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Full Length Research Paper

# Optimum plot size for experiments with papaya genotypes in field

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The objective of this study was to determine a suitable plot size for field experiments with papaya genotypes. Two experiments were carried out using a randomized complete block design with 11 and 12 papaya genotypes, respectively. In both experiments, plots consisted of one row, with 10 plants each. Spacing between rows was 3.5 m, with 1.5 m between plants. The characteristic evaluated was fruit production in t ha<sup>-1</sup> in first year of cultivation, and the basic unit used was one plant. Suitable plot size was estimated using Lin and Binns, and Hatheway's methods. These methods are complementary and should be used together in the determination of the optimum plot size. The results of these tests showed that the optimum plot size for the evaluation of yield in papaya was four plants by plot with four replications each assuming 30% of the precision for establishing differences among the means of two genotypes.

Key words: Breeding, Carica papaya, intrablock correlation.

## INTRODUCTION

Papaya is one of the main tropical fruits produced in the world. World papaya production reached 12.6 million tonnes in 2014, with India, Brazil, Indonesia, Nigeria and Mexico as its main producers (FAO, 2014).

Field experimentation with papaya has been carried out quite frequently in order to implement new technologies

for the crop (Cortes et al., 2017; Santos et al., 2017) and to evaluate plant productivity (Oliveira et al., 2014; Dantas et al., 2015; Luz et al., 2015) and disease resistance (Poltronieri et al., 2017) of new genotypes. In experiments carried out to evaluate the productivity of new genotypes, Oliveira et al. (2014) used 5 plants per

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> plot, while Dantas et al. (2015) used 6 plants per plot and Luz et al. (2015) 10 plants per plot, both with 4 replications in a randomized block design. This variable number suggests the convenience in carrying out a sound investigation to establish optimum plot size for papaya field experiments.

Several authors emphasize the importance of plot size determination in experiments for the evaluation of new genotypes obtained in plant breeding (Leite et al., 2006; Casler, 2013; Silva et al., 2016). Many methods have been assessed with this aim, either from blank test (Meier and Lessman, 1971; Paranaíba et al., 2009; Lorentz et al., 2012) or from experiments involving analyses of variance (Pimentel-Gomes, 1984; Lin and Binns, 1984; Barbosa et al., 2001). The method proposed by Lin and Binns (1984) is used to estimate intra-block correlation and heterogeneity index, which is easier to perform than the methods involving blank assays. This method is commonly used in conjunction with Hatheway's method to determine optimum plot size in experiments involving the evaluation of genotypes as has been assayed in bean (Storck et al., 2007) and soybean (Storck et al., 2009).

Khan et al. (2017) mention that, Hatheway's method is one of the best options for calculating the size of the plot, since this method determines the optimum plot size considering the experimental design, the number of treatments, the coefficient of variation, the expected difference between treatments and the number of replicates (Alves and Seraphim, 2004). This is because there is no linear relationship between the variability measured by the coefficient of variation and the optimum plot size (Khan et al., 2017), and therefore the methodology used should consider this factor as Hatheway's method does (Alves and Seraphim, 2004).

In papaya, the experimental plot size has been determined for comparing seedlings performance in greenhouses (Lima et al., 2007; Brito et al., 2012; Celanti et al., 2016 a, b) and also for adult plants in the open field (Schmildt et al., 2016), but all these works were performed with blank test, test that evaluates only one genotype at a time.

The aim of this work is to determine the optimum plot size for experiments involving several genotypes of papaya in the field, using the methods proposed by Lin and Binns (1984) and Hatheway (1961).

#### MATERIALS AND METHODS

The plot size determination was realized for two papaya experiments which are part of the partnership between the Federal University of Espírito Santo and the company Caliman Agrícola S.A. aiming to obtain new papaya cultivars (Silva et al., 2017). The experiments were performed from 15 July 2012 to 15 July 2013 at the Santa Terezinha Farm of Caliman Agrícola S.A., sited 150 km away from the town of Vitória, in the state of Espírito Santo (ES), Brazil (19°11' 49" S, 40°05' 52" W, at an altitude of 30 m.a.s.l.).

In both experiments, the experimental design was a randomized block with four replications. The first experiment was carried out with 11 papaya genotypes (New hybrids: CR3 x SSAM; CR3 x

UENF/Caliman 01; CR3 x JS 12; CR3 x Improved Sunrise Solo Line 72/12; CR3 x Progeny Tainung; CR1 x Sekati; CR1 x Progeny Tainung; CR1 x JS 12; CR2 x SS32; JS12 x SSAM. Commercial hybrid: UENF/Caliman 01), while in the second experiment 12 genotypes were used (New hybrids: CR1 x São Mateus; CR1 X Improved Sunrise Solo Line 72/12; CR2 x São Mateus; CR3 x São Mateus; CR1 x Maradol; CR2 x Sekati; CR3 x Maradol; CR1 x UENF/Caliman 01; CR3 x Sekati; CR1 x SSAM; BSA x Golden PC. Commercial variety: Golden THB). In order to obtain the new hybrids, the genotypes CR1, CR2, CR3, JS12, Sekati and Maradol are from the Formosa group and the other genotypes are from the Solo group.

