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Optimum plot size and number of replications for experiments with the chickpea[1]

Tamanho ótimo de parcelas e número de repetições para experimentos com grão-de-bico

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ABSTRACT - In agricultural experimentation, use of the optimum plot size is an important way of increasing experimental precision; however, studies of this type are scarce for the chickpea, a legume that has been conquering both the market and consumers throughout the world. The aim of this study, therefore, was to determine the optimum plot size for evaluating experiments with the chickpea, in scenarios comprising combinations of the number of treatments, number of replications and levels of precision. Two blank experiments were carried out, comprising eight crop rows, 7 m in length, at a spacing of 50 cm between rows and 10 cm between plants; the six central rows were evaluated, giving a total of 60 plants per row. The index of soil heterogeneity was determined, and the optimum plot size was estimated using the Hatheway method in scenarios formed by a combination of *i* treatments (i = 4, 8, 12 and 16), *r* replications (r = 3, 4, 5, 6, 7 and 8) and levels of precision (LSD = 25%, 30% and 40%). The index of soil heterogeneity was greater than 0.7 for each of the variables under evaluation. The Hatheway method makes it possible to estimate different plot sizes based on the conditions and limitations of the experimental area. In experiments with the chickpea including 4 to 16 treatments, 25% LSD and six replications, plots of 25 basic units are sufficient to identify significant differences between the mean values of the treatments with a probability of 5%.

Key words: *Cicer arietinum* L. Agricultural experimentation. Blank experiment. Hatheway.

RESUMO - Na experimentação agrícola, a utilização do tamanho ótimo de parcela é uma estratégia importante, para aumentar a precisão experimental, e trabalhos desta natureza são escassos para a cultura do grão-de-bico, leguminosa que vem conquistando o mercado e consumidores em todo o mundo. Assim, o objetivo deste trabalho foi determinar o tamanho ótimo de parcela, para avaliar experimentos com a cultura do grão-de-bico, em cenários formados por combinações de números de tratamentos, números de repetições e níveis de precisão. Dois experimentos em branco foram realizados, compostos por oito linhas de cultivo, de 7 m de comprimento, espaçadas em 50 cm entre linhas e 10 cm entre plantas; foram avaliadas as seis linhas centrais, totalizando 60 plantas por linha de cultivo. Foi determinado o índice de heterogeneidade do solo e o tamanho ótimo de parcela foi estimado por meio do método de Hatheway, em cenários formados pelas combinações de i tratamentos (i = 4, 8, 12 e 16), r repetições (r = 3, 4, 5, 6, 7 e 8) e níveis de precisão (DMS = 25%, 30% e 40%). O índice de heterogeneidade do solo foi superior a 0,7 para todas as variáveis avaliadas. O método de Hatheway possibilita estimar diferentes tamanhos de parcelas, de acordo com as condições e limitações da área experimental. Em experimentos com o grão-de-bico, com 4 a 16 tratamentos, DMS de 25% e seis repetições, as parcelas de 25 unidades básicas são suficientes para identificar, com probabilidade de 5%, diferenças significativas entre as médias dos tratamentos.

Palavras-chave: *Cicer arietinum* L.. Experimentação agrícola. Experimento em branco. Hatheway.

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INTRODUCTION

The chickpea (*Cicer arietinum* L.) is a legume of global economic importance, the third most cultivated grain legume, with India the largest producer (FAOSTAT, 2020). In Brazil, the use of cultivars that are more adaptable to the local climate conditions has resulted in satisfactory productivity in agricultural experiments. The semi-arid region of Brazil has shown the potential for obtaining yields in the chickpea that are above the national average (AVELAR *et al*., 2018; FONSECA *et al*., 2020; HOSKEM *et al*., 2017; PEGORARO *et al*., 2018). However, the crop is still little studied, and there is a lack of research to identify the best type of management and the use of experimental techniques that would allow gains in experimental precision.

According to Storck *et al*. (2011), experimental precision is hampered by the heterogeneity of the experimental units, which is due to various factors such as variations in soil fertility, drainage, leveling, texture and soil structure, among others. In experimental planning, choosing the number of treatments, replications and plot size is an important step, as it contributes to reliable results and valid conclusions.

In blank experiments, the experimental area can be divided into plots of the smallest possible size, so that each is compatible with the treatments to be evaluated (STORCK *et al*., 2011). From the data collected in the plots, it is possible to estimate the coefficient of variation (CV) and the Smith index of soil heterogeneity (b) (1938). The index of soil heterogeneity usually varies between planting sites and sowing time, among other variables. As such, repeating the experiment adds greater reliability to the results.

The methodology proposed by Hatheway (1961) can be used to calculate the optimum plot size (X) by estimating the CV and the index of soil heterogeneity, taking into consideration the experimental design, number of treatments, number of replications and desired experimental accuracy. Once the experimental design and number of treatments have been established, the researcher can then choose the best combination of plot size, number of replications and level of experimental precision applicable to the available experimental area.

