Genetic diversity and nitrogen fixation in underutilized tropical legumes

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Thesis submitted in fulfilment of the requirements for the degree

Doctor of Philosophy in Biology

at the North-West University

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Graduation ceremony: July 2019

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DECLARATION

I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agriculture Sciences, Department of Microbiology, and the work contained herein are my original work with exception of the citations and that this work has not been submitted at any other University in part or entirety for the award of any degree.

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DEDICATION

This thesis is dedicated to my wonderful supervisors; Prof. Olubukola O. Babalola and Prof. Michael Abberton for carving a career pathway for me in the study of underutilized legumes.

ACKNOWLEDGEMENTS

I give all glory, honour, and adoration to Almighty God for his mercies and abundant grace in my life. The work presented in this thesis has been possible only through the contribution of many. I would like to express appreciation to management and staff of the Genetic Resources Center-IITA through the provision of a graduate research scholarship and North-West University for Institutional Bursary awards. Many good people at Genetic Resources Center contributed to my work in many ways, but special thanks are due to Dr Olaniyi Oyatomi for continuous interest, encouragement, and valuable discussions.

I thank my supervisors, Prof. Babalola, Prof. Abberton, Dr Aziz, and Dr Dianda, from whom I have garnered a wealth of knowledge. Your enthusiasm, insight, criticism, and encouragement were invaluable throughout my PhD journey. The roles played by Prof. Babalola during the fellowship application cannot be overemphasized. She provided the link for me to study in IITA. Prof. Babalola also provided all the needed financial, moral, and emotional support by visiting the field and laboratories to monitor the research activities in Nigeria. I shall be eternally grateful for your constant engagement. I cannot thank Prof. Abberton enough for guidance and ideas, also for always having 'regular meetings' for me even at the busiest times. Dr Aziz left mid-way for the United States but served as mentor and field assistant by helping with all field related activities including conveying us from the field to the office and providing office space and a desktop computer for me in the Soil Microbiology lab. I greatly benefited from his selfless research activism. Dr Dianda facilitated the field meeting with Prof. Ken Giller (author of the famous book on Nitrogen fixation in tropical cropping systems) and offered useful insights towards the improvements of the study. It was great pleasure to work with you all.During my PhD journey, I learnt so much from different individuals. Mr Ben Faloye (Field bank Manager) and Mr Sam Korie taught me experimental field design, mapping, and layout. Mr Ofodile taught me statistical

package using SAS and was also ready to help when I ran into confusion. Mr Adebowale and Mr Adekunle provided implementation support during the two years of field study with the kind assistance of the GRC screen house team. I would also like to thank Mr Eniola, Mr Williams, Ms Olaoluwa, Mr Nwachukwu, and Mr Innocent for teaching me how to analyze soil samples and conduct plant sample analysis and other microbiology related activities. At the Food and Nutrition Science Laboratory, Dr Maziya-Dizon, Mr Adesokan, Mrs Olaniyan, and Mr Akin provided useful assistance and technical expertise. At the Bioscience Center, Dr Andreas (IITA Bioinformatician) and Mrs Iwu taught me bioinformatics and molecular biology techniques. The brilliant people I was privileged to call my colleagues included Tesleem, Adenike, Moses, Sani, Khomotso, Comfort, Darkwa Kwabena, and Cobes. Special thanks to Dr and Dr (Mrs) Aremu for friendship, advice, and good homor and to Dr Olayiwola for their technical inputs into Chapters 4 and 7. Prof. Steven Bouillon of the Division of Soil and Water Management, KU Leuven provided training support on the use of 15N natural abundance technique in his laboratory. The staff at the Knowledge Center was very useful as well (Mr Louis, Mr Austin, and Mrs Ayinla) for providing regular space and other necessary assistance. I also appreciate the research support provided by the Communications Unit led by K. Lopez and her team including Mr Juba. Rev. (Dr) and Mrs Abiodun Aina provided decent accommodation in Akingbile, Ibadan, when I was in Nigeria. I appreciate the immense support provided by the Deputy-Director General, IITA, Dr May-Guri Saethre, Prof. Olawoye, and Mrs Badejo to the graduate fellowship program and by extension to the association I led.

Many people outside IITA helped me along the way. I would like to say a big thank you to peers in the Microbial Biotechnology Laboratory as well as to all the members and staff of the Department of Microbiology, the past and the present HODs, Prof. Emmanuel Mukvepho and Prof. Collins Ateba as well as all faculty and staff members of the Faculty of Natural and

Agricultural Sciences, North West University, for their immense support. My gratitude goes to my family in North West University, for their encouragement. Special appreciation is due to Dr Mobolaji Adegboye, (University of Waterloo, Canada), Dr Bernard Ojuederie (NWU, South Africa) and Dr Omotola Fashola (LASU, Nigeria) for reviewing the literature review and for other technical assistance. My thanks go also to Musanchi, Pule Pule, Sandra Horn, and Tedy for help with registration and bursary clearance among other things. My past and present teachers at the Olabisi Onabanjo University Ago-Iwoye and University of Lagos Nigeria were very supportive of my plans to study abroad. I thank Prof. Odunaike (Dean of Science, OOU), Prof. Adebajo, Prof. Efuntoye, and Dr Agunbiade who provided with visa support. Comrade Egberongbe (SSANU Chairman) and Dr Egberongbe also provided relocation assistance. Dr and Dr (Mrs) Gboyega Ilusanya; and Dr Adesetan provided additional Naira and Rand for use at the airport. The supportive roles provided by Prof. Sade Ogunsola (Deputy Vice-Chancellor) and Dr Tenny Eqwuatu at the University of Lagos cannot be overemphasized. The exemplary academic mentorship support provided from the high school to university days by the Academic Excellence Initiative (AEI) is worthy of mention. Mr Ayilara, Prof. Adedeji (Uganda), Dr G. Olumuyiwa (Abuja), Engr. Faheed, Dr Bello (USA), Mr Tijani, Pharm. Bakare and Pharm. Abideen. Last, I salute the cooperation of my family during my study, and my Dad, Engr. Olasunkanmi for his constant inspirational pieces of advice. Emotional support was provided by Fehintoluwa, Modupeoluwa, Toyyibah, Imam Nasir, Tunde, Isiwat and other siblings from the Adegboyega, Akanni, and Adelani families for always being there for me. I specially appreciate my wonderful treasure Omolola during my long absence from home and kind assistance to keep the priorities straight and maintain a proper perspective. I owe you all big time.

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GENERAL ABSTRACT

Legumes in some cases are underutilized and form only a relatively small proportion of human diets. In general they fix atmospheric nitrogen which may provide an economic advantage for smallholder farmers. By appropriate utilization of legumes, food security and soil fertility can be significantly achieved. During the 2016/2017 and 2017/2018 cropping seasons at the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria, field and laboratory experiments were conducted to determine the genetic diversity and nitrogen fixation of two underutilized tropical legumes, winged bean (*Psophocarpus tetragonolobus* (L.) DC.) and African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms). Twenty-five accessions of each crop were used for these experiments without rhizobia inoculation or N fertilization. In each season, randomized complete block design (RCBD) was used for the filed experiments in three replications. This study confirms characters that can be used to improve African yam bean germplasm include dry pod weight, number of seeds per pod, leaf rachis, terminal petiole length, and seed length. African yam bean fix N and nodulate with indigenous soil bacteria. TSs77 fixed the highest amount of N at 22.47 kg ha^{-1} followed by TSs30 at 20.91 kg ha^{-1} and TSs101 at 19.80 kg ha^{-1}. These top three accessions can be identified for breeding programs as superior N-fixing accessions. The protein content of the accessions showed significant differences. For instance, TSs104 had the highest protein content of 25.08%; followed by TSs76 (24.82%), TSs1 (24.52%), TSs4 (24.31%), and TSs67 (24.24%) while the accession with the lowest protein contents in the processed seeds was TSs30 (22.02%). However in the unprocessed seeds, protein content ranged between TSs38 (24.93%) and TSs11 (19.13%). Other proximate analyses evaluated showed differences among the accessions; there were reductions in the unprocessed seeds for phytate and tannin contents. Evidence of the nutritional content of these crops as observed in this study implied that they can be utilized in various dishes for adults and children, to reduce malnutrition in sub-Saharan Africa. No rhizobia were isolated but other isolated root nodule-associated bacteria

were analyzed using morphological, biochemical and 16S rRNA. The molecular analysis revealed the presence of *Kosakonia oryzae; Enterobacter asburiae*; *E. cloacae*; *Ralstonia pickettii; Variovorax* sp. and *Hydrocarboniphaga effuse*. The specific roles of these associated bacteria were not ascertained but previous reports suggest they may assist in plant growth and development. The $\delta^{15}N$ signatures of the legume differed among accessions and varied from 2.52 (TSs61) to 0.24 (TSs44) in the shoots and from 2.70 (TSs98) to 0.82 (TSs16) in the roots. Significant differences were recorded among the reference plants used for estimating the percentage N derived from the atmosphere (Ndfa) of African yam bean shoots. TSs76 had the highest Ndfa of 66.73%, 51.83%, and 63.48% followed by TSs4 with 66.18%, 51.03%, and 62.87% while the lowest was TSs1 with 40.07%, 13.22% and 34.21% when *Eleusine indica*, *Zea mays,* and *Tridax procumbens* were used respectively for estimation. The $\delta^{13}C$ values of shoots were much greater (i.e., less negative) while the values for the roots also varied considerably. Consequently, the $\delta^{13}C$ values of African yam bean shoots ranged from -31.49 (TSs98) to -30.93 (TSs4) and from -31.16 (TSs68) to -30.20 (TSs4) for the roots. The observed variation indicated differences in water-use efficiency among the accessions. The carbon and N ratio (C/N) values were lower than 24 gg^{-1} and the reference plants had over 24 $gg^{1.}$ These outcomes support the opinion that photosynthetic activities in the underutilized legume were stimulated by N nutrition. TSs44 has a significantly higher number of nodules than other accessions at 169.67 while TSs23 had the lowest number at 58.42. The use of ^{15}N natural abundance method in determining N fixation and water-use efficiency in African yam bean is the first report to the best of my knowledge.

The winged bean accessions evaluated possessed the potential to fix nitrogen and also nodulated with indigenous soil bacteria. GCV were high for pod length, dry pod weight, estimated number of seeds per pod, total number of seeds and seed weight. The high GCV suggests that these characters can easily be selected for improvement. In the processed seeds,

Tpt17 had the highest protein content of 40.30%, followed by Tpt11 (39.72%), Tpt43 (39.35%), Tpt15-4 (39.21%), and Tpt4 (38.88 %); the lowest was recorded in Tpt48 (34.18%). In the unprocessed seeds, Tpt17 also recorded the highest crude protein content at 31.13%, followed by Tpt4 (31.02%), Tpt15-4 (30.84%), and Tpt42 (30.62%) while the lowest was contained in Tpt125 (28.43%). Other proximate composition analyses suggested that winged bean could serve as a complementary item in human diets and animal feed. In the swollen roots (tubers) and seeds, processing was observed to lower the levels of anti-nutrients. The $\delta^{15}N$ values of winged bean showed great differences among the accessions and varied from 3.34 (Tpt18) to 0.86 (Tpt3-B) in the shoots and from 3.07 (Tpt15) to 0.49 (Tpt32) for roots. Among the reference plants used for estimation the percentage Ndfa of winged bean shoots also varied significantly between 66.12% (Tpt3-B) and 24.3% (Tpt18). Differences were seen in the estimation of the roots. The amount of nitrogen fixed differed significantly ($p \leq 0.05$) among accessions. The amount fixed (kgN ha^{-1}) in the shoots varied among the accessions with Tpt32 fixing 27.16 kg $ha^{-1,}$ followed by Tpt15-4 at 25.66 kg ha^{-1} and the accession fixing least was Tpt30 that measured 9.02 kg ha^{-1} with a considerably lower amount fixed in the root. Variation exists in the carbon and N ratio among the winged bean accessions studied when compared with other parameters analyzed. Overall, the C/N ratio for the shoots ranged from 15.87 (Tpt51) to 11.97 (Tpt32) and from 18.33 (Tpt12) to 17.83 (Tpt53) for the roots. The $\delta^{13}C$ values of winged bean shoots ranged from -30.60 (Tpt48) to -29.62 (Tpt19) and from -30.17 (Tpt53) to -19.19 (Tpt6) for the roots. The values obtained showed these accessions were generally stable in their expression of water-use efficiency. Winged bean root nodule-associated bacteria isolated from winged bean roots were *Enterobacter asburiae*; *E. bugandensis*; *E. cloacae*; *Enterobacter* sp; *Enterobacteriaceae bacterium; Pseudomonas cremoricolorata* and *P. fluorescens. Others are P. montellii; P. putida; Kosakonia oryzae; Ralstonia* sp*;* and an uncultured bacterium clone. Rhizobia

recovered from winged bean nodules include *Rhizobium mayense, R. multihospitium, R. pusense,* and several other *rhizobia* sp. The rhizobia isolated have been previously confirmed as playing key roles in nodulation and N fixation. This outcome reveals the importance of incorporating legumes in tropical agriculture for crop intensification. Finally, the study provides evidence that African yam bean and winged bean accessions can be improved in a pre-breeding program with respect to the following traits; N-fixing potential, nodulation capacity, proximate and anti-nutritional composition, and diversity in bacteria nodulating the roots..

LIST OF PUBLICATIONS

Chapter 2: Positioning African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) as a valuable crop.

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M.; Oyatomi, O.A.; and Babalola, O.O. *This chapter has been formatted for publication in Global Food Security*

Candidate's Contributions: designed the study, managed the literature searches, and wrote the first draft of the manuscript.

Chapter 3: Increasing productivity of the underutilized winged bean in tropical agriculture: Prospects for Interdisciplinary research.

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M; Oyatomi, O.A., and Babalola, O.O. *This chapter has been formatted for publication in Plant Genetic Resources*

Candidate's Contributions: designed the study, managed the literature searches, and wrote the first draft of the manuscript.

Chapter 4: Genetic variability for seed yield components character in African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) and winged bean (*Psophocarpus tetragonolobus* (L.) DC.). *This chapter has been formatted for publication in Plant Genetic Resources.*

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M.; Oyatomi, O.A.; and Babalola, O.O.

Candidate's Contributions: managed the literature searches, did all the wet laboratory bench work, field work, performed all the analyses, interpreted the results, and wrote the first draft of the manuscript.

Chapter 5: Nodulation, Nitrogen fixation, and water-use efficiency in African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) *This chapter has been formatted for publication in Plant and Soil.*

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M; Oyatomi, O.A., and Babalola, O.O.

Candidate's Contributions: managed the literature searches, did all the wet laboratory bench work, field work, performed all the analyses, interpreted the results, and wrote the first draft of the manuscript.

Chapter 6: Nodulation, Nitrogen fixation, and water-use efficiency in winged bean (*Psophocarpus tetragonolobus* (L.) DC.) *This chapter has been formatted for publication in Agriculture, Ecosystems, and Environment.*

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M; Oyatomi, O.A., and Babalola, O.O.

Candidate's Contributions: managed the literature searches, did all the wet laboratory bench work, field work, performed all the analyses, interpreted the results and wrote the first draft of the manuscript.

Chapter 7: Morphological description, proximate and anti-nutritional composition of raw and processed seeds of underutilized tropical legumes.

This chapter has been formatted for publication in Journal of Food Security

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir, A.H.; Dianda, M; Maziya-Dixon, B; Oyatomi, O.A; and Babalola, O.O.

Candidate's Contributions: managed the literature searches, did all the wet laboratory bench work, performed all the analyses, interpreted the results, and wrote the first draft of the manuscript.

Chapter 8: Isolation and characterization of nodule associated bacteria in underutilized tropical legumes.

This chapter has been formatted for publication in Phytobiomes

Authors: Adegboyega, T.T.; Abberton, M.T; AbdelGadir A.H.; Dianda, M; Oyatomi, O.A.; and Babalola, O.O.

Candidate's Contributions: managed the literature searches, did all the wet laboratory bench work, field work, performed all the analyses, interpreted the results and wrote the first draft of the manuscript.

LIST OF ABBREVIATIONS

BNF----------Biological nitrogen fixation

N----------Nitrogen

C----------Carbon

P----------Phosphorus

GA----------Genetic advance

GCV----------Genotypic coefficient of variation

PCV---------- Phenotypic coefficient of variation

GAM----------Genetic advance as percentage of mean

GRC----------Genetic Resources Center

RCBD----------Randomized complete block design

IITA----------International Institute of Tropical Agriculture

AYB---------- African yam bean

WB----------Winged bean

PCR----------Polymerase chain reaction

CHAPTER ONE

General Introduction

1.1 Introduction to this chapter

The relevance of legumes in sustainable cropping systems has been extensively studied (Siddique *et al*., 2012). Intercropping or rotation of grain legumes with cereals or other non-leguminous crops has many benefits including enhanced yield, increased efficiency of nitrogen (N) use, reduced occurrence of disease, and in some cases improved access to other essential elements such as phosphorus (Foyer *et al*., 2016a). The N-fixing ability of legumes affords opportunities for natural soil fertilization. Grain legumes can reduce greenhouse gas emissions in agricultural cropping systems. About 21 Mt of nitrogen is fixed annually by crop legume–rhizobia symbioses (Foyer *et al*., 2016b) returning 5–7 Mt of nitrogen to soils from about 190 million ha of grain legumes and saving US$8–12 billion (Reeves *et al*., 2016).

Consequently, N-fixing legumes provide opportunities for reducing future nitrogen use. The inclusion of grain legumes in cropping systems can enhance annual productivity as well as increasing diversity in cropping systems, thereby reducing reliance on a cereal monoculture (Malik *et al*., 2016).

The cultivation of legumes is a very promising way for resource-poor farmers to increase income, especially when the comparatively low input cost compared with cereals is considered (Siddique *et al*., 2012). For example, a formal survey of farmers in Bangladesh indicated their awareness of the economic advantages of using integrated crop management practices for chickpea (Foyer *et al*., 2016b).

Agricultural lands in Africa have been experiencing continuous degradation with considerable effects on the gross domestic product estimated at 18% (Nkonya *et al*., 2016) owing to nutrient depletion and poor agronomic practices (Tittonell and Giller, 2013). Nitrogen limits crop productivity and consequently food production. The use and production of N fertilizers in global agriculture is expensive for the growth of crops (Mus *et al*., 2016). There are potential benefits to be derived from reducing dependence on N fertilizers in agriculture in both developed and developing countries as well as renewed interest in research on biological nitrogen fixation (BNF) and prospects for increasing its importance in agriculture (Mus *et al*., 2016).

Global food insecurity and increases in food prices are consequences of the inability to maintain global food production in line with population growth (Abberton *et al.*, 2016). Many of the less-studied crops, such as African yam bean *(Sphenostylis stenocarpa*), bambara groundnut *(Vigna subterranea),* winged bean *(Psophocarpus tetragonolobus)* and pigeon pea *(Cajanus cajan),* have prospects in terms of distinctive nutritional profiles and environmental adaptations when compared with widely cultivated crops. Therefore, investigations into these minor crops (otherwise known as "underutilized crops") have the potential to advance crops and crop varieties that could be applied in future agriculture and crop breeding for improved varieties devoid of the current limitations with available landraces for health and nutritional security (Varshney *et al*., 2010).

Therefore, traditional crops have a role to play in most smallholder farms in Africa owing to the fact that most indigenous crops are capable of growing well on eroded land, leached, or marginal soils. African yam bean is a crop with considerable nutritional potential but currently faced with poor awareness about its taxonomy, agronomy,

genetics, medicinal value, and productive potential of the crop may be partly due to limited research on it. The subsistence nature of its production may have been occasioned by its limited acceptability as a valuable crop among middle-aged farmers in Africa. Research information on the crop is patchy in old and poorly accessible literature (Daniel and Celestina, 2013).

Winged bean is also a tropical legume found growing abundantly in hot and humid equatorial countries including India, Burma, Sri Lanka, Thailand, and the Philippines (Vatanparast *et al.*, 2016). It is also called a wonder legume as it has high protein content in the seeds and is considered as versatile. Seeds of winged bean contain some pharmacologically active anti-nutrients such as trypsin and chymotrypsin inhibitors, haematoglutins and amylase inhibitors which may have adverse physiological effects when consumed by humans or animals (Mohanty *et al*., 2013).

Diversity of diets based on different crops assures better nutrition and greater health with additional benefits for human productivity and livelihoods. Underutilized crops which rely on the biological functioning of the ecosystem require a low input of synthetic fertilizers, pesticides, and irrigation, and could be promoted as an alternative for ensuring food and nutritional security particularly in Africa (Modi and Mabhaudhi, 2013). However, information is scarce on nitrogen fixation among landraces in areas where the crops are grown.

1.2 Problem Statement

African yam bean and winged bean are tropical legumes largely considered orphan crops (Subuola *et al*., 2012). They both have untapped potential for improvement both in quantity and quality of production. Little work has been carried out on morphological characterization and none on the nitrogen fixation of landraces conserved at the Genetic Resources Center (GRC) of the International Institute of Tropical Agriculture (IITA) collected from different

parts of sub-Saharan Africa. Policymakers, the international and national research communities, and industries have not invested in the improvement of these crops. Small-holder farmers are losing interest in cultivation, owing in part to a number of agronomic limitations. Both legumes may face being phased out of cultivation due to high values placed on other legumes such as soybean, cowpea, and groundnut. Hence, they receive little research and commercial attention in tropical countries (Ndidi *et al*., 2014). Therefore, to contribute towards filling some of the research gaps, this study was conducted to determine the variation in nitrogen fixation and nodulation; the diversity of root nodulating bacteria; evaluation of the nutritional composition and anti-nutrients present in the seeds and swollen roots, as well as exploration of the genetic variability for component characters in seed yield. The data generated will be useful in a pre-breeding program of the crops for optimal utilization.

1.3 Justification

The current global reliance on a small number of staple crops has its own economic, ecological, nutritional and agronomic risks and limits the potential contributions of underutilized tropical legumes such as African yam bean and winged bean. The potential uses of BNF cannot be overemphasized in the face of the current high application of synthetic N fertilizers and global population growth. Reports has it that synthetic N fertilizers provide less than fifty percent of present crop yields and will need to be increased with projected population increase; high cost of purchase, high energy cost of production, negative environmental impacts (loss of biodiversity, eutrophication, and run-off into water bodies) (Erisman *et al*., 2008; Heffer and Prud'homme, 2015; Ladha *et al*., 2016; Nejat *et al*., 2015; Storkey *et al*., 2015). The prospects of the socio-economic gains through a boost in the production and utilization of underutilized legumes are huge. Consequently, public perception of the advantages to health and wellbeing of diets rich in underutilized legumes

may be a significant factor of cultural modification in considering African yam bean and winged bean as a key to food security.

1.4 General Objective

The aim of this study is to determine the genetic diversity and variation in nodulation including yield, N-fixing potential, and nodule-associated bacteria of different accessions of African yam bean and winged bean.

1.4.1 Specific Objectives

This study is aimed at achieving the following objectives:

1. To analyze the genetic variability for seed yield and component characters in African yam bean and winged bean.
2. To compare the nutritional composition and anti-nutritional factors of African yam bean and winged bean seeds and tubers.
3. To analyze the diversity of nodule-associated bacteria isolated from African yam bean and winged bean.
4. To determine the variation in terms of biological N-fixing and nodulation potential of African yam bean and winged bean landraces.

1.5 Research Questions

1) What is the nature and extent of variation of African yam bean and winged bean for seed yield and component characters?

2) What is the nutritional and anti-nutritional make-up of African yam bean and winged bean seeds and tubers?

3) What are the indigenous soil bacteria in African yam bean and winged bean with the potential to fix nitrogen?

4) What is the extent and nature of N-fixing ability of African yam bean and winged bean landraces?

CHAPTER TWO

Positioning African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) as a valuable crop for the future of agriculture in sub-Saharan Africa

Abstract

Several crops in Africa with the potential to reduce the challenges of malnutrition are currently largely overlooked by scientific research and remain underutilized. African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) is a food crop with nutritional, medicinal, and economic value. Limitations with respect to this crop include knowledge of agronomic value, inadequate understanding concerning factors important for tuber formation, and low awareness about its use. Factors limiting use include prolonged cooking time for the seeds and the presence of anti-nutritional components in the seeds and swollen roots. Studies are required to bridge knowledge gaps so identified. There is also need to gain greater knowledge of BNF in this crop and its contribution to soil-N economy. It may be a crop for the coming generations due its inherent traits if utilized to address food insecurity and feed the increasing population in sub-Saharan Africa.

Keywords: African yam bean, nitrogen fixation, genetic diversity, sub-Saharan Africa.

2.1 Introduction

In Africa, several challenges work against food security. These include low soil fertility, high cost of fertilizers, and low uptake of improved varieties (Jiri *et al*., 2015). At the same time, the inability to support global agriculture food production with the rising population is seen by unstable food prices (Abberton *et al*., 2016). Traditional crops may play important roles in the profitability of many smallholder farms in Africa. This is due to the fact that most indigenous crops are capable of growing well on eroded land, leached, or marginal soils. African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms) is a crop with untapped potentials. There is very limited information on its overall benefits to humans and animals.

The role played by nitrogen in agriculture cannot be overemphasized. It is an essential nutrient, a principal component of nucleic acids and a part of chlorophyll. It greatly affects yields, the pattern of growth of plants, and their chemical composition (Amoo and Babalola, 2017). Some economic benefits can be derived from reduced use of nitrogenous fertilizers in agriculture. Renewed support for the use of BNF and its importance in agriculture is a step in this direction. BNF is the translation of nitrogen from the atmosphere to ammonia in the form that can be assimilated by plants (Mus *et al*., 2016). Many underutilized crops do not need a high input of fertilizers or pest control as well as irrigation, and may be suitable for advancement in some areas of the world, particularly in sub-Saharan Africa (Modi and Mabhaudhi, 2013).

In plant roots, bacteria live in the root outgrowths called nodules. It is within the nodules that they fix nitrogen and the plant absorbs the ammonia. It has been established that nodulation is also one of the best developed models of symbiotic association (Tellah *et al*., 2016). Legumes

in partnership with the rhizobia can fix considerable amounts of nitrogen from the atmosphere. The rhizobium-legume symbiosis can therefore increase yield and has also proven useful in improving soil fertility (Nigam and Xaxlo, 2017).

There is little information on the N fixing abilities of African yam bean landraces and their contribution to crop productivity in areas where the crop is grown. The N budget of the crop through BNF is not known. This review makes available information on the current position of the crop aimed at increasing its utilization and improvement. A previous review on African yam bean (Adewale *et al*., 2012) focused on cultivation, distribution, morphological traits, genetic diversity, and pest management. This effort, without overlooking these aspects, explores BNF, utilization as food, market values, and the conservation of genetic resources.

2.2 African yam bean: underused

In the world today an estimated 800 million people are suffering from malnutrition (McGuire, 2015). The majority of these individuals are from developing countries and are mostly rural dwellers (Chivenge *et al*., 2015; McGuire, 2015). Genetic erosion and the loss of plant bio-cultural diversity are being experienced owing to the over-dependence of agricultural systems on a very few crop species (Stamp *et al*., 2012). Therefore to ensure that the diets consumed by the populace are balanced there is a need for improvement in the living conditions for farming communities fortified with stronger farming systems. At the same time, the appropriate use of agricultural biodiversity will be a step towards food security, advancing the sources of nutritious foods and promoting sustainable agriculture (Ayenan and Ezin, 2016).

Neglected and underutilized crop species suffer conservation challenges. Farmers that show interest in the production of these species have limited knowledge on their economic potential, processing, and other related utilization concerns (Alozie *et al*., 2009). The ability

is known of these species to adapt to low-input system of farming and could provide a significant contribution to creating a food surplus in various parts of the globe (Nnamani *et al*., 2017b). One of these crop species is African yam bean. It is an annual legume with the capacity to produce seeds that are produced in a pod with varying patterns and colors (Asoiro and Ani, 2011). Other than the seeds, farmers can also harvest swollen roots often referred to as tubers which resemble sweet potato. The swollen roots that come in various forms (Adewale and Dumet, 2011) have high nutritional composition and mature after five to eight months (Chinedu and Nwinyi, 2012). Omeire (2012) reports significantly high levels in the content of amino acids (lysine, histidine, arginine, and valine) in the seeds.

2.3 Nitrogen fixation, soil fertility status and African yam bean

In a study examining the effect of some indigenous legumes on soil physico-chemical status using an-Anultisol soil type in South-East Nigeria, it was found that African yam bean improved fertility levels while maize depleted soil fertility (Anikwe and Eze, 2010). Fertility remained virtually stagnant in the plots left bare. However, African yam bean improved soil properties (for example, organic carbon, ammonium, and nitrate) more than other legumes such as *Mucuna cochinchinensis* and *Cajanus cajan*. This led to an increase in both grain yield and stover weight of maize in plots where African yam bean had been planted in the previous season. In addition, growth parameters of the subsequent maize were highly improved. The study therefore recommended that indigenous legumes, especially African yam bean, be used by maize growers and mostly smallholder farmers in South-East Nigeria. The result also showed that it can be used in relay cropping or incorporated as green manure in ultisols especially when maize or other cereal crops are to be subsequently planted (Anikwe and Eze, 2010).

The N-budget of African yam bean and the contribution of BNF are not yet known. Currently, progress is being made to understand the capacity of the landraces to fix nitrogen

with the aim of improving yields. Despite this, yields still remain low on most fields compared with other legumes such as cowpea in Africa (Muthuri, 2013). Methods that may enhance BNF should be adopted such as the use of microbial inoculants, etc., (Mathenge, 2017).

Furthermore Tettey (2014) reported that data are sparse on fixation and nodulation with naturally occurring strains of rhizobia except for the highly popular legumes. There are several factors which have not been well studied with respect to the capacity of African yam bean to fix N and improve soil fertility (Tettey, 2014). There is also a need to ascertain the true potential of BNF and its contribution to soil-N fertility management in ensuring a sustainable environment with this crop.

2.4 Effects of fertilizer application on African yam bean

Tettey (2014) states that N fertilization has inhibitory effects on the ability of the crop to nodulate and fix nitrogen. Nodule number decreased sharply in fertilized soils in Ghana. The low quantity fixed with supplementary N may mean that the crop did not tolerate mineral N and fix more N for its use. In another study conducted in Makurdi, North-Central Nigeria, Ogbaji (2016), reports that irrespective of the rate of N application (0, 45, 90 kg/ha) on African yam bean, growth and yield were enhanced. He also reports a significant relationship between fertilizer and accession ($p \leq 0.05$) plant shoot and height at 4, 6, and 8 weeks after planting. The conclusion was that N fertilization has a considerable effect on the crop's yield as well as growth (Ogbaji, 2016).

2.5 Geographical distribution of African yam bean

African yam bean is known to tolerate a wide range of geographical, climatic, and edaphic ecologies (Daniel and Celestina (2013). The extent of the environment where it is found lie

within the latitudes of 15º North to 15º South and the longitudes of 15º West to 40º East of Africa. Available evidence supports the view that Africa is the crop's origin (Fig. 2.1).

Figure 2.1: The centre of diversity for African yam bean

Source: Daniel and Celestina (2013)

Presently, there is considerable information on morphological, molecular assessment for yield (Adewale *et al*., 2012; Adewale *et a*l., 2010; Daniel and Celestina, 2012), biochemical profiles (Arogundade *et al*., 2014; Rapport and Lee, 2003)), physico-chemical seed quality (Olisa *et al*., 2010) and genetic diversity but not in all areas identified in Figure 2.1.

2.5.1 Cultivation

Literature reports show that the crop was introduced to Ghana in 1958 from Togo, a neighboring country (Adansi, 1975). Farmers in the region indicated that it had been grown well before this date with several uses among the population and no wild relatives existed (KIu *et al*., 2001). Several landraces are grown in the Nkwanta and Ho West Districts particularly with the use of multi-colored seeds. The crop is usually found in association with other crops without fertilizer application (KIu *et al*., 2001).

In various locations in Benue State, southern Guinea savanna zone of Nigeria, several varieties of African yam bean are grown ranging from Makurdi (brown), Oju (Light grey), Gboko (red), to Ado (golden stripe) (Ogbaji and Okeh, 2016). In addition, it is cultivated in various States in South-East and South-South Nigeria (Nnamani et al., 2017b) and other locations in South-West Nigeria (Adewale *et al*., 2015). The crop has a plethora of names in local communities where it is found particularly in Ghana and Nigeria (Nnamani *et al.*, 2017b).

Figure 2.2: African yam bean on the field

Photo: T.T. Adegboyega, IITA (2017)

Figure 2.3: Different pod sizes of African yam bean

Photo: T.T. Adegboyega, IITA (2017)

2.6 Nutritional composition, anti-nutritional factors, and processing

2.6.1 Nutritional composition

The most prominent of all the constraints to African yam bean utilization is low awareness of its full potential. In studying the seeds of nine different varieties, it was confirmed from the varying levels of protein content (28.63-30.43%) among the varieties studied that the crop can be used by adults and infants as a good substitute for animal protein (Abioye et al., 2015b). A few other studies also reported significant differences in the nutritional composition of the seeds (Ajibola *et al*., 2016; Igbabul *et al*., 2015; Ndidi *et al*., 2014).

