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Optimal plot size in the evaluation of papaya scions: proposal and comparison of methods¹

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ABSTRACT

Evaluating the quality of scions is extremely important and it can be done by characteristics of shoots and roots. This experiment evaluated height of the aerial part, stem diameter, number of leaves, petiole length and length of roots of papaya seedlings. Analyses were performed from a blank trial with 240 seedlings of “Golden Pécíolo Curto”. The determination of the optimum plot size was done by applying the methods of maximum curvature, maximum curvature of coefficient of variation and a new proposed method, which incorporates the bootstrap resampling simulation to the maximum curvature method. According to the results obtained, five is the optimal number of seedlings of papaya “Golden Pécíolo Curto” per plot. The proposed method of bootstrap simulation with replacement provides optimal plot sizes equal or higher than the maximum curvature method and provides same plot size than maximum curvature method of the coefficient of variation.

Key words: *Carica papaya* L.; experimental design; experimental precision.

RESUMO

Tamanho ótimo de parcela na avaliação de mudas de mamoeiro: proposta e comparação de métodos

Em experimentos com mudas de mamoeiro é de extrema importância que se avalie a qualidade das mudas o que é feito por características da parte aérea e radicular. Foram avaliados altura da parte aérea, diâmetro do caule, número de folhas, comprimento do pecíolo e comprimento da maior raiz em plântulas de mamoeiro com objetivo de estimar o tamanho ótimo de parcelas. As análises foram feitas a partir de ensaio em branco com 240 plântulas de ‘Golden Pécíolo Curto’. A determinação do tamanho ótimo de parcela foi feita aplicando-se os métodos de máxima curvatura, da máxima curvatura do coeficiente de variação e um método proposto, que incorpora a simulação bootstrap de reamostragem ao método da máxima curvatura. O número ótimo de plantas por parcela para avaliação de mudas de mamoeiro ‘Golden Pécíolo Curto’ é de cinco. O método proposto de simulação bootstrap com reposição proporciona tamanhos ótimos de parcela iguais ou superiores ao método da máxima curvatura e, proporciona mesmo tamanho de parcela do método da máxima curvatura do coeficiente de variação.

Palavras-chave: *Carica papaya* L.; precisão experimental; planejamento experimental.

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INTRODUCTION

In every experiment, one of the main objectives is to reduce the error. In general, the experimental unit should be chosen to minimize the experimental error, which is the measure of variation that exists among observations of experimental units equally treated throughout the experiment (Steel *et al.*, 1997; Storck *et al.*, 2011).

Although one considers that the larger the plot size, the lower the experimental error and, consequently, the greater the accuracy of the experiment, this relationship is not linear (Smith, 1938; Paranaíba *et al.*, 2009; Barbin, 2013). The increase in the size of the plot initially leads to a decrease of the experimental error up to some extent, from which the precision gain is very small (Paranaíba *et al.*, 2009; Storck *et al.*, 2011).

An adequate experimental design involves the determination of the plot size and it will also depend on the crop, number of treatments, and environmental conditions of each experiment (Federer, 1977; Storck *et al.*, 2011).

Several methods have been reported in the literature for estimation of the size of the plot. The most commonly used method is the modified maximum curvature, according to Meier & Lessman (1971). Another method widely used in the last years is the method of maximum curvature of the coefficient of variation (Paranaíba *et al.*, 2009) which reduces the calculations to determine the optimum plot size as the great advantage over the previous methods. Even so, the blank trial is still needed, and in this experiment, the plants need to be set in rows, and evaluated in the exact sequence in which they are found, in order to estimate the coefficient of spatial autocorrelation of the first order.

Recently, Santos *et al.* (2012) and Storck *et al.* (2014) incorporated the bootstrap simulation to the method of maximum curvature of coefficient of variation proposed by Paranaíba *et al.* (2009) and, Brito *et al.* (2014) to the linear response plateau method. However, the incorporation of simulation to the Meier & Lessman (1971) method was not found in the literature.

To determine the optimum plot size in a simpler manner is expected since the formation of clusters would be made from the simulations. The issue on flaws in the final stand (Brum *et al.* 2016), which is common in experiments involving seedlings, could also be contoured from the use of bootstrap simulation.

