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Biochemical and agro-morphological characterization of wild, under-exploited vigna species and their utilization

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BIOCHEMICAL AND AGRO-MORPHOLOGICAL CHARACTERIZATION OF WILD, UNDER-EXPLOITED *VIGNA* SPECIES AND THEIR UTILIZATION

Difo Voukang Harouna

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ABSTRACT

According to the Food and Agriculture Organization (FAO) of the United Nations recent report, the immense challenge of achieving the Zero Hunger target by 2030 is still persistent. Therefore, the search for alternative food and feed sources for human and animal nutrition to feed the exponentially growing human population is a daunting task. It is imperative that 70% more food is needed to cover the gap between suppy and demand of food. Exploring the new, innovative methods of crop production remains the most viable and sustainable option available to most researchers to achieve food and nutrition security. This study explored the biochemical, agro-morphological, cooking cookability and farmers' preference of 160 accessions of four wild unexplored *Vigna* species in order to reveal information leading to their future domestication and utilization. The agro-morphological study was conducted in two agro-ecological zones in Tanzania, namely: The mid-altitude agro-ecological zone (Kisongo, Arusha region) and the high altitude agro-ecological zone (Lyamungo, Kilimanjaro region). The augmented block design study was used. An explorative survey and a focus group discussion (FGD) were performed to assess the farmers' preferences, perception and prospective use of the wild *Vigna* legumes while the Mattson Bean Cooker was used to evaluate the cooking characteristics of the legumes. Standard procedures and methods approved by the Association of Official Analytical Chemists (AOAC) were used in carrying out the biochemical characteristics tests of the wild *Vigna* legumes. The study revealed that the wild *Vigna* legumes are less known by many farmers but can be accepted as food, feed, cover crop or organic fertilizer although thre is need for improvement. Furthermore, it was demonstrated that the wild *Vigna* species possesses a large variation range of agro-morphological, biochemical and consumption characteristics which could be exploited in the improvement and/or domestication of species. It was also found that some individual wild accessions have higher nutrient, mineral content and best cooking time as compared with domesticated ones which could be advantageous for bio-fortification or domestication. Indications relating to the candidate accessions favorable for domestication, based on the agro-morphological, socio-cultural practice, cooking and biochemical characteristics were revealed. The study concluded that the genus *Vigna* (wild and domesticated species) presents a considerably high diversity in terms of agro-morphological, socio-cultural practice, cooking and biochemical characteristics. However, despite their under-exploitation for human benefits, the wild *Vigna* legumes demonstrated important agro-morphological, socio-cultural practice, cooking and biochemical characteristics comparable with the domesticated ones that

attracted some farmers' preferences. Therefore, the evaluation of other important agro-morphological traits, biochemical characteristics, socio-economic implication of wild *Vigna* utilization, toxicity studies and prospects of a patent/prototype establishment for promising accessions are among other recommendations for further studies.

DECLARATION

I, Difo Voukang Harouna do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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Dr. Athanasia O. Matemu

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CERTIFICATION

The undersigned certify that they have read this thesis titled "Biochemical and Agro-Morphological Characterization of Wild, Under- exploited *Vigna* Species and their Utilization" submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy (PhD) in Life Sciences and Bio-Engineering (Food and Nutritional Sciences).

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DEDICATION

To you and to them;

***You**, who gave birth to them in my absence and,*

***Them**, who set their eyes on their father for their first time in life after knowing everyone else…*

Dearest wife and two sons, my physical absence has no excuse but sincere apologies: I didn't prefer the 3 years of study over you, but believe me; I sincerely did it for you and made it because of you

*[**Balkissou, Tayssir & Abdul Hafeez**]*

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LIST OF ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrophotometry
AGG	Australian Grain Genebank
AHC	Agglomerative Hierarchical Clustering
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
FAME	Fatty Acid Methyl Esther
FAO	Food and Agriculture Organization
GC-MS	Gas Chromatography – Mass Spectroscopy
GLM PROC	Generalized Linear Model Procedure
GRC	Genetic Resource Center
HPLC	High Performance Liquid Chromatography
IFAD	International Fund for Agricultural Development
IITA	International Institute of Tropical Agriculture Australian
IPGRI	International Plant Genetic Resource Institute
MBC	Mattson Bean Cooker
NBPGR	National Bureau of Plant Genetic Resources
NIAS	National Institute of Agrobiological Sciences
PCA	Principal Component Analysis
PEM	Protein Energy-malnutrition
RDA	Recommended Dietary Allowance
SAS	Statistical Analysis System
TaCRI	Tanzania Coffee Research Institute
TARI	Tanzania Agricultural Research Institute
TMSH	Trimethyl Sulfonium Hydroxide
UNICEF	United Nations Children's Fund
WFP	World Food Program
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

The present study evaluated some agro-morphological and biochemical characteristics of 160 accessions of wild and under-exploited legumes of the genus *Vigna* (*V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata*) and explored some preliminary aspect of their utilization such as their cooking properties and farmers' perception, prior knowledge and acceptability as useful resource in the food chain value. An accession simply refers to a group of related plant material from a single species which is collected at one time from a specific location. Each accession is an attempt to capture the diversity present in a given population of plants.

Legumes are well recognized for their important nutritional value for both humans food and animals feed and are sometimes refered to as the "poor man's meat". The *Phaseolus* (Common bean) and the *Vigna* (Cowpea) groups are the most recognized and consumed legumes genera in addition to soybean (*Glycine max*) (Garcia *et al*., 1997; Gepts, 2001). Among these two genera of legumes, there exist many domesticated edible species of beans as well as many wild under-exploited (non domesticated species with no commercial names yet), under-utilized (domesticated but non improved varieties known as farmers local varieties possessing local names) and unexplored species (species with very little human attention).

It has been documented that the genus *Vigna* (in the family *Fabaceae*) is comprised of more than 100 wild species within which very few species have been domesticated (Tomooka *et al*., 2014). Crop species with little attention or completely ignored by agricultural researchers, plant breeders, and policymakers which are wild or semi-domesticated varieties and non-timber forest species adapted to particular local environments are defined as neglected and underutilized species (Padulosi *et al*., 2013). The term under-exploited wild Vigna species in this context denote those Vigna species which have not yet been domesticated. They do not possess commercial names since they have not got a common popular use by people or group of people. Thus, they should be differentiated from some domesticated Vigna species such as Bambara groundnuts (*Vigna subterranea*), considered as under-utilized crops. They are regarded as wild and under-exploited species of *Vigna* which are collected from their natural

agro-ecological environment and kept in research genebanks for breeding purposes. It is curiously noted that neglected and underutilized species present tremendous opportunities for fighting poverty, hunger and malnutrition (Padulosi *et al*., 2013). In addition, it is also reported that wild plant relatives present uncontestable potential genetic resources for crop improvement and an avenue for exploring alternative production systems.

According to the Food and Agriculture Organization (FAO) of the United Nations recent report, the immense challenge of achieving the Zero Hunger target by 2030 is still persistent. Therefore, the search for alternative food and feed sources for human and animal nutrition to feed the exponentially growing human population is a daunting task. It is imperative that 70% more food is needed to cover the gap between suppy and demand of food. Exploring the new, innovative methods of crop production remains the most viable and sustainable option available to most researchers to achieve food and nutrition security.

Some species of wild under-exploited *Vigna* genus have been reported with good agronomic characteristics such as disease resistance (Oyatomi *et al*., 2016a), important nutrients and mineral elements (Difo *et al*., 2015; Macorni *et al*., 1997) as well as nutraceuticals (Bhat & Karim, 2009). On the other hand, the challenges faced by the cultivated legumes varieties are beginning to raise serious concerns to the scientific community (Ojiewo *et al*., 2018). It is revealed in a recent report that domesticated legume crop production is challenged by a number of biotic (diseases and pests) and abiotic stresses (heat, frost, drought and salinity), edaphic factors (associated with soil nutrient deficits) and policy issues (where less emphasis is put on legumes compared to priority starchy staples) (Ojiewo *et al*., 2018). This might be one of the key motivating factors that led to many attempts in breeding and bio-fortification.

The yield of many legumes such as cowpea is affected negatively by different biotic and abiotic factors. Yield losses in cowpea associated with parasitic weed, Striga gesnerioides have been reported to be as high as 83% upto 100% (Cardwell & Lane, 1995). In Addition, according to (Mamiro *et al*., 2011), the contribution of micro- and macro- nutrients is significant for both improved lines and local varieties of cowpea in Tanzania. Moreover, leaves have higher mineral content than the grains and a low nutrient intake per capita among the citizen, which may not meet the recommended dietary allowance (RDA) for most nutrients.

Food insecurity, protein-energy malnutrition (PEM), hidden hunger, increase demand for food and food-feed competition are among the major challenges for the developing countries (Bhat & Karim, 2009; Riley, 2016). These challenges, coupled with the negative effect of mono-cropping and climate change, increase the necessity for crop improvement. In addition, the low genetic diversity of crops which hinders crops domestication and artificial selection, presents a potential challenge for crop improvement.

This study focuses on the genus *Vigna*. The genus *Vigna* (in the family Fabaceae) is comprised of more than 100 wild species (Tomooka *et al*., 2014). Within that genus, only ten species have been domesticated (Harouna *et al*., 2018; Tomooka *et al*., 2014) while some species such as *Vigna racemosa* and *Vigna reticulata* have not been domesticated. They are regarded as wild relatives, under-exploited and unexplored species of *Vigna* which are collected and kept in research gene banks for breeding purposes. However, very few reports describing these wild species especially in terms of their biochemical characteristics and utilization have been published (Harouna *et al*., 2018). Few accessions (maximum five) were considered in the chemical evaluation of seven species (*Vigna vexillata*, *Vigna vexillata macrosperma*, *Vigna oblongifolia*, *Vigna unguiculata dekindtiana*, *Vigna racemosa*, *Vigna reticulate* and *Vigna ambacensis*) reported earlier (Macorni *et al*., 1997). Fermentation characteristics and fortification value of *Vigna racemosa* have also been reported (Difo *et al*., 2015; Folashade *et al*., 2017). However, out of the one hundred existing wild species, much is yet to be explored and exploited for the benefit of mankind either in terms of food variety addition or to biodiversity conservation (Harouna *et al*., 2018; Harouna *et al*., 2019b).

Therefore, this study examined the biochemical and agro-morphological characteristics of 160 accessions of four wild unexplored *Vigna* species (42 of *V. ambacensis,* 5 of *V. racemosa,* 52 of *V. reticulata* and 61 of *V. vexillata*) and reveal information leading to their future domestication and utilization.

1.2 Statement of the Problem

According to FAO, 70% more food is needed over the next four decades to adequately nourish the human population projected to exceed 9 billion by 2050 (FAO, 2009). Besides this fact, humankind now depends on a reduced amount of agricultural biological diversity for its food supplies due to agricultural modernization, changes in diets and population

density. Therefore, a need to screen the hitherto wild species for an attempt to domesticate more food crops could be a judicious method that contributes in mitigating this challenge.

In Africa, some species of wild under-exploited *Vigna* species have been reported with good agronomic characteristics such as disease resistance (Oyatomi *et al*., 2016a), important nutrients and mineral elements (Difo *et al*., 2015; Mamiro *et al*., 2011) as well as nutraceuticals compounds (Bhat & Karim, 2009). However, the existing and mainly cultivated varieties of *Vigna* species such as cowpea (*Vigna unguculata*) and mung beans (*Vigna radiata*) are facing challenges both in terms of agronomic characteristics and nutritional content due to several biotic, abiotic and physiological constraints which led to many attempts to solve these constraints through breeding and bio-fortification (Quiroz *et al*., 2016; Singh, 2016). Therefore, there is a need to check the diversity and biochemical constituents of the wild relatives of the *Vigna* species in order to provide useful information that will help to improve the cultivated varieties or to domesticate the wild ones.

Lastly, considering the utilization of legumes, it has been reported to have some arguments regarding producing food to feed animals versus humans especially hungry people (Capper *et al*., 2013). In fact, it has been reported that most ingredients of the livestock feeds are also used for human nutrition in East Africa and this has led to competition between humans and animals. Therefore, there is a need for researchers to first evaluate the potential of the wild/underutilized beans (*Vigna* species) in order to help identify and set the type of *Vigna* species to be directed for animal use and contribute in policy formulation.

1.3 Rationale of the Study

More than 820 million people in the world were hungry in 2018, underscoring the immense challenge of achieving the Zero Hunger target by 2030 (FAO, 2019; IFAD, 2019; UNICEF, 2019; WFP, 2019; WHO, 2019). Additionally, it is unanimously recognized by many researchers and organizations that only 12 crops contribute most to the current global food production, with only three crops (rice, wheat and maize) providing more than 50% of the world's calories (Singh *et al*., 2019). A rapid reduction of the gene pool in both plant and animal genetic resources is observed as only a dozen species of animals provide 90 percent of the animal protein consumed globally and just four crop species provide half of the plant-based calories in the human diet (FAO, 2009). Paradoxically, more than a thousand wild

species of plants including legumes exist and are not exploited for their domestication as food crops.

The yield of many legumes such as cowpea is affected negatively by different biotic and abiotic factors. Yield losses in cowpea associated with parasitic weed, *Striga gesnerioides* have been reported to be as high as 83% upto 100% (Cardwell & Lane, 1995). In Addition, according to (Mamiro *et al*., 2011), the contribution of micro- and macro- nutrients is significant for both improved lines and local varieties of cowpea in Tanzania. Moreover, leaves have higher mineral content than the grains and a low nutrient intake *per capita* among the citizen, which may not meet the recommended dietary allowance (RDA) for most nutrients.

The major livestock (including poultry) feed ingredients have been facing market competition with human food demands in some East African countries (Mengesha *et al*., 2011). This is due to increased demands for feeds so as to increase production of animal proteins, especially for ingredients with relatively good protein and energy values. One of the easiest methods to search for alternative source of food or feed could be by screening the hitherto wild under-exploited or under-utilized *Vigna* species.

Accessions of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* seeds were readily available for dispatch from the Genetic Resources Center of the International Institute of Tropical Agriculture, (IITA), Ibadan- Nigeria and the Australian Grain Genebank (AGG) at the start of this study which justify their choice. In addition the passport data from these genetic resource centers revealed abundance and geographical diversity at the collection sites.

1.4 Objectives

1.4.1 General Objective

To explore the biochemical, agro-morphological, cooking characteristics and farmers acceptability perspectives of 160 accessions of four wild unexplored *Vigna* species in order to reveal information leading to their future domestication and utilization.

1.4.2 Specific Objectives

(i) To elucidate some qualitative and quantitative agro-morphological characteristics of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(ii) To identify the farmers' preferences, perception and prospective uses of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(iii) To determine the cooking characteristics of seeds of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(iv) To evaluate some biochemical characteristics (proximate, mineral and fatty acid composition) of seeds of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

1.5 Research Questions

(i) Can some accessions of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* possess qualitative and quantitative agro- morphological characteristics that can be domesticated or benefit some locally cultivated accessions for crop improvement programs?

(ii) What could be the farmers' preferences, perception and prospective uses of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions that can be good for their uses and domestication?

(iii) Are the cooking characteristics of grains of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions comparable to those of domesticated ones?

(iv) Do some accessions of *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* possess significant nutritional qualities that could make them edible or domesticated?

1.6 Significance of the Study

This study intended to significantly impact on food and nutrition security in the society. Some of the knowledge gaps that will be addressed and those which show the importance of this study are as follows:

(i) It revealed useful information that will help in wild *Vigna* (*V. ambacensis, V. racemosa, V. reticulata and V. vexillata*) domestication and utilization. For instance, accessions for domestication have been identified. Their potential characteristics in terms of agro-morphology, farmers' acceptability, cooking and some biochemical

components have been revealed. This will greatly help further research and ease the domestication of selected wild accessions.

(ii) It has also provided useful information for *Vigna* genus breeding program through agronomic and nutritional characteristics. The agglomerative hierarchical clustering has grouped some wild accessions together with domesticated accession. This is also an indication that there could be compatibility of breeding with the wild accessions. Therefore, some important traits like mineral content could be transferred to the domesticated accessions that lack such traits. Meaning that the information from this point can help in biofortification for example.

(iii) It showed the genetic diversity of wild *Vigna* legumes. The study has revealed the various expressions of each character that could be exploited in a genetic study to elucidate the various alleles of each character.

(iv) It will bring about the domestication of many wild *Vigna* species and increase the cultivated varieties of *Vigna* species and impact on foods security level of poor societies.

(v) It provided useful information that can help policy makers to decide on which type of *Vigna* species to be directed to specific uses as per farmers' preferences.

(vi) It revealed the nutritional potential of some wild and under-exploited *Vigna* species. This study has thus exposed the nutritional components that have been for sometimes not well known and therefore, this will motivate researchers to give more attention on this aspect of the wild *Vigna* legumes.

1.7 Delineation of the Study

This study was design and carried out to cover the following scope:

(i) The qualitative and quantitative agro-morphological characteristics of wild Vigna legumes, namely *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(ii) The identification of farmers' preferences, perception and prospective uses of the wild *Vigna* legumes, namely *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(iii) The determination of the cooking characteristics of seeds of of the wild *Vigna* legumes, namely *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

(iv) The evaluation of some biochemical characteristics (proximate, mineral and fatty acid composition) of seeds of of the wild *Vigna* legumes, namely *V. ambacensis, V. racemosa, V. reticulata* and *V. vexillata* accessions.

The following are some of the aspects were regarded as out of the scope of this study:

- The study focused only on four wild *Vigna* species and limited number of accessions based on the available information on the literature and their availability from the genebanks.
- The study did not cover the aspects of Invivo and invitro toxicity study as it was not designed to address that.
- Other essential minerals apart from Fe, Zn, Mn and Cu and Amino acids were not investigated as they were not designed to cover the scope of this study.
- The examination of the carbohydrates, protein and fiber fractions to ascertain the digestibility are not included as part of this study.
- Determination of Phytochemicals/antinutrients in the wild legumes was not included in the scope of this study.
- Evaluation of disease resistance and effects of abiotic and abiotic factors was not part of this study.
- The socio-economic implication (cost analysis) of the utilization of the wild *Vigna* legumes.
- Studies of high protein and high lipid genes were also not included in this study.
- The consumer response, including sensory characteristics of these legumes were out of the scope of this study.

- The feed formulation and responses of animals fed on feed formulated with wild legumes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The domestication of many wild plants for thousands of years since the beginning of human civilization is the result of the contant search of food and feed for human and animal nutrition. Crops have been manipulated in order to possess new and desirable characteristics. Due to artificial selection processes based on phenotypes over the centuries (Acquaah, 2007), new species and/or varieties have been derived. These new varieties have become genetically different from their original progenies over time. In some cases, such domesticated varieties have received very limited economic importance in the global market, hence the term orphan or underutilized crops attributed to them (GFAR, 2001).

Food insecurity, protein-energy malnutrition (PEM), hidden hunger, increase demand for food and food-feed competition are among the major challenges for the developing countries (Bhat & Karim, 2009; Riley, 2016). These challenges, coupled with the negative effect of mono-cropping and climate change, increase the necessity for crop improvement. In addition, the low genetic diversity of crops which hinders crops domestication and artificial selection, presents a potential challenge for crop improvement.

It is generally known that legumes have very important nutritional value for both humans and animals and are referred to as the "poor man's meat''. The two widely consumed types of legumes belong to two different genera namely the *Phaseolus* and the *Vigna* commonly referred to as common beans and peas, respectively (Garcia *et al*., 1997; Gepts, 2001). Among these two genera of legumes, there exist many domesticated edible species as well as under-exploited wild species. This review focuses on the genus *Vigna*.

The genus *Vigna* is categorized into seven sub-genera and sixteen sections (Fatokun *et al*., 1997). The seven sub-genera include *Ceratotropis, Haydonia, Lasiocarpa, Macrorhycha, Plectotropis, Sigmoidotropis* and *Vigna* (Boukar *et al*., 2013). All domesticated and cultivated *Vigna* varieties belong to only three sub-genera, namely *Ceratotropis, Plectotropis* and *Vigna* (Pandiyan *et al*., 2012). The subgenus *Ceratotropis* is well known as Asian *Vigna* and the sub-genus *Vigna* commonly called African *Vigna* are the most known sub-genera containing most popular legumes like cowpeas, black gram and green gram (Boukar *et al*., 2013; Pandiyan *et al*., 2012). Studies have shown differences between the three sub-genera

and also revealed that *Vigna vexillata* is an intermediate species between Asian and African *Vigna* (Boukar *et al.*, 2013). Successful crosses between members of the three sub-genera have been reported, though some failure of inter-specific hybridization involving members of the Asian and African *Vigna* have also been reported and attributed to post-fertilization events (Fatokun *et al.*, 1997).

It has been documented that the genus *Vigna* (in the family Fabaceae) is comprised of more than 100 wild species within which very few species have been domesticated (Tomooka *et al.*, 2014). Crop species with little attention or completely ignored by agricultural researchers, plant breeders, and policymakers which are wild or semi-domesticated varieties and non-timber forest species adapted to particular local environments are defined as neglected and underutilized species (Padulosi *et al.*, 2013). The term under-exploited wild *Vigna* species in this context denote those *Vigna* species which have not yet been domesticated. They do not possess commercial names since they have not got a common popular use by people or group of people. Thus, they should be differentiated from some domesticated *Vigna* species such as Bambara groundnuts (*Vigna subterranea*), considered as under-utilized crops. They are regarded as wild and under-exploited species of *Vigna* which are collected from their natural agro-ecological environment and kept in research genebanks for breeding purposes. It is curiously noted that neglected and underutilized species present tremendous opportunities for fighting poverty, hunger and malnutrition (Padulosi *et al.*, 2013). In addition, it is also reported that wild plant relatives present uncontestable potential genetic resources for crop improvement and an avenue for exploring alternative production systems (Dwivedi *et al.*, 2008; Couch, 2004).

Some species of wild under-exploited *Vigna* genus have been reported with good agronomic characteristics such as disease resistance (Oyatomi *et al.*, 2016a), important nutrients and mineral elements (Difo *et al.*, 2015; Macorni *et al.*, 1997) as well as nutraceuticals (Bhat & Karim, 2009). On the other hand, the challenges faced by the cultivated legumes varieties is beginning to raise serious concerns to the scientific community (Ojiewo *et al.*, 2018). It is revealed in a recent report that domesticated legume crop production is challenged by a number of biotic (diseases and pests) and abiotic stresses (heat, frost, drought and salinity), edaphic factors (associated with soil nutrient deficits) and policy issues (where less emphasis is put on legumes compared to priority starchy staples) (Ojiewo *et al.*, 2018). This might be one of the key motivating factors that led to many attempts in breeding and bio-fortification

(Quiroz *et al*., 2016; Singh, 2016). Therefore, studies on the genetic diversity and biochemical constituents of the wild and domesticated wild relatives of the *Vigna* species are necessary in order to provide useful information that will help to improve the cultivated varieties or to domesticate the wild ones.

In an attempt to contribute to bringing possible solutions to these challenges and information that expose the knowledge gaps in this area, this review focuses on the genetic, agronomic, nutritional, biochemical potentials and various utilization of the under-exploited wild *Vigna* species in human and animal nutrition with reference to the domesticated ones.

2.2 An overview of Domesticated *Vigna* Species with their Potentials

Reports have shown that about 100 species of the genus *Vigna* (Leguminosae plant family) exist and are widely distributed in the tropical and subtropical areas of the world (Tomooka *et al*., 2002). So far, only ten (Takahashi *et al*., 2016) species of the genus *Vigna* have been domesticated and being widely utilized as human food though some are still considered domesticated but under-utilized. In this section, a brief review of the ten domesticated species with respect to their level of production, yield, availability, utilization as human foods and the level of genetic diversity are given.

2.2.1 Azuki Bean (*Vigna angularis* var. angularis)

This is recently considered as the second most important legume in Japan after soybean (Kaga *et al*., 2008). It is a widely consumed dietary legume crop in Eastern Asia with an annual cultivation area estimated to be 670 000; 120 000; 30 000 and 20 000 ha for China, Japan, the Korean peninsula and Taiwan respectively (Kang *et al*., 2015; Rubatzky & Yamaguchi, 1997). This clearly shows how much important it is for human nutrition and consequently the impact of its domestication on food security. The crop average seed yields is estimated to be in the range of 1-2.5 t/ha (Ecocrop, 2007). It is estimated to be 1450 kg/ha in Taiwan, 1900 kg/ha in Japan, 500-600 kg/ha in Kenya and 1340-2240 kg/ha in New Zealand (Ecocrop, 2007; Jansen, 2006a). It is assumed that a variety of azuki bean with a yield of 2160 kg/ha could be able to uptake 74 kg N, 18 kg P and 50 kg K per ha (Jansen, 2006a). The real geographical location (country) where this bean was domesticated is not well known. However, the wide distribution of its presumed wild ancestor, *Vigna angularis* var. *nipponensis* in Japan suggests Eastern Asia as its domestication place. It is generally present there as a crop complex where the cultivated, wild, and weedy forms are widely

distributed and encountered (Kaga *et al*., 2008; Tomooka *et al*., 2002). The genetic diversity of its seed coat and seed size (Fig. 1A) further demonstrate its impact on the global food security level by adding food varieties.

2.2.2 Bambara Groundnut (*Vigna subterranea* (L.) Verdc)

This is documented to originate in West Africa with a considerable genetic diversity (Mohammed *et al*., 2016). It is an important food legume grown widely in semi-arid Africa which is closely related to cowpea (*Vigna unguiculata*) with which it shares much of its cultivation areas as well as origins of genetic diversity (Basu *et al*., 2007). In many parts of Africa, it is the third most important legume after groundnut (*Arachis hypogaea*) and cowpea (Basu *et al*., 2007). The seed yield varies and depends mainly on rainfall. It can reach up to 4 t/ha under field conditions. However, an average seed yield ranging from 300- 800 kg/ka is commonly reported under farmers conditions (Brink *et al*., 2006). Nutrition wise, it is rich in carbohydrate and protein, making it an important benefit to the diets of people who cannot afford expensive animal protein (Hillocks *et al*., 2012). Botanical wise, it consists of two taxonomic forms; var. *spontanea* comprising the wild forms, which are restricted to an area from Nigeria to Sudan with a centre of diversity around Cameroon and var. *subterranea* comprising the cultivated forms, found in many parts of the tropics and particularly sub-Saharan Africa (Basu *et al*., 2007). The relatively high genetic identity between wild and domesticated forms suggests that wild form (*Vigna subterranea* var. *spontanea*) is likely to be the true progenitor of cultivated forms (Pasquet *et al*., 1999). The higher genetic diversity in wild materials as compared with domesticated forms makes the *spontanea* forms important potential sources of beneficial genes for Bambara groundnut breeding and improvement (Pasquet *et al*., 1999). The most important and frequently used part of the crop as human food is the seed. Those seeds possess diverse identifiable morphological features, such as seed texture, colour, seed shape, seed eye and hilum colour and pattern. Figure 1B illustrates some of the morphological features of seed genetic diversity. The morphological features of Bambara groundnut can be utilized for its genetic improvement upon classification into homogenous seed material (Mohammed *et al*., 2016). Thus, future research should focus on the pre-breeding and breeding of this crop to its genetic potential, followed by the dissemination of seed of improved varieties to farmers.

2.2.3 Black Gram (*Vigna mungo var. mungo* (L.) Hepper)

This is also known as urd, urad, or mash and is another important grain legume mainly grown and consumed in South and Southeast Asian countries, like Afghanistan, Bangladesh, India, Pakistan, Nepal, etc. (Kaewwongwal *et al*., 2015). It originated from central Asia and now found in many tropical areas of Asia, Africa and Madagascar while in the USA and Australia it is mostly cultivated as a fodder crop (Jansen, 2006b). Global its growing area is estimated to be higher than 5 Mha with India being the largest producer (about 3 Mha), followed by Myanmar (about 1 Mha) and Pakistan (0.5 Mha) (Kaewwongwal *et al*., 2015). The average dry seed yield is about 350–800 kg/ha but under good management it can reach 1500–2500 kg/ha (Jansen, 2006b). The seeds contain about 25% protein and 65% carbohydrates and are mainly consumed as soup. The seed flour is used as a major ingredient for several kinds of foods, such as cakes, biscuits, snacks, cookies and doughnuts. Sprouts produced from black gram are also consumed as a vegetable source of vitamins and minerals. It is believed to have been domesticated in India about 4500 years ago from its wild progenitor, *Vigna mungo* var. *silvestris* (Fuller & Harvey, 2006). Many breeding programs for this crop exist in India, Pakistan and Thailand. However, to increase its potential as food and feed, it is necessary to study and exploit its genetic diversity. There has been less research on this crop, especially in terms of molecular genetic diversity as compared with cowpea and mung bean though some efforts have been noticed (Ghafoor & Ahmad, 2005; Ghafoor *et al*., 2001; Gupta *et al*., 2001; Singh *et al*., 2009; Sivaprakash *et al*., 2004; Souframanien & Gopalakrishna, 2004). However, these studies have provided little information on the extent of genetic diversity in this crop because most of them, employed less than 150 accessions, and the germplasm used originated from only a single geographical region (country). Though very well known as ‘’black’’ gram, the genetic variations (diversity) of the seeds based on color, size and texture for this crop exist (Fig. 1C) confirming that many cultivated varieties do exist (Vyas *et al*. 2016) and hence their potential is promising.

2.2.4 Cowpea (*Vigna unguiculata* (L.) Walp.)

This is one of the most important grain legume crops in the world with a larger zone of occurrence and cultivation which covers the semiarid regions of Africa, Asia, Southern Europe, Southern United States, and Central and South America (Diouf, 2011; Singh, 2006; Timko *et al.*, 2007). Its cultivation covers 14.5 million hectares with an annual production of 5.5 million tons worldwide. Presently, Nigeria is the largest producer of cowpea followed by

Niger, Burkina Faso, Myanmar, Cameroon and Mali (Simon *et al*., 2007). It is cultivated not only for its seed but also for its leaves which serves both as a human food as well as animal feed. It is assumed that leaves yield of about 400 kg/ha can be obtained without noticeable reduction of seed yields (Madamba *et al*., 2006). The world average yield is estimated at 240 kg/ha and 500 kg/ha for its dry seed and fodder (air-dried leafy stems) respectively (Madamba *et al*., 2006). Its average yield of dry seeds under subsistence agriculture in tropical Africa is estimated at 100–500 kg/ha (Madamba *et al*., 2006). This crop plays a crucial role in human nutrition due to its grain protein content (23–32 %) of high quality (rich in lysine, tryptophan) and high nutritional value. The grains also contain a substantial amount of mineral and vitamins (like folic acid and vitamin B) and the hay is used for feeding animals during the dry season in many parts of West Africa (Badiane *et al*., 2014). In the poorer areas, cowpea is a valuable source of protein cheaper than animal protein (fish, meat, or poultry), thus helping to fight malnutrition for the low-income farmers. Both the leaves and the grains of this crop have found various uses especially as human food since they are processed into various foods products such as Akara, Moin-moin, Koki, Couscous, Red-Red Stew, Ndambe, Thiebou Kathiakh, Cake, Bread and Cookie and used as ingredients in complementary food formulation for children (Badiane *et al*., 2014; Hallensleben, *et al*., 2009; Mamiro *et al*., 2011). Although mainly cultivated and consumed in West Africa, it is believed it was first domesticated in East Africa and then transported to West Africa (Badiane *et al*., 2014). The seed also present a certain range of genetic diversity with phenotypic attributes of color (white, cream, green, buff, red, brown, or black), texture (smooth, rough, or wrinkled) and uniformity (solid, speckled, to patterned) (Badiane *et al*., 2014). Much research attention is being given to this crop as compared with other legumes of the genus *Vigna* and this may be due to its wider distribution and uses. Evaluation of genetic diversity, variation, and genetic distance in cowpea genotypes has been conducted in several studies according to morphological and physiological markers (Ntundu *et al*., 2006; Siise & Massawe, 2013; Stoilova & Pereira, 2013), and molecular markers such as Amplified Fragment Length Polymorphism (AFLP) (Coulibaly *et al*., 2002; Tosti & Negri, 2002). Nevertheless, much is still to be done to cover the existing gaps concerning this crop. Morphological identification of cowpea seeds is illustrated in Fig. 1D.

2.2.5 Creole Bean (*Vigna reflexo-pilosa* var. glabra = *Vigna glabrescens*)

This is the only tetraploid species in genus *Vigna* and the little-known cultivated species of the sub-genus *Ceratotropis* (Muthaiyan *et al*., 2011). For that reason, information on its area of production and yield data are very scanty and almost not documented. The ancestral species that make up this allotetraploid species have not conclusively been identified, although previous studies suggested that a donor genome for this crop is *V. trinervia* (Chankaew *et al*., 2014). It was first found used as a forage crop in West Bengal, Mauritius, and Tanzania, while it was used as a pulse in the same ways as mung bean in Vietnam and Philippines (Tomooka *et al*., 2002). The crop is considered to have been domesticated in Southeast Asia from its possible wild ancestor, *Vigna reflexo-pilosa* var. *reflexo-pilosa* (Egawa & Tomooka, 1994). The wild form has a wide geographical distribution ranging from East, Southeast and South Asia, and across the islands from the west to the north Pacific Islands. The cultivated form is very close to mung bean as it was recognized in the past as a glabrous variety of mung bean, *V. radiata* var. *glabra* which was later treated as a distinct species, *V. glabrescens* (Chankaew *et al*., 2014). It is distinguished from its wild progenitor mainly through the thick glabrous stem and erect-growth habit (Tomooka *et al*., 2002). It has been reported that this crop possesses a high potential as a gene source for breeding other *Vigna* crops (Chankaew *et al*., 2014). It also presents morphological variations from its seeds as shown in Fig. 1G.

2.2.6 Minni Payaru (*Vigna stipulacea*)

This is a newly recognized as a domesticated Indian *Vigna* species and therefore has very limited published information (Muthaiyan *et al*., 2011). The name *V. stipulacea* has not been used in the Indian literature and this species seems to have been included in the description of *V. trilobata* (Muthaiyan *et al*., 2011). It was first known to be a wild *Vigna* species until recently when a survey was conducted in India; researchers realized that there was a semi-domesticated form of *V. Stipulacea* in Tamil Nadu (India) and many other areas around that region (Tomoka, 2008). For many farmers, it has different utilization and was cultivated for different purposes such as animal feeding, manure production as well as an ingredient for cake baking (Muthaiyan *et al*., 2011). Further studies are still needed to unveil the possible potential of this crop. Figure 1J shows the seed structure of the crop as there is few literature showing different seed variations.

2.2.7 Moth Bean (*Vigna aconitifolia* (Jacq.) Marechal)

This is a minor legume crop and considered to be the most drought and heat tolerant cultigen among Asian *Vigna* (Tomooka *et al*., 2011). It is widely grown in India and the Far East and has been qualified as a possibly more significant food source for the future (Adsule, 1996). It is considered to have been domesticated in India, Pakistan, Myanmar or Ceylon (NARO Genebank, 2017). India's driest state, Rajasthan, is the major moth bean growing state contributing almost 86% of the area of the country's under its cultivation (Gupta *et al*., 2016). Its average seeds yield is estimated to be in the range of 70–270 kg/ha, though yields of up to 2600 kg/ha have been recorded in the United States and Australia with experimental seeds (Brink & Jansen, 2006). The Yield of its green matter for forage has also been estimated to be at about 37–50 t/ha and that of its hay at about 7.5–10 t/ha (Brink & Jansen, 2006). It is generally also known by common names such as dew bean, dew gram, moth, mat, mat bean and matki. Its nutritional content is also well appreciable for human consumption as it possesses very important nutrients. The nutritional content consists of 24.3% protein, 68.0% carbohydrates, 3.9% lipids, 3.8% ash, 133 mg/100g calcium, 356 mg/100g phosphorus, 183 mg/100g magnesium, 11 mg/100g iron, 0.50 mg/100g thiamine, 0.10 mg/100g riboflavin and 1.7 mg/100 g niacin (Adsule, 1996). The wild ancestral form and cultivated form have not been distinguished taxonomically. However, the existence of a putative wild ancestral form in Tamil has been recognized (Tomoka, 2008). Researchers have found that there is a substantial genetic variation between moth bean germplasms which could be used in crop improvement (Gupta *et al*., 2016). Few accessions of moth beans kept at the National Institute of Agro-biological Sciences genebank, Japan is as shown in Fig. 1H. Further research on these accessions is needed to shed more light on their genetic potential in legume improvement programs.

2.2.8 Mung Bean (*Vigna radiata* (L.) Wilczek)

This is another important grain legume, especially in Asia (India, South East-Asia, and East Asia) but also eaten in Southern Europe and in the Southern USA (Heuzé *et al*., 2015). It is mainly produced in Asia (90%) with India being the largest producer (more than 50% of world production) and consumer of its entire production. China also produces large amounts, representing 19% of its legume production, but Thailand remains the main exporter of its production which increased by 22% per year between 1980 and 2000 (Lambrides & Godwin, 2006). Although this crop is also produced in many African countries, it is known as a minor

crop (Mogotsi, 2006). Reliable statistics for its production are difficult to obtain, as it is often lumped together with that of other *Vigna* and *Phaseolus* spp. It is reported that China produced 891 000 t (19% of her total pulse production) from 772 000 ha in 2000 (Mogotsi, 2006). Its average yields are estimated at 300–700 kg/ha, though yields of 1.25 t/ha were obtained under irrigation in Kenya (Mogotsi, 2006). Such productivity also attests its importance as food for human nutrition and food security impact. It is rich in crude protein (25-31%), iron (4-6 mg/100 g), crude fiber (1-5%) and many other biochemical constituents (Anwar *et al*, 2007). It is believed that the seeds represent an invaluable source of digestible protein for humans in places where meat is lacking or where people are mostly vegetarian (Heuzé *et al*., 2015). It can be cooked fresh or dry and be eaten whole or made into flour, soups, porridge, snacks, bread, noodles and ice-cream (Heuzé *et al*., 2015). The crop is known to have originated from India. Based on the archaeobotanical evidence, both south-eastern India and western Himalayan foothills are probably the places where domestication of this crop could have occurred (Fuller & Harvey, 2006). Its presumed progenitor is the wild form (*Vigna radiata* var. *sublobata* (Roxb) Verdcourt), which is widely distributed across the world tropics from western Africa to northern Australia and Papua New Guinea (Tomooka *et al*., 2002). The cultivated form was introduced to southern and eastern Asia, Africa, Austronesia, the Americas and the West Indies. It is now widespread throughout the tropics and found from sea level up to an altitude of 1850 m in the Himalayas (Mogotsi, 2006). Both the cultivated and the wild forms of this crop also possess a very large pool of genetic diversity which is conserved in genebanks around the world as genetic resources (Tomooka *et al*., 2002). The World Vegetable Center with about 5600 accessions possesses the largest collection of genetic resources for this crop. The number of wild genetic resources (var. *sublobata*) has significantly increased due to the particular interest by Australian and Japanese scientists for the crop recently (Lawn *et al*., 2002; Vaughan *et al*., 2006). The use of genetic resources of wild and cultivated germplasm efficiently for research and breeding through both morphological and molecular approaches is continuously gaining interests nowadays. This crop can easily be identified through its various seeds morphological traits as shown in Fig. 1E.

2.2.9 Rice Bean (*Vigna umbellate* (Thunb.) Ohwi & Ohashi)

This is a multipurpose legume as well as a neglected and under-utilized legume (Joshi *et al*., 2008). It was found naturally in India, Central China and in Southeast Asia and was

introduced to Egypt, the East Coast of Africa and the islands of the Indian Ocean. It is now cultivated in tropical Asia, Fiji, Australia, tropical Africa, the Indian Ocean Islands as well as in the Americas (USA, Honduras, Brazil, and Mexico) (Rajerison, 2006). Compared with cowpea (*Vigna unguiculata*), adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiata*), it is a less important crop. However, it represents a locally important contributor to human nutrition in parts of India and South-East Asia (Joshi *et al*., 2008; Tomooka *et al*., 2011). All parts of the plant are edible and used in culinary preparations (Heuzé *et al*. 2016). It is also used as an important fodder and a green manure (Tomooka *et al*., 2002). The dry seeds are boiled and eaten with rice or sometimes replace rice in stews or soups. In Madagascar, they are ground to make nutritive flour included in the children's food (Heuzé *et al*., 2016). The annual productivity and area of production for this crop is not documented enough (Rajerison, 2006). This may be due to its limited utilization and consumption despite its importance in some parts of the world. Its average seed yield is only 200–300 kg/ha which is assumed to be low due to its very short cycle, though experimental yields of up to 2500 kg/ha have been obtained in India (Rajerison, 2006). Fresh fodder yields of up to 35 t/ha has also been obtained (Rajerison, 2006). It is believed that it originated from Southeast Asia and was probably domesticated in Thailand and neighboring regions (Tomooka *et al*., 2011). Though it can better tolerate harsh conditions (drought, waterlogging and acid soils, etc.) than cowpea, it is still considered as an underutilized legume. There is a very limited number of breeding programs to improve this crop, therefore compelling farmers to rely on landraces rather than on cultivars (Joshi *et al*., 2008). Recently, the genetic diversity in cultivated and wild forms of this crop from Thailand, India and Nepal using molecular markers was studied (Muthusamy *et al*., 2008; Seehalak *et al*., 2006). However, studies of genetic diversity using many cultivated and wild accessions from many countries have not been conducted, and the level and geographic cline of genetic diversity remain unknown. A view of the seed morphological diversity of this crop can be seen in Fig. 1I.

2.2.10 Tuber Cowpea (*Vigna vexillata*)

This is another recently recognized as domesticated *Vigna* species as it was found cultivated in Bali and Timor, Indonesia (Karuniawan *et al*., 2006). That domesticated form was discovered with some important agronomic characteristics such as prominent seed size increase, loss of pod shattering and loss of seed dormancy (Tomooka *et al*., 2011). It is cultivated for its tuber but the seeds are also used as human food. Its root protein content is

15% which is about 2.5 times higher than that of yam (6%), 3 times higher than that of potato (5%) and sweet potato (5%) and 5 times higher than that of cassava (3%) (Tomooka *et al*., 2011). Earlier reports have described the use of wild *Vigna vexillata* as edible tuber and sometimes edible seeds in Africa (Senegal, Ethiopia, Sudan and South Africa), East and North East India, northern Australia and Southeast Asia (Tomooka *et al*., 2011). The limited use of this other domesticated legume can explain its limited productivity and commercialization and therefore the limited availability of documented information. However, forage dry matter yields ranging from 300—1100 kg/ha have been obtained in northern Australia while dry matter yields of 2780 kg/ha have been achieved in Zambia (PROTA, 2018). Seed yields of 500—1250 kg/ha have also been reported while fresh tuber yields of 1.44 t/ha have been obtained in Nigeria (PROTA, 2018). The wild form is an extremely polymorphic species and several taxonomic varieties exist (Tomooka *et al*., 2011). It also presents a considerable diversity in terms of seed morphology as shown below (Fig. 1F).

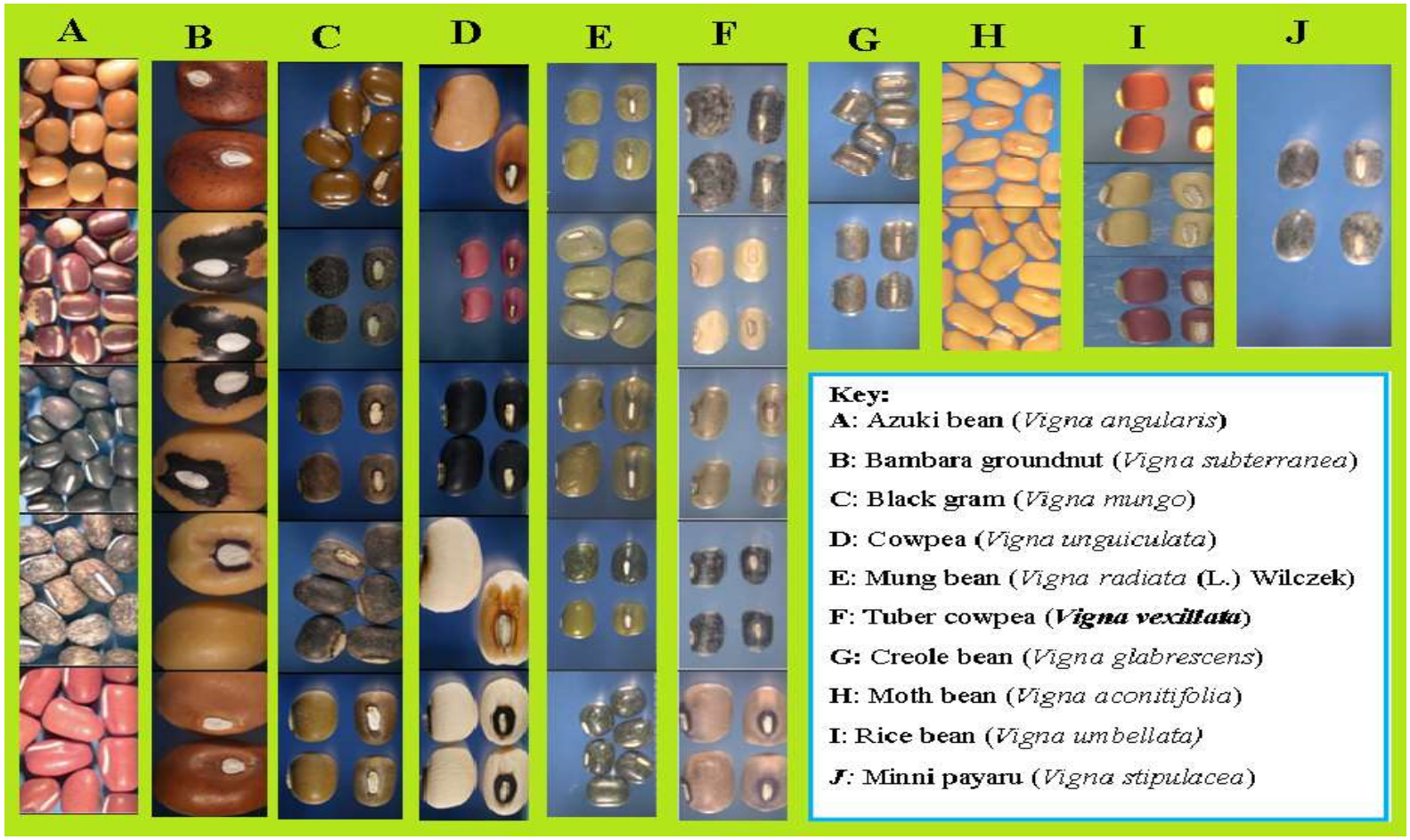


Figure 1: Illustration of Domesticated *Vigna* Species Depicting the Diversity in Seed Morphology. Source: Images Compiled from A (*Isemura et al.*, 2011), B, C, D, E, G, H, I (NARO Genebank, 2017), Tomooka *et al.*, 2011)

From the above description of the utilization of the domesticated *Vigna* species, it can be noticed how much the domestication has gained popularity and usages especially in terms of human as well as animal nutrition. However, a greater impact may be possible with more domesticated *Vigna* species existence as foods and feeds. Due to the lack of information and intensive research on the wild *Vigna* species, it is important to acknowledge the efforts of some international organizations in trying to genetically classify them and catalog them in genebanks.

2.3 The Diversity, Genetic Resources and Agronomic Characteristics of Wild Under-exploited *Vigna* Species

The world wild *Vigna* genetic resources, as well as cultivars, landraces, breeding populations, are maintained and cataloged by several international and national research programs and genebanks. Table 1 contains a summary of some of the wild *Vigna* species from different genebanks and databases. Most genebanks possess accessible databases, making the wild *Vigna* genetic resources available to breeders searching for new sources of genetic diversity, such as resistance or tolerance to abiotic stresses (drought, heat, waterlogged soils, acidic soils, zinc-deficient soils, and soils with toxic levels of boron) and biotic stresses (diseases, insects and weeds).

The Genetic Resources Center of the International Institute of Tropical Agriculture, (IITA), Ibadan-Nigeria possess the largest reservoir for the wild *Vigna* species originated from Africa (Table 1). The National Bureau of Plant Genetic Resources (NBPGR), India (Bisht *et al*., 2005) and The Australian Grains Genebank (AGG) also possesses a quite number of good wild *Vigna* accessions, while the genebank project of the National Institute of Agrobiological Sciences (NIAS), Japan mainly possess wild *Vigna* species of the domesticated correspondent. Access to some information from the genebanks requires a formal request depending on the information needed. Therefore, this section reveals some genetic resources of these wild *Vigna* species based on the information obtained from three genebank (Table 1): The Australian Grains Genebank, the Genetic Resources Center of the International Institute of Tropical Agriculture, (IITA), Ibadan- Nigeria and the National Bureau of Plant Genetic Resources (NBPGR), India.

Table 1: Origin and availability of wild *Vigna* Species existing in Genebanks

S/N	Species Name	Countries of Origin of accession
1	*Vigna aconitifolia*	India
2	*Vigna adenantha*	Nigeria, Argentina, Brazil, Colombia, Paraguay, Malawi, United States of America, Belgium , Equatorial Guinea, Sierra Leone
3	*Vigna adenatha*	Nigeria
4	*Vigna ambacensis*	Nigeria, Ghana, South Africa, Benin, Cameroon, Tanzania, Rwanda, Zaire, Malawi, Chad, Zambia, Mali, Niger, Republic of the Congo, Central African Republic, Botswana, Burundi, Gabon
5	*Vigna angivensis*	Madagascar
6	*Vigna antillana*	Brazil
7	*Vigna baoulensis*	Nigeria
8	*Vigna benuensis*	Cameroon
9	*Vigna bourneae*	India
10	*Vigna candida*	Brazil, Palau, Mexico, Colombia
11	*Vigna caracalla*	Uruguay
12	*Vigna comosa*	Senegal, Gabon, Republic of the Congo
13	*Vigna dalzelliana*	India
14	*Vigna davyi*	South Africa, Swaziland
15	*Vigna dekindtiana*	Nigeria
16	*Vigna dolomitica*	Zaire
17	*Vigna filicaulis*	Senegal, Chad, Central African Republic
18	*Vigna fischeri*	Botswana
19	*Vigna friesiorum*	India, Colombia, South Africa
20	*Vigna frutescens*	Tanzania, Rwanda, Malawi
21	*Vigna gentryi*	Colombia, Mexico
22	*Vigna glabrescens*	Philipines, India
23	*Vigna gracilis*	Cote d'Ivoire, Rwanda, Gabon
24	*Vigna hainiana*	India
25	*Vigna heterophylla*	Kenya
26	*Vigna hosei*	Indonesia, Tanzania, Malaysia, Botswana, Rwanda, Cameroon, Colombia, Benin, South Africa, Ghana, Zimbabwe, Niger
27	*Vigna juruana*	Cameroon
28	*Vigna kirkii*	Tanzania, Zaire, Malawi

S/N	Species Name	Countries of Origin of accession
29	*Vigna khandalensis*	India
30	*Vigna lasiocarpa*	Belgium, Colombia, Guam, Costa Rica, Brazil
31	*Vigna laurentii*	Burundi
32	*Vigna linearis*	Colombia, Belize, Puerto Rico, Brazil, Venezuela
33	*Vigna lobatifolia*	Namibia
34	*Vigna longiloba*	Congo
35	*Vigna longifolia*	Guam, Colombia
36	*Vigna luteola*	Australia, Brazil, Botswana, Central African Republic, Niger, Rwanda, Kenya, Mexico, Chad, South Africa, Colombia, Tanzania,
37	*Vigna macrosperma*	Mozambique
38	*Vigna mariana*	Republic of the Congo, Gabon, Mozambique, Benin, Equatorial Guinea
39	*Vigna membranacea*	Kenya, Ghana, Somalia, Ethiopia, Colombia
40	*Vigna minima*	Indonesia, India
41	*Vigna monophylla*	Tanzania, Zimbabwe
42	*Vigna multinervis*	Cote d'Ivoire, Gabon, Nigeria, Cameroon, Republic of the Congo
43	*Vigna mungo*	India, Japan
44	*Vigna nervosa*	Swaziland, Zimbabwe, South Africa
45	*Vigna nigritia*	Nigeria, Zaire, Liberia, Gabon, Benin, Ghana, Cameroon, Republic of the Congo, Congo,
46	*Vigna oblongifolia*	Kenya, Tanzania, Costa Rica, Rwanda, Zaire, Nigeria, Malawi, Botswana, Zambia, South Africa, Republic of the Congo, Zimbabwe, Namibia,
47	*Vigna parkeri*	Kenya, Colombia
48	*Vigna peduncularis*	Brazil, Colombia
49	*Vigna pilosa*	India
50	*Vigna platyloba*	Tanzania, Malawi, Zambia
51	*Vigna racemosa*	Zaire, Cameroon, Nigeria, Ghana, Botswana, Malawi, Central African Republic, Niger, Tanzania, Gabon, Colombia, Benin, Mali, Uganda, Belgium, Zambia,
52	*Vigna radiata*	Cameroon, Brazil, Madagascar, Ghana, Colombia, India, Australia
53	*Vigna reticulata*	Cote d'Ivoir, Zambia, Zaire, Burundi, Nigeria, Ghana, Tanzania, Chad, Republic of the Congo, Central African Republic, Malawi, Gabon , Kenya, Cameroon, Zimbabwe, Gambia, Kenya
54	*Vigna schimperi*	Zaire
55	*Vigna speciosa*	Colombia, Mexico

S/N	Species Name	Countries of Origin of accession
56	*Vigna spontanea*	Nigeria
57	*Vigna subterranea*	Nigeria
58	*Vigna trilobata*	India, Belgium
59	*Vigna triphylla*	Zaire
60	*Vigna umbellata*	China, India
61	*Vigna unguculata*	Zaire, Nigeria, Tanzania, Ghana, Central African Republic, Kenya, Chad, Botswana, Zambia, Malawi, Cameroon, Burkina Faso, Swaziland, The Republic of the Congo, Congo , Niger, Mozambique, South Africa, Senegal, Mali, Belgium, UK, Colombia, Laos, Philippines, Namibia
62	*Vigna venulosa*	Nigeria, Cameroon, Liberia
63	*Vigna vexillata*	Australia, Suriname, Zaire, Costa Rica, Rwanda, Brazil, Nigeria, Tanzania, Cameroon, Central African Republic, Malawi Chad, Zambia, Kenya, Swaziland, Niger, Ghana, Republic of the Congo, Botswana, Somalia, Zimbabwe, South Africa, Gabon, Benin, Mozambique, Panama, Colombia, India, Sudan, Mexico, Columbia, Senegal, Dominican Republic, Peru, Indonesia, Niger, Venezuela, Belize, Cuba
64	*Vigna wittei*	Tanzania, Zaire, Zambia, Congo, Republic of the Congo, South Africa, Gabon, Nigeria
65	*Species not identified (no species name mentioned)*	Tanzania, Nigeria, Mali, Botswana, Brazil, Colombia, Ghana, Democratic Republic of Congo, China, Republic of the Congo, Cameroon, Benin, Zambia, Burundi, Congo, Malawi, Mexico, Mozambique, South Africa, Niger

*****The number in front of the genebank is the number of accession in that genebank. The number of accession from the NBPGR here is based on the number used by Bisht (Bisht *et al*., 2005). Source: Compiled by authors based on 1. Wild *Vigna* Passport data of ‘’The Genetic Resources Center, International Institute of Tropical Agriculture, (IITA), Ibadan- Nigeria"; http://my.iita.org/accession2/index.jspx. 2. Purposely requested passport data for the three mentioned species from The Australian Grains genebank (AGG), link: http://www.seedpartnership.org.au/associates/agg. 3. National Bureau of Plant Genetic Resources (NBPGR), India (Bisht *et al*., 2005)

From Table 1 above, it is clear that the origin of the wild *Vigna* species is so diverse and there exist (65) identified species and about several other unidentified species comprising about 84 different accessions. The information gathered here may be limited to what could be accessed due to the difficulties in getting full access to the passport data of some of the genebanks and other existing genebanks. It should be also noted that some information about these species is not well organized and synthesized or lacking in the passport data of some of the genebanks.