Figure 2.4: Seeds of different landraces of African yam bean

Photo: T.T. Adegboyega, IITA (2017)

If utilized in this manner, it will reduce the heavy demand on other legumes. The high contents of protein (28.63-30.43%), carbohydrate (50.80-53.57%), and crude fiber (2.40-3.03%) could make the crop well positioned in reducing protein deficiency. Policymakers and nutritionists could incorporate the seeds for various product developments (Abioye et al.,

2015a). Previous study showed significant differences in the mineral composition of the seeds and tubers. The seeds had a higher percentage of magnesium in TSs148 (788.9±10.3), followed by potassium in TSs19 (770±10.3) with iron content in TSs41 (28.5±0.1) being the lowest. Calcium levels were highest in AYB34 (70.1±0.4). Phosphorus levels were not significantly different in accessions AYB70B (250±0.0), TSs152 (255±7.1), and TSs66 (255±7.1). In the tubers, the calcium content was significantly highest in AYB45 (51.6±1.1) and phosphorus was lowest in AYB45 (1.5±0.0) (Ojuederie and Balogun, 2017b).

The swollen roots are also rich in protein (15.5%) and minerals such as: potassium, magnesium, and iron which are essential to humans. Ojuederie and Balogun (2017a), suggested that the swollen roots (tubers) could be used as a staple food in West Africa where only the seeds are currently consumed as they are highly nutritious. Advocacy should therefore be made on the nutritional contribution as an important driving factor in promoting the value of African yam bean.

2.6.2 Anti-nutritional factors

Anti-nutrients are natural or synthetic compounds that interfere with the absorption of vitamins and minerals; these are found in some foods particularly grains and legumes. They may block the pathway of digestive enzymes which are necessary for nutrient absorption. Common examples of anti-nutrients studied in the crop include oxalate, saponin, phytate, and alkaloids. Others are tannin, trypsin, and hydrogen cyanide (Ajibola and Olapade, 2016b). In a recent study of nine varieties of the crop, very low content of tannin was recorded in AYB 95 (6 mg/100 g) as against 18 mg/100 g of tannin content obtainable in the seeds of other varieties. Suggestions have been made that processing could reduce the level of anti-nutrients to levels which may not be harmful to humans and animals. Adequate seed manipulation has been recommended as a means of eliminating or lowering anti-nutrients to a level that can be

tolerated when consumed. For example, procedures such as heating, soaking, or fermentation can be employed.

2.6.3 Food and market values of African yam bean

Several food products can be made from the seeds. The assessment of the socio-economic situation of the traders in the five States of South-East Nigeria showed that a relatively high income could be obtained from the sale of products, seeds, and the array of foods derived from African yam bean. The highest income of US $180 equivalent to ₦64, 980 was generated monthly, particularly in Abia State, from the sales of prepared food, sold in the open markets and from sellers along the highway; the lowest income was recorded from Ebonyi State. This amount derived is more than three times the amount paid to workers as the minimum wage (US$49 or ₦18,000) by the Federal Government of Nigeria (Nnamani *et al*., 2017b). Some African smallholder farmers make use of indigenous plants as a source of income between cropping seasons as well as for food sustainability. In South-eastern Nigeria, it has been established that potential sources of a high level of nutrients can be obtained from these plants to help in maintaining good health. They are positioned to reduce poverty and build a prosperous life (Nnamani *et al*., 2017a).

2.7 Threats, conservation status, and post-management

2.7.1 Threats

African yam bean has suffered neglect in many of the areas where it was previously grown. In Nigeria, for example, it is gradually disappearing with several factors identified for its being abandoned. These include the need to use stakes as it has been confirmed Ogah (2013) that the crop performs better when staked and planted earlier in May of each planting season with a greater seed/tuber yield than from those that were not staked and were planted later either in June or July of each season. Others are intensive labor, reduced market value, low seed yield, and non-availability of improved varieties. Therefore, we may be right to

conclude that the crop is undergoing genetic erosion. There are no current statistics of the cultivation and utilization of the crop in the Northern and South-western agro-ecological zones of Nigeria and other growing areas in Africa.

2.7.2 Conservation status

Currently, the GRC of (IITA has been able to collect and conserve only about 200 accessions. Other organizations in Nigeria have limited germplasm collections namely, the National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan; Institute of Agriculture and Research (IAR &T), Ibadan; Ebonyi State University Abakaliki, and Germplasm Screening Laboratory, Obafemi Awolowo University, Ile-Ife. This is a significant factor limiting the promotion and improvement of the crop's productivity (Normah *et al*., 2012). Appropriate investment in the release of improved varieties is a viable option for increasing productivity (De Donato *et al*., 2013). There is an urgent need to embark on a wide collection of new germplasm followed by characterization and evaluation in its known center of diversity in Africa.

2.8 Genetic diversity

Few studies have analyzed the genetic variation in African yam bean. Seed coat colors and its pattern alone cannot be adequate as means of classification (Babasola, 2011). Therefore, the classification based on phenotypic description may have limited information but current characterization does not look only at seed coat color and pattern. In a study Ojuederie *et al*. (2015a), variations were reported in the strength of more than 40 traits in distinguishing among accessions studied. The result showed significant differences in the traits studied (over a period of two years (2011-2012) and that some of the traits could be important for yield improvement (Ojuederie *et al*., 2015a). Molecular techniques have been employed to analyze intra-specific diversity, e.g., random amplified polymorphic DNA (RAPD) technique (Moyib

et al., 2008); amplified fragment length polymorphism (AFLP) (Ojuederie *et al*., 2014) and simple sequence repeat (SSR) cowpea derived (Shitta *et al.*, 2016). The RAPD primers assessed diversity in 24 accessions from Nigeria, Five AFLP primers revealed the genetic diversity among 80 accessions from Nigeria and other countries revealing different levels of information.

Variation also exists in the shapes of tubers which include round, oval, spindle, and irregular. The identified wide genetic base of African yam bean is a promising factor for genetic gain in an integrated breeding platform. Consequently, molecular characterization of germplasm collections and the use of important agronomic traits could be employed in marker trait association studies to facilitate the selection of promising genotypes.

2.9 Improvement of African yam bean productivity: potential for yield increase

There is little information on the physiology of tubers and the factors affecting their formation in the crop. Therefore, there is a need to investigate whether environment and other factors such as soil types and the effects of moisture and temperature have specific roles to play within the different accessions. Why do some accessions produce tubers and others do not? What is the average amount of the tuber population per accession? Other important data on swollen root formation that may be useful in understanding the physiology include the number of tubers, weight, width, length to width ratio, shapes, skin color, and branching as well as the nutritional composition. Up till this point, there has been no breeding program aimed at boosting the research potential of African yam bean for various aspects of selection intensity, accuracy, and genetic variance. A way out of this challenge will require increase funding from states, donor agencies, and research consortia on orphan crop promotion (Stamp *et al*., 2012).

2.10 Conclusion and prospects for the future

This review sheds light on the potential of the crop, ways to promote its cultivation in the growing areas, and the role of BNF. Identifying African yam bean accessions with high N fixing ability across a wide range of environments could be a key finding as an environmentally friendly and low-cost strategy towards improving its germplasm productivity, utilization, and establishing low-input cropping systems. In addition, identification of genomic regions, and ultimately gene (s), conditioning BNF in African yam bean can be useful for breeding programs targeted at improving nitrogen fixation. It may be a worthy adventure in Africa to properly collect, characterize, and conserve available germplasm. Furthermore, public support should be sought in breeding platforms to contribute to the development of improved varieties. Accessions which have high N-fixing capacity could be used for land reclamation and their significantly wide adaptability may be additional qualifications for improved utilization. The crop holds promise of a bright future in ameliorating poverty and nutritional deficiencies among Africans. To achieve these ends, attention must be directed towards research and promotion.

CHAPTER THREE

Increasing productivity of the underutilized legume winged bean (*Psophocarpus tetragonolobus* (L.) DC.) in tropical agriculture: prospects for interdisciplinary research

Abstract

Despite the increasing search for cheap sources of protein-rich foods worldwide, research attention has not been focused on underutilized legumes whose seeds contain high amounts of protein and other vital mineral constituents. Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is a food crop that can be used in addition in the commonly available diets of the vast majority of people. The present areas of attention required to increase the crop's cultivation are as follows: (a) complete germplasm characterization (b) accurate information on the crop's history for quality genetic resource management (c) use of the current genetic approach to develop breeding programs; and (d) information on the N-fixing capacity of landraces and their nodulation efficiencies as the means of evaluating their contributions to agricultural systems. Identifying winged bean landraces with high N-fixing ability would be a valuable step in developing environmentally friendly and low cost approaches to the improvement of the crop.

Keywords: Complementary food security crop, genetic diversity, sub-Saharan Africa**,** winged bean.

3.1 Introduction

Psophocarpus tetragonolobus (L.) DC., commonly referred to as winged bean, is a well-known N-fixing legume exhibiting a vigorous twining habit and swollen root production (Vatanparast *et al*., 2016). It grows in major parts of the world (the hot, humid equatorial countries) (Lepcha *et al*., 2017). The attention of the public was first drawn to the significance of winged bean in 1975 by the U.S. Academy of Science in a publication entitled "The winged bean: a high protein crop for the tropics". For some years, there was a series of spontaneous research efforts in understanding the legume, particularly with respect to its nutritional, anti-nutritional, and value-added products, but later these interests faded away (Lepcha *et al*., 2017).

Today, nearly four decades after the first publication by the NAS, much more is still required to be done in terms of crop improvement. Four areas need urgent attention: (a) complete germplasm characterization to identify best populations (b) examination of the crop's history to enhance further exploration and genetic resource management (c) utilization of modern approaches to strengthen breeding programs, and (d) assessment of the N-fixing capacity of landraces and nodulation efficiencies as the means of evaluating their contributions to the sustainability of agricultural systems. This review examines current knowledge, constraints, and future perspectives towards germplasm and crop improvements.

3.2 Plant Description

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.*)* belongs to the *Phaseoleae* tribe and subfamily *Papilionoideae* (Egan *et al*., 2016). It is mostly cultivated as an annual crop despite its perennial nature, produces purple, white, or blue flowers, and could be 4 m in height (Fig. 3.1). Pods possess varying sizes and numbers of seeds (Figs 3.2a and 3.2b). Some accessions also produce tubers of different sizes and diameter (Lepcha *et al*., 2017).

Figure 3.1: Flower pattern of winged bean
Photo: T.T. Adegboyega, IITA (2017).

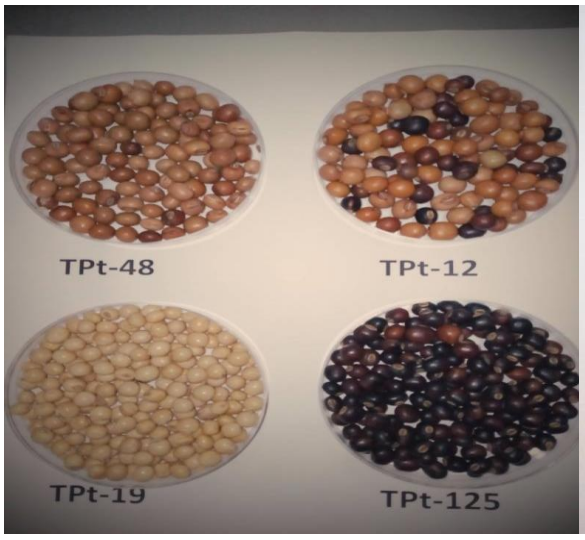

Figure 3.2a: Different landraces of winged bean **Figure 3.2b: Basic seed forms**
Photo: T.T. Adegboyega, IITA (2017).

Different parts of the crop are used for specific dishes. For instance, in India, immature pods are consumed raw as vegetables. In other areas, seeds that are not ripe are used in making delicious meals while mature seeds are first roasted and then consumed like peanuts. In Papua New Guinea, Ghana, and Burma (Myanmar), the swollen roots are used for diverse culinary and confectionary preparations. In Indonesia and Thailand, the seeds are used as ingredients for making traditional foods such as *tempeh* and *kecipir* and snacks (Lepcha *et al*., 2017). Another economic importance of the crop is its antimicrobial effectiveness in treating common bacterial infections (Khalili *et al*., 2013; Nazri *et al*., 2011). For industrial application, winged bean seed extracts have been reported to contain an active bioactive peptide that has angiotensin-converting enzyme inhibitor capacity (Mohtar *et al*., 2013).

3.3 Origin, Distribution, and Cultivation

Controversy still surrounds the origin of this species. In general, four centers have been suggested as possible areas of origin (i) Asia (ii) Indo-Malaya (iii) Papua New Guinea which hosts large collections, and (iv) Africa. In all, the African origin received greater support owing to closer similarity with *P. grandifloras (*Fatihah *et al.,* 2012*).* The figure below shows winged bean growing on the field (Fig. 3.3).

Figure 3.3: A mature winged bean plant on the field

Photo: T.T. Adegboyega, IITA (2017).

Winged bean is being increasingly recognized as an important plant because of its distinctive features. Table 3.1 shows the protein content, fat, ash, and other parameters of different plant parts of the crop. The percentage of protein (29.8-39.0) in the dried bean is among the highest in the legume group, besides being highly digestible. The young pods are considered very appetizing by those who regularly eat them and contain a high percentage of protein. The protein content is comparable to that of other vegetables with edible young fruits, but the others do not compare in flavour with the winged bean. There has also have been a report of a high percentage of protein in the flowers that are eaten in some parts of Papua New Guinea. but their use has not spread to other areas where the plant is cultivated. Several characters of wild plants still exist in most of the winged bean accessions, *viz. vining* and indeterminate habit of growth, photosensitivity, pod shattering (when dry on the vine), presence of toxic substances in the raw dry beans and in the tubers, an uneven germination rate, low yields, and tremendous diversity. To overcome these problems, conventional plant breeding techniques targeting nitrogen fixation can play a significant role (Koshy *et al*., 2013).

In general, this crop is grown in a traditional manner. It resembles the climbing or indeterminate common bean which requires stakes for climbing so that the aerial parts of the plant are situated for ease of harvest. However, winged bean can also be grown as a creeping vine. These vines climb only when they encounter another plant or some other type of support (such as stakes). Good results have been obtained using winged bean in this way as a cover crop. It is very common to find winged bean in association with other crops as part of a farming system. It often occupies a secondary position. It is also found as part of a rotation, especially with sweet potato (*Ipomoea batatas*) as alternate crop. Rice, followed by winged bean, followed by sugar cane, is a common rotation in South-east Asia (Myanmar). In Papua New Guinea, it is very common to find winged bean in association with maize. The maize and bean are planted at the same time or the legume is planted later and uses the dry stalks for support. The winged bean is also found in association with *Leucaena leucocephala*. It climbs this plant like a stake while it is growing (Rahman *et al*., 2014).

3.4 Nutritional and Anti-nutritional Composition

3.4.1 Nutritional Composition

The nutrients, mineral composition, and physico-chemical properties of winged bean have considerable potential (Table 3.1). The seeds, pods, swollen roots, flowers, and foliage are rich in macronutrients (Table 3.2, (Makeri *et al*., 2017) and micronutrients (Table 3.3, (Lepcha *et al*., 2017).

Table 3. 1: Nutritional composition of winged bean parts

Content (Mean)	Flowers	Leaves	Immature pods	Unripe seeds	Ripe seeds	Tubers
Water[a]	84.2-87.5	64.2-85.0	76.0-93.0	35.8-88.1	8.7-24.6	54.9-65.0
Energy (mJ)[b]	0.17	0.20	0.19	0.10-0.71	1.61-1.89	0.63
Protein	2.8-5.6	5.0-7.6	1.9-4.3	4.6-10.7	29.8-39.0	3.0-15.0
Fat	0.5-0.9	0.5-2.5	0.1-3.4	0.7-10.4	15.0-20 4	0.4-1.1
CHO	3.0-8.4	3.0-8.5	1.1-7.9	5.6-42.1	23.9-42	27.2-30.5
Fiber	-	3.0-4.2	0.9-3.1	1.0-2.5	3.7-16.1	1.6-17.0
Ash	0.8	1.0-2.9	0.4-1.9	1.0	3.3-4.9	0.9-1.7

[a] g per 100 g fresh weight [b] mJ = megajoules; CHO=carbohydrate

Source: Modified from National Academy of Science, 1981

Table 3. 2: Nutrients, minerals, and physico-chemical features of winged bean seeds

Parameter(s)	Value	Parameter(s)	Value
Moisture (%)	9.22 ± 0.18	Calcium (mg/kg)	889.86 ± 0.63
Total ash (%)	4.91 ± 0.01	Sodium (mg/kg)	1972.34 ± 0.69
Fat (%)	17.51 ± 0.35	Potassium (mg/kg)	4219.30 ± 0.81
Crude Fiber (%)	12.23 ± 0.13	Peroxide value (meg/kg)	11.41 ± 0.30
Crude Protein (%)	33.83 ± 0.61	Saponification value (mgKOH/g)	190.34 ± 0.64
Carbohydrate (%)	22.30 ± 0.82	Unsaponification matter (g/kg)	16.36 ± 0.64
Magnesium (mg/kg)	36, 476 ± 0.04	Acid value (mgKOH/g)	0.71 ± 0.01
Zinc (mg/kg)	36, 476 ± 0.64	Iodine value	144.57 ±0.53
Copper (mg/kg)	90.79 ± 0.72	Refractive index at 25˚C	1.47 ± 0.01

Source: Modified from Lepcha *et al*. (2017)

Table 3. 3: Fatty acid content of selected food oils and winged bean oil

Food Lipid	PUFA[a]	Mono-saturated FA[b]	Saturated FA	P/S [c] value
1. Maize oil	53.00	32.00	11.00	4.80
2. Soybean oil	59.00	20.00	15.00	3.90
3. Peanut oil	29.00	47.00	18.00	1.60
4. Chicken fat	26.00	38.00	32.00	0.80
5. Egg yolk	12.00	49.00	32.00	0.40
6. Pork fat	09.00	49.00	38.00	0.20
7. Beef fat	03.00	44.00	48.00	0.06
8. Coconut oil	Trace	07.00	86.00	-
9. Winged bean oil	30.70	39.00	30.30	1.00

a=Polysaturated fatty acid; b=Fatty acid; c=The ratio of PUFA to saturated FA

Source: Modified from Lepcha *et al*. (2017)

The seed oil content and fatty acids composition may be explored for different industrial uses (Table 3.3). Oil obtained from winged bean has been described as better than soybean oil owing to its efficient heating capacity which makes it suitable for frying food. What needs to be done now is to scale up its utilization (Makeri *et al*., 2016).

3.4.2 Anti-nutritional Composition

Anti-nutritional factors (ANFs) have also been studied. Common examples include tannins, phytate, trypsin inhibitors, chymotrypsin inhibitors, and hemagglutinins. A study by Kortt (1984) described variation in trypsin and chymotrypsin levels of the seeds. The trypsin discovered was lower within the diversity from Malaysia (23.4 g,) and Indonesia (28 g) and different from that originating in Papua New Guinea (36 g) and/or Myanmar (33 g). The trypsin inhibitor targets of the varieties was within 11,300 IU/g to 74,700 IU/g (Vatanparast *et al*., 2016). Generally, the toxic level of the seeds is related to the hemagglutinin content. Jaffe and Korte (1976) confirmed death within two weeks of rats that consumed unprocessed

beans. When seeds are autoclaved for 30 minutes, complete destruction of the trypsin and chymotrypsin inhibitors and hemagglutinin potential is observed and returned 50–66% of insoluble protein. Phytate constituents present in winged bean are calculated to fall within 6.1–7.5 mg of phytate phosphorus/g of bean, uniform with that of soybean (Lepcha *et a*l., 2017).

3.5 Pests and Diseases

- Caterpillars of *Aphis craccivora* (black bean aphid), *Henosepilachna signatipennis* (ladybird), *Ophiomyia phaseoli* (bean fly), *Lampides boeticus* (pea blue butterfly), *Nezara viridula* (southern green sucking pest), *Podalia* spp., *Polyphagotarsonemus latus,* and *Tetranychus urticae* have been known to destroy shoots, leaves, and flowers of winged bean (Reddy, 2015). In Kpouebo, Ivory Coast, spot ring mosaic virus has been revealed to cause between 10-20% of produce loss and leaf curl infection (Reddy, 2015). In a another study, endornavirus 1 has been provisionally identified by Okada *et al*. (2017). Furthermore, five major fungal infections have also been reported and include false rust (*Synchytrium psophocarpi*), dark leaf spot (*Pseudocercosa psophocarpi*), powdery mildew (*Oidium* sp.; *Erysiphe cichoracearum*), collar rot (*Rhizoctonia solani, Macrophomina phaseolina, Fusarium semitectum*, *F. equiseti*) and *choanephora* blight (*Choanephora cucurbitarum*) (Reddy, 2015).

3.6 Biological N fixation and winged bean productivity

The importance of nitrogen to plants cannot be overemphasized. It greatly affects yields on fields, the pattern of growth of plants, and their chemical composition (Amoo and Babalola, 2017). Winged bean is suitable for cultivation in soils with low fertility as it combines with and fixes nitrogen with the aid of bacterial symbionts. It has been used as a cover crop and is also used for intercropping systems (Wong *et al*., 2017).

3.7 Cytogenetics and biotechnology

Cytological studies of *Psophocarpus* genus have established the simple chromosome number of x = 9. Therefore *P. tetragonolobus* and the whole additional species have 2n = 2× = 18. The changing with initial maturing, high yielding, dwarf, erect, with not shattering pod support plants with diminished anti-nutritional agents should contains important hybrid motives in winged bean. Morphometric measurement may reveal best changes for a number of traits in germplasm characterization, containing varieties size of the leaf and structure and pod color in Asiatic germplasm. Genetic researches show the dominance of purple above green for the stem color, calyx color, and/or pod wing color, and rectangular above flat pod structure. Useful variations in contents on nutritional and/or biochemical traits such as protein and oil have been well revealed (Lepcha *et al*., 2017).

3.8 Molecular marker developments in winged bean

Winged bean has received little attention with respect to molecular breeding or genomic studies. Mohanty *et al*. (2013) have evaluated 24 accessions together and conclude that ISSR markers are excellent when compared to RAPD markers. In addition, Chen *et al*. (2015) also used the performance of ISSR markers to ascertain genetic diversity among 45 accessions and report just a small genetic base in the germplasm group. Chapman (2015) further revealed seedling transcriptome from the winged bean genotype Ibadan Local-1 revealed mining of ~1900 microsatellite and ~1800 conserved orthologous set loci to another exertion enhancing genomics aided breeding programs on winged bean. Wong *et al*. (2017) created 9,682 genic SSR markers via a transcriptome developed from Malaysian accessions which verified 18 SSRs within nine accessions. Therefore, sustained efforts are required towards further development in terms of molecular advancement.

3.9 Conclusion

The effort to generate genetic resources and improved lines of winged bean would help to hasten a breeding program as the lack of improved varieties suitable for specific environments has been one of the limiting factors preventing wider use. We suggest a very strong interdisciplinary approach to increase productivity.

CHAPTER FOUR

Genetic variability for character of seed yield components in African yam bean and winged bean

Abstract

Knowledge of the genetic variability of a crop is very important for its genetic improvement. Two underutilized legumes, winged bean (*Psophocarpus tetragonobolus* (L.) DC.) and African yam bean (*Sphenostylis stenocarpa*, (Hoechst ex. A. Rich.) Harms.), were evaluated for genetic variability. Twenty-five accessions of each of the crops were grown on the field for two successive seasons. Data were collected on yield traits: number of pods per plant, pod length, seed thickness, seed width, seed length, total number of seed, seed per pod and 100 seed weight. Analysis of variance for 14 morphological traits showed variation among the accessions for all the traits measured. Total seed weight, dry pod weight, number of seeds per pod and 100-seed weight exhibited high genotypic (s^2g) and phenotypic variances (s^2p). Results obtained revealed statistically significant ($p \leq 0.05$) differences among the accessions tested for important characters showing the scope for improvement of the crops. This study implied that a significant yield response could be obtained through use of an appropriate direct selection scheme.

Keywords: African yam bean, genetic advance, genotypic coefficient, phenotypic coefficient, variance, underutilized crop, winged bean.

4.1 Introduction

The ability to identify agronomic traits is of great significance in understanding the approach to improve crop yield (Andualem *et al.,* 2013). The selection for superior genotypes based on yield alone is less efficient due to the complexity of the yield and its dependence on yield components. Direct selection has also been observed to be ineffective owing to the large environment-genotype interaction (Narolia *et al*., 2017). For a successful breeding program, there must be genetic differences among plants to be evaluated by the breeder. Thereafter the desirable combination of genes can be selected (Aziz and Osman, 2015). Desirable traits such as yield, protein content, and quality are under the control of many genes and environmental factors. The environment affects the expression of the character and there will be no separate classes of phenotype but continuous variation for the character (Aziz and Osman, 2015).

The measure of the relationship between breeding values and phenotypic values is referred to as heritability (Falconer *et al*., 1996). Thus, it plays a predictive role in breeding, expressing the reliability of the phenotype as a guide to its breeding value. It is the breeding value which determines how much of the phenotype would be passed onto the next generation. There is a direct relationship between heritability and response to selection, which is referred to as genetic advance. A high genetic advance with high heritability estimates offers the most effective condition for selection (Wolie *et al*., 2013).

However, to carry out effective selection, available genetic variation among African yam bean accessions, the nature of the component traits on which selection would be made effective, and the influence of environmental factors on each trait all need to be known (Jaleta *et al*., 2011).

Information on the nature and magnitude of variability and heritability in a population is one of the prerequisites for a successful breeding program in selecting genotypes with desirable

characters (Wolie *et al*., 2013). Every breeder should therefore be able to know the heritability of the agronomical characters to improve the yield of the crop being studied appropriately.

African yam bean is an annual legume with the capacity to produce bean seeds in a pod with varying seed patterns and colors (Asoiro and Ani, 2011). Other than the seeds, farmers can also harvest swollen roots, often referred to as tubers, which resemble sweet potato. The swollen roots mature in five to eight months (Chinedu and Nwinyi, 2012) and come in various shapes (spindle, round, oval, and irregular) (Adewale and Dumet, 2011). The edible swollen roots have been shown to have high nutritional values. Omeire (2012) reports higher levels in the content of amino acids (lysine, histidine, arginine, valine) in African yam bean. All farmers not show the same interest in its production owing to limited knowledge on the economic potential, processing, and other related utilization concerns (Alozie *et al*., 2009). The crop's ability to adapt to a low-input system of farming is known and it could provide a significant contribution to food surplus in tropical agriculture (Nnamani *et al*., 2017b).

Winged bean *(Psophocarpus tetragonolobus)* (L.) DC. is a legume exhibiting a vigorous twining habit and production of swollen roots (Vatanparast *et al*., 2016). It grows largely in many parts of hot, humid, and equatorial countries (Lepcha *et al*., 2017). The fact that all parts of the plant are edible at every stage of its growth earns it titles such as 'one species mall' and 'supermarket on a stalk'.

Knowledge is limited of the extent and pattern of variability, heritability of the traits, and genetic gain present in the germplasm. Therefore, this study was done with the objective of assessing genetic variability that may contribute to the improvement of African yam bean and winged bean.

4.2 Materials and Methods

4.2.1 Location of study

On-station field experiments were conducted at IITA (Latitude 7°30'8''; Longitude 3° 54' 37'') Ibadan, Nigeria in a randomized complete block design (RCBD) with three replications on an Alfisol soil of the Egbeda series. Monthly rainfall ranged between 0.05 and 86.5 mm while the minimum and maximum temperatures ranged between 20- and 27 °C and from 24 to 35.2 °C respectively between 2016 and 2017 cropping seasons.

4.2.2 Planting materials

Twenty-five accessions each of the crops were obtained from the GRC of the IITA Ibadan. Seeds were planted on 5 m ridges, spaced 1 m apart. Each accession was planted on two rows at 1 m intra-row spacing. Initially, two seeds were planted per hill and later thinned to one plant per hill to give a total of 10 plants per accession in each plot. The experiment was prepared in three replicates. Data were taken on germination rate and number of seeds that germinated. Four weeks after planting, the seedlings were staked with Japanese plastic stakes (GradStak 16 mm). Manual weeding was done regularly to keep the field weed-free and insects were controlled with cyperdiforce applied at the rate of 35-60 mL in 20 L of water at intervals of 2 weeks during the flowering period and later every 2 weeks for a total of three applications before harvesting. Neither fertilizers nor inoculants were used during the seasons. A total of 14 agro-morphological variables were recorded using IITA phenotypic descriptors for African yam bean (Adewale and Dumet, 2011). The testa basal colors were determined using the Methuen Handbook of Color (Kornerup and Wanscher, 1978).

4.2.3 Data Collection

From five individual plants, data for morphological studies were generated within the row of each accession. Quantitative characters were measured in cm and by counting. The qualitative characters were scored by nominal codes using available descriptors.

4.2.4 Statistical Analysis

General linear model (GLM) procedure of the Statistical Analysis System computer software version 9.4 (SAS, 2014) was used for data analysis and LSD at $P \leq 0.05$ was used for mean separation. The mean values obtained were from two years of field data. Genotypic and phenotypic components of variance were calculated by the formula of Burton and Devane (1953), broad sense heritability and genetic advance were computed respectively as a percentage of the mean following the methods of Johnson *et al.* (1955). Genetic advance as percentage of the mean was classified as follows; 0-10%: Low, 10-20%: Moderate, and 20% and above: High. The value of k was taken as 2.06 assuming 5% selection intensity.

4.3 Results

The detailed description of the accessions used in this study is presented in Tables 4.1 and 4.2. Analysis of variance for 14 morphological traits showed variation among the accessions for all the traits under study for African yam bean and winged bean. In African yam bean, accessions exhibited a wide range of variability for most of the characters (Table 4.3). The analysis of variance revealed that accessions under study differed significantly for all measured characters. Total seed weight, dry pod weight, seeds per pod, and 100-seed fresh weight exhibited high genotypic (s^2g) and phenotypic (s^2p) variances. Total number of seeds per accession, 100-seed fresh weight, total seed weight, terminal petiole length, dry pod weight, and number of seeds per pod also exhibited a high genotypic (GCV) and phenotypic (PCV) coefficient of variances (Table 4.4). The values estimated for the phenotypic coefficient of variability (PCV) ranged from 7.53 for flower width to 127.08 for dry pod weight while the genotypic coefficient of variability (GCV) ranged from 2.90 for flower width and 90.80 for dry pod weight. Furthermore, the PCV values were observed to be higher than their counterpart GCV values for all the traits evaluated (Table 4.4).

