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# Assessment of genetic diversity among 32 pigeon pea genotypes using morphological traits and simple sequence repeat markers in Northern Ghana

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**Abstract** Thirty-two pigeon pea (*Cajanus cajan*) genotypes comprising of 26 exotic genotypes and six landraces were characterised with morphological and molecular markers to assess the extent of inherent genetic variability and agronomic performance in the Guinea Savannah ecology of northern Ghana. Twenty morphological traits (10 qualitative and 10 quantitative) and alongside 10 simple sequence repeat (SSR) markers were used for the characterisation. Result indicated highly statistical significant ($p < 0.001$) genotypic differences for ten quantitative traits. Grain yield ranged between 501.30 kg and 1280.00 kg with a mean of 653.32 kg. Pearson correlation analysis revealed the strongest character association ($r = 0.99$) between days to 50 % flowering (DFPF) and days to pod initiation (DPI) implying these traits can be selected together. Principal component analyses of the morphological traits revealed the first three principal components (PC's) accounting for 87.87% of the total variation with all the traits being captured by $PC_2$, apart from 100 seed weight. A total of 15 alleles with a range of 2 to 3 and a mean of 2.5 alleles per marker were generated from six polymorphic markers out of the 10 SSR markers tested. Polymorphic information content (PIC) ranged from 0.11 to 0.37 with mean of 0.25 for the six polymorphic markers. A dendrogram derived from Jaccard coefficient analysis of the morphological traits revealed two main clusters at 0.6 similarity level whilst for the molecular markers, four main clusters were obtained at 0.6 similarity level. Genotype ICEAP 00673-1 formed a solitary cluster based on both morphological and molecular data indicating its uniqueness at both levels of classification. The study revealed ample genetic diversity within the germplasm to warrant selection for further improvement in the breeding programme.

**Keywords:** Jaccard coefficient analysis, Morphological, Molecular markers, Genetic diversity, Polymorphic information content

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## Introduction

Pigeon pea (*Cajanus cajan* [L.] Millsp.) is an essential leguminous crop grown widely in the tropical and subtropical regions of the world. It is being cultivated worldwide on 6.99 million hectares area with a production of 5.96 million tonnes (FAOSTAT, 2018). It is a multi-purpose leguminous crop that can produce grain for human consumption, mulch or green manure for soil fertility maintenance and the fodder is being used as supplementary livestock feed for domestic livestock during the dry seasons (December-May). In Northern Ghana, pigeon pea is usually grown as a minor crop in the traditional crop mixtures but constitutes a major component of traditional farming systems (Padi, 2003). In addition, it is one of the crops that contributes significantly to fuel wood for many households in Northern Ghana. It is used as stakes when intercropped with yam thereby reducing deforestation as a result of cutting stakes for yam. This multipurpose leguminous crop can thrive under a wide range of edaphic factors such as stony to heavy clay loams of coarse texture and high moisture conditions if there is no stagnant water on the surface of the soil or excessive salinity of the soil (Tiwari *et al.*, 2012). This partly accounts for its cultivation in Northern Ghana where mostly the soils are poor, gravely, hilly and phosphorus and nitrogen deficient (Kombiok *et al*., 2012).

Pigeon pea productivity in northern Ghana is limited and constrained resulting to low yields (600-700 kg/ha), (Padi, 2003) as opposed to potential yield of 2000-3000 kg/ha reported elsewhere (Sharma and Jodha, 1982). This could be due to the inavailability of improved varieties in Ghana, low yield potential and long maturity periods of landraces (7-9 months), drought, inadequate and imbalanced fertilization, susceptibility to pests, flower drop, poor seed quality, poor taste and long cooking time (Padi, 2003). In order to increase the yield of the crop in Northern Ghana, there is the need to develop and make available to farmers as the new improved varieties that fit into the cropping season and with high yielding potential.

The genetic improvement of any crop largely depends on the magnitude of useful genetic differences within the germplasm for the traits of interest (Carvalho and Schaal, 2001; Ojuederie *et al.*, 2014), association of traits and selection of superior parents for hybridization and selection procedure used (Pandey *et al*., 2016). An effective breeding program hinges on parental diversity to obtain variable genes useful in genetic improvement of a cultivar. Therefore, at the beginning of any breeding programme, it is important to understand and assess the magnitude of the genetic diversity present in the available germplasm.

