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Phenotypic Divergence Analysis in Pigeonpea [*Cajanus cajan* (L.) Millspaugh] Germplasm Accessions

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Abstract: Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is an important source of grain protein for low-income countries such as Malawi. Knowledge of the genetic diversity in pigeonpea is essential for an effective breeding program. The study objective was to assess the genetic diversity among diverse pigeonpea accessions to select complementary and unique genotypes for breeding. Eighty-one pigeonpea accessions were evaluated in six environments in Malawi using a 9 × 9 alpha-lattice design with two replications. The cross-tabulation analysis revealed a significant genotype variation on plant growth, flower, and seed traits. The combined analysis of variance identified genotypes MWPLR 14, ICEAP 01170, ICEAP 871091, and ICEAP 01285 as early maturing varieties, while Kachangu, MWPLR 16, TZA 5582, No. 40, and MWPLR 14 were identified as high-yielding genotypes. The correlation analysis revealed a significant positive correlation between grain yield and a hundred seed weight (HSWT) ($r = 0.50$, $p < 0.01$), suggesting the usefulness of this trait for selection. The nonlinear principal component analysis identified grain yield (GDY), days to 50% flowering (DTF), days to 75% maturity (DTM), number of pods per plant (NPP), number of racemes per plant (NRP), 100 seed weight (HSWT), leaf hairiness (LH), and number of seeds per pod (NSP) as the most discriminated traits among the test genotypes. The cluster analysis using morphological traits delineated the accessions into three clusters. The selected high-yielding and early-maturing genotypes may be recommended as parental lines for breeding and grain yield improvement in Malawi or similar agro-ecologies.

Keywords: agronomic performance; correlation analysis; malawi; pigeonpea; yield stability

1. Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh, 2n = 2x = 22) is an essential cash and food crop in the tropical and subtropical regions of the world. It is a multi-purpose crop that is cultivated mainly for its edible grains that are high in dietary protein and essential amino acids such as leucine (16.48 g/kg), tyrosine (14.77 g/kg), and arginine (13.51 g/kg) [1].

Pigeonpea is an essential component of the agriculture systems in semi-arid ecologies due to its adaptation to growing with relatively low rainfall and with poor soil fertility. It has a deep root system and a unique ability to maintain optimal osmotic adjustment under limited water conditions [2]. Pigeonpea can fix atmospheric nitrogen in the soils through symbiosis with species of *Rhizobium* bacteria depositing up to 200 kg of nitrogen per hectare in agricultural lands [3,4]. Thus, pigeonpea has important roles in enhancing food security and livelihoods, especially during drought years, and providing ecosystem services through nitrogen fixation and soil health improvement.

Pigeonpea accounts for 5% of the world's pulse production [5]. India is the largest producer of pigeonpea, accounting for 25% of the world's production, followed by Myanmar and Malawi [6]. In Malawi, pigeonpea accounts for more than 22% of total legume production and ranks as the 3rd most important legume crop after groundnut and common beans. The grain productivity of pigeonpea in Malawi is low (≈700 kg ha^{-1}) compared to its potential yield of 2500 kg ha^{-1} [7]. The yield gap is due to various constraints, including insect pests and diseases, drought stress, and a lack of improved cultivars. The breeding and deployment of improved cultivars can enhance pigeonpea production and productivity. The successful development of improved cultivars with the client and market-preferred traits depends on the availability of adequate genetic variation.

Reportedly, modern pigeonpea cultivars and varieties exhibit relatively low levels of genetic diversity [8]. The loss of genetic diversity is due to continuous artificial selection and breeding for a few targeted economic traits to meet the market requirements [9]. Hence, there is a need to initiate pre-breeding programs in the target production environments through divergence breeding involving modern and obsolete cultivars, landraces, and wild relatives that possess desirable traits. This will broaden the genetic diversity of pigeonpea through gene recombination and effective selection [9]. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and various national and regional improvement programs are actively involved in genetic improvement and conservation of the pigeonpea. Diverse pigeonpea collections are preserved globally, including by ICRISAT, the International Institute of Tropical Agriculture (IITA), and the Svalbard Global Seed Vault in Norway. These genetic resources can be used for pigeonpea improvement and breeding programs globally [10].

To date, only seven pigeonpea cultivars have been released in Malawi. These cultivars were introductions from ICRISAT [7] developed in Kenya with germplasm from eastern and southern Africa (ESA). The ESA region is recognized as a secondary center of genetic diversity for pigeonpea. The introduced cultivars are poorly adapted to local farming conditions in Malawi and lack farmer-preferred traits such as good cooking quality, resistance to pod borers, and high yield potential. Therefore, the development of high performance, locally adapted pigeonpea cultivars is an important target in Malawi. This requires a range of genetic resources and crosses to integrate adaptive and functional traits, according to the needs and preferences of farmers and the value chain. Introduced germplasm can provide useful genetic resources that can be introgressed into locally adapted germplasm to improve economic traits such as high yield, early maturity, and pest and disease resistance, among others [9]. Evaluating accessions maintained by the public and private breeding sectors within the ESA region provides an opportunity to identify stable and high-yielding genotypes for selection.

Many pigeonpea genotypes have been collected and maintained at the Department of Agricultural Services in Malawi for breeding purposes. The genotypes are adapted to the ESA region and possess valuable attributes including good cooking quality, insect pests, and disease resistance, but they are limited by their poor yield performance. The key traits present in the local and introduced germplasm should be assessed for pre-breeding and breeding purposes. Hence, the objectives of the study were to determine the genetic diversity among pigeonpea accessions in selected target production environments in Malawi to select complementary and unique genotypes for breeding.

2. Materials and Methods

2.1. Plant Materials

The study evaluated 81 pigeonpea genotypes comprising 28 landraces, 6 released cultivars, and 47 advanced elite lines (Table 1), which were sourced from the Department of Agricultural Research Services (DARS)/Malawi and the Tanzania Agriculture Research Institute (TARI) and the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)/Kenya. The landraces were included as checks for adaptation to local conditions and possessing farmers' traits, while the elite lines provide important genetic resources, since Tanzania and Kenya have more advanced pigeonpea-breeding programs. The released cultivars provided a benchmark against commercial standards that are currently in production.

Table 1. Description of the pigeonpea genotypes used in the study.

