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Vigna Legumes: Exploring Some Biochemical Constituents of the Wild Species for Potential Neo-Domestication Candidates

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Abstract

Due to the foresight of food system collapse, the search for alternative food and feed sources for human and animal nutrition becomes a daunting task. In addition, achieving Zero Hunger target by the year 2030 as set by the FAO is challenging. Re-exploring the less explored foods, coupled to refining less refined traits, cultivating the uncultivated, and popularizing the unpopular food crops are steps to achieve the domestication of wild plants for food and nutrition security. In that line of thought, this study explored the proximate composition of 87 accessions of four wild unexplored *Vigna* species in order to reveal information leading to their future domestication and utilization. Standard procedures and methods approved by AOAC were used in carrying out the proximate composition of the wild *Vigna* legumes. The study revealed that the wild *Vigna* species possess a large variation range of nutrient characteristics which could be exploited in the improvement of domesticated species or guide their domestication. It was also found that some individual wild accessions have higher nutrient content 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 nutrient characteristics were revealed.

1. Introduction

The Challenges of achieving the Zero Hunger target by 2030 as set by the FAO to fight global Food and nutrition insecurity are now surfacing¹. Additionally, only 12 crops contribute most to the current global food production, with only three of them (rice, wheat and maize) providing more than 50% of the world's calories². Reports have alerted that there is a reduction of the gene pool in both plant and animal genetic resources globally³. The world is notified that 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 diet³. Hence, directing researchers attention to plant protein sources becomes a necessity as the animal protein sources face challenges of preference (for vegetarians) and affordability. As such, the contribution of legumes as plant protein source is unanimously uncontested and domestication of new species is becoming an imperative due the challenges faced by the domesticated ones^{2,4,5}.

Legumes (family: Fabaceae) constitute the third largest family of flowering plants⁶. They have uncontestedly proven their nutritional importance for both humans and animals and have been appreciably qualified as the "poor man's meat". The domesticated, cultivated and commercialized legumes such as soybeans, cowpeas, common beans and others have demonstrated considerable contribution to the global food security⁴. *Phaseolus* and *Vigna* genera comprises the most widely consumed legumes, namely common beans (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*)^{4,7,8}. Within each genus, there are fewer domesticated edible species as compared with the numerous non-domesticated wild species. Some domesticated or semi-domesticated species have been termed as neglected and underutilized species due to the little attention paid to them or the complete ignorance of their existence by agricultural researchers, plant breeders, and policymakers⁹.

The genus *Vigna* is categorized into seven subgenera and sixteen sections¹⁰. The seven genera include *Ceratotropis*, *Haydonia*, *Lasiocarpa*, *Macrorhycha*, *Plectotropis*, *Sigmoidotropis* and *Vigna*¹¹. All domesticated and cultivated *Vigna* varieties belong to only three subgenera, namely *Ceratotropis*, *Plectotropis*, and *Vigna*¹². The subgenus *Ceratotropis* is well known as Asian *Vigna* and the subgenus *Vigna* commonly called African *Vigna* are the most known subgenera containing most popular legumes like cowpeas, black gram and green gram^{11,12}.

The *Vigna* genus furthermore comprise more than 100 wild species, which do not have common names, apart from their scientific appellation¹³. They are given different denotations, such as the under-exploited wild *Vigna* species, undomesticated *Vigna* species, wild *Vigna* or alien species, depending on the scientist^{4,14}. These wild species constitute the core subject of interest in this research, as very little information about them is reported. For the purpose of this study, accessions of *V. racemosa*, *V. ambacensis*, *V. reticulata*, and *V. vexillata* were first considered for investigations of some of their biochemical constituents based on the very little information gathered about them and their availability in the nearest gene bank. Earlier reports have demonstrated their potential usages, farmers' preferences, cooking and agro-morphological characteristics¹⁵⁻¹⁷.

Limited reports on the biochemical composition of wild *Vigna* legumes have been noticed. So far, only eight wild *Vigna* species have been given quantitatively evaluated in terms of chemical composition out of the more than a hundred species existing. These

species are *V. vexillata*, *V. vexillata macrosperma*, *V. luteola*, *V. oblongifolia*, *V. unguiculata dekindtiana*, *V. racemosa*, *V. reticulata* and *V. ambacensis*¹⁸. The flavonoid content of some species has also been qualitatively assessed through HPLC method¹⁹.

It is very important to note here 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 *V. racemosa* were reported in a recent study²⁰. Another study also reported recently on the chemical changes during open and controlled fermentation of *V. racemosa* seed collected from their natural environment, regardless of their genetic specification²¹. Other studies focusing on qualitative evaluation of bioactive compounds of *V. kirki*, *V. marina*, *V. gracilis*, *V. heterophylla*, *V. parkeri*, *V. hosei*, *V. adenantha*, *V. venusta*, *V. minima*, *V. glabrascens*, and *V. triphylla* have revealed the presence biochemicals such as Robinin, Kaempferol-3-rutinoside, Isorhamnetin-3- rutinoside, Hyperoside, Delphinidin and Cyanidin^{18,19,22}.

Information regarding the mineral content, protein fractions, lipid profile and functional potential of the wild *Vigna* species 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 to also be investigated to enhance their usages. Therefore, this study aimed at exploring the proximate composition, some mineral content (Cu, Zn, Mn and Fe) and fatty acid composition of some wild *Vigna* species (*V. racemosa*, *V. ambacensis*; *V. reticulata*, and *V. vexillata*) in order to detect potential accessions for domestication and/or crop improvement.

2. Materials And Methods

2.1. Seeds Preparation for Proximate Composition Analysis

One hundred and six (106) accessions of the four species of wild *Vigna* legumes (*V. racemosa*, *V. ambacensis*; *V. reticulata*, and *V. vexillata*) were obtained from genebanks as presented in appendix 1. Approximately 20–100 seeds of each accession were supplied by the genebanks and planted in an experimental plot, following the augmented block design arrangement, and allowed to grow until full maturity as described in an earlier study¹⁶.

The experimental research, the plant material collection and field studies on plants (either cultivated or wild) were performed in accordance with the relevant institutional, national, and IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. The plant materials were collected, identified and issued to us by the relevant international institutions (the Australian Grain Genebank and the International Institution for Tropical Agriculture, IITA) with due respect of international guidelines and regulations. The plant materials were deposited in the Nelson Mandela African Institution of Science and Technology herbarium for identification and authentication under the voucher number NM-AIST/P.220/CAM.

The seeds were planted at the Tanzania Agricultural Research Institute (TARI), Selian in the Arusha region, located in the northern part of Tanzania. TARI-Selian 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.).

Eighty seven (87) accessions of matured seeds of wild *Vigna* species of legumes harvested were selected based on their productivity in the field to carry out the proximate composition. 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*).

