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Evaluation of cowpea germplasm lines for protein and mineral concentrations in grains

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Abstract

Cowpea, an indigenous crop to sub-Saharan Africa, is found mainly in the dry savanna regions along with cereals such as millets and sorghum. Cowpea is grown primarily for human consumption of the grains, which are rich in protein, carbohydrates and contain some minerals. The development and deployment of cowpea varieties with higher nutritional value will be of immense benefit to consumers. As a first step in the enhancement of mineral content in cowpea grains, several germplasm lines (1541) of different origins and obtained from the genetic resources unit at IITA were sown in the experimental field in Minjibir, Kano State, Nigeria. The grains were analysed for protein and nine mineral contents. However, in this study, we shall report data on crude protein, Fe, Zn, Ca, Mg, K and P. The data generated from the chemical analysis were subjected to the 'mixture of normal distributions' clustering method, which distributed the cowpea lines into nine groups. Groups G7 and G9 contained 174 genotypes and these were characterized by high mineral concentrations. The mean nutritional content values for group G9 were 24.7% for protein, and 58.9, 41.5, 1107, 2132, 15,282 and 5664 mg/kg for Fe, Zn, Ca, Mg, K and P, respectively. On the other hand, the 363 lines making up groups G2, G3 and G6 showed low amounts of nutrients in their grains. Some of the lines in G7 and G9 would be good as parents to use in breeding programmes that aimed at developing nutrient-dense cowpea varieties.

Keywords: cowpea; diversity; mineral composition; nutritional value; *Vigna unguiculata*

Introduction

Cowpea is an important grain legume found mainly in the savanna regions of sub-Saharan Africa, where it is grown in intercropping system with cereals such as millets and sorghum. This indigenous crop to sub-Saharan Africa thrives relatively better than other crops in the drought-prone areas of the region. The average grain yield of cowpea in the region is estimated to be about 470 kg/ha (FAO, 2008) and the potential yield is up to 2.3 t/ha. Nigeria and Niger Republic are the two highest cowpea producers worldwide at 3.04 million and 0.69 million tons with average yield of 0.69 and 0.17 t/ha, respectively. Cowpea is grown primarily for human consumption of the grains, which are rich in protein, carbohydrates and contain some minerals as well. In many homes in sub-Saharan Africa, cowpea, being a legume, is a major source of dietary protein. Most cowpea varieties being grown contain between 22 and 25% protein in their grains. Following evaluation of eight lines, Bressani (1985) and Elias et al. (1964) reported that cowpea contains 24% protein and about 62% soluble carbohydrates and small amounts of other nutrients. Bliss (1975), however, reported that depending on the genotype, cowpea grains may contain between 23 and 30% protein. In a study involving 100 improved cowpea breeding and germplasm lines, protein content in grains ranged from 22 to 30% (Fatokun, unpublished IITA data). These latter reports reveal genetic differences in protein content

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among cowpea lines, which suggest the possibility of increasing the protein level in cowpea grains through selection. Carnovale *et al.* (1990) studied the nutritional properties of a number of cowpea lines and found that the means (± standard errors) for Ca, Zn, P and K contents in grains were 37(±5), 4.7(±0.3), 430(±20) and 125(±15) mg/100 g (fresh weight basis), respectively. When compared with other edible grain legumes, they found that cowpea grains contained 8.85(±0.55) mg/100 g iron while common bean, faba bean and chickpea contained 8.43(±0.28), 6.10(±1.13) and 4.96(±0.82) mg/100 g respectively.

Recent studies carried out using laboratory animals showed that grain legumes such as beans could have positive impact on human health. According to Thompson *et al.* (2009), cooked dry bean (*Phaseolus vulgaris* L.) powder diets when fed to rats reduced cancer incidence from 95 to 67% and the number of cancer tumours per animal was also reduced. Their study also showed that efficacies of the dry bean lines on cancer incidence depended on their centre of origin. Although no such study has been reported in cowpea, it is conceivable that, as a grain legume, cowpea could also have attributes that are similarly beneficial to health.

