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Genetic parameters and combined selection for phosphorus, phytate, iron, and zinc in Mesoamerican common bean lines

Parâmetros genéticos e seleção combinada para fósforo, fitatos, ferro e zinco em linhagens de feijão Mesoamericano

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ABSTRACT

The development of common bean cultivars that contain satisfactory minerals and phytate concentrations for the different nutritional requirements of consumers is a new strategy of breeding programs. This work aimed to obtain estimates of genetic parameters for the concentrations of phosphorus, phytate, iron, and zinc in a recombinant inbred line (RIL) population of Mesoamerican common bean, to study the correlations between these traits, and to select common bean lines for the biofortification program and for diets that require the decrease in the intake of these minerals. The RIL were obtained from the cross between BRS Esteio and SCS 205 Riqueza. Genetic variability and transgressive segregation were detected for all traits evaluated. Heritability estimates for the concentrations of phosphorus, phytate, iron, and zinc ranged from intermediate (h^2: 30.31%) to high (h^2: 98.68%) magnitude, and quantitative inheritance was observed. The phosphorus concentration showed an intermediate correlation estimate with iron (r = 0.4157) and zinc (r = 0.5693) concentrations. Cultivar BRS Expedito and line L 56-17 have a low phytate concentration (≤ 1.29%) and a high iron concentration (≥ 95 mg kg^{-1} of dry matter - DM), and will be selected by the common bean biofortification program. Lines L 59-17, L 31-17, and L 26-17 and cultivars IPR Siriri and BRS Valente have a high phytate concentration (≥ 2.57%) and a low zinc concentration (≤ 26 mg kg^{-1} DM) and will be selected for diets that aim at using the beneficial properties of phytate and reducing the zinc intake.

Index terms: *Phaseolus vulgaris;* heritability; inheritance pattern; Pearson's correlation; selection index.

RESUMO

O desenvolvimento de cultivares de feijão com concentração de minerais e de fitatos que atendam aos diferentes requerimentos nutricionais dos consumidores é uma nova estratégia dos programas de melhoramento. Os objetivos desse trabalho foram obter estimativas de parâmetros genéticos para a concentração de fósforo, fitatos, ferro e zinco em uma população de linhagens homozigotas recombinantes (LHR) de feijão Mesoamericano, estudar as correlações entre esses caracteres e selecionar linhagens de feijão para o programa de biofortificação e para dietas que necessitam diminuir a ingestão desses minerais. A LHR foi obtida pelo cruzamento entre BRS Esteio e SCS 205 Riqueza. Variabilidade genética e segregação transgressiva foram observados para todos os caracteres avaliados. Estimativas de herdabilidade para a concentração de fósforo, fitatos, ferro e zinco foram de intermediária (h^2: 30,31%) a alta magnitude (h^2: 98,68%) e herança quantitativa foi observada. A concentração de fósforo mostrou intermediária correlação com as concentrações de ferro (r= 0,4157) e zinco (r= 0,5693). A cultivar BRS Expedito e a linhagem L 56-17 apresentam baixa concentração de fitatos (≤ 1,29%) e alta concentração de ferro (≥ 95 mg kg^{-1} de matéria seca - MS) e serão selecionadas pelo programa de biofortificação do feijão. As linhagens L 59-17, L 31-17 e L 26-17 e as cultivares IPR Siriri e BRS Valente possuem alta concentração de fitatos (≥ 2,57%) e baixa concentração de zinco (≤ 26 mg kg^{-1} de MS) e serão selecionadas para uso em dietas que visam as propriedades benéficas dos fitatos e a redução da ingestão de zinco.

Termos para indexação: *Phaseolus vulgaris;* herdabilidade; padrão de herança; correlação de Pearson; índice de seleção.

INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is a staple food in several countries and is found in local recipes that use fresh or dried grains. Common beans are healthy food due to high concentrations of several minerals and low concentrations of toxic elements in the grains (Di Bella et al., 2016).

The mineral concentrations in common bean cultivars has been described in the literature as having a wide genetic variability (Akond et al., 2011; Hossain et al., 2013; Ribeiro et al., 2013a; Martins et al., 2016). This fact has enabled the development of common bean lines with high concentrations of phosphorus (Blair et al., 2009a, 2012; Maziero; Ribeiro; Facco, 2016), iron

(Blair et al., 2009b, 2010; Jost et al., 2013; Ribeiro et al., 2013a,b; Martins et al., 2016), and zinc (Blair et al., 2009b, 2010; Ribeiro et al., 2013a; Martins et al., 2016; Maziero; Ribeiro; Facco, 2016). Breeding programs that aim at the biofortification of the common bean must also consider the phytate concentration. This compound represents 40-72% of the phosphorus stored in the common bean grains, and this form of phosphorus is less available (Coelho et al., 2002). Phytate also shows a high affinity for polyvalent cations, such as iron and zinc, interfering with the intestinal absorption of these elements (Schlemmer et al., 2009). Therefore, lowering the phytate concentration is imperative for the common bean biofortification.

However, phytate also has beneficial health properties, such as the prevention of several types of cancer (lung, liver, breast, prostate, and skin) and the antioxidant and anticalcification activities (Schlemmer et al., 2009). Also, some patients need to reduce iron intake due to hereditary hemochromatosis (Cançado; Chiattone, 2010). Similarly, a low-zinc intake diet is recommended for patients who need to normalize blood HDL levels (Guerrero-Romero; Rodríguez-Morán, 2005). In these cases, the use of common bean cultivars with high phytate concentration and low iron and zinc concentrations could represent health benefits.

