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PLANEJAMENTO EXPERIMENTAL NA CULTURA DA BERINJELA

Santa Maria, RS
2018

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Dissertação apresentada ao Curso de Pós-Graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Agronomia.**

Orientador: Prof. Dr. Alessandro Dal'Col Lúcio

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Aprovado em 28 de fevereiro de 2018:

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Santa Maria, RS
2018

DEDICATÓRIA

Aos meus pais Nelvir Luís Krysczun e Eunice Ireis Ketzer Krysczun, minha irmã Diovana Ketzer Krysczun e meu cunhado Gabriel Ferrando dos Santos, pelos ensinamentos de vida, exemplo de caráter e amor incondicional, dedico-lhes este trabalho.

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Treine enquanto eles dormem, estude enquanto eles se divertem, persista enquanto eles descansam, e então, viva o que eles sonham.

(Provérbio japonês)

RESUMO

PLANEJAMENTO EXPERIMENTAL NA CULTURA DA BERINJELA

AUTOR: Dionatan Ketzer Krysczun
ORIENTADOR: Alessandro Dal'Col Lúcio

O consumo de berinjela aumentou nos últimos anos, estimulado pelos seus múltiplos benefícios a saúde humana. Pesquisas devem ser realizadas com esta espécie hortícola com objetivo de fornecer recomendações técnicas para o aumento da produção e qualidade de frutas. Nesse sentido, os objetivos desse trabalho foram determinar a influência do tamanho do ensaio de uniformidade sobre a estimativa do tamanho de parcela e estimar o tamanho de amostra, de parcela e o número de repetições suficientes para ensaios com a cultura da berinjela. Dois ensaios de uniformidade foram realizados com berinjela em túnel plástico. Em cada unidade experimental básica e em cada colheita foram avaliadas a massa fresca de frutos e o número de frutos. As colheitas foram analisadas individualmente e agrupadas. Em cada túnel e variável foram planejados 25 ensaios de uniformidade de diferentes tamanhos por fileira de cultivo e colheita (individual e agrupada). Para cada ensaio de uniformidade planejado foram realizadas 3.000 reamostragens bootstrap com reposição. Em cada ensaio de uniformidade planejado foram estimados 3.000 tamanhos de parcela pelo método da curvatura máxima do coeficiente de variação e calculada a média e a amplitude do intervalo de confiança de 95%, pela diferença entre os percentis 97,5% e 2,5%. A heterogeneidade foi encontrada entre as linhas de colheita e as colheitas individuais na mesma fila. No entanto, quando as colheitas foram agrupadas, esse fato não foi observado. O tamanho do ensaio de uniformidade influencia a estimativa do tamanho de parcela, pois ensaios com baixo número de unidades experimentais básicas apresentam alta variabilidade experimental e estimativas pouco precisa. Quando as colheitas são agrupadas, houve uma redução no tamanho de parcela e a amostra necessária, o agrupamento de seis colheitas apresentou o menor tamanho de amostra e tamanho de parcela. Para estimativa do tamanho de parcela em experimentos com berinjela são necessários ensaios de uniformidade compostos por 16 unidades experimentais básicas para avaliar a variável massa fresca de frutos e, 14 unidades experimentais básicas, para a variável número de frutos. O tamanho de parcela é de cinco plantas, a amostra é de nove plantas na direção da linha de colheita com intervalo de confiança de 20%. Com o objetivo de alcançar uma diferença mínima significativa no teste de Tukey de 30% do tratamento, são necessárias três repetições com cinco plantas cada parcela.

Palavras-chave: *Solanum melongena* L. Ambiente protegido. Precisão experimental. Controle experimental. Bootstrap.

ABSTRACT

EXPERIMENTAL PLANNING IN BERINJELA CULTURE

AUTHOR: Dionatan Ketzer Krysczun
ADVISOR: Alessandro Dal'Col Lúcio

Eggplant consumption has increased in recent years, boosted by its multiple benefits to human health. Research should be carried out with this horticultural species in order to provide technical recommendations for increasing fruit production and quality. In this sense, the objectives of this work were to determine the influence of the size of the uniformity trial on the plot size estimation and to estimate the sample size, plot size and number of replications sufficient for trials with eggplant culture. Two uniformity trials were performed with eggplant in plastic tunnel. The fresh fruit mass and the number of fruits were evaluated in each basic experimental unit and in each harvest. Crops were analyzed individually and pooled. In each tunnel and variable, 25 trials of uniformity of different sizes were planned per crop and crop row (individual and grouped). For each planned uniformity trial, 3,000 bootstrap resampling was performed with replacement. In each planned uniformity trial, 3,000 plot sizes were estimated by the maximum curvature of the coefficient of variation method and the mean and 95% confidence interval were calculated by the difference between the 97.5% and 2.5%. The heterogeneity was found between the harvest rows and the individual crops in the same row. However, when harvests were pooled, this fact was not observed. The size of the uniformity trial influences the plot size estimation because trials with low number of basic experimental unit's present high experimental variability and inaccurate estimates. When the harvests are grouped, there was a reduction in plot size and the required sample, the clustering of six harvests presented the smallest sample size and plot size. In order to estimate the plot size in experiments with eggplant, uniformity trials are required composed of 16 basic experimental units to evaluate the fresh fruit mass variable and 14 basic experimental units for the variable number of fruits. The plot size is five plants, the sample is nine plants in the direction of the harvest row with a confidence interval of 20%. In order to reach a significant minimum difference in the Tukey test of 30% of the treatment, three replications with five plants per plot are required.

Key words: *Solanum melongena* L. Protected environment. Experimental precision. Quality control. Bootstrap

SUMÁRIO

1 INTRODUÇÃO

A berinjela (*Solanum melongena* L.) é uma hortaliça anual, amplamente cultivada nas áreas de clima tropical e subtropical. Necessita de temperaturas elevadas ao longo do seu desenvolvimento vegetativo e reprodutivo para desenvolver-se adequadamente. As plantas apresentam porte arbustivo, caule semi-lenhoso e ereto, altura entre 50 cm a 180 cm e sistema radicular bem desenvolvido, podendo alcançar profundidades acima de 100 cm. É classificada como uma olerícola de múltiplas colheitas, devido ao seu hábito de crescimento indeterminado.

A cultura da berinjela está entre as principais espécies olerícolas (*Solanum tuberosum, Solanum lycopersicum* e *Capsicum ssp.*) economicamente importante da família da *Solanaceae* (Morris e Taylor, 2017). Nos últimos, anos a berinjela vem sendo utilizada como alimento funcional, devido a presença de metabólitos secundários (polifenóis), que contribuem na prevenção de doenças degenerativas, como câncer e doenças cardiovasculares (Cao et al., 1996; Wang et al., 1996; Manach et al., 2004; Niño-Medina et al., 2017).

Desta forma, pesquisas com esta espécie olerícola vem sendo realizadas visando proporcionar recomendações técnicas, com foco no aumento da produção e qualidade de frutos (Moncada et al., 2013; Douds et al., 2017). Na condução de experimentos, os pesquisadores devem minimizar a variação residual (erro experimental) para que os resultados gerados sejam minimamente confiáveis. Para conseguir isso, os experimentos devem ser bem planejados e implementados (Lúcio et al., 2017). Na fase de planejamento, é essencial a escolha do tamanho de parcela, amostra e número de repetição, que visam assegurar a precisão experimental, confiabilidade dos resultados gerados nos experimentos e redução dos recursos materiais, financeiros e humanos que são geralmente restritos.

A estimativa do tamanho de parcela, amostra e número de repetições é realizada a partir de ensaios de uniformidade, sem a aplicação de tratamentos, e depende diretamente da magnitude da variabilidade experimental (Schwertner et al., 2015a; Schwertner et al., 2015b; Lúcio e Benz, 2017). Lorentz e Lúcio (2009) observaram que, a variabilidade dos dados em culturas olerícolas de múltiplas colheitas, como a berinjela, está relacionada a diversos fatores tais como: (i) sombreamento na área experimental devido ao posicionamento incorreto das estruturas; (ii) germinação e mudas desuniformes; (iii) lesões nas plantas dura’nte a implementação, condução e colheita do experimento; (iv) insetos, patógenos e ervas daninhas; (v) irrigação e adubação desuniformes; (vi) maturação desuniforme e ponto de colheita subjetivo, o que leva à variabilidade entre plantas e colheitas e a um excesso de zeros no banco de dados.