Table 4. 1: Description of twenty-five accessions of African yam bean

SN	Accession	Origin	Source	Methuen Code	Seed colour
1	TSs1	Nigeria	IITA	7F8	Dark brown
2	TSs3	Nigeria	IITA	6C4	Brown
3	TSs4	Nigeria	IITA	5B4	Greyish orange
4	TSs9	Nigeria	IITA	8F7	Dark brown
5	TSs11	Nigeria	IITA	4B3	Greyish yellow
6	TSs16	Nigeria	IITA	6F8	Dark brown
7	TSs23	Nigeria	IITA	6E5	Brown
8	TSs24	Nigeria	IITA	6D5	Light brown
9	TSs27	Nigeria	IITA	5C4	Brownish orange
10	TSs30	Nigeria	IITA	5B4	Greyish orange
11	TSs33	Nigeria	IITA	6E6	Brown
12	TSs38	Nigeria	IITA	5D4	Dark blond
13	TSs44	Nigeria	IITA	4D3	Olive brown
14	TSs61	Nigeria	IITA	7D5	Light brown
15	TSs66	Bangladesh	IITA	7F7	Dark brown
16	TSs67	Bangladesh	IITA	18F3	Dark violet
17	TSs68	Zaire	IITA	6D5	Light brown
18	TSs76	Ghana	IITA	1B2	Greenish grey
19	TSs77	Ghana	IITA	7F7	Dark brown
20	TSs81	Nigeria	IITA	6D6	Light brown
21	TSs82	Nigeria	IITA	6F8	Dark brown
22	TSs98	Nigeria	IITA	5C4	Reddish blond
23	TSs101	Nigeria	IITA	7C4	Brownish orange
24	TSs104	Nigeria	IITA	4D4	Olive brown
25	TSs109	Nigeria	IITA	7F8	Dark brown

Table 4. 2: Description of twenty-five accessions of winged bean used for the study

SN	Origin	Source	Accession No	Methuen Code	Seed Color
1	No Identity	IITA	Tpt2	7D6	Light brown
2	Costa Rica	IITA	Tpt4	8FC	Dark brown
3	Indonesia	IITA	Tpt6	6D6	Light brown
4	Sri Lanka	IITA	Tpt10	7E2	Brownish grey
5	Nigeria	IITA	Tpt11	5B4	Greyish orange
6	Sri Lanka	IITA	Tpt12	7E8	Brown
7	No Identity	IITA	Tpt14	6EC	Brown
8	No Identity	IITA	Tpt15	9F4	Dark brown
9	Indonesia	IITA	Tpt16	5B5	Greyish orange
10	Trinidad and Tobago	IITA	Tpt17	5B4	Light brown
11	No Identity	IITA	Tpt18	7E8	Brown
12	Nigeria	IITA	Tpt19	8F5	Dark brown
13	No Identity	IITA	Tpt30	8F8	Brownish orange
14	Liberia	IITA	Tpt32	7E7	Brown
15	No Identity	IITA	Tpt33	6D6	Light brown
16	No Identity	IITA	Tpt42	8E7	Reddish brown
17	Bangladesh	IITA	Tpt43	8FE	Dark brown
18	No Identity	IITA	Tpt48	4B4	Greyish yellow
19	Bangladesh	IITA	Tpt51	5B4	Greyish orange
20	Nigeria	IITA	Tpt53	7F8	Dark brown
21	No Identity	IITA	Tpt125	6B5	Light brown
22	Nigeria	IITA	Tpt126	7EB	Brown
23	No Identity	IITA	Tpt154	5B4	Greyish orange
24	No Identity	IITA	Tpt15-4	5C4	Reddish blond brownish orange
25	No Identity	IITA	Tpt3-B	5D4	Yellowish dark blond

Table 4. 3: Comparison of the means and standard error (SE) for 14 traits of African yam bean and winged bean germplasm

Crop	Character	Mean value	SE (±)	Crop	Character	Mean value	SE (±)
S. stenocarpa	Terminal petiole length (cm)	8.14	0.39	*P. tetragonolobus*	Terminal petiole length (cm)	13.35	0.37
S. stenocarpa	Leaf Rachis	10.89	0.50	*P. tetragonobolus*	Leaf Rachis	18.09	0.39
S. stenocarpa	Terminal leaf length (cm)	10.78	0.21	*P. tetragonolobus*	Terminal leaf length(cm)	11.32	0.25
S. stenocarpa	Terminal leaf width (cm)	4.10	0.16	*P. tetragonolobus*	Terminal leaf width (cm)	9.73	0.28
S. stenocarpa	Flower width (cm)	3.45	0.03	*P. tetragonolobus*	Flower width (cm)	3.50	0.03
S. stenocarpa	Dry pod weight (g)	57.52	5.23	*P. tetragonolobus*	Dry pod weight (g)	248.22	13.59
S. stenocarpa	Seeds/pod	23.03	0.44	*P. tetragonolobus*	Seeds/pod	14.66	0.34
S. stenocarpa	Seed Length	10.60	0.59	*P. tetragonolobus*	Seed Length	9.15	0.09
S. stenocarpa	Seed Thickness	18.86	0.75	*P. tetragonolobus*	Seed Thickness	7.56	0.09
S. stenocarpa	Pod Length	7.66	0.17	*P. tetragonolobus*	Pod Length	16.13	0.44
S. stenocarpa	100-Seed Fresh Weight (g)	6.46	0.10	*P. tetragonolobus*	100-Seed Fresh Weight (g)	32.75	0.37
S. stenocarpa	Seed Width (cm)	6.13	0.12	*P. tetragonolobus*	Seed Width (cm)	8.63	0.08
S. stenocarpa	Total seed weight (g)	248.37	32.52	*P. tetragonolobus*	Total seed weight (g)	2495.03	195.13
S. stenocarpa	Total number of seeds	985.85	118.51	*P. tetragonolobus*	Total number of seeds	7759.28	566.24

Table 4. 4 :Estimates of components of variance, heritability, and genetic advance for seed yield and yield components in African yam bean

Characters	s^2g	s^2p	GCV (%)	PCV (%)	h^2 (%)	GA	GAM
Terminal petiole length (cm)	4.31	11.36	25.44	41.42	37.9	2.63	32.34
Leaf Rachis	7.49	18.41	25.07	39.40	40.71	3.6	33.04
Terminal leaf length (cm)	0.31	3.20	5.10	16.51	9.55	0.35	3.25
Terminal leaf width (cm)	0.04	1.96	4.88	34.15	2.22	0.06	1.56
Flower width (cm)	0.01	0.07	2.90	7.53	10.83	0.06	1.68
Dry pod weight (g)	2728.59	5352.42	90.80	127.08	50.98	76.8	133.46
Seed/pod	29.2	64.85	50.95	75.96	45.03	7.47	70.47
Seed Length	2.01	5.01	18.40	29.09	40.13	1.84	24.05
Seed Thickness	0.56	2.32	12.08	24.49	24.22	0.75	12.22
Pod Length	10.77	34.30	14.24	25.41	31.39	3.78	16.43
100-Seed Fresh Weight (g)	22.81	94.56	25.29	51.53	24.13	4.83	25.61
Seed Width (cm)	0.30	1.63	8.36	19.67	18.26	0.48	7.40
Total seed Weight (g)	1842.02	51887.7	17.28	91.68	3.55	16.7	6.70
Total Number of seeds	0.00	723648.39	0.00	86.28	0.00	0.00	0.00

s^2g =Genotypic variance; s^2p =Phenotypic variance; GCV=Genotypic coefficient of variation, PCV=Phenotypic coefficient of variation; h^2= Broad sense heritability; GA=Genetic advance; GAM= Genetic advance over mean

Table 4. 5: Estimates of mean, components of variance, heritability, and genetic advance for seed yield and yield components in winged bean

Traits	s^2g	s^2p	GCV (%)	PCV (%)	h^2 (%)	GA	GAM
Terminal petiole length (cm)	0.90	10.07	7.11	23.78	8.94	58.46	437.95
Leaf Rachis	0.19	11.60	2.41	18.82	1.64	11.49	63.49
Terminal leaf length (cm)	0.40	4.61	5.62	18.97	8.79	38.85	343.36
Terminal leaf width (cm)	0.00	5.76	0.00	24.66	0.00	0.00	0.00
Flower width (cm)	0.01	0.06	2.22	7.14	9.64	4.95	141.71
Dry pod weight (g)	11455.07	31779.21	43.12	71.82	36.05	13237.12	5332.86
Seed/pod	17.47	24.18	28.50	33.54	72.23	731.69	4989.77
Seed Length	0.40	1.29	6.90	12.40	30.97	72.38	790.74
Seed Thickness	0.29	1.32	7.07	15.22	21.59	51.14	676.70
Pod Length	20.43	36.66	28.02	37.54	55.74	695.20	4309.92
100-Seed Fresh Weight (g)	1.38	20.60	3.59	13.86	6.69	62.59	191.10
Seed Width (cm)	0.18	1.07	4.88	11.97	16.63	35.41	410.14
Total seed weight (g)	1097451.14	1321501.84	41.99	46.07	83.05	196661.13	7882.11
Total Number of seeds	8568620.93	11484969.33	37.73	43.68	74.61	520850.67	6712.62

s^2g =Genotypic variance; s^2p =Phenotypic variance; GCV=Genotypic coefficient of variation, PCV=Phenotypic coefficient of variation; h^2= Broad sense heritability; GA=Genetic advance; GAM= Genetic advance over mean.

4.4 Discussion

According to Narolia and Reddy (2012) a PCV higher than the GCV means that observed variation was due not only to accessions studied but also due to environmental influence. It is generally agreed in literature that PCV and GCV values roughly more than 20% are regarded as high; values lower than 10% are regarded as ;ow, and when between 10 and 20% they are referred to as Mmdium (Singh *et al*., 2012). Based on this classification, PCV values were low for flower width; medium for terminal leaf length and seed width, and high for every other trait. The GCV values were low for terminal leaf length, terminal leaf width, flower width, seed width, and total number of seeds; medium for seed length, seed thickness, pod length, and total seed weight, and high for terminal petiole length, leaf rachis, dry pod weight, number of seeds per pod, and 100-seed fresh weight (Table 4.4). The high GCV values of these characters suggested the possibility of improving these traits through selection. The results of this investigation thus supported these earlier report (Akande, 2008).

The difference between values for PCV and GCV was high for terminal leaf width, dry pod weight, seeds per pod, 100-seed fresh weight, and total seed weight, indicating the influence of environment. Although, this variation was low for terminal leaf length, seed length, seed thickness, pod length, and seed width, suggesting minimal environmental influence on the expression of the characters and thereby the highest estimates of heritability. Similar results were found by Wolie *et al*. (2013).

The broad sense heritability ranged from 24.13% for 100-seed fresh weight to 50.98% for dry pod weight (Table 4.4). Singh (2001) was of the view that if the heritability of a character is very high, 80% or more, selection for such a character would be done with ease. This is because there would be a close correspondence between the accession and the phenotype due to the relatively small contribution of the environment to the phenotype. However, for characters with low heritability, 40% or less, selection may be considerably difficult due to the effect of environment. Based on these criteria, the heritability values were moderate (31-

50) for terminal petiole length, leaf rachis, dry pod weight, seeds per pod, and seed length, and low at less than 30 for terminal leaf length, terminal leaf width, flower width, and seed thickness.

Genetic advance (GA) has been described as the improvement of traits in genotypic value for a new population when compared with the base population at a given selection intensity in one cycle of selection (Singh, 2001). The estimates of GA for total seed weight was 16.7 g, which means that when we intend to select the best 5% high yielding accessions as parent, the mean total seed weight of progenies could be improved from 248.37 g to 265.07 g (Table 4.4). Maximum genetic advance as a percentage of the mean (GAM) at 5% selection intensity was recorded for dry pod weight (133.46%), number of seeds per pod (70.47%, leaf rachis (33.04%), terminal petiole length (32.34%), followed by seed length (24.05%). It was minimal for terminal leaf width (1.56) and flower width (1.68).

Johnson *et al.* (1955), suggested that a high heritability estimate along with high genetic advance are usually more helpful in predicting gain under selection than heritability estimate alone. The present investigation reveals moderate heritability coupled with high expected genetic advance as the percentage mean for dry pod weight, number of seeds per pod, leaf rachis, terminal petiole length, and seed length. Moderate heritability was observed with relatively higher genetic gain for terminal petiole length, leaf rachis, dry pod weight, number of seeds per pod, and seed length. Therefore these traits could be improved more easily than others characters. This observation was in agreement with the outcome of previous work by Adewale *et al*. (2010) on seed metrics for genetic and shape determination and by Ibirinde and Aremu (2013) for selected African yam bean grown in south-western Nigeria.

In winged bean, pod length, dry pod weight, total seed weight, total number of seeds, seed per pod, and 100-seed fresh weight exhibited high genotypic (s^2g) and phenotypic (s^2p) variances. The total number of seeds per accession, total seed weight, terminal leaf length, terminal leaf width, pod length, dry pod weight, and number of seeds per pod also exhibited a high genotypic (GCV) and phenotypic (PCV) coefficient of variances (Table 4.5).

PCV values were from 7.14 for flower width to 71.822 for dry pod weight while the GCV values were from 2.22 for flower width to 43.12 for dry pod weight. Furthermore, PCV values were observed to be higher than their counterpart GCV for all the traits evaluated (Table 4.5). Based on this classification, the PCV value was low for flower width; medium for terminal leaf length, leaf rachis, seed length, seed thickness, seed width, and 100-seed fresh weight. It was high for terminal leaf length, terminal leaf width, pod length, dry pod weight, seed per pod, total seed weight, and total number of seeds.

GCV values were low for terminal petiole length, leaf rachis, terminal leaf length, flower width, seed length, seed thickness, and 100-seed fresh weight; no trait was in the medium range. Values were high for dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds (Table 4.5). The high GCV values of these characters suggest the possibility of improving these traits through selection.

The PCV and GCV values was high only for terminal leaf width and dry pod weight which reveals environments could influence these traits. Although, this difference was low for flower width, number of seeds per pod, seed length, seed thickness, pod length, 100-seed fresh weight, total seed weight, and total number of seeds, suggesting less environmental influence on the expression of the characters that thereby have the highest estimates of heritability. In the study by Rajeshwar *et al*. (2009) on winged bean, a high genetic advance was observed for almost all the characters under study. A high heritability coupled with high GA was recorded for pods per plant, dry pod weight and 100 seed weight. It suggests the

preponderance of additive gene action which can be exploited by effective selection methods for the development of elite genotypes of winged bean.

The broad sense heritability ranged from 83.05% for total seed weight to 1.64% for leaf rachis (Table 4.5). However for traits with low heritability, 40% or less, selection may be difficult due to environmental influence. Based on these criteria, the broad sense heritability estimate was high for total seed weight (83.05); moderate (75-55) for total number of seeds, seed per pod, and pod length; low with less for 55 for dry pod weight, seed length, and other traits. The estimates of GA for seed per pod was 731.69 indicating that whenever we select the best 5% high yielding accessions as parents, the mean total seed weight of progenies can be improved from 731.69 to 746.35. Other improvements could be possible in parameters such as total number of seeds from 520, 850.67 to 528, 609.95; total seed weight from 196,661.13 to 199,156.13; 100-seed fresh weight from 62.59 to 95.34, and dry pod weight from 13,237.12 to 13,485.338 (Table 4.5).

The genetic advance as a percentage of mean (GAM) at 5% selection intensity was recorded for total seed weight (7882.11%); total number of seeds (6712.62%); dry pod weight (5332.86%); seed per pod (4989.77%), pod length (4309.92%), and 100-seed fresh weight (191.10%). The present study reveals moderate heritability coupled with high expected genetic advance as a percentage for mean for total number of seeds, total seed weight, dry pod weight, and pod length while moderate heritability was observed with relatively higher genetic gain for terminal petiole length, leaf rachis, number of seeds per pod, and seed length. Therefore, these characters could be improved more easily than others. The result obtained in this study was similar to that of Prasanth *et al*. (2015) who recorded substantial differences among winged bean genotypes evaluated for variability.

In the accessions studied, the PCV value was low for flower width; medium for terminal leaf length, leaf rachis, seed length, seed thickness, seed width, and 100-seed fresh weight. It was

high for terminal leaf length, terminal leaf width, dry pod weight, seed per pod, pod length, total seed weight, and total number of seeds. In a related study, correlation and path analysis studies conducted on pod yield and component characters in winged bean revealed that pod yield per plant had a significantly high and positively correlated with pod length, pod weight, and pods per plant at both genotypic and phenotypic levels, indicating more possibility of improvement in these traits through simultaneous selection (Prasanth *et al*., 2016).

GCV values were low for terminal petiole length, leaf rachis, terminal leaf length, flower width, seed length, seed thickness, and 100-seed fresh weight; no trait was at the medium range. Values were high for dry pod weight, seed per pod, pod length, total seed weight, and total number of seeds. The high GCV values of these characters suggest the possibility of improving these traits through selection. The difference between PCV and GCV values was high only for terminal leaf width and dry pod weight indicating the influence of environment on these characters. However, this difference was low for flower width, number of seeds per pod, seed length, seed thickness, pod length, 100-seed fresh weight, total seed weight, and total number of seeds, suggesting less influence of environment on the expression of the characters and thereby the highest estimates of heritability. Therefore these characters could be improved more easily than other characters in winged bean germplasm. Our result is similar to previous studies by Prasanth *et al*. (2015) and by Kant and Nandan (2018).

Finally, the investigation revealed different traits that can be selected in germplasm of both African yam bean and winged bean. For instance, the PCV and GCV values was high in African yam bean for terminal leaf width, dry pod weight, number of seeds per pod, 100-seed fresh weight, and total seed weight while in winged bean it was high for terminal leaf width and dry pod weight.

CHAPTER FIVE

Nodulation, N fixation, and water-use efficiency of different accessions of African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.)

Abstract

In this study, the natural isotopes of N (^{15}N) and C (^{13}C) were employed to determine symbiotic N fixation and water-use efficiency in the underutilized tropical legume, African yam bean. On-station field experiments were conducted at IITA Ibadan Nigeria in a randomized complete block design with three replications on an Alfisol soil of the Egbeda series with *Zea mays* and two weed species (*Tridax procumbens* and *Eleusine indica*) as reference plants. Significant differences were seen in all parameters measured including nodulation and growth. TSs44 had a significantly higher number of nodules than other accessions at 169.67; TSs23 had the lowest number at 58.42. Shoot dry matter ranged from 107 to 344 g plant^{-1}; $\delta^{15}N$ from 0.82 to 2.70. TSs77 fixed the highest amount of nitrogen at 22.47 kg ha^{-1} and at the same time produced the highest number of seeds during harvest; followed by TSs30 at 20.91 kg ha^{-1} and TSs101 at 19.80 kg ha^{-1}. C/N evaluated ranged from 18-23 to 15.44; $\delta^{13}C$ ranged from -30.93 to -31.50; %N from 2.59 to 2.22, and Total N kg/ha from 34450 to 29518. The amount of nitrogen fixed (kg N ha^{-1}) varied among the reference plants used. On the other hand, root dry matter ranged from 27.30 to 9.80 g plant^{-1}, $\delta^{15}N$ from 2.52 to 0.24; percentage BNF varied among the accessions. The amount of nitrogen fixed (kg N ha^{-1}) also differed remarkably.

Keywords: African yam bean, BNF, nodules, N contribution, $\delta^{15}N$, underutilized legume, sub-Saharan Africa.

5.1 Introduction

In global agriculture, deficiency of nitrogen (N) and phosphorus (P) limits plant growth and yield. The use of synthetic fertilizers negatively impacts soil health and causes environmental pollution. Furthermore, they are very expensive and inaccessible to smallholder farmers who possess limited access to a financial credit facility in sub-Saharan Africa (Mohale *et al*., 2014).

When these challenges are considered, they reduce application of chemical inputs by farmers who hitherto have limited access to finance. Therefore, substituting N_2-fixing legumes for N fertilizers is a viable way of obtaining atmospheric N_2 targeted at improved yields. Studies have shown that legumes such as cowpea, groundnut and soybean can supply considerable amounts of N to Africa's cropping system (Mohale *et al.*, 2014; Pule-Meulenberg *et al*., 2010).

Carbon isotope discrimination ($\delta^{13}C$) is seen as an important evaluation tool for determining water-use efficiency in a plant such as African yam bean (Mohale *et al*., 2014). When there is water shortage, stomata closure can occur, leading to an intercellular CO_2 concentration that is enough to support photosynthesis. When this happens, less $\delta\ ^{13}C$ observation is noticed, and little quantity of water is also lost. This forms the basis for the use of $\delta\ ^{13}C$ as a tool for determining water-use efficiency in C_3 species such as African yam bean and winged bean, etc. (Mohale *et al*., 2014).

African yam bean is a crop with tremendous nutritional potential. The poor level of awareness about the taxonomy, agronomy, genetics, medicinal value, and productive potential of the crop may be due largely to limited research on it. The subsistence nature of its production may have been occasioned by its poor acceptability as a valuable crop among middle-aged farmers in Africa (Daniel and Celestina, 2013).

The total number and spread of nodules throughout the root system may affect root growth and root system architecture as both the availability and distribution of nitrogen influence lateral root growth (Ferguson *et al*., 2013). Biotechnology and crop improvement programs in recent years have therefore recognized the importance of using the root system of legumes (Smith and Read, 2010) and the enhanced capacity of nutrient uptake through symbiosis with N-fixing bacteria (Shtark *et al*., 2010). There is scarce data on the nodulation ability of African yam bean when grown on the field with naturally occurring indigenous soil bacteria. Although African yam bean is positioned as being drought tolerant, there is scarcity of data on its water-use determination under field condition. This study investigate variation in nodulation, N fixation, and water-use efficiency of African yam bean sampled from the field without application of fertilizers and inoculants using ^{15}N and ^{13}C natural abundance. To our knowledge, this is the first report on the use of isotopic studies involving the crop.

5.2 Material and methods

5.2.1 Experimental site, plant sample collection, and processing

On-station field experiments were conducted in IITA Ibadan, Nigeria, (Latitude 7°30'8'';; Longitude 3° 54' 37''). Monthly rainfall ranged between 0.05 and 86.5 mm while the minimum and maximum temperatures ranged between 20 and 27 °C and from 24 to 35.2°C respectively. Seeds of 25 accessions of African yam bean were collected from the Gene bank of IITA, Ibadan, as follows:TSs1, TSs3, TSs4, TSs9, TSs11, TSs16, TSs23, TSs24, TSs27, TSs30, TSs33, TSs38, TSs44, TSs61 and TSs66. TSs67, TSs68, TSs76, TSs77, TSs81, TSs82, TSs98, TSs101, TSs104, and TSs109. Two seeds were sown per hill. The experiment was set up in a randomized complete block design (RCBD) with three replications. Seeds were manually scarified with a sterile surgical blade. At 50% flowering, plant shoots were cut off at the base with the roots carefully washed under a running tap. Nodule number, nodule fresh and dry weights, fresh and dry shoot weights, fresh and dry root weights were recorded.

Plant shoots were oven dried and the leaves were separated into stems and petioles and ground. The plant tissues with the leaves were used in plant N determination. The plant shoots and roots were oven dried at 60 °C for 48 hr. Thereafter, they were weighed, and milled to fine powder (0.85 mm sieve) for ^{15}N and ^{13}C isotope analysis. The analysis of the plant materials for ^{15}N and ^{13}C was carried out by mass spectrometry at the Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven, Belgium.

5.2.2 Reference plants

Non-fixing plants, *Zea mays* (maize) and two weed species (*Tridax procumbens* and *Elusine indica*) were samples concurrently from the field and processed for ^{15}N and ^{13}C isotope analysis as described for the legume.

5.2.3 Soil analysis

Before planting, soil samples were collected from a depth of 0 to 15 cm. The soil was an Alfisol of the Egbeda series. Some of the soil analysis data obtained are in Table 5.1.

5.2.4 Measurement of N_2 fixation

$^{15}N/^{14}N$ analysis

The plant material was weighed into Al tin capsules (0.5-1 mg) and analyzed for %N and $^{15}N/^{14}N$ ratio using a Thermo EA-1110 elemental analyzer (FORNO EA/NA ThermoQuest, Italia, S.P.A) coupled to a mass spectrometer via Conflo II open-split device. ^{15}N natural abundance is expressed as δ (delta) notation and is the per mille deviation of the ^{15}N natural abundance of the sample from atmospheric (atm) N_2 (0.36637 atom % ^{15}N).

The isotopic composition (δ15N) as described by Unkovich *et al*. (2008)

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)\ atm} \times 1000$$

Where $^{15}N/^{14}N$ sample is the abundance ratio of ^{15}N and ^{14}N in the shoot and root samples while the $^{15}N/^{14}N$atm is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

5.2.5 Percentage N derived from atmosphere

The percentage N derived from atmospheric N_2 was calculated by the equation (Unkovich *et al*. (2008)

$$\% \text{ Ndfa} = \frac{\delta^{15}\text{N ref} - \delta^{15}\text{Nleg}}{\delta^{15}\text{N ref} - B} \times 100$$

δ^{15}N ref represents ^{15}N natural abundance of reference plants, δ^{15}N leg is the ^{15}N of legumes, and the B value is the ^{15}N of the test legume solely reliant on N_2 fixation. The B value replaces the value of atmospheric N_2 (Unkovich *et al*., 2008). In this study, the B value used was −1.54‰ as explained by Unkovich *et al*. (2008).

The quantity of N-fixed was estimated as described by Unkovich *et al*. (2008);

$$\text{N fixed} = \text{amount of N} \times \% \text{ NdfA}$$

5.2.6 $^{13}C/^{12}C$ isotopic analysis

To analyze for $^{13}C/^{12}C$ ratio, shoot and root samples (0.5-1mg) followed the same procedures as described for $^{15}N/^{14}N$ isotopic ratio. The ratio of $^{13}C/^{12}C$ in the sample was used to calculate the ^{13}C ($\delta^{13}C$) as described (Stout and Rafter, 1978).

$$\delta^{13}C = \frac{\left(\left(^{13}C/^{12}C\right)_{\text{sample}} - \left(^{13}C/^{12}C\right)_{\text{standard-1}}\right)}{\left(^{13}C/^{12}C\right)_{\text{standard}}} \times 1000$$

$^{13}C/^{12}C$ is the isotopic ratio of the sample, and $^{13}C/^{12}C$ standard is the isotopic ratio of the known universally accepted standard for C.

5.2.7 Statistical analysis

Analysis of variance (ANOVA) using Statistical analysis software (SAS, version 9.4) was used to analyse data obtained. Where significant differences were found, the means were separated using Fisher's least significance difference (LSD) test at $p \leq 0.05$.

5.3 Results

Table 5. 1: Soil analysis of study site

Soil parameters	Value
Sand (%)	78.00
Silt (%)	9.00
Clay (%)	13.00
pH (H_2O)	7.30
Organic Carbon (%)	1.97
Total N (%)	0.04
Available P mg kg^{-1}	45.97
Ca^{2+} (cmol kg^{-1})	2.36
Mg^{2+} (cmol kg^{-1})	0.69
K^{+} (cmol kg^{-1})	0.14
Na^{+} (cmol kg^{-1})	0.57
Zn (ppm)	5.10
Cu (ppm)	1.35
Mn (ppm)	168.95
Fe (ppm)	31.40

Table 5. 2: Means of nodulation parameters

Accession	NON	NFW(g)	NDW(g)
TSs1	109.67	12.94	2.72
TSs101	84.17	7.71	1.16
TSs104	92.50	9.43	1.87
TSs109	122.50	8.92	1.59
TSs11	96.08	11.13	2.15
TSs16	136.08	9.66	2.12
TSs23	58.42	7.21	1.57
TSs24	103.58	8.09	1.84
TSs27	101.00	8.02	1.80
TSs3	113.33	10.24	1.89
TSs30	77.08	8.58	1.75
TSs33	114.67	7.91	1.69
TSs38	64.83	6.91	1.48
TSs4	103.58	7.94	1.88
TSs44	169.67	9.83	2.07
TSs61	97.08	9.73	2.06
TSs66	85.25	8.16	1.42
TSs67	104.17	7.00	1.41
TSs68	91.92	6.72	1.06
TSs76	104.00	6.24	1.40
TSs77	100.75	9.40	1.65
TSs81	117.67	12.13	2.71
TSs82	74.00	8.94	1.80
TSs9	94.00	8.78	1.47
TSs98	124.33	10.35	2.04
S.E	6.2	0.6	0.2

KEY: SE=Standard error; NON =Number of nodules; NFW =Nodule fresh weight;

NDW=Nodule dry weight; FSW = Fresh shoot weight; FRW =Fresh root weight; DSW =

Dry shoot weight; DRW= Dry root weight; TBM = Total biomass.

Table 5. 3: Mean estimates of plant growth parameters

Accession	FSW (g)	FRW (g)	DSW (g)	DRW (g)	TBM (g)
TSs1	382.51	62.11	168.97	7.52	176.49
TSs101	429.60	60.52	107.43	9.56	116.99
TSs104	268.27	63.35	115.39	6.93	122.32
TSs109	301.06	58.43	140.51	8.49	149.00
TSs11	324.99	62.09	156.93	9.27	166.20
TSs16	374.69	46.56	156.77	5.61	162.38
TSs23	392.70	60.26	154.39	11.86	166.25
TSs24	299.70	60.93	118.65	10.09	128.74
TSs27	308.89	76.72	123.59	11.96	135.55
TSs3	294.85	75.08	174.32	10.15	184.47
TSs30	296.89	52.49	101.46	7.13	108.59
TSs33	364.35	68.19	170.65	9.85	180.50
TSs38	336.49	64.98	125.58	8.34	133.92
TSs4	235.58	52.68	60.08	7.71	67.79
TSs44	423.97	88.09	181.28	7.69	188.97
TSs61	406.26	53.01	147.71	11.83	159.54
TSs66	406.82	73.52	185.05	10.76	195.81
TSs67	516.04	76.90	219.10	14.35	233.45
TSs68	330.41	64.46	160.71	9.39	170.10
TSs76	385.81	67.90	161.31	11.85	173.16
TSs77	185.43	52.09	129.49	7.51	137.00
TSs81	272.58	66.93	136.97	9.97	146.94
TSs82	385.57	54.33	137.16	10.80	147.96
TSs9	446.12	66.92	189.70	8.77	198.47
TSs98	512.77	72.09	184.25	13.41	197.66
S.E	40	4.8	13	0.6	8.5

Key: SE=Standard Error; FSW= Fresh shoot weight; FRW=Fresh root weight; DSW=Dry shoot weight; DRW=Dry root weight; TBM =Total biomass.

Table 5. 4: Pearson correlation coefficients **of nodulation and growth parameters**

	NON	NFW	NDW	FSW	FRW	DSW	DRW	TBM
NON	1	0.415*	0.433*	0.094^{ns}	0.365^{ns}	0.302^{ns}	-0.174	0.283^{ns}
NFW		1	0.882***	-0.117	-0.041	0.104^{ns}	-0.278	0.083^{ns}
NDW			1	-0.163	-0.058	-0.029	-0.216	-0.041
FSW				1	0.398*	0.685*	0.545*	0.699***
FRW					1	0.567*	0.426*	0.577*
DSW						1	0.43098*	0.99849***
DRW							1	0.479*
TBM								1

*p≤0.05 significant, ***≤0.001 is highly significant

KEY: NON=Number of nodules; NFW=Nodule fresh weight; NDW=Nodule dry weight; FSW=Fresh shoot weight; FRW=Fresh root weight; DSW= Dry shoot weight; DRW=Dry root weight; TBM=Total biomass

Table 5. 5: Contribution of principal component axis (pca) to the variation of nodulation and plant growth parameters of African yam bean

Trait	PC1	PC2
Fresh shoot weight	0.88	-
Dry shoot weight	3.12	-
Total Biomass	32.60	-
Number of nodules	-	34.90
Fresh root weight	-	11.20
Eigen Value	7803.69	1121.35
Difference	6682.33	676.71
Proportion	0.82	0.11
Cumulative	0.82	0.94
Contribution (%)	82.70	11.90

Table 5. 6: Comparison of percentage N derived from the atmosphere of shoot and root

	Shoot	Shoot	Shoot	Root	Root	Root
ACCESSION	**M ± SE**	**E ± SE**	**T ± SE**	**M ± SE**	**E ± SE**	**T ± SE**
TSs1	13.22±25.08	40.07±17.32	34.21±19.01	60.75±18.77	55.37±21.34	65.98±16.27
TSs101	48.42±13.71	64.37±9.47	60.9±10.40	28.47±13.45	18.67±15.29	38±11.66
TSs104	27.90±19.80	50.2±13.67	45.34±15.01	48.82±5.51	41.8±6.27	55.64±4.78
TSs109	50.60±12.43	65.88±8.58	62.55±9.42	24.88±27.92	14.58±31.75	34.89±24.2
TSs11	34.32±18.01	54.64±12.44	50.21±13.66	43.47±26.23	35.72±29.82	51±22.74
TSs16	23.09±16.97	46.88±11.72	41.69±12.87	32.39±13.96	23.13±15.87	41.4±12.1
TSs23	23.16±14.48	46.93±10.00	41.75±10.98	24.44±6.69	14.09±7.61	34.51±5.8
TSs24	42.35±16.78	60.18±11.59	56.29±12.72	34.79±7.59	25.85±8.63	43.48±6.58
TSs27	43.41±6.68	60.91±4.61	57.1±5.06	25.28±6.93	15.04±7.88	35.23±6.01
TSs3	40.18±15.06	58.68±10.4	54.65±11.42	26.3±6.88	16.19±7.83	36.11±5.97
TSs30	47.12±3.26	63.48±2.25	59.91±2.47	58.88±18.01	53.24±20.48	64.36±15.61
TSs33	46.53±11.07	63.07±7.64	59.46±8.39	40.71±22.46	32.58±25.53	48.61±19.47
TSs38	22.21±9.03	46.27±6.24	41.03±6.85	35±18.76	26.08±21.33	43.65±16.26
TSs4	51.03±13.56	66.18±9.36	62.87±10.28	46.9±5.78	39.62±6.57	53.97±5.01
TSs44	41.6±1.67	59.67±1.15	55.73±1.27	70.63±12.5	66.6±14.22	74.54±10.84
TSs61	17.46±4.77	42.99±3.3	37.42±3.62	16.77±20.89	5.36±23.76	27.86±18.11
TSs66	18.23±15.21	43.53±10.5	38.01±11.53	21.56±21.77	10.81±24.75	32.01±18.87
TSs67	36.31±2.13	56.01±1.47	51.71±1.61	39.77±21.6	31.51±24.56	47.79±18.72
TSs68	45.87±9.51	62.61±6.57	58.96±7.21	31.75±13.1	22.39±14.9	40.84±11.35
TSs76	51.83±4.44	66.73±3.07	63.48±3.37	34.7±15.87	25.75±18.05	43.4±13.76
TSs77	50.06±9.36	65.51±6.46	62.14±7.09	32.76±26.7	23.54±30.37	41.71±23.15
TSs81	30.12±5.72	51.73±3.95	47.02±4.33	41.6±21.29	33.59±24.21	49.38±18.45
TSs82	34.59±1.83	54.82±1.26	50.41±1.39	19.85±16.84	8.86±19.14	30.53±14.59
TSs9	26.95±8.59	49.55±5.93	44.62±6.51	42.01±14.1	34.06±16.03	49.73±12.22
TSs98	35.40±19.91	55.38±13.75	51.03±15.09	45.63±8.32	38.18±9.46	52.88±7.21
F Statistics	0.64	0.64	0.64	0.12	0.11	0.11

