

Article

Agro-Morphological Variability of Wild *Vigna* Species Collected in Senegal

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Abstract: The domesticated *Vigna* species still need some of the beneficial characters that exist in the wild *Vigna* species, despite the improvements obtained so far. This study was carried out to enhance our understanding of the Senegalese wild *Vigna* diversity by exploring the agro-morphological characteristics of some accessions using 22 traits. The phenotyping was carried out in a shaded house for two consecutive rainy seasons (2021 and 2022) using the alpha-lattice experimental design with 55 accessions. Multiple correspondence analysis was carried out based on the qualitative traits, which showed considerable variability for the wild species (*Vigna unguiculata* var. *spontanea*, *Vigna racemosa*, *Vigna radiata* and the unidentified accession). The quantitative traits were subjected to statistical analysis using descriptive statistics and ANOVA. Our results revealed that ninety-five percent (95%) pod maturity ranged from 74.2 to 125.8 days in accession 3 of *V. unguiculata* and in accession 92 (*V. racemosa*), respectively. In addition, accession 14 of *V. radiata* recorded the highest weight for 100 seeds with a value of 4.8 g, while accession 18 of *V. unguiculata* had the lowest (1.48 g). The ANOVA showed significant differences for the accessions during each season ($p \leq 0.05$). Seasonal effects (accession $\times$ season) were observed for some quantitative traits, such as the terminal leaflet length and width, time to 50% flowering and 95% pod maturity, pod length and 100-seed weight. Principal component analysis showed that reproductive traits, such as the time to 50% flowering, number of locules per pod, pod length, pod width and 100-seed weight, were the major traits that accounted for the variations among the wild *Vigna* accessions. The genetic relationship based on qualitative and quantitative traits showed three clusters among the wild *Vigna* accessions. Indeed, the diversity observed in this study could be used to select parents for breeding to improve the cultivated species of *Vigna*.

Keywords: agro-morphological traits; wild *Vigna* species; cluster; breeding; Senegal



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1. Introduction

The self-pollinating diploid crop belonging to the family of Fabaceae, cowpea (*Vigna unguiculata* (L.) Walp.), is one of the most important grain legumes growing in the tropical and subtropical regions [1]. It is cultivated worldwide for food and/or a cash crop [2]. According to Xiong et al. [3], the cowpea origin has been linked to Africa, based on the important diversity existing among the germplasm lines and the preponderance of wild relatives distributed in several parts of this continent. Cowpea has a major socio-economic impact in Sahelian countries including Senegal, where the crop is growing on

289,895 hectares with an annual production over 253,897 tons in 2021 [4]. In addition, being a legume, cowpea has the ability to fix nitrogen in association with Bradyrhizobia and to grow in low-fertility soil [5,6]. It is tolerant to high temperatures and drought compared to other legume crops [7]. For the wild species, each accession has unique traits that can be useful, but, in the *Vigna* genus, only a few of them are domesticated, such as (*V. radiata* (L.) Wilczek), (*V. mungo* (L.) Hepper), (*V. aconitifolia* (Jacq.) Maréchal), (*V. angularis* (Willd.) Ohwi and Ohashi), (*V. umbellata* (Thunb.) Ohwi and Ohashi), (*V. subterranea* (L.) Verdc.) and (*V. unguiculata* (L.) Walp.) [8]. The wild *Vigna* species are reported to have important nutritional elements [9] and adaptability to unusual edaphic conditions, such as acidic soils, deserts and wetlands [10]. On top of that, it has also been reported that some wild species are used as supplements in traditional diets, fodder feeds or medicine. Through the domestication process, cowpea underwent many phenotypic changes compared to its wild progenitor (*Vigna unguiculata* var. *spontanea*). For many cultivated species, the loss of genetic diversity, in part due to the breeding programs associated with modern agricultural practices, has been dramatic [11], which is unlike their wild ancestors that present a very wide range of variability both in terms of important agronomic characteristics and genetic diversity. This statement is in accordance with the conclusions of Benjamin et al. [12] who reported a great agro-morphological variability among the wild accessions collected from Nigeria. Popoola et al. [13] again reported, using morphometric analysis of some species in the genus *Vigna*, considerable variabilities of the accessions in their growth habits, vegetative traits and flowering and reproductive attributes. In addition to this, Joshua et al. [14], in their work, evaluated cowpea landraces and their wild relative for growth and yield attributes in Bauchi, Northern Guinea. They reported a wide variability among the genotypes evaluated with respect to the traits studied, such as the number of seeds per pod and one hundred seed weight. Furthermore, the narrow genetic basis of the elite germplasm has increased their vulnerability to biotic and abiotic stress. Based on previous studies, cultivated cowpea has a narrow genetic basis, resulting from a single domestication event [15–18] that has limited its ability to respond to climate change or pathogen attacks [19]. Therefore, the conservation and characterization of a plant's genetic resources are a prerequisite for breeding programs in order to improve yield, pest and disease tolerance and resistance to other biotic and abiotic constraints [20]. Morphological traits are used in plant breeding for the description, classification of genetic material and the selection of intended genotypes [21]. Those traits are used in order to estimate diversity and select parental lines for crossing [22]. For years, these traits have formed a tool for breeders to attempt to check and capture the phenotypic differences among several crops worldwide [22,23] but also in cowpea [24]. According to Krichen et al. [21], morphological and agronomic traits remain imperative for breeders plants despite the extensive use of molecular markers in diversity studies [25]. Some of the wild *Vigna* species exhibit significant agro-morphological diversity, which could be utilized in crop improvement and domestication efforts. Thus, some authors have suggested to broaden the genetic basis of cultivated cowpea by using interspecific hybridization [26]. For this purpose, it would be very interesting to explore the agro-morphological diversity of the wild species for identifying the traits that can be used to improve the cultivated cowpea. Therefore, this study aimed to enhance our understanding of the genetic diversity of the Senegalese wild *Vigna* for identifying the relevant traits that can be used in cowpea breeding programs.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of 55 accessions that were collected in different regions of Senegal between September and December 2016. The collection included 43 accessions of *Vigna unguiculata* var. *spontanea*, 1 hybrid accession of *V. unguiculata*, 9 accessions of *V. racemosa*, 1 accession of *V. radiata* and 1 unidentified accession. The *Vigna unguiculata* var. *spontanea* accessions were provided by the national wild germplasm, carried out by Sarr et al. [27] (Supplementary Table S1).