The vegetative morphology and important agronomic characteristics of some of these wild *Vigna* species present a wide range of diversity which could be exploited in domestication and crop improvement. For example, it was revealed that most wild *Vigna* species under the subgenus *Ceratotropis* (also known as Asian *Vigna*) and some other subgenera of the genus *Vigna* present epigeal germination and sessile first and second leaves making these characters key distinguishing features for the wild *Vigna* species (Bisht *et al*., 2005).

2.4 Potentials of Under-exploited Wild *Vigna* Species as Promising Food Crops for the Future

2.4.1 Agronomic, Environmental and Climatic Potentials

The wild *Vigna* species present a very wide range of variability both in terms of important agronomic traits and genetic diversity, which makes it an important source of information for crop improvement and an important food and animal feed source for the future. In an earlier study about the genetic diversity of the *Vigna* species, it was demonstrated that the cultigens (domesticated forms) of the con-specific wild *Vigna* species present better agronomic characteristics than their wild relatives (Bisht *et al*., 2005). It was shown that the domesticated accessions were more robust in growth, with large vegetative parts and often of erect growth type with three to five fold increase in seed size and seed weight, except *V. aconitifolia*, which has still retained the wild-type morphology to a greater extent. This can be normally understood because domesticated crops are improved crops to suit human preferences through selection and breeding processes. On the other hand, some researchers still suggest that some desirable agronomic characters found in wild *Vigna* species should be explored for genetic improvement of domesticated *Vigna* species such as cowpea (Popoola *et al*., 2015). This means that the domesticated *Vigna* species still need some beneficial traits offered by the wild *Vigna* species despite the improvement done so far. Such characters are hairiness, abundant pod production, a high number of locules and seeds per pod, longer

lifespan and extensive branching habit which could lead to higher seed yield (Popoola *et al*., 2017). These observations have been reported for *Vigna unguiculata* (cowpea), *Cajanus cajan* (pigeon pea) and *Sphenostylis stenocarpa* (African Yam Bean) (Popoola *et al*., 2011). It is also believed that there is a high level of genome homology among the various cultivated varieties of cowpea for example, due to the non-exploitation of the genomes of the cowpea's wild relatives during the development of such cultivated varieties (Fatokun *et al*., 1997). This could present another challenge in individual recognition and characterization of individual varieties and their specific challenges in order to improve them. In addition, continuous efforts are being made through crossing processes to address susceptibility to some insect pests like pod borer (*Maruca vitrata*), sucking bug complex and storage pests which can cause high yield losses in some cultivated cowpea varieties (Baker *et al*., 1989; Fatokun *et al*., 1997).

Apart from the agronomic characteristics which have been addressed in a considerable level to suit human desires, the improved domesticated *Vigna* species now face climatic and environmental challenges. It is remarkably noticed that under harsh climatic and environmental conditions (biotic and abiotic stress), the domesticated *Vigna* species find it difficult to resist and perform well while their wild under-exploited relatives find no problem to such conditions. It is reported that wild *Vigna* species are adapted to various habitats including harsh environments such as sandy beaches, acid soils, limestone rocks, deserts and wetlands (Tomooka *et al*., 2014). For example, *V. marina* was found growing well in a saline land, *V. vexillata* in an acidic land, *V. exilis* in alkaline lands, *V. trilobata* in drought-prone lands, *V. luteola* in flood-prone lands and *V. stipulacea* in a pest and disease-prone environment (Tomooka *et al*., 2014).

Therefore, studies aiming at clarifying the mechanisms of adaptation to these extreme environments present great potential towards enhancing world food production. Moreover, reports have shown the potentials of the under-exploited *Vigna* as a disease-resistant genetic resource. It is the case for example for some wild *Vigna* species which were identified as highly resistant to *S. gesnerioides* though most of them were not members of section *Catiang*, where cultivated cowpea (*V. unguiculata*) belongs (Oyatomi *et al*., 2016a).

It is more appropriate to domesticate these wild species well adapted to environmental stress than improving stress resistance of existing domesticated species, due to the low levels of resistance to environmental stress (Tomooka *et al*., 2014). Unfortunately, there are very

limited numbers of studies attempting the domestication of these wild species. Furthermore, it is also noted that very few or almost nil studies that address individual species with an attempt of relating their agronomic features and genetic diversity with their biochemical and nutritional constituents as well as their acceptability by farmers and utilization as feed ingredients. Hopefully, this concern may attract the interest of the scientific community in the nearest future because of the positive impact made by other neglected underutilized species on improving rural livelihood (Gotor *et al.*, 2013; Padulosi *et al*., 2014).

Most of the recent studies are directed towards elucidating the genetic diversity of the wild *Vigna* species especially through morphological and molecular means (Illakkiam *et al*., 2014; Pandiyan *et al*., 2012). It is also supported that the taxonomic affinities and genetic diversity among the *Vigna* species are of great importance because they present a great potential utilization as nutritious human food, fodder for ruminant animals, cover crop for rotational farming and more importantly genetic improvement of cowpea (Popoola *et al*., 2015). The seed size, color, and shape of these wild *Vigna* species are also diverse (Fig. 2) that they can represent other important selection criteria for consumers' acceptances and preferences as well as for crop improvement programs.

Therefore, more research has to be focused on feed experiments to reveal their potentialities in animal nutrition and palatability studies for consumer acceptability of cooked seeds. Considering the level of food and feed challenges coupled to the unpredictable climatic and environmental constraints in some parts of the world, it is necessary to also screen and investigates some types of food crops that can adapt to such unpredictable conditions. There is a very limited source of information on the human or animal uses of these wild species. In addition, most of the previous studies were carried out on a limited number of accessions and species of the wild *Vigna* genus which limit the available information on the genetic diversity, agronomic features and biochemical constituents of many of these wild *Vigna* species. The limited uses, perception, and knowledge of these wild species by researchers and local communities (consumers and farmers) can explain the limited and almost no documented information about some of them. The seed morphology of these wild species best illustrates their genetic diversity and displays some of their admirable characters (Fig. 2).

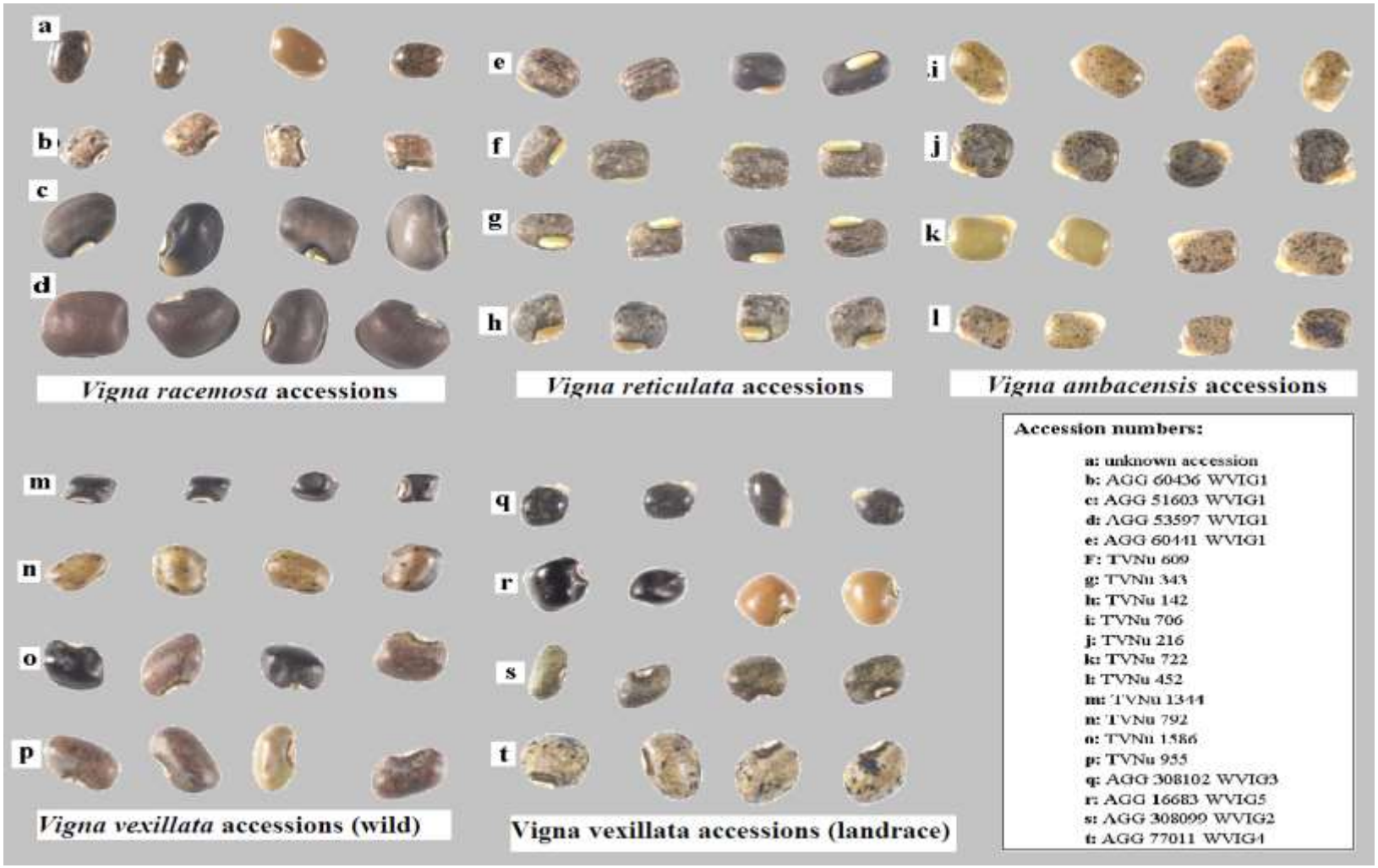


Figure 2: Photograph Depicting Diversity in Seed Morphology of some Wild *Vigna* Species. Source: Images Taken and Compiled by the Authors based on Seeds Requested from the Australian Grain Genebank (AGGB) (a, b, c, d, e, q, r, s, t) and the Genetic Resources Center, International Institute of Tropical Agriculture, (IITA), Ibadan- Nigeria (f, g, h, i, j, k, l, m, n, o, p)

2.4.2 The Nutritional and Biochemical Potentials of Under-exploited Wild *Vigna* Species

Very few reports in this line of research have been published. So far, only eight wild *Vigna* species have been quantitatively evaluated in terms of nutritional and biochemical composition out of the more than a hundred species present as shown in Table 1. These species are *Vigna vexillata, Vigna vexillata macrosperma, Vigna luteola, Vigna oblongifolia, Vigna unguculata dekindtiana, Vigna racemosa, Vigna reticulate* and *Vigna ambacensis* (Macorni *et al*., 1997). The flavonoid content of some species has also been qualitatively assessed through HPLC method (Lattanzio *et al*., 1997). The biochemical parameters so far accessed through published literature are summarized in Table 2.

Table 2: Some Quantitatively Evaluated and Reported Biochemical Constituents of Wild *Vigna* Species

S/N	*Vigna* Species	Protein (g/100g)	Trypsin Inhibitors (TIU/mg protein)	Lectin (HA)	Phytic acid (g/100g)	Tanin (g/100g)	Starch Fractions				Bioactive compounds
							TS (g/100g)	RDS (%TS)	SDS (%TS)	RS (%TS)	
1	***Vigna mugo*** **(black gram)**	23.6	-	-	-	3.98	37.9	-	-	3.40	Daidzein
2	***Vigna radiata*** **(green gram)**	24.5	-	-	-	2.25	39.9	-	-	4.18	Rutin, Kaempferol-3-rutinoside
3	*Vigna unguculata dekindtiana*	26.5	65	231	1.173	0.829	41.39	2.995	32.13	64.92	Hyperoside (Quercetin-3-galactoside), Robinin (Kaempferol-3-robinoside-7-rhamnoside)
4	*Vigna vexillata*	27.9	126	574	1.009	2.025	29.72	1.48	25.20	73.35	Rutin
5	*Vigna vexillata macrosperma*	25.5	151	517	1.01	0.359	41.62	7.90	22.06	70.04	Rutin
8	*Vigna reticulata*	23.1	29	5233	1.012	1.630	49.69	4.99	20.04	74.76	-
9	*Vigna ambacensis*	23.1	27	967	0.564	0.957	46.87	5.38	30.72	63.90	Kaempferol-3-rutinoside
10	*Vigna luteola*	22.2	213	16000	0.827	2.993	28.92	1.45	24.34	74.24	Rutin, Robinin

Note: "-" Means not found values from literature, TS means total starch, RDS: Rapidly digestible starch, SDS: Slowly digestible starch and RS: Resistant starch (RS) = TS - (RDS + SDS) for the wild *Vigna* species. Source: Compiled by the authors from (Bravo *et al*., 1999; Lattanzio *et al*., 1997; Macorni *et al*., 1997)

Table 2 shows that a very few species and accessions have been evaluated for a few biochemical parameters as compared with the existing number (Table 1). From the eight species quantitatively evaluated, *V. vexillata* present the highest protein content of 29.3% and all accessions of the studied species present a high sulfur amino acids content ranging from 2.05 - 3.63g per 16g N with a resistant starch content ranging from 64 - 75% (Macorni *et al*., 1997). This is an exceptionally important potential that could be exploited in cowpea bio-fortification for essential amino acids. In comparison with other domesticated *Vigna* species, the wild *V. vexillata* reported here presents a slightly higher protein content of about 5% over black gram (23.6%) and green gram (24.5%). Similar comparison with other domesticated edible crops shows that the lowest protein value as 22.2% (*V. luteola*) (Table 2) which is two times higher than the protein content of maize (10.2%) and wheat (14.3) and three times higher than that of rice (7.6%) (FAO, 2013). This shows how important the *Vigna* genus is as a source of proteinous food. There exists a wide variability in terms of trypsin inhibitor, tannin and lectins contents with *V. luteola* having the highest levels while *V. unguculata dekindtiana, V. reticulata* and *V. ambacensis* present very low levels (Macorni *et al*., 1997). From Table 2, the phytic acid content varies from 1.173 to 0.564% for *Vigna unguculata dekindtiana* and *Vigna ambacensis* respectively. These values are within the range of phytic acid concentrations in most legumes (1-3%) (Arendt *et al*., 2013) and hence prove once again that wild *Vigna* species can be exploited as potential human foods.

It is very important to note that information on studies purposely focusing on the chemical composition of wild *Vigna* species accessions is scanty or not well documented. The proximate composition, fatty acid composition, total phenolic content, antioxidant activity and amino acid profile of an unknown accession of *Vigna racemosa* were reported in a recent study (Omowaye *et al*., 2015). Another study also reported recently on the chemical changes during open and controlled fermentation of *Vigna racemosa* seed collected from their natural environment, regardless of their genetic specification (Difo *et al*., 2015). Other studies focusing on qualitative evaluation of bioactive compounds of *Vigna kirki, Vigna marina, Vigna gracilis, Vigna heterophylla, Vigna parkeri, Vigna hosei, Vigna adenantha, Vigna venusta, Vigna minima, Vigna glabrascens,* and *Vigna triphylla* have revealed the presence of biochemicals such as Robinin, Kaempferol-3-rutinoside, Isorhamnetin-3- rutinoside, Hyperoside, Delphinidin and Cyanidin (Bravo *et al*., 1999; Lattanzio *et al*., 1997; Macorni *et al*., 1997).

Information regarding the mineral content, the protein fractions, the lipid profile and functional potential of the wild *Vigna* species listed in Table 1 may still be under investigation by researchers. Such information might be a breakthrough in crop improvement (bio-fortification) activities leading to nutrients (such as minerals, proteins, lipids and vitamins) increase in legumes which is highly solicited nowadays in fighting hidden hunger in developing countries. Many other biochemical parameters of these wild *Vigna* species need also to be investigated to enhance their usages. It is also noted that the previous studies were carried out only on a few accession of these legumes. The chemical composition of the other parts of the *Vigna* species such as leaves and stems have not yet been given attention by scientific community either as roughage in animal nutrition or as human food and this may be due to their limited utilization (cultivation) and attention.

2.5 Potential Uses of Wild Under-exploited *Vigna* Species and Constraints

From the very few reports, it is apparent that only very few non-domesticated wild *Vigna* species have been used as human food in some parts of the world namely Australia (*Vigna marina*) (Tomooka *et al*., 2011), Southern Asia (*Vigna vexillata*) (Karuniawan *et al*., 2006), and Nigeria (*Vigna racemosa*) (Folashade *et al*., 2017). The increasing interest in preserving the genetic resources of these wild *Vigna* species in genebanks may suggest their important use as sources of valuable genes for the improvement of domesticated legumes. Unfortunately, the process of crop improvement is usually very limited by the phenomena of cross-species compatibility and low genetic diversity. Therefore, an emphasis on the domestication of new wild species will be of greater importance to global food security than the improvement of the domesticated species. Domestication of new crop species presents a tremendous advantage of diversifying food choices and uses for the benefit of human nutrition while conserving species biodiversity at the same time.

It is suggested that further studies are necessary in order to investigate if these underutilized/wild species could also be explored for their effective uses as animal feeds (ruminants and non-ruminants) or as a protein supplement in fish farming (Bhat & Karim, 2009). However, an attempt to exploit the wild *Vigna* species in animal feed experiments is very scanty. Curiously, soybean, a legume with an uncontestable importance in human nutrition due to its considerable oil and protein concentration as well as diverse uses as an ingredient of human foods. Also, it is highly used in the animal feed industry, creating a food-feed competitive relationship. Soybean production has increased substantially over the

past two decades to meet the rising oil demands for the human food market and meal for the animal feed market (Ravindran, 2013). It represents an important ingredient in the diet of poultry, pigs, cattle and aquatic animals by providing about 43% crude protein (Dei, 2011). This amount of protein could also be provided by some species of wild under-exploited *Vigna*, if adequate exploitation is carried out since some accessions have been proven to contain up to about 22- 29% protein and other important amino acids (Macorni *et al*., 1997).

As mentioned earlier, the wild *Vigna* species offer a diverse potential in terms of agronomic, ecological and biochemical characteristics. However, they present a very limited utilization in the crop improvement programs for the benefit of human social life as they are cross-species incompatible with the domesticated species. Secondly, demographic explosion, coupled with high food demand and production (yield) challenges which have accelerated the need for crop breeding and improvement to suit human desires and ensure food security by promoting the conservation, supply and distribution of bred and improved crops (Mba *et al*., 2014). This might have gradually led to the abandoning of the wild relatives and landraces of modern crops by the local people to the extent that they could be later not recognized and considered as weeds to the newly created plants in the farm leading to disappearance of the original crops. This could explain why scientists are now realizing that the wild relatives of modern crops deemed crucial for food security are being pushed to the brink of extinction (Briggs, 2017). Thirdly, the consumption of these wild legumes could be limited because of the presence of some toxic biochemicals in their most edible parts (seeds). This was also pointed out by an earlier review which emphasized that the greatest impediment to the consumption of most under-utilized legumes is the presence of antinutrients which could be removed or deactivated by proper processing techniques (Bhat & Karim, 2009). Lastly, the rapid evolution, distribution and spreading of improved bred crop varieties in order to respond to food security challenges are being associated with the disappearance of wild crop relatives (Briggs, 2017; Mba *et al*., 2014). From that perspective, one could imagine and question the awareness, believes and preferences of some generation of people about the origin of the consumed modern crops. This may explain the stigma about the consumption and even the existence of these wild legumes and therefore their rejection as food while they have been used as such in the past.

According to the Food and Agriculture Organization of the United Nations (FAO), 70% more food is needed over the next four decades to nourish adequately the human population

projected to exceed 9 billion by the year 2050 (FAO, 2009). Besides this fact, humankind now depends on a reduced amount of agricultural biological diversity for its food supplies due to agricultural modernization, changes in diets and population density. A rapid reduction of the gene pool in both plant and animal genetic resources is observed as only a dozen species of animals provide 90% of the animal protein consumed globally and just four crop species provide half of the plant-based calories in the human diet (FAO, 2009). Therefore, the domestication, adoption and industrial utilization of the wild *Vigna* species presented in Table 1 could be of utmost importance in contributing to achieving the FAO expectation to increase food production by 70% more by the year 2050. Secondly, it is generally known that maize and soya beans are two widely consumed crops for both humans and animals. These crops are highly cultivated and solicited globally, especially for food and feed formulations making them a subject of competition between humans and animals. Maize (corn) represents the most commonly used energy source, and soybean meal is a common plant protein source for the poultry industry for example. This raises the issue of whether it is necessary to first produce crops for animal feed before thinking of man or is it necessary to feed the animals with the same types of crops used by humans. The exploration and exploitation of some wild *Vigna* species in animal feed formulation could orient the policy makers towards the right crop species to direct to animal and human uses according to their preferences. This could help to shape the future better and contribute greatly to ensuring global food security.

2.6 Concluding Remarks of Literatures Reviewed

Both wild and domesticated *Vigna* species presents a very high genetic diversity in terms of seed morphology, physiological and agronomic characteristics. Though very limited in number as compared with wild species, the domesticated species have demonstrated enormous impact in both human and animal nutrition especially in parts of the world where meat is less affordable. Considering how fast the domesticated species have become popular, these wild *Vigna* species may represent a promising tool to contribute in reducing food insecurity and hidden hunger and a good food and/or feed material for the future. Based on the potentials presented by their genetic diversity, it is now strongly believed that wild *Vigna* species can be the 'new model plant species' for the genetic study of natural adaptation to stresses such as salt, acid, alkali, drought, flood, pests and diseases. Therefore, more attention should be given to the wild species since they present huge opportunities for crop bio-fortification, food variety addition (domestication), food value addition, crop improvement

and biodiversity conservation. For it is worth thinking about the future food and feed materials to be used in case of complications that may arise from unpredictable situations such as climate change, to avoid more surprising difficult global food challenges.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Agro-morphological Exploration of Wild Unexplored *Vigna* Species for Domestication

3.1.1 Sample Collection and Preparation

One hundred and sixty (160) accessions of wild *Vigna* species of legumes were obtained from gene banks, as presented in Appendix 1. Approximately 20–100 seeds of each accession were supplied by the gene banks and planted in pots, which were placed in screen houses at the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania during the period of November 2017–March 2018. The pot experiment only allowed for seed multiplication and the preliminary observation of the growth behaviour of the wild legumes, prior to experimentation in the field. In the field, all the accessions were planted in an experimental plot, following the augmented block design arrangement (Bisht *et al.*, 2005), and allowed to grow until full maturity. In addition to the wild accessions, three domesticated *Vigna* legumes—that is, cowpea (*V. unguiculata*), rice bean (*V. umbellata*), and a semi-domesticated landrace (*V. vexillata*)—were used as checks. The checks were obtained from the Genetic Resource Center (GRC-IITA), Nigeria (cowpea), the National Bureau of Plant Genetic Resources (NBPGR), India (rice bean), and the Australian Grain Gene bank (AGG), Australia (semi-domesticated landrace V. *vexillata*).

3.1.2 Study Sites and Meteorological Considerations

The study was conducted in two agro-ecological zones, located at two research stations in Tanzania, during two main cropping seasons (March–September 2018 and March–September 2019).

The first research station (site A) was at the Tanzania Agricultural Research Institute (TARI), Selian in Arusha region, located in the northern part of Tanzania. The Tanzania Agricultural Research Institute, Selian branch lies at a latitude of 3°21′50.08” N and longitude of 36°38′06.29″ E at an elevation of 1390 m above sea level (a.s.l.) (Fig. 3).

The second site (site B) was at the Tanzania Coffee Research Institute (TaCRI), located in the Hai district, Moshi, Kilimanjaro region (latitude 3°13′59.59″ S, longitude 37°14′54″ E). The site is at an elevation of 1681 m above sea level (Fig. 3).

The meteorological characteristics (monthly rainfall and temperature dynamics) of the two study sites for the two cropping seasons were obtained from the Tanzania Meteorological Agency and are summarized in Fig. 4.

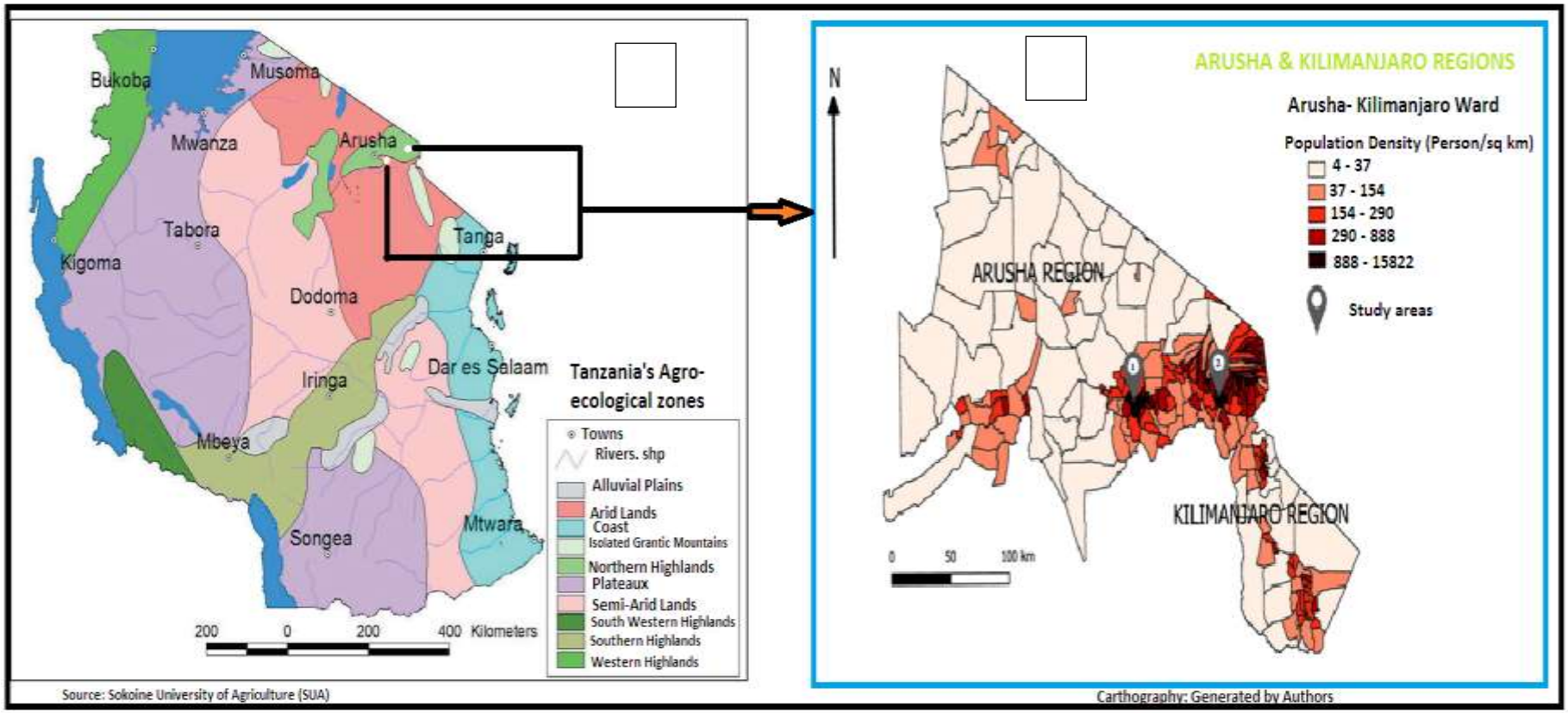


Figure 3: Tanzania Map Showing Agro-ecological Zones of Tanzania (A) (MAFSC, 2014) and the Study Sites (B): 1 = Arusha District (Arusha Region) and 2 = Hai District (Kilimanjaro Region)

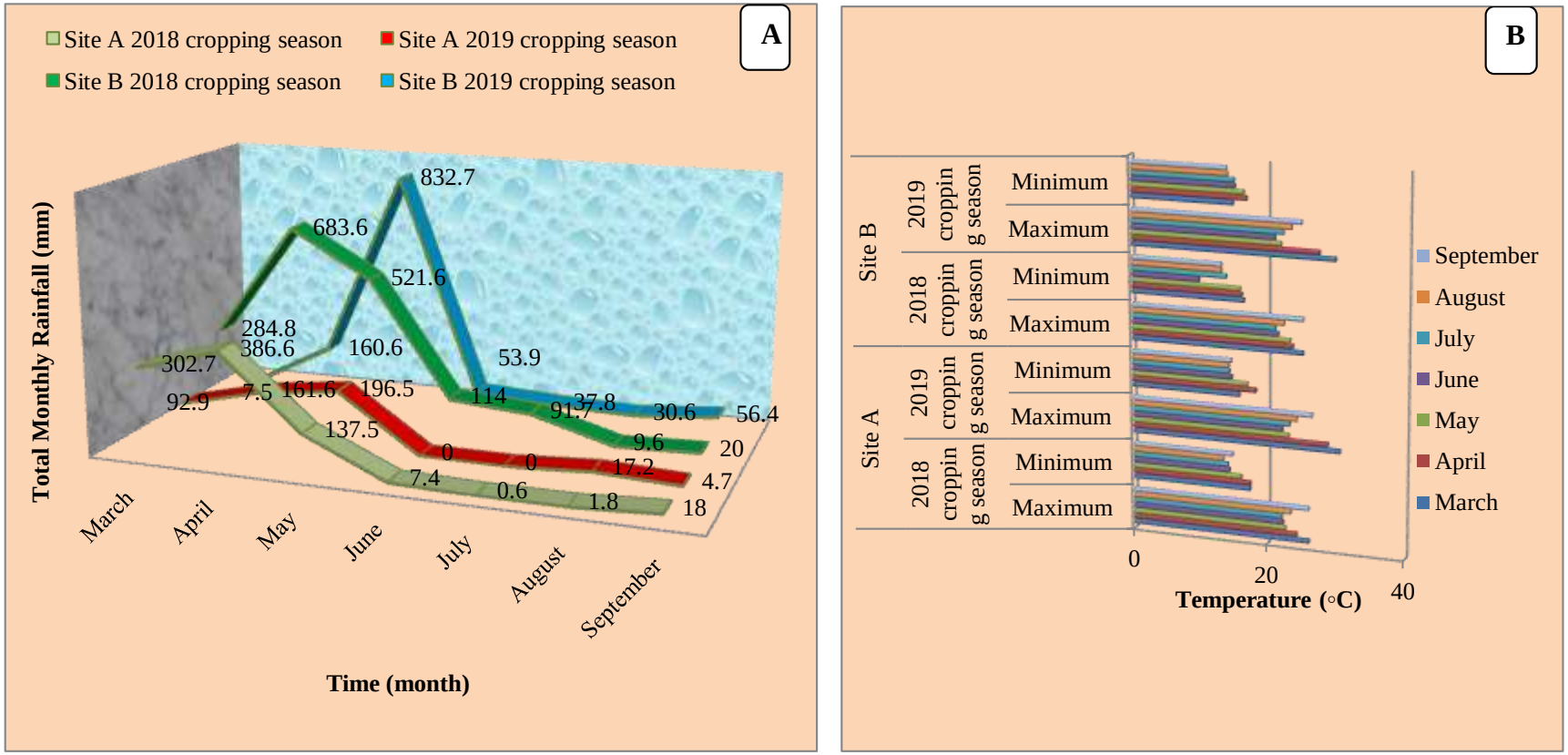


Figure 4: Meteorological Characteristics of the Two Study Sites. (A) Rainfall Dynamics of the Study Sites; (B) Temperature Dynamics of the Study Sites

3.1.3 Experimental Design and Planting Process

The 160 accessions of wild *Vigna* legumes were planted in an augmented block design field layout, following the randomization generated by the statistical tool on the website developed by the Indian Agricultural Research Institute (IASRI, 2019), with three checks. The software generated a total of 208 experimental plots, with 8 blocks each containing 26 experimental plots. Each plot represents a line of different wild accession or a check. Each check was repeated two times within a block. Ten seeds from each accession were planted in a line of 5 m in length, with a distance of 50 cm between each seed. The distances between the accessions (lines) within a block, as well as the distance between the blocks, were 1 m each. Data were then collected from five randomly selected plants in each line using the wild *Vigna* descriptors (Bisht *et al*., 2005).

3.1.4 Data Collection and Analysis

Fifteen qualitative and fifteen quantitative characters were observed and recorded using both International Plant Genetic Resource Institute (IPGRI) and National Bureau of Plant Genetic Resources (NBPGR) descriptors (Bisht *et al*., 2005). The 15 qualitative and 15 quantitative characters were recorded. The descriptors used in this study are shown in Table 3. Data on the quantitative traits were recorded for five randomly selected individuals per accession.

The generalized linear model procedure (GLM PROC) of the SAS software (SAS University Edition, SAS Institute Inc., North Carolina State, USA) was used to analyze the accession and the block and block vs. accession effects, while the two-way analysis of variance (two-way ANOVA), agglomerative hierarchical clustering (AHC) and principle component analysis (PCA) of XLSTAT were used to analyze the accession × site and accession × season interactions, as well as the clustering and variations among accessions. The SAS University Edition version and the XLSTAT-Base version 21.1.57988.0 were used.

Table 3: Important Descriptors for the Characterization of the Wild *Vigna* Species Germplasm

Parameters	Descriptors
	Qualitative Traits
Seed germination habit	1. Epigeal, 2. Hypogeal
Attachment of primary leaves (at two-leaf stage)	1. Sessile, 2. Sub-sessile, 3. Petiolate
Growth habit (recorded at first pod maturity)	1. Erect, 2. Semi-erect, 3. Spreading, 4. Semi-prostrate, 5. Prostrate, 6. Climbing
Leafiness (at 50% flowering)	1. Sparse, 2. Intermediate, 3. Abundant
Leaf pubescence	1. Glabrous, 2. Very sparsely pubescent, 3. Sparsely pubescent, 4. Moderately Pubescent, 5. Densely pubescent
Petiole pubescence	1. Glabrous, 2. Pubescent, 3. Moderately pubescent, 4. Densely pubescent
Lobing of terminal leaflet (at first pod maturity)	1. Unlobed, 2. Shallow, 3. Intermediate, 4. Deep 5. Very deep
Terminal leaflet shape	1. Lanceolate, 2. Broadly ovate, 3. Ovate, 4. Rhombic, 5. Others
Stipule size	1. Small, 2. Medium, 3. Large
Hypocotyl color	1. Green; 2. Purple, 3. Others
Stem pubescence	1. Glabrous, 2. Sparsely pubescent, 3. Moderately pubescent, 4. Highly pubescent
Pod attachment to peduncle	1. Erect, 2. Horizontal, 3. Horizontal-pendent 4. Pendent, 5. Others
Pod pubescence	1. Glabrous, 2. Sparsely pubescent, 3. Moderately pubescent, 4. Densely pubescent
Pod curvature	1. Straight, 2. Slightly curved, 3. Curved (sickle shaped)
Constriction of pod between seeds	1. Absent, 2. Slight, 3. Pronounced
Pod cross section	1. Semi flat, 2. Round, 3. Others
	Quantitative Traits
1	Germination time (days)
2	Terminal leaflet length (cm)
3	Terminal leaflet width (cm)
4	Petiole length (cm)
5	Days to flowering
6	Flower bud size (cm)
7	Number of flowers per raceme
8	Peduncle length (cm)
9	Pods per peduncle
10	Pod length (cm)
11	Pods per plant
12	Seeds per pod
13	Seed size (mm^2)
14	100-Seed weight (g)
15	Yield (Kg/ha)

3.2 Farmers' Perceptions, Preferences and Prospective Uses of Wild *Vigna* Species for Human Exploitation

3.2.1 Explorative Survey on Prior Knowledge of Wild *Vigna* Legume Species

The explorative survey was conducted among legume farmers in a mid-altitude agro-ecological zone (Arusha Region) and a high altitude agro-ecological zone (Kilimanjaro Region) of Tanzania where legume cultivation is intensified, as shown in Fig. 3A (Binagwa *et al*., 2016). A purposive sampling from a crop-growing population of 0.13% (37 985) from Arusha (MAFC *et al*., 2012a) and 0.17% (56,710) from Kilimanjaro (MAFC *et al*., 2012b) were used to obtain a representative sample size. The total number of farmers involved in legume improvement programs included 50 from the Seliani Agricultural Institute (TARI), Arusha and 100 from the Tanzania Coffee Research Institute (TaCRI), Moshi, Kilimanjaro regions, respectively (Fig. 3B). A systematic selection of farmers who had at least two years of trying locally improved legume varieties was performed. An individual face-to-face interview with the help of a semi-structured questionnaire prior to participant experimental plot visit was executed to obtain a broad range of individual opinions and explore their awareness of wild legumes. The questionnaire consisted of 24 items including socio-demographic characteristics. The items were categorized and analyzed to assess the socio-demographic characteristics of participants, their prior knowledge/awareness about wild legumes, and the uses of wild legumes as known by experienced farmers as well as some challenges faced by legume farmers.

3.2.2 Farmers' Preferences and Perceptions of Wild *Vigna* Legumes

Participants of the previous study (the explorative survey) in the Arusha (N_1 = 50) and Kilimanjaro (N_2 = 100) regions also participated in this study. Field visits were done in groups of five participants. A trained research assistant was recruited to guide the participants around the experimental field from the first to the last block or vice versa. A semi-structured questionnaire was used to collect information on the most preferred accessions (at least 10), and reasons for each selection were given. Every accession was assigned a number to ease participant selection. The number of times each accession was selected was divided by the total number of selections and multiplied by 100 to give the percentage of selection of each accession.

3.2.3 Focus Group Discussion

Participants in their respective regions were further grouped into two groups based on their gender, men and women, giving a total of four group interviews. Each group was invited to participate in an animated video-recorded focus group interview to ascertain their opinion about wild *Vigna* legumes, as obtained in the previous studies. The recorded videos (04) were transcribed verbatim and translated from Swahili language to English. The transcripts were cross-checked with the recordings by the interviewers to align transcripts with notes on non-verbal responses. A coding framework was developed based on the interview objectives and the interview guide. The qualitative data analysis package NVivo 11 was used to code and organize the data systematically as described by other workers (De Beukelaar *et al*., 2019). Key concepts and categories were identified.

3.2.4 Data Analysis

The collected information during the explorative survey was grouped, coded, organized, and analyzed using the statistical package IBM SPSS Statistic 20.0 (New York, USA). Analysis consisted of the descriptive statistics as well as the binary logistic regression to test for the relationship between the prior knowledge about the wild *Vigna* legumes and the farmers' socio-demographic characteristics. In the case of farmers' preferences and perceptions of wild *Vigna* legumes study, data were coded and entered in the statistical package IBM SPSS Statistic 20.0 and analyzed. Analysis included descriptive statistics and likelihood ratio test of X^2 to determine the relationship between the preferences and the farmers' gender, farming experience and research location (Mchugh, 2013).

3.3 Assessment of Water Absorption Capacity and Cooking Time of Wild Under-Exploited *Vigna* Species Towards their Domestication

3.3.1 Assessment of Water Absorption Capacity: Seed Soaking Process

Eighty-four accessions were selected from the agro-morphological study (Objective 1) based on the availability of seeds after maturity for this study. The seeds were soaked according to the method adopted from McWatters and modified by Shafaei (Watters *et al*., 2002; Shafaei *et al*., 2016).

Ten seeds of each accession were randomly selected and weighed, then placed in glass beakers containing 200 mL distilled water and allowed to stand at room temperature (25 °C) for 24 h. The weight of water absorbed by various seeds was measured after 24 h, as it is the soaking time generally practiced by most consumers at home. After reaching required time, the soaked samples were removed from the beakers and placed on a blotter paper (Analytik Jena US LLC - Upland, Canada) to eliminate the excess water, and then weighed. A precision electronic balance (Model GF400, Taunton, MA, USA) was used to measure weight of sample before and after immersion. All tests were performed in triplicate. The weight of water absorbed was determined using the formula below (MCWatters *et al*., 2002; Shafaei *et al*., 2016):

$$\mathrm{Wa} = \frac{Wf - Wi}{Wi}$$

Where, ***Wa*** is the water absorption, ***Wf*** is weight of seeds after immersion (g), and ***Wi*** is weight of seeds before immersion (g).

3.3.2 Cooking Process on a Mattson Bean Cooker

A Mattson Bean Cooker (MBC) apparatus was used to record the mean cooking time of each accession of wild *Vigna* legume. The apparatus consists of 25 plungers and a cooking rack with 25 reservoir-like perforated saddles, each of which holds a grain and a plunger calibrated to a specific weight. Each plunger weighs 90 g and terminates in a stainless-steel probe of 1.0 mm in diameter (Wang & Daun, 2005). The cooking proceeded by immersing MBC in a beaker with boiling water (98 °C) over a hotplate. The 50% cooked point, indicated by plungers dropping and penetrating 13 (approximately 50% of the 25 individual seeds) of the individual beans, corresponds to the sensory preferred degree of cooking, according to methodology adapted from Proctor and Watts (Proctor and Watts, 2013; Siqueira *et al*., 2013). A digital chronometer (Zhangzhou Deheng Electronic Co., Ltd., Fujian, China) was used to record the cooking time during the process.

3.3.3 Data Analysis

The values for water absorption capacity and cooking time were recorded in triplicate and presented as mean ± standard error using XLSTAT. The data were subjected to two-way analysis of variance (ANOVA), correlation coefficients, and Tukey's test. A $p < 0.05$ was

considered statistically significant. Agglomerative Hierarchical Clustering (AHC) analysis was performed to examine similarities between accessions. Descriptive statistics for the yield traits as well as cooking time and water absorption capacity were also computed using XLSTAT. All the data were entered in an excel sheet and analyzed using XLSTAT-Base version 21.1.57988.0.

3.4 Biochemical Characterization of Wild *Vigna* Species

3.4.1 Seeds Preparation for Biochemical Characterization

The 87 accessions of matured seeds of wild *Vigna* species of legumes harvested from the study on agro-morphological characterization (Objective 1) provided enough seeds to carry out the biochemical characterization. Cowpea (*V. unguiculata*), rice bean (*V. umbellata*) and a semi-domesticated landrace (*V. vexillata*) were also harvested from the agro-morphological characterization study and used in this part.

The matured fruits were harvested with their pods, sun-dried and the seeds were removed from the pods, threshed and winnowed, then freed from broken seeds, dust and other foreign materials to obtain clean seeds. The seeds were then stored in plastic bags at room temperature (27 – 30°C) for subsequent analysis.

After that, the seed samples were grinded using a kitchen blender (3 in 1 Electric Chopper Juice Blender HB-38, 350W, Jar Capacity: 1.5L, Guangdong, China) and sieved and the 1 mm fraction were collected for analysis.

3.4.2 Proximate Composition Exploration of Seeds of Wild *Vigna* Species

(i) Moisture Content Determination

The method employed for the determination of moisture content in the sample was based on the measurement of the loss in weight due to drying at a temperature of about 105°C as described in the AOAC methods (method 950.46) (AOAC, 2000). A watch glass was washed and dried in an oven at about 105°C for 3 h, it was cooled and weighed empty.

About 2.0 g of sample was weighed into a clean watch glass. The watch glass and its content were dried in an Air-circulated oven (DRY-Line 56, STEP Systems GmbH, Nuremberg,

Germany) at about 105°C to constant weight. The watch glass and its content was cooled in desiccators and reweighed.

The percentage moisture was obtained using the expression:

$$\% \text{ Moisture} = \frac{Loss\ in\ weight\ on\ drying\ \times 100}{Initial\ sample\ weight\ (g)}$$

(ii) Ash Content Determination

The term ash refers to the residue left after combustion of the oven dried sample and is a measure of the total mineral content. Determination of the ash content was done according to AOAC method 923.03 (AOAC, 2000).

Three different crucibles were preheated in a muffle furnace at about 550°C. Each crucible was cooled in a desiccator and weighed. Approximately 2.0 g of each sample was weighed into different crucible. The crucibles and their contents were transferred into a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) set at 550°C and allowed to stay for 6 h. After cooling the heated crucibles, the weights of crucible and their content were taken and recorded. The percentage ash was calculated using the following expression:

$$\% \text{ Ash} = \frac{weight\ of\ ash\ (g)}{weight\ of\ dry\ sample} \times 100$$

(iii) Crude Lipid (Fat) Content Determination

The crude lipid content of wild legumes samples was determined according to the AOAC method 960.39. A Soxtec ™ extraction system (Model 2043 Extraction Unit; Tecator, Sweden) and 30 mL of Petroleum ether (Mallinckrodt, Paris, KE, USA) were used to extract the oil from the samples. The amount of extracted oil was determined gravimetrically.

The percentage of lipid was obtained following the equation below:

$$\% \text{ Lipid } = \frac{Weight_{(extraction\ cup+residue)} - Weight_{(extraction\ cup)}}{weight\ of\ dry\ sample}\ X\ 100$$

(iv) Crude Protein Content Determination

The protein content of the wild *Vigna* legume samples was analyzed according to the AOAC method 928.08 (AOAC, 2000). The samples were digested with concentrated sulfuric acid (Pharmco- AAPER, USA), Hydrogen peroxide (Fisher Scientific, Fair Lawn, NJ, USA), and two Kjeldahl catalyst tablets (FisherTab ST-35; Fisher Scientific, Sweden) using a Kjeltec block digester unit (Model 2020 Digester; Tecator, Sweden). The total nitrogen amount in the sample was determined by distillation and titration of the extracts using a Kjeltec instrument (Kjeltic™ 8200 Auto Distillation Unit) (Ng *et al.*, 2008). A conversion factor of 6.25 was used to convert the amount of nitrogen to amount of protein present in the samples.
The amount of protein in the samples (dry basis) is calculated from the following formula:

$$\% \text{ Protein} = \frac{(T - B) \times M \times 14.007 \times 100 \times 6.25 \times MCF}{weight\ of\ dry\ sample}$$

Where,

T = Volume (ml) of the standard hydrochloric acid used in the sample titration.

B = Volume (ml) of the standard hydrochloric acid used in the blank titration.

M = Molarity of the acid in four decimal places.

W = mass of the sample used in the determination in milligrams.

6.25 = *factor used to convert percent N to percent crude protein. Most proteins contain 16% N, so* ***the*** *conversion factor is 6.25 (100/16 = 6.25).*

MCF = Moisture Correction Factor = 100/ (100-% Moisture)

(v) Crude Fiber Content Determination

Triplicate fat-free grinded samples of 1.0 g were weighed into a clean pre-weighed crucible. The crucible with sample was then transferred into the hot-extraction unit and the sample was left to digest for 30 min with 150 mL of solution containing 12.5% Sulphuric acid and 0.25 mL of Octanol. The condenser was switched off after 30 min and allowed to cool. The acid solution was filtered and washed with hot distilled water using suction. Then the samples were digested for 30 min with 150 mL alkali solution (12.5% NaOH) and 0.25 mL of octanol

to dissolve the alkali-soluble matter from the samples. The porcelain crucibles' final residues were dried at 105 ℃ in an oven for 1 h, cooled in a desicator and then weighed. The final residues were dried at 105^{o}C in an oven for 60 min. The residues were ignited in a pre-heated muffle furnace (Carbolite, UK) at 550 $^{\circ}$C for 3 h and weighed. The percent of crude fiber content was calculated using the following equation:

$$\% \text{ Fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

Where,

$\mathbf{W_1}$ = Sample weight, W_2 = Crucible weight with ash, $\mathbf{W_3}$ = Empty crucible weight

(vi) Carbohydrate Content Determination

The percentage carbohydrate was obtained by difference (AOAC, 2000)

Percentage carbohydrate = 100 – (% moisture + %protein + %fat + %ash)

3.4.3 Mineral Content Evaluation of Wild *Vigna* Legume Species

The following minerals: Copper (Cu), Manganese (Mn), Zinc (Zn), and Iron (Fe), were determined using atomic absorption spectrophotometry (AAS) as described by AOAC (AOAC, 2000).

(i) Sample Digestion

Prior to the reading of the absorbance of the samples using the AAS, the samples were first digested using the dry ashing technique which was reported to be suitable for plant materials (Paul *et al*., 2016). Two grams of each sample was weighed into a pre-heated crucible and properly ashed for 6 h through a Muffle Furnace (Nabertherm GmbH, Lilienthal, Germany) and cooled in a desiccator. After ashing, the residue was dissolved in 30 mL of concentrated Hydrochloric acid (HCl) and allowed to stand for 24 h. The volume of the solution was made up to 100 mL with distilled water and then filtered through a Watman filter paper No 4. The filtered solution was used for reading the absorbance of the samples on the AAS with specific wavelengths (Table 4).

(ii) Mineral Evaluation

The mineral elements in wild *Vigna* seeds were measured using an AAS with flame atomization (Model 210 VGP, Buck Scientific Inc., East Norwalk, Connecticut, USA) operating under the working conditions summarized in Table 4 (Fernandez-Hernandez *et al*., 2010; Salman *et al*., 2014; Waziri *et al*., 2013). The measurements were made in hold mode with air- acetylene flame, where the air (as oxidant) was maintained at a flow of 50 mL min⁻1 and the acetylene (as fuel) was maintained at a flow of 20 mL min^{-1}, to reach a flame temperature of 2600 °C. The hollow-cathode lamps were specific for each element analyzed. Previously, to achieve maximum sensitivity and precision, the equipment was equilibrated by alignment of the lamp and lighter and adjustment of the selected wavelength. Table 4 shows the instrumental conditions for each mineral element evaluated in the samples.

Table 4: Instrumental Conditions for the Mineral Element Determination in Wild *Vigna* Seeds

Element	Wavelength (nm)	Band- pass (nm)	Lamp current (mA)	Optimum working range (mg/kg)
Cu	324.8	0.7	10	0.1- 10.0
Zn	213.9	0.7	10	0.2- 10.0
Mn	279.5	0.2	20	0.1- 10.0
Fe	248.3	0.2	30	1.0- 40.0

3.4.4 Fatty Acid Content Evaluation of Wild *Vigna* Legume Species

(i) Lipid Extraction

The total lipids were extracted by mixing chloroform–methanol (1:1 v/v) with the samples using a slightly modified version of Lee's method (Lee *et al*., 2010). One gram of each sample was measured in a 250 mL conical flask and 30 mL of the chloroform-methanol solution was added and allowed to stand for 48 hours. The mixtures were transferred into a separatory funnel and shaken for 5 min. The lipid fraction was then separated from the separatory funnel and the solvent evaporated using a rotary evaporator. The weight of the crude lipid obtained from each sample was measured using an electronic scale.

(ii) Lipid Methylation: Preparation of Fatty Acid Methyl Esters (FAMEs)

Total lipids extracted were further converted to fatty acid methyl esters (FAMEs) by using 0.25 M trimethylsulfonium hydroxide (TMSH) in methanol.

For every 1.0 mL of sample, 200 µL of trimethylsulfonium hydroxide (TMSH) was added. After waiting for at least 10 min (to allow the fatty acids to convert to methyl esters), 1.0 µL was injected into the GC-MS for analysis (Omowaye *et al*., 2015).

(iii) Determination of the Fatty Acid Methyl Esters (FAMEs)

Fatty acid methyl esters were determined by capillary Gas Chromatography on GC-MS (Shimadzu 2010, Kyoto, Japan) equipped with flame ionization detector and capillary HP-88 column (100 m × 0.25 mm ×0.20 µm, J & W Scientific, USA). Separation and detection were performed under the following temperature program: initial temperature 125°C, rate 10°C min^{-1} to 175°C, hold 10 min, rate 5°C min^{-1} to 210°C, hold 5 min, rate 2°C min^{-1} to final temperature of 230°C, hold 12 min. Total analysis time was 50.5 min. The injector and detector temperatures were 250°C and 280°C, respectively; split ratio 1:50; volume 1 µL; carrier gas, N2, 1.33 mL/min; makeup gas, N2, 30 mL/min; detector gases, H2, 40 mL/min; synthetic air, 400 mL/min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA) (Milićević *et al*., 2014).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Agro-morphological Exploration of Wild Unexplored *Vigna* Species for Domestication

(i) Qualitative Traits Exploration of the Wild Unexplored *Vigna* Species

Figure 5 gives a pictorial description of some distinguishing morphological characteristics of the wild *Vigna* legumes, studied based on the physical phenotypic observations during the pot experimental phase. Other qualitative characteristics were studied in the field at different stages of the plants' growth, i.e., the germination, vegetative, podding and maturity stages.