Key: M=Maize; T=*Tridax procumbens*; E=*Eleusine indica;* SE= Standard Error

Table 5. 7: Comparison of N fixed (kg N/ha) of shoot and root

	Shoot	Shoot	Shoot	Root	Root	Root
ACCESSION	**E ± SE**	**T ± SE**	**M ±SE**	**E ± SE**	**T ± SE**	**M ± SE**
TSs1	12.76±5.51	10.9±6.03	4.24±7.93	0.03±0.00	1.54±0.33	0.03±0.00
TSs101	20.91±3.59	19.8±3.84	15.85±4.77	0.06±0.01	2.02±0.76	0.06±0.01
TSs104	15.89±4.19	14.29±4.68	8.56±6.45	0.04±0.01	2.14±0.78	0.04±0.01
TSs109	20.81±2.80	19.76±3.05	16±3.95	0.04±0.01	0.92±0.83	0.04±0.01
TSs11	17.22±4.19	15.8±4.56	10.68±6.00	0.04±0.01	1.93±0.8	0.04±0.01
TSs16	14.98±3.43	13.29±3.83	7.22±5.28	0.03±0.01	1.18±0.54	0.03±0.01
TSs23	14.86±3.09	13.19±3.42	7.21±4.63	0.04±0.01	1.47±0.49	0.04±0.01
TSs24	19.1±3.71	17.87±4.07	13.44±5.37	0.04±0.01	1.59±0.26	0.04±0.01
TSs27	18.39±1.37	17.23±1.51	13.1±2.00	0.05±0.01	1.51±0.04	0.05±0.01
TSs3	19.91±4.24	18.58±4.55	13.8±5.65	0.04±0.00	1.42±0.28	0.04±0.00
TSs30	20.97±1.48	19.79±1.45	15.57±1.42	0.03±0.01	2.23±1.07	0.03±0.01
TSs33	19.56±2.50	18.45±2.72	14.44±3.54	0.04±0.02	1.91±1.23	0.04±0.02
TSs38	14.32±1.67	12.68±1.89	6.8±2.68	0.04±0.02	1.86±0.95	0.04±0.02
TSs4	20.66±3.19	19.65±3.45	16±4.39	0.03±0.01	1.54±0.29	0.03±0.01
TSs44	18.7±0.40	17.46±0.41	13.03±0.48	0.03±0.02	2.55±1.59	0.03±0.02
TSs61	13.45±0.97	11.71±1.08	5.44±1.48	0.03±0.00	0.74±0.43	0.03±0.00
TSs66	13.38±3.03	11.67±3.35	5.54±4.51	0.07±0.03	1.41±0.43	0.07±0.03
TSs67	18.42±0.70	17.01±0.73	11.95±0.84	0.08±0.02	3.13±0.33	0.08±0.02
TSs68	20.25±3.02	19.1±3.15	14.98±3.63	0.04±0.00	1.55±0.46	0.04±0.00
TSs76	19.57±0.80	18.6±0.69	15.12±0.62	0.07±0.01	2.79±0.77	0.07±0.01
TSs77	22.47±1.91	21.3±2.12	17.1±2.95	0.03±0.00	1.5±0.78	0.03±0.00
TSs81	17.28±1.29	15.7±1.39	10.02±1.86	0.02±0.01	1.17±0.95	0.02±0.01
TSs82	17.76±0.56	16.33±0.56	11.2±0.63	0.05±0.01	1.29±0.60	0.05±0.01
TSs9	14.81±1.75	13.34±1.93	8.05±2.56	0.04±0.01	1.87±0.17	0.04±0.01
TSs98	17.33±4.02	15.95±4.45	10.99±5.99	0.04±0.01	2.06±0.29	0.04±0.01
F Statistics	0.575	0.575	0.613	0.0587	0.547	0.0587

Key: M=Maize; T=*Tridax procumbens*; E=*Eleusine indica;* S.E= Standard Error

Table 5.8: Comparison of δ ^{15}N, %N, dry weights, and total N of shoot and root

	Shoot				Root			
ACCESSION	**δ^{15}N ±SE**	**% N ± SE**	**SDW(g) ±SE**	**Total N (kg/ha) ±SE**	**δ^{15}±SE**	**% N ± SE**	**RDW (g)±SE**	**Total N (kg/ha) ±SE**
TSs1	2.7±1.23	2.39±0.03	211.83±60.49	31790.27±463.51	0.66±0.79	1.91±0.22	9.87±1.36	2514.14±475.22
TSs101	0.98±0.67	2.42±0.08	101.33±59.73	32206.83±1077.74	2.03±0.57	2.23±0.05	18.67±4.24	5582.54±1372.60
TSs104	1.99±0.97	2.43±0.14	202.63±66.69	32254.13±1906.07	1.16±0.23	2.15±0.17	12.4±2.63	3661.36±1048.30
TSs109	0.88±0.61	2.37±0.05	136.23±31.64	31534.26±604.12	2.18±1.18	2.01±0.17	14.7±4.42	3752.81±856.52
TSs11	1.67±0.88	2.39±0.20	250.4±78.47	31829.33±2699.14	1.39±1.11	2.01±0.09	13.9±1.81	3742±639.41
TSs16	2.22±0.83	2.43±0.08	183.9±87.81	32327.63±1125.64	1.86±0.59	2.31±0.35	9.8±3.50	2694.22±725.81
TSs23	2.22±0.71	2.4±0.12	232.77±28.59	31938.4±1601.58	2.2±0.28	2.11±0.12	14.03±2.67	4024.87±928.77
TSs24	1.28±0.82	2.39±0.04	184.27±53.61	31747.69±536.98	1.76±0.32	2.15±0.10	12.77±1.17	3687.01±517.09
TSs27	1.23±0.33	2.27±0.01	226.97±54.37	30189.26±96.59	2.16±0.29	2.05±0.17	17.47±4.77	4557.64±814.84
TSs3	1.39±0.74	2.52±0.08	249.73±77.85	33530.87±1107.3	2.12±0.29	2.08±0.06	14.03±0.67	3883.71±248.30
TSs30	1.05±0.16	2.48±0.15	140.33±9.62	33024.18±2007.56	0.74±0.76	2.13±0.23	10.63±2.39	3140.86±1036.35
TSs33	1.07±0.54	2.33±0.06	200.07±93.1	31003.67±790.3	1.51±0.95	2.09±0.2	12.87±4.11	3772.19±1538.94
TSs38	2.26±0.44	2.34±0.05	193.17±29.79	31103.31±609.76	1.75±0.79	2.15±0.09	14.3±5.49	4124.75±1648.99
TSs4	0.85±0.66	2.34±0.04	106.53±27.54	31085.8±529.51	1.25±0.24	2.03±0.14	10.93±2.75	2996.4±810.96
TSs44	1.32±0.08	2.36±0.05	344±114.62	31351.38±621.59	0.24±0.53	2.21±0.21	9.67±5.46	2996.73±1889.86
TSs61	2.5±0.23	2.36±0.05	301.1±114.79	31334.7±684.06	2.52±0.88	2.14±0.08	10.47±1.93	2943.42±442.54
TSs66	2.46±0.74	2.32±0.06	325.63±90.43	30899.16±843.92	2.32±0.92	2.39±0.19	20.27±6.68	6781.29±2772.71
TSs67	1.57±0.1	2.47±0.04	282.8±104.39	32877.37±474.88	1.55±0.91	2.2±0.10	27.3±6.03	8158.86±2112.54
TSs68	1.11±0.47	2.41±0.14	268.33±85.63	32026.41±1907.58	1.89±0.55	2.21±0.1	12.63±0.29	3717.22±175.60
TSs76	0.82±0.22	2.22±0.17	200.3±28.21	29518.57±2317.07	1.76±0.67	2.18±0.19	22.57±1.65	6600.2±1001.25
TSs77	0.9±0.46	2.59±0.11	119.83±39.74	34450.91±1424.65	1.84±1.13	2.28±0.06	10.8±1.36	3296.8±491.29
TSs81	1.88±0.28	2.52±0.12	165.6±40.33	33508.96±1532.99	1.47±0.90	1.99±0.12	8.3±4.18	2317.78±1184.37
TSs82	1.66±0.09	2.44±0.06	263.29±27.75	32394.93±789.37	2.39±0.71	2.09±0.06	17.5±3.48	4819.74±865.22
TSs9	2.03±0.42	2.25±0.01	249.2±86.03	29904.37±71.76	1.45±0.6	2.22±0.09	14.33±3.34	4184.97±883.73
TSs98	1.62±0.97	2.37±0.06	312.67±18.08	31497.25±733.01	1.3±0.35	2.08±0.06	15±3.72	4211.12±1165.74
F Statistics	0.635^{ns}	0.718 ^{ns}	0.289^{ns}	0.718^{ns}	0.106 ^{ns}	0.836 ^{ns}	0.050 ^{ns}	0.058 ^{ns}

Key: SDW=Shoot dry weight; RDW=Root dry weight: SE=Standard Error; N=Nitrogen; ns=Not significant.

Table 5.9: Comparison of C/N and δ ^{13}C of shoot and root

ACCESSION	Shoot C/N	Shoot δ^{13}C	Root C/N	Root δ ^{13}C
TSs1	16.74	-31.15	21.48	-30.39
TSs101	16.56	-31.07	17.98	-30.53
TSs104	16.62	-31.30	18.88	-31.06
TSs109	16.88	-31.15	20.18	-30.67
TSs11	16.98	-31.26	20.02	-30.91
TSs16	16.50	-31.45	18.12	-30.70
TSs23	16.75	-31.32	19.08	-30.96
TSs24	16.77	-31.21	18.65	-30.90
TSs27	17.62	-31.38	19.78	-30.96
TSs3	15.90	-31.03	19.27	-30.82
TSs30	16.23	-31.11	19.14	-30.32
TSs33	17.18	-31.28	19.46	-30.46
TSs38	17.12	-31.31	18.64	-30.87
TSs4	17.12	-30.93	19.87	-30.20
TSs44	16.98	-31.31	18.43	-30.80
TSs61	16.99	-31.08	18.75	-30.63
TSs66	17.24	-31.22	16.95	-30.80
TSs67	16.19	-31.39	18.25	-30.80
TSs68	16.74	-31.37	18.15	-31.16
TSs76	18.23	-31.26	18.62	-30.81
TSs77	15.49	-31.06	17.55	-30.93
TSs81	15.95	-31.50	20.21	-30.95
TSs82	16.44	-31.24	19.15	-30.71
TSs9	17.79	-31.29	18.10	-30.73
TSs98	16.91	-31.49	19.27	-30.60
F Statistics	0.80^{ns}	0.87 ^{ns}	0.14 ^{ns}	0.71 ^{ns}
C.V (%)	7.09	-1.38	10.54	-1.75

Key: C/N=Carbon Nitrogen ratio; C= Carbon**.**

Table 5.10: Comparison of the mean of $\delta^{15}N$, $\delta^{13}C$, C/N of reference plants

Plant part	**C/N**	**δ^{13}Ccorr**	**δ^{15}N**	**%C**	**%N**	**Reference plant species**
Shoot	31.0	-12.9	3.4	40.0	1.3	*Zea mays*
Root	99.9	-12.5	3.2	40.0	0.4	*Z.mays*
Shoot	12.1	-18.9	5.5	40.0	3.4	*Eleusine indica*
Root	25.3	-24.0	2.7	40.0	1.6	*E. indica*
Shoot	12.1	-24.4	4.9	40.0	4.0	*Tridax procumbens*
Root	26.5	-19.3	3.9	40.0	1.6	*T. procumbens*

Table 5.11: Correlation analysis of African yam bean accessions

	CN	δ13Ccorr	δ15N	N	SDW(g)	Total Nha	δ15NE	NdfAE	ENFkgNha	δ15NM	NdfAM	MNFkgNha	δ15T	NfAT	TNFkgNha
CN	1	-0.33*	-0.188*	-0.980*	-0.421*	-0.597*	-0.408*	-0.026*	-0.437*	-0.408*	0.207*	-0.287*	-0.408*	0.136*	-0.426*
δ^{13}Ccorr		1	0.215*	0.334*	-0.215*	-0.227*	-0.391*	-0.388*	-0.319*	-0.391*	-0.206*	-0.265*	-0.391*	-0.275*	-0.285*
δ^{15}N			1	0.202*	0.057^{NS}	0.026^{NS}	-0.040*	-0.845*	-0.290*	-0.040*	-0.996*	-0.510*	-0.040*	-0.982*	-0.419*
N				1	0.420*	0.598*	0.392*	0.011^{NS}	0.428*	0.392*	-0.219*	0.270*	0.392*	-0.150*	0.412*
SDW(g)					1	0.804*	0.796*	0.356*	0.709*	0.796*	0	0.488*	0.796*	0.062^{NS}	0.648*
Total N ha						1	0.962*	0.441*	0.902*	0.962*	-0.064*	0.665*	0.962*	0.102*	0.844*
δ^{15}NE							1	0.507*	0.915*	1*	0.001*	0.693*	1*	0.170*	0.852*
NdfAE								1	0.615*	0.507*	0.842*	0.625*	0.507*	0.925*	0.683*
ENF kg N ha									1	0.915*	0.235*	0.915*	0.915*	0.372*	0.984*
δ^{15}NM										1	0.001*	0.693*	1*	0.171*	0.852*
NdfAM											1	0.446*	0.001*	0.983*	0.365*
MNF kg N ha												1	0.693*	0.523*	0.950*
δ^{15}T													1	0.171*	0.852*
NfAT														1	0.486*

5.4 Discussion

The experimental site had suitable levels of carbon, nitrogen, phosphorus, calcium, magnesium and iron (Table 5.1). There were significant differences among accessions in nodulation parameters. TSs44 had a significantly higher number of nodules than other accessions at 169.67 while TSs23 had the lowest number of nodules at 58.42. TSs1**,** though not significantly different from some other accessions, had the highest nodule fresh weight at 12.94 and TSs76 had the lowest at 6.24. Also, TSs1 also had the highest nodule dry weight at 2.72 while TSs76 had the lowest at 2.71 (Table 5.2).

There were significant differences among accessions regarding growth parameters of African yam bean (Table 5.3). TSs67 had a significantly higher fresh shoot weight, dry shoot weight, and dry root weight than other genotypes at 516.00 g, 219.1 g, and 14.70 g with a total biomass of 233.45 g. Significant variation was also found in the fresh and dry root weights among the accessions with TSs44 having the highest value, 88.09 g, and TSs16 the lowest 46.56 g. However, TSs77 had the lowest value for fresh shoot weight at 185.43 g and TSs44 had the lowest total biomass at 67.79 g (Table 5.3).

The number of nodules was strongly and positively correlated with nodule fresh weight (r= 0.415) and nodule dry weight (r=0.433) (Table 5.4). Nodule fresh weight was positively and strongly associated with nodule dry weight at r= 0.882. The relationship between fresh shoot weight and other growth parameters was positively and significantly correlated. Fresh shoot weight correlated with fresh root weight at r=0.398; dry shoot weight at r= 0.685; dry root weight at r=0.545, and total biomass at r=0.699. Fresh root weight also correlated positively and significantly with dry shoot weight at r=0.567, dry root weight at r=0.426, and total biomass at r=0.577. Dry shoot weight also significantly and positively correlated with dry root weight at r=0.430 and total biomass at r=0.988 while dry root weight correlated significantly and positively with total biomass at r=-0.479.

The principal component axis (PCA) obtained from the nodulation and plant growth parameters of African yam bean showed variations in Eigen value and proportion as 0.82 (82%) and 0.11 (9.4%) indicating the first two PCAs when the cut-off was at 10% while other components 3 - 8 were less than 4% (Table 5.5).

The first PCA contributed the maximum towards variability (82 %) and was closely related to plant growth parameters. The second PCA was related to the variation among African yam bean accessions owing to the number of nodules (34.9) and fresh root weight (11.2).

A positive correlation that also exists between number of nodules and fresh shoot weight indicated that the nodules were actively fixing N which in turn led to an increase in plant biomass. The highest proportion and Eigen vector were accounted for by the variation of African yam bean nodulation in PRIN 1 which should be considered in crop improvement purposes. It also confirms the varying responses of accessions to nodulation.

In this study nodulation was done manually by counting the total nodules excised from the roots through the use of a laboratory counter. This approach is prone to errors and may not provide information about the distribution of nodules in the root system. Therefore there is a need to use electronic device for data capturing and estimating nodule distribution patterns and basic structure of the plant by using an image analysis device. The electronic device should be able to clearly show phenotypic differences in the pattern of nodulation among genetically diverse accessions in environmental conditions. This strategy may make it feasible to determine the relationships among nodulation patterns, root system, and eventually plant yield.

There was variability in the nodulation and plant growth ability of the African yam bean accessions evaluated. The nodulation capability is high when compared with other legumes. The low N content of the soil during planting indicates that the majority of the N content

observed was derived from the atmosphere by the activity of the N-fixing bacteria in the nodules. The high N content of the plant makes it an additional protein source for tropical African countries. Adequate protein and other mineral constituents of the crop can be obtained from the seeds and since the seeds and swollen roots are edible, appropriate protein and other vital nutrients can be obtained as most of the N in legume plants has been observed to be exported to the grain.

A comparison of growth in African yam bean between the studied accessions showed they had differences in dry weight but not statistically different from one another in shoot and root dry weights measured. The shoot dry weight ranges from 344 g^{-1} in TSs44 to 101.3 g^{-1} in TSs101 while the dry root weight for TSs67 and TSs81 recorded the highest values at 27.3 to 8.3 g^{-1} (Table 5.3).

The carbon (C) and nitrogen (N) ratio is an indicator of C and N relationships in plants. It is also used in determining photosynthetic N use-efficiency. Significant differences were recorded in C/N ratios among the accessions studied. In general, carbon (C) and nitrogen (N) ratio ranged from 21.5 (TSs1) to 17 (TSs66) for the shoots and from 18.23 (TSs76) to 15.49 (TSs77) (Table 5.9) for the roots.

The $\delta^{15}N$ signatures of the legume differed among accessions and varied from 2.52 (TSs61) to 0.24 (TSs44) in the shoots and from 2.70 (TSs98) to 0.82 (TSs16) for the roots. The percentage Ndfa of African yam bean shoots also varied significantly among the reference plants used for estimation. TSs76 had the highest percentage Ndfa of 66.73, 51.83, and 63.48; followed by TSs4 with 66.18, 51.03, and 62.87 while the lowest was TSs1 with 40.07, 13.22, and 34.21 when *E. indica*, *Z. mays*, and *T. procumbens* were used for measurement respectively (Table 5.6). For the root, percentage Ndfa was observed to be highest in TSs44 with 66.6, 74.54, and 70.63 and lowest for TSs61 at 5.36, 27.86, and 16.77 for *E. indica*, *Z.*

mays, and *T. procumbens.* This shows that the legume obtains a considerable larger amount of N from the atmosphere in the shoot than in the root.

Two weed plant species and maize were used as reference plants. Recent studies have shown that weeds can be used to determine legume's dependence on N fixation for its own N nutrition (Mohale *et al*., 2014). $\delta\ ^{15}N$ and other parameters analyzed for the reference plants are shown in Table 5.10.

The quantity of N fixed differed significantly ($p \leq 0.05$) between accessions. For example, in the shoot, TSs77 had the highest amount of N fixed at 22.47 kg ha^{-1} and the lowest was TSs1 at 12.76 kg ha^{-1}. In the root TSs67 had the highest at 0.08 kg ha^{-1} and TSs81 had the lowest at 0.02 kg ha^{-1} (Figs 6.3 & 6.4). Similar results was obtained by (Kermah et al., 2017a) where legumes fixed an average of 11 and 31 kg ha^{-1} more N_2 than in poorly fertile fields in the southern Guinea savanna (SGS) and northern Guinea savanna (NGS) of Ghana. The amount of N_2 fixed varied significantly between sites and among legume species. N_2 fixed by cowpea and groundnut was 13 and 9 kg ha^{-1} respectively larger in the SGS than in the NGS while 31 kg ha^{-1} more N_2 was fixed by soybean in the SGS. The amount of fixed-N in soybean shoots also varied significantly across 37 farmers' fields from 22 kg ha^{-1} for PAN1583 at Bergville field 2 to 298 kg ha^{-1} for PAN1666 at Potchefstroom field 3. The observed N contribution by soybean was highest in Potchefstroom and Parys in the North-West Province of South Africa (Mapope and Dakora, 2016).

In a related study by Mohale *et al*. (2014), the amount of N-fixed on farmers' field differed remarkably when Bambara groundnut farms were surveyed. Symbiotic N contribution of Bambara groundnut ranged between 4 and 200 kg ha^{-1} across 26 fields in South Africa.

There was no difference in C concentration among the accessions. The average value obtained was 40%. Analysis of variance of $\delta^{13}C$ from the shoots and roots revealed a

significantly marked variation among accessions under investigation. The $\delta^{13}C$ values of shoots less negative (much greater) while the values for the roots also varied considerably. However, the accessions that showed much lower values were observed to be -30.93 (TSs4) and -31.03 (TSs3) in the shoots. Consequently, the $\delta^{13}C$ values of shoots ranged from -31.49 (TSs98) to -30.93 (TSs4) and those of roots from -31.16 (TSs68) to-30.20 (TSs4) (Table 5.4). These findings are similar to that obtained by Mohale *et al.*, (2014); Mapope and Dakora, (2016);(Kermah et al., 2017a; Mapope and Dakora, 2016; Mohale et al., 2014). Percentage N correlated positively with dry weight (r= 0.42*); Total N (r=0.59*) with $\delta^{15}N$ (r=0.20*). The $\delta^{13}C$ correlated positively with percentage N (r=0.33*), with $\delta^{15}N$ (r= 0.21*). N-fixed and percentage N was positively correlated (r= 0.42*) while dry weight also correlates positively with Total N/ha (r=0.804*); $\delta^{15}T$; $\delta^{15}NE$; $\delta^{15}NM$ (r=0.79*) (Table 5.11).

Carbon accumulation in plant species is a function of its photosynthetic activity (Belane *et al.*, 2011). The C concentrations for the legume were 40% which is comparable to those reported for other symbiotic legumes (Mohale *et al*., 2014). The lower C accumulation in plants is maintained by N nutrition. The C and N ratio is undoubtedly a good estimate of the N status. Because bacteriodes in root nodules of symbiotic legumes reduce atmospheric N_2 to NH_3, N_2-fixing species tend to have C and N values less than 24 gg^{-1} and non-legumes with higher values (Mohale *et al*., 2014). The non-legumes used as reference plants had over 24 gg^{-1} while the accessions analysed had lower values (Table 5.9) results which are similar with previous studies (Mohale *et al*., 2014). While the $\delta^{15}N$ values were decreasing, there were corresponding increase in the C and N ratio (Table 5.9). These outcomes support the opinion that photosynthetic activities in the underutilized legume were stimulated by symbiotic N nutrition.

The $\delta^{13}C$ of C3 plants is a known measure of water-use efficiency in those species (Mohale *et al.*, 2014). In this study, the variation in $\delta^{13}C$ of the underutilized legume ranged from -31.16 to -30.20 for the shoots and from -31.49 to -30.93 for roots, thereby determining the level of water usage of the accessions. Surprisingly, TSs68 showed higher values than TSs 77 which recorded the highest N contribution to the site. This means that there is a normal relationship between water-use and N_2 fixation, and ultimately nodule performance. There was positive correlation between $\delta^{13}C$ and dry weight (r=-0.21*); N content, and dry weight yield (r=0.42*), as well as a perfect correlation between N-fixed and Total Nha content (r=0.90*; 0.67* and 0.84*). When all these are considered, the results suggest strong relationships between N nutrition and water-use, N_2 fixation and photosynthesis, and water-use and plant growth in the underutilized legume.

Based on drought activities, there are usual increase in $\delta^{13}C$ of C3 plants (Vandoorne *et al*., 2012) in the same way that they increase their $\delta^{13}C$ values when faced with low water supply (Hartman and Danin, 2010). It can be suggested that the high $\delta^{13}C$ values of the underutilized legume were due to low soil moisture due to uneven rainfall. When appropriate plant density is used, the legume has the potential to become an important food crop with considerable biofertilizer effects in sub-Saharan Africa. It is also important to screen and identify African yam bean landraces with superior N fixing capacity and water-use efficiency for increased yield particularly in the current climate situation.

The importance of nodulation and N fixation to agriculture, natural ecosystems, and the global N cycle is indisputable (O'Rourke *et al*., 2013). The applications of large quantities of N fertilizers are evident in environmental degradation such as leaching of nitrates into the groundwater and development of soil acidity (Ridley *et al*., 2004). Most farmers cannot afford these fertilizers owing to exorbitant prices except in cases where Governments subsidize them. Judging by the negative environmental concerns and associated cost of the increased use of N fertilizers in agriculture, there is a renewed mandate that alternatives be urgently provided (Aliyu *et al*., 2013).

The result of the study showed that TSs44, TSs16, TSs98, TSs109, TSs81, TSs67, and TSs3 all had a very high mean numbers of nodules. The significant mean squares of number of

nodules indicated variability among the accessions used in this study with regard to nodulation capability. Significant differences in the number and biomass of nodules among crop landraces of the same species have been reported (Egbe *et al*., 2013). TSs67 had the highest biomass production which was reflected in its high value for both dry shoot weight and dry root weight. A strong and positive correlation between dry shoot weight and dry root weight indicated that the shoot mass production is affected by the root mass in African yam bean and therefore it can be said that as the root mass increases, the shoot mass increases, and vice versa.

CHAPTER SIX

Nodulation, N fixation, and water-use efficiency potential of different accessions of winged bean (*Psophocarpus tetragonolobus* (L.) DC.)

Abstract

Underutilized legumes are domesticated legumes with beneficial attributes, but unfortunately have largely gone igonored by researchers and the industries. Their ability to adapt to extreme soils; varying climatic conditions; and to tolerate abiotic stresses, potentially position them to combat global climate associated stresses. In this study, the natural isotopes of N and C were used to determine N-fixing potential and water-use efficiency of selected accessions of field-grown winged bean (*Psophocarpus tetragonolobus* (L.) DC.)**.** The field experiment was set up in a randomized complete block design (RCBD) with three replications on an Alfisol soil of the Egbeda series. *Zea mays* and two weed species (*Tridax procumbens* and *Eleusine indica)* were sampled as reference plants. The data obtained revealed significant differences in all the parameters measured in the plant shoots and roots. The amount of N fixed, C/N ratio, and $\delta^{13}C$ varied among the selected accessions. The results showed that natural isotopes can be used to assess N fixation, water-use efficiency and N-nutrition in field-grown accessions of winged bean.

Keywords: BNF, nodules, $\delta^{15}N$, underutilized legume, sub-Saharan Africa, winged bean,

6.1 Introduction

Underutilized legumes are a set of orphan crops that tend to be regionally significant but not traded globally and therefore have received less attention from research. They are widely distributed beyond the centers of their origin and are important for the subsistence of local populations yet they remain poorly documented and neglected by mainstream research and development programs (Cullis and Kunert, 2017).

Several issues have been raised about what makes a crop either underutilized or orphan. The crops are usually linked with the traditional heritage of their places of origin, poorly documented as to their use and cultivation, and also adapted to specific agro-ecological niches with a weak seed supply system. They can also be used as animal feed and other agricultural applications to provide income for resource-poor farmers (Cullis and Kunert, 2017). Owing to their lack of strong economic value, they have been abandoned by both the international scientific community and by industry compared with other commodities such as maize, rice, and wheat (Foyer *et al*., 2016a). This neglect has led to a weak genetic improvement program giving rise to lower yield.

Winged bean is a self-pollinating orphan crop. It possesses a twining habit, swollen tuberous roots, and pods with both annual and perennial growth forms (Vatanparast *et al*., 2016). Every part of the plant is edible. This exceptional nutritional quality and the fact that the plant provides sources suitable for human food at all stages of its life cycle make it a promising candidate for more widespread use in protein deficient parts of the world. The young pods contain 2.4 g of protein per 100 g; the dried tubers contain 8–20% of protein and the seeds 34% , as well as high oil contents (18%) (Amoo *et al*., 2006). If both seed and swollen tuberous yields are combined, it can out-yield many other legume crops that are conventionally grown in the tropics and thus offer a cheap nutritional food source. Therefore, it is projected as a promising alternative to soybean in areas where soybean cultivation

success is marginal. It is very challenging to find a high rainfall-adapted tropical legume with as many desirable features as this crop (Vatanparast *et al*., 2016). Consequently, efforts need to be geared up in terms of breeding activities, particularly in developing determinate, self-supporting varieties, producing large numbers of small pods with nutritious seeds and swollen roots that will be resistant to environmental stresses. Above all, the genetic improvement of underutilized crop such as winged bean can be enhanced with the use of current molecular tools that would provide an opportunity for the release of newly improved varieties (Sharma *et al.*, 2014).

Studies on genomic resource development that provides information on genetics, evolution, ecology, and molecular breeding programs are lacking in winged bean. Hence the need to explore genomic technologies to support significant possibilities for winged bean crop improvement (Egan *et al*., 2012). Furthermore, the incorporation of legumes in tropical agriculture is a way of providing a sustainable and cheap way of benefiting from atmospheric N. Reports have shown legumes such as cowpea, groundnut and soybean can supply suitable amounts of N (Mohale *et al*., 2014; Pule-Meulenberg *et al.*, 2010). The aim of this study was to evaluate N fixation and water-use efficiency on field-grown accessions of winged bean.

6.2 Material and Methods

6.2.1 Experiment site, plant sample collection, and processing

On-station field experiments were conducted at IITA Nigeria (Latitude 7°30'8'';; Longitude 3° 54' 37''). Monthly rainfall ranged between 0.05 and 86.5 mm while the minimum and maximum temperatures ranged between 20 and 27 °C and from 24 to 35.2 °C respectively. Seeds of 25 accessions of winged bean were collected from the GRC of IITA (Table 6.1). Two seeds manually scarified with a sterile surgical blade were sown per hill in a randomized complete block design (RCBD) with three replications. Twelve weeks after planting, one plant was dug out from each replicate for nodule count, nodule fresh weight, fresh shoot weight, and fresh root weight. The process involved initially breaking the soils around the plants to a depth of about 20 cm with a garden fork making sure their roots were not disturbed. The plants were then pulled out gently and the root region cut out with a sharp cutlass; shoot and root from individual plant were labelled and packed separately, roots into paper bags and shoots into net bags. Roots were taken to the Soil Microbiology Laboratory of IITA and washed with water to remove soil particles. The nodules on the roots and those that had broken off in the course of washing were picked, counted, and weighed to obtain fresh nodule weight. These were put in smaller envelopes and oven-dried at 70 ^{0}C for 72 hr after which they were weighed on a sensitive electronic balance for dry nodule weight. The roots were also bagged separately, weighed to obtain fresh root weight, and oven-dried at 70 ^{o}C for 72 hr to obtain the dry root weight. The same procedure was used to obtain the shoot dry weight; the total biomass of each plant was calculated.

Plant shoots were oven dried and the leaves were separated into stems and petioles and ground. The tissues with the leaves were used in plant N determination. The shoots and roots were oven dried at 60 °C for 48 hr. Thereafter, they were weighed, and milled to fine powder (0.85 mm sieve) for ^{15}N and ^{13}C isotope analysis. The analysis of the plant materials for ^{15}N

and ^{13}C was carried out by mass spectrometry at the Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven, Belgium.

6.2.2 Planting materials and reference plants

Winged bean seeds of 25 accessions were collected from the GRC of IITA as follows: Tpt2,Tpt4,Tpt6,Tpt10,Tpt11,Tpt12,Tpt14,Tpt15,Tpt16,Tpt17,Tpt18,Tpt19,Tpt30, and Tpt32. Others are Tpt33, Tpt42, Tpt43, Tpt48, Tpt51, Tpt125, Tpt126, Tpt154, Tpt15-4, and Tpt3-B. Non-fixing plants, *Zea mays* (maize) and two weed species (*Tridax procumbens* and *Elusine indica*) were samples concurrently from the field and processed for ^{15}N and ^{13}C isotope analysis as described for the legume.

6.2.3 Measurement of N_2 fixation

$^{15}N/^{14}N$ analysis

The finely prepared plant material was weighed into Aluminium tin capsules (0.5-1 mg) and analyzed for %N and $^{15}N/^{14}N$ ratio using a Thermo EA-1110 elemental analyzer (FORNO EA/NA ThermoQuest, Italia, S.P.A) coupled to a mass spectrometer via Conflo II open-split device. ^{15}N natural abundance is expressed as δ (delta) notation and is the per mille deviation of the ^{15}N natural abundance of the sample from atmospheric (atm) N_2 (0.36637 atom % ^{15}N).

The isotopic composition (δ15N) was determined as described by Unkovich *et al*. (2008)

$$\delta^{15}N = \frac{(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{atm}}{(^{15}N/^{14}N)\ atm} \times 1000$$

Where $^{15}N/^{14}N$ sample is the abundance ratio of ^{15}N and ^{14}N in the shoot and root samples. The $^{15}N/^{14}N$atm is the abundance ratio of ^{15}N and ^{14}N in the atmosphere.

6.2.4 Percentage N derived from atmosphere

The percentage N derived from atmospheric N_2 was calculated using the equation of Unkovich *et al*. (2008)

$$\%\ Ndfa = \frac{\delta^{15}N\ ref - \delta^{15}N leg}{\delta^{15}N\ ref - B} \times 100$$

where the $\delta^{15}N$ ref is the ^{15}N natural abundance of reference plants, $\delta^{15}N$ leg is the ^{15}N of the legume, and the B value is the ^{15}N of the test legume solely dependent on N_2 fixation. The B value replaces the value of atmospheric N_2 (Unkovich *et al*., 2008). In this study, the B value used was −1.54‰ as explained by Unkovich *et al*. (2008). The amount of N-fixed was as estimated by Unkovich *et al*. (2008)

$$Nfixed = amount\ of\ N \times \%\ NdfA$$

6.2.5 $^{13}C/^{12}C$ isotopic analysis

The ratio of $^{13}C/^{12}C$ in each sample was used to calculate the ^{13}C natural abundance ($\delta^{13}C$) as described for $^{15}N/^{14}N$ analysis (Stout and Rafter, 1978).

$$\delta^{13}C = \left(\frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{standard-1}}{(^{13}C/^{12}C)_{standard}} \right) 1000$$

$^{13}C/^{12}C$ sample is the isotopic ratio of the sample, and $^{13}C/^{12}C$ standard is the isotopic ratio of known universally accepted standard for C.

