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Brief Report

Diversity in Selected Grain Mineral and Protein among Pigeonpea Landraces

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Abstract: Pigeonpea (*Cajanus cajan*) is an important grain legume that provides highly nutritious food for human consumption. It contains high amounts of protein, carbohydrates, fats as well as both macro- and micronutrients. This study examined the genetic diversity of grain mineral and protein content among fourteen pigeonpea landraces. There were highly significant differences ($p \leq 0.001$) among the landraces for most of the mineral elements including calcium (Ca), copper (Cu), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P) and zinc (Zn). The K and P content ranged from 8874.21 to 15,817.38 mg/kg and 2899.23 to 4945.12 mg/kg, respectively. Relatively high amounts of Ca (2103.43 mg/kg) and Mn (73.11 mg/kg) were observed in 'G-03', but 'G-09' attained the highest content of K (15,817.38 mg/kg) and Zn (38.56 mg/kg). Highly significant ($p \leq 0.001$) negative correlations were observed between Mn and Cu. The principal component analysis showed that three landraces ('G-03', 'G-04' and 'G-05') were highly associated with Ca, P, Mg and Mn. The three landraces ('G-03' for Ca and Mn; 'G-04' for Mg and P; 'G-09' for Cu, K and Zn) possessing high grain mineral and protein ('G-10') contents can be utilized in pigeonpea breeding programs that are aimed at improving the grain's traits.

Keywords: correlation; diversity; grain protein; local landrace; mineral content

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1. Introduction

Pigeonpea (*Cajanus cajan*) is a drought-tolerant food legume which is cultivated widely in tropical countries. The grain of pigeonpea sustains the livelihoods of rural communities as a source of affordable protein in many parts of Africa and Asia [1,2]. Moreover, it plays a vital role in food security and income generation through trading in both formal and informal markets. In southern Africa, it is often consumed as fresh green bean or boiled dry grain [3]. The main pigeonpea-producing countries in southern Africa include Malawi, Mozambique and Tanzania. In South Africa, it is cultivated as a minor crop largely in the eastern coastal region of the country.

Pigeonpea grain contains abundant levels of carbohydrates, minerals and proteins [2]. It possesses about 20–26% protein, 65% carbohydrates and 2% fats [4,5]. It can be used as a supplement to cereal-based diets that are deficient in protein, vitamin B and beta-carotenes [6]. The range of minerals in the grain of improved pigeonpea genotypes includes calcium (Ca) (130.00 mg/100 g), potassium (K) (1329.00 mg/100 g), iron (Fe) (5.23 mg/100 g) and zinc (Zn) (2.76 mg/100 g) as well as thiamine (0.64 mg/100 g) and niacin (2.96 mg/100 g) [7]. Medicinally, it is used to treat measles, hepatitis, diabetes and liver dysfunction [8,9]. In addition, pigeonpea possesses considerable amounts of natural antimicrobial compounds such as tannins, flavonoids and alkaloids and a broad spectrum of phytochemical, anticarcinogenic, anti-inflammatory and antidiabetic properties [10–13].

Grain extracts appeared to reduce red blood cell sickling, suggesting the potential to benefit people with sickle cell anaemia [13,14].

In many parts of Africa where poor rural communities typically depend on starch-based diets leading to nutrient deficiencies such as anaemia, hypocalcaemia as well as kwashiorkor, pigeonpea is an affordable source of good-quality protein [15,16]. Biofortification and dietary diversification with nutrient-rich legumes such as pigeonpea can reduce nutrient deficiencies. However, previous research efforts placed little emphasis on the nutrient profiles of the pigeonpea [17–19]. Likely, this was due to inadequate funding. Therefore, the objective of this study was to determine the diversity in grain mineral and protein content among pigeonpea landraces that are available in the pool maintained at the University of Venda with a view to exploit their potential in human diet in future.

2. Materials and Methods

2.1. Location, Planting and Genetic Materials

This study was conducted at the University of Venda (22°56′59″ S; 30°28′59″ E, 724 m a.s.l.). Plants were raised in the shedhouse during the summer growing season (October–March). The mean daily temperatures at the location range between 26 and 38 °C in summer and between 18 and 26 °C in winter [20]. The average annual rainfall is about 650 mm. The red soils at the location have an apedal structure: they are deep (>1500 mm), dystrophic and well-drained.

Fourteen pigeonpea genotypes selected randomly from a germplasm pool maintained at the University of Venda were utilized in this study (Table 1). Prior to planting, pots measuring 35 cm × 30 cm × 35 cm were filled with field topsoil. At planting, during the first week of December 2020, two seeds of each genotype were placed in a hole 3 cm deep in the pot and subsequently covered with soil and allowed to germinate. At physiological maturity, between July and August 2021, the grain was harvested manually and stored in the refrigerator until analysis. The pigeonpea plants were raised without application of chemical fertilizers to simulate typical agronomic practices that prevail in the smallholder production systems in the area. To address the heterogeneity associated with landraces, each one of them was previously selfed repeatedly for several filial generations to attain uniformity.

Table 1. Pigeonpea landraces that were utilized in the study (For grain size, 100-grain weight: small $\leq$ 11.0 g; medium = 11.1–16.0 g; large > 16.0 g).

