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Onion culture: experimental techniques for carrying out high precision experiments

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ABSTRACT: The cultivation of onion *Allium cepa* L. is of extreme economic importance globally, and, because of this, research must be constantly updated in order to increase productivity. This study was designed to estimate the optimal plot size, sample size and number of repetitions for experiments evaluating variables in the onion crop. Uniformity tests were carried in the years 2016 and 2017 in the experimental area of the Crop Science Department at Universidade Federal de Santa Maria, Santa Maria (RS), Brazil. Each plant was considered a basic experimental unit. Bulb mass, bulb height, and bulb diameter were measured for each basic experimental unit. The optimal plot size, sample size, and number of repetitions were estimated by the method of maximum curvature of the variation coefficient. For onion culture, the optimal plot size to evaluate the bulb mass, bulb height and bulb diameter is eight, six and six, respectively. The optimal sample size for evaluating the bulb mass of the onion crop is six plants, while for the height and diameter variables the optimal sample size is four plants in the direction of the row considering a semi-amplitude of the confidence interval (D%) equal to 20% of the average. Bulb mass variables require 10 repetitions to assess up to 20 treatments in a randomized block design for the least significant difference of the Tukey's test, expressed as a percentage of the average of 35%. Bulb height and bulb diameter require just six repetitions for the same assessment.

Key words: *Allium cepa*, plot size, sample size, number of replications.

INTRODUCTION

The onion crop (*Allium cepa* L.) is of extreme economic importance, being among the most relevant vegetable crops in the world (Faostat 2021). The world's largest producers of onions are China, India and the United States of America, with 24.775.344, 22.071.000 and 3.284.420 tons, respectively (Faostat 2021). The onion is consumed fresh, processed and industrialized and gives rise to many products that are used as condiments in human food (Costa and Resende 2007).

Research to qualify cultural practices and increase their productivity for onion cultivation is essential, as it is an economically important crop. Several factors interfere with onion production, including the use of quality seeds and of associated production technologies (Pannacci et al. 2020), necessary nutrition (Okada et al. 2020), solar radiation, luminosity and water availability (Esmaeilzadeh et al. 2020), among others. In some onion-producing countries, average productivity is very low (Ghosh et al. 2018). Therefore, research that promotes the best growth and development of culture must be constantly updated.

For agricultural research to provide high experimental precision and reliable results for farmers, experiments must follow some concepts of experimental statistics. These concepts guide the researcher to plan, execute, analyse the data obtained and interpret the results of experiments correctly. The experimental planning phase is one of the fundamental steps for the success of the experiment, as it is essential that the data obtained in the experiments be a reflection of the effect of the tested treatments (Krysczun et al. 2018; Lúcio et al. 2020; Tartaglia et al. 2021).

When experimental planning is carried out carefully, the data obtained are more reliable and precise, and can generate useful technical recommendations that can be generalized to a variety of settings. The number of repetitions per treatment, adequate sample and plot sizes, and choice of experimental design that controls variability are all important factors that minimize experimental error and provide greater experimental precision. In addition, there are practical advantages in reducing the working time, labor and cost necessary to carry out the experiment (Tartaglia et al. 2021).

The shape and size of the plot and the number of repetitions needed in an experiment are important in order to identify significant differences between treatment (Silva 2014[2]; Storck et al. 2011; Tartaglia et al. 2021). The size of the plot depends on the experimental material, the number of treatments, the area, the cost and the labor available (Lúcio and Sari 2017). The greater the number of repetitions, the lower the residual variance of the treatments and, consequently, the lower the mean square of the error (Lúcio and Sari 2017; Tartaglia et al. 2021).

In many cases, when the experiments are large, sampling in the plot can be used to facilitate their evaluation. However, this practice can inflate the mean square of the error (Lúcio and Sari 2017). Thus, it is necessary to use an appropriate sample size.

Several studies have estimated the optimum plot and sample size, in addition to the number of repetitions in various cultures, as for *Helianthus annuus* (Sousa et al. 2016), *Cucurbita pepo* (Lúcio and Benz 2017), *Solanum melongena* (Krysczun et al. 2018), and *Cucumis sativus* (Lúcio et al. 2020). For *A. cepa*, Boyhan et al. (2003) estimated optimum the size and number of plots to optimize the variables yield, seedstem formation, purple blotch and/or *Stemphylium* e botrytis leaf blight. While these are important variables for optimization, other variables are also relevant.

