Number of experiments necessary to more accurately differentiate common bean genotypes for grain physical traits and minerals in cluster analysis[1]

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ABSTRACT

The number of experiments that provides greater detail in the differentiation of common bean genotypes for grain physical traits and minerals in cluster analysis is not known. This study was undertaken to determine the number of experiments necessary to more accurately differentiate common bean genotypes for grain physical traits and minerals in cluster analyses. Seven traits of grain physical quality and the concentration of six minerals were evaluated in 17 common bean genotypes with carioca (9) and black (8) grains. Statistical analyses were performed in data obtained from one, two, three and four experiments. A significant genotype × experiment interaction occurred for all traits, except for the potassium concentration. Tocher's and the unweighted pair group method with arithmetic mean (UPGMA) cluster analyses were efficient in differentiating common bean genotypes by grain type when the data obtained from one experiment were considered. However, the use of data obtained from four experiments made it possible to recognize differences regarding grain lightness and brightness as well as the other traits. Four experiments are need for the Tocher's and the UPGMA cluster analyses to more accurately differentiate carioca and black bean genotypes for grain physical traits and minerals.

Keywords: *Phaseolus vulgaris*; genotype × experiment interaction; clustering methods; Mahalanobis' generalized distance.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has an important roles in food security, being a source of carbohydrates, proteins and minerals in the human diet (Câmara *et al.*, 2013). Because common bean grains have a great diversity of colors and sizes, their consumption has become popular among consumers from several countries who accept different grain types in their diet. However, 90% of the common bean produced in Brazil is restricted to two grain types: carioca (beige seed coat with brown streaks) and black (Lemos *et al.*, 2015). These grain types are highly appreciated by consumers and, for this reason, breeding programs have put greater efforts into the development of carioca and black bean cultivars with high grain physical quality and mineral concentration. This is because characteristics such as color, size, cooking time and nutritional value of beans are analyzed by consumers who are mindful of its quality.

Carioca and black bean cultivars growing at Brazil show high genetic similarity because in the process of developing of these cultivars were performed crosses between parents of both grain types, which difficulty the separating the genotypes by grain type (Veloso *et al.*, 2015). For this

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reason, cluster analyses are shown to be potential tools to identify promising parents for use in crosses as well as recognize very similar, i.e., duplicate, genotypes. Several clustering methods have been described in the literature (Cruz & Carneiro, 2014), but Tocher's optimization and the UPGMA hierarchical cluster analyses are widely employed in common-bean breeding programs.

Tocher's method is widely used in differentiating common bean genotypes regarding agronomic traits evaluated in one experiment (Lima *et al.*, 2012; Gonçalves *et al.*, 2016; Santos *et al.*, 2019). However, when data of agronomic traits obtained individually in two (Kumar *et al.*, 2009; Coelho *et al.*, 2010) or four (Ceolin *et al.*, 2007) experiments were used, there was variation in the number of groups and in the composition of the groups formed by Tocher's method. Most agronomic traits determined in common bean genotypes showed a significant genotype × experiment interaction effect, therefore the use of data obtained from six experiments were suggested by Carg nelutti Filho *et al.* (2009) to identify divergent common bean cultivars by Tocher's method. For common bean genotypes no previous work was found with the definition of the necessary number of experiments to be recommended in Tocher's cluster analysis for traits of grain physical quality and minerals.

The UPGMA method has been efficient in differentiating common bean genotypes based on different morphological and/or agronomic traits evaluated in a single experiment (Kloster *et al.*, 2011; Grahic *et al.*, 2013; Bertoldo *et al.*, 2014; Hegay *et al.*, 2014; Veloso *et al.*, 2015; Gonçalves *et al.*, 2016; Canci *et al.*, 2019; Santos *et al.*, 2019; Long *et al.*, 2020) or on the average of two (Guidoti *et al.*, 2018; Arteaga *et al.*, 2019; Savic *et al.*, 2019) or three (Cabral *et al.*, 2011) experiments. None of these studies described the criterion used to define the number of experiments employed in the UPGMA cluster analysis. For agronomic and grain physical quality traits and minerals, no recommendations were found in the literature as to the necessary number of experiments to be conducted to achieve greater accuracy in differentiating common bean genotypes using the UPGMA method.