This methodology has been used to estimate the optimum plot size in various crops, among which we mention the velvet bean (CARGNELUTTI FILHO *et al*., 2014), black oats and common vetch (CARGNELUTTI FILHO *et al*., 2020a), and buckwheat (CARGNELUTTI FILHO *et al*., 2020b); however, studies on experimental design for characteristics of the chickpea are scarce in the literature.

The aim of this study, therefore, was to determine the optimum plot size for experiments with the chickpea in scenarios formed by a combination of the number of treatments, number of replications and levels of precision.

MATERIAL AND METHODS

Location and characterization of the experimental area

Two experiments were conducted, from May to September 2019, on the experimental farm of the Federal University of Minas Gerais, Montes Claros Campus, (16°40’59.15” S and 43°50’17.8” W). According to the Köppen classification, the climate is type Aw, tropical semi-arid (ALVARES *et al*., 2013). Soil samples were collected at a depth of 0-20 cm for a chemical and physical characterization of the experimental area before setting up the experiments, as per methodologies proposed by Teixeira *et al*. (2017).

The results for the granulometric composition of the soil were sand: 220 g kg^{-1}, silt: 460 g kg^{-1} and clay: 320 g kg^{-1}. For the chemical attributes, the results were organic matter (OM): 30.3 g kg^{-1}, pH ($H_2$0): 6.70, P (Mehlich-1): 13.74 mg dm^{-3}, K (Mehlich-1): 152 mg dm^{-3}, Ca: 7.85 cmol$_c$ dm^{-3}, Mg: 1.41 cmol$_c$ dm^{-3}, Al (KCl): 0.00 cmol$_c$ dm^{-3}, H+Al: 1.19 cmol$_c$ dm^{-3}, SB: 9.50 cmol$_c$ dm^{-3}, t: 9.50 cmol$_c$ dm^{-3}, T: 10.84 cmol$_c$ dm^{-3}, base saturation (V): 89.

Setting up the experiment

Two blank experiments (with no treatments) were conducted using a cultivar from the desi group, code CNPH 003, planted on two sowing dates, 15 May and 22 May 2019. Each experiment included eight crop rows, 7 m in length. The working area comprised the six central rows, disregarding 0.5 m from the ends of each row (border). The spacing between the rows was 0.5 m, with 0.1 m between plants.

The seeds were previously treated with Protreat fungicide (Carbendazim + Thiram), at a concentration of 5 mL kg^{-1} seeds. The experiments were set up in soils classified as haplic cambisols. Planting was carried out manually, placing two seeds per furrow. Thinning was performed 30 days after emergence, maintaining 10 plants linear m^{-1}.

Fertilizer was applied close to the crop row when planting, using 300 kg ha^{-1} simple superphosphate, 160 kg ha^{-1} potassium chloride and 300 kg ha^{-1} ammonium sulfate. Twenty-five days after emergence, 56 kg ha^{-1} ammonium sulfate were used as topdressing, as per Nascimento *et al*. (2016).

Phytosanitary treatments and irrigation were carried out based on the needs of the crop and the technical recommendations for the region (NASCIMENTO *et al.*, 2016). Irrigation was via a micro-sprinkler system, at an irrigation frequency of four days. Weeds were controlled manually when necessary.

Characteristics under evaluation

The plots were evaluated per basic unit (BU), comprising one plant, where the number of seeds (NS), seed weight (SW) and shoot dry mass (SDW) were evaluated. The NS was obtained by counting, the SW was obtained by drying the seeds in an oven at 105 °C for 24 hours to determine the moisture, which was then corrected to 13%. Finally, to obtain the SDW in g plant^{-1}, a forced air circulation oven was used at 65 °C to constant weight. All the evaluations were made at the end of the crop cycle, 120 days after sowing.

Statistical analysis

The experimental trials were simulated for an experiment in randomized blocks, comprising a combination of *i* treatments (i = 4, 8, 12 and 16), *r* replications (r = 3, 4, 5, 6, 7 and 8) and the least significant difference between the mean values of these treatments, which were detected as significant at 5% probability and expressed as a percentage (LSD = 25%, 30% and 40%). The statistical analysis was carried out using the R statistical software (R DEVELOPMENT CORE TEAM, 2019), employing the *ggplot2* (PEDERSON *et al*., 2020) and *directlabels* packages (HOCKING, 2021).

To determine the optimum plot size, the formula proposed by Hatheway (1961) was used, given by:

$$X_0 = \sqrt[b]{\frac{2(t_1 + t_2)^2 CV^2}{rLSD^2}} \quad (1)$$

where0: x_0 is the optimum plot size in BUs; *b* is the Smith index of soil heterogeneity (1938); t_1 is the critical value of Student's t-distribution for tests of significance (bilateral at 5%); t_2 is the critical value of Student's t-distribution, corresponding to 2(1-*p*), where *p* is the probability of obtaining a significant result, ($p = 0.80$ in this study), t_1 and t_2 with degrees of freedom (DF). The degrees of freedom (DF) were obtained from the expression: DF=(i-1)×(r-1) for a randomized block design, where *i* is the number of treatments and *r* is the number of replications.

