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## Experimental planning for conducting experiments with cucumber

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### ABSTRACT

In order to be considered highly reliable (showing very accurate results), an experiment needs to be very well planned. Defining the experimental plot size and number of replicates is fundamental to control the experimental error at the beginning of the experiment. The aim of this study was to estimate the plot size and the number of replicates sufficient to perform experiments with *Cucumis sativus*. A uniformity trial was installed in the first week of January 2017. The spacing used was 0.3 m between plants and 1 m between rows, resulting in 12 plants in each of the 12 cultivation rows and each basic experimental unit was composed of one plant. The variables observed in 18 harvests were average fruit mass (MMF, in g), average fruit length (CMF, in cm) and average fruit diameter (DMF, in cm). The harvests were analyzed individually and grouped to reduce experimental variability. The number of replicates and the plot size were estimated using the method of maximum curvature of the coefficient of variation. The plot size and the number of replicates were influenced by the variability in the rows and between the harvests. We recommend plots consisting of four plants per cultivation row with six replicates for the minimum significant difference by Tukey's test, expressed in 25% the means percentage.

**Keywords:** *Cucumis sativus*, experimental accuracy, number of replicates, plot size, experimental variability.

### RESUMO

#### Planejamento experimental para condução de experimentos com pepino

Para que um experimento apresente alta confiabilidade e precisão em seus resultados há a necessidade de seu adequado planejamento. Nesta etapa, a definição do tamanho da parcela experimental e do número de repetições é fundamental para que o erro experimental já seja controlado no início do experimento. Desta forma o objetivo do trabalho foi estimar o tamanho de parcela e o número de repetições suficientes para realização de experimentos com *Cucumis sativus*. A implantação do ensaio de uniformidade ocorreu na primeira semana de janeiro de 2017. O espaçamento utilizado foi de 0,3 m entre plantas e 1 m entre fileiras, resultando em 12 plantas em cada uma das 12 fileiras de cultivo e cada unidade experimental básica foi composta de uma planta. As variáveis observadas nas 18 colheitas foram massa média de frutos (MMF, em g), comprimento médio de frutos (CMF, em cm) e diâmetro médio de frutos (DMF, em cm). As colheitas foram analisadas individualmente e agrupadas para a redução da variabilidade experimental. Foram estimados o número de repetições e o tamanho de parcela pelo método da curvatura máxima do coeficiente de variação. O tamanho de parcela e o número de repetições são influenciados pela variabilidade existente nas fileiras de cultivo e entre as colheitas. Para uma diferença mínima significativa do teste de Tukey expressa em percentagem da média de 25%, recomenda-se parcelas de quatro plantas por fileira de cultivo com seis repetições.

**Palavras-chave:** *Cucumis sativus*, precisão experimental, número de repetições, tamanho de parcela, variabilidade experimental.

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Cucumber (*Cucumis sativus*) is a vegetable of great social and economic importance in horticulture sector, since it shows high fruit productivity and economic profitability per hectare (Conab, 2019). Moreover, according to Carvalho *et al.* (2013), cucumber stands out due to its high nutritional value and its nutraceutical properties. Considering food demand

and that cucumbers have numerous health benefits, studies should be carried out in order to provide technical recommendations aiming to increase productivity and quality of cucumber fruits (Vieira Neto & Gonçalves, 2016).

In order to generate reliable technical recommendations for cucumber crop, the variability among plots should be a result of a true effect of treatments

(Lúcio & Sari, 2017). In order to obtain this result, the experimental error should be minimized. It is essential that researchers adopt appropriate and sufficient estimates of size and shape of the plot, as well as, the number of replicates to minimize the experimental error (Storck *et al.*, 2016). These estimates are directly influenced by variability related to the experiment

(Steel *et al.*, 1997; Storck *et al.*, 2016); this variability should be studied so that the experimental planning will be appropriate, generating unbiased estimation of treatment effect (Krysczun *et al.*, 2018).

One of the researcher's problems is the variability among vegetable cultivars, mainly those with multiple-harvest characteristic, such as the cucumber crop. Physiological characteristics and indeterminate growth habit (uneven growth and flowering) result in uneven fruit maturation (Carpes *et al.*, 2010). The consequence of this phenomenon is overdispersion in database due to excess zeros (absence of fruits suitable for harvesting) and heterogeneity among plants within the same crop (Sari *et al.*, 2018). Moreover, other factors which cause variability can be noticed: the heterogeneity of soil fertility, plant damage in the experiment due to intensive management, uneven irrigation, occurrence of pests, diseases and weeds (Lúcio *et al.*, 2010; Lúcio & Benz, 2017).

