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Research article

Nutritional and phytochemical compositions and their interrelationship in succulent pods of pigeonpea (*Cajanus cajan* [L.] Millsp.)

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ABSTRACT

Malnutrition remains one of the major human health issues affecting millions of people in sub-Saharan Africa (SSA). Hence, the objective of this study was to quantify the nutritional and phytochemical compositions of immature pods of pigeonpea genotypes to select promising lines with unique nutritional quality for production and cultivar development. Seven preliminarily tested and identified pigeonpea genotypes were grown under field conditions using a randomized complete block design with three replicates to quantify the nutritional and phytochemical contents in the immature pods. Significant ($P \leq 0.01$) genotype effect was detected for the assessed nutritional and phytochemical compositions. Relatively higher contents of iron (15.53 mg/100g), zinc (1.59 mg/100g), magnesium (114.60 mg/100g) and total flavonoid (8.47 mg CE/g) were present in genotype Ilonga_14m1 ICEAP-0054. Higher compositions of beta-carotene (2.84 mg/100g), total phenolics (20.42 mg GAE/g), and vitamin-C (95.84 mg/100g) were detected in genotypes, Mali ICEAP-00046, PigeonP-3018 and Kiboko ICEAP-00932, respectively. Cluster analysis allocated the tested genotypes into three main groups. Significant ($P \leq 0.05$) positive correlations were recorded among the assessed nutritional and phytochemical compositions that will allow direct and indirect selection of the evaluated genotypes for nutritional and phytochemical quality improvement. The principal component analysis resolved four components that cumulatively explained 76.85% of the total genetic variation in nutritional and phytochemical compositions among the tested genotypes of pigeonpea. Genotype PigeonP-3021 exhibited high levels of beta-carotene and vitamin C, while Kiboko ICEAP-00932 and PigeonP-3018 had high contents of aluminium, iron, phosphorus and total phenolics. Genotype Ilonga_14m1 ICEAP-0054 had high compositions of zinc, potassium, magnesium, copper and calcium. Unique pigeonpea genotypes (i.e., PigeonP-3021, Kiboko ICEAP-00932, and PigeonP-3018) were identified for quality breeding or direct production with promising nutrient profiles for food and nutrition security.

1. Introduction

Legumes such as Bambara groundnut, groundnut or peanut, cowpea, chickpea, lentil, pea, common bean, faba bean, lima bean, mung bean, soybean, lupine, and pigeonpea are the cheapest and alternative sources of plant-derived protein. Hence legume production offers greater opportunities to circumvent protein malnutrition and to enhance soil-fertility through atmospheric nitrogen fixation and integration in the soil systems. These make legumes a crop of choice in crop rotation or intercrop systems and mitigate climate change. The food and feed quality (e.g. nutritional values and taste) and use is dependent on the legume species (Ton et al., 2021). Pigeonpea (*Cajunus cajan* [L.] Millsp.; ($2n = 2x = 22$), a member of the Fabaceae family, is the sixth most globally produced food legume ranked after dry bean, chickpea, field pea, cowpea and lentil (Seleman et al., 2016; FAOSTAT 2020). According to global estimates (FAOSTAT 2017), about 6.8 million tons of pigeonpea are produced per annum from ~7.0 million hectares of land. South Asia accounts for nearly 90% of world pigeonpea production. Pigeonpea grows under various abiotic stresses including heat and drought, poor soil nitrogen fertility, and salinity (Choudhary et al., 2011). Pigeonpea is

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relatively resistant to several biotic stresses such as weeds, insect pests, viruses, bacteria and fungi (Choudhary et al., 2013; Liu et al., 2021). These attributes make pigeonpea an ideal crop for production in resource-poor communities using low input agricultural production systems. Despite previous breeding efforts to improve pigeonpea productivity across diverse agro-ecological zones, the actual pigeonpea yield of around 800 kg/ha is still below the crop's potential yield of 3 t/ha (Varshney et al., 2012). Hence, the United Nations Sustainable Development Goal 2 aims to end hunger, achieve food security and improve nutrition and promote sustainable agriculture globally (UNSDG, 2015).