For the present study, the values of t_1 and t_2 were obtained using the Microsoft Office Excel software, employing the following functions t_1=INVT(0.05;DF) and t_2=INVT(0.4;DF), respectively. CV is the coefficient of variation expressed as a percentage (%) for plots one basic unit (BU) in size; *r* is the number of replications and LSD is the least significant difference to be detected between the mean values of the treatments, expressed as a percentage (%).

The index of soil heterogeneity (b) was estimated after logarithmic transformation and linearization of the Smith equation (1938):

$$VU_X = \frac{V_1}{X^b} \quad (2)$$

i.e. by logarithmic transformation, $\log(VU_x) = \log(V_i) - b.\log(X)$, where VU(x) is the variance in BUs of plots comprising X BUs, V_1 is the variance of plots comprising one BU, and X is the number of BUs that make up the plot (plot size).

RESULTS AND DISCUSSION

The heat maps for number of seeds (NS), shoot dry weight (SDW) and seed weight (SW) were generated to verify the variability between them (Figure 1). The heat map showed no pattern in variability for any of the analyzed variables, presenting results with random variations. Similar behavior can be seen between the variables on the respective sowing dates.

In the two blank experiments, a reduction in variance can be seen per BU with the increase in plot size, which confirms the possibility of increasing the experimental precision using larger plots. This behavior was also seen by Cargnelutti Filho *et al.* (2014), Celanti *et al.* (2016) and Cargnelutti Filho *et al.* (2020a), in studies with the velvet bean, papaya and black oats, and common vetch, respectively. A significant reduction in variance was clearly seen up to 25 BUs, after which VU(x) tended to stabilize (Figure 2). The high values for the coefficient of determination (r^2) indicate that VU(x) can be adequately explained by BUs using the chosen function.

For NS, SDW and SW, the index of soil heterogeneity (b) was, respectively, 1.0407, 0.9993 and 0.9125 in the first experiment and 0.9592, 0.7574 and 1.2081 in the second experiment. Considering the Hatheway expression (1961), when fixing the values of the variables pertaining to the roots, and using the values found above for *b*, the optimum plot size (X) decreases in the following order: SW, SDW and NS, for Experiment I, and SDW, NS and SW for Experiment II.

The index of soil heterogeneity (b) was greater than 0.7 for each of the variables under analysis, and for two of the variables was greater than one unit. In the literature, estimates for the value of *b* greater than one are common (CARGNELUTTI FILHO *et al*., 2020a; LÚCIO *et al*., 2011; SANTOS *et al*., 2012; SOUSA; SILVA; ASSIS, 2016). Values of *b* close to 1 indicate high soil heterogeneity, revealing a lack of correlation between adjacent BUs.

Figure 1 - Heat maps for number of seeds (NS), shoot dry weight (SDW) and seed weight (SW) in the chickpea in two blank experiments (I and II)