Thus, minimizing variability sources to obtain accurate responses in the experiments is essential. In this sense, increasing the number of replicates and/or reduction of plot size is one way to minimize the experimental error and, consequently, to increase the result accuracy (Lúcio & Sari, 2017).

Grouping harvests has been an efficient method to decrease experimental variability, since it decreases the presence of null values in database. As a consequence, the results obtained tend to be more similar when grouped, being possible for the researcher to use smaller plots and lower number of replicates in the experiments, without losing experimental accuracy. Some researchers, such as Carpes *et al.* (2010) and Lúcio *et al.* (2016), also reported similar results.

Several studies had already been done in order to estimate the plot size and number of replicates for several horticultural crops, such as, green pepper (Lorentz *et al.*, 2005), lettuce (Lúcio *et al.*, 2011) and eggplant (Krysczun *et al.*, 2018). However, few studies can be found for cucumber

crop. That's why researchers, who work with vegetable crops, use the plots of the most varied sizes and, due to lack of information, they decide to use empirical research methodologies. For instance: Macedo Junior *et al.* (2001) used 12 plants to build plots and Santi *et al.* (2013) used plots composed of four plants. Santana *et al.* (2010) used just one plant to build the plot. This situation must have affected directly the quality of the experiment since the plot size is not sufficient (small) or increasing the experimental variability due to the loss of the panelist's efficiency during the measurement of variables, when the plots were too big. Also, in the case of plots bigger than necessary, a higher demand of experimental area, labor and financial resources is noticed. Thus, further studies aiming to improve the quality of experiments with this crop are necessary.

Therefore, this study aims to estimate the plot size and number of replicates sufficient to carry out experiments with *Cucumis sativus*.

## MATERIAL AND METHODS

### Local description and experimental design

Uniformity trial with cucumber crop was carried out in a plastic greenhouse Pampean-arch type, oriented the north-south direction and covered with low density polyethylene (LDPE) film, 150 microns thick and anti-UV additive, located in coordinates 29°42'23''S, 53°43'15''W and 95 m altitude. The region climate is Cfa type, according to Köppen classification (Alvares *et al.*, 2013). The experimental soil is classified as arenic dystrophic Red Argisol (Streck *et al.*, 2008).

The trial was implemented in the first week of January, 2017. Spacing was 0.3 m between plants and 1 m between rows, consisting of 12 plants in each of the 12 cultivation rows. Each cucumber plant was conducted with one stem and it was considered a basic experimental unit (UEB), following the recommendation of Federer (1977) and Steel *et al.* (1997), totalizing 12 UEB in each cultivation row and uniformity trial

with 144 UEB. The authors used hybrid Primepack Plus®, canned / salad type. Harvests were performed twice a week, when the fruits were approximately 12 cm.

In each UEB of one plant and in each of 18 harvests, we evaluated the following variables: average fruit mass (MMF, in g), average fruit length (CMF, in cm) and average fruit diameter (DMF, in cm). Harvests (H) were analyzed individually (H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18) and grouped (H1+H2, H1+H2+H3, H1+...+H4, H1+...+H5, H1+...+H6, H1+...+H7, H1+...+H8, H1+...+H9, H1+...+H10, H1+...+H11, H1+...+H12, H1+...+H13, H1+...+H14, H1+...+H15, H1+...+H16, H1+...+H17, H1+...+H18). We analyzed harvests as shown above in order to characterize the variability in cucumber crop. The grouping of multiple successive harvests was also carried out to reduce variability between UEB, decreasing overdispersion and mitigating the negative effect of excess zeros in the database, according to Lúcio & Sari (2017).

### Statistical analyses

One descriptive analysis was done for each variable, in each row in individual and grouped harvests. The authors calculated means, variance, standard deviation, standard error and coefficient of variation (data non shown). We tested variance homogeneity between cultivation rows for each harvest (individual and grouped) and between harvests (individual and grouped) for each cultivation line for all the tested variables. For these analyses, Bartlett's test at 5% error probability was used (Steel *et al.*, 1997).

