



# Evaluation of Protein and Micronutrient Levels in Edible Cowpea (*Vigna Unguiculata* L. Walp.) Leaves and Seeds

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Cowpea is the most important seed legume in Africa. Its leaves and seed are consumed to meet the dietary requirements of protein and micronutrient in rural African communities. In this study, leaf protein of 32 cowpea genotypes was 23–40% at Taung (South Africa), 28–40% at Wa and 24–35% at Manga (Ghana). Seed protein level was also up to 40% in landrace Bengpla and more than 30% in nine other genotypes planted at Taung. Trace elements in cowpea leaves showed markedly high concentrations of Fe (2,011  $\mu\text{g.g}^{-1}$ ), Zn (150  $\mu\text{g.g}^{-1}$ ), Mn (325  $\mu\text{g.g}^{-1}$ ), and B (43  $\mu\text{g.g}^{-1}$ ) in genotype Apagbaala, in contrast to the very low levels of Fe (273  $\mu\text{g.g}^{-1}$ ), Zn (40  $\mu\text{g.g}^{-1}$ ), Mn (219  $\mu\text{g.g}^{-1}$ ), and B (32  $\mu\text{g.g}^{-1}$ ) in genotype Encore. Leaf Fe concentration was highest in genotype Apagbaala (2,011  $\mu\text{g.g}^{-1}$ ), followed by Fahari (2,004  $\mu\text{g.g}^{-1}$ ), Iron Gray (1,302  $\mu\text{g.g}^{-1}$ ), Line 2020 (944  $\mu\text{g.g}^{-1}$ ), Bensogla (927  $\mu\text{g.g}^{-1}$ ), Omondaw (605  $\mu\text{g.g}^{-1}$ ), IT96D-1951 (591  $\mu\text{g.g}^{-1}$ ), IT93K-452-1 (574  $\mu\text{g.g}^{-1}$ ), Ngonji (569  $\mu\text{g.g}^{-1}$ ), and Mchanganyika (566  $\mu\text{g.g}^{-1}$ ), and lowest in Bechuana white (268  $\mu\text{g.g}^{-1}$ ). Cowpea seed also showed greater concentrations of Fe in genotype Soronko (67  $\mu\text{g.g}^{-1}$ ), IT93K-452-1 (67  $\mu\text{g.g}^{-1}$ ), Brown Eye (65  $\mu\text{g.g}^{-1}$ ), Bensogla (61  $\mu\text{g.g}^{-1}$ ), and TVU11424 (62  $\mu\text{g.g}^{-1}$ ). Trace elements in cowpea seed differed among genotypes, and ranged from 45.1 to 67.0  $\mu\text{g.g}^{-1}$  for Fe, 33.9 to 69.2  $\mu\text{g.g}^{-1}$  for Zn, 10.1 to 17.4  $\mu\text{g.g}^{-1}$  for Mn, 14.7 to 21.4  $\mu\text{g.g}^{-1}$  for B, and 5.2 to 8.1  $\mu\text{g.g}^{-1}$  for Cu. Genotypes Apagbaala, Fahari, Iron Gray, and Line 2020, respectively, exhibited 34.2-, 34.0-, 22.5-, and 18.3-fold higher Fe concentration in leaves than seed, and 3.5-, 2.0-, 2.0-, and 3.5-fold greater Zn in leaves than seed (in that order). The genotypes that accumulated significantly high levels of protein and trace elements in cowpea leaves and seed, were generally high  $\text{N}_2$ -fixers, thus suggesting a link between  $\text{N}_2$  fixation and cowpea's ability to synthesize protein and accumulate nutrient elements in leaves and seed. Therefore, identifying cowpea genotypes that can enhance protein accumulation and micronutrient density in edible leaves and seed through breeding has the potential to overcome protein-calorie malnutrition and trace element deficiency in rural Africa.

**Keywords:** cowpea, food, sustainability, breeding, micronutrients

## INTRODUCTION

Food and nutritional insecurity remain a major problem facing Africa, as about 239 million people are suffering from protein-calorie malnutrition (Fanzo, 2012), and another 232 million from micronutrient deficiency (Andrea and Rose, 2015). In Africa, hunger is the result of food insecurity due to low crop yields stemming from soil moisture deficit from low rainfall, farmer use of nutrient-poor soils for agriculture and unimproved crop varieties, as well as the effects of biotic stress such as insect pests and diseases (Dakora and Keya, 1997). Although N fertilizers can be used to overcome soil infertility and increase crop yields, they are expensive and inaccessible in Africa. On average, only about 8.8 kg NPK fertilizer is applied per hectare by smallholder farmers in Africa (Hena and Baanante, 2006).

Due to low nutritious food production, protein-calorie malnutrition is highly prevalent in African children, and is the outcome of low protein and calorie intake. Although the consumption of meat, dairy products and seafood can overcome protein-calorie malnutrition, these foods are expensive to resource-poor households in rural Africa. So the use of protein-rich plant foods has been the main option for many poor African communities. Leafy vegetables, for example, are a good source of dietary protein (Aletor et al., 2002), however, nodulated legumes are even better due to their ability to fix  $N_2$  when in symbiosis with soil bacteria termed “rhizobia.” Here,  $N_2$ -fixing bacteroids in root nodules are able to reduce atmospheric  $N_2$  to  $NH_3$ , which is incorporated into amino acids and protein, and stored in leaves and seeds. This explains why the edible leaves and seed of legumes (or pulses) are a very high source of dietary protein. Of the cultivated legumes used as food, seed protein is as high as 40% in soybean (Zarkadas et al., 2007), 33% in cowpea (Ddamulira and Santos, 2015), 20–25% in common bean (BroUGHTON et al., 2003), 20.6% in Bambara groundnut (Mazahib et al., 2013), 21.3% in Kersting’s bean (Ayenan and Ezin, 2016), 27–29% in pigeonpea (Saxena et al., 1987), 21–31% in mungbean (Yi-shen et al., 2018), 21.8–25.8% in chickpea (Xu et al., 2016) and 20–30% in groundnut (Toomer, 2018). Additionally, cowpea also contain 34.9% of protein in edible leaves (Enyiukwu et al., 2018).

In addition to protein, the edible leaves and seeds of legumes also contain high levels of dietarily-important mineral nutrients, which are needed for human nutrition and health, especially for overcoming trace element deficiency and promoting brain development. For example, mineral concentrations are also reported to be 142–626 and 60–99  $mg.kg^{-1}$  for Fe, 49–104 and 44–65  $mg.kg^{-1}$  Zn, 196–394 and 5–32  $mg.kg^{-1}$  Mn, 8.6–19.7 and 8.3–14.7  $mg.kg^{-1}$  Cu and 42–55 and 10–22  $mg.kg^{-1}$  B in cowpea leaves and seeds, respectively (Belane and Dakora, 2011a). Other studies have reported 22.6  $mg.kg^{-1}$  Fe, 33.1  $mg.kg^{-1}$  Zn, 6.7  $mg.kg^{-1}$  Mn, and 7.5  $mg.kg^{-1}$  Cu for groundnut seed (Toomer, 2018), as well as 500.0  $mg.kg^{-1}$  Fe, 405.0  $mg.kg^{-1}$  Zn, 480.0  $mg.kg^{-1}$  Mn, and 85.0  $mg.kg^{-1}$  Cu for chickpea seed (Xu et al., 2016).

Given the inherently low infertility of African soils, as well as the high cost of chemical fertilizers and their polluting effect on the environment, there is a need to develop sustainably green and affordable technologies for increasing the nutritional quality

of food legumes for use by resource-poor, smallholder farmers in Africa. The aim of this study was to assess protein level and trace element density in edible leaves and seed of 30–32 cowpea genotypes grown in the field at Wa and Manga in Ghana, and at Taung in South Africa.

