

MORPHOMETRIC DIVERSITY AND GENETIC DISPOSITION EVALUATION OF SOME GREEN GRAM (*Vigna radiata*) VARIETIES

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Abstract

Diversity study on the variability level of nutritional and anti-nutritional contents across available accessions could also be a key factor to the selection of superior genotypes for breeding program. This study identified morphological variability in twenty accessions of Vigna radiata through the multivariate- Principal Component Analysis PCA, Cluster analysis and correlation between yield and yield-related parameters. Evaluation of nutritional and anti-nutritional contents through the analysis of protein and Lectin were also adopted for the variability study. According to the principal component analysis, four principal components had Eigenvalues greater than 1, and accounted for 77% of the total variations using 13 variables. Amongst the first four Principal components, PC1 contributed a high proportion of the total variation (37.2%), while others contributed 13.83%, 8.22%, and 7.81% respectively. Hierarchical clustering method separated twenty accessions into three clusters containing six, five and nine accessions respectively. The total protein and hemagglutinating ability of Mung bean extracts from both seeds and leaves investigated revealed low Lectin in leaves and zero level of Lectin in the dried seeds. The result showed that accessions such as TVr56, TVr53, TVr43, and TVr59 should be selected during the breeding programme for Mung bean due to their high protein, low leave Lectin and zero seed Lectin. More importantly, genetic compatibility study between the evaluated and other improved cultivars from other countries should be encouraged.

Keywords: multivariate analysis, green gram, Principal Component Analysis, lectin, hemagglutinating ability, protein, variability

Introduction

Mung bean (*Vigna radiata* 'R. Wilczek') is an important annual legume. It is cultivated in the tropical, subtropical, and temperate Asia. This species has been widely domesticated in Africa with scanty and insufficient information (Brishti *et al.*, 2017). It is an important short duration grain legume that can be grown in varying environmental conditions. It is an annual, erect or semi-erect plant, reaching approximately 0.15-1.25 meters in height. It has branching stems which may sometimes be wining at the tips (Chauhan & Williams,

2018). The leaves are alternate, trifoliate with elliptical to ovate leaflets, 5-18 cm long and 3-15 cm in breadth. The mature seeds are rich sources of digestible protein for humans in places where meat is scarce (Deraz & Khalil, 2008). Mung beans are cooked fresh or dried and can be eaten whole or made into flour, soups, porridge. The germinated seeds have high nutritional value with approximately 25 to 28 per cent protein, significant percentage of ash, carbohydrates and rich sources of antioxidant components necessary to combat chronic diseases. However, the plant has low concentration of amino acid methionine and

cysteine (Brishti *et al.*, 2017).

Mung bean is grown primarily as an intercrop with wheat, maize, potato and other crops during the monsoon season and as a monoculture at other times (Kheroar *et al.*, 2016). It has a deep and well-spread root system and can tolerate drought. It is known to produce nitrogenous fodder for the livestock. Developing high yielding varieties by improving or incorporating the yield attributing traits are necessary for increasing the current production and productivity level (Mohan & Dharendra, 2014).

Mung bean has been getting its space gradually in plateau state and some Northern parts of the country since its introduction to Nigeria (Chauhan & Williams, 2018), due to the little knowledge of its nutritional content. As a future food crop, orphan crops have predominantly been investigated by scientists in developing countries with the help of some international bodies including the International Institute for Tropical Agriculture (IITA), Ibadan Nigeria, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Laboratory of Genetics, Horticulture and Seeds Sciences. Typical orphan legume crops include Bambara groundnut (*V. subterranea*), Mung bean (*Vignaradiata*), horse gram (*Macrotyloma uniflorum*), moth bean (*V. aconitifolia*), dolichos (*Lablab purpureus*), and marama bean (*Tylosema esculentum*) (Cullis & Kunert, 2017).

Mung bean as a legume, has the characteristic nature of fixing atmospheric nitrogen through the process of symbiosis with *Rhizobium* in the soil (Chadha, 2010; Khan *et al.*, 2012).

Many sources of information stated that the world population has been projected to reach 9.8 billion in 2050, and 11.2 billion in 2100 and this increase is mainly expected in developing countries like Africa. Food and

nutrition security will likely be the greatest problems that will face these countries (Karim & Zahurul, 2016). To keep pace with the trend, agricultural production will need to increase.

Variability study helps breeders to understand the genetic relationships among accessions and to select the superior accession more systemically and effectively in order to produce quality varieties (Altınordu *et al.*, 2014; Cullis & Kunert, 2017).