Defining the number of experiments that provides greater detail in the differentiation of common bean genotypes based on traits of grain physical quality and minerals in cluster analyses will allow greater efficiency in identifying promising genotypes for use in controlled crosses. This information is unprecedented and constitutes an important innovation for common-bean breeding programs. Therefore, this study was conducted to determine the number of experiments necessary to more accurately differentiate common bean genotypes for grain physical traits and minerals in cluster analyses.

MATERIAL AND METHODS

Description of the experiments

Four experiments were established in two consecutive years - 2016 and 2017 - and in the two seasons recommended for the cultivation of common bean in the southern region of Brazil: rainy and dry, which correspond to sowing carried out in the months of October and February, respectively. The experimental area is located on the Universidade Federal de Santa Maria (UFSM), in Santa Maria - RS, Brazil (29º42´S latitude, 53º49´W longitude and 95 m altitude). The region is characterized by its humid subtropical climate, with hot summers and no clearly defined dry season.

A randomized block design with three replicates was used in all experiments. Each experimental plot consisted of 4-m four rows, spaced 0.5 m apart and a usable area of 4 m^2. A total of 17 common bean genotypes (lines and cultivars) were evaluated: SM 0312, BRS MG Uai, CNFC 15 097, LEC 02-16, GEN 45-2F-293P, LP 09-33, LEC 01-16, Pérola, Carioca, IAC Netuno, LP 11-117, TB 02-19, CHP 04-239-52, CHP 01-182-48, TB 03-11, BRS Valente and Guapo Brilhante. All genotypes have grains of the Mesoamerican gene pool and were developed by different public research institutions that release new carioca and black bean cultivars for cultivation in the southern region of Brazil.

The soil in the experimental area is a typic alitic Argisol, Hapludalf, which was prepared by the conventional cultivation system. All experiments were conducted observing the minimum requirements for determining the Value for Cultivation and Use (VCU) of common bean cultivars (Brasil, 2006). The management practices were implemented following the guidelines of the technical recommendations for the cultivation of common bean in the southern region of Brazil (CTSBF, 2012).

Evaluation of grain physical quality and mineral concentration

The physical quality of the common bean grains was analyzed based on seven traits, namely, L*, a*, b*, absorption, normal grains, cooking time and mass of 100 grains. The values of L*, a* and b* characterized grain color and were evaluated using a portable colorimeter. The L* parameter measures the variation from black (0) to white (100); a* quantifies the intensity of the colors from green (-60) to red (+60); and b* indicates the color spectrum between blue (-60) and yellow (+60).

The traits of absorption, normal grains and cooking time were determined in a sample of 25 grains after soaking in 50 mL of distilled water for 8 h, at room temperature (20 ± 2 ºC). Absorption was obtained by the difference in weight of the grains after and before soaking relative to the initial weight of the grains, and expressed in %. Normal grains differed from hard grains in their ability to absorb water, and were expressed in %. Cooking time was evaluated using a Mattson cooker, by following the methodology described in Ribeiro *et al.* (2021). Mass of 100 grains was quantified in three random 100-grain samples, whose moisture was standardized to 13%, and expressed in g.

The concentration of six minerals (potassium, phosphorus, calcium, magnesium, iron and copper) was analyzed according to the methodology described by Miyazawa *et al.* (2009). Reading of these minerals were carried out in an atomic absorption spectrophotometer, except for potassium, which was obtained on a flame photometer, and phosphorus, which was quantified using an optical emission spectrophotometer.

Statistical analyses

The obtained data were subjected to analysis of variance for the following number of experiments: one: experiment I (2016 rainy season crop); two: experiments I and II (2016 rainy and 2017 dry season crops); three: experiments I, II and III (2016 rainy, 2017 dry and 2017 rainy season crops); and four: experiments I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops). In combined analyses of variance, all effects were considered random, except for the genotype effect, which was analyzed as fixed. The homogeneity of residual variances was analyzed by Hartley's maximum F-test. The degrees of freedom of the error and of the genotype × experiment interaction were adjusted for traits with heterogeneous residual variances (Cruz, 2016).