Most poor rural and urban communities in SSA are predominantly dependent on starch- and carbohydrate-based products as their sources of food and nutrition due to the high cost and unavailability of protein, vitamin and mineral-rich and balanced diets. Pigeonpea is a vital food crop cultivated to derive various products (e.g. as leaf, and pod vegetable and dry grains). Fresh and succulent leaves and pods contain essential nutrients required for human nutrition. For example, nutrient analyses revealed significant quantities of carbohydrate (54.36%–60.1%), protein (19.28%–25.79%), fat (0.993%–1.75%), fibre (2.28%–3.06), energy (326.8–345.23 kcal) (Anjulo et al., 2021) and water-soluble vitamins such as vitamin A (302.94 μg/100g) and vitamin C (23.51 mg/100) in pigeonpea (Ojwang et al., 2021). Micro and micro-nutrients (in mg/kg dry weight) range from 105.17 to 144.07 for potassium (K), 8.95 to 12.67 for magnesium (Mg), 7.74 to 12.27 for calcium (Ca), 0.247 to 0.543 for iron (Fe), 0.122 to 0.313 for zinc (Zn), 0.061 to 0.432 for manganese (Mn) and 0.087 to 0.134 for copper (Cu) (Anjulo et al., 2021). The immature grain and green pods contain 10 times more fat, five times more vitamin ‘A’ and three times more vitamin ‘C’ than dry grains (Kimani, 2000).

Additionally, the grain is regarded as a medicinal pulse that possesses phytochemical compounds with various properties such as anti-inflammatory, antimicrobial, antioxidant, neuroprotective, anticancer and antidiabetic agents (Dinore and Farooqui, 2020). The following phytochemicals were reported in pigeonpea seed: alkoloid (34%), flavonoid (46%), sterol (22%) and phenol (44%) (Igboabuchi 2021; Pal et al., 2011). The positive nutritional and phytochemical attributes identify pigeonpea as an essential food and nutrition security crop and medicinal plant. In order to keep up with the escalating global human population pressure coupled with demand for healthy food, an effort is required to increase the deployment of pigeonpea cultivars possessing enhanced nutritional and phytochemical composition. Furthermore, the green leaves from plants are used as animal fodder (Jeevarathinam and Chelladurai, 2020).

Genetic improvement for enhanced nutritional and phytochemical attributes in pigeonpea is key to extend the significance of the crop for food and nutrition security, and for human health. Currently, there is limited research targeted for nutritional quality improvement in the immature pods of pigeonpea for enhanced nutritional and phytochemical composition. Pigeonpea has not been widely evaluated for nutritional and phytochemical attributes to develop new cultivars with enhanced nutritional and phytochemical attributes. Hence, seven genetically diverse and elite pigeonpea genotypes were sourced from the University of KwaZulu-Natal (UKZN) gene bank for evaluation. These genotypes require nutritional and phytochemical composition screening for nutritional and phytochemical composition to enhance nutritional and phytochemical attributes in the immature pods. Therefore, the objective of this study was to quantify the nutritional and phytochemical compositions of immature pods of pigeonpea genotypes to select promising lines with unique nutritional quality for production and cultivar development for nutritional quality.

Table 1. Information of pigeonpea genotypes evaluated in the study.

Entry No.	Genotype name	Source	Country
1	Ilonga_14m1 ICEAP-0054	ICRISAT	Kenya
2	Kiboko ICEAP-00932	ICRISAT	Kenya
3	Mali ICEAP-00046	ICRISAT	Kenya
4	PigeonP-3014	ICRISAT	Kenya
5	PigeonP-3018	ICRISAT	Kenya
6	PigeonP-3021	ICRISAT	Kenya
7	Tumia ICEAP-00068	ICRISAT	Kenya

ICRISAT = International Crops Research Institute for the Semi-Arid Tropics.

2. Materials and methods

2.1. Plant materials

Seven elite pigeonpea genotypes obtained from the University of KwaZulu-Natal (UKZN) germplasm collection were used for this study (Table 1).

2.2. Study site, experimental design and data collection

The experiment was conducted at the Roodeplaat research station in Gauteng Province, South Africa (25.6740° S, 28.3395° E, 1168 m above sea level), during the 2017/2018 cropping season. Roodeplaat is characterised by average annual rainfall of 772 mm and average temperature of 19.93 °C. The soil type at Roodeplaat is generally clay loam with pH between 5.0 and 6.2. The field experiment was carried out using randomised complete block design with three replications. Each plot consisted of 4 m rows, with inter and intra-row spacing of 1 m and 30 cm, respectively. Two seeds were sown and later thinned to one seedling per stand. The trial was planted under rainfed conditions and irrigation was supplied sparingly to avoid wilting and drought stress. Agronomic management practices have been carried out as recommended to the crop. Hence, immature pods were harvested at flowering stage prior to proper seed development.

