DE GRUYTER Open Agriculture. 2019; 4: 487-499

Research Article

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Phenotypic diversity for agro-morphological traits in pigeon pea landraces [(*Cajanus cajan* L.) Millsp.] cultivated in southern Benin

https://doi.org/10.1515/opag-2019-0046
received September 20, 2018; accepted June 18, 2019

Abstract: Pigeon pea is an important but unfortunately neglected grain legume crop in Benin. In this study, the phenotypic diversity in 84 landraces from Benin and four advanced varieties were evaluated at two locations during two consecutive rainy seasons. Twelve quantitative and ten qualitative variables were recorded. A wide range of variation was observed in qualitative traits as well as in quantitative variables. The overall Shannon–Weaver diversity index ranged from 0.41 (pod hairiness) to 1.51 (seed colour). Analysis of variance revealed highly significant differences among accessions for all quantitative traits, except the number of seeds per pod, the days to maturity and the seed yield per plant. In all quantitative traits, phenotypic coefficient of variation was higher than genotypic coefficient of variation indicating a substantial environmental effect on their variability. However, some traits (days to flowering, pod width, number of secondary branches and 100 seed weight) showed great value of heritability. Hierarchical clustering on principal components subdivided the collection from Benin into two groups that were clearly separated from the group of advanced varieties. The results gathered from this study have important implications for pigeon pea germplasm characterization and conservation while the inferred phenotypic groups could be used for parental selection and heterotic crossings in pigeon pea breeding in Benin.

Keywords: Benin; *Cajanus cajan*; Heritability; Phenotypic diversity; agro-morphological traits

1 Introduction

Pigeon pea (*Cajanus cajan* [L.] Millspaugh) is an important food legume (or pulse) crop that is predominantly cultivated in tropical and subtropical regions of the world (Varshney et al. 2010). The crop plays an important role in food and nutritional security for rural communities in developing countries. The seed of pigeon pea is eaten as a green vegetable and dry pulse and is an important source of protein, vitamin B, carotene, and ascorbic acid (Odeny 2007, Choudhary et al. 2013). According to Varshney et al. (2010), pigeon pea seeds have 20–22% protein. It is generally planted by smallholder farmers in low input and rainfed conditions (Khoury et al. 2015; Saxena et al. 2018). Pigeon pea is a hardy and drought tolerant crop assuring sustainable returns from marginal lands with minimal inputs, hence it is considered as a very suitable crop for subsistence agriculture (Odeny 2007).

Unfortunately, pigeon pea is considered as an “orphan crop” in many countries in Sub Saharan Africa (Odeny 2007; Varshney et al. 2012). In Benin, the crop is neglected and underutilized (Dansi et al. 2012; Zavinon et al. 2018). The annual planted area amounts in average 4059 ha with a production of 2799 tons (INSAE 2015; Ayenan et al. 2017a). The major constraint of pigeon pea production in the country is the lack of high yielding varieties (Zavinon et al. 2018). The cultivars produced by the farmers are mainly landraces and have a long maturity cycle and very low grain yield (Ayenan et al. 2017b; Zavinon et al. 2018).

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It has retained little attention by research and conservation in Benin, where its potential value is underestimated and underexploited (Dansi et al. 2012). Although classified among the nineteen most important neglected and underutilized priority crop species which should merit attention and support in Benin (Dansi et al. 2012; Zavinon et al. 2018), little or no scientific study was devoted to this crop until recently. A concerted research effort is therefore needed for pigeon pea crop improvement.

The knowledge of different landraces and their evaluation are necessary for improvement strategy development in any crop (Adoukonou-Sagbadja et al. 2007,;Gbaguidi et al. 2018), as these traditional landraces are the potential donor parents for improved varieties (Upadhyaya et al. 2007). In Benin no characterization of pigeon pea has been carried out, although recent efforts were made to collect and conserve the local cultivars across the major growing area in the country (Ayenan et al 2017a; Zavinon et al. 2018). However, for making a rational conservation plan of these collections, it is necessary to assess the level of morphological variability present within and among the landraces.

The present study aimed to (1) evaluate the phenotypic diversity in a collection of pigeon pea landraces cultivated in Benin using agro-morphological traits, (2) quantify the genetic contribution in the phenotypic variability observed, (3) estimating the heritability and genetic advance of studied traits in the collection. These goals will enable to identify the most efficient landraces for development and research programs.

2 Material and methods

2.1 Description of the trial sites

Two trials were conducted consecutively during the cropping seasons 2016 and 2017. The first trial was installed at the experimental site of AfricaRice at Abomey-Calavi (latitude: N 6° 25’ 260’’ and longitude: E 2° 19’ 682’’) in Benin. It was repeated during the 2017 cropping season in a reference farmer’s field at Pobè, one of a district in the Plateau department belonging to the agro-ecological zone VII or “*depression zone*”. Both experimental sites are located in southern Benin characterized by a sub-equatorial climate with two rainy seasons and two dry seasons. Annual mean temperatures range from 26 to 28°C and annual rainfall varies between 800 to 1400 mm (Yabi and Afouda 2012). The main difference between these two experimental sites resides in the types of soil. Indeed, on the AfricaRice experimental site at Abomey-Calavi, the soils are deep, ferralitic with a red colour. They are soils formed on the sandy continental terminal with a pH of about 6.2 and organic matter content averaging 1.5% (Agbossou 2014). In contrast, the soils at the Pobè site are vertisols rich in swelling clay. Their structure varies according to the water content. They are porous and swollen in rainy weather, but cracked and dry during periods where there is a lack of rain and often with shrinkage slots during desiccation. In general, they are black soils very rich in organic matter.

