



# **Path Analysis in Cowpea Reveals Protein Accumulation Dependency of Mineral Nutrients Accumulation**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors FCLC and AROM designed the study, wrote the protocol, Authors FCLC and MFM performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## **ABSTRACT**

This work aimed at evaluating the effects of the nutrients on the protein content of cowpea grains. The trial was carried out in completely randomized design, in the Instituto Federal do Ceará (Federal Institute of Ceará), Limoeiro do Norte, Ceará State, Brazil, between October and November 2018. Grains of 10 cowpea cultivars were evaluated about its mineral nutrient phosphorus, potassium, calcium, magnesium, sulfur, chlorine, iron, zinc, copper, manganese, boron, sodium and protein contents. The seeds of the cultivars were obtained from the farmers market in three municipalities of the Ceará State, Brazil, in the crop year 2017. The components of variances within and between families were computed by analysis of variance, and the genetic variance and correlation were therefrom estimated. The statistical analyses of variance, Pearson's correlation and Path analysis were carried out. The protein content broad sense heritability was 60.47%, and the other cowpea traits evaluated had high broad heritability values, ranging from 49.91% (sulfur content) to 99.69%

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(zinc content). No mineral nutrient content presented any genotype correlation with protein content, that is, no gene function is related to mineral nutrients and protein accumulation. Potassium (0.44), chlorine (0.38) and calcium (0.35) presented the higher path coefficients in protein of cowpea accumulation but are still weak indices (<0.50) to be indicated for screening. In screening cowpea cultivars for protein content, potassium and related traits are not the most important but present some degree of dependency with protein accumulation in the grains, resulting from path effects.

*Keywords: Biometrics trials; cowpea protein; path effect; plant nutrition.*

## 1. INTRODUCTION

Protein content is a major trait for breeding programs of cowpea. Path analysis can measure how much plant mineral nutrients enhance protein content in cowpea. Brazil is a major producer of cowpea in the world. Since 2015, Midwest Region afforded the second major region producer in the rank of cowpea production, followed by Northeast [1], due to many efforts in achieving completely mechanized cropping, whereby cropping fields and grain yield has been enlarging mainly in Mato Grosso state [2]. Currently, the decreasing of cowpea productivity in Northeast region is due to inappropriate technological agricultural practices, such as low-density plant population in the field, sowing non-bred seeds, and mainly cropping in nitrogen deficient soils [3,4,5]. But, in North and Northeast regions the seeds used by farmers are mainly the grains the producers save from the previous year production, without regards to the vigor of the seeds that must be substituted by five and five years. In this way, the cultivars can lose their vigor to produce high nutritional contents, such as protein, iron, and zinc etc., that are provided mainly to the poor populations by cowpea [6]. Then, the lacking information about protein content of cultivars grown by family farmers concerning its high probability of the losing vigor and the way these chief nutritional contents interact with others can reduce the efficiency of the plant breeding programs.

The interaction between nutrients reveals deficiencies and toxicity symptoms in many plants. Absorption, distribution, and function of nutrients undergo changes when it plays interactions over other mineral nutrients [7], such as competitive inhibition, potentiating of a nutrient by applying a given nutrient or no effect occurs [8] over protein content expression. One way to assess protein content interactions with mineral nutrients contents is by genetic correlations and path analysis [9]. Several monovalent ions are required for the synthesis of proteins such as rubidium, cesium, ammonium,

sodium, lithium, and among them potassium, which is required in larger quantities than in enzymatic activation [10]. To enzymatic activation, potassium is required at least for 60 different enzymes related to plant growth [8]. Chlorine also participates in the many enzyme activation, such as asparagine synthetase, amylase, and ATPase. It can also occur as in chlorinated organic compounds [11]. In protein synthesis, ribosomal enzymes are stopped at high concentrations of chlorine. This blocks growth and development of the plant, in a way, competitive inhibition can occur between chlorine and phosphorus, chlorine and nitrogen, two essential nutrients for protein synthesis [12].

Concerning the nutritional quality of cowpea, few works were achieved about genetic variability of nutritional components [13,14,6,15], and assessments concerning genetic resources used by farmers about its nutritional components are scarce. Breeding for nutritional quality and biofortification is an efficient way to avoid malnutrition and mineral deficiency instead of artificially supplying programs that have proven to be unsustainable in developing countries due to high operational cost. Cowpea turns these problems easier to solve because it has higher mineral and protein contents, is more adaptive to a range of climates, soils. Cowpea is widely spread in many countries mostly in rural areas, therefore primary nutrients (protein, iron, and zinc) must come from agricultural products [16].

