

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

*Vigna vexillata* (L.) is a wild relative of cowpea characterized by heavy pubescence of the leaves, stems and pods that confer resistance to insect pest on it, which could be utilized for genetic improvement of cowpea, *V. unguiculata* (L.) Walp. Twenty-six (26) accessions originally collected from six African countries; Cameroun, Zaire, Ghana, Swaziland, Congo and Nigeria, obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were used for the study. Twenty-four morphological characters were pooled for the evaluation of intraspecific variabilities among the accessions. Mitotic chromosome counts and meiotic behavior were studied using root tip cells and pollen mother cells from young flower buds. 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 ( $n = 11$ ). The study revealed that the *V. vexillata* accessions studied showed tremendous intra-specific variabilities in their morphological characters which demonstrated the plasticity of their genomes, but 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.

30 **Key words:** Chromosome count, Cluster Analysis, Host Plant Resistance, Morphological diversity,  
31 *Vigna vexillata*.

32  
33 **INTRODUCTION**

34  
35 *Vigna vexillata* (L.) A. Richard belongs to the genus *Vigna* which contains cultivated cowpea *V.*  
36 *unquiculata* and its wild relatives that are considered important in the world of agriculture [1,2]. It is an  
37 annual tuberous twinner or prostrate herb characterised by heavy pubescence on leaves, stems and pods  
38 [3]. *V. vexillata* is well distributed in tropical Africa, Asia and Australia [4] and is cultivated for its edible  
39 tuberous roots. A cultivated variety of *V. vexillata* was reported in Bali, Indonesia, where it is grown mainly  
40 for its tuberous root as food and forage and to control erosion [5, 6]. The fresh young shoots, green seeds  
41 and tubers are used as vegetables [1,3, 5, 7]. Several reports indicate that *V. vexillata* contains  
42 genes/traits that confer resistance or tolerance to insect pests, diseases, drought, heat and other abiotic  
43 factors that are lacking in the cultivated species or the high yielding improved varieties of cowpea [8].  
44 Generally, wild *Vigna* and its wild relatives possess great potentials that could be manipulated for the  
45 improvement of cowpea [3,9]. In addition, quantitative inheritance of resistance to powdery mildew  
46 caused by the fungus *Erysiphe polygoni* in *V. vexillata* and Mung bean (*V. radiata* (L.) Wilczek) have  
47 shown that the species are genetically possessing important traits that might be of interest for breeding  
48 improved varieties [4, 10]. Gogile *et al.* [11] screened selected genotypes of cowpea for salt tolerance  
49 during seedling growth stage and showed the presence of broad intraspecific genetic variation in cowpea  
50 varieties for salt stress with respect to their early biomass production. Wild *Vigna* species are also  
51 considered as pasture cover crops, fibre plants, green manure and erosion control plants [10,12]. Several  
52 attempts have been made to cross between the two species so as to explore the genetic attributes and  
53 other agronomic importance of *V. vexillata*, but all efforts to hybridise with *V. unquiculata* have been  
54 unsuccessful. The cultivation and utilization of the species are also on the decline, while genetic variability  
55 in the species has not been properly explored and utilized. Recently, novel genetic resources in the  
56 genus *Vigna* was unveiled from gene bank accessions using DNA sequences of nuclear rDNA-ITS and  
57 chloroplast atpB-rbcL spacer regions [13]. The objective of this study was to evaluate some accessions of  
58 *V. vexillata* collected from six African countries to assess their level of intraspecific relationships and

59 identify areas of taxonomic overlap which could be used for genetic improvement of cowpea and other  
60 beneficial purposes.