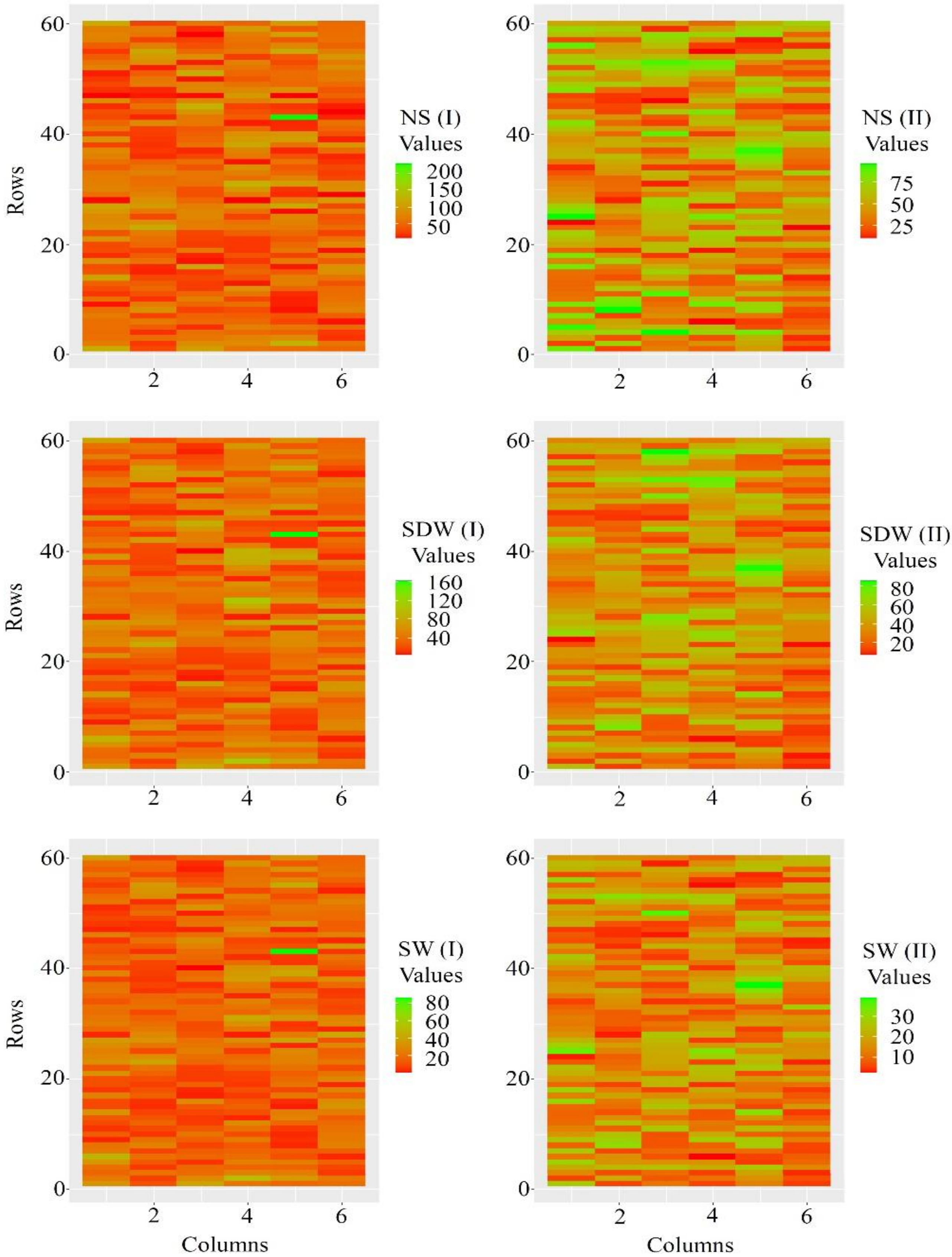
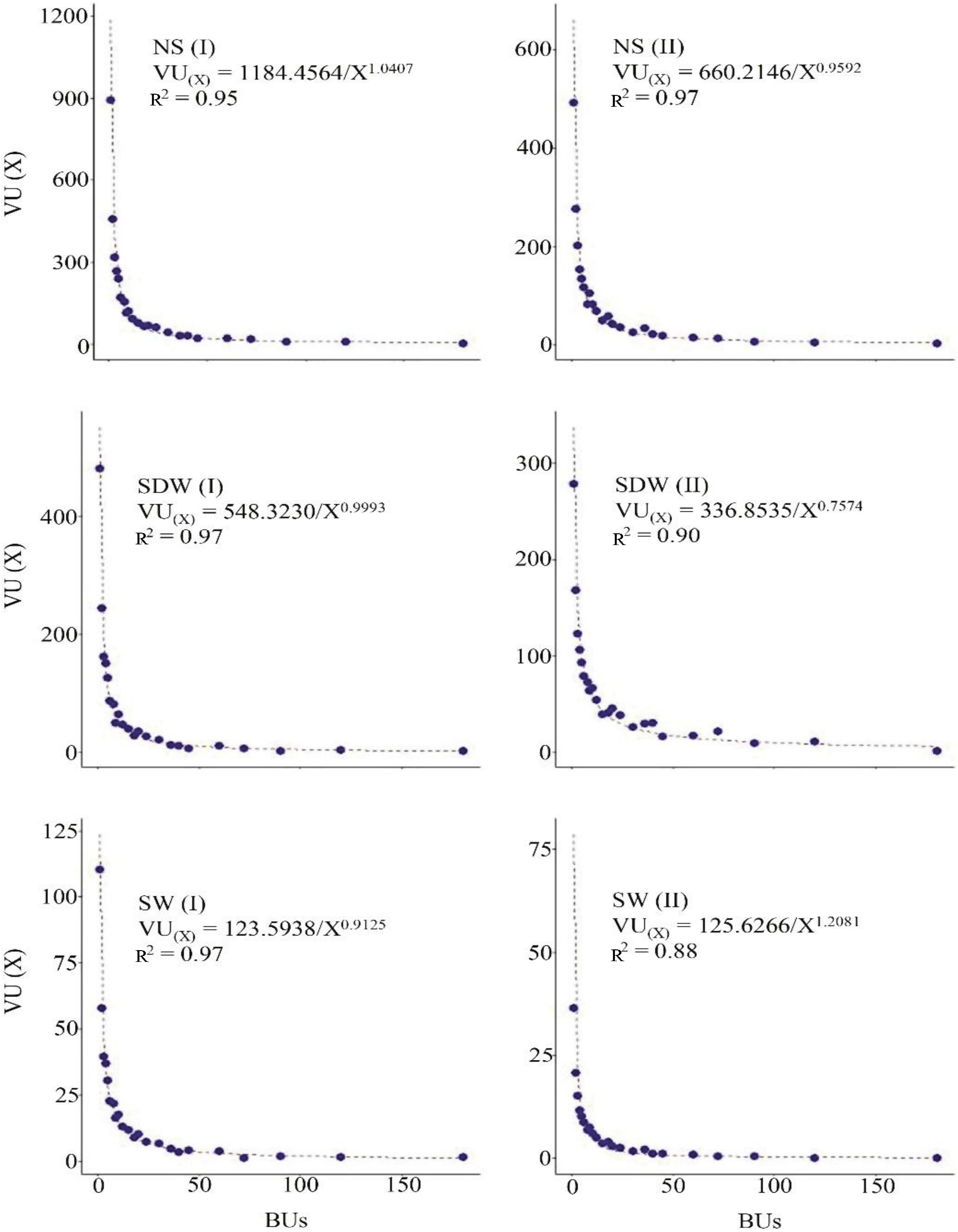


