African Journal of Rural Development, **Vol. 8 (3): July-September 2023:** ISSN 2415-2838

Protein and Iron composition and stability of selected Cowpea (*Vigna unguiculata* (l.) Walp.) Magic population lines in Uganda

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual legume grown widely in Sub-Sahara Africa (SSA), having several advantages over other legumes, such as high protein content (23-32%), drought resilience, and high nitrogen fixation ability. Cowpea grains, leaves, and immature pods are important sources of plant-based proteins and essential nutrients for human health. Nutrient deficiencies are key public health concerns in SSA, including Uganda where 22.4% of children below five years are malnourished, and 28.5% of women of reproductive age are anemic. This study focuses on cowpea MAGIC (multi-parent advanced generation inter-cross) population developed from eight genetically diverse founder parents selected for a mix of important traits. The study aimed to contribute to the improvement of the nutritional composition of cowpea grain genotypes in Uganda. Seeds of 166 cowpea MAGIC population lines were quantified for protein and iron concentrations using the micro-kjeldahl and thiocyanate colorimetry methods, respectively at the Animal Nutrition laboratory at Makerere University and correlation among the traits examined. Thirty genotypes were selected by index selection method, field grown in three environments and evaluated for influence of genotype by environment interaction on the traits. Data generated from the laboratory were subjected to additive main effects and multiplicative interaction (AMMI), and genotype and genotype-by-environment (GGE) biplot analyses in R software to quantify the effects of genotypes. Estimates of best linear unbiased estimates (BLUEs) and best linear unbiased predictors (BLUPs) were employed to identify superior cowpea lines. The two genotypes MAGIC078 and MAGIC119 that recorded the highest levels of protein and Iron content are recommended for future cultivation and breeding to supplement protein and iron thus to combat nutrient deficiencies and malnutrition plaguing SSA.

Key Words: AMMI, Cowpea grains, Iron, MAGIC population, Protein, Uganda

RÉSUMÉ

Cowpea (*Vigna unguiculata* (L.) Walp) is an annual legume grown widely in Sub-Sahara Africa (SSA), having several advantages over other legumes, such as high protein content (23-32%), drought resilience, and high nitrogen fixation ability. Cowpea's grains, leaves, and immature pods are important sources of plant-based proteins and essential nutrients for human health. Nutrient deficiencies are key public health concerns in SSA, including Uganda

Cite as: Nadege, L., Mbeyagala, E., Amponsah Adjei, e., Ibanda, A., Arfang, B., Edema, R. and Nuwamanya, E.2023 Protein and Iron composition and stability of selected Cowpea (*vigna unguiculata (l.) Walp.*) Magic population lines in Uganda. *African Journal of Rural Development* 8 (3):

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Introduction

Humans require both macro- and micronutrients to carry out their regular physiological processes (Gerrano *et al.,* 2019). The extent of malnutrition and its impact on human health have been studied. Deficits in protein and micronutrients are a contributing factor to malnutrition in children and pregnant women (Bain *et al.,* 2013). Lack of protein causes wasting, stunting, weight loss and lowered immunity to illnesses, which increases health risks (Gegios *et al.,* 2010). Iron is a component of blood hemoglobin which plays a role in the transport of oxygen, and its deficiency can lead to diseases such as anemia (Freitas *et al.,* 2022). Malnutrition is widely prevalent in Sub-Sahara Africa (SSA), especially in Uganda, where 22.4% of children below five years are malnourished, 28.5% of women of reproductive age are anemic (Ministry of Health, 2021). Minerals and protein nutrition derived from plants is the cheapest alternative to circumvent malnutrition. Therefore, improving protein and mineral content in staple crops such as cowpea is paramount in the fight against malnutrition, especially in developing countries.

Cowpea (*Vigna unguiculata* (L.) Walp) is an indigenous, self-pollinating diploid legume belonging to the Fabaceae family. It is mostly grown in SSA (Sindhu *et al.,* 2019), including Uganda. The grain of cowpea is the most important component of the plant for human consumption (Singh, 2015). Cowpea is a significant dietary plant-based protein source for millions of individuals in poor countries. It complements nutritionally deficient cereal and tuber crop staples (Ddungu *et al.,* 2015).

The IITA's gene bank maintains 15,003 cultivars of cowpea from 89 different nations. Amongst this germplasm is the cowpea MAGIC (multi-parent advanced generation inter-cross) population developed from eight founder parents with high genetic diversity, possessing many traits, and thus providing a broad genetic base for trait discovery (Huynh *et al.,* 2017).

