Genetic parameters and breeding strategies for high levels of iron and zinc in *Phaseolus vulgaris* L.

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ABSTRACT. One of the current focus of common bean breeding programs in Brazil is to increase iron (FeC) and zinc content (ZnC) in grains. The objectives of this study were to estimate genetic parameters for FeC and ZnC in common bean, verify the need for conducting multi-site evaluation tests, identify elite lines that combine high FeC and ZnC with good adaptability, stability, and agronomic potential, and examine the genetic association between FeC and ZnC. Elite lines (140) were evaluated for important agronomic traits in multiple environments. In one trial, FeC and ZnC were evaluated and genetic parameters were estimated. Based on the high heritability estimates and significant selection gains obtained, the conditions for a successful selection was favorable. Of the 140 evaluated lines, 17 had higher FeC and ZnC, and were included in the validation test (2013, five environments), specifically for the evaluation of FeC and ZnC. The line by environment interaction for FeC and ZnC was detected, but it was predominantly simple. The environmental effect strongly influenced FeC and ZnC . The environment Brasília/rainy season was selected as

the best evaluation site for preliminary tests for FeC and ZnC, because it resulted in similar conclusions as the mean of the five environments. The lines CNFP 15701 and CNFC 15865 had higher FeC and ZnC and were highly adaptable and stable, and are recommended for utilization in breeding programs. The lines CNFC 15833, CNFC 15703, and CNFP 15676 showed excellent combined agronomic and nutritional traits, and were selected for the development of biofortified cultivars. Additionally, the genetic association between FeC and ZnC was detected.

Key words: Common bean; Elite lines; Biofortification; Line by environment interaction

INTRODUCTION

Low levels of iron and zinc in the human body are among the most common nutritional deficiencies in the world (Eguílaz et al., 2010). Therefore, is an imminent need for quality, low-cost foods that meet nutritional demands, especially in developing countries (Ribeiro, 2011). Breeding can increase the bioavailability of mineral concentrations in the edible portions of plants (White and Broadley, 2005), thereby providing enhanced nutritional quality, without additional food costs. Common bean (*Phaseolus vulgaris* L.) is an excellent option for this breeding because it is the most widely consumed legume in the world, and part of the daily diet of the Brazilian people (Broughton et al., 2003).

The availability of common bean cultivars with nutritional qualities, along with important agronomic traits, would contribute to the sustainability of the common bean production chain. To obtain a cultivar with all these properties, the best strategy is to assess the nutritional composition of the elite lines of breeding programs, which already have favorable agronomic traits. However, one of the current focus of common bean breeding programs in Brazil is to increase the iron (FeC) and zinc content (ZnC); therefore, more genetic variability studies are required to estimate genetic and phenotypic parameters, the genetic associations between these traits, and the genotype by environment interactions.

Several studies have reported the presence of genetic variation, indicating the possibility of selecting lines with higher content (Araújo et al., 2003; Ribeiro et al., 2008; Silva et al., 2012a). Although some lines with high FeC and ZnC have been identified, there are few reports of breeding programs that obtained improved lines for these minerals (Blair et al., 2009; Silva et al., 2012b; Pereira et al., 2014).

It is necessary to determine the correlation between FeC and ZnC in the grain, since this association can facilitate or hamper the selection of lines with high concentrations of both these minerals. Some studies reported positive intermediate correlations between FeC and ZnC (Cichy et al., 2009; Blair et al., 2009, 2010), but these were phenotypic and not genetic correlations. Most studies indicated that the inheritance of FeC and ZnC is quantitative (Cichy et al., 2009; Blair et al., 2009, 2010), although another study identified monogenic inheritance for ZnC (Cichy et al., 2005).

A previous study noted that there was a relevant genotype by environment interaction for these two minerals (Cichy et al., 2009), while other studies detected importance only for ZnC (Ribeiro et al., 2008; Blair et al., 2011) or FeC (Araújo et al., 2003). In contrast, the

interaction has also been reported to be absent, or of little significance, for the two traits or only FeC (Ribeiro et al., 2008; Blair et al., 2011). Additionally, most of the studies addressing the identification of potential parents for these traits were evaluated in only a few environments. Since these traits are possibly quantitative, it is expected that the effect of the environment is strong, and that the genotype by environment interaction is also important.

Based on the above, the objectives of this study were: to estimate genetic and phenotypic parameters for FeC and ZnC in common bean grains; to detect effects of genotype by environment interaction; to determine the relevance of performing tests in different environments for selection of nutritionally superior lines; to identify elite lines that combine high FeC and ZnC with good adaptability and stability, and agronomic potential; and to detect genetic associations between FeC and ZnC in common bean grains.

