CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Optimization of the assessment of anthracnose severity in artificially inoculated common beans

Jussara Mencalha[1*], Mariana Andrade Dias[2], Elaine Aparecida de Souza[2], Vinicius Quintão Carneiro[2], Fernanda Aparecida Castro Pereira[2], Pedro Crescêncio Souza Carneiro[1] and José Eustáquio de Souza Carneiro[1]

Crop Breeding and Applied Biotechnology 24(2): e47552426, 2024
Brazilian Society of Plant Breeding. Printed in Brazil
http://dx.doi.org/10.1590/1984-70332024v24n2a19

Abstract: *The aim was to estimate the minimum number of plants required per plot to assess the anthracnose severity by artificial inoculation of V2 stage plants. Seventy-eight carioca common bean cultivars were inoculated in 13 experiments to assess its reaction to races 65, 73, 81, and 89 of Colletotrichum lindemuthianum. Sample sizes ranged from one to nine plants were adopted to setting the number of plants per plot. A total of 1000 samplings were performed for each sample size. The following parameters were estimated in each sampling: accuracy, coefficient of variation and Pearson's correlation. The mean values of each parameter in the 1000 samplings for each sample size were subjected to quadratic regression with a plateau as a function of sample size for each experiment. Six plants per plot were needed to assess the severity of anthracnose by artificially inoculating the pathogen on common bean plants at the V2 stage.*

Keywords: Phaseolus vulgar*is L.,* Colletotrichum lindemuthianu*m, experimental precision, number of plants, V2 stage.*

INTRODUCTION

Anthracnose caused by *Colletotrichum lindemuthianum* (Saac. and Magnus) Briosi and Cavara is one of the most serious diseases affecting bean crops. It can cause crop losses of up to 100% when contaminated seeds and/or susceptible cultivars are used under favorable environmental conditions (Gonçalves-Vidigal et al. 2020, Paulino et al. 2022). The strategies developed to control anthracnose include the use of healthy seeds, fungicides, and resistant cultivars. The use of resistant cultivars is considered the most effective, and economical alternative to manage the disease (Miklas et al. 2006, Ferreira et al. 2013). Therefore, breeding programs have focused on the development of resistant bean cultivars (Moreira et al. 2006, Ragagnin et al. 2009).

The bean breeding program of Bioagro/ Universidade Federal de Viçosa developed Rudá-R, a dry bean line with 'carioca-type' grains containing the following disease resistant alleles: *Co*-4, *Co-6*, and *Co-10* (anthracnose); *Phg-1* (angular leaf spot); and *Ur-ON* (rust) (Ragagnin et al. 2009). The cultivar BRSMG Pioneiro was also developed by this program using the backcross method assisted by molecular markers involving the parents Rudá and Ouro Negro (Moreira et al. 2006). An efficient method in obtaining progenies resistant to anthracnose has been the recurrent selection through artificial inoculation of seedlings with *C. lindemuthianum* isolates (Costa et al. 2020).

***Corresponding author:**
E-mail: jumencalha@gmail.com
ORCID: 0000-0001-5782-0673

Received: 13 November 2023
Accepted: 27 February 2024
Published: 01 March 2024

[1] Universidade Federal de Viçosa, Avenida PH Rolfs, s/n, Campus Universitário, 36570-900, Viçosa - MG, Brazil
[2] Universidade Federal de Lavras, Trevo Rotatório Professor Edmir Sá Santos, Universidade Federal de Lavras, 37203-202, Lavras - MG, Brazil

The choice of the parents and the accurate selection of resistant lines is crucial for the success of breeding programs that aim to obtain cultivars that are resistant to the main races of *C. lindemuthianum* (Mencalha et al. 2022). Selection of lines is performed based on the assessment of genotypes for anthracnose severity. The assessment in field experiments has not been accurate and precise because the disease occurs in patches in the field due to the spread of the pathogen over short distances. To make the assessment more efficient, artificial inoculation of the pathogen has been carried out in a greenhouse setup using host plants at their V2 (Alzate-Marin et al. 2006, Calil et al. 2008, Celin et al. 2012, Barcelos et al. 2013, Celin et al. 2013) or V3 (Vidigal Filho et al. 2007, Gonçalves-Vidigal et al. 2012, Vidigal Filho et al. 2020, Martiniano-Souza et al. 2021) stages. The plants at the V2 stage are easier to handle as artificial inoculation can be carried out in plastic trays, which makes it possible to assess a greater number of genotypes while saving time and resources.

In the final stage of breeding programs or when one is interested in characterizing germplasm banks, many genotypes need to be assessed. However, the availability of time, manpower, financial and human resources are limiting factors for efficient germplasm characterization. These limitations exist even in the case of greenhouse experiments where bean seedlings are assessed for anthracnose severity; such experiments have either been carried out without any replication (Alzate-Marin et al. 2006, Calil et al. 2008, Celin et al. 2013) or with fewer replications (Barcelos et al. 2013). Celin et al. (2012) assessed the resistance of 117 carioca bean genotypes to six races of *C. lindemuthianum* by inoculating eight plants of each genotype for each *C. lindemuthianum* race without using a statistical design. In a study carried out by Barcelos et al. (2013), 500 bean accessions from the UFLA germplasm bank were assessed for resistance to races 65 and 81 of *C. lindemuthianum* by inoculating nine seeds of each cultivar with two replications, which adds up to 18 seeds per cultivar for each *C. lindemuthianum* race.

