Genetic Variability and Heritability Estimates of Nutritional Composition in the Leaves of Selected Cowpea Genotypes [*Vigna unguiculata* (L.) Walp.]

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Additional index words. genetic parameter, mineral, principal component, protein

Abstract. Cowpea is indigenous to the African continent and is grown for its leaves and grain in different countries of the world. The objective of this study was to determine the variability and heritability of mineral and crude protein contents in the leaves of selected cowpea genotypes grown in South Africa. The trials consisted of twenty five cowpea genotypes evaluated for two cropping seasons. The combined mean values showed wide genetic variation in the mineral elements evaluated. The mean values of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), and zinc (Zn) varied from 2.23 to 3.69 mg·kg⁻¹; 6.96 to 14.15 mg·kg⁻¹; 311.30 to 1049.95 mg·kg⁻¹; 1.13 to 1.74 mg·kg⁻¹; 0.36 to 0.70 mg·kg⁻¹; 130.08 to 186.42 mg·kg⁻¹; 126.62 to 307.87 mg·kg⁻¹; 0.27 to 0.39 mg·kg⁻¹; and 27.76 to 43.55 mg·kg⁻¹, respectively. The total protein content varied from 21.39% to 33.45%. The correlation analysis revealed significant degree of association between and among mineral elements and total protein content. Biometrical analysis revealed that the phenotypic variances were higher than the genotypic variances. High values of heritability estimates were also recorded for most of the evaluated traits. The principal component analysis (PCA) showed that the first three principal components contributed 71.93% of total variation among the genotypes. The study revealed that there is an ample genetic variability that can be exploited for use in breeding for nutritional quality in cowpea leaves.

Cowpea is an important legume crop in different parts of Africa. It is an underused crop with a high potential for food and nutritional security. In South Africa, cowpea is neglected and is mainly grown by subsistence farmers for its leafy vegetable and grain legumes for human consumption (Jansen van Rensburg et al., 2007). In arid and semiarid tropical regions of Africa, it is also been used as livestock feed and as a valuable component of the cropping systems (Singh et al., 2002). Cowpea is cultivated for the production of grain seeds, immature pods, fresh and dried leaves, as well as hay for livestock feed (Xu et al., 2009). It is a good source of protein, vitamins, and minerals (Mamiro et al., 2011).

Cowpea production and productivity is generally low due to insufficient availability of improved varieties and locally adapted cultivars (Ishiyaku et al., 2005). Santos and Boiteux (2013) reported that increased nutritional quality in terms of mineral and total protein content of cowpea genotypes are considered as an important component of global intervention programs that are focused on alleviating human malnutrition. Little information

is available on the nutritional quality of the leaves of cowpea genotypes grown in South Africa. A better understanding and knowledge of the nutritional composition of the leaves of these genotypes is an important step toward the development of improved varieties with higher nutritional content. Improvement of nutritional quality of cowpea genotypes, however, requires information on the genetic variability that exists among available germplasm. Information on the genetic heritability and genetic advance of the targeted traits is also important for efficient selection. This study was therefore carried out to determine the level of variability of the protein and mineral composition in the leaves of selected genotypes. The genotypic and phenotypic variances as well as the heritability estimates of the nutritional components were also determined.

Material and Methods

Plant materials and description of study area. Seeds of 25 cowpea genotypes (Table 1) were obtained from Agricultural Research Council—Roodeplaat Vegetable and Ornamental Plants (ARC-VOP) gene-bank, Pretoria, South Africa. The experiment was conducted at the Research Farm of ARC-VOP (25.604 S 28.345 E), during the 2012/13 and 2013/14 cropping seasons at an altitude of 1168 m above sea level. The location received a total rainfall of 498.86

and 610.30 mm during the experiment periods in 2012/13 and 2013/14, respectively. The minimum and maximum recorded temperatures during the growth periods were 7.14 to 9.11 °C and 35.56 to 36.37 °C in 2012/2013 and 2013/2014, respectively.

Experimental design and planting. The seeds of the 25 genotypes were sown directly in the demarcated field plots laid out in a randomized complete block design with three replications. Each plot measured 4 m in length and 2 m in width. Spacing was 1 m between the rows and 0.40 m within the rows. The distance between replications was 1.5 m. Two seeds were sown in each hole and the seedlings were thinned to one at 2 weeks after planting when fully established keeping 40 seedlings in a plot. Agronomic management practices such as weeding and supplementary irrigation were applied when required; however, no fertilizer was applied to simulate low-input conditions.