## MATERIALS AND METHODS

### Site Description

Field trials were conducted in Ghana and South Africa in 2005 and 2006. In Ghana, these field experiments were carried out at Dokpong and Bamahu near Wa in the Upper West Region, and at Manga in the Upper East Region, in 2005 and 2006, while in South Africa, these trials were conducted at Taung. Details of the experimental environments in the countries (altitude, longitude, mean annual rainfall, soil characteristics, cropping history, etc.) have been described elsewhere (Belane and Dakora, 2009, 2010, 2011b; Belane et al., 2011).

### Origin of Cowpea Genotypes

The cowpea genotypes used for this study were collected from Ghana, Tanzania, South Africa, and the International Institute of Tropical Agriculture (IITA) in Nigeria, as indicated by Belane and Dakora (2010). The 30 genotypes exhibited different useful biological traits ranging from number of days to 50% flowering and number of days to physiological harvest, to levels of  $N_2$  fixation, pest resistance, and seed yield (Belane and Dakora, 2010).

### Field Design, Planting, and Pest Management

The experimental design used in this study has been described elsewhere (Belane and Dakora, 2009, 2010, 2011b; Belane et al., 2011), and involved the use of a randomized complete block design with four replicate plots per cowpea genotype in all the experiments. Each plot measured 3 m  $\times$  5 m (i.e., 15 m<sup>2</sup>). The experiments were planted in mid-July each year, with a row-to-row spacing of 60 and 20 cm within-row. Weeds were controlled with a hoe. Two low-dose sprays of lambda cyhalothrin (Karate 2.5 EC) insecticide were applied at flowering and at pod formation to control pests.

### Plant Harvest and Processing

Healthy young trifoliolate leaves were harvested from 12 plants per plot at 46 and 72 DAP in 2005 and 2006 to assess for any changes in mineral density close to physiological maturity. The leaf samples were oven-dried (60°C), weighed, and ground to fine powder (0.85 mm) for mineral analysis. At physiological maturity, cowpea seeds were harvested and similarly processed for analysis of nutrient elements.

### Protein Analysis in Cowpea Leaves and Seed

The percent N in cowpea leaves and seeds was determined using mass spectrometry, as described by Belane and Dakora (2010). The protein in leaves and seeds was estimated as %N of organ  $\times$  6.25 (Jones, 1941; Mariotti et al., 2008).

## Determination of Micronutrients in Cowpea Leaves and Seeds

Trace elements such as Fe, Zn, Cu, Mn, and B in cowpea leaves and seeds were measured, as described by Belane and Dakora (2011a). Briefly, 1 g of ground cowpea leaf or seed sample was ashed in a porcelain crucible at 500°C overnight, the ash was dissolved in 5 ml of 6 M HCl (analytical grade) and placed in an oven at 50°C for 30 min, after which 35 ml of de-ionized water was added. The mixture was filtered through Whatman No. 1 filter paper, and mineral concentrations determined in leaf and seed extracts from four replicate samples using inductively coupled plasma mass spectrometry (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA) (Ataro et al., 2008).

## Correlation Analysis

Correlation analyses were performed for the levels of micronutrients in leaves and seeds of cowpea genotypes to ascertain any relationships that may exist in the translocation of trace elements between the two organs.

## Statistical Analysis

The data on protein and micronutrient levels in cowpea leaves and seed were subjected to analysis of variance (ANOVA) using a STATISTICA analytical software program version 7.1. A one-way ANOVA was used to compare protein and micronutrient levels among genotypes. Where significant differences were found, the Duncan's multiple range test was used to separate treatment means at  $p \leq 0.05$  or  $p \leq 0.001$ .

## RESULTS

### Leaf Protein Levels of Cowpea Genotypes

The leaf protein of cowpea genotypes used in this study varied markedly between and among genotypes irrespective of location. The leaf protein of 30 cowpea genotypes grown at Wa in the Guinea savanna of the Upper West Region in Ghana also differed significantly, and ranged from about 28% for genotype ITH98-46 to 40% for Soronko (Figure 2). Of the 30 cowpea genotypes tested at Wa, 29 recorded more than 30% protein in their leaves (Figure 2).

Leaf protein was also assessed for 30 cowpea genotypes planted at Manga in the Sudano-Sahelian savanna in the Upper East Region of Ghana. Leaf protein levels also differed among the cowpea genotypes at Manga, and were found to vary from 24 to 35% (Figure 3). Some 12 out of the 30 genotypes studied recorded more than 30% protein in their leaves at Manga, Ghana.

### Seed Protein of Cowpea Genotypes

The concentration of protein in cowpea seed was determined for only the 32 genotypes planted at the Taung site in South Africa. The data revealed marked differences in seed protein, which ranged from about 20% in Soronko to 40% in Bengpla (Figure 4). Ten cowpea genotypes, including Bengpla, recorded more than 30% protein in their seed when planted at Taung in South Africa (Figure 4).

## Micronutrient Density in Cowpea Leaves

The levels of micronutrients in edible leaves of cowpea genotypes were assessed using ICP-MS analysis for only the 32 cowpea genotypes planted at Taung (Table 1), but not Wa or Manga in Ghana. The concentration of micronutrients in the leaves varied hugely between and among genotypes. As show in Table 1, the level of Fe in cowpea leaves ranged from 268  $\mu\text{g.g}^{-1}$  in Bechuana white to 2,011  $\mu\text{g.g}^{-1}$  in Apagbaala landrace. Other genotypes with markedly high leaf Fe levels included Fahari (2,005  $\mu\text{g.g}^{-1}$ ), Iron Gray (1,302  $\mu\text{g.g}^{-1}$ ), Line 2020 (945  $\mu\text{g.g}^{-1}$ ), and Bensogla (927  $\mu\text{g.g}^{-1}$ ). In contrast, the genotypes which showed the lowest leaf Fe concentrations were Bechuana white (268  $\mu\text{g.g}^{-1}$ ), Encore (273  $\mu\text{g.g}^{-1}$ ), IT94D-437-1 (314  $\mu\text{g.g}^{-1}$ ), and TVU11424 (313  $\mu\text{g.g}^{-1}$ ).

The distribution of Zn in cowpea leaves also differed markedly, and ranged from 37  $\mu\text{g.g}^{-1}$  in Vallenga to 150  $\mu\text{g.g}^{-1}$  for Apagbaala (which also recorded the highest Fe concentration; Table 1). Other cowpea genotypes with high levels of Zn in leaves included Line 2020 (132  $\mu\text{g.g}^{-1}$ ), Iron Gray (90  $\mu\text{g.g}^{-1}$ ), Fahari