Reducing the sample size (SS) is an effective strategy that would help reduce the costs of anthracnose severity experiments as well as allow the assess of many lines with replications. However, a small SS can affect experimental precision (Guimarães et al. 2021) which would consequently affect selection accuracy. Therefore, adequate sample size is essential to achieve a desirable level of experimental precision (Krysczun et al. 2018, Bittencourt et al. 2023). Currently, there is lack of information on the number of plants required per plot to obtain a precise assessment and accurate selection. The aim of this study was to estimate the minimum number of plants required per plot to assess the anthracnose severity using artificial inoculation at V2 stage.

MATERIAL AND METHODS

Seventy-eight carioca common bean cultivars were used for the assessment of anthracnose severity. Due to the large number of cultivars for assessment and the space required in the greenhouse, these were divided into two groups. Forty-one cultivars were assessed for their reaction to *C. lindemuthianum* races 65, 81 and 89, and thirty-seven cultivars assessed for their reaction to races 65, 73, 81, and 89. Two experiments were performed to assess each race of the pathogen, except the assessment of the forty-one cultivars regarding the reaction to race 81 in which one experiment was carried out. Thus, a total of thirteen trials was conducted.

All cultivars were obtained from common bean breeding programs conducted in Brazil by public institutions (Empresa Brasileira de Pesquisa Agropecuária: EMBRAPA; Empresa de Pesquisa Agropecuária de Minas Gerais: EPAMIG; Instituto Agronômico de Campinas: IAC; and Instituto Agronômico de Pesquisa do Paraná: IAPAR) and universities (Universidade Federal de Viçosa: UFV; Universidade Federal de Lavras: UFLA).

The experimental setup involved a randomized block design with three replications and nine plants per plot. The experiments were conducted in a greenhouse at the UFLA (lat 21° 14’ S, long 44° 59’ W, alt 910 m asl), Lavras, MG, Brazil from 2020 to 2021.

The seeds were germinated in polystyrene trays with 162 cells, each containing the commercial substrate Tropstrato HA Hortaliças (Vida Verde ®). To obtain spore suspension, mycelial discs of the *C. lindemuthianum* races were grown on petri dishes containing potato agar dextrose (PDA) medium. After ten days a mycelial fragment was transferred to sterile pods partially immersed in water-agar medium in test tubes. The test tubes were incubated in Bio-Oxygen Demand (BOD) at 22 °C for 15 days (Costa et al. 2017). At the end of 15 days, 6 ml sterile distilled water was added to the tubes and the surface of the pods was scraped aseptically with a brush to release the conidia. The homogenate was then filtered through two layers of cheesecloth to remove mycelial fragments. The concentration of the conidial suspension

was obtained using a hemocytometer and adjusted to 1.2×10^6 conidia mL^{-1} (Costa et al. 2017).

Plants with fully expanded primary leaves (V2 stage) were inoculated with conidial suspensions by spraying over both leaf surfaces and stems. The inoculated plants were placed in a nebulization chamber for 48 h with controlled temperature and humidity (20 ± 1 °C and >95%) with a 12h photoperiod. The seedlings were then transferred to a greenhouse with a temperature of approximately 24 ºC and 95% relative humidity, and scored after 12 days of inoculation according to the severity scale of 1 to 9 (Van Schnoonhoven and Pastor-Corrales 1987), where 1 = plants with no visible symptoms; and 9 = dead plants. Each plant of plot was assigned an anthracnose severity score.

Different sample sizes (SS) that ranged from one to nine plants were adopted to setting the ideal number of plants per plot in assessment of anthracnose severity in common bean in stage V2. Plants were sampled randomly within each plot to sampling each SS. The severity score of the plot was estimated by the mean of the scores of the plants sampled in each SS. The sampling process for each SS was repeated 1000 times.

Individual analyses of variance were performed for each one of the 1000 samplings of each SS. The following model was adopted: $Y_{ij} = m + B_j + C_i + \varepsilon_{ij}$, where Y_{ij} is the value observed in the cultivar i (i = 1, 2, 3,... 41) in block j (j = 1, 2, 3); m is the general mean; B_j is the random effect of block j; C_i is the fixed effect of cultivar i; ε_{ij} is the experimental error associated with Y_{ij}. These procedures were performed for each of the 13 trials conducted to assess the severity of anthracnose.

The estimates of accuracy ($\hat{r}_{\hat{g}g}$) and coefficient of variation (CV) obtained in each of the 1000 samplings were used as a criterion to set the minimum number of plants per plot. Accuracy was estimated based on the formula: $\hat{r}_{\hat{g}g} = (1 - \frac{1}{F})^{1/2}$, where F is the value of the variance ratio for the effects of cultivars associated with the analysis of variance in each sampling (Resende and Duarte 2007). The CV(%) was obtained according to the following expression: $CV(\%) = 100 \frac{\sqrt{MSE}}{\overline{y}}$, where MSE is the mean square of error and $\overline{y}$ is the mean of the scores in the experiment.

The means of the cultivars severity scores obtained with SS of nine plants were adopted as comparison parameter in relation to the other SS (1 to 8). Therefore, the Pearson correlation (r) was also estimated between the means of the cultivars in each SS and the means obtained using nine plants per plot. This correlation was estimated in each of the 1000 samplings of each SS.

