Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2022v43n4p1787

Genotype by environment interaction in common bean cultivars for iron and zinc concentration in grains

Interação entre genótipos e ambientes em cultivares de feijão para concentração de ferro e zinco nos grãos

Poliana Regina Carloni[1]; Patrícia Guimarães Santos Melo[2]; Leonardo Cunha Melo[3]; Luís Cláudio de Faria[3]; Thiago Lívio Pessoa Oliveira de Souza[3]; Válter Martins de Almeida[4]; Hélio Wilson Lemos de Carvalho[5]; Israel Alexandre Pereira Filho[6]; Marcelo Sfeir de Aguiar[3]; Helton Santos Pereira[3]*

Highlights

The environmental effect has a great influence on the concentrations of iron and zinc.
Genotype by environment interaction influences the concentrations of iron and zinc.
Cultivar BRS Sublime has all of the desirable traits.
Iron and zinc concentrations are positively correlated.

Abstract

Iron and zinc deficiencies in humans can cause serious health problems. Increasing the iron (IC) and zinc (ZC) concentrations in common bean (*Phaseolus vulgaris* L.) grains using genetic breeding can be an effective strategy to prevent these problems. Thus, the aims of this study were i) to investigate the importance of genetic, environmental, and genotype-by-environment interaction effects on IC and ZC in common bean grains; ii) to select cultivars that have high mean values and phenotypic stability for IC and ZC, as well as high protein concentration (PC), high yield, and standard commercial grain quality; and iii) to investigate whether there is a genetic relationship between these traits. A total of 34 cultivars were evaluated in 19 different environments. Genetic variability was observed among the genotypes for all traits. For IC and ZC, the environmental effect represented most of the total variation (63% and 65%, respectively), and the effect of the G × E interaction was 18% for IC and 15% for ZC. The genetic correlations between IC, ZC,

[1] Dra, CNPq Industrial Technological Development Scholarship - Level B, Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Embrapa Algodão, Santo Antônio de Goiás, GO, Brazil. E-mail: polianacarloni@gmail.com
[2] Profa Dra, School of Agronomy, Universidade Federal de Goiás, UFG, Goiânia, GO, Brazil. E-mail: pgsantos@gmail.com
[3] Drs. Researcher, EMBRAPA, Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil. E-mail: leonardo.melo@embrapa.br; luis.faria@embrapa.br; thiago.souza@embrapa.br; marcelo.sfeir@embrapa.br; helton.pereira@embrapa.br
[4] M.e, Researcher, Empresa Matogrossense de Pesquisa Assistência e Extensão Rural, EMPAER, Várzea Grande, MT, Brazil. E-mail: almeida-walter@uol.com.br
[5] M.e, Researcher, EMBRAPA, Embrapa Tabuleiros Costeiros, Aracaju, SE, Brazil. E-mail: helio.carvalho@embrapa.br
[6] M.e, Researcher, EMBRAPA, Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil. E-mail: israel.pereira@embrapa.br
* Author for correspondence

Received: Aug. 23. 2021 - Approved: May 17, 2022

and PC were positive, indicating that the selection of one of these traits resulted in gains for the other. The genetic correlations between IC or ZC with yield or 100-grain weight were low or intermediate, but negative, indicating that it is important to evaluate these traits at all stages of breeding programs that focus on developing cultivars with high IC and ZC. The cultivar BRS Sublime, with the carioca grain type, combined high IC, ZC, and PC, high yield, and grains of commercial size.
Key words: *Phaseolus vulgaris* L. Adaptability. Correlation. Stability. Biofortification.

Resumo

A deficiência de ferro e zinco, em humanos, causam sérios problemas de saúde. O aumento da concentração de ferro (CFe) e concentração de zinco (CZn) em grãos de feijão (*Phaseolus vulgaris* L.) por melhoramento genético pode ser uma estratégia eficaz para prevenir esses problemas. Assim, os objetivos deste estudo foram: i) verificar a importância do efeito genético, ambiental e da interação entre genótipos e ambientes para CFe e CZn, em grãos de feijão; ii) selecionar cultivares de feijoeiro-comum que associem altas médias e estabilidade fenotípica para CFe e CZn, além de alta concentração de proteína (CPt), alta produtividade e grãos com padrão comercial; iii) verificar se existe relação genética entre esses caracteres. Foram avaliadas 34 cultivares de feijão, em 19 ambientes. Houve variabilidade genética entre os genótipos para todos os caracteres. Para CFe e CZn, o efeito de ambientes representou a maior parte da variação total, 63% e 65%, respectivamente, o efeito de interação representou 18% para CFe e 15% para CZn. As correlações entre CFe, CZn e CPt foram positivas, o que indica que a seleção de um desses caracteres resulta em ganhos para o outro. As correlações entre CFe e CZn com produtividade e massa de 100 grãos, foram baixas ou intermediárias, mas negativas, o que indica a importância de avaliar esses caracteres em todas as fases do programa de melhoramento com foco no desenvolvimento de cultivares com altas CFe e CZn. A cultivar BRS Sublime, com grão tipo carioca, reuniu altas CFe, CZn, CPt, produtividade e grãos com tamanho comercial.
Palavras-chave: *Phaseolus vulgaris* L. Adaptabilidade. Correlação. Estabilidade. Biofortificação.

Introduction

The common bean (*Phaseolus vulgaris* L.) has considerable potential for biofortification of iron concentration (IC) and zinc concentration (ZC) in the grains because it is the most important pulse crop and leguminous plant for direct human consumption (Beebe et al., 2000) and has relatively high concentrations of these minerals. In addition, various authors have indicated genetic variability in these traits (Amongi et al., 2018; Caproni et al., 2020; Martins et al., 2016; Mukamuhirwa & Rurangwa, 2018; Pereira et al., 2014; Philipo et al., 2020; Silva et al., 2012). In Brazil, most studies have been conducted using precommercial lines. However, it is important to check the variability present between the cultivars that are available in the market for farmers, as these would be immediate sources for supplying greater quantities of these minerals.

IC and ZC have quantitative genetic control, with several genes acting to control traits (Beebe et al., 2000; Blair et al., 2009). These traits can be strongly influenced by the environment and by the genotype-environment interaction, as in the case of grain yield. Genotype-by-environment interaction occurs when there is a differential response of genotypes in different environments. This allows the identification of cultivars with specific adaptations to certain environments and/or cultivars with broad adaptations, which present satisfactory performance in most environments.

The few studies that have addressed these points in common bean indicate that these effects are present (Martins et al., 2016; Mukamuhirwa & Rurangwa, 2018; Nchimbi-Msolla & Tryphone, 2010; Pereira et al., 2014; Philipo et al., 2020; Silva et al., 2012) for IC and ZC. However, the number of environments evaluated in previous studies has always been below five, which can be considered small in the case of quantitative traits with a high influence of environmental factors. Because of this, evaluation in various environments (locations, years, sowing times, and types of cropping system) is essential for identifying genotypes that are truly superior, as well as for allowing a better understanding of the effect of the interaction.

