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Selection in cowpea genotypes for nutritional traits[1]

Seleção de genótipos de feijão-caupi para caracteres nutricionais

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HIGHLIGHTS:

Cowpea genotypes show variability in nutritional traits.
Cowpea genotypes with high nutrient concentration are indicated for genetic improvement.
Cowpea genotypes have favorable chemical properties and nutritional value for cultivar development.

ABSTRACT: Cowpea is a worldwide consumed legume due to its high nutrient concentrations. Selecting genotypes with high nutrient concentrations can contribute to developing biofortified cultivars. This study aimed to evaluate the nutritional potential and indicate genotypes of *Vigna unguiculata* for genetic improvement based on nutritional traits. Forty-three cowpea genotypes belonging to the Active Germplasm Bank of the Federal University of Ceará were used in the study. The analyses of dry matter, ash, ether extract, proteins, and minerals (phosphorus, potassium, calcium, magnesium, sulfur, sodium, copper, iron, zinc, manganese, and boron) were performed in triplicate. The data were subjected to analysis of variance, means comparison test, genetic parameters, nutritional quality indexes, and the sum of ranks. Genetic variances predominated concerning environmental variation for the nutrient concentrations of cowpea can be transmitted to future generations. The CE-0151, CE-0189, CE-0207, CE-0228, CE-0248, CE-0542, CE-0685, CE-0686, CE-0796, CE-0997, and CE-1002 genotypes are indicated for selection for continuing the biofortified cowpea breeding program.

Key words: *Vigna unguiculata*, biofortification, plant breeding, selection of genotypes

RESUMO: O feijão-caupi é uma leguminosa consumida mundialmente pelo seu alto teor de nutrientes. A seleção de genótipos com alto teores nutricionais podem contribuir para o desenvolvimento de cultivares biofortificadas. Este trabalho teve como objetivo avaliar o potencial nutricional e indicar genótipos de *Vigna unguiculata* para o melhoramento genético com base em caracteres nutricionais. Foram utilizados 43 genótipos de feijão-caupi pertencentes ao Banco Ativo de Germoplasma da Universidade Federal do Ceará. As análises de matéria seca, cinzas, extrato etéreo e proteínas e de minerais (fósforo, potássio, cálcio, magnésio, enxofre, sódio, cobre, ferro, zinco, manganês e boro) foram feitas em triplicatas. Os dados foram submetidos à análise de variância, teste de médias, parâmetros genéticos e aos índices de qualidade nutricional e soma de postos. Existe variação genética entre os genótipos avaliados, podendo ser transmitida para próximas gerações. Os genótipos CE-0151, CE-0189, CE-0207, CE-0228, CE-0248, CE-0542, CE-0685, CE-0686, CE-0796, CE-0997 e CE-1002 são indicados para seleção para continuar o programa de melhoramento de feijão-caupi biofortificado.

Palavras-chave: *Vigna unguiculata*, biofortificação, melhoramento vegetal, seleção de genótipos

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Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a legume species native to Africa and belonging to the family Fabaceae; showing high concentrations of proteins, fats, carbohydrates, minerals, vitamins (Rengadu et al., 2020), and starch, which can be used to produce gels for industrial use (Oyeyinka et al., 2020). This species is one of the most important vegetable protein sources in developing countries and is mainly grown in Western and Central Africa, Latin America, and Southeast Asia (Nardi & Ozcan, 2022).

The varieties of cowpea differ in terms of their morphology and proximate quality, which are fundamental aspects of breeding programs for the species (Gerrano et al., 2022). These variations can be explored to benefit human health by fighting hidden hunger (Silva et al., 2021).

Hidden hunger (lack of nutrients) is a reality in several developing countries, affecting around two billion people worldwide (Loureiro et al., 2018). Cowpea breeding programs are necessary to improve the nutritional grain quality in cowpea cultivars and assist in hunger reduction (Lovato et al., 2018).

Legumes have been consumed in various ways for thousands of years worldwide. These plants contain various nutritional components, such as proteins, minerals, and vitamins essential to human health (Kumar & Pandey, 2020). Therefore, breeding programs aimed at increasing the concentrations of these components can reduce the risk of several diseases.