Anti-nutritional contents called lectins were first described in 1888 (as extracts) by Stillmark working with castor bean extracts (Movafagh *et al.*, 2013). They have an affinity for carbohydrates and they can agglutinate cells of various types. Lectin was reported as a carbohydrate-binding protein present in most plants, especially in seed, tubers like potatoes, and also in animals. (Hamid *et al.*, 2013; Shi *et al.*, 2007) underlined that dietary lectins act as protein antigens that bind to surface glycoprotein on erythrocytes or lymphocytes.

Lectins are found in abundance in the legume seeds (Hamid *et al.*, 2013). Recent on hemagglutinating variation in seeds and leaves of okra spp (*Abelmoschus esculentus* and *caillei*) suggested further improvement programme as the studied species was discovered to have low protein and less Lectin contents (Ajiboye *et al.*, 2019). The study recommended analysis of genetic compatibility between the two well-recognised species of okra (*A. esculentus* and *A. caillei*) as an important selection procedure for breeders. The high content of Lectin consumption as reported by (Hamid & Masood, 2009) can cause three primary physiological reactions viz: severe intestinal damage, distrustful digestion and nutrient deficiencies.

They can bind to the erythrocyte of the individual and simultaneously cause hemagglutination. Hamid *et al.*, (2013) further established that Lectins are toxic; causing inflammation, nausea, vomiting and diarrhoea. Lectins can cause food poisoning that

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sometimes get past the gut wall and deposit in distant organs (Hamid *et al.*, 2013). With legume seeds, whose Lectin content is appreciable, this presents serious problems in nutritional practice.

Considering the above-mentioned points, the present study was carried out to assess the genetic diversity and subsequently isolate the suitable parents from the accessions evaluated using multivariate analysis and also dissected the nutritional and anti-nutritional constituents of green gram. The research targets contributing to number two of the United States Developmental Goal (USDG) which underlined zero hunger in the whole world by 2030.

Therefore, this research-work is aimed at characterizing accessions, adopting the morphometric traits, nutritional and anti-nutritional attributes of Green gram.

Methodology

Materials and Methods

Collection of plant material

Seeds of Mung bean used in this study were collected from the Genetic resources unit (GRC), International Institute of Tropical Agriculture- IITA, Ibadan, Nigeria (Table 1).

Experimental setup

Morphometric traits evaluation

Phenotyping of twenty (20) Mung bean accessions was performed at the experimental site of Faculty of Science, Federal University Oye-Ekiti, Ekiti State, Nigeria. The experiment was conducted in a Simple lattice square design with two replications. Each accession was sown with a spacing of 30 × 30 cm and all recommended agronomical practices were followed to raise the crop.

Nutritional and anti-nutritional constituents

The variability study of protein and the

hemagglutinating analysis of the seeds and leaves were efficiently carried out at the standardized Enzymology laboratory, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria

Table 1- List of accessions and their sources

S/N	Species name	Accession codes	Source
1	<i>Vignaradiata</i> (Mung bean)	TVr17	IITA
2	<i>Vignaradiata</i> (Mung bean)	TVr19	IITA
3	<i>Vignaradiata</i> (Mung bean)	TVr21	IITA
4	<i>Vignaradiata</i> (Mung bean)	TVr31	IITA
5	<i>Vignaradiata</i> (Mung bean)	TVr32	IITA
6	<i>Vignaradiata</i> (Mung bean)	TVr34	IITA
7	<i>Vignaradiata</i> (Mung bean)	TVr37	IITA
8	<i>Vignaradiata</i> (Mung bean)	TVr43	IITA
9	<i>Vignaradiata</i> (Mung bean)	TVr44	IITA
10	<i>Vignaradiata</i> (Mung bean)	TVr46	IITA
11	<i>Vignaradiata</i> (Mung bean)	TVr48	IITA
12	<i>Vignaradiata</i> (Mung bean)	TVr49	IITA
13	<i>Vignaradiata</i> (Mung bean)	TVr52	IITA
14	<i>Vignaradiata</i> (Mung bean)	TVr53	IITA
15	<i>Vignaradiata</i> (Mung bean)	TVr56	IITA
16	<i>Vignaradiata</i> (Mung bean)	TVr59	IITA
17	<i>Vignaradiata</i> (Mung bean)	TVr82	IITA
18	<i>Vignaradiata</i> (Mung bean)	TVr90	IITA
19	<i>Vignaradiata</i> (Mung bean)	TVr94	IITA
20	<i>Vignaradiata</i> (Mung bean)	TVr97	IITA

Source: International Institute of Tropical Agriculture (IITA) (2020)

Data Collection

Twenty accessions of Mung bean were collected from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Morphometric data such as days to 50 percent flowering, days to maturity, plant height, leaf length, leaf width, petiole length, pod length, pod yield per plant, seeds per pod, seed yield per plant, harvest index, stem girth, number of primary branches, hundred-seed weight and days to germination were collected. The mean values were subjected to statistical analysis using the R-software version 3.6.2.