2.2. Site of Study and Meteorological Conditions

The trials were conducted in the Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS) during two consecutive rainy seasons (2021 and 2022) from July to November. CERAAS is located in Thiès (latitude of 14°45'57" N and longitude of 16°53'31" W), Senegal. The meteorological characteristics (monthly rainfall) during the two rainy seasons were recorded. The total rainfall of the study site was 538 and 546.5 mm during the rainy seasons of 2021 and 2022, respectively.

2.3. Experimental Design and Sowing Process

Fifty-five (55) wild *Vigna* accessions were sown using the alpha-lattice design with 3 replications and 11 blocks per replication for two consecutive rainy seasons (2021 and 2022). The blocks contained 10 rows of 1.5 m each. The inter- and intra-row spacing were 50 cm. Each accession was sown in two rows. Two seeds were sown per pocket, and three weeks after sowing, they were thinned in one plant per pocket. Each accession was represented by 8 plants. For each accession, scarification was performed using a pair of scissors by cutting the seed coat. The scarified seeds were placed in Petri dishes containing soaked paper and left at room temperature. Radicle emergence was noticed 24 h after sowing.

2.4. Data Collection and Analysis

Twenty-two (22) traits, including 14 qualitative and 8 quantitative characters, were recorded during two consecutive rainy seasons (2021 and 2022) using both International Board for Plant Genetic Resources [28] and International Plant Genetic Resource Institute descriptors [29] (Table 1). These traits were chosen because of their usefulness for *Vigna* morphological description. The qualitative traits were measured based on visual scoring, while the quantitative characters were evaluated using a metric ruler or weighing balance. Twelve plants were analyzed for each accession in each rainy season. All the collected data were analyzed using R software version 4.1.2 [30]. Multiple correspondence analysis (MCA) was performed to reveal the most discriminant characters and relationships between the qualitative traits. To examine the relationships between accessions, the hierarchical cluster analysis (HCA) was carried out based on those traits. The quantitative traits were subjected to statistical analysis using descriptive statistics based on the mean of the two seasons. Pearson coefficient correlation was performed among the various quantitative traits using R software version 4.1.2. The quantitative data normality was checked with the Shapiro test for normality. To test for differences between accessions in order to indicate the accession effect, replicate effect and block effect, an analysis of variance (ANOVA) was calculated for each of these quantitative traits. In order to establish the interactions between the accessions and the seasons (years), a two-way ANOVA was performed. A principal component analysis (PCA) that was based on the quantitative traits was conducted to identify the relationships between variables and similarities between accessions. The construction of the plots and graphics was performed using R software version 4.1.2.

Table 1 provides information on the 8 quantitative and 14 qualitative traits related to the vegetative characteristics, flowers, pods and seeds used for the characterization of the wild *Vigna* accessions studied during the two rainy seasons based on IBPGR [28] and IPGRI [29] descriptors.

Table 1. List of the 22 morphological characters studied in the wild *Vigna* accessions.

Parameters		Descriptors
Qualitative traits		
Hypocotyl color		Green; Purple; Others
Leaf color		Pale green; Intermediate green; Dark green
Leaf texture		Cariaceous; Intermediate; Membranous
Growth habit		Erect; Intermediate; Prostrate; Climbing
Terminal leaflet shape		Globose; Sub-globose; Sub-hastate; Hastate; Others
Plant pigmentation		None; Moderate
Plant hairiness		Glabrescent; Short appressed hairs
Flower color		Violet; Dark blue; Yellow
Pod dehiscence		No shattering; Pods opened and twisted
Pod texture		Smooth; Rough
Seed texture		Smooth; Rough
Seed coat color		Grey; Marbled; brown; red; green; black
Eye color		White; Black
Seed shape		Kidney; Ovoid; Crowder; Globose; Rhomboid; Others
Quantitative traits		
Parameters	Code	Unit
Terminal leaflet length	Tlfl	cm
Terminal leaflet width	Tlfw	cm
Time to 50% flowering	T50%fw	day
Time to 95% pod maturity	T95%Rp	day
Pod length	Pdl	cm
Pod width	Pdw	cm
Number of locules per pod	Nlpd	-
100-seed weight	HSdw	g

3. Results

3.1. Qualitative Traits of the Wild *Vigna* Species

3.1.1. Morphological Variability of the Whole Set of *Vigna* Accessions

A wide range of variability was noticed among the morphological characters in the accessions used during this experiment (Figure 1), depending on their developmental stage. Figure 1 gives a pictorial description of some distinctive morphological traits of the wild *Vigna* accessions, studied on the basis of phenotypic observations made during the developmental stage and after harvest.

All accessions of *V. racemosa* as well as the unidentified accession showed a purple hypocotyl color. The *Vigna unguiculata* (var *spontanea* and hybrid) and *V. radiata* accessions showed a green hypocotyl color. The leaf color was dark green for all the *V. unguiculata* accessions, intermediate green for *V. racemosa* and *V. radiata* and pale green for the unidentified accession. The leaf texture was cariaceous for the *V. unguiculata* and *V. radiata* accessions, while it was membranous for *V. racemosa* and the unidentified accession (Supplementary Table S2). Among the *V. unguiculata* accessions, the terminal leaflet shapes registered were globose, sub-globose, hastate, sub-hastate and lanceolate. It was oval in *V. racemosa*, deltoid in the *V. radiata* accession and elliptical in the unidentified accession (Figure 1). All the *V. unguiculata* accessions and the unidentified accession showed a purple pigmentation on the stem and petiole, unlike those of *V. racemosa* and *V. radiata* that were unpigmented. For the plant hairiness, all the *V. racemosa*, *V. radiata* and unidentified accessions presented moderate pubescence. The *V. unguiculata* accessions were glabrous (Supplementary Table S2).