Biofortification can be performed by conventional breeding methods (Buratto & Moda-Cirino, 2017). Thus, the selection of cowpea genotypes is essential to identify germplasms with high nutritional potential, which can be used in breeding programs to develop biofortified cultivars. In this scenario, genetic parameter estimates are reliable indicators for improving genetic traits through selection (Kumar et al., 2015). Furthermore, selection indexes also assist in efficiently predicting selection gains by establishing a linear combination of several traits, thus enabling efficient simultaneous selection (Cruz, 2016). Therefore, this study aimed to evaluate the nutritional potential and indicate genotypes of *Vigna unguiculata* for genetic improvement based on nutritional traits.

Material and Methods

The experiment was conducted in the Horticulture Sector of the Department of the Agricultural Sciences Center of the Federal University of Ceará (CCA/UFC), Pici Campus (3º44'24.4" S, 38º34'32.0" W, and altitude of 19.5 meters) in Fortaleza, Ceará, Brazil. The experiment was conducted under rainfed conditions. During the experimental period, from February to May 2020, the cumulative rainfall was 1,111.8 mm, whereas the mean temperature was 27.3 ºC.

The experimental design was completely randomized, with 43 treatments (39 genotypes and four commercial cultivars) and three replicates. The treatments consisted of 43 cowpea genotypes (*V. unguiculata*) and four commercial cultivars (BRS Juruá, BRS Tumucumaque, BRS Guariba, and BRS Aracê). BRS Juruá and BRS Tumucumaque are biofortified commercial varieties belonging to the Active Germplasm Bank (BAG) of the Plant Science Department of CCA/UFC, according to Table 1.

Table 1. Common name, origin, class, and subclass of cowpea genotypes

ID bag	Common ID	Class	Subclass
CE-0002	Bengala	colored	manteiga
CE-0024	Cowpea-535	mixed	misturado
CE-0061	_	colored	vinagre
CE-0068	Lampião	colored	rajado
CE-0070	Quarenta dias - 1	colored	mulato
CE-0114	Texas Purple Hull - 49	mixed	misturado
CE-0151	1304	colored	mulato
CE-0155	1571	brown	mulato
CE-0164	2380	white	fradinho
CE-0165	2381	black	preto
CE-0172	4280 (*V. sesquipedales*)	black	preto
CE-0189	Costa Rica V-10	colored	mulato
CE-0199	Coleção Pernambuco V-8	white	fradinho
CE-0205	V-24	white	fradinho
CE-0206	V-33	white	fradinho
CE-0207	V-34	colored	mulato
CE-0228	Guerreiro 105	mixed	misturado
CE-0243	Malhado Preto	colored	corujinha
CE-0244	Tvu 2000	black	preto
CE-0248	Tvu 91	colored	vinagre
CE-0253	TVu191	colored	mulato
CE-0313	Tvu 2000	colored	mulato
CE-0337	Tvu 4538	colored	mulato
CE-0398	TVu 200	colored	mulato
CE-0542	ER-7	white	fradinho
CE-0685	CNCx666-21E	black	preto
CE-0686	CNCx666-26E	black	preto
CE-0688	CNCx666-26E	black	preto
CE-0689	CNCx666-31E	black	preto
CE-0796	CNCx251-60E	colored	mulato
CE-0925	Tvu 4552	white	olho-marrom
CE-0957	MNC-01627D-65-1	white	branco liso
CE-0958	IT 91K-118-2	colored	manteiga
CE-0964	MNC-06-887B-561	white	fradinho
CE-0986	IT 81D-1032	colored	vinagre
CE-0997	IT 81D-1073	colored	mulato
CE-0999	MNC 03-720-C-31	white	fradinho
CE-1002	MNC 01-627F-14-5	white	branca
CE-1007	MNC-01-625D	white	branco liso
CE-0942	BRS Juruá	colored	verde
CE-0978	BRS Tumucumaque	white	branco liso
CE-0934	BRS Guariba	white	branco liso
CE-0943	BRS Aracê	colored	verde

The total experimental area had 214 m², containing ten plants per plot. The soil of the area was classified as Ultisols (United States, 2014), which corresponds to the Argissolo Vermelho Distrófico in the Brazilian soil classification system (EMBRAPA, 2018). The area was conventionally prepared by plowing and harrowing. Fertilization was performed based on soil analysis (Table 2) and considering the crop requirements at sowing with the use of phosphorus (super simple phosphate) and potassium (potassium chloride) and at top dressing (15 days after the emergence) with nitrogen (Urea). The spacing was 1.0 m between rows and 0.50 m between plants in the row. Three seeds were sown per hole, and the plants were thinned to two plants per hole 15 days after sowing.