Blood typing

Standard serological procedure was followed. The finger was sterilized and pricked with a

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sterilized lancet. 0.5 ml and blood samples were collected in 0.85% NaCl (physiological saline) in a test tube. RBCs pellet was washed thrice with physiological saline by centrifuging it at 6000 rpm. 2% RBCs suspension was prepared in physiological saline. Blood typing was done by normal serological method using 2% RBCs suspension.

Estimation of crude protein

Preparation of crude extracts: Fresh leaves and seeds of *V. radiata* were air-dried and properly grounded into powder with the aid of mortar and pestle. The grounded seeds and leaves were then transferred to a well-labeled tube.

Protein determination via leaves

0.8 grams of the samples of each accession of *V. radiata* were weighed and homogenized with 15ml of the buffer (0.1M solution, pH=6.5) and stored in the freezer for 12hrs. The samples were centrifuged at 6000rpm for 10 minutes and the supernatant was collected into a well labeled universal tube.

Protein determination via seeds

1.5 grams of the grounded seeds were weighed and transferred to the well labeled universal tubes and 5ml of the prepared buffer (0.1M solution, pH=6.5) was added to each tube and were left in the freezer overnight. The samples were transferred from the universal bottle to the plastic test tubes and then centrifuged at 6000rpm for 10 minutes. Supernatant were later decanted into the universal tubes.

Determination of protein using the Bradford method

100ul of distilled water (H₂O) was pipetted into a well-labeled test tube with 500ul of Bradford reagents as blank. 100ul of the extracts of each accession were pipetted into

small test tubes and 500ul of Bradford reagent were also added into the test tube containing each accession. The sample containing both extracts and Bradford were then checked under Vis-Spectrophotometer at 595 wavelengths. Blue coloration indicates the presence of protein in each accession and protein concentration can be detected under U.V spectrophotometer at 595 wavelengths (Pedrol, 2001)

Data Analysis

The data collected from the experimental field were subjected to analyses using the latest version of R software version 4.2 (R Core Team, 2019). All quantitative traits compiled mean data was computed for Analysis of variance (ANOVA) to detect the variability present among the twenty accessions of Mung bean. The coefficient of variation (CV) was analyzed for all the traits observed using the mean value and standard deviation in equation 1.

$$CV (\%) = \text{Mean/Standard deviation} \times 100\% \dots\dots\dots (1)$$

This test helped to reveal the level of heterogeneity during the field experiment. Pearson's test was conducted to detect the positive and negative correlation among the quantitative traits observed. The variables generated were then subjected to Principal Component Analysis-PCA, which revealed the variables that contributed mainly to the variation observed among accessions used in this study. This was generated using packages such as 'FactoMineR', ggplot2 and FactoExtra to group accessions into different categories. Hierarchical clustering was adopted for the grouping of accessions based on the performance of the accession on the field.

Hemagglutination attributes

The dried seed and leaves extract of *V. radiata* were thawed at room temperature just before

the beginning of the experiments. Microtitre plates of 12 columns and 8 rows of holes were used for the hemagglutination tests. 25µl red blood was added to an equal amount of extracts of the samples. After 25 minutes, results were recorded following (Koley, 2019) method. When agglutination occurs, cross-linked red blood cells form a network that prevents the red blood cells from forming sediments to the bottom of the well. They appeared like a carpet covering the whole microtiter plate well. When no agglutination occurred, no red blood cells sediment would be formed on the bottom of the well.

Result

Analysis of Variance

The mean, standard deviation, minimum and maximum values of all characters observed are shown in Table 2. The analysis of variance conducted for some quantitative traits exhibited significant differences (P<0.05) among the accessions, suggesting the existence of appreciable diversity among the twenty accessions for different yield and yield-related traits. Before ANOVA test, the assumption of normality was evaluated and determined to be satisfied. It was found out that the variables showed a high degree range of variation across the twenty accessions studied. Table 3 shows the Pod length, 100- seed weight, Number of pods per plant, seed yield per plant, yield per plant, days to 50% flowering, days to maturity and the harvest index imply significant variability between the twenty accessions (Table 3). Other variables studied like plant height, petiole length, leaf girth, leaf length, stem girth showed no variation across the accessions of *V. radiata* studied. This suggests the presence of a substantial amount of variability among the twenty accessions evaluated (Table 3)

