences (SPSS) version 23 program. Using Pearson's Chi-squared test, an association/dependence test without parametric data was carried out.

Figure 1. 2 The researcher (me) with some of the farmers during my studies at Mbapuka village in the Mukwe constituency. (All pictures taken with the participants consent).

1.3. Results and discussion

The study identified favoured characteristics in the Bambara groundnut farming systems and farmers' perceptions of production constraints in the two constituencies as presented in Tables 1.1 to Table 1.5.

1.3.1. Demographic data of participants

No statistically significant correlation was observed between constituency and the farmers age, according to a cross-tabulation of constituencies by socio-demographics ($\chi 2$=4.243; $P < 0.374$). The study found that, 31.7% of those interviewed were 60 years of age or older, 30.2% were in the 50–59 age range, 22.2% in the 40–49 age range, 11.1% in the 30–39 age range, and 4.8% in the 20–29 age range. These results are comparable to Valombola *et al*. (2021), who reported that the age of the majority of the participants in their study ranged in the category between 46–60 years. The fact that the majority of young people moved to urban regions to look for work may have contributed to the respondents' age range of 40 to 60 in the present study. On the other hand, older individuals continued to work as farmers in rural areas. It is argued that one of the main causes of rural teenagers' migration to urban areas is their negative attitudes toward agricultural pursuits. Horn *et al*. (2015), however, said that youth migration to cities could be reduced if they are motivated to engage in agriculture where they can produce for income generation purposes. An association was detected amongst gender, however, no association was detected among household size (Table 1.1).

Table 1.1: Socio-demographics of Bambara groundnut farmers that were interviewed

Variables		**% of farmers**	**df**	χ^2	***p*-value**
	Female	87.3	1	10.279	0.001
Gender	Male	12.7			
	20-29	4.8			
	30-39	11.1			
	40-49	22.2	4	4.243	0.374
	50-59	30.2			
Age (in years)	≥60	31.7			
	≤10	46.0			
	11-20	46.0	2	0.639	0.727
Household size	≥21	7.9			

According to the demographic findings, only 12.7% of men compare to a high number (87.3%) of women interviewed. This result concurred with findings from Karunaratne *et al*. (2015) and Valombola *et al*. (2021). This could be due to the fact that men are not interested in field cultivation for crop production, as they prioritize other income-generating activities or occupations that are perceived as more profitable or prestigious than Bambara groundnut cultivation. Furthermore, in some societies, certain agricultural activities are traditionally assigned to specific genders. Bambara groundnut cultivation is perceived as a task traditionally associated with women or not aligned with traditional male roles, hence, men less likely participate. Consequently, addressing the gender disparity in Bambara groundnut cultivation may require interventions that tackle these underlying factors. This could include promoting gender equality and challenging gender stereotypes, providing training and resources to enhance men's agricultural knowledge and skills, , and raising awareness about the economic potential and nutritional value of Bambara groundnut cultivation. Engaging men as active participants in promoting sustainable agriculture and food security is crucial for achieving inclusive agricultural development.

1.3.2. Bambara groundnuts farming systems

The Bambara groundnut farming systems in two constituencies in Kavango East Region are shown in Table 1.2 below. Statistically significant differences were recorded among farm sizes in the two constituencies at (P = 0.016), with 65.1% of farmers reported with farm sizes between two (2) and five (5) ha, while 11.1% of farmers reported of having farms sizes larger than five (5) ha while 23.8% of the farmers with 1ha or less farm sizes. There were no statistically significant differences in land sizes between the two constituencies with regards to the area designated for Bambara groundnut growing ($P < 0.576$). According to the data analysis, majority of farmers (77.8%) indicated that they only allocate a small portion of land (less than a ha) for Bambara groundnut cultivation, while 22.2% indicated that they allocate more than 1ha. Horn *et al*. (2015) reported that farmers frequently allocate cropping area based on their preferred or most important crops, and as a result, majority of farmers in the two constituency could have interest in other crops than in Bambara as suggested that larger farming areas are usually dedicated to the crops that are most valuable by the farmer. This was also corroborated by Flesseiner (2006) finding that 67% of farmers in the Kavango region were involved in Bambara groundnut cultivation, which was ranked the sixth important crop in that region. Additionally, in Zimbabwe, the crop also was frequently reported to be receiving a lower priority when land is allocated among the villages (Hillocks *et al.*, 2012).

The annual average yields of Bambara groundnut were also found to be significantly different at ($P < 0.006$). About 42.9% of the farmers reported that they usually harvest less than 50 kg of Bambara groundnut from their portions annually, 33.3% harvest 100 kg or more, while 23.8% harvest 50 to 100 kg. The on-farm production level was still unknown and is anticipated to be substantially low. This is in line with Fleissner (2006) who outlined that the grain yield levels on research stations in the Kavango region ranged from 400 to 600 kg/ha. Local farmers in Namibia, especially in Kavango region have been cited in Grönemeyer *et al.* (2016) as having to deal with low yields and declining soil fertility which could have a negative impact on the farming communities. Therefore, it is crucial to look at measures to increase yield and guarantee food security. These expectations cannot be met sustainably unless soil fertility and production in previously degraded fields are restored. The utilization of green and farm yard manures, composts, and crop residues, as well as crop management options such as natural fallow, intercropping, relay cropping, cover crops, crop rotations, and dual-purpose legumes, has the potential to reverse the deterioration in soil health. Among these techniques, legumes are widely recognized as soil fertility builders and restorers, owing to their relationship with symbiotic nitrogen fixation (Yakubu *et al*., 2010; Chanway *et al*., 2014; Musa *et al.*, 2016). Consequently, microbial inoculants have been used for generations to improve agricultural production, soil health, and plant health (Saad *et al.*, 2020). Many studies have demonstrated that microbial inoculants can boost legumes nodulation, nitrogen fixation, and grain yield in the fields of real-world subsistence farmers (Thilakarathna *et al.*, 2019; Ibny *et al*., 2019; Allito *et al.*, 2021; Gedamu *et al*., 2021). According to a study done in the Kavango East Region by Luchen *et al.*, (2018), cowpeas that were subjected to bio-inoculant treatments yielded a greater grain production in kg ha^{-1} when compared to the negative control and fertilizer treatments. This clearly shows that bio-inoculants may provide local subsistence farmers with a less expensive eco-friendly way to improve output of Bambara groundnuts and other crops.

Table 1.2: Bambara groundnut farming systems in the two constituencies in Kavango East region in Namibia

Variables		**% of farmers**	**df**	**χ2**	***p*-value**
Size of the farm	≤ 1ha	23.8	2	8.304	0.016
	2-5 ha	65.1			
	≥5ha	11.1			
Area planted with BGN	≤ 1ha	77.8	1	0.313	0.576
	≥1ha	22.2			
Average yield per year	≤50kg	42.9			
	50-100kg	23.8	2	10.171	0.006
	≥100Kg	33.3			
Main uses	Home consumption	85.7	1	2.922	0.087
	Income generation	14.3			
Seed color preference	Black	20.6			
	Brown	6.3			

	Red	17.5	4	18.898	0.001
	Cream	54			
	Chocolate	1.6			
Reasons for seed preference	Early maturing	27	4	27.592	0.000
	Easy to cook	17.5			
	High yielder	22.2			
	Size (big)	17.5			
	other	15.9			

It was discovered that Bambara groundnut was grown by 85.7% of farmers just for home consumption while 14.3% was for income generation. The results revealed that only a small number of farmers grow Bambara groundnut for the purpose of selling and income generation, while the majority grow the legume for home consumption. It was also reported that some farmers sell their fresh produce or seeds unofficially at open marketplaces to other farmer during planting seasons.

According to the findings and comparisons with other nations, Bambara groundnut is significantly underutilized and does not receive the attention it merits in Namibia. Temegne *et al.* (2018), for instance, noted that Bambara groundnut seeds are fed to pigs and chickens while leafy stems are used as animal fodder in Eastern Nigeria. Additionally, the IRAD (Institute of Agricultural Research for Development) in Cameroon mixes Bambara groundnut flour with cereal to create "baby lion" porridge. However, there is not a single commercial Bambara groundnut by-product available in Namibian stores.

Another factor that farmers considered during evaluation in all of the constituencies was on the choice of colour preferred at sowing. A high statistically significant difference was observed between constituencies with regard to preferred seed colour of Bambara groundnut ($\chi2$=18.898; $P < 0.001$). Indicating that the favoured seed colour of the Bambara groundnut varied between the two constituencies. In Ndiyona, for example, the majority of farmers chose the black seeded Bambara groundnut, followed by the red coloured Bambara groundnut, however in Mukwe, no farmer favoured the brown coloured seeds, with the majority preferring the cream-coloured Bambara groundnut. Overall, cream-coloured seeds were most popular among Bambara groundnut consumers (54%), followed by black (20.6%), and chocolate (1.6%). Similar findings were made by Valombola *et al*. (2021), who also claimed that cream seeds were the most popular in that area. In contrast, Hillocks *et al.* (2012) reported on the red seeds variety popularity in Zimbabwe than cream seeds variety, and was reported to have high market value than the cream coloured.

Farmers reported that they choose preferred seed colour based on the variety of characteristics, including early maturation (27%), easy cooking (17.5%), high yielder (22.2%), size (large), and other factors including the shoot's resistance to wilting, notably for the red colour Bambara groundnut. According to the results of the Chi-square test, there was a highly significant association ($\chi2 = 27.592$; $P \leq 0.000$) between the constituencies' seed selection criteria. The farmers explained that some Bambara groundnut colours, like the red ones, are bitter and difficult when cooking or hard when crushing to make relish. According to Mubaiwa *et al.* (2017) and Khan *et al* (2021), the hard-to-cook phenomenon is the main issue restricting the use of Bambara groundnut.

1.3.3. Traditional dishes prepared from Bambara groundnut

Bambara groundnut is consumed in a variety of ways at various stages of maturity in the two constituencies in the Kavango East Region (Table 1.3). After boiling for nearly an hour, newly collected pods are consumed as a snack.

Table 1.3: Traditional dishes prepared from Bambara groundnut in the two constituencies in Kavango East Region

Traditional dishes from Bambara groundnut in Kavango	**Preparation**
1.Stiff porridge "mugombakapindi"	1.1.Boiled dry seeds with mahangu flour
2.Relish/soup	2.1.Fresh seeds/ dry roasted seeds, boiled into soup
3.Snacks "shikuvira"	3.1.Roasted dry seed flour mixed with peanut flour
4. Boiled seeds	4.1.Boiled seeds when fresh or dry, mixed with corn
5.Instant porridge "rutura"	5.1.roasted dry seed flour with warm water

Dry Bambara groundnuts can be mashed to make soup or relish or roasted and consumed as a snack. Additionally, a blend of Mahangu flour and boiled dry Bambara groundnut seeds is used to make the stiff porridge "mugombakapini." Bambara groundnuts are roasted, crushed into flour, and then used to produce "rutura," an instant porridge. It is hard to find recipe of these important cuisine in Namibia as most of the information is not documented. Even though the number of foods made in Kavango is less than in other African countries, other countries also prepare some of these dishes. For instance, Mubaiwa *et al.* (2017) reported that Bambara flour has been used to make both stiff and thin porridge in Eastern Africa. Dry Bambara groundnut seeds are pulverized and converted into a delicacy in Eastern Nigeria known as "okpa," which is wrapped in banana leaves before cooking and consumed as breakfast, lunch, or dinner by a typical low socioeconomic family (Obidiebube *et al.*, 2019).

1.3.4. Inputs for Bambara groundnut farming

The Chi-square test indicated that there was a statistically significant association ($\chi2$ = 3.592; $P \leq 0.058$) in farmers' usage of fertilizer in the two constituencies. Indicating that the usage of fertilizer differed among the two constituencies. For instance, 100% of farmers in Mukwe constituencies indicated that they do not use fertilizer in their Bambara groundnut fields, while 8.7% of farmers in the Ndiyona constituencies indicated that they use fertilizer. The farmers who use fertilizer were identified as one of the leading farmers in the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) programs, hence, they are given fertilizer and are trained on how to use it (Table 1.4).

Table 1.4: Farmers' response on the usage of fertilizer in the two constituencies in Kavango East Region

Constituency	**% of farmers with fertilizer usage**	**% of farmers without fertilizer usage**	**df**	**χ^2**	***P*-value**
Mukwe	0	100	1	3.592	0.058
Ndiyona	8.7	91.3			

It was clear, nonetheless, that majority of farmers do not utilize fertilizers at ($\chi2$ = 6.86; $P \leq 0.032$). While all farmers in Mukwe constituency reported that they do not use fertilizer, 42.5% of them cited unavailability and affordability as the reasons why they do not use it, while 17.4% of the farmers in Ndiyona cited unavailability only. Fertilizer is not required, according to 57.5% of farmers in Mukwe and 73.9% of farmers in Ndiyona. This is due to several factors, including the fact that their forefathers never applied fertilizer on their field crops including Bambara groundnut which they believed that it did not need fertigation (Table 1.5). The conclusions regarding fertilizer consumption are comparable to those of Horn *et al*. (2015) who reported that most farmers do not have access to synthetic fertilizers for enhancing soil fertility. Furthermore, these fertilizers are believed to increase soil acidity and may result in a high reliance on N fertilizer for crop production (Lindström and Mousavi, 2020). As a result, more efficient techniques are required. One of the techniques is the use of rhizobacteria inoculants to increase leguminous crop production while reducing the need of synthetic N fertilizer (Laurette *et al.*, 2015). Rhizobial inoculation is a less expensive and often more successful agricultural strategy for guaranteeing adequate N supply (Yakubu *et al*., 2010).

Table 1.5: Reasons for not using fertilizer in the two constituencies in the Kavango East Region

Constituency	**Reason for farmers not using fertilizer**		**df**	**χ^2**	***P*-value**
	% of farmers who do not need fertilizer	**% of farmers who do not use fertilizer due to availability**			
Mukwe	57.5	42.5	1	6.86	0.032
Ndiyona	73.9	17.4			

1.3.5. Bambara groundnut production constraints

High costs and field pests were cited as major production constraints in Bambara groundnut production (Table 1. 6). In addition, high labour costs were cited as a constraint on the production of Bambara groundnuts by 62.5% of farmers and high seed costs were cited by 37.5% of farmers in the Mukwe constituency. On the other hand, 60.9% of farmers in Ndiyona constituency, responded that high labour costs, of 30.4% claimed high seed costs, and 8.7% said high fertilizer costs were a production limitation for Bambara groundnuts. Due to low yield of Bambara groundnut, most farmers finishes their harvested Bambara groundnut before the next planting and harvest, sparing nothing for planting the following season. As a result, they struggle to obtain enough seeds for planting, and their only alternative is to borrow or buy from informal markets or neighbours. This is supported by Valombola *et al*. (2021) who noted that, the seed production and marketing system for this crop is not formalised, as farmers continue to acquire seeds informally by using their own seeds from prior harvests. Some of the farmers claimed that the reasons for not expanding their production was due to the lack of necessary harvesting equipment. Some claimed that the cultivation by hand hoeing was limiting their production and urged for government to avail tractors or oxen to them. Where there is no means of labour to land tilling, farmers reserve their energy for the cultivation of other vital crops like Mahangu instead of Bambara groundnut. Grönemeyer and Reinhold-Hurek (2018) reported that several factors, such as unpredictable yields, the danger of crop failure, and a lack of financial resources contribute to food insecurity in these rain-fed agriculture systems in the Kavango Region. This means that farmers also need to be provided with the required resources to maximize productivity, in addition to the use of microbial inoculants to boost production or yield. According to Mfilinge *et al*. (2014) and Santos *et al*. (2019), using appropriate inoculant strains in nitrogen-deficient soils may provide an excellent opportunity for increasing legume growth and development.

Table 1.6: Bambara groundnut production constraints in the two constituencies (Mukwe and Ndiyona) in Kavango East

Bambara groundnut production constraints	Constituency		Df	χ^2	*P*-value
	% of farmers in Mukwe	% of farmers in Ndiyona			
High cost					
fertilizer	0.0	8.7			
seed	37.5	30.4	2	3.693	0.158
labor	62.5	60.9			
Field pest					
Stem borer	7.5	4.3			
Greenburg's	0.0	21.7	3	13.719	0.003
Aphids (runwamaghadhi)	92.5	65.2			
Stem and stalk borers	0.0	8.7			

There were statistically significant association between constituencies and field pests ($\chi2 = 13.719$; $P \leq 0.003$), that is, the response on field pests as a contributing factor to yield losses every year was different among the two constituencies (Table 1.6). Most of the farmers in both constituencies deals with aphids, also known as "runwamaghadhi" locally (Fig 1.3). In addition, they also reported of rodents, termites, ants, and cutworms (*Agrotis*) as major pests in their field crops. Similar to this finding, Mukakalisa (2010) and Tlankka *et al*. (2020) found that insect pests such as aphids (*Aphis* sp.), leafhoppers (*Hilda patruelis*), and bruchids (*Callosobruchus maculatus,* and *C. subinnotatus*) limit the output of Bambara groundnuts. Therefore, efficient agriculturally beneficial microbes (microbial inoculants) have the potential to play a role in sustainable crop production due to their immense plant growth promoting attributes, as well as plant protection metabolites (biopesticides) (Singh *et al.*, 2016; Gedamu *et al*., 2021). The use of biopesticudes will be of good use to the farmers as they are less expensive and safer than chemical pesticides (Babalola *et al.*, 2017; Tsegaye *et al*., 2017).

Figure 1.3: Aphids (left) and termites (right), pests observed among Bambara ground, during growing and harvesting periods.

1.4. Conclusion

This study provided insights on Bambara groundnut farming systems, farmers preferred seed colour and the reason for their preference, Bambara groundnut farming input, and production constraints in the two constituencies of the Kavango East Region, in Namibia. The availability of better seeds and production inputs, which includes fertile soils were found to be critical factors in increasing Bambara groundnut yield in the country. Therefore, a healthy and productive soil is essential for agricultural sustainability, food security, and energy renewal. It is believed that the development of the underutilized Bambara groundnut is expected to be greatly aided by the adoption of modern technology, such as inoculants, to improve production and feed the world's expanding population. Based on the information presented this study, the Kavango Regions could serve as the Bambara groundnut production hub and could supply the entire Namibia and beyond. However, this could only be realised if the country's Bambara groundnut productivity is supported with modern production practices and inputs as well as provision of quality seeds amongst others.