Genotype		Type	Grain Size and Color
Designation	**Code**		
G-01	SST	Exotic landrace	Small, brown
G-02	ENT-3	Exotic landrace	Small, brown
G-03	MP-BLK	Local landrace	Medium, black
G-04	HBR	Exotic landrace	Large, red
G-05	LW-AM	Local landrace	Medium, grey
G-06	MJ-ORIG	Exotic landrace	Large, red
G-07	T-POD	Exotic landrace	Medium, cream/white
G-08	MP-BRN-SPEC	Local landrace	Medium, brown
G-09	UG-22	Exotic landrace	Medium, cream/white
G-10	I-557	Improved genotype	Large, cream/white
G-11	DC	Exotic landrace	Large, cream/white
G-12	MJ-HBR	Exotic landrace	Large, red
G-13	L-POD-YLW	Exotic landrace	Medium, cream/white
G-14	EX-ML-2	Exotic landrace	Large, red

2.2. Grain Mineral and Protein Determination

Dry pigeonpea grain samples (weighing 5.0 g each) were ground to a fine powder using a mortar and pestle and sieved through a 1.0 mm stainless steel sieve (mesh number 18) to obtain a homogenized sample. For the determination of mineral elements, about

1.0 g of each sample was ashed at 450 °C for 4 h, after which a few drops of dH_2O followed by 2.0 mL of hydrochloric acid (HCl) were added [21]. The samples were evaporated to dryness in a water bath prior to addition of 2.5 mL of freshly prepared 1:9 HCl solution to each sample and then filtered using Whatman filter paper discs thereafter. The filtered sample was diluted with de-ionized water at a ratio of 5:20 and analysed for mineral elements using a Varian 720 Inductively Coupled Plasma Emission Spectrometer (ICP-OES, Frankfurt, Germany).

For crude protein, total nitrogen was determined using Kjeldahl's method. About 1.0 g of each seed sample was mixed with 20.0 mL of concentrated sulphuric acid (H_2SO_4) in heating tubes. Two Kjeldahl selenium catalyst tablets were added to each tube and the mixture was boiled inside a fume cupboard and subsequently distilled using a Buchi distillation unit K-350. The liberated NH_4-N was collected in a beaker containing 20.0 mL 4% boric acid (H_3BO_3), and three droplets of methyl red indicator was added prior to titration with 0.1 M HCl using a Mettler Toledo DL15 autotitrator with a pH electrode. Percentage crude protein was calculated as total nitrogen × 6.25. Replicate samples were analysed for each nutrient per genotype.

2.3. Experimental Design and Data Analysis

The experiment was laid as a completely randomized block design with two replications. Quantitative data sets were subjected to analysis of variance (ANOVA) using Minitab version 19.0 followed by mean separation at $p \leq 0.05$ using Tukey's test procedure. Prior to the ANOVA, the evaluation of the protein and all the mineral elements, except for Fe, Mg and Mn, complied with the requirements for normality of data and the homogeneity of variances test and indicated normal skewness between -0.5 and 0.5 as well as a platykurtic distribution (Kurtosis < 3.0), respectively. Hence, the data sets for Fe, Mg and Mn were transformed using the BoxCox standard method. To determine the strength of the linear association between nutritional traits, Pearson's correlation coefficient test was performed. The principal component analysis (PCA) was used to determine the significant variables that contributed to the variation observed among the pigeonpea genotypes.

3. Results

3.1. Analysis of Variance for Selected Mineral and Grain Contents

There were very highly significant ($p \leq 0.001$) differences among the genotypes for most of the mineral elements including Ca, Cu, K, Mg, P and Zn (Table 2). The P content (4031.94 mg/kg) was more than double that of both Ca (1257.46 mg/kg) and Mg (1409.94 mg/kg), respectively. Similarly, the Fe content (45.24 mg/kg) was three-fold more than the that of Cu. The mean crude protein content was 21.50%. The local landrace 'G-03' attained the highest (2103.43 mg/kg) Ca content followed by 'G-04' (1606.27 mg/kg) and 'G-02' (1552.62 mg/kg) (Table 3). The Mg content ranged between 1231.21 and 1829.99 mg/kg. In addition, the exotic landrace 'G-04' showed a comparatively high Mg (1829.99 mg/kg) and P (4945.12 mg/kg) contents. The exotic landrace 'G-09', attained the highest K content (15,817.38 mg/kg) which was only 6.7% higher than in the check (improved genotype) 'G-10' (14,793.03 mg/kg) (Table 3).

Among the micro-nutrients, the Fe and Zn content ranged between 32.99 and 66.05 mg/kg and 20.48 and 38.56 mg/kg, respectively (Table 3). The genotype 'G-03' attained a significantly high Mn content (73.11 mg/kg), which was >30.0% higher than that observed in the rest of the genotypes. The average crude protein was 21.50% but the check ('G-10') achieved 23.50% protein. Nonetheless, two landraces (namely 'G-08' and 'G-01') attained similar protein contents: 23.25% and 23.41%, respectively.

Table 2. Analysis of variance for selected mineral and protein content in the grain of pigeonpea landraces.

Source	df	Mean Squares								
		Ca	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**P**	**Zn**	**Protein**
Replication	1	16,756.88	0.23	283.37	755,162.79	8727.94	3.73	33,662.84	2.40	20.04
Genotype	13	264,715.48 ***	4.66 ***	261.04	10,281,201.98 ***	60,253.00 ***	718.69 ***	702,416.16 ***	65.67 ***	5.10
Mean	-	1257.46	11.24	45.24	13,014.14	1409.94	23.34	4031.94	28.05	21.50
C.V. (%)	-	29.06	13.65	36.29	17.37	12.50	80.30	14.82	20.33	9.66
R^2 (%)	-	95.89	95.77	50.51	97.47	94.46	98.60	95.12	97.47	74.23

*** = very highly significant; highly significant and significant at the 0.1%, 1.0% and 5.0% probability levels, respectively. (Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; P = phosphorus; Zn = zinc).

