GMR
The Original by FUNPEC-RP

Genetic control and selection of common bean parents and superior segregant populations based on high iron and zinc contents, seed yield and 100-seed weight

P.R.C. Di Prado[1], L.C. Faria[2], T.L.P.O. Souza[2], L.C. Melo[2], P.G.S. Melo[1] and H.S. Pereira[2]

[1] Universidade Federal de Goiás, Goiânia, GO, Brasil
[2] Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brasil

Corresponding author: H.S. Pereira
E-mail: helton.pereira@embrapa.br

Genet. Mol. Res. 18 (1): gmr18146
Received September 13, 2018
Accepted March 06, 2019
Published March 28, 2019
DOI http://dx.doi.org/10.4238/gmr18146

ABSTRACT. The objectives of this study were to select promising common bean populations for high iron and zinc contents (FeC and ZnC), seed yield and 100-seed weight, to study the genetic control of these traits and to select parents for high FeC and ZnC based on a diallele analysis. Fifteen populations were obtained from crosses among six parents selected for high FeC and ZnC using a complete diallel scheme. The 15 populations were evaluated together with two control cultivars/lines (Pérola and Piratã 1). The following traits were evaluated: FeC and ZnC in four environments/generations and seed yield and 100-seed weight, in three environments/generations. Individual and joint analyzes of variance were performed for the four traits and individual and joint diallel analyzes for FeC and ZnC. Significant differences were found among populations for all traits, which demonstrates genetic variability that can be exploited. For FeC, the additive effects were more important and the white seeded line G 2358 stood out for high general combining ability (5.63), being for this reason indicated as a parent. For ZnC, both additive and non-additive effects were important. The populations selected as simultaneously superior for the four traits were Porto Real x G 2358 and BRSMG Majestoso x G 2358, with at least one parent with *carioca* seeds; and BRS Requinte x BRSMG Majestoso, Porto Real x BRS Requinte and Porto Real x BRSMG Majestoso, with *carioca* seeds. The BRS Requinte x G 2358 population is recommended to obtain lines with high FeC and ZnC.

Key words: *Phaseolus vulgaris*; Biofortification; Diallel; Plant breeding

INTRODUCTION

The common bean (*Phaseolus vulgaris*) is the most important legume with direct human consumption, especially in Eastern Africa and Latin America (Blair et al., 2009). It is nutritionally rich in proteins, vitamins and micronutrients, such as iron, zinc, copper and manganese. Brazil has stood out as one of the largest producers and consumers of common bean in the last years, with an annual production of approximately 2.5 million tons (Feijão, 2019).

Micronutrient and vitamin deficiency affect approximately 3.5 billion people worldwide; it is considered a public health issue (Blair et al., 2009). Deficiency in iron and zinc affects the world population, in rich and poor countries (WHO, 2009). Iron is essential for the formation of hemoglobin and its deficiency causes anemia. Zinc deficiency can disrupt children’s growth, lead to immune system problems, dermatitis, diarrhea, delay in sexual maturation, pregnancy problems, and neurobehavioral changes, such as mood swings and depression (Levenson and Morris, 2011; Gibson, 2012).

Some alternatives proposed to combat micronutrient deficiency are diet diversification, supplementation, fortification and biofortification. Biofortification is characterized by the increase in nutrient content in the food by genetic breeding, at no additional costs and without the need for stimulating consumption (Ribeiro, 2010). Various researchers have pointed out that in the long term, the biofortification strategy is the most feasible and efficient (Frossard et al., 2000; Bouis, 2003; Rios et al., 2009; Ribeiro, 2010; Levenson and Morris, 2011; Gibson, 2012).

For the success of a breeding program, genetic variability among lines is essential. Several studies indicate the genetic variability for iron and zinc contents (FeC and ZnC) in common bean seeds (Ribeiro et al., 2008; Nchimbi-Msolla and Tryphone, 2010; Silva et al., 2012; Pereira et al., 2014; Martins et al., 2016). However, there are few reports of breeding programs that have developed improved lines with higher levels of these minerals (Blair et al., 2009, Jost et al., 2009; Mukamuhirwa et al., 2015). Most of the studies reporting the development of breeding lines using parents with contrasting FeC and ZnC, mainly with the objective of QTL mapping. The FeC and ZnC are quantitative traits (Beebe et al., 2000; Cichy et al., 2009; Blair et al., 2009, 2010). Consequently, the best way to obtain lines with higher levels of these minerals is through crosses between parents with high levels of these minerals (Ramalho et al., 2012).

The three key stages in a breeding program aiming at the development of new elite lines are: selection of parents, achievement of the segregating population and choice of the method of conducting the segregating population (Ramalho et al., 2012). Methods for selecting parents may involve only information from the parents, such as those using their own mean of the trait or genetic divergence or using progeny performance. Among these, diallel crosses have been widely used in common bean breeding for various traits (Machado et al., 2002; Pereira et al., 2007; Silva et al., 2013; Mukamuhirwa et al., 2015; Vale et al., 2015).

There are few studies that have used diallel analysis for FeC and ZnC. Mukamuhirwa et al. (2015) reported the predominance of additive gene action for FeC. Silva et al. (2013) indicate that both additive and non-additive effects are important for FeC. For ZnC, both indicate predominance of additive gene action.

