

# Recurrent selection for high iron and zinc concentrations in black bean grain

Lara Rodrigues de Queiroz<sup>1</sup> , Ludivina Lima Rodrigues<sup>1</sup> , Saulo Muniz Martins<sup>2</sup> , Thiago Lívio Pessoa Oliveira de Souza<sup>2</sup> , Leonardo Cunha Melo<sup>2</sup> , Helton Santos Pereira<sup>2,\*</sup> 

1. Universidade Federal de Goiás – Escola de Agronomia – Melhoramento de Plantas – Goiânia (GO), Brazil.

2. Embrapa Arroz e Feijão – Pesquisa – Melhoramento de Feijão – Santo Antônio de Goiás (GO), Brazil.

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\***Corresponding author:** [helton.pereira@embrapa.br](mailto:helton.pereira@embrapa.br)

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**ABSTRACT:** The iron concentration (FeC) and zinc concentration (ZnC) in common bean grain are quantitative traits, and appropriate breeding techniques are required to achieve genetic gain. The aim of this study was to obtain a recurrent selection population of black bean to increase the FeC and ZnC in the grain and to select the superior progenies for formation of the next cycle and obtain lines. The base population was formed by crosses among ten parents. A total of 351 progenies were obtained, and, after two generations of selection, the 27 best progenies were evaluated in two field trials for FeC, ZnC, 100 seed weight and yield. Analyses of variance were carried out and genetic parameters were estimated. The heritability estimates ranged from 59 to 94% for the four traits. The estimates of expected gain from direct selection for each trait (3 to 21%) and simultaneous selection (1 to 4%) indicate success from selection. The eight progenies, selected based on simultaneous selection, have superior mean values, including to those of 'BRS Supremo' (10% for FeC, 8% for ZnC, 5% for 100 seed weight and 3.8% for yield), the Brazilian black bean cultivar with the highest FeC and ZnC. The recurrent selection population shows high genetic variability and potential for obtaining lines superior to the cultivars currently on the market, allying high agronomic performance and high FeC and ZnC in the grain. Furthermore, this population shows potential for generating a new recurrent selection cycle, from recombination of the eight superior progenies.

**Key words:** *Phaseolus vulgaris*, biofortification, yield, 100 seed weight.

## INTRODUCTION

Common bean or dry edible bean (*Phaseolus vulgaris* L.) is the legume of greatest direct consumption in the world. Its prominence is also established by its high nutritional value, constituting an excellent source of protein, iron and zinc (FAO 2020); it is considered a staple food in South America, Central America and East Africa. Brazil is one of the main consumers and producers of common bean, producing approximately 2.7 million tons annually on 1.7 million hectares, with mean yield of 1,553 kg·ha<sup>-1</sup> (Embrapa 2020). The black commercial group is the second most consumed in Brazil, representing approximately 20% of the total common bean produced annually (540,000 tons), in 340,000 hectares (Pereira et al. 2012). Therefore, this type of grain has great economic and social importance.

Iron and zinc deficiency is recognized as a problem for health throughout the world, especially affecting needy families that do not have access to foods with high protein and nutrient content. Iron is essential in forming hemoglobin, and its deficiency causes anemia (Welch 2002), afflicting approximately 26% of the world population (WHO 2020). Zinc is an essential nutrient with numerous structural, biochemical and regulatory functions in human physiology. After iron, it is the micromineral of most abundant distribution in the human body. At least 17% of the world population has inadequate ingestion of zinc (Wessells and Brown 2012).



Biofortification is a technique in which agricultural crops are modified or improved to accumulate more nutrients in their edible parts (Pérez-Massot et al. 2013), and it constitutes one of the main strategies for diminishing undernutrition. Thus, through plant breeding, a product of superior nutritional quality is achieved that has low cost of production and is accessible to the population. Common bean is a crop with a high potential for biofortification, because it is one of the vegetables with the highest iron concentration in its edible part (Pereira et al. 2014).

In Brazil, people consume 50 g of beans daily (Wander 2007). An average adult needs 14 mg of iron and 7 mg of zinc per day (Institute of Medicine, 2001). Consumption of grains of the cultivar 'Pérola', the most planted in Brazil, provides 24% of iron and 27% of zinc daily demand. The supply can be expanded with the development of biofortified cultivars.

