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CROP PRODUCTION

Plot and sample sizes for biometric variables in lettuce seedlings

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ABSTRACT: In order to ensure reliable inferences in scientific experiments, sampling criteria should be determined and defined during the experimental design phase. Determining plot and sample sizes without scientific criteria could result in resource and labor inefficiencies with no gain in experimental precision. In this work we estimate plot and sample sizes for biometric variable analyses in lettuce seedlings. The seedlings were sown in four expanded polystyrene foam transplant trays with 128 and 200 cells. Foliar area, stem diameter, shoot height, root length, root volume, root area, fresh and dry shoot and root masses. The methods described by Paranaiba (PARANAIBA et al., 2009) and Cochran (COCHRAN, 1977) were used for plot size and sample size estimations, respectively. Sample size varied according to the variables analysed and transplant tray cell number. Root variables required the highest number of samples. For a 10% margin of error, 12 seedlings were necessary for evaluations in a 128 cell tray, while 11 seedlings were required for analysis in 200 cell trays.
Key words: *Lactuca sativa*, crop vegetables, experimental planning.

Tamanhos de parcelas e amostras para variáveis biométricas em mudas de alface

RESUMO: A fim de garantir inferências confiáveis em experimentos científicos, os critérios de amostragem devem ser determinados e definidos durante a fase de planejamento experimental. Determinar tamanhos de parcelas e amostras sem critérios científicos pode resultar em ineficiências de recursos e mão de obra sem ganho de precisão experimental. Neste trabalho estimamos tamanhos de parcelas e amostras para análises de variáveis biométricas em mudas de alface. As mudas foram semeadas em quatro bandejas de transplante de espuma de poliestireno expandido com 128 e 200 células. Área foliar, diâmetro do caule, altura da parte aérea, comprimento da raiz, volume da raiz, área da raiz, parte aérea fresca e seca e massas radiculares. Os métodos descritos por Paranaíba (PARANAÍBA et al., 2009) e Cochran (COCHRAN, 1977) foram utilizados para estimativas de tamanho de parcela e tamanho de amostra, respectivamente. O tamanho da amostra variou de acordo com as variáveis analisadas e o número de células da bandeja de transplante. As variáveis de raiz exigiram o maior número de amostras. Para uma margem de erro de 10%, foram necessárias 12 mudas para avaliação em bandeja de 128 células, enquanto 11 mudas foram necessárias para análise em bandeja de 200 células.
Palavras-chave: *Lactuca sativa*, hortaliças, planejamento experimental.

INTRODUCTION

Seedling production is one of the most important stages for high quality vegetable production. Currently, few vegetable producers continue to produce their own seedlings, as acquiring them from a specialized nursery is an established practice in Olericulture and the production of seedlings is one part of the production chain that has experienced significant growth (JORGE et al., 2016). Seedling production directly affects plant performance and there is a direct relation between high quality seedlings and productive plants in the field.

Certain aspects of seedling production management (CLÁUDIO et al., 2020; VETRANO et al., 2020) determine seedling quality and consequently their production. In a work assessing the effect of seedling production tray cell volumes on the quality of seedlings, CECCHERINI et al. (2020) found that factors related to the cell volume affect their quality and production performance. These results highlight the importance of high quality seedlings and exemplify the need for studies determining transplant quality.

In agricultural experimentation it is necessary to balance the need for samples that adequately represent the population with restraints related to labor, time and financial resource costs (COCHRAN, 1977). Therefore, researchers should establish an optimal sample size permitting the estimation of average variable values

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with appropriate precision (HELL et al., 2017). There are reports on sample size concerning cultures such as beans (CARGNELUTTI FILHO et al., 2018), soybeans (SOUZA et al., 2022) and cauliflower (BITTENCOURT et al., 2022). With respect to seedlings sample size assessments, there are works with eggplant, gilo (HELL et al., 2017), papaya (CELANTI et al., 2016) and cassava (SCHOFFEL et al., 2020) in the literature.

