**Morphological plasticity of economical traits in pigeonpea genotypes grown in South Africa**

*Mofokeng Maletsema Alina 1, Bello Zaid 1, Mashingaidze Kingstone 1 and Abe Shegro Gerrano 2,3\**

*1 Agricultural Research Council-Grain Crops, Private Bag X 1251, Potchefstroom, 2520, South Africa*

*2 Agricultural Research Council - Vegetables, Industrial and Medicinal Plants, Private Bag X 293, Pretoria, 0001. South Africa*

*3 Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Mmabatho, South Africa*

*\*Corresponding author’s E-mail address: agerrano@arc.agric.za*

*Corresponding author: ORCID: http://orcid.org/0000-0001-7472-8246*

**Abstract**

Pigeonpea (*Cajunus cajan*) is among the most important leguminous crops cultivated globally. However, it is regarded a neglected pulse in southern Africa in terms of research and production. The objective of the study was to determine the existence agro-mophological traits among tested pigeonpea genotypes using multivariate analysis. The studies were conducted at Mafikeng and Nelspruit located in North West and Mpumalanga Provinces of South Africa. The trials were laid out in a randomized complete block design with three replications. Field phenotyping data were recorded and data were analysed using analysis of variance (ANOVA), Pearson’s correlations, principal component analysis (PCA), biplots constructed using principal coordinate analysis, Shannon Weaver diversity indices and frequencies. Significant genotype effect was observed for plant height (PH), pod bearing (PDB) and seed number per pod (SNP) among the studied genotypes. Seed yield (SY) was positively correlated with seed number per pod (SNT), seed number per plant (SNP) and pod weight (PWT) whereas PBD was negatively associated with hundred seed weight (HSW). PCA revealed five significant principal components (PCs), which accounted for 84.70% of phenotypic variation among the studied genotypes. The Shannon weaver indices ranged from 0.98‑1.00, indicating the presence of variation among the qualitative traits measured. The clustering grouped genotypes into three groups, with ICEAP00554, ICEAP000979-1, ICEAP00540, and Karatu-1 being the most diverse and singletons. The multivariate analyses revealed the existence of morphological variation among the pigeonpea genotypes, which is a pre-requisite in breeding programs.

**Keywords:** Agro-morphology, Characterization, Cluster, PCA, Pigeonpea, Variation

**Introduction**

Pigeonpea (*Cajunus cajan*) is a diploid (2n = 2x = 22) legume (Van der Maesen, 1990) that is considered as an underutilised food and fodder crop despite its importance. It has great potential to enhancefood and nutritionsecurity (Lin-Qi 2014). The crop improves the fertility of the soil through atmospheric nitrogen fixation (Adebowale and Maliki 2011; Choudhary *et al*. 2013). The crop can be intercropped with other crop species (Lin-Qi, 2014) especially cereal based cropping system. Pigeonpea is a good source of mineral elements and vitamins (Saxena *et al*. 2010). This crop has high potential to cope with climate smart agriculture and providing nutritional and food security. It has the ability to survive and give good economic benefits when planted under dryland farming conditions, when there is limitation of rainfall and sustain the livelihood of resource poor rural populations in tropical and sub-tropical regions of the African continent. Furthermore, the crop helps in protecting the environment from soil erosion and degradation, improve the fertility of the soil, increase crop production and productivity at marginal crop lands towards soil and food security strategies.

The seed of the crop can be eaten as a green vegetable and dry pulse and is an important source of nutritional components (Choudhary *et al*. 2013). It has been reported that the seeds of pigeonpea contains 21% of protein, 48.4% starch, 8.2 % crude fibre, 2.3% fat, 94.6% Calcium, 113.7% Magnesium, 1.4% Copper, 4.6% Iron, 2.5% Zinc (Faris *et al*. 1987). The green pods and foliage of the plant are mainly used as livestock feed (Mallikarjuna *et al.* 2011). It is climate smart crop that adapt to the current climate change, which is tolerant to heat, drought, diseases and insect pests (Odeny 2007). The crop is cultivated by the resource poor small scale farmers with the low input agriculture in South Africa. Hence, genetic improvement of this crop is important to increase production and productivity of the crop.

For an efficient evaluation and utilisation of the plant genetic materials, understanding and knowledge about genetic diversity, and information on collection and classification are important and the basis for crop improvement programs (Khan *et al*. 2014; Syafii *et al.* 2015), which is elucidated through different marker systems such as agro-morphological, biochemical and molecular markers. Among these, agro-morphological characterisation is considered as the initial step for designing breeding programs (Smith *et al*. 1989; Kahn *et al*. 2014) although influenced by the growing environmental condition unlike with DNA-based markers. Variability existed in pigeonpea using agronomic and morphological traits evaluated which is still paramount important for plant breeders and curators because they will be able to select potential parents based on yield and its components, and farmer preferred agronomic traits for production and utilization. Yohane *et al*. (2020) reported the existence of widest variability based on the agro-morpholocal performance of among test pigeonpea accessions in Malawi. Assessing genetic variability helps to study heterosis (Virky *et al*. 2003), selection of transgressive breeding segregants and genes of novelty, and has a role in collection and conservation of germplasm for crop improvement (Duran *et al*. 2009). In order to have all these done, sound statistical tools are required for data analysis for assessment of genetic divergence (Syed *et al*. 2019) and result in heterotic expression of the progenies. In order to reduce the large number of data set and identify a key agronomic traits that account for the majority of the variance, data must be subjected to multivariate analysis (Immad *et al*. 2018) for selection and breeding.