Genetic variability for these traits has been reported in various studies on this crop (Araújo et al. 2003; Beebe et al. 2000; Di Prado et al. 2019; Gouveia et al. 2014; Martins et al. 2016; Ribeiro et al. 2008; Silva et al. 2012), which indicates potential for increasing these concentrations. Regarding the interaction between genotypes and environments, some papers reported that there was a relevant genotype by environment interaction for these two minerals (Cichy et al. 2009; Martins et al. 2016), while other studies detected importance only for zinc concentration (ZnC) (Blair et al. 2011; Ribeiro et al. 2008) or iron concentration (FeC) (Araújo et al. 2003).

In addition to nutritional quality, agronomic traits such as yield, grain weight, disease resistance and others must be considered in the breeding steps because they are essential for acceptance of new cultivars by producers and consumers (Menezes Júnior et al. 2008). In plant breeding programs, most agronomic traits have quantitative inheritance, which means that they are controlled by various genes of lesser effects and are highly affected by environments (Ramalho et al. 2012). In common bean, inheritance of FeC and ZnC has also been described as polygenic (Blair et al. 2009, 2010; Cichy et al. 2009; Izquierdo et al. 2018).

Thus, breeding methods that take the complex nature of these traits into account are required for continuous genetic gain. The recurrent selection method was initially proposed for allogamous plants; however, it has been successfully used in autogamous plants, especially in common bean for various traits, such as yield, reaction to angular leaf spot, plant architecture and early maturity (Alves et al. 2015; Arantes et al. 2010; Menezes Júnior et al. 2008, 2013; Pires et al. 2014; Silva et al. 2007). Nevertheless, for nutritional traits, such as FeC and ZnC, there are no studies using recurrent selection in common bean.

Furthermore, information on genetic parameters is essential for evaluating genetic progress in each selection cycle, as well as the genetic variability obtained, and, thus, for designing efficient strategies for development of cultivars with higher nutritional value. Therefore, the aim of this study was to obtain a recurrent selection population for increasing the FeC and ZnC in common bean grain, check the effectiveness of recurrent selection in generating progenies with higher concentrations of these minerals, and select the best progenies for recombination and formation of the next recurrent selection cycle and for obtaining lines.

## MATERIAL AND METHODS

The base population was formed by crosses between ten parents that had high FeC and ZnC in the grain ('Xamego'/'Piratã 1'/'Iapar 5'/'G6495'/'BRS Grafite'/'G6492'/'4'/'BRS Supremo'/'Brasil 0001'/'Milionário'/'BRS Esplendor'/'Xamego'/'Piratã 1'); one parent was from the mulatinho commercial group ('Piratã 1'), one of brown color ('Brasil 0001'), and the others from the black commercial group (Pereira et al. 2014; 2018). The crosses and other steps of development of the progenies were performed in Santo Antônio de Goiás under protective screening in 2012 and 2013, up to formation of the base population.

In 2014, around 500 seeds obtained from the base population were sown, forming the  $C_0S_0$  generation in the winter crop season in the field in 24 4 m rows. At harvest, 351 plants were selected and harvested individually, forming the  $C_0S_{0:1}$  generation. The 351  $C_0S_{0:1}$  progenies were sown in the field in 2015, each one in a plot consisting of one 3 m row, inserting a check cultivar ('BRS Supremo') after every nine progenies. Of these progenies, 250 were discarded for not exhibiting the agronomic standard desired (i.e., the plants had very prostrate plant architecture and were susceptible to the main diseases) or for not having black grain at harvest.

Seeds of the 101  $C_0S_{0.1}$  progenies selected and of the check cultivars were sent for analysis of FeC and ZnC. From the results obtained, 64 progenies with higher FeC and ZnC were selected. These 64 progenies were once more evaluated for FeC and ZnC in the 2016 winter crop season in the  $C_0S_{0.2}$  generation. Considering the FeC and ZnC obtained in the two generations together, 27 progenies were selected with higher FeC and ZnC, which, in the  $C_0S_{0.3}$  generation, composed two experiments, including three check cultivars: 'BRS Esteio', 'BRS Supremo' and 'Xamego'. The 'BRS Supremo' and 'Xamego' cultivars have higher FeC and ZnC in the grain compared to the cultivars currently available on the market (Pereira et al. 2014); 'BRS Esteio' (Pereira et al. 2013) is currently the black bean cultivar most grown in Brazil, with excellent agronomic performance and commercial grain quality.