2.2 Plant material and experimental design

The plant material constituted of 88 accessions of pigeon pea in which eighty four were local landraces sampled in the four major pigeon pea growing departments (Couffo, Collines, Plateau and Zou) in southern Benin and four (04) improved pigeon pea genotypes obtained from the International Institute of Tropical Agriculture (IITA) Nigeria/Ibadan. The landraces are the pigeon pea local varieties traditionally cultivated by the farmers in southern Benin. They were recently sampled during an ethno-botanical survey and reported to differ in many morphological attributes among which their maturity cycle, seed colour but also their geographic origin (Zavinon et al. 2018). The four advanced varieties obtained from IITA were characterized by their short maturity and high grain yield potential. They were used in the experimentation as check varieties.

During the first year (2016) the accessions were planted in an augmented experimental design (Federer 1956). This design has been reported early on pigeon pea (Upadhyaya et al. 2007) and on other species such as rice (Sanni et al. 2012), sorghum (Aruna and Audilakshmi 2008). It has been used because of the large number of varieties to be tested (88) and the limited quantity of seed available. We used four blocks of 25 plots each to sow the 88 pigeon pea accessions. The four varieties used as control were randomly replicated in each block while the landraces were also randomly assigned to the plots in each block without replication. Plots were 1.5 m length and inter row and intra row spacing were 1 m and 0.5 m, respectively. In contrast, during the cropping season (2017), a randomized complete block with two (02) replications was adopted for the experiment conducted at the Pobè site. The plots in each block were represented by four rows of 1.5 m length with row to row spacing of 1 m and plant to plant of 0.5 m. At all the experimental sites, the sowing was direct and two or three weeks after emergence, the plants were thinned to one plant per hill.

2.3 Data collection

In total, 22 characters including 12 quantitative and 10 qualitative were recorded. IBPGR/ICRISAT Descriptors (1993) were used for data collection. The 10 qualitative traits (plant vigor, growth habit, stem colour, stem thickness, petal colour, flowering pattern, pod colour, pod form, pod hairiness, seed colour pattern) were recorded on individual plants within the plot according to Manyassa et al. (2008). Concerning the 12 quantitative traits, days to flowering and maturity, 100-seed weights were taken on a plot basis (Upadhyaya et al. 2007; Manyassa et al. 2008). Data on number of primary branches, number of secondary branches, plant height, pods per plant, pod bearing length, and seed yield per plant were taken on four randomly selected plants in each plot except at the Pobè experimental site where these data were recorded on three randomly selected competitive plants of a genotype in a plot in each replication. The quantitative traits like pod length, pod width and number of seeds per pod were recorded on ten pods selected randomly from the competitive plants. All the 12 quantitative traits were recorded at both sites except pods per plant, seed yield per plant and days to maturity which were not recorded at AfricaRice site. Description of all traits and the different codes used is given in table 1.

2.4 Statistical data analysis

Quantitative data were analyzed through the calculation of basic statistical parameters (range, mean, standard deviation, and coefficient of variation) and analysis of variance (ANOVA) for assessing variation within pigeon pea accessions for each experimental location¶. ANOVA was also performed with the combined data across locations. For that analysis, the means of each genotype under each environment was used to perform ANOVA following the method described by Sharma (2006). This determined environmental as well as interaction of genotypes × location effects on different traits. The combined site variance components were used to estimate broad sense heritability (H^2), expected genetic advance (*GA*), phenotypic coefficient of variance (*PCV*) and genotypic coefficient of variance (*GCV*). These genetic parameters were calculated according to Burton et al. (1953) as follow:

a – Phenotypic coefficient of variance (PCV)

$$PCV = \sqrt{\sigma_P^2/_{\bar{X}}} \times 100$$

b – Genotypic coefficient of variance (GCV)

$$GCV = \sqrt{\sigma_G^2/_{\bar{X}}} \times 100$$

c– Heritability (H^2)

$$H^2 = {\sigma_G^2}/_{\sigma_P^2}$$

d – Expected genetic advance (GA)

$$GA = k \times \frac{\sqrt{\sigma_P^2}}{\bar{X}} \times H^2 \times 100$$

The parameters σ_P^2, σ_G^2 and σ_E^2 are respectively phenotypic, genotypic and environmental variance. They were obtained according to Mazid et al. (2013) as:

$$\sigma_E^2 = \frac{MSe}{r} \quad \text{and} \quad \sigma_G^2 = \frac{MSg - MSe}{r}; \quad \sigma_P^2 = \sigma_G^2 + \sigma_E^2$$

where MSp, MSg, MSe are mean squares of phenotypes, genotypes, and error, respectively; r was number of replication and $\bar{X}$ is mean of the trait.