Table 3. Mean values for selected mineral content and protein in the grain of pigeonpea landraces.

Genotype	Nutritional Element								
	Ca (mg/kg)	**Cu (mg/kg)**	**Fe (mg/kg)**	**K (mg/kg)**	**Mg (mg/kg)**	**Mn (mg/kg)**	**P (mg/kg)**	**Zn (mg/kg)**	**Protein (%)**
G-03	2103.4 ^{a} ± 7.3	8.7 ^{f} ± 0.5	41.8 ^{a} ± 4.5	12,227.7 ^{cd} ± 328.6	1620.6 ^{ab} ± 66.6	73.1 ^{a} ± 5.8	4710.3 ^{ab} ± 87.8	28.4 ^{cde} ± 0.6	20.0 ^{a} ± 0.2
G-04	1606.3 ^{b} ± 70.6	9.1 ^{ef} ± 0.2	36.5 ^{a} ± 1.5	8874.2 ^{e} ± 360.9	1830.0 ^{a} ± 105.9	32.2 ^{c} ± 2.5	4945.1 ^{a} ± 8.1	27.2 ^{def} ± 0.3	21.9 ^{a} ± 0.2
G-02	1552.6 ^{b} ± 76.1	11.4 ^{bcd} ± 0.5	34.8 ^{a} ± 3.0	13,988.9 ^{abc} ± 16.8	1231.2 ^{d} ± 2.7	15.8 ^{de} ± 1.0	2899.2 ^{f} ± 31.2	21.7 ^{g} ± 1.6	21.2 ^{a} ± 3.5
G-05	1431.6 ^{bc} ± 189.4	10.0 ^{def} ± 0.7	36.7 ^{a} ± 3.7	9975.8 ^{e} ± 526.1	1563.3 ^{bc} ± 30.4	34.1 ^{c} ± 34.1	4837.5 ^{ab} ± 98.4	32.5 ^{bc} ± 0.6	23.1 ^{a} ± 2.8
G-11	1414.2 ^{bcd} ± 158.4	10.1 ^{def} ± 0.1	66.0 ^{a} ± 40.7	13,444.4 ^{bc} ± 189.8	1423.2 ^{bcd} ± 89.7	11.7 ^{e} ± 2.0	4059.4 ^{bcde} ± 31.0	22.8 ^{fg} ± 0.9	19.4 ^{a} ± 2.8
G-12	1351.1 ^{bcde} ± 78.7	11.7 ^{bcd} ± 0.9	55.2 ^{a} ± 20.8	13,602.7 ^{bc} ± 362.3	1316.0 ^{cd} ± 30.8	14.9 ^{de} ± 0.2	3714.9 ^{de} ± 93.8	30.7 ^{bcd} ± 2.7	21.3 ^{a} ± 1.8
G-13	1306.5 ^{bcdef} ± 7.5	12.7 ^{ab} ± 0.1	62.2 ^{a} ± 30.2	15,025.5 ^{ab} ± 924.8	1550.2 ^{bc} ± 58.2	13.0 ^{de} ± 0.2	3704.9 ^{de} ± 428.7	35.5 ^{ab} ± 0.77	21.7 ^{a} ± 1.4
G-06	1266.7 ^{bcdef} ± 124.5	12.0 ^{bc} ± 0.2	33.0 ^{a} ± 9.4	15,278.9 ^{ab} ± 3.4	1290.5 ^{d} ± 24.6	12.4 ^{e} ± 0.4	4198.8 ^{abcde} ± 224.1	24.8 ^{efg} ± 0.53	21.4 ^{a} ± 2.5
G-09	1030.4 ^{cdefg} ± 116.9	14.3 ^{a} ± 0.3	52.9 ^{a} ± 7.8	15,817.4 ^{a} ± 46.9	1321.9 ^{cd} ± 37.0	13.9 ^{de} ± 0.3	3548.8 ^{ef} ± 43.3	38.6 ^{a} ± 1.4	22.4 ^{a} ± 0.1
G-01	1002.3 ^{cdefg} ± 19.9	12.7 ^{ab} ± 0.4	37.4 ^{a} ± 7.6	14,274.4 ^{abc} ± 797.2	1263.1 ^{d} ± 13.7	9.8 ^{e} ± 0.5	3887.6 ^{cde} ± 257.4	20.5 ^{g} ± 2.2	23.4 ^{a} ± 1.2
G-08	994.0 ^{defg} ± 114.0	10.4 ^{cdef} ± 0.2	36.7 ^{a} ± 8.9	14,283.1 ^{abc} ± 511.5	1271.9 ^{d} ± 15.2	9.5 ^{e} ± 0.3	3631.7 ^{def} ± 265.3	22.7 ^{fg} ± 0.4	23.2 ^{a} ± 2.6
G-10	961.9 ^{efg} ± 114.3	12.2 ^{bc} ± 0.6	35.6 ^{a} ± 3.2	14,793.0 ^{ab} ± 268.9	1409.1 ^{bcd} ± 53.5	9.0 ^{e} ± 0.1	4310.9 ^{abcd} ± 105.9	22.5 ^{fg} ± 1.0	23.5 ^{a} ± 0.3
G-07	898.0 ^{fg} ± 69.5	11.4 ^{bcd} ± 0.2	46.8 ^{a} ± 2.3	9922.9 ^{e} ± 506.7	1255.8 ^{d} ± 97.7	25.0 ^{cd} ± 2.5	4550.8 ^{abc} ± 291.1	31.8 ^{bcd} ± 0.5	20.2 ^{a} ± 1.1
G-14	685.3 ^{g} ± 162.2	10.6 ^{cde} ± 0.4	57.9 ^{a} ± 23.3	10,689.0 ^{de} ± 1216.0	1392.3 ^{bcd} ± 106.9	52.2 ^{b} ± 0.4	3447.0 ^{ef} ± 86.8	32.9 ^{bc} ± 2.0	18.2 ^{a} ± 0.3

Means followed by the same letter in a column are not significantly different ($p \leq 0.05$) (Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; P = phosphorus; Zn = zinc).