The trials were conducted in 2017 in Brasília (DF) and Santo Antônio de Goiás (GO) in the winter crop season (sowing in May/June and harvest from August to October). The experimental design used was randomized blocks with three replications, and plots were constituted by three 3 m rows and between-row spacing of 0.5 m. The grain from the plots was weighed for yield determination, given in  $g\cdot plot^{-1}$  and transformed into  $kg\cdot ha^{-1}$ . From each plot, 100 bean grains were removed at random for determination of 100 seed weight in  $g\cdot 100\ seeds^{-1}$ . After that, these seeds were used for determination of the FeC and ZnC in the grain.

The FeC and ZnC analyses were carried out a single time by acid digestion of the organic matter (with a 2:1 nitric-perchloric acid mixture) according to the flame atomic absorption spectrophotometry technique, adapted from the Association of Official Analytical Chemists (AOAC 1995). To check the accuracy of the analyses, for each 40 samples, one was performed in triplicate and two were laboratory control samples with known concentrations. The grain was rapidly washed with deionized water and subsequently dried in a laboratory oven at 60 °C for 12 h (to 6% moisture content). The grain was ground ( $\leq 200$  mesh) in a zirconium oxide ball mill (Retsch MM200). The sample was predigested with an acid mixture (50 °C/30 min.), followed by digestion proper (100 °C/30 min.; 170 °C/3 h; cooling at ambient temperature, and new addition of 2 mL of acid mixture and digestion at 170 °C/3 h). The extract obtained was adequately diluted and read in an atomic absorption spectrophotometer (AGILENT/VARIAN model SpectrAA 50 B) calibrated with a standard curve for iron and zinc.

The statistical analyses were processed with the aid of the Genes software (Cruz 2013). Individual analyses of variance were carried out for all the traits, and joint analyses involving the data from the two environments. The effects of genotypes and environments were considered fixed. The experimental coefficient of variation (CV[%]) and selective accuracy (SA) (Resende and Duarte 2007) were estimated to evaluate the accuracy and the informativeness of the experiments.

Genetic and phenotypic parameters were estimated, such as genetic variance, heritability and gains expected from selection, with selection intensity of 30% of the best progenies. As the genotype effect was considered fixed, it should be noted that the heritability calculated refers to the genotypic coefficient of determination. The estimates of the confidence intervals of heritability were obtained through expressions developed by Knapp et al. (1985).

For simultaneous selection, the "weight-free" and "parameter-free" multiplicative index proposed by Elston (1963) was used, in which minimum or maximum values are pre-established. For FeC and ZnC, the minimum value was the overall mean of the experiments; and for yield and 100 seed weight, the minimum values were those of the 'Xamego' check cultivar. Gain based on the combined traits was then calculated. The eight best progenies were selected for recombination.

## RESULTS AND DISCUSSION

The mean values of the 101 progenies in the  $C_0S_{0.1}$  generation were 71.4  $mg\cdot kg^{-1}$  for FeC and 39.4  $mg\cdot kg^{-1}$  for ZnC, which were lower than the mean values of the check cultivar 'BRS Supremo' (72.7  $mg\cdot kg^{-1}$  for FeC and 40.6  $mg\cdot kg^{-1}$  for ZnC) (Table 1). However, the mean values of the 64 selected progenies were superior to those of 'BRS Supremo' (75.5  $mg\cdot kg^{-1}$  for FeC, ranging from 56.5 to 93.9  $mg\cdot kg^{-1}$  and 40.1  $mg\cdot kg^{-1}$  for ZnC, ranging from 30.2 to 51.4  $mg\cdot kg^{-1}$ ). Some progenies that exhibited a very high concentration for one of the minerals and lower than that of the check cultivar for the other were also selected, which explains minimum values below the mean of the check cultivar.

**Table 1.** Summary of the results of evaluation and selection of the progenies for iron concentrations (FeC) and zinc concentrations (ZnC) in the grain in different generations.

