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Diversity among selected pigeon pea accessions conserved at Genetic Resources Research Institute (GeRRI) in Kenya.

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Abstract: A study was carried out in 2022-2023 at KALRO-Perkerra, Marigat in Baringo County in Kenya to regenerate, bulk and characterize pigeon pea accessions conserved in the cold- room at the Genetic Resources Research Institute (GeRRI). Three hundred and fifty-two accessions were planted although two 246 germinated and grew to maturity. Quantitative data were collected on plant height, number of primary branches per plant, number of secondary branches per plant, days from planting to 50% flowering, days from planting to first mature pod, 100-seed weight and seeds per pod. Qualitative traits recorded were plant growth habit, terminal leaflet shape, leaf hairiness, flowering pattern, pod hairiness, seed shape and base seed colour. Most of the accessions (72.36%) had an indeterminate flowering habit. Plant height ranged from 105.8 to 441.8 cm with a mean of 297.98 cm. Highest variance was found in plant height followed by days to 50% flowering and days from planting to first mature pod. There was strong and positive correlation between plant height and days to 50% flowering (0.6145), plant height and days to first mature pod (0.6091) and between days to 50% flowering and days to first mature pod (0.9940). Only the first three principal components (PCs) were significant and they explained 69.165% of total variation. These three PCs were positively and strongly correlated with plant height, number of primary branches, number of secondary branches, days to 50% flowering, days to first mature pod and seeds per pod. Clustering based on the principal components grouped the pigeon pea accessions into two major clusters. The smaller cluster consisting of 51 accessions was positively correlated to PC1. The pigeon pea accessions conserved at GeRRI have significant variability among them. This variability can be used in classification of genotypes and selection of candidate parents for breeding purposes.

Keywords: Characterization; Pigeon peas; Principal Component Analysis; Quantitative traits.
Abbreviations: KALRO= Kenya Agricultural and Livestock Research Organization

Introduction

Pigeon peas, *Cajanus cajan* [L.] Millspaugh is a perennial legume belonging to the family fabaceae. India is the center of origin and primary center of diversity for pigeonpea (Saxena et al., 2014; Kaoneka et al., 2016); the crop moved to Africa about 4,000 years ago. It is mainly grown for its edible pods and seeds. The seeds can be harvested and eaten green or dry. Dried and split seeds are used to make a popular traditional Indian dish called toor dal. Pigeon peas are predominantly cultivated in the developing countries of tropical and subtropical environments (Suman et al., 2017). In India, pigeon pea stands next only to chickpea in terms of importance as a mainstream pulse crop (Bishnoi et al., 2019). In most African countries, pigeon pea seeds are used as food (mostly combined with cereals) or are grown for commercial purposes (Odeny, 2007; Ayenan et al., 2017).

Pigeon peas take a long time to produce flowers and then pods. They can survive for up to four or five years (making them "short-lived perennials") in good soil and weather conditions although they produce the best and highest yields in the first year. Pigeon peas grow well in hot humid climates with temperatures of between 18 and 38 ^{0}C (Odeny, 2007).

In Kenya, pigeon pea is widely grown in the Rift Valley, along the Coast, and in the dry Eastern and south eastern parts of the country. The traditional pigeon pea landraces take up to nine months to mature. Local plant breeders in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) have been able to develop medium duration varieties (maturing in 6 months) and short duration ones (maturing in 4 months) (CIMMYT, 2024). The medium and late maturing varieties have been hugely embraced by most famers in the dry parts of Kenya that are prone to frequent droughts and crop failures. Genetic Resources Research Institute (GeRRI) of the Kenya Agricultural and Livestock Research Organization (KALRO) has been conserving pigeon pea accessions collected from different parts in Kenya. The accessions have been conserved in the cold room at -20^{0}C for a long time without regeneration or characterization. Regeneration/characterization activities are normally carried out depending on availability of funds and the interests of the funding organization. Viability tests conducted at the GeRRI laboratory showed viability levels of the conserved pigeon pea accessions to be between 65 and 5%. With funding from World Bank through the Kenya Climate Smart Agriculture Project (KCSAP), the management at GeRRI decided to regenerate and characterize these pigeon pea accessions to forestall genetic loss. Therefore, a regeneration/bulking exercise was carried out at KALRO-Perkerra to renew, increase seeds quantities and characterize old pigeon pea accessions held at GeRRI coldroom.