Multivariate analytic tools plays a great role in identification and selection of parental lines for crop improvement (Malik *et al*. 2014). The tools include principal component analysis and cluster analysis among others. These tools are effective for studying the variability and relationships between and within accessions (Mondal *et al*. 2003; Ajmal *et al*. 2013). The principal component analysis (PCA) includes the total variance of variables, explains maximum of variance within a data set, and is a function of primary variables. PCA shows which of the traits are decisive in genotype differentiation (Kovacic 1994). It enables in understanding the similarity and differences among the test traits of interest (Immad *et al.* 2018).

Cluster analysis identifies and classifies individuals or variables on the basis of their similarity of the characteristics they possess, so the degree of association will be strong between members of the same cluster and weak between members of different clusters. It aims to allocate a set of individuals to a set of mutually exclusive, exhaustive groups such that the individuals within a particular group are similar to one another, while the individuals in the different groups are dissimilar. This will help in parental identification for population development (Immad *et al.* 2018) in the breeding programme. Multivariate analysis clustered tested genotypes based on their similarity and differences in agro-morphological characteristics (Mohammadi and Prasanna 2003; Peeters and Martinelli 1989; Rachovska *et al*. 2002), which can help to develop heterotic group in the breeding programme. The knowledge and understanding of various crop species and their evaluation are necessary for improvement strategy development in any crop (Gbaguidi *et al*. 2018), as these traditional landraces are the potential donor parents for improved varieties (Upadhyaya *et al*. 2007). Hence, the objective of the study was to determine the presence of agro-morphological diversity using multivariate analyses techniques.

**Materials and Methods**

**Plant Material and Trial Sites**

The pigeonpea genotypes were obtained from ICRISAT, Kenya and Tanzania (Table 1) for this study.

**Table 1:** Pigeonpea germplasm used in the study.

|  |  |  |
| --- | --- | --- |
| Number | Genotype Name | Origin/source |
| 1 | ICEAP 01147 | ICRISAT |
| 2 | ICEAP 01154-2 | ICRISAT |
| 3 | ICEAP 01150-1 | ICRISAT |
| 4 | ICEAP 01179 | ICRISAT |
| 5 | ICEAP 00979-1 | ICRISAT |
| 6 | ICEAP 01172-2-4 | ICRISAT |
| 7 | ICEAP 01159 | ICRISAT |
| 8 | ICEAP 01544-2 | ICRISAT |
| 9 | ICEAP 00540 | ICRISAT |
| 10 | ICEAP 00554 | ICRISAT |
| 11 | ICEAP 00557 | ICRISAT |
| 12 | ICEAP 00850 | ICRISAT |
| 13 | Ilonga 14-M1 | Tanzania |
| 14 | Mali | Tanzania |
| 15 | Ilonga 14-M2 | Tanzania |
| 16 | Karatu-1 | Tanzania |
| 17 | Kiboko | Tanzania |
| 18 | Komboa | Tanzania |
| 19 | Tumia | Tanzania |

The experimental trials were conducted at the North West University research farm at Mafikeng (25° 48ʹ S, 45° 38ʹ E; 1012 m. a. s. l.) in North West Province and Nelspruit (25.49o 89’ S, 31.35o37’ E; 670 m. a. s. l.) in Mpumalanga Provinces during 2019/2020 cropping season in South Africa. Pigeonpea is widely grown predominantly in this two Province in South Africa and have extreme variations in agro-climatic conditions. The soils on the North West University farm belongs to the Hutton series, with sandy loam and a yellow sand alternating (Molope 1987; Kasirivu e*t al*. 2011), while the Nelspruit field consisted of sandy loam soil. Mafikeng receives a summer rainfall, with an annual mean of 571 mm during the cropping season. The mean annual maximum temperature is 37 °C, while the mean minimum temperature is 9 °C. The field in Nelspruit is characterised by mean maximum temperature of 28 oC. The mean annual minimum temperature is12.5 oC with an annual precipitation of about 796 mm during the cropping season.

**Trial Design and Management**

The trials were laid out in a randomized complete block design replicated three times with a plot consisting of two rows of 4 m length. The inter- and intra-row spacing’s were 90 cm and 60 cm, respectively. The experiment was conducted during summer cropping season in rained condition based on the farmers practice. Weeding was done manually. No fertilizer was applied to simulate low input cropping system in the region (Gerrano *et al*. 2015).

**Data Collection**

Data were recorded according to standard descriptor list for pigeonpea (IBPGR 1994). Data were recorded from three randomly selected plants in the middle of each rows per replications. The qualitative data recorded included base flower colour, second flower colour, vigour at 50% flowering, pod form, seed colour pattern, seed shape, and pattern of streaks. The list of quantitative traits studied is presented in Table 2.

**Statistical Data Analysis**

The recorded quantitative data were analysed using analysis of variance (ANOVA), principal component analysis (PCA), and Pearson correlations. The qualitative data were analysed using frequencies, spearman correlations, and Shannon Weaver diversity index. The biplots were generated using principal coordinate analysis in SAS version 9.6 (2021). A dendrogram was constructed using Genstat 18th edition (VSN International, Hempstead, UK)

**Table 2:** A list of economical traits measured, abbreviations and definitions.

|  |  |  |
| --- | --- | --- |
| Trait | Abbreviation | Measurement/definitions |
| Plant height (cm) | PHT | Height of a plant from the base of the stem to the tip of the plant at harvest |
| Days to 50% flowering | DFF | Number of days from planting until 50% of the plants have flowered in a plot |
| Pod bearing (cm) | PDB | Distance from lowest to the top most of the plant |
| Leaf length mm) | LFL | Length from the tip of the leaf to the leaf petiole ( |
| Leaf width (mm) | LFW | Length in the middle of the leaf from one tip to the other tip |
| Branch number | BRN | Number of branches per plant |
| Stem diameter (cm) | STD | Diameter of plant stem |
| Pod length (mm) | PDL | Length of the pod from bottom end to top end at harvest |
| Pod width (mm) | PDW | Length at the centre of the pod from one end to the other end/diameter |
| 100 Seed weight (g) | HSW | Weight of 100 seed picked randomly for each genotype |
| Pod weight (g) | PWT | Weight of dry pods harvested from each genotype |
| Seed number per pod | SNT | Number of seeds in a pod (average of 10 pods) |
| Seed number per plant | SNP | A number of seeds produced by a single plant. |
| Seed yield (g) | SYD | Weight of seeds produced per plant |

