



DANILO ARAUJO GOMES

**OTIMIZAÇÕES NA FENOTIPAGEM POR IMAGENS
DE LINHAGENS DE FEIJÃO COMUM QUANTO A COR,
TAMANHO E FORMATO DAS SEMENTES**

**LAVRAS – MG
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Prof. Dr. Vinícius Quintão Carneiro
Orientador

**LAVRAS – MG
2025**

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**OTIMIZAÇÕES NA FENOTIPAGEM POR IMAGEM DE LINHAGENS DE FEIJÃO
COMUM QUANTO AO TAMANHO E COR DE SEMENTES**

**OPTIMIZATIONS IN HIGH-THROUGHPUT PHENOTYPING OF COMMON BEAN
LINES FOR SEED COLOR, SIZE AND SHAPE**

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética e Melhoramento de Plantas, para obtenção do título de Doutor.

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O presente trabalho foi realizado com apoio do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

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RESUMO GERAL

O feijão (*Phaseolus vulgaris* L.) é uma espécie que apresenta enorme variabilidade quanto ao aspecto das sementes, principalmente em relação à coloração, brilho, tamanho e formato. Esses caracteres são fundamentais na comercialização das sementes dessa cultura, uma vez que determinam a aceitação de uma nova cultivar no mercado consumidor. Assim, o aspecto das sementes é um dos principais caracteres avaliados em um programa de melhoramento de feijoeiro. O procedimento padrão de avaliação dessa característica limita-se a avaliações visuais ou medições manuais por meio de paquímetros ou colorímetros. Com o objetivo de contribuir para a melhoria dessas avaliações nos programas de melhoramento do feijoeiro, foram realizados dois estudos que exploram metodologias de visão computacional destinadas para a avaliação do aspecto das sementes de feijão-comum. O primeiro artigo foca no uso da visão computacional para avaliar a cor do tegumento de feijões vermelhos. Esse estudo baseou-se na avaliação de quarenta e sete linhagens de feijão vermelho quanto à cor do tegumento, utilizando-se escala de notas e visão computacional. A análise de imagens demonstrou maior precisão em comparação à avaliação visual, com correlações inferiores a -0,95 entre os valores médios dos pixels nos canais R*, L* e a* e as notas visuais atribuídas. Destaca-se que o canal a* apresentou maior acurácia na diferenciação das linhagens, confirmando o potencial da visão computacional como ferramenta eficiente para auxiliar a seleção em programas de melhoramento do feijoeiro. O segundo artigo buscou determinar o número ideal de sementes por parcela na avaliação por imagens de linhagens de feijão quanto aos caracteres relacionados ao tamanho e formato das sementes. Essa determinação baseou-se na precisão experimental e na acurácia de seleção. Para isso, este trabalho utilizou de cinco diferentes experimentos com linhagens pertencentes aos grupos comerciais carioca e preto. Constatou-se a possibilidade de avaliação de um pequeno número de sementes por parcela para que as avaliações de tamanho e formato dos grãos sejam precisas e as seleções acuradas. Ambos os estudos destacaram que a fenotipagem de linhagens de feijão comum por meio de imagens digitais, quanto à coloração e aos caracteres físicos das sementes, é uma alternativa viável, pois é mais precisa, acurada, rápida e automatizada.

Palavras-chave: *Phaseolus vulgaris* L.; fenotipagem por imagem; tamanho amostral

GENERAL ABSTRACT

Common bean (*Phaseolus vulgaris* L.) exhibits great variability in seed traits, particularly in color, gloss, size, and shape. These traits are fundamental for seed commercialization, as they determine the acceptance of a new cultivar in the consumer market. Thus, seed appearance is one of the main traits evaluated in bean breeding programs. The standard procedure for evaluating these traits is limited to visual assessments or manual measurements using calipers or colorimeters. Aiming to improve these evaluations in bean breeding programs, two studies were conducted exploring computer vision methodologies for assessing common bean seed appearance. The first article focuses on the use of computer vision to evaluate the seed coat color of red common beans. Forty-seven red bean lines were evaluated for seed coat color using scoring scales and computer vision. Image analysis proved to be more precise than visual assessment, with correlations lower than -0.95 between the mean pixel values in the R*, L*, and a* channels and the assigned visual scores. The a* channel showed the highest accuracy in differentiating lines, confirming the potential of computer vision as an efficient tool to support selection in bean breeding programs. The second article aimed to determine the optimal number of seeds per plot for image-based evaluation of common bean lines regarding traits related to seed size and shape. This determination was based on experimental precision and selection accuracy. To this end, five experiments were carried out with lines from the carioca and black commercial groups. Results indicated that it is possible to evaluate a reduced number of seeds per plot while still obtaining precise measurements of seed size and shape, allowing accurate selection. Both studies highlighted that digital image-based phenotyping of common bean lines for seed coat color and physical seed traits is a viable alternative, as it is more precise, accurate, rapid, and automated.

Keywords: *Phaseolus vulgaris* L.; image-based phenotyping; sample size

INDICADORES DE IMPACTO

A presente tese avança no desenvolvimento e aplicação de metodologias de fenotipagem de alto rendimento baseada em imagens para avaliação de caracteres determinantes na aceitação do feijão-comum pelo mercado consumidor. Foram exploradas duas frentes complementares: (i) o uso da visão computacional para avaliação da cor do tegumento de sementes de feijões do grupo vermelho; e (ii) a determinação do número ótimo de sementes por parcela para análises digitais de tamanho e formato em feijão-comum. Essas inovações metodológicas ampliam a eficiência, a acurácia e a velocidade do processo seletivo. No primeiro capítulo, a análise por imagens demonstrou alta acurácia experimental, elevada correlação com avaliações visuais e alta capacidade de discriminação de linhagens de feijão-vermelho, confirmando seu potencial de aplicação em programas de melhoramento. No segundo capítulo, foi possível estabelecer que a fenotipagem digital de caracteres de tamanho e forma pode ser realizada com 17 sementes por parcela, garantindo precisão estatística e decisões de seleção confiáveis. Assim, a tese fornece protocolos que podem ser aplicados rotineiramente para a obtenção de cultivares mais competitivas e alinhadas às preferências de consumidores. Do ponto de vista científico, a tese contribui para a consolidação da fenotipagem digital como ferramenta estratégica no melhoramento genético do feijoeiro, oferecendo referências práticas e protocolos de aplicação imediata. No aspecto tecnológico, representa um avanço para a modernização dos programas de melhoramento do feijoeiro, aproximando-os de tendências globais em agricultura de precisão e análise de imagens. Além das aplicações técnico-científicas, destaca-se que todo o trabalho envolveu a participação ativa de estudantes de graduação e pós-graduação, favorecendo a formação de recursos humanos altamente qualificados na área de melhoramento genético de plantas. Essa inserção acadêmica contribuiu para o desenvolvimento de competências em metodologias inovadoras de fenotipagem digital, fortalecendo a integração entre pesquisa, ensino e capacitação profissional. Dessa forma, a tese não apenas gerou avanços científicos e tecnológicos, mas também promoveu impacto direto na formação de especialistas capazes de aplicar e difundir essas ferramentas em diferentes contextos agrícolas.

IMPACT INDICATORS

This thesis advances the development and application of high-throughput image-based phenotyping methodologies for evaluating traits that determine consumer acceptance of common bean. Two complementary approaches were explored: (i) the use of computer vision for assessing seed coat color of red common bean lines; and (ii) the determination of the optimal number of seeds per plot for digital analyses of seed size and shape in common bean. These methodological innovations enhance the efficiency, accuracy, and speed of the selection process. In the first chapter, image analysis demonstrated high experimental accuracy, strong correlation with visual assessments, and high discriminatory capacity among red bean lines, confirming its potential application in breeding programs. In the second chapter, it was established that digital phenotyping of seed size- and shape-related traits can be conducted with 17 seeds per plot, ensuring statistical precision and reliable selection decisions. Thus, the thesis provides protocols that can be routinely applied to achieve the development of more competitive cultivars aligned with consumer preferences. From a scientific perspective, the thesis contributes to consolidating digital phenotyping as a strategic tool in common bean breeding, offering practical references and protocols with immediate applicability. From a technological standpoint, it represents an advance in the modernization of common bean breeding programs, bringing them closer to global trends in precision agriculture and image-based analysis. Beyond scientific and technological applications, the thesis also stands out for involving undergraduate and graduate students, thereby promoting the training of highly qualified human resources in plant breeding. This academic engagement fostered the development of skills in innovative digital phenotyping methodologies, strengthening the integration of research, teaching, and professional training. In this way, the thesis not only generated scientific and technological advances but also contributed directly to the training of specialists capable of applying and disseminating these tools in different agricultural contexts.

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PRIMEIRA PARTE

1. INTRODUÇÃO

Common bean (*Phaseolus vulgaris* L.) is an important source of protein for human nutrition, contributing to food security. Its nutrient-rich seeds, relatively accessible to a large portion of the population, provide proteins and micronutrients such as iron and zinc to millions of people worldwide, especially in Latin America and East/Southern Africa, where consumption of this legume can reach up to 66 kg per capita annually (Keller et al., 2020). Thus, common bean is a valuable food for more than half a billion people, particularly in developing countries (Winham et al., 2008; Diaz et al., 2020). According to estimates from Embrapa Arroz e Feijão (2023), the apparent per capita consumption of common bean in Brazil in 2021 was 12.2 kg person⁻¹. National bean production in the 2022 growing season reached 2,553,264 tons, with an average yield of 1,571 kg ha⁻¹ (EMBRAPA, 2023).

Different commercial groups of common bean are produced in Brazil. Among them, the carioca, black, red, rosinha, and jalo groups stand out (Carneiro et al., 2014). Distribution of beans consumed in Brazil is composed of 70% carioca beans, 12% black beans, and 3% special beans (EMBRAPA, 2023), with carioca beans being the most consumed and produced in the country. This consumer preference for carioca beans has guided bean breeding programs toward developing new cultivars of this commercial group. As a result, carioca bean cultivars account for the largest number of bean cultivars recommended in Brazil (Barili et al., 2016; Lemos et al., 2020; Capanema et al., 2019). Breeding programs in Brazil have focused on developing lines with high yield potential, upright plant architecture, resistance to major pathogens, earliness, and seed quality traits that meet market demands (Botelho et al., 2011; Costa et al., 2010; Cunha et al., 2005; Ramalho et al., 2005; Silva et al., 2008).

Bruno et al. (2018) highlighted that consumer acceptance of new common bean cultivars is determined by traits such as cooking time, quality, and flavor, in addition to seed size, color, and tolerance to seed coat darkening during storage. Thus, new lines to be recommended must present seeds consistent with the desirable standards of the respective commercial group. For example, for carioca and black beans, the most consumed in Brazil, desirable traits include the absence of a yellow halo, absence of flattened seeds, absence of gloss, elliptical shape, and 100-seed weight between 23 and 25 g (Ramalho and Abreu, 2015).

In terms of technological aspects, seed quality can be divided into commercial, culinary, and sensory attributes. Commercial quality refers to seed appearance, including color, gloss,

shape, and size. Culinary quality refers to seed hydration capacity, short cooking time, and production of a thick broth. Sensory quality refers to desirable traits such as flavor and texture (Bassinello et al., 2003). Seed size and shape are traits considered by both packers and consumers when choosing a commercial bean brand. Consumers associate larger seeds with greater expansion after cooking and, consequently, with better cooking yield (Perina et al., 2010). Seed size varies according to seed type and cultivar, but currently, seeds with 100-seed weight between 23 and 25 g are preferred (Ramalho and Abreu, 2015). Thus, cultivars with lower values may face restrictions in market adoption.

The selection of genotypes with desirable seed appearance is a major challenge since evaluations for this trait are mostly based on visual scoring scales (Ramalho et al., 1998). Therefore, success in selection depends on evaluator precision, making the process highly subject to errors. Although fast and practical, visual evaluation lacks precision, which makes selection less accurate. Moreover, in visual evaluation, criteria are assessed jointly, making the evaluation less detailed. When the goal is to evaluate these criteria individually, additional equipment such as colorimeters and calipers are used to assess seed color and size, respectively. However, these methods are time-consuming, which makes their use impractical for evaluating a large number of seeds and genotypes.

Silva et al. (2024) verified that seed coat color evaluation of carioca bean cultivars based on digital image analysis showed coefficients of variation about four times lower than those obtained with visual evaluation using the scoring scale proposed by Silva et al. (2008). Furthermore, they found that the proposed methodology allowed better discrimination among the cultivars evaluated. These results demonstrate the superiority of computer vision for this trait. Similarly, Cardoso (2022) showed that seed size and shape evaluation in common bean using digital images provides experimental precision comparable to that obtained with calipers. The author also highlighted that image-based evaluation of these traits is faster and enables automation of processes in breeding programs.

The use of large-scale phenotyping techniques based on digital image analysis in common bean breeding, especially for seed commercial appearance, constitutes an important tool to assist in the selection of superior genotypes. However, there are few studies employing digital image analysis for common bean seed evaluation, particularly with a breeding focus. Therefore, the objective of this thesis was to contribute to improving evaluations of common bean seed appearance in breeding programs. The thesis is composed of two chapters, which provide valuable information regarding the evaluation of common bean seed appearance using computer vision.

The first article of this thesis, entitled “*High-throughput phenotyping of seed coat color of red common beans*”, addresses the use of a digital methodology based on computer vision to evaluate red bean lines for seed coat color. The second article, entitled “*Optimization of image-based phenotyping of common bean lines for seed size and shape*”, aimed to determine the optimal number of seeds per plot for the evaluation of seed size-related traits in common bean using digital images.

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SEGUNDA PARTE – ARTIGOS

Artigo 1 - Redigido conforme as normas da revista Euphytica - Versão preliminar	
Título do artigo:	High-throughput phenotyping of seed coat of red commom beans
Autores:	Danilo Araujo Gomes Jussara Mencialha Guilherme Almeida Domingos Jaque José Eulário Lampi Dique Constantino Rico Raimundo Artur Vinícius Quintão Carneiro
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ARTIGO 1 - High-throughput phenotyping of seed coat of red common beans

Abstract

Seed coat color is one of the main traits that determines consumer acceptance of red common beans. Bean breeding programs have directed their attention to the seed coat color of beans from red common bean commercial group lines. Selection of lines for this trait has been based on visual assessment, which is subject to errors. Computer vision is an alternative that helps provide more accurate assessment of seed coat color. Therefore, the objective of this study was propose the use of a digital methodology based on computer vision to assess red common bean lines regarding seed coat color. The beans from forty-seven red common bean lines were assessed for seed coat color using scoring scales and computer vision. The mean intensity of the image pixels in the R*, L*, and a* channels of the seed coat images was measured for each plot. Image analysis of seed coat color was highly accurate and more precise than visual analysis. The correlations between the visual assessment of seed coat color and the mean values of the pixels in the R*, L*, and a* channels were below -0.95, confirming strong association of pixel values and visual assessment scores. The mean pixel values in the a* channel showed greater accuracy in assessing seed coat color and better ability in differentiating the lines. Computer vision proved to be effective in assisting assessment of the seed coat color of red common bean lines and thus can be routinely used in bean breeding programs to optimize the selection process.

Keywords: *Phaseolus vulgaris* L., computer vision, plant breeding

1. Introduction

A new common bean cultivar must meet the demands of producers, industry and the consumer market. Consumer acceptance of common beans in different commercial groups is predominantly determined by cooking time, flavor, size, shape, and seed coat color (Ribeiro et al., 2019; Kläsener et al., 2020). Bean cultivars for the red commercial group must produce seeds with a light and bright red seed coat, an elliptical shape, and a 100-seed weight between 22 and 24 g for wider acceptance in the market (Menezes Júnior et al., 2013; Melo et al., 2020). A bright red seed coat color is one of the main attractions for consumers when they purchase beans of this commercial group. Therefore, bean breeding programs have directed their attention to assessment of this trait in selection of red bean lines.

The selection of bean lines within different commercial groups for seed coat color and grain appearance is a major challenge in bean breeding, since these traits are mainly assessed using visual grading scales (Melo et al., 2020; Pereira et al., 2021; Santana et al., 2023). Therefore, appropriate selection of lines regarding these traits depends on the skill of the evaluators, which makes the assessment subject to errors even if performed by experienced evaluators. Image analysis via colorimeters for assessment of seed coat color is more precise and accurate than visual analysis (Ribeiro et al., 2019; Ribeiro et al., 2020; Campa et al., 2023). However, some limitations, such as the small sample size assessed and the time required for assessment, hinder rapid decision making and assessment of the large number of lines that are constantly developed by breeding programs.

Computer vision has considerable potential to assist selection of bean lines by providing a new approach in seed coat color assessment that is more accurate, precise, and agile than the visual and colorimetric assessments currently performed by bean breeding programs (Mendoza et al., 2017; Sadohara et al., 2022; Silva et al., 2024). The implementation of computer image analysis will increase the accuracy of genetic control studies and line selection for seed traits to make breeding programs more efficient. However, image analysis for assessment of seed coat color of red common beans has not been widely used by bean breeding programs. Therefore, the objective of this study was evaluate the use of computer vision to assess the seed coat color of beans from red common bean lines.

