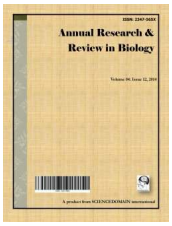

Annual Research & Review in Biology

19(5): 1-12, 2017; Article no.ARRB.36173
ISSN: 2347-565X, NLM ID: 101632869

Morphological Diversity and Cytological Studies in Some Accessions of *Vigna vexillata* (L.) A. Richard

Jacob O. Popoola[1*], Adesola Adebambo[2], Samuel Ejoh[1], Paterne Agre[3], Adegoke E. Adegbite[2] and Conrad A. Omonhinmin[1]

[1]Department of Biological Sciences, College of Science and Technology, Covenant University, P.M.B. 1023, Canaanland Ota, Ogun State, Nigeria.
[2]Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.
[3]Bioscience and Yam breeding Unit, International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author JOP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PA and CAO managed the analyses of the study. Authors AA and SE managed the literature searches and data collection. Author AEA reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/36173
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) S. A. C. N. Perera, University of Peradeniya, Sri Lanka.
(2) Nehemie Donfagsiteli Tchinda, Institute of Medical Research and Medicinal Plants Studies, Cameroon.
(3) Héla El Ferchichi Ouarda, University of Carthage, Tunisia.
Complete Peer review History: http://www.sciencedomain.org/review-history/21912

Original Research Article

Received 16th August 2017
Accepted 18th September 2017
Published 15th November 2017

ABSTRACT

Aim: The objectives of this study were to characterize and evaluate intraspecific relationship among twenty-six accessions of *Vigna vexillata* (L.) and work out interrelationship among the morphological traits which could be used for genetic improvement of cowpea, *V. unguiculata* (L.) Walp.
Study Design: Field experiment was laid out in blocks of five buckets per accession in a row giving a total of 260 plants.
Place and Duration of Study: At the experimental field of the Department of Biological Sciences, Covenant University, Ota, Ogun state, Nigeria, during the dry planting season (September – December, 2012).

**Corresponding author: E-mail: jacob.popoola@covenantuniversity.edu.ng;*

Methodology: A total of 26 traits comprising 18 quantitative and 8 qualitative traits of the vegetative, floral, pod and seed were evaluated using descriptive statistics, Pearson Correlation Coefficient (PCC), Principal Component Analysis (PCA) and Cluster Analysis (CA). Mitotic chromosome counts and meiotic behaviour were studied using root tip cells and pollen mother cells from young flower buds.

Results: The analysis of variance showed that all quantitative morphological characters were significantly different among the accessions (P = 0.01) except stipule length and width. There were significant correlations among characters such as calyx lobe length, standard petal length and width, peduncle length, days to 50% flowering, days to 50% pod maturity, pod length and width, number of locules per pod, number of seeds per pod, and 100-seed weight which could be used for breeding and conservation purposes. The first six principal components accounted for 89.84% of the total variance. The cluster analysis segregated the 26 accessions into three main clusters; cluster I (15 accessions), cluster II (10 accessions) and cluster III (1 accession). Mitotic chromosome counts of 2n = 22 were recorded for all the accessions and meiosis was observed to be normal with the formation of eleven bivalents (n = 11).

Conclusion: The intraspecific variabilities indicates plasticity in the genomes of the studied accessions, with high correlations among the morphological characters which are common to all accessions, thus justifying their grouping as a species. The morphological and reproductive attributes displayed by accessions TVnu93 and TVnu97 in terms of plant vigour, early flowering and pod maturity, longer pods and relatively high 100-seed weight made them good potential candidates in breeding for host plant resistance in cowpea.

Keywords: Chromosome count; cluster analysis; morphological diversity; Vigna vexillata.

1. INTRODUCTION

Vigna vexillata (L.) A. Richard belongs to the genus *Vigna* which contains cultivated cowpea *V. unguiculata* and its wild relatives that are considered important in the world of agriculture [1,2]. It is an annual tuberous twinner or prostrate herb characterised by heavy pubescence on leaves, stems and pods [3]. *V. vexillata* is well distributed in tropical Africa, Asia and Australia [4] and is cultivated for its edible tuberous roots. A cultivated variety of *V. vexillata* was reported in Bali, Indonesia, where it is grown mainly for its tuberous root as food and forage and to control erosion [5,6]. The fresh young shoots, green seeds and tubers are used as vegetables [1,3,5,7]. Several reports indicate that *V. vexillata* contains genes/traits that confer resistance or tolerance to insect pests, diseases, drought, heat and other abiotic factors that are lacking in the cultivated species or the high yielding improved varieties of cowpea [8]. Generally, wild *Vigna* and its wild relatives possess great potentials that could be manipulated for the improvement of cowpea [3,9]. In addition, quantitative inheritance of resistance to powdery mildew caused by the fungus (*Erysiphe polygoni* DC) in *V. vexillata* and Mung bean (*V. radiata* (L.) Wilczek) have shown that the species are genetically possessing important traits that might be of interest for breeding improved varieties [4,10]. Gogile et al. [11] screened selected genotypes of cowpea for salt tolerance during seedling growth stage and showed the presence of wide intraspecific genetic variation in cowpea varieties for salt stress with respect to their early biomass production. Wild *Vigna* species are also considered as pasture cover crops, fibre plants, green manure and erosion control plants [10,12]. Several attempts have been made to cross between the two species so as to explore the genetic attributes and other agronomic importance of *V. vexillata*, but all efforts to hybridise with *V. unguiculata* have been unsuccessful [6,13]. The cultivation and utilization of the species are also on the decline, while genetic variability in the species has not been properly explored and utilized. Recently, novel genetic resources in the genus *Vigna* was unveiled from gene bank accessions using DNA sequences of nuclear rDNA-ITS and chloroplast atpB-rbcL spacer regions [13]. The objective of this study was to evaluate some accessions of *V. vexillata* collected from six African countries to assess their level of intraspecific relationships and identify areas of taxonomic overlap which could be used for genetic improvement of cowpea and other beneficial purposes such as medicinal uses and as cover crop to control erosion.