In early generations of a breeding program, Zemolin et al. (2016) selected recombinants of Andean common bean with low iron and zinc concentrations. However, the development of common bean cultivars with high phytate concentration and low mineral concentrations is still unprecedent in the literature. No previous studies regarding the genetic parameters for the concentrations of phosphorus, phytate, iron, and zinc in a single recombinant inbred line population of Mesoamerican common bean were found. The combined selection for the development of common bean cultivars that meet the different nutritional requirements of consumers is a new strategy of breeding programs.

Thus, this work aimed to estimate genetic parameters for the concentrations of phosphorus, phytate, iron, and zinc in a recombinant inbred line population of Mesoamerican common bean, to study the correlations between these traits, and to select common bean lines for the biofortification program and for diets that require the decrease in the intake of these minerals.

MATERIAL AND METHODS

Obtaining of the recombinant inbred line population

The common bean cultivars BRS Esteio (Brazilian Agricultural Research Corporation, 2012; Pereira et al., 2013) and SCS 205 Riqueza (Agricultural Research and Rural Extension Corporation, 2016; Kavalco et al., 2017) were crossed in 2012 to develop a recombinant inbred line population. BRS Esteio has black grains and was originally denominated as line CNFP 10104. SCS 205 Riqueza has carioca grains (beige seed coat with brown streaks) and was originally denominated as line CHC 01-175. These cultivars represent the two most produced grain types in Brazil and belong to the Mesoamerican gene pool. These cultivars were previously selected by Ribeiro et al. (2013a) due to differences observed for iron and zinc concentrations in the grains.

The recombinant inbred line population was grown in a greenhouse by the Single-Seed Descent method (Brim, 1966). The seeds obtained in each plant of the F_5 generation were collected individually for the evaluation of the lines in field experiments.

Field experiments and determination of mineral and phytate concentrations

The recombinant inbred line population was evaluated in two experiments installed in the field area of the Common Bean Breeding Program of the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil (lat. 29º42'S; long. 53º43'W; alt. 95 m asl.). The region has a humid subtropical climate, with hot summers and undefined dry season, according to Köppen classification (Kuinchtner; Buriol, 2001).The soil is classified as a typical alitic Argisol, Hapludalf, and showed the following chemical composition before the experiments installation: organic matter - 1.80%; base saturation - 73.90%; pH (H_20) - 6.10; effective cation exchange capacity - 9.90 cmolc dm^{-3}; potassium - 80.00 mg dm^{-3}; phosphorus - 12.70 mg dm^{-3}; calcium: - 6.70 cmolc dm^{-3}; iron - 2.026.50 mg dm^{-3}; copper - 1.20 mg dm^{-3}; and zinc - 0.50 mg dm^{-3}. The soil was prepared in a conventional manner, and fertilization was based on the interpretation of the soil chemical analysis. At furrow sowing 275 kg ha^{-1} of the $N-P_2O_5-K_2O$ 5-20-20 formula (urea - 45% nitrogen; triple superphosphate - 18% P_2O_5; and potassium chloride - 60% K_2O) were applied. In addition, 67 kg ha^{-1} of urea (45% nitrogen) were distributed in the growth stage of the first trifoliate leaf. Fertilizers were not added with micronutrients.

The $F_{5:6}$ generation was evaluated in the dry season (sowing on March 9, 2017), in an augmented block design, with two replications. Treatments consisted of 103 common bean genotypes, with 100 lines at the $F_{5:6}$ generation (non-common treatments), two parent cultivars (BRS Esteio and SCS 205 Riqueza), and cultivar Pérola (control). The $F_{5:7}$ generation was sown on October 28, 2017, in the rainy season, in a 11 x 11 simple lattice design. This experiment evaluated 121 common bean genotypes, consisting of 100 lines at the $F_{5:7}$ generation, two parent cultivars, and 19 common bean cultivars (controls). In both experiments, the experimental plot was composed of a 1 m row, spaced at 0.50 m, with 15 seeds per linear meter.

Management practices were the same for both experiments. Seeds were treated with the fungicide Maxim XL (Fludioxonil and Metalaxyl−M) and the insecticide Cruiser (Thiamethoxam), both at a dose of 200 mL 100 kg^{-1} of seeds. The insecticide Engeo™ Pleno (Thiamethoxam and Lambda-cyhalothrin) was applied at a dose of 125 mL ha^{-1}, whenever the insect infestation caused approximately 5% damage to common bean plants. Weeds were eliminated by mechanical control to prevent competition with the crop. Fungicides were not applied during the development of common bean plants.

Plants harvesting and grain processing were performed manually at the maturation stage to avoid samples contamination. The grains were dried in the sun, and, if needed, oven-dried at 40 ºC, until reaching 13% humidity. A random sample of 10 g grains of each treatment was ground in an analytical knife micro-mill (Q298A21, Quimis, São Paulo, Brazil) to obtain not-sieved particles smaller than 1 mm in diameter.

Mineral concentrations (phosphorus, iron, and zinc) were determined in 0.5 g aliquots of the raw bean flour, which were digested in nitric and perchloric solution (HNO_3 + $HClO_4$, in a 3:1 ratio by volume), as described by Jost et al. (2013). The phosphorus concentration was obtained in an optical emission spectrophotometer (AA-7000, Shimadzu, São Paulo, Brazil), with a wavelength of 660 nm. Iron and zinc concentrations were determined in an atomic absorption spectrophotometer (AAS, Perkin Elmer AAnalyst 200, Waltham, USA), with a wavelength of 248.30 nm and 213.90 nm, respectively.

Phytate concentration was quantified in 2.0 g aliquots of the raw bean flour, added with 40 ml of hydrochloric acid (HCl), at a concentration of 2.4% (0.65 N), following the methodology developed by Latta and Eskin (1980). Afterward, the phytate concentration was obtained in a UV/visible spectrophotometer, in the 500 nm range (SP-220, Bioespectro, São Paulo, Brazil).

