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Multivariate analysis applied to the evaluation of genetic variability for the physiological quality trait of common bean seeds

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ABSTRACT: Knowledge of the genetic variability and of the variables for evaluating common bean lines for the physiological seed quality trait is important for the selection of promising common bean genotypes for this trait in breeding programs. Through multivariate analysis, the objective of this study was to evaluate the efficiency of physical and physiological tests for studies of genetic variability in seeds of common bean lines. Twenty lines of common bean were evaluated for thousand-seed weight, first count, germination, tetrazolium, electrical conductivity, accelerated aging, field emergence and seedling performance. The experimental data were subjected to analysis of variance, analysis of genetic parameters and multivariate analysis of principal components. Genetic variability was observed among the common bean lines, and two groups of lines with superior physiological performance according to the germination and seed vigor tests were identified. First germination count, germination, seedling length in the field, accelerated aging and emergence speed index tests are the most promising for characterizing the physiological potential of seeds of common bean lines.

Index terms: genetic diversity, *Phaseolus vulgaris* L., principal component analysis, vigor tests.

RESUMO: O conhecimento da variabilidade genética e das variáveis para avaliação de linhagens de feijoeiro para a característica de qualidade fisiológica de sementes, é importante para a seleção de genótipos de feijão promissores, para esta característica, em programas de melhoramento. Por meio da análise multivariada, objetivou-se neste estudo, avaliar a eficiência de testes físicos e fisiológicos para estudos de variabilidade genética em sementes de linhagens de feijão comum. Foram avaliadas vinte linhagens de feijão comum quanto à massa de mil sementes, primeira contagem, germinação, tetrazólio, condutividade elétrica, envelhecimento acelerado, emergência em campo e desempenho de plântulas. Os dados experimentais foram submetidos à análise de variância, parâmetros genéticos e a análise multivariada de componentes principais. Verificou-se a existência de variabilidade genética entre as linhagens de feijão e a identificação de dois grupos de linhagens com desempenho fisiológico superior de acordo com os testes de germinação e de vigor de sementes. A primeira contagem de germinação, teste de germinação, comprimento de plântula em campo, envelhecimento acelerado e o índice de velocidade de emergência, são os testes mais promissores para a caracterização do potencial fisiológico das sementes das linhagens de feijão.

Termos para indexação: diversidade genética, *Phaseolus vulgaris* L., análise de componentes principais, vigor de sementes.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) establishment depends on the germination capacity of the seeds used and on the subsequent initial performance of the plants. Thus, the physiological potential of the seeds is the first step for the development of the morphological traits of common bean in the environment (Sun et al., 2007; Borém and Carneiro, 2015).

However, little emphasis has been given to the selection of traits associated with seed quality and performance within genetic improvement, which primarily targets production and adaptation traits. On the other hand, traits related to germination, field emergence and seed vigor have high heritability and variability (Maia et al., 2011; Martins et al., 2016). Knowledge on which traits have greater genetic variability would help breeding programs in selection programs aimed at improving the quality of common bean seeds, which, despite the importance for the establishment of the crop and for obtaining high yields, is a challenge in the common bean production chain.

Knowledge on genetic variability and evaluation of the dissimilarity between lines in breeding programs, which work with a large number of lines/lots, can facilitate the selection and inference about the differentiation of promising common bean genotypes in relation to the physiological quality of seeds. Dissimilarity is identified by multivariate analysis, through which the multiple variables that are important for the crop are quickly and simultaneously analyzed (Cruz and Souza, 2014). In wheat, multivariate analysis allowed detecting the factors that most contribute to the identification of seed vigor associated with environmental factors during production (Bagateli et al., 2022). In soybean, multivariate analysis helped identifying seed vigor attributes for the pre-selection of genotypes tolerant to weathering-induced deterioration in the pre-harvest phase of seeds (Pinheiro et al., 2022).

Among the multivariate methods, principal component analysis is one of the most widely used (Hair et al., 2009). Applying this methodology in seed analysis allows the formation of clusters according to the parameters presented in the germination and vigor tests of seeds, facilitating a more accurate physiological, qualitative and behavioral characterization as well as management of the lines. In this analysis, the effects of contributions from the characteristics are considered jointly and not only individually, being of great value for selecting superior genotypes for high quality of seeds of legume species such as soybean (Barbosa et al., 2013; Martins et al., 2016; Szareski et al., 2018).

In common bean, principal component analysis was efficient in the pre-selection of genotypes with agronomic traits of interest, such as yield, grains per pod and other production components (Araujo and Vivas, 2018). However, the method has not yet been explored in the pre-selection of common bean genotypes aimed at obtaining seeds with high quality.