**Results**

**Genotype by Environment Interaction**

ANOVA showed genotype (G), site (S), and genotype × site interaction (GEI) effects on quantitative traits is presented in Table 3. There were significant (P ≤ 0.05) differences between sites based on days to flowering, plant height, branch number, stem diameter, pod bearing, pod length, pod weight and significant differences for seed number per pod. There were significant (P ≤ 0.01) differences between genotypes based on pod length and pod weight. There was a significant (P ≤ 0.05) site × genotype interaction based on plant height, pod bearing and seed number per pod.

**Table 3:** Combined analysis of variance for quantitative traits among the studied pigeonpea genotypes.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of variation | d.f | DFF | PHT | BRN | STD | LLT | LWT | PDB | 100SW | PDL | PDW | SNP | PWT | SEP | SYD |
| Site (S) | 1 | 85323.90\*\* | 175005.38\*\* | 1141.91\*\* | 11556.66\*\* | 0.21 | 722.55 | 41198.18\*\* | 83.37 | 1957.27\*\* | 10.92 | 138.27\* | 142.24\*\* | 1.34 | 45.9124209 |
| Genotype (G) | 18 | 76.16 | 1908.74 | 15.61 | 13.51 | 1.86 | 327.19 | 1417.32 | 163.29 | 507.82\*\* | 3.70 | 57.00 | 46.02\*\* | 8.34 | 20.04 |
| S × G | 18 | 72.88 | 2867.50\*\* | 16.66 | 14.72 | 2.05 | 325.35 | 2733.83\* | 165.91 | 322.15 | 4.04 | 61.20\* | 30.78 | 8.87 | 17.94 |

d.f. = degree of freedom; DFF = Days to 50% flowering; PHT = plant height; BRN = Branch number; LLT = Leaf length; LWT = Leaf width; PDB = Pod bearing; 100SW = hundred seed weight; PDL= Pod length; PDW = Pod width; SNP = Seed number per pod; PWT = Pod weight; SEP = Seed number per plant; STD = Stem diameter; SYD = seed yield per plant; \* = significantly different from zero at p ≤ 0.05; \*\* = significantly different from zero at p ≤ 0.01

**Pearson’s Correlation Analysis**

Pearson’s correlations (*r*) of 14 quantitative traits measured in the study are shown in Table 4. Days to flowering was significantly and positively correlated with plant height, branch number, stem diameter, and hundred seed weight. Similarly, days to flowering was significantly and positively correlated with pod weight and negatively correlated with pod bearing. Plant height was highly significant and positively correlated with branch number per plant, stem diameter, and hundred seed weight, and negatively associated with pod bearing. Branch number had a negative and significant association with stem diameter and pod bearing, and a positive correlation with hundred seed weight. Stem diameter had a significant and positive correlation with leaf length, whereas pod bearing showed a negative association with hundred seed weight. Leaf length showed positive and significant correlations with leaf width and pod bearing. Leaf width had a negative association with seed number per pod. Pod bearing had a highly significant negative correlation with hundred seed weight. Pod length showed a positive association with seed number per pod, pod weight, seed number per plant, seed yield. Pod width showed a positive and highly significant correlations with seed number per plant and seed yield. Seed number per pod was positively correlated with pod weight, seed number per plant, and seed yield. Pod weight had positive correlations with seed number per plant and seed yield. Seed number per plant was highly significant and positively correlated with seed yield (Table 4).

**Table 4:** Pearson correlations for quantitative traits among the studied pigeonpea genotypes.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variable | DFF | PHT | BRN | STD | LLT | LWT | PDB | 100SW | PDL | PDW | SNP | PWT | SEP |
| DFF | 1.00 |  |  |  |  |  |  |  |  |  |  |  |  |
| PHT | **0.701\*\*\*** | 1.00 |  |  |  |  |  |  |  |  |  |  |  |
| BRN | **0.625\*\*\*** | **0.751\*\*\*** | 1.00 |  |  |  |  |  |  |  |  |  |  |
| STD | **-0.900\*\*\*** | **-0.667\*\*\*** | **-0.492\*\*\*** | 1.00 |  |  |  |  |  |  |  |  |  |
| LLT | -0.089 | 0.017 | 0.040 | **0.241** | 1.00 |  |  |  |  |  |  |  |  |
| LWT | -0.034 | -0.019 | 0.075 | 0.169 | **0.672\*\*\*** | 1.00 |  |  |  |  |  |  |  |
| PDB | **-0.498\*\*\*** | **-0.405\*\*\*** | **-0.341\*\*\*** | **0.504** | **0.190\*** | -0.056 | 1.00 |  |  |  |  |  |  |
| 100SW | **0.525\*\*\*** | **0.431\*\*\*** | **0.296\*\*** | **-0.574** | -0.053 | -0.003 | **-0.353\*\*\*** | 1.00 |  |  |  |  |  |
| PDL | 0.183 | 0.046 | -0.011 | -0.136 | 0.117 | 0.095 | -0.010 | 0.159 | 1.00 |  |  |  |  |
| PDW | 0.063 | 0.083 | 0.114 | -0.024 | 0.003 | -0.110 | 0.014 | -0.060 | 0.018 | 1.00 |  |  |  |
| SNP | 0.086 | 0.133 | 0.089 | -0.076 | -0.085 | **-0.202\*** | 0.020 | -0.063 | **0.436\*\*\*** | 0.135 | 1.00 |  |  |
| PWT | **0.189\*** | 0.068 | 0.013 | -0.139 | 0.102 | 0.055 | -0.006 | 0.135 | **0.986\*\*\*** | 0.161 | **0.526\*\*\*** | 1.00 |  |
| SEP | 0.183 | 0.107 | 0.064 | -0.130 | 0.060 | -0.037 | 0.005 | 0.072 | **0.858\*\*\*** | **0.453\*\*\*** | **0.669\*\*\*** | **0.932\*\*\*** | 1.00 |
| SYD | 0.183 | 0.096 | 0.042 | -0.136 | 0.065 | -0.013 | 0.001 | 0.092 | **0.928\*\*\*** | **0.248\*\*\*** | **0.694\*\*\*** | **0.974\*\*\*** | **0.976\*\*\*** |