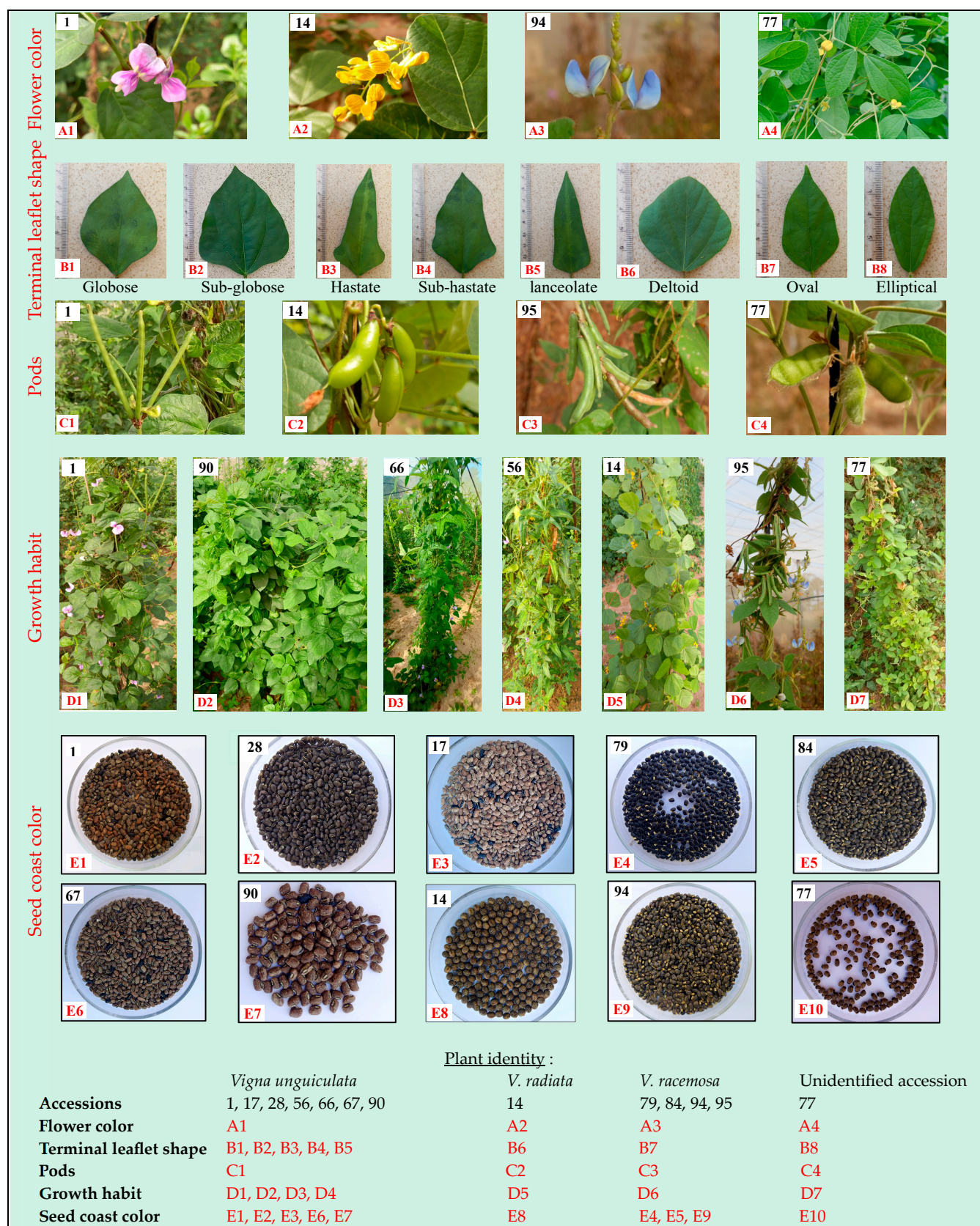


Figure 1. Qualitative trait variation in the wild *Vigna* species.

All the *V. unguiculata* accessions had violet flowers. The *V. radiata* and the unidentified accessions had yellow flowers. In *V. racemosa*, all accessions had dark blue flowers. The

seed shapes observed were rhomboid in *V. unguiculata* and *V. racemosa*, globose in *V. radiata* and cylindrical in the unidentified accession. The seed eye color was white for all wild *Vigna* species except the unidentified accession that showed a dark color (Figure 1). All the *Vigna* accessions had twining growth habits and dehiscent pods. The unidentified accession had not dehiscent pods (Supplementary Table S2).

3.1.2. Multiple Correspondence Analysis

The first three axes of the multiple correspondence analysis (MCA) were the most appropriate for interpreting the observed variance. These axes explained 67.66% of the total variance (Table 2). Nine qualitative traits that were correlated with the first three axes contributed strongly to the description of the observed variation. Most qualitative traits were correlated with these axes.

Table 2. Multiple correspondence analysis for 14 qualitative traits.

Factor Axes	Dim 1	Dim 2	Dim 3
Variance	0.712	0.517	0.259
% of var.	32.385	23.497	11.773
Cumulative % of var.	32.385	55.882	67.665

The multiple correspondence analysis showed that the qualitative traits, which accounted for more variability in the Dim1, Dim2 and Dim3, were leaf color, terminal leaflet shape, hypocotyl color, plant hairiness, pigmentation on the stem and petiole, flower color, leaf texture, seed shapes and dehiscent pod (Figure 2).

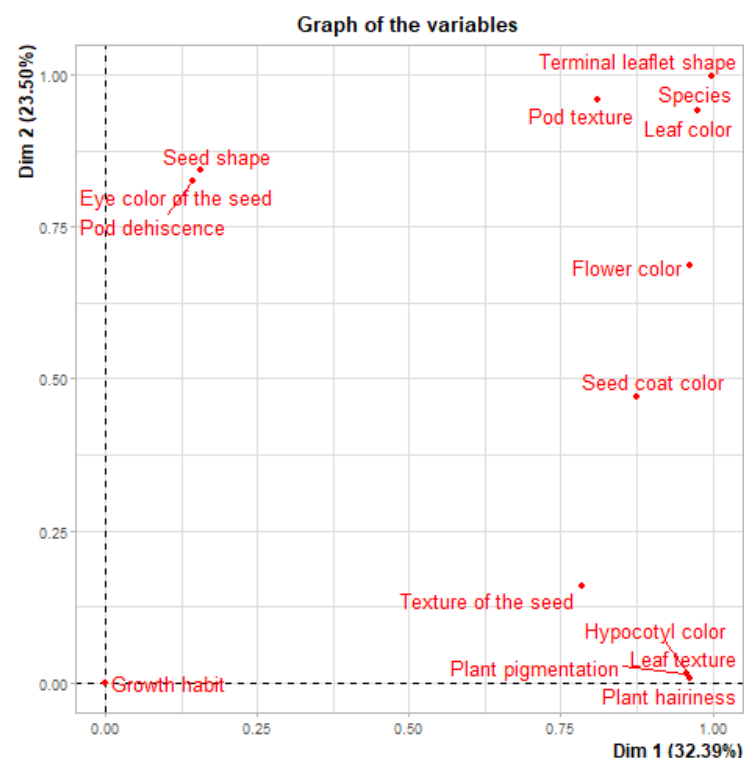


Figure 2. Multiple correspondence analysis on the basis of qualitative traits using R software (version 4.1.2).

