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Characterization of cowpea cultivars for grain size, color, and biofortification

Caracterização de cultivares de feijão-caupi para tamanho, cor e biofortificação de grão

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ABSTRACT - Cowpea crops have high socioeconomic importance in the Northeast region of Brazil. These crops generate employment and income; in addition, it is an excellent source of protein, minerals, vitamins, and dietary fiber, and contributes to the food security of thousands of people. Biofortification of cowpea with iron, zinc, and proteins can contribute to prevent the higher hunger. The objective of this work was to assess iron, zinc, and protein contents in cowpea grains of different cultivars and the effect of grain size and color on the contents of these nutrients. Twenty-four cultivars with variation in size and color of the seed coat were evaluated. Iron contents ranged from 7.12 to 8.60 mg 100 g^{-1}, with an overall mean of 7.75 mg 100 g^{-1}. Zinc contents ranged from 4.46 to 4.93 mg 100 g^{-1}, with an overall mean of 4.71 mg 100 g^{-1}. Protein contents ranged from 31.50 to 36.24 g 100 g^{-1}, with an overall mean of 33.57 g 100 g^{-1}. Grain size ranged from 13.55 to 37.88 g, with an overall mean of 21.50 g. The cultivars Caldeirão, BRS-Guariba, and Pingo-de-Ouro-1-2 have higher iron contents; BRS-Aracê and BRS-Imponente have higher zinc contents; BR-14-Mulato, BRS-Guariba, BRS-Aracê, and BRS-Inhuma, have higher protein contents; and BR-3-Tracuateua and BRS-Imponente have larger grain sizes. The size and color of the seed coat do not influence the iron, zinc and protein contents of the evaluated cowpea cultivars.

Keywords: *Vigna unguiculata*. Iron. Zinc. Protein. Grain weight. Grain color.

RESUMO - O feijão-caupi é uma cultura de grande importância socioeconômica na região Nordeste do Brasil. Além de ser um gerador de emprego e renda, é uma excelente fonte de proteínas, minerais, vitaminas e fibras alimentares, contribuindo para a segurança alimentar de milhares de pessoas. A biofortificação do grão de feijão-caupi com ferro, zinco e proteínas pode contribuir na prevenção da fome oculta. Este trabalho teve como objetivo caracterizar os teores de ferro, zinco e proteínas nos grãos de cultivares de feijão-caupi e verificar a influência da cor e tamanho do grão nos teores desses nutrientes. Foram avaliadas 24 cultivares com variação no tamanho e cor do grão. O teor de ferro variou de 7,12 a 8,60 mg 100g^{-1} e média geral de 7,75 mg 100g^{-1}. O teor de zinco apresentou variação de 4,46 a 4,93 mg 100 g^{-1} e média geral de 4,71 mg 100 g^{-1}. O teor de proteínas variou de 31,50 a 36,24 g 100g^{-1}, com média geral de 33,57 107 g 100g^{-1}. O tamanho do grão variou de 13,55 a 37,88 g e apresentou média geral de 21,50 g. As cultivares Caldeirão, BRS Guariba e Pingo de Ouro 1-2 apresentam maior teor de ferro; BRS Aracê e BRS Imponente, maior teor de zinco; BR 14-Mulato, BRS Guariba, BRS Aracê e BRS-Inhuma, maior teor de proteínas; e BR 3-Tracuateua e BRS Imponente, maior tamanho de grão. O tamanho e a cor do tegumento grão não influenciam nos teores de ferro, zinco e proteínas das cultivares de feijão-caupi avaliadas.

Palavras-chave: *Vigna unguiculata*. Ferro. Zinco. Proteína. Massa de grão. Cor do grão.

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Cowpea (*Vigna unguiculata* L. Walp.) is known in Brazil as feijão-fradinho, feijão-de-corda, feijão-macassar, and feijão-caupi. Cowpea crops have high socioeconomic importance in Brazil, mainly in the North and Northeast regions. Considering the context of perspectives of climate change and the need for food in the world, cowpea crops have current strategic potential due to their plasticity, adaptation to a wide range of environments in tropical and subtropical regions, and high nutritional value; in addition, it is a staple food in more than 65 countries (FREIRE FILHO, 2011; FREIRE FILHO et al., 2017).

The expansion of cowpea crops to the Central-West region of Brazil and emergence of large crop areas using high technology has occurred due to the development of cultivars with earlier and more uniform maturation, and more compact architecture, which combined with new management techniques, enabled fully mechanized cultivation and high yields.