6.2.6 Statistical analysis

Analysis of variance (ANOVA) using Statistical analysis software (SAS, version 9.4) was used to analyze data obtained. Where significant differences were found, the means were separated using Fisher's least significance difference (LSD) test at $p \leq 0.05$.

6.3 Results

Table 6.1: Mean number of nodules, nodule dry and fresh weights

Accession	NON	NFW(g)	NDW(g)
Tpt15	607.42	21.25	4.54
Tpt18	477.75	17.45	4.14
Tpt126	469.42	21.78	5.03
Tpt30	464.50	20.89	4.77
Tpt32	434.92	39.74	3.67
Tpt15-4	400.50	11.01	3.10
Tpt4	392.08	14.30	3.76
Tpt19	381.08	14.41	4.20
Tpt11	368.08	15.16	3.86
Tpt48	359.42	11.98	2.98
Tpt12	352.17	16.02	4.15
Tpt2	332.17	12.91	3.54
Tpt6	327.50	14.33	3.39
Tpt16	326.83	14.07	3.79
Tpt42	320.83	12.66	2.90
Tpt125	312.08	12.50	2.86
Tpt33	301.92	11.64	3.19
Tpt14	295.83	10.52	2.94
Tpt17	293.25	13.36	3.00
Tpt10	290.67	15.09	3.29
Tpt43	288.58	17.50	4.06
Tpt53	276.17	14.02	3.54
Tpt51	251.07	12.67	3.48
Tpt154	219.25	10.85	3.16
Tpt3-B	207.33	10.75	2.54
F Statistics	0.05^{ns}	0.25^{ns}	0.59^{ns}
C.V (%)	71.90	122.64	64.40

Key: NON=Number of nodules; NFW=Nodule fresh weight; NDW=Nodule dry weight

Table 6.2: Means of plant growth parameters

Accession	FSW (g)	DSW(g)	FRW (g)	DRW(g)	TBM(g)
Tpt15	1015.62	329.38	101.61	17.06	118.67
Tpt18	1106.11	403.23	111.68	25.60	137.28
Tpt126	811.97	261.91	95.57	20.22	115.79
Tpt30	893.62	279.37	134.60	19.50	154.10
Tpt32	594.09	205.16	95.39	16.79	112.18
Tpt15-4	608.09	285.17	126.17	15.22	141.39
Tpt4	613.00	258.72	130.94	20.98	151.92
Tpt19	366.99	149.73	106.52	15.57	122.08
Tpt11	759.25	226.80	119.71	19.52	139.23
Tpt48	503.50	255.10	104.11	14.87	118.98
Tpt12	710.14	173.17	97.40	15.45	112.84
Tpt2	565.50	275.99	139.34	18.32	157.66
Tpt6	703.10	210.38	106.87	13.80	120.67
Tpt16	901.13	260.47	111.57	14.85	126.41
Tpt42	799.31	174.50	173.16	36.47	209.63
Tpt125	847.32	118.32	117.57	24.00	141.57
Tpt33	879.71	259.36	88.56	17.84	106.40
Tpt14	769.09	232.87	115.35	24.11	139.46
Tpt17	691.83	176.83	74.95	14.20	89.15
Tpt10	613.11	211.61	123.82	19.71	143.52
Tpt43	924.03	256.50	100.40	16.77	117.17
Tpt53	674.83	137.82	78.24	14.98	93.21
Tpt51	708.63	152.55	109.33	18.66	128.00
Tpt154	608.86	149.79	69.75	10.37	80.12
Tpt3-B	931.07	154.45	81.71	28.75	110.46
F Statistics	0.86^{ns}	0.01*	0.95^{ns}	0.51^{ns}	0.52^{ns}
C.V (%)	96.60	78.00	96.50	103.40	90.50

Key: FSW=Fresh shoot weight; FRW= Fresh root weight; DSW=Dry shoot weight; DRW=Dry root weight; TBM=Total biomass.*=Mean values of accessions are significantly different at $p \leq 0.05$ and ns=not-significant.

Table 6.3: Pearson correlation coefficients of nodulation and growth parameters

	NON	NFW	NDW	FSW	FRW	DSW	DRW	TBM
NON	1	0.56*	0.71**	0.24^{ns}	0.23^{ns}	0.68*	-0.01^{ns}	0.67*
NFW		1	0.51^{ns}	0.06^{ns}	-0.07^{ns}	0.2^{ns}	-0.09^{ns}	0.19^{ns}
NDW			1	0.21^{ns}	0.03^{ns}	0.45*	-0.22^{ns}	0.43*
FSW				1	-0.01^{ns}	0.44*	0.41*	0.47*
FRW					1	0.24^{ns}	0.56*	0.29^{ns}
DSW						1	0.02^{ns}	0.99**
DRW							1	0.11^{ns}
TBM								1

Key: *p ≤0.05 significant, **p≤0.0001 is highly significant

FSW = Fresh shoot weight; FRW= Fresh root weight; DSW= Dry shoot weight; DRW= Dry root weight;

TBM =Total biomass.

Table 6.4: Contribution of principal component axis (pca) to the variation of nodulation and plant growth parameters of winged bean

Trait	Prin1	Prin2	Prin3	Prin4
NON	0.47	-	-	-
NFW	-	-	-	0.50
NDW	0.38			
FSW	-	-	-	0.53
FRW	-	-	0.58	-
DSW	0.47	-	-	-
DRW	-	0.63	-	0.41
TBM	0.48	-	-	-

Key: FSW=Fresh shoot weight; FRW=Fresh root weight; DSW=Dry shoot weight; DRW= Dry root weight; TBM=Total biomass.

Table 6.5: Eigen values of the correlation matrix

Principal axis	Eigen value	Difference	Proportion	Cumulative
1	3.47	1.66	0.43	0.43
2	1.80	0.76	0.22	0.65
3	1.04	0.15	0.13	0.79
4	0.89	0.44	0.11	0.90

Table 6.6: Comparison of %N derived from the atmosphere and N-fixed (kg N/ha)

ACCESSION	%NFAT	%NFAM	%NFAE	ACCESSION	EFIXED	MFIXED	TFIXED
Tpt3-B	62.81	50.95	66.12	Tpt32	27.16	20.75	25.76
Tpt32	62.13	50.04	65.50	Tpt15-4	25.66	19.09	24.23
Tpt15-4	60.03	47.28	63.59	Tpt12	25.62	18.69	24.11
Tpt12	58.86	45.74	62.53	Tpt53	24.43	16.41	22.68
Tpt51	55.07	40.74	59.07	Tpt51	23.54	16.30	21.96
Tpt154	54.85	40.44	58.86	Tpt3-B	22.25	17.12	21.13
Tpt125	54.17	39.55	58.25	Tpt154	22.62	15.60	21.09
Tpt14	54.09	39.45	58.18	Tpt125	21.58	14.85	20.11
Tpt53	52.88	37.84	57.07	Tpt19	20.91	13.49	19.29
Tpt48	51.26	35.71	55.60	Tpt14	20.75	14.06	19.29
Tpt19	51.15	35.57	55.50	Tpt48	20.90	13.46	19.28
Tpt11	47.43	30.66	52.11	Tpt43	20.68	11.55	18.69
Tpt4	47.34	30.53	52.02	Tpt11	19.89	11.88	18.14
Tpt43	45.56	28.19	50.41	Tpt4	19.66	11.50	17.88
Tpt10	43.49	25.47	48.52	Tpt2	19.65	9.67	17.47
Tpt2	42.06	23.58	47.22	Tpt10	17.55	9.38	15.77
Tpt16	41.59	22.96	46.79	Tpt16	16.83	8.31	14.97
Tpt42	35.67	15.14	41.39	Tpt17	16.39	4.58	13.81
Tpt33	33.09	11.74	39.04	Tpt42	15.98	5.83	13.77
Tpt17	32.93	11.53	38.90	Tpt15	16.38	3.81	13.64
Tpt126	32.43	10.88	38.44	Tpt33	15.48	3.90	12.95
Tpt6	32.04	10.36	38.09	Tpt6	15.02	4.08	12.63
Tpt15	30.75	8.65	36.91	Tpt126	13.91	3.88	11.72
Tpt30	24.97	1.03	31.64	Tpt18	11.93	0.05	9.34
Tpt18	24.31	0.17	31.05	Tpt30	11.49	0.17	9.02
F Statistics	0.03*	0.03*	0.03*	*F Statistics*	0.07^{ns}	0.06^{ns}	0.07^{ns}
C.V (%)	33.30	71.62	27.40	C.V (%)	29.60	75.75	35.60

Key: NFAT=*Tridax procumbens*; E=*Eleusine indica*; M= Maize; EFIXED=*E. indica*; T=*T. procumbens*; M=maize

Table 6.7: Comparison of $\delta^{15}N$, percentage N, dry weight, and total N of winged bean shoot and root

ACCESSION	Shoot-$\delta^{15}N$	N	SDW	TOTAL NHA	Root- $\delta^{15}N$	N	RDW	TOTAL NHA
Tpt10	2.10	2.69	311.57	35773.89	2.52	2.75	25.10	9668.29
Tpt11	1.85	2.84	268.80	37781.49	2.51	2.65	24.73	8804.23
Tpt12	1.11	3.09	267.93	41094.34	2.22	2.19	19.27	5559.89
Tpt125	1.42	2.75	58.87	36624.92	2.26	2.60	27.30	9479.04
Tpt126	2.82	2.73	182.93	36287.07	2.43	2.55	29.80	10180.94
Tpt14	1.42	2.68	344.57	35706.25	1.04	1.70	30.10	7710.93
Tpt15	2.93	3.34	417.87	44441.75	3.07	2.82	28.23	10849.16
Tpt15-4	1.04	3.03	324.30	40341.40	2.58	2.85	22.27	8678.21
Tpt154	1.37	2.88	238.40	38294.29	1.80	2.33	13.53	3793.75
Tpt16	2.23	2.70	299.77	35863.13	1.73	2.61	26.87	9483.20
Tpt17	2.79	3.21	216.17	42752.44	1.90	2.31	12.13	3747.34
Tpt18	3.34	2.89	391.83	38464.83	2.17	2.60	23.17	8076.50
Tpt19	1.61	2.82	277.53	37501.12	0.99	2.56	15.10	5366.11
Tpt2	2.20	3.15	391.03	41925.39	2.52	2.71	15.30	5543.66
Tpt3-B	0.86	2.53	228.60	33694.16	1.96	2.33	23.93	7357.62
Tpt30	3.30	2.77	336.23	36774.71	2.56	2.69	29.10	10350.88
Tpt32	0.90	3.12	225.36	41471.63	0.49	2.47	14.93	4902.89
Tpt33	2.78	3.11	229.00	41336.25	2.37	2.60	25.60	8752.89
Tpt4	1.86	2.85	380.03	37861.05	2.73	2.85	28.57	10834.99
Tpt42	2.61	2.91	217.17	38652.51	1.81	2.62	26.27	9200.24
Tpt43	1.97	3.09	338.43	41064.04	1.44	2.62	19.73	6856.06
Tpt48	1.60	2.82	359.10	37513.24	2.31	2.69	18.17	6389.20
Tpt51	1.36	2.98	205.93	39696.18	2.59	2.80	11.30	4315.01
Tpt53	1.50	3.18	297.67	42318.94	1.59	2.26	21.23	6367.13
Tpt6	2.84	2.97	206.53	39454.30	1.78	1.72	22.77	7906.69
F Statistics	*0.03**	*0.02**	*0.40^{ns}*	*0.02**	*56.90^{ns}*	*19.70^{ns}*	*46.40^{ns}*	*52.50^{ns}*
C.V (%)	48.00	8.60	48.00	8.60	0.70	0.30	0.50	0.50

Key: N=Nitrogen; SDW=shoot dry weight; RDW=root dry weight; *p ≤0.05 significant; ns=not significant.

Table 6.8: Comparison of C/N and δ13C of winged bean shoots and roots

ACCESSION	Shoot CN	δ13C	Root-CN	δ13C
Tpt10	15.07	-29.94	14.94	-29.29
Tpt11	14.12	-30.18	15.08	-28.99
Tpt12	12.98	-30.00	18.33	-28.84
Tpt125	14.58	-30.41	15.41	-29.60
Tpt126	14.68	-30.03	15.77	-29.10
Tpt14	14.90	-30.06	10.91	-19.28
Tpt15	11.97	-30.11	14.31	-29.23
Tpt15-4	13.21	-30.24	14.21	-29.65
Tpt154	13.91	-30.43	17.41	-29.21
Tpt16	14.87	-30.39	15.64	-29.61
Tpt17	12.50	-30.12	17.47	-29.20
Tpt18	13.84	-29.95	15.38	-29.19
Tpt19	14.22	-29.62	15.68	-29.08
Tpt2	12.84	-30.16	14.81	-29.54
Tpt3-B	15.87	-30.20	17.66	-29.39
Tpt30	14.57	-30.03	14.95	-29.67
Tpt32	12.84	-30.19	16.57	-29.17
Tpt33	13.20	-29.83	15.58	-29.22
Tpt4	14.11	-30.32	14.03	-29.26
Tpt42	13.78	-30.22	15.31	-29.61
Tpt43	13.11	-30.46	15.34	-29.63
Tpt48	14.18	-30.60	14.88	-29.70
Tpt51	13.44	-30.36	14.31	-29.38
Tpt53	12.70	-30.59	17.83	-30.17
Tpt6	13.49	-30.15	10.36	-19.19
F Statistics	0.03*	0.01*	20.80^{ns}	-16.50^{ns}
C.V (%)	8.55	-0.90	0.40	0.40

Key: CN=Carbon Nitrogen; C=Carbon; *p ≤0.05 significant; ns=not significant.

6.4 Discussion

The soil used for the study is an Alfisol of the Egbeda series. Soil properties determined at a depth of 0-15 cm, were pH (H_20), 7.3; organic C, 1.97%, total N, 0.04%, and extractable P (Bray P), 45.97 mg/kg. Others were Ca, 2.36 cmol/kg; Mg, 0.69, cmol/kg; K,0.14 (cmol/kg); Na, 0.57 (cmol/kg); Mn, 168.95 ppm, Fe, 31.4 ppm, Cu, 1.35 ppm, and Zn, 5.1 ppm.

There were significant differences of accession on nodulation parameters of winged bean. Tpt15 had a significantly higher number of nodules than other accessions at 607.42 while Tpt3-B had the lowest at 207.33. Tpt32**,** though not significantly different from some other accessions, had the highest nodule fresh weight at 39.74 and Tpt14 had the lowest at 10.52. Also Tpt126 had the highest nodule dry weight at 5.03 while Tpt3-B had the lowest at 2.54 (Table 6.1).

Significant differences exist among accessions on some growth parameters of winged bean as shown in the table below. Tpt18 had significantly higher fresh shoot weight, dry shoot weight, and total biomass than other accessions. Statistically significant variation were recorded in the fresh and dry root weights among the accessions with Tpt42 and Tpt154 having the highest values of 173.16 g and 36.47 g. However, Tpt19 had the lowest value for fresh shoot weight at 366.99 g and Tp125 had the lowest total biomass at 142.33 g (Table 6.2).

Correlation of nodulation and growth parameters showed that the number of nodules was strongly and positively correlated with nodule fresh weight (r= 0.56); nodule dry weight (r=0.71); dry shoot weight (r=0.68), and total biomass (r=0.67). There was a positive correlation with fresh shoot weight (r=0.24) and fresh root weight (r=0.23). Nodule dry weight also correlated positively with dry shoot weight at r=-0.45; total biomass at r=-0.43. Fresh shoot weight correlated positively with dry shoot weight at r=0.44; dry root weight at r=0.41, and total biomass at r=0.47. Fresh root weight also correlated positively with dry root weight at r=0.56. Dry shoot weight positively correlates with total biomass at r=0.99 while dry root weight correlated non-significantly at r=0.02 (Table 6.3).

The principal component axis (PCA) obtained from the nodulation and plant growth parameters of winged bean showed variations in Eigen value and proportion as 0.63 (63%) and 0.38 (38%) indicating the first four PCAs when the cut-off was at 10%; other components 5-8 were less than 4% (Table 6.4) while the overall cumulative contribution of the principal component was 90% (Table 6.5).

The first PCA contributed the most towards variability and was highly related to plant growth parameters. The second PCA was related to variation among accessions owing to number of nodules and fresh root weight.

In current agricultural practices, the use of chemical fertilizers is on the increase with its resultant negative impact on the soil and environment. The increase in the amount of these chemical inputs has greatly affected the sustainability of the crop production systems and increased the cost of cultivation, thereby posing a very great danger to maintaining global food security amid a rising population. Therefore, there is the need to employ a potential environmentally friendly approach such as nodulation and N fixation in tackling some of the challenges in a bid to improve soil health, manage crop yield, and protect the environment (Singh *et al*., 2016). The result of the study showed that Tpt15, Tpt18, Tpt126, Tpt30, Tpt32, and Tpt15-4 all had a very high mean number of nodules. The significant mean squares of numbers of nodules indicated variability among the winged bean accessions used in this study with regard to nodulation capability. Significant differences in the number and biomass of nodules among crop landraces of the same species have been reported (Egbe *et al*., 2013). Tpt18 had the highest biomass production which was reflected in its high value for both dry shoot weight and dry root weight. A strong and positive correlation between dry shoot weight and dry root weight indicated that the shoot mass production is affected by the root mass in winged bean and therefore it can be said that as the root mass increases, the shoot mass increases and vice versa.

A positive correlation that also exists between number of nodules and fresh shoot weight indicated that the nodules were actively fixing N which thereby leading to increase in plant biomass. The highest proportion and Eigen vector were accounted for by the variation of winged bean nodulation in PRIN 1 and this should be considered in crop improvement purposes. It also confirms the varying response of accessions to nodulation.

There was variability in the nodulation and plant growth ability of the winged bean accessions evaluated. The nodulation capability is high when compared to other legumes as can be seen from the result of this study (Abaidoo *et al*., 2017; Waluyo and Lie, 2016); Abaidoo *et al*., 2017). The high N yield observed in this work could be attributed to the high number of nodules as the bacteria in the nodules can be said to actively fixing nitrogen. The low-N content of the soil during planting indicates that most of the content observed was derived from the atmosphere by the activity of the N-fixing bacteria in the nodules. The high N content of the plant makes it an alternative protein source for tropical African countries. The study also showed winged bean nodulates with indigenous soil bacteria.

Two weed plant species and maize were used as reference plants. Recent report have shown that weeds can be used to estimate a legume's dependence on N_2 fixation (Kermah et al., 2017b). $\delta\ ^{15}N$ and other parameters analyzed for the reference plants are shown in Table 6.9.

Table 6.9: Comparison of the mean of $\delta^{15}N$, $\delta^{13}C$, C/N. of reference plants

Plant part	C/N	δ^{13}Ccorr	δ^{15}N	%C	%N	Reference plant species
Shoot	31.0	-12.9	3.4	40.0	1.3	*Zea mays*
Root	99.9	-12.5	3.2	40.0	0.4	*Z. mays*
Shoot	12.1	-18.9	5.5	40.0	3.4	*Eleusine indica*
Root	25.3	-24.0	2.7	40.0	1.6	*E. indica*
Shoot	12.1	-24.4	4.9	40.0	4.0	*Tridax procumbens*
Root	26.5	-19.3	3.9	40.0	1.6	*T. procumbens*

Key: CN=Carbon Nitrogen; C=Carbon; N=Nitrogen

A comparison of winged bean growth between the studied accessions showed differences in dry weight but these were not statistically different from one another in shoot and root dry weights measured. The accession with the highest dry shoot weight was Tpt18 (403.23 g^{-1}) while the lowest was Tpt125 (118.32 g^{-1}) while Tpt 42 and Tpt154 had the highest and lowest dry root weight of 36.47 and 10.37 g^{-1} respectively (Table 6.2).

The carbon (C) and N ratio is an indicator of the C and N relationships in plants. It is also used in determining photosynthetic N-use efficiency. Significant differences were recorded in C and N ratio among the accessions studied. Overall, the C/N ratio ranged from 15.87 (Tpt51) to 11.97 (Tpt32) for the shoots; it ranged from 18.33 (Tpt12) to 17.83 (Tpt53) for the roots (Table 6.7).

The $\delta^{15}N$ value of winged bean showed great differences among the accessions. It varied from 3.34 (Tpt18) to 0.86 (Tpt3-B) in the shoots and from 3.07 (Tpt15) to 0.49 (Tpt32) for roots. The percentage Ndfa of winged bean shoots also varied significantly among the reference plants used for estimation between 66.12% (Tpt3-B) and 24.3% (Tpt18). Differences also existed for the roots estimation (Table 6.5).

The amount of N fixed differed significantly ($p \leq 0.05$). The quantity of N fixed (kg ha^{-1}) in the shoots varied among the accessions with Tpt32 fixing 27.16 kg $ha^{-1,}$ followed by Tpt15-4 at 25.66 kg ha^{-1} . The lowest fixed accession was Tpt30 that measured 9.02 kg ha^{-1} with considerable lower amounts fixed in the roots (Table 6.5). Similar results involving legumes were obtained by other researchers (Abaidoo et al., 2017; Chalk, 2016; Kermah et al., 2017b); Polania et al. (2016a)).

Table 6.10: Correlation analysis

	CN	δ^{13}CCORR	δ^{15}N	N	SDW	TOTALNHA	NDFAE	ENFIXED	NDFAM	MNFIXED	NFAT	TNFIXED
CN	1	0.08	-0.13^{ns}	-0.99*	-0.19^{ns}	-0.99^{ns}	0.13^{ns}	-0.17^{ns}	0.13^{ns}	0.02^{ns}	0.13^{ns}	-0.12^{ns}
d^{13}CCORR		1	0.34^{ns}	-0.06^{ns}	0.04^{ns}	-0.06^{ns}	-0.34^{ns}	-0.37^{ns}	-0.34^{ns}	-0.37^{ns}	-0.34^{ns}	-0.37^{ns}
d^{15}N			1	0.14^{ns}	0.21^{ns}	0.14^{ns}	-1*	-0.94*	-1*	-0.99*	-1*	-0.96
N				1	0.19^{ns}	1*	-0.14^{ns}	0.16^{ns}	-0.14^{ns}	-0.03^{ns}	-0.14^{ns}	0.11^{ns}
SDW					1	0.19^{ns}	-0.21^{ns}	-0.14^{ns}	-0.21^{ns}	-0.17^{ns}	-0.21^{ns}	-0.16^{ns}
TOTALNHA						1	-0.14	0.16	-0.14^{ns}	-0.03^{ns}	-0.14^{ns}	0.11^{ns}
NDFAE							1	0.95*	1*	0.99*	1*	0.96*
ENFIXED								1	0.95*	0.98*	0.95*	0.99*
NDFAM									1	0.99*	1*	0.96*
MNFIXED										1	0.99*	0.99*
NFAT											1	0.96*
TNFIXED												1

The $\delta^{13}C$ of C3 plants such as winged bean is often used as a biological measure of water-use efficiency (Lawson and Pike, 2017). Analysis of variance of $\delta^{13}C$ from winged bean shoots and roots revealed significantly marked variation among accessions under investigation. The $\delta^{13}C$values of shoots were much greater (i.e., less negative) while the values for the roots also varied considerably. Consequently, the $\delta^{13}C$ values of shoots ranged from -30.60 (Tpt48) to -29.62 (Tpt19) and from -30.17 (Tpt53) to -19.19 (Tpt6) for the roots (Table 6.4). The values obtained show these accessions are generally stable in their expression of water-use efficiency irrespective of agronomic practice. Water-use efficiency is known to decrease with the availability of soil-water and increase with soil moisture deficit (Fang *et al*., 2010; Hartman and Danin, 2010; Lawson and Pike, 2017; Mapope and Dakora, 2016)**.** $\delta^{15}N$ correlated positively with percentage N derived from the atmosphere, Nfixed, percentage N also correlated significantly with Total N (Table 6.9).

The extent of C in plants is linked to photosynthetic activity (Belane *et al*., 2011). In this study, C concentrations for the legume were 40% which was in agreement to previous reports for other legumes (Mohale *et al*., 2014; Polania *et al*., 2016b). A higher C content indicates photosynthetic C accumulation and accounts for a substantial amount of plant biomass (Mohale *et al.*, 2014). The lower C content in plants is moderated by N nutrition. The C and N ratio is undoubtedly a good estimate of the N status (Mohale *et al*., 2014). The non-legumes used as reference plants had over 24 gg^{-1}, all other accessions used exhibited C and N ratios below 24 gg^{-1} (Table 6.7) which is similar with outcomes from previous researches (Adams *et al.*, 2016; Mohale *et al*., 2014; Ulm *et al.*, 2017). In all cases, the C and N ratio value increased with decreasing $\delta^{15}N$ values (Table 6.7). These outcomes support the opinion that photosynthetic activities in the underutilized legumes was mediated by N nutrition, as accessions producing low C and N-fixed ratios yielded high N.

In this study, the variation in $\delta^{13}C$ of the underutilized legume ranged from -30.60 to -29.62 for the shoots and from -30.17 to -19.19 for the roots, therefore showing differences in the water-use potential among the accessions. Tpt48 showed higher values than Tpt19 which made the recorded the highest N contribution to the site. This means that there is a relationship between water-use potential and N_2 fixation, and nodule performance. This suggestion was further established by the finding that $\delta^{13}C$ also correlated with the amount of N-fixed (r= -0.319; -0.265; -0.285*) depending on the reference plant used. Furthermore, there was a positive correlation between $\delta^{13}C$ and dry weight yield (r=-0.21*); N content, and dry weight yield (r=0.420*), as well as a perfect correlation between N-fixed and Total Nha content (r=0.90*; 0.67* and 0.84*). Based on drought pattern, studies have shown C3 plant increase their $\delta^{13}C$ (Franco *et al*., 2018; Vandoorne *et al*., 2012) and in the same manner they increase their $\delta^{13}C$ values when in low water conditions (Hartman and Danin, 2010). It can be suggested that the high $\delta^{13}C$ values of winged bean were due to reduced soil moisture. In conclusion, the ^{15}N and ^{13}C natural abundance used to assess N fixation and water relations in the underutilized tropical legume grown on IITA soils proved useful. The sometimes small symbiotic N yield per ha obtained in this study for the roots may be due to plant density other than to less symbiotic functioning. The $\delta^{13}C$ of winged bean shoots varied among the accessions showing better water-use efficiency.

CHAPTER SEVEN

Morphological characterization, proximate and anti-nutritional composition of African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) and winged bean (*Psophocarpus tetragonolobus* (L.) DC.)

Abstract

Many people in sub-Saharan Africa suffer from protein malnutrition with negative health and economic impact. Underutilized legumes such as African yam bean (*Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms.) and winged bean (*Psophocarpus tetragonolobus* (L.) DC.) may contribute to the alleviation of the problem of malnutrition owing to their high protein content. In this study, the proximate compositions (fat, moisture, crude protein, ash, and carbohydrate) and anti-nutritional contents (tannin and phytate) of African yam bean and winged bean (processed and unprocessed) were determined using standard methods with 50 accessions being analyzed for each legume. Statistical significant variation ($p \leq 0.05$) were observed among the accessions and parameters evaluated. For instance in winged bean processed seeds, Tpt17 had the highest protein content of 40.30% and the lowest was recorded in Tpt48 (34.18%). For unprocessed seeds, Tpt17 also recorded the highest crude protein content at 31.13%, followed by Tpt4 (31.02%) while the lowest was contained in Tpt125 (28.43%). In the processed seeds, the highest value recorded was in Tpt51 (2.57%) and the lowest in Tpt43 (1.81%) while in the unprocessed seeds, the highest was 3.43 in Tpt32 and lowest value obtained 1.36% in Tpt30. The phytate content also recorded significant differences. The highest content in the processed seeds was found in Tpt19 (9.38) and the lowest in Tpt4 (3.78) while the values were from 9.96 (Tpt42) to 4.09 (Tpt19). In the tubers, Tpt42 recorded the highest protein content of 19.07% followed by Tpt4 (17.34%) while the lowest was found in Tpt10 (12.26%).

In the processed seeds of African yam bean, TSs104 had the highest protein content of 25.08%; while lowest protein value was in TSs30 (22.02%). At the same time, in the

unprocessed seeds, protein content was highest in TSs38 (24.93%) and lowest in TSs11 (19.13%). Available samples could analyze only the protein and ash content in the tubers. In this case, TSs27 recorded the highest protein of 9.30% and TSs9 recorded the lowest of 3.59%. The phytate content showed TSs1 with the highest content at 7.08%; TSs66 had the lowest value of 2.95% for processed seeds while TSs30 and TSs67 had the highest (5.86%) and lowest (3.18%) values for unprocessed seeds. The tannin content for processed seeds was highest in TSs23 (2.45%) and lowest in TSs98 (0.08%) while for unprocessed seeds, the highest and lowest values were obtained in TSs67 (3.88%) and TSs82 (0.66%) respectively. Morphological descriptions also showed variation among traits such as the 100-seed weight, total seed weight, and seed colour. In conclusion, evidence of the nutritional content of these crops as observed in this study implied that they can be utilized in various dishes for adults and children, to reduce malnutrition in sub-Saharan Africa.

Keywords: Food security, nutrients, underutilized legumes.

7.1 Introduction

Legumes are a crucial source of a variety of phyto-chemicals that are important for human health. These include protein, carbohydrates, fiber, minerals, vitamins, carotenoids, and polyphenols (Kouris-Blazos and Belski, 2016). Consequently, they hold a near-unique position among foodstuffs because of their health-giving properties (Foyer *et al*., 2016b). Studies have been conducted to emphasize the importance of legumes to mortality reduction among the population (Chang *et al*., 2012; Foyer *et al*., 2016b). Observational studies suggest that legumes can reduce risks of cardiovascular disease. Intervention studies show that this is mediated via improvements in blood pressure, lipid profile, inflammation, blood sugar metabolism, and body weight (Hashemi *et al*., 2014; Kouris-Blazos and Belski, 2016).

Underutilized legumes are crops found in many developing countries but their economic potential is constrained by a number of factors (Cullis and Kunert, 2017). They can be used as animal feed and in other agricultural activities to generate income for small-holder farmers in sub-Saharan Africa. They are grown to support families and can offer high nutritional value but they are currently neglected by research and poorly documented. (Cullis and Kunert, 2017). African yam bean is one of such crops with tremendous nutritional potentials. The low awareness about the taxonomy, agronomy, genetics, medicinal value, and productive potential of the crop may be due to limited research on it. The subsistence production of the crop may have been occasioned by its poor acceptability as a valuable crop among middle-aged farmers in Africa (Daniel and Celestina, 2013). *Psophocarpus tetragonolobus* (L.), popularly known as winged bean, is also a tropical legume found growing abundantly in hot, humid equatorial countries, such as India, Burma, Sri Lanka, Thailand, and the Philippines. It is also called a wonder

legume as it has high protein content in the seeds and is therefore considered as a versatile legume. Seeds of winged bean contain some pharmacologically active anti-nutrients such as trypsin and chymotrypsin inhibitors, haematoglutins, and amylase inhibitors (Mohanty *et al*., 2013). In this present study, seeds and swollen roots (tubers) of these two underutilized legumes were analyzed. The main objective of this study was to evaluate the proximate and anti-nutritional composition of African yam bean and winged bean seeds and tubers (processed and unprocessed).

7.2 Materials and Methods

Grain of African yam bean and winged bean was processed into flour using the method described by Alamu *et al*. (2016). Seeds were cleaned and sorted to remove impurities before being roasted slightly under low heat (autoclaving) until light brown in color and the seed coat could be removed by hand. The roasted grain was coarse-milled and winnowed to remove seed coats. The decorticated grain was milled into fine powder and sieved for processed samples. The unprocessed samples were cleaned and milled until fine flour was also obtained. The samples were stored in airtight containers at 4-6 °C and appropriately labelled for analysis. The tubers harvested from the field were peeled, rinsed with water, and dried in the oven. The dried samples were then milled, labelled, and packaged in airtight containers for analysis. The proximate and anti-nutritional analyses were conducted at the Food and Nutrition Sciences Laboratory, IITA Ibadan, Nigeria.

7.2.1 Proximate Composition

7.2.1.1 Moisture Content

For the analysis 3g of flour sample was weighed out separately into moisture canisters in duplicates. The sample was placed in a drying oven (Memmert, GmbH, Model-30-750) at 105 °C and dried to a constant weight, then cooled in a bench top desiccator; adequate care was ensured when the sample was re-weighed. The percentage moisture content was calculated using the formula:

$$\% \text{ Moisture Content} = \frac{M_1 - M_2}{M_1 - M_0} X\ 100$$

Where M_o = Weight in g of dish and lid (g)

M_1 = Weight in g of dish, lid and sample before drying (g)

M_2 = Weight in g of dish, lid and sample after drying (g)

7.2.1.2 Crude Fats

In the analysis 3 g of flour sample was weighed into filter paper and inserted in a dry extraction thimble which was placed in the Foss Soxtec 2055 fat extractor. The fat was extracted from the sample with petroleum ether in the extracting can and evaluated as a percentage of the weight before the solvent evaporated. The defatted sample was kept and used in determining crude fiber. The percentage crude fat content was estimated using the formula:

$$\% \text{ Fat/Oil} = \frac{(W_3 - W_2)}{W_1} \times 100$$

Where:

Weight of the can with the extracted oil = W_3.

Weight of the empty can = W_2

Weight of sample = W_1

7.2.1.3 Ash Content

The sample (2 g) was weighed in duplicates into a clean dry crucible, placed in a muffle furnace (Vulcan, Model-3-1750) at 550 °C and ashed to a constant weight. It was cooled in a bench-top desiccator and weighed again.

