Journal of Underutilized Legumes
Vol. 6 (1): 31 – 42 (April, 2024)

Society for Underutilized Legumes
https://sulegumes.org/
e-ISSN: 2705-3776

Phenotypic diversity within winged bean (*Psophocarpus tetragonolobus* L.) accessions .

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Received November 19, 2023
Accepted for publication April 16, 2024
Published April 20, 2024

Abstract

Exploring the potentials of winged bean for supply of plant protein, edible oil, minerals and anti-oxidants will make significant impacts in achieving a food and nutritionally secure world. Nineteen winged bean accessions assembled from diverse origins were assessed in an experiment in which entries were arranged in a randomized complete block design and replicated thrice. Twenty-five physiological, chronological, and morphometric traits assessed in the 19 accessions were subjected to principal component and cluster analyses based on Ward's method. Genetic correlation coefficients were calculated among the recorded traits. The first seven principal component axes whose eigenvalues or latent roots exceeded 1.0 explained 82.8% of total variance. The 16 genetically correlated traits were delineated into four groups as revealed on Ward's dendrogram. The biplot of the first two principal axes separated the 19 accessions into three groups while the cluster analysis based on minimum variance coefficients produced a dendrogram which, when truncated arbitrarily at 0.60 similarity level, resulted in four distinct clusters. The genetically correlated traits within each cluster will elicit correlated response where selection for one implies indirection selection for others in a winged bean improvement program. The accessions from each country were mostly grouped together implying that crosses among accessions between different countries rather than within a country may yield better results in winged been hybridization programs.

Keywords: Winged bean, principal component analysis, dendrogram, eigenvalues, IITA

Introduction

The quest for increasing global agricultural output to meet the demand for food and other basic needs of life particularly for the world's vulnerable is urgent. Surprisingly, the world still relies on an abysmally low number of animal and plant species for food and nutritional security. Only 30 plant species supply > 90% of the world's calorific needs and 60% of these human food energy needs are being sourced from rice, wheat, maize and potato only [1]. Yet, there exists a long list of underutilized edible legumes like hyacinth bean, grasspea, winged bean, Bambara groundnut, jack bean, African yam bean, etc., with enormous potentials for contributing to the nutrition and health benefits of vulnerable peoples globally, and particularly in the developing economies. Apart from their unique nutritional profiles, a handful of the underutilized legumes have great merits in supporting eco-friendly living environment when compared to the widely grown conventional crops [2].

Winged bean [*Psophocarpus tetragonolobus* (L.) DC., Fam. Fabaceae; 2n=2x=18], commonly named by different people as four-angled bean, Princess pea, asparagus pea, dambala, manila bean, kok-tau, or goa bean, is one of

the under-exploited tropical legumes that possess appreciable levels of nutritional constituents such as essential amino acids and fatty acids in their seeds [3,4]. It is ubiquitous in the countries around the equator with hot and tropical climate such as India, Burma, Sri Lanka, Malaysia, Thailand, Philippines, China, Indonesia and several south Pacific Islands. Papua New Guinea may be the centre of diversity for winged bean because the crop is most endemic there [5-9], and in few places in Africa like Nigeria. Winged bean is important for its high economic value to the local people and the international community because nearly all the parts of the crop are edible and profitably useable [10] - the tender leaves, flowers, and pods; the green and dried seeds; and the roots of tuber-producing accessions. The tubers have an exceptionally high protein content ranging between 8 and 20% (dry weight) compared with other tuberous crops like *Dioscorea spp* (2%), *Manihot esculenta* (1%), and *Ipomoea batatas* (2%) [10,11]. Apart from crude protein content of the winged bean seed (29.8-37.5%), which is comparable to that of other legumes, it is also well-endowed with carbohydrates (4.3 g calories per 100 g), edible oil (15-20%), and vitamin A (300-900 IU) [10,11]. Despite the numerous attractive attributes of the winged bean seeds, they contain few undesirable anti-nutrients that inhibit the activities of digestive enzymes containing trypsin, chymotrypsin, haematoglutins and amylase [12,9]. These unwanted anti-nutritional substances have undesirable consequence on the popularity and acceptability of the crop among farmers [4].