References

1. Aviara, N. A. *et al.* (2013) 'Bambara groundnut processing, storage and utilization in North Eastern Nigeria', *Continental J. Engineering Sciences*, 8(1), pp. 28–36. doi: 10.5707/cjengsci.2013.8.1.28.36.
2. Babalola, O. O. *et al.* (2017) 'Biological nitrogen fixation: The role of underutilized leguminous plants', in Deepak, G. P. et al. (eds) *Microorg. Green Revol.* Singapore: springer nature, pp. 431–443. doi: 10.1007/978-981-10-6241-4.
3. Fleissner, E. W. K. (2006) *The improvement of Bambara groundnut production in Northern Namibia by means of breeding strategies and agronomic investigations*. Windhoek.
4. Gedamu, S. A., Tsegaye, E. A., & Beyene, T. F. (2021). Effect of rhizobial inoculants on yield and yield components of faba bean (Vicia fabae L .) on vertisol of Wereillu District , South Wollo, Ethiopia. *CABI Agriculture and Bioscience*, 2(8), 1–10. https://doi.org/10.1186/s43170-021-00025-y
5. Gopalakrishnan, S. *et al.* (2015) 'Plant growth promoting rhizobia : challenges and opportunities', *3 Biotech*, 5, pp. 355–377. doi: 10.1007/s13205-014-0241-x.
6. Grönemeyer, J. L. *et al.* (2016) 'Bradyrhizobium vignae sp. nov., a nitrogen-fixing symbiont isolated from effective nodules of Vigna and Arachis', *International Journal of Systematic and Evolutionary Microbiology*, 66(1), pp. 62–69. doi: 10.1099/ijsem.0.000674.
7. Grönemeyer, J. L. and Reinhold-Hurek, B. (2018) 'Diversity of bradyrhizobia in subsahara Africa: A rich resource', *Frontiers in Microbiology*, 9(2194), pp. 1–8. doi: 10.3389/fmicb.2018.02194.
8. Hillocks, R. J. *et al.* (2012) 'Bamnara nut: A review of utilisation, market potential and crop improvent', *African Crop Science Journal*, 20(1), pp. 1–16.
9. Horn, L., Shimelis, H. and Laing, M. (2015) 'Participatory appraisal of production constraints, preferred traits and farming system of cowpea in the northern Namibia: Implications for breeding', *Legume Research*, 38(5), pp. 691–700. doi: 10.18805/lr.v38i5.5952.
10. Karunaratne, A. S., Walker, S. and Ruane, A. C. (2015) 'Modelling Bambara

groundnut yield in Southern Africa : towards a climate-resilient future', *Climate Research*, 65, pp. 193–203. doi: 10.3354/cr01300.

11. Khan, M. M. H. *et al.* (2021) 'Bambara groundnut (Vigna subterranea L. Verdc): A crop for the new millennium, its genetic diversity, and improvements to mitigate future food and nutritional challenges', *Sustainability*, 13(10), pp. 1–27. doi: 10.3390/su13105530.
12. Laurette, N. N. *et al.* (2015) 'Isolation and Screening of Indigenous Bambara Groundnut (Vigna Subterranea) Nodulating Bacteria for their Tolerance to Some Environmental Stresses', *American Journal of Microbiological Research*, 3(2), pp. 65–75. doi: 10.12691/ajmr-3-2-5.
13. Lindström, K. and Mousavi, S. A. (2020) 'Effectiveness of nitrogen fixation in rhizobia', *Microbial Biotechnology*, 13(5), pp. 1314–1335. doi: 10.1111/1751-7915.13517.
14. Luchen, C. C. *et al.* (2018) 'Evaluating the Yield Response to Bio-Inoculants of Vigna unguiculata in the Kavango Region in Namibia', *Journal of Plant Pathology & Microbiology*, 9(10), pp. 1–5. doi: 10.4172/2157-7471.1000456.
15. Mfilinge, A., Mtei, K. and Ndakidemi, P. A. (2014) 'Effects of Rhizobium inoculation and Supplementation with P and K , on Growth , Leaf chlorophyll content and Nitrogen Fixation of Bush bean varieties', *American Journal of Research Communication*, 2(10), pp. 49–87.
16. Mubaiwa, J. *et al.* (2017) 'Hard-to-cook phenomenon in bambara groundnut (Vigna subterranea (L.) Verdc.) processing: Options to improve its role in providing food security', *Food Reviews International*, 33(2), pp. 167–194. doi: 10.1080/87559129.2016.1149864.
17. Mukakalisa, C. (2010) *Molecular, Environmental and Nutritional Evaluation of Bambara Groundnut (Vigna subterranea (L.) Verdc.) for Food Production in Namibia*. The University of Namibia.
18. Obidiebube, E. *et al.* (2019) 'Effect of spacing on the growth and yield of Bambara groundnut (Vigna subterrananea (L) Verde.) in Asaba Delta State', *Global Journal of Agricultural Research*, 7(3), pp. 9–13. Available at: www.eajournals.org.
19. Saad, M. M. *et al.* (2020) 'Tailoring plant-associated microbial inoculants in

agriculture: A roadmap for successful application', *Journal of Experimental Botany*, 71(13), pp. 3878–3901. doi: 10.1093/jxb/eraa111.

20. Santos, M. S., Nogueira, M. A. and Hungria, M. (2019) 'Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture', *AMB Express*, 9(1), pp. 1–22. doi: 10.1186/s13568-019-0932-0.
21. Singh, J. S. *et al.* (2016) 'Book Review: Microbial Inoculants in Sustainable Agricultural Productivity- Vol. II: Functional Application', in *Frontiers in Microbiology*, pp. 1–2. doi: 10.3389/fmicb.2016.02105.
22. Temegne, N. *et al.* (2018) 'Origin and Ecology of Bambara Groundnut (Vigna Subterranea (L.) Verdc: A Review', *J. ecol. nat. resour.*, 2(4), pp. 1–10. doi: 10.23880/jenr-16000140.
23. Thilakarathna, M. S. *et al.* (2019) 'Evaluating the Effectiveness of Rhizobium Inoculants and Micronutrients as Technologies for Nepalese Common Bean Smallholder Farmers in the Real-World Context of Highly Variable Hillside Environments and Indigenous Farming Practices', *Agriculture*, 9(20), pp. 1–17. doi: 10.3390/agriculture9010020.
24. Tlankka, N. S., Mbega, E. R. and Ndakidemi, P. A. (2020) 'Potential of Indigenous Pesticidal Plants in the Control of Field and Post-Harvest Arthropod Pests in Bambara Groundnuts (Vigna subterranea (L.) Verdc.) in Africa: A Review', *American Journal of Plant Sciences*, 11(05), pp. 745–772. doi: 10.4236/ajps.2020.115054.
25. Tsegaye, Z., Assefa, F. and Beyene, D. (2017) 'Properties and Application of Plant Growth Promoting Rhizobacteria', *International Journal of Current Trends in Pharmacobiology and Medical Sciences*, 2(1), pp. 30–43.
26. Valombola, S. J., Awala, K. S. and Hove, K. (2021) 'Farmers' preferences, seed source, production constraints and improvement needs assessment of Bambara groundnut (Vigna subterranea [l.] verdc.) northern rural of Namibia', *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 21(3), pp. 789–798.

27. Yakubu, Kwari and Ngala (2010) 'N 2 Fixation by Grain Legume Varieties as Affected By Rhizobia Inoculation in The Sandy Loam Soil Of Sudano-Sahelian Zone of North Eastern Nigeria .', *Nigerian Journal of Basic and Applied Science*, 18(2), pp. 229–236.

Chapter 2: A Review of the literature

Prospects of rhizobial inoculant technology on Bambara groundnut crop production and growth

Abstract

After peanuts and cowpeas (*Vigna unguiculata*), the Bambara groundnut (*Vigna subterranea* (L.) Verdc) is the third most significant food legume in Africa. It is characteristically grown in marginal soils, is drought tolerant, and also has the potential for nitrogen (N) fixation. Despite that, year-on-year Bambara groundnut yields are on a gradual decline due to a combination of abiotic and biotic stresses such as uneven annual rainfall and climate-induced changes in soil microbial community compositions, negatively impacting food security. Thus, the application of rhizobial inoculants at planting significantly improves yields in many leguminous crops. Moreover, symbiotic inoculants are well established in developed countries for improving N fixation and productivity in grain legumes. Sub-Saharan African countries, however, still under-utilise the above practice. In crop production, N is the most frequently deficient nutrient since it stimulates root and shoots growth. Whereas N fertilisers can be used to supplement soil N levels, they are, however, also costly, at times inadequate, may not be timely in supply and may have deleterious environmental consequences. Hence, rhizobial inoculants are seen as a cheaper, easier, and safer method for improving N-fixation and crop productivity in grain legumes. As a result, smallholder farming systems are food secure. Thus, identifying the most efficient rhizobial strains for biofertiliser production for Bambara groundnut is of utmost importance to the farming communities.

Keywords: Bambara groundnuts, biofertiliser, food security, legumes (Fabaceae), productivity, rhizobia

2.1. Introduction

Leguminosae, which has more than 770 genera and 19,500 species, is the third-largest land plant family (Liu et al., 2011; Ren, 2018). Additionally, leguminous are the second-most produced crops after cereal (Belete et al., 2019). There are many native legume species in Africa where the highest levels of global legume diversity are found (Sprent et al., 2010). According to Mfilinge et al. (2014), Büchi et al. (2015), Phillips and Saunders (2016), and other sources, legumes are grown as fodder, seed crops, as cover crops along cash crops or in conjunction with other crops. The capacity of legumes to fix atmospheric N has been credited with their ability to dominate many unfavourable and disturbed ecosystems by enabling the plants to flourish in nutrient-poor soils (Andrews and Andrews, 2017; Jaiswal and Dakora, 2019; Van Wyk, 2019).

The Bambara groundnut (*Vigna subterranea* (L.) Verdc), which is one of the historically grown legume food in Africa, is significant in several areas, including nutrition, medicine, and agronomical value, yet it is neglected and underutilised (Musa et al., 2016; Babalola et al., 2017; Gbaguidi et al., 2018; Ikenganyia et al., 2018; Khan et al., 2021). After the well-known groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*), it is the third-grain legume crop grown in the tropical lowlands of Africa (Egbe et al., 2013). The grains show a variety of colours (Figure 2.1). Bambara groundnut varieties are enjoyed by many people, especially in Africa, and are consumed in various forms, including dried, freshly cooked, or in combination with other grains as food (Kanonge-Mafaune et al., 2018). In addition to other nutritional content, the grain of Bambara is made up of a high percentage of protein (20.6%), carbohydrate (56.5%), fat (6.6%), and fibre (6.3%) (Hillocks et al., 2012; Mubaiwa et al., 2017; Ibny et al., 2019). According to Mubaiwa et al. (2017), the Bambara groundnut has a higher protein quality since its protein score recorded was 80% as opposed to 74% for soybean, 65% for groundnut, and 64% for cowpea. The lack of information regarding better seed systems, breeding, agronomic techniques, processing, and utilisation are amongst the factors restricting the utilisation of Bambara groundnut (Mubaiwa et al., 2018; Tan et al., 2020). It is well known that Bambara groundnut cultivation contributes to the preservation of soil fertility through the symbiotic fixation of N by root nodule-associated bacterial symbionts called rhizobia (Karunaratne et al., 2015). Despite its contribution to soil wellbeing, symbiotic N-fixation is affected by some climatic conditions such as poor rainfall, prolonged drought, as well as high temperatures that affect rhizobium populations in the soil, and consequently reduce nodulation and yields of locally cultivated Bambara groundnuts (Grönemeyer et al., 2014). As a countermeasure strategy, studies have indicated that coating Bambara groundnut seeds with rhizobial inoculants before planting tends to increase yields (Hillocks et al., 2012; Ibny et al., 2019). Motivated by the need for adaptation to climate change and to sustainably stabilise and improve yield of nutritious food in smallholder agriculture facing challenges to apply N fertiliser (Pulido-Suárez et al., 2021), this review discusses benefits of Bambara groundnut and potential of rhizobial inoculation in Bambara groundnut crop production, and urges on developing adapted inoculants. As a search strategy, we used the terms ‘Bambara groundnut’, ‘rhizobia’, ‘biofertiliser/inoculants’ or ‘Biological Nitrogen Fixation’, to screen the relevant journals for recent articles from 2010 to 2022, with the exception of one article from 1997. The quality areas of selection bias, suitable data collection and analysis, and generalizability were used to evaluate each study’s quality.

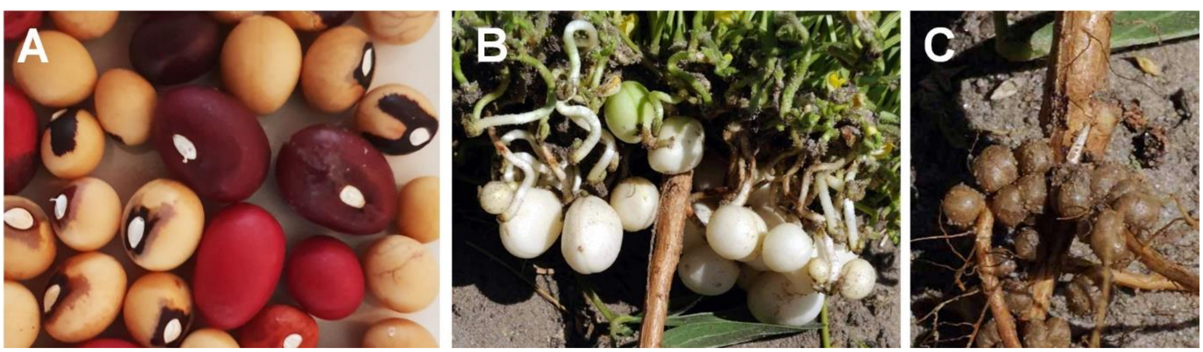


Figure 2.4: Examples of Bambara groundnuts. **(A)** Dehulled grains in various colours, here from cream to dark brown. **(B)** Freshly harvested Bambara groundnuts. **(C)** Symbiotic root nodules of field-grown Bambara groundnut.

2.2. Origin and distribution of Bambara groundnut

Bambara groundnut is a native African legume grown for generations in sub-Saharan Africa, primarily in semi-arid areas such as Namibia (Ikenganyia et al., 2017; Temegne et al., 2018). According to Nassé et al. (2019); Mayes et al. (2019) and Khan et al. (2021), Bambara groundnut was initially discovered in West Africa and appears to have moved southwards throughout sub-Saharan Africa. Literature has suggested that the Bambara groundnut originated in an area named "BAM-BARA", which is home to an agriculturist tribe that resided largely in the state of Bambara near Timbukutu in the west African region of central Mali (Tan et al., 2020), hence the name Bambara groundnut. Currently, the Bambara groundnut is grown extensively in most of West to southern Africa, Central Africa, Indonesia, Malaysia, India, Sri Lanka, Philippines, South Pacific, sections of northern Australia, Papua New Guinea, Central and South America (Aviara et al., 2013; Karunaratne et al., 2015).

Several semi-arid and sub-Sahara African countries, including Nigeria, Ghana, Cameroon, Togo, and Mali are now well-known for cultivating the crop. The primary growing regions for Bambara nuts in Southern Africa, are South Africa and Zimbabwe, while Southeast Asia, especially Thailand, Indonesia, and Malaysia, make up the secondary growing region (Khan et al., 2021). The world's annual production is estimated to be around 330 thousand tons, with the west African countries - Burkina Faso, Cameroon, Mali, Niger, Togo, and the Democratic Republic of the Congo being the major growing regions, with annual production at about 300 000 million tons and with Burkina Faso providing the most extensive yield at around 100 000 million tons per year (Khan et al., 2021). The lower yields in Sub-Saharan Africa emphasise the need for Bambara groundnut breeding to enrich varieties, as well as inoculant use to improve agronomic practices and enhance yields.

2.3. Biological nitrogen fixation

Biological Nitrogen Fixation (BNF) is a fascinating biological phenomenon that involves the interaction between soil microorganisms, in particular bacteria, and higher plants (Santos et al., 2019). When the bacteria are in a symbiotic relationship, the BNF process is mediated by bacteria and the product is readily available to plants (Santi et al., 2013). During this process atmospheric N_2 is reduced to ammonia (NH_3) by the action of the enzyme nitrogenase (Liu et al., 2016). BNF occurs in the rhizosphere of non-leguminous plants and, in the root nodules of leguminous plants in the soil (Ahemad and Kibret, 2014).

Rhizobia in the root nodule obtain carbon sources and thus energy from the higher plant and use them to reduce usable N which is then directly supplied to the plant to produce biomass and food (Puozaa et al., 2017; Mahmud et al., 2020). Jaiswal et al. (2021) reported that legume-rhizobia symbiosis is responsible for between one-third and one-half of the total N added to agricultural land. Similar to this, Kanonge-Mafaune et al. (2018) claimed that under ideal conditions, grain legumes may fix up to 200 kg of N ha^{-1}, considerably lowering the need for inorganic N fertilisers. Ibny et al. (2019) and Pulido-Suárez et al. (2021) also argue that the N fixed by legumes can be as high as the amounts of N fertilisers used in conventional farming practices. This is congruent with Egbe et al. (2013) arguing that nearly half of the amount of N in inorganic N fertilisers is through symbiotic N fixation, according to estimates. According to some estimations, Bambara groundnuts may fix up to 28.42 kg N ha^{-1} in the Sudano-Sahelian zone of Nigeria (Yakubu et al., 2010). Furthermore, Yusif et al. (2016) noted that fixation by rhizobia recruited from the soil alone provided additional N of 61.1 mg $plant^{-1}$ but soil and inoculated rhizobia (HISTICK) together gave an additional N input of 124.7 mg $plant^{-1}$ in groundnut in Nigeria. While the above estimates differ in magnitude, the overall observation is that BNF significantly contributes to soil N build-up, making it a reliable substitute for inorganic fertilizer application.

Table 2.1 presents some of the benefit increases of legume yields or nitrogen inputs with different inoculants. The highest N increases have been recorded by Denton et al. (2017) in Faba bean and Lupin. Furthermore, several reports have as well indicated increased grain yield with increased N content in the soil. The highest grain yield improvements have been recorded in cowpea which was inoculated with *Bradyrhizobium* strains BR 3267 and BR 3262 (EMBRAPA BRAZIL). From the review, it is evident that Bambara groundnut has been ignored by research given that there are few reports on inoculant use on Bambara groundnut. However, the information emanating from research findings on other legume crops is giving support to inoculant technology and its benefit to the plants.

Table 2.7: Benefit increase of legumes by inoculants

Common name of Legume crop	Variety	rhizobia inoculant	Benefits increase	References
Cowpea	Bornoji red	Three *Bradyrhizobium* strains (collected from the same legume cultivars during the previous experiment)	42.68 kg N ha^{-1} (N fixation)	Yakubu, Kwari and Ngala (2010)
Groundnut	KoljiKanuri		27.19 kg N ha^{-1} (N fixation)	
	Mallum			
Bambara groundnut	Karekare		32.53 kg N ha^{-1} (N fixation)	
Soy bean	N/A	Legumefix with *Bradyrhizobium japonicum* strain 532 C (Becker Underwood, UK), Biofix with *B. japonicum* strain USDA 110 (MEA, Kenya)	19 and 12% (grain yield)	
Cowpea	N/A	*Bradyrhizobium* strain BR 3267 and BR 3262 (EMBRAPA BRAZIL)	46 and 37% (grain yield)	Ulzen *et al.* (2016)
			grain yield from 0.48 to 1.94 t ha^{-1} (grain yield)	
Faba bean	N/A	Peat- based carrier (BASF, Southbank, Australia)	46 to 280 kg N ha^{-1} (N fixation)	
Lupin	N/A		175 kg N ha^{-1} (N fixation)	Denton *et al.* (2017)
Faba bean	Moti	NSFBR-15 and NSFBR-12	206.9 and 99.3% (shoot dry weight)	Allito *et al.* (2021)

Common name of Legume crop	Variety	rhizobia inoculant	Benefits increase	References
Faba bean	N/A	Carrier peat-based *Rhizobium* strains of faba bean, EAL 1018 obtained from Soil Microbiology Laboratory of Holeta Agricultural Research Centre (HARC)	45.6, 27 and 11.6% (grain yield)	Gedamu, Tsegaye and Beyene (2021)
Groundnut	Chinese	KNUST 1031	24% (seed yield)	Wilson *et al.* (2021)
Groundnut	Samnut 22	KNUST 1002	16% (seed yield)	

2.4. The rhizobial symbionts

According to Tsukanova et al. (2017), rhizobia are soil-borne bacteria that are part of the *Proteobacteria* families α, ß, and δ with the ability to develop nodules on the roots, and occasionally the stems of host plants, which are mostly legumes (Ahemad and Kibret, 2014; Raissa et al., 2020). The alpha-proteobacteria harbour most bacterial genera of nodule-forming microsymbionts; for example, the major genera are *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Mesorhizobium*, *Ensifer (Sinorhizobium), Neorhizobium*, *Pararhizobium*, and *Allorhizobium*, collectively referred to as "rhizobia" (Jaiswal et al., 2021), which can form symbiotic associations with a variety of legumes.