Figure 2 - Graphical representation of the relationship between variance per basic unit (BU), between plots of X BUs in size and the planned plot size in BUs, and estimates for the parameters of the Smith (1938) function $VU_X = V_1 / X^b$. Data for the variables number of seeds (NS), shoot dry weight (SDW) and seed weight (SW) in the chickpea in two blank experiments (I and II)



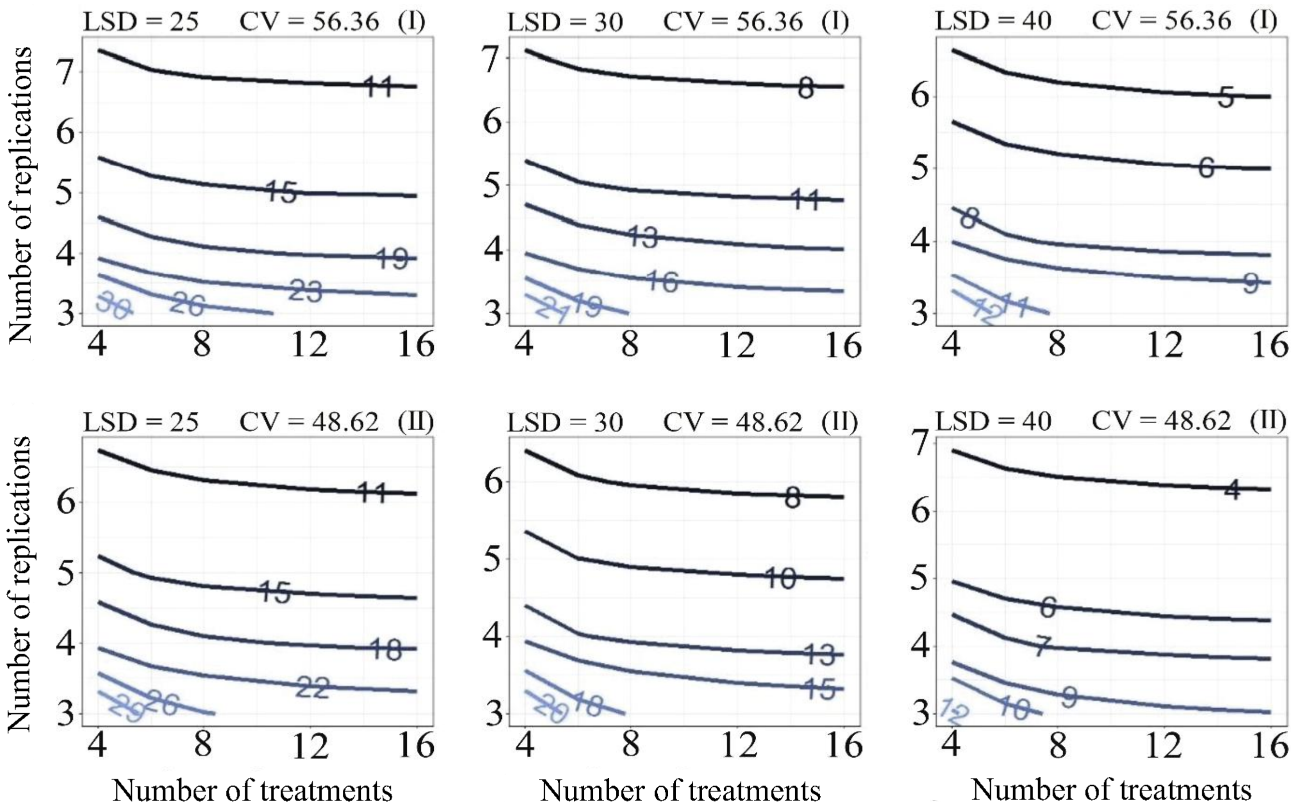
According to Lin and Binns (1986), for *b* less than 0.2, an increase in the number of replications is more efficient in improving experimental precision. When *b* ranges from 0.2 to 0.7, changes in plot size and in the number of replications can be used together to obtain gains in experimental precision; for *b* greater than 0.7, an increase in plot size is the most efficient method of improving precision. In this context, for the results obtained in this study, the best strategy for increasing the experimental precision is to increase the plot size.

