Genetic parameters and selection of micromineral-biofortified Andean bean lines with high technological quality

Parâmetros genéticos e seleção de linhagens de feijão Andino com alta qualidade tecnológica e biofortificadas para microminerais

Nerinéia Dalfollo Ribeiro[1]*; Henrique Caletti Mezzomo[2]; Greice Rosana Kläsener[2]

Highlights

Micromineral-biofortified Andean bean lines are unprecedented.
These traits showed high heritability ($h^2 \geq 75.57\%$).
Lines DFA 39-18 and DFA 01-18 are iron-biofortified and have technological quality.
Lines DFA 03-18, DFA 27-18 and DFA 44-18 have high technological quality.

Abstract

The development of new micromineral-biofortified Andean bean cultivars with a grain pattern that meets consumers' requirements is unprecedented. This study proposes to obtain estimates of genetic parameters for technological-quality traits and micromineral concentration and to select superior Andean bean lines. The cross between cultivars Hooter and Cal 96 generated the recombinant inbred line population that was evaluated in the $F_{5:6}$ and $F_{5:7}$ generations. Technological quality was analyzed based on grain color determined in a colorimeter: L* (dark to light), a* (green to red) and b* (blue to yellow) values; and mass of 100 grains. Micromineral concentration (iron and zinc) was obtained by acid digestion. A significant genotype effect was found for all evaluated traits, except for zinc concentration in the $F_{5:7}$ generation. L*, a* and b* showed high heritability ($h^2 \geq 90.85\%$) and qualitative inheritance. High heritability ($h^2 \geq 75.57\%$) was also observed for mass of 100 grains and iron and zinc concentrations, which exhibited quantitative inheritance. Grains of the cranberry bean lines DFA 39-18 and DFA 01-18 are very light (L* ≥ 52.18), with light red (a* ≤ 9.49) and light yellow (b* ≤ 16.00) shades; large (mass of 100 grains > 40 g); and have high iron concentrations (Fe ≥ 95.00 mg kg^{-1} dry matter). Grains of the red mottled bean lines DFA 03-18, DFA 27-18 and DFA 44-18 exhibited high technological quality, i.e., they are dark (33.11 < L* < 37.28), light red (16.24 < a*< 18.72) and very light yellow (b* ≤ 9.45) in shade, and large (mass of 100 grains > 40 g). These lines will be selected by the breeding program.
Key words: Color pattern. Frequency distribution. Heritability. Scott-Knott. Selection index. *Phaseolus vulgaris.*

[1] Profª Drª, Department of Plant Science, Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil. E-mail: nerineia@hotmail.com
[2] Students of the Master´s Course of the Pós-Graduate Program in Agronomy, UFSM, Santa Maria, RS, Brazil. E-mail: hc_mezzomo@hotmail.com; rosanaklasener@hotmail.com
* Author for correspondence

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

517

Resumo

O desenvolvimento de novas cultivares de feijão Andino biofortificadas para microminerais e com padrão de grãos que atenda a demanda dos consumidores é inédito. Os objetivos desse trabalho foram obter estimativas de parâmetros genéticos para caracteres da qualidade tecnológica e concentração de microminerais e selecionar linhagens de feijão Andino superiores. O cruzamento entre as cultivares Hooter e Cal 96 gerou a população homozigota recombinante que foi avaliada nas gerações $F_{5:6}$ e $F_{5:7}$. A qualidade tecnológica foi analisada pela coloração dos grãos determinada em um colorímetro pelos valores de L* (escuro a claro), a* (verde a vermelho) e b* (azul a amarelo) e pela massa de 100 grãos. A concentração de microminerais (ferro e zinco) foi obtida por digestão ácida. Efeito significativo para genótipo foi verificado para todos os caracteres avaliados, exceto para a concentração de zinco na geração $F_{5:7}$. Os valores de L*, a* e b* apresentaram alta herdabilidade ($h^2 \geq 90,85\%$) e herança qualitativa. Alta herdabilidade ($h^2 \geq 75,57\%$) também foi observada para a massa de 100 grãos e a concentração de ferro e zinco, que mostraram herança quantitativa. Grãos das linhagens de feijão *cranberry* DFA 39-18 e DFA 01-18 são muito claros (L* ≥ 52,18), com tonalidades vermelho claro (a* ≤ 9,49) e amarelo claro (b* ≤ 16,00); grandes (massa de 100 grãos > 40 g); e têm alta concentração de ferro (Fe ≥ 95,00 mg kg^{-1} de matéria seca). Grãos das linhagens de feijão *red mottled* DFA 03-18, DFA 27-18 e DFA 44-18 mostram alta qualidade tecnológica, ou seja, são escuros (33,11 < L* < 37,28), com tonalidades vermelho claro (16,24 < a*< 18,72) e amarelo muito claro (b* ≤ 9,45) e grandes (massa de 100 grãos > 40 g). Essas linhagens serão selecionadas pelo programa de melhoramento.
Palavras-chave: Padrão de cor. Distribuição de frequência. Herdabilidade. Skott-Knott. Índice de seleção. *Phaseolus vulgaris.*

Introduction

Common bean (*Phaseolus vulgaris* L.) is cultivated in several countries in tropical, subtropical and temperate regions of the world (Broughton et al., 2003). This crop is often the major source of protein, fibers and minerals in the human diet (Suárez-Martínez et al., 2016). Additionally, genetic variability has been reported in the iron and zinc concentrations in common bean genotypes (Blair, Astudillo, Grusak, Graham, & Beebe, 2009; Cichy, Caldas, Snapp, & Blair, 2009; N. D. Ribeiro, Steckling, Mezzomo, & Somavilla, 2019b).

In Brazil, the most widely produced and consumed common bean grain types are carioca (beige seed coat with brown streaks) (70%) and black (20%) (Lemos, Mingotte, & Farinelli, 2015), both belonging to the Mesoamerican gene pool. However, to increase the likelihood of a new common bean cultivar being accepted, breeding programs should observe grain color and size. In common bean, grain color can be assessed quantitatively by using a colorimeter, which measures the L*, a* and b* values. In the case of carioca bean cultivars, grains must be very light to be accepted by consumers. Arns et al. (2018) recommended the following grain color pattern for carioca beans: greater lightness (L* ≥ 55.00), light red (a* ≤ 7.00) and light yellow (b* ≤ 16.00) shades. Black bean cultivars, in turn, should have very dark grains, i.e., L* ≤ 22.00 (N. D. Ribeiro, Possebom, & Storck, 2003) and a* and b* values near zero, which would indicate the lack of a secondary color (Possobom, Ribeiro, Zemolin, & Arns, 2015; N. D. Ribeiro, Mezzomo, & Santos, 2019a). Because these color patterns are associated with newly

518

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

harvested and fast-cooking grains, they would be more appreciated by consumers. According to Carbonell, Chiorato, Gonçalves, Perina and Carvalho (2010), carioca and black bean grains must also be medium-sized (25 to 40 g 100 grains^{-1}) to meet the market demand.

Cranberry (light cream seed coat with red streaks and patches) and red mottled (dark red seed coat with cream streaks) common bean grains are still grown on a small scale in Brazil. However, the production of these grain types can increase the income of family farms due to their high commercial value. Additionally, in highly technified production systems, the cultivation of these grain types would represent significant advances to the export of grains highly demanded in the international market. This scenario suggests a need to develop Andean bean cultivars with cranberry and red mottled grains that are adapted to the growing conditions in Brazil. Nevertheless, no studies have been found that propose grain color or size patterns for these types of grain while also meeting the preference of bean consumers.