DFF=Days to 50% flowering, PHT = plant height, BRN= Branch number, LLT= Leaf length, LWT= Leaf width, PDB=Pod bearing, 100SW= hundred seed weight, PDL=Pod length, PDW=Pod width, SNP Seed number per pod, PWT= Pod weight, SEP= Seed number per plant, STD = Stem diameter, SYD=seed weight per plant.

**Principal Component Analysis**

Five most important PCs were identified contributing 32.9%, 24.9%, 12.7%, 8.3% and 5.9%, to the total variation of 84.7%, respectively (Table 5). The first PC had pod length, pod weight, seed number per plant and seed yield contributing to this variation. In the second PC, days to flowering, plant height, branch number, stem diameter contributed the most to variation. Leaf length, and leaf width contributed the most variation in third PC. In the fourth PC, pod width was the most contributor to variation whereas in the fifth PC, pod width and seed number per pod were the traits that contributed the most variation.

**Table 5:** Factor loadings of the most important PCs for agro-morphological traits among the studied pigeonpea genotypes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Traits | PC1 | PC2 | PC3 | PC4 | PC5 |
| DFF | 0.57 | **-0.72** | 0.05 | -0.02 | 0.01 |
| PHT | 0.47 | **-0.70** | 0.12 | 0.24 | -0.25 |
| BRN | 0.37 | **-0.64** | 0.19 | 0.37 | -0.31 |
| STD | -0.52 | **0.74** | 0.12 | 0.13 | -0.07 |
| LLT | -0.00 | 0.21 | **0.87** | 0.18 | -0.04 |
| LLW | -0.04 | 0.08 | **0.91** | -0.02 | 0.07 |
| PDB | -0.27 | 0.58 | -0.01 | 0.17 | -0.22 |
| HSW | 0.37 | -0.54 | 0.09 | -0.35 | 0.32 |
| PDL | **0.82** | 0.42 | 0.13 | -0.30 | 0.03 |
| PDW | 0.27 | 0.11 | -0.15 | **0.78** | **0.53** |
| SNP | 0.612 | 0.31 | -0.26 | 0.15 | **-0.45** |
| PWT | **0.87** | 0.44 | 0.07 | -0.17 | 0.06 |
| SEP | **0.88** | 0.44 | -0.05 | 0.14 | 0.10 |
| SYD | **0.89** | 0.45 | -0.02 | -0.07 | -0.02 |
| Eigenvalue | 4.62 | 3.50 | 1.78 | 1.163 | 0.82 |
| Explained variance (%) | 32.97 | 24.96 | 12.694 | 8.307 | 5.87 |
| Cumulative variance (%) | 32.968 | 57.931 | 70.625 | 78.932 | 84.80 |

DFF = Days to 50% flowering, PHT = plant height, BRN = Branch number, LLT = Leaf length, LWT = Leaf width, PDB = Pod bearing, 100SW = hundred seed weight, PDL= Pod length, PDW = Pod width, SNP = Seed number per pod, PWT = Pod weight, SEP = Seed number per plant, STD = Stem diameter, SYD =– seed weight per plant.

**Principal Coordinate Analysis**

The principal component (PC) biplot of the quantitative traits showing grouping of genotypes superimposed with traits is presented in Figure 1, PC1 had 31.35 % and PC2 had 0.26% variances with the total contributing variation of 51.61%. Gerrano et al. (2022) reported that the angles lesser than 45o between the vector lines of the two respective variables indicate positive and high trait correlation and revealed the ability to discriminate the test genotypes for breeding. Genotypes ICEAPO1150-1, ICEAPO1154-2, ilonga14-M2, ICEAPO1172-2-4, ICEAPO1544-2, Mali, ICEAPO4459 and longa14-M1 were grouped together based on high SYD, HSW, SNT, PWT, PDW and PDL. Further, ICEAPO1179 and Tumia were identified as best genotypes for BRN, PHT and SNP. The genotype ICEAP01147, Kiboko, and ICEAP00850 were associated with the variables LLF, DFF, and LLW, while the genotypes ICEAP00557, Karatu-1, ICEAP00554 and Komboa revealed less association to the variables recorded indicating that the genotypes were less responsive to the variables. Genotype ICEAP00850 was associated to PDB. Genotype ICEAP00540 is peculiar genotypes that was found far from the rest of genotypes from the scatter biplot (Figure 1), which can be considered for further evaluation in the breeding program. Stem diameter and pod bearing were negatively correlated with plant height, branch number, seed yield, and 100 seed weight. Seed number per pod, pod length, pod width, pod weight, seed yield, and seed number per plant were positively correlated with hundred seed weight, while branch number and plant height were highly positively correlated. The same traits were also correlated with stem diameter, pod bearing, leaf width and leaf length.