A collection of more than 15,000 cowpea accessions is maintained in the genebank at IITA. This germplasm collection is designed to represent the genetic diversity of the species and valuable resource to ensure long-term gains from selection. The germplasm collection could be evaluated for the purpose of identifying lines that may be sources of useful genes. This study was initiated to determine the protein and mineral concentrations in grains of several cowpea germplasm lines collected from different locations and thereafter identify those with high mineral contents, which could be used as parents in breeding nutrient-dense varieties.

Materials and methods

Of the over 15,000 cowpea germplasm accessions collected from different countries and maintained at the genebank at IITA, 1541 were selected randomly and sown at the IITA experimental farm in Minjibir, Kano State, Nigeria. Minjibir (12°08.997′N, 8°39.733′E) is located in the Sudan savanna agroecology, where the bulk of the crop is being produced. Planting was carried out in June 2006 on ridges spaced 80 cm apart. Spacing within row was 20 cm. Three seeds of each germplasm accession were sown and later thinned at 2 weeks after planting to two plants per stand. The experiment consisted of single 4 m long row observation plots without field replication. The plots were weeded by hand as necessary while the plants were sprayed with the insecticide 'Act Force' (Chlorpyrifos 40% EC) at the rate of 1.2 l/ha at vegetative stage, flower opening and once during pod maturing. The pods from all the plants in each experimental plot were harvested at plant maturity, and a random sample of 20 pods per cowpea accession was taken to the laboratory, where they were washed with detergent, rinsed with distilled water and allowed to dry further before threshing by hand with gloves to avoid contamination. Threshed seeds (about 25 g) were placed in seed envelopes, sealed and shipped in well-packaged containers to analytical laboratory at the Waite University, Australia, for chemical analysis. The seeds were further washed and oven dried before chemical analysis was carried out in Australia. Seeds from each genotype were divided into two lots for the chemical analysis. The seeds were analysed for protein, Fe, Zn, Ca, Mg, P and K. Samples were digested with nitric/perchloric acid and solutions were analysed by radial inductively coupled plasma atomic emission spectrometry for the determination of the mineral contents. For the total nitrogen analysis, Dumas' method using nitrogen analyser manufactured by LECO (St. Joseph, MI, USA) was employed. Samples were analysed as received and reported at 65°C oven dry basis. The limit of detection (the lowest concentration that can be reported using the stated method) is 0.03%. Crude protein content is determined by multiplying the measured amino-nitrogen value by a protein conversion factor 6.25 as discussed by Simonne *et al.* (1997).

Statistical analysis

A linear model was used to estimate variance components due to the differences between genotypes and the adjusted means were used in the next steps. From the adjusted means, a correlation analysis between the variables was performed using all data and within-group data. To identify groups of genotypes showing different mineral concentrations, a mixture of normal distribution two-stage clustering method (Franco *et al.*, 1998) was utilized. As a criterion to evaluate the protein and mineral contents, we used the 75th percentile (P75) for each nutrient obtained from the whole dataset. Since this P75 indicates that only 25% of accessions have a performance better than this value, it is a strict comparison criterion. A two-dimensional graphical representation of groups was done using canonical variables analysis (Mardia *et al.*, 1979).

The steps performed in the analysis were (1) statistical description of all the mineral and protein contents, (2) variance components estimation and estimation of adjusted means for protein and the minerals, (3) estimation of associations between variables using coefficients of correlation, (4) grouping of accessions

Table 1. Minimum and maximum values of nutrient content in 1541 accessions from the cowpea germplasm collection.

Variable	Minimum	Maximum	Mean	Std	CV (%)
Protein	17.5	32.5	25.0	0.4	9.2
Fe	33.6	79.5	53.2	6.4	12.0
Zn	22.1	58.0	38.1	5.1	13.3
Ca	310	1395	826	161.5	19.6
Mg	1515	2500	1915	157	8.2
K	11,400	18,450	14,890	993	6.7
P	3450	6750	5055	471	9.3
No. of samples	1541				

Std: Standard deviation and CV: coefficient of variation

by a model-based cluster analysis technique and (5) description of the groups or clusters using canonical variables, descriptive statistics and the P75 criterion.

