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Optimum plot size for field experiments in sesame

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Abstract

This work aimed to determine the appropriate plot size for field experiments in sesame. We performed a complete randomized block design experiment, using 14 sesame varieties and four replicates. The plots were composed of four rows of 0.8 m long, spaced 0.6 m apart, and 0.1 m between plants. The useful plot area (0.72 m^2), which was the two central rows, was divided into 12 basic units with one plant (0.06 m^2) each. The measures of sesame production were taken from the useful plot area. The appropriate size of the experimental plot was estimated using the intraclass correlation coefficient method and calculated the detectable difference between treatments. The optimum plot size for evaluation of sesame seed yield was 0.18 m^2 (useful area), taking into account the one-row border on the sides. Gains in experimental precision (12%) were occurred with increments in plot size up to eight basic units (0.48 m^2), using five replicates and four or more varieties. The increase in the number of replicates and plot size was more efficient than the increase in varieties number to increase the experimental precision.

Key words: *Sesamum indicum* L, Intraclass correlation coefficient, Experimental precision, Experimental unit.
Abbreviations: ρ _intraclass correlation coefficient method; d_detectable difference between treatments.

Introduction

Sesame (*Sesamum indicum* L.) is the ninth most cultivated oilseed in the world and its cultivation has great economic potential, due to the possibilities of exploitation, both in the national and international market (Mesquita et al., 2013). World production is estimated at 3.16 million tons, obtained on eight million hectares, with a productivity of 481.4 kg ha^{-1}. Brazil is characterized as a small sesame producer with 15 thousand tons produced in an area of 25 thousand hectares and yields around 600.0 kg ha^{-1}, as it is planted in poor soils (Queiroga et al., 2007). After the fall in cotton production caused by the cotton boll weevil (*Anthonomus grandis*) cotton's breeding program is developing sesame studies to recommend varieties suitable for cultivation in the Northeast region (Queiroz and Beltrão, 2013). However, for a breeding program success, it is necessary to detect small variations among varieties during experiments, since the tendency is to decrease the difference among the new varieties. The challenge of breeders is to increase the experiment precision, allowing for genetic advances and, consequently, more productive and better quality materials (Silva, 2009). Thus, the execution of high precision experiments requires planning. Therefore, one of the fundamental questions is the appropriate size of the plot or experimental units.

Plot sizes tend to increase with the progress of the breeding program, whereas the more advanced populations need larger plot size for experiments. With the advancement of generations, there is a reduction in the variation between the selected materials, requiring a higher number of plants to detect variation and make the selection. When the increase of plot size does not result in more precision, additional increases in accuracy will be obtained with the use of more replicates (Cargnelutti Filho et al., 2012).

Several factors are involved in choosing the size and shape of experimental plots. Among them, soil heterogeneity is a crucial factor. Thus it is essential to have information about the area, in which the experiments will be carried out (Storck et al., 2016).

Several methods have been used to estimate the optimal plot size, such as the modified maximum curvature method and the linear model segmented with plateau (Ferreira, 2007), either from uniformity assays or experiments that include treatment effects. The intraclass correlation coefficient method stands out among the estimation methods that take advantage of experimental data from trials with treatments (Pimentel Gomes, 1984). This method was initially applied to trees, but the theory also applies to annual plants. In this method researcher considers single subplots or lines instead of trees, and the number of unit subplots may be pointed out from the intraclass correlation coefficient, giving the minimum variance of the average in a treatment.

The literature on plot size comprises many cultures and distinct situations. However, no studies were found on the subject for the sesame crops. This work aimed to define the ideal plot size for experiments in sesame using the method proposed by Pimentel Gomes (1984).

Results and discussion

The analysis of variance (Table 2) calculated the intraclass correlation coefficient ($\hat{\rho}$), and then we estimated the optimal number of useful basic units per plot (Table 3).

Significant reductions in d values (gains in experimental accuracy) was occurred with increases size of small plots (Table 4).

The mean square of residue between plots was higher than within the plots (Table 4), showing more variability between plots than among the basic units (BU) within the plot, which resulted in a positive and non-close to zero value ($\hat{\rho}$ = 0.4852) of intraclass correlation coefficient ($\hat{\rho}$) for the basic units within plot. This result reveals some correlation between the basic units within the plot, suggesting the use of reasonably small plots, in this case with 3.00 useful BU (0.18 m^2).

In a previous experiment, researchers used plots with a useful area of 2.00 m² to evaluate the growth and productivity of sesame (Mesquita et al., 2013). However, according to our results, the size of the plot could be significantly reduced without compromising the information obtained, since plots with 0.18 m² of useful area were satisfactory for evaluation of the production of sesame seeds.

