Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Genetics/ Original Article

Genetic parameters and validation of microsatellite markers associated with iron and zinc in common bean

Abstract – The objective of this work was to estimate the genetic parameters, evaluate the agronomic performance, and validate the microsatellite molecular markers (SSRs) linked with quantitative trait loci (QTLs) for Fe and Zn concentrations in grains of common bean, in order to select superior lines. One hundred and sixteen lines from two populations ('BRS Requinte' × 'Porto Real' and 'BRS Requinte' × G2358) and five check genotypes were evaluated in three environments. The parents and lines were genotyped with 20 SSRs. In the simultaneous selection of the lines for the four evaluated traits, the gains from selection were 4.7% for Fe concentration, 2.8% for Zn concentration, 3.9% for yield, and 0.9% for 100-seed weight. Therefore, there is the possibility of selection of lines that combine desirable phenotypes for the traits of interest. The only polymorphic marker is BM 154 in the 'BRS Requinte' × 'Porto Real' population, indicating that the QTLs linked with the markers may already be fixed or that the markers are not associated in the used populations. The single-marker analysis of QTL mapping shows an association between BM 154 and Fe concentration in only one environment, explaining 14.5% of phenotypic variation, which indicates the occurrence of the interaction of QTLs with environments.

Index terms: *Phaseolus vulgaris*, biofortification, heritability, 100-seed weight, yield.

Parâmetros genéticos e validação de marcadores microssatélites associados com ferro e zinco em feijão comum

Resumo – O objetivo deste trabalho foi estimar os parâmetros genéticos, avaliar o desempenho agronômico e validar os marcadores moleculares microssatélites (SSRs) ligados a loci de caracteres quantitativos (QTLs) para concentrações de Fe e Zn em grãos de feijão comum, para a seleção de linhagens superiores. Cento e dezesseis linhagens oriundas de duas populações ('BRS Requinte' × 'Porto Real' e 'BRS Requinte' × G2358) e cinco genótipos testemunhas foram avaliadas em três ambientes. Os genitores e as linhagens foram genotipados com 20 SSRs. Na seleção simultânea das linhagens para os quatro caracteres avaliados, os ganhos com a seleção foram de 4,7% para concentração de Fe, 2,8% para concentração de Zn, 3,9% para produtividade e 0,9% para massa de 100 grãos. Desta forma, há possibilidade de seleção de linhagens que reúnam fenótipos desejáveis para os caracteres de interesse. O único marcador polimórfico é o BM 154 na população 'BRS Requinte' x 'Porto Real', o que indica que os QTLs ligados aos marcadores já podem estar fixados ou que os marcadores não estão associados nas populações utilizadas.

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Received
February 06, 2023

Accepted
May 29, 2023

How to cite
CARLONI, P.R.; SOUZA, T.L.P.O. de; AGUIAR, M.S. de; MELO, L.C.; MELO, P.G.S.; PEREIRA, H.S. Genetic parameters and validation of microsatellite markers associated with iron and zinc in common bean. **Pesquisa Agropecuária Brasileira**, v.58, e03267, 2023. DOI: https://doi.org/10.1590/S1678-3921.pab2023.v58.03267.

A análise de mapeamento de QTL por marca simples mostra associação entre BM 154 e concentração de Fe em apenas um ambiente, a qual explica 14,5% da variação fenotípica, o que indica a presença de interação de QTLs com ambientes.

Termos para indexação: *Phaseolus vulgaris*, biofortificação, herdabilidade, massa de 100 grãos, produtividade.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the legume mostly used for direct human consumption worldwide (Izquierdo et al., 2018). In Brazil, it is grown in all regions and is of great economic and social importance as it is the main source of plant protein in food for humans and is rich in minerals such as iron and zinc, which makes it a crop with high potential for biofortification (Pereira et al., 2014). Through breeding, biofortification increases the concentration of minerals available in plants, representing a potentially sustainable strategy for combatting iron and zinc deficiency in populations with common bean in their diets (Bouis & Saltzman, 2017).

For a successful breeding program, the genetic properties of the population under study, such as variability and genetic parameters related to the traits of interest, should be investigated to allow of the identification of superior lines for selection (Ramalho et al., 2012). One of the most important genetic parameters is heritability, which measures the reliability of the phenotypic value as an indicator of the reproductive value. In the literature, heritability estimates ranging from low to high magnitude have been reported for iron and zinc concentrations (Mukamuhirwa et al., 2015; Martins et al., 2016; Caproni et al., 2020; Zanotti et al., 2020; Queiroz et al., 2021).