2.3. Nutritional and phytochemical traits quantification

Following lyophilisation, the harvested materials were ground to fine powder. Beta-carotene and vitamin C (ascorbic acid) were extracted and quantified using high performance liquid chromatography methods as described by Moyo et al. (2018). For the determination of total phenolic and flavonoid contents, the samples were extracted as described by Amoo et al. (2012). The Folin and Ciocalteu colorimetric method (Singleton and Rossi 1965) with slight modifications outlined by Fawole et al. (2009) was used to quantify total phenolic content, while the aluminium chloride colorimetric method (Zhishen et al., 1999) was used to quantify flavonoid content. Gallic acid and catechin were used for plotting the calibration curves in the quantification of total phenolic and flavonoids, respectively. Each determination was done in triplicate.

For the mineral element analysis, the samples were digested as described by Ang and Lee (2005). Briefly, 0.5 g of dried pigeonpea immature pods was weighed into a Teflon beaker, followed by the addition of 9 ml of Aqua-regia [HCl: HNO_3, (3:1)]. The sample-acid mixture was heated on a hot plate at 95 °C for 2 h. The inner walls of the beaker were washed with at least 2 ml deionized water (18 Ω, Millipore, Opurite System, Lasec, South Africa) and the content was carefully transferred into a 100 ml calibrated volumetric flask. The beaker was further rinsed several times with deionised water and each time the content was transferred into the same volumetric flask.

The flask was filled to the mark with deionised water. The samples were digested in triplicate and analysed using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES 9820, Shimadzu, Japan). The instrument was equipped with mini-torch and the flame was operated in both axial and radial view position. Scandium (Sc) at wavelength 361.384 nm was used as an internal standard and each element was monitored at specific wavelength with no or minimum interferences. Multi-elements standards ranging from 0.0016 ppm to 1000 ppm were used to generate calibration curve and the amount of elements in the sample was expressed in mg/100 g dry weight.

2.4. Data analysis

The data were subjected to analysis of variance (ANOVA) using GenStat 18th edition (VSN International, Hempstead, UK). Mean values were separated using least significant differences (LSD). Pearson's correlation coefficients (r) were determined using RStudio Version 3.2.1 (R Development Core Team, 2008). Principal component analysis (PCA) was conducted to identify quality traits explaining most phenotypic variation among the studied genotypes. Principal component (PC) biplot was constructed to visualise association between test variables using RStudio Version 3.2.1. Further, cluster analysis was conducted based on neighbour-joining algorithm and average Euclidian distance methods using XLSTAT (2016).

3. Results

3.1. Effects of genotype on nutritional and phytochemical traits

ANOVA revealing the effect of genotype on the responses of nutritional and phytochemical traits is presented in Table 2. Significant ($P \leq 0.01$) genotype main effect was observed for all evaluated traits except beta-carotene, flavonoids, total phenolics and vitamin-C, indicating presence of genetic diversity among the test pigeonpea genotypes.

3.2. Mean performance of pigeonpea genotypes for nutritional and phytochemical traits

Mean values of nutritional and phytochemical traits among the tested pigeonpea genotypes are presented in Table 3. The genotype Ilonga_14m1 ICEAP-0054 recorded the highest concentrations for all the macro- and micronutrients as well as flavonoid. The genotypes Mali ICEAP-00046, Kiboko ICEAP-00932, and PigeonP-3018 had the highest beta-carotene, vitamin C and total phenolic contents, in that order. With the exception of aluminium, copper and vitamin C, the genotype PigeonP-3014 recorded the lowest concentrations for all assessed quality traits. The lowest aluminium and vitamin C concentrations were recorded in genotypes Mali ICEAP-00046 and PigeonP-3021, respectively, while genotypes PigeonP-3018 and PigeonP-3021 had the lowest copper content.