In field experiments involving papaya crops, several useful plot sizes are found, arbitrarily set since there are no studies reporting which plot size should be used. There are reports of the use of only one plant per plot (Pratissoli *et al.*, 2007; Melo *et al.*, 2009) to 20 plants per plot (Vivas *et al.*, 2011). In trials with production of papaya seedlings in nursery, useful sizes of plots follow the same arbitrariness,

also due to the lack of studies on plot designs. Evaluation of experiments with plots sizes of four (Melo *et al.*, 2007), six (Sá *et al.*, 2013), 10 (Paixão *et al.*, 2012; Mengarda *et al.*, 2014) and 12 (Serrano *et al.*, 2010) seedlings per plot are reported.

The objective of this study was to comparatively determine the optimal size of plots for evaluation of papaya seedlings by the method of maximum curvature of Meier & Lessman (1971), by the method of maximum curvature of the coefficient of variation according to Paranaíba *et al.* (2009) and by a new method that incorporates the bootstrap simulation to the method of Meier & Lessman (1971).

MATERIAL AND METHODS

The data used in this study were obtained from a greenhouse at the Experimental Farm of CEUNES/UFES in São Mateus, state of Espírito Santo, between parallels 18°40'19.6" South latitude and 39°51'23.7" West longitude. The climate according to Köppen classification is Aw (tropical humid), with rains in summer and dry winter.

The optimal size of plots was determined using papaya seedlings (*Carica papaya* L.) cv. Golden Pécíolo Curto, whose seeds were obtained from the Caliman Agrícola S. A. company. The blank test was carried out using three black polyethylene trays containing 10x14 tubes of 50 cm³. The trays were allocated together to provide 14 rows of 30 tubes, totaling 420 tubes. All 420 tubes were sown in summer with a single seed, being utilized for evaluating only the seedlings in the eight central rows, corresponding to 240 seedlings. The tubes were filled with Bioplant[®] substrate, adding the slow release fertilizer Basacot mini 3M[®] at a dose of 10 g dm⁻³ substrate (Paixão *et al.*, 2012).

The characters evaluated 30 days after sowing were as follow: SH: seedling height - determined with the aid of a centimeter graduated ruler, by measuring the base of the stem to the apex of the last leaf; SD: stem diameter - obtained with a digital caliper (mm) measured in the middle region of the stem; NL: number of leaves - counting of full grown leaves; PL: petiole length - obtained by measuring with centimeter graduated ruler from the connection point in the plant to the insertion point on the leaf; and LLR: length of the longest root - determined by measuring from the base of the seedling to its end, with a centimeter graduated ruler.

By using the five characters, it was determined the optimum size of the plot using the following methods: maximum curvature method, according to Meier & Lessman (1971); method of maximum curvature of the coefficient of variation according to Paranaíba *et al.* (2009); maximum curvature method according to Meier & Lessman (1971) using bootstrap simulation, which is a proposal made in this work.

To determine the optimum plot size by the method of maximum curvature of Meier & Lessman (1971), 240 seedlings from the blank trial were structured in basic experimental units (BEU), where each BEU was composed of a seedling. The BEU were grouped using the exact dividing seedling number of the total number of seedlings from the blank trial, ranging from 1 BEU to 60 BEU, providing 12 clusters. For each specific cluster, all the possibilities of clustering composition were evaluated, characterizing different compositions (Table 1).

For each X_i BEU, it was calculated: $m(X_i)$, mean of the plots with X_i UEB in size; $V(X_i)$, variance among plots with X_i UEB in size; $CV_{(X_i)}$, coefficient of variation among plots with X_i UEB in size; and $VU_{(X_i)} = V_{(X_i)} / X_i^2$, variance per BEU among plots of X_i BEU in size. From the cluster of 12 data of X_i and $CV_{(X_i)}$ the constants $(\hat{\beta}_0)$ and the regression coefficient $(\hat{\beta}_1)$ were estimated by log transformation of the function $CV_{(X_i)} = \hat{\beta}_0 X_i^{-\hat{\beta}_1}$ weighing it by degrees of freedom associated to the number of applicable plots with X_i UEB in size for each size of the designed plot in the uniformity test (Steel *et al.*, 1997). Similarly, Smith's (1938) heterogeneity index (b) was estimated from the relationship between $VU_{(X_i)}$ and X_i . By using the values of $\hat{\beta}_0$ and $\hat{\beta}_1$ the optimal size of the plot was calculated given by $X_0ML = [\hat{\beta}_0^2 \hat{\beta}_1^2 (2\hat{\beta}_1 + 1) / \hat{\beta}_1 + 2]^{(1/2 + 2 + 2\hat{\beta}_1)}$.