Technological quality, grain color and size define the acceptance or rejection of a new common bean cultivar. If the common bean cultivar is biofortified for microminerals, it can be used as a food to solve nutrition problems affecting people of all ages and social classes, especially in Africa, Asia and Latin America. Breeding programs have put greater efforts into developing new iron- and zinc-biofortified Mesoamerican common bean lines (Blair et al., 2009; Jost et al., 2013; Martins et al., 2016; Maziero, Ribeiro, & Facco, 2016; N. D. Ribeiro et al., 2019b). Results for iron- and zinc-biofortification in Andean beans have been obtained mostly in highly heterozygous generations (Mukamuhirwa, Tusiime, & Mukankusi, 2015; Possobom et al.,

2015; Zemolin, Ribeiro, Casagrande, Silva, & Arns, 2016).

The greater the knowledge about the phenotypic parameters of those traits, the more efficient the development of new micromineral-biofortified Andean bean cultivars of high technological quality. Estimates of the genetic parameters of heritability, coefficient of genetic variation and b coefficient are useful to investigate genetic variability in the population and predict gains with the selection. Studies providing these estimates for the traits of color (L*, a* and b*), mass of 100 grains, and iron and zinc concentrations in highly homozygous populations of Andean bean are unprecedented. Moreover, no studies exist that propose combined selection for high technological quality and micromineral biofortification in Andean bean. The release of new mineral-biofortified Andean bean cultivars with cranberry and red mottled grains with a grain pattern that meets consumers' preference represents an important innovation for breeding programs. Therefore, the present study was carried out to obtain estimates of genetic parameters for technological quality traits and micromineral concentration and to select biofortified Andean bean lines with cranberry and red mottled grains with high technological quality.

Materials and Methods

Origin of Andean bean lines

The 52 F$_{5:7}$ recombinant inbred lines (RIL) were developed from a cross made in 2012 between two common bean cultivars of the Andean gene pool: Hooter × Cal 96. Hooter has a determinate (type I) growth

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

519

habit, cranberry type grains, and was released in 2009 by the Seminis Seed Company for cultivation in the United States. Cal 96 has an indeterminate (type II) growth habit with short guides, red mottled type grains, and was released in 1998 by the National Agricultural Research Organization for cultivation in Uganda. These cultivars showed to be adapted to the growing conditions found in the southern region of Brazil (N. D. Ribeiro, Domingues, Gruhn, Zemolin, & Rodrigues, 2014a) and to differ in iron and zinc concentrations (N. D. Ribeiro et al., 2014b).

The seeds obtained from this cross were advanced up to the F_5 generation by the Single-Seed Descent method. However, three seeds of each F_2 plant were sown and only one plant was kept after emergence. This procedure was repeated up to the F_5 generation so that all genetic variability observed in the F_2 generation would be preserved in a highly homozygous generation. The seeds of each F_5 plant were harvested individually and packed in paper bags. Only the plants that exhibited a sufficient number of seeds for the development of a field experiment were evaluated. Each F_5 plant generated was considered a RIL.

Evaluation of Andean bean RILs

Two experiments were conducted in the Common-Bean Breeding Program area at the Federal University of Santa Maria, in Santa Maria, Rio Grande do Sul (RS), Brazil (latitude 29º42'S, longitude 53º43'W, and 95 m above sea level). The climate of the region is a humid subtropical type with hot summers, without a clearly defined dry season. The soil in the experimental area is a typic alitic Argisol,

Hapludalf, which was prepared conventionally, with one plowing (by harrow) and one disking operation (to level the plot).

The $F_{5:6}$ RILs were evaluated in an experiment established on November 13, 2017, which corresponds to the rainy-season crop period. The experiment was laid out in an augmented block design with three replicates. Treatments consisted of 61 Andean common bean genotypes, represented by 57 $F_{5:6}$ RILs, two parental cultivars (Hooter and Cal 96), and two control cultivars (Pérola and Iraí). The RILs were evaluated only once throughout the experiment (regular treatments) and the parental and control cultivars were included in the three replicates (common treatments). Thus, the means of the regular treatments were adjusted in relation to the blocks in analysis of variance.

The $F_{5:7}$ RILs were sown on February 27, 2018, during the dry-season crop period of 2018. The experiment was carried out in an 8 × 8 simple lattice design with two replicates, since a larger amount of seeds was available. Sixty-four Andean common bean genotypes were evaluated, consisting of 52 $F_{5:7}$ RILs, two parental cultivars (Hooter and Cal 96), and 10 control cultivars (Iraí, BRS MG Realce, Pérola, Carioca, BRS Estilo, Fepagro Garapiá, IAC Imperador, IPR Tangará, IPR Siriri and SCS Riqueza). In the second experiment, a higher number of control cultivars was included to keep the design balanced. Moreover, because only two Andean common bean cultivars are registered for cultivation in the RS state (Iraí and BRS MG Realce), it was also necessary to include control cultivars of Mesoamerican common bean with carioca beans that represent the most widely cultivated and consumed common bean type in Brazil.

520

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

In both experiments, the experimental plot consisted of a 1-m row, spaced at 0.5 m, with 15 seeds sown per linear meter. The management practices employed in the two experiments were uniform and similar and followed the technical recommendations for the common bean crop in RS state (Comissão Técnica Sul Brasileira de Feijão [CTSBF], 2012).

The plants were harvested and threshed manually at maturity (R9 stage) to prevent mechanical damage and grain contamination. The grains were then oven-dried at 40ºC until reaching 13% moisture. Subsequently, they were packed in paper bags, which were kept refrigerated (temperature: 5 ºC; air relative humidity: 75%) throughout the evaluation period.

Determination of technological quality traits and micromineral concentration

Immediately after processing, the grains were evaluated for color and mass of 100 grains. Grain color was measured using a portable colorimeter (CR 410; Konica Minolta, Osaka, Japan), adopting the scale proposed by the International Commission on Illumination (CIE). This scale comprises three color axes: (1) L*, which indicates luminosity, ranging from 0 (dark) to 100 (light); (2) a*, which determines the degree of the colors green and red, with a negative a* value indicating a greener color and a positive b* value characterizing a redder color; and (3) b*, which measures the degree of the colors blue and yellow, with a negative b* value indicating a bluer color and a positive b* value denoting a yellower color. Mass of 100 grains was determined by weighing three random 100-grain samples. Color and mass of 100 grains were evaluated in triplicate in each experimental plot.

Micromineral concentration was analyzed in samples of 10 g of raw grains, which were ground in a micromill (Q298A21; Quimis, São Paulo, Brazil), without any previous treatment, until a fine (unsieved) flour was obtained. A 0.5-g aliquot of this flour was used for nitric-perchloric acid digestion, which was performed according to the methodology described by Miyazawa, Pavan, Muraoka, Carmo and Melo (2009). The final product of the digestion process was transferred to 50-mL Falcon tubes, which were kept refrigerated (temperature: 5 ºC; air relative humidity: 75%), until the micromineral concentration was read. The iron and zinc concentrations were measured using an atomic absorption spectrophotometer (AAS Perkin Elmer AAnalyst 200; Waltham, United States) at the wavelengths of 248.30 and 213.0 nm, respectively; results were expressed as milligrams per kilogram of dry matter (mg kg^{-1} DM).