Table 2-Descriptive statistics for trait variability in 20 Mung bean genotypes

Variables	Mean±SD	Min	Max	CV (%)
PH	49.15±8.43	41.44	74.67	17
LG	11.53±1.0	9.84	13.28	9
LL	9.06±0.71	7.92	10.39	8
PL	6.4±0.7	5.11	7.46	11
SG	0.35±0.002	0.32	0.38	5
D50F	31±2.15	28	34	7
DTM	73.66±1.83	71	76	2
NOB	3.64±0.69	2.5	5	18
NPP	26.75±2.32	21.5	30.5	9
PODL	6.51±1.07	4.3	8.63	16
X100SW	3.78±0.4	2.83	4.43	10
SYPP	4.44±0.89	3.32	6.16	20
YPP	14.48±2.08	10.66	17.89	14
HI	29.41±4.94	22.55	37.98	17
DOG	3.05±0.05	3	3.1	2

Min- Minimum, Max-Maximum, SD-Standard deviation, CV- Coefficient of variation

PH- Plant height, LG-Leaf girth, LL- Leaf length, PL-Petiole length, SG-Stem girth, D50F- Days to 50% flowering, DTM- Days to maturity, NOB-Number of primary branches, NPP-Number of pod per plant, PODL-pod length, X100SW-One hundred seed weight, SYPP-Seed yield per plant, YPP-Yield per plant, HI-Harvest index, DOG- Date of germination.

Table 3; Table showing Analysis of variance of the quantitative variables and their significant difference across accessions of Green gram.

Variables	Accessions (Ddl=19)	Residuals (Ddl= 19)
PH	67.96 ^{ns}	77.07
LG	0.675 ^{ns}	1.300
LL	0.305 ^{ns}	0.687
D50F	9.474 ^{***}	0.000
DTM	6.819 ^{***}	0.056
PL	0.3092 ^{ns}	0.686
SG	0.0000178 ^{ns}	0.000
NOB	0.8352 ^{***}	0.083
NPP	9.868 ^{***}	1.183
PODL	2.3255 ^{***}	0.009
X100SW	2.3255 ^{***}	0.045
SYPP	1.631 ^{***}	0.000
YPP	8.864 ^{***}	0.000
HI	50.14 ^{***}	0.000
DOG	0.005 ^{***}	0.000

ns- non-significant, P<0.005 *, P<0.01 **, P< 0.001 ***

PH- Plant height, LG-Leaf girth, LL- Leaf length, PL-Petiole length, SG-Stem girth, D50F- Days to 50% flowering, DTM- Days to maturity, NOB-Number of branches, NPP-Number of pod per plant, PODL-pod length, X100SW-One hundred seed weight, SYPP-Seed yield per plant, YPP-Yield per plant, HI-Harvest index, DOG- Date of germination.

Correlation Matrix between traits

The Pearson’s correlation of variation analysis between variables showed significant (P<0.05)

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relationships (Figure 1). Weak negative and significant correlation were detected among hundred-seed weight (X100SW) and Petiole length (PL) ($r=-0.37$), DOG with SYPP ($r=0.46$), SYPP ($r=-0.43$), HI ($r=-0.47$) and YPP ($r=-0.39$). In contrast to the negative relationship between hundred-seed weight (X100SW) and Petiole length (PL), X100SW showed a positive correlation with Days of germination-DOG ($r=0.43^{**}$). A strong positive correlation was observed between plant height PH ($P<0.001$) and variables such as Leaf girt-LG, Leaf length-LL, Petiole length- PL and Stem girth-SG. A weak negative correlation was also observed between the Number of pods per plant, NPP and the leaf length LL ($r=0.28$). Table 4 also showed a non-significant correlation between some observed quantitative variables. Variables like Plant height showed no significant correlation with NOB-(Number of branches), NPP-(Number of pods per plant), PODL-(Pod length), X100SW (Hundred-seed weight), SYPP (Seed yield per plant), YPP (Yield per plant), HI (Harvest index), DOG (Days of germination). Highly significant ($P<0.001$) and strong positive correlation were observed between Leaf girt (LG) and Leaf length (LL) ($r=0.94$), Leaf length (LL) and Petiole length (PL) ($r=0.92$), Seed yield per plant (SYPP) and Yield per plant (YPP) ($r=0.52$), Seed yield per plant (SYPP) and Harvest index (HI)-($r=0.75$). Most importantly (Figure 1) revealed a negative correlation between pod length and YPP (Yield per plant) ($r=-0.37$). However, no significant positive correlation was detected for pod length (PODL) (Figure 1).