Estimates of genetic parameters and combined selection

Data were subject to analysis of variance, according to the augmented block and single lattice designs for the $F_{5:6}$ and $F_{5:7}$ generations, respectively. The experimental precision was evaluated by the coefficient of experimental variation (CEV, %) and by the selective accuracy (SA). The SA was obtained according to Resende and Duarte (2007). The efficiency of the lattice design in relation to the randomized block design was evaluated as described in Jost et al. (2013).

The following estimates of genetic parameters were obtained for the two generations: phenotypic variance, environmental variance, genetic variance, heritability, coefficient of genetic variation (CGV, %), and CGV/CEV ratio. The heritability was obtained based on the variance components, using the means of treatments, by the formula $h^2 = \frac{\sigma^2 G}{\sigma^2 P}$, where σ^2G is the genetic variance and σ^2P is the phenotypic variance. At the $F_{5:6}$ and $F_{5:7}$ generations, the homozygosis is high, and the genetic variance corresponds to the additive variance. Therefore, the estimates obtained are equivalent to the narrow-sense heritability.

Data normality was evaluated by the Shapiro-Wilk test (p-value <0.05). Frequency distribution graphs for each trait evaluated were generated at the $F_{5:6}$ and $F_{5:7}$ generations. In each graph, the number of classes was established by the expression $\sqrt{n}$, where n is the number of observations. The distribution type (continuous or discontinuous) was analyzed for each trait and generation evaluated.

The Pearson's linear correlation analysis was performed for the four traits evaluated at the $F_{5:7}$ generation, considering the phenotypic matrix data. The significance of the coefficients was verified by the Student's t-test (p-value <0.05).

The index based on the rank sum (Mulamba; Mock, 1978) was applied to the selection of the 24 superior Mesoamerican common bean lines for the four traits evaluated at the $F_{5:7}$ generation (20% of the total lines evaluated). The selection was performed to increase the concentrations of phosphorus, iron, and zinc and decrease the phytate concentration, aiming at the development of biofortified common bean cultivars. Thus, weight 1 was attributed to all the traits, except the iron concentration, which received weight 2. Common bean lines with low concentrations of phosphorus, iron, and zinc and high phytate concentration also were selected, aiming at directing them to diets that require the reduction of these minerals and using the antioxidant and cancer prevention benefits of phytate. In this case, all traits received weight 1, except for the phytate concentration, which received weight 2. Analyses were performed using a Microsoft Office Excel spreadsheet and the Genes software (Cruz, 2016).

RESULTS AND DISCUSSION

General results

The analysis of variance revealed a significant treatment effect for all traits evaluated at the $F_{5:6}$ and $F_{5:7}$ generations (Table 1). Therefore, from the cross between cultivars BRS Esteio and SCS 205 Riqueza, Mesoamerican common bean lines with genetic variability for the concentrations of phosphorus, phytate, iron, and zinc were generated. Mesoamerican common bean lines with great variation for the concentrations of phosphorus and zinc (Maziero; Ribeiro; Facco, 2016), phosphorus and phytate (Blair et al., 2009a, 2012), and iron and zinc (Blair et al., 2009b, 2010) were previously obtained from crosses between contrasting parents for these traits. These results show the possibility of developing common bean cultivars with concentrations of phosphorus, phytate, iron, and zinc that meet the different nutritional requirements of consumers.

The $F_{5:6}$ generation had the lowest values for coefficient of experimental variation (1.66 to 7.82%) and the highest values of selective accuracy (≥ 0.98), providing higher experimental precision in the evaluation of the traits. However, the highest experimental precision was expected for the $F_{5:7}$ generation when the experiment was conducted in a lattice design. The lattice design required a larger field area due to the use of replications for all treatments. Larger experimental areas tend to show heterogeneity in the soil chemical or physical composition, and this fact contributed to a larger experimental error in the evaluation of the lines at the $F_{5:7}$ generation. Nevertheless, the efficiency of the lattice design varied from 101.11 (iron concentration) to 148.29% (zinc concentration), indicating that the use of this design was adequate. Ramalho, Ferreira and Oliveira et al. (2000) recommended the evaluation of the efficiency of the lattice design in relation to the randomized block design; if the result is higher than 100%, the analysis of variance according to the single lattice design should be maintained.

Genetic parameters of phosphorus and phytate concentrations

The decomposition of the phenotypic variance evidenced the greatest contribution of the genetic variance in relation to the environmental variance for the phosphorus and phytate concentrations at the $F_{5:6}$ and $F_{5:7}$ generations (Table 1). Thus, heritability estimates ranged from intermediate to high magnitude for the phosphorus concentration (h^2: 30.31-98.68%) and of high magnitude for the phytate concentration (h^2: 73.63-97.47%) were obtained. In $F_{6:8}$ lines of Mesoamerican common bean, Maziero, Ribeiro and Facco (2016) observed that the heritability of the phosphorus concentration ranged among the populations evaluated (h^2: 23.90-61.90%). The heritability estimates of the phosphorus concentration in Mesoamerican common bean may vary according to the genetic variability of the population evaluated (Maziero; Ribeiro; Facco, 2016) and of the generation analyzed, as observed in the present study. For the phytate concentration, no previous work has addressed the obtainment of genetic parameters in segregating or high-homozygosis generation in Mesoamerican common bean.

The relationship between the coefficient of genetic variation and the coefficient of environmental variation (CGV/CEV ratio) was higher than the unity for the phosphorus ($F_{5:6}$ generation) and phytate ($F_{5:6}$ and $F_{5:7}$ generations) concentrations. CGV/CEV ratio close to or greater than unity indicate a favorable selection condition, with higher possibilities of selection gain (Jost et al., 2013). However, at the $F_{5:7}$ generation, the CGV/CEV ratio was 0.47 for the phosphorus concentration. CGV/CEV ration lower than 0.6 were previously obtained for the phosphorus concentration in $F_{6:8}$ lines of four Mesoamerican common bean populations (Maziero; Ribeiro; Facco, 2016), indicating that the environmental variance had higher expression in the phenotype variance. In the present study, the lower heritability and the CGV/CEV ratio values confirmed a greater non-controllable variation for the phosphorus concentration evaluated at the $F_{5:7}$ generation.

