Repeatability and number of measurements for productive variables in vegetable crops experiments

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ABSTRACT

One of the ways to optimize the evaluation of the experiment and obtain high precision of the results is with the use of an adequate number of measurements for each variable observed in the experiment. In this sense, the objective of this study was to estimate the repeatability coefficient and the number of measurements necessary for the analysis of characters of production of zucchini, cucumber, pout pepper and pea. In the experiments carried out with zucchini and cucumber the variables number of fruits, fruit mass, fruit length, largest fruit diameter and smallest fruit diameter were evaluated. For pea the number, length and mass of pods, number and mass of grains per pod and for pout pepper number and mass of fruits, largest fruit diameter and length were evaluated. The repeatability coefficient (r) was estimated by the principal component method using the correlation matrix and the covariance matrix. The number of measurements for each variable was also determined based on the coefficients of determination of 0.80, 0.85, 0.90, 0.95 and 1.00. The estimated repeatability coefficient obtained the highest estimates using the covariance matrix, ranging from 0.67 to 0.84 for zucchini, from 0.66 to 0.97 for cucumber, from 0.47 to 0.70 for pea and from 0.57 to 0.71 for pout pepper. To evaluate the fruit mass in zucchini, cucumber, pea and pout pepper, are exactly, respectively, 7, 4, 8 and 8 measurements for a coefficient of determination of 95%.

Keywords: Capsicum chinense, Cucumis sativus, Cucurbita pepo, Pisum sativum, variability, experimental precision.

RESUMO

Repetibilidade e número de mensurações para caracteres produtivos em experimentos com olerícolas

Uma das formas de otimizar a avaliação do experimento e obter elevada precisão dos resultados é com o uso de quantidade adequada de mensurações para cada variável observada no experimento. Neste sentido, o objetivo deste estudo foi estimar coeficiente de repetibilidade e o número de mensurações necessárias para análise de caracteres de produção de abobrinha, pepino, pimenta biquinho e ervilha. Os experimentos foram realizados no Departamento de Fitotecnia da Universidade Federal de Santa Maria. Para abobrinha e pepino avaliaram-se as variáveis número, massa e comprimento de frutos, maior e menor diâmetro dos frutos. Para ervilha avaliou-se o número, massa e comprimento de vagens, número e massa de grãos, e para pimenta biquinho avaliou-se número, massa, maior diâmetro e o comprimento dos frutos. O coeficiente de repetibilidade (r) foi estimado pelo método dos componentes principais usando a matriz de correlação e a matriz de covariâncias. Determinou-se ainda, o número de mensurações para cada variável com base nos coeficientes de determinação de 0.80, 0.85, 0.90, 0.95 e 1.00. O coeficiente de repetibilidade estimado obteve as maiores estimativas com o uso da matriz de covariâncias, variando de 0.67 a 0.84 para abobrinha, de 0.66 a 0.97 para pepino, de 0.47 a 0.70 para ervilha e de 0.57 a 0.71 para pimenta biquinho. Para avaliar a massa de frutos em abobrinha, pepino, ervilha e pimenta biquinho, são necessárias, respectivamente 7, 4, 8 e 8 mensurações para um coeficiente de determinação de 95%.

Palavras-chave: Cucurbita pepo, Cucumis sativus, Capsicum chinense, Pisum sativum, variabilidade, precisão experimental.

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Vegetables in general have multiple benefits that help human health in preventing diseases (Canella et al., 2018), in addition to contributing to the generation of employment and income around the world. Given the vast economic and social importance conferred by vegetable industries, the need for research in the most diverse areas is essential, aiming at increasing productivity and product quality.

Generally, when conducting experiments, it seeks to measure as many variables as possible to try to identify and elucidate the influence and response of plants about the evaluated treatments. This condition requires therefore high demand for labor, laboratory materials, and time, and result in a high number of measurements. This could be minimized by using a smaller number of measurements during the evaluation of variables, ensuring the same reliability and accuracy of the
One way to optimize the evaluation of the experiment and reducing experimental variability to obtain high accuracy of the results is through the use of adequate measurements for each variable observed in the experiment. In this sense, the repeatability coefficient is a statistical method used in genetic improvement to measure the expression of a specific treatment over time (Cruz et al., 2012). The repeatability coefficient of each variable makes it possible to estimate the number of measurements of this variable. Thus, there are the possibility of not wasting time, labor, and financial resources necessary to better discriminate the effects of different treatments under evaluation (Della Bruna et al., 2012; Diel et al., 2020a).