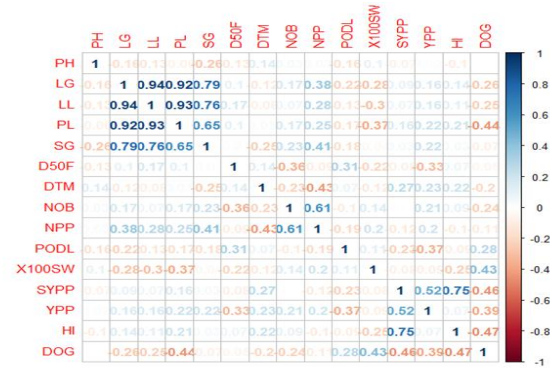


Figure 1: Correlation Matrix for 13 quantitative variables observed

Table 4- Eigen values and contribution of first four principal components axes to variation

Descriptors	PC 1	PC 2	PC 3	PC 4
PH	0.85	0.18	-0.08	-0.22
LG	0.93	0.07	-0.26	0.02
LL	0.87	-0.05	-0.39	0.01
PL	0.94	-0.24	-0.14	0.00
SG	0.73	0.45	-0.06	0.08
D50F	0.03	-0.33	-0.69	0.40
DTM	-0.17	-0.71	0.21	-0.13
NOB	0.32	0.53	0.48	0.35
NPP	0.48	0.71	0.13	0.32
PODL	-0.50	-0.09	-0.31	0.51
X100SW	-0.54	0.47	0.39	0.23
SYPP	0.32	-0.55	0.58	0.24
YPP	0.49	-0.08	0.57	-0.27
HI	0.35	-0.56	0.31	0.51
DOG	-0.68	0.41	-0.22	-0.09
Eigen Value	5.58	2.73	2.07	1.17
Variance				
Percentage	37.17	18.22	13.83	7.81
Cumulative				
Percentage	37.17	55.39	69.22	77.03

at $P<0.05$, **, $P<0.01$, *** $P<0.001$.

PH- Plant height, LG-Leaf girth, LL- Leaf length, PL-Petiole length, SG-Stem girth, D50F- Days to 50% flowering, DTM- Days to maturity, NOB-Number of branches, NPP-Numbr of pod per plant, PODL-pod length, X100SW-One hundred seed weight, SYPP-Seed yield per plant, YPP-Yield per plant, HI-Harvest index, DOG- Date of germination.

Principal Component Analysis

Principal Component Analysis (PCA) using accessions means of 15 descriptors was performed to determine which of the character strongly explained each of the principal components. The criterion was based on the selection of the principal component whose eigenvalues are >1 . The principal component analysis performed on the fifteen 13 variables across the accessions revealed that the first four principal components (PCs) with Eigen value >1 accounted for 77% of the total variability. The significant variables in each

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PC was determined using >0.5 significant correlation (Akohoue et al., 2019). The percentage of the total variance within the first four PC axes are 37.17, 55.39, 69.22 and 77.03 respectively. The PC1 to PC4 have the Eigenvalue 5.58, 2.73, and 2.07 and 1.17. Since the yield parameters are correlated to these principal components explained, hierarchical clustering was performed using the four components

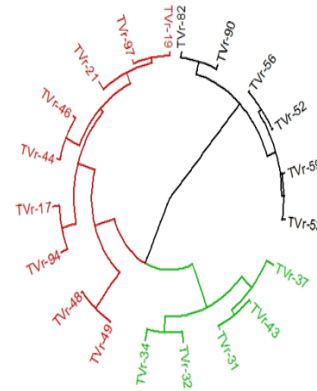


Figure 2- Hierarchical clustering structure of twenty (20) accessions of Green gram

Cluster analysis

To evaluate and utilize germplasm effectively, there is a need to investigate level of the diversity of the available accessions. Hierarchical clustering analysis revealed three different clusters of mung bean accessions evaluated (Figure 2). The three clusters were significantly distant from one another. The entries in this study were grouped into three circular clusters containing accessions that were highly similar in terms of the morphological attributes considered. Cluster 1 consisted of six accessions with the accession codes-TVr-82, TVr-90, TVr-56, TVr-52, TVr-59, TVr-53 which are further separated into two subgroups. Cluster 2 consists of TVr-37, TVr-43, TVr-31, TVr-32, TVr-34 which are also separated into two subgroups. Finally, the third cluster contains TVr-49, TVr-97, TVr-21, TVr-46, TVr-46, TVr-44, TVr-17, TVr-94, TVr-48 and TVr-49.

Accessions in cluster three were also further grouped based on the level of similarities between their descriptive variables (Table 5) From the study, Cluster one (I), cluster two (II) and cluster three (III) are composed of 30%, 45% and 25% accessions respectively in this study. Cluster 2 was composed of high yielding accessions with the highest hundred- seed weight. All accessions in all clusters showed no dissimilarities in the number of days to 50% flowering and days to maturity.

