

Genetic dissimilarity of common bean lines for agronomic and biofortification traits

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ABSTRACT. The evaluation of the genetic dissimilarity of common bean lines obtained by the breeding program is significant to identify duplicate accessions, to select superior lines, and to plan new hybrid combinations. The objectives of this study were to evaluate the genetic dissimilarity of common bean genotypes for agronomic and mineral biofortification traits, select superior lines, and identify promising hybrid combinations for biofortification programs. For this purpose, 169 common bean genotypes ($F_{6,8}$ inbred lines and parental cultivars) were evaluated in two experiments conducted in a simple lattice design in Santa Maria-RS, Brazil. The following agronomic traits were evaluated: insertion of the first pod, insertion of the last pod, cycle, mass of 100 grains, grain yield, and concentrations of potassium, phosphorus, zinc, and copper in grains. The genotypes assessed presented genetic variability for all traits, except for copper concentration in grains. The traits that showed the highest contribution to the genetic dissimilarity were the concentrations of phosphorus, potassium, and zinc in grains. In cluster analyses, six groups by principal components and 33 groups by the Tocher optimization method were identified. These methods

partially agreed in the formation of different groups of common bean genotypes. Controlled crossings between the lines 17 and 162 with lines of groups XX, XXI, XXIV, and XXXIII are recommended to obtain recombinants with high grain yield and biofortified for phosphorus, potassium, and zinc.

Key words: *Phaseolus vulgaris*; Agronomic performance; Minerals; Cluster analysis

INTRODUCTION

Brazil is the largest country in area in South America and a large bean producer. The common bean (*Phaseolus vulgaris* L.) is the species most cultivated in this country. Two types of grains of the Mesoamerican gene pool are grown, Carioca type (beige seed coat with brown streaks), accounting for 50% of the cultivated land, and the black type, with 25% of the cultivated land in the country. Blair et al. (2013) proposed that these two types of grains constitute the sub-races Carioca (Brazilian cream beans) and Veracruz (small black beans) of the race Mesoamerica.

As Carioca and black beans are also the types of grains most consumed in Brazil, they have received more attention from breeding programs. Therefore, the genetic variability observed in the landraces and the genotypes developed for breeding programs requires a more detailed characterization. Genetic dissimilarity and cluster analyses have been widely used to determine the genetic variability of common bean genotypes that have been grown in a given region or country (Bonett et al., 2006; Chiorato et al., 2006; Gonçalves Ceolin et al., 2007; Elias et al., 2007; Barelli et al., 2009; Coelho et al., 2010; Freitas et al., 2011; Blair et al., 2013; Grahic et al., 2013; Gonçalves et al., 2014; Hegay et al., 2014; Sofi et al., 2014). Thus, accession duplicates are identified, and hybrid combinations are planned based on the identification of genotypes with favorable traits for selection.

The main clustering methods used to identify similarities and differences among common bean genotypes are principal components (Freitas et al., 2011; Blair et al., 2013; Grahic et al., 2013; Hegay et al., 2014; Sofi et al., 2014) and the Tocher optimization method (Bonett et al., 2006; Gonçalves Ceolin et al., 2007; Elias et al., 2007; Barelli et al., 2009; Coelho et al., 2010; Gonçalves et al., 2014). Genetic dissimilarity of common bean genotypes has been evaluated by agronomic descriptors (Bonett et al., 2006; Barelli et al., 2009; Freitas et al., 2011; Hegay et al., 2014; Sofi et al., 2014) and seed characteristics (Blair et al., 2013; Grahic et al., 2013; Gonçalves et al., 2014). Elias et al. (2007) evaluated the genetic dissimilarity of common bean cultivars by agronomic and nutritional descriptors (crude protein and dietary fiber), using the Tocher optimization method. However, no previous studies assessing the genetic dissimilarity of common bean lines obtained by breeding programs, considering morphological, phenological, production, and mineral biofortification traits, were found in the literature.

Development of common bean cultivars with high agronomic performance and biofortified for minerals is a new strategy in the breeding programs. For this purpose, it is necessary to evaluate and to identify genotypes that have an erect plant architecture, with growth cycle and grain size that meet market acceptance and high grain yield. If these genotypes are biofortified for minerals, this represents technological advantages for common bean growers. Besides, the use of biofortified foods can contribute to improved consumers' health.

Potassium can lower blood pressure and reduce cardiovascular diseases (He and MacGregor, 2008). Phosphorus contributes to diminishing fatigue, anxiety, and skin sensibility symptoms (Martínez-Ballesta et al., 2010). Zinc helps control dermatitis (Gibson, 2012), and copper has been used to control hypochromic anemia (Huskisson et al., 2007).