The aim of this study, therefore, was to estimate the optimal plot size, the sample size and the number of repetitions for the variables bulb mass, bulb height and bulb diameter in order to minimize the experimental error and increase the precision of experiments with onion culture.

MATERIAL AND METHODS

Site description and experimental design

The uniformity trials were conducted in the experimental area of the Crop Science Department at Universidade Federal de Santa Maria, Santa Maria (RS), Brazil (latitude 29°42'23"S; longitude 53°43'15"W, and 95 m altitude). The region's climate is of the Cfa type, according to Köppen's classification (Alvares et al. 2013), and the soil in the experimental area is classified as Typic Hapludalf (Streck et al. 2008).

Seedling transplant took place in two different seasons. The first set was transplanted in the last week of June 2016 into six beds with four rows of plants each, spaced 20 cm apart and 20 cm between plants, totaling 55 plants per row in an area of 200 m^2. In the second season, seedlings were transplanted in the first week of July 2017 into six beds with five rows of plants each, spaced 20 cm apart and 20 cm between plants, totaling 100 plants per row, in an area of 300 m^2. Each plant was considered a basic experimental unit (BEU). Bulb mass (BM, in g), bulb height (BH, in cm) and bulb diameter (BD, in cm) were evaluated in each BEU.

Statistical analysis

A homogeneity test of variances between cultivation rows was performed for each bed and for each variable using the homogeneity test of variances proposed by Bartlett (1937).

For each cultivation row, plot sizes (number of plants) were estimated using the maximum curvature of the variation coefficient method proposed by Parnaiba et al. (2009), by Eq. 1:

$$\widehat{X_0} = \frac{10\sqrt[3]{2(1-\hat{\rho}^2)s^2\bar{Y}}}{\bar{Y}} \quad (1)$$

[2] Silva, W. C. (2014). Estimativa de tamanho ótimo de parcelas experimentais para a cultura do taro (Colocasia esculenta). Universidade Federal de Viçosa. 2014 59 f. Tese (Doutorado). Viçosa: UFV.

in which: $\widehat{X_0}$ = the appropriate plot size; s^2 = the variance in the row of cultivation; $\bar{Y}$ = the average of the BEU in the row of cultivation; $\hat{\rho}$ = the first-order spatial autocorrelation estimated by Eq. 2:

$$\rho = \frac{\sum_{i=1}^{n}(\hat{\varepsilon_i} - \bar{\varepsilon})(\hat{\varepsilon}_{i-1}\varepsilon)}{\sum_{i-1}^{rc}(\hat{\varepsilon_i} - \bar{\varepsilon})^2} \tag{2}$$

in which: $\hat{\varepsilon}$ = the experimental error associated with the observation of each i BUE; $\bar{\varepsilon}$ = average of the experimental errors.

The experimental error was estimated by Eq. 3:

$$\varepsilon_i = \rho\varepsilon_i\text{-}1 + U_i \tag{3}$$

in which: U_i = the pure experimental error, regardless of $U_i \sim N(0,\sigma^2)$.

The sample size (number of plants) estimated for each cultivation row was performed using the methodology proposed by Cochran (1977) (Eq. 4):

$$n = \frac{t^2_{\propto/2}(CV\%)^2}{(D\%)^2} \tag{4}$$

in which: n = the sample size; $t^2_{\alpha/2}$ = the value of Student's t-table with n-1 degrees of freedom at 5% probability of error;

CV% = the coefficient of variation of the variable considered calculated by Eq. 5:

$$CV\% = \frac{100\sqrt{s^2}}{\bar{X}} \tag{5}$$

in which: s^2 = the sample variance; $\bar{X}$ = the mean of each variable; D% = the semi-amplitude of the mean confidence interval (D% = 5, 10, 15 and 20).

The correction for the finite population was carried out according to the Cochran recommendation (1977). For this, Eq. 6 was applied:

$$nc = \frac{n}{1 + \frac{n}{N}} \tag{6}$$

in which: nc = the corrected sample size; N = the population size for each crop row (55 for the year 2016 and 100 for the year 2017); *n* = the sample size for infinite population.

