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Comparison of Methodologies to Determine the Optimum Plot Size for Okra Seedlings Evaluation

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Abstract

The production of okra using seedlings is a practice increasingly used by farmers. However, this system still lacks further research involving substrates, tray types, cell volume, pest control and disease. For this it is important to determine the optimum size of the plots, in order to reduce the experimental errors and the expenses with the experiment. The objective of this work was to determine the optimum plot size for experiments involving okra seedlings produced in Styrofoam trays of 128 cells using different methods. The methods were the maximum curvature, the maximum curvature with bootstrap simulation and the maximum curvature of the coefficient of variation. The evaluated characteristics were aerial part height, stem diameter, aerial dry matter, root dry matter, total dry matter and quality of seedlings as measured by Dickson quality index. The results showed that the optimum plot size is different between the evaluated characteristics and for characteristics there is no significant difference in the optimum plot size between the three different methods. The optimum size for evaluating okra seedlings produced in Styrofoam trays of 128 cells is 10 seedlings per plot and is indicated the use of the maximum curvature method using a bootstrap simulation.

Keywords: *Abelmoschus esculentus* cv. Santa Cruz 47, experimental planning, experimental precision, experimental design

1. Introduction

The okra (*Abelmoschus esculentus* L.) is a vegetable adapted to tropical and subtropical weather conditions being widely cultivated in Brazil, especially by small farmers and one of the most cultivated cultivar is the 'Santa Cruz' (Purquerio, Lago, & Passos, 2010). According to Paula Júnior and Venzon (2007) the favorable temperature range for okra planting varies from 18 °C to 35 °C, and temperatures below 18 °C compromise the development, delay the beginning of production and cause fruit abortion.

The planting of this crop can be done in the form of direct sowing in the plant beds (Meena, Dubey, Jain, Tiwari, & Negi, 2017) or seedlings previously produced in bags (Agwu & Ezigbo, 2005) or trays (Modolo, Tessarioli Neto, & Ortigozza, 2001; Benício, Reis, & Rodrigues, 2011). The production of okra seedlings in trays has been a good alternative for a reduction of costs with seeds, since it can reach 8 kg ha^{-1} of seeds in the field planting (Modolo et al., 2001). Besides other advantages of this type of production, according to Sousa, Lédo, and Silva (2007), seedlings are more uniform, there is greater control of diseases and pests, a high index of establishment in the field, economy with labor, substrate, pesticides, water, space, better planting scheduling, more comfortable, hygienic and safe working conditions, and improved final product.

Benicio et al. (2011) point out that although the production of okra from seedlings produced in trays is a reality the studies of this nature are still scarce. It is verified that there is no consensus among researchers regarding the number of plants to be used in each experimental plot, and, thus, in okra experiments conducted in trays the number of plants per plot has been variable, from eight (Modolo & Tessarioli Neto, 1999) up to 36 (Silva et al., 2013).

Any experiment conduction must begin with a good initial planning. In this planning, after the determination of the characteristics that will be studied and the design that is going to be adopted, the researcher will quantify how much material is necessary to carry out the test and then determine the size of each plot (Firmino, Cogo, Almeida, Campos, & Morais, 2012).

Although most of researchers still choose to determine the plot size in an arbitrarily way, however, the ideal is to make such a choice based on scientific criteria, which involve the use of uniformity tests, also called blank test. In these essays it is shown that there is a nonlinear relationship between experimental error and plot size (Smith, 1938; Meier & Lessman, 1971). Although the researcher wishes to reduce the experimental error increasing the plot size, this criterion should be used with caution, since given a certain increase in plot size the reduction of the experimental error is very low (Pimental-Gomes, 2009), considering that it is not linear (Sousa et al., 2018) leading to expenditures for experimental material and unnecessary physical space usage. Then, it is desirable to find the optimum plot size (Meier & Lessman, 1971; Paranaíba, Ferreira, & Morais, 2009; Celanti, Schmildt, Schmildt, Alexandre, & Cattaneo 2016a).

