

Genetic variability and diversity analysis in pod and seed characters of some neglected and underutilized legumes (NULs).

Abstract

In response to the paucity of information challenge on the neglected and underutilized legumes, this paper explored pods and seeds morphological data of the twenty-four accessions of these crops with a view to establishing the occurrence of genetic variability and diversity analysis among the studied taxa. Twenty-four accessions of neglected and underutilized legumes (NULs) obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were assessed for genetic variability and diversity analysis through Pod and seed morphological characters. Each accession was planted into plot of 5 ridges of 5 meter long, spaced 1meter apart and replicated three times at the teaching and research farm of the Federal University Oye Ekiti, Ekiti State, Nigeria. Descriptive statistics was employed to evaluate differences in the mean values of the accessions while discriminatory traits among accessions were identified by Principal Component Analysis (PCA). Similarities among the studied plants were assessed by cluster analysis. The first two principal component axes explained 72% of the total variation. Pod length, pod width and 100-seed weight were traits that

contributed most of the variations in the legume accessions. There was a display of intra-species similarities and inter-specific genetic diversity among the studied accessions.

Keywords: Variability; diversity; analysis; pod and seed; NULs; PCA

Introduction

Neglected and underutilized legumes are so called because of their existence in a wide range of diversity as cultivated or wild species across various regions of the world and majorly cultivated by traditional farmers (Klu *et al.*, 2001; Adebowale and Lawal, 2004; Adewale, 2010^b; Adewale and Odoh, 2013; Nnamani *et al.*, 2017; Agbolade, *et al.*, 2019). Different regions of the world cultivate different forms of these crops (NULs). For example, African yam bean (*Stenophyllis stenocarpa*), bambara groundnut (*Vigna subterranean* (L.) Thouars), lablab bean (*Lablab purpureus*), lima bean (*Phaseolus lunatus*) and the pigeon pea (*Cajanus cajan* (L.) DC) are the commonly cultivated species of these legumes in sub-sahara Africa (Agbolade *et al.*, 2019).

The neglected and underutilized legumes have unusual characteristics that differentiate them from the common pea. These peculiarities are reflected in their growth habit, fruiting pattern, seed sizes and their utilities (Agbolade *et al.* 2013). They also have special significance in agriculture because of their ability to enrich the soil through symbiotic nitrogen fixation (King, 1963; Agbolade and Komolafe, 2016).

Genetic variability is either the presence of or the generation of genetic differences and could be explained as the formation of individuals differing in genotypes or the presence of genotypically different individuals in contrast to environmentally induced differences which has a rule cause only temporary non-heritable changes of the phenotype (Richard, 1978). Genetic variability in a population is important for biodiversity (Churchill, 1974).

Genetic diversity deals with the total number of genetic characteristics in the genetic make-up of a species (Ammar *et al.*, 2015; Agbolade *et al.*, 2019). Its data in a given species allows the plants to adapt to various environmental conditions, such as fluctuation in climates and soil conditions. In addition, genetic diversity in a population of plant species, including cultivars, accessions, landraces and wild individuals is a crucial resource for increasing food production and for development of sustainable agricultural practices (Esquinas-Alcazar, 2005).

Until recently, neglected and underutilized legumes are group of crops which have been lightly esteemed and ignored by research, technology, marketing systems and conservation; although their cultivation and consumption serve as livelihood options for the poor (Karikari, 2000; Ofori *et al.*, 2017). There is still paucity of information on these crops (NULs), despite the fact that a great number of people make a living on them (Padulosi *et al.*, 2000, Ofori, 2017). Hence, this current study is aimed at exploring pods and seeds morphological data of the twenty-four accessions of neglected and underutilized legume species with a view to establishing the

occurrence of genetic variability diversity analysis among the studied taxa. Genetic diversity data is useful for crop improvement and breeding plan (Ariyo, 1993, Agbolade and Komolafe, 2016; Agbolade *et al*, 2019).

Materials and Methods

Seed acquisition

Seeds of 24 accessions of twelve species of miscellaneous legumes were obtained from the Genetic Resources Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo-state, Nigeria for screening.

Seed cultivation

Each accession was planted into plot of 5 ridges of 5 meter long, spaced 1meter apart and replicated three times at the teaching and research farm of the Federal University Oye Ekiti, Ekiti State, Nigeria. Initially, two seeds were planted per hill and later thinned to one plant per hill, to give a total of twelve plants per accession per plot. Three replicates of the plots were prepared in the two locations.