3.1.3. Relationship between the Wild *Vigna* Accessions

The dendrogram, based on the qualitative traits including hypocotyl color, leaf color and texture, growth habit, terminal leaflet shape, plant pigmentation and hairiness, flower

color, seed texture, coat color, shape and eye color, showed that 55 *Vigna* accessions were clustered into four major classes (Figure 3). Cluster 1 was formed with only the *V. radiata* accession. Cluster 2 gathered the hybrid accession of *V. unguiculata* (90) and all the *V. unguiculata* var *spontanea* accessions. Cluster 3 was formed by the unidentified accession, and cluster 4 consisted of the nine accessions of *V. racemosa* (Figure 3).

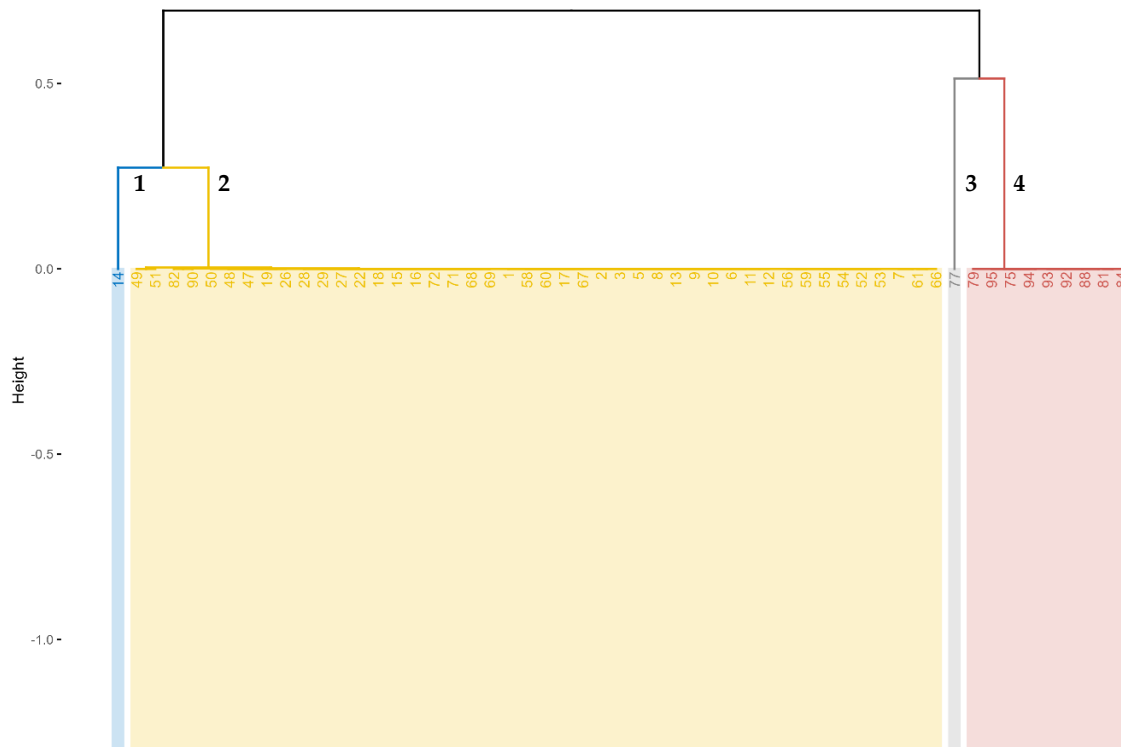


Figure 3. Dendrogram of the wild *Vigna* accessions based on qualitative traits with R software (version 4.1.2).

3.2. Diversity Description Based on Quantitative Traits

3.2.1. Descriptive Statistics Analysis

The terminal leaflet length ranged from 4.16 cm in the unidentified accession to 12.52 cm in the *V. unguiculata* var *spontanea* accession 7 (Figure 4A). Among the *V. unguiculata* accessions (hybrid and var *spontanea*), this trait ranged from 7.77 cm (accession 18) to 12.52 cm (accession 7). Among the *V. racemosa* accessions, the values of the terminal leaflet length ranged between 6.26 cm for accession 92 and 7.68 cm in accession 94. The terminal leaflet width ranged from 2.33 cm in the unidentified accession to 6.56 cm in the accession hybrid of *V. unguiculata* (number 90). Among *V. unguiculata*, it ranged from 2.95 cm in accession 56 to 6.56 cm (hybrid accession, 90). Among *V. racemosa*, the terminal leaflet width variations range from 3.3 cm for accession 94 to 4.28 cm in accession 93 (Figure 4B).

Fifty (50%) percent flowering varied widely from 52.2 days in accession 13 of *V. unguiculata* var *spontanea* to 112.3 days for *V. racemosa* accession 79 (Figure 4C). For 95% pod maturity, it ranged from 74.2 in accession 3 of *V. unguiculata* var *spontanea* to 125.8 days in *V. racemosa* (accession 92) (Figure 4D). The pod length varied from 2.36 cm in the unidentified accession to 10.4 cm in the hybrid accession of *V. unguiculata* (number 90) (Figure 4E). There was also variation in the 100-seed weight; accession 14 (*V. radiata*) recorded the highest value (4.8 g), while accession 18 of *V. unguiculata* var *spontanea* had the lowest value (1.48 g) (Figure 4F).