3.3. Correlation analysis among nutritional and phytochemical traits

Pearson's correlation coefficients (r) among studied nutritional and phytochemical traits are indicated in Table 4. Aluminium was significantly and positively correlated with copper ($r = 0.73$, $p < 0.01$), iron ($r = 0.57$, $p < 0.01$), magnesium ($r = 0.47$, $p < 0.05$) and phosphorus ($r = 0.60$, $P < 0.05$). Calcium was positively and significantly correlated with copper ($r = 0.53$, $p < 0.01$), iron ($r = 0.58$, $p < 0.01$), potassium ($r = 0.58$, $p < 0.01$), magnesium ($r = 0.84$, $p < 0.01$) and phosphorus ($r = 0.83$, $p < 0.01$). Similarly, copper was positively correlated with iron ($r = 0.47$, $p < 0.05$), potassium ($r = 0.49$, $p < 0.05$), magnesium ($r = 0.76$, $p < 0.01$) and phosphorus ($r = 0.60$, $p < 0.01$). In similarly fashion, iron was significantly correlated with potassium ($r = 0.71$, $p < 0.01$), phosphorus ($r = 0.78$, $p < 0.01$), magnesium ($r = 0.75$, $p < 0.01$), flavonoids ($r = 0.49$, $p < 0.05$) and total phenolics ($r = 0.54$, $p < 0.05$). Potassium recorded a positive correlation with magnesium and phosphorus ($r = 0.91$, $p < 0.01$ and $r = 0.74$, $p < 0.01$), respectively. Positive correlation was observed between magnesium with phosphorus ($r = 0.81$, $p < 0.01$) and flavonoids ($r = 0.48$, $p < 0.01$), whereas flavonoids positively and significantly correlated with total phenolics ($r = 0.86$, $p < 0.01$) (Table 4). Non-significant and weak negative correlations were identified for calcium with beta-carotene ($r = -0.01$), as well as between vitamin C with iron ($r = -0.03$), flavonoids ($r = -0.24$) and total phenolics ($r = -0.23$).

3.4. Cluster analysis

The level of diversity and similarity among the pigeonpea genotypes for their nutritional and phytochemical characteristics was determined using cluster analysis (Figure 1). Furthermore, Table 5 revealed variabilities among the clusters by summarising cluster means for the nutritional and phytochemical traits. The cluster analysis divided the test genotypes into two main clusters and a singleton (G1, Ilonga_14-m1 ICEAP-0054) with peculiar alleles.

The dendrogram grouped the genotypes into two main clusters (I and II) at 80% genetic dissimilarity, while G1 was differentiated at a genetic dissimilarity of 75% (Figure 1). Cluster II contained three genotypes (G5-Tumia ICEAP-00068, G6-Kiboko ICEAP-00932, and G7-PigeonP-3018), while cluster I consisted of three genotypes (G2-PigeonP-3021, G3-Mali ICEAP-00046 and G4-PigeonP-3014). Cluster I contained genotypes with the lowest averages for all the evaluated nutritional and phytochemical traits, whereas cluster II consisted of genotypes with intermediate averages for all the traits. Accession Ilonga_14-m1 ICEAP-0054 was not included in any of the two clusters (clusters I and II), but grouped as a singleton and stood individually as a separate cluster (cluster III) formed at a genetic distance of about 0.80. This indicates that it was dissimilar from the other test accessions. It had the highest levels for all the nutritional and phytochemical traits (Table 5).

3.5. Principal component analysis

Principal component (PC) biplot showing grouping of pigeonpea genotypes with nutritional and phytochemical traits is presented in Figure 2. The principal component analysis revealed two significant PCs with eigenvectors greater than one, which cumulatively accounted for 76.85% of the total variation. The PC biplot offers a summary of the similarities and differences among the test genotypes and the correlations between the measured traits. Angles lesser than 45^{o} between the vector lines of study variables indicate high trait associations and ability to discriminate genotypes. Genotypes excelling in a particular trait are plotted closer, while those with the lowest concentrations were plotted furthest to the vector line. Most of the nutritional and phytochemical traits were closely associated with genotype G1. Beta-carotene and vitamin C were associated with the genotype G6, while G2 and G5 were associated with aluminium, iron, phosphorus and total phenolics. Further, G1 was associated with zinc, potassium, magnesium, cupper and calcium. The genotypes located at the quadrants 2 and 3 in the left-hand

Table 2. Analysis of variance showing mean square values and significant tests for nutritional and phytochemical traits among studied pigeonpea genotypes.

Traits	Replications d.f = 2	Genotype d.f = 6	Error d.f. = 12
Aluminium	1.91	6.99**	0.31
Calcium	6.50	386.04**	8.21
Copper	0.00	0.51**	0.01
Iron	0.20	70.71**	0.10
Potassium	1576.02	114183.30**	398.60
Magnesium	4.31	3042.43**	2.12
Phosphorus	1097.00	11447.00**	1248.00
Zinc	0.24	0.76**	0.05
Beta-carotene	0.04	0.06 ns	0.43
Flavonoids	9.70	4.16 ns	2.21
Total phenolics	8.70	21.23 ns	15.80
Vitamin C	42.00	286 ns	1825.00

Note: ** = significant at $P \leq 0.01$; d.f. = degree of freedom; ns = non-significant.

Table 3. Mean values for nutritional and phytochemical traits of seven pigeon pea genotypes.