Another important parameter is expected gain from selection. For Fe and Zn concentrations, estimates ranging from 2.7 to 37.9% and from 2.7 to 16.1%, respectively, have already been found (Martins et al., 2016; Zanotti et al., 2020; Queiroz et al., 2021). In most of these studies, the parameters were estimated for lines generated from crosses between parents with high contrasts for Fe and Zn concentrations. However, in the case of traits with quantitative genetic control, as Fe and Zn concentrations (Blair et al., 2009, 2010, 2011; Cichy et al., 2009; Izquierdo et al., 2018; Caproni et al., 2020), the breeding populations with the greatest potential for obtaining lines are generated by the cross between parents with high phenotypic mean values. For this reason, it is also important to estimate these parameters in elite breeding populations.

In the process of selecting superior bean lines for high Fe and Zn concentrations, it is also necessary to carry out simultaneous selection for other agronomically and commercially important traits, such as yield and 100-seed weight in Brazil, in order to increase the chances of acceptance and use of a new cultivar (Ribeiro et al., 2013).

To pinpoint promising lines, molecular marker-assisted selection (MAS) can be quite a useful process in breeding programs. However, for it to be effective, after the identification of a connection with genes of interest, the markers must be validated in breeding populations. Some studies have already identified markers linked with quantitative trait loci (QTLs) that control Fe and Zn concentrations in common bean (Blair et al., 2009, 2010, 2011; Cichy et al., 2009; Caproni et al., 2020). However, these markers still need to be validated in breeding populations for an effective use of MAS.

The objective of this work was to estimate the genetic parameters, evaluate the agronomic performance, and validate the microsatellite molecular markers (SSRs) linked with QTLs for Fe and Zn concentrations in grains of common bean, in order to select superior lines.

Materials and Methods

The lines used in the present study came from the 'BRS Requinte' × 'Porto Real' and 'BRS Requinte' × G2358 segregating populations selected from the 15 populations obtained and evaluated by Di Prado et al. (2019). Population 'BRS Requinte' × 'Porto Real', formed by two parents well adapted to Brazilian growing conditions, belongs to the carioca commercial group type (beans with cream-colored seed coat with brown streaks, which are the main type consumed in Brazil) and showed good performance for Fe and Zn concentrations, yield, and 100-seed weight. The 'BRS Requinte' × G2358 population had the highest Fe and Zn concentrations, with an intermediate yield and 100-seed weight.

In the F_4 generation, obtained in bulk, the populations were grown in a field in the municipality of Santo Antônio de Goiás, in the state of Goiás, Brazil (16°29'8"S, 49°18'32"W, at 819 m altitude), in the 2013 rainy crop season (sowing in December). A random sample of 58 plants was harvested from each population to obtain the lines. A total of 116 lines and five check genotypes ('BRS Requinte', 'Porto Real', 'Pérola', Piratã-1, and CNFP 8348) were evaluated in an 11×11 triple-lattice experimental design, with plots of one 3.0 m length row, in the 2014 winter crop season (sowing in May) in the same municipality. After that, the experiment was repeated in the 2014 rainy season (sowing in December) also in Santo Antônio de Goiás and, then, in the 2015 dry season (sowing in January) in the municipality of Ponta Grossa, in the state of Paraná, Brazil (25°5'40"S, 50°9'48"W, at 956 m altitude).

The evaluated traits were: Fe and Zn concentrations in two environments, i.e., in the 2014 winter season and 2014 rainy season in Santo Antônio de Goiás; and yield and 100-seed weight in three environments, that is, in the 2014 winter season and 2014 rainy season in Santo Antônio de Goiás and in the 2015 dry season in Ponta Grossa. Yield was obtained in grams per plot and then converted into kilogram per hectare. From each plot, one random sample of 100 seeds was collected for weighing and determination of 100-seed weight in grams.

After weighed, the samples were used to determine Fe and Zn, a single time, by acid digestion of organic matter using a 2:1 nitric-perchloric mixture, following the flame atomic absorption spectrophotometry technique, adapted from Association of Official Analytical Chemists (AOAC) (Cunniff, 1995). To verify the accuracy of the laboratory analysis, for every 40 samples, one was performed in triplicate, and two control samples with pre-established values were used for verification. The data of Fe and Zn concentrations were expressed in a dry basis (mg kg^{-1}), based on the water content of the sample obtained by the gravimetric method at 105ºC until reaching a constant weight.