The genetic inheritance of protein content in cowpea appeared to be controlled by recessive genes [14], which can harden the breeding of protein content in cowpea. Otherwise, it was demonstrated some combining specific ability in cowpea [13], denoting the evidence of maternal effect over the protein content of cowpea genotypes. Breeding on a single trait is a point to be considered yet [17], since some pleiotropy and gametic disequilibrium in loci which can affect other traits, they also can reduce their potential of the target trait under screening [18]. Genotypic correlations are mainly due to

pleiotropy, but linked genes can occur some way rarely [19]. Path analysis consists of modeling individual direct and indirect effects to a target variable [20], such as the influence of the mineral nutrients to protein content accumulation in cowpea, whose estimates are obtained by regression equations with previously variables standardization [19]. To assess the relationship between mineral nutrients and its effect on protein content of cowpea cultivars was the aim of this study.

## 2. MATERIALS AND METHODS

The grains of 10 cowpea cultivars were assessed by measuring the contents of crude protein according to [21], phosphorus, potassium, calcium, magnesium, sulfur, chlorine, iron, zinc, copper, manganese, boron and sodium according to [22] with three replications. The cultivars of cowpea namely 'Azulão', 'Branco', 'Corujinha', 'Epace-10', 'Feijão-da-Bahia', 'Paulistinha', 'Pingo-de-ouro', 'Pujante', 'Rabo-de-cobra' and 'Sempre-verde' were obtained from farmers market in the municipalities of Limoeiro do Norte, Tabuleiro do Norte and Morada Nova, Ceará State, Brazil, grown in crop year 2017, carrying out in completely randomized design under the assumption of completely random breeding. The components of variances within and between families were computed by analysis of variance, and the genetic variance and correlation were therefrom estimated, according to the second method reported by [23].

The data obtained from chemical analysis were transformed to the model  $\sqrt[3]{x+1}$  so that all variables approached the normal distribution. Data were analyzed by the software Genes [24]. The parameters evaluated were the variance components: genotypic variance (1), environmental variance (2); genotypic coefficient of variation (3), coefficient b (4), broad sense heritability (5), according to the following equations:

$$s_g^2 = \frac{(GMS-RMS)}{r} \quad (1)$$

$$s_a^2 = \frac{\sqrt{RMS}}{m} \quad (2)$$

$$GCV = \frac{\sqrt{GMS}}{m} \quad (3)$$

$$b = \frac{GCV}{RCV} \quad (4)$$

$$H^2 = \frac{s_a^2 + s_d^2}{s_a^2 + s_d^2 + s_i^2 + s_e^2} \quad (5)$$

Where GMS: genotypic mean square; RMS: residual mean square; r: number of replications; m: mean; GCV: genotypic coefficient of variation; RCV: residual coefficient of variation;  $s_a^2$ : additive variance,  $s_d^2$ : dominant variance,  $s_i^2$ : epistatic variance, and  $s_e^2$ : environmental variance.

Genotypic correlations and path analysis coefficient were estimated aimed at protein content to assess direct, and indirect effects of mineral components. Phenotypic (6), genotypic (7) and environmental (8) correlations were calculated by the following equations:

$$r_f = \frac{TMP_{xy}}{\sqrt{TMS_x TMS_y}} \quad (6)$$

$$r_g = \frac{s_{gxy}}{\sqrt{s_{gx}^2 s_{gy}^2}} \quad (7)$$

$$r_a = \frac{EMP_{xy}}{\sqrt{EMS_x EMS_y}} \quad (8)$$

where TMP: Treatment mean product; EMP: environmental mean product; TMS: treatment mean square; EMS: environmental mean square;  $s_{gx}^2$  and  $s_{gy}^2$ : genotypic covariance estimators between the characters x and y.