The variation range observed for the phosphorus and phytate concentrations was different between the generations evaluated. Mesoamerican common bean lines of the $F_{5:6}$ generation had phosphorus concentration ranging from 1.72 to 4.30 g kg^{-1} of dry matter - DM (Figure 1A) and phytate concentration ranging from 0.22 to 2.70% (Figure 1C). Conversely, Mesoamerican common bean lines of the $F_{5:7}$ generation had phosphorus concentration ranging from 3.27 to 4.64 g kg^{-1} DM (Figure 1B) and phytate concentration ranging from 0.68 to 3.39% (Figure 1D). Crosses between parents of the Mesoamerican and Andean gene pools generated common bean lines at the $F_{5:8}$ (Blair et al., 2009a) and $F_{7:11}$ (Blair et al., 2012) generations, with similar value for phosphorus concentration and lower value for phytate concentration when compared with those found in this study. Maziero, Ribeiro and Facco (2016) generated $F_{6:8}$ lines of Mesoamerican common bean from crosses between different parents, with phosphorus concentration ranging from 4.1 to 5.9 g kg^{-1} DM. In the present study, even using convergent crosses for phosphorus and phytate concentrations, transgressive segregation was detected, *i.e.*, common bean lines with phosphorus and phytate concentrations lower and higher than those of the parents were obtained in the two generations evaluated.

Table 1: Analysis of variance and estimates of genetic parameters for the concentrations of phosphorus, phytate, iron, and zinc found in Mesoamerican common bean lines at the $F_{5:6}$ and $F_{5:7}$ generations.

Sources of variation	DF	Mean square			
		Phosphorus	Phytate	Iron	Zinc
		g kg^{-1}	%	mg kg^{-1}	mg kg^{-1}
		$F_{5:6}$ generation			
Block	1	1.563	0.208	221.706	3.159
Treatment (adjusted)	102	0.214*	0.306*	45.296*	30.722*
Residue	2	0.003	0.008	1.676	0.713
General mean		3.19	1.13	70.53	30.36
Common mean - control		3.52	0.96	69.36	33.85
No common mean - lines		3.18	1.14	70.60	30.15
CEV (%)[1]		1.66	7.82	1.83	2.80
Selective accuracy		0.99	0.99	0.98	0.99
Phenotypic variance		0.212	0.316	48.575	28.803
Environmental variance		0.003	0.008	1.676	0.713
Genetic variance		0.209	0.308	46.899	29.090
Heritability (%)		98.68	97.47	96.55	97.61
CGV (%)[2]		14.40	48.51	9.70	17.89
CGV/CVE ratio[3]		8.64	6.20	5.29	6.39
		$F_{5:7}$ generation			
Replications	1	0.045	0.017	1005.476	331.157
Block/replications (adjusted)	20	0.181	0.235	93.669	23.182
Treatment (adjusted)	120	0.142*	0.612*	147.735*	8.828*
Residue	100	0.099	0.161	74.803	5.165
Lattice efficiency (%)		107.44	102.98	101.11	148.29
General mean		3.95	1.71	68.65	23.20
Control mean		3.94	1.99	70.00	22.72
Lines mean		3.95	1.70	68.61	23.22
CEV (%)[1]		7.97	23.50	12.60	9.79
Selective accuracy		0.55	0.86	0.70	0.64
Phenotypic variance		0.071	0.306	73.867	4.414
Environmental variance		0.005	0.081	37.401	2.583
Genetic variance		0.021	0.225	36.466	1.831
Heritability (%)		30.31	73.63	49.37	41.49
CGV (%)[2]		3.72	27.77	8.80	5.83
CGV/CEV ratio[3]		0.47	1.18	0.70	0.59

[1]Coefficient of environmental variation. [2]Coefficient of genetic variation. [3]Coefficient of genetic variation and coefficient of environmental variation ratio. *Significant by F test at 0.05 probability.