There exists a collection of winged bean accessions from Trinidad and Tobago, Papua New Guinea, Nigeria, Indonesia, Costa Rica, Bangladesh, and a few from unknown sources in the genebank of the Genetic Resources Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Earlier studies have shown that in-depth and well-documented assessment of the existing phenotypic and morphological variation through measurement of quantitative traits within a set of new genetic acquisitions is the starting point for crop improvement programs [13-15]. Multivariate techniques involving principal component analysis (PCA) and complete cluster linkage analysis have been successfully deployed for phenotypic characterization of underutilized legumes like winged bean [9], lima bean [16] and a wild cowpea relative known as *Vigna ambacensis* [15]. Paucity of information on the agronomic, morphological, and physiological attributes of the available winged bean germplasm has greatly undermined improvement programs using genetic, cytogenetic, and molecular principles. Therefore, the objective of this study was to assess the pattern of phenotypic variability among 19 IITA winged bean accessions.

Materials and Methods

Germplasm and pre-planting seed treatment

Seeds of 19 winged bean accessions sourced from seven different countries (Table 1) were obtained from the IITA GRC genebank in May 2015. The seeds were scarified mechanically in preparation for sowing by excising the section of the outer cover of the seed that is directly opposite the micropyle with the aid of a scalpel blade in order to enable the seed to absorb water for adequate germination.

Field experiment and maintenance

Scarified seeds were sown in a trial conducted in the Teaching and Research (T&R) Farm, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, located in the derived savanna agro-ecology of southwest Nigeria (latitude 8º10' N, longitude 4º10' E, altitude 341 metres above sea level). The annual mean rainfall and daily temperature of the site are 1,000-1,200 mm and 28-30 ºC, respectively. The soils of the site were classified as alfisols (FAO classification).

Accessions were arranged in RCBD in three replications. Each plot (accession) had five rows per replicate and each row was 5-m long. The rows were planted with a spacing of 1.0 m between them and the hills were spaced 1.0 m within a row. Two seeds were sown in a hill and later thinned to one plant per stand 10 days after sowing. Staking was done at three weeks after planting (3 WAP) to facilitate the growth and development of the crop. A mixture of premextra and metaphor, each 5 l ha^{-1}, was applied a day after planting as pre-emergence weed control.

Subsequently, the experimental plot was kept free of weeds by hand weeding. No fertilizer supplementation was done.

Table 1: Codes and countries of origin of the 19 winged bean accessions used for this study

Accession	Source
TPt-2	Nigeria
TPt-3	Nigeria
TPt-4	Trinidad and Tobago
TPt-5	Nigeria
TPt-6	Nigeria
TPt-10	Papua New Guinea
TPt-11	Costa Rica
TPt-12	Liberia
TPt-14	Indonesia
TPt-15	Indonesia
TPt-16	Indonesia
TPt-17	Indonesia
TPt-19	Nigeria
TPt-21	Papua New Guinea
TPt-22	Papua New Guinea
TPt-26	Nigeria
TPt-31	Indonesia
TPt-51	Bagladesh
TPt-53	Bagladesh

Country-based distribution of the 19 accessions: Nigeria = 6, Trinidad and Tobago = 1, Papua New Guinea = 3, Costa Rica = 1, Liberia = 1, Indonesia = 5, Bagladesh = 2

Data collection and analyses

i. Physiological traits

Normalized difference vegetation index (NDVI) scores were captured weekly from two to six weeks after planting (WAP) using a handheld portable spectroradiometer known as the Greenseeker according to the procedures described in [17]. The Greenseeker handheld optical sensor unit was fitted with a red sensor to emit both the red (RED) waveband centered at 650 ± 10 nm and near infra-red (NIR) band centered at 770 ± 15 nm. The device was held at approximately 60 cm above the crop canopy as each plot was navigated from start to end of each row. The data collected in log plots mode were used to compute the NDVI value based on the algorithm:

$$NDVI = (R_{NIR} - R_{RED})/(R_{NIR} + R_{RED})$$

where R_{NIR} = the fraction of emitted NIR radiation returned from the sensed area, and R_{RED} = the fraction of the emitted red radiation returned from the sensed area [18,19]. Average NDVI values were manually recorded for each plot.

ii. Chronological and morphometric traits

Days to first flower and 50% flowering were estimated as the numbers of days from planting until when the first plant and 50% of plants in a plot flowered, respectively. Days to first pod and 50% pods were counted as the

numbers of days from planting to when the first plant and 50% of the plants in a plot produced pods, respectively. Number of leaves on 1-m length secondary vine was counted and recorded. Measurements were taken on leaf, flower, and seed characters using a metre rule and Vernier calipers. Flower and seed colors were assessed using a standard colour chart. A representative plant in each plot was cut at the ground level and separated into pods and shoot to determine the fresh pod, fresh shoot, and total fresh weights per plot. The pods and shoot harvested from each plot were dried separately in a dryer for 48 hours at 68°C. A 100-seed weight (grams) was taken for each plot using a sensitive scale.

