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Sample size, plot size and number of replications for trials with *Solanum melongena* L.

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ABSTRACT

Due to the increasing consumption of eggplant (*Solanum melongena* L.), research should be carried out with this horticultural species aiming at providing technical recommendations for increasing production and quality of fruits. The aim of this study was to estimate the plot and sample size, as well the number of replications suitable for eggplant trials. Two uniformity trials were performed with eggplant in a plastic tunnel. The fresh fruit weight and the total number of fruits were assessed in each basic unit and in each harvest. The harvests were analyzed individually and grouped. Heterogeneity was found among crop rows and individual harvests within the same row. However, when the harvests were grouped, this fact was not observed. When the harvests are grouped, there was a reduction in plot size and sample needed the grouping of six harvests presented the smallest sample size and plot size. The plot size is five plants, the sample is nine plants in the direction of the crop row with a half-range of the 20% confidence interval. Aiming at achieving a minimum-significant difference in the Tukey's test of 30% of treatment mean, three replications with five plants each plot is needed.

1. Introduction

The eggplant (*Solanum melongena* L.) is an annual vegetable widely cultivated in tropical and subtropical climate areas. The world consumption of eggplant has shown an increased in the last years, a fact promoted by the innumerable benefits of this crop, in evidence, the presence of metabolites that significantly contribute to a healthy eating (Sun et al., 2015). Thus, research with this vegetable crop has been conducted to provide technical recommendations, focusing on increasing production and fruit quality (Çolak et al., 2017; Douds et al., 2017).

To ensure a high experimental precision and credibility in research, it is essential that experimental planning, conducting research, data analysis and interpretation of results be performed in a careful and appropriate manner. In the planning phase, it is essential to choose the plot size, sample size and number of replications that increase the experimental precision and reliability of the results generated in the experiments. The plot size, sample size and number of replications are directly influenced by the variability in the experiment (Lúcio et al., 2017). This variability inflates the estimate of experimental error, interfering on statistics of hypothesis testing and on comparison of treatments, leading the researcher to wrong interpretations and conclusions.

The variability of the data in multiple-harvest vegetable crop trials (such as eggplant) may be related to several factors such as: (i) the heterogeneity of soil fertility; (ii) uneven irrigation; (iii) occurrence of pests, diseases and weeds; (iv) uneven maturation of fruits; (v) presence or absence of suitable fruits to be harvested in a given crop; and (vi) variability between the cropping rows (Lorentz and Lúcio, 2009). Thus, there is a need to minimize the sources of variability in these experiments. In this regard, grouping the results of several harvests can reduce variation caused by timing and maturity, and choosing appropriate plot size, sample size and number of replications can reduce spatial variations due to soil, disease and irrigation.

Recommendations for sample size, plot size and number of replications have been obtained for several vegetable crops, zucchini (Lúcio et al., 2008), tomato (Lúcio et al., 2010) and green beans (Santos et al., 2012). However, for the eggplant crop, this information has not been obtained. Thus, the aim of this study was to estimate the plot size, sample size and number of replications sufficient for trials with eggplant crop.

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2. Material and methods

2.1. Site description and experimental design

Two uniformity tests with eggplant were conducted in the experimental area of the Crop Science Department of the Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil (S: 29° 42'23 "W: 53° 43 ' '15' 'and 95 m above sea level). The climate of the region is Cfa, according to the Koppen's classification (Alvares et al., 2013). The soil of the experimental area is classified as Alfisols (Soil Survey Staff, 1999).

A chemical fertilization was carried out according to the soil analysis and recommendation of fertilization for eggplant crop. The trials were performed in plastic tunnels (protected environment) covered with a 150 µm thickness low-density polyethylene film with anti-UV protection.

The cultivar employed was the 'Longe Purple' and the implementation of the trials occurred in the third week of October, with seedlings acquired in local commerce. The spacing used was 0.7 m between plants and 1 m between rows, resulting in 28 plants in each of the three rows of cultivation. Considering each plant as a basic experimental unit (BU), each row presented 28 BUs.

A dripper irrigation system with one drip tubing each crop row and emitters spaced at 0.3 m was used. The hydraulic nominal pressure of the system was 8 m water column providing a water flow of 3.4 l h^{-1} emmitter^{-1}. Thus, it was possible to obtain a continuous wetting width. Both irrigation interval and amount of water to be applied was estimated according to Supplemental Tables 1 and 2, respectively, considering the technical information of the irrigation system. All other cultural management were carried out according to the recommendation for the crop.

The fruits were harvested in both tunnels when they had 18 cm length, with a bright color and soft pulp. The fruits harvested in each BU were stored in identified plastic bags and sent to the laboratory for counting and weighing in an analytical balance with of accuracy 0.01 g.

The variables evaluated in each BU and in the six harvests were: fresh fruit weight (FFW, in g) and total number of fruits (TNF). The harvests (H) were analyzed individually (H1, H2, H2, H3, H4, H5, and H6) and grouped (H1 + H2, H1 + H2 + H3, H1 + H2 + H3 + H4, H1 + H2 + H3 + H4 + H5 and H1 + H2 + H3 + H4 + H5 + H6).