Cowpea crop area, production, and yield in Brazil were 1,352,100 ha, 720,900 Mg, and 533 kg ha^{-1}, respectively, in the 2020/2021 agricultural year. The state of Mato Grosso had the highest national production, 153,600 Mg, in an area of 152,800 ha, reaching a yield of 1,005 kg ha^{-1}, well higher than the national mean (CONAB, 2021).

Studies have shown that cowpea grains have higher zinc contents than common beans (COELHO et al., 2021; GERRANO et al., 2019) and cowpea germplasms presents high variability in grain zinc, iron, and protein contents (CARVALHO et al., 2012; DIAS-BARBOSA et al., 2020, 2021; SANTOS; BOITEUX, 2013, 2015; OLIVEIRA et al., 2017; WENG et al., 2019; CARDONA-AYALA et al., 2021). Variability in iron and zinc contents assists in the selection of superior lines and in the development of biofortified cultivars with these micronutrients (DIAS-BARBOSA et al., 2020, 2021).

The Brazilian Agricultural Research Corporation (Embrapa Meio-Norte) has developed biofortified cowpea cultivars (BRS-Aracê, BRS-Tumucumaque, and BRS-Xiquexique) with high grain iron and zinc levels (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017). These cultivars have been considered biofortified because they have iron and zinc contents higher than 6.0 and 4.0 mg 100 g^{-1} dry weight, respectively, whereas conventional cowpea cultivars have lower contents of these micronutrients (FREIRE FILHO, 2011). New biofortified cowpea cultivars are potential vehicles to improve iron and zinc status of populations with prevalence of deficiency of these micronutrients (COELHO et al., 2021).

In general, cowpea consumers prefer larger grains with white coat and wrinkled texture; however, the preference in terms of grain color varies. Grain size and color are characters that define the price of the product on the market (internal and external). The mean grain size of Brazilian cultivars is approximately 17 grams 100 $grains^{-1}$ (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017); however, consumers and markets prefer grains with weights higher than 20 g 100 $grains^{-1}$ (FREIRE FILHO, 2011).

Few studies found in the literature have evaluated the effect of grain size and color on nutrient contents. The objective of cowpea breeding programs in Brazil is to develop cultivars with superior grains in grain size and nutrient contents; however, the correlation between these characters needs to be further investigated, to better guide breeders in choosing the best strategy for simultaneous selection for these characters.

Thus, the objective of this work was to assess iron, zinc, and protein contents in cowpea grains of different cultivars and the effect of grain size and color on the contents of these nutrients.

MATERIAL AND METHODS

Twenty-four cowpea cultivars from the Active Bank of Cowpea Germplasm of the Brazilian Agricultural Research Corporation (Embrapa Meio-Norte) were evaluated (Table 1).

Table 1. Cowpea cultivars and their respective classes, grain color, and commercial subclasses.

Cultivar	Commercial class/ Grain color	Commercial subclass
Sempre-Verde	Colors/Brown	Evergreen
Caldeirão	Colors/Brown	Mulatto
BR-3-Tracuateua	White/White	Rough White
BR-14-Mulato	Colors/Brown	Mulatto
BR-17-Gurguéia	Colors/Brown	Evergreen
BRS-Rouxinol	Colors/Brown	Evergreen
BRS-Urubuquara	White/White	Rough White
BRS-Milênio	White/White	Rough White
BRS-Novaera	White/White	Rough White
BRS-Guariba	White/White	Plain White
BRS-Marataoã	Colors/Brown	Mulatto
BRS-Pujante	Colors/Brown	Mulatto
BRS-Xiquexique	White/White	Plain White
BRS-Tumucumaque	White/White	Plain White
BRS-Pajeú	Colors/Brown	Mulatto
BRS-Potengi	White/White	Plain White
BRS-Itaim	White/White	Black Eye
BRS-Aracê	Colors/Green	Green
BRS-Acauã	Colors/Brown	Crowder
BRS-Carijó	White/White	Black Eye
BRS-Tapaihum	Black/Black	Black
BRS-Imponente	White/White	Rough White
BRS-Inhuma	Colors/Brown	Crowder
Pingo-de-Ouro-1-2	Colors/Brown	Crowder

The experiment was conducted under greenhouse conditions at the Experimental Field of the Embrapa Meio-Norte, in Teresina, Piauí, Brazil (05°21'42"S, 41°44'26"W, and altitude of 424 m), from September 2017 to January 2018.