Protein concentration (PC) is another important trait related to the nutritional quality of common bean (Buratto et al., 2009). In addition to the nutritional value of grains, other agronomic traits, such as yield and 100-grain weight (W100), are determining factors for the release of a common bean cultivar. Ribeiro et al. (2013) indicate that selection of genotypes for yield, grain quality, and nutritional quality in common bean is recent, and that studies are still at an early stage. Therefore, we evaluated whether there was an association among these traits by estimating phenotypic, genetic, and environmental correlations.

Thus, the aims of this study were i) to investigate the importance of the genetic and environmental effects, and the genotype by environment interaction for IC and ZC in common bean grains; ii) to select cultivars that have high mean values and phenotypic stability for IC and ZC, as well as high PC, yield, and standard commercial grain quality; and iii) to investigate whether there is a genetic relationship between these traits.

Materials and Methods

Genotypes, environments, and experimental design

We evaluated 34 common bean genotypes from different market classes [18 *carioca* (beige with brown stripes), 12 black, three *mulatinho* (cream), and one brown], which included five pre-commercial lines, 24 principal cultivars from different institutions, and five controls with IC and ZC know (Brasil 0001, G 6492, Piratã 1, IAC Una, and Xamego) (Pereira et al., 2014). The genotypes were evaluated in Brazil at 10 locations in the Distrito Federal and in the states of Goiás, Mato Grosso, Mato Grosso de Sul, Minas Gerais, Paraná, Pernambuco, and Sergipe in 2012, 2013, and 2014. The evaluations were conducted in the dry (sowing from January to March), winter (April to June), and rainy growing seasons (April to May, or October to December, depending on the region), resulting in a total of 19 environments (combination of location/season/year).

The experiments were carried out in randomized complete blocks with two replicates and plots of two 3.0 m rows were conducted according to technical recommendations for the common bean crop, but without disease control.

Phenotyping

IC and ZC were evaluated in all 19 environments, grain yield and W100 in 17 environments, and PC in five environments. Grain yield was obtained in g plot^{-1}, harvesting all the plants in the plot, with later conversion to kg ha^{-1} and adjustment to 13% grain moisture. A 100-grain sample was collected from each plot, at random, for weighing and determination of W100 in g 100 seeds^{-1}; after weighing, these 100-grain samples were sent for IC and ZC analyses.

Analyses of IC and ZC were carried out by acid digestion of organic matter (with a 2:1 nitric perchloric mixture), according to the flame atomic absorption spectrophotometry technique adapted from the Association of Official Analytical Chemists [AOAC] (2005). To check the precision of the laboratory analysis, for every 40 samples, one was carried out in triplicate, and two were control samples with pre-established values for verification. The common bean grains were rapidly washed with deionized water and dried in a laboratory oven at 60 °C for 12 h (to 6% moisture). The grain samples were ground (≤200 mesh) in a zirconium oxide ball mill (Retsch MM200) and placed in containers of PTFE (polytetrafluoroethylene) to avoid contamination from metallic elements. The samples were weighed one day after grinding to achieve a moisture equilibrium. The samples were pre-digested with an acid mixture (50 °C for 30 min), followed by digestion (100 °C/30 min; 170 °C/3 h, cooling at room temperature, and addition of 2.0 mL of acid mixture and digestion at 170 °C for 3 h. The obtained extract was adequately diluted and read in an atomic absorption spectrophotometer (Agilent/Varian model SpectrAA 50 B), which was previously calibrated with a standard curve for iron and zinc.

Analyses of PC were performed based on bean meal (grains ground in a ball mill), according to the Kjeldahl method (AOAC, 2005). This method quantifies the total nitrogen concentration contained in the sample and uses a factor of 6.25 for conversion into protein. The data for IC and ZC were expressed on a dry matter basis (mg kg^{-1}) based on the moisture concentration of the sample obtained by the gravimetric method at 105 °C until constant weight (Instituto Adolfo Lutz [IAL], 2008). PC data were expressed as percentages (%). The glassware and materials used in the analysis were subjected to a special washing process with a decontamination step in 5% nitric or chloridic acid solution (V:V) to avoid contamination.

Statistical analysis

Statistical analyses were performed using GENES (Cruz, 2013) and Statistical Analysis System Institute [SAS Institute] (2008). A single-environment analysis of variance was performed for each trait. In joint analysis, the model used was: $y_{ijk} = \mu + b_{j(k)} + g_i + a_k + g \times a + e_{ijk}$; where Y_{ijk} is the observation of genetic treatment i, in block j, evaluated in environment k; μ is a constant inherent to all observations; $b_{j(k)}$ is the fixed effect of block j within trial k; gi is the fixed effect of genotype i; ak is the random effect of environment (location, year and crop combination); "g x a" is the interaction between genotypes and environments; and e_{ijk} is the experimental error associated with the ijk-th observation, assuming e_{ijk}~N(0,σ_e^2).

Genetic and phenotypic correlations among all traits were estimated according to the procedure reported by Cruz et al. (2012), considering the values of the plots for each environment evaluated. The coefficients of determination (R^2 %) of genotypes, environments, and the genotype-environment interaction were also estimated by the relative contribution of the sum of squares of each source of variation in relation to the total sum of squares; R^2 makes inferences about which of these sources of variation are most important for the expression of each trait. The experimental coefficient of variation (CV%) and selective accuracy (SA) were estimated (Resende & Duarte, 2007) to evaluate the accuracy of the experiments. SA corresponds to a linear correlation between genotypic and phenotypic values.

The mean values of the genotypes were compared using the Scott and Knott (1974) test at 10% probability. The Scott and Knott (1974) test was used to avoid the high level of overlapping groups of genotypes in the classical multiple comparisons test. A significance level of 10% was used to reduce the probability of absence of discrimination among genotypes due to type II errors (Zimmermann, 2014). This procedure is recommended when small differences between treatments are expected, such as those among cultivars.

Analyses of adaptability and stability were performed using the method described by Nunes et al. (2005) for IC and ZC. Initially, the mean values for each trait of the cultivars in the different environments were standardized through the expression $z_{ij} = \frac{\left(\bar{y}_{ij} - \bar{y}_{.j}\right)}{s_{.j}}$, where z_{ij} is the value of the standardized variable corresponding to genotype *i* in environment *j*; $\bar{y}_{ij}$ is the mean of genotype *i* in environment *j*; $\bar{y}_{.j}$ is the mean of environment *j;* and is the phenotypic standard deviation among the mean values of the genotypes in environment *j*, given by $s_{.j} = \sqrt{\sum_{i=1}^{t} \frac{\left(\bar{y}_{ij} - \bar{y}_{.j}\right)^2}{t-1}}$. As the standardized variable z_{ij} assumed both positive and negative values, a constant *k* was added (k=3.0) to the values of z_{ij} so that all of them would be positive.