Há necessidade de se minimizar as fontes de variabilidade existentes nestes experimentos. Nesse sentido, a determinação de um tamanho de parcela, de amostra e de um número de repetições, que levam a uma precisão experimental, é essencial para que as conclusões obtidas nestes experimentos sejam precisas e confiáveis. O tamanho de parcela, de amostra e do número de repetições já foi definido para diversas culturas olerícolas de múltiplas colheitas, tais como: o pimentão (Lúcio et al., 2003; Lorentz and Lúcio, 2009), abobrinha italiana (Carpes et al., 2010), tomate salada (Lúcio et al., 2012), tomate cereja (Lúcio et al., 2016), feijão-de-vagem (Santos et al., 2012) e alface (Lúcio et al., 2011; Santos et al., 2012a).

Nos trabalhos que estimaram o tamanho de parcela em culturas olerícolas, como o pimentão (Lorentz et al., 2005; Lorentz e Lucio, 2009), abobrinha italiana (Carpes et al., 2010), tomate (Lúcio et al. ., 2011), feijão (Santos et al., 2012), rabanete (Silva et al., 2002), tomate cereja (Lúcio et al., 2016) e brócolis (Brum et al., 2016), os autores não investigaram a influência do tamanho do ensaio de uniformidade sobre a estimativa do tamanho de parcela. Storck et al. (2006) investigando na cultura da batata a influência do tamanho do ensaio de uniformidade sobre a estimativa do tamanho de parcela concluiu que o tamanho do ensaio de uniformidade não influencia na estimativa do tamanho de parcela. Entretanto, Cargnelutti Filho et al. (2011) trabalhando com nabo forrageiro, Schwertner et al. (2015a) com tomate salada, feijão-de-vagem e abobrinha italiana e Schwertner et al. (2015b) com alface e pimentão, afirmaram que ensaios de uniformidade com baixo número de unidades experimentais básicas levam a estimativas pouco precisas do tamanho de parcela, sendo importante a elucidação da influência do tamanho do ensaio de uniformidade sobre a estimativa do tamanho de parcela.

Para a cultura da berinjela, os trabalhos sobre o planejamento experimental são limitados, de difícil acesso, específicos e desatualizados. Hell et al. (2017), é um exemplo de trabalho especifico, pois seu objetivo é estimar o tamanho amostra para avaliar características de qualidade de plântulas de berinjela. Deste modo, é importante que seja realizado estudos na área do planejamento experimental na cultura da berinjela, para que os pesquisadores tenham informações mínimas que possibilitam um planejamento adequado. Sendo assim, a dissertação está organizada em dois artigos. O primeiro teve objetivo de estimar o tamanho do ensaio de uniformidade e sua relação com a estimativa do tamanho de parcela na cultura de berinjela. O segundo teve por objetivo estimar o tamanho de parcela, de amostra e o número de repetições suficientes para ensaios com a cultura da berinjela.

2 ARTIGO I - THE SIZE OF THE UNIFORMITY TRIAL AFFECTS THE ACCURACY OF PLOT SIZE ESTIMATION IN EGGPLANT

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The size of the uniformity trial affects the accuracy of plot size estimation in eggplant

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Abstract: The plot size estimation is based on uniformity trials, however little is known about and how the size of uniformity trial affect the estimate of the plot size. To test this hypothesis, two uniformity trials were performed with the eggplant culture in a plastic tunnel. The fresh mass of fruit and number of fruits were assessed in six harvests. For each trial, 25 uniformity trials of different sizes were planned per harvest and harvest row (individual and grouped) since they presented heteroscedasticity. For each planned uniformity trial, bootstrap procedure was used to estimate 3,000 plot sizes by the maximum coefficient of variation curvature method. The mean and 95% confidence interval width was calculated by the difference between the 97.5th and 2.5th percentiles. The size of the uniformity trial affects accuracy the plot size estimation because trials with few number of basic experimental units present high experimental variability and inaccurate estimates. For plot size estimation in eggplant experiments, it is suggested that the uniformity trials be composed of at least 16 basic

experimental units for the fresh mass of fruits and 14 basic experimental units the variable number of fruits.

Key words: *Solanum melongena* L., bootstrap, experimental planning, reduction of experimental variability

1. Introduction

The experimental design aims to ensure the experimental accuracy and credibility of the results generated by the research (Lúcio and Benz, 2017). An important step of experimental planning is the correct choice of plot size that aiming at increasing the experimental accuracy and reliability of the results generated in the experiments and, at the same time, reducing the use of material, financial and human resources that are generally restricted (Lúcio et al., 2016).

The estimation of plot size is based on uniformity trials without treatments (Schwertner et al., 2015a, 2015b) and depends directly on the magnitude of the experimental variability (Lúcio and Benz, 2017). The variability of the data in multiple-harvests horticultural crops, such as *Solanum melongena* L., is related to several factors, such as the heterogeneity of soil fertility, plant damage in the experiment by intensive management, unequipped application of irrigation, fertilization and agrochemicals, occurrence of pests, diseases and weeds, uneven maturity of fruits and the presence or absence of suitable fruits to be harvested and the variability between the cropping rows (Lúcio and Sari, 2017; Krysczun et al., 2018).

Several studies are found in the literature aiming at estimating the plot size for horticultural crops such as pepper (Lorentz et al., 2005; Lorentz and Lucio, 2009), italian zucchini (Carpes et al., 2010), tomato (Lúcio et al., 2011), bean (Santos et al., 2012), radish (Silva et al., 2002), cherry tomato (Lúcio et al., 2016) and broccoli (Brum et al., 2016). However, in these studies the authors did not have the concern to investigate the accuracy of the plot size estimates obtained in the uniformity trials.

The influence of size of the uniformity trial size on plot size estimation was studied in the potato crop (Storck et al., 2006) who have concluded that the size of the uniformity trial did not influence the plot size estimation. On the other hand, studies that evaluated turnip forage (Cargnelutti Filho et al., 2011), tomato, green beans and Italian zucchini (Schwertner et al., 2015a) and lettuce and pepper (Schwertner et al., 2015b) pointed out that uniformity trials with a few number of basic experimental units (BEU) lead to inaccurate estimates of plot size, and it is important to elucidate the influence of uniformity trial size on plot size estimation.

Information regarding the effect of size of uniformity trial on plot size estimates are scarce for the eggplant crop, thus, there would seem to be value in an investigation to clarify this problem. Based on the observed evidences, our hypothesis is that uniformity trials with few basic experimental units present high variability, reducing the accuracy of plot size estimation. In this context, the aim of this study was to determine the influence of uniformity trial size on the estimation of plot size in the eggplant crop.

2. Material and methods

2.1. Site description and experimental design

Two uniformity trials were conducted with the eggplant crop in the city of Santa Maria, Rio Grande of Sul, Brazil (S: 29º42'23" W: 53º43'15"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).

The trials were carried out in the third week of October and were performed in two plastic tunnels (20-m long, 5-m wide and 3-m high central foot) covered with 150-µm tick low-density polyethylene film with anti-UV protection. The cultivar used was the "Longe Purple", and in both trials the plants were arranged in three cultivation rows (R1, R2 and R3) spaced at 1-m. Each row had 28 plants spaced at 0.7-m where each plant was considered an BEU.

The chemical fertilization was carried out according to the soil analysis and recommendation of fertilization of the eggplant crop. The irrigation was performed by a drip system with one drip tube each row and emitters spaced at 0.30-m. The nominal hydraulic pressure of the system was 8-m of water column providing a water flow of 3.4 l h^{-1} $emitter^{-1}$. Thus, it was possible to obtain a continuous wetting width. Both the irrigation interval and the amount of water to be applied were estimated according to evapotranspiration of eggplant (mm day^{-1}) and stages phenological, respectively, considering the technical information of the irrigation system. All other cultural practices were carried out according to the culture's recommendation.

The fruits were harvested when they had approximately 18-cm length, with a bright color and soft pulp. The fruits harvested in each BEU (plant) were stored in identified plastic bags and sent to a laboratory for counting and weighing in an analytical balance with readout of 0.01 grams (g).

Six harvests were carried out. In each harvest, the fresh mass of fruit (FMF, in g $plant^{-1}$) and the number of fruits (NF, $plant^{-1}$) were assessed in each BEU. The harvests were analyzed individually (H1, 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), where each ordinal number corresponds to one harvest.

2.2. Statistical analysis

For both assessed variables and analyzed harvests (individually and grouped), between and within-row variance homogeneity was analyzed by Bartlett’s test (Bartlett, 1937) since the data followed normal distribution according to the previous Shapiro-Wilk test (Shapiro and Wilk, 1965). For both tunnels, cultivation row, harvests (individually and grouped) and assessed variables, the following statistics were estimated for each BEU: minimum, average, maximum, percentage of plants with zero values and coefficient of variation (CV%).

