



## RESEARCH ARTICLE

# Exploration of quantitative and qualitative traits of pigeonpea [*Cajanus cajan* (L.) Milsp.] to understand the genetic diversity

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## Abstract

Pigeonpea [*Cajanus cajan* (L.) Milsp.] is an important leguminous crop with high protein content and nutritional value in tropical and subtropical regions. To assess the genetic diversity and correlation among qualitative and quantitative phenotypic traits in 73 pigeon pea Minicore (MC) collection of pigeon pea experiments was laid out under field and rain shelter conditions for two years. Principal component analysis contributed 85.9% of the overall variation in quantitative traits and four components contributed 76.6% in qualitative traits to total variation. Eigenvalues indicated that a pod length, seed weight and specific gravity contributed to total diversity in quantitative traits. Among the quantitative traits, the highest coefficient (CV%) was found in specific gravity (81.4%), followed by a number of primary branches (65.3%), while pod length, pod width, plant height, branch angle, seed weight and stem width showed more than 20% coefficient of variability. In cluster analysis, qualitative traits grouped the genotypes into three clusters and quantitative traits in four clusters, signifying quantitative traits offer better clustering of genotypes. The overall Shannon weaver diversity index ranged from 2.47 (seed coat color) to 0.243 (seed size). The present study indicated that MC93, MC116, MC2 displayed a higher number of seeds/pod, better pod length and pod width, whereas MC109, MC90, MC117 and MC120 showed higher seed weight. These traits seem to be an important contributor to seed yield and must be considered in the selection for high seed yield. The findings may facilitate genebank curators to understand the Mini Core (MC) collection within and among the cores, develop appropriate germplasm conservation policies, and help in crop improvement.

**Keywords:** Genetic diversity, Minicore collection, Pearson correlation, Quantitative and qualitative traits

## Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important multipurpose legume crop grown in semiarid regions of Asia, Africa, Latin America, and the Caribbean (Kumar et al. 2021). *C. cajan* is the only domesticated species among Cajanea family. The crop has the ability to produce an economic yield under low moisture conditions, making it an important crop in dry areas. As food, it is a rich source of proteins (20-22%), and as a component of production systems, it contributes to the improvement of soil fertility through biological nitrogen fixation (Hemavathy et al. 2019; Bopape et al. 2021). This legume is considered to have originated in India and it is cultivated in many parts of the world, including Southern Africa, particularly the region across Kenya, Malawi, Mozambique, and Tanzania (Kaoneka et al. 2016). The major constraint of pigeon pea production in the country is the lack of high yielding varieties. Farmer's cultivars are mostly landraces with a long maturity cycle and a very low grain yield (Ayenan et al. 2017). The study on genetic diversity in pigeonpeas is required for effective breeding and germplasm conservation. Previous research looked at the crop's genetic diversity through morphological

and agronomic traits (Njung'e et al. 2016). For an effective evaluation, use of the genetic materials and for a breeding program, detailed knowledge such as agro-morphological, biochemical and molecular markers is required (Nyirenda et al. 2020). It was reported that the genetic diversity of existing

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pigeonpea cultivars and variations is relatively low (Rupika et al. 2014). The loss of genetic diversity is due to continuous artificial selection and breeding for a few targeted economic traits to meet the market requirements. As a result, it is necessary to start pre-breeding programs in the intended production locations using divergent breeding with current and extinct cultivars, landraces, and wild relatives that have desirable features. Through gene recombination and efficient selection, there will be an improvement in the genetic diversity of pigeonpeas (Nyirenda et al. 2020; Zavinon et al. 2020). Global organizations like ICRISAT, the International Institute of Tropical Agriculture (IITA), and the Svalbard Global Seed Vault in Norway all maintain extensive collections of pigeonpeas. The ICRISAT gene bank has 13,632 pigeonpea accessions, including 555 wild relatives from 74 countries. At ICRISAT, a pigeonpea core collection of 1,290 farmed pigeonpea accessions from 53 countries was developed. In crop production, a recent tendency is to investigate differences from entire gene bank collections, donor selection, and pre-breeding of selected donors in order to facilitate breeding programs/workshops (Dhakal et al. 2020; Roy et al. 2020). To determine the effect of different genes on phenotypes, available Mini Core (MC) collection of pigeon peas must be characterized and evaluated for various agro-morphological and biochemical traits. Different highyielding MC collections of pigeonpeas were developed and tested (Arumugam et al. 2018), identified varied markers and validated the developed highyielding lines (Singh et al. 2020). Genes and gene networks were also decoded (Chaudhary et al. 2018; Yvoz et al. 2020). Despite its nutritional and economic significance, a lack of comprehensive knowledge about its genomic diversity and resources prohibits it from being wisely utilized through molecular breeding programs and biotechnological intervention (Singh et al. 2024).

Multidimensional techniques and methodologies have played an important role in crop improvement, allowing us to make use of this subset of core and to understand their mode of activity in order to increase nutritional aspects, cut hunger and overcome malnutrition challenges (Shannon and Weaver 1949). To address crop improvement concerns, efforts were made to extend the genetic basis by collecting and preserving MC collection of pigeonpea throughout the world, resulting in significant collections at national and international gene banks. In the present study, the focus was made on 73 MC pigeon pea genotypes to determine the presence of genotypic diversity using 13 quantitative and 10 qualitative traits and to identify divergent and superior MC pigeonpea germplasms using statistical analytic tools.

## Materials and methods

### *Plant material, trial design and management*

The study comprises 73 pigeonpea Mini Core (MC) collections collected from National Institute of Plant Biotechnology

(NIPB), ICAR, New Delhi. The material was planted in two replicas 2019-20 and 2020-21, in the Phenomics Rainout shelter at ICAR, New Delhi and harvested from April to May, depending on the maturity of the plant. The trial was laid out and organized in a lane design, replicated five times in a single row consisting of 73 rows of 5 m length in each row. The spacing between the rows was 100 cm. The insect pests that were prevalent (pod borer and aphids) were controlled by insecticides used on legumes. Plants were irrigated twice in a week. Weeding was done manually using hand hoes.