Percentage ash content was calculated using the formula;

$$\% \text{ ASH} = \frac{(\text{weight of crucible + ash}) - (\text{weight of empty crucible}) \times 100}{\text{Sample weight}}$$

7.2.1.4 Crude Fiber

The defatted sample was used in determining the crude fiber. For the analysis 1 g of the sample was digested in H_2SO_4 and NaOH solutions and the residue was calcined. The difference in weight after calcination indicated the quantity of fiber present. The defatted dry sample was weighed and placed in the flask (200 ml) and 80 ml of H_2SO_4 was added and boiled for 30 min. The mixture was filtered and the residue was transferred to the flask; 80 ml of NaOH was added and boiled for 30 min. The hydrolyzed mixture was carefully filtered and the residue was washed with distilled water. Washing was finished off with three washes in petroleum ether. The residue was placed in a clean dry crucible and weighed. Thereafter, the crucible together with the residue was placed in a muffle furnace at 550 °C and ashed to a constant weight, cooled in a desiccator, and weighed. The percentage of crude fiber content was calculated as:

$$\text{Crude fiber content } \% = \frac{(A - B)}{C} \times 100$$

Where:

A = Weight of crucible with dry residue (g)

B = Weight of crucible with ash (g)

C = Weight of sample (g)

7.2.1.5 Crude Protein

The Kjeldahl method of protein analysis was used. For this, 0.2 g of the flour sample was weighed out separately into digestion tubes in duplicates. The sample was first digested using the Foss Tecator^{TM} Digestor, distilled using Kjectec 2200 distillation apparatus, and titrated using the automated Titre equipment. The blank titre was also carried out and recorded. The percentage protein content was displayed automatically on the screen and recorded in the laboratory tablet.

7.2.1.6 Total Carbohydrate

This was determined by difference, which is the addition of a percentage of moisture, protein, fat, and ash contents were subtracted from 100%.

7. 2.2 Anti-nutritional factors in flour samples

The samples were analyzed for phytate and tannin.

7.2.2.1 Phytate determination: The phytate was analyzed using 1 g of the sample. The extraction and precipitation of phytic acid was done as described by Wheeler and Ferrel (1971). Iron in the precipitate was then measured according to the method of Makeover (1970). A 4:6 Fe/P atomic ratio was used to determine the phytic acid content of the samples.

7.2.2.2 Tannin determination: Tannin was determined following the method described by Adegunwa *et al.* (2011). 0.5 g of sample was dispensed in 50 ml of distilled water and mixed thoroughly. The mixture was allowed to stand for 30 min at 28^{o}C before filtration through Whatman No. 42 filter paper. About 2 ml of the extract was dispensed into a 50 ml volumetric flask. Standard tannin solution (2 ml) and 2 ml of distilled water were put in separate volumetric flasks to serve as standards; Folins reagent was added to each of the flasks and 2.5 ml of saturated Na_2CO_3 solution. The content of each flask was made up to 50 ml with distilled water and incubated at 28^{o}C for 90 min. Their respective absorbance was measured in a spectrophotometer (ThermoFisher Scientific, Model, G10 UV-ViS) at 260 nm using the reagent blank to calibrate the instrument at zero.

7.2.3 Statistical Analysis

Analysis of variance (ANOVA) using Statistical Analysis System software (SAS, version 9.4) was used to evaluate data obtained. The clustering of accessions was performed by the average linkage method using eight traits that were found to be significantly different among the accessions.

7.3 Results

Table 7.1: Morphological description of African yam bean and winged bean used for the study

Winged bean	100 seed weight (g)	Total seed weight (g)	Seed color	African yam bean	100 seed weight (g)	Total seed weight (g)	Seed color
Tpt2	31.10	3728.00	Light brown	TSs1	20.10	324.80	Dark brown
Tpt4	31.60	2787.00	Dark brown	TSs3	17.00	219.10	Brown
Tpt6	32.80	3412.20	Dark brown	TSs4	27.30	321.10	Greyish orange
Tpt10	30.10	4124.00	Brownish grey	TSs9	19.20	65.00	Dark brown
Tpt11	33.50	3554.50	Greyish orange	TSs11	18.00	24.80	Greyish yellow
Tpt12	43.10	4198.00	Brown	TSs16	15.90	28.70	Dark brown
Tpt14	33.50	2991.90	Light brown	TSs23	17.50	153.20	Brown
Tpt15	32.00	3806.00	Dark brown	TSs24	21.20	89.60	Light brown
Tpt16	37.50	4298.30	Dark brown	TSs27	27.10	19.70	Brownish orange
Tpt17	31.10	4009.50	Light brown	TSs30	22.90	42.80	Greyish orange
Tpt18	33.00	3455.50	Dark brown	TSs33	24.90	104.20	Brown
Tpt19	33.20	3370.50	Light brown	TSs38	23.90	30.40	Dark blond
Tpt30	33.70	3313.70	Reddish blond	TSs44	18.00	379.70	Olive brown
Tpt32	33.30	2755.70	Brown	TSs61	19.10	51.90	Light brown
Tpt33	32.20	3610.00	Dark brown	TSs66	27.00	164.60	Dark brown
Tpt42	33.50	4342.00	Reddish brown	TSs67	22.90	210.00	Dark violet
Tpt43	37.80	4246.00	Brown	TSs68	24.10	236.10	Light brown
Tpt48	33.10	3921.10	Greyish yellow	TSs76	17.90	12.40	Greenish grey
Tpt51	34.00	4040.0	Dark brown	TSs77	21.10	743.00	Dark brown
Tpt53	31.10	4396.30	Greyish orange	TSs81	21.00	205.60	Light brown
Tpt125	33.00	4075.00	Light brown	TSs82	22.00	104.10	Dark brown
Tpt126	32.00	3728.00	Yellowish brown	TSs98	24.30	102.50	Reddish blond
Tpt154	34.20	4979.00	Greyish orange	TSs101	23.90	144.80	Brownish orange
Tpt15-4	32.00	2787.00	Reddish blond brownish orange	TSs104	20.00	70.90	Olive brown
Tpt3-B	30.00	4467.00	Yellowish dark	TSs109	25.20	102.70	Dark brown

Table 7.2: Proximate composition (mean) of African yam bean flour

Accession	Sample	M.C. (%)	ASH (%)	FAT (%)	CRUDE PROTEIN (%)	CRUDE FIBER (%)	CHO (%)
TSs1	Processed	5.66	3.71	2.38	24.52	2.78	29.14
TSs1	Unprocessed	4.14	3.16	1.61	19.78	5.27	66.04
TSs101	Processed	8.69	3.84	1.40	23.94	5.66	56.48
TSs101	Unprocessed	9.95	3.64	2.18	22.74	6.25	55.23
TSs104	Processed	9.04	3.35	1.42	25.08	5.13	55.99
TSs104	Unprocessed	10.03	3.10	1.77	19.39	5.35	60.35
TSs109	Processed	9.60	3.37	1.16	23.32	10.16	52.41
TSs109	Unprocessed	10.28	3.42	1.58	21.39	5.26	58.08
TSs11	Processed	5.24	3.70	0.83	22.79	8.13	59.32
TSs11	Unprocessed	3.31	3.06	1.55	19.13	5.89	67.06
TSs16	Processed	8.22	3.70	1.24	23.78	5.60	57.47
TSs16	Unprocessed	10.06	3.42	1.70	21.37	5.59	57.87
TSs23	Processed	8.32	3.70	1.24	21.30	5.69	59.77
TSs23	Unprocessed	9.89	3.51	1.80	21.93	7.69	55.19
TSs24	Processed	7.78	3.67	0.92	21.37	5.04	61.23
TSs24	Unprocessed	10.54	3.41	2.62	21.34	5.35	56.72
TSs27	Processed	8.30	3.30	1.58	22.99	6.07	57.77
TSs27	Unprocessed	10.34	3.25	1.50	20.33	5.50	59.07
TSs3	Processed	5.61	3.63	2.08	23.66	0.00	0.00
TSs3	Unprocessed	4.19	3.06	1.91	19.15	4.66	67.03
TSs30	Processed	8.49	3.32	1.56	22.02	5.57	59.05
TSs30	Unprocessed	9.75	3.37	1.85	21.04	4.79	59.20
TSs33	Processed	8.12	3.67	1.11	23.69	5.67	57.76
TSs33	Unprocessed	9.36	3.32	2.10	20.76	5.31	59.16
TSs38	Processed	9.40	3.42	1.34	22.50	8.33	55.03

TSs38	Unprocessed	9.83	3.99	1.34	24.93	5.68	54.23
TSs4	Processed	5.39	3.37	1.83	24.31	4.97	60.14
TSs4	Unprocessed	4.21	3.08	1.31	19.26	4.77	67.36
TSs44	Processed	4.38	3.51	1.60	22.62	6.01	29.16
TSs44	Unprocessed	9.88	3.38	1.42	21.16	4.76	59.41
TSs61	Processed	8.22	3.82	1.65	22.78	4.66	58.89
TSs61	Unprocessed	10.10	3.67	1.62	22.95	5.98	55.68
TSs66	Processed	8.49	3.53	1.46	20.79	5.52	60.22
TSs66	Unprocessed	10.07	3.42	2.00	21.40	5.42	57.69
TSs67	Processed	8.30	3.94	0.49	24.24	5.44	57.62
TSs67	Unprocessed	9.99	3.47	1.87	21.71	5.06	57.90
TSs68	Processed	8.79	3.83	0.37	20.50	5.67	60.85
TSs68	Unprocessed	9.63	3.64	1.83	22.73	5.22	56.96
TSs76	Processed	8.65	3.60	0.39	24.82	5.24	57.31
TSs76	Unprocessed	9.86	3.59	1.53	22.45	3.96	58.60
TSs77	Processed	8.19	3.20	1.65	22.37	20.86	43.74
TSs77	Unprocessed	10.83	3.63	1.54	22.69	5.04	56.27
TSs81	Processed	8.75	3.71	1.54	23.97	5.91	56.14
TSs81	Unprocessed	10.04	3.55	1.94	22.19	3.99	58.30
TSs82	Processed	8.72	3.43	1.51	23.97	5.55	56.84
TSs82	Unprocessed	9.60	3.48	1.80	21.78	4.90	58.44
TSs9	Processed	4.84	3.32	1.16	22.96	4.79	62.93
TSs9	Unprocessed	4.62	3.19	1.81	19.96	3.20	67.21
TSs98	Processed	8.39	3.47	1.06	22.37	4.59	60.13
TSs98	Unprocessed	10.45	3.21	1.77	20.03	4.86	59.68
F Statistics		**10.71*** **	**4.03*** **	**1.71***	**1.59^{ns}**	**2.98***	**1.33^{ns}**
C.V (%)		13.90	4.75	25.20	5.74	38.10	20.19

M.C.=Moisture Content; CHO=Carbohydrate; Significant level at p ≤0.0001 (***), or p ≤0.05 (*).

Table 7.3: Proximate composition (mean) of winged bean flour

Accession	Sample	M.C. (%)	ASH (%)	FAT (%)	CRUDE PROTEIN (%)	CRUDE FIBER (%)	CHO (%)
Tpt10	Processed	6.84	4.71	16.07	37.71	11.13	23.56
Tpt10	Unprocessed	4.63	4.75	18.50	29.69	5.43	37.00
Tpt11	Processed	6.92	4.92	14.91	39.72	10.79	22.76
Tpt11	Unprocessed	4.75	4.73	18.28	29.55	4.83	37.87
Tpt12	Processed	6.89	4.75	17.38	35.08	9.88	26.03
Tpt12	Unprocessed	5.45	4.87	18.35	30.47	5.18	35.67
Tpt125	Processed	8.38	4.87	18.66	36.76	10.40	20.94
Tpt125	Unprocessed	7.19	4.55	15.76	28.43	6.26	37.82
Tpt126	Processed	8.10	4.93	15.01	38.29	11.47	22.20
Tpt126	Unprocessed	7.37	4.59	17.84	28.68	6.29	35.24
Tpt14	Processed	6.78	4.68	15.59	37.80	11.64	23.52
Tpt14	Unprocessed	3.76	4.88	18.42	30.47	5.93	36.54
Tpt15	Processed	6.75	4.65	15.14	37.51	10.62	25.34
Tpt15	Unprocessed	4.05	4.71	19.01	29.41	5.71	37.11
Tpt15-4	Processed	8.34	4.45	15.71	39.21	11.02	21.29
Tpt15-4	Unprocessed	5.34	4.93	17.75	30.84	5.78	35.37
Tpt154	Processed	8.44	4.67	15.10	38.29	10.47	23.03
Tpt154	Unprocessed	7.35	4.60	17.00	28.72	6.30	36.02
Tpt16	Processed	6.77	4.80	16.16	36.26	10.73	25.29
Tpt16	Unprocessed	4.62	4.77	18.27	29.82	3.09	18.43
Tpt17	Processed	6.97	4.88	16.21	40.30	10.60	21.06
Tpt17	Unprocessed	4.07	4.98	18.33	31.13	0.00	0.00
Tpt18	Processed	6.72	4.65	17.12	36.30	10.87	24.36
Tpt18	Unprocessed	5.97	4.76	18.14	29.74	6.86	34.52
Tpt19	Processed	8.10	4.61	16.63	36.01	10.96	23.70
Tpt19	Unprocessed	7.45	4.69	15.99	29.33	4.61	37.94
Tpt2	Processed	6.74	4.91	17.65	35.23	11.75	23.74
Tpt2	Unprocessed	5.12	4.87	17.81	30.25	5.70	36.26
Tpt3-B	Processed	7.69	4.67	17.60	36.25	7.50	26.30

Tpt3-B	Unprocessed	6.55	4.66	13.87	29.10	6.06	39.76
Tpt30	Processed	8.05	4.78	16.18	36.24	11.14	23.63
Tpt30	Unprocessed	5.29	4.75	17.09	29.72	4.99	38.16
Tpt32	Processed	8.35	4.73	14.21	38.70	10.99	23.05
Tpt32	Unprocessed	6.16	4.72	17.12	29.47	6.19	36.35
Tpt33	Processed	8.26	4.60	16.59	36.81	10.15	23.60
Tpt33	Unprocessed	6.15	4.72	17.09	29.48	0.00	0.00
Tpt4	Processed	6.72	4.75	16.20	38.88	11.13	22.34
Tpt4	Unprocessed	5.77	4.96	18.39	31.02	5.22	34.63
Tpt42	Processed	8.51	4.54	17.12	36.48	12.12	21.24
Tpt42	Unprocessed	5.89	4.90	16.47	30.62	6.61	35.52
Tpt43	Processed	8.51	4.60	14.09	39.35	11.41	22.06
Tpt43	Unprocessed	6.67	4.63	17.28	28.93	5.85	36.64
Tpt48	Processed	8.11	4.61	15.75	34.18	11.18	26.19
Tpt48	Unprocessed	6.90	4.57	17.44	28.54	6.83	35.71
Tpt51	Processed	8.17	4.63	18.91	36.24	11.94	20.13
Tpt51	Unprocessed	6.26	4.69	15.82	29.34	7.29	36.61
Tpt53	Processed	8.31	4.63	18.79	34.33	11.15	22.80
Tpt53	Unprocessed	8.53	4.64	17.17	29.00	6.60	34.06
Tpt6	Processed	6.72	4.79	17.65	36.83	13.82	20.21
Tpt6	Unprocessed	4.74	4.78	17.76	29.88	5.49	37.34
F Statistics		**13.86*** **	**1.40^{ns}**	**1.04^{ns}**	**4.17*** **	**3.35*** **	**2.54***
C.V. (%)		7.37	3.13	7.78	2.94	15.26	24.31

Table 7.4: Means of anti-nutritional composition of processed and unprocessed flour of winged bean and African yam bean

Accession	Sample	Tannin (%)	Phytate (mg/100g)	Accession	Tannin (%)	Phytate (mg/100g)
Tpt10	Processed	1.95	6.73	TSs1	2.23	7.08
Tpt10	Unprocessed	2.55	6.61	TSs1	2.32	4.09
Tpt11	Processed	2.01	8.07	TSs101	1.34	3.29
Tpt11	Unprocessed	2.55	5.83	TSs101	1.53	4.40
Tpt12	Processed	1.98	8.70	TSs104	0.00	5.27
Tpt12	Unprocessed	2.33	8.25	TSs104	2.13	4.23
Tpt125	Processed	2.38	8.56	TSs109	0.00	4.66
Tpt125	Unprocessed	1.92	7.11	TSs109	2.84	4.41
Tpt126	Processed	2.23	9.26	TSs11	1.87	6.07
Tpt126	Unprocessed	1.92	7.45	TSs11	2.24	4.45
Tpt14	Processed	2.03	8.17	TSs16	1.02	5.42
Tpt14	Unprocessed	2.41	5.96	TSs16	2.47	4.29
Tpt15	Processed	2.05	7.57	TSs23	2.45	4.52
Tpt15	Unprocessed	2.76	5.75	TSs23	1.26	3.95
Tpt15-4	Processed	2.25	8.09	TSs24	0.69	4.06
Tpt15-4	Unprocessed	1.82	8.28	TSs24	3.20	3.58
Tpt154	Processed	2.32	5.74	TSs27	1.79	5.47
Tpt154	Unprocessed	1.92	9.02	TSs27	3.26	3.68
Tpt16	Processed	1.89	8.71	TSs3	2.31	5.30
Tpt16	Unprocessed	2.56	8.37	TSs3	3.00	5.43
Tpt17	Processed	2.52	6.73	TSs30	2.09	4.72
Tpt17	Unprocessed	2.94	6.27	TSs30	2.38	5.86
Tpt18	Processed	2.16	9.24	TSs33	0.56	4.59
Tpt18	Unprocessed	2.45	5.76	TSs33	2.49	3.38
Tpt19	Processed	1.93	9.38	TSs38	0.55	5.29
Tpt19	Unprocessed	1.39	4.09	TSs38	1.48	4.08

Tpt2	Processed	1.88	5.28	TSs4	2.07	6.57
Tpt2	Unprocessed	2.25	6.67	TSs4	2.24	5.00
Tpt3-B	Processed	2.22	7.05	TSs44	0.08	5.86
Tpt3-B	Unprocessed	2.70	8.95	TSs44	3.58	3.75
Tpt30	Processed	1.69	9.25	TSs61	0.00	5.23
Tpt30	Unprocessed	1.36	5.59	TSs61	2.60	4.48
Tpt32	Processed	1.94	9.36	TSs66	0.00	2.95
Tpt32	Unprocessed	3.43	6.84	TSs66	2.69	3.45
Tpt33	Processed	1.99	8.32	TSs67	0.00	4.36
Tpt33	Unprocessed	2.81	9.41	TSs67	3.88	3.18
Tpt4	Processed	2.40	3.78	TSs68	0.00	5.16
Tpt4	Unprocessed	2.52	9.11	TSs68	0.88	3.38
Tpt42	Processed	2.31	7.01	TSs76	0.00	5.87
Tpt42	Unprocessed	1.96	9.96	TSs76	2.99	3.24
Tpt43	Processed	1.81	7.60	TSs77	0.00	4.16
Tpt43	Unprocessed	2.59	9.09	TSs77	2.29	3.38
Tpt48	Processed	2.49	6.73	TSs81	0.12	3.29
Tpt48	Unprocessed	1.71	9.06	TSs81	2.45	4.00
Tpt51	Processed	2.57	8.89	TSs82	0.00	4.40
Tpt51	Unprocessed	2.89	7.48	TSs82	0.66	4.33
Tpt53	Processed	2.48	9.16	TSs9	2.11	5.81
Tpt53	Unprocessed	1.72	7.62	TSs9	2.56	4.25
Tpt6	Processed	1.79	5.05	TSs98	0.08	4.73
Tpt6	Unprocessed	1.76	8.65	TSs98	2.16	3.64
F Statistics		**2.51***	**1.25^{ns}**		**2.65***	**4.57*****
C.V (%)		16.72	19.25		45.50	13.60

M.C.=Moisture Content; CHO=Carbohydrate; Significant level at p ≤0.0001 (***), or p ≤0.05 (*).

7.5: Means of African yam bean and winged bean tuber flour

Accession	MC	ASH	FAT	PROTEIN
TSs104	0.00	0.00	0.00	0.00
TSs109	0.00	2.80	0.00	9.08
TSs24	0.00	1.38	0.00	5.08
TSs27	0.00	3.19	0.00	9.30
TSs30	0.00	3.22	0.00	4.22
TSs38	0.00	3.09	0.00	0.00
TSs67	0.00	0.00	0.00	4.61
TSs76	0.00	0.00	0.00	0.00
TSs82	0.00	1.26	0.00	0.00
TSs9	0.00	1.28	0.00	3.59
Tpt10	6.83	2.66	0.53	12.26
Tpt11	7.13	2.62	0.49	17.29
Tpt12	7.79	1.89	0.90	16.10
Tpt125	5.76	2.35	0.53	16.21
Tpt126	5.94	2.36	0.23	15.51
Tpt15	7.11	2.20	0.38	15.34
Tpt15-4	6.96	2.43	0.31	14.29
Tpt154	4.67	3.31	0.34	14.85
Tpt16	6.16	2.92	0.21	16.44
Tpt18	7.67	2.13	0.41	16.41
Tpt19	6.99	2.37	0.45	16.22
Tpt2	3.32	1.57	0.45	12.44
Tpt3-b	7.81	2.59	0.51	13.61
Tpt30	3.62	2.78	1.17	0.00
Tpt32	3.26	1.27	0.00	15.01
Tpt33	6.96	2.63	4.53	15.80
Tpt4	6.82	2.76	0.54	17.38
Tpt42	7.69	1.54	4.38	19.07
Tpt43	1.40	1.10	0.00	0.00
Tpt48	6.10	2.04	1.04	14.38
Tpt51	5.45	1.48	0.27	14.69
Tpt53	5.26	2.40	0.51	14.41
Tpt6	6.70	3.03	0.49	16.01
F Statistics	**9.54*** **	**1.99***	**32.19*** **	**19.21*** **
C.V (%)	34.10	44.30	46.46	19.26

M.C. =Moisture Content; TSs=Tropical *Sphenostylis stenocarpa*; Tpt=Tropical *Psophocarpus tetragonobolus*; significant level at p ≤0.0001 (***), or p ≤0.05 (*).

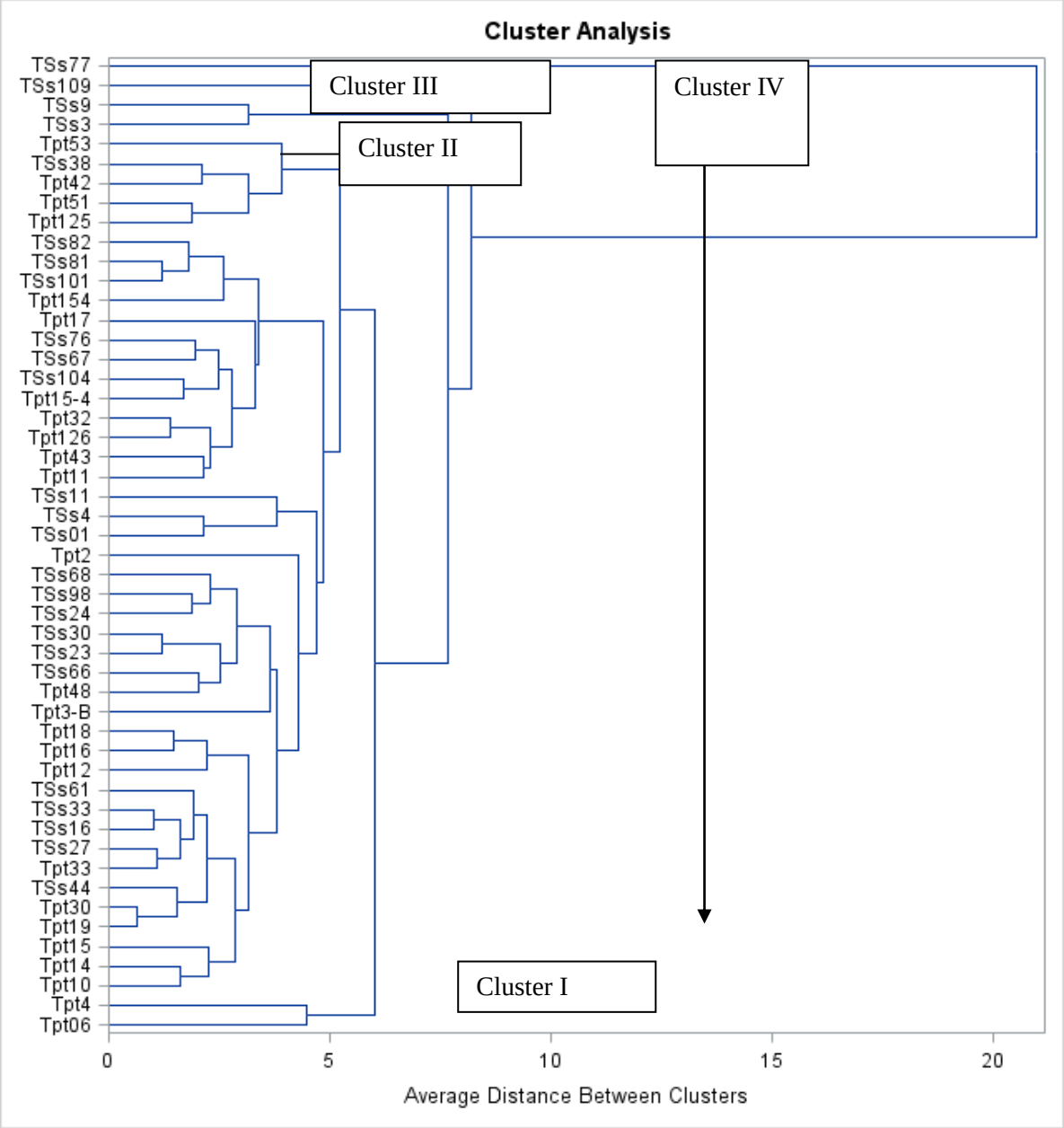

Figure 7.1: Dendrogram illustrating hierarchical clustering patterns of processed seeds of 50 accessions of African yam bean and winged bean belonging to four clusters based on proximate and anti-nutritional composition.

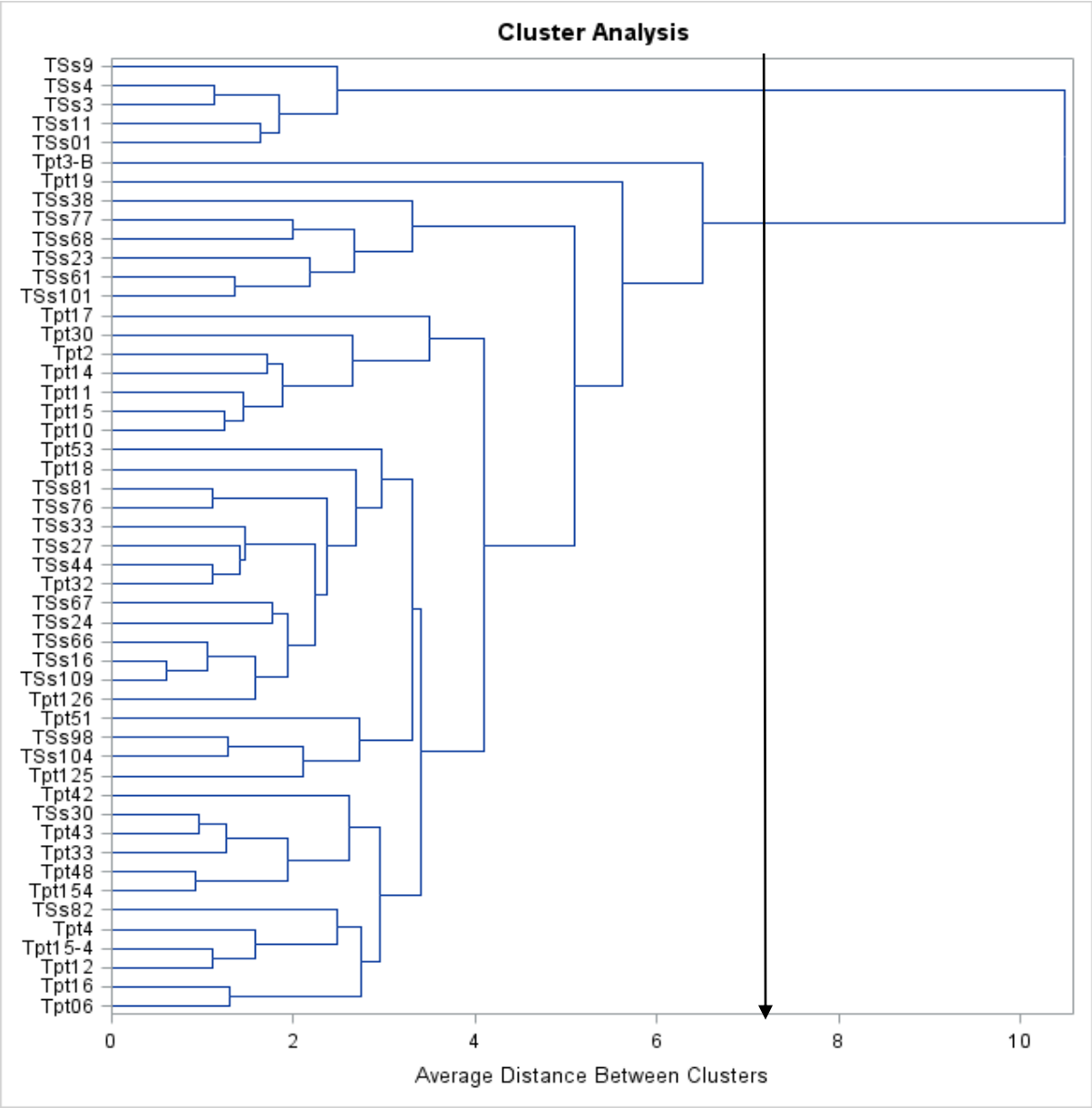

Figure 7.2: Dendrogram illustrating hierarchical clustering patterns of unprocessed seeds of 50 seeds accessions of African yam bean and winged bean belonging four clusters based on proximate and anti-nutritional composition.

Table 7.6: Clustering of African yam bean for proximate and anti-nutritional composition

Cluster Number	Sample	Accession	Percentage Similarity	Features
I	Processed	TSs1,TSs101,TSs104, TSs11, TSs16, TSs23, TSs23, TSs24, TSs27, TSs30, TSs33, TSs38, TSs4, TSs44, TSs61, TSs66, TSs67, TSs76, TSs81, TSs82 and TSs98.	92	High fat and protein, moderate tannin and phytate
II	Processed	TSs3	4	Moderate proximate composition, low tannin and phytate
II	Processed	TSs9	4	Moderate proximate composition, low tannin and phytate
III	Processed	TSs109	2	High moisture, less fat content, moderate tannin
IV	Processed	TSs77	2	Moderate proximate composition and low phytate and tannin
I	Unprocessed	TSs101, TSs 104, TSs 109, TSs 16, TSs 23, TSs 24, TSs 27, TSs 30, TSs 33, TSs 38, TSs 44, TSs 61, TSs 66, TSs 67, TSs 68, TSs 76, TSs 77, TSs 81, TSs 82 and TSs 98.	86	High fat and protein, moderate tannin and phytate
II	Unprocessed	TSs1, TSs 11, TSs 3, TSs 4 and TSs 9	10	Moderate proximate composition, low tannin and phytate

Table 7.7: Clustering of winged bean for proximate and anti-nutritional composition

Cluster Number	Sample	Accession	Percentage Similarity	Features
I	Processed	Tpt6,Tpt 10,Tpt11,Tpt 12,Tpt125,Tpt126,Tpt14,Tpt15,Tpt15-4,Tpt154,Tpt16,Tpt17,Tpt 18,Tpt19,Tpt2,Tpt 3-B,Tpt30,Tpt 32,Tpt 33,Tpt 4,Tpt 42,Tpt 43, Tpt48,Tpt 51 and Tpt 53.	92	High fat and protein, moderate tannin and phytate
I	Unprocessed	Tpt6,Tpt10, Tpt11,Tpt 12,Tpt125,Tpt 126,Tpt 14,Tpt15,Tpt15-4,Tpt 154,Tpt16,Tpt17, Tpt18,Tpt2,Tpt30,Tpt32,Tpt 33,Tpt 4,Tpt 43,Tpt48,Tpt 51 and Tpt 53.	86	High fat and protein, moderate tannin and phytate
III	Unprocessed	Tpt19	2	High proximate composition and low phytate and tannin
IV	Unprocessed	Tpt3-B	2	High fat and protein, moderate tannin and phytate

7.4 Discussion

For African yam bean, TSs4 had the highest 100-seed weight of 27.3 g, followed by TSs27 (27.1 g) while the accession with the lowest 100-seed weight was TSs16 at 15.9 g. For total seed weight; TSs77 had the highest weight of 743 g while the lowest was TSs30 with 42.8 g. The seed color varied from brown, dark brown, and greyish orange to yellowish dark blond (Table 7.1). As suggested by Ojuederie *et al.* (2015b) the weight of total seeds and 100-seed weight are two of the important determinant factors to be considered for improvement in the yield of African yam bean.