2. Materials and Methods

Two experiments assessing 47 common bean lines from the red commercial group (Supplementary Table 1) were conducted in the Center for Scientific and Technological

Development of the Federal University of Lavras (UFLA) (21°58' S, 42°22' W; 918 m asl) in the 2021 winter and rainy seasons. A randomized block experimental design was used, with three repetitions. The experimental plots consisted of two 2.0-m rows, with a 0.6-m spacing between rows. The cultivars Vermelhinho, BRSMG Marte, Safira, Ouro Vermelho, and the OVR line were used as controls in these experiments. The management practices of fertilization and pest control followed the technical recommendations of Carneiro et al. (2015) for growing common bean in the state of Minas Gerais.

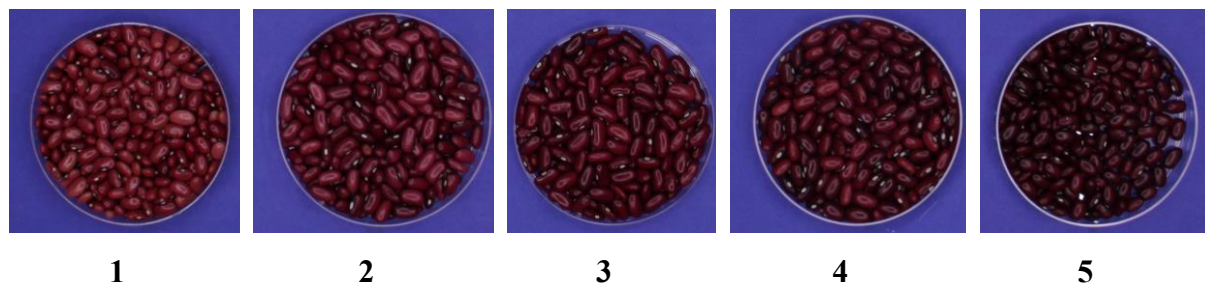


Fig. 1. Rating scale adapted from Silva et al. (2007) for assessment of the seed coat color of red beans.

The seed coat color of the beans from the lines was also assessed using images. The same randomized block design structure used in conducting the field experiments was used to acquire images of the common beans from each plot. The beans from the lines of each plot were placed in Petri dishes, filling the entire bottom of the dish. The images were obtained using a computer vision system (Fig. 2) in the Phenomics Laboratory of the Department of Biology at UFLA. This system consists of a Canon EOS 60D digital camera and Canon EF-S 18-35 mm Zoom Lens attached to a photo studio. It has an artificial lighting system equipped with 4 LED lamps, 9W of power, luminous flux of 900 lm, and cold white color temperature (6500 K). The camera configuration was set at ISO 200, exposure time 1/20, and F-Stop 1/10.

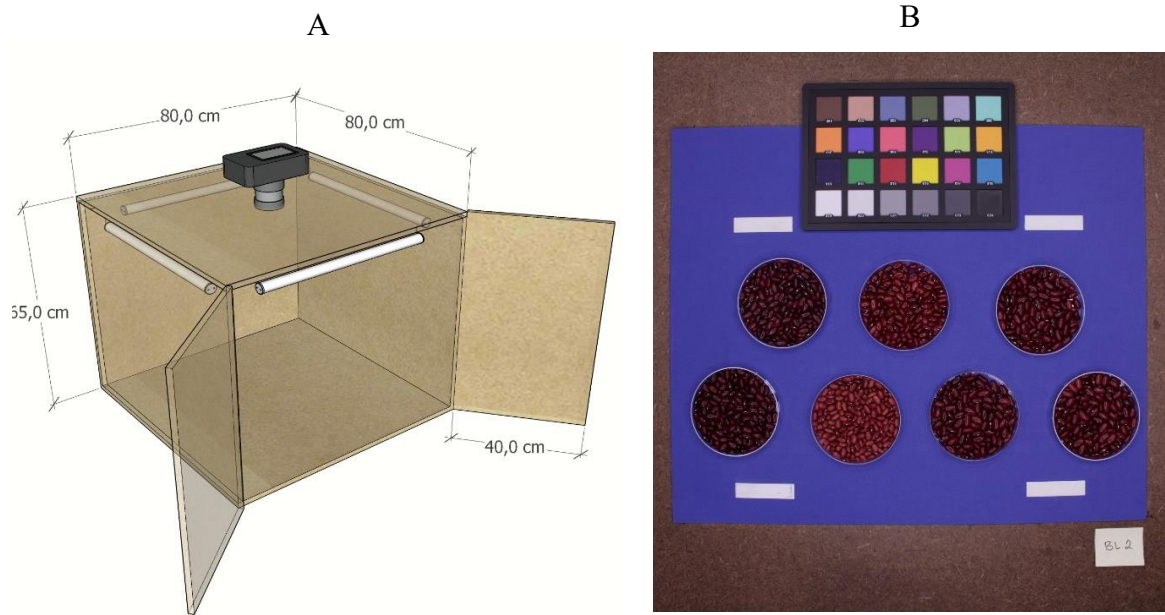


Fig. 2. Model of the photographic studio used to capture images (A) and an example of an image of beans from seven plots (B) of the experiments for assessment of red bean lines.

The images captured from each plot of each experiment were segmented to obtain only the region of the dishes and eliminate the background from the image. The image obtained in the RGB color space was converted to the CIELAB color space for segmentation purposes. This was carried out using the b^* channel of the CIELAB color space by the method of Otsu (1975). After segmenting the Petri dishes of beans from each experimental plot, the mean values of the pixels of the beans from each plot were obtained in the R^* channel of the RGB color space and in L^* and a^* of the CIELAB color space. All image processing was carried out with the aid of the Python-OpenCv (Bradski and Kaehler, 2008).

Individual analysis of variance was performed on the visual and image assessment data from each experiment involving the 47 red bean lines, according to the following statistical model: $Y_{ij} = \mu + B_j + L_i + e_{ij}$, where Y_{ij} is the value observed in the plot which contains line i ($i = 1, 2, 3, \dots, 47$) in block j ($j = 1, 2, 3$); μ is the overall average of the experiment; B_j is the random effect of block j ; L_i is the fixed effect of line i ; e_{ij} is the experimental error associated with Y_{ij} . Joint analysis of variance was also performed on data from visual and image assessments of the lines, according to the following model: $Y_{ijk} = \mu + B/S_{jk} + L_i + S_k + LS_{ik} + e_{ijk}$, where Y_{ijk} is the value observed in the plot that received line i ($i = 1, 2, 3, \dots, 47$) in block j ($j = 1, 2, 3$) in season k ($k = 1, 2$); μ is the overall average of the experiments; B/S_{jk} is the random effect of block j in season k ; L_i is the fixed effect of line i ; S_k is the fixed effect

of season k ; LS_{ik} is the fixed effect of the interaction between line i and season k ; and e_{ij} is the experimental error associated with Y_{ijk} . The coefficient of variation (CV, %) was obtained from the expression proposed by dos Anjos et al. (2019): $CV(\%) = 100 \frac{\sqrt{MSE}}{|\bar{S}_{ui} - u|}$, where MSE is the mean square error, $\bar{S}_{ui}$ is the mean of the scores in the experiment, and u is the numerical value of the score assigned to the undesirable phenotype. Accuracy was estimated based on the formula proposed by Resende and Duarte (2007): $\hat{r}_{gg} = (1 - \frac{1}{F})^{1/2}$, where F is the value of the ratio between the mean square of lines and the mean square error of the analysis of variance.

Pearson correlations between the mean values of the lines for traits assessed visually and by images were estimated in each season and in the mean of the two seasons. In individual and joint analyses of variance in which the effect of lines was significant, the Scott-Knott (1974) cluster test was performed on the means of the lines. The Tukey's least significant difference (LSD) was also estimated according to Bittencourt et al. (2023). The LSD for each trait was expressed as a percentage of the mean of the respective experiment as a form of standardization in order to compare the LSD values for the traits assessed visually and through images.

When there was a significant line-by-season interaction, this interaction was decomposed into its complex part, according to Cruz and Castoldi (1991). The fraction of the interaction for the two seasons was obtained by the expression: $C(\%) = 100 \times \left(\frac{C}{Q_{ML \times S}} \right)$, where $C = \sqrt{(1 - r)^3 Q_W Q_R}$ and $Q_{ML \times S}$ is the mean square of the interaction between lines and the two seasons assessed. In this expression, r is the correlation between the mean performance of the lines in the two seasons; and Q_W and Q_R are the mean squares between lines in the winter and rainy seasons, respectively. The analyses were carried out using the ExpDes package (Ferreira et al., 2013) and the R software (R Core Team 2023).

3. Results

The effect of lines was significant ($P < 0.01$) for the GA and SCC traits and for the intensities of the pixels in the R*, L*, and a* channels of the seed coats obtained in the two seasons for assessing the lines (winter/2021 and rainy/2021) (Table 1). Therefore, these results show that at least one mean of the lines differs from the others for each of the traits assessed. The accuracy for GA in the winter season was 0.93 and 0.94 in the rainy season. The value of this parameter for SCC was 0.96 in the winter season and 0.98 in the rainy season. Accuracy estimates for the traits assessed by images were greater than 0.94 in both seasons.

Coefficients of variation were 25.51 for GA and 23.62 for SCC in the winter season, and 27.27 for GA and 17.99 for SCC in the rainy season (Table 1). The estimates of the coefficients of variation observed in the assessments carried out using images (R^* , L^* and a^*) were lower than those obtained in the visual assessments of the beans (GA and SCC). The estimates of this parameter for these traits ranged from 1.27 to 15.51 in the winter season (Table 1). In the rainy season, they ranged from 0.77 to 8.45. The lowest CV values in both seasons (1.27 and 0.77) were observed for mean intensity in the a^* channel.

The LSD value was 52% for GA and 65% for SCC in the winter season, and 36% for GA and 43% for SCC in the rainy season. The LSD values for the traits assessed by images (R^* , L^* , and a^*) were lower in magnitude, except for intensity in the L^* channel in the winter season experiment, where an LSD of 52% was observed. The intensity in the a^* channel showed lower LSD values, below 4% in the two seasons assessed.

Table 1. Summary of individual analysis of variance for appearance scores (GA) and for seed coat color (SCC), mean of pixels in the R*, L*, and a* channels of the experiment for assessment of red bean lines in the 2021 winter and rainy seasons.

Source of variation	df	Mean square									
		Winter					Rainy				
		GA	SCC	R*	L*	a*	GA	SCC	R*	L*	a*
Blocks	2	0.87	0.25	281.34	164.95	12.76	0.43	0.27	120.55	26.87	20.88
Lines	46	1.76**	4.08**	534.30**	212.65**	63.21**	1.28**	3.55**	526.60**	195.86**	67.38**
Error	92	0.23	0.28	51.58	28.10	3.61	0.15	0.14	14.70	8.14	1.28
Mean		3.12	2.76	63.11	34.18	149.18	3.58	2.92	60.15	33.74	147.19
$\hat{r}_{gg}^a$		0.93	0.96	0.95	0.94	0.97	0.94	0.98	0.99	0.98	0.99
CV, % ^b		25.51	23.62	11.38	15.51	1.27	27.27	17.99	6.37	8.45	0.77
LDS, % ^c		52.00	65.00	38.00	52.00	4.00	36.00	43.00	21.00	28.00	2.00

^a $\hat{r}_{gg}$, accuracy. ^bCV, %, Coefficient of variation. ^cLDS, %, Least significant difference. **Significant at the 0.01 probability level by the F test.

The effect of lines was significant ($P < 0.01$) in joint analyses of variance of all the traits assessed, while the effect of seasons was significant only for GA ($P < 0.01$) and for the intensity means in the a* channel ($P < 0.05$) (Table 2). The line-by-season interaction was significant for all visual (GA and SCC) and image (R*, L*, and a*) assessments, which highlights the differential response of lines throughout the seasons for all the traits assessed (Table 2). This interaction was predominantly of a simple nature for SCC, R*, L*, and a*, which exhibited correlation estimates between seasons greater than or equal to 0.84, and the percentage of the complex part below 40% (Table 2). The line-by-season interaction for GA was predominantly complex in nature, with a percentage of 58.87%, and the correlation between the two seasons was 0.63. The mean intensity in the a* channel of the seed coats of the lines had a percentage of the complex part and correlation estimates closer to the values of these parameters for SCC.

Table 2. Summary of joint analysis of variance for grain appearance (GA) scores and seed coat color (SCC), mean of pixels in R*, L*, and a* channels of the experiments for assessment of red bean lines.

Source of variation	df	Mean square				
		GA	SCC	R*	L*	a*
Blocks/seasons	4	0.65	0.25	200.95	95.91	16.82
Lines (L)	46	2.47**	7.30**	995.32**	376.23**	124.93**
Seasons (S)	1	14.70**	1.80 ^{ns}	620.33 ^{ns}	13.09 ^{ns}	279.31*
L x S	46	0.57**	0.33*	65.56**	32.28**	5.64**
Error	184	0.19	0.21	33.13	18.11	2.44
Mean		3.35	2.84	61.63	33.96	148.18
$\hat{r}_{gg}^a$		0.96	0.98	0.98	0.97	0.99
CV, % ^b		26.33	21.36	9.33	12.53	1.05
Complex L x S, % ^c		58.87	28.25	35.15	39.45	29.15
Correlation (WS/RS) ^d		0.63 ⁺⁺	0.92 ⁺⁺	0.88 ⁺⁺	0.84 ⁺⁺	0.91 ⁺⁺

^a $\hat{r}_{gg}$, accuracy. ^bCV, %, Coefficient of variation. ^cComplex L x S, %, percentage of the complex part of the L x S interaction. ^dCorrelation (WS/RS), Pearson correlation between means of intensity of seed coat color in the winter season (WS) and rainy season (RS). **Significant at the 0.01 probability level by the F test. *Significant at the 0.05 probability level by the F test. ns, nonsignificant. ++Significant at the 0.01 probability level by the t test.

The correlations between all traits assessed in the two seasons and in the mean of the seasons were significant ($P < 0.05$) and of high magnitude. The correlation estimate between the two visual assessments (GA and SCC) was 0.82 in the winter season and 0.66 in the rainy season (Figure 3). In the mean of the two experiments, the estimate of this parameter was 0.84. The means of intensity in the R*, L*, and a* channels had the highest estimates of the correlation coefficient with the visual assessment of the seed coat color (SCC) in the two experiments for assessment of lines and in the mean of the experiments ($r > |0.94|$). The

correlation estimates between GA and the traits assessed by images were of high magnitude, but they were lower than those observed between the traits assessed by images (R^* , L^* and a^*) and SCC. The correlations between the traits assessed by images had estimates close to 1 in each season assessed, as well as in the mean of the seasons.

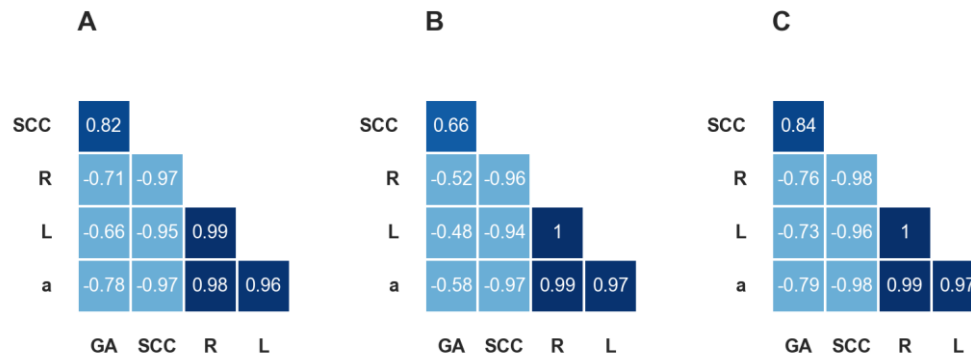


Fig. 3. Pearson correlation estimates between the traits assessed visually (GA and SCC) and by images (R^* , L^* , a^*) in the 2021 winter (A) and rainy (B) seasons and in the mean of these two seasons (C).

The mean GA scores of the lines were clustered by the Scott-Knott test into three groups in the winter season and in the mean of the seasons, while in the rainy season, five groups were observed (Figure 4). The mean values of SCC of the lines were allocated to four groups in the winter season and in the mean of the seasons, and to five groups in the rainy season. The mean pixels in the R^* and L^* channels were allocated to four groups in the winter season and the mean of the seasons, and to six groups in the rainy season. However, the means of the lines for the mean intensity of the pixels in the a^* channel were allocated to five, eight, and six groups in the winter season, in the rainy season, and in the mean of these seasons, respectively.