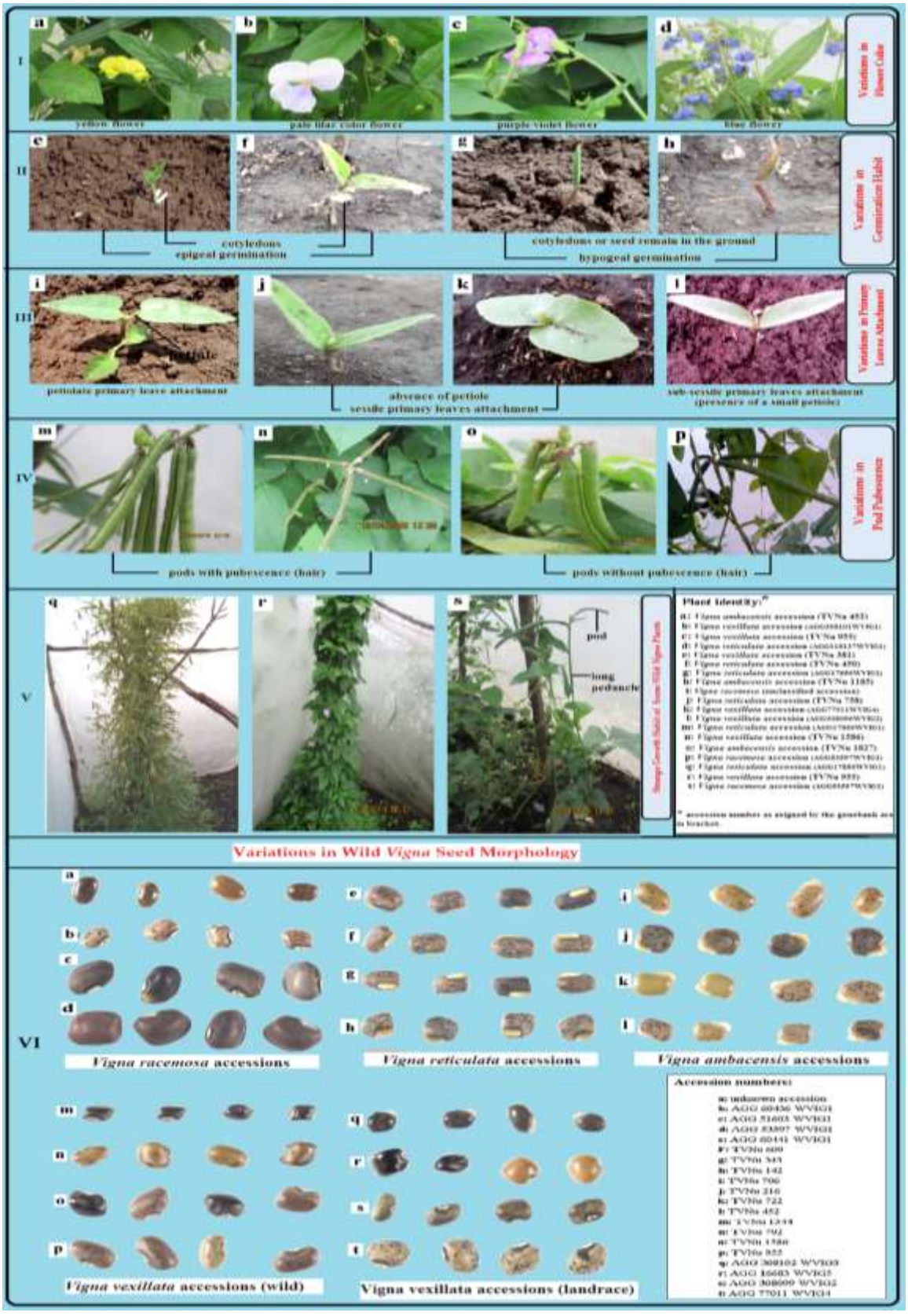


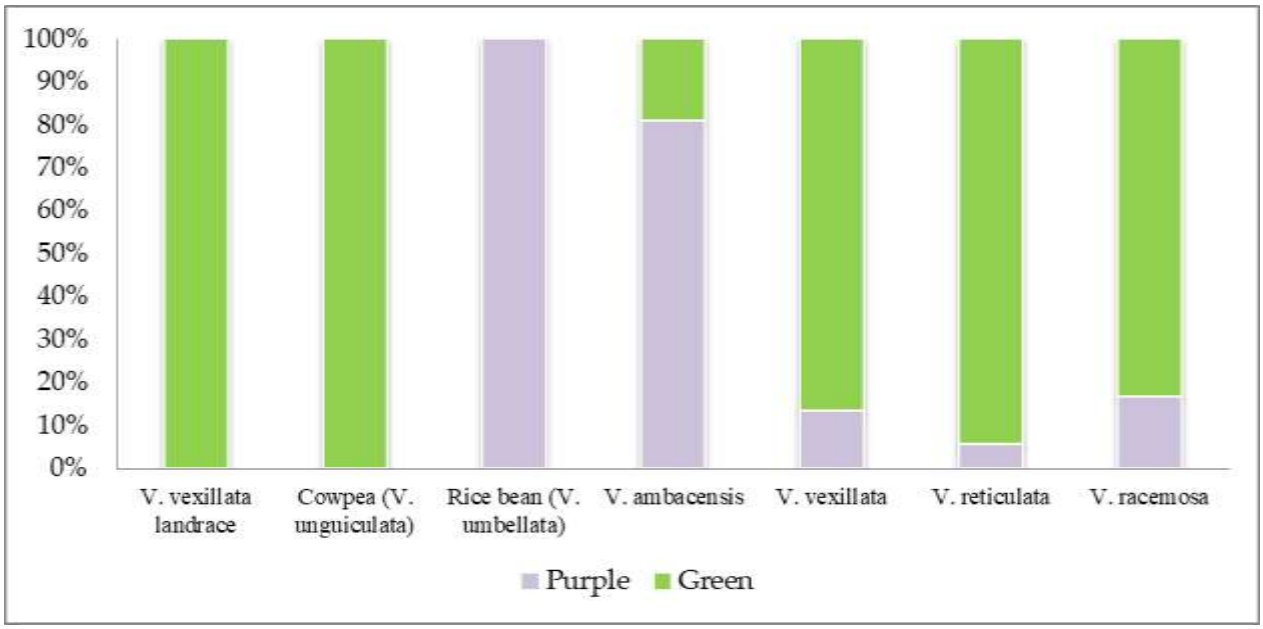
Figure 5: Some Qualitative Morphological Characteristics of the Studied Wild *Vigna*

- **Germination Stage**

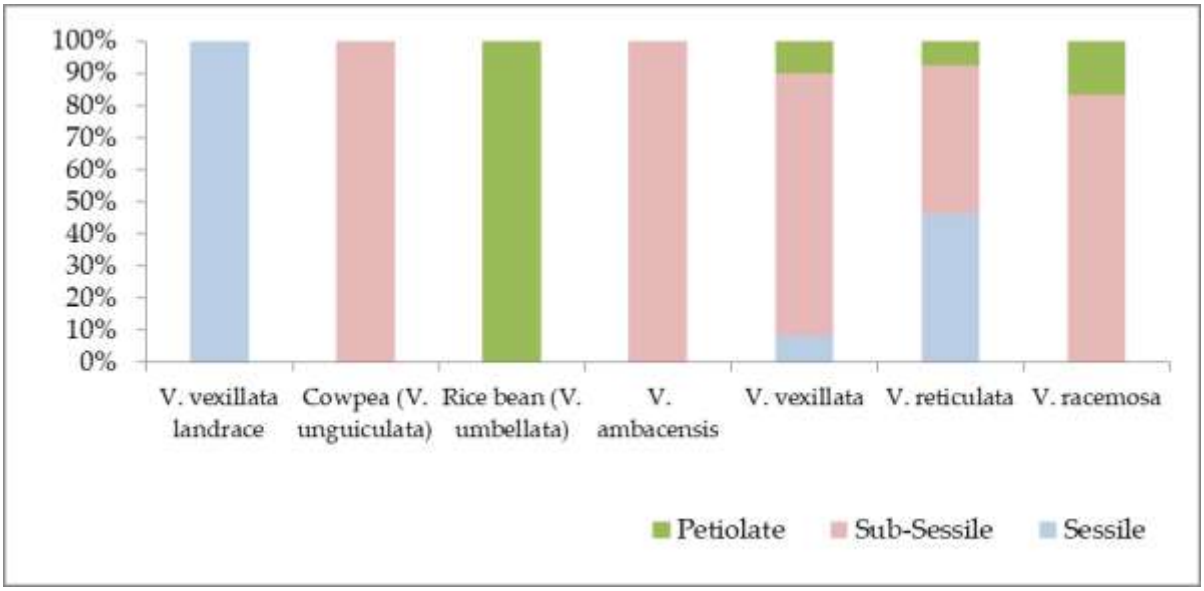
The hypocotyl colour, primary leaf attachment (at the two-leaf stage), and germination habit were the traits monitored at this growth stage. Figure 6 shows the percentage of the distribution of accessions for each trait variation. All the checks showed homogenous phenotypic characteristics, while the variations among accessions of the same species were observed for the wild species. *V. ambacensis* accessions showed a higher percentage of purple hypocotyl colour, which they share with rice bean (*V. umbellata*). On the other hand, *V. vexillata*, *V. reticulata*, and *V. racemosa* showed higher percentages of green hypocotyl colour, similar to cowpea and the landrace of *V. vexillata*.

The *V. vexillata*, *V. ambacensis*, and *V. racemosa* accessions showed a resemblance in the primary leaf attachment trait (sub-sessile) to cowpea, presenting a high percentage of accessions for the trait, while the *V. reticulata* accessions shared the sessile phenotype with the landrace of *V. vexillata*.

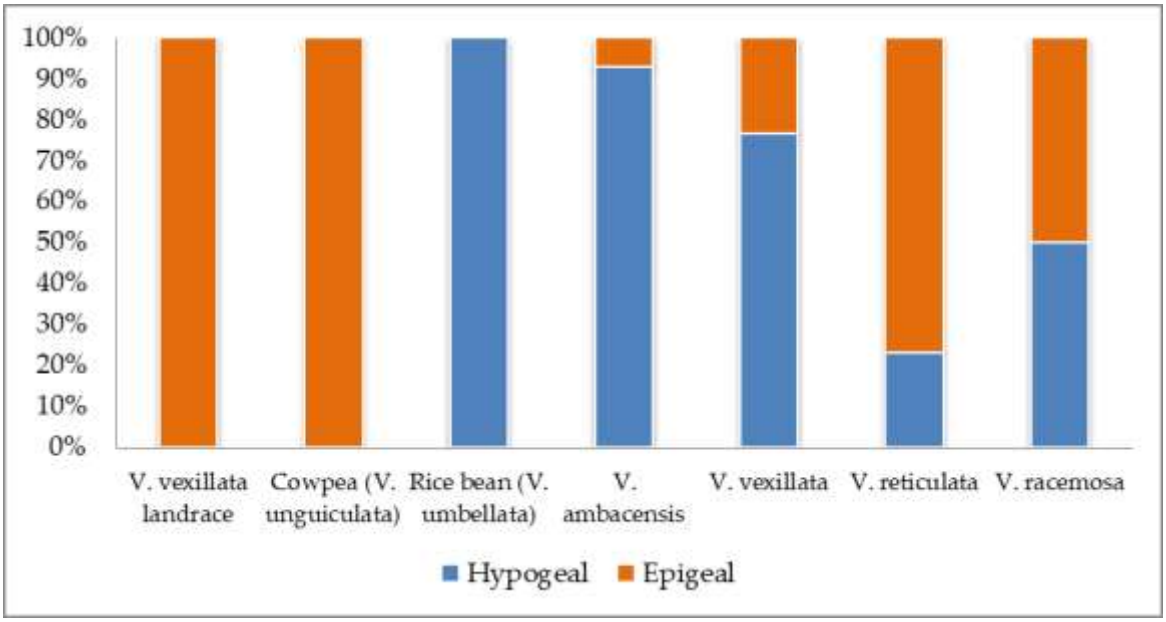
Both cowpea and the landrace of *V. vexillata* presented an epigeal germination habit, which they have in common with most accessions of *V. reticulata* and *V. racemosa*, while most accessions of *V. ambacensis* and *V. vexillata* shared a common phenotype (hypogeal) with rice bean.



(a). Variations in hypocotyl color



(b). Attachment of primary leaves (at the two-leaf stage)



(c). Variations in germination Habit

Figure 6: The Variations of Some Selected Qualitative Traits, at the Germination Stage of the Four *Vigna* Species Evaluated (a) Hypocotyl Color; (b) Attachment of Primary Leaves (at the two-leaf stage); (c) Germination Habit

- **Vegetative Stage**

The frequencies of distributions of variations for the qualitative traits examined at the vegetative stage are presented in Fig. 7. The leafiness, leaf pubescence, petiole pubescence, lobing of terminal leaflet, terminal leaflet shape, stipule size and stem pubescence traits were monitored to characterize the wild accessions of *Vigna* legumes at this stage.

Most *V. ambacensis* (76%) and *V. vexillata* (63%) accessions presented a sparse leafy character, which was not the case with any of the checks (Fig. 7a). Most *V. racemosa* and *V. reticulata* accession shared a common feature of intermediate leafiness with cowpea. Rice bean and the landrace of *V. vexillata* presented an abundant leafiness, which they had in common with few *V. reticulata, V. racemosa* and *V. vexillata*.

High variations were observed for the leaf pubescence traits among the wild accessions of all the species. A higher percentage (59%) of *V. ambacensis* presented moderate leaf pubescence, as found in rice bean, while the other species presented less than 50% accession for the same trait variation. Cowpea had a glabrous leaf pubescence, while the landrace of *V. vexillata* was very sparsely pubescent (Fig. 7b).

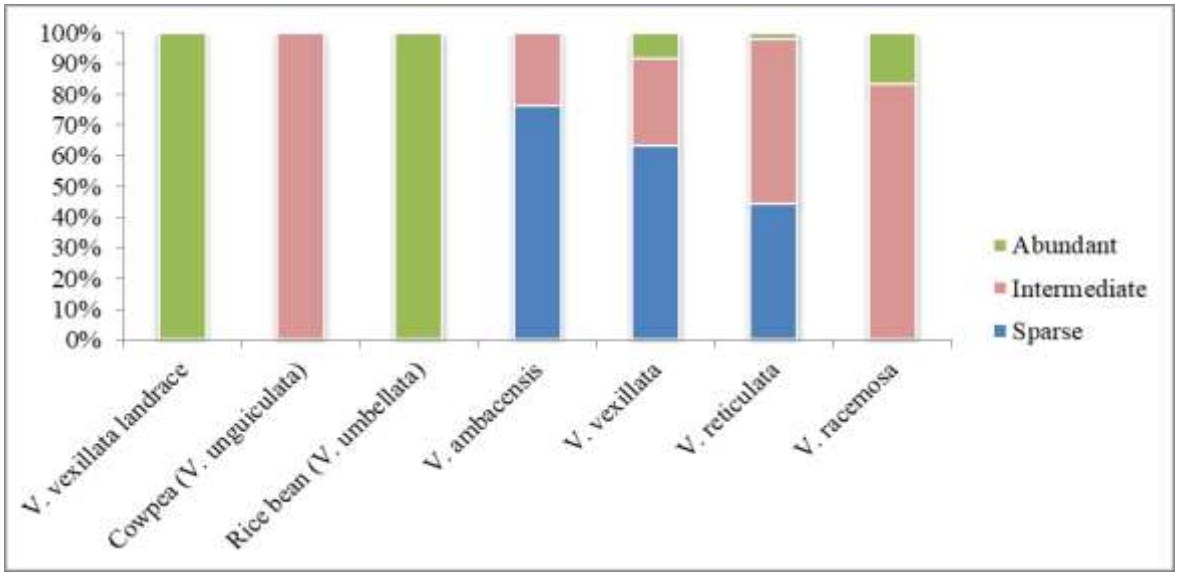
Considerable variations were also observed in the petiole pubescence trait (Fig. 7c). Only the *V. racemosa* accession significantly (33%) shared the common feature of globrous petiole pubescence with cowpea and *V. vexillata* landrace, and 50% of the *V. ambacensis* accession showed a moderately pubescent characteristic, which is similar to that found in rice bean. On the other hand, 50% of the *V. vexillata* accessions were pubescent and did not share the trait intensity with any of the checks. The *V. reticulata* accessions presented the highest percentage (42%) of the densely pubescent variant within the trait and a considerable percentage (36%) of the moderately pubescent variant.

Lobing of the terminal leaflet trait varied little among the studied wild accessions (Fig. 7d). The majority (more than 90%) of all the accessions of the studied wild species presented an unlobed variant of the trait, like cowpea and *V. vexillata* landrace (Fig. 7d).

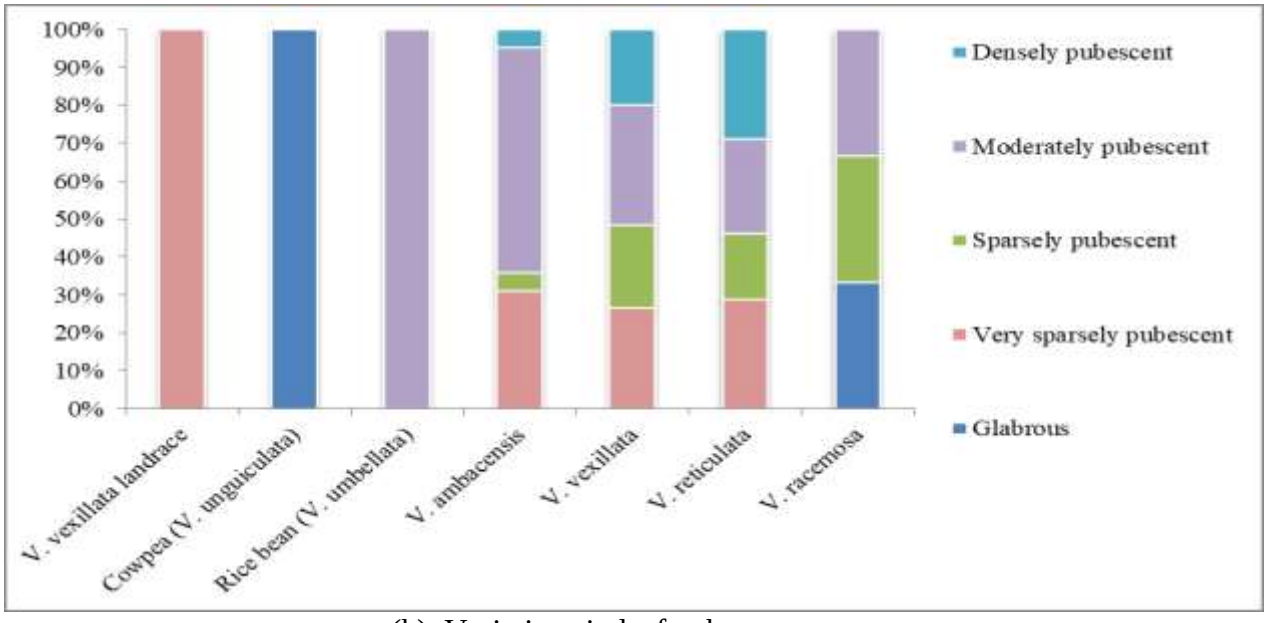
Most *V. racemosa, V. ambacensis*, and *V. reticulata* (66, 83 and 53% respectively) presented an ovate variant of the terminal leaflet shape trait, which is the same variant found in cowpea (Fig. 7e). The broadly ovate variant of this trait was only found in the *V. vexillata* landrace, while the rice bean presented an irregular variant (with lobes), which was found in few accessions of *V. reticulata* and *V. vexillata*. The lanceolate variant of the trait was also found in *V. racemosa* (33%), *V. ambacensis* (17%), *V. vexillata* (50%), and *V. reticulata* (38%).

All three of the checks showed a large variant of the stipule size trait, while 67% of *V. racemosa* had the medium stipule size variant, as well as 56% of the *V. reticulata* accessions (Fig. 7f). The small size variant was observed in 45 and 52% of *V. ambacensis* and *V. vexillata* respectively.

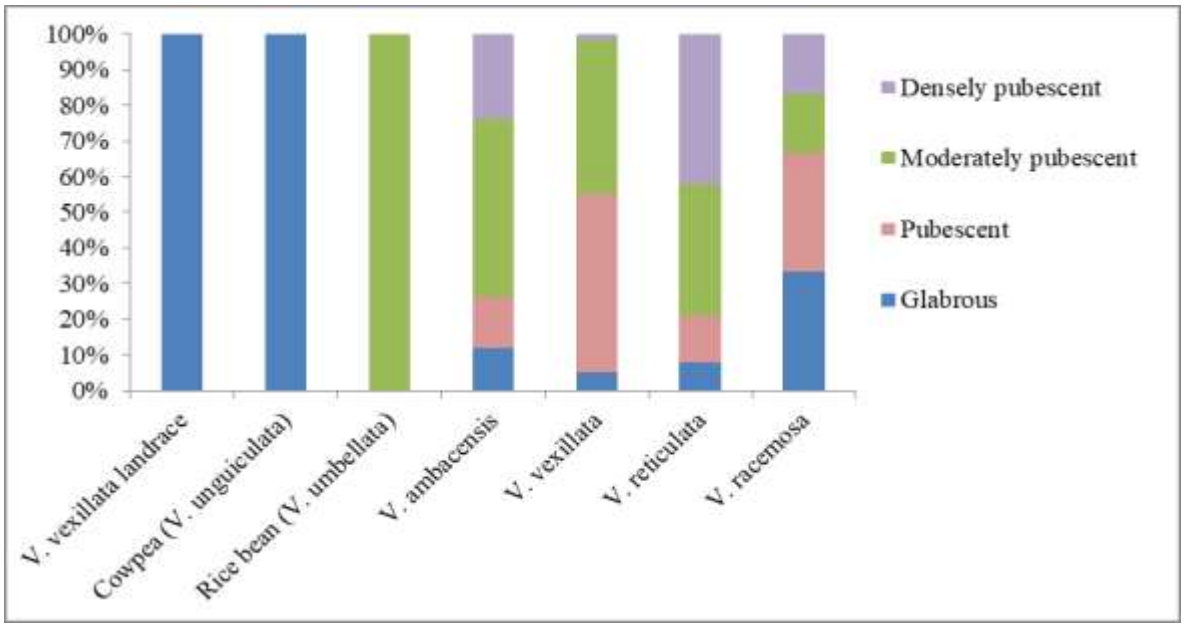
The stem pubescence trait also varied significantly among the wild accessions (Fig. 7e). The *V. vexillata* landrace presented the glabrous variant of the trait, which matched with 15.00, 17.31 and 33% of *V. vexillata*, *V. reticulata* and *V. racemosa* respectively. The stem of rice bean presented the highly pubescent variant of the stem pubescent trait, as found in 33, 18, and 38% of *V. ambacensis*, *V. vexillata* and *V. reticulata* respectively. A moderately pubescent variant was found in 31, 33, 25 and 17% of *V. ambacensis*, *V. vexillata*, *V. reticulata* and *V. racemosa* respectively. Finally, 50% of the *V. racemosa* accessions had the sparsely pubescent variant, while only 36, 33 and 19% of *V. ambacensis*, *V. vexillata* and *V. reticulata* were found to have the same variant.



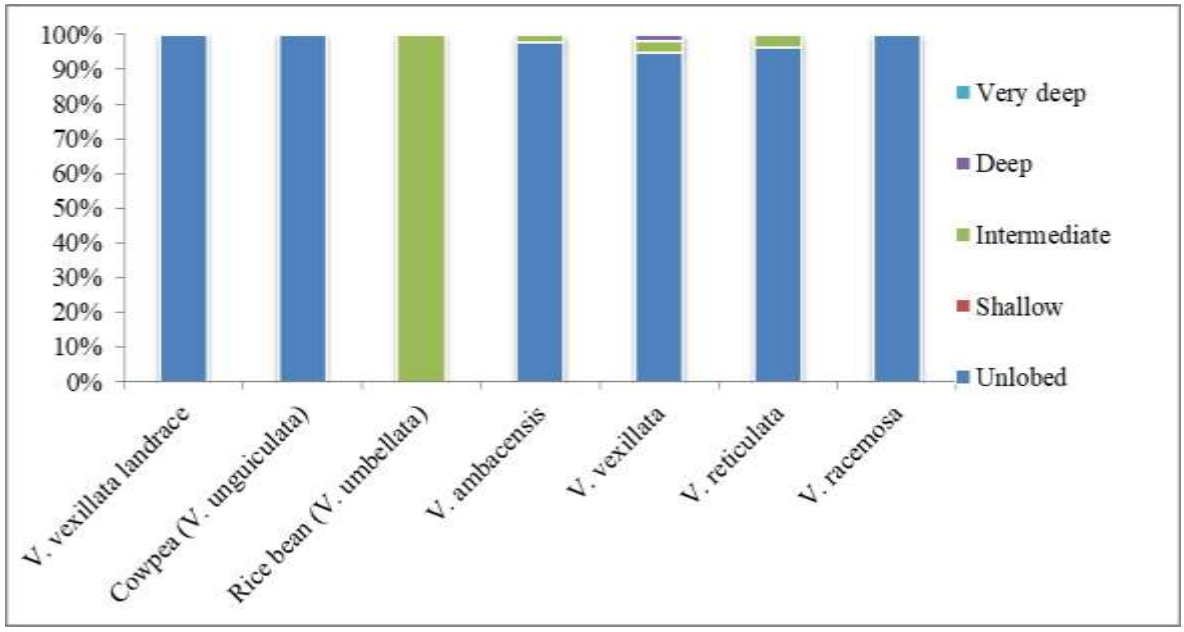
(a). Variations in leafiness (at 50% flowering)



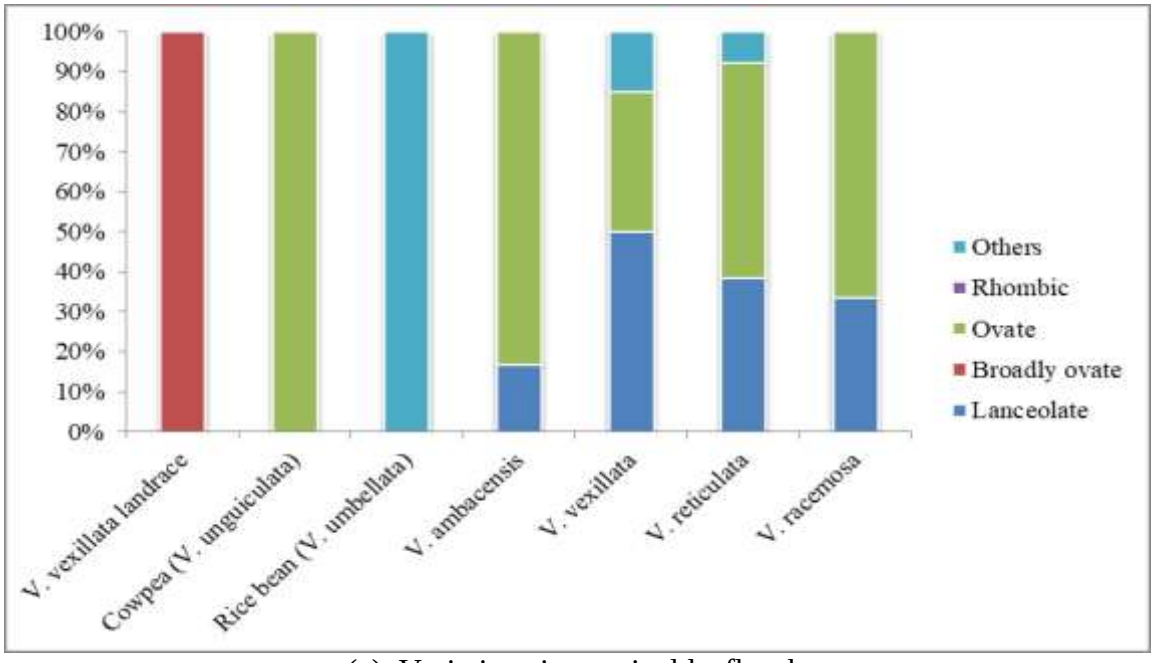
(b). Variations in leaf pubescence



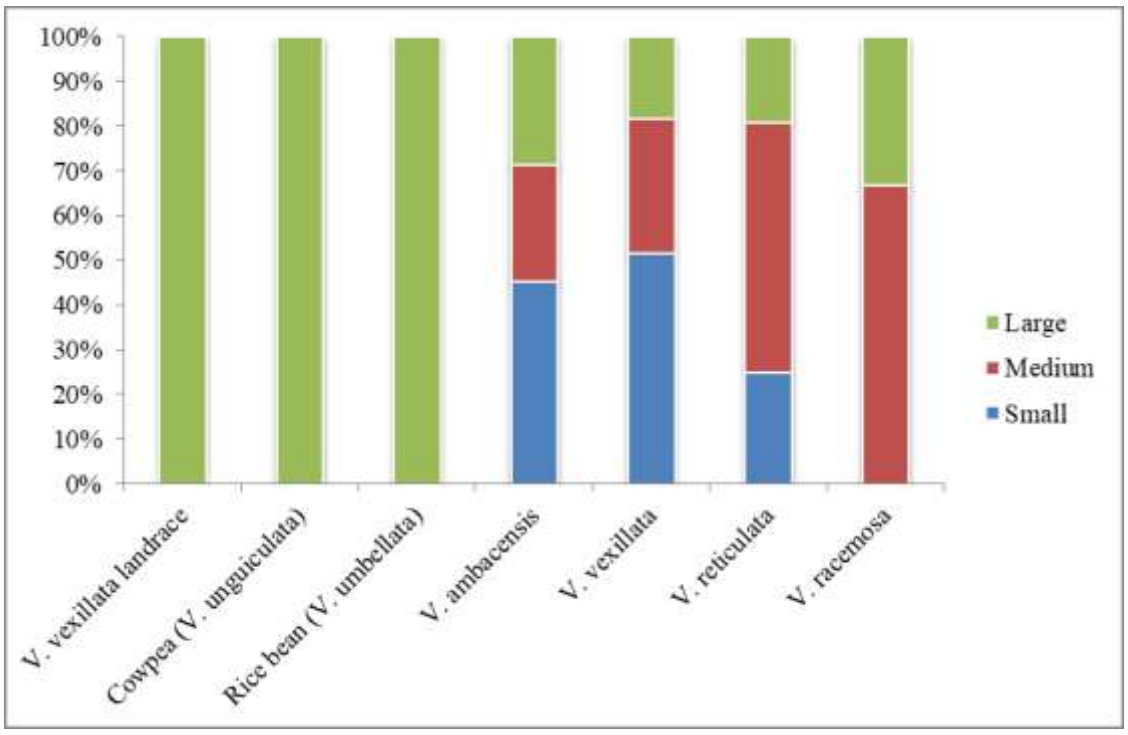
(c). Variations in petiole pubescence



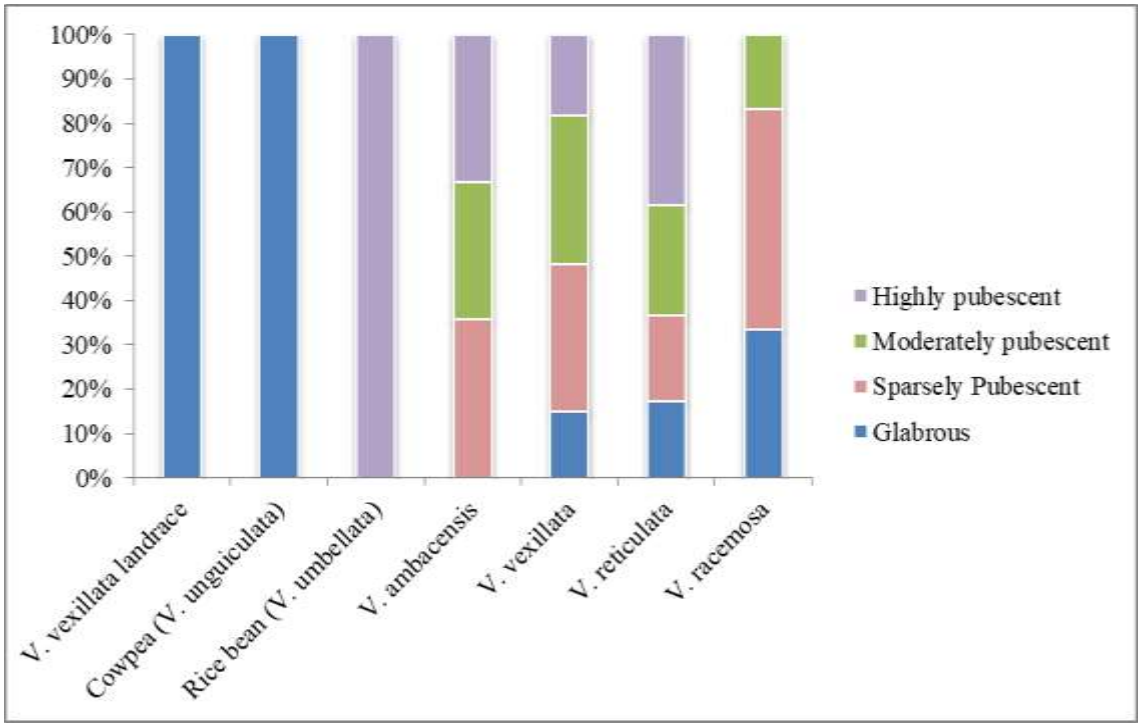
(d). Variations in Lobing of the terminal leaflet (at first pod maturity)



(e). Variations in terminal leaflet shape



(f). Variations in stipule size



(g). Variations in stem pubescence

Figure 7: Variations in Some Selected Qualitative Traits, Evaluated at the Vegetative Stage. (a) Leafiness (at 50% flowering); (b) Leaf Pubescence; (c) Petiole pubescence; (d) Lobing of the Terminal Leaflet (at First Pod Maturity); (e) Terminal Leaflet Shape, (f) Stipule Size; (g) Stem Pubescence

- **Pod Formation and Maturity Stage**

At this stage, the following traits were observed and recorded for wild accessions and checks under study: pod attachment to peducle, pod pubescence, pod curvature, constriction of the pod between seeds and pod cross section (Fig. 8).

For the pod attachment to peducle trait, most accessions of *V. ambacensis* (71%) and *V. racemosa* (50%) were similar to rice bean for the "pendent" variant of the trait, and 48% of *V. vexillata* and 35% of *V. reticulata* accessions presented the "horizontal'' variant of the trait, which is similar to cowpea. The "erect" variant of the trait was found in *V. vexillata* landrace and a few accessions of *V. vexillata* and *V. reticulata* (Fig. 8a). The horizontal-pendent form of the trait was commonly found in all the wild accessions, with 24, 2, 31 and 33% in *V. ambacensis*, *V. vexillata*, *V. reticulata* and *V. racemosa* respectively.

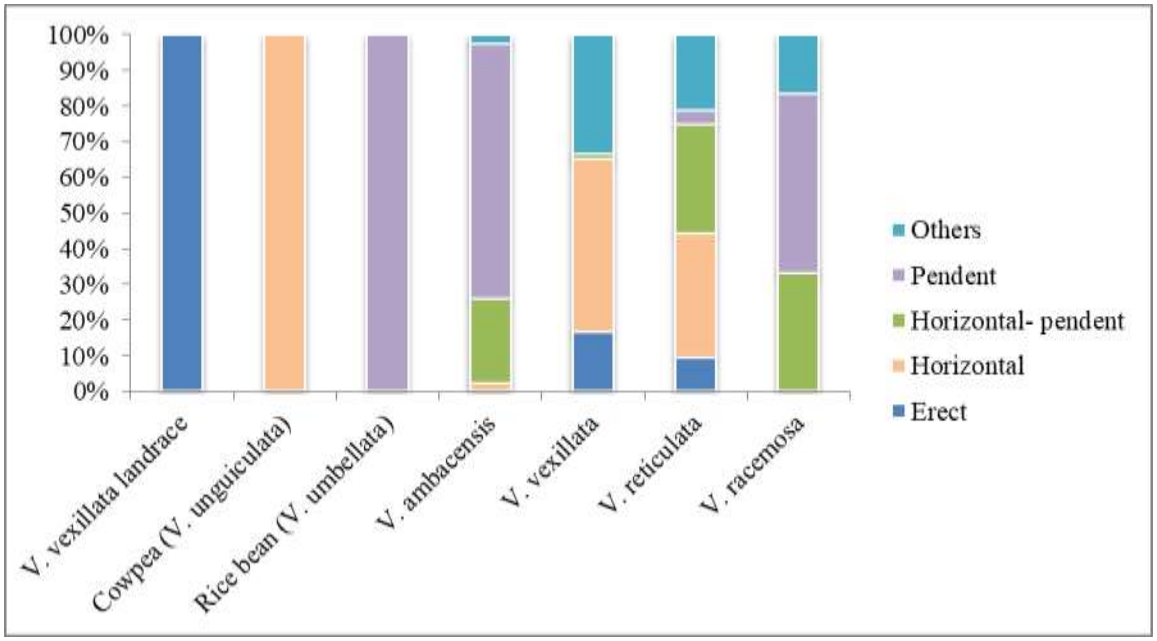
All the checks had a glabrous form for the pod pubescence trait (Fig. 8b). Only *V. racemosa* (50%) accessions were found to be similar to the checks for this trait. Moreover, 5, 43, 27%, and 17% of *V. ambacensis*, *V. vexillata*, *V. reticulata* and *V. racemosa* respectively were

moderately pubescent, while 86% of *V. ambacensis*, 50% of *V. vexillata*, 29% of *V. reticulata* and 33% of *V. racemosa* were sparsely pubescent. Only 42% of the accessions of *V. reticulata* and 10% of those of the *V. vexillata* pods were densely pubescent.

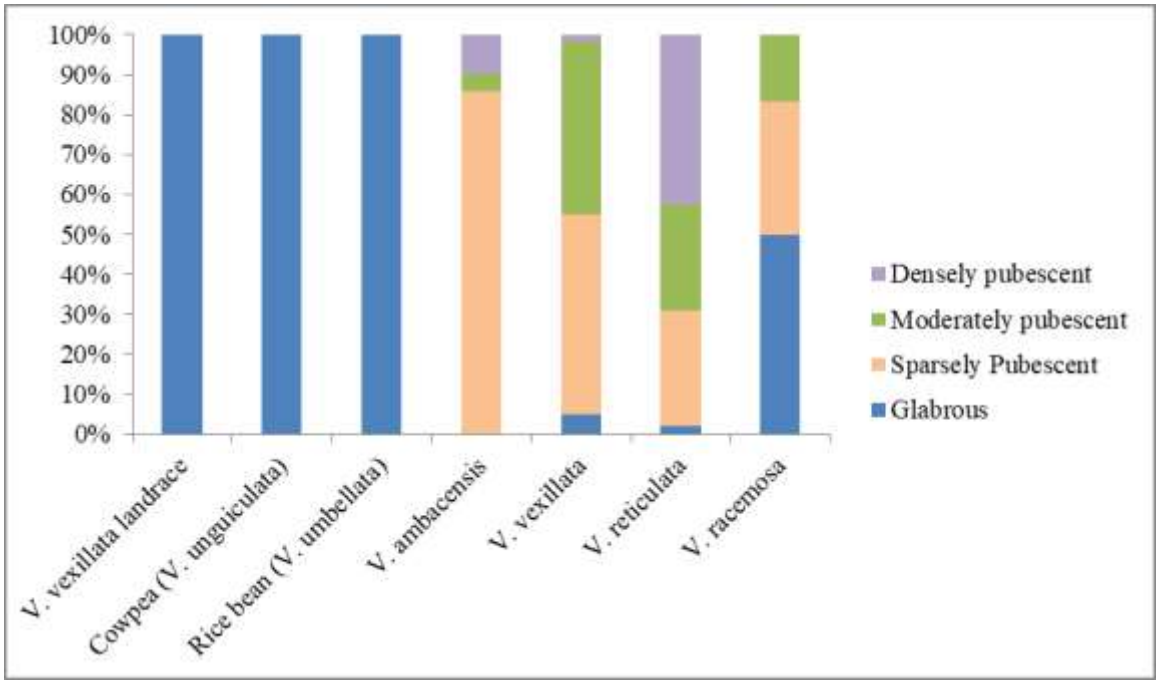
More than 50% of the studied wild accessions presented the "slightly curved'' form of the pod curvature trait, which was similar to cowpea (Fig. 8c). Rice bean and the *V. vexillata* landrace commonly shared the "straight'' form of the trait with 14% of *V. ambacensis*, 10% of *V. vexillata* and and 48% of *V. reticulata* accessions. On the other hand, only *V. vexillata* (38%) and *V. racemosa* (33%) accessions showed the "curved'' form of the pod curvature trait (Fig. 8c).

Most of the studied wild *Vigna* accessions (more than 50%) had no constriction of the pod between seeds (variant: "absent") as found in *V. vexillata* landrace (Fig. 8d). The trait was found in a "pronounced" form only in rice bean and 15% of *V. ambacensis*, as well as 12% of *V. reticulata* accessions. A "slight'' constriction of the pod between seeds was the form found in cowpea and 24% of *V. ambacensis*, 23% of *V. reticulata* and 33% of *V. racemosa* accessions (Fig. 8d).

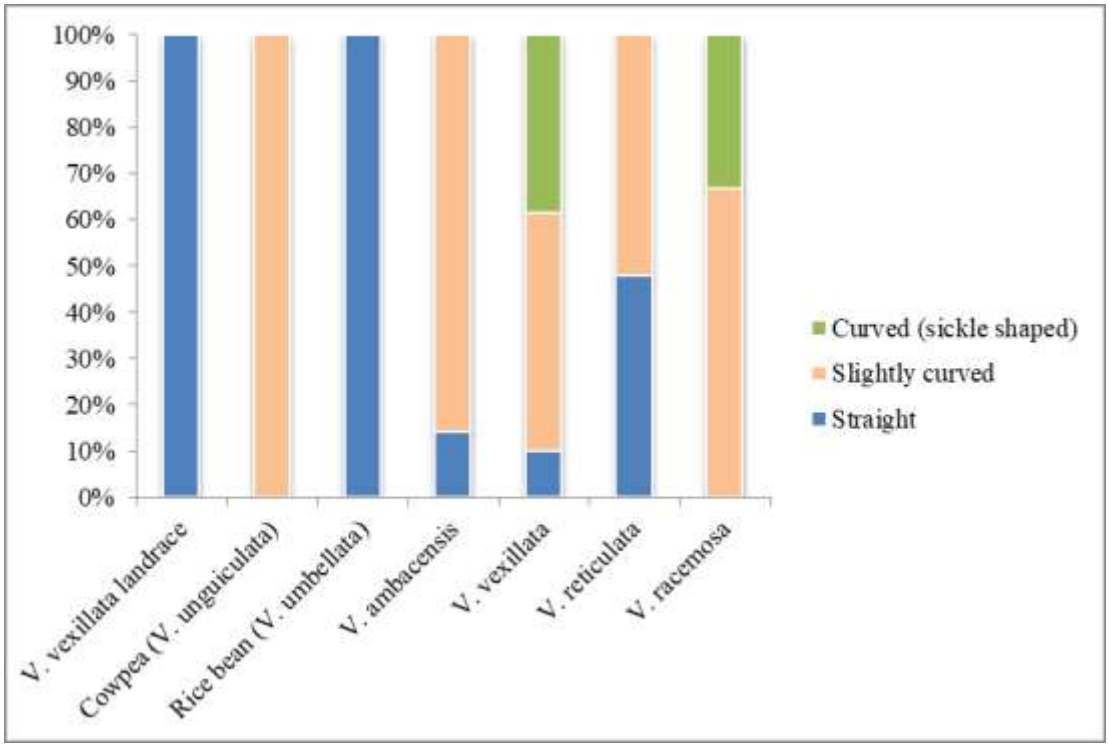
Cowpea and *V. vexillata* landrace presented a "semi-flat'' form of the pod cross section trait, together with 33% of *V. ambacensis*, 18% of *V. vexillata*, 19% of *V. reticulata*, and 67% of *V. racemosa* accessions (Fig. 9e). Rice bean presented a flat (''other'') form of the trait, together with 14% of *V. ambacensis*, 38% of *V. vexillata* and 15% of *V. reticulata* accessions. The "round'' variant of the trait was observed in 52% of *V. ambacensis*, 43% of *V. vexillata*, 65% of *V. reticulata* and 33% of *V. racemosa* accessions (Fig. 8e).



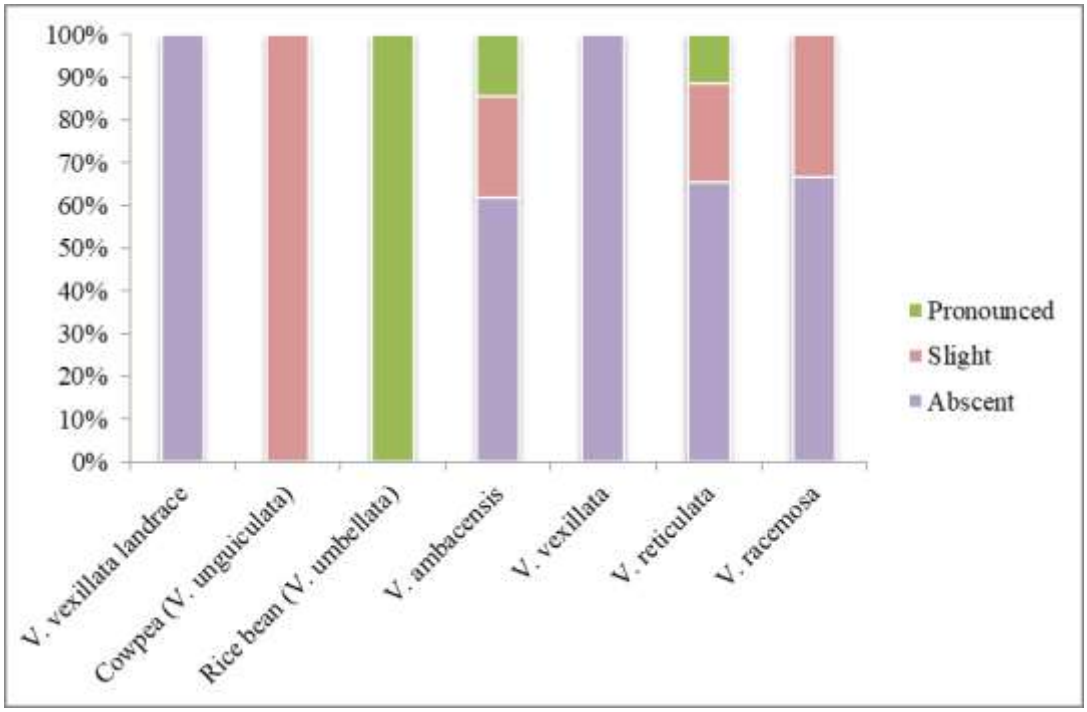
(a). Variation in pod attachment to peduncle



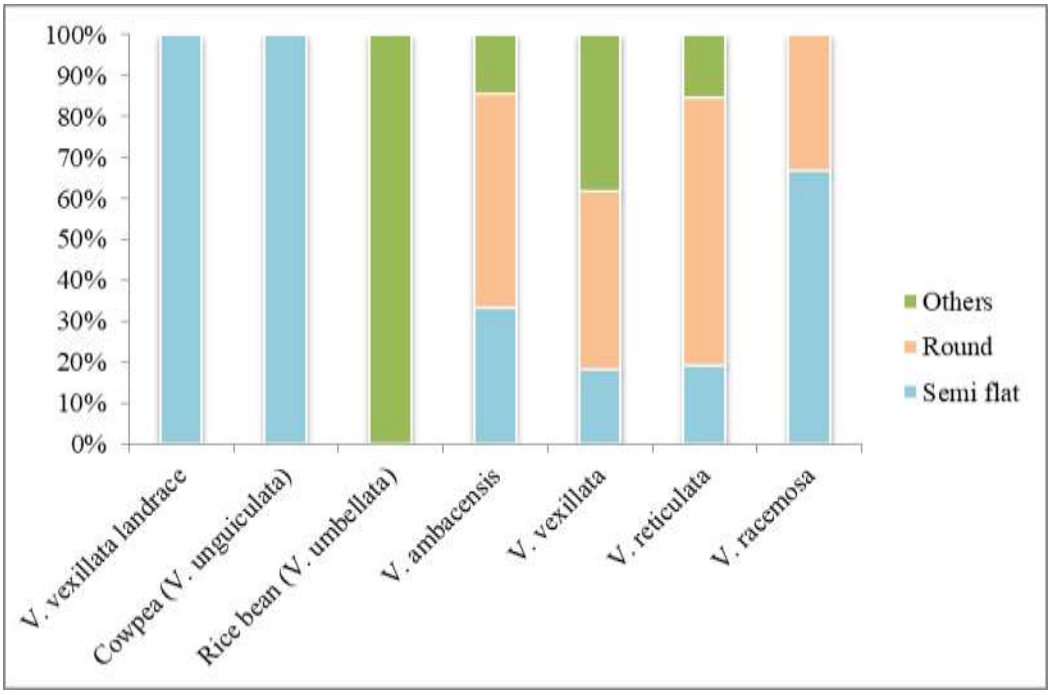
(b). Variations in pod pubescence



(c). Variations in pod curvature



(d). Variations in constriction of the pod between seeds



(e). Variations in pod cross section

Figure 8: Variations in Some Selected Qualitative Traits, Evaluated at the Maturity and Podding Stages. (a) Pod Attachment to Peduncle; (b) Pod Pubescence; (c) Pod Curvature; (d) Constriction of the Pod Between seeds; (e) Pod Cross Section

(ii) Quantitative Traits Exploration of the Wild Unexplored *Vigna* Species

The means, ranges and coefficients of variation for the selected quantitative traits studied at site A, selian and B, Tacri during the two cropping seasons are summarized in Appendix 2. Furthermore, the adjusted mean values for the studied traits are summarized per species in Appendix 3. The two tables show the results for only one season (the 2018 cropping season) for site A (Appendices 2 and 3).

To understand the variations of the means for the various traits studied within the cropping sites and seasons, the generalized linear model procedure (glm proc) of the SAS University Editions was run, and the results are summarized in Tables 5–8. One-way analysis of variance (ANOVA) and type III Sum of Squares Analysis, as well as the analysis of differences, helped to indicate the accession effect, block effect and the differences among the accessions, checks and check vs. accession.

The results from site A study during the 2018 cropping season show that there was a significant difference ($p < 0.05$) between the checks and the wild accessions for all the analyzed traits (Table 5a). Accession effects were also found for all the traits, except for the number of flowers per raceme trait (trait 7) (Table 5a). Block effects were only found for the

terminal leaflet width (trait 3), days to flowering (trait 5), number of flowers per raceme (trait 7), pods per peduncle (trait 9), pod length (trait 10) and seed size (trait 13) traits (Table 5a). Significant differences among the accessions, checks and check vs accession were observed, as shown in Table 5b for all the traits. Exceptionally, the seed size trait showed no significant difference among the checks ($p > 0.05$) (Table 5b).

Similarly, the results from site B during the 2018 cropping season show that there was a significant difference ($p < 0.05$) between the checks and the wild accessions for all the analyzed traits (Table 6a). Accession effects were also found for all the traits, with no exception, like in the case of trait 7 at site A (Table 6a). Block effects were only found for the terminal leaflet width (trait 3), days to flowering (trait 5), number of flowers per raceme (trait 7), pods per peduncle (trait 9), pod length (trait 10), seeds per pod (trait 12) and seed size (trait 13) traits (Table 6a). Significant differences among the accessions, checks and check vs accession were observed as shown in Table 6b for all the traits. Exceptionally, the seed size trait showed no significant difference among the checks ($p > 0.05$) (Table 6b).

A similar pattern of results was observed in the site B study area during the second cropping season (2019 cropping season) (Table 7). It was found that there was a significant difference ($p < 0.05$) between the checks and the wild accessions for all the analyzed traits (Table 7a). Accession effects were also found for all the traits, with no exception, like in the case of trait 7 at site A (Table 7a). Block effects were only found for the terminal leaflet width (trait 3), days to flowering (trait 5), number of flowers per raceme (trait 7), pods per peduncle (trait 9), pod length (trait 10), seeds per pod (trait 12) and seed size (trait 13) traits (Table 7a). Significant differences among the accessions, checks and check vs accession were observed, as shown in Table 7b for all the traits. Exceptionally, the seed size trait showed no significant difference among the checks ($p > 0.05$) (Table 7b).

Table 8 shows that out of the 15 quantitative traits examined, only the days to flowering, pods per plant, hundred seed weight and the yield were affected by their growing environment (accession x site effect), while only the number of flowers per raceme and the pods per plant were affected by the cropping season (accession x season effect) (Table 8). All the quantitative traits showed significant differences among the accessions for each site and each season. The same result was observed among the checks, except for the seed size trait.

To determine whether some of the wild *Vigna* accessions share common quantitative traits and can be grouped together, an agglomerative hierarchical clustering analysis was performed, and a dendrogram of three clusters was obtained based on 138 accessions out of the 160 planted due to the exclusion of 22 accessions which did not germinate or did not perform well (Fig. 9). The various accessions forming each cluster are presented in Table 9. Cluster I, which is made up of the majority of wild accessions also included two checks, the *Vigna vexillata* landrace and cowpea (*V. unguiculata*). Cluster II was made up of only check 3 (rice bean, *V. umbellate*), while cluster III contained 50 accessions of the wild *Vigna* species.

Furthermore, to examine the relationship that could exist between the quantitative traits and the accessions, as well as the relationship between the accessions themselves, a principal component analysis (XLSTAT) was performed using the adjusted means values, obtained earlier. A correlation circle, combined with an observation chart, was obtained, as shown in Fig.10. The analysis showed that the first (F1 = 45.39%) and second (F2 = 14.22%) PCA dimensions represent 59.61% of the initial information, which is the best combination and explains the variation among the accessions and traits. It was found that there is a positive correlation between the traits, except for the 'days to flowering' trait, which is due to the angles between their vectors (Fig. 10). It was also noted that all the checks, together with a set of wild accessions, are found on the right side of the F1 axis, forming a group of accessions with higher values for the examined quantitative traits, except for the days to flowering trait. Those accessions shared common features with the checks. A second group, made up of only wild accessions, was found on the left side of the F1 axis, representing the accessions with lower values for the evaluated traits. These accessions also present lower values for the 'days to flowering' trait on the F2 axis (Fig. 10).

Table 5: Morphological Traits* of Accessions Observed at the Tanzania Agricultural Research Institute (TARI, Arusha Region) during the 2018 Cropping Season

(a)

Traits	ANOVA					Type III Sum of Squares Analysis									
	Model					Block Effect					Accession Effect				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
1	172	230.50	1.34	0.00	<0.0001	7	0.00	0.00	-	-	165	230.19	1.40	0.00	<0.0001
2	172	2513.26	14.61	11.86	<0.0001	7	36.22	5.17	4.20	0.0019	165	1512.31	9.17	7.44	<0.0001
3	172	685.22	3.98	2.99	0.0001	7	15.18	2.17	1.63	0.1600	165	675.48	4.09	3.07	0.0001
4	172	1999.94	11.63	6.57	<0.0001	7	72.68	10.38	5.86	0.0001	165	1908.16	11.56	6.53	<0.0001
5	172	41,888.19	243.54	269.88	<0.0001	7	2.67	0.38	0.42	0.8818	165	41,253.80	250.02	277.07	<0.0001
6	172	92.42	0.54	21.64	<0.0001	7	0.63	0.09	3.65	0.0046	165	75.72	0.46	18.48	<0.0001
7	172	5907.56	34.35	14.23	<0.0001	7	24.98	3.57	1.48	0.2069	165	165	4000.12	24.24	10.05
8	172	16,033.67	93.22	68.36	<0.0001	7	25.65	3.66	2.69	0.0245	165	12,837.61	77.80	57.05	<0.0001
9	172	759.23	4.41	4.96	<0.0001	7	10.55	1.51	1.69	0.1427	165	539.13	3.27	3.67	<0.0001
10	172	4990.46	29.01	27.94	<0.0001	7	13.24	1.89	1.82	0.1139	165	3894.89	23.61	22.73	<0.0001
11	172	298,644.75	1736.31	365.40	<0.0001	7	81.81	11.69	2.46	0.0367	165	221,943.38	1345.11	283.07	<0.0001
12	172	3427.67	19.93	Infini	<0.0001	7	0.00	0.00			165	2597.62	15.74	Infini	<0.0001
13	172	26,079.22	151.62	29.99	<0.0001	7	49.43	7.06	1.40	0.2377	165	23,910.77	144.91	28.66	<0.0001
14	172	6155.01	35.78	14.06	<0.0001	7	60.79	8.68	3.41	0.0070	165	5923.05	35.90	14.11	<0.0001
15	172	225 200 114.2	1 309 303.0	14.99	<0.0001	7	2 007 022.1	286 717.4	3.28	0.0087	165	218 001 052.5	1 321 218.5	15.13	<0.0001

(b)

Traits	Contrast (Differences)														
	Among Accessions					Among Checks					Check vs. Accession				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
1	53	36.59	0.69	Infini	<0.0001	3	16.00	5.33	Infini	<0.0001	1	49.51	49.51	Infini	<0.0001
2	53	397.53	7.50	6.09	<0.0001	3	80.92	26.97	21.89	<0.0001	1	49.72	49.72	40.35	<0.0001
3	53	181.78	3.43	2.58	0.0019	3	28.15	9.38	7.05	0.0008	1	130.73	130.73	98.16	<0.0001
4	53	779.54	14.71	8.31	<0.0001	3	132.98	44.33	25.03	<0.0001	1	343.41	343.41	193.94	<0.0001
5	53	17,494.29	330.08	365.79	<0.0001	3	55.09	18.36	20.35	<0.0001	1	1095.79	1095.79	1214.34	<0.0001
6	53	17.56	0.33	13.34	<0.0001	3	0.29	0.10	3.84	0.0178	1	0.29	0.29	11.70	0.0016
7	53	984.25	18.57	7.70	<0.0001	3	137.32	45.77	18.97	<0.0001	1	159.39	159.39	66.05	<0.0001
8	53	1790.74	33.79	24.78	<0.0001	3	240.59	80.20	58.81	<0.0001	1	1751.07	1751.07	1284.07	<0.0001
9	53	141.29	2.67	3.00	0.0004	3	27.70	9.23	10.38	<0.0001	1	10.15	10.15	11.41	0.0018
10	53	822.94	15.53	14.95	<0.0001	3	516.15	172.049	165.66	<0.0001	1	91.85	91.85	88.45	<0.0001
11	53	98,712.64	1862.50	391.96	<0.0001	3	1092.09	364.03	76.61	<0.0001	1	6392.80	6392.80	1345.35	<0.0001
12	53	377.84	7.13	Infini	<0.0001	3	288.00	96.00	Infini	<0.0001	1	82.51	82.51	Infini	<0.0001
13	53	8853.77	167.05	33.04	<0.0001	3	5.64	1.88	0.37	0.7736	1	1092.99	1092.99	216.20	<0.0001
14	53	2595.73	48.98	19.24	<0.0001	3	59.56	19.85	7.80	0.0004	1	379.86	379.86	149.26	<0.0001
15	53	103 443 899.9	1 951 771.7	22.35	<0.0001	3	13 494 835.8	4 498 278.6	51.52	<0.0001	1	5 333 366.9	5 333 366.9	61.08	<0.0001

(a) Analysis of variance (ANOVA) and type III sum of squares analysis for the selected quantitative traits at the Tanzania Agricultural Research Institute (TARI, Arusha region) during the 2018 cropping season; (b) analysis of the differences and interactions between accessions and checks for the selected traits (all species)*

1: Germination time; **2:** Terminal leaflet length; **3:** Terminal leaflet width; **4:** Petiole length; **5:** Days to flowering; **6:** Flower bud size; **7:** Number of flowers per raceme; **8:** Peduncle length; **9:** Pods per peduncle; **10:** Pod length; **11:** Pods per plant; **12:** Seeds per pod; **13:** Seed size; **14:** 100-Seed weight; **15:** Yield.

