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Optimal plot size in lettuce seed germination experiments

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Abstract: Lettuce in works under controlled conditions is widely used in experiments with a direct focus on the species itself, or as an indicator species used in allelopathy works. But despite its frequent use, there is no support in the literature for using a single plot size and, therefore, there are works from the use of 5 to 100 seeds per experimental plot. It is understood that the works have been developed using different experimental precisions. The low experimental precision caused by a smaller-than-optimal plot size can lead to a high residual value in the analysis of variance and consequent difficulties in detecting statistical difference between treatments. On the other hand, the use of a high number of seeds per plot can lead to unnecessary expenditure and does not contribute to improving experimental precision. The objective of this work was to determine the optimal plot size in seed germination experiments under controlled conditions. The modified maximum curvature method according to Meier and Lessman was used. After the subsequent analysis, the high variations between different sizes and the factors that can be interfered because of this were observed, and the optimal plot size was defined as six seeds for the biogerminative test of lettuce by petri dishes of 10 cm in diameter.

Keywords: *Lactuca sativa* L. Statistical analysis. Experimental planning. Data analysis. Biogerminative tests. Allelopathy.

Adherence to the scope of BJEDIS: The research proposes the discussion about the optimal plot size (OPS), when conducted with lettuce seeds, generally used in allelopathic tests. OPS is a way to reduce experimental error, maximize the data obtained in the experiment and increase the chances of identifying statistical differences. Therefore, the definition of an OPS for lettuce seeds (*Lactuca sativa* L.) contributes to the research process of other professionals and helps researchers in the area.

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1. INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in the world. In commercial production, its cultivation can take place in the open field or in a protected system, usually hydroponics. In both cases, seedling preparation is necessary and, therefore, good seed emergence and plant vigor are essential. At the same time, germination work under controlled conditions is carried out frequently. There are reports in the literature of several experiments that study the effects of different variables on lettuce germination and vigor, as shown by Rodrigues et al. (1), who evaluated the effects of high temperatures associated with the use of sodium hypochlorite solution, Pereira et al. (2), who analyzed the phytotoxicity of lead, Sousa et al. (3), who evaluated the presence of pesticide residues in soil, Catão et al. (4), who evaluated the influence of temperature, and Teodoro et al. (5), who evaluated germination before and after imbibition in sodium hypochlorite solution. It also includes several studies involving allelopathy, such as those addressed by Silveira et al. (6), Oliveira et al. (7), Oliveira et al. (8), and Silveira et al. (9).

The realization of any experiment must start with good planning. In this planning, after determining the characteristics that will be studied, and which design will be adopted, the researcher begins to quantify how much material will be needed to carry out the test, and for this he must determine the size of each plot (10, 11)

Ribeiro-Oliveira et al. (12), when studying the germination of seeds of the species *Bowdichia virgilioides* Kunth, from three seed lots, found that the sample size is essential to draw valid conclusions. Continuing the work with the same species, now applying statistical methods to determine the size of the experimental plot, Ribeiro-Oliveira et al. (13) conclude that it is possible to carry out studies of this nature using smaller amounts of seeds in relation to what has been used in most scientific works. With the realization that there was a lack of scientific information regarding the size of the sample or experimental plots, Ribeiro-Oliveira and Ranal (13) continued this type of study with six species from the Brazilian cerrado biome, and found that the size of the sample can affect inferences about the germination process especially the germination speed of the seeds, and that, however, the germination percentage is less affected by the size of the experimental sample. This same behavior may not be the same for other species, especially lettuce. Schmildt et al. (14), when classifying the precision of experiments based on the evaluation of 37 articles on germination and vigor in lettuce, found that the variability measured by the coefficient of variation for the germination percentage is as high as for the germination speed. However, it is necessary that this conclusion be made based on scientific research for the main varieties in current cultivation.

Although most researchers still choose to determine the size of the plot arbitrarily, the ideal is to make such a choice based on scientific criteria, which usually involve the use of uniformity tests, also called blank assays. In these tests, it is demonstrated that there is a non-linear relationship between experimental error and plot size (15, 16). Although the researcher wants to reduce the experimental error and, for this, increase the plot size, this criterion must be done with caution, since, after a certain increase in the plot size, the decrease in experimental error is very small (16), leading to unnecessary expenditure of experimental material and physical space. Thus, the optimal plot size is sought (17, 18).