A dataset comprising 45 chronological, agronomic, morphological and physiological traits was subjected to preliminary analysis of variance (ANOVA) using the general linear model, PROC GLM, procedures in SAS [20] to detect the traits for which the accessions exhibited true differences. Accessions showed significant variation for 25 traits that are described in more details in Table 2.

With the aid of SAS software, principal component analysis was conducted on the 25 traits using PROC PRIN to determine the contributions of measured traits to the pattern of variation observed among studied accessions. Cluster analysis [21] was performed with PROC CLUSTER to determine similarity coefficients based on Unweighted Pair Group Method using Arithmetic Averages (UPGMA). The pattern of phenotypic diversity among the 19 winged bean accessions was visualized in a dendrogram constructed with PROC TREE using the output of the cluster analysis. Thereafter, genetic correlation coefficients were computed for all paired traits using the META-R software (R for Windows, version 5.0) platform [22].

Results

Results of eigenvalues of the correlation matrix for the PCA based on the measured 25 phenotypic traits showed that the first seven principal component axes whose eigenvalues or latent roots exceeded 1.0 were able to explain 82.8% of the total variance (Table 3). The first principal component (PRIN1) that explained 22.5% of the total variance was positively and strongly associated with STDL, KLL, DTP, DTF, WGW, DPP, KLW, WGL, FWL, KSL, and DFF, but weakly associated with NDVI_1, PEDL, and TLL (see meanings of acronyms in Table 2) in decreasing order of importance (Table 4). The second principal component (PRIN2) explained 20.4% of the total variance and was positively and strongly associated with STDW, NDVI_2, WGL, SWT, SDL, NDVI_I, but weakly associated with STDL, SDW, and KLL, also in decreasing order of importance. Only TLW, DSW, and SDPP were negatively associated with both PRIN1 and PRIN2 (Table 4).

The genetic correlation analysis revealed that 16 out of the 25 measured traits were significantly correlated with one another. The genotypic correlation matrix of the 16 traits is presented in Table 5. Visualizing a dendrogram of genetic associations among the traits based on Ward's method showed that the 16 variables were grouped into four distinct groups, each group comprising four traits (Figure 1). The traits in Group 1 (G1) are chronological in nature except FWL. Positive and significant genetic correlations were recorded between FWL and each of DFF (0.32***), DTP (0.95***) and DFP (1.00***) (Table 5). Expectedly, DTF in Group 2 had strong and significant correlation with each member of Group 1 (FWL = 0.67**, DFF = 0.74***, DTP = 0.86*** and DFP = 1.00***) but had no genetic relationship with members of its own group except with SDW (1.00***). In Group 3, the two physiological traits, NDVI1 and NDVI2, were significantly and strongly associated (0.73**). NDVI1 also exhibited a strongly significant and positive correlation with PDW (0.93***) and PEDL (0.82***) while NDVI2 exhibited significant but moderate genetic association with SDPP (0.55*). In Group 4, SDPP was genetically correlated with PDL (0.88**), TLW (0.76**), and TLL (1.00***). Interestingly, the genetic association between TLL and TLW (0.25ns) was not significant (Table 5).

Table 2: Trait acronyms and descriptions of 25 agronomic, physiological and morphological traits measured on 19 winged bean accessions

S/N	Trait code	Description
1	NDVI_1	NDVI measured 2WAP.
2	NDVI_2	NDVI measured 3WAP.
3	DFF	Days to first flower.
4	DTF	Days to 50% flower.
5	DFP	Days to first pod.
6	DTP	Days to 50% pod
7	TLL	Mean length of 5 top leaflets (cm).
8	TLW	Mean width of 5 top leaflets (cm).
9	NLM	Mean number of leaves on 1m vine length.
10	FWL	Mean length of 5 unopened flowers (mm).
11	FSL	Mean length of 5 flower stalks (cm).
12	STDL	Mean length of 5 flower standards (mm).
13	STDW	Mean width of 5 flower standards (mm).
14	WGL	Mean length of 5 flower wings (mm).
15	WGW	Mean width of 5 flower wings (mm).
16	KLL	Mean length of 5 flower keels (mm).
17	KLW	Mean width of 5 flower keels (mm).
18	PEDL	Mean length of 5 peduncles (cm).
19	PDL	Mean length of 5 pods (cm).
20	PDW	Mean width of 5 pods (cm).
21	SDPP	Mean number of seeds per pod.
22	SDL	Mean length of 5 seeds (mm).
23	SDW	Mean width of 5 seeds (mm).
24	SWT	Mean 100-seed weight (g)
25	DSW	Dry shoot weight (g).