In this context, the objective of this study was to evaluate the efficiency of germination and vigor tests for seeds of common bean lines, through multivariate analysis, for studies of genetic variability aiming at high seed quality.

MATERIAL AND METHODS

The experiment was carried out at the Seed Technology Laboratory and at the *Fazenda Experimental de Ciências Agrárias* (FAECA) (latitude: 22°13’15”, longitude: 54°48’21”, and altitude: 430 m), both belonging to the *Universidade Federal da Grande Dourados* (UFGD) in the municipality of Dourados, Mato Grosso do Sul, Brazil. Twenty lines of common bean (*Phaseolus vulgaris* L.), from the germplasm bank of the plant breeding and biotechnology program of UFGD, were evaluated. In the 2020 season, seeds at the R9 stage were manually harvested to avoid injury, placed in single-layer paper packaging and stored in an air-conditioned chamber (15 ± 3 °C and RH 45± 5%) during the experimental period. The following tests were used to study the genetic variability, aiming at the high quality of the seeds:

Thousand-seed weight: 100 seeds of eight replications for each line were weighed on a precision scale, and the results were expressed in grams (Brasil, 2009). Moisture content in the seeds was determined in four replications of 4.5 g ± 0.5 g of seeds for each line, by the oven method at 105 °C, for 24 hours, and the results were expressed as percentage (wet basis) (Brasil, 2009).

Germination test: conducted in four replications of 50 seeds in a roll of paper moistened with distilled water in the proportion of 2.5 times the dry paper mass. The rolls were kept at a temperature of 20-30 °C and under constant light. First count was performed at five days after the beginning of the test, and germination percentage was computed at nine days after the beginning of the test (Brasil, 2009). In parallel, daily counts of normal seedlings were performed to calculate the germination speed index (Maguire, 1962).

Electrical conductivity test: conducted in four replications of 50 seeds. Seeds were placed in containers with 75 mL of distilled water, and then the samples were kept in a B.O.D. chamber at 25 °C for 24 hours. After that, readings were performed with a conductivity meter (Tecnal, TEC-4 MP) and the results were expressed in $\mu S.cm^{-1}.g^{-1}$ (ISTA, 2016).

Tetrazolium test: performed to evaluate the viability of the seeds, in two replications of 100 seeds of each line, previously kept between moistened paper for 24 h. Afterwards, the samples were immersed in the 0.1% tetrazolium solution and kept for 3 h at 40 °C. For the evaluation, the seeds were cut longitudinally through the embryo to observe essential structures. The results were expressed as percentage of viable and non-viable seeds (Brasil, 2009).

Accelerated aging test: performed by arranging the seeds in a single layer on a screen coupled to a Gerbox-type plastic box, with 40 mL of water at the bottom. The containers with the seeds were kept for 48 hours at 41 °C. Seed moisture content was determined after this period, and the seeds were subjected to the germination test. At five days after the beginning of the test, the percentage of normal seedlings was recorded (Medeiros et al., 2019).

Seedling emergence in the field: four replications with 50 seeds of each line were sown in four 4-m-long rows, in a *Latossolo Vermelho distroférrico* (Oxisol). The number of seedlings at V1 was recorded daily to calculate the emergence speed index (Maguire, 1962). The number of seedlings emerged until the complete stabilization of the stand was expressed as a percentage.

Seedling performance: it was evaluated by determining seedling length and dry mass in the laboratory and in the field. In the laboratory, the test was conducted in four replications with 20 seeds arranged in the upper third of the germitest paper moistened with distilled water and kept at 20-30 °C. After a period of five days, the total length of normal seedlings (Brasil, 2009) was measured using a millimeter ruler, and the results were expressed in centimeters. Seedling length evaluation was also carried out in the field, in a *Latossolo Vermelho Distroférrico* (Oxisol), with the sowing of 50 seeds of each line in four 4-m-long rows. At 20 days after sowing, the length of seedlings that emerged was determined as the distance between the apical bud and the tip of the root. The results were expressed in centimeters.

After determining seedling length in the laboratory and in the field, the cotyledons were removed and the seedlings were placed in paper bags and kept in an oven with forced air circulation regulated at 60 °C, until reaching constant weight. After drying, seedling dry mass was determined on a precision scale (0.001 g). The results were expressed in grams.