Trait	Measurement	Generation		
		C <sub>0</sub> S <sub>0.1</sub>	C <sub>0</sub> S <sub>0.2</sub>	C <sub>0</sub> S <sub>0.3</sub>
FeC	No. of progenies	101	64	27
	Selection intensity	63%	42%	30%
	Mean of the Progenies	71.4	78.8	68.4
	Variance	53.2	139.2	14.3
	Mean of BRS Supremo	72.7	77.4	64.3
	Mean of those selected	75.5	87.8	71.6
ZnC	Mean of the Progenies	39.4	42.8	44.3
	Variance	8.0	18.4	4.1
	Mean of BRS Supremo	40.6	40.7	42.1
	Mean of those selected	40.1	44.8	45.8

In evaluation of the C<sub>0</sub>S<sub>0.2</sub> generation, as expected, the mean values of the 64 progenies (78.8 mg·kg<sup>-1</sup> for FeC and 42.8 mg·kg<sup>-1</sup> for ZnC) were higher than those of 'BRS Supremo' (77.4 mg·kg<sup>-1</sup> for FeC and 40.7 mg·kg<sup>-1</sup> for ZnC) (Table 1). In the mean of the two evaluations, the 64 progenies had mean values of FeC (75.2 mg·kg<sup>-1</sup>) and of ZnC (41.1 mg·kg<sup>-1</sup>) higher than those of 'BRS Supremo' (74.6 and 40.5 mg·kg<sup>-1</sup>, respectively) (Table 1), which indicates the possibility of obtaining lines with concentrations higher than those of that cultivar. As already mentioned, 'BRS Supremo' is currently the black bean cultivar with the highest concentrations of these minerals (Pereira et al. 2018).

This is important because the final aim of any recurrent selection program in the case of autogamous plants should be to obtain lines superior to the existing cultivars for the trait in question, for use as new cultivars. From these results, 27 progenies with FeC ranging from 75.4 to 91.4 mg·kg<sup>-1</sup> and ZnC ranging from 37.3 to 45.6 mg·kg<sup>-1</sup> were selected for evaluation in experiments with replications in different environments. These progenies exhibited even higher mean values (81.6 mg·kg<sup>-1</sup> for FeC and 42.4 mg·kg<sup>-1</sup> for ZnC).

Considering evaluation of the progenies in the experiments, the estimates of the coefficient of variation (CV) ranged from 6.4 to 10.9% for FeC and from 5.1 to 7.4% for ZnC, indicating good experimental accuracy (Table 2). The estimates of selective accuracy (SA) for these traits were considered to be of moderate to high magnitude, ranging from 0.61 to 0.73 for FeC and from 0.74 to 0.75 for ZnC, confirming the good discrimination capacity of the treatments. These results were similar to those obtained by Di Prado et al. (2019), Martins et al. (2016), Pereira et al. (2014) and Silva et al. (2012).

**Table 2.** Summary of the individual analyses of variance for iron and zinc concentrations (mg·kg<sup>-1</sup>), 100 seed weight (grams), and grain yield (kg·ha<sup>-1</sup>) of the check cultivars and of the 27 progenies evaluated in Santo Antônio de Goiás (STA) and Brasília (BSB), winter/2017.

Source of variation	DF	Iron concentration		Zinc concentration		100 seed weight		Grain yield
		STA	BSB	STA	BSB	STA	BSB	BSB
		MS	MS	MS	MS	MS	MS	MS
Blocks	2	41.4	22.3	42.2	21.9	1.4	6.70	16862
Genotypes	29	46.3 <sup>**</sup>	78.7 <sup>ns</sup>	11.0 <sup>**</sup>	25.8 <sup>**</sup>	11.3 <sup>**</sup>	7.61 <sup>**</sup>	158543 <sup>**</sup>
Progenies (P)	26	43.0 <sup>*</sup>	86.0 <sup>*</sup>	10.0 <sup>**</sup>	28.3 <sup>**</sup>	10.9 <sup>**</sup>	7.84 <sup>**</sup>	168230 <sup>**</sup>
Check (C)	2	69.3 <sup>*</sup>	18.2 <sup>ns</sup>	4.9 <sup>ns</sup>	6.0 <sup>ns</sup>	18.4 <sup>**</sup>	2.21 <sup>ns</sup>	105631 <sup>ns</sup>
P vs. C	1	84.5 <sup>ns</sup>	7.2 <sup>ns</sup>	49.6 <sup>**</sup>	0.9 <sup>ns</sup>	8.6 <sup>**</sup>	12.47 <sup>**</sup>	12516 <sup>ns</sup>
Residue	58	21.5	48.7	4.7	11.6	0.3	1.58	38927
Mean		72.6	64.1	42.5	45.8	23.7	20.2	964
CV (%) <sup>1</sup>		6.4	10.9	5.1	7.4	2.2	6.2	19.9
SA <sup>2</sup>		0.73	0.61	0.75	0.74	0.98	0.89	0.88