Multicollinearity diagnostics was based in the combined analysis of variance of four experiments, using the phenotypic correlation matrix for the 13 traits evaluated. The collinearity classes were evaluated based on the criteria proposed by Montgomery *et al.* (2012).

The cluster analyses were implemented for data obtained from one, two, three and four experiments. For this, the residual variance and covariance matrices obtained in the analyses of variance of these experiments were used to generate matrices of genetic dissimilarity using Mahalanobis' generalized distance with standardized means (D^2). The traits that most contributed to genetic divergence were identified in the Mahalanobis' generalized distance analysis.

Cluster analyses were performed using two methods: Tocher and 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 measure the consistency of the clustering pattern. All statistical analyses were carried out using Genes software (Cruz, 2016).

RESULTS AND DISCUSSION

Individual and combined analyses of variance

The genotype effect was significant for 57.69% of the traits evaluated in the different analyses of variance (Table 1), which shows that the common bean genotypes differed for most traits of grain physical quality and minerals. Previous works also found expressive variation for the traits of grain physical quality (Rivera *et al.*, 2016; Herrera-Hernández *et al.*, 2018; Dias *et al.*, 2021) and mineral concentration (McClean *et al.*, 2017; Steckling *et al.*, 2017; Yeken *et al.*, 2019; Delfini *et al.*, 2020; Jan *et al.*, 2021) in common bean genotypes. The existence of genetic variability allows the execution of cluster analyses. Results obtained in these analyses help breeders identify promising parents for use in controlled crosses and recognize genotypes with very similar traits of grain physical quality and minerals, which characterizes a duplication of accessions.

Table 1: Results of the F test of analysis of variance for the traits of L*, a*, b*, absorption (abs., %), normal grains (normal, %), cooking time (time, min:s), mass of 100 grains (mass, g) and concentrations of potassium (K, g kg^{-1} dry matter - DM), phosphorus (P, g kg^{-1} DM), calcium (Ca, g kg^{-1} DM), magnesium (Mg, g kg^{-1} DM), iron (Fe, mg kg^{-1} DM) and copper (Cu, mg kg^{-1} DM) obtained in 17 common bean genotypes evaluated in experiments I (2016 rainy season crop), I and II (2016 rainy and 2017 dry season crops), I, II and III (2016 rainy, 2017 dry and 2017 rainy season crops) and I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops)

Experiment	**L***	**a***	**b***	**Abs.**	**Normal**	**Time**	**Mass**	**K**	**P**	**Ca**	**Mg**	**Fe**	**Cu**
Genotype (G)													
I	467.51*	199.87*	679.80*	27.47*	17.93*	5.07*	2.86*	1.35^{ns}	1.86^{ns}	1.95*	2.36*	1.56^{ns}	6.37*
I and II	210.09*	18.09*	252.75*	1.79^{ns}	4.91*	0.60^{ns}	3.08*	1.57^{ns}	0.67^{ns}	1.93^{ns}	1.22^{ns}	1.21^{ns}	2.87*
I, II and III	392.85*	39.33*	373.71*	2.22*	4.11*	0.69^{ns}	4.19*	2.52*	1.01^{ns}	1.76^{ns}	1.12^{ns}	0.94^{ns}	1.03^{ns}
I, II, III and IV	540.38*	48.60*	207.27*	2.25*	2.45*	0.61^{ns}	5.86*	1.76^{ns}	1.56^{ns}	1.99*	0.90^{ns}	0.86^{ns}	1.65^{ns}
Experiment (E)													
I and II	63.77*	26.69*	222.55*	0.04^{ns}	9.30*	207.87*	0.15^{ns}	0.95^{ns}	11.00*	0.16^{ns}	10.37*	6.15^{ns}	24.71*
I, II and III	9.84*	3.54^{ns}	4.28^{ns}	20.81*	7.05*	18.71*	2.91^{ns}	8.86*	3.00^{ns}	51.13*	567.95*	24.24*	833.18*
I, II, III and IV	9.51*	8.54*	11.18*	77.77*	20.76*	30.26*	52.63*	8.27*	2.63^{ns}	102.45*	762.91*	72.91*	521.55*
G x E													
I and II	6.72*	16.69*	4.85*	1.62*	9.31*	8.94*	3.58*	1.07^{ns}	1.28^{ns}	1.17^{ns}	1.81*	1.81*	2.56*
I, II and III	3.92*	6.00*	2.88*	7.67*	9.02*	4.98*	3.23*	0.76^{ns}	2.11*	1.77*	1.80*	1.82*	4.70*
I, II, III and IV	4.27*	5.90*	4.84*	7.45*	10.70*	5.07*	3.16*	1.38^{ns}	1.71*	2.43*	2.02*	1.91*	3.83*