Establishing optimal plot size increases experimental precision and the reliability of information. In experiments, precision and reliability are correlated with issues related to variability in the experimental material used (PIRES et al., 2016). Thus, plot size should be estimated for each culture and each variable. Even so, the number of samples to be evaluated (sample size) must be defined within each plot. For example, in a plot containing 20 samples, 15 samples must be evaluated. Such planning is important in order to prevent material waste and optimize the planting space by dividing the same tray into several plots. For this reason, this study aims to estimate the ideal plot and sample sizes for the many different biometric variables related to lettuce seedling shoot and root growth.

MATERIALS AND METHODS

The experiment made use of four expanded polystyrene foam trays with 128 cells and four expanded polystyrene trays with 200 cells, all filled with commercial substrate for vegetable seedlings enriched with 80 g of NPK 4-14-8 per 25 kg of the substrate. Three seeds per cell of the 'Cinderela' lettuce cultivar were used. Sowing was carried out on May 24th, 25th, 26th, and 27th, 2019 with two trays sown per day and starting with trays of 128 cells. Trays were kept under protected environment (greenhouse) on tray stands at 1 m from ground, since sowing until transplant evaluation. Water was disposed through an automatic system of irrigation in a schedule of three to four times a day, with 2.5 minutes per irrigation. To keep one plant per cell a thinning was carried out at nine and ten days after sowing.

Seedlings were harvested 21 days after emergence. All the individuals in each tray were evaluated, totaling 1312 seedlings. After harvesting, roots were washed in running water using a sieve to avoid root losses. Then seedlings were placed on absorbent paper to remove excessive water and afterwards the total length - TL (cm) and aerial length - AL (cm) were determined. Root length - RL (cm) was defined as the difference between TL and AL. The stem diameter SD - (mm) was measured in the middle portion of the transplant stems. Next total fresh - TFM (g), aerial - FSW (g) and root fresh - FMR (g) masses were determined.

To obtain digital images from shoots and roots, the materials were placed between transparent acetate sheets and scanned. Leaf area - LA (cm^2) was measured using the Digimizer® software (version 5.3.5 Medical Software Brolkstraat, 2019), while root volume - RV (mm^3) and root area - RA (mm^2) were measured with Safira® software (EMBRAPA, 2010). Afterwards, the material was dried in oven with forced air circulation at 65ºC until reaching a constant mass. Then shoot (SDW) and root (DRW) dry weight were determined. To estimate the plot size (number of cells to be used in the tray, considering cell area of 12.25 cm^2 in 128 cell trays and 9 cm^2 in 200 cell trays), the maximal curvature of the coefficient of variation method proposed by PARANAIBA et al., (2009), using the following expression:

$$\hat{X}_0 = \frac{10\sqrt[3]{2(1-\hat{p}^2)s^2\overline{X}}}{\overline{X}} \quad (1)$$

Where: $\hat{X}_0$ = adequate plot size; s^2 = the variance in the growing tray; $\overline{X}$ = average of basic units in the growing tray; $\hat{p}$ = first order spatial self-correlation, estimated by the expression (2):

$$\hat{\rho} = \frac{\sum_{i=2}^{n}(R_i)(R_{i-1})}{\sum_{i=1}^{n}(R_i)^2} \quad (2)$$

Where: R = residual.

After plot size estimation, the number of samples per plot was estimated using the expression (3) (COCHRAN, 1977), as follows:

$$n = \frac{t^2_{\alpha/2}(CV\%)^2}{(D\%)^2} \quad (3)$$

Where: n = sample size; $t^2_{\alpha/2}$ = Student t value for n-1 degrees of freedom with a probability of error p=0.05; D% = is the half-width of the mean's confidence interval (D% = 5, 10, 15, 20%); CV% = the variation coefficient of each variable, calculated through the expression (4), as follows:

$$CV\% = \frac{100\times\sqrt{s^2}}{\overline{X}} \quad (4)$$

Where: s^2 = sample variance; $\overline{X}$ = average of each variable.