Table 6: Morphological Traits* of Accessions Observed at the Tanzania Coffee Research Institute (TaCRI, Kilimanjaro Region) during the 2018 Cropping Season

(**a**)

Traits	ANOVA					Type III Sum of Square Analysis									
	Model					Block Effect					Accession Effect				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
	172	2564.76	14.91	Infini	<0.0001	7	0.00	0.00			165	1217.73	7.38	Infini	<0.0001
	172	2986.01	17.36	11.86	<0.0001	7	43.03	6.15	4.20	0.0019	165	1796.78	10.89	7.44	<0.0001
	172	829.12	4.82	2.99	0.0001	7	18.36	2.62	1.63	0.1600	165	817.33	4.95	3.07	0.0001
	172	2121.73	12.33	6.57	<0.0001	7	77.10	11.01	5.86	0.0001	165	2024.37	12.27	6.53	<0.0001
	172	45758.01	266.03	233.27	<0.0001	7	4.33	0.62	0.54	0.80	165	44869.16	271.93	238.44	<0.0001
	172	133.09	0.77	21.64	<0.0001	7	0.91	0.13	3.65	0.0046	165	109.03	0.66	18.48	<0.0001
	172	4330.26	25.18	8.55	<0.0001	7	22.38	3.20	1.09	0.3929	165	3090.50	18.73	6.36	<0.0001
	172	17010.12	98.90	68.36	<0.0001	7	27.21	3.89	2.69	0.0245	165	13619.42	82.54	57.05	<0.0001
	172	748.83	4.35	4.82	<0.0001	7	10.10	1.57	1.74	0.1314	165	538.18	3.26	3.61	<0.0001
	172	4792.84	27.87	27.94	<0.0001	7	12.72	1.817	1.82	0.1139	165	3740.65	22.67	22.73	<0.0001
	172	60475.56	351.60	365.40	<0.0001	7	16.57	2.37	2.46	0.0367	165	44943.53	272.39	283.07	<0.0001
	172	3387.31	19.69	236.32	<0.0001	7	0.58	0.08	1.00	0.4478	165	2581.24	15.64	187.73	<0.0001
	172	23536.50	136.84	29.99	<0.0001	7	44.61	6.37	1.40	0.2377	165	21579.47	130.78	28.66	<0.0001
	172	4993.97	29.03	14.40	<0.0001	7	48.15	6.88	3.41	0.0070	165	4836.06	29.31	14.54	<0.0001
	172	182274678.9	1059736.5	15.32	<0.0001	7	1589762.2	227108.9	3.28	0.0087	165	177020077.70	1072849.00	15.51	<0.0001

(**b**)

Traits	Contrast (Differences)														
	Among Accessions					Among Checks					Check vs. Accession				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
	53	67.20	1.27	Infini	<0.0001	3	16.00	5.33	Infini	<0.0001	1	118.26	118.26	Infini	<0.0001
	53	472.30	8.91	6.09	<0.0001	3	96.14	32.04	21.89	<0.0001	1	59.07	59.07	40.35	<0.0001
	53	219.95	4.15	2.58	0.0019	3	34.07	11.36	7.05	0.0008	1	158.18	158.18	98.16	<0.0001
	53	827.02	15.60	8.31	<0.0001	3	141.08	47.03	25.03	<0.0001	1	364.33	364.33	193.94	<0.0001
	53	17480.49	329.82	289.20	<0.0001	3	58.84375	19.61	17.20	<0.0001	1	872.40	872.40	764.95	<0.0001
	53	25.28	0.47	13.34	<0.0001	3	0.41	0.14	3.84	0.0178	1	0.42	0.42	11.70	0.0016
	53	805.51	15.20	5.16	<0.0001	3	125.78	41.93	14.25	<0.0001	1	169.17	169.17	57.48	<0.0001
	53	1899.80	35.84	24.78	<0.0001	3	255.25	85.08	58.81	<0.0001	1	1857.71	1857.71	1284.07	<0.0001
	53	138.13	2.61	2.89	0.0006	3	29.55375	9.85	10.92	<0.0001	1	12.05	12.05	13.35	0.0008
	53	790.35	14.91	14.95	<0.0001	3	495.71	165.24	165.66	<0.0001	1	88.22	88.22	88.45	<0.0001
	53	19989.31	377.16	391.96	<0.0001	3	221.15	73.72	76.61	<0.0001	1	1294.54	1294.54	1345.35	<0.0001
	53	377.20	7.12	85.40	<0.0001	3	276.38	92.13	1105.50	<0.0001	1	78.07	78.070	936.85	<0.0001
	53	7990.53	150.76	33.04	<0.0001	3	5.093673	1.70	0.37	0.7736	1	986.42	986.43	216.20	<0.0001
	53	2134.87	40.28	19.98	<0.0001	3	47.18	15.73	7.80	0.0004	1	324.85	324.85	161.15	<0.0001
	53	83831969.77	1581735.28	22.87	<0.0001	3	10689259.48	3563086.49	51.52	<0.0001	1	4556054.50	4556054.50	65.87	<0.0001

(**a**) Analysis of variance (ANOVA) and type III sum of squares analysis for the selected quantitative traits at the Tanzania Coffee Research Institute (TaCRI, Kilimanjaro region) during the 2018 cropping season; (**b**) analysis of the differences and interactions between accessions and checks for the selected traits (all species). *

1: Germination time; **2:** Terminal leaflet length; **3:** Terminal leaflet width; **4:** Petiole length; **5:** Days to flowering; **6:** Flower bud size; **7:** Number of flowers per raceme; **8:** Peduncle length; **9:** Pods per peduncle; **10:** Pod length; **11:** Pods per plant; **12:** Seeds per pod; **13:** Seed size; **14:** 100-Seed weight; **15:** Yield

Table 7: Morphological Traits* of Accessions Observed at the Tanzania Coffee Research Institute (TaCRI, Kilimanjaro Region) During the 2019 Cropping Season

(a)

Traits	ANOVA					Type III Sum of Square Analysis									
	Model					Block Effect					AccessionEffect				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
1	172	1886.23	10.97	Infini	<0.0001	7	0.00	0.00			165	1236.69	7.50	Infini	<0.0001
2	172	2312.36	13.44	11.86	<0.0001	7	33.32	4.76	4.20	0.0019	165	1391.43	8.43	7.44	<0.0001
3	172	627.56	3.65	2.99	0.0001	7	13.90	1.99	1.63	0.1600	165	618.64	3.75	3.07	0.0001
4	172	2185.86	12.71	6.57	<0.0001	7	79.43	11.35	5.86	0.0001	165	2085.55	12.64	6.53	<0.0001
5	172	45758.01	266.03	233.27	<0.0001	7	4.33	0.62	0.54	0.7960	165	44869.16	271.93	238.44	<0.0001
6	172	155.23	0.90	21.64	<0.0001	7	1.07	0.15	3.65	0.0046	165	127.18	0.77	18.48	<0.0001
7	172	108256.43	629.40	8.55	<0.0001	7	559.500	79.93	1.09	0.3929	165	77262.23	468.26	6.36	<0.0001
8	172	18046.03	104.92	68.36	<0.0001	7	28.86	4.12	2.69	0.0245	165	14448.84	87.57	57.05	<0.0001
9	172	1643.58	9.56	4.62	<0.0001	7	26.73	3.82	1.84	0.1094	165	1181.19	7.16	3.46	<0.0001
10	172	4918.26	28.59	27.94	<0.0001	7	13.05	1.86	1.82	0.1139	165	3838.54	23.26	22.73	<0.0001
11	172	216024.75	1255.96	365.40	<0.0001	7	59.18	8.45	2.46	0.0367	165	160542.80	972.99	283.07	<0.0001
12	172	4479.72	26.04	236.32	<0.0001	7	0.77	0.11	1.00	0.4478	165	3413.69	20.69	187.73	<0.0001
13	172	24009.58	139.59	29.99	<0.0001	7	45.51	6.50	1.40	0.2377	165	22013.22	133.41	28.66	<0.0001
14	172	5554.90	32.30	14.06	<0.0001	7	54.86	7.84	3.41	0.0070	165	5345.55	32.40	14.11	<0.0001
15	172	203243103.1	1181645.9	14.99	<0.0001	7	1811337.5	258762.5	3.28	0.0087	165	196745949.9	1192399.7	15.13	<0.0001

(b)

Traits	Contrast (Differences)														
	Among Accessions					Among Checks					Check vs. Accession				
	DF	Sum of Squares	Mean Squares	F	Pr > F	DF	Sum of Squares	Mean Squares	F	*p*	DF	Sum of Squares	Mean Squares	F	*p*
1	53	44.13	0.83	Infini	<0.0001	3	16.00	5.33	Infini	<0.0001	1	103.45	103.45	Infini	<0.0001
2	53	365.75	6.90	6.09	<0.0001	3	74.45	24.82	21.89	<0.0001	1	45.74	45.74	40.35	<0.0001
3	53	166.48	3.14	2.58	0.0019	3	25.78	8.59	7.05	0.0008	1	119.73	119.73	98.16	<0.0001
4	53	852.01	16.08	8.31	<0.0001	3	145.34	48.45	25.03	<0.0001	1	375.34	375.34	193.94	<0.0001
5	53	17480.49	329.82	289.20	<0.0001	3	58.84	19.61	17.20	<0.0001	1	872.40	872.40	764.95	<0.0001
6	53	29.49	0.56	13.34	<0.0001	3	0.48	0.16	3.84	0.0178	1	0.49	0.49	11.70	0.0016
7	53	20137.78	379.96	5.16	<0.0001	3	3144.59	1048.20	14.25	<0.0001	1	4229.18	4229.18	57.48	<0.0001
8	53	2015.50	38.03	24.78	<0.0001	3	270.79	90.26	58.81	<0.0001	1	1970.84	1970.84	1284.07	<0.0001
9	53	295.70	5.58	2.70	0.0013	3	63.23	21.08	10.18	<0.0001	1	25.27	25.27	12.21	0.0013
10	53	811.04	15.30	14.95	<0.0001	3	508.68	169.56	165.66	<0.0001	1	90.53	90.53	88.45	<0.0001
11	53	71403.81	1347.24	391.96	<0.0001	3	789.97	263.32	76.61	<0.0001	1	4624.23	4624.23	1345.35	<0.0001
12	53	498.84	9.41	85.40	<0.0001	3	365.51	121.84	1105.50	<0.0001	1	103.25	103.25	936.85	<0.0001
13	53	8151.14	153.80	33.04	<0.0001	3	5.20	1.70	0.37	0.7736	1	1006.25	1006.25	216.20	<0.0001
14	53	2342.65	44.20	19.24	<0.0001	3	53.76	17.92	7.80	0.0004	1	342.83	342.83	149.26	<0.0001
15	53	93358119.63	1761473.96	22.35	<0.0001	3	12179089.35	4059696.45	51.52	<0.0001	1	4813363.60	4813363.60	61.08	<0.0001

(**a**) Analysis of variance (ANOVA) and type III sum of squares analysis for the selected quantitative traits at the Tanzania Coffee Research Institute (TaCRI, Kilimanjaro region) during the 2019 cropping season; (**b**) analysis of the differences and interactions between accessions and checks for the selected traits (all species). *

1: Germination time; **2:** Terminal leaflet length; **3:** Terminal leaflet width; **4:** Petiole length; **5:** Days to flowering; **6:** Flower bud size; **7:** Number of flowers per raceme; **8:** Peduncle length; **9:** Pods per peduncle; **10:** Pod length; **11:** Pods per plant; **12:** Seeds per pod; **13:** Seed size; **14:** 100-Seed weight; **15:** Yield

Table 8: Results of the Two-way Analysis of Variance Showing the Accession Interactions due to the site and Season for the Studied Quantitative Traits

S/N	Traits	*p*-Values for Site Effects			*p*-Values for Season Effects		
		Site	Accession	Site x Accession	Season	Accession	Accession x Season
1	Germination time (days)	<0.0001	0.000	0.153	0.097	0.000	0.979
2	Terminal leaflet length (cm)	<0.0001	<0.0001	1.000	<0.0001	<0.0001	0.961
3	Terminal leaflet width (cm)	0.000	<0.0001	1.000	<0.0001	<0.0001	0.998
4	Petiole length (cm)	0.000	<0.0001	1.000	0.009	<0.0001	1.000
5	Days to flowering	<0.0001	<0.0001	0.032	<0.0001	<0.0001	1.000
6	Flower bud size (cm)	<0.0001	<0.0001	0.078	<0.0001	<0.0001	0.899
7	Number of flowers per raceme	<0.0001	<0.0001	0.995	<0.0001	0.000	0.003
8	Peduncle length (cm)	0.003	<0.0001	1.000	0.003	<0.0001	1.000
9	Pods per peduncle	0.742	<0.0001	0.973	<0.0001	<0.0001	0.054
10	Pod length (cm)	0.194	<0.0001	1.000	0.371	<0.0001	1.000
11	Pods per plant	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
12	Seeds per pod	0.894	<0.0001	1.000	<0.0001	<0.0001	0.712
13	Seed size (mm^2)	<0.0001	<0.0001	0.052	0.013	<0.0001	0.506
14	100-Seed weight (g)	<0.0001	<0.0001	0.037	<0.0001	<0.0001	0.068
15	Yield (Kg/ha)	<0.0001	<0.0001	0.032	<0.0001	<0.0001	0.055

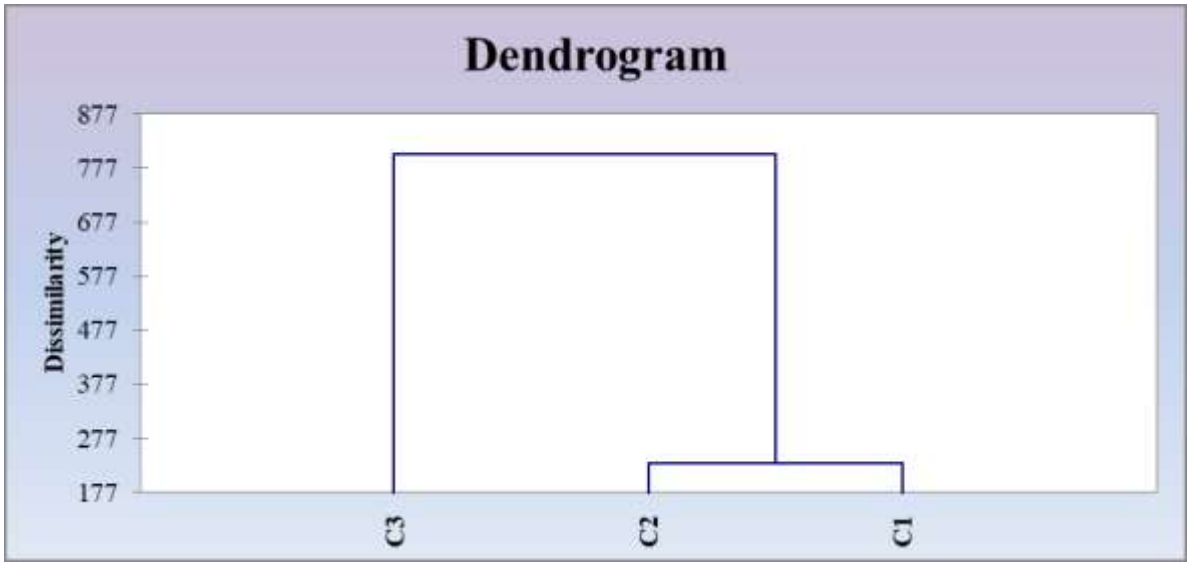


Figure 9: Tree Diagram Showing the Quantitative Traits of the Studied Wild *Vigna* Species, Specically Accessions

Table 9: Distribution of the Accessions, According to the Clusters Generated from the Agglomerative Hierarchical Analysis (AHC)

Class	1	2	3
Objects	91	2	49
Sum of weights	91	2	49
Within-class variance	241116.782	591.362	24460.953
Minimum distance to centroid	15.782	17.195	21.321
Average distance to centroid	339.857	17.195	145.418
Maximum distance to centroid	1877.573	17.195	192.887

Cluster I		Cluster II	Cluster III
Check 1	TVNu- 608 (VRe)	Check 3	TVNu- 1185 (VA)
Check 2	TVNu- 781 (VV)		TVNu- 1792 (VA)
	TVNu- 1476 (VV)		TVNu- 1644 (VA)
	TVNu- 1624 (VV)		TVNu- 148 (VA)
TVNu- 450 (VRe)	AGG17856WVIG 1 (VRe)		TVNu- 216 (VA)
TVNu- 1804 (VA)	AGG62154WVIG 1 (VV)		TVNu- 557 (VA)
TVNu- 593 (VV)	TVNu- 1796 (VV)		TVNu- 313 (VA)
TVNu- 1805 (VRe)	TVNu- 1825 (VRe)		TVNu- 1678 (VA)
TVNu- 293 (VV)	TVNu- 56 (VRe)		AGG308103WVIG 3 (VV)
AGG308096 WVIG 2 (VV)	AGG51603WVIG 1 (VRa)		AGG308101WVIG 1 (VV)
TVNu- 758 (VRe)	TVNu- 302 (VRe)		TVNu- 1546 (VV)
TVNu- 3 (VA)	TVNu- 1359 (VV)		TVNu- 1212 (VA)
TVNu- 1191 (VRe)	TVNu- 604 (VRe)		TVNu- 1125 (VA)
TVNu- 877 (VA)	TVNu- 350 (VRe)		AGG53597WVIG 1 (VRa)
TVNu- 1121 (VV)	TVNu- 325 (VRe)		AGG16683WVIG 5 (VV)
TVNu- 312 (VRe)	TVNu- 342 (VA)		TVNu- 1593 (VV)
TVNu- 219 (VA)	TVNu- 324 (VRe)		TVNu- 1840 (VA)
TVNu- 1590 (VV)	TVNu- 1852 (VRe)		TVNu- 1586 (VV)
Unknown *Vigna* reticulata	TVNu- 602 (VRe)		TVNu- 1585 (VV)
TVNu- 1701 (VV)	TVNu- 629 (VA)		AGG308098WVIG 2 (VV)
TVNu- 739 (VRe)	TVNu- 1213 (VA)		TVNu- 1378 (VV)
TVNu- 1369 (VV)	TVNu- 1748 (VV)		TVNu- 142 (VRe)
TVNu- 738 (VRe)	TVNu- 1591 (VV)		TVNu- 792 (VV)
TVNu- 1631 (VA)	TVNu- 1827 (VA)		TVNu- 1529 (VV)
TVNu- 605 (VRe)	TVNu- 1394 (VRe)		TVNu- 1843 (VA)
TVNu- 1150 (VA)	AGG308099WVIG 2 (VV)		TVNu- 1360 (VV)
TVNu- 178 (VV)	TVNu- 1808 (VRe)		AGG52867WVIG 1 (VRa)
TVNu- 1791 (VA)	TVNu- 374 (VA)		TVNu- 1069 (VA)
TVNu- 375 (VA)	TVNu- 1344 (VV)		TVNu- 1621 (VV)
TVNu- 832 (VV)	TVNu- 1092 (VV)		TVNu- 1628 (VV)
TVNu- 1388 (VRe)	TVNu- 1617 (VV)		TVNu- 1781 (VA)
TVNu- 765 (VA)	TVNu- 333 (VV)		TVNu- 1851 (VA)
TVNu- 223 (VA)	TVNu- 1522 (VRe)		TVNu- 141 (VRe)
AGG60441WVIG 1 (VRe)	TVNu- 524 (VRe)		TVNu- 349 (VRe)
TVNu- 452 (VA)	TVNu- 1186 (VA)		TVNu- 1156 (VRe)
AGG308100WVIG 3 (VV)	TVNu- 379 (VRe)		TVNu- 1679 (VA)

	TVNu- 343 (VRe)	TVNu- 1443 (VV)	TVNu- 259 (VRe)
	TVNu- 138 (VRe)	TVNu- 955 (VV)	TVNu- 636 (VV)
	TVNu- 1594 (VV)	TVNu- 837 (VV)	Unknown *Vigna*
	TVNu- 607 (VRe)	TVNu- 224 (VRe)	TVNu- 969 (VV)
	TVNu- 1405 (VRe)	TVNu- 1582 (VV)	TVNu- 947 (VA)
	AGG308097WVIG 1 (VV)		TVNu- 1691 (VA)
	TVNu- 932 (VRe)		TVNu- 706 (VA)
	AGG60436WVIG 1 (VRa)		AGG308102WVIG 3 (VV)
	TVNu- 120 (VV)		TVNu- 1677 (VA)
	TVNu- 491 (VRe)		TVNu- 479 (VV)
	AGG58678WVIG 2 (VV)		TVNu- 722 (VA)
	TVNu- 1698 (VRe)		AGG118137WVIG 1 (VRe)
	AGG308107WVIG 2 (VV)		TVNu- 1112 (VRe)
	TVNu- 1718 (VV)		TVNu- 1185 (VA)
			TVNu- 1792 (VA)

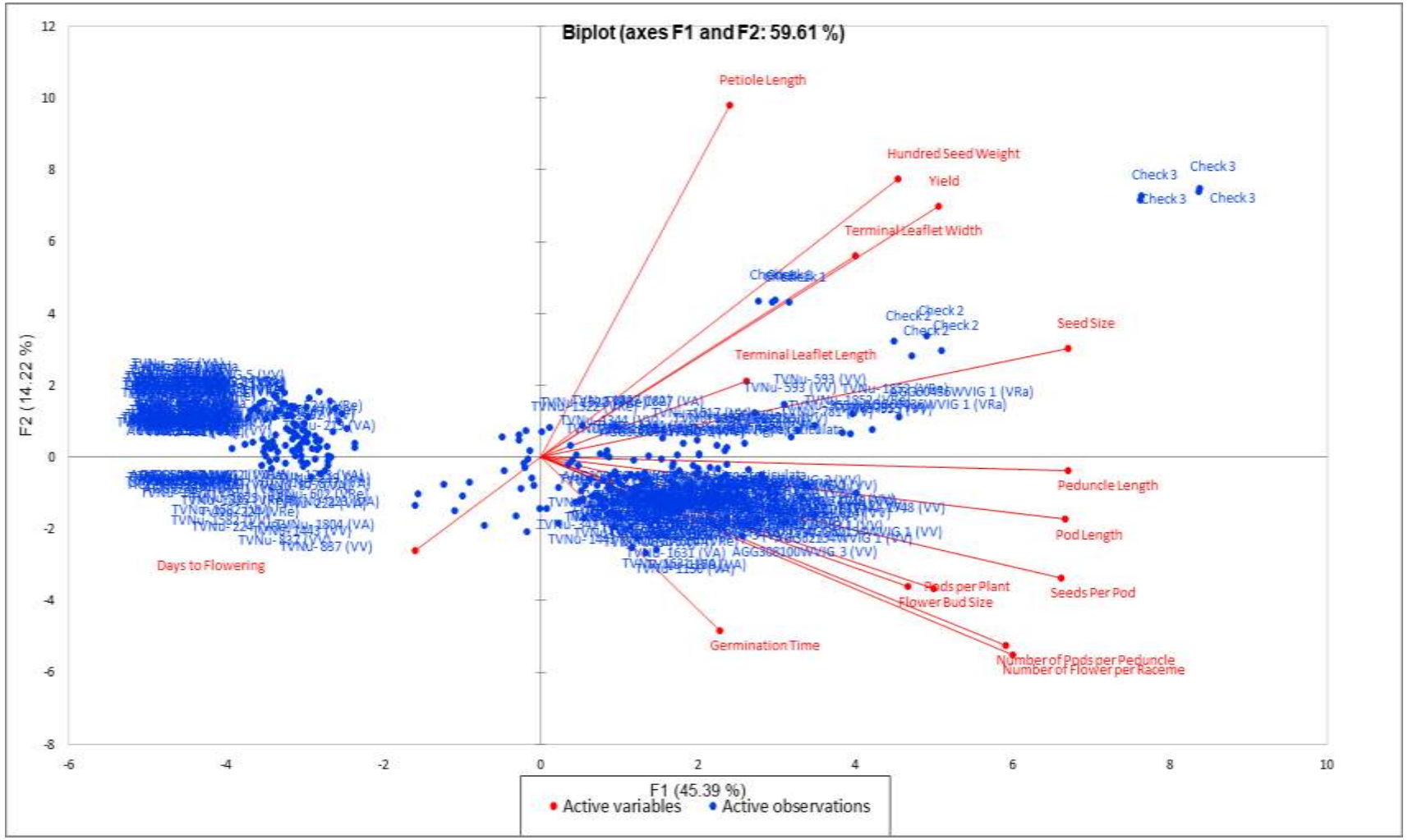


Figure 10: Principal Component Analysis Result, Showing the Relationship between the Traits and the Accessions

4.1.2 Farmers' Perceptions, Preferences and Prospective Uses of Wild *Vigna* Species for Human Exploitation

(i) Socio-demographic Characteristics of Participants

The results from the socio-demographic characteristics showed that 64 and 36% were female and male farmers respectively (Fig. 11a). Most of the participants were above 45 years old, with the highest level of education being primary (Kilimanjaro) and secondary school (Arusha). Furthermore, most of the farmers had a reasonable number of years of experience farming legumes, varying from two to more than 35 years (Fig. 11d). The intervals of years of farming experience and the percentages of participants with the longest farming experience were 6–10 and 16–20% respectively (Fig. 11d).

(ii) Prior Knowledge/Awareness about Wild Legumes

Less than 30% (28 and 26% in both study sites) of the experienced participants involved in the study were aware of the existence of wild legumes (Fig. 12). According to the binary logistic regression analysis (Table 10), the model including the farmers' socio-demographic characteristics as explanatory variables and prior knowledge of legumes as a dependent variable is a good fit with the data as p = 0.633 > 0.05 (*Hosmer and Lemeshow test*). This explains that the variance in the outcome is significant (X^2 = 40.632, df = 19, p.003*)* (Omnibus Tests of Model Coefficients). The results show that there is no significant association between the prior knowledge about wild legumes and the overall gender (Wald = 0.495, df = 1, p > 0.05) (Table 10). However, there is a slight effect associated with being a female farmer and prior knowledge (B = 0.303, p = 0.482). No significant relationship existed between the overall farmers' age groups and their prior knowledge of wild legumes (Wald = 7.061, df = 6, p = 0.315 > 0.05), although there is a slight significance relationship with the youngest age group [15–20] (Wald = 4.113, df = 1, B = 2.982, p = 0.043) as shown in Table 10. Similarly, the test shows that the education level (Wald = 3.962, df = 4, p = 0.411) as well as their farming experience (Wald = 5.462, df = 7, p = 0.604) do not have any influence to their prior knowledge about wild legumes. On the contrary, the location (research site) has a significant effect on their prior knowledge of wild legumes (Wald = 9.884, df = 1, B = 1.687, p = 0.002).

(iii) Prior Uses of Wild Legumes

A few participants who had prior knowledge of wild legumes mentioned several uses attributed to them which they had seen before. Some of the uses mentioned were livestock feed, human food and soil fertility ingredients as well as botanical pesticides (Table 11).

(iv) Challenges Faced by Legume Farmers

Diseases and drought (or reduced rainfall) were the most challenges faced by the farmers in both mid and high altitude agro-ecological zones (Fig. 13). Apart from diseases and reduced rainfall issues, other reported challenges were related to market access, pest and storage (Fig. 13). Taste and cooking aspects were not of very serious concern to the farmers in the two zones, since most of them seemed to be comfortable with the taste and cooking aspects of their legumes.

(v) Farmers' Preferred Accessions of Wild *Vigna* Legumes

The study shows that 74 accessions out of the 160 planted grown to an appreciable level at the time of screening and were selected based on the participants' personal preferences (Fig. 15). In the high-altitude zone (Kilimanjaro), only five (5) accessions (TVNu-293, TVNu-758, AGG308107WVIG 2, AGG308101WVIG 1, and TVNu-1546) were selected by the farmers more than half of the time, while in the mid-altitude zone (Arusha), none of the accessions had up to 50% selection (Fig. 14). The five most selected accessions in the mid-altitude zone—TVNu-293 (36%), TVNu-758 (36%), AGG51603WVIG 1 (30%), AGG308099WVIG 2 (40%), and AGG53597WVIG 1 (34%)—were different from those selected in the high-altitude zone, except for TVNu-293 and TVNu-758.

The likelihood ratio test revealed that the wild *Vigna* selection (preferences) significantly depended on the farmers' gender ($G^2 = 130.813$, df = 73, $p < 0.000$), farming experience ($G^2 = 669.196$, df = 511, $p < 0.000$), and location ($G^2 = 1110.606$, df = 73, $p < 0.000$).

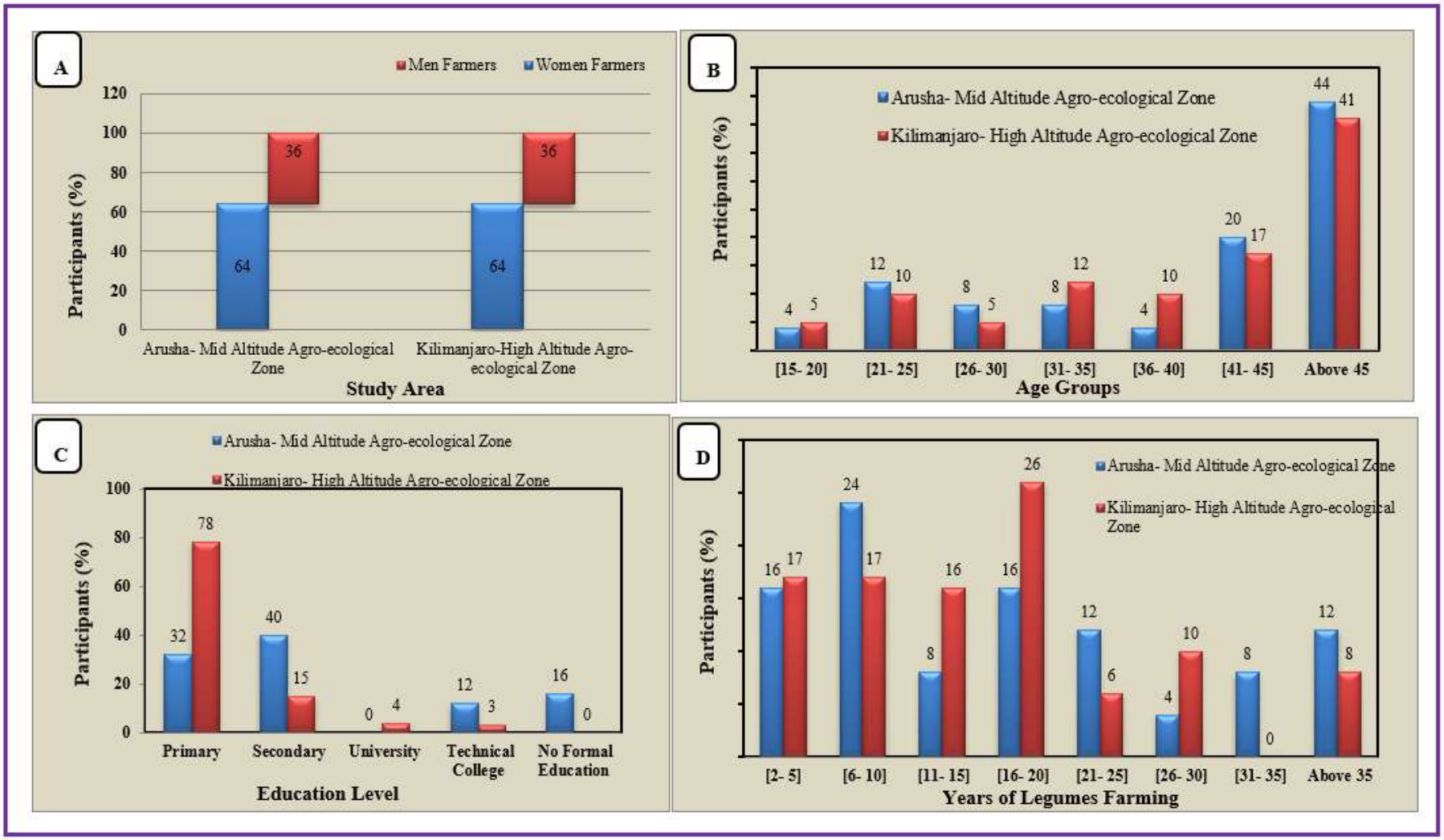


Figure 11: Socio-demographic Characteristics of Participants (A): Participants' Gender per Study Area (%); (B): Participants' Age Groups; (C): Participants' Education Level; and (D): Participants' Legumes Farming Experience

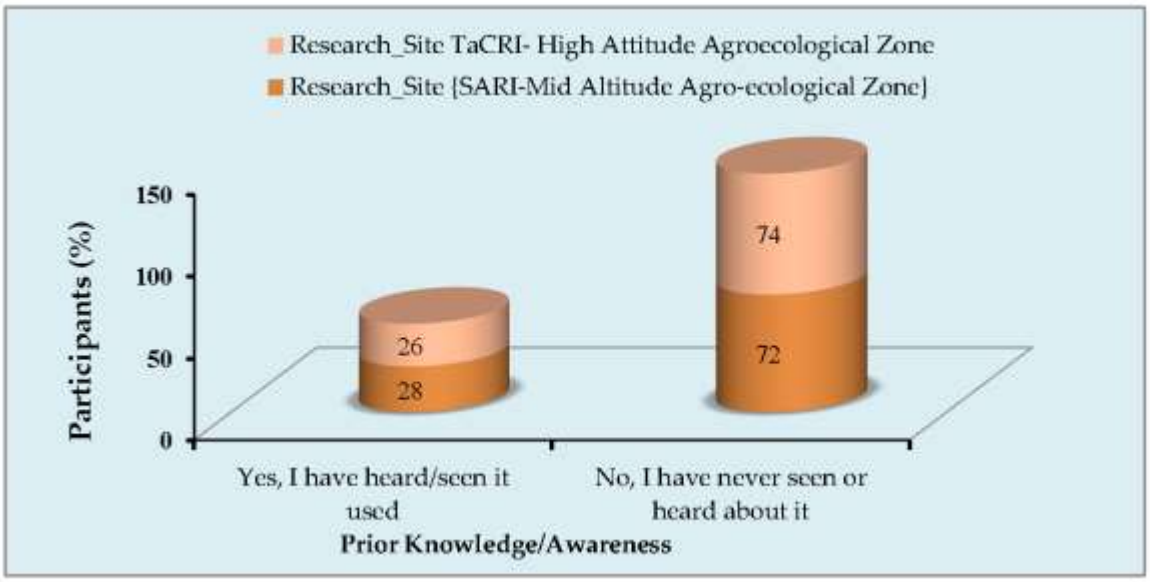


Figure 12: Participants' Prior Knowledge of Wild Legumes

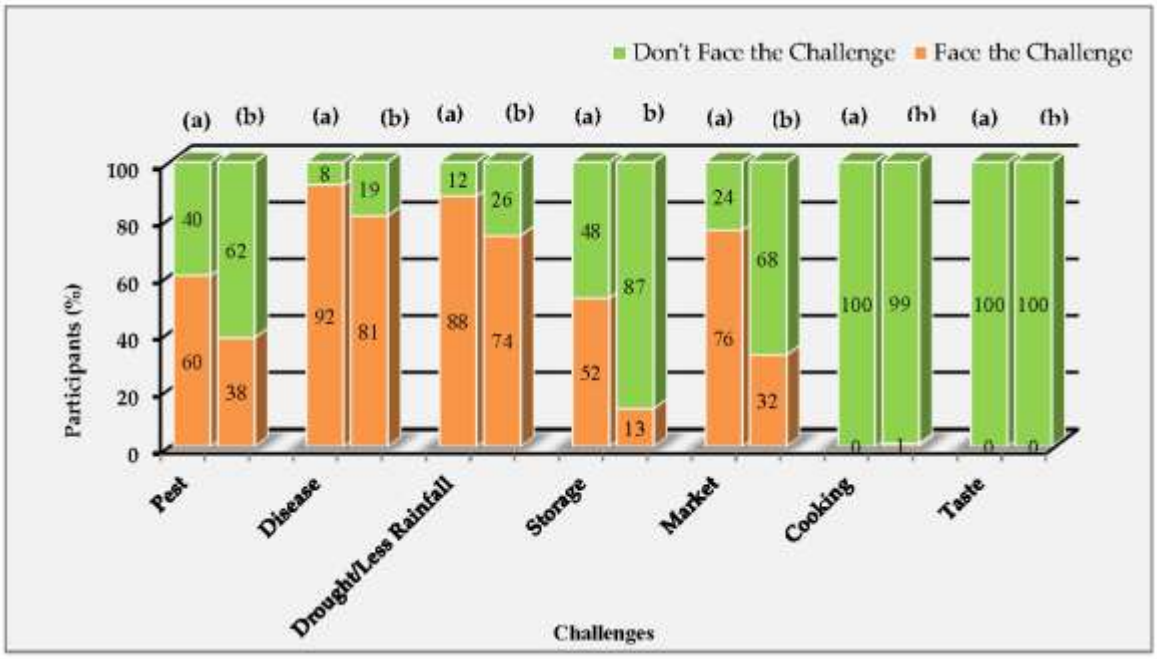


Figure 13: Participants' Challenges Faced during Legumes Cultivation in the Two Study Areas: (a) Arusha and (b) Kilimanjaro

Table 10: Binary Logistic Analysis Result

Variables in the Equation									
			B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
									Lower	Upper
Step 1 [a]	**Gender (1)**		0.303	0.431	0.495	1	0.482	1.354	0.582	3.153
	Age				7.061	6	0.315			
		Age (1)	2.982	1.471	4.113	1	0.043	19.732	1.105	352.281
		Age (2)	1.162	1.010	1.325	1	0.250	3.197	0.442	23.123
		Age (3)	1.755	1.124	2.440	1	0.118	5.786	0.639	52.342
		Age (4)	1.154	0.876	1.733	1	0.188	3.171	0.569	17.668
		Age (5)	1.010	0.798	1.601	1	0.206	2.745	0.575	13.111
		Age (6)	−0.255	0.622	0.168	1	0.681	0.775	0.229	2.620
	Education_Level				3.962	4	0.411			
		Level (1)	1.817	1.269	2.049	1	0.152	6.155	0.511	74.087
		Level (2)	2.334	1.285	3.299	1	0.069	10.316	0.831	127.995
		Level (3)	1.694	1.763	0.923	1	0.337	5.439	0.172	172.291
		Level (4)	1.407	1.504	0.876	1	0.349	4.084	0.214	77.805
	Research_Site (1)		1.687	0.537	9.884	1	0.002	5.402	1.887	15.460
	Farming_Experience				5.462	7	0.604			
		Experience (1)	−1.005	1.216	0.683	1	0.408	0.366	0.034	3.966
		Experience (2)	−1.245	1.118	1.242	1	0.265	0.288	0.032	2.573
		_Experience (3)	−1.222	1.022	1.430	1	0.232	0.295	0.040	2.183
		_Experience (4)	0.121	0.873	0.019	1	0.890	1.129	0.204	6.248
		_Experience (5)	0.409	1.025	0.159	1	0.690	1.505	0.202	11.216
		_Experience (6)	−0.559	0.998	0.313	1	0.576	0.572	0.081	4.046
		_Experience (7)	21.259	194,50.255	0.000	1	0.999	1,708,644,034.887	0.000	.
	Constant		−2.586	1.058	5.975	1	0.015	0.075		

[a]. Variable(s) entered on step 1: Gender, Age, Education_Level, Research_Site, Farming_Experience.B: represent the values for the logistic regression equation for predicting the dependent variables from the independent variables; S.E.: Standard errors associated with coefficients; Wald: Wald Chi-Square value; df: Degree of freedom for each of the tests of the coefficients; Sig.: Significance level (p-value); EXP(B): Exponentiation of the coefficients (odd ratios for the predictors); C.I.: Confidence Interval

Table 11: Wild Legumes Uses as known by Participants with Prior Knowledge of Wild Legumes *

	Percentage (%)	Livestock Feed	Human Food	Soil Fertility Ingredient	Traditional Botanical Pesticides
Participants in a mid-altitude agro-ecological zone	28	12 Animal feed = 'Chakula cha mifugo', 'chakula cha ng'ombe'	16 Human food = 'Chakula cha binadamu', Vegetable = 'Mboga'	0	0
Participants in a high-altitude agro-ecological zone	26	4 Animal feed = ''Chakula cha mifugo', 'chakula cha ng'ombe'	4 Human food = 'chakula cha binadamu' Vegetable = 'Mboga'.	14 Rattlepod (Crotalaria ochroleuca) = 'Marejea' used as fertilizer = 'mbolea', Nourish the soil = 'Hurutubisha ardhi', Cover crop = 'Kutandaza shambani'	4 Pesticide = 'kunyunyuzia shambani', 'kutengeza dawa ya kunyunyuzia shambani'

***** Words in single quotation marks (' ') are exact expressions given by participants in Swahili, which has been translated

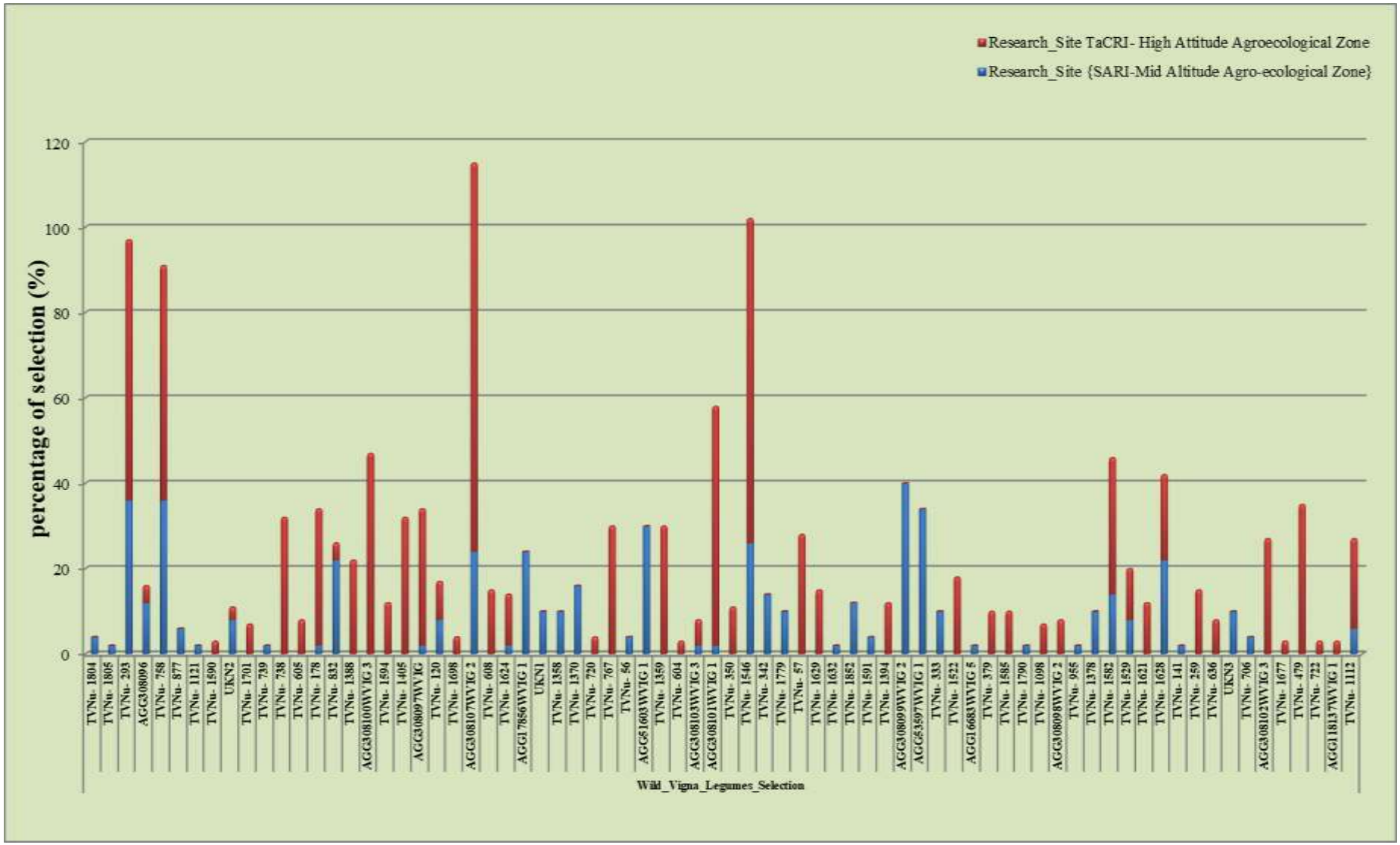


Figure 14: Wild *Vigna* Legumes Preferred (selected) by Participants from the Two Agro-ecological Zones

(vi) Prospective Uses of Farmers' Preferred Accessions of Wild *Vigna* Legumes

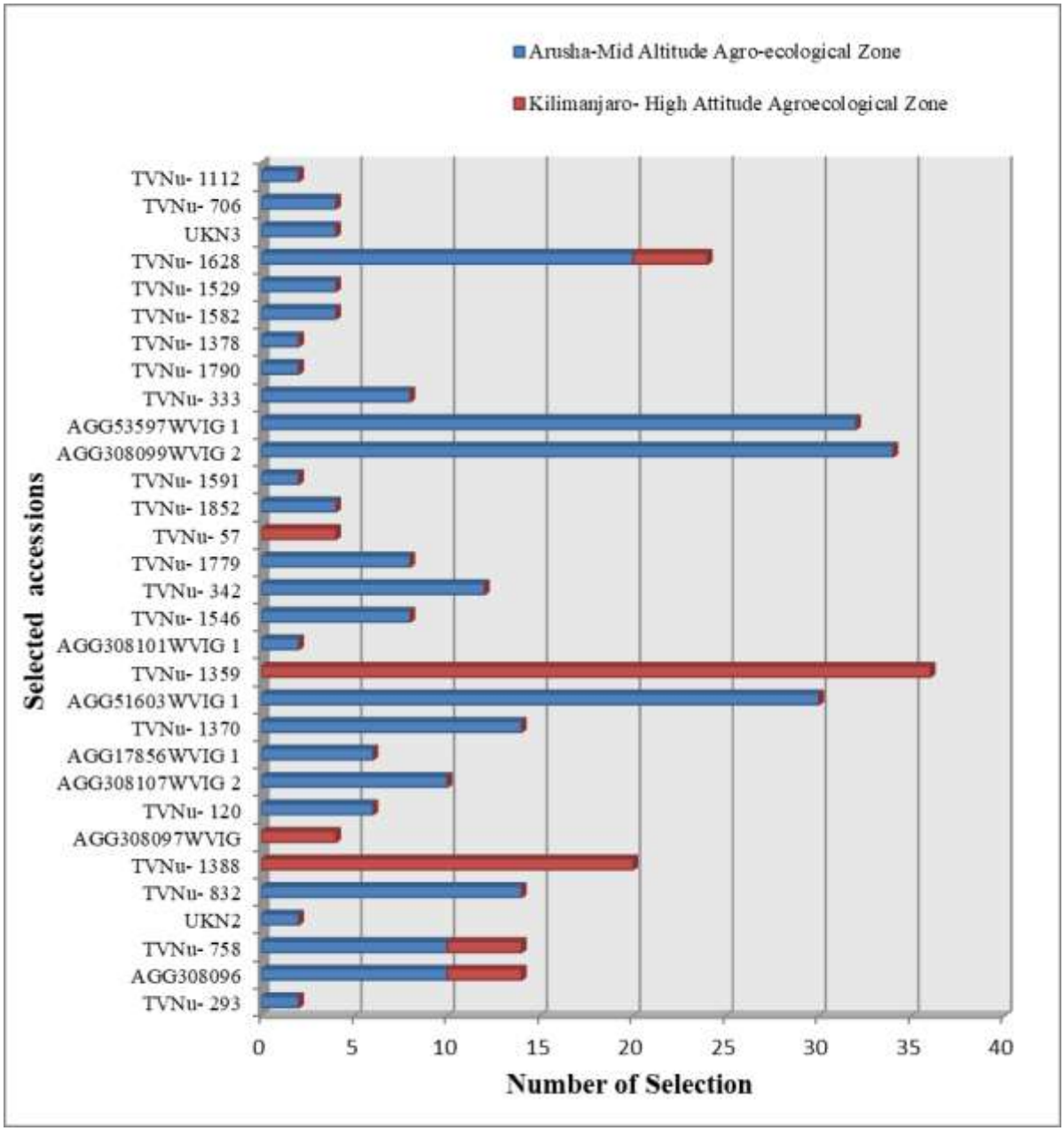
The suggested uses of selected accessions were based on their personal assessment and preferences. Some accessions were selected for more than one use, and the number of selections for every accession is shown on Fig. 15a–e. Other uses were proposed by farmers that better suited the accession of their choice. Four main uses (human food, animal feed, forage, and cover crop) were proposed as a result of farmer's preferences and perceptions. Therefore, a total of 31 accessions were preferred as human food (Fig. 15a), 49 were preferred as animal feed (Fig. 16b), 27 were preferred as forage (Fig. 15c), 28 were preferred as cover crop (Fig. 15d) and 44 were given specific personal uses (Fig. 15e) respectively.

Four accessions were selected at least 30 times or more as human food, while 27 accessions were selected less than 30 times for the same purpose (Fig. 15a). The four most selected accessions for this purpose were TVNu-1359 (36), AGG308099WVIG 2 (34), AGG53597WVIG 1 (32) and AGG51603WVIG 1 (30) respectively.

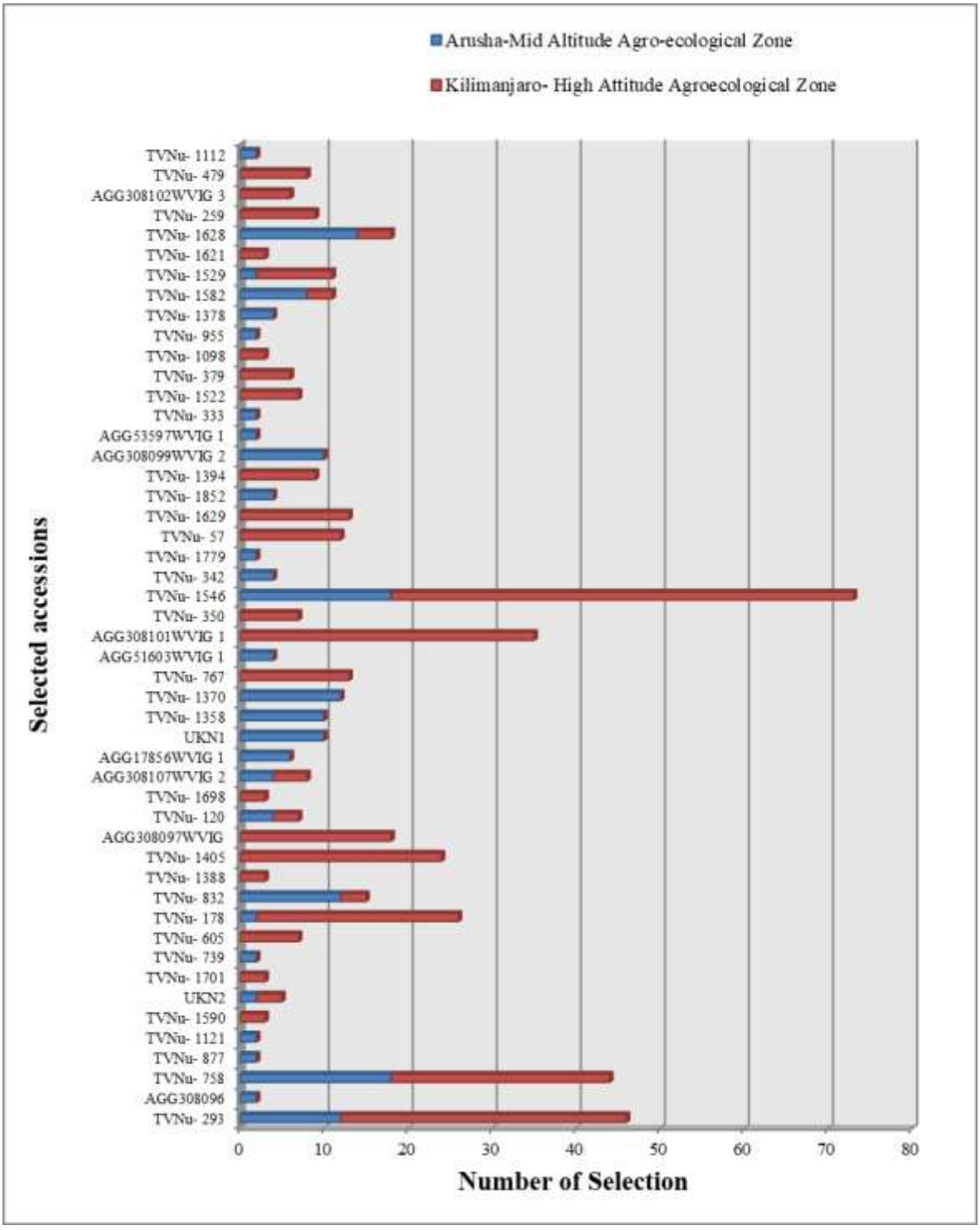
Four other accessions were also selected at least 30 times or more by participants as animal feed in the two study sites combined. The selected accessions were TVNu-1546 (18 + 55), TVNu-293 (12 + 34), TVNu-758 (18 + 26) and AGG308101WVIG 1 (35) respectively (Fig. 15b).