For each variable, cultivation row and harvests (individually and grouped), 25 UTs of sizes 3, 4, …, 28 BEU were planned, since it was observed heteroscedasticity between the harvests. For each planned uniformity trial, 3,000 plot sizes were estimated by using bootstrap procedure with replacement. For this, a plant was randomly selected in each cultivation row and the adjacent plants were used to setup the uniformity trial. For example, considering a planned uniformity trial of three BEU, let *S* = {1, 2, ..., 28} be the sample space to be sampled; assuming that the sampled BEU was the BEU 3, then the first uniformity trial would consist of the BEUs {3, 4, and 5}. In the second bootstrap cycle, a new BEU was randomly selected, and assuming that the selected BEU was the BEU 23, the uniformity trial would consist of the BEUs {23, 24 and 25}. The same procedure was followed for the remaining 2,998 cycles with three BEUs and the other planned uniformity trials.

For each bootstrap cycle, the plot sizes was estimated by the maximum curvature method of the coefficient of variation proposed by Paranaiba 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 plot size; s^2: is the variance of the sample; $\overline{Y}$: is the mean of the BEUs; $\hat{\rho}$: is the first-order spatial autocorrelation, estimated by the equation: $\hat{\rho} = \frac{\sum_{i=1}^{rc}(\hat{\varepsilon}_i - \bar{\varepsilon})(\hat{\varepsilon}_{i-1} - \bar{\varepsilon})}{\sum_{i=1}^{rc}(\hat{\varepsilon}_i - \bar{\varepsilon})^2}$, where $\hat{\varepsilon}$ is the experimental error associated with each observation.

For each one of the 3300 different scenarios {25 UTs × 3 cultivation rows × 2 tunnels × 2 variables × 11 harvests (individually and grouped)} the following statistics were calculated with the 3000 values of PS ($\hat{X}_0$) estimated by the bootstrap procedures: mean and amplitude of the 95% confidence interval (AIC95%). The AIC95% was estimated by the difference between the

97.5th and 2.5th percentiles. The size of the uniformity trial (in BEU) was determined starting from the initial uniformity trial size (three BEU) and considering as the sufficient uniformity trial size the number of BEUs from which the AIC95% was less than or equal to two BEUs. The uniformity trial size determined from *bootstrap* confidence intervals, with replacement, is a suitable procedure, mainly because it is independent of the probability distribution of the data. All analyzes were performed using the R 3.4.2 software (R Core Team, 2017).

3. Results

3.1. Variance homogeneity

The analyzes revealed heterogeneous variances for the analyzed variables between the crop rows considering individual harvest in both tunnels. Thus, when the harvests were grouped, there was no heteroscedasticity.

3.2. Estimates of descriptive statistics

When analyzing the individual harvests, it can be seen high percentages of plants with zeros values, reaching 46% in tunnel 1 and 42% in tunnel 2, and high CV% in relation to the grouped harvests, for all variables, cultivation rows and tunnels (Table 1 and 2).

Considering the individual harvests, the maximum and minimum number of fruits were eight and zero fruits per plant^{-1}, respectively (Table 1). For the grouped harvests, these values were 20 and 3 fruits per plant^{-1}, respectively.

Analyzing the fresh mass of fruit in the individual harvests, maximum and minimum fruit production were of 3105 and zero g plant^{-1}, respectively (Table 2). For the grouped harvests (at the end of the productive cycle), these values were of 8015 and 1190 g plant^{-1}, respectively.

By observing the percentage of zeros (Table 1 and 2), for the first grouped harvest (H1 + H2), the percentage of zero was null, that is, no observation with zeros values were observed.

Harvesting grouping is a simple and effective way of reducing the variability in the dataset of experiments with multiple-harvests horticultural crops.

3.3. Confidence interval

The AIC95% and plot size averages were higher in individual harvests than grouped harvests, regardless of the variables, tunnels and cultivation row (Figure 1 to 4). This result is due to the high amounts of observations with null values (% of 0) between the individual crops (Table 1 and 2).

As the size of the planned uniformity trial increased, it was found that, regardless of the variables, tunnels, cropping rows and harvests (individually and grouped), there was a reduction of the AIC95% of the plot size. However, the mean PS did not change significantly with increasing UT size (Figure 1 to 4).

3.4. Size of the uniformity trial

The size of the uniformity trial required to estimate plot size with an AIC95% less than or equal to two BEUs of the mean presented a large variation between the variables, cultivation rows and tunnels (Table 3). However, when the harvests were grouped there is a trend to reduce the sizes of the uniformity trials.

At the individual harvests the size of the uniformity trial ranged from 13 BEU to 25 BEU (for FFM) and 12 BEU to 23 BEU (for NF), independently on the tunnel and cultivation row. For the grouped harvests (H1 + H2 + H3 + H4 + H5 + H6), a lower size of uniformity trial size was observed, ranging from 8 BEU to 16 BEU (for FFM) and 7 BEU to 14 BEU (for NF), independently on tunnel and cultivation row.

4. Discussion

4.1. The size of the uniformity trial influences the plot size estimation in Solanum melongena L.?

It is possible to affirm that yes. To confirm this affirmation will be used as an example the second cultivation row of the individual harvest H6 (Figure 1). The AIC95% of plot size in the 3,000 samples of the uniformity trial with size of three BEUs was 18.17 BEU, while for the trial with the size of 27 BEU it was solely 0.38 BEU. However, when the same cultivation row (R2) was analyzed in the grouped harvests, the AIC95% considering three BEU trial was 4.89 BEU, whereas considering 27 BEU 27 the AIC95% was 0.27 BEU. Therefore, the following statements can be inferred: (i) the AIC95% for plot size estimation is larger in trials with few BEUs due to the large variability within the cultivation row. Thus, the plot size estimation will present low precision; (ii) the variability between individual crops and within each crop row is minimized by grouping the harvests.

The results found in this study are consistent with Cargnelutti Filho et al. (2011), Schwertner et al. (2015a, 2015b) who concluded that small uniformity trials provide plot size estimates with high variability and low accuracy. However, Storck et al. (2006) state that the size of uniformity trials does not influence the plot size estimation, this result is attributed to the great size of the trial of initial uniformity planned, which was 288 EUB.

In this way, it is possible to define a uniformity trial size that is economically feasible to be conducted and provide to the researcher the desired accuracy in plot size estimation.

4.2. Reduction of experimental variability

The eggplant is a horticultural species of multiple harvests, presents numerous harvests throughout its productive phase. When working with this characteristic (of multiple harvests), the researchers may find the absence of fruit suitable to be harvested in a particular harvest. This absence is bad from the statistical point of view, because it generates an excess of zeros values in the database, thus increasing the experimental variability.

To minimize the excess of zeros values in the database it is recommended to use the grouping harvests crop. According to the results, the grouping of harvests reduced the variability among the plants in the same cultivation row due to the reduction of observations with null values in the dataset. Null values are frequently observed due to the uneven maturation of fruits or the absence of fruit suitable for harvesting. This result matches those found by Lucio and Benz (2017) and Krysczun et al. (2018).

The variability between crop rows in protected environments had already been reported for crops such pepper (Lorentz et al., 2005), Italian zucchini (Lúcio et al., 2008) and eggplant (Krysczun et al., 2018). The heterogeneity between the crop rows may be related to the proximity to the sides of the protected environment structure (Feijó et al., 2008) and the conditions of limitation of climatic factors such as air temperature and sunshine hours (Lúcio et al., 2008), a fact that may have occurred in this study, due to periods with low air temperature observed during the tests (< 18 ºC). Minimal air temperature below recommended to the eggplant crop, slows plant growth and affects productivity; affected plants rarely recover, even if they return to ideal temperature conditions.

4.3. Recommendations

That research ensures researchers the possibility to predict in advance the accuracy of plot size estimates for each uniformity trial size planned at the individual and grouped harvest. Thus, researchers must be careful of the size of the uniformity trial used in estimating plot size in horticultural crops, as the low number of BEUs will provide inaccurate estimates. Future research with horticultural crops of multiple harvests should be used, whenever possible, the largest number of harvests grouped, because the variability will be minimized, independent of the statistical analysis used.

In order to estimate the plot size, it is recommended to use the grouped harvests (H1 + H2 + H3 + H4 + H5 + H6) and that the size of uniformity trial be composed of at least 16 EUB for the FMF variable and 14 EUB for the variable NF with an AIC95% less than or equal to two EUBs of the plot size average. Other benefits of these results are the reduction of humans, financial and material resources, as well as the experimental area required to perform the uniformity trial and to maximize the use of space (which is limited in the protected environment). In this way, in the same experimental area, the researcher can conduct more than one of uniformity trial, being able to use different times of cultivation, cultivars, horticultural crops, cultural management and ensure that the plot size estimate is accurate.

For eggplant crop the size of the uniformity trial affects accuracy the plot size estimation. Grouping the harvests is a simple and effective procedure to reduce the variability in the dataset. To estimate the plot size for trials with eggplant, it is suggested that the uniformity trials be composed of at least 16 basic experimental units for the variable fresh mass of fruit and 14 basic experimental units for the variable number of fruits.