To estimate the number of repetitions (row of cultivation), the minimum significant difference (d) of the Tukey's test was used, expressed as a percentage of the test mean (Eq. 7)

$$\mathrm{d} = \left(\frac{\mathrm{q}_{\propto(\mathrm{i;DFE})}\frac{\sqrt{MSE}}{\mathrm{r}}}{\bar{\mathrm{Y}}}\right) \mathrm{x}\ 100 \tag{7}$$

in which: $q\alpha$("i;DFE") = the critical value of the Tukey's test at level α of probability of error (α = 0.05 adopted in this research); i = the number of simulated treatments (two to 20 treatments); DFE = the number of degrees of freedom of error for the randomized block design, that is, (i-1) (r-1), in which r is the number of repetitions defined as 24 for the year 2016 and 30 for the year 2017; MSE = the mean square of the error and the mean of the experiment.

Each row of cultivation constituted a block due to the significant heterogeneity between the rows of cultivation. Thus, replacing the expression of the experimental coefficient of variation (CV) in percentage in the expression for the calculation of the isolating r, there is Eq. 8:

$$r = \left(\frac{q_{\propto(i;DFE)}\ CV}{d}\right)^2 \quad (8)$$

In this study, the CV was expressed as a percentage and corresponded to the CVXo, because this was the expected CV for the experiment with the plot size (Xo) calculated previously. Through the highest coefficient of variation in plot size (CVXo) of the total grouping of harvests, the number of repetitions (r) was determined, in an iterative fashion until convergence, for experiments in randomized block design in scenarios formed by combinations of i (i = 2, 3, 4, ..., 20) and d (d = 5%, 10%, 15%, ..., 50%). All analyses were performed with the R software version 3.5.3 (R Development Core Team 2019) and with Office Excel® application.

RESULTS AND DISCUSSION

Experimental variability

In some beds, there was heterogeneity between the crop rows (Table 1), as seen through Bartlett's homogeneity test of variances carried out in each season. It indicates that the optimal experimental design should use randomized blocks, following the recommendation of Lúcio and Sari (2017), for whom each block / replication is composed of a row of cultivation.

Table 1. p-value of the Bartlett's test between the rows of each plot, plot size (X_o, in plants) and coefficient of variation in plot size in parentheses (CV X_o, in %) of each row in each plot, for the bulb mass (BM, in grams), bulb height (BH, in centimeters), and onion bulb diameter (BD, in centimeters) variables, for the growing season in 2016 and 2017.

Bed	X_o	2016					2017					
		1	2	3	4	p-value*	1	2	3	4	5	p-value*
1	BM	6 (14)	5 (11)	6 (13)	5 (11)	0,019	6 (13)	6 (13)	6 (13)	6 (13)	6 (14)	0,001
	BH	3 (6)	3 (7)	3 (7)	2 (5)	0,418	3 (7)	3 (7)	4 (9)	3 (7)	3 (7)	0,579
	BD	3 (8)	3 (6)	3 (7)	3 (6)	0,007	3 (7)	3 (7)	4 (9)	3 (7)	4 (8)	0,064
2	BM	6 (14)	6 (13)	5 (11)	6 (14)	0,078	7 (16)	7 (16)	7 (16)	7 (16)	7 (15)	0,033
	BH	3 (7)	3 (6)	2 (5)	3 (7)	0,055	4 (9)	5 (11)	4 (9)	5 (11)	4 (10)	0,455
	BD	4 (8)	4 (8)	3 (6)	3 (7)	0,110	4 (9)	5 (11)	5 (11)	5 (12)	4 (10)	0,053
3	BM	5 (12)	6(13)	7 (15)	5 (11)	0,258	6 (14)	7 (16)	8 (18)	8 (18)	8 (17)	0,236
	BH	3 (6)	3 (7)	3 (7)	3 (6)	0,198	4 (8)	4 (10	5 (12)	6 (14)	6 (14)	0,014
	BD	3 (6)	3 (7)	4 (9)	3 (7)	0,007	4 (8)	5 (11)	6 (13)	6 (14)	6 (14)	0,248
4	BM	6 (14)	5 (12)	6 (13)	6 (13)	0,741	6 (14)	7 (16)	6 (14)	7 (15)	7 (15)	0,007
	BH	4 (9)	3 (7)	3 (6)	3 (6)	0,030	3 (8)	4 (8)	4 (10)	5 (11)	5 (11)	0,624
	BD	4 (9)	3 (7)	3 (8)	3 (7)	0,097	4 (8)	4 (9)	5 (11)	5 (11)	5 (11)	0,666
5	BM	6 (12)	6 (14)	6 (13)	6 (13)	0,426	6 (14)	6 (14)	6 (14)	6 (14)	6 (13)	0,040
	BH	2 (5)	2 (5)	2 (5)	2 (5)	0,632	5 (11)	4 (8)	4 (9)	3 (7)	4 (9)	$7{,}05\text{X}10^{-8}$
	BD	4 (9)	4 (9)	4 (9)	4 (8)	0,347	4 (10)	4 (9)	4 (10)	3 (8)	4 (9)	0,571
6	BM	5 (12)	5 (12)	7 (15)	6 (14)	0,403	7 (15)	7 (15)	6 (14)	6 (14)	6 (14)	0,031
	BH	3 (7)	3 (6)	3 (7)	3 (8)	0,122	3 (7)	4 (9)	4 (9)	4 (9)	3 (7)	0,001
	BD	3 (7)	3 (7)	3 (8)	4 (10)	0,413	3 (8)	4 (10)	4 (10)	4 (10)	3 (8)	0,665