2. MATERIALS AND METHODS

2.1 Acquisition of Materials, Study Site and Cultivation

Seeds of twenty-six accessions of *Vigna vexillata* representing collections from six different African countries were obtained from the Genetic Resources Centre (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan. The study was carried out during the dry planting season (September – December) 2012. The experimental site is located within the coordinates 6.6699º N, 3.1574º E with an elevation of 55 m above the sea level of guinea and derived savanna. The mean annual rainfall varies from 1000 mm to 2000 mm with average temperature of 27ºC. The soils are rich in clay, sand, humus and minerals which support savanna vegetation. The seeds were scarified before planting to enhance germination. Four seeds were planted in each plastic bucket filled with top soil and thinned to two plants after seedling establishment. The experiment was laid out in blocks of five buckets per accession in a row giving a total of 260 plants in buckets. The plants were watered regularly and kept free of weeds throughout the period of the study. The collections comprised 8 accessions from Nigeria, 13 from Cameroun, 2 from Ghana and 1 each from Swaziland, Democratic Republic of Congo (DRC) and Congo. The sources and seed characters of the *Vigna vexillata* accessions studied are shown in Table 1.

2.2 Morphological Characterization and Evaluation

A total of 26 traits comprising 18 quantitative and 8 qualitative traits of the vegetative, floral, pod and seed (Table 2) were evaluated using standard descriptors for *Vigna* species and *Vigna* database descriptors [14,15]. Qualitative traits were scored based on rating and coding according to the descriptors while quantitative traits were measured in SI units using the metric ruler, weighing balance and counter (PCE Instruments, Alcante, Spain). Ten measurements were taken from 5 middle plants of each accession and their means calculated. Colors were determined using the Methuen Handbook of Colors [16]. Phenology was observed every 3 – 5 days and dates recorded for the appearance of first flowers on 50% of the plants; end of flowering; first ripe pods and maximum dry weight of the seeds.

Table 1. Sources and seed characters of *Vigna vexillata* accessions studied

S/N	Accession no	Country	Texture	Colour	Size
1	TVnu 80	DRC	Smooth	Black	Small
2	TVnu 84	Nigeria	Smooth	Brown	Small
3	TVnu 93	Nigeria	Smooth	Brown	Small
4	TVnu 97	Nigeria	Smooth	Black	Small
5	TVnu 143	Nigeria	Smooth	Black	Small
6	TVnu 160	Nigeria	Smooth	Black	Small
7	TVnu 178	Nigeria	Smooth	Brown	Small
8	TVnu 180	Nigeria	Smooth	Black	Small
9	TVnu 201	Nigeria	Smooth	Brown	Small
10	TVnu 226	Cameroun	Smooth	Brown	Small
11	TVnu 318	Cameroun	Smooth	Black	Small
12	TVnu 381	Cameroun	Smooth	Brown	Small
13	TVnu 384	Cameroun	Smooth	Dark Brown	Small
14	TVnu 391	Cameroun	Smooth	Dark Brown	Small
15	TVnu 392	Cameroun	Smooth	Black	Small
16	TVnu 518	Swaziland	Smooth	Dark Brown	Small
17	TVnu 563	Ghana	Smooth	Black	Small
18	TVnu 576	Cameroun	Smooth	Black	Small
19	TVnu 635	Congo	Smooth	Dark Brown	Small
20	TVnu 831	Cameroun	Smooth	Dark Brown	Small
21	TVnu 832	Cameroun	Smooth	Brown	Small
22	TVnu 834	Cameroun	Smooth	Black	Small
23	TVnu 837	Cameroun	Smooth	Dark Brown	Small
24	TVnu 977	Cameroun	Smooth	Dark Brown	Small
25	TVnu 1109	Cameroun	Smooth	Black	Small
26	TVnu 1701	Ghana	Smooth	Black	Small

DRC: Democratic Republic of Congo

Table 2. Qualitative and quantitative traits used for evaluation of the 26 accessions of *V. vexillata*

Qualitative traits	Quantitative traits
Leaf shape	Terminal leaflet length (TLL)
Leaf 'V' marking	Terminal leaflet width (TLW)
Leaf texture	Petiole length (PL)
Stem texture	Rachis length (RL)
Raceme position	Stipule length (STL)
Flower colour	Stipule width (STW)
Pod texture	Days to 50% flowering (DF)
Pod colour	Standard petal length (SPL)
	Standard petal width (SPW)
	Calyx lobe length (CLL)
	Peduncle length (PDL)
	Days to 50% pod maturity (DPM)
	Number of pods per peduncle (NPPP)
	Pod length (PODL)
	Pod width (PODW)
	Number of locules per pod (NLPP)
	Number of seeds per pod (NSPP)
	100-seed weight (SW)

2.3 Cytological Studies

All the chemical reagents used were of analytical reagent grade. Mitotic and meiotic studies were carried out on all the accessions. For mitosis, root tips were collected from the sprouted seeds plated on moistened filter paper in petri-dishes and pre-treated with 0.04% Colchicine solution for 3 hours between 9.00 am and 12.00 noon. The pre-treated root tips were then fixed in Carnoy fluid (3 ethanol: 1 acetic acid) for 24 hours and preserved in refrigerator in 70% ethanol. The roots were hydrolyzed in 1 N HCL for 5 minutes and rinsed with clean water. Slides were prepared by squashing the tips of the hydrolyzed roots with a mounted needle while irrigating with the fixative until a homogenous solution was obtained. A drop of FLP-orcein stain was added and covered with cover slip. Excess stain was tapped out of the preparation with the aid of the blunt end of a biro pen while holding the slide within a fold of filter paper. Photomicrographs of good mitotic stages were taken at X1000 magnification under oil immersion using a Leica 2000 phase contrast microscope.