Individual variance analyses were carried out for each trait in each environment, as well as joint analyses of variance, considering the effect of treatments as random and that of environments as fixed. The following genetic parameters were estimated: genetic variance, variance of the interaction between lines and environments, heritability, gain expected from direct selection for each trait, and gain from the simultaneous selection of traits (Ramalho et al., 2012). For simultaneous selection, a cut-off line was defined for each trait. For Fe and Zn concentrations, the minimum value established for selection was the overall mean. For yield and 100-seed weight, the minimum value established was 90% of the overall mean, since the mean was affected by the check genotypes with a high yield and larger beans.

The coefficients of variation determined in each experiment and selective accuracy were used to evaluate experimental accuracy and the informativeness of the experiments, respectively. The statistical analyses were processed with the aid of the GENES software (Cruz, 2016).

In the literature, 43 microsatellite markers (SSRs) were linked with QTLs that control Fe and Zn concentrations in the common bean grain (Blair et al., 2003, 2006, 2009, 2010, 2011; Cichy et al., 2009). It was possible to obtain the sequences of the primers of 20 of these markers (BMd 1, BMd 10, BMd 16, BMd 22, BMd 27, BMd 33, Bng 91, Bng 95, BM 137, BM 154, BM 158, BM 165, BM 170, BM 184, BM 185, BM 201, BM 218, BM 239, BMc 127, and BMc 248), which were used to check for the existence of polymorphism among the three parents ('BRS Requinte', 'Porto Real', and G2358). After that, the polymorphic markers were used for genotyping of the lines. For this, samples of young leaves from ten plants collected in the trial carried out in the winter of 2014, in Santo Antônio de Goiás, were used to compose a bulk of each one of the parents and of the 116 lines. DNA was extracted based on the CTAB protocol, as described by Alvares et al. (2019).

The Master Mix commercial product of the QIAGEN Multiplex PCR Kit (QIAGEN N.V., Venlo, Netherlands) was used for the amplification of the SSRs, according to the methodology proposed by Alvares et al. (2019). The reactions were conducted in a thermocycler programmed for an initial denaturation at 92°C for 5 min, followed by 30 denaturation cycles at 92°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, and final extension at 72°C for 5 min. Vertical electrophoresis in 6.0% polyacrylamide gel was performed about 2 hours on the PCR amplification products stained with 1.0% silver nitrate, following Alvares et al. (2019). Genotyping of the 'BRS Requinte', 'Porto Real', and G2358 parents was carried out by allelic polymorphism, based on band size in comparison with 10 bp DNA ladders (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).

The polymorphic SSRs between parents 'BRS Requinte' × 'Porto Real' and 'BRS Requinte' × G2358 were selected for use in the amplification reactions with the DNA extracted from the lines of the respective cross. The primers selected were marked with fluorescence (6-FAM) and analyzed through capillary electrophoresis. The amplification reactions were equal to those described previously for the parents, and the products with the amplification reactions were separated by capillary electrophoresis on the platform of the 3500 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using the D filter for fluorescence detection. The data were processed with the Data Collection v.2.0 software and then genotyped with GeneMapper v.3.5 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

The segregation of the marker was evaluated by the chi-square test, considering the frequency expected in the F_4 generation (43.75% AA: 12.5% Aa: 43.75% aa). When the segregation was confirmed according to the expected pattern, the single-marker analysis of QTL mapping was used on the data obtained from the phenotypic and molecular evaluation of the lines. The coefficient of determination was estimated from the relationship between the sum of squares of genotypes and the total. Selection efficiency (SE) was estimated using the expression: $SE (\%) = (1 - 4rf^2) \times 100$, where rf is the recombination frequency. Analyses were performed with the aid of the Genes software (Cruz, 2016).

Results and Discussion

The joint analyses of variance indicated the presence of genetic variability among the lines for all traits (Table 1), an essential factor for a successful selection. The effect of environments was also significant for every trait, except for Fe concentration, since the evaluation was carried out in the same location (Santo Antônio de Goiás) and year (2014), with variation only in the crop season (winter and rainy). In the literature, several studies have reported a highly significant effect of environments for Fe and Zn concentrations (Silva et al., 2012; Pereira et al., 2014; Martins et al., 2016; Di Prado et al., 2019; Philipo et al., 2020; Queiroz et al., 2021; Carloni et al., 2022).