Genotype	Al	Ca	Cu	K	Mg	P	Fe	Zn	β-carotene	Vitamin C	Flavonoid (mg CE/g)	Total phenolics (mg GAE/g)
	(mg/100 g)											
Ilonga_14m1 ICEAP-0054	**9.76**	**36.00**	**1.23**	**1008.67**	**114.60**	**288.67**	**15.53**	**1.59**	2.71	87.12	**8.47**	19.40
Kiboko ICEAP-00932	6.16	20.15	0.11	845.33	61.53	172.33	7.51	1.16	2.74	**95.84**	7.39	18.61
Mali ICEAP-00046	*6.09*	15.17	0.10	711.33	32.60	129.73	5.01	0.55	**2.84**	81.32	5.71	13.83
PigeonP-3014	7.70	*4.34*	0.31	*375.33*	*11.87*	*108.60*	*2.63*	*0.11*	*2.45*	80.67	*5.28*	*13.50*
PigeonP-3018	7.71	10.15	*0.09*	691.33	44.20	191.4	14.63	0.32	2.59	74.51	7.75	**20.42**
PigeonP-3021	7.03	31.73	*0.09*	774.00	54.27	236.27	10.41	0.87	2.48	*67.28*	7.56	17.52
Tumia ICEAP-00068	9.05	23.60	0.39	830.67	59.20	211.33	12.13	0.77	2.67	71.20	6.27	16.88
Overall Mean	7.64	20.16	0.33	748.10	54.04	191.19	9.69	0.77	2.64	79.71	6.92	17.16
LSD (0.05)	0.99**	5.09**	0.16**	35.52**	2.59**	62.84**	0.57**	0.38**	1.17**	75.99**	2.64**	7.07**
CV%	6.90	4.80	3.3	2.00	1.50	6.50	1.70	24.10	30.00	3.10	17.00	6.50

CV % = percentage coefficient of variation LSD = least significant difference; Al = aluminium; Ca = calcium; Cu = copper, Fe= Iron, K = potassium, Mg = magnesium, P = phosphorus, Zn = zinc. Boldly-written values indicate the highest value for each trait.

Table 4. Correlation coefficient analysis showing association between the nutritional and phytochemical traits in the immature pods of pigeonpea genotypes.

Trait	Al	Beta-carotene	Ca	Cu	Fe	K	Mg	P	Flavonoids	Total phenolic	Vitamin C
Al	-										
Beta-carotene	0.02 ^{ns}	-									
Ca	0.34 ^{ns}	-0.01 ^{ns}	-								
Cu	0.73**	0.06 ^{ns}	0.53**	-							
Fe	0.57**	0.03 ^{ns}	0.58**	0.47*	-						
K	0.26 ^{ns}	0.15 ^{ns}	0.83**	0.49*	0.71**	-					
Mg	0.47*	0.08 ^{ns}	0.84**	0.76**	0.75**	0.91**	-				
P	0.60**	0.07 ^{ns}	0.83**	0.60**	0.78**	0.74**	0.81**	-			
Flavonoids	0.02 ^{ns}	0.01 ^{ns}	0.32 ^{ns}	0.21 ^{ns}	0.49*	0.41 ^{ns}	0.48*	0.39 ^{ns}	-		
Total phenolics	0.09 ^{ns}	0.03 ^{ns}	0.22 ^{ns}	0.10 ^{ns}	0.54**	0.37 ^{ns}	0.41 ^{ns}	0.40 ^{ns}	0.86**	-	
Vitamin C	0.03 ^{ns}	0.36 ^{ns}	0.06 ^{ns}	0.09 ^{ns}	-0.03 ^{ns}	0.07 ^{ns}	0.09 ^{ns}	0.04 ^{ns}	-0.24 ^{ns}	-0.23 ^{ns}	-
Zn	0.33 ^{ns}	0.01 ^{ns}	0.81 ^{ns}	0.59 ^{ns}	0.46 ^{ns}	0.83 ^{ns}	0.84 ^{ns}	0.75 ^{ns}	0.29 ^{ns}	0.27 ^{ns}	0.10^{ns}

Note: Al = aluminium; Ca = calcium; Cu = copper, Fe= Iron, K = potassium, Mg = magnesium, P = phosphorus, Zn = zinc; ** = significant at $P \leq 0.01$; * = significant at $P \leq 0.05$; ns = non-significant.