*p-value less than 0.05 indicates heterogeneous variances between cultivation rows within each bed.

After the previous test of homogeneity of the variances, it was found that some rows were heterogeneous. Soil fertility, irregular irrigation or the presence of pests, diseases, and weeds influence variability (Lúcio et al. 2010; Lúcio and Benz 2017). For these and other factors that may impact the experimental conditions, randomized block designs are recommended. These designs allow greater control of the experimental variability of the area studied, which minimizes the potential for inflation of the experimental error and the variation coefficient. This was also recommended by Marodim et al. (2000), and Lúcio and Sari (2017). Using the CV as a measure of experimental quality allows the comparison of results from different experiments involving the same response variable or species, enhancing the precision and the reliability of the results (Steel et al. 1997).

Plot size

Optimal plot sizes were larger for variable bulb mass in both seasons. In 2016, the plot size ranged from five to seven plants, with a CV in plot size of 11 and 15%, respectively, whereas in 2017 the plot size was six and eight plants, with a CV of 13 and 18%, respectively. This larger plot size in 2017 is due to the greatest variability of the experimental database in this growing year. For the variables bulb height and bulb diameter in both years (Table 1), the plot sizes were smaller, ranging from two to six plants, with a variation coefficient of 5 and 14%, respectively.

The plot size is mainly influenced by the variability of the experimental area. The effect of this variability can be reduced by correct dimensioning and the use of the experimental design (Storck et al. 2006).

For experiments with the onion crop, the largest plot size indicated in this work (eight plants for bulb mass, six plants for bulb height and bulb diameter) is recommended, as this plot size allows coverage of the variability across rows and other variables, to provide more reliable results and accurate estimates of treatment effects. These portion sizes are in accordance with those recommended by Boyhan et al. (2003) to evaluate several variables with production, trunk and seed formation, number of pairs and disease incidence, which vary from four to 10 plants per plot. However, for the variables analyzed in this work, no data were found in the literature. In addition, due to the heteroscedasticity of the cultivation rows, a randomized block design, with each row, a block is recommended. This was seen and recommended by researchers working with other cultures such as *Capsicum annuum* (Lorentz and Lúcio 2009), *Pisum sativum* (Tartaglia et al. 2021), *Solanum lycopersicum* (Lúcio et al. 2016), *Solanum melongena* (Krysczun et al. 2018) and *Cucumis sativus* (Lúcio et al. 2020).

Sample size

The optimal sample size (in number of plants) varied according to the variable analyzed, with the minimum differences between means (D%) and between cultivation rows (Table 2). Furthermore, as it seeks to identify the minimum differences between the means of the largest treatments, the number of plants needed to carry out the sampling may be less. In 2016, for a semi-amplitude of the 95% confidence interval (D%) equals to 5% of the average sample size, the bulb mass variable ranged from five to six plants between rows, while for the variables bulb height and bulb diameter ranged from two to four plants between rows. For a semi-amplitude of the confidence interval (D%) equals to 20% of the mean, the sample size for the bulb mass variable ranged from three to five plants, and for the bulb height and bulb diameter variables from one to two plants between the rows (Table 2).