MATERIAL AND METHODS

Preliminary tests

Preliminary tests are a step in the common bean breeding program of the Embrapa Rice and Beans, and are performed to evaluate breeding lines previously selected for various traits. These tests are used for the selection of lines that will eventually constitute the trials used to select cultivars, and therefore are evaluated in various environments and with plot sizes adequate for agronomic evaluation. One hundred and forty elite lines with different grain types, and derived from different crosses, were evaluated, and were grouped by grain type: carioca (cream with brown stripes), 68 lines; black, 30 lines; mulatinho (cream-colored grain), 16 lines; early-cycle carioca, 14 lines; and purple, 12 lines. The tests were conducted in 2011 at five environments (dry season: Carira-SE, Lavras-MG, and Lambari-MG; rainy season: Ponta Grossa-PR; and winter: Santo Antônio de Goiás-GO), using a randomized complete block design (three replicates) and plots 4.0 m long with two rows. Phenotypic evaluations were conducted for grain yield, plant architecture, lodging tolerance, and reaction to anthracnose (caused by *Colletotrichum lindemuthianum*) and angular leaf spot (caused by *Phaeoisariopsis griseola*), as described by Melo (2009). The FeC and ZnC in the grain were evaluated only in the Santo Antônio de Goiás trial, using samples of 50 grains from each plot (two replicates).

Validation test

Based on the results of the preliminary tests, 17 high FeC and ZnC elite lines/cultivars were selected, and validation tests were conducted to confirm the FeC and ZnC of the lines, and the effects of the genotype by environment interaction. In addition to these lines, five control genotypes were used (Pereira et al., 2014), three of which had high FeC and ZnC (G 6492, BRS Supremo, and BRS Cometa) and two cultivars had intermediate levels (Pérola and BRS Agreste).

The experiment was designed as a randomized block design (two replicates), with 3 m long one-row plots. In 2013, validation tests were established in five environments: Ponta Grossa-PR (dry season); Santo Antônio de Goiás-GO (winter season); and Ponta Grossa-PR, Santo Antônio de Goiás-GO, and Brasília-DF (rainy season). In these tests, only FeC and ZnC content was evaluated. Agronomic evaluation was not carried out in validation test because in preliminary tests these evaluations were performed with appropriately sized plots.

Determining grain concentrations of iron and zinc

FeC and ZnC was determined from one sample per plot. This sample was analyzed by acid digestion of organic matter using a nitric:perchloric (2:1) mixture and atomic absorption flame spectrophotometry, adapted from the methods by the Association of Official Analytical Chemists (1995). After harvest, the grains were rapidly washed with deionized water and then dried (to 6.0% moisture) in an oven at 60°C for 12 h. The dry beans were ground in a zirconium oxide ball mill (Retsch, MM200), sieved (≤200 mesh), and stored in polytetrafluoroethylene containers to prevent contamination of the metallic elements. The samples were weighed the day after grinding, to determine moisture balance, and then pre-digested with the acid mixture (50°C/30 min), followed by the acid digestion process (100°C/30 min; 170°C/3 h; cooling at room temperature; new addition of 2.0 mL of acid mixture; and digestion at 170°C/3 h). The extract was adequately diluted and read on an atomic absorption spectrophotometer (Agilent/Varian SpectrAA 50 B model), pre-calibrated with the standard curve for iron and zinc. Mean data were expressed on dry basis, based on moisture concentration of the sample, and obtained by the gravimetric method at 105°C to constant weight (Instituto Adolfo Lutz, 1995). The glassware and equipment used in the analysis were treated with a special washing process, including a decontamination step with a 5.0% nitric or hydrochloric acid solution (v/v) to eliminate any sources of contamination.

Genetic and statistical analysis

In the preliminary tests, individual variance analyses (ANOVA) were performed for all traits in all environments, using the F test. In the analyses of variance for FeC and ZnC, except for the mean effect, all effects of the model were considered random. The genotype effect was considered random because the lines had never undergone selection for these nutrients and represented a population of elite lines obtained from the Embrapa breeding program. For the analysis of agronomic traits, the effects of means and lines were considered fixed. The joint analysis was performed only for grain yield, and considered the environmental effect as fixed.

For the other traits, the evaluations were carried out in only one replication per environment. The assessment in a particular environment was considered as one replication in a single randomized block analysis. For FeC and ZnC, genetic and phenotypic parameters were estimated, such as genetic variances, environmental variances, genetic variation coefficients, index b, heritability and associated error, and expected gains with selection, with a selection intensity of 20% of the best lines (Ramalho et al., 1993).

In the validation test, individual and joint analyses of variance were performed for FeC and ZnC. The environmental and line effects were considered fixed, because the lines had already been selected for these traits in the preliminary tests and, consequently, no longer represented the original variability of the population. Means were compared using the Scott-Knott test at 10% of probability (Scott and Knott, 1974). The experimental precision was evaluated using selection accuracy (SA; Resende and Duarte, 2007).