The crop management practices for weed control consisted of manual hoeing in the seedling emergence period and close to flowering. Insecticides were applied to control pests during plant development (20 and 40 days after emergence, Decis® 25 EC). The pods were collected and threshed during harvest,

Table 2. Chemical attributes of the soil of the experimental area

pH	O.M. (g kg^{-1})	P	K^+	Na^+	H^++Al^{3+}	Al^{3+}	Ca^{2+}	Mg^{2+}	BS	CEC
		(mg dm^{-3})		(cmol$_c$ dm^{-3})						
7.0	4.6	27.89	28.33	0.06	0.61	0.00	1.13	0.53	1.80	2.41

OM - Organic matter; BS - Base saturation; CEC - Cation exchange capacity

depending on the cycle of each genotype (Pessoa et al., 2022), and the seeds were selected for nutrient analysis.

The nutrient analyses were performed by washing the raw, dry grains with distilled water and placing them in a forced-air oven at 60 °C for 48 hours. After this procedure, the grains were ground in an electric coffee grinder to obtain a powder used in the analyses. The powder was stored in hermetically sealed polyethylene bags and kept under refrigeration (4 °C) while it was used in the analyses. The chemical analyses were performed in triplicate.

The analyses of dry matter, ash, ether extract, and protein were performed at the Laboratory of Food Analysis and Animal Nutrition of the Center of Agricultural Sciences of the Federal University of Paraíba, following the recommendations of the Association of Official Analytical Chemists (AOAC, 2022).

Mineral characterization (phosphorus, potassium, calcium, magnesium, sulfur, sodium, copper, iron, zinc, manganese, and boron) was performed at the Laboratory of Soils of Embrapa Agroindústria Tropical, according to the methodologies described by Miyazawa et al. (2009).

The NQI (Nutritional Quality Index) was calculated according to Carvalho et al. (2012) and Pereira (2013), with slight modifications. This index was calculated by considering the arbitrarily determined weights of four for protein, three for the minerals Fe (iron) and Zn (zinc), and two for Ca (calcium) and Mg (magnesium). The arbitrary weight attributed to each component was multiplied by the difference calculated between each value of the respective component and their overall mean, followed by the algebraic sum of each term. The result of this sum was divided by the sum of weights according to the following Eq. 1:

$$NQI = \frac{\left\{\begin{array}{c}\left[4\left(Pb - XPb\right)\right] + \left[3\left(Fe - XFe\right)\right] + \\ \left[3\left(Zn - XZn\right)\right] + \left[2\left(Mg - XMg\right)\right] + \\ \left[2\left(Ca - XCa\right)\right]\end{array}\right\}}{4+3+3+2+2} \quad (1)$$

The sum of ranks index of Mulamba & Mock (1978) was determined by considering all nutrients according to the following expression:

$$Ii = \Sigma_K ar_{ik} \quad (2)$$

where:

Ii - index of the ith genotype.

Σ_{Ka} - sum of the weight assigned to each variable.

r_{ik} - rank of progeny i for trait k. All analyses were performed using the software Genes (Cruz, 2016).

The data were subjected to analysis of variance. The means were grouped by the Scott-Knott clustering algorithm at p ≤ 0.05. The estimates of heritability, genetic variance, and the relationship between genetic and environmental variation coefficients were also calculated.

Results and Discussion

Genetic variability is essential for selecting individuals for pre-improvement and generation advancement (Carvalho et al., 2021). From this perspective, the present study revealed significant differences between genotypes for most traits evaluated, except dry matter (Table 3), indicating variability between genotypes for the grain nutrient concentrations, enabling the increase in micro and macronutrient levels through conventional breeding. Freitas et al. (2022) also reported cowpea variability for iron, zinc, and protein.