Except for a few studies that have demonstrated that species of the genus *Bradyrhizobium* nodulate Bambara groundnut, there is little information available on the biodiversity of rhizobia nodulating Bambara groundnut in African soils (Ibny et al., 2019). According to Jaiswal and Dakora (2019), *Bradyrhizobium* is regarded as one of the most cosmopolitan and diverse bacterial groups responsible for nodulation in a variety of host legumes including Bambara groundnut in Africa. However, it is important to remember that the distribution patterns of rhizobial species vary depending on the geographic region (Ikenganyia et al., 2017). Hence, they might be other rhizobial symbionts associated with Bambara groundnut which have not been explored. Despite the need for increased food legume production, it has been reported that the diversity and distribution of the African nodulating indigenous legumes that form symbioses with bradyrhizobia and other rhizobia are not well understood (Ajayi et al., 2020). Nonetheless, research has revealed that bradyrhizobia nodulates several African dietary legumes (Ibny et al., 2019; Ajayi et al., 2020; Dlamini et al., 2021). This could be explained by the distinctive edaphoclimatic conditions of the continent's diverse habitats (Jaiswal and Dakora, 2019). To find rhizobia that can efficiently nodulate and promote the growth of significant yet underutilised crop species like Bambara groundnut, it is crucial to constantly investigate new geographic regions. Further research to facilitate identifying of numerous unique bradyrhizobia residing in African soils are required, to better understand the biogeography of *Bradyrhizobium* strains and their potential for inoculant production. Examples of some of the rhizobia that have been isolated from different legume crops and more specifically on

Bambara groundnut are presented in Table 2.2. Amongst at least 10 species of *Bradyrhizobium* spp. isolated from nodules or nodulating Bambara groundnut in the laboratory, there are several that were Namibian isolates recently described as novel species, such as *B. subterraneum* (Grönemeyer et al., 2015a), *B. kavangense* (Grönemeyer et al., 2015b), *B. namibiense* (Grönemeyer et al., 2017), or *B. vignae* (Grönemeyer et al., 2016). Thus, this host plant appears to be quite promiscuous towards *Bradyrhizobium* spp., suggesting that inoculants that are well-adapted to varieties and environmental conditions may have to be developed for maximum benefit of yields.

Table 2.8: Examples of rhizobia isolated from different legumes at different locations

Rhizobia species	Common name of legumes	Latin name	Crop part	Location of isolation	Reference
Ensifer fredii *Rhizobium tropici*	Soybean	*Glycine max* (*L.*)	virgin and cultivated soils	Zambia	Kapembwa *et al.* (2016)
Rhizobium anhuiense, R. fabae, R. vallis, R. sophorae, Agrobacterium radiobacter	Faba bean	*Vicia faba* (*L.*)	Root nodule	Panxi, China	Chen *et al.* (2018)
Bradyrhizobium pachyrhizi, B. yuanmingense and *B. daqingensees*	Cowpea, Bambara Groundnut, peanut, Hyacinth bean, and common bean.	*Vigna unguiculata, V. subterranea, Arachis hypogaea, Lablab purpureus, and Phaseolus vulgaris*	Root nodules	Namibian Mashare and the Angolan Cusseque areas	Grönemeyer *et al.* (2014)
Bradyrhizobium kavangense	Cowpea and Bambara groundnut	*Vigna unguiculata and Vigna subterranea*	Root nodules	Subsistence farmers' fields in the Kavango region, Namibia	Grönemeyer, Hurek and Reinhold-Hurek, (2015)
Bradyrhizobium subterraneum	Bambara groundnut and Peanuts	*Vigna subterranean and Arachis hypogaea*	Root nodules	Subsistence farmers' fields in the Kavango region, Namibia	Grönemeyer, Chimwamurombe and Reinhold-Hurek (2015)

Rhizobia species	Common name of legumes	Latin name	Crop part	Location of isolation	Reference
Bradyrhizobium vignae .	Cowpea, Bambara groundnut and peanuts	*Vigna unguiculata*, *Vigna subterranea* and *Arachis hypogaea*	Root nodule	Subsistence farmers' Fields in the Kavango region of Namibia	Grönemeyer *et al* (2016)
Bradyrhizobium namibiense	Hyacinth bean	*Lablab purpureus*	Root nodules	Namibia	Grönemeyer, Bünger and Reinhold-Hurek (2017)
Bradyrhizobium vignae, B. kavangense, B. subterraneum, B. elkanii and *B. pachyrhizi*.	Bambara groundnut	*Vigna subterranea*	Root nodules	Ghana and South Africa	Puozaa, Jaiswal and Dakora, (2017)
Bradyrhizobium pachyrhizi B. yuanmingense, B. arachidis, B. subterraneum	Bambara groundnut	*Vigna subterranea*	Root nodule	South Africa and Nougani Mali	Ibny *et al.* (2019)
Bradyrhizobium yuanmingense, Rhizobium alamii, R. sullae, and *Ensifer* sp.	Bambara groundnut	*Vigna subterranea*	Root nodules	Côte D'ivoire	Raiss*a et al*. (2020)

Rhizobia species	Common name of legumes	Latin name	Crop part	Location of isolation	Reference
Bradhyrhizobium spp	Bambara groundnut	*Vigna subterranea*	Root nodule	Nigerian soils	Ajayi, Dianda and Fagade, (2020)
Bradyrhizobium arachidis, *B. manausense*, *B. guangdongense*, *B. elkanii*, and *B. pachyrhizi*.	Bambara groundnut	*Vigna subterranea* (*L*)	Root nodule	Eswatini	Dlamin*i et al*. (2021)

2.5. Mechanisms of nitrogen fixation by rhizobia

Nitrogen fixation happens through three different processes, including root hair penetration and infection thread formation, or rhizobia can infect their legume hosts and induce root or stem nodule formation (Liu et al., 2011). In some plants such as peanuts, where the infection process does not involve root hairs, the rhizobia can also enter the host through wounds or lateral root emergence (also known as "crack" entry) (Boogerd and Van Rossum, 1997; Ren, 2018); or penetration of root primordia found on the stem of some plants such as in *Sesbania* can result in entry (Froussart et al., 2016). But based on the information at hand, it appears that most species of legumes have their roots infected by rhizobia through root hair infection (Perrine-Walker et al., 2014; Wang et al., 2018*)*. According to Lindström and Mousavi (2020), the *nod*, *nif* and *fix* genes dominantly regulate symbiotic nitrogen fixation in rhizobia. Additionally, other sets of genes in the bacteria regulate various aspects of the nodulation process (Hong et al., 2012). The "*nif*" genes which encode the nitrogen-fixing enzyme nitrogenase subunits and additional proteins required for nitrogenase synthesis, regulation, and function, and the "*nod*" genes, which encode proteins for Nod factor synthesis that induce various symbiotic responses on legume roots, are the main symbiosis genes being studied (Gopalakrishnan et al., 2015). According to Li et al. (2017), particular *nod* genes have been demonstrated to be important determinants of legume host specificity. Furthermore, Andrews and Andrews (2017) noted that because rhizobium strains are specialised, one rhizobium strain may infect some species of legumes but not others. The *nif* and *nod* genes, with the exception of *Bradyrhizobium,* are frequently carried on plasmids or symbiotic islands. These genes can be transferred (lateral transfer) between several bacterial species within a genus but seldom across genera (Shin et al., 2016).

The exchange of signals that enables mutual recognition and the activation of a signalling cascade is the first step in symbiotic interactions (Lindström and Mousavi, 2020). In reaction to the flavonoids released in plant root exudates, rhizobial lipochito-oligosaccharide signal molecules known as Nod factors are produced (Froussart et al., 2016). Nodule organogenesis begins when the plant detects the Nod factors. This is done by the root hair tips curling, which creates an infection thread, mitotic division of root cortical cells, and the formation of a nodule primordium (Rao, 2014).

Thousands of living rhizobial symbionts, the majority of which are in the distorted form known as bacteroids, essentially colonise each root nodule (Ren, 2018). These bacteroids are encircled by membrane fragments from plant cells. N is fixed in these structures, known as symbiosomes-like stuctures, which may include numerous bacteroids or simply one (Babalola et al., 2017). Rhizobia differentiate into N_2-fixing bacteroids that use nitrogenase to catalyze the conversion of atmospheric N into ammonia (Mahmud et al., 2020; Jaiswal et al., 2021).

2.6. Rhizobia inoculation and its constraints

Various researchers have reported on the application of microbial consortia or single microbes as inoculum that could be employed in agriculture to remedy degrading soil conditions; however, the effective transfer of microbial inoculants from the lab to the field remains a challenge that requires urgent attention (Ahemad and Kibret, 2014; Pulido-Suárez et al., 2021). The transfer of microbial inoculants to the field is challenged by numerous factors such as crop species and crop variants, varying climatic conditions between fields, as well as by the exponential rise in the number of microbial isolates in recent years (Mfilinge et al., 2014; Ikenganyia et al., 2017).

According to Sajid et al. (2011); Singh et al. (2016), and Jaiswal and Dakora (2019), often regions regarded as geographic origins of legumes correlate with diversity hotspots of their respective symbionts. In addition, the performance of isolates on various local hosts varied, according to Grönemeyer et al. (2014), who found that most Namibian isolates were more effective in inducing nodulation on peanut and hyacinth bean grown in Namibia as opposed to the Angolan strains when used on the same varieties. Rhizobial strains frequently perform poorly in environments that are distinct from their original habitats, and Bünger et al. (2021) recently found that their efficacy depends on environmental parameters like soil temperature and soil texture, amongst others. Furthermore, Allito et al. (2020) suggested that soil pH and consequentially the amount of accessible phosphorus have sizable effects on inoculant success. However, Kanonge-Mafaune et al. (2018) pointed out that optimising the concentration of rhizobial cells per unit seed three times in cowpea and four times in beans has improved responses to inoculation and grain productivity. Comparing the usage of inoculants in consortia form to individual forms, Moreira et al. (2010) observed that the use of inoculant in consortia form increased growth of the woody legume species

Albizia lebbeck (L.) Benth, *Enterolobium contortisiliquum* (Vell.) Morong, and *Leucaena leucocephala*. It has also been discovered that seed coat compounds play a significant role in nodule development (Redjeki et al., 2013). This could be true as reported by Ibny et al. (2019) that variation in Bambara groundnut seed colour influences the choice of microsymbiont partners, through the attraction of native and familiar rhizobia with potential symbiotic efficiency within crop species.

2.7. Concluding remarks

It is anticipated that the adoption of contemporary technology will play a crucial part in the progress of the underutilised Bambara groundnut in the world and Africa in particular to increase production to feed the World's growing populations. Rhizobial inoculants are one way to boost the production of Bambara groundnut that needs to be explored and developed for the benefit of the agricultural sector. There is also a need to develop better strains of inoculants that suits the local environmental conditions and replace/solve the problem of exotic rhizobial strains whose survival rates in local soils are low due to severe and difference in the environmental and soil conditions. Since Bambara is a native crop to Africa, an abundance of native rhizobia that are well adapted and capable of forming symbiotic relationships with the crop to efficiently fixing N may be available in local soils. It is suggested that temperature-resistant *Bradyrhizobium* strains should be isolated and selected on local soils (Grönemeyer and Reinhold-Hurek, 2018). Therefore, it is highly recommended for a comprehensive approach to the use of 'inoculants' and this should include diagnostics of the field environment in relation to the desired crop. Furthermore, a holistic approach that investigate to determine the best agricultural practices, screening of available culture collections for inoculants, research in the area of microbiomes, and, integration of all the possible solutions into large-scale industrial production and field applications can potentially lead to sustainable agriculture and contribute to the global food security.

References

1. Ahemad M., Kibret M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King. Saud. Univ. Sci.* 26, 1–20. doi: 10.1016/j.jksus.2013.05.001
2. Ajayi O. O., Dianda M., Fagade O. E. (2020). Evaluation of some nodulation genes found in bambara symbiotic *Rhizobia* strains. *Int. J. Innov. Sci. Res.* 5, 42–46. Available at: https://www.hilarispublisher.com/open-access/evaluation-of-some-nodulation-genes-found-in-bambara-symbiotic-rhizobia-strains.pdf.
3. Allito B. B., Ewusi-Mensah N., Logah V. (2020). Legume-rhizobium strain specificity enhances nutrition and nitrogen fixation in faba bean (*Vicia faba* l.). *Agronomy.* 10, 1–21. doi: 10.3390/agronomy10060826
4. Andrews M., Andrews M. E. (2017). Specificity in legume-rhizobia symbioses. *Int. J. Mol. Sci.* 18, 1–39. doi: 10.3390/ijms18040705
5. Aviara N. A., Lawal A., Atiku A., Haque M. (2013). Bambara groundnut processing, storage and utilization in north Eastern Nigeria. *cont. J. eng. Sci.* 8, 28–36. doi: 10.5707/cjengsci.2013.8.1.28.36
6. Belete S., Bezabih M., Abdulkadir B., Tolera A., Mekonnen K., Wolde-meskel E. (2019). Inoculation and phosphorus fertilizer improve food-feed traits of grain legumes in mixed crop-livestock systems of Ethiopia. *Agric. Ecosyst. Environ.* 279, 58–64. doi: 10.1016/j.agee.2019.04.014
7. Boogerd F. C., Van Rossum D. (1997). Nodulation of groundnut by *Bradyrhizobium*: A simple infection process by crack entry. *FEMS Microbiol. Rev.* 21, 5–27. doi: 10.1016/S0168-6445(97)00004-1
8. Büchi L., Gebhard C. A., Liebisch F., Sinaj S., Ramseier H., Charles R. (2015). Accumulation of biologically fixed nitrogen by legumes cultivated as cover crops in Switzerland. *Plant Soil.* 393, 163–175. doi: 10.1007/s11104-015-2476-7
9. Bünger W., Sarkar A., Grönemeyer L. J., Zielinski J., Revermann R., Hurek T., et al. (2021). Root nodule rhizobia from undomesticated shrubs of the dry woodlands of southern Africa can nodulate Angolan teak *Pterocarpus angolensis*, an important source of timber. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.611704

10. Babalola O. O., Olanrewaju S. O., Dias T., Ajilogba F. C., Cruz C. (2017). Biological nitrogen fixation: The role of underutilized leguminous plants. In Deepak G. P., Yogeshvari K. J., Rajababu V. V., Shelat N. H.(Eds). *Microorg. Green Revol.* 6:431–443. springer nature. doi: 10.1007/978-981-10-6241-4

11. Chen, Y. X., Zou, L., Penttinen, P., Chen, Q., Li, Q. Q., Wang, C. Q., & Xu, K. W. (2018). Faba bean (Vicia faba L.) nodulating rhizobia in Panxi, China, are diverse at species, plant growth promoting ability, and symbiosis related gene levels. *Frontiers in Microbiology*, *9*(1338), 1–10. https://doi.org/10.3389/fmicb.2018.01338

12. Denton M. D., Phillips L. A., Peoples M. B., Pearce D. J., Swan A. D., Mele P. M., et al. (2017). Legume inoculant application methods: effects on nodulation patterns, nitrogen fixation, crop growth and yield in narrow-leaf lupin and faba bean. *Plant Soil.* 419, 25–39. doi: 10.1007/s11104-017-3317-7

13. Dlamini S. T., Jaiswal S. K., Mohammed M., Dakora F. D. (2021). Studies of phylogeny, symbiotic functioning and ecological traits of indigenous microsymbionts nodulating bambara groundnut (*Vigna subterranea* l. verdc) in eswatini. *Microb. Ecol.* 82:688–703. doi: 10.1007/s00248-021-01684-0

14. Egbe M. O., Alhassan G. A., Ijoyah M., Onyilo M. (2013). Nodulation, nitrogen yield and fixation by bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces intercropped with cowpea and maize in southern Guinea savanna of Nigeria. *J. Agric. Sci.* 1, 15–28. doi: 10.12735/as.v1i4p15

15. Froussart E., Bonneau J., Franche C., Bogusz D. (2016). Recent advances in actinorhizal symbiosis signaling. *Plant Mol. Biol.* 90, 613–622. doi: 10.1007/s11103-016-0450-2

16. Gbaguidi A. A., Dansi A., Dossou-Aminon I., Gbemavo D. S. J. C., Orobiyi A., Sanoussi F., et al. (2018). Agromorphological diversity of local bambara groundnut (*Vigna subterranea* (L.) verdc.) collected in Benin. *Genet. Resour. Crop Evol.* 65, 1159–1171. doi: 10.1007/s10722-017-0603-4

17. Gedamu, S. A., Tsegaye, E. A., & Beyene, T. F. (2021). Effect of rhizobial inoculants on yield and yield components of faba bean (Vicia fabae L .) on vertisol of Wereillu District , South Wollo, Ethiopia. *CABI Agriculture and Bioscience*, *2*(8), 1–10. https://doi.org/10.1186/s43170-021-00025-y

18. Gopalakrishnan S., Sathya A., Vijayabharathi R., Varshney R., Gowda L., Krishnamurthy L. (2015). Plant growth promoting rhizobia : challenges and opportunities. *3. Biotech.* 5, 355–377. doi: 10.1007/s13205-014-0241-x

19. Grönemeyer J. L., Hurek T., Reinhold-Hurek B. (2015b). *Bradyrhizobium kavangense* sp. nov., a symbiotic nitrogen-fixing bacterium from root nodules of traditional Namibian pulses. *Int. J. Syst. Evol. Microbiol.* 65, 4886–4894. doi: 10.1099/ijsem.0.000666

20. Grönemeyer J. L., Bünger W., Reinhold-Hurek B. (2017). *Bradyrhizobium namibiense* sp. nov., a symbiotic nitrogen-fixing bacterium from root nodules of *Lablab purpureus*, hyacinth bean, in Namibia. *Int. J. Syst. Evol. Microbiol.* 67, 4884–4891. doi: 10.1099/ijsem.0.002039

21. Grönemeyer J. L., Chimwamurombe P., Reinhold-Hurek B. (2015a). *Bradyrhizobium subterraneum* sp. nov., a symbiotic nitrogen-fixing bacterium from root nodules of groundnuts. *Int. J. Syst. Evol. Microbiol.* 65, 3241–3247. doi: 10.1099/ijsem.0.000403

22. Grönemeyer J. L., Hurek T., Bünger W., Reinhold-Hurek B. (2016). *Bradyrhizobium vignae* sp. nov., a nitrogen-fixing symbiont isolated from effective nodules of *Vigna* and *Arachis*. *Int. J. Syst. Evol.* 66, 62–69. doi: 10.1099/ijsem.0.000674

23. Grönemeyer J. L., Kulkarni A., Berkelmann D., Hurek T., Reinhold-hurek B. (2014). Rhizobia indigenous to the okavango region in Sub-Saharan Africa : Diversity, adaptations, and host specificity. *Appl. Environ. Microbiol.* 80, 7244–7257. doi: 10.1128/AEM.02417-14

24. Grönemeyer J. L., Reinhold-Hurek B. (2018). Diversity of bradyrhizobia in Sub Sahara Africa: A rich resource. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.02194

25. Hillocks R. J., Bennett C., Mponda O. M., Maritime C. (2012). Bambara nut: A review of utilisation, market potential and crop improvent. *Afr. Crop Sci. J.* 20, 1–16. Available at: http://www.bioline.org.br/pdf?cs12001.