For each genotype i there is a mean value of z_{ij} in the j environments ($\bar{z}_i$), which is considered a measure of adaptability of the genotype. The CV of the z_{ij} for genotype i in j environments (CV_{Zi}) is a measure of stability of the genotype. Genotypes that exhibited $CV_{Zi} \leq$ 20% were considered very stable, 20% < CV_{Zi} ≤ 30% as stable, and CV_{Zi} > 30% as unstable. The standardized values z_{ij} were used to develop graphs for each genotype, and the dimensions of the axes (environments) were equivalent to the values of z_{ij} (Nunes et al., 2005).

Results and Discussion

Iron and zinc concentration

Experimental quality diagnosis

In this type of work, possible sampling problems should be considered since the sample used to determine the mineral concentration was very small. Sampling problems can lead to poor accuracy in determining the parameters. During the mineral analysis, procedures were carried out to eliminate contamination and ensure homogeneity of the samples. From a genetic point of view, as cultivars were used, which, in the case of common beans, are homozygous lines, there is less chance of sampling problems when compared to segregating materials (populations and progenies). In addition, in the case of this type of problem, estimates of experimental precision would be affected, as a higher CV and lower estimates of selective accuracy would be expected. The estimates obtained indicated excellent experimental precision, with CVs always below 19% in each environment and because the mean selective accuracy was 0.62 for IC and 0.70 for ZC. Therefore, no sampling problems were identified.

Genetic variation in IC and ZC

Joint analyses for IC and ZC revealed significant differences among the genotypes, indicating genetic variability (Table 1). The overall mean values of the genotypes ranged from 51.9 mg kg^{-1} (BRS Estilo) to 67.5 mg kg^{-1} (Piratã 1) for IC (Table 2). The Scott and Knott (1974) method formed three groups, and the group with the highest mean values was composed of 11 genotypes, including four controls with high IC: Piratã 1, Brasil 0001, G 6492, and Xamego, which repeated the good performance observed by Pereira et al. (2014). The seven cultivars that had high IC were BRS Cometa, BRS Sublime, and BRS FC402 from the *carioca* (beige grain coat with brown stripes) market class, BRS Marfim from *mulatinho* market class, and BRS Supremo, CNFP 11995, and BRS Esplendor from the black-market class (Table 2).

The mean values of the genotypes for ZC ranged from 27.7 mg kg^{-1} (IAC Diplomata) to 35.7 mg kg^{-1} (Brasil 0001) (Table 2). Five groups of genotypes were formed, with the control Brasil 0001 having the highest mean value. The second group included the controls Xamego and Piratã 1. These three controls confirmed the high ZC values reported by Pereira et al. (2014). The third group consisted of nine genotypes and the fourth group consisted of eight genotypes. The genotypes of these four groups were considered to have good ZC because they had higher concentrations than those of the cultivars Pérola and BRS Estilo, which are two of the most grown cultivars in Brazil and have intermediate ZC (Martins et al., 2016; Pereira et al., 2014).

Table 1

Summary of combined analyses of variance of the 34 genotypes evaluated in 19 environments

Source of Variation	IC[a]			ZC[b]			Yield		
	DF	MS	R^2	DF	MS	R^2	DF	MS	R^2
Blocks/Environments	19	130	0.01	19	56	0.02	17	476,367	0.00
Genotypes (G)	33	327**	0.05	33	113**	0.08	33	2,279,387**	0.03
Environments (E)	18	6967**	0.63	18	1687**	0.65	16	127,524,322**	0.85
G x E	425+	85**	0.18	398+	17**	0.15	257+	752,881**	0.08
Residual	441+	55	0.12	412+	12	0.10	264+	315,382	0.03
Mean		60.3			29.9			2,186	
CV (%)		12.4			11.5			25.7	
SA		0.86			0.92			0.82	
Source of Variation	100-grain weigth			PC[c]					
	DF	MS	R^2	DF	MS	R^2	-	-	-
Blocks/Environments	17	1.1	0.01	5	8.6	0.02	-	-	-
Genotypes (G)	33	145.3**	0.33	33	14.8**	0.08	-	-	-
Environments (E)	16	386.1**	0.42	4	372.8**	0.65	-	-	-
G x E	361+	7.2**	0.18	132	4.1**	0.15	-	-	-
Residual	376+	2.7	0.07	165	2.2	0.10	-	-	-
Mean		21.9			29.9			-	
CV (%)		7.4			11.5			-	
SA		0.98			0.92			-	

[a]IC, iron concentration; [b]ZC, zinc concentration; [c]PC, protein concentration; *CV* experimental coefficient of variation; SA selective accuracy; R^2, ratio between the sum of squares of the respective effect and the total sum of squares; + degrees of freedom adjusted according to Cochran (1954); ** significant at 1% probability, by F test.

Table 2

Mean values of iron (mg kg^{-1}), zinc (mg kg^{-1}), and protein (%) concentrations in common bean seeds, seed yield (kg ha^{-1}), 100-seed weight (g), and parameters of adaptability and stability of 34 genotypes evaluated in 19 environments

Genotype	CC*	Iron concentration			Zinc concentration			Yield	W100	PC
		Mean	Z_i**	CV_{Zi}***	Mean	Z_i	CV_{Zi}	Mean	Mean	Mean
BRS Cometa	C	64.2 a	3.62	28.0	30.7 c	3.27	19.4	1,962 c	22.2 d	26.0 b
BRS Sublime	C	63.4 a	3.54	16.5	30.9 c	3.33	17.6	2,256 b	23.1 c	25.6 b
BRS FC402	C	61.8 a	3.25	21.2	30.8 c	3.25	23.0	2,354 b	20.6 f	26.7 b
Porto Real	C	60.6 b	2.94	30.9	31.1 c	3.40	16.9	2,005 c	20.5 f	25.5 b
BRS Madrepérola	C	60.6 b	3.11	26.1	30.7 c	3.25	25.4	1,964 c	22.3 d	24.7 c

continue...

continuation...