In the joint analysis, the contribution of each source of variation to the total variation was estimated using the coefficient of determination (Ramalho et al., 1998). Additionally, stability and adaptability for FeC and ZnC of the lines were analyzed by the method proposed by Nunes et al. (2005). This method standardizes the mean of the lines in the different environments by using 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 line i in environment j, $\bar{y}_{ij}$ is the mean of line i in environment j, $\bar{y}_{.j}$ is the mean of environment j, and $S_{.j}$ is the phenotypic standard deviation between the means of lines in environment j, given by:

$$S_{.J} = \sqrt{\sum_{i=l}^{t} \left(\frac{\bar{y}_{ij} - \bar{y}_{.j}}{t-1}\right)^2}$$

Then, a constant (two) was added to the obtained z_{ij} values to eliminate negative values. In this way, the mean of the z_{ij} values for one line is a measure of adaptability of the line (z_i), and the coefficient of variation for z_{ij} is an estimator of stability for each line (CV_i). For the interpretation of stability, the stability estimator was considered excellent for lines with CV_i below 20, good for lines between 20 and 30, and poor for lines above 30.

The importance of the simple part of the genotype by environment interaction was determined by estimating the Spearman correlations between the means of FeC and ZnC in the different environments. The association between FeC and ZnC was assessed by estimating the phenotypic and genetic correlations between FeC and ZnC based on the mean of 22 lines tested in each of the five environments, and also based on the general mean of the lines (Vencovsky and Barriga, 1992). SAS and GENES (Cruz, 2013) software were used for the statistical and genetic analyses.

RESULTS AND DISCUSSION

Preliminary tests

For the carioca, black, and mulatinho types, there was genetic variability between lines for FeC and ZnC, showing the presence of at least one contrast statistically different from zero ($P \leq 0.01$). For the other grain type, there was not genetic variability. Regarding to the genetic parameters, the genetic variance in relation to the environmental variance was high for all grain types. The heritability estimates were different from zero (52.6 - 83.8% for FeC, and 41.7 - 95.7% for ZnC; medium to high magnitude), indicating the possibility of successful selection for FeC and ZnC in grain (Table 1).

These heritability values were similar to those reported in other studies, which showed estimates from 29.0 - 84.0% for both traits, considering lines obtained from different parents in biparental crosses (Cichy et al., 2005; Jost et al., 2009; Rosa et al., 2010; Buratto, 2012). However, Silva et al. (2012a) reported higher heritability estimates (97.9% for FeC and 97.4% for ZnC) in lines from different genetic backgrounds.

The genetic variation coefficients (5.58 - 9.00% and 8.51 - 9.04% for ZnC and FeC, respectively) indicated favorable conditions for selection of these two nutrients (Table 1). Index b ranged from 0.85 - 4.72 for ZnC and from 1.05 - 2.28 for FeC, being considered adequate (Resende and Duarte, 2007). The expected selection gain ranged from 5.05% (carioca, Zn) to 12.52% (mulatinho, Zn; Table 1) and these values are similar to those reported by Jost et al. (2009), Rosa et al. (2010), and Buratto (2012). Overall, as a result of the genetic variability and high heritability of these traits, there is good potential for selection of lines with higher FeC and ZnC.

Table 1. Estimates for genetic variance (σ^2_g), phenotypic variance (σ^2_F), environmental variance (σ^2_e), narrow-sense heritability (h^2), associated error [$S(h^2)$], coefficient of genetic variation (CV_g), index b, and expected gain with selection (GS%), for iron and zinc concentrations in common bean grains, tested in Santo Antônio de Goiás during the winter of 2011.

	σ^2_g	σ^2_F	σ^2_e	h^2	$S(h^2)$	CV_g	b	GS (%)	Mean
Iron concentration									
Carioca	25.02	47.53	22.51	52.64	11.40	8.51	1.05	9.07	58.74
Black	30.54	36.44	5.89	83.82	5.81	9.04	2.28	11.22	61.14
Mulatinho	30.92	38.65	7.73	79.99	10.00	8.43	2.00	11.92	65.93
Zinc concentration									
Carioca	2.66	6.37	3.71	41.70	14.04	5.58	0.85	5.05	29.21
Black	6.35	9.13	2.78	69.55	10.94	7.33	1.51	8.04	34.38
Mulatinho	9.99	10.44	0.45	95.70	2.15	9.00	4.72	12.52	35.11

Validation tests

The coefficients of variation (CV) values were low for FeC and ZnC (4.1 - 9.3%), showing good experimental precision, which could be confirmed by the estimates of selective accuracy (SA), considered as high or very high (SA ≥ 0.70) in four environments for FeC and in five environments for ZnC (Resende and Duarte, 2007). These results are similar to those obtained by Pereira et al. (2014) for the same traits.

The means for FeC in the trials ranged from 58.1 - 82.0 mg/kg, representing a variation of 41% and indicating great diversity among the environments. For ZnC, the variation between environments was 29.2-37.3 mg/kg (i.e., a difference of approximately 28%). This wide variation can be explained by the geographical differences among the evaluation locations, which represent great climatic diversity: Santo Antônio de Goiás (823 m a.s.l., latitude 16°29'W, longitude 49°18'S); Brasília (1171 m a.s.l., latitude 15°46'W, longitude 47°55'S); and Ponta Grossa (969 m a.s.l., latitude 25°05'W, longitude 50°09'S).