Common bean is the main source of plant protein consumed on a daily basis in Latin America and Africa. For this reason, bean biofortification is an important alternative to be used in the control of nutritional deficiencies. New common bean cultivars biofortified for minerals and of high agronomic performance represent technologic innovation in bean production system. Thus, the objectives of this study were to evaluate the genetic dissimilarity of common bean genotypes for agronomic and mineral biofortification traits, to select superior lines for these traits, and to identify promising hybrid combinations to obtain recombinants with high agronomic performance and biofortified for minerals.

MATERIAL AND METHODS

Plant material

The $F_{6,8}$ common bean lines were obtained from bi-parental crossings between the cultivars IAPAR 44 x Guapo Brilhante, BRS Expedito x BRS Valente, Pérola x Guapo Brilhante, TPS Nobre x Guapo Brilhante, IAPAR 44 x IAPAR 31, and Diamante Negro x TPS Bonito (Table 1). Parents were selected based on high agronomic performance and contrasting concentrations of potassium (Poersch et al., 2011), phosphorus (Ribeiro et al., 2011), zinc (Rosa et al., 2010), and copper in grains (Poersch et al., 2013). The parents and lines obtained are of the Mesoamerican gene pool and represent the types of grains most consumed in Brazil, Carioca (beige seed coat with brown streaks) and black.

Table 1. Genealogy, growth habit (GH), grain type (GT), breeder, and year of the register of nine common bean cultivars used as parents to obtain segregating populations for the concentrations of potassium, phosphorus, zinc, and copper.

Cultivar	Genealogy ¹	GH ²	GT ³	Breeder ⁴	Year
BRS Valente	(Emgopa 201-Ouro / Ônix) // AN 512586	II	B	EMBRAPA	2001
BRS Expedito	CNF 5491 / FT Tarumã	II	B	EMBRAPA	2006
Diamante Negro	XAN 87 / A 367	II	B	EMBRAPA	1991
Guapo Brilhante (BR FEPAGRO 44)	XAN 125 // [BAT 336 (A83 x ICA Pijao)]	II	B	EMBRAPA	1995
IAPAR 31	BAC 4/RAI46//BAC 2/IGUAÇU/3/BAT/BAC 4	II	C	IAPAR	1988
IAPAR 44	BAC 2/ RAI 12// Rio Tibagi/ Cornell 49242	II	B	IAPAR	1990
Pérola	Selection in the Aporé cultivar	III	C	EMBRAPA	1996
TPS Bonito	IAPAR 14 / IAC Carioca	II	C	FT Sementes	1999
TPS Nobre	FT 120/ FT 84-1806// FT 84-424	II	B	FT Sementes	1996

¹/: single cross; //: double cross. ²GH: growth habit - II: indeterminate with short guides; III: indeterminate with great guides. ³GT: grain type: C - Carioca and B - black. ⁴Breeder: EMBRAPA: Empresa Brasileira de Pesquisa Agropecuária; IAPAR - Instituto Agrônomo do Paraná; FT Sementes - Francisco Terasawa Pesquisa e Sementes Ltda.

Progenies obtained were advanced until the F_6 generation by the single-seed descent method (SSD). Plants were cultivated in a greenhouse (not artificially climatized) of the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul (RS), Brazil. The $F_{6,7}$ lines and parents were multiplied in the field in the rainy season of 2011 to produce a larger amount of seeds for the experiments.

Evaluation of agronomic and mineral biofortification traits in common bean genotypes

The experiments were carried out in the field area of the Bean Breeding Program of the UFSM (latitude 29°42'S, longitude 53°49'W, and 95 m above sea level), in two growing seasons: the rainy season of 2012 and the dry season of 2013. Sowing was carried out in October (rainy season of 2012) and March (dry season of 2013), which are the traditional seasons for growing beans in RS. The climate in the region is Cfa humid subtropical with hot summers and no defined dry season.

The experimental design used was a 15 x 15-simple lattice design (rainy season of 2012) and 13 x 13-simple lattice design (dry season of 2013). In the rainy season of the 2012 experiment, 225 genotypes were evaluated: 212 F_{6,8} lines, nine parents and four control cultivars (BRS Campeiro, Carioca, FTS Magnífico, and Rio Tibagi). In the dry season of the 2013 experiment, 160 F_{6,8} lines and nine parents were assessed, totaling 169 genotypes. This was necessary because some lines did not produce a sufficient number of seeds for both experiments. Experimental plots consisted of two 1.0-m long rows, 0.50 m apart. Sowing density was 15 seeds/m.

