

Article

Genotype-by-Environment Interaction for the Contents of Micro-Nutrients and Protein in the Green Pods of Cowpea (*Vigna unguiculata* L. Walp.)

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Abstract: Cowpea (*Vigna unguiculata* L. Walp.) is a drought-tolerant legume crop widely cultivated in arid and semi-arid regions of sub-Saharan Africa (SSA), including South Africa. The leaves, young and immature pods, and grains of cowpea are a vital source of plant-based proteins and essential nutrients for human wellbeing. The objective of this study was to determine the effect of genotype-by-environment interaction (GEI) on the contents of micro-nutrients and protein content of the green pods of cowpea to recommend superior genotypes for cultivation and breeding. Fifteen genetically diverse cowpea genotypes were evaluated across six test environments in South Africa, using a randomized complete block design replicated three times. Micro-nutrients such as iron (Fe), manganese (Mn), zinc (Zn), and total protein (TP) content were determined in the immature pods of cowpea. Data were subjected to additive main effects and multiplicative interaction (AMMI) analysis. Significant ($p < 0.05$) genotype (G) differences were detected for Fe, Mn, Zn and TP, suggesting the presence of genetic divergence for selection. Furthermore, a significant ($p < 0.05$) environment (E) effect was recorded for all studied nutrient, indicating the impact of the test environments on nutrient compositions. The GEI effect was significant for all the assessed nutrients, indicating that specific and broadly adapted genotypes could be identified. Based on best linear unbiased estimates (BLUEs) and best linear unbiased predictors (BLUPs) analyses, the following ranges of nutrient compositions were observed: Fe (83.70–109.03 and 69.77–134.16 mg/kg), Mn (20.60–33.83 and 18.75–36.83 mg/kg), Zn (33.79–40.53 and 28.81 mg/kg), and TP (22.37–24.54 and 21.44–25.25 mg/kg), respectively, across the tested environments. The AMMI test procedure (F_R -test) identified the first interaction principal component axis (IPCA-1) to be a significant ($p < 0.05$) component of the GEI, explaining >91% of phenotypic variation in nutrient contents among the tested genotypes across environments. Cowpea genotypes Meterlong Bean and TVU-14196 were identified for their high Fe, Zn and Mn contents and recommended for cultivation in Mafikeng, Potchefstroom and Roodeplaat in South Africa. For TP, genotypes Meterlong Bean and Kisumu Mix had stable performance and are recommended for production at all the test environments. The identified genotypes are recommended for future cultivation and breeding to supplement micro-nutrients and protein and combat nutrient deficiencies and malnutrition in South Africa.

Keywords: AMMI; BLUEs; BLUPs; cowpea; green pods; nutrient analysis

1. Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.; $2n = 2x = 22$) is widely grown for its green pods, fresh leaves and dry grains [1–3]. Approximately, 820 million people worldwide suffer from hidden hunger due to low intake of essential nutrients [4]. Malnutrition causes various health complications, including an impaired immune system, stunted physical and intellectual growth, and in extreme cases, death [4–6]. Zinc deficiency in the human diet leads to anaemia and immune system disorders [7,8]. Zn deficiency causes stunted growth and abnormal brain development, diarrhoea, and pneumonia [9]. According to [10], 13.8% of children between the ages of 5 and 12 in South Africa suffer from Fe deficiency, whereas 27.7% lack Zn. This is despite the availability of nutritious leguminous crops, such as cowpea, which are economical and vital sources of macro- and micro-nutrients and protein for combating malnutrition.

Higher quantities of micro-nutrients such as potassium (K) (19,743.74 mg/kg), calcium (Ca) (4175.56 mg/kg), phosphorus (P) (4525.56 mg/kg), magnesium (Mg) (3588.18 mg/kg), sodium (Na) (231.76 mg/kg), iron (Fe) (71.43 mg/kg), manganese (Mn) (30.55 mg/kg), boron (B) (26.54 mg/kg), aluminium (Al) (30.78 mg/kg), zinc (Zn) (39.29 mg/kg) and copper (Cu) (6.53 mg/kg) were reported in immature pods of cowpea [11]. Protein content was found to vary between 21 and 40% in immature pods [11]. Therefore, cowpea serves as a vital source of essential nutrients to combat nutritional insecurity and related illnesses. Promotion of cowpea as a food and nutritional security crop and a livestock fodder in SSA requires dedicated breeding efforts to develop and deploy nutrient-enriched cultivars for small- and large-scale production.

Genetic variability exists in cowpea pods for the concentration of macro- and micro-nutrients [12–15], allowing the biofortification of these nutrients. There are considerable breeding efforts to develop cowpea varieties with improved nutritive value. In Africa, the International Institute of Tropical Agriculture (IITA) maintains the largest cowpea germplasm collection, integral in the development and distribution of candidate cowpea genotypes with desirable attributes, including high yield potential and tolerance to drought and diseases for cultivation [16,17]. In South Africa, the Agricultural Research Council (ARC) has benefitted from the IITA germplasm collection, sourcing promising genotypes for production and commercialization [13,17–20]. Additionally, the ARC maintains the largest collection, either newly bred or sourced from other breeding programs, including the IITA. These cowpea accessions have been found to possess optimum concentrations of essential nutrients [18,20] and other desirable agronomic attributes, such as high yield potential and drought tolerance [17,21]; they are suitable for large-scale production in the diverse and water-restricted agro-ecologies of South Africa to combat both food and nutrition insecurities. However, there has been limited studies on the influence of genotype-by-environment interaction (GEI) on micro-nutrient composition in cowpea, which limited the recommendation of suitable genotypes for production in targeted production environments of South Africa.