For calculations of the optimal size of the plot by the method of maximum curvature of the coefficient of variation according to Paranaíba *et al.* (2008), the 240 seedlings of the blank trial received sequential numeration from 1 to 240, in which the five characteristics were measured in these appropriately identified seedlings. From the values of those characteristics, it was determined the sample mean (m), the sample variation (s^2) and the estimate of the coefficient of the spatial autocorrelation of the first order

($\hat{\rho}$), in which $\hat{\rho} = \sum_{i=2}^{240} E_i E_{i-1} / \sum_{i=1}^{240} E_i^2$ and $E_i = x_i - \bar{x}$ where x_i is the value observed in the plant i. It was also determined the coefficient of variation, which is given by $CV_{(X_i)}P = 100\sqrt{(1-\hat{\rho}^2)s^2/m^2} / \sqrt{X_i}$, where X_i indicates the number of BUE. Finally, it was determined the plot optimal size, given by $X_0P = (10/m) \left(\sqrt[3]{2(1-\hat{\rho}^2)s^2m} \right)$.

The proposed method is based on the maximum curvature method, according to Meier & Lessman (1971) with the proposed modification of clustering the X_i BEU (Table 1) by bootstrap simulation with replacement (Efron, 1979; Martinez & Louzada Neto, 2001).

For the simulations, 12 sample sizes were designed (1, 2, 3, 4, 5, 6, 10, 12, 15, 30 and 60 UEB) for each character. Then, for each designed sample size of each characteristic, 2,000 simulations were performed by resampling with replacement. For each simulated sample, the mean was estimated. Thus, for each sample size of each characteristic, 2,000 mean estimates were obtained (Ferreira, 2009) and from these, a coefficient of variation was obtained for each designed sample size, which we named $CV_{(X_i)}boot$.

From the set of 12 data of X_i and $CV_{(X_i)}boot$, the constant $\hat{\beta}_0$ and the regression coefficient $\hat{\beta}_1$ were estimated by log transformation of the function $CV_{(X_i)}boot = \hat{\beta}_0 X_i^{-\hat{\beta}_1}$. By using the values $\hat{\beta}_0$ and $\hat{\beta}_1$, the optimum plot size was calculated given by $X_0MLboot = [\hat{\beta}_0^2 \hat{\beta}_1^2 (2\hat{\beta}_1 + 1) / \hat{\beta}_1 + 2]^{(1/2 + 2\hat{\beta}_1)}$. The heterogeneity index (b) was calculated by log transformation of the function $VU_{(X_i)}boot = V_i / X_i^b$ according to Smith (1938).

Performance of each of the three methods was demonstrated graphically by the relationship between the coefficients of variation and the number of BEU and the presentation of the optimum plot size. Data were analyzed

Table 1: Size of the designed plot ($X_i = WR \times BR$), in basic experimental units (BEU), plot shape (WR = within the row; BR = between rows) and total number of plots for the several clustering in blank trial of papaya seedlings (*Carica papaya* L.) cv. Golden Peciolo Curto, in a greenhouse in the municipality of São Mateus, ES

Clustering	Size (X_i)	Shape (WRxBR)	Number of plots
1	1	(1x1)	240
2	2	(1x2), (2x1)	120
3	3	(3x1)	80
4	4	(4x1), (1x4), (2x2)	60
5	5	(5x1)	48
6	6	(6x1), (3x2)	40
7	10	(10x1), (5x2)	24
8	12	(6x2), (3x4)	20
9	15	(15x1)	16
10	20	(10x2), (5x4)	12
11	30	(30x1), (15x2)	8
12	60	(30x2), (15x4)	4

using the computational resources of the R software (R Development Core Team, 2014). Because it is a discrete random variable, the optimum plot size was presented by a full number, rounding up to the higher entire number.