Table 3: Eigenvalues of the correlation matrix for the 25 traits measured on 19 winged bean accessions from seven different countries

Principal Component Axis	Eigenvalue	Percent variation	Cumulative variance (%)
1	5.61259	22.45	22.45
2	5.0982	20.39	42.84
3	2.94302	11.77	54.62
4	2.37377	9.5	64.11
5	1.971	7.88	71.99
6	1.51407	6.06	78.05
7	1.18533	4.74	82.79
8	0.9711	3.88	86.68
9	0.74649	2.99	89.66
10	0.68144	2.73	92.39
11	0.63012	2.52	94.91
12	0.34586	1.38	96.29
13	0.33452	1.34	97.63
14	0.21462	0.86	98.49
15	0.17435	0.7	99.19
16	0.11864	0.47	99.66
17	0.0587	0.23	99.9
18	0.02618	0.1	100

Table 4: Eigenvectors (or latent vectors) of PRIN1 and PRIN2 for the 25 phenotypic traits measured on the 19 winged bean accessions sourced from seven countries.

Trait	PRIN1	PRIN2
NDVI_1	0.136696	0.250839
NDVI_2	-0.081606	0.286318
DFF	0.238663	-0.276035
DTF	0.305077	-0.068778
DFP	0.279394	-0.206704
DTP	0.307433	-0.218794
FWL	0.244952	0.017852
STDL	0.340548	0.172392
STDW	0.003537	0.314662
WGL	0.269204	0.281406
WGW	0.304625	0.089063
KLL	0.332326	0.11652
KLW	0.276729	0.004014
TLL	0.133257	-0.145346
NLM	0.030974	-0.289823
TLW	-0.001088	-0.07506
FSL	0.241257	-0.025124
PDL	-0.041736	-0.31471
PDW	0.033332	-0.181528
PEDL	0.136488	0.019279
SDL	-0.045016	0.2802
SDW	0.096124	0.120064
SDPP	-0.000228	-0.150293
DSW	-0.117403	-0.124977
SWT	0.017857	0.280938

See Table 2 for meanings of acronymns

Table 5: Genotypic correlation matrix of 16 measured traits of 19 winged bean accessions evaluated in LAUTECH in 2015.

Trait	NDVI1	NDVI2	DFF	DTF	DFP	DTP	FWL	TLL	TLW	PDL	PDW	PEDL	SDL	SDW	SDPP
NDVI2	0.73**														
DFF	-0.22^{ns}	-0.55*													
DTF	0.11^{ns}	-0.01^{ns}	0.74***												
DFP	0.30^{ns}	-0.70**	1.00***	1.00***											
DTP	-0.12^{ns}	-0.61**	0.87***	0.86***	1.00***										
FWL	-0.37^{ns}	-0.97***	0.32***	0.67**	1.00***	0.95***									
TLL	-0.54*	-0.45^{ns}	0.55*	0.32ns	0.40ns	0.51*	0.13ns								
TLW	-1.00**	-0.13^{ns}	0.24ns	-0.03ns	-0.29ns	0.30ns	-0.18ns	-0.25ns							
PDL	-0.02^{ns}	-0.24^{ns}	0.52*	0.31ns	0.56*	0.36ns	-0.64**	0.24ns	0.03ns						
PDW	0.93***	0.44^{ns}	0.33ns	0.36ns	0.47*	0.32ns	-1.00***	-0.50*	0.31ns	0.64**					
PEDL	0.82***	0.15^{ns}	0.12ns	0.37ns	0.47*	0.34ns	0.04ns	0.26ns	-0.89***	0.10ns	-0.05ns				
SDL	-0.29^{ns}	0.39^{ns}	-0.53*	0.35ns	-0.82***	-0.45ns	-0.45ns	-0.42ns	-0.53*	-0.21ns	-0.82***	-0.01ns			
SDW	-1.00***	-0.07^{ns}	0.32ns	1.00***	1.00***	0.74**	0.02**	-0.72**	-1.00***	-0.61**	-0.99***	-0.67**	0.93***		
SDPP	0.23^{ns}	0.55*	0.66**	0.16ns	-0.55*	0.24ns	-0.73ns	1.00***	0.76**	0.88***	1.00***	0.03ns	-0.72**	-1.00***	
DSW	-0.99***	-0.31^{ns}	0.14ns	0.07ns	0.59**	-0.19ns	0.51ns	-0.07ns	-0.15ns	0.02ns	0.81***	-0.45ns	0.43ns	1.00***	-0.75**