In addition to the works mentioned above regarding the size of plots for seed germination, Ribeiro-Oliveira and Ranal (19) can also be mentioned, who studying the process in varieties of corn, soybean, beans, wheat and sunflower, concluded that, in general, to study this process in an easier, safer and more standardized way, seven diaspores are needed per plot. Gomes et al. (20) when evaluating germination of atemoya seeds in BOD concluded that the optimal plot size is 10 seeds. References to the optimal plot size in experiments on lettuce germination and vigor were not found in the literature, with studies using from 5 (21, 22) to 50 seeds per plot (23, 24, 25) in petri dishes and, from 10 (26) to 100 seeds per plot (27, 28) on germination in transparent gerbox.

As for the method for determining the optimal plot size, the literature reports more than a dozen, and the most frequently used in recent research are the maximum curvature method modified according to Meier and Lessman (29), the maximum curvature of the coefficient of variation (17) and the iterative method proposed by Nesi et al. (30), in which, in both cases, a blank test is required. In the case of seed germination, Ribeiro-Oliveira et al. (13, 12) used the method of Meier and Lessman (29) and Gomes et al. (20) used a method based on the stabilization of the means, which considers the stabilization of the means through the mean test, in this case the Tukey test.

Given the high demand for scientific studies on germination, emergence and vigor with lettuce and considering the scarcity of scientific studies that emphasize the size of experimental plots, the objective of this work was to determine the optimal size of experimental plots in the germination of lettuce seeds.

2. MATERIALS AND METHOD

The experiment was carried out on the premises of the plant improvement laboratory of Centro Universitário Norte do Espírito Santo (LMP/CEUNES) located in the city of São Mateus, state of Espírito Santo.

The optimal plot size was performed by means of an experiment of seed germination and seedling vigor of *Lactuca sativa* L. (lettuce) according to the literature (31)**.** Seeds of the smooth-leaf variety 'Vitória de Santo Antão' were used. The experiment was carried out in Petri dishes with a diameter of 10 cm, containing two sheets of filter paper. Each plate received three milliliters of distilled water before seed placement and were incubated at 25 °C, under fluorescent light (8 x 40 W), for a period of 12 days, in BOD, and at the end of this period, the lengths of root, shoot height, length of the largest leaf, in millimeters, and total fresh matter, weighted in g and also percentage of germination. For the present study, the plot size was performed for the mentioned characteristics, with the exception of the germination percentage. To this end, only the seed lot that guarantees at least 90% of germinated seeds was used in the experiment, as this is the guarantee of most companies when they sell the seeds.

The blank test consisted of a 10 cm diameter petri dish containing 50 seeds, because it meets the different plot sizes adopted in the literature, from 5 seeds (21, 22) to 50 seeds per plot (23, 24, 25) and also meets the recommendation of Ribeiro-Oliveira et al. (26) who used the same dimension in the blank trials for the study of species of agricultural interest. The afore mentioned blank test was repeated five times with the same seed lot. This procedure was carried out with three different batches of seeds of the variety in order to take advantage of maximum variability, as shown by Ribeiro-Oliveira et al. (19), the quality of the seed lot can interfere with the sample size.

For each seed lot, the optimal plot size was determined by the modified maximum curvature method of Meier and Lessman (29), using bootstrap simulation with 2000 resamplings as suggested by Celanti et al. (32). For this, each seedling was individually weighed on a precision balance, constituting a basic experimental unit (BEU) and the simulated plot sizes had a minimum size of one BEU and a maximum size of 50 BEU (X_i = 1; 2; 3; 4; 5; ... 50 BEU = 1; 2; 3; 4; 5; ... 50 seedlings). Then, for each planned sample size, 2,000 simulations were performed, through resampling, with replacement. For each simulated sample, the mean was estimated. Thus, for each sample size, 2,000 average estimates were obtained (33) and from these, a coefficient of variation was obtained for each planned sample size, which we denote by $CV_{(X_i)}boot$. For the other characteristics evaluated, the same procedure was carried out as described for the fresh weight of the seedlings.