Procedure for determining $CV_{(x_i)}$ and $VU_{(x_i)}$ from bootstrap simulation in software R is described in script below for the designed plots with one and two seedlings from the “golden peciolo curto” file for the characteristic seedling height (SH). For other designed plot sizes (3; 4; 5; 6; 10; 12; 15; 20; 30; 60), it proceeds in a similar manner.

```
X<-read.table("e:\\data\\golden peciolo curto.txt",
header=T) # data import
```

```
R = 2000 # number of resamplings
```

```
boot.means = numeric(R)
```

```
for (i in 1:R) { boot.sample = sample(X$AP, 1, replace=T)
```

```
boot.means[i] = mean(boot.sample) }
```

```
m1<-mean(boot.means) #mean
```

```
d1<-sd(boot.means) # standard deviation
```

```
cv1 =(d1*100)/m1 # coefficient of variation
```

```
v1<-d1^2 # variance
```

```
vu1<-v1/1^2 # variance per beu
```

```
R = 2000 # number of resamplings
```

```
boot.means = numeric(R)
```

```
for (i in 1:R) { boot.sample = sample(X$AP, 2, replace=T)
```

```
boot.means[i] = mean(boot.sample) }
```

```
m2<-mean(boot.means) #mean
```

```
d2<-sd(boot.means) # standard deviation
```

```
cv2 =(d2*100)/m2 # coefficient of variation
```

```
v2<-(2*d2)^2 # variance
```

```
vu2<-v2/2^2 # variance per beu
```

RESULTS AND DISCUSSION

The results of the analysis of the coefficients of variation in function of the different plot sizes, measured by the number of basic experimental units (BEU) from 240 seedlings for height, stem diameter, number of leaves, petiole length and length of the longest root in papaya seedlings (*Carica papaya* L.) cv. Golden Peciolo Curto are shown in Figures 1 to 5, respectively. For all 15 adjusted curves (three methods x five characters), a decrease in the coefficient of variation decreased as the size of the plot increased, a result expected from the statistical point of view (Barbin, 2013). It is noteworthy that, although the method of maximum curvature of the coefficient of variation according to Paranaíba *et al.* (2009) does not require BEU clustering to estimate the optimum plot size, the method allows the determination of the coefficient of variation according to the different sizes of the plots, and therefore

it also provides a graphic representation and adjustment of a power model nonlinear regression.

It is observed that the optimum plot size estimated was four seedlings per plot when using seedling height (Figure 1) and stem diameter (Figure 2) and five seedlings per plot when using number of leaves (Figure 3), petiole length (Figure 4), and length of the longest root (Figure 5). Thus, it is pointed the number of five seedlings per plot as optimum size for ‘Golden Peciolo Curto’. Different estimates of sample sizes for different characters of the same plants were also detected in the production of coffee seedlings “Catuaí Amarelo” (Firmino *et al.*, 2012) and coffee “Rubi” (Cipriano *et al.*, 2012). In papaya, with the lack of scientific