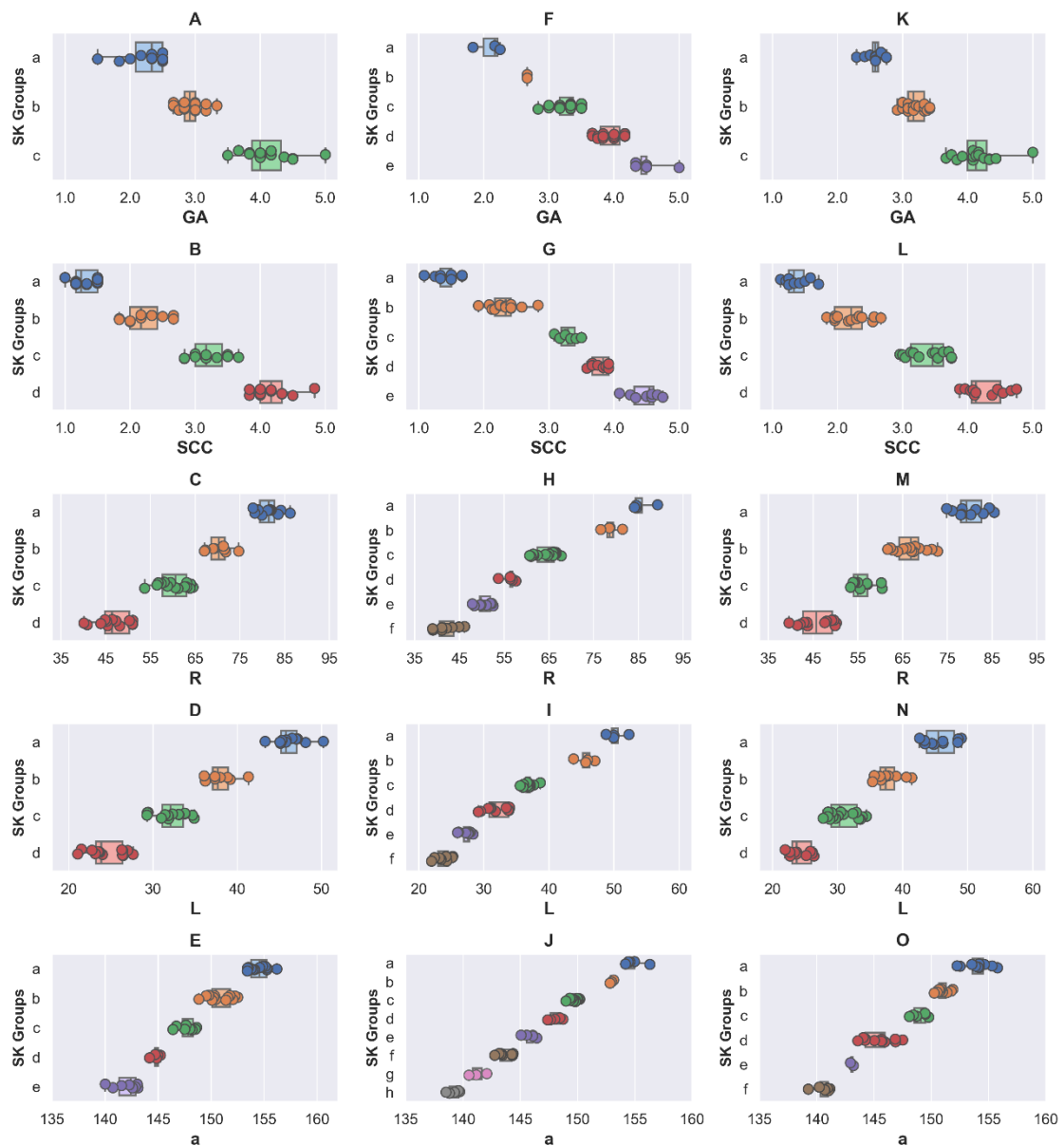


Fig. 4. Grouping by the Scott-Knott test and dispersion of the means of the lines for traits assessed visually (GA and SCC) and by images (R*, L*, a*) in the winter season (A, B, C, D, E), in the rainy season (F, G, H, I, J), and in the mean of the two seasons (K, L, M, N, O).

The lines belonging to the group with the lowest means for GA had scores lower than 2.5, 2.25, and 2.75 in the winter season, in the rainy season, and in the mean of the two seasons, respectively (Figure 4; Supplementary Tables 2, 3, and 4). The cultivars BRSMG Marte and Ouro Vermelho were allocated to the group with the lowest mean in the rainy season and in the mean of the seasons, while in the winter season, only the BRSMG Marte cultivar, with a GA score of 2.33, was allocated to the group with the lowest mean. The group with the lowest means for SCC had lines with scores lower than 1.8 in both of the seasons assessed and in their mean. The controls Vermelinho, Ouro Vermelho, and OVR were allocated to the group with the

lowest means in the rainy season and in the mean of the seasons, with scores less than or equal to 1.5. The BRSMG Marte cultivar, with a SCC score of 1.5, was allocated to the group of lowest means, together with the cultivars Vermelhinho, Ouro Vermelho, and the OVR line in the winter season.

The coincidence of the lines allocated to the groups with the lowest means for GA and SCC was 63.63% in the winter season and in the mean of the two seasons. This statistic for the rainy season was 33.33%. The percentage of coinciding lines allocated to the group with the lowest mean for SCC and in the groups with the highest mean for pixels in the R*, L*, and a* channels was greater than 83% in the winter season and in the mean of the two seasons. In the rainy season, this percentage was less than 55%. The lines belonging to the group with the lowest mean for SCC had mean pixels in the R*, L*, and a* channels greater than or equal to 72.82, 41.4, and 151.88 in the mean of the two seasons, respectively (Supplementary Table 4).

4. Discussion

The results of individual analysis of variance confirmed the significance ($P < 0.01$) of the effect of lines both in visual assessment of seed coat color and in image assessments (R*, L*, and a*). Thus, the mean of at least one line differed from the others for color-related traits (SCC, R*, L*, and a*) in the two seasons assessed. This shows the possibility of replacing the visual assessment of seed coat color of red bean lines with image assessment. Furthermore, the accuracies of these assessments were similar to those obtained by visual assessments, with values greater than 0.90 (Table 1). Such values indicate that there is very high correlation between the true genotypic values of the lines and those observed in experiments. According to Resende and Duarte (2007), accuracy values such as those observed for the image assessments in this study show very high experimental accuracy.

The assessments of R*, L*, and a* showed a reduction in the CV of 51.82, 34.33, and 94.62 %, respectively, in relation to this parameter for SCC in the winter season. The reduction in the rainy season was 64.59, 53.02, and 95.72%. Therefore, image assessment proved to be more precise than visual assessment in both seasons. Furthermore, this form of assessment allowed us to capture smaller differences between lines assessed for color, as the LSD values were lower than those observed in visual assessments. Under selection conditions in bean breeding programs, where differences between elite lines are increasingly smaller, image assessment exhibited greater potential to capture these differences than visual assessments. An example of this is seen for the mean value of the pixels in the a* channel, which had an LSD of

4% in the winter season and 2% in the rainy season, whereas the LSD for SCC were 65% and 43% in these seasons.

The agreement between the results obtained in visual assessment and image assessment was also observed for the effect of lines in joint analysis of variance (Table 2). This was also observed for the non-significant effect of seasons for the SCC, R^* , and L^* traits. However, this effect was significant for the mean of the pixels of the seed coats in the a^* channel. This can be explained by the low residual variation observed for this trait. In a way similar to what was observed in the individual analyses of variance, image assessments of seed coat color had accuracies similar to SCC, and lower values of CV and of LSD for this trait. This highlights the feasibility of using image analysis for assessment of the seed coat color of red bean lines in one or more seasons.

The visually assessed seed coat color (SCC) had a predominantly simple line-by-season interaction. However, the interaction was of a complex nature for the GA trait, with correlation values between the two seasons of 0.63, and the percentage of the complex part of the line-by-season interaction was 58.87%. This complex nature can be explained by the fact that the GA assessment considers not only color, but also the shape and size of the grain. This trait has proven to be more affected by the environment than traits related to seed coat color. Furthermore, the fact that GA is assessed using a rating scale can lead to errors that affect the results obtained in the lines-seasons interaction. Therefore, it is important to also use image assessment of the size and shape of beans. These assessments, together with color assessment, allow for accurate selection of lines for GA.

The predominance of the line-by-season interaction of a simple nature observed for SCC was also observed for all traits assessed by images (R^* , L^* and a^*). The significant correlations between seasons for each trait related to seed coat color (SCC, R^* , L^* , and a^*) were of high magnitude ($r \geq 0.84$), and the percentage of the complex part of the line-by-season interaction was lower than 50% as well (Table 2). These values are indicators that there was no pronounced change in the ranking of the lines regarding these traits in the two seasons (Cruz et al., 2012; Elias et al., 2016; Leon et al., 2016; Begna, 2022). Therefore, the assessment by images (R^* , L^* , and a^*) exhibited an line-by-season interaction pattern similar to that observed in the SCC assessment, especially the mean trait of the pixels of the seed coat in the a^* channel, which had correlation values between the two seasons and percentage of the complex part of the line-by-season interaction very close to that observed for SCC.

The correlations between the means of the lines for SCC and the means of the R^* , L^* , and a^* traits were significant and very close to -1, both in individual seasons and in their mean

(Figure 3). These correlations are negative, as higher SCC scores are associated with lower R^* , L^* , and a^* values. The higher values in the R^* and a^* channels are related to greater red intensity in the images, while the higher the pixel value in the L^* channel, the lighter the object in the image (Léon et al., 2006; Lucak and Plataniotis, 2018). Therefore, the rankings of the lines based on image assessments are similar to those observed in visual assessment. These results show that selection regarding seed coat color can be based on assessments made through images.

The correlation of the means of the lines for R^* , L^* , and a^* with GA were significant, but of lower magnitude. These correlations were lower than -0.48 both in individual seasons and in their mean, which demonstrates that there is an association between seed coat color and GA. This was also reinforced by the correlations that were significant and greater than 0.66 between SCC and GA. These correlations were positive, because the lower the values of these traits, the better the commercial appearance, preferentially light red beans of desirable size and shape. Therefore, it appears that color greatly affects the assessment of GA; consequently, selection based only on color may successfully provide lines with good commercial grain appearance.

The rainy season provided the best discrimination of lines, due to the formation of the largest number of groups in the Scott-Knott test for all the traits assessed (Figure 4). The GA trait was that which least discriminated the lines, mainly in the winter season and in the mean of seasons, when only three groups were observed. This lower discrimination may have occurred due to the lower experimental accuracy of this trait because of the higher CV and LSD values (Table 1). Furthermore, the complex interaction of line-by-season may be consuming part of the variability between lines in the mean of the seasons, resulting in less discrimination under these conditions. The clustering pattern of the means of the SCC scores of the lines was similar to the clustering of the means of the pixels in the R^* and L^* channels. However, the use of the mean of the pixels in the a^* channel showed greater ability in discriminating the lines compared to the other traits obtained visually and by images. This may be related to the lower CV and LSD values observed for this trait in each of the seasons and in their mean.

The assessments of the intensities in the R^* , L^* , and a^* channels demonstrated high experimental accuracy, high correlation with the SCC assessment, and capacity superior to visual assessment in discriminating red bean lines. However, these channels have their own particularities that make them useful under different conditions. The L^* channel, representing the variation between black and white, has potential in assessing seed coat darkening. This channel has been used to evaluate the seed coat darkening of bean lines from the carioca

commercial group using colorimeters (Siqueira et al., 2014; Arns et al., 2018; Spitti et al., 2019; Almeida et al., 2021) and images (Silva et al., 2024). Laurent et al. (2010) found that the L* channel has the potential to assist in assessing the seed coat darkening of red bean lines.

The main particularity of the R* and a* channels is to represent variations in the intensity of the red color (Léon et al., 2006; Lucak and Plataniotis, 2007). Unlike the R* channel of the RGB color space, the a* channel is free from the effect of image luminosity, because in the CIELAB color space, the L* channel represents only luminosity, and channels a* and b* represent only color variations. This may be one of the reasons why the mean of the a* channel of the seed coats is the most precise and accurate measurement and provides greater discrimination of lines.

Red bean breeding aims to obtain high yielding lines, beans with good commercial appearance, and upright plants that allow mechanized harvesting, resistance to diseases, and adaptation to different locations, years, and crop seasons. As such, recommending a new cultivar of this commercial type is a complex process. Among the various traits of importance in this process, grain appearance stands out, as commercial acceptance will determine the success of a new cultivar. Studies show that this trait is decisive when consumers purchase beans (Ribeiro et al., 2019; Kläsener et al., 2020). Selection of lines in red bean breeding programs must be highly accurate for success in obtaining superior lines. Yet selection of lines for commercial appearance of the beans can be biased when based on visual assessment. Therefore, the use of methodologies, such as the use of images, to achieve greater accuracy in the process is of great importance.

5. Conclusions

Image assessment showed high experimental accuracy, high correlation with visual assessments, and high capacity for discriminating red bean lines. Therefore, this form of assessment has potential for use in assisting red bean breeding programs in selection of lines with desirable seed coat color.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Table 1. Origin of the red common bean lines and cultivars evaluated in the two experiments conducted in the municipality of Lavras during the 2021 winter and rainy seasons.

ID	Lines	Origin ^a	ID	Lines	Origin ^a
1	CIAT 20	CIAT	25	CIAT 2	CIAT
2	Vermelhinho	CRIOULA	26	VR 25	UFV
3	CIAT 16	CIAT	27	CIAT 15	CIAT
4	CIAT 10	CIAT	28	UFV 16	UFV
5	UFV 1	UFV	29	CIAT 9	CIAT
6	UFV 11	UFV	30	CIAT 8	CIAT
7	VR 22	UFV	31	UFV 5	UFV
8	CIAT 13	CIAT	32	CIAT 7	CIAT
9	UFV 8	UFV	33	OVR	UFV
10	UFV 3	UFV	34	CIAT 3	CIAT
11	BRSMG MARTE	CONVÊNIO	35	CIAT 14	CIAT
12	Safira	EMBRAPA	36	UFV 2	UFV
13	Ouro Vermelho	CONVÊNIO	37	CIAT 6	CIAT
14	UFV 15	UFV	38	VR 26	UFV
15	UFV 6	UFV	39	CIAT 5	CIAT
16	CIAT 23	CIAT	40	CIAT 11	CIAT
17	UFV 4	UFV	41	CIAT 4	CIAT
19	UFV 14	UFV	42	UFV 13	UFV
20	CIAT 12	CIAT	43	CIAT 22	CIAT
21	CIAT 17	CIAT	44	CIAT 18	CIAT
22	CIAT 21	CIAT	45	UFV 7	UFV
23	UFV 10	UFV	46	UFV 12	UFV
24	CIAT 19	CIAT	47	UFV 9	UFV

^aCIAT, International Center for Tropical Agriculture; UFV, Federal University of Viçosa; EMBRAPA, Brazilian Agricultural Research Corporation; AGREEMENT, collaborative agreement established among the Federal University of Viçosa, Federal University of Lavras, Brazilian Agricultural Research Corporation, and Agricultural Research Company of Minas Gerais.

Supplementary Table 2. Means of seed coat appearance (GA) and color (SCC), and pixel values in the R (RGB), L, and a (Lab) channels from the evaluation experiment of red common bean lines in the 2021 winter season.