Code	Genotype Designation	Description	Source	Origin	Code	Genotype Designation	Description	Source	Origin
G1	ICEAP 0673/1	Breeding line	ICRISAT	Kenya	G42	ICEAP 87105	Cultivar	ICRISAT	Kenya
G2	ICEAP 00554	Breeding line	ICRISAT	Kenya	G43	MWPLR 16	Landrace	GENEBANK	Malawi
G3	ICEAP 01164/1	Breeding line	ICRISAT	Kenya	G44	TZA 2496	Landrace	TARI	Tanzania
G4	MWPLR 19	Landrace	GENEBANK	Malawi	G45	TZA 5582	Landrace	TARI	Tanzania
G5	MWPLR 22	Landrace	GENEBANK	Malawi	G46	TZA 5596	Landrace	TARI	Tanzania
G6	ICEAP 01170	Breeding line	ICRISAT	Kenya	G47	Chitedze Pigeonpea 2	Cultivar	DARS	Malawi
G7	ICEAP 01169	Breeding line	ICRISAT	Tanzania	G48	MWPLR 7	Landrace	GENEBANK	Malawi
G8	TZA 2439	Landrace	TARI	Tanzania	G49	Babati	Landrace	TARI	Tanzania
G9	MWPLR 9	Landrace	GENEBANK	Malawi	G50	TZA 5557	Landrace	TARI	Tanzania
G10	MWPLR 6	Landrace	GENEBANK	Malawi	G51	MWPLR 14	Landrace	ICRISAT	Kenya
G11	MWPLR 17	Landrace	GENEBANK	Malawi	G52	ICEAP 01101/1	Breeding line	ICRISAT	Kenya
G12	TZA 253	Landrace	TARI	Tanzania	G53	TZA 2456	Landrace	TARI	Tanzania
G13	MWPLR 1	Landrace	GENEBANK	Malawi	G54	TZA 5464	Landrace	TARI	Tanzania
G14	MWPLR 18	Landrace	GENEBANK	Malawi	G55	ICEAP 01101/2	Breeding line	ICRISAT	Kenya
G15	TZA 2464	Landrace	TARI	Tanzania	G56	ICEAP 01285	Breeding line	ICRISAT	Kenya
G16	ICEAP 00604	Breeding line	ICRISAT	Kenya	G57	MWPLR 25	Landrace	GENEBANK	Malawi
G17	TZA 2509	Landrace	GENEBANK	Malawi	G58	ICEAP 87091	Breeding line	ICRISAT	Kenya
G18	ICEAP 01146/1	Breeding line	ICRISAT	Kenya	G59	TZA 2692	Landrace	TARI	Tanzania
G19	MWPLR 11	Landrace	GENEBANK	Malawi	G60	TZA 2807	Landrace	TARI	Tanzania
G20	TZA 5555	Landrace	TARI	Tanzania	G61	ICEAP 00068	Breeding line	ICRISAT	Kenya
G21	No. 40	Landrace	TARI	Tanzania	G62	TZA 2785	Landrace	TARI	Tanzania
G22	ICEAP 01150	Breeding line	ICRISAT	Kenya	G63	MWPLR 10	Landrace	GENEBANK	Malawi
G23	MZ2/9	Breeding line	TARI	Tanzania	G64	ICEAP 00612	Breeding line	ICRISAT	Kenya
G24	ICEAP 01172/1	Breeding line	ICRISAT	Kenya	G65	MWPLR 21	Landrace	GENEBANK	Malawi
G25	ICEAP 01103/1	Breeding line	ICRISAT	Kenya	G66	TZA 2514	Landrace	TARI	Tanzania
G26	MWPLR 24	Landrace	GENEBANK	Malawi	G67	TZA 2466	Landrace	TARI	Tanzania
G27	ICEAP 01155	Breeding line	ICRISAT	Kenya	G68	ICEAP 01179	Breeding line	ICRISAT	Kenya
G28	ICEAP 01180/2	Breeding line	ICRISAT	Malawi	G69	MWPLR 13	Landrace	GENEBANK	Malawi
G29	MWPLR 4	Landrace	GENEBANK	Malawi	G70	MWPLR 2	Landrace	GENEBANK	Malawi
G30	Kachangu	Cultivar	DARS	Malawi	G71	TZA 250	Landrace	DARS	Malawi
G31	Mwayiwathualimi	Cultivar	DARS	Kenya	G72	MWPLR 3	Landrace	GENEBANK	Malawi
G32	MWPLR 8	Landrace	ICRISAT	Malawi	G73	TZA 5541	Landrace	TARI	Tanzania
G33	ICEAP 01154/2	Breeding line	ICRISAT	Kenya	G74	MWPLR 23	Landrace	GENEBANK	Malawi
G34	Chitedze Pigeonpea 1	Cultivar	DARS	Malawi	G75	ICEAP 00979/1	Breeding line	ICRISAT	Kenya
G35	ICEAP 01164	Breeding line	ICRISAT	Kenya	G76	TZA 197	Landrace	TARI	Tanzania
G36	Bangili	Landrace	TARI	Tanzania	G77	MWPLR 20	Landrace	GENEBANK	Malawi
G37	ICEAP 00053	Breeding line	ICRISAT	Kenya	G78	HOMBOLO	Landrace	TARI	Tanzania
G38	MWPLR 12	Landrace	GENEBANK	Malawi	G79	ICEAP 86012	Breeding line	ICRISAT	Kenya
G39	TZA5463	Landrace	TARI	Tanzania	G80	ICEAP 01106/1	Breeding line	ICRISAT	Kenya
G40	MWPLR 5	Landrace	GENEBANK	Malawi	G81	Sauma	Cultivar	DARS	Malawi
G41	MWPLR 15	Landrace	GENEBANK	Malawi					

ICRISAT = International Crops Research Institute for the Semi-Arid Tropics, DARS = Department of Agricultural Research Services, TARI = Tanzania Agricultural Research Institute.

2.2. Study Sites

Field experiments were conducted in Malawi at three sites, Bvumbwe, Chitedze, and Makoka Research Stations, during the 2017/18 and 2018/19 cropping seasons. The geographic location, altitude, weather, and soil characteristics of the study locations are presented in Table 2. Each season and site combination presented unique environmental conditions due to variations in temperature, rainfall, and agronomic practices. Therefore, due to site × season combinations, six environments were identified for evaluating the genotypes: Bvumbwe during 2017/18 (Environment 1), Bvumbwe in 2018/19 (Environment 2), Chitedze in 2017/18 (Environment 3), Chitedze in 2018/19 (Environment 4), Makoka in 2017/18 (Environment 5), and Makoka in 2018/19 (Environment 6).

Table 2. Physical and weather characteristics of the study locations.

Site	Latitude	Longitude	Altitude (Masl)	Soil Texture	Rainfall (mm)		Min Temp (°C)		Max Temp (°C)	
					2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
Bvumbwe	15°55′ S	35°04′ E	1228	Sandy clay loam	975.2	1442	16.2	17.9	22.6	24.9
Chitedze	13°59′ S	33°38′ E	1146	Sandy clay	929.8	693.4	18.5	20.2	24.7	29.4
Makoka	15°32′ S	35°11′ E	1029	Sandy clay loam	566.6	1184.8	16.3	15.6	23.2	28.2

Masl = meters above sea level, mm = millimeters, min = minimum, max = maximum, temp = temperature, °C = degrees Celsius.

2.3. Experimental Design and Data Collection

The experiment at each site was laid out in an alpha-lattice design with two replications. Each genotype was planted on a plot consisting of two rows. The rows were 5 m in length and 0.90 m apart, giving a plot size of 4.5 m^2. Seeds were planted at 0.75 m apart within a row. Three seeds were planted per planting station and thinned to one plant two weeks after emergence. All agronomic practices were applied following standard practices for pigeonpea production in Malawi [7]. Both qualitative and quantitative phenotypic traits' data were collected as presented in Table 3 according to pigeonpea descriptors of the International Board for Plant Genetic Resource (IBPGR) and International Centre for Research Institute for Semi-Arid Tropics (ICRISAT) [11].

2.4. Statistical Analysis

Data collected on qualitative traits (Table 3) were subjected to frequency distribution and cross-tabulation analyses using SPSS for Windows 25.0 [12].

The quantitative data from each variable were tested for homogeneity of variances using Bartlett's test and data normality using the Shapiro–Wilkes test before the analysis of variance (ANOVA). Subsequently, the data were pooled across sites and subjected to a combined analysis of variance following the alpha lattice procedure in Genstat 18th edition [13]. The total variance was partitioned into genotype (σ^2g), environment (σ^2e), and genotype by environment (σ^2ge) components based on the mean squares derived from the partial analysis of variance adapted from [14]. Correlation and principal component analyses were performed using Genstat 18th edition [13] to determine influential components and trait relationships. A nonlinear principal component analysis was conducted in SPSS (SPSS 2016). The nominal variables (qualitative traits) were transformed using the categorical principal component analysis (CATPCA) procedure described by [15]. The nonlinear PCA can standardize both quantitative and qualitative data to deduce their associations and identified the most important components.

Table 3. Descriptors for the pigeonpea qualitative and quantitative traits.