Genetic diversity in crops can be assessed at both the molecular and morphological levels (Adjebeng-Danquah *et al*., 2020; Adjebeng-Danquah *et*

*al*., 2016; Asare *et al*., 2011; Elameen *et al*., 2011 and Khoury *et al*., 2010). Morphological evaluation enables the breeder to assess the traits of agronomic importance for which improvement is necessary. Morphological characters are easy and rapid to score, and are very useful during the initial assessment of large number of germplasm (Asare *et al*., 2011; Elameen *et al*., 2011; Khoury *et al*., 2010). Morphological characters have successfully been utilised in studying genetic differences in several crops including, cowpea (Mafakheri *et al*., 2017), mucuna (Sathyanarayana *et al*., 2012), sweetpotato (Elameen *et al*., 2011), African yam bean (Ojuederie *et al.*, 2014) and cassava (Adjebeng-Danquah *et al*., 2016; Asare *et al*., 2011). However, the most morphological descriptors especially quantitative traits are highly influenced by the environment and genotype by environment interaction, they are not found to be reliable as markers (Adjebeng-Danquah *et al*., 2020).

A combination of both morphological and molecular characterisation gives better estimate of the genetic diversity within the population with minimal errors that normally evolve from environmental influence. Denwar *et al*. (2019) found that combining molecular characterization with phenotypic characters was more informative than either molecular or morphological characterization alone, especially when knowledge of population and identification of key phenotypic traits associated with diversity for hybridization and selection.

Therefore, this study was undertaken to ascertain the genetic diversity among 32 pigeon peas germplasm based on their morphological and genetic information and select genotypes useful in improvement of pigeon pea varieties for smallholder farmers in Northern Ghana.

## Materials and methods

### *Plant materials*

The planting materials consisted of twenty-six (26) exotic pigeon pea genotypes, which were obtained from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 2011), India and six landraces assembled from Northern Ghana (Table 1).

### *Experimental area and field evaluation*

The research was carried out at CSIR-Savanna Agricultural Research Institute experimental fields. This area locates in the Guinea Savannah Zone of Ghana at an altitude of 183 m at latitude 9° 25’’N and longitude 0°58’’W of the equator. The area is characterised with a single season of rainfall with an annual mean of 1000-1200 mm which usually begins in April and end in November but with July to September being the months of heavy and recurrent rains. The

soil is sandy loam developed from the Voltaian sandstone known as the Nyankpala series (CSIR-SARI, 2016). There is uniform temperature with a monthly mean of 23.4℃ as the minimum and 34.5℃ as the maximum. The relative humidity ranges from 46% to 76.8%. The planting was done in 2015 with three replicates using a randomised complete block design (RCBD). Each plot consisted of two rows of 6 m long, with 1.0 m X 0.60 m spacing between rows and within rows, respectively. Two seeds were planted per hole but later thinned to one per stand at three weeks after seedling emergence. Fertiliser was applied two weeks after sowing at the rate of 25- 60-30 kg/ha of N-$P_2O_5$-$K_2O$, respectively. Weed control was done manually using hoe after four weeks of planting and as and when was needed.

**Table 1.** List of pigeon pea genotypes used in this study and their source

| Entry | Genotype | Source | Special attribute(s) |
|---|---|---|---|
| 1 | ICEAP 01101-1 | ICRISAT, India | Early maturing |
| 2 | ICPL 87091 | ICRISAT, India | Multiple harvest within growing cycle |
| 3 | ICPL 86012 | ICRISAT, India | Early maturing |
| 4 | ICEAP 00652-2 | ICRISAT, India | Early maturing |
| 5 | ICEAP 01107-6 | ICRISAT, India | Early maturing |
| 6 | ICEAP 01284 | ICRISAT, India | Early maturing |
| 7 | ICEAP 01106-1 | ICRISAT, India | Early maturing |
| 8 | ICEAP 01101-2 | ICRISAT, India | Early maturing, big seed size |
| 9 | ICEAP 00604 | ICRISAT, India | Early maturing |
| 10 | ICEAP 01107-5 | ICRISAT, India | Early maturing, small seed size |
| 11 | Tamale 0002 | Tamale, Ghana | Taste good after cooking |
| 12 | Tamale 001 | Tamale, Ghana | Shrub is good for firewood |
| 13 | ICEAP 01130-3 | ICRISAT, India | Early maturing, high $N_2$ fixer |
| 14 | ICEAP 01133-1 | ICRISAT, India | High yielding |
| 15 | Nanton 001 | Nanton, Ghana | Tall and good for firewood |
| 16 | ICEAP 00659 | ICRISAT, India | High yielding |
| 17 | ICEAP 00661 | ICRISAT, India | Medium maturing |
| 18 | Kpalsogu 001 | Kpalsogu, Ghana | Big seed size, high biomass |
| 19 | ICEAP 011225 | ICRISAT, India | Medium maturing, high biomass |
| 20 | Nyankpala 002 | Nyankpala, Ghana | High biomass, tall plant |
| 21 | Nyankpala 001 | Nyankpala, Ghana | High biomass, drought tolerant |
| 22 | ICEAP 01179 | ICRISAT, India | Small seed size |
| 23 | ICEAP 01159 | ICRISAT, India | Big seed size |
| 24 | ICEAP 00902 | ICRISAT, India | High biomass, drought tolerant |
| 25 | ICEAP 01147 | ICRISAT, India | Drought tolerant, $N_2$ fixer |
| 26 | ICEAP 00673-1 | ICRISAT, India | Early maturing, high yielding |
| 27 | ICEAP 00540 | ICRISAT, India | Medium maturing |
| 28 | ICEAP 01154-2 | ICRISAT, India | Medium maturing |
| 29 | ICEAP 01172-2 | ICRISAT, India | Medium maturing |
| 30 | ICEAP 00911 | ICRISAT, India | Medium maturing |
| 31 | ICEAP 00557 | ICRISAT, India | Indeterminate growth habit |
| 32 | KAT 60/8 | ICRISAT, India | Tolerance to wilt and leaf spot disease |