LINHAGENS	GA	SCC	R	L	a
VR 25	1.50 a	1.33 a	71.85 b	37.97 b	153.41 a
CIAT-6	1.83 a	1.17 a	77.92 a	43.30 a	154.57 a
VR 26	2.00 a	1.17 a	81.70 a	45.76 a	155.26 a
CIAT-18	2.17 a	2.33 b	63.09 c	33.07 c	150.24 b
BRSMG Marte	2.33 a	1.50 a	78.33 a	45.06 a	153.43 a
CIAT-5	2.33 a	1.83 b	69.22 b	37.35 b	152.05 b
CIAT-9	2.33 a	2.00 b	71.51 b	38.84 b	152.51 b
CIAT-2	2.50 a	1.00 a	79.07 a	45.13 a	153.95 a
CIAT-4	2.50 a	1.17 a	81.97 a	46.50 a	155.33 a
VR 22	2.50 a	1.33 a	81.20 a	45.29 a	155.09 a
UFV-13	2.50 a	3.00 c	57.39 c	29.40 c	148.20 c
Vermelhinho	2.67 b	1.17 a	84.15 a	46.98 a	156.20 a
UFV-5	2.67 b	1.50 a	74.73 b	41.32 b	153.52 a
OVR	2.67 b	1.50 a	81.45 a	47.13 a	153.78 a
CIAT-12	2.75 b	3.67 c	53.71 c	27.63 d	146.40 c
CIAT-19	2.83 b	1.83 b	71.30 b	39.17 b	152.24 b
CIAT-14	2.83 b	2.17 b	68.98 b	37.34 b	151.78 b
CIAT-11	2.83 b	2.17 b	63.82 c	33.79 c	150.33 b
CIAT-8	2.83 b	2.33 b	67.05 b	36.06 b	151.39 b
UFV-11	2.83 b	2.50 b	63.99 c	33.77 c	150.45 b
CIAT-20	2.83 b	2.83 c	59.49 c	30.99 c	148.85 b
CIAT-7	2.83 b	3.17 c	58.80 c	31.42 c	148.00 c
Ouro Vermelho	3.00 b	1.00 a	86.22 a	50.21 a	155.23 a
CIAT-21	3.00 b	2.00 b	79.90 a	45.78 a	154.11 a
UFV-7	3.00 b	2.67 b	62.61 c	33.03 c	149.79 b
UFV-14	3.00 b	2.83 c	60.58 c	32.30 c	148.64 c
CIAT-10	3.00 b	3.00 c	61.15 c	31.78 c	149.54 b

CIAT-15	3.00 b	3.00 c	63.10 c	34.88 c	148.52 c
CIAT-22	3.00 b	3.17 c	56.46 c	29.27 c	147.63 c
CIAT-1	3.17 b	1.50 a	83.53 a	48.11 a	154.85 a
CIAT-3	3.17 b	2.67 b	64.51 c	34.68 c	150.08 b
CIAT-23	3.33 b	3.50 c	56.74 c	29.31 c	147.52 c
UFV-8	3.33 b	4.00 d	50.30 d	26.28 d	144.87 d
UFV-10	3.50 c	3.83 d	47.28 d	23.69 d	144.19 d
CIAT-16	3.67 c	4.00 d	50.99 d	26.40 d	145.22 d
UFV-12	3.83 c	3.83 d	51.06 d	26.98 d	144.73 d
UFV-1	3.83 c	4.33 d	45.43 d	22.74 d	143.11 e
UFV-6	4.00 c	3.33 c	67.26 b	36.20 b	151.58 b
CIAT-17	4.00 c	4.00 d	50.95 d	26.36 d	144.98 d
CIAT-13	4.00 c	4.17 d	46.45 d	23.99 d	143.04 e
UFV-15	4.00 c	4.17 d	44.84 d	23.59 d	142.27 e
UFV-2	4.17 c	4.00 d	45.20 d	23.46 d	142.59 e
UFV-4	4.17 c	4.50 d	40.88 d	21.05 d	140.74 e
UFV-3	4.37 c	4.83 d	40.12 d	21.49 d	140.01 e
UFV-16	4.50 c	4.33 d	48.13 d	26.43 d	142.85 e
UFV-9	4.50 c	4.83 d	43.88 d	23.14 d	141.55 e
Safira	5.00 c	3.50 c	58.08 c	31.82 c	146.72 c

Supplementary Table 3. Means of seed coat appearance (GA) and color (SCC), and pixel values in the R (RGB), L, and a (Lab) channels from the evaluation experiment of red common bean lines in the 2021 rainy season.

LINHAGENS	GA	SCC	R	L	a
CIAT-1	1.83 a	1.92 b	66.13 c	37.09 c	149.65 c
Ouro Vermelho	2.17 a	1.50 a	65.92 c	36.65 c	149.81 c
BRSMG Marte	2.25 a	2.42 b	64.68 c	36.08 c	149.32 c
CIAT-2	2.67 b	2.17 b	66.58 c	37.68 c	149.80 c
UFV-5	2.67 b	2.42 b	66.12 c	36.70 c	149.97 c
CIAT-5	2.83 c	2.08 b	62.42 c	33.94 d	148.99 c
VR 22	3.00 c	1.33 a	81.45 b	47.06 b	154.19 a
CIAT-18	3.00 c	2.25 b	61.50 c	33.44 d	148.72 d
CIAT-19	3.17 c	2.17 b	64.21 c	35.58 c	149.35 c
UFV-7	3.17 c	2.42 b	60.69 c	33.96 d	147.64 d
CIAT-22	3.17 c	3.08 c	57.62 d	30.92 d	147.39 d
VR 25	3.33 c	1.33 a	84.10 a	48.78 a	154.97 a
CIAT-6	3.33 c	1.33 a	78.69 b	45.65 b	153.15 b
OVR	3.33 c	1.50 a	84.60 a	49.94 a	154.57 a
CIAT-11	3.33 c	2.25 b	66.40 c	36.97 c	150.19 c
CIAT-8	3.33 c	2.33 b	67.75 c	38.67 c	149.95 c
UFV-11	3.33 c	2.83 b	61.64 c	33.85 d	148.46 d
CIAT-20	3.33 c	3.17 c	61.00 c	33.73 d	148.01 d
UFV-8	3.33 c	3.25 c	56.39 d	30.68 d	146.44 e
CIAT-23	3.33 c	3.58 d	51.91 e	28.34 e	144.37 f
VR 26	3.50 c	1.08 a	89.29 a	52.30 a	156.31 a
CIAT-4	3.50 c	1.67 a	78.49 b	45.95 b	152.84 b
CIAT-10	3.50 c	2.13 b	66.63 c	37.08 c	150.02 c
CIAT-9	3.50 c	2.33 b	65.88 c	36.77 c	149.49 c
CIAT-14	3.50 c	2.58 b	65.01 c	36.00 c	149.63 c
CIAT-17	3.67 d	3.92 d	48.33 e	25.95 e	143.14 f
CIAT-15	3.67 d	3.92 d	47.88 e	26.11 e	142.77 f

Vermelhinho	3.75 d	1.25 a	84.51 a	49.98 a	154.42 a
UFV-16	3.75 d	3.75 d	52.27 e	29.15 d	144.32 f
CIAT-21	3.83 d	1.67 a	76.58 b	43.79 b	152.97 b
UFV-13	3.83 d	3.33 c	52.55 e	27.70 e	145.56 e
UFV-14	3.83 d	3.67 d	53.71 d	29.51 d	145.07 e
UFV-10	4.00 d	3.67 d	51.01 e	27.92 e	144.26 f
CIAT-7	4.00 d	3.83 d	49.57 e	27.21 e	143.45 f
CIAT-12	4.00 d	4.08 e	46.14 f	25.15 f	142.08 g
UFV-2	4.00 d	4.25 e	39.00 f	21.96 f	138.81 h
CIAT-3	4.17 d	3.42 c	56.34 d	31.11 d	146.09 e
CIAT-16	4.17 d	3.50 c	56.83 d	31.79 d	146.09 e
UFV-9	4.17 d	4.50 e	43.35 f	24.19 f	140.52 g
UFV-15	4.17 d	4.75 e	41.07 f	23.21 f	139.60 h
UFV-6	4.33 e	2.58 b	66.06 c	36.84 c	150.15 c
UFV-1	4.33 e	3.88 d	49.76 e	27.62 e	143.30 f
UFV-12	4.50 e	4.33 e	44.89 f	25.23 f	141.16 g
CIAT-13	4.50 e	4.58 e	41.06 f	23.44 f	139.26 h
UFV-4	4.50 e	4.58 e	42.09 f	24.30 f	139.71 h
UFV-3	4.50 e	4.67 e	39.09 f	22.41 f	138.52 h
Safira	5.00 e	3.92 d	49.81 e	27.57 e	143.35 f

Supplementary Table 4. Means of seed coat appearance (GA) and color (SCC), and pixel values in the R (RGB), L, and a (Lab) channels from the evaluation experiments of red common bean lines in the 2021 winter season.

LINHAGENS	GA	SCC	R	L	a
VR 26	2.75 a	1.13 a	85.50 a	49.03 a	155.78 a
BRSMG Marte	2.29 a	1.96 b	71.50 b	40.57 b	151.38 b
VR 25	2.42 a	1.33 a	77.98 a	43.37 a	154.19 a
CIAT-1	2.50 a	1.71 a	74.83 a	42.60 a	152.25 a
Ouro Vermelho	2.58 a	1.25 a	76.07 a	43.43 a	152.52 a
CIAT-6	2.58 a	1.25 a	78.31 a	44.48 a	153.86 a
CIAT-2	2.58 a	1.58 a	72.82 b	41.40 b	151.88 b
CIAT-5	2.58 a	1.96 b	65.82 b	35.64 b	150.52 b
CIAT-18	2.58 a	2.29 b	62.29 b	33.26 c	149.48 c
UFV-5	2.67 a	1.96 b	70.43 b	39.01 b	151.74 b
VR 22	2.75 a	1.33 a	81.33 a	46.18 a	154.64 a
CIAT-9	2.92 b	2.17 b	68.70 b	37.81 b	151.00 b
CIAT-4	3.00 b	1.42 a	80.23 a	46.22 a	154.08 a
OVR	3.00 b	1.50 a	83.03 a	48.54 a	154.17 a
CIAT-19	3.00 b	2.00 b	67.76 b	37.37 b	150.80 b
CIAT-11	3.08 b	2.21 b	65.11 b	35.38 b	150.26 b
CIAT-8	3.08 b	2.33 b	67.40 b	37.37 b	150.67 b
UFV-7	3.08 b	2.54 b	61.65 b	33.49 c	148.71 c
UFV-11	3.08 b	2.67 b	62.82 b	33.81 c	149.46 c
CIAT-20	3.08 b	3.00 c	60.24 c	32.36 c	148.43 c
CIAT-22	3.08 b	3.13 c	57.04 c	30.09 c	147.51 d
CIAT-14	3.17 b	2.38 b	67.00 b	36.67 b	150.71 b
UFV-13	3.17 b	3.17 c	54.97 c	28.55 c	146.88 d
Vermelhinho	3.21 b	1.21 a	84.33 a	48.48 a	155.31 a
CIAT-10	3.25 b	2.56 b	63.89 b	34.43 c	149.78 c
CIAT-15	3.33 b	3.46 c	55.49 c	30.50 c	145.64 d
CIAT-23	3.33 b	3.54 c	54.32 c	28.82 c	145.95 d

UFV-8	3.33 b	3.63 c	53.35 c	28.48 c	145.66 d
CIAT-12	3.38 b	3.88 d	49.93 d	26.39 d	144.24 d
CIAT-21	3.42 b	1.83 b	78.24 a	44.79 a	153.54 a
UFV-14	3.42 b	3.25 c	57.14 c	30.91 c	146.85 d
CIAT-7	3.42 b	3.50 c	54.19 c	29.32 c	145.72 d
CIAT-3	3.67 c	3.04 c	60.42 c	32.89 c	148.09 c
UFV-10	3.75 c	3.75 c	49.15 d	25.80 d	144.23 d
CIAT-17	3.83 c	3.96 d	49.64 d	26.16 d	144.06 d
CIAT-16	3.92 c	3.75 c	53.91 c	29.09 c	145.65 d
UFV-1	4.08 c	4.10 d	47.60 d	25.18 d	143.20 e
UFV-2	4.08 c	4.13 d	42.10 d	22.71 d	140.70 f
UFV-15	4.08 c	4.46 d	42.96 d	23.40 d	140.94 f
UFV-16	4.13 c	4.04 d	50.20 d	27.79 c	143.59 d
UFV-6	4.17 c	2.96 c	66.66 b	36.52 b	150.86 b
UFV-12	4.17 c	4.08 d	47.98 d	26.10 d	142.94 e
CIAT-13	4.25 c	4.38 d	43.75 d	23.71 d	141.15 f
UFV-4	4.33 c	4.54 d	41.49 d	22.68 d	140.23 f
UFV-9	4.33 c	4.67 d	43.62 d	23.67 d	141.03 f
UFV-3	4.43 c	4.75 d	39.60 d	21.95 d	139.27 f
Safira	5.00 c	3.71 c	53.95 c	29.69 c	145.04 d

Artigo 2 - Redigido conforme as normas da revista Crop Science - Versão preliminar	
Título do artigo:	Optimization of image-based phenotyping of common bean lines for seed size and shape.
Autores:	Danilo Araujo Gomes Jussara Mencalha Guilherme Almeida Domingos Jaque José Eulário Lampi Dique Constantino Rico Raimundo Artur Vinícius Quintão Carneiro
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ARTIGO 2 - Optimization of image-based phenotyping of common bean lines for seed size and shape

ABSTRACT

Seed size and shape are key traits for the commercialization of new common bean lines, as they determine the acceptance of a new cultivar in the consumer market. The evaluation of these traits in breeding programs is traditionally performed using calipers. Although precise, this approach is labor-intensive and time-consuming. Image analysis is an alternative to accelerate this evaluation; however, information on how to properly conduct such assessments is lacking. Therefore, the objective of this study was to determine the optimal number of seeds per plot for image-based evaluation of common bean lines with respect to seed size- and shape-related traits. Common bean lines from the carioca and black commercial groups were evaluated in five different experiments. In each experiment, images of 100 seeds per plot were captured, and seed length, width, length-to-width ratio (J index), area, and perimeter were assessed. Subsequently, sample sizes ranging from 1 to 100 seeds per plot were generated. For each of these sample sizes, 1,000 resamplings were performed in each experiment, in which seeds were randomly drawn from each plot. Accuracy and the coefficient of variation were calculated for each of the 1,000 resamplings for each sample size. In addition, Pearson's correlation between the mean of each line in each resampling and the mean obtained with 100 seeds per plot was estimated. The average values of each parameter across the 1,000 resamplings were subjected to quadratic plateau regression as a function of the number of seeds per plot. The gradual increase in sample size resulted in accuracy, coefficient of variation, and Pearson's correlation estimates progressively closer to those observed with 100 seeds per plot. However, the experiments showed that image-based phenotyping of bean lines for seed length, width, J index, area, and perimeter should be conducted with at least 17 seeds per plot to ensure precise evaluations and reliable selection decisions.

Keywords: *Phaseolus vulgaris* L.; computer vision; sample size

1 INTRODUCTION

The large variability inherent to traits related to common bean (*Phaseolus vulgaris* L.) seeds has been consistently reported. Substantial differences are observed among bean lines derived from the Mesoamerican and Andean gene pools. These differences range from the type of phaseolin present in the seeds to traits associated with seed appearance (Singh et al., 1991;

Chiorato et al., 2006; Valentini et al., 2018; Carvalho Paulino et al., 2021). Furthermore, within these beans there are commercial groups that differ mainly in seed coat color, presence of mottling, size, and shape (Delfini et al., 2017). These traits are essential for the commercialization of common bean seeds, as they determine the acceptance of a new cultivar in the consumer market (Ribeiro et al., 2019). Therefore, seed appearance is one of the most important traits in common bean.

New lines to be recommended must present seeds according to the desirable standards of the respective commercial group. For example, in carioca and black beans, which are the most consumed in Brazil, the preferred characteristics include the absence of yellow halo, absence of flattened seeds, absence of gloss, elliptical shape, and a mean 100-seed weight ranging from 23 to 25 g (Ramalho and Abreu, 2015). The 100-seed weight is an important yield component, showing positive correlations with grain yield (Katuuramu et al., 2020), and is also associated with higher volumetric expansion and cooking yield (Perina et al., 2018). Although particularities exist among different commercial groups of Mesoamerican beans, in Brazil, consumer preference is generally for Mesoamerican seeds with elliptical shape, semi-full grain filling, and medium-to-large seed size (Carbonell et al., 2010; Ribeiro et al., 2019).

Bean breeding programs, attentive to consumer market demands, are continuously dedicated to evaluating and selecting progenies and lines based on seed appearance (Dias et al., 2021; Portilla et al., 2022; Martins et al., 2023; Jamanca-Gonzales et al., 2024). Visual assessment is the main approach employed by breeding programs to select lines with better seed appearance (Rocha et al., 2019; Melo et al., 2020; Martins et al., 2023). Due to its rapidity and practicality, even when a large number of lines must be evaluated, this methodology is widely used. However, one of the limitations of visual seed appearance assessment is the need for experienced evaluators, since this trait consists of a combination of attributes such as seed coat color and darkening, presence or absence of yellow halo, presence or absence of gloss, seed size, and shape (Ramalho et al., 1998).

The evaluation of seed size and shape can also be conducted individually using calipers (Mambrin et al., 2015). Although this method provides high experimental precision (Santos et al., 2016; Cardoso, 2022; Kläsener et al., 2022), it is highly time- and labor-demanding (Li et al., 2019; Liu et al., 2020; Marrano and Moyers, 2022; Yang et al., 2021), which limits its application when a large number of lines must be assessed. Consequently, most studies evaluating bean seed size and shape have been carried out with small sample sizes and a limited number of lines (Mambrin et al., 2015; Ribeiro et al., 2021; Kläsener et al., 2022).

The computer vision approach has shown potential for assessing several traits relevant to plant breeding, such as seed color and size (Kara et al., 2013; Miranda et al., 2023), fruits (Nagamatsu et al., 2021; Rifna et al., 2021), tubers (Haque et al., 2021; Ccopi et al., 2024), among other structures. Silva et al. (2024) developed a computer vision methodology to evaluate carioca bean lines for seed coat color and darkening, which proved to be more precise and accurate than visual evaluation of these traits. Similarly, Cardoso (2022) demonstrated that the evaluation of bean seed size and shape using digital images achieves experimental precision comparable to that of caliper-based measurements. Moreover, this author highlighted that image-based evaluation of these traits is faster and allows automation of processes in breeding programs.