GEI refers to differential responses of genotypes across the target production environments [22]. This results in variable genotypic performance, making it necessary to identify specifically or widely adapted genotypes for adoption by farmers for small- and large-scale production. To quantify the effects of genotype, environment and GEI, the additive main effects and multiplicative interaction (AMMI) and genotype and genotype-by-environment (GGE) biplot analyses [23,24] are widely used. The AMMI uses ANOVA for additive main effects followed by principal component analysis to explain the multiplicative interaction effects [23–25]. For effective use of AMMI, AMMI model diagnosis has been suggested to identify the most appropriate AMMI model family for accuracy gain and delineate mega-environments (MGE) [23,24,26]. AMMI analysis has been widely used in cowpea, and this allowed for the identification of genotypes with narrow and wide adaption for production in various environments [20,27,28]. Estimates of best linear unbiased estimates (BLUEs) and best linear unbiased predictors (BLUPs) are useful indicators of genotype stability [29,30]. Furthermore, BLUPs analysis provides comparison of genotype mean to

the population mean, revealing superior and poor genotypes [14,29,31]. GEI analysis for nutrient composition in cowpea is crucial for recommending the most suitable genotypes for adoption and, improve the intake of essential nutrients, and combat food and nutrition insecurities. The objective of this study was to determine the influence of GEI on the contents of micro-nutrients and protein content of the green pods of cowpea to recommend superior genotypes for cultivation and breeding.

2. Materials and Methods

2.1. Plant Materials

Fifteen cowpea genotypes were used in the present study (Table 1). The genotypes were obtained from the Agricultural Research Council (ARCP) gene bank collections based in Pretoria.

Table 1. List of cowpea genotypes used in the study (Source: [11,21]).

Entry No.	Genotype Code	Genotype Name	Origin	Grain Yield (t ha ⁻¹)	Growth Habit	Seed Colour	Reference(s)
1	G1	Veg cowpea 1	South Africa	0.43	Semi-upright	Purple	[11,21]
2	G2	Ukaluleni	South Africa	0.42	Prostrate	Brown	[11,21]
3	G3	VCDC	Nigeria	0.42	Upright	White	[11,21]
4	G4	TVU-5431	Nigeria	0.46	Upright	Cream	[11,21]
5	G5	Chappy	South Africa	0.36	Prostrate	Cream	[11,21]
6	G6	Mamlaka	South Africa	0.37	Semi-upright	Brown	[11,21]
7	G7	IT96D-602	Nigeria	0.34	Upright	Cream	[11,21]
8	G8	98K-5301	Nigeria	0.39	Upright	White	[11,21]
9	G9	ITOOK-1060	Nigeria	0.30	Upright	Cream	[11,21]
10	G10	TVU-14196	Nigeria	0.26	Semi-erect	White	[11,21]
11	G11	Veg cowpea 2	South Africa	0.34	Semi-upright	White	[11,21]
12	G12	Meter long bean	South Africa	0.41	Prostrate	Brown	[11,21]
13	G13	Vigna Onb	South Africa	0.57	Prostrate	Purple	[11,21]
14	G14	Kisumu Mix	Kenya	0.42	Prostrate	Brown	[11,21]
15	G15	M217	South Africa	0.34	Upright	Purple	[11,21]

2.2. Study Sites

Field experiments were conducted at Mafikeng (25.8560° S, 25.6403° E, 1369 m above sea level), Potchefstroom (26.7145° S, 27.0970° E, 1349 m above sea level) and Roodeplaat (25.6740° S, 28.3395° E, 1168 m above sea level), in South Africa during the 2016/17 and 2017/18 summer cropping seasons. These provide six test environments, namely: E1 = Mafikeng, 2016/2017; E2 = Mafikeng, 2017/2018; E3 = Potchefstroom, 2016/2017; E4 = Potchefstroom, 2017/2018; E5 = Roodeplaat, 2016/2017; E6 = Roodeplaat, 2017/2018. The description of environmental conditions for the tested locations is presented in Table 2.

Table 2. Description of the locations used in the study.

Location	Geographic Co-Ordinates	Latitude	Soil Type	Soil pH	Annual Precipitation	Average ai Temperature
Mafikeng	25.8560° S, 25.6403° E	1369 m	Sandy	4.5–5.2	443 mm	27.51 °C
Potchefstroom	26.7145° S, 27.0970° E	1349 m	Sandy clay loam	5.6	604 mm	18.12 °C
Roodeplaat	25.6740° S, 28.3395° E	1168 m	Clay loam	5.0–6.2	772 mm	19.93 °C

2.3. Field Trial Design and Cultural Practices

The genotypes were planted in a randomised complete block design (RCBD) with three replications. Planting was carried out during the summer cropping season (October to March) in the two years 2016/2017 and 2017/2018. Each genotype was planted on a 4 m² plot, with 20 cm intra-row and 50 cm inter-row spacing. Two seeds were planted per hole and thinned to one plant two weeks after emergence. Weed, insect pests and diseases were controlled as recommended for the crop using a combination of chemical and cultural practices. Manual weeding was carried out twice prior to flowering. The crops were raised under rainfed conditions, and irrigation was only supplied sparingly at the establishment to avoid wilting.

2.4. Data Collection

Green and immature pods of cowpea genotypes from five randomly selected plants at the middle of the rows of each plot were harvested 40 days after planting. The green pods were oven-dried at 60 °C for 24 h to reduce moisture content to ~12.5%, ground to powder using an electric blender, sieved to pass through a 0.5 mm mesh, and further ground using a pestle and mortar. Flour samples of about 0.5 g for each genotype under each test environment were analysed for Fe, Mn and Zn mineral contents at the Agricultural Research Council (ARC) analytical laboratory in Pretoria, South Africa. Mineral contents were measured using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES 9820, Shimadzu Corporation, Kyoto, Japan) as described by use the reference from the first review. The protein content was determined by the UV-Vis spectrophotometer method adapted from [32]. The nutrients were measured in triplicates and reported in mg/kg.

2.5. Data Analysis

2.5.1. AMMI Model Diagnosis for Studied Nutritional Traits

AMMI model diagnosis was performed using AMMISOFT version 1.0 to select the best AMMI model family for each nutritional trait, and to delineate MGEs [26]. The F_R -test was used to diagnose the best AMMI model family for each nutrient and identify significant interaction principal component axes (IPCAs), according to [33]. The yield ratio for AMMI winning genotypes in each environment was calculated by dividing by the yield of the overall winner [26]. The ratio guides the significance of narrow adaptations due to GEI effects, whereby a ratio of 1 represents a winning genotype across locations and a ratio of more than a unit indicate narrow adaptation [26].