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

2.2. Moisture Content Determination

The method employed for the determination of moisture content in the sample based on the measurement of the loss in weight due to drying at a temperature of about 105°C as describe in the AOAC methods (method 950.46) ²³. A watch glass was washed and dried in an oven (DRY-Line 56, STEP Systems GmbH, Nuremberg, Germany) at about 105°C for 3 h, it was cooled in a desiccator 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 a desiccator and reweighed.

The percentage moisture was obtained using the expression below;

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

2.3. 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 ²³.

Three different crucibles were preheated in a Muffle furnace (Nabertherm GmbH, Lilienthal, Germany) at about 550°C. Each crucible was cooled in a desiccator and weighed. Approximately 2.0 g of each sample was weighed into different crucibles. 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 crucibles and their content were taken and recorded. The percentage ash was calculated using the following expression;

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

2.4. Crude Lipid Content Determination

The crude lipid content of wild *Vigna* 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;

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

2.5. Crude Protein Content Determination

The protein content of the wild *Vigna* legume samples was analyzed according to the AOAC method 928.08 ²³. 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 Kjeltac instrument (Kjeltic™ 8200 Auto Distillation Unit)²⁴. 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:

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

Where,

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

B = Volume 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)

2.6. Crude Fiber Content Determination

The fiber content was evaluated using AOAC method²³.

Fat-free grinded wild *Vigna* 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 (Fibertec M6, 200-230V, FOSS, Denmark) and the sample was left to digest for 30 min with 150 mL of solution containing 12.5% Sulphuric acid (Sigma Aldrich, Germany) and 0.25 mL of octanol (Sigma Aldrich, Germany). 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) (Sigma Aldrich, Germany) and 0.25 mL of octanol to dissolve the alkali-soluble matter from the samples. The porcelain crucibles' final residues were dried at 105°C in an oven (DRY-Line 56, STEP Systems GmbH, Nuremberg, Germany) for 1 h, cooled in a desiccator and then weighed. The final residues were dried at 105°C in an oven for 60 min. The residues were ignited in a pre-heated Muffle furnace (Carbolite, UK) at 550°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,

W₁ = Sample weight, **W₂** = Crucible weight with ash, **W₃** = Empty crucible weight

2.7. Carbohydrate Content Determination

The percentage carbohydrate was obtained by difference²³

Percentage carbohydrate = 100 - (% Moisture + %Protein + %Fat + %Ash + %Crude fiber)

2.8. Mineral Content Evaluation of Wild *Vigna* Species

The mineral elements were determined using Atomic Absorption Spectrophotometry (AAS) as described by AOAC, (2000).

2.8.1. 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 as reported to be suitable for plant materials²⁵. 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 Whatman filter paper No. 4. The filtered solution was used for reading the absorbance of the samples on the AAS with specific wavelength.

2.8.2. Minerals 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 2,600°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 1 shows the instrumental conditions for each mineral element evaluated in the samples.

2.9. Fatty acid Content Evaluation of Wild *Vigna* Species

2.9.1. 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 h. 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.

2.9.2. 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)²⁰.

2.9.3. Determination of the Fatty Acid Methyl Esters (FAMES)

FAMES were determined by capillary Gas Chromatography Mass Spectrophotometry 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)³⁰.

2.10. Data Analysis

The data on proximate composition parameters were collected in triplicate. The one-way analysis of variance (ANOVA), the hierarchical clustering analysis (HCA) and principal component analysis (PCA) were performed using XLSTAT.

3. Results

3.1. Proximate Composition Domesticated *Vigna* Species Used as Checks

The proximate composition for the various *Vigna* species studied is summarized in Tables 1–4. 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: a semi-domesticated 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 significantly 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 1–4).

3.2. Proximate Composition of *Vigna ambacensis* Accessions

Table 1 summarizes the proximate composition of *V. ambacensis* accessions. It shows that the lipid content of all the wild accessions is significantly similar to those of Check 1 and 2 while it is significantly ($p < 0.05$) higher than that of Check 3. All the accessions showed significant lower ash content than the three checks except for accessions TVNu219 and TVNu877 which is comparable to that of the checks. The moisture and protein content of the wild accessions are significantly lower than that of the checks while the carbohydrate and fiber content of the wild accessions were higher than that of the checks.

Table 1
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 ± 0.264bc	8.376 ± 0.243ef	21.173 ± 0.315de	6.360 ± 0.413ab	59.444 ± 0.765e
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 ± 0.782e
TVNu219	1.268 ± 0.043a	3.555 ± 0.543ab	8.658 ± 0.312ef	19.914 ± 0.312efg	6.754 ± 0.418a	59.851 ± 0.765de
TVNu877	1.263 ± 0.045a	3.286 ± 0.431bc	8.982 ± 0.263def	19.328 ± 0.326fgh	6.243 ± 0.432ab	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 ± 0.974bc
TVNu1804	1.258 ± 0.056a	3.016 ± 0.213cd	10.476 ± 0.443bcd	18.760 ± 0.319gh	5.731 ± 0.413bc	60.759 ± 0.765cde
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 ± 0.965a
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(<i>V. ambacensis</i> accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

3.3. Proximate Composition of *Vigna reticulata* Accessions

Table 2 summarizes the proximate composition of *V. reticulata* accessions. Results for the lipid content of most of the wild accessions were not statistically significant from those of Check 1 and Check 2. Four accessions (TVNu1394_VRe, TVNu324_VRe, TVNu57_VRe, and TVNu141_VRe) exhibited comparably lipid content to Check 3. 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. All the accessions showed lower moisture content than the three checks. The accession TVNu1112_VRe (31.074%) had substantially higher protein content which is significantly higher than that of all the checks. On the other hand, five accessions (TVNu350_VRe, TVNu1852_VRe, TVNu324_VRe, TVNu57_VRe, and TVNu141_VRe) had protein content comparable to that of Check 1 and Check 2. The rest of the accessions had low protein content which is lower than that of Check 3. It was noticed that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents respectively as compared to the checks.

Table 2
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

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
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
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(<i>V. reticulata</i> Accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

3.4. Proximate Composition of *Vigna vexillata* Accessions

Table 3 presents the proximate composition of *Vigna vexillata* accessions. It was found that the lipid content of most the wild accessions is significantly lower from that of Checks 1 and 2, except for few accessions (AGG308096WVIG2, TVNu333, TVNu293, and TVNu 832) which are statistically higher than that of Check 3. Similar to the *Vigna reticulata* species, all the accessions showed

comparable ash content 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, AGG308101WVIG2 and AGG308099WVIG2 exhibited significant higher protein content than that of all the checks. On the other hand, ten accessions (AGG308097WVIG2, TVNu1378, TVNu1529, TVNu1344, TVNu333, TVNu293, TVNu178, TVNu781, TVNu120, and TVNu1629) had protein content comparable to that of Check 1 and Check 2. The rest of the accessions had low protein content which is statistically lower than that of Check 3. It is similarly noticed that the greater number of wild accessions present a significantly higher fiber and carbohydrates contents as compared to the checks.