Results

Variations in protein and mineral concentrations

There were variations among the 1541 germplasm lines in protein and mineral concentrations in their grains. Protein content ranged from 17.5 to 32.5% with a mean of 25.0 among the accessions evaluated. The maximum value obtained was almost twice that of the accession with the lowest value (Table 1). The Fe content in grains ranged from 33.6 to 79.5 mg/kg, while Zn ranged from 22.1 to 58.0 mg/kg. The Fe and Zn concentrations in the accessions with the highest values were more than double the values for the accessions with the lowest amounts of both minerals. The results also indicated that cowpea is rich in K with values ranging from 11,400 to 18,450 mg/kg. The low coefficient of variation (CV) values (less than 20 in all of them) associated with protein and mineral concentrations in cowpea indicated low differences between samples in the laboratory.

The germplasm accessions with the lowest and highest concentrations of each mineral were identified. None of the accessions identified showed lowest value in more than one mineral and protein contents. On the other hand, accession TVu-2723 was among lines with the highest amounts of Fe (79.53 mg/kg), Mg (2450 mg/kg), K (18,200 mg/kg) and P (6350 mg/kg). Three accessions TVu-10342, TVu-526 and TVu-1877 were among those with highest amounts in two minerals each. Accession TVu-10342 had 76.98 mg/kg Fe and 57.95 mg/kg Zn, while TVu-526 had 78.12 mg/kg Fe and 1320 mg/kg Ca and accession TVu-1877 had 54.0 mg/kg Zn and 2450 mg/kg Mg. The variance components due to differences between accessions were significant ($P \leq 0.01$) compared with the laboratory sample variance for all nutrients (Table 2). The statistical analysis showed that the differences among accessions explained from 84.4 to 96.1% of the total observed variation. The minerals Zn, Ca, P and Mg explained more of the differences between the tested accessions than did protein, Fe and K, while P, Zn, Mg and protein were the most important in differentiating the groups.

Table 2. Analysis of variance (ANOVA) to identify the more relevant variables in the comparison of accessions: percentage of the total variance explained by the accessions, order of importance of the variables into the accessions differentiation (order acc), order of importance of the variables into the groups differentiation (order gr) and CV between samples

	ANOVA	Variance	Explained				
Variable	*F* value	Component	Variance (%)		Order acc	Order gr	CV (%)
Protein	11.81	0.1	84.4	**	7	4	3.8
Fe	17.47	38.7	89.2	**	5	6	4.1
Zn	50.42	25.2	96.1	**	1	2	2.6
Ca	40.61	25,438.0	95.2	**	2	5	4.3
Mg	23.88	23,499.0	92.0	**	4	3	2.4
K	12.29	90,6047.5	85.0	**	6	7	2.7
P	32.12	215,321.1	93.9	**	3	1	2.3

** Significance at $P \leq 0.01$; the null hypothesis is 'the variance among accessions is equal to the between-samples variance'.

Cluster analysis

The multivariate cluster analysis technique was used to group the different cowpea accessions based on the values for all seven nutrients in their grains. The 'mixture of normal distributions' clustering method identified nine groups with each group containing different number of accessions (Table 3). Group G4 had the highest number of accessions (571), which represented about 37% of total number of accessions evaluated. This was followed by G3 with 229 members, while G2 and G9 had the lowest numbers of 59 and 55 members, respectively. Groups G1, G5, G7 and G9 contained the genotypes with generally high protein and mineral concentrations.

Group G5 (180 accessions) showed average value greater than the 75% of all the tested accessions (the percentile P75 from the whole collection, Table 3) for protein, Fe, Zn, Mg and P; group G9 (55 accessions) showed average value greater than the P75 for Fe, Zn, Ca, Mg and P; group G7 (119 accessions) showed mean value higher than P75 for Fe, Zn, Mg and P; and groups G1 and G8 (134 and 119 accessions, respectively) showed means greater than P75 for K (Table 3). Also Fig. 1 reveals that groups G5, G7 and G9 had the highest average values for protein, Fe, Zn, Ca, Mg and P, group G5 had the highest mean values for protein and Fe, group G7 had the highest mean values for P and Zn, group G9 for Ca and Mg and group G1 for K. In summary, groups G5, G7 and G9 (354 accessions) showed the most promising accessions in terms of their protein and mineral contents.