2.5.2. Genotype-by-Environment Interaction Analysis Using AMMI

The AMMI analysis was conducted to deduce the effects due of G, E and GEI [23,24,26] using AMMISOFT Version 1.0 according to the following formula:

$$\bar{Y}_{ijk} = \mu + G_i + E_j + \sum_{k=1}^m \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij} \quad (1)$$

where \bar{Y}_{ijk} = the yield of the i th genotype in the j th environment, μ = the grand mean, G_i = the mean of the i th genotype minus the grand mean, E_j = the mean of the j th environment minus the grand mean, λ_k = the square root of the eigenvalue of the k th IPCA axis, α_{ik} and γ_{jk} = the principal component scores for IPCA axis k of the i th genotypes and the j th environment, and ρ_{ij} = the deviation from the model.

2.5.3. Estimates of BLUEs and BLUPs for Nutritional Traits

BLUEs and BLUPs for the nutrients were computed using Multi Environment Trial Analysis with R (META-R) Version 6.0 [30] using a random-effects model according to [29].

The linear model for combined analysis across environments based on RCBD is as follows:

$$Y_{ijk} = \mu + Env_i + Rep_j (Env)_I + Gen_k + Env_i \times Gen_k + \varepsilon_{ijk} \quad (2)$$

where μ is the overall mean, the terms Env_i and $Env_i \times Gen_k$ are the effects of the i th environment and the environment \times genotype ($G \times E$) interaction, respectively. Rep is the replication effect and ϵ_{ijk} is the residual/error terms. The replications and blocks were treated as fixed factors, whereas genotypes, environment, and interactions were treated as random factors.

2.5.4. Correlations among Environments and Nutritional Traits

Genetic correlations among studied nutrients were estimated using META-R Version 6.0 [30]. Significant genetic correlations between a pair of nutrients indicate that the nutrients can be simultaneously selected during breeding.

3. Results and Discussion

AMMI model diagnosis distinguishes GEI signals causing real narrow adaptations and GEI noise [26]. The F_R test using AMMISOFT diagnosed AMMI-1 model family for all nutrients (Table 3). GEI signal and noise of 58.45 and 41.55%, respectively, were recorded for Fe content. The sum of squares (SS) for GEI-signal was 1.77 times higher than that for G main effects, indicating narrow adaptation for Fe. For manganese content, GEI signal and noise were 50.48 and 49.52%, respectively. Higher SS were recorded for GEI-signal, which was 0.67 times higher than that for the G-signal. For zinc content, 73.29% GEI signal and 26.71% noise were detected. High SS, attributable to the GEI effects, was observed; 2.17 times higher than SS due to G effects. AMMI model diagnosis revealed 29.71 and 70.29% of the GEI-signal and noise, respectively, for protein content. The SS for GEI-signal of 0.53 times higher than G-signal indicated the importance of narrow adaptations and location-specific selection for protein content in the tested cowpea genotypes. The high GEI SS observed in the present study indicates that nutrient content in the evaluated cowpea genotypes was influenced by the test environment. This necessitates the recommendation of well-suited cowpea genotypes based on specific adaptation.

Table 3. Additive main effects and multiplicative interaction analysis of variance for nutrient content of 15 cowpea genotypes evaluated in six environments of South Africa.

Source of Variation	df	Iron			Manganese			Zinc			Total Protein Content		
		F_R -Test MS	TV † (%)	GEI Ex-plained	F_R -Test MS	TV (%)	GEI Ex-plained	F_R -Test MS	TV (%)	GEI Ex-plained	F_R -Test MS	TV (%)	GEI Ex-plained
Total Treatment	269	3230.18			175.01			350.14			9.17		
Genotype (G)	89	7497.26 **			442.18 **			949.54 **			18.01 **		
Environment (E)	14	3866.65 **	6.41		300.11 **	9.15		288.80 **	12.42		19.23 **	10.92	
GxE	5	89,788.94 **	51.67		5898.67 **	62.65		13,696.41 **	21.02		171.30 **	34.73	
Error	70	2345.40 **	18.71		80.85 **	11.80		171.20 **	36.82		6.82 *	19.36	
Blocks/E	180	1120.34			42.91			53.77			4.79		
Pure Error	12	3161.69 **			83.14*			199.42 **			5.61 ns		
IPCA1	168	974.53			40.04			45.73			4.74		
Residual	18	5529.58 **	97.14		168.13 **	95.52		480.16 **	98.96		13.93 *	91.44	
	10	292.92 ns			15.97 ns			9.10 ns			2.34 ns		

† TV, total variance; GEI, genotype-by-environment interaction; IPCA1, interaction principal component axis 1; MS, mean squares; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ns, non-significant.

The AMMI analysis of variance, showing mean squares, an explanation of percentage variation and significance tests of IPCA1 for nutrient content among cowpea genotypes tested across six environments, is presented in Table 3. There was a low genotype effect for Fe (6.41%), Mn (9.15%), Zn (12.42%) and TP (10.92%), suggesting the presence of low genetic diversity and heritable variation for the assessed nutrients among the studied cowpea genotypes. These represent a limitation regarding the genetic improvement of these nutritional attributes using the current cowpea germplasm. The environment (E) effect was significant ($p \leq 0.01$), explaining >50% of the variation for Fe and Mn. For Zn and TP, E effect was low (i.e., 21.02 and 34.73%, respectively), indicating that most variation for this trait was due to the genotype effect. In some instances, the high E effect

suggests that nutrient composition is mainly affected by environmental conditions. The environmental effect is reported to account for a large proportion of total variation in nutritional composition in multi-environment studies [27,34]. GEI effects were significant ($p < 0.05$) and accounted for the lowest variation ($\leq 37\%$) for Fe, Mn, Zn and TP. The low GEI effect implied that the assessed nutritional traits were not largely influenced by the genotype by environment interaction, suggesting that the mean genotype performance was consistent irrespective of test conditions. IPCA1 explained more than 91% GEI affects for the assessed nutrients, indicating the best model fit for GEI analysis. Furthermore, the significant GEI implied the importance of selection and recommendation of suitable genotypes for specific environments selected in the present study.