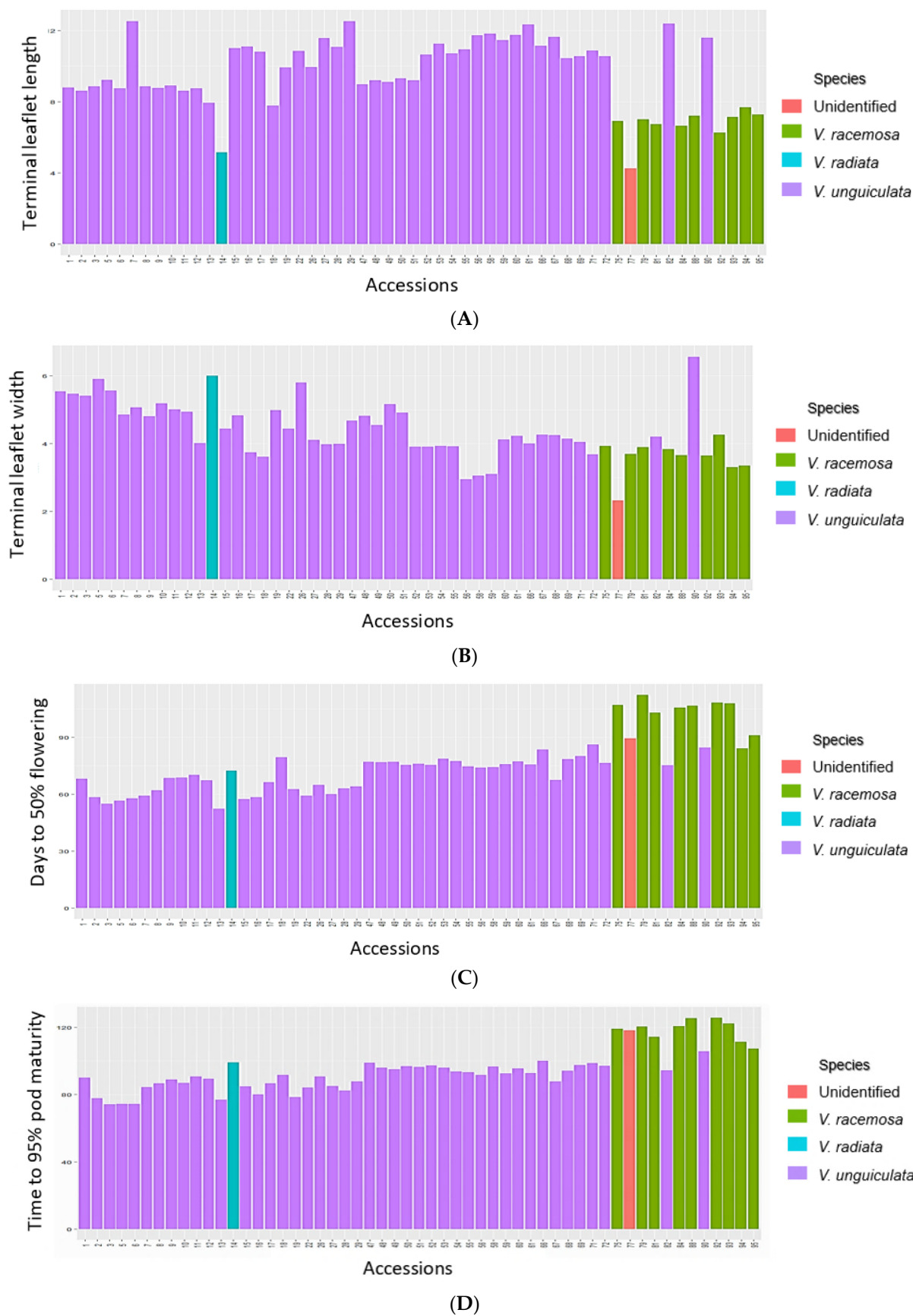


Figure 4. Cont.

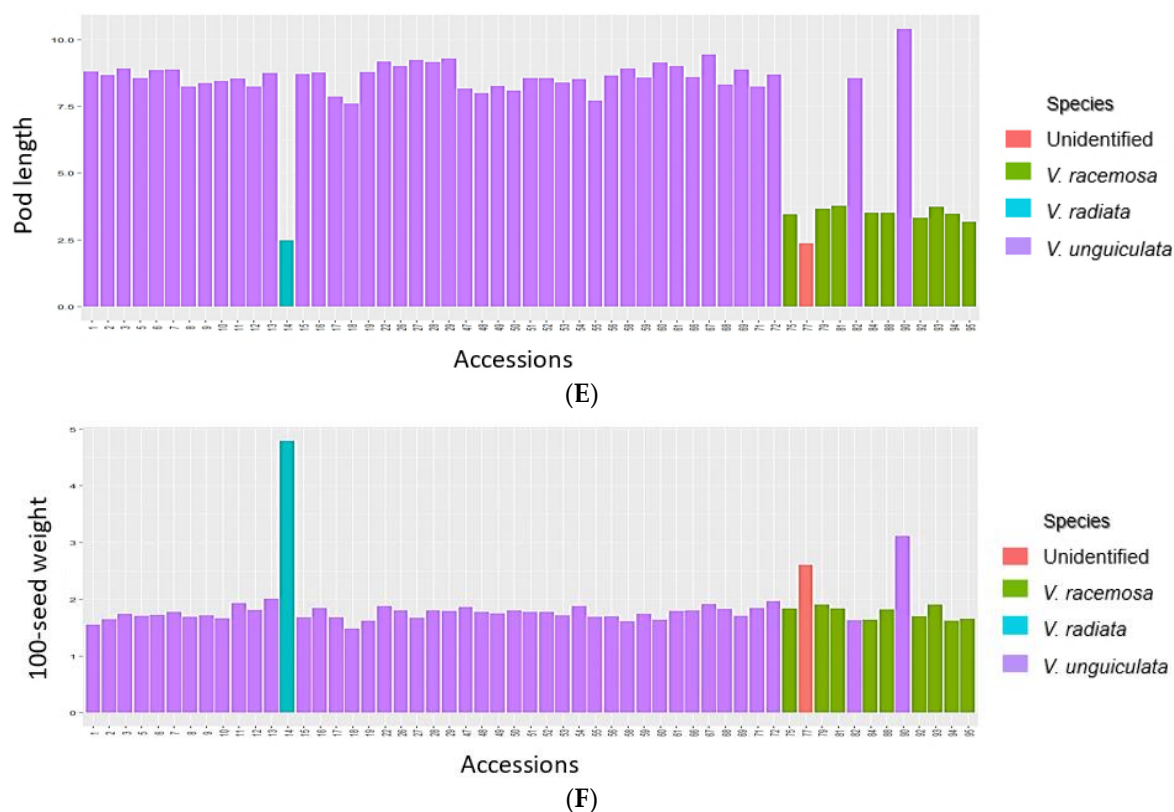


Figure 4. Descriptive statistics of some quantitative traits studied during the two seasons. Graphs generated with R software (version 4.1.2). Figures (A–F) showed the means of some quantitative traits during the two study seasons. (A): terminal leaflet length; (B): terminal leaflet width; (C): time to 50% flowering; (D): time to 95% pod maturity; (E): pod length and (F): 100-seed weight. Each species is represented by a color.