The implementation of new methodologies, such as those based on image analysis, requires well-established protocols. Seed size and shape measurements using calipers have been performed with 10 (Ribeiro et al., 2021; Kläsener et al., 2022) to 50 seeds (Steckling, 2023). Nevertheless, a minimum of 10 seeds is recommended to characterize seed size and flattening degree in beans when using calipers (Steckling, 2023). However, this type of information is not yet available for measurements based on digital image analysis. Therefore, the objective of this study was to determine the optimal number of seeds per plot for evaluating seed size- and shape-related traits in common bean lines using digital images.

2 MATERIALS AND METHODS

2.1 Experimental description

The study involved the evaluation of common bean lines belonging to the carioca and black commercial groups, assessed in five different cropping seasons during 2019 and 2020 (Table 1). The experiments were conducted at the Scientific and Technological Development Center of the Federal University of Lavras (UFLA), located at 918 m altitude, 21°58' S latitude, and 42°22' W longitude. The experimental design was a randomized complete block design with three replications. Plots consisted of two 2-m rows, with a row spacing of 0.6 m and a planting density of 15 seeds per meter. Fertilization and crop management practices were performed according to the recommendations for common bean cultivation in the state of Minas Gerais, as described by Carneiro et al. (2015).

Table 1. Identification of the experiments, cropping season and year, experimental location, and commercial group of common bean lines used in the analyses.

Experiment ID	Season/year	N° of genotypes	Location	Comercial group
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1	Winter/2019	63	Lavras	Carioca
2	Winter/2019	63	Lavras	Carioca
3	Rainy/2019	50	Lavras	Preto
4	Dry/2020	64	Lavras	Carioca
5	Dry/2020	48	Lavras	Preto

2.2 Image acquisition and processing

The evaluation of seed traits including area, perimeter, length, width, and length-to-width ratio (J index) was performed using images of each plot, with a sample of 100 seeds. Images were obtained at the Image Analysis Laboratory (LAS-UFLA) of the Seed Sector, Department of Agriculture, Federal University of Lavras (UFLA). Images, in “.jpg” format and 5184×3456 resolution, were captured using the GroundEye® system, model S800 (Figures 1a and 1b) (Andrade et al., 2024; Reis et al., 2023). This device consists of a capture module with an acrylic tray, a high-resolution camera, and uniform illumination.

The “.jpg” images captured were subjected to segmentation to isolate the seed region and remove the background (Figure 1c). At this stage, the image initially obtained in the RGB color system (additive color model formed by red, green, and blue tones) was converted to the Lab color space (represented by luminosity, red/green coordinates, and yellow/blue coordinates) to facilitate segmentation. The image matrix from the b channel was used as the basis for applying the thresholding segmentation technique proposed by Otsu (1975). This method was employed to determine the threshold value that minimized the sum of intraclass variances of image pixels, thereby separating pixels into seed and background groups.

Thus, from the image containing only the seeds, it was possible to isolate each seed and obtain measurements of area, perimeter, length, width, and J index in pixel units (Figure 7d). Subsequently, these measurements were converted to millimeters for length, width, and perimeter, and to mm^2 for seed area. For this conversion, a white reference square of known size ($20 \text{ mm} \times 20 \text{ mm}$) was included in the image, which enabled quantification of pixel dimensions and determination of a conversion factor to the desired unit. All image processing was carried out using the OpenCV library (Howse, 2013) and the Scikit-Image library (Van der Walt et al., 2014) in the Python programming language (Van Rossum and Drake, 2014).

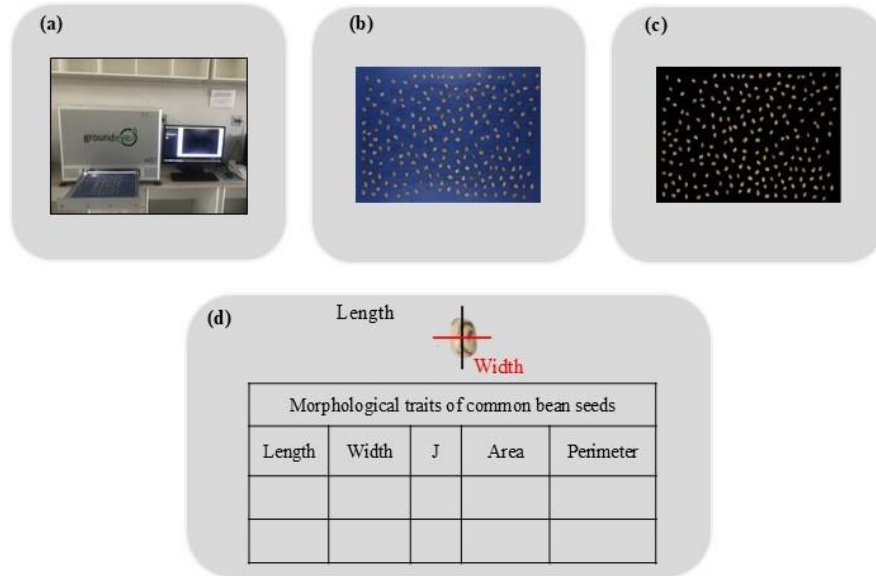


Figure 1. Overview of the image acquisition process of common bean line seeds (a–b), image segmentation (c), and calculation of seed morphological traits (d).

2.3 Bootstrapping procedure and precision statistics

From the data of 100 seeds per plot in each experiment for seed length, width, J index, area, and perimeter, resamplings were performed with sample sizes ranging from 1 to 100 seeds. For each of these sample sizes, 1000 resamplings were carried out in each experiment, in which seeds were randomly drawn from each plot. The information for each trait at the plot level was represented by the mean of the seeds sampled in each resampling.

Based on the plot means obtained for different sample sizes, individual analyses of variance were performed for each trait in each experiment according to the following model:

$$Y_{ij} = m + B_j + C_i + \varepsilon_{ij},$$

where Y_{ij} is the observed mean value of genotype i in block j ($j = 1, 2, 3$); m is the overall constant; B_j is the random effect of block j ; C_i is the fixed effect of genotype i ; and ε_{ij} is the experimental error associated with the observation Y_{ij} .

For each resampling, the following parameters were estimated: accuracy ($\hat{r}_{gg}$), coefficient of variation (CV), and Pearson's correlation (PC) between the line means from each resampling at each plot size and the genotype means obtained with 100 seeds per plot. Accuracy was estimated using the formula: $\hat{r}_{gg} = \left(1 - \frac{1}{F}\right)^{1/2}$, according to Resende (2009), where F is the ratio between the mean squares of genotype and residual from the individual analysis of variance in each resampling. The CV was calculated using the expression: $CV (\%) = 100 \frac{\sqrt{MSR}}{\bar{S}}$ where MSR is the mean square of the residual, and $\bar{S}$ is the overall mean of the genotypes for

the trait under evaluation. These parameters were used as criteria to determine the minimum number of seeds per plot.

2.4 Plot size definition

The mean values of the parameters ($\hat{r}_{\hat{g}g}$, CV, and PC) across the 1000 resamplings were subjected to a quadratic plateau regression as a function of plot size. This procedure allowed identifying the minimum plot size that maintains the same accuracy, experimental precision, and ranking consistency as the evaluation performed with 100 seeds per plot for each of the traits analyzed. The following quadratic plateau regression model was adopted:

$$Y = \begin{cases} \beta_0 + \beta_1 X + \beta_2 X^2 + \varepsilon, & \text{se } X \leq X_0 \\ Y_0 + \varepsilon, & \text{se } X > X_0 \end{cases}$$

$$X_0 = -\frac{\beta_1}{2\beta_2}$$

$$Y_0 = \frac{\beta_1^2}{4\beta_2}$$

where Y is the value of the parameter for X seeds per sample; β_0 , β_1 and β_2 are the regression coefficients for the quadratic segment; X is the number of seeds per sample ($i=1,2,3,\dots, 100$); X_0 is the number of seeds at which the quadratic model levels off into a plateau; Y_0 is the parameter value on the y axis corresponding to the plateau; and $\varepsilon_i \sim N(0, \sigma^2)$ is the random error associated with Y (Kaps & Lamberson, 2009). Model fit was evaluated using the coefficient of determination (R^2).

The minimum number of seeds per sample in each experiment was defined based on the x -axis point (number of seeds per sample) located at the junction between the quadratic segment and the regression plateau. Statistical analyses were performed using the *easynls* package (Arnhold, 2017) and the R software (R Core Team, 2020).

3 RESULTS

The effect of lines was significant for all traits analyzed in all individual analyses of variance when considering 100 seeds per sample in all experiments. Under these conditions, accuracies ranged from 0.79 to 0.98 for seed length, 0.89 to 0.98 for width, 0.76 to 0.98 for the J index, 0.87 to 0.97 for seed area, and 0.74 to 0.98 for seed perimeter. Regarding CVs, values were equal to or lower than 19.23% across all traits evaluated in the five experiments. CVs ranged from 1.67 to 9.28% for length, 1.52 to 3.97% for width, 1.10 to 15.42% for the J index,

2.96 to 8.41% for seed area, and 1.56 to 19.23% for seed perimeter (Supplementary Figures 1–5).

The effect of lines was also significant in all 1000 resamplings for sample sizes equal to or greater than three seeds per plot for area, length, and width, and eight seeds for perimeter and J index. Thus, in part of the individual analyses of variance from resamplings with fewer than three and eight seeds per plot, respectively, a nonsignificant line effect was observed.

Values of $r\hat{r}_{gg}$ increased as the number of seeds evaluated per plot increased (Supplementary Figures 1–5). This increase in the number of seeds per plot also resulted in reduced CVs. The mean values of $r\hat{r}_{gg}$ and CV from the 1000 resamplings showed greater variation when the sample size was fewer than 12 seeds. Resamplings with five or more seeds presented accuracies greater than 0.70 across all traits and experiments. The highest mean accuracy values, when using 12 or more seeds per plot, were observed in Experiment 4, with accuracies above 0.95. In the same experiment, when employing this number of seeds per resampling, the lowest CVs were observed, with values below 4.40%. Except for perimeter and the J index in Experiments 1 and 2, all other traits in all experiments presented CVs below 9.7% when eight or more seeds per plot were used (Supplementary Figures 1–5).

The number of seeds in each experimental unit also affected the ranking of lines. The mean Pearson's correlations between line means obtained with different numbers of seeds per experimental unit and those obtained with 100 seeds increased as the number of seeds per plot increased. PC values from the 1000 resamplings were greater than 0.90 when using at least 14 seeds for width, 8 seeds for area and J index, and 7 seeds for perimeter and length (Supplementary Figures 1–5).

All quadratic plateau models fitted for the parameters ($r\hat{r}_{gg}$, CV, PC) across all experiments presented coefficients of determination greater than 0.91. The regression coefficients (β_0 , β_1 e β_2) of these models were statistically different from zero ($p < 0.01$) according to the t -test (Supplementary Tables 1–5).

The regression models for accuracy of seed length showed plateau values between 8.56 and 9.50 seeds per sampling (Figure 2). The accuracies estimated by the regression models at these plateau values were higher than 0.79. Moreover, when considering the mean accuracy of the 1000 resamplings using between 8 and 10 seeds per plot, values also remained above 0.79 in all experiments (Supplementary Figure 1). The regression models for the coefficient of variation of seed length reached a plateau between 8.09 and 15.25 seeds per plot (Figure 2), with estimated CV values ranging from 1.85 to 9.4%. When evaluating between 9 and 16 seeds

per sampling, the mean coefficients of variation from the 1000 resamplings ranged from 2.32 to 9.69% across all experiments (Supplementary Figure 1). The plateaus estimated in the regression models for PC of seed length varied from 8.46 to 9.88 seeds per plot (Figure 2). The correlation coefficients estimated by the regression models at these plateau values were 0.99. Similar results were observed for the mean of this parameter from the 1,000 resamplings when 10 seeds per plot were used to represent the plot mean (Supplementary Figure 1).

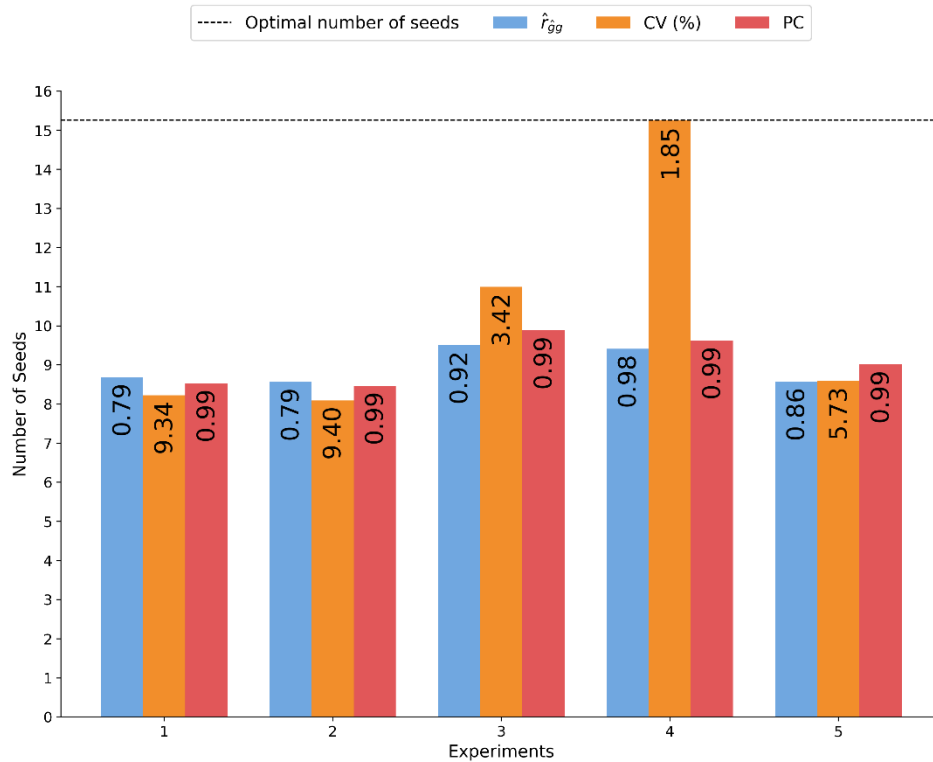


Figure 2. Plateau (number of seeds) of the regression models for mean accuracy ($\hat{r}_{gg}$), mean coefficient of variation (CV), and Pearson's correlation (PC) from 1000 resamplings for each number of seeds per plot in five experiments evaluating common bean seed length based on digital image analysis.

The numbers of seeds observed at the plateaus of the regression models for accuracy of seed width ranged from 9.12 to 13.86 (Figure 3). Within this range, the accuracies estimated by the models varied from 0.89 to 0.97. The mean accuracies of the 1000 resamplings for seed width, considering between 10 and 14 seeds per plot, ranged from 0.87 to 0.95 (Supplementary Figure 2). The regression models for CV of seed width showed a plateau between 9.84 and 16.13 seeds per plot (Figure 3), with estimated CV values ranging from 1.69 to 4.08. When using between 10 and 17 seeds per plot, the mean coefficients of variation of the 1,000 resamplings for seed width ranged from 2.09 to 4.57 (Supplementary Figure 2). For PC, the plateaus observed for seed width ranged from 9.55 to 14.04 (Figure 3). The correlation

coefficients estimated by the regression models at these plateau values were 0.99. Moreover, in all experiments, whenever 15 seeds per plot were used, correlations above 0.90 were observed.

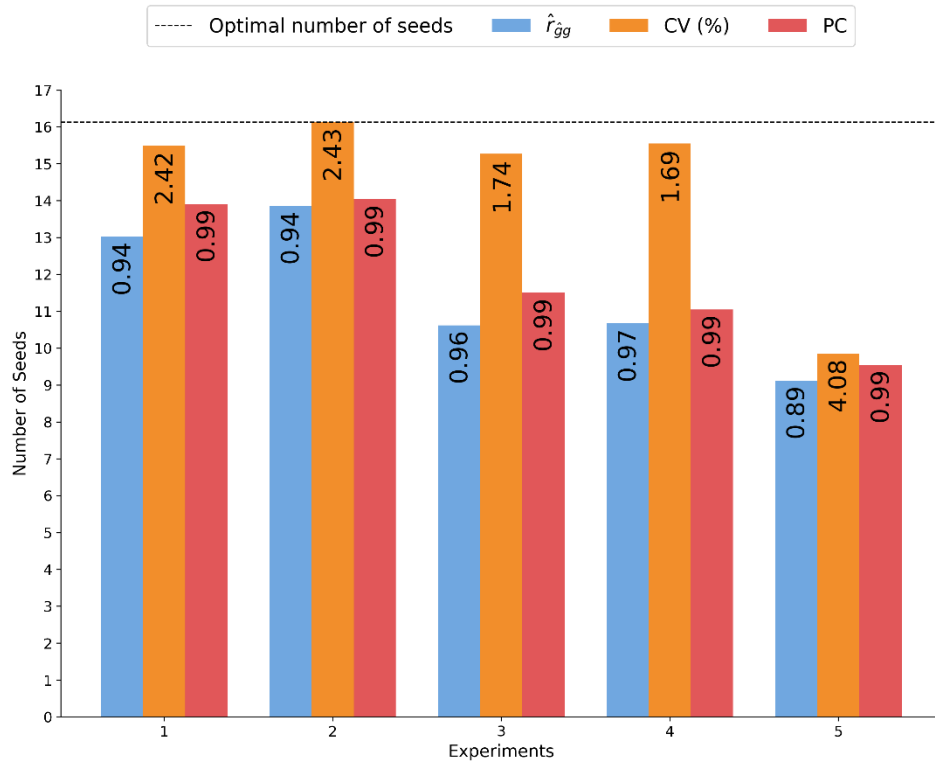


Figure 3. Plateau (number of seeds) of the regression models for mean accuracy ($\hat{r}_{gg}$), mean coefficient of variation (CV), and Pearson's correlation (PC) from 1000 resamplings for each number of seeds per plot in five experiments evaluating common bean seed width based on digital image analysis.