In addition, differences, especially for Fe and Zn, were observed in the chemical composition of the sampled soils from the experimental areas. The highest FeC value (82.0 mg/kg) was noted for the Ponta Grossa/rainy season environment (2013), which had the second highest soil iron concentration and the most acidic soil of the environments tested. In general, acidic soils favor Fe absorption (Moraghan et al., 2002). For ZnC, the highest mean value for the grain (37.3 mg/kg) was noted for the Brasília/rainy season environment (2013), where the soil zinc concentration was highest. Cichy et al. (2005) reported that ZnC in common bean grains is changed by the soil zinc concentration. According to Corte et al. (2003), the nutritional quality of common bean grains is determined by the genotype, and influenced by the prevailing environmental conditions during the plant cycle and grain development. Thus,

changes in the chemical composition of common bean grains can be expected in response to soil conditions (soil type, pH, fertility, texture, and organic matter) and to climatic variations (temperature, precipitation, photoperiod, and radiation; Ribeiro, 2010).

The joint analysis detected the presence of variability between lines for FeC ($P \leq$ 0.01) and ZnC (Table 2), indicating that even after the selection of lines with higher FeC and ZnC in preliminary tests, variation was still observed among the 22 elite lines. Several studies reported the presence of genetic variability for FeC and ZnC (Araújo et al., 2003; Ribeiro et al., 2008; Silva et al., 2012a). However, most evaluated accessions from genebanks or lines derived from biparental crosses (Beebe et al., 2000; Gelin et al., 2007; Ribeiro et al., 2008; Silva et al., 2012a; Carvalho et al., 2012; Pereira et al., 2014). Variability between lines with high agronomic potential has been rarely reported in the literature.

Table 2. Summary of the joint analysis of variance for iron and zinc grain concentration (mg/kg), using 22 common bean lines evaluated in five environments[1] in validation tests during 2013.

SV[2]	d.f.[3]	Iron concentration				Zinc concentration			
		SS[4]	MS[5]	P value	R[2][6]	SS	MS	P value	R[2]
Block/environment	5	168.2	33.6	-	0.01	136.6	27.3	-	0.02
Lines (L)	21	4,014.1	191.1	0.001	0.15	1,428.7	68.0	0.001	0.26
Environments (E)	4	17,094.8	4,273.5	0.001	0.64	2,709.6	677.4	0.002	0.49
L x E	84	3,310.0	39.4	0.001	0.12	679.1	8.1	0.059	0.12
Residue	105	2,059.0	19.6	-	0.08	615.4	5.9	-	0.11
Total	219	26,645.5	-	-		5,569.5	-	-	
Mean			65.4				33.56		
CV[7] (%)			6.8				3.70		
SA[8]			0.95				0.96		

[1]Five environments: dry (Ponta Grossa-PR); winter (Santo Antônio de Goiás-GO); rainy (Ponta Grossa-PR, Santo Antônio de Goiás-GO, and Brasília-DF); [2]SV: source of variation; [3]d.f.: degrees of freedom; [4]SS: sum of squares; [5]MS: mean square; [6]R[2]: coefficient of determination, obtained by ratio of the sum of squares of the respective effect by the total sum of squares; [7]CV: coefficient of variation; [8]SA: selection accuracy.

There were also significant differences between environments, confirming the results from the individual analyses. The environmental effect accounted for 64% of the total variation for FeC and 49% for ZnC (Table 2), indicating that the cultivation environment should be considered in the selection of biofortified cultivars to enable the expression of the full genetic potential of the lines.

Silva et al. (2012b) and Nchimbi-Msolla and Tryphone (2010) also detected a strong environmental effect on the expression of these traits. According to the authors, the line by season interaction has an influence, although low, on the grouping of lines with high and low nutrient levels. In Rio Grande do Sul, Brazil, Ribeiro et al. (2008) found no significant effect of the interaction of cultivar by location for FeC, but observed significance for ZnC. In this study, the line by environment interaction was significant for FeC and ZnC ($P \leq 0.06$). In other words, the lines had different responses to environmental variations (Table 2), in agreement with results for FeC in Paraná (Araújo et al., 2003) and for FeC and ZnC in Goiás, Brazil (Pereira et al., 2014). Most research that has focused on the genetic control of FeC and ZnC in grains has reported that the inheritance is quantitative (Cichy et al., 2009; Blair et al., 2009, 2010). A pronounced genotype by environment interaction is therefore also expected, which is considered important for most studies with these traits (Araújo et al., 2003; Cichy et al., 2009; Pereira et al., 2014).

Genetic and environmental factors, and the genotype by environment interaction, affected FeC and ZnC in common bean grains. Thus, the identification of elite lines with

adaptability and stability for these traits is extremely important, particularly since common bean is grown in most Brazilian states in three growing seasons per year, and using different levels of technology.

Six and five elite lines had particularly high FeC and ZnC, respectively, with higher means than the controls for high FeC and ZnC (BRS Supremo, G 6492, and BRS Cometa; Table 3). The four superior lines for FeC (CNFP 15701, CNFRx 15602, CNFC 15865, and CNFM 15632) are worth mentioning, not only because they had the highest means, but also because they had the highest adaptability indices (Z_{ij}) and represented four different types (black, roxinho, carioca, and mulatinho). These four lines were also superior in the preliminary tests and repeated their good performance in the validation testing. The mean of the first four lines was 7% higher than the overall mean and 11% above the mean of the cultivar Pérola, which is the most widely cultivated cultivar in Brazil (Table 3).