The minimum, maximum and mean values of $\hat{r}_{\hat{g}g}$, CV and r of the 1000 samplings for each SS were estimated for each one of the 13 trials (Table 1). The mean data of these parameters ($\hat{r}_{\hat{g}g}$, CV e r) were submitted a quadratic-plateau regression as a function of SS in each one of the 13 trials. The following quadratic-plateau regression model was adopted:

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

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

$$Y_0 = \beta_0 - \frac{\beta_1^2}{4\beta_2}$$

where Y is the value of the statistical parameter ($\hat{r}_{\hat{g}g}$, CV, r); X is the number of plants in the plot (i = 1, 2, 3, … 9); β_0 is the intercept, β_1 is the slope, β_2 is the quadratic term; X_0 is the number of plants for which the quadratic model fits on a plateau (critical point in X); Y_0 is the Y-axis value that corresponds to the plateau (maximum value for Y); and ε is the random error associated with Y. The degree of model fit was measured using the coefficient of determination (r^2).

The minimum number of plants per plot in each experiment was defined with the aid of the value point on the x-axis (number of plants per plot) located at the junction of the quadratic segment and the regression plateau. The value of the three parameters ($\hat{r}_{\hat{g}g}$, CV, r) on the y-axis associated with the plateau was also estimated in each experiment. All analyses were performed using the easynls package and R software (R Core Team 2022).

RESULTS AND DISCUSSION

The significant effect of cultivars for anthracnose severity were observed in all 13 experiments conducted to assess the reaction to races 65, 73, 81, and 89 of *C. lindemuthianum* when data from all nine plants in the plots were used.

Table 1. Minimum (Min), maximum (Max), and mean accuracies of 1000 samplings performed for each sample size (SS) in 13 experiments to assess carioca common bean cultivars for anthracnose severity

Exp	Sample Size								
	1	2	3	4	5	6	7	8	9[c]
1	0.93[a] (0.85-0.98)[b]	0.96 (0.93-0.98)	0.97 (0.94-0.98)	0.97 (0.95-0.98)	0.97 (0.96-0.99)	0.98 (0.97-0.98)	0.98 (0.97-0.98)	0.98 (0.97-0.98)	0.98 (0.98-0.98)
2	0.89 (0.81-0.95)	0.93 (0.88-0.97)	0.94 (0.92-0.97)	0.95 (0.93-0.97)	0.96 (0.94-0.97)	0.96 (0.95-0.97)	0.96 (0.95-0.97)	0.96 (0.96-0.96)	0.96 (0.96-0.96)
3	0.94 (0.87-0.98)	0.96 (0.92-0.99)	0.97 (0.95-0.98)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.98 (0.97-0.99)	0.98 (0.98-0.99)	0.98 (0.98-0.98)	0.98 (0.98-0.98)
4	0.94 (0.86-0.98)	0.96 (0.93-0.98)	0.97 (0.96-0.99)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.98 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)
5	0.90 (0.79-0.96)	0.94 (0.89-0.97)	0.95 (0.92-0.97)	0.95 (0.93-0.97)	0.96 (0.94-0.97)	0.96 (0.95-0.97)	0.96 (0.95-0.97)	0.96 (0.96-0.96)	0.96 (0.96-0.96)
6	0.94 (0.87-0.98)	0.96 (0.93-0.98)	0.97 (0.95-0.99)	0.97 (0.96-0.98)	0.98 (0.97-0.98)	0.98 (0.97-0.99)	0.98 (0.98-0.98)	0.98 (0.98-0.98)	0.98 (0.98-0.98)
7	0.93 (0.83-0.98)	0.96 (0.92-0.98)	0.97 (0.95-0.98)	0.97 (0.96-0.98)	0.98 (0.97-0.98)	0.98 (0.97-0.98)	0.98 (0.98-0.98)	0.98 (0.98-0.98)	0.98 (0.98-0.98)
8	0.88 (0.68-0.95)	0.93 (0.87-0.96)	0.94 (0.89-0.97)	0.95 (0.92-0.97)	0.95 (0.94-0.97)	0.96 (0.94-0.97)	0.96 (0.95-0.97)	0.96 (0.96-0.97)	0.96 (0.96-0.96)
9	0.96 (0.70-0.88)	0.97 (0.85-0.92)	0.97 (0.89-0.94)	0.97 (0.91-0.95)	0.97 (0.93-0.95)	0.97 (0.94-0.96)	0.97 (0.95-0.96)	0.97 (0.96-0.96)	0.96 (0.96-0.96)
10	0.92 (0.83-0.98)	0.96 (0.92-0.98)	0.97 (0.95-0.99)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.98 (0.98-0.99)	0.98 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)
11	0.91 (0.82-0.97)	0.95 (0.91-0.98)	0.96 (0.94-0.98)	0.97 (0.95-0.98)	0.97 (0.96-0.98)	0.98 (0.97-0.98)	0.98 (0.97-0.98)	0.98 (0.98-0.98)	0.98 (0.98-0.98)
12	0.94 (0.88-0.98)	0.97 (0.93-0.99)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.98 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)
13	0.96 (0.92-0.99)	0.97 (0.95-0.99)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)