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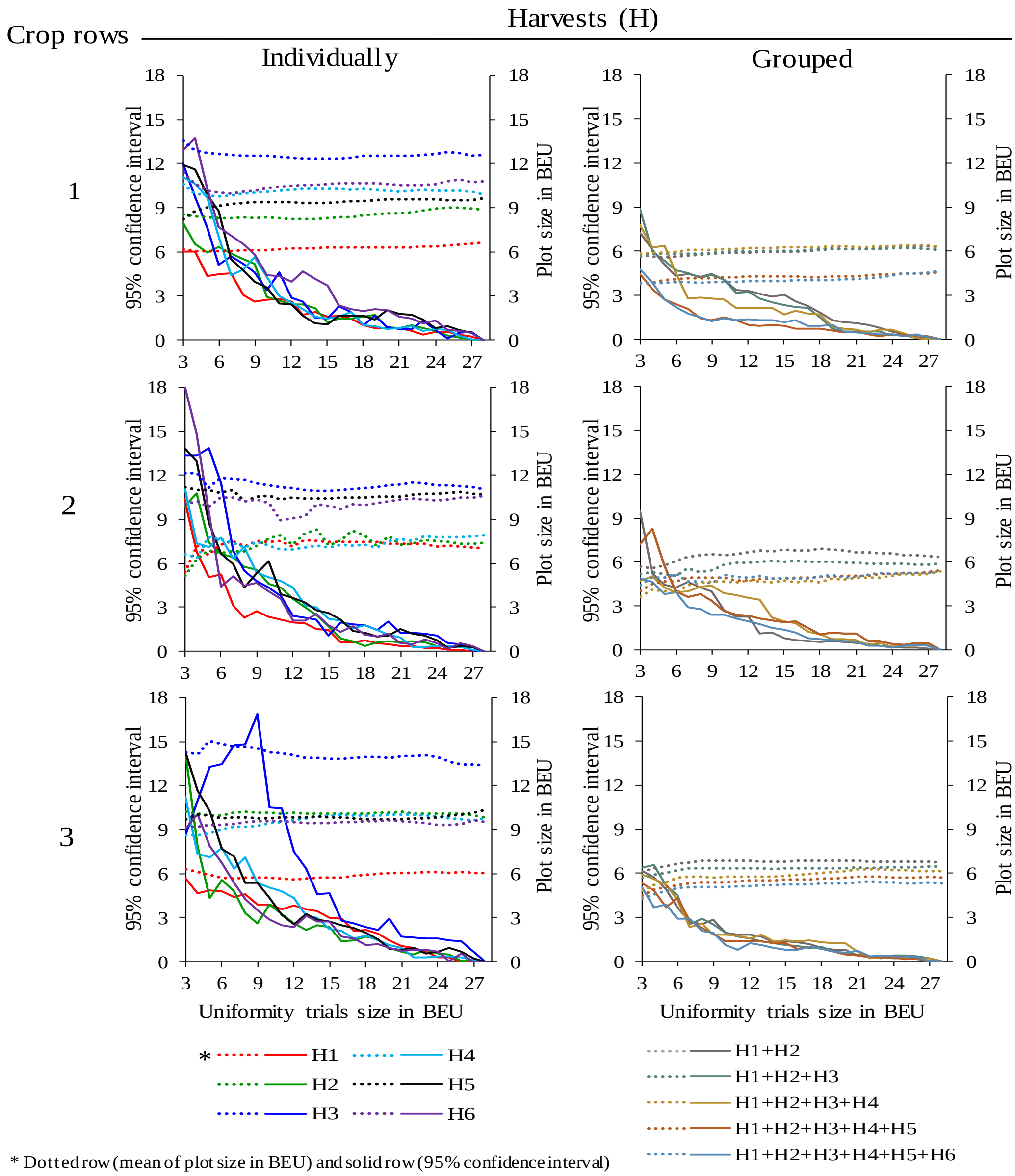

Figure 1 - Amplitude of the 95% confidence interval and mean of the 3,000 plot size estimates, in basic experimental unit (BEU), to evaluate the number of fruits in the uniformity trial performed in the plastic tunnel 1.

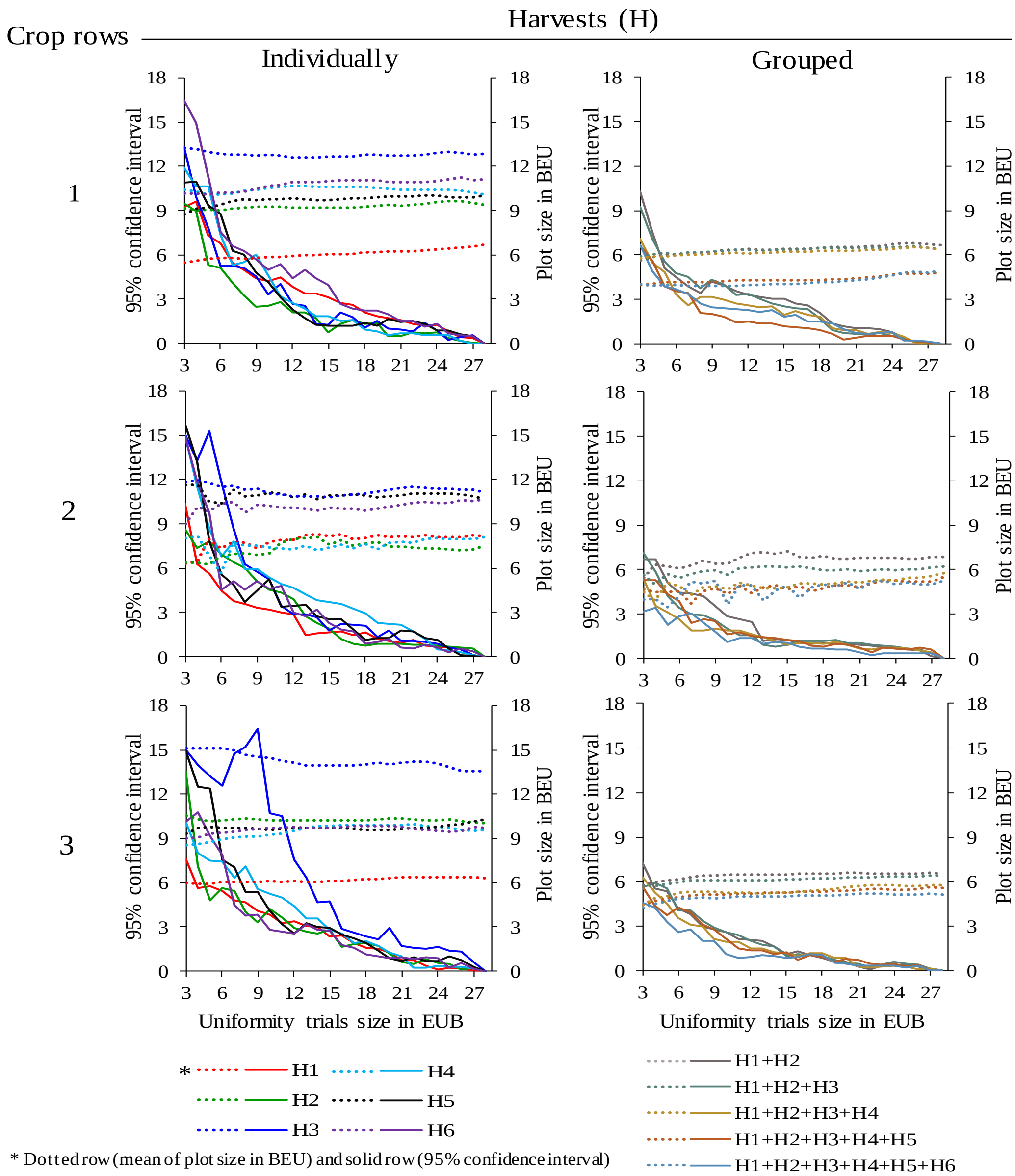

Figure 2 - Amplitude of the 95% confidence interval and mean of the 3,000 plot size estimates, in basic experimental unit (BEU), to evaluate the fresh mass of fruit (FMF, in g plant^{-1}) in the uniformity trial performed in the plastic tunnel 1.