The most stable lines were CNFM 15632 and CNFC 15703, with CV_i lower than 20. For line CNFRx 15602, despite the high FeC and good adaptability, it was not very stable as it had inconstant performance in the different environments. The lines CNFP 15701, CNFC 15865, and CNFM 15632 had high mean, high adaptability, and good phenotypic stability (Table 3).

For ZnC, the four superior lines (CNFRx 15602, CNFP 15701, CNFRx 15865, and CNFC 15598) had high means and the highest adaptability indices (Z_{ij}). These first four lines also stood out in the preliminary tests. The mean of the first four lines was 10% higher than the overall mean and 20% higher than the control, Pérola (Table 3). The most stable lines, having an ideal CV_i ($CV_i < 20$), were CNFP 15701, CNFC 15833, CNFC 15865, and CNFRx 15602. Therefore, the four lines cited initially (CNFRx 15602, CNFP 15701, CNFRx 15865, and CNFC 15598) associated high mean ZnC with high adaptability and good stability.

Table 3. Iron and zinc grain concentrations (means; mg/kg) and adaptability and stability parameters of 22 common bean lines evaluated in five environments in validation tests during 2013.

Line	GT[1]	Iron concentration			Zinc concentration		
		Means[2]	Z_i[3]	CV_i[4]	Means	Z_i	CV_i
CNFP 15701	Black	71.7[a]	3.18	21.8	37.3[a]	3.27	11.7
CNFRx 15602	Purple	71.1[a]	2.75	41.1	37.1[a]	3.29	17.8
CNFC 15865	Carioca	70.9[a]	3.16	25.4	37.2[a]	3.13	16.5
CNFM 15632	Mulatinho	69.0[a]	2.76	16.4	34.9[b]	2.45	32.6
CNFP 15685	Black	68.8[a]	2.55	35.3	36.1[a]	2.64	38.3
CNFP 15676	Black	68.8[a]	2.46	20.7	33.4[b]	1.98	26.6
CNFC 15833	Carioca	68.1[b]	2.56	39.5	34.5[b]	2.32	14.9
BRS Esplendor	Black	67.8[b]	2.42	33.9	34.2[b]	2.29	26.6
CNFP 15678	Black	67.3[b]	2.29	25.1	34.6[b]	2.39	20.7
BRS Supremo[5]	Black	67.0[b]	2.30	22.4	34.0[b]	2.16	26.7
CNFC15704	Carioca	66.1[b]	2.11	33.3	30.1[d]	0.82	40.1
G 6492[5]	Black	66.1[b]	2.14	24.1	35.2[b]	2.45	37.2
CNFC 15703	Carioca	65.8[b]	2.04	16.7	32.6[c]	1.73	35.6
CNFM 15647	Mulatinho	65.2[b]	2.06	25.3	34.3[b]	2.36	27.3
CNFRx 15598	Purple	64.8[b]	1.89	22.5	36.4[a]	2.88	25.7
BRS Agreste[5]	Mulatinho	63.2[c]	1.71	31.6	29.8[d]	0.79	74.4
Pérola[5]	Carioca	62.8[c]	1.57	39.2	29.8[d]	0.86	38.9
IAC Centauro	Mulatinho	61.0[c]	1.18	22.9	29.5[d]	0.75	52.0
CNFC 15852	Carioca	60.8[c]	1.17	77.7	32.1[c]	1.51	30.3
CNFC 15839	Carioca	59.1[d]	0.72	105.3	31.9[c]	1.43	24.7
BRS Cometa[5]	Carioca	58.5[d]	0.73	59.3	32.8[c]	1.73	20.3
BRS MG Tesouro	Purple	55.4[d]	0.25	225.3	29.9[d]	0.75	122.8
Means		65.4			33.6		

[1]Grain type; [2]Means followed by the same letter do not differ significantly at the level of 10% (Scott-Knott procedure). [3]Z_i: value of the standard variable (adaptability parameter); [4]CV_i: coefficient of variation of the standard variable for each line (stability parameter). [5]Control lines.