From the set of 50 data from the X_i and the regression $CV_{(X_i)}boot$ constant $\hat{\beta}_0$ and coefficient were estimated $\hat{\beta}_1$ via logarithmization of the function $CV_{(X_i)}boot = \hat{\beta}_0 X_i^{-\hat{\beta}_1}$. . Using the values $\hat{\beta}_0$ and $\hat{\beta}_1$ the optimal plot size given by

$$X_0 MLboot = \left[\hat{\beta}_0^2\,\hat{\beta}_1^2\left(2\hat{\beta}_1 + 1\right)/\hat{\beta}_1 + 2\right]^{\left(1/2+2\hat{\beta}_1\right)}.$$

The optimal plot size was the largest among the sizes verified for the four characteristics evaluated and was graphically represented in the relationship between $CV_{(X_i)}boot$ and the number of BEU. Data were analyzed using the computational resources of the R software (34) from scripts developed by Celanti et al. (32).

3. RESULTS AND DISCUSSION

The germination percentage of the seeds of the three different batches of lettuce seeds for the different germination and vigor experiments are shown in Figure 1 Experiments 1 and 2 of batch 3 of lettuce seeds (*Lactuca sativa* L. Vitória de Santo Antão) were used to determine the optimal plot size (OPS) because they had the best germination rate. Figure 2 illustrates experiments 1 and 2 of lot 3 at the evaluation stage 12 days after sowing.

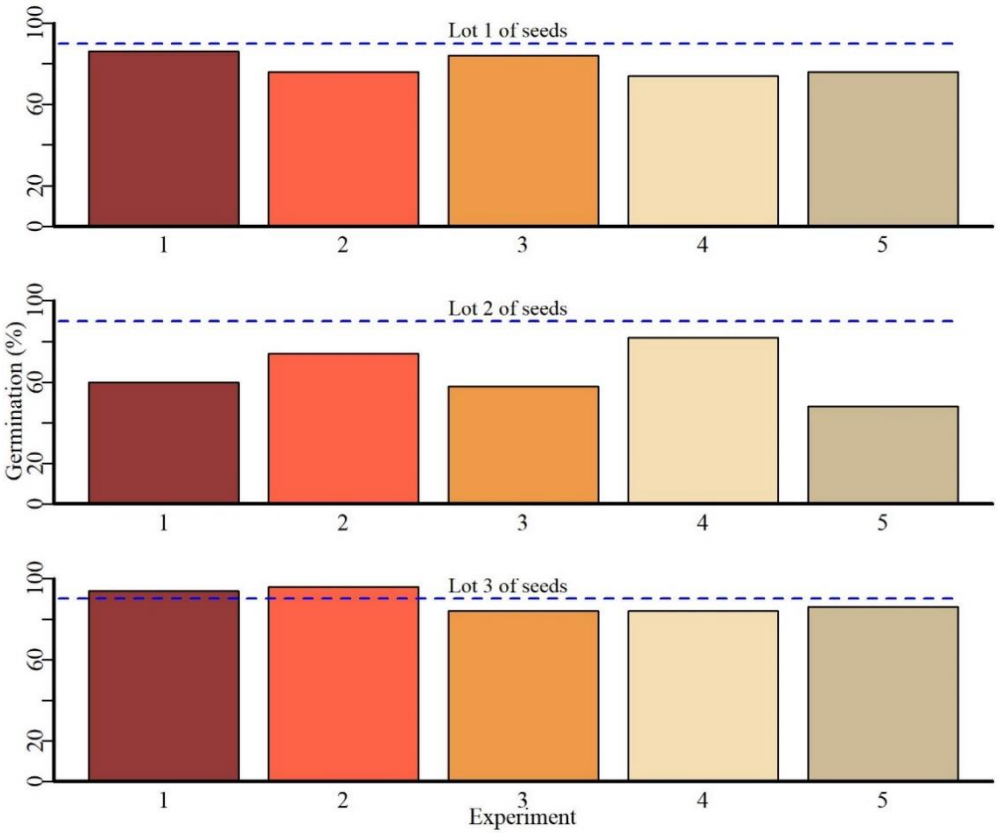

Figure **1.** Percentage of lettuce (*Lactuca sativa* L. Vitória de Santo Antão) seed germination in five experiments for three different seed lots, twelve days after sowing. The dashed line represents the percentage value of the seed lot.

