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Scientific Article
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Genetic divergence and selection of bean cultivars of different grain types based on physical traits[1]

Divergência genética e seleção de cultivares de feijão de diferentes tipos de grãos baseada em características físicas

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ABSTRACT - Genetic divergence analysis and combined selection for various physical grain traits are unprecedented for common bean cultivars. The objectives of this study were to examine the genetic variability of common bean cultivars for 12 physical grain traits; study the correlations between these traits; analyze the genetic divergence; and select superior common bean cultivars. Two experiments were carried out in 2019 in which 22 common bean cultivars of different grain types were evaluated. Significant effects of genotype, grain type and genotype × environment interaction were obtained for all physical grain traits, indicating the existence of genetic variability. High correlations were observed between grain length and width (r = 0.844), normal grains and uptake (r = 0.796) and uptake and cooking time (r = -0.651). The L* (grain lightness) and a* (variation between green and red hue) values had a greater contribution to the differentiation between common bean cultivars. Four and three groups of cultivars were formed by the analyses of canonical variables and unweighted pair group method with arithmetic mean, respectively. Both cluster analyses are efficient in separating the cultivars into homogeneous groups based on the L* and a* values, although the canonical variables analysis provides more detailed information. The cranberry bean cultivars BRS Executivo, BRS MG Realce and Hooter and the black bean cultivars IPR Tiziu, BRS Esteio and Guapo Brilhante have superior physical grain traits and will thus be selected by the breeding program.

Key words: *Phaseolus vulgaris* L. Genetic variability. Correlation analysis. Cluster analysis. Selection index.

RESUMO - A análise da divergência genética e a seleção combinada para várias características físicas dos grãos é inédita para cultivares de feijão. Os objetivos desse trabalho foram avaliar a variabilidade genética de cultivares de feijão para 12 características físicas dos grãos, estudar as correlações entre essas características, analisar a divergência genética e selecionar cultivares de feijão superiores. Dois experimentos foram conduzidos em 2019, sendo avaliadas 22 cultivares de feijão de diferentes tipos de grãos. Efeitos significativos de genótipo, tipo de grão e interação genótipo x ambiente foram obtidos para todas as características físicas dos grãos, evidenciando variabilidade genética. Alta correlação foi observada entre comprimento e largura dos grãos (r = 0,844), grãos normais e absorção (r = 0,796) e absorção e tempo de cozimento (r = -0,651). Os valores de L* (claridade dos grãos) e a* (variação entre as tonalidades verde a vermelho) mostraram maior contribuição para a diferenciação entre as cultivares de feijão. Nas análises de variáveis canônicas e de ligação média dento de grupo com média aritmética foram formados quatro e três grupos de cultivares, respectivamente. As duas análises de agrupamento são eficientes para separar as cultivares de feijão em grupos homogêneos pelos valores de L* e a*, sendo que a análise de variáveis canônicas fornece informações mais detalhadas. As cultivares de feijão cranberry BRS Executivo, BRS MG Realce e Hooter e as cultivares de feijão preto IPR Tiziu, BRS Esteio e Guapo Brilhante apresentam características físicas de grãos superiores e serão selecionadas pelo programa de melhoramento.

Palavras-chave: *Phaseolus vulgaris* L.. Variabilidade genética. Análise de correlação. Análise de agrupamento. Índice de seleção.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely used in food worldwide. However, preferences regarding the color, size and shape of grains used in the diet differ between countries, states and municipalities. In addition, some people like to vary the type of common bean they consume.

In Brazil, the most produced and consumed grain types of common bean are carioca (beige seed coat with brown streaks) and black. Nevertheless, in some regions of the country, cultivation areas of red and cranberry (cream seed coat with red streaks and spots) beans are increasingly expanding due to the greater market demand for these grain types. In this scenario, breeding programs have intensified the selection of common bean cultivars with superior physical grain traits in an effort to attain greater consumer acceptance.

Physical grain traits in common bean include color, mass of 100 grains, the dimensions of length, width and thickness, shape, degree of flatness, and cooking time. Standards for these traits have been defined by breeding programs. Grain color has been determined using a colorimeter, based on the L* value, which measures grain lightness. For carioca beans, grains should exhibit high lightness ($L^* \geq 55.00$), as recommended by Arns *et al*. (2018), whereas black beans should have low-lightness grains ($L^* \leq 22.00$), as suggested by Ribeiro, Possebom and Storck (2003). Carioca and black bean grains should be medium in size (mass of 100 grains from 25 to 30 g) and exhibit an elliptical shape, half-full flatness (CARBONELL *et al*., 2010) and fast cooking (≤ 25 min) (SANTOS; RIBEIRO; MAZIERO, 2016). For red and cranberry bean grains, no references to established standards for physical grain traits have been found in the literature.

Selection for various physical grain traits has been a great challenge for common-bean breeding programs, since significant effects of the genotype × environment interaction have been reported (ARNS *et al*., 2018; DELFINI *et al*., 2017; RIBEIRO *et al*., 2014; RIBEIRO; KLÄSENER, 2020). This fact has an impact on analyses of genetic divergence and combined selection for physical grain traits, as these analyses must consider different environments for the proper interpretation of results.

Breeding programs have extensively analyzed genetic divergence in common bean cultivars based on agronomic and seed traits (ARTEAGA *et al*., 2019; BAREKE, 2019; HEGAY *et al*., 2014; NADEEM *et al*., 2020; YEKEN *et al*., 2019). However, few studies analyzed genetic divergence in common bean cultivars based on physical grain traits (BOROS; WAWER, 2018; RIVERA *et al*., 2016), and these works were restricted the evaluation performed in one environment. Nonetheless, the evaluation of genetic divergence between common bean cultivars based on physical grain traits in experiments carried out in different growing seasons is unprecedented.

Combined selection for physical grain traits was successfully employed for carioca lines (ARNS *et al*., 2018) and for carioca and black lines (RIBEIRO; KLÄSENER, 2020) of common bean. However, no previous studies were found in the literature addressing combined selection for various physical grain traits in common bean aiming at the selection of cultivars of different grain types. Therefore, the present study proposes to investigate genetic variability in common bean cultivars of different grain types for 12 physical grain traits; study the correlations between these traits; analyze the genetic divergence of the cultivars; and select superior common bean cultivars based on physical grain traits.