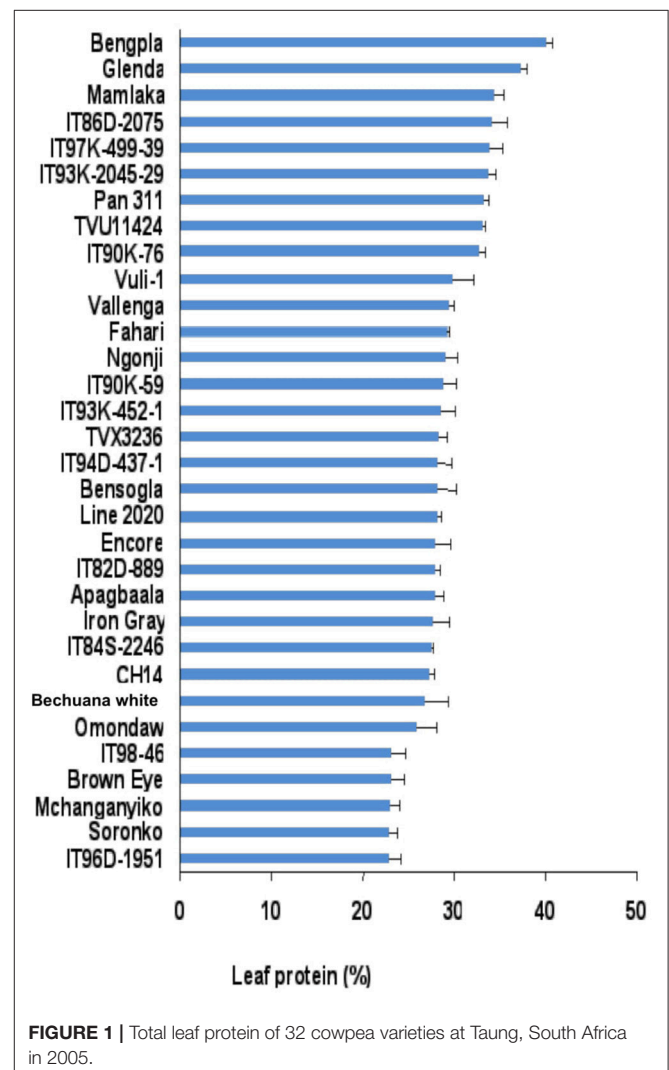


FIGURE 1 | Total leaf protein of 32 cowpea varieties at Taung, South Africa in 2005.

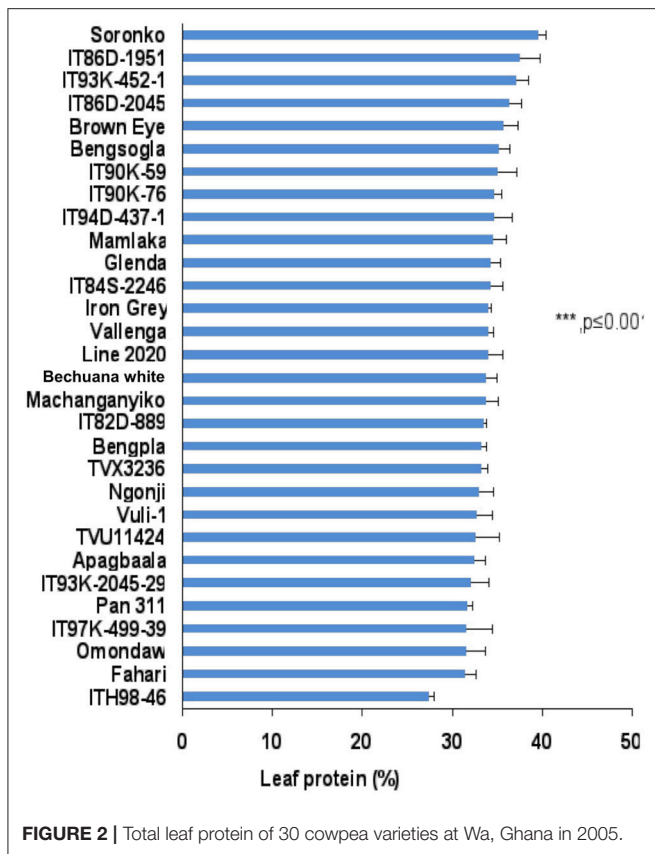


FIGURE 2 | Total leaf protein of 30 cowpea varieties at Wa, Ghana in 2005.

(80  $\mu\text{g.g}^{-1}$ ), and Bengsogla (73  $\mu\text{g.g}^{-1}$ ), which incidentally also recorded high Fe concentrations in cowpea leaves. However, the genotypes with the least Zn concentration in leaves included Vallenga (37  $\mu\text{g.g}^{-1}$ ), Bechuana white (38  $\mu\text{g.g}^{-1}$ ), IT82D-889 (39  $\mu\text{g.g}^{-1}$ ), and Encore (40  $\mu\text{g.g}^{-1}$ ).

The density of Mn in edible cowpea leaves similarly differed among the genotypes, and ranged from 165  $\mu\text{g.g}^{-1}$  in IT86D-2075 to 404  $\mu\text{g.g}^{-1}$  in Line 2020 (Table 2). Other genotypes with increased Mn in leaves included Iron Gray (364  $\mu\text{g.g}^{-1}$ ) and Apagbaala (325  $\mu\text{g.g}^{-1}$ ). Leaf concentration of B also differed with cowpea genotype, with levels ranging from 31  $\mu\text{g.g}^{-1}$  in Bechuana white to 50  $\mu\text{g.g}^{-1}$  in Bengpla (Table 2). The concentration of Cu in cowpea leaves was similar for all 32 genotypes (Table 2).

### Micronutrient Density in Cowpea Seed

The concentrations of trace elements (Fe, Zn, Cu, Mn, and B) in cowpea seed were generally lower relative to leaves. As shown in Table 2, Fe levels in seed differed among the genotypes tested, and ranged from 45  $\mu\text{g.g}^{-1}$  for Bengpla to 67  $\mu\text{g.g}^{-1}$  in Soronko and IT95K-452-1. Other genotypes with high levels of Fe in seed included Brown Eye (65  $\mu\text{g.g}^{-1}$ ), IT98-46 (64  $\mu\text{g.g}^{-1}$ ), TVU11424 (62  $\mu\text{g.g}^{-1}$ ), IT86D-2075 (62  $\mu\text{g.g}^{-1}$ ), and Bengsogla (61  $\mu\text{g.g}^{-1}$ ). In contrast, the genotypes with low levels of Fe in seed were Bengpla (45  $\mu\text{g.g}^{-1}$ ), followed by Mamlaka (50  $\mu\text{g.g}^{-1}$ ).

As shown in Table 2, the Cu levels in cowpea seed varied from 5.20  $\mu\text{g.g}^{-1}$  in genotype IT82D-889 to 8.11  $\mu\text{g.g}^{-1}$  for genotype IT96D-1951 and 8.06  $\mu\text{g.g}^{-1}$  for Vallenga (Table 2). Other genotypes with increased levels of Cu in cowpea seed included Bengsogla (7.90  $\mu\text{g.g}^{-1}$ ), Iron Gray (7.86  $\mu\text{g.g}^{-1}$ ), Brown Eye (7.76  $\mu\text{g.g}^{-1}$ ), and Pan 311 (7.49). Similarly, Zn concentration in cowpea seed was different for the 32 genotypes tested (Table 3). Genotype TVU11424 recorded the highest levels of Zn (69.15  $\mu\text{g.g}^{-1}$ ), followed by Soronko (53.88  $\mu\text{g.g}^{-1}$ ), and IT90K-59 (49.78  $\mu\text{g.g}^{-1}$ ). In contrast, the lowest Zn concentration was found in Bengpla (33.89  $\mu\text{g.g}^{-1}$ ), followed by Mamlaka (34.64  $\mu\text{g.g}^{-1}$ ), and Line 2020 (37.86  $\mu\text{g.g}^{-1}$ ).