Selection of the best segregating populations allows focusing on the development of elite lines in these populations, which optimizes resources and maximizes successful results. These populations should harbor important crop production traits, as well as aspects related to mineral contents, to increase the probability of obtaining lines that will become future cultivars (Mendes et al., 2009). In the common bean, seed yield is the main trait considered during the development of a new cultivar (Faria et al., 2013). Another important trait is the 100-seed weight (W100), which is directly related to the size of the seeds, fundamental to the commercial acceptance of a new cultivar (Carbonell et al., 2010).

The main goals of this work were to obtain promising common bean populations for high FeC and ZnC, seed yield and 100-seed weight, in addition to examining genetic control of these traits and to select parents for high FeC and ZnC, based on a diallel analysis.

MATERIAL AND METHODS

Fifteen segregant populations were obtained from crosses among six common bean parents by following the complete diallel scheme proposed by Griffing (1956), in which only the hybrids are considered (model IV). Out of these six parents, four are cultivars highly adapted to the Brazilian growing conditions: BRS Cometa, BRS Requinte, BRSMG Majestoso and Porto Real, from *Carioca* market class, which are the most consumed class in Brazil, with high seed yield, besides other desirable phenotypes and high FeC and ZnC compared to other *Carioca*-seeded cultivars; and two lines introduced from other countries, G 2358 and G 14378, both with white seeds, low adaptation to the common bean growing in Brazil, but with high FeC and ZnC (Pereira et al., 2014). The crosses were carried out in Santo Antônio de Goiás, Goiás, Brazil, in May of 2011. The F_1 generation was sown in February/2012, aiming to obtain the F_2 seeds.

The 15 segregant populations and two controls (Pérola – cultivar with *carioca* seeds most grown in Brazil and with intermediates FeC and ZnC, and Piratã 1 – cultivar with “mulatinho” seeds and with high FeC and ZnC) (Pereira et al., 2014; Martins et al., 2016) were evaluated in field trials using a randomized block design with three replicates and four row-plots with 4.0 m in length, spaced by 0.5 m between rows. It was used the technologies recommended for the crop considering the different environments and growing systems, but without control of diseases.

The field trials were carried out in Santo Antônio de Goiás (SAG), in the winter growing season of 2012 (May, F_2 generation), and 2013 (June, F_3 generation) and rainy growing season of 2013 (December, F_4 generation), and in Brasília, Federal District in the rainy growing season of 2013 (December, F_4 generation). As a result, the effect of generations (F_2, F_3 and F_4) was mixed with the effect of environments and, therefore, it will be treated by environments/generations.

The evaluated traits were: FeC and ZnC, in the four environments; seed yield and W100 in three environments (SAG I/12, SAG I/13 and Brasília A/13). Seed yield was obtained in g plot^{-1} by collecting all the seeds from the two central rows, with subsequent conversion to kg.ha^{-1}. From each plot, a sample of 100 seeds was randomly collected for weighing and determination of the W100, in g 100 seeds^{-1}.

The analysis of FeC and ZnC was performed by acid digestion of the organic matter (with 2:1 nitro-perchloric mixture), using a flame atomic absorption spectrophotometry technique, adapted from the Association of Official Analytical Chemists (AOAC, 1995). To verify the accuracy of the laboratory analysis, for every 40 samples, one sample was performed in triplicate, and two control samples were added, with pre-established values, for checking. The seeds were submitted to a rapid washing with deionized water and then dried in an oven at 60ºC for 12 h (to 6% humidity). The dry seeds were ground (≤200 mesh) in a zirconium oxide ball mill (Retsch MM200) and PTFE (polytetrafluoroethylene) containers to avoid contamination with metallic elements, in addition to weighing the sample one day after milling for moisture balance. The pre-digestion of each sample was performed with the acid mixture (50ºC / 30min), followed by a digestion step (100ºC / 30 min; 170ºC / 3 h; cooling at room temperature and a new addition of 2 mL of acid mixture and digestion at 170°C / 3 h). The extract obtained was properly diluted and read in an atomic absorption spectrophotometer (AGILENT/VARIAN model Spectra 50 B) previously calibrated with a standard curve for iron and zinc. The FeC and ZnC data were expressed as dry basis (mg.kg^{-1}), based on the moisture contents of the sample obtained by gravimetric method at 105ºC until constant weight (Instituto Adolfo Lutz, 1995). The glassware and materials used in the analysis underwent a special washing process with a decontamination step using 5% nitric or hydrochloric acid solution (V: V) to avoid contamination.

Analyzes of variance were carried out for each trait in each trial (environment/generation). In order to carry out the joint analyzes, the homogeneity of the variances was verified by means of the 7: 1 ratio of the residual mean squares, as reported by Pimentel-Gomes (2009). The coefficients of experimental variation (CV%) and selective accuracy were also estimated, as proposed by Resende and Duarte (2007).

The diallel analysis was accomplished only for FeC and ZnC, using method IV proposed by Griffing (1956), in which the effects of general combining ability (g_i) of the parents and specific combining ability (s_{ij}) of the populations are estimated. The errors associated with these estimates were obtained as described by Ramalho et al. (1993). The coefficients of determination (R²%) were also estimated by means of the relative contribution of the squared sum of the SCA and GCA, in relation to the total sum of squares.

The means were grouped by the Scott-Knott method (Scott and Knott, 1974), at 10% probability. This level of significance was used to reduce the probability of type II error (Zimmerman, 2014). The statistical analyzes were processed using the GENES software (Cruz, 2013).