The data were subjected to analysis of variance in a completely randomized design. Analyses of variances and covariances of each variable were used to estimate the following variables:

Environmental variance - $\hat{\sigma}_a^2$ = MSR (residual mean square);

Genotypic variance - $\hat{\sigma}_g^2$ = (MSL-MSR), where MSL (mean square between lines) = $\sigma_e{}^2 + r\sigma_l^2$, where r =number of replications;

Phenotypic variance - $\hat{\sigma}_f^2 = \hat{\sigma}_g^2 + \hat{\sigma}_a^2$;

Broad-sense heritability coefficient - $h_x^2 = \hat{\sigma}_g^2 / \hat{\sigma}_f^2$;

Coefficient of genetic variation - CVg = 100 $\sqrt{\hat{\sigma}_g^2}$ / m, where m = overall mean of the experiment;

Variation index - CVg/CVe ; where CVe = coefficient of experimental variation.

Multivariate analysis was performed using the principal components method to estimate the genetic diversity among the lines, based on seed germination and vigor tests. The analyses were processed using the Rbio statistical software (Bhering, 2017).

RESULTS AND DISCUSSION

Moisture content in the seeds of the lines was, on average, 7.8 ± 0.6%, which characterizes uniformity between the values and constitutes a primary factor for the standardization and reliability of the tests to assess the quality of common bean seeds (Marcos-Filho, 2015).

Significant differences were observed in the physiological quality of the seeds of the evaluated lines through all the tests employed, indicating the existence of genetic variability for this trait (Table 1). Genetic variability in relation to seed quality has also been reported in other studies with common bean (Michels et al., 2014; Barroso et al., 2019) and soybean (Martins et al., 2016), assisting in the process of selecting lines for the production of seeds with high quality.

The average percentages of germination and seedling emergence were 83% and 81%, respectively. Seed germination in the laboratory is one of the most important characteristics for the evaluation of seed quality, as the results are used to characterize genotypes and serve as an approval parameter for seeds suitable for commercialization (Powell, 2022).

The estimated values of heritability combined with the genetic variances reflect a favorable selection condition. Heritability values, in general, ranged from 57% to 97%, and the estimated values of the coefficients of genetic variation (σ^2G) were higher than those observed for the environmental variance (σ^2E), indicating the existence of a high potential of the genetic component in the expression of the phenotype for the physiological quality trait, evaluated by means of germination and vigor tests, except for the evaluation of seedling dry mass in the field (Table 1). The degradation of substances present in the reserve tissues into soluble compounds and the transport of these products to the growth

Table 1. Summary of the analysis of variance and estimates of genetic parameters for the characteristics of thousand-seed weight (TSW, g), seed moisture content (MC, %), electrical conductivity (EC, µS cm^{-1}g^{-1}), viability by tetrazolium test (TZ, %), accelerated aging (AA, %), seed germination (G, %), first germination count (FGC, %), germination speed index (GSI), seedling length (SL, cm), seedling dry mass (SDM, g), seedling emergence in the field (SEF, %), emergence speed index (ESI), seedling length in the field (SLF, cm), seedling dry mass in the field (SDMF, g), in 20 common bean lines.

V	Mean	MSL (19)[1]	h_x^2	σ_f^2	$\hat{\sigma}_g^2$	$\hat{\sigma}_a^2$	CV_g%	I_v	Accuracy
TSW	176.75	2280.52**	94.63	570.13	539.56	30.56	13.14	2.10	97,28
MC	7.63	0.23**	87.29	0.058	0.051	0.007	2.96	1.31	93.43
EC	70.21	1036.52**	83.24	259.13	215.72	43.40	20.91	1.11	91.24
TZ	80.10	189.08**	90.72	47.27	42.88	4.38	8.17	1.56	95.24
AA	57.45	786.60**	91.97	196.57	180.79	15.78	23.40	1.69	95.90
G	82.75	679.94**	89.49	169.98	152.13	17.85	16.28	1.45	94.60
FGC	47.77	596.36**	89.03	149.09	132.73	16.35	24.11	1.42	94.35
GSI	8.66	20.54**	71.20	5.13	3.65	1.47	22.06	0.78	84.38
SL	26.82	8.53**	57.50	2.13	1.22	0.90	4.12	0.58	75.84
SDM	51.53	229.37**	80.23	57.34	46.01	11.33	13.16	1.00	89.57
SEF	81.1	228.17**	63.80	57.04	36.39	20.64	8.18	0.66	79.87
ESI	3.44	0.89**	74.42	0.222	0.16	0.057	11.81	0.85	86.26
SLF	26.18	42.86**	71.41	10.72	7.66	3.06	10.57	0.79	84.51
SDMF	1123.74	171783.14*	48.61	42945.78	20876.35	22069.42	12.85	0.48	69.61

[1] Degrees of freedom. ^{ns}, **, *; not significant, significant at 1% and 5% probability levels, respectively, by F test. V: variables; MSL: mean square between lines; h_x^2: broad-sense heritability; σ_f^2: phenotypic variance; $\hat{\sigma}_g^2$: genetic variance; $\hat{\sigma}_a^2$: environmental variance; CVg%: coefficient of genetic variation; I_v: Index of variation.

points of the embryonic axis (Dan et al., 1987) occur in a critical period of the plant's life, which is the growth of the seedling in the field, so there may be variations due to the differences of sites and edaphoclimatic conditions.