For meiotic studies, young flower buds were collected and fixed in Carnoy fluid for 24 hours, and then stored under refrigeration in 70% ethanol. Meiotic cells were obtained by squashing anthers in a drop of FLP-orcein stain. Meiotic phases were observed with the aid of light microscope and meiotic behaviour recorded. Photomicrographs of meiotic dividing cells were taken at X1000 magnification under oil immersion using a Leica 2000 phase contrast microscope.

2.4 Data Analysis

Mean values of all characters of the 26 accessions of *V. vexillata* were estimated using Excel Microsoft (2013). Data were analyzed using descriptive statistics, one way ANOVA, coefficients of variation (CV %), Pearson Correlation Coefficient (PCC), Principal Component Analysis (PCA) and Cluster Analysis (CA). All statistical analyses were performed using Minitab® 18 Statistical software (Minitab Inc.) and Paleontological Statistics Software package (version 3.15 for Windows: Ohio, USA). Statistical significance was set at $P < 0.01$. Principal Component Analysis (PCA) was used to determine relationships among morphological characters of *V. vexillata* accessions and scatter plot of PC1 and PC2 generated. Ward method of Cluster Analysis (CA) was performed based on Euclidean Distance using Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) [17].

3. RESULTS

3.1 Qualitative Characteristics of *V. vexillata* Studied

The accessions showed indeterminate growth habit with thickly hairy stems and leaves. Among the studied accessions, four terminal leaflet shapes of ovate, ovate-elliptic, lanceolate and heterophitic were observed. Lanceolate leaflet shape was dominant (50%) in thirteen accessions, ovate leaflet shape (34.61%) in nine accessions, ovate-elliptic shape (11.53%) in three accessions while only one accession had heterophitic shape (ovate – lanceolate leaflet) (3.84%) (Table 3) (Plate 1). Over 95% of the accessions (25 accessions) had leaf "V" marking on their leaves while one accession TVnu 1701 had white patches along the midrib on the adaxial surface of the leaf. Pod shattering was observed to be a common character in all the accessions. Pod and seed samples of some of the accessions are shown in Plate 2.

Table 3. Observations on the vegetative, floral, pod and seed qualitative characters of the *Vigna vexillata* accessions

S/N	Acc no	Leaf shape	Leaf 'V' marking	Flower colour	Pod colour
1	TVnu 80	Ovate elliptic	Present	Purple	Brown
2	TVnu 84	Lanceolate	Present	Violet	Brown
3	TVnu 93	Lanceolate	Present	Light purple	Brown
4	TVnu 97	Ovate	Present	Violet	Black
5	TVnu 143	Ovate	Present	Violet	Black
6	TVnu 160	Ovate	Present	Purple	Brown
7	TVnu 178	Ovate	Present	Violet	Brown
8	TVnu 180	Lanceolate	Present	Purple	Black
9	TVnu 201	Ovate	Present	No flowering	
10	TVnu 226	Ovate elliptic	Present	Violet	Black
11	TVnu 318	Ovate elliptic	Present	Purple	Black
12	TVnu 381	Lanceolate	Present	Pink	Brown
13	TVnu 384	(Heterophitic) Ovate, Lanceolate	Present	Pink	Black
14	TVnu 391	Lanceolate	Present	Violet	Dark brown
15	TVnu 392	Lanceolate	Present	Pink	Black
16	TVnu 518	Ovate	Present	Pink	Dark brown
17	TVnu 563	Lanceolate	Present	Pink	Black
18	TVnu 576	Ovate	Present	Pink	Black
19	TVnu 635	Lanceolate	Present	Light purple	Dark brown
20	TVnu 831	Ovate	Present	Pink	Dark brown
21	TVnu 832	Lanceolate	Present	Purple	Brown
22	TVnu 834	Lanceolate	Present	Pink	Black
23	TVnu 837	Lanceolate	Present	Pink	Dark brown
24	TVnu 977	Lanceolate	Present	Violet	Black
25	TVnu 1109	Ovate	Present	Purple	Black
26	TVnu 1701	Lanceolate	Absent*	Pink	Black

**With white patches along the mid-rib*

3.2 Quantitative Characteristics of *V. vexillata* Studied

One way ANOVA showed that all quantitative characters were significantly different among the accessions ($P < 0.01$) except stipule length and width (Table 4). The terminal leaflet length varied from 5.31 cm in TVnu 518 to 13.81 cm in TVnu 977 while the terminal leaflet width varied from 1.74 cm in TVnu 635 to 7.01 cm inTVnu 318. The petiole length ranged from 2.46 cm in TVnu 518 to 9.04 cm in TVnu 226 whereas rachis length varied from 0.64 cm in TVnu 518 to 2.16 cm in TVnu 392. The days to 50% flowering ranged from 41 days in TVnu 97 to 99 days in TVnu 226 with an average value of 64.19 while TVnu 201 did not produce flower throughout the period of study, and thus no record for other reproductive characters. The days to pod maturity also varied considerably, which ranged from 0 day in TVnu201 to 99 days in TVnu 226 with an average mean value of 78.81 days. Pod length ranged from 7.40 cm in TVnu178 to 13.44 cm in TVnu 93 while pod width ranged between 0.20 cm and 0.40 cm. The number of seeds per pod ranged from 6 to 19. The coefficient of variation ranged from 20.38% in stipule length to 37.46% in days to flowering (Table 4). Accession TVnu 93 recorded higher 100-seed weight value of 2.95 g, followed by accession TVnu 837 with 2.84 g.