The best 50 accessions, all of which belonged to the three above-mentioned groups (G5, G7 and G9), were identified (data not shown). To rank the accessions based on their nutrient contents, the value for each nutrient was standardized by its mean and variance (to avoid scale differences) and the standard scores were summed up.

The best ten accessions in terms of nutrient contents showed values greater than the P75 for all the nutrients measured except Ca. These best ten accessions [group, protein %, Fe, Zn, Ca, Mg, K and P in mg/kg] included TVu-2723 (9, 27.6, 79.5, 52.7, 1260, 2450, 18,200, 6350), TVu-1829 (5, 30.4, 69.4, 47.7, 980, 2150, 17,000, 5850), TVu-3638 (9, 30.7, 62.1, 45.8, 1305, 2400, 17,950, 5950), TVu-2508 (5, 32.2, 61.8, 47.1, 750, 2150, 16,450, 6200), TVu-2356 (5, 30.0, 78.7, 51.4, 710, 2000, 15,500, 5900), TVu-526 (9, 27.5, 78.1, 48.4, 1320, 2350, 16,300, 5700), TVu-2880 (5, 29.2, 72.3, 47.4, 810, 2200, 15,800, 5450), TVu-408 (5, 30.8, 58.4, 49.6, 835, 2200, 15,800, 5750), TVu-7654 (5, 26.5, 71.5, 44.7, 745, 2250, 17,250, 5500), and TVu-8810-1 (5, 30.1, 69.6, 43.6, 735, 2250, 15,500, 5750). Three of the lines (TVu-2723, TVu-3638 and TVu-526) belonged to G9 and all of them showed individual values higher than P75 for all nutrients. The other seven belonged to G5 and one of them, accession TVu-2508, showed the highest value for protein content (32.2%) of all the accessions used in this study. On the other hand, groups G2, G3 and G6, containing a total of 363 accessions, had the lowest mean values for protein and mineral concentrations in their grains. The average mineral concentration values per group ranked from 1 to 9, with 1 being the lowest and 9 the highest mean values. The ranking for the best three groups (G5, G7 and G9) and the worst two groups (G2 and G6) is shown in Fig. 1. Figure 1 exhibits how the groups compare with one another pertaining to

Table 3. Group average values for the nutrient content, number of accessions per groups (size) and percentage of accessions forming each group (%)[a]

Group	Protein %	Fe (mg/kg)	Zn (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	P (mg/kg)	Size	%
G1	24.6	55.3	37.6	871	1912	16,366[b]	5340	134	9
G2	23.8	45.6	30.2	797	1752	13,905	4138	59	4
G3	22.6	51.4	41.1	705	1844	14,636	5256	229	15
G4	25.4	51.2	36.0	899	1883	14,451	4791	571	37
G5	27.1[b]	60.6[b]	41.6[b]	798	2059[b]	14,807	5372[b]	180	12
G6	22.6	48.9	35.2	652	1722	14,870	4786	75	5
G7	23.4	58.3[b]	46.5[b]	759	2102[b]	15,118	5679[b]	119	8
G8	25.7	51.1	33.6	760	1906	16,030[b]	4859	119	8
G9	24.7	58.9[b]	41.5[b]	1107[b]	2132[b]	15,281	5665[b]	55	4
Mean	24.8	53.2	38.1	826	1915	14,880	5055		
L	24.7	52.9	37.9	818	1907	14,840	5032		
U	24.9	53.5	38.4	834	1923	14,940	5079		
P75	26.3	57.2	41.4	925	2000	15,500	5350		
Total								1541	100

L, lower value; *U*, upper value; P75, percentile 75 values.
[a] Statistics for the whole collection: average value (mean), confidence interval for the mean (95% *L* and *U* values) and P75.
[b] The group mean is greater than the P75 of the whole collection.