Mineral analysis and protein contents determination. Young leaves of plants from five randomly selected plants at the middle row of each plot were harvested at 6 weeks after planting. The leaves were oven dried, ground into fine powder, and 0.5 g samples were drawn for analysis. The analysis involved the determination of total protein content and the amount of nine essential mineral elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn). The analysis was carried out in the laboratory of the Institute for Soil, Climate and Water, ARC, Pretoria, South Africa. Mineral contents were determined by atomic absorption spectrophotometry (SpectraAA 300, South Africa). Total protein content (nitrogen × 6.25) was determined by the combustion method (Leco® model, FP-528, St. Joseph, MI).

Statistical data analysis. Nutritional data were analyzed by means of analysis of variance (ANOVA) using Agrobases Generation II (2008) statistical computer software. Estimates of genetic variability for total protein and mineral elements among the studied genotypes were determined. Furthermore, genetic parameters such as genetic and phenotypic variances, genetic and phenotypic cvs, expected genetic advance/genetic gain, as well as percentage of genetic advance were estimated using the functions suggested by Farshadfar et al. (2013) and Comstock and Robinson (1952) as follows:

$$\sigma^2_e = \text{Mse}$$

$$\sigma^2_g = \frac{\text{Mg1} - \text{Mg2}}{r \times s}$$

$$\sigma^2_p = \text{VG} + \text{VE}$$

$$\text{PCV}(\%) = \frac{\sqrt{\sigma^2_p}}{x} \times 100$$

$$\text{GCV}(\%) = \frac{\sqrt{\sigma^2_g}}{x} \times 100$$

$$\text{ECV}(\%) = \frac{\sqrt{\sigma^2_e}}{x} \times 100$$

$h^2_{bs} = \frac{\sigma^2_g}{\sigma^2_p} \times 100$, h^2_{bs} = broad sense heritability; σ^2_g = genetic variance; σ^2_p = phenotypic variance.

Received for publication 8 July 2015. Accepted for publication 28 Aug. 2015.

We are grateful to the Agricultural Research Council, Pretoria, South Africa for the financial support for the experiment.

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Table 1. List of cowpea genotypes evaluated showing their sources of origin and growth habit.

No	Genotype ^z	Origin ^y	Growth habit
1	Veg cowpea 1	South Africa	Semierect
2	Veg cowpea 2	South Africa	Semierect
3	Fahari	Tanzania ^y	Semierect
4	Embu buff	South Africa	Semierect
5	Glenda	South Africa	Semierect
6	Kisumu mix	Kenya	Prostrate
7	Makatini	South Africa	Erect
8	Ukaluleni	South Africa	Prostrate
9	Veg cowpea 3	South Africa	Erect
10	Tatro mix	Kenya	Prostrate
11	MLBP	South Africa	Prostrate
12	VCDR	South Africa	Erect
13	VCDC	South Africa	Erect
14	Oukawa	South Africa	Prostrate
15	Vigna Onb	South Africa	Prostrate
16	TVU7778	Nigeria	Erect
17	5431	South Africa	Erect
18	Chappy	South Africa	Prostrate
19	2460	South Africa	Prostrate
20	MA2	South Africa	Erect
21	M346	South Africa	Erect
22	MA1	South Africa	Erect
23	M217	South Africa	Erect
24	IT96D602	Nigeria	Erect
25	Vuli	Tanzania ^y	Prostrate

^zMLBP = Meter Long Bean Piet; VCDR = Vegetable cowpea dakama red; VCDC = Vegetable cowpea dakama cream.

^yAVRDC = The World Vegetable Center.

$$GG = \left(\frac{i \cdot \sigma^2_g}{\sqrt{\sigma^2_p}} \right) 100/x$$

GG (%) = GA × 100/x, where σ^2_e = environmental variation, Mse = error mean square, Vg = genetic variation, r = number of replication, s = number of seasons, VP = phenotypic variation, σ^2_g = genetic variance, σ^2_p = phenotypic variance, PCV = phenotypic coefficient of variance, GCV = genotypic cv, ECV = environmental cv, h^2_{bs} = broad sense heritability, GG = genetic gain, GG (%) = percentage of genetic gain, the standard selection differentials (i) for 5% selection intensity was 2.06.