### *DNA extraction and SSR genotyping*

Young fresh trifoliate leaves from 3 week-old plants were harvested from each pigeon pea genotype and bulked in transparent Ziploc bags and sent to the laboratory. Silica gel was used in drying the leaf samples at room temperature for 3 days with bead beaters used for grinding the samples into powdered form. The ground plant tissue (about 0.02 g) were transferred into Eppendorf tubes (2-ml) and the genomic deoxyribonucleic acid (DNA) was extracted from the plant tissue using the cetyl trimethylammonium bromide (CTAB) method (Tiwari *et al.*, 2012). A 2% of agarose gel stained with ethidium bromide was used to check the quality and quantity of the extracted DNA. The DNA samples were then diluted to 50 ng/µl prior to polymerase chain reaction (PCR) amplification. A set of 10 simple sequence repeats (SSRs) markers, with nucleotide length between 20 and 25 (Table 2) were used to genotype the pigeon pea accessions. Amplification of DNA was carried out in a 10-µl PCR reaction volume (5 µl of x2 VWR Red *Taq* DNA Polymerase master mix, 3 µl nuclease-free water, 1 µl of 10 mM primer [0.5 µl each of forward and reverse] and 1 µl of genomic DNA). The reaction was amplified in an ABI thermal cycler with the conditions of denaturation (94℃ /30 s), annealing X℃ [depending on primer (Table 2)]/30 s, and extension 72℃/30 s for a cycle length of 35. PCR products were resolved on a horizontal 6% polyacrylamide gel electrophoresis system at 120V for 3 hr. Ethidium bromide staining was employed and the image was captured for analysis.

**Table 2.** List of SSR markers and their sequence used in this investigation

| SSR name | Primer sequence |
|---|---|
| **PKS30** | F: AAGTGTGACACCCTCTACCC |
| | R: TGACATCGGGACATAGATAGAA |
| **CCac006** | F: ACATGTGTGGCGTAGTGTGA |
| | R: GCAAAACCGTTCCATAAAAA |
| **CCtc009** | F: ACAAATCCGGTGACCCATAA |
| | R: CCGAGAACAAAAACATTGAACA |
| **CCttc018** | F: ACAATTACTCAAATGCTCTCAACG |
| | R: TAAATGTCGCTTCCTATGATAGACC |
| **CCac029** | F: CGTGGACTAATCATCCCGTAA |
| | R: ATAATGCCAAAGGGGGAGAA |
| **CCB4** | F: GGAGCTATGTTGGAGGATGA |
| | R: CCTTTTTGCATGGGTTGTAT |
| **CCtta015** | F: AACACGCACCTCAATTCCA |
| | R: GAATGAGGAATGAAGGGACAAA |
| **CCttat001** | F: TACAGCAGCCACATCAAAGC |
| | R: TGAACCGTGAAAGTGGGATT |
| **CCB7** | F: CAACATTTGGACTAAAAACTG |
| | R: AGGTATCCAATATCCAACTTG |
| **CCB10** | F: CCTTCTTAAGGTGAAATGCAAGC |
| | R: CATAACAATAAAAGACCTTGAATGC |

### *Data collection and analysis*

#### Morphological data

Data were collected on four randomly selected and tagged plants in the middle of the two rows, using the International Board for Plant Genetic Resources (IBPGR, 1981) Descriptor List for pigeon pea. Seed coat colour, flower colour, stem colour, leaf shape, growth habit, leaf base shape, seed eye colour, pod colour, grain yield, 100 seed weight, days to first flower formation, days to 50 % flowering, days to first pod initiation, days to maturity, height at flowering, height at maturity were recorded for quantitative traits.