The plateau of the regression model for accuracy of the J index ranged from 6.89 to 10.33 seeds per plot (Figure 4), with estimated values for this parameter ranging from 0.76 to 0.98, respectively. The mean accuracy of the 1000 resamplings for this trait, when considering between 7 and 11 seeds per plot, ranged from 0.75 to 0.96 across all experiments (Supplementary Figure 3). The regression models for the CV of the J index presented plateaus between 6.35 and 16.55 seeds per plot (Figure 4), with estimated values of this parameter ranging from 1.25 to 15.50. When considering between 7 and 17 seeds per plot, the mean coefficients of variation of the 1000 resamplings ranged from 1.62 to 15.63 (Supplementary Figure 3). The plateaus estimated in the regression models for PC of this trait ranged from 7.28 to 10.57 seeds per plot (Figure 4). The PC values estimated by the regression models at these plateau values, as well as those obtained from the mean of the 1000 resamplings using 7 or more seeds per plot, were approximately equal to 0.99 (Supplementary Figure 3).

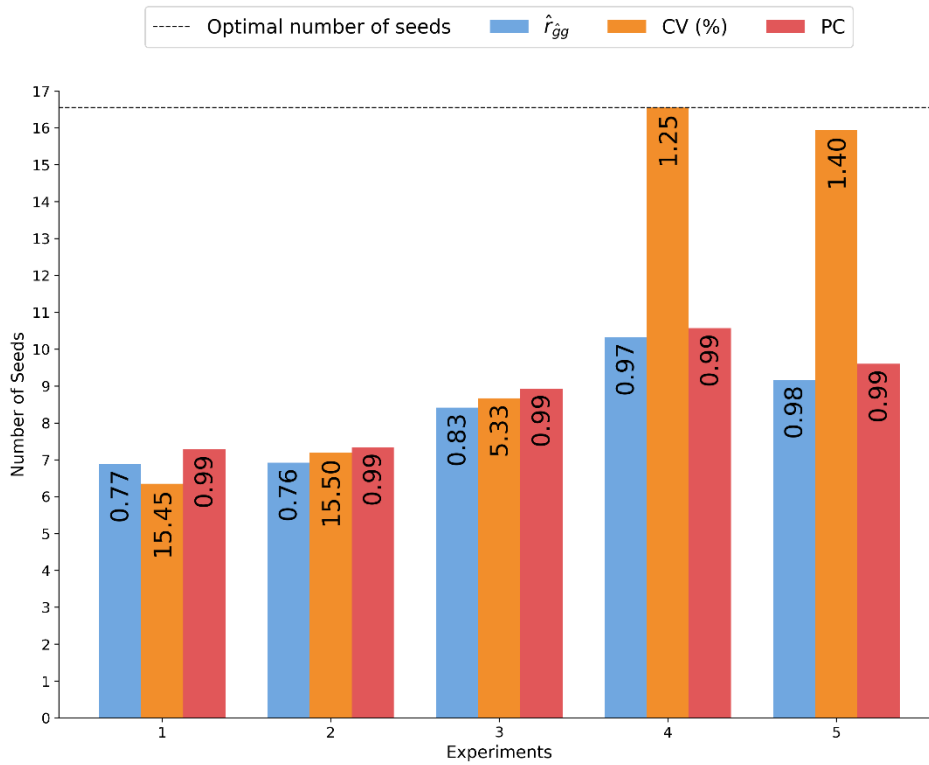


Figure 4. Plateau (number of seeds) of the regression models for mean accuracy ($\hat{r}_{gg}$), mean coefficient of variation (CV), and Pearson's correlation (PC) from 1000 resamplings for each number of seeds per plot in five experiments evaluating the J index of common bean seeds based on digital image analysis.

The regression models for accuracy of seed area showed plateau values between 8.8 and 10.1 seeds per plot (Figure 5). The accuracies estimated by the regression models at these plateau values were higher than 0.87. Moreover, when considering the mean accuracy of the 1000 resamplings using between 9 and 11 seeds per plot, values for this parameter also remained above 0.84 (Supplementary Figure 4). The regression models for the CV of this trait reached a plateau between 10 and 14.78 seeds per plot (Figure 5), with estimated CV values ranging from 3.27 to 8.63, respectively. When evaluating between 10 and 15 seeds per plot, the mean coefficients of variation of the 000 resamplings ranged from 4.12 to 9.51 (Supplementary Figure 4). The plateau estimated in the regression models for PC of this trait across experiments ranged from 9.67 to 10.63 seeds per plot (Figure 5). The correlation coefficients estimated by the regression models at these plateau values were 0.99. Similar results, with correlations above 0.92, were observed for the mean of this parameter from the 1000 resamplings when 11 seeds per plot were used to represent the plot mean (Supplementary Figure 4).

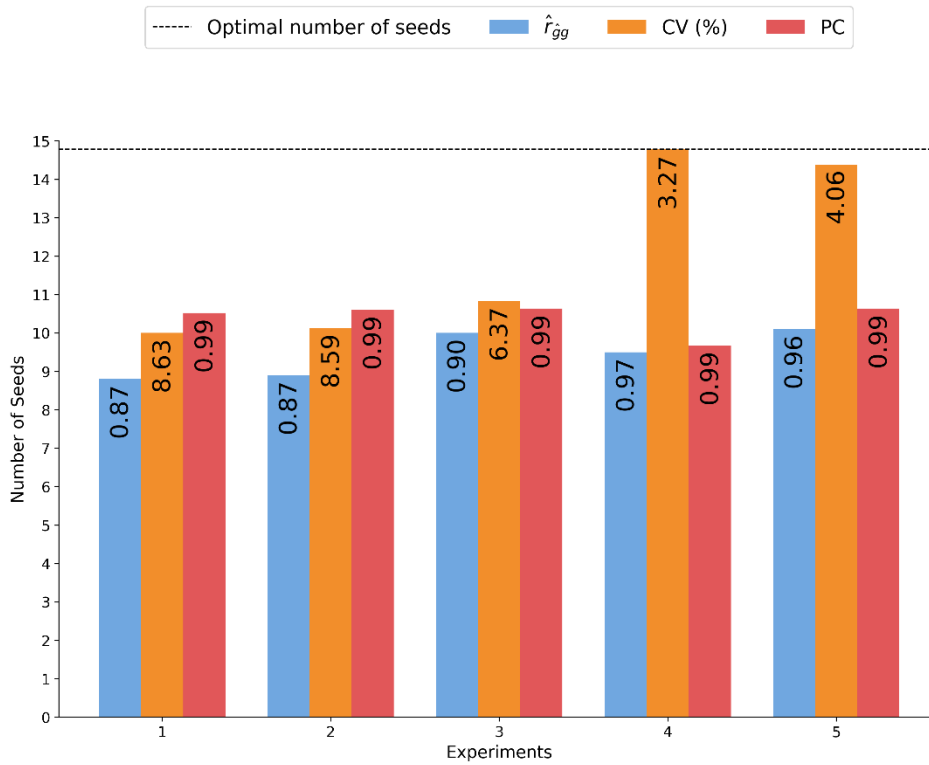


Figure 5. Plateau (number of seeds) of the regression models for mean accuracy ($\hat{r}_{gg}$), mean coefficient of variation (CV), and Pearson's correlation (PC) from 1000 resamplings for each number of seeds per plot in five experiments evaluating common bean seed area based on digital image analysis.

A similar trend to that observed for the other traits was also found for seed perimeter, with plateau values in the regression models for accuracy ranging from 7.4 to 9.61 seeds per plot (Figure 6). The accuracies estimated for these seed numbers ranged from 0.74 to 0.98. The mean accuracy of the 1000 resamplings for seed perimeter, when considering between 8 and 10 seeds per plot, ranged from 0.74 to 0.96 (Supplementary Figure 5). The regression models for CV of this trait reached a plateau between 7.25 and 14.9 seeds per plot (Figure 6), with estimated CV values ranging from 1.73 to 19.25%. When evaluating between 8 and 15 seeds per plot, the mean coefficients of variation of the 1000 resamplings for seed perimeter ranged from 2.19 to 19.34 (Supplementary Figure 5). The plateaus estimated in the regression models for PC of perimeter ranged from 7.15 to 9.85 seeds per plot (Figure 6). The correlation coefficients estimated by the regression models at these plateau values were 0.99.

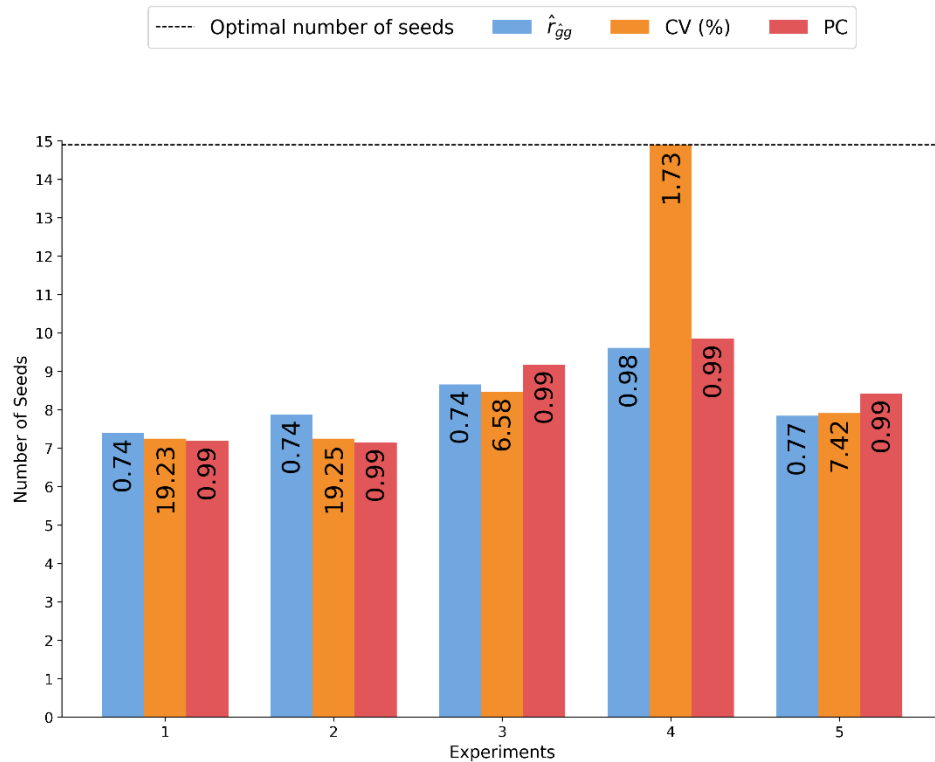


Figure 6. Plateau (number of seeds) of the regression models for mean accuracy ($\hat{r}_{gg}$), mean coefficient of variation (CV), and Pearson's correlation (PC) from 1000 resamplings for each number of seeds per plot in five experiments evaluating common bean seed perimeter based on digital image analysis.

4 DISCUSSION

Accuracy estimates for all traits and experiments, when using 100 seeds per plot, were higher than 0.79. According to Resende and Duarte (2007), such values indicate that the evaluation of physical traits of common bean seeds by digital image analysis, using 100 seeds per plot, provides high to very high experimental precision. High accuracy values indicate a strong correlation between the genetic values estimated from phenotypic data and the true values of the lines, thus providing greater reliability in selection. Ribeiro et al. (2019) and Kläsener et al. (2022), evaluating common bean cultivars for seed length, width, thickness, and shape using a digital caliper, reported accuracy values higher than 0.86. These results demonstrate that the use of 100 seeds per plot in image-based evaluations allows accurate selection of common bean lines for these traits.

When using 100 seeds per plot across all experiments, the lowest CV estimates were observed for seed width and length. Santos et al. (2016), in evaluating 14 common bean genotypes, reported values close to those observed in the present study, with CVs of 4.84% for width and 3.19% for length when evaluating 10 seeds per plot using a digital caliper. Steckling (2023), in evaluating 50 seeds of 105 common bean cultivars with a digital caliper across three

different seasons, reported CVs ranging from 4.46 to 5.49 for length, 4.55 to 5.35 for width, and 4.98 to 4.46 for the J index. Despite differences in the equipment used for evaluation, these results indicate the possibility of reducing the number of seeds per plot for assessing the physical traits of bean seeds.

In the present study, CV values for the J index below 5.27% were observed in most experiments. Cardoso (2022) reported that CV values obtained using both caliper and digital image analysis for the J index, seed length, and width were very similar, evidencing comparable precision between the two methodologies. The J index is an important trait in common bean that enables the classification of seed shape in lines, as proposed by Puerta Romero (1961). This author classified genotypes by seed shape as follows: spherical (1.16–1.42), elliptical (1.43–1.65), oblong/short kidney (1.66–1.85), oblong/medium kidney (1.86–2.00), and oblong/long kidney (> 2.00). Consumer preference is mainly for beans with elliptical seed shape (Ribeiro et al., 2019).

Similar results to those observed in the present study regarding CV for seed area and perimeter were reported by Duc et al. (2023), who evaluated 164 soybean accessions through digital image analysis, finding CVs of 12.47% for area and 6.72% for perimeter. Supporting our results, Cardoso (2022), aiming to validate the GroundEye system (model S800) for digital image-based measurement of seed size and shape in common bean, concluded that phenotyping of seed size and shape using images, in addition to providing speed and efficiency, is as precise and accurate as phenotyping using calipers.

In this context, one of the main objectives of common bean breeding programs regarding seed appearance is the search for cultivars with larger seeds, which are more accepted by consumers (Lemos et al., 2020). Thus, an alternative to the simultaneous use of seed length and width in selection is the use of seed area and perimeter. These measurements allow determination of whether a line has seeds of sufficient size to meet the demands of producers, traders, and consumers. The evaluation of seed area and perimeter is only feasible through digital image phenotyping, since calipers do not directly provide these measurements. This limitation reinforces the importance of digital phenotyping in the analysis of common bean seeds. The CV values obtained indicate that seed physical traits were determined with high experimental precision, contributing to greater efficiency in the selection of superior common bean cultivars. Overall, the values were consistently low, which is indicative of adequate precision for evaluating these traits.

The effect of lines was significant in the individual analyses of variance when using 100 seeds per sampling in all experiments. This effect was also significant in the individual analyses

of variance of the 1000 resamplings when using eight or more seeds per plot, considering all experiments and traits evaluated. These results indicate that even with a reduced number of seeds per plot, it was possible to detect at least one contrast among line means different from zero. The nonsignificant effect observed in part of the analyses was due to the high estimate of residual variance, resulting from the large variation among plot means that received the same treatment. This phenomenon occurred when fewer than eight seeds per plot were used. In this scenario, high CV values were also observed, which is a consequence of the increased residual variance (Supplementary Figures 1–5).

Different statistical approaches have been employed to determine sample size. The experimental coefficient of variation (Mencalha et al., 2024a; Mencalha et al., 2024b; Bittencourt et al., 2023; Steckling, 2023; Tartaglia et al., 2021) and accuracy (Mencalha et al., 2024a; Mencalha et al., 2024b; Steckling, 2023; Souza et al., 2022) have been the most commonly used metrics in recent studies. CV, defined as the residual standard deviation relative to the mean, is widely used to verify the precision of experiments. Low-precision experiments increase the likelihood of type II error, that is, failing to detect differences among treatments when they actually exist (Faria Filho et al., 2016). Accuracy, in turn, represents the correlation between the true genetic value of a line and the phenotypic value obtained in the experiments (Resende and Duarte, 2007). These parameters are influenced both by environmental variation affecting each plot and by plot size. Mencalha et al. (2024b) used the parameters $\hat{r}_{gg}$, CV, and PC to determine the optimal number of replications and plants per plot for assessing angular leaf spot severity in bean lines under artificial inoculation.