The Mn distribution in cowpea seed ranged from 10.05  $\mu\text{g.g}^{-1}$  in Bechuana white to 17.43  $\mu\text{g.g}^{-1}$  in CH14 (Table 2). The highest Mn concentrations in cowpea seed were recorded by genotypes CH14 (17.43  $\mu\text{g.g}^{-1}$ ), Iron Gray (17.06  $\mu\text{g.g}^{-1}$ ), Bechuana white (16.85  $\mu\text{g.g}^{-1}$ ), Fahari (16.46  $\mu\text{g.g}^{-1}$ ), and IT86D-2075 (16.21  $\mu\text{g.g}^{-1}$ ). By contrast, the lowest Mn levels were produced by Bechuana white (10.05  $\mu\text{g.g}^{-1}$ ) and IT82D-889 (10.09  $\mu\text{g.g}^{-1}$ ). The B levels in cowpea seed also differed among genotypes, and varied from 14.71  $\mu\text{g.g}^{-1}$  for IT82D-889 to Brown Eye (21.44  $\mu\text{g.g}^{-1}$ ). The highest concentration of B was found in Brown Eye (21.44  $\mu\text{g.g}^{-1}$ ), followed by IT94D-437-1 (21.30  $\mu\text{g.g}^{-1}$ ), Encore (19.81  $\mu\text{g.g}^{-1}$ ), IT90K-59 (19.20  $\mu\text{g.g}^{-1}$ ), Bechuana white (19.11  $\mu\text{g.g}^{-1}$ ), and IT93K-2045-29 (19.00  $\mu\text{g.g}^{-1}$ ). However, the lowest B levels were recorded by IT82D-889 (14.71  $\mu\text{g.g}^{-1}$ ), followed by Bengsogla (15.57  $\mu\text{g.g}^{-1}$ ) and Bengpla (15.71  $\mu\text{g.g}^{-1}$ ).

### Correlation Analysis of Micronutrients in Cowpea Leaves and Seeds

Leaf Fe was positively correlated with seed Fe, leaf Zn, leaf Mn, and seed Mn (Table 4). Seed Fe was also correlated positively with seed Zn and leaf B, but negatively with seed Cu and seed B. Seed Zn correlated with positively leaf Cu, seed Cu and seed B, but negatively with leaf Mn. Similarly, leaf Mn correlated significantly with seed Mn but negatively with seed Cu, leaf B and seed B, and seed Cu correlated with seed B (Table 4).

## DISCUSSION

### Leaf and Seed Protein of Cowpea Genotypes

Food and nutritional insecurity remain a major problem facing Sub-Saharan Africa, as about 239 million people are currently suffering from protein-calorie malnutrition (Fanzo, 2012; Andrea and Rose, 2015). In rural Africa, food/nutritional security and micronutrient deficiency are met through the consumption of leafy vegetables and seed legumes (Belane and Dakora, 2011a), as animal protein is too expensive for resource-poor households. In Sub-Saharan Africa, cowpea is the major food grain legume, cultivated and consumed by the majority of smallholder farming communities and is very important as a food crop in meeting dietary protein requirements, and overcoming micronutrient deficiency.



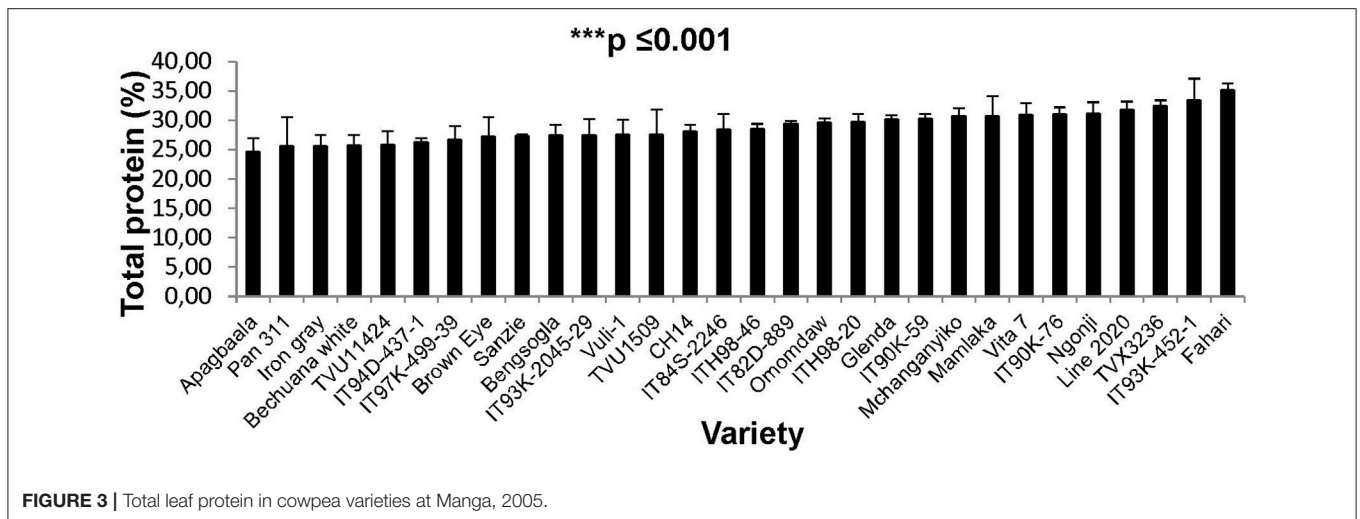


FIGURE 3 | Total leaf protein in cowpea varieties at Manga, 2005.

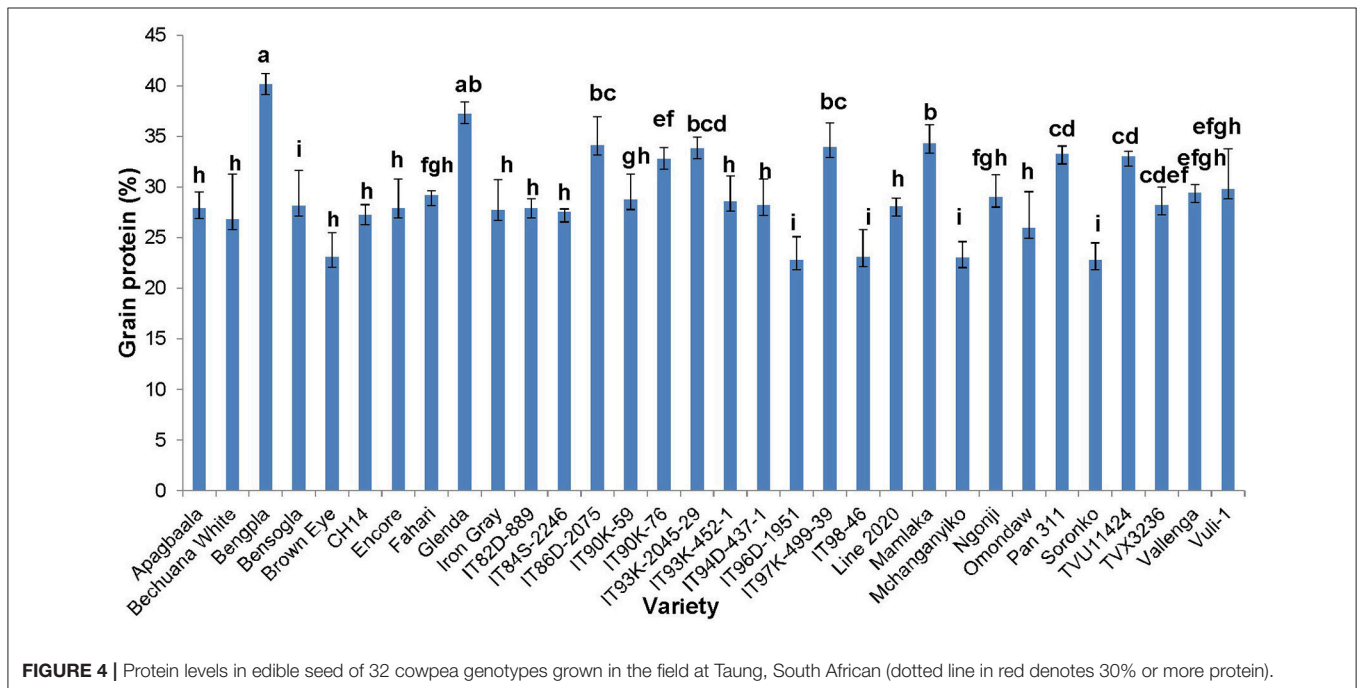


FIGURE 4 | Protein levels in edible seed of 32 cowpea genotypes grown in the field at Taung, South African (dotted line in red denotes 30% or more protein).