**Fig. 1:** PC biplot for quantitative traits among the studied pigeonpea genotypes. PC1=first principal component; PC2=second principal component

The biplot for the qualitative traits, the PC1 showed 40.27% and F2 had 26.41% (Figure 2). The first quadrant showed base flower colour and vigour at 50% flowering, which are positively correlated in this quadrant and are associated with the genotypes Ilonga 144-M1, ICEAP 00850, and ICEAP 01159, while the second quadrant showed seed shape that was associated with the genotypes positioned in this quadrant. The 3rd quadrant had pod form and seed colour pattern that are positively correlated to each other. The genotypes Kiboko and Mali had similar pod form and seed colour pattern in this quadrant. The 4th quadrant consists of only second flower colour. All the genotypes scattered in this quadrant were grouped together based on this qualitative trait (Figure 2). In Figure 2, the genotypes that are circled have similar values for PC1 and PC2 scores, which made them to be positioned on one dot.

**Fig. 2:** PCA biplot for qualitative traits among the studied pigeonpea genotypes.

**Frequencies of Qualitative Traits**

The frequencies of eleven qualitative traits measured are shown in Table 6. Vigorousness at flowering was high with 71.4% of plants being vigorous and intermediate was 23.2%. The base flower colour was dominated by yellow flowers followed by orange-yellow. The second flower colour was predominantly composed of red flowers (71.4%). The pattern of streaks was dominated by sparse streaks (35.1%), followed by uniform coverage of second colour and dense streaks. All plants of various genotypes had 100% stems thicker than 13 mm with green stems dominating (63.2%). The growth habit of the crop was predominantly composed of spreading types (75.4%) followed by erect and compact at 22.8%. The genotypes were dominated by cylindrical pods 96.40 with speckled seed colour pattern at 71.4% followed by mottled and speckled at 17.9%. The shape of the seed was predominantly globular (64.3%) with oval shape being 21.4%.

**Shannon Weaver Diversity**

Shannon weaver diversity indices are shown in Table 6. The diversity indices range from 0.96 (second flower colour) to 1.00 (flowering pattern and stem thickness). All traits showed significant variation except for flowering pattern and stem thickness.

**Table 6.** Frequency percentages of qualitative traits for medium duration pigeonpea

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Trait | Score | Frequency (%) | Cumulative frequency (%) | H’ |
| Vigour at 50% flowering | Low | 5.36 | 5.36 | 0.99 |
|  | Intermediate | 23.21 | 28.57 |  |
|  | High | 71.43 | 100 |  |
| Base flower colour | Light yellow | 19.65 | 19.65 | 0.97 |
|  | Yellow | 51.78 | 71.43 |  |
|  | Orange-yellow | 28.57 | 100 |  |
| Second flower colour | Red | 71.43 | 71.43 | 0.96 |
|  | Purple | 28.57 | 100 |  |
| Pattern of streaks | Sparse | 35.09 | 35.09 | 0.97 |
|  | Medium amount | 15.79 | 50.88 |  |
|  | Dense | 22.81 | 73.68 |  |
|  | Uniform coverage of second color | 26.32 | 100 |  |
| Flowering pattern | Determinate | 100 | 100 | 1.00 |
| Stem Thickness rating | Thick (>13 mm) | 100 | 100 | 1.00 |
| Growth habit | Erect and compact | 22.81 | 22.81 | 0.98 |
|  | Semi spreading | 1.75 | 24.56 |  |
|  | Spreading | 75.44 | 100 |  |
| Stem color | Green | 63.16 | 63.16 | 0.98 |
|  | Sun Red | 36.84 | 100 |  |
| Pod form | Flat | 3.64 | 3.64 | 0.99 |
|  | Cylindrical | 96.36 | 100 |  |
| Seed color pattern | Plain | 3.57 | 3.57 | 0.99 |
|  | Mottled | 7.14 | 10.71 |  |
|  | Speckled | 71.43 | 82.14 |  |
|  | Mottled and speckled | 17.86 | 100 |  |
| Seed shape | Oval | 21.43 | 21.43 | 0.98 |
|  | Globular | 64.29 | 85.71 |  |
|  | Square | 14.29 | 100 |  |

H’ = Shannon Weaver Index

**Hierarchical Clustering**

A dendrogram was constructed using hierarchical clustering to present interrelationships among the studied pigeonpea genotypes (Figure 3). The dendrogram grouped genotypes into three clusters and four singletons. The first cluster was composed of six genotype, Longa14-M1, Mali, ICEAP00557, Ilonga14-M2, ICEAP01159, and ICEAP00850. The second cluster was composed of four genotypes, ICEAP0050-1, ICEAP01179, kmboa and ICEAP01147. The genotypes, Kiboko, ICEAP01154-2, Tumia, ICEAP01172-2-4 and ICEAP01544-2 were grouped in cluster three. Four genotypes were identified as most divergent and grouped as singletons (ICEAP00554, ICEAP000979-1, ICEAP00540, and Karatu-1). These genotypes were far and distantly related with the rest of the test genotypes.

Chart, box and whisker chart

Description automatically generated

**Fig. 3:** A dendrogram showing interrelationships and divergence among nineteen pigeonpea genotypes based on quantitative traits.

**Discussion**

The knowledge of morphological variation for a trait and trait correlations are important components of any breeding objective. There were highly significant differences for sites based on days to flowering, plant height, branch number, stem diameter, pod bearing, pod length, pod weight and significant differences for seed number per pod (Table 3). This indicates that the expression of the significant traits varied with the growing environmental conditions they were tested in. Their performance was not stable across sites. The presence of highly significant differences in genotypes based on pod length and pod weight highlights the presence of genotypic variation among the genotypes evaluated based on the two traits which can be exploited for cultivar improvement in future breeding programmes. The significant differences on genotype x site interaction could be attributed to the different reactions of the accessions to sites or due to differences between the sites. In each environment, phenotypic manifestation is the result of the action of the genotype under the influence of the environment. However, when considering a series of growing environments, in addition to the genetic and environmental effects, an additional effect can be detected from their interaction (Des Marais 2013; Nunes *et al*. 2014). Significant genotype × environment interaction on yield and yield components in this study concur with the results reported previously (Vales *et al*. 2012; Kimaro *et al*. 2016; Gerrano *et al*. 2020).