### *Data collection*

Data were recorded according to the standard descriptor list of pigeonpea (Sameer et al. 2017; IBPGR 1993). A total of 23 traits, including 10 qualitative and 13 quantitative, were recorded (Supplementary Table S1). The data on qualitative traits recorded were pod color, leaf shape, stem color, foliage, clustering, spreading, susceptibility to SMV, seed coat color, seed size and seed shape, whereas the quantitative traits were seeds per pod, pod length (cm), pod width (cm), plant height (cm), no. of 1° branch, branch angle (°), leaf length (cm), leaf width (cm), stem width (cm), specific gravity, 100 seed weight (g), seed length (cm), seed width (cm). The water displacement technique was used to calculate specific gravity while the seeds were soaking for the night before germination. At the last harvest, the height of the plant was determined by measuring the length of the highest vein. The acquired information was arranged in a matrix for further usage with the Microsoft Excel 2019 programme.

### *Statistical analysis*

The recorded data of quantitative and qualitative traits were analyzed using ANOVA, principal component analysis (PCA) and Pearson's correlations. A one-sample variance test was used to determine if the variation was significant or not. Using the similarity matrix, Principal Component Analysis (PCA) was conducted to identify groups and determine the axes and attributes that contributed significantly to the variance. The collected data was organized into a matrix in Microsoft Excel 2019 programme. For qualitative traits, identity numbers were given to each character to calculate frequencies, Shannon Weaver diversity index (Supplementary Tables S1 and S2). Different descriptive statistics such as range, mean, and standard deviation of various quantitative characters and frequency percentage of qualitative characters were calculated using XLSTAT (Addinsoft, www.xlstat.com). The Shannon and Weaver diversity index (H) was used to calculate phenotypic diversity for each characteristic. Agglomerative Hierarchical Clustering (AHC) uses Ward's minimal variance technique as a clustering algorithm and squared Euclidean distances as a measure of dissimilarity. Clustering graph plotted using the 'ggplot2' package in R software. The first two principal components, which accounted for the largest variance, were

used to generate two-dimensional scatter plots. The biplot were constructed using Minitab and R-studio using 'dplyr' and 'tidyverse' packages.

## Results and discussion

Phenotypic traits have been used for a while to investigate interactions between plant genotypes and calculate their genetic diversity (Mofokenget al. 2022; Rai et al. 2010). Descriptor list has been found to be useful for the collection of phenotypic data that has been used in various crops such as rice (*Oryza sativa*) (Chaudhary et al. 2021; Dhakal et al. 2020); mungbean (*Vigna radiata*), black gram (*Vigna mungo*) (Ghafoor et al. 2001) and sugarcane (*Saccharum officinarum*) (Njung'e et al. 2016; Bohra et al. 2017). Diversity analysis is important step to find out how the available genetic resources are interlinked genetically. The quantitative phenotypic data were pooled after applying tests for variance and normality. The results on various quantitative and qualitative traits are presented here below:

### Quantitative traits

The evaluated 73 pigeonpea genotypes of MC collection varied significantly for the 13 quantitative traits (Table 1). The descriptive statistics and ANOVA analysis show that there was a significant variance (at  $p < 0.05$ ) in all of the traits except plant height, leaf length, number of primary branches and seed width.

An average pod length recorded was 4.84 cm, ranging from 0.5 to 9 cm, with 25.7% CV. The average pod width was 0.6 cm and the range found in between 0.2 to 2.2 cm. The highest pod length was found in MC116 and MC83, while the lowest occurred in MC130 and MC99. Similarly, the highest pod width found in MC82 and MC116 while the lowest was found in MC130 and MC99, respectively. The diversified pod yield in the MC collection may be caused by all of these

traits. This indicates the genotypic heterogeneity that can be benefited to enhance cultivars in upcoming breeding programs across the MC collection that was investigated based on the traits.

Leaf length ranged from 4.5 to 11 cm with an average mean value of 7.77 and 19.3% of CV. The highest leaf length was found in MC65, MC50 and MC89 and the lowest in MC84 and MC3. The mean value of leaf width was 3.21, ranging from 2 to 4.5 cm, with 18.1% CV. MC82, MC65 and MC19 had the longest leaf width, while the shortest was recorded in MC3, MC121 and MC130. Leaf length and width exhibited non-significant differences among assessed genotypes. However, there were marked genotype differences in plant height that had a high standard deviation (52.81) as it ranged from 70 cm (in MC120) to 260 cm (in MC18) with an average mean value of 192.4 cm. MC120, MC109, MC111 and MC118 displayed short stature, while MC82, MC77, MC66 and MC82 were comparatively very tall exceeding even 200cm.

Table 1 summarizes the coefficient of variance of specific gravity (81.4%), no. of primary branches (65.26%) and pod width (49.62%) having high coefficient of variability. Any breeding goal must include knowledge of genetic variance for a trait as well as their correlations. This implies that the conditions in which the materials were evaluated affected the traits significantly, as has been reported earlier (Kimaro 2016). Minicore collections were pooled from different regions of the world, including India, Myanmar, West Africa, Bangladesh and Tanzania. These MC collections showed huge diversity among each other and, hence, revealed great variation in their phenotypic traits. Higher values represent a greater degree of relative variability.

For the number of seeds per pod, the mean value of 3.95 was recorded and the range varied from 3 to 6, with 14.2% CV. The highest number of seeds per pod was recorded in MC83 and the lowest was found in MC18. The mean weight