The performance of these lines is shown in Figure 1, with above-mean performance in practically all environments, forming a circumscribed circle in the shape of a "full ball". This shows that they responded efficiently to environmental stimuli and with adequate performance predictability. In contrast, cultivar Pérola had less ability to accumulate FeC and ZnC in the grain than the other lines, forming a circumscribed circle in the shape of a "slack ball".
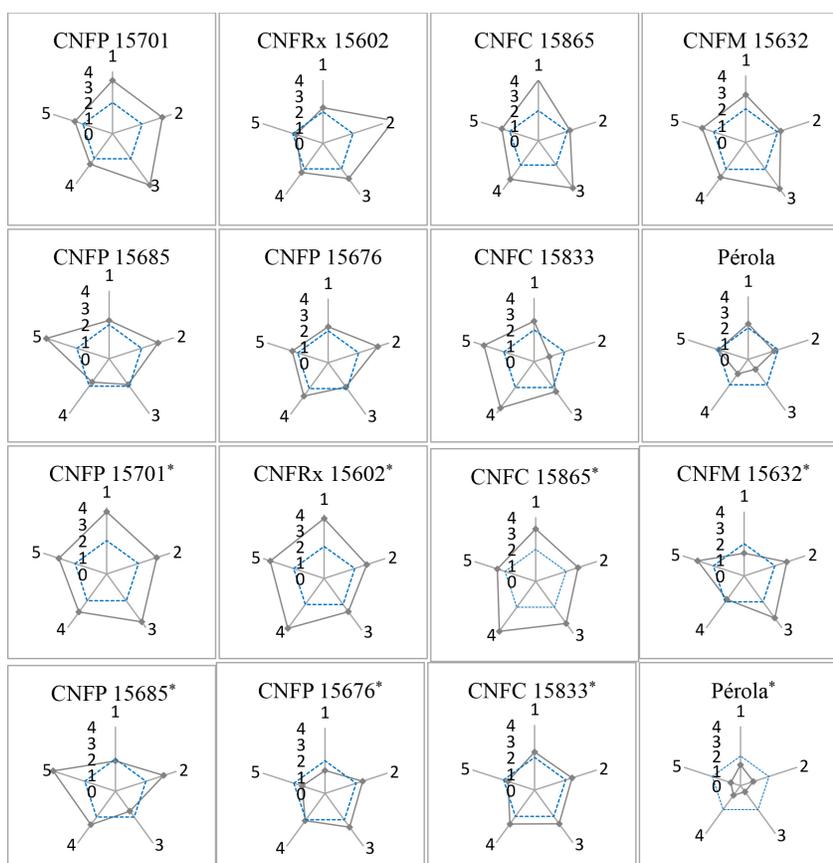

Figure 1. Performance graphs for Fe (FeC) and Zn content (ZnC) of the superior elite lines and the cultivar Pérola (control) using the diagram method (Nunes et al., 2005). 1: Ponta Grossa-PR/dry season; 2: Santo Antônio de Goiás-GO/winter; 3: Brasília-DF/rainy season; 4: Ponta Grossa-PR/rainy; 5: Santo Antônio de Goiás-GO/rainy. *ZnC.

Considering both nutritional traits, the lines CNFP 15701, CNFRx 15602, CNFC 15865, and CNFP 15685 were associated with the highest FeC and ZnC. Lines CNFP 15701 and CNFC 15865 were also highly adaptable and had good phenotypic stability (Table 3).

Elite lines facilitate the development of cultivars as they represent an outstanding group among the products of a breeding program, after undergoing several selection cycles, over several generations, for several traits. Among these, it is therefore possible to identify lines with an appropriate configuration, that is, lines that combine the different traits of interest.

For the identification of elite lines with high FeC and ZnC and superior agronomic traits, the FeC and ZnC data of the validation tests were used, together with the agronomic data from the preliminary tests. Thus, the lines were compared with controls with the same grain type, and comparisons of agronomic traits were only performed for lines with proven high FeC and ZnC.

Line CNFC 15865 had an excellent performance for FeC and ZnC in the validation tests, better than the cultivars Pérola and BRS Cometa, as well as in the preliminary test (Table 4). For grain yield, the performance of CNFC 15865 was similar to that of BRS Cometa and poorer to that of Pérola. In terms of tolerance to lodging, architecture, and reaction to anthracnose, line CNFC 15865 was superior to cultivar Pérola and similar to BRS Cometa. The lines CNFC 15833 and CNFC 15703 were also noteworthy for their intermediate nutritional performance, and with better performance for agronomic traits than all the cultivars (Table 4 and Figure 1).

Table 4. Means of elite lines and control cultivars for iron (FeC) and zinc grain concentration (ZnC), based on validation test, and grain yield (Prod; kg/ha), lodging (Lod), reaction to anthracnose (An), architecture (Arq), and reaction to angular leaf spot (Ls), (scores: 1 - 9), based on the preliminary tests.