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

However, a significant genotype × experiment (environment) interaction effect for 34 of the 39 tested combinations (13 traits × 3 combined analyses of variance) was obtained. Therefore, the common bean genotypes exhibited variation for all traits of grain physical quality and five minerals when cultivated in different environment, confirming previous results described by Steckling *et al.* (2017), Ribeiro & Kläsener (2020), Dias *et al.* (2021) and Ribeiro *et al.* (2021). In this case, the formation of groups of common bean genotypes based in genetic dissimilarity will be specific for each environment and the selection of common bean parents for use in controlled crosses will be different for each environment. Thus, the environmental variability between growing years and seasons is very important in cluster analyses in the evaluation of the genetic dissimilarity of the common bean germplasm.

With regard to the potassium concentration, the F test was significant for the genotype effect only in the combined analysis variance of three experiments (I, II and III). For this reason, potassium concentration was excluded of the cluster analyses. However, multicollinearity diagnostics showed a condition number (CN) equal to 5,698.14, indicating severe collinearity, by the classes proposed by Montgomery *et al.* (2012). Thus, highly correlated traits and with a greater weight in the last eigenvectors had to be discarded before cluster analyses were performed. The exclusion of the traits of b*, a* and normal grains resulted in a CN = 16.82, that is, weak multicollinearity. Cruz & Carneiro (2014) recommended deletion the multicollinear variables to prevent that these variables implicitly receiving greater weights in the cluster analyses, which allows the proper interpretation of the results obtained in the analyses.

Tocher's cluster analysis

The L* value was the trait that most contributed to the differentiation between common bean genotypes, according to the results obtained from Mahalanobis' generalized distance (Table 2). The percentage contribution of the L* value to the discrimination of genotypes ranged from 90.11% (experiment I) to 96.78% (experiments I, II, III and IV), i.e., this trait had a major participation in the formation of the different groups in cluster analyses. The visual assessment of grain color was also efficient to distinguish different groups of common bean landraces evaluated in Spain (Arteaga *et al.*, 2019) and in Turkey (Canci *et al.*, 2019). However, no previous studies were found including the L* value in cluster analyses of common bean genotypes. The present study is the first record that grain color, as quantified by the L* value, is an efficient descriptor to differentiate carioca and black bean genotypes, suggesting great potential for use in cluster analyses.

Table 2: Relative contribution (S.j) of the traits of L* value, absorption (%), cooking time (time, min:s), mass of 100 grains (mass, g) and concentrations of phosphorus (P, g kg^{-1} of dry matter - DM), calcium (Ca, g kg^{-1} DM), magnesium (Mg, g kg^{-1} DM), iron (Fe, mg kg^{-1} DM) and copper (Cu, mg kg^{-1} DM), obtained from Mahalanobis' generalized distance, in 17 common bean genotypes evaluated in experiments I (2016 rainy season crop), I and II (2016 rainy and 2017 dry season crops), I, II and III (2016 rainy, 2017 dry and 2017 rainy season crops) and I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops)