For each, individual and grouped harvests, and in each cultivation row, the authors estimated the plot sizes using the method of maximum curvature of the coefficient of variation, proposed by Parnaíba *et al.* (2009), by the expression

$$\hat{X}_0 = \frac{10^3 \sqrt{2(1-\hat{\rho}^2)} s^2 \bar{Y}}{\bar{Y}}$$

in which  $\hat{x}_0$  is the appropriate plot size,  $s^2$  is the variance in cultivation row,  $\bar{Y}$  is the UEB means in cultivation

row,  $\hat{\rho}$  is the first-order spatial autocorrelation, estimated by the expression:

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

where  $\hat{\varepsilon}$  is the experimental error associated with observation of each  $i$  UEB and  $\bar{\varepsilon}$  means of experimental errors.

In order to estimate the number of replicates, we used the minimum significant difference (d) by Tukey's test, expressed as percentage of the trial means:

$$d = \left( \frac{Q_{\alpha(i;GLE)} \sqrt{\frac{QME}{r}}}{\bar{Y}} \right) \times 100$$

in which  $Q_{\alpha(i;GLE)}$  is the critical value of Tukey's test at  $\alpha$  level of probability error ( $\alpha=0.05$  used in this study),  $i$  is the number of simulated treatments (2 to 20 treatments),  $GLE$  is the number of degrees of freedom error for randomized block design,  $(i-1)(r-1)$ , where  $r$  is the number of replicates defined in 12 where each cultivation row consisted of one block, since significant heterogeneity between cultivation rows was noticed;  $QME$  is the mean square error and  $\bar{Y}$  is the experiment means. Thus, replacing the expression of the experimental coefficient of variation in percentage, in expression to calculate d, and isolating r, we have

$$r = \left( \frac{Q_{\alpha(i;GLE)} CV}{d} \right)^2$$

In this study, CV was expressed in percentage, and corresponds to  $CV_{X_o}$ , since this is the CV expected for this experiment with the previously calculated plot size ( $X_o$ ). With the higher coefficient of variation for the plot size ( $CV_{X_o}$ ) of total grouping of harvests, we determined the number of replicates (r), using iterative process until convergence, for experiments in randomized block design, scenarios formed by combinations of  $i$  treatments ( $i= 2, 3, 4, \dots, 20$ ) and d ( $d= 5\%, 10\%, 15\%, \dots, 50\%$ ). In each harvest (individual and grouped), the highest estimate of plot size between cultivation rows was used. All analyses were performed with the aid of R software (R Development Core Team, 2019) and Office Excel® application.

## RESULTS AND DISCUSSION

Evaluating the variance heterogeneity among cultivation rows, in each individual and grouped harvest,

### Experimental variability

**Table 1.** *P-value* of Bartlett's test, plot size ( $X_o$ , in plants) and coefficient of variation in plot size in parentheses ( $CV_{X_o}$ , in %), between individual and grouped harvests, for average fruit mass (MMF, in grams), average fruit length (CMF, in cm) and average fruit diameter (DMF, in cm) of cucumber. Santa Maria, UFSM, 2018.