3.2.2. Analysis of Variance

Table 3a,b indicate the accession effect, block effect and replicate effect based on the analysis of variance (ANOVA). The results of the ANOVA indicate the existence of a significant difference ($p < 0.05$) between the accessions for all the measured traits from one season to the next. Replicate effects were only found ($p < 0.05$) for the time to 95% pod maturity, terminal leaflet length and width in 2021 (Table 3a).

Table 3. Analysis of variance for 8 quantitative traits in 2021 and 2022.

a. Analysis of Variance for 8 Quantitative Traits in 2021						
2021	Accessions Effect		Repetition Effect		Block Effect	
Traits	Mean Sq	Pr(>F)	Mean Sq	Pr(>F)	Mean Sq	Pr(>F)
T50%fw	760.100	$<2 \times 10^{-16}$	29.000	0.063	11.100	0.369
T95% Rp	675.800	$<2 \times 10^{-16}$	25.500	0.026	8.900	0.157
Tlfl	8.446	$<2 \times 10^{-16}$	3.319	1.4×10^{-6}	0.192	0.577
Tlfw	1.615	$<2 \times 10^{-16}$	0.233	0.029	0.098	0.064
Pdl	14.050	$<2 \times 10^{-16}$	0.123	0.384	0.085	0.889
Pdw	0.005	$<2 \times 10^{-16}$	0.000	0.239	0.000	0.617
Nlpd	34.890	$<2 \times 10^{-16}$	0.990	0.127	0.390	0.722
HSdw	1.051	$<2 \times 10^{-16}$	0.020	0.310	0.015	0.604

Table 3. Cont.

b. Analysis of Variance for 8 Quantitative Traits in 2022						
2022	Accessions Effect		Repetition Effect		Block Effect	
Traits	Mean Sq	Pr(>F)	Mean Sq	Pr(>F)	Mean Sq	Pr(>F)
T50%fw	656.800	$<2 \times 10^{-16}$	177.600	7.3×10^{-6}	9.600	0.816
T95% Rp	252.570	$<2 \times 10^{-16}$	115.040	8.1×10^{-6}	7.870	0.509
Tlfl	15.773	$<2 \times 10^{-16}$	0.535	0.138	0.140	0.973
Tlfw	2.844	$<2 \times 10^{-16}$	0.204	0.114	0.079	0.653
Pdl	15.197	$<2 \times 10^{-16}$	0.134	0.041	0.029	0.828
Pdw	0.005	$<2 \times 10^{-16}$	0.000	0.225	0.000	0.442
Nlpd	42.740	$<2 \times 10^{-16}$	0.420	0.056	0.150	0.362
HSdw	0.479	$<2 \times 10^{-16}$	0.012	0.577	0.027	0.211

The results during the 2022 rainy season show that there were replicate effects ($p < 0.05$) for the time to 50% flowering, time to 95% pod maturity and pod length (Table 3b). Any block effect and significant difference have been obtained during the two rainy seasons ($p > 0.05$).

The quantitative traits studied showed significant differences for all the species during the two rainy seasons (Table 4). Among these, only six (time to 50% flowering, time to 95% pod maturity, terminal leaflet length and width, pod length and 100-seed weight) were affected by the season (accession $\times$ season).

Table 4. Analysis of variance for the interactions due to the season for the studied quantitative traits.

p-Values for Season Effects				
Code	Trait	Season	Accession	Season $\times$ Accession
T50%fw	Time to 50% flowering	0.160	$<2 \times 10^{-16}$ ***	2.77×10^{-13} ***
T95% Rp	Time to 95% pod maturity	0.188	$<2 \times 10^{-16}$ ***	$<2 \times 10^{-16}$ ***
Tlfl	Terminal leaflet length	2×10^{-16} ***	$<2 \times 10^{-16}$ ***	2.33×10^{-14} ***
Tlfw	Terminal leaflet width	$<2 \times 10^{-16}$ ***	$<2 \times 10^{-16}$ ***	8.24×10^{-10} ***
Pdl	Pod length	0.0001 ***	$<2 \times 10^{-16}$ ***	1.3×10^{-12} ***
Pdw	Pod width	0.332	$<2 \times 10^{-16}$ ***	0.401
Nlpd	Number of locules per pod	0.0003 ***	$<2 \times 10^{-16}$ ***	2.98×10^{-7} ***
HSdw	Weight of 100 seeds	0.002 **	$<2 \times 10^{-16}$ ***	$<2 \times 10^{-16}$ ***

***, ** Significant at the 0.001 and 0.01 levels, respectively.

3.2.3. Principal Component Analysis

The two axes of the principal component analysis (PCA) explained 73.02% of the total variation and were retained to analyze the agro-morphological variability among the wild *Vigna* accessions (Figure 5). The time to 50% flowering (T50%fw), terminal leaflet length (Tlfl), number of locules per pod (Nlpd) and pod length (Pdl) were the traits with the highest contribution to the PC1. These traits explained 46.86% of the total variation to the PC1. Agronomic traits, such as pod width (Pdw), 100-seed weight (HSdw) and terminal leaflet width (Tlfw), were the most important traits that contributed to the variation in PC2.

A significant positive correlation ($p = 0.000$) was observed between the 100-seed weight (HSdw) and pod width (Pdw). The number of locules per pod (Nlpd) was significantly correlated with pod length (Pdl) ($p = 0.000$). The 100-seed weight was negatively correlated with pod length ($p = 0.023$) and number of locules per pod ($p = 0.010$) (Figures 5 and 6 and Table 5).