The experiment was conducted in a randomized complete block design, with two replications. The total area of the experiment was 100.8 m^2 (8.4 × 12.0 m), the block area was 38.40 m^2 (12.0 × 3.2 m), and the plot area (evaluation area) was 1.60 m^2 (0.5 × 3.2 m). The plot was represented by one row with 16 plants, which were used to collect the data. The spacing use was 0.50 m between rows and 0.20 m between plants within the rows. The plants were irrigated using a micro-sprinkler system.

The planting was carried out manually into holes dug using a hoe, placing 4 seeds per hole. A thinning was carried out 15 days after sowing, leaving one plant per hole. Insect pest control was carried out by spraying insecticides according to the insecticide manufacturer's recommendations when the levels for control were reached. Weed control was carried out using two manual weeding during the experiment. The harvest was carried out in the entire evaluation area of the plot, when all pods were dry; the pods were then threshed and the grains packed into plastic bags and kept in a refrigerator.

Grain size and iron, zinc and protein contents were evaluated. Grain size was evaluated indirectly, based on the weight of 100 grains (g) randomly collected from the evaluation area of each plot.

Analyses of grain protein, iron, and zinc contents were carried out from January to March 2018. A sample of 10 grams of grain of each cultivar was used. The grains were ground using a zirconia mill to obtain a flour, which was used for the analyses. The samples were homogenized with 5 mL of a perchloric acid solution. The mixture was heated at 200 °C for 2 hours for drying, the digested samples were diluted into distilled water, and iron and zinc contents were determined using a flame atomic absorption spectrometer, as described by Sarruge and Haag (1974). Grain protein contents were obtained based on the determination of nitrogen contents by the Kjeldahl method, as described by AOAC (2016), using a conversion factor of 6.25. All nutritional analyses were performed in triplicate. The protein results were expressed as g 100 g^{-1} of dry weight and the minerals iron and zinc as mg 100 g^{-1} of dry weight.

The data were subjected to analysis of variance and the means of the cultivars were grouped by the Tocher's clustering method, which groups the means based on an intergroup distance limit. Overall means of commercial classes were compared by the Tukey's test ($p<0.05$). Genotypic correlations between characters were estimated using the Pearson's correlation coefficient. The significance of the coefficients of correlation was evaluated by the t-statistic. The analyses were carried out using the Genes software (CRUZ, 2013) and the R statistical environment (R DEVELOPMENT CORE TEAM, 2014).

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance showed that the cultivars differed from each other by the F-test for all evaluated characters: iron, zinc, and protein contents ($p<0.05$) and grain size ($p<0.01$) (Table 2). This result indicated the existence of genetic variability among cultivars for these characters.

Table 2. Analysis of variance for iron (TFe), zinc (TZn), and protein (TPr) contents and grain size (TGr) of cowpea cultivars.

Source of ariation	Freedom degree	Mean square			
		TFe	TZn	TPr	TGr
Blocks	1	389.99	1550.19	19.80	15.30
Cultivars	23	146.23*	25.97*	7.71*	58.37**
Residue	23	71.35	18.14	3.19	2.78
Coefficient of Variation (%) Variation		10.89	9.05	5.32	7.73

* and ** = significant at 5% and 1% probability by the F-test, respectively.

The existence of genetic variability for iron, zinc, and protein contents is a necessary condition for the selection of superior genotypes and biofortification of cowpea grains. In this sense, Dias-Barbosa et al. (2020, 2021), evaluating a group of cowpea lines for grain iron and zinc contents, and Santos and Bouteux (2013), evaluating 86 F_6 cowpea progenies for iron, zinc, and protein contents, also found variability among cowpea genotypes and possibility of genetic gains with selection for biofortification of grains with these nutrients.

Grouping of means

Mean grain iron contents were grouped by the Tocher's clustering method into 10 groups, with an intergroup distance limit of 0.90 mg 100 g^{-1} (Table 3).

Table 3. Mean iron (TFe) and zinc (TZn) contents obtained from the evaluation of 24 cowpea cultivars.