Statistical analyses

The obtained data were subjected to individual analysis of variance. In the augmented block design experiment, the residual degree of freedom was obtained from the common treatments, since the regular treatments did not influence the estimate of the experimental error. In the second experiment, the efficiency of the lattice design in relation to the randomized block design was evaluated as described by Ramalho, Ferreira and Oliveira (2000).

Phenotypic parameters were obtained from the analysis of variance performed in each evaluated generation. The coefficient of experimental variation (CEV) and selective accuracy (SA) statistics were calculated

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

521

as described by N. D. Ribeiro et al. (2017) and considered in the evaluation of the experimental precision of the evaluated traits. The following estimates of genetic parameters were obtained: heritability (h^2), coefficient of genetic variation (CGV) and b coefficient. The h^2 was estimated considering the mean of the treatments, using the formula described next:

$$h^2 = \frac{\sigma^2 G}{\sigma^2 P},$$

where $\sigma^2 G$ is the genetic variance and $\sigma^2 P$ is the phenotypic variance. The $F_{5:6}$ and $F_{5:7}$ RILs were highly homozygous; therefore, all genetic variation can be attributed to additive variance, i.e., the estimated h^2 corresponded to narrow-sense heritability. The CGV was obtained by the following expression:

$$CGV = 100 \frac{\sqrt{GMS}}{m},$$

where GMS corresponds to the genotype mean square and m is the overall mean of the experiment. The b coefficient was estimated as the ratio between CGV/CEV.

Frequency distribution for the means of the traits evaluated in the F5:6 and F5:7 generations was tested for normality by the Lilliefors test at the 5% probability level. Frequency distribution histograms were constructed for each evaluated trait in each generation, with the number of classes defined by the expression $\sqrt{n}$, where n = number of observations.

In order to select superior Andean common bean lines for technological quality traits and micromineral concentration, the data obtained in the $F_{5:7}$ generation were subjected to Scott-Knott test at the 5% probability level to evaluate the groups formed and to the selection index (multiplicative index). These analyses were performed only for the traits for which a significant genotype effect was observed in analysis of variance. For the multiplicative index (Subandi, Compton, & Empig, 1973), a 10% selection intensity was applied which resulted in the selection of six superior genotypes. Selection was performed with the aim of selecting common bean lines with cranberry grains (Hooter pattern), i.e., direct selection for L* value, mass of 100 grains, and iron concentration and inverse selection for the a* and b* values. Selection was also performed for common bean lines with red mottled grains (Cal 96 pattern), applying direct selection for mass of 100 grains and iron concentration and inverse selection for the L*, a* and b* values. Frequency distribution graphs were also constructed using the Office Excel electronic spreadsheet and statistical analyses were carried out using Genes software (Cruz, 2016).

Results and Discussion

Overall results

A significant genotype effect (p-value < 0.05) was observed for all the evaluated traits, except zinc concentration in the $F_{5:7}$ generation (Table 1). Therefore, there is genetic variability for grain color (L*, a* and b* values), mass of 100 grains, and iron concentration in the two evaluated generations and for zinc concentration in the $F_{5:6}$ generation. Similarly, common bean lines with a broad variation for L* (E. H. Ribeiro, Pereira, Coelho, & Freitas, 2009; Erfatpour, Navabi, & Pauls, 2018), a* and b* (Erfatpour et al., 2018), mass of 100 grains (E. H. Ribeiro et al., 2009; Blair et al., 2010a), and iron and zinc concentrations (Blair et al., 2009, 2010a; Cichy et al., 2009; Martins et al., 2016) were previously obtained from crosses

522

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

between parents that are contrasting for those traits. These findings suggest the possibility of developing new micromineral-biofortified Andean bean cultivars of the cranberry and red mottled grain types with high technological quality.

The evaluated traits showed CEV values lower than 13.83% and SA values ranging from 0.52 to 0.99. Similar CEV and SA values have been described for L*, a* and b* (Arns et al., 2018; N. D. Ribeiro et al., 2019a), mass of 100 grains (Arns et al., 2018), and iron and zinc concentrations (Martins et al., 2016; N. D. Ribeiro et al., 2019b) in experiments evaluating common bean lines. In the present study, the CEV $\leq$ 13.83% indicated high experimental precision in the evaluation of technological quality traits and micromineral concentration in experiments with RILs of common bean conducted in the augmented block and simple lattice designs. However, when the SA statistic was considered, very high experimental precision (SA $\geq$ 0.90) was obtained for all traits,

except zinc concentration in the $F_{5:7}$ generation, which exhibited moderate experimental precision (0.50 < SA < 0.65) according to the classes established by de Resende and Duarte (2007). Zinc concentration in the $F_{5:7}$ generation showed a F test value for genotype lower than 2.00, and thus no significant difference was detected for genotype and no genetic parameter estimates were obtained for that trait. Therefore, SA showed to be more discriminatory than CEV to evaluate the experimental precision of technological quality traits and micromineral concentration in experiments with RILs of Andean bean conducted as incomplete designs.

In the $F_{5:7}$ generation, the efficiency of the simple lattice design ranged from 92.74 (L*) to 115.52% (zinc concentration), averaging 100.16% when the six evaluated traits were considered. These results warrant maintaining analysis of variance in the lattice design, as recommended by Ramalho et al. (2000).

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

523

Table 1

Analysis of variance, estimates of mean and of phenotypic parameters for seed coat color (L*, a* and b* values), mass of 100 grains (M100G, g), and iron (Fe) and zinc (Zn) concentrations (mg kg^{-1} dry matter) obtained in the Andean common bean lines in the $F_{5:6}$ and $F_{5:7}$ generations

Sources of variation	DF[1]	Mean square					
		L*	a*	b*	M100G	Fe	Zn
		$F_{5:6}$ generation					
Block	2	55.62	7.87	11.81	92.67	558.12	58.32
Genotype (adjusted)	60	107.98*	31.00*	13.72*	42.19*	170.16*	24.86*
Residue	6	2.10	0.31	1.09	7.27	30.97	5.83
CEV (%)[2]		2.84	4.89	9.05	5.94	6.74	6.69
Selective accuracy		0.99	0.99	0.96	0.91	0,90	0.87
General mean		51.26	11.01	11.60	44.58	81.10	35.61
No common mean - lines		51.10	11.46	11.54	45.39	82.60	36.08
Common mean - control		52.02	8.86	11.89	40.74	74.01	33.42
Phenotypic variance		103.96	29.19	11.93	29.78	165.66	27.95
Environmental variance		2.10	0.31	1.09	7.27	30.97	5.83
Genetic variance		101.86	28.88	10.84	22.50	134.69	22.12
Heritability (%)		97.98	98.92	90.85	75.57	81.30	79.14
CGV (%)[3]		19.75	46.88	28.54	10.45	14.05	13.04
Coefficient b[4]		6.96	9.58	3.15	1.76	2.08	1.95
		$F_{5:7}$ generation					
Replicate	1	0.87	1.41	0.28	42.90	73.55	30.22
Block/replicate (adjusted)	14	3.06	1.05	2.96	32.78	36.21	35.07
Genotype (adjusted)	63	197.63*	58.10*	35.85*	235.30*	303.22*	22.84^{ns}
Residue	49	4.55	1.37	1.90	35.27	51.02	16.73
CEV (%)[2]		4.11	11.28	10.19	11.51	9.75	13.83
Selective accuracy		0.99	0.98	0.97	0.92	0.91	0.52
Lattice efficiency (%)		92.74	94.86	105.87	98.43	93.55	115.52
General mean		51.96	10.37	13.52	51.61	73.23	29.56
No common mean - lines		50.93	11.24	13.29	55.72	73.30	29.92
Common mean - control		56.45	6.62	14.57	33.79	74.27	28.58
Phenotypic variance		98.81	29.05	17.92	117.65	151.61	-
Environmental variance		2.27	0.68	0.95	17.64	25.51	-
Genetic variance		96.54	28.37	16.97	100.01	126.10	-
Heritability (%)		97.70	97.64	94.70	85.01	83.17	-
CGV (%)[3]		18.91	51.34	30.46	19.38	15.33	-
Coefficient b[4]		4.60	4.55	2.99	1.68	1.57	-