The heritability values were high, above 70% for most traits (Table 3). The highest mean values were observed for ash (96.22%), ether extract (93.68%), proteins (98.54%), phosphorus (85.25%), calcium (88.40%), sodium (91.50%), zinc (92.01%), manganese (94.13%), and boron (94.85%). These data will serve to support the choice of genotypes for selection. The heritability coefficient defines the proportion of the selection differential that will be transmitted to the following generation, enabling the selection of more promising genotypes for desired traits (Leite et al., 2015), i.e., the higher this coefficient, the higher the success of selection for a given trait (Marialva et al., 2019).

Knowledge about genetic parameters, such as heritability, is required in breeding programs aimed at obtaining new bean cultivars with high grain nutrient concentrations (Buratto & Moda-Cirino, 2017), as reported in the present study.

Table 3. Summary of the analysis of variance: mean squares, heritability (h^2%), and the relationship between the genetic and environmental coefficients (CVg/CVe) for cowpea nutrient descriptors

S.V.	Traits/Mean squares				
	DM	AH	EE	PB	P
Treatments	0.54^{ns}	1.50**	0.33**	43.76**	0.37 **
h^2 (%)	0.00	96.22	93.68	98.54	85.25
CVg/CVe	0.00	2.61	2.22	5.61	1.38
CV (%)	0.96	7.45	11.89	2.76	6.02
F.V	Traits/Mean squares				
	Ca	Mg	S	Na	Cu
Treatments	0.03**	0.07**	0.04**	0.10**	2.74*
h^2 (%)	88.40	76.58	67.63	91.50	58.00
CVg/CVe	1.59	1.04	0.83	1.89	0.69
CV (%)	11.25	7.57	7.70	20.69	16.79
F.V.	Traits/Mean squares				
	K	Fe	Zn	Mn	B
Treatments	3.17*	436.87**	85.13**	13.46**	23.81**
h^2 (%)	58.32	72.67	92.01	94.13	94.85
CVg/CVe	0.68	0.94	1.95	2.31	2.48
CV (%)	9.04	20.02	5.96	6.40	7.01

*, ** - Significant at p ≤ 0.05 and 0.01 by the F-test, respectively; ^{ns} - Non-significant; DM – Dry matter; AH – Ash, EE – Ether extract; PB – Proteins; P – Phosphorus, Ca – Calcium; Mg – Magnesium; S - Sulfur, Na – Sodium; Cu – Copper; K – Potassium; Fe – Iron; Zn – Zinc; Mn – Manganese; and, B - Boron

The relationship between the coefficients of genetic and environmental variation (CVg/CVe) was higher than one for most traits (Table 2), e.g., ash (2.61), ether extract (2.22), proteins (5.61), phosphorus (1.38), calcium (1.59), magnesium (1.04), sodium (1.89), zinc (1.95), manganese (2.31), and boron (2.48), corroborating the highest heritability values and favoring selection since the highest contribution to the next generation is of genetic origin. The relationship between the coefficients of genetic and environmental variation is used to quantify the genetic variability available in the population when determining its potential for breeding purposes (Araújo et al., 2014).

The Scott-Knott test allowed clustering genotypes into two to 11 distinct groups, varying according to the trait analyzed and showing that the genotypes had varying nutrient concentrations (Table 4). Similar to this study, Melo et al. (2017) reported that the chemical and nutrient compositions of cowpea vary among cultivars, assisting the selection and breeding of accessions with high nutrient concentrations.

The trait with the highest data variation corresponded to proteins, forming 11 different groups with mean values ranging from 17.14 (CE-0248) to 33.88 (CE-0686), showing the highest variability among the studied genotypes. In that regard, genotypes CE-0686 (33.88), CE-0165 (31.30), and CE-1002 (30.78) are recommended for selection for showing the highest protein concentrations (Table 4). Developing cultivars with high protein concentrations is one of the main objectives when breeding vegetable species such as cowpea (Frota et al., 2017).