Quantitative data were also analyzed by means of the multivariate methods. Therefore, in order to assess the relationship among the 12 different traits, Pearson's correlation coefficients were estimated. Hierarchical clustering on Principal components (HCPC) was carried out following Cericolla et al. (2013) to determine patterns of variation and the genetic relationship existing between accessions in the collection. For this analysis, the principal components (PCs) with eigen-values above 1 were considered for the analysis. Moreover, cluster analysis was performed on principal components to assess the level of dissimilarity among the accessions. Ward's minimum variance clustering method was used to classify accessions in different clusters on the basis of a Euclidean distances matrix. The first two PCs were plotted and the 88 accessions were projected in the system of axes formed by these two PCs in order to appreciate the dispersion and the grouping of accessions in different clusters.

Variability due to the qualitative traits was assessed by the calculation of frequencies of each modality in each character. Based on the phenotypic frequencies of qualitative traits Shannon–Weaver diversity indices (*H'*) as described by Jain et al (1975) and reported in Manyassa et al. (2008) was calculated following the formula:

$$H' = -\sum_{i=1}^{n} pi\ logpi$$

Table 1: List of morphological traits evaluated and their description and abbreviations used

Morphological traits	Codes	Description
Qualitative variables		
Growth habit	GHa	Related to the stem direction. 1) compact, 2) spreading
Plant vigor	PtVg	1) Low, 2) medium, 3) High. Recorded at 50% flowering
Stem color	StCo	Color of the stem (green ; purple; mixed)
Pod form	Pofo	1) flat, 2) cylindrical
Pod color	PoCo	Primary color of pod (green ; purple ; mixed)
Stem thickness	StT	1) Thin (< 5mm), 2) intermediate (5-13 mm), 3) thick (>13mm)
Petal color	PtCo	Main color of petals (Yellow ; red ; striped red)
Sepal Color	SpCo	Main color of sepals (green ; dark purple)
Seed Color	SeCo	Dry seed color (Light grey; Red; Brown; Mottled; Black)
Pod hairiness	PoH	1) glabrous, 2) pubescent
Quantitative variables		
Plant height (cm)	PtHe	Ground to tallest canopy point
Days to 50% flowering	DF	Number of days from sowing to when 50% of plant flowering
Primary branches	PBr	Number of primary branches per plant
Secondary branches	SBr	Number of secondary branches per plant
Days to 75% maturity	DM	Number of days from sowing to 75 % physiological maturity
Pod length (cm)	PoL	Length of pod in cm (at maturity)
Pod width (mm)	PoW	Width of pod in cm (at maturity)
Pod number	NPo	Number of pods per plant (at maturity)
Seeds per pod	Se/Po	Number of seeds per pod taken from 10 randomly selected pods
Pod bearing length (cm)	PoBL	Distance (in cm) between lowest and topmost pod on the plant
100-seed weight (g)	100SW	Weight of air dried 100 seeds (in grams)
Seed yield per plant (g)	Yield/Pt	Grain yield per plant (in grams)

where pi is the proportion of the accessions in the i^{th} class of an n-class character.

Data management and all statistical analyses were performed using the following packages (Agricolae; FactoMineR, factoextra) in the software R (R development core team, 2016).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Variation in qualitative traits

Frequency distribution of different classes within each qualitative character is given in table 2. In the collection, the accessions analyzed are generally vigorous. A predominance of class of high vigor was observed(67%) while the individuals with medium vigor were accounted for 33%. Concerning the stem thickness, the majority of landraces had big stems (diameter > 13 mm) while the intermediate and thin phenotypic classes accounted for 13.63% and 9.09%, respectively. In general, the branching pattern of the stem of pigeon pea may vary from bush type to compact upright type and indicates its growth habit**.** Two main classes of growth habit were observed in the collec-

Table 2: Frequency distribution and Shannon-Weaver diversity index of qualitative traits

Qualitative Traits	Modalities	Frequency (%)	Diversity index (*H'*)
Growth habit	*Erect*	55.68	0.68
	Spreading	44.32	
Plant vigor	*Medium*	33	0.63
	High	67	
Stem thickness	*Thin*	9.09	0.68
	Intermediate	13.63	
	Thick	77.27	
Pod hairiness	*Non hairy*	81.81	0.47
	Hairy	18.19	
Pod form	*Cylindrical*	71.6	0.59
	Flat	28.4	
Pod color	*Green*	35.22	0.75
	Mixed	64.77	
	Purple	2.27	
Stem color	*Green*	59.09	0.87
	Purple	7.95	
	Dark purple	32.95	
Sepal color	*Green*	39.77	0.67
	Dark purple	60.23	
Petal color	*Yellow*	20.45	0.76
	Red	7.95	
	Striped Red	71.6	
Seed color	*Cream*	14.77	1.51
	Light grey	47.72	
	Red	11.36	
	Brown	9.09	
	Mottled	14.77	
	Black	4.45	

tion: erect (55.68%) and spreading habit (44.32%). Many of the landraces (71.6%) presented a cylindrical pod shape while 28.4% had flat pods. It was observed that most of the landraces (81.81) had pubescent pods while 18.19% of accessions did not present any hair on their pods. The colour of different organs such as stem, flower, pods and seed were also recorded. The plant pigmentation recorded as stem colour varied between green, purple and mixed. The majority of accessions (59.09%) in the collection had a green stem. Individuals with purple and mixed stem colour represented respectively 7.95% and 32.95%. The collection was composed of 35.23% of individuals with green pods, 62.50% with mixed pods and only 2.27% presented purple pods. The sepal had two main colours: green and dark purple. The frequency of individual with green sepal was 39.77% against 60.23% with dark purple sepal. The corolla pigmentation varied through three different colours yellow (20.45%); red (7.95%) and striped red (71.6%). The variation observed in seed colour showed six classes in which the most represented was light grey (47.72%) followed by cream and mottled, accounting each for 14.77%. The accessions with red, brown and black seed colours represented respectively 11.36%, 9.09% and 4.45% of individuals in the collection. Based on the Shannon-Weaver diversity index, the ten qualitative traits presented a relatively low diversity. The diversity index ranged from 0.47 (pod hairiness) to 1.51 (seed colour). Seed colour was the most diversified (1.51) followed by stem colour (0.87).