*. **, *** Correlation coefficients significant at 5, 1, and 0.01% probability levels, respectively; ns = correlation coefficients not significant

NDVI1 and NDVI2=NVDI measured 4 and 5 weeks after planting, respectively; DFF=Days from planting to first flower; DTF=Days from planting to 50% flower; DFP=Days from planting to first pod; DTP=Days from planting to 50% pods; FWL=Mean length of 5 unopened flowers (mm); TLL= Mean length of 5 top leaflets (cm); TLW= Mean width of 5 top leaflets (cm); PDL= Mean length of 5 pods (cm); PDW= Mean width of 5 pods (cm); PEDL= Mean length of 5 peduncles (cm); SDL= Mean length of 5 seeds (mm); SDW= Mean width of 5 seeds (mm); SDPP= Mean number of seeds per pod; DSW=Dry shoot weight (g)

A plot of PRIN2 versus PRIN1 produced an eclipse that delineated the 19 accessions into three main groups (Figure 2). Group 1 had 10 loosely connected accessions (TPt16, TPt51, TPt11, TPt21, TPt22, TPt53, TPt10, TPt3, TPt5, TPt14) when compared to Group 2 that had eight closely connected accessions (TPt6, TPt15, TPt17, TPt31, TPt2, TPt26, TPt19 and TPt4). Group 3 has only one accession, namely TPt12 (Figure 2). Results of UPGMA-based cluster analysis resulted in a dendrogram that, when truncated arbitrarily at 0.60 similarity level, grouped the 19 accessions into four distinct clusters (Figure 3). Cluster I composed of five accessions. Clusters II and IV had six accessions apiece while Cluster III had only two accessions. The five accessions in Cluster I included TPt2 and TPt6 from Nigeria, TPt15 from Indonesia, TPt10 from Papua New Guinea, and TPt12 from Liberia. In Cluster II, the only accession from Costa Rica shared similarity with TPt14 and TPt16 from Indonesia, and three other accessions, TPt3, TPt5 and TPt19, from Nigeria. Cluster III comprised the only two accessions from Bangladesh, TPt51 and TPt53. Cluster IV had TPt21 and TPt22 from Papua New Guinea, TPt17 and TPt31 from Indonesia, TPt26 from Nigeria, and TPt4, being the only accession from Trinida and Tobago.

Three (TPts 2, 6 and 10) out of the five accessions in Cluster I, five (TPts 3, 11, 5, 16, and 14) out of the six accessions in Cluster II, and the two (TPts 51 and 53) accessions in Cluster III of Figure 3 were captured in Group 1 of Figure 2, amounting to correspondence levels of 66, 88, and 100%, respectively. Also, three (TPts 4, 26, and 31) out of the four accessions in Cluster IV of Figure 3 were found in Group 2 of Figure 2, translating into 75% concurrence level.