AMMI model family showing best-performing cowpea genotypes in the test environments of South Africa for studied nutritional traits is shown in Tables 4 and 5. Winning cowpea genotypes and MGEs identified by different AMMI model families (i.e., AMMI 0, 1, 2, 3, 4 and F [full model]) are shown in Table 4. In Table 5, genotypes with superior performance in either one or more environments based on AMMI-1 and AMMI-F model families are displayed. According to [26], AMMI-1 model family is useful for delineating MGE. On the contrary, AMMI-0 captures no GEI signal and noise, whereas AMMI-1 and AMMI-2 discard relatively large GEI-noise and detect large GEI-signal. As a result, AMMI model families 1 and 2 are the most predictive [26]. In the present study, the AMMI-1 model family was the most accurate and predictive. Iron content in the pods ranged between 83.70 and 109.03 mg/kg based on BLUPs among the tested cowpea genotypes (Table 6), which is higher than the range of 60.05–97.78 mg/kg reported elsewhere [11]. The high Fe nutrient status in the present study could be attributed to enhanced genotype potential for Fe accumulation. AMMI-1 identified Meter long bean as a winning genotype in four environments (E6, E2, E4 and E5) for Fe content (Tables 4 and 5). This genotype was suitable for cultivation in diverse production environments (i.e., Mafikeng, Potchefstroom and Roodeplaat) to improve dietary Fe. TVU-14196 was the winning genotype for Fe content in two environments, namely E1 and E3 (Tables 4–6). AMMI-1 delineated two MGEs for Fe content (Table 4) of which MGE 1 consisted of environments E2, E6, E4 and E5, and MGE 2 was comprised of environments E1 and E3 (Table 5). Estimates of BLUEs and BLUPs for 15 cowpea genotypes evaluated across six test environments of South Africa for nutritional traits are presented in Table 6. Fe content for the winning genotypes (i.e., Meter long bean and TVU-14196) in environments E1, E2, E3 and E6 varied between 90.72 and 180.54 based on BLUPs values much higher than that of improved and bio-fortified cultivars BRS Aracê, BRS Xiquexique and BRS (≥ 60 mg/kg) grown in Brazil [13]. Additionally, [5] reported Fe content of 51 mg/kg in cowpea cultivar BRS Xiquexique, which was also lower than Fe recorded in the present study. The identified genotypes are potential candidates as useful genetic resources to develop cowpea varieties with enhanced Fe concentrations.

Table 4. Winning cowpea genotypes for nutritional composition and mega-environment delineation based on additive main effects and multiplicative interaction (AMMI) model families.

Nutritional Traits	Genotype	Mega-Environments	AMMI Model Family					
			0	1	2	3	4	F
Iron	G13						1	1
	G12		6	4	4	4	3	2
	G9							1
	G10			2	2	1	1	1
	G15					1	1	1
	Mega-environments		1	2	2	3	4	5
Manganese	G12		6	6	6	6	5	5
	G10						1	1
	Mega-environments		1	1	1	1	2	2
Zinc	G12		6	4	3	3	1	1

Table 4. Cont.

Nutritional Traits	Genotype	Mega-Environments	AMMI Model Family						
			0	1	2	3	4	F	
	G6							1	
	G15								2
	G9				1	1	1	1	1
	G10			2	1	1	1	2	1
	G5				1	1	1	1	1
		Mega-environments	1	2	4	4	5	5	5
Total protein	G14			3	2	2	2	2	2
			Mega-environments						
Nutritional traits	Genotype	Mega-environments	0	1	2	3	4	F	
	G8				1	1	1	1	
	G12		6	3	3	2	2	2	
	G10					1	1	1	
		Mega-environments	1	2	3	4	4	4	

See genotype (G) codes in Table 1. Only winning genotypes are shown in the table above.

Table 5. Winning cowpea genotypes evaluated across six test environments of South Africa for nutrient composition.

Nutritional traits	Environment (E)	Ratio	AMMI-1 Ranks					AMMI-F Ranks				
			1	2	3	4	5	1	2	3	4	5
Iron	E6	1.00	G12	G13	G8	G5	G4	G13	G12	G8	G4	G3
	E2	1.00	G12	G13	G8	G4	G5	G12	G10	G3	G13	G2
	E4	1.00	G12	G8	G13	G10	G4	G12	G6	G8	G5	G14
	E5	1.00	G12	G10	G15	G4	G9	G9	G5	G12	G14	G10
	E1	1.02	G10	G12	G15	G9	G14	G10	G4	G8	G7	GG5
	E3	1.34	G10	G15	G9	G14	G7	G15	G9	G10	G1	G14
Manganese	E2	1.00	G12	G8	G14	G15	G10	G12	G8	G14	G15	G5
	E1	1.00	G12	G9	G8	G14	G15	G12	G8	G10	G6	G4
	E3	1.00	G12	G9	G8	G14	G15	G12	G14	G15	G10	G7
	E4	1.00	G12	G9	G8	G14	G15	G12	G9	G7	G8	G2
	E6	1.00	G12	G9	G8	G7	G15	G9	G4	G12	G8	G5
	E5	1.00	G12	G9	G1	G7	G10	G12	G9	G15	G8	G13
Zinc	E2	1.00	G12	G3	G8	G15	G6	G12	G3	G8	G15	G6
	E1	1.00	G12	G10	G15	G7	G8	G10	G12	G15	G6	G13
	E3	1.00	G12	G10	G15	G7	G8	G15	G10	G12	G9	G7
	E4	1.00	G12	G10	G15	G7	G8	G9	G12	G7	G6	G8
	E5	1.05	G10	G12	G15	G7	G5	G15	G12	G9	G5	G11
	E6	1.02	G10	G12	G15	G7	G1	G5	G10	G4	G12	G11
Total protein	E2	1.11	G12	G8	G14	G3	G9	G12	G14	G3	G10	G8
	E4	1.05	G12	G14	G8	G3	G9	G12	G8	G14	G7	G3
	E5	1.01	G12	G14	G8	G3	G15	G8	G12	G9	G15	G3
	E6	1.00	G14	G8	G10	G3	G15	G14	G10	G5	G8	G3
	E1	1.00	G14	G10	G6	G8	G15	G10	G15	G6	G14	G8
	E3	1.00	G14	G10	G6	G15	G8	G14	G6	G10	G3	G7

See genotype (G) codes in Table 1.

Table 6. Best linear and unbiased predictions (BLUPs) and best linear and unbiased estimates (BLUEs) of 15 cowpea genotypes evaluated for nutrient content among six environments in South Africa.