Table 3
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 ± 0.153cdefg	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 ± 0.150bcde	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

Accessions	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
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
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 ± 0.600ij	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
Significant	Yes	Yes	Yes	Yes	Yes	Yes

3.5. Proximate composition of *Vigna racemosa* Accessions

Table 4 summarizes the proximate composition of *V. racemosa* accessions. It was found that the lipid content of AGG53597WVIG1 and AGG51603WVIG1 accessions was significantly comparable to that of Check 1 and Check 2 whereas “Unknown_ *Vigna racemosa*”, AGG52867WVIG1 and ‘Unknown *Vigna*’ showed comparable lipid content to that of Check 3. Similar to the *V. 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 the checks. Accession AGG53597WVIG1 showed higher moisture content as compared with Check 1, Check 3, and all the other wild accessions. Moreover, it was comparable to that of Check 2. The accession AGG51603WVIG1 (36.689%) showed substantially significant higher protein content than that of all the checks whereas accession AGG53597WVIG1 (28.852%) had a protein content comparable to that of Check 1. The rest of the accessions had significant low protein content which is comparable to that of Check 3. Furthermore, the greater number of wild accessions presents significantly higher fiber and carbohydrates contents as compared to the checks.

Table 4
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_ <i>Vigna racemosa</i>	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 <i>Vigna</i>	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(<i>V. racemosa</i> Accessions)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes	Yes	Yes

3.6. Concomitant Analysis of the Four *Vigna* Species

In order to appreciate the proximate composition of the wild accessions per species, the mean of each component for all the accessions belonging to each species was calculated. Figure 1 shows the means of proximate composition of wild *Vigna* accessions per species. Looking at the variations in the proximate composition taking globally all accessions per species, it was revealed that there is no significant difference between species vis-à-vis other species and the checks.

Besides, 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. 2). It reveals that based on the proximate composition, both wild accessions and checks can form three groups (clusters (C)) of legumes in which the three checks belong to the same group (Cluster 1) with some wild accessions (Table 5).

Table 5

Accessions belonging to clusters as grouped on the dendrogram based on their 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 WWIG2
	Check 2	TVNu720_Va	AGG308107WWIG2
	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
	AGG308101WWIG1	TVNu1698_VRe	TVNu479
	TVNu120	TVNu1808_VRe	AGG52867WWIG1_Vra
	AGG308097WWIG 1	TVNu607_VRe	Unknown <i>Vigna</i>
	TVNu1593	TVNu379_VRe	
	TVNu1370	TVNu1852_VRe	
	TVNu1629	TVNu739_VRe	
	AGG308099WWIG2	TVNu138_VRe	
	TVNu1344	TVNu1405_VRe	
	AGG62154WWIG_1	TVNu349_VRe	
	TVNu1529	TVNu325_VRe	
	TVNu1546	TVNu758_VRe	
	TVNu1586	Unknown <i>_Vigna reticulata</i>	
	TVNu1378	TVNu1394_VRe	
	AGG53597WWIG1_Vra	TVNu1825_VRe	

Class	1	2	3
	AGG51603WVIG1_Vra	TVNu- 224_VRe	
	Unknown_Vigna_racemosa	TVNu1191_VRe	
		TVNu1779_VRe	
		TVNu491_VRe	
		TVNu524_VRe	
		TVNu1520_VRe	
		AGG17856WVIG_1_VRe	
		TVNu343_VRe	
		TVNu1388_VRe	
		TVNu767_VRe	
		TVNu161_VRe	
		TVNu738_VRe	
		TVNu1790_VRe	
		TVNu916_VRe	
		TVNu605_VRe	

The Principal Component Analysis (PCA) function of XLSTAT was ran using the means values for nutrient component in each accession in order to explore the relationship that could exist between the proximate composition and the accessions, as well as the relationship between the accessions themselves. A correlation circle with an observation chart was obtained, as shown in Fig. 3. The best combination that explains the variation among the accessions and traits (proximate composition) were revealed by the analysis as the the first (F1 = 49.74%) and second (F2 = 24.75%) PCA dimensions represent 74.49% of the initial information. It was confirmed that there is a positive correlation between the traits ash, moisture and protein, except for the lipid, fiber and carbohydrate traits, which could be due to the angles between their vectors (Fig. 3 and Table 6). It was also observed 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. 3).

Table 6
A correlation matrix for the proximate composition (Pearson (n))

Variables (%)	Lipid	Ash	Moisture	Protein	Fiber	Carbohydrates
Lipid	1	-0.395	-0.129	-0.336	0.434	0.169
Ash	-0.395	1	0.399	0.564	0.375	-0.740
Moisture	-0.129	0.399	1	0.362	0.040	-0.580
Protein	-0.336	0.564	0.362	1	-0.031	-0.899
Fiber	0.434	0.375	0.040	-0.031	1	-0.320
Carbohydrates	0.169	-0.740	-0.580	-0.899	-0.320	1
<i>Values in bold are different from 0 with a significance level alpha = 0,95</i>						

3.7. Mineral Contents of the Wild *Vigna* Species Accessions

3.7.1. Mineral Contents of *V. ambacensis* Accessions

The mineral contents for *V. ambacensis* accessions with their mean values are summarized in Table 7. Accessions TVNu-1185 (0.951 mg/100 g) and TVNu-1792 (0.918 mg/100 g) had the highest copper (Cu) contents as compared with all the checks and other accessions. Check 2 present the highest concentrations for manganese (Mn) and zinc (Zn) while Check 2 and Check 3 dominate in iron (Fe) concentration. The appendix 1 shows the dynamics of mineral elements in *V. ambacensis* accessions.

Table 7
Mean values of the mineral contents of *V. ambacensis* accessions (mg/100 g)

Accessions	Cu	Zn	Mn	Fe
Check 1	0.718cdef	2.363a	1.301b	3.823b
TVNu1185	0.951a	1.936ab	1.064c	2.350c
Check 2	0.428h	2.386a	1.571a	5.734a
TVNu-1644	0.812abcd	2.007ab	1.020cd	2.289c
TVNu-1699	0.718cdef	1.889ab	0.931cde	2.392c
TVNu-1804	0.841abc	1.822ab	0.971cde	2.289c
Check 3	0.493gh	1.650b	1.600a	5.870a
TVNu-219	0.659defg	1.976ab	0.999cde	2.080c
TVNu-1840	0.690cdef	1.906ab	0.961cde	2.224c
TVNu-223	0.583efgh	1.859ab	0.912cde	2.370c
TVNu-720	0.635efg	1.778b	0.970cde	2.314c
TVNu-1792	0.918ab	1.750b	0.832e	2.032c
TVNu-877	0.744bcde	1.715b	0.887de	2.109c
TVNu-342	0.562fgh	1.673b	0.885de	2.203c
Pr > F(Model)	< 0.0001	0.001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes

3.7.2. Mineral Contents for the *V. reticulata* Accessions

The mineral contents for the *V. reticulata* accessions can be observed with their mean values summarized in Table 8. It is noted that accession TVNu1808_VRe (1.253 mg/100 g) present the highest Cu concentration which is significantly higher than that of all other accessions and the checks. The accessions TVNu758_VRe (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) outperformed the checks as for Zn concentration (Table 2). The appendix 2 shows the dynamics of mineral elements in *V. reticulata* accessions.