By analyzing $\hat{r}_{gg}$, CV, and PC statistics in response to increasing seed numbers per plot, it was observed that the variation between the minimum and maximum values of the 1000 resamplings decreased when 12 or more seeds per plot were used (Supplementary Figures 1–5). According to Bittencourt et al. (2023), the smaller the sample size, the farther the lower and upper limits deviate from the true values, creating larger intervals that favor overestimation and/or underestimation of results. Thus, it was observed that excessive reduction in the number of seeds per plot may result in less precise evaluations and, consequently, less accurate selections. Supporting this study, Souza et al. (2022), in evaluating the influence of the number of soybean plants sampled per experimental unit on grain yield determination, reported that as the number of plants increased, the maximum and minimum values of these statistics approached the true values. In the present study, mean CV values decreased as the number of seeds per sampling increased, while mean accuracy and correlation coefficient values increased

(Supplementary Figures 1–5). Thus, the gradual increase in sample size brought the estimates of $\hat{r}_{gg}$, CV, and PC closer to the estimates obtained when using 100 seeds per plot.

The number of seeds evaluated also affected the ranking of lines for all traits analyzed in this study. The correlation between line means obtained with different sample sizes and the mean obtained with 100 seeds was higher when more seeds were used per plot. The mean correlation of the 1000 resamplings, when employing at least 14 seeds per plot, was greater than 0.90 across all experiments and traits, indicating that the ranking of cultivars was not strongly affected when 14 or more seeds per plot were used. Thus, selection for seed physical traits can be based on image-based evaluations containing at least 14 seeds.

The quadratic plateau regression models showed a high degree of fit for the mean values $\hat{r}_{gg}$, CV, and PC as a function of plot size in all experiments. The coefficients of determination of the models for these three parameters were greater than 0.90 (Supplementary Tables 1–5). These results indicate that 90% of the variation in each parameter was explained by plot size, thus demonstrating the robustness of the models adopted for decision-making regarding the number of seeds per plot in the use of image-based phenotyping of bean seeds for analyzing seed physical traits.

The sample sizes associated with the plateau, considering all parameters and experiments, ranged from 9.5 to 15.25 for seed length, 13.92 to 16.13 for width, 10.33 to 16.55 for the J index, 10.1 to 14.78 for area, and 9.61 to 14.9 for seed perimeter. Thus, variations in the number of seeds per sampling were small across experiments and traits, indicating that plot size is more strongly associated with the parameters evaluated than with the growing season in which the experiment was conducted. Supporting the present study, Mencalha et al. (2024a), aiming to determine the minimum number of plants per plot required to assess anthracnose severity in 78 bean cultivars, reported the need for at least 5 to 6 plants when considering $\hat{r}_{gg}$, CV, and PC parameters. Similarly to what was observed in the present study, they found small variation in the number of plants to be evaluated per plot.

The maximum number of seeds observed at the plateaus of the regression models obtained for all traits in all experiments represents the sufficient number of seeds to evaluate them via digital image analysis. As the sample size varied according to the parameter and trait evaluated, the maximum value obtained across all traits was defined as the sufficient number of seeds per plot for evaluating common bean lines for seed physical traits. Therefore, in evaluations of common bean lines for seed physical traits, it is recommended to use 17 seeds per plot. The results indicate that it is possible to use images containing fewer than 100 seeds

per plot, enabling rapid analysis of a large number of common bean lines, potentially increasing population size and selection intensity, ensuring adequate genetic diversity and precise estimates of genetic value, and maximizing genetic gain in breeding programs. Supporting this study, Neu et al. (2023), comparing three models for estimating the required sample size to evaluate multicollinearity in rye morphological traits, reported that the quadratic plateau model showed the best fit for all multicollinearity indicators, with adjusted coefficients of determination above 0.90, indicating a more accurate modeling of the relationship between sample size and multicollinearity degree.

Seed visual appearance is decisive in the commercialization of common bean. This is one of the attributes of seed commercial quality, involving traits such as seed length, width, size, shape, and color (Ribeiro et al., 2019). In this sense, bean breeders have sought to develop lines that, in addition to agronomic traits, also present good seed commercial appearance (Bezerra et al., 2021). The results of this study showed that image-based phenotyping of common bean seeds, in addition to providing speed and efficiency, is precise and accurate, and therefore can be routinely used by bean breeding programs. Researchers generally use about 10 seeds per replication to evaluate seed physical traits of bean lines using calipers (Santos et al., 2016; Kläsener et al., 2022). This sample size per plot may not be representative of the line and may cause errors in selecting superior lines. However, there are not enough studies dedicated to this determination. In the present study, based on resamplings conducted in five experiments, it was identified that 17 seeds per plot are necessary for accurate and precise evaluation of common bean lines for seed physical traits using images.

Phenotyping of lines by image analysis for seed physical traits proved to be a viable alternative for line selection, as it is precise, accurate, rapid, and automated, allowing the evaluation of a larger number of lines and enabling the assessment of traits that cannot be directly obtained using calipers. The information generated in this study aims to optimize experimental design, not only highlighting the importance of image-based phenotyping of common bean seeds but also emphasizing the importance of a sufficient sample size to allow reliable statistical inference, optimizing evaluation time, labor allocation, and making decision-making more accurate.

5 CONCLUSION

Image-based phenotyping of common bean lines for seed length, width, J index, area, and perimeter should be conducted with at least 17 seeds per plot to ensure precise evaluations and allow accurate selection.

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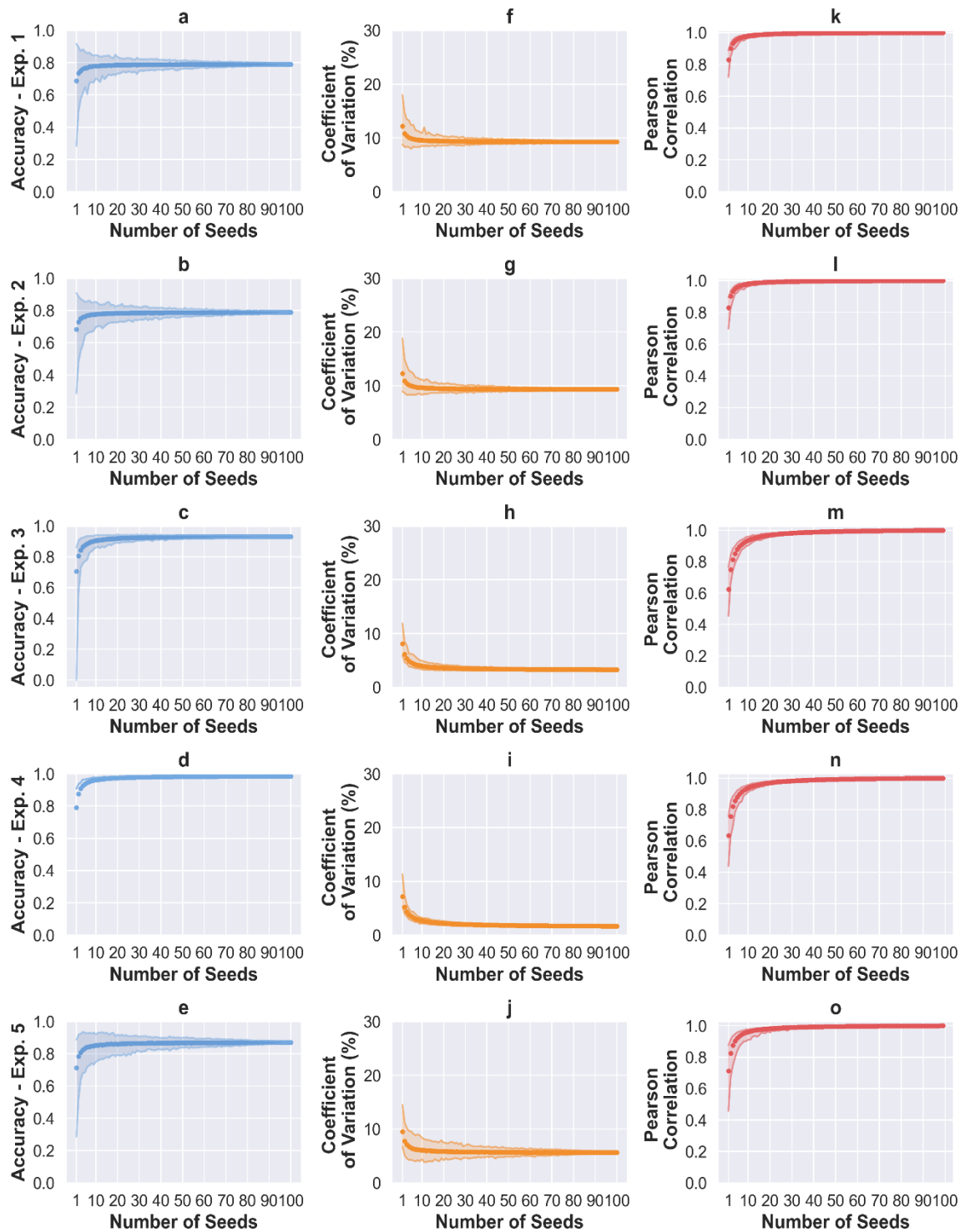
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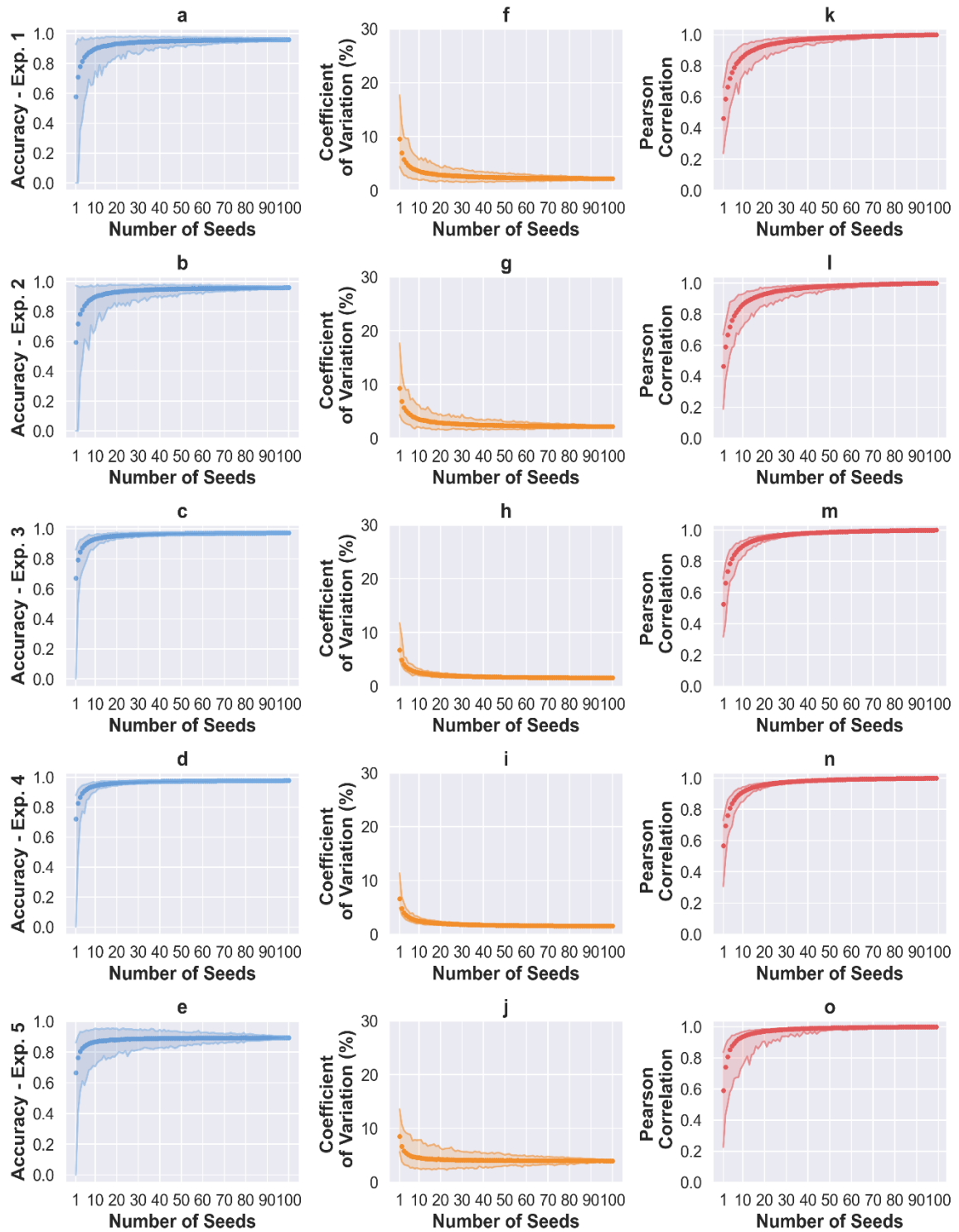
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Supplementary material

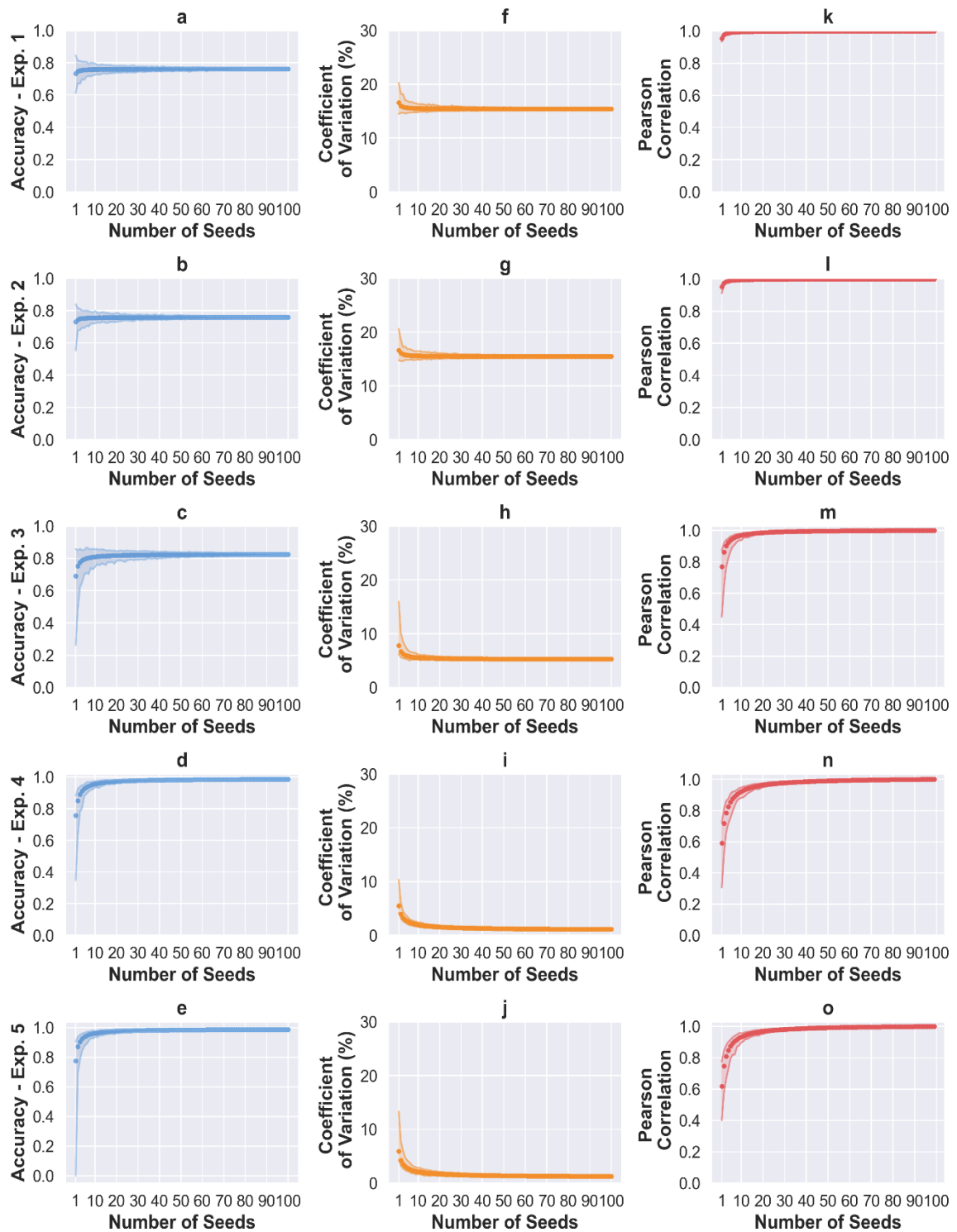
Artigo 2 - Optimization of image-based phenotyping of common bean lines for seed size and shape.