Traits	Code	Description
Qualitative Traits		
Plant habit	PH	1 = Compact (erect), 2 = semi-spreading (semi-erect) or 3 = spreading
Flower streak pattern	FSP	0 = no streaks, 1 = sparse, 2 = medium and 3 = dense streaks, 4 = uniform coverage of second color
Flower base/main color	FBC	1 = ivory (green white), 2 = light yellow, 3 = yellow, 4 = orange, 5 = red, 6 = purple
Leaf shape	LS	1 = ovate, 2 = triangular, 3 = trullate
Leaf hairiness	LH	1 = hairy, 2 = non-hairy
Pod form	PF	1 = flat, 2 = cylindrical
Pod color	PC	1 = green, 2 = purple, 3 = mixed (green +purple) and 4 = dark purple
Seed color pattern	SCP	1 = plain, 2 = mottled, 3 = speckled, 4 = mottled and speckled, 5 = ringed
Seed main color	SMC	1 = white (yellow white), 2 = cream (gray white), 3 = orange, 4 = brown, 5 = grey, 6 = purple, 7 = black
Seed eye color	SEC	1 = purple, 2 = light brown, 3 = reddish brown, 4 = gray/dark, 5 = cream/white
Seed shape	SSH	1 = Oval, 2 = pea-shape, 3 = square/angular, 4 = elongate
Quantitative Traits		
Plant height	PH	Measured in cm from plant base to the tip of the main stem
Days to 50% flowering	DTF	Number of days from sowing until when 50% of the plants have at least one open flower
Primary branches	PBR	The average number of primary branches of 10 randomly selected and tagged plants
Secondary branches	NSB	The average number of secondary branches of 10 randomly selected and tagged plants
Days to 75% maturity	DTM	Number of days from sowing until when 75% of the pods in a plot turn brown
Number of seeds per pod	NSP	The average number of pods per plant from 10 randomly selected and tagged pods
Number of pods per plant	NPP	The average number of pods from 10 randomly selected and tagged plants
Number of racemes per plant	NRP	The average number of racemes from 10 randomly selected and tagged plants
Grain yield (t/ha)	GYD	Weight of the grain harvested in a plot extrapolated to t/ha
100 seed weight (g)	HSWT	Weight of a random sample of 100 grain

3. Results

3.1. Genotype Variation Based on Qualitative Traits

Significant variations were exhibited among genotypes for all assessed qualitative traits ($p < 0.001$) such as growth habit, flower main color, flower streak pattern, pod color, and seed traits (Table 4, Figure 1A–D). A large proportion of test genotypes (61.9%) were semi-spreading, followed by spreading (26.6%) and compact (11.5%) in growth habits. A majority of the test genotypes (64.9%) had yellow flower color (Table 4, Figure 1A), while 16.8% had purple flowers, 13.6% had ivory flowers, and 7.4%

had light yellow flowers (Table 4, Figure 1A). A large population of the genotypes (60.5%) had no flower streaks, and the rest of the genotypes had sparse, medium, dense, and uniform coverage streaks at 8.1%, 1.9%, 14.5%, and 15%, respectively (Table 4, Figure 1B). About 48.7% of the genotypes had a green pod color, while 33.9% had a mixed pod color and 7.1% had purple pods (Table 4, Figure 1C). A majority of the genotypes (76.8%) had a cream seed coat color, while 11% had a brown seed coat color and the rest had gray, orange, and purple seed coat colors (Table 4, Figure 1D). About 70.2% of the test genotypes had a brown seed eye, and 20.7% had a purple seed eye, while the remainder had gray or cream seed eyes. The most common seed shape was square or angular shapes, which were exhibited by 69.3% of the test genotypes.

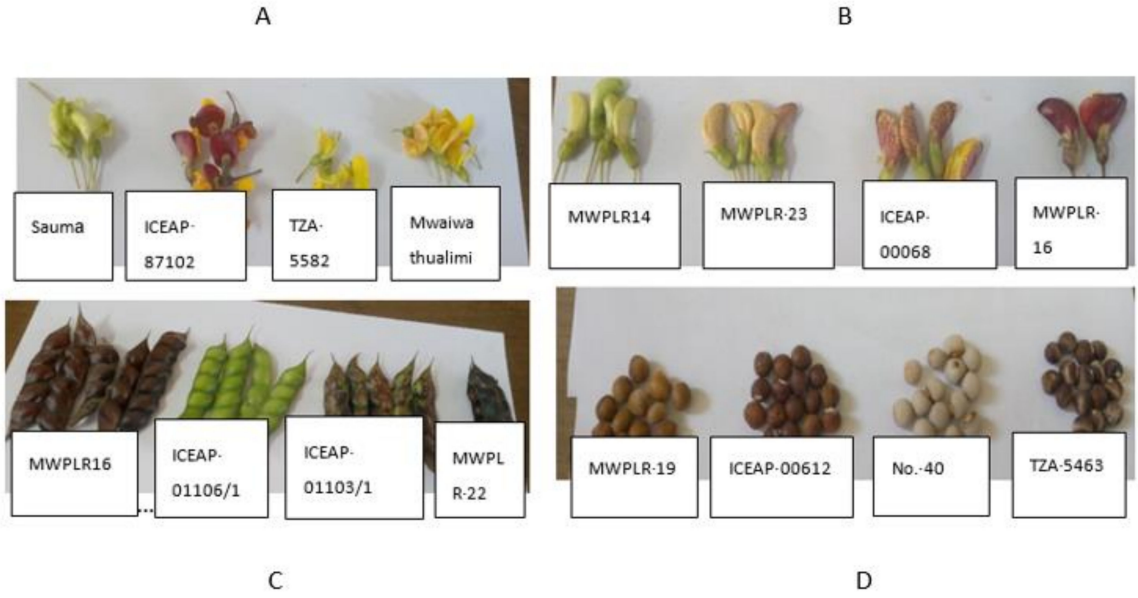

Figure 1. Genetic variability for some qualitative traits in pigeonpea genotypes: (**A**) flower color: genotype Sauma (ivory), ICEAP 87105 (purple), TZA 5582 (yellow), Mwaiwathualimi (light yellow); (**B**) flower streak pattern: genotype MWPLR 14 (no streak), MWPLR 23 (medium streaks), ICEAP 00068 (dense streaks), MWPLR 16 (uniform coverage); (**C**) pod color: genotype MWPLR 16 (purple), ICEAP 01106/1 (green), ICEAP 01103/1 (mixed), MWPRL 22 (dark purple) and (**D**) seed coat color: genotype MWPLR 19 (orange), ICEAP 00612 (brown), No. 40 (cream), and TZA 5463 (purple).

3.2. Genotype and Environment Variances for Quantitative Traits

The quantitative agronomic data were pooled across sites after applying tests for homogeneity of variance and normality. The genotype × environment interaction effects were significant ($p < 0.001$) for grain yield (GYD), days to 50% flowering (DTF), days to 75% maturity (DTM), plant height (PH), number of primary branches (NPB), number of pods per plant (NPP), number of racemes per plant (NRP), 100 seed weight (HSWT), and number of secondary branches per plant (NSB) (Table 5). The genotype and environment had significant ($p < 0.001$) effects on all assessed traits except the NSP. The site × season × type interaction effects were significant ($p \leq 0.001$ and $p = 0.05$, respectively) for DTM, PH, and NSP (Table 6). The site × type interaction effects were only significant ($p = 0.01$ and $p = 0.05$) for DTM, NPP, NRP, and HSWT, respectively. However, season × type interaction effects were not significant for all the variables except for GYD, which was highly significant.

Table 4. Frequency distribution and significance tests among 81 pigeonpea genotypes assessed based on qualitative traits.