Correlation and principal component analyses. The correlation coefficients for mineral elements and protein contents, as well as the PCA, was carried out using NCSS (2004) computer software.

Results and Discussion

Genetic variability. Significant differences ($P \leq 0.01$) were observed for the concentration of most of the mineral elements and protein content of the genotypes for the 2012/2013 and 2013/2014 cropping seasons (Tables 2 and 3). The ANOVA of the combined data over seasons also showed highly significant ($P \leq 0.01$) differences for total protein content and all evaluated mineral elements in the cowpea leaves except K and Mn (Table 4). This variability offers a wide scope for the selection of potential parents for the improvement of the nutritional contents of the leaves. According to Inuwa et al. (2012) and Gerrano et al. (2015), the existence of wide genetic variability is a prerequisite to effective selection for traits of

interest in a plant breeding improvement program. A highly significant interaction between seasons and genotypes was observed for some of the mineral elements, which indicated that genotypes reacted differently in different seasons (Tables 2 and 3). This might probably be due to the seasonal variation of the environmental conditions as well as the differences in the mineral mining and use abilities of the genotypes.

The mean values of the mineral elements are shown in Table 4. The Ca concentration ranged from 2.23 to 3.69 mg·kg⁻¹ with genotypes MA1, Makatini, and M217 among the highest. Genotypes that ranked high in Cu concentration were M217 (14.15 mg·kg⁻¹), MA1 (13.50 mg·kg⁻¹), and Embo buff (13.45 mg·kg⁻¹). Genotype Meter Long Bean Piet (MLBP) had an unusually high iron concentration (1049.95 mg·kg⁻¹) compared with the rest of genotypes. It also recorded the highest Zn concentration (43.55 mg·kg⁻¹). The differences in the concentration of the mineral elements among the genotypes might be due to the differences in the size of the biomass of the plants (Gerrano et al., 2015). With the very high level of Fe and Zn deficiency and its associated health problems in Africa, this genotype can be very valuable in supplying these essential micronutrients especially among the rural poor. The overall average value for Zn was similar to the magnitude of the Zn content reported by Mamiro et al. (2011). The highest Mg, P, and total protein contents were recorded in genotype Kisumu mix. This genotype is also therefore, a good candidate for use as parental line in the cowpea improvement program. Genotype MA2 showed the highest Na concentration (307.87 mg·kg⁻¹). The total protein content ranged from 21.39% to 33.45% with a mean

of 27.35% among the genotypes evaluated. The highest value was recorded in genotypes Kisumu mix followed by genotypes Vegetable cowpea dakama cream (VCDR) and Vigna Onb with the values 30.35% and 30.81%, respectively. These values were relatively higher than what was reported in Tanzania (Mamiro et al., 2011), Nigeria (Nielsen et al., 1997), and Uganda (Okonya and Maass, 2014). The total protein content in current findings was, however, in the range of what Santos and Boiteux (2013) reported in cowpea genotypes in Brazil. These variations might be due to genotypic differences as well as differences in the soil and climatic conditions. Most mineral elements are generally deficient in African diets despite their importance for growth and development particularly in young children where they are required on daily basis for metabolic activities. High protein and mineral elements contents recorded in the young leaves of these genotypes, although does not necessarily indicate their bioavailability, is an indication of their potentials to contribute in meeting the daily dietary requirements of protein and essential minerals in South Africa. According to Belane (2010), many rural communities in South Africa are already relying on cowpea leaves to meet their daily mineral and protein requirements.