Harvests	<i>p-value</i> <sup>1</sup>			$X_o$		
	MMF	CMF	DMF	MMF	CMF	DMF
H1	0.000	0.000	0.000	29 (64)	29 (64)	29 (64)
H2	0.000	0.000	0.000	29 (64)	29 (64)	29 (64)
H3	0.001	0.001	0.080	28 (64)	28 (63)	28 (64)
H4	0.000	0.000	0.000	28 (64)	28 (63)	28 (64)
H5	1.319×10 <sup>-13</sup>	0.145	0.032	29 (64)	29 (64)	29 (64)
H6	0.003	0.983	0.198	11 (26)	11 (25)	12 (28)
H7	0.000	0.000	0.000	29 (64)	29 (64)	29 (64)
H8	0.003	0.971	0.903	22 (49)	22 (49)	22 (49)
H9	0.003	1.860×10 <sup>-9</sup>	1.660×10 <sup>-5</sup>	6 (14)	6 (13)	6 (13)
H10	0.003	0.999	0.919	17 (37)	19 (43)	19 (43)
H11	0.968	6.860×10 <sup>-9</sup>	0.033	8 (18)	6 (13)	8 (19)
H12	6.892×10 <sup>-6</sup>	0.9063	0.697	15 (33)	11 (25)	11 (25)
H13	0.004	9.640×10 <sup>-10</sup>	6.540×10 <sup>-6</sup>	10 (23)	5 (10)	7 (14)
H14	0.060	0.239	0.325	11 (24)	10 (22)	10 (22)
H15	3.133×10 <sup>-5</sup>	0.006	0.009	19 (43)	16 (35)	16 (36)
H16	0.920	0.996	0.996	12 (27)	10 (27)	10 (22)
H17	0.014	0.570	0.716	29 (64)	29 (64)	29 (64)
H18	0.928	0.999	0.999	19 (42)	18 (41)	18 (41)
H1+H2	1.517×10 <sup>-7</sup>	0.055	0.179	22 (50)	29 (64)	29 (64)
H1+...+H3	2.554×10 <sup>-9</sup>	0.000	1.920 10 <sup>-6</sup>	24 (54)	19 (42)	17 (37)
H1+...+H4	8.527×10 <sup>-14</sup>	1.580×10 <sup>-5</sup>	0.001	25 (55)	16 (36)	15 (33)
H1+...+H5	2.875×10 <sup>-9</sup>	0.055	9.760×10 <sup>-12</sup>	15 (33)	14 (31)	13 (28)
H1+...+H6	4.289×10 <sup>-9</sup>	1.140×10 <sup>-14</sup>	2.560×10 <sup>-7</sup>	12 (26)	10 (22)	7 (16)
H1+...+H7	1.322×10 <sup>-8</sup>	3.160×10 <sup>-14</sup>	2.50×10 <sup>-7</sup>	12 (27)	10 (22)	7 (17)
H1+...+H8	6.448×10 <sup>-9</sup>	2.330×10 <sup>-17</sup>	1.44×10 <sup>-9</sup>	11 (24)	9 (19)	7 (17)
H1+...+H9	1.136×10 <sup>-7</sup>	1.080×10 <sup>-22</sup>	1.060×10 <sup>-13</sup>	8 (19)	7 (15)	6 (14)
H1+...+H10	0.000	6.790×10 <sup>-20</sup>	1.280×10 <sup>-12</sup>	8 (18)	6 (14)	7 (15)
H1+...+H11	0.016	0.059	0.688	8 (17)	5 (12)	5 (11)
H1+...+H12	0.476	0.093	0.688	6 (14)	5 (11)	4 (10)
H1+...+H13	0.144	0.295	0.189	5 (12)	4 (10)	4 (8)
H1+...+H14	0.510	0.301	0.214	4 (10)	4 (9)	3 (8)
H1+...+H15	0.732	0.345	0.664	5 (10)	4 (9)	4 (9)
H1+...+H16	0.567	0.538	0.630	4 (10)	4 (9)	4 (8)
H1+...+H17	0.545	0.576	0.484	4 (10)	4 (9)	4 (8)
H1+...+H18	0.258	0.380	0.049	4 (10)	4 (9)	4 (9)

<sup>1</sup>*p-values* lower than 0.05 show heterogeneous variances between rows of cultivation within each individual or grouped harvest.

the authors identified 78% heterogeneity in individual harvests and 59% in grouped harvest for average fruit mass (MMF); 50% heterogeneity in individual harvests and 41% in grouped harvest for average fruit length (CMF), and 45% heterogeneity in individual harvests and 53% in grouped harvest for average fruit diameter (DMF) (Table 1). This fact shows that the randomized blocks should be the experimental design adopted, since the use of a completely randomized design demands total homogeneity among experimental plots (Steel *et al.*, 1997), and this was not observed when using the Barlett's test (Table 1). Thus, according to Lúcio & Sari (2017), each block/replicate should be composed of one cultivation row.

CV% ranged from zero (when no plants in the row produced fruits) to 331 when plants in rows showed very wide range in production values when estimated in individual harvests. As the

harvests are grouped, the coefficient of variation between the plants in the rows decreases, as well as the variability between the cultivation rows. These results were already expected and they are consequence of a reduction of null values in databank (plants which did not show fruits to be harvested).

Due to management intensity of vegetable crops, since the cultivations are carried out in rows, and also with the results obtained after using Bartlett's test (Table 1), the authors recommend the randomized block designs in order to control the experimental area variability properly, regardless of how individual or grouped harvests are assessed. Following this procedure, we aim to avoid increasing the estimate of the experimental error and, consequently, the experimental precision is increased. This recommendation was carried out, for instance, by Carpes *et al.* (2010), Lúcio *et al.* (2016, 2017) and Krysczun

*et al.* (2018) for other vegetable crops.

**Plot size**

Regardless of the evaluated variable, the plot sizes shown are larger in individual harvests than in grouped ones (Table 1). This result shows, again, that the greatest variability observed in the harvests analyzed individually (see CV% in Table 1) directly interferes with plot size estimates.