Data were taken on germination rate and the number of seeds that germinated. The plants were sprayed at one week interval with karate at a concentration of 0.5% starting from the period of flower bud initiation to pod maturity. The experimental plots were kept clean by weeding with hand hoes and cutlasses throughout the period of the study.

Pod and seed characters

The qualitative pod and seed characters scored include pod colour, pattern of pigmentation on green pods, pod texture, seed coat colour, seed coat texture etc. Quantitative characters measured include pod length, pod width, number of pods per peduncles, numbers of locules per pods, number of seeds per pod, seed length, seed width and 100-seed weight.

Ten measurement of each quantitative character were taken with the aid of graduated meter rule in centimeters from ten randomly selected samples of each accession and their mean values were calculated and recorded. Photographs of the pods and seeds of the accessions studied were taken.

Data were generated from five individual plants within the row of each accession. Fourteen morphological (quantitative and qualitative) characters were scored on each of the miscellaneous legume accessions. Quantitative characters were determined by measurements in centimeters and counting. The qualitative characters which were determined visually were scored by nominal codes. No descriptors had been developed for most miscellaneous legumes yet; however, descriptors for cowpea were used as guide

to develop the descriptor list for the present morphological characterization. Duncan multiple range tests were employed to analyze the similarities and differences in the mean values of the quantitative characters.

The multivariate statistical method employed were principal component analysis (PCA) and cluster analysis (CA). The principal component analysis produced vector loadings for variables on principal component (PC) axes while cluster analysis yielded dendrogram. Pearson correlation coefficient was employed to identify the dependence of characters on one another.

Result

The accessions of various NULs studied showed considerable intra and inter-specific variability in the pod and seed characters (Table 1). Number of pods per peduncle ranged from 7.60cm in TLn 29 to 1.30cm both in TVsu 1415 and TKg 6. Days to pod maturity was in TPtu 1 (145.70cm) and lowest in TVn 1 (75.10cm). The pod length ranged between 29.86cm and 1.11cm TCg 1 and TKg 6 respectively, while the pod width ranged between 4.11cm 0.36cm in accessions TCg 1 and TCe 3 respectively. TKg 6 and TKg 12 produced the lowest number of locules per pod (1.00cm) while the highest number of locules per pod and seed per pod were 14.70cm and 14.40cm in TPt 18. Seed length also ranged from 2.76cm to 0.40cm in TCg 4 and TVr 1001 while seed width ranged from 1.92cm in TCg 1 to 0.33cm in TVr 145 and TVr 1001.

Table 1: Data on Pod and Seed Characters of the Neglected and Underutilized (NULs) Legumes Studied.

Scientific name	Common name	Accession number	DPM	NPP	PODL	PODW	NLPP	NSPP	SL	SW	100WT
<i>Canavalia gladiata</i>	Sword bean	TCg1	102.20 ¹	2.40 ^{bc}	29.86 ^k	4.11 ⁱ	9.20 ^h	9.20 ^{ht}	2.57 ⁱ	1.92 ^k	311.10 ^o