Figure **2.** Illustration of experiments 1 (left) and 2 (right) of batch 3 of lettuce seeds (*Lactuca sativa* L. Vitória de Santo Antão), twelve days after sowing.

As for the descriptive characteristics of experiment 1 of batch 3, the variability of the data, measured by the coefficient of variation (CV%), among the four characteristics evaluated, the one with the lowest dispersion was shoot height (SH) with CV=20, 30%, very high, and the one that presented the greatest was the root length (RL) with CV=28.11%, also very high, according to the classification proposed by Schmildt et al. (14). In the descriptive characteristics of experiment 2 of lot 3, the characteristic that had the lowest dispersion was the shoot height (SH) with CV=16.65%, very high, and the one that presented the greatest dispersion was the root length (RL) with CV=24.56%, high, according to Schmildt et al. (14) (Table 1). It can be seen that there is high variation when using 50 seeds in a petri dish, as Bulegon et al. (23), Paula et al. (24) and Souza et al. (25), this result occurs because the sample size interferes in the percentage and speed of germination of lettuce (*Lactuca Sativa* L.) (14). Regarding the data normality test, only the root length characteristic, from experiment 1, does not present a normal distribution, according to the Shapiro-Wilk test (Table 1).

Table **1.** Descriptive statistics with minimum, maximum, mean values, standard deviation (SD), coefficient of variation (CV) and p-value of the Shapiro-Wilk (SW) normality test from lettuce seedlings (*Lactuca sativa* L. Vitória de Santo Antão), evaluated 12 days after sowing in petri dishes for different seed germination experiments.

Characteristic[1/]	Minimum	Maximum	Average	PD	CV (%)	SW [2/]
Lot 3, experiment 1						
RL	8.32	35.22	24.65	6.93	28.11	< 0.01
SH	5.72	23.35	16.96	3.44	20.30	0.25
LLL	2.94	8.90	5.83	1.36	23.32	0.39
TFM	0.0086	0.0239	0.0160	0.0036	22.52	0.86
Lot 3, experiment 2						
RL	10.73	32.43	20.68	5.08	24.56	0.38
SH	12.58	30.42	21.44	3.57	16.65	0.40
LLL	2.69	7.99	5.00	1.22	24.40	0.35
TFM	0.0067	0.0214	0.0144	0.0030	21.38	0.98

[1/] RL, root length, in mm; SH, shoot height in mm; LLL, length of the largest leaf, in mm; TFM, total fresh matter in g.

[2/] p value greater than 0.05 indicates normal distribution of data by the Shapiro-Wilk normality test.

Table 2 shows the optimal plot size (X_0 MLboot) for CR, APA, CMF and MFT, for the two experiments in batch 3, using the method of Meier and Lessman (12) with 2000 bootstrap simulations proposed by Celanti et al. al. (32) on lettuce seedlings (*Lactuca sativa* L. Vitória de Santo Antão), twelve days after sowing. This table can be consulted if the researcher wants to carry out the analysis of a specific characteristic. It is also possible to perceive different values than those used in the literature by other researchers (24). This OPS value, however, between 4 and 6 seeds per petri dish considering at least 90% germination, is similar to the number of seeds used by Pardócimo et al. (21) and Bittencourt et al. (22) in petri dishes. In this way, it is understood that it is possible to work with a smaller number of seeds than is occasionally worked (13). In this way, this material will serve for future consultations for researchers, mainly for works with allelopathy.

Table **2.** Estimated equation, coefficient of determination (R^2), Smith's heterogeneity index (b), optimal plot size (X_0 MLboot) and coefficient of variation for X_0 (CV_{Xo}), using the method of Meier and Lessman (29) with 2000 bootstrap simulations for four traits in lettuce seedlings (*Lactuca sativa* L. Vitória de Santo Antão), twelve days after sowing.