**Table 1. The descriptive statistics and analysis of 13 quantitative phenotypic traits of MC collection**

Variables	Mean	Standard deviation	CV%	Range	p-value
Seed per pod (SPP)	3.959	0.564	14.2%	3	<0.0001
Pod length (PL) (cm)	4.845	1.246	25.7%	8.5	0.0206
Pod width (PW) (cm)	0.603	0.299	49.6%	2.1	<0.0001
Plant height (PH) (cm)	192.493	52.819	27.4%	190	0.2294
No. of primary branches (NBR)	1.986	1.296	65.3%	4	0.636
Branch angle (BA) (°)	47.589	10.581	22.2%	65	0.0401
Leaf length (LL) (cm)	7.773	1.501	19.3%	6.5	0.298
Leaf width (LW) (cm)	3.212	0.581	18.1%	2.5	<0.0001
Stem width (STW) (cm)	2.178	0.831	38.1%	3.7	<0.0001
Specific gravity (SG)	0.656	0.534	81.4%	3.55	0.0148
Seed weight (SWT) (g)	8.926	2.601	29.1%	11.48	0.0434
Seed length (SL) (cm)	0.560	0.082	14.7%	0.4	<0.0001
Seed width (SW) (cm)	0.394	0.076	19.3%	0.35	0.442

of 10 seeds was 8.92 g, ranging from 4.82 to 16.30 g, with CV 29.1%. MC81, MC83 showed lowest and MC109, MC90 and MC89 produced the highest seed weight (>0.6gm/10 seed). The average seed length and seed width were 0.56 cm and 0.39 cm. The seed length varied from 0.4 to 0.8 cm with 14.7% of CV, while seed width ranged between 0.25 and 0.6 cm with 19.3% CV. The highest seed length was found in MC109, MC118 and MC114, while the lowest in MC30 and MC9. The highest seed width was measured in MC77 and MC109 and the lowest were in MC99 and MC87. The evaluated germplasm could illustrate important genetic variation that underpins the morphological variations. There was a substantial difference in quantitative traits among 73 pigeonpea MC accessions like growth habit, no. of seed per pod and seed weight, which are critical for developing cultivars with desired expectations. For instance, diversity in growth habits is significant for identifying genotypes with compact growth habits that may be suitable for intercropping in moisture-limited conditions to maximize space utilization and productivity (Sarkar et al. 2023; Jaggal et al. 2012).

### Qualitative traits

The qualitative traits and the relative percent frequency of evaluated pigeonpea MC collection is listed in the Supplementary Table S2. Green-brown and Green constitute the major type of pod color with 89% and 8.2% variation, while the remaining genotypes had brown and red coloured pod. About 76.7% had most common elliptic leaf shape and the remainder had linear leaf shape. As per the seed shape of pigeonpea MC collection, semi-spheroid and spheroid

constitute 47% and 40% of total, while semi-deltoid, fusiform and ovoid shape constitutes 8%, 3% and 3%, respectively. Major variation has been observed in seed color where copper (28.8%), russet (11%), white (11%), cream-copper (8.2%) and tawny (6.8%) are the major types recorded among the studied pigeon pea genotypes. Based on the Shannon-Weaver diversity index, the 10 qualitative traits presented a relatively large variation. The diversity index ranged from 0.243 (seed size) to 2.47 (seed coat color). Seed color was the most diversified trait, followed by spreading (1.38), seed shape (1.12) and susceptibility to SMV disease (1.124). Breeding cultivars that match farmers' expectations require a variety that shows a better growth pattern with suitable seed coat color. Pollinators need colorful blooms and pigment in plants in order to encourage cross-pollination. Additionally, a wide range of defense-related molecules known as pigmentation chemicals protect foliage against UV radiation, diseases, and insects (Sharma et al. 2019).

### Correlation coefficient

Pearson's correlation coefficient analysis revealed significant ( $p = 0.05$ ) positive or negative connections between numerous factors in the pigeonpea germplasm (Tables 2 and 3). The 13 quantitative phenotypic traits of pigeon pea MC collection showed significant genetic differences ( $p = 0.05$ , one-sample variance z-test).

In the present study, the no. of seeds per pod and pod length ( $r = 0.61$ ) is highly significant and shows a positive correlation with pod length and width (Table 3). Similarly, leaf length, leaf width, stem width show a positive correlation with plant height and weak negative correlation

**Table 2. Pearson correlations of the quantitative traits measured on MC collection of pigeonpea**

Traits	SPP	PL (cm)	PW (cm)	PH (cm)	NBR	BA (°)	LL (cm)	LW (cm)	STW (cm)	SG	100 SW(g)	SL (cm)	SW (cm)
SPP	1												
PL (cm)	0.61	1											
PW (cm)	0.44	0.53	1										
PH (cm)	-0.03	-0.04	-0.04	1									
NBR	0.06	0.29	0.13	-0.11	1								
BA (°)	-0.09	-0.13	-0.05	0.15	0.14	1							
LL (cm)	0.12	-0.06	-0.27	0.39	-0.3	-0.2	1						
LW (cm)	0.01	-0.01	-0.26	0.45	-0.2	-0.2	0.77	1					
STW (cm)	0.05	0.15	0.22	0.58	0.09	-0.2	0.19	0.2	1				
SG	0.19	0.17	0.03	0.03	-0.1	-0.1	0.07	0.11	0.13	1			
100 SW(g)	0.2	0.04	-0.09	-0.39	-0.1	-0.3	0.15	0.11	-0.26	0.22	1		
SL (cm)	-0.14	-0.12	-0.02	-0.51	0.02	-0.1	-0.15	-0.13	-0.35	0.02	0.53	1	
SW (cm)	0.09	-0.1	-0.03	0.08	-0.1	-0.1	0.19	0.13	0.12	0.07	0.36	0.04	1

\*SPP = Seed per pod, PL = Pod length, Pw = Pod width, PH = Plant height, NBR = No. of 1° branches, BA = Branch angle, LL = Leaf length, LW = Leaf width, SW = Stem width, SG = Specific gravity, 100 SW = Seed weight, SL = Seed length and SW = Seed width.

with seed weight and seed length (Between 0 and  $-0.3$ ). The positive correlations of most traits indicate that multiple trait selection is viable, but the poor correlations among the traits indicate an inefficient selection or minimal genetic gain (Nyirenda et al. 2020; Sumbele et al. 2021). The limited connections between the qualities might however, lead to ineffective selection or modest genetic gains. In contrast, Hemavathy et al. (2017) found a negative correlation between seed weight and grain yield. A strong correlation ( $r = 0.858$ ) between grain yield and the number of pods per plant was reported (Hanumanthappa et al. 2020).

Branch angle had a negative correlation with leaf length, leaf width and seed related traits. In contrast, stem width represents a negative correlation with seed weight and seed width, respectively. The number of primary branches had a positive correlation with pod length and pod width (Table 3).