Correction for a finite population was performed according to COCHRAN (1977) using the following expression (5):

$$nc = \frac{n}{1+\frac{n}{N}} \quad (5)$$

Where: nc = adjusted sample size; N = population size (128 and 200 cells); n = sample size for an infinite population.

RESULTS AND DISCUSSION

Plot size and sample size (inside a plot size) were different for each studied variable and assumed sample errors (Table 1). For the trays with 128 cells, sample sizes within a plot ranged from 3 to 12 seedlings, while for those of 200 cells, there was a variation of 3 to 10 seedlings. For both cell volumes, the results indicate that root variables require larger plot sizes, while for aerial parts, small sizes are adequate. RVati, for example, requires 12 seedlings in the 128 trays, and for CT and CP only two seedlings are necessary in the same size tray, assuming D%=10.

For the 200 cell trays, sample size differences for all analyzed variables (Table 2) were also observed. In this cell size, the RV variable required 11 seedlings per plot; the CT required two seedlings per plot. As observed for the 128 cell tray, the variables with the highest variability were those related to the root system.

The smaller the CV, the smaller the sample size, since some characteristics with high variability will require the analysis of a higher number of seedlings to diminish the experimental error. Thus, it was expected that the smallest sample sizes will be those that get the smaller CV (CELANTI et al., 2016).

The highest CVs observed in this work were 26.7 and 23.91 for the VR variable in both trays; i.e., 128 and 200 cells, respectively (Tables 1 and 2). High CVs are attributed to root variables since they showed increased variability both in growth as well as in the process of material sampling. The process of removing and washing roots leads to material losses, which causes wide variability. Another factor that could increase the variability is the way the root variable area and root variable volume are obtained. Because roots, for example, may overlap during imaging, resulting in high variability, higher plot and sample sizes are required.

For the variables of length, the results showed a pattern of reduced plot and sample size. Although seedlings showed differences and their measurement requires simple instruments, like a ruler, there was a trend of uniformity, resulting in homogeneity and, consequently, low variability.

Sample sizes were different between trays, and for the same number of cells, differences were also observed according to the biometric variable analyzed. Similar results were noticed for *Passiflora edulis* (COELHO et al., 2011), *Passiflora foetida* fruits (SCHMILDT et al., 2017), eggplant and gilo (HELL et al., 2017).

When studying seedlings, determinations of adequate sample and plot sizes can reduce costs and increase research efficiency (BACCHETTI et al., 2008). Our results demonstrate that for the 128 cell trays a plot with at least 12 seedlings is necessary to evaluate root variables. In this way, the tray space is optimized. For example, instead of using a tray with 128 cells as a single plot and single repetition, the same tray could be used with different plots (containing a minimum of 12 seedlings each) and within these plots a minimum of 12 seedlings could be used for evaluation (simple size) of that specific variable. This practice could save analysis time and research costs by reducing reagent, substrate, seeds and other materials.

In 128 cell trays it is possible to achieve 10 plots with 12 seedlings each, totaling 120 seedlings. Another possibility is to divide the tray into 8 plots with 16 seedlings. In each 16 seedling plot, only 12 should be evaluated for that variable with the highest sample size in the plot, in our case root variables, and so on. The seedlings in a plot must be selected randomly.

For the 200 cell trays, the minimum plot size required for root evaluations is 11 seedlings. As exemplified for the 128 cell trays, all of the tray could be used. Each 200 cell tray could be divided into 18 plots with 11 seedlings each, with 2 unused seedlings per tray. It is also possible to get 10 plots of 20 seedlings each. The sample size should be in accordance with table 2.

For biometrical variables from aerial parts of the seedlings, it is possible to use smaller plot sizes, increasing the number of repetitions per tray. In this case, using the 128 cell trays, at least 4 seedlings should be evaluated. So, it is possible to get up to 32 plots in this cell size. However, when using 200 cell trays, 5 seedlings are necessary, so each tray could contain up to 40 plots. For the evaluation of both variables, 10 plots containing 12 seedlings or 8 plots containing 16 seedlings would be recommended for the 128-cell tray, while 18 plots with 11 seedlings or 10 plots with 20 seedlings would be called for the 200-cell tray since the root variables require a larger sample size.