Only one accession was selected up to 30 times to serve as forage (Fig. 15c), while none of the preferred as cover crop accessions were chosen up to 30 times by the participants in both study sites (Fig. 16d).

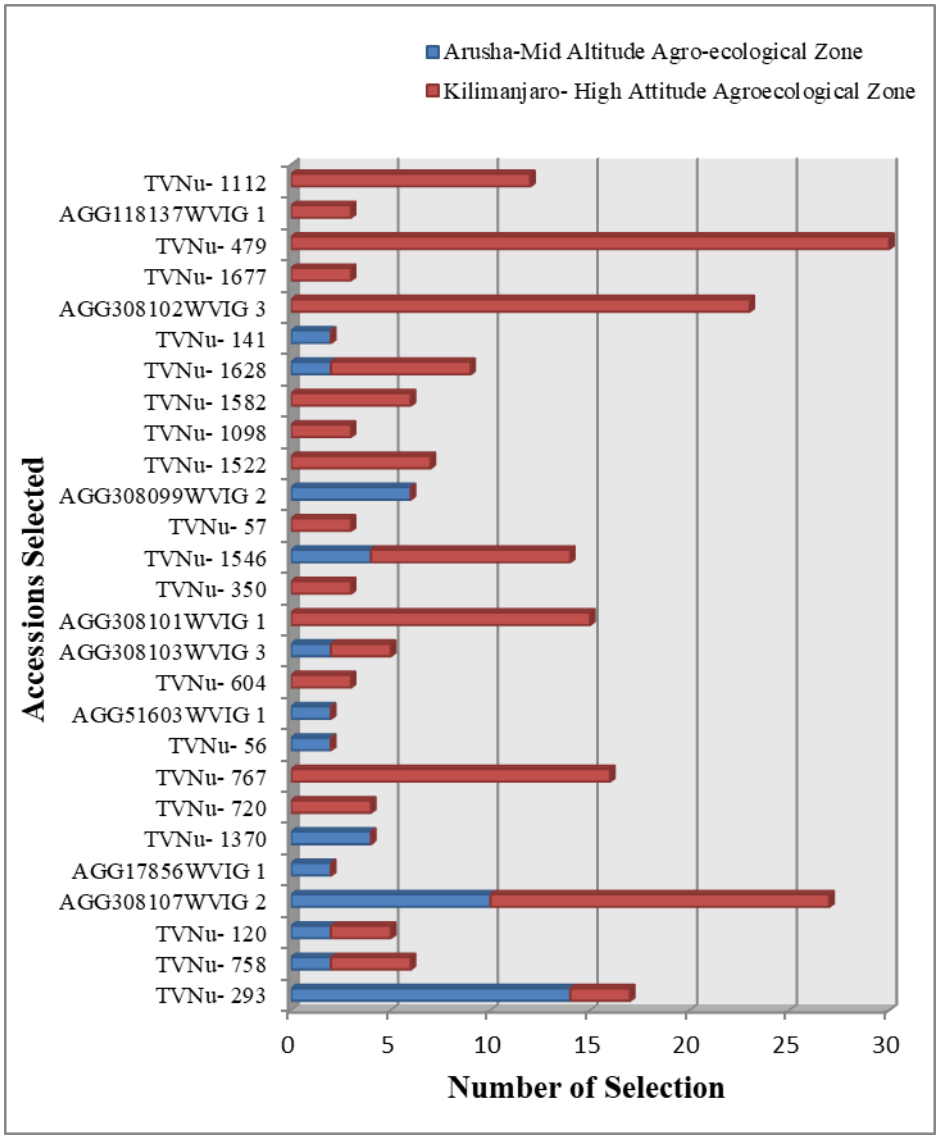
Out of the 44 selected accessions with specified uses, only two accessions—AGG308107WVIG 2 (35) and AGG308100WVIG 3 (36)—were selected more than 30 times (Fig. 15e).



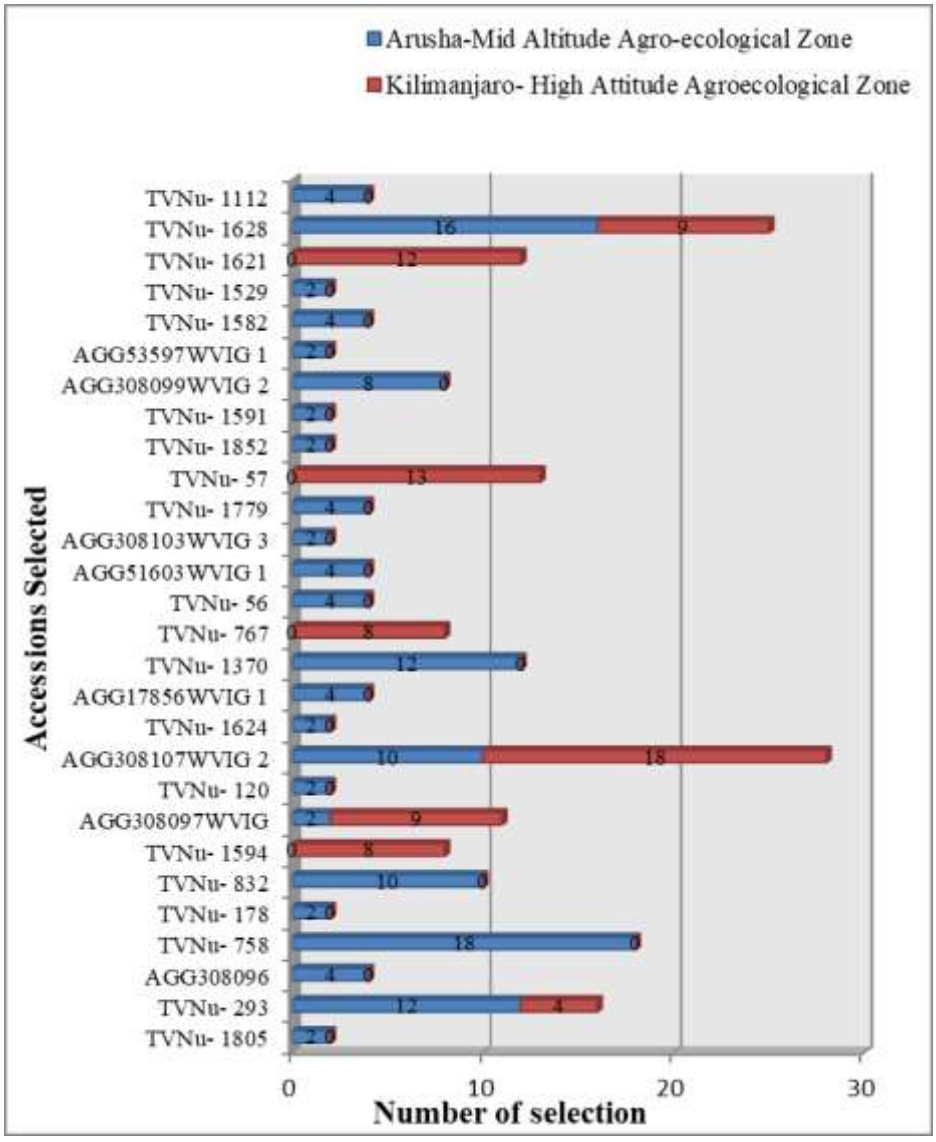
(**a**) Wild *Vigna* legumes suggested as a human food



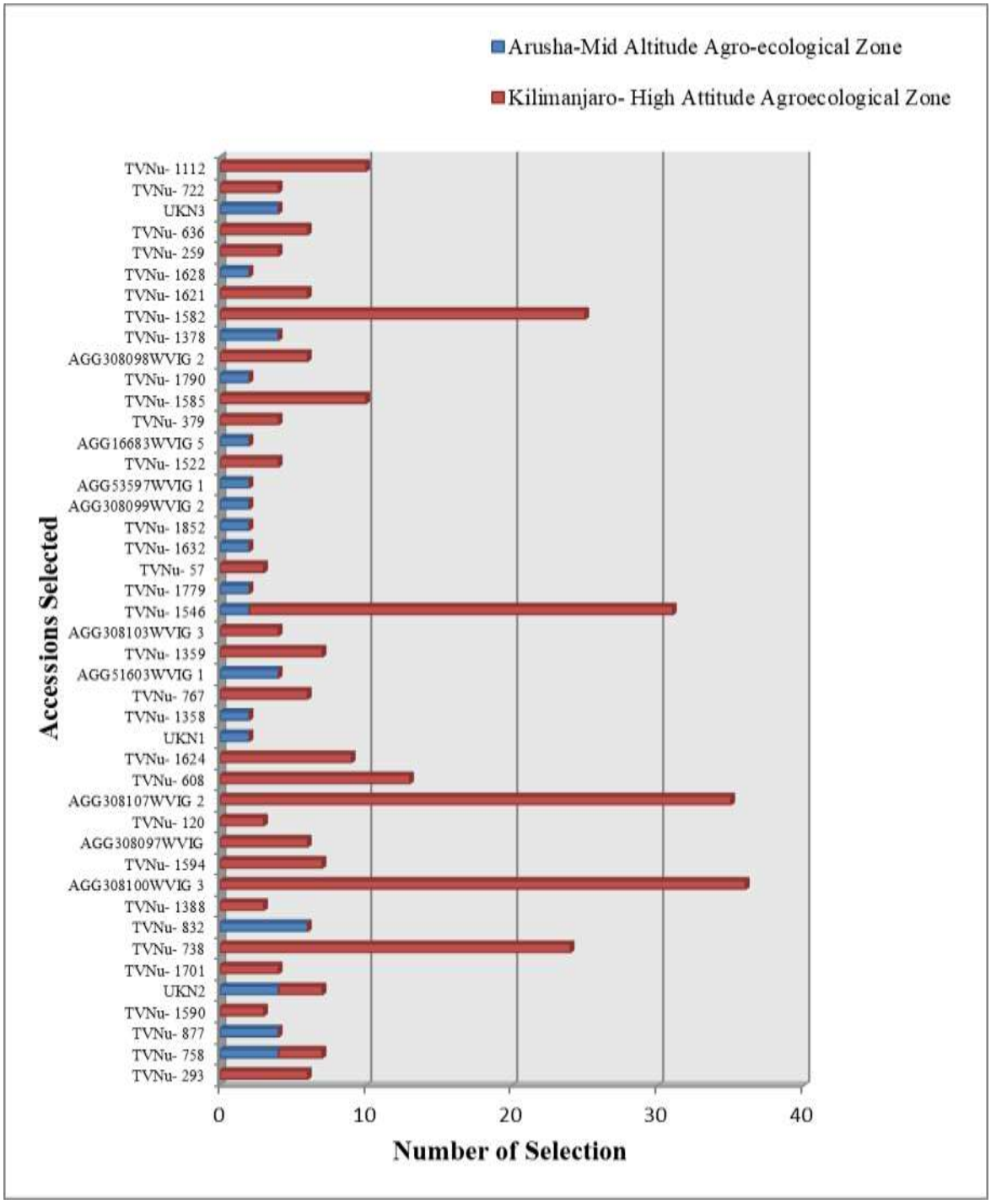
(**b**) Wild *Vigna* legumes suggested as animal feed



(**c**) Wild *Vigna* legumes suggested as forage



(**d**) Wild *Vigna* legumes suggested as cover crop



(e) Wild *Vigna* legumes given specified uses

Figure 15: (a) Wild *Vigna* Legumes Suggested as a Human Food; (b) Wild *Vigna* Legumes Suggested as Animal Feed; (c) Wild *Vigna* Legumes Suggested as Forage; (d) Wild *Vigna* Legumes Suggested as Cover Crop; and (e) Wild *Vigna* Legumes Given Specified Uses

All of the non-domesticated wild *Vigna* legumes subjected to this study belonged to four species, *V. racemosa*, *V. reticulata*, *V. vexillata* and *V. ambacensis*. In summary, it has been shown that the *V. vexillata* accessions were more preferred, followed by *V. reticulata* and *V.*

racemosa (Fig. 16). Despite the higher number of *V. ambacensis* accessions as compared with *V. racemosa*, it was less selected than *V. racemosa*.

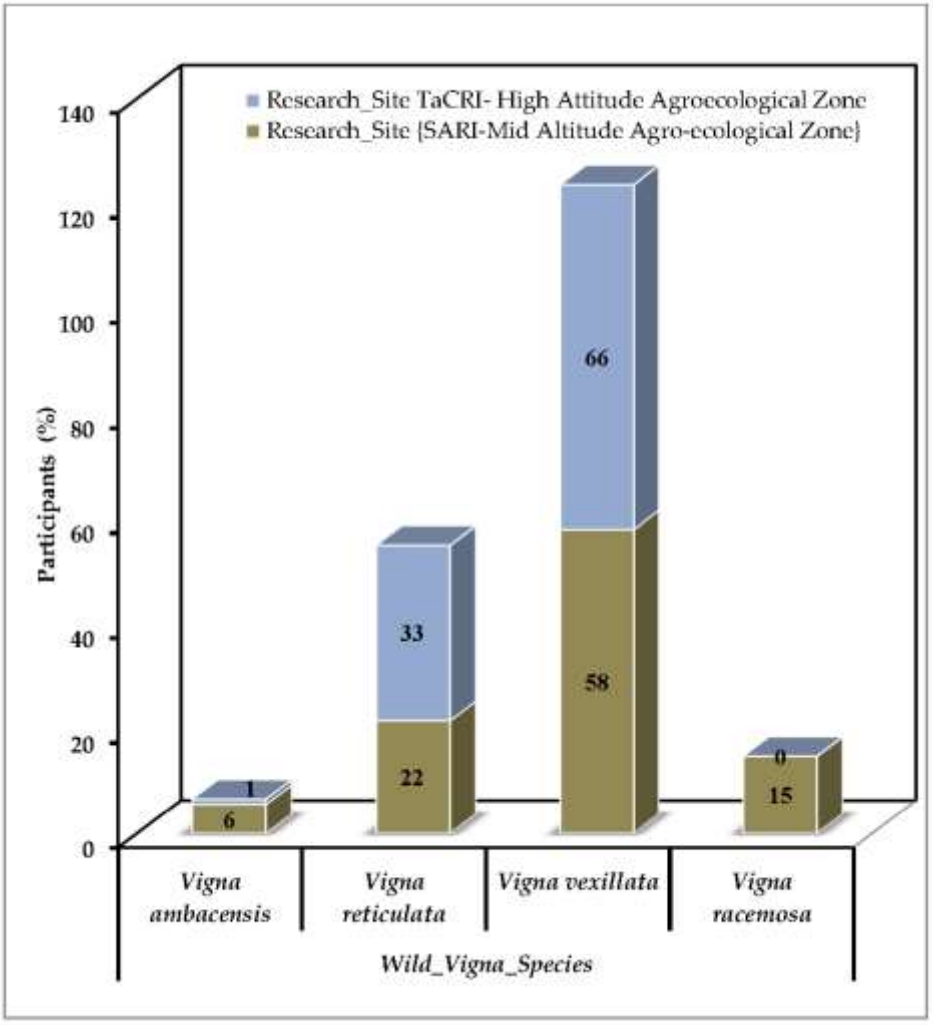


Figure 16: Wild *Vigna* Legumes Selected According to their Species

From their sight and appraisal of the wild *Vigna* legumes, other uses could be organic manure (locally known as '*Mbolea*'—fertilizer), business use, medicinal uses, prevention of soil erosion and vegetable food for accessions with nice leaves (Fig. 17). For personal uses, none of the accessions was selected up to 30 times or more. However, five accessions were selected more than 20 times at least for a specific use. The selected accessions were AGG308100WVIG 3 (24) and TVNu-738 (24) for soil erosion mitigation and TVNu-1582 (22), TVNu-1546 (26) and AGG308107WVIG 2 (28) for soil fertility as an organic manure agent respectively (Fig. 17).

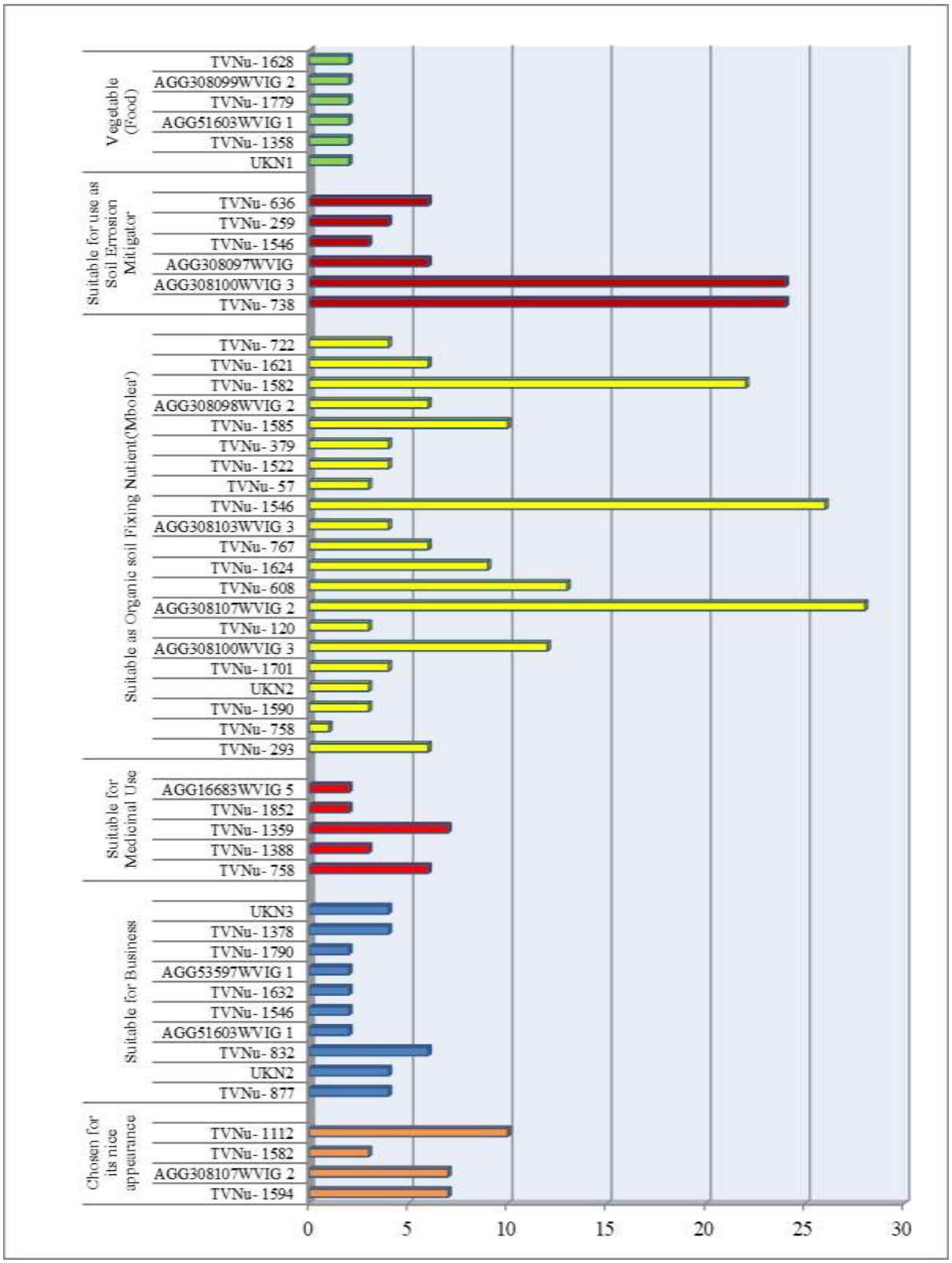


Figure 17: Specified uses of Wild *Vigna* Legumes as Proposed by Farmers in the Two Agro-ecological Zones of Tanzania

(vii) Farmers' Perception of Wild *Vigna* Legumes

From the focus group discussion, most farmers perceived some accessions of wild *Vigna* legumes as good material for future promising business in the field of agriculture due to their high seed production, resistance to drought conditions, and high production of leaves, which can benefit both humans and animals as forage. For example, a male farmer from the Arusha region during the group discussion enthusiastically responded when the interviewer asked whether they would be willing to adopt some of the wild *Vigna* legumes presented to them for the first time. He said: "Yes, I like some of these beans because many people don't know about them, they are found in the bush but people don't know that they can be useful, so if we discover their usefulness, this can be a great source of good business because they seem to have a higher productivity as compared with other known beans." To support the view, another voice rose in the hall and said: "Yes, I also like some, because after seeing these crops planted in the farm (referring to the wild legumes of study), I discovered that there are other new varieties of legumes, and this may be another source of food. I also realized that some of them have nice leaves that can be used as vegetables, and some can help us feed our cattle."

A smaller proportion of farmers (represented by 26 and 28% in study I, as shown in Fig. 12), who curiously noticed the existence of wild legumes before the study, confirmed having seen some of the planted legumes of the study and having consumed them or used them as medicine for animals and even humans. One of the most interesting views that supported this point was from one of the old female farmers in the Arusha region, who said: "This variety with [a] large number of leaves lying on the ground (referring to one of the varieties of the study with a spreading growth habit), I have consumed them several times when I was a kid. Back then, our mothers used to go to the bush and harvest their leaves, and then go to town and buy maize and come back to cook them together. Myself, I have eaten them and we used to call that meal (*Ngolowo*), which is very delicious and when we mix it with milk, it looks similar to another meal called (*Rojo*). So for that one, it is not a poison, because I have eaten it before, it is a food, the leaves are eaten and the seeds are also eaten; it is called (*Ngolowo*)". All her mates in the hall during the group discussion listened to her speech with very attuned ears and clapped at the end. A similar view came from the group of men, which was articulated in these terms: "I have seen these beans before growing in the bush and we were using them as food and feed for animals; then, when I saw it here, I just confirmed that it is

edible. Animals enjoy them so much. We used to take them from the bush and consume them and we had no health problems with them, and after I saw it here in the farm, I just realized that it is a normal food. It has never affected our people negatively after consuming them.

However, most of the participants in general proposed that more research and improvements were needed, especially in terms of the toxicity and nutritional benefits, as well as the seed color of the legumes to increase their acceptability for efficient exploitation and utilization. "One of the varieties I saw in the farm numbered 132 looks nice; it looks similar to (Choroko, Swahili word for Mung bean). So, I think that if it can be improved, it will be good for business because it has high productivity and nice leaves, but we don't know if it is not toxic or can negatively affect our health'', said a participant who was supported by another one, who said: "similar to this one (participant showing some seeds harvested from the experimental fields), if the color can be improved, it will be very nice, because people in the market don't like buying black-colored beans. Their reason is that the black-colored seeds turn the cooking water black and that is not preferable for them. The black-colored seed beans are only preferred during hunger seasons; that is, seasons where less rainfall has affected the crop yield in the community."

4.1.3 Assessment of Water Absorption Capacity and Cooking Time of Wild Under-exploited *Vigna* Species towards their Domestication

(i) Cooking Time and Water Absorption Capacity of Domesticated Legumes

The values for both water absorption and cooking time showed no significant difference between agro-ecological zones and between the three species and therefore no environment × species interaction (Table 12a). A detailed presentation of the interactions between species (*V. vexillata* landrace, *V. unguiculata*, and *V. umbellate*) as replicated within locations is shown in Tables 12b and 12c. It shows that there is no replicate interaction effect between species within locations for the water absorption capacity trait in all the tested combinations. However, replicate interaction effects were significant ($p < 0.05$) when tested within locations between species for the cooking time trait except when tested across locations (Table 12c).

Table 12: Results of the Cooking Time and Water Absorption Capacity for the Domesticated Legume Seeds

(a)

	Water Absorption Capacity		Cooking Time (min)	
Checks	**Site A**	**Site B**	**Site A**	**Site B**
Landrace of *Vigna vexillata*	1.33 ± 0.11 [a]	1.32 ± 0.13 [a]	10.24 ± 0.15 [a]	10.26 ± 0.15 [a]
Cowpea (*Vigna unguiculata*)	1.27 ± 0.08 [a]	1.27 ± 0.08 [a]	16.29 ± 0.15 [c]	16.31 ± 0.15 [c]
Rice Bean (*Vigna umbellata*)	1.16 ± 0.06 [a]	1.16 ± 0.06 [a]	13.20 ± 0.12 [b]	13.23 ± 0.12 [b]

Analysis of Variance (ANOVA)

	Water Absorption Capacity					Cooking Time (min)				
Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Model	5	1.263	0.253	1.134	0.343	5	1582.515	316.503	356.710	<0.0001
Error	258	57.475	0.223			258	228.919	0.887		
Corrected Total	263	58.738				263	1811.434			

Type III Sum of Squares Analysis

Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Location (Site) Effect	1	0.001	0.001	0.004	0.950	1	0.044	0.044	0.050	0.823
Species Effect	2	1.262	0.631	2.833	0.061	2	1582.470	791.235	891.749	<0.0001
Location X Species	2	0.000	0.000	0.000	1.000	2	0.000	0.000	0.000	1.000

Results are represented as the mean value of triplicates ± standard error. Different letters in the same column represent statistically different mean values (p = 0.05). Site A: TARI-Selian; Site B: TaCRI. DF: Degree of freedom; F: F-ratio; p: p-value

(b)

Location x Species/Tukey (HSD)/Analysis of the Differences between the Categories with a Confidence Interval of 95% (Water Absorption Capacity)					
Contrast	**Difference**	**Standardized Difference**	**Critical value**	**Pr > Diff**	**Significant**
Location-Site AxSpecies-Check 1 vs. Location-Site BxSpecies-Check 3	0.173	1.675	2.871	0.550	No
Location-Site AxSpecies-Check 1 vs. Location-Site AxSpecies-Check 3	0.172	1.661	2.871	0.559	No
Location-Site AxSpecies-Check 1 vs. Location-Site BxSpecies-Check 2	0.063	0.620	2.871	0.990	No
Location-Site AxSpecies-Check 1 vs. Location-Site AxSpecies-Check 2	0.059	0.584	2.871	0.992	No
Location-Site AxSpecies-Check 1 vs. Location-Site BxSpecies-Check 1	0.006	0.054	2.871	1.000	No
Location-Site BxSpecies-Check 1 vs. Location-Site BxSpecies-Check 3	0.167	1.619	2.871	0.587	No
Location-Site BxSpecies-Check 1 vs. Location-Site AxSpecies-Check 3	0.166	1.605	2.871	0.596	No
Location-Site BxSpecies-Check 1 vs. Location-Site BxSpecies-Check 2	0.057	0.563	2.871	0.993	No
Location-Site BxSpecies-Check 1 vs. Location-Site AxSpecies-Check 2	0.054	0.527	2.871	0.995	No
Location-Site AxSpecies-Check 2 vs. Location-Site BxSpecies-Check 3	0.114	1.160	2.871	0.855	No
Location-Site AxSpecies-Check 2 vs. Location-Site AxSpecies-Check 3	0.112	1.145	2.871	0.862	No
Location-Site AxSpecies-Check 2 vs. Location-Site BxSpecies-Check 2	0.004	0.038	2.871	1.000	No
Location-Site BxSpecies-Check 2 vs. Location-Site BxSpecies-Check 3	0.110	1.122	2.871	0.872	No
Location-Site BxSpecies-Check 2 vs. Location-Site AxSpecies-Check 3	0.108	1.107	2.871	0.878	No
Location-Site AxSpecies-Check 3 vs. Location-Site BxSpecies-Check 3	0.001	0.014	2.871	1.000	No
Tukey's d critical value			4.061		

Check 1: Landrace of *Vigna* vexillata; Check 2: Cowpea (*Vigna unguiculata*); Check 3: Rice Bean (*Vigna umbellata*)

(c)

Location x Species/Tukey (HSD)/Analysis of the Differences between the Categories with a Confidence Interval of 95% (Cooking Time)

Contrast	Difference	Standardized Difference	Critical value	Pr > Diff	Significant
Location-Site BxSpecies-Check 2 vs. Location-Site AxSpecies-Check 1	6.074	29.913	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 2 vs. Location-Site BxSpecies-Check 1	6.048	29.785	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 2 vs. Location-Site AxSpecies-Check 3	3.109	15.908	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 2 vs. Location-Site BxSpecies-Check 3	3.083	15.775	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 2 vs. Location-Site AxSpecies-Check 2	0.026	0.135	2.871	1.000	No
Location-Site AxSpecies-Check 2 vs. Location-Site AxSpecies-Check 1	6.048	29.785	2.871	<0.0001	Yes
Location-Site AxSpecies-Check 2 vs. Location-Site BxSpecies-Check 1	6.022	29.657	2.871	<0.0001	Yes
Location-Site AxSpecies-Check 2 vs. Location-Site AxSpecies-Check 3	3.083	15.775	2.871	<0.0001	Yes
Location-Site AxSpecies-Check 2 vs. Location-Site BxSpecies-Check 3	3.057	15.642	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 3 vs. Location-Site AxSpecies-Check 1	2.991	14.514	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 3 vs. Location-Site BxSpecies-Check 1	2.965	14.388	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 3 vs. Location-Site AxSpecies-Check 3	0.026	0.131	2.871	1.000	No
Location-Site AxSpecies-Check 3 vs. Location-Site AxSpecies-Check 1	2.965	14.388	2.871	<0.0001	Yes
Location-Site AxSpecies-Check 3 vs. Location-Site BxSpecies-Check 1	2.939	14.262	2.871	<0.0001	Yes
Location-Site BxSpecies-Check 1 vs. Location-Site AxSpecies-Check 1	0.026	0.122	2.871	1.000	No
Tukey's d critical value			4.061		

Check 1: Landrace of *Vigna vexillata*; Check 2: Cowpea (*Vigna unguiculata*); Check 3: Rice Bean (*Vigna umbellata*). (**a**) Means, analysis of variance and type III sum of square analysis for the cooking time and water absorption capacity traits of domesticated legume seeds. (**b**) Details of interactions within locations effects for water absorption capacity trait. (**c**) Details of interactions within locations effects for cooking time trait

There is a significant difference between the three domesticated varieties ($p < 0.05$). Pearson correlation analysis shows that there is no correlation between the water absorption capacity and cooking time considering only the three seed varieties ($r = -0.030$ for site A, 0.029 for site B) (Fig. 18). Cowpea has a higher cooking time than rice bean which also cook longer than the landrace of *V. vexillata*.

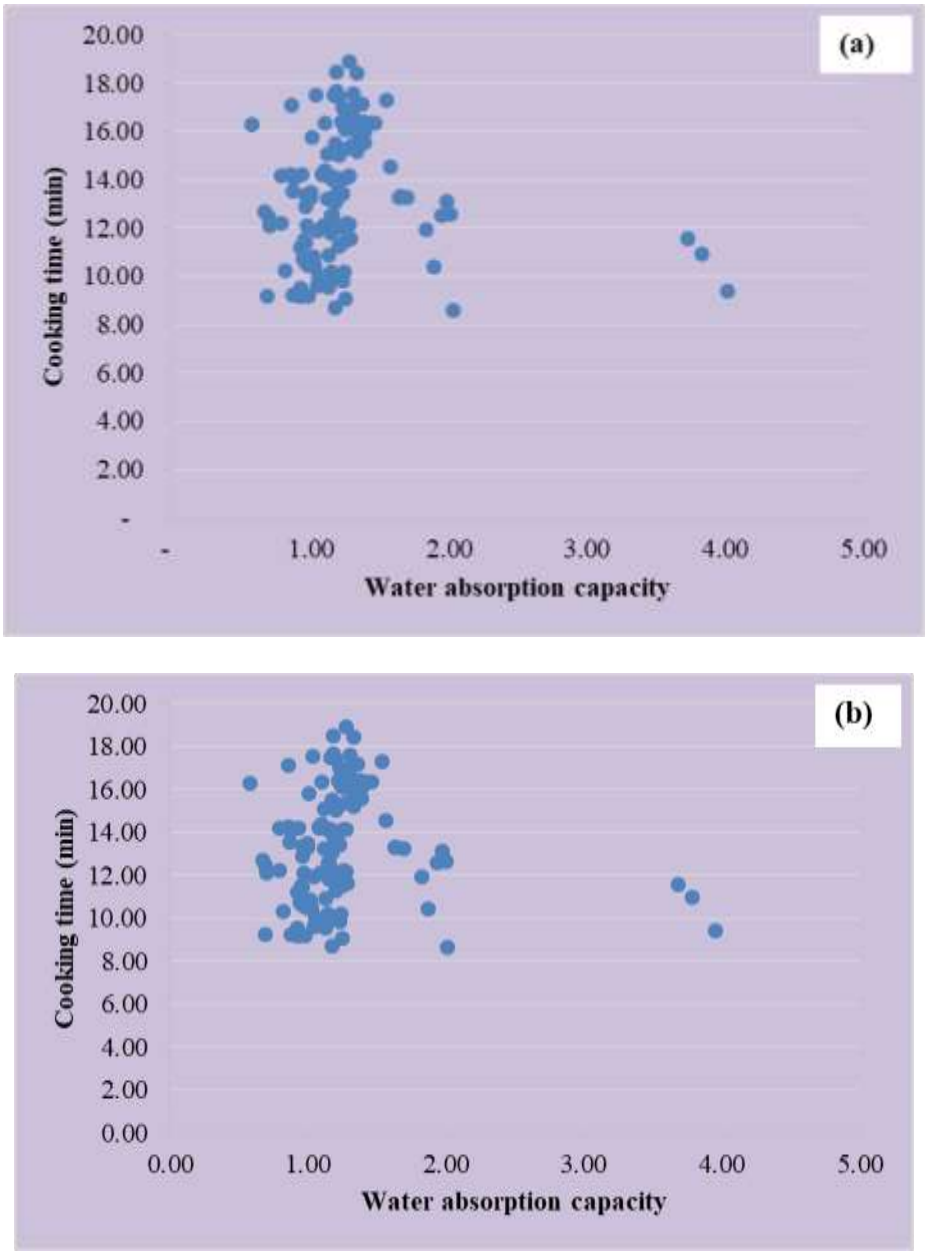


Figure 18: Correlation between Water Absorption and Cooking Time for the Three Checks. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

(ii) Cooking Time and Water Absorption Capacity of *Vigna* ambacensis Accessions

The water absorption capacities and the cooking times for 11 accessions of wild *Vigna ambacensis* are presented in Table 13. There was no significant difference ($p > 0.05$) between the absorption capacity and cooking times for the 11 accessions of the wild *Vigna* when compared to their corresponding accession harvested in the other agro-ecological zone (Table 13).

Considering the water absorption capacity, all the wild accessions exhibited significantly low values as compared with all three checks. The water absorption capacity of wild accessions varied from 0.08 ± 0.01 to 0.47 ± 0.01 (Table 13) in both site A and B. Accession TVNu342 showed no significant difference in water absorption capacity with three checks and with accession TVNu219.

The cooking time of the wild accessions varied from 23.02 ± 0.50 to 24.26 ± 0.07 min in both sites (Table 13). All the wild accessions possessed significantly higher cooking time values compared with the three checks. None of the accessions cooked faster than the checks. Additionally, there was no correlation between the water absorption capacity and cooking time considering only the 11 accessions studied ($r = -0.025$ for site A and $r = -0.024$ for site B) (Fig. 19).

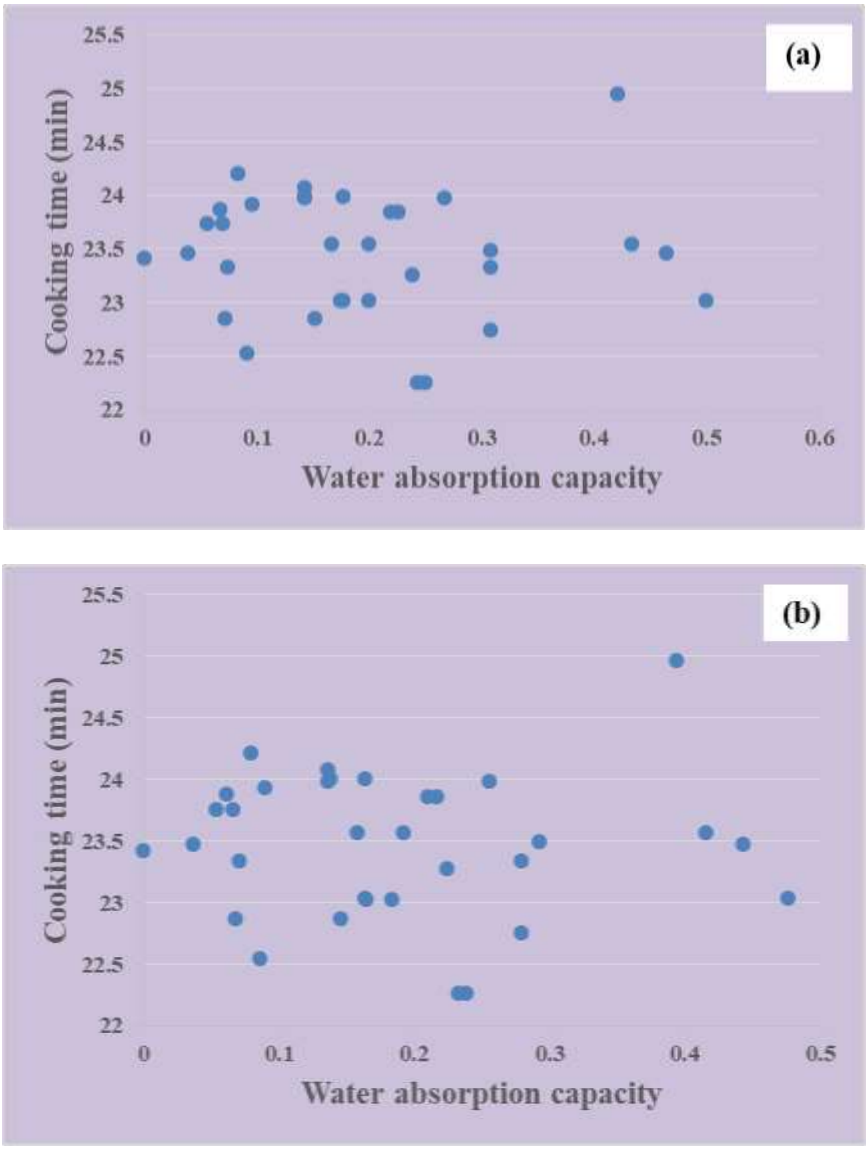


Figure 19: Correlation between Water Absorption and Cooking Time for the *Vigna ambacensis* Accessions. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

Table 13: Cooking Time and Water Absorption of *Vigna ambacensis* Accessions

Species/Accession Number	Water Absorption Capacity		Cooking Time (min)	
	Site A	Site B	Site A	Site B
Landrace of *Vigna vexillata*	1.33 ± 0.11 [a]	1.32 ± 0.13 [a]	10.24 ± 0.15 [a]	10.26 ± 0.15 [a]
Cowpea (*Vigna unguiculata*)	1.27 ± 0.08 [a]	1.27 ± 0.08 [a]	16.29 ± 0.15 [b]	16.31 ± 0.15 [b]
Rice bean (*Vigna umbellata*)	1.16 ± 0.06 [a]	1.16 ± 0.06 [a]	13.20 ± 0.12 [c]	13.23 ± 0.12 [c]
TVNu1699	0.14 ± 0.01 [c]	0.13 ± 0.01 [c]	24.26 ± 0.07 [d]	23.87 ± 0.10 [d]
TVNu342	0.47 ± 0.01 [a,b]	0.45 ± 0.01 [a,b]	23.34 ± 0.16 [d]	23.35 ± 0.18 [d]
TVNu877	0.22 ± 0.01 [c]	0.21 ± 0.01 [c]	24.10 ± 0.19 [d]	23.71 ± 0.22 [d]
TVNu223	0.21 ± 0.01 [c]	0.21 ± 0.02 [c]	23.35 ± 0.55 [d]	23.36 ± 0.50 [d]
TVNu720	0.22 ± 0.01 [c]	0.20 ± 0.01 [c]	23.02 ± 0.50 [d]	23.03 ± 0.45 [d]
TVNu219	0.28 ± 0.02 [b,c]	0.26 ± 0.01 [b,c]	24.06 ± 0.49 [d]	24.08 ± 0.50 [d]
TVNu1840	0.11 ± 0.01 [c]	0.10 ± 0.01 [c]	23.36 ± 0.21 [d]	23.37 ± 0.30 [d]
TVNu1804	0.09 ± 0.01 [c]	0.08 ± 0.01 [c]	23.55 ± 0.52 [d]	23.56 ± 0.50 [d]
TVNu1792	0.23 ± 0.01 [c]	0.09 ± 0.01 [c]	23.28 ± 0.22 [d]	23.30 ± 0.30 [d]
TVNu1644	0.09 ± 0.01 [c]	0.21 ± 0.01 [c]	23.12 ± 0.10 [d]	23.13 ± 0.15 [d]
TVNu1185	0.12 ± 0.01 [c]	0.11 ± 0.01 [c]	23.34 ± 0.33 [d]	23.35 ± 0.30 [d]

Analysis of Variance (ANOVA)

	Water Absorption Capacity					Cooking Time (min)				
Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Model	27	60.707	2.248	11.756	<0.0001	27	6864.480	254.240	313.317	<0.0001
Error	302	57.761	0.191			302	245.057	0.811		
Corrected Total	329	118.469				329	7109.537			

Type III Sum of Squares Analysis

Source	**DF**	**Sum of squares**	**Mean squares**	**F**	***p***	**DF**	**Sum of Squares**	**Mean Squares**	**F**	***p***
Location (Site)	1	0.002	0.002	0.011	0.916	1	0.007	0.007	0.008	0.929
Genotype (Accessions)	13	60.704	4.670	24.414	<0.0001	13	6864.433	528.033	650.730	<0.0001
Location × Genotype	13	0.001	0.000	0.001	1.000	13	0.002	0.000	0.000	1.000

Results are represented as the mean value of triplicates ± standard error. Mean values without any letter in common within each column are significantly different ($p = 0.05$). Site A: TARI-Selian; Site B: TaCRI. DF: Degree of freedom; F: F-ratio; p: p-value

(iii) Cooking Time and Water Absorption Capacity of *Vigna vexillata* Accessions

The result for water absorption capacity and cooking time for 35 accessions of wild *Vigna vexillata* is shown in Table 14. The values for water absorption capacity and cooking time show no significant difference ($p > 0.05$) when compared with the values of their corresponding accessions harvested in the other agro-ecological zone.

The Water Absorption Capacity in all the wild accessions with exception of TVNu781 and TVNu837 showed significant low values compared with the three checks. The water absorption capacity of the wild *V. vexillata* accessions varied from 0.04 ± 0.00 to 1.10 ± 0.03 in both site A and B.

Considering the cooking time, there is a high diversity in differences among the accessions. The cooking time varied from 16.22 ± 0.23 to 31.04 ± 0.33 min in site A and from 16.24 ± 0.20 to 31.06 ± 0.31 min in site B (Table 13). Accessions TVNu781, AGG308107WVIG2, AGG308097WVIG1, and TVNU1624 exhibited relatively similar cooking time with check 2 (Cowpea) (Table 14). Conversely, cooking time for all other remaining accessions was significantly higher than all the checks. Pearson correlation analysis shows that there is a weak negative correlation between the water absorption capacity and cooking time considering the wild *V. vexillata* tested ($r = -0.31$ for site A and $r = -0.32$). Furthermore, the regression analysis shows that the water absorption capacity and cooking time are related by the equation: $Y = -5.12x + 27.15$ with $R^2 = 0.094$ (Fig. 20).

Table 14: Cooking Time and Water Absorption Capacity of *Vigna vexillata* Accessions

Species/Accession Number	Water Absorption Capacity		Cooking Time (min)	
	Site A	Site B	Site A	Site B
Landrace of *Vigna vexillata*	1.33 ± 0.11 [a]	1.32 ± 0.13 [a]	10.24 ± 0.15 [n]	10.26 ± 0.15 [n]
Cowpea (*Vigna unguiculata*)	1.27 ± 0.08 [a,b,c]	1.27 ± 0.08 [a,b,c]	16.29 ± 0.15 [l]	16.31 ± 0.15 [l]
Rice Bean (*Vigna umbellata*)	1.16 ± 0.06 [a,b,c]	1.16 ± 0.06 [a,b,c]	13.20 ± 0.12 [m]	13.23 ± 0.12 [m]
TVNu781	1.10 ± 0.02 [abcd]	1.10 ± 0.01 [abcd]	31.04 ± 0.33 [a,b]	31.06 ± 0.31 [a,b]
TVNu837	1.07 ± 0.01 [abcd]	1.05 ± 0.01 [abcd]	29.34 ± 0.32 [a,b,c,d,e,f,g]	29.35 ± 0.01 [a,b,c,d,e,f,g]
TVNu1582	0.73 ± 0.01 [abcd]	0.67 ± 0.02 [abcd]	16.25 ± 0.24 [l]	16.26 ± 0.30 [l]
TVNu1358	0.57 ± 0.01 [abcd]	0.53 ± 0.01 [abcd]	17.37 ± 0.26 [l]	17.38 ± 0.28 [l]
AGG308107WVIG2	0.43 ± 0.01 [abcd]	0.41 ± 0.01 [abcd]	26.32 ± 0.49 [f,g,h,i,j]	26.33 ± 0.51 [f,g,h,i,j]
TVNu1593	0.42 ± 0.01 [abcd]	0.38 ± 0.01 [bcd]	16.22 ± 0.23 [l]	16.24 ± 0.20 [l]
TVNu1591	0.41 ± 0.01 [abcd]	0.38 ± 0.01 [bcd]	26.38 ± 0.40 [f,g,h,i,j]	26.39 ± 0.43 [f,g,h,i,j]
TVNu120	0.40 ± 0.01 [abcd]	0.38 ± 0.01 [bcd]	31.10 ± 0.31 [a]	30.71 ± 0.34 [a]
TVNu333	0.40 ± 0.02 [abcd]	0.37 ± 0.02 [bcd]	26.28 ± 0.40 [f,g,h,i,j]	26.30 ± 0.35 [f,g,h,i,j]
TVNu1546	0.39 ± 0.02 [bcd]	0.37 ± 0.01 [bcd]	29.07 ± 0.13 [a,b,c,d,e,f]	29.08 ± 0.15 [a,b,c,d,e,f]
AGG308101WVIG1	0.37 ± 0.01 [bcd]	0.34 ± 0.01 [bcd]	29.36 ± 0.50 [a,b,c,d,e]	29.37 ± 0.47 [a,b,c,d,e]
TVNu1701	0.35 ± 0.01 [bcd]	0.33 ± 0.01 [cd]	24.59 ± 0.50 [j]	24.60 ± 0.57 [j]
AGG308096 WVIG2	0.34 ± 0.01 [cd]	0.32 ± 0.01 [cd]	26.47 ± 0.59 [f,g,h,i,j]	26.49 ± 0.60 [f,g,h,i,j]
TVNu1629	0.33 ± 0.01 [cd]	0.32 ± 0.02 [cd]	28.19 ± 1.15 [b,c,d,e,f,g,h,i]	28.20 ± 1.20 [b,c,d,e,f,g,h,i]
TVNu293	0.33 ± 0.01 [cd]	0.31 ± 0.01 [cd]	26.32 ± 0.28 [f,g,h,i,j]	26.33 ± 0.30 [f,g,h,i,j]
TVNu832	0.32 ± 0.01 [cd]	0.30 ± 0.01 [cd]	25.46 ± 0.36 [h,i,j]	25.47 ± 0.36 [h,i,j]
TVNu1796	0.32 ± 0.01 [cd]	0.30 ± 0.01 [cd]	27.38 ± 0.48 [c,d,e,f,g,h,i,j]	27.39 ± 0.50 [c,d,e,f,g,h,i,j]
TVNu1529	0.32 ± 0.01 [cd]	0.30 ± 0.01 [cd]	27.29 ± 0.64 [c,d,e,f,g,h,i,j]	27.30 ± 0.64 [c,d,e,f,g,h,i,j]
TVNu1628	0.30 ± 0.01 [cd]	0.28 ± 0.01 [cd]	26.30 ± 0.36 [f,g,h,i,j]	26.31 ± 0.33 [f,g,h,i,j]
TVNu1344	0.29 ± 0.01 [cd]	0.28 ± 0.01 [cd]	30.03 ± 0.44 [a,b,c]	30.04 ± 0.44 [a,b,c]
TVNu1632	0.29 ± 0.01 [cd]	0.28 ± 0.01 [cd]	29.41 ± 0.52 [a,b,c,d]	28.25 ± 0.50 [a,b,c,d]
TVNu1370	0.28 [cd]	0.26 ± 0.02 [cd]	28.23 ± 0.39 [l]	29.43 ± 0.40 [l]
TVNu1360	0.28 [cd]	0.25 ± 0.01 [cd]	27.05 ± 0.71 [d,e,f,g,h,i,j]	27.60 ± 0.72 [d,e,f,g,h,i,j]
TVNu1624	0.25 [cd]	0.23 ± 0.01 [d]	26.28 ± 0.46 [f,g,h,i,j]	26.29 ± 0.46 [f,g,h,i,j]
TVNu1621	0.25 ± 0.01 [cd]	0.23 ± 0.01 [d]	17.24 ± 0.47 [l]	17.26 ± 0.48 [l]
AGG62154WVIG_1	0.20 ± 0.01 [d]	0.19 ± 0.01 [d]	21.33 ± 0.17 [k]	21.34 ± 0.17 [k]
TVNu1092	0.19 ± 0.01 [d]	0.18 ± 0.01 [d]	29.02 ± 0.23 [a,b,c,d,e,f,g]	29.04 ± 0.55 [a,b,c,d,e,f,g]
TVNu479	0.18 ± 0.01 [d]	0.17 ± 0.01 [d]	26.50 ± 0.56 [e,f,g,h,i,j]	26.52 ± 0.20 [e,f,g,h,i,j]
AGG308097WVIG 1	0.17 ± 0.01 [d]	0.16 ± 0.01 [d]	28.56 ± 0.50 [a,b,c,d,e,f,g]	28.58 ± 0.50 [a,b,c,d,e,f,g]
TVNu178	0.17 ± 0.01 [d]	0.16 ± 0.01 [d]	17.03 ± 0.54 [l]	16.64 ± 0.01 [l]
TVNu955	0.11 ± 0.01 [d]	0.11 ± 0.01 [d]	25.28 ± 0.47 [i,j]	25.29 ± 0.47 [i,j]
TVNu1378	0.11 ± 0.01 [d]	0.10 ± 0.00 [d]	16.29 ± 0.45 [l]	16.31 ± 0.47 [l]

Species/Accession Number	Water Absorption Capacity		Cooking Time (min)	
	Site A	Site B	Site A	Site B
TVNu1586	0.06 ± 0.00 [d]	0.05 ± 0.01 [d]	28.39 ± 0.29 [a,b,c,d,e,f,g]	28.41 ± 0.30 [a,b,c,d,e,f,g]
TVNu381	0.04 ± 0.00 [d]	0.042 ± 0.00 [d]	25.41 ± 0.63 [h,i,j]	25.42 ± 0.64 [h,i,j]
AGG308099WVIG2	0.042 ± 0.01 [d]	0.04 ± 0.01 [d]	26.16 ± 0.48 [h,i,j]	26.17 ± 0.50 [h,i,j]

Analysis of Variance (ANOVA)

	Water Absorption Capacity					Cooking Time (min)				
Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Model	75	111.003	1.480	9.649	<0.0001	75	22,437.582	299.168	368.513	<0.0001
Error	398	61.050	0.153			398	323.106	0.812		
Corrected Total	473	172.052				473	22,760.688			

Type III Sum of Squares Analysis

Source	**DF**	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Location (Site)	1	0.018	0.018	0.117	0.732	1	0.012	0.012	0.015	0.903
Genotype (Accessions)	37	110.978	2.999	19.554	<0.0001	37	22,437.529	606.420	746.983	<0.0001
Location × Genotype	37	0.013	0.000	0.002	1.000	37	0.005	0.000	0.000	1.000

Results are represented as the mean value of triplicates ± standard error. Mean values without any letter in common within each column are significantly different (p = 0.05). Site A: TARI-Selian; Site B: TaCRI. DF: Degree of freedom; F: F-ratio; p: p-value

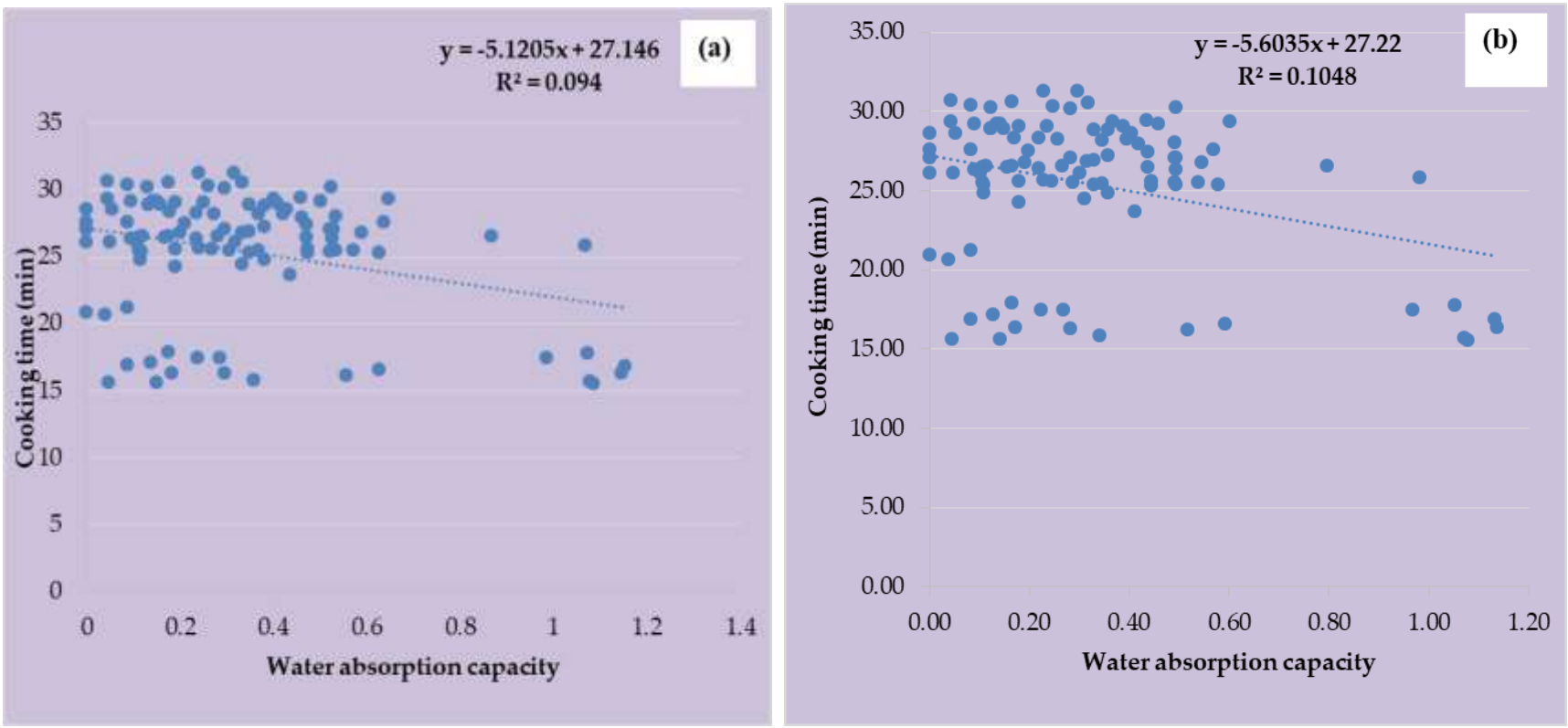


Figure 20: Correlation between Water Absorption and Cooking Time for the *Vigna vexillata* Accessions. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

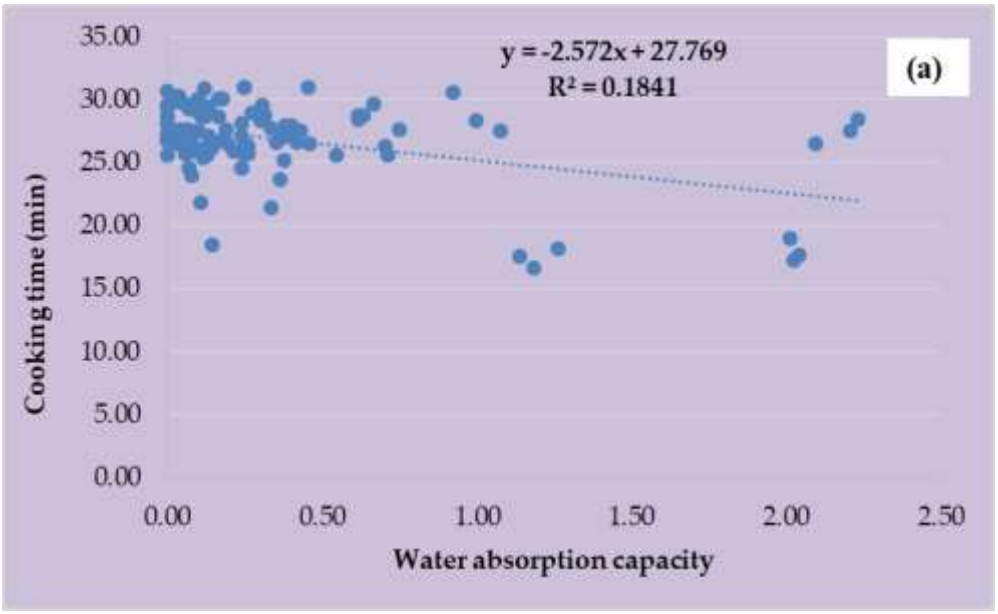
(iv) Cooking Time and Water Absorption capacity of *Vigna reticulata* Accessions

Table 15 shows the various values for water absorption capacity and cooking time for 32 accessions of wild *Vigna reticulata*. The values for water absorption capacity and cooking time showed no significant difference ($p > 0.05$) when compared with the values of their corresponding accessions harvested in the other agro-ecological zone.