<sup>1</sup>Experimental coefficient of variation; <sup>2</sup>Selective accuracy. ns, \*, \*\*not significant, significant at p < 0.05 and significant at p < 0.01, respectively.

For grain yield, the CV was 19.9% (Table 2) considering only the trial in Brasília, given that, in the trial in Santo Antônio de Goiás, the estimates of the experimental quality parameters were not satisfactory (CV = 34%). The estimate of SA was 0.88, which is considered high for grain yield. For 100 seed weight, the CV ranged from 2.2 to 6.2%, and the SA ranged from 0.89 to 0.98, considered very high. These estimates indicated good experimental precision for the two traits and they are similar to those reported by Chiorato et al. (2015) and Santos et al. (2018).

Considering the individual analyses of variance (Table 2) and joint analyses of variance (Table 3), there was a significant difference for the four traits, indicating genetic variability among the progenies. Even after a previous step of selection, which led to the group of 27 progenies, this result shows that there is still the possibility of selection of progenies with greater concentrations of these minerals for recombination and formation of a new recurrent selection cycle.

**Table 3.** Summary of the joint analyses of variance for iron and zinc concentrations in the grain ( $\text{mg}\cdot\text{kg}^{-1}$ ) and 100 seed weight (grams) of the check varieties and of the 27 progenies, evaluated in Santo Antônio de Goiás and Brasília in winter/2017.

Source of variation	DF	Iron concentration		Zinc concentration		100 seed weight	
		MS	P value	MS	P value	MS	P value
Genotypes (G)	29	80.8	0.001	23.2	0.001	15.4	0.001
Progenies (P)	26	85.2	0.001	24.4	0.001	15.2	0.001
Check cultivars (C)	2	54.0	0.220	10.8	0.270	16.1	0.001
P vs. C	1	21.2	1.000	18.5	0.130	20.8	0.001
Environments (E)	1	3255.5	0.001	484.6	0.020	536.5	0.001
G × E	29	44.1	0.200	13.6	0.030	3.5	0.001
P × E	26	43.9	0.210	13.9	0.030	3.6	0.001
C × E	2	33.6	1.000	0.1	1.000	4.5	0.010
(P vs C) × E	1	70.5	0.161	32.0	0.050	0.2	1.000
Residue	116	35.1	-	8.2	-	0.9	
Overall mean			68.3		44.2		22.0
CV (%) <sup>1</sup>			8.7		6.5		4.4

<sup>1</sup>Experimental coefficient of variation.

There was also a significant effect of the environments, which highlights the heterogeneity between the two locations for the three traits (Table 3). The overall mean values of the progenies in the environments ranged from 64.1 to 72.6  $\text{mg}\cdot\text{kg}^{-1}$  for FeC, from 42.5 to 45.8  $\text{mg}\cdot\text{kg}^{-1}$  for ZnC, and from 20.2 to 23.7 g for 100 seed weight, confirming the direct effect of environments on the traits evaluated (Table 2). This indicates the importance of knowing which environmental factors and how environmental factors affect expression of FeC and ZnC in the grain, so that cultivars can be recommended in a reliable manner.

Environmental factors, such as type of soil, pH, organic matter content and soil moisture, can affect both the uptake and the accumulation of these micronutrients in the grain (Ribeiro 2010). In geographic terms, there is a considerable difference in altitude between Santo Antônio de Goiás at 823 m and Brasília at 1,171 m, which leads to a considerable difference of temperature in the period in which the experiments were carried out (May to August). This is a preponderant factor in development of the common bean crop and can help explain the environmental effect detected.