[a] Mean accuracy; [b] (Minimum accuracy-maximum accuracy); [c] Analysis carried out with all plants on the plot; Exp: Experiment

The accuracies of these experiments were ≥0.96 (Table 1), indicating a high correlation between the true genotypic value of the cultivars and that obtained using experimental data (Resende and Duarte 2007). Furthermore, 77% of these experiments showed CV values equal to or lower than 22% when all nine plants in the plot were assessed (Table 2). These accuracy and coefficient of variation values were similar to those obtained in studies assessment the severity of anthracnose through the inoculation of plants at stage V2. Pereira et al. (2019) observed accuracies greater than 0.96 for anthracnose severity when assessing common bean lines. In study carried out by Tosquy-Valle et al. (2020) a coefficient of variation equal to 22.14% was observed when assessing the severity of anthracnose under artificial inoculation.

The significant effect of cultivars was observed in all 1000 samplings that were performed for each SS, that is, even with a reduction in the number of plants assessed per plot, genetic variability was observed between the cultivars. The mean accuracy of the 1000 samplings increased with an increase in the number of plants assessed per plot (Table 1). The smallest SS showed greater variation of accuracy values in the 1000 samplings; the highest variation in accuracy ranged between 0.68 and 0.95 in the case of experiment 8, where the SS was one plant per plot. According to Bittencourt et al. (2023), the smaller the SS, the farther the lower and upper limits are from the real values, creating wider intervals that leave room for biased, overestimated, or underestimated estimates. SS >3 plants showed accuracy greater than 0.91 in all experiments and in all 1000 samplings. According to Resende and Duarte (2007), mean accuracy values ≥ 0.90 indicate high precision, indicating the possibility of reducing the number of plants to be assessed without the loss of genetic variability.

Experimental precision was influenced by the number of plants assessed per plot. An increase in the SS resulted in a reduction in the mean CV of the 1000 samplings per SS, which in turn resulted in an increase in experimental precision. This increase in experimental precision as a function of SS has been observed in other studies (Padrón et al. 2018, Alves et al. 2022, Bittencourt et al. 2023). The highest variation among the 1000 samplings for the CV was observed when one plant per plot was used (Table 2). Experiment 8 showed the highest variation among all the samplings for this parameter,

Table 2. Minimum (Min), maximum (Max), and mean coefficients of variation of 1000 samplings performed for each sample size (SS) in 13 experiments to assess carioca common bean cultivars for anthracnose severity

Exp	Sample Size								
	1	2	3	4	5	6	7	8	9[c]
1	31.4[a] (18.8-41.1)[b]	24.3 (16.8-30.5)	21.3 (15.5-26.5)	19.6 (14.9-26.4)	18.5 (14.6-22.3)	17.9 (15.3-20.5)	17.3 (14.9-19.7)	16.9 (15.5-18.7)	16.5 (16.5-16.5)
2	39.8 (30.4-49.5)	31.0 (23.7-38.1)	27.4 (21.8-32.6)	25.5 (21.8-30.1)	24.3 (21.2-27.2)	23.5 (21.1-26.3)	22.9 (21.5-24.5)	22.5 (21.8-23.5)	22.4 (22.4-22.4)
3	34.0 (20.4-47.5)	26.3 (18.1-37.5)	23.2 (18.0-29.8)	21.3 (17.1-27.2)	20.1 (16.1-24.0)	19.4 (16.8-22.6)	18.8 (16.5-20.7)	18.4 (17.5-19.6)	18.3 (18.3-18.3)
4	32.5 (20.8-43.7)	24.1 (18.0-31.4)	20.6 (15.3-26.6)	18.7 (13.5-23.4)	17.3 (13.8-21.6)	16.3 (13.5-19.9)	15.6 (13.5-17.8)	15.1 (13.8-16.5)	14.8 (14.8-14.8)
5	48.3 (34.3-59.7)	38.4 (30.0-45.9)	34.7 (28.5-40.6)	32.6 (27.6-37.9)	31.3 (27.0-35.5)	30.3 (26.5-33.2)	29.7 (27.7-31.7)	29.4 (28.3-30.6)	29.2 (29.2-29.2)
6	41.8 (28.1-54.1)	32.3 (22.6-41.0)	28.4 (21.3-35.6)	26.3 (20.4-31.2)	24.8 (21.4-29.5)	23.9 (20.6-26.8)	23.2 (20.9-25.4)	22.7 (21.4-23.8)	22.6 (22.6-22.6)
7	34.8 (20.9-47.8)	26.0 (18.1-33.5)	22.2 (16.7-28.1)	20.2 (15.9-25.2)	18.9 (16.0-22.5)	18.0 (15.6-21.0)	17.5 (16.4-18.8)	17.2 (16.7-17.7)	17.1 (17.1-17.1)
8	48.6 (34.6-64.4)	37.9 (29.0-47.5)	33.5 (25.6-41.8)	31.1 (25.8-37.9)	29.6 (24.3-34.4)	28.5 (25.2-31.8)	27.7 (25.5-30.1)	27.2 (25.9-28.8)	26.9 (26.9-26.9)
9	43.4 (30.8-56.1)	33.4 (23.6-42.4)	29.4 (21.3-37.2)	27.2 (21.4-33.0)	25.8 (21.2-30.4)	24.8 (21.0-28.0)	24.2 (22.0-26.4)	23.7 (22.5-24.9)	23.4 (23.4-23.4)
10	39.6 (24.0-52.8)	28.3 (19.3-39.9)	23.8 (16.7-30.6)	20.7 (15.0-27.5)	18.9 (15.4-24.0)	17.8 (15.5-20.9)	17.0 (15.5-18.5)	16.6 (16.1-17.2)	16.5 (16.5-16.5)
11	33.0 (23.2-43.0)	24.6 (17.7-31.6)	21.3 (16.5-26.7)	19.4 (15.3-24.0)	18.1 (15.3-21.3)	17.3 (14.9-19.5)	16.7 (15.3-18.3)	16.4 (16.0-16.9)	16.3 (16.3-16.3)
12	34.7 (22.9-45.4)	25.0 (17.1-35.8)	20.9 (15.1-26.9)	18.6 (14.0-23.0)	17.1 (13.7-20.4)	16.1 (13.8-19.3)	15.3 (13.9-17.2)	14.9 (14.4-15.8)	14.8 (14.8-14.8)
13	30.4 (18.6-39.2)	23.0 (13.9-31.0)	20.2 (14.1-27.3)	18.4 (13.0-24.3)	17.4 (12.6-22.1)	16.6 (13.2-20.4)	16.1 (13.8-19.2)	15.8 (14.5-17.5)	15.6 (15.6-15.6)