MATERIAL AND METHODS

Grain production from the common bean cultivars

Twenty-two common bean cultivars were characterized in terms of physical grain quality in two consecutive experiments carried out in the year 2019 in the summer (dry season) and spring (rainy season) crops. The experiments were established in the area of the Bean Breeding Program at the Federal University of Santa Maria (UFSM), located in Santa Maria, Rio Grande do Sul, Brazil (latitude 29º42´S, longitude 53º49´W and 95 m altitude). The climate of the region is a humid subtropical type with hot summers and no clearly defined dry season.

The soil in the experimental area is a typical alitic Argisol, Hapludalf, which was prepared by the conventional cultivation system, with one subsoiling and two harrowing operations. No soil acidity correction was required, as the pH value of the soil in the experimental area was close to the reference pH of the crop.

The experiments were carried out in a randomized block design with three replicates. Plots consisted of two 4-m-long rows, spaced 0.50 m apart, totaling a usable area of 4 m^2. The sowing density adopted was 15 seeds per linear meter. The evaluated treatments were 22 common bean cultivars of different grain types, namely, five carioca grains, six black grains, six red grains and five cranberry grains (Figure 1). These cultivars characterize the grain types most produced and consumed in Brazil, representing the technological advances of different research institutions.

Sowing was carried out manually. The management practices employed in fertilization, seed treatment, irrigation and control of weed plants, insects and diseases were uniform in both experiments and were implemented according to technical recommendations for the cultivation of common bean in the southern region of Brazil (COMISSÃO TÉCNICA SUL-BRASILEIRA DE FEIJÃO, 2012).

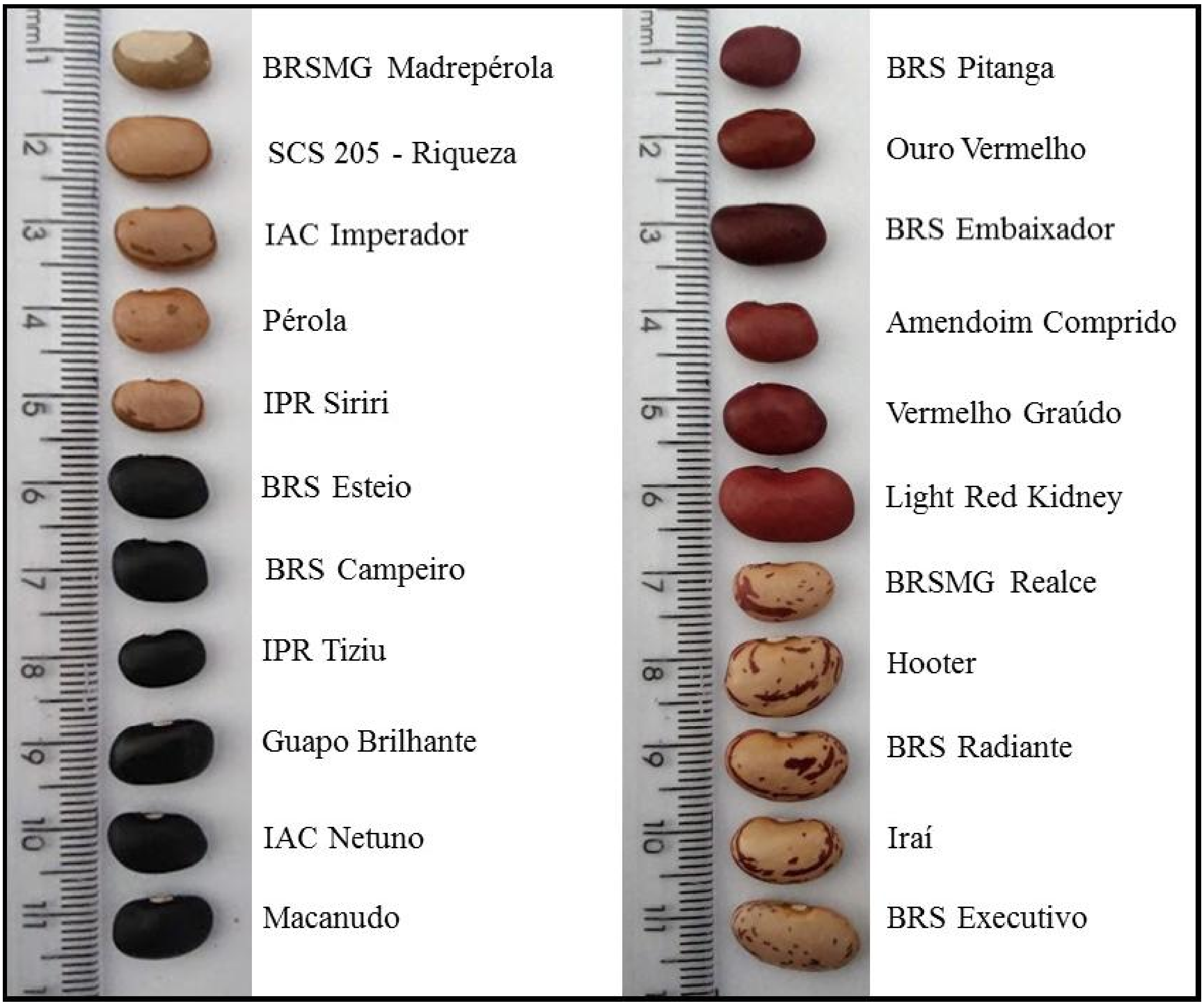
The harvest was also performed manually, at maturity stage, when the pods were dry and the grains showed the typical color of each cultivar. Grains were processed manually to avoid mechanical damage and were kept under refrigeration (temperature of 5 ºC and relative humidity of 75%) throughout the evaluation period.