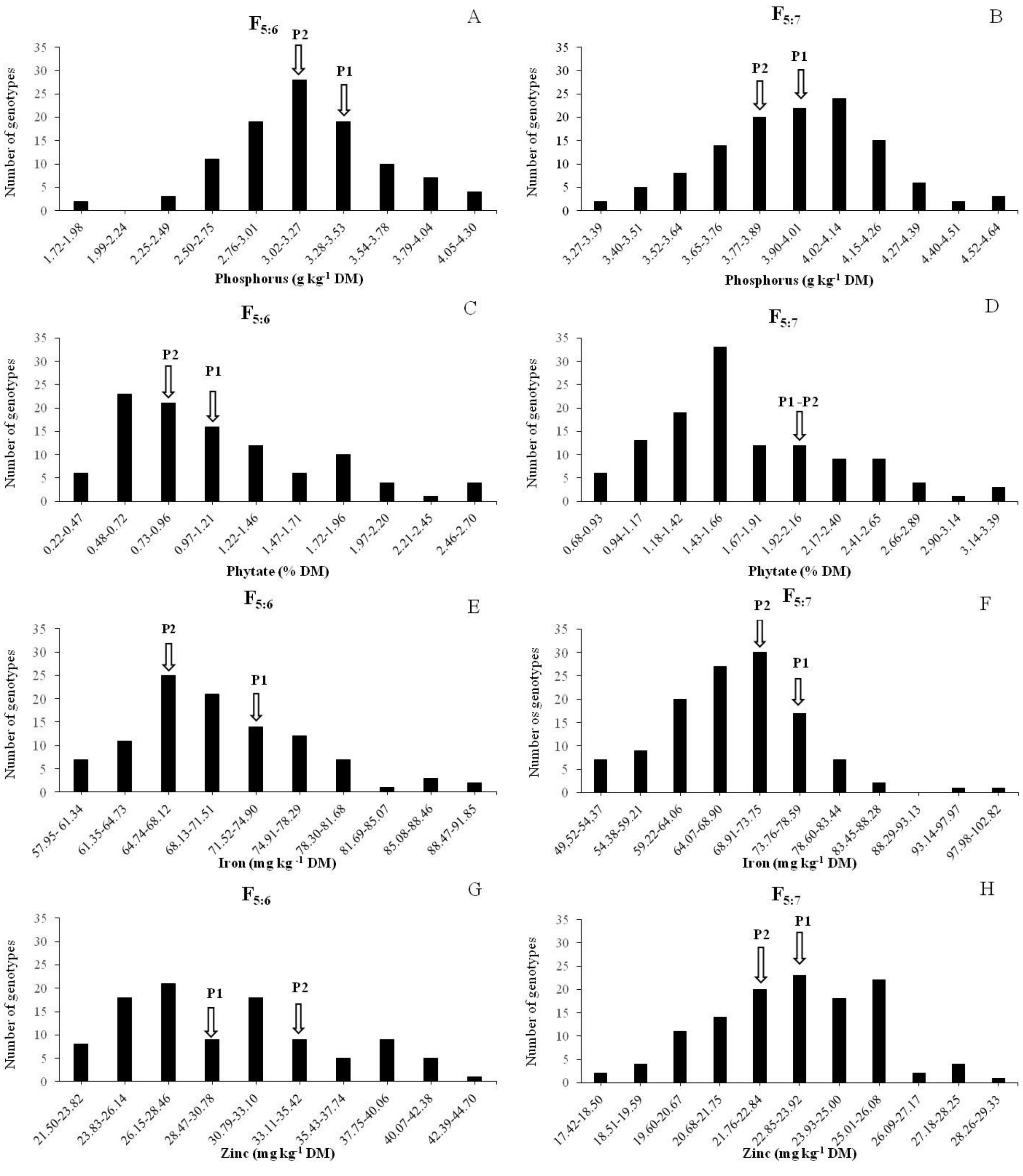

Figure 1: Frequency distribution for the concentrations of phosphorus, phytate, iron, and zinc found in Mesoamerican common bean lines at the $F_{5:6}$ and $F_{5:7}$ generations. P1 and P2 are the parent cultivars: P1 (BRS Esteio) and P2 (SCS 205 Riqueza).

Phosphorus and phytate concentrations in $F_{5:6}$ and $F_{5:7}$ lines of Mesoamerican common bean showed a continuous distribution, close to normal, characterizing quantitative inheritance (Figure 1A, 1B, 1C, and 1D). The continuous distribution for the phosphorus and phytate concentrations in common bean lines of the $F_{5:8}$ (Blair et al., 2009a) and $F_{7:11}$ (Blair et al., 2012) generations has been previously described in crosses between parents of the Mesoamerican and Andean gene pools. These results confirm that phosphorus and phytate concentrations in common bean have a quantitative inheritance in high homozygote population studied.

Genetic parameters of iron and zinc concentrations

The $F_{5:6}$ generation revealed high heritability estimates for the iron (h^2: 96.55%) and zinc (h^2: 97.61%) concentrations in Mesoamerican common bean (Table 1). This result can be attributed to the higher value of the genetic variance in relation to the environmental variance of these minerals at the $F_{5:6}$ generation. However, values of environmental variance exceeded the values of genetic variance at the $F_{5:7}$ generation, resulting in heritability estimates of intermediate magnitude for the iron (h^2: 49.37%) and zinc (h^2: 41.49%) concentrations. Similarly, heritability estimates from intermediate to high magnitude were detected for the iron concentration in Mesoamerican common bean lines at the $F_{3:4}$, $F_{3:5}$, and $F_{3:6}$ generations (Teixeira et al., 2015) and in common beans of grain types carioca, black, and mulatinho (cream-colored grain) (Martins et al., 2016). For the zinc concentration, Maziero, Ribeiro and Facco (2016) verified heritability estimates of low to high magnitude in common bean populations obtained from different crosses between Mesoamerican parents. Traits with high heritability estimates have higher prospects of success with selection since most of the variation will be of genetic nature. Heritability estimates provide a better evaluation of the genetic variability of the population and allow predicting selection gains. Therefore, they are essential for the breeding program.

The values of CGV/CEV ratio were higher than the unity for the iron and zinc concentrations only at the $F_{5:6}$ generation, indicating a higher probability of selection success. Martins et al. (2016) observed CGV/CEV ratio close to or greater than the unity for iron and zinc concentrations in Mesoamerican common bean inbred lines, demonstrating favorable conditions for the selection of these two minerals. However, CGV/CEV ratio ≤ 0.70 for the iron and zinc concentrations were obtained at the $F_{5:7}$ generation. CGV/CEV ratio lower than the unity does not prevent obtaining a genetic gain in the selection of a trait. Although the value of CGV/CEV ratio is lower than the unity for iron concentration in Mesoamerican common bean lines at the F_7 generation, Jost et al. (2013) observed positive genetic gain estimates for the different selection indices evaluated. Maziero, Ribeiro and Facco (2016) reported similar results for zinc concentration in Mesoamerican common bean lines at the $F_{6:8}$ generation. In this case, the values of CGV/CEV ratio were lower than 0.70, but the genetic gain of all the four populations evaluated had a favorable magnitude for the selection of common bean lines with high zinc concentration.