In this study, we evaluated 32 field-grown cowpea genotypes at Taung in South Africa, and 30 each at Wa and Manga in Ghana for leaf and seed protein, as well as for micronutrient density in the two organs at Taung. The results revealed marked differences in the levels of protein in cowpea leaves independent of location (Figures 1–4), as well as of seed protein and micronutrient density in plant parts at Taung (Tables 1, 2). The leaf protein of 32 cowpea genotypes grown at Taung (South Africa) ranged from 23% for genotype IT96D-1951 to 40% for Bengpla, with nine genotypes recording more than 30% leaf protein (Figure 1). At Wa in the Guinea savanna of Ghana, cowpea leaf protein ranged from 28 to 40% for ITH98-46 and Soronko, respectively, with 29 genotypes accumulating more than 30% protein in their

leaves (Figure 2). Similarly, at Manga in the Sudano-Sahelian savanna of Ghana, leaf protein levels varied from 24 to 35%, with 12 out of 30 genotypes recording more than 30% protein in their leaves (Figure 3). The leaves of N<sub>2</sub>-fixing legumes such as cowpea are very rich in N due to the species ability to reduce N<sub>2</sub> into NH<sub>3</sub> and subsequently into nitrogenous solutes for plant use (Belane et al., 2014). In plants, N is required for the synthesis of macromolecules such as chlorophyll needed for harvesting light photon energy during photosynthesis and formation of the enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), which reduces CO<sub>2</sub> during photosynthesis. Because Rubisco accounts for over 90% of leaf N (Belane and Dakora, 2015), most of the protein found in green leaves of

**TABLE 1** | Micronutrients in edible leaves of field-grown cowpea varieties harvested at 76 DAP at Taung, South Africa in 2005.

Genotype	Fe	Cu	Zn $\mu\text{g}\cdot\text{g}^{-1}$	Mn	B
Apagbaala	2011.29 ± 183.51a	14.77 ± 1.70	150.00 ± 4.44a	325.00 ± 32.14a-c	43.24 ± 3.94a-d
Bechuana white	267.88 ± 15.03e	7.10 ± 0.94	38.25 ± 4.32i	257.23 ± 27.04cde	31.24 ± 2.35h
Bengpla	480.73 ± 12.69cde	15.95 ± 0.90	51.63 ± 3.40f-i	253.06 ± 13.54cde	50.11 ± 1.17a
Bensogla	927.01 ± 99.60bcd	12.25 ± 0.82	73.44 ± 1.44b-e	255.22 ± 22.78cde	39.00 ± 0.38b-h
Brown Eye	344.27 ± 13.99de	11.28 ± 0.44	49.16 ± 4.95f-i	287.64 ± 5.74bcd	39.85 ± 0.52b-h
CHI14	462.17 ± 106.26cde	8.02 ± 0.42	48.81 ± 1.54f-i	251.05 ± 23.46cde	31.24 ± 3.69h
Encore	272.84 ± 8.52e	9.11 ± 2.08	40.07 ± 3.47hi	218.98 ± 20.33def	32.07 ± 4.01gh
Fahari	2004.59 ± 110.75	32.49 ± 24.17	80.00 ± 9.35bc	263.58 ± 13.99cde	40.72 ± 2.66b-g
Glenda	346.69 ± 9.55de	11.74 ± 1.44	53.92 ± 3.96e-i	253.96 ± 27.68cde	46.32 ± 2.33b-g
Iron Gray	1302.40 ± 130.35b	11.10 ± 1.01	89.89 ± 7.69b	364.27 ± 19.39ab	45.83 ± 1.77a-c
IT82D-889	340.15 ± 29.22de	8.26 ± 0.19	39.38 ± 1.02hi	280.03 ± 16.78b-e	33.14 ± 2.62e-h
IT84S-2246	349.24 ± 30.87cde	10.77 ± 1.41	44.31 ± 3.31f-i	273.65 ± 27.35cde	42.68 ± 4.92a-d
IT86D-2075	344.41 ± 28.13de	9.57 ± 0.59	47.69 ± 1.33f-i	165.15 ± 14.07f	35.48 ± 1.03d-h
IT90K-59	393.48 ± 12.62cde	12.44 ± 0.99	62.24 ± 0.92c-f	292.55 ± 23.59bcd	45.39 ± 1.83a-c
IT90K-76	457.94 ± 34.17cde	12.74 ± 2.68	48.67 ± 4.34f-i	267.32 ± 5.63cde	41.42 ± 0.99a-f
IT93K-2045-29	413.83 ± 33.57cde	8.96 ± 1.23	51.15 ± 3.06f-i	220.06 ± 18.22def	37.78 ± 3.07c-h
IT93K-452-1	574.39 ± 57.99cde	11.45 ± 0.98	53.56 ± 4.76e-i	319.96 ± 19.83abc	38.93 ± 0.71b-h
IT94D-437-1	314.16 ± 11.81e	20.40 ± 10.05	52.84 ± 5.22f-i	314.90 ± 33.46bc	45.01 ± 2.59a-c
IT96D-1951	591.92 ± 36.71cde	12.46 ± 1.73	61.11 ± 5.74c-g	310.81 ± 15.44bc	41.60 ± 0.99a-f
IT97K-499-39	342.46 ± 9.27de	20.11 ± 9.11	43.57 ± 1.00f-i	259.17 ± 6.22cde	38.19 ± 0.57c-h
IT98-46	434.47 ± 82.20cde	10.99 ± 1.76	52.2 ± 3.01f-i	250.12 ± 11.45cde	42.63 ± 0.17a-d
Line 2020	944.65 ± 82.20bc	20.07 ± 2.73	131.84 ± 9.83a	403.86 ± 6.58a	47.70 ± 0.77ab
Mamlaka	332.59 ± 0.16de	12.71 ± 4.446	50.54 ± 4.91f-i	242.63 ± 9.00c-f	41.82 ± 0.32a-e
Mchanganyiko	565.51 ± 53.26cde	6.58 ± 0.54	41.92 ± 1.62g-i	264.58 ± 16.88cde	39.03 ± 1.23b-h
Ngonji	569.34 ± 13.80cde	11.33 ± 2.00	63.05 ± 5.70c-f	195.48 ± 9.04ef	32.39 ± 0.32fgh
Omondaw	605.09 ± 39.12cde	13.03 ± 2.81	75.25 ± 6.68bcd	284.29 ± 25.93bcd	43.85 ± 5.90a-d
Pan 311	468.27 ± 46.05cde	16.12 ± 5.61	50.91 ± 4.96f-i	269.63 ± 24.82cde	46.94 ± 5.53a-c
Soronko	462.37 ± 44.75cde	9.13 ± 0.56	43.96 ± 1.56f-i	271.71 ± 13.36cde	42.54 ± 0.54a-d
TVU11424	313.13 ± 0.86e	10.53 ± 0.49	46.80 ± 4.19f-i	210.74 ± 20.23def	39.12 ± 0.99b-h
TVU3236	466.71 ± 34.79cde	14.07 ± 1.38	58.94 ± 4.77d-h	268.78 ± 23.74cde	45.90 ± 1.15a-c
Vallenga	355.36 ± 19.68cde	9.25 ± 4.63	37.19 ± 1.61i	214.59 ± 17.29def	38.22 ± 11.71c-h
Vulli-1	328.23 ± 20.51e	7.73 ± 0.74	44.17 ± 2.30f-i	215.93 ± 14.18def	34.71 ± 0.46d-h
F statistics	4.24	0.92	12.44	2.53	2.38
Significance level	0.001	0.58	0.001	0.001	0.001

Values (Means ± S.E) followed by dissimilar letters are significant at  $P \leq 0.05$ .

monocots and dicots consists of Rubisco. Thus, a culturable form of this protein could be a biotech spin-off for enhanced nutritional security.