RESULTS AND DISCUSSION

Individual analysis of variance showed significant differences among populations for all traits in all environments/generations, except for ZnC and seed yield in one environment/generation (Table 1). These results highlight the existence of genetic variability between the populations for the four traits and the potential success with the selection. The coefficients of variation (CV) in the environments/generations ranged from 4.7% to 8.8% for FeC and from 4.9 to 7.4% for ZnC, indicating high experimental accuracy. Selective accuracy was considered high (≥0.70 and <0.90) or very high (≥0.90) in all environments/generations, indicating that the experiments were really informative. These estimates were similar to those found for these traits by Silva et al. (2012), Pereira et al. (2014) and Martins et al. (2016).

The mean values of FeC in the environments/generations ranged from 53.6 to 73.6 $mg.kg^{-1}$, which indicates significant environmental effects (Table 1), similar to the results reported by Pereira et al. (2014) and Martins et al. (2016). However, means of ZnC in the environments/generations ranged from 30.4 to 36.2 $mg.kg^{-1}$, indicating a lower environmental influence on ZnC than for FeC, similar to the results found in other studies (Silva et al., 2012; Mukamuhirwa et al., 2015).

Table 1. Summary of analysis of variance for iron and zinc contents in the bean seeds ($mg.kg^{-1}$), evaluated in the generations F_2 (Santo Antônio de Goiás - SAG, winter of 2012), F_3 (SAG, winter of 2013) and F_4 (SAG and Brasília, rainy of 2013).

		Iron content											
Source of variation		**F_2 (SAG I/12)**			**F_3 (SAG I/13)**			**F_4 (SAG A/13)**			**F_4 (Brasília A/13)**		
	DF	**MS**	**P-value**	**R²%**	**MS**	**P-Value**	**R²%**	**MS**	**P-Value**	**R²%**	**MS**	**P-Value**	**R²%**
Blocks	2	26.9	-	-	62.6	-	-	5.8	-	-	11.9	-	-
Treatments	16	524.4	0.000	-	192.3	0.000	-	28.8	0.001	-	35.7	0.001	-
Controls (C)	1	2144.4	0.000	-	343.4	0.007	-	6.5	0.375	-	10.1	0.326	-
Populations (P)	14	420.0	0.000	-	150.0	0.001	-	31.9	0.001	-	39.8	0.001	-
GCA[1]	5	462.0	0.000	39%	131.0	0.020	31%	44.3	0.001	50%	51.0	0.002	46%
SCA[2]	9	396.7	0.000	61%	160.6	0.002	69%	25.0	0.008	50%	33.6	0.006	54%
Contrast C vs P	1	365.7	0.000	-	632.8	0.000	-	8.7	0.304	-	3.7	0.552	-
Residue	32	17.9	-	-	41.6	-	-	8.0	-	-	10.2	-	-
Overall mean		59.6			73.6			60.0			53.6		
CV[3] (%)		7.1			8.8			4.7			6.0		
SA[4]		0.98			0.88			0.85			0.85		
		Zinc content											
Sources of variation		F_2 (SAG I/12)			F_3 (SAG I/13)			F_4 (SAG A/13)			F_4 (Brasília A/13)		
	GL	QM	P-valor	R²%	QM	P-valor	R²%	QM	P-valor	R²%	QM	P-valor	R²%
Blocks	2	23.0	-	-	1.7	-	-	27.0	-	-	10.2	-	-
Treatments	16	25.9	0.000	-	10.0	0.001	-	19.1	0.007	-	14.4	0.042	-
Control (C)	1	135.1	0.000	-	52.4	0.000	-	4.7	0.417	-	68.1	0.004	-
Populations (P)	14	19.8	0.000	-	6.4	0.027	-	14.7	0.038	-	10.7	0.157	-
GCA[1]	5	24.6	0.000	44%	5.4	0.119	30%	21.8	0.020	53%	5.3	0.593	18%
SCA[2]	9	17.2	0.000	56%	7.0	0.028	70%	10.7	0.173	47%	13.8	0.079	82%
Contrast C vs P	1	1.7	0.455	-	17.7	0.018	-	96.2	0.001	-	11.6	0.209	-
Residue	32	3.0	-	-	2.8	-	-	6.9	-	-	7.0	-	-
Overall mean		35.4			30.4			35.4			36.2		
CV[3] (%)		4.9			5.5			7.4			7.3		
SA[4]		0.94			0.84			0.80			0.71		

[1]General combining ability; [2]Specific combining ability; [3]Experimental coefficient of variation; [4]Selective accuracy.

Considering the joint analysis, the effect of environments/generations was significant for FeC and ZnC, confirming the importance of the environmental effect on the expression of these traits (Table 2). In addition, significant differences between the controls and between the populations were found, which confirms the existence of genetic variability. The interactions of controls and populations with environments/generations were significant for the two traits, indicating a differential effect of the environment on the genotypes. This same interaction has been reported in previous studies (Araújo et al., 2003;

Nchimbi-Msolla and Tryphone, 2010; Silva et al., 2012; Pereira et al., 2014; Martins et al., 2016).

The diallel analysis showed that the effects of the general combining ability (GCA) and the specific combining ability (SCA) were significant in all environments/generations for FeC and in the joint analysis, which indicates additive and non gene action in trait expression (Tables 1 and 2). In the first generations, the dominance effect was greater compared to the additive effect, which can be explained by the greater proportion of heterozygotes in the first generations. In the joint analysis, there was a predominance of additive effects (66%) (Table 2). Zemolin et al. (2016) evaluated two crosses between Andean common bean lines and observed heterosis for FeC and ZnC in the first generations; but these authors pointed out difficulties of obtaining common bean hybrids. Dominance can be observed in the first generations, but it is reduced at every self-fertilization generation.