In both experiments, the optimum plot size for NS varied according to the number of treatments, number of replications and least significant difference (Figure 3). In the second experiment, there was a reduction in the CV, but the indicated plot sizes were similar to those recommended in Experiment I. By fixing the number of treatments there is a reduction in plot size for an increase in the number of replications. Furthermore, with fixed values for the number of replications and the same LSD, there is a more modest reduction in plot size as the number of treatments increases (Figure 3).

The optimum plot size (X) estimated by the Hatheway method (1961) with a fixed number of treatments (i) and replications (r) decreases with a reduction in the desired precision (LSD). For example, if the researcher wishes to evaluate NS in an experiment with eight treatments and four replications, and a precision of LSD equal to 25%, the plot should comprise 19 plants in Experiment I and 18 plants in Experiment II. However, if less precision is desired when comparing the treatments (LSD = 40%), eight plants would be sufficient for Experiment I and seven plants for Experiment II.

This behavior was found by Celanti *et al*. (2016), Padrón, Lopes and Renedo (2018), and Sousa, Silva and Assis (2016), in studies with the papaya, pepper and sunflower, respectively. According to Storck *et al*. (2011), lower estimates for LSD ensure greater experimental precision and make it possible to identify small differences between the mean values of the treatments. However, working with low values for LSD (greater experimental precision) is often impractical due to the very large plot size. Therefore, for greater precision, using smaller plots together with a higher number of replications is often a more efficient way of using the experimental area (CARGNELUTTI FILHO *et al*., 2014; STORCK *et al*., 2011).

Figure 3 - Optimum plot size in basic units (BUs), for combinations of (i) treatments, (r) replications and (LSD) least significant difference, for the number of chickpea seeds in two blank experiments (I and II)



Cargnelutti Filho *et al*. (2020a) point out, however, that an increase in the number of replications will lead to a greater demand for labor, financial resources and time. If the characteristic is difficult to measure or takes a long time to evaluate, using a larger plot size and a smaller number of replications may be more advantageous, provided the experimental area can accommodate larger plots. Therefore, depending on the experimental area available, the number of treatments and the desired precision, the researcher can decide which combination of plot size and number of replications best applies to his experiment.

An increase in LSD leads to a reduction in plot size for shoot dry weight (Figure 4). For example, in the first experiment, for eight treatments and five replications, we have 18 plants per plot (LSD = 25%), 13 plants per plot (LSD = 30%) and seven plants per plot (LSD = 40%). In the second experiment, considering the same combinations, we have 27 plants per plot (LSD = 25%), 16 plants per plot (LSD = 30%) and eight plants per plot (LSD = 40%). Comparing the results of the two experiments, we find a lower CV for Experiment II and an increase in the proposed plot size. Considering the coefficient of variation separately, such a result was not expected, as high variability generally indicates larger plot sizes, however the Hatheway formula (1961) considers several other factors, and the low estimate for the heterogeneity index in Experiment II can explain this unexpected result.

In the second experiment, there is a reduction in CV compared to the first experiment, together with a reduction in the estimated optimum plot size (Figure 5). Plot size in the first experiment ranged from 59 BUs (i = 4, r = 3 and LSD = 25%) to eight BUs (i = 16, r = 6 and LSD = 40%). In the second experiment, the plot size varied between 16 BUs (i = 4, r = 3, LSD = 25%) and three BUs (i = 16, r = 7, LSD = 40%) (Figure 5).

The coefficient of variation went from 46.76% to 61.6%, which, among the variables under analysis are considered very high values according to the classification of Pimentel-Gomes (2009), where such high estimates can be explained as a result of evaluating individual plants. In addition, these results show variability between the BUs, an important characteristic in experiments of this nature, as it reflects actual conditions in the field (CARGNELUTTI FILHO *et al*., 2014). In the literature, when studying different crops, many studies have also found a CV greater than 40% in plots comprising one BU (LUCIO *et al*., 2011; OLIVEIRA *et al*., 2011; PADRÓN; LOPES; RENEDO, 2018).

Figure 4 - Optimum plot size in basic units (BUs), for combinations of (i) treatments, (r) replications and (LSD) least significant difference, for shoot dry weight in the chickpea in two blank experiments (I and II)