Trait	Description	Frequency (%)	DF	Chi-Square	Genotype Code [a]
Growth habit	Compact	11.5	160	304.52 **	G53, G2, G1, G27, G26
	Semi-spreading	61.9			G63, G50, G28, G70, G76, G80, G51, G78, G49, G32, G62, G39, G67, G5, G8, G13, G72, G24, G74, G3,32, G22, G4, G40, G30, G52, G56, G48, G79, G36, G23,G16, G77, G7, G71, G44, G67, G46, G69, G33, G54, G20, G43, G42, G71, G62, G65,G39, G69, G17, G18, G59
	Spreading	26.6			G45, G41, G29, G49, G56, G64, G37, G60, G15, G11, G65, G75, G81, G44, G67, G11, G46
Flower color	Ivory	13.6	240	910.08 ***	G78, G40, G36, G27, G33, G80, G51
	Light yellow	7.4			G13, G5, G31
	Yellow	64.9			G50, G45, G70, G53, G76, G72, G24, G74, G3, G22, G4, G58, G68, G18, G19, G17, G9, G62, G29, G32, G65, G21, G52, G1, G56, G37, G48, G79. G23, G16, G61, G77, G7, G71, G44, G15, G67, G11, G69, G65, G75, G20, G43, G26, G71, G44, G15, G67, G62, G11, G46, G65
	Purple	16.8			G63, G28, G41, G56, G60, G25, G46, G54, G26, G42
Flower streak pattern	No streaks	60.5	320	589.69 ***	G17, G53, G36, G12, G15, G37, G20, G60, G9, G54, G11, G66, G55, G80, G81, G71, G73, G23, G1, G65, G21, G18, G7, G13, G51, G62, G48, G49, G58, G14, G32, G16, G2, G27, G22, G6, G57, G10, G31, G8, G39, G30
	Sparse streaks	8.1			G49, G69, G42, G33, G28, G5, G70
	Medium sparse	1.9			G72, G74
	Dense streaks	14.5			G47, G61, G29, G60, G34, G40, G45, G67, G45, G68, G63, G77, G19
	Uniform coverage	15			G79, G50, G76, G59, G25, G46, G78, G38, G51, G75, G26, G35, G52, G56, G41, G43
Pod color	Green	48.7	240	647.43 ***	G73, G42, G1, G24, G74, G75, G52, G16, G65, G21, G18, G7, G13, G62, G17, G47, G61, G15, G20, G29, G44, G72, G60, G64, G9, G11, G66, G55, G80, G71, G58, G14, G27, G6, G57, G10, G8, G19
	Purple	7.1			G76, G45, G67, G38
	Mixed (green + purple)	33.9			G81, G70, G53, G36, G61, G43, G37, G34, G54, G79, G50, G40, G25, G33, G46, G42, G51, G4, G68, G26, G49, G3, G35, G32, G69, G2, G63, G22, G56, G77, G41, G30
	Dark purple	10.3			G31, G28, G39, G48, G59, G43

Table 4. *Cont.*

Trait	Description	Frequency (%)	DF	Chi-Square	Genotype Code [a]
Seed color pattern	Plain	56.6	240	841.57 ***	G59, G80, G5, G18, G6, G53, G65, G62, G35, G34, G67, GG4, G60, G66, G21, G70, G36, G42, G40, G14, G50, G66, G20, G79, G49, G2, G3, G69, G56, G81, G47, G72, G15, G44
	Mottled	15.3			G41, G25, G34, G48, G28, G78, G23, G31, G9, G37, G57
	Speckled	22.2			G75, G68, G43, G38, G10, G19, G52, G58, G51, G73, G59, G76, G16, G29, G13, GG3, G17, G8, G54, G1, G24, G7, G71, G27, G12, G22, G55, G77
	Mottled + speckled	5.9			G46, G33, G30, 632, G39, G45, G26
Seed main color	Cream	76.8	320	1049.31 ***	G75, G68, G59, G43, G5, G18, G6, G38, G10, G53, G65, G63, G35, G19, G34, 52, G72, G15, G44, G22, G55, G57, G77, G60, G58, G78, G32, G73, G51, G70, G36, G16, G29, G42, G40, G23, G14, G17, G8, G50, G66, G20, G49, G54, G2, G3, G69, G1, G24, G45, G7, G9, G71, G81, G12, G47
	Orange	3			G4, G46, G25
	Brown	11			G64, G76, G63, G30, G34, G48, G28, G31, G37, G26
	Gray Purple	6.2 3			G80, G66, G67, G56 G39, G33, G41
Seed shape	Oval	30.7	80	480.21 ***	G75, G22, G5, G25, G38, G53, G35, G34, G28, G73, G51, G70, G36, G29, G42, G40, G31, G8, G18, G49, G3, G45, G37, G28, G27, G12, G55, G57
	Square/angular	69.3			G15, G44, G22, G77, G68, G59, G43, G46, G80, G18, G33, G30, G41, G6, G10, G65, G62, G19, G34, G67, G4, G52, G48, G60, G58, G66, G32, G64, G76, G21, G16, G13, G23, G14, G63, G17, G39, G52, G66, G79, G54, G2, G69, G1, G24, G56, G7, G9, G71, G81
Seed eye color	Purple	20.7	240	848.32 ***	G68, G5, G34, G25, G60, G78, G51, G64, G76, G21, G16, G29, G42, G40, G31, G50, G49, G2, G69, G24, G81, G55, G57
	Light brown	70.2			G75, G59, G43, G46, G18, G33, G30, G41, G6, G10, G53, G65, G62, G35, G19, G34, G67, G52, G48, G58, G28, G66, G32, G73, G36, G23, G14, G17, G39, G74, G20, G79, G54, G1, G46, G45, G9, G71, G37, G27, G12, G47, G15, G44, G22
	Gray/dark	1.2			G25
	Cream	7.5			G80, G38, G63, G8, G7, G26

DF = degrees of freedom, ** and *** = significance at 0.01 and 0.001 levels, respectively; [a] see genotype codes (G1–G81) in Table 1.

Table 5. Mean squares and significant tests for grain yield and yield components measured in 81 pigeonpea genotypes across six environments in Malawi.

Source of Variation	DF	DTF	DTM	PH	NPB	NSB	NRP	NPP	NSP	GYD	HSWT
Location	2	9024.2 ***	8735.4 ***	54,965 ***	114.4 ***	93.7 *	226.9 ***	3236 **	22.5 ***	5,968,860 ***	1008.1 ***
Replication (Rep)	1	701.9 ns	289 ns	118 ns	1.2 ns	105.4 *	14,646 ns	9810 *	0.45 ns	1,663,232 *	9.5 ns
Block (Rep)	8	3168.5 ***	5703.4 ***	7710.9 ns	52.9 *	93.7 *	9099 *	6433.6 **	2.4 *	16,534,356.5 ***	72.2 **
Genotype (G)	80	879.2 ***	1234.9 ***	2137 ***	12.5 *	30.9 *	5004.9 *	1990.3 *	0.8 ns	351,745.3 *	16.8 *
Season (S)	1	3370.5 **	2945.3 *	447 ns	409.6 ***	650.1 ***	2,023,492 ***	437.5 ***	31.5 ***	30,308,789 ***	50.2 *
G × L	160	243 *	361.9 *	1106 *	18 *	35.6 *	6150.9 *	1916.1 *	0.9 *	360,816.9 *	20.7 **
G × S	80	3610.3 ns	606.9 ns	1198 ns	17.9 *	34.7 *	4642.7 ns	1060.3 *	0.9 ns	400,468.2 *	14.9 ns
G × L × S	160	330.6 ns	484.9 ns	744 ns	15.2*	34.5 *	6110.9 ns	1502.8 *	0.7 ns	919,105.3 ns	16.2 ns
Residual	469	345.4	585.8	1243.1	14.5	11.8	5822.9	5667.2	0.8	313,554	15.4

DF = degrees of freedom, Rep = replication, DTF = days to 50% flowering, DTM = days to 75% maturity, PH = plant height, NPB = number of primary branches, NSB = number of secondary branches per plant, NRP = number of racemes per plant, NPP = number of pods per plant, NSP = number of seeds per pod, GYD = grain yield, HSWT = 100 seed weight, *, ** and *** = significance at 0.05, 0.01 and 0.001 probability levels, respectively.

Table 6. Mean squares and significant tests for grain yield and yield components among the three categories of pigeonpea genotypes.