The moisture content recorded TSs109 at 9.60% and TSs44 at 4.38% with the highest and lowest moisture contents for processed seeds; in the unprocessed seeds, it ranged between TSs77 (10.83%) to TSs11 (3.31%). The ash content also showed significant variation. TSs67 had the highest content of 3.94% followed by TSs101 (3.84%) while the lowest content for ash was found in processed seeds for TSs77 (3.20%). In the unprocessed seeds, it ranged between TSs38 (3.99%) and TSs11 (3.06%).

The fat content was low in percentage if African yam bean is compared with other legumes. Percentage fat in the processed seeds was between 2.38% (TSs1) and 0.37% (TSs68); in the unprocessed seeds, it ranged between 2.62% (TSs24) and 1.31% (TSs4).

The protein contents of the accessions in the two forms in which the seeds were analyzed showed markedly significant differences. TSs104 had the highest protein content of 25.08% followed by TSs76 (24.82%); TSs1 (24.52%); TSs4 (24.31%), and TSs67 (24.24%); the accessions with the lowest percentage of protein contents in the processed seeds were TSs30 (22.02); TSs23 (21.37); TSs23 (21.30); TSs66 (20.79), and TSs68 (20.50). However in the unprocessed seeds, protein content ranged between TSs38 (24.93%) and TSs11 (19.13%). The crude fiber content also showed statistical significant variation. TSs77 contained the

highest quantity of crude fiber in the processed seeds (20. 86%) while the lowest was in TSs1 (2.78%), in the unprocessed seeds, the contents ranged between TSs23 (7.69%) and TSs9 (3.20%). The carbohydrate contents of both processed and unprocessed seeds of African yam bean were consistently high. For processed seeds, TSs9 had the highest percentage carbohydrate of 62.93% and TSs1 recorded the lowest at 29.64%. For unprocessed seeds, it ranged between TSs4 (67.36%) and TSs38 (54.23%). Our results were consistent and similar to previous studies (Abioye et al., 2015b; Ajibola and Olapade, 2016a; Igbabul et al., 2015; Ojuederie and Balogun, 2017b; Okoye et al., 2017; Onuoha et al., 2017).

Only nine out of the 25 African yam bean accessions could be processed for swollen root (tuber) proximate analysis owing to nematode infestation on the field. Available samples could analyze only the protein and ash content. TSs27 recorded the highest protein of 9.30%, followed by TSs109 (9.08%); TSs24 (5.08%) and TSs9 recorded the lowest of 3.59%. The ash content also showed TSs30 with the highest value of 3.22%, followed by TSs27 (3.19%), and TSs38 (3.09%)' the lowest ash content was contained in TSs82 (1.26%). These outcomes are agreements with the results of Ojuederie and Balogun (2017b) in selected African yam bean tubers.

TSs1 had the highest phytate content at 7.08 while TSs66 had the lowest value of 2.95 for processed seeds while TSs30 and TSs67 had the highest and lowest values of 5.86 and 3.18 respectively for unprocessed seeds. Our result does not agree with the higher results foundby Abioye et al. (2015b). They reported phytate content values ranged from 16.66 to 41.67% for AYB 61 and AYB 45. Phytate causes a reduction in protein availability in the body. It also decreases calcium bioavailability and forms calcium phytate complexes that inhibit the absorption of iron. Tannin content for processed seeds ranged between TSs23 (2.45) and TSs98 (0.08); for unprocessed seeds, it ranged between TSs67 (3.88) and TSs82 (0.66). This

finding is similar to the report by ((Abioye et al., 2015b; Ajibola and Olapade, 2016a; Ndidi et al., 2014; Onuoha et al., 2017).

There was also variation among the accessions with respect to morphological characters. Tpt12 had the highest 100-seed weight at 43.1 g while the lowest weight was found in Tpt3-B at 30 g. The total seed weight (kg) ranged between Tpt154 (4.9 kg) and Tpt4 (2.7 kg). The seed color also exhibited great variation from brown, dark brown, greyish orange, light brown to yellowish dark blond (Table 7.1). The moisture content in the processed seeds ranged between 8.51% (Tpt42) and 6.72 % (Tpt6); in the unprocessed seeds, it ranged between 8.53% (Tpt53) and 3.76 % (Tpt14). This outcome differed from the findings of Singh (2012) that reported moisture content for fully mature seeds to be 86.10% and 60.10% in the swollen roots. Differences were also seen for ash content. In the processed seeds, the values of ash ranged between Tpt126 (4.93) and Tpt15-4 (4.45) while in the unprocessed seeds, it ranged between Tpt17 (4.98) and Tpt125 (4.55).

Variability was observed in fat content indicating its suitability for both domestic and industrial application if well packaged. In the processed seeds, the fat contents are as follows: Tpt51 (18.91), Tpt53 (18.79), and Tpt125 (18.66); the least was in Tpt43 (14.09). In the unprocessed seeds, the values ranged between Tpt15 (19.01), Tpt10 (18.50), and Tpt14 (18.42); the least fat content was recorded in Tpt3-B (13.87). The values obtained for crude fat were higher than that reported by Singh (2012) who recorded crude fat content of 0.47% in the fully mature seeds and 0.56% in the tubers. Due to high thermal conductivity, winged bean oil is valuable as a frying medium in addition to its oxidative stability which is much higher than that of soybean oil (Makeri *et al*., 2016).

Variations were seen in the crude protein content of winged bean accessions. In the processed seeds, Tpt17 had the highest content of 40.30%, followed by Tpt11 (39.72%), Tpt43

(39.35%), Tpt15-4 (39.21%), Tpt4 (38.88) and the lowest was recorded in Tpt48 (34.18%). In the unprocessed seeds, Tpt17 also recorded the highest crude protein content at 31.13%, followed by Tpt4 (31.02%), Tpt15-4 (30.84%), and Tpt42 (30.62%) while the lowest was contained in Tpt125 (28.43%).

The winged bean crude fiber in the processed samples ranged between 13.82 (Tpt6) and 10.40 (Tpt125) in the unprocessed seeds, it ranged between 7.29 in Tpt51 and 4.83 in Tpt11. This finding is consistent with the report of Singh (2012) who recorded 12.65% in fully mature seeds and 2.76% in the tubers.

Unlike African yam bean, winged bean showed relatively low contents of carbohydrate. In the processed seeds, the values obtained ranged between 26.30 (Tpt3-B) and 20.94 (Tpt125); in the unprocessed seeds, it showed much higher values than in the processed samples, from 39.76 in Tpt3-B to 34.53 in Tpt18.

The tannin content showed varying differences among the accessions. In the processed seeds, it ranged from Tpt51 (2.57) to Tpt43 (1.81); in the unprocessed seeds, it ranged between 3.43 in Tpt32 and 1.36 in Tpt30 (Table 7.3). The phytate content also recorded significant differences. The highest content was found in Tpt19 (9.38) and the lowest in Tpt4 (3.78) in the processed seeds while the values ranged between 9.96 in Tpt42 and 4.09 in Tpt19 (Table 7.4).

Twenty-four winged bean accessions analyzed produced swollen roots (tubers). Owing to nematode infestation of the roots on the field, few proximate estimates could be evaluated with the available processed samples. They include statistically significant differences in moisture, ash, fat, and protein among the accessions on these parameters. The moisture content ranged from 7.81 recorded in Tpt42 to 1.40 in Tpt43. The ash content also varied among the accessions from Tpt154 (3.31) to Tpt43 (1.10). The fat content was low compared

to that obtained in the seeds. It ranged between 4.53 (Tpt33) and 0.21 (Tpt16). Tpt42 recorded the highest protein content of 19.07% followed by Tpt4 (17.34), Tpt11 (17.29), Tpt16 (16.44), and Tpt18 (16.41); the lowest protein content was found in Tpt10 (12.26).

In general, the proximate and anti-nutritional assessments on winged bean seeds and swollen roots were similar to those of previous studies (Afe Dwiani *et al*., 2014; Lepcha *et al*., 2017; Makeri *et al*., 2016; Yea *et al*., 2014)

The average linkage technique produced four clusters of African yam bean and winged bean accessions, where individuals within any cluster were more closely related for all the proximate and anti-nutrients examined than were individuals in different clusters. For the processed samples, cluster I was the largest and included 46 (92%) accessions. Cluster II comprised two (2%) accessions which were African yam bean only while clusters III and IV contained one accession each of African yam bean (4%). For the unprocessed samples, Cluster I contained the largest numbers and also included 43 accessions (86%) of both African yam bean and winged bean. Cluster II contained only five accessions of African yam bean (10%) while Clusters III and IV each contained winged bean only (4%) (Figs 7.5 and 7.6).

7.5 Conclusion

The percentages of anti-nutritional contents in flours produced from African yam bean and winged bean are reduced when processed with increase in their nutrient composition. The beans merit wider use in tropical countries. This study provided evidence that there are variations in the nutritional and anti-nutritional values. The protein contents were very high and compare well with other legumes and could replace them in meals for protein enrichment. These protein levels are higher than that of soybean (32%) (Alamu *et al*., 2017) indicating that winged bean in particular, with above 30% protein, could be a replacement in

various food formulations where soybean had been used. The crude fiber contents of the seeds were higher than those of most other legumes which indicated that the seeds are positioned as a functional food with health benefits associated with both soluble and insoluble fiber. We also observed the anti-nutritional composition was low. This could mean that these accessions, depending on the nature in which the seeds are used, have reduced quantities of anti-nutritional levels and could mitigate the constraints from the anti-nutritional factor in the utilization of African yam bean and winged bean seeds.

Chapter EIGHT

Isolation and characterization of nodule-associated bacteria in underutilized tropical legumes

Abstract

In tropical agriculture, soils have been generally regarded as diverse in bacterial population despite the pressure on the agricultural systems coupled with unfriendly climatic conditions that affect soil health. There is gradual approach in the application of rhizobia-inoculant in place of fertilizer by smallholder farmers in sub-Saharan African agriculture systems which have led to the conduct of diversity studies on tropical rhizobia strains. There is paucity of data on the nodule-associated bacteria in underutilized tropical legumes particularly winged bean and African yam bean. In this study, bacterial isolates from winged bean and African yam bean root nodules were characterized. 16S rRNA gene analysis showed winged bean root nodule-associated bacteria as *Enterobacter asburiae*; *E. bugandensis*; *E. cloacae*; *Enterobacter* sp; *Enterobacteriaceae bacterium; Pseudomonas cremoricolorata* and *P. fluorescens. Others are P. montellii; P. putida; Kosakonia oryzae; Ralstonia* sp*;* and an uncultured bacterium clone. Rhizobia recovered from winged bean nodules include *Rhizobium mayense, R. multihospitium, R. pusense,* and several other *rhizobia* sp. While none was isolated in African yam bean except other classes of bacteria. The rhizobia isolated have been previously confirmed as playing key roles in nodulation and N fixation. This outcome reveals the importance of incorporating legumes in tropical agriculture for crop intensification.

Keywords: Bacteria, diversity, underutilized legumes, 16S rRNA genes

8.1 Introduction

The roles played by bacteria in the fixation of atmospheric nitrogen (N_2) into ammonia are significant for plant productivity particularly in N-poor soils. It is estimated that about 60% of the fixed N on earth are obtained from BNF while manufactured fertilizers contribute 25% (Martínez-Hidalgo and Hirsch, 2017). It has been proposed that rhizobia and the 'other' bacteria act in concert as a community within the root nodules to enhance plant health and survive, more especially under environmental stress conditions. For several years, rhizobia were thought to be the only N-fixing organism found in the nodules of legumes. Microbial culture technique may have also confirmed this understanding. However, other bacteria which are not rhizobia are usually found within nodules obtained from the soil thereby indicating the presence of a phyto-microbiome where the interaction among the individuals is not only complex but also likely to affect the conduct and vigor of host plants (Martínez-Hidalgo and Hirsch, 2017).

The microorganism that colonizes everything that is connected to the plant body and all associated endophytes and epiphytes is regarded as the plant microbiome (Quiza *et al*., 2015). Therefore, it is an important aspect of the phytobiome, which has been identified as plants, their environment, and the organisms they interact with, to provide an opportunity to influence plant health and productivity (Busby *et al*., 2017).

Rhizobia, a class of bacteria, have been shown to help in the release of phyto-hormones, and mineral uptake in contaminated agricultural soils while also supporting plant growth (Karthik *et al*., 2017). Current practice in agriculture has moved to the use of natural methods that has less negative consequences on the ecosystem, cheap and cost effective for smallholder farmers who have difficulty in obtaining credit facilities from financial houses. Owing to proven benefits of cost saving, increase yield and associated soil health improvements, the

use of biofertilizers in tropical agriculture has increased tremendously now compared with the past decades (Meng *et al*., 2015).

It has also been observed that the relationship between legumes and rhizobia is specific (Batista *et al*., 2015). The environments can also play a role in the distribution and diversity of the organism. The alpha group of rhizobia forms the majority while the beta group which interacts with Mimosa genus (Koskey *et al*., 2018). Recent classification has been made of 40 rhizobia species belonging to the seven genera of Alpha-proteobacteria (Lemaire *et al*., 2015). However, there are other N-fixing bacteria, which have been recently identified from Beta and Gamma proteobacteria that form symbiotic relationships with legumes inhabiting the nodules. Each organism is known to nodulate and fix N with specific legumes. For instance, *rhizobium* strains are largely associated with chickpea; *Bradyrhizobium* strains are often found to nodulate soybean, cowpea and green gram (Koskey *et al*., 2018). However, the specific strain that can nodulate and fix N in winged bean and African yam bean has not been fully studied. In the study of microbes in root nodules, advanced molecular approaches have been developed to complement the usual methods of identification (morphological and cultural) (Ismail *et al*., 2013). This study utilizes PCR and 16S rRNA gene sequencing on the root nodule isolates.

To achieve the crop improvement of underutilized tropical legumes such as winged bean and African yam bean, there is a greater need to create awareness on N-fixation and researches that target the different bacteria associated with their root nodules that can used as biofertilizers. There is paucity of data on the diversity of bacteria that nodulate with the various accessions of African yam bean and winged bean in tropical agriculture. Hence, there is the need to carry out this study as part of the effort towards a pre-breeding program targeting selection of accessions with high nodulation and N-fixing ability. The aim of this

study was to isolate and identify bacteria associated with the root nodules of field-grown African yam bean and winged bean on an Alfisol soil of the Egbeda series in Ibadan, Nigeria.

8.2 Materials and Methods

8.2.1 Field nodule harvest

The field trapping of isolates was carried out between May and August 2017 during the first rainy season with winged bean and African yam bean as the host plants. Twelve weeks after planting, one plant of each was dug out for nodule estimation. The process involved initially breaking the soils around the plants to a depth of about 20 cm with a garden fork, making sure their roots were not disturbed. The plant was then pulled out gently and the root region cut out with the use of a sharp cutlass; individual plants were separated into shoots and roots and labelled and packed separately with the shoots going into net bags. The roots were put into paper bags and taken to the Soil Microbiology Laboratory of IITA to be washed with water to remove soil particles. The nodules on the roots and those that broke off in the course of washing were picked, counted, and weighed to obtain fresh nodule weight. They were put in smaller envelopes and oven-dried at 70 ^{0}C for 72 hr after which they were weighed on a sensitive electronic balance for dry nodule weight. The roots were also bagged separately, weighed to obtain fresh root weight, and oven-dried at 70 ^{o}C for 72 hr to obtain the dry root weight. Nodules were taken at random from each accession and preserved using silica gel. Nodule-associated bacteria were cultured and characterized morphologically, biochemically, and molecularly. Extraction was done a day after nodule harvest.

8.2.2 Bacterial isolation from the root nodules

The isolation of nodule bacteria and maintenance was done following procedures previously described (Muthini et al., 2014; Somasegaran and Hoben, 1994; Vincent, 1970a). A total of 37 isolates were obtained, maintained in vials containing 25% glycerol-Yeast extract mannitol broth (YEMB) and preserved at −20 °C.

8.2.3 Morphological and biochemical characterization of the isolates

Based on standard microbiological techniques described by Somasegaran and Hoben (1994) the isolates were characterized for morphological parameters (Kawaka *et al*., 2014; Liu, 2014). The wet smears of the isolate were air-dried, heat fixed, and then Gram stained as described by Beck *et al*. (1993) and viewed under the microscope. The production of acid or alkali was determined in YEMA medium containing bromothymol blue (YEMA-BTB). The plates were incubated at 28 °C for 7 days in the dark. The isolates that changed the green color of YEMA-BTB to yellow were identified as acid producers and fast growers and blue were known as alkaline producers and slow growers.

8.2.4 DNA extraction

Thirty-seven pure isolates were genetically characterized using 16S rRNA gene approach. Genomic DNA was extracted from pure bacterial isolates using ZR Bacterial DNA MiniPrep^{TM} kit according to the manufacturer's instruction (Zymo Research Corp, South Africa). The concentration and purity of DNA were estimated using a NanodropTM Lite Spectrophotometer (Thermo Scientific Inc, USA) at 260-280 nm and by horizontal gel electrophoresis (Thistle Scientific Ltd, USA) on a 0.8% (w/v) agarose gel at 100 V for 30 min and visualized under UV after staining with GelRed TM (Thermo Scientific Inc, USA).

The PCR cocktail mix consist of 2.5 µl of 10x PCR buffer,1µl of 25mM MgCl2, 1 µl each of forward primer (27F:AGAGTTTGATCMTGGCTCAG), 1 µl of DMSO, 2 µl of 2.5mMDNTPs, 0.1 µl of 5 µ/µl Taq DNA polymerase, and 3 µl of 10ng/µl DNA. The total reaction volume was made up to 25 µl using 13.4 µl of Nuclease-free water. PCR was done in Gene Amp PCR System 9700 thermocycler (Applied Biosystems) with an initial denaturation at 94 ˚C for 5 min, followed by 36 cycles of denaturation at 94 ˚C for 30 sec, annealing at 56 ˚C for 30 sec and elongation at 72 ˚C for 45 sec followed by a final elongation step at 72 ˚C for 7 min and a hold temperature at 10 ˚C forever. Amplified

fragments were visualized on Safe view-stained 1.5% agarose electrophoresis gels. PCR products were purified using ethanol and the EDTA precipitation protocol developed in IITA, Bioscience Center. Purified PCR products were sequenced at the Inqaba, South Africa, using ABI 3500 genetic analyzer (Applied BioSystem, (Thermo Fisher Scientific Inc, USA). Sequences generated were analyzed using Geneious version 9.1.7.

8.2.5 Data Analysis

Assembled sequences were transferred to Geneious software for analysis. Sequences were submitted to the NCBI BLAST portal (www.ncbi.blast.lnm.nih.gov) for a sequence similarity search, and sequences with greater than 75% similarity were retrieved for phylogenetic analysis.

8.3 Results

8.3.1 Physico-chemical Characteristics of soils

- Soil pH of the experimental site was 7.3 while soil organic matter was 1.97%. The total mean of soil N was 0.04%. The available P content was 2.36, the soil is 78% sandy. Analysis showed that the soils are acidic while other parameters studied are moderate.

8.3.2 Morphological Characterization

The nodule bacteria estimation using winged bean and African yam bean plants, 37 pure isolates were obtained based on morphological and biochemical characterization-fast growers (62.16%) and slow growers (37.83%) (Table 8.1). The isolates were Gram negative rods and never utilize Congo red dye. Color observed ranged from creamy to white on the culture plates. Glucose and arabinose as a source of carbon were used by all the isolates but only 37.83% were able to utilize lactose.

Table 8.1: Morphological features of isolates from winged bean and African yam bean

Isolate Code	pH Reaction	Cell Shape	Gram Stain	Growth	BTB	GL	AR	LAT
W1	Acid	R	Neg.	Fast	Yellow	G	G	NG
W6	Acid	R	Neg.	Fast	Yellow	NG	G	NG
W12	Acid	R	Neg.	Fast	Yellow	G	G	G
W13	Acid	R	Neg.	Fast	Yellow	G	NG	NG
W14	Acid	R	Neg.	Fast	Yellow	G	NG	NG
W16	Acid	R	Neg.	Nil	NC	G	G	G
W17	Acid	R	Neg.	Fast	Yellow	G	G	G
W19	Acid	R	Neg.	Slow	Blue	G	NG	G
W21	Acid	R	Neg.	Slow	Blue	G	G	G
W25	Acid	R	Neg.	Slow	Blue	G	G	G
W26	Acid	R	Neg.	Slow	Blue	G	G	G
W27	Acid	R	Neg.	Slow	Blue	G	Partial G	NG
W28	Acid	R	Neg.	Fast	Yellow	Partial G	G	G
W29	Acid	R	Neg.	Slow	Blue	G	NG	NG
W30	Acid	R	Neg.	Slow	Blue	Partial G	NG	NG
W31	Acid	R	Neg.	Fast	Yellow	G	G	G
W32	Acid	R	Neg.	Fast	Yellow	G	G	G
W33	Acid	R	Neg.	Fast	Yellow	NG	NG	G
W35	Acid	R	Neg.	Fast	Yellow	G	G	G
W36	Acid	R	Neg.	Fast	Yellow	Partial G	G	NG
W39	Acid	R	Neg.	Slow	Blue	G	G	NG
W40	Acid	R	Neg.	Slow	Blue	NG	NG	NG
W41	Acid	R	Neg.	Fast	Yellow	Partial G	G	NG
W42	Acid	R	Neg.	Fast	Yellow	G	NG	NG
W44	Acid	R	Neg.	Fast	Yellow	G	NG	NG
W45	Acid	R	Neg.	Fast	Yellow	G	G	NG
W48	Acid	R	Neg.	Fast	Yellow	G	G	NG
W50	Acid	R	Neg.	Fast	Yellow	NG	G	NG
A115	Acid	R	Neg.	Fast	Yellow	G	G	NG
A113	Acid	R	Neg.	Fast	Yellow	G	G	G
A122	Acid	R	Neg.	Slow	Blue	NG	G	G
A142	Acid	R	Neg.	Fast	Yellow	NG	G	G
A120	Acid	R	Neg.	Fast	Yellow	G	G	NG
A124	Acid	R	Neg.	Slow	Blue	NG	G	NG
A103	Acid	R	Neg.	Slow	Blue	NG	G	NG
A143	Acid	R	Neg.	Slow	Blue	G	NG	NG
A131	Acid	R	Neg.	Slow	Blue	G	G	NG

Key: W=winged bean; A= African yam bean; G=Growth; NG=No growth; BTB=Bromothymol blue; GL=Glucose; Ar=Arabinose; Lat=Lactose; R=Rod; Neg.=Negative;

8.3.3 16S rRNA gene analysis

Analysis of the 16S rRNA gene sequences showed mostly non-rhizobia nodule bacteria, 85% and above similarity indices, with 8 out of 37 showing rhizobia while others could be classified as nodule-inhabiting isolates. The sequences from this study and those with the greatest homologues from the NCBI genebank used in phylogenetic analysis are listed in Table 8.2. The DNA and PCR gel pictures of the isolates are shown (Figs 8.1; 8.2; 8.4, and 8.5) while the phylogenetic trees for winged bean and African yam bean are shown in Figs 8.3 and 8.6.

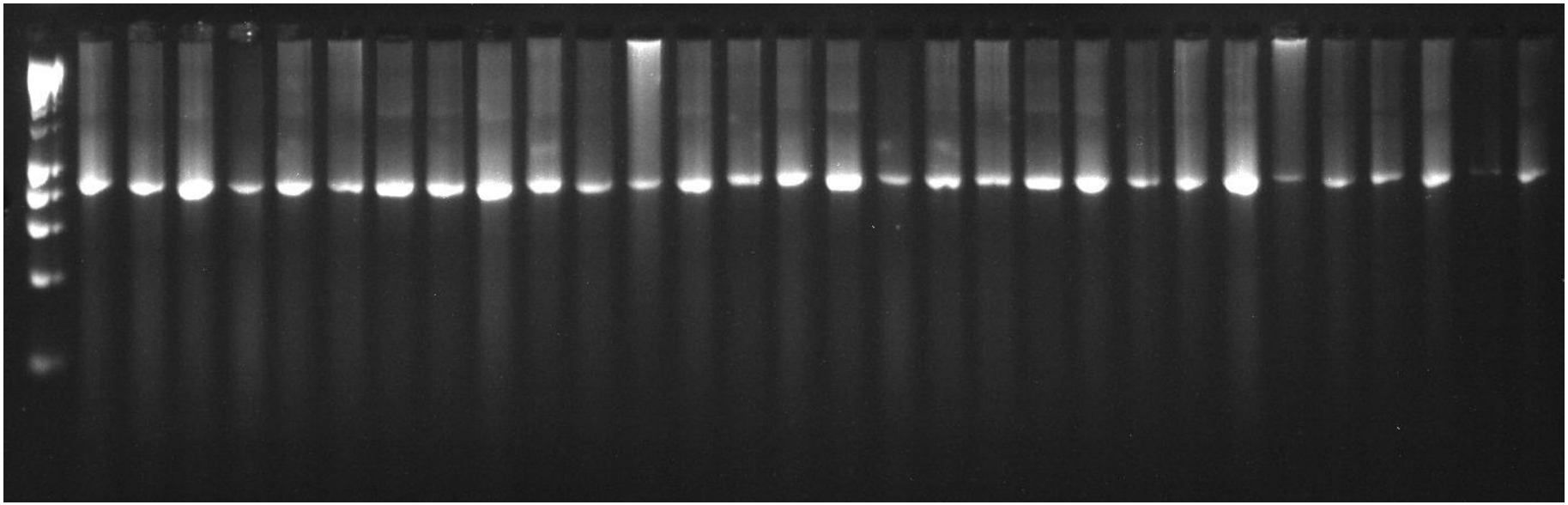

Figure 8.1: PCR gel pictures of winged bean bacterial isolates

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

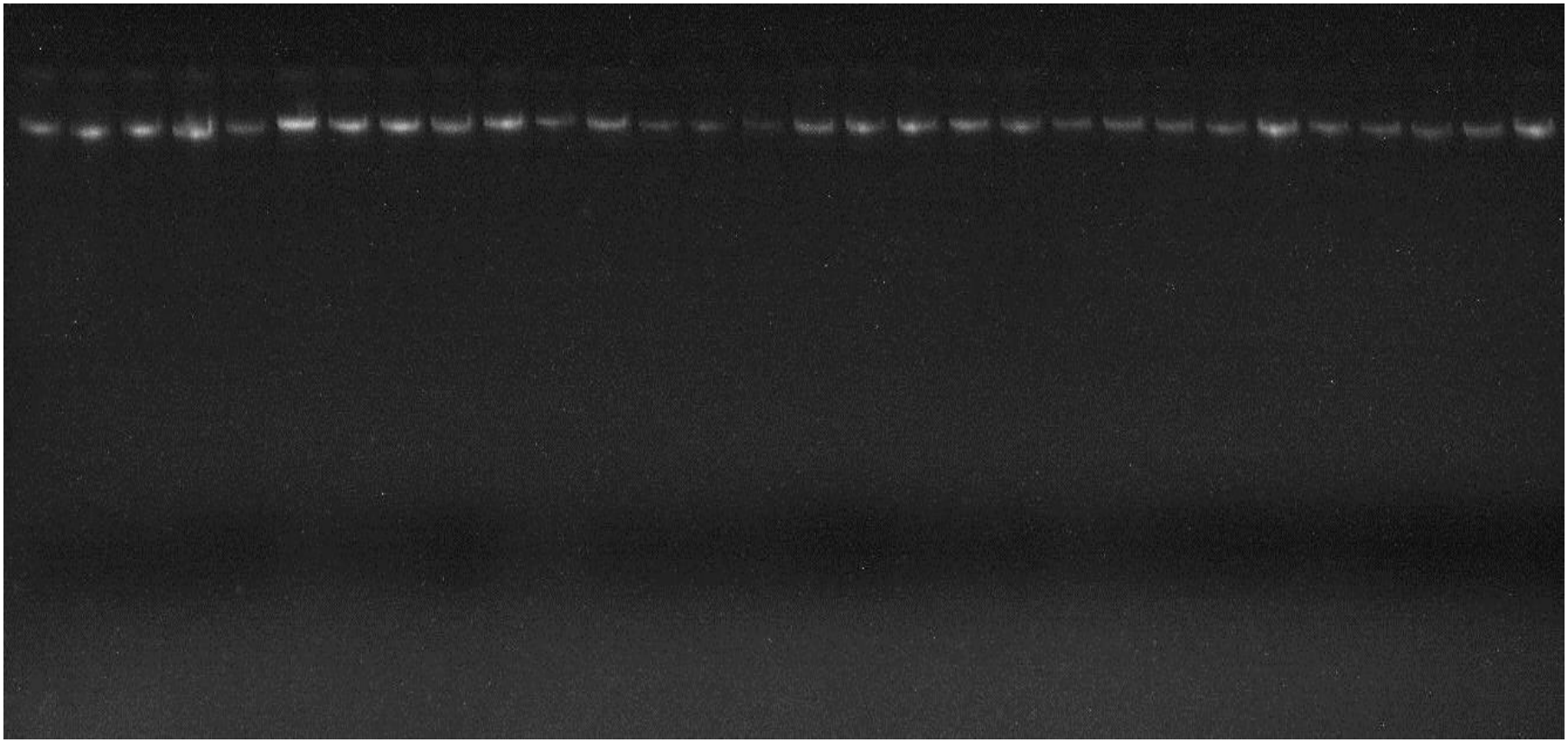

Figure 8.2: DNA samples of winged bean bacterial isolates

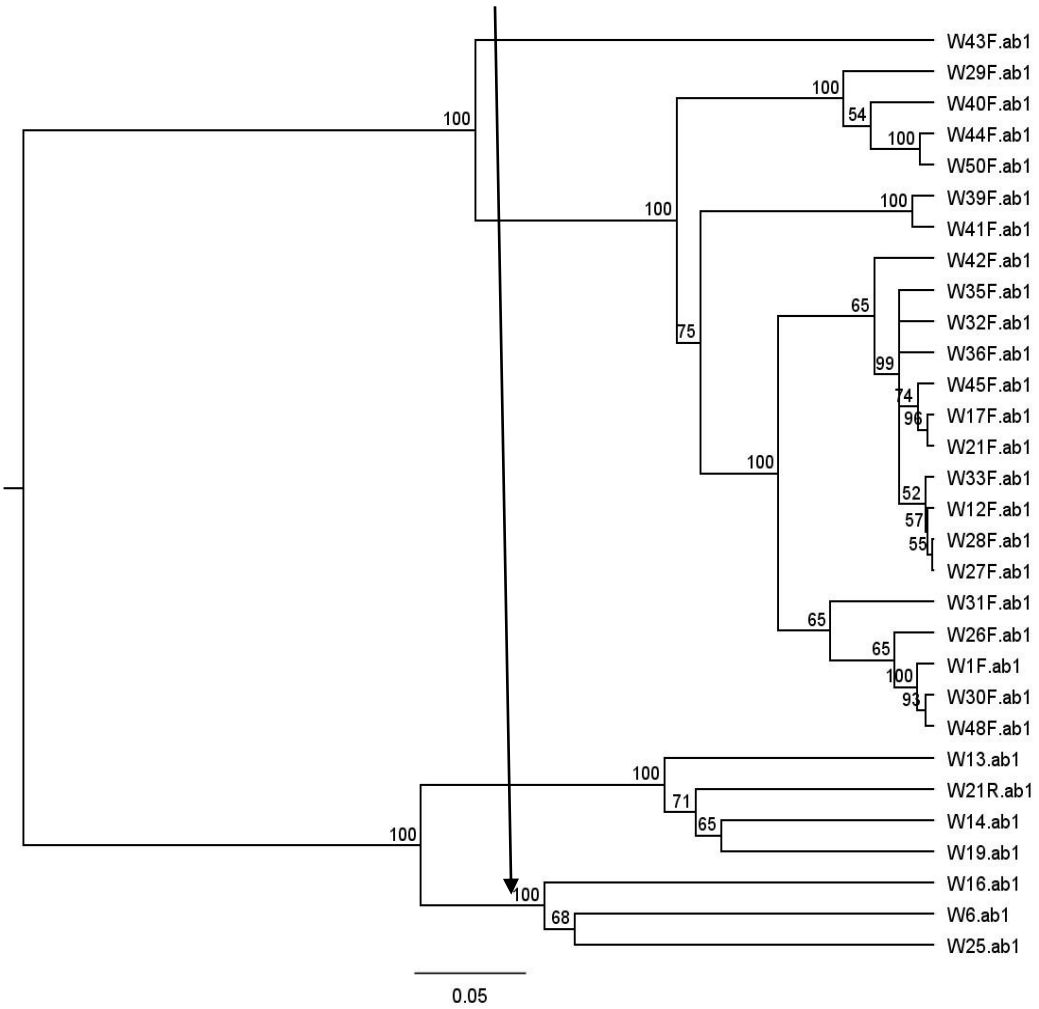

Figure 8.3: A neighbor-joining phylogenetic tree built using 16S rRNA gene sequences obtained from root nodule bacteria isolated from winged bean