Path analysis was achieved with one chain, with protein content being the dependent variable, hypothesizing the mineral nutrients as the explicative variables enhancing the dependent variable, calculated by the equation (9):

$$r_{ix} = P_{ix} + \sum_{j \neq i}^n r_{jP_{ix}} \quad (9)$$

where  $r_{ix}$  the correlation between the pairs of dependent and each the explicative variables;  $P_{ix}$  the direct effect of the i-variable, and  $r_{jP_{ix}}$  the indirect effect of the i-variable by j-variable under the dependent variable. The base equations matrix system to estimate the final effects are calculated by the equation (10), [19]:

$$X'Y = X'X\hat{\beta} \quad (10)$$

## 3. RESULTS

All cultivars presented genetic variability to protein content and mineral nutrients contents, except to chlorine and sulfur (Table 1), proving the ability of the genotypes of cowpea to be screened for improving nutritional quality, and biofortification. The nutrient demand, uptake, and accumulation vary among cultivars even among

plants of the same species, denoting feasibility to plant breeding programs.

When greater than 10%, the genotypic coefficient of variation is profitable for the selection of cultivars. Although less than 10%, the genotype coefficients of variation of protein content, phosphorus, potassium, calcium, sulfur and chlorine contents may be profitable yet. The protein content heritability was high (60.47%), even the coefficient b (0.71) indicating some environmental effect; when greater than 1, coefficient b indicates more contribution of a genetic effect than environmental effect. Besides that, most of the traits had the genotype effect greatest, as it is reinforced by heritability >50%, and coefficient b > 1.

The significant genotypic correlations were low (<0.5), presenting mostly higher genotypic correlation than environmental correlation, with some discrepancy between the signs of phenotypic and genotypic correlations (Table 2). The significant correlations were (P and Mg), (P and Cl), (K and Mg), and (Mg and Fe). Protein content was not correlated with any of the mineral nutrient content. The phosphorus content of cowpea was positively correlated with magnesium content ( $P = .01$ ). However, a negative genetic correlation ( $P = .01$ ) was noted, which suggests the most environmental contribution to nutrient accumulation, as the content of phosphorus in the grain is forwarded

to decrease as the soil bases (Ca and K) [25], because the P-phytate binds such bases, disabling their ionic activity.

Path analysis provided useful information for the selection to improve the protein content, such as that total contribution of potassium, calcium, sulfur, chlorine and iron present bigger total effects on protein content. The influence of phosphorus, manganese, and sodium disfavored protein content of cowpea. Many ions affect the potassium activity but required for protein synthesis. The scores of phenotypic correlations between protein and mineral contents coincide with the scores of the total effects of the respective coefficients of the path analysis, in the way the path analysis specifies the contribution of each mineral nutrient compound (Table 3).

#### 4. DISCUSSION

Variability of protein content is due to differences mainly on the protein storage such as albumins, globulins, prolamins, and glutelins. Plants can change the root architecture to enhance or inhibit the potassium uptake. It happens according to potassium availability in the soil solution. In addition to the dual response, the root cells can present so high or low concentration, respectively: (i) high-affinity operation to low  $K^+$  concentrations ( $K^+/H^+$  symporter enhances potassium uptake); (ii) low affinity when in high

**Table 1. Components of variance: Genotypic Mean Square (GMS), Residual Mean Square (RMS), Genotypic Coefficient of Variation (GCV), coefficient b and broad sense heritability (H<sup>2</sup>) of protein content and chemical components of ten cowpea cultivars**

Traits	GMS		RMS	GCV	Coefficient b	H <sup>2</sup>
PY	3.3455	*	2.1873	4.31	0.71	60.47
P <sup>1</sup>	0.0304	**	0.0003	5.77	5.96	99.07
K	6.8952	**	0.1124	8.88	4.52	98.40
Ca <sup>1</sup>	0.0024	*	0.0010	1.99	0.89	70.36
Mg	0.1233	**	0.0015	10.89	5.31	98.83
S <sup>1</sup>	0.0010	ns	0.0010	1.28	0.58	49.91
Cl <sup>1</sup>	0.0008	ns	0.0006	1.54	0.68	57.99
Fe	93.0673	**	3.0928	17.25	3.17	96.78
Zn	154.3034	**	0.4722	23.16	10.44	99.69
Cu	0.6067	**	0.0557	11.64	1.91	91.60
Mn	16.4740	**	0.2474	18.50	4.71	98.52
B	12.7503	**	0.3839	11.40	3.33	97.08
Na <sup>1</sup>	2.2164	**	0.1055	22.59	2.65	95.46

<sup>1</sup>Data adjusted to the model  $\sqrt[3]{x+1}$ , \* $P = .05$ , \*\* $P < .01$ , ns: not significant at 5% probability by test F; PY: protein content, Protein, and Cl: (%); P, K, Ca, Mg, and S: ( $g\ kg^{-1}$ ); Fe, Zn, Cu, Mn, B, and Na: ( $mg\ kg^{-1}$ ).