All the wild accessions showed significantly low water absorption capacity values compared with the checks except for TVNu1520, and TVNu325 (Table 15). The water absorption capacity of the wild *V. reticulata* accessions varied from 0.06 ± 0.01 to 1.27 ± 0.08 in site A and from 0.06 ± 0.01 to 1.32 ± 0.13 in site B. No significant location and genotype × location interactions ($p > 0.05$) were observed for both water absorption capacity and cooking time traits in these accessions. However, only significant genotype interaction was observed for both traits ($p < 0.05$).

Regarding cooking time, there is a high diversity in differences of means among the accessions. Twenty-five accessions showed significant higher cooking time values. Check 2 showed no significant difference in cooking time with TVNu325 and the unknown *V. reticulata* accession only. The cooking times for all accessions varied from 17.41 ± 0.44 to 30.25 ± 0.41 min in site A and from 17.42 ± 0.45 to 30.26 ± 0.42 min in site B (Table 15).

Pearson correlation analysis shows that there is a weak negative correlation between the water absorption and cooking time considering the wild *V. reticulata* tested ($r = -0.43$ for site A and $r = -0.45$) (Fig. 22). Furthermore, the regression analysis shows that the water absorption and cooking time are related by the equation: $Y = -2.57x + 27.77$ with $R^2 = 0.18$ (Fig. 21).



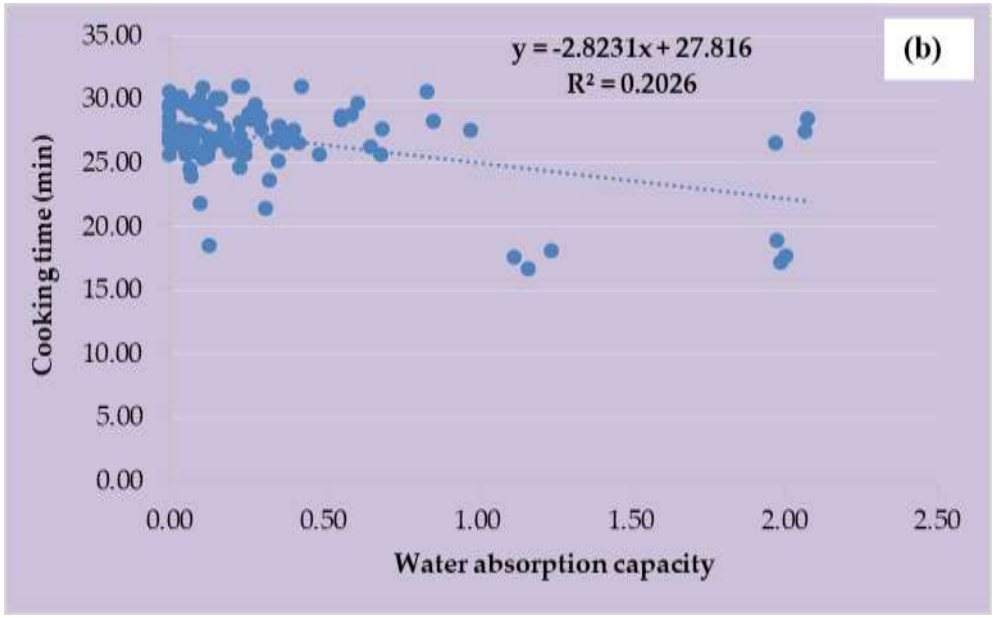


Figure 21: Correlation Between Water Absorption and Cooking Time for the *Vigna reticulata* Accessions. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

Table 15: Cooking Time and Water Absorption Capacity of *Vigna reticulata* Accessions

	Water Absorption Capacity		Cooking Time (min)	
Species/Accession Number	**Site A**	**Site B**	**Site A**	**Site B**
Landrace of *Vigna vexillata*	1.33 ± 0.11 ^{a,b,c}	1.32 ± 0.13 ^{a,b,c}	10.24 ± 0.15 ^{h}	10.26 ± 0.15 ^{h}
Cowpea *(Vigna unguiculata)*	1.27 ± 0.08 ^{a,b,c,d}	1.27 ± 0.08 ^{a,b,c,d}	16.29 ± 0.15 ^{f}	16.31 ± 0.15 ^{f}
Rice Bean *(Vigna umbellata)*	1.16 ± 0.06 ^{a,b,c,d}	1.16 ± 0.06 ^{a,b,c,d}	13.20 ± 0.12 ^{g}	13.23 ± 0.12 ^{g}
TVNu324	0.49 ± 0.02 ^{c,d}	0.47 ± 0.02 ^{c,d}	26.51 ± 0.47 ^{a,b,c,d}	26.53 ± 0.48 ^{a,b,c,d}
TVNu325	2.03 ± 0.02 ^{a,b}	1.99 ± 0.01 ^{a,b}	17.92 ± 0.51 ^{f}	17.93 ± 0.52 ^{f}
Unknown _*Vigna reticulata*	1.20 ± 0.02 ^{a,b,c,d}	1.18 ± 0.02 ^{a,b,c,d}	17.41 ± 0.44 ^{f}	17.42 ± 0.45 ^{f}
TVNu343	0.19 ± 0.01 ^{c,d}	0.18 ± 0.01 ^{c,d}	30.25 ± 0.41 ^{a}	30.26 ± 0.42 ^{a}
TVNu767	0.12 ± 0.01 ^{c,d}	0.11 ± 0.01 ^{c,d}	29.18 ± 0.99 ^{a,b,c,d}	29.20 ± 1.00 ^{a,b,c,d}
TVNu1520	2.18 ± 0.03 ^{a}	2.04 ± 0.03 ^{a}	27.46 ± 0.91 ^{a,b,c,d}	27.48 ± 0.92 ^{a,b,c,d}
TVNu349	0.31 ± 0.02 ^{c,d}	0.29 ± 0.01 ^{c,d}	29.14 ± 0.74 ^{a,b,c,d}	28.76 ± 0.75 ^{a,b,c,d}
TVNu379	0.77 ± 0.01 ^{c,d}	0.71 ± 0.02 ^{c,d}	29.38 ± 0.46 ^{a,b,c,d}	28.99 ± 0.44 ^{a,b,c,d}
TVNu524	0.17 ± 0.01 ^{c,d}	0.17 ± 0.01 ^{c,d}	25.57 ± 0.57 ^{c,d,e}	25.58 ± 0.58 ^{c,d,e}
TVNu1698	0.12 ± 0.01 ^{c,d}	0.11 ± 0.01 ^{c,d}	26.34 ± 0.56 ^{b,c,d,e}	26.36 ± 0.57 ^{b,c,d,e}
TVNu1191	0.22 ± 0.01 ^{c,d}	0.21 ± 0.01 ^{c,d}	25.38 ± 1.00 ^{d,e}	25.39 ± 0.99 ^{d,e}
TVNu1394	0.82 ± 0.02 $^{b,\ c,d}$	0.75 ± 0.01 $^{b,\ c,d}$	28.21 ± 0.99 ^{a,b,c,d}	27.82 ± 0.97 ^{a,b,c,d}
TVNu-224	0.19 ± 0.01 ^{c,d}	0.18 ± 0.01 ^{c,d}	25.55 ± 0.51 ^{c,d,e}	25.57 ± 0.52 ^{c,d,e}
TVNu739	0.15 ± 0.01 ^{c,d}	0.14 ± 0.01 ^{c,d}	28.50 ± 0.46 ^{a,b,c,d}	28.52 ± 0.47 ^{a,b,c,d}
TVNu56	0.24 ± 0.02 ^{c,d}	0.22 ± 0.02 ^{c,d}	27.01 ± 2.73 ^{a,b,c,d}	26.62 ± 2.70 ^{a,b,c,d}
TVNu1405	0.29 ± 0.02 ^{c,d}	0.26 ± 0.02 ^{c,d}	30.03 ± 0.64 ^{a,b}	29.64 ± 0.62 ^{a,b}
TVNu607	0.08 ± 0.01 ^{d}	0.08 ± 0.01 ^{d}	27.33 ± 0.49 ^{a,b,c,d}	26.38 ± 0.47 ^{a,b,c,d}
TVNu916	0.12 ± 0.01 ^{c,d}	0.11 ± 0.01 ^{c,d}	26.37 ± 0.52 ^{b,c,d,e}	27.84 ± 0.55 ^{b,c,d,e}
AGG17856WVIG 1	0.16 ± 0.01 ^{c,d}	0.15 ± 0.01 ^{c,d}	28.23 ± 1.00 ^{a,b,c,d}	27.35 ± 0.97 ^{a,b,c,d}
TVNu1790	0.32 ± 0.02 ^{c,d}	0.29 ± 0.02 ^{c,d}	28.43 ± 0.47 ^{a,b,c,d}	28.44 ± 0.47 ^{a,b,c,d}
TVNu491	0.15 ± 0.01 ^{c,d}	0.14 ± 0.01 ^{c,d}	28.44 ± 0.93 ^{a,b,c,d}	28.45 ± 0.92 ^{a,b,c,d}
TVNu1808	0.16 ± 0.01 ^{c,d}	0.15 ± 0.01 ^{c,d}	29.36 ± 0.42 ^{a,b,c}	29.38 ± 0.43 ^{a,b,c}
TVNu738	0.12 ± 0.01 ^{c,d}	0.12 ± 0.01 ^{c,d}	26.42 ± 0.39 ^{a,b,c,d}	26.43 ± 0.40 ^{a,b,c,d}
TVNu1779	0.19 ± 0.02 ^{c,d}	0.17 ± 0.01 ^{c,d}	26.12 ± 2.04 ^{c,d,e}	25.74 ± 2.01 ^{c,d,e}

	Water Absorption Capacity		Cooking Time (min)	
Species/Accession Number	**Site A**	**Site B**	**Site A**	**Site B**
TVNu605	0.42 ± 0.02 ^{c,d}	0.36 ± 0.02 ^{c,d}	29.16 ± 0.51 ^{a,b,c,d}	29.18 ± 0.51 ^{a,b,c,d}
TVNu57	0.06 ± 0.01 ^{d}	0.06 ± 0.01 ^{d}	28.00 ± 0.55 ^{a,b,c,d}	27.61 ± 0.48 ^{a,b,c,d}
TVNu138	0.23 ± 0.01 ^{c,d}	0.21 ± 0.01 ^{c,d}	27.19 ± 0.62 ^{a,b,c,d}	26.80 ± 0.60 ^{a,b,c,d}
TVNu161	0.18 ± 0.01 ^{c,d}	0.16 ± 0.01 ^{c,d}	30.02 ± 0.77 ^{a,b}	29.64 ± 0.76 ^{a,b}
TVNu758	0.16 ± 0.01 ^{c,d}	0.15 ± 0.01 ^{c,d}	27.10 ± 0.30 ^{a,b,c,d}	27.11 ± 0.30 ^{a,b,c,d}
TVNu1825	0.25 ± 0.02 ^{c,d}	0.23 ± 0.02 ^{c,d}	25.50 ± 0.91 ^{d,e}	25.51 ± 0.91 ^{d,e}
TVNu1522	0.19 ± 0.01 ^{c,d}	0.17 ± 0.01 ^{c,d}	22.56 ± 0.57 ^{e}	22.57 ± 0.57 ^{e}
TVNu1388	0.18 ± 0.01 ^{c,d}	0.16 ± 0.01 ^{c,d}	26.53 ± 0.69 ^{a,b,c,d}	26.54 ± 0.70 ^{a,b,c,d}

Analysis of Variance (ANOVA)

	Water Absorption Capacity					Cooking Time (min)				
Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Model	69	131.740	1.909	11.989	<0.0001	69	22845.864	331.099	225.891	<0.0001
Error	386	61.473	0.159			386	565.779	1.466		
Corrected Total	455	193.213				455	23411.643			

Type III Sum of Squares Analysis

Source	**DF**	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Location (Site)	2	0.025	0.013	0.080	0.924	2	0.052	0.026	0.018	0.982
Genotype (Accessions)	34	88.722	2.609	16.385	<0.0001	34	12987.598	381.988	260.610	<0.0001
Location × Genotype	33	0.033	0.001	0.006	1.000	33	0.000	0.000	0.000	1.000

Results are represented as the mean value of triplicates ± standard error. Mean values without any letter in common within each column are significantly different ($p = 0.05$). Site A: TARI-Selian; Site B: TaCRI. DF: Degree of freedom; F: F-ratio; p: p-value

(v) Cooking Time and Water Absorption of *Vigna racemosa* Accessions

The results for water absorption capacity and cooking time for accessions of wild *Vigna racemosa* are shown in Table 16. The values for water absorption capacity and cooking time tested showed no significant difference ($p > 0.05$) when compared with the values of their corresponding accession harvested in the other agro-ecological zone through two-way analysis of variance (ANOVA).

The water absorption capacity of some of the wild accessions showed significant difference to each other and to the three checks. The unknown *Vigna racemosa* and unknown *Vigna* legume accessions displayed significantly low values similar to the three checks (Table 16). The water absorption capacity of the wild *V. racemosa* accessions varied from 0.08 ± 0.01 to 1.35 ± 0.03 in site A and from 0.08 ± 0.00 to 1.32 ± 0.13 in site B (Table 16).

On the other hand, non-significant difference in cooking time between AGG51603WVIG1, AGG52867WVIG1 accessions and check 1 was observed. Besides, they were all significantly different from check 2, check 3 and the other accessions. Generally, AGG53597WVIG1 exhibited superior low cooking time compared with the three checks. The cooking time for all accessions varied from 8.26 ± 0.42 to 30.33 ± 0.48 min in site A and from 7.87 ± 0.40 to 30.34 ± 0.50 min in site B.

Pearson correlation analysis shows that there is a strong negative correlation between the water absorption and cooking time considering the wild *V. racemosa* accessions tested ($r = -0.91$ for site A and $r = -0.92$ for site B). Furthermore, the regression analysis shows that the water absorption capacity and cooking time are related by the equation: $Y = -17.17x + 32.10$ with $R^2 = 0.84$ (Fig. 22)

Table 16: **Cooking Time and Water Absorption Capacity of *Vigna racemosa* Accessions**

Species/Accession Number	Water Absorption		Cooking Time (min)	
	Site A	Site B	Site A	Site B
Landrace of *Vigna vexillata*	1.33 ± 0.11 [a]	1.32 ± 0.13 [a]	10.24 ± 0.15 [d]	10.26 ± 0.15 [d]
Cowpea (*Vigna unguiculata*)	1.27 ± 0.08 [a]	1.27 ± 0.08 [a]	16.29 ± 0.15 [b]	16.31 ± 0.15 [b]
Rice Bean (*Vigna umbellata*)	1.16 ± 0.06 [a]	1.16 ± 0.06 [a]	13.20 ± 0.12 [c]	13.23 ± 0.12 [c]
AGG53597WVIG1	1.35 ± 0.03 [a]	1.33 ± 0.02 [a]	8.26 ± 0.42 [d]	7.87 ± 0.40 [d]
AGG51603WVIG1	1.29 ± 0.01 [a]	1.27 ± 0.02 [a]	10.15 ± 0.22 [d,e]	10.17 ± 0.25 [d,e]
AGG52867WVIG1	1.04 ± 0.04 [a]	1.02 ± 0.00 [a]	11.27 ± 0.41 [d]	11.28 ± 0.42 [d]
Unknown *Vigna* legume	0.43 ± 0.01 [a,b]	0.39 ± 0.02 [a,b]	29.35 ± 0.31 [a]	28.97 ± 0.30 [a]
Unknown *Vigna racemosa*	0.08 ± 0.01 [b]	0.08 ± 0.00 [b]	30.33 ± 0.48 [a]	30.34 ± 0.50 [a]

Analysis of Variance (ANOVA)

Source	Water Absorption Capacity					Cooking Time (min)				
	DF	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Model	15	13.441	0.896	4.279	<0.0001	15	4957.993	330.533	386.632	<0.0001
Error	278	58.223	0.209			278	237.663	0.855		
Corrected Total	293	71.664				293	5195.656			

Type III Sum of Squares Analysis

Source	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***	***DF***	**Sum of Squares**	**Mean Squares**	***F***	***p***
Location (Site)	1	0.004	0.004	0.017	0.896	1	0.006	0.006	0.007	0.934
Genotypes (Accessions)	7	13.436	1.919	9.165	<0.0001	7	4957.947	708.278	828.489	<0.0001
Location × Genotype		0.003	0.000	0.002	1.000	7	0.001	0.000	0.000	1.000

Results are represented as the mean value of triplicates ± standard error. Mean values without any letter in common within each column are significantly different (p = 0.05). Site A: TARI-Selian; Site B: TaCRI. DF: Degree of freedom; F: F-ratio; p: p-value

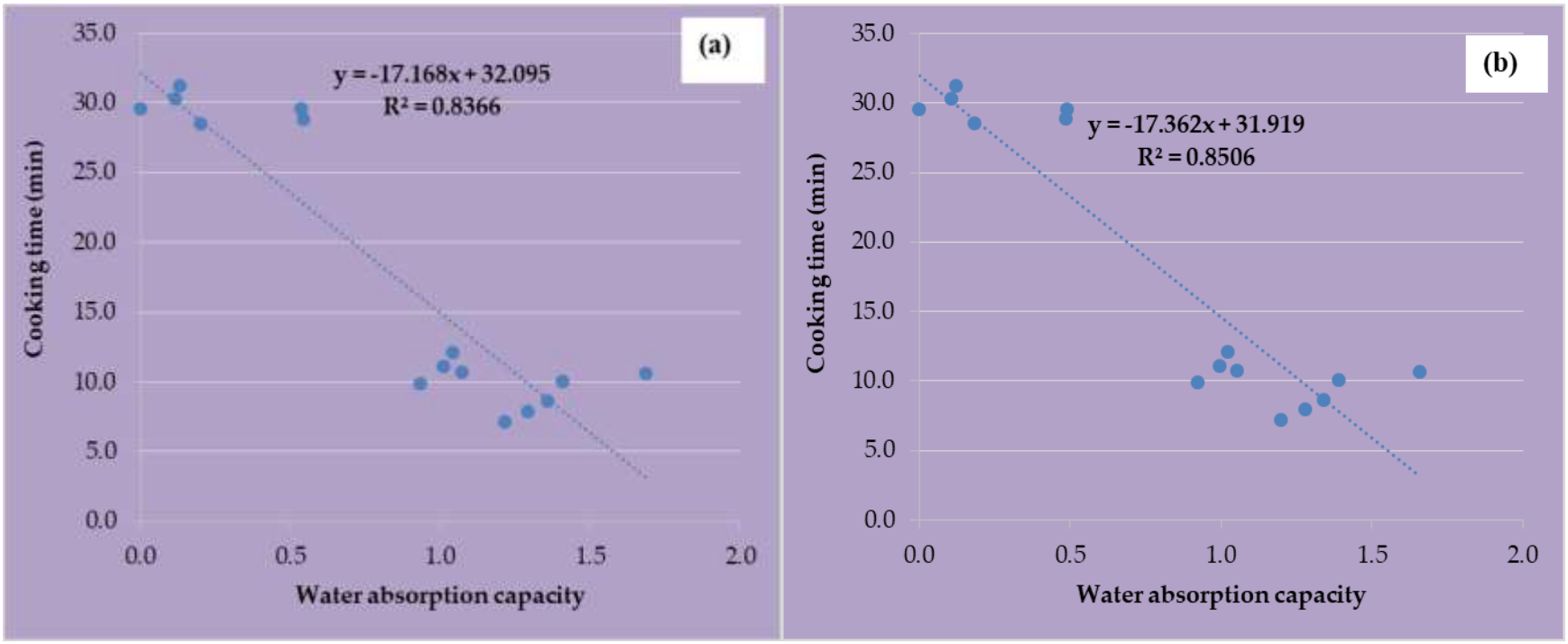


Figure 22: Correlation between Water Absorption and Cooking Time for the *Vigna racemosa* Accessions. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

(vi) Water Absorption Capacity, Cooking Time and Clustering Analysis of the Four *Vigna* species for Domestication and Crop Improvement

Figure 23 shows the pattern of evolution of water absorption as a function of cooking time to depict the existing relationship between the two parameters for the eighty four accessions from the four wild *Vigna* species (*V. ambacensis, V. reticulata, V. vexillata* and *V. racemosa*) and three domesticated species. It shows that the relationship is a strong negative correlation (−0.69 for site A and −0.70 for site B) between the water absorption and the cooking time which follows the equation: $Y_A = -7.99X + 26.52$ ($R^2 = 0.48$) or $Y_B = -8.21X + 26.57$ ($R^2 = 0.50$) (Fig. 23).

Agglomerative Hierarchical Clustering (AHC) analysis performed on all the four *Vigna* species taking water absorption capacity, cooking time and their individual weights before any processing as variable traits revealed seven classes (Fig. 24). Details of various accessions belonging to each class are provided in Table 17. Class 1 consists of nineteen accessions of *V. reticulata*, sixteen accessions of *V. vexillata*, and all the eleven accessions of *V. ambacensis*. Class 2 consists of only eight accessions of *V. reticulata* and ten accessions of *V. vexillata* while class 3 consists of two accessions of *V. reticulata*, one accession of *V. vexillata*, three accessions of *V. racemosa* and check 2 and 3. The class 4 consists of one accession of *V. vexillata* and check 3 only, while one accession makes up class 5. Class 6 is made up of four accessions of *V. vexillata* and class 7 of two *V. reticulata* and two *V. vexillata*.

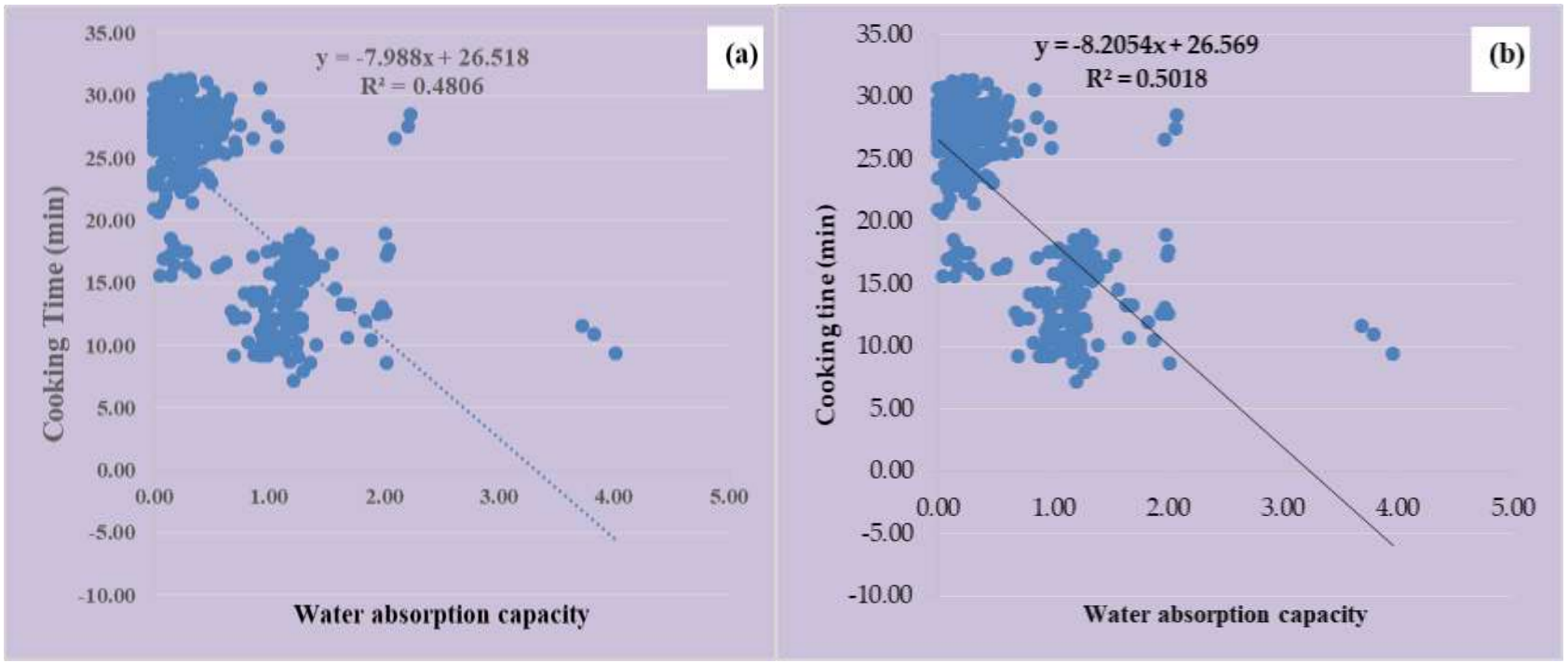


Figure 23: Correlation Between Water Absorption and Cooking Time for the *Vigna* Species Studied. (a) Plotted with Data from Site A; (b) Plotted with Data from Site B

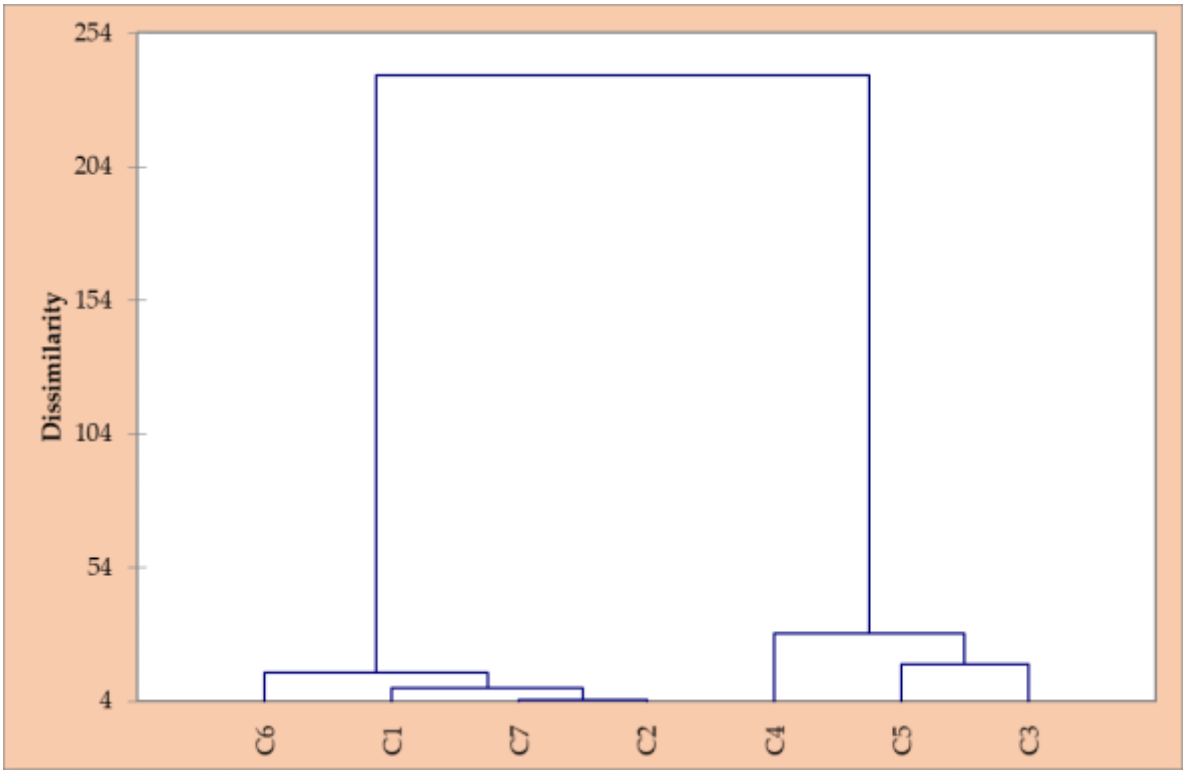


Figure 24: Dendrograms Showing Relationship among 84 Accessions of the Four Wild *Vigna* Species and Three Domesticated Varieties Regarding their Weights before Soaking, Water Absorption and Cooking Time

Table 17: Details of Classes from the Dendrogram for Cooking Time and Water Absorption Capacity*

Class	1	2	3	4	5	6	7
Object	47	20	8	2	1	4	4
	TVNu324_VRe	TVNu1632_VV	TVNu325_VRe	Check 3	TVNu1520_VRe	AGG308107WVIG2 _VV	TVNu379_VRe
	TVNu342_VA	TVNu1701_VV	Check 2	TVNu781_VV		AGG62154WVIG_1_VV	TVNu1582_VV
	AGG308101WVIG1 _VV	TVNu1629_VV	Unknown _*Vigna reticulata*			TVNu1624_VV	TVNu1358_VV
	TVNu1344_VV	TVNu767_VRe	AGG51603WVIG1_VRa			AGG308097WVIG 1_VV	TVNu1394_VRe
	AGG308096 WVIG2_VV	TVNu343_VRe	AGG53597WVIG1_VRa				
	TVNu120 _VV	TVNu333_VV	Check 1				
	TVNu1529_VV	TVNu1370_VV	TVNu837_VV				
	TVNu720_VA	TVNu349_VRe	AGG52867WVIG1_VRa				
	TVNu223_VA	TVNu1378_VV					
	TVNu1546_VV	TVNu1405_VRe					
	TVNu1698_VRe	TVNu1593_VV					
	TVNu877_VA	Unknown *Vigna*					
	TVNu524_VRe	TVNu381_VV					
	TVNu1699_VA	TVNu479_VV					
	TVNu1191_VRe	TVNu605 _VRe					
	TVNu1621_VV	TVNu1360_VV					
	TVNu607_VRe	TVNu1790_VRe					
	TVNu56_VRe	TVNu1808_VRe					
	TVNu- 224_VRe	Unknown_*Vigna_racemos*					
	TVNu739_VRe	TVNu161_VRe					
	TVNu916_VRe						
	TVNu955_VV						
	TVNu1092 _VV						
	TVNu1591_VV						
	TVNu178_VV						
	TVNu293_VV						
	TVNu1840_VA						
	AGG17856WVIG_1_VRe						
	TVNu738_VRe						
	TVNu1796_VV						
	TVNu1792_VA						
	TVNu832_VV						
	TVNu219_VA						
	TVNu491_VRe						
	TVNu1628_VV						
	TVNu1779_VRe						
	TVNu138_VRe						
	AGG308099WVIG2_VV						
	TVNu1804_VA						
	TVNu1586_VV						

Class	1	2	3	4	5	6	7
Object	**47**	**20**	**8**	**2**	**1**	**4**	**4**
	TVNu57_VRe						
	TVNu1825_VRe						
	TVNu1644_VA						
	TVNu758_VRe						
	TVNu1388_VRe						
	TVNu1522_VRe						
	TVNu1185_VA						

*Abbreviations put beside the accession names serves to identify species: VA stands for *V. ambacensis*, VV for *V.vexillata*, VRe for *V. reticulata*, and VRa for *V. racemos*

(vii) Descriptive Statistics and Yield Traits of the Wild *Vigna* Species

Table 18 shows results of the means values for water absorption capacity, cooking time and yield traits of the four wild species studied. *Vigna ambacensis* present mean values of 0.20, 23.45 min and 1.74 g for water absorption capacity, cooking time and yield per plant respectively in site A while in site B the mean values are 0.18, 23.43 min, and 0.78 g for water absorption capacity, cooking time and yield per plant respectively. In *Vigna vexillata*, the values of 0.34, 25.42 min and 16.84 g were found for water absorption capacity, cooking time and yield per plant respectively in site A and 0.32, 25.40 min and 12.54 g for water absorption capacity, cooking time and yield per plant respectively in site B. For *Vigna reticulata*, the mean values are 0.39, 26.77 min and 10.60 g for water absorption capacity, cooking time and yield per plant respectively in site A while in site B the mean values are 0.37, 26.78 min and 6.78 g for water absorption capacity, cooking time and yield per plant, respectively. Finally, *Vigna racemosa* present mean values of 0.84, 17.70 min and 28.25 g for water absorption capacity, cooking time and yield per plant respectively in site A, while in site B, the mean values are 0.81, 17.72 min and 18.28 g for water absorption capacity, cooking time and yield per plant respectively. The yield values varied from 13.45 g (*V. vexillata* landrace) to 86.04 g (rice bean) in site A, while it varied from 7.62 g (*V. vexillata* landrace) to 61.92 g (rice bean) in site B for the domesticated legumes. For the wild legumes, it varied from 1.74 g (*Vigna ambacensis*) to 28.25 g (*Vigna racemosa*) in site A and from 0.78 g (*Vigna ambacensis*) to 18.28 g (*Vigna racemosa*) in site B.

Table 18: Descriptive Statistic and Yield Traits of the Wild *Vigna* Species

Species	Descriptive Parameters	Water Absorption Capacity		Cooking Time (min)		Yield per Plant (g)	
		Site A	Site B	Site A	Site B	Site A	Site B
Landrace of *Vigna vexillata*	Mean	1.33	1.32	10.24	10.26	13.45	7.62
	CV (%)	9.50	9.37	1.46	1.45	4.59	6.34
	Range	0.69–4.01	0.70–3.96	8.56–11.89	8.59–11.91	9.00–26.55	4.94–17.31
Cowpea (*Vigna unguiculata*)	Mean	1.27	1.27	16.29	16.31	52.690	26.657
	CV (%)	1.85	1.82	0.93	0.93	5.48	5.42
	Range	0.58–1.58	0.58–1.57	14.06–18.84	14.09–18.87	28.80–106.08	14.71–53.35
Rice Bean (*Vigna umbellata*)	Mean	1.16	1.16	13.20	13.23	86.04	61.92
	CV (%)	4.05	4.02	0.92	0.91	2.378	2.361
	Range	0.67–2.02	0.68–2.00	11.73–14.98	11.76–15.01	60.27–109.76	43.51–78.86
Vigna ambacensis	Mean	0.20	0.18	23.45	23.43	1.74	0.78
	CV (%)	11.21	11.20	0.44	0.42	22.36	14.25
	Range	0.00–0.50	0.00–0.58	22.25–24.95	22.26–24.96	0.72–5.36	0.43–1.65
Vigna vexillata	Mean	0.34	0.32	25.42	25.40	16.84	12.54
	CV (%)	7.80	7.95	1.73	1.70	9.48	6.77
	Range	0.00–1.15	0.00–1.13	15.54–31.28	15.55–31.30	9.48–63.00	7.61–35.26
Vigna reticulata	Mean	0.39	0.37	26.77	26.78	10.60	6.78
	CV (%)	13.83	14.04	1.20	1.27	10.55	10.62
	Range	0.00–2.24	0.00–2.08	16.60–30.98	16.58–40.00	4.32–30.36	2.58–17.69
Vigna racemosa	Mean	0.84	0.81	17.70	17.72	28.25	18.28
	CV (%)	16.70	17.06	14.83	14.81	37.02	38.37
	Range	0.00–1.69	0.00–1.66	7.11–31.19	7.12–31.22	2.08–49.00	1.21–34.70

CV: Coefficient of variation; Range (Minimum−Maximum)

4.1.4 Biochemical Characterization of Wild *Vigna* Species

(i) Proximate Composition Exploration of seeds of Wild *Vigna* Species

The proximate composition for the various *Vigna* species studied is summarized in Tables 19-22. The proximate composition of the three domesticated legumes included in this study for comparison is presented in the tables presenting the results for each species in order to ease the appreciation. The three domesticated legumes used here as checks are the same as the ones used on previous objectives, they are: A landrace of *Vigna vexillata* (Check 1), cowpea (Check 2), and rice bean (Check 3). A keen examination of the proximate composition of these three checks shows that there is no significant difference in lipid, fiber and carbohydrate content of Check 1 and Check 2 which are significantly different ($p<0.05$) from that of check 3. Their lipid content is significantly higher than that of Check 3 while their carbohydrate and fiber contents are lower than that of Check 3. The ash and moisture contents of the three checks are apparently similar. This can be elucidated by evaluating the individual minerals. The protein content of the three checks is significantly different with Check 1 having the highest protein content (Table 19-22).

(ii) Proximate Composition of *Vigna* Ambacensis Accessions

Table 19 summarizes the proximate composition of *Vigna ambacensis* accessions. It shows that the lipid content of all the wild accessions was not different from those of Check 1 and 2 while it was significantly ($p<0.05$) higher than that of Check 3. All the accessions show lower ash content than the three checks except for accessions TVNu219 and TVNu877. The moisture, fiber, protein and carbohydrate content of the wild accessions are significantly lower than that of the checks.

Table 19: Proximate Composition of *Vigna ambacensis* Accessions (g/100g)

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
Check 1	1.127 ±0.128a	3.729 ±0.126ab	11.052 ±0.281abc	27.313 ±0.597a	3.654 ±0.832f	53.125 ±1.323g
Check 2	1.215 ±0.150a	3.716 ±0.229ab	12.663 ±0.665a	25.935 ±0.938b	3.641 ±0.678f	52.829 ±1.023g
Check 3	0.636 ±0.015b	3.833 ±0.167a	11.706 ±0.615ab	22.800 ±1.046c	4.637 ±0.589de	56.388 ±1.234f
TVNu342	1.300 ±0.032a	3.347±0.264 bc	8.376 ±0.243ef	21.173 ±0.315de	6.360 ±0.413ab	59.444 ±0.765 e
TVNu720	1.295 ±0.081a	3.078 ±0.432cd	9.769 ±0.345cde	20.550 ±0.318def	5.848 ±0.410bc	59.460 ±0.725e
TVNu1840	1.305 ±0.035a	2.762 ±0.143de	9.504 ±0.253cdef	21.814 ±0.215cd	5.248 ±0.412cd	59.367 ±0.782 e
TVNu219	1.268 ±0.043a	3.555 ±0.543ab	8.658 ±0.312ef	19.914 ±0.312efg	6.754 ±0.418a	59.851±0.765 de
TVNu877	1.263 ±0.045a	3.286 ±0.431bc	8.982 ±0.263def	19.328 ±0.326fgh	6.243±0.432 ab	60.898 ±0.874cde
TVNu1644	1.284 ±0.054a	2.537 ±0.143ef	9.451 ±0.345cdef	19.359 ±0.368fgh	4.820 ±0.400de	62.549 ±0.879bc
TVNu1699	1.289 ±0.065a	2.808 ±0.145de	8.103 ±0.124ef	19.946 ±0.245efg	5.335 ±0.412cd	62.520±0.974 bc
TVNu1804	1.258 ±0.056a	3.016 ±0.213cd	10.476 ±0.443bcd	18.760 ±0.319gh	5.731 ±0.413bc	60.759 ±0.765 cde
TVNu1185	1.134 ±0.078a	3.281 ±0.243bc	9.434 ±0.363cdef	18.208 ±0.313h	6.234 ±0.487ab	61.708 ±0.895bcd
TVNu223	1.273 ±0.079a	1.992 ±0.043g	9.197 ±0.296def	20.517 ±0.317def	3.786 ±0.401f	63.235 ±0.891b
TVNu1792	1.279 ±0.087a	2.265 ±0.123fg	7.885 ±0.384f	18.790 ±0.314gh	4.304 ±0.411ef	65.478 ±0.965 a
F	10.603	40.530	16.830	110.097	50.763	77.787
Pr > F(Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pr > F(*V. ambacensis* Accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

(iii) Proximate Composition of *Vigna reticulata* Accessions

Table 20 summarizes the proximate composition of *Vigna reticulata* accessions. It shows that the lipid content of most of the wild accessions is not significantly different from that of Check 1 and 2 except for four accessions (TVNu1394_VRe, TVNu324_VRe, TVNu57_VRe, TVNu141_VRe) which are comparable to Check 3 (lowest lipid content among checks). All the accessions show ash content comparable to that of the three checks indicating that none of the accessions had higher ash content than that of the checks. All the accessions showed moisture content lower than that of the three checks. The accession TVNu1112_VRe (31.074%) had the highest protein content which is significantly higher than that of all the checks while five accessions (TVNu1852_VRe, TVNu141_VRe, TVNu57_VRe, TVNu324_VRe, TVNu350_VRe) had protein content comparable to that of Check 1 and Check 2. The rest of the accessions had very low protein content which is lower than that of Check 3. It was noticed that the greater number of wild accessions had a significantly higher fiber and carbohydrates contents respectively as compared to the checks.

Table 20: Proximate Composition of *Vigna reticulata* Accessions (g/100g)

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
Check 1	1.127 ±0.128abcd	3.729 ±0.126abcde	11.052 ±0.281bcde	27.313 ±0.597b	3.654 ±0.832j	53.125 ±1.323 klmn
Check 2	1.215 ±0.150abcd	3.716 ±0.229abcde	12.663 ±0.665a	25.935 ±0.938bc	3.641±0.678 j	52.829 ±1.023lmn
Check 3	0.636 ±0.015f	3.833 ±0.167abc	11.706 ±0.615 ab	22.800 ±1.046fghijk	4.637 ±0.589ghij	56.388 ±1.234efghijk
TVNu350_VRe	1.132 ±0.212abcd	4.130 ±0.313a	10.610 ±0.600bcdefg	24.910 ±0.800cde	7.433 ±0.760a	51.785 ±1.005no
TVNu56_VRe	1.419 ±0.223a	3.509 ±0.313abcdef	10.175 ±0.602cdefghijk	23.182 ±0.800fghi	6.317 ±0.505abcdefg	55.397 ±1.034ghijklm
TVNu1522_VRe	1.108 ±0.215abcd	3.749 ±0.310abcde	11.585 ±0.623abc	23.115 ±0.802fghij	6.749 ±0.525abcd	53.694 ±1.006jklmn
TVNu1698_VRe	1.391 ±0.210ab	3.513 ±0.311abcdef	10.060± 0.600defghijkl	22.405 ±0.800ghijkl	6.324 ±0.500abcdefg	56.307 ±1.055fghijkl
TVNu1808_VRe	1.015±0.200 cde	3.935 ±0.310ab	9.910 ±0.600defghijklm	23.823 ±0.801efg	7.083 ±0.515ab	54.234 ±1.321ijklmn
TVNu607_VRe	1.374 ±0.214ab	3.562 ±0.300abcdef	9.008 ±0.600jklmn	23.525 ±0.802efgh	6.411 ±0.508abcdefg	56.119 ±1.520fghijkl
TVNu379_VRe	1.123 ±0.200 abcd	3.513 ±0.315abcdef	10.263 ±0.643cdefghij	23.632 ±0.800efgh	6.324 ±0.507abcdefg	55.145 ±1.045ghijklmn
TVNu1852_VRe	1.170 ±0.223abcd	3.375±0.313 abcdefg	10.927 ±0.614bcdef	26.597 ±0.868b	6.076 ±0.500abcdefgh	51.856 ±1.000mno
TVNu739_VRe	1.341 ±0.200 abc	3.782 ±0.310abc	9.312 ±0.601ghijklmn	22.126 ±0.800hijklm	6.808 ±0.503abc	56.630 ±1.068cdefghijk
TVNu138_VRe	1.369 ±0.200 ab	3.275±0.312 abcdefg	10.507±0.615 bcdefghi	22.833 ±0.800fghijk	5.895 ±0.500abcdefgh	56.121 ±1.098fghijkl
TVNu1405_VRe	1.380±0.200 ab	2.939 ±0.300bcdefgh	10.221± 0.615cdefghij	24.238 ±0.812def	5.290 ±0.500bcdefghij	55.931 ±1.075fghijkl
TVNu349_VRe	1.154 ±0.200 abcd	3.762 ±0.302abcd	8.053 ±0.600n	22.748 ±0.800fghijk	6.771 ±0.500abcd	57.512 ±1.645bcdefghi
TVNu325_VRe	1.425 ±0.200 a	3.217 ±0.301abcdefg	9.576 ±0.600fghijklm	22.113 ±0.805hijklm	5.791 ±0.489abcdefgh	57.878 ±1.067bcdefgh
TVNu758_VRe	1.195 ±0.221abcd	3.491 ±0.304abcdef	10.147 ±0.630cdefghijkl	20.231 ±0.700n	6.285 ±0.500abcdefg	58.651 ±1.740bcdefg
Unknown _*Vigna reticulata*	1.335±0.210 abc	3.496 ±0.303abcdef	9.660 ±0.600efghijklm	21.476 ±0.800klmn	6.293 ±0.500abcdefg	57.739 ±1.054bcdefghi
TVNu1394_VRe	0.755 ±0.200 ef	3.657 ±0.300abcdef	9.334 ±0.600ghijklmn	22.756 ±0.807fghijk	6.583 ±0.505abcdef	56.916 ±1.061cdefghij
TVNu1825_VRe	1.414 ±0.200 a	2.924 ±0.300bcdefgh	8.596 ±0.600mn	23.229 ±0.820fghi	5.262 ±0.500bcdefghij	58.576 ±1.008bcdefg
TVNu- 224_VRe	1.146 ±0.200 abcd	3.476 ±0.300abcdef	8.556 ±0.600mn	23.444 ±0.808efgh	6.256 ±0.516abcdefg	57.121 ±1.056cdefghij
TVNu1191_VRe	1.330 ±0.200 abc	3.210 ±0.300abcdefg	11.267 ±0.643abcd	20.844 ±0.800lmn	5.777 ±0.500abcdefgh	57.572 ±1.075bcdefghi
TVNu1112_VRe	1.066 ±0.200 bcde	3.686 ±0.300abcdef	8.547 ±0.600mn	31.074 ±0.867a	6.634 ±0.505abcde	48.994 ±1.005o
TVNu1779_VRe	1.436 ±0.205a	2.796 ±0.300cdefgh	8.483 ±0.601mn	23.014 ±0.807fghijk	5.032 ±0.500cdefghij	59.239 ±1.569abcdef
TVNu491_VRe	1.386 ±0.200 ab	3.227 ±0.305abcdefg	8.764 ±0.600klmn	21.746 ±0.800ijklmn	5.808 ±0.500abcdefgh	59.070 ±1.056abcdef
TVNu524_VRe	1.115 ±0.200 abcd	3.227±0.301 abcdefg	10.904 ±0.604bcdef	22.428 ±0.802ghijkl	5.808 ±0.502abcdefgh	56.518 ±1.055defghijk
TVNu1520_VRe	1.430 ±0.200 a	2.840 ±0.300cdefgh	9.013±0.612 jklmn	22.631 ±0.805fghijk	5.112 ±0.500cdefghij	58.973 ±1.016bcdef

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
AGG17856WVIG_1_VRe	1.397 ±0.200 ab	2.719 ±0.300defgh	10.193 ±0.621cdefghijk	20.679 ±0.800mn	4.894 ±0.500defghij	60.119 ±1.850abc
TVNu324_VRe	0.747 ±0.280 ef	3.371 ±0.300abcdefg	9.917 ±0.600defghijklm	24.933 ±0.804cde	6.068 ±0.500abcdefgh	54.964 ±1.000hijklmn
TVNu343_VRe	1.363 ±0.214 ab	2.988 ±0.267bcdefgh	8.715 ±0.600lmn	22.162 ±0.800hijklm	5.378 ±0.500bcdefghij	59.395 ±1.075abcdef
TVNu57_VRe	0.739 ±0.100 ef	3.084±0.301 abcdefgh	10.536 ±0.605bcdefgh	25.845 ±0.832bcd	5.551±0.500 abcdefghi	54.246 ±1.045ijklmn
TVNu1388_VRe	1.358 ±0.240 abc	2.699 ±0.300efgh	10.164 ±0.615cdefghijk	21.510 ±0.800jklmn	4.859 ±0.500efghij	59.410 ±1.087abcdef
TVNu767_VRe	1.408 ±0.200 ab	2.632 ±0.267fgh	9.133 ±0.600hijklmn	22.146 ±0.800hijklm	4.738 ±0.500fghij	59.944 ±1.060abcde
TVNu161_VRe	1.402 ±0.210 ab	2.340 ±0.285gh	9.703 ±0.600efghijklm	21.494 ±0.800klmn	4.211±0.500 hij	60.849 ±1.095ab
TVNu738_VRe	1.346 ±0.220 abc	2.120 ±0.241h	9.891 ±0.600defghijklm	22.797 ±0.802fghijk	3.816 ±0.500ij	60.030 ±1.068abcd
TVNu1790_VRe	1.131 ±0.200 abcd	2.901 ±0.300bcdefgh	9.659 ±0.600efghijklm	22.930 ±0.821fghijk	5.222 ±0.500bcdefghij	58.157 ±1.055bcdefgh
TVNu916_VRe	1.138 ±0.230 abcd	3.189 ±0.300abcdefg	9.091 ±0.600ijklmn	22.249 ±0.800ghijklm	5.740 ±0.502abcdefgh	58.593 ±1.078bcdefg
TVNu141_VRe	0.948 ±0.200 def	2.723 ±0.298defgh	9.649 ±0.600efghijklm	27.025 ±0.845b	4.902 ±0.500defghij	54.753 ±1.324hijklmn
TVNu605 _VRe	1.352 ±0.200 abc	2.410 ±0.254gh	8.480 ±0.600mn	20.877 ±0.800lmn	4.338 ±0.500hij	62.542 ±1.058a
F	12.978	6.535	16.452	56.192	8.277	20.420
Pr > F(Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pr > F(*V. reticulate* Accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

(iv) Proximate Composition of *Vigna vexillata* Accessions

Table 21 presents the proximate composition of *Vigna vexillata* accessions. It was found that the lipid content of most of the wild accessions was significantly lower than that of Checks 1 and 2 except for few accessions (AGG308096WVIG2, TVNu333, TVNu293 and TVNu 832) which were higher than that of Check 3. Similarly all the accessions from *Vigna reticulata* species showed comparable ash contents to that of the three checks. A significant number of accessions showed comparable moisture content to that of the checks indicating phenotypic similarity in moisture content. The accessions TVNu832, TVNu1701, TVNu1546, AGG308101 WVIG2 and AGG308099WVIG2 had the highest protein content which is significantly higher than that of all the checks. On the other hand, ten accessions (AGG308097WVIG2, TVNu1378, TVNu1529, TVNu1344, TVNu333, TVNu293, TVNu178, TVNu781, TVNu120, TVNu1629) had protein content comparable to that of Check 1 and Check 2. The rest of the accessions had very low protein content which is lower than that of Check 3. It is similarly noticed here that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents as compared to the checks.