Table 5-Descriptive statistics of clusters of Mung bean accessions

Descriptors	Cluster 1	Cluster 2	Cluster 3
	Mean± SD	Mean ± SD	Mean± SD
PH	49.89±12.39	49.94±8.46	49.55±9.47
LG	10.85±0.28 ^a	11.6±0.24 ^a	12.22±0.3 ^b
LL	8.63±0.21 ^a	9.07±0.14 ^a	9.57±0.19 ^b
PL	5.97±0.24 ^a	6.43±0.19 ^a	6.86±0.22 ^a
SG	0.34±0 ^a	0.36±0.01 ^a	0.36±0.01 ^a
D50F	31.5±1.76	29.44±1.67	33.2±1.1
DTM	74.33±1.86	73.14±1.92	73.8±1.79
NOB	3.21±0.43	4.11±0.57	3.3±0.41
NPP	24.42±1.66 ^a	28.19±1.86 ^a	26.95±0.41 ^a
PODL	7.14±1	6.15±1.24	6.39±0.55
X100SW	3.92±0.35 ^a	3.92±0.33 ^a	3.37±0.81 ^a
SYPP	4.26±0.43	4.57±1.03	4.42±1.2
YPP	12.74±1.84	15.99±1.38	13.88±1.71
HI	28.78±3.15	28.6±5.44	31.65±6.25
DOG	3.08±0.04	3.04±0.05	3.02±0.04

*Significant correlation coefficients are shown in bold

PH- Plant height, LG-Leaf girth, LL- Leaf length, PL-Petiole length, SG-Stem girth, D50F- Days to 50% flowering, DTM- Days to maturity, NOB-Number of branches, NPP-Number of pod per plant, PODL-pod length, X100SW-One hundred seed weight, SYPP-Seed yield per plant, YPP-Yield per plant, HI-Harvest index, DOG- date of germination.

Protein determination

The result of the analyses revealed a high level of variation in the protein level found in the seeds and leaves of the accessions. Figure 3 showed variation in protein level across accessions of Green gram. TVr56, TVr53, TVr17, TVr37, TVr43, TVr59 revealed a high-level of protein compared to other accessions. The second group of accessions that revealed a high-level of protein are TVr82, TVr90, TVr21, TVr34, TVr19, TVr46, and TVr44. Accessions with very low level of protein are TVr52, TVr32, TVr48, TVr94 and TVr97. Accession TVr 56 has the highest protein

group B. However, the whole accessions evaluated in this study agglutinated at the lowest level of strength (1:1) mainly with Blood groups O and B (Tables 6 and 7).

Table 7: Hemagglutinating reaction pattern of *Vigna radiata* seeds with the blood groups

Accession		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
TVr 17	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 19	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 21	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 31	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 32	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 34	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 37	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 43	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 44	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 46	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 48	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 49	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 52	B	-	-	-	-	-	-	-	-	-	-	-	-
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TVr 53	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 56	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 59	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 82	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 90	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 94	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-
TVr 97	B	-	-	-	-	-	-	-	-	-	-	-	-
	O	-	-	-	-	-	-	-	-	-	-	-	-

Discussion

Variability study and characters association are pre-requisites for the improvement of any crop for the selection of superior genotypes and improvement of any traits (Tiwarei *et al.*, 2019). The result of morphometric evaluation showed significant differences between traits observed across the studied accessions of *V. radiata*. Phenotypic coefficient of variation showed homogeneity and low experimental error within the blocks and replicates during the experiments.

Principal Component Analysis showed a significant correlation which could help in the manipulation of traits in Green gram. Hierarchical cluster analysis revealed three clusters of accessions in contrast to (Mehandi *et al.*, 2015) who reported four clusters in accessions of mung bean in India. These findings revealed that the number of pods and seed-weight of Mung bean contributed to the first two principal components which can be utilized during the selection of parent lines for improvement program. The result corresponds with (Feteme, 2012) which revealed the strong contribution of the number of pods per plant in the characterization of green gram. Waniale *et al.*, (2014) found out that the first two principal components accounted for 90% which is contrary to what was gotten in this study. The first four principal components contributed 76% of the total variation. It was revealed by (Waniale *et al.*, 2014) that plant height contributed mainly to the variation in PC1 which denotes that it revealed the most variation among the genotype studied. This is in line with this study because Plant height contributed to the variation but not the main contributor. Knowledge of the relationship between yield and yield-related traits are key to the proper selection of the best accession in the breeding program. The correlation observed among the variables evaluated is in line with the report of (Pandey, 2010). In accordance with this report, the pod length evaluated exhibited no significant (P<0.005) correlation with yield per plant. Grouping of genotypes by multivariate methods in this study is of practical value to the breeders of Mung bean accessions evaluated based on the traits of interest from a particular group for hybridization programs with other improved cultivars from the other parts of the world. The accessions were evaluated not only based on their morphological traits but also considered was the nutritional and anti-nutritional analyses of

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protein and lectin embedded in them respectively. This will facilitate better selection and more research attention to fully establish a better genotype with so many important traits to contribute mainly to food security and sufficiency.