In addition to the environmental effect, the genotype by environment interaction was observed for ZnC and 100 seed weight, which refers to the differential response of the genotypes between the environments. For ZnC, similar results have been reported in the literature (Blair et al. 2010; Ribeiro et al. 2008). For 100 seed weight, this interaction does not always appear (Di Prado et al. 2019). For FeC, interaction was not detected, which diverges from what is normally reported in the literature (Araújo et al. 2003; Martins et al. 2016). This is considered a good result, since the ordering of the progenies is similar in the environments without distortions in the genotype responses. However, the fact of having been evaluated in only two environments in one crop season alone may explain the lack of the interaction effect.

For all the traits, the estimates of genetic variance among the progenies, both in individual analyses and in joint analyses, confirmed the existence of genetic variability (Table 4). All the estimates of the confidence interval for heritability, except for FeC evaluated in Brasília, exhibited lower limits greater than zero, indicating that there is still the possibility of success from selection. The heritability estimates based on joint analyses were 59% (43 to 50% in the environments) for FeC and 67% (52 to 59% in the environments) for ZnC, considered as moderate to high, which indicates a good possibility of success from selection, considering the quantitative nature of these traits.

**Table 4.** Estimates of genetic parameters in relation to the iron and zinc concentrations ( $\text{mg}\cdot\text{kg}^{-1}$ ), 100 seed weight (grams) and grain yield ( $\text{kg}\cdot\text{ha}^{-1}$ ), evaluated in the 2017 winter crop season in Santo Antônio de Goiás (STA) and Brasília (BSB).

Parameter	Iron concentration			Zinc concentration			100 seed weight			Grain yield
	STA	BSB	JOIN	STA	BSB	JOINT	STA	BSB	JOINT	BSB
Overall mean	72.6	64.1	68.3	42.5	46.0	44.2	23.7	20.2	22.0	964
Mean of selected progenies	75.6	67.6	71.6	43.8	47.8	45.8	23.9	20.7	22.3	1012
$\sigma^2g^1$	7.2	12.4	8.3	1.7	5.6	2.7	3.5	2.1	2.4	43791
$h^2$ (%) <sup>2</sup>	50.0	43.3	58.7	52.4	59.2	66.6	97.5	80.0	94.0	76.8
CI $h^2$ (%) <sup>3</sup>	10–51	3–44	26–59	13–53	25–60	40–66	95–98	63–80	89–94	58–77
GS (30%) <sup>4</sup>	2.7	4.2	3.5	2.7	4.4	3.0	8.3	7.9	7.4	21.4
GSS (30%) <sup>5</sup>	-	-	2.7	-	-	2.3	-	-	1.0	3.8

<sup>1</sup>Genetic variance; <sup>2</sup>heritability; <sup>3</sup>confidence interval of heritability; <sup>4</sup>expected gain from selection; <sup>5</sup>expected gain from simultaneous selection.

Some authors obtained heritability estimates for FeC below those found in the present study (Jost et al. 2009); others obtained superior estimate (83.8%) (Martins et al. 2016). This can be explained by the fact that heritability consists of an estimate intrinsic to the population under study, and that its magnitude depends on the genetic variability of the genotypes, number of environments and number of replications under evaluation. In relation to heritability for ZnC, other studies have detected estimates (from 53 to 70%) similar to those obtained in the present study (Martins et al. 2016; Rosa et al. 2010; Zemolin et al. 2016) (Table 4).

The heritability estimates for 100 seed weight were of high magnitude: 94% (80 to 97%) (Table 4), as reported in other studies (Alvares et al. 2016; Di Prado et al. 2019; Melo et al. 2004), indicating high possibility of success from selection. For grain yield, heritability was 73%, which was considered above average, since this trait normally has lower heritability (Ramalho et al. 2012). In general, the heritability estimates reinforce the potential of this population for selection of the four traits.