[1]Degrees of freedom. [2]Coefficient of environmental variation. [3]Coefficient of genetic variation. [4]Ratio of coefficient of genetic variation to coefficient of environmental variation. *Significant by the F test at 0.05 probability.

524

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

Genetic parameters and inheritance of technological quality traits in Andean bean

High heritability ($h^2 \geq 75.57\%$) was obtained for L*, a*, b*, and mass of 100 grains (Table 1). The L*, a* and b* values also showed high h^2 when evaluated in a highly heterozygous generation of Andean and Mesoamerican bean populations (Possobom et al., 2015) and in a highly homozygous generation of a Mesoamerican bean population (N. D. Ribeiro et al., 2019a). Similarly, mass of 100 grains showed a high h^2 in different F_3 populations obtained from crosses between Andean and Mesoamerican parents (Mukamuhirwa et al., 2015). Traits with high h^2 can obtain greater gains with selection, since most part of the observed variation will be of genetic nature.

Greater contribution from genetic variation to the expression of L*, a*, b*, and mass of 100 grains was confirmed by the high CGV values (10.45 to 51.34%). No previous studies have been found that evaluate CGV for technological quality traits in Andean bean. However, high CGV values for L* and mass of 100 grains were observed when 185 RILs were evaluated in a F_7 generation of Mesoamerican bean with different grain types (E. H. Ribeiro et al., 2009). N. D. Ribeiro et al. (2019a) also observed high CGV values ($\geq 42.89\%$) for L*, a* and b* in 100 RILs in $F_{5:6}$ and $F_{5:7}$ generations of Mesoamerican bean of the carioca and black grain types.

All technological quality traits exhibited b coefficients ≥ 1.68, indicating great genetic variability for grain color (L*, a* and b* values) and mass of 100 grains among the Andean bean lines. Studies using the b coefficient as an estimate of genetic variation of technological quality traits were found only evaluating Mesoamerican beans. E. H. Ribeiro et al. (2009)

observed b coefficients > 1.0 for L* and mass of 100 grains in 185 RILs of common bean in the F_7 generation, whereas N. D. Ribeiro et al. (2019a) obtained such b coefficients for L*, a* and b* in 100 RILs of common bean in the $F_{5:6}$ and $F_{5:7}$ generations. Traits with b coefficients > 1.0 represent a very favorable situation for selection, according to Vencovsky and Barriga (1992).

High magnitude estimates of the genetic parameters of h^2, CGV and b coefficient were obtained for the L*, a* and b* values, and mass of 100 grains in Andean bean, indicating the existence of genetic variability among the evaluated lines. Thus, selection of Andean bean lines with cranberry and red mottled grains of high technological quality would be possible.

The L*, a* and b* values did not show normal distribution in the $F_{5:6}$ and $F_{5:7}$ generations (Figures 1A, 1B, 1C, 1D, 1E and 1F). The frequency distribution histograms obtained in the two evaluated generations demonstrate the presence of few phenotypic classes for the L*, a* and b*, indicating that few genes act on the expression of color in Andean bean grains. Similar results were observed by Possobom et al. (2015) in F_2 progeny of Andean and Mesoamerican beans, revealing that L*, a* and b* exhibited qualitative inheritance, regardless of gene pool. In a highly homozygous generation ($F_{5:6}$ and $F_{5:7}$) of a population of Mesoamerican bean, N. D. Ribeiro et al. (2019a) described discontinuous variation, that is, the occurrence of few classes for L*, a* and b*.

For mass of 100 grains, normal distribution was detected by the Lilliefors test in the two evaluated generations (Figures 2A and 2B). Therefore, Andean bean lines with a mass of 100 grains lower and higher than those

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

525

of their parents were obtained. The continuous variation indicates that mass of 100 grains in Andean bean is under polygenic control, i.e., quantitative inheritance.

In the present study, the L*, a* and b* values showed qualitative inheritance and mass of 100 grains exhibited quantitative inheritance in Andean bean. Therefore, greater ease is expected in the selection of cranberry and red mottled lines with grain colors of greater acceptance by the market.