Determination of physical grain quality

Physical grain quality assessments were performed immediately after the grains were processed. Grain color was determined using a portable colorimeter (CR 410, Konica Minolta, Osaka, Japão), which was previously calibrated with a standard white ceramic plate, following the manufacturer's recommendations. The L* a* b* scale was selected, as it considers the following color coordinates: L*, ranging from black (lower values) to white (higher values); a*, which evaluates the hues from green (negative values) to red (positive values); and b*, which analyzes the hues from blue (negative values) to yellow (positive values). On this scale, higher L*, a* and b* values indicate high lightness, and more reddish and more yellowish grains, respectively. All determinations were performed in triplicate, for each replicate.

Mass of 100 grains was evaluated in three samples of 100 grains with 13% moisture, which were collected at random in each replicate. The grain samples were placed in petri dishes and weighed on a precision scale. Grain dimensions (length, width and thickness) were measured with a digital caliper on 10 grains sampled at random. Length was determined parallel to the hilum; width was measured from the hilum to the opposite side; and

Figure 1 - Common bean cultivars of different grain types evaluated



thickness was measured perpendicular to the length and width. The shape and degree of flatness of the common bean grains were considered qualitative traits, which were obtained as described in Puerta Romero (1961). The ratio between the grain length and width was used to characterize grain shape into the following categories: spherical = 1.16 to 1.42; elliptical = 1.43 to 1.65; oblong/kidney-shaped short = 1.66 to 1.85; oblong/kidney-shaped medium = 1.86 to 2.00; or oblong/kidney-shaped long > 2.00. Lastly, the degree of flatness was calculated as the thickness-to-width ratio, as follows: flat < 0.69; half-full = 0.70 to 0.79; and full > 0.80.

Cooking-related traits (normal grains, uptake and cooking time) were evaluated in a sample of 25 grains per replicate. These were soaked in 50 mL of distilled water for eight hours, at room temperature (20 ± 2 ºC). Subsequently, the water was removed and the grains were partially dried with a paper towel. The grains that absorbed water increased in size (i.e. swelled) and were considered normal grains. The number of normal grains was counted and results were expressed in %. Uptake was calculated by the following expression: [(weight of grains after uptake - weight of grains before uptake)/weight of grains before uptake] × 100.

Cooking time was determined using a Mattson cooker (Embrapa Instrumentação, São Paulo, Brazil). The cooker uses a stainless steel rack with 25 perforated depressions over which the grains and 25 metal plungers are distributed. Each plunger weighs 90 g and is 1.0 mm in diameter at its tip, which lies above the grains. The apparatus was placed inside a 7-L pan with 3 L of hot distilled water and cooking was carried out on a domestic stove, over a medium flame. The grain was considered to be cooked when the plunger dropped, piercing it. The drop time of each plunger was recorded and the average drop time of the first 13 plungers of the cooker was considered the cooking time of each sample.

Statistical analyses

The obtained data were subjected to individual and combined analyses of variance, considering the randomized-block model. The homogeneity of the residual variances was checked by Hartley's maximum F test, following the criterion that the ratio between the highest and lowest residual mean squares was not greater than seven (CRUZ; REGAZZI, 1997). In the combined analysis of variance, the effects of genotype (G), environment (E) and G × E interaction were analyzed as fixed, whereas the experimental error was considered random. The genotype effect was decomposed into grain types by the hierarchical method. The significance level was assessed by the F test ($p < 0.05$) for all traits. Results expressed in percentage terms (normal grains and uptake) were transformed using the expression $\sqrt{x+0.5}$, where x is the trait value.

Multicollinearity diagnostics were performed with the phenotypic correlation matrix obtained in the combined analysis of variance for all evaluated traits, except for those of qualitative nature. The collinearity assessment followed the criteria established by Montgomery and Peck (1981). To obtain weak collinearity - a requirement for the correlation, cluster and selection-index analyses, highly correlated traits and traits with a greater weight in the last eigenvectors were excluded.

Pearson's linear correlation coefficients were estimated from the genotype mean matrix of combined analysis of variance. The significance of Pearson's correlation coefficients was evaluated by Student's *t* test (p value < 0.05).

The residual variance and covariance matrices from combined analysis of variance were used to generate the genetic dissimilarity matrix between common bean cultivars by Mahalanobis' generalized distance (D^2) and by canonical variables. For this purpose, the original means of the traits were transformed into standardized variables by a pivotal condensation process, which is characterized by showing zero residual covariance and residual variances equal to one (CRUZ; REGAZZI, 1997). The analysis of the relative importance of the traits for genetic dissimilarity between the common bean cultivars was based on the canonical variables analysis. Cluster analyses were performed using two methods, namely, canonical variables and the hierarchical unweighted pair group method with arithmetic mean (UPGMA). The cophenetic correlation coefficient (CCC) was established from Pearson's linear correlation between the elements of the cophenetic matrix and the elements of the dissimilarity matrix to check the consistency of the clustering pattern.

The multiplicative index (SUBANDI; COMPTON; EMPIG, 1973) was used to obtain estimates of heritability and selection gain as well as for the simultaneous selection of common bean cultivars of different grain types with superior physical grain quality. Selection was performed with a view to selecting the three light-grain cultivars with the best physical quality. For this, direct selection was applied for the L* value, mass of 100 grains, length, width, normal grains and uptake and inverse selection was carried out for the a* value and cooking time. The three dark-grain cultivars with superior physical quality were also selected, considering inverse selection for the L* and a* values and cooking time and direct selection for the remaining traits. Analyses were performed using the Office Excel spreadsheet and Genes software (CRUZ, 2016).

RESULTS AND DISCUSSION

Overall results

The ratio between the highest and lowest residual mean square of the individual analysis of variance was less than seven for all evaluated traits, except for the b value*. In this case, the degrees of freedom of the error and of the G × E interaction for the b* value also had to be calculated, as described in Cochran (1954). In this way, homogeneous residual variances were obtained for all physical grain traits, thus allowing the combined analysis of variance.