Lines	GT[1]	FeC	ZnC	FeC[+]	ZnC[+]	Prod	Lod	An	Arq	Ls
CNFC 15865	c	70.93[a]	37.21[a]	78.05	32.40	1.933[d]	3.8[a]	2.0[a]	4.2[a]	4.0[a]
CNFC 15833	c	68.05[b]	34.51[b]	73.80	33.25	2.357[b]	4.0[b]	3.5[a]	4.7[b]	5.0[a]
CNFC 15703	c	65.80[b]	32.63[c]	62.85	28.35	2.337[a]	2.5[a]	1.0[a]	4.0[a]	-
Pérola*	c	62.80[c]	29.77[d]	66.25	29.70	2.656[a]	4.5[b]	6.0[b]	5.0[b]	4.3[a]
BRS Cometa*	c	58.49[d]	32.83[c]	62.05	30.90	1.911[d]	3.8[a]	2.5[a]	3.2[a]	4.0[a]
CNFP 15701	p	71.71[a]	37.35[a]	71.51	40.81	2.117[c]	4.4[b]	3.5[a]	3.6[b]	6.0[b]
CNFP 15676	p	68.84[a]	33.41[b]	65.15	37.84	2.557[a]	2.8[a]	1.0[a]	3.2[a]	1.0[a]
BRS Esplendor	p	67.80[b]	34.20[b]	82.64	37.74	1.685[e]	4.4[b]	1.0[a]	3.6[b]	5.5[b]
BRS Supremo*	p	67.03[b]	33.97[b]	-	-	-	-	-	-	-
G 6492*	p	66.07[b]	35.20[b]	-	-	-	-	-	-	-
CNFM 15632	m	69.01[a]	34.89[b]	73.37	39.57	2.857[b]	4.7[a]	2.0[a]	4.7[a]	-
BRS Agreste*	m	63.19[c]	29.76[d]	70.08	30.49	2.753[b]	3.0[a]	2.0[a]	3.7[a]	-
CNFRx 15602	pp	71.06[a]	37.66[a]	62.05	33.40	2.662[a]	3.0[a]	3.5[a]	4.0[a]	-
BRS MG Tesouro	pp	55.35[d]	29.89[d]	64.85	29.25	2.146[a]	3.0[a]	3.0[a]	5.0[a]	-

[1]GT Grain type; c: carioca; p: black; m: mulatinho; and r: purple.*Controls. The cultivars used in the tests are underlined. For each GT, means followed by the same letter in a column do not differ significantly at the level of 10% (Scott-Knott procedure). [+]FeC and ZnC in the preliminary tests.

For FeC and ZnC in both validation and preliminary tests, the black seeded line, CNFP 15701, performed better than the other lines (Table 4). The performance for grain yield was superior or similar for lodging tolerance, architecture, and reaction to angular leaf spot for the CNFP 15701 line compared to the BRS Esplendor line. Line CNFP 15676 on the other hand showed intermediate performance for FeC and ZnC (Figure 1), but performed better for all agronomic traits compared to the controls and other tested lines.

Line CNFRx 15602 (roxinho type) had excellent performance for FeC and ZnC in the validation tests (Table 3). In the preliminary test, no significant differences were detected for any agronomic trait, between CNFRx 15602 and BRSMG Tesouro (control for the roxinho type), suggesting a high agronomic potential of CNFRx 15602 (Table 4).

In the mulatinho grain group, the best line was CNFM 15632, which had excellent values for FeC and good values for ZnC in the validation tests (Table 3). In the preliminary test, this line performed very well, better than the control cultivar BRS Agreste, confirming the good nutritional performance. In terms of agronomic traits, only grain yield differed significantly, and the grain yield of line CNFM 15632 was similar to that of BRS Agreste.

For the development of any biofortified cultivar, it is recommended to combine

agronomic and nutritional traits, as well as adaptability and phenotypic stability. The lines CNFC 15833, CNFC 15703, and CNFP 15676 were selected for their potential as biofortified cultivars, based on their high agronomic potential, superiority in iron and zinc accumulation, great adaptability, and good stability (Tables 3 and 4; Figure 1).

Identification of the environment for preliminary assessment of nutritional traits

Once the genotype by environment interaction is detected for FeC and ZnC in the grains, the predominant type of interaction (simple or complex) must be identified, since this directly influences the selection process. From a practical point of view, it must be known whether only one environment is enough to identify promising lines or if multi-site tests are needed, which are cost-intensive.

For FeC, the Spearman correlation estimates between environments were significant, positive, and moderate to strong, ranging from 0.40 ($P \leq 0.05$) to 0.72 ($P \leq 0.01$) (Table 5). The mean correlation between the different pairs of environments was 0.51, indicating that the interaction is predominantly simple and that the classification of lines for FeC will not be significantly changed.

Table 5. Estimates of the Spearman correlation between the tested pairs for common bean and in the joint analysis, for Fe (below the diagonal line) and Zn grain concentration (above the diagonal), in mg/kg.

Environment	PG/dry[1]	SAG/win[2]	BRA/rainy[3]	PG/rainy[4]	SAG/rainy[5]	Mean[6]
PG/dry	-	0.68**	0.70**	0.70**	0.76**	0.85**
SAG/win.	0.42**	-	0.62**	0.59**	0.77**	0.76**
BRA/rainy	0.72**	0.57**	-	0.73**	0.63**	0.83**
PG/rainy	0.50**	0.40*	0.48**	-	0.70**	0.88**
SAG/rainy	0.46**	0.51**	0.48**	0.53**	-	0.90**
Mean[7]	0.72**	0.83**	0.80**	0.67**	0.69**	-

[1]PG/dry = Ponta Grossa, dry season; [2]Santo Antônio de Goiás, winter season; [3]BRA/rainy = Brasília, DF, rainy season; [4]PG/rainy = Ponta Grossa, rainy season; [5]SAG/rainy = Santo Antônio de Goiás, rainy season; [6]correlation between means of the joint analysis and means of the specific environment, for ZnC; [7]correlation between means of the joint analysis and means of the specific environment, for FeC. * and **: significant at 5% and 1% (*t*-test), respectively.