Trait	**Experiment I**		**Experiments I and II**		**Experiments I, II and III**		**Experiments I, II, III and IV**	
	S.j	**Value,%**	**S.j**	**Value,%**	**S.j**	**Value,%**	**S.j**	**Value,%**
L*	52,691.41	90.11	82,245.65	95.96	54,560.66	95.81	56,294.54	96.78
Absorption	2,613.27	4.47	967.58	1.13	1,202.29	2.11	748.77	1.29
Time	391.95	0.67	325.08	0.38	70.70	0.12	62.32	0.11
Mass	458.52	0.78	603.89	0.70	468.92	0.82	456.98	0.78
P	136.32	0.23	100.69	0.12	70.70	0.12	64.62	0.11
Ca	438.12	0.75	105.06	0.12	-13.07	-0.02	75.77	0.13
Mg	81.43	0.14	93.31	0.11	64.37	0.11	34.94	0.06
Fe	149.73	0.26	41.80	0.05	71.81	0.13	52.20	0.09
Cu	1,512.08	2.59	1,226.19	1.43	451.39	0.79	378.43	0.65

The number of groups formed and the genotypes belonging to each clustering generated by Tocher's method were different if we consider data obtained from one, two, three or four experiments (Table 3). Similar results were described for agronomic traits evaluated in two consecutive years (Kumar *et al.*, 2009; Coelho *et al.*, 2010) and in four experiments (Ceolin *et al.*, 2007), and for nine nutritional traits analyzed in two years (Pereira *et al.*, 2011). In all these papers were described differences regarding the clustering pattern obtained by Tocher's method when were used data of individually experiments. The most evaluated traits in the present study showed a significant genotype × experiment interaction effect (Table 1). Therefore, the formation of groups of common bean genotypes obtained by Tocher's method will be specific for each environment for traits of grain physical quality and minerals. The use of data obtained from individual experiments result in low coincidence in the stratification of groups of common bean genotypes based on genetic dissimilarity.

The use of data from one experiment resulted in the formation of three groups by Tocher's method (Table 3). Group 1 comprised the nine genotypes of carioca beans. The black bean genotypes were clustered into groups 2 (opaque grains) and 3 (grains of intermediate brightness and bright). None previous study was found of genetic dissimilarity analysis for traits of grain physical quality and minerals evaluated in common bean genotypes using Tocher's method. For agronomic traits, the Tocher's method was applied with efficient to differentiate common bean genotypes based on data obtained from a single experiment (Lima *et al.*, 2012; Veloso *et al.*, 2015; Gonçalves *et al.*, 2016; Santos *et al.*, 2019). In the present study, Tocher's method allowed differentiate the genotypes of carioca and black beans, based on analysis performed with data obtained from a single experiment. Nevertheless, not was efficient for the identification of differences with respect to grain lightness and brightness in common bean genotypes of the same grain type. A similar response was observed when the Tocher's method was applied to data from two or three experiments.

Table 3: Common bean genotypes classified in each group obtained by Tocher's optimization method, from Mahalanobis' generalized distance, in experiments I (2016 rainy season crop), I and II (2016 rainy and 2017 dry season crops), I, II and III (2016 rainy, 2017 dry and 2017 rainy season crops) and I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops)

Group	Cluster of genotypes based in number of experiments			
	I	I and II	I, II and III	I, II, III and IV
1	LEC 02-16 LEC 01-16 GEN 45-2F-293P LP 09-33 CNFC 15 097 Carioca Pérola SM 0312 BRS MG Uai	CHP 04-239-52 TB 03-11 BRS Valente CHP 01-182-48 IAC Netuno LP 11-117 TB 02-19	CHP 04-239-52 TB 03-11 CHP 01-182-48 IAC Netuno LP 11-117 BRS Valente	Pérola CNFC 15 097 Carioca BRS MG Uai LP 09-33 LEC 02-16 LEC 01-16 GEN 45-2F-293P
2	LP 11-117 TB 03-11 IAC Netuno CHP 04-239-52 CHP 01-182-48 BRS Valente	LEC 02-16 LP 09-33 LEC 01-16 GEN 45-2F-293P Carioca BRS MG Uai CNFC 15 097 Pérola SM 0312	Pérola CNFC 15 097 Carioca BRS MG Uai SM 0312 LP 09-33 LEC 01-16 LEC 02-16 GEN 45-2F-293P	CHP 01-182-48 TB 03-11 CHP 04-239-52 BRS Valente IAC Netuno LP 11-117
3	TB 02-19 Guapo Brilhante	Guapo Brilhante	TB 02-19	Guapo Brilhante
4			Guapo Brilhante	TB 02-19
5				SM 0312

Tocher's clustering has also been performed based on the average data from two (Maziero *et al.*, 2017) or several experiments (Pereira *et al.*, 2019) for agronomic traits. Cargnelutti Filho *et al.* (2009) suggested the use of data of six experiments so that common bean genotypes can be more accurately differentiated in the Tocher's method based on agronomic traits. For traits of grain physical quality and minerals, no definition was found on the minimum number of experiments to achieve greater accuracy in the differentiation of common bean genotypes in the Tocher's cluster analysis.