The repeatability coefficient (r) can be estimated by several methodologies, including structural analysis, determined based on the theoretical eigenvalue of the correlation matrix, principal components obtained from correlation or covariance matrices, and the method based on the analysis of variance (Cruz et al., 2012), and these present differences in the results. Diel et al. (2020a), studying the repeatability for strawberry variables, indicated that the r-values were better estimated by the principal component analysis method, being the most efficient one that uses the covariance matrix.

Some studies have estimated the plot size and the number of replications for the cultures of Cucumis sativus (Lúcio et al., 2020), Cucurbita pepo (Santos et al., 2014), and Pisum sativum spp. arvense (Cargnelutti Filho et al., 2015). However, it is important to estimate the repeatability coefficient and the number of measurements required for high experimental precision in these cultures, a fact that has not yet been studied. Given the above, the present work aims to estimate the repeatability coefficient and the number of measurements necessary to collect observations of production variables in experiments with zucchini (Cucurbita pepo), cucumber (Cucumis sativus), pout pepper (Capsicum chinense) and pea (Pisum sativum).

**MATERIAL AND METHODS**

**Site description**

The experiments were conducted in the experimental area of the Crop Science Department at Federal University of Santa Maria (29°42′23″S; 53°43′15″W, 95 m altitude). The climate of this region is the Cfa type, according to the Köppen’s classification (Alvares et al., 2013). The soil in the experimental area is classified as Typic Hapludalf (Streck et al., 2008).

The soil preparation of the experimental area, in all experiments, was carried out with the rotary hoe and the basic fertilization for each of the crops according to soil analysis (CQFS, 2016). The other cultural managements were carried out according to recommendations for each studied species and by good agricultural practices.

**Experimental design of zucchini**

The experiments were conducted in the years 2018 and 2019. Sowing was held on September 26, 2018 for the spring-summer season (S/S) and February 2, 2019 for the summer-autumn season (S/A), in 128 cell trays containing Carolina® substrate. The seedling transplantation was carried out in beds on October 24, 2018 for S/S and March 10, 2019 for S/A (28 and 19 days after sowing, respectively), with 0.70 m spacing between plants.

A randomized block design with eight replications and plots composed of seven plants was used, which were composed of three treatments, being evaluated the genotypes: Caserta, PX13067051, and Tronco.

The plants were individually tutored and irrigation was performed by drip according to crop necessity. The harvests were carried out in all plants of the plot, 25 days after the transplant, being repeated every three days. The fruit size for the harvest was standardized at 15 cm in average. The evaluated variables were the number of fruits per plant (NF), fruit mass (FM, g), fruit diameter (FD, cm), and fruit length (FL, cm). For the variables, FD and FL, five fruits from each plant were sampled and later represented by the average due to the high number of fruits produced per plant.

**Experimental design of pea**

The uniformity test was conducted in the field in 2016, without the use of irrigation. The used cultivar was Ervilha Grão 40; it was implemented on March 5, 2016 in four beds with two rows of cultivation and 0.45 m spacing between plants and 0.80 m between rows. Each row was composed of 30 pits, containing four plants per pit and each one was considered a basic unit (BU).

The pods were harvested in all BUs when they had a light green color. After collected, they were packed in identified plastic bags and sent to the laboratory for counting and weighing on a digital scale. Five BUs of each row were randomly obtained to measure the number of pods per plant (NP, un.), the mass of pods (MP, g), pod length (PL, cm), the number of grains per pod (NGP, un.) and mass of grains per pod (MGP, g). For the variables MGP, MP, and PL, 5 plants were sampled randomly from each row for measurements, and then the mean values were calculated.
Experimental design of cucumber

The experiment was conducted in 2019 in a randomized block design with six replications and four plants per plot. The treatments consisted of three cultivars: Atlântico, Caipira, and Fuyu. Sowing was carried out in trays on January 4, 2019 using the substrate Carolina®. The seedlings were transplanted into beds on January 28, 2019 spaced 0.80 m between plants and between beds.