Estimates of  $\text{N}_2$  fixation by cowpea plants sampled from the same field experiments as the materials used in this study showed 43 to 93% N derived from atmospheric  $\text{N}_2$  fixation at Taung (Belane et al., 2011), 8 to 60% at Manga (Belane and Dakora, 2009) and 64 to 87% at Wa (Belane and Dakora, 2010). Cowpea from farmers' fields could also derive about 30 to 99% of their N nutrition from symbiotic fixation at Wa in the Upper West Region of Ghana (Naab et al., 2009). Similar results from Botswana have shown that field-grown cowpea plants obtained between 19 and 92% of their N nutrition from symbiosis (Pule-Meulenberg et al., 2010). Clearly, the levels of  $\text{N}_2$  fixation reported in those studies (Belane and Dakora, 2009, 2010, 2011b;

Naab et al., 2009) can help to explain the strong variation in protein concentration found in the edible leaves and seed of the cowpea material used in this study.

At Taung in South Africa, cowpea genotypes Fahari, Glenda, IT93K-2045-29, Mamlaka, Pan311, and TVu11424 were among those with the highest amounts of N-fixed (Belane et al., 2011). Coincidentally, however, the same genotypes also revealed more protein in cowpea seed in this study (Figure 4), clearly indicating a direct link between protein concentration in cowpea seed and cowpea symbiotic efficiency, as well as the levels of N-fixed. In the same manner,  $\text{N}_2$  fixation and photosynthesis are metabolically interlinked at the level of the plant's N and C economy, especially where  $\text{N}_2$  reduced to  $\text{NH}_3$  by the enzyme nitrogenase is incorporated with *de novo* photosynthate into amino acids, needed for protein biosynthesis. In this study, the

**TABLE 2** | Micronutrients in seed of field-grown cowpea varieties harvested at 150 DAP in Taung.

Genotype	Fe	Cu	Zn $\mu\text{g}\cdot\text{g}^{-1}$	Mn	B
Apagbaala	58.82 ± 4.52a-i	6.70 ± 0.66b-j	43.40 ± 0.94c-f	15.58 ± 0.90a-e	18.21 ± 0.49a-c
Bechuana white	53.06 ± 1.7e-j	6.55 ± 0.29c-i	41.94 ± 1.32c-g	16.85 ± 0.44a-c	19.11 ± 0.35a-c
Bengpla	45.14 ± 7.39j	6.94 ± 0.97a-i	33.89 ± 4.53g	10.05 ± 1.99f	15.71 ± 2.25ef
Bensogla	61.37 ± 1.14a-g	7.90 ± 0.56ab	41.12 ± 1.17c-g	15.87 ± 0.61a-e	15.57 ± 0.73ef
Brown Eye	65.08 ± 2.86a-c	7.76 ± 0.28a-c	45.70 ± 1.82b-e	13.93 ± 0.49c-e	21.44 ± 2.65a
CHI14	55.27 ± 3.31c-i	6.05 ± 0.33f-m	39.80 ± 1.82e-g	17.43 ± 1.07a	16.71 ± 0.31b-f
Encore	56.99 ± 3.41a-i	5.66 ± 0.37k-m	40.07 ± 2.63d-g	13.68 ± 1.28de	19.81 ± 0.25ab
Fahari	58.88 ± 2.61a-i	6.25 ± 0.43d-m	40.78 ± 3.07c-g	16.46 ± 0.21a-d	17.10 ± 0.72b-f
Glenda	51.96 ± 2.56f-j	5.40 ± 0.43k-m	42.17 ± 3.22c-g	14.84 ± 1.59a-e	18.63 ± 0.25a-e
Iron Gray	57.90 ± 1.32a-i	7.87 ± 0.25ab	44.39 ± 0.83c-e	17.06 ± 1.04ab	18.48 ± 0.50a-e
IT82D-889	50.81 ± 5.38h-j	5.20 ± 0.43m	42.15 ± 6.68c-g	10.09 ± 0.93f	14.71 ± 1.83f
IT84S-2246	55.82 ± 4.14c-i	6.99 ± 0.65a-i	42.92 ± 1.77c-g	13.54 ± 0.62de	18.42 ± 1.23a-e
IT86D-2075	62.19 ± 1.63a-e	5.29 ± 0.17lm	41.86 ± 1.43c-g	16.21 ± 0.78a-d	18.76 ± 0.32a-e
IT90K-59	57.15 ± 3.46a-i	6.15 ± 0.40e-m	49.78 ± 9.26bc	15.54 ± 0.87a-e	19.20 ± 0.99a-c
IT90K-76	53.90 ± 0.22d-j	7.03 ± 0.69a-h	40.96 ± 0.14c-g	14.28 ± 1.71b-e	18.46 ± 0.59a-e
IT93K-2045-29	60.17 ± 1.50a-i	5.66 ± 0.13k-m	40.62 ± 0.46c-g	13.89 ± 0.69c-e	19.00 ± 0.52a-d
IT93K-452-1	66.73 ± 3.23ab	7.25 ± 0.17a-g	43.62 ± 0.62c-f	15.23 ± 0.65a-e	17.14 ± 1.00b-f
IT94D-437-1	60.90 ± 1.74a-h	7.32 ± 0.33a-f	42.93 ± 0.98c-g	15.32 ± 0.84a-e	21.30 ± 0.52a
IT96D-1951	57.72 ± 2.83a-i	8.11 ± 0.70a	42.22 ± 1.12c-g	14.77 ± 0.81a-e	18.65 ± 1.62a-e
IT97K-499-39	58.91 ± 3.12a-i	6.00 ± 0.13g-m	49.38 ± 3.24b-e	14.37 ± 0.95a-e	17.59 ± 0.77b-f
IT98-46	63.53 ± 7.81a-d	5.95 ± 0.61h-m	49.69 ± 5.77bc	15.82 ± 2.00a-e	19.76 ± 0.81ab
Line 2020	51.65 ± 7.88g-j	5.74 ± 0.81j-m	37.86 ± 4.11e-g	13.90 ± 1.90c-e	16.18 ± 2.73c-f
Mamlaka	50.32 ± 6.50ij	5.33 ± 0.14k-m	34.64 ± 4.12fg	14.64 ± 2.12a-e	17.10 ± 2.03b-f
Mchanganyiko	57.19 ± 1.50a-i	6.18 ± 0.62e-m	43.72 ± 1.34c-f	14.60 ± 0.66a-e	17.91 ± 0.39b-f
Ngonji	57.91 ± 0.91a-i	6.04 ± 0.14f-m	40.95 ± 0.55c-g	14.72 ± 0.28a-e	18.75 ± 0.12a-e
Omondaw	57.80 ± 1.59a-i	7.36 ± 0.57a-e	39.85 ± 1.99e-g	15.77 ± 0.75a-e	15.84 ± 0.94d-f
Pan 311	60.27 ± 2.78a-i	7.49 ± 0.27a-d	46.21 ± 0.95b-e	13.42 ± 0.61de	17.26 ± 1.50b-f
Soronko	66.95 ± 2.25a	6.75 ± 0.25b-j	53.88 ± 4.04b	13.94 ± 0.67c-e	16.27 ± 0.54c-f
TVU11424	61.77 ± 3.07a-f	5.88 ± 0.33h-m	69.15 ± 7.46a	15.59 ± 1.77a-e	18.44 ± 0.14a-e
TVU3236	59.58 ± 1.08a-i	5.59 ± 0.30c-k	49.41 ± 3.05b-e	14.16 ± 0.74b-e	17.78 ± 0.59b-f
Vallenga	56.79 ± 1.18b-i	8.06 ± 0.33a	44.72 ± 0.86b-e	13.00 ± 0.13e-f	16.65 ± 0.75b-f
Vulli-1	58.51 ± 0.22a-i	6.40 ± 0.49d-m	42.76 ± 1.08c-g	14.99 ± 0.40a-e	17.71 ± 0.81b-f
F statistics	1.78	3.67	3.43	2.22	1.79
Significance level	0.01	0.001	0.001	0.001	0.01