Protein estimation has proven to be a crucial determining factor in the variability study of species and is also used by breeders for the proper selection of highly nutritive accessions in the breeding program (Aremu et al., 2019). Accessions TVr 90 and TVr 97 showed high level of protein in the leaves of accessions studied. The protein in the seeds showed more accessions having high protein concentration. Accessions TVr53, TVr56, TVr17, TVr59, TVr44 and TVr37 exhibited best protein level in the seeds of green gram. Accessions with high nutritional contents should be incorporated and used as parents in breeding program of legumes (Aremu et al., 2019).

Twenty different accessions used in this study were screened for identification of Lectin hemagglutinating ability when subjected to two human blood groups (O and B). From the result, the extracts of Mung bean seeds evaluated tested negative to the presence of lectin. This is contrary to what Pandey, 2010 reported for *Vigna radiata*.

Arpad and George, 1998 emphasized deeply that when species are found to contain high amount of lectin, there is serious need to properly denature the lectin before consumption. On this note, these accessions observed in this study can be consumed without passing through much denaturation process. This suggestion is due to the little and zero appearance of lectin observed in these accessions.

Conclusion and Recommendation

The result of this study revealed that yield and yield-related variables are effective tools in the grouping of Mung bean accessions.

The principal components grouped the accessions observed to four components which open the variability level of the accessions studied. The clusters of Mung bean generated can be used for the selection of parents which will be useful in the development of new varieties in the future Mung bean breeding program.

In this study, the principal component analysis and correlation matrix suggest improvement of the following traits among others. Number of pods per plant; leaf length, leaf girth and seed yield per plant in Mung bean for food sufficiency and security in Nigeria. It is recommended to select accessions TVr56, TVr53, TVr43, and TVr59 during the breeding program for Mung bean due to the very high protein, low Lectin in leaves and zero Lectin in seeds. Most importantly, genetic compatibility study between the accessions and other improved cultivars from other countries should be considered.

However, investigating the association between molecular markers and phenotype of these accessions of Mung bean evaluated would help breeders to make accurate decision regarding genotype performance.

References

- Ajiboye, A. A., Adekoya, M. ., Isiaka, A. ., Komolafe, R. ., David, A. ., Chukwuma, D. ., & Ajiboye, M. . (2019). Comparative Assessment of Lectin Content in Leaves and Seeds of Two Comparative Assessment of Lectin Content in Leaves and Seeds of Two Species of Okra , *Abelmoschus esculentus* and *Abelmoschus caillei*. *Asian Journal of Applied Sciences*, 07(November), 565–568. <https://doi.org/10.24203/ajas.v7i5.5909>
- Akohoue, F., Achigan-Dako, E. G., Coulibaly, M., & Sibiya, J. (2019). Correlations, path coefficient analysis and phenotypic diversity of a West African germplasm of Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Maréchal & Baudet]. *Genetic Resources and*