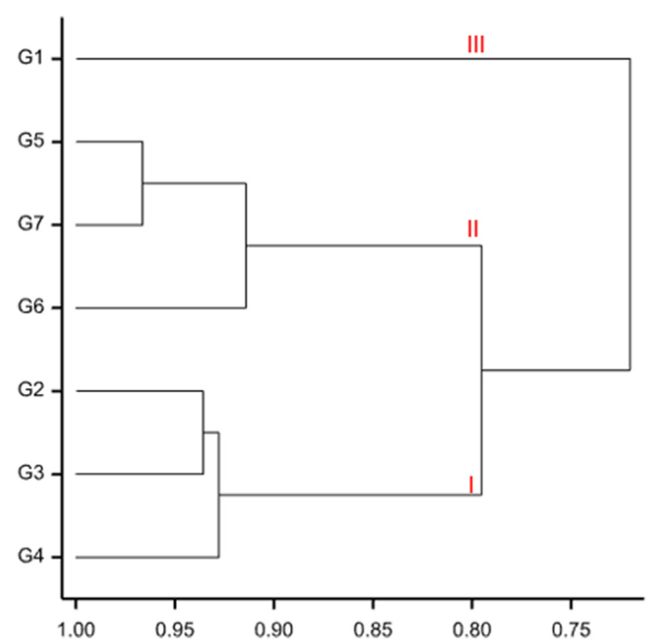

Figure 1. Dendrogram of the seven pigeonpea genotypes generated by average Euclidian distance based on the nutritional traits. G1 = Ilonga_14-m1 ICEAP-0054, G2 = PigeonP-3021, G3 = Mali ICEAP-00046, G4 = PigeonP-3014, G5 = Tumia ICEAP-00068, G6 = Kiboko ICEAP-00932, G7 = PigeonP-3018.

side (i.e., G3 and G4) were not associated with any of the studied nutritional and phytochemical compositions.

4. Discussion

Pigeonpea is a rich source of minerals, vitamins, proteins, and carbohydrates (Saxena et al., 2010) that are essential for human growth and development. The present study quantified nutritional and phytochemical traits in the immature pods of pigeonpea genotypes in order to select promising lines with suitable nutritional quality for production and cultivar development. In the present study, significant genotype effect was observed for Al, Ca, Cu, Fe, K, Mg, P and Zn nutrients, indicating genetic variability for nutrient composition among the test germplasms. This variation among the genotypes plays a significant role in improving selection gain for the traits of interest. Genetic diversity for nutritional traits in immature pods of pigeonpea genotypes was reported elsewhere (Singh et al., 2018). In the current study, genotype Ilonga_14-m1 ICEAP-0054 contained the highest concentrations for all the nutritional factors as well as flavonoids, indicating its superiority and potential to contribute to nutrition security.

Phenolic compounds such as flavonoids and phenolic acids serve as radical scavengers and as metal chelators (Rani et al., 2014). In this study, the concentration of total phenolics ranged from 13.50 mg GAE/g -20.42 mg GAE/g with the highest value recorded for Pigeon P-3018 and lowest value recorded by Pigeon P-3014. This range is higher than the previous range of between 3.0 to 18.3 mg/g reported by Saxena et al. (2010). The flavonoid content ranged from 5.28 mg CE/g to 8.47 mg

Table 5. Summary of mean values for nutritional and phytochemical traits among piegonpea genotypes allocated in three clusters.

Traits	Cluster mean value			Mean
	I	II	III	
Aluminium	6.94	7.64	9.76	8.11
Beta-carotene	2.59	2.67	2.71	2.66
Calcium	17.08	17.97	36.00	23.68
Copper	0.17	0.20	1.23	0.53
Iron	6.02	11.42	15.53	10.99
Potassium	620.22	789.11	1008.67	806.00
Magnesium	32.91	54.98	114.60	67.50
Phosphorus	158.20	191.69	288.67	212.85
Flavonoids	6.18	7.14	8.47	7.27
Total phenolics	14.95	18.64	19.40	17.66
Vitamin C	76.42	80.52	87.12	81.35
Zinc	0.51	0.75	1.59	0.95
Mean	78.52	98.56	132.81	103.30

CE/g, which were recorded in genotypes PigeonP-3014 and Ilonga_14m1 ICEAP-0054, in that order (Table 3). Genotypes identified with low total phenolic and flavonoid contents may be beneficial for their nutritional value while the genotypes with high total phenolic and flavonoid contents may contain good antioxidant compounds.