3.2 Variation in quantitative traits

3.2.1 Phenotypic divergence in the collection

The accessions analyzed were significantly different for a large number of quantitative traits measured. As revealed by the descriptive statistics and ANOVA results (Table 3), a significant variation existed (at $P < 0.05$) in all the characters except days to 75% maturity, number of seeds per pod and seed yield per plant. However, seed yield per plant showed a high coefficient of variation (CV = 30.89). It varied from 28.73g to 133.9g indicating there was a wide range of variability in the germplasm for this character even though the difference between accessions was not significant. At all experimental sites, the number of primary branches and secondary branches showed a high value of coefficient of variation. A high variability exists among the traditional pigeon pea varieties for these two characters. The coefficients of variation of primary branches were respectively 23.34 and 28.76 at the Pobè and Abomey-Calavi experimental site while it was 38.64 and 35.01 for the secondary branches respectively at the Pobè and Abomey-Calavi experimental site. Another trait which exhibited a high variability (CV = 35.76) in the germplasm was number of pods per plant. It varied from 105.3 to 691.1 pods per plant. The traits such as plant height, days to

Table 3: Descriptive statistics and P value of ANOVA of the 12 quantitative variables measured

Traits	Pobè					Abomey-Calavi				
	Range	**Mean**	**SD**	**CV**	**P > (F)**	**Range**	**Mean**	**SD**	**CV**	**P > (F)**
PtHe	194.5 - 401	303.46	40.56	13.37	< 0.000	169.50 - 290	226.94	25.17	11.09	0.028
PBr	14.5 – 58.5	38.27	8.9	23.34	0.003	18 – 78.75	29.31	8.42	28.73	< 0.000
SBr	24.5 – 187.9	88.54	34.21	38.64	< 0.000	28.75 – 163.25	91.74	32.12	35.01	< 0.000
DF	132.5 - 228	191.99	16.22	8.45	< 0.000	110 - 214	175.79	20.84	11.85	< 0.000
PoL	4.25 – 8.2	5.15	0.56	10.98	0.025	3.96 – 7.76	5.33	0.80	15.03	0.007
PoW	6.5 – 10.8	8.31	0.8	10.31	<0.000	5.20 - 12	9.83	0.94	9.58	0.006
Se/Po	3.1 – 4.82	4.04	0.35	8.83	**0.244**	3 - 5.33	4.24	0.52	12.25	**0.134**
PoBL	31.71 – 74.94	49.92	8.05	16.14	0.004	38.09 – 68.43	52.92	6.53	12.35	0.011
100SW	5.3 – 12.05	9.65	1.17	12.21	< 0.000	5.70 -12.80	9.57	1.21	12.66	< 0.000
DM	209.5 – 309.5	277.06	18.34	6.62	**0.251**	-	-	-	-	-
NPo	105.3 – 691.1	380	135.9	35.76	0.002	-	-	-	-	-
Yield/Pt	28.73 – 133.9	83.54	25.81	30.89	**0.083**	-	-	-	-	-

50% flowering, pod length, pod width, pod bearing length and 100 seed weight showed significant differences ($P < 0.05$) among the accessions analyzed, but low variation (CV < 20%) whatever the experimental site.

3.2.2 Components of phenotypic variance and estimation of genetic parameters

The mean values of nine quantitative traits recorded at both experimental sites were used in a combined analysis of variance to estimate the different genetic variability parameters (Genotypic variance, phenotypic variance and environmental variance). Based on these variance components, phenotypic and genotypic coefficient of variation, heritability and genetic advance were estimated and presented in table 4. The phenotypic and genotypic coefficients of variation were generally low for all the nine traits studied. Phenotypic coefficient of variation (*PCV*) varied from 7.87 for pod width to 28.91 for number of secondary branches whereas genotypic coefficient of variation (*GCV*) varied from 3.21 for pod bearing length to 17.81 for number of secondary branches. The highest value of phenotypic and genotypic coefficients of variation was observed in a number of secondary branches revealing an important magnitude of variability for this character. In all the characters, phenotypic coefficient of variation was found to be higher than genotypic coefficient of variation. Heritability estimates ranged from 9.65 to 69.06 and the genetic advance ranged from 16.21 to 59.57. The highest value (69.06) of heritability was observed in days to 50% flowering while pod bearing length exhibited the lowest (9.65) value of heritability. Concerning genetic advance, the highest value was observed in number of secondary branches and the lowest value of genetic advance was observed in pod width. A moderate value (42.15) of heritability was observed in pod width, while the characters like number of secondary branches and 100 seed weight exhibited a relatively moderate value of heritability. It was estimated to be 37.95% and 34.84% for the number of secondary branches and 100 seed weight, respectively. The rest of the characters (plant height; number of primary branches; pod length; number of seed per pod) showed lower heritability ($H^2 < 25\%$). Among the nine quantitative traits studied, only the number of secondary branches exhibited a great value of heritability coupled with a high value of genetic advance.