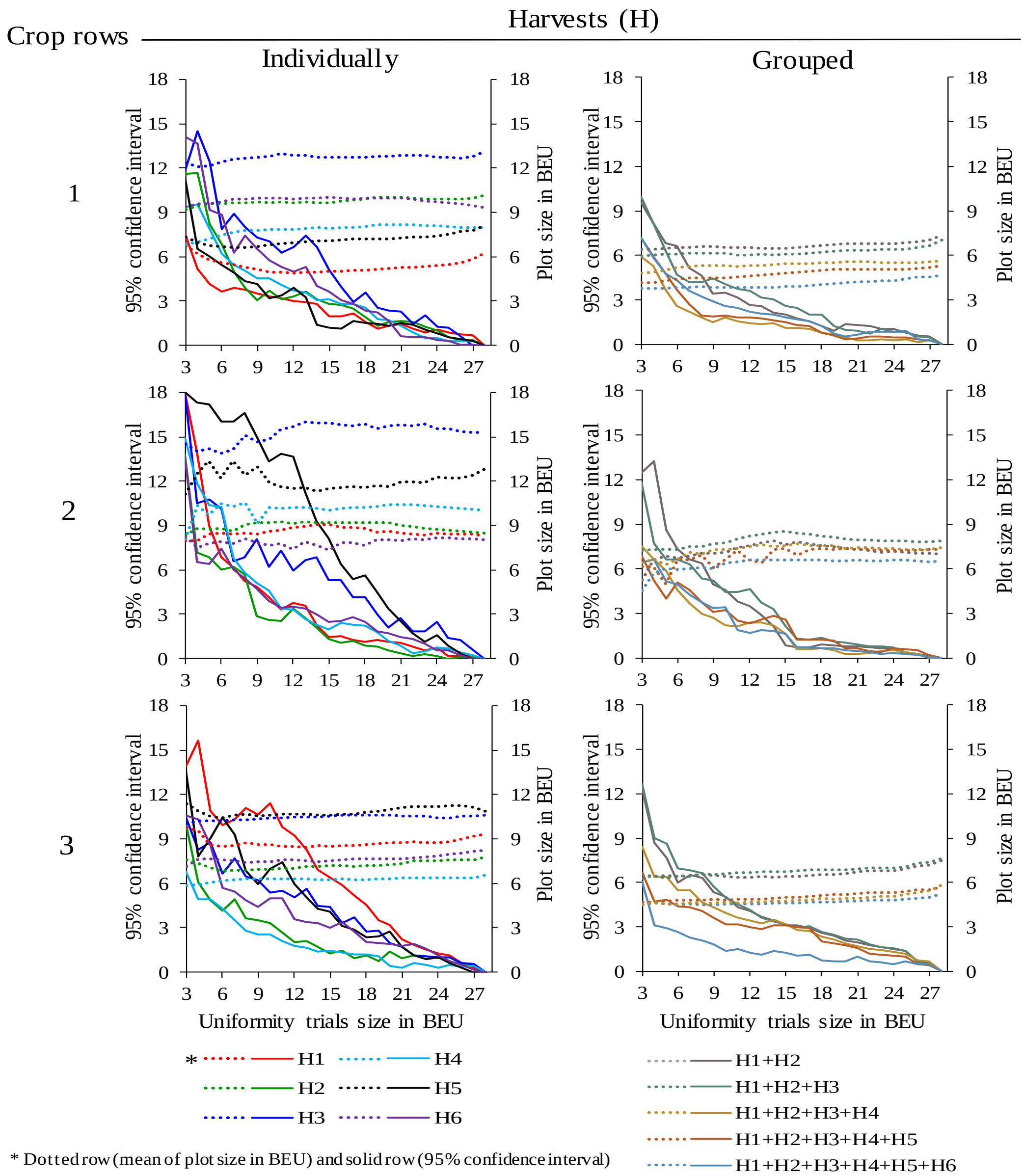

Figure 3 – Amplitude of the 95% confidence interval and mean of the 3,000 plot size estimates, in basic experimental unit (BEU), to evaluate the number of fruits in the uniformity trial performed in the plastic tunnel 2.

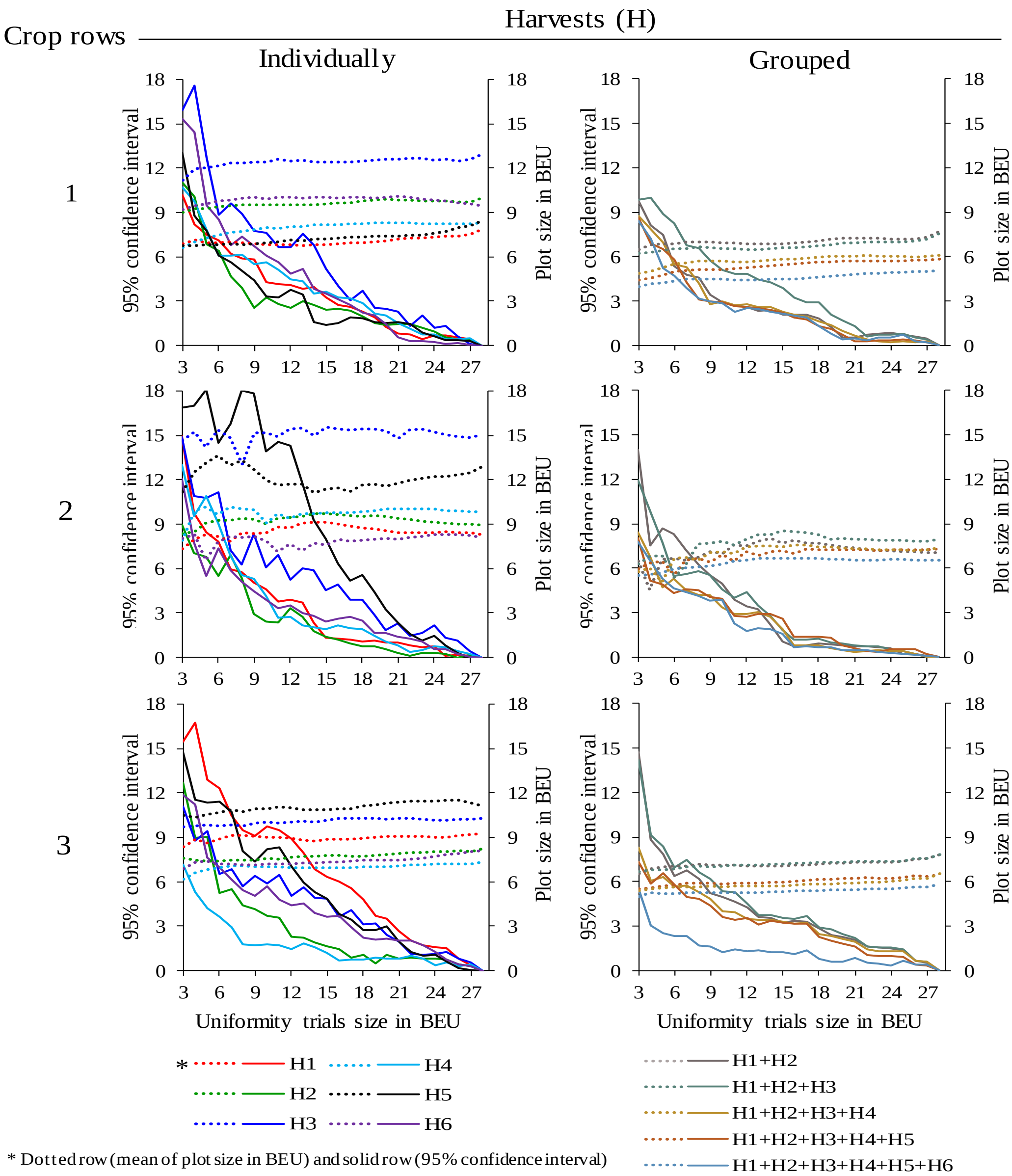

Figure 4 – Amplitude of the 95% confidence interval and mean of the 3,000 plot size estimates, in basic experimental unit (BEU), to evaluate the fresh mass of fruit (FMF, in g plant^{-1}) in the uniformity trial performed in the plastic tunnel 2.

Table 1 - Estimates of the statistics minimum values (Min), maximum values (Max), percentage of zero (% of 0), coefficient of variation (CV%) and mean of number of fruits obtained in the individual and grouped harvests in the two plastic tunnels.