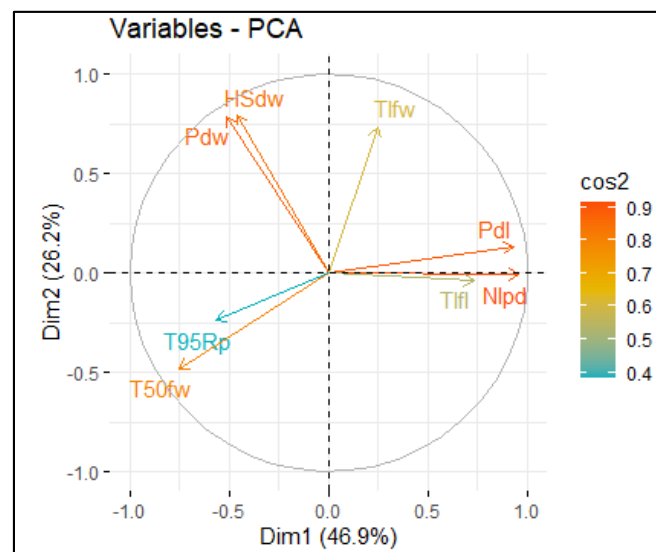


Figure 5. Principal component analysis of wild *Vigna* accessions using 8 quantitative traits under R (version 4.1.2). Variables with high cos2 values are colored orange. Variables with low cos2 values are colored blue.

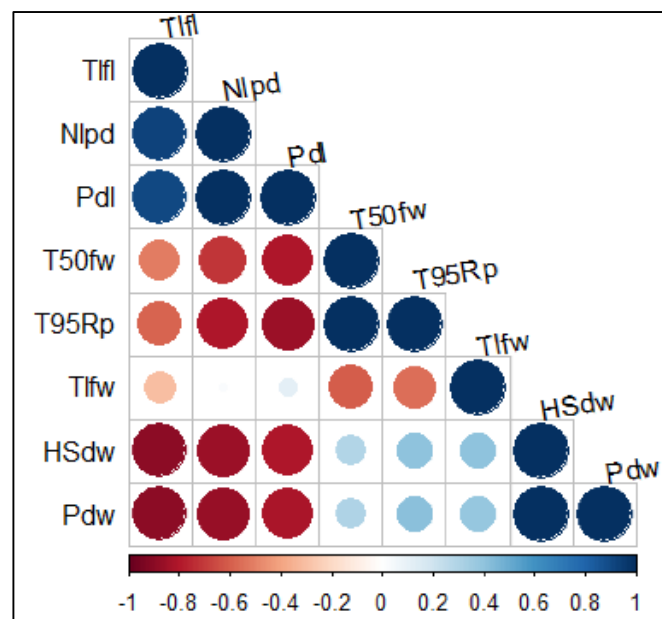


Figure 6. Correlation of the quantitative traits studied. Graph generated using 8 quantitative traits under R (version 4.1.2).

Table 5. Correlation between quantitative traits.

Traits	T50fw	T95Rp	HSdw	Nlpl	Pdl	Pdw	Tlfl	Tlflw
T50fw								
T95Rp	0.000							
HSdw	0.152	0.100						
Nlpl	0.000	0.000	0.010					
Pdl	0.000	0.000	0.023	0.000				
Pdw	0.205	0.140	0.000	0.013	0.029			
Tlfl	0.000	0.000	0.010	0.000	0.000	0.013		
Tlflw	0.003	0.005	0.741	0.056	0.032	0.685	0.138	

Tlfl: terminal leaflet length; Tlflw: terminal leaflet width; T50fw: time to 50% flowering; Pdl: pod length; Pdw: pod width; T95Rp: time to 95% pod maturity; Nlpl: number of locules per pod; HSdw: 100-seed weight.

3.2.4. Cluster Analysis Showing the Grouping of the Accessions Based on the Quantitative Traits

Three clusters were generated using the quantitative traits recorded on the wild *Vigna* accessions. Cluster 1 contained only the *V. radiata* accession. Cluster 2 encompassed the *V. racemosa* accessions and the unidentified accession (77). Cluster 3 was the largest and included only the *V. unguiculata* accessions (Figure 7). Cluster 3 included the hybrid accession of *V. unguiculata* (90) and all the *V. unguiculata* var *spontanea* accessions.



Figure 7. Grouping of the wild *Vigna* species based on 8 quantitative traits. Graph generated with R version 4.1.2.

Based on the quantitative traits, the wild *Vigna* were divided into three clusters. Cluster 1 included the *V. radiata* accession (14) with a high value of the 100-seed weight and a large pod compared to the overall average. Similarly, cluster 2 included the accessions with a low number of locules per pod and that were late flowering and late maturing compared to the overall average for each of these traits. Cluster 3 encompassed the accessions with long pods and a high number of locules per pod and that flowered earlier and matured earlier compared to the overall average for each of these traits (Table 6).

Table 6. Description of each cluster by quantitative variables.

Cluster 1				Cluster 2				Cluster 3			
Traits	Mean in Category	Overall Mean	p-Value	Traits	Mean in Category	Overall Mean	p-Value	Traits	Mean in Category	Overall Mean	p-Value
Pdw	0.700	0.405	0	T50fw	101.500	75.701	0	Pdl	8.495	7.625	0
HSdw	4.795	1.853	0	T95Rp	97.190	91.249	0.013	Nlpd	14.508	13.133	0
Tlfw	5.998	4.365	0.05	Tlfw	3.592	4.365	0.001	Tlfl	10.285	9.542	0
Tlfl	5.161	9.542	0.025	Tlfl	6.710	9.542	0	Tlfw	4.503	4.365	0.015
Pdl	2.485	7.625	0.009	Nlpd	8.214	13.133	0	Pdw	0.400	0.405	0.046
Nlpd	2	13.133	0	Pdl	4.310	7.625	0	T95Rp	89.719	91.249	0.007
								T50fw	69.913	75.701	0