Table 8
Mean values of the mineral contents of *V.reticulata* accessions (mg/100 g)

Accessions	Cu	Zn	Mn	Fe
TVNu758_VRe	0.903bcdefg	2.129abcd	1.079cde	4.894ab
TVNu1852_VRe	0.814cdefghi	2.667a	1.072cde	2.781d
TVNu1191_VRe	1.001bc	1.976bcde	1.092cd	2.398d
Check 1	0.718fghij	2.363abc	1.301b	3.823c
Check 2	0.428l	2.386ab	1.571a	5.734a
TVNu1808_VRe	1.253a	2.357abc	0.915defg	2.766d
TVNu57_VRe	0.772cdefghi	1.922bcde	1.206bc	2.676d
TVNu343_VRe	0.855bcdefgh	2.048abcd	1.046cdef	2.335d
TVNu141_VRe	0.828cdefghi	2.673a	0.996defg	2.317d
TVNu325_VRe	0.922bcdef	1.650def	1.067cde	2.530d
AGG17856WVIG_1_VRe	0.452kl	2.056abcd	1.050cdef	4.596bc
TVNu1522_VRe	0.635hijkl	2.170abcd	1.031cdefg	2.542d
TVNu324_VRe	0.810cdefghi	1.856bcde	1.050cdef	2.352d
Unknown _Vigna reticulata	0.886bcdefg	1.860bcde	0.996defg	2.335d
TVNu1825_VRe	0.795cdefghi	1.747bcdef	1.036cdefg	2.463d
Check 3	0.493jkl	1.650def	1.600a	5.870a
TVNu- 224_VRe	0.867bcdefgh	1.590def	1.035cdefg	2.366d
TVNu138_VRe	0.756defghi	1.927bcde	0.955defg	2.441d
TVNu1520_VRe	0.993bcd	1.730cdef	1.005defg	2.238d
TVNu491_VRe	0.726fghij	1.945bcde	0.985defg	2.269d
TVNu738_VRe	0.693fghij	2.016bcd	1.025cdefg	2.123d
TVNu607_VRe	0.669ghijk	1.814bcdef	0.995defg	2.361d
TVNu349_VRe	0.923bcdef	1.676def	0.945defg	2.227d
TVNu605_VRe	0.613ijkl	1.897bcde	0.935defg	2.418d
TVNu916_VRe	0.815cdefghi	1.746bcdef	0.986defg	2.164d
TVNu161_VRe	0.686fghijk	1.595def	0.979defg	2.656d
TVNu379_VRe	0.719fghij	1.696def	1.026cdefg	2.252d
TVNu767_VRe	0.738efghi	1.830bcdef	0.922defg	2.350d
TVNu1394_VRe	0.860bcdefgh	1.785bcdef	0.872g	2.179d
TVNu1388_VRe	0.966bcde	1.786bcdef	0.853g	2.073d
TVNu1779_VRe	1.06ab	1.347ef	0.946defg	1.980d
TVNu56_VRe	0.856bcdefgh	1.361ef	0.975defg	2.179d
TVNu524_VRe	0.676ghijk	1.530def	0.941defg	2.393d
TVNu739_VRe	0.784cdefghi	1.750bcdef	0.909defg	2.152d

Accessions	Cu	Zn	Mn	Fe
TVNu1698_VRe	0.642hijkl	1.830bcdef	0.899efg	2.075d
TVNu1405_VRe	0.592ijkl	1.707def	0.908defg	2.248d
TVNu1790_VRe	0.766cdefghi	1.196f	0.936defg	2.120d
Pr > F(Model)	< 0,0001	< 0,0001	< 0,0001	< 0,0001
Significant	Yes	Yes	Yes	Yes

3.7.3. Mineral Contents for the *V. vexillata* Accessions

The mean values for the mineral contents of *V. vexillata* accessions are summarized in Table 9. 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 TVNu-333 (2.756 mg/100 g), TVNu-781 (2.407 mg/100 g) and TVNu-1370 (2.496 mg/100 g) outperformed the checks. The accession AGG308099WVIG2 (3.180 mg/100 g) presented the highest concentration of Zn outperforming all the checks and other wild accessions. On the other hand, accessions TVNu-1582 (6.563 mg/100 g), TVNu-832 (6.229 mg/100 g), and TVNu-333 (6.411 mg/100 g) had best Fe concentration beyond that of all the checks. The appendix 3 shows the dynamics of mineral elements in *V. vexillata* accessions.

Table 9
Mean values of the mineral contents of *V. vexillata* accessions (mg/100 g)