The majority of the qualitative traits, such as pod color, leaf shape, foliage and clustering, were found to be significantly associated with other traits. Pod color is negatively associated with seed coat color ( $r = -0.24$ ) with each other. The size and color of pods are important factors in determining the marketability of a crop. The activity and interplay of several parts culminate in the complicated property known as a crop's pod yield. In order to produce the elite MC subset with higher yield or other attributes, the correlation of pod yield or weight with other qualities must be explored for the breeding strategy (Khatun et al. 2022). However, pod color shows a negative association with foliage, spreading and clustering. A significant negative association was found between leaf shape and seed coat color ( $r = -0.24$ ), and leaf shape shows a positive correlation with pod color ( $r = 0.23$ ) (Table 3). Other quantitative traits showed either positive (Green colored) and negative but weak (Blue colored) to moderate (Orange colored in Table

3) association. The positive correlations of numerous traits in pigeon pea MC collection demonstrate the effectiveness of the traits for crop improvement and can be utilized to boost seed production (Rahaman et al. 2015). Overall, compared to qualitative traits, quantitative traits showed strong associations.

### Principal Component Analysis (PCA)

Principal component analysis was used to identify the distinct factors/components that had a significant impact on the overall phenotypic traits, which included 10 qualitative and 13 quantitative traits. Eigenvalues, variability contribution rate, and cumulative contribution rate as shown in Table 4. The Eigenvalue of seed weight (0.57), which significantly increased total diversity and also has a CV of over 20%, suggests that it is appropriate for use in the breeding of genotypes with high yields. Breeders may have recently promoted short-duration and determinate types (with large seeds) suitable for a variety of cropping systems, which may have resulted in high seed weight in the extra-early group (Sarkar et al. 2023). There is a mild negative association between seed weight and seed length and a positive correlation between leaf length, leaf width, stem width and plant height.

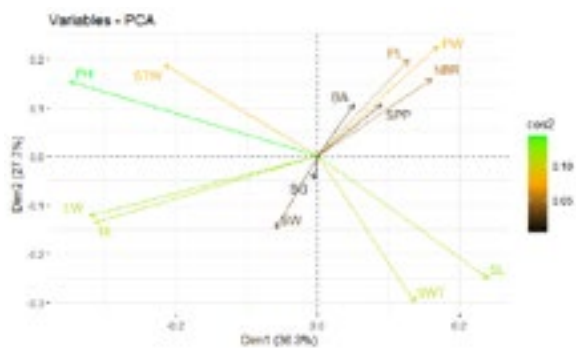
The PCA biplot of quantitative traits, P1 (Dim1) had 36.3% and P2 (Dim2) had 27.7% (Fig. 1). PCA analysis revealed that four of the thirteen principal components significantly contributed to nearly 85.9% of total quantitative variations. The first component (P1) possesses the highest variance (36.3%), followed by P2, which accounted for 27.7%, while P3 and P4, accounted for 16.5 and 5.5% of the total quantitative trait variation, respectively.

Plant height, leaf length, leaf width, and stem width are moderately loaded in PC1 component. This component was

**Table 3. Correlation matrix for 10 qualitative traits of MC collection. The different colour shows an association between variables**

Traits	SCC	SS	PC	SSH	LSH	SC	FO	CL	SP	SUS
SCC	1									
SS	-0.03	1								
PC	-0.24	-0.08	1							
SSH	0.10	0.04	0.06	1						
LSH	-0.15	0.06	0.23	0.04	1					
SC	0.05	-0.05	0.04	-0.08	-0.16	1				
FO	0.09	0.18	-0.15	0.00	-0.17	0.10	1			
CL	0.09	0.18	-0.15	0.00	-0.17	0.10	1	1		
SP	0.10	0.15	-0.17	0.08	0.11	0.16	0.31	0.31	1	
SUS	0.01	0.02	-0.01	-0.03	0.25	-0.20	0.07	0.07	-0.09	1

\*SCC = Seed coat color, SS = Seed size, PC = Pod color, SSH = Seed shape, LSH = Leaf shape, SC = Stem color, FO = Foliage, CL = Clustering, SP = Spreading and SUS = Susceptible to SMV.

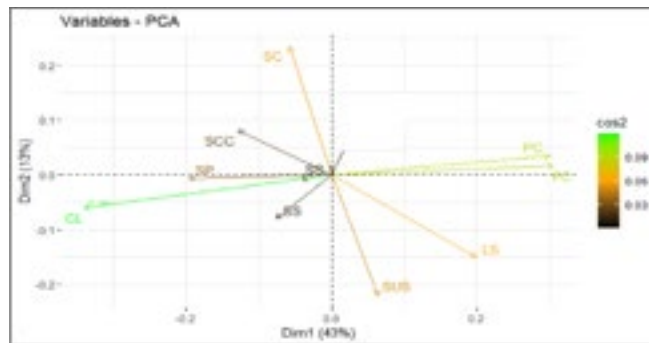


**Fig. 1.** Principal components (PCs) correlations between PCs and 13 quantitative traits

positively contributed with all traits except seed width and specific gravity, whose contribution was ignorable. PC2 is negatively contributed by seed per pod, pod length and pod width while moderately contributed by seed weight and seed length (Table 4). All variables were loaded positively in PC3 except plant height, number of primary branches, branch angle and stem width. On the other hand, seed width and stem width show a strong positive contribution in PC4. This implies that these traits are useful for selection. According to other studies, trait contribution to distinct PCs varies based on genetic diversity within the examined genotypes and the number of traits evaluated (Sarkar et al. 2023).

PCA identified that four principal components (PC1, PC2, PC3 and PC4) significantly contributed 76.61% of the total variation in 10 qualitative traits. The biplot of qualitative traits, the P1(Dim1) had 43% and P2(Dim2) had 13.02%. The second quadrant showed seed coat colour and seed shape. These traits show a positive correlation in 2<sup>nd</sup> quadrant. Similarly, stem color, pod color and specific gravity are positively correlated with one another in the third quadrant. PC1 was described as having the highest variation (43%) followed by PC2, PC3, PC4 and PC5, accounting for 13.02, 12.3, 8.2 and 7.3% of the total qualitative variations, respectively. These four components, were therefore, considered for further analysis of pigeonpea MC collection.