In 2017, for a semi-amplitude of the 95% confidence interval (D%) equals to 5% of the average sample size, the bulb mass variable ranged from five to eight plants between rows, while for variables bulb height and bulb diameter it varied from three to six plants between rows. For a semi-amplitude of the confidence interval (D%) equals to 20% of the mean, the sample size for the variable bulb mass varied from four to six plants, and for variables bulb height and bulb diameter from one to four plants between the rows (Table 2).

As a single sample size for the onion variables bulb mass, bulb height and bulb diameter, six plants are the recommended amount, which is the largest sample size considered in this study. With this recommendation, 75% of the plants in each row of cultivation will be sampled, resulting in reduction in evaluation time, labor, and costs. If the researcher chooses to evaluate only the variables bulb height and bulb diameter to identify the minimum differences between the means of the largest treatments, the number of plants necessary for sampling may be less (four plants per plot). For this reason, adequate sampling is extremely important as the researcher will be able to carry out a greater number of evaluations in the same period of time (Fernandes and Silva 1996). In studies with *Phaseolus vulgaris* (Haesbaert et al. 2011), *Solanum lycopersicum* (Lúcio et al. 2012) and *Solanum melongena* (Krysczun et al. 2018), similar sample sizes were recommended.

Table 2. Sample size (number of plants) and minimum differences between means (D = 5, 10, 15 and 20%) for each row in each bed, for the variables bulb mass (BM), bulb height (BH), and bulb diameter (BD) for the growing season in 2016 and 2017.

Bed	Row	2016												2017											
		BM				BH				BD				BM				BH				BD			
		5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
1	1	6	5	5	4	3	3	2	1	2	2	1	1	6	5	4	4	3	2	1	1	3	2	2	1
	2	5	4	4	3	2	2	1	1	3	2	2	1	6	5	5	4	3	2	1	1	3	2	2	1
	3	6	5	5	4	3	2	2	1	3	2	2	1	6	5	5	4	4	3	3	2	4	3	3	2
	4	5	4	4	3	2	2	1	1	2	1	1	1	5	5	4	4	3	2	1	1	3	2	2	1
	5	-	-	-	-	-	-	-	-	-	-	-	-	5	5	4	4	3	2	1	1	3	2	2	1
2	1	6	5	4	4	3	3	2	1	3	2	1	1	6	6	5	5	3	2	1	1	3	2	2	1
	2	6	5	4	4	3	3	2	2	3	2	1	1	6	6	5	5	3	3	2	1	4	3	3	2
	3	5	4	4	3	3	2	1	1	2	1	1	1	7	6	6	5	3	2	2	1	4	3	3	2
	4	6	5	5	4	3	3	2	1	3	2	2	1	7	6	6	5	4	4	3	3	4	4	3	3
	5	-	-	-	-	-	-	-	-	-	-	-	-	7	6	6	5	4	4	3	3	4	4	3	3
3	1	5	5	4	4	2	2	1	1	2	1	1	1	6	5	5	4	3	3	2	2	3	3	2	2
	2	6	5	5	4	3	2	2	1	3	2	2	1	7	6	6	5	4	4	3	3	4	4	3	3
	3	6	6	5	4	4	3	2	2	2	2	1	1	8	8	7	6	5	5	4	4	5	5	4	4
	4	5	4	4	3	3	3	2	1	2	2	1	1	8	7	7	6	6	5	5	4	6	5	5	4
	5	-	-	-	-	-	-	-	-	-	-	-	-	7	7	6	6	5	5	4	4	5	5	4	4
4	1	6	5	4	4	4	3	3	2	4	3	3	2	6	6	5	5	3	3	2	1	4	3	2	2
	2	5	5	4	4	3	3	2	1	3	2	1	1	7	6	6	5	3	3	2	1	4	3	3	2
	3	6	5	4	4	3	3	2	1	2	2	1	1	6	6	5	4	4	4	3	3	5	4	3	3
	4	5	5	4	4	3	2	2	1	3	2	1	1	7	6	6	5	5	4	3	3	5	4	4	3
	5	-	-	-	-	-	-	-	-	-	-	-	-	7	6	6	5	5	4	4	3	5	4	4	3
5	1	5	5	4	4	4	3	3	2	2	1	1	1	6	6	5	4	5	4	4	3	4	3	3	3
	2	6	6	5	4	4	3	2	2	2	1	1	1	6	6	5	4	3	3	2	2	4	3	2	2
	3	6	5	5	4	3	3	2	2	2	1	1	1	6	6	5	5	4	3	3	2	4	3	3	2
	4	6	5	5	4	3	3	2	2	2	1	1	1	6	6	5	4	3	2	2	1	3	3	2	1
	5	-	-	-	-	-	-	-	-	-	-	-	-	6	5	5	4	4	3	3	2	4	3	3	2
6	1	5	5	4	4	3	2	2	1	3	2	1	1	6	6	5	5	3	2	2	1	3	3	2	1
	2	5	4	4	3	3	2	2	1	2	2	1	1	6	6	5	5	4	3	3	2	4	3	3	2
	3	6	6	5	5	3	3	2	1	3	3	2	1	6	6	5	4	4	3	2	2	4	3	3	2
	4	6	5	4	4	3	3	2	2	3	2	1	1	6	6	5	4	4	3	2	2	4	3	3	2
	5	-	-	-	-	-	-	-	-	-	-	-	-	6	6	5	4	3	2	2	1	3	2	2	1