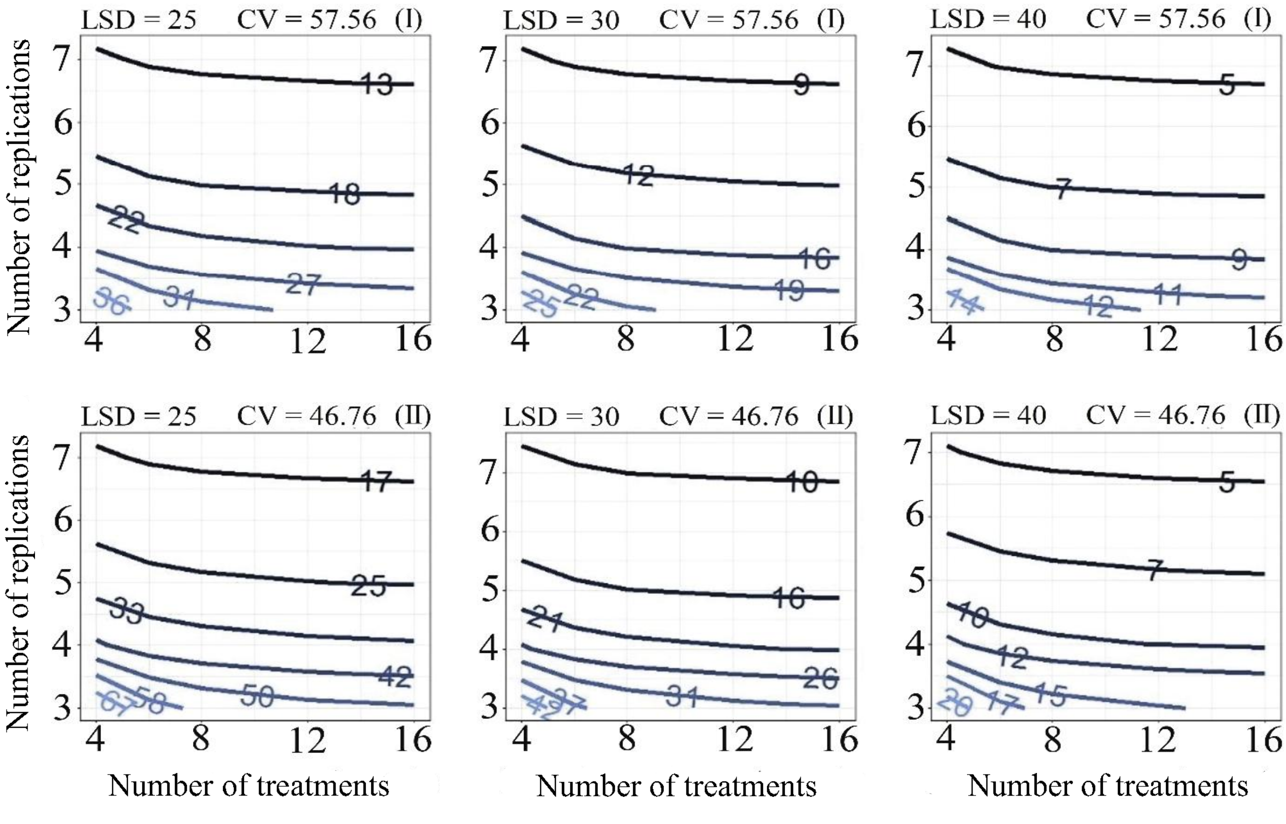
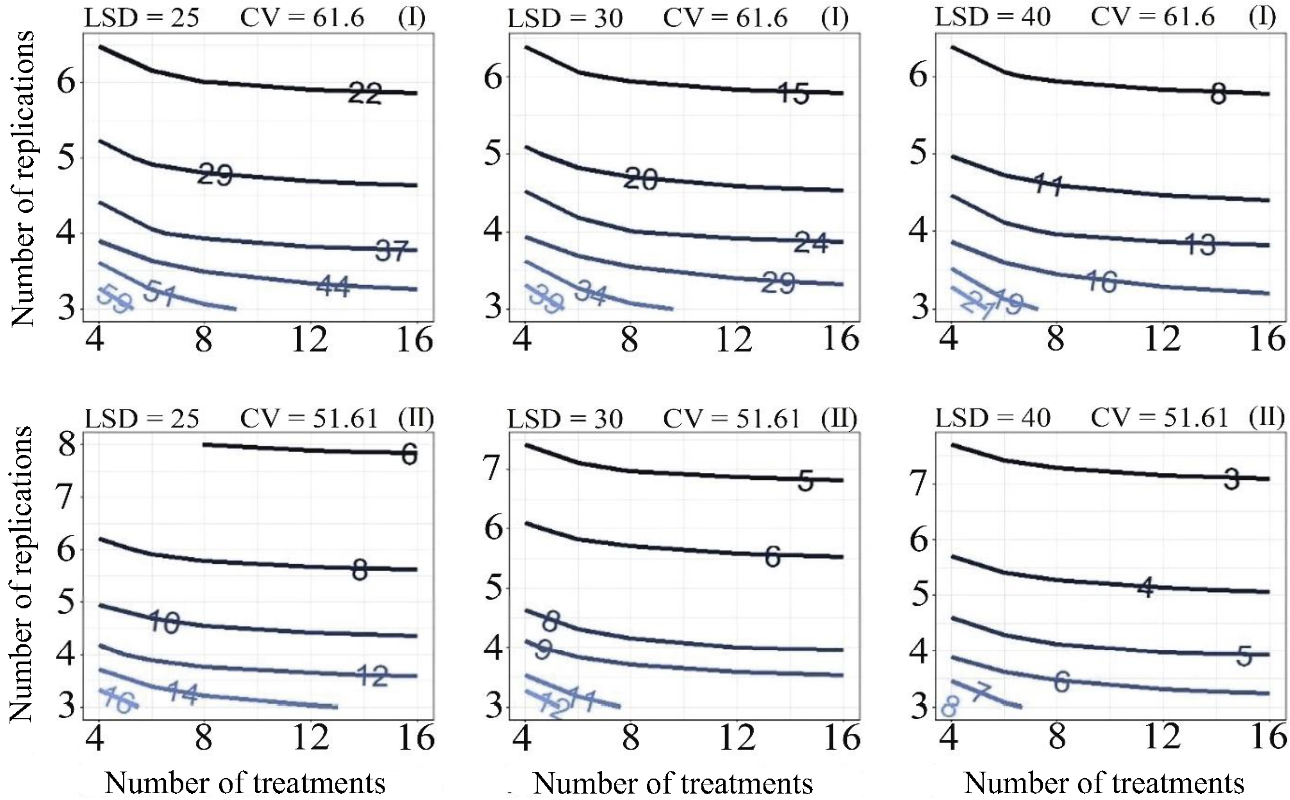