2.2. Statistical analysis

For both variables, the homogeneity of the variances among the crop rows and within each row was evaluated, considering both individual and grouped harvest. For these analyzes the Bartlett test (Steel et al., 1997) was used, since the data adhered to the normal distribution according to the previous Shapiro-Wilk test.

For each of the individual and grouped harvests and in each crop row, plot sizes were estimated by the maximum curvature method of the coefficient of variation proposed by Paranaíba et al. (2009): $\hat{X}_0 = \frac{10\sqrt[3]{2(1-\hat{\rho}^2)s^2\overline{Y}}}{\overline{Y}}$, where $\hat{X}_0$: is the appropriate plot size, s^2: is the variance in the crop row, $\overline{Y}$: is the mean of the BUs in the crop row, $\hat{\rho}$: is the first-order spatial autocorrelation, estimated by the equation: $\hat{\rho} = \frac{\sum_{i=2}^{n}(R_i)(R_{i-1})}{\sum_{i=1}^{n}(R_i)^2}$.

The estimation of the sample size to achieve a specific confidence interval for the mean for each of the individual and grouped harvest and in each crop row was performed by the expression: $n = \frac{t^2_{\alpha/2}(CV\%)^2}{(D\%)^2}$ (Cochran, 1977), where "n" is the sample size; $t^2_{\alpha/2}$ is the value of the Student's-t table with n-1° d.f. at 5% probability error; CV% is the coefficient of variation of the considered variable ($CV\% = \frac{100\times\sqrt{s^2}}{\overline{Y}}$, on that s^2 is the sample variance, $\overline{Y}$ is the mean of each variable); and D% is the half-width of the mean's confidence interval (D% = 5, 10, 15, and 20). The correction for finite population was performed by $nc = \frac{n}{1+\frac{n}{N}}$ (Cochran, 1977), "nc" is the corrected sample size, "N" is the population size for each crop row (28 plants), and "n" is the sample size for infinite population (estimated sample size).

For the estimation of the number of replications, it was considered the minimum significant difference (d) of the Tukey's test, expressed as a percentage of the mean of the trails: $d = (q\alpha(i; DF)\sqrt{MSE/r})/\overline{Y} \times 100$, whereq $\alpha(i;GLE)$ is the critical value of the Tukey's test at the α probability of error (α = 0,05), i is the number of treatments, DF is the number of degrees of freedom of error, that is, (i-1)(r-1) for the randomized block design, MSE is the mean square error, r is the number of replications and m is the mean of the experiment. Thus, replacing the expression of the experimental coefficient of variation ($CV = \sqrt{QME}/\overline{Y} \times 100$) in the expression for the calculation of d, and isolating r, have $r = (q\alpha(i;GLE)CV/d)^2$.

In the work, the CV was expressed as a percentage and corresponds to the CV_{Xo}, as this is the expected CV for the experiment with the plot size (X_o) previously calculated. With the highest coefficient of variation of plot size (CV_{Xo}) of the grouping (H1 + H2 + H4 + H5 + H6), the number of replications (r) was determined, by iterative process up to convergence, for experiments in the randomized block design, in scenarios formed by the combinations of "i" (i = 2, 3, 4, …, 15) and "d" (d = 5%, 10%, 15%, …, 50%). All analyzes were performed with software R version 2.15.1 (R Development Core Team, 2014) and Office Excel® application.

3. Results

3.1. Experimental variability

In both tunnels, there was heteroscedasticity among the crop rows and among individual harvests inside the same row. However, when the harvests were grouped heteroscedasticity was not observed.

3.2. Plot size

The plot size was smaller when the harvests were grouped, independently of tunnels or variables (Table 1). Thus, it is indicated to measure the variables FFM and TNF of eggplant that the harvests are grouped and that the plot be composed of at least five plants in the direction of the crop row. However, if the researcher chooses using plot size for individual and/or grouped harvests, it will be necessary to use a plot size higher than that indicated in this study.

3.3. Sample size

The grouped harvests showed smaller sample sizes than individual harvests (Tables 2 and 3). For the FFM, regardless of the tunnel and crop row for a D = 5% of the mean, the sample size ranged from 25 to 28 plants among individual harvests and 22–26 for grouped harvested (Table 2). For a D = 20% of the mean, the sample size ranged from 5 to 13 plants between the individual harvests and 9–23 for grouped harvests.

For the TNF (Table 3), regardless of the tunnel and crop row, for a D = 5% of the mean, with a confidence level of 95%, the sample size ranged from 24 to 28 plants among individual harvests and 21–26 for grouped harvests. For a D = 20% of the mean, the sample size ranged from 8 to 24 plants between individual harvests and 4–26 for grouped harvests (Table 4).

3.4. Number of replications

For the evaluation of FFM and TNF, the number of replications