Mainly because the genotypes evaluated are different, there is the possibility of significant gains aimed at improving the physiological quality of seeds among them. The greater the genetic effect contained in a given trait, the greater its heritability, indicating that the expression of physiological attributes related to the genotype may be manifested in subsequent cycles (Vasconcelos et al., 2012). The indices of variation that occur in relation to the coefficients of genetic and environmental variation, in general, were higher than or close to the unit, ranging from 0.66 to 2.10, except for SDMF and SL, contributing to indicating that the existing variability is explained by the genotype (Cruz and Souza, 2014).

According to the multivariate analyses, the first two principal components explained about 53.60% of the total accumulated variance; the first principal component (PC1) explained 35.50% and the second principal component (PC2) explained 18.10% of the original variability of the data (Table 2). The third principal component was not considered as it did not add relevant information. Similar results were observed through principal component analysis for the segregation of common bean cultivars with high seed quality, where PC1 explained 45.18% and PC2 explained 33% of the variability (Padilha et al., 2020).

The discriminatory power of the variables given to each principal component is measured through the correlation between each variable and a principal component (Barbosa et al., 2013; Hongyu et al., 2015). Therefore, based on the values of correlation (Table 2), the variables first germination count (0.788), germination test (0.749), seedling length in the field (0.756), accelerated aging (0.710), emergence speed index (0.707), seedling emergence in the field (0.647), seedling dry mass in the field (0.639), seedling dry mass in the laboratory (0.589) and germination speed index (0.548) were correlated with PC1 (Table 2). Similar results were observed by Pinheiro et al. (2022) for these same variables, which correlated in the principal component analysis in a study with soybean genotypes.

The correlated variables with the highest discriminatory power for PC2 were electrical conductivity (0.680), tetrazolium (0.630) and thousand-seed weight (0.280) (Table 2), and the positive correlations are responsible for discriminating the lines located in the upper part of PC2 (Figure 1).

Table 2. Correlation between each principal component and evaluation of the physiological performance of the seeds of 20 lines of common bean.

Principal components (PC)	PC1	PC2
Contribution of PC (%)	35.50	18.10
Cumulative contribution (%)	35.50	53.60
Thousand-seed mass	0.151	0.280
Electrical conductivity	-0.344	0.680
Tetrazolium (viability)	0.364	0.630
Accelerated aging	0.710	-0.396
Seed germination	0.749	-0.413
First germination count	0.788	-0.342
Germination speed index	0.548	-0.514
Seedling length	0.316	-0.225
Seedling dry mass	0.589	0.175
Seedling emergence in the field	0.647	0.409
Emergence speed index	0.707	0.366
Seedling length in the field	0.756	0.455
Seedling dry mass in the field	0.639	0.372