Estimates of genotypic, phenotypic, and environmental cvs, broad sense heritability, genetic variance, and phenotypic variances as well as genetic gain are presented in Table 5. The values obtained for genetic and phenotypic coefficients of variation were close for most of the characters evaluated. This phenomenon implies that the improvement of the evaluated traits can be achieved through selection. It also suggested that greater contribution of genotype rather than environment and that the selection can be based only on the phenotypic values as observed by Girish et al. (2006). Iron, copper, and sodium had high values of genetic and phenotypic cvs. Calcium, iron, magnesium, sodium, and phosphorus had high values for heritability and high genetic gain showing that the selection for these traits will be very effective and reliable. On the other hand, Mn, K, and Zn had lower values for genetic gain, which indicate that it will require many generations of crossings to accumulate the relevant gene/allele. According to Shukla et al. (2006) and Mobina et al. (2014), genetic cv together with heritability estimates and genetic gain provide a reliable indication and estimate of the expected amount of improvement through selection for the traits of interest.

Correlation analysis. The Pearson correlation coefficient showed that there was significant ($P \leq 0.01$; $P \leq 0.05$) correlation among some of the traits evaluated (Table 6). Potassium was significant and positively correlated with Mg ($r = 0.51$), P ($r = 0.66$), protein ($r = 0.70$), and Zn ($r = 0.45$). Magnesium was also significantly and positively correlated with P ($r = 0.63$), protein ($r = 0.64$), and Zn ($r = 0.40$). Phosphorus was significantly and positively correlated with

Table 2. Means, mean squares, least significant differences, and cv for mineral elements and protein content in cowpea leaves during 2012/13 cropping season.

No	Genotype ^z	Mineral element concn (mg·kg ⁻¹) dry mass								%	
		Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	Protein
1	Veg cowpea 1	3.35	7.82	349.73	1.36	0.51	241.03	165.13	0.25	26.23	22.20
2	Veg cowpea 2	2.24	12.12	526.97	1.75	0.61	198.40	175.90	0.36	41.45	29.66
3	Fahari	3.74	6.19	425.13	1.41	0.68	209.03	176.87	0.29	33.91	24.81
4	Embo buff	4.25	14.98	427.77	1.77	0.61	131.13	168.57	0.37	37.27	27.07
5	Glenda	3.55	9.15	424.67	1.51	0.49	194.57	172.33	0.30	35.44	27.20
6	Kisumu mix	2.75	10.68	268.70	1.99	0.69	174.50	150.17	0.38	41.87	33.42
7	Makatini	4.91	13.15	783.90	1.31	0.47	204.13	183.07	0.28	34.88	23.09
8	Ukaluleni	3.50	13.67	519.83	1.30	0.44	186.93	137.37	0.29	30.44	22.65
9	Veg cowpea 3	3.50	7.99	559.27	1.52	0.50	180.27	132.13	0.29	32.24	26.13
10	Tatro mix	2.56	10.08	345.60	2.20	0.60	137.53	199.20	0.31	41.85	27.99
11	MLBP	3.96	9.34	267.57	1.80	0.49	182.63	177.63	0.33	40.97	24.61
12	VCDR	4.16	12.85	348.40	0.99	0.52	105.73	171.70	0.30	29.48	23.21
13	VCDC	3.43	10.77	552.67	1.73	0.48	178.17	167.70	0.34	37.92	29.18
14	Oukawa	3.41	10.38	334.47	1.67	0.52	184.87	177.73	0.30	32.58	27.01
15	Vigna Onb	4.13	13.50	652.67	1.68	0.49	187.00	178.43	0.33	41.26	28.13
16	TVU7778	2.77	9.59	359.67	1.62	0.68	161.83	174.67	0.33	36.19	28.57
17	5431	4.14	8.58	689.63	1.43	0.46	211.87	175.13	0.31	36.37	19.28
18	Chappy	4.58	10.27	588.67	1.40	0.56	131.23	172.33	0.32	33.08	23.52
19	2460	4.53	9.72	477.53	1.69	0.53	170.80	166.47	0.32	31.80	26.56
20	MA2	2.34	10.62	355.13	1.84	0.52	167.97	439.50	0.33	37.87	28.12
21	M346	3.39	12.18	544.23	1.30	0.62	179.90	165.67	0.29	37.88	23.95
22	MA1	5.10	15.43	455.33	1.17	0.40	170.10	174.07	0.31	35.25	19.91
23	M217	4.66	17.47	546.93	1.31	0.60	189.93	160.83	0.29	35.36	22.27
24	IT96D 602	3.78	12.79	624.00	1.15	0.47	193.80	153.10	0.26	27.11	19.97
25	Vuli	2.80	7.34	311.27	1.68	0.55	185.90	162.23	0.32	32.15	26.80
	MS for genotype	1.796**	22.232**	55,563.318**	0.236*	0.018**	2,484.131 ^{NS}	9,413.689**	0.003 ^{NS}	58.776 ^{NS}	35.274**
	Residual	0.11	1.94	2,132.29	0.13	0.00	1,979.05	124.36	0.00	44.47	15.33
	LSD	0.54	2.28	75.81	0.60	0.10	73.03	18.31	0.10	10.95	6.43
	SED	0.27	1.14	37.70	0.30	0.05	36.32	9.11	0.05	5.44	3.20
	cv (%)	8.99	12.57	9.80	23.49	11.62	24.94	6.23	18.77	18.93	15.41
	GM	3.66	11.07	471.03	1.54	0.05	178.37	179.12	0.31	35.23	25.41