Significant genotype and grain type effects (p value < 0.05) were obtained for all physical grain traits (Table 1). Therefore, there was genetic variability between the common bean cultivars and between the four grain types evaluated (carioca, black, red and cranberry). The carioca bean cultivars differed for L* value, grain width, grain thickness, degree of flatness, normal grains and uptake. Similarly, previous results showed genetic variability between carioca bean cultivars for grain dimensions and degree of flatness (DELFINI *et al*., 2017) and between carioca bean lines for L* value and uptake (ARNS *et al*., 2018). The black bean cultivars, in turn, differed for all traits, except for the L*, a* and b* values and mass of 100 grains. Delfini *et al*. (2017) evaluated 19 black bean cultivars and also observed genetic variability for grain dimensions, shape and degree of flatness. These results favor the selection of carioca and black bean cultivars with superior physical grain traits.

In the red bean cultivars, a significant effect was observed for all traits. Therefore, these cultivars showed genetic variability for the physical grain traits, which is in accordance with the observations made by Wani *et al.* (2017) in red bean cultivars produced in India. The cranberry bean cultivars, on the other hand, differed for all evaluated traits, except for grain color (L*, a* and b* values). In the cranberry bean lines grown in Brazil, significant differences were also described for mass of 100 grains and cooking time (RIBEIRO *et al*., 2014). The results obtained for the red and cranberry bean cultivars demonstrate that it is possible to select superior cultivars for physical grain traits, which represents significant advances for breeding programs.

A significant G × E interaction was observed for all physical grain traits (Table 1). Similar results were described for grain color as evaluated using a colorimeter (ARNS *et al*., 2018; RIBEIRO; KLÄSENER, 2020), mass of 100 grains, grain dimensions, shape, degree of flattening (CABRAL *et al*., 2011; DELFINI *et al*., 2017) and cooking-related traits (ARNS *et al*., 2018; GARCIA *et al*., 2012; RIBEIRO; KLÄSENER, 2020) evaluated in common bean genotypes grown in several environments. These results showed that the physical traits of common bean grains vary according to genotype, environment and G × E interaction. Therefore, analyses of genetic divergence and of selection of common bean cultivars based on physical grain traits must consider different environments for the proper interpretation of results.

Table 1 - Combined analysis of variance containing the degrees of freedom (DF), mean squares, mean, coefficient of experimental variation (CEV%) and selective accuracy (SA) for the following traits: L* value (L*), a* value (a*), b* value (b*), mass of 100 grains (M100G, g), grain length (LENGTH, mm), grain width (WIDTH, mm), grain thickness (THICK., mm), grain shape (SHAPE), degree of flatness (FLAT.), normal grains (NORMAL, %), uptake (%) and cooking time (CT, s) of 22 common bean cultivars evaluated in the two growing seasons (dry season of 2019 and rainy season of 2019)

	DF	Mean square					
		L*	a*	b*	M100G	LENGTH	WIDTH
Block/environment	4	0.38	1.24	1.93	2.94	0.10	0.01
Genotype (G)	21	1644.72*	511.35*	283.18*	530.22*	42.13*	8.76*
Grain type (T)	3	11356.92*	3469.88*	1870.08*	1931.54*	124.83*	22.42*
G/Type	18	26.02*	18.27*	18.70*	296.66*	28.34*	6.48*
T/Carioca	4	12.97*	4.72^{ns}	1.03^{ns}	5.98^{ns}	1.10^{ns}	0.71*
T/Black	5	1.31^{ns}	2.79^{ns}	4.70^{ns}	14.14^{ns}	38.65*	14.79*
T/Red	5	77.53*	53.79*	60.25*	581.59*	46.22*	3.25*
T/Cranberry	4	5.57^{ns}	6.75^{ns}	1.92^{ns}	584.34*	20.36*	5.92*
Environment (E)	1	86.67*	1.74^{ns}	242.30*	803.15*	0.97*	0.33*
G x E	21	14.30*	12.22*	12.56*	61.95*	2.72*	0.63*
Error	84	1.81	0.93	4.00	4.06	0.12	0.03
Mean		40.85	10.40	9.00	30.82	10.95	6.32
CEV(%)		3.29	9.27	22.22	6.54	3.13	2.89
SA		0.999	0.999	0.993	0.996	0.999	0.998

Continuation table 1

	DF	THICK.	SHAPE	FLAT.	NORMAL	UPTAKE	CT
Block/environment	4	0.01	0.00	0.00	0.21	0.31	142.20
Genotype (G)	21	5.89*	0.26*	0.01*	4.73*	13.76*	455802.31*
Grain type (T)	3	14.60*	0.47*	0.00*	9.74*	8.78*	530395.03*
G/Type	18	4.43*	0.22*	0.01*	3.89*	15.46*	443370.19*
T/Carioca	4	0.66*	0.00^{ns}	0.00*	3.19*	7.85*	20452.63^{ns}
T/Black	5	7.84*	0.03*	0.01*	4.47*	9.72*	131987.58*
T/Red	5	1.13*	0.70*	0.00*	5.11*	29.28*	975852.24*
T/Cranberry	4	8.08*	0.08*	0.04*	2.37*	12.98*	589913.45*
Environment (E)	1	0.00^{ns}	0.07*	0.00*	1.23^{ns}	0.25^{ns}	309043.70*
G x E	21	0.42*	0.02*	0.00*	2.02*	4.10*	161797.72*
Error	84	0.03	0.00	0.00	0.22	0.37	7165.05
Mean		4.62	1.72	0.73	8.94	8.19	1124.23
CEV(%)		3.87	2.78	3.79	5.23	7.40	7.53
SA		0.997	0.996	0.970	0.977	0.987	0.992

* : Significant by F test at 0.05 probability. ^{ns}: non-significant

Coefficients of variation ranging from 2.78 to 22.22% were obtained for all evaluated traits. The observed amplitude of variation is comparable to the coefficient of variation values found for physical grain traits determined in common bean genotypes (ARNS *et al*., 2018; CABRAL *et al*., 2011; DELFINI *et al*., 2017; RIBEIRO; KLÄSENER, 2020; SANTOS; RIBEIRO; MAZIERO, 2016). Selective accuracy values ≥ 0.970 were detected for all physical grain traits, indicating very high experimental precision, according to the classes proposed by Resende and Duarte (2007). High selective accuracy values have been previously described for physical grain traits evaluated in common bean genotypes (ARNS *et al*., 2018; RIBEIRO; KLÄSENER, 2020). Therefore, the physical grains traits were determined with high experimental precision, which contributes to greater efficiency in the selection of superior common bean cultivars.