<i>Canavalia gladiata</i>	Sword bean	TCg4	98.80 ^c	2.60 ^{cd}	28.00 ^j	3.43 ^h	9.60 ^h	9.60 ⁱ	2.67 ⁱ	1.63 ^j	311.20 ^o
<i>Pachyrhizus tuberosus</i>	Mexican yam bean	TPtu1	145.70 ⁿ	2.90 ^{cdc}	6.25 ^c	1.03 ^d	4.00 ^c	3.40 ^c	1.03 ^g	0.98 ^h	24.10 ^g
<i>Pachyrhizus tuberosus</i>	Mexican yam bean	TPtu5	146.40 ^a	2.80 ^{cdc}	6.26 ^c	1.00 ^d	4.10 ^c	3.40 ^c	0.71 ^{ci}	0.98 ^h	23.10 ^f
<i>Psophocarpus tetragonolobus</i>	Winged bean	TPt12	123.70 ^j	3.40 ^{cfg}	15.14 ^g	2.06 ^g	14.50 ⁱ	14.00 ^{im}	0.71 ^{cd}	0.63 ^d	31.02 ^h
<i>Psophocarpus tetragonolobus</i>	Winged bean	TPt18	116.70 ^g	3.80 ^{fg}	13.96 ^f	2.05 ^g	14.70 ⁱ	14.10 ^m	0.77 ^{dc}	0.97 ^h	30.73 ^h
<i>Canavalia ensiformis</i>	Jack bean	TCc1	116.90 ^s	1.60 ^a	23.25 ⁱ	0.40 ^{ab}	8.40 ^{fg}	8.30 ^{fg}	1.95 ^h	1.26 ⁱ	122.03 ⁿ
<i>Canavalia ensiformis</i>	Jack bean	TCc3	118.40 ^{sh}	1.96 ^{ab}	22.98 ⁱ	0.36 ^a	8.10 ^{ef}	8.00 ^{ef}	1.93 ^a	1.28 ⁱ	120.99 ^m
<i>Vigna angularis</i>	Rice bean	TVa1	75.10 ^a	3.90 ^{fg}	9.60 ^{de}	0.40 ^{ab}	11.00 ⁱ	10.40 ^j	0.70 ^{cd}	0.44 ^b	7.45 ^c
<i>Vigna angularis</i>	Rice bean	TVa1173	78.60 ^a	3.90 ^{fg}	9.41 ^{de}	0.48 ^{ab}	8.56 ^{fg}	8.30 ^{fg}	0.60 ^{bc}	0.41 ^b	8.73 ^d
<i>Vigna mungo</i>	Mung bean	TVm12	76.60 ^a	3.40 ^{efg}	5.18 ^{bc}	0.50 ^b	8.50 ^{fg}	8.10 ^{fg}	0.59 ^{bc}	0.50 ^c	4.46 ^b
<i>Vigna mungo</i>	Mung bean	TVm13	18.00 ^c	2.60 ^{cd}	5.19 ^{bc}	0.40 ^{ab}	9.00 ^{gh}	8.70 ^{gh}	0.57 ^b	0.41 ^b	4.09 ^{ab}
<i>Lablab purpureus</i>	Lablab bean	TLn21	103.20 ^f	6.70 ^h	4.30 ^b	1.71 ^f	3.80 ^a	3.50 ^c	0.89 ^{ci}	0.71 ^e	16.58 ^e
<i>Lablab purpureus</i>	Lablab bean	TLn29	103.44 ^f	7.60 ⁱ	4.24 ^b	1.79 ^f	3.80 ^a	3.60 ^e	0.88 ^{ef}	0.71 ^e	16.75 ^e
<i>Sphenostylis stenocarpa</i>	African yam	TSs137	136.40 ⁱ	3.90 ^g	20.78 ^h	1.09 ^{de}	7.00 ^d	6.70 ^d	0.88 ^{ef}	0.71 ^e	41.14 ^j
<i>Sphenostylis stenocarpa</i>	African yam	TSs156	143.70 ^m	4.00 ^s	21.42 ^h	1.20 ^a	7.60 ^{da}	7.50 ^a	0.89 ^{ef}	0.81 ^f	37.20 ⁱ
<i>Vigna subterranean</i>	Bambara groundnut	TVsu1126	123.40 ⁱ	1.40 ^a	1.16 ^a	1.02 ^d	1.00 ^a	1.00 ^e	1.93 ^{fg}	0.81 ^f	59.03 ^k
<i>Vigna subterranean</i>	Bambara groundnut	TVsu1415	125.10 ^j	1.30 ^a	1.17 ^a	0.99 ^d	1.00 ^e	1.00 ^e	0.98 ^{fg}	0.91 ^a	61.89 ⁱ
<i>Kerstingiella geocarpa</i>	Kersting groundnut	TKg6	127.80 ^k	1.30 ^a	1.11 ^a	0.99 ^d	1.00 ^a	1.00 ^e	0.99 ^{fg}	0.71 ^e	23.32 ^{fg}
<i>Kerstingiella geocarpa</i>	Kersting groundnut	TKg12	128.00 ^k	1.40 ^a	1.16 ^a	1.00 ^d	1.00 ^a	1.00 ^a	0.96 ^{fg}	0.71 ^e	24.16 ^g
<i>Vigna radiata</i>	Green gram	TVr45	88.30 ^d	3.10 ^{de}	8.68 ^d	0.40 ^{ab}	13.80 ^k	13.70 ⁱ	0.42 ^a	0.33 ^a	3.61 ^{ab}
<i>Vigna radiata</i>	Green gram	TVr1001	89.30 ^d	3.30 ^{ef}	9.87 ^e	0.40 ^{ab}	13.00 ^j	12.90 ^k	0.40 ^a	0.33 ^a	3.52 ^a
<i>Cajanus cajan</i>	Pigeon pea	TCc8127	120.60 ⁱ	1.40 ^a	5.47 ^e	0.80 ^a	3.00 ^b	2.80 ^b	0.70 ^{cd}	0.51 ^c	8.02 ^{cd}
<i>Cajanus cajan</i>	Pigeon pea	TCc8156	119.70 ^{hi}	1.40 ^a	5.74 ^e	0.80 ^a	2.80 ^b	2.70 ^b	0.80 ^{de}	0.50 ^c	7.51 ^c
		Total mean	112.08	3.00	10.84	1.18	7.81	6.79	1.02	0.80	54.21