The soil is classified as typical alitic Argisol (Hapludalf) and was conventionally prepared. Fertilization was performed according to the needs indicated by chemical analysis of soil. Fertilizer applied at the base was 275 kg/ha of the 5-20-20 commercial formula (urea: 45% nitrogen, superphosphate: 18% P₂O₅, and potassium chloride: 60% K₂O). In the first trifoliate leaf stage (V3), 20 kg/ha urea (45% nitrogen) was distributed. The insect control was carried out with the application of 100 mL/ha Engeo™ Pleno (Thiamethoxam and lambda-cyhalothrin). Weeds were mechanically removed when necessary to prevent competition with the crop. Disease control was not carried out in the experiments.

Insertion of the first pod and insertion of the last pod were assessed at the maturity stage, in 10 plants harvested at random in the experimental plot. The distance between the cotyledonal node and the insertion of the first and last pod were measured in centimeters, without consideration of the lodging degree of the plants. The cycle was determined by the number of days from emergence (V1) to the maturity (R9) stage of 51% of the plants in the experimental plot.

Mass of 100 grains and grain yield were evaluated at 13% of average humidity. Mass of 100 grains was obtained by weighing of three samples of 100 grains. Grain yield was determined by extrapolating the weight of the product obtained in each plot per hectare. As the plant stands were heterogeneous, it was necessary to adjust the plant stands for grain yield in all experimental units by the covariance - ideal stand method (Schmidt et al., 2001).

Samples of 50 g of harvested grains from each experimental unit were ground in a knife micro-mill (Q298A21, Quimis, Diadema, SP, Brazil) to produce particles smaller than 1 mm and not sieved. A sample of 0.5 g of the bean flour was used to perform nitric-perchloric digestion (HNO₃ + HClO₄, in a 3:1 ratio by volume), according to the procedure described by Jost et al. (2013). Mineral concentrations were determined by the Miyazawa and Pavan (2009) method as follows: potassium by flame photometer (B642, MICRONAL S.A., São Paulo, Brazil); phosphorus by UV-VIS spectrophotometer (UV-Visible Spectrophotometer T60, PG Instruments Ltd., Leicestershire, UK); zinc and copper by atomic absorption spectrophotometer (XplorAA DUAL, GBC Scientific Equipment Pty. Ltd., Braeside, Australia).

Genetic dissimilarity analyses

Joint analysis of variance was carried out with the genotypes common to both experiments, after testing for the efficiency of simple lattice design, compared to a randomized block design. Effects of genotypes (G) and growing season (S) were considered as fixed, and the others as random effects (block, G x S interaction and experimental error). The homogeneity of residual variances was verified by the Hartley Fmax test.

Multicollinearity diagnosis was made using the phenotypic correlation matrix, and its effect was assessed by the number of conditions, as defined by Montgomery and Peck (1981). The mean data obtained in both experiments were used for the genetic dissimilarity and cluster analyses. Principal component analysis was performed using standardized data to identify the traits with greater and lesser contribution to the total variation and cluster analysis. For this purpose, a dispersion graph was generated from the first two principal components. The first principal component was represented by the x-axis and the second principal component by the y-axis, allowing visualization of the groups formed.

The Mahalanobis generalized distance matrix was used as a measure of dissimilarity for the genotype clustering analysis, which was performed according to the Tocher optimization method. The means of the genotype groups formed by the Tocher method were compared by the Scott-Knott test at 0.05 of probability. The Genes software was used to carry out the statistical analyses (Cruz, 2013).

RESULTS

Joint analysis of variance

The simple lattice design showed an efficiency from 99.54% (mass of 100 grains) to 120.95% (phosphorus concentration), compared to the randomized block design (Table 2). The variance of the experimental error was homogeneous for all traits evaluated in both growing seasons ($P > 0.05$), which allowed making a joint analysis of variance. In the joint analysis of variance, a significant genotype x growing season interaction was observed for insertion of the first pod, insertion of the last pod, cycle, mass of 100 grains, grain yield, and zinc concentration in grains (Table 2).

Table 2. Joint analysis of variance containing the mean squares, degrees of freedom (d.f.), mean, coefficient of variation (CV), and efficiency of the lattice design (ELD) for insertion of the first pod (IFP), insertion of the last pod (ILP), cycle, mass of 100 grains (M100G), grain yield (Yield), and concentrations of potassium (K), phosphorus (P), zinc (Zn), and copper (Cu) in grains of 169 common bean genotypes evaluated in the rainy and dry seasons.