Results and Discussion

Most of the pigeon peas had an erect and compact growth pattern (Figure 1). Zavinon et al. (2019) found that most of pigeon pea landraces from Benin had compact and erect growth habits (55.68%). Most plants in the current study had either lanceolate or narrow-elliptic terminal leaflet (Figure 2). The narrow leaves (lanceolate and narrow-elliptic) could be due to genetics or adaptation to high temperatures to minimize water loss through transpiration. The temperatures in the field could go as high as 35^{0}C. Over 72% of the accessions had indeterminate flowering pattern. Manyasa et al. (2008) found most Tanzanian pigeon pea landraces (59%) to have indeterminate flowering pattern. Almost half of the pigeon pea accessions in the current study had hairy leaves and pods (Table 1) although the two traits had a weak, positive correlation of 0.011915. Most seeds were globular in shape (42.68%) followed by oval (30.49%) while elongated shape was the least at 8.13% (Table 1). Daudu et al. (2020) found that most of the pigeon pea accessions had globular seeds (37%) followed by elongate and oval shapes both at 25% while the least were square shaped at 13%. Maletsema et al. (2021) found globular seed shape to be the most common (64.29%) among pigeon pea accessions sourced from ICRISAT in Kenya and Tanzania. Yohane et al. (2020) found the most common seed shapes to be square or angular, which were exhibited by 69.3% of the test genotypes sourced from Malawi, Tanzania and Kenya. Adegboyegun et al. (2020) found oval to be the most common seed shape (50%) among pigeon pea seeds sourced from ICRISAT in Niamey, Nigeria. Manyasa et al. (2008) found that oval seeds shape was the most common (48%) followed by globular shape (39%) among pigeon pea landraces collected in Tanzania. There is a wide diversity in popular pigeon pea seed colour in different regions of the world based mostly on the farmers' preferences. In the current study, cream and orange were the dominant seed colours at 29.67% and 28.05%, respectively (Table 1). Ayenan et al. (2017) reported a predominance of white seeded pigeon pea varieties in Benin; varieties with white or cream as the primary seed colour were the most grown not only in terms of allocated area but also in terms of number of households cultivating them. Yohane et al. (2020) found cream to be the major seed colour and was exhibited by 76.8% of the pigeon pea accessions collected from different sources. Manyasa et al. (2008) found cream to be the most common base seed colour (94%) among pigeon pea landraces collected from different regions in Tanzania. Zavinon et al. (2019) found light gray to be the most common seed colour (47.72%) among pigeon peas cultivated in Benin. Kinhoégbè et al. (2020) found cream coloured and oval-shaped seeds to be dominant among pigeonpea landraces grown in Benin. This suggests that landraces with these traits have been selected by farmers for a long period of time. Generally, heterogeneity for qualitative traits in pigeon pea germplasm depends often on its natural out-crossing rate (Upadhyaya et al. 2007) which ranges from 3% to 26% and varies according to locations, genotype, and the intensity of the insect population and time of flowering (Reddy et al. 2004). The highest level of phenotypic variation among quantitative traits in the current study was found in days to 50% flowering (CV = 43). Other traits which exhibited high variability among the accessions were number of primary branches and days to first mature pod (CV = 38) (Table 2). The wide variability was expected as these pigeon peas were collected from different places in Kenya. The number of primary branches were similar to those observed by Manyasa et al. (2008) among pigeon pea landraces from different regions in Tanzania. Most of the pigeon pea accessions in the current study were more than 200cm tall at 50% flowering stage (Figure 3). The great plant height was probably due to genetic constitution of the pigeon pea accessions enhanced by the high temperatures at Marigat. Silim et al. (1995) reported that there is reduction in pigeonpea plant height with decrease in temperature during the cropping season. Days from planting to 50% flowering peaked 61-80 days after planting (Figure 4). There was also strong and positive correlation between plant height and days to 50% flowering (0.6145), plant height and days to first mature pod (0.6091) and between days to 50% flowering and days to first mature pod (0.9940) (Table 3). Most tall plants took long to flower. The number of seeds per pod and 100-seed weight were negatively correlated (-0.1416). This implies that most pigeon pea accessions that had fewer seeds per pod had heavier seeds; however, this is not always the case as the correlation was weak. On the contrary, Bishnoi et al. (2019) found positive and significant correlation (0.375; $P<0.001$) between number of seeds per pod and 100 seed weight among pigeon pea accessions. In a previous study, Zavinon et al. (2019) found high and positive correlation ($r = 0.803$) between days to 50% flowering and plant height among pigeon pea accessions in Benin. Maletsema et al. (2021) found strong and positive correlation (0.701) between plant height and days to 50% flowering in pigeon peas. Bishnoi et al. (2019) also found positive and significant (0.428, $P<0.001$) correlation between plant height and days to 50% flowering. Kesha et al. (2016) found positive and significant correlation (0.345, $P<0.01$) between plant height and days to 50% flowering while Yohane et al. (2020) found moderate and significant correlations ($r = 0.44$, $P < 0.01$) between days to 50% flowering and plant height among pigeon pea accessions. Kinhoégbè et al. (2020) found positive and significant correlation between plant height and pod length ($r=0.92$, $P<0.001$), plant height and number of pods per plant ($r=0.91$, $P<0.001$), plant height and number of seeds per pod ($r=0.89$, $P<0.001$), and between plant height