Table 1 - Average, Plot size (X0), coefficient of variation of X0 (CV X0) and sample size (n) inside the plot, for confidence interval semi-widths of 5, 10, 15 and 20% precision, for the variables total length - TL (cm), root length - RL (cm), shoot length - SL (cm), stem diameter - SD (mm), total fresh mass - TFM (g), fresh mass of the root - FMR (g), fresh shoot weight - FSW (g), dry root weight - DRW (g), dry shoot weight - DSW (g), root volume - RV (mm²), area of the root - RA (mm²) and leaf area - LA (cm²) in 128 cell lettuce trays). Campos dos Goytacazes, UENF, 2019.

	Trays	Average	X0	CV X0	D = 5%	D = 10%	D = 15%	D = 20%
	T1	18.72	3	4.94	2	2	1	2
TL	T2	18.54	3	5.47	2	2	1	2
	T3	18.60	3	5.38	2	2	1	2
	T4	16.62	3	4.66	3	2	1	1
	T1	8.35	3	7.23	3	2	2	1
RL	T2	7.69	4	7.99	4	3	2	2
	T3	8.12	3	7.34	3	2	2	1
	T4	7.55	4	8.01	4	3	2	2
	T1	10.37	3	6.28	3	2	1	1
SL	T2	10.84	3	7.13	3	2	2	1
	T3	10.48	3	6.64	3	2	1	1
	T4	9.07	3	5.19	3	2	2	1
	T1	1.47	4	9.47	4	3	3	2
SD	T2	1.56	4	8.22	4	3	2	2
	T3	1.43	3	7.57	3	2	2	1
	T4	1.45	4	8.57	4	3	3	2
	T1	0.93	4	9.26	4	3	3	2
TFM	T2	1.05	4	9.46	4	3	3	2
	T3	1.04	4	9.00	4	3	3	2
	T4	0.77	3	7.30	3	2	2	2
	T1	0.14	8	18.08	8	8	7	7
FMR	T2	0.14	7	14.20	7	6	5	5
	T3	0.16	11	24.04	11	11	10	9
	T4	0.16	11	23.85	11	11	10	9
	T1	0.79	4	8.43	4	3	2	2
FSW	T2	0.91	4	9.57	4	3	3	2
	T3	0.88	5	10.14	5	4	3	3
	T4	0.69	4	8.05	4	3	3	2
	T1	0.01	5	11.78	5	4	4	3
DRW	T2	0.01	7	14.53	7	6	6	5
	T3	0.01	6	13.18	6	5	5	4
	T4	0.01	4	9.03	4	4	3	3
	T1	0.04	4	9.90	4	3	3	2
DSW	T2	0.04	5	10.16	5	4	3	3
	T3	0.04	4	9.53	4	3	3	2
	T4	0.04	4	9.05	4	3	3	2
	T1	0.12	9	20.36	9	9	8	7
RV	T2	0.13	8	18.58	8	7	7	6
	T3	0.23	12	26.72	12	12	11	11
	T4	0.20	8	18.04	8	8	7	7
	T1	0.45	9	18.90	9	8	8	7
RA	T2	0.48	8	17.42	8	7	7	6
	T3	0.38	10	21.40	10	9	9	8
	T4	0.32	8	17.22	8	8	7	7
	T1	3.62	4	7.94	4	3	2	2
LA	T2	4.12	4	8.81	4	3	3	2
	T3	3.87	4	8.73	4	3	3	2
	T4	3.26	4	7.50	4	3	3	2

Table 2 - Average, Plot size (X0), coefficient of variation of X0 (CV X0) and sample size (n) inside the plot, for confidence interval semi-widths of 5, 10, 15 and 20% precision, for the variables total length - TL (cm), root length - RL (cm), shoot length - SL (cm), stem diameter - SD (mm), total fresh mass - TFM (g), fresh mass of the root - FMR (g), fresh shoot weight - FSW (g), dry root weight - DRW (g), dry shoot weight - DSW (g), root volume - RV (mm²), area of the root – RA (mm²) and leaf area - LA (cm²) in 200 cell lettuce trays). Campos dos Goytacazes, UENF, 2019.