#### Gene diversity

Scoring of bands were employed to generate data for gene diversity. The DNA bands obtained at each locust were converted into one (1) as being present and zero (0) as being absent for the different base pairs (bp) that were obtained through the amplification. Cluster analysis using complete link and the Jaccard coefficient were performed to generate dendrogram of the different genotypes of pigeon peas.

#### Data analysis

Analysis of Variance (ANOVA) was performed on data collected on quantitative traits and Duncan's Multiple Range Test (DMRT) employed for assessing variability among accessions. Correlation analysis was performed to determine the degree of association between and among the traits. Cluster analysis were performed using Canberra, Furthest Neighbour Similarity Matrix to generate a dendrogram depicting genetic relationships among the genotypes. GenStat Statistical Software (version 12.0), Microsoft Excel Spreadsheet were used for both the morphological and gene diversity data. Genetic diversity parameters such as polymorphic information content (PIC), major allele frequency, heterozygosity and number of alleles per markers were obtained using PowerMarker V3.2.5 (Liu and Muse, 2005).

## Results

### *Quantitative traits of pigeon pea genotypes grown in Nyankpala*

In general, the genotypes exhibited wide range of variations for most of the characters measured (Table 3). Wider ranges were observed for days to flower initiation, days to pod initiation, days to 50 % flowering, height at flowering, height at maturity and grain yield per hectare whereas low ranges were recorded for number of branches at 14 weeks after planting (WAP), 100

seed weight and days to maturity. Grain yield ranged from 501.3 kg $ha^{-1}$ to 1280 kg $ha^{-1}$ with a mean of 653.3 kg $ha^{-1}$ and that of 100 seed weight ranged from 7.0 g-14.0 g (a mean value of 10.5g). Generally, the ANOVA for the ten traits showed extensive variability between the genotypes. There were highly significant ($P \leq 0.01$) differences among the tested genotypes signifying the existence of variability which can be exploited through selection. The coefficient of variations (CV) ranged from 0.7-18.8 % and could be ascribed to high levels of genotypic variation detected in most of the traits for the different genotypes. This correlates well with indices of experimental reliability (Gomez and Gomez, 1984).

**Table 3.** Phenotypic variability in quantitative traits of pigeon pea genotypes grown Nyankpala in 2015

| Trait | Minimum | Maximum | Mean | Std. | F.prob | CV% |
|---|---|---|---|---|---|---|
| Days to flower initiation | 51.0 | 148.0 | 103.79 | 31.87 | <.001 | 3.5 |
| Days to pod initiation | 65.0 | 159.0 | 127.85 | 34.78 | <.001 | 2.4 |
| Days to 50 % flowering | 79.0 | 160.0 | 131.17 | 31.25 | <.001 | 1.9 |
| No. of branches @14WAP | 22.3 | 51.0 | 36.13 | 7.56 | <.001 | 10.3 |
| Height @ flowering (cm) | 83.0 | 202.5 | 144.60 | 33.68 | <.001 | 10.8 |
| Height @ maturity (cm) | 94.7 | 276.0 | 192.84 | 45.31 | <.001 | 10.5 |
| 100 seed weight (g) | 7.0 | 14.0 | 10.46 | 1.58 | <.001 | 12.0 |
| No. of plants harvested | 2.0 | 61.0 | 10.56 | 8.44 | <.001 | 18.8 |
| Days to maturity | 180.0 | 202.0 | 191.71 | 5.64 | <.001 | 0.7 |
| Grain yield (kg/ha) | 501.3 | 1280.0 | 653.32 | 185.19 | <.001 | 17.4 |

<.001= *highly Significant at 1%, CV = Coefficient of variation*

### *Qualitative traits variations within the assembled pigeon pea germplasm*

Cluster analysis (CA) was performed using seed colour, base flower colour, stem colour, leaf shape, growth habit, leaf base, seed colour pattern, seed second colour, seed eye colour, pod colour and a dendrogram depicting variabilities and similarities amongst the 32 genotypes studied were plotted. Two major clusters of the genotypes were formed by the CA at a coefficient of similarity of 0.6. Cluster two had four genotypes while, cluster one contained 28 genotypes. Cluster one was further grouped into two sub-cluster at 0.66 similarity coefficient, of which cluster 1B had three genotypes, and 1A had 25 genotypes. There was no cluster formed at 1.0 similarity coefficient indicating that the genotypes used in this study were unique without duplications (Figure 1).