26. Hong Y., Ma Y., Wu L., Maki M., Qin W., Chen S. (2012). Characterization and analysis of *nif*H genes from *Paenibacillus sabinae* T27. *Microbiol.* 167, 596–601. doi: 10.1016/j.micres.2012.05.003

27. Ibny F., Jaiswal S., Mustapha M., Dakora F. D. (2019). Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of

bambara groundnut (*Vigna subterranea* l. verdc.) in Africa and their relationship with phylogeny. *Sci. Rep.* 9, 1–17. doi: 10.1038/s41598-019-48944-1

28. Ikenganyia E., Anikwe M., Ngwu O. (2017). Influence of rhizobacteria inoculant application methods and phosphate fertilizer rates on dry matter accumulation, yield of bambara groundnut [*Vigna subterranea* (L.) verdc] and soil total nitrogen content in a degraded ultisol in southeast Nigeria. *Agrotech.* 6, 1–7. doi: 10.4172/2168-9881.1000165
29. Ikenganyia E., Anikwe M. A. N., Ngwu O. (2018). Impacts of plant growth promoting rhizobacteria inoculation configurations on vegetative growth, nodulation and yield of bambara groundnut [*Vigna subterranea* (L.) verdc.]. *Int. J. Hortic. Agric. Food Sci.* 2, 54–69. doi: 10.22161/ijhaf.2.3.5
30. Jaiswal S. K., Dakora F. D. (2019). Widespread distribution of highly adapted *Bradyrhizobium* species nodulating diverse legumes in Africa. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.00310
31. Jaiswal S. K., Mohammed M., Ibny F. Y. I., Dakora F. D. (2021).). rhizobia as a source of plant growth-promoting molecules: Potential applications and possible operational mechanisms. *Front. Sustain. Food Syst.* 4. doi: 10.3389/fsufs.2020.619676
32. Kanonge-Mafaune G., Chiduwa M. S., Chikwari E., Pisa C. (2018). Evaluating the effect of increased rates of rhizobial inoculation on grain legume productivity. *Symbiosis.* 75, 217–227. doi: 10.1007/s13199-018-0550-7
33. Kapembwa, R., Mweetwa, A. M., Ngulube, M., Yengwe, J., & Yengwe, J. (2016). Morphological and Biochemical Characterization of Soybean Nodulating Rhizobia Indigenous to Zambia. *Sustainable Agriculture Research*, *5*(3), 84–92. https://doi.org/10.5539/sar.v5n3p84
34. Karunaratne A. S., Walker S., Ruane A. C. (2015). Modelling bambara groundnut yield in southern Africa : towards a climate-resilient future. *Clim. Res.* 65, 193–203. doi: 10.3354/cr01300
35. Khan M. M. H., Rafii M. Y., Ramlee S. I., Jusoh M., Al-Mamun M. (2021). Bambara groundnut (*Vigna subterranea* l. verdc): A crop for the new millennium, its genetic diversity, and improvements to mitigate future food and nutritional challenges. *Sustainability* 13, 5530. doi: 10.3390/su13105530

36. Lindström K., Mousavi S. A. (2020). Effectiveness of nitrogen fixation in rhizobia. *Microb.* 13, 1314–1335. doi: 10.1111/1751-7915.13517

37. Li H.-B., Singh R. K., Singh P., Song Q. Q., Xing Y. X., Yang L. T., et al. (2017). Genetic diversity of nitrogen-fixing and plant growth promoting *Pseudomonas* species isolated from sugarcane rhizosphere. *Front. Microbiol.* 8. doi: 10.3389/fmicb.2017.01268

38. Liu J., Kelley M. S., Wu W., Banerjee A., Douvalis A. P., Wu J., et al. (2016). Nitrogenase-mimic iron-containing chalcogels for photochemical reduction of dinitrogen to ammonia. *Proc Natl Acad Sci.* 113, 5530–5535. doi: 10.1073/pnas.1605512113

39. Liu Y., Wu L., Baddeley J. A., Watson C. A. (2011). Models of biological nitrogen fixation of legumes. a review. *Agron. Sustain. Dev.* 31, 155–172. doi: 10.1051/agro/2010008

40. Mahmud K., Makaju S., Ibrahim R., Missaoui A. (2020). Current progress in nitrogen fixing plants and microbiome research. *Plants.* 9, 1–17. doi: 10.3390/plants9010097

41. Mayes S., Ho W. K., Chai H. H., Gao X., Kundy A. C., Mateva K. I., et al. (2019). Bambara groundnut : an exemplar underutilised legume for resilience under climate change. *Planta.* 250, 803–820. doi: 10.1007/s00425-019-03191-6

42. Mfilinge A., Mtei K., Ndakidemi P. A. (2014). Effects of *Rhizobium* inoculation and supplementation with p and K, on growth, leaf chlorophyll content and nitrogen fixation of bush bean varieties. *Am. J. Res. Commun.* 2, 49–87. doi: 10.4236/as.2014.514152

43. Moreira F. M., de S., de Carvalho T. S., Siqueira J. O. (2010). Effect of fertilizers, lime, and inoculation with rhizobia and mycorrhizal fungi on the growth of four leguminous tree species in a low-fertility soil. *Biol. Fertil.* 46, 771–779. doi: 10.1007/s00374-010-0477-5

44. Mubaiwa J., Fogliano V., Chidewe C., Linnemann A. R. (2017). Hard-to-cook phenomenon in bambara groundnut (*Vigna subterranea* (L.) verdc.) processing: Options to improve its role in providing food security. *Food Rev. Int.* 33, 167–194. doi: 10.1080/87559129.2016.1149864

45. Mubaiwa J., Fogliano V., Chidewe C., Linnemann A. R. (2018). Bambara groundnut (*Vigna subterranea* (L.) verdc.) flour: A functional ingredient to

favour the use of an unexploited sustainable protein source. *PloS One* 13, 1–19. doi: 10.1371/journal.pone.0205776

46. Musa M., Massawe F., Mayes S., Alshareef I., Singh A. (2016). Nitrogen fixation and n-balance studies on bambara groundnut (*Vigna subterranea* l. verdc) landraces grown on tropical acidic soils of Malaysia. *Commun. Soil Sci. Plant Anal.* 47, 533–542. doi: 10.1080/00103624.2016.1141928

47. Nassé G., Raissa K., Ibrahim K., Sidiky B. (2019). Nodulation and agronomic performance of indigenous rhizobia isolated from bambara groundnut (*Vigna subterranean* l.) nodules in dalao, côte d'Ivoire. *Int. J. Res. Stud. Microbiol. Biotechnol.* 5, 13–18. doi: 10.20431/2454-9428.0502004

48. Perrine-Walker F. M., Lartaud M., Kouchi H., Ridge R. W. (2014). Microtubule array formation during root hair infection thread initiation and elongation in the *Mesorhizobium-lotus* symbiosis. *Protoplasma.* 251, 1099–1111. doi: 10.1007/s00709-014-0618-z

49. Phillips S., Saunders R. (2016). The role of seed coatings in enhancing rhizobium colonisation and yield increases in pulse crops in the northern mallee of south Australia. *Solutions. to. Improve. Nitro. Use Efficiency. World.* 4, 5–6. Available at: http://agronomyaustraliaproceedings.org/images/sampledata/ini2016/pdf-papers/INI2016_Phillips_Shane.pdf.

50. Pulido-Suárez L., González-Rodríguez Á.M., Cubas J., del Arco-Aguilar M., Martín-Esquivel J. L., León-Barrios M. (2021). Applying agronomic principles of rhizobial inoculation to the conservation of a keystone legume species in a high mountain ecosystem on an oceanic island. *Front. Agron.* 3. doi: 10.3389/fagro.2021.660574

51. Puozaa D. K., Jaiswal S. K., Dakora F. D. (2017). African Origin of *Bradyrhizobium* populations nodulating bambara groundnut (*Vigna subterranea* l. verdc) in ghanaian and south African soils. *PloS One* 12, 1–23. doi: 10.1371/journal.pone.0184943

52. Raissa G. N. K., Ibrahim K., Kaoutar T., Issouf B., Maxwell B. G. A., Sélastique A. D., et al. (2020). Molecular and symbiotic efficiency characterization of rhizobia nodulating bambara groundnut (*Vigna subterranea* l.) from agricultural soils of daloa localities in côte d'ivoire. *Int. J. Syst. Evol. Microbiol.* 9, 507–519. doi: 10.20546/ijcmas.2020.907.056

53. Rao D. L. (2014). Recent advances in biological nitrogen fixation in agricultural systems. *Proc. Indian Natl. Sci.* 80, 359–378. doi: 10.16943/ptinsa/2014/v80i2/55114

54. Redjeki E. S., Mayes S., Azam-Ali S. (2013). Evaluating the stability and adaptability of bambara groundnut (*Vigna subterranea* (L.) verd.) landraces in different agro-ecologies. *Acta Hort.*, 979:389–400. doi: 10.17660/ActaHortic.2013.979.42

55. Ren G. (2018). *The evolution of determinate and indeterminate nodules within the papilionoideae subfamily* (Wageningen, Netherlands:Wageningen University). Available at: https://library.wur.nl/WebQuery/wda/2232324.

56. Sajid M., Rab A., Noor S., Shah M., Jan I., Khan M. A., et al (2011). Influence of rhizobium inoculation on growth and yield of groundnut cultivars. *Sarhad. J. Agric.* 27, 573–576. Available at:https://www.aup.edu.pk/sj_pdf/INFLUENCE%20OF%20RHIZOBIUM%20INOCULATION%20ON%20GROWTH.PDF.

57. Santi C., Bogusz D., Franche C. (2013). Biological nitrogen fixation in non-legume plants. *Ann. Bot.* 111, 743–767. doi: 10.1093/aob/mct048

58. Santos M. S., Nogueira M. A., Hungria M. (2019). Microbial inoculants: Reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB. Express.* 9, 1–22. doi: 10.1186/s13568-019-0932-0

59. Shin W., Islam R., Benson A., Joe M. M., Kim K., Gopal S., et al. (2016). Role of diazotrophic bacteria in biological nitrogen fixation and plant growth improvement. *Korean. J. Soil Sci. Fert.* 49, 17–29. doi: 10.7745/kjssf.2016.49.1.017

60. Singh J. S., Koushal S., Kumar A., Vimal S. R., Gupta V. K. (2016). Book review: Microbial inoculants in sustainable agricultural productivity- vol. II: functional application. in. *Front. Microbiol* 7. doi: 10.3389/fmicb.2016.02105

61. Sprent J. I., Odee D. W., Dakora F. D. (2010). African Legumes: A vital but under-utilized resource. *J. Exp. Bot.* 61, 1257–1265. doi: 10.1093/jxb/erp342

62. Tan X. L., Azam-ali S., Goh E., Von Mustafa M., Chai H. H., Ho K. W., et al. (2020). Bambara groundnut : An underutilized leguminous crop for global food security and nutrition. *Front. Nutr.* 7. doi: 10.3389/fnut.2020.601496

63. Temegne N., Gouertoumbo W., Wakemm G., Nkou F., Youmbi E., Ntsombo-Ntsefong G. (2018). Origin and ecology of bambara groundnut (*Vigna subterranea* (L.) verdc: A review. *J. Ecol. Nat. Resour*. 2, 1–10. doi: 10.23880/jenr-16000140

64. Tsukanova K. A., Chebotar V., Meyer J. J. M., Bibikova T. N. (2017). Effect of plant growth-promoting rhizobacteria on plant hormone homeostasis. *S. Afr. J. Bot.*, 113:91–102. doi: 10.1016/j.sajb.2017.07.007

65. Ulzen, J., Abaidoo, R. C., Mensah, N. E., Masso, C., & Abdelgadir, A. H. (2016). Bradyrhizobium Inoculants Enhance Grain Yields of Soybean and Cowpea in Northern Ghana. *Frontiers in Plant Science*, *7*(1770), 1–9. https://doi.org/10.3389/fpls.2016.01770

66. Van Wyk B. E. (2019). The diversity and multiple uses of southern African legumes. *Aust. Syst. Bot.* 32, 519–546. doi: 10.1071/SB19028

67. Wang Q., Liu J., Zhu H. (2018). Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.00313

68. Wekesa C. S., Furch A. C. U., Oelmüller R. (2021). Isolation and characterization of high-efficiency rhizobia from Western Kenya nodulating with common bean. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.697567

69. Yakubu H., Kwari J. D., Ngala A. L. (2010). N_2 fixation by grain legume varieties as affected by rhizobia inoculation in the sandy loam soil of sudano-sahelian zone of north Eastern Nigeria. *Nig. J. Basic Appl. Sci.* 18, 229–236. doi: 10.4314/njbas.v18i2.64325

70. Yusif S., Muhammad I., Hayatu N., Sauwa M., Tafinta I., Mohammed M., et al. (2016). Effects of biochar and rhizobium inoculation on nodulation and growth of groundnut in sokoto state, Nigeria. *J. Appl. Life Sci.* 9, 1–9. doi: 10.9734/jalsi/2016/27297

Chapter 3

Evaluation of the effect of *Bradyrhizobium* inoculation on nodulation and growth parameters of three Bambara groundnut varieties in the Kavango East Region

Abstract

Bambara groundnut (*Vigna subterranea* (L.) Verdc) can efficiently fix atmospheric N, making it available to plants. Application of rhizobial inoculants have been demonstrated to greatly increase the yields of several different leguminous crops. There is, however, limited research on the effect of inoculating grain legumes with rhizobial bacteria in developing countries like Namibia. To this extent, a study was conducted at the ZERI greenhouse, University of Namibia during 2020/2021 cropping season, using soil from the Kavango region to ascertain how well the various *Bradyrhizobium* strains promote nodulation and growth parameters among three varieties of Bambara groundnuts. The study also evaluated the strains' competitive ability to colonise the roots and promote nodulation in a given period. All the Bambara groundnut varieties (brown, cream and red) were grown under three treatments namely, treatment one in which the groundnuts were treated with a single strain (*Bradyrhizobium* 9-5), treatment two in which the groundnuts were treated with a mixed inoculant consisting of seven *Bradyrhizobium* strains [1-7; 3B 4-1; 9-5, 36 1-1; 36 3-2; 55 1-1 and 60 2-1], and treatment three was a negative control. The treatments were set up in a randomized complete block design with three replications. The findings indicate that the utilization of the two inoculants resulted in superior performance compared to non-inoculated cultivars, as evidenced by significant increases in both plant height and shoot dry weight. Var02 (cream), Var03 (red) and Var01 (brown) treated with the mixed inoculant (MK) had the highest shoot dry weight with 1.71, 1.50, and 1.24 g plant^{-1} respectively. Nevertheless, Var01, which was not inoculated, produced 3.7 nodules. When compared to the single strain 9-5, the mixed strains were found to be more productive and contagious at 0.05 level of significance.

Keywords*:*

Bambara groundnuts, biofertilizer, legumes, productivity, rhizobia

3.1. Introduction

While several agricultural research projects have typically concentrated on staple crops, scientists in industrialized nations have paid attention to underutilized and neglected crop species (Ajayi *et al*., 2020). One such example of an underutilized crop is the Bambara groundnut (*Vigna subterranea* (L.) Verdc), a legume crop grown for human consumption in many parts of the world, particularly in Africa (Nassé *et al.*, 2019). The Bambara groundnut, like other legumes, can fix N (Hillocks *et al.*, 2012). This feature gives legumes an advantage over other plant species in N limited soils because they can convert atmospheric N into forms that plants can use through their symbiotic relationship with rhizobia (Allito *et al.*, 2021; Zielinski and Reinhold-hurek, 2021). The symbiotic process can provide all or some of the N required by the legume. It can also help build up the soil's N that can be used by succeeding non-legume crops (Mohammed *et al.*, 2021).

Despite its importance, the production of Bambara groundnut is hindered by poor crop establishment, improper planting depth and plant spacing, the use of unimproved seeds, insufficient soil fertility, and a lack of efficient nodulation technology (Ikenganyia *et al*., 2018). For instance, it has been reported that local farmers in Namibia have experienced low yields and declining soil nutrient levels, leading to low natural nodulation rates in pulses like Bambara groundnut (Grönemeyer *et al*,. 2014). According to Grönemeyer *et al*. (2014), the decline in soil nutrients is most likely attributed to heat and drought conditions in recent years. Consequently, inoculation with an effective and suitable rhizobial strain is necessary to boost Bambara groundnut output and improve symbiotic nitrogen fixation. It has been reported that inoculation affects the microbial community by increasing the amount of the chosen rhizobial strain in the rhizosphere (Mohammed *et al.*, 2021; Pulido-Suárez *et al.,* 2021). Various researchers including Shin *et al.* (2016) have reported that rhizobacteria inoculants could significantly improve yield in many leguminous crops. Moreover, the use of rhizobia inoculants for improvement in nitrogen fixation and productivity of grain legumes has been well-established in developed countries (Sajid *et al.*, 2011).

In order for inoculant strains to successfully establish themselves, they must be able to endure the soil environment and make use of the ecological niche that host plant roots supply (Ibny *et al.,* 2019). Should ineffective rhizobial strains be used for inoculation, they would be outcompeted by native rhizobial strains. This is supported by Allito *et* *al.* (2021) and Sánchez-cañizares *et al.* (2021) that some native rhizobia in the soil may be ineffective at root colonisation, and hence nodulation. In such circumstances, nodulation can be enhanced by inoculating seeds with more potent strains. This strengthens the hypothesis that strain competitiveness is necessary for successful inoculation in field settings.

Although some strains of rhizobacteria have been isolated from Namibia (Grönemeyer *et al.*, 2014; Grönemeyer *et al*., 2015a; Grönemeyer *et al*., 2015b; Grönemeyer *et al.*, 2016; Grönemeyer *et al*., 2017), this technique has not been implemented in Namibia yet, nor has there been a study to determine the most competitive strain to colonise Bambara groundnut roots, as well as those of related crops. This study consequently sought to assess and examine the effects of seed inoculation with different *Bradyrhizobium* strains on nodulation and yield components of Bambara groundnut crops grown in Namibia.

3.2. Material and methods

3.2.1. Study site and Planting materials

Top soil was collected from the Mashare Research Station of the Ministry of Agriculture, Water and Land Reform (GPS coordinates of 17° 54′ 08.13″ S and 20° 08′ 00.99″ E) on a site where no legume crops had been previously cultivated, and no fertilizer applied. In this experiment, three local Bambara groundnut varieties (brown, cream and red), henceforth referred to as Var01 (brown), Var02 (cream) and Var03 (red), were used. *Bradyrhizobium* sp. 1-7, *Bradyrhizobium vignae* 3B 4-1, *Bradyrhizobium vignae* 9-5, *Bradyrhizobium* sp. 36 1-1, *Bradyrhizobium vignae* 36 3-2, *Bradyrhizobium subterraneum* 55 1-1, *Bradyrhizobium subterraneum* 60 2-1 isolated by Grönmeyer *et al*. (2014) in Namibia were used as inoculants in this study in two forms. The first form consisted of a single strain (9-5) (Figure 3.1), the second form was the mixture (Mix) of all the 7 strains.