As for the method to determine the optimum plot size, there are in the literature more than a dozen proposals, and the most frequently used in Brazil with several crops are those of maximum modified curvature (Meier & Lessman, 1971) and maximum curvature of the coefficient of variation (Paranaíba et al., 2009). Recently, Celanti et al. (2016a) proposed the use of a bootstrap simulation to determine optimum plot size with the advantage of being fast for the analysis since the researcher does not need to identify the position of the plant in the blank test area.

Researches to determine optimum plot size has been done for coffee seedlings ‘Rubi’ (Cipriano, Cogo, Campos, & Almeida, 2012), coffee seedlings ‘Catuaí Amarelo 2SL’ (Firmino et al., 2012), papaya ‘Golden PC’ (Celanti et al., 2016a) and papaya ‘Baixinho de Santa Amália’ (Celanti, Schmildt, Alexandre, Cattaneo, & Schmildt, 2016b). For okra, however, no work was found in the literature about determining the optimum plot size in seedling production. In this way the objective of this work is to determine and compare the optimum plot size for experiments involving okra seedlings produced in trays using the methods by Meier and Lessman (1971), Paranaíba et al. (2009) and Meier and Lessman (1971) using bootstrap simulation, according to Celanti et al. (2016a).

2. **Methods**

The study was performed in a greenhouse, in a controlled environment, at the Federal University of Espírito Santo (UFES) at the north campus (CEUNES) at the city of São Mateus-ES between parallels 18°40′19.6″ south latitude and 39°51′23.7″ west longitude. The weather of the region according to the classification of Köppen is Aw (tropical humid) with rains in summer and dry winter (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2014).

The determination of the optimum plot size was done with okra (*Albelmoschus esculentus* L.) cv. Santa Cruz 47. The uniformity test was performed using Styrofoam tray containing 128 cells in a 16 × 8 array (Figure 1). Seeds were seeded on August 4, 2015, using three seeds per cell, and if more than one seed germinated was realized the thinning leaving only the most vigorous seedling. The substrate Bioplant® was used. The greenhouse used has a plastic transparent covered ceiling of 100 microns and 50% shading on the sides. The irrigation was performed by sprinkling once a day after 17:00 hours, irrigating about 10 ml of water per cell. The minimum mean and maximum mean temperature were of 21.1 °C and 29.4 °C, respectively, whereas the minimum relative and maximum relative humidity were of 56.9% and 96.5%, respectively.

Figure 1. Seedlings of okra on styrofoam tray at the E2 development stage

The seedlings were evaluated at the E5 development stage when 50% of the seedlings presented the beginning of the fourth final leaf, which occurred 30 days after sowing. The characteristics evaluated in each plant were: aerial part height (APH) determined with ruler graduated in centimeters measuring from the apex of the last leaf to soil distance; stem diameter (SD) obtained with digital caliper in millimeters; aerial dry matter (ADM) in grams; root dry matter (RDM) in grams; total dry matter (TDM) in grams; and Dickson quality index (DQI) given by the equation 1 according to Dickson, Leaf, and Hosner (1960).

$$DQI = TDM/[(APH/SD) + (ADM/RDM)] \tag{1}$$

Before determining the optimum plot size, the six characteristics were analyzed by the descriptive statistics of all 128 seedlings. In this way, the mean (m), the standard deviation (s), the variance (s^2) and the coefficient of variation (CV) were calculated.

For each of the six characteristics evaluated, the optimum plot size was determined by three methods: maximum curvature method, according to Meier and Lessman (1971); maximum curvature method of the coefficient of variation, according to Paranaíba et al. (2009); maximum curvature method, according to Meier and Lessman (1971) using bootstrap simulation, according to Celanti et al. (2016a).

For the determination of the optimum plot size by the Meier and Lessman's (1971) maximum curvature method the 128 seedlings contained in the tray (blank test) were structured in basic experimental units (BEU), and each BEU is consisted of one seedling. In each BEU, the evaluation of the six characteristics were carried out in the greenhouse. Then with the data available and with the exact identification of each seedling, the analysis was performed. For this, the BEU were grouped using number of exact dividing seedlings of the total number of the blank test, ranging between 1 to 64 BEU, constituting seven groups. For each specific group, all possibilities of grouping were verified by evaluating the characteristics of the seedlings within each of the eight lines (WL) of 16 seedlings or of the 16 seedlings between the eight rows (BL). Thus, the sizes of the groups (Xi) were in BEU: X1 = 1 (1WL × 1BL); X2 = 2 (1WL × 2BL, 2WL × 1BL); X4 = 4 (4WL × 1BL; 1WL × 4BL & 2WL × 2BL); X8 = 8 (8WL × 1BL; 1WL × 8BL; 4WL × 2BL & 2WL × 4BL); X16 = 16 (16WL × 1BL; 8WL × 2BL & 4WL × 4BL); X32 = 32 (16WL × 2BL; 8WL × 4BL & 4WL × 8BL); X64 = 64 (16WL × 4BL & 8WL × 8BL).