LEGEND: DPM- Days to pod maturity, NPP- Number of pod per peduncle, PODL- pod length, PODW- pod width, NLP-Number of locules per pod, NSPP-Number of seed per pod, SL-Seed length, SW – Seed width, 100W – 100 Seed weight.

Principal Component Analysis

Table 2 presents the Eigen values, percentage variance of the principal component (PC) axes and the Eigen values of nine pod and seed morphological traits.

These out of the nine PC axes had Eigen values >0.1 percentage variances reduced from PC1 to PC9 in a progressive manner: percentages of the total variance within the first three PC axes were 46.04, 26.26 and 11.65 respectively. The Eigen values for each the first two PC-axes were >2.0 and explained 72.3% of the total variation.

In Table 2, pod and seed morphological traits with Eigen values >0.2 were considered significant in their contribution to loading each PC-axis. Five characters namely, pod length, pod width, seed length, seed width and 100-seed weight of the taxa loaded PC1; all made positive contribution. None out of the five characters except pod length re-featured in PC2. Characters such as number of pod per peduncle, number of locules per pod, number of seed per pod; all featured in PC2 with the day to pod maturity which made negative contribution to the loading.

Figure 1 is indicative of degree of relatedness and diverseness among the 12 species of 24 accessions of the miscellaneous legumes based on their pod and seed characters presented as a two dimensional spatial configuration from the first two principal axes. The configuration explained about 72% of the total variation among the species and accessions. Approximately, six distinct sub specific clusters were identifiable from the configuration. The accessions of *Canavalia gladiata* (coded 1&2) appeared to distinct themselves from all other accessions and fell loosely at the right place. Accessions coded 22,22; 9, 10; 11, 12 (TVr145, TVr1001, TVa1173; TVm12, TVm13), respectively formed a cluster and fell at the last plane. The two accessions of the Mexican yam bean

(*Pachyrhizus tuberosus*) coded 3 and 4 appeared not to be much close based on pod and seed behaviors. However, the two accessions of

Bambara groundnut (*Vigna subterranean*) and one of Kersting groundnut (TKg6) (*Kerstingiella geocarpa*) appeared very closely related. This further lends credence to similarities observed in the clustering patterns of vegetative and floral characters.