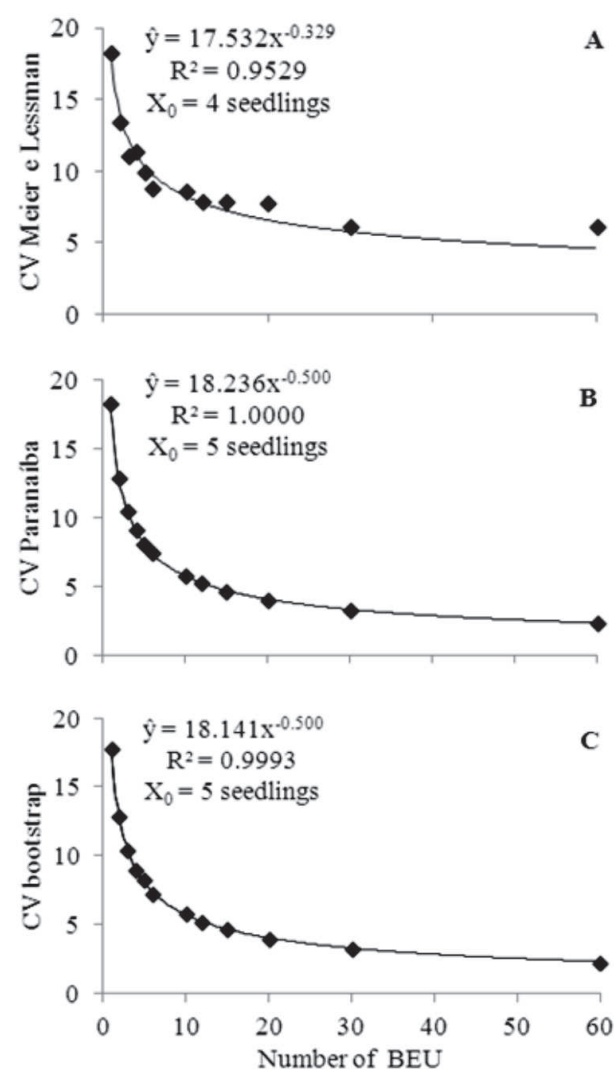


Figure 1: Relationship between coefficient of variation (CV) and size of the designed plot, in UEB and estimates of the optimum size of the plot (X_0) by three methods using the trait height of seedlings of papaya (*Carica papaya* L.) cv. Golden Peciolo Curto in a trial for uniformity with 240 BEU. Methods: A – maximum curvature according to Meier and Lessman (1971); B – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); C – maximum curvature according to Meier and Lessman (1971) using bootstrap simulation.

results on optimum plot size in the production of seedlings, we verified great variation since experiments in which plots were used with four seedlings (Melo *et al.*, 2007) up experiments whose parcels contained 12 seedlings (Serrano *et al.*, 2010). The use of appropriate plot size in the experiments is crucial for reducing experimental error and a consequent increase in experimental precision (Catapatti *et al.*, 2008). Therefore, the researcher who is using more plants in the plot than the recommended may be spending more than necessary for his or her experimentation with technical, physical or financial resources.

When the three methods for determining the sample size are compared, it can be seen that the coefficient values of $\hat{\beta}_0$ and $\hat{\beta}_1$ and the optimum plot size are similar among

the methods of maximum curvature of coefficient of variation and the maximum curvature method with bootstrap simulation to SH (figures 1B, 1C), SD (figures 2B, 2C), NL (figures 3B, 3C), PL (figures 4B, 4C) and LLR (figures 5B, 5C), and the values of the coefficient get closer to 0.5. Thus, it is clear that the bootstrap simulation with replacement leads to similar results to the method of maximum curvature of coefficient of variation presented by Paranaíba *et al.* (2009), and with the advantage of not needing to identify the sequence of plots in the uniformity test since the bootstrap simulation the drawing is at random.

The means of the characters are presented in Table 2, where it is observed that the mean of the 2,000 estimates