Values (Mean ± S.E) followed by dissimilar letters are significantly different at  $P \leq 0.05$ .

relationship was however not so clear between percent N derived from fixation and protein levels in cowpea leaves, as found for the seeds. For example, cowpea genotypes Ngonji (56%), Mamlaka (51%) IT90K-59 (48%), IT93K-452-1 (46%), IT90K-59 (48%), and Mchanganyiko (44%), which derived relatively higher N from fixation at Manga in Ghana (Belane and Dakora, 2009), also produced significantly much greater leaf protein in this study (Figure 3). In contrast, Bechuana white, which obtained the highest N from symbiosis (60%) at Manga, was the fourth lowest in leaf protein production, while Fahari, which derived only 25% of its N from fixation, produced the highest leaf protein (Figure 3). The observed anomaly in the relationship between percent N derived from fixation and leaf protein concentration in cowpea genotypes appears to depend on the traffic and pathways of symbiotic N transported to leaves from  $\text{N}_2$ -fixing bacteroids in

root nodules, and the subsequent incorporation of fixed-N into protein. The variation in seed protein found among the cowpea genotypes tested in this study is consistent with a recent report by Gerrano et al. (2019).

### Trace Element Density in Cowpea Leaves and Seeds

In Africa, about 232 people are suffering from trace element deficiency (Andrea and Rose, 2015), a problem that can be addressed through studies of nodulated legumes that have the ability to accumulate micronutrients in organs. In this study, there were marked differences in the uptake and accumulation of the micronutrients Fe, Zn, Cu, Mn B in leaves of the 32 cowpea genotypes grown in the field at Taung in South Africa (Table 1). Of the five trace elements (Fe, Zn, Cu, Mn, and B)

**TABLE 3** | Correlation among micronutrients in cowpea leaves and seeds.

	Seed Fe	Leaf Zn	Seed Zn	Leaf Mn	Seed Mn	Leaf Cu	Seed Cu	Leaf B	Seed B
Leaf Fe	0.41***	0.55***	0.09	0.25*	0.22*	0.1	-0.47	0.02	-0.27
Seed Fe		0.21	0.27*	-0.01	-0.06	0.01	-0.25*	0.51***	-0.39***
Leaf Zn			-0.03	0.31**	0.06	0.09	-0.19	0.06	-0.19
Seed Zn				-0.39***	0.00	0.26*	0.33**	0.13	0.31**
Leaf Mn					0.37***	0.01	-0.7***	-0.26*	-0.27*
Seed Mn						0.06	-0.27*	-0.19	-0.06
Leaf Cu							0.05	0.03	0.18
Seed Cu								0.11	0.35**
Leaf B									-0.21

\*, \*\*, and \*\*\* denote statistical significance at 0.05, 0.01 and 0.001 levels.

**TABLE 4** | Amount of edible cowpea leaf to consume to meet the recommended daily dietary intake of Fe and Zn.

Genotype	Leaf DM	Recommended intake	Fe and Zn level in cowpea leaf	Amount of leaf DM to eat to meet daily intake of micronutrient
	mg.leaf <sup>-1</sup>	mg.day <sup>-1</sup>	%	μg.mg <sup>-1</sup> DM
<b>Fe</b>				
Fahari	28.4	8	0.2005	8.0 μg per 4 mg leaf DM
Apagbaala	30.9	8	0.2011	8.0 μg per 4 mg leaf DM
Iron gray	24.7	8	0.1302	8.1 μg per 6.2 mg leaf DM
Bechuana white	27.2	8	0.0279	8.0 μg per 30 mg leaf DM
<b>Zn</b>				
Apagbaala	30.9	11	0.0150	11.4 μg per 76 mg leaf DM
Line 2020	25.9	11	0.0132	11.1 μg per 85 mg leaf DM
Iron Gray	24.7	11	0.0090	11.0 μg per 124 mg leaf DM
Vallenga	19.8	11	0.0037	11.1 μg per 300 mg leaf DM

Calculated using leaf micronutrient concentration and leaf dry matter.

that were measured in cowpea leaves, Fe concentration was highest in genotype Apagbaala (2,011 μg.g<sup>-1</sup>), followed by Fahari (2,004 μg.g<sup>-1</sup>), Iron Gray (1,302 μg.g<sup>-1</sup>), Line 2020 (944 μg.g<sup>-1</sup>), Bensogla (927 μg.g<sup>-1</sup>), Omondaw (605 μg.g<sup>-1</sup>), IT96D-1951 (591 μg.g<sup>-1</sup>), IT93K-452-1 (574 μg.g<sup>-1</sup>), Ngonji (569 μg.g<sup>-1</sup>), and Mchanganyika (566 μg.g<sup>-1</sup>), and lowest in Bechuana white (268 μg.g<sup>-1</sup>). In that order, the 10 cowpea genotypes were 7.51-, 7.48-, 4.86-, 3.52-, 3.46-, 2.26-, 2.21-, 2.14-, 2.12-, and 2.11-fold greater in leaf Fe concentration than Bechuana white, the lowest Fe accumulation (Table 2).

However, the leaf concentrations of Zn, Mn and B in cowpea were much lower than that of Fe, with Zn distribution ranging from 37.2 μg.g<sup>-1</sup> for Vallenga to 150 μg.g<sup>-1</sup> for Apagbaala (Table 1). Nine out of the 32 cowpea genotypes studied showed