In the present study, five groups were formed when Tocher's method was analyzed using the data obtained from four experiments. Group 1 contained the carioca bean genotypes with the highest grain lightness (L* = 56.20) and the highest absorption (94.67%), namely, Pérola, CNFC 15 097, Carioca, BRS MG Uai, LP 09-33, LEC 02-16, LEC 01-16 and GEN 45-2F-293P (Tables 3 and 4). Group 2 included the black bean genotypes of dark grains with an opaque seed coat (L* = 21.35) and with the highest average calcium concentration (1.60 g kg^{-1} of dry matter - DM), as follows: CHP 01-182-48, TB 03-11, CHP 04-239-42, BRS Valente, IAC Netuno and LP 11-117.

Table 4: Means of the traits of L* value, absorption (%), cooking time (time, min:s), mass of 100 grains (mass, g) and concentrations of phosphorus (P, g kg^{-1} dry matter - DM), calcium (Ca, g kg^{-1} DM), magnesium (Mg, g kg^{-1} DM), iron (Fe, mg kg^{-1} DM) and copper (Cu, mg kg^{-1} DM) obtained in each of the five groups established by Tocher's optimization method, from Mahalanobis' generalized distance, in experiments I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops)

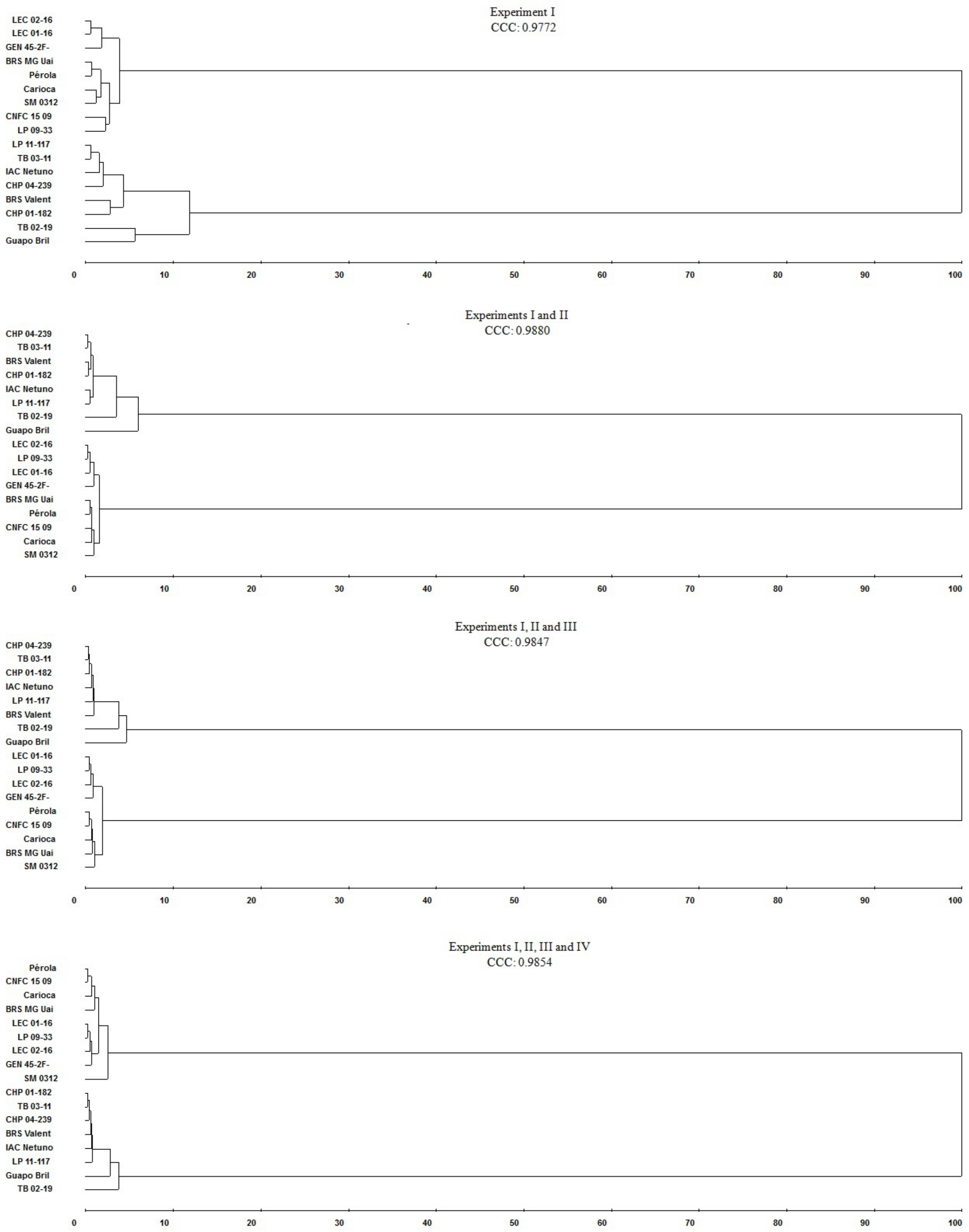
Group	L*	Absorption	Time	Mass	P	Ca	Mg	Fe	Cu
1	56.20	94.67	16:45	26.25	3.09	1.52	2.30	56.98	7.31
2	21.35	89.03	16:46	24.46	3.08	1.60	2.32	58.19	7.72
3	21.17	57.59	17:00	22.05	3.47	1.60	2.27	60.46	7.40
4	22.27	78.49	17:29	32.93	3.08	1.36	2.23	65.81	8.88
5	51.84	86.86	16:14	26.83	3.36	1.39	2.25	61.75	6.99