1 2 3 4 5 6 7 8 9 10

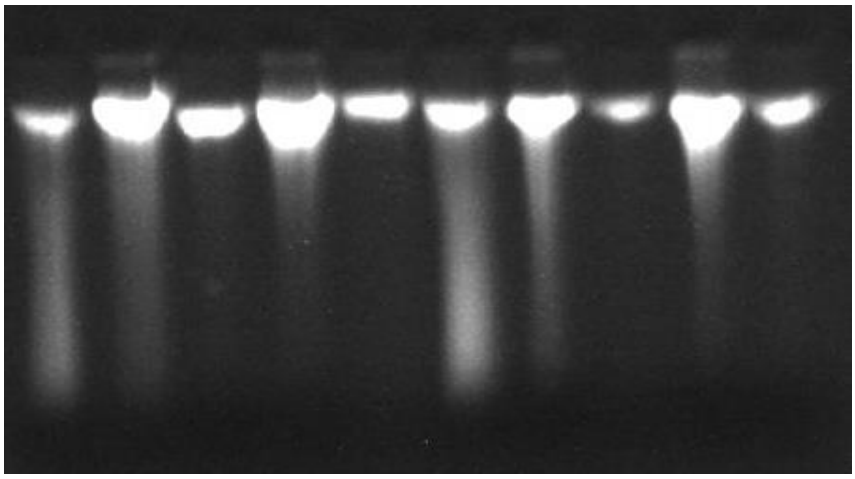

Figure 8.4: DNA image from selected African yam bean bacterial isolates

M 1 2 3 4 5 6 7 8 9 10

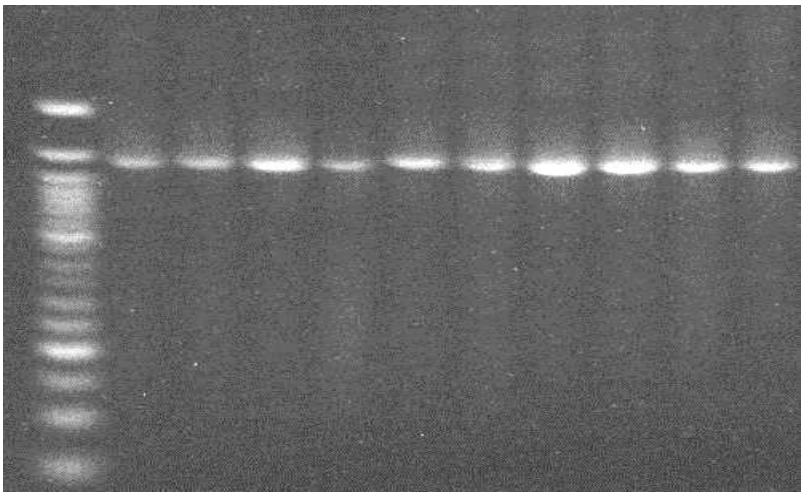

Figure 8.5: PCR 16S gene from selected African yam bean bacterial isolates

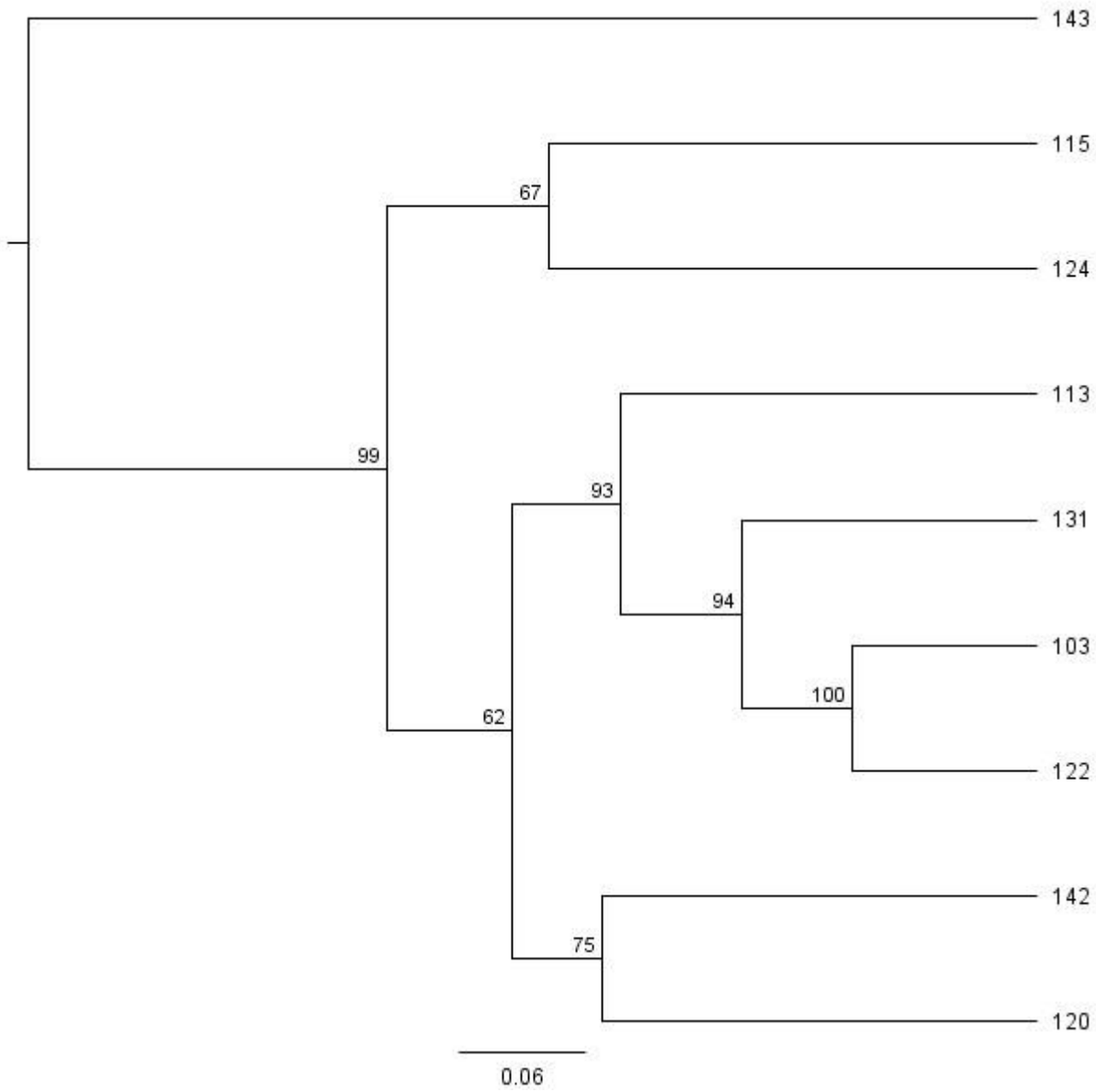

Figure 8.6: Phylogenetic tree of African yam bean bacterial isolates

Table 8.2: Identities of eight isolates based on 16S rRNA gene sequences and accession numbers of sequences with highest similarity values

Isolate Identity	Accession	16S rRNA gene identification	Percentage Identity	NCBI sequences with greatest similarity
103	KX380909	*Ralstonia pickettii*	98.90%	KX380909
143	KJ184862	*Variovorax* sp.	92.90%	KJ184862
113	NR_029102	*Hydrocarboniphaga effuse*	96.20%	NR_029102
115	JF690881	*Enterobacter asburiae*	82.40%	JF690881
120	LT799040	*Kosakonia oryzae*	80.70%	LT799040
122	KU598759	*Ralstonia* sp.	86.00%	KU598759
131	KX380909	*Ralstonia pickettii*	85.50%	KX380909
142	CP017184	*E. cloacae*	90.70%	CP017184

Table 8.3: Identities of isolates based on 16S rRNA gene sequences and accession numbers of sequences with highest similarity values

Isolate Identity	Accession	16S rRNA gene identification	Percentage identity	NCBI sequences with greatest similarity
W19	JX155406	*Enterobacter asburiae*	92.20	JX155406
W45	KY951337	*E. asburiae*	97.80	KY951337
W12	LT992502	*E. bugandensis*	98.40	LT992502
W27	CP027618	*E. cloacae*	99.20	CP027618
W42	CP026975	*E. cloacae*	85.90	CP026975
W14	KX450915	*E. cloacae*	94.10	KX450915
W28	CP027618	*E. cloacae*	98.30	CP027618
W17	CP010512	*E. cloacae*	98.60	CP010512
W13	KF303801	*E. sp.*	94.70	KF303801
W36	HQ204310	*E. sp.*	99.00	HQ204310
W35	GU459203	*Enterobacteriaceae bacterium*	90.10	GU459203
W32	MF109030	*Kosakonia oryzae*	96.30	MF109030
W33	CP014007	*K. oryzae*	91.60	CP014007
W31	KM272805	*Pseudomonas cremoricolorata*	79.70	KM272805
W26	KJ511902	*P. fluorescens*	85.90	KJ511902
W48	MF045810	*P. monteilii*	98.70	MF045810
W30	CP023299	*P. mosselii*	99.20	CP023299
W1	JX514407	*P. putida*	97.50	JX514407
W39	KT183537	*Ralstonia sp.*	97.50	KT183537
W16	KR107936	*Rhizobium mayense*	88.50	KR107936
W29	JN896359	*R. multi hospitium*	99.50	JN896359
W6	MH236187	*R. pusense*	93.70	MH236187
W40	LC368035	*R. sp.*	83.70	LC368035
W44	FN645726	*R. sp.*	99.50	FN645726
W21	KF008228	*R. sp.*	94.20	KF008228
W50	GQ483458	*R. sp.*	98.90	GQ483458
W25	AF514801	*R. sp.*	90.70	AF514801
W41	GQ359968	Uncultured bacterium clone	99.30	GQ359968

8.4 Discussion

Winged bean and African yam bean were used as trap plants in this study and resultant root nodule bacterial isolates were characterized. The results showed a high level of diversity within the 37 isolates obtained both phenotypically and by the 16S RNA gene classification. The results of the isolates Gram staining and growth on yeast extract mannitol agar-congo red (YEMA-CR) and yeast extract mannitol agar-bromothymol blue media, preliminarily support the morphological and cultural features of bacterial species that nodulate winged bean and African yam bean (Somasegaran and Hoben, 1994; Vincent, 1970b). The outcomes of this study on the morphological and biochemical characterization of indigenous bacterial isolates nodulating winged bean and African yam bean were similar to the results obtained from studies in Kenya (Kawaka *et al*., 2014; Muthini *et al*., 2014) and in Ecuador (Torres-Gutiérrez *et al*., 2017) on common beans. 62.16% of the isolates had very fast growing colonies on YEMA and all were acidic. The remaining 37.83% were tested as slow growers. They all utilized glucose and arabinose as a source of carbon; only 37.83 % used lactose (Table 8. 2). However, growth on BTB showed the majority of the isolates producing yellow colonies indicating their capacity to survive in an acid environment.

The comparison of the 16S rRNA gene sequences with those already deposited in the NCBI genebank database indicated that in its natural habitat, winged bean and African yam bean form symbioses with root nodule bacteria of different genera. The wide diversity of the bacteria established in this study confirms the previous study showing them to be capable of nodulation with different groups of bacteria (Benson *et al*., 2015). One good outcome of this study was the occurrence of eight rhizobia strains as N-fixing associates of winged bean. These strains could be helpful as potential biofertilizers when effectiveness is fully tested and may show that winged bean are rhizobia-compatible. Further investigations on their symbiotic performance under different environments might indicate the actual preferences of winged bean. We did not report any rhizobia for African yam bean except other classes of bacteria (Tables 8.4 and 8. 5).

Different titles have been given to other bacteria found in legume nodules. They include the following; non-rhizobia endophytes (NRE) (De Meyer *et al*., 2016); nodule endophytes (Velázquez *et al*., 2013) and nodule-associated bacteria (NAB) (Rajendran *et al*., 2012). Most of these bacteria have been reported to be generally non-pathogenic while some have been implicated as mammalian pathogens. For example, *Burkholderia* and *Staphyllococcus* sp. and *Bordetella avium* have been isolated from nodules (Larrainzar *et al*., 2014). Nodules of Hedysarum, a forage legume, were found to harbor *Enterobacter cloacae, E. kobei*, *Escherichia vulneris*, *Pantoa agglomerans*, and *Leclercia adecarboxylata (*Muresu *et al*., 2008*).* This is because it had long been known that co-inoculation of rhizobia and other bacteria particularly Bacillus species promotes not only nodulation but also N availability in sustainable agricultural systems (Rajendran *et al*., 2012). It is generally agreed that most of the non-pathogenic bacteria found within the nodules in plant may be safe and provide effective support for enhancing N-fixation (Martínez-Hidalgo and Hirsch, 2017).

N-fixing bacteria comprise the bulk of the microbial population found in the nodules of legumes; both α-rhizobia (Alpha proteobacteria for example, *Rhizobium* and *Bradyrhizobium*) and β-rhizobia (Beta proteobacteria, e.g., *Cupriavidus* and *Burkholderia*) (Gyanshenar *et al.*, 2011) are the frequent and most studied residents of legume nodules. In addition, the α and β-rhizobia are evolutionary different, their symbiotic genes (*nod* and *nif*) have been reported to be highly similar (Martínez-Hidalgo and Hirsch, 2017). However, legume root nodules contain many other microbial inhabitants. A study by Rodríguez-Caballero *et al*. (2017), showed that inoculating plants with arbuscular mycorrhiza fungi changed the bacterial population and improved plant growth particularly because of improved shoot N, P, and K levels. However abundant isolated members of Gram-positive and Gram-negative bacteria, some of which have been proven to have N-fixing capacity. Nodulation of leguminous crops largely depends on the presence of a specific and compatible soil for a particular legume.

The phylogenetic tree showed different clusters and sub-clusters. *Ralstonia pickettii* isolated in the samples was similar to the finding by Birlutiu *et al*. (2017); the *Enterobacter cloacae* and other *Enterobacter* species (Kryuchkova *et al*., 2014) have been observed to possess plant growth-promoting properties such as fixation of atmospheric N, solubilisation of phosphates, and synthesis of phytohormone indole-acetic acid (Kryuchkova *et al*., 2014), *Variovorax* species identified were similar to those in the study by Bravo *et al*. (2015); the *Pseudomonas* species have been reported to exhibit high degrees of plant growth-promoting activities thereby being classified as plant growth-promoting rhizobacteria (Kaushal and Wani, 2016). The rhizobia species identified were similar to those in previous reports particularly *Phaseolus vulgaris* (Baginsky *et al.*, 2015; Koskey *et al*., 2018; Román-Ponce *et al*., 2016). For example, the *R. mayense* isolated was the same as those found in *Calliandra grandiflora (*Shamseldin *et al.*, 2017*), R. pusense (*Ribeiro *et al.*, 2015*), R. multihospitium* in

alfalfa *(*Jia *et al.,* 2015*;* Moe *et al.,* 2015*)*. The rhizobia strains have been confirmed as N-fixers in the previous studies highlighted. *Kosakonia oryzae* was similar to what Li *et al*. (2017) isolated in China while genus *Hydrocarboniphaga effuse* belongs to the class of Gamma proteobacteria as reported by Liu *et al*. (2011).

Cluster I describes other members of the Beta proteobacteria and only one member (*Hydrocarboniphaga effusa*) of Gamma proteobacteria (Fig. 8.3) for winged bean. For African yam bean, two major Clusters were formed. Cluster I contains only two isolates (115 and 124) while Cluster II contains the remaining related bacteria species (Fig. 8.4). This study has revealed the level of heterogeneity within isolated populations resident in the nodules of winged bean and African yam bean. Our observations showed that diverse species of bacteria were found in the nodules. Therefore, we need to further characterize isolates from nodules as well as the soils in which the crops are grown. This will enable us to differentiate clearly if the species are responsible for N- fixation and nodulation and other plant growth-promoting activities. There is also the need to test the performance of the eight rhizobia strains in controlled and field experiments to really show competition with any available commercial strain.

8.5 Conclusion

This study showed the presence of bacteria associated with nodules of African yam bean and winged bean. The rhizobia isolated have been previously confirmed to play key roles in nodulation and N-fixation. Further studies could explain their functions in N-fixation, plant growth, and yield development. The plant activities of the other isolates also require further assessment so as to confirm if they possess any plant growth-promoting potential.

Chapter NINE

9.1 Conclusions and Recommendations

The present study provides evidence that African yam bean and winged bean accessions show variations and can be improved in a pre-breeding program with regard to N-fixing potential, nodulation capacity, proximate and anti-nutritional composition, and diversity in root nodulating bacteria.

9.2 African yam bean

African yam bean fix nitrogen and nodulate with indigenous soil bacteria. TSs77 fixed the highest amount of N at 22.47 kg ha^{-1} followed by TSs30 at 20.91 kg ha^{-1} and TSs101 at 19.80 kg ha^{-1}. This means that these top three accessions can be identified for breeding programs as superior N-fixing accessions. The protein contents of the accessions showed markedly significant differences. For instance, TSs104 had the highest protein content of 25.08%; followed by TSs76 (24.82%); TSs1 (24.52%); TSs4 (24.31%), and TSs67 (24.24%). The accession with the lowest protein contents in the processed seeds was TSs30 (22.02%). However, in the unprocessed seeds, protein content ranged between TSs38 (24.93) and TSs11 (19.13). Other proximate analyses evaluated showed differences among the accessions while there were reductions in the anti-nutritional composition. The evidence showed African yam bean can be optimally utilized in various dishes for adults and children in attempts to raise awareness about the crop and alleviate malnutrition in sub-Saharan Africa. Root nodule-associated bacteria isolated were analyzed using morphological and biochemical methods and 16S rRNA. Molecular analysis revealed the presence of *Kosakonia oryzae; Enterobacter asburiae*; *E. cloacae*; *Ralstonia pickettii; Variovorax* sp and *Hydrocarboniphaga effuse* as root-associated bacteria. The specific roles of these associated bacteria were not ascertained. The $\delta^{15}N$ signatures of the legumes differed among accessions and varied from 2.52 (TSs61)

to 0.24 (TSs44) in the shoots and from 2.70 (TSs98) to 0.82 (TSs16) for the roots. The percentage nitrogen derived from the atmosphere (Ndfa) of African yam bean shoots also varied significantly among the reference plants used for estimation. TSs76 had the highest percentage Ndfa of 66.73, 51.83, and 63.48; followed by TSs4 with 66.18%, 51.03% and 62.87%; the lowest was TSs1 with 40.07%, 13.22%, and 34.21 % when *E. indica*, *Z. mays*, and *T. procumbens* were used respectively for estimation. The $\delta^{13}C$ values of shoots were much greater (i.e., less negative) while the values for the roots also varied considerably. Consequently, the $\delta^{13}C$ values of African yam bean shoots ranged from -31.49 (TSs98) to -30.93 (TSs4) and from -31.16 (TSs68) to -30.20 (TSs4) for the roots. The observed variations indicate levels in water-use potential among the accessions. The C and N values were less than 24 gg^{-1} and the reference plants had over 24 $gg^{1.}$ These outcomes support the opinion that photosynthetic activities in the underutilized legume were stimulated by N nutrition. TSs44 has a significantly higher number of nodules than other accessions at 169.67; TSs23 had the lowest number at 58.42. This study, to the best of my knowledge, provided the first report on the use of the ^{15}N natural abundance method in determining N-fixation and water-use efficiency in African yam bean.

9.3 Winged bean

The winged bean accessions evaluated possessed the potential to fix N and also nodulated with indigenous soil bacteria. GCV were high for dry pod weight, number of seeds per pod, pod length, total seed weight, and total number of seeds. The high GCV suggest that these traits can be improved by selection. In the processed seeds, Tpt17 had the highest protein content of 40.30%, followed by Tpt11 (39.72%), Tpt43 (39.35%), Tpt15-4 (39.21%), and Tpt4 (38.88 %) and the lowest was recorded in Tpt48 (34.18%). In the unprocessed seeds, Tpt17 also recorded the highest crude protein content at 31.13%, followed by Tpt4 (31.02%), Tpt15-4 (30.84%), Tpt42 (30.62%) while the lowest was contained in Tpt125 (28.43%).

Other proximate composition analyses suggested that winged bean could serve as complementary item in human diets and animal feed. Processing was observed to reduce the levels of anti-nutrients in the seeds and swollen roots. The $\delta^{15}N$ values of winged bean showed great differences among the accessions and varied from 3.34 (Tpt18) to 0.86 (Tpt3-B) in the shoots and from 3.07 (Tpt15) to 0.49 (Tpt32) for roots. The percentage Ndfa of winged bean shoots also varied significantly among the reference plants used for estimation between 66.12% (Tpt3-B) to 24.3% in Tpt18. Differences also exist for the root estimation. The amount of N fixed differed significantly ($p \leq 0.05$) among accessions. The amount of N fixed kg N ha^{-1} in the shoots varied among the accessions with Tpt32 fixing 27.16 kg $ha^{-1,}$ followed by Tpt15-4 at 25.66 kg ha^{-1}. The lowest fixed accession was Tpt30 that measured 9.02 kg ha^{-1} with a considerable lower amount fixed in the root. Significant differences were observed in C and N ratios among the winged bean accessions studied. In all, the C and N ratio ranged from 15.87 (Tpt51) to 11.97 (Tpt32) for the shoots and from 18.33 (Tpt12) to 17.83 (Tpt53) for the roots. The $\delta^{13}C$ values of winged bean shoots ranged from -30.60 (Tpt48) to -29.62 (Tpt19) and from -30.17 (Tpt53) to -19.19 (Tpt6) for the roots. The values obtained showed these accessions are generally stable in their expression of water-use efficiency. Bacteria associated with winged bean root nodules included *Enterobacter asburiae*; *E. bugandensis*; *E. cloacae*; *Enterobacter* sp; *Enterobacteriaceae bacterium; Kosakonia oryzae; Pseudomonas cremoricolorata* and *P. fluorescens.* Others were *P. monteilii* sp*; P. putida* sp*; Ralstonia* sp*; Rhizobium mayense; R. multihospitium; R. pusense;* several Rhizobium species and an uncultured bacterium clone. The rhizobia species obtained have been previously confirmed in other studies to be responsible for nodulation and N-fixation. This further reiterates the importance of the incorporation of legumes in tropical agriculture for crop intensification.

Finally, the study provides evidence that African yam bean and winged bean accessions can be improved in a pre-breeding program with respect to N-fixing potential, nodulation capacity, proximate and anti-nutritional composition, and diversity in root-nodulating bacteria.

9 .4 Recommendations for future work

1. More studies are needed to screen the underutilized legumes with superior symbiosis and water-use efficiency in different agroecologies.
2. Assessment of thermal conductivity tests on winged bean oil.
3. Nutritional product developments from Africa yam bean and winged bean seeds and swollen roots.
4. Investigation into the practical utilization of winged bean for food frying application and production of fat products
5. The use of electronic device for recording and estimating nodule distribution arrangements and structures by using image analysis devices
6. There is a need to further determine how the rhizobia strains isolated from winged bean would perform when inoculated on the field.
7. There is a need to study the link between diversities and metabolic activities of bacteria to establish N-fixation and nodulation.
8. Plant growth-promoting traits induced by the crops need to be evaluated in association with the root-nodulating bacteria.
9. Wider germplasm collection, exploration, and exploitation across the various centers of diversity.

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APPENDICES

Appendix 1: Genotypic and phenotypic components of variance

Genotypic Variance ($\sigma^2 g$) = $\frac{MSg - MSe}{r}$

Where, MSg = Mean square of genotype, MSe = Mean square of error, r = number of replications

Phenotypic Variance, $\sigma^2 p = \sigma^2 g + \frac{\sigma^2 e}{r}$

Where, $\sigma^2 e$ = MSe and $\sigma^2 g$ = genotypic variance

Genotypic Coefficient of Variation (GCV)

$$\mathbf{GCV} = \frac{\sqrt{\sigma^2 \mathbf{g}}}{\overline{\mathbf{X}}} x \mathbf{100}$$

Where, $\sigma^2 g$ = genotypic variance and $\bar{X}$ = mean of the character

Phenotypic Coefficient of Variance (PCV) =

$$\mathbf{PCV} = \frac{\sqrt{\sigma^2 \mathbf{P}}}{\overline{\mathbf{X}}} x \mathbf{100}$$

Where, $\sigma^2 P$ = phenotypic variance, and $\bar{X}$ = mean of a character

Heritability (h^2): heritability in for all characters was computed as suggested by Hansen *et al*. (1956). We may ignore dominance and consider the difference between the lines as purely additive genetic variance and the heritability as narrow sense heritability.

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} X100$$

Genetic Advance (GA): The extent of genetic advance to be expected from selecting five percent of the superior genotypes was calculated by using the following formula:

$GA = (k)\ (\sigma_p)\ (h^2)$

Where k = Intensity of selection, h^2 = Heritability in broad sense and σ_p = Phenotypic standard deviation (Phenotypic variance)$^{0.5}$. The value of k was taken as 2.06 assuming 5% selection intensity.

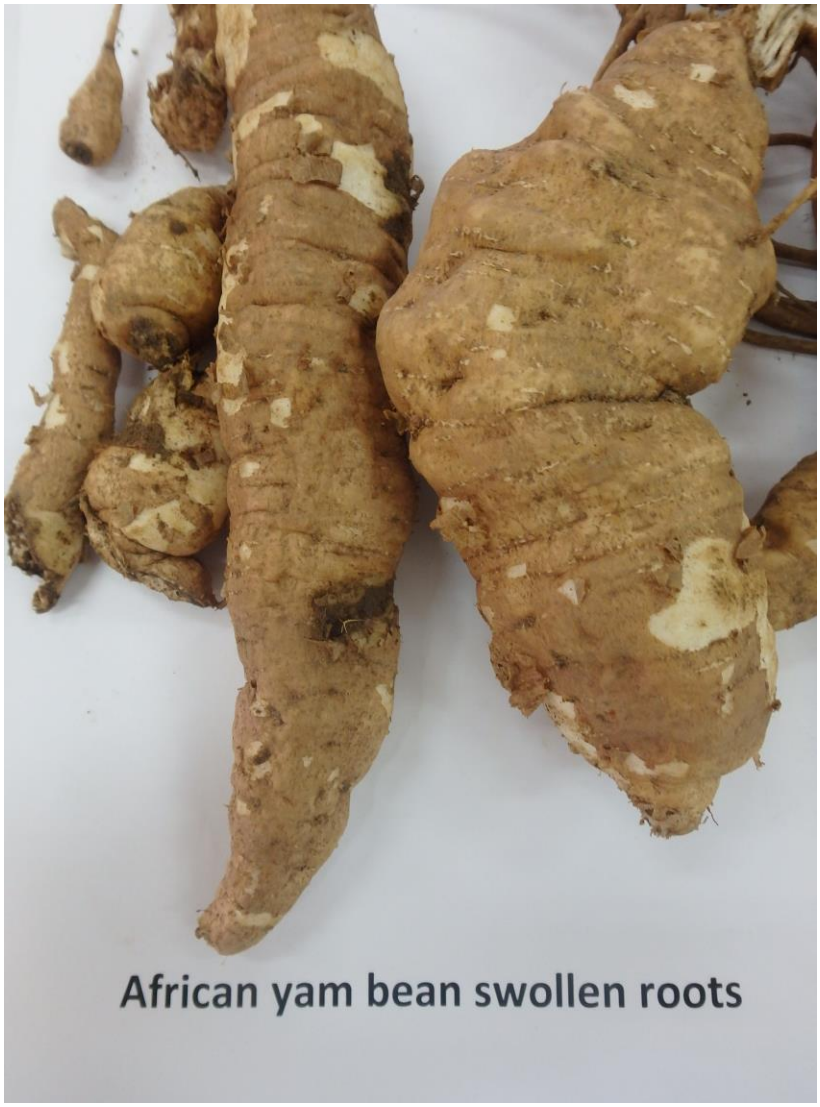
Genetic Advance as percentage of the Mean (GAM)

GAM was estimated using the following formula: $GAM = \frac{GA}{X} \times 100$

Where GA= Genetic advance and x= Grand mean of the character

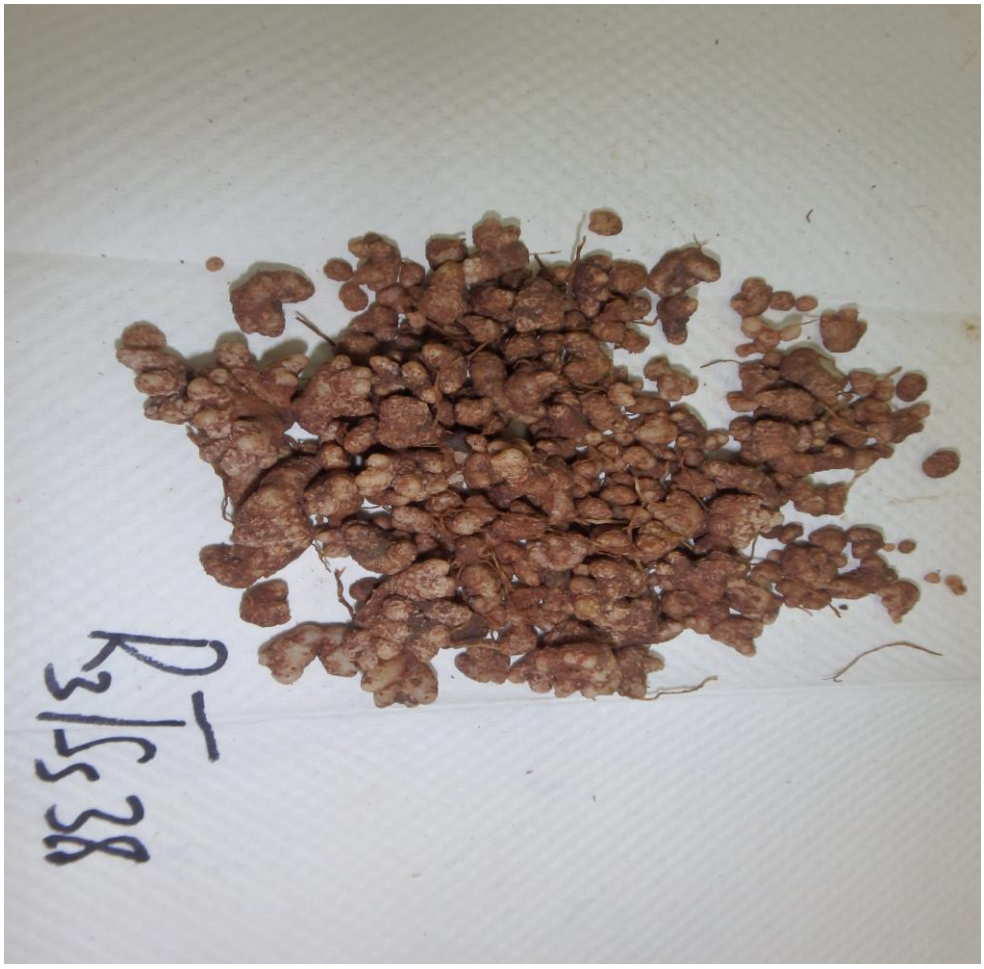
Genetic advance as a percentage of the mean was categorized as low, moderate and high as given by Johnson *et al*. (1955) as 0-10%: Low, 10-20%: Moderate, and 20% and above: High

Appendix 2: Selected tubers from African yam bean harvested from the field



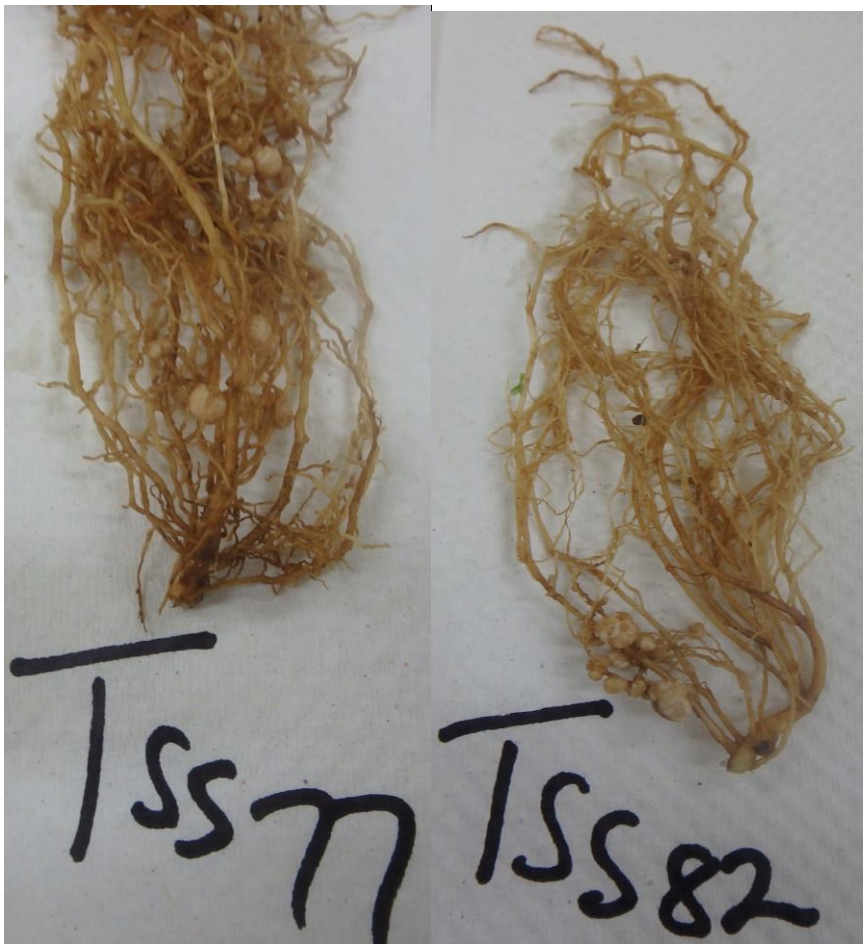
Appendix 3: Selected tubers from winged bean harvested from the field

Appendix 4: Detached nodules from African yam bean root harvested from the field



Appendix 5: Detached nodules from winged bean root harvested from the field

Appendix 6: African yam bean roots containing nodules



Appendix 7: Winged bean roots containing nodules