Calcium is an important mineral element in the human body, which helps in maintaining strong bones and teeth, blood clotting, neurotransmission, muscular movements, hormonal activities and normal heartbeat (Katosh 2013). In the present study, calcium content ranged between 4.34 mg/100 g to 36.0 mg/100 g. This highest level of calcium content recorded with genotype Ilonga_14-m1 ICEAP-0054 is lower than what was previously reported in pigeonpea green pods (Saxena et al., 2010). High concentrations of Fe (15.53 mg/100g), K (1008.67 mg/100g), Mg (114.60 mg/100g) and P (288.67 mg/100g) were observed for genotype Ilonga_14-m1 ICEAP-0054. Similar findings were reported by Sekhon et al. (2017) and Singh et al. (2018).

The highest level recorded for copper and magnesium was comparable to what was previously reported in pigeonpea green pods (Saxena et al., 2010). On the other hand, the highest iron content reported in the current study is at least three-fold of what was previously reported in pigeonpea green pods (Saxena et al., 2010). Iron is required for haemoglobin synthesis and its deficiency causes iron-deficiency anaemia, which is a problem in women and children. The use of the superior genotype (Ilonga_14-m1 ICEAP-0054) in biofortification and crop improvement holds a potential in fighting the scourge of iron deficiency especially in the vulnerable groups. This genotype also contains the highest levels of potassium and phosphorus. Potassium is a key mineral element in the human body that acts as a vasodilator and it reduces blood constriction and blood pressure, whilst phosphorus is important in the structure and function of the human body. Zinc is an important trace mineral element, essential for the body's immune system, cell division, wound healing and sense of smell and taste (Stefanidou et al., 2006). The highest value for Zn concentration was recorded for the genotype Ilonga_14-m1 ICEAP-0054 (1.59 mg/100 g), whereas Pigeon P-3014 recorded the lowest reading (0.11 mg/100 g). Singh et al. (2018) reported a range of 0.8 mg/100 g to