3.3 Pearson Correlation Coefficients of the *Vigna vexillata* Accessions

Significant correlations were found among reproductive characters such as floral, seed and pod characters while low or no correlations were found among vegetative characters (Table 5). Days to 50% flowering was fairly correlated with terminal leaflet length ($r = 0.52$) and rachis length ($r = 0.50$) while calyx lobe length was highly significantly correlated with days to flowering ($r = 0.73$), standard petal length (0.84) and width ($r = 0.85$). Days to pod maturity was also significantly correlated with days to flowering at $r = 0.99$, standard petal length (0.63), calyx lobe length (0.79) and peduncle length (0.76). Number of pods per plant was correlated with standard petal length and width (0.69 and 0.68, respectively) and peduncle length with 0.55. Pod length was significantly correlated with standard petal length, width, calyx lobe length, peduncle length, days to pod maturity and number of pods per plant (Table 5).

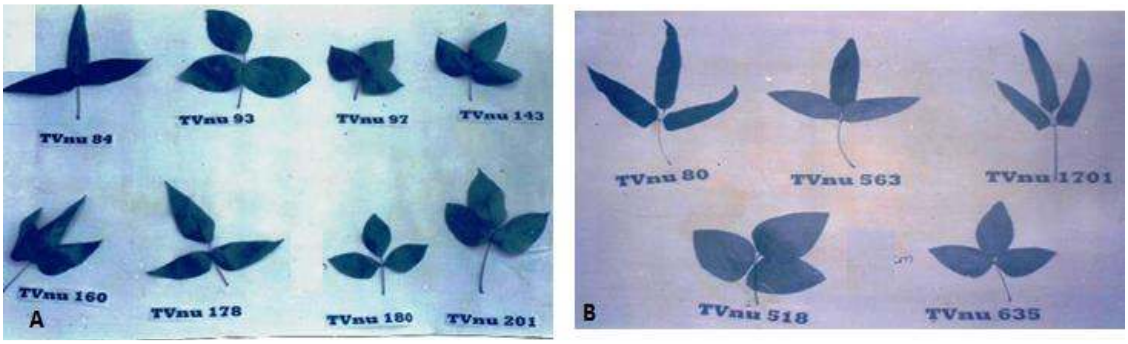

Plate 1. Variability in leaf shape and size.
A: Accessions TVnu84, 93, 97, 143, 160, 178, 180 and 201
B: Accessions TVnu80, 563, 1701, 518 and 635

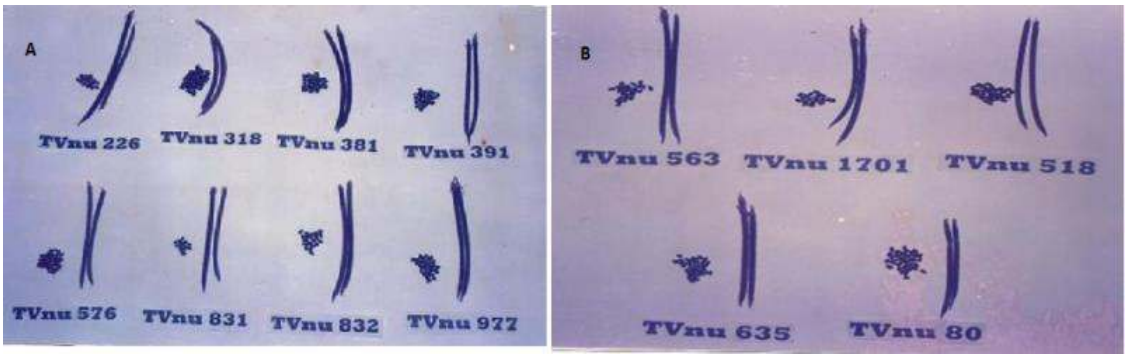

Plate 2. Variability in the size and shape of pods and seeds in some of the *Vigna vexillata* accessions studied.
A: Accessions T*Vnu*226, 318, 381, 391, 576, 831, 832 and 977
B: Accessions T*Vnu*563, 1701, 518, 635 and 80

3.4 Principal Component Analysis of the *Vigna vexillata* Accessions Studied

The eighteen morphological characters were considered for PCA analysis with a Measure of Sample Adequacy greater than 0.5. The first six principal components accounted for 89.84% of the total variance among accessions with Eigen values >1 (Table 6). Only PC1 and PC2 are informative enough to discriminate the 26 accessions of *V. vexillata*. The first principal component (PC1) explained 49.80% of the total variance influenced by days to flowering (DF), standard petal length and width (SPL, SPW), calyx lobe length (CLL) and peduncle length (PDL). Other characters that influenced the variation in PC1 include: days to pod maturity (DPM), number of pods per plant (NPPP), pod length (PODL), pod width (PODW), number of locules per pod (NLPP), number of seeds per pod (NSPP) and seed weight (SWT). The Eigenvalue ranged from 0.55 in PC6 to 8.96 in PC1. The reproductive characters effectively discriminate the accessions. PC2 accounted for 16.38% of the variance of which only vegetative characters of terminal leaflet length, petiole length, rachis length, stipule length and width contributed to the variation. Floral, pod and seed characters did not contribute to the variation hence lower value of percentage variation and Eigenvalues. The loading plot is shown in Fig. 1 reflecting the contributions of the characters to PC1 and PC2.

3.5 Cluster Analysis

The 26 accessions of the *Vigna vexillata* were segregated into three clusters (Fig. 2). Cluster 1 consists of 15 accessions subdivided into two groups: cluster group 1a and cluster group 1b. Cluster 2 comprised of 10 accessions while cluster 3 has one accession.