Figure 3.5: Bambara groundnut (BGN) varieties used in the study (left) and Inoculant 9-5 with peat as a carrier, then packed in Whirl-Pack® sample bags (right)

3.2.2. Experimental layout and inoculation

The three Bambara groundnut varieties were grown in experimental pots under the greenhouse environment of the Zero-Emission Research Initiative (ZERI) at the University of Namibia's main campus (GPS coordinates of 22° 36' 23.99" S and 17° 03' 16.20" E). The experiment was performed in a randomized complete block design with a three-by-three factorial design under three replications. The surface sterilization of Bambara groundnut seeds was performed following the guidelines of Somasegaran and Hoben (1994). The observation was done on a daily basis for 60 days and data on the parameters of interest was captured and analysed.

3.2.3. Data collection

Parameters such as the number of nodules per plant, plant height (cm) and shoot weight were collected 60 days after planting.

3.2.3.1. Plant height, shoot, and nodules

Plant height was determined at 30 and 60 days after planting (DAP). Plant height was determined by measuring the plant from the crown to the last leaf. Root nodules were examined at 60 DAP. The roots were removed using a hand trowel without causing any nodule damage. The number of young nodules was counted, and the total nodules per plant recorded. Sampled nodules were then collected and dehydrated in silica gel-filled vials that were kept at 4 °C until needed. Plant shoots were determined by cutting out the root and the shoots were weighed immediately to determine the shoot fresh weight and after 72-hour oven drying period at 65 °C, on an electronic balance to determine their dry weight.

3.2.3.2. Bacterial Identification

Identification of bacteria was done by 16S-23S rRNA internally transcribed spacer (ITS) genotyping. To extract the DNA, dried nodules from each of the three Bambara groundnut varieties were rehydrated by soaking in sterile distilled water and surface-sterilized as described by Puozaa, Jaiswal and Dakora (2017). Single nodules were then crushed in a drop of sterile deionised distilled water in sterilised 2-ml microcentrifuge tubes using a sterile plastic pestle. Following this, bacterial genomic DNA was extracted directly from the crushed nodules using NucleoSpin® Tissue **(**from MACHEREY-NAGEL: # 740952.50) kit according to the manufacturer's instructions.

The ITS was amplified using the non-degenerate primer pairs: FGPS 130 (5'–CCGGGTTTCCCCATTCGG-3': 18-mer; Tm 60.5°C) and FGPS 1490 (5'–TGCGGCTGGATCACCTCCTT-3'; 20-mer; Tm 61.4°C) as described by Laguerre *et al. (*1996) with increased annealing temperature at 58°C. The primer pair covers mostly α-Proteobacteria but is highly specific for Rhizobiaceae.

3.3. Data analysis

The statistical software Genstat® (14th edition, VSN International, UK) was used to determine significant differences between treatments using the Analysis of Variance (ANOVA) function. The data were further subjected to mean comparisons and correlation analysis on all the traits.

3.4. Results

The analysis of variance for days to emergence, plant height, days to flowering, number of nodules per plant, shoot fresh weight and shoot dry weight of Var01, Var02 and Var03 of Bambara groundnut is presented in Table 3.1. The results of this study indicated significant interactions ($P \leq 0.05$) among the agronomic traits studied (Table 3.1). The mean square values and significance levels ($P \leq 0.05$) are indicated in the analysis of variance (ANOVA) tables to discern significant differences. Corresponding means and ranges, standard errors, least significant differences and coefficient of variations for various traits are presented in Tables 3.2, and 3.3 respectively.

Table 3.9: The mean-square values and significance tests of three varieties of Bambara groundnut treated with *Bradyrhizobium* strains as bio-inoculant evaluated in pots under greenhouse condition.

Source of variation	df	DTE		PHT				DTF		Nodule_Plant		SFW		SDW	
				30 DAP		60 DAP									
Replication	2	0.59		7.56		15.38		3.37		43.37		0.05		0.00	
Variety	2	21.81	***	13.54	ns	46.70	**	59.70	***	9.93	ns	2.44	***	0.09	***
Inoculant	2	9.04	**	6.57	ns	2.13	ns	9.59	*	170.48	**	26.09	***	0.72	***
Variety x Inoculant	4	0.93	ns	1.80	ns	7.74	ns	6.26	ns	15.54	ns	1.13	***	0.07	***
Residual	16	0.84		4.31		5.22		2.66		18.45		0.54		0.00	

d.f.= degrees of freedom; DTE=Days to emergence; PHT= plant height; DTF, Days to flowering; Nodule_plant, Number of nodules per plant; SFW, Shoot fresh weight; SDW, Shoot dry weight; *, ** and *** denote significant at 5%, 1% and 0.1% probability level, respectively; ns = non-significant

3.4.1. Effect of different *Bradyrhizobium* strains on Agro-morphological Traits in Bambara groundnut.

3.4.1.1. Nodulation

For the number of nodules statistical analysis, there were statistically significant differences across the means of the different treatments at the 0.05 level of significance. On the contrary, there were no statistically significant differences recorded among the means of the variety and among the interaction of treatment and variety with nodule number per plant. Furthermore, increased number of nodules were observed (Figure 3.2) in Var03 treated with inoculant MK (12 nodules plant^{-1}), followed by Var03 inoculated with 9-5K with 11.3 nodule plant^{-1}. The non-inoculated Var02 and Var03 showed no nodules. However, nodules were observed in Var01 which was not inoculated (Table 3.2).

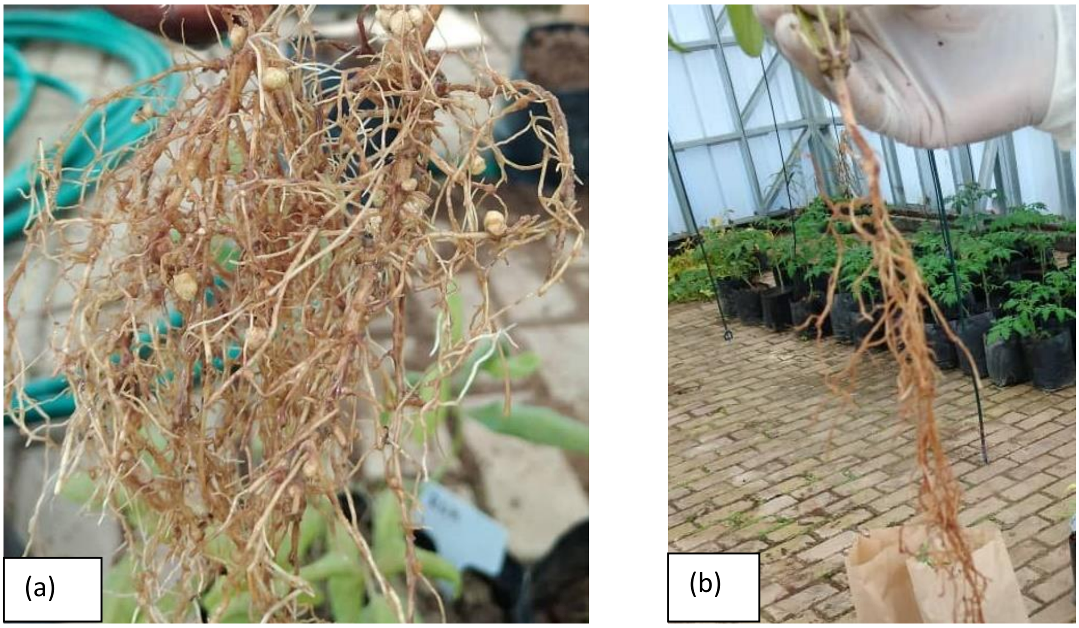


Figure 3.6 Nodules on selected Bambara ground nut roots, including (a) nodules observed in the inoculated plants (b) roots without nodules in the non-inoculated plants

3.4.1.2. Shoot biomass and plant height

The statistical analysis of shoot fresh weight and shoot dry weight revealed that there were statistically significant differences at the (0.05) level of significance among varieties, treatments, and the treatment-variety interaction (Table 3.1). Plants inoculated with MK had the highest shoot dry weight, relative to those treated with 9-5K and the negative control. Var02 with inoculant MK had the highest shoot dry weight and Var02 with inoculant 9-5K had the lowest, with 1.71 and 0.874 g plant^{-1}, respectively (Table 3.2).

Furthermore, as shown in Table 4.1 there were statistically significant differences in plant height at 60 DAP among varieties at the 0.05 level of significance. However, there were no statistically significant differences in the interaction between treatment and variety at the 0.05 level of significance.

Table 3.10: The mean values for agro-morphological traits of three Bambara groundnut varieties treated with *Bradyrhizobium* strains as bio-inoculant evaluated in pots under greenhouse condition.

Variety	Inoculant	DTE	PHT		DTF	Nodule_plant	SFW	SDW
			30DAP	60 DAP				
	0K	11.30^{bc}	13.90^{b}	14.60^{bc}	60.70^{abc}	3.70^{bc}	6.00^{e}	0.90^{g}
Var01	9-5K	9.00^{d}	14.50^{ab}	14.30^{bc}	62.00^{ab}	7.70^{ab}	6.50^{d}	1.00^{f}
	MK	10.70^{bc}	14.50^{ab}	12.20^{c}	59.30^{bc}	7.70^{ab}	9.20^{b}	1.20^{c}
Mean		10.3	14.3	13.7	60.7	6.3	7.2	1.0
	0K	10.00^{cd}	15.30^{ab}	15.60^{abc}	62.70^{a}	0.00^{c}	7.70^{c}	1.00^{e}
Var02	9-5K	8.70^{d}	14.10^{ab}	14.30^{bc}	60.00^{abc}	5.70^{abc}	6.00^{e}	0.90^{h}
	MK	10.00^{cd}	17.10^{ab}	18.00^{ab}	58.00^{cd}	8.70^{ab}	10.00^{a}	1.70^{a}
Mean		9.6	15.5	16.0	60.2	4.8	7.9	1.2
	0K	14.00^{a}	16.80^{ab}	17.70^{ab}	55.70^{d}	0.00^{c}	7.30^{c}	1.10^{d}
Var03	9-5K	11.70^{b}	15.80^{ab}	17.80^{ab}	56.30^{d}	7.30^{abc}	7.40^{c}	1.10^{d}
	MK	12.00^{b}	17.80^{a}	19.20^{a}	56.00^{d}	13.00^{a}	10.00^{a}	1.50^{b}
Mean		12.6	16.8	18.2	56.0	6.8	8.3	1.2
Grand mean		10.8	15.5	16.0	59.0	6.0	7.8	1.2
SED		0.75	1.69	1.87	1.33	3.51	0.19	0.01
LSD (5%)		1.59	3.59	3.96	2.82	7.44	0.40	0.03
CV (%)		8.5	13.4	14.3	2.8	72.0	2.9	1.5

Note: Var01 =brown, Var02 =cream, Var03=red, DTE= Days to emergence; PHT= plant height; DTF= Days to flowering; Nodule_plant= Number of nodules per plant; SFW= Shoot fresh weight; SDW= Shoot dry weight; SED= Standard error of difference; LSD= least significant difference; CV= coefficient of variation; Values followed by the same letters are not statistically different LSD test at P < 0.05

3.4.2. Correlation Analysis

Table 3.3 presents pair-wise correlation coefficients among agro-morphological traits with their levels of significance. In both cases, plant height had positive and significant association with shoot fresh weight, shoot dry weight at 0.01.and 0.05 level respectively. However, plant height had negative correlation with the number of days to flowering and days to emergence.

Table 3.11: Pair-wise phenotypic correlation coefficients of agro-morphological traits among three Bambara groundnut varieties treated with *Bradyrhizobium* strains as bio-inoculant evaluated in pots under greenhouse condition.

Variables	**DTE**	**PHT-30DAP**	**PHT-60DAP**	**DTF**	**Nodule_plant**	**SFW**	**SDW**
DTE	1	0.539499	0.52384	-.737*	-0.1941	0.194951	0.15469
PHT-30DAP	0.539499	1	.895**	-.730*	0.312151	.726*	.796*
PHT-60DAP	0.52384	.895**	1	-.700*	0.215294	0.421118	0.581084
DTF	-.737*	-.730*	-.700*	1	-0.32073	-0.44385	-0.51565
Nodule_plant	-0.1941	0.312151	0.215294	-0.32073	1	0.547754	0.60212
SFW	0.194951	.726*	0.421118	-0.44385	0.547754	1	.918**
SDW	0.15469	.796*	0.581084	-0.51565	0.60212	.918**	1

Traits details given in Table1, *. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

3.4.3. Phylogenetic characterization of rhizobia

The ITS sequences of the bacterial isolates were aligned with reference nucleotide sequences (7 *Bradyrhizobium* strains used as inoculants). The phylogenetic position of the bacterial isolates was evaluated by constructing a phylogenetic tree using the Maximum Likelihood (ML) method. This method placed the bacterial isolates in different clades encompassing members of their species; this was supported with bootstrap values. Bootstrap values based on 100 replications were listed as percentages at the branching points

It was found that more isolates from the root nodules clustered with *Bradyrhizobium subterraneum* 60 2-1, indicating that this strain is the most competitive to reach the roots and produce nodulation within a certain time frame. An isolate from non-inoculated Var01 was observed clustering with *Bradyrhizobium* sp. 1-7. Additionally, most isolates from non-inoculated Var01 nodules are in a different clade from the reference strains, and even more intriguing is that some isolates from inoculated nodules are not in the same clade as the reference strains.

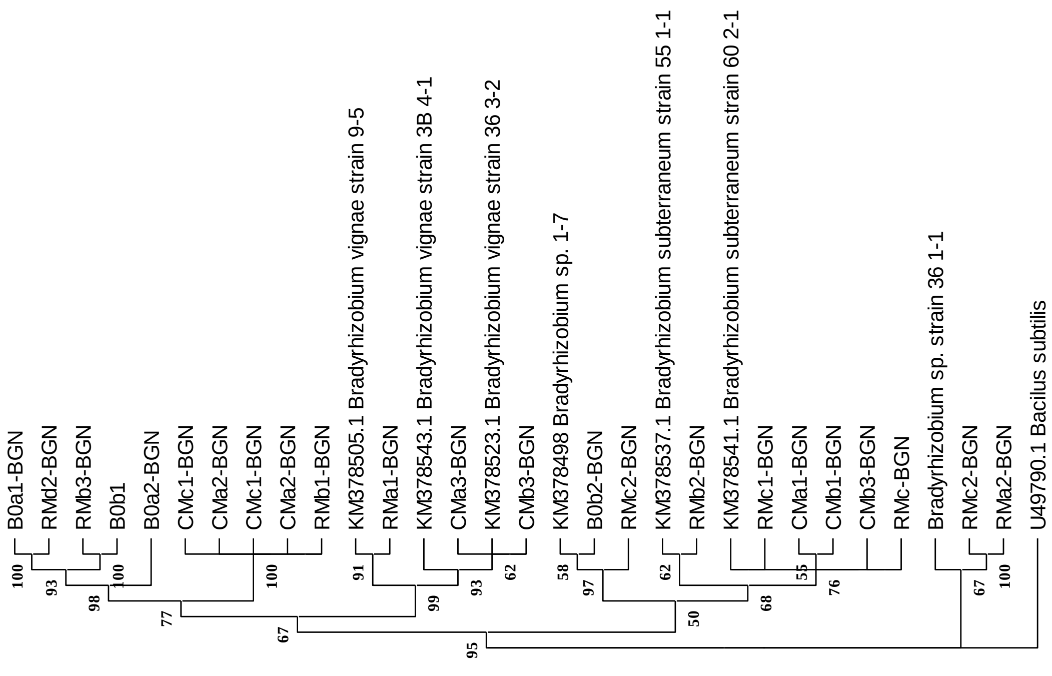


Figure 3.7: Phylogenetic tree based on ITS sequences using Maximum Likelihood method for bacterial isolates from root nodules and the strains used as inoculants in the study

The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). The bootstrap consensus tree inferred from 100 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test 100 replicates are shown next to the branches (Felsenstein, 1985). Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 31 nucleotide sequences. There were a total of 2175 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021).

3.5. Discussion

The *Bradyrhizobium* strains induced nodulation among the inoculated varieties as compared to the non-inoculated. This was congruent with the findings of Chen *et al.* (2018) who reported that inoculation enhances the number of nodules produced by ensuring that the presence of the desirable rhizobial strains is close to the root of the plant. However, nodules were also observed in the non-inoculated var01, which provided evidence of the existence of infective resident rhizobia strains in the Kavango soil. Moreover, the differences in nodule number per plant among the three varieties of Bambara groundnut indicate that the tested Bambara groundnut varieties respond differently to the different *Bradyrhizobium* strains. This result confirms earlier reports that seed coat compounds have also been noted to play a significant role in nodule formation (Redjeki *et al*., 2013). Ibny *et al.* (2019) claimed that variation in Bambara groundnut seed colour influences the choice of microsymbiont partners by luring various rhizobia with various levels of symbiotic efficiency, highlighting the significant influence of variety within a crop species.

Additionally, the use of native *Bradyrhizobium* strains for inoculation and the likely low population densities of native soil -borne bradyrhizobia could have contributed to the positive results obtained in the study. Studies carried out in the savanna region of the Benin Republic revealed that the indigenous rhizobia populations in the soil was inversely connected to the Bambara groundnut reaction to inoculation and that only response was shown when the population was < 5 rhizobia cells/g soil (Yakubu *et al*., 2010).

The data on shoot dry weight (Table 3.2) revealed that the highest shoot dry weights were found in Bambara groundnuts Var02, Var03 and var01 treated with inoculant MK with 1.71, 1.50, and 1.24 g plant^{-1}, respectively. The findings in this study are similar to Gomoung *et al.* (2017) who reported that inoculation of Bambara groundnut with rhizobial strains induced a considerable increase in plant length, fresh weight, and dry weight. Additionally, it was noted by Gedamu *et al*., (2021) that strain inoculation enhances plant dry weight. It was observed that inoculating plants with *Bradyrhizobium* strain CB756 and (NC-92) peat-based commercial inoculants led to considerably increased dry matter yields in South Africa and northern Punjab, Pakistan, compared to un-inoculated controls (Latif *et al.*, 2014; Mbah and Dakora, 2018). On the other hand, no increase in shoot biomass was observed in Var02 and Var01 treated with 9-5K. This might be because the introduced strain (9-5) did not fix enough N at the time of sampling. Another reason could be that strain 9-5 failed to colonize the roots. The phylogenetic tree shows that *Bradyrhizobium subterraneum* 60 2-1 was the most competitive of the seven strains utilized in this investigation, as more isolates from the root nodules clustered with 60 2-1. Grönemeyer *et al*. (2015) reported that *Bradyrhizobium subterraneum* are responsible for the nodulation of Bambara groundnut.

It is generally believed that many African soils contain a diverse group of indigenous populations of *Bradyrhizobia* spp. that can nodulate and fix atmospheric nitrogen in several legumes (Ibny *et al.*, 2019; Ajayi, Dianda and Fagade, 2020; Dlamini *et al.*, 2021). From the phylogenetic analysis the isolates in the first and second Clade did not group with any reference type strain. This suggests that there may be unidentified rhizobia capable of nodulating Bambara ground nut in the Kavango soil.