Table 1. Frequency distribution of some qualitative traits in pigeon pea accessions.

Qualitative trait	Modalities	Frequency (%)
Flowering pattern	Indeterminate	72.36
	Determinate	27.64
Pod hairiness	Glabrous	51.63
	Pubescent	48.37
Leaf hairiness	gGabrous	44.72
	Pubescent	55.28
seed shape	Oval	30.49
	Globular	42.68
	Square	18.70
	Elongate	8.13
Base seed colour	Cream	29.67
	Grey	8.94
	Orange	28.05
	Light brown	21.95
	Reddish brown	11.38

Table 2. Variability among quantitative traits of pigeon peas.

Statistic	Plant height (cm)	Number of primary branches	Number of secondary branches	Days to 50% flowering	Days to first mature pod	100 seed weight (g)	Seeds per pod
Max	441.8	24	45	191	210	23.6	8
Min	105.8	4	4	34	42	8.23	3
Mean ± SD	297.98±78.49	10±4	23±8	90±39	100±38	15.33±3.52	5±1
Variance	6160.90	14	59	1503	1419	12.40	1
% CV	26.34	38	33	43	38	22.98	22

SD=Standard Deviation

Table 3. Pearson correlation coefficients among quantitative traits in pigeon pea accessions.

	Plant height	No. of primary branches	No. of secondary branches	Days to 50% flowering	Days to first mature pod	100 seed weight (g)	Seeds per pod
Plant height	1.0000						
No of primary branches	-0.0475	1.0000					
Number of secondary branches	0.2352	0.1500	1.0000				
Days to 50% flowering	0.6145	0.0132	-0.0004	1.0000			
Days to first mature pod	0.6091	0.0395	-0.0028	0.9940	1.0000		
100 seed weight (g)	0.1318	-0.0808	-0.0487	0.1567	0.1537	1.0000	
Seeds per pod	-0.0623	0.0401	-0.0370	-0.1068	-0.0998	-0.1416	1.0000

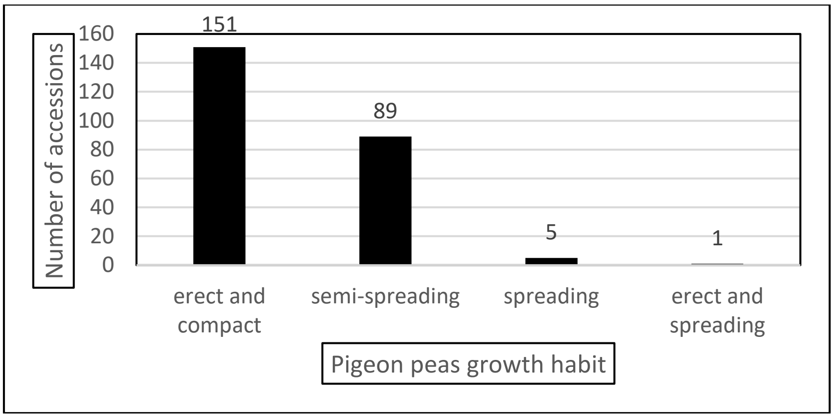

Figure 1. Growth patterns of the pigeon pea accessions.