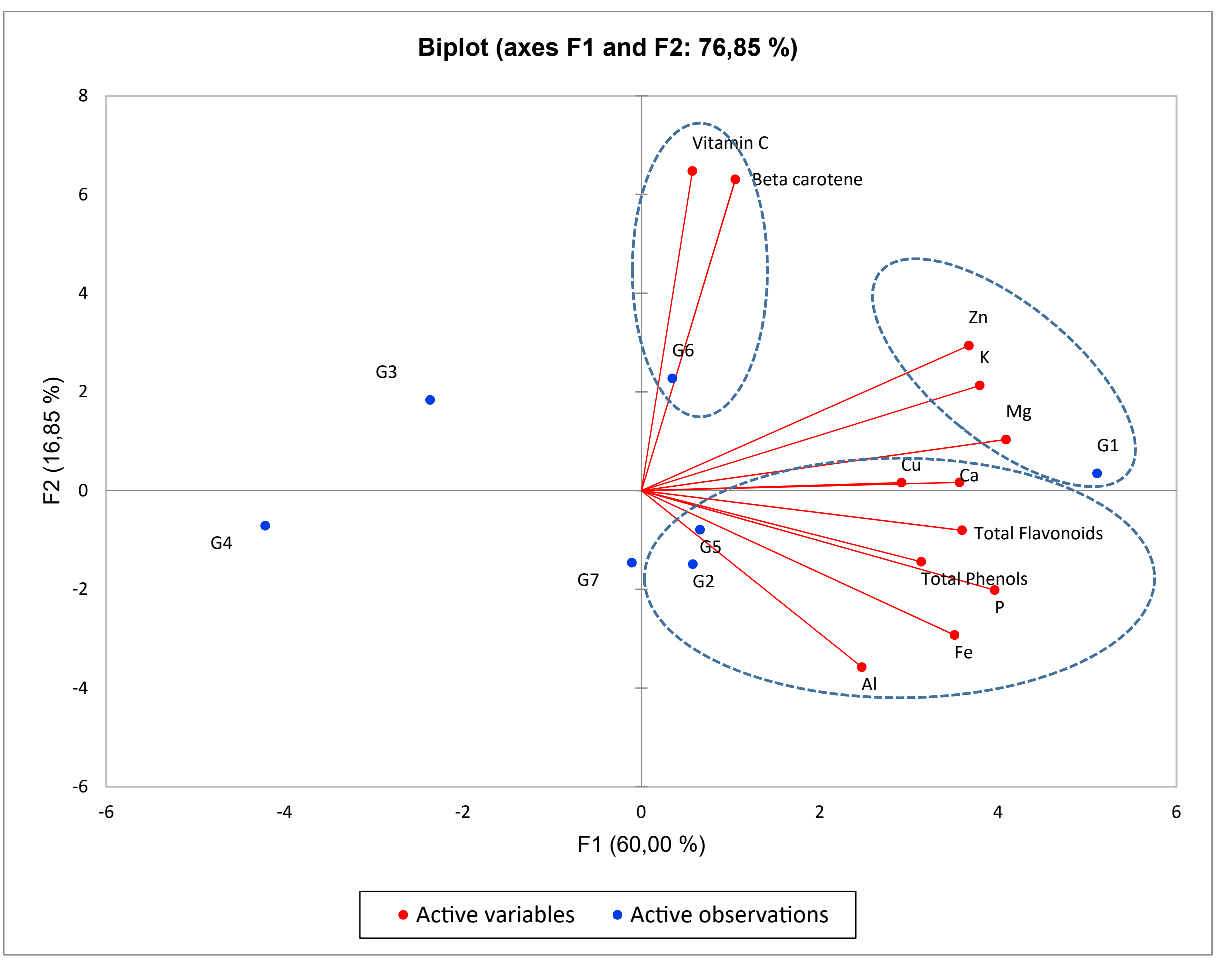

Figure 2. Principal component biplot of nutritional and phytochemical traits for the evaluated pigeonpea genotypes. Note: G1 = Ilonga_14-m1 ICEAP-0054, G2 = PigeonP-3021, G3 = Mali ICEAP-00046, G4 = PigeonP-3014, G5 = Tumia ICEAP-00068, G6 = Kiboko ICEAP-00932, G7 = PigeonP-3018.

3.6 mg/100 g for zinc while Saxena et al. (2010) reported zinc concentration of 2.5 mg/100 g in pigeonpea green immature pods. The results revealed that the investigated lines appeared to be rich sources of iron, copper, manganese and zinc, and this indicates that the lines can effectively contribute towards meeting the daily recommended dietary intake.

Correlation analysis is important to measure the association among major selection traits and plays a vital role in determining effective procedures in breeding programme. In the present study, positive and significant ($P \leq 0.05$) correlations were observed between Cu, Fe, Mg and P, Ca with Cu, Fe, K, Mg and P, Cu with Fe, K, Mg, and P, Fe with K, Mg, total flavonoids and total phenolics, K with Mg and P, Mg and P, and total flavonoids and total phenolics (Table 4 and Figure 2). Total phenols and total flavonoids had significant positive relationship, and this association will be important in selecting for enhanced medicinal properties. Pele et al. (2016) reported that low bioavailability of nutrients is caused by availability of anti-nutrients such as phytate and polyphenols. These results are important, as they will contribute in selection of lines with intention of improving the nutrition in the pigeonpea breeding program. Non-significant and weak negative correlations were computed for beta-carotene and vitamin C with most of the nutritional and phytochemical traits. Hence, these traits could be discarded in the selection of these genotypes for crop improvement. Non-significant correlation was observed among most of the traits, which indicated that selection for high concentration of the traits of interest does not always mean that it leads to greater concentration of the other trait, and that selection for both traits should perhaps be performed simultaneously.

Cluster analysis was used to differentiate the genotypes into groups in order to give a better view of the similarities and differences that exist between them. The dendrogram partitioned the genotypes into three main groups revealing the existence of high variation among the genotypes and thus validating the extensive variability patterns observed (Figure 1). Similar results were reported by Nwanekezi et al. (2017) that cluster analysis differentiated genotypes between the Japan and the North Korean origins. The results indicated the presence of genetic diversity existing among the clusters as revealed by the distant relationships exhibited by certain genotypes. The existence of genetic diversity in the gene pool assists breeders in selecting the best parental genotypes based on nutritional traits to breed for traits of interest.

5. Conclusion

Genotypic variation was observed for most evaluated nutritional and phytochemical traits. This variation indicated the existence of genetic potential for selection of parental lines for nutrition quality improvement in the pigeonpea breeding programs. Genotype PigeonP-3021 was selected for Beta-carotene and vitamin C, while Kiboko ICEAP-00932 and PigeonP-3018 were superior for aluminium, iron, phosphorus and total phenolics. Ilonga_14m1 ICEAP-0054 was selected for suitable zinc, potassium, magnesium, cupper and calcium. Genotypes possessing suitable concentrations of most nutritional traits (i.e., PigeonP-3021, Kiboko ICEAP-00932, PigeonP-3018 and Ilonga_14m1 ICEAP-0054) could be used as potential parental lines in recombination breeding for improved nutritional value in vegetable pigeonpea.

Declarations

Author contribution statement

Abe Shegro Gerrano: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Abueng Moalafi: Performed the experiments; Wrote the paper.

Hlabana A. Seepe: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Stephen Amoo; Hussein Shimelis: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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