As a precaution, it is therefore recommended for FeC that in the final tests, where the number of lines is restricted and the selection pressure is strong, the evaluations be carried out in several environments. On the other hand, in preliminary tests, with lower selection pressure and a high number of lines, any of the five environments can be used as evaluation sites, since all were correlated with each other. The environment Brasília-DF/rainy stood out, since, of the five elite lines selected based on the combined mean, three coincided with the selection in this environment, indicating that this environment would have selected the same lines as the mean of the five environments.

For ZnC, the Spearman correlations were also significant, positive, and moderate to strong, ranging from 0.59 ($P \leq 0.05$) to 0.77 ($P \leq 0.01$), with slightly higher magnitude than FeC (Table 5). The mean correlation between the different pairs of environments was 0.69, indicating the predominance of simple interaction. For ZnC, the simple interaction type was even more predominant, indicating that the genotype classification should not be changed.

All environments were strongly correlated with the mean, and any can be used for selection for increased ZnC in the grain. Since the evaluation for FeC and ZnC in the grain

is performed simultaneously, Brasília-DF/rainy is recommended as the main preliminary evaluation site for elite lines.

Relationship between iron and zinc concentrations in the grains

The phenotypic correlation between FeC and ZnC, based on the overall means of the lines, was significant ($P \leq 0.01$), positive, and high (0.74). This was confirmed by the estimates of the five tests, ranging from 0.43 ($P \leq 0.05$) to 0.83($P \leq 0.01$). The magnitude of the correlation in the different experiments varied from intermediate to high, also indicating environmental influence on the association of these traits (Table 6). Our results were similar to those of other studies (Gelin et al., 2007; Cichy et al., 2009; Blair et al., 2009, 2010, 2011), all with recombinant inbreed lines derived from crosses between one parent with high and one with low FeC and ZnC. In other studies using lines with different genetic backgrounds, a relationship between the two minerals was either refuted (Ribeiro et al., 2008) or confirmed (Nchimbi-Msolla and Tryphone, 2010; Silva et al., 2012a; Pereira et al., 2014). In general, the estimates of the above studies averaged 0.52 (ranged from -0.08 to 0.75).

Table 6. Estimates of the phenotypic (r_f), genetic (r_g), and environmental correlations (r_a) between the iron and zinc grain concentrations, based on the means of 22 common bean elite lines evaluated in each one of the five environments in validation tests during 2013 and based in general means of the lines.

Environment	r_f	r_g	r_a
Ponta Grossa-PR/dry season	0.46*	0.46 ns	0.45+
Santo Antônio de Goiás-GO/winter	0.60**	0.75++	0.13 ns
Brasília-DF/rainy season	0.83**	0.96++	0.66++
Ponta Grossa-PR/rainy	0.43*	0.54+	-0.18 ns
Santo Antônio de Goiás-GO/rainy	0.46*	0.60 ns	0.33 ns
General means	0.74**	0.81++	0.22++

*,**Significant at 5 and 1% (*t*-test), respectively; + and ++significant at 5 and 1% by the resampling by bootstrapping (Cruz, 2013).

There are few studies on the estimates of genetic correlation between FeC and ZnC in bean grains. Only the genetic correlation involves the inheritable nature of associations, which is of key interest in breeding programs (Falconer, 1987). Estimates of genetic correlations were similar to the phenotypic correlations, being positive, and intermediate to high, but significant in only three environments (Table 6). The mean genetic correlation was 0.81 ($P \leq 0.01$), indicating high association, exceeding the values observed by Silva et al. (2012a) and Pereira et al. (2014), 0.54 and 0.62 ($P \leq 0.01$), respectively. In other words, some of the genes that control these traits are linked and/or pleiotropic. The association between the two traits indicates the possibility of common metabolic pathways in the accumulation of the two minerals in common bean grain. The accumulation of both minerals in grain is controlled by the same quantitative trait loci (Blair et al., 2010).

Based on the results obtained, there is genetic variability in the FeC and ZnC between common bean elite lines from carioca, mulatinho, and black types and the selection conditions of elite lines with higher FeC and ZnC in the grains are favorable, because high heritability and significant gains with selection were observed. Additionally, the line by environment interaction for FeC and ZnC in common bean is predominantly simple; however, the environmental effect

strongly influences FeC and ZnC in bean grains. The location Brasília-DF, in the rainy season, was identified as the best location for assessments of preliminary tests.

Furthermore, the lines CNFP 15701 and CNFC 15865 were not only superior in accumulating Fe and Zn in the grains, but also had high adaptability and good phenotypic stability. Once the agronomic and nutritional traits were combined, the lines CNFC 15833, CNFC 15703, and CNFP 15676 were selected as candidate biofortified cultivars.

Conflicts of interest

The authors declare no conflict of interest.

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