**Table 2. Phenotypic ( $r_p$ ), genotypic ( $r_g$ ) and environmental ( $r_a$ ) correlation coefficients between the chemical components of 10 cowpea cultivars**

Traits		P	K	Ca	Mg	S	Cl	Fe	Zn	Cu	Mn	B	Na			
PY	$r_p$	-0.12	0.44	0.35	0.26	0.30	0.38	0.30	0.21	0.10	-0.10	0.28	-0.11			
	$r_g$	-0.18	0.59	0.58	0.35	0.46	0.34	0.38	0.27	0.15	-0.10	0.38	-0.15			
	$r_a$	0.22	-0.15	-0.08	-0.16	0.11	0.43	0.10	0.07	-0.09	-0.33	-0.10	0.02			
P	$r_p$		0.63	0.53	0.01	**	0.79	-0.16	*	-0.66	0.47	-0.43	0.01	-0.53	0.15	
	$r_g$		0.63	0.56	-0.01		0.80	-0.27		-0.89	0.48	-0.43	0.01	-0.54	0.16	
	$r_a$		0.31	0.20	0.16		0.40	0.12		0.24	0.54	-0.02	0.16	0.14	-0.38	
K	$r_p$			0.07	0.35	**	0.93	0.49		-0.11	0.50	0.07	0.14	-0.47	0.26	
	$r_g$			0.08	0.37		0.94	0.66		-0.14	0.50	0.07	0.15	-0.48	0.26	
	$r_a$			0.05	0.49		0.32	-0.09		0.51	0.44	0.03	0.21	0.22	-0.20	
Ca	$r_p$				0.02		-0.31	-0.01		0.38	0.46	-0.02	0.07	0.06	0.26	
	$r_g$				0.02		0.48	-0.69		0.06	0.53	-0.03	0.07	0.06	0.32	
	$r_a$				0.03		0.26	-0.14		0.22	0.10	0.11	0.12	-0.10	-0.17	
Mg	$r_p$						0.30	0.54		-0.31	*	0.65	0.04	0.23	-0.43	0.31
	$r_g$						0.41	-0.40		0.65	0.55	0.04	0.23	-0.44	0.31	
	$r_a$						0.16	-0.09		0.30	0.49	0.21	0.43	0.16	-0.04	
S	$r_p$						0.62	-0.26		-0.18	0.28	0.24	-0.03	0.08		
	$r_g$						1.06	-0.33		-0.35	0.36	0.34	-0.05	0.09		
	$r_a$						0.10	0.41		0.60	0.04	0.05	0.11	-0.14		
Cl	$r_p$							-0.29		-0.57	0.31	0.38	0.54	0.40		
	$r_g$							-0.41		-0.77	0.41	0.57	0.72	0.51		
	$r_a$							0.15		0	-0.17	-0.14	0.12	0		
Fe	$r_p$									0.14	0.60	0.10	0.06	0.45		
	$r_g$									0.14	0.64	0.11	0.06	0.47		
	$r_a$									0.41	-0.09	0.05	-0.09	-0.39		
Zn	$r_p$									0.50	0.41	0.25	0.28			
	$r_g$									0.52	0.29	0.41	0.26			
	$r_a$									0.43	0.34	0.16	-0.54			
Cu	$r_p$										0.29	0.63	0.29			
	$r_g$										0.30	0.68	0.33			
	$r_a$										0.08	-0.19	-0.26			
Mn	$r_p$											0.56	-0.07			
	$r_g$											0.56	-0.07			
	$r_a$											0.53	-0.18			
B	$r_p$												0.21			
	$r_g$												0.22			
	$r_a$												-0.16			

PY: protein content, \* significant at  $P = .05$ . \*\* significant at  $P = .01$ . Data without asterisk mark means non-significant at 5% probability by  $t$ -test

concentrations (channels). Then, transmembrane proteins (transport, channel, and pump) quantity, and protein conformation input genetic variability to cowpea cultivars through minerals accumulations into the grains [10,26]. Found as an ion within cytosol, vacuoles, and vessels, potassium does not constitute any structure in the plant; it works as an osmotic regulator, controlling the stomata

closure, and as a cofactor of chemical reactions of the plant metabolism. Moreover, potassium has high mobility in the plant and provides adequate ionic cell environment [10,25,27]. Therefore, this ion is essential for respiration, photosynthesis, transpiration, loading, and unloading of the phloem, and consequently for filling proteins in cowpea grains.