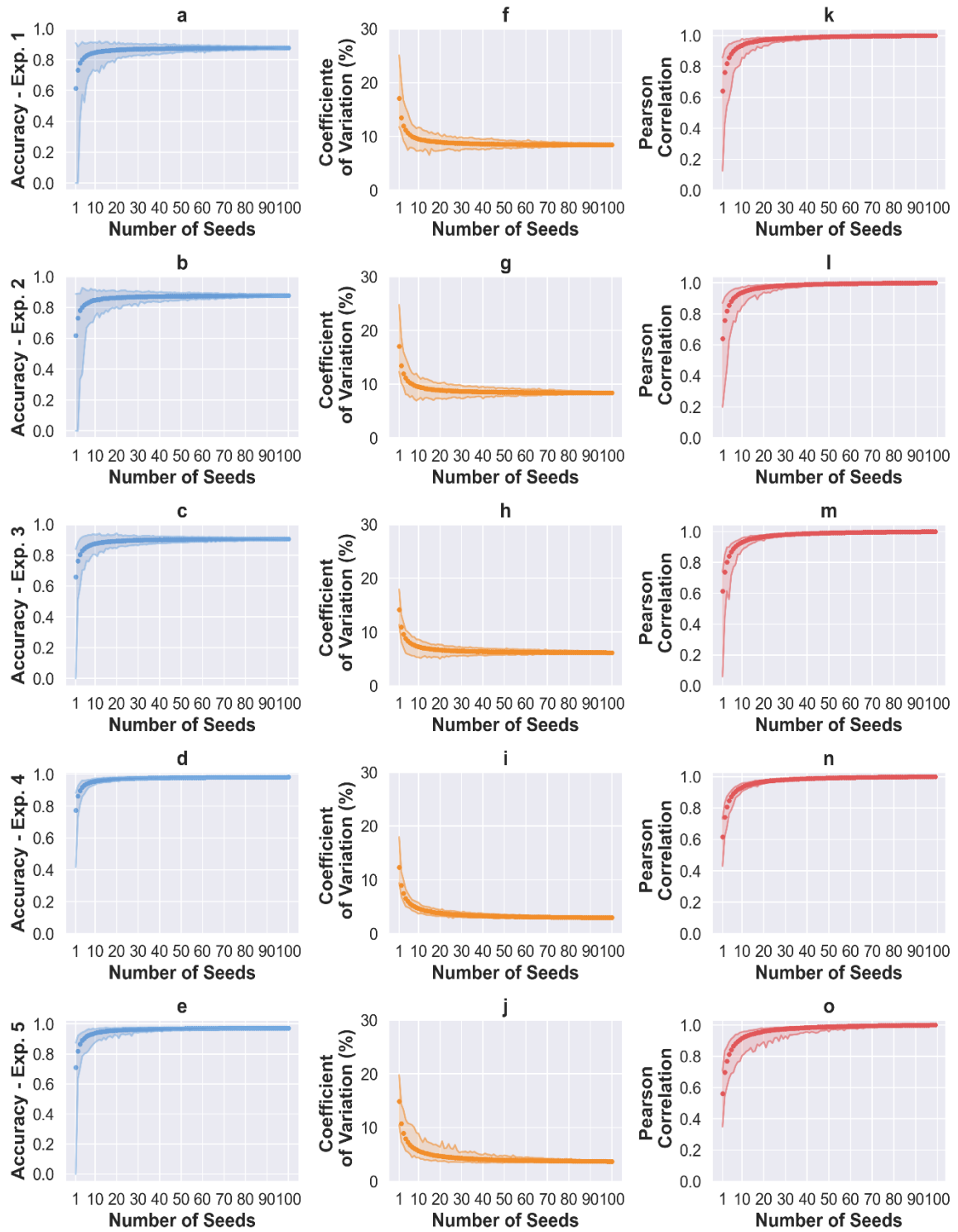
Supplementary Figure 1. Minimum, mean, and maximum values of accuracy (a, b, c, d, e), coefficient of variation (f, g, h, i, j), and Pearson's correlation (k, l, m, n, o) for the length trait, based on 1000 resamplings for each number of seeds per plot in five experiments. The central line indicates the mean of the parameters across 1000 resamplings per number of seeds per plot, and the outer lines indicate the minimum and maximum values.



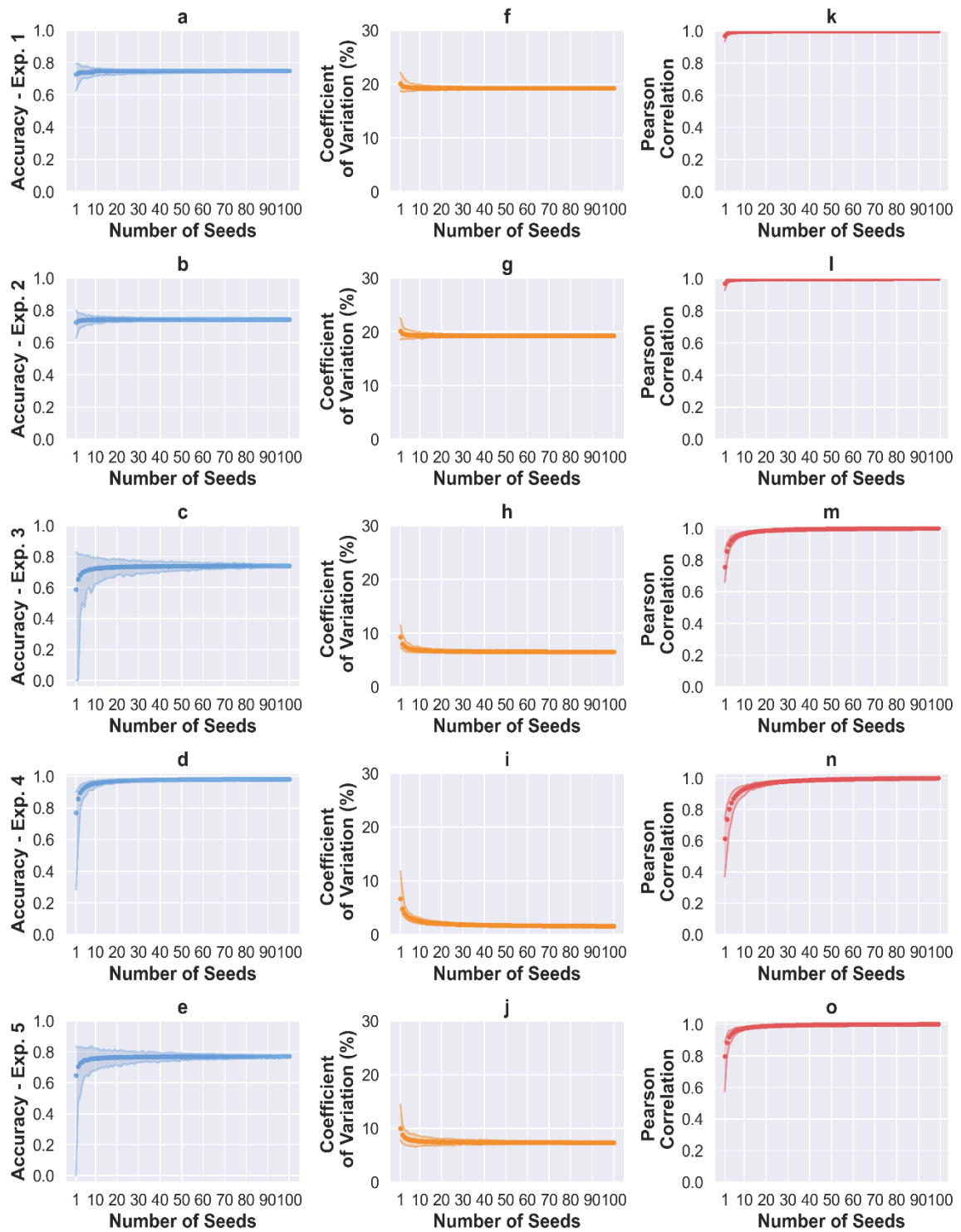
Supplementary Figure 2. Minimum, mean, and maximum values of accuracy (a, b, c, d, e), coefficient of variation (f, g, h, i, j), and Pearson's correlation (k, l, m, n, o) for the width trait, based on 1000 resamplings for each number of seeds per plot in five experiments. The central line indicates the mean of the parameters across 1000 resamplings per number of seeds per plot, and the outer lines indicate the minimum and maximum values.



Supplementary Figure 3. Minimum, mean, and maximum values of accuracy (a, b, c, d, e), coefficient of variation (f, g, h, i, j), and Pearson's correlation (k, l, m, n, o) for the J coefficient trait, based on 1000 resamplings for each number of seeds per plot in five experiments. The central line indicates the mean of the parameters across 1000 resamplings per number of seeds per plot, and the outer lines indicate the minimum and maximum values.



Supplementary Figure 4. Minimum, mean, and maximum values of accuracy (a, b, c, d, e), coefficient of variation (f, g, h, i, j), and Pearson's correlation (k, l, m, n, o) for the area trait, based on 1000 resamplings for each number of seeds per plot in five experiments. The central line indicates the mean of the parameters across 1000 resamplings per number of seeds per plot, and the outer lines indicate the minimum and maximum values.



Supplementary Figure 5. Minimum, mean, and maximum values of accuracy (a, b, c, d, e), coefficient of variation (f, g, h, i, j), and Pearson's correlation (k, l, m, n, o) for the perimeter trait, based on 1000 resamplings for each number of seeds per plot in five experiments. The central line indicates the mean of the parameters across 1000 resamplings per number of seeds per plot, and the outer lines indicate the minimum and maximum values.

Supplementary Table 1. Quadratic plateau regression models for mean accuracy ($\hat{r}_{gg}$), coefficient of variation

Exp ^a	$\hat{r}_{gg}$				CV (%)				PC			
	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2
1	0.60**	0.06**	-0.003**	0.91	17.37**	-1.75**	0.09**	0.91	0.76**	0.04**	-0.002**	0.91
2	0.60**	0.06**	-0.003**	0.91	17.29**	-1.71**	0.08**	0.91	0.76**	0.04**	-0.002**	0.91
3	0.64**	0.05**	-0.002**	0.91	14.16**	-1.44**	0.06**	0.91	0.75**	0.05**	-0.002**	0.92
4	0.76**	0.04**	-0.002**	0.91	11.33**	-1.09**	0.04**	0.90	0.79**	0.04**	-0.002**	0.91
5	0.70**	0.05**	-0.002**	0.91	13.72**	-1.34**	0.05**	0.89	0.74**	0.04**	-0.002**	0.91

(CV), and Pearson's correlation (PC) based on 1000 resamplings for each number of seeds per plot in five experiments for the evaluation of seed area.

^aExperiments.

β_0 , β_1 , e β_2 : Regression coefficients of the quadratic plateau regression model.

** Significant at the 1% probability level by the t-test.

r^2 : Coefficient of determination of the model.

Supplementary Table 2. Quadratic plateau regression models for mean accuracy ($\hat{r}_{gg}$), coefficient of variation (CV), and Pearson's correlation (PC) based on 1000 resamplings for each number of seeds per plot in five experiments for the evaluation of seed perimeter.

Exp ^a	$\hat{r}_{gg}$				CV (%)				PC			
	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2
1	0.72**	0.01**	-0.0003**	0.91	20.22**	-0.27**	0.018**	0.91	0.97**	0.01**	-0.0004**	0.91
2	0.72**	0.01**	-0.0003**	0.93	29.29**	-0.28**	0.019**	0.92	0.97**	0.01**	-0.0004**	0.91
3	0.57**	0.04**	-0.002**	0.91	9.57**	-0.70**	0.040**	0.91	0.81**	0.04**	-0.002**	0.91
4	0.75**	0.04**	-0.002**	0.91	6.07**	-0.58**	0.020**	0.89	0.78**	0.04**	-0.002**	0.91
5	0.63**	0.03**	-0.002**	0.91	10.37**	-0.74**	0.040**	0.91	0.84**	0.03**	-0.002**	0.91

^aExperiments.

β_0 , β_1 , e β_2 : Regression coefficients of the quadratic plateau regression model.

** Significant at the 1% probability level by the t-test.

r^2 : Coefficient of determination of the model.

Supplementary Table 3. Quadratic plateau regression models for mean accuracy ($\hat{r}_{gg}$), coefficient of variation (CV), and Pearson's correlation (PC) based on 1000 resamplings for each number of seeds per plot in five experiments for the evaluation of seed length.

Exp ^a	$\hat{r}_{gg}$				CV (%)				PC			
	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2
1	0.67**	0.02**	-0.001**	0.91	12.52**	-0.77**	0.050**	0.92	0.88**	0.02**	-0.001**	0.91
2	0.67**	0.02**	-0.001**	0.91	12.64**	-0.80**	0.040**	0.91	0.87**	0.02**	-0.001**	0.91
3	0.69**	0.04**	-0.002**	0.91	8.05**	-0.84**	0.030**	0.90	0.77**	0.04**	-0.020**	0.91
4	0.77**	0.04**	-0.002**	0.91	6.53**	-0.61**	0.020**	0.89	0.80**	0.04**	-0.002**	0.91
5	0.70**	0.04**	-0.002**	0.91	9.90**	-0.97**	0.050**	0.91	0.81**	0.04**	-0.002**	0.91

^aExperiments.

β_0 , β_1 , e β_2 : Regression coefficients of the quadratic plateau regression model.

** Significant at the 1% probability level by the t-test.

r^2 : Coefficient of determination of the model.

Supplementary Table 4. Quadratic plateau regression models for mean accuracy ($\hat{r}_{gg}$), coefficient of variation (CV), and Pearson's correlation (PC) based on 1000 resamplings for each number of seeds per plot in five experiments for the evaluation of seed width.

Exp ^a	$\hat{r}_{gg}$				CV (%)				PC			
	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2
1	0.59**	0.05**	-0.002**	0.91	8.65**	-0.80**	0.025**	0.90	0.66**	0.04**	-0.001**	0.92
2	0.61**	0.04**	-0.001**	0.91	8.42**	-0.74**	0.020**	0.90	0.67**	0.04**	-0.001**	0.92
3	0.66**	0.05**	-0.002**	0.91	6.14**	-0.57**	0.010**	0.89	0.72**	0.04**	-0.002**	0.91
4	0.72**	0.04**	-0.002**	0.91	5.98**	-0.55**	0.010**	0.90	0.75**	0.04**	-0.002**	0.91
5	0.65**	0.05**	-0.002**	0.91	8.72**	-0.94**	0.050**	0.91	0.73**	0.05**	-0.002**	0.91

^aExperiments.

$\beta_0, \beta_1, \text{ e } \beta_2$: Regression coefficients of the quadratic plateau regression model.

** Significant at the 1% probability level by the t-test.

r^2 : Coefficient of determination of the model.

Supplementary Table 5. Quadratic plateau regression models for mean accuracy ($\hat{r}_{gg}$), coefficient of variation (CV), and Pearson's correlation (PC) based on 1000 resamplings for each number of seeds per plot in five experiments for the evaluation of the seed J coefficient.

Exp ^a	$\hat{r}_{gg}$				CV (%)				PC			
	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2	β_0	β_1	β_2	r^2
1	0.72**	0.01**	-0.001**	0.91	16.90**	-0.46**	0.036**	0.90	0.96**	0.01**	-0.001**	0.91
2	0.72**	0.01**	-0.001**	0.91	16.82**	-0.37**	0.025**	0.91	0.96**	0.01**	-0.001**	0.91
3	0.67**	0.03**	-0.002**	0.91	8.01**	-0.61**	0.030**	0.91	0.84**	0.03**	-0.001**	0.91
4	0.75**	0.04**	-0.002**	0.91	4.84**	-0.43**	0.013**	0.89	0.77**	0.04**	-0.002**	0.91
5	0.76**	0.04**	-0.002**	0.91	5.29**	-0.48**	0.015**	0.89	0.79**	0.04**	-0.002**	0.91

^aExperiments.

$\beta_0, \beta_1, \text{ e } \beta_2$: Regression coefficients of the quadratic plateau regression model.

** Significant at the 1% probability level by the t-test.

r^2 : Coefficient of determination of the model.

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FINAL CONSIDERATIONS

The strategies analyzed in this thesis for evaluating common bean seed traits reveal significant advances in the use of digital methodologies to support breeding programs. The use of computer vision to evaluate seed coat color in red bean lines demonstrated high precision, with a strong association between pixel values and the assigned visual scores. The prominence of the *a* channel in differentiating lines confirms the potential of digital phenotyping as an efficient tool to optimize the selection process in breeding programs.

Similarly, the investigation of the minimum number of seeds required for image-based evaluations of size- and shape-related traits showed that analyses can be conducted with smaller sample sizes without compromising experimental precision. Defining 17 seeds per plot as the sufficient number for consistent evaluations contributes to resource optimization, ensuring speed, reliability, and greater capacity for large-scale assessments.

Thus, the results of this thesis demonstrate that digital phenotyping applied to common bean seed appearance is a viable, precise, and automated alternative. These advances reduce the time and labor required for evaluations while providing greater detail and accuracy in seed characterization. Therefore, the studies presented here reinforce the importance of integrating image-based methodologies into bean breeding programs, supporting the selection of superior genotypes and the adoption of cultivars that meet consumer market demands.