Genetic parameters and inheritance of micromineral concentration in Andean bean

The iron ($F_{5:6}$ and $F_{5:7}$ generations) and zinc concentrations ($F_{5:6}$ generation) in Andean bean showed high heritability ($h^2 \geq 79.14\%$) (Table 1), confirming the results observed by Mukamuhirwa et al. (2015) in a F_3 generation obtained from a 6 × 6 diallel cross involving Andean and Mesoamerican parents. However, Zemolin et al. (2016) evaluated the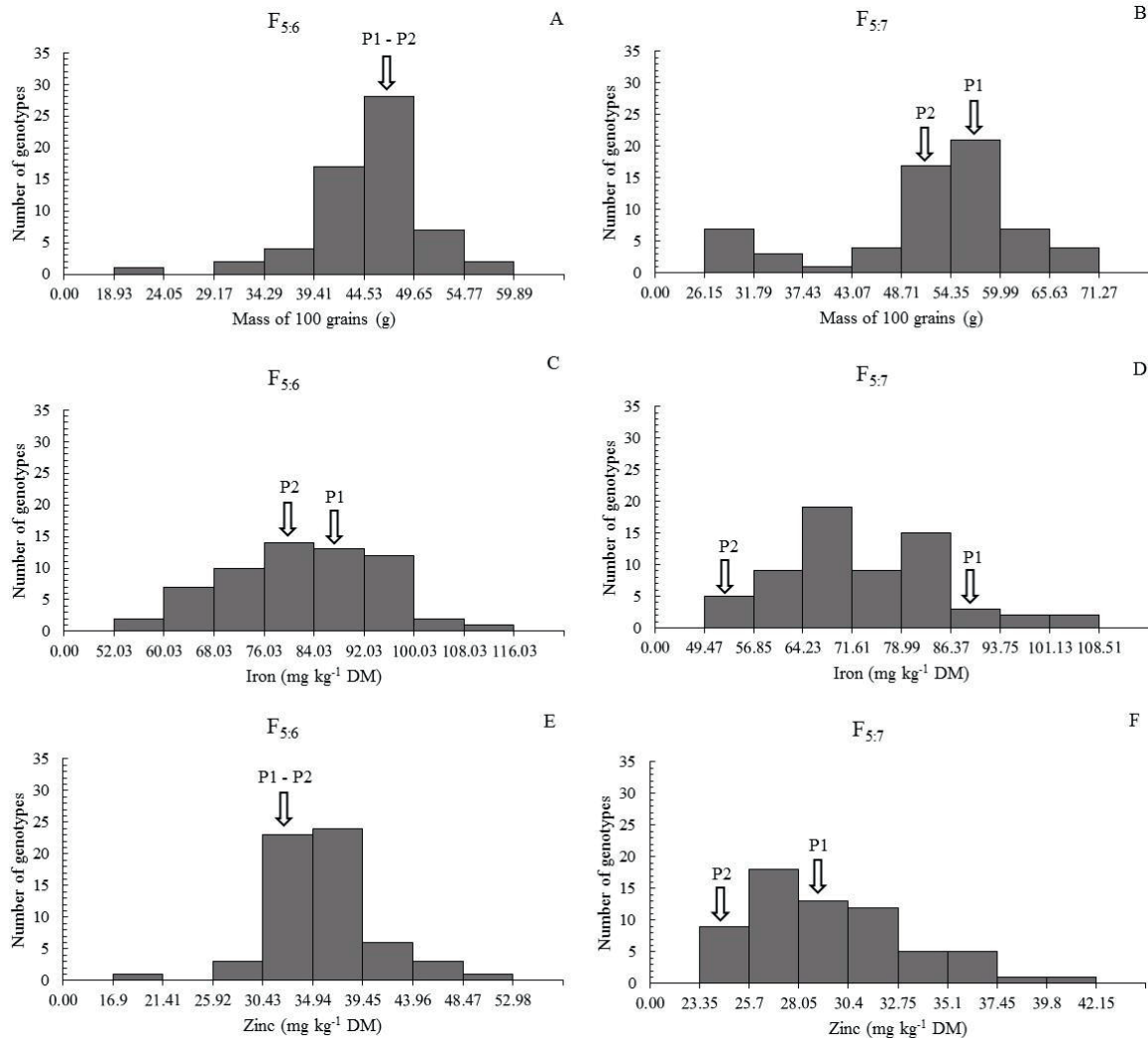 h^2 of iron and zinc concentrations in a F_2 generation developed from different crosses between Andean parents and reported values between 19.04 and 63.60%, which characterize estimates of low to high magnitude according to the classification proposed by Soltani et al. (2016). Katuuramu et al. (2018), in turn, observed intermediate h^2 values for the iron and zinc concentrations determined in cooked grains of 206 Andean bean genotypes evaluated in the United States. The observed differences can be explained by the method used to estimate heritability, evaluated generation, genetic diversity of the population and use of raw or cooked grains in the determination of the micromineral concentration in common bean genotypes. Heritability estimates are useful to evaluate the available genetic variability and predict gains with selection. High h^2 values ($\geq 60.00\%$) are associated with greater genetic variability and with greater gains with selection, i.e., increased chances of success in the selection of superior genotypes for micromineral concentration.

526

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021
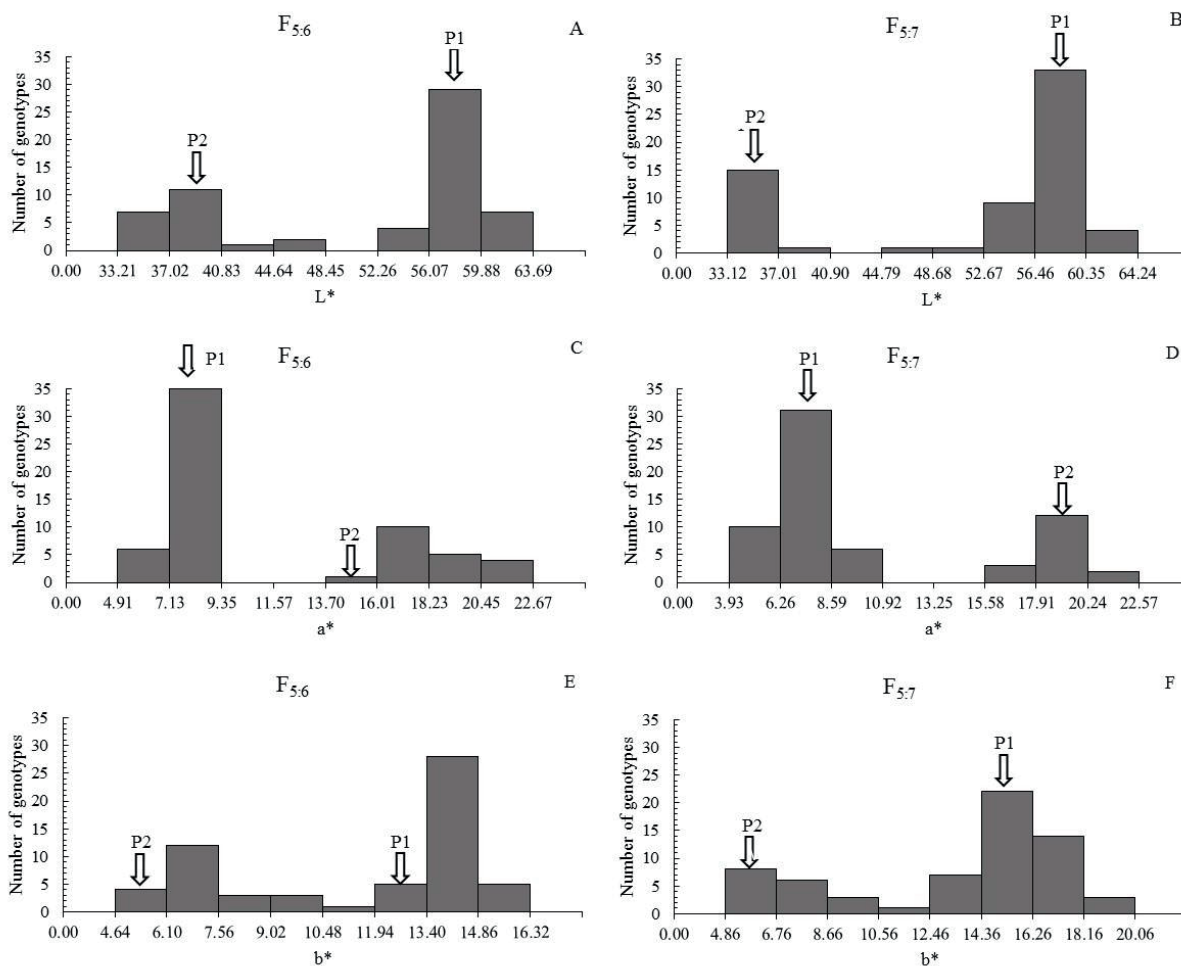
Figure 1. Frequency distribution histograms for seed coat color (L*, a*, and b* values) obtained in the Andean common bean lines in the $F_{5:6}$ and $F_{5:7}$ generations. P1 and P2 are the parent cultivars: P1 (Hooter) and P2 (Cal 96).