The plants were tutored individually, the beds were covered with mulching plastic-type and irrigation was performed by drip hoses. The evaluated variables were the number of fruits per plant (NF), fruit mass (FM, g), fruit length (FL, cm), the largest fruit diameter (LFD, cm) and the smallest fruit diameter (SFD, cm), measured in all fruits harvested in the experiment.

Estimation of repeatability coefficients

The repeatability coefficient (r) was estimated using two methodologies. The first method was that of principal components analysis using the correlation matrix (PCAcov), which consists of obtaining a correlation matrix between the genotypes in each measurement pair, determining the eigenvalues and normalized eigenvectors.

\[
\eta \sum_{j=1}^{\eta} \frac{\lambda_j}{\lambda_j} (j = 1, 2, \ldots, \eta)
\]

where \( \eta \) is the number of periods evaluated and \( \lambda_j \) is the eigenvalue associated with the eigenvector, whose elements have the same sign and similar magnitudes (Cruz et al., 2012). The second method used was the principal component analysis using the covariance matrix (PCAcov), which is an alternative technique that can be applied in the phenotypic covariance matrix, as follows:

\[
\Gamma = \sigma_g^2 \begin{bmatrix}
1 & \rho & \ldots & \rho \\
\rho & 1 & \ldots & \rho \\
\rho & \rho & \ldots & \rho \\
\rho & \rho & \rho & 1
\end{bmatrix}
\]

where

\[
V(y_g) = V(Y_g) = \sigma_g^2 + \sigma^2 = \sigma_r^2,
\]

and

\[
Cov(y_g, y_g) = (\sigma_g^2 + \sigma^2) \rho = \rho \sigma_r^2.
\]

The first eigenvalue is given by

\[
\lambda_1 = \sigma_g^2 [1 + (\eta - 1) \rho]
\]

thus, the coefficient repeatability is given by

\[
r = \rho \frac{\hat{\lambda} - \sigma_g^2}{\sigma_r^2 (\eta - 1)}
\]

being \( \hat{\lambda} \), the eigenvalue \( \Gamma \) associated with the eigenvector whose elements have the same sign and similar magnitudes.

Number of measurements

For all estimates, the determination coefficient (R²) and the minimum number of measurements (\( \eta_m \)) necessary to predict the real genotypic value associated with preset R² magnitudes were estimated.

The determination coefficient was estimated based on the averages of the measurements and in the estimation of the repeatability coefficient (r), according to the following expression

\[
R^2 = \frac{\eta_r}{1 + r (\eta - 1)}
\]

The minimum number of measurements was estimated by the expression

\[
\eta_m = \frac{R^2 (1 - r)}{1 - R^2 r}
\]

based on the preset determination coefficients of 0.80, 0.85, 0.90, 0.95, and 1.0.

All analyses were performed using the Genes and R statistical software, using the ggplot2 package (R Development Core Team, 2019).

RESULTS AND DISCUSSION

The coefficients of determination (R²) estimates were above 0.90, independent of the adopted estimation repeatability coefficient, except for pea in the method of adopting correlation matrix (PCAcov). This condition shows the high capacity of the methods to predict the treatment characteristic except for pea, where for the variables number of pods, mass of pods, and number of grains per plant, they presented low values of R² (0.4924, 0.5251 and 0.6356 respectively), estimated by the PCAcov method. However, using the PCAcov estimation method, high values of the coefficient of determination and repeatability are observed (Table 1).

The repeatability coefficient increased in all variables when the estimation method used was PCAcov. When using this way, the coefficient of determination also increased for all variables and species evaluated, indicating its greater precision about PCAcov (Table 1).

The lower the repeatability coefficient (r), the greater the number of measurements needed to be made to achieve the required level of precision, that is, more measurements must be done for a given genotype to express its full potential. With this increase in the number of measurements, there will be greater demands on time, labor, and financial resources to experiment. In this idea, one should choose the estimation method that presents the highest “r” and, consequently, the lowest number of measurements. Thus, the number of measurements to evaluate the production variables of the studied species were lower for the PCAcov than for the PCAcov method (Table 1), and you should choose to use one of them.