The lines responded differently to environmental variation for all traits, except for Zn concentration (Table 1), as frequently observed for Fe concentration (Silva et al., 2012; Pereira et al., 2014; Martins et al., 2016; Di Prado et al., 2019; Philipo et al., 2020; Queiroz et al., 2021). The effect of environments and of the genotype and environment interaction was also reported for yield (Torga et al., 2010; Alvares et al., 2016; Torres et al., 2022). However, for 100-seed weight, most of the time, the effect of environments is observed, but not always of the interaction (Alvares et al., 2016; Torres et al., 2022).

The mean values of the Fe and Zn concentrations of the lines of the 'BRS Requinte' × G2358 population were 64.4 and 34.6 mg kg^{-1}, respectively, higher than those of 60.6 and 33.7 mg kg^{-1} of the 'BRS Requinte' × 'Porto Real' population (data not shown). Furthermore, the lines of the 'BRS Requinte' × G2358 population had lower mean values of 2,019 kg ha^{-1} for yield and of 19.8 g for 100-seed weight, compared with those of 2,189 kg ha^{-1} and 21.3 g, respectively, obtained for the 'BRS Requinte' × 'Porto Real' population, confirming the results found for these populations by Di Prado et al. (2019). In addition, the mean Fe and Zn concentrations in the lines of the 'BRS Requinte' × 'Porto Real' and 'BRS Requinte' × G2358 populations were 26 and 17% and 34 and 20% higher, respectively, than those of the Pérola cultivar, which is one of the most planted in Brazil and has intermediate Fe and Zn concentrations (Carloni et al., 2022). The superiority of the studied lines in relation to the Pérola cultivar confirms the potential of the used populations for the extraction of biofortified lines, as reported by Di Prado et al. (2019).

The high estimates of genetic variance and heritability confirm the existence of variability among the lines (Table 2), shown in the analyses of variance (Table 1). The estimate of heritability for Fe concentration in the joint analysis was 69.5%, ranging from 58.5% for 'BRS Requinte' × G2358 to 68.7% for 'BRS Requinte' × 'Porto Real', which indicates a greater possibility of success in the selection of lines from the latter population. These values are similar to those found in several studies, but higher than those reported by Caproni et al. (2020) and Queiroz et al. (2021) and lower than those obtained by Mukamuhirwa et al. (2015), Martins et al. (2016), and Zanotti et al. (2020). For Zn concentration, the estimate of heritability was 53.1%, similar in the two populations (Table 2), being lower than most values reported in the literature (Mukamuhirwa et al., 2015; Martins et al., 2016;

Zanotti et al., 2020; Queiroz et al., 2021) but higher than that found by Caproni et al. (2020), indicating that both populations have a similar potential for obtaining gains from selection.

The estimates of heritability for yield of the lines of the two populations did not vary significantly, with values of 70.2 and 75.7%, respectively, (Table 2), which were similar to those reported by Torga et al. (2010) and Torres et al. (2022) but higher than those obtained by Alvares et al. (2016). For 100-seed weight, the estimates of heritability were high and similar between both populations, with values of 93.6 and 90.6%, respectively, which are similar to those of 81.7 to 93.1% found by Alvares et al. (2016) and of 90 to 93.6% by Torres et al. (2022). These high values reflect the considerable genetic effect on the expression of this trait compared with the others.

The gain expected from the direct selection of the 24 best lines, regardless of the population of origin and based on the combined analysis, was 7.4% for Fe

Table 1. Summary of joint analyses of variance for Fe and Zn concentrations in the grain, grain yield, and 100-seed weight of common bean (*Phaseolus vulgaris*).