Seven distinct groups were formed based on the nutrient manganese, with the CE genotypes 0206, 0689, 0313, 0199, 0244, 0686, 0243, 0253, 0997, 0207, 0248, 0685, 0964, 0164, 0070, 0114, and 978 showing the highest mean values (Table 4). CE-0689 also showed the highest ash, phosphorus, and potassium concentrations among these genotypes. The presence of these nutrients in cowpea makes this crop potentially important in the human diet from a nutritional perspective (López-Morales et al., 2020) since genotypes with high concentrations of macro

Table 4. Mean nutrient values in cowpea genotypes

ID bag	AH	EE	PB	P	K	Ca	Mg	S
	(g 100g^{-1})			(g kg^{-1})				
CE-1002	3.64 b	1.10 d	30.78 b	4.12 b	10.90 b	0.51 c	1.64 b	1.51 c
CE-0155	4.02 a	1.20 c	28.02 e	4.03 c	12.88 b	0.50 c	1.82 b	1.54 c
CE-0206	4.07 a	0.95 d	25.64 f	4.00 c	12.43 b	0.66 b	1.90 a	1.38 c
CE-0024	3.35 b	1.02 d	24.71 g	3.56 c	11.66 b	0.55 c	1.63 b	1.49 c
CE-0689	4.11 a	1.02 d	31.36 b	4.68 a	13.31 a	0.48 c	2.23 a	1.65 b
CE-0986	3.27 b	1.01 d	26.25 f	3.70 c	11.63 b	0.35 d	1.56 b	1.34 c
CE-0172	3.83 a	1.07 d	30.27 c	4.26 b	12.83 b	0.53 c	1.79 b	1.63 b
CE-0002	3.96 a	1.93 b	29.84 c	4.10 b	13.31 a	0.55 c	1.73 b	1.50 c
CE-0313	4.36 a	1.17 c	25.57 f	4.24 b	13.59 a	0.63 b	1.94 a	1.64 b
CE-0199	4.01 a	1.07 d	27.00 e	4.26 b	12.09 b	0.63 b	1.99 a	1.48 c
CE-0542	3.84 a	1.25 c	23.21 h	4.12 b	12.23 b	0.65 b	1.78 b	1.32 c
CE-0244	4.03 a	1.24 c	28.89 d	3.81 c	13.69 a	0.62 b	1.94 a	1.69 b
CE-0686	3.73 b	1.21 c	33.88 a	4.76 a	11.80 b	0.60 b	1.92 a	1.70 b
CE-0205	3.65 b	1.30 c	26.20 f	3.85 c	12.50 b	0.43 d	1.87 b	1.53 b
CE-0151	3.62 b	1.22 c	28.64 d	3.94 c	12.36 b	0.41 d	1.58 b	1.69 b
CE-0243	3.89 a	1.22 c	30.03 c	3,73 c	13.11 a	0.66 b	2.06 a	1.65 b
CE-1007	3.63 b	1.24 c	29.47 c	3.73 c	11.19 b	0.46 d	1.76 b	1.50 c