4. Discussion

The characterization and evaluation of cultivated and wild accessions are one of the most important activities for plant genetic resource management, leading to the discovery of relevant traits that can be used for crop adaptation. In the cowpea, the wild species remain the main reservoir of traits of interest that are usable in crop improvement due to their adaptation to a wide range of environments and their resistance to several diseases. So, their exploration in terms of genetic and agro-morphological study is essential for understanding the process of domestication. The present investigation of the agro-morphological variability of the wild *Vigna* accessions that was based on fourteen qualitative and eight quantitative traits, which included vegetative, floral, pod and seed traits, revealed that there is variation within the same species and among the *Vigna* accessions. These results are in agreement with the findings of Popoola et al. [13] and Joshua et al. [14]. Based on the traits examined, we discovered that there is more agro-morphological variability in *Vigna unguiculata* compared to that in the other *Vigna* species. In addition, some traits described in the *V. racemosa* accessions, such as the hairiness, that disappeared during the domestication process are desirable characters that could be also used in a breeding program. These traits could have potential use, since they are thought to be responsible for some benefits, such as the resistance to pests and diseases [31,32]. Similar findings were reported in previous studies [33–35] in the *Vigna unguiculata* var *spontanea* wild relatives and [36] in *Cajanus cajan* and *Sphenostylis stenocarpa*. In this work, sub-hastate, globose, hastate and sub-globose were the most occurring terminal leaflet shapes, unlike the lanceolate form, which was the least frequent. These morphological differences in terminal leaflet shape have been observed in *V. unguiculata* var *spontanea*. However, the terminal leaflet shapes oval (*V. racemosa*), deltoid (*V. radiata*) and elliptic (unidentified accession) were also observed in our study. Nicotra et al. [37] reported that the oval and elliptic terminal leaflets may help capture sunlight and carry out photosynthesis efficiently. Multiple correspondence analysis (MCA) suggests that morphological characters, such as leaf color, terminal leaflet shape, hypocotyl color, plant hairiness, pigmentation on the stem and petiole, flower color, leaf texture, seed shapes and dehiscent pod, have the potential to discriminate the wild *Vigna* accessions. The MCA, based on the qualitative phenotypic traits, was confirmed with an analysis of the Hierarchical Ascending Classification, which was used to construct a dendrogram. The dendrogram showed variation among the accessions for the traits studied. It also revealed the importance of morphological markers to differentiate and classify the wild *Vigna* species. Wild accessions with similar traits were found in the same group. This information would help breeders to make the right decision and choice in a cowpea breeding program. This study showed significant differences among the accessions for most of the agro-morphological characters evaluated. These results are in agreement with the report of Moalafi et al. [38]. The *V. unguiculata* var *spontanea* accession 13 reaches 50% flowering at the earliest time (52.2 days after sowing). The significant correlation among some traits, such as days to 50% flowering and time to 95% pod maturity, could be exploited in breeding to improve cultivated cowpea. Furthermore, the accessions with longer pods (accession 90) possessed a higher number of locules per pod and are potential raw materials to boost seed production of cowpea. However, for most of the *V. racemosa* accessions (such as accessions 79 and 92), we observed a late time to 50% flowering (approximately 112.3 days after sowing) during the two seasons; this could be due to a response of these accessions to photoperiod. Indeed, depending on photoperiod, flowering can be delayed in some genotypes [39]. During this study, the *V. radiata* accession (14) had the highest 100-seed weight, 4.8 g. The seed weight is a yield indicator [13,40] and, therefore, useful to increase the production. The analysis of variance (ANOVA) in the first and second season showed accession effects with significant differences for all the analyzed quantitative traits. For the same trait, the accessions differed significantly over one year. Similar results were described by Adewale et al. [41] in their studies of eleven cowpea genotypes. The *Vigna* accessions involved in this study revealed different phenotypic and probably genetic characteristics. Most of the quantitative traits, such as terminal leaflet length and width, pod length, number of

locules per pod and 100-seed weight, were affected by the season (year). These variations in some quantitative traits for the same accession from season to season are likely due to the heavier precipitation in the 2022 season compared to that in the 2021 season and others environmental factors. The repetition effect observed could be due to specific factors such as soil characteristics. The significant effect of the season (accession $\times$ year effect) on some quantitative traits examined, such as the days to 50% flowering, time to 95% pod maturity and 100-seed weight, has also been reported by Adewale et al. [41] in their study of genotypic variability and stability of some grain yield components of cowpea. The principal component analysis (PCA), based on the quantitative traits, confirmed a high agro-morphological relationship among the wild *Vigna* accessions (Figures 5 and 6). High diversity among related accession groups is a good factor for the genetic improvement of species in the group. The cluster analysis, based on the eight quantitative traits studied, classify the accessions into three distinct groups. The cluster analysis clearly separated the accessions based on yield components, mainly those related to pods and seeds, as well as flowering, pod maturity and some vegetative traits. These quantitative traits are important in explaining the diversity of the *Vigna* accessions. Cluster 3 encompasses the largest number of accessions, while cluster 2 contains some accessions, and cluster 1 contains one accession (Figure 7), showing wide inter-cluster divergence, which is desirable for future hybridization programs. These are clear indications that these wild *Vigna* accessions could be made useful, and pre-breeding material (for example, a marker-assisted backcross) must be developed and evaluated in order to identify some useful traits. Thus, the combination of this agro-morphological information with the molecular information obtained with SSR markers by Sarr et al. [27] may provide added value for the use of some of these wild *Vigna* accessions in breeding programs. According to our study, despite the limited number of samples, the Senegalese wild *Vigna* accessions have a high level of morphological diversity, corroborating a previous genetic analysis [27], which showed that the wild forms remain more diverse than the cultivated.

5. Conclusions

This study revealed a high level of agro-morphological diversity between and within the wild *Vigna* species. Some particular traits of agronomic importance, including hairiness, a large length and width of the terminal leaflet, early maturity, a longer pod, a high number of locules per pod and a high weight of 100 seeds, can be targeted for subsequent hybridization for cowpea improvement. The high agro-morphological variability observed in this study suggests that these wild *Vigna* accessions constitute a true reservoir. Despite the influence of environmental factors (on some traits), the agro-morphological traits used in this study allowed for the characterization of the wild *Vigna* accessions. This study sets the basis for the genetic improvement of cultivated cowpea using wild relatives, since the observed diversity can be exploited in breeding and varietal improvement programs. However, further characterization that focuses on the study of the photoperiod in the wild *Vigna* species may be of great value. This would provide more information for characterizing diversity.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13112761/s1>. Table S1: List of the wild *Vigna* accessions used in this study and their region of provenance. Table S2: Agro-morphological characteristics of 55 wild *Vigna* accessions.

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