Yohane et al. (2020) reported the existence of positive correlations for most of secondary traits that revealed multiple trait identification and selection for simultaneous trait improvement, while the weak correlations among the traits would result in an inefficient selection or low genetic gains that will take long time to fix the traits of interest. In this study seed yield was positively correlated with seed number per pod, seed number per plant and pod weight whereas pod bearing was negatively associated with hundred seed weight. The positive correlations of various traits in this study showed the usefulness of the traits for selection in crop improvement and they can further be used for improvement of seed yield (Saroj *et al*. 2013; Ojwang *et al*. 2016). Similar trends were reported by Sodavadiya *et al*. (2009) and Linge *et al*. (2010) and Prasad *et al*. (2013) in pigeonpea studies. Furthermore, Yohane et al. (2020) reported a significant and positive correlation between important economic traits such as grain yield and a hundred seed weight. Similarly, Kinhoégbè *et al*. (2020) reported positive association of some of the economical traits in pigeonpea that grown in Benin. These findings suggest the usefulness of these traits for selection and are in accordance with the correlations in this study.

The Principal component analysis over sites revealed five most important PCs with pod length, pod weight, seed number per plant, seed yield, leaf length, leaf width, days to flowering, plant height, and stem diameter being the most contributing traits to the total variation observed. This suggests that these traits are useful for selection. Other reports have indicated that trait contribution to different PCs varies with genetic diversity within the tested germplasm and the number of traits evaluated (Upadhyaya *et al*. 2007). The biplot also showed the different grouping of pigeonpea genotypes based on specific traits. These findings suggested that both qualitative and quantitative variables data set can reveal diversity among the tested genotypes but complementary information for breeding.

The most of pigeonpea accessions in the current study showed a strong tendency to spreading growth habit, yellow based flower colour, with red second flower colour, sparse pattern of streaks, green stems, with globular and speckled seed color pattern. The results are in contrast with the results of Kinhoégbè *et al*. (2020) where these authors reported genotypes with semi-spreading growth habit, lanceolate leaflet shape, light yellow base flower colour, and plain seed colour pattern. Similar results have been reported in the morphological variability of Tanzanian pigeonpea germplasm (Manyasa *et al*. 2008) tested, which might be due to the genetic background of the crop. Furthermore, similar results were reported in the previous studies in world-wide collection of pigeonpea for above qualitative traits of this crop (Rupika and Bapu 2014). Shannon Weaver indices also confirmed the presence of genetic divergence based on qualitative traits. Thus, in spite of the influence of prevalent environmental factors, qualitative variables can be used to characterize pigeonpea genetic resources.

The pigeonpea genotypes were clustered into three major groups, indicating that there genotypes in the three groups are distantly related. The ones in the same cluster are closely related and they maybe of the same source or origin. Selection of genotypes within these clusters may not be desirable to get higher yield and economic benefits and transgressive segregants (Muniswamy *et al*. 2014; Rupika and Bapu 2014). Therefore, for crop hybridization programs, the choice of suitable diverse parents based on their genetic differentiation would be more fruitful than the choice based on the geographical distances. ICEAP 00540, ICEP00979-1, Karatu-1 and ICEAP00554 would be the ideal genotypes for use as parents in any pigeonpea breeding programme for agronomic improvement. The identified genotypes in different clusters show that their interrelationship may be due to free exchange of materials that may have overlapped in the previous diversity distribution pattern of the domesticated species (Jaradat and Shahid 2006; Aghaee *et al*. 2010). Niranjana *et al*. (2014) also reported three clusters in their findings on pegion pea. Reddy and Jayamani (2019) reported seven major groups of the sixteen pigeonpea genotypes studied for genetic diversity assessment using multivariate analysis. Qutadah *et al*. (2019) also reported seven clusters in their pigeonpea genetic diversity study. Other cluster groups were revealed by various researchers (Singh *et al*. 2014; Kinhoégbè *et al*. 2020) in different parts of the world.

In conclusion, the study revealed the existence of genetic diversity among the pigeonpea genotypes studied based on the analysis of variance and multivariate tools used for analyses. The results indicated that the higher level of genetic diversity observed within the acquired genotypes from ICRISAT and Tanzania would enable efficient utilisation and pigeonpea improvement in breeding programs in South Africa and other countries. The variability among the genotypes also will help to identify and select the potential parents for hybridization. The selection accessions for single trait and improvement for this trait would require more breeding work therefore it is suggested that selection of accession for multiple traits as well as directly correlated traits would accelerated pigeonpea breeding for improvement of traits of interest simultaneously. Further characterization using molecular techniques as well as conservation attention for these local germplasms should be conducted.

**Acknowledgements**

The first author would like to thank the Department of Agriculture, Land Redistribution and Rural Development for funding. Additionally, the authors would like to thank the technical assistance and trial management of Paul Rantso, Dinah Scott, Deon Du Toit, and Theodora Mathobisa.

**Disclosure statement**

The authors have not declared any conflict of interests.

**Funding**

This work was supported by the Department of Agriculture, Land Redistribution and Rural Development.

**Notes on contributors**

**Dr. Maletsema Alina Mofokeng** is a Researcher in Plant Breeding at the Agricultural Research Council-Grain Crops, Potchefstroom, South Africa: Design, planting, trial monitoring, data recording, analysis and final drafting of the manuscript.

**Dr. Zaid Bello** is a Researcher in Agronomy department of Agricultural Research Council-Grain Crops, Potchefstroom, South Africa: Trial management, and review the manuscript.

**Dr Kingstone Mashingaidze** is a Senior Research Manager in the Agricultural Research Council-Grain Crops, Potchefstroom, South Africa: Review the manuscript.