Environment E1								
Genotype code	BLUPs_Fe †	BLUEs_Fe	BLUPs_Mn	BLUEs_Mn	BLUPs_Zn	BLUEs_Zn	BLUPs_TP	BLUEs_TP
G1	79.74	77.75	20.29	18.08	29.43	27.47	23.04	23.48
G2	86.27	85.19	23.84	23.69	31.19	30.29	22.58	21.12
G3	71.12	67.95	22.25	21.19	32.34	32.13	22.70	21.74
G4	110.90	113.20	24.49	24.72	29.53	27.64	22.63	21.41
G5	95.61	95.81	23.33	22.88	29.57	27.70	22.67	21.61
G6	88.09	87.26	27.11	28.85	33.62	34.17	23.24	24.52
G7	96.21	96.50	22.82	22.09	33.30	33.66	22.95	23.03
G8	110.15	112.35	27.77	29.89	32.37	32.17	23.06	23.58
G9	86.96	85.97	22.51	21.59	33.12	33.37	22.87	22.60
G10	149.32	156.91	27.12	28.87	37.66	40.62	23.36	25.14
G11	78.00	75.77	23.37	22.95	30.94	29.89	22.77	22.12
G12	90.72	90.24	29.33	32.36	35.68	37.46	22.70	21.75
G13	81.47	79.72	20.53	18.47	33.32	33.69	22.85	22.55
G14	95.02	95.14	23.04	22.43	33.23	33.54	23.21	24.37
G15	92.59	92.38	23.66	23.41	35.24	36.75	23.32	24.93
H ²	0.71		0.37		0.36		0.07	
V _G	399.13		10.88		8.91		0.35	
V _E	164.90		18.89		15.95		4.35	
Grand mean	94.14		24.10		32.70		22.93	
CV	13.64		18.03		12.21		9.10	
LSD	20.14	21.48	5.78	7.27	5.29	6.68	1.54	3.49
G significance	**	**	*	*	*	**	ns	ns
Environment E2								
Genotype code	BLUPs_Fe	BLUEs_Fe	BLUPs_Mn	BLUEs_Mn	BLUPs_Zn	BLUEs_Zn	BLUPs_TP	BLUEs_TP
G1	26.03	10.85	10.59	2.42	10.65	3.51	23.84	22.41
G2	108.43	110.41	30.26	30.01	47.61	47.41	24.67	23.92
G3	112.31	115.10	30.18	29.89	57.71	59.40	26.91	28.03
G4	107.19	108.91	31.49	31.73	51.39	51.90	24.46	23.53
G5	94.84	93.99	31.93	32.34	50.09	50.36	25.18	24.86
G6	89.20	87.18	28.65	27.75	52.84	53.63	24.68	23.94
G7	95.59	94.90	31.12	31.21	50.71	51.10	25.87	26.13
G8	103.99	105.05	38.53	41.60	55.87	57.22	26.09	26.52
G9	95.19	94.42	29.37	28.75	50.82	51.22	25.53	25.50
G10	119.00	123.18	31.77	32.12	52.60	53.34	26.15	26.63
G11	54.32	45.03	22.85	19.62	27.16	23.12	24.89	24.32
G12	180.54	197.55	46.52	52.81	70.91	75.09	28.52	30.98
G13	109.25	111.40	27.50	26.13	44.57	43.80	23.90	22.52
G14	92.45	91.10	37.91	40.73	52.33	53.02	27.47	29.06
G15	95.41	94.68	34.74	36.29	54.89	56.06	25.33	25.13
H ²	0.62		0.45		0.64		0.29	
V _G	1297.08		86.70		225.47		3.16	
V _E	810.47		104.60		126.93		7.88	
Grand Mean	98.92		30.89		48.68		25.57	
CV	28.78		33.11		23.14		10.98	
LSD	43.32	47.61	14.45	17.10	17.29	18.84	3.47	4.70
G significance	**	**	*	**	**	**	ns	ns

Table 6. Cont.

Environment E3								
Genotype code	BLUPs_Fe	BLUEs_Fe	BLUPs_Mn	BLUEs_Mn	BLUPs_Zn	BLUEs_Zn	BLUPs_TP	BLUEs_TP
G1	136.23	139.24	30.38	30.36	42.92	42.74	21.67	21.35
G2	110.99	108.51	31.10	31.76	39.09	37.93	22.47	22.65
G3	131.61	133.62	29.00	27.66	39.03	37.86	22.94	23.43
G4	125.17	125.79	27.75	25.23	37.22	35.59	21.56	21.15
G5	76.66	66.71	26.98	23.73	37.78	36.29	21.35	20.81
G6	121.04	120.75	27.03	23.83	38.62	37.34	23.30	24.01
G7	121.01	120.71	31.26	32.07	46.04	46.65	22.66	22.96
G8	107.23	103.94	28.18	26.07	43.77	43.81	22.00	21.88
G9	140.44	144.38	30.26	30.12	46.63	47.39	21.44	20.96
G10	138.62	142.15	32.78	35.05	52.02	54.15	23.02	23.56
G11	119.95	119.42	28.36	26.42	43.74	43.77	22.43	22.59
G12	129.67	131.26	36.13	41.56	50.87	52.70	19.88	18.41
G13	85.15	77.05	27.35	24.44	40.31	39.47	22.23	22.27
G14	132.78	135.04	35.43	40.20	44.31	44.48	24.09	25.31
G15	158.93	166.88	34.07	37.55	52.24	54.42	21.72	21.42
H ²	0.61		0.26		0.57		0.34	
V _G	540.72		17.95		32.78		1.63	
V _E	353.11		51.16		24.93		3.15	
Grand Mean	122.36		30.40		43.64		22.18	
CV	15.36		23.53		11.44		8.00	
LSD	28.48	31.43	8.57	11.96	7.46	8.35	2.32	2.97
G significance	**	**	ns	*	**	**	*	*
Environment E4								
Genotype code	BLUPs_Fe	BLUEs_Fe	BLUPs_Mn	BLUEs_Mn	BLUPs_Zn	BLUEs_Zn	BLUPs_TP	BLUEs_TP
G1	81.74	78.40	30.26	29.72	43.07	43.38	22.45	22.05
G2	82.20	79.13	31.41	31.31	39.55	38.45	22.54	22.18
G3	77.89	72.30	30.31	29.79	39.07	37.78	23.95	24.41
G4	76.85	70.65	28.12	26.77	37.86	36.09	21.07	19.88
G5	95.90	100.85	29.58	28.78	40.98	40.45	22.95	22.83
G6	108.82	121.34	29.83	29.13	45.52	46.80	22.68	22.40
G7	82.96	80.33	34.53	35.62	46.28	47.87	24.30	24.95
G8	97.29	103.05	31.82	31.88	43.83	44.44	24.53	25.32
G9	79.43	74.73	37.29	39.42	47.38	49.40	23.45	23.61
G10	71.61	62.33	27.72	26.22	40.30	39.50	21.22	20.12
G11	85.72	84.71	28.71	27.58	39.83	38.85	23.39	23.52
G12	122.43	142.91	45.57	50.85	47.05	48.94	24.90	25.90
G13	83.78	81.63	29.71	28.96	40.73	40.11	22.59	22.27
G14	88.02	88.35	29.86	29.17	40.60	39.92	24.34	25.01
G15	77.05	70.97	30.47	30.01	42.45	42.51	23.02	22.94
H ²	0.36		0.47		0.46		0.37	
V _G	291.35		28.60		13.24		2.02	
V _E	511.77		32.60		15.81		3.45	
Grand Mean	87.45		31.69		42.30		23.16	
CV	25.87		18.02		9.40		8.02	
LSD	30.05	37.84	8.13	9.55	5.62	6.65	2.48	3.11
G significance	*	**	**	**	**	**	*	*