Multicollinearity diagnostics revealed a condition number (CN) of 325.47, characterizing moderate to strong collinearity, according to the criteria established by Montgomery and Peck (1981). To obtain weak collinearity, pairs of highly correlated traits were evaluated and traits with a greater weight in the last eigenvectors were eliminated. After excluding the traits of grain thickness and b* value, a CN = 44.25 was obtained, that is, weak collinearity. This prevented multicollinear variables from being implicitly receiving a greater weight in the correlation, cluster and selection-index analyses (CRUZ; CARNEIRO, 2006).

Correlations between physical grain traits

Grain length and width were highly correlated (r = 0.844) (Table 2). Among the grains types evaluated in the present study, the longer grains were also wider, confirming previous results obtained by Bareke (2019), Canci *et al*. (2019) and Pérez-Vega *et al*. (2010), in common bean genotypes. However, a low correlation between grain length and width in common bean was described by Nadeen *et al.* (2020), Rana *et al.* (2015) and Yeken *et al*. (2019). Thus, the length-to-width ratio can vary between common bean cultivars of different grain types.

Among the cooking-related traits, the correlations between normal grains and uptake (r = 0.796) and between uptake and cooking time (r = -0.651) are noteworthy. This is because higher percentages of normal grains were associated with greater uptake and greater uptake resulted in a shorter cooking time, which is in agreement with the observations of Ribeiro and Kläsener (2020) for carioca and black bean lines. A negative correlation between uptake and cooking time for carioca and black bean lines was also described by Santos, Ribeiro and Maziero (2016). In this case, selection for greater uptake can be recommended for the indirect selection of fast-cooking common bean cultivars.

Low or non-significant correlation estimates were obtained for the other evaluated physical grain traits. A lack of correlation between two traits indicates that there are no linked genes or pleiotropic effects (BALESTRE *et al*., 2013). Therefore, ease is expected in the selection of superior common bean cultivars, as the observed genetic values for most of the physical grain traits were independent.

Analysis of genetic divergence for physical grain traits in common bean

The first two canonical variables explained 79.55% of the total variation in the data (Table 3). The first canonical variable accounted for 52.04% of the obtained variation, with the L* value (1.00) and grain width (0.45) being the most important physical traits in differentiating between the cultivars. The second canonical variable represented 27.50% of the total variation and the most relevant traits to identify the differences between the cultivars were the a* value (0.85) and grain width (0.58). Thus, the color of common bean grains, determined quantitatively by the L* and a* values, was the trait of greatest contribution in the analysis of phenotypic differences between cultivars of different grain types. The qualitative assessment of grain color, performed by visual observation, was also efficient to distinguish different groups of common bean landraces evaluated in Spain (ARTEAGA *et al*., 2019). However, no previous studies of genetic divergence between common bean cultivars have been found considering the quantitative assessment of grain color.

The clustering performed using the first two canonical variables was represented in a two-dimensional scatter plot of the obtained scores (Figure 2). As shown in the plot, four groups were formed. Group 1 was constituted by the black bean cultivars (lower L* and a* values): BRS Esteio, Guapo Brilhante, IAC Netuno, BRS Campeiro, Macanudo and IPR Tiziu. Group 2 consisted of the red bean cultivars (intermediate L* and high a* values): BRS Embaixador, Ouro Vermelho, Amendoin Comprido, Light Red Kidney, BRS Pitanga and Vermelho Graúdo. Group 3 was composed predominantly of carioca bean cultivars (high L* and low a* values): Pérola, IAC Imperador, BRS Executivo, SCS 205 Riqueza, IPR Siriri and BRSMG Madrepérola. Group 4 contained the cranberry bean cultivars (high L* and intermediate a* values): BRS Radiante, Iraí, Hooter and BRSMG Realce. The L* and a* values made it possible to clustered similar common bean cultivars for grain lightness and red hue in the same group. However, the clustering carried out by canonical variables was not very informative regarding the other physical grain traits that differentiate common bean cultivars.

Table 2 - Pearson's phenotypic correlation coefficients obtained between the traits L* value (L*), a* value (a*), mass of 100 grains (M100G), grain length (LENGTH), grain width (WIDTH), normal grains (NORMAL), uptake and cooking time (CT) obtained from 22 common bean cultivars evaluated in two experiments carried out in the year 2019