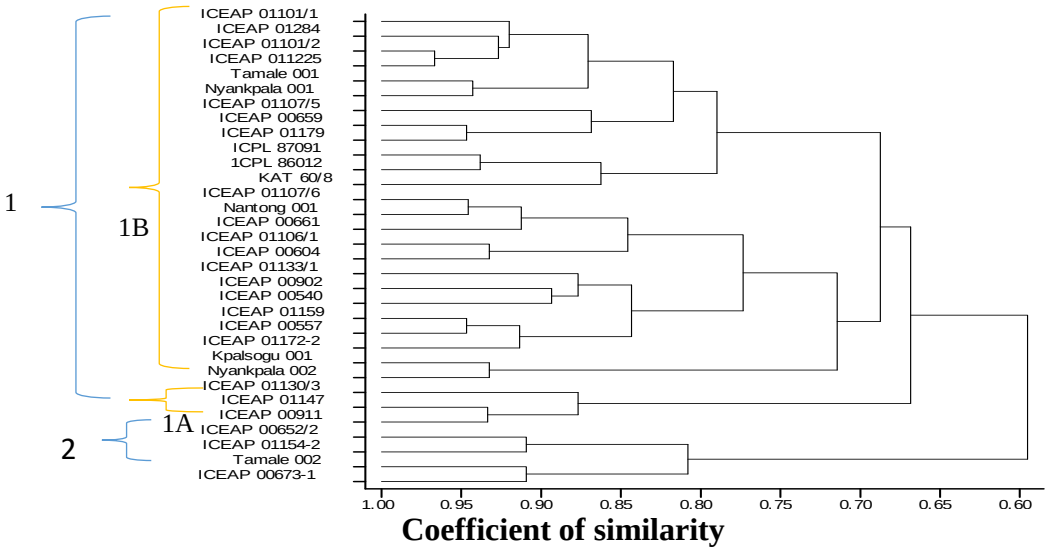


**Figure 1**. A dendrogram showing genetic relationships among 32 pigeon pea genotypes based on their morphological traits

***Correlation analysis of agronomic traits***

All the studied traits depicted positive associations (Table 4). Days to 50 % flowering (DFPF) positively correlated with all the other traits (r = 0.99). Height at maturity (HM) significantly and positively correlated (r = 0.80) with days to flower initiation (DFI). Number of branches (NB) significantly correlated with DFPF (r = 0.79); days to maturity (DM) (r = 0.76); DFI (r = 0.80) and height at flowering (HF, r = 0.77). However, DFPF was poorly correlated with grain yield (GY) (r = 0.23) and 100 seed weight (HSW) (r = 0.08), respectively. Similarly, NB was positively and significantly associated with HF (r = 0.89), HM (r = 0.86), DPI (r = 0.78), DFI (r = 0.66), and DM (r = 0.59), respectively. DM was positively and significantly correlated with four of the traits at $P \leq 0.05$. GY, HM and HF were all positively correlated with the other traits studied. Generally, GY and HSW were weakly correlated with all the traits studied.

***Principal component analysis of quantitative traits***

The first three principal components (PCs) accounted for 87.87 % of total variance with the first principal component (PC1) recording the highest (57.77 %). The second and third principal components (PC2 and PC3) accounted for 20.89 % and 9.20 % of the total genetic variance, respectively (Table 4). The Eigen values that show the relative discriminating power of the principal axes, which was relatively high for PC1 (5.77), medium for PC2

(2.09) and low (0.96) for PC3. PC1, which accounted for the highest proportion (57.77 %) of total variation mostly correlated with days to flowering initiation, days to pod initiation, days to 50 % flowering, branches at 14 WAP, height at flowering, height at maturity and days to maturity. PC2 accounted for 20.86 % of the total variation and was associated with number of plants harvested and grain yield. PC3 axis also contributed 9.20 % and was linked with days to pod initiation and 100 seed weight (Table 5).

**Table 4.** Pearson's correlations among nine quantitative traits of *Cajanus cajan*

| **Traits** | **DFPF** | **NB** | **DM** | **DPI** | **DFI** | **GY** | **HM** | **HF** | **HSW** |
|---|---|---|---|---|---|---|---|---|---|
| **DFPF** | - | | | | | | | | |
| **NB** | 0.79* | - | | | | | | | |
| **DM** | 0.76* | 0.59* | - | | | | | | |
| **DPI** | 0.99** | 0.78* | 0.77* | - | | | | | |
| **DFI** | 0.80* | 0.66* | 0.74* | 0.81* | - | | | | |
| **GY** | 0.23 | 0.41 | 0.07 | 0.22 | 0.09 | - | | | |
| **HM** | 0.80* | 0.86* | 0.71* | 0.79* | 0.73* | 0.27 | | | |
| **HF** | 0.77* | 0.89* | 0.69* | 0.75* | 0.70* | 0.27 | 0.89* | - | |
| **HSW** | 0.08 | 0.16 | 0.07 | 0.08 | 0.09 | 0.03 | 0.29 | 0.21 | - |