Table 4. Measurements of the quantitative characters of the *Vigna vexillata* accessions studied

Quantitative traits	Mean	SD	Range	Variance	Coef. of variation %	Sig. between accession
TLL	9.55	2.36	5.31(Tvnu518) - 13.81(TVnu977	5.56	24.71	p < 0.01
TLW	3.55	1.30	1.74(TVnu635) - 7.01(TVnu318)	1.69	36.61	p < 0.01
PL	4.88	1.64	2.46(TVnu518) - 9.04(TVnu226)	2.70	33.71	p < 0.01
RL	1.25	0.32	0.64(TVnu518) - 2.16(TVnu392)	0.10	25.70	p < 0.01
SL	0.62	0.13	0.15(TVnu832) - 0.80(Tvnu201)	0.02	**20.96**	ns
SW	0.28	0.09	0.22(TVnu80) - 0.72(TVnu832)	0.01	32.55	ns
DF	64.19	24.04	0 (TVnu201) - 99.00(TVnu226)	578.08	**37.46**	p < 0.01
SPL	1.99	0.41	0 (TVnu201)- 2.25(TVnu180)	0.17	20.59	p < 0.01
SPW	3.99	0.82	0(TVnu201) - 4.56(TVnu576)	0.67	20.55	p < 0.01
CLL	1.47	0.36	0 (TVnu201) - 2.00(TVnu384)	0.13	24.22	p < 0.01
PDL	8.67	2.01	0 (TVnu201) - 11.54(TVnu318)	4.03	23.16	p < 0.01
DPM	78.81	25.19	0 (TVnu201)- 115.00(TVnu226)	634.48	31.96	p < 0.01
NPPP	2.33	0.69	0 (TVnu201)- 3.50(TVnu381)	0.48	29.74	p < 0.01
PODL	10.04	2.33	0 (TVnu201)- 13.44(TVnu93)	5.41	23.16	p < 0.01
PODW	0.30	0.07	0(TVnu201) - 0.38(TVnu84)	0.00	22.77	p < 0.01
NLPP	13.79	3.40	0 (TVnu201)- 17.10(TVnu178)	11.56	24.66	p < 0.01
NSPP	11.65	3.62	0(TVnu201) - 15.00(TVnu318)	13.08	31.05	p < 0.01
SWT	2.32	0.56	0(TVnu201) - 2.95(TVnu93)	0.32	24.27	p < 0.01

ns: Non-significant

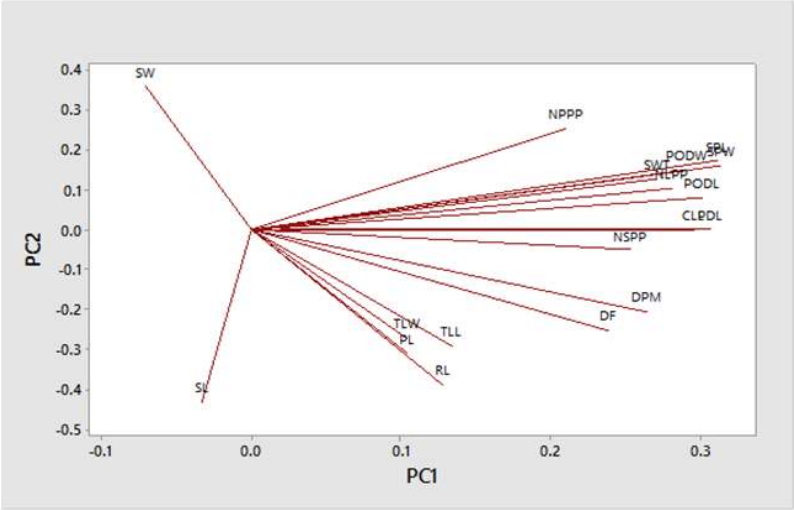

Fig. 1. Loading plot of quantitative characters along the first and second components

Table 5. Correlations of the quantitative characters studied

Traits	TLL	TLW	PL	RL	SL	SW	DF	SPL	SPW	CLL	PDL	DPM	NPPP	PODL	PODW	NLPP	NSPP	SWT
TLL	0																	
TLW	0.01	0																
PL	0.51	0.14	0.00															
RL	0.71	0.24	0.51	0.00														
SL	0.15	0.34	0.15	0.38	0.00													
SW	0.16	-0.38	-0.08	-0.32	-0.70	0.00												
DF	**0.52**	0.42	0.49	**0.50**	0.02	-0.22	0.00											
SPL	0.26	0.12	0.14	0.16	-0.29	-0.01	0.54	0.00										
SPW	0.24	0.14	0.15	0.17	-0.29	-0.04	0.56	0.97	0.00									
CLL	0.41	0.25	0.32	0.27	-0.25	-0.19	0.73	0.84	0.85	0.00								
PDL	0.24	0.37	0.29	0.31	-0.15	-0.22	**0.68**	**0.85**	**0.89**	**0.81**	0.00							
DPM	0.47	0.45	0.48	0.46	-0.02	-0.20	**0.99**	**0.63**	**0.65**	**0.79**	**0.76**	0.00						
NPPP	0.13	-0.06	0.02	0.11	-0.41	0.12	0.24	**0.69**	**0.68**	0.57	0.55	0.33	0.00					
PODL	0.25	0.30	0.27	0.23	-0.13	-0.08	0.49	**0.86**	**0.87**	**0.72**	**0.81**	**0.60**	**0.55**	0.00				
PODW	0.24	0.19	0.07	0.20	-0.14	-0.04	0.42	**0.90**	**0.86**	**0.66**	**0.76**	0.52	**0.66**	**0.81**	0.00			
NLPP	0.18	0.14	0.09	0.18	0.02	-0.22	0.36	**0.80**	**0.80**	**0.62**	**0.71**	0.45	0.59	**0.82**	0.85	0.00		
NSPP	0.14	0.35	0.07	0.22	0.24	-0.50	0.41	0.62	0.64	0.59	**0.67**	0.48	0.34	**0.71**	**0.67**	**0.88**	0.00	
SWT	0.23	0.09	0.05	0.23	-0.09	-0.09	0.37	**0.82**	**0.83**	0.64	0.66	0.44	0.44	**0.84**	**0.80**	**0.76**	**0.65**	0.00