Source of Variation	DF	DTF	DTM	PH	NPB	NSB	NRP	NPP	NSP	GYD	HSWT
Site	2	9167 ***	8020 ***	55,114 ***	108.6 ***	93 *	1,309,332 ***	118,174 ***	22.54 ***	1.658 ***	80.16 ***
Rep	1	717	328	111	2.7	109	14184	9836	0.45	1.651	0.26
Rep (Block)	16	123	82	9769	33.3	71	14908	21,509 ***	1.35	0.206	11.78
Season	1	3797 **	3625 *	407	433.3 ***	4672 ***	2,043,617 ***	440,237 ***	31.51 ***	3.092 ***	0.04
Type	2	1629 *	4725 **	44,433 **	18.5	14	4686	5891 *	40.2 ***	2.087 ***	20.09
Site*Season	2	2523 **	700	55,081 ***	910.5 ***	253 ***	1,018,464 ***	149,039 ***	36.09 ***	6.38 ***	39.42 *
Site*Type	4	114	4385 **	1257	17	29	988,914 *	8167 ***	0.41	0.078	45.83 *
Season*Type	2	161	388	3023	9.9	2	642	380	1.94	2.006 ***	6.44
Site*Season*Type	4	676	7883 ***	65,810 ***	3.1	44	1771	2032	30.17 *	0.2	1.82
Residual	937	386	619	1177	15.5	31	5823	1682	0.79	0.149	12.45

DF = degrees of freedom, Rep = replication, DTF = days to 50% flowering, DTM = days to 75% maturity, PH = plant height, NPB = number of primary branches, NSB = number of secondary branches per plant, NRP = number of racemes per plant, NPP = number of pods per plant, GYD = grain yield, HSWT = 100 seed weight, *, ** and *** = significance at 0.05, 0.01 and 0.001 probability level.

3.3. Mean Performance of Pigeonpea Genotypes across the Test Environments

Tables 7–10 summarize the mean values and statistics for eight quantitative traits recorded from three locations in two seasons. The tables presents the best ten, and the bottom five genotypes on DTF, DTM, PH, NPB, NRP, NPP and HSWT ranked on grain yield response. The mean DTF and DTM were 112 and 157 days, respectively (Table 7). Genotype MWPLR 14 was the earliest to attain 50% flowering and maturity at 74 and 113 days, which was followed by ICEAP 01170 at 85 and 125 days, ICEAP 87091 at 85 and 132 days, ICEAP 01285 at 87 and 133 days, and ICEAP 01169 at 91 and 137 days, respectively. Sauma was among the latest genotypes to flower and mature at 145 and 205 days, respectively. There were marked genotype differences in plant height that varied from 125.3 to 202.4 cm (Table 8). The mean plant height of the test genotypes was 167.5 cm. The shortest genotype across the testing environments was ICEAP 87105. The tallest genotypes with plant heights exceeding 180 cm were Kachangu, No. 40, ICEAP 01106/3, ICEAP 00068, TZA 5596, MWPLR 6, Sauma, and ICEAP 00053. The mean number of the primary branch of the test genotypes was 15 (Table 8). The most productive genotypes with many primary branches per plant were MWPLR 12, MWPLR 20, ICEAP 01170, and MWPLR 23, with 19, 18, 17, and 17 primary branches per plant, in that order. The mean number of pods per plant varied from 67 to 144, with a grand mean of 94 pods per plant (Table 9). The highest number of pods per plant was 144, 134, 126, 124, and 123 observed on the genotypes Kachangu, MWPLR 16, TZA 5582, No. 40, and MWPLR 14, in that order. The number of seeds per pod exhibited non-significant differences among the assessed genotypes. The mean number of grains per pod was five. There was a wide genetic variation for grain yield ranging from 0.5 to 1.8 t ha^{-1} with a mean of 1.1 t ha^{-1} (Table 10). Accessions No. 40, MWPLR 14, and MWPLR 16 were the three best performing genotypes with mean yields of 1.8, 1.7, and 1.7 t ha^{-1}, respectively. The lowest grain yield response was 0.5 t/ha recorded for the genotypes ICEAP 00604 and ICEAP 01285. The 100 seed weight ranged from 11.0 to 17.3 g/100 seed (Table 10). Accessions MWPLR 22, TZA 5582, and MWPLR 14 expressed the highest HSWT ≥ 17 g/100 seed.

Table 7. Mean values for 10 quantitative traits among the ten top best and five bottom performing genotypes after evaluating 81 genotypes in six environments in Malawi.

Genotype	DTF							DTM						
	Y1			YII			Mean	YI			YII			Mean
	S1	S2	S3	S1	S2	S3		S1	S2	S3	S1	S2	S3	
Top Ten Genotypes														
21	129	131	141	124	131	132	131	173	191	211	158	176	176	181
43	125	105	119	117	105	105	113	177	166	172	156	161	154	164
51	63	65	64	87	67	98	74	95	105	102	127	116	132	113
30	100	97	118	128	116	118	113	133	150	164	159	159	164	155
45	107	96	91	128	101	124	108	143	158	146	170	153	165	156
81	163	127	155	132	165	130	145	215	201	254	171	211	178	205
17	147	120	125	109	120	106	121	182	167	174	156	160	147	164
66	120	95	115	116	108	116	111	155	151	170	154	158	161	158
74	118	78	123	113	115	118	110	163	145	166	153	165	163	159
20	116	120	129	122	120	127	122	143	163	175	156	160	172	161
Bottom Five Genotypes														
39	113	90	131	85	90	88	99	149	144	195	127	150	122	147
13	126	117	109	116	107	115	115	167	166	153	145	154	155	156
50	117	77	107	116	77	115	101	141	136	156	155	137	149	145
42	114	102	127	120	102	120	114	145	154	172	164	166	162	160
79	124	101	122	117	127	119	118	168	153	165	152	179	161	163
Mean	117.8	102.8	115.5	110.6	106.1	113.1	110.6	154.7	156.5	163.2	148.7	155.7	154.3	155.3
STD	17.9	18.2	15.1	13.0	16.9	12.3	10.5	22.0	22.0	21.1	13.7	18.4	14.9	11.9
SED±	2.0	2.0	1.7	1.4	1.9	1.4	1.2	2.4	2.4	2.3	1.5	2.0	1.7	1.3
CV (%)	15.2	17.7	13.1	11.8	15.9	10.8	9.5	14.2	14.0	12.9	9.2	11.8	9.6	7.7

STD = standard deviation, SED = standard error of difference, CV = coefficient of variation, S1 = site 1 (Bvumbwe), S2 = site 2 (Chitedze), S3 = site 3 (Makoka), Y1 = year 1 (2017/18), Y11 = year 2 (2018/19), DTF = days to flowering, DTM = days to 75% maturity, See genotype codes (G1–G81) in Table 1.

Table 8. Mean values for plant height and number of primary branches among the ten top best and five bottom performing genotypes after evaluating 81 genotypes in six environments in Malawi.

Genotype	PH							NPB						
	Y1			Y11			Mean	Y1			Y11			Mean
	S1	S2	S3	S1	S2	S3		S1	S2	S3	S1	S2	S3	
Top Ten Genotypes														
21	166.5	220.0	193.0	160.0	212.8	193.0	190.9	19	19	17	14	18	12	16
43	113.5	147.5	127.5	96.5	146.7	148.0	163.7	14	15	17	14	17	11	15
51	151.5	109.0	158.0	234.5	209.4	149.0	168.6	13	12	14	18	13	11	13
30	229.5	188.5	204.0	170.0	218.5	204.0	202.4	15	13	18	15	16	15	15
45	139.5	144.5	173.0	161.5	169.4	197.5	164.2	15	13	22	15	17	14	16
81	163.0	222.0	191.0	160.5	168.1	194.5	183.2	13	17	19	18	12	14	15
17	163.5	164.0	163.5	100.0	152.1	156.0	149.9	15	14	21	17	16	13	16
66	181.5	177.5	164.0	161.5	156.8	149.5	165.1	12	13	13	14	16	12	13
74	156.0	195.0	185.5	124.5	178.7	164.0	167.3	15	18	17	20	18	12	17
20	152.5	163.0	168.5	138.5	247.5	166.5	172.8	10	12	20	12	18	11	14
Bottom Five Genotypes														
39	203	154.5	174	157.5	200	151.5	173.4	16	18	17	15	12	12	15
13	169	171.5	134	134.5	203.3	156.5	161.5	18	12	18	15	10	15	14
50	119	101.5	149.5	130.5	218.5	166.5	147.6	18	13	14	15	17	13	15
42	140	153	175.5	104.5	207.7	120	125.3	14	9	16	14	13	13	13
79	174	165.5	167.5	120.5	201.4	148	162.8	11	18	23	13	14	13	15
Mean	168.0	166.7	166.2	143.4	195.5	166.1	167.3	14.6	13.6	18.0	14.9	14.6	12.8	14.5
STD	23.9	34.5	22.1	23.0	27.0	23.1	12.6	2.7	4.4	2.7	2.4	3.2	2.0	1.3
SED±	2.7	3.8	2.5	2.6	3.0	2.6	1.4	0.3	0.5	0.3	0.3	0.4	0.2	0.1
CV (%)	14.2	20.7	13.3	16.0	13.8	13.9	7.5	18.7	32.1	15.0	16.3	22.0	15.6	9.1