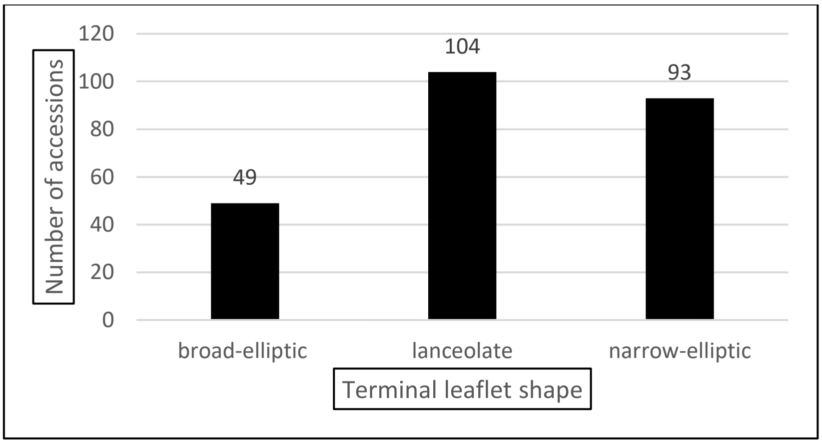

Figure 2. Terminal leaflet shape of the pigeon pea accessions.

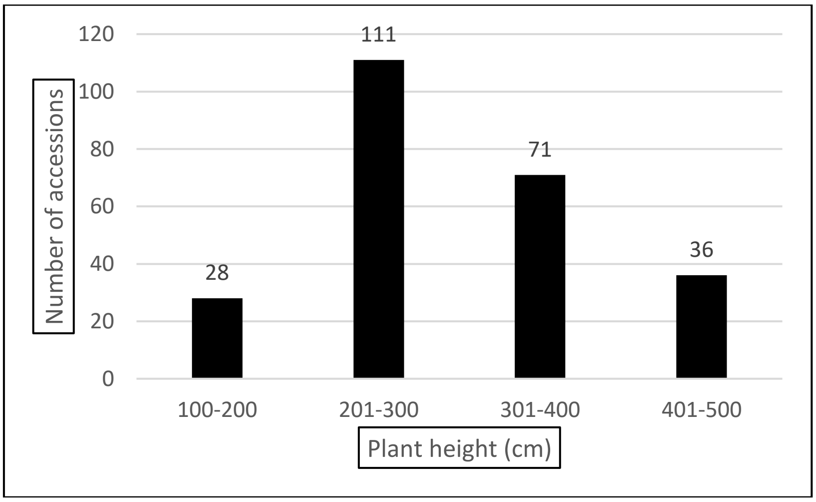

Figure 1. Plant height (cm) of pigeon pea accessions at 50% flowering.

Table 4. Eigenvalues for the pigeon pea accessions characterized.

Number	Eigenvalue	% variation explained	Cumulative % variation
1	2.5658	36.654	36.654
2	1.2204	17.435	54.089
3	1.0553	15.076	69.165
4	0.9090	12.985	82.150
5	0.8412	12.017	94.167
6	0.4026	5.752	99.919
7	0.0056	0.081	100.000

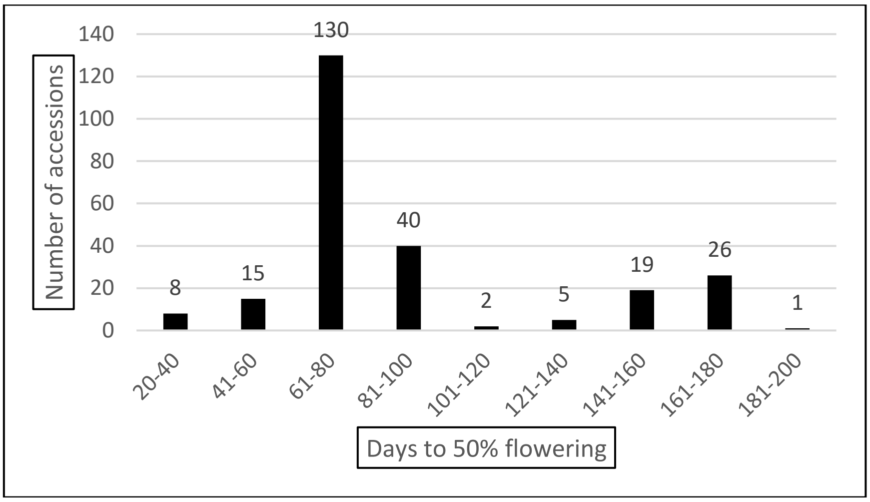

Figure 2. Days from planting to 50% flowering of the pigeon pea accessions.