Table 6. Cont.

Genotype code	Across environments							
	BLUPs_Fe	BLUEs_Fe	BLUPs_Mn	BLUEs_Mn	BLUPs_Zn	BLUEs_Zn	BLUPs_TP	BLUEs_TP
G1	83.70	69.77	20.63	18.75	33.79	28.81	22.65	22.32
G2	89.97	85.72	24.94	24.65	35.70	33.51	22.37	21.90
G3	92.97	93.34	23.85	23.16	37.17	37.11	23.84	24.18
G4	95.20	98.99	25.44	25.34	36.25	34.86	22.08	21.44
G5	91.20	88.83	24.85	24.53	37.36	37.57	23.06	22.96
G6	92.74	92.74	24.69	24.31	37.43	37.76	23.22	23.22
G7	88.70	82.49	26.23	26.42	38.07	39.33	23.36	23.44
G8	96.62	102.61	27.71	28.44	37.94	39.00	24.05	24.49
G9	91.32	89.14	27.34	27.93	37.18	37.14	23.15	23.10
G10	96.10	101.28	26.29	26.50	38.98	41.55	23.56	23.74
G11	85.87	75.30	22.69	21.58	35.14	32.14	22.83	22.61
G12	109.03	134.16	33.83	36.83	40.53	45.38	23.75	24.04
G13	95.15	98.87	23.14	22.19	36.52	35.51	22.57	22.21
G14	91.00	88.33	27.32	27.90	37.40	37.68	24.54	25.25
G15	91.43	89.42	26.85	27.27	38.64	40.73	23.44	23.56
H ²	0.16		0.47		0.48		0.14	
V _G	84.51		12.18		6.53		0.69	
V _{GE}	456.96		13.60		41.82		0.70	
V _E	974.53		40.04		45.73		4.74	
Grand Mean	92.73		25.72		37.21		23.23	
CV	33.66		24.60		18.17		9.37	
LSD	20.35	32.20	5.17	6.00	5.60	8.70	1.40	1.74
GE Significance	**	**	*	*	*	*	ns	ns

See genotype codes in Table 1. † BLUEs, best linear and unbiased estimates; BLUPs, best linear and unbiased predictions; Fe, iron; Mn, manganese; Zn, zinc; TP, total protein; V_G, genetic variance; V_E environmental variance; V_{GE} genotype-by-environment interaction variance; CV, coefficient of variation; LSD, least significant difference; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ns, non-significant.

Cowpea genotype Meter long bean was the overall winner across all environments for Mn content. A single MGE was delineated for Mn content (Tables 4 and 5). The Meter long bean recorded high values of Mn of >45 mg/kg in E2 and E4, in that order, based on BLUPs values (Table 6). This genotype is suitable for cultivation across the diverse production environments of South Africa. However, Mn content of 71.43 mg/kg previously reported by [19] for Meterlong Bean indicates limited breeding gains for genetic enhancement of Mn content using this genotype. Regarding Zn content, two MGE were delineated based on AMMI-1, whereas four MGE were delineated by AMMI-2 and AMMI-3, and five MGE by AMMI-4 (Table 4). The genotype Meter long bean won in four environments (E2, E1, E3 and E4), whereas TVU-14196 won in two environments (E5 and E6) for Zn content (Tables 4 and 5). High BLUPs values of >50 mg/kg were recorded for Zn content under E2 (70.91 mg/kg), E3 (50.81 mg/kg) and E6 (53.10 mg/kg), based on BLUPs (Table 6). Zn content ranging from 23.9 to 30.1 mg kg⁻¹ was reported in cowpea [35]. Additionally, Meter long bean can be used as breeding parent in improvement programs for high Zn content. ITOOK-1060 and Chappy were winner genotypes based on AMMI-2 and AMMI-3 (Table 4).

Total protein ranged between 22.37 and 24.54% based on BLUPs values across studied environments (Table 6). Comparable to the findings of the present study, protein content ranging between 20.65 and 28.54% was reported in the green pods of cowpea by [19]. Kisimu Mix was a winner genotype for TP in three environments, including E6, E1 and E3 based on AMMI-1 (Tables 4 and 5). Additionally, Meter long bean was a winner genotype in three environments (E2, E4 and E5) for TP according to AMMI-1 (Tables 4

and 5). These genotypes were identified as sources of high grain protein content (>27%) and recommended for wide-area cultivation due to their broader adaptation (Tables 4–6). Two MGE were allocated for TP content comprising of E2, E4 and E5, and E6, E1 and E3, respectively (Tables 4 and 5). Ratios above a unit were computed for Fe content in environments such as E1 and E2, E5 and E6 for Zn, and E2, E4 and E5 for TP (Table 5), signifying the importance of narrow adaptation for the accumulation of these nutrients in the specified environments (i.e., Fe, Zn and TP).