BRS Ametista	C	59.7 b	2.82	26.4	29.2 d	2.69	31.7	2,180 b	24.7 b	25.6 b
IAC Alvorada	C	59.3 b	2.85	17.5	30.0 d	3.02	32.8	2,041 c	25.9 a	26.3 b
BRS Requinte	C	59.2 b	2.86	29.7	29.3 d	2.82	28.0	2,225 b	20.0 g	26.5 b
Pérola	C	59.1 b	2.75	25.0	28.8 e	2.66	30.2	2,391 b	24.2 b	24.4 c
BRS Pontal	C	59.0 b	2.76	28.0	29.1 e	2.66	25.6	2,346 b	22.6 d	26.0 b
IPR Tangará	C	58.9 b	2.86	29.5	30.1 d	3.00	24.9	1,994 c	23.6 c	25.1 c
IAPAR 81	C	58.8 b	2.76	33.9	28.8 e	2.57	41.1	1,828 c	22.2 d	24.9 c
IAC Formoso	C	58.6 b	2.63	41.8	27.9 e	2.34	30.7	2,360 b	23.9 c	25.4 b
CNFC 10729	C	58.1 b	2.55	42.4	29.8 d	3.00	27.1	2,350 b	25.8 a	25.6 b
BRS Notável	C	57.7 b	2.51	27.9	28.3 e	2.44	25.6	2,332 b	22.2 d	24.7 c
IPR 139	C	57.5 b	2.57	33.4	28.6 e	2.48	47.0	2,247 b	22.9 d	25.7 b
CNFC 10467	C	56.6 b	2.41	35.1	29.4 d	2.85	28.6	1,863 c	21.5 e	25.9 b
BRS Estilo	C	51.9 c	1.57	69.9	28.7 e	2.55	26.7	2,309 b	22.8 d	25.2 b
BRS Marfim	M	62.2 a	3.31	26.5	30.0 d	3.07	29.4	2,316 b	22.5 d	24.0 c
BRS Agreste	M	60.3 b	2.9	30.2	28.2 e	2.48	30.7	1,972 c	20.5 f	24.8 c
BRS Supremo	B	64.4 a	3.71	26.9	31.7 c	3.68	27.9	2,118 c	19.6 g	25.6 b
CNFP 11995	B	63.9 a	3.55	30.3	30.8 c	3.27	20.8	2,571 a	23.4 c	24.6 c
BRS Esplendor	B	61.5 a	3.20	33.9	30.6 c	3.24	29.6	2,571 a	18.4 h	24.9 c
IAC Diplomata	B	60.7 b	3.06	33.3	27.7 e	2.23	28.2	2,064 c	21.6 e	26.7 b
IPR Uirapuru	B	60.4 b	3.02	28.0	28.5 e	2.54	31.2	2,437 a	21.0 f	24.0 c
BRS Esteio	B	59.4 b	2.86	32.8	28.8 e	2.57	35.5	2,552 a	21.5 e	23.8 c
IPR Tuiuiu	B	58.5 b	2.75	26.7	29.9 d	2.98	22.9	2,231 b	20.8 f	23.4 c
BRS Campeiro	B	58.0 b	2.68	32.0	28.8 e	2.56	31.0	2,407 b	23.6 c	25.8 b
BRS FP403	B	57.9 b	2.61	25.7	28.7 e	2.63	28.6	2,597 a	24.8 b	24.6 c
Piratã 1[a]	M	67.5 a	4.18	15.9	32.9 b	4.01	19.8	1,918 c	20.8 f	27.1 b
Brasil 0001[a]	M	64.5 a	3.74	24.9	35.7 a	4.98	23.1	1,433 d	17.9 i	30.3 a
G 6492[a]	B	64.1 a	3.63	23.7	32.2 c	3.74	20.3	1,818 c	19.6 g	26.1 b
Xamego[a]	B	63.2 a	3.55	29.6	33.1 b	4.04	23.4	2,064 c	17.5 i	25.9 b
IAC Una[a]	B	60.5 b	2.88	31.9	28.1 e	2.42	31.8	2,249 b	20.8 f	25.8 b
Mean	-	60.3	3.00	29.9	29.9	3.00	27.6	2,186	21.9	25.5

*Commercial classes: *carioca* (C), *mulatinho* (M), and black (B); **Z_i: parameter of adaptability; ***CV_{Zi} coefficient of variation of the standardized variable (parameter of stability). [a]Controls with known iron and zinc concentrations. Mean values followed by the same letter do not differ statistically among themselves according to the Scott and Knott (1974) test at 10% probability.

Environment effect in IC and ZC

The environmental effect was significant for the two minerals, representing 63% of the total variation for IC and 65% for ZC, which confirmed the environmental effect on the manifestation of these traits (Table 1). Martins et al. (2016) also found that the environmental effect was pronounced for both minerals (64% for IC and 49% for ZC). This indicates that, in addition to using a cultivar with high genetic potential for IC and ZC, it is necessary to work in favorable environments to obtain the maximum IC and ZC in common bean grains. Therefore, understanding which environmental factors are responsible for increasing or decreasing IC and ZC is essential.

On the other hand, Philipo et al. (2020) found an environmental effect representing a small part of the total variation for IC (1.7%), which differs from the results found in the present study and in the literature. This can be explained by the greater diversity of genotypes evaluated, the number of environments, and the difference in the region where the evaluations were carried out (Tanzania). Philipo et al. (2020) confirmed the importance of environmental variation (39.7 %) in ZC.

The mean of IC in the 19 environments ranged from 44.5 to 81.2 mg kg^{-1}, representing a variation of 82.5%, confirming that there is large environmental variation. For ZC, the mean value ranged from 23.5 to 39.9 mg kg^{-1}, i.e., a variation of 69.8%. These variations are higher than those reported in the literature (Martins et al., 2016; Mukamuhirwa & Rurangwa, 2018; Pereira et al., 2014; Philipo et al., 2020). As the number of environments evaluated increased, the variation in the two traits increased, which underscores the importance of environmental variation and evaluation in various environments.

Genotype by environment interaction in IC and ZC

The effect of the genotype × environment interaction (G × E) was significant for IC and ZC and represented 18% of the total variation for IC and 15% for ZC (Table 1). Some studies have identified the effect of the G × E interaction as important for the two traits. However, in these studies, the number of environments evaluated was relatively small, ranging from two to ten (Martins et al., 2016; Mukamuhirwa & Rurangwa, 2018; Nchimbi-Msolla & Tryphone, 2010; Pereira et al., 2014; Philipo et al., 2020; Silva et al., 2012). In this study, 19 environments were used, which permitted more accurate and consistent conclusions with respect to the environmental effect and effect of the G × E interaction.

Stability for IC and ZC

As the G × E interaction was confirmed, it is important to identify the genotypes that combine a high mean for these minerals with adaptability and phenotypic stability. The genotypes that had the highest indices of adaptability (Z_i) for IC were the controls Piratã 1, Brasil 0001, and G 6492 and the cultivars BRS Supremo and BRS Cometa. The genotypes with the greatest adaptability were considered (Table 2). In general, the 11 genotypes that had the highest mean values of IC also had the highest mean values of Z_i, indicating that the mean value and Z_i are strongly correlated. The 14 genotypes with had values of Z_i above the mean were considered to have good adaptability for IC.