The expected gain from direct selection (GS) of the eight best progenies for each trait in isolation was 3.5% for FeC, 3.0% for ZnC, 7.4% for 100 seed weight and 21.4% for grain yield (Table 4), from which it can be inferred that there is still the possibility of selection of progenies with higher concentrations of these minerals and with favorable agronomic phenotypes. The mean values of the eight best progenies for FeC ( $71.6 \text{ mg}\cdot\text{kg}^{-1}$ ) and for ZnC ( $45.8 \text{ mg}\cdot\text{kg}^{-1}$ ) were higher than the mean values of the 'Xamego' cultivar, the check cultivar that had the highest concentrations of these minerals ( $70.3 \text{ mg}\cdot\text{kg}^{-1}$  for FeC and  $44.7 \text{ mg}\cdot\text{kg}^{-1}$  for ZnC), and also higher than those of 'BRS Supremo' (Table 5). The 'Xamego' cultivar, though it has high FeC and ZnC, not only in this study but in others (Pereira et al. 2014), is no longer used commercially. The mean value of the progenies selected was 4.6% greater than the overall mean ( $68.4 \text{ mg}\cdot\text{kg}^{-1}$ ) for FeC and 3.3% greater ( $44.2 \text{ mg}\cdot\text{kg}^{-1}$ ) for ZnC.

The eight best progenies not only had nutritionally superior grain, but they also stood out for their agronomic traits, with mean grain yield ( $1,012 \text{ kg}\cdot\text{ha}^{-1}$ ) and 100 seed weight (22.32 g) higher than the mean of the 'Xamego' cultivar ( $750 \text{ kg}\cdot\text{ha}^{-1}$  and 19.19 g) (Table 5). Considering all 27 progenies, 85% of them were better than the 'BRS Supremo' check cultivar for FeC, 89% for ZnC, 67% for yield and 74% for 100 seed weight. This indicates the high frequency of favorable alleles in this recurrent selection population and confirms the possibility of obtaining superior lines in the future.



**Table 5.** Mean values of the 27 progenies and three check cultivars, overall mean, mean values of the best 30% of the progenies, and mean value of the progenies selected simultaneously for all the traits studied: iron concentration (FeC), zinc concentration (ZnC) ( $\text{mg}\cdot\text{kg}^{-1}$ ), 100 seed weight (100SW) (grams) and grain yield ( $\text{kg}\cdot\text{ha}^{-1}$ ).

Progeny	FeC	ZnC	100SW	Grain yield
216206943*	70.2	45.2	21.1	980
216206961*	70.0	45.1	24.2	866
216206962*	70.4	45.8	24.2	1192
216206977	71.3	43.0	19.8	923
216206978	65.9	40.0	23.2	1096
216206987*	68.3	47.2	20.9	920
216206993	59.2	38.9	21.7	1706
216207006*	72.7	46.4	20.9	1042
216207007	67.8	43.2	22.7	1125
216207008	69.9	46.1	22.1	595
216207009*	74.3	44.6	21.2	829
216207012	68.2	44.5	22.5	1075
216207013	61.2	41.4	22.1	641
216207015	72.6	46.9	19.3	687
216207017	68.1	45.0	21.6	858
216207018	65.9	42.6	23.0	1085
216207019	67.7	44.8	20.3	930
216207023	64.3	42.2	21.9	1384
216207025	72.3	44.1	22.1	950
216207026	66.6	45.6	22.4	628
216207027	70.4	43.3	27.4	879
216207029 <sup>†</sup>	73.3	46.4	22.5	1137
216207034 <sup>†</sup>	73.5	45.8	23.5	1129
216207037	63.2	45.4	21.0	795
216207041	66.9	44.1	22.0	863
216207042	65.2	45.4	21.9	875
216206944	68.2	43.7	20.6	835
'XAMEGO'	70.3	44.7	19.2	750
'BRS ESTEIO'	67.2	42.9	22.4	1123
'BRS SUPREMO'	64.3	42.1	21.2	901
Overall mean	68.3	44.2	22.0	960
Mean of the progenies	68.4	44.3	22.1	964
Mean of the 8 progenies selected	71.6	45.8	22.3	1012

<sup>†</sup>Progenies selected to be recombined to form cycle 1.

For a cultivar to be adopted by both farmers and consumers, high concentrations of FeC and ZnC must be accompanied by other satisfactory agronomic and commercial requirements. For that reason, it is important to perform simultaneous selection based on the four traits to identify the eight best progenies to be recombined, aiming to form a new generation of the recurrent selection program, as well as to obtain lines for the purpose of recommending new cultivars. Thus, the expected gain from simultaneous selection for all the traits was 2.7% for FeC, 2.3% for ZnC, 3.8% for yield and 1.0% for 100 seed weight (Table 4). This result reinforces that it is possible to select lines with high FeC and ZnC, together with high yield and larger grain.