3.6. Conclusion

The greenhouse experiment is useful as it is the only way to determine the performance of pure strains used as inoculants under similar conditions, and thus complement the findings of field experiments. However, the validity of the greenhouse experiment is limited, because it does not represent varying soil and environmental conditions (Allito *et al.*, 2021). These results led to the conclusion that a field experiment was required. Since each *Bradyrhizobium* cluster contained a specific isolate from the root nodule, the mixed inoculant used in the greenhouse experiment was going to be applied to the seeds as a single strain to accurately assess the efficacy of each of the seven *Bradyrhizobium* strains in the field.

References

1. Ajayi, O. O., Dianda, M. and Fagade, O. E. (2020) 'Evaluation of some Nodulation Genes found in Bambara Symbiotic Rhizobia Strains', *International Journal of Innovative Science and Research Technology*, 5(5), pp. 42–46.

2. Allito, B. B. *et al.* (2021) 'Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (Vicia faba L.)', *Scientific Reports*, 11(1), pp. 1–13. doi: 10.1038/s41598-021-83235-8.

3. Chen, Y. X. *et al.* (2018) 'Faba bean (Vicia faba L.) nodulating rhizobia in Panxi, China, are diverse at species, plant growth promoting ability, and symbiosis related gene levels', *Frontiers in Microbiology,* 9(1338), pp. 1–10. doi: 10.3389/fmicb.2018.01338.

4. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791.

5. Gedamu, S. A., Tsegaye, E. A. and Beyene, T. F. (2021) 'Effect of rhizobial inoculants on yield and yield components of faba bean (Vicia fabae L .) on vertisol of Wereillu District , South Wollo, Ethiopia', *CABI Agriculture and Bioscience*, 2(8), pp. 1–10. doi: 10.1186/s43170-021-00025-y.

6. Gomoung, D. *et al.* (2017) 'Influence of Cross-Inoculation on Groundnut and Bambara Groundnut- Rhizobium Symbiosis : Contribution to Plant Growth and Yield in the Field at Sarh (Chad) and Ngaoundere (Cameroon)', *American Journal of plant sciences*, 8, pp. 1953–1966. doi: 10.4236/ajps.2017.88131.

7. Grönemeyer, J. L. *et al.* (2014) 'Rhizobia Indigenous to the Okavango Region in Sub-Saharan Africa : Diversity , Adaptations , and Host Specificity', *Applied and Environmental Microbiology*, 80(23), pp. 7244–7257. doi: 10.1128/AEM.02417-14.

8. Grönemeyer, J. L., Chimwamurombe, P. and Reinhold-Hurek, B. (2015) 'Bradyrhizobium subterraneum sp. nov., a symbiotic nitrogen-fixing bacterium from root nodules of groundnuts', *International Journal of Systematic and Evolutionary Microbiology*, 65(10), pp. 3241–3247. doi:

10.1099/ijsem.0.000403.

9. Hillocks, R. J. *et al.* (2012) 'Bamnara nut: A review of utilisation, market potential and crop improvent', *African Crop Science Journal*, 20(1), pp. 1–16.

10. Ibny, F. *et al.* (2019) 'Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (Vigna subterranea L . Verdc .) in Africa and their relationship with phylogeny', *Scientific Reports*, 9(1), pp. 1–17. doi: 10.1038/s41598-019-48944-1.

11. Ikenganyia, E., Anikwe, M. A. N. and Ngwu, O. (2018) 'Impacts of Plant Growth Promoting Rhizobacteria Inoculation Configurations on Vegetative Growth , Nodulation and Yield of Bambara groundnut [Vigna subterranea (L .) Verdc .]', *International journal of Horticulture, Agriculture and Food science(IJHAF)*, 2(3), pp. 54–69. doi: 10.22161/ijhaf.2.3.5.

12. Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.

13. Latif, R. *et al.* (2014) 'Significance of Groundnut Inoculation and NP Fertilizer Application on Yield , Nitrogen Uptake , Fixation and Soil N Balance Sheet under Rainfed Conditions', *Journal of Biology and Medical Sciences*, 2, pp. 7–14.

14. Mohammed, M. *et al.* (2021) 'Bradyrhizobium Inoculation of Field-Grown Kersting ' s Groundnut [Macrotyloma geocarpum (Harms) Marechal & Baudet] Increased Grain Yield and N 2 Fixation , Measured Using the Ureide , and 15 N Natural Abundance Techniques', *Frontiers in Sustainable Food Systems*, 5(672247), pp. 1–18. doi: 10.3389/fsufs.2021.672247.

15. Nassé, G. *et al.* (2019) 'Nodulation and Agronomic Performance of Indigenous Rhizobia Isolated from Bambara Groundnut (Vigna Subterranean L.) Nodules in Dalao, Côte d'Ivoire', *International Journal of Research Studies in Microbiology and Biotechnology*, 5(2), pp. 13–18. doi: 10.20431/2454-9428.0502004.

16. Redjeki, E. S., Mayes, S. and Azam-Ali, S. (2013) 'Evaluating the stability and

adaptability of Bambara groundnut (Vigna subterranea (L .) Verd .) landraces in Different agro-ecologies', in *Acta Hort*, pp. 389–400.

17. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msab120.

18. Sajid, M. *et al.* (2011) 'Influence of Rhizobium Inoculation on growth and yield of groundnut cultivars', *Sarhad J. Agric*, 27(4), pp. 573–576.

19. Shin, W. *et al.* (2016) 'Role of Diazotrophic Bacteria in Biological Nitrogen Fixation and Plant Growth Improvement', *Korean Journal of Soil Science and Fertilizer*, 49(1), pp. 17–29. doi: 10.7745/kjssf.2016.49.1.017.

20. Somasegaran P, Hoben HJ (1994) "Handbook for Rhizobia. Methods in Legume-Rhizobium Technology. New York: Springer-Verlag, pp 240-58.

21. Weihenstephan, W. *et al.* (2017) 'African origin of Bradyrhizobium populations nodulating Bambara groundnut (Vigna subterranea L . Verdc) in Ghanaian and South African soils', *PLOS ONE*, 12(9), pp. 1–23. doi: 10.3389/fsufs.2021.728014.

22. Yakubu, Kwari and Ngala (2010) 'N 2 Fixation by Grain Legume Varieties as Affected By Rhizobia Inoculation in The Sandy Loam Soil Of Sudano-Sahelian Zone of North Eastern Nigeria .', *Nigerian Journal of Basic and Applied Science*, 18(2), pp. 229–236.

Chapter 4

Influence of *Bradyrhizobium* Inoculants on Dry Matter Accumulation, and Yield of Bambara Groundnut [*Vigna subterranea* (L.) Verdc] in Namibia

Abstract

In comparison to several other legumes throughout Africa, the symbiosis between the legume Bambara groundnut (*Vigna subterranea* (L.) Verdc.) and rhizobia bacteria has not been sufficiently explored to enable economic exploitation of the relationship. A field study was conducted from 2021 to 2022 at the Ministry of Agriculture, Water and Land Reform (MAWRL), Mashare Agricultural Developments Institute (MADI), in the Kavango East Region. The goal was to determine the effect of *Bradyrhizobium* strains on nodulation rate and yield and yield component of three different Bambara groundnut varieties (brown, cream and red Bambara). The three Bambara groundnut varieties were grown under three treatments as follows: chemical fertilizer (urea), *Bradyrhizobium* strains [1-7; 3B 4-1; 9-5, 36 1-1; 36 3-2; 55 1-1 and 60 2-1], and negative control where the Bambara groundnuts were grown without any nutritional supplement. The Bambara groundnuts were harvested 130 days after planting, and several yield metrics were evaluated. The control Var03 (red) yielded the greatest grain at 1311.7 kg ha^{-1}, followed by Var01 (brown) treated with In1-7 at 1237.3kg ha^{-1}, while Var01 and Var02 (cream) inoculated with In36 3-2 yielded 1171.3kg ha^{-1} each. Inoculating the Bambara groundnut seeds with *Bradyrhizobium* strains before planting positively influenced nodulation, grain yield, and biomass production compared to treating the groundnuts with urea fertiliser. However, Var03 was highly compatible with native soil rhizobia, giving it an edge over some of the introduced strains. This implies that the unidentified strains should be investigated as possible bio-inoculants.

Keywords: Bambara groundnuts, food security, legumes (Fabaceae), productivity, biofertiliser

4.1. Introduction

Crop production is threatened by the world population growth and climate change, especially in dry areas (Santos *et al*., 2019; Saad *et al.*, 2020; Khaitov *et al.*, 2020). Namibia is one of the driest countries in the world which is depended on subsistence farming for food production. Agriculture, especially crop production is mostly practiced in the northern communal areas including the Kavango region where it is predominantly dominated by smallholder and subsistence farming, as is typical for many Sub-Saharan African (SSA) regions (Luchen *et al.*, 2018; Valombola *et al.*, 2019). According to Grönemeyer and Reinhold-Hurek (2018) about 99, 88, and 59% of households in Angola, Namibia, and Botswana, practice arable agriculture respectively. Factors such as variable yields, crop failure risk, a lack of financial resources, and nitrogen (N) poor, low-fertility soils all contribute to food insecurity under this rain fed agriculture system (Horn *et al*., 2015; Grönemeyer and Reinhold-Hurek, 2018; Valombola *et al*., 2021). Therefore, there is an urgent need to implement new management technologies to improve crop quality and increase grain yield. One of the technologies is to improve soil N content which can be improved through the incorporation of pulses and legume green manure with biological nitrogen fixation (BNF) of root nodule symbioses (Grönemeyer and Reinhold-Hurek, 2018). Symbiotic nitrogen (N_2) fixation is a vital biological activity in legumes that promotes plant development and the production of high-protein seed and forage (Denton *et al.*, 2017).

BNF by rhizobial-legume symbioses offers an environmentally friendly and less expensive option compared to chemical N fertilizers for legume crops (Thilakarathna *et al.*, 2019; Gedamu *et al*., 2021). Therefore, it is thought that inoculating legumes with effective rhizobia will boost the yield and yield-related traits of legumes while preserving soil health (Hillocks *et al.*, 2012; Singh *et al.*, 2016; Ibny *et al*., 2019; Allito *et al.*, 2021; Gedamu *et al*., 2021).

It is however known that inoculants often fail to outcompete native rhizobia for nodule occupancy, despite the reduced N fixation capacity of the native rhizobia, ultimately resulting in low yields (Ulzen *et al.,* 2016). Strains that perform well under controlled conditions are often selected as inoculants, although it is uncommon to look at how much faster native strains occupy nodules. According to Sánchez-cañizares *et al.* (2021), the rhizobial inoculants were often found to perform better in the field when they are based on native strains with a high capacity to fix N attributable to their genetic adaptations to the local environment. The effectiveness of native populations chosen as elite strains may therefore be exploited. Specific host cultivar-rhizobial strain combinations may be bred using information from laboratory studies, and assessing competition and understanding how diverse rhizobial strains behave in combination with assays conducted under field conditions. The objective of this field study was to assess how three different Bambara groundnut varieties grown in the Kavango region of Northern Namibia would respond to locally isolated *Bradyrhizobium* strains in terms of growth and yield.

Figure 4.8 showing the Bambara groundnut varieties used in the study, from left, Var01 (cream), Var02 (brown) and Var03 (red)

4.2. Materials and methods

A field experiment was carried out at Mashare Agricultural Developments Institute (MADI), Ministry of Agriculture, Water and Land Reform (MAWRL) (GPS coordinates of 17° 54′ 08.13″ S and 20° 08′ 00.99″ E.) in Kavango East Region. The experiment was performed under rain fed settings in order to stimulate the area's natural environmental conditions during the cropping season of 2021/2022. Deep ploughing, harrowing, and levelling were used to prepare the soil. The space was then ridged and divided into 4 m by 2 m plot sizes in a split plot design with three replications in a randomized complete block design (RCBD). Three Bambara groundnut varieties Var01 (brown), Var02 (cream), and Var03 (red) shown in Figure 4.1 above, were subjected to three distinct treatments, the first of which involved treatment with chemical fertilizer (urea), the second treatment with seven *Bradyrhizobium* strains, while the third was a no treatment negative control.

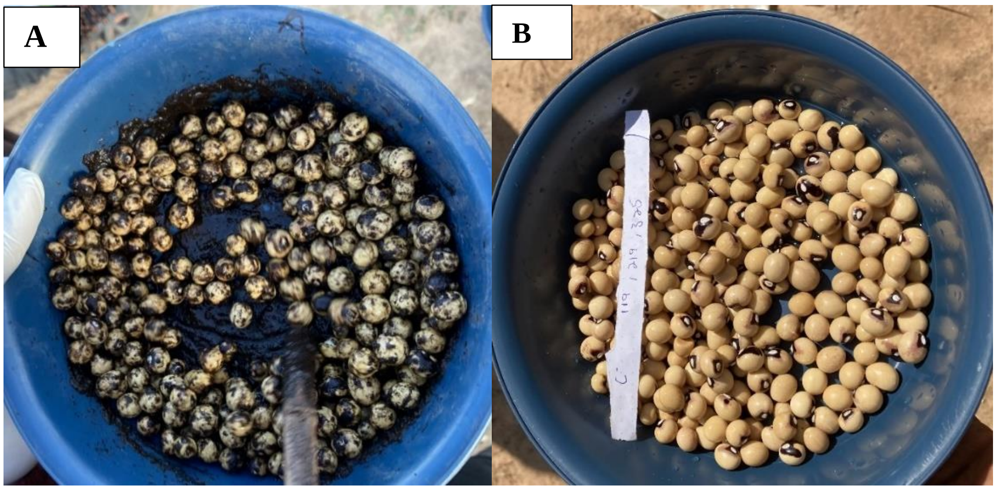


Figure 4.9: Bradyrhizobium inoculated (A) and un-inoculated (B) Bambara groundnut seeds

The inoculant treatment was carried out by obtaining a large number of bacterial inoculant strains named as *Bradyrhizobium* 1-7; 3B 4-1; 9-5, 36 1-1; 36 3-2; 55 1-1; and 60 2-1 (Table 4.1).

Table 4.12: Seven (7) *Bradyrhizobium* strains, preselected for bio-inoculant formulation for Bambara cultivation

ID	Reference isolate	Host plant	Country	Temp (°C)
1-7	*Bradyrhizobium* sp. 1-7	PEA	NAM	35
3B 4-1	*Bradyrhizobium vignae* 3B 4-1	BGN	NAM	38
9-5	*Bradyrhizobium vignae* 9-5	BGN	NAM	40
36 1-1	*Bradyrhizobium* sp. 36 1-1	BGN	NAM	35
36 3-2	*Bradyrhizobium vignae* 36 3-2	BGN	NAM	38
55 1-1	*Bradyrhizobium subterraneum* 55 1-1	BGN	NAM	35
60 2-1	*Bradyrhizobium subterraneum* 60 2-1	BGN	NAM	38

The strains were grown in freshly prepared Modified Arabinose Gluconate Medium with peat as a carrier before being placed in Whirl-Pack® sample bags. The bags were kept at room temperature and away from direct sunlight for long periods of time. To sustain the viability of bacterial cells, the inoculation technique was performed before planting under shade. The inoculant tick slurry was carefully mixed with dried seed so that all of the seeds received a thin covering of the inoculant (Figure 4.2). After allowing the seeds to air dry for a few minutes, they were planted at the appropriate seeding rate and spacing. To avoid contamination, un-inoculated seeds were planted first. To maximize the efficacy of the *Bradyrhizobium* strains and avoid cell death caused by solar radiation, the seeds were immediately covered with soil after sowing. 50 kg N ha^{-1} of Urea (CH_4N_2O) was applied to the soils where the N fertilizer treatment was to be performed. In addition, all plots were first treated with a low dose of phosphorus (superphosphate) at a rate of 20 kg ha^{-1}. This is because plants require phosphorus for growth, nodulation, and seed formation, as well as for N fixation, which is an energy-intensive process (Ikenganyia *et al*., 2017). All the plots were kept free from weeds by weeding with a typical hand hoe and the plants were thinned at the 4-6 leafy stage to achieve the desired plant density.

4.2.1. Yield assessment

4.2.1.1. Data collection

The yield of the different Bambara groundnut varieties under the three treatments was examined by comparing the physiological maturity of the groundnuts.

4.2.1.1.1. Root nodules

Randomly selected plants were gently removed from the gross area in each plot using a spade during the flowering phase to acquire data on nodulation. To avoid nodule loss, the entire root system was exposed before uprooting. The soil sticking to the roots was then gently washed away with water. Nodules from each plant's roots were removed, collected, and counted. Nodule samples were collected dehydrated in silica gel-filled vials and kept at 4 °C until needed. Nodules from selected plants were oven-dried for 72 hours at 65 °C, and then dry weights recorded after.

4.2.1.1.2. Plant shoot

Shoot biomass data was collected during the flowering phase and at maturity. Shoot biomass was calculated by randomly choosing 5 plants from a net plot of each treatment. Sampled plants were separated into roots and shoots, and the plant shoots were weighed on an electronic balance to determine their fresh weight. The shoots were then placed in paper envelopes and oven-dried for 72 hours at 65 °C for the dry matter determination of shoots.

4.2.1.1.3. Soil Plant Analysis Development: leaf chlorophyll measurement (SPAD)

The amount of chlorophyll present in the leaves was determined by a SPAD-502 meter, taking three young, enlarged leaves of each plant soon before harvest. Three SPAD value were taken from a single leaf, one about the midway of the leaf blade and the other two around 1 cm apart from the midpoint. The mean SPAD value of each leaf was determined by taking the average of the three readings. The means of three leaf data points were determined for each plant.

4.2.1.1.4. Number of pods

Number of fresh pods were determined at harvest. In determining the number of fresh pods, a spade was used to carefully scoop out the soil containing the plant roots from the middle rows in each subplot and the number of fresh pods per plant were then manually counted. Based on the number of plants on that subplot, this was used to calculate the average number of pods per plant. This was done for every plot. Fresh pods per plot were packed in a paper envelope and oven dried for 72 hours at 65 °C, and the pod dry weights were recorded.

4.2.1.1.5. Number of seeds per pod and 100 seed weight

From fresh pods harvested, 40 were chosen at random and the number of seeds in these 40 pods were counted to determine the average number of seeds per pod. Finally, 100 seeds were chosen from the 40 pod seeds and weighed to determine the 100 seed weight per subplot. The seed weight per subplot was likewise recorded, and the procedure described above was repeated for all subplots.

4.2.1.1.6. Grain yield

Seed yield expressed in Kg ha^{-1} was evaluated by the following formula as in (Gomoung et al., 2017):

$$Y = ((P \times 10000))/(S.E)$$

Where, Y = dry weight of seeds in Kg ha^{-1}; P = dry weight of seeds per unit of experimental area; S.E = Experimental surface area (m^2); 1 ha = 10,000 m^2

4.3. Data analysis

The statistical software Genstat® (14th edition, VSN International, UK) was used to determine significant differences between treatments using the Analysis of Variance

(ANOVA) function. The data were further subjected to mean comparisons and correlation analysis on all the traits.

4.4. Results and discussion

The results of this study revealed a significant interactions (p 0.05) among the agronomic traits studied. The mean square values and significance probability level at 5%, 1% and 0.1% are indicated in the analysis of variance (ANOVA) tables to discern significant differences.