The iron concentration ranged from 57.95 to 91.85 mg kg^{-1} DM at the $F_{5:6}$ generation (Figure 1E) and from 49.52 to 102.82 mg kg^{-1} DM at the $F_{5:7}$ generation (Figure 1F). Transgressive segregation was observed in both generations. Common bean lines generated from the cross between parents of the Mesoamerican gene pool had lower variation range for iron concentration at the $F_{3:6}$ generation (Teixeira et al., 2015). Conversely, other studies showed values for the F_7 (Ribeiro et al., 2014) and F_{10} (Blair et al., 2010) generations similar to those found in the present study.

The cultivars BRS Esteio and SCS 205 Riqueza showed values relatively close for iron and zinc concentrations (Figures 1E, 1F, 1G, and 1H), in contrast with the observations of Ribeiro et al. (2013a). In this case, the differences in the soil pH, soil micromineral content and amount of precipitation during the growing season explain the variation that was observed in the iron and zinc concentrations (Petry et al., 2015; Possobom et al., 2015). When non-contrasting parents for the iron and zinc concentrations are used in a directed cross, common bean lines with lower variation range for this mineral are expected, as was verified by Teixeira et al. (2015). Nevertheless, this is not a rule, as detected in the present study, and in the work of Zemolin et al. (2016) in Andean common bean lines. Common bean lines with wide variation for the iron and zinc concentrations were obtained in the two generations evaluated, from the cross between parents with little variation for the concentration of these mineral, allowing the selection of superior lines.

The zinc concentration had a greater variation range at the $F_{5:6}$ generation (21.50-44.70 mg kg^{-1} DM, Figure 1G), in relation to the $F_{5:7}$ generation (17.42-29.33 mg kg^{-1} DM, Figure 1H). Therefore, even when using convergent crosses for the zinc concentration, a wide segregation was observed for this mineral in the $F_{5:6}$ and $F_{5:7}$ generations, comparable to that observed previously by Zemolin et al. (2016) in segregants obtained from crosses between Andean parents. In the two generations were obtained common bean lines with lower and higher zinc concentration than that of the parents, respectively, which is typical of transgressive segregation. Previous studies have shown that transgressive segregation occurs for zinc concentration in Mesoamerican common bean at the $F_{3:6}$ (Teixeira et al., 2015), $F_{7:11}$ (Blair et al., 2009b), and F_{10} (Blair et al., 2010) generations.

The frequency distribution observed for the iron and zinc concentrations at the $F_{5:6}$ and $F_{5:7}$ generations was continuous (Figure 1E, 1F, 1G, and 1H). Therefore, the iron and zinc concentrations in the Mesoamerican common bean had quantitative inheritance, confirming the previous results obtained for this gene pool at the $F_{7:11}$ (Blair et al., 2009b) and F_{10} (Blair et al., 2010) generations. Thus, the iron and zinc concentrations in a recombinant inbred line population of Mesoamerican common bean have a quantitative inheritance. Quantitative inheritance traits presented a large number of genes and the high environmental effect on their expression (Ramalho et al., 2012). This fact indicates difficulties for the selection of Mesoamerican common bean lines by the iron and zinc values.

Correlations between the concentrations of phosphorus, phytate, iron, and zinc

The evaluation of the correlations between minerals and phytate may represent gains by indirect selection. The phosphorus concentration had a positive and low magnitude correlation with the phytate concentration (r = 0.1956) and an intermediate magnitude correlation with iron (r = 0.4157) and zinc (r = 0.5693) concentrations (Table 2). Previous studies have also verified that the phosphorus and phytate concentrations in common bean of different gene pools have a low magnitude correlation (Blair et al., 2009a, 2012). However, no correlation was observed between the phosphorus and iron concentrations in common bean lines (Silva et al., 2012; Hossain et al., 2013). Already, an intermediate magnitude correlation between phosphorus and zinc concentrations in common bean was described previously by Silva et al. (2012) and Hossain et al. (2013).

Table 2: Pearson's correlation estimates between the concentrations of phosphorus, phytate, iron, and zinc found in Mesoamerican common bean lines at the $F_{5:7}$ generation.

	Phytate	Iron	Zinc
Phosphorus	0.1956*	0.4157*	0.5693*
Phytate		0.0975^{ns}	0.1537^{ns}
Iron			0.3977*

*Significant by the Student's t-test at 0.05 probability. ^{ns}Non-significant.

The other pairs of correlations evaluated were not significant (iron - phytate and zinc phytate) or were of low magnitude (iron - zinc), confirming the results previously observed by Akond et al. (2011) in common bean inbred lines. This fact suggests the absence of linked genes or pleiotropic effects between iron and zinc concentrations and between these minerals and phytate concentration. According to Balestre et al. (2013), when this phenomenon occurs, the genetic values of the traits are independent, and therefore, the selection of superior genotypes is supposed to be easier.

In the present study, correlation estimates between phosphorus and iron concentrations (r = 0.4157) and phosphorus and zinc concentrations (r = 0.5693) were of intermediate magnitude. Thus, the selection of common bean lines with high phosphorus concentration will also be efficient to increase the iron and zinc concentrations in the Mesoamerican common bean lines.

Combined selection to increase concentrations of phosphorus, iron, and zinc and decrease phytate concentration

The total sum of gains obtained by the rank sum index was 5.98%, showing favorable individual gains for the development of common bean cultivars with biofortified grains for phosphorus (1.88%), iron (6.58%), and zinc (3.62%), and with low phytate concentration (-6.10%), (Table 3). Of the 24 superior genotypes selected by the rank sum index, none had high phosphorus concentration, *i.e.*, ≥ 5.00 g kg^{-1} DM, according to Steckling et al. (2017). Similarly, none genotype selected had high zinc concentration, which should be ≥ 31.00 mg kg^{-1} DM, as indicated by Tryphone and Nchimbi-Msolla (2010).