Cultivar	TFe (mg 100 g^{-1})	TZn (mg 100 g^{-1})
Sempre-Verde	7.65 e	4.72 c
Caldeirão	8.59 a	4.72 c
BR-3-Tracuateua	7.79 d	4.72 c
BR-14-Mulato	7.65 e	4.68 c
BR-17-Gurguéia	7.08 j	4.78 b
BRS-Rouxinol	7.51 f	4.81 b
BRS-Urubuquara	7.96 c	4.74 c
BRS-Milênio	8.07 b	4.71 c
BRS-Novaera	7.42 g	4.55 d
BRS-Guariba	8.60 a	4.68 c
BRS-Marataoã	7.28 h	4.80 b
BRS-Pujante	7.37 g	4.69 c
BRS-Xiquexique	7.61 e	4.79 b
BRS-Tumucumaque	7.79 d	4.68 c
BRS-Pajeú	7.90 c	4.62 d
BRS-Potengi	7.96 c	4.70 c
BRS-Itaim	8.11 b	4.67 c
BRS-Aracê	7.90 c	4.87 a
BRS-Acauã	7.28 h	4.79 b
BRS-Carijó	8.03 b	4.69 c
BRS-Tapaihum	7.12 j	4.46 e
BRS-Imponente	7.19 i	4.93 a
BRS-Inhuma	7.73 d	4.48 e
Pingo-de-Ouro-1-2	8.52 a	4.68 c
IDL	0.90	0.67
OM	7.75 a	4.71 a
$OM_{Colors+Black}$	7.66 a	4.70 a
OM_{White}	7.85 a	4.67 a

Means followed by the same letter in the columns belong to the same group by the Tocher's clustering method; Overall means followed by the same letter in the columns are not different from each other by the Tukey's test ($p<0.05$); IDL = Intergroup distance limit; OM = Overall mean of cultivars; $OM_{Colors+Black}$ = Overall mean of cultivars of the colors and black classes; OM_{White} = Overall mean of cultivars of the white class.

Iron contents ranged from 7.12 to 8.60 mg 100 g^{-1} with an overall mean of 7.75 mg 100 g^{-1}. Dias-Barbosa et al. (2021) evaluated a group of 33 cowpea genotypes and found higher variation (4.70 to 7.96 mg 100 g^{-1}) and lower overall mean (6.05 mg 100 g^{-1}). Similarly, Cardona-Ayala et al. (2021), evaluated 10 cowpea genotypes in 10 environments in Northeastern Colombia and found higher variation (4.97 to 7.18 mg 100 g^{-1}) and lower overall mean (6.01 mg 100 g^{-1}).

Group A included the cultivars with the greatest iron contents, formed by Caldeirão, BRS-Guariba, and Pingo-de-Ouro-1-2, with 8.59, 8.60, and 8.52 mg 100 g^{-1}, respectively, differing from the cultivars of the other groups. These values were higher than those found by Carvalho et al. (2012) for the cultivars BRS-Guariba and Pingo-de-Ouro-1-2; they evaluated a group of 30 cowpea genotypes and found mean iron contents from 6.9 to 6.4 mg 100 g^{-1}, respectively. These differences in iron contents were probably due to environmental factors and connected to differences in soil conditions, management, and climate, since the genotypes are homozygous for these characters, i.e., they do not present genetic variation. The lowest iron contents were found for cultivars in group J, formed by BR-17-Gurguéia and BRS-Tapaihum, with 7.08 and 7.12 mg 100 g^{-1}, respectively. This result indicates that these two cultivars are the least suitable among the evaluated cultivars for using as parents in crosses focused on increasing iron contents in cowpea grains.

Seeds from biofortified cowpea cultivars have iron contents higher than 6.0 mg 100 g^{-1} (FREIRE FILHO, 2011;

COELHO et al., 2021). All cowpea cultivars evaluated in the present work presented iron contents higher than 6.0 mg 100 g^{-1} and thus are considered biofortified with iron.

The comparison between commercial classes of cowpea cultivars showed no differences in overall mean for iron contents, indicating similar grain iron contents. BRS-Tumucumaque and BRS-Xiquexique are biofortified cultivars with high iron contents (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017) that belong to the white class. However, two out of the three cultivars with the highest grain iron contents found in the present study belong to the colors class (BRS-Caldeirão and Pingo-de-Ouro-1-2). This result indicates that the selection of lines of both classes provides a high chance of obtaining iron-biofortified cultivars.