LSD = least significant difference; MS = mean squares; GM = grand mean.

^zMLBP = Meter Long Bean Piet; VCDR = Vegetable cowpea dakama red; VCDC = Vegetable cowpea dakama cream; SED = standard error of the difference.

NS, *, **Nonsignificant or significant at 5% and 1% level of probability, respectively.

Table 3. Means, mean squares, least significant differences, and cv for mineral elements and protein content in cowpea leaves during 2013/14 cropping season.

No.	Genotype ^z	Mineral element concn (mg·kg ⁻¹) dry mass								%	
		Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	Protein
1	Veg cowpea 1	2.22	7.95	438.27	1.31	0.31	126.10	263.33	0.28	29.29	26.98
2	Veg cowpea 2	2.21	8.87	489.57	1.35	0.32	135.87	179.83	0.33	32.24	29.14
3	Fahari	2.47	7.72	503.80	1.33	0.63	162.57	245.57	0.42	41.59	33.23
4	Embo buff	2.40	11.92	539.60	1.31	0.46	146.17	182.90	0.34	31.64	32.67
5	Glenda	2.14	8.28	449.27	1.19	0.47	143.27	176.80	0.31	34.06	29.30
6	Kisumu mix	2.41	9.21	353.90	1.34	0.71	158.93	237.70	0.39	37.25	33.47
7	Makatini	2.35	7.40	486.60	1.27	0.34	132.40	171.50	0.31	26.94	29.33
8	Ukaluleni	2.41	9.29	274.40	1.19	0.52	166.60	115.87	0.32	35.41	28.51
9	Veg cowpea 3	2.09	8.26	420.90	1.28	0.41	125.13	189.07	0.39	35.17	30.03
10	Tatro mix	2.20	8.25	475.00	1.29	0.58	138.30	171.20	0.36	32.75	28.57
11	MLBP	1.54	11.94	1832.30	1.47	0.45	169.30	161.40	0.38	46.13	28.85
12	VCDR	2.13	10.16	331.67	1.31	0.47	154.43	253.03	0.36	41.74	30.41
13	VCDC	2.05	9.38	437.80	1.16	0.48	135.77	169.83	0.40	34.00	31.52
14	Oukawa	2.36	11.06	506.07	1.24	0.47	145.63	210.77	0.35	35.22	30.16
15	Vigna Onb	2.59	9.03	445.57	1.29	0.49	144.13	188.77	0.39	34.19	33.49
16	TVU7778	2.49	11.11	653.70	1.26	0.38	159.03	248.33	0.34	37.57	30.55
17	5431	2.14	7.34	396.53	1.24	0.59	160.97	220.40	0.32	35.35	28.84
18	Chappy	2.40	9.92	635.50	1.42	0.55	163.40	186.63	0.38	44.19	31.67
19	2460	2.27	7.00	1,046.30	1.29	0.40	151.07	176.57	0.31	30.77	26.20
20	MA2	2.33	11.79	472.77	1.23	0.35	131.00	176.23	0.34	39.58	28.91
21	M346	2.28	11.63	1,125.30	1.18	0.32	152.53	220.20	0.29	38.37	25.22
22	MA1	2.28	11.57	608.77	1.09	0.31	139.40	253.43	0.30	36.55	22.86
23	M217	2.57	10.83	670.10	1.22	0.31	142.70	134.83	0.29	31.70	24.40
24	IT96D 602	2.27	12.06	480.07	1.40	0.50	171.00	176.07	0.34	30.59	28.65
25	Vuli	2.31	7.88	881.33	1.17	0.50	183.40	220.83	0.33	40.39	28.97
	MS for genotype	0.130*	8.560**	323,263.859**	0.023 ^{NS}	0.035**	690.617*	4,426.507**	0.005**	66.61**	21.961**
	Residual	0.073	1.170	1,809.461	0.048	0.002	328.495	237.230	0.002	26.370	8.608
	LSD	0.444	1.775	69.833	0.361	0.076	29.754	25.286	0.074	8.430	4.817
	SED	0.221	0.883	34.732	0.179	0.038	14.799	12.576	0.037	4.190	2.396
	cv (%)	11.89	11.27	7.11	17.26	10.23	12.12	7.81	13.09	14.38	10.02
	GM	2.276	9.595	598.207	1.272	0.452	149.564	197.244	0.343	35.710	29.276