The degree of genetic variation for seed protein and iron content in cowpea seeds have been investigated by several researchers. Singh and Jauhar, (2006) evaluated 52 cowpea varieties and reported protein and iron contents ranging from 20% to 26% and 56ppm to 95.8ppm. Ddamulira *et al.* (2015) reported protein levels ranging from

23.9% to 30.9% in cowpea seeds of 29 cowpea genotypes. Dakora and Belane (2019) evaluated 32 cowpea genotypes and reported seed protein and iron levels of up to 40% and seed iron content of up to 67 µg.g^{-1}. Furthermore, Ddamulira *et al.* (2015) evaluated 29 cowpea genotypes in three locations where combined analysis over the locations showed significant genotype, environments and G x E interaction for cowpea grain yield and protein content. The AMMI analysis for the grain yield indicated that G x E interaction effect was highly significant and partitioned in the first principal component axis (IPCA) where the IPCA1 explained 17.6% of the interaction sums implying that interaction of cowpea genotypes within six environments was predicted by the first component of genotypes and environment. The AMMI analysis of protein content showed that the G x E interaction, genotype and environment accounted for 43.9%, 49.6% and 9.5% of the sums of squares, respectively. The effect of each genotype and environment on protein content was illustrated by AMMI (IPCA vs means) where the values closer to the origin of the axis (IPCA1) provided a smaller contribution to the interaction than those that were far away. Gerrano *et al.* (2022) evaluated 15 genetically diverse cowpea genotypes across six test environments for stability of contents of protein and micro nutrients. A significant component of the GEI, the first interaction principal component axis (IPCA-1) explained >91% of the phenotypic diversity in nutrient contents among the genotypes evaluated across environments, according to the AMMI test procedure. For Iron content, GEI signals and noises were measured at 58.45 and 41.55%, respectively. The diagnostic of the AMMI model for protein Content showed that the GEI-signal and noise, respectively, were 29.71 and 70.29%. Estimates of BLUEs and BLUPs revealed two wining genotypes for Iron content in four environments varying between 90.72 and 180.54 based on BLUPs. Based on BLUPs data, the total protein varied between 22.37 and 24.54% across the studied environments.

The cowpea MAGIC population has not yet been evaluated to measure the level and diversity of its seed nutritional content, hence the difficulty in identifying genotypes with high levels of protein and iron in this population. Additionally, the magnitude of genotype by environment interaction for protein and iron has not been assessed thus making selection for superior genotypes for protein and iron difficult. Therefore, the aim of this study was to contribute to the improvement of the nutritional composition of cowpea grain genotypes in Uganda. Specifically, the study identified cowpea genotypes with high concentration of protein and iron in the MAGIC population and determined the influence and magnitude of Genotype by environment interaction on these nutritional components. The study tested the hypothesis that the cowpea MAGIC population encompasses genotypes with high levels of protein and iron content, and these nutritional components are sufficiently stable across environment, warranting a fast breeding progress and variety release processes in Uganda.

Methodology

Protein and iron quantification in the cowpea MAGIC population. Laboratory analysis for both Protein and Iron were carried out at the Animal Nutrition Laboratory at Makerere University. The protein content within the dry cowpea seeds of each of the 166 accessions of the MAGIC population was determined using Micro-Kjeldhal method (AOAC, 2012) in two replicates.

A sample of 50g of dried cowpea seeds from each genotype was ground using a laboratory seed grinder to obtain fine powder, then mixed with five millimeters of digestion solution. Each sample was digested in a digester block with the temperature gradually increasing up to 370 °C ensuring that all the protein nitrogen was completely converted into ammonia in the form of ammonium sulphate. The protein nitrogen was then quantified by distillation and removal of the nitrogen as ammonia in 2% boric acid which created borate ions that was subsequently used during titration with standard sulphuric acid. Nitrogen content was then multiplied by conversion factor of 5.45 to establish the quantity of crude protein present.