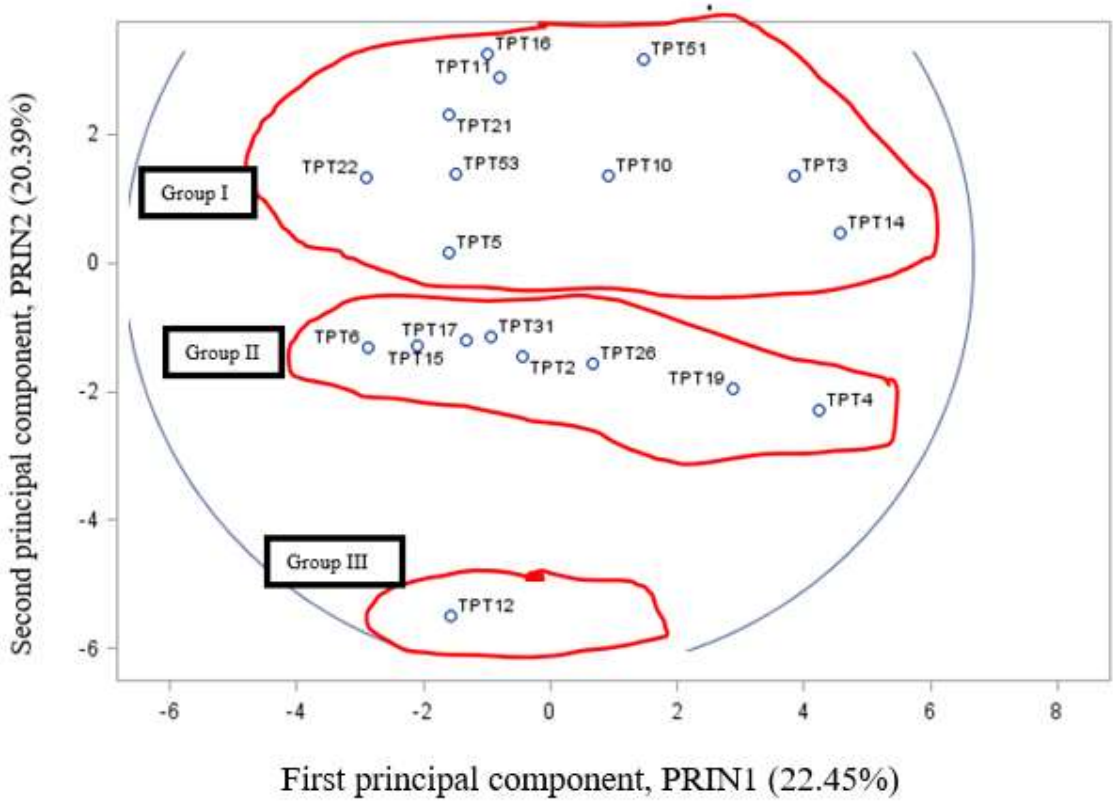

Figure 2: A biplot of PRIN2 against PRIN1 delineating the 19 winged bean accessions into three distinct groups (Group I-III)

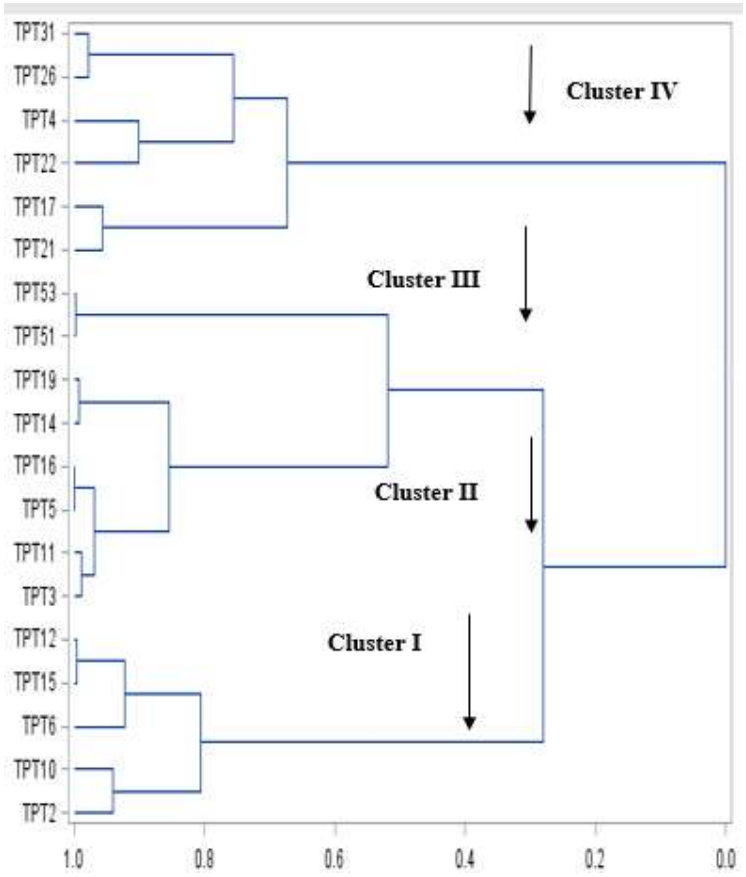

Figure 3: A dendrogram displaying the clustering pattern of 19 winged bean accessions based on Ward's Minimum Variance Coefficients