Source of variation(1)	DF	Fe (mg kg^{-1})		Zn (mg kg^{-1})		DF	Yield (kg ha^{-1})		100-seed weight (g)	
		MS	p-value	MS	p-value		MS	p-value	MS	p-value
Treatments	120	156.0	0.001	28.5	0.001	120	962802	0.001	25.3	0.001
Check genotypes (C)	4	472.0	0.001	38.0	0.022	4	925402	0.004	43.0	0.001
Lines (L)	115	143.5	0.001	28.0	0.001	115	939862	0.001	24.1	0.001
L1	57	139.6	0.001	27.1	0.001	57	790910	0.001	22.6	0.001
L2	57	105.5	0.001	27.1	0.001	57	973323	0.001	15.4	0.001
L1 vs L2	1	2,535.3	0.001	134.6	0.001	1	7522907	0.001	600.3	0.001
C vs L	1	321.9	0.007	51.4	0.049	1	3750498	0.001	92.8	0.001
Environments (E)	1	9.3	0.624	5855.7	0.001	2	31236588	0.001	55.8	0.001
Treatments × E	120	61.1	0.009	15.7	0.107	240	628488	0.001	2.6	0.001
C × E	4	32.7	0.560	8.1	0.651	8	931412	0.001	1.1	0.642
L × E	115	62.4	0.006	16.0	0.088	230	621788	0.001	2.6	0.001
L1 × E	57	54.9	0.111	10.3	0.872	114	573362	0.001	2.4	0.001
L2 × E	57	69.5	0.006	20.9	0.006	114	651797	0.001	2.8	0.001
(L1 vs L2) × E	1	86.4	0.161	57.0	0.038	2	1671541	0.001	7.7	0.005
(C vs L) × E	1	22.2	0.477	11.3	0.354	2	187291	0.453	5.9	0.017
Residue	420	43.8	-	13.1	-	630	236122	-	1.5	-
Overall mean(2)	-	-	62.4	-	34.1	-	-	2,116	-	20.6
SAG1 (2014 winter season)	-	-	62.5	-	40.3	-	-	2,758	-	21.5
SAG2 (2014 rainy season)	-	-	62.3	-	27.9	-	-	2,007	-	20.3
PG (2015 dry season)	-	-	-	-	-	-	-	1,584	-	20.0
Experimental CV (%)	-	-	10.6	-	10.6	-	-	23.0	-	5.9
Selective accuracy	-	-	0.85	-	0.73	-	-	0.87	-	0.97

(1)L1, 'BRS Requinte' × 'Porto Real'; and L2, 'BRS Requinte' × G2358. (2)SAG1 and SAG2, in the municipality of Santo Antônio de Goiás, in the state of Goiás, Brazil, in the winter and rainy seasons, respectively; and PG, in the municipality of Ponta Grossa, in the state of Paraná, Brazil. DF, degrees of freedom; MS, mean squares; and CV, coefficient of variation.

Table 2. Estimates of genetic variance (σ^2_g), heritability (h^2), and variance of the line × environment interaction (σ^2_{gps}) related to iron and zinc concentrations in the grain, yield, and 100-seed weight of common bean (*Phaseolus vulgaris*), based on the joint analysis.

Genotypes	Fe concentration			Zn concentration			Yield			100-seed weight		
	σ^2_g	h^2	σ^2_{gps}	σ^2_g	h^2	σ^2_{gps}	σ^2_g	h^2	σ^2_{gps}	σ^2_g	h^2	σ^2_{gps}
Lines(1)	16.6	69.5	3.11	2.5	53.1	0.47	78,193	74.9	42,852	2.5	94.0	0.13
Population 1	16.0	68.7	1.86	2.3	51.4	0.00	61,643	70.2	37,471	2.3	93.6	0.10
Population 2	10.3	58.5	4.30	2.3	51.4	1.29	81,911	75.7	46,186	1.6	90.6	0.15

(1)Population 1, 'BRS Requinte' × 'Porto Real'; and Population 2, 'BRS Requinte' × G2358.

concentration (Table 3). Of the selected lines, 17 are from the 'BRS Requinte' × G2358 population, which confirms its greater potential for this trait, and 7 are from 'BRS Requinte' × 'Porto Real'. The gain from selection of the 12 best lines of the former population was 7.1%, greater than that of 5.5% of the latter.

Since, in breeding programs, bean grain type is a determinant in the selection of lines, the carioca and black bean commercial types, representing 75 and 10% of Brazilian production, were taken into account (Pereira et al., 2021). Of the 17 lines selected from the 'BRS Requinte' × G2358 population, none have carioca grain type and only 3 have black grain type. However, the 7 lines of the 'BRS Requinte' × 'Porto Real' population have carioca grain type.

Regarding Zn concentration, the gain expected from selection was 4.7% (Table 3). As for Fe concentration, 17 of the 24 lines selected were from the 'BRS Requinte' × G2358 population, but only one of had carioca grain type. In contrast, the 7 selected lines of the 'BRS Requinte' × 'Porto Real' population had carioca grain type. Once more, the gain expected from the selection of the 12 best lines of the former population was greater (5.2%) than that of the latter (3.6%).

The estimates of gain from selection obtained in the present study were generally lower than those of 9.0 to 38% reported for Fe concentration and of 5.0 to 16% for Zn concentration (Martins et al., 2016, Zanotti et al., 2020, Queiroz et al., 2021). This difference can be attributed to the fact that the parents of the lines used here already have high Fe and Zn concentrations, and, consequently, the genetic variability generated is less than that expected in crosses with contrasting parents.