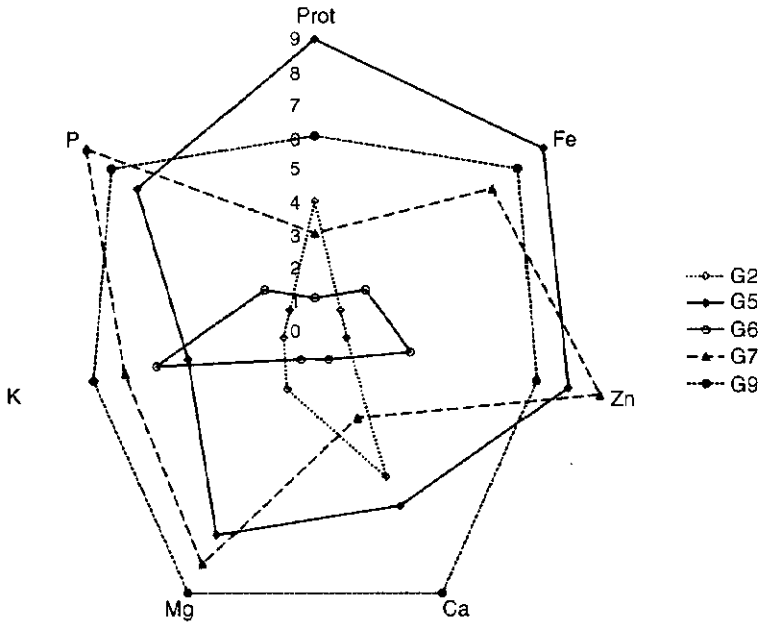

Fig. 1. Nutrient content ranked average values per group (1 = lowest, 9 = highest) for the three better (G5, G7, G9) and the two worst (G2, G6) groups found by cluster analysis. Prot, protein.

their mean protein and mineral concentrations. The farther from the centre of the figure a group is, the higher is the mean value in its mineral and protein contents. For example, the mean values for Ca and Mg for G9 were highest, while G2 had the lowest mean values for P, K, Zn and Fe. From the figure, it can be observed that the highest mean protein and Fe values were associated with group G5. Closely related groups in terms of mineral or protein concentration in grains would have similar shape and size.

Figure 2 shows the results of canonical variable analysis, an analysis that allows an optimal graphical representation of the distances between groups in a two dimensional space. The first canonical variable (Can1), which explained 62% of the ratio of 'variance between groups:variance within groups', was associated mainly with high values for P and Zn. The second (Can2) explained 16% of the ratio and was associated with high values for all the minerals considered. Group G9 revealing average values greater than the percentile P75 for all nutrients except protein, G5 with fairly similar mineral concentration except Ca and P, and G7 showing the highest values for Zn and P are located to the right and upper sections along Can1 and Can2 axes, respectively (Fig. 2). The best 50 accessions appeared in the first quadrant and the best ten (accession numbers are shown in the figure) are located at right and upper part of the canonical representation. On the other hand, groups G2 and G6 appeared on the third quadrant in contrast to the best groups. (A supplementary table (Table S1) showing the mean values of protein and mineral contents for all the tested germplasm lines and their respective groups can be found online at journals.cambridge.org/pgr).

Correlation coefficients

Coefficients of correlation calculated on the sampled 1541 cowpea accessions showed low but significant values between nutrient content (protein and mineral concentrations) except the relationship between Ca and K, which was not significant. The results (Table 4) showed that Fe, Zn, Mg and P were positively associated. Zn and P showed the highest correlation ($r = 0.66$, $P \leq 0.05$) followed closely by Fe and Zn and Mg and P. There were significant negative correlations between Zn and Ca and Ca and P. Correlation coefficients were determined within each of the various groups, i.e. G1–G9. Coefficients of correlation were lowest when calculated for group G2 (59 accessions), highest and generally positive for group G9 (55 accessions), one of the three best groups, and intermediate when calculated for group G5 (180 accessions). The averaged values of the absolute values (to avoid the sum of negatives and positives, and considering that a high negative is as important as a high positive) of the correlation coefficients were 0.19, 0.22, 0.28 and 0.43 for G2, G5, all 1541 accessions and G9, respectively (Table 4).