Direct selection of these traits exhibits superior results (Table 5) compared to simultaneous selection. Nevertheless, the efficiency is lower because the traits are handled individually, making it difficult to obtain lines with the various traits of interest together; yet these would be the lines that have potential for becoming cultivars. The eight progenies selected were 10% superior for FeC, 8% for ZnC, 5% for seed weight, and 11% for yield in relation to 'BRS Supremo', aggregating high grain nutritional quality (FeC and ZnC) and good agronomic quality.

Considering the estimates of genetic parameters obtained (high heritability and expressive gains from selection), the recurrent selection population formed shows wide variability and genetic potential for generation of lines with higher FeC and ZnC in the grain. The eight progenies chosen based on simultaneous selection were used for recombination and formation of a base population for a new recurrent selection cycle (C1S0 generation). An alternative that allows an increase in genetic variability in a recurrent selection population is adding parents outside of the recurrent selection program in the step of recombination for formation of a new cycle (Ramalho et al. 2012). In this respect, two lines of common bean with black seed coat from different origins were also included in the recombination, CNFP 18131 and CNFP 15701.

The CNFP 18131 line was developed at Embrapa Arroz e Feijão specifically for high FeC and ZnC, from a population generated at CIAT (Colombia) (parents MIB154/SER14//SEN22/SER7). This line showed high FeC (80.9 mg·kg<sup>-1</sup>), ZnC (36.9 mg·kg<sup>-1</sup>), yield (1,728 kg·ha<sup>-1</sup>) and 100 seed weight (24.9 g) in the mean of nine evaluation environments. In relation to 'BRS Supremo', CNFP 18131 showed superiority of 8.5% for FeC, 7.9% for ZnC, 8.5% for yield and 28% for 100 seed weight (personal communication, Helton Pereira, unpublished data).

The other line that was inserted in recombination is the elite line CNFP 15701. It was not developed specifically for higher FeC and ZnC, but it was selected in the preliminary trials for black bean of Embrapa Arroz e Feijão in 2013 as an elite line that combined high FeC and ZnC (71.7 and 37.3 mg·kg<sup>-1</sup>) and good yield (2,117 kg·ha<sup>-1</sup>) with excellent adaptability and good phenotypic stability. This line showed 7% superiority for FeC and 10% for ZnC in relation to 'BRS Supremo', with yield similar to 'BRS Esplendor' (Martins et al. 2016).

In parallel, lines were obtained from these eight progenies selected, which will be evaluated for FeC, ZnC and other traits of agronomic and commercial importance and, then, after some selection steps, they will be evaluated in multilocation trials to check for potential in becoming biofortified cultivars.

## CONCLUSION

The recurrent selection population formed from the parents 'Brasil 0001', 'BRS Esplendor', 'BRS Grafite', 'BRS Supremo', 'G6492', 'G6495', 'IAPAR 65', 'Milionário', 'Piratã 1' and 'Xamego' shows high genetic variability and potential for obtaining lines superior to the cultivars currently on the market, allying high agronomic performance and high iron and zinc concentrations in the grain. Furthermore, this population shows potential for generating a new recurrent selection cycle, which already was obtained from recombination of the eight superior progenies and two external parents.

## AUTHORS' CONTRIBUTION

**Conceptualization:** Pereira H. S.; **Methodology:** Pereira H. S., Melo L. C. and Souza, T. L. P. O.; **Investigation:** Pereira H. S., Melo L. C., Souza T. L. P. O., Queiroz L. R., Rodrigues L. L. and Martins S. M.; **Writing – Original Draft:** Pereira H. S., Queiroz L. R., Rodrigues L. L. and Martins S. M.; **Writing – Review and Editing:** Pereira H. S., Queiroz L. R., Rodrigues L. L. and Martins S. M.; **Funding Acquisition:** Pereira H. S., Melo L. C. and Souza, T. L. P. O.; **Resources:** Pereira H. S. and Melo L. C.; **Supervision:** Pereira H. S.

## DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.



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