[a] Mean coefficient of variation; [b] (Minimum coefficient of variation-maximum coefficient of variation); [c] Analysis carried out with all plants on the plot; Exp: Experiment

which ranged between 34.6 and 64.4%, averaging at 48.55%. When the SS was eight plants per plot, the mean CV was 27.2%, with a narrow variation range of 25.9 to 28.8%; these values were close to those observed for the SS of nine, where the CV was 26.9%. The other experiments showed a similar pattern for the CV.

We observed that the SS marginally influenced the ranking of cultivars on the basis of their anthracnose severity after inoculation with *C. lindemuthianum* races 65, 73, 81, and 89. Pearson correlations obtained between the mean severity of the cultivars in each sample for each number of plants per plot and the mean severity of the cultivars using nine plants were significant at 5% probability. The correlation of the mean of the assessed cultivars considering the different SS with the mean in the assessment condition of all plants increased with an increase in the number of assessed plants per plot (Table 3). The greatest variation among the 1000 samplings was observed in experiment 8, when one plant per plot was used and the values ranged from 0.79 to 0.98. When compared with all other experiments, samplings with correlation values <0.90 were observed only for the SS with one plant. The mean correlation of all 1000 samplings regardless of SS was >0.90, which indicates that the cultivar ranking was not strongly altered with respect to different SS.

The quadratic-plateau regression models showed a high degree of fit for the mean values of accuracy, CV, and correlation coefficient as a function of the SS in all experiments. The model determination coefficients for these three parameters were higher than 0.97 (Table 4). This result indicates that 97% of the variation in each parameter was explained by the sample size. In these models, t-test analyses showed that all regression coefficients (β0*,* β1 *and* β2) were significant at 1% probability.

In the 13 trials, the regression models for accuracy showed a plateau between 4.44 and 4.79 plants per plot (Table 4), indicating that it would be necessary to assess at least 5 plants to obtain an accuracy similar to the assessment performed with 9 plants per plot. The estimated accuracies in all the experiments using these models and for these plateau values were >0.96. Furthermore, in all experiments, accuracies >0.93 were observed for this SS among the

Table 3. Minimum (Min), maximum (Max), and mean Pearson correlations of 1000 samplings performed for each sample size (SS) in 13 experiments to assess carioca common bean cultivars for anthracnose severity

Exp	Sample Size							
	1	2	3	4	5	6	7	8
1	0.95[a] (0.87-0.98)[b]	0.97 (0.94-0.99)	0.98 (0.97-0.99)	0.99 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)
2	0.92 (0.82-0.97)	0.96 (0.90-0.98)	0.98 (0.96-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)
3	0.95 (0.90-0.99)	0.98 (0.94-0.99)	0.99 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
4	0.95 (0.84-0.98)	0.97 (0.93-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)
5	0.94 (0.87-0.97)	0.97 (0.93-0.99)	0.98 (0.96-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
6	0.95 (0.86-0.98)	0.98 (0.95-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
7	0.94 (0.87-0.98)	0.97 (0.94-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (1.00-1.00)
8	0.92 (0.79-0.98)	0.96 (0.90-0.98)	0.98 (0.95-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)
9	0.91 (0.77-0.97)	0.96 (0.92-0.98)	0.97 (0.95-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)
10	0.93 (0.83-0.97)	0.97 (0.93-0.98)	0.98 (0.96-0.99)	0.99 (0.98-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
11	0.93 (0.85-0.97)	0.97 (0.94-0.99)	0.98 (0.96-0.99)	0.99 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
12	0.95 (0.88-0.98)	0.97 (0.95-0.99)	0.98 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	0.99 (0.99-1.00)	1.00 (0.99-1.00)
13	0.96 (0.91-0.99)	0.98 (0.95-0.99)	0.99 (0.97-0.99)	0.99 (0.98-0.99)	0.99 (0.99-0.99)	0.99 (0.99-0.99)	0.99 (0.99-1.00)	1.00 (0.99-1.00)

[a]: Mean Pearson correlation; [b]: (Minimum Pearson correlation-maximum Pearson correlation); Exp: Experiment

1000 samplings performed (Table 1). In terms of the mean accuracy of these samplings with five plants per plot, the experiments showed values >0.95. These results denote a high correlation between the true genotypic value of the cultivar and that estimated from the experimental data (Resende and Duarte 2007), indicating that we could possibly reduce the number of assessed plants per plot from nine to five.