PCA and scree plot (Supplementary Table S3) analysis of qualitative traits showed that foliage, clustering and spreading of branches were strong. The stem color (sc), seed size (sc) and seed coat colour (scc) were moderately loaded in PC1, suggesting that parameters included in PC1 were mainly related to seed-related traits. PC2 showed moderate association with seed coat colour and seed shape (ss) and negative association with other traits. Leaf shape (ls) and susceptibility to SMV disease were observed to be positive, but stem colour was not. Pod colour was found to be negatively associated in PC3 (Table 5). The PC4 and PC5 positively loaded to foliage, clustering and susceptible to SMV and negatively associated with seed shape, spreading (SP), and seed coat colour.



**Fig. 2.** Principal components (PCs) correlations between PCs and 10 qualitative traits

### **Hierarchical clustering**

Cluster analysis is a prominent statistical approach for categorizing things into clusters with substantial similarity to other clusters. The clusters will be useful for future heterotic breeding since different sets of alleles can alter their traits and performance (Twumasi and Tetteh 2017). The agglomerative hierarchical clustering (AHC) study was carried out using the Ward approach. Figures 3 and 4 show the resulting dendrogram for quantitative and qualitative traits for the examined pigeonpea MC collection. To describe the existing variability in the collection and to study the resemblances between individuals' valuation of the phenotypic diversity using qualitative and quantitative traits outlined the genotypes into four distinct clusters based on the 13 quantitative traits (Fig. 3). The quantitative traits are classified as 30, 3, 27, and 13 germplasm in clusters I, II, III, and IV, respectively. Cluster I is the largest collection of quantitative traits, followed by Cluster III.

For all quantitative variables except seed length and width pod width, the comparative study of phenotypic mean values revealed significant variance among clusters. This finding suggests that the collection has a high level of genetic diversity. This distinct separation between the MC collection of pigeonpeas supports their distinction and proves to the collection's longstanding legacy. Therefore, it is essential to develop a successful plan for maintaining this MC collection, which may contain valuable genes for pigeon pea breeding. However, due to the decade of sympatric agriculture or other agricultural practices used by the local pigeon pea producers, the groups established by the collection of pigeon pea seem closer, indicating a high level of gene flow among them (Nietsche et al. 2015).

Clustering results in three clusters for qualitative qualities, cluster I, cluster II, and cluster III, with 40, 32, and 1 genotype in each. It is advised to hybridize between various clusters with the maximum genetic divergence as parental lines in order to create a genotype or variety with the desired traits. According to reports, there is significant variability and segregation of features with strong heterotic effects in rice crop populations also (Dhakal et al. 2020).



**Table 4.** Eigenvalues, the proportion of variability and the cumulative rate of the 13 quantitative traits of MC collection of pigeon pea contributed to the first six principal components (PCs)

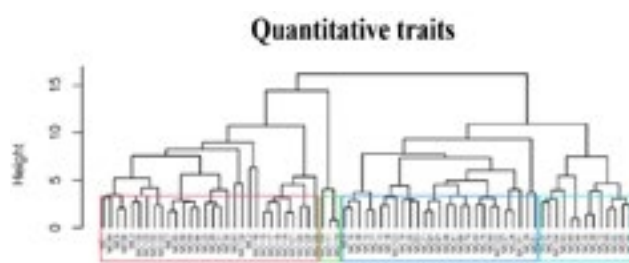
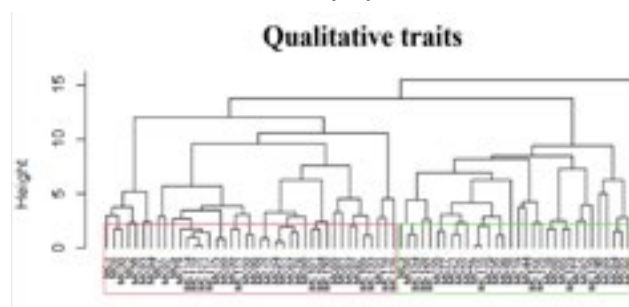
Component	PC1	PC2	PC3	PC4
Seed per pod (SPP)	0.386	0.356	0.716	0.278
Pod length (PL)	0.526	0.683	0.617	0.241
Pod width (PW)	0.690	0.775	0.317	-0.172
Plant height (PH)	-1.177	0.516	-0.355	-0.060
No. of primary branch (NBR)	0.671	0.528	-0.517	-0.009
Branch angle (BA)	0.294	0.334	-1.194	0.314
Leaf length (LL)	-1.061	-0.527	0.156	0.304
Leaf width (LW)	-1.085	-0.472	0.106	0.294
Stem width (STW)	-0.704	0.644	0.035	-0.496
Specific gravity (SG)	0.053	-0.203	0.260	-0.030
Seed weight (SWT)	0.571	-1.114	0.257	-0.037
Seed length (SL)	0.973	-0.952	-0.405	-0.040
Seed width (SW)	-0.138	-0.568	0.007	-0.586
Eigenvalues	0.532	0.406	0.242	0.080
Proportion of variance	0.363	0.277	0.165	0.055
Cumulative proportion	0.3629	0.6400	0.8052	0.8597

Red (positive correlation) and blue (negative correlation) color indicated positive and negative contribution where the intensity of color showing their contribution. Darker the color, higher the loading to principal component.

**Table 5.** Eigenvalues, the proportion of variability and the cumulative rate of the 10 qualitative traits of pigeon pea MC collection contributed to the first four principal components (PCs)

Components	PC1	PC2	PC3	PC4
Seed coat color (SCC)	-0.178	0.203	-0.453	0.357
Seed size (SS)	-0.105	-0.199	0.002	-0.153
Pod color (PC)	0.414	0.087	0.386	-0.194
Seed shape (SSH)	-0.056	-0.020	-0.358	-0.485
Leaf shape (LSH)	0.274	-0.376	-0.116	-0.157
Stem color (SC)	-0.080	0.584	0.176	-0.031
Foliage (FO)	-0.469	-0.150	0.408	0.048
Clustering (CL)	-0.469	-0.150	0.408	0.048
Spreading (SP)	-0.270	-0.015	-0.078	-0.464
Susceptibility to SMV (SUS)	0.088	-0.554	-0.037	0.425
Eigenvalue	0.525	0.1590	0.1504	0.1010
Proportion	0.4300	0.1302	0.1232	0.0827
Cumulative	0.4300	0.5602	0.6834	0.7661

Blue and red color indicated positive and negative contribution where the intensity of color showing their contribution. Darker the color, higher the loading to principal component.