Iron quantification was also carried in two replicates using thiocyanate colorimetry(RSC, 2012). This method involved four stages which were; Preparation of Fe3+ standard solutions, Preparation of 1 mol $L-^{1}$ ammonium thiocyanate solution, Preparation of cowpea seeds where 2g of seeds from each sample was transferred to a crucible and heated, reducing the sample entirely to ash. After cooling, the sample ash was crushed to fine powder, 10ml of 1 mol L^{-1} of HCL added and stirred to ensure that all the ash soaked. Then 5ml of distilled water was added and the solution filtered into a 100ml conical flask and used for colorimetric analysis. In colorimetric analysis, 10ml of the filtered solution obtained was transferred into a clean test tube then 10ml of every $Fe3^{+}$ standard solution was transferred into different boiling tubes in ascending sequence of concentration beginning with 2 × 10−5 mol L^{-1} standard. Subsequently 10ml (1 mol L^{-1}) of ammonium thiocyanate solution was placed into small clean containers and 10ml of thiocyanate solution was then poured into each of the iron solutions as fast as possible, mixed and allowed to stand for 15 minutes then the absorbance of each solution was then measured at a wavelength of 490nm using the colorimeter. The absorbance results obtained for $Fe3^{+}$ standard solutions were used to prepare a graph of $Fe3^{+}$ (in mol $L-^{1}$) and absorbance (at 490 nm) then a line was drawn that went through the origin to best fit the data points. Points on the line were identified of best fit which corresponded to the absorbance measured for an iron sample. The concentration of $Fe3^{+}$ in a sample solution was determined by drawing a vertical line to the horizontal axis. This concentration was then used to calculate the mass of iron in the sample.

Data analysis. Data on protein and iron content obtained from the laboratory were subjected to analysis of variance using R software and means were separated using Fisher's projected Least significant difference at 5%. A simple linear model was employed following the formula:

$$Y_{ij} = \mu + Gen_i + Rep_j + \epsilon_{ij}$$

where, Y_{ij} is the concentration of the protein or iron of the i^{th} genotype ($Gen_i)$ in the j^{th} replication ($Rep_j)$, μ is the overall mean, and ϵ_{ij} is the residual error term. Pearson's correlation coefficient was computed to ascertain the degree and direction of the relationship between protein and iron content.

A multi-trait selection index was computed using the formula: $I = b^T x$, where b is the vector of selection index coefficients, *T* represents the transpose of the vector $b,$ and x is the vector of mean standardized trait values per genotype. The index coefficients were derived by solving the equation: $b = G^{-1}w$, where G is the genetic (or phenotypic) variance-covariance matrix weighted by heritabilities, and w is the vector of economic weights (2.0 and 1.0 for protein and iron concentrations, respectively (Falconer and Mackay, 1996). This multi-trait selection index was used to select the 30 highest index genotypes from the MAGIC population to be evaluated in in the multi-environment trial to assess the influence and magnitude of genotype by environment interaction on protein and iron content.

Results and Discussion

Analysis of variance indicated significant (P<0.001) differences in the protein and iron levels of the cowpea MAGIC population lines (Table 1). The data revealed marked differences in seed protein with means ranging from 15.1% to 26.8% and the Iron content ranged from 10.6mg/kg to 62.5mg/kg. The results showed that genotype MAGIC078 had the highest mean Protein content of 26.8% as compared to all the checks. However, four checks outperformed some of the cowpea lines. Narocowpea 2A, Narocowpea 2B, Narocowpea 3 and Narocowpea4 revealed mean protein contents of 23.1% ,20.7% ,25.0% and 22.4%, respectively, surpassing other cowpea lines like MAGIC 266 which registered a mean protein content of 18.3%. Regarding the iron contents, MAGIC119 had the highest mean iron levels of 62.5mg/kg greatly surpassing the four checks namely: Narocowpea 2A, Narocowpea 2B, Narocowpea 3 and Narocowpea 4 who registered iron contents of 45.2mg/kg, 43.0mg/kg, 46.2mg/kg and 35.5mg/kg,respectively (Table 2).

Cowpea protein and iron contents exhibited variability among genotypes likely, due to differences in genetic attributes as well as extraction and determination techniques (Afiukwa *et al.*, 2013). Seed protein and iron content showed a weak negative correlation coefficient of -0.06. This suggests that if both protein and iron content are to be improved simultaneously, advanced breeding techniques such as marker-assisted selection (MAS) or genomic selection will need to be employed in order to break the negative correlation and develop varieties with enhanced levels of both traits.

Table 1. Analysis of Variance for Protein and iron content of one hundred and sixty-six (166) Cowpea MAGIC population lines

Source of variation	DF	MS	
		CP	FE
REP	1	0.003	12.6
GEN	165	7.06***	142.54***
Residuals	165	0.29	0.22

***Significant at 0.001, DF = degrees of freedom, CP= Crude Protein, FE= Iron, MS = mean square

Thirty cowpea lines were selected by index selection and advanced for GEI analysis on their protein and iron contents. MAGIC070 had the highest selection index of 126 corresponding to 24.3% and 58.8% protein and iron contents, respectively. Amongst the selected genotypes were two checks Narocowpea 3 and Narocowpea 2A which recorded selection indices of 113 and 109, respectively (Tables 2 and 3). Index selection method allows for the simultaneous selection of a set of variables, independent of any association between them, by combining data on several agronomic traits with the genetic characteristics of the evaluated population. Rather than selecting for each feature separately, this method enables breeders to concentrate on improving many characteristics simultaneously while taking trade-offs into account (Carneiro *et al.,* 2021)