Silva et al. (2013) reported values close to the relative contribution of additive and non-additive effects for FeC in the F_2 generation, with predominance of relative contribution of SCA (54%), presenting a value close to that found in our work (61%) (Table 1). Mukamuhirwa et al. (2015) observed that the relative contribution of GCA for FeC in a F_3 generation was 64%, which is greater than that found in the same generation in our study (31%). According to Cruz et al. (2012), when parents with previous selection are used, such as in our work, the differential of the additive effects can be reduced and, consequently, the importance of the non-additive effects may increase.

Table 2. Summary of joint analyses of variance for iron and zinc contents in the bean seeds ($mg.kg^{-1}$), seed yield ($kg.ha^{-1}$) and100-seed weight (g).

Sources of variation	Iron content				Zinc content			Seed yield			100-seed weight	
	DF	MS	P-value	R^2(%)	MS	P - value	R^2(%)	DF	MS	P-value	MS	P-value
Blocks/environments	8	26.8	-	-	15.4	-	-	6	201500	-	6.0	-
Treatments	16	291.5	0.000	-	29.8	0.000	-	16	1758316	0.000	37.5	0.000
Controls (C)	1	1245.9	0.000	-	214.6	0.000	-	1	2964613	0.000	162.0	0.000
Population (P)	14	242.0	0.000	-	18.5	0.000	-	14	1592726	0.000	25.5	0.000
GCA[1]	5	445.6	0.000	66%	27.6	0.000	53%	-	-	-	-	-
SCA[2]	9	128.9	0.000	34%	13.5	0.006	47%	-	-	-	-	-
Contrast C vs P	1	29.0	0.224	-	2.8	0.456	-	1	2870286	0.000	81.2	0.000
Environments (E)	3	3663.4	0.000	-	355.3	0.000	-	2	26611245	0.000	290.6	0.000
Treatments x E	48	163.2	0.000	-	13.2	0.000	-	32	414764	0.008	2.3	0.109
Controls x E	3	419.8	0.000	-	15.4	0.029	-	2	1121249	0.007	3.2	0.150
Populations x E	42	133.3	0.000	-	11.0	0.000	-	28	347787	0.046	2.4	0.100
GCA x E	15	80.9	0.000	-	9.8	0.021	-	-	-	-	-	-
SCA x E	27	162.3	0.000	-	11.7	0.001	-	-	-	-	-	-
Contrast (C vs P) x E	3	325.5	0.000	-	41.2	0.000	-	3	430643	0.120	1.0	0.000
Residue	128	19.4	-	-	4.9	-	-	96	216029	-	1.6	-
Mean		61.7			34.4				2973		24.8	
CV[3]		7.1			6.5				15.6		5.2	
SA[4]		0.96			0.91				0.93		0.97	

[1] General combining ability; [2] Specific combining ability; [3] Experimental coefficient of variation; [4] Selective accuracy

For ZnC, GCA and SCA effects were significant in one environment/generation (SAG I/12 F_2), and in the joint analysis the relative contributions of GCA (53%) and SCA (47%) were close (Table 1 and 2). Silva et al. (2013) found significant effects only for GCA, evaluating only the F_2 generation, which indicates a predominance of additive gene action for ZnC. This study found values close to those of the relative contribution of the additive and non-additive effects in a F_2 generation, with the predominance of relative contribution of SCA (56%). On the other hand, Mukamuhirwa et al. (2015) observed a greater contribution of the GCA effects (82%), evaluating only the F_3 generation. In our work, GCA was not significant in the F_3 generation.

For FeC, the importance of the relative contribution of GCA and SCA varied over the generations, and in the joint analysis GCA was predominant. Therefore, the additive effects are considered more important for this trait. However, for ZnC, in an environment/generation and in the joint analysis, the relative contributions of SCA and GCA were close. So, both additive and nonadditive effects are important in ZnC expression.

Melo et al. (1997) evaluated the occurrence of interactions of combining abilities during two generations (F_3 and F_4) for seed yield at two locations. They found interactions mainly for GCA, highlighting the importance of carrying out the diallel analysis in several locations/ environments and generations to obtain accurate and reliable estimates.

Among the parents, G 2358 was the only one with a positive and significant g_i estimate in all environments/generations and in the joint analysis (5.63) for FeC, which shows its potential to generate populations with high means (Table 3). As a result, G 2358 should be indicated as a parent in crosses aiming to increase FeC in common bean seeds. However, this line has less adaptation to the Brazilian growing conditions, presenting white seeds and smaller size in relation to the *Carioca* type seed, which is preferred in Brazil. Pereira et al. (2014) also evaluated this line and concluded that it contains high FeC.

Table 3. Estimates of the general combining ability (g_i) of six common bean parents for iron and zinc contents in the seeds evaluated in the generations F_2 (Santo Antônio de Goiás - SAG, winter of 2012), F_3 (SAG, winter of 2013) and F_4 (^{1}SAG and ^{2}Brasília, rainy season of 2013) based on the joint analysis.