**Fig. 3.** Hierarchical cluster dendrogram showing genetic similarity matrix of 73 pigeonpea MC collection evaluated on 13 quantitative traits. Cluster 1, in orange colour, cluster 2 in green colour, cluster 3 in blue colour and cluster four in purple colour**Fig. 4.** Agglomerative hierarchical clustering (AHC) dendrogram analysis using Euclidean distance into different cluster as per ward method for 10 qualitative traits of 73 pigeonpea MC collection. Cluster I, cluster II and cluster III with red colour, green colour and

blue colour. The genotypes detected in distinct clusters indicate that their interaction may be due to the accessible exchange of materials that may have overlapped in the earlier diversity distribution pattern of the domesticated species (Rupika and Bapu, 2014; Muniswamy et al. 2014). The cluster analysis separated the accessions into several groups, indicating that there is significant genetic variation among the genotypes. However, the geographical proximity could explain the association of these MC genotypes in each group.

### Supplementary material

Supplementary Tables S1 to S3 are provided and can be accessed at [www.isgpb.org](http://www.isgpb.org)

### Authors' contribution

Conceptualization of research (NKS, NS, MS, M); Designing of the experiments (NKS, MS, M); Contribution of experimental materials (NKS); Execution of field/lab experiments and data collection (NS); Analysis of data and interpretation (NKS, MS, NS); Preparation of manuscript (NKS, M, MS, NS).

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**Supplementary Table S1. A list of material and quantitative traits studied**

Genotype	origin	SPP	PL	PW	PH	NBR	BA	LL	LW	STW	SG	SWT	SL	SW
MC1	India	4	5	0.7	180	3	55	5	2.5	4	0.35	7.75	0.6	0.4
MC10	India	4	5.5	0.8	240	2	50	8	3.5	3	0.05	7.37	0.5	0.5
MC101	India	3	4.5	0.4	180	4	50	6.7	2.8	0.8	0.25	5.63	0.55	0.35
MC104	India	5	5	0.7	142	3	45	8.4	3.3	2	0.55	5.46	0.5	0.45
MC109	India	3	0.5	0.1	90	0	55	7.5	2.9	0.5	0.25	16.30	0.8	0.55
MC111	India	3	0.5	0.1	90	0	55	7.5	2.9	0.5	0.25	10.68	0.65	0.4
MC114	India	3	0.5	0.1	90	0	55	7.5	2.9	0.5	0.25	9.23	0.7	0.4
MC115	India	4	5.5	0.5	110	3	40	6.1	3	1.2	0.60	12.02	0.6	0.4
MC116	India	5	9	1.5	107	3	50	6.4	3.1	1.9	1.65	14.16	0.65	0.4
MC117	India	4	5.5	0.5	110	3	40	6.1	3	1.2	0.60	14.74	0.6	0.5
MC118	India	4	5.6	0.4	102	3	40	7	2.4	1.2	0.70	14.25	0.75	0.4
MC119	India	4	5.6	0.4	102	3	40	7	2.4	1.2	0.70	10.89	0.65	0.3
MC12	Myanmar	4	4.5	0.6	255	3	50	8.3	3.5	3.5	0.65	7.45	0.5	0.55
MC120	India	4	5.6	0.4	70	0	30	7.5	3.4	1.5	2.00	13.54	0.7	0.35
MC121	India	4	5.6	0.4	102	3	40	7	2.4	1.2	0.70	8.96	0.6	0.4
MC122	India	4	5.5	0.5	146	3	50	7.1	3.2	1.5	1.10	7.68	0.55	0.4
MC123	India	3	3.4	0.4	202	4	40	8.9	3.5	2	1.00	8.93	0.55	0.3
MC125	India	5	6	1	130	3	50	6.9	2.9	2	0.70	11.06	0.6	0.4
MC126	India	4	4.5	0.6	170	3	45	7.3	2.9	2.5	1.05	12.77	0.65	0.5
MC128	India	4	5.5	0.5	180	4	45	9	3	2.5	0.90	11.70	0.7	0.4
MC13	India	3	4.5	0.6	220	3	40	7	3.5	2.5	0.70	8.43	0.5	0.4
MC130	India	4	5	1	180	3	55	6.5	2.5	2.5	0.60	8.85	0.7	0.45
MC132	India	4	5.4	0.6	179	4	60	8.3	2.5	3	0.50	6.42	0.45	0.4
MC133	India	4	5	1	180	3	55	6.5	2.5	2.5	0.60	8.72	0.5	0.4
MC134	India	4	6	0.4	180	4	75	9	3.7	0.8	0.05	10.21	0.55	0.35
MC18	India	3	3.4	0.3	260	2	30	7.7	4	2.9	0.70	7.46	0.5	0.45
MC19	India	3	4	0.2	235	2	55	10	4.3	3	0.50	7.82	0.65	0.3
MC2	India	4	4.5	2.2	170	2	50	5	2.2	2.5	0.45	7.07	0.65	0.4
MC20	India	3	5.3	0.6	245	3	40	9.5	4.2	3.5	0.40	7.33	0.6	0.4