Table 2. Comparative of performance of (30) cowpea MAGIC lines selected for high Protein and Iron concentration

Genotype	CP	Genotype	FE
MAGIC078	26.8	MAGIC119	62.5
MAGIC149	26.6	MAGIC173	61.5
IT89KD-288	25.5	MAGIC015	60.9
MAGIC001	25.5	MAGIC095	60.2
MAGIC144	25.2	MAGIC070	58.8
MAGIC284	25.2	MAGIC098	58.3
NAROCOWPEA 3	*25*	MAGIC048	57.8
MAGIC301	24.7	MAGIC197	57.5
MAGIC011	24.6	MAGIC096	56.2
MAGIC039	24.6	MAGIC006	55.8
MAGIC085	24.4	*NAROCOWPEA 3*	*46.2*
NAROCOWPEA 2A	*23.1*	*NAROCOWPEA 2A*	*45.2*
NARO COWPEA 4	*22.4*	*NAROCOWPEA 2B*	*43*
NAROCOWPEA 2B	*20.7*	*NARO COWPEA 4*	*35.5*
MAGIC266	18.3	MAGIC279	27.8
MAGIC194	18.2	MAGIC107	27.7
MAGIC220	18.1	MAGIC089	27.4
MAGIC276	17.7	MAGIC243	27.1
MAGIC248	17.6	MAGIC099	25.7
MAGIC104	17.5	MAGIC010	24.6
MAGIC223	17.5	MAGIC293	24.4
MAGIC197	17.3	MAGIC240	21.7
MAGIC293	17.2	MAGIC001	19.9
MAGIC251	15.1	MAGIC149	10.6

Table 3. Comparative of performance of (30) cowpea MAGIC lines selected for high Protein and Iron concentration

S/N	Genotype	Crude Protein (%)	Iron (mg/kg)	Index	S/N	Genotype	Crude Protein (%)	Iron (mg/kg)	Index
1	MAGIC070	24.3	58.8	126	16	NARO-COWPEA2A	23.6	45.2	109
2	MAGIC119	21.8	62.5	124	17	MAGIC024	21.9	48.8	109
3	MAGIC011	24.6	54.4	122	18	MAGIC051	23	46	108
4	MAGIC048	22.6	57.8	121	19	MAGIC301	24.7	42.2	108
5	MAGIC095	21.5	60.2	120	20	MAGIC205	20.3	51	107
6	MAGIC173	20.6	61.4	120	21	MAGIC020	22.5	46	107
7	MAGIC015	20.6	60.9	119	22	MAGIC078	26.8	36.1	106
8	MAGIC098	21.4	58.3	118	23	MAGIC039	24.6	40.6	106
9	MAGIC068	23.2	52.9	116	24	MAGIC126	21.4	47.2	106
10	NAROCOWPEA3	25	46.2	113	25	MAGIC174	21.7	46.5	106
11	MAGIC085	24.4	47.2	113	26	MAGIC328	23.6	42	105
12	MAGIC147	22.3	50.7	112	27	MAGIC049	22.7	44	105
13	IT89KD-288	25.5	42.8	111	28	MAGIC179	21.3	46.5	105
14	MAGIC225	23.1	46.4	109	29	MAGIC060	21.8	45.3	104
15	MAGIC185	23.9	44.4	109	30	MAGIC075	20.8	37.6	99

Conclusion

The current study revealed a broad range of genetic variation amongst the 166 accessions of the cowpea MAGIC population lines that were assessed for seed crude protein and iron concentration. The bulk of cowpea accessions had medium protein and iron content, few of the cowpea population had high protein and high iron content of 25.9% and 13.9%, respectively, based on the grouping used. In order to increase the fraction of high protein and iron cowpea genotypes, more breeding efforts are necessary. In breeding projects for enhanced cowpea varieties based on protein and iron content, the study offers guidance on choosing superior parental genotypes. The genotypes MAGIC078 and MAGIC119 revealed highest levels of crude protein and iron concentrations, respectively. With the focus on improved grain nutrition composition, these genotypes are valuable genetic resources for breeding programs and cultivar release for developing cowpea genotypes that exhibit high protein and iron content.

Acknowledgement

This paper is an output of the First Author’s MSc study at Makerere University, Kampala, Uganda. The Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and the Makerere University Regional Centre for Crop Improvement (MaRCCI) funded the study.

Statement of No-Conflict of Interest

The Authors declare no conflict of interest in the paper.

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