The lines L 39-17 and L 12-17 exhibited phytate concentration similar to the value observed in the common bean mutants evaluated by Cominelli et al. (2018), which have a low phytate concentration. Phytate represents 40-72% of the phosphorus stored in the common bean grains (Coelho et al., 2002). Therefore, decreasing the phytate concentration in the grains without reducing the phosphorus concentration is an important goal of the common bean breeding program. Common bean genotypes with the lowest phytate concentration ($\leq$ 1.29%) were also those that have the highest phosphorus concentration values (3.79-4.41 g kg^{-1} DM): BRS Expedito, L 17-17, L 92-17, IAC Milênio, L 5-17, L 52-17, L 39-17, L 36-17, L 56-17, L 12-17, L 38-17, and L 77-17. Phosphorus contributes to the maintenance of bones and teeth (Pravst, 2011), and common bean is an important phosphorus source (Silva et al., 2012; Maziero; Ribeiro; Facco, 2016).

Table 3: Mean of the original population (Xo), mean of the selected population (Xs), heritability (h²), genetic gain (GG), and percentage of genetic gain (GG%) with simultaneous selection by the rank sum index for the 24 Mesoamerican common bean lines (20% of the total lines evaluated) with high concentrations of phosphorus (g kg^{-1} of dry matter - DM), iron (mg kg^{-1} DM), and zinc (mg kg^{-1} DM) and low phytate concentration (%) at the $F_{5:7}$ generation.

	Xo	Xs	h² (%)	GG	GG (%)
Phosphorus	3.95	4.19	30.31	0.07	1.88
Phytate	1.71	1.57	73.63	-0.10	-6.10
Iron	68.65	77.79	49.37	4.51	6.58
Zinc	23.20	25.23	41.49	0.84	3.62
Total gain				5.32	5.98

Selected lines $F_{5:7}$	Phosphorus	Phytate	Iron	Zinc
BRS Expedito	4.40	1.29	96.95	29.16
L 56-17	4.54	1.46	74.39	26.36
L 17-17	4.11	1.24	79.24	24.51
L 66-17	4.32	1.79	79.82	25.51
L 92-17	4.41	1.16	72.78	24.47
L 89-17	4.46	2.58	81.50	27.56
L 61-17	4.54	1.91	74.63	26.72
L 15-17	4.04	1.59	77.38	27.59
IAC Milênio	3.94	1.23	76.87	25.46
L 5-17	4.15	1.17	73.70	24.33
L 52-17	4.41	1.27	70.28	25.88
L 34-17	4.54	2.72	81.59	25.49
L 39-17	4.16	0.97	73.93	23.10
L 79-17	4.17	2.45	79.89	26.73
L 99-17	4.17	1.54	74.54	24.16
L 36-17	4.24	1.25	72.33	23.67
L 4-17	4.08	1.46	74.84	23.61
L 54-17	4.21	2.55	81.13	24.79
L 56-17	3.79	1.28	102.08	23.32
L 12-17	3.99	0.92	71.23	25.19
L 16-17	3.89	1.31	82.61	22.65
L 19-17	4.23	2.12	74.75	24.41
L 38-17	3.78	1.07	72.55	24.97
L 77-17	4.11	1.29	68.08	25.84

The cultivar BRS Expedito and the line L 56-17 have high iron concentration, *i.e.,* higher than 95 mg kg^{-1} DM, as previously defined by Ribeiro et al. (2013b). The use of common bean cultivars with biofortified grains for iron may represent health benefits. The consumption of iron-rich foods is essential to prevent symptoms of iron deficiency, which, according to Camaschella (2015), consist of anemia, weakness, fatigue, difficulty in concentration, and low productivity at work.

The cultivar BRS Expedito and the line L 56-17 also showed low phytate concentration. Phytate is considered as antinutrient because it may inhibit the absorption of iron and zinc, especially in unbalanced diets, leading to the deficiency of these minerals (Schlemmer et al., 2009). Therefore, decreasing phytate concentration is crucial to increase the bioavailability of iron and zinc in common bean grains. The cultivar BRS Expedito and the line L 56-17 are important for the common bean biofortification program and may represent benefits to the diets of people diagnosed with iron deficiency.

Combination selection to decrease the concentrations of phosphorus, iron, and zinc and increase the phytate concentration in common bean

Negative genetic gain estimates were obtained for the concentrations of phosphorus (-1.64%), iron (-5.79%), and zinc (-2.37%) (Table 4). Conversely, positive genetic gain estimate was detected for the phytate concentration (16.03%). These estimates favor the selection of Mesoamerican common bean lines with low concentrations of phosphorus, iron, and zinc and high phytate concentration, that are indicated for diets that aim at reducing the intake of these minerals and using the beneficial properties of bioactive compounds that are beneficial to health (Chávez-Mendoza; Sánchez, 2017). Andean common bean lines with low concentrations of iron and zinc were previously selected in F_2 generation by Zemolin et al. (2016). However, the selection of Mesoamerican common bean lines with low concentrations of phosphorus, iron, and zinc and high phytate concentration is still unprecedent in the literature.

Among the genotypes selected by the rank sum index, L 59-17, IPR Siriri, L 31-17, L 26-17, and BRS Valente showed phytate concentration similar to the value found in a wild bean genotype by Cominelli et al. (2018), characterizing high phytate concentration. Increasing the phytate concentration in food is a recent trend in industrialized countries for people who adopt balanced diets. In this case, phytate presents several health benefits, such as anticancer (lung, liver, breast, prostate, and skin), antioxidant, and anticalcification activities (Schlemmer et al., 2009). The phytate concentration of genotypes L 59-17, IPR Siriri, L 31-17, L 26-17, and BRS Valente range from 2.57 to 3.32%. These variation range was from twice to three times higher that those values found in this study for the genotypes BRS Expedito, L 17-17, L 92-17, IAC Milênio, L 5-17, L 52-17, L 39-17, L 36-17, L 56- 17, L 12-17, L 38-17, and L 77-17 selected for low phytate concentration (Table 3). However, phosphorus concentration was very similar in these genotypes, which can be justified by the low correlation between phosphorus and phytate concentrations (Table 2). Cominelli et al. (2018) verified a 75% reduction in the phytate concentration in mutant common bean lines (low phytate concentration) in relation to the wild bean genotype (high phytate concentration), without a significant difference for phosphorus concentration in these genotypes. Therefore, Mesoamerican common bean cultivars with low or high phytate concentration can be obtained, without changing the phosphorus concentration of the grains.