For group G9, all coefficients were positive and only two were non-significant (Ca–Mg and Ca–P). These results are interesting as they suggested that selecting

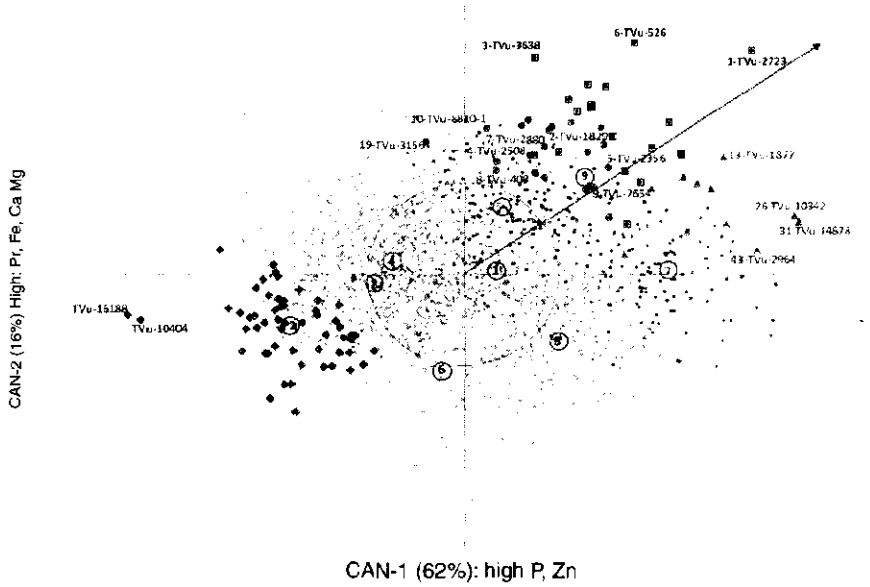

Fig. 2. Display of the nine groups along the first (Can1) and second (Can2) canonical variables: groups located at right and upper section of the figure show the highest nutrient content values. Four groups are showed in detail: G2 (dark red), G5 (red), G7 (green) and G9 (blue). Accessions into the 'best' 20 are marked with big circles (G5), big triangles (G7) and big squares (G9), 'best' ten accessions are identified by their name. Pr, protein. A colour version of this figure can be found online at journals.cambridge.org/pgr

for one nutrient could improve simultaneously the others when accessions within G9, a group with the highest mean values for Ca and Mg (Table 3) are used as parents. Similar responses should be expected when members of group G5 (the group with highest values for protein) are used as parents. The exception would be when selecting for either Ca- or P-rich lines because there was inverse relationship ($r = -0.19$, $P \leq 0.05$) between the two minerals.

Discussion

Cowpea grains are appreciated mainly for their relatively high protein content, which in most improved varieties hovers slightly below 25% (Carnovale *et al.*, 1990). In sub-Saharan Africa where cost of meat could be high, relative to income, people will need to obtain protein from additional sources to supplement their needs for this important nutrient. Cowpea remains a cheap source of protein in the people's diets. It will be of immense benefit to cowpea consumers if the present levels of protein in grains can be further increased. This is particularly important because the digestibility of cowpea is approximately 80.9% (Marconi *et al.*, 1990), implying that not all the protein in the consumed cowpea grain is available for the body's uptake. The extent of genetic diversity observed among the cowpea germplasm lines evaluated in this study suggested that there is a potential to genetically enhance the present levels of protein in the grains. While most varieties and breeding lines have slightly less than 25% on average and there are among germplasm lines those with up to 32% protein, it should be possible to obtain, through breeding, varieties with higher level. Following a diallel analysis among ten cowpea breeding lines, protein content was found to be associated with high general combining ability (Fatokun, unpublished IITA data). This implies that lines with high protein concentration in their grains can transfer this attribute to their progeny. The cowpea germplasm lines with relatively high protein content and which could be potential parents to cross while developing new varieties that will be characterized by enhanced protein levels have been revealed in this study.