## 61 2. Materials and Methods

### 62 2.1 Acquisition of Materials and Cultivation

63 Seeds of twenty-six accessions of *Vigna vexillata* representing collections from six different African  
64 countries were obtained from the Genetic Resources Centre (GRC) of the International Institute of  
65 Tropical Agriculture (IITA), Ibadan. The study was carried out during the dry planting season (September  
66 – December) 2012. The seeds were scarified before planting to enhance germination. Four seeds were  
67 planted in each plastic bucket filled with top soil and thinned to two plants after seedling establishment.  
68 The experiment was laid out in blocks of five buckets per accession in a row giving a total of 260 plants in  
69 buckets. The plants were watered regularly and kept free of weeds throughout the period of the study.  
70 The collections comprised 8 accessions from Nigeria, 13 from Cameroun, 2 from Ghana and 1 each from  
71 Swaziland, Zaire and Congo. The sources and seed characters of the *Vigna vexillata* accessions studied  
72 are shown in Table 1.

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

S/N	Accession No	Country	Texture	Colour	Size
1	TVnu 80	Zaire	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

74

75 **2.2 Morphological characterization and evaluation**

76 A total of 26 traits comprising 18 quantitative and 8 qualitative traits of the vegetative, floral, pod and seed  
 77 (Table 2) were evaluated using standard descriptors for *Vigna* species and *Vigna* database descriptors  
 78 [14, 15]. Qualitative traits were scored based on rating and coding according to the descriptors while  
 79 quantitative traits were measured in SI units using the metric ruler, weighing balance and counter (PCE  
 80 Instruments, Alcante, Spain). Ten measurements were taken from 5 middle plants of each accession and  
 81 their means calculated. Colors were determined using the Methuen Handbook of Colors [16]. Phenology  
 82 was observed every 3 – 5 days and dates recorded for the appearance of first flowers on 50 % of the  
 83 plants; end of flowering; first ripe pods and physiological maturity.

84 **Table 2:** Qualitative and Quantitative Traits Used For Evaluation of the 26 Accessions of *V. vexillata*  
 85

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)

86

### 87 **2.3 Cytological Studies**

88 All the chemical reagents used were of analytical reagent grade. Mitotic and meiotic studies were carried  
89 out on all the accessions. For mitosis, root tips were collected from the sprouted seeds plated on  
90 moistened filter paper in petri-dishes and pre-treated with 0.04% Colchicine solution for 3 hours between  
91 9.00 am and 12.00 noon. The pre-treated root tips were then fixed in Carnoy fluid (3 ethanol: 1 acetic  
92 acid) for 24 hours and preserved in refrigerator in 70% ethanol. The roots were hydrolyzed in 1 N HCL for  
93 5 minutes and rinsed with clean water. Slides were prepared by squashing the tips of the hydrolyzed  
94 roots with a mounted needle while irrigating with the fixative until a homogenous solution was obtained. A  
95 drop of FLP-orcein stain was added and covered with cover slip. Excess stain was tapped out of the  
96 preparation with the aid of the blunt end of a biro pen while holding the slide within a fold of filter paper.  
97 Photomicrographs of good mitotic stages were taken at X1000 magnification under oil immersion using a  
98 Leica 2000 phase contrast microscope.

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

### 104 **2.4 Data analysis**

105 Mean values of all characters of the 26 accessions of *V. vexillata* were estimated using Excel Microsoft  
106 (2013). Data were analyzed using descriptive statistics, one way ANOVA, coefficients of variation (CV %),  
107 Pearson Correlation Coefficient (PCC), Principal Component Analysis (PCA) and Cluster Analysis (CA).

108 All statistical analyses were performed using Minitab® 18 Statistical software (Minitab Inc.) and  
 109 Paleontological Statistics Software package (version 3.15 for Windows: Ohio, USA). Statistical  
 110 significance was set at  $P < 0.01$ . Principal Component Analysis (PCA) was used to determine  
 111 relationships among morphological characters of *V. vexillata* accessions and scatter plot of PC1 and PC2  
 112 generated. Ward method of Cluster Analysis (CA) was performed based on Euclidean Distance using  
 113 Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) [17].