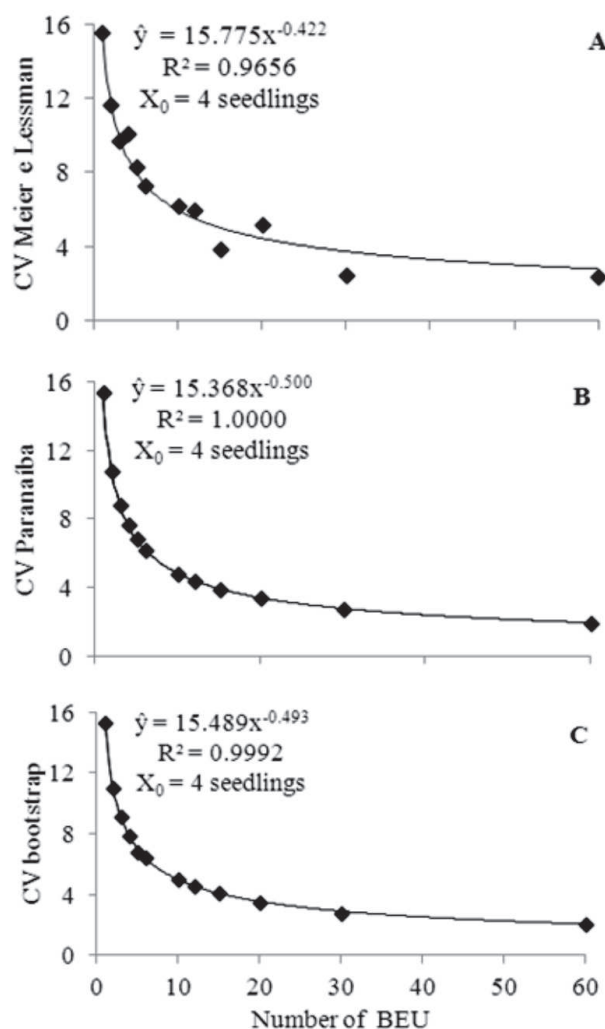


Figure 2: Relationship between coefficient of variation (CV) and size of the designed plot, in BEU and estimates of the optimum size of the plot (X_0) by three methods using the trait stem diameter of papaya seedlings (*Carica papaya* L.) cv. Golden Peciolo Curto in a trial for uniformity with 240 UEB. Methods: A – maximum curvature according to Meier and Lessman (1971); B – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); C – maximum curvature according to Meier and Lessman (1971) using bootstrap simulation.

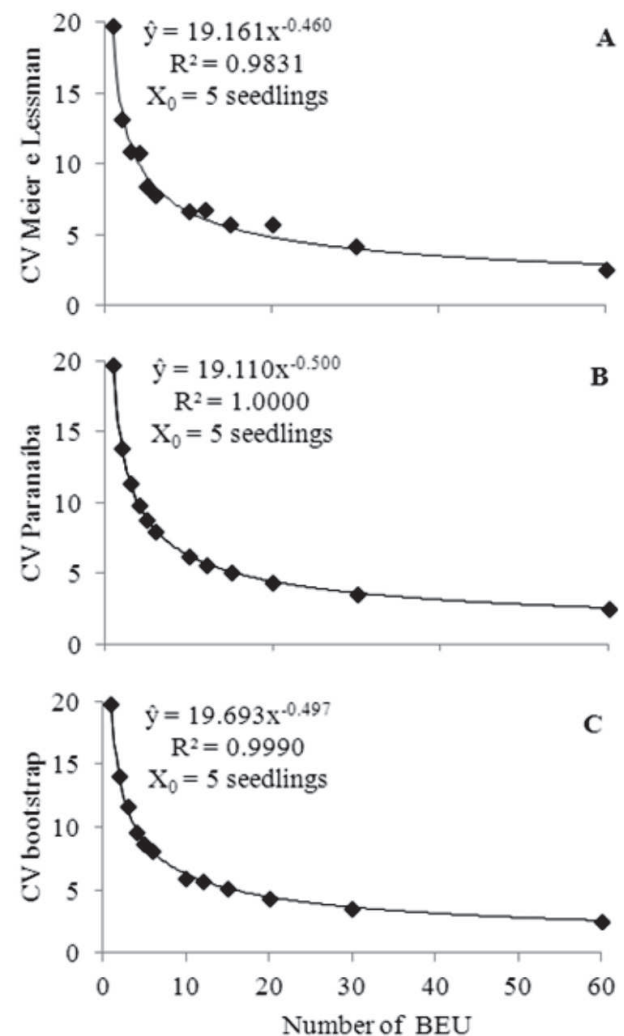


Figure 3: Relationship between coefficient of variation (CV) and size of the designed plot, in BEU and estimates of the optimum size of the plot (X_0) by three methods using the trait number of leaves papaya tree plantlet (*Carica papaya* L.) cv. Golden Peciolo Curto in a trial for uniformity with 240 UEB. Methods: A – maximum curvature according to Meier and Lessman (1971); B – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); C – maximum curvature according to Meier and Lessman (1971) using bootstrap simulation.

by bootstrap present values very close to the real values presented in the methods of Meier & Lessman (1971) and Paranaíba *et al.* (2009). This is because the resampling is done thousands of times and the bootstrap technique with replacement allows the same probability of drawing to all values of the sample (Ferreira, 2009).