Row	Harvests	Tunnel 1					Tunnel 2				
		Max	Min	% of 0	CV%	Mean	Max	Min	% of 0	CV%	Mean
1	H1	3	1	0	37	1.86	2	0	4	33	1.67
	H2	4	0	11	58	1.71	4	0	14	75	1.71
	H3	2	0	39	98	0.71	3	0	32	95	0.88
	H4	4	0	25	72	1.82	5	0	4	48	2.42
	H5	8	0	14	66	3.18	4	0	4	43	2.21
	H6	3	0	25	79	1.18	5	0	14	68	2.13
	H1+H2	6	1	0	35	3.57	6	0	4	45	3.38
	H1+…+H3	7	1	0	33	4.29	8	0	4	40	4.25
	H1+…+H4	10	1	0	35	6.11	10	3	0	30	6.67
	H1+…+H5	14	6	0	22	9.29	13	4	0	27	8.88
	H1+…+H6	16	7	0	22	10.46	18	6	0	23	11.00
2	H1	3	0	4	41	1.89	3	0	11	54	1.64
	H2	4	0	4	44	2.00	3	0	14	61	1.40
	H3	2	0	32	81	0.86	4	0	42	127	0.80
	H4	4	0	7	51	2.25	5	0	14	70	1.96
	H5	4	0	21	77	1.50	4	0	32	90	1.44
	H6	3	0	21	76	1.46	4	0	7	55	2.16
	H1+H2	6	1	0	35	3.89	5	0	7	44	3.04
	H1+…+H3	8	2	0	32	4.75	9	0	7	50	3.84
	H1+…+H4	12	4	0	28	7.00	14	1	0	46	5.80
	H1+…+H5	15	4	0	28	8.50	17	2	0	43	7.24
	H1+…+H6	16	6	0	27	9.96	20	3	0	38	9.40
3	H1	3	1	0	32	2.00	3	0	21	54	1.74
	H2	3	0	21	69	1.67	4	0	4	42	2.04
	H3	1	0	46	109	0.46	4	0	14	72	1.52
	H4	5	0	14	67	1.92	5	1	0	35	2.96
	H5	5	0	11	67	2.04	3	0	19	76	1.30

H6	5	0	14	66	2.08	6	0	7	55	2.43
H1+H2	6	1	0	39	3.67	6	1	0	36	3.78
H1+…+H3	7	1	0	39	4.13	9	1	0	37	5.30
H1+…+H4	11	3	0	33	6.04	11	4	0	23	8.26
H1+…+H5	13	4	0	30	8.08	13	4	0	25	9.57
H1+…+H6	17	5	0	26	10.17	18	7	0	23	12.00

Table 2 - Estimates of the statistics minimum values (Min), maximum values (Max), percentage of zero (% of 0), coefficient of variation (CV%) and mean of fresh mass of fruits (FMF, in g plant^{-1}) obtained in the individual and grouped harvests in the two plastic tunnels.

Row	Harvests	Tunnel 1					Tunnel 2				
		Max	Min	% de 0	CV%	Mean	Max	Min	% de 0	CV%	Mean
1	H1	2045	302	0	38	1078	1551	0	4	48	858
	H2	1655	0	11	64	727	1578	0	14	69	638
	H3	931	0	39	101	293	1088	0	32	102	298
	H4	1780	0	25	74	733	2245	0	4	53	972
	H5	3105	0	14	70	1134	1957	0	4	54	888
	H6	1412	0	25	82	507	2295	0	14	64	972
	H1+H2	3301	574	0	38	1806	2762	0	4	47	1496
	H1+…+H3	3351	574	0	36	2098	3782	0	4	45	1793
	H1+…+H4	4844	574	0	36	2832	4177	1116	0	33	2765
	H1+…+H5	5770	1922	0	24	3966	5847	1507	0	31	3654
	H1+…+H6	7149	1922	0	25	4473	7701	1896	0	25	4626
2	H1	2228	0	4	51	1130	1470	0	11	53	782
	H2	1824	0	4	46	802	1140	0	14	59	518
	H3	867	0	32	82	332	1378	0	42	128	287
	H4	1605	0	7	53	802	1741	0	14	68	659
	H5	1300	0	21	77	557	1588	0	32	102	513
	H6	1504	0	21	78	615	1644	0	7	51	929
	H1+H2	3331	288	0	40	1931	2361	0	7	41	1300
	H1+…+H3	4127	633	0	35	2263	3739	0	7	49	1587
	H1+…+H4	5732	1568	0	31	3064	5480	925	0	44	2246
	H1+…+H5	6853	1568	0	30	3622	6597	925	0	44	2759
	H1+…+H6	7243	2468	0	28	4237	8015	1190	0	37	3688
3	H1	1955	437	0	35	1148	1759	0	21	66	823
	H2	1288	0	21	72	631	1423	0	4	57	688
	H3	577	0	46	110	194	1460	0	14	73	556
	H4	1867	0	14	65	771	1838	290	0	45	936
	H5	2019	0	11	73	768	1437	0	19	81	551

H6	2544	0	14	68	852	2761	0	7	51	1093
H1+H2	3182	465	0	37	1778	2767	0	0	48	1511
H1+…+H3	3532	500	0	36	1972	3908	0	0	48	2067
H1+…+H4	4437	1209	0	31	2743	4673	290	0	37	3003
H1+…+H5	6204	1767	0	29	3511	5449	736	0	36	3473
H1+…+H6	6496	2182	0	26	4362	7918	1635	0	30	4547

Table 3 - Size of the uniformity trial (in basic experimental units) to estimate the fresh mass of fruits (FMF, in g plant^{-1}) and number of fruits (NF) for individually and grouped harvests, cultivation row in the two tunnels.

Harvests	Tunnel 1						Tunnel 2					
	FMF			NF			FMF			NF		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
H1	19	16	17	14	12	19	19	15	22	15	16	22
H2	14	15	18	19	15	18	18	14	14	18	14	14
H3	17	20	21	17	16	21	23	25	21	23	23	20
H4	14	22	18	17	18	18	21	18	13	19	19	12
H5	13	17	18	18	21	18	17	22	21	17	22	21
H6	20	16	16	18	17	16	19	19	23	20	19	19
H1+H2	19	13	14	18	14	9	18	15	22	16	15	21
H1+…+H3	18	11	13	18	15	9	20	15	22	18	18	22
H1+…+H4	17	10	10	16	15	10	17	15	21	8	15	20
H1+…+H5	10	10	11	10	15	10	16	16	20	8	17	19
H1+…+H6	16	9	9	7	12	10	16	13	8	14	13	9

3 ARTIGO II - SAMPLE SIZE, PLOT SIZE AND NUMBER OF REPLICATIONS FOR TRIALS WITH *Solanum melongena* L.

Submetido para o periódico: Scientia Horticulturae
Situação: Publicado

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.

Keywords: *Solanum melongena* L.; Experimental planning; Experimental precision; Quality control

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.

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 meters 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 Paranaiba et al. (2009): $\hat{X}_0 = \frac{10\sqrt[3]{2(1-\hat{\rho}^2)s^2\bar{Y}}}{\bar{Y}}$, where $\hat{X}_0$: is the appropriate plot size , s^2 : is the variance in the crop row , $\bar{Y}$: is the mean of the BUs in the crop row, $\hat{\rho}$: is the first-order spatial autocorrelation, estimated by the equation: $\hat{\rho} = \frac{\sum_{i=1}^{rc}(\hat{\varepsilon}_i - \bar{\varepsilon})(\hat{\varepsilon}_{i-1} - \bar{\varepsilon})}{\sum_{i=1}^{rc}(\hat{\varepsilon}_i - \bar{\varepsilon})^2}$.

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 degrees of freedom at 5% probability error; CV% is the coefficient of variation of the considered variable ($CV\% = \frac{100 \times \sqrt{s^2}}{\bar{Y}}$, on that s^2 is the sample variance , $\bar{Y}$ 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: $d = \left(q_{\alpha(i;DF)}\sqrt{MSE/r}\right)/\bar{Y} \times 100$, where $q_{\alpha(i;GLE)}$ 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 $(CV = \sqrt{QME}/\bar{Y} \times 100)$ 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.

Table 1

Plot size (Xo, in plants) and coefficient of variation in plot size (within parentheses), between individual and grouped harvests in each crop row (CR) for fresh fruit weight (FFW) and total number of fruits (TNF).