4.4.1. Effect of inoculation on the three different Bambara ground varieties used in this experiment

4.4.1.1. Nodule number

The number of nodules was evaluated between the three treatments (fertilizer, bio-inoculant, and the control with no treatment). Tables 4.2 shows the findings of these comparisons. A statistically significant differences was observed between treatments and varieties at the 0.05 level of significance. However, there were no statistically significant differences recorded among interaction between treatments and varieties, implying that the Bambara groundnut varieties responses to the treatments was the same in terms of number of nodules recorded.

The highest nodule number was observed in Var03 inoculated with In55 1-1 with 53.25 nodules, followed by Var02 inoculated with In36 3-2 with 43.00 nodules, and Var01 inoculated with In55 1-1 with, 36.00 average nodules number per plant, respectively (Table 4.3). These result shows that variety inoculation with bradyrhizobia, as opposed to treating the Bambara groundnuts with urea fertiliser, may be more suited to increase the nodule number, particularly for Var02 and Var01 in the study area. The results were expected as it is well known that, symbiotic relationships between rhizobacteria and Bambara groundnut plants usually results in the initiation and growth of root nodules, which increases the amount of nitrogen fixation in the crop (Ntambo *et al.*, 2017). The result from this study is comparable to the findings of Yakubu *et al*. (2010) and Raissa *et al.* (2020), who reported that inoculating Bambara groundnut with rhizobia increased the number of nodules compared to the control. Allito *et al.* (2021) also discovered a considerable rise in *Vicia faba*, commonly known as faba bean nodulation after rhizobium inoculation. On the other hand, it is sometimes possible for the inoculant not to have any effect on the nodulation ability of the variety especially in the presence of superior native rhizobia than the introduced strains. This has been reported by Nyaga and Njeru (2020), who outlined that in the presence of superior native rhizobia, than the introduced strains will have no influence on nodulation as compared to un-inoculated varieties. This could be the reasons why the un-inoculated Var03 in this study had a high number of nodules.

4.4.1.2. Nodule dry weight

A statistically significant difference was recorded across the means of the different varieties, treatments, and the interaction among varieties and treatments at the 0.05 level of significance for the nodule dry weights, indicating that the response of the Bambara groundnut varieties to the treatments was different in terms of nodule dry weight (Table 4.2). Var02 inoculated with strain In60 2-1 showed a highest nodule dry weight with 0.1915 g $plant^{-1}$, followed by Var03 inoculated with In36 3-2 with 0.1733 g $plant^{-1}$ and Var01 inoculated with In36 3-2 with 0.1210 g $plant^{-1}$, respectively (Table 4.2). This study revealed that inoculating Bambara groundnut with *Bradyrhizobium* [60 2-1, 36 3-2] increases nodule dry weight as compared with the control. The current study's findings are congruent with those of Bejandi *et al.* (2012), Ulzen *et al.* (2016) and Allito *et al.* (2021), who reported that using rhizobial strains resulted in significantly greater number of nodules and dry weight in their studies. The increase in nodule dry weight could be attributed to large size and increased number of nodules formed by the infection threads of the infected Bambara groundnut.

It has also been noted that when the availability of soil N or application of N fertilizer is increased, the nodule quantity and size are usually reduced. As a result, the decrease in nodule dry weight of plants treated with urea fertilizer demonstrates the soil's high N concentration. Ntambo *et al.* (2017) also reported that an increase in N levels reduces nodule quantity as well as nodule dry weight per plant in inoculated plants.

Table 4. 13: Mean-square values and significance tests of three varieties of Bambara groundnut treated with different *Bradyrhizobium* strains as bio-inoculant.

Source of variation	**df**	**PHT**		**Nr_Nod**	**NDW**	**SFW1**	**SDW**		**SPAD**	**Nr_pods**	**Seeds_40**	**100_SW**	**PDW**	**C_N**	**Grain_yield**
		30DAP	**60DAP**				**60 DAP**	**Mat**							
Replication	2	14.548	0.138	556.2	0.004992	978.5	40.22	46.37	22.56	3129.7	43.81	5329	36.31	4.356	58096
Variety	2	46.544^{***}	28.01^{***}	1334.7^{***}	0.004303^{*}	100.8^{ns}	2.85^{ns}	89.66^{*}	121.79^{**}	4638.7^{***}	93.37^{*}	26905^{***}	102.96^{*}	20.001^{***}	164732^{*}
Inoculants	8	5.099^{**}	5.445^{*}	272.8^{*}	0.003267^{**}	1581.4^{***}	101.33^{***}	22.42^{ns}	25.21^{ns}	383.7^{ns}	7.94^{ns}	1959^{ns}	70.62^{*}	3.124^{*}	112991^{*}
Variety x Inoculants	16	1.006^{ns}	3.375^{ns}	117.7^{ns}	0.003872^{*}	482.5^{ns}	26.94^{ns}	79.83^{***}	27.61^{ns}	557.6^{ns}	41.33^{*}	2023^{ns}	110.95^{***}	3.604^{**}	177513^{***}
Residual	52	1.391	2.552	103.6	0.001316	376.1	17.86	25.43	19.35	435.2	21.74	12224	27.78	1.469	44454

d.f= degrees of freedom; PHT=plant height; Nr_Nod=Number of nodules per plant; SFW1= Shoot fresh weight at 60days after planting; SDW=Shoot dry weight; SPAD value, leaf chlorophyll content; Nr_pods= pod number per plant; seeds_40= number of seeds in 40 pods; 100_SW= 100 seed weight; PDW= Pod dry weight; C_N= carbon: Nitrogen*, ** and *** denote significant at 5%, 1% and 0.1% probability level, respectively; ns = non-significant

4.4.2. Effect of inoculations and micronutrients on agronomic parameters

Figure 4.10: Depicting the Bambara groundnut experimental layout in the field at Mashare during 2021/2022.

4.4.2.1. Plant height

The plant height was recorded 30 and 60 days after planting. Bambara groundnuts inoculated with *Bradyrhizobium* strains had significantly enhanced plant height (Table 4.3) at 30 DAP. A statistically significant difference was recorded at the 0.05 level of significance in plant height across treatments and among varieties (Table 4.2). As a result, the tallest plants were Var03-In3B 4-1 and Var02-In3B 4-1 with 13.93 cm and 13.13 cm plant^{-1}, respectively. This implies that strain 3B 4-1 may have contributed to the synthesis of growth hormones that enhanced plant growth in the varieties. The findings are comparable to those of Gomoung *et al.* (2017) who discovered that inoculating cowpea plants with two strains of rhizobia significantly increased the plant height.

Fertilizer, on the other hand, increased plant height at 60 DAP with 18.73 cm and 18.10 cm in Var03-N and Var01-N, respectively, when compared to the inoculated and negative controls (Table 4.3). According to Hasan *et al.* (2018), applying N fertilizer to the Bambara groundnut enhances plant height and aids vegetative development. Similar results were obtained in this investigation, which could be attributed to an insufficient amount of N fixed by the introduced strains at the time of sampling hence, plant height was increased in the plants were fertilizer was applied.

4.4.2.2. Soil Plant Analysis Development: leaf chlorophyll measurement (SPAD) values

As shown in Table 4.2, there were no statistically significant differences observed between the means of different treatments and the mean of the interaction of treatment and variety among SPAD values. However, there were statistically significant differences in the means of distinct varieties with SPAD values at the 0.05 level of significance. The plant health and N concentration are indicated by chlorophyll content. Although not statistically significant, the greatest SPAD values were recorded for Var03-In36 1-1 with 38.40, which is close to the 40.3 found by Musa *et al.* (2016). This study's findings contradict those of Bejandi *et al.* (2012), who reported a higher SPAD values of inoculated plants higher than those of un-inoculated plants. This demonstrated that the inoculant boosted nitrogen supply in their study.

4.4.2.3. Number of pod/plants

Inoculating Bambara groundnut with *Bradyrhizobium* strains did not significantly increase the number of pods per plant (Table 4.2). However, there were statistically significant differences of $p<.001$ of means among different varieties recorded at the 0.05 level of significance. In addition, there were no statistically significant differences among the mean of interaction of treatment and variety with number of pods per plant observed. This means that there were no differences in the number of pods recorded between the inoculated, fertilizer-treated, and negative control.

This suggests that, despite the use of native strains in the study area, the increased number of pods could be attributed to efficient wild rhizobia strains that interacted quickly with the host plant, whereas the introduced inoculants were less competitive, as was the case with Gomoung *et al.* (2017).

In contrast, Nassé *et al.* (2019) observed that indigenous strains boosted Bambara groundnut yield both the number and weight of pods in their experiment. Furthermore, Gedamu *at al*. (2021) reported that inoculation with *Rhizobium* strains increased the number of pods per plant in their study.

4.4.2.4. Number of seed/pods

There were statistically significant differences recorded at 0.05 level of significance across the means of the different varieties and in the mean of the interaction of treatment and varieties in terms of the number of seeds per pod. On the contrary, there were no statistically significant differences among various treatments in terms of the number of seeds per pod (Table 4.2). Var03 produced most seeds per 40 number of pods with 46.15, followed by Var01 with 43.52 and 42.56 seeds recorded for Var02, respectively (Table 4.3). Mfilinge *et al*. (2014) reported that inoculation with *Rhizobium* significantly affected yield components, especially the number of seeds per pod and 1000-seed weight in common bean and soybean respectively.

Table 4. 14: Mean values for agro-morphological traits of three varieties of three Bambara groundnut varieties treated with *Bradyrhizobium* bio-inoculant

Variety	**Inoculant**	**PHT**		**Nr Nod**	**NDW (g)**	**SFW1 (g)**	**SDW (g)**		**SPAD_values**	**Nr_pods**	**Seeds_40_pods**	**100_SW (g)**	**PDW (g)**	**C_N**	**Grain_yield (kg/ha)**
		30 DAP	**60 DAP**				**60 DAP**	**Mat**							
	In1-7	11.67	15.73	28	0.06	57	20.39	14.73	25.27	52.21	42.67	220	30.93	14.37	1237.26
	In3B 4-1	10.73	15.93	25.83	0.1	82.33	20.36	25.53	27.08	75.98	49	205	28.8	13.95	1152.17
	In9-5	9.7	13.67	20.75	0.1	45.33	12.41	14.04	27.81	66.77	41	185	12.14	15.83	485.71
	In36 1-1	9.63	14.4	26.25	0.11	45	13.06	17.38	26.01	56.69	43	207.5	15.79	14.92	631.67
Var01	In36 3-2	9.58	14.27	26.42	0.12	55.33	15.86	22.41	24.5	77.11	40.33	223.33	29.28	15.23	1171.33
	In55 1-1	8.63	13.73	36	0.12	40	9.85	18.28	23.97	87.21	43.67	185	23.2	16.33	928.08
	In60 2-1	10.03	13.73	18.75	0.07	55	14.88	17.76	24.1	62.71	41.33	208.33	25.88	15.49	1035
	N	10.57	18.1	32.5	0.06	39	11.31	17.43	23.6	74.44	44.67	195	17.37	15.28	694.66
	O-	11.93	16.6	31.83	0.1	85.67	20.79	13.27	24.69	48.69	46	231.67	21.95	13.92	877.86
Mean		10.28	15.13	27.37	0.09	56.07	15.43	17.87	25.23	66.87	43.52	206.76	22.82	15.04	912.64
	In1-7	11.49	14.93	29.75	0.15	61.67	17.24	20.34	22.25	41.32	40.67	220	16.21	15.59	648.48
	In3B 4-1	13.13	15.67	28.25	0.1	70	18.31	18.79	23.62	70.75	41.67	247.5	22.32	16.04	892.6
	In9-5	11.6	17.27	28	0.1	61	15.71	30.64	25.24	69.71	40	261.67	25.39	14.77	1015.57
	In36 1-1	11.17	14.63	24.17	0.09	40.67	11.31	20.34	21.15	53.86	50.33	240	25.94	14.8	1037.71
Var02	In36 3-2	11.33	16.2	43	0.1	41	10.75	19.18	21.93	72.38	41.33	275	29.28	15.4	1171.29
	In55 1-1	10.3	14.33	42.75	0.07	26.67	9.1	16.87	23.03	42.79	40	240	26.06	15.1	1042.4

	In60 2-1	10.74	15.87	35	0.19	57.67	19.01	16.65	25.08	54.41	41.67	250	25.41	13.95	1016.33
	N	11.87	15.13	19.83	0.06	61.67	13.69	12.99	22.68	55.58	42	245	18.44	15.15	737.79
	O-	12.6	15.2	26.25	0.13	73.67	20.39	13.91	22.5	58.76	45.33	232.5	27.38	16.2	1095.12
Mean		11.58	15.47	30.78	0.11	54.89	15.06	18.86	23.05	57.73	42.56	245.74	24.05	15.22	961.92
	In1-7	13.4	18	30.25	0.16	59.33	18.49	18.69	26.74	48.9	49.67	192.5	24.56	19.89	982.29
	In3B 4-1	13.93	17.7	43.83	0.1	60.33	15.47	14.83	26.89	44	41.67	322.5	21.13	16.15	845.04
	In9-5	12.67	17	43.33	0.11	40	11.31	13.79	23.9	25.87	49.67	307.5	19.19	17.01	767.78
	In36 1-1	12.52	16.23	31.83	0.08	33	10.86	10.16	38.4	27.45	42.67	282.5	18.4	15.73	735.87
Var03	In36 3-2	12.83	16.73	45.08	0.17	46.67	12.97	10.75	25.06	36.3	47.67	266.67	14.02	15.82	560.96
	In55 1-1	11.68	16.4	53.25	0.08	32	9.25	12.12	28.53	26.87	51	257.5	17.85	17.94	714.01
	In60 2-1	13.72	15.93	32.08	0.09	57	17.33	15.37	29.73	36.72	44.33	253.33	12.49	15.7	499.56
	N	12.43	18.73	36.33	0.12	90	22.6	23.37	22.39	56.44	45	293.33	21.57	16.41	862.88
	O-	12.93	16.62	52	0.16	52.33	14.8	18.84	24.07	66.63	43.67	247.5	32.79	14.86	1311.71
Mean		12.9	17.04	40.89	0.12	52.3	14.79	15.32	27.3	41.02	46.15	269.26	20.22	16.61	808.9
Grand mean		11.59	15.88	33.01	0.11	54.42	15.09	17.35	25.19	55.21	44.07	240.59	22.36	15.62	894.49
SED		0.963	1.304	8.312	0.03	15.834	3.45	4.117	3.591	17.033	3.807	28.571	4.304	0.99	172.152
LSD (5%)		1.932	2.618	16.679	0.059	31.774	6.924	8.262	7.207	34.179	7.639	57.332	8.636	1.986	345.448
CV (%)		10.2	10.1	30.8	33.8	35.6	28	29.1	17.5	37.8	10.6	14.5	23.6	7.8	23.6

Traits details given in Table 4.2

SED= Standard error of difference; LSD= least significant difference; CV=coefficient of variation

4.4.2.5.100 seed weight

As shown in Table 4.2, there were no statistically significant differences between the means of different treatments and the mean of the treatment-varieties interaction. However, at the 0.05 level of significance, there were statistically significant differences in means of different varieties in 100 seed weight recorded. As a result, Var03 had a high 100 seed weight with 269.26 g, followed by Var02 with 245.74 g and least was recorded in Var01 with 206.76 g (Table 4.3). The weight difference obtained in this experiment could be linked to the influence of N biological fixation on grain filling ability. Moreover, it is possible that the small size of the grains limited the potential for inoculation to increase seed size, despite the positive correlation between 100 seed weight and seed size. Other factors such as environmental conditions and nutrient availability may also play a role in determining seed size (Gedamu *et al*., 2021).

4.4.2.6. Biomass

The plant biomass was measured during early flowering and harvest. There were no statistically significant differences in shoot dry weight at early flowering across means of different varieties or the mean of the treatment-variety interaction. However, there were statistically significant differences in means of different treatments at the 0.05 level of significance in shoot dry weight (60 DAP). Var03-N had the greatest shoot dry weight (22.60g plant^{-1}), followed by Var01-O- (20.79g plant^{-1}) and Var02-O- (20.39g plant^{-1}). This suggests that inoculation had no observable effect on the dry weight of the shoots during flowering. In contrast, Nyaga and Njeru (2020) found that rhizobia inoculation boosted cowpea shoot dry weight at flowering when compared to control plants.

There were statistically significant differences recorded across the means of different varieties, means of different treatments, and means of the interaction of treatment and varieties for shoot dry weight at maturity. Var02-In9-5 had the highest shoot dry weight at maturity, followed by Var01-In3B 4-1 and Var03-N, with 30.64, 25.53, and 23.37 g plant^{-1}, respectively. Ikenganyia *et al*. (2017) obtained similar results, reporting that inoculation greatly increased plant biomass of Bambara groundnut. These findings indicate that it is better to use bio-inoculants than to use chemical fertiliser to increase shoot biomass in Var01 and Var02.

In terms of plant biomass yield, it was discovered that the resulting shoot dry weight measurements varied significantly depending on the type of variety, with a significant P-value of less than 0.05. Var02 exhibited the highest shoot dry weight when subjected to all three treatments when compared to the other varieties. Variety 02 could be recommended for forage or animal feed production due to its high biomass value.

4.4.2.7. C/N ratio

The carbon to nitrogen (C/N) ratio is important in composting because microorganisms require a proper balance of C and N (range from 25 to 35) to survive (Büchi *et al.*, 2015). High C/N ratios can cause composting to take longer, whereas low C/N ratios increase nitrogen loss. The C: N in the seeds was evaluated in this study, and there were statistically significant differences observed at 0.05 level of significance across the means of different varieties, means of different treatments, and means of the interaction of treatment and varieties. The C/N ratio in the present study varied between 13.92 and 19.89. Var01 which was not treated (negative control) displayed a lowest C/N ratio of 13.92, followed by Var02-In60 2-1 with 13.95, Var01-In3B 4-1 with 13.95, and Var01-In1-7 with 14.37. This is comparable to the findings of Büchi *et al.* (2015), who found that the mean C/N varied between species from 9.8 in *Vicia pannonica* to 32.5 in *Lupinus albus*.

4.4.2.8. Grain yield

There were statistically significant differences between the means of different varieties, the means of different treatments, and the mean of the interaction of treatment and varieties at 0.05 level of significance (Table 3.1). A significant interaction response implies that bacteria performance differed depending on variety. The un-inoculated Var03 yielded the most grain at 1311.7 kg ha^{-1}, followed by Var01-In1-7 at 1237.3kg ha^{-1}, and Var01 and Var02 inoculated with In36 3-2 yielded 1171.3kg ha^{-1} apiece. Var01-In9-5 had the lowest grain production at 485.7 kg ha^{-1}. This indicates that for Var01 and Var02, the increments in grain yield after inoculation is due to the reason that the soils N is a limiting factor and the existing rhizobia bacteria may not be capable to supply N through BNF to the two varieties. Thus, the grain yield could be strongly improved by means of inoculation. This was not the case for Var03 as more yield were observed in the negative control.

Grain yield in this study ranged between 485.7 and 1311.7 kg ha^{-1}, higher than the range reported in Chad, with weight between 491.25 and 910.375 kg ha^{-1} (Gomoung *et al.*, 2017). The findings of this study are in line with the findings of Wilson *et al.* (2021) who reported that inoculating groundnut cultivars with efficient native strains increased grain yield. Although inoculation is site specific, two basic scenarios are known where rhizobia inoculation is likely to produce a response: when compatible rhizobia of the host legume are absent and when the native rhizobia population is low in the experimental area (Ulzen *et al.*, 2016). In this study, it was clear that the introduced strains were not compatible with native strains for Var03.