When ρ≤ 0.15, the solutions are excellent, while low positive values (ρ≤ 0.15) may overestimate the number of useful plants per plot of optimum size. Then, one may need to estimate useful rows as well (Pimentel Gomes, 1988). Silva et al. (2003) confirmed the information above, when performed clonal tests of eucalyptus. They attributed the possibility of inconsistent values to the presence of a single coefficient (ρ), when plot effectively analyzed. Such procedure may underestimate useful plants in the plot, confusing the expected intraclass correlation coefficient in an experiment with a small number of trees and a different degree of competition concerning the plot used in the calculation of ρ.

On the other hand, the increase in accuracy was slight with the increase in areas of large plots. Our results corroborate with those obtained by several authors (Henriques Neto et al., 2004; Martin et al., 2004; Brum et al., 2008; Donato et al., 2008; Lúcio et al., 2011; Lúcio et al., 2012; Santos et al., 2012; Sousa et al., 2015).

The highest gains in experimental accuracy (reduction of d values) with increments in plot size were occurred up to 8 BU (0.48 m²). Differences around 20% between cultivar means can be detected using plots with 4 BU (0.24 m²), 5 replicates, and 4 cultivars; or plots with 6 BU (0.36 m²), 3 replications and 4 and 8 cultivars. Differences around 15% can be detected in plots with 4 BU (0.24 m²), 7 replicates, and 4 or more cultivars; plots with 6 BU (0.36 m²), 5 replicates, and 4 or more cultivars; 8 BU (0.48 m²), 3 replicates, and 8 or more cultivars; 10 BU (0.60 m²), 3 replicates, and 4 and 8 cultivars; or 12 BU (0.72) and 3 replicates.

The increase in number of replicates was more efficient in reducing the value of d (increase of the experimental accuracy) when compared to the addition in number of cultivars, a fact also shown by Storck, Bisognin and Oliveira (2006), Storck et al. (2007), Donato et al. (2008) and Sousa et al. (2015).

Materials and methods

Place of study

The data collection was occurred in an experiment carried out in the city of Barbalha, CE, located 415 meters high, with geographical coordinates 7°18'20"S and 39°18'9"W.

We developed an experiment in a randomized block design with 14 sesame cultivars and four replicates. The plots comprised of four rows of 0.8 meters in length each, with an area of 1.92 m² (2.4 m x 0.8 m). The row spacing was 0.6 m and between plants 0.1 m. The plot area consisted of two central rows, eliminating a plant from the ends, making up an area of 0.72 m² (1.2 m x 0.6 m). To collect data on the production of sesame seeds, we divided the plot area into 12 basic units, each consisting of one plant in the row, with an area of 0.06 m².

Based on Pimentel Gomes (1984), the following statistical model was assumed:

$$Y_{ijk} = m + c_i + b_j + e_{ij} + e_{ijk} \quad (1)$$

where k = number of samples (basic units) per plot, Y_{ijk} = the seed yield in the K basic unit, of the i cultivar, in j block; m = general average; c_i = effect of cultivar i (i = 1, 2, ..., I cultivars); b_j = effect of block j (j = 1, 2, ..., J blocks); e_{ij} = experimental error between plots; and e_{ijk} = experimental error between basic units within the plot (k = 1, 2, ..., K basic units per plot). From the statistical model, the analysis of variance was performed, considering the experimental error between plots (residue (a)) and between basic units within the plot (residue (b)) (Table 1).

[1]V_1 = mean square of residue between plots; V_2 = mean square of residue within the plot; $\sigma^2 =$ variance relative to the experimental error between the basic units within the plot; ρ= intraclass correlation coefficient due to the basic units within the plot.

From the analysis of variance (Table 1), the intraclass correlation coefficient was estimated equalizing the residual mean squares to the respective mathematical expectations, obtaining the following formula:

$$\hat{\rho} = \frac{V_1 - V_2}{V_1 + (K-1)V_2} \quad (2)$$

Where; $\hat{\rho}$= estimation of the intraclass correlation coefficient; V_1 = mean square of the residue between plots; V_2 = average square of the residue between basic units within the plot; and K = number of basic plot units (12 basic units).

Pimentel Gomes (1984) proposes to choose the optimal plot size from plots with K basic units, complete border, and a double line of useful plants. In this case, the number of

Table 1. Design of the analysis of variance with K basic units per plot and mathematical expectation of mean squares.