Coefficients of genetic variation ≥ 13.04% and b coefficients ≥ 1.57 were detected for the iron concentration in the two evaluated generations and for the zinc concentration in the $F_{5:6}$ generation, thus validating the hypothesis of greater contribution from genetic variation to the expression of those traits and favorable conditions for selection. The use of CGV and the b coefficient as measures to estimate genetic variation in the iron and zinc concentrations in RILs is restricted to Mesoamerican bean. In this gene pool, more promising populations were identified for the selection of micromineral-biofortified common bean lines when the CGV and b coefficient values were interpreted (Martins et al., 2016; Maziero et al., 2016; N. D. Ribeiro et al., 2019b). The populations that showed higher CGV and b coefficients ≥ 1.00 exhibited greater genetic variability for the iron and zinc concentrations and were consequently the most promising for the selection of superior lines for the common-bean biofortification program.

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

527

Figure 2. Frequency distribution histograms for mass of 100 grains and iron and zinc concentrations (mg kg^{-1} dry matter) obtained in the Andean common bean lines in the F$_{5:6}$ and F$_{5:7}$ generations. P1 and P2 are the parent cultivars: P1 (Hooter) and P2 (Cal 96).

According to the Lilliefors test, the iron and zinc concentrations exhibited normal distribution close to the mean in both generations (Figures 2C, 2D, 2E and 2F). The frequency distribution histograms revealed a continuous variation for the iron and zinc concentrations, which is characteristic of quantitative inheritance. Quantitative inheritance was previously described for the iron and zinc concentrations in RILs obtained from a cross between two Andean bean lines in the F$_{5:7}$ generation (Cichy et al., 2009) and from one cross between two lines of different gene pools in the F$_{7:11}$ generation (Blair et al., 2009). Quantitative inheritance suggests that multiple loci are involved in homeostasis, accumulation and/or retention of those minerals in common bean (Katuuramu et al., 2018).

In the lines obtained from the cross between cultivars Hooter and Cal 96, the iron

concentration ranged from 52.03 to 116.03 mg kg^{-1} DM in the $F_{5:6}$ generation (Figure 2C) and from 49.47 to 108.51 mg kg^{-1} in the $F_{5:7}$ generation (Figure 2 D). For zinc, the observed variation range was from 16.90 to 52.98 mg kg^{-1} DM and from 23.35 to 42.15 mg kg^{-1} DM for the $F_{5:6}$ and $F_{5:7}$ generations, respectively (Figures 2E and 2F). Andean bean lines with iron and zinc concentrations higher than those present in their parents were obtained in the two evaluated generations, although transgressive segregation was not very evident. Likewise, Blair et al. (2010a) observed that the concentration of those microminerals did not exhibit a characteristic transgressive segregation in RILs in the F_{10} generation obtained from a cross between two lines of Mesoamerican bean.

In the present study, the iron and zinc concentrations in Andean bean lines obtained from the Hooter × Cal 96 cross showed greater genetic variability, i.e., high h^2, higher CGV values and b coefficient ≥ 1.00. However, those traits exhibited quantitative inheritance, which may complicate the selection of micromineral-biofortified Andean bean lines with cranberry and red mottled grains.

Selection of micromineral-biofortified Andean bean lines with high technological quality

The Scott-Knott test resulted in the formation of three groups of genotypes for L*, namely: (1) genotypes with greater lightness – L*: 62.24 to 52.18, comprising all common bean genotypes with cranberry and carioca beans; (2) genotypes with intermediate lightness – L*: 45.65, including only the line DFA 33-18 with red mottled beans; and (3) genotypes with lower lightness – L*: 37.28 to 33.11, grouping the other common bean genotypes with red mottled beans (Table 2). A similar variation range was previously observed for L* determined in common bean genotypes with cranberry (N. D. Ribeiro et al., 2014a), carioca (Arns et al., 2018) and kidney (red seed coat) beans (Parmar, Singh, Kaur, & Thakur, 2017).

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

529

Table 2

Means[1] of seed coat color (L*, a* and b* values), mass of 100 grains (M100G, g), and iron (Fe) and zinc (Zn) concentrations (mg kg^{-1} dry matter) obtained in the Andean common bean lines in the F$_{5:7}$ generation

Genotype	Bean type	L	a*	b*	M100G	Fe	Zn
DFA 32-18	cranberry	64.24a*	5.97e*	14.71b*	60.62a*	75.52c*	33.23^{ns}
IAC Imperador	carioca	61.10a	4.41e	14.95b	26.14b	72.07c	25.71
DFA 16-18	cranberry	60.45a	8.11d	16.82a	61.96a	85.62b	29.35
IPR Siriri	carioca	60.44a	5.08e	15.80b	26.28b	71.42c	27.54
Carioca	carioca	59.88a	4.15e	14.61b	30.41b	62.22c	25.65
DFA 26-18	cranberry	59.57a	7.10d	13.83b	58.90a	79.52b	25.88
DFA 19-18	cranberry	59.44a	8.78d	18.11a	55.77a	85.92b	28.03
Hooter	cranberry	59.11a	6.80d	15.91b	57.83a	90.57b	28.72
DFA 01-18	cranberry	59.04a	7.30d	15.90b	58.18a	107.17a	35.34
DFA 18-18	cranberry	59.04a	8.37d	19.20a	55.08a	79.22b	28.90
DFA 48-18	cranberry	59.03a	8.54d	15.71b	49.76a	70.97c	30.38
DFA 04-18	cranberry	58.67a	7.59d	15.01b	66.42a	76.57b	32.28
DFA 05-18	cranberry	58.55a	5.77e	11.82b	45.53a	84.32b	30.96
DFA 39-18	cranberry	58.51a	6.98d	15.42b	71.23a	108.52a	33.64
DFA 34-18	cranberry	58.48a	7.24d	15.37b	53.50a	64.37c	27.02
DFA 17-18	cranberry	58.25a	8.58d	16.55a	52.70a	71.52c	31.42
DFA 31-18	cranberry	58.24a	7.01d	16.00b	60.69a	79.42b	29.63
DFA 20-18	cranberry	58.16a	6.72d	14.99b	51.06a	82.77b	28.68
DFA 36-18	cranberry	58.08a	8.10d	17.14a	60.11a	86.12b	28.36
DFA 37-18	cranberry	58.04a	8.36d	18.59a	60.73a	68.97c	32.71
BRS Estilo	carioca	58.01a	4.55e	15.73b	33.84b	56.57c	25.64
DFA 40-18	cranberry	57.85a	7.70d	16.68a	53.63a	76.52b	27.25
DFA 43-18	cranberry	57.78a	7.22d	15.14b	47.18a	74.82c	27.37
SCS 205 Riqueza	carioca	57.76a	5.67e	17.26a	28.64b	74.12c	27.36
DFA 47-18	cranberry	57.76a	8.23d	15.42b	56.44a	79.57b	30.07
Iraí	cranberry	57.65a	7.97d	13.62b	32.85b	74.57c	28.43
DFA 02-18	cranberry	57.63a	7.37d	16.73a	52.69a	65.02c	32.29
IPR Tangará	carioca	57.45a	3.93e	14.27b	27.87b	67.07c	27.72
DFA 13-18	cranberry	57.44a	7.78d	12.65b	48.15a	65.82c	34.54
DFA 51-18	cranberry	57.40a	8.21d	16.00b	59.16a	69.32c	27.66
DFA 42-18	cranberry	57.30a	8.13d	13.48b	49.87a	72.92c	27.69
Fepagro Garapiá	carioca	57.34a	5.00e	17.27a	29.11b	82.72b	38.63
DFA 21-18	cranberry	57.27a	7.04d	15.19b	57.00a	62.67c	32.54
DFA 22-18	cranberry	57.14a	9.49d	17.45a	58.54a	55.97c	32.09
Pérola	carioca	57.02a	4.55e	15.44b	29.18b	83.22b	28.23
DFA 45-18	cranberry	57.00a	7.32d	15.45b	54.70a	95.37a	32.34

contuation...