Table 21: Proximate Composition of *Vigna vexillata* Accessions (g/100g

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
Check 1	1.127 ±0.128ab	3.729 ±0.126abcdefg	11.052 ±0.281abcde	27.313 ±0.597cde	3.654 ±0.832cdefg	53.125 ±1.323 ijk
Check 2	1.215 ±0.150a	3.716 ±0.229abcdefg	12.663 ±0.665a	25.935 ±0.938defgh	3.641 ±0.678cdefg	52.829 ±1.023ijk
Check 3	0.636 ±0.015ghijk	3.833 ±0.167abcdefg	11.706 ±0.615abc	22.800 ±1.046jklmn	4.637±0.281 abcdefg	56.388 ±1.234defghi
TVNu1701	0.851 ±0.153 cdefg	4.846 ±0.300a	10.153 ±0.650bcdefghi	30.287 ±0.850b	5.864 ±0.500a	47.999 ±1.003lm
TVNu333	0.916 ±0.155bcd	4.106 ±0.315abcde	11.070 ±0.685abcde	24.915 ±0.803efghijk	4.968 ±0.503abcde	54.024 ±1.001hijk
TVNu293	0.961 ±0.151bc	4.180 ±0.307abcd	9.618 ±0.606defghij	25.341 ±0.825efghij	5.058 ±0.506abcd	54.843 ±1.005ghijk
TVNu1582	0.837 ±0.152cdefgh	4.114 ±0.305abcde	10.357 ±0.608bcdefgh	23.651 ±0.805ghijklm	4.978 ±0.515abcde	56.063 ±1.003defghij
TVNu832	0.893 ±0.150 bcde	3.716 ±0.315abcdefg	10.263 ±0.615bcdefghi	33.593 ±0.858a	4.496 ±0.500abcdefg	47.039 ±1.003m
TVNu178	0.801 ±0.150cdefghi	4.288 ±0.321abc	8.759 ±0.600hij	24.849 ±0.801efghijk	5.188 ±0.508abc	56.116 ±1.003defghi
TVNu781	0.795 ±0.150cdefghi	4.248 ±0.308abc	9.685 ±0.602defghij	24.892 ±0.800efghijk	5.140 ±0.518abc	55.239 ±1.056efghij
AGG308101WVIG1	0.878 ±0.150cdef	3.673 ±0.300abcdefg	10.151 ±0.658bcdefghi	30.289 ±0.852b	4.445 ±0.505abcdefg	50.564 ±1.052klm
TVNu120	0.665 ±0.150efghijk	4.228 ±0.300abc	10.270 ±0.635bcdefghi	24.412 ±0.802efghijkl	5.116 ±0.508abc	55.309 ±1.075efghij
AGG308097WVIG 1	0.920 ±0.151bcd	3.606 ±0.300abcdefg	10.026 ±0.652bcdefghij	26.748 ±0.800cdef	4.363 ±0.521abcdefg	54.337 ±1.008ghijk
TVNu1593	0.543 ±0.110 jk	4.475 ±0.331ab	11.836 ±0.6085ab	22.740 ±0.750jklmn	5.414 ±0.505ab	54.993 ±1.035fghij
TVNu1370	0.679 ±0.110defghijk	4.285 ±0.300abc	10.101 ±0.605bcdefghi	21.120 ±0.728mn	5.184 ±0.515abc	58.631 ±1.051bcdefg
AGG308096 WVIG2	0.904 ±0.150 bcde	3.694 ±0.307abcdefg	9.187 ±0.600defghij	20.357 ±0.689n	4.470 ±0.500abcdefg	61.387 ±1.050abc
TVNu1629	0.532 ±0.120k	4.043 ±0.351abcdef	10.790 ±0.606abcdefg	25.140 ±0.801efghijk	4.892 ±0.500abcdef	54.603 ±1.003ghijk
AGG308099WVIG2	0.663 ±0.103efghijk	3.978 ±0.321abcdef	9.687 ±0.600defghij	28.656 ±0.802bcd	4.814 ±0.500abcdef	52.202 ±1.003ijkl
TVNu1344	0.804 ±0.105cdefghi	3.657 ±0.305abcdefg	9.118 ±0.605efghij	26.153 ±0.781defg	4.425±0.500 abcdefg	55.843 ±1.003defghij
AGG308107WVIG2	0.871 ±0.150 cdefg	3.235 ±0.308bcdefg	10.947 ±0.600abcdef	22.281 ±0.815klmn	3.915 ±0.500bcdefg	58.752 ±1.051bcdefg
TVNu1358	0.850 ±0.150cdefg	3.323 ±0.315bcdefg	9.812 ±0.600cdefghij	22.484 ±0.800jklmn	4.021 ±0.501bcdefg	59.510 ±1.068bcde
AGG62154WVIG_1	0.662 ±0.121efghijk	3.601 ±0.250abcdefg	9.943 ±0.600bcdefghij	25.347 ±0.800efghij	4.357 ±0.508abcdefg	56.090 ±1.056defghi
TVNu1529	0.684 ±0.106defghijk	3.640 ±0.300abcdefg	9.579 ±0.600defghij	25.811 ±0.808defghi	4.404 ±0.506abcdefg	55.881 ±1.015defghij
TVNu1546	0.774 ±0.102cdefghijk	3.150 ±0.300cdefg	11.091 ±0.682abcd	29.520 ±0.850bc	3.811±0.500cdefg	51.655±1.003 jkl
TVNu1092	0.895 ±0.109bcde	3.235 ±0.334bcdefg	8.581 ±0.601hij	23.870 ±0.805fghijklm	3.915 ±0.501bcdefg	59.503 ±1.003bcde
TVNu1586	0.593 ±0.101ijk	3.716 ±0.309abcdefg	9.520 ±0.600defghij	23.622 ±0.817ghijklm	4.497 ±0.505abcdefg	58.051 ±1.009cdefgh
TVNu1632	0.694 ±0.104defghijk	3.252 ±0.301bcdefg	9.848 ±0.608cdefghij	23.662 ±0.832ghijklm	3.935 ±0.500bcdefg	58.609 ±1.015bcdefg
TVNu1378	0.681 ±0.103defghijk	3.574 ±0.300abcdefg	8.864 ±0.600ghij	26.699 ±0.850cdef	4.325±0.509abcdefg	55.858 ±1.003defghij

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
TVNu1624	0.725 ±0.105cdefghijk	3.417 ±0.300bcdefg	9.661 ±0.609defghij	22.718 ±0.805jklmn	4.135 ±0.507bcdefg	59.344 ±1.085bcdef
TVNu381	0.776 ±0.135cdefghij	2.673 ±0.210g	10.125 ±0.600bcdefghi	23.141 ±0.807hijklmn	3.234 ±0.500g	60.051 ±1.095bcd
TVNu1360	0.761 ±0.108cdefghijk	3.220 ±0.305bcdefg	8.475 ±0.615hij	24.293 ±0.808fghijkl	3.897±0.500 bcdefg	59.354 ±1.065bcdef
TVNu1621	0.846 ±0.150 cdefgh	2.849 ±0.250efg	9.166 ±0.600defghij	22.864 ±0.805ijklmn	3.448 ±0.500efg	60.826 ±1.055abc
TVNu837	0.755 ±0.121cdefghijk	3.199 ±0.300bcdefg	8.343±0.600 ij	25.129 ±0.850efghijk	3.871 ±0.500bcdefg	58.703 ±1.003bcdefg
TVNu1628	0.590 ±0.105ijk	3.360 ±0.300bcdefg	8.902 ±0.600ghij	21.520 ±0.801lmn	4.065 ±0.500bcdefg	61.564 ±1.057abc
TVNu1796	0.604 ±0.115hijk	2.896 ±0.251defg	9.709 ±0.607defghij	23.134 ±0.850hijklmn	3.505 ±0.487defg	60.152±1.075 bcd
TVNu1591	0.720 ±0.131cdefghijk	2.616 ±0.205g	8.988 ±0.613fghij	22.270 ±0.852klmn	3.165 ±0.495g	62.241 ±1.354abc
TVNu955	0.648 ±0.105fghijk	3.087±0.305 cdefg	8.469 ±0.605hij	21.559 ±0.850lmn	3.735 ±0.500cdefg	62.502 ±1.352ab
TVNu479	0.732 ±0.115cdefghijk	2.793 ±0.208fg	8.124 ±0.600j	20.254±0.850 n	3.380 ±0.502fg	64.717 ±1.435a
F	11.955	5.365	8.867	31.181	5.720	25.612
Pr > F(Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pr > F(V. vexillata Accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

(v) Proximate Composition of *Vigna racemosa* Accessions

Table 22 summarizes the proximate composition of *Vigna racemosa* accessions. It was found that the lipid content of two wild accessions (AGG53597WVIG1, AGG51603WVIG1) was comparable to that of check 1 and 2 while the accessions 'Unknown_*Vigna_racemosa*', AGG52867WVIG1, 'Unknown *Vigna*' had comparable lipid content to that of Check 3. Similar to the *Vigna reticulata* species, all the accessions showed comparable ash content to that of the three checks indicating that none of the accessions had higher ash content than that of the checks. Accession AGG53597WVIG1 showed higher moisture content as compared with Check 1, Check 3 and all the other wild accessions. However, it is comparable to that of Check 2. The accession AGG51603WVIG1 (36.689%) had the highest protein content which is significantly higher than that of all the checks while accession AGG53597WVIG1 (28.852%) had a protein content comparable to that of Check 1. The rest of the accessions had very low protein content which is comparable to that of Check 3. It is similarly noticed here that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents as compared to the checks.

Table 22: Proximate Composition of *Vigna racemosa* Accessions (g/100)

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
Check 1	1.127 ±0.128a	3.729 ±0.126b	11.052 ±0.281cde	27.313 ±0.597bc	3.654 ±0.832c	53.125 ±1.323c
Check 2	1.215 ±0.150a	3.716 ±0.229b	12.663 ±0.665ab	25.935 ±0.938cd	3.641 ±0.678c	52.829 ±1.023c
Check 3	0.636 ±0.015d	3.833 ±0.167b	11.706 ±0.615bcd	22.800 ±0.589e	4.637 ±0.589b	56.388 ±1.234b
AGG53597WVIG1	1.041 ±0.342ab	3.871 ±0.432b	13.167 ±1.457a	28.852 ±0.765b	4.684 ±0.752b	48.384 ±1.398d
AGG51603WVIG1	0.981 ±0.035abc	6.196 ±1.432a	11.722 ±0.765bc	36.689 ±0.681a	6.691 ±0.532a	37.721 ±1.281e
Unknown_*Vigna_racemosa*	0.770 ±0.123cd	3.856 ±0.456b	10.201 ±0.657ef	23.048 ±0.356e	4.666 ±0.831b	57.459 ±1.532ab
AGG52867WVIG1	0.818 ±0.281bcd	3.310 ±0.532b	10.547 ±0.557de	24.492 ±0.345de	4.005 ±0.05bc	56.827 ±1.982ab
Unknown *Vigna*	0.711 ±0.0432d	3.438 ±0.346b	9.331 ±0.327f	23.052±0.312 e	4.160 ±0.281bc	59.307 ±1.881a
F statistics	14.946	48.604	28.543	147.317	44.083	180.733
Pr > F(Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Pr > F(*V. racemosa Accessions*)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

To have a quick view on the proximate composition of the wild accessions in order to appreciate their content per species, the mean of each component for all the accessions belonging to each species was calculated. Figure 25 shows the means of proximate composition of wild *Vigna* accessions per species. It reveals that looking at the variations in proximate composition globally per species, there is no significant difference between species vis-à-vis other species and the checks.

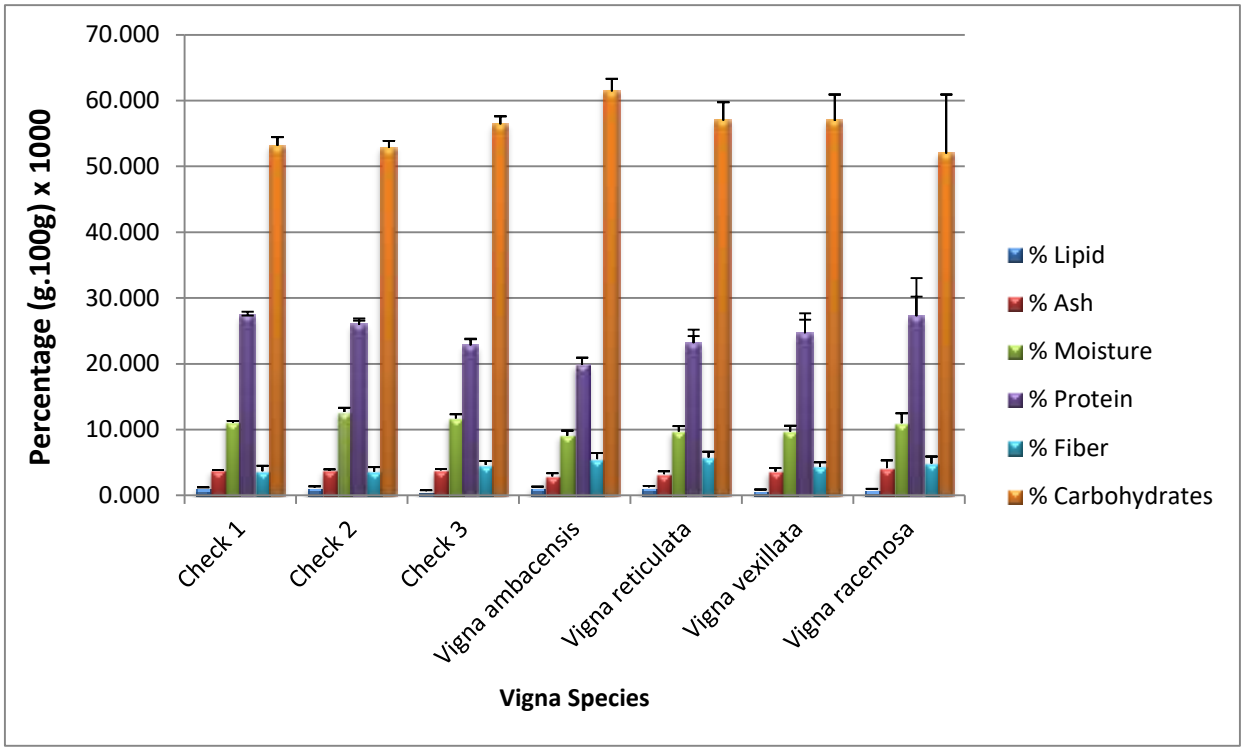


Figure 25: Means of Proximate Composition of Wild *Vigna* Accessions per Species

In order to group the accessions based on their phenotypic similarities in terms of the proximate composition, the agglomerative hierarchical clustering of XLSTAT was run to obtain a dendrogram (Fig. 26). It reveals that based on the proximate composition, both wild accessions and checks can form three groups of legumes in which the three checks belong to the same group with some wild accessions (Table 23).

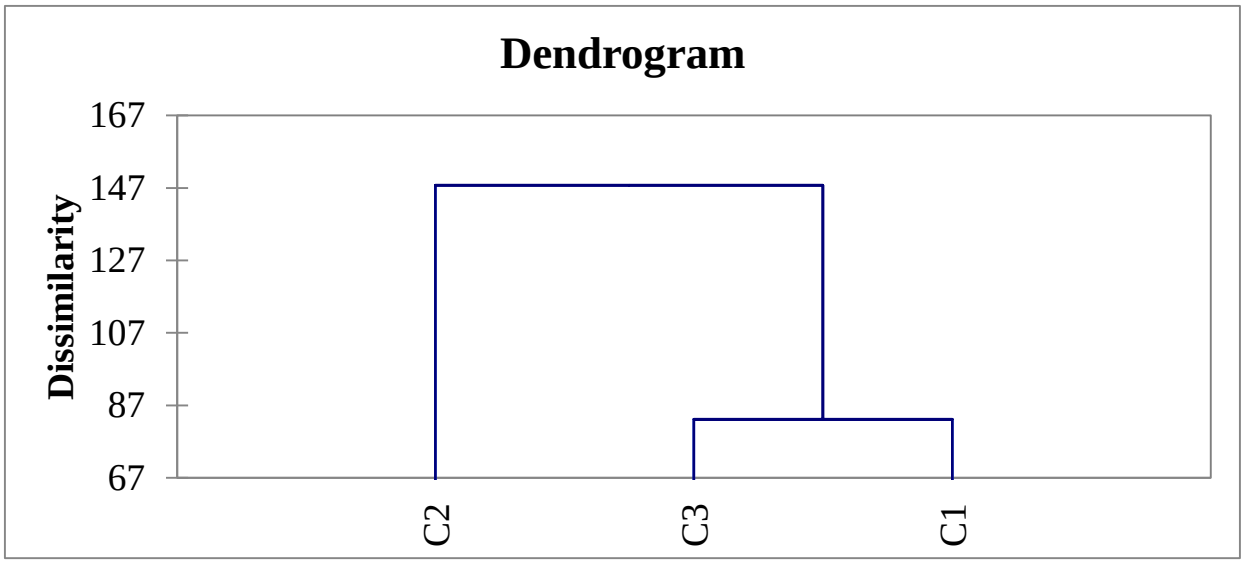


Figure 26: Dendrogram Showing Slusters of Wild *Vigna* Accessions Based on Proximate Composition

Table 23: Accessions Belonging to Classes as Grouped on the Dendrogram Based on Proximate Composition

Class	1	2	3
Objects	30	43	17
Sum of weights	30	43	17
Within-class variance	30.959	13.130	6.113
Minimum distance to centroid	1.120	0.592	0.695
Average distance to centroid	4.300	3.046	2.079
Maximum distance to centroid	18.995	8.220	5.336
	Check 1	TVNu342_Va	AGG308096 WVIG2
	Check 2	TVNu720_Va	AGG308107WVIG2
	Check 3	TVNu1840_Va	TVNu1358
	TVNu1112_VRe	TVNu219_Va	TVNu1092
	TVNu324_VRe	TVNu877_Va	TVNu1632
	TVNu57_VRe	TVNu1644_Va	TVNu1624
	TVNu141_VRe	TVNu1699_Va	TVNu381
	TVNu1701	TVNu1804_Va	TVNu1360
	TVNu333	TVNu1185_Va	TVNu1621
	TVNu293	TVNu223_Va	TVNu837
	TVNu1582	TVNu1792_Va	TVNu1628
	TVNu832	TVNu350_VRe	TVNu1796
	TVNu178	TVNu56_VRe	TVNu1591
	TVNu781	TVNu1522_VRe	TVNu955
	AGG308101WVIG1	TVNu1698_VRe	TVNu479
	TVNu120	TVNu1808_VRe	AGG52867WVIG1_Vra
	AGG308097WVIG 1	TVNu607_VRe	Unknown *Vigna*
	TVNu1593	TVNu379_VRe	
	TVNu1370	TVNu1852_VRe	
	TVNu1629	TVNu739_VRe	
	AGG308099WVIG2	TVNu138_VRe	
	TVNu1344	TVNu1405_VRe	
	AGG62154WVIG_1	TVNu349_VRe	
	TVNu1529	TVNu325_VRe	
	TVNu1546	TVNu758_VRe	
	TVNu1586	Unknown *_Vigna* reticulata	
	TVNu1378	TVNu1394_VRe	
	AGG53597WVIG1_Vra	TVNu1825_VRe	
	AGG51603WVIG1_Vra	TVNu- 224_VRe	
	Unknown_*Vigna*_racemosa	TVNu1191_VRe	
		TVNu1779_VRe	
		TVNu491_VRe	
		TVNu524_VRe	
		TVNu1520_VRe	

Class	1	2	3
		AGG17856WVIG_1_VRe TVNu343_VRe TVNu1388_VRe TVNu767_VRe TVNu161_VRe TVNu738_VRe TVNu1790_VRe TVNu916_VRe TVNu605 _VRe	

Furthermore, to examine the relationship that could exist between the proximate composition and the accessions, as well as the relationship between the accessions themselves, a principal component analysis (PCA) (XLSTAT) was performed using the means values for nutrient component in each accession. A correlation circle, combined with an observation chart, was obtained, as shown in Fig. 27. The analysis showed that the first (F1 = 49.74%) and second (F2 = 24.75%) PCA dimensions represent 74.49% of the initial information, which is the best combination and explains the variation among the accessions and traits. It was found that there is a positive correlation between the traits ash, moisture and protein, except for the lipid, fiber and carbohydrate traits, which is due to the angles between their vectors (Fig. 27). It was also noted that all the checks, together with a set of wild accessions, are found on the left side of the F1 axis, forming a group of accessions with lower values for the examined nutrients traits, except for the lipid, fiber and carbohydrates. Those accessions could share common features with the checks. A second group, made up of only wild accessions, was found on the right side of the F1 axis, representing the accessions with higher values for the evaluated traits (Fig. 27).

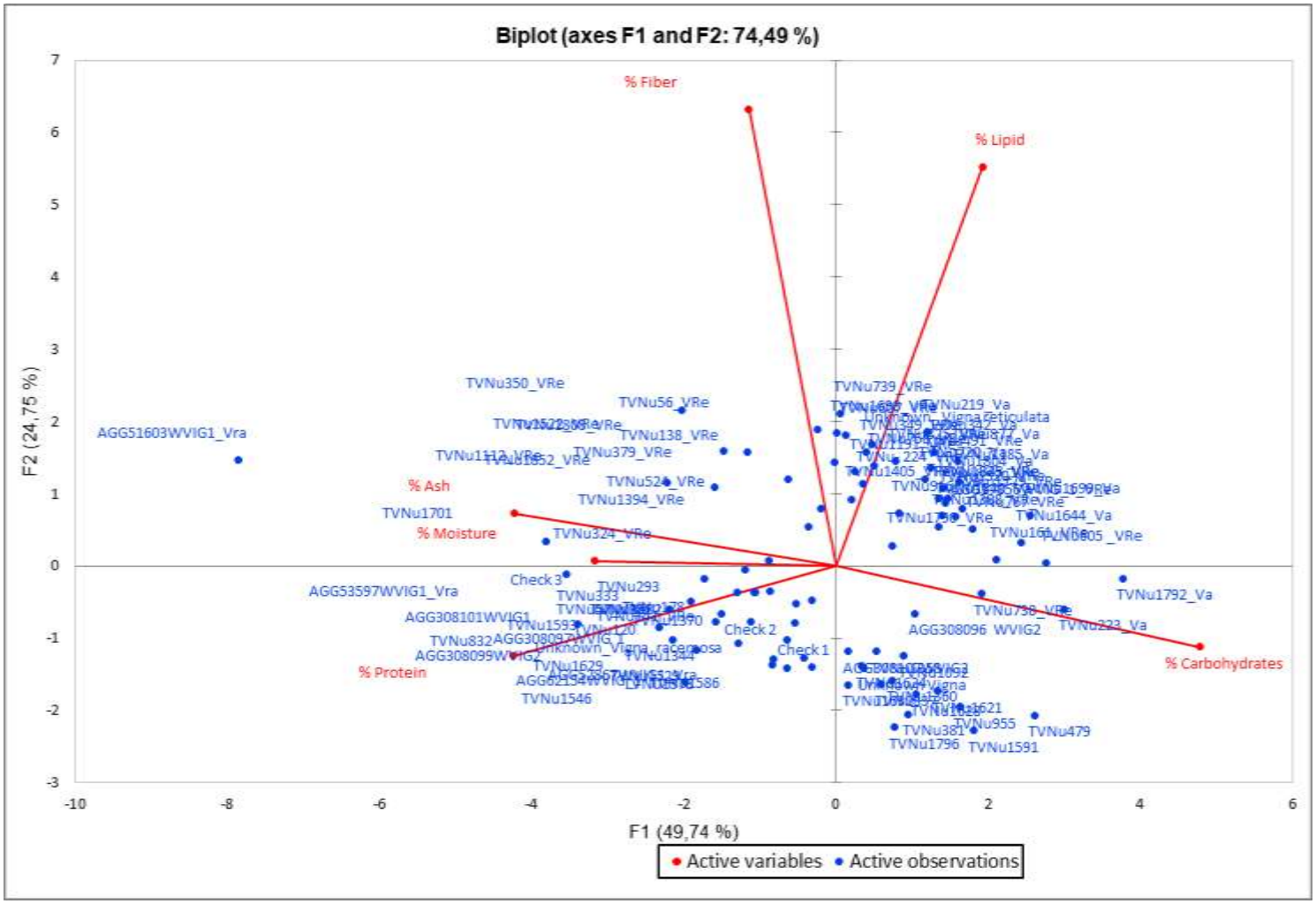


Figure 27: Result of the PCA Analysis Showing Correlations between Nutrients Contents and Wild *Vigna* Accessions

(vi) Mineral Content Evaluation of Wild *Vigna* Legume Species

Mineral Content of V. ambacensis Accessions

Figure 28 shows the mineral content of *V. ambacensis* accessions. Accessions TVNu- 1185 (0.951 mg/100 g) and TVNu-1792 (0.918 mg/100 g) have the highest copper (Cu) contents as compared with all the checks and other accessions. Check 1 present the highest concentrations for Manganese (Mn) and Zinc (Zn) while check 3 dominate in Iron (Fe) concentration.

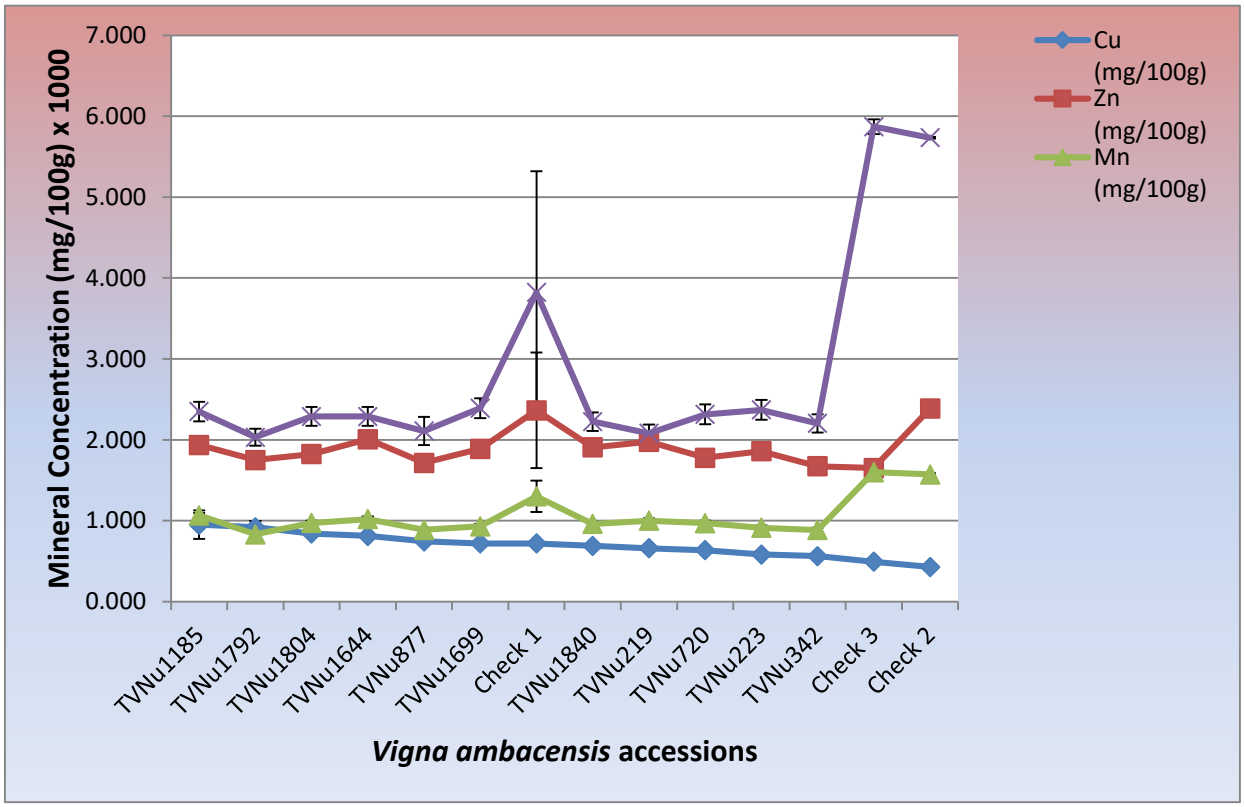


Figure 28: Mineral Content of *Vigna ambacensis* Accessions

Mineral Content of the V. reticulata Accessions

On Fig. 29, the dynamics of mineral content for the *V. reticulata* accessions can be observed. It is noted that accession TVNu1808 (1.253 mg/100 g) present the highest Cu concentration which is significantly higher than that of all the checks. The accessions TVNu758 (4.894 mg/100 g) has the highest Fe concentration among the wild accessions; however, that concentration is not significantly higher than that of Check 2 (5.734 mg/100 g) and Check 3 (5.870 mg/100 g) respectively. The checks dominated the Mn concentration though the

accession TVNu57 (1.206 mg/100 g) showed a comparable concentration to that of Check 1 (1.301 mg/100 g). TVNu-141 (2.673 mg/100 g) and TVNu1852 (2.667 mg/100 g) out-performed the checks with respect to Zn concentration.

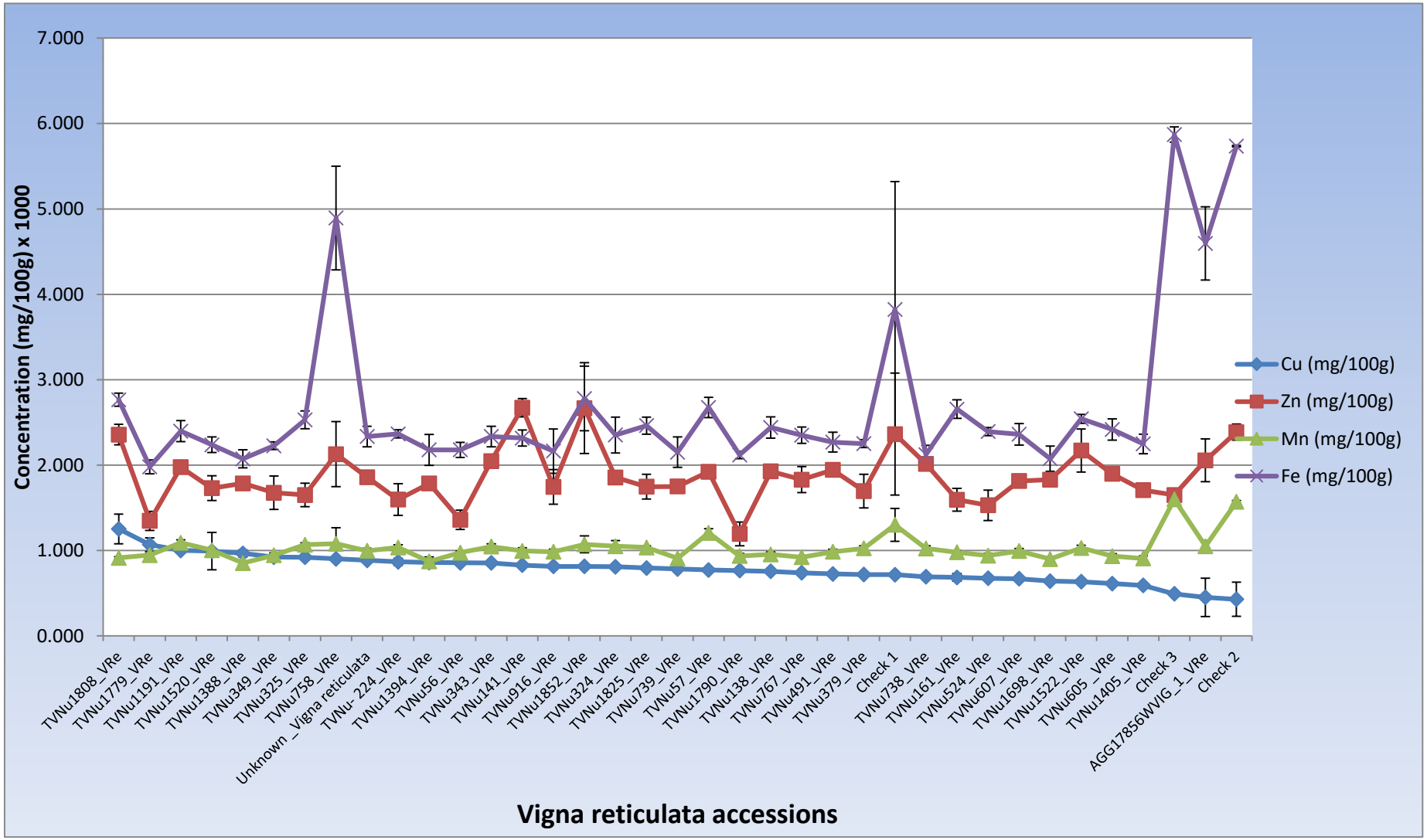


Figure 29: Mineral Content of *Vigna reticulata* Accessions

Mineral Content of the V. vexillata Accessions

For *V. vexillata* accessions (Fig. 30), it can be noticed that accessions TVNu-370 (0.807 mg/100 g) and TVNu-1628 (0.758 mg/100 g) had the highest Cu concentrations. In terms of Mn, accessions TVNu333 (2.756 mg/100 g), TVNu781 (2.407 mg/100 g) and TVNu1370 (2.496 mg/100 g) out-performed the checks. The accession AGG308099WVIG2 (3.180 mg/100 g) presented the highest concentration of Zn out-performing all the checks and other wild accessions. On the other hand, accessions TVNu1582 (6.563 mg/100 g), TVNu832 (6.229 mg/100 g) and TVNu333 (6.411 mg/100 g) had best Fe concentration beyond that of all the checks.

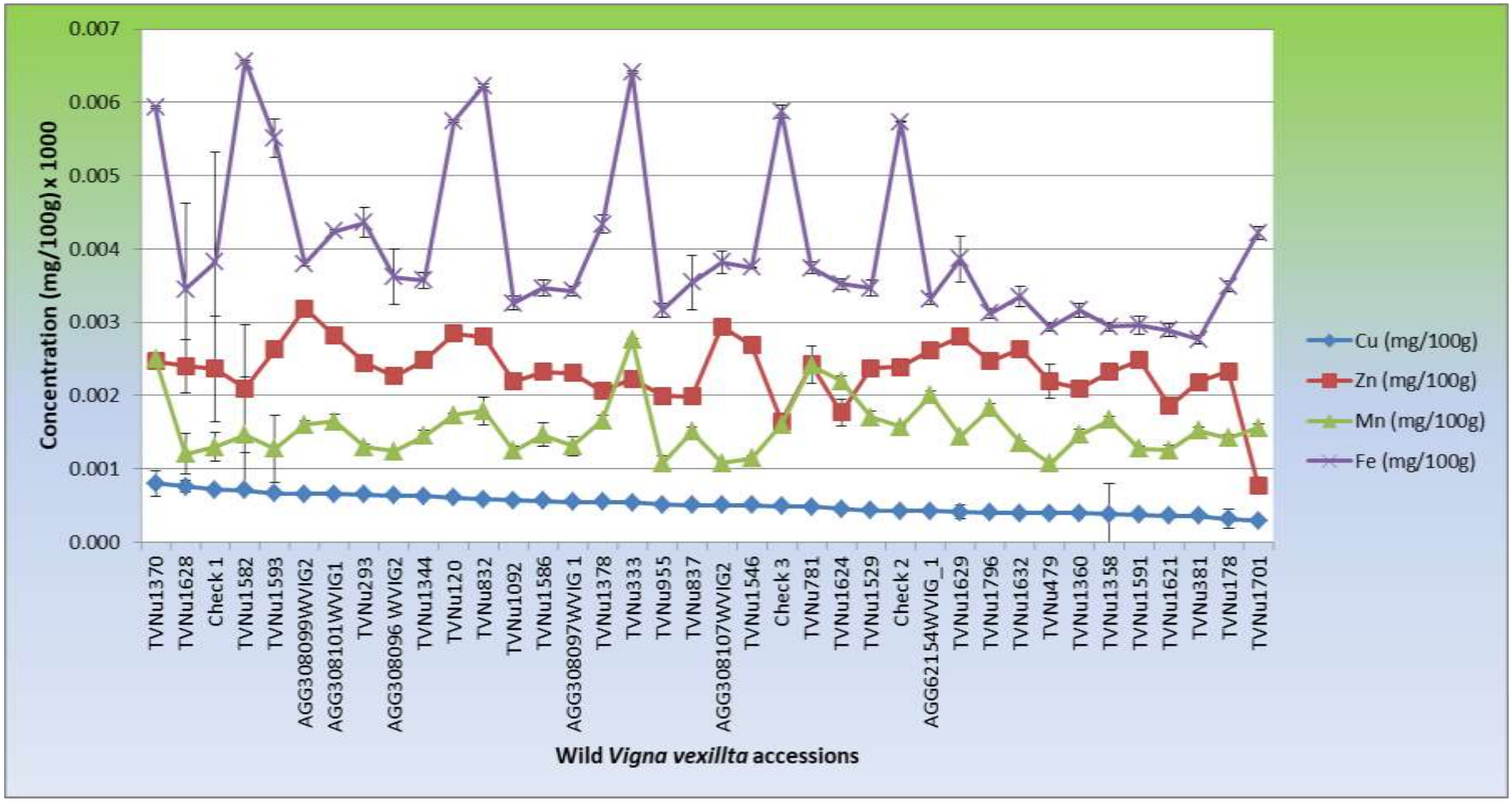


Figure 30: Mineral Content of *Vigna vexillata* Accessions

Mineral Content of the V. racemosa Accessions

A very remarkable accession (AGG51603WVIG1) among the *V. racemosa* accession (Fig. 31) presented the highest concentration in Zn (3.355 mg/100g), Mn (2.133 mg/100 g) and Fe (7.614 mg/100 g) respectively, out-performing all the checks and the other accessions. The mean value for each mineral taken in bulk as per species is presented in Fig. 32. The figure shows that the checks out-perform all the other wild accessions.

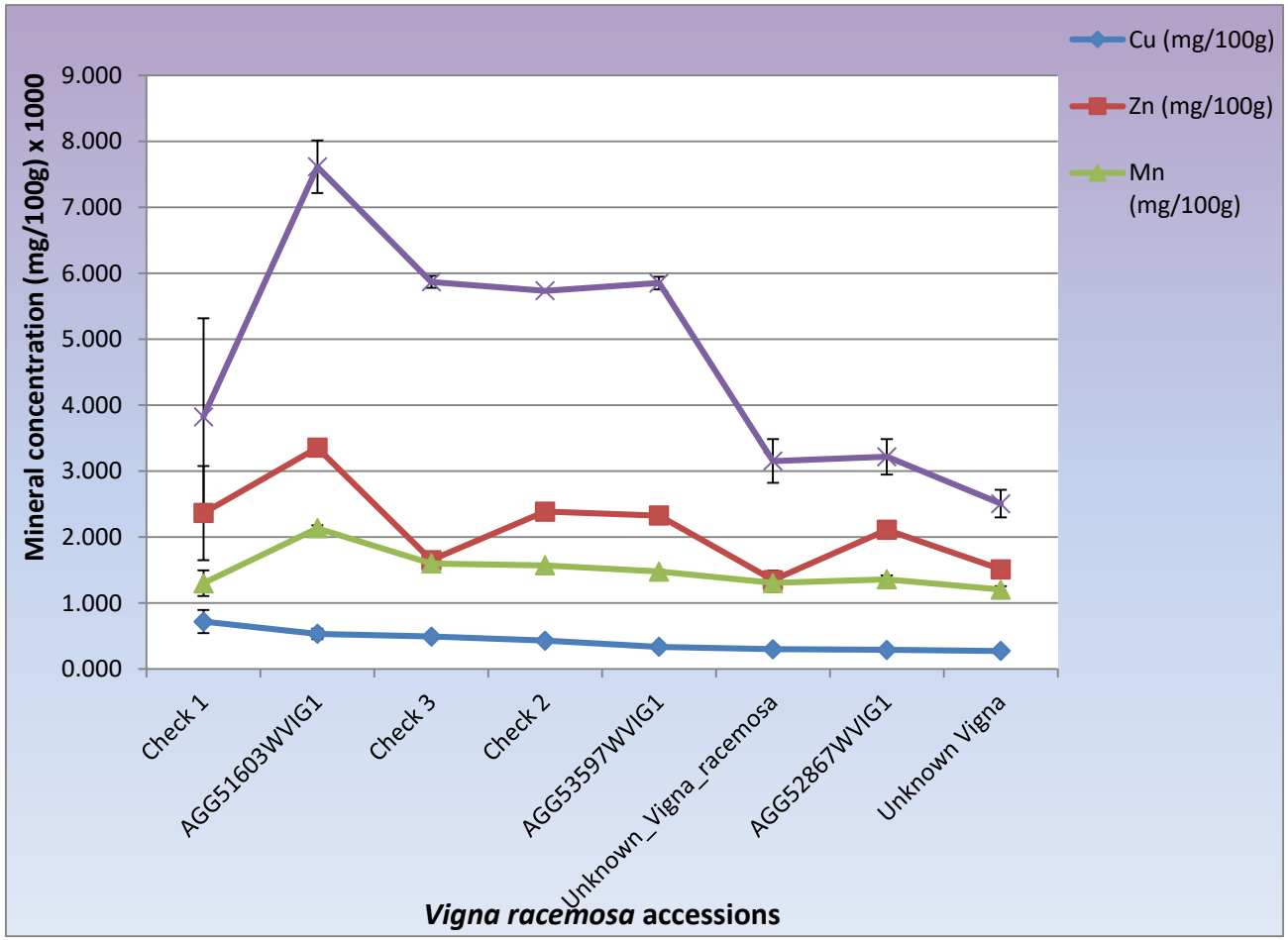


Figure 31: Mineral Content of *Vigna racemosa* Accession

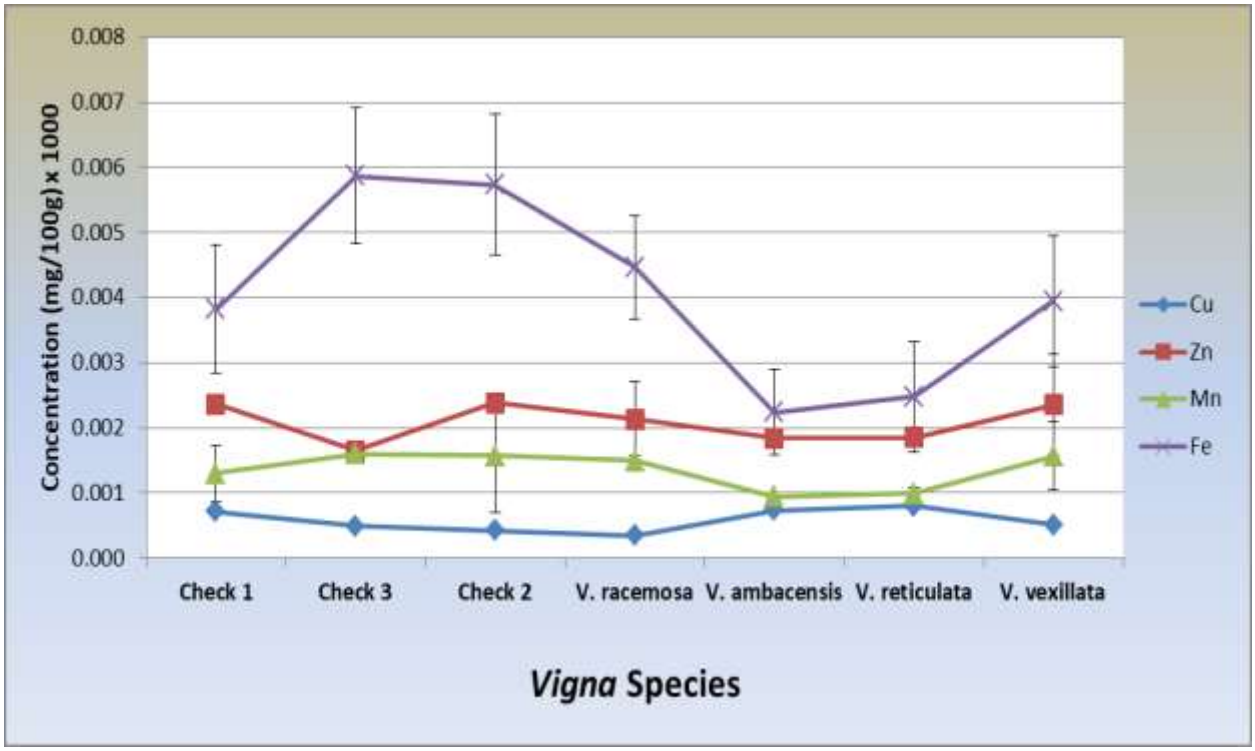


Figure 32: Mineral Content of *Vigna* Species

In order to group the accessions based on their phenotypic similarities in terms of the studied mineral composition, the agglomerative hierarchical clustering of XLSTAT was run to obtain a dendrogram (Fig. 33). The dendrogram shows clusters of wild *Vigna* accessions based on mineral compositions (Mn, Zn, Cu and Fe) studied. It reveals that based on the mineral content analysed, both wild accessions and checks can form three groups of legumes in which the two checks (Check 2 and 3) belong to the same group with some nine wild accessions (Table 24). Table 24 shows the details on the number of accessions belonging to the classes as grouped on the dendrogram.

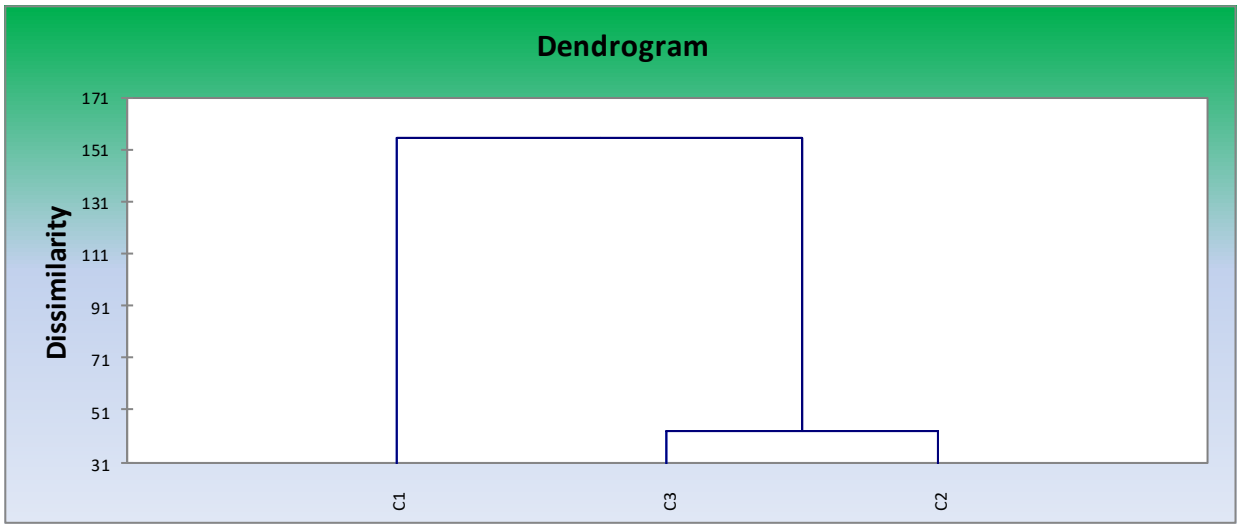


Figure 33: Dendrogram Showing Clusters of Wild *Vigna* Accessions Based on Mineral Composition

Table 24: Accessions Belonging to Classes as Grouped on the Dendrogram for Minerals

Class	1	2	3
Objects	43	34	11
Sum of weights	43	34	11
Within-class variance	0.137	0.562	0.971
Minimum distance to centroid	0.056	0.113	0.398
Average distance to centroid	0.303	0.651	0.832
Maximum distance to centroid	0.958	1.667	1.862
	TVNu1185_Va	Check 1	Check 3
	TVNu1792_Va	Unknown_*Vigna_racemosa*	Check 2
	TVNu1804_Va	AGG52867WVIG1_Vra	AGG51603WVIG1_Vra
	TVNu1644_Va	Unknown *Vigna*	AGG53597WVIG1_Vra
	TVNu877_Va	AGG17856WVIG_1_VRe	TVNu758_VRe
	TVNu1699_Va	TVNu1628	TVNu1370
	TVNu1840_Va	AGG308099WVIG2	TVNu1582
	TVNu219_Va	AGG308101WVIG1	TVNu1593
	TVNu720_Va	TVNu293	TVNu120
	TVNu223_Va	AGG308096 WVIG2	TVNu832
	TVNu342_Va	TVNu1344	TVNu333
	TVNu1808_VRe	TVNu1092	
	TVNu1779_VRe	TVNu1586	
	TVNu1191_VRe	AGG308097WVIG 1	
	TVNu1520_VRe	TVNu1378	
	TVNu1388_VRe	TVNu955	
	TVNu349_VRe	TVNu837	
	TVNu325_VRe	AGG308107WVIG2	
	Unknown _*Vigna reticulata*	TVNu1546	
	TVNu- 224_VRe	TVNu781	
	TVNu1394_VRe	TVNu1624	
	TVNu56_VRe	TVNu1529	
	TVNu343_VRe	AGG62154WVIG_1	
	TVNu141_VRe	TVNu1629	
	TVNu916_VRe	TVNu1796	
	TVNu1852_VRe	TVNu1632	
	TVNu324_VRe	TVNu479	
	TVNu1825_VRe	TVNu1360	
	TVNu739_VRe	TVNu1358	
	TVNu57_VRe	TVNu1591	
	TVNu1790_VRe	TVNu1621	
	TVNu138_VRe	TVNu381	
	TVNu767_VRe	TVNu178	
	TVNu491_VRe	TVNu1701	
	TVNu379_VRe		

Class	1	2	3
	TVNu738_VRe		
	TVNu161_VRe		
	TVNu524_VRe		
	TVNu607_VRe		
	TVNu1698_VRe		
	TVNu1522_VRe		
	TVNu605 _VRe		
	TVNu1405_VRe		

(vii) Fatty Acids Composition of Wild *Vigna legume* Species

The tables 25-27 show the distribution of the predominant fatty acids present in *V. reticulata*, *V. vexillata* and *V. racemosa* accessions. *V. ambacensis* accessions were not assessed for fatty acids because of the availability of very limited amount of samples to cover all the biochemical characterization. It is found that five fatty acids predominantly makeup the lipid composition of the studied *Vigna* species accessions. From the results, it is apparent of the three fatty acids found, three of them are saturated fatty acids (Hexadecanoic acid, Stearic acid, Heptadecanoic acid) while the other two are unsaturated fatty acids (9, 12-Octadecadienoic acid (Z, Z), 9, 12, 15-Octadecatrienoic acid).

Remarkably, the wild accessions are predominated by the saturated fatty acids while the unsaturated fatty acids are mainly found in the checks (domesticated legumes). However, the *V. racemosa* accessions show a predominance in unsaturated fatty acid too. Few accessions of wild *Vigna* species were also found with saturated fatty acids.

Table 25: Fatty acid Composition of *V. reticulata* Accessions (% composition)

Accessions	Hexadecanoic acid,	Stearic acid	Heptadecanoic acid	9,12-Octadecadienoic acid (Z, Z)	9,12,15-Octadecatrienoic acid, (Z, Z, Z)
Check 1	-	-	39.77	27.28	23.77
Check 2	-	-	47.82	26.53	16.55
Check 3	-	-	34.88	23.57	15.26
TVNu141_VRe	60.73	33.70	-	-	-
TVNu1112_VRe	21.40	40.87	30.52	-	-
TVNu1825_VRe	86.34	13.08	-	-	-
TVNu1698_VRe	-	-	46.05	21.76	18.83
TVNu350_VRe	58.30	32.35	-	-	-
TVNu56_VRe	20.54	39.23	29.30	-	-
TVNu1522_VRe	82.89	12.56	-	-	-
TVNu1808_VRe	-	-	44.21	20.89	18.08
TVNu607_VRe	57.69	32.01	-	-	-
TVNu379_VRe	20.33	38.82	29.00	-	-
TVNu1852_VRe	82.03	12.43	-	-	-
TVNu739_VRe	-	-	43.75	20.68	17.89
TVNu138_VRe	54.65	30.33	-	-	-
TVNu1405_VRe	19.26	36.78	27.47	-	-
TVNu349_VRe	77.71	11.77	-	-	-
TVNu325_VRe	-	-	41.45	19.59	16.95
TVNu758_VRe	52.83	29.32	-	-	-
TVNu1394_VRe	18.61	35.55	26.56	-	-
TVNu- 224_VRe	75.12	11.38	-	-	-
TVNu1191_VRe	-	-	40.06	18.93	16.39
TVNu1779_VRe	48.58	26.96	-	-	-
TVNu491_VRe	17.12	32.69	24.42	-	-
TVNu524_VRe	69.07	10.47	-	-	-
TVNu1520_VRe	-	-	36.84	17.41	15.07

Accessions	Hexadecanoic acid,	Stearic acid	Heptadecanoic acid	9,12-Octadecadienoic acid (Z, Z)	9,12,15-Octadecatrienoic acid, (Z, Z, Z)
AGG17856WVIG_1_VRe	75.98	11.51	-	-	-
TVNu324_VRe	-	-	40.52	19.15	16.57
TVNu343_VRe	53.44	29.65	-	-	-
TVNu57_VRe	18.83	35.96	26.86	-	-
TVNu1388_VRe	75.98	11.51	-	-	-
TVNu767_VRe	-	-	40.52	19.15	16.57
TVNu738_VRe	51.62	28.64	-	-	-
TVNu1790_VRe	18.19	34.74	25.95	-	-
TVNu916_VRe	73.39	11.12	-	-	-
TVNu605 _VRe	-	-	39.14	18.50	16.01

Table 26: Fatty Acid Composition of *V. vexillata* Accessions (% composition)

Accessions	Hexadecanoic acid	Stearic acid	Heptadecanoic acid	9, 12-Octadecadienoic acid (Z,Z)	9,12,15-Octadecatrienoic acid, (Z,Z,Z)
Check 1	-	-	39.77	27.28	23.77
Check 2	-	-	47.82	26.53	16.55
Check 3	-	-	34.88	23.57	15.26
TVNu120	52.74	34.02	-	-	-
TVNu1624	85.27	12.37	-	-	-
TVNu1370	56.29	34.57	-	-	-
TVNu1378	81.40	15.42	-	-	-
TVNu1529	-	-	37.43	26.03	10.75
TVNu1701	50.63	32.66	-	-	-
TVNu333	81.86	11.87	-	-	-
TVNu293	54.04	33.19	-	-	-
TVNu1582	78.14	14.81	-	-	-
TVNu832	-	-	35.56	24.73	10.21
TVNu178	50.10	32.32	-	-	-
TVNu781	81.01	11.75	-	-	-
AGG308101WVIG1	53.47	32.85	-	-	-
AGG308097WVIG 1	73.26	13.88	-	-	-
TVNu1593	-	-	33.69	23.42	9.67
AGG308096 WVIG2	47.46	30.62	-	-	-
TVNu1629	76.74	11.13	-	-	-
AGG308099WVIG2	48.97	30.08	-	-	-
TVNu1344	70.82	13.42	-	-	-
AGG308107WVIG2	-	-	32.57	22.64	9.35
TVNu1358	45.88	29.60	-	-	-
AGG62154WVIG_1	68.22	9.90	-	-	-
TVNu1546	45.03	27.66	-	-	-

Accessions	Hexadecanoic acid	Stearic acid	Heptadecanoic acid	9, 12-Octadecadienoic acid (Z,Z)	9,12,15-Octadecatrienoic acid, (Z,Z,Z)
TVNu1092	65.12	12.34	-	-	-
TVNu1586	-	-	29.95	20.82	8.60
TVNu1632	46.41	29.94	-	-	-
TVNu381	75.04	10.88	-	-	-
TVNu1360	49.53	30.42	-	-	-
TVNu1621	71.63	13.57	-	-	-
TVNu837	-	-	32.94	22.90	9.46
TVNu1628	46.41	29.94	-	-	-
TVNu1796	72.48	10.51	-	-	-
TVNu1591	47.84	29.39	-	-	-
TVNu955	69.19	13.11	-	-	-
TVNu479	-	-	31.82	22.12	9.14

Table 27: Fatty Acid Composition of *V. racemosa* Accessions (% composition)

Accessions	Hexadecanoic acid	Stearic acid	Heptadecanoic acid	9,12-Octadecadienoic acid (Z,Z)	9,12,15-Octadecatrienoic acid, (Z,Z,Z)
Check 1	-	-	39.77	27.28	23.77
Check 2	-	-	47.82	26.53	16.55
Check 3	-	-	34.88	23.57	15.26
AGG53597WVIG1	-	-	39.77	27.28	23.77
AGG51603WVIG1	-	-	47.82	26.53	16.55
Unknown_*Vigna_racemosa*	-	-	34.88	23.57	15.26
AGG52867WVIG1	-	-	39.77	27.28	23.77
Unknown *Vigna*	-	-	-	26.53	16.55

4.2 Discussions

4.2.1 Agro-morphological Exploration of Wild Unexplored *Vigna* Species for Domestication

The qualitative exploration of the wild *Vigna* species showed that there are variations in their characteristics for the same trait within the same species (section 4.1.1.), while all the checks expressed the same form of a particular trait throughout the experiments. Some of these qualitative characters are expressions of a genetic variation within the genome of the plant. A recent taxonomic differentiation was established between two wild *Vigna* species (*V. stipulacea* and *V. trilobata*) based on their morphological characteristics, such as germination habit, primary leave attachment and so on (Gore *et al*., 2019). Therefore, the variations observed could be due to the heterogenous nature of the wild accessions, which have been homogenized in the checks through selection and breeding processes. It is a common opinion of many researchers that wild crop populations are much older and more diverse than domesticated crops, having undergone millennia of recombination, genetic drift and natural selection (Smýkal *et al*., 2018a). Some of the trait forms found in the wild accessions might have only disappeared from the domesticated one during the domestication process. Some of the unique traits of the wild accessions, such as their leaf, stem and petiole pubescence, which are not found in the checks, might have existed in those checks but disappeared with time due to purposeful selection against the traits during the domestication process. They could have a potential use, if they are domesticated, since they are thought to be responsible for some beneficial traits, such as the resistance to diseases and pests (Oyatomi *et al*., 2016b; Popoola *et al*., 2017). Therefore, it might be time to start examining some of the traits from the wild species that have disappeared in order to domesticate new species. The qualitative characteristics of the wild *Vigna* accessions found in this study were in line with most of the characteristics found in earlier works, carried out on other wild *Vigna* species (Bisht *et al*., 2005; Gore *et al*., 2019).