Groups 3, 4 and 5 were characterized by having only one common bean genotype. Group 3 was composed of cultivar Guapo Brilhante, which has black grains with a bright seed coat (L* = 21.17), the lowest absorption value (57.59%) and the highest average calcium concentration (1.60 g kg^{-1} DM). Group 4 allocated the line TB 02-19, which exhibited lighter black grains (L * = 22.27), the longest cooking time (17 min and 29 s), the largest mass of 100 grains (32.93 g) and the highest concentrations of iron (65.81 mg kg^{-1} DM) and copper (8.88 mg kg^{-1} DM). Group 5 was formed by the line SM 0312, which has carioca grains with black streaks (L* = 51.84) and the shortest cooking time (16 min and 14 s). The common bean genotypes that belonged to groups 3, 4 and 5 may showed restrictions to the sale of grains, since cultivar Guapo Brilhante and the line TB 02-19 exhibited the lowest absorption values and the longest cooking time among the genotypes. Lines TB 02-19 and SM 0312 have L* values that did not meet the established standards of breeding programs for the black (L* ≤ 22; Ribeiro *et al.*, 2003) and carioca (L* ≥ 55; Arns *et al.*, 2018) beans, respectively. The greater grain lightness of the line TB 02-19 and the lower lightness of the line SM 0312 are associated with long storage periods. For this reason, they can be highly rejected by consumers.

The results of data obtained from four experiments enabled the differentiation of common bean genotypes by grain type (carioca and black), based on the L* value, with greater detail in terms of grain lightness and brightness as well as other traits of grain physical quality and minerals. Thus, the use of data obtained from four experiments was more discriminative in the formation of groups by Tocher's method for traits of grain physical quality and minerals evaluated in common bean genotypes.