Sources of variation	d.f.	Mean square								
		IFP (cm)	ILP (cm)	Cycle (days)	M100G (g)	Yield (kg/ha)	K (g/kg) (dry matter)	P (g/kg) (dry matter)	Zn (mg/kg) (dry matter)	Cu (mg/kg) (dry matter)
Block	1	7.76	311.81	603.84	38.30	418,143.79	15.34	24.17	450.30	0.53
Genotype (G)	168	18.47*	90.53*	51.70*	25.94*	466,773.56*	3.40*	0.60*	37.21*	13.17 ^{ns}
Season (S)	1	18,449.89*	172,960.33*	112,201.81*	61.86*	11,615,546.72*	88.20*	16.88*	9541.31*	804.16*
G x S	168	14.10*	44.45*	37.22*	9.15*	389,708.35*	2.08 ^{ns}	0.35 ^{ns}	23.15*	15.38 ^{ns}
Residue	337	4.96	28.86	15.34	4.92	125,356.11	1.75	0.33	18.43	19.78
Mean		16.64	39.95	93.18	20.75	1864.01	16.74	4.90	27.30	13.40
CV (%)		13.38	13.45	4.20	10.69	18.99	7.89	11.69	15.72	33.18
ELD (%)		101.62	104.82	99.88	99.54	107.15	116.94	120.95	104.26	114.57

*Significant by the F-test ($P = 0.05$); ns = non-significant.

A significant effect of genotypes on potassium and phosphorus concentrations in grains was obtained. No significant effect of the genotype x growing season interaction and genotypes was observed to copper concentration in grains. For the copper concentration in grains, there was no genetic variability, and high coefficient of variation was obtained (CV% = 33.18%). For this reason, the values of copper concentration were not used for the genetic dissimilarity and clustering analyses.

Dissimilarity by principal components

The first three principal components explained 66.515% of the total variation (Table 3). The first principal component accumulated the highest percentage of total variation (30.992%). In this study, it was possible to identify the traits that most contributed to the genetic dissimilarity between the genotypes evaluated: concentration of phosphorus (0.506) followed by potassium (0.424) and zinc (0.413).

Table 3. Estimation of eigenvalues (root and % cumulative) and relative importance of the traits insertion of the first pod (IFP), insertion of the last pod (ILP), cycle, mass of 100 grains (M100G), grain yield (yield), concentrations of potassium (K), phosphorus (P), and zinc (Zn) in grains obtained in each principal component (PC) to estimate genetic dissimilarity among 169 common bean genotypes evaluated in the rainy and dry seasons.

PC	Root (%)	Cumulative (%)	IFP	ILP	Cycle	M100G	Yield	K	P	Zn
PC 1	30.992	30.992	0.235	0.030	0.194	-0.354	-0.419	0.424	0.506	0.413
PC 2	25.878	56.870	0.452	0.581	0.473	0.381	0.264	0.130	-0.046	0.005
PC 3	9.645	66.515	-0.594	-0.230	0.415	0.181	0.300	0.505	0.176	-0.113
PC 4	9.264	75.779	-0.236	-0.056	0.305	0.007	0.123	-0.365	-0.244	0.800
PC 5	8.012	83.791	0.048	-0.178	-0.183	0.598	-0.482	0.381	-0.400	0.193
PC 6	6.679	90.470	-0.087	0.341	-0.589	-0.156	0.454	0.432	-0.109	0.317
PC 7	4.993	95.463	0.306	-0.418	-0.241	0.449	0.348	-0.145	0.541	0.193
PC 8	4.536	100.000	-0.477	0.532	-0.189	0.335	-0.300	-0.250	0.433	0.030

Insertion of the last pod presented eigenvector with higher magnitude; thus, this trait contributed less to the genetic dissimilarity among the genotypes evaluated. Insertion of the last pod was not effective in discriminating the differences among the common bean genotypes and, for this reason, was not used in the cluster analyses.

The first two principal components separated the common bean genotypes into six groups (Figure 1). A large group (group 6) containing 156 genotypes, group 5 was formed by seven genotypes, and group 1, by three genotypes. The remaining three genotypes, which did not cluster with each other, constitute the groups 2, 3, and 4. The genotypes of the group 1 (lines 17, 19, and 163) showed the highest values of grain yield and mass of 100 grains. Pérola cultivar (group 2), line 120 (group 3), and line 33 (group 4) presented the highest mass of 100 grains.

Dissimilarity by Tocher optimization method

The Tocher optimization method, based on Mahalanobis generalized distance matrix, separated the common bean genotypes into 33 groups (Table 4). The number of genotypes in each group varied from one (in 15 groups: XIX to XXXIII) to 51 (group I). The group I comprised 51 genotypes, corresponding to 30.18% of the genotypes evaluated. In this group, the lines and parents showed similarity for insertion of the first pod, cycle, mass of 100 grains, grain yield, and concentrations of potassium, phosphorus, and zinc in grains.