a very high concentration of Zn in edible leaves. These included Apagbaala (150.0 μg.g<sup>-1</sup>), Line 2020 (131.8 μg.g<sup>-1</sup>), Iron Gray (89.9 μg.g<sup>-1</sup>), Fahari (80.0 μg.g<sup>-1</sup>), Omondaw (75.3 μg.g<sup>-1</sup>), Bensogla (73.4 μg.g<sup>-1</sup>), Ngonji (63.1 μg.g<sup>-1</sup>), IT90K-59 (62.2 μg.g<sup>-1</sup>), and TVU3236 (58.9 μg.g<sup>-1</sup>), which (in that order) were 4.0-, 3.5-, 2.4-, 2.2-, 2.0-, 2.0-, 1.7-, 1.7-, and 1.6-fold higher in leaf Zn concentration than Vallenga, the genotype with the lowest Zn accumulation. Similarly, leaf Mn concentration was markedly greater in Line 2020 (403 μg.g<sup>-1</sup>), followed by Iron Gray (365 μg.g<sup>-1</sup>), Apagbaala (325 μg.g<sup>-1</sup>), IT93K-421-1 (320 μg.g<sup>-1</sup>), IT94D-437-1 (315 μg.g<sup>-1</sup>), and IT96D-1951 (311 μg.g<sup>-1</sup>); and these were, respectively, 2.5-, 2.2-, 2.0-, 1.9-, 1.9-, and 1.9-fold higher than genotype IT86D-2075, which recorded the least Mn in leaves (165 μg.g<sup>-1</sup>). Boron concentration in leaves was also much greater in Bengpla (50.1 μg.g<sup>-1</sup>), followed by Line 2020 (47.7 μg.g<sup>-1</sup>), Pan 311 (46.9), Glenda (46.3 μg.g<sup>-1</sup>), TVU3236 (45.9 μg.g<sup>-1</sup>), Iron Gray (45.8 μg.g<sup>-1</sup>), IT90K-59 (45.4 μg.g<sup>-1</sup>), IT94D-437-1 (45.0 μg.g<sup>-1</sup>), Omondaw (43.9 μg.g<sup>-1</sup>), and Apagbaala (43.2 μg.g<sup>-1</sup>), and lowest in Bechuana white (31.2 μg.g<sup>-1</sup>). As a result, these genotypes were, respectively, 1.60-, 1.53-, 1.50-, 1.48-, 1.47-, 1.47-, 1.45-, 1.44-, 1.40-, and 1.38-fold higher in B than Bechuana white.

Taken together, the results of this study have shown that the concentrations of Fe and Zn (the two most important trace elements) were highest in the leaves of genotypes Apagbaala, Fahari, Iron gray, Line 2020, Bensogla and Omondaw. Furthermore, Fe, but not Zn, was also higher in the leaves of genotypes IT96D-1951 and IT93K-452-1, while conversely Zn, but not Fe, showed increased distribution in Ngonji, IT90K-59 and TVU3236. Interestingly, the leaves of cowpea genotypes Apagbaala, Line 2020, Iron Gray, IT94D-437-1, and Omondaw were similarly very rich in Mn and B, as found for Fe and Zn. These results agree with the findings of Belane and Dakora (2012), and suggest that, with little effort, breeders can easily identify cowpea genotypes with the ability to accumulate high levels of the micronutrients Fe, Zn, Mn, and B in edible leaves for use by farmers to overcome trace element deficiency in Africa.

In this study, the distribution of micronutrients in cowpea seed also differed markedly among the genotypes studied, with a range of 45.1 to 67.0 μg.g<sup>-1</sup> for Fe, 33.9 to 69.2 μg.g<sup>-1</sup> for Zn, 10.1 to 17.4 μg.g<sup>-1</sup> for Mn, 14.7 to 21.4 μg.g<sup>-1</sup> for B,



and 5.2 to 8.1  $\mu\text{g}\cdot\text{g}^{-1}$  for Cu (Table 2). The strong variation in cowpea micronutrient distribution found in this study is consistent with a report by Gerrano et al. (2019). Furthermore, we found that the cowpea genotypes with higher micronutrient accumulation, recorded much greater concentrations in leaves than seed. In fact, genotypes Apagbaala, Fahari, Iron Gray, and Line 2020, which showed an ability to increase micronutrient density, respectively, exhibited 34.2-, 34.0-, 22.5-, and 18.3-fold higher Fe concentration in leaves than seed, just as the same genotypes (in that order) revealed 3.5-, 2.0-, 2.0-, and 3.5-fold greater Zn distribution in leaves than seed. These results are consistent with the findings of an earlier study which showed that trace element concentration was much greater in cowpea leaves than seed (Belane and Dakora, 2011a). Our data also suggest that the assimilation and translocation of mineral nutrients from leaves to developing ovules to form seed differed among the cowpea genotypes probably as a result of traffic barriers to solute transport. Whatever the case, the observed differences in leaf micronutrient density seems to suggest that, depending on the cowpea genotype, a greater or lesser amount of leaf material must be consumed in order to meet the recommended daily intake of trace elements such as Fe and Zn (Table 3). Thus, a higher concentration of the micronutrients in cowpea leaves generally led to a lower amount (on dry matter basis) of the leaf material needed for consumption in order to meet the daily dietary intake of each trace element, and *vice versa*. Another factor that seems to define the level of mineral accumulation in nodulated legumes is the symbiotic efficiency of  $\text{N}_2$ -fixing bacteria in root nodules. It has been shown that high  $\text{N}_2$ -fixing legumes generally tend to accumulate more nutrient elements in shoots than their low-fixing counterparts (Belane et al., 2014). In this study, genotypes such as Apagbaala, Fahari, Iron Gray, Line 2020, Bengsogla and Omondaw, which accumulated significantly high concentrations of trace elements in leaves and seed, were earlier reported to be high  $\text{N}_2$ -fixers, and to accumulate large amounts of symbiotic N in their biomass (Belane and Dakora, 2009, 2010).

Correlation analysis revealed some physiological relationships between micronutrients in leaves and seeds (Table 3). During organ development, ovules are generally regarded as sinks for nutrients stored in leaves as sources. The significant correlation (Table 3) between leaf Fe and seed Fe ( $r = 0.41^{***}$ ), or leaf Mn and seed Mn ( $r = 0.37^{***}$ ) attest to this source/sink relationship between leaves and seeds (developed ovules) when it comes to nutrient uploading in the phloem and its translocation to ovules that are developing into seeds. It however seems there was co-transport of Zn and Mn from xylem to leaves, just as there was synergy in the translocation of Fe and Mn to seeds. This was

evidenced by the positive correlation between leaf Fe and leaf Zn ( $r = 0.55^{***}$ ), leaf Fe and leaf Mn ( $r = 0.25^*$ ), and/or leaf Mn and seed Mn ( $r = 0.37^{***}$ ). Conversely, seed Fe was negatively correlated with seed Cu and seed B, and leaf Mn with seed Cu, seed B, and leaf B. This inverse relationship implies that when seed Fe was increasing, seed Cu and B were decreasing; just as when leaf Mn was accumulating, seed Cu, seed B, and leaf B were decreasing (Table 3). However, whether these synergies and/or antagonisms can be usefully exploited during cowpea breeding, remains to be seen.

The recommended daily dietary intake of the micronutrients Fe and Zn is 8 and 11  $\text{mg}\cdot\text{day}^{-1}$ , respectively (Ross et al., 2011). Assuming cowpea leaves to be the sole source of dietary Fe and Zn (on dry weight basis), the estimated amount to consume in order to meet the daily intake of 8 and 11  $\text{mg}\cdot\text{day}^{-1}$  for Fe and Zn was found to vary with cowpea genotype (Table 4). Smaller leaf material of the genotypes with higher leaf concentration of Fe and Zn was needed to meet the daily intake relative to their counterparts with low levels of Fe and Zn. Coincidentally, however, the genotypes with increased Fe and Zn in leaves were reported to be high  $\text{N}_2$ -fixers in different studies (Belane and Dakora, 2009, 2010; Belane et al., 2011). Clearly, the cowpea/rhizobia symbiosis seems to be a major determinant of leaf and seed protein biosynthesis in cowpea, as well as the accumulation of dietary mineral nutrients in edible parts of this species. Therefore, enhancing these traits in cowpea genotypes through breeding has the potential to overcome protein-calorie malnutrition and trace element deficiency in rural Africa.

## DATA AVAILABILITY

All datasets generated for this study are included in the manuscript/supplementary files.

## AUTHOR CONTRIBUTIONS

FD designed the experiment and wrote the manuscript. AB undertook the field experiments, collected, analyzed the data, and was a doctoral student of FD.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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