Parent	Iron content					Zinc content				
	F_2	F_3	F_4^1	F_4^2	Joint	F_2	F_3	F_4^1	F_4^2	Joint
G 2358	11.40*	5.38*	1.78*	3.96*	5.63*	2.88*	1.15*	-0.31	0.27	1.00
BRS Requinte	2.00*	2.45	0.77	-0.70	1.13	-0.73	-0.28	-0.53	-0.69	-0.56
Porto Real	-0.81	-0.87	0.60	-1.87*	-0.74	-0.18	0.38	2.58*	0.93	0.93
BRSMG Majestoso	-3.95*	-2.42	1.62*	-0.86	-1.40	-0.51	-0.69	0.24	-0.19	-0.29
G 14378	-2.72*	-3.43*	-2.02*	0.34	-1.96	-0.93*	-0.10	-0.91	-0.75	-0.67
BRS Cometa	-5.92*	-1.12	-2.76*	-0.88	-2.67*	-0.53	-0.46	-1.07	0.42	-0.41
$\sigma^2 (g_i)^3$	1.93	2.94	1.29	1.46	2.01	0.79	0.77	1.20	1.21	1.01
$\sigma^2 (g_i - g_j)^4$	3.00	4.56	2.00	2.26	3.12	1.22	1.19	1.86	1.88	1.57

^{3}Error associated with general combining ability; ^{4}Error associated to the difference between g_i of the parent *i* with parent *j*.

On the other hand, cultivar BRS Cometa presented a negative and significant g_i estimate (-2.67), which is undesirable since it indicates that this parent will produce populations with lower means. Cultivar BRS Cometa was evaluated with other Brazilian cultivars of *Carioca* market class and some controls with high contents of iron in multiple environments and presented high FeC, besides high adaptability and stability for this trait (Di Prado, 2017). The poor performance of BRS Cometa to generate superior populations may be explained by epistasis among genes that control this trait, since its gene set that expresses high FeC should have a favorable effect and, when recombination occurs by crosses, this gene arrangement is lost.

Estimates of g_i of the other parents did not differ from zero in the joint analysis, indicating that, in general, these parents formed intermediate populations. In evaluations performed by Di Prado (2017), BRS Requinte and Porto Real presented FeC considered good, but below that obtained with BRS Cometa.

For ZnC, the mean estimates of g_i for the genotypes did not differ from zero (Table 3). However, the G 2358 parent obtained a g_i estimate (1.00) close to significant (1.01) and presented positive and significant estimates (2.88 and 1.15) in two environments/generations, which shows that this parent may be superior to others, in some situations, to generate populations with high ZnC.

Estimates of s_{ij} for FeC of the populations, considering the average of all environments/generations, did not differ from zero for most populations (Table 4). Only BRS Requinte x G 2358 population had a positive and significant s_{ij} estimate (3.46). Observing the s_{ij} estimates of this population in the environments/generations, it is found that the mean value was influenced by a significant and very high estimate (18.34) in an environment/generation. In another environment/generation, the s_{ij} estimate became significant and negative (-2.26), showing the influence of the environment on the estimate of s_{ij}. On the other hand, BRS Cometa x BRS Requinte population was the only one that presented mean s_{ij} estimate significant and negative (-6.08) in three of the four environments/generations. This result confirms that the performance of this population was lower to that expected by the g_i estimates of the parents. For ZnC, the s_{ij} mean estimates of the populations were all equal to zero.

Table 4. Means of iron and zinc contents (FeC and ZnC) ($mg.kg^{-1}$) in the seeds, seed yield (Yield, $kg.ha^{-1}$) and 100-seed weight (W100, g), in addition to estimates of the effects of average specific combining ability (s_{ij}) for FeC and ZnC.

Population / Control	**FeC**		s_{ij}	**ZnC**		s_{ij}	**Yield**		**W100**	
BRS Requinte x G 2358	72.1	a	3.46*	34.9	b	0.19	2586	c	23.0	e
Porto Real x G 2358	67.9	b	1.20	36.4	a	0.21	2969	b	25.4	c
Piratã 1	67.9	b	-	37.7	a	-	2943	b	23.8	d
BRSMG Majestoso x G 2358	64.7	c	-1.35	34.2	b	-0.84	3281	b	25.2	c
G 2358 x G 14378	64.2	c	-1.35	35.0	b	0.37	2768	c	21.1	f
BRS Requinte x BRSMG Majestoso	64.0	c	2.47	34.6	b	1.12	2846	b	24.7	c
BRS Cometa x G 2358	62.8	c	-1.96	35.0	b	0.08	2212	d	24.6	c
BRS Requinte x G 14378	62.3	c	1.28	33.3	c	0.23	3204	b	21.9	f
Porto Real x BRS Requinte	61.1	d	-1.12	33.8	c	-0.90	3113	b	24.8	c
BRS Cometa x Porto Real	60.8	d	2.39	34.3	b	-0.48	2303	d	26.8	b
BRS Cometa x G 14378	60.6	d	3.38	34.5	b	1.28	3046	b	23.7	d
BRS Cometa x BRSMG Majestoso	60.1	d	2.27	33.4	c	-0.24	2328	d	27.0	b
Porto Real x BRSMG Majestoso	58.4	d	-1.28	36.4	a	1.50	2941	b	26.7	b
Porto Real x G 14378	58.0	d	-1.19	34.2	b	-0.34	3275	b	24.0	d
BRSMG Majestoso x G 14378	56.4	e	-2.12	31.8	d	-1.54	3666	a	24.2	d
BRS Cometa x BRS Requinte	54.2	e	-6.08*	32.7	c	-0.65	3314	b	25.4	c
Pérola	53.5	e	-	31.7	d	-	3755	a	29.8	a
$\sigma^2 (s_{ij})^1$			3.41			1.72	-		-	
$\sigma^2 (s_{ij} - s_{ik})^2$			5.40			2.72	-		-	
$\sigma^2 (s_{ij} - s_{kl})^3$			4.41			2.22	-		-	

Means followed by the same letter did not differ statistically from each other by the Scott-Knott method at 10% probability. [1]Error associated with s_{ij}; [2]Error associated with the difference in the specific combining ability when a parent is the same; [3]Error associated with the difference in the specific combining ability when parents are different.