114  
 115 **3. RESULTS**

116  
 117 **3.1 Qualitative Characteristics of *V. vexillata* studied**

118  
 119 The accessions showed indeterminate growth habit with thickly hairy stems and leaves. Among the  
 120 studied accessions, four terminal leaflet shapes of ovate, ovate-elliptic, lanceolate and heterophytic were  
 121 observed. Lanceolate leaflet shape was dominant (50 %) in thirteen accessions, ovate leaflet shape  
 122 (34.61 %) in nine accessions, ovate-elliptic shape (11.53 %) in three accessions while only one accession  
 123 had heterophytic shape (ovate – lanceolate leaflet) (3.84 %) (Table 3) (Plate 1). Over 95 % of the  
 124 accessions (25 accessions) had leaf “V” marking on their leaves while one accession TVnu 1701 had  
 125 white patches along the midrib on the adaxial surface of the leaf. Pod shattering was observed to be a  
 126 common character in all the accessions. Pod and seed samples of some of the accessions are shown in  
 127 Plate 2.

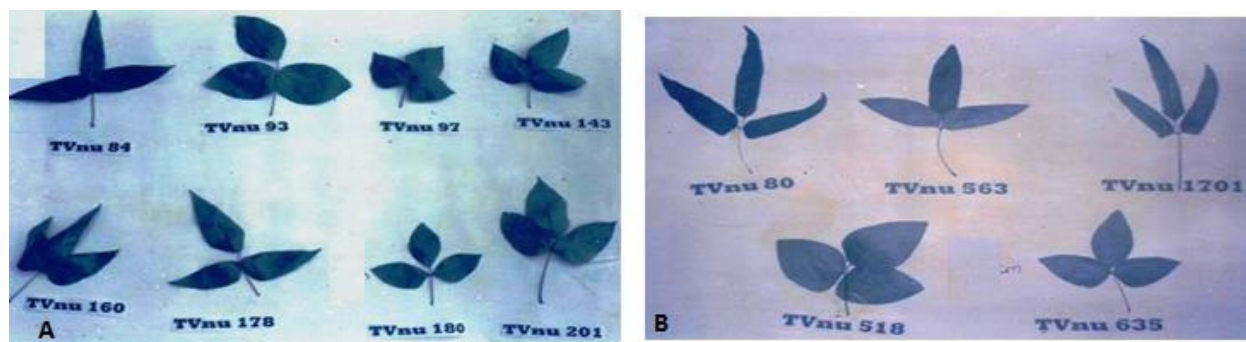
128 Table 3: Observations on the Vegetative, Floral, Pod and Seed Qualitative Characters of the *Vigna*  
 129 *vexillata* Accessions.

S/N	ACCESSION NO	Leaf Shape	Leaf 'V' Marking	Raceme Position	Flower Colour	Pod Texture	Pod Colour
1	TVnu 80	Ovate elliptic	Present	Above canopy	Purple	Pubescent	Brown
2	TVnu 84	Lanceolate	Present	Above canopy	Violet	Pubescent	Brown
3	TVnu 93	Lanceolate	Present	Above canopy	Light purple	Pubescent	Brown
4	TVnu 97	Ovate	Present	Above canopy	Violet	Pubescent	Black
5	TVnu 143	Ovate	Present	Above canopy	Violet	Pubescent	Black
6	TVnu 160	Ovate	Present	Above canopy	Purple	Pubescent	Brown
7	TVnu 178	Ovate	Present	Above canopy	Violet	Pubescent	Brown
8	TVnu 180	Lanceolate	Present	Above canopy	Purple	Pubescent	Black
9	TVnu 201	Ovate	Present	Above canopy	No flowering		
10	TVnu 226	Ovate elliptic	Present	Above canopy	Violet	Pubescent	Black