DFA 35-18	cranberry	56.78a	7.99d	16.30a	56.39a	60.12c	26.08
DFA 50-18	cranberry	56.18a	7.26d	13.10b	50.95a	79.67b	26.68
DFA 41-18	cranberry	55.99a	8.45d	17.36a	60.45a	79.80b	28.16
DFA 49-18	cranberry	55.82a	7.53d	15.33b	54.35a	67.02c	28.61
DFA 29-18	cranberry	55.51a	8.49d	17.45a	58.46a	69.72c	29.50
DFA 11-18	cranberry	55.36a	8.60d	17.53a	51.03a	87.47b	41.54
DFA 15-18	cranberry	55.36a	7.89d	17.62a	58.68a	79.42b	28.73
BRS MG Realce	cranberry	55.30a	8.96d	13.42b	34.17b	91.37b	29.99
DFA 07-18	cranberry	55.01a	7.79d	20.46a	54.77a	65.42c	31.44
DFA 09-18	cranberry	54.50a	9.30d	17.72a	57.40a	72.87c	30.80
DFA 23-18	cranberry	52.18a	8.65d	14.96b	58.02a	64.32c	28.41
DFA 33-18	red mottled	45.65b	18.05c	8.17c	56.52a	64.02c	28.47
DFA 14-18	red mottled	37.28c	22.53a	9.45c	65.65a	66.92c	33.92
DFA 06-18	red mottled	36.82c	19.44b	6.92c	53.71a	69.22c	33.14
DFA 10-18	red mottled	36.55c	16.99c	7.00c	60.48a	52.12c	34.81
DFA 52-18	red mottled	36.52c	16.30c	7.63c	51.66a	79.92b	28.41
CAL 96	red mottled	36.28c	18.38c	6.85c	49.10a	49.47c	25.39
DFA 28-18	red mottled	36.00c	19.53b	6.65c	54.79a	60.47c	25.49
DFA 24-18	red mottled	35.93c	21.44a	8.96c	51.78a	61.42c	25.01
DFA 38-18	red mottled	35.82c	20.04b	5.75c	39.52b	60.92c	26.66
DFA 03-18	red mottled	35.46c	16.24c	5.07c	69.30a	52.77c	26.91
DFA 08-18	red mottled	35.11c	19.42b	5.97c	53.76a	65.92c	25.55
DFA 25-18	red mottled	34.97c	18.72c	6.80c	56.31a	65.77c	27.04
DFA 46-18	red mottled	34.56c	18.50c	7.17c	47.38a	60.67c	25.86
DFA 12-18	red mottled	34.24c	20.14b	9.38c	54.21a	99.87a	36.17
DFA 30-18	red mottled	33.73c	19.14b	7.01c	56.45a	63.92c	28.98
DFA 44-18	red mottled	33.23c	18.21c	5.54c	51.61a	66.62c	27.74
DFA 27-18	red mottled	33.11c	18.64c	5.77c	54.18a	70.77c	28.23
Mean		51.96	10.37	13.52	51.61	73.23	29.56
CV(%)		4.10	11.28	10.19	11.51	9.75	13.84

[1]Means followed by same letter in a column constitute a homogeneous group according to the Scott-Knott test at 0.05 probability. ns = non-significant.

Cranberry and carioca bean genotypes must have very light grains (> L* value) to be accepted by consumers, which associate greater lightness with newly harvested and fast-cooking grains. For carioca beans, N. D. Ribeiro, Storck, & Poersch (2008) proposed L* ≥ 55.00 as a standard for grain lightness. For cranberry beans, no proposal of a L* standard that meets the consumer preference was found. In the present study, the cranberry bean lines and the three control cultivars of this type of grain (Hooter, Iraí and BRS MG Realce) did not

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

531

differ for the L* value. In this way, L* ≥ 52.18 can be recommended as a standard to be adopted in the selection of cranberry bean lines, as this value corresponds to the lightness of the grains of cultivars Hooter, Iraí and BRS MG Realce, which are already sold in the market.

Red mottled bean genotypes showed darker grains (< L*) when compared to the carioca and cranberry bean genotypes. However, there is not a standard L* value reference used in the selection of red mottled bean lines better accepted by consumers. In this study, the L* value of cultivar Cal 96, the only red mottled bean cultivar evaluated, did not differ from those of the other lines with that grain type, except line DFA 33-18 (L* = 45.65). Thus, it can be stated that red mottled bean lines with L* values from 33.11 to 37.28 have great chances of meeting the demands of common bean consumers, since their grain lightness is similar to that of cultivar Cal 96, which is widely present in the human diet. Therefore, a L* value from 33.11 to 37.28 is proposed for the selection of red mottled bean lines.

However, grain color in common bean cannot be evaluated exclusively based on the L* value, since it did not differ between the cranberry and carioca bean genotypes, which confirms the results observed by Soares, Caliari, Bassinello, Fernandes and Becker (2012). Thus, it is important to also analyze the a* and b* values. When the common bean genotypes were evaluated for a*, five groups were formed, whereas when b* was measured, the common bean genotypes were clustered in three groups. Therefore, a* was the most discriminatory grain color parameter in the differentiation between the cranberry, carioca and red mottled bean genotypes. Erfatpour et al. (2018) also found that a* was the most efficient parameter to be used in the differentiation of grain color between the cranberry and pinto bean genotypes.

For carioca beans, Arns et al. (2018) recommended that, in addition to greater lightness (L* ≥ 55.00), grains should exhibit light red (a* ≤ 7.00) and light yellow (b* ≤ 16.00) shades. All evaluated carioca bean cultivars met this standard, except cultivars SCS 205 Riqueza and Fepagro Garapiá, which showed b* ≥ 17.26. For cranberry beans, the lines which showed the lightest red (a* ≤ 9.49) and yellow (b* ≤ 16.00) shades did not differ from the control cultivars with this type of grain, namely, Hooter, Iraí and BRS MG Realce. Therefore, when aiming at the selection of cranberry bean lines with lighter grains, the following standards are recommended: L* ≥ 52.18, a* ≤ 9.49 and b* ≤ 16.00. However, red mottled beans are darker, as represented by lower L* and b* values and higher a* value. The red mottled bean lines which did not differ from the control cultivar Cal 96 exhibited 33.11 < L* < 37.28, 16.24 < a* < 18.72 and b* ≤ 9.45, which are the recommended grain color standards in the selection of superior lines.

The common bean genotypes were clustered in two groups for mass of 100 grains: (1) lower mass of 100 grains (≤ 39.52 g) included all carioca bean cultivars, the cranberry bean cultivars Iraí and BRS MG Realce and the red mottled bean line DFA 38-18; and (2) higher mass of 100 grains (≥ 45.53 g) comprised all other evaluated genotypes of cranberry and red mottled beans. Therefore, the Scott-Knott test divided the common bean genotypes by grain size into medium (25 to 40 g 100 grains^{-1}) and large (> 40 g 100 grains^{-1}), according to the classes represented by Blair, González, Kimani and Butare (2010b). Because all evaluated carioca bean cultivars showed medium-sized grains, they meet the demands of consumers

532

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

of this type of grain, as stated by Carbonell et al. (2010). However, there is a higher market demand for cranberry and red mottled bean cultivars with large grains, since consumers relate a larger grain size to increased yield after cooking. All cranberry and red mottled bean genotypes have large grains, except cultivars Iraí and BRS MG Realce and the line DFA 38-18.