3.2.3 Relationships among agro-morphological characters

The mean values from the two experimental sites were used to predict the relationship between the quantitative traits. Pearson's correlation coefficient between pairs of traits and probability associated were given in table 5. In all the 12 quantitative traits ten high positive and significant correlations were observed. The highest correlation (r = 0.803) was recorded between days to 50% flowering and plant height, followed by plant height and pod

Table 4: Estimation of variance components, phenotypic and genotypic coefficient of variation heritability and genetic advance

Traits	Variance Components			Phenotypic and Genotypic Coefficient of Variation		Heritability and Genetic Advance	
	σ_P^2	σ_G^2	σ_E^2	PCV	GCV	H^2	GA
PtHe	637.5	135.5	502	9.52	4.38	21.25	19.61
PBr	40.65	5.95	34.7	18.86	7.21	14.63	38.88
SBr	679.5	257.9	421.6	28.91	17.81	37.95	59.57
DF	266.2	183.85	82.35	8.87	7.37	69.06	18.27
PoL	0.27	0.06	0.21	9.91	4.67	22.22	20.42
PoW	0.51	0.215	0.295	7.87	5.11	42.15	16.21
Se/Po	0.11	0.025	0.085	8.01	3.81	22.72	16.50
PoBL	28.28	2.73	25.55	10.34	3.21	9.65	21.30
100SW	0.86	0.3	0.56	9.64	5.69	34.88	19.87

Table 5: Pearson's correlation coefficient among 12 quantitative traits of 88 pigeon pea accessions

	PtHe	PBr	SBr	DF	PoL	PoW	Se/Po	PoBL	100SW	DM	NPo	Yield/Pt
PtHe	1											
PBr	0.581^{***}	1										
SBr	0.619^{***}	0.643^{***}	1									
DF	0.803^{***}	0.496^{***}	0.585^{***}	1								
PoL	-0.017^{NS}	0.045^{NS}	0.013^{NS}	-0.057^{NS}	1							
PoW	0.178^{NS}	0.042^{NS}	0.065^{NS}	0.115^{NS}	0.012^{NS}	1						
Se/Po	0.053^{NS}	-0.077^{NS}	0.043^{NS}	-0.046^{NS}	0.397^{***}	0.1^{NS}	1					
PoBL	0.797^{***}	0.508^{***}	0.636^{***}	0.731^{***}	0.035^{NS}	0.187^{NS}	0.104^{NS}	1				
100SW	0.452^{***}	0.35^{**}	0.302^{**}	0.328^{**}	0.065^{NS}	0.151^{NS}	0.025^{NS}	0.414^{***}	1			
DM	-0.146^{NS}	-0.026^{NS}	-0.126^{NS}	0.063^{NS}	-0.106^{NS}	-0.023^{NS}	-0.103^{NS}	-0.099^{NS}	-0.247^{*}	1		
NPo	0.191^{NS}	0.313^{**}	0.369^{**}	0.215^{*}	-0.198^{NS}	-0.126^{NS}	-0.174^{NS}	0.188^{NS}	0.043^{NS}	-0.032^{NS}	1	
Yield/Pt	0.281^{*}	0.198^{NS}	0.318^{**}	0.201^{NS}	-0.073^{NS}	-0.160^{NS}	-0.027^{NS}	0.144^{NS}	0.094^{NS}	-0.078^{NS}	0.656^{***}	1

^{NS} – not significant * $p < 0.05$; ** $p < 0.01$; *** $P < 0.001$

bearing length (r = 0.797) and days to 50% flowering and pod bearing length (r = 0.731). As expected, the number of primary branches was positively associated to the number of secondary branches (r = 0.643) and these two characters showed a positive and highly significant correlation with plant height on one hand and with the pod bearing length on the other hand. Pearson's correlation coefficient between plant height and number of primary and secondary branches were respectively 0.581 and 0.619 while between pod bearing length and number of primary and secondary branches, Pearson's correlations coefficient was respectively 0.508 and 0.636. Positive and highly significant correlation (r = 0.656) was also detected between seed yield per plant and number of pods. Negative associations were also observed between some pairs of characters. In total twenty negative correlations were detected. They were not significant ($p > 0.05$) except the association between days to 75% maturity and 100 seed weight which was significant at 5%.