11	TVnu 318	Ovate elliptic	Present	Above canopy	Purple	Pubescent	Black
12	TVnu 381	Lanceolate	Present	Above canopy	Pink	Pubescent	Brown
13	TVnu 384	(Heterophitic) Ovate, Lanceolate	Present	Above canopy	Pink	Pubescent	Black
14	TVnu 391	Lanceolate	Present	Above canopy	Violet	Pubescent	Dark brown
15	TVnu 392	Lanceolate	Present	Above canopy	Pink	Pubescent	Black
16	TVnu 518	Ovate	Present	Above canopy	Pink	Pubescent	Dark brown
17	TVnu 563	Lanceolate	Present	Above canopy	Pink	Pubescent	Black
18	TVnu 576	Ovate	Present	Above canopy	Pink	Pubescent	Black
19	TVnu 635	Lanceolate	Present	Above canopy	Light purple	Pubescent	Dark brown
20	TVnu 831	Ovate	Present	Above canopy	Pink	Pubescent	Dark brown
21	TVnu 832	Lanceolate	Present	Above canopy	Purple	Pubescent	Brown
22	TVnu 834	Lanceolate	Present	Above canopy	Pink	Pubescent	Black
23	TVnu 837	Lanceolate	Present	Above canopy	Pink	Pubescent	Dark brown
24	TVnu 977	Lanceolate	Present	Above canopy	Violet	Pubescent	Black
25	TVnu 1109	Ovate	Present	Above canopy	Purple	Pubescent	Black
26	TVnu 1701	Lanceolate	Absent*	Above canopy	Pink	Pubescent	Black