UPGMA cluster analysis

The CCC ranged from 0.9772 (experiment I) to 0.9880 (experiments I and II) (Figure 1), with significance detected at 1% probability by the *t* test. These values were higher than the CCC obtained in UPGMA cluster analysis, considering agronomic traits evaluated in one (Veloso *et al.*, 2015; Gonçalves *et al.*, 2016), two (Arteaga *et al.*, 2019) and three (Cabral *et al.*, 2011) experiments with different common bean genotypes. The CCC values obtained indicate a high adjustment between the cophenetic matrix and the dissimilarity matrix based on Mahalanobis' generalized distance (Cabral *et al.*, 2011). In the present study, CCC values ≥ 0.9772 are associated with greater reliability in the clustering pattern provided by the UPGMA method for traits of grain physical quality and minerals analyzed in one, two, three and four experiments.


Experiment I
CCC: 0.9772

Experiments I and II
CCC: 0.9880

Experiments I, II and III
CCC: 0.9847

Experiments I, II, III and IV
CCC: 0.9854

Figure 1: Dendrograms and cophenetic correlation coefficient (CCC) of the unweighted pair group method with arithmetic mean (UPGMA), from Mahalanobis' generalized distance, obtained in 17 common bean genotypes evaluated in experiments I (2016 rainy season crop), I and II (2016 rainy and 2017 dry season crops), I, II and III (2016 rainy, 2017 dry and 2017 rainy season crops) and I, II, III and IV (2016 rainy, 2017 dry, 2017 rainy and 2018 dry season crops).

In the four dendrograms obtained by the UPGMA method, two groups were formed: 1 - comprising the carioca bean genotypes; and 2 - containing all black bean genotypes, adopting 90% similarity as a criterion for the definition of the groups. Similarly, common bean genotypes were clustered into different groups according to grain type (Kloster *et al.*, 2011) and to grain color (Canci *et al.*, 2019) by the UPGMA method when were evaluated different morphological descriptors. The UPGMA method has been efficient in differentiating common bean genotypes based on morphological (Kloster *et al.*, 2011; Grahic *et al.*, 2013; Hegay *et al.*, 2014; Canci *et al.*, 2019) and agronomic (Bertoldo *et al.*, 2014; Gonçalves *et al.*, 2016; Santos *et al.*, 2019; Long *et al.*, 2020) traits evaluated in single experiment. However, differentiating common bean genotypes using the UPGMA method based in grain physical quality traits and minerals is unprecedented in common bean.

It was only possible to differentiate the line SM 0312 (black-streak carioca) from the other carioca common bean genotypes in group 1 when data obtained from four experiments were used. For the black common bean genotypes, cultivar Guapo Brilhante (bright grains) and line TB 02-19 (lighter black grains) clearly stood out among the genotypes present in group 2. Therefore, the use of data obtained from four experiments was more discriminative in the stratification of groups by the UPGMA method, similar to the observations with Tocher's method for traits of grain physical quality and minerals. Previous studies have shown that the UPGMA method has also been used to differentiate common bean genotypes based on morphological and/or agronomic traits evaluated in two (Guidoti *et al.*, 2018; Arteaga *et al.*, 2019; Savic *et al.*, 2019) and three (Cabral *et al.*, 2011) experiments. None of these studies defined the number of experiments that should be used in cluster analysis. The number of experiments to be employed in UPGMA cluster analysis to provide a better differentiation between common bean genotypes for traits of grain physical quality and minerals was not found in literature.

In the present study was observed that the most traits of grain physical quality and minerals showed a significant genotype × experiment interaction effect (Table 1). This result shows that the effects of years and seasons in the same growing location need to be considered in cluster analyses. The use of data from four experiments in the Tocher's and the UPGMA cluster analyses always greater efficiency in the differentiation between carioca and black bean genotypes, especially for recognition differences in grain lightness and brightness (L* value) and other traits of grain physical quality and minerals (Table 3 and Figure 1). Thus, the use of four experiments in the Tocher's and the UPGMA cluster analyses is recommended for common-bean breeding programs with an emphasis on traits of grain physical quality and minerals. This strategy provides greater detail about the differences between common bean genotypes, thereby allowing a more accurate identification of promising parents for use in controlled crosses as well as of duplicate accessions.

CONCLUSIONS

Tocher's and the UPGMA cluster analyses are efficient in differentiating common bean genotypes by grain type using data obtained from one experiment.

Four experiments are need for the Tocher's and the UPGMA cluster analyses to more accurately differentiate carioca and black bean genotypes for grain physical traits and minerals.

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