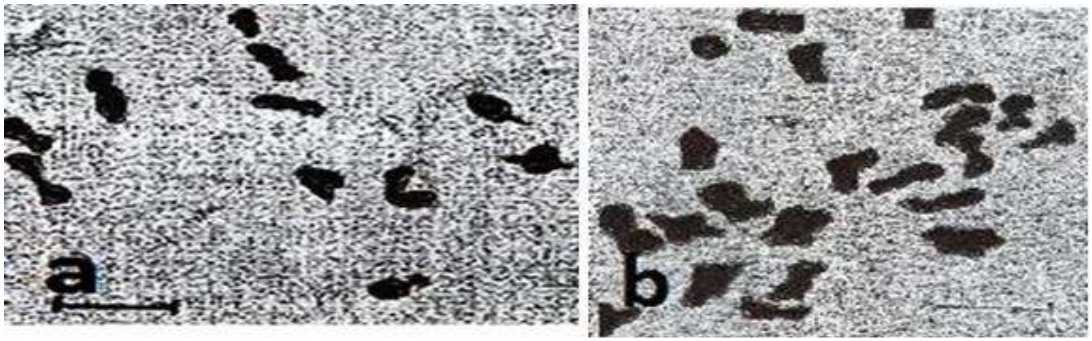

Plate 3. Meiotic and mitotic chromosomes in *Vigna vexillata* (TVnu84)
a. Meiotic metaphase I in *Vigna vexillata* (TVnu84) (n = 11)
b. Mitotic metaphase in *Vigna vexillata* (TVnu84) (2n = 22)
Scale line represents 3.50 µm

Table 6. Eigen values, % variation and the first six PCs of *Vigna vexillata* accessions studied

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Terminal Leaflet Length (TLL)	0.40	0.50	0.46	0.46	-0.12	0.01
Terminal Leaflet Width (TLW)	0.31	0.47	-0.31	***-0.60***	0.20	0.38
Petiole Length (PL)	0.31	***0.53***	0.48	0.08	0.37	-0.18
Rachis Length (RL)	0.38	***0.67***	0.23	0.37	0.01	0.33
Stipule Length (SL)	-0.10	***0.74***	***-0.54***	0.23	0.07	0.02
Stipule Width (SW)	-0.21	***-0.61***	***0.58***	-0.05	0.36	0.14
Days to Flowering (DF)	***0.71***	0.43	0.33	-0.31	-0.13	-0.10
Standard Petal Length (SPL)	***0.93***	-0.29	0.04	0.02	-0.02	-0.03
Standard Petal Width SPW)	***0.94***	-0.27	0.02	-0.02	-0.02	-0.06
Calyx Lobe Length (CLL)	***0.88***	0.00	0.18	-0.15	-0.23	-0.15
Peduncle Length (PDL)	***0.92***	0.00	0.00	-0.19	-0.02	0.00
Days to Pod Maturity (DPM)	***0.79***	0.35	0.28	-0.33	-0.08	-0.07
Number of Pods Per Plant (NPPP)	***0.63***	-0.43	0.14	0.14	-0.28	0.39
Pod Length (PODL)	***0.90***	-0.14	-0.09	0.02	0.30	-0.01
Pod Width (PODW)	***0.87***	-0.26	-0.13	0.14	0.09	0.16
Number of Locule Per Pod (NLPP)	***0.84***	-0.18	-0.34	0.22	0.06	-0.03
Number of Seeds Per Pod (NSPP)	***0.76***	0.09	-0.53	0.06	-0.01	-0.12
Seed Weight (SWT)	***0.81***	-0.22	-0.17	0.23	0.19	-0.10
Eigenvalue	8.96	2.95	1.91	1.17	0.63	0.55
% Variation	49.80	16.38	10.59	6.52	3.48	3.07

Measure of Sample Adequacy (MSA) < 0.5 influenced the variation

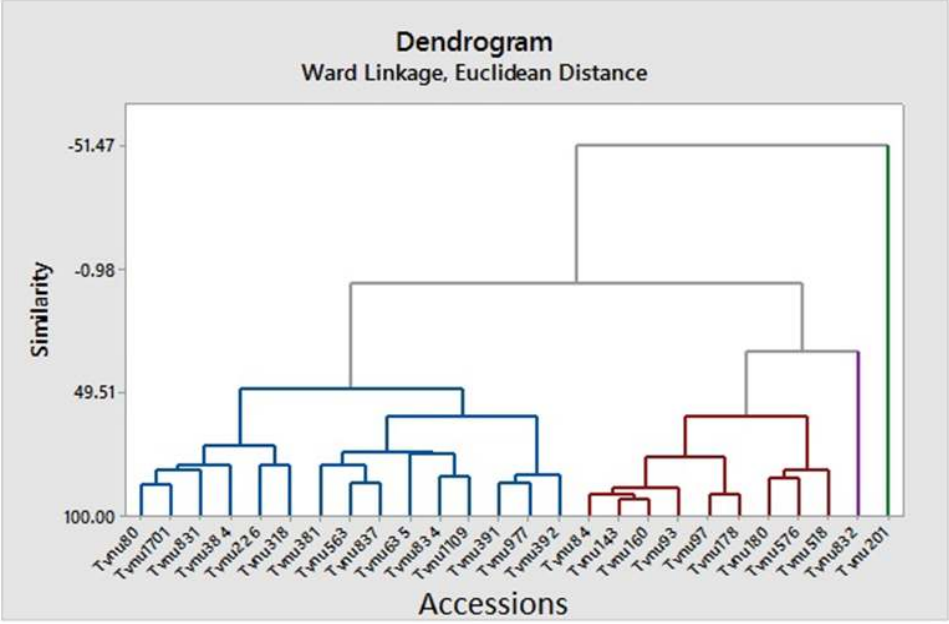

Fig. 2. Cluster Analysis showing the grouping of the *V. vexillata* accessions based on the correlation of all the morphological characters.