*$P \leq 0.05$;* Significant; ** Highly significant*

DFPF- days to 50 % flowering, NB- Number of branches at 14WAP, DM- Days to maturity, DPI- Days to pod initiation, DFI- Days to flower initiation, GY- Grain Yield (kg), HM- Height at maturity, HF- Height at flowering, HSW- 100 seed weight

**Table 5**. Principal components analysis of 10 quantitative characters showing their contributions to total variation among the 32 pigeon pea genotypes

| **Traits** | **$PC_1$** | **$PC_2$** | **$PC_3$** |
|---|---|---|---|
| Days to flower initiation | 0.377* | -0.188 | 0.218 |
| Days to pod initiation | 0.376* | -0.069 | 0.305* |
| Days to 50% flowering | 0.385* | -0.052 | 0.276 |
| Branches@14WAP | 0.353* | 0.241 | -0.302 |
| Height @flowering | 0.347* | 0.017 | -0.206 |
| Height @ maturity | 0.362* | 0.025 | -0.174 |
| 100 seed wt (g) | -0.220 | -0.040 | 0.718* |
| Plants harvested | 0.084 | 0.639* | 0.212 |
| Days to maturity | 0.364* | -0.234 | 0.191 |
| Grain yield (kg/ha) | 0.061 | 0.658* | 0.144 |
| Eigenvalue | **5.77** | 2.09 | **0.92** |
| % Variance | **57.77** | 20.89 | **9.20** |
| % Cumulative Variance | 57.77 | 78.67 | **87.87** |

*Values bolded and asterisked made substantial contribution to total variance in the respective axes. Maximum and least discriminating power (Eigen value), maximum and least percentage variance and maximum cumulative percentage variance values are bolded*

***Cluster analysis using molecular data***

Cluster analysis was obtained using complete link and the Jaccard coefficient shows two major clusters at 0.0 of similarity coefficient (Figure 2). Cluster two was solitary having one genotype (ICEAP 00673-1) and the other (cluster one) had the remaining 31 genotypes indicating these may be of the same ancestral or pedigree lines. Again, at 0.2 similarity coefficient, the cluster one was sub-grouped into 1A and 1B, where 1B contained one genotype (ICEAP 01107/5) and 1A contained the remaining 30 genotypes. At 0.5 similarity coefficient, cluster 1A again sub-clustered the genotypes into two, 1Ai which contained 23 genotypes and 1Aii which contained 7 genotypes which included the local genotype Nanton 001. At 1.0 similarity, sub-cluster 1Ai was again divided into six clusters in which the local accessions were found in a combination of two, three or solitary in a cluster.

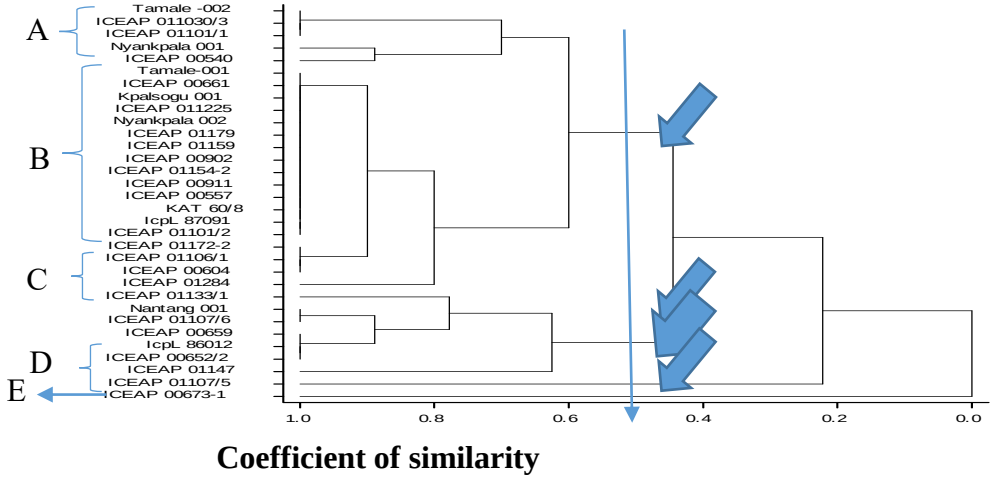


**Figure 2**. A dendrogram showing clustering pattern of 32 pigeon pea using present and absent of band in the amplification