Harvests	Tunnel 1						Tunnel 2					
	FFM (g)			TNF			FMM (g)			TNF		
	CR1	CR2	CR3	CR1	CR2	CR3	CR1	CR2	CR3	CR1	CR2	CR3
H1	5 (12)	7 (16)	5 (11)	5 (12)	6 (13)	5 (11)	6 (14)	7 (16)	8 (19)	4 (10)	7 (17)	8 (19)
H2	9 (20)	6 (14)	10(22)	8 (18)	6 (14)	10 (22)	9 (20)	8 (17)	8 (19)	9 (21)	8 (17)	8 (17)
H3	12 (27)	10 (23)	13 (30)	12 (27)	10 (23)	13 (29)	12 (27)	15 (33)	9 (21)	12 (28)	15 (34)	10 (22)
H4	10 (22)	7 (16)	9 (19)	10 (22)	7 (15)	9 (19)	7 (15)	9 (19)	6 (12)	6 (14)	9 (20)	5 (10)
H5	9 (20)	10 (22)	9 (20)	9 (19)	9 (21)	9 (20)	7 (16)	12 (26)	10 (23)	7 (15)	12 (26)	10 (22)
H6	11 (25)	10 (22)	9 (21)	10 (23)	10 (22)	9 (20)	9 (20)	7 (15)	7 (16)	9 (20)	7 (26)	7 (16)
H1+H2	5 (12)	6 (12)	5 (12)	5 (10)	5 (11)	5 (12)	6 (13)	6 (12)	6 (14)	6 (12)	6 (12)	6 (13)
H1+...+H3	5 (11)	5 (11)	5 (12)	4 (10)	4 (10)	5 (12)	6 (13)	7 (15)	6 (14)	5(11)	7 (15)	6 (13)
H1+...+H4	5 (11)	5 (11)	4 (9)	5 (12)	5 (12)	5 (11)	4 (10)	6 (14)	5 (12)	4 (9)	7 (15)	5 (10)
H1+...+H5	4 (7)	5 (10)	4 (8)	3 (7)	3 (7)	4 (9)	4 (9)	6 (14)	5(11)	3 (8)	6 (14)	4 (10)
H1+...+H6	3 (8)	5 (9)	3 (7)	3 (7)	3 (7)	3 (8)	3 (7)	5 (11)	4 (9)	3 (6)	5 (11)	3 (8)

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 to 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 to 23 for grouped harvests.

Table 2

Sample size (in number of plants) and confidence intervals for means (D = 5, 10, 15, and 20%), in individual and grouped harvests in each crop row of the fresh fruit weight.

Tunnel	Row	D %	H1	H2	H3	H4	H5	H6	H1+H2	H1+...+H3	H1+...+H4	H1+...+H5	H1+...+H6
1	1	5	25	27	28	27	27	27	25	25	25	22	22
		10	19	24	26	25	25	26	19	19	18	13	14
		15	14	21	24	22	22	23	14	13	13	8	8
		20	10	17	22	19	19	20	10	9	9	5	5
	2	5	26	26	27	26	27	27	25	25	24	24	23
		10	22	21	25	23	25	25	20	18	17	16	15
		15	18	16	23	18	22	22	14	12	11	10	9
		20	14	12	20	14	19	19	10	9	8	7	6
	3	5	25	27	28	27	27	27	25	25	24	23	22
		10	18	25	27	24	25	25	19	18	16	16	14
		15	13	22	25	21	22	21	13	13	11	10	9
		20	9	18	23	17	19	18	9	9	7	7	6
2	1	5	26	27	28	26	26	27	26	26	24	24	22
		10	22	25	26	23	23	24	21	21	17	17	14
		15	17	21	24	18	18	21	17	16	12	11	8
		20	13	18	22	14	15	17	13	12	8	8	5
	2	5	26	27	28	27	28	26	26	26	26	26	25
		10	23	24	27	24	26	22	20	22	21	21	19
		15	18	10	26	21	24	18	15	17	16	16	13
		20	14	16	24	18	22	14	11	13	12	12	9
	3	5	27	27	27	26	27	26	26	26	25	25	24
		10	24	23	25	21	25	22	22	22	19	19	16
		15	21	19	22	16	23	18	17	17	13	13	11
		20	17	15	19	12	20	14	13	13	9	9	7

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 to 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 to 26 for grouped harvests.

Table 3

Sample size (in number of plants) and confidence interval for means (D = 5, 10, 15, and 20%), for individual and grouped harvests in each crop row of the total number of fruit.

Tunnel	Rows	D %	H1	H2	H3	H4	H5	H6	H1+H2	H1+...+H3	H1+...+H4	H1+...+H5	H1+...+H6
1	1	5	25	27	28	27	27	27	25	24	25	21	21
		10	19	23	26	25	24	25	18	17	18	12	12
		15	14	19	24	22	21	23	12	12	13	7	7
		20	10	16	22	18	17	20	9	8	9	4	4
	2	5	25	26	27	26	27	27	25	24	25	21	21
		10	20	21	25	22	25	25	18	17	18	12	12
		15	15	16	23	18	22	22	13	11	13	7	7
		20	11	12	20	14	19	19	9	8	9	4	4
	3	5	24	27	28	27	27	27	25	25	24	24	23
		10	17	25	26	24	25	24	19	19	18	16	15
		15	12	21	25	21	22	21	14	13	12	11	9
		20	8	18	23	18	19	17	10	10	8	7	6
2	1	5	25	27	28	26	26	27	26	26	24	23	21
		10	18	25	26	22	22	24	21	20	16	15	12
		15	13	22	25	17	18	21	16	15	11	9	7
		20	9	18	22	13	14	17	12	11	7	6	4
	2	5	26	27	28	27	28	26	25	26	26	26	25
		10	23	23	27	25	26	22	20	22	21	21	19
		15	18	19	26	21	24	18	15	17	16	16	13
		20	15	15	24	18	22	14	11	13	12	12	9
	3	5	27	26	27	25	27	26	26	26	24	24	23
		10	24	22	25	19	25	22	21	21	16	16	14
		15	21	18	22	13	23	18	16	17	11	11	9
		20	17	14	19	10	20	14	12	13	7	7	6

3.4. Number of replications

For the evaluation of FFM and TNF, the number of replications ranged between 1 (3 treatments with d = 50%) and 180 (2 treatments with d = 15%) in scenarios formed by combinations of treatments (i = 2, 3, 4, ..., 15) and d minimum differences among treatment averages (d = 5%, 10%, 15%, ..., 50%) to be detected as significant at 5% probability by the Tukey's test.

Table 4

Number of replications for experiments in the randomized block design, in scenarios formed by the combinations of i treatments (i = 2, 3, 4, ..., 15) and minimum differences between averages of treatments to be detected as significant at 5% of (D = 5, 10, 15, ..., 50%), for the variables fresh weight of fruits and total number of fruits, from the plot size (X_o = 5 plants) and coefficient of variation in plot size (CV_{X_o} = 11%).

i	5%	10%	15%	20%	25%	30%	35%	40%	45%	50%
2	180	45	20	11	7	5	4	3	2	2
3	123	31	14	8	5	3	3	2	2	1
4	116	29	13	7	5	3	2	2	1	1
5	116	29	13	7	5	3	2	2	1	1
6	117	29	13	7	5	3	2	2	1	1
7	119	30	13	7	5	3	2	2	1	1
8	121	30	13	8	5	3	2	2	1	1
9	122	31	14	8	5	3	2	2	2	1
10	124	31	14	8	5	3	3	2	2	1
11	126	32	14	8	5	4	3	2	2	1
12	131	33	15	8	5	4	3	2	2	1
13	130	32	14	8	5	4	3	2	2	1

14	133	33	15	8	5	4	3	2	2	1
15	131	33	15	8	5	4	3	2	2	1

4. Discussion

4.1. Reduction of experimental variability

In short, nowadays there is great interest in reducing production variability in greenhouses, both for commercial and statistical reasons. From the commercial point of view, the reduction in production variability provide to farmers a competitive advantage in marketing its production. On the other hand, from the statistical point of view, grouping harvests has proven to be an efficient technique in reducing variability between plants in the same crop row due to the reduction of null values in the dataset due to uneven maturity of fruits or the absence of fruit suitable for harvesting. This result is consistent with those found by Lúcio et al. (2017). The reduction of productive variability of chicory grown in a greenhouse also provided better goodness-of-fit of growth models, which is important in developing production strategies (Olivoto et al., 2018a).

The variability among crop rows in protected environments had already been reported for pepper crops (Santos et al., 2012) and Italian zucchini (Lúcio et al., 2008). Feijó et al. (2008) pointed out the heterogeneity among crop rows may be related to the proximity to the sides of the protected environment structure. In addition, Lúcio et al. (2008) reported the heterogeneity among the rows may also be related to the climatic conditions and the type of cultural management carried out.

All the results obtained in this study corroborate with the results presented by these authors. In this context, it should be noted that plot and sample size will be directly impacted by variability, and, as we have shown, grouping harvests is an efficient technique for minimizing the effects of this variability.

4.2. Plot size

Choice of plot size and use of appropriate experimental design are ways to control experimental error and variability inherent in an experiment (Storck et al., 2006). In the case of experiments with eggplant, due to the variability between the rows of protected cultivation, it is recommended to adopt a randomized block design, each row being a block. In a study with salad and cherry tomatoes (Lúcio et al., 2016, 2010), and peppers (Lorentz and Lúcio, 2009), they found similar values of plot size to those recommended in this study were found.

4.3. Sample size

Sample size planning is an important step that must be carefully considered when installing an experimental research (Anderson et al., 2017). Recent studies focused on this subject have revealed the importance of the correct sample size planning in reducing the confidence interval of parameter estimates (Olivoto et al., 2018b). Although studies focused on sample size planning is more popular for world-important crops (e.g., maize, soybean, rice), the way in choosing the sufficient sample size for fitting nonlinear growth models for horticultural crops was proposed by Elli et al. (2018). For eggplant, however, were still lacked in literature a study focused on experimental planning. Our findings fill this gap.