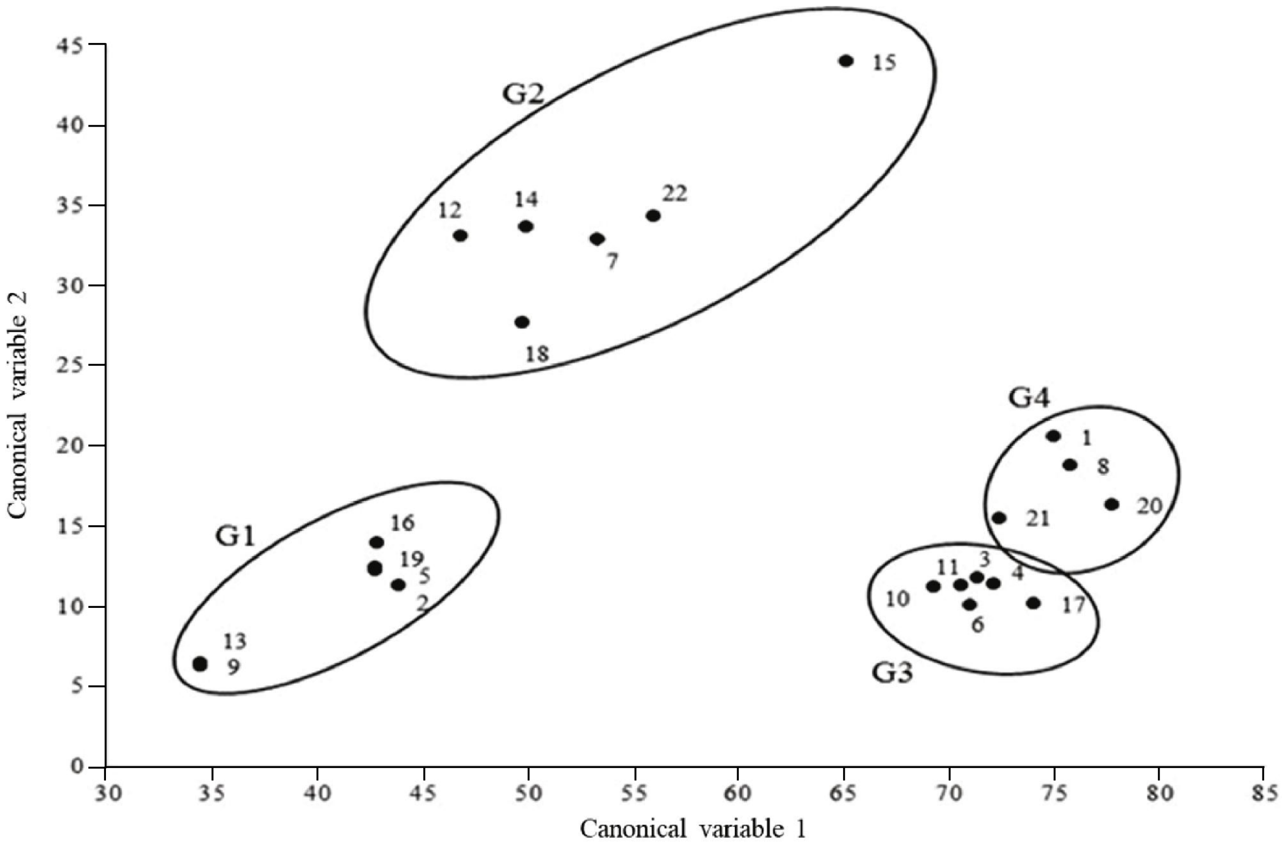
	a*	M100G	LENGTH	WIDTH	NORMAL	UPTAKE	CT
L*	-0.098	0.291	0.241	0.223	0.290	-0.047	0.075
a*		0.371	0.575*	0.514*	0.345	0.196	0.078
M100G			0.474*	0.169	0.219	-0.074	0.405
LENGTH				0.844*	-0.013	-0.311	0.443*
WIDTH					0.135	-0.101	0.222
NORMAL						0.796*	-0.439*
UPTAKE							-0.651*

* Significant by t test at 0.05 probability

Table 3 - Canonical variables (CV) established by the linear combination of standardized variables, observed variance (OV., %), accumulated variance (AV., %) and relative importance of traits for canonical variables of L* value (L*), a* value (a*), mass of 100 grains (M100G), grain length (LENGTH), grain width (WIDTH), normal grains (NORMAL), uptake and cooking time (CT) obtained from 22 common bean cultivars evaluated in two experiments carried out in the year 2019

CV	OV.	AV.	Relative importance of traits							
			L*	a*	M100G	LENGTH	WIDTH	NORMAL	UPTAKE	CT
CV1	52.04	52.04	1.00	-0.12	0.30	0.14	0.45	0.27	-0.13	0.15
CV2	27.50	79.55	-0.19	0.85	-0.11	-0.17	0.58	0.07	-0.00	0.21
CV3	9.51	89.06	-0.25	-0.47	0.25	-0.50	0.82	0.26	-0.42	0.68
CV4	6.48	95.54	-0.10	-0.29	-0.40	0.92	-0.09	-0.17	0.11	-0.01
CV5	2.50	98.04	-0.13	-0.18	0.84	0.73	-0.58	0.23	0.25	0.08
CV6	1.27	99.31	0.05	0.15	-0.15	0.25	-0.49	0.31	-0.27	0.79
CV7	0.52	99.83	-0.02	-0.09	-0.28	-0.27	0.34	0.45	0.66	0.43
CV8	0.17	100.00	-0.04	-0.03	-0.06	-0.04	0.03	0.98	-0.67	-0.08

Figure 2 - Dispersion graph obtained from the physical grain traits of 22 common bean cultivars for the canonical variable 1 and the canonical variable 2



G1 (2= BRS Esteio; 5= Guapo Brilhante; 9= IAC Netuno; 13= BRS Campeiro; 16= Macanudo; and 19= IPR Tiziu), G2 (7= BRS Embaixador; 12= Ouro Vermelho; 14= Amendoim Comprido; 15= Light Red Kidney; 18= BRS Pitanga; and 22= Vermelho Graúdo), G3 (3= Pérola; 4= IAC Imperador; 6= BRS Executivo; 10= SCS 205 – Riqueza; 11= IPR Siriri; and 17= BRSMG Madrepérola) and G4 (1= BRS Radiante; 8= Iraí; 20= Hooter; and 21= BRSMG Realce)

The obtained CCC was 0.84, which was significant at 1% probability by the t test. This result is comparable to the CCC values found by Arteaga *et al*. (2019), Cabral *et al*. (2011) and Veloso *et al*. (2015) and using cluster analysis by the UPGMA method, considering morphological traits of common bean cultivars. Cophenetic correlation coefficients close to unity indicate high adjustment between the cophenetic matrix and the dissimilarity matrix based on Mahalanobis' generalized distance (CABRAL *et al*., 2011). Therefore, in the present study, high reliability was achieved in the clustering pattern obtained by the UPGMA method.

Cluster analysis performed using the UPGMA method separated the common bean cultivars into three groups, namely: group 1 - formed by cultivar Light Red Kidney (light red grains); group 2 - including all black bean cultivars and cultivars with dark red grains; and group 3 - containing all the carioca and cranberry bean cultivars (Figure 3). This is explained by the fact that the L* (43.51%) and a* (21.83%) values were the two traits that most contributed to the differentiation between the common bean cultivars, also identified by Mahalanobis' generalized distance. Based on the UPGMA method, most cultivars were grouped into two large groups: dark grains (black and red) and light grains (carioca and cranberry). Likewise, Arteaga *et al.* (2019) observed the formation of two groups of common bean cultivars by the UPGMA method, with group 1 including 75% of brown-grain cultivars and group 2 containing 55% of white-grain cultivars, from an analysis of 32 traits of flowers, pods and grains. However, the use of the UPGMA method did not allow the separation of common bean lines of different grain types in the analysis of molecular markers by Veloso *et al*. (2015). The authors attributed this fact to the great genetic similarity between the carioca, black, red and cranberry bean lines developed by different research institutions in Brazil.