STD = standard deviation, SED = standard error of difference, CV = coefficient of variation, S1 = site 1 (Bvumbwe), S2 = site 2 (Chitedze), S3 = site 3 (Makoka), Y1 = year 1 (2017/18), Y11 = year 2 (2018/19), PH = plant height (cm), NPB = number of primary branches, see genotype codes (G1–G81) in Table 1.

Table 9. Mean values for number of racemes and number of pods per plant among the ten top best and five bottom performing genotypes after evaluating 81 genotypes in six environments in Malawi.

Genotype	NRP							NPP						
	Y1			Y11			Mean	Y1			Y11			Mean
	S1	S2	S3	S1	S2	S3		S1	S2	S3	S1	S2	S3	
Top Ten Genotypes														
21	214	402	71	130	61	47	154	157	270	66	61	92	98	124
43	138	173	97	117	95	58	113	119	315	98	72	110	90	134
51	260	155	146	113	80	51	134	167	231	109	65	76	90	123
30	178	430	134	132	73	52	166	127	362	95	97	83	101	144
45	191	647	160	151	88	83	220	96	261	106	81	92	122	126
81	200	536	85	89	69	40	170	140	240	69	61	70	82	110
17	184	258	96	139	94	61	139	102	158	65	35	112	89	93
66	148	168	108	119	76	49	111	69	186	82	26	78	64	84
74	196	414	98	125	84	81	166	128	112	64	46	40	94	81
20	126	259	106	148	130	73	140	115	177	78	38	157	45	101
Bottom Five Genotypes														
39	161	465	103	145	55	60	165	128	125	93	38	61	82	88
13	155	228	80	119	99	52	122	98	195	55	37	60	95	90
50	116	321	199	195	81	46	159	79	78	60	59	96	84	76
42	122	150	87	151	80	62	109	99	78	90	62	67	70	78
79	98	552	70	131	163	54	178	53	226	51	26	165	90	102
Mean	174.1	312.3	99.0	161.6	91.8	58.9	149.4	114.6	148.2	80.0	51.0	80.9	86.7	93.4
STD	43.9	146.5	27.7	39.8	30.0	12.1	26.2	30.5	56.7	22.1	16.1	33.4	19.7	14.1
SED±	4.9	16.3	3.1	4.4	3.3	1.3	2.9	3.4	6.3	2.5	1.8	3.7	2.2	1.6
CV (%)	25.2	46.9	28.0	24.7	32.7	20.6	17.5	26.6	38.2	27.7	31.5	41.3	22.8	15.1

STD = standard deviation, SED = standard error of difference, CV = coefficient of variation, S1 = site 1 (Bvumbwe), S2 = site 2 (Chitedze), S3 = site 3 (Makoka), Y1 = year 1 (2017/18), Y11 = year 2 (2018/19), NRP = number of racemes per plant, NPP = number of pods per plant, see genotype codes (G1–G81) in Table 1.

Table 10. Mean values for grain yield and hundred seed weight among the ten top best and five bottom performing genotypes after evaluating 81 genotypes in six environments in Malawi.

Genotype	GYD							HSWT						
	Y1			Y11			Mean	Y1			Y11			Mean
	S1	S2	S3	S1	S2	S3		S1	S2	S3	S1	S2	S3	
Top Ten Genotypes														
21	2.1	0.9	2.3	2.4	1.3	1.7	1.8	16.0	16.5	10.0	10.5	12.5	15.5	13.5
43	1.7	1.7	1.6	1.8	1.6	1.9	1.7	17.0	14.5	14.0	17.0	22.5	13.0	16.3
51	1.8	1.0	2.1	2.1	1.7	1.7	1.7	16.5	17.5	14.5	18.5	21.5	13.5	17.0
30	2.3	1.6	1.2	1.2	1.4	1.8	1.6	17.5	17.0	15.0	16.0	16.0	12.0	15.6
45	1.5	0.9	1.4	1.5	2.3	1.9	1.6	18.4	19.0	15.5	16.0	16.5	18.0	17.2
81	1.3	0.5	1.5	1.6	2.3	2.3	1.6	19.5	16.0	15.5	19.0	15.0	11.0	16.0
17	1.1	0.5	0.7	1.4	2.5	3.0	1.5	18.5	14.0	11.0	17.5	20.0	15.5	16.1
66	2.4	1.5	1.2	1.2	1.4	1.5	1.5	15.5	15.5	15.0	17.5	17.5	13.5	15.8
74	2.2	1.6	1.1	1.0	1.1	1.8	1.5	14.5	14.5	15.5	16.9	20.0	13.5	15.8
20	1.2	0.9	1.7	1.7	1.7	1.2	1.4	16.0	12.5	15.0	18.5	15.0	14.0	15.2
Bottom Five Genotypes														
39	0.4	0.4	1.1	1.1	1.2	0.9	0.8	15.5	14.5	14.5	16	15	16	15.3
13	0.8	0.2	0.5	1.4	0.5	0.3	0.6	12.5	15	14	15	16.5	16	14.8
50	0.9	0.5	0.4	0.5	0.4	0.7	0.6	13	10.5	17.5	21	19	14.5	15.9
42	0.6	0.4	0.9	0.5	0.4	0.4	0.5	12	12.5	14	19	20	14.5	15.3
79	0.8	0.3	0.4	0.5	0.3	0.5	0.5	13	16.5	14.5	17.5	17.5	14	15.5
Mean	1.1	0.6	1.3	1.3	1.5	1.3	1.2	15.9	13.9	13.5	17.6	12.9	14.2	14.7
STD	0.4	0.3	0.4	0.4	0.5	0.4	0.2	2.4	3.2	2.4	2.3	4.5	2.5	1.3
SED±	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.3	0.4	0.3	0.3	0.5	0.3	0.1
CV (%)	37.3	43.3	32.8	32.1	31.3	33.5	20.5	15.1	22.9	18.0	13.2	35.1	17.5	8.9

STD = standard deviation, SED = standard error of difference, CV = coefficient of variation, S1 = site 1 (Bvumbwe), S2 = site 2 (Chitedze), S3 = site 3 (Makoka), Y1 = year 1 (2017/18), Y11 = year 2 (2018/19), GYD = grain yield (t ha^{-1}), HSWT = 100 seed weight (g), see genotype codes (G1–G81) in Table 1.

3.4. Correlation Analysis among Phenotypic Traits

Assessed traits exhibited variable degrees of associations with grain yield (Table 11). Grain yield was moderately correlated with HSWT (r = 0.50, $p < 0.01$). A number of secondary traits exhibited variable pairwise correlations. DTF and DTM exhibited the strongest correlation (r = 0.79, $p < 0.01$). There were moderate correlations between DTF and PH (r = 0.44, $p < 0.01$), NPB and NSP (r = 0.41, $p < 0.01$), and DTM and PH (r = 0.41, $p < 0.01$). Relatively, HSWT exhibited weak correlations (r < 0.30) with NPB and NPP.

Table 11. Phenotypic correlation coefficients among the ten quantitative traits of 81 pigeonpea genotypes evaluated in six environments.