None of the lines selected by the rank sum index exhibited iron concentration lower than 42.00 mg kg^{-1} DM, which is the reference proposed by Tryphone and Nchimbi-Msolla (2010) to characterize low iron concentration in common bean. However, all genotypes selected have low zinc concentration ($\leq$ 26.00 mg kg^{-1} DM), as proposed by Tryphone and Nchimbi-Msolla (2010). The identification of low-iron common bean lines is important for diets that require the reduction of iron intake due to hereditary hemochromatosis (Cançado; Chiattone, 2010). In this case, the intestinal iron absorption by the human organism may increase, causing an iron overload, triggering a series of diseases, such as fibrosis, functional insufficiency, sclerosis, diabetes, and cardiomyopathies (Cançado; Chiattone, 2010; Pietrangelo, 2010). Moreover, low-zinc common bean lines could be beneficial to normalize blood HDL levels (Guerrero-Romero; Rodríguez-Morán, 2005). In early generations of a breeding program, Zemolin et al. (2016) selected recombinants of Andean common bean with low iron and zinc concentrations. No previous studies have addressed the selection of Mesoamerican or Andean common bean lines with a low concentration of these minerals in generations with high homozygosis.

The lines L 59-17, L 31-17, and L 26-17 and the cultivars IPR Siriri and BRS Valente stood out for the high phytate concentration and low zinc concentration. These common bean genotypes are indicated for balanced diets, aiming at using the beneficial properties of phytate and reducing zinc intake. The development de common bean lines with the most consumed grain types in Brazil that attend different nutritional requirements of consumers is a new strategy of breeding programs. For decades phytates has been regarded as an antinutrient. However, beneficial properties of phytates has been described (Schelemmer et al., 2009), and bioactive compounds are beneficial to health (Chávez-Mendoza; Sánchez, 2017). The results obtained in the present study need to be validated for biovailability of minerals and protective role analysis of the common bean lines selected.

Table 4: Mean of the original population (Xo), mean of the selected population (Xs), heritability (h^2), genetic gain (GG), and percentage of genetic gain (GG%) with simultaneous selection by the rank sum index for the 24 Mesoamerican common bean lines (20% of the total lines evaluated) with low concentrations of phosphorus (g kg^{-1} of dry matter - DM), iron (mg kg^{-1} DM), and zinc (mg kg^{-1} DM) and high phytate concentration (%) at the $F_{5:7}$ generation.

	Xo	Xs	h^2 (%)	GG	GG (%)
Phosphorus	3.95	3.73	30.31	-0.06	-1.64
Phytate	1.71	2.08	76.63	0.27	16.03
Iron	68.65	60.59	49.37	-3.98	-5.79
Zinc	23.20	21.88	41.49	-0.55	-2.37
Total gain					

Selected lines $F_{5:7}$	Phosphorus	Phytate	Iron	Zinc
L 59-17	3.36	2.65	62.59	22.22
Guapo Brilhante	3.50	2.21	60.12	21.25
L 7-17	3.27	1.66	52.62	18.53
IPR Siriri	3.95	2.68	59.39	21.56
L 31-17	3.83	2.57	59.22	22.82
L 100-17	3.54	2.05	54.18	23.30
L 6-17	3.76	2.07	59.82	22.07
L 85-17	4.07	2.49	57.73	21.23
L 64-17	3.61	1.61	57.37	20.34
L 40-17	3.82	1.86	60.40	20.30
L 26-17	4.16	2.99	64.77	21.73
Fepagro Garapiá	3.69	1.94	52.39	23.68
L 96-17	3.81	2.04	66.16	21.68
L 58-17	3.58	1.99	67.92	22.34
L 84-17	3.77	2.17	63.67	23.37
IPR Juriti	3.74	2.34	63.30	24.52
L 91-17	3.74	1.59	59.60	21.48
BRS Valente	4.05	3.32	73.19	22.15
L 18-17	3.88	2.17	71.69	22.05
L 28-17	3.60	1.34	58.82	19.43
L 97-17	3.66	1.66	62.82	23.28
L 63-17	3.53	1.57	56.65	23.54
IPR Tiziu	4.00	1.62	58.03	21.14
BRS Estilo	3.72	1.35	51.82	21.10

CONCLUSIONS

Heritability estimates from intermediate (h^2: 30.31%) to high (h^2: 98.68%) magnitude are obtained for concentrations of phosphorus, phytates, iron, and zinc, and occurs transgressive segregation and quantitative inheritance in the recombinant inbred line population of Mesoamerican common bean. The indirect selection by the highest phosphorus concentration will also be efficient to increase the iron and zinc concentrations in the Mesoamerican common bean lines. The cultivar BRS Expedito and the line L 56-17 have a low phytate concentration ($\leq$ 1.29%) and high iron concentration ($\geq$ 95 mg kg^{-1} DM) and will be selected by the common bean biofortification program. The lines L 59-17, L 31-17, and L 26-17 and the cultivars IPR Siriri and BRS Valente have high phytate concentration ($\geq$ 2.57%) and low zinc concentration ($\leq$ 26 mg kg^{-1} DM) and will be selected for use in diets that aim at using the beneficial properties of phytate and require the reduction of zinc intake.

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