Regarding the quantitative traits studied, Appendices 2 and 3 summarized the means, ranges and coefficients of variation at site A for only one season (the 2018 cropping season). This is due to the fact that during the 2019 cropping season at site A, the rainfall was not enough (as per the pattern shown in Fig. 4) to allow for germination and the growth of certain accessions. Most seeds did not germinate during that season, and those that did germinate (mainly checks) could not resist the harsh conditions. Figure 4 shows that the rainfall started at a very

low rate (92.9 mm), then achieved its peak value (196.6 mm) and stopped. This amount of rainfall might have not been sufficient for the soil to allow the germination of the wild accessions. It is also known that the seed structure could influence the germination of seeds (Smykal *et al.*, 2014; Swanson *et al.*, 1985). However, the characteristics of the seed structure of wild legumes are still yet to be reported.

The first cropping season at site A showed significant differences ($p < 0.05$) between the checks and the wild accessions for all the analyzed traits (Table 5a). Accession effects were found for all the traits, except for the number of flowers per raceme (trait 7) (Table 5a). This shows that the different accessions and species involved in the study possess different phenotypic and probably genetic characteristics. The number of flowers per raceme seemed to have no significant difference among the accessions and the checks. This might have been influenced by other agro-climatic conditions of the environment at that moment, which could affect some accessions, but probably not all. It has been reported that simple shading can affect the number of flowers per raceme (Jiang & Egli, 1993). The block effect observed at that site could be due to some particular factors of the field, ranging from agro-climatic to soil characteristics. The most probable explanation could be that the soil was heterogeneous in the same field and differently affected the checks. The ability of a plant to respond to soil characteristics can affect some of its physiological and phenotypic characteristics (Morgan & Connolly, 2013). In addition, such block effect might be due to some differences in slopes of the blocks which could have affected soil water retention capacity and variation in nutrients especially nitrogen.

A similar pattern of results was observed during the two cropping seasons at site B. The observed phenomenon could have the same explanation as in the case of site A, mentined above. Based on the result shown in Table 8, only the days to flowering, pods per plant, hundred seed weight and the yield were affected by their growing environment (accession x site effect), while only the number of flowers per raceme and the pods per plant were affected by the cropping season. These effects might be explained by the agro-climatic characteristics of each crooping site and season. As shown in Fig. 4, site A had lower and shorter rainfall characteristics, which can affect the days to flowering. This is in line with earlier reports that predicted that the changes in the flowering time are associated with a reduction in precipitation (Kigel *et al.*, 2011). The effect of the yield and yield parameters, such as the pods per plant and hundred seed weight traits, has been reported before in relation to other legumes, and these reports do not contradict the present findings (Sabaghnia *et al.*, 2008;

Satish *et al*., 2017). Therefore, it should be recommended that these traits be taken into consideration during any attempt to domesticate or improve wild legumes. The number of flowers per raceme and the pods per plant were the only traits affected by the cropping season. These two traits are closely related, as confirmed by the positive correlation that exists between the two as shown in Fig. 10. They could also be directly or indirected affected by the variations in temperature and rainfall, as per the earlier explanation (Kigel *et al*., 2011; Morgan & Connolly, 2013). The significant effect of the season on the number of flowers per raceme and pods per plant has also been reported in relation to the landraces of *Phaseolus vulgaris* and cowpea (*Vigna unguiculata*) (Adewale *et al*., 2010; Arteaga *et al*., 2019). These two traits also need to be considered in any attempt at domestication.

Figure 9 revealed that the wild *Vigna* accessions could be grouped into three clusters, with one larger cluster (cluster I), including two checks as shown in Table 9. This shows that some of the wild accessions share common features and probably genetic characteristics. Cluster I, containing the checks, could offer a clear orientation for the selection of candidates for domestication. Cluster 1 could also offer recommendations pertaining to the cooking time and water absorption capacity traits as reported earlier (Harouna *et al*., 2019a). These are clear indications that these wild legumes could be domesticated and made useful, as the preliminary finding showed that farmers would be interested in utilizing them for various purposes (Harouna *et al*., 2019b). In fact, it has recently been reported that *Vigna* stipulacea, another wild legume species with biotic resistance traits could be domesticated (Takahashi *et al*., 2019). However, it is also necessary to note that domestication process could also affect the nutritional and health characteristics of the domesticated product as warned by some researchers(Smýkal *et al*., 2018b). Therefore, the choice of *V. vexillata, V. reticulata, V. ambacensis* and *V. racemosa* species in this study was first based on their availability in genebanks and from the little preliminary information obtained from previous investigations (Harouna *et al*., 2019a; Harouna *et al*., 2018; Harouna *et al*., 2019b).

Figure 10 provides further insights relating to the domestication of these wild legumes by grouping them based on their quantitative agro-morphological traits. It was shown that most of the quantitative traits are positively correlated, and there is a degree of commonality between the checks and a group of some wild species.

4.2.2 Farmers' Perceptions, Preferences and Prospective Uses of Wild *Vigna* Species for Human Exploitation

The explorative survey above shows that women were more engaged in legume farming in the two zones compared with men. Similarly, the contribution of women in agricultural activities is well-known in Africa (Njuki *et al*., 2013). In this study, no statistical significance was found between gender influence and prior knowledge about legumes. This means that being a woman or a man does not influence the probability of being aware of wild legumes.

Legume farming was mainly practiced by the older participants (Fig. 11b). This indicates that the younger generations in the legume growing areas were not very interested in legume-farming activities or farming other crops. In general, belonging to any age group did not influence the prior knowledge about the legumes, due to the long period of disappearance of the wild genotypes, which led to the ignorance of many generations (Briggs, 2017; Harouna *et al*., 2018; Tomooka *et al*., 2014). However, belonging to the 15 to 20-year-old age group showed a slight influence on the prior knowledge of wild legumes. This may suggest that farmers in this age range may possess some understanding of wild legumes.

The education level of farmers and their farming experience showed no significant influence on their prior knowledge of wild legumes, which meant that being educated or well experienced in farming legumes did not influence the knowledge of wild legumes. This showed that both experienced and non-experienced farmers as well as educated and non-educated farmers might have the same perception and prior background about wild legumes. In addition, it implied that both farming experience and level of education may not be necessary when making policy decisions about the implementation or adoption of a wild legume as a new crop. However, this is in contradiction with other studies carried out using other domesticated crops such as rice and maize which found that farming experience and education level are important factors to consider in introducing improved varieties (Himire *et al*., 2015; Hussein *et al*., 2015). Then, it is necessary for further research to try such experiences with other wild crops in other parts of the world to ascertain this fact.

From the results, the location (research site) has a significant effect on the prior knowledge of wild legumes, meaning that being in the Arusha region increased the chance of knowing wild legumes. Decision making regarding the adoption of wild *Vigna* legumes needs to take the location of farmers into consideration. This is in line with earlier reports (Mwangi *et al*.,

2015). This could be explained by the fact that Arusha region is more populated by a certain ethnic group of people (the Maasai) who are well-known in Tanzania for their indigenous ethno-medical knowledge of plants (Ibrahim & Ibrahim, 1998; Ibrahim *et al*., 2012). They are also found in the high-altitude agro-ecological zone (Kilimanjaro), but they are more concentrated in the mid-altitude agro-ecological zone of Arusha (Ibrahim & Ibrahim, 1998).

The ignorance of the wild legumes by the majority of participants in the two study sites may be due to the high and long-term distribution of bred, improved and landrace varieties of legumes that led to the disappearance, rejection and negligence of the original wild legumes (Harouna *et al*., 2018a). However, the numerous challenges (biotic, abiotic, and policy) faced by the improved varieties have recently raised scientific concerns (Ojiewo *et al*., 2018). Therefore, it might be important to go back to the wild and investigate other legumes with good characteristics in relation to their acceptability in order to mitigate the global food insecurity challenge, as pointed out by earlier reports (Porch *et al*., 2013).

It is noted from this study that despite the high ignorance noted by the majority, the wild legumes are still used for various purposes, including human consumption by a minority. It has also been noted that ignorance or knowledge/awareness of wild legumes significantly depends on the location of the farmers rather than their gender, age group, or farming experience. This could be explained by the fact that some ethnic groups of people with significant traditional and indigenous knowledge of plants are concentrated in some parts of the world (Ibrahim & Ibrahim, 1998). Then, it would be wise to carry out more investigation on such legumes in order to domesticate more varieties possessing resistance to the current legumes challenges. From this study, the main challenges experienced by legume farmers in the two study sites were diseases and low rainfall, which might definitely be due to climate change, as it is global challenge (Boukar *et al*., 2016). Therefore, alternatives varieties of legumes with resistance to climate variability and diseases would be of great benefit to such similar communities. The study also attempted to screen some accession of choice by the same farmers based on the general appearance, pods, and seeds of some of the wild legumes in order to select varieties for domestication.

Furthermore, it was observed that the prior knowledge about wild legumes is independent of gender, age, education level, and farming experience, but dependent on the farmers' location. However, it is curiously noted that after carefully sighting the wild *Vigna* legumes performing in the field by participants, it is revealed that there is a significant relationship

between the farmers' preferences and their gender, farming experience, and location (likelihood ratio test). This could be explained that the knowledge of wild legumes increases farmers' attraction and preferences of wild legumes depending on their gender, farming experience, and location. Less than 50% (74 out of 160) of the planted accessions were preferred by farmers in both research sites (Fig. 14), showing that several accessions had common preferences depending on the locations. Although this could be influenced by the number of accessions that reached an appreciable growth level by the selection period, the selection should depend on other parameters such as farmers' gender (G^2 = 130.813, df = 73, $p < 0.000$), farming experience (G^2 = 669.196, df = 511, $p < 0.000$), and location (G^2 = 1110.606, df = 73, $p < 0.000$), as confirmed by the X^2 test. In a similar study, significant correlations between preferences of male and female farmers in an on-farm trial indicated that both groups have similar criteria for the selection of rice varieties in India (Burman *et al*., 2017). Experiments investigating farmers' knowledge about unknown or wild food crops are lacking or almost non-existent in the literature (Beukelaar *et al*., 2019). The wild *Vigna* species are not well-known legumes, which could be the reason taxonomic characterizations have still been under investigation by scientists until recently (Gore *et al*., 2019).

The ignorance of wild legumes by the majority explains the few uses suggested by the farmers as compared with the uses suggested after field visits to farms with wild *Vigna* legumes (Fig. 12 and Table 11). Several uses have been suggested by farmers after sighting the wild *Vigna* legumes in farms, showing their interest and motivation to adopt some of the wild crops for human benefit. This is in accordance with findings from earlier research studies carried out with domesticated legumes possessing characteristics that are not well-known (Bekele, 2016; Bruno *et al*., 2018). It was observed that the farmers were willing to adopt some of the crops for several human exploitation purposes, although some need more improvement. It is also noted that some farmers even had experience consuming some of the wild *Vigna* legumes. Therefore, farmers generally perceived the wild *Vigna* legumes as exploitable resources for a variety of purposes that lack awareness and scientific attention. A recent report also demonstrated participant eagerness to adopt wild vegetables (duckweed) as human food upon first-time observations from a picture (Beukelaar *et al*., 2019).

This study also shows that there is a high probability that any sample of farmers taken in Tanzania and any other region of the world would ignore the existence of wild legumes. Therefore, considering food insecurity levels in the developing world, the dependence on a

few accessions of legumes, and the challenges faced by farmers and consumers regarding domesticated legumes, there is a need to further study these un-exploited legumes and orient their utilization. Very limited reports on approaching the assessment of participants, farmers, or consumers' perception, appreciation, or adoption of wild plants as human food exist.

4.2.3 Assessment of Water Absorption Capacity and Cooking Time of Wild Under-Exploited *Vigna* Species towards their Domestication

The values for water absorption capacity and cooking time showed no significant difference when compared with the values of their corresponding accessions harvested in the other agroecological zone for all the accessions tested. This could be due to the existence of a very slight difference in the characteristics of the two agroecological zones that could not significantly affect the genetic performance of the *Vigna* genus regarding the weight, water absorption, and cooking time. This is further justified by the fact that the interaction effect (location × genotype) showed that the differences observed for cooking time and water absorption capacities do not depend on location in all the accessions tested in this study (Tables 12a, 13–16). In the same line, a recent report revealed that the agroecological conditions could affect some nutrients like amino acids, protein, and minerals in quinoa but have no effect on their saponin and fiber content (Reguera *et al*., 2018). Furthermore, this study also demonstrates that the replication of the same species within the same location does not depend on the other species for the water absorption capacity trait (Table 12b.), while for cooking time trait, there is an interaction with other species within the same location (Table 12c). This could be an important characteristic to be exploited in breeding programs.

The non-significant or significant changes observed in the mean seed weights of some accessions when compared before and after soaking depicted here by their water absorption capacity values could be explained by the fact that some accessions possess a more water permeable seed coat than others (Tables 12–16). The seed coat water permeability as a phenotype possesses a crucial role in legumes cooking properties and germination (Smykal *et al*., 2014). However, the development of legume seed coat has not yet been characterized at a molecular level to strongly support its genetic implication (Thompson *et al*., 2009). A study involving legume showed that the water absorption of dry beans differs between varieties (Zamindar *et al*., 2013).

Looking at the *V. ambacensis* species, all the wild accessions exhibited significantly lower water absorption capacity values as compared with all three checks (Table 13). The accession TVNu342, with a water absorption capacity not significantly different from the checks exhibited a higher cooking time. This could imply that not only the water absorption capacity is directly or indirectly linked to cooking times of legumes and requires further physiological investigation. The genus *Vigna* possess a very large number of species in which very few have been studied extensively. The *V. ambacensis* is among the not very much studied species (Harouna *et al*., 2018). The very first comprehensive web genomic resource of the genus *Vigna* has just recently been published and that covered only three commercially domesticated species (Jasrotia *et al*., 2019; Smykal *et al*., 2014). Taxonomic re-arrangements are also still under investigation (Gore *et al*., 2019) and efforts to domesticate some of the selected wild *Vigna* species is in progress (Harouna *et al*., 2018; Harouna *et al*., 2019b). Pearson correlation analysis shows that there is no correlation between the water absorption and cooking time considering only the three domesticated species ($r = -0.025$). This could be due to some individual physiological differences or similarities among the tested accessions which requires further examination at molecular level as reports on *V. ambacensis* studies are very scanty and need to be addressed for proper exploitation of its full potential towards domestication (Harouna *et al*., 2018).

For the *V. vexillata* species, Table 14 showed that there are some phenotypic similarities between the wild accessions with the cowpea and rice bean with regard to their water absorption capacity values as many accessions show no significant different values with those for the two checks. Henceforth, it requires further investigations at molecular level involving phylogenetic analysis to establish a strong relationship between the accessions. In this regard, it is noted that the genetic diversity and structure of *V. vexillata* as well as many wild *Vigna* legumes are still under investigation (Corrêa *et al*., 2010; Gore *et al*., 2019; Harouna *et al*., 2018; Thompson *et al*., 2009). The idea is also supported by an earlier report that stipulated that domestication of the commercial *V. vexillata* (zombie pea) is not certain and it took place more than once in different regions (Dachapak *et al*., 2017). Concerning the cooking time (Table 14), there is a high diversity in differences among the accessions. This could also explain why there is a weak negative correlation between the water absorption and cooking time considering the wild *V. vexillata* tested ($r = -0.31$) (Fig. 22).

Wild *V. reticulata* species revealed that there is no significant difference between accessions regarding water absorption capacity (Table 15) with cowpea and rice bean except for TVNu1520, TVNu325 and the *V. vexillata* landrace. This demonstrates a considerable variability among the accessions as far as water absorption capacity is concerned as a phenotypic trait. Considering the cooking time (Table 15), a high diversity in differences of means among the accessions is noticed. Twenty-five accessions show no significant difference to each other but they are significantly different from the *V. vexillata* landrace and rice bean, while cowpea showed no significant difference to TVNu325 and the unknown *V. reticulata* accession. Curiously, scanty information about *V. reticulata* is also noticed. The genotype interactions in both water absorption capacity and cooking time phenotypic traits simply demonstrate the phenotypic diversity of these accessions which is very important in breeding.

Though very few accessions were included in this study, *V. racemosa* species present more phenotypic similarities with the *V. vexillata* landrace and cowpea with respect to the water absorption capacity and cooking time traits studied. It was revealed from the results that there is no significant difference between the means of the following wild accessions (AGG51603WVIG1, AGG53597WVIG1, AGG52867WVIG1) regarding their weights before soaking and the *V. vexillata* landrace and cowpea. The weights taken after the soaking process revealed a similar phenomenon while the water absorption shows closeness to rice bean. In the cases of cooking time, two accessions seem to be related to the *V. vexillata* landrace. All these assumptions need further investigations as *V. racemosa* also suffer from scanty information.

This study also showed that there is a strong negative correlation between the water absorption and the cooking time with a correlation coefficient of $r = -0.69$ which follows the equation: $Y = -7.99\,x + 26.52$ ($R^2 = 0.48$) for site A and $Y = -8.21x + 26.57$ ($R^2 = 0.50$) for the site B (Fig. 23). This result is in line with previous reports. For example, an early report proved that the cooking time was longer in bean varieties without prior soaking (Corrêa *et al*., 2010). A similar result was found within classes of oriental noodle, in which cooking time was significantly shortened with increase in water absorption (Hatcher *et al*. 1999). This could be an important parameter to guide the breeding of legumes with regards to cooking time when knowing their water absorption capacity.

Agglomerative hierarchical clustering (AHC) analysis performed on all the four species revealed the existence of seven classes when weight of accessions before the soaking process, water absorption capacity, and cooking time are taken as parameters (Fig. 24). Details of various accessions belonging to each class are provided in Table 17. The analysis shows that some accessions between the four species can be grouped together in the same cluster as they present similar traits or relationship. It is in line with what the first comparison based on Tukey analysis showed in this study. For example, class 1 consists of *V. vexillata*, *ambacensis* and *reticulata* accessions while class 2 is mainly *V. vexillata* with few *V. reticulata*. It is also noted that all *V. ambacensis* are grouped in class 1. This can simply imply that there are phenotypic trait similarities of the accessions within species with each other and with checks. However, further molecular investigations are needed to fully investigate assumptions of any genetic relationship within and between species. The classifications of the *Vigna* species remain a continuous and evolving process as their origin are still a subject of speculations. For example, it is reported that the Asian *Vigna* were still belonging to the genus *Phaseolus* until 1970 (Tomooka *et al*., 2011). It is generally speculated that the *Vigna* might have originated from Africa and evolved from the African genus *Wajira* as it is basal compared with *Vigna* and *Phaseolus* (Tomooka *et al*., 2011). Although, little attention has been paid to the conservation of the African wild *Vigna* species as more than 20 species are apparently not conserved in any ex-situ collection despite their several ethnobotanical uses (Tomooka *et al*., 2011). Therefore, it could be speculated from this study that accessions in groups 3, 4 and 5 are likely candidates for domestication since these groups contain the check lines, though further investigations are required.

Based on a general assessment view, the values of yield per plant for the wild *Vigna* species studied here are lower than those of the domesticated species, especially cowpea and rice bean (Table 18). A similar finding was reported by an earlier report (Shafaei *et al*., 2016). However, it might be important to note that the yield per plant for these wild legume accessions may be influenced by their seed characteristics because some of them could have a high number of seeds per plant with a surprising low weight as compared with the domesticated ones that produced fewer numbers of seed. The low seed weights in wild accessions could be attributed to their small seed sizes compared to domesticated ones with bigger seed sizes. The domesticated species here could have certainly acquired bigger seed sizes during the domestication process. Copious amount of small sized seed is one of the characteristic of weeds to enhance their reproduction chances and survival. Seed size is one

of the important domestication traits (Schlautman *et al*., 2018) that should be considered by breeders in the course of improvement and domestication of these wild legumes as they all presented smaller seed sizes by mere looking. From this study, yield, water absorption capacity and cooking time are apparently not related, though they are very important traits that need to be considered in breeding and selection of wild candidates for domestication. This may be due to the fact that yield mainly depends on seed physical characteristics such as seed size, seed weight, and seed number, while cooking time and water absorption capacity depends on seed physiological characteristics such as seed coat biosynthesis (Smykal *et al*., 2014). This could also be supported by the high variation in yield between locations as compared with low variations in cooking time and water absorption capacity (Table 18). In the same vein, it is also noted that the domesticated legumes possess high values of yield per plant in addition to their low cooking time and high water absorption capacity values as compared with the wild ones. Such characteristics might be among the factors that hinders their utilization as earlier reported (Harouna *et al*., 2019b). Yield is a very important trait in crop domestication. However, these wild legumes with multipurpose utilizations as suggested by farmers in our earlier investigation (Harouna *et al*., 2019b) fit well as candidates for domestication considering the domestication criteria established by researchers recently (Schlautman *et al*., 2018). Crop domestication of novel species is becoming one of the potential alternatives to mitigate the global food security challenge.

4.2.4 Biochemical Characterization of Wild *Vigna* Species

(i) Proximate Composition Exploration of Seeds of Wild *Vigna* Species

Check 1 and Check 2 might be related in terms of the lipid, fiber and carbohydrates content though they are of different species (*V. vexillata* and *V. unguiculata*). In addition, it was noted that Check 1 is landrace of *V. vexillata* which has not yet been fully domesticated as it is noticed that taxonomic arrangements within the *Vigna* genus are not completed (Gore *et al*., 2019). Phylogenic proximity between *V. vexillata* and *V. unguiculata* has also been reported (Boukar *et al*., 2013). However, the differences observed between the three checks or between Checks 1 and 2 with Check 3 for the nutrients evaluated can simply be attributed to their species differences. This can be the most probable explanation to the results obtained showing differences and similarities in terms of some nutrients between the three checks.

According to Table 19, the lipid content of all the wild accessions of *V. ambacensis* is similar to those of check 1 and 2 while it is significantly ($p<0.05$) higher than that of check 3. This is in line with reports that support the idea of constituents reduction in legumes due to domestication (Marín *et al.*, 2014). Other differences among the checks and the wild accessions may be due to species differences and phylogenic relationships. It is suggested that accessions with higher nutrients than that of the domesticated species should be further investigated for breeding or domestication.

From Table 20, the lipid content of most of the wild accessions of *V. reticulata* is not significantly different from that of Check 1 and 2 except for few accessions (TVNu1394_VRe, TVNu324_VRe, TVNu57_VRe, TVNu141_VRe) which are comparable to Check 3. All the accessions show comparable ash content to that of the three checks indicating that none of the accessions had higher ash content than that of the checks. This can be due to species or phylogenic proximity of *V. reticulate* with Check 1 and 2 (Table 23). All the accessions showed lower moisture content than that of the three checks. The low moisture content observed in wild accessions can be related to the seed characteristics and probably the genetic makeup of the *V. reticulata* accessions. As it was earlier reported seed characteristics of wild legumes affect their composition and cooking characteristics (Altuntas & Demirtola, 2007; Ereifej, 2004; Harouna *et al.*, 2019a). The accession with highest protein content (TVNu1112_VRe, 31.074%) might be a suitable genetic material for domestication or breeding and therefore should be further investigated through molecular marker as its high protein content might be due to its genomic difference. This might have been acquired based on the growing environmental conditions. For the accessions with protein content comparable to that of check 1 and check 2 (TVNu1852_VRe, TVNu141_VRe, TVNu57_VRe, TVNu324_VRe, TVNu350_VRe), phylogenic studies as well as breeding and improvement is recommended. The rest of the accessions with very low protein content which is lower than that of check 3 should be exploited for other nutritional elements. It is noticed that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents as compared to the checks. This is in line with earlier reports on wild legumes (Difo *et al.*, 2015; Macorni *et al.*, 1997). It might be due to the biosynthesis of many polysaccharides by the wild legumes in order to protect the embryo and survive in harsh environments (Smykal *et al.*, 2014). Therefore, it should be recommended to carry-out sound examination of the carbohydrates and fiber fraction to ascertain the digestibility and clear nutritive contribution of the carbohydrates and fiber contained in these seeds.

The proximate composition of *Vigna vexillata* accessions displayed in Table 21 shows that the lipid content of most of the wild accessions is significantly lower from that of Check 1 and 2 except for a few accessions (AGG308096WVIG2, TVNu333, TVNu293 and TVNu 832) which are higher than that of Check 3. This could be attributed to species and genomic differences as explained in the case of *V. reticulata*. Similar to the *Vigna reticulata* species, all the accessions showed comparable ash content to that of the three checks which can be explained by the same reasons as elaborated earlier. A significant number of accessions showed comparable moisture content to that of the checks indicating phenotypic similarity in moisture content.

The accessions with highest protein content within this species (TVNu1701, AGG30801WVIG1) are speculated to be suitable genetic materials candidate for domestication or breeding and therefore should be further investigated through molecular marker as their high protein content might be due to their genomic difference. This might have been acquired based on the environmental condition they were grown. For the accessions with protein content comparable to that of Check 1 and Check 2, phylogenic studies as well as breeding and improvement is recommended. The rest of the accessions with very low protein content which is lower than that of Check 3 should be exploited for other nutritional elements. As it was also noticed that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents as compared to the checks, it concurred with earlier reports on wild legumes (Difo *et al*., 2015; Macorni *et al*., 1997). It might be due to the biosynthesis of many polysaccharides by the wild legumes in other to protect the embryo and survive in harsh environments (Smykal *et al*., 2014) as explained earlier.

In the case of *Vigna racemosa* accessions (Table 22), the same trend of result of proximate composition was observed as in the cases of *V. reticulata* and *V. vexillata*. Therefore, same explanation could be attributed to the variations observed in their proximate composition.

To have a quick view on the proximate composition of the wild accessions in order to appreciate their content per species, the mean of each component for all the accessions belonging to each species was presented (Fig. 25). It reveals that looking at the variations in proximate composition globally per species, there is no significant difference between species vis-à-vis other species and the checks. This is simply due to a mathematical effect that

buffered the highest and lowest values of the means of each nutrient for each separate species.

From Fig. 26 it is apparent that the wild *Vigna* accessions could be grouped into three classes based on the proximate composition, with class I (C1) including all the three checks (Table 23). This shows that some of the wild accessions share common features and probably genetic characteristics. Class I, containing the checks, is speculated to offer a clear orientation for the selection of candidates for domestication. This result is in line with previous findings on the same types of wild legumes pertaining to the cooking time and water absorption capacity as well as agro-morphological traits as reported earlier (Harouna *et al*., 2020; Harouna *et al*., 2019a). These are clear indications that these wild legumes could be domesticated and made useful, as the preliminary finding showed that farmers would be interested in utilizing them for various purposes (Harouna *et al*., 2019b). In fact, it has recently been reported that *Vigna* stipulacea, another wild legume species with biotic resistance traits could be domesticated (Takahashi *et al*., 2019). However, it is also necessary to note that domestication process could also affect other nutritional and health characteristics of the domesticated product as opined by some researchers(Smýkal *et al*., 2018b). Also, Fig. 27 provides further indications relating to the domestication of these wild legumes by grouping them based on their quantitative proximate composition traits analysed through PCA. It was shown that most of the nutrients analysed are positively correlated, and there is a degree of commonality between the checks and a group of some wild species.

(ii) Mineral Content Evaluation of Wild *Vigna legume* Species

Examination of the mineral content of the wild and domesticated *Vigna* legumes as shown in Fig. 28-33 demonstrated that some wild accession possess a considerable amount of a specific or a combination of minerals that can point out their relevance in human and animal nutrition. This is in line with speculations earlier suggested and recommended by researchers (Harouna *et al*., 2020, 2018; Harouna *et al*., 2019). For instance, an accession like TVNu-1792 (0.918 mg/100 g) having the highest amount of Cu could satisfy the recommended dietary allowance (RDA) of a male adult just by consuming 100 g of it as the recommended dietary requirement is known as 900 µg/d (NCBI, 2020). Another tangible example to demonstrate the importance of these wild species in terms of minerals is the case of Fe. The RDA of Fe for an adult male is about 8 mg/day, therefore, the consumption of just 150 g of

accession AGG51603WVIG1 (7.614 mg/100 g) if maintained and all absorbed after digestion can satisfy this need.

Agronomic bio-fortification is unanimously emphasized by many researchers and organizations nowadays as a measure to fight against hidden hunger due to micro-nutrients deficiencies in humans (de Valença *et al*., 2017). On the other hand, it is found that many of the domesticated crops (including legumes) suffer from low concentration of one or more minerals. To this end, is added the challenge of breeding the wild with the domesticated accessions to improve the mineral content (Takahashi *et al*., 2019). Therefore, domestication of the wild accessions is one of the good methods to improve the micro-nutrient composition of crops and add more varieties for human nutrition and biodiversity conservation (Singh *et al*., 2019). Move to recommendation section.

The reason for higher concentration of some minerals in these wild accessions is not yet elucidated but it could be speculated as adaptive mechanisms to cope is edaphic stresses like salinity. It can also be attributed to the genetic makeup of seeds or due to their environmental origin. However, this needs to be investigated as very few reports exist in documentation of the biochemical characteristic of the wild *Vigna* legumes.

(iii) Fatty Acid Composition of *Wild Vigna legume* Species

It was found that five fatty acids predominantly makeup the lipid composition of the studied *Vigna* species accessions. The important roles played by fatty acids in human nutrition are undeniable (FAO, 2010). From the tables, it can also be noticed that three of the fatty acids found are saturated fatty acids (Hexadecanoic acid, Stearic acid, Heptadecanoic acid) while the two others are unsaturated fatty acids (9, 12-Octadecadienoic acid (Z, Z), 9,12,15-Octadecatrienoic acid. Evidence of the presence of saturated and unsaturated fatty acids in underutilized legumes have been reported (Ade-Omowaye *et al*., 2015). The predominance of saturated fatty acids in wild accessions may simply be attributed to the genetic differences among the accessions and species which need to be examined through genome sequencing of the wild accessions. This study has henceforth unveiled that the wild *Vigna* accession could also contain important fatty acid found in oil seed and which are of health importance. This could confer the functional potential to the wild legumes.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Based on the results from this stud, the genus *Vigna* (wild and domesticated species) presents a considerably high diversity in terms of agro-morphological, socio-cultural practice, cooking and biochemical characteristics. Though very limited in number as compared with wild species, the domesticated species have demonstrated enormous impact in both human and animal nutrition especially in parts of the world where meat is less affordable and preferable.

However, despite their under-exploitation for human benefits, the wild *Vigna* legumes demonstrated important agro-morphological, socio-cultural practice, cooking and biochemical characteristics comparable with the domesticated ones. Therefore, the study revealed that the wild *Vigna* legumes are less known by many farmers but can be accepted as food, feed, cover crop or in soil fertilization though need some improvement. Furthermore, it was demonstrated that the wild *Vigna* species possesses a large variation range of agro-morphological, biochemical and utilization characteristics which could be exploited in the improvement and/or domesticated species or guide their domestication. It was also found that some individual wild accessions have higher nutrient, mineral content and best cooking time as compared with domesticated ones which could be advantageous for bio-fortification or domestication. Indications relating to the candidate accessions favorable for domestication, based on the agro-morphological, socio-cultural practice, cooking and biochemical characteristics were revealed.

Some accessions were noticed as best performing based on some specific parameters studied. The best accessions with exceptional characteristics on farmers' preferrences, perception and acceptability, were TVNu293, TVNu758, AGG308107WVIG2, AGG308101WVIG1, TVNu1546, AGG51603WVIG1, AGG308099WVIG 2 and AGG53597WVIG 1.

In terms of cooking time, the following seven accessions were instead the best: TVNu325, Unknown *Vigna reticulata*, TVNu837, TVNu781, AGG52867WVIG1, AGG51603WVIG1 and AGG53597WVIG 1. It is noted here that among the seven best accessions for cooking time, only two (AGG52867WVIG1, AGG51603WVIG1) belong to the group of accessions selected by farmers. Following the proximate composition, the protein content was the

criterion used for selection of accessions because it is the major component of legumes. Therefore, based on the protein content, the best accessions were: TVNu832, TVNu1701, AGG51603WVIG 1, AGG53597WVIG 1, TVNu1112. At this point, only one accession (AGG51603WVIG 1) could be found as best in farmers' acceptability, cooking time and protein content. The mineral elements analysed also indicated the following thirteen accessions as best: AGG51603WVIG1 (Fe, Mn and Zn), TVNu1185 (Cu), TVNu1792 (Cu), TVNu1808 (Cu), TVNu 758 (Fe), TVNu-141 and TVNu1852 (Zn), TVNu370 and TVNu1628 (Cu, Mn), AGG308099WVIG2 (Zn), TVNu1582, TVNu832, TVNu-333 (Fe). Among these, AGG51603WVIG1 accession was the only selected for the three first criteria (farmers' acceptability, cooking time and protein content) and belongs to the best in Fe, Mn and Zn. Therefore, this accession is the best suited for domestication. However, other selected accessions should not be neglected and should be subjected to more study to unveil their potentials.

5.2 Recommendations

The following recommendations should be taken into consideration in order to unveil all the useful potentials of wild *Vigna* species:

(i) As only four *Vigna* species and limited number of accessions were involved in this study, it should be recommended to explore more species and a huge number of accessions in order to elucidate more agro-morphological traits that will lead to the exploitation of the *Vigna* genus.

(ii) Improvement and toxicity study is recommended by farmers in order to ascertain the non harmful effects of these wild legumes upon human consumption.

(iii) More essential minerals and Amino acids should be investigated in order to unveil some hidden nutritionl potentials of these wild legumes that sometimes lack in our most preferred domesticated foods.

(iv) Additionally, it is recommended to carryout sound examination of the carbohydrates and fiber fraction to ascertain the digestibility and clear nutritive contribution of the carbohydrates and fiber contained in these seeds.

(v) Phytochemicals/antinutrients should be investigated. This will unveil the functional and medicinal properties of these wild legumes.

(vi) *Invivo* and *In vitro* toxicity studies should be carried out in order to establish the LD_{50} and the level at which the consumption of these wild legumes can be toxic. This can also greatly lead to the safe adoption of the wild legumes as food.

(vii) More disease resistance and effects of abiotic factors should be investigated. This may reveal more disease resistant accessions and accessions with abiotic resistant genes that will lead to the breeding of some domesticated ones or the domestication of more resistant accessions.

(viii) Protein fractions should be investigated in order to detect the presence of some bioactive or nutritive proteins and peptides in comparison with the checks and other protein-rich food plants.

(ix) The socio-economic implication (cost analysis) of the utilization of the wild legumes should be investigated in order to check if the cultivation of wild legumes can be more profitable due to its low cost of production as compared with the domesticated ones.

(x) Some high protein and high lipid genes should be investigated in order to verify if such genes exist as some accessions produced high protein levels comparables to that of soy bean. It should be noted that some high protein and lipid genes have been discovered in soy bean.

(xi) The consumer response, including sensory characteristics of these legumes should also be investigated in order to reveal some characteristics such as taste, flavour and mouth feel as compared to domesticated legumes. The results of such a finding can lead to the marketability of the product because the sensory characteristics are the main factors of food preference.

(xii) The feed formulation and responses of animals fed on feed formulated with wild legumes should be tested in order to see if some of these accessions can replace soy bean in animal feed formulation as an ingredient. This will reduce the use of soy

bean only and bring an alternative of soy bean in animal industry. Therefore, this could contribute in reducing food-feed competition.

(xiii) It is also recommended that eventually creating a patent/prototype for some of the accessions with high potential impact in human life should be envisaged. After proper screening of an accession and propably inproving some of its weak or less desired characteristics either genetically or by classical breeding, such accession will be a good crafted material of the researchers who will succed in the crafting and that should deserve a patent.

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APPENDICES

Appendix 1: Wild *Vigna* Legumes Accessions Used in the Study

S/N	Accession Number	Species Name	Genebank
1	TVNu- 313	*Vigna ambacensis*	GRC, IITA
2	TVNu- 557	*Vigna ambacensis*	GRC, IITA
3	TVNu- 1186	*Vigna ambacensis*	GRC, IITA
4	TVNu- 375	*Vigna ambacensis*	GRC, IITA
5	TVNu- 1212	*Vigna ambacensis*	GRC, IITA
6	TVNu- 1792	*Vigna ambacensis*	GRC, IITA
7	TVNu- 947	*Vigna ambacensis*	GRC, IITA
8	TVNu- 1679	*Vigna ambacensis*	GRC, IITA
9	TVNu- 1840	*Vigna ambacensis*	GRC, IITA
10	TVNu- 219	*Vigna ambacensis*	GRC, IITA
11	TVNu- 720	*Vigna ambacensis*	GRC, IITA
12	TVNu- 877	*Vigna ambacensis*	GRC, IITA
13	TVNu- 706	*Vigna ambacensis*	GRC, IITA
14	TVNu- 216	*Vigna ambacensis*	GRC, IITA
15	TVNu- 722	*Vigna ambacensis*	GRC, IITA
16	TVNu- 1631	*Vigna ambacensis*	GRC, IITA
17	TVNu- 1677	*Vigna ambacensis*	GRC, IITA
18	TVNu- 1791	*Vigna ambacensis*	GRC, IITA
19	TVNu- 765	*Vigna ambacensis*	GRC, IITA
20	TVNu- 1843	*Vigna ambacensis*	GRC, IITA
21	TVNu- 629	*Vigna ambacensis*	GRC, IITA
22	TVNu- 452	*Vigna ambacensis*	GRC, IITA
23	TVNu- 1185	*Vigna ambacensis*	GRC, IITA
24	TVNu- 342	*Vigna ambacensis*	GRC, IITA
25	TVNu- 1125	*Vigna ambacensis*	GRC, IITA
26	TVNu- 1678	*Vigna ambacensis*	GRC, IITA
27	TVNu- 223	*Vigna ambacensis*	GRC, IITA
28	TVNu- 1644	*Vigna ambacensis*	GRC, IITA
29	TVNu- 1781	*Vigna ambacensis*	GRC, IITA
30	TVNu- 1851	*Vigna ambacensis*	GRC, IITA
31	TVNu- 1069	*Vigna ambacensis*	GRC, IITA
32	TVNu- 456	*Vigna ambacensis*	GRC, IITA
33	TVNu- 148	*Vigna ambacensis*	GRC, IITA
34	TVNu- 3	*Vigna ambacensis*	GRC, IITA
35	TVNu- 1827	*Vigna ambacensis*	GRC, IITA
36	TVNu- 1691	*Vigna ambacensis*	GRC, IITA
37	TVNu- 1804	*Vigna ambacensis*	GRC, IITA

S/N	Accession Number	Species Name	Genebank
38	TVNu- 1699	*Vigna ambacensis*	GRC, IITA
39	TVNu- 1184	*Vigna ambacensis*	GRC, IITA
40	TVNu- 374	*Vigna ambacensis*	GRC, IITA
41	TVNu- 1150	*Vigna ambacensis*	GRC, IITA
42	TVNu- 1213	*Vigna ambacensis*	GRC, IITA
43	AGG52867WVIG 1	*Vigna racemosa*	AGG
44	AGG51603WVIG 1	*Vigna racemosa*	AGG
45	AGG53597WVIG 1	*Vigna racemosa*	AGG
46	AGG60436WVIG 1	*Vigna racemosa*	AGG
47	*Unknown Vigna racemosa*	*Vigna racemosa*	Self- collected
48	AGG60441WVIG 1	*Vigna reticulata*	AGG
49	AGG17856WVIG 1	*Vigna reticulata*	AGG
50	AGG118137WVIG 1	*Vigna reticulata*	AGG
51	TVNu- 259	*Vigna reticulata*	GRC, IITA
52	TVNu- 302	*Vigna reticulata*	GRC, IITA
53	TVNu- 161	*Vigna reticulata*	GRC, IITA
54	TVNu- 1790	*Vigna reticulata*	GRC, IITA
55	TVNu- 138	*Vigna reticulata*	GRC, IITA
56	TVNu- 604	*Vigna reticulata*	GRC, IITA
57	TVNu- 1112	*Vigna reticulata*	GRC, IITA
58	TVNu- 312	*Vigna reticulata*	GRC, IITA
59	TVNu- 224	*Vigna reticulata*	GRC, IITA
60	TVNu- 1394	*Vigna reticulata*	GRC, IITA
61	TVNu- 995	*Vigna reticulata*	GRC, IITA
62	TVNu- 1405	*Vigna reticulata*	GRC, IITA
63	TVNu- 1522	*Vigna reticulata*	GRC, IITA
64	TVNu- 379	*Vigna reticulata*	GRC, IITA
65	TVNu- 609	*Vigna reticulata*	GRC, IITA
66	TVNu- 1191	*Vigna reticulata*	GRC, IITA
67	TVNu- 766	*Vigna reticulata*	GRC, IITA
68	TVNu- 343	*Vigna reticulata*	GRC, IITA
69	TVNu- 349	*Vigna reticulata*	GRC, IITA
70	TVNu- 916	*Vigna reticulata*	GRC, IITA
71	TVNu- 758	*Vigna reticulata*	GRC, IITA
72	TVNu- 491	*Vigna reticulata*	GRC, IITA
73	TVNu- 767	*Vigna reticulata*	GRC, IITA
74	TVNu- 608	*Vigna reticulata*	GRC, IITA
75	TVNu- 1808	*Vigna reticulata*	GRC, IITA

S/N	Accession Number	Species Name	Genebank
76	TVNu- 1825	*Vigna reticulata*	GRC, IITA
77	TVNu- 1852	*Vigna reticulata*	GRC, IITA
78	TVNu- 1698	*Vigna reticulata*	GRC, IITA
79	TVNu- 932	*Vigna reticulata*	GRC, IITA
80	TVNu- 450	*Vigna reticulata*	GRC, IITA
81	TVNu- 524	*Vigna reticulata*	GRC, IITA
82	TVNu- 605	*Vigna reticulata*	GRC, IITA
83	TVNu- 1156	*Vigna reticulata*	GRC, IITA
84	TVNu- 607	*Vigna reticulata*	GRC, IITA
85	TVNu- 1779	*Vigna reticulata*	GRC, IITA
86	TVNu- 325	*Vigna reticulata*	GRC, IITA
87	TVNu- 324	*Vigna reticulata*	GRC, IITA
88	TVNu- 57	*Vigna reticulata*	GRC, IITA
89	TVNu- 56	*Vigna reticulata*	GRC, IITA
90	TVNu- 1520	*Vigna reticulata*	GRC, IITA
91	TVNu- 602	*Vigna reticulata*	GRC, IITA
92	TVNu- 1388	*Vigna reticulata*	GRC, IITA
93	TVNu- 141	*Vigna reticulata*	GRC, IITA
94	TVNu- 738	*Vigna reticulata*	GRC, IITA
95	TVNu- 739	*Vigna reticulata*	GRC, IITA
96	TVNu- 350	*Vigna reticulata*	GRC, IITA
97	TVNu- 142	*Vigna reticulata*	GRC, IITA
98	TVNu- 1805	*Vigna reticulata*	GRC, IITA
99	*Unknown Vigna reticulata*	*Vigna reticulata*	Self- collected
100	AGG308102WVIG 3	*Vigna vexillata*	AGG
101	AGG308105WVIG 2	*Vigna vexillata*	AGG
102	AGG308098WVIG 2	*Vigna vexillata*	AGG
103	AGG16683WVIG 5	*Vigna vexillata*	AGG
104	AGG308099WVIG 2	*Vigna vexillata*	AGG
105	AGG308097WVIG 1	*Vigna vexillata*	AGG
106	AGG308101WVIG 1	*Vigna vexillata*	AGG
107	AGG308100WVIG 3	*Vigna vexillata*	AGG
108	AGG58678WVIG 2	*Vigna vexillata*	AGG
109	AGG308103WVIG 3	*Vigna vexillata*	AGG
110	AGG308107WVIG 2	*Vigna vexillata*	AGG
111	AGG308096 WVIG 2	*Vigna vexillata*	AGG
112	AGG62154WVIG 1	*Vigna vexillata*	AGG
113	TVNu- 1098	*Vigna vexillata*	GRC, IITA

S/N	Accession Number	Species Name	Genebank
114	TVNu- 1629	*Vigna vexillata*	GRC, IITA
115	TVNu- 1718	*Vigna vexillata*	GRC, IITA
116	TVNu- 1590	*Vigna vexillata*	GRC, IITA
117	TVNu- 1378	*Vigna vexillata*	GRC, IITA
118	TVNu- 120	*Vigna vexillata*	GRC, IITA
119	TVNu- 178	*Vigna vexillata*	GRC, IITA
120	TVNu- 1796	*Vigna vexillata*	GRC, IITA
121	TVNu- 1529	*Vigna vexillata*	GRC, IITA
122	TVNu- 1092	*Vigna vexillata*	GRC, IITA
123	TVNu- 1546	*Vigna vexillata*	GRC, IITA
124	TVNu- 1370	*Vigna vexillata*	GRC, IITA
125	TVNu- 1626	*Vigna vexillata*	GRC, IITA
126	TVNu- 1358	*Vigna vexillata*	GRC, IITA
127	TVNu- 1624	*Vigna vexillata*	GRC, IITA
128	TVNu- 1585	*Vigna vexillata*	GRC, IITA
129	TVNu- 1617	*Vigna vexillata*	GRC, IITA
130	TVNu- 1621	*Vigna vexillata*	GRC, IITA
131	TVNu- 479	*Vigna vexillata*	GRC, IITA
132	TVNu- 1344	*Vigna vexillata*	GRC, IITA
133	TVNu- 1628	*Vigna vexillata*	GRC, IITA
134	TVNu- 381	*Vigna vexillata*	GRC, IITA
135	TVNu- 792	*Vigna vexillata*	GRC, IITA
136	TVNu- 1586	*Vigna vexillata*	GRC, IITA
137	TVNu- 1582	*Vigna vexillata*	GRC, IITA
138	TVNu- 293	*Vigna vexillata*	GRC, IITA
139	TVNu- 1359	*Vigna vexillata*	GRC, IITA
140	TVNu- 955	*Vigna vexillata*	GRC, IITA
141	TVNu- 1591	*Vigna vexillata*	GRC, IITA
142	TVNu- 1701	*Vigna vexillata*	GRC, IITA
143	TVNu- 1443	*Vigna vexillata*	GRC, IITA
144	TVNu- 832	*Vigna vexillata*	GRC, IITA
145	TVNu- 1121	*Vigna vexillata*	GRC, IITA
146	TVNu- 636	*Vigna vexillata*	GRC, IITA
147	TVNu- 1476	*Vigna vexillata*	GRC, IITA
148	TVNu- 1748	*Vigna vexillata*	GRC, IITA
149	TVNu- 781	*Vigna vexillata*	GRC, IITA
150	TVNu- 969	*Vigna vexillata*	GRC, IITA
151	TVNu- 1592	*Vigna vexillata*	GRC, IITA
152	TVNu- 1632	*Vigna vexillata*	GRC, IITA

S/N	Accession Number	Species Name	Genebank
153	TVNu- 333	*Vigna vexillata*	GRC, IITA
154	TVNu- 1360	*Vigna vexillata*	GRC, IITA
155	TVNu- 1594	*Vigna vexillata*	GRC, IITA
156	TVNu- 1369	*Vigna vexillata*	GRC, IITA
157	TVNu- 593	*Vigna vexillata*	GRC, IITA
158	TVNu- 1593	*Vigna vexillata*	GRC, IITA
159	TVNu- 837	*Vigna vexillata*	GRC, IITA
160	***Unknown***	***Vigna***	**Self- collected, NM-AIST, Tanzania**

GRC, IITA: Genetic Resource Center, Germplasm Health Unit, International Institute of Tropical Agriculture (IITA), Headquarters, PMB 5320, Oyo Road, Idi-Oshe, Ibadan, Nigeria. AGG: Australian Grain Genebank, Department of Economic Development, Jobs, Transport and Resources, Private Bag 260, Horsham, Victoria 3401

Appendix 2: Means, Ranges and Coefficients of Variation for the Selected Quantitative Traits[a], Analyzed at Site A and B During the Two Cropping Seasons

S/N	Selected Quantitative traits	Site A (2018 Season)			Site B (2018 Season)			Site B (2019 Season)		
		Mean	Range	CV (%)	Mean	Range	CV (%)	Mean	Range	CV (%)
1.	Germination time (days)	6.31	5–9	0	7.03	5–12	0	7.37	5–10	0
2.	Terminal leaflet length (cm)	7.72	1.2–17.36	14.38	8.41	2.16–16.67	14.38	7.4	1.15–16.65	14.38
3.	Terminal leaflet width (cm)	3.58	0.9–10.1	32.24	3.94	0.99–11.11	32.24	3.43	0.86–9.67	32.24
4.	Petiole length (cm)	4.07	0.7–15	32.7	4.19	0.72–15.45	32.73	4.25	0.73–15.68	32.7
5.	Days to flowering	51.33	20–86	1.85	79.02	48–114	1.35	54.02	23.63–89.33	1.98
6.	Flower bud size (cm)	0.88	0.3–2.3	17.97	1.05	0.36–2.76	17.97	1.14	0.39–2.98	17.97
7.	Number of flowers per raceme	6.23	3–21	24.95	5.03	1.0–19	34.13	25.13	5–95	34.13
8.	Peduncle length (cm)	10.2	4.1–28	11.45	10.5	4.22–28.84	11.45	10.82	4.35–29.71	11.45
9.	Pods per peduncle	2.22	1–7	42.52	2.19	1.0–5.20	43.36	3.25	1.5–10.50	44.24
10.	Pod length (cm)	5.46	3.5–15	18.68	5.35	3.43–14.7	18.68	5.42	3.47–14.89	18.68
11.	Pods per plant	38.18	5–190	5.71	17.18	2.25–85.5	5.71	32.47	4.25–161.60	5.71
12.	Seeds per pod	4.89	6–15	0	4.87	5–15	5.93	5.6	5.75–17.25	5.93
13.	Seed size (mm^2)	10.6	4.83–43.82	21.2	10.07	4.59–41.63	21.2	10.18	4.63–42.05	21.2
14.	100-Seed weight (g)	3.87	1.1–22.40	41.23	3.33	0.98–19.94	42.66	3.68	1.05–21.28	41.23
15.	Yield (Kg/ha)	688.99	28.7–4390.40	42.89	590.7	25.54–3366.69	44.52	654.54	27.27–4170.88	42.89

Appendix 3: Adjusted Mean Values for Selected Suantitative Traits per Species

Species	Season/Site	Mean														
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Check 1	Site A (2018 Season)	6.50	8.37	7.14	9.79	44.31	1.31	3.69	26.00	1.36	5.59	28.13	6.00	20.85	7.04	573.71
	Site B (2018 Season)	6.50	9.12	7.85	10.09	72.56	1.58	2.21	26.78	1.30	5.48	12.66	6.00	19.81	6.26	510.60
	Site B (2019 Season)	6.50	8.03	6.83	10.24	47.56	1.70	11.03	27.58	2.01	5.55	23.92	6.90	20.01	6.69	545.02
Check 2	Site A (2018 Season)	5.50	11.51	5.30	5.72	42.13	1.49	7.46	20.56	3.20	13.63	39.69	12.00	20.03	9.74	1870.80
	Site B (2018 Season)	5.50	12.54	5.83	5.89	70.13	1.79	5.76	21.18	3.19	13.35	17.86	11.88	19.03	8.67	1665.01
	Site B (2019 Season)	5.50	11.04	5.07	5.98	45.13	1.94	28.81	21.81	4.78	13.53	33.75	13.66	19.22	9.26	1777.26
Check 3	Site A (2018 Season)	8.50	12.07	6.79	11.52	20.81	1.15	12.79	16.63	5.28	10.19	69.63	7.50	40.04	18.95	3714.20
	Site B (2018 Season)	8.50	13.16	7.47	11.86	48.81	1.38	10.76	17.12	5.21	9.98	31.33	7.50	38.04	16.87	3305.64
	Site B (2019 Season)	8.50	11.58	6.50	12.04	23.81	1.49	53.78	17.64	7.63	10.11	59.22	8.63	38.42	18.00	3528.49
V. ambacensis	Site A (2018 Season)	6.71	6.56	2.05	2.40	55.01	0.51	5.23	4.51	1.80	2.66	14.74	3.13	5.44	1.10	164.89
	Site B (2018 Season)	7.84	7.15	2.26	2.47	82.77	0.61	4.42	4.64	1.79	2.62	6.65	3.16	5.17	0.89	130.63
	Site B (2019 Season)	7.68	6.29	1.96	2.51	57.77	0.66	21.98	4.78	2.67	2.66	12.57	3.63	5.22	1.05	156.64
V. Vexillata	Site A (2018 Season)	7.15	7.40	2.84	2.48	56.41	0.97	6.95	9.11	2.39	6.35	51.46	4.73	6.80	1.79	454.34
	Site B (2018 Season)	8.17	8.07	3.12	2.55	84.40	1.16	5.64	9.38	2.38	6.22	23.16	4.70	6.46	1.47	377.83
	Site B (2019 Season)	8.12	7.10	2.72	2.59	59.40	1.26	28.21	9.66	3.55	6.30	43.76	5.40	6.49	1.70	431.62
V. reticulate	Site A (2018 Season)	7.75	6.48	2.89	2.38	56.44	1.16	8.55	10.53	2.75	5.60	58.71	5.43	8.46	2.46	448.54
	Site B (2018 Season)	8.45	7.07	3.11	2.39	83.02	1.34	6.72	10.33	2.62	5.23	25.16	5.17	7.66	1.94	352.58
	Site B (2019 Season)	8.17	6.22	2.71	2.43	58.02	1.44	33.61	10.64	3.90	5.30	47.55	5.94	7.73	2.32	421.50
V. racemose	Site A (2018 Season)	7.75	6.73	2.65	2.98	62.88	1.09	4.84	6.93	1.63	4.37	27.56	5.25	7.44	3.04	653.85
	Site B (2018 Season)	10.00	7.34	2.92	3.07	90.96	1.31	4.00	7.14	1.60	4.28	12.40	5.23	7.07	2.71	581.93
	Site B (2019 Season)	9.50	6.46	2.54	3.11	65.96	1.42	19.99	7.35	2.37	4.34	23.44	6.01	7.14	2.89	621.16

[a]**1:** Germination time; **2:**Terminal leaflet length; **3:** Terminal leaflet width; **4:** Petiole length; **5:** Days to flowering; **6:** Flower bud size; **7:** Number of flowers per raceme; **8:** Peduncle length; **9:** Pods per peduncle; **10:** Pod length; **11:** Pods per plant; **12:** Seeds per pod; **13:** Seed size; **14:** 100-Seed weight; **15:** Yield. Check 1: Landrace of *Vigna* vexillata; Check 2: Cowpea (*Vigna unguiculata*); Check 3: Rice Bean (*Vigna umbellata*).