The highest values of Z_i for ZC were for the controls Brasil 0001, Xamego, Piratã 1, and G 6492 (Table 2). The other 13 genotypes also showed good adaptability ($Z_i \geq 3.00$). The 12 genotypes with the highest mean values of ZC also had the highest values of Z_i.

In terms of stability, the estimates of CV_{Zi} for IC ranged from 15.9 to 69.9%, which showed a large difference in stability among the genotypes (Table 2). In addition to the control Piratã 1 (CV_{Zi}=15.9%), the cultivars BRS Sublime and IAC Alvorada were very stable ($CV_{Zi} \leq 20.0\%$). Another 17 genotypes were considered stable (20.0% < $CV_{Zi} \leq 30.0\%$). Pereira et al. (2014) obtained estimates of CV_{Zi} for the genotypes Piratã 1 (13.6%) and G 6492 (20.6%) near those obtained in the present study. The estimates of CV_{Zi} for ZC ranged from 16.9 to 47.0% (IPR 139) (Table 2). The genotypes Piratã1, BRS Sublime, BRS Cometa, and Piratã 1 were highly stable. The other 19 genotypes were considered stable.

The control Piratã 1 had above average performance in 18 of the 19 environments for IC, while Brasil 0001 performed above average for IC in 15 of the 19 environments, forming the "well-inflated" graph in Figure 1. For ZC, these two controls also formed well-inflated graphs (Figure 2), with superior performances in 18 environments for Piratã 1 and 17 for Brasil 0001. The genotypes that also had well-inflated graphs for IC were BRS Supremo and G 6492 (black), and BRS Cometa and BRS Sublime (*carioca*) (Figure 1). These genotypes perform efficiently in response to environmental stimuli with high predictability. In contrast, the cultivar BRS Estilo had a performance considered undesirable for IC, with an "uninflated" graph.

The black-seeded cultivars Xamego and BRS Supremo and the carioca seeded cultivars BRS Sublime and BRS Cometa stood out for their efficiency in response to environmental variations, that is, they have high predictability for ZC. The cultivars Pérola and BRS Estilo had poor performances for ZC, similar to IC, and formed deflated graphs (Figure 2).

A genotype that can be considered good should have a high mean for the target trait, good adaptability ($Z_i \geq 3.00$), and good stability ($CV_{Zi} \leq 30.0$). As expected, the controls Piratã 1, Brasil 0001, G 6492, and Xamego stood out because of their high mean values for IC and ZC, adaptability, and stability. In addition to the controls, the cultivars that stood out for IC and ZC simultaneously were BRS Cometa, BRS Sublime, BRS FC402 (*carioca*), BRS Supremo (black), and BRS Marfim (*mulatinho*).

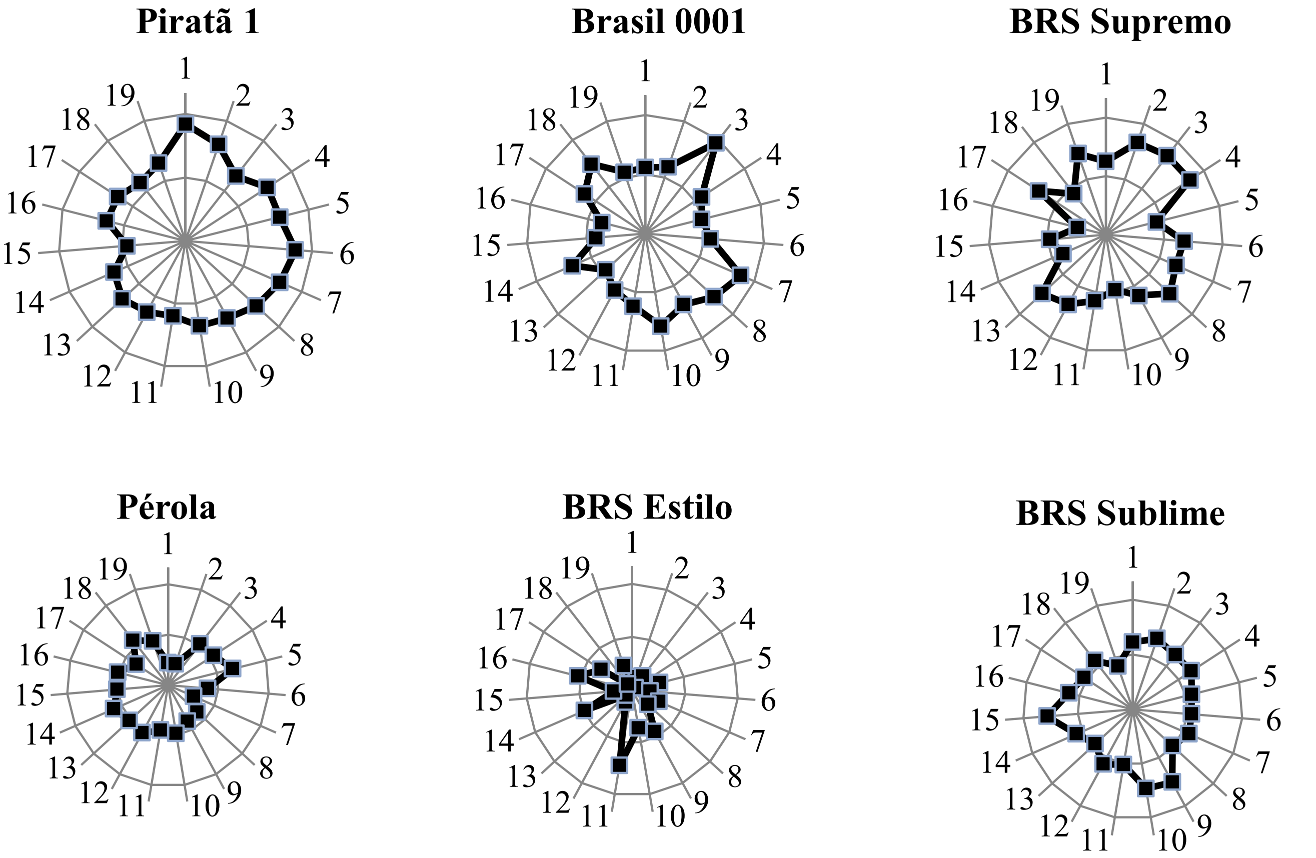

Figure 1. Graphical representation of genotype performance for iron concentration in common bean seeds using the graph method (Nunes et al., 2005). The center circle represents the mean of the environment associated with the standardized value Z_{ij}, (3), and the axes refer to the 19 environments.