Since every subsistence farmer's goal is to boost the grain yield, it is best to advise Bambara groundnut farmers to make use of bio-inoculant rather than the mineral fertilizer. This is because the bio-inoculants are both eco-friendly and also a cheaper alternative. Furthermore, following the bio-inoculant treatment, Var01 and Var02 yielded the highest grain yield of 961.92 and 912.64 kg ha^{-1}, respectively. As a result, the findings of this study are relevant in terms of providing subsistence farmers with bio-inoculants capable of effectively fixing biological nitrogen while in symbiosis with Bambara groundnut in a low-rainfall climate.

Table 4. 15: Pair-wise phenotypic correlation coefficients of agro-morphological traits among three Bambara groundnut varieties treated with *Bradyrhizobium* strains as bio-inoculant

Variables	**PHT1**	**PHT2**	**Nr_Nod**	**NDW**	**SFW1**	**SDW1**	**SDW2**	**SPAD**	**Nr_pods**	**Seeds_40**	**100_SW**	**PDW**	**C_N**	**Grain_yield**
PHT1	1	.662**	0.333	0.167	0.296	0.336	-0.236	0.193	-.533**	0.279	.623**	-0.098	0.34	-0.098
PHT2	.662**	1	.443*	0.153	0.282	0.284	0.086	0.099	-0.25	0.345	.507**	-0.029	0.29	-0.029
Nr_Nod	0.333	.443*	1	0.271	-0.332	-0.32	-0.224	0.04	-0.364	0.252	.527**	0.042	0.251	0.042
NDW	0.167	0.153	0.271	1	0.211	0.294	0.1	-0.131	-0.003	0.11	0.032	0.071	0.159	0.071
SFW1	0.296	0.282	-0.332	0.211	1	.920**	0.344	-0.222	0.234	0.01	0.015	0.186	-0.177	0.186
SDW1	0.336	0.284	-0.32	0.294	.920**	1	0.281	-0.126	0.128	-0.014	-0.035	0.256	-0.157	0.256
SDW2	-0.236	0.086	-0.224	0.1	0.344	0.281	1	-0.35	.591**	-0.176	-0.151	.438*	-0.195	.438*
SPAD	0.193	0.099	0.04	-0.131	-0.222	-0.126	-0.35	1	-.405*	0.044	0.123	-0.333	0.145	-0.333
Nr_pods	-.533**	-0.25	-0.364	-0.003	0.234	0.128	.591**	-.405*	1	-0.342	-.524**	.445*	-0.308	.445*
Seeds_40	0.279	0.345	0.252	0.11	0.01	-0.014	-0.176	0.044	-0.342	1	0.041	-0.085	.409*	-0.085
100_SW	.623**	.507**	.527**	0.032	0.015	-0.035	-0.151	0.123	-.524**	0.041	1	-0.046	0.062	-0.046
PDW	-0.098	-0.029	0.042	0.071	0.186	0.256	.438*	-0.333	.445*	-0.085	-0.046	1	-0.246	1.000**
C_N	0.34	0.29	0.251	0.159	-0.177	-0.157	-0.195	0.145	-0.308	.409*	0.062	-0.246	1	-0.246
Grain_yield	-0.098	-0.029	0.042	0.071	0.186	0.256	.438*	-0.333	.445*	-0.085	-0.046	1.000**	-0.246	1

Traits details given in Table 4.2; **. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

4.4.3. Correlation analysis

Table 4.2 shows pair-wise correlation coefficients and levels of significance for agro-morphological parameters. The plant height and the number of nodules per plant were both positively and significantly correlated with 100 seed weight, however, the 100 seed weight showed a negative correlation with the number of pods per plant. The shoot dry weight showed positive correlation with number of pods and grain production at maturity. This is comparable to the findings of Gomoung *et al.* (2017) who discovered a positive and significant correlation between the number of seeds and the dry weight of the pods of Bambara at Ngaoundere.

4.5. Conclusion

The response of Bambara groundnut varieties to inoculated strains differed greatly, which could be attributed to bradyrhizobia-plant genotype specificity. Inoculation with strain In55, for example, increased nodulation in Var03 compared to Var02. Furthermore, nodulation in the untreated Var03 (negative control) was higher than in the negative controls of Var01 and Var02. This demonstrates that, despite the low natural rhizobia population, Var03 was extremely compatible with native soil rhizobia, providing them with a competitive advantage over some of the introduced strains. The outcome from this finding shows that bradyrhizobia inoculation is an efficient and practical method of increasing Bambara groundnut production in Namibia. The utilization of the most competitive strains as bio-inoculants can be adopted in Namibia, providing the local farmers with a less expensive and more environmentally friendly alternative to mineral fertilizers. This will enhance profitability and crop yield because less money will be spent on chemicals to enrich the poor soils.

5. Overall conclusion of the study

The productivity of Bambara groundnut is decreasing in subsistence farming systems due to biotic and abiotic climatic factors and socioeconomic limitations. In chapter one, a study was conducted in Namibia's Kavango East Region to assess factors contributing to the diminishing output of Bambara groundnut. Results showed that most farmers allocate less than a hectare for Bambara groundnut planting each year, and yields were low, averaging between 1 and 100 kg per season. Farmers preferred cream-seeded Bambara groundnuts and cited field pests, low yield, lack of improved varieties, expensive labor, and seed costs as production barriers. Breeding for improved varieties and inoculant application were suggested as approaches to boost output and add nitrogen to the soil for succeeding cropping. In the second chapter, a literature review was conducted regarding the topic Rhizobial inoculants are seen as a cheaper, easier, and safer method for improving nitrogen fixation and crop productivity in grain legumes. Identifying efficient rhizobial strains for biofertilizer production for Bambara groundnut is essential to smallholder farming systems' food security. In chapter 3 a presentation of a. study conducted at the University of Namibia to evaluate the effect of two inoculants (a single strain and a mixed inoculant) on the nodulation and growth parameters of three Bambara groundnut varieties. The results indicated that both inoculants significantly increased plant height and shoot dry weight compared to non-inoculated cultivars. The mixed inoculant outperformed the single strain in terms of productivity and competitiveness, and the cream and red varieties produced the highest shoot dry weight. In the final chapter results of a field experiment conducted at MADI in Namibia to determine the effect of different *Bradyrhizobium* strains on the nodulation rate and yield of three Bambara groundnut varieties was presented. The results showed that inoculating the seeds with *Bradyrhizobium* strains before planting positively influenced nodulation, grain yield, and biomass production compared to treating with urea fertilizer. Var03 had the highest grain yield, indicating that it was highly compatible with native soil rhizobia, while the other varieties showed better performance with introduced strains. The study suggests that further investigation into unidentified strains is necessary to develop possible bio-inoculants for Bambara groundnut.

References

1. Allito, B. B. *et al.* (2021) 'Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (Vicia faba L.)', *Scientific Reports*, 11(1), pp. 1–13. doi: 10.1038/s41598-021-83235-8.

2. Bejandi, T. K. *et al.* (2012) 'Effects of plant density, Rhizobium inoculation and microelements on nodulation, chlorophyll content and yield of chickpea', *Annals Biological Research*, 3(2), pp. 951–958.

3. Büchi, L. *et al.* (2015) 'Accumulation of biologically fixed nitrogen by legumes cultivated as cover crops in Switzerland', *Plant and Soil*, 393(1–2), pp. 163–175. doi: 10.1007/s11104-015-2476-7.

4. Denton, M. D. *et al.* (2017) 'Legume inoculant application methods: effects on nodulation patterns, nitrogen fixation, crop growth and yield in narrow-leaf lupin and faba bean', *Plant and Soil*, 419(1–2), pp. 25–39. doi: 10.1007/s11104-017-3317-7.

5. Gedamu, S. A., Tsegaye, E. A. and Beyene, T. F. (2021) 'Effect of rhizobial inoculants on yield and yield components of faba bean (Vicia fabae L .) on vertisol of Wereillu District , South Wollo, Ethiopia', *CABI Agriculture and Bioscience*, 2(8), pp. 1–10. doi: 10.1186/s43170-021-00025-y.

6. Gomoung, D. *et al.* (2017) 'Influence of Cross-Inoculation on Groundnut and Bambara Groundnut- Rhizobium Symbiosis : Contribution to Plant Growth and Yield in the Field at Sarh (Chad) and Ngaoundere (Cameroon)', *American Journal of plant sciences*, 8, pp. 1953–1966. doi: 10.4236/ajps.2017.88131.

7. Grönemeyer, J. L. and Reinhold-Hurek, B. (2018) 'Diversity of bradyrhizobia in subsahara Africa: A rich resource', *Frontiers in Microbiology*, 9(2194), pp. 1–8. doi: 10.3389/fmicb.2018.02194.

8. Hasan, M. *et al.* (2018) 'Nitrogen and phosphorus management for Bambara groundnut (Vigna subterranea) production-A review', *Legume Research*, 41(4), pp. 483–489. doi: 10.18805/LR-379.

9. Hillocks, R. J. *et al.* (2012) 'Bamnara nut: A review of utilisation, market potential and crop improvent', *African Crop Science Journal*, 20(1), pp. 1–16.

10. Horn, L., Shimelis, H. and Laing, M. (2015) 'Participatory appraisal of production constraints, preferred traits and farming system of cowpea in the northern Namibia: Implications for breeding', *Legume Research*, 38(5), pp. 691–700. doi: 10.18805/lr.v38i5.5952.

11. Ibny, F. *et al.* (2019) 'Symbiotic effectiveness and ecologically adaptive traits of native rhizobial symbionts of Bambara groundnut (Vigna subterranea L . Verdc .) in Africa and their relationship with phylogeny', *Scientific Reports*, 9(1), pp. 1–17. doi: 10.1038/s41598-019-48944-1.

12. Ikenganyia, E., Anikwe, M. and Ngwu, O. (2017) 'Influence of Rhizobacteria Inoculant Application Methods and Phosphate Fertilizer Rates on Dry Matter Accumulation , Yield of Bambara Groundnut [Vigna subterranea (L .) Verdc] and Soil Total Nitrogen Content in a Degraded Ultisol in Southeast Nigeria', *Agrotechnology*, 6(165), pp. 1–7. doi: 10.4172/2168-9881.1000165.

13. Luchen, C. C. *et al.* (2018) 'Evaluating the Yield Response to Bio-Inoculants of Vigna unguiculata in the Kavango Region in Namibia', *Journal of Plant Pathology & Microbiology*, 9(10), pp. 1–5. doi: 10.4172/2157-7471.1000456.

14. Mfilinge, A., Mtei, K. and Ndakidemi, P. A. (2014) 'Effects of Rhizobium inoculation and Supplementation with P and K , on Growth , Leaf chlorophyll content and Nitrogen Fixation of Bush bean varieties', *American Journal of Research Communication*, 2(10), pp. 49–87.

15. Musa, M. *et al.* (2016) 'Nitrogen Fixation and N-Balance Studies on Bambara Groundnut (Vigna subterranea L. Verdc) Landraces Grown on Tropical Acidic Soils of Malaysia', *Commun Soil Sci Plant Anal.*, 47(4), pp. 533–542. doi: 10.1080/00103624.2016.1141928.

16. Nassé, G. *et al.* (2019) 'Nodulation and Agronomic Performance of Indigenous Rhizobia Isolated from Bambara Groundnut (Vigna Subterranean L.) Nodules in

Dalao, Côte d'Ivoire', *International Journal of Research Studies in Microbiology and Biotechnology*, 5(2), pp. 13–18. doi: 10.20431/2454-9428.0502004.

17. Ntambo, M. S. *et al.* (2017) 'The effect of rhizobium inoculation with nitrogen fertilizer on growth and yield of soybeans (Glycine max L.)', *International Journal of Biosciences (IJB)*, 10(3), pp. 163–172. doi: 10.12692/ijb/10.3.163-172.

18. Nyaga, J. W. and Njeru, E. M. (2020) 'Potential of Native Rhizobia to Improve Cowpea Growth and Production in Semiarid Regions of Kenya', *Frontiers in Agronomy*, 2, pp. 1–13. doi: 10.3389/fagro.2020.606293.

19. Raissa, G. N. K. *et al.* (2020) 'Molecular and symbiotic efficiency characterization of rhizobia nodulating Bambara groundnut (Vigna subterranea L.) from Agricultural Soils of Daloa Localities in Côte D'ivoire', *Int.J.Curr.Microbiol.App.Sci (2020)*, 9(7), pp. 507–519. doi: 10.20546/ijcmas.2020.907.056.

20. Sánchez-cañizares, C. *et al.* (2021) 'Competition , Nodule Occupancy , and Persistence of Inoculant Strains : Key Factors in the Rhizobium -Legume Symbioses', *Frontiers in Plant Science*, 12(690567), pp. 1–26. doi: 10.3389/fpls.2021.690567.

21. Santos, M. S., Nogueira, M. A. and Hungria, M. (2019) 'Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture', *AMB Express*, 9(1), pp. 1–22. doi: 10.1186/s13568-019-0932-0.

22. Thilakarathna, M. S. *et al.* (2019) 'Evaluating the Effectiveness of Rhizobium Inoculants and Micronutrients as Technologies for Nepalese Common Bean Smallholder Farmers in the Real-World Context of Highly Variable Hillside Environments and Indigenous Farming Practices', *Agriculture*, 9(20), pp. 1–17. doi: 10.3390/agriculture9010020.

23. Ulzen, J. *et al.* (2016) 'Bradyrhizobium Inoculants Enhance Grain Yields of Soybean and Cowpea in Northern Ghana', *Frontiers in Plant Science*, 7(1770),

pp. 1–9. doi: 10.3389/fpls.2016.01770.

24. Wilson, G. *et al.* (2021) 'Native Rhizobia Strains Enhance Seed Yield of Groundnut Varieties in Northern Ghana', *Frontiers in Agronomy*, 3(653044), pp. 1–10. doi: 10.3389/fagro.2021.653044.

25. Yakubu, Kwari and Ngala (2010) 'N 2 Fixation by Grain Legume Varieties as Affected By Rhizobia Inoculation in The Sandy Loam Soil Of Sudano-Sahelian Zone of North Eastern Nigeria .', *Nigerian Journal of Basic and Applied Science*, 18(2), pp. 229–236.

Appendices

Ethical clearance certificate

UNAM

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SOS-0099 Date: 18 July 2022

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: EFFECTS OF DIFFERENT RHIZOBIUM INOCULANT STRAINS ON BAMBARA GROUNDNUT SEED RHIZOSPHERIC NITROGEN LEVELS, NODULATION AND APICAL PLANTS GROWTH IN NAMIBIA

Student: FELICITAS FWANYANGA

Student Number: 201608437

Supervisor(s): DR. LYDIA HORN
PROF. TIMOTHY SIBANDA

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Questionnaire

Universität Bremen

UNAM

A research in partial fulfilment of the requirements for master of science. Program in biological sciences at the university of Namibia. Questionnaire on Bambara groundnuts production systems in the northern communal areas of Namibia

Bambara groundnuts (*Vigna subterranea (L.) Verdc*) production systems in the Northern communal areas of Namibia

Survey Questionnaire

A. General information   Date____________

Region_______________________   Constituency_____________________

Village/sublocation__________________

Name of respondent__________________   ☐M   ☐F

Age (range) _______________________

Number of Household_________

B. Bambara groundnuts farming systems

1. What is the approximate size of your farm?
   a) Less than 1 hectare ☐
   b) 2-5 hectare ☐
   c) More than 5 hectare ☐
2. On how many hectares do you plant Bambara groundnuts?
3. Do you plant any improved Bambara groundnuts varieties
   a) Yes
   b) No
4. If yes, what improved varieties do you plant- Names?
   a) Kahandje ☐
   b) Likorova ☐
   c) Mandonga ☐
   d) Mauta gharumbamba ☐
   e) All four ☐
   f) Any other than the list above?---

   ----
5. Why do you prefer the improved varieties
   a) Early maturing ☐
   b) High yielders ☐

1. Easy to handle ☐

2. Other point---

----

1. What is your average Bambara groundnuts yield approximately (bag = 50 kg)?

____

____

2. What are the main uses of the Bambara groundnuts you grow?

1. Home consumption

2. Animal feed

3. Income generation

4. Other ____________________________--

------

5. How many bags of Bambara groundnuts do you require for the family consumption every year?

1. Less than 5 bags

2. 6 to 10 bags

3. More than 10 bags

4. What type of food do you prepare from Bambara groundnuts ?----------------------
----

----

5. Do you sell your Bambara groundnuts?

a) Yes

b) No

6. If yes, in which form (wet or dry)?

----

7. Does seed colour play an important role in selecting a variety for you?

a) Yes

b) No

8. If yes, why--

----

9. What seed colour would you prefer and why?

1. Black
2. Brown
3. Red
4. Cream
5. Chocolate
6. Others?..............................

7. Reasons why you prefer those colours---

----

8. Farm inputs

   1. Do you apply fertilizer to your Bambara groundnuts?

      a) Yes

      b) No

   2. If no, why not?..

   3. If yes, what type of fertilizer do you apply and method of application?

Fertilizer	Quantity of bags	Method of application
Kraal Manure		
NPK		
Lime		
Basal fertilizer		
Other		

Options 1. Drilling 2. Broadcast 3. Other

4. How much fertilizer do you put per hectare? ____________________

5. How many times do you weed each growing season? _

   ____

6. What is the cost of labour per weeding? ____________________

1. Bambara groundnuts production Constraints

Bambara groundnuts production constraints Tick appropriately			
1	High cost of inputs	Fertilizers	
		Seed	
		Labor	
2	Storage pests	Weevils (*Callosobruchus maculatus*)	
		Larger grain borer	
		Moths	
3	Field pests	stem borer	
		Greenbugs	
		Aphids	
		Stem and stalk borers	
		Amanita	
4	Diseases	Downy mildew	
		Bacterial leaf spot	
		Rust	
		Stem rots	
		Storage rots	
	Parasitic weeds	Striga	
	Other	Birds	

5	Abiotic	Drought	
		Soil fertility and acid soil	
		Heat	
6	Policies	Low market prices	

2. Do you experience any Bambara groundnuts diseases on your farm during the growing seasons?

1. Yes

2. No

3. Which diseases are these?

___

___

___

4. To what extent do the diseases affect the Bambara groundnuts yield?

1. Significantly

2. slightly

3. Dismally

4. In which season do you see the symptoms?

1. Prolonged rains--

2. Short rains--

3. Both long and short rains--

4. Other (specify)---

5. In which season are the symptoms most severe?

1. Prolonged rains______________

2. Short rains______________

3. Both long and short rains

4. Other (specify)_____________

5. Do you control the diseases and pests?

1. Yes

2. No

Control measures		
1	Chemicals	
2	Rotation (cultural measures)	
3	Early planting (cultural measures)	
4	Timely weeding (cultural measures)	
5	Resistant varieties	
6	Others...	

3. How effective are the control measures that you use? tick one

1. Very effective ___________

2. Effective ___________

3. Not effective at all__________

4. I do not know

5. Does aphid attack the varieties you grow differently?

1. Yes

2. No

3. If so, which varieties are attacked most?

    1. Improved varieties

    2. Local varieties

Farmers' expectation from Sustainable solutions to Bambara groundnuts productions

4. In your opinion, what should be the focus of these research efforts on Bambara groundnut research?

____

____

____