The regression models adjusted for the CV as a function of SS showed a plateau between 5.05 and 5.72 plants (Table 4). For these plateau values, the models estimated CV of 30.1 and 15.6%, respectively. Mencalha et al. (2022) and Tosquy-Valle et al. (2020) observed similar CV values when using artificial inoculation of bean plants under the same conditions. Except experiments 5 and 8, most experiments showed CV values <25% when six plants per plot were assessed. Although higher CV values were observed in experiments 5 and 8 in the samplings with six plants per plot, these experiments showed a high CV even when nine plants per plot were assessed (Table 2). Therefore, these results reinforce the possibility of reducing the SS to six plants and maintaining the experimental precision obtained with nine plants per plot in the assessment of anthracnose severity.

The plateau values obtained in the regression models for the correlation coefficient in each experiment varied between 4.56 and 4.84 plants per plot. The correlation coefficients estimated by the models for these SS were >0.99 (Table 4). Furthermore, it was observed that all 1000 samplings for a SS equal to 5 presented correlation coefficients >0.98 in all experiments (Table 3). These results indicate that the ranking of the cultivars assessed with five plants per plot was similar to that observed with a SS of nine plants. Thus, it is possible to reduce the number of plants assessed for anthracnose severity to five using correlation coefficient as a criterion.

The minimum number of plants required to assess the severity of anthracnose was five with respect to the accuracy (Table 4) and correlation coefficient parameters (Table 4), and six plants with respect to the CV parameter (Table 4). All samplings with six plants per plot presented accuracies and correlation coefficients above 0.94 and 0.99, respectively.

Table 4. Quadratic-plateau regression models for mean accuracy, mean coefficient of variation, and mean Pearson correlation of 1000 samplings performed for each sample size in 13 experiments to assess carioca common bean cultivars for anthracnose severity

Exp	Accuracy						Coefficient of variation						Pearson correlation					
	β_0	β_1	β_2	r^2	NP	Plateu	β_0	β_1	β_2	r^2	NP	Plateau	β_0	β_1	β_2	r^2	NP	Plateu
1	0.90**	0.03**	-0.01**	0.97	4.72	0.98	37.27**	-7.21**	0.65**	0.98	5.52	17.4	0.92**	0.03**	-0.01**	0.97	4.65	0.99
2	0.85**	0.05**	-0.01**	0.98	4.60	0.96	47.86**	-9.67**	0.95**	0.98	5.10	23.2	0.89**	0.05**	-0.01**	0.98	4.70	0.99
3	0.92**	0.03**	-0.01**	0.98	4.64	0.98	40.45**	-7.90**	0.73**	0.98	5.43	19.0	0.94**	0.03**	-0.01**	0.98	4.60	0.99
4	0.91**	0.03**	-0.01**	0.97	4.61	0.98	39.06**	-8.16**	0.71**	0.97	5.72	15.7	0.92**	0.03**	-0.01**	0.97	4.58	0.99
5	0.87**	0.04**	-0.01**	0.97	4.61	0.96	57.27**	-10.77**	1.07**	0.98	5.05	30.1	0.91**	0.04**	-0.01**	0.98	4.67	0.99
6	0.91**	0.03**	-0.01**	0.97	4.62	0.98	49.76**	-9.73**	0.90**	0.98	5.41	23.4	0.93**	0.03**	-0.01**	0.98	4.61	0.99
7	0.89**	0.04**	-0.01**	0.98	4.44	0.98	42.68**	-9.50**	0.91**	0.98	5.24	17.8	0.92**	0.04**	-0.01**	0.98	4.56	0.99
8	0.85**	0.05**	-0.01**	0.98	4.79	0.96	57.49**	-10.94**	1.01**	0.98	5.40	28.0	0.89**	0.05**	-0.01**	0.98	4.84	0.99
9	0.83**	0.05**	-0.01**	0.98	4.73	0.96	51.82**	-10.33**	0.97**	0.98	5.31	24.4	0.88**	0.05**	-0.01**	0.98	4.74	0.99
10	0.88**	0.04**	-0.01**	0.98	4.53	0.98	48.89**	-11.40**	1.03**	0.98	5.54	17.3	0.90**	0.04**	-0.01**	0.98	4.61	0.99
11	0.88**	0.04**	-0.01**	0.98	4.54	0.98	40.05**	-8.65**	0.81**	0.98	5.33	17.0	0.90**	0.04**	-0.01**	0.98	4.59	0.99
12	0.91**	0.03**	-0.01**	0.98	4.46	0.99	42.77**	-9.87**	0.90**	0.98	5.51	15.6	0.93**	0.03**	-0.01**	0.98	4.61	0.99
13	0.94**	0.02**	-0.01**	0.97	4.54	0.99	36.50**	-7.51**	0.70**	0.98	5.38	16.3	0.95**	0.02**	-0.01**	0.98	4.63	0.99