The mean daily consumption of common beans in Brazil is 42 g per person day-1 (Feijão, 2019). The Pérola cultivar consumed in this amount will provide approximately 20.3% of the daily iron requirement and 19.8% of the daily zinc requirement. The BRS Estilo cultivar provided 17.8% iron and 19.7% zinc. Disregarding the bioavailability issues, which were not evaluated in the present study because they are extremely complex, and considering the genotypes identified as having higher concentrations, such as Piratã 1, 23.1% of the daily requirement of iron and 22.6% of zinc would be provided, representing a 13.8% and 14.1% increase in the daily requirements of iron and zinc in relation to Pérola. The *carioca* seeded cultivar BRS Cometa provides 8.6% more iron than Pérola and 23.7% more iron than BRS Estilo, and 6.6% more zinc than Pérola and BRS Estilo, which can represent an immediate increase in the ingestion of iron and zinc by the population. Considering the black-seeded cultivars, the use of BRS Supremo instead of IPR Uirapuru, which is more often grown in Brazil, will provide 6.8% more iron and 11.3% more zinc.

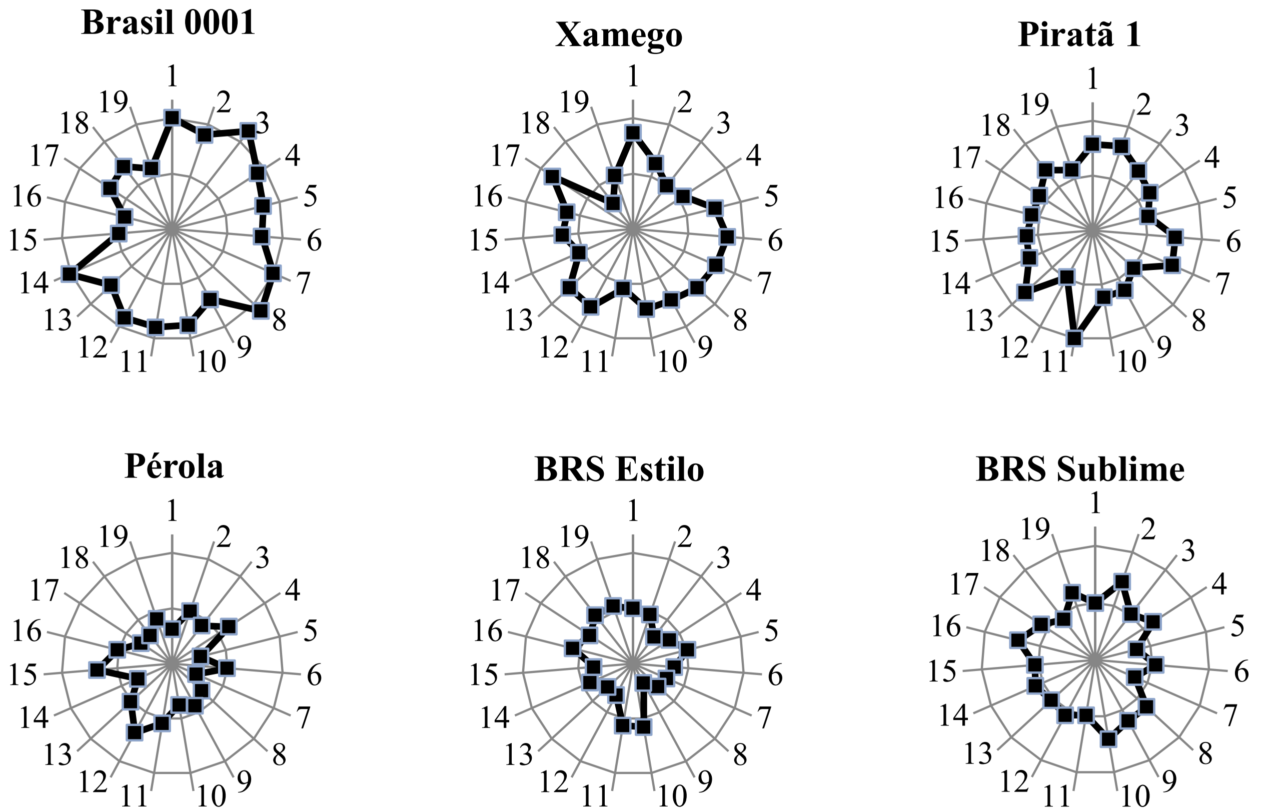

Figure 2. Graphical representation of genotype performance for zinc concentration in common bean seeds using the graph method (Nunes et al., 2005). The center circle represents the mean of the environment associated with the standardized value Zij, (3), and the axes refer to the 19 environments.

Grain yield, 100-grain weight, and protein concentration

In addition to IC and ZC, other traits are nutritionally important, such as PC in grains, and commercially important, such as grain yield and W100. For a biofortified cultivar to be successful, it should also have a good phenotypic performance for these traits.

Joint analyses for PC, grain yield, and W100 detected significant differences among genotypes, environments, and G × E interactions (Table 1). For the three traits, the environment effect also represented a large part of the total variation, at 51% for PC, 85% for grain yield, and 42% for W100, similarly to those obtained in other studies (Buratto et al., 2009; Pereira et al., 2012, 2017). In comparison, the environmental effect was the most important for grain yield, which has also been reported in other studies (Pereira et al., 2012, 2017). Environmental differences were confirmed by the variation in the mean values of the environments: from 23.0 to 29.2% for PC, from 709 to 5,189 kg ha^{-1} for grain yield, and from 17.3 to 26.9 g for W100.

The mean PC values of the genotypes ranged from 23.4 to 30.3% (Table 2). The amplitude of variation obtained was greater than that reported in other studies (Buratto et al., 2009), probably because of genetic differences among the genotypes evaluated. Brasil 0001 exhibited the highest PC and the other 20 genotypes, with PC ranging from 25.2% (BRS Estilo) to 27.1% (Piratã 1), were considered to have good PC levels. In addition to having the highest PC, the genotypes G 6492, Xamego, and BRS Supremo from the black-market class; BRS Cometa, BRS Sublime, and BRS FC402 from the *carioca* market class; and Piratã 1 from the *mulatinho* market class and Brasil 001 also had high IC, ZC, and high adaptability and stability for IC and ZC (Table 2).

For grain yield, the mean values of the genotypes ranged from 1,433 to 2,597 kg ha^{-1}, representing 81% variation among the genotypes (Table 2). Four groups of mean values were calculated. The first was composed of BRS FP403, BRS Esplendor, CNFP 11995, BRS Esteio, and IPR Uirapuru, all black-seeded cultivars. The second group was composed of 15 genotypes, including the cultivars BRS Estilo and Pérola, which have a high yield potential (Pereira et al., 2012). Thus, it can be considered that these 20 genotypes exhibited adequate grain yield for commercial production. The cultivars BRS FC402 and BRS Sublime, with high IC, ZC, and PC, adaptability, and stability for IC and ZC, also stood out with high yields.