LSD = least significant difference; MS = mean squares; GM = grand mean; SED = standard error of the difference.

^zMLBP = Meter Long Bean Piet; VCDR = Vegetable cowpea dakama red; VCDC = Vegetable cowpea dakama cream.

NS, *, **Nonsignificant or significant at 5% and 1% level of probability, respectively.

Table 4. Analysis of variance revealing mean mineral elements, total protein contents, and mean squares for different quality traits in cowpea leaves over two cropping seasons.

		Mean of the mineral element content (mg·kg ⁻¹) and total protein									
No.	Genotype ^z	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	% Protein
1	Veg cowpea 1	2.79	7.89	394.00	1.34	0.41	183.57	214.23	0.27	27.76	24.59
2	Veg cowpea 2	2.23	10.50	508.27	1.55	0.47	167.13	177.87	0.35	36.84	29.40
3	Fahari	3.11	6.96	464.47	1.37	0.65	185.80	211.22	0.36	37.75	29.03
4	Embo buff	3.33	13.45	483.68	1.54	0.54	138.65	175.73	0.35	34.45	29.87
5	Glenda	2.85	8.72	436.97	1.35	0.48	168.92	174.57	0.31	34.75	28.25
6	Kisumu mix	2.58	9.95	311.30	1.67	0.70	166.72	193.93	0.39	39.56	33.45
7	Makatini	3.63	10.28	635.25	1.29	0.41	168.27	177.28	0.29	30.91	26.21
8	Ukaluleni	2.96	11.48	397.12	1.25	0.48	176.77	126.62	0.30	32.92	25.58
9	Veg cowpea 3	2.79	8.13	490.08	1.40	0.46	152.70	160.60	0.34	33.70	28.08
10	Tatro mix	2.35	9.17	410.30	1.74	0.59	137.92	185.20	0.34	37.30	28.28
11	MLBP	2.75	10.64	1,049.95	1.64	0.47	175.96	169.52	0.36	43.55	26.73
12	VCDR	3.15	11.50	340.03	1.15	0.49	130.08	212.37	0.33	35.61	26.81
13	VCDC	2.74	10.08	495.23	1.44	0.48	156.97	168.77	0.37	35.96	30.35
14	Oukawa	2.88	10.72	420.27	1.46	0.50	165.25	194.25	0.33	33.90	28.58
15	Vigna Onb	3.36	11.26	549.12	1.49	0.49	165.57	183.60	0.36	37.72	30.81
16	TVU7778	2.63	10.35	524.68	1.44	0.53	160.43	211.50	0.33	36.88	29.56
17	5431	3.11	7.96	543.08	1.33	0.52	186.42	197.77	0.31	35.85	24.06
18	Chappy	3.49	10.10	612.08	1.41	0.55	147.32	179.48	0.35	38.64	27.59
19	2460	3.40	8.36	761.93	1.49	0.47	160.93	171.52	0.32	31.28	26.38
20	MA2	2.41	11.20	413.95	1.53	0.43	149.48	307.87	0.33	38.73	28.52
21	M346	2.83	11.91	834.78	1.24	0.47	166.22	192.93	0.29	38.12	24.58
22	MA1	3.69	13.5	532.05	1.13	0.36	154.75	213.75	0.31	35.90	21.39
23	M217	3.52	14.15	608.52	1.26	0.45	166.32	147.83	0.29	33.53	23.33
24	IT96D 602	2.96	12.43	552.03	1.28	0.48	182.40	164.58	0.30	28.85	24.31
25	Vuli	2.61	7.61	596.30	1.43	0.53	184.65	191.53	0.33	36.27	27.89
	SS for season (S)	71.42	81.20	606,527.78	2.76	0.28	31,118.11	12,321.60	0.04	8.48	559.51
	SS for rep (R)	0.36	11.11	16,679.03	0.52	0.03	62,185.77	365.80	0.01	31.03	109.43
	SS for genotype (G)	23.65	535.10	3,745,252.26	3.38	0.77	34,516.93	155,530.49	0.13	1,724.38	1,048.99
	SS for S × G	22.57	203.92	5,346,599.99	2.83	0.51	41,693.64	176,634.23	0.05	1,284.65	324.66
	SS for pooled error	8.701	149.08	189,203.95	8.62	0.29	110,762.07	17,356.27	0.26	3,416.26	1,149.12
	MS for S	71.42**	2.80**	606,527.78**	2.76**	0.28**	31,118.11**	12,321.60**	0.04**	8.48**	559.51**
	MS for R	0.09	2.80	4,169.76	0.13	0.01	15,546.44	91.50	0.003	15.51	27.40
	MS for G	0.99**	22.30**	156,052.18**	0.14 _{NS}	0.03**	1,438.21 _{NS}	6,480.44**	0.01**	71.85**	43.71**
	MS for S × G	0.94**	8.50**	222,775.00**	0.12 _{NS}	0.02**	1,737.26 _{NS}	7,359.76**	0.00 _{NS}	53.53*	13.53 _{NS}
	MS for pooled error	0.091	1.55	1,970.88	0.09	0.003	1,153.77	180.79	0.00	34.86	11.97
	LSD	0.345	1.428	50.88	0.34	0.063	38.93	15.41	0.06	6.765	3.97
	SED	0.174	0.7195	25.63	0.17	0.03	19.611	7.76	0.03	3.409	2.00
	cv (%)	10.15	12.06	8.30	21.29	11.11	20.72	7.15	15.93	16.60	12.65
	GM	2.97	10.33	534.62	1.41	0.5	163.97	188.18	0.33	35.47	27.35