Discussion

Adequate phenotypic characterization of an under-exploited crop such as winged bean is key to understanding the extent of exploitable variation existing within the germplasm in a preliminary breeding program. Germplasm characterization through phenotypic diversity assessment is usually aimed at achieving efficient and effective preservation and sustainable exploitation of existing plant germplasm [9]. It also forms the basis for elucidating the genetic variability in new germplasm pools [23]. Assessment of phenotypic and/or morphological variation using multivariate tools have been successfully deployed for characterizing germplasms of leguminous crops such as *Vigna ambacensis* [15], *Archis pintoi* [23], lima bean [16], and common bean [24].

In the present study, the 16 genetically correlated traits were either positively or negatively associated with the first two principal component axes, suggesting that they are the important traits contributing the most to the agronomic and morphological variation observed in the studied winged bean accessions and are, therefore, to be measured in characterization studies of the crop. Estimating the genetic relatedness among measured traits is helpful for assessing the correlated response in a primary trait of interest, which is achievable through indirect selection of a secondary trait [25]. The NDVI measured at four weeks after planting (NDVI1) can be selected with correlated response for thicker pods and longer peduncles. However, it is interesting to observe in this study that NDVI1, which is a measure of the crop's physiological aspects like chlorophyll content, green biomass and water status [17,26] had a strong and negative correlation with dry shoot weight. This might suggest that winged bean accessions with high green biomass accumulate more water and less dry matter content. The genetic correlation

coefficient of ≥ 0.95 existing between mean length of unopened flowers (FWL) and days to first pod and 50% pods (DFP and DTP) is considered biologically meaningful, suggesting that it takes relatively longer time for long flowers to open and form pods. Earlier workers have opined that only genetic correlations with absolute values of > 0.71, translating to 50% of the total variation in one trait being explained by the other trait should be considered biologically meaningful [23,27]. Hence, variation in floral length can be exploited in selection for time to pod formation and ultimately for productivity.

The observed patterns of clustering based on the genetic relationships among the measured traits largely revealed the effectiveness of the Ward's clustering method. Other workers have used similar morphological, chronological and physiological traits that were assessed in this study for phenotypic characterization of other leguminous crops such as *Phaseolus lunatus* [16], *Arachis pintoi* [23], and several wild *Archis* accessions [16,28] attributed the relationships among the quantitative characters to genetic linkage or pleiotropy. Hence, the association among the three chronological characters involving winged bean pods in Group 1 similar to the seed and flower characters in Group 2 and the physiological normalized difference vegetation index in Group 3, could be likened to the association among seed weight, length and width of lima bean reported by [16]. The implication for crop improvement is that selection based on one trait implies indirect selection for the others in a particular group because the relationships within each group is genetic rather than phenotypic. However, two traits in the same group may not be improved in opposite directions especially where genetic linkage is involved.

The clustering patterns of the 19 winged bean accessions visualized through the two methods, namely principal component analysis (PCA) and Ward's Minimum Variance Coefficients showed high degrees of concurrence ranging between 60 and 100%. The clustering patterns, which revealed accessions from the same country being grouped together showed that there is less variability among such accessions. It is, however, imperative to note that when crosses are made among accessions from different clusters that possess different combinations of the measured morphological, chronological and physiological traits, the resultant progenies would combine the varied genetic backgrounds of the parents for the improvement of traits of interest. The outcome of this study will assist plant breeders in planning hybridization programs aimed at improving the studied winged bean accessions.

Author's Contribution

Adebayo, M.A.: Conceptualization, Supervision, experiment planning, Data analysis and interpretation, writing original draft, Reviewing, and Editing. **Adebayo M.A. and Shonde T.E**.: Carried out the experiments and Data analysis. **Adebayo M.A.:** Writing-Reviewing and Editing. **Adebayo M.A.:** Literature review and editing, **Adebayo M.A and Shonde T.E.**: Technical Expertise, Guidance, Reviewing and Editing.

Acknowledgements

The authors declare are grateful to Professor Michael Abberton, Head of the Genetic Resources Center (GRC), IITA, Ibadan, for making the winged bean germplasm available for this study. We also thank the GRC staff for technical support.

Declaration of Competing Interest

The authors declare that there is no competing interest that could have appeared to influence the work reported in this paper.

Funding

This research work did not receive funding.

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