Characteristic[1/]	Equation	R^2 (%)	B	X0 MLboot	CV_{xo}
Lot 3, experiment 1					
RL	$\hat{Y} = 28.0831x^{-0.5057}$	0.9995	1.0099	5.4	12.09
SH	$\hat{Y} = 19.8938x^{-0.4984}$	0.9993	0.9983	4.3	9.45
LLL	$\hat{Y} = 23.2165x^{-0.5024}$	0.9992	0.9992	4.8	10.61
TFM	$\hat{Y} = 22.1529x^{-0.4994}$	0.9993	0.9996	4.6	10.45
Lot 3, experiment 2					
RL	$\hat{Y} = 23.8747x^{-0.4944}$	0.9977	0.9913	4.8	10.59
SH	$\hat{Y} = 16.6641x^{-0.5060}$	0.9993	1.0141	3.8	8.60
LLL	$\hat{Y} = 24.4274x^{-0.5018}$	0.9991	1.0021	4.9	11.08
TFM	$\hat{Y} = 21.4721x^{-0.5008}$	0.9995	1.0037	4.5	10.07

[1/] RL, root length, in mm; SH, shoot height in mm; LLL, length of the largest sheet, in mm; TFM, total fresh matter in g.

Figure 3 shows a relationship between the coefficient of variation and the number of seeds for the Root Length (RL) characteristic.

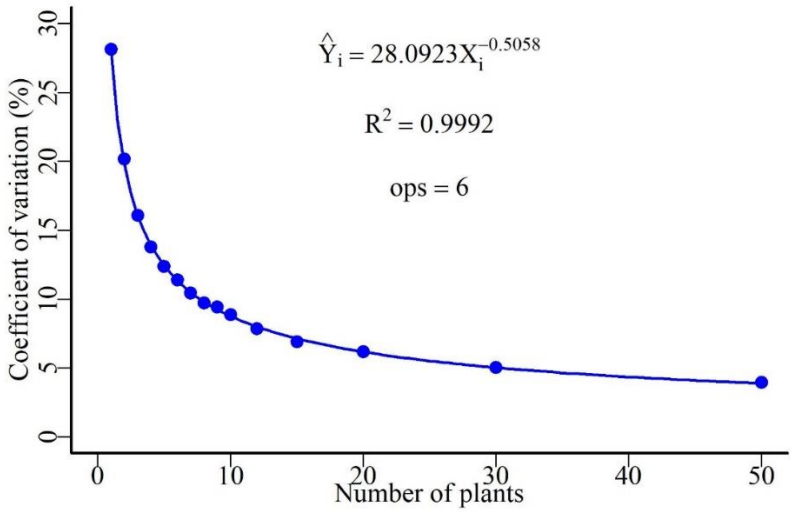

Figure **3.** Graphic representation between the coefficient of variation relationship and the planned plot size, in BEU and estimates of the optimal plot size (X_0) for the root length characteristic in experiment 1 of lettuce seed lot 3 (*Lactuca sativa* L. Vitória de Santo Antão), twelve days after sowing.

CONCLUSION

The optimal plot size for the cultivation of lettuce (*Lactuca sativa* L. Vitória de Santo Antão) in biogerminative tests is six seedlings per 10cm diameter petri dish.

CONFLICT OF INTEREST

There is no conflict of interest.

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Sample CRediT author statement

Rubens Neres Araújo: Conceptualization, Bibliographic research, Data Analysis, Preparation of the original draft, Editing and Revision. **Nathan Ribeiro de Castro**: Preliminary Studies, Data Analysis, Editing and Review. **Édlen dos Santos Bonelá**: Conceptualization, Supervision, Editing and Review. **Karina Tiemi Hassuda dos Santos**: Conceptualization, Supervision, Editing and Review. **Gleyce Pereira Santos**: Conceptualization, Supervision, Editing and Review. **Ana Valeria Lima Santos**: Conceptualization, Editing and Review. **Vinicius Souza Oliveira**: Conceptualization, Editing and Review. Omar Schmildt: Conceptualization, Supervision and Review. **Edilson Romais Schmildt**: Conceptualization, Supervision, Data Analysis and Review.

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