130 \*With white patches along the mid-rib

131

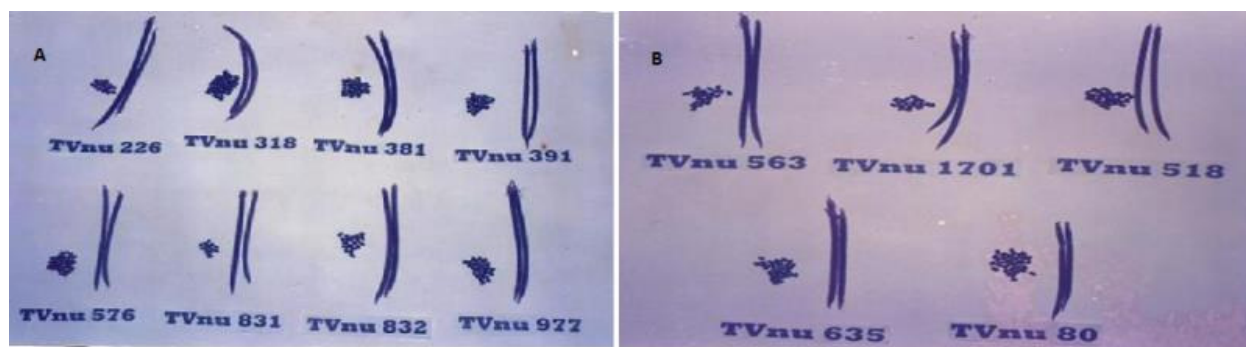


132

133 Plate 1: Variability in leaf shape and size.

134 A: Accessions TVnu84, 93, 97, 143, 160, 178, 180 and 201

135 B: Accessions TVnu80, 563, 1701, 518 and 635.



136

137 Plate 2: Variability in pod and seed shape and size in some of the *Vigna vexillata* accessions studied.

138 A: Accessions TVnu226, 318, 381, 391, 576, 831, 832 and 977

139 B: Accessions TVnu563, 1701, 518, 635 and 80.

140

141 **3.2 Quantitative characteristics of *V. vexillata* studied**

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143 One way ANOVA showed that all quantitative characters were significantly different among the  
 144 accessions ( $P < 0.01$ ) except stipule length and width (Table 4). The terminal leaflet length varied from  
 145 5.31 cm in TVnu 518 to 13.81 cm in TVnu 977 while the terminal leaflet width varied from 1.74 cm in  
 146 TVnu 635 to 7.01 cm in TVnu 318. The petiole length ranged from 2.46 cm in TVnu 518 to 9.04 cm in  
 147 TVnu 226 whereas rachis length varied from 0.64 cm in TVnu 518 to 2.16 cm in TVnu 392. The days to  
 148 50 % flowering ranged from 41 days in TVnu 97 to 99 days in TVnu 226 with an average mean value of  
 149 64.19 while TVnu 201 did not produce flower throughout the period of study, and thus no record for other  
 150 reproductive characters. The days to pod maturity also varied considerably, which ranged from 0 day in  
 151 TVnu201 to 99 days in TVnu 226 with an average mean value of 78.81 days. Pod length ranged from  
 152 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  
 153 number of seeds per pod ranged from 6 to 19. The coefficient of variation ranged from 20.38 % in stipule  
 154 length to 37.46 % in days to flowering (Table 4). Accession TVnu 93 recorded higher 100-seed weight  
 155 value of 2.95 g, followed by accession TVnu 837 with 2.84 g.

156

157



158 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	24.68	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	<b>20.38</b>	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	<b>37.46</b>	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

159 ns: Non-significant

160

161 **3.3 Pearson Correlation Coefficients of the *Vigna vexillata* Accessions**

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163 Significant correlations were found among reproductive characters such as floral, seed and pod  
 164 characters while low or no correlations were found among vegetative characters (Table 5). Days to 50 %  
 165 flowering was fairly correlated with terminal leaflet length ( $r = 0.52$ ) and rachis length ( $r = 0.50$ ) while calyx  
 166 lobe length was highly significantly correlated with days to flowering ( $r = 0.73$ ), standard petal length  
 167 (0.84) and width ( $r = 0.85$ ). Days to pod maturity was also significantly correlated with days to flowering at  
 168  $r = 0.99$ , standard petal length (0.63), calyx lobe length (0.79) and peduncle length (0.76). Number of  
 169 pods per plant was correlated with standard petal length and width (0.69 and 0.68, respectively) and  
 170 peduncle length with 0.55. Pod length was significantly correlated with standard petal length, width, calyx  
 171 lobe length, peduncle length, days to pod maturity and number of pods per plant (Table 5).

172 Table 5: Correlations of the Quantitative Characters studied.

Traits	TLL	TLW	PL	RL	SL	SW	DF	SPL	SPW	CLL	PDL	DPM	NPP P	POD L	POD W	NLP P	NSP P	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	<b>0.52</b>	0.42	0.49	<b>0.50</b>	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	<b>0.68</b>	<b>0.85</b>	<b>0.89</b>	<b>0.81</b>	0.00							
DPM	0.47	0.45	0.48	0.46	-0.02	-0.20	<b>0.99</b>	<b>0.63</b>	<b>0.65</b>	<b>0.79</b>	<b>0.76</b>	0.00						
NPPP	0.13	-0.06	0.02	0.11	-0.41	0.12	0.24	<b>0.69</b>	<b>0.68</b>	0.57	0.55	0.33	0.00					
PODL	0.25	0.30	0.27	0.23	-0.13	-0.08	0.49	<b>0.86</b>	<b>0.87</b>	<b>0.72</b>	<b>0.81</b>	<b>0.60</b>	<b>0.55</b>	0.00				
PODW	0.24	0.19	0.07	0.20	-0.14	-0.04	0.42	<b>0.90</b>	<b>0.86</b>	<b>0.66</b>	<b>0.76</b>	0.52	<b>0.66</b>	<b>0.81</b>	0.00			
NLPP	0.18	0.14	0.09	0.18	0.02	-0.22	0.36	<b>0.80</b>	<b>0.80</b>	<b>0.62</b>	<b>0.71</b>	0.45	0.59	<b>0.82</b>	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	<b>0.67</b>	0.48	0.34	<b>0.71</b>	<b>0.67</b>	<b>0.88</b>	0.00	
SWT	0.23	0.09	0.05	0.23	-0.09	-0.09	0.37	<b>0.82</b>	<b>0.83</b>	0.64	0.66	0.44	0.44	<b>0.84</b>	<b>0.80</b>	<b>0.76</b>	<b>0.65</b>	0.00