3.2.4 Cluster analysis

Hierarchical clustering on principal components (HCPC) was used to describe the existing variability in the collection and to study the resemblances and differences between individuals based on the 12 quantitative traits. HCPC is a multivariate method which combines principal component analysis and clustering methods for defining stable morphologic groups. Therefore a principal compo-

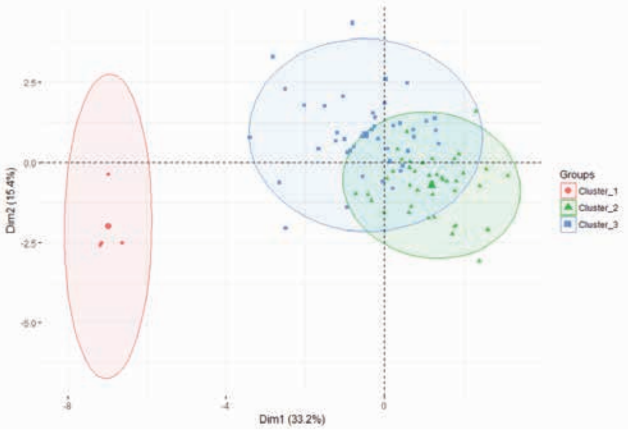

Figure 1: Scatter plot of HCPC for first two principal components showing the grouping of accessions into three clusters

Table 6: Principal components' (PCs) characteristics and correlations between PCs and quantitative traits.

	Dim.1	**Dim.2**	**Dim.3**	**Dim.4**
Eigen-value	3.98	1.84	1.37	1.04
Variance (%)	33.16	15.4	11.41	8.71
Cumul of variance (%)	33.16	48.57	59.99	68.7
Correlation between variables and PCs				
PtHe	**0.892**	0.155	-0.119	0.023
PBr	**0.748**	-0.087	-0.010	0.107
SBr	**0.817**	-0.084	0.084	0.117
DF	**0.823**	0.094	-0.260	0.136
PoL	-0.004	**0.491**	0.593	0.318
PoW	0.168	**0.416**	-0.183	-0.309
Se/Po	0.02	0.496	0.584	0.308
PoBL	**0.85**	0.201	-0.085	0.073
100SW	**0.547**	0.201	0.113	-0.453
DM	-0.127	-0.111	-0.501	0.704
NPo	0.325	-0.769	0.274	0.004
Yield/Pt	0.306	**-0.673**	0.453	0.03

nents analysis (PCA) was performed based on the 12 quantitative traits and the results (Table 6) showed that the first four principal components (PCs) had an eigenvalue above 1. The percentage of variation explained by these four principal components was 68.7 of the total variability. The PC1 was the most important and accounted for 33.16% followed by the second PC which accounted for 15.4%. The associations between morphological characters and the principal components (Table 6) showed that the PC1 was positively correlated with the plant height, number of primary and secondary branches, days to 50% flowering, pod bearing length and 100 seed weight indicating that this PC was determined by the vegetative parameters. However, PC2 was negatively correlated with seed yield per plant and number of pod but moderate association was observed between PC2 and the characters pod length, pod width and number of seeds per pod. PC2 was not defined by the grain yield but it contrasted pod dimensions with seed yield per plant and its component number of pods.

The clustering carried out on the principal components grouped the 88 pigeon pea accessions into three major clusters (Figure 1). The projection of individuals into the axis system formed by the two first PCs showed a clear separation between the four advanced varieties from the landraces. The four advanced varieties formed cluster_1 which was negatively correlated with the two first PCs. The 84 pigeon pea landraces sampled in Benin were almost grouped together, separated by the first PC into two clusters (cluster_2 and cluster_3) as shown on the individual factor map (Figure 1). The majority of individuals in cluster_2 were positively associated with PC1 indicating that this cluster was composed of individuals with a high value for the vegetative parameters while cluster_3 was positively correlated with PC2 suggesting that this cluster was composed of individuals with a low grain yield. The characteristics of each cluster were detailed in table 7.

The clustering pattern indicated the existence of a significant amount of variability among the pigeon pea collection. The comparative analysis of phenotypic mean values showed significant variation among clusters for all quantitative variables except pod width. Cluster_1 consisting only of the four advanced varieties was characterized by a very short cycle, the shortest plants and much less branching. The second group (cluster_2) consisted of 41 accessions characterized by the tallest plants with many branches. The individuals of this cluster had a large number of pods and therefore a high grain yield potential. Late maturity cycle, a moderate number of pods, and moderate seed yield per plant were the main characteristics of cluster_3. This cluster was also characterized by the individuals with intermediate days to flowering.

4 Discussion

Determination of the genetic diversity of any crop species is a suitable precursor for crop improvement. In the case of pigeon pea, several studies were conducted previously for estimating its genetic diversity. Various techniques such as morphological descriptors (Upadhyaya et al. 2007;

Table 7: Mean performance of quantitative traits within the different clusters of pigeon pea

Variables	**Cluster 1 (N = 4)**	**Cluster 2 (N =41)**	**Cluster 3 (N= 43)**	**F**
PtHe	117.77 ±17.20	276.73 ± 19.32	254.21 ± 19.89	122.7***
PBr	19.40 ± 1.77	35.16 ±6.25	32.31 ± 5.87	13.38***
SBr	24.29 ± 4.80	99.86 ± 21.72	80.88 ± 20.39	28.16***
DF	73.19 ± 4.05	188.46 ± 11.48	179.53 ± 15.96	127.08***
PoL	5.38 ± 0.49	5.42 ± 0.32	5.69 ± 0.44	5.38**
PoW	8.40 ± 0.54	9.12 ± 0.46	9.02 ± 0.73	2.53^{NS}
Se/Po	3.77 ± 0.41	4.04 ± 0.33	4.23 ± 0.30	6.26**
PoBL	88.50 ± 8.46	207.41 ± 20.62	193.84 ± 20.87	61.89***
100SW	8.14 ± 0.25	9.90 ± 0.73	9.33 ± 0.98	10.30***
DM	-	272.27 ± 16.47	281.63 ± 19.04	5.78*
NPo	-	468..4 ± 111.1	295.7 ± 99.2	56.55***
Yield/Pt	-	100.07 ± 20.77	67.79 ± 19.63	53.62***