Accessions	Cu	Zn	Mn	Fe
TVNu-1370	0.807a	2.466bcdef	2.496ab	5.931a
TVNu-832	0.587abcdefgh	2.806abc	1.796cdef	6.229a
TVNu-120	0.607abcdefg	2.844abc	1.731cdefg	5.744a
AGG308101WVIG1	0.659abcde	2.817abc	1.647cdefgh	4.244bc
AGG308099WVIG2	0.660abcde	3.187a	1.607defghi	3.803bcde
TVNu-333	0.538abcdefghi	2.230cdefgh	2.756a	6.411a
TVNu-1593	0.663abcd	2.632abcde	1.276efghi	5.511a
TVNu-1582	0.712abc	2.099defgh	1.465defghi	6.563a
TVNu-293	0.653abcde	2.449bcdefg	1.300efghi	4.360b
TVNu-781	0.483cdefghi	2.428bcdefg	2.407ab	3.744bcde
TVNu-1344	0.629abcdef	2.481bcdef	1.452defghi	3.575bcde
Check 1	0.718abc	2.363bcdefg	1.301efghi	3.823bcde
Check 2	0.428defghi	2.386bcdefg	1.571defghi	5.734a
TVNu-1378	0.551abcdefghi	2.065defgh	1.664cdefgh	4.340b
TVNu-1629	0.414defghi	2.801abc	1.443defghi	3.865bcde
AGG62154WVIG_1	0.428defghi	2.622abcde	2.005bcd	3.317bcde
AGG308107WVIG2	0.506bcdefghi	2.942ab	1.077i	3.819bcde
TVNu-1529	0.431defghi	2.372bcdefg	1.705cdefgh	3.468bcde
Check 3	0.493bcdefghi	1.650h	1.600defghi	5.870a
TVNu-1628	0.758ab	2.402bcdefg	1.205ghi	3.451bcde
TVNu-1586	0.562abcdefgh	2.324bcdefgh	1.467defghi	3.468bcde
TVNu-1546	0.503bcdefghi	2.686abcd	1.148hi	3.745bcde
TVNu-1796	0.402defghi	2.468bcdef	1.830cde	3.122de
AGG308096 WVIG2	0.633abcdef	2.265bcdefgh	1.237fghi	3.618bcde
TVNu-1624	0.454cdefghi	1.770gh	2.197abc	3.524bcde
TVNu-1632	0.395defghi	2.636abcde	1.358efghi	3.349bcde
AGG308097WVIG 1	0.552abcdefghi	2.308bcdefgh	1.309efghi	3.430bcde
TVNu-837	0.507bcdefghi	1.986efgh	1.512defghi	3.542bcde
TVNu-1358	0.379fghi	2.323bcdefgh	1.670cdefgh	2.938e
TVNu-1092	0.574abcdefgh	2.195cdefgh	1.245fghi	3.263bcde
TVNu-1701	0.292i	0.772i	1.560defghi	4.219bcd
TVNu-178	0.320hi	2.334bcdefgh	1.423efghi	3.493bcde
TVNu-1591	0.378fghi	2.481bcdef	1.278efghi	2.963e
TVNu-1360	0.393efghi	2.099defgh	1.462defghi	3.165cde

Accessions	Cu	Zn	Mn	Fe
TVNu-381	0.357ghi	2.186cdefgh	1.524defghi	2.765e
TVNu-955	0.513bcdefghi	1.992efgh	1.070i	3.165cde
TVNu-479	0.394defghi	2.191cdefgh	1.070i	2.934e
TVNu-1621	0.359ghi	1.857fgh	1.253fghi	2.889e
Pr > F (Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes

3.7.4. Mineral Contents for the *V. racemosa* Accessions

The mean values for the mineral contents of *V. racemosa* accessions are presented in Table 10. A very remarkable accession, AGG51603WVIG1 among the *V. racemosa* accessions presented the highest concentration in all Zn (3.355 mg/100 g), Mn (2.133 mg/100 g) and Fe (7.614 mg/100 g) respectively, outperforming all the checks and the other accessions (Appendix 4). The appendix 4 shows the dynamics of mineral elements in *V. racemosa* accessions.

Table 10
Mean values of the mineral contents of *V. racemosa* accessions (mg/100 g)

Accessions	Cu	Zn	Mn	Fe
AGG51603WVIG1	0.524ab	3.354a	2.133a	7.543a
Check 2	0.428bc	2.386b	1.571bc	5.734a
Check 3	0.493b	1.650bc	1.600b	5.870a
AGG53597WVIG1	0.336bc	2.327b	1.478bcd	5.851a
Check 1	0.718a	2.363b	1.301de	3.823b
AGG52867WVIG1	0.289bc	2.110bc	1.361bcde	3.218b
Unknown_ <i>Vigna_racemosa</i>	0.301bc	1.348c	1.305cde	3.152b
Unknown <i>Vigna</i>	0.272c	1.511c	1.204e	2.505b
Pr > F (Model)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant	Yes	Yes	Yes	Yes

3.8. Concomitant Analysis of the Four *Vigna* Species

The mean value for each mineral taken in bulk as per species is presented in Fig. 4. The figure shows that the checks outperform all the other wild accessions.

In order to group the accessions based on their phenotypic similarities in terms of the studied mineral composition, the Agglomerative Hierarchical Clustering (AHC) of XLSTAT was run to obtain a dendrogram (Fig. 5). The dendrogram show clusters (C) of wild *Vigna* accessions based on mineral compositions (Mn, Zn, Cu, and Fe) studied. Based on the minerals evaluated, it reveals that both wild accessions and checks can form three groups of wild *Vigna* accessions in which the two checks (Check 2 and Check 3) belong to the same group with some nine wild accessions (Table 11). Table 11 shows the details on the number of wild *Vigna* accessions belonging to the clusters as grouped on the dendrogram.

Table 11

Wild *Vigna* accessions belonging to classes as grouped on the dendrogram for mineral composition

Clusters	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_ <i>Vigna_racemosa</i>	Check 2
	TVNu1804_Va	AGG52867WVIG1_Vra	AGG51603WVIG1_Vra
	TVNu1644_Va	Unknown <i>Vigna</i>	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_ <i>Vigna reticulata</i>	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	

Clusters	1	2	3
	TVNu739_VRe	TVNu1358	
	TVNu57_VRe	TVNu1591	
	TVNu1790_VRe	TVNu1621	
	TVNu138_VRe	TVNu381	
	TVNu767_VRe	TVNu178	
	TVNu491_VRe	TVNu1701	
	TVNu379_VRe		
	TVNu738_VRe		
	TVNu161_VRe		
	TVNu524_VRe		
	TVNu607_VRe		
	TVNu1698_VRe		
	TVNu1522_VRe		
	TVNu605_VRe		
	TVNu1405_VRe		

To analyze the relationship between the evaluated mineral elements to each other and vis-à-vis the accessions, the Principal Component Analysis (PCA) was ran (Fig. 6) and the Pearson correlation results are as shown on Table 12. It shows that Fe, Zn and Mn are positively related to each other and to a group of wild *Vigna* accessions including the checks while they are not related to Cu (Table 12 & Fig. 6).

Table 12
The Pearson correlation matrix (n):

Variables (mg/100g)	Cu	Mn	Fe
Cu	1	-0.215	-0.468
Zn	-0.215	1	0.536
Mn	-0.468	0.467	1
Fe	-0.357	0.536	0.715

3.9. Fatty Acids Composition of Wild *Vigna* Species

Tables 12–14 below show the distribution of the predominant fatty acids present in *V. reticulata*, *V. vexillata* and *V. racemosa* accessions. *Vigna ambacensis* accessions were not assessed for fatty acids because of the availability of very limited amount of samples to cover all the biochemical characterization. Both saturated, mono- and poly-unsaturated fatty acids were reported. From the results, Hexadecanoic acid (C16:0), Stearic acid (C18:0), and Heptadecanoic acid (C17:0) were the major saturated fatty acids. On the other hand, 9, 12- Octadecadienoic acid (C18:2n-6), and 9, 12, 15-Octadecatrienoic acid, (C18:3n-3) were the predominant unsaturated fatty acids (Table 12–14).