3.6 Cytological Studies

All the accessions of *Vigna vexillata* studied showed somatic chromosome counts of 2n = 22 and meiotic counts of n = 11 (Plate 3). Meiosis was observed to be normal in all the accessions with regular formation of 11 bivalents and normal separation and movement of chromosomes to the poles. The mitotic metaphase chromosomes are very small and are mostly metacentrics and sub- metacentrics.

4. DISCUSSION

The present study re-evaluated the phenotypic intraspecific and cytological relationships among twenty-six (26) accessions of *Vigna vexillata* collected from six African countries; Cameroun,

DRC, Ghana, Swaziland, Congo and Nigeria as a pilot study to further explore the potentials of the species for varied needs. In the present study, all the accessions exhibited considerable intraspecific variation in most of their morphological characters, though some traits were observed to be common to all the accessions which could be regarded as diagnostic characters of the species. Such characters include hairiness of the stem, leaves and pods, elongated peduncle, prominent and showy flowers. The glabrescent to densely pubescent texture of the stem, leaves and pods are important characters that could be exploited for genetic improvement of the cultivated species. The flower colour also varied from pink, violet, light purple to purple. All the accessions studied were observed to be highly prolific in pod production with the exception of accession TVnu201 that neither flowered nor podded. The *V. vexillata* accessions exhibited densely hairy pods that are least attacked by insect pests. The hairs on the pods provide protection against pest attack on the pods. This trait is partly responsible for the high level of resistance to insect pests observed in *V. vexillata*. This trait is therefore a desirable character that can be used in breeding for host-plant resistance to insect pests of cowpea. One of the major constraints of cowpea is insect pest attack on the plant on the field. This problem can be ameliorated in cowpea by presence of hairs on cowpea as observed for *V. vexillata.* Similar findings were reported in previous studies on *Vigna unguiculata* and wild relatives [3,6,7,18].

The use of heritable morphological characters and chromosome counts in the characterization of plant species are indispensable for breeding and genetic improvement of species [18,19,20]. The accessions with shorter days of flowering (TVnu 97) resulted in earlier maturity days with potential for higher pod and seed yield. Generally, all the accessions produced long pods and thus higher number of seeds per pod and seed set percentages. The agronomic importance of fruits/pods per plant, seeds per fruit pod and seed weight have been severally highlighted among plant species especially those characterized by long pods [21,22]. The higher pod size and number of seeds per pod recorded in this study, can be attributed to the reproductive mechanisms, flowering pattern and dual pollination mechanisms in the species. In addition, *V. vexillata* is an outcrossing species highly adapted to a mixed system of selfing (autogamy, cleistogamy) and outcrossing (xenogamy) capable of viable pollens exposed to multiple means of dispersal [23].

The results of the correlations and relationships among the morphological characters revealed that the reproductive and yield related characters are very useful in the selection of parent accessions for hybridization trials. Number of seeds per pod showed highly significant and positive correlation with pod length, pod width and number of locules per pod. In this study, days to pod maturity was significantly correlated with days to flowering at $r = 0.99$, and other floral characters such as standard petal length and calyx lobe length. This implies that days to flowering is a useful character for breeders to select. Accession TVnu 97 stood out with shorter days to flowering which could be used for heterosis breeding.

The results of PCA with a Measure of Sample Adequacy (MSA) greater than 0.5 indicated that the variations expressed among the 26 accessions of *V. vexillata* were greatly influenced by the floral, pod and seed characters highlighted in Table 6. This suggests the heritability, stability and consistency of the characters in the assessment of the variability among the 26 accessions of *V. vexillata*. The cluster analysis also aligned with the result of PCA which segregated the 26 accessions into three major cluster groups based on character delimitations. Though the degree of similarity was high, the analysis showed that considerable degree of morphological differentiation exists among the accessions based morphometric traits such as days to flowering, pod maturity, fruit size, and number of seeds per pod and seed weight. Such traits have been consistently implicated in the interrelationship of characters as useful traits that can be exploited in breeding program of *Vigna* species [7,21]. However, based on the cluster analysis, only accession TVnu 201 was grouped in cluster 3, which could be suggested as a potential parental line in breeding trials with *V. unguiculata,* but the non-flowering attribute could be a setback. The failure of accession TVnu 201 to flower throughout the period of study may be connected to incompatibility of gametes and poor pollination. Gurmu et al. [24] asserted that self - and cross - incompatibilities are genetic factors that limit combination of desirable traits from candidate parents particularly if the accessions belong to the same incompatibility group.

The chromosome count of $2n = 22$ recorded in this study is consistent with the earlier reports of

2n = 22 for *V. unguiculata* and some related wild species [6,25,26]. Meiosis was observed to be normal with formation of eleven bivalents (n = 11) which possibly explain the generally high pod production recorded in all the accessions. Cytological data on some wild tropical *Vigna* species and cultivars from cowpea and Asparagus bean also revealed a diploid chromosome number ranging from 20 to 24 counts [27]. Understanding the genetic variability and karyogamy among *Vigna* species particularly between the wild relatives and *V. unguiculata* are highly important to design and accelerate genetic improvement programs.

5. CONCLUSION

The present study significantly highlights the morphological diversity and cytological similarity in some accessions of *V. vexillata* (L.) that can be beneficial for genetic improvement of cowpea, *V. unguiculata* (L.) Walp. The study clearly revealed the presence of heavy pubescence on all the accessions of *V. vexillata* studied, which is a character that can be transferred to cultivated cowpea to reduce insect pest attack on cowpea on the field to boost its yield. The study suggests direct selection of traits like days to 50% flowering, days to 50% pod maturity, number of pods per peduncle, pod length, number of locules per pod, number of seeds per pod and 100-seed weight for trial hybridization for crop improvement breeding programs. In addition, accessions TVnu93 and TVnu97 are good potential candidates in breeding for host plant resistance in cowpea. On the whole, this study is a timely contribution considering the genetic implication of the species to cowpea improvement, its utilization for food, pasture cover crops, fibre plants, and green manure and as erosion control plant for environmental restoration.