3.2. Relationships between Selected Grain Mineral and Protein Content

There was a highly significant ($p \leq 0.01$) negative association between Cu and Mn (Table 4). Nonetheless, a highly significant ($p \leq 0.01$) strong positive linear relationship was observed between Cu and K, suggesting that increasing the content of either mineral will result in a concomitant improvement in the content of the other mineral. In contrast, Ca showed a negative correlation with Cu but a significant ($p \leq 0.05$) positive correlation with Mg (Table 4). Similarly, a significant ($p \leq 0.05$) negative linear relationship existed between Mg and Cu but a positive linear relationship was observed between Mg and P. Significant ($p \leq 0.05$) negative correlations were observed between protein and both Fe and Mn (Table 4).

Table 4. Pearson's correlation coefficients (r) indicating the association between selected grain mineral and protein contents in pigeonpea landraces. (Ca = calcium; Cu = copper; Fe = iron; K = potassium; Mg = magnesium; Mn = manganese; P = phosphorus; Zn = zinc).

Variable	Ca	Cu	Fe	K	Mg	Mn	P	Zn	Protein
Ca	1.000								
Cu	−0.541 *	1.000							
Fe	−0.148	0.157	1.000						
K	−0.139	0.704 **	0.074	1.000					
Mg	0.557 *	−0.573 *	0.021	−0.523	1.000				
Mn	0.424	−0.646 **	0.011	−0.592 *	0.507	1.000			
P	0.348	−0.514	−0.275	−0.579 *	0.647 *	0.377	1.000		
Zn	−0.105	0.246	0.502	−0.171	0.223	0.287	0.043	1.000	
Protein	−0.086	0.347	−0.575 *	0.332	−0.04	−0.532 *	0.104	−0.226	1.000

**; * = highly significant and significant at the 1.0% and 5.0% probability levels, respectively.

3.3. Principal Component and Cluster Analysis for Selected Grain Mineral and Protein Contents

The first three principal components (PCs) accounted for 76.50% of the variation among the pigeonpea genotypes with eigenvalues of more than one (Table 5). Three variables, namely Mg, Mn and P, were associated with PC1 with Mn, contributing the most variation to this component. The second principal component (PC2) explained 22.84% of the variance, reflecting positive loadings of protein and P but negative correlations for Fe and Zn. However, the third principal component (PC3) explained only 12.49% of the variance, with negative loadings for both Zn and protein. The pigeonpea landraces were scattered randomly across all the quadrants on the biplot (Figure 1). Nevertheless, the improved genotype 'G-10' along with the local 'G-03' and exotic 'G-04', 'G-01', 'G-14' and 'G-09' were clustered at the extreme margins of the cartesian plane. Genotypes 'G-05', 'G-04' and 'G-03' were closely associated with P, Ca, Mg and Mn. In addition, protein as well as K and Cu appeared to be associated markedly with five landraces, namely 'G-01', 'G-02', 'G-06', 'G-08' and 'G-10', which were located in the top-left quadrant (Figure 1). In contrast, <30.0% of the landraces ('G-07', 'G-11', 'G-12' and 'G-13') were closely associated with both Fe and Zn. Moreover, a negative relationship was detected between protein and each of these two micro-elements as indicated by the obtuse angle between the vector for protein and that of Fe and Zn. Furthermore, both 'G-09' and 'G-14' were positioned conspicuously far away from the origin, suggesting that they were the most genetically divergent landraces for the eight elements and grain proteins that were evaluated. Several nutritional traits including Ca, Mg, Mn, P and Zn revealed a negative and significant association with traits such as Cu, protein and K, as indicated by a large angle between the traits (Figure 1). Three genotypes ('G-01, 'G-08' and 'G-10') were associated with high levels of protein and K. Furthermore, both 'G-09' and 'G-14', which were positioned distantly from the origin, also suggested that they possessed unique alleles for the grain mineral elements and protein contents that were evaluated.

Table 5. The eigenvectors and eigenvalues for grain mineral and protein content among pigeonpea landraces.

Parameter	Principal Components		
	PC1	**PC2**	**PC3**
Ca	0.315	0.170	0.136
Cu	−0.449	−0.112	−0.349
Fe	−0.034	−0.608	−0.028
K	−0.413	0.053	0.047
Mg	0.415	0.046	−0.350
Mn	0.421	−0.184	0.125
P	0.369	0.234	−0.375
Zn	0.068	−0.481	−0.597
Protein	−0.191	0.514	−0.471
Eigenvalue	3.70	2.05	1.12
Proportion (%)	41.17	22.84	12.49
Cumulative (%)	41.17	64.01	76.50