MC21	India	4	4.5	0.6	255	3	50	8.3	3.5	3.5	0.65	6.42	0.5	0.3
MC25	West Africa	4	6	0.6	104	3	40	7.2	3.5	1.5	0.50	8.37	0.6	0.3
MC26	India	4	5.5	0.8	119	2	30	7.5	2.9	2	0.25	8.61	0.6	0.4
MC27	India	3	4	0.6	250	2	55	7.5	2.9	2.9	0.30	6.94	0.4	0.3
MC28	India	3	3.4	0.4	220	2	60	7	2.7	2.5	0.40	6.32	0.5	0.3
MC3	India	4	5.7	0.8	162	2	60	5	2	1.5	0.10	9.21	0.6	0.45
MC30	India	4	4.8	0.7	170	2	30	6.5	3	3	0.40	5.46	0.4	0.25
MC31	Bangladesh	4	6.2	0.8	110	1	30	7	3.1	2.5	0.50	7.96	0.5	0.3
MC34	India	5	5.6	0.7	170	2	40	7.5	3.4	2	0.55	9.12	0.5	0.3
MC35	India	4	4.5	0.4	200	2	45	10	4.1	2.5	0.70	7.30	0.45	0.4
MC36	India	4	4.5	0.4	200	2	45	10	4.1	2.5	0.70	8.33	0.45	0.5
MC38	Trinidad and Tobago	4	5	0.9	220	2	30	7.5	3.1	3	0.60	10.24	0.55	0.45
MC39	India	4	4.8	0.7	215	2	50	6.5	2.9	2.5	2.50	8.29	0.5	0.45
MC44	India	4	5	0.8	220	0	50	8.5	2.9	1.5	0.85	7.21	0.5	0.45
MC48	India	4	5	0.5	250	0	50	8	3.8	3.1	0.55	8.80	0.6	0.4
MC50	India	4	5	0.6	250	0	45	10.5	4.2	2.5	1.00	11.43	0.6	0.35
MC51	India	4	4.5	0.5	255	0	55	8	3.5	3.5	3.60	9.12	0.55	0.35
MC56	India	4	5.5	0.6	195	2	60	7	2.9	1.2	0.55	5.50	0.5	0.3
MC6	India	4	4.2	0.6	230	4	45	7	3	2.9	0.20	5.62	0.6	0.3
MC63	India	3	5	0.4	230	2	50	8	4.2	2	0.45	9.42	0.65	0.45
MC65	India	4	5	0.5	234	0	45	11	4.4	2.5	0.40	7.87	0.55	0.5
MC66	India	4	4.5	0.4	250	0	45	7.9	3	2.5	0.25	9.91	0.45	0.4
MC69	India	4	4.6	0.6	230	0	50	7.9	2.7	2	0.45	7.69	0.5	0.3
MC7	India	4	5.9	0.8	172	1	55	5.5	2.4	2.5	0.30	6.99	0.55	0.3
MC70	India	5	5.4	0.6	236	2	50	9.2	3.7	2.9	0.70	9.80	0.5	0.4
MC72	India	4	3.5	0.3	220	0	25	9	3.4	2	0.65	11.17	0.45	0.5
MC76	India	4	4.5	0.6	208	2	50	8.2	3.6	1.4	0.75	6.89	0.6	0.45
MC77	India	4	5	0.4	255	2	50	9	3	4	0.85	9.21	0.5	0.6
MC79	India	4	3.4	0.3	235	0	60	7.5	2.8	2	0.60	7.69	0.5	0.45
MC80	India	4	6	0.8	245	0	30	10	4	2.5	0.85	9.16	0.55	0.45

MC81	India	4	4.4	0.5	260	1	55	9	3.8	2.5	0.60	4.82	0.5	0.4
MC82	India	4	4.5	0.5	245	0	30	10.5	4.5	1.8	0.70	12.28	0.6	0.4
MC83	Nepal	4	4.8	0.5	240	3	90	4.5	3	0.3	0.50	5.39	0.45	0.3
MC84	Nepal	4	5	0.4	240	3	45	4.5	3	2	0.55	8.08	0.6	0.4
MC85	India	4	6.2	0.9	180	0	50	7	2.8	2	0.15	6.27	0.55	0.3
MC86	India	4	5	0.8	180	0	45	7.5	2.9	1.2	0.55	6.81	0.6	0.3
MC87	India	4	5	0.6	230	2	60	10	3.4	2	0.05	6.35	0.55	0.3
MC89	India	5	5.2	0.7	220	2	45	10.2	4.2	2.2	0.55	14.74	0.55	0.4
MC9	India	4	5.2	0.8	220	3	44	6.5	2.8	3.4	0.25	8.04	0.45	0.5
MC90	India	5	5.2	0.7	220	2	45	10.2	4.2	2.2	0.55	15.64	0.6	0.45
MC93	Tanzania, Africa	6	7	0.9	220	2	40	10.1	3.3	1.8	1.15	9.57	0.5	0.45
MC94	India	4	5	0.4	210	2	60	8	3	1.8	1.40	9.51	0.5	0.4
MC97	India	4	3.8	0.5	230	3	50	9	3.4	2.5	0.60	7.71	0.5	0.35
MC99	India	4	4.5	0.6	200	4	50	6	2.6	2.5	0.35	7.45	0.7	0.25

**Supplementary Table S2. Frequency percentages of 10 qualitative traits for MC collection of pigeonpea**

Trait	Score	Frequency (%)	Cumulative Frequency (%)	Shannon Weaver (H')
Pod color	Green + Brown	89.0%	65%	0.426271368
	Green	8.2%	71%	
	Brown	1.4%	72%	
	Red	1.4%	73%	
Leaf shape	Elliptic	76.7%	56%	0.542729009
	Linear	23.3%	73%	
Stem color	Green + Brown	13.7%	10%	0.399454802
	Green	86.3%	73%	
Foliage	High	17.8%	13%	1.271713571
	Medium	42.5%	44%	
	Low	28.8%	65%	
	No	11.0%	73%	
Clustering	High	17.8%	14%	1.271713571
	Medium	42.5%	44%	
	Low	28.8%	65%	
	No	11.0%	73%	
Spreading	Broad	20.5%	15%	1.384210248
	Semi	42.5%	46%	
	Erect	9.6%	53%	
	Erect + Compact	23.3%	70%	
	No	4.1%	73%	
Susceptibility	High	1.4%	1%	1.124598469
	Moderate	21.9%	17%	
	Low	41.1%	47%	
	No	35.6%	73%	
Seed coat color	Copper	28.8%	21%	2.470969515
	Almond	2.7%	23%	
	Black	2.7%	25%	
	Caramel	2.7%	27%	
	Cream + copper dotted	8.2%	33%	
	Russet	11.0%	41%	
	Peacan	1.4%	42%	
	Tawny	6.8%	47%	
	White	11.0%	55%	
	Syrup	1.4%	56%	
	Mustard	4.1%	59%	
	Caramel + Hickory dotted	1.4%	60%	
	Orange Peach	4.1%	63%	
Gingerbread	2.7%	65%		