CE-0957	3.83 a	1.23 c	29.00 d	4.37 b	11.77 b	0.49 c	1.76 b	1.50 c
CE-0165	3.78 a	1.16 c	31.30 b	4.52 a	12.59 b	0.37 d	1.62 b	1.86 a
CE-0999	3.47 b	1.30 c	25.74 f	3.78 c	10.68 b	0.46 d	1.64 b	1.51 c
CE-0068	3.64 b	1.20 c	21.90 i	3.82 c	14.07 a	0.49 c	1.85 b	1.47 c
CE-0253	3.66 b	1.38 c	21.89 i	3.81 c	13.05 a	0.55 c	1.98 a	1.51 c
CE-0997	3.26 b	1.69 b	22.08 i	4.53 a	14.84 a	0.45 d	2.05 a	1.56 c
CE-0958	3.26 a	1.69 d	22.08 e	4.51 a	13.49 a	0.47 c	1.85 b	1.57 c
CE-0207	3.98 a	0.88 d	23.01 h	3.69 c	12.36 b	0.57 b	1.89 a	1.57 c
CE-0061	3.50 b	1.00 d	20.69 j	4.05 c	12.57 b	0.57 b	1.87 b	1.49 c
CE-0189	2.01 b	1.30 c	21.04 j	3.43 c	11.19 b	0.64 b	1.74 b	1.57 c
CE-0248	1.92 b	1.39 c	17.14 k	3.74 c	13.22 a	0.52 c	2.03 a	1.47 c
CE-0685	2.52 c	1.12 d	28.18 e	4.87 a	12.72 b	0.44 d	2.06 a	1.61 b
CE-0964	2.34 c	2.12 a	22.88 h	4.16 b	13.40 a	0.60 b	2.21 a	1.86 a
CE-0398	2.29 c	1.92 b	18.97 k	3.48 c	12.72 b	0.57 b	1.81 b	1.60 b
CE-0337	2.56 c	1.80 b	21.30 j	4.09 b	12.59 b	0.49 c	1.77 b	1.49 c
CE-0164	2.32 c	2.24 a	21.80 i	3.90 c	13.81 a	0.66 b	2.14 a	1.82 a
CE-0228	1.83 d	2.00 b	22.01 i	3.47 c	12.40 b	0.87 a	1.80 b	1.47 c
CE-0688	2.68 c	0.96 d	25.88 f	4.63 a	13.39 a	0.48 c	2.05 a	1.57 c
CE-0796	2.49 c	0.98 d	23.94 g	4.19 b	15.10 a	0.45 d	1.69 b	1.58 b
CE-0070	2.31 c	1.03 d	21.76 i	3.87 c	14.40 a	0.61 b	1.97 a	1.45 c
CE-0114	2.64 c	1.06 d	20.56 j	4.10 b	14.43 a	0.79 a	1.96 a	1.54 c
CE-0925	2.46 c	0.89 d	20.79 j	3.77 c	12.29 b	0.52 c	1.78 b	1.68 b
BRS Juruá	3.78 a	1.13 d	22.44 i	4.28 b	14.56 a	0.67 b	1.85 b	1.61 b
BRS Tumucumaque	3.59 b	1.36 c	23.42 h	4.24 b	12.56 b	0.70 b	1.89 a	1.63 b
BRS Guariba	3.26 b	1.12 d	22.06 i	3.84 c	12.20 b	0.58 b	1.86 b	1.60 b
BRS Aracê	3.73 b	1.46 c	23.25 h	4.03 c	13.08 a	0.60 b	1.82 b	1.68 b