173

174 **3.4 Principal Component Analysis of the *Vigna vexillata* Accessions Studied**

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

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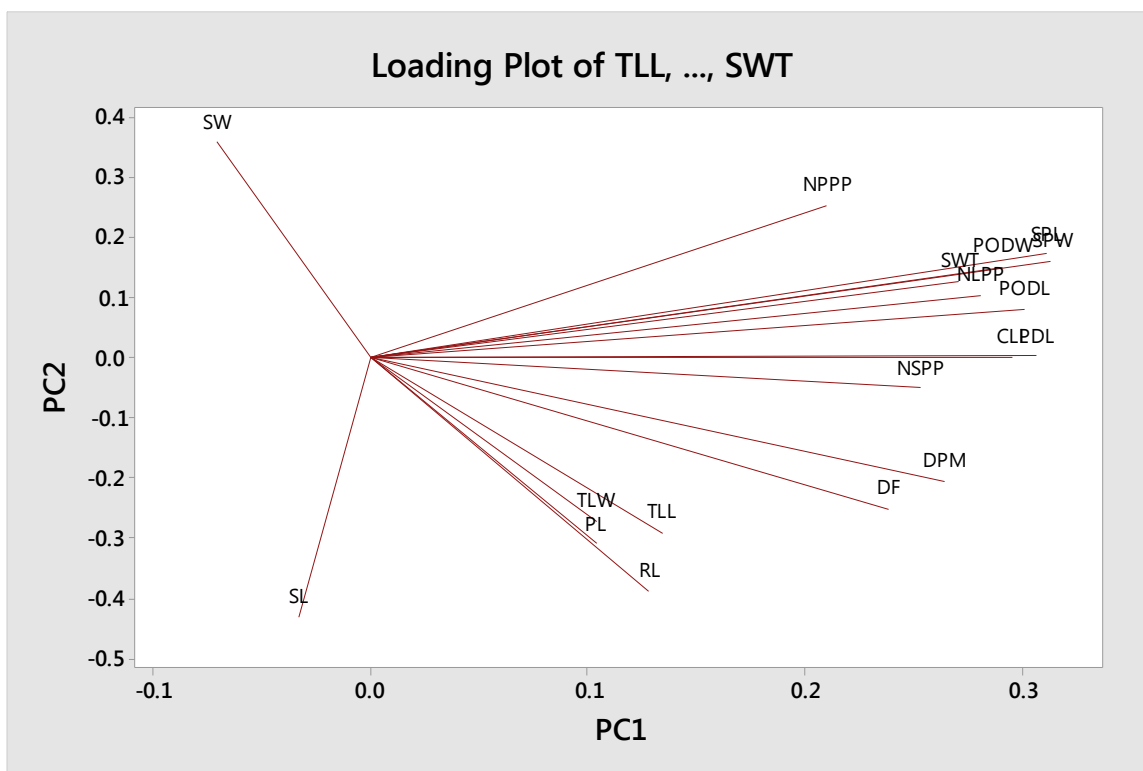
191 Table 6: Eigen Values, % Variation and the First Six PCs of *Vigna vexillata* Accessions Studied

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

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

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196 Fig 1: Loading plot of all the characters for PC1 and PC2

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200 **3.5 Cluster Analysis**

201 The 26 accessions of the *Vigna vexillata* were segregated into three clusters (Figure 2). Cluster 1 consists

202 of 15 accessions subdivided into two groups: cluster group 1a and cluster group 1b. Cluster 2 comprised

203 of 10 accessions while cluster 3 has one accession.