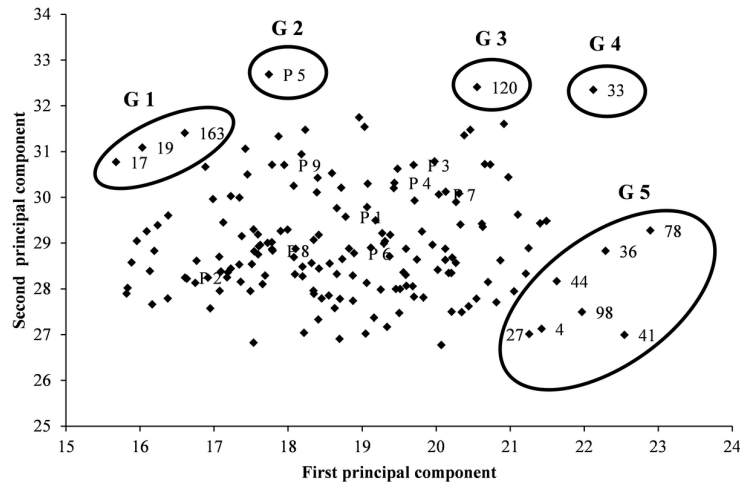


Figure 1. Dispersion graph obtained from the agronomic and biofortification descriptors of 169 common bean genotypes for the first two principal components. P: parent; P1: TPS Nobre; P2: TPS Bonito; P3: IAPAR 31; P4: BRS Expedito; P5: Pérola; P6: Guapo Brilhante; P7: BRS Valente; P8: Diamante Negro; P9: IAPAR 44.

Table 4. Clustering analysis formed by the Tocher optimization method, based on the Mahalanobis generalized distance, for agronomic and biofortification descriptors of 169 common bean genotypes.

Group	Genotype number	Genotypes
I	51	18, 28, 20, 110, 50, TPS Nobre*, 158, IAPAR 44*, 160, 64, 55, 113, 123, 26, 85, 164, 169, 6, 21, 142, 125, 52, 8, 102, 151, 159, 130, 61, 128, 166, 149, 126, 10, 121, 131, 16, 99, 141, 9, 106, 153, 88, 92, 93, 48, 74, 109, 80, 76, 168, and 73
II	14	BRS Valente*, Diamante Negro*, 62, 155, 14, 35, 51, BRS Expedito*, 2, 31, 147, 7, 34, and 57
III	15	22, 79, 157, 53, 75, 116, 117, 101, 136, 13, 114, TPS Bonito*, 46, 119, and 54
IV	16	89, 112, 154, 77, 108, 150, 84, 144, 145, 70, 32, 129, 87, Guapo Brilhante*, 25, and 59
V	7	65, 140, IAPAR 31, 163, 23, 86, and 127
VI	5	42, 66, 103, 111, and 107
VII	8	115, 167, 44, 27, 4, 60, 39, and 56
VIII	9	1, 3, 12, 36, 30, 72, 98, 96, and 91
IX	4	94, 135, 152, and 40
X	3	11, 63, and 24
XI	4	81, 133, 15, and 37
XII	3	47, 146, and 69
XIII	5	132, 148, 105, 67, and 49
XIV	2	122 and 124
XV	2	90 and 100
XVI	2	5 and 19
XVII	2	82 and 97
XVIII	2	156 and 165
XIX	1	120
XX	1	33
XXI	1	95
XXII	1	143
XXIII	1	162
XXIV	1	137
XXV	1	17
XXVI	1	Pérola*
XXVII	1	68
XXVIII	1	138
XXIX	1	78
XXX	1	38
XXXI	1	58
XXXII	1	139
XXXIII	1	41

*Parent.

However, based on the crosses performed, groups of genotypes with striking differences for some traits were obtained. In the groups XXIII and XXV, the lines of highest grain yield were identified, 162 (2713.4 kg/ha) and 17 (3040.8 kg/ha), outperforming the other lines and cultivars evaluated (Tables 4 and 5). In the groups XII, XVI, and XXII, the lines 47, 146, 69, 5, 19, and 143 were identified as having a mass of 100 grains equal or superior to 25 g, which was not significantly different from the Pérola cultivar (group XXVI).

Table 5. Mean*, minimum (Min), and maximum (Max) values obtained in each group formed by the Tocher optimization method for insertion of the first pod (IFP), cycle, mass of 100 grains (M100G), grain yield (Yield), and concentrations of potassium (K), phosphorus (P), and zinc (Zn) in grains of 169 common bean genotypes evaluated in the rainy and dry seasons.