ACKNOWLEDGEMENT

The authors acknowledge the management of Covenant University and Centre for Research, Innovation and Discovery of the University for Publication Support. We also extends thanks to Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for providing the seeds used for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fatokun CA, Danesh D, Young ND, Stewart EL. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor Appl Genet. 1993;86:97-104.
2. Leu YL, Hwang TL, Kuo PC, Liou KP, Huang BS, Chen GF. Constituents from *Vigna vexillata* and their anti-inflammatory activity. International Journal of Molecular Sciences. 2012;13(8):9754-68.
3. Padulosi S, Ng NQ. Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp. In Singh BB, Dashiell KE, Jackai LEN (eds), ed., Advances in Cowpea Research, Ibadan, IITA-JIRCAS. 1997;1-12.
4. Koona P, Osisanya EO, Jackai LE, Tamo M, Markham RH. Resistance in accessions of cowpea to the coreid pod-bug *Clavigralla tomentosicollis* (Hemiptera: Coreidae). Journal of Economic Entomology. 2002;6:1281-1288.
5. Karuniawan A, Iswandi A, Kale PR, Heinzemann J, Grüneberg WJ. *Vigna vexillata* (L.) A. Rich. cultivated as a root crop in Bali and Timor. Genetic Resources and Crop Evolution. 2006;53(1):213-217.
6. Damayanti F, Lawn RJ, Bielig LM. Genetic compatibility among domesticated and wild accessions of the tropical tuberous legume *Vigna vexillata* (L.) A. Rich. Crop and Past Sci. 2010a;61(10):785-797.
7. Damayanti F, Lawn RJ, Bielig LM. Expression of qualitative and quantitative traits in hybrids between domesticated and wild accessions of the tropical tuberous legume *Vigna vexillata* (L.) A. Rich. Crop and Past Sci. 2010b;61(10):798–811.
8. Marechal R. Studies in phaseolinae. In Proceeding of IITA Collaborators' Meeting on Grain Legume Improvement. 1976;35-38.
9. Agbagwa IO, Datta S, Patil PG, Singh P, Nadarajan N. A protocol for high-quality genomic DNA extraction from legumes. Genet Mol Res. 2012;1(4):4632-9.
10. James AT, Lawn RJ. Inheritance of selected traits in accessions of *Vigna vexillata* (L) a Rich of Australian and African origin. Australian Journal of Botany. 1991;39(5):415-29.
11. Gogile A, Andargie M, Muthuswamy M. Screening selected genotypes of cowpea

[*Vigna unguiculata* (L.) Walp.] for salt tolerance during seedling growth stage. Pak J Biol Sci. 2013;16(14):671-9.
12. Dachapak S, Somta P, Poonchaivilaisak S, Yimram T, Srinives P. Genetic diversity and structure of the zombi pea (*Vigna vexillata* (L.) A. Rich) gene pool based on SSR marker analysis. Genetica. 2017;145(2):189-200.
13. Takahashi Y, Somta P, Muto C, Iseki K, Naito K, Pandiyan M, Natesan S, Tomooka N. Novel genetic resources in the genus *Vigna* unveiled from gene bank accessions. PloS One. 2016;11(1):e0147568.
14. International Board for Plant Genetic Resources (IBPGR). Descriptors for cowpea; 1983.
Available:http://www.bioversityinternational.org/uploads/tx_news/Descriptors_for_cowpea_377.pdf
(Accessed May 21, 2017)
15. IITA Accession 2, Descriptors cowpea characterization.
16. Kornerup A, Wanscher JH. Methuen handbook of colour (3rd ed.). Methuen. London, England; 1978.
17. Sneath PHA, Sokal RR. Numerical taxonomy — the principles and practice of numerical classification. W. H. Freeman: San Francisco; 1973.
18. Marubodee R, Ogiso-Tanaka E, Isemura T, Chankaew S, Kaga A, Naito K, Ehara H, Tomooka N. Construction of an SSR and RAD-marker based molecular linkage map of *Vigna vexillata* (L.) A. Rich. PloS One. 2015;10(9):e0138942.
19. Adesoye AI, Nnadi NC. Mitotic chromosome studies of some accessions of African yam bean *Sphenostylis stenocarpa* (Hochst. Ex. A. Rich.) Harm. African Journal of Plant Science. 2011;5(14):835-41.
20. Popoola JO, Adegbite EA, Obembe OO. Cytological studies on some accessions of African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich. Harms). Inter Res J Plant Sci. 2011;2(8):249-253.
21. Popoola JO, Aremu BR, Daramola F, Ejoh AS, Adegbite AE. Morphometric analysis of some species in the genus *Vigna* (L.) Walp: Implication for utilization for genetic improvement. Journal of Biological Sciences. 2015;15(4):156-66.
22. Aremu CO, Ibirinde DB. Bio-diversity studies on accessions of African yarn bean (*Sphenostylis stenocarpa*). Inter J of Agric Res. 2012;7(2):78-85.
23. Popoola JO, Bello OA, Obembe OO. Phenotypic intraspecific variability among some accessions of Drumstick (*Moringa oleifera* Lam.). Canad J of Pure and Applied Sci. 2016;10(1):3681-3693.
24. Gurmu F, Hussein S, Laing M. Self-and cross-incompatibilities in sweet potato and their implications on breeding. Australian Journal of Crop Science. 2013;13:2074.
25. Shambhu B. Studies on flower visitors of field bean *Lablab purpureus (L.)* sweet and their role in pollination and pod set (Doctoral dissertation); 2013.
26. Adetula OA, Fatokun CA, Obigbesan G. Centromeric banding pattern of mitotic chromosomes in *Vigna vexillata* (TVNu 73). Afric J of Biotech. 2005;4(5):400-402.
27. Frahm-Leliveld JA. Cytological data on some wild tropical *Vigna* species and cultivars from cowpea and asparagus bean. Euphytica. 1965;14(3):251-270.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21912