**Table 3. Path analysis coefficients with direct and indirect effects of explanatory chemical components on protein content from ten cowpea cultivars**

Traits	Direct effects	Indirect effects to protein content through											Total effects	
		P	K	Ca	Mg	S	Cl	Fe	Zn	Cu	Mn	B		Na
P	10.63		5.24	-0.02	-7.94	1.74	-14.22	1.75	-0.69	-0.01	0.65	-1.33	4.10	-0.12
K	8.36	6.66		-0.38	-9.34	-5.19	-2.42	1.85	0.12	-0.25	0.58	-2.25	2.71	0.44
Ca	-5.25	0.04	0.60		-0.20	3.26	-0.25	1.71	-0.03	-0.13	-0.07	-2.22	2.89	0.35
Mg	-10.03	8.42	7.78	-0.11		-3.17	-6.65	2.39	0.07	-0.41	0.52	-2.65	4.11	0.26
S	-10.60	-1.75	4.09	1.61	-3.00		13.21	-0.65	0.45	-0.43	0.04	-0.65	-2.01	0.30
Cl	21.44	-7.05	-0.95	0.06	3.11	-6.53		-1.08	0.50	-0.68	-0.65	-3.41	-4.39	0.38
Fe	3.70	5.02	4.17	-2.42	-6.48	1.88	-6.24		0.22	-0.18	-0.07	-3.89	4.59	0.30
Zn	1.62	-4.55	0.62	0.11	-0.41	-2.92	6.65	0.51		-0.89	-0.50	-2.20	2.15	0.21
Cu	-1.78	0.09	1.18	-0.37	-2.30	-2.54	8.17	0.38	0.81		-0.35	-5.42	2.24	0.10
Mn	-1.22	-5.64	-3.95	-0.31	4.30	0.31	11.47	0.22	0.66	-0.51		-4.86	-0.57	-0.10
B	-8.65	1.64	2.17	-1.35	-3.08	-0.80	8.46	1.66	0.41	-1.12	-0.68		1.61	0.28
Na	7.68	5.67	2.95	-1.97	-5.37	2.78	-12.26	2.21	0.46	-0.52	0.09	-1.81		-0.11

Phosphorus, manganese, and sodium inhibit protein content, then the lower content of these nutrient plant need for keeping homeostasis on, the higher protein content can be achieved. Phosphorus and chlorine correlated genotype negatively ( $P = .05$ ), potassium and magnesium correlated phenotype and genotype positively ( $P = .01$ ). Magnesium and iron correlated phenotype negatively and genotype positively, also assuring the environmental effects on these variables ( $P = .05$ ). The differences in signs of the phenotype and genotype correlations indicate environmental effects. Genetic correlations must assess pleiotropy or some disbalance in the loci of the gametes [18]. Phosphorus accumulation can be used as a competitor indicator of nitrogen acquisition in plants through metabolic models, such as the nitrogen content decreases whilst phosphorus content increases in the grain [28], then protein content also decreases, since protein content is straightly proportional to nitrogen content. Since screening in breeding programs is based on the phenotype, while protein and chemical components networks are rarely considered, in the way many ions are essential for protein synthesis, such as potassium, chlorine [10,12], but phosphorus, manganese, and sodium have contrary effects on protein content.

Some ions may increase in concentration in the cell. This is not the case of potassium enhancing iron uptake and transport [8]. Probably, potassium depolarizes root cell membrane surface helping iron complexes uptake by plants. Many enzymes belonging to pathways related to protein synthesis require potassium and chlorine as cofactors, amounting protein content. Protein soluble content tends to be positive for the potassium/sodium ratio. Also, high protein content may indicate genotypes of high tolerance to salinity [29]. But, not obligately this insight represents a biological function. Knowledge of these pathways open way to biotechnology approaches to enhance these proteins accumulation in cowpea since the most protein-rich genotype of cowpea related to date was from 20 to 30% for Brazilian genotypes [30], 39% reported for the genotype Achusuru in Nigeria [31].

Besides high phosphorus content has no benefits to seedlings, the environmental gain of a program to breed a genotype to reduce phosphorus load is of great interest in agricultural systems [32]. Storing phosphorus in the grain is a very environment dependent function in the

plant. To screen cultivars that are efficient in the use of nutrients such as potassium and chlorine must also be part of a breeding program, so that, quantifying these nutrients can be used instead of identifying cultivars that produce high levels of proteins [8].