Remarkably, variation of both saturated and unsaturated fatty acids were found in the wild *Vigna* accessions. On the other hand, unsaturated fatty acids were mainly found in the checks (domesticated legumes). Besides, for the *V. racemosa* accessions, unsaturated fatty acids were predominant (Table 14). Furthermore, a good number of wild *Vigna* species accessions were also rich in unsaturated fatty acids comparable to the domesticated legumes (checks).

Table 12
Fatty acid composition of *V. reticulata* accessions (% composition)

Accessions	Hexadecanoic acid (C16 :0)	Octadecanoic acid (C18 :0)	Heptadecanoic acid (C17 :0)	9,12-Octadecadienoic acid (C18:2n-6)	9,12,15-Octadecatrienoic acid, (C18:3n-3)
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
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

Accessions	Hexadecanoic acid (C16 :0)	Octadecanoic acid (C18 :0)	Heptadecanoic acid (C17 :0)	9,12-Octadecadienoic acid (C18:2n-6)	9,12,15-Octadecatrienoic acid, (C18:3n-3)
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 13
Fatty acid composition of *V. vexillata* accessions (% composition)

Accessions	Hexadecanoic acid (C16 :0)	Octadecanoic acid (C18 :0)	Heptadecanoic acid (C17 :0)	9,12-Octadecadienoic acid (C18:2n-6)	9,12,15-Octadecatrienoic acid, (C18:3n-3)
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	-	-	-
AGG308097WVIG1	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	-	-	-
AGG62154WVIG1	68.22	9.90	-	-	-
TVNu1546	45.03	27.66	-	-	-
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

Accessions	Hexadecanoic acid (C16 :0)	Octadecanoic acid (C18 :0)	Heptadecanoic acid (C17 :0)	9,12-Octadecadienoic acid (C18:2n-6)	9,12,15-Octadecatrienoic acid, (C18:3n-3)
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 14
Fatty acid composition of *V. racemosa* accessions (% composition)

Accessions	Hexadecanoic acid (C16 :0)	Octadecanoic acid (C18 :0)	Heptadecanoic acid (C17 :0)	9,12-Octadecadienoic acid (C18:2n-6)	9,12,15-Octadecatrienoic acid, (C18:3n-3)
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_ <i>Vigna_racemosa</i>	-	-	34.88	23.57	15.26
AGG52867WVIG1	-	-	39.77	27.28	23.77
Unknown <i>Vigna</i>	-	-	-	26.53	16.55

4. Discussion

4.1. Proximate Contents Evaluation of Wild *Vigna* Species

The proximate composition for the various *Vigna* species studied is summarized in Tables 1–4. Check 1 and Check 2 might be related in terms of the phenotypic traits lipid, fiber and carbohydrates content though they are of different species (*V. vexillata* and *V. unguiculata*). In addition, it should be 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 completed³¹. Phylogenetic proximity between *V. vexillata* and *V. unguiculata* has also been reported¹¹. 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. These can be the most probable explanation to the result obtained showing differences and similarities in terms of some nutrients between the three checks.

According to Table 1, the lipid content of all the wild accessions of *V. ambacensis* is significantly 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 domestication³². Other differences among the checks and the wild accessions may be due to species differences and phylogenetic relationships. It is therefore suggested that accessions with higher nutrients than that the domesticated species should be further investigated for breeding or domestication.

Following the Table 2, 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, and 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 phylogenetic proximity of *V. reticulata* with Check 1 and 2 (Table 5). 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 low moisture content could also be a factor of good storing quality of the seeds. 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 environmental background. For the accessions with protein content comparable to that of Check 1 and Check 2 (TVNu1852_VRe, TVNu141_VRe, TVNu57_VRe, TVNu324_VRe, and TVNu350_VRe), phylogenetic 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. The greater number of wild accessions presented a significantly higher fiber and carbohydrates contents as compared to the checks. This is in line with earlier reports on wild legumes^{18,21}. It might be due to the biosynthesis of many polysaccharides by the wild legumes in order to protect the embryo and survive in harsh environments³⁵. Therefore, it should be recommended to carry out sound examination of the carbohydrates and fiber fractions to ascertain the digestibility and clear nutritive contribution of these seeds.

In Table 3, the proximate composition of *V. vexillata* accessions is displayed. It was found that the lipid content of most of the wild accessions is significantly lower than that of Check 1 and Check 2 except for a few accessions (AGG308096WVIG2, TVNu333, TVNu293, and TVNu832) 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 *V. 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 TVNu1701, and AGG30801WVIG1 with highest protein content are speculated to be suitable candidate genetic materials for domestication or breeding. Therefore further investigation should be done through molecular marker as its high protein content might be due to its genomic difference. Furthermore, this might have been acquired based on the environmental background. For the accessions with protein content comparable to that of Check 1 and Check 2, phylogenetic studies as well as breeding and improvement are 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^{18,21}. It might be due to the biosynthesis of many polysaccharides by the wild legumes in order to protect the embryo and survive in harsh environments³⁵ as explained earlier. Therefore, it is 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.

In the case of *V. racemosa* accessions (Table 4), 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. 1). 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.

The Fig. 2 specifies that the wild *Vigna* accessions could be clustered into three classes based on the proximate composition, with class I (C1) including all the three checks (Table 5). This indicates that some of the wild accessions share common features and probably genetic characteristics. Therefore, the class I which contains the checks presents an 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^{16,17}. 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 purposes³⁶. In fact, it has recently been reported that *V. stipulacea*, another wild legume species with biotic resistance traits is domesticable⁵. Moreover, it is also necessary to note that the domestication process could also affect other nutritional and health characteristics of the domesticated product as alerted by some researchers³⁷. The PCA further provides indications relating to the domestication of these wild legumes by grouping them based on their quantitative proximate composition

traits (Fig. 3). 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.

4.2. Mineral Contents Evaluation of Wild *Vigna* Species

A scrupulous examination of the mineral contents of the wild *Vigna* accessions and domesticated *Vigna* legumes studied demonstrated that a considerable amount of a specific or a combination of minerals that can point out its relevance in human and animal nutrition. The superior mineral content observed in the checks is supported by³⁸ with cowpea (*Vigna* spp.) showing a better mineral pattern. In an earlier study, presence of considerable amounts of Fe, Ca, Na, K and P from wild bean (*V. sublobata*) was reported³⁹. This is in line with speculations earlier suggested and recommended by researchers (Harouna et al., 2020, Harouna et al., 2019). For instance, an accession like TVNu-1792 (0.918 mg/100g) 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/d⁴¹. 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/100g) if maintained and all absorbed after digestion can satisfy the need. Likewise, two little known *Vigna* species (*V. capensis* and *V. sinensis*) were reported to be rich source of K, Ca, Mg, P and Fe when compared with RDA⁴². The positive correlation between Fe, Zn and Mn can be due several factors such as growing environment, growing season and/or genetic makeup of the various seed which may need to be investigated.