LSD = least significant difference; MS = mean squares; SS = sum of squares; GM = grand mean.

^zMLBP = Meter Long Bean Piet; VCDR = Vegetable cowpea dakama red; VCDC = Vegetable cowpea dakama cream; SED = standard error of the difference.

NS, *, **Nonsignificant or significant at 5% and 1% level of probability, respectively.

Table 5. Estimates of genetic parameters for the mineral concentration and total protein contents in cowpea leaves.

Genetic parameters	Mineral elements and total protein									
	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn	Protein
GM	2.97	10.33	534.62	1.41	0.50	163.97	188.18	0.33	35.47	27.35
σ ² g	0.97	22.04	155,723.70	0.13	0.03	1,245.92	6,450.31	0.01	66.04	41.72
σ ² p	1.07	23.59	157,694.60	0.22	0.03	2,399.69	6,631.10	0.01	100.90	53.69
PCV (%)	34.73	47.05	74.28	33.33	34.27	29.88	43.27	30.30	28.31	26.81
GCV (%)	33.04	45.40	73.81	25.53	34.27	21.53	42.68	30.30	22.92	23.62
GG (%)	71.12	89.94	38.29	107.14	70.85	86.80	10.80	62.42	159.64	6.60
ECV	10.11	12.00	8.30	21.28	0.00	20.72	7.15	0.00	16.63	12.65
h ² bs (%)	91.46	46.87	98.75	58.14	90.77	51.92	97.27	100.00	65.45	77.70
GG	65.73	90.44	151.10	38.86	72.07	31.95	86.71	62.42	38.20	42.87
GG (%)	2,213.13	3,045.12	5,087.54	1,308.42	2,426.60	1,075.76	2,919.53	2,101.68	1,286.20	1,443.43

GM = grand mean; σ²g = genetic variance; σ²p = phenotypic variance; PCV = phenotypic coefficient variance; GCV = genotypic cv; ECV = Environmental cv; h²bs = broad sense heritability; GG = genetic gain.