^{NS} – not significant * p < 0.05; ** p < 0.01; *** P < 0.001

Manyassa et al. 2008) biochemical characterization (Joshi et al. 2009) and molecular marker analysis (Saxena et al. 2010; Njung'e et al. 2016; Bohra et al. 2017) were used in the pigeon pea diversity assessment. However, because morphological characters are influenced by the environment, genetic diversity estimation in pigeon pea based on phenotypic traits is lesser done. Although morphological characterization is susceptible to environmental effects, it is the first step in the description and classification of the germplasm. The present study is the first report on genetic diversity study in a large scale collection of pigeon pea landraces from Benin using agro-morphological characters.

The study revealed a wide ranging variation in qualitative traits as well as in quantitative variables. Among the 10 qualitative traits measured in this study, seed colour pattern was highly diverse followed by the plant pigmentation as revealed by the Shannon-Weaver diversity index. This result is in congruence with the finding of Upadhyaya et al. (2007) who, also based on the same diversity index, found the highest diversity in primary seed colour. But in contrast with our results, Manyassa et al. (2009) in a similar study reported the highest diversity for qualitative traits in flowering pattern and seed eye colour; but they observed nonetheless a moderate diversity in seed colour. In general, the heterogeneity observed for qualitative traits in pigeon pea germplasm depends often on its natural out-crossing rate (Upadhyaya et al. 2007) which ranges from 3% to 26% and varies according to locations, genotype, and the intensity of the insect population and time of flowering (Saxena et al. 1990; Reddy et al. 2004). In addition, different agricultural practices and seed management modes, such as seed exchange and poly-varietal cultivation adopted by Beninese pigeon pea producers (Zavinon et al. 2018), are other main factors which increase heterogeneity in pigeon pea germplasm for qualitative traits. The predominance of cream and light grey seed types in the collection supports the farmers' preference of landraces with these seed colours reported by Ayenan et al. (2017a) and the low frequency observed in black, red or brown seed colour agree the finding of Zavinon et al. (2018) that cultivation of local landraces with black, red or brown seed colour are being abandoned. The germplasm of Beninese pigeon pea landraces was dominated by highly vigorous plant with big stem. The world pigeon pea collection analyzed by Upadhyaya et al. (2007) was also dominated by highly vigorous accessions with thick stems. Although the majority of the accessions were compact and erect, the proportion (44.32%) of spreading pigeon pea types in the collection was not less significant. In contrast with the predominance of semi-spreading pigeon pea in the Tanzanian collection (Manyassa et al. 2008), pigeon pea accessions of spreading growth habit were not suitable for the intercropping system in which pigeon pea is often produced in association with cereals and other legumes. Indeed, these highly branching pigeon pea types do not enable the development of other crops cultivated in association. Mean performance observed in a number of primary branches and secondary branches is a reflection of the highly branching pigeon pea types of the collection. Indeed, the performance recorded in a number of primary and secondary characters was higher than that reported earlier in the Ugandan collection (Manyassa et al. 2009), Tanzanian collection (Manyassa et al. 2008) as well in the world collection (Upadhyaya et al. 2007). The high value observed in a number of primary and secondary branches could be explained by the large inter-row and intra-row spacing (low population density) adopted in our study and is in congruence with the findings of Mula et al. (2011); under low population density pigeon pea was known to produce greater biomass (branches). The large phenotypic plasticity observed in a number of primary and secondary branches indicate that these two characters would be highly influenced by the environment and agree with the highest values of phenotypic coefficient of variation observed here in these two characters.

Whatever the experimental site, ANOVA revealed significant variation among the accessions for quantitative characters except number of seed per pod, days to 75% maturity and seed yield per plant. High phenotypic divergence in pigeon pea collections was also reported in previous studies (Manyassa et al. 2008; Upadhyaya et al. 2007; Vange and Egbe 2009). This result indicates that Beninese pigeon pea germplasm presented a valuable source of genetic diversity that would be useful in a pigeon pea breeding program. However, the non-significance in number of seeds per pod was identical with the finding of Mayassa et al. (2008) in the Tanzanian collection evaluated at Illonga site, indicating that the accessions analyzed here produced almost the same number of seeds per pod. According to Upadhyaya et al. (2007), variations in days to 75% maturity were not significant in the late pigeon pea maturity group. This result is in congruence with that observed here, as the collection analyzed was dominated by long duration landraces. On the other hand, it can also be explained by the effects of Harmattan. Indeed, based on farmers' knowledge, this wind blowing in West Africa would favour fruiting and maturation of pigeon pea accessions almost in the same period.