The protein, Fe, Zn, Ca, and P contents in grains of the cowpea germplasm lines tested in this study are within the ranges reported among 21 improved IITA breeding lines by Carnovale *et al.* (1990). The results that we obtained showed that there exists remarkable genetic diversity in protein and mineral concentrations in grains of cowpea. White and Broadley (2009) also reported the existence of genetic diversity in the mineral concentrations of many crop species. In common bean, a legume-like cowpea, Beebe *et al.* (2000) reported a wide range in mineral concentrations while evaluating a core collection made up of more than 1000 accessions. Bänziger and Long (2000) measured Zn and Fe concentrations in kernels of 1814 maize core germplasm and breeding populations, and reported significant variations among them for these minerals. They attributed

Table 4. Coefficients of correlation between variables for all tested cowpea accessions (4a, lower diagonal, $n = 1{,}541$), G2 (4a, upper diagonal, $n = 59$), G5 (4b, lower diagonal, $n = 180$), and G9 (4b, upper diagonal, $n = 55$)

	4a							4b						
	Pr	Fe	Zn	Ca	Mg	K	P	Pr	Fe	Zn	Ca	Mg	K	P
Pr		0.1	0.24	0.07	0.34*	0.28*	0.29*		0.59*	0.57*	0.47*	0.41*	0.51*	0.58*
Fe	0.40*		0.49*	0.06	0.01	0.1	−0.01	0.30*		0.70*	0.40*	0.52*	0.38*	0.43*
Zn	0.14*	0.58*		0.05	−0.1	0.02	0.06	0.53*	0.45*		0.29*	0.40*	0.42*	0.32*
Ca	0.22*	0.07*	−0.06*		−0.1	0.22	−0.47*	0.04	−0.01	0.21*		0.23	0.41*	0.04
Mg	0.31*	0.46*	0.40*	0.23*		0.29*	0.35*	0.16*	0.02	0.06	0.29*		0.51*	0.38*
K	0.15*	0.20*	0.11*	0.01	0.21*		0.29*	0.35*	0.21*	0.16*	−0.08	0.21*		0.52*
P	0.16*	0.48*	0.66*	−0.11*	0.52*	0.37*		0.54*	0.16*	0.36*	−0.19*	0.06	0.33*	

Pr, protein.
* Significant at $P \leq 0.05$; this significance depends on the number of observations (degrees of freedom), which were different for each group.

the variations observed to both genetic and environmental conditions of where the maize plants were grown.

We observed significant and positive relationship between protein and Fe concentration in grains when all of the 1541 accessions tested were considered. The implication of this observation is that the concentrations of both protein and Fe can be increased without adverse effect on each other. Similarly, there were positive and significant correlations between Fe and Mg, Fe and P as well as between Zn and P, indicating that the densities of these minerals in cowpea can be increased concurrently through breeding. However, we observed negative but significant relationship between Ca and P as Townsend *et al.* (1999) also reported earlier in alfalfa a forage legume plant. The concentrations of both minerals cannot be increased concurrently through selection. In general, it can be stated that breeding activities aimed to enhance the levels of some of these minerals in cowpea grains could also lead to the improvement of the others. Given the level of variation detected in this study, sufficient diversity exists in the cowpea germplasm to necessitate further investigations into genes and/or genetic markers that could be deployed in the development of nutrient-dense cowpea varieties through breeding. The wide range found in the concentration of each of the minerals among the cowpea germplasm lines evaluated provides further evidence that responses to selection should be expected while breeding nutrient-dense varieties.

It is important to note that increased mineral content in the grains does not guarantee increased nutrient status for the consumer. There are numerous compounds such as phytates in plant-based diets that could reduce nutrient absorption. Therefore, studies of bioavailability of important nutrients in cowpea-based diets would be essential. It is necessary to demonstrate the efficacy of nutrient enrichment of plant foods towards improving the nutritional health of targeted populations. According to Graham *et al.* (2001), this requires that the bioavailability of Fe, Zn, provitamin A carotenoids and other micronutrients in selected micronutrient-enriched genotypes of staple plant foods be demonstrated, to ensure a human health impact before advancing genotypes in breeding programmes.

The cluster analysis has helped to group the cowpea germplasm accessions into classes based on their levels of protein and mineral concentrations. From this study, it was concluded that members of some groups such as G5 and G9, which included TVu-2723, TVu-3638 and TVu-2508, would be potential sources of genes for enhancing protein and mineral concentrations in improved cowpea varieties. These lines would therefore be selected and used in crossing for generating segregating populations from where selections can be made for newly developed nutrient-dense cowpea varieties.

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