The level of association among the studied nutritional traits can enhance the selection and development of cowpea genotypes, combining excellent nutritional attributes for breeding and/or production. Genetic correlation coefficients among nutritional composition traits across test environments are presented in Table 7. High positive and significant correlations were recorded between Fe and both Mn and Zn, suggesting that simultaneous improvement of these nutritional traits is possible. Moreover, positive correlations between Zn and both Mn and TP indicated the possibility to jointly improving their concentrations. On the contrary, the low and poor association between Fe and TP would allow for independent selection and genetic improvement. Overall, correlation analysis suggested that breeding cowpea genotypes with the desired micro-nutrients and protein content simultaneously is achievable.

Table 7. Genetic correlations among nutritional composition traits evaluated in 15 cowpea genotypes across test environments.

Traits	Fe †	Mn	Zn	TP
Fe	-			
Mn	1.00 **	-		
Zn	1.00 **	1.00 **	-	
TP	0.32 ^{ns}	0.58 *	0.84 **	-

† Fe, iron; Mn, manganese; Zn, zinc; TP, total protein; *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$. ^{ns}, non-significant.

4. Conclusions

The present study determined the genotype-by-environment interaction effect on cowpea nutritional composition to identify and recommend suitable genotypes for adoption and cultivation, as well development of a new breeding population, in South Africa and similar agro-ecologies. The nutritional composition was significantly influenced by the genotype-by-environment interaction effect. BLUPs values for iron, manganese, zinc and total protein were in the ranges 83.70–109.03 mg/kg, 20.60–33.83 mg/kg, 33.79–40.53 mg/kg, and 22.37–24.54 mg/kg, respectively, across the studied environments. Genotype Meterlong Bean was selected with suitable iron, manganese and TP, and recommended for production in Mafikeng, Potchefstroom and Roodeplaat environments. Genotypes TVU-14196 and Kisumu Mix were selected with high iron, zinc and TP, and recommended for Mafikeng and Potchefstroom environments. The selected genotypes are recommended for South Africa or related agro-ecologies. Furthermore, these genotypes could provide novel genes to benefit subsequent improvement and cultivar development programs for increased cowpea nutrient composition.

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References

- Awurum, A.N.; Enyiukwu, D.N. Evaluation of the seed dressing potential of phytochemicals from *Carica papaya* and *Piper guineense* on the germination of cowpea (*Vigna unguiculata* L. Walp) seeds and incidence of the seed-borne fungi. *J. Agric. Sci.* **2013**, *7*, 29–35.
- Okello, J.J.; Hutchinson, M.J.; Mwang'ombe, A.; Ambuko, J.; Olubayo, F.; Mwakangalu, M. Consumer demand for value-added products of African indigenous vegetables in coastal Kenya: The case of sun-dried and frozen cowpea leaves. *J. Agric. Food Syst. Community Dev.* **2015**, *6*, 189–207. [[CrossRef](#)]
- Owade, J.O.; Abong, G.; Okoth, M.; Mwangombe, A.W. A review of the contribution of cowpea leaves to food and nutrition security in east Africa. *Food Sci. Nutr.* **2020**, *8*, 36–47. [[CrossRef](#)] [[PubMed](#)]
- FAOSTAT. Food and Agriculture Data. Available online: <http://www.fao.org/faostat/en/#home> (accessed on 22 November 2021).
- Pereira, E.J.; Carvalho, L.M.J.; Dellamora-Ortiz, G.M.; Cardoso, F.S.N.; Carvalho, J.L.V.; Viana, D.S.; Freitas, S.C.; Rocha, M.M. Effects of cooking methods on the iron and zinc contents in cowpea (*Vigna unguiculata*) to combat nutritional deficiencies in Brazil. *Food Nutr. Res.* **2014**, *58*, 20694. [[CrossRef](#)]
- Rana, N.; Bansal, R.; Sharma, S.; Sharma, Y.; Sonah, H.; Deshmukh, R.; Sharma, T.R. Global perspectives on agriculture: Food security and nutrition. In *Advances in Agri-Food Biotechnology*; Springer: Berlin/Heidelberg, Germany, 2020; pp. 1–27. [[CrossRef](#)]
- Bailey, R.L.; West, J.K.P.; Black, R.E. The Epidemiology of global micronutrient deficiencies. *Ann. Nutr. Metab.* **2015**, *66*, 22–33. [[CrossRef](#)]
- Platel, K.; Krinivasan, S. Bioavailability of micronutrients from plant foods: An update. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1608–1619. [[CrossRef](#)]
- White, P.J.; Broadley, M.R. Physiological limits to zinc biofortification of edible crops. *Front. Plant Sci.* **2011**, *2*, 8. [[CrossRef](#)]
- Visser, M.; Van Zyl, T.; Hanekom, S.M.; Baumgartner, J.; van der Hoeven, M.; Taljaard-Krugell, C.; Smuts, C.M.; Faber, M. Nutrient patterns and their relation to anemia and iron status in 5- to 12-y-old children in South Africa. *Nutr. J.* **2019**, *62*, 194–200. [[CrossRef](#)]
- Gerrano, A.S.; Jansen van Rensburg, W.; Adebola, P. Nutritional composition of immature pods in selected cowpea [*Vigna unguiculata* (L.) Walp.] Genotypes in South Africa. *Aust. J. Crop Sci.* **2017**, *11*, 134–141. [[CrossRef](#)]
- Teka, T.A.; Retta, N.; Bultosa, G.; Admassu, H.; Astatkie, T. Protein fractions, in vitro protein digestibility and amino acid composition of select cowpea varieties grown in Ethiopia. *Food Biosci.* **2020**, *36*, 100634. [[CrossRef](#)]
- Coelho, R.C.; Barsotti, R.C.F.; Maltez, H.F.; Júnior, C.A.L.; de Sousa Barbosa, H. Expanding information on the bioaccessibility and bioavailability of iron and zinc in biofortified cowpea seeds. *Food Chem.* **2021**, *347*, 129027. [[CrossRef](#)]
- Mbusu, N.W.; Gerrano, A.B.; Lebaka, N.; Amoo, S.; Mofokeng, A.; Labuschagne, M. Variability in the concentration of mineral elements and phytochemical contents of cowpea genotypes for crop improvement. *Acta Agric. Scand. B Soil Plant Sci.* **2021**, *71*, 132–144. [[CrossRef](#)]
- Mohammed, S.B.; Dzidzienyo, D.K.; Yahaya, A.; Umar, L.M.; Ishiyaku, M.F.; Tongoona, P.B.; Gracen, V. High soil phosphorus application significantly increased grain yield, phosphorus content but not zinc content of cowpea grains. *Agronomy* **2021**, *11*, 802. [[CrossRef](#)]
- Togola, A.; Boukar, O.; Servant, A.; Chamarthi, S.; Tamo, M.; Fatokun, C. Identification of sources of resistance in cowpea mini core accessions to *Aphis craccivora* Koch (Homoptera: Aphididae and their biochemical characterization). *Euphytica* **2020**, *216*, 88. [[CrossRef](#)]
- Gerrano, A.S.; Thungo, Z.G.; Mavengahama, S. Phenotypic description of elite cowpea (*Vigna unguiculata* L. Walp) genotypes grown in drought-prone environments using agronomic traits. *Heliyon* **2022**, *8*, e08855. [[CrossRef](#)]
- Owusu, E.Y.; Amegbor, I.K.; Mohammed, H.; Kusi, F.; Atopkle, I.; Sie, E.K.; Ishahku, M.; Zakaria, M.; Iddrisu, S.; Kendey, H.A. Genotype × environment interactions of yield of cowpea (*Vigna unguiculata* (L.) walp) inbred lines in the Guinea and Sudan Savanna ecologies of Ghana. *J. Crop Sci. Biotechnol.* **2020**, *23*, 453–460. [[CrossRef](#)]
- Gerrano, A.S.; Jansen van Rensburg, W.S.; Vente, S.L.; Shargie, N.G.; Amelework, B.A.; Shimelis, H.A.; Labuschagne, M.T. Selection of cowpea genotypes based on grain mineral and total protein content. *Acta Agric. Scand.-B Soil Plant Sci.* **2019**, *69*, 155–166. [[CrossRef](#)]
- Gerrano, A.S.; van Rensburg, W.S.J.; Mathew, I.; Shayanowako, A.I.; Bairu, M.W.; Venter, S.L.; Swart, W.; Mofokeng, A.; Mellem, J.; Labuschagne, M. Genotype and genotype × environment interaction effects on the grain yield performance of cowpea genotypes in dryland farming system in South Africa. *Euphytica* **2020**, *216*, 80. [[CrossRef](#)]
- Gerrano, A.; Adebola, P.; Jansen van Rensburg, W.; Venter, S. Genetic variability and heritability estimates of nutritional composition in the leaves of selected cowpea genotypes [*Vigna unguiculata* (L.) Walp.]. *J. Am. Soc. Hortic. Sci.* **2015**, *50*, 1435–1440. [[CrossRef](#)]
- Yan, W.; Kang, M.S. *GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists, and Agronomists*; CRC Press: Boca Raton, FL, USA, 2003; pp. 63–88.
- Gauch, H.G. Statistical analysis of yield trials by AMMI and GGE. *Crop Sci.* **2006**, *46*, 1488–1500. [[CrossRef](#)]
- Gauch, H.G.; Piepho, H.P.; Annicchiarico, P. Statistical analysis of yield trials by AMMI and GGE: Further considerations. *Crop Sci.* **2008**, *48*, 866–889. [[CrossRef](#)]