For W100, the mean values of the genotypes ranged from 17.5 to 25.9 g, confirming the existence of genetic variability (Table 2). IAC Alvorada, CNFC 10729, BRS FP403, Pérola, and BRS Ametista had the highest numbers of grains. Another 13 genotypes also presented grains with W100 equal to or better than that of BRS Estilo, which has a grain size that is highly accepted commercially (Pereira et al., 2012).

Identification of superior cultivar for the five traits

In terms of recommendation to Brazilian farmers, among the existing common bean cultivars, BRS Sublime, of the *carioca* grain type, presents excellent agronomic and commercial potential, high IC, ZC, and PC, and phenotypic stability, being the most suitable for cultivation in order to obtain grains with higher nutritional quality and high economic return to the farmers.

Another important conclusion is that studies are needed to identify which environmental factors (soil and climate) are important in the expression of IC and ZC, so that appropriate environmental conditions can be identified to maximize the genetic potential of cultivars with higher IC and ZC.

Correlations among the traits

In terms of genetic relationships among the traits evaluated, the estimate of phenotypic correlation between IC and ZC was significant and positive (0.70), indicating a strong association between these minerals (Table 3). Some authors have found associations between IC and ZC ranging from absent to strong (Amongi et al., 2018; Blair et al., 2009; Caproni et al., 2020; Guzmán-Maldonado et al., 2003; Martins et al., 2016; Mukamuhirwa et al., 2015; Nchimbi-Msolla & Tryphone, 2010; Pereira et al., 2014; Silva et al., 2012). In general, the correlations between IC and ZC ranged from -0.08 to 0.75, with a mean of 0.50, based on the 23 estimates obtained in the studies cited.

The estimate of the genetic correlation between IC and ZC was significant and positive (0.75), similar to that of the phenotypic correlation (Table 3). The genetic correlation between IC and ZC has been estimated by various studies and all obtained significant and positive estimates: 0.42 (Silva et al., 2012), 0.62 (Pereira et al., 2014), and 0.85 (Martins et al., 2016). These values indicate that the genes that control these traits are linked and/or pleiotropic, or a result of selection or other evolutionary forces.

Table 3
Estimates of phenotypic (superior diagonal) and genetic (inferior diagonal) correlations between iron concentration (IC), zinc concentration (ZC), seed yield, 100-grain weight (W100), and protein (PC) concentration in common bean

Trait	IC	ZC	PC	Yield	W100
IC	-	0.70**	0.37*	-0.34*	-0.45**
ZC	0.75**	-	0.67**	-0.54**	-0.53**
PC	0.44**	0.75**	-		-0.34*
Yield	-0.39*	-0.59**	-0.62**	-	0.35*
W100	-0.50**	-0.56**	-0.38*	0.37*	-

** and * significant at 1% and 5% probability, respectively, by the *t* test.

As the estimates of phenotypic (rf) and genetic (rg) correlations were similar, the estimates of genetic correlations were emphasized, which is more important from the breeding perspective. The association between IC and PC was significant, positive, and intermediate, and that between ZC and PC was significant, positive, and high. Guzmán-Maldonado et al. (2003) did not find significant association between IC and PC, but found significant, positive, and low association between ZC and PC (0.25). The positive associations observed between IC, ZC, and PC are desirable because they facilitate the selection of genotypes that combine high concentrations of these nutrients.

The genetic correlation between IC and grain yield was significant, negative, and of low magnitude, which indicates an inverse association (Table 3), similar to the results of Ribeiro et al. (2014). The estimate of the genetic correlation between ZC and yield was significant, negative, and of intermediate magnitude. In contrast, the estimate of the correlation by Ribeiro et al. (2013) was significant, positive, and of low magnitude (0.35) between these traits.

The estimates of genetic correlation between IC and W100 and between ZC and W100 were significant, negative, and of intermediate magnitudes (Table 3), indicating higher concentrations of iron and zinc in smaller common bean grains. Guzmán-Maldonado et al. (2003) and Mukamuhirwa et al. (2015) found no significant association between IC and W100 but found a significant, negative, and intermediate association (-0.56) between ZC and W100.

The differences between the correlations obtained in this study and those of other authors can be attributed to the origin of the genetic material. It should be highlighted that, in this study, the controls with high IC and ZC had low grain yields and W100. These are genotypes with lower adaptation to Brazilian growing conditions, which may explain the positive correlation between IC and ZC and PC, and the negative correlation between IC and ZC with grain yield and W100 (Table 2).

This reinforces the importance of evaluating grain yield and W100 in breeding programs with a view toward obtaining cultivars that have not only high IC and ZC but also high yield and commercial grain size. Associations of low magnitude indicate that it is possible to overcome or "break" this association and join desirable phenotypes for the traits in a single genotype, as in the cultivar BRS Sublime, which joins high yield, commercial grain size, and high IC and ZC.

Conclusions

There is genetic variability among the common bean genotypes for iron, zinc, and PC; grain yield, and W100, indicating the possibility of obtaining cultivars with high agronomic potential and grains with higher nutritional quality. The environmental effect is important in the expression of these traits, but it is more important for grain yield and iron, zinc, and protein concentrations. The interaction between genotype and environment is important for the expression of these traits.

The cultivar BRS Sublime, with carioca grain type, combined grains with high iron, zinc, and protein concentrations, high W100, and high grain yield, is the most suitable for cultivation in order to obtain grains with higher nutritional quality and high economic return to the farmers.

There were positive genetic correlations between iron, zinc, and protein concentrations, indicating that the selection for high iron concentrations results in high zinc and protein concentrations.

Acknowledgments

Our thanks to the Embrapa Arroz e Feijão and to the Harvest Plus Program for funding this study. To the CNPq for funding the study and granting scholarships of productivity in technological development and innovative extension to TLPOS, LCM, PGSM, and HSP. We would like to thank the Fundação de Amparo à Pesquisa do Estado de Goiás for granting a doctoral scholarship to PRCP and the Embrapa Produtos e Mercado (branch offices of Ponta Grossa and Brasília), the Universidade Federal de Goiás, the Empresa Mato-grossense de Pesquisa, Assistência e Extensão Rural, the Instituto Agronômico de Pernambuco, the Embrapa Agropecuária Oeste, the Embrapa Milho e Sorgo, and the Embrapa Tabuleiros Costeiros for support in carrying out field trials.