Figure 5 - Optimum plot size in basic units (BUs) for combinations of (i) treatments, (r) replications and (LSD) least significant difference, for seed weight in the chickpea in two blank experiments (I and II)



According to Nascimento *et al*. (2016), the number of pods can vary from a few to three hundred per plant. This high variability also explains the high values of the coefficient of variation for the variables seed number and weight. The high variability in shoot dry weight can be explained by the high number of evaluations that are necessary to obtain the end result.

There is no consensus among researchers concerning a definition of the optimum plot size for the chickpea in agricultural experiments, and different sizes and numbers of plants evaluated per plot were found for determining plant height: an area of 4.5 m², evaluating ten plants (ARTIAGA *et al*., 2015); an area of 5 m², evaluating ten central plants (ALMEIDA NETA *et al*., 2021); and an area of 15 m², evaluating five plants per plot (JOSHI *et al*., 2021). The number of BUs recommended in this study was higher than the number of plants evaluated in the above studies, which may mean a loss of experimental precision in those studies.

According to Cargnelutti Filho *et al*. (2020b), it is essential to determine the ideal plot size to ensure correct evaluation of the treatments and not compromise the reliability of the results. For the characteristics under evaluation, different measures of variability and, consequently, different estimates of plot size for the same experimental precision were also found between characteristics of the lettuce (LUCIO *et al*., 2011), velvet bean (CARGNELUTTI FILHO *et al*., 2014), bell pepper (PADRÓN; LOPES; RENEDO, 2018), forage palm (GUIMARÃES *et al*., 2020) and tomato (OLIVEIRA *et al*., 2021).

The information in this study includes a finite number of scenarios formed by combinations of *i* treatments (i = 4, 8, 12 and 16), *r* replications (r = 3, 4, 5, 6, 7 and 8) and least significant differences, detected as significant at 5% probability (LSD = 25%, 30% and 40%). However, for the three variables under evaluation, other scenarios can be simulated using the expression proposed by Hatheway (1961), based on the mean value of *b* and the mean value of CV. The following estimates can be used in the expression: NS (b = 0.9999 and CV = 52.49%), SDW (b = 0.8784 and CV 52.16%) and SW (b = 1.0603 and CV = 56.61%).

As an example in this context, to evaluate the NS for five treatments with four replications in a randomized block design and LSD equal to 25%, we have: $DF = (5-1)\times(4-1) = 12$, $t1 = INVT(0.05;12) = 2.178813$ and $t2 = INVT(0.40;12) = 0.872609$, therefore the optimum plot size will be: $X_0 = \sqrt[0.99995]{2(2.178813+0.872609)^2 \times 52.49^2 / 4 \times 25^2} = 20.52 BUs \cong 21$ plants per plot.

Considering the mean value of *b* and the mean value of CV for the entire experiment, we have the following estimates: CV = 53.75% and *b* = 0.979533. From these estimates, we can simulate the optimum plot size for five treatments, four replications in a randomized block design and LSD equal to 25%. Under these conditions, we have $GL = (5-1)\times(4-1) = 12$, $t1 = INVT(0.05;12) = 2.178813$ and $t2 = INVT(0.40;12) = 0.872609$, therefore the optimum plot size will be: $X_0 = \sqrt[0.979533]{2(2.178813+0.872609)^2 \times 53.75^2 / 4 \times 25^2} = 22.95 UBs \cong 23$ plants per plot.

Definition of the plot size and the number of replications in the chickpea is therefore up to the researcher and should be defined considering the availability of the experimental area and the number of treatments to be evaluated. However, it can generally be said, that for experiments in randomized blocks, plots comprising 25 plants are sufficient to identify significant differences between the treatments under evaluation, relating this information to the number of replications and treatments, and the precision possible in the experimental area.

CONCLUSION

In experiments with the chickpea including 4 to 16 treatments, 25% LSD and six replications, plots of 25 basic units are sufficient to identify significant differences between the mean values of the treatments with a probability of 5%.

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