25. Yan, W.; Kang, M.S.; Ma, B.; Woods, S.; Cornelius, P.L. GGE biplot vs. AMMI Analysis of genotype-by-environment data. *Crop Sci.* **2007**, *47*, 643–655. [[CrossRef](#)]
26. Gauch, H.G. A Simple protocol for ammi analysis of yield trials. *Crop Sci.* **2013**, *5*, 1860–1869. [[CrossRef](#)]
27. Ddamulira, G.; Santos, C.A.F.; Alanyo, O.M.; Lwanga, C.K. Grain yield and protein content of Brazilian cowpea genotypes under diverse Ugandan environments. *Am. J. Plant Sci.* **2015**, *6*, 2074. [[CrossRef](#)]
28. Iseki, H.; Ikazaki, K.; Batiemo, J.B. Cowpea yield variation in three dominant soil types in the Sudan savanna of west Africa. *Field Crops Res.* **2021**, *261*, 108012. [[CrossRef](#)]
29. Piepho, H.P. Best linear unbiased prediction (BLUP) for regional yield trials: A Comparison to additive main effects and multiplicative interaction (AMMI) analysis. *Theor. Appl. Genet.* **1994**, *89*, 647–654. [[CrossRef](#)]
30. Alvarado, G.; Roodríguez, F.M.; Pacheco, A.; Burgueño, J.; Crossa, J.; Vargas, M.; Pérez-Rodríguez, P.; Lopez-Cruz, M.A. META-R: A Software to analyse data from multi-environment plant breeding trials. *Crop J.* **2020**, *8*, 745–756. [[CrossRef](#)]
31. Henderson, C.R. Best linear unbiased estimation and prediction under a selection model. *Biometrics* **1975**, *31*, 423–439. [[CrossRef](#)]
32. Okoronkwo, N.E.; Mba, K.C.; Nnorom, I.C. Estimation of protein content and amino acid compositions in selected plant samples using UV-Vis spectrophotometric method. *Am. J. Food Sci. Health* **2017**, *3*, 41–46.
33. Cornelius, P.L. Statistical tests and retention of terms in the additive main effects and multiplicative interaction model for cultivar trials. *Crop Sci.* **1993**, *33*, 1186–1193. [[CrossRef](#)]
34. Gauch, H.G., Jr.; Zobel, R.W. Identifying mega-environments and targeting genotypes. *Crop Sci.* **1997**, *37*, 311–326. [[CrossRef](#)]
35. Manzeke, M.G.; Mtambanengwe, F.; Nezomba, H.; Watts, M.J.; Broadley, M.R.; Mapfumo, P. Zinc fertilization increases productivity and grain nutritional quality of cowpea (*Vigna unguiculata* [L.] Walp.) under integrated soil fertility management. *Field Crops Res.* **2017**, *213*, 231–244. [[CrossRef](#)]