***Genetic diversity parameters on 32 pigeon pea genotypes as shown by six polymorphic SSR markers***

Six out of the ten SSR markers representing 60% were polymorphic while the other 4 (40%) were monomorphic. The six polymorphic markers successfully amplified the polymorphic loci in all the 32 pigeon pea genotypes characterised. A total of 15 alleles were produced by six polymorphic markers with a range of 2 to 3 and a mean of 2.5 alleles per marker (Table 6). Out of the 6 polymorphic markers, 3 representing 50% were biallelic (2 alleles/locus)

while, the remaining 50% were multi-allelic (>2 alleles/locus). Major allele frequency for the six polymorphic markers ranged from 0.73 to 0.94 with marker CCtta015 recording the highest (0.94) whiles, CCB10 recorded the least (0.73). All the six polymorphic markers used in the study recorded major allele frequency above 0.70 and an average of 0.83. Gene diversity ranged from 0.12 for marker Cctta015 to 0.42 for marker CCB10 with an average gene diversity of 0.28. None of the markers recorded gene diversity above 0.5. Marker Ccac006 had the highest heterozygosity (0.41) whereas, the lowest (0.09) was recorded by CCB10. Polymorphic information content (PIC) ranged from 0.11 to 0.37 with means of 0.25 for the six markers which were polymorphic (Table 6). None of the six polymorphic markers had a PIC value greater than 0.5.

**Table 6**. Genetic diversity parameters on 32 pigeon pea genotypes as shown by six polymorphic SSR markers

| Marker | Major Allele Frequency | Allele Number | Gene Diversity | Heterozygosity | PIC |
|---|---|---|---|---|---|
| PKS30 | 0.88 | 3.00 | 0.22 | 0.19 | 0.21 |
| CCac029 | 0.87 | 2.00 | 0.23 | 0.27 | 0.21 |
| CCac006 | 0.80 | 2.00 | 0.32 | 0.41 | 0.27 |
| CCB4 | 0.79 | 3.00 | 0.34 | 0.39 | 0.29 |
| CCtta015 | 0.94 | 2.00 | 0.12 | 0.13 | 0.11 |
| CCB10 | 0.73 | 3.00 | 0.42 | 0.09 | 0.37 |
| Mean | 0.83 | 2.50 | 0.28 | 0.25 | 0.25 |

## Discussion

The presence of significant genetic variations among individuals for a trait in a population provides opportunity for selection for that trait of interest (Govindaraj *et al.,* 2015). The expression of variability is crucial for any program for crop improvement and the extent of variability for any character mostly defines the success of genetic improvement for specific traits (Singh *et al.,* 2015). Therefore, the occurrence of differences for quantitative and qualitative traits could be deployed for improvement of new and improved cultivars that are better performing. To control existing variability effectively, it is important to characterise germplasm for phenotypic and yield traits precisely. Morphological evaluation offers an easy and fast way for assessing the extent of diversity (Asare *et al.,* 2011).

In the present study, the phenotypic variability in quantitative traits of the different genotypes showed the existence of highly significant difference ($p \leq 0.001$) for all the 10 traits studied amongst the 32 pigeon pea. These findings indicate the presence of ample genetic variation amongst the evaluated pigeon pea genotypes, which provides the possibility for improvement through hybridisation and selection. This indicates that pigeon pea genotypes are

genetically diverse for quantitative traits and corroborates the findings of Bramel *et al.* (2004) detected significant differences in quantitative traits amongst 638 pigeon pea accessions. Several other studies have reported similar levels of genetic variability in crops like sesame, pigeon pea and okra (Menzir, 2008; Arameshwarappa *et al.,* 2009; Spandana *et al.,* 2011; Desawi *et al.,* 2014; Anokye *et al.,* 2014).

The cluster analysis grouped the genotypes into two major clusters and this might be attributed to the two sources from where the germplasms were collected. Cluster two contained four genotypes, of which one was from Ghana and the remaining three were from ICRISAT-Indian. Cluster one, which was sub-clustered into 1A and 1B had the genotypes from Ghana all clustered in 1B but in pairs. This may be attributed to the similarity in their geographical origins of collection or related ancestry. A similar observation was made in a genetic diversity of okra accessions in Ghana (Amoatey *et al.,* 2015). The genotypes ICEAP 00673-1, ICEAP 01101/1 and Tamale 002 were the most divergent and may provide variable genes useful in future pigeon pea improvement programmes through hybridisation.