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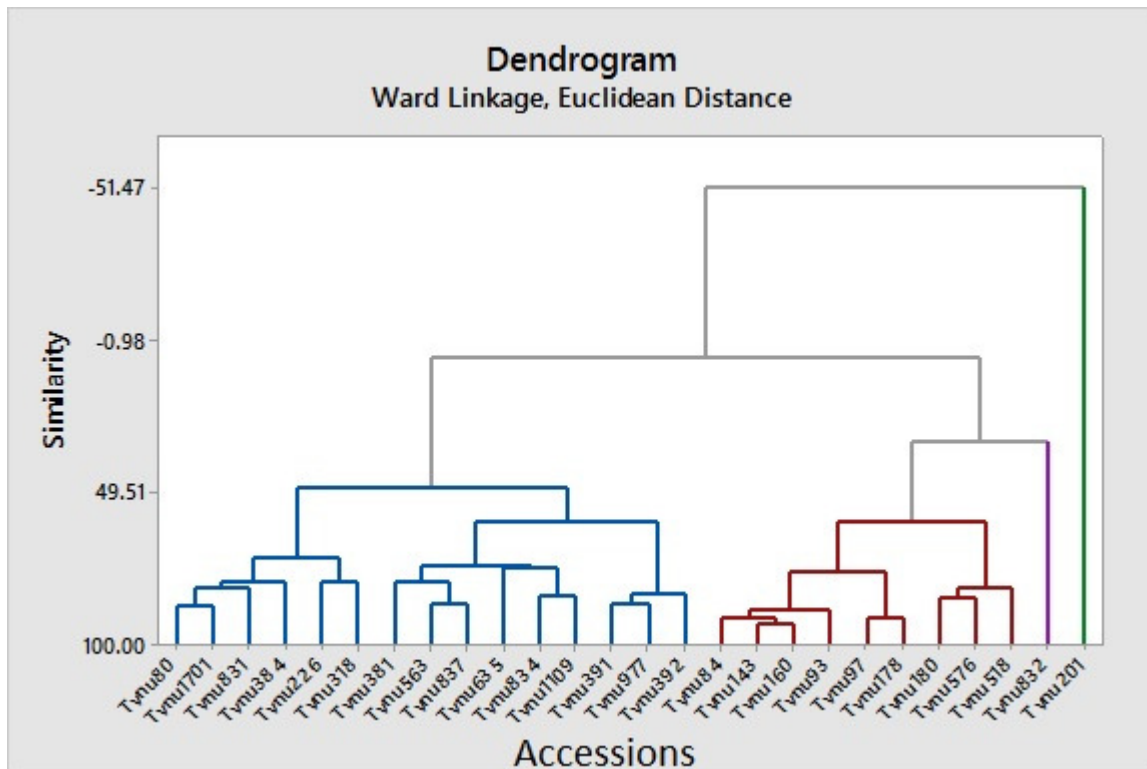
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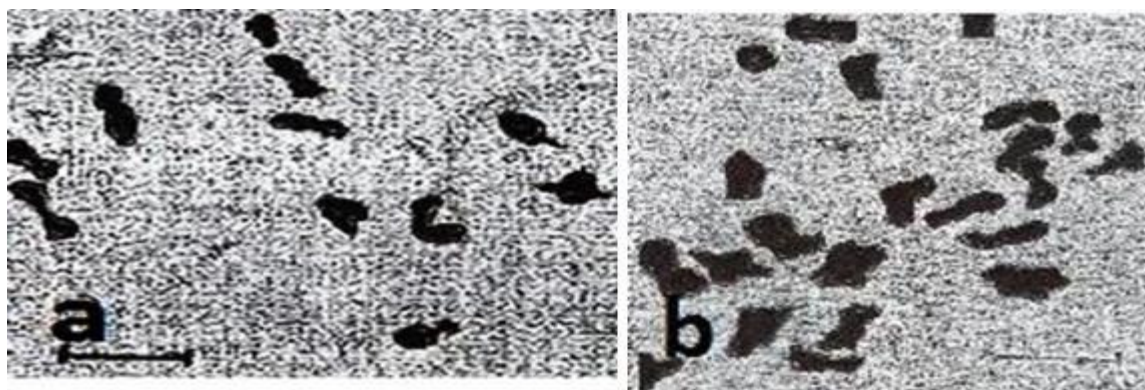
215