For each of the Xi BEU calculated: $m_{(X_i)}$, mean of the plots with Xi BEU of size; $V_{(X_i)}$ variance between plots with Xi BEU of size; $CV_{(X_i)}$ coefficient of variation between plots with Xi BEU of size; and $VU_{(X_i)} = V_{(X_i)}/X_i^2$ variance by BEU between plots of size Xi BEU. From the set of seven data of Xi and $CV_{(X_i)}$ the constant ($\hat{\beta}_0$) and the regression coefficient ($\hat{\beta}_1$) were estimated by logarithmizing the function $CV_{(X_i)} = \hat{\beta}_0 X_i^{-\hat{\beta}_1}$ $CV_{(X_i)}$ = weighted by the degrees of freedom associated with the number of cabling plots of size Xi BEU for each plot size planned in the uniformity test (Steel, Torrie, & Dickey, 1997). Using the values $\hat{\beta}_0$ and $\hat{\beta}_1$ the optimum plot size (X_0) was calculated by Meier and Lessman's (1971) maximum curvature method, according to Equation 2.

$$X_0 = \left[\hat{\beta}_0^2 \cdot \hat{\beta}_1^2 \cdot \left(2\hat{\beta}_1 + 1\right)/\hat{\beta}_1 + 2\right]^{\left(1/2+2\hat{\beta}_1\right)} \tag{2}$$

The coefficient of variation for the optimum sample size X0 was also determined, according to Equation 3.

$$CV_{X_0} = \left[100 \cdot \left(s/\sqrt{X_0}\right)\right]/m \tag{3}$$

To verify the influence degree of the seedlings on the others, for each characteristic, the heterogeneity index (b) was determined by logarithmizing of the function $VU_{(X_i)} = V_1/X_i^b$, according to Smith (1938).

The maximum curvature method, according to Meier and Lessman (1971), using simulation, differs from the original to make the groupings of the X_i BEU by bootstrap simulation with replacement (Efron, 1979), according to Celanti et al. (2016a). We symbolized this methodology by ML_{boot}, to differ of Meier and Lessman (1971) proposal, which named ML.

For the simulations, six sample size were planned (1; 2; 4; 8; 16; 32; and 64 BEU) for each characteristic. Then, for each planned sample size on each character, 2000 simulations were made, by means of resampling with replacement. For each simulated sample, the mean was estimated. Thus, for each sample size of each character, 2000 mean estimation (Ferreira, 2009) were obtained and used to obtain a coefficient of variation for each planned sample size. Other determinations are the same adopted in the method proposed by Meier and Lessman (1971).

For the optimum plot size calculation by maximum curvature method of the coefficient of variation, according to Paranaíba et al. (2009), the 128 seedlings of the blank test received sequential numeration from 1 to 128, and the six characteristics measured in these seedlings properly identified, 1 to 16 in the first row, 17 to 32 in the second row, and so on to the seedling 128 in the eighth row. From the values of these characteristics, the first order spatial autocorrelation coefficient estimation ($\hat{\rho}$) was determined, whose $\hat{\rho} = \sum_{i=2}^{128} E_i E_{i-1} / \sum_{i=1}^{127} E_i^2$ and $E_i = x_i - \bar{x}$, where, x_i is the observed value in the seedling i. Finally, the optimum plot size was determined, according to Equation 4.

$$X_0 = (10/m)\left[\sqrt[3]{2\left(1-\hat{\rho}^2\right)s^2 m}\right] \quad (4)$$

The coefficient of variation was also determined for the optimum plot size, according to Equation 5.

$$CV_{(X_0)} = 100\sqrt{\left(1-\hat{\rho}^2\right)s^2/m^2}/\sqrt{X_0} \quad (5)$$

The data were analyzed using computational resources from the software R (R Development Core Team, 2018), whose script for the determinations by bootstrap simulation is described by Celanti et al. (2016a).