In the present study, the two cluster analyses were efficient in separating the common bean cultivars into homogeneous groups by grain color (L* and a* values). Canonical variables analysis was more detailed in the formation of groups based on different grain types as compared with the UPGMA analysis. However, the two cluster analyses were not very informative with regard to the other physical grain traits that differentiate common bean cultivars of different grain types.

Selection of superior light-grain common bean cultivars for physical traits

The use of the multiplicative index allowed the selection of superior light-grain (carioca and cranberry) and dark-grain (black and red) common bean cultivars for physical grain traits. When selection was carried out for the light-grain cultivars, high heritability estimates were obtained ($h^2 \geq 68.20\%$) for all physical grain traits (Table 4). The range of variation observed for heritability is comparable to values described for grain color (ARNS *et al*., 2018), mass of 100 grains (ARNS *et al*., 2018; NADEEM *et al*., 2020; RANA *et al*., 2015), grain dimensions (BAREKE, 2019; NADEEM *et al*., 2020; RANA *et al*., 2015) and cooking-related traits (ARNS *et al*., 2018) evaluated in common bean genotypes. The magnitude of heritability is governed mainly by genetic variability. High heritability is a result of the lesser effect of the environment on the expression of the trait and is, therefore, related to greater genetic variability. Therefore, high heritability indicates greater genetic variability, which increases the chances of success in the selection of superior common bean cultivars for physical grain traits.

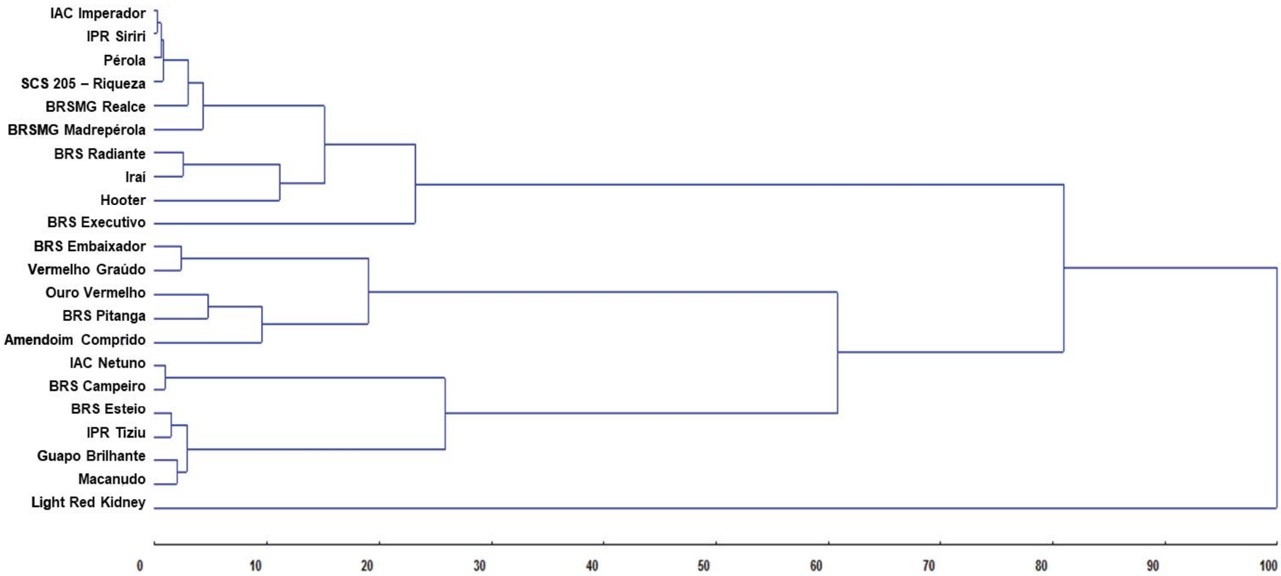
The highest genetic gain estimates were observed for mass of 100 grains (37.60%), uptake (14.10%) and normal grains (6.62%). Similarly, Arns *et al*. (2018) reported that the highest genetic gain values were found for mass of 100 grains and uptake when the multiplicative index was applied for the selection of carioca common bean lines with high physical grain quality. For the other physical grain traits, genetic gain estimates favorable to the objectives of the selection of light-grain cultivars were not obtained.

The use of the multiplicative index allowed the selection of superior light-grain cultivars for physical grains traits, namely, BRS Executivo, BRSMG Realce and Hooter. These three common bean cultivars have cranberry grains with a L* values ranging from 56.80 to 58.47 and a* values from 7.81 to 9.74, i.e., their grains are very light and slightly red. Similar L* values were observed in cranberry bean lines evaluated in Brazil by Ribeiro *et al*. (2014).

Cultivars BRS Executivo and Hooter have large grains (≥ 40 g), while BRSMG Realce has medium grains (25 to 40 g), based on the classes of mass of 100 grains described by Blair *et al*. (2010). These three common bean cultivars a had grain size characteristic of the Andean gene pool, which was also validated by the grain length and width dimensions, as proposed by Pereira *et al*. (2009). In addition, the three selected cranberry bean cultivars stood out with fast cooking, which was defined by Santos, Ribeiro and Maziero (2016) as a cooking time less than 25 min. Fast-cooking cranberry bean cultivars were previously selected by Ribeiro *et al*. (2014).

Cranberry bean cultivars BRS Executivo, BRSMG Realce and Hooter stood out over the other evaluated light-grain cultivars due to their greater grain lightness, larger grain size and fast cooking. These cultivars have superior physical grain traits and will thus be selected by the breeding program.