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 humans⁴³. On the other hand, it was found that many of the domesticated crops (including legumes) suffer from low concentration of one or more minerals. To this challenge, is added the bottleneck challenge of breeding the wild with the domesticated one to improve the mineral content⁵. Therefore, domestication of the undomesticated is one of the adequate methods to improve the micro-nutrient composition of crops and add more varieties for human nutrition and biodiversity conservation². Further studies leading to the domestication of these wild species is hereby highly recommended.

The reason for higher concentration of some minerals in these wild accessions is not yet elucidated and can be attributed to the genetic makeup of seeds or due to their environmental origin. The variation in mineral contents from the same species may be attributed by the genetic makeup, geographical location and soil fertility⁴⁴. However, this needs to be investigated as very few reports exist in documentation of the biochemical characteristic of the wild *Vigna* legumes.

4.3. Fatty Acid Composition of Wild *Vigna* 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 undeniable⁴⁵. Other than saturated fatty acids, both checks and a good number of wild *Vigna* accessions, exhibited significant concentration of functional fatty acids Octadecadienoic acid (C18:2n-6) (Omega 6) and Octadecatrienoic acid (C18:3n-3) (Omega 3). Similarly, linoleic and linolenic, palmitic and stearic acids were the fatty acids reported from two little known species of *Vigna* (*V. capensis* and *V. sinensis*)⁴². Evidence of the presence of saturated and unsaturated fatty acids in underutilized legumes have been reported^{20,44}. 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* accessions could also contain important fatty acids found in oil seeds and which are of health importance. This could confer the functional potential to the wild legumes.

5. Conclusion

The wild *Vigna* species studied presents a considerably high diversity in terms proximate composition. Despite their under-exploitation for human benefits, the wild *Vigna* legumes demonstrated nutrient characteristics comparable with the domesticated ones. The study also demonstrated that the wild *Vigna* species possesses a large variation range of nutrient 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 as compared with domesticated ones which could be advantageous for bio-

fortification or domestication. The candidate accessions favorable for domestication, based on the proximate characteristics were revealed. Therefore, based on the protein content, the best accessions were: TVNu832, TVNu1701, AGG51603WVIG 1, AGG53597WVIG 1, and TVNu1112. Therefore, these accessions are the best suited for domestication. However, other selected accessions should not be neglected and should be subjected to more study to unveil their potentials. The chemical composition of the other parts of the *Vigna* species such as leaves and steams 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.

Declarations

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Figures

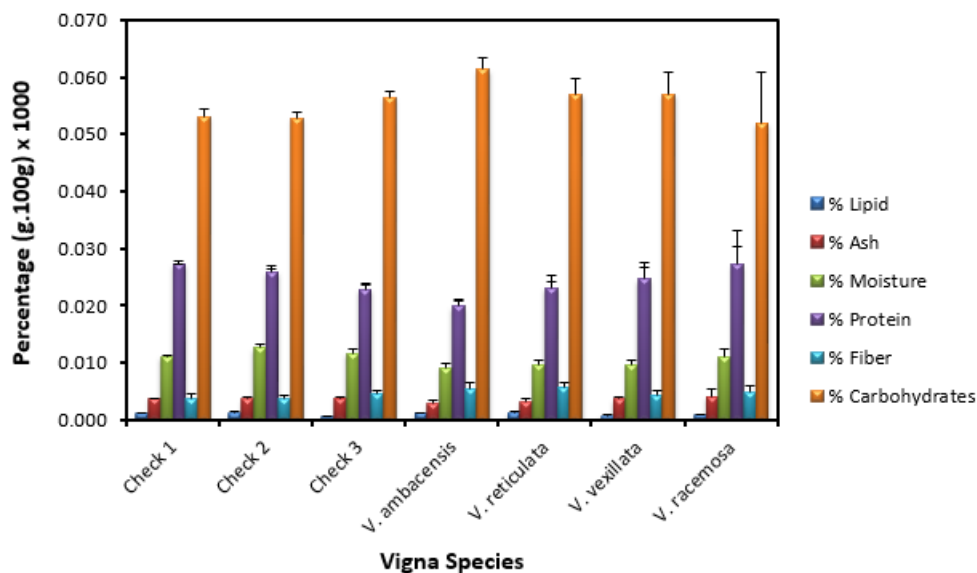


Figure 1

Means of proximate composition of wild *Vigna* accessions per species

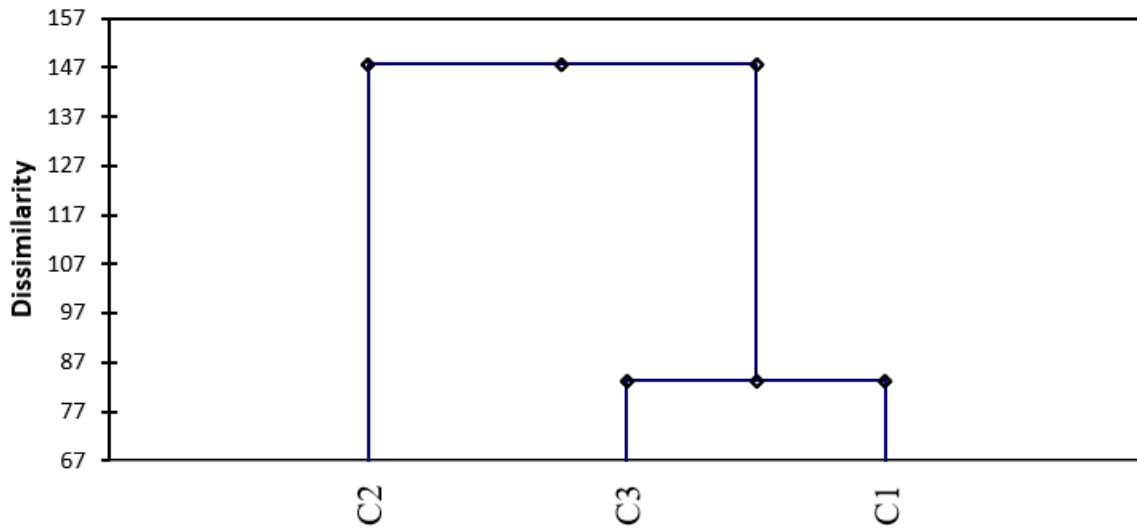


Figure 2

Dendrogram showing clusters of wild *Vigna* accessions based on their proximate composition