Recent studies revealed that 32, 8, 4 and 2 plants are enough to detect a treatment difference of 5%, 10%, 15% and 20% of the mean, respectively, for leaf number of eggplant (Leonardo et al., 2017). Our results revealed that considering the same deviations, the sample size needed for estimating FFM and TNF is 25, 19, 13 and 9 plants (maximum number of plants across rows with harvest grouped). The largest number of plants to be assessed found in this study compared to study by Leonardo et al. (2017) is related to the characteristic of the assessed traits. Production-related variables are highly influenced by environmental factors due to the polygenic inheritance (Portis et al., 2014); thus, due to the higher variation in productivity a largest number of plants is needed for achieving a giving precision.

It can be seen from this recommendation that only 32% of the plants in each crop row will be sampled, that is, there will be a reduction in evaluation time, labor and costs. For these reasons, an adequate sampling is of extreme importance because the researcher can carry out a greater number of evaluation, maximizing his research.

4.4. Number of replications

The number of replicates has a direct, highly predictable, repeatable, and tangible effect on the ability to detect differences among treatments. Casler, (2015) pointed out that few agronomic researchers have ever conducted a preliminary analysis with the goal of designing a better experiment. Here, we demonstrated that considering a plot size of five plants (X_o= 5), the researchers might then establish the relationship between *i* (treatments), *d* (differences between treatment means) aiming at determining the number of replicates to be used in eggplant trials. In addition, important factors such as the size of the experimental area, availability of labor, financial resources should also be considered when planning the number of replicates.

For the estimated plot size (X_o= 5 plants) with d= 30%, it is observed that the number of replications estimated was three. Koller et al. (2016) also recommended three replications for trials with eggplant, however, their recommendation was empirical, based on the expert opinions of the various authors. Here, we demonstrated that the number of replicates to be used must be chosen considering both the number of treatments as well as the differences among treatment means (in percentage of overall mean). It is evident that the greater the number of treatments, the greater the number of replications needed. In this context, the correct choice of the number of treatments to be tested also has a direct effect on detection of differences among treatments. For example, for detecting differences among treatments of 30% of the mean, three replications are enough when evaluating up to 10 treatments. A research with 11 treatments, however, will need four replicates to detect the same difference among treatment means.

In the trials performed in the tunnels this was the number of crop rows adopted, depending on the dimensions of each plastic tunnel. In this way, it can be recommended that for experiments with eggplant in protected environments and with a minimum significant difference of the Tukey test expressed as a percentage of the mean of 30%, it is possible to adopt plots of five plants per crop row, each one of the three rows considered a block.

5. Conclusions

For eggplant trials, the plot and sample size are influenced by the variability in harvests and crop rows. Grouping harvests has proven to be an effective method to reduce that variability. 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.

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4 DISCUSSÃO GERAL

Os objetivos propostos para esse trabalho foram alcançados. O banco de dados utilizados para realizar esse estudo apresenta elevada variabilidade, conforme as análises descritiva e de homogeneidade de variâncias. Devido a esse problema, empregou-se o agrupamento de colheita no qual tem por objetivo reduzir o número de observações com valores zero no banco de dados e, consequentemente, a variabilidade experimental, conforme já discutido nos artigos.

Em ambos os artigos, os melhores resultados obtidos para os objetivos propostos foi quando utilizou todas as colheitas (H1+H2+H3+H4+H5+H6), porém a partir do primeiro agrupamento já se observou resultados superiores às colheitas avaliadas individualmente. Assim, ficou evidente a importância do agrupamento de colheitas para culturas de múltiplas colheitas afim de minimizar a variabilidade, assegurar a precisão experimental e a confiabilidade dos resultados gerados nos experimentos.

Os resultados obtidos no Artigo 1 servem como subsídio para pesquisadores da área de experimentação vegetal que visam estimar o tamanho de parcela em culturas olerícolas. Assim, devem ficar atento ao tamanho do ensaio de uniformidade utilizado para estimativa do tamanho de parcela, pois o baixo número de unidades experimentais básicas leva a estimativas pouco precisa.

No Artigo 2, as estimativas do tamanho de parcela, de amostra e do número de repetição têm por objetivo auxiliar no planejamento experimental na cultura da berinjela, a fim de reduzir o uso de recursos humanos, materiais e financeiros, e assegurar confiabilidade dos resultados gerados nos experimentos.

5 CONCLUSÃO GERAL

O agrupamento de colheitas é um procedimento simples e eficaz para reduzir a variabilidade no banco de dados em ensaios com berinjela. O tamanho do ensaio de uniformidade influência a estimativa do tamanho de parcela. Para estimar tamanhos de parcelas em ensaios com berinjela são necessários ensaios de uniformidade compostos de no mínimo 16 unidades experimentais básicas para a variável massa fresca de frutos e 14 unidades experimentais básicas para a variável número de frutos.

A amostragem de plantas em ensaios de berinjela em túneis plásticos, considerando uma semiamplitude do intervalo de confiança da média de 20%, deverá ser de nove plantas no sentido da fileira de cultivo. Para uma diferença mínima significativa do teste de Tukey expressa em percentagem da média de 30%, o tamanho de parcela para ensaios com berinjela é de cinco plantas com três repetições.

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APÊNDICE

Tabelas suplementares

Table 1. Evapotranspiration of eggplant (mm day^{-1}) irrigated by dripper irrigation system according to the crop stage and daily historical of air average temperature (24 hours) and air relative humidity (ARU)

ARU (%)	Air temperature (°C)								
	14	16	18	20	22	24	26	28	30
	Initial[a] and final[b] stages								
40	3.8	4.2	4.7	5.1	5.6	6.1	6.6	7.1	7.6
50	3.2	3.5	3.9	4.3	4.6	5.0	5.5	5.9	6.4
60	2.6	2.8	3.1	3.4	3.7	4.0	4.4	4.7	5.1
70	1.9	2.1	2.3	2.6	2.8	3.0	3.3	3.5	3.8
80	1.3	1.4	1.6	1.7	1.9	2.0	2.2	2.4	2.5
90	0.6	0.7	0.8	0.9	0.9	1.0	1.1	1.2	1.3
	Vegetative stage[c]								
40	5.5	6.1	6.7	7.3	8.0	8.6	9.4	10.1	10.9
50	4.6	5.0	5.5	6.1	6.6	7.2	7.8	8.4	9.1
60	3.7	4.0	4.4	4.9	5.3	5.8	6.2	6.7	7.3
70	2.7	3.0	3.3	3.6	4.0	4.3	4.7	5.1	5.4
80	1.8	2.0	2.2	2.4	2.7	2.9	3.1	3.4	3.6
90	0.9	1.0	1.1	1.2	1.3	1.4	1.6	1.7	1.8
	Productive stage[d]								
40	4.5	5.0	5.5	6.0	6.6	7.1	7.7	8.3	0.0
50	3.8	4.2	4.6	5.0	5.5	5.9	6.4	7.0	7.5
60	3.0	3.3	3.7	4.0	4.4	4.8	5.1	5.6	6.0
70	2.3	2.5	2.7	3.0	3.3	3.6	3.9	4.2	4.5
80	1.5	1.7	1.8	2.0	2.2	2.4	2.6	2.8	3.0
90	0.8	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5

[a]Initial stage: Transplanting up to the seedling establishment (7-10 days); [b]Final stage: last harvest; [c]Vegetative stage: Seedling establishment up to the flowering stage; [d]Productive stage:

Flowering up to the last harvest. Values not shown can be estimated by using a simple linear interpolation. Source: Adapted from Marouelli et al. (2014).

Table 2. Irrigation interval for the eggplant regarding the evapotranspiration (ET), root depth, and soil texture.

ET(mm day^{-1})	**Rood depth**								
	10 cm			**30 cm**			**50 cm**		
	Sand	**Silt**	**Clay**	**Sand**	**Silt**	**Clay**	**Sand**	**Silt**	**Clay**
1	1	2	3	2	5	8	--	--	--
2	2×day	1	1	1	3	4	2	4	7
3	2×day	1	1	1	2	3	1	3	4
4	3×day	2×day	1	1	1	2	1	2	3
5	4×day	2×day	2×day	2×day	1	2	1	2	3
6	4×day	3×day	2×day	2×day	1	1	1	1	2
7	4×day	3×day	2×day	3×day	1	1	2×day	1	2
8	--	--	--	3×day	1	1	2×day	1	2
9	--	--	--	3×day	2×day	1	2×day	1	1
10	--	--	--	4×day	2×day	1	2×day	1	1

Source: Adapted from: Marouelli et al. (2014).

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