	Cream	1.4%	66%	
	Carob	1.4%	67%	
	Almond + Copper dotted	2.7%	69%	
	White + Black dotted	1.4%	70%	
	Cinnamon	4.1%	73%	
Seed size	Large	4.1%	3%	0.243210322
	Medium	94.5%	72%	
	Small	1.4%	73%	
Seed shape	Spheroid	39.7%	29%	1.125103517
	Semi-Spheroid	46.6%	63%	
	Semi-Deltoid	8.2%	69%	
	Fusifiform	2.7%	71%	
	Ovoid	2.7%	73%	

**Supplementary Table S3. Identity number given to each character given to calculate frequencies and diversity index**

Genotype	Origin	SCC	SS	PC	SS	LS	SC	FO	CL	SP	SUS
MC1	India	1	2	1	1	1	1	2	2	1	4
MC2	India	2	2	1	2	1	1	1	1	1	4
MC3	India	3	2	1	2	1	2	3	3	1	3
MC6	India	4	2	1	3	1	1	2	2	2	4
MC7	India	5	2	2	2	1	2	1	1	3	2
MC9	India	4	2	1	1	1	2	3	3	1	2
MC10	India	6	2	1	1	1	2	2	2	2	4
MC12	India	7	2	1	1	1	2	2	2	1	4
MC13	India	6	2	1	2	1	2	1	1	2	2
MC18	India	1	2	1	1	2	2	2	2	4	3
MC19	India	1	2	2	4	1	2	4	4	2	3
MC20	India	5	2	1	1	1	2	4	4	4	3
MC21	Mayanmar	5	2	1	2	1	2	4	4	2	3
MC25	India	8	2	1	1	1	2	4	4	2	3
MC26	India	9	3	1	1	1	2	3	3	2	4
MC27	India	1	2	1	1	1	2	4	4	2	3
MC28	India	8	2	1	2	1	2	3	3	2	3
MC30	India	6	2	1	3	1	1	2	2	1	3
MC31	India	9	1	1	2	1	2	2	2	2	4
MC34	India	10	2	1	2	1	2	2	2	3	4
MC35	India	1	2	1	1	1	2	3	3	3	4
MC36	India	1	2	1	2	1	2	3	3	3	4
MC38	India	11	2	1	2	1	1	2	2	1	4
MC39	India	11	2	1	1	1	2	2	2	3	3
MC44	India	1	2	3	3	2	2	3	3	3	3

MC48	India	12	2	1	5	1	2	4	4	4	4
MC50	India	1	2	1	1	1	2	2	2	4	3
MC51	India	13	2	1	2	1	2	2	2	3	4
MC56	India	9	2	1	1	1	2	3	3	2	3
MC63	India	14	2	1	1	1	2	2	2	2	4
MC65	West Africa	1	2	1	2	2	2	3	3	4	4
MC66	India	1	2	1	2	2	2	2	2	4	4
MC69	India	15	2	1	4	2	2	2	2	2	3
MC70	India	6	2	1	2	1	2	3	3	4	3
MC72	India	5	2	1	2	2	1	3	3	4	3
MC76	India	19	2	1	2	1	2	2	2	2	4
MC77	Bangladesh	13	2	1	1	1	2	4	4	4	3
MC79	India	1	2	1	2	1	2	2	2	2	3
MC80	India	11	2	1	2	1	2	1	1	4	4
MC81	India	16	2	1	2	2	2	3	3	4	3
MC82	Trinidad and Tobago	17	2	1	1	2	1	3	3	4	3
MC83	India	17	2	1	2	1	2	2	2	4	2
MC84	India	5	2	1	2	1	2	3	3	4	2
MC85	India	5	2	1	2	1	2	3	3	5	3
MC86	India	14	2	1	2	1	2	2	2	5	3
MC87	India	18	2	1	2	1	2	3	3	4	3
MC89	India	3	2	1	5	1	2	2	2	4	2
MC90	India	1	2	1	1	1	2	2	2	4	2
MC93	India	1	2	2	2	2	1	1	1	2	3
MC94	India	1	2	1	2	2	1	1	1	2	4
MC97	India	1	2	1	1	1	2	1	1	2	1
MC99	India	6	2	1	2	1	2	1	1	1	2
MC101	India	8	2	1	2	2	2	1	1	1	3
MC104	India	9	2	1	3	2	2	1	1	1	4
MC109	India	19	2	1	3	1	2	2	2	2	2
MC111	India	9	2	1	1	1	2	2	2	2	2
MC114	India	6	2	1	2	1	2	2	2	2	2
MC115	India	9	2	1	2	1	2	3	3	2	3
MC116	India	1	2	4	1	2	2	1	1	1	4
MC117	India	1	2	1	1	1	2	2	2	2	2
MC118	India	8	1	1	1	1	2	2	2	1	4
MC119	Nepal	2	2	1	2	1	2	2	2	2	2
MC120	Nepal	13	2	1	2	1	1	4	4	2	3
MC121	India	1	2	1	2	1	2	2	2	2	2
MC122	India	9	2	2	2	1	2	3	3	1	3
MC123	India	1	2	1	2	2	2	3	3	5	3



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MC125	India	1	2	2	2	1	2	2	2	2	3
MC126	India	8	1	2	1	1	2	1	1	1	2
MC128	India	1	2	1	1	2	2	2	2	1	4
MC130	Tanzania, Africa	6	2	1	1	2	2	1	1	2	4
MC132	India	6	2	1	1	2	2	3	3	2	4
MC133	India	19	2	1	2	1	2	2	2	2	2
MC134	India	9	2	1	1	1	2	3	3	2	4

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