The values of the coefficient of variation in the evaluated characters also present similarity among each other in the comparison of three methodologies. It is noteworthy that, for the calculation of this statistic by the method proposed by Paranaíba *et al.* (2009), the numerator of the equation $CV_{(X_i)}P = 100\sqrt{(1-\hat{\rho}^2)s^2/m^2}/\sqrt{X_i}$ contains the spatial autocorrelation coefficient of the first

order ($\hat{\rho}$), which ranges from -1 to +1. Algebraically, when the CV method proposed by Paranaíba *et al.* (2009) will have a value close to the CV of the method by Meier & Lessman (1971), as it can be observed for the five characters (Table 2). The autocorrelation close to zero indicates random distribution between the seedlings, which is what happened to the five evaluated characters, which can be explained by the fact that each seedling is contained in a different tube.

It can be seen in Table 2 that the heterogeneity index of Smith (1938), (b) is as twice as the value of the coefficient (Figures 1A, 2A, 3A, 4A, 5A) estimated in the equation that determines the optimum plot size by

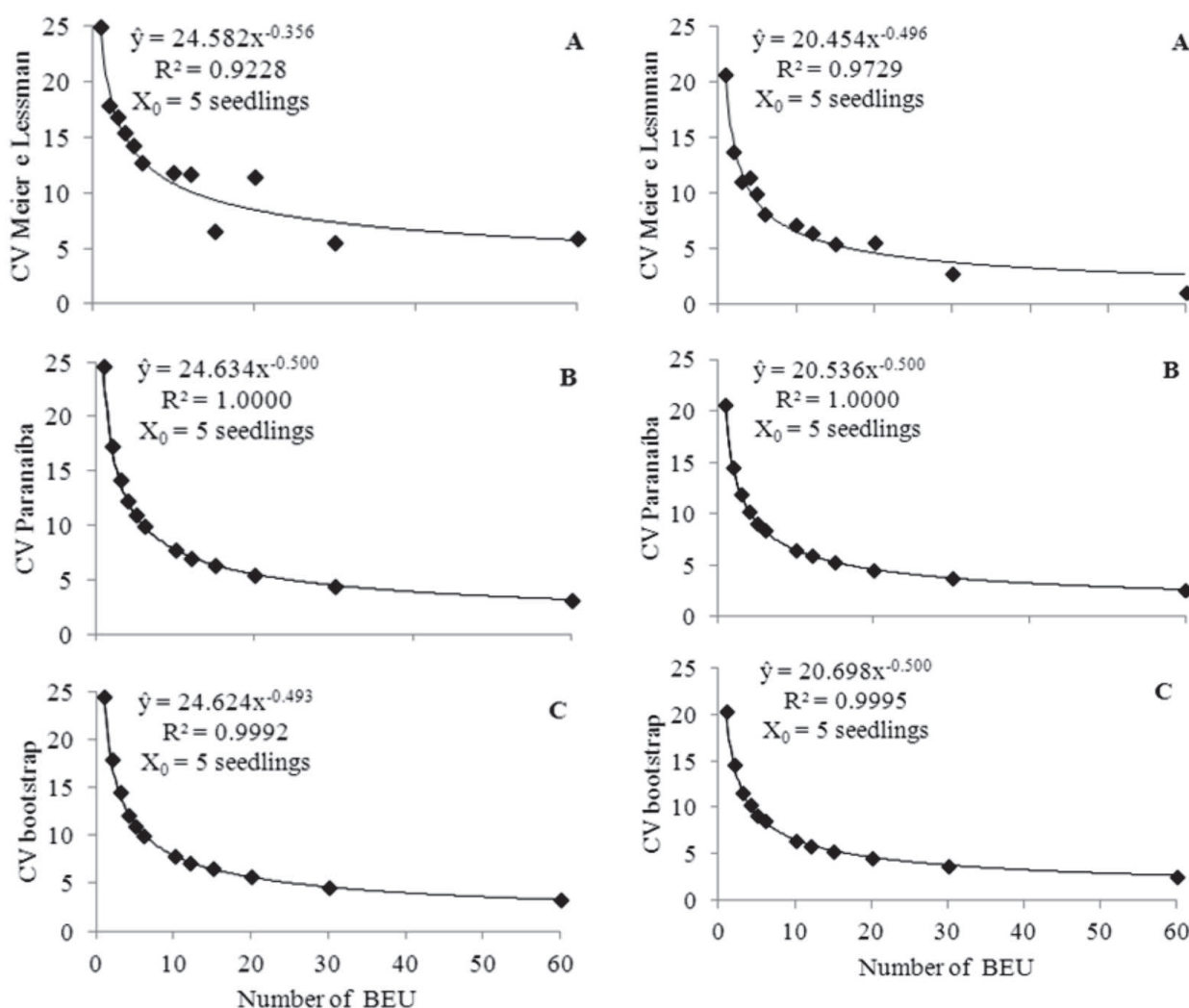


Figure 4: Relationship between coefficient of variation (CV) and size of the designed plot, in BEU and estimates of the optimum size of the plot (X_0) by three methods using the trait length of petiole of papaya seedlings (*Carica papaya* L.) cv. Golden Pecíolo Curto in a trial for uniformity with 240 BEU. Methods: A – maximum curvature according to Meier and Lessman (1971); B – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); C – maximum curvature according to Meier and Lessman (1971) using bootstrap simulation.

Figure 5: Relationship between coefficient of variation (CV) and size of designed plot, in BEU and estimates of the optimum plot size (X_0) by means of three methods, using the trait length of the longest root of papaya tree plantlet (*Carica papaya* L.) cv. Golden Pecíolo Curto in a uniformity trial with 240 BEU. Methods: A – maximum curvature according to Meier and Lessman (1971); B – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); C – maximum curvature according to Meier and Lessman (1971) using bootstrap simulation.

Table 2: Estimates of means (m), coefficient of variation (CV), spatial autocorrelation coefficient of the first order and heterogeneity index (b) obtained by different methods using height (SH), stem diameter (SD), number of leaves (NL), petiole length (PL) and length of the longest root (LLR) of 240 seedlings of papaya (*Carica papaya* L.) cv. Golden Peciolo Curto produced in a greenhouse