For pea, the lowest repeatability coefficient was found for the variable number of pods (NP), estimated by the PCAcov method (r = 0.24). This result increases the number of measurements so that the results of the experiment are more accurate.

The repeatability coefficient is widely used in the area of genetic improvement to predict results and select higher genotypes (Cruz et al., 2012). In this sense, several studies have already been carried out, such as Dutra Filho et al. (2020) who used repeatability analysis methods for the selection of Saccharum officinarum genotypes, with greater longevity in the stalks. Azevedo et al. (2020) estimated the repeatability coefficients for the number of chestnut fruits and determined the appropriate number of measurements necessary for an efficient selection of promising chestnut trees (Bertholletia excelsa). Diel et al. (2020a) estimated the repeatability coefficient and the number of measurements required for...
Table 1. Repeatability coefficients (r) and coefficient of determination (R²) estimated by the principal component analysis methods based on the correlation matrix (PCAcor) and the covariance matrix (PCAcov) for production variables of different vegetable crop species. Santa Maria, UFSM, 2021.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PCAcov</th>
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<th>PCAcov</th>
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<tr>
<td></td>
<td>r</td>
<td>R²</td>
<td>r</td>
<td>R²</td>
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<tr>
<td>Zucchini S/S¹</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Number of fruits</td>
<td>0.62</td>
<td>0.96</td>
<td>0.68</td>
<td>0.97</td>
</tr>
<tr>
<td>Fruit mass</td>
<td>0.61</td>
<td>0.96</td>
<td>0.77</td>
<td>0.98</td>
</tr>
<tr>
<td>Fruit length</td>
<td>0.71</td>
<td>0.98</td>
<td>0.84</td>
<td>0.99</td>
</tr>
<tr>
<td>Largest fruit diameter</td>
<td>0.61</td>
<td>0.96</td>
<td>0.67</td>
<td>0.97</td>
</tr>
<tr>
<td>Smallest fruit diameter</td>
<td>0.61</td>
<td>0.96</td>
<td>0.67</td>
<td>0.97</td>
</tr>
<tr>
<td>Zucchini S/A¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fruits</td>
<td>0.74</td>
<td>0.99</td>
<td>0.76</td>
<td>0.99</td>
</tr>
<tr>
<td>Fruit mass</td>
<td>0.67</td>
<td>0.98</td>
<td>0.73</td>
<td>0.98</td>
</tr>
<tr>
<td>Fruit length</td>
<td>0.72</td>
<td>0.98</td>
<td>0.84</td>
<td>0.99</td>
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<tr>
<td>Largest fruit diameter</td>
<td>0.68</td>
<td>0.98</td>
<td>0.82</td>
<td>0.99</td>
</tr>
<tr>
<td>Smallest fruit diameter</td>
<td>0.68</td>
<td>0.98</td>
<td>0.79</td>
<td>0.99</td>
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<td>Cucumber</td>
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<tr>
<td>Number of fruits</td>
<td>0.65</td>
<td>0.95</td>
<td>0.66</td>
<td>0.95</td>
</tr>
<tr>
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<td>0.63</td>
<td>0.95</td>
<td>0.79</td>
<td>0.98</td>
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<tr>
<td>Pea</td>
<td></td>
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<tr>
<td>Number of pods</td>
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<td>0.49</td>
<td>0.54</td>
<td>0.78</td>
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<tr>
<td>Mass of pods</td>
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<tr>
<td>Pod length</td>
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<td>Number of grains</td>
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<td>0.64</td>
<td>0.47</td>
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<td>Mass of grains per pod</td>
<td>0.52</td>
<td>0.84</td>
<td>0.62</td>
<td>0.89</td>
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<tr>
<td>Pout pepper</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of fruits</td>
<td>0.52</td>
<td>0.94</td>
<td>0.72</td>
<td>0.98</td>
</tr>
<tr>
<td>Fruit mass</td>
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<td>0.93</td>
<td>0.71</td>
<td>0.97</td>
</tr>
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<td>0.68</td>
<td>0.97</td>
</tr>
<tr>
<td>Fruit length</td>
<td>0.56</td>
<td>0.95</td>
<td>0.57</td>
<td>0.95</td>
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</tbody>
</table>

¹ S/S: Spring/Summer growing season; S/A: Summer/Autumn growing season.