Table 5. Eigenvectors (loading matrix) for the pigeon pea accession characterized.

Phenotypic characters	Principal components						
	1	2	3	4	5	6	7
Plant height	0.79223	0.15623	-0.04183	-0.29733	0.05431	-0.50483	-0.00022
No of primary branches	0.00029	0.65272	-0.07606	0.73395	0.12671	-0.11589	0.00142
Number of secondary branches	0.11304	0.67111	-0.52336	-0.43423	0.14096	0.23346	-0.00028
Days to 50% flowering	0.95499	-0.01586	0.17485	0.09472	-0.07954	0.19758	0.05316
Days to first mature pod	0.95238	-0.00098	0.18045	0.11683	-0.07419	0.19609	-0.05303
100 seed weight (g)	0.26892	-0.48860	-0.41264	0.16409	0.70088	0.02267	-0.00000
Seeds per pod	-0.18437	0.28394	0.73518	-0.20921	0.54706	0.04308	0.00031

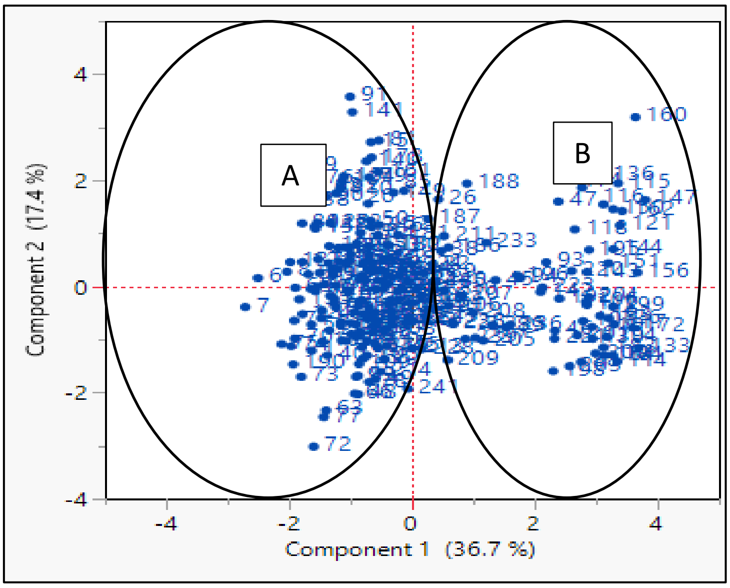

Figure 3. Scatter plot for first two principal components grouping pigeon pea accessions into two clusters.

Table 6. Mean performance of quantitative traits in different clusters of pigeon pea accessions.

Cluster	Plant height	No of primary branches	No of secondary branches	Days to 50% flowering	Days to 1st mature pod	Hundred seed weight	Seeds per pod
B	385.895	10.3025	23.41	162.19	170.3675	15.885	5.1925
A	274.595	9.244	23.571	72.373	82.626	15.242	5.221

and seed yields(tons/ha) (r=0.98, P<0.001). The first three principal components explained 69.165% of total variation (Table 4).