Using sampling induces a source of experimental variation through sampling error. The more variable the data are relative to the average, the larger the sample size must be (Silva et al. 2019). With an increase in the number of plants sampled, sampling error is reduced, and the reliability and precision of the results increase (Cargnelutti Filho et al. 2018).

Number of repetitions

In 2016 the number of repetitions ranged from two (three treatments with d = 50%) to 334 (two treatments with d = 5%), while in 2017 the number of repetitions ranged from three (three treatments with d = 50%) to 481 (two treatments with d = 5%), both scenarios formed by the combinations of i treatments (i = 2, 3, 4, ..., 20) and *d* minimum differences between treatment means (d = 5%, 10%, 15%, ..., 50%), to be detected as significant at 5% probability by the Tukey's test (Table 3).

Table 3. Number of repetitions for experiments in randomized block design, in scenarios formed by combinations of i treatments (i = 2, 3, 4, ..., 20), and minimum differences between the means of treatments to be detected as significant at 5% probability, by the Tukey's test, expressed as a percentage of the experiment mean (d = 5, 10, 15, ..., 50%), for the variable bulb mass (BM), bulb height (BH) and bulb diameter (BD), being the largest plot size (X_0 = 7, 4 and 4 plants) and coefficient of variation in plot size (CV_{Xo} = 15, 9 and 10%), respectively, for the number of repetitions in the year 2016. For the year 2017, the largest plot size for the variables BM, BH and BD was eight, six, and six plants, and the coefficient of variation calculated in the size of the plot was 18, 14 and 14%, respectively.