	Trays	Average	X0	CV X0	D = 5%	D = 10%	D = 15%	D = 20%
	T1	17.90	3	6.25	3	2	2	1
TL	T2	17.26	3	6.41	3	2	2	1
	T3	17.22	3	5.72	3	2	1	2
	T4	16.18	3	6.46	3	2	1	1
	T1	7.90	4	7.80	4	3	2	2
RL	T2	7.79	3	7.05	3	2	2	1
	T3	7.57	3	7.52	3	2	2	1
	T4	6.85	4	9.50	4	3	3	2
	T1	10.00	4	8.26	4	3	3	2
SL	T2	9.47	4	9.43	4	3	3	2
	T3	9.66	3	7.04	3	2	2	1
	T4	9.33	3	6.99	3	2	2	1
	T1	2.21	5	10.32	5	4	3	3
SD	T2	1.70	5	10.64	5	4	3	3
	T3	1.66	4	9.33	4	3	3	2
	T4	1.53	4	9.02	4	3	3	2
	T1	1.00	8	6.00	8	8	7	7
TFM	T2	0.84	6	12.91	6	5	5	4
	T3	0.78	4	9.02	4	3	3	2
	T4	0.72	4	9.22	4	3	3	2
	T1	0.17	8	18.69	8	7	7	6
FMR	T2	0.18	5	11.69	5	4	4	3
	T3	0.13	7	15.13	7	6	6	5
	T4	0.09	7	16.34	7	7	6	5
	T1	0.83	10	21.26	10	10	9	9
FSW	T2	0.66	6	14.07	6	5	5	4
	T3	0.65	4	8.78	4	3	3	2
	T4	0.63	4	8.96	4	3	3	2
	T1	0.01	5	12.14	5	4	4	3
DRW	T2	0.01	6	14.07	6	5	5	4
	T3	0.01	6	12.97	6	5	5	4
	T4	0.01	6	13.79	6	5	5	4
	T1	0.04	5	10.52	5	4	4	3
DSW	T2	0.03	6	13.61	6	5	5	4
	T3	0.03	5	10.11	5	4	3	3
	T4	0.03	5	10.67	5	4	4	3
	T1	0.26	9	19.08	9	8	8	7
RV	T2	0.37	10	21.75	10	9	9	8
	T3	0.24	10	21.82	10	10	9	9
	T4	0.17	11	23.91	11	11	10	10
	T1	0.40	8	17.84	8	7	7	6
RA	T2	0.56	9	19.11	9	8	8	7
	T3	0.38	10	21.67	10	10	9	9
	T4	0.27	10	21.19	10	10	9	8
	T1	3.22	4	8.81	4	3	3	2
LA	T2	2.91	6	13.13	6	5	5	4
	T3	2.94	4	8.37	4	3	3	2
	T4	2.75	4	7.99	4	3	2	2

Our research findings are useful for studies concerning the quality of plug seedlings of lettuce, the most cultivated leafy vegetable worldwide. These results inform how many plots could be distributed in trays with different cell volumes and how many seedlings are necessary for biometric evaluations according to the desired significance level stipulated in the research plan. They also demonstrate that it is not necessary to use extremely large sample sizes and suggest arrangements for a set of biometric variables, promoting efficiency and reducing costs and labor.

CONCLUSION

The plot and sample sizes (within a single plot) vary according to the number of cells in a tray and the biometric variables considered. Root variables will require the highest sample size, while for aerial parts analysis smaller sample sizes are required. Since the highest plot sizes are 12 and 11, respectively, for 128 and 200 cell trays, it is possible to increase repetition number per tray, dividing it into plots, and thus optimizing research efficiency.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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