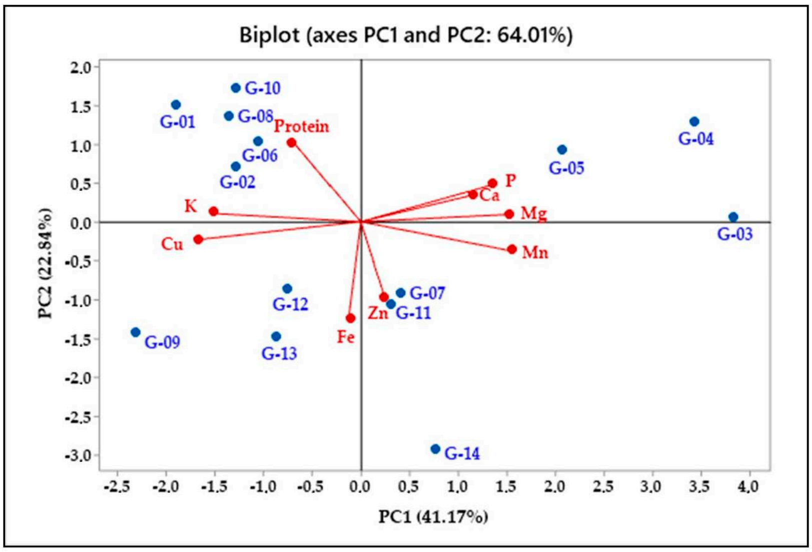

Figure 1. Biplot analysis for grain mineral and protein content among pigeonpea landraces.

The cluster analysis revealed three clusters out of fourteen pigeonpea landraces (Table 6). ‘G-01′ was clustered together with the local landrace ‘G-08′ in sub-cluster I-a, but sub-cluster I-c consisted of ‘G-02′ as a singleton joining the two subclusters at a similarity level of 81.70%, based on Euclidean and correlation distance, using complete linkage clustering. Compared to other landraces in the cluster, a singleton is the most superior within the cluster. The improved check genotype ‘G-10′ was clustered together with ‘G-06′ in subcluster II-a and ‘G-13′ was the most diverse member of the cluster. Cluster III consisted of five landraces including ‘G-03′ and ‘G-14′.

Table 6. Groups of pigeonpea landraces determined via cluster analysis based on grain mineral and protein content.

Cluster	Sub-Cluster	Number of Genotypes	Total	Genotypes	Type
I	a	2	5	G-01	Exotic
				G-08	Local
	b	2		G-11	Exotic
				G-12	Exotic
	c	1		G-02	Exotic
II	a	3	4	G-06	Exotic
				G-10	Improved
				G-13	Exotic
	b	1		G-09	Exotic
III	a	2	5	G-03	Local
				G-14	Exotic
	b	3		G-04	Exotic
				G-05	Local
				G-07	Exotic

4. Discussion

The genotypic variability observed for the various minerals including Ca, Cu, Mg, Mn, P and Zn indicated the potential for exploiting the landraces in pigeonpea genetic improvement programs that are aimed at the optimization of the grain of such minerals. Similar findings of genetic variation in nutritional characteristics were previously reported in improved and vegetable pigeonpea varieties [22,23]. In a similar study involving common bean, differences in individual minerals and protein were attributed to fertilizer applications and edaphic factors [24]. For instance, the grain content of Zn and Ca was due to soil and/or foliar applications of chemical fertilizers containing these minerals [25,26]. Nonetheless, the pigeonpea genotypes in this study were grown without the use of fertilizers. In the current study, genotype 'G-03' attained the highest concentrations for most mineral elements, thus demonstrating its superiority and potential for exploitation in future pigeonpea programs for enhancing grain nutrients.

The grain mineral elements in these pigeonpea landraces are essential for the optimal functioning of the immune system [23,27]. For example, calcium is important for normal heartbeat and muscle functioning, bone health as well as neurotransmission [28]. The highest Ca content observed in genotype 'G-03' (2103.43 mg/kg) was double that in similar studies [29]. Moreover, the Ca content which was observed in this study for 'G-03' was higher than in common bean [24]. The high levels (>15,000 mg/kg) of K accumulated by pigeonpea landraces such as 'G-13', 'G-06' and 'G-09' in this study also suggested the potential to reduce the risk of cardiovascular diseases and diabetes when included in the diet [30]. The presence of K in the body is also associated with an increase in iron utilization and hypertension control [31]. In comparison with other leguminous species, the P content in the pigeonpea landraces was higher than in both lentil (*Lens culinaris*) (299.45 mg/100 g) and garden pea (*Pisum sativum*) (352.79 mg/100 g). On the other hand, the Mg content observed in this study was almost two-fold that observed in a similar study in Nigeria (84.31 mg/100 g), suggesting that the accumulation of this mineral depends on both genetic and environmental factors since distinct genotypes were used in the studies [32]. Mg acts as a cofactor for more than 300 enzymes, thus regulating multiple fundamental functions such as glycaemic control, type 2 diabetes, myocardial contractions and osteoporosis [33,34].

Among the micronutrients in this study, iron was the most abundant microelement (ranging between 32.99 and 66.05 mg/kg), and was similar to the content observed in chickpea (48.6–55.6 mg/kg), but was significantly lower than in lentil (75.6–100 mg/kg) [35,36]. The landraces 'G-11' and 'G-13', which showed relatively high grain Fe content, could be used in biofortification aimed at fighting anaemia in vulnerable groups such as women and children. Some of the pigeonpea landraces also contained significantly higher Zn than chickpea (21.1–28.3 mg/kg). Zn is essential for different biological functions such as wound healing, protection from oxidative damage by reactive oxygen species and prevention of both pancreatic and prostate cancer [37]. The two landraces ('G-13' and 'G-09') which contained significantly high Cu and Zn could also be useful in mineral biofortification in pigeonpea. Various studies reported successful improvements in micronutrients such as Fe and Zn in legumes including pigeonpea through biofortification [38–41]. The highest grain protein content (23.50%), which was attained by the landrace 'G10', was comparable but lower than the variability (23.35–29.50%) which was observed among 600 pigeonpea genotypes from a regional genebank in India [42]. Nonetheless, this study identified useful landraces that contain a good balance of protein and mineral content; hence, they can be use as donor parental materials in pigeonpea breeding programs aimed at enhancing grain protein content to benefit end-users, particularly in poor rural communities that are prone to malnutrition.