Figure 3 - Dendrogram representing genetic dissimilarity among the 22 common bean cultivars obtained by the unweighted pair group method with arithmetic mean (UPGMA), using Mahalanobis' generalized distance, based on physical grain traits evaluated in two experiments carried out in the year 2019



Selection of superior dark-grain common bean cultivars for physical traits

The multiplicative index was also applied for the selection of superior dark-grain common-bean cultivars for physical grain traits. This selection resulted in high heritability estimates ($h^2 \geq 96.17\%$) for all evaluated traits. High heritability estimates indicate genetic variability between black and red bean cultivars for physical grain traits, allowing the selection of superior common bean cultivars.

Genetic gain estimates favorable to the selection of dark-grain cultivars were observed for the L* (-18.36%) and a* (-90.14%) values and for grain width (1.45%). The a* value showed the highest magnitude of genetic gain among the evaluated traits. Arns *et al*. (2018) also found a high negative genetic gain estimate for the a* value, in an experiment with carioca bean lines. Reducing the a* value is important in carioca beans, as this will result in a less intense red hue. In black beans, an a* value close to zero indicates the absence of a secondary color, which gives greater commercial value to the grains. For this reason, the magnitude of the genetic gain estimate obtained for the a* value, in the present study, shows that it is possible to select dark-grain common bean cultivars with a grain color that meets the market demand.

The following dark-grain cultivars were selected by the multiplicative index due to their superior physical grain traits: IPR Tiziu, BRS Esteio and Guapo Brilhante. These three common bean cultivars have black grains that stand out with low lightness (L*: 21.09 to 22.26) and a slightly red hue (a*: 1.13 to 1.37). The selected cultivars exhibited L* values similar to those observed in the black bean lines evaluated by Ribeiro and Kläsener (2020) and within the recommended range for black bean lines (RIBEIRO; POSSEBOM; STORCK, 2003).

Table 4 - Average of the original population (X_0), average of selected cultivars (Xs), heritability (h^2), genetic gain (GG) and percentage of genetic gain (GG%) with simultaneous selection by the multiplicative index for the traits L* value (L*), a* value (a*), mass of 100 grains (M100G, g), grain length (LENGTH, mm), grain width (WIDTH, mm), normal grains (NORMAL, %), uptake (%) and cooking time (CT, min) and the three light-grain and three dark-grain cultivars selected based on the best physical quality obtained from 22 common bean cultivars evaluated in two experiments carried out in the year 2019

Light-grain cultivars								
Trait	X_0	X_S	h^2%	GG	GG%	Selected cultivars		
						BRS Executivo	BRSMG Realce	Hooter
L*	58.03	57.87	68.20	-0.11	-0.19	58.47	56.80	58.35
a*	7.38	8.79	98.19	1.39	18.82	7.81	8.83	9.74
M100G	33.01	45.53	99.12	12.41	37.60	52.33	33.55	50.72
LENGTH	11.27	10.80	99.07	-0.47	-4.18	8.78	11.68	11.94
WIDTH	6.48	6.04	98.95	-0.43	-6.62	4.59	6.62	6.93
NORMAL	83.91	96.43	92.86	0.61	6.62	95.65	95.84	97.81
UPTAKE	65.93	86.68	96.14	1.14	14.10	97.41	94.28	69.89
CT	19.02	19.10	98.36	5.02	0.44	15.82	17.30	24.17
Total gain				19.56	66.59			
Dark-grain cultivars								
Trait	X_0	X_s	h^2%	GS	GS%	Selected cultivars		
						IPR Tiziu	BRS Esteio	Guapo Brilhante
L*	26.54	21.65	99.75	-4.87	-18.36	21.09	22.26	21.61
a*	12.92	1.26	99.85	-11.65	-90.14	1.27	1.13	1.37
M100G	29.00	24.16	99.33	-4.81	-16.58	22.45	24.55	25.48
LENGTH	10.67	9.95	99.82	-0.72	-6.74	10.30	9.63	9.93
WIDTH	6.19	6.28	99.77	0.09	1.45	6.41	6.46	5.98
NORMAL	76.56	67.57	96.17	-0.51	-5.78	75.00	79.92	49.98
UPTAKE	68.06	57.76	98.01	-0.63	-7.67	79.92	59.60	37.82
CT	18.50	20.72	98.56	130.35	11.74	18.90	21.47	21.75
Total gain				107.25	-132.08			

Cultivars IPR Tiziu and BRS Esteio have small grains (≤ 25 g), while Guapo Brilhante has medium grains (25 to 40 g), based on the classification of mass of 100 grains presented by Blair *et al*. (2010). According to Carbonell *et al*. (2010), black bean cultivars must have medium-sized grains to meet the demand of Brazilian consumers. Only the cultivar Guapo Brilhante has a grain size of greater acceptance for black bean cultivars produced in Brazil. The three selected common bean cultivars showed grain size, length and width characteristic of Mesoamerican beans, considering the classification proposed by Pereira *et al*. (2009).

Black bean cultivars IPR Tiziu, BRS Esteio and Guapo Brilhante had a cooking time ≤ 21.75 min, which was defined as fast cooking by Santos, Ribeiro and Maziero (2016). Lines of fast-cooking black beans were previously selected by Ribeiro and Kläsener (2020) and Santos, Ribeiro and Maziero (2016). The selection of fast-cooking common bean cultivars meets the demand of consumers who have little time available to prepare the meal.

The three selected black bean cultivars (IPR Tiziu, BRS Esteio and Guapo Brilhante) stood out due to their fast cooking and grain color that meet the market demand for this type of grain. Because these common bean cultivars showed superior physical grain traits, their selection is recommended for the breeding program.

CONCLUSIONS

1. Common bean cultivars of different grain types have genetic variability for physical grain traits. Selection for greater uptake is recommended for the indirect selection of fast-cooking common bean cultivars;
2. The L* and a* values are the traits that most contribute to the differentiation between common bean cultivars of different grain types, making them decisive for the formation of homogeneous groups in terms of grain color. The number of groups formed and the cultivars clustered in each group differ in the analyses of canonical variables and unweighted pair group method with arithmetic mean;
3. The cranberry bean cultivars BRS Executivo, BRSMG Realce and Hooter and the black bean cultivars IPR Tiziu, BRS Esteio and Guapo Brilhante have superior physical grain traits and will thus be selected by the breeding program.

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