The estimates of phenotypic coefficients of variation (*PCV*) were higher than those of genotypic coefficients of variation (*GCV*) for all characters, suggesting that the greatest variability observed in quantitative traits was in part due to environmental factors. This tendency was general in phenotypic diversity studies and has been well reported in pigeon pea (Vange and Egbe 2009; Sreelakshmi and Shivani 2015; Kumara et al. 2014) and other crops like maize (Ogunniyan and Olakojo 2015). While *PCV* was higher than *GCV* for all characters, the magnitude between these two genetic parameters was low for the character days to 50% flowering and moderate for pod width, number of secondary branches and 100 seed weight. These characters were lesser influenced by environmental effects and this explains therefore the high value of heritability observed in days to 50% flowering (69.06) and even the moderate values of heritability in pod width (42.15), number of secondary branches (37.95) and 100 seed weight (34.88). According to Manyassa et al. (2008) the characters with high heritability values could be used for pigeon pea germplasm classification. Therefore, the characters days to flowering, pod width, number of secondary branches and 100 seed weight could be useful in germplasm characterization and selection for pigeon pea breeding in Benin.

Knowledge of correlations among different traits is essential to design an effective breeding strategy for any crop (Mazid et al. 2013). Among the 88 pigeon pea accessions studied and based on 12 quantitative traits, two major groups of correlations were observed. Firstly, highly significant and positive correlations were observed between the vegetative characters such as plant height, pod bearing length, number of primary and secondary branches and days to 50% flowering. The second major group of correlations was established between seed yield and its component number of pods per plant. The positive association between the vegetative parameters was also reported by Pandey et al (2016) in their recent genetic association studies for yield and yield related traits in pigeon pea. Upadhyaya et al. (2007) detected significant positive correlation between number of pods and seed yield within the world pigeon pea collection. Since seed yield is a complex trait difficult to handle in breeding, its positive correlation observed in the present study with the number of pods is an interesting asset as it can be indirectly improved by focussing on the latter trait which is easier to assess. Apart from these two major groups of observed positive correlations among the characters, a significantly negative correlation was also observed between days to 75% maturity and 100 seed weight. This result indicates that long duration landraces had low seed weight while high seeds mass corresponds to landraces with short maturity duration. A simple explanation of this result would be that, long cycle duration favours vegetative biomass development to the detriment of reproductive traits and therefore reduction of agronomic performance.

A multivariate method combining hierarchical clustering and principal component analysis was used here to describe the relationships between the accessions. The same multivariate tools were also used by Manyassa et al. (2008) and Upadhyaya et al. (2007) to find out the relative importance of different traits in the collections they have analyzed. As revealed by the principal component analysis, the first four principal components had an eigenvalue above 1 and accounted for 68.7% of the total variation. A similar result was also reported by Manyassa et al. (2008). The first two principal components PC1 and PC2 which accounted for most of the variability were highly correlated with the vegetative and agronomic traits, respectively. Thus, among the 12 quantitative traits(plant height, number of primary and secondary branches, days to flowering, pod bearing length, 100 seed weight, number of pods and seed yield per plant), eight were strongly associated with these two first PCs and revealed their importance in explaining the variation. The same characters were also highlighted by Upadhyaya et al. (2007) and Manyassa et al. (2008) as important characters for characterization of pigeon pea germplasm accessions. Cluster analysis based on principal components subdivided the 88 pigeon pea accessions into three major clusters (Figure 1). This result indicates the existence of wide genetic variability in the collection. As expected, the four improved varieties formed an independent cluster clearly separated from those formed by the landraces. This clear delineation between improved varieties and landraces supports their differentiation and confirms the traditional status of the local varieties used in the present study. It is therefore crucial to define an efficient strategy for conservation of such landraces which could be a reservoir of interesting genes for pigeon pea breeding. However, the two groups formed by the landraces seem closer suggesting a high level of gene flow among them due to the decade of sympatric cultivation or other agricultural practices adopted by the local pigeon pea producers in Benin (Zavinon et al. 2018).

5 Conclusion

The present study on pigeon pea aimed to contribute to valorization and conservation of this important but neglected genetic resource in Benin. The results showed a wide morphological variability in the collection. Based on qualitative traits, seed colour was the most diversified trait followed by plant pigmentation. The collection was dominated by the accessions with white seed while the frequency of accessions with coloured seed was very low. A great variability was mainly observed in quantitative traits such as plant height, primary and secondary branches, days to flowering, pod bearing length, 100 seed weight, number of pod and seed yield per plant that are found to be suitable in pigeon pea germplasm characterization. The quantification of genetic and environmental components of phenotypic variability indicated that days to 50% flowering, pod width, number of secondary branches and 100 seed weight were less influenced by the environment conditions. The existing variability in the collection enabled the classification of the accessions into three different clusters. The group of improved varieties characterized by early accessions was clearly separated from the two other clusters that composed of the landraces characterized by their late maturity cycle but differed to each other by their seed yield potential. Phenotypic diversity observed in this study can be used to drive valuable information about genomic structure and genetic control of useful traits in developing mapping populations for QTL analyses. Therefore, it is important to develop efficient strategies for the conservation of genetic resources of pigeon pea, a neglected and underutilized crop in Benin. The study will help in the selection of the most efficient landraces which can be used in the development of new varieties by the research program.

Acknowledgements: This study was financed by Ministry of Higher education and Scientific Research (Benin Republic). The authors sincerely acknowledge Fabienne Adigoun and Biopaix Ahoyo of the Laboratory of Genetic Resources and Molecular Breeding (LaREGAME) and all others who helped during trials installation and data collection.

Conflict of interest: The authors declare that they have no conflict of interest.

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