**Dr Abe Shegro Gerrano** is a Senior Research Specialist in the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants, Pretoria, South Africa: Experimental design, data analysis, and review the manuscript.

**In Loving Memory**

This article is dedicated to our colleague Dr. Maletsema Alina Mofokeng who passed away on 21 April 2022.

**References**

Adebowale OJ, K Maliki (2011). Effect of fermentation period on the chemical composition and functional properties of pigeonpea seed flour. Int Food Res J 18: 1329-1333.

Aghaee M, R Mohammadi, S Nabovati (2010). Agro-morphological characterization of durum wheat accessions using pattern analysis. Aust J Crop Sci 4: 505-514.

Ajmal SU, MN Minhas, A Hamdani, A Shakir, M Zubair, Z Ahmad (2013). Multivariate analysis of genetic divergence in wheat (*Triticum aestivum*) germplasm. Pakistan J Bot 45: 643-1648.

Choudhary AK, S Kumar, BS Patil, JS Bhat, M Sharma (2013). Narrowing yield gaps through genetic improvement for *Fusarium* wild resistance in three pulse crops of the semi-arid tropics. SABRAO J. Breed. Genet 45: 341-370.

Des Marais LD, KM Hernandez, E Juenger (2013). Genotype- by-environment interaction and plasticity: exploring genomic responses of plants to the abiotic environment. Annu Rev Ecol Evol Syst: 44: 5-29.

Duran C, N Appleby, D Edwards, J Batley (2009). Molecular genetic markers: discovery,

applications, data storage and visualisation. Curr Bioinform 4: 16-27.

Faris DG, KB Saxena, S Mazumdar, U Singh (1987). Vegetable Pigeonpea: A promising crop for India. ICRISAT, Patancheru.

Gbaguidi AA, A Dansi, I Dossou-Aminon, DSJC Gbemavo, A Orobiyi, F Sanoussi,

H Yedomonhan (2018). Agromorphological diversity of local Bambara groundnut (*Vigna subterranea* (L.) Verdc.) collected in Benin. Genet Resour Crop Evol 65: 1159–1171.

Gerrano AS, WS Jansen van Rensburg, PO Adebola (2015). Genetic diversity of amaranthus species in South Africa. S Afr J Plant Soi 32: 39-

46.

Gerrano AS, WSJ Van Rensburg, I Mathew, AIT Shayanowako, MW Bairu, SL Venter, W Swart, A Mofokeng, JJ Mellem, M Labuschagne. (2020). Genotype and genotype x environment interaction effects on the grain yield performance of cowpea genotypes in dryland farming system in South Africa. Euphytica, 216: 80. https://doi.org/10.1007/s10681-020-02611 z.

Gerrano AS, A Moalafi, HA Seepe, S Amoo, H Shimelis. (2022). Nutritional and phytochemical ompositions and their interrelationship in succulent pods of pigeonpea (*Cajanus cajan* L.] Millsp.). Heliyon 8: e09078.

IBPGR (1994). International Board for Plant Genetic Resources, Rome, Italy.

Immad AS, KV Imran, AM Shakeel, MS Pukhta, AD Zahoor, L Ajaz (2018). Genetic Diversity by Multivariate Analysis Using R Software. Int J Pure Appl Biosci 6: 181-190.

Jaradat AA, MA Shahid (2006). Patterns of phenotypic variation in a germplasm collection of (*Carthamus tinctorius* L.) from the Middle East. Genet. Resour. Crop Evol 53: 225-

244.

Khan SA, J Iqbal, H Khurshid, N Saleem, MA Rabbani, M Zia, ZK Shinwari (2014). The extent of intra-specific genetic divergence in *Brassica napus* L. population estimated through various agro-morphological traits. Eur J Acad Res 2: 2255-2275.

Kasirivu J, S Materechera, M Dire (2011). Composting ruminant animal manure reduces emergence and species diversity of weed seedlings in a semi-arid environment of South Africa. South. S Afr J Plant Soil 28: 228-235.

Kimaro D (2016). Genetic Improvement of pigeonpea (*Cajanus cajan* (L.) Millsp.) for Fusarium wilt resistance in Tanzania. Ph.D. Thesis, University of Kwazulu-Natal, Pietermaritzburg, South Africa.

Kinhoégbè G, G Djèdatin, LEY Loko, RI Agbo, RK Saxena, RK Varshney, C Agbangla, A Dansi A (2020). Agro-morphological characterization of ppigeonpea (*Cajanus cajan* L. Millspaugh) landraces grown in Benin: Implications for breeding and conservation. J Plant Breed Crop Sci 12: 34-49.

Kovacic Z (19940. Multivariate analysis. Faculty of Economics. University of Belgrade. (In

Serbian). P. 293.

Linge SS, hv Kalpande, SL Sawargaonlar, BV Hudge, HP Thanki (2010). Study of enetic variability and correlation in interspecific derivatives of pigeonpea (*Cajanus*

*cajan* (L.) Millsp.). Electron J Plant Breed 1: 929-935.

Malik R, H Sharma, I Sharma, S Kundu, A Verma, S Sheoran S, R Kumar, R Chatrath (2014). Genetic diversity of agro-morphological characters in Indian wheat varieties using GT biplot. Aust J Crop Sci 8: 1266-1271.

Mallikarjuna N, KB Saxena, DR Jadhav (2011). *Cajanus.* In: Kole, C. (Ed.), Wild Crop Relatives: Genomic and Breeding Resources, Legume Crops and Forages. Springer-

erlag, Berlin, Heidelberg, pp. 21-33.

Mohammadi SA, BM Prasanna (2003). Analysis of genetic diversity in crop plants-salient

statistical tools and considerations. Crop Sci 43: 1235-1248.