The zinc contents in the cowpea cultivars were grouped by the Tocher's clustering method into five groups, with an intergroup distance limit of 0.67 mg 100 g^{-1} (Table 3). Zinc contents ranged from 4.46 to 4.93 mg 100 g^{-1} with an overall mean of 4.71 mg 100 g^{-1}. Group A included the cultivars with the highest zinc contents, formed by BRS-Aracê and BRS-Imponente, with 4.87 and 4.93 mg 100 g^{-1}, respectively, differing from the cultivars of the other groups. The existence of variability among cultivars for this character indicates the possibility of gains in zinc contents with selection.

The overall mean found for zinc contents was lower than that found by Oliveira et al. (2017), who evaluated zinc contents in 12 cowpea genotypes in four locations in the states of Piauí and Maranhão, Brazil, and found a mean of 4.82 mg 100 g^{-1} for the cowpea cultivar BRS-Xiquexique; zinc contents varied according to the cultivation environments from 4.11 to 5.66 mg 100 g^{-1}. The zinc contents found in the present study were higher than those found by Cardona-Ayala et al. (2021), who evaluated 10 cowpea genotypes in 10 environments in northeastern Colombia and found zinc contents ranging from 3.16 to 4.70 mg 100 g^{-1}, with an overall mean of 4.09 mg 100 g^{-1}; they reported that zinc contents may vary according to the crop edaphoclimatic conditions due to the existence of genotype × environment interaction. This result explains the differences in zinc contents found for the cultivar BRS-Xiquexique in the present work and in the work of Oliveira et al. (2017).

Seeds from biofortified cowpea cultivars have zinc contents higher than 4.0 mg 100 g^{-1} (FREIRE FILHO, 2011; COELHO et al., 2021). All cowpea cultivars evaluated in the present work presented zinc contents higher than 4.0 mg 100 g^{-1} and thus are considered biofortified for zinc.

The absence of differences in overall means found for zinc contents between commercial classes indicates that the commercial class is not a factor that determines zinc contents in cowpea grains. The cultivar BRS-Aracê is part of the colors class, green subclass, and presents high grain zinc contents (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017; COELHO et al., 2021). However, the cultivar BRS-Imponente belongs to the white class and presented the highest grain zinc contents. This result indicates that the selection of lines of both commercial classes provides a high chance of obtaining zinc-biofortified cultivars. Zinc-biofortified cowpea cultivars, or foods derived from them, can be used as a strategy to combat malnutrition and diseases connected to the immune system in populations with zinc deficiency.

Regarding protein contents, the Tocher's clustering method grouped the cowpea cultivars into four groups, with an intergroup distance limit of 0.94 g 100 g^{-1} (Table 4). Protein contents ranged from 31.50 to 36.24 g 100 g^{-1}, with an overall mean of 33.57 g 100 g^{-1}.

Cultivars in groups A and B had the highest protein contents, presenting the following ascending order: BR-14-Mulato, BRS-Guariba, BRS-Aracê, and BRS-Inhuma, with 36.24, 35.22, 35.30, and 34.85 g 100 g^{-1}, respectively. The protein contents and overall mean found were higher than those found by Dias-Barbosa et al. (2021), who evaluated 12 cowpea genotypes and found protein contents from 19.21 to 23.80 g 100 g^{-1}, with an overall mean of 22.14 g 100 g^{-1}. Thus, the cowpea cultivars in group A are indicated for use in nutritional interventions in protein-deficient populations.

The lowest protein contents were found for cultivars in Group D, namely, Sempre-Verde, Caldeirão, BRS-Milênio, BRS-Novaera, BRS-Marataoã, BRS-Itaim, and BRS-Carijó. The cultivar BRS-Novaera presented the lowest protein content (31.50 g 100 g^{-1}). which was higher than that found for this cultivar by Gonçalves et al. (2020): 22.49 g 100 g^{-1}. The differences in protein contents between cultivars can be explained by differences in cultivation and cultural practices and climate, which can affect protein contents of cowpea cultivars.

No differences were found when comparing the overall mean protein contents between commercial classes of cowpea cultivars. This result indicates that the evaluated cultivars present similar dynamics regarding grain protein contents, regardless of the commercial type, indicating that the selection of genotypes from both classes studied provides a high chance of obtaining protein-biofortified cultivars.