Year	i	BM										BH										BD									
		5	10	15	20	25	30	35	40	45	50	5	10	15	20	25	30	35	40	45	50	5	10	15	20	25	30	35	40	45	50
2016	2	334	83	37	21	13	9	7	5	4	3	120	30	13	8	5	3	2	2	1	1	148	37	16	9	6	4	3	2	2	1
	3	229	57	25	14	9	6	5	4	3	2	82	21	9	5	3	2	2	1	1	1	102	25	11	6	4	3	2	2	1	1
	4	216	54	24	14	9	6	4	3	3	2	78	19	9	5	3	2	2	1	1	1	96	24	11	6	4	3	2	2	1	1
	5	215	54	24	13	9	6	4	3	3	2	77	19	9	5	3	2	2	1	1	1	96	24	11	6	4	3	2	1	1	1
	6	217	54	24	14	9	6	4	3	3	2	78	20	9	5	3	2	2	1	1	1	96	24	11	6	4	3	2	2	1	1
	7	221	55	25	14	9	6	5	3	3	2	79	20	9	5	3	2	2	1	1	1	98	25	11	6	4	3	2	2	1	1
	8	224	56	25	14	9	6	5	4	3	2	81	20	9	5	3	2	2	1	1	1	100	25	11	6	4	3	2	2	1	1
	9	228	57	25	14	9	6	5	4	3	2	82	20	9	5	3	2	2	1	1	1	101	25	11	6	4	3	2	2	1	1
	10	231	58	26	14	9	6	5	4	3	2	83	21	9	5	3	2	2	1	1	1	103	26	11	6	4	3	2	2	1	1
	11	235	59	26	15	9	7	5	4	3	2	85	21	9	5	3	2	2	1	1	1	104	26	12	7	4	3	2	2	1	1
	12	243	61	27	15	10	7	5	4	3	2	88	22	10	5	4	2	2	1	1	1	108	27	12	7	4	3	2	2	1	1
	13	241	60	27	15	10	7	5	4	3	2	87	22	10	5	3	2	2	1	1	1	107	27	12	7	4	3	2	2	1	1
	14	248	62	28	16	10	7	5	4	3	2	89	22	10	6	4	2	2	1	1	1	110	28	12	7	4	3	2	2	1	1
	15	244	61	27	15	10	7	5	4	3	2	88	22	10	5	4	2	2	1	1	1	109	27	12	7	4	3	2	2	1	1
	16	250	62	28	16	10	7	5	4	3	2	90	22	10	6	4	2	2	1	1	1	111	28	12	7	4	3	2	2	1	1
	17	256	64	28	16	10	7	5	4	3	3	92	23	10	6	4	3	2	1	1	1	114	28	13	7	5	3	2	2	1	1
	18	260	65	29	16	10	7	5	4	3	3	94	23	10	6	4	3	2	1	1	1	116	29	13	7	5	3	2	2	1	1
	19	265	66	29	17	11	7	5	4	3	3	96	24	11	6	4	3	2	1	1	1	118	29	13	7	5	3	2	2	1	1
	20	270	68	30	17	11	8	6	4	3	3	97	24	11	6	4	3	2	2	1	1	120	30	13	8	5	3	2	2	1	1
2017	2	481	120	53	30	19	13	10	8	6	5	291	73	32	18	12	8	6	5	4	3	291	73	32	18	12	8	6	5	4	3
	3	329	82	37	21	13	9	7	5	4	3	199	50	22	12	8	6	4	3	2	2	199	50	22	12	8	6	4	3	2	2
	4	311	78	35	19	12	9	6	5	4	3	188	47	21	12	8	5	4	3	2	2	188	47	21	12	8	5	4	3	2	2
	5	310	77	34	19	12	9	6	5	4	3	187	47	21	12	7	5	4	3	2	2	187	47	21	12	7	5	4	3	2	2
	6	312	78	35	20	12	9	6	5	4	3	189	47	21	12	8	5	4	3	2	2	189	47	21	12	8	5	4	3	2	2
	7	318	79	35	20	13	9	6	5	4	3	192	48	21	12	8	5	4	3	2	2	192	48	21	12	8	5	4	3	2	2
	8	323	81	36	20	13	9	7	5	4	3	195	49	22	12	8	5	4	3	2	2	195	49	22	12	8	5	4	3	2	2
	9	328	82	36	20	13	9	7	5	4	3	198	50	22	12	8	6	4	3	2	2	198	50	22	12	8	6	4	3	2	2
	10	333	83	37	21	13	9	7	5	4	3	202	50	22	13	8	6	4	3	2	2	202	50	22	13	8	6	4	3	2	2
	11	338	85	38	21	14	9	7	5	4	3	205	51	23	13	8	6	4	3	3	2	205	51	23	13	8	6	4	3	3	2
	12	350	88	39	22	14	10	7	5	4	4	212	53	24	13	8	6	4	3	3	2	212	53	24	13	8	6	4	3	3	2
	13	348	87	39	22	14	10	7	5	4	3	210	53	23	13	8	6	4	3	3	2	210	53	23	13	8	6	4	3	3	2
	14	357	89	40	22	14	10	7	6	4	4	216	54	24	14	9	6	4	3	3	2	216	54	24	14	9	6	4	3	3	2
	15	352	88	39	22	14	10	7	5	4	4	213	53	24	13	9	6	4	3	3	2	213	53	24	13	9	6	4	3	3	2
	16	360	90	40	22	14	10	7	6	4	4	218	54	24	14	9	6	4	3	3	2	218	54	24	14	9	6	4	3	3	2
	17	368	92	41	23	15	10	8	6	5	4	223	56	25	14	9	6	5	3	3	2	223	56	25	14	9	6	5	3	3	2
	18	375	94	42	23	15	10	8	6	5	4	227	57	25	14	9	6	5	4	3	2	227	57	25	14	9	6	5	4	3	2
	19	382	96	42	24	15	11	8	6	5	4	231	58	26	14	9	6	5	4	3	2	231	58	26	14	9	6	5	4	3	2
	20	389	97	43	24	16	11	8	6	5	4	235	59	26	15	9	7	5	4	3	2	235	59	26	15	9	7	5	4	3	2

For the bulb height variable, the number of repetitions for the year 2016 ranged from one (two treatments with d = 50%) to 120 (two treatments with d = 5%), while in 2017 the number of repetitions varied from two (three treatments d = 50%) to 291 (two treatments with d = 5%) (Table 3).