In principal component analysis, the first principal component (PC1) explained 36.654% of the total variation among the pigeon pea accessions while the second one explained 17.435% of total variation. If we consider only eigenvalues >1 as significant (Kaiser's rule) (Ng'uni, 2011; Sneath and Sokal, 1973; Jeffers, 1967), then only the first three principal components were significant and they explained 69.165% of the total variation. A high eigenvector loading for a trait points to the relatedness of that trait to the respective PC. The first principal component (PC1) showed strong positive correlation with plant height (0.79223), days to 50% flowering (0.95499) and days from planting to first mature pod (0.95238) (Table 5). Most of variation in PC1 was due to these traits. This PC is associated with tall plants; these plants took long to reach 50% flowering and to bear the first mature pod. In a previous study, Zavinon et al. (2019) found that PC1 was positively correlated with plant height (0.892), days to 50% flowering (0.823), number of primary branches 0.748), number of secondary branches (0.817), and 100-seed weight (0.547) in pigeon pea accessions cultivated in Benin. In the current study, the second principal component (PC2) was positively correlated with number of primary branches and number of secondary branches while the third principal component (PC3) was strongly correlated with number of seeds per pod (Table 5). These first three PCs represent traits that are associated with big pigeon pea accessions that take long to mature. In most cases, late maturity results in high yields as the accessions have longer time to accumulate dry matter. In addition, more branches produce more racemes/clusters and hence more pods, and seeds. Furthermore, the indeterminate flowering pattern prevalent in these accessions (72%) means that more pods are produced over time hence more yields. Consequently, traits with high coefficients in PC1 to PC3 should be considered as more important in phenotyping pigeon pea accessions since these principal axes explained 69.165% of the total variation. These traits could be used to indirectly select high yielding accessions; especially if these traits have high heritability and are positively correlated. In the current study there was positive correlation between plant height and days to 50% flowering, plant height and days to first mature pod, plant height and number of secondary branches, and between plant height and 100 seed weight. Consequently, these traits can be improved together. The negative correlation between plant height and number of primary branches report (-0.0475) was very weak and may not have significant effects during selection. Zavinon et al. (2019) found positive correlation between plant height and number of primary branches (r= 0.581, P<0.001) and between plant height and number of secondary branches (r= 0.619, P<0.001). They also found found high broad-sense heritability for days to 50% flowering (69.06%) and moderate heritability for number of secondary branches (37.95%) and 100-seed weight (34.88%) among pigeon pea landraces in Benin. Pushpavalli et al. (2018) found high broad-sense heritability for days to 50% flowering (89.4%), days to maturity (89.5%) and 100 seed weight (61.7%), and moderate heritability for plant height (48.0 %), number of primary branches (49.7%) and number of secondary branches (41.5%). Manyasa et al. (2008) found medium broad-sense heritability for number of primary branches (0.58) and 100 seed mass (0.63), and high heritability for days to 50% flowering (0.75) and plant height (0.82). Bishnoi et al. (2019) found high broad sense heritability estimates for number of seeds per pod (0.94), plant height (0.88), days to 80% maturity (0.88) and days to 50% flowering (0.87), moderate for primary branches per plant (0.52) and low for 100 seed weight (0.14). In addition, they found high genetic advance for pod length (24.55), number of primary branches per plant (21.28) and plant height (19.47). Traits with high heritability are the most reliable as germplasm descriptors (Abu-Alrub et al., 2004), since they are less influenced by environmental effects. Consequently, plant height, days to 50% flowering, number of primary branches and 100 seed mass could be used for pigeonpea germplasm classification and selection; in addition to the more stable qualitative traits. Ayenan et al. (2017) reported that seed colour, maturity and plant height were the predominant criteria used by farmers in Benin to classify and identify pigeonpea varieties. Manyasa et al. (2009) reported seed size and maturity as the most important criteria used by Ugandan pigeonpea producers to discriminate their varieties. Clustering based on the principal components grouped the pigeon pea accessions into two major clusters (Figure 5). The smaller cluster (B) consisting of 51 accessions was positively correlated to PC1. This means that most accessions in this cluster were tall and took more days to reach 50% flowering and to produce first mature pod (Table 6). The bigger cluster (A) showed no clear correlation to any of the principal components. Accessions 91 and 141 showed strong positive correlation to PC2. Accession 91 (GBK 004286) had a mean of 24 primary branches and 40 secondary branches while accession 141 (GBK 042198) had a mean of 20 primary branches and 34 secondary branches. Accession 160 (GBK 042104) had a strong positive correlation to both PC1 and PC2. This accession was one of the tallest at 407 cm, had 19 primary branches and 45 secondary branches. In addition, it took 178 days to reach 50% flowering and 188 days from planting to produce the first mature pod. In contrast, accession 72 (GBK 004263) was negatively correlated to both PC1 and PC2. This accession was 121 cm tall at 50% flowering, it had 9 primary branches and 4 secondary branches. In addition, it took 64 days from planting to 50% flowering and 78 days from planting to first mature pod. The two clusters (A and B) were not different from each other in terms of number of primary branches, number of secondary branches, 100 seed weight and number of seeds per pod (Table 6).