- Crop Evolution*, 66(8), 1825–1842. <https://doi.org/10.1007/s10722-019-00839-w>
- Altınordu, F., Martin, E., Hamzaoğlu, E., & Çetin, Ö. (2014). New chromosome counts, karyotype analyses and asymmetry indices in some taxa of genus *Senecio* L. and related genera *Tephrosia* (Rchb.) Rchb. and *Turanecio* Hamzaoğlu belong to tribe *Senecioneae* (Asteraceae) from Turkey. *Plant Systematics and Evolution*, 300(10), 2205–2216. <https://doi.org/10.1007/s00606-014-1042-8>
- Aremu, C. O., Ojuederie, O. B., Ayo-vaughan, F., Dahunsi, O., Adekiya, A. O., Olayanju, A., Adebisi, O. T., Sunday, I., Inegbedion, H., Asaleye, A. J., Abolusoro, S., Aboyeji, C. M., Ajiboye, B. O., & Obaniyi, S. (2019). Morphometric analysis and characterization of the nutritional quality in African yam bean accessions. *Plant Physiology Reports*, November. <https://doi.org/10.1007/s40502-019-00472-w>
- Arpad, P. and George, G. (1998). Assessment of lectin inactivation by heat and digestion. *Methods in molecular medicine*, 9:505-14.
- Brishti, F. H., Zarei, M., Muhammad, S. K. S., Ismail-Fitry, M. R., Shukri, R., & Saari, N. (2017). Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. *International Food Research Journal*, 24(4), 1595–1605.
- Chadha, M. L. (2010). Short Duration Mungbean: A New Success in South Asia. In *The World Vegetable Center (AVRDC) Report* (pp. 2–43).
- Chauhan, Y. S., & Williams, R. (2018). Physiological and agronomic strategies to increase mungbean yield in climatically variable environments of Northern Australia. *Agronomy*, 8(6). <https://doi.org/10.3390/agronomy8060083>
- Cullis, C., & Kunert, K. J. (2017). *Unlocking the potential of orphan legumes*. 68(8), 1895–1903. <https://doi.org/10.1093/jxb/erw437>
- Deraz, S. F., & Khalil, A. A. (2008). Strategies to Improve Protein Quality and Reduce Antinutritional Factors in Mung Bean. *Food*, 2(1), 25–38.
- Feteme, A. (2012). Estimation of genetic diversity of mungbean (*Vigna radiata* L. Wilczek) in Malaysian tropical environment. *African Journal of Microbiology Research*, 6(8), 1770–1775. <https://doi.org/10.5897/ajmr11.1133>
- Hamid, R., & Masood, A. (2009). Dietary Lectins as Disease Causing Toxicants. *Pakistan Journal of Nutrition*, 8(3), 293–303. <https://doi.org/10.3923/pjn.2009.293.303>
- Hamid, R., Masood, A., Wani, I. H., & Rafiq, S. (2013). Lectins: Proteins with Diverse Applications. *Journal of Applied Pharmaceutical Science*, 3(4), 93–103. <https://doi.org/10.7324/JAPS.2013.34.S18>
- Karim, S., & Zahurul, K. (2016). World's Demand for Food and Water: The Consequences of Climate Change. *Intech*, 225–240. <https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics>
- Khan, M., Naveed, K., Ali, K., Ahmad, B., & Jan, S. (2012). Impact of mungbean-maize intercropping on growth and yield of mungbean. *Pakistan Journal of Weed Science Research*, 8(2), 191–200.
- Kherwar, S., Banga, U., Vishwavidyalaya, K., Patra, B., Chandra, B., & Viswavidyalaya, K. (2016). Advantages of Maize-Legume Intercropping Systems. *Journal of Agricultural Science and Technology*, 3(8), 733–744.

- Koley, S. (2019). *International Journal of Health Sciences and Research*. February.
- Mehandi, S., Singh, I. P., Bohra, A., & Singh, C. M. (2015). Multivariate analysis in Mung bean [*Vigna radiata* (L .) Wilczek]. *Legume Research*, 38(6), 758–762. <https://doi.org/10.18805/lr.v38i6.6720>
- Mohan, L., & Dhirendra. (2014). Utilization of Genetic diversity and its association characters in Mungbean [*Vigna radiata* (l .) Wilczek]. *Legumes Research*, 37(6), 679–681. <https://doi.org/10.5958/0976-0571.2014.00696.1>
- Movafagh, A., Ghanati, K., Amani, D., Mahdavi, S. M., Hashemi, M., Abdolahi, D. Z., Darvish, H., Gholami, M., Mosammami, S., Safari, S., Darehgazani, R., Naini, N. S., Motlagh, M. G., & Zamani, M. (2012). The structure Biology and Application of Phytohemagglutinin (PHA) in Phytomedicine: With special up-to-date references to lectins. *Journal of Paramedical Sciences*, 4(2013), 126–141.
- Pandey, P. (2010). Genetic association and path coefficient analysis in. *Advance in Agriculture & Botany*, 2(3), 251–258.
- Pedrol, N. (2001). Protein Content Quantification by Bradford Method. In *Handbook of Plant Ecophysiology Techniques* (Issue 01, pp. 283–295). <https://doi.org/10.1007/0-306-48057-3>
- Shi, J., Jun, S., Kakuda, Y., Ilic, S., & Kim, D. (2007). *Isolation and characterization of lectins from kidney beans (Phaseolus vulgaris)*. 42, 1436–1442. <https://doi.org/10.1016/j.procbio.2007.07.015>
- Tiwari, D. N., Tripathi, S. R., Tripathi, M. P., Khatri, N., & Bastola, B. R. (2019). Genetic Variability and Correlation Coefficients of Major Traits in Early Maturing Rice under Rainfed Lowland Environments of Nepal. *Advance in Agriculture*, 2019, 9. <https://doi.org/10.1101/520338>
- Waniale, A., Wanyera, N., & Talwana, H. (2014). Morphological and Agronomic traits variations for Mungbean variety selection and improvement in uganda Mungbean (*Vigna radiata* l . wilczek). *African Journal of Crop Science*, 22(2), 123–136.