For grain yield, the gain expected from selection was 15.2%, similar for the two populations and greater than that found for Fe and Zn concentrations (Table 3). Fifteen of the 24 lines selected are from the 'BRS Requinte' × 'Porto Real' population and all have carioca grain type. Of the 9 lines selected from the 'BRS Requinte' × G2358 population, only one has carioca grain type. For 100-seed weight, the gain expected from selection was 10.8%, also higher than that for Fe and Zn concentrations, and the 'BRS Requinte' × 'Porto Real' population stood out with an estimate of 14.2% and a higher mean yield of 23.6 g. Therefore, in general, the 'BRS Requinte' × G2358 population stood out for Fe and Zn concentrations, whereas the 'BRS Requinte' × 'Porto Real' population showed better yield and 100-seed weight, which is in agreement with the results found by Di Prado et al. (2019). Torres et al. (2022) obtained gains expected from selection of 10.8% for yield and 8.8% for 100-seed weight, near those observed in the present study.

The gain expected from simultaneous selection for the four evaluated traits was 4.7% for Fe concentration, 2.8% for Zn concentration, 3.9% for yield, and 0.9% for 100-seed weight (Table 3), indicating that it is possible to select lines that combine desirable phenotypes for these traits. The gains expected from direct selection for each trait individually showed greater estimates than those from simultaneous selection, but with a reduced direct practical effect since the lines must combine various traits of interest to be released as cultivars. Therefore, simultaneous selection proved to be effective in the selection of lines with greater chances of becoming cultivars (Alvares et al., 2016; Torres et al., 2022).

Table 3. Mean values of the 24 selected lines and of the 12 lines selected from each common bean (*Phaseolus vulgaris*) population, expected gain from direct and simultaneous selection (GS), and number of lines selected from each population (NL) among the 24 best for Fe and Zn concentrations in the grain, yield, and 100-seed weight, based on the joint analysis.

Selected lines	Fe (mg kg^{-1})			Zn (mg kg^{-1})			Yield (kg ha^{-1})			100-seed weight (g)		
	Mean	GS (%)	NL	Mean	GS (%)	NL	Mean	GS (%)	NL	Mean	GS (%)	NL
						Direct selection						
Lines	69.2	7.4	24	37.2	4.7	24	2,530	15.2	24	22.9	10.8	24
'BRS Requinte' × 'Porto Real'	67.5	5.5	7	36.7	3.6	7	2,530	14.2	15	23.6	14.2	19
'BRS Requinte' × G2358	70.2	7.1	17	37.6	5.2	17	2,510	14.7	9	21.6	4.9	5
						Simultaneous selection						
Lines	66.6	4.7	-	35.9	2.8	-	2,225	3.9	-	20.8	0.9	-
Overall mean	62.4	-	-	34.1	-	-	2,116	-	-	20.6	-	-

Of the 24 lines selected for the four traits together, 10 are from the 'BRS Requinte' × 'Porto Real' population and have carioca grain type (Table 4). Among them, lines 'BRS Requinte' × 'Porto Real'.8, 'BRS Requinte' × 'Porto Real'.39, and 'BRS Requinte' × 'Porto Real'.32 stood out. Of the 14 selected lines of the 'BRS Requinte' × G2358 population, 'BRS Requinte' × G2358.1 and 'BRS Requinte' × G2358.7. have carioca grain, whereas 'BRS Requinte' × G2358.53 and 'BRS Requinte' × G2358.29 have black grain and show good performance. These seven lines have greater Fe and Zn concentrations than the cultivars already in use in Brazil and also have agronomic and commercial potential to be evaluated in multiple environments, aiming their recommendation as new cultivars.

The genetic correlation between Fe and Zn concentrations was 0.89, positive and of high magnitude, similar to that of 0.85 reported by Martins et al. (2016). Other authors found genetic correlation ranging from 0.42 (Silva et al., 2012) to 0.62 (Pereira et al., 2014), considered weak to moderate. High estimates make it easier to select genotypes that combine high concentrations of those nutrients, indicating the occurrence of pleiotropy or a connection between the genes that control the expression of these traits. The genetic correlation between Fe or Zn concentrations and yield was negative and of moderate magnitude, with estimates of -0.49 and -0.47, respectively. As for the Fe/Zn and 100-seed weight correlation, the estimates of -0.33 and -0.23, respectively, were negative and of weak magnitude. These associations can be explained,

Table 4. Mean values of the common bean (*Phaseolus vulgaris*) check genotypes and 24 lines selected for iron and zinc concentrations in the grain, yield, 100-seed weight, and grain type.