References

Amongi, W., Mukankusi, C., Sebuliba, S., & Mukamuhirwa, F. (2018). Iron and zinc grain concentrations diversity and agronomic performance of common bean germplasm. *African Journal of Food, Agriculture, Nutrition and Development, 18*(3), 13717-13742. doi: 10.18697/ajfand.83.17070

Association of Official Analytical Chemist (2005). *Official methods of analysis* (18nd ed.). AOAC International.

Beebe, S., Gonzalez, A. V., & Rengifo, J. (2000). Research on trace minerals in the common bean. *Food and Nutrition Bulletin, 21*(5), 387-391. doi: 10.1177/156482650002100408

Blair, M. W., Astudillo, C., Grusak, M. A., Graham, R., & Beebe, S. (2009). Inheritance of seed iron and zinc concentrations in common bean (Phaseolus vulgaris L.). *Molecular Breeding, 23*(11), 197-207. doi: 10.1007/s11032-008-9225-z

Buratto, J. S., Moda Cirino, V., Scholz, M. B. S., Langame, D. E. M., Fonseca, N. S., Jr., & Préte, C. E. C. (2009). Variabilidade genética e efeito de ambiente para teor de proteína em grãos de feijão. *Acta Scientiarum, 31*(4), 593-597. doi: 10.4025/actasciagron.v31i4.910

Caproni, L., Raggi, L., Talsma, E. F., Wenzl, P., & Negri, V. (2020). European landrace diversity for common bean biofortification: a genome-wide association study. *Nature, 10*(19775), 1-13. doi: 10.1038/s41598-020-76417-3

Cochran, W. G. (1954). The combination of estimates from different experiments. *Biometrics, 10*(1), 101-129. doi: 10.2307/3001666

Cruz, C. D. (2013). GENES- a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum, 35*(3), 271-276. doi: 10.4025/actasciagron.v35i3.21251

Cruz, C. D., Regazzi, A. J., & Carneiro, P. C. S. (2012). *Modelos biométricos aplicados ao melhoramento genético* (4a ed.). UFV.

Feijão (2019). *Dados conjunturais do feijão no Brasil de 1985 a 2019.* http://www.cnpaf.embrapa.br/ socioeconomia/index.htm

Guzmán-Maldonado, S. H., Martínez, O., Acosta-Gallego, A., Guevera-Lara, F., & Paredes-López, O. (2003). Putative quantitative trait loci for physical and chemical components of common bean. *Crop Science, 43*(3), 1029-1035. doi: 10.2135/cropsci2003.1029

Instituto Adolfo Lutz (2008). *Métodos físico-químicos para análise de alimentos.* Instituto Adolfo Lutz.

Martins, S. M., Melo, P. G. S., Faria, L. C., Souza, T. L. P. O., Melo, L. C., & Pereira, H. S. (2016). Genetic parameters and breeding strategies for high levels of iron and zinc in *Phaseolus vulgaris* L. *Genetics and Molecular Research, 2*(15), 1-14. doi: 10.4238/gmr.15028011

Mukamuhirwa, F., & Rurangwa, E. (2018). Evaluation for high iron and zinc content among selected climbing bean genotypes in Rwanda. *Advances in Crop Science and Technology, 2*(6), 1-6. doi: 10.4172/2329-88 63.1000344

Mukamuhirwa, F., Tusiime, G., & Mukankusi, M. C. (2015). Inheritance of high iron and zinc concentration in selected bean varieties. *Euphytica, 205*(2), 349-360. doi: 10.1007/s10681-015-1385-4

Nchimbi-Msolla, S., & Tryphone, G. M. (2010). The effects the environment on iron and zinc concentrations and performance of common beans (Phaseolus vulgaris L.) genotypes. *Asian Journal of Plant Sciences, 9*(8), 455-462. doi: 10.3923/ajps.2010.455.462

Nunes, J. A. R., Ramalho, M. A. P., & Abreu, A. F. B. (2005). Graphical method in studies of adaptability and stability of cultivars. *Annual Report of the Bean Improvement Cooperative, 48*, 182-183.

Pereira, H. S., Almeida, V. M., Melo, L. C., Wendland, A., Faria, L. C., Del Peloso, M. J., & Magaldi, M. C. S. (2012). Influência do ambiente em cultivares de feijoeiro-comum em cerrado com baixa altitude. *Bragantia, 71*(2), 165-172. doi: 10.1590/S0006-87052012005000024

Pereira, H. S., Alvares, R. C., Silva, F. C., Faria, L. C., & Melo, L. C. (2017). Genetic, environmental and genotype x environment interaction effects on the common bean grain yield and commercial quality. *Semina: Ciências Agrárias, 38*(3), 1241-1250. doi: 10.5433/1679-0359.2017v38n3p1241

Pereira, H. S., Del Peloso, M. J., Bassinello, P. Z., Guimarães, C. M., Melo, L. C., & Faria, L. C. (2014). Genetic variability for iron and zinc content in common bean lines and interaction with water availability. *Genetics and Molecular Research, 3*(13), 6773-6785. doi: 10.4238/2014.August.28.21

Philipo, M., Ndakidemi, P. A., & Mbega, E. R. (2020). Environmental and genotypes influence on seed iron and zinc levels of landraces and improved varieties of common bean (Phaseolus vulgaris L.) in Tanzania. *Ecological Genetics and Genomics, 15*, 1-10. doi: 10.1016/j.egg.2020.100056

Resende, M. D. V., & Duarte, J. B. (2007). Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesquisa Agropecuária Tropical, 37*(3), 182-194.

Ribeiro, N. D., Jost, E., Maziero, S. M., Storck, L., & Rosa, D. P. (2014). Selection of common bean lines with high grain yield and high grain calcium and iron concentrations. *Revista Ceres, 61*(1), 77-83. doi: 10.1590/S0034-737X2014000100010

Ribeiro, N. D., Mambrin, R. B., Storck, L., Prigol, M., & Nogueira, C. W. (2013). Combined selection for grain yield, cooking quality and minerals in the common bean. *Revista Ciência Agronômica, 44*(4), 869-877. doi: 10.1590/S1806-66902013000400025

Statistical Analysis System Institute (2008). *SAS technical report SAS/STAT software.* SAS Institute.

Scott, A. J., & Knott, M. (1974). A cluster analysis method for grouping means in the analysis of variance. *Biometrics, 30*(3), 507-512. doi: 10.2307/2529204

Silva, C. A., Abreu, A. F. B., Ramalho, M. A. P., & Corrêa, A. D. (2012). Interaction genotype by season and its influence on the identification of beans with high content of zinc and iron. *Bragantia, 71*(3), 336-341. doi: 10.1590/S0006-87052012005000037

Zimmermann, F. J. P. (2014). *Estatística aplicada à pesquisa agrícola.* EMBRAPA Arroz e Feijão.