Sources of Variation	Degrees of Freedom	Mean Square (MS)	Expectation (MS)
Blocks	J-1		
Cultivars	I-1		
Residue (a)	(J-1)(I-1)	V1	$\sigma^2[1+(K-1)\rho]$
Residue (b)	JI(K-1)	V2	$\sigma^2(1-\rho)$

Table 2. Summary of analysis of variance of the production of sesame seeds.

Sources of Variation	Degrees of Freedom	Mean Squares
Blocks	3	232.66**
Cultivars	13	800.43**
Residue (a)	39	688.52
Residue (b)	616	55.94

[1]**: significant at 1% probability according to the F test.

Table 3. Optimum plot size in basic units (BU) for the production of sesame seeds, estimated by the intraclass correlation coefficient method[1].

Mean Square of Residual Between Parcels	Mean Square of Residual Inside Plot	Intraclass Correlation Coefficient ($\hat{\rho}$)	Optimum Number of Useful Basic Units per Plot (k)
688.52	55.94	0.4852	3.00

[1]Basic unit = 0.6 m x 0.1 m.

Table 4. Difference between averages of two cultivars (% of the mean) expected to be detected in the evaluation of the sesame production, considering different plot sizes (BU), cultivar numbers and replicates, estimated by the Hatheway method[1].

	Number of Cultivars											
	4			8			12			16		
BU	Number of replicates			Number of replicates			Number of replicates			Number of replicates		
	3	5	7	3	5	7	3	5	7	3	5	7
	Differences Between Cultivar Average in % of Mean (d)											
2	39.91	28.14	23.26	35.86	26.76	22.36	34.90	26.42	22.18	34.47	26.26	22.11
4	26.63	18.77	15.52	23.93	17.86	14.92	23.28	17.63	14.80	23.00	17.52	14.75
6	21.01	14.82	12.25	18.88	14.09	11.78	18.38	13.91	11.68	18.15	13.83	11.64
8	17.77	12.53	10.35	15.96	11.91	9.95	15.53	11.76	9.87	15.34	11.69	9.84
10	15.60	11.00	9.09	14.01	10.46	8.74	13.64	10.33	8.67	13.47	10.26	8.64
12	14.02	9.89	8.17	12.60	9.40	7.86	12.26	9.28	7.79	12.11	9.22	7.77

[1]BU (Basic Unit) = 0.06 m^2 (0.6 m x 0.1 m); b (coefficient of soil heterogeneity) = 1.1676; CV of plots with 1 BU of size = 21.85%.

useful basic units (k) was: a) If $\hat{\rho} \geq 0.50$, so k = 2; b) if $0 < \hat{\rho} < 0.50$, use the equation:

$$k = 2\sqrt{\frac{(1-\hat{\rho})}{\hat{\rho}}} \quad (3)$$

Where; k is a natural number or one of the values of k natural numbers even closer to the value of the root; c) when p < 0, k should be as large as possible, compatible with a reasonable number of degrees of freedom for the residue (usually at least 10 df).

The difference between two cultivar averages were expressed as a percentage of the mean expected to be detected was calculated using the Hatheway (1961) method:

$$d = \sqrt{\frac{2(t_1+t_2)^2 CV_1^{\,2}}{rX^b}} \quad (4)$$

Where; t_1 is the critical value of t in Student's distribution at the 5% level, t_2 is the t-value from the table at level 2(1-P), P the probability of obtaining a significant result (80%), CV_1 the coefficient of variation of plots with 1 BU (basic unit), r the number of repetitions, and b the coefficient of soil heterogeneity, obtained after the linearization of the equation of Smith (1938):

$$V_x = \frac{V_1}{X^b} \quad (5)$$

Where; V_x is the variance per unit area of plots made up of X BU of size, V_1 is the variance of the plots constituted of 1 BU, and X is the number of BU that make up the plot (plot size). The value of b and CV1 were estimated in each of the 56 plots (14 cultivars and four replicates), using the 12 BU of the plot area.

To estimate the detectable difference (d), we experimented with a randomized block design comprising 4, 8, 12, and 16 cultivars; 3, 5, and 7 replicates; and parcel sizes of 2, 4, 6, 8, 10, and 12 BU.

Conclusions

The optimum plot size for evaluating the yield of sesame seeds was 0.18 m² (useful area), considering a one-row border on the sides. This size is smaller than the one generally used in research with the sesame crop (2.00 m²).

The highest gains in experimental precision (12%) with increments in plot size was occurred up to 8 Basic Units (0.48 m²), using 5 replicates, and 4 or more cultivars.

The Increase in number of replicates and plot size were more efficient to increase experimental accuracy than the increase in the number of cultivars.

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