Materials and Methods

Site description

The study was conducted at the Apiculture and Beneficial Insects Research Institute (ABIRI)'s experimental farm located at Perkerra (KALRO-Perkerra) in Marigat area of Baringo County, Kenya. The ABIRI is one of the 16 semi-autonomous institutes of KALRO. Marigat and the surrounding areas experience arid and semi-arid climatic conditions. The rainfalls are erratic with an average of 450-700 mm while potential evapotranspiration is 1360 mm. The area traditionally has bimodal rainfall pattern, a long rainy season occurs from March to May while intermittent short rains fall in September, October, November, and December. The mean air temperature ranges from 16.8 to 32.4 ^{0}C with an average of 24.6 ^{0}C. The high temperature increases evaporation in the region, making it dry for a better part of the year (Okuku et al., 2024). Consequently, crop production at KALRO-Perkerra and the neighbouring Perkerra Irrigation Scheme depend mainly on water from river Perkerra. Furrow irrigation is the norm. The soils at KALRO-Perkerra are volcanic fluvisols of sandy/silty clay loam texture (Muthoni et al., 2024).

Plant materials

Three hundred and fifty-two pigeon pea accessions conserved in the cold at GeRRI were identified for regeneration/bulking. The accessions were selected because they were old with very low viability and had few seeds per accession. They had been conserved for over 15 years and had not been bulked/regenerated although the seed samples were small.

Field management

The seeds were planted at KALRO-Perkerra, Marigat in Baringo County between 2nd and 6th of August 2022. Pigeon peas were planted on the shoulder of irrigation ridges at a spacing of 1 meter between the ridges and 1 meter from plant to plant within a ridge. Each plot consisted of four ridges five meters long giving a population of 24 plants per plot. Small shallow holes were dug along the ridge shoulders at a spacing of 1 meter. Di-ammonium phosphate (DAP) was applied into the planting holes at a rate of 10 grams per hole and mixed thoroughly with the soil before planting. Two pigeon pea seeds were planted in each hole and covered lightly with soil. Three days before planting, the field was irrigated because it had not rained; furrow irrigation is the only one used at KALRO-Perkerra. The rains came two days after planting was completed which assured us of good crop emergence. Despite the good rains that fell intermittently for the next four days, the crop germination was uneven and field establishment was poor. This necessitated gapping after the first weeding. The gapped seeds also had uneven germination and poor crop establishment. This could be due to low seed viability as the seeds were really old. Consequently, only two hundred and forty-six accessions grew to maturity in the field. In the early stages of crop growth, weeding was done severally because the weeds were growing fast and vigorously due to the high temperatures. There was need to keep the field weed-free as the young plants had low vigour. Furrow irrigation was done as needed. Pests and diseases were controlled using appropriate pesticides and agrochemicals.

Data collection and analysis

In addition to regeneration/bulking, some basic characterization was done to enhance the value of the accessions. It has been reported that lack of basic characterization/evaluation information about the accessions conserved in genebanks is one of the reasons for the low uptake/utilization of the germplasm by the clientele (Khoury et al., 2010). The following data were collected when 50% of the plants in a given accession had flowered: plant height, number of primary branches per plant, number of secondary branches per plant, days from planting to 50% flowering, plant growth habit, terminal leaflet shape, leaf hairiness and flowering pattern. Days from planting to 50% flowering was taken on the whole plot while data on the other traits were recorded from ten randomly selected plants in each plot. Terminal leaflet shape was taken on the terminal leaflet of the third primary branch from the ground on the selected plants. Other traits recorded at different times were days from planting to first mature pod, pod hairiness, base seed colour, seed shape, seeds per pod and 100 seed weight (g). Base seed colour, seed shape and 100 seed weight (g) were taken after seed harvesting and shelling. Characterization was carried out according to the descriptors for pigeon peas (IBPGR and ICRISAT, 1993).

Data were then entered in excel and analyzed using the SAS Statistical software (SAS, 2003). Descriptive statistics (frequency, range, mean, standard deviation, and coefficient of variation) were computed. In addition, correlation coefficients were analysed in order to assess the relationship among the 7 quantitative traits. Quantitative data were standardized and subjected to principal component analysis (PCA). The PCA and correlation matrices were used to explore the links between the quantitative traits and the principal components. Cluster analysis was performed on principal components to assess the level of dissimilarity among the accessions.

Conclusion

The pigeon pea accessions conserved at GeRRI have significant variability among them. Highest variance was found in plant height followed by days to 50% flowering and days from planting to first mature pod. The strong positive correlation between plant height and days to 50% flowering and between plant height and days to first mature pod means these traits can be improved together. Only the first three principal components were significant and they explained 69.165% of total variation. These three PCs were positively and strongly correlated with plant height, number of primary branches, number of secondary branches, days to 50% flowering, days to first mature pod and seeds per pod. Variability in these traits can be used in classification of genotypes and selection of candidate parents for breeding purposes.

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