In a breeding program, it is important to select the best populations for obtaining elite lines, since the operational capacity is limited by several factors, such as size of experimental areas, labor and financial resources. In order to better perform the selection process, it is important to observe the means and the results of the diallel analysis, as suggested by Cruz et al. (2012). For FeC, BRS Requinte x G 2358 population presented the highest mean (72.1 $mg.kg^{-1}$), being even higher than the best control Piratã 1 (67.9 $mg.kg^{-1}$).

This population was developed by a parent with a positive g_i estimate (G 2358) and presented a positive s_{ij} estimate (3.46) in the joint analysis (Tables 3 and 4). Porto Real x G 2358 population can also be considered very promising because it presented FeC (67.9 $mg.kg^{-1}$) similar to that of the control Piratã 1, and it was formed by a parent with a positive g_i estimate (G 2358). Other 11 populations presented FeC ranging from 58.0 $mg.kg^{-1}$ and 64.7 $mg.kg^{-1}$, lower than that of Piratã 1 (67.9 $mg.kg^{-1}$), but higher than that of cultivar Pérola (53.5 $mg.kg^{-1}$), which is the most grown cultivar in Brazil. These results indicate that these populations also have the potential to develop elite lines with higher FeC as long as they also present other important agronomic phenotypes. This confirms what was reported by Frossard et al. (2000), who indicated that plant breeding to increase iron and zinc contents in seeds should be effective. Additionally, these authors mention that the increase of the mineral content in the seeds must be accompanied by an increase in bioavailability, so that there is actually greater absorption by the consuming organism.

For ZnC, as no parent or population presented positive estimates of g_i and s_{ij}, respectively, the selection of the best populations was carried out according to the means. Consequently, the populations Porto Real x BRSMG Majestoso (36.4 $mg.kg^{-1}$) and Porto Real x G 2358 (36.4 $mg.kg^{-1}$) should be highlighted, since they presented ZnC means similar to the Piratã 1 (37.7 $mg.kg^{-1}$) (Table 4). Twelve other populations with ZnC ranging from 32.7 to 35.0 $mg.kg^{-1}$ were superior to cultivar Pérola (31.7 $mg.kg^{-1}$), presenting potential for production of elite lines with high ZnC.

In addition to the mineral content, other traits essential for the acceptance of a new common bean cultivar should be considered to select the best populations, such as seed yield and W100. W100 is indicative of the size of the seeds. For seed yield, CV values ranged from 14.8 to 15.7% and AS was high in all environments/generations. For W100, CV values ranged from 4.3 to 5.9% and AS was always very high ($\geq$0.90). Consequently, the experimental accuracy was considered very good for both traits, and the trials were informative. There was variation between the environments for seed yield and W100, with averages varying from 2356 to 3768 $kg.ha^{-1}$ and from 22.4 to 27.1 g, respectively. The importance of environmental variation was confirmed by the joint analyzes, which detected significant effects of environments/generations for both traits. Several studies reported the importance of the environmental effect in these traits (Carbonell et al., 2010; Pereira et al., 2012). On the other hand, the interactions of populations and controls with environments/generations were significant only for seed yield (Table 2), which is frequently reported (Pereira et al., 2012; Vale et al., 2015). For W100, the interactions were not significant, indicating that, although the environment influences the expression of W100, it does not differentially affect the genotypes (Table 2).

As the main objective of our study was to develop and select populations with high potential for extraction of elite lines with high FeC and ZnC, these traits play a major role in the selection process. For seed yield, BRSMG Majestoso x G 14378 (3666 $kg.ha^{-1}$) population was highlighted, with a mean similar to that of the cultivar Pérola (3755 $kg.ha^{-1}$) (Table 4). Another 12 populations presented statistically similar means (2586 $kg.ha^{-1}$ to 3314 $kg.ha^{-1}$), which is similar to the general mean of the trials (2973 $kg.ha^{-1}$), but lower than the average for Pérola. Considering that the cultivar Pérola has a pattern of high agronomic performance, these populations can be considered as the second option for lines selection.

Regarding W100, cultivar Pérola presented the highest mean (29.8 g), based on joint analysis of the environments/generations (Table 4). None of the populations presented W100 similar to that of the Pérola cultivar. However, several cultivars available on the market present seeds with W100 slightly lower than the seeds of Pérola, such as: BRS Requinte, Porto Real, BRS Cometa, BRS Estilo, BRSMG Madrepérola and BRS Pontal (Pereira et al., 2012; Di Prado, 2017). Nine populations presented W100 equal to or greater than 25 g, which is the acceptable standard for the Brazilian internal market for *Carioca* seeds (Pereira et al., 2012). In addition, they presented higher means than Piratã 1, and they could be considered suitable for line extraction.