Trait	DTF	DTM	PH	NPB	NSB	NRP	NPP	NSP	GYD	HSWT
DTF	1	0.787 **	0.442 **	0.069	0.006	0.063	0.121	−0.134	0.232 *	−0.021
DTM		1	0.409 **	0.066	0.037	0.034	0.121	−0.020	0.131	0.023
PH			1	0.057	0.149	0.249 *	0.190	−0.123	0.123	0.021
NPB				1	0.044	0.261 *	0.145	0.406 **	0.174	0.350 **
NSB					1	0.024	0.152	−0.101	0.214	0.090
NRP						1	0.191	0.262 *	0.177	0.124
NPP							1	0.099	0.354 **	0.307 **
NSP								1	0.051	0.173
GYD									1	0.498 **
HSWT										1

**. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level (2-tailed), DTF = days to 50% flowering, DTM = days to 75% maturity, PH = plant height, NPB = number of pods per plant, NSB = number of secondary branches per plant, NRP = number of racemes per plant, NPP = number of primary branches per plant, GYD = grain yield, HSWT = 100 seed weight.

3.5. Nonlinear Principal Component (PC) and Cluster Analysis

The nonlinear principal component analysis was performed to identify the most discriminative variables among the pigeonpea genotypes. A total of 98% of the variation explained by the qualitative and quantitative traits were explained by the first three principal components (Table 12). In general, traits such as GYD, DTF, DTM, leaf hairiness (LH), leaf shape (LS), and NRP contributed much to the phenotypic variation in the PCs. However, GYD, LH, NPP, HSWT, and NSP were the highest contributors (with contributions of 0.86, 0.63, 0.63, 0.51, and 0.45, respectively) on PC1. The second principal component accounted for 73% of the total variation, with NRP and pod form (PF) being the highest (0.74 and 0.62) positive contributors. Conversely, traits including LS, flowering pattern (FP), and flower main color (FMC) negatively correlated with PC2 exhibiting negative (−0.63, −0.57, and −0.44, respectively) PC scores. DTF and DTM were the positive contributors to the observed phenotypic variation on PC3 with PC loadings of 0.83 and 0.79, respectively.

Table 12. Principal components showing variation and contribution by 24 phenotypic traits among 81 pigeonpea genotypes assessed in six environments in Malawi.

Trait	Dimension		
	1	2	3
FMC	−0.026	−0.435	−0.269
FP	0.016	−0.568	0.050
FSC	−0.101	−0.172	0.291
FSP	0.357	−0.095	−0.080
GH	−0.077	0.227	0.151
LH	0.629	−0.435	−0.112
LS	−0.386	−0.626	0.143
PC	0.010	0.175	−0.310
PF	0.203	0.616	0.043
SCP	−0.050	0.327	−0.235
SEC	−0.038	0.236	−0.345
SMC	0.020	−0.144	−0.060
SSH	0.082	−0.134	0.186
STC	−0.042	0.000	0.023
DTF	0.186	−0.069	0.827
DTM	0.236	−0.190	0.793
PH	0.294	−0.357	0.118
NPB	0.037	0.208	0.239
NSB	0.160	0.398	0.204
NPP	0.626	0.086	−0.073
PL	0.252	0.353	0.104
NRP	0.001	0.735	−0.117
NSP	0.476	0.076	−0.075
HSWT	0.508	0.219	−0.084
GYD	0.863	0.146	−0.109
Eigen value	3.404	2.967	2.163
Variance %	39	34	25
Cumulative	39	73	98

DTF = days to 50% flowering, DTM = days to 75% maturity, PH = plant height, NPB = number of primary branches, NSB = number of secondary branches per plant, NRP = number of racemes per plant, NPP = number of pods per plant, GYD = grain yield, HSWT = 100 seed.

Figure 2 shows the variable correlation plot showing positive association between PC1 and DTF traits such as DTM, GYD, HSWT, NSP, plant height (PH), and pod length (PL). Conversely, seed eye color (SEC), pod color (PC), number of secondary branches per plant (NSB), and FMC exhibited negative associations with PC1. Quantitative traits such as plant habit (PH), NPP, HSWT, DTF, DTM, NRP, and GYD were positively correlated to each other as exhibited by their vectors, which were in the same direction and separated by acute angles between them. Similarly, the qualitative traits such as SEC, PC, and FMC were positively correlated to each other. However, the quantitative traits were positively correlated with GYD, while the qualitative traits were negatively correlated with GYD.

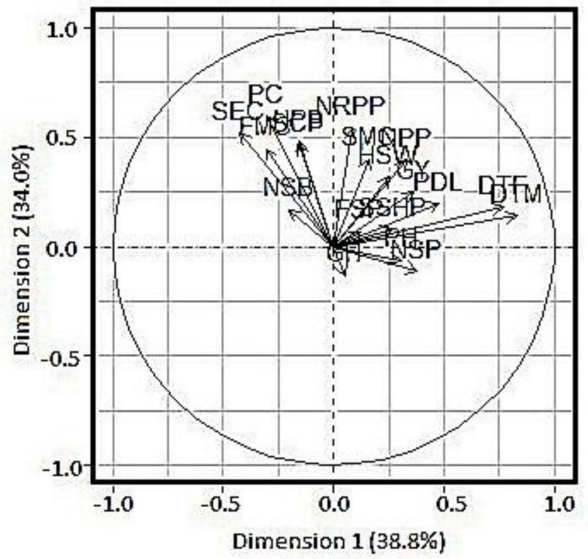

Figure 2. Trait biplot showing the relationship among quantitative and qualitative traits in 81 pigeonpea genotypes evaluated in six environments in Malawi. Dim 1 = dimension 1, Dim2 = dimension 2, PCA = principal component analysis. For trait code description, refer to Table 3.

Assessment of the phenotypic diversity using morphological attributes delineated the genotypes into three distinct clusters (Figure 3). The first cluster had the highest number (51) of genotypes. The second cluster had 27 genotypes, and the third cluster had three genotypes. However, the composition of genotypes in all the three clusters consisted of mixtures of landraces, breeding lines, and cultivars.

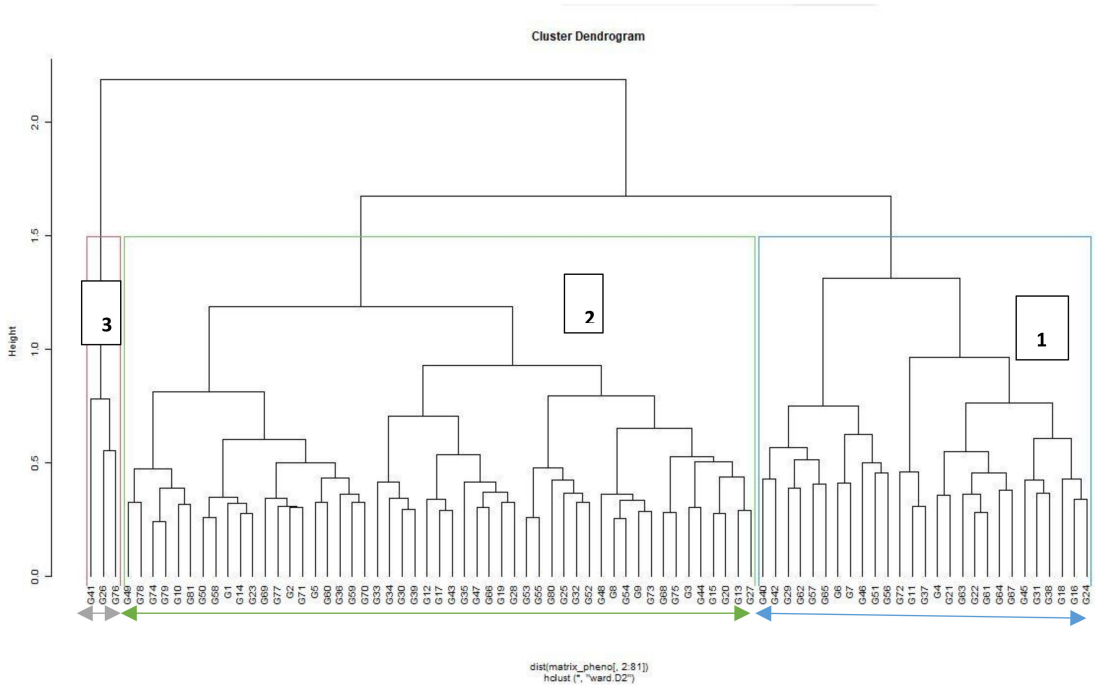

Figure 3. Hierarchical cluster dendogram showing genetic similarity matrix of 81 genotypes evaluated in six environments in Malawi based on phenotypic traits. Cluster 1, in pink color, cluster 2 in green color and cluster 3 in blue color. See Table 1 for the genotypes codes.