Group	IFP (cm)		Cycle (days)		M100G (g)		Yield (kg/ha)		K (g/kg) (dry matter)		P (mg/kg) (dry matter)		Zn (mg/kg) (dry matter)	
I	16.8	c	92.1	b	20.1	b	1790.2	c	16.6	a	4.9	a	27.1	a
II	18.1	b	95.5	b	22.7	a	1786.6	c	17.8	a	5.0	a	27.1	a
III	14.3	c	90.7	b	22.6	a	2054.5	b	16.1	a	4.6	a	24.3	a
IV	15.3	c	93.2	b	20.1	b	2328.2	b	15.9	a	4.8	a	27.5	a
V	19.6	b	96.0	b	24.7	a	2038.6	b	16.3	a	4.8	a	25.9	a
VI	14.0	c	90.1	b	20.1	b	1580.7	c	16.0	a	5.1	a	28.8	a
VII	15.4	c	90.8	b	18.0	c	1466.6	c	18.1	a	5.1	a	28.1	a
VIII	18.5	b	90.5	b	17.3	c	1466.5	c	17.2	a	5.3	a	30.7	a
IX	13.2	c	94.9	b	19.0	b	1734.2	c	17.4	a	4.9	a	25.7	a
X	13.8	c	90.9	b	21.7	a	1381.2	c	16.5	a	4.2	a	29.4	a
XI	20.3	a	94.1	b	20.0	b	1987.7	b	16.6	a	4.7	a	29.1	a
XII	16.4	c	89.3	b	25.0	a	2436.7	b	17.1	a	4.6	a	25.8	a
XIII	16.6	c	101.4	a	19.5	b	1665.3	c	16.9	a	5.1	a	29.1	a
XIV	14.2	c	89.5	b	24.0	a	1752.3	c	16.8	a	4.7	a	20.3	a
XV	16.2	c	89.8	b	17.3	c	1565.2	c	15.4	a	5.4	a	24.1	a
XVI	17.9	b	93.8	b	27.3	a	2134.4	b	16.6	a	4.1	a	26.5	a
XVII	18.9	b	98.6	a	24.2	a	1967.1	b	15.6	a	5.4	a	28.8	a
XVIII	18.0	b	104.1	a	20.9	b	2053.5	b	18.0	a	5.0	a	27.4	a
XIX	22.6	a	103.3	a	21.3	a	1860.7	c	17.7	a	5.2	a	26.6	a
XX	19.6	b	103.0	a	23.3	a	1695.2	c	19.6	a	5.4	a	32.7	a
XXI	17.6	b	105.3	a	19.3	b	2335.5	b	16.9	a	5.1	a	34.6	a
XXII	17.2	c	100.0	a	25.2	a	1834.9	c	18.0	a	5.6	a	29.4	a
XXIII	16.3	c	94.5	b	20.0	b	2713.4	a	18.1	a	4.6	a	21.2	a
XXIV	17.6	b	97.3	a	17.4	c	2048.5	b	17.0	a	5.6	a	35.5	a
XXV	15.7	c	95.3	b	24.0	a	3040.8	a	16.7	a	4.2	a	25.3	a
XXVI	22.1	a	98.3	a	27.2	a	2317.9	b	16.3	a	4.7	a	28.0	a
XXVII	15.7	c	90.8	b	17.9	c	1874.1	c	16.7	a	4.4	a	29.6	a
XXVIII	21.2	a	94.5	b	22.9	a	1252.1	c	16.4	a	5.0	a	30.2	a
XXIX	21.0	a	96.0	b	16.0	c	1224.9	c	18.0	a	5.9	a	28.0	a
XXX	15.7	c	91.3	b	22.0	a	1773.6	c	16.7	a	4.9	a	36.0	a
XXXI	11.7	c	93.8	b	22.4	a	2067.3	b	15.5	a	4.8	a	29.6	a
XXXII	16.5	c	87.8	b	19.6	b	2371.5	b	15.7	a	4.3	a	20.5	a
XXXIII	17.4	b	89.5	b	13.5	c	1509.5	c	17.6	a	5.9	a	32.6	a
Min.	11.7		87.8		13.5		1224.9		15.4		4.1		20.3	
Max.	22.6		105.3		27.3		3040.8		19.6		5.9		36.0	

*Means not followed by the same letter in the column differ by the Scott-Knott test ($P = 0.05$).

Plant architecture was characterized by the insertion of the first pod. Lines of groups XI, XIX, XXVIII, and XXIX showed the highest insertion of the first pod, not significantly different from the Pérola cultivar (group XXVI). The insertion of the first pod of line 58 (group XXXI) is 11.7 cm. In all the other lines evaluated, insertion of the first pod was over 12 cm. Regarding the plant's cycle, the application of the Scott-Knott test enabled the separation of groups formed by the Tocher method into normal cycle (letter *b*) and late cycle (letter *a*).