Considering the four traits together, the populations Porto Real x G 2358, BRSMG Majestoso x G 2358, BRS Requinte x BRSMG Majestoso, Porto Real x BRS Requinte and Porto Real x BRSMG Majestoso deserve special mention as they simultaneously presented good performance for the four traits (Table 4). In a similar way, Ribeiro et al. (2014) were able to select lines that had higher iron contents and higher grain yield, obtained from one population. In common bean breeding, the color of the seed is an important trait and is related to its commercial quality, which directly interferes in the acceptance or not of the cultivar by the market. As previously mentioned, line G 2358 has white seeds of smaller size. Generally, the cross between lines with white seeds and *Carioca* seed lines generates great segregation for seed color, and it is difficult to recover seeds with the desirable commercial standard for the *Carioca* market class. This can be explained by the fact that the genetic control of seed color in beans is very complex, with a large number of genes involved, as well as interactions between these genes and their alleles (McClean et al., 2002). In addition, G2358 presents less adaptation to the Brazilian farming conditions.

Consequently, out of the five selected populations, BRS Requinte x BRSMG Majestoso, Porto Real x BRS Requinte and Porto Real x BRSMG Majestoso originated from crosses between two *Carioca* seeded parents, which facilitates the recovery of seeds with a commercial pattern and increases the chance of obtaining elite lines with potential to become cultivars. On the other hand, the populations Porto Real x G 2358 and BRSMG Majestoso x G 2358, which originate from crosses between a parent with white seeds and a lower adaptation (G 2358) and another one with *carioca* seeds, should generate lines with higher potential for FeC and ZnC, but which will probably not include the other desirable phenotypes for recommendation as a new cultivar. However, these lines may be useful for new crosses, since they will have incorporated the favorable alleles for FeC and ZnC from the less adapted parent.

BRS Requinte x G 2358 population, despite presenting lower seed yield (2586 $kg.ha^{-1}$) and smaller seeds than the commercial standard (23.0 g), presented the highest mean for FeC (72.1 $mg.kg^{-1}$), being the only one superior to the Piratã 1 (67.9 $mg.kg^{-1}$), which shows high levels (Di Prado, 2017). In addition, this population presented good performance for ZnC (34.9 $mg.kg^{-1}$; Table 4). Thus, this population can be used to obtain lines with high FeC and ZnC, with the objective of incorporating favorable alleles for FeC and ZnC in lines with *carioca* seeds, for further crosses.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank Embrapa Rice and Beans, the Harvest Plus Program, and the Brazilian Council for Scientific and Technological Development (CNPq) for financial support. TLPOS, LCM, PGSM and HSP were also supported by CNPq. PRCP was supported by Fundação de Amparo à Pesquisa do Estado de Goiás (Fapeg) which granted a Ph.D. scholarship. We also thank the Embrapa Business Office in Brasília for the support to carrying out the final field trials.

REFERENCES

Araújo R, Miglioranza E, Montalvan R, Destro D, et al. (2003). Genotype x environment interaction effects on the iron content of common bean grains. *Crop Breed. Appl. Biotechnol.* 3: 269-274.

Association of Official Analytical Chemists (1995). The Official Methods of Analysis. AOAC International, Gaithersburg.

Beebe S, Gonzalez AV and Rengifo J (2000). Research on trace minerals in the common bean. Food Nutr. *Bull. Suppl.* 21: 387-391.

Blair MW, Astudillo C, Grusak M, Graham R, et al. (2009). Inheritance of seed iron and zinc content in common bean (*Phaseolus vulgaris* L.). *Mol. Breed.* 23: 197-207.

Blair MW, Medina J, Astudillo C, Rengifo J, et al. (2010). QTL for seed iron and zinc concentrations and content in a Mesoamerican common bean (*Phaseolus vulgaris* L.) population. *Theor. Appl. Genet.* 121: 1059-1079.

Bouis HE (2003). Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? *Proc. Nutri. Soc.* 62: 403-411.

Carbonell SAM, Chiorato AF, Gonçalves JGR, Perina EF, et al. (2010). Tamanho de grão comercial em cultivares de feijoeiro. *Cienc. Rural.* 40: 2067-2073.

Cichy KA, Caldas GV, Snapp SS and Blair MW (2009). QTL analysis of seed iron, zinc and phosphorus levels in an Andean bean population. *Crop Sci.* 49: 1742-1750.

Cruz CD (2013). GENES – a software package for analysis in experimental statistics and quantitative genetics. *Acta Sci. Agron.* 35: 271-276.

Cruz CD, Regazzi AJ and Carneiro PCS (2012). Modelos Biométricos aplicados ao melhoramento genético. Universidade Federal de Viçosa, Viçosa.

Di Prado PRC (2017). Melhoramento genético para altos teores de ferro e zinco em feijoeiro-comum. Doctoral thesis, Universidade Federal de Goiás, Goiânia. Available at [https://repositorio.bc.ufg.br/tede/handle/tede/7294].

Faria AP, Moda-Cirino V, Buratto JS, Silva CFB, et al. (2009). Interação genótipo x ambiente na produtividade de grãos de linhagens e cultivares de feijão. *Acta Sci. Agron.* 31: 579-585.

Feijão (2019). Dados conjunturais do feijão (área, produção e rendimento) - Brasil - 1985 a 2017. Available at [http://www.cnpaf.embrapa.br/socioeconomia/index.htm]. Assessed 16 Jan. 2019.