3. Results and Discussion

The okra seedlings showed good development, with average height of 12 cm and stem diameter close to 2 mm (Table 1), whose values are equivalent to observed for other researchers (Benício et al., 2011; Silva et al., 2013) in experimentation involving okra seedlings. From 128 cells containing three seeds, four cells only did not show formation of seedling. For purpose of this research, the values of the missing cells were obtained by mean of the two neighboring seedlings in the row, as recommended by Brum, Brandelero, Vargas, Storck, and Zanini (2016). Therefore, for low frequency of fault and good development of seedlings, the data obtained are suitable for study proposed.

Table 1. Mean (m), standard deviation (s), variance (s^2) and coefficient of variation (CV) for six characteristics evaluated from 128 okra seedlings produced in Styrofoam trays

Characteristic	m	s	s^2	$CV_{(\%)}$
Aerial part height	12.3961	1.6038	2.5722	12.8805
Stem diameter	1.8086	0.3224	0.1039	17.4554
Aerial dry matter	0.0465	0.0250	6.25×10^{-4}	54.3645
Root dry matter	0.0262	0.0161	2.592×10^{-4}	61.7647
Total dry matter	0.0727	0.0275	7.56×10^{-4}	38.0228
Dickson quality index[(1)]	0.0078	0.0042	8.4×10^{-6}	56.0003

Note. [(1)] Dickson quality index (DQI), according to Dickson et al. (1960).

The variability was greater between the seedlings, measured by coefficient of variation (CV), for characteristics dry matter and Dickson quality index (DQI) (Table 1). Thus, it is expected that the optimum plot size is different for the six characteristics, and mainly, greater for the characteristics of greater variability, since the parameter CV and variance (s^2) are the main determinants of the optimum plot size by Meier and Lessman's (1971) and Paranaíba et al.'s (2009) method, respectively. Indeed, the optimum size (X_0) ranged from 4 plants per plot for aerial part height and stalk diameter to 10 plants per plot for root dry matter (Table 2). Different plot size for different characteristics were also detected to determine the optimum plot size in experiments with others researchers in others cultures (Cipriano et al., 2012; Firmino et al., 2012; Celanti et al., 2016a, 2016b).

Table 2. Estimated equation, determination coefficient (R^2), heterogeneity index (b), first order spatial autocorrelation coefficient ($\hat{\rho}$), optimum plot size (X_0) and coefficient of variation for X_0 (CV_{Xo}), using three methods for six characteristics to okra seedling produced in Styrofoam trays of 128 cells

Method[(1)]	Equation	R^2(%)	b[(2)]	$\hat{\rho}$	X_0	CV_{Xo}
Aerial part height						
ML	$\hat{Y} = 11.7320x^{-0.2670}$	97.00	0.5340	-	2.11	8.02
ML_{boot}	$\hat{Y} = 12.9600x^{-0.5009}$	98.89	1.0059	-	3.23	7.20
Paranaíba	-	-	-	0.2481	3.16	7.06
Stem diameter						
ML	$\hat{Y} = 17.7380x^{-0.4160}$	99.78	0.832	-	3.72	9.23
ML_{boot}	$\hat{Y} = 17.8888x^{-0.5012}$	99.86	1.0058	-	4.00	8.91
Paranaíba	-	-	-	0.0980	3.98	8.89
Aerial dry matter						
ML	$\hat{Y} = 54.6640x^{-0.6100}$	99.60	1.2200	-	8.40	18.55
ML_{boot}	$\hat{Y} = 54.0262x^{-0.5013}$	99.88	1.0062	-	8.36	18.61
Paranaíba	-	-	-	-0.1305	8.29	18.53
Root dry matter						
ML	$\hat{Y} = 60.0030x^{-0.4570}$	99.41	0.914	-	8.91	20.47
ML_{boot}	$\hat{Y} = 61.5114x^{-0.4986}$	99.88	1.0010	-	9.11	20.42
Paranaíba	-	-	-	-0.0218	9.12	20.40
Total dry matter						
ML	$\hat{Y} = 37.5590x^{-0.5610}$	99.87	1.122	-	6.63	14.56
ML_{boot}	$\hat{Y} = 37.4770x^{-0.4983}$	99.89	1.0013	-	6.55	14.77
Paranaíba	-	-	-	-0.0868	6.57	14.69
Dickson quality index (DQI) according to Dickson et al. (1960)						
ML	$\hat{Y} = 52.7250x^{-0.4030}$	98.11	0.806	-	7.98	19.98
ML_{boot}	$\hat{Y} = 54.3236x^{-0.5060}$	99.88	1.0058	-	8.30	18.79
Paranaíba	-	-	-	-0.0049	8.37	18.72