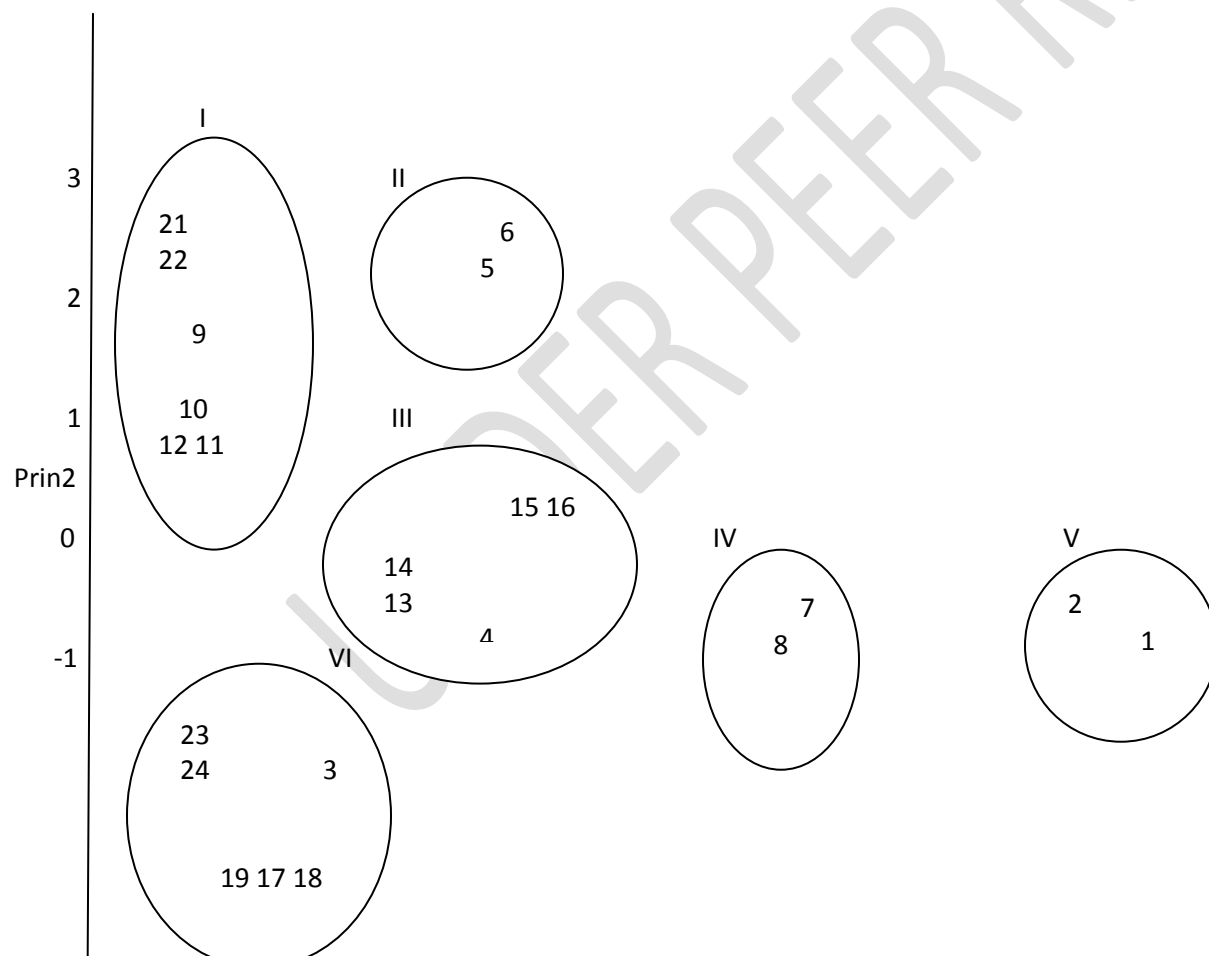
Table 2: Eigen values, Variance Proportion of Five Pc-Axes and Eigenvectors of Nine Pod and Seed Characters

	Principal Component Axes				
	PC1	PC2	PC3	PC4	PC5
Eigen values	4.1439	2.3629	1.0483	0.8955	0.3604
% variance per Pc-axes	0.4604	0.2626	0.1165	0.0995	0.0400
% cumulative variance across pc-axes	0.4604	0.7230	0.8395	0.9390	0.9790
Morphological traits	Eigenvectors				
Days to pod maturity	0.049	-0.249	-0.464	0.829	-0.001
Number of pod per peduncle	-0.056	0.300	0.714	0.452	0.389
Pod length	0.420	0.203	-0.189	0.086	0.471
Pod width	0.383	-0.028	0.374	0.250	-0.703
Number of locules per pod	0.130	0.608	-0.204	0.001	-0.137
Number of seeds per pod	0.138	0.601	-0.225	0.018	-0.147
Seed length	0.455	-0.179	0.021	-0.147	0.269
Seed width	0.452	-0.184	0.084	-0.067	0.110

100 seeds weight	0.473	-0.091	0.021	-0.113	-0.086
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NB: Eigenvectors ≥ 0.2 are in bold.

UNDER PEER REVIEW



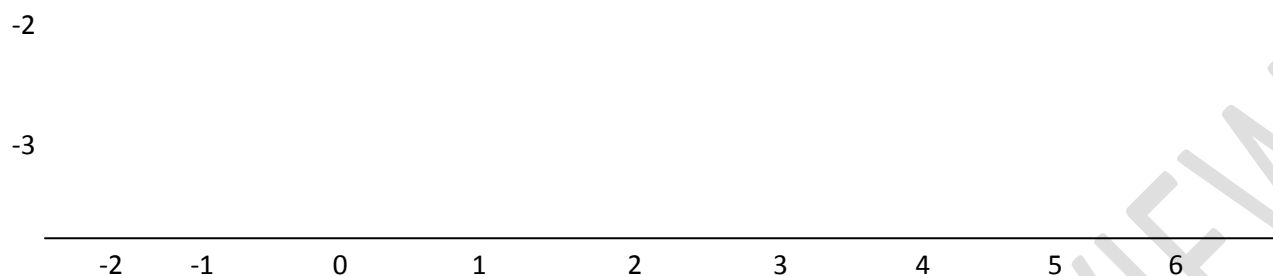


Figure 1: Two dimensional spatial configurations of the twelve species of twelve- four accessions of the miscellaneous legumes plotted by vectors from the first two PC-axes according to their pod and seed characters