Exp: Experiment; NP: Number of plants. β_0, β_1 and β_2: Regression coefficients of the quadratic-plateau regression model. ** Significant at the 0.01 probability level using t-test r^2: coefficient determinant of the model

Most samplings with the SS of six plants showed CV values <25%. According to Bittencourt et al. (2023), the sample size varies according to the statistical procedure used, and the maximum value obtained among all the statistics represents the number of plants sufficient for reliable assessment of the trait. Thus, for the assessments to be as accurate and precise as those performed with a SS of nine plants, it would be necessary to assess at least six plants. According Guimarães et al. (2021), a small SS can affect experimental precision which would consequently affect selection accuracy. Therefore, adequate sample sizing is essential to maintain accuracy and experimental precision (Bittencourt et al. 2023).

Previous studies that were carried out to assess the reaction of cultivars to the races of *C. lindemuthianum* involved a SS of eight (Calil et al. 2008, Celin et al. 2012, Celin et al. 2013) or nine plants (Barcelos et al. 2013, Oliveira et al. 2015). Celin et al. (2012) assessed the resistance of 117 carioca bean genotypes to six races of *C. lindemuthianum* by inoculating eight plants of each genotype for each *C. lindemuthianum* race without using a statistical design. In a study carried out by Barcelos et al. (2013), 500 bean accessions from the UFLA germplasm bank were assessed for resistance to races 65 and 81 of *C. lindemuthianum* by inoculating nine seeds of each cultivar with two replications, which adds up to 18 seeds per cultivar for each *C. lindemuthianum* race. Although there is no information regarding the adequate SS, this study demonstrates that the experiments were carried out with a sufficient number of plants to achieve high precision and accuracy. The use of six plants per plot in the assessment of anthracnose severity would allow a 33% reduction in the cost of experiments of this nature and would make it possible to assess a greater number of genotypes.

CONCLUSION

Six plants per plot is the minimum number needed to assess bean lines with high precision and accuracy regarding the severity of anthracnose through artificial inoculation of plants at the V2 stage.

ACKNOWLEDGEMENTS

The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

REFERENCES

Alves SRM, Lopes R, Meneses C, Valente MSF, Martins CC, Ramos SF, Oliveira I, Fraxe TJP, Costa L and Lopes MTG (2022) Morpho-agronomic characterization, sample size, and plot size for the evaluation of *Capsicum chinense* genotypes. **Horticulturae 8**: 785.

Alzate-Marin AL, Souza TLPO, Arruda KMA, Silva MGM, Chagas JM, Barros

EG and Moreira MA (2006) Reaction of the common bean cultivar "Vermelhinho" to rust, anthracnose and angular leaf spot. **Revista Ceres 56**: 164-170.

Barcelos QL, Souza EA, Pádua PF, Ishikawa FH, Costa LC, Pereira R, Abreu AFB and Ramalho MAP (2013) Investigation of sources of resistance to antracnose disease in a *Phaseolus vulgaris* germplasm collection in Brazil. **Annual Report of the Bean Improvement Cooperative 56**: 37-38.

Bittencourt KC, Souza RR, Pazetto SB, Toebe M, Toebe ICD and Cargnelutti Filho A (2023) How many cauliflower seedlings are necessary to estimate experimental precision statistics reliably? **Scientia Horticulturae 310**: 111788.

Calil IP, Sanglard DA, Carneiro JES, Arruda KMA, Celin EF, Barros EG and Carneiro PCS (2008) Characterization of accessions of *Phaseolus vulgaris* L. for reaction to the fungus *Colletotrichum lindemuthianum*. **Annual Report of the Bean Improvement Cooperative 51**: 178-179.

Celin EF, Paula RG, Pereira AC, Oliveira BM, Paula Júnior TJ, Vieira RF, Carneiro PCS and Carneiro JES (2013) Reaction of common bean genotypes to five races of *Colletotrichum lindemuthianum* in Brazil. **Annual Report of the Bean Improvement Cooperative 56**: 35-36.

Celin EF, Pereira AC, Oliveira BM, Poersch NL, Paula RG, Carneiro LF, Vieira RF and Carneiro JES (2012) Sources of resistance to *Colletotrichum lindemuthianum* in carioca common bean class. **Annual Report of the Bean Improvement Cooperative 55**: 137-138.

Costa LC, Ramalho MAP, Abreu AFB, Costa KC and Souza EA (2020) Effectiveness of recurrent selection for anthracnose resistance in common bean. **Annual Report of the Bean Improvement Cooperative 63**: 73-74.

Costa LC, Nalin RS, Ramalho MAP and Souza EA (2017) Are duplicated genes responsible for anthracnose resistance in common bean? **Plos One 12**: e0173789.

Ferreira JJ, Campa A and Kelly JD (2013) Organization of genes conferring resistance to anthracnose in common bean. In Varshney RK and Tuberosa R (eds) **Translational genomics for crop breeding: biotic stress**. John Wiley & Sons, New York, p. 151-176.

Gonçalves-Vidigal, Gilio TAS, Valentini G, Vaz-Bisneta M, Vidigal Filho PS, Song Q, Oblessuc PR and Melotto M (2020) New Andean source of resistance to anthracnose and angular leaf spot: Fine-mapping of disease-resistance genes in California Dark Red Kidney common bean cultivar. **Plos One 15**: e0235215.