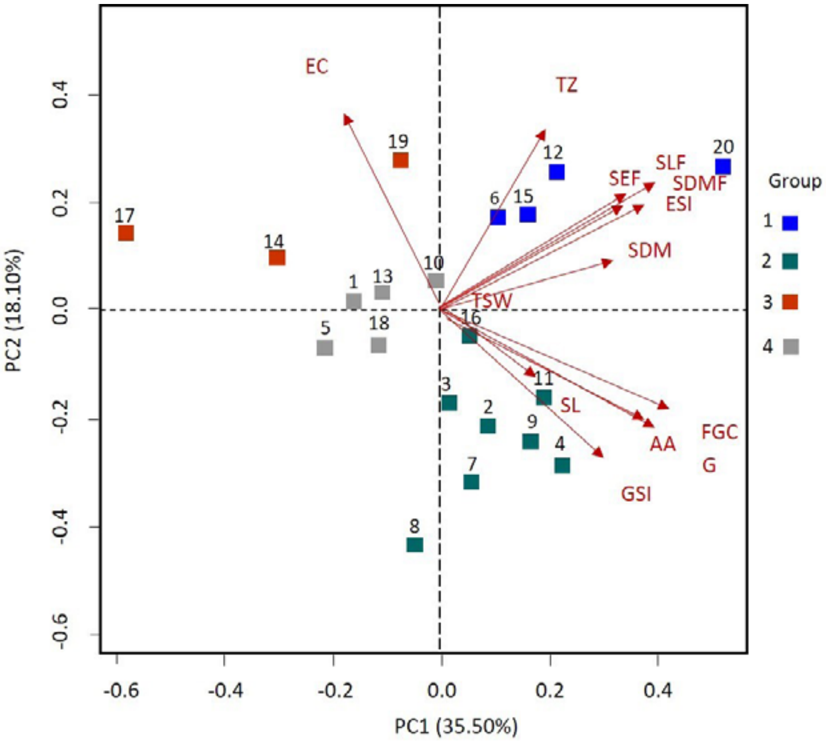

Figure 1. Graphic dispersion in relation to the first two principal components, based on the physiological performance of seeds of 20 lines of common bean according to the variables: Thousand-seed weight (TSW), electrical conductivity (EC), tetrazolium (TZ), accelerated aging (AA), seed germination (G), first germination count (FGC), germination speed index (GSI), seedling length (SL), seedling dry mass (SDM), seedling emergence in the field (SEF), emergence speed index (ESI), seedling length in the field (SLF), seedling dry mass in the field (SDMF).

The positive correlations of PC1 in the right quadrant of the figure are mainly associated with seed vigor evaluated under field conditions (Figure 1). The association between the groups of variables and the common bean lines indicated in the upper right quadrant of PC1 express group 1, which consists of the lines L20, L12, L6 and L15, which have the potential to show high performance in the field, determined by emergence and emergence speed index, as well as plants with higher growth and capacity to transfer dry matter to embryonic axis growth (Figure 1).

Regarding PC2, a higher potential for transfer of embryo reserves for seedling formation was observed in lines of group 1, determined by the performance of seedlings in the field and higher seed viability. It is worth mentioning that the establishment of the stand constitutes a solid basis for obtaining high yield and is the first opportunity for the producer to evaluate the initial performance of the seeds acquired. The close relationship between rapid and uniform emergence of seedlings and obtaining the desired plant population per area reinforces the importance of using seeds with high physiological quality to increase crop yield (Waterworth et al., 2019).

In the lower quadrant of PC1, there is the formation of group 2, comprising the lines L2, L3, L4, L7, L8, L9, L11 and L16, which were characterized by having seeds with high physiological performance under favorable conditions, as exemplified in the variables obtained with the germination test. However, the accelerated aging test, which exposes seeds to abiotic stress and is widely applied in the selection of soybean seed lots (Marcos-Filho, 2015), was grouped together with the evaluations that were not determinant in the assessment of the performance of the common bean lines.

It can also be inferred that group 3 (PC2) comprised the lines L17, L14 and L19 with lower seed vigor compared to the other lines, given the position in the quadrant opposite to the variables of group 2, due to the high results of electrical conductivity. It is worth mentioning that the large amount of electrolytes leached in the electrical conductivity test is inversely proportional to the quality of the sample analyzed. Similar results were reported by Silva et al. (2019), who identified that the high results of electrical conductivity also led to the formation of a group with lower quality of *Brachiaria decumbens* seeds through multivariate analysis.

In relation to PC2, group 4 also includes the lines L1, L5, L10, L13 and L18, with seeds with lower potential for transfer of reserves, determined by the low results of dry mass, given the position in the quadrant opposite to the variables of group 1, which contains the groups with higher growth and dry matter translocation capacity. Similar results were observed in the segregation of common bean cultivars with high suitability for using embryo reserves for seedling growth, through principal component analysis (PCA) (Padilha et al., 2020). According to Ebone et al. (2020), seeds with reserves available for embryo growth are essential to ensure plant stand uniformity and, consequently, high grain yields.

According to the results, the multivariate analysis showed that the existing variability for the physiological quality trait of seeds of the studied lines is explained by the genotype. The results and multivariate trends obtained in this study are important to define the effects of the genotype x seed quality interaction in order to strategically position common bean genotypes to obtain seeds with high physiological performance. These parameters are important for the selection of genotypes aimed at obtaining seeds with high quality and for the differentiation of levels between common bean seed lots.

CONCLUSIONS

Multivariate principal component analysis is efficient to evaluate the existence of genetic variability for the physiological quality trait of seeds among common bean lines.

Principal component analysis allows the identification of groups of common bean lines that differ in terms of seed physiological performance.

The variables first germination count, germination test, seedling length in the field, and emergence speed index are promising for the selection of common bean genotypes regarding the physiological potential of the seeds.

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