As harvests are grouped, the estimates of plot size and CVs decrease (Table 1). This reduction is due to a decrease of null values in dataset (Lúcio *et al.*, 2010; Krysczun *et al.*, 2018). Plot size estimates ( $X_0$ ) ranged from 29 to four plants, in cultivation row regardless the evaluated variable, whereas CV% ranged from 64 to 10%, 64 to 9% and 64 to 8% respectively for average fruit mass, average fruit length and average fruit diameter (Table 1). The similar amplitude of CV% is due to the standardization performed at the time of fruit harvest favoring similar responses observed in three variables, in relation to the experimental variability observed in this study.

Grouping the first 15 harvests made plot size stabilize in four UEB/plants and  $CV\% \leq 10$ , showing good experimental accuracy. As the uniformity test was performed with 12 UEB in each cultivation row, using a plot size composed of four plants, the researcher can test a maximum of  $i=$  three treatments. If the researcher chooses to carry out an experiment with a larger number of treatments and does not have an experimental area, labor and financial resources available, he/she will have to use smaller plot sizes.

**Number of replicates**

Number of replicates was determined using the variation coefficient ( $CV=10\%$ ), of grouped harvests in three evaluated variables, using a four-plant plot size.

In order to evaluate MMF, CMF and DMF the number of replicates ranged from one (two treatments with  $d=50\%$ ) to 148 (2 treatments with  $d=5\%$ ) (Table 2), in scenarios formed by combinations of  $i$  treatments ( $i=2, 3, 4, \dots, 20$ ) and  $d$

**Table 2.** Number of replicates for experiments using randomized block design, in scenarios formed by combinations of  $i$  treatments ( $i=2, 3, 4, \dots, 20$ ) and  $d$  minimum differences between treatment means to be identified as significant at 5% probability, by Tukey's test, expressed in percentage of the experiment means ( $d=5, 10, 15, \dots, 50\%$ ), for average mass, length and diameter average of cucumber fruits, using the plot size ( $X_0=4$  plants) and coefficient of variation in plot size ( $CV_{X_0}=10\%$ ). Santa Maria, UFSM, 2018.

<b>i</b>	<b>5%</b>	<b>10%</b>	<b>15%</b>	<b>20%</b>	<b>25%</b>	<b>30%</b>	<b>35%</b>	<b>40%</b>	<b>45%</b>	<b>50%</b>
2	148	37	16	9	6	4	3	2	2	1
3	102	25	11	6	4	3	2	2	1	1
4	96	24	11	6	4	3	2	2	1	1
5	96	24	11	6	4	3	2	1	1	1
6	96	24	11	6	4	3	2	2	1	1
7	98	25	11	6	4	3	2	2	1	1
8	100	25	11	6	4	3	2	2	1	1
9	101	25	11	6	4	3	2	2	1	1
10	103	26	11	6	4	3	2	2	1	1
11	104	26	12	7	4	3	2	2	1	1
12	108	27	12	7	4	3	2	2	1	1
13	107	27	12	7	4	3	2	2	1	1
14	110	28	12	7	4	3	2	2	1	1
15	109	27	12	7	4	3	2	2	1	1
16	111	28	12	7	4	3	2	2	1	1
17	114	28	13	7	5	3	2	2	1	1
18	116	29	13	7	5	3	2	2	1	1
19	118	29	13	7	5	3	2	2	1	1
20	120	30	13	8	5	3	2	2	1	1

minimum differences among treatment means ( $d= 5\%$ ,  $10\%$ ,  $15\%$ , ...,  $50\%$ ), being verified as significant at  $5\%$  probability, using Tukey's test.

Considering four-plant plots in an experiment with two treatments ( $i= 2$ ), six replicates are necessary in order to consider  $25\%$  the minimum significant difference by Tukey's test (Table 2). Thus, the authors recommend that for experiments with cucumber cultivation in a protected environment, and with a minimum significant difference using Tukey's test ( $25\%$  average), the researcher should adopt plots with four plants per row of cultivation, with six replicates/blocks.

The authors highlight that the smaller the minimum significant differences that the researcher intends to obtain between treatments, the greater the number of replicates needed and the greater the need for an experimental area, even keeping the four-plant plot size in the cultivation row.

When planning the experiment, the researcher must take into consideration the minimum significant differences between the treatments to be verified, the size of the experimental area, availability of labor, financial resources and the number of treatments to be evaluated.

Considering experiments with cucumber crop, plot size and number of replicates are influenced by the variability in cultivation rows and harvests.

For the minimum significant difference by Tukey's test, expressed in means percentage the  $25\%$ , we recommend four plant plots per cultivation row with six replicates, for

experiments with *Cucumis sativus*.

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