Three cranberry bean lines (DFA 01-18, DFA 39-18 and DFA 45-18) and one red mottled bean line (DFA 12-18) showed high iron concentration, which were previously defined by N. D. Ribeiro, Domingues, Zemolin and Possobom (2013) as values above 95.00 mg kg^{-1} DM. Including iron-biofortified bean cultivars in the human diet is important for the prevention of clinical manifestations related to iron deficiency. Camaschella (2015) described the main recurring symptoms of iron deficiency in the human organism as being anemia, weakness, fatigue, difficulty concentrating and low productivity at work.

Although the genotype effect was not significant for the zinc concentration in the F$_{5:7}$ generation (Table 1), 13 cranberry bean lines and four red mottled bean lines had a zinc concentration ≥ 31.00 mg kg^{-1} DM (Table 2). This is a reference value for zinc biofortification programs, as it characterizes a high zinc concentration in common bean, according to Tryphone and Nchimbi-Msolla (2010). Clinical manifestations of zinc deficiency in the human organism are delayed growth, immune dysfunction and cognitive impairment (Prasad, 2014). Thus, including zinc-biofortified bean cultivars in the human diet may provide health benefits.

Genetic gains favorable to the objectives of selection of cranberry bean lines (Hooter pattern) were obtained for the traits of

L* (14.81%), a* (-34.98%), mass of 100 grains (11.71%), and iron concentration (20.10%) (Table 3). Nonetheless, a positive gain was obtained for b* (7.51%), which is undesirable for this grain type.

The multiplicative index allowed the selection of the six superior cranberry bean genotypes for all evaluated traits, as follows: DFA 39-18, DFA 32-18, DFA 05-18, DFA 01-18, Hooter, and DFA 26-18. All selected genotypes exhibited a high L* (≥ 52.18), which characterizes very light grains; low a* (≤ 9.49), indicating a light red shade; low b* (≤ 16.00), characterizing a light yellow shade; and large grains (mass of 100 grains > 40 g), i.e., they possess high technological quality. However, only the lines DFA 39-18 and DFA 01-18 were biofortified for iron. The multiplicative index was successfully applied in the simultaneous selection of mineral-biofortified Mesoamerican bean lines of high agronomic performance (Jost et al., 2013; Maziero, Ribeiro, & Storck, 2015). Nevertheless, the use of the multiplicative index in the selection of iron-biofortified Andean bean lines with high technological quality is unprecedented in common bean.

When the multiplicative index was applied for the selection of red mottled bean lines (Cal 96 pattern), genetic gain estimates favorable to selection were observed for the traits of L* (-10.61%), b* (-25.24%), mass of 100 grains (11.09%) and iron concentration (9.61%) (Table 3). However, only three of the selected lines showed red mottled beans: DFA 03-18, DFA 27-18 and DFA 44-18. This may be explained by the lower number of red mottled bean lines evaluated.

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

533

Table 3
Means of the original population (X_0), mean of the selected population (Xs), heritability (h^2), genetic gain (GG), and percentage of genetic gain (GG, %) with simultaneous selection by the multiplicative index for the six Andean common bean genotypes (10% of the total lines evaluated) with cranberry beans (Hooter pattern) and red mottled beans (Cal 96 pattern) for seed coat color (L*, a* and b* values), mass of 100 grains (M100G, g), and iron concentration (Fe, mg kg^{-1} dry matter) in the $F_{5:7}$ generation

Trait	X_0	X_s	h^2 (%)	GG	GG (%)	
Phenotypic parameters for cranberry beans						
L*	51.96	59.84	97.70	7.69	14.81	
a*	10.37	6.66	97.64	-3.63	-34.98	
b*	13.52	14.60	94.70	1.02	7.51	
M100G	51.61	58.72	85.01	6.04	11.71	
Fe	73.23	90.94	83.17	14.72	20.10	
Total gain				25.85	19.15	
Trait	Selected genotypes of cranberry beans					
	DFA 39-18	DFA 32-18	DFA 05-18	DFA 01-18	Hooter	DFA 26-18
L*	58.51	64.24	58.56	59.04	59.12	59.57
a*	6.99	5.98	5.78	7.31	6.80	7.11
b*	15.42	14.71	11.82	15.90	15.91	13.83
M100G	71.24	60.63	45.53	58.19	57.84	58.91
Fe	108.52	75.52	84.32	107.17	90.57	79.52

Trait	X_0	X_s	h^2%	GG	GG%	
Phenotypic parameters for red mottled beans						
L*	51.96	46.32	97.70	-5.51	-10.61	
a*	10.37	12.19	97.64	1.78	17.12	
b*	13.52	9.92	94.70	-3.41	-25.24	
M100G	51.61	58.34	85.01	5.72	11.09	
Fe	73.23	81.69	83.17	7.04	9.61	
Total gain				5.61	1.97	
Trait	Selected genotypes of red mottled beans					
	DFA 33-18	DFA 39-18	DFA 27-18	DFA 44-18	DFA 05-18	DFA 01-18
L*	35.47	58.51	33.11	33.23	58.56	59.04
a*	16.24	6.99	18.64	18.21	5.78	7.31
b*	5.07	15.42	5.77	5.54	11.82	15.90
M100G	69.31	71.24	54.18	51.62	45.53	58.19
Fe	52.77	108.52	70.77	66.62	84.32	107.17

534

Semina: Ciênc. Agrár. Londrina, v. 42, n. 2, p. 517-538, mar./abr. 2021

The superior red mottled bean lines DFA 03-18, DFA 27-18 and DFA 44-18 stood out for high technological quality, with low L* (33.11 < L* < 37.28), characterizing dark grains; low a* (16.24 < a* < 18.72), indicating a light red shade; low b* (≤ 9.45), denoting a very light yellow shade; and large grains (mass of 100 grains > 40 g). However, none of those lines were biofortified for iron.

Conclusion

Grain color (L*, a* and b* values), mass of 100 grains and iron and zinc concentrations show high heritability (h^2 ≥ 75.57%) in the recombinant inbred line population of Andean bean, and qualitative inheritance is only observed for grain color.

Grains of the cranberry bean lines DFA 39-18 and DFA 01-18 are very light (L* ≥ 52.18), with light red (a* ≤ 9.49) and light yellow (b* ≤ 16.00) shades; large (mass of 100 grains > 40 g) and have high iron concentration (Fe ≥ 95.00 mg kg^{-1} dry matter). The selection of these lines for use in human nutrition and in breeding programs is therefore recommended.

Grains of the red mottled bean lines DFA 03-18, DFA 27-18 and DFA 44-18 have high technological quality: they are dark (33.11 < L* < 37.28), with light red (16.24 < a* < 18.72) and very light yellow (b* ≤ 9.45) shades, and large (mass of 100 grains > 40 g). Therefore, they will be selected by the breeding program.

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535

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536

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537

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538

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