Mineral concentrations in grains varied from 15.4 to 19.6 g/kg dry matter (DM) for potassium, from 4.1 to 5.9 g/kg DM for phosphorus, and from 20.3 to 36.0 mg/kg DM for zinc in the groups formed by the Tocher method (Table 5). However, although there was a

significant difference detected by the F-test for genotypes regarding the concentrations of potassium, phosphorus, and zinc (Table 2), the application of the Scott-Knott test did not result in stratification between the groups, at the same significance level.

All groups formed by the Tocher method presented potassium concentration higher than 12.0 g/kg DM, characterizing high potassium concentration in grains. Groups II, VI, VII, VIII, XIII, XV, XVII, XVIII, XIX, XX, XXI, XXII, XXIV, XXVIII, XXIX, and XXXIII showed high phosphorus concentration in grains (>5.0 g/kg DM). These groups were constituted by 55 common bean genotypes. The groups with high zinc concentration in grains, i.e., superior to 31 mg/kg DM were: groups XX, XXI, XXIV, XXX, and XXXIII. Five common bean lines with high zinc concentration in grains were obtained, namely lines 33, 95, 137, 38, and 41. Therefore, common bean cultivars biofortified for potassium, phosphorus, and zinc can be obtained for breeding programs.

DISCUSSION

The simple lattice design is used to evaluate experiments with many treatments, in which the effectiveness of local control can be reduced if complete blocks were used. However, its efficiency, compared to a randomized block design, can be tested to define which statistical analysis will be carried out. The efficiency of the simple lattice design was above 100% for the most traits evaluated, i.e., a low efficiency was observed. In this case, analysis of variance can be made in a randomized block design, using the adjusted mean squares of treatments and the effective error of the lattice (Jost et al., 2013).

A significant genotype x growing season interaction for all traits evaluated was observed, except for the concentrations of potassium, phosphorus, and copper in grains. Therefore, the agronomic performance of the common bean genotypes was not the same for both growing seasons, corroborating with previous studies reported by Cabral et al. (2011), Moura et al. (2013), Ribeiro et al. (2014), and Mambrin et al. (2015). Preliminary studies also found a significant genotype x environment interaction for zinc concentration in grains of common bean genotypes (Silva et al., 2012; Hossain et al., 2013).

We found genetic variability for all traits, except for copper concentration in grains. This allows the selection of superior common bean lines for agronomic and biofortification traits. Besides, multicollinearity diagnosis revealed a condition number of 6.83, indicating weak collinearity, according to the classification proposed by Montgomery and Peck (1981). Therefore, the use of agronomic and biofortification traits that presented a significant mean square for genotypes and/or a significant genotype x environment interaction is an appropriate procedure for cluster analysis.

The highest proportion of the total variation (66.515%) was explained by the first three principal components. This value was higher than those found in previous studies, where morphological and/or phenological and/or production descriptors (Chiorato et al., 2006; Hegay et al., 2014; Sofi et al., 2014) were used to differentiate common bean genotypes by principal components. The number of descriptors, the discriminative capacity of descriptors, and the genetic diversity of the germplasm assessed can justify the varying percentages of variability detected by principal components in these studies.

The concentrations of phosphorus, potassium, and zinc were the descriptors that most contributed to the differentiation of the genotypes evaluated. However, Pereira et al. (2011) found that phosphorus concentration was the third trait that most contributed to the genetic

dissimilarity among 34 common bean genotypes, using only nutritional quality descriptors.

The cluster analysis by principal components separated the common bean genotypes into six groups. There is a genetic dissimilarity among the groups. The group 6 concentrated 156 genotypes, corresponding to 92.30% of the genotypes evaluated. Therefore, in this group, the common bean genotypes showed high genetic similarity for the agronomic and mineral biofortification traits. The use of graphic dispersion by principal components based on the morphological and/or phenological and/or production traits has been applied to differentiate common bean genotypes efficiently (Freitas et al., 2011; Blair et al., 2013; Grahic et al., 2013; Hegay et al., 2014). However, this method enables the formation of few groups, and one of these groups usually concentrates the highest amount of germplasm evaluated (Freitas et al., 2011; Grahic et al., 2013). However, the groups formed using morphological traits are very similar to those obtained by microsatellite molecular markers, when the graphic dispersion by principal components is used in beans (Blair et al., 2013; Hegay et al., 2014).

The Tocher clustering analysis allowed greater discrimination of genotypes than the principal component analysis. The common bean genotypes were separated into 33 groups by the Tocher optimization method. Similarly, the use of the Tocher method allowed the formation of a considerable number of groups based on similarities (within the group) and differences (among the groups) regarding the morphological, phenological, and production traits of common bean genotypes (Bonett et al., 2006; Elias et al., 2007; Barelli et al., 2009).