For the measurement of the bulb diameter variable in 2016, the number of repetitions ranged between one (two treatments with d = 50%) and 148 (two treatments with d = 5%) (Table 3).

From the plot size (X_o), the researcher can establish the relationship between i (treatments), d (differences between treatment means) and number of repetitions. For example, considering plots of eight onion plants, for the bulb mass variable in an experiment with three treatments (i = 3), seven repetitions are necessary for the minimum difference of 35% to be considered significant by the Tukey's test. Onion culture experiments are recommended to use eight plants per cultivation row, with seven repetitions, to show a difference between treatments on the Tukey's test.

The smaller minimum significant differences intended to be shown between treatments, the larger number of repetitions will be required. For example, for the bulb mass variable, if the researcher intends to evaluate three treatments (i = 3) to find minimum significant differences between treatments of 15%, the experiment must include at least 37 repetitions (Table 3), with eight plants constituting each plot. These many repetitions may not be financially or practically feasible with respect to the size of the experimental area or the human resources required. Therefore, when planning the experiment, the researcher must take into account the minimum significant differences between treatments desired to be detected, the size of the experimental area, availability of labor, financial resources and the number of treatments to be evaluated (Krysczun et al. 2018).

For a minimum significant difference of the Tukey's test expressed as a percentage of the average of 35%, experiments with *A. cepa* L. are recommended to use plots of eight plants per row of cultivation with ten replicates for the bulb mass variable. While to evaluate bulb height and bulb diameter variables, six repetitions are necessary. These values are close to those ones suggested by (Boyhan et al. 2003), two to five repetitions, to evaluate production, stem and seed formation, number of pairs and disease incidence in onion culture. Studies with other vegetable crops have been conducted with similar results. For example, with *Capsicum annuum*, considering a minimum difference in percentage of the mean between treatments (d) of 20% and an estimated number of repetitions equals to eight (Lúcio et al. 2004). This illustrates the great challenge in identifying small differences between treatments with a low number of repetitions for these cultures (Catapatti et al. 2008).

When the objective of an experiment is to demonstrate statistical significance for small differences between treatments, using smaller plot sizes with a greater number of repetitions is recommended (Nagai et al. 1978; Igue et al. 1991; Henriques Neto et al. 2004; Sousa et al. 2016). Such experimental designs reduce experimental error, increasing the precision of the experiment and providing more reliable research results (Rossetti 2002).

While this research provides some recommendations, each individual researcher is best positioned to identify the plot size, sample and the number of repetitions that is optimal for their experiment, taking into account the variables to be analyzed, the availability of experimental area, the human and financial resources, as well as the number of treatments to be analyzed and the minimum differences between treatments that are of interest.

CONCLUSION

For onion culture, the optimal plot size to evaluate the bulb mass, bulb height and bulb diameter is eight, six and six plants, respectively.

The optimal sample size for evaluating the bulb mass of the onion crop is six plants, while for the height and diameter variables the optimal sample size is four plants in the direction of the row considering a semi-amplitude of the confidence interval (D%) equals to 20% of the average.

Bulb mass variables require 10 repetitions to assess up to 20 treatments in a randomized block design for the least significant difference of the Tukey's test, expressed as a percentage of the average of 35%. Bulb height and bulb diameter require just six repetitions for the same assessment.

AUTHORS' CONTRIBUTION

Conceptualization: Lambrecht, D. M. and Lúcio, A. D. C.; **Methodology:** Lambrecht, D. M., Krysczun, D. K. and Tischler, A. L.; **Investigation:** Lambrecht, D. M., Tartaglia, F. L. and Tischler A. L.; **Writing – Original Draft:** Lambrecht, D. M., Diel, M. I. and Tartaglia, F. L.; **Writing – Review and Editing:** Lambrecht, D. M., Diel, M. I. and Lúcio, A. D. C.; **Funding Acquisition:** Lúcio, A. D. C.; **Supervision:** Lúcio, A. D. C..

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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