Gonçalves-Vidigal MC, Vidigal Filho PS, Nogueira LM, Reck SAC, Oliveira MB, Motomiya WR, Lacanallo GF, Cruz AS, Grunvald AK, Nunes MPBP, Sousa LL and Pastor-Corrales MA (2012) Reaction of common bean cultivars inoculated simultaneously with *Colletotrichum lindemuthianum* and *Pseudocercospora griseola*. **Annual Report of the Bean Improvement Cooperative 55**: 97-98.

Guimarães BVC, Carvalho AJ, Aspiazú I, Silva LS, Silva RRP, Pimenta AML and Moura MMA (2021) Optimal plot size for experimentation of common beans (*Phaseolus vulgaris* L.) in the northern region of Minas Gerais, Brazil. **Revista FCA UNCuyo 53**: 55-63.

Krysczun DK, Lúcio AD, Sari BG, Diel MI, Olivoto T, Santana CS, Ubessi C and Schabarum DE (2018) Sample size, plot size and number of replications for trials with *Solanum melongena* L. **Scientia Horticulturae 233**: 220-224.

Martiniano-Souza MC, Gonçalves-Vidigal MC, Costa AF, Vidigal Filho PS, Martins VSR, Vaz Bisneta M, Lacanallo GF, Coêlho M, Dartibale GB and Valentini G (2021) Virulence and genetic diversity of *Colletotrichum lindemuthianum* and resistance of local common bean germplasm to anthracnose in Pernambuco State, Brazil. **European Journal of Plant Pathology 159**: 727-740.

Mencalha J, Dias MA, Souza EA, Carneiro PCS, Carneiro VQ and Carneiro JES (2022) Potential parents of carioca bean for use in breeding aiming resistance to *Colletotrichum lindemuthianum*. **Agronomy Journal 114**: 2159-2168.

Miklas PN, Kelly JD, Beebe SE and Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. **Euphytica 147**: 105-131.

Moreira MA, Barros EG, Carneiro JES, Faleiro FG, Faria LC, Carneiro GES, Del Peloso MJ, Paula Júnior TJ, Abreu AFB, Ramalho MAP, Melo LC, Santos JB, Rava CA, Costa JGC, Sartorato A and Faria JC (2006) BRSMG Pioneiro: New carioca common bean cultivar resistant to anthracnose and rust, for the southern of Brazil. **Annual Report of the Bean Improvement Cooperative 49**: 279-280.

Oliveira BM, Clelin EF, Pereira AC, Carneiro PCS, Carneiro JES, Vieira RF and Paula Júnior TJ (2015) Resistance to *Colletotrichum lindemuthianum* in inbred common bean lines from the Ouro negro x Meia noite cross. **Annual Report of the Bean Improvement Cooperative 58**: 33-34.

Padrón RAR, Lopes SJ and Renedo VSG (2018) Estimation of the optimal plot size and number of replications in a field pepper crop experiment with varying irrigation depths and application frequencies. **Scientia Horticulturae 237**: 96-104.

Paulino PPS, Gonçalves-Vidigal MC, Bisneta MV, Vidigal Filho PS, Nunes MPBA, Xavier LFS, Martins VSR and Lacanallo GF (2022) Occurrence of anthracnose pathogen races and resistance genes in common bean across 30 years in Brazil. **Agronomy Science and Biotechnology 8**: 1-21.

Pereira LA, Costa LC, Pádua PF and Ramalho MAP (2019) Variability for angular leaf spot and anthracnose resistance among common bean progenies with different levels of endogamy. **Tropical Plant Pathology 44**: 275-283.

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at <https://www.R-project.org/>. Accessed on August 01, 2023.

Ragagnin VA, Souza TLPO, Sanglard DA, Arruda KMA, Costa MR, Alzate-Marin AL, Carneiro JES, Moreira MA and Barros EG (2009) Development and agronomic performance of common bean lines simultaneously resistant to anthracnose, angular leaf spot and rust. **Plant Breeding 128**: 156-163.

Resende MDV and Duarte JB (2007) Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical 37**: 182-194.

Tosquy-Valle OH, Garrido-Ramírez ER, Ibarra-Pérez FJ, Esqueda-Esquivel VA and Rodríguez-Rodríguez JR (2020) Reaction of tropical black bean breeding lines to the artificial inoculation of rust and anthracnose in Southeas Mexico. **Annual Report of the Bean Improvement Cooperative 63**: 123-124.

Van Schnoonhoven A and Pastor-Corrales MA (1987) **Standard system for the evaluation of bean germplasm**. CIAT, Cali, 87p.

Vidigal Filho PS, Gonçalves-Vidigal MC, Kelly JD and Kirk WW (2007) Sources of resistance to anthracnose in traditional common bean cultivars from Paraná, Brazil. **Journal of Phytopathology 155**: 108-113.

Vidigal Filho PS, Gonçalves-Vidigal MC, Vaz-Bisneta M, Lima LRL, Martins VSR, Oblessuc PR and Melotto M (2020) Sources of resistance to *Colletotrichum lindemuthianum* and *Pseudocercospora griseola* in common bean from Brazil. **Annual Report of the Bean Improvement Cooperative 63**: 77-78.