Correlation analysis is critical for detecting the relationship between nutritional features that can be used to determine effective breeding strategies [43]. In this regard, positively correlated mineral elements could make concomitant biofortification with multiple minerals easy. However, significant negative correlations were observed between grain protein and Fe and Mn. Nonetheless, the improvement of Ca content in pigeonpea is favoured by Mg selection since a significant positive correlation between Ca and Mg was evident among the landraces. Various studies reported that Fe and Mn are cofactors in several enzymes (such as arginase, glutamine synthetase, Mn superoxide dismutase and pyruvate carboxylase), thus suggesting that, perhaps, deficiencies in these minerals could diminish grain protein content [44,45]. In contrast, excess Mn was reported to decrease some proteins associated with signalling pathways as well as negatively affect the utilization of other minerals such as Ca and Fe and eventually cause oxidative stress [45].

Principal component analysis (PCA) is an important tool for determining the significance of various traits and genotypes based on their contribution to the overall variation. Mineral elements such as Ca, Mg, Mn and P could be considered = the most informative for evaluating genetic diversity for the grain and protein content among the pigeonpea landraces due to their strong association with the first principal component (PC1). In this regard, the landraces such as 'G-03', 'G-04' and 'G-05' which were strongly associated with Ca, P, Mg and Mn could be considered for introgressing genes that control the accumulation of these nutrient elements. Similar studies involving PCA showed that pigeonpea and lentil were characterized by high calcium and protein contents, respectively [35,42]. The six landraces ('G-03', 'G-04', 'G-10', 'G-01', 'G-09' and 'G-14') which were positioned far from the origin of the biplot indicated that they were the most distinct and probably possessed unique genes associated with the grain traits.

The cluster analysis partitioned the landraces into three major groups, revealing considerable intra-cluster variability. Two landraces ('G-04' and 'G-09'), which were grouped as singletons, were among the most divergent for the traits. Similar studies in pigeonpea also revealed high genetic diversity based on cluster analysis [42]. The pigeonpea genotypes that were grouped together may not be recommended for hybridization as they could be related. However, molecular tools can be used to validate the genetic similarity of the landraces with regard to the grain mineral and protein content. This is partly because phenotypic expression is influenced by the environment and provides a proxy indicator of genetic variation, but molecular markers are more reliable.

5. Conclusions

This study revealed the variability in a range of mineral elements among pigeonpea landraces, which provided suggestions for selecting superior landraces for pigeonpea genetic enhancement programs. This study also identified three landraces ('G-03', 'G-01' and 'G-13') that possess high grain mineral and protein contents. Such genetic materials are recommended for utilization in pigeonpea breeding programs that are aimed at improving the grain's nutritional traits such as its mineral and protein content. The development of molecular markers for these traits could also be useful in the genetic improvement of pigeonpea.