Note. [(1)] Methods: ML = maximum modified curvature, according to Meier and Lessman (1971); ML_{boot} = maximum modified curvature, according to Meier and Lessman (1971) using resampling bootstrap simulation with 2000 simulations; Paranaíba = maximum curvature method of the coefficient of variation, according to Paranaíba et al. (2009).

[(2)] b: Smith heterogeneity index (1938).

Whereas in the experimental evaluation of seedlings the interest is focused on characteristics of aerial part and root, optimum size is 10 seedlings per plot. This size is in accordance with experiments which used okra seedlings in trays carried out by Benício et al. (2011), Souza, Souza, Pires, Cordeiro, and Alves (2014), however differs from the plot size used for other researchers (Modolo & Tessarioli Neto, 1999; Silva et al., 2013).

In comparison between methods, for each characteristic, a good accordance is verified in the results of the optimum plot size and in the value of the coefficient of variation for the optimum size (CV_{Xo}) (Table 2). The CV_{Xo} to optimum plot size ranged from 8% for aerial part height to 20% for root dry matter. Similar results were verified by Brum et al. (2016) in the evaluation of two characteristics in broccoli using Paranaíba et al.'s (2009) method. Such a behavior is explained due to the fact that the CV_{Xo} is dependent on X_0, as can be seen in the equation 3 for Meier and Lessman's (1971) method, and equation 5, for Paranaíba et al.'s (2009) method.

To emphasize the importance of the results, the characteristic root dry matter is taken as basis, using the equation 5 and classification of CV according Pimentel-Gomes (2009) which considers as low values up to 10%, average values between 10 and 20%, high to values between 20% and 30% and very high values above 30%. For the optimal plot size (X_0) of 10 plants per plot, presented in Table 2, the coefficient of variation is 20% (CV_{Xo} =

20%), what is considered mean for experiments involving plants (Pimentel-Gomes, 2009). On the other hand, if 16 plants per plot were used, the coefficient of variation will be of 16%, occurring, therefore, a low gain in experimental precision, once it will continue being classified as CV mean, with needless spending of seedlings.

From this blank test realized that there is little correlation between the plots verified by the values of Smith (1938) heterogeneity coefficient close to unit, using plots grouping by Meier and Lessman's (1971) method. For this same method, using simulation, the values of b are equal to unit, as demonstrated by Celanti et al. (2016a). In practice, this means that the biometric values verified, for each characteristic, of the seedling contained in a Styrofoam tray cell, poor interfere in the biometric values of the neighboring seedlings. Such a behavior can be also evaluated by the values of the first order spatial autocorrelation coefficient ($\hat{\rho}$) by Paranaíba et al.'s (2009) method, which are close to zero. Indeed, as the seedlings are in separate cells, it is expected that the performance of one seedling does not influence the performance the others.

From the results presented in this work, encourage to use Meier and Lessman's (1971) method with bootstrap simulation, according to Celanti et al. (2016a), emphasized because of being, among the methods used, the one who is less costly due to the fact that the position identification of the seedlings in the trays is unnecessary. Celanti et al.'s (2016a) article describes the script to use the methodology in the software R.

5. Conclusion

The optimum plot size to evaluate characteristics of aerial part and root as well as the quality of okra seedlings produced in Styrofoam trays of 128 cells is of 10 seedlings per plot.

The maximum curvature method proposed by Meier and Lessman is indicated using bootstrap simulation.

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