Cluster Analysis

The quantitative Dendrogram obtained from the cluster analysis for pod and seed characters on the 12 species of 24 accessions of the miscellaneous legumes is presented in figure 2. The analysis also produced 23 morphotypes or clusters; the cluster history is represented in Table 2. The two accessions (TKg 6 and TKg 12) were the most similar phenotypically based on the nine pod and seed morphological characteristics employed to discriminate among the taxa. They both had the least distance 0.0095 (Figure 1). The diversity of the 24 accessions spanned a distance between 0.009 and 2.0758 (Figure 1). All the accessions were also unique at the inflection point of zero (Figure 2) and became a single subfamily entity of Pappillonoideae at approximately 2.2 similarity level distance. Four main clusters were noticeable 0.50 level of similarity (Figure 2). Pods and Seeds characters clustered all the Vigna species together at nearly the same similarity level. In fact, all accessions of the same species were grouped accordingly with the two accessions of *Pachyrizus tuberosus* (TPtu 1 and TPtu 5).

UNDER PEER REVIEW

Discussion

Occurrence of variability in organisms gives assurance of evolutionary survival and the possibilities to improve species on significant characters. Variation data is a guide for selection, which is a tool for effective classification; hence knowledge of the within species variation is a fundamental track to effective classification and improvement (Chedda and Fatokun, 1982; Agbolade and Komolafe, 2016). The characters examined differentiated accessions of the NULs evaluated. Variation in qualitative characters revealed different genetic basis for the expressed phenotypic traits among the accessions. The quantitative characters showed wide range of differences by high coefficient of variation in the mean values among the species; indicative of wide genetic variability.

Principal Component Analysis (PCA) revealed pod length, pod width and number of seed per plant as phenotypic characters with significant contribution to detecting variation among the studied accessions. Agreement between the PCA and the cluster analysis lends credence to the existing variability among the NULs species and accessions, justifying their classification.

Variability in pod and seed characters observed include days to pod maturity, number of pod per peduncle, pod length, pod width, number of locules per pod, number of seeds per pod, seed length, seed width and weight of one hundred seeds. Accessions of the same species recorded closer values for both qualitative and quantitative characters; indicating more intra-specific relatedness. This corroborates Popoola *et al.*, 2011 and Agbolade and Komolafe, 2016. Similarity in pod and seed types was common among subspecies. For example, all the *Vigna* species had smooth and medium seed type; the two species of *Canavalia* possessed straight and elongated pod while the two accessions of *Kerstingiella geocarpa* shared features in common with *Vigna subterranean*. These

similarities are pointers to their classification as sub family *Pappillonoideae*. Statistical analyses of mean values for each ten measurements taken at random per plant also confirmed similarities the quantitative characters studied. The number of locules per pod correlated positively with the number of seeds per pod which might possibly account for high seed set percentages in all the species and accessions. The two accessions each of *Canavalia gladiate*, *Canavalia ensiformis*, *Sphenostylis stenocarpa* and *Psophocarpus tetragonolobus* showed very good performance in some agronomic traits, having values very high in pod length, number of locules per pod and number of seeds per pod.

Dendrogram revealed broad groupings resulting in fewer groups than the original number of species and accessions studied; which enabled drawing an easy and logical reference. The observed variability observed could be concluded to be basically genetic since the plants were raised under the same environment. Our observation is in line with Adewale and Odoh, 2013; Popoola, *et al.*, 2011 and Agbolade *et al.*, 2019.

Conclusion

Though this is a sectional study on the genetic variability and diversity analysis among the study accessions, results clearly indicate variability in pod and seed characters useful for maintenance of diversity, conservation and future breeding work. Understanding of variation and its pattern among crop's germplasm is crucial for crop improvement. This study has undertaken degrees of morphological variation in pods and seeds characters of the twenty-four accessions of twelve species of these legumes and also

evaluated the relative importance of both types of traits in their variability determination. The use of combined / additive characters of the species and newer genetic markers such as GBS and SNP will enhance the understanding of the genetic diversity available among the species valuable for genetic manipulation and breeding of better quality variants in terms of nutrition and yield

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UNDER PEER REVIEW