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References

1. Ganguly, K.; Gulati, A. Pulses Value Chain-Pigeon Pea and Gram. In *Agricultural Value Chains in India, India Studies in Business and Economics*; Gulati, A., Ganguly, K., Wardhan, H., Eds.; Springer: Singapore, 2022. [CrossRef]
2. Talari, A.; Shakappa, D. Role of Pigeonpea (*Cajanus cajan*) in Human Nutrition and Health: A Review. *Asian J. Dairy Food Res.* **2018**, *37*, 212–220.
3. Gwata, E.T. Potential Impact of Edible Tropical Legumes on Crop Productivity in the Small-Holder Sector in Sub-Saharan Africa. *J. Food Agric. Environ.* **2010**, *8*, 939–944.
4. Ojwang, D.; Nyankanga, R.; Imungi, J.; Rao, G.; Olanya, M.; Kumar, V.; He, Z. Effects of Processing and Storage on the Nutrient Composition of Green Vegetable Pigeonpea. *J. Food Process. Preserv.* **2021**, *45*, e15714. [CrossRef]
5. Janeczko, A.; Dziurka, M.; Ostrowska, A.; Koscielniak, J. Improving Vitamin Content and Nutritional Value of Legume Yield Through Water and Hormonal Seed Priming. *Legum. Res.* **2015**, *38*, 185–193. [CrossRef]
6. Abebe, K.B. The Dietary Use of Pigeonpea for Human and Animal Diets. *Sci. World J.* **2022**, *2022*, 4873008. [CrossRef]
7. Dinore, J.M.; Farooqui, M. GC-MS and LC-MS: An Integrated Approach Towards the Phytochemical Evaluation of Methanolic Extract of Pigeonpea (*Cajanus cajan*) Leaves. *Nat. Prod. Res.* **2020**, *36*, 2177–2181. [CrossRef]
8. Oladunmoye, M.K.; Kehinde, F.Y. Ethnobotanical Survey of Medicinal Plants Used in Treating Viral Infections Among Yoruba Tribe of Southwestern Nigeria. *Afr. J. Microbiol. Res.* **2011**, *5*, 2991–3004. [CrossRef]
9. Hosseinpour-Niazi, S.; Mirmiran, P.; Hedayati, M.; Azizi, F. Substitution of Red Meat with Legumes in the Therapeutic Lifestyle Change Diet Based on Dietary Advice Improves Cardiometabolic Risk Factors in Overweight Type 2 Diabetes Patients: A Cross-over Randomized Clinical Trial. *Eur. J. Clin. Nutr.* **2015**, *6*, 592–597. [CrossRef]
10. Luo, M.; Liu, X.; Zu, Y.; Fu, Y.; Zhang, S.; Yao, L.; Efferth, T. *Cajanol*, A Novel Anticancer Agent from Pigeonpea Roots, Induces Apoptosis in Human Breast Cancer Cells Through A ROS-Mediated Mitochondrial Pathway. *Chem. Biol. Interact* **2010**, *188*, 151–160. [CrossRef]
11. Vo, T.T.; Yang, N.C.; Yang, S.E.; Chen, C.L.; Wu, C.H.; Song, T.Y. Effects of *Cajanus cajan* Roots Extracts on the Antioxidant and Anti-Inflammatory Activities. *Chin. J. Physiol.* **2020**, *63*, 137–148. [CrossRef]
12. Grover, J.K.; Yadav, S.; Vats, V. Medicinal Plants of India with Anti-Diabetic Potential. *J. Ethnopharmacol.* **2002**, *81*, 81–100. [CrossRef] [PubMed]
13. Verma, H. Phytochemical Characterization of Twelve Medicinal Plants Used for Sickle Cell Disease Management in Chattisgarh. *Int. J. Pharm. Biol. Sci.* **2015**, *6*, 1062–1070.
14. Ekeke, G.; Shode, F. The Reversion of Sickled Cells by *Cajanus cajan*. *Planta Med.* **1985**, *51*, 504–507. [CrossRef] [PubMed]
15. Pele, G.I.; Oladiti, E.O.; Bamidele, P.O.; Fadipe, E.A. Influence of Processing Techniques on the Nutritional and Anti-Nutritional Properties of Pigeonpea (*Cajanus cajan*). *Int. J. Eng. Appl. Sci.* **2016**, *3*, 92–94.
16. Majili, Z.S.; Nyaruhucha, C.; Kulwa, K.; Mutabazi, K.; Rybak, C.; Sieber, S. Preferences and Consumption of Pigeonpeas Among Rural Households as Determinants for Developing Diversified Products for Sustainable Health. *Sustainability* **2020**, *12*, 6130. [CrossRef]