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

218

219 **3.6 Cytological Studies**

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



224

225 Plate 3: Meiotic and mitotic chromosomes in *Vigna vexillata* (TVnu84)226 a. Meiotic metaphase I in *Vigna vexillata* (TVnu84) ( $n = 11$ )227 b. Mitotic metaphase in *Vigna vexillata* (TVnu84) ( $2n = 22$ )228 Scale line represents 3.50  $\mu\text{m}$ .

229

230 **3.7 Discussion**

231

232 The present study re-evaluated the phenotypic intraspecific and cytological relationships among twenty-

233 six (26) accessions of *Vigna vexillata* collected from six African countries; Cameroun, Zaire, Ghana,

234 Swaziland, Congo and Nigeria as a pilot study to further explore the potentials of the species for varied

235 needs. In the present study, all the accessions exhibited considerable intraspecific variation in most of

236 their morphological characters, though some traits were observed to be common to all the accessions

237 which could be regarded as diagnostic characters of the species. Such characters include hairiness of the

238 stem, leaves and pods, elongated peduncle, prominent and showy flowers. The glabrescent to densely

239 pubescent texture of the stem, leaves and pods are important characters that could be exploited for

240 genetic improvement of the cultivated species. The flower colour also varied from pink, violet, light purple

241 to purple. All the accessions studied were observed to be highly prolific in pod production with the

242 exception of accession TVnu201 that neither flowered nor podded. The *V. vexillata* accessions exhibited

243 densely hairy pods that are least attacked by insect pests. The hairs on the pods provide protection

244 against pest attack on the pods. This trait is partly responsible for the high level of resistance to insect

245 pests observed in *V. vexillata*. This trait is therefore a desirable character that can be used in breeding for

246 host-plant resistance to insect pests of cowpea. One of the major constraints of cowpea is insect pest

247 attack on the plant on the field. This problem can be ameliorated in cowpea by presence of hairs on  
248 cowpea as observed for *V. vexillata*. Similar findings were reported in previous studies on *Vigna*  
249 *unguiculata* and wild relatives [3, 6, 7, 18].

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

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

268 The results of PCA with a Measure of Sample Adequacy (MSA) greater than 0.5 indicated that the  
269 variations expressed among the 26 accessions of *V. vexillata* were greatly influenced by the floral, pod  
270 and seed characters highlighted in Table 6. This suggests the heritability, stability and consistency of the  
271 characters in the assessment of the variability among the 26 accessions of *V. vexillata*. The cluster  
272 analysis also aligned with the result of PCA which segregated the 26 accessions into three major cluster  
273 groups based on character delimitations. Though the degree of similarity was high, the analysis showed  
274 that considerable degree of morphological differentiation exists among the accessions. However, only

275 accession TVnu 201 was grouped in cluster 3 which is distinct and thus could be used as a parental line  
276 in breeding trials with *V. unguiculata*, but the non-flowering attribute could be a setback.

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

## 285 **Conclusion**

286 The present study significantly highlights the morphological diversity and cytological similarity in some  
287 accessions of *V. vexillata* (L.) that can be beneficial for genetic improvement of cowpea, *V. unguiculata*  
288 (L.) Walp. The study clearly revealed the presence of heavy pubescence on all the accessions of *V.*  
289 *vexillata* studied, which is a character that can be transferred to cultivated cowpea to reduce insect pest  
290 attack on cowpea on the field to boost its yield. On the whole, this study is a timely contribution  
291 considering the genetic implication of the species to cowpea improvement, its utilization as food, pasture  
292 cover crops, fibre plants, green manure and erosion control plant.

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