Group I concentrated 30.18% of the genotypes evaluated. Earlier studies also mentioned that a large number of genotypes with similarity for agronomic and/or seed descriptors were grouped into a single group, when clustering analysis was carried out by the Tocher optimization method (Bonett et al., 2006; Elias et al., 2007; Barelli et al., 2009; Gonçalves et al., 2014). Veloso et al. (2015), using microsatellite markers associated with quantitative trait loci for agronomic traits, verified that some parents in common were used in crossings to obtain new common bean cultivars in use in Brazil. This resulted in a great genetic similarity among the Mesoamerican common bean genotypes being cultivated in Brazil.

Groups of genotypes with striking differences for some traits were obtained by the Tocher optimization method. The groups XII (lines 47, 146, and 69), XVI (lines 5 and 19), XXII (line 143), and XXVI (Pérola) presented genotypes with a mass of 100 grains from 25.0 to 27.2 g. These genotypes presented medium-sized grains, according to classification presented by Blair et al. (2010). Therefore, the selection of these genotypes is recommended for breeding programs because medium-sized Carioca common beans (25 to 30 g) are better accepted by Brazilian consumers (Carbonell et al., 2010).

Insertion of the first pod higher than 12 cm has been associated with bean plants that have an erect architecture (Melo, 2009). In this study, all lines evaluated presented insertion of the first pod over 12 cm, except the line 58 (group XXXI).

The genotypes evaluated presented normal or late cycles, according to the classification described by Del Peloso et al. (2009). In the present study, the cycle was obtained using the mean data of two growing seasons. In the dry season, the lowest minimum and maximum mean air temperature values were recorded during the pod-filling to maturity stages. In this condition, the thermal sum that is required to reach maturity takes more time to be accumulated in common bean genotypes. Therefore, the cycle is longer in the dry season, which contributes to an increase in the average number of days to maturity for all genotypes under study.

However, when the Tocher cluster analysis was made, using data for each environment, the genotype groups formed were different for each growth environment

(Gonçalves Ceolin et al., 2007; Coelho et al., 2010). This occurs because there is a significant genotype x environment interaction for agronomic traits and, consequently, the genotypes performance differs. For this reason, Cargnelutti Filho et al. (2009) recommended the use of mean data obtained in six experiments of common bean to perform clustering analysis by the Tocher method, so that more reliable inferences can be made from the groups formed when morphological, phenological, and production descriptors are used. However, using the mean data of six experiments to evaluate duplicate accessions, select superior genotypes, and planning crossings based on dissimilarity among groups is unviable in the routine of common bean breeding program. In this regard, the use of mean data obtained in two different growing seasons or 2 years of cultivation, in the same location, represents the cultivation environmental variability and provides more robust information. For this reason, it was implemented in this study as well as by Bonett et al. (2006), Freitas et al. (2011), and Sofi et al. (2014).

Potassium concentration in grains superior to 12.0 g/kg DM was observed in the 33 groups formed by the Tocher method. These potassium values are high for common bean genotypes, according to Maziero et al. (2015). Potassium controls the high blood pressure and cardiovascular diseases (He and MacGregor, 2008). Thus, the development of common bean cultivars biofortified for potassium is important to prevent cardiovascular diseases that can be observed when there is a low potassium intake in the diet.

High phosphorus concentration in grains (>5.0 g/kg DM) was obtained in 55 common bean genotypes. Phosphorus deficiency causes painful bone, anxiety, fatigue, and skin sensitivity (Martínez-Ballesta et al., 2010). These symptoms should be reduced by using common bean cultivars biofortified for phosphorus in the diet.

The lines 33, 95, 137, 38, and 41 presented zinc concentration in grains over 31 mg/kg DM. According to Tryphone and Nchimbi-Msolla (2010), this value represents high zinc concentration in common bean grains. Therefore, these five common bean lines were selected by the breeding program. Common bean lines biofortified for zinc may contribute to the improvement of zinc deficiency-related symptoms, such as changes in the immune system, dermatitis, and decreased taste sensitivity (Gibson, 2012).

The Tocher and principal component clustering methods were partially in agreement regarding the formation of common bean genotype groups based on agronomic and biofortification traits. Crossing between the common bean lines 17 and 162 with lines of groups XX, XXI, XXIV, and XXXIII are recommended to obtain recombinants with high grain yield and biofortified for potassium, phosphorus, and zinc. In the present study, it is expected that the recombination makes it possible to obtain common bean inbred lines with high agronomic performance and biofortified for minerals.

Conflicts of interest

The authors declare no conflict of interest.

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