Frossard EM, Bucher M, Machler F, Mozafar A, et al. (2000). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* 80: 861-879.

Gibson R (2012). Zinc deficiency and human health: etiology, health consequences, and future solutions. *Plant Soil* 361: 291-299.

Griffing B (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Austr. J. Biol. Sci.* 9: 463-493.

Jost E, Ribeiro ND, Cerutti T, Poersch NL, et al. (2009). Potencial de aumento do teor de ferro em grãos de feijão por melhoramento genético. *Bragantia*. 68: 35-42.

Machado CF, Santos JB, Nunes GHS and Ramalho MAP (2002). Choice of commom bean parents based on combining ability estimates. *Genet. Mol. Bio.* 25: 179-183.

Martins SM, Melo PGS, Faria LC, Souza TSPO, et al. (2016). Genetic parameters and breeding strategies for high levels of iron and zinc in *Phaseolus vulgaris* L. *Genet. Mol. Res.* 2: 1-14.

McClean PE, Lee RK, Otto C, Gepts P, et al. (2002). Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). *J. Hered.* 93: 148-152.

Melo LC, Santos JB and Ramalho MAP (1997). Choice of parentes to obtain common bean (*Phaseolus vulgaris*) cultivars tolerant to low temperatures at the adult stage. *Braz. J. Genet.* 20: 283-292.

Mendes FF, Ramalho MAP and Abreu AFB (2009). Índice de seleção para escolha de populações segregantes de feijoeiro-comum. *Pesq. Agropec. Bras.* 44: 1312-1318.

Mukamuhirwa F, Tusiime G and Mukankusi MC (2015). Inheritance of high iron and zinc concentration in selected bean varieties. *Euphytica*. 205: 349-360.

Nchimbi-Msolla S and Tryphone GM (2010). The effects of the environment on iron and zinc concentrations and performance of common beans (*Phaseolus vulgaris* L.) genotypes. *Asian J. Plant Sci.* 9: 455-462.

Pereira HS, Almeida VM, Melo LC, Wendland A, et al. (2012). Influência do ambiente em cultivares de feijoeiro-comum em cerrado com baixa altitude. *Bragantia.* 71: 165-172.

Pereira HS, Del Peloso MJ, Bassinello PZ, Guimarães CM, et al. (2014). Genetic variability for iron and zinc content in common bean lines and interaction with water availability. *Genet. Mol. Res.* 3: 6773-6785.

Pereira HS, Santos JB, Abreu ADFB and Couto KR (2007). Informações fenotípicas e marcadores microssatélites de QTL na escolha de populações segregantes de feijoeiro. *Pesq. Agropec. Bras.* 42: 707-713.

Pimentel Gomes FP (2009). Curso de estatística experimental. Nobel, São Paulo.

Ramalho MAP, Abreu AFB, Santos JB and Nunes JAR (2012). Aplicação da genética quantitativa no melhoramento de plantas autógamas. Universidade Federal de Lavras, Lavras.

Ramalho MAP, Santos JB and Zimmermann MJO (1993). Genética quantitativa em plantas autógamas: aplicações ao melhoramento do feijoeiro. Universidade Federal de Goiás, Goiânia.

Resende MDV and Duarte JB (2007). Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesqui. Agropecu. Trop.* 37: 182-194.

Ribeiro ND (2010). Potencial de aumento da qualidade nutricional do feijão por melhoramento genético. *Semina: Cienc. Agrar.* 31: 1367-1376.

Ribeiro ND, Domingues LS, Zemolin AEM and Possobom MTDF (2014). Selection of common bean lines with high agronomic performance and high calcium and iron concentrations. *Rev. Ceres*. 48: 77-83.

Ribeiro ND, Jost E, Cerutti T, Maziero SM, et al. (2008). Composição de microminerais em cultivares de feijão e aplicações para o melhoramento genético. *Bragantia.* 67: 267-273.

Rios DAS, Alves KR, Costa NMB and Matino HSD (2009). Biofortificação: culturas enriquecidas com micronutrientes pelo melhoramento genético. *Rev. Ceres.* 56: 713-718.

Scott AJ and Knott M (1974). A cluster analysis method for grouping means in the analysis of variance. *Biometrics.* 30: 507-512.

Silva CA, Abreu ADFB, Ramalho MAP and Maia LCS (2012). Chemical composition as related to seed color of common bean. Crop Breed. *Appl. Biotechnol.* 12: 132-137.

Silva CA, Abreu AFB and Ramalho MAP (2013). Genetic control of zinc and iron concentration in common bean seeds. *Afri. J. Agric. Res.* 11: 1001-1008.

Vale NM, Barili LD, Oliveira HM, Carneiro JES, et al. (2015). Escolha de genitores quanto à precocidade e produtividade de feijão tipo carioca. *Pesq. Agropec. Bras.* 50: 141-148.

WHO Global Database Anaemia (2009). Worldwide prevalence of anemia 1993-2005. Available at [http://whqlibdoc.who.int/publications/2008/9789241596657 eng.pdf]. Assessed: 15 Jan. 2019.

Zemolin AEM, Ribeiro ND, Casagrande CR, Silva MJ, et al. (2016). Genetic parameters of iron and zinc concentrations in Andean common beans seeds. *Acta Sci., Agron.* 38: 439-446.

Zimmermann FJP (2014). Estatística aplicada à pesquisa agrícola. Embrapa Arroz e Feijão, Santo Antônio de Goiás.