Statistics	Methods ⁽³⁾	Characters				
		SH	SD	NL	PL	LLR
m ⁽¹⁾	ML	9.92	2.57	4.53	2.41	12.88
	P	9.92	2.57	4.53	2.41	12.88
	MLboot	9.97	2.56	4.54	2.42	12.82
CV ⁽¹⁾	ML	18.25	15.52	19.76	24.95	20.59
	P	18.23	15.36	19.72	24.64	20.55
	MLboot	18.18	15.29	19.58	24.57	20.38
$\hat{\rho}$	P	0.0401	0.1414	-0.0627	0.1563	-0.0555
b ⁽²⁾	ML	0.655	0.840	0.912	0.708	0.976
	MLboot	1.007	1.010	0.991	0.995	1.011

⁽¹⁾ Statistics obtained from a total of 240 seedlings individually assessed.

⁽²⁾ b was obtained by the method of Smith (1938).

⁽³⁾ Method: ML – maximum curvature according to Meier and Lessman (1971); P – maximum curvature of coefficient of variation according to Paranaíba *et al.* (2009); MLboot – maximum curvature methods, according to Meier and Lessman (1971) by using bootstrap simulation.

using the modified maximum curvature method (Meier & Lessman, 1971). This relationship, also was reported by Lorentz *et al.* (2012), and it can also be verified in the proposed method; however, the values are close to 0.500 and the b values are close to 1.000. Considering that Smith's b values (1938) range from zero to one and that values closer to one indicate heterogeneity in crop environment, it is clear that the method proposed by bootstrap simulation is valuing the maximum of heterogeneity. Santos *et al.* (2012) report that when heterogeneity is large, the plots are less related to each other and in this case, they should be larger to obtain the same degree of experimental precision. Thus, it is expected that the proposed method will present optimal plot size equal to or greater than the method for maximum curvature modified by Meier & Lessman (1971) and this is interesting from the practical point of view since that method sometimes determines optimal size of plots smaller than a plant (Leite *et al.*, 2006), which is a criticism of the method.

Taking as an example the tray model used in this experiment (10x14 = 140 tubes) for further studies, a tray would be enough to allocate 28 plots of 5 seedlings.

CONCLUSIONS

The optimum number of plants per plot for evaluation of 'Golden Peciolo Curto' papaya seedlings is five.

The method proposed by bootstrap simulation with replacement provides optimum sizes of plots equal to or higher than the method of maximum curvature. It also provides the same size of plot than the method of maximum curvature of the coefficient of variation.

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