Cowpea cultivars were grouped into two grain size groups, with an intergroup distance limit of 8.57 g 100 $grains^{-1}$, determined by the Tocher's clustering method (Table 4). Grain size ranged from 13.55 to 37.88 g 100 $grains^{-1}$, with an overall mean of 21.50 g 100 $grains^{-1}$. This range is greater than that found by Marinho et al. (2021), who evaluated 14 cowpea genotypes in the state of Acre, Brazil, from 2016 to 2018 and found a range of 19.94 to 34.03 g 100 $grains^{-1}$; however, the mean was similar (21.88 g 100 $grains^{-1}$). The largest grain sizes were found for cultivars in group A, namely, BR-3-Tracuateua and BRS-Imponente, with 29.31 and 37.88 g 100 $grains^{-1}$, respectively. This result confirms the extra-large grain size of the cultivar BRS-Imponente (ROCHA; DAMASCENO-SILVA; MENEZES-JÚNIOR, 2017). However, Marinho et al. (2021) found grain sizes ranging from 31.50 to 35.50 g 100 $grains^{-1}$ for the cultivar BRS-Imponente, smaller than the means found in the present study.

Table 4. Mean protein contents (TPr) and grain size (TGr) of 24 cowpea cultivars.

Cultivar	TPr (g 100 g^{-1})	TGr (g 100 $grains^{-1}$)
Sempre-Verde	32.23 d	18.87 b
Caldeirão	32.40 d	16.12 b
BR-3-Tracuateua	33.78 c	29.31 a
BR-14-Mulato	36.24 a	13.55 b
BR-17-Gurguéia	34.05 c	14.74 b
BRS-Rouxinol	34.18 c	15.65 b
BRS-Urubuquara	33.99 c	23.03 b
BRS-Milênio	32.72 d	24.22 b
BRS-Novaera	31.50 d	24.12 b
BRS-Guariba	35.22 b	21.22 b
BRS-Marataoã	32.30 d	19.98 b
BRS-Pujante	33.45 c	21.36 b
BRS-Xiquexique	34.14 c	18.27 b
BRS-Tumucumaque	33.79 c	23.88 b
BRS-Pajeú	33.36 c	19.50 b
BRS-Potengi	33.10 c	23.12 b
BRS-Itaim	32.51 d	26.45 b
BRS-Aracê	35.30 b	17.07 b
BRS-Acauã	33.97 c	21.03 b
BRS-Carijó	32.11 d	24.74 b
BRS-Tapaihum	33.08 c	20.88 b
BRS-Imponente	33.45 c	37.88 a
BRS-Inhuma	34.85 b	22.55 b
Pingo-de-Ouro-1-2	33.79 c	20.03 b
IDL	0.94	8.57
OM	33.57 a	21.56 b
$OM_{Colors+Black}$	33.78 a	18.56 c
OM_{White}	33.43 a	24.26 a

Means followed by the same letter in the columns belong to the same group by the Tocher's clustering method; Overall means followed by the same letter in the columns are not different from each other by the Tukey's test ($p<0.05$); IDL = Intergroup distance limit; OM = Overall mean of cultivars; $OM_{Colors+Black}$ = Overall mean of cultivars of the colors and black classes; OM_{White} = Overall mean of cultivars of the white class.

Grain size is little affected by environmental factors and most of the observed variation is due to genetic causes, which facilitates the selection of superior genotypes for this character (MARINHO et al., 2021). However, grain size is more affected by the environment than nutritional characters. Thus, another factor that can explain the differences in grain size found for BRS-Imponente is the genotype × environment interaction, which can affect the phenotype, increasing or decreasing the grain size. Moreover, Marinho et al. (2021) conducted a study in Acre, North region of Brazil, whereas the present work was conducted in Piauí, Northeast region of Brazil; these Brazilian regions have very different soil and climate conditions, which can significantly affect the phenotypes of the cultivars, including grain size.

The comparison between the overall mean grain size of cultivars of the white class and the overall mean of cultivars of the colors + black class showed superiority for the white class, which differed from the colors + black classes and the overall mean of the cultivars. This result confirms those of the Freire Filho (2011), who reported occurrence of larger grain sizes for cultivars of the white class; in addition, Marinho et al. (2021) reported larger grain sizes and rough texture for the cultivars BR-3-Tracuateua and BRS-Imponente. The market demand for cowpea cultivars with larger grains is high in Brazil; thus, cultivars with larger grains have higher prices. However, the preference of consumers varies according to the region and state of the country and commercial type, highlighting the white class. Thus, cowpea breeding programs have focused on the development of cultivars with large or extra-large grains.