Line	Fe concentration	Zn concentration	Yield	100-seed weight	Grain type
	-----------------(mg kg^{-1})	-----------------	(kg ha^{-1})	(g)	
'BRS Requinte' × 'Porto Real'.39	72.8	35.7	2,197	20.2	Carioca
'BRS Requinte' × 'Porto Real'.8	72.3	38.7	2,499	20.3	Carioca
'BRS Requinte' × G2358.53	72.1	35.6	2,151	20.3	Black
Piratã-1(1)	71.8	35.0	2,851	20.7	*Mulatinho*
'BRS Requinte' × G2358.13	70.8	35.2	2,196	21.9	White
'BRS Requinte' × G2358.40	68.7	38.5	1,904	18.6	Beige-black streak
'BRS Requinte' × 'Porto Real'.29	68.1	35.6	2,422	21.0	Carioca
'BRS Requinte' × G2358.43	68.1	36.3	2,091	19.4	Beige-black streak
'BRS Requinte' × 'Porto Real'.32	67.8	38.1	2,062	21.9	Carioca
'BRS Requinte' × G2358.48	67.7	35.8	2,275	22.7	White
'BRS Requinte' × 'Porto Real'.4	67.0	35.2	2,124	22.6	Carioca
'BRS Requinte' × G2358.41	66.0	36.2	1,907	21.1	Beige-black streak
'BRS Requinte' × 'Porto Real'.25	65.8	36.5	2,527	22.2	Carioca
'BRS Requinte' × G2358.1	65.7	34.4	2,222	20.0	Carioca
'BRS Requinte' × G2358.14	65.3	34.2	2,539	19.9	White
'BRS Requinte' × G2358.9	64.8	36.7	1,991	20.3	Black
'BRS Requinte' × 'Porto Real'.28	64.7	36.9	2,214	21.7	Carioca
'BRS Requinte' × 'Porto Real'.58	64.7	34.4	2,082	20.6	Carioca
'BRS Requinte' × G2358.42	64.2	36.7	2,024	20.9	Beige-black streak
'BRS Requinte' × G2358.7	64.0	34.5	1,911	20.9	Carioca
'BRS Requinte' × 'Porto Real'.17	64.0	34.3	2,144	19.4	Carioca
'BRS Requinte' × G2358.44	63.7	35.4	2,091	19.5	Beige-black streak
'BRS Requinte' × G2358.36	63.2	35.2	2,986	20.4	Carioca
'BRS Requinte' × G2358.33	63.2	34.2	2,058	20.0	Black
'BRS Requinte' × 'Porto Real'.19	63.1	36.8	2,780	23.4	Carioca
'Porto Real'(1)	61.8	34.6	2,151	22.5	Carioca
CNFP 8348(1)	59.8	33.8	2,051	20.2	Black
'BRS Requinte'(1)	54.3	31.8	2,387	21.0	Carioca
'Pérola'(1)	48.1	28.9	2,554	25.6	Carioca

(1)Check genotypes.

in part, by the fact that one of the parents (G2358) of half of the lines has high Fe and Zn concentrations and a lower yield and 100-seed weight. In practical terms, these negative associations indicate that yield and 100-seed weight must be evaluated during the development process of lines with high Fe and Zn, aiming selection with desirable phenotypes for the four traits.

The 20 SSRs previously identified as linked with QTLs were tested in parents 'BRS Requinte', 'Porto Real', and G2358. BM 154 was the only polymorphic marker and occurred only between 'BRS Requinte' (allele 276) and 'Porto Real' (allele 262). It is common to find a low number of polymorphic SSRs between improved lines of the same gene pool (Pereira et al., 2007), as in the present study. Other authors also evaluated Mesoamerican genotypes and obtained polymorphism rates ranging from 2.0 to 25% (Blair et al., 2006; Pereira et al., 2007; Torga et al., 2010; Alvares et al., 2019).

The chi-square test for monogenic segregation indicated that the deviations were not significant and, therefore, the BM 154 marker has Mendelian segregation. Single-marker analysis of QTL mapping in Santo Antônio de Goiás, in the 2014 winter season, did not detect a link between the marker and QTLs that control Fe (p-value = 0.75) and Zn (p-value = 0.41) concentrations. In Santo Antônio de Goiás, in the 2014 rainy season, likewise, no association was found between the marker and Zn concentration (p-value = 0.26). However, there was an association with Fe concentration (p-value = 0.01), which indicates a high probability of the BM 154 marker being linked to a QTL that controls the concentration of this nutrient. In the combined analyses, no association was observed for either Fe concentration (p-value = 0.42) and Zn concentration (p-value = 0.23).