The repeatability and number of measurements for productive variables in vegetable crops experiments shows differences between the PCAcov and PCAcov methods. For the PCAcov method, the $\eta_m$ needed to evaluate the experiments were lower about PCAcov in all situations (Table 1). The greater the precision of the results sought by the researcher, the greater the number of measurements required. When seeking to obtain 100% accuracy of the results of the experiment, the number of measurements required is high and practically impossible to carry out in practice, due to the time, the human and financial resources they demand, which might make the execution of the experiment unfeasible.

For the S/S season in the experiment with zucchini the $\eta_m$ required for the evaluation of FL, LFD, SDF, FM, and NF variables are, respectively, approximately 4, 9, 9, 6, and 9 measurements by the PCAcov method. By the PCAcov method, approximately 8, 12, 12, 12, and 12 measurements are required, respectively, both estimated from R² of 0.95 (Figure 1).

For the S/A season in the experiment with zucchini the $\eta_m$ required to obtain 95% confidence level was 4, 4, 5, 7, and 6 measurements for the evaluation of the FL, LFD, SFD, FM, and NF variables by the PCAcov method, while for PCAcov method the number of measurements required was 7, 9, 9, 7, and 7, respectively (Figure 2).

For cucumber, with a 95% confidence level, the $\eta_m$ necessity to evaluate the variables FL, LFD, SFD, FM, and NF was 1, 6, 5, 4 and 10 measurements respectively by the PCAcov and 4, 11 method, 11, 8 and 10 measurements, respectively, for the same variables, using the PCAcov method (Figure 3).

In the culture of pea, due to the low values of the repeatability coefficient, the values $\eta_m$ were higher when compared with the other evaluated species. We observed that by the PCAcov method, the number of measurements necessary to assess PL, MGP, MP, NGP, and NP, with 95% confidence, is 8, 12, 8, 21, and 15 respectively. As for the PCAcov method, the $\eta_m$ increases it even more for the same variables, using the PCAcov method (Figure 3).

In the culture of pea, due to the low values of the repeatability coefficient, the values $\eta_m$ were higher when compared with the other evaluated species. We observed that by the PCAcov method, the number of measurements necessary to assess PL, MGP, MP, NGP, and NP, with 95% confidence, is 8, 12, 8, 21, and 15 respectively. As for the PCAcov method, the $\eta_m$ increases it even more for the same variables, using the PCAcov method (Figure 3).

Repeatability and number of measurements for productive variables in vegetable crops experiments character evaluation in the culture of Fragaria x ananassa, when grown on substrates. However, there are still few studies in the literature that provide this information on the number of measurements needed to evaluate variables in experiments with vegetable crops from multiple harvests with adequate experimental precision and accuracy.

Resende (2002) proposes the following classification for the repeatability coefficient: high repeatability (r≥0.60), medium repeatability (0.30<r<0.60), and low repeatability (r≤0.30). In the present work, the results of the repeatability coefficient can be considered high, except for the experiment with pea by the PCAcov method, which should be considered average.

The number of measurements required for evaluation ($\eta_m$) of agricultural experiments with the species analyzed in this study, based on the coefficient of determination (R²)
researcher chooses the 100% confidence level (Figure 4).

For pout pepper, using the PCAcov method, the ƞm evaluation of FL, FD, FM, and NF variables is 14, 9, 8, and 7, respectively. However, considering the estimated by PCAcor there increases considerably, mainly for the variables FL, FD, FM, and NF, being necessary 15, 13, 20, and 17 measurements for these variables FL, FD, FM, and NF respectively (Figure 5).

**Figure 1.** Number of measurements required for different coefficients of determination (R²) estimated by the methods of principal component analysis based on the correlation matrix (PCAcor) and the covariance matrix (PCAcov) for the variables fruit length (FL, cm), largest fruit diameter (LDF, cm), smallest fruit diameter (SDF, cm), fruit mass (FM, g/plant) and the number of fruits (NF, un.) in zucchini in spring-summer season. Santa Maria, UFSM, 2021.