Correlations between characters

Genotypic correlations between grain size and iron, zinc and protein contents are shown in Figure 1. The correlation between grain size and iron contents was negative, but low and not significant (r_g = -0.0678), which denotes absence of correlation between grain size and iron contents and shows that increases in grain size are not affected by increases or decreases in grain iron contents. This result is consistent with those found by Moura et al. (2012) regarding direction of the correlation, but not regarding magnitude; they found a negative and significant correlation (r_f= -0.6132), however, via phenotypic correlation.

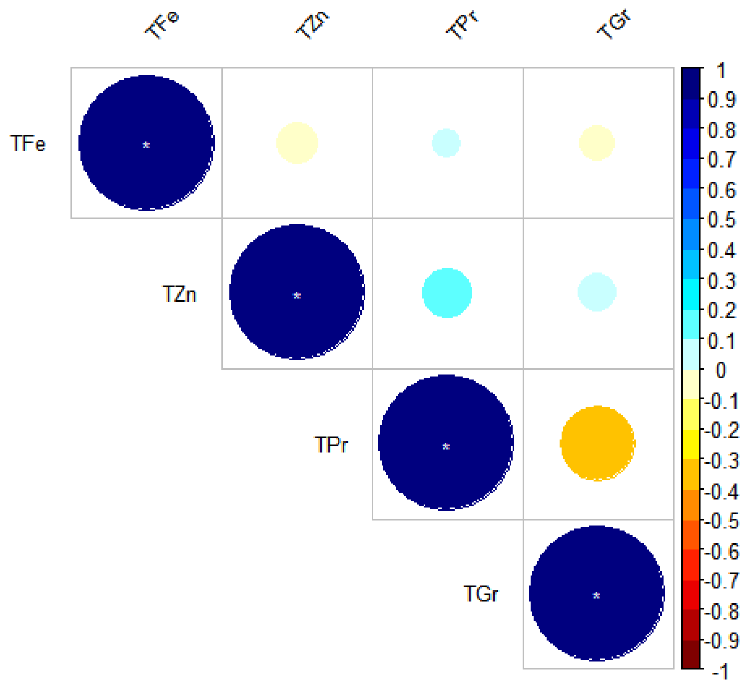

Figure 1. Genotypic correlations (r_g) between iron (TFe), zinc (TZn), and protein contents (TPr) and grain size (TGr) of 24 cowpea cultivars.

The correlation between grain size and zinc contents was positive, but low and not significant (r_g = 0.0855) (Figure 1). This result denotes no correlation between grain size and zinc contents and indicates that increases in grain size are not affected by increases or decreases in grain iron contents. Moura et al. (2012) evaluated phenotypic correlation in cowpea populations and found similar results regarding direction of the correlation, but different magnitude, which was negative and significant (rf = -0.6132).

The correlation between grain size and protein contents was positive, but low and not significant (r_g = 0.3160) (Figure 1). This result denotes no correlation between grain size and protein contents and indicates that increases in grain size are not affected by increases or decreases in grain protein contents. This result is consisted with those found by Moura et al. (2012), who found a negative, high, and significant correlation (r_f= -0.7221), however, via phenotypic correlation.

The correlation between iron and zinc contents was negative, but low and not significant; Dias-Barbosa et al. (2021) found similar result, with a non-significant but positive correlation. The correlations between iron and zinc contents and protein contents were positive, but low and not significant (r_g = 0.048, rg = 0.0141), different than those found by Dias-Barbosa et al. (2021), who found a negative and significant correlation between iron contents and protein contents (r_f = -0.51) and negative and non-significant correlation between zinc contents and protein contents (r_f = -0.21); however, they used phenotypic correlation.

In the context of breeding programs focused on biofortification of cowpea grains with iron, zinc, and proteins, the results of the correlations found in the present work indicate the possibility of obtaining cultivars biofortified with these nutrients, regardless of the grain size, i.e., using large or small-grain cultivars. The lack of correlation between iron, zinc, and protein contents indicates that selection for improvement of one of these characters does not interfere with the improvement of the other.

CONCLUSIONS

The cowpea cultivars Caldeirão, BRS-Guariba, and Pingo-de-Ouro-1-2 have higher iron contents; BRS-Aracê and BRS-Imponente have higher zinc contents; BR-14-Mulato, BRS-Guariba, BRS-Aracê, and BRS-Inhuma have higher protein contents; and BR-3-Tracuateua and BRS-Imponente have larger grain sizes.

Grain size does not affect iron, zinc, and proteins contents and these characters are not affected by the commercial class of the evaluated cowpea cultivars.

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