Phenotypic correlations among the 32 pigeon pea genotypes for 10 traits showed intrinsic positive association between the variables. Days to pod initiation (DPI) exhibited strong positive correction with days to 50 % flowering (DFPF) (r = 0.99), number of branches (NB) (r = 0.78) and days to maturity (r = 0.77). This indicates that genotypes that flowered earlier will mature earlier, thus; maturity days of pigeon pea depend on when they flower. Pushpavalli *et al*. (2018) also reported similar findings when they studied genetic diversity in 49 pigeon pea genotypes. Grain yield exhibited strong but intermediate association with number of branches (r = 0.41). Similar results were previously reported by Sharma *et al.* (2012). Height at flowering positively and significantly correlated with number of branches (NB), height at maturity, days to 50 % flowering (DFPF), days to pod initiation (DPI), days to flower initiation (DFI) and days to maturity. This indicates that these yield-related traits can be selected, explored and exploited for developing high yielding pigeon pea genotypes by breeders. The yield-related traits were highly associated with each other confirming the findings of Sodavadiya *et al.* (2009) and Thanki *et al.* (2010). This suggests that it may be more effective to conduct indirect selection for yield-related traits via selection for a correlated trait than to select directly.

In this study, the first three PCs had Eigen values of 8.82 and explains 87.87 % of the total variation among the 32 genotypes. The plant traits that accounted for variation in the genotypes along the PC1 were morphological and phenological characters. Similar observations were reported by Hamid *et al*. (2011); Rekha *et al.* (2013) and Hemavathy *et al*. (2017) in pigeon pea. Yield

characteristics and seed yield such as grain yield, number of plants harvested, days to t pod initiation and 100 seed weight were the main traits that accounted for the variations observed in PC2 and PC3 axes, respectively and contributing significantly to the total variation in the genotypes.

The cluster analysis of genetic similarity obtained using complete link and the Jaccard coefficient from the molecular diversity study separated the genotypes into two main groups of clusters at 0% similarity. Similar trend was reported by Khoiriyah *et al. (*2018). One of the genotype formed solitary cluster which shows clear genetic separation from other clusters. At 100% similarity, the local accessions (Tamle 002, Nyankpala 001, Tamale 001, Kpalsogu 001, Nyankpala 002 and Nanton 001) were in the combinations of two, three or lonely in clusters A, B and C. It was observed that Tamale 002 and Nyankpala 001 were linked together in one cluster likewise Tamale 001, Kpalsogu 001 and Nyankpala 002. On the other hand, Nanton 001 was standing alone in a different cluster. The local accessions, Tamale 001 and Tamale 002 were collected from the Tamale Municipal, which shares boundary with Tolon District on the West where local accessions Nyankpala 001, Nyankpala 002 and Kpalsogu 001 were also collected. From this study, it may be concluded that these local accessions are of the same genetic background and were distributed among famers and their families across the two districts. Nonetheless, Nanton 001, which was collected from Nanton District does not share boundaries with these two districts hence was standing alone in a cluster without any of the local accessions shows its distinctiveness from the other local accessions linked in a cluster. The results of the present work corroborate the findings of Muniswamy *et al.* (2019) which indicated that genetic diversity could result from ecological differences or due to limited genetic crossability. The genotype ICEAP 00673-1, which formed the solitary cluster was obtained from ICRISAT in India and is characterised by early maturity, high yielding and higher 100 seed weight compared to the other genotypes. The distinctiveness of this genotype separated it from the other 31 genotypes indicating it can be a good resource for genetic improvement in the pigeon pea breeding programme.

The SSR markers, which successfully amplified the loci in the 32 pigeon pea genotypes had major allele frequencies ranging from 0.73 to 0.94. Hence, they can be classified as being polymorphic markers since they fulfil the requirement of being polymorphic markers. The least allele numbers identified by polymorphic SSRs was 2. This is in consonance with and Muniswamy *et al.* (2019) when they used SSR markers in pigeon pea molecular studies.

It concluded that the 32 pigeon pea genotypes (*Cajanus cajan*) exhibited significant genetic diversity in the both morphological and molecular levels based on their quantitative and qualitative traits studies. The cluster analysis grouped the genotypes into two major clusters at both morphological and

molecular levels. ICEAP 00673-1 was unique for both levels of classification and was distinct from all the other genotypes. The genotypes ICEAP 01101-1, ICEAP 00673-1 and Tamale 002 were the most divergent at both morphological and molecular levels. According to the principal component analysis, the major traits of importance that accounted for the greatest variability (PC1) were flowering initiation, days to pod initiation, days to 50 % flowering, branches at 14 WAP, height at flowering, height at maturity and days to maturity. These traits can be helpful in the selection of genotypes for the improvement of pigeon pea varieties for smallholder farmers in Northern Ghana.

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