Molope M (1987). Soil Aggregate Stability. The Contribution of Biological and Physiological

Processes. S Afr J Plant Soil 4: 121-126.

Mondal MAA (2003). Improvement of potato (*Solanum tuberosum* L.) through hybridization and in vitro culture technique. A PhD Thesis. Rajshahi University, Rajshahi, Bangladesh.

Muniswamy S, R Lokesha, P Dharmaraj, S Yamanura, JR Diwan (2014). Morphological

characterization and assessment of genetic diversity in minicore collection of pigeonpea

(*Cajanus Cajan* (L.) Millsp). Eur J Pharm Biopharm 5: 179-186.

Niranjana KB, PS Dharmaraj, VB Wali (2014). Genetic diversity and variability studies of

advanced breeding lines of pigeonpea (*Cajanus cajana*. L). Int J adv pharm Biol Sci 3: 404-409.

Nunes HF, FFH Freire, VQ Ribeiro, RLF Gomes (2014). Grain yield adaptability and stability of blackeyed cowpea genotypes under rainfed agriculture in Brazil. Afr J Agric Res 9: 255-261.

Odeny DA (2007). The potential of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa, Natural resources forum. Wiley Online Library.

Ojwang JD, RO Nyankanga, OM Olanya, DO Ukuku, J Imungi (2016). Yield components of vegetable pigeonpea cultivars. Trop Subtrop Agric Environ 67: 1-12.

Prasad Y, K Kumar, SB Mishra (2013). Studies on genetic parameters and interrelationships among yield and yield contributing traits in pigeonpea [*Cajanus cajan* (L.) Millsp.]. The Bioscan 8: 207-211.

Peeter J, JA Martinelli (1989). Hierarchical cluster analysis as a tool to manage variation in

germplasm collections. Theor Appl Genet 78: 42-48.

Qutadah SM, S Mehandi, IP Singh, F Singh (2019). Assessment of Genetic Diversity for Polygenic Traits in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. Int J Curr Microbiol Appl Sci 8: 1581-1588.

Rachovska G, d Dimova, B Bojinov (2003). Application of cluster analysis and principal

component analysis for evaluation of common winter wheat genotypes. Proc. Scientific session of jubilee 2002- Sadovo, 3: 68-72 (Bg).

RCoreTeam (2019). R: A Language and Environment for Statistical Computing, (“Action of the

Toes”) R Foundation for Statistical Computing, Vienna, Austria.

Reddy DSE, P Jayamani (2019). Genetic diversity in land races of pigeonpea (*Cajanus cajan* (L.) Millsp.). Electron J Plant Breed 10: 667-672.

Rupika K, KJR Bapu (2014). Assessment of genetic diversity in pigeonpea germplasm

collection using morphological characters. Eur J Pharm Biopharm 5: 781- 785.

Saxena, K.B., Kumar, R.V., Sultana, R. 2010. Quality nutrition through pigeonpea-

review. ICRISAT. 2: 1335-1344.

Saroj, S.K., Singh, M.N., Kumar, R., Singh, T., Singh, M.K. 2013. Genetic variability, correlation and path analysis for yield attributes in pigeonpea. The Bioscan 8: 941-

944.

SAS Institute. 2021. SAS/STAT user’s guide, version 9.2 SAS Institute, Cary, North Carolina,

USA.

Singh AK, S Swain, RK Gautam, PK Singh, AK Betal, T Bharathimeena, N Kumar, SD Roy (2014). Agro-morphological characterization of Bay Islands ppigeonpea (Cajanus cajan) landraces and advanced lines using under Islands conditions. 3rd International Conference on Agriculture and Horticulture, October 27-29, 2014 Hyderabad International Convention Centre, India.

Smith JSC, OS Smith (1989). The description and assessment of distance between inbred

lines of maize. The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34: 151-161.

Sodavadiya MS, JJ Pithia, AG Savaliya, A Pansuriya, VK Korat (2009). Studies on characters association and path analysis for seed yield and its components in pigeonpea (*Cajanus cajan* (L) Millsp). Legume Res 32: 203-205.

Syafii M, I Cartika, D Ruswandi (2015). Multivariate analysis of genetic diversity among some maize genotypes under maize-albizia cropping system in Indonesia. Asian J Crop Sci 7: 244-255.

Syed MQ, M Suhel, IP Singh, S Farindra (2019). Assessment of Genetic Diversity for Polygenic Traits in Pigeonpea [*Cajanus cajan* (L.) Millspaugh]. Int J Curr Microbiol Appl Sci 8: 1581-1588.

Upadhyaya HD, KN Reddy, CLL Gowda, S Singh (2007). Phenotypic diversity in the

pigeonpea (*Cajanus cajan*) core collection. Genet Resour Crop Evol 54: 1167-1184.

Van der Maesen LGJ (1990). Pigeonpea: origin, history, evolution and taxonomy. In: Nene L, Hall, SD, and. Sheilla VK (eds.), The pigeonpea, 15-46. C.A.B. International, Wallingford, UK. ISBN 0-85198:657-9.

Vales M, R Srivastava, R Sultana, S Singh, I Singh, G Singh, S Patil, K Saxena (2012). Breeding for earliness in pigeonpea: Development of new determinate and non-

determinate lines. Crop ScI 52: 2507-2516.

Virk PS, GS Khush, SS Virmani (2003). Breeding strategies to enhance heterosis in rice. In: Hybrid rice for food security, poverty alleviation and environmental protection, Virman, S.S., Mao, C.X., Hardy, B., (Eds.). International Rice Research Institute Los Banos, Philippines, 21-29.

Yohane EN, H Shimelis, M Laing, I Mathew, A Shayanowako (2020). Phenotypic divergence analysis in pigeonpea [*Cajanus cajan* (L.) Millspaugh] germplasm accessions. Agronomy, 10, 1682, https://doi.org/10.3390/agronomy10111682.