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ID bag	Na (g kg^{-1})	Cu	Fe	Zn	Mn	B	NQI	Sum of Ranks
		(mg kg^{-1})						
CE-1002	0.25 d	6.00 b	68.33 b	49.33 b	15.33 c	9.88 b	55.01	5
CE-0155	0.30 d	5.00 b	53.00 b	40.00 c	12.33 d	10.82 b	7.32	34
CE-0206	0.43 c	6.00 b	53. 00 b	40.33 c	14.33 c	8.24 c	5.54	36
CE-0024	0.67 b	5.00 b	57.67 b	40.33 c	13.33 d	7.31 c	11.01	28
CE-0689	0.22 d	5.33 b	60.33 b	43.67 c	13.33 d	5.41 d	23.52	22
CE-0986	0.39 c	4.33 b	49.33 b	36.67 d	11.33 d	5.31 d	-17.98	39
CE-0172	0.42 c	7.33 a	54.67 b	46.33 b	12.00 d	6.23 d	17.79	27
CE-0002	0.42 c	6.67 a	61.67 b	46.67 b	13.67 d	6.01 d	32.84	16
CE-0313	0.41 c	5.00 b	51.67 b	45.00 b	12.67 d	5.11 d	7.73	33
CE-0199	0.43 c	7.67 a	50.33 b	46.00 b	14.67 c	2.46 e	7.29	35
CE-0542	0.54 b	6.00 b	42.00 b	39.33 c	10.67 e	3.13 e	-28.14	41
CE-0244	0.59 b	5.33 b	45.33 b	39.00 c	12.67 d	7.42 c	-13.46	38
CE-0686	0.32 d	5.00 b	66.67 b	45.67 b	17.67 b	10.60 b	48.39	7
CE-0205	0.31 d	5.67 b	50.00 b	35.00 d	10.00 f	7.83 c	21.43	24
CE-0151	0.64 b	7.67 a	58.67 b	51.67 a	13.00 d	12.94 a	46.17	8
CE-0243	0.44 c	6.33 b	52.00 b	46.33 b	11.33 e	5.42 d	10.78	29
CE-1007	0.20 d	6.67 a	46.33 b	46.67 b	15.33 c	11.09 b	7.81	32
CE-0957	0.27 d	7.33 a	59.33 b	53.67 a	15.67 c	7.23 c	43.85	9
CE-0165	0.44 c	6.33 b	56.67 b	51.67 a	13.67 d	9.17 b	36.87	13
CE-0999	0.37 c	7.00 a	43.00 b	44.33 b	12.67 d	11.65 b	-4.33	37
CE-0068	0.85 a	5.67 b	55.67 b	45.00 b	13.67 d	8.60 c	21.87	23
CE-0253	0.15 d	5.33 b	53.00 b	48.67 b	14.67 c	7.63 c	21.32	25
CE-0997	0.65 b	6.33 b	65.00 b	53.00 a	14.33 c	7.06 c	56.44	3
CE-0958	0.46 c	6.33 b	60.33 b	51.00 a	14.33 c	12.33 a	49.18	6
CE-0207	0.78 a	7.00 a	41.67 b	40.00 c	13.00 d	3.31 e	-25.42	40
CE-0061	0.71 b	7.00 a	61.00 b	46.00 b	12.67 d	5.49 d	28.78	19
CE-0189	0.56 b	6.67 a	43.00 b	33.33 d	8.33 g	11.32 b	-28.35	42
CE-0248	0.45 c	8.00 a	36.33 b	34.33 d	13.33 d	8.62 c	-38.14	43
CE-0685	0.18 d	6.00 b	76.67 b	45.67 b	19.33 a	10.7 b	70.78	2
CE-0964	0.36 c	6.33 b	64.67 b	42.67 c	13.00 d	11.61 b	39.79	11
CE-0398	0.91 a	8.00 a	58.67 b	49.00 b	13.33 d	6.85 c	32.73	17
CE-0337	0.69 b	6.00 b	57.00 b	51.33 a	12.67 d	5.47 d	30.68	18
CE-0164	0.38 c	6.00 b	51.33 b	43.33 c	11.67 e	8.07 c	8.24	31
CE-0228	0.45 c	6.00 b	68.33 b	46.67 b	13.67 d	13.40 a	55.97	4
CE-0688	0.40 c	6.00 b	67.00 b	41.67 c	17.00 b	6.05 d	35.89	15
CE-0796	0.43 c	7.00 a	113.33 a	56.33 a	17.00 b	5.56 d	158.35	1
CE-0070	0.62 b	7.00 a	61.00 b	47.67 b	13.33 d	8.48 c	38.68	12
CE-0114	0.41 c	7.00 a	59.00 b	48.00 b	15.33 c	8.03 c	36.02	14
CE-0925	0.63 b	8.33 a	58.00 b	51.33 a	14.67 c	10.23 b	41.46	10
BRS Juruá	0.35 c	5.33 b	52.00 b	47.67 b	15.67 c	10.47 b	24.29	21
BRS Tumucumaque	0.20 d	5.00 b	61.67 b	40.33 c	9.67 f	13.16 a	27.74	20
BRS Guariba	0.25 d	5.33 b	54.67 b	44.00 b	13.00 d	5.23 d	10.07	30
BRS Aracê	0.29 d	5.33 b	53.00 b	48.00 b	15.67 c	6.62 c	19.27	26