The fact that the BM 154 marker is associated with Fe concentration in one environment and not in another indicates an interaction of QTLs with environments, which has already been reported in the literature (Pereira et al., 2007; Torga et al., 2010; Margarido et al., 2015). The interaction of QTLs with environments can be characterized by the differential expression of the QTL due to environmental changes, by an absence of the expression of the QTL in some of the evaluated environments (representing an inconsistency in the detection of QTLs in different environments), and by the variation in their effects. The estimate of the coefficient of determination for Fe concentration in Santo Antônio de Goiás, in the 2014 rainy season, indicates that that marker explains 14.8% of the phenotypic variation. Gelin et al. (2007) evaluated 68 SSRs and identified only two associated with Zn concentration (BM 154 and BM 184), concluding that BM 154 explained 15.3% of the phenotypic variation for Zn concentration. In the present study, the BM 154 marker showed an association with Fe concentration, which confirms that the same QTL can control the two traits simultaneously and that it is affected by the interaction with environments.

Izquierdo et al. (2018) carried out a meta-analysis joining seven populations that identified molecular markers linked to QTLs of Fe and Zn concentrations, concluding that these genomic regions had not yet been successfully used in MAS. The authors found eight meta-QTLs that, together, control Fe and Zn concentrations in common bean, distributed in 7 of 11 chromosomes, which reflects the genetic complexity of the accumulation of Fe and Zn concentrations in common bean seeds and the difficulty of aggregating these eight regions in a single biofortified line, given the low probability of success, estimated at 0.39%. The BM 154 marker is located on chromosome 9, near the location of the meta-QTL 9.1., which is related to genes that participate in mineral absorption, transport to leaves, and translocation to seeds, embryos, and endosperms.

In the environment of Santo Antônio de Goiás, in the 2014 rainy season, in which the BM 154 marker showed an association with Fe concentration, the mean value of the heterozygous lines (262/276) was 64.5 mg kg^{-1}, higher than that of 61.1 and 53.6 mg kg^{-1} of lines with genotypes 262/262 and 276/276, respectively. The 'Porto Real' parent, with the 262 allele, had a mean Fe concentration of 61.8 mg kg^{-1}, higher than that of 54.3 mg kg^{-1} of 'BRS Requinte', which has the 276 allele. The higher mean value of the heterozygous lines can be explained by the effect of dominance or overdominance, indicating an interaction between alleles.

The selection efficiency of a marker measures its effectiveness in discriminating a trait. The greater the selection efficiency, the greater the contribution of the marker will be for assisting in decision making in a breeding program. For Fe concentration in the environment of Santo Antônio de Goiás, in the 2014 rainy season, the selection efficiency of the BM 154 marker was 69.9%, considered high.

However, for BM 154 to be used for MAS, evaluations in other environments are necessary to confirm the association of this marker with QTLs of Fe concentration since it was useful in only one environment. It is also important to consider the interaction between QTLs and genetic backgrounds.

In general, the markers identified in the literature had a low efficiency for MAS in the populations evaluated here. The populations of the cited studies are different from those developed for breeding purposes, which are constituted from parents with a high mean value, as those in the present work. Therefore, it is necessary to identify and validate new molecular markers linked to Fe and Zn concentrations for their incorporation in MAS routines. To increase the chance of success, it is important to carry out mapping for Fe and Zn concentrations with lines used as parents in the breeding program.

Conclusions

1. The estimates of heritability and of gain expected from selection are high, indicating the possibility of success in the selection of common bean (*Phaseolus vulgaris*) lines for high iron and zinc concentrations, yield, and 100-seed weight.
2. Twenty-four lines, simultaneously, show high iron and zinc concentrations, good yield, and commercial-standard grain size.
3. The BM 154 microsatellite marker is polymorphic for the 'BRS Requinte' × 'Porto Real' population and shows an association with iron concentration in one environment, being a potential candidate for marker-assisted selection.

Acknowledgments

To Embrapa Arroz e Feijão and to the Harvest Plus Program, for funding (process numbers 20.18.01.009.00 and 20.19.00.118.00, respectively); to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), for funding (process number 471719/2012-9) and for scholarships of productivity in technological development and innovative extension (process number 313274/2019-3); to Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG), for a doctoral scholarship; and to Luana Alves Rodrigues of Embrapa Arroz e Feijão, for technical support.

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