The carbon isotope discrimination approach ( $\Delta$ ) identifies alternatively genotypes efficient for the use of water, by quantifying the ration  $^{13}\text{C}/^{12}\text{C}$ . Alternatively, since  $\Delta$  is an expensive methodology that cannot be routinely used, it is possible to quantify the potassium content [33], but it works specifically to each cropped species. Thus, it can be inferred that an efficient plant for the use of water can also be more productive in terms of protein content since potassium enhances it. Potassium may not be considered solely for selection of genotypes for protein production, since this is a very complex character that depends on gene interactions, with maternal effect and some degree of pleiotropy.

Chlorine gives the third higher direct contribution to protein content in cowpea. Through potassium, only chlorine and manganese contents affect negatively the protein content in cowpea. Chlorine presented a high direct effect counteracted by indirect effects of the other nutrients that reduced its ability for protein content accumulation, due to its reducing ability for photosynthesizing, yield, protein biosynthesis, and quality [12]. Phosphorus gives a very good direct effect on protein content, but it is very counteracted by chlorine indirect effect. Some mineral nutrients have a very low total effect such as zinc, and copper.

Protein content in the grains is not affected by the storage of the mineral nutrients. As minerals and proteins storage progressively is achieved, when the need for mineral nutrients has been supplied, the plant prioritizes the production and storage of proteins. Despite the path coefficients of the nutrient content potassium, calcium, magnesium, sulfur, chlorine, iron, zinc, copper and boron pointed some effect on protein content, these are not the main roles, as the highest path coefficient was presented by potassium content (0.44). This information is firstly reported on the scientific literature regarding the storage of mineral nutrients over the plant ability to enhance the protein content and storage in the grains. Some information previously reported the effect of the minerals iron and zinc contents on the productivity traits of grain yield and 100-grain weight of cowpea and

protein content [34]. Differently, protein content was positively correlated with iron content in other studies [9], suggesting these traits may be used in screening for breeding programs of cowpea.

Protein content and its accumulation in the grains are such a complex phenomenon depending on many cell components, such as enzymes, carbohydrates, DNAs, RNAs, ribosomes besides the environmental effect. Only three mineral nutrients presented a total negative effect over protein content (P, Mn, Na). It can be understood as cell working toward plant homeostasis. Protein content is a trait depending on gene interaction, under maternal effect (cytoplasmic factors), additive and non-additive gene effects, some pleiotropy as its correlations with some mineral nutrients as iron [9,14,35]. Mineral nutrients are important traits contributing to protein content in cowpea. Protein content genes of cowpea need yet to be studied, as the interest of cowpea protein production, added such a number of physiological processes relating to protein content.

Path analysis is useful for screening the roles of the mineral nutrients not regarded yet for the biotechnology approaches, as they may be considered for discovering secondary genes to protein content or potential genes silenced by the mineral nutrient roles of cowpea, for instance. Moreover, genetic engineering techniques have the potential to increase protein content of the grains. Fluxomics and metabolomics approaches would help to understand all components of the cell processes and its pathway to protein synthesis and regulation. Also, transcriptomics approaches may demonstrate the functioning of the mRNAs and help to identify the genes involved in protein content and mineral nutrients functioning such as enzyme catalysis. The start signaling transduction pathways to activate genes coding to protein content can be considered in future works concerning metabolic mapping of protein content what would be useful for genotypes selection in cowpea breeding programs.

## 5. CONCLUSION

The protein content of cowpea did not present any genotypic correlation with the mineral nutrients of the grains. Genotype correlations frequently indicate pleiotropy or linkage genes. So, it is necessary to carry out studies to find out such effects. Breeding works concerning

biochemical traits inheritance should be taken because they are very related to cropping traits, such as potassium for water stress tolerance and calcium as a signaling ion in abiotic stress. The low path coefficients of the mineral nutrient contents reveal that its main roles are weakly related to protein content of cowpea. The mineral nutrients phosphorus, manganese and sodium presented negative effect on protein content. It is recommended to construct a selection index including these variables, from a database including a considerable number of cowpea accessions. Knowledge of Fluxomics Approaches is still necessary for supporting metabolic engineering approaches about protein production and regulation of cowpea. In screening cowpea cultivars for protein content, potassium and related traits are not the most important but present some degree of dependency with protein accumulation in the grains, resulting from path effects.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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