AH – Ash; EE – Ether extract; PB – Proteins; P – Phosphorus; Ca – Calcium; Mg – Magnesium. S - Sulfur; Na – Sodium; Cu – Copper; K – Potassium; Fe – Iron; Zn – Zinc; Mn – Manganese; B - Boron. Means followed by the same letter in the column belong to the same group by the Scott-Knott clustering algorithm at $p \leq 0.05$

and micronutrients have the potential to be used against malnutrition, a problem that affects many people.

Five groups were formed only by boron, with genotypes CE-0151, CE-0958, CE-0228, and CE-0978 showing the highest performances. Several cowpea biofortification studies aim to increase zinc and iron concentrations (Melo et al., 2017; Kumar & Dhaliwal, 2021). However, selecting genotypes with high concentrations of various elements (Fe, Zn, Mn, for example) is essential for breeding programs since it will be possible to develop a cultivar rich in several nutrients, including boron.

The ether extract (CE-0964 and CE-0164), calcium (CE-0114), sodium (CE-0068, CE-0207, and CE-0398), and zinc (CE-0151, CE-0957, CE-0165, CE-0997, CE-0958, CE-0337, CE-0796, and CE-0925) showed for different groups, with some genotypes showing the highest mean values (Table 4). Haider et al. (2021) reported variability among genotypes of *Vigna radiata* for the grain Zn concentrations, ranging from 15 to 45 mg kg^{-1}. However, the genotypes of the present study showed higher values, highlighting the potential to initiate a breeding program aimed at biofortification. In addition to zinc, the ether extract, calcium, and sodium also showed variability. Since the environmental conditions were the same for all plants in the present study, the variability observed among the genotypes is inherent to genetic aspects, as observed in Table 3.

Three groups were formed for the ash concentration, with 15 genotypes showing the best performances (CE-0155, CE-0206, CE-0689, CE-0172, CE-0002, CE-0313, CE-0199, CE-0542, CE-0244, CE-0246, CE-0957, CE-0165, CE-0958, CE-0207, and 942). Phosphorus showed the highest concentrations in seven genotypes (CE-0686, CE-0165, CE-0997, CE-0958, CE-0685, and CE-0688). Finally, the highest performances for the sulfur concentration were observed in three genotypes (CE-0165, CE-0964, and CE-0164) (Table 4).

The lowest data variation was observed in the concentrations of potassium, magnesium, copper, and iron (Table 3). Genotype CE-0796 showed a higher iron concentration than the biofortified commercial varieties (BRS Juruá and BRS Tumucumaque) and is indicated for selection. Therefore, selecting genotypes with the highest potassium, magnesium, copper, and iron concentrations is an interesting approach, even if they show slight variation, which can be used in breeding. Cowpea biofortification has been mostly used to increase the concentrations of Fe and Zn, as reported by Guillén-Molina et al. (2021) and Haider et al. (2021).

The sum of ranks of the genotypes based on the studied traits ranged from -38.14 (CE-0248) to 158.35 (CE-0796) for the nutritional quality index (NQI) and from 1 (CE-0796) to 43 (CE-0248) for the Mulamba & Mock index (Table 4). Selection based on these indexes enables the identification of divergent parents with the highest means regarding the traits meant to be improved (Bertini et al., 2010).

The cowpea genotypes CE-0796, CE-0685, CE-0997, CE-0228, and CE-1002 stood out based on the NQI considering nutritional characteristics (Table 4). Due to their high nutrient concentrations, these genotypes are recommended for selection to be used in breeding programs to develop biofortified cultivars. The selection indexes enable the selection of superior genotypes by simultaneously selecting various traits, resulting in more success in selection (Silva et al., 2020). Freitas et al. (2022) also identified the best cowpea genotypes based on their nutrient profile (iron, zinc, and protein concentrations) using the nutritional quality index.

Genotypes CE-0248, CE-0189, CE-0542, and CE-0207 showed the highest nutrient values based on the Mulamba & Mock (1978) index. This method is based on the sum of ranks of the evaluated traits, classifying genotypic materials by order of interest according to their performance (Ramos et al., 2022). The genotypes selected by the two methods (indexes) showed no coincidences among the selected individuals since different methodologies were used, thus increasing the number of genotypes indicated for selection.

Conclusions

1. Genetic variances predominated over environmental variation for the nutrient concentrations of cowpea.

2. The CE-0151, CE-0189, CE-0207, CE-0228, CE-0248, CE-0542, CE-0685, CE-0686, CE-0796, CE-0997, and CE-1002 genotypes are indicated for selection to continue the biofortified cowpea breeding program.

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