Zn ($r = 0.67$), K ($r = 0.66$), Mg ($r = 0.63$), and protein ($r = 0.81$). These positive correlation among the traits suggested a close genetic and inherent association among them and implies that both pair of traits can be improved simultaneously. The result of this study therefore, implies that high protein content is associated with high content of Mg, K, and P and can be improved simultaneously.

Principal component analysis. The first three principal components explained 71.93% of the total variation among the cowpea genotypes (Table 7). PC1 had an eigenvalue of 4.37 and accounted for 43.65% of total variation. This component was associated with K, Mg, P, and protein, which were important in contributing to the variability among the genotypes. PC2 had an eigenvalue of 1.53, contributing 15.27% of

genetic variation and had Cu and Mn concentrations as the main contributing factor.

PC3 had eigenvalues of 1.30, and indicated that Ca, Fe, and Na contributed 13.01% of the variation. Sodium contributed the most to variation in this PC.

The biplot separated the cowpea genotypes into three different groups (Fig. 1) and showed that there was a wide genetic variability among them. Genotypes VCDR,

Table 6. Pearson correlation coefficients among selected mineral elements and total protein content in the leaves of 25 cowpea genotypes.

Mineral element and total protein content	Ca	Cu	Fe	K	Mg	Mn	Na	P	Protein
Ca	1.00								
Cu	0.33	1.00							
Fe	0.21	0.09	1.00						
K	-0.55**	-0.26	0.04	1.00					
Mg	-0.28	-0.34	-0.27	0.51**	1.00				
Mn	-0.07	-0.37	0.23	-0.14	0.01	1.00			
Na	-0.25	-0.10	-0.24	0.08	-0.04	-0.18	1.00		
P	-0.28	-0.15	-0.09	0.66**	0.63**	-0.28	0.05	1.00	
Protein	-0.45*	-0.26	-0.32	0.70**	0.64**	-0.22	0.10	0.81**	1.00
Zn	-0.29	0.02	0.27	0.45*	0.40*	-0.19	0.25	0.67**	0.41*

*, **Significant at 5% and 1% level of probability, respectively.

Table 7. Principal component analysis for mineral concentration and total protein in cowpea leaves revealing eigenvalue, total variance, and eigenvector and contribution to total variation explained by the first three PC axes.

PC	Eigenvalue	Total variance		Eigenvectors (loading) for								
		Individual (%)	Cumulative (%)	Ca	Cu	Fe	K	Mg	Mn	Na	P	Protein
1	4.37	43.65	43.65	0.28	0.18	0.16	-0.38	-0.36	0.1	-0.08	-0.4	-0.45
2	1.53	15.27	58.92	0.22	0.56	-0.25	-0.08	-0.13	-0.7	0.18	0.12	0.08
3	1.3	13.01	71.93	0.35	0.26	0.51	0.15	0.13	-0.02	-0.66	0.26	0.07

Fig. 1. The principal component analysis score plot of first and second principal components showing the overall genetic variation in nutritional composition in the leaves of cowpea.

Embu buff, MA1, MA2, and M217 were clearly separated from the rest and form a distinct group. Similarly, genotype MLBP, Veg cowpea1, 5431, Vulii, and Fahari formed another group. The third group consist of the rest of the genotypes that clustered around the origin showing that these genotypes were genetically similar for the traits evaluated and need to broaden the genetic base of the materials by including unrelated cowpea genotypes (Gerrano et al., 2015). This groupings provided a very valuable information for the selection of suitable parents for the improvement of the evaluated traits.

Conclusions

These results showed the existence of wide genetic variability in the concentrations of total protein and mineral elements in the young leaves of cowpea genotypes offering a wide scope for selection. All characters showed high to very high broad-sense heritability values and genetic advance, which indicate that the evaluated traits are highly heritable. From the results of this study, the following cowpea genotypes could be selected and incorporated into the cowpea improvement program: genotypes MA1 and Makatini for Ca; genotype M217 for Cu;

Meter Long Bean Piet for Fe, K, and Zn; Tatro mix for K; Kisumu mix for Mg, P, and protein; genotypes 5431 and Fahari for Mn; and genotype MA2 for Na.

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