4. Discussion

The current study evaluated 81 pigeonpea genotypes across six environments to assess the genetic diversity and yield stability, and to select complementary and unique genotypes for breeding. The genotypes exhibited wide and significant variation in qualitative traits (Table 4), which indicated that the tested germplasm could harbor important genetic variation that underpins the morphological variation. Similarly, [16] reported significant variation in qualitative traits among pigeonpea accessions sourced from ICRISAT's international genebank. The variation in qualitative traits such as growth habit and seed color is important for breeding cultivars that meet farmer expectations. For instance, the variation present in growth habit is important to identify genotypes with compact growth habit for intercropping to maximize space utilization and productivity in moisture-limited environments. Farmers often intercrop pigeonpea with cereal crops such as maize and sorghum, and legumes such as groundnuts. Hence, pigeonpea genotypes with a spreading growth habit may not be suitable for mixed cropping systems [17]. The diversity in pigeonpea seed color helps to identify genotypes that are preferred by local farmers. For instance, farmers in Malawi prefer pigeonpea varieties with a cream seed color, which they associate with good cooking quality. Similar findings were reported by [18], who reported a predominance of cream and light gray pigeonpea varieties in Benin, reflecting the farmers' color preferences. Knowledge of variability in qualitative traits among the accessions and understanding farmer preferences are important as a basis for the development of direct breeding objectives and appropriate breeding strategies.

The significant genetic variation exhibited in the quantitative traits (Table 5) highlights the genetic diversity available for exploitation during cultivar development. The genotype performances were also affected by significant genotype × environment interactions, suggesting that genotype performances were not consistent in all the environments. Genotypic variation is underpinned by differences in genetic constitution among the genotypes, which is important for crop improvement [19]. The environment influences phenotypic expression through variation in factors such as temperature, humidity, and soil fertility. The significant impact of the environment on phenotypic expression is known to reduce genotype–phenotype correlation [20], which complicates the identification of stable and superior genotypes. However, significant genotype × environment interaction on yield and yield components of legumes such as common bean, cowpea, and pigeonpea has been previously reported [21–23]. In the present study, the genotypes that matured early were shorter with low numbers of branches and pods per plant and low grain yields compared to the medium to late maturing genotypes that grew taller, produced more branches and pods per plant, and had higher grain yields. Similarly, [24] reported that cultivars with higher numbers of primary branches, secondary branches, number of pods per plant, and taller plant height had higher grain yields.

There was limited genetic variation among pigeonpea landraces, cultivars, and breeding lines in this study (Table 6). This could be attributed to gene flow arising from the exchange of germplasm between Malawi and Tanzania. In addition, there could be high level of genotype relatedness since the breeding lines and cultivars were developed from the landraces collected from Malawi and Tanzania by ICRISAT. However, the genotype performance in terms of days to maturity and plant height were affected by significant genotype × environment interactions (Tables 7 and 8), suggesting that genotype performances were not consistent in all the environments. This could be because the landraces, cultivars, and breeding lines belong to three maturity groups: early, medium, and late duration. The early maturity exhibited by the ICRISAT genotypes could be a result of selection for earliness at ICRISAT in Kenya, which has advanced pigeonpea breeding programs and has developed a number of elite breeding lines that have been distributed in several East and Southern African countries for evaluation. The TARI and DARS genotypes are comprised of landraces and cultivars that are medium to late maturing. Similarly, [21] also reported that traditionally grown pigeonpea cultivars and landraces are represented by varieties from medium to long maturity groups (150 to 280 days), which are high yielding but very sensitive to photoperiod.

The positive and moderate correlation between GYD and HSWT (r = 0.50, $p < 0.01$) (Table 11) indicated that HSWT could be used for the direct selection for GYD. The moderate positive correlation between DTF, DTM, and PH revealed that selection for earliness can be based on the plant height. Although pigeonpea is relatively drought-tolerant, there is a need to develop early flowering and maturing cultivars to fit in the cropping cycles of sub-Saharan Africa, which are becoming progressively shorter due to climate change. The positive correlations exhibited by most secondary traits show that multiple trait selection would be possible. However, the weak correlations among the traits would result in an inefficient selection or low genetic gains. A strong correlation (r = 0.858) between grain yield and the number of pods per plant was reported by [25]. In addition, [26] reported moderate to weak correlations between grain yield and days to 50% flowering (r = 0.58), days to maturity (r = 0.59), and plant height (r = 0.42). Conversely, [27] and [28] reported a negative association between 100 seed weight and grain yield. The significant relationship between DTF, DTM, HSWT, PH, NPP, and GYD is useful when selecting for high grain yield [16]. Direct selection for these traits would result in yield improvement in pigeonpea.

The nonlinear principal component analysis enabled the identification of important traits with high variability among the genotypes. In this study, GDY, DTF, DTM, NPP, NRP LH, HSWT, and NSP were identified as the most important traits due to their high contribution on PC1 and PC2 (Table 12). This suggests that these traits are useful for selection. Accessions that exhibit high and desirable mean performances based on the target traits would be selected for improvement. Other reports indicated that trait contribution to different PCs varies with genetic diversity within the tested germplasm and the number of traits evaluated [16,25]. The results further revealed that DTF, NPP, NPB, NSB, PH, PL, and HSWT are important secondary traits for the indirect selection for GYD due to their positive association with GYD and their high contribution on the PCs.

The cluster analysis delineated the accessions into three groups (Figure 3), suggesting the presence of considerable genetic variation among the genotypes. However, a mixture of breeding lines, landraces, and cultivars in each group could be attributed to the geographical proximity between Malawi and Tanzania (where some of the landraces were collected). The level of natural outcrossing in pigeonpea is very high and varies from 5 to 70% depending on the prevailing weather conditions and insect activities for pollination [29]. In addition, the breeding lines from ICRISAT were developed using some parents selected from the landraces from Tanzania and Malawi. East Africa is known as a center of diversity for pigeonpea—hence the close genetic relatedness. The present finding is in agreement with [30], who reported little variation among the cultivated pigeonpea collected in Africa based on diversity array technology (DArT) markers.

5. Conclusions

The study examined 81 pigeonpea genotypes for their diversity and yield stability. The genotypes exhibited a wide genetic variation in qualitative traits such as growth habit, flower main color, flower streak pattern, pod color, and seed traits. The combined analysis revealed significant genotype × environment interaction effects for most traits, suggesting the need for selection for specific adaptation. A lack of significant variation in quantitative traits among landraces, cultivars, and breeding lines indicate that there is potentially high gene flow among the different categories of germplasm, which could present genetic bottlenecks during breeding. Traits such as GDY, DTF, DTM, NPP, NRP, HSWT, LH, and NSP with high scores on PC1 and PC2 are useful selection indices for pigeonpea improvement. Accessions that exhibited high and desirable mean performances in the target traits such as early maturing (MWPLR 14, ICEAP 01170, ICEAP 871091, ICEAP 01285) and high yielding (Kachangu, MWPLR 16, TZA 5582, No. 40, and MWPLR 14) would be recommended as parental lines for the breeding program. The genetic diversity analysis using morphological traits has enabled the identification of promising parents and heterotic clusters for breeding.

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the experiments and wrote up the manuscript. All authors have read and agreed to the published version of the manuscript.

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