17. Gwata, E.T.; Silim, S.N. Utilization of Landraces for The Genetic Enhancement of Pigeonpea In Eastern and Southern Africa. *J. Food Agric. Environ.* **2009**, *7*, 803–806.
18. Gwata, E.T.; Siambi, M. Genetic Enhancement of Pigeonpea for Latitude Areas in Southern Africa. *Afr. J. Biotechnol.* **2009**, *8*, 4413–4417.
19. Tadele, Z.; Bartels, D. Promoting Orphan Crops Research and Development. *Planta* **2019**, *250*, 675–676. [CrossRef]
20. Mzezewa, J.; Misi, T.; Van Rensburg, L.D. Characterisation of Rainfall at Semi-Arid Ecotope in The Limpopo Province (South Africa) and Its Applications for Suitable Crop Production. *Water SA* **2012**, *36*, 19–26.
21. Thovhogi, F.; Mchau, G.R.A.; Gwata, E.T.; Ntushelo, N. Evaluation of Leaf Mineral, Flavonoid, and Total Phenolic Content in Spider Plant Germplasm. *Molecules* **2021**, *26*, 3600. [CrossRef]
22. Singh, S.K.; Jadhav, P.; Nadanwar, R. Assessment of Nutritional Quality Parameters in Selected Vegetable Type Pigeonpea Genotypes. *J. Pharmacogn. Phytochem.* **2018**, *1*, 1446–1450.
23. Singh, U.; Jambunathan, R.; Saxena, K.; Subrahmanyam, N. Nutritional Quality Evaluation of Newly Developed High-Protein Genotypes of Pigeonpea (*Cajanus cajan*). *J. Sci. Food Agric.* **1990**, *50*, 201–209. [CrossRef]
24. Murube, E.; Beleggia, R.; Pacetti, D.; Nartea, A.; Frascarelli, G.; Lanzavecchia, G.; Bellucci, E.; Nanni, L.; Gioia, T.; Marciello, U.; et al. Characterization of Nutritional Quality Traits of a Common Bean Germplasm Collection. *Foods* **2021**, *10*, 1572. [CrossRef] [PubMed]
25. Frossard, E.; Bucher, M.M.; Achler, F.; Mozafar, A.; Hurrell, R. Potential for Increasing the Content and Bioavailability of Fe, Zn and Ca in Plants for Human Nutrition. *J. Sci. Food Agric.* **2000**, *80*, 861–879. [CrossRef]
26. Yang, S.E.; Vo, T.T.; Chen, C.L.; Yang, N.C.; Chen, C.I.; Song, T.Y. Nutritional Composition, Bioactive Compounds and Functional Evaluation of Various Parts of *Cajanus cajan*. *Agriculture* **2020**, *10*, 558. [CrossRef]
27. Peters, B.S.E.; Martini, L.A. Nutritional Aspects of the Prevention and Treatment of Osteoporosis. *Arq. Bras. Endocrinol. Metabol.* **2010**, *54*, 179–185. [CrossRef]
28. Singh, U.; Jain, K.C.; Jambunathan, R.; Faris, D.G. Nutritional Quality of Vegetable Pigeonpeas (*Cajanus cajan*): Mineral and Trace Elements. *J. Food Sci.* **1984**, *49*, 645–646. [CrossRef]
29. Susmitha, D.; Kalaimagal, T.; Senthil, R.; Vetriventhan, M.; Anitha, S.; Manonmani, S.; Jeyakumar, P.; Reddymalla, S.; Peerzada, O.; Arveti, V.N.; et al. Calcium-Rich Pigeonpea Seed Coat: A Potential by Product for Food and Pharmaceutical Industries. *Sustainability* **2022**, *14*, 4918. [CrossRef]
30. Stone, M.; Martyn, L.; Weaver, C. Potassium Intake, Bioavailability, Hypertension, and Glucose Control. *Nutrients* **2016**, *8*, 444. [CrossRef]
31. Sica, D.A.; Struthers, A.D.; Cushman, W.C.; Wood, M.; Banas, J.S., Jr.; Epstein, M. Importance of Potassium in Cardiovascular Disease. *J. Clin. Hypertens.* **2002**, *4*, 198–206. [CrossRef]
32. Adepoju, O.T.; Dudulewa, B.I.; Bamigboye, A.Y. Effect of Cooking Methods on Time and Nutrient Retention of Pigeonpea (*Cajanus cajan*). *Afr. J. Food Agric. Nutr. Dev.* **2019**, *19*, 14708–14725.
33. Bertinato, J.; Xiao, C.W.; Ratnayake, W.M.N.; Fernandez, L.; Lavergne, C.; Wood, C.; Swist, E. Lower Serum Magnesium Concentration is Associated with Diabetes, Insulin Resistance, and Obesity in South Asian and White Canadian Women but not Men. *Food Nutr. Res.* **2015**, *59*, 25974. [CrossRef] [PubMed]
34. Gröber, U.; Schmidt, J.; Kisters, K. Magnesium in Prevention and Therapy. *Nutrients* **2015**, *7*, 8199–8226. [CrossRef] [PubMed]
35. Ray, H.; Bett, K.; Tar'an, B.; Vandenberg, A.; Thavarajah, D.; Warkentin, T. Mineral Micronutrient Content of Cultivars of Field Pea, Chickpea, Common Bean, and Lentil Grown in Saskatchewan, Canada. *Crop Sci.* **2014**, *54*, 1698–1708. [CrossRef]
36. Langyan, S.; Yadava, P.; Khan, F.N.; Bhardwaj, R.; Tripathi, K.; Bhardwaj, V.; Bhardwaj, R.; Gautam, R.K.; Kumar, A. Nutritional and Food Composition Survey of Major Pulses Towards Healthy, Sustainable, and Biofortified Diets. *Front. Sustain. Food Syst.* **2022**, *6*, 878269. [CrossRef]
37. Costello, L.C.; Franklin, R.B. Decreased Zinc in the Development and Progression of Malignancy: An Important Common Relationship and Potential for Prevention and Treatment of Carcinomas. *Exp. Opin. Ther. Targets* **2017**, *21*, 51–66. [CrossRef] [PubMed]
38. Hanumanthappa, D.; Vasudevan, S.N.; Maruthi, J.B.; Shakuntala, N.M.; Macha, S.I. Enrichment of Iron and Zinc Content in Pigeonpea Genotypes through Agronomic Biofortification to Mitigate Malnutrition. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 4334–4342.
39. Sellamuthu, K.M.; Malathi, P. Biofortification of Crops to Overcome Malnutrition in India. *Biotica Res. Today* **2021**, *3*, 402–405.
40. Dhaliwal, S.S.; Sharma, V.; Shukla, A.K.; Verma, V.; Kaur, M.; Shivay, Y.S. Biofortification—A Frontier Novel Approach to Enrich Micronutrients in Field Crops to Encounter Nutritional Security. *Molecules* **2022**, *27*, 1340. [CrossRef]
41. Behera, S.K.; Shukla, A.K.; Tiwari, P.K.; Tripathi, A.; Singh, P.; Trivedi, V. Classification of Pigeonpea (*Cajanus cajan*) Genotypes for Zinc Efficiency. *Plants* **2020**, *9*, 952. [CrossRef]
42. Susmitha, D.; Kalaimagal, T.; Senthil, R.; Vetriventhan, M.; Manonmani, S.; Jeyakumar, P.; Anita, B.; Reddymalla, S.; Choudhari, P.L.; Nimje, C.A.; et al. Grain Nutrients Variability in Pigeonpea Genebank Collection and Its Potential for Promoting Nutritional Security in Dryland Ecologies. *Front. Plant Sci.* **2022**, *13*, 934296. [CrossRef] [PubMed]
43. Boorboori, M.R.; Eradatmand Asli, D.; Tehrani, M. The Effect of Dose and Different Methods of Iron, Zinc, Manganese and Copper Application on Yield Components, Morphological Traits and Grain Protein Percentage of Barley Plant (*Hordeum vulgare*) in Greenhouse Conditions. *Adv. Environ. Biol.* **2012**, *6*, 740–746.

44. Rai, S.; Singh, P.K.; Mankotia, S.; Swain, J.; Satbhai, S.B. Iron Homeostasis in Plants and its Crosstalk with Copper, Zinc, and Manganese. *Plant Stress* **2021**, *1*, 100008. [CrossRef]
45. Millaleo, R.; Reyes-Diaz, M.; Ivanov, A.G.; Mora, M.L.; Alberdi, M. Manganese as Essential and Toxic Element for Plants: Transport, Accumulation and Resistance Mechanisms. *J. Soil Sci. Plant Nutr.* **2010**, *10*, 476–494. [CrossRef]