**Figure 2.** Number of measurements required for different coefficients of determination (R²) estimated by methods of principal component analysis based on the correlation matrix (PCAcor) and the covariance matrix (PCAcov) for the variables fruit length (FL, cm), largest fruit diameter (LFD, cm), smallest fruit diameter (SFD, cm), fruit mass (FM, g/plant) and the number of fruits (NF) in zucchini in summer-autumn season. Santa Maria, UFSM, 2021.
According to Cruz et al. (2014), the higher the repeatability coefficient, the smaller the number of measurements needed to predict the real value of individuals. On the other hand, when the repeatability coefficient is low, it indicates that a large number of measurements is needed to predict the real effect of treatments (Della Bruna et al., 2012).

According to Manfio et al. (2011), the use of the principal component method allows isolating the effect of the alternation, which is not possible in the analysis of variance, where this component is included in the experimental error. Cruz et al. (2014) mention that, when the estimated
The use and indication of the number of measurements for variables in different cultures are important from the point of view of rationing resources, mainly labor in evaluations of agricultural experiments. Diel et al. (2020a) emphasized that, if the objective of the study is to differentiate cultivars, a smaller number of measurements can be performed. The same does not happen if the total fruit production in the cycle is to be determined, being important that the researcher previously determines his objectives before the beginning of the evaluations of the variables.

The number of measurements made by each researcher is distinct in different agricultural species. Pagoto et al. (2020), evaluating the efficiency of different forms of pollination for zucchini with or without fertilization with boron, when harvesting the fruits selected the commercial quality ones, and measured the diameter, length, and average mass of the fruits. According to the present study, these measures could have been carried out only four times for the diameter of the fruit, and nine times for the length and mass of the fruit, reducing the time, labor, and financial resources used in the evaluations.

For the culture of pout pepper, Diel et al. (2020b) evaluated the production of two cultivars in different growing seasons. The authors carried out 16 harvests for the first growing season, 12 harvests for the second growing season, and only six harvests for the third growing season, as this showed the death of plants due to frost. As the author’s objective was to indicate which cultivar is most productive and which is the time of best production, the measurements of the first and second seasons would already be more than necessary proposed by the present study (eight measurements for FM by the PCAcov method). In compensation, for the third season evaluated by the authors, the measures taken would not be enough for the treatments to show their full potential since only six harvests were carried out.

For the culture of pea, Zárate et al. (2012) carried out experiments with different treatments between plant spacing and ground cover with poultry litter, which consisted of evaluating the fresh and dry mass of pods, grains, number of pods, length, width and thickness of the pods. Based on the results of the present study, so that researchers could find significant differences between their treatments with precision and reliability of the results, they should perform 8, 12, 8, and 15 measurements, respectively, to assess PL, MGP, MP, and NP.

Sediyama et al. (2014) studied the types of pruning in cucumber, from the Aodai, Japonês, and Caipira groups, which underwent 12 harvests to detect differences in productivity between hybrids and choose the most productive in the different treatments. However, according to the present study, the researchers could have performed only eight harvests for FM to obtain differences between their treatments, thus reducing 33% of the number of evaluations performed.

These are some examples of experiments where the number of measurements is not previously considered before the beginning of the evaluations of the variables, further evidencing the importance of research to estimate the number of adequate measurements for each species and observed variables.

For vegetable crops with multiple...
harvests or when more than one measurement of a given character is needed in the same individual, these questions must be taken into account, allowing the researcher to promote studies with the greatest precision and in some cases, reduce the time, labor and waste of financial resources to carry out the evaluations of the variables in the experiment.

The repeatability coefficient estimated by the PCAcov method obtained the highest estimates for experiments with zucchini, cucumber, pout pepper and pea.

For the experiment with zucchini, in the S/S season, 4, 9, 8, and 7, respectively, and in experiments with pepper, the \( \eta_m \) necessity to evaluate the variables FL, LFD, SFD, FM, and NF, respectively. For experiments with cu, the \( \eta_m \) necessity to evaluate the variables FL, LFD, SFD, FM, and NF is 1, 6, 5, 4, and 10 measurements, respectively.

For pea, 8, 12, 8, 21, and 15 measurements are needed to evaluate the PL, MGP, MP, NGP, and NP, respectively, and in experiments with pout, the \( \eta_m \) necessity to evaluate the variables FL, FD, FM, and NF is 14, 9, 8 and 7, respectively.

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