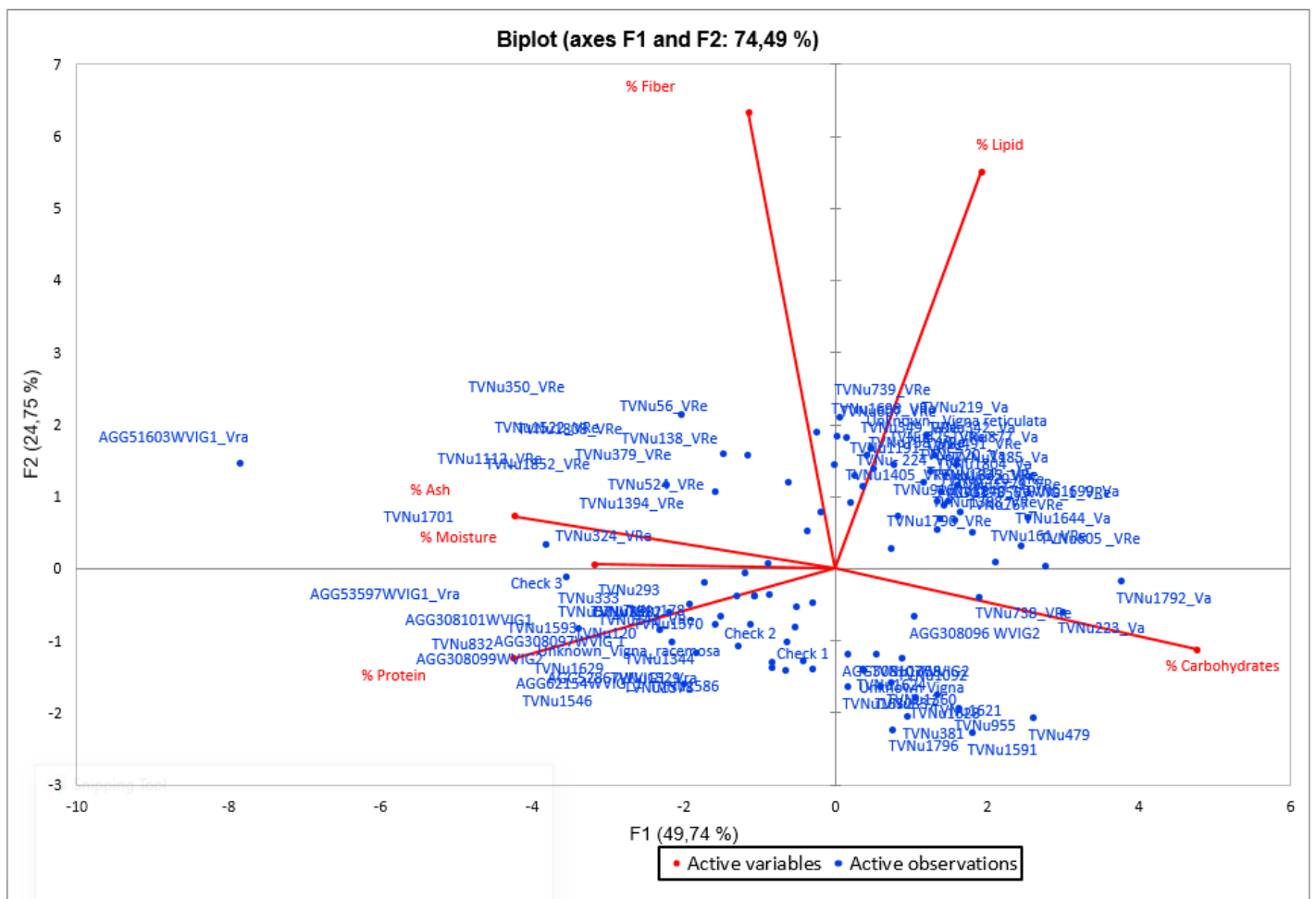


Figure 3

The PCA analysis showing correlations between nutrients contents and wild *Vigna* accessions

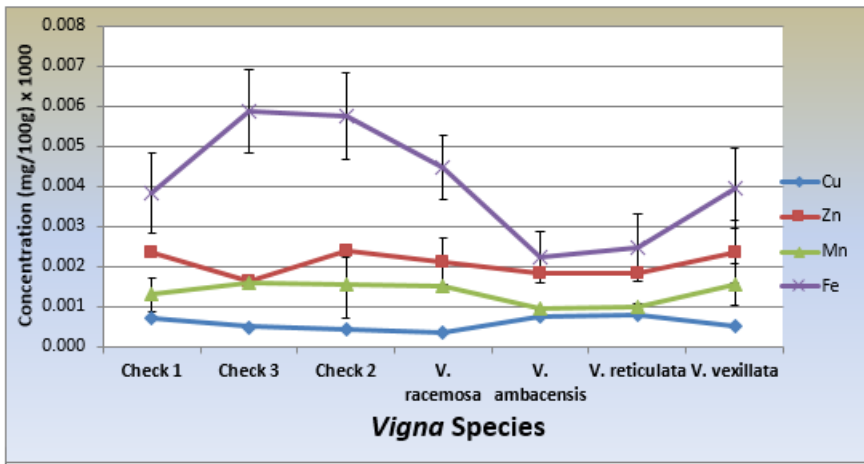


Figure 4

Mean mineral contents of the *Vigna* species

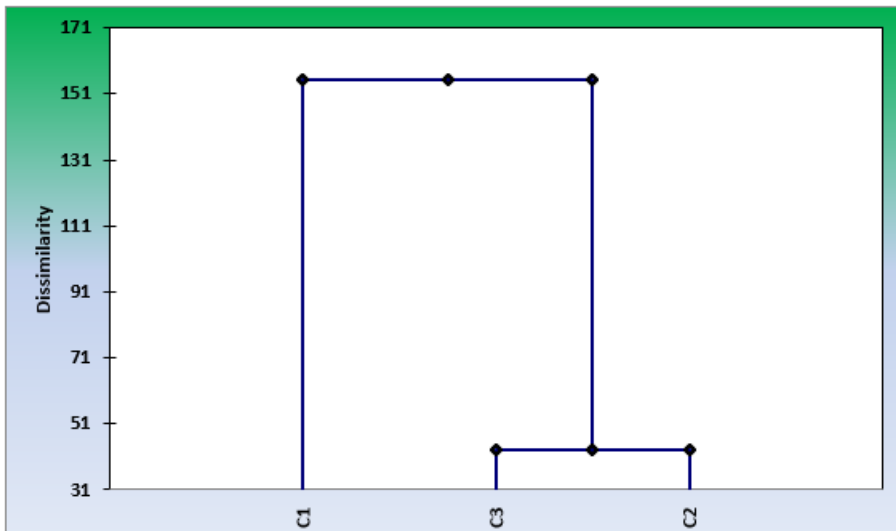


Figure 5

Dendrogram showing clusters of wild *Vigna* accessions based on the mineral composition