The plots consist of one row of 10 plants. Spacing between rows was 3.5 m, with 1.5 m between plants. The characteristic evaluated was fruit production in t ha<sup>-1</sup> in the first year. The basic unit (BU) was one plant. Based on Lin nd Binns (1984), the statistical model adopted in both experiments, referring to a randomized block design, was (Equation 1):

$$Y_{ij} = m + g_i + b_j + e_{ij} \tag{1}$$

Where:  $Y_{ij}$  = the yield obtained from genotype i, in block j; m = general mean;  $g_i$  = effect from genotype i (i = 1, 2, ..., I genotypes);  $b_j$  = effect from block j (j = 1, 2, ..., J blocks);  $e_{ij}$  = experimental error.

From the adopted statistical model, an analysis of variance was carried out considering the fixed model (Cruz, 2016) according to Table 1. From the analysis and components of variance (Table 1), the intra-block correlation ( $\hat{\rho}$ ) was estimated according to the Equation 2:

$$\hat{\rho} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma^2}$$
<sup>(2)</sup>

From of Equation 2, the heterogeneity index (b) of Lin and Binns (1984) was determined according to the Equation 3:

$$b = \frac{\log[I - (I - 1)(I - \hat{\rho})]}{\log(I)}$$
(3)

Where: log = logarithm to the base 10; I = treatments number;  $\hat{\rho}$  = the intra-block correlation.

From the analysis of variance, the  $\rm CV_{exp}$  that is the estimate of the experimental coefficient of variation in percentage, was determined by Equation 4:

$$CV_{exp} = 100 \frac{\sqrt{MSR}}{\overline{Y}}$$
(4)

Where: MSR = mean square residual showed in the Table 1;  $\overline{Y}$  = the general mean.

The plot size ( $X_0$ ) in the evaluation of yield in the papaya was calculated using Hatheway's method (Hatheway, 1961), by Equation 5:

| Table 1. Variance analysis and mathematical expectations of the mean squares to fixe | ed model |
|--|----------|
|--|----------|

| Source of variation | Degrees of freedom | Mean square (MS) | Expectation (MS)                        | F                               |
|---------------------|--------------------|------------------|---|---------------------------------|
| Blocks (B)          | (J – 1)            | MSB              | $\sigma^2 + I \sigma_b^2$               | -                               |
| Genotypes (G)       | (I – 1)            | MSG              | $\sigma^2 + \frac{J}{I-1} \sum_i c_i^2$ | $\frac{\text{MSG}}{\text{MSR}}$ |
| Residual (R)        | (J-1)(I-1)         | MSR              | $\sigma^2$                              | -                               |
|                     |                    |                  |   |                                 |

 $\sigma_b^2$  = variance between blocks obtained by  $\sigma_b^2 = (MSB - MSR)/I$ ;  $\sigma^2$  = variance relative to the experimental error.

$$X_{0} = b \sqrt{\frac{2(t_{1} + t_{2})^{2} C V_{exp}^{2}}{J d^{2}}}$$
(5)

Where: b and  $CV_{exp}$  are defined by Equations 3 and 4, respectively; J = the number of replications considered; d = the difference between genotypes mean to be detected as significant at the 5% probability, and expressed as a percentage of the expected detected mean; t<sub>1</sub> = the critical value of Student's t distribution for the level of significance of the test (type I error) of  $\alpha$  = 5% (bilateral test at 5%), with df degrees of freedom and t<sub>2</sub> = the critical value of the Student t distribution, corresponding to 2 (1-*P*) (bilateral test), where *P* is the probability of obtaining a significant result, that is, the power of the test (*P* = 0.80, in this study), with df degrees of freedom.

The tabulated values of t distribution were obtained with residual degrees of freedom, according to the treatments I and J replications, where df = (I-1)(J-1) for a randomized block design. As reported by Cargnelutti Filho et al. (2014), the parameter d measures the precision, being that a small percentage of d indicates greater precision; in other words, small differences between treatments means will be considered significant. In the simulations, the criteria for combinations take into consideration d values as 20, 30 and 40% and the other criteria were used according to Celanti et al. (2016b): the lowest number of treatments was three (I = 3), whereas the detection of the difference between two means can now be made by analysis of variance; the smallest number of replications was 2 (J = 2), because this is the minimum for detecting the experimental error; the I treatments and J replications were combined to provide a minimum of 20 plots per according experiment. to **Pimentel-Gomes** (2009)recommendations; since this is a discrete random variable, the optimum plot size was presented by integer number, rounding to the closest whole number.

For a better understanding of the variability of the studied genotypes, the comparison of the average productivity was shown by Scott-Knott's clustering test. Statistical analyses were performed using Genes (Cruz, 2016) and Excel<sup>®</sup> software.

#### **RESULTS AND DISCUSSION**

The coefficients of variation in this experiment were  $CV_{exp}$  = 19.92 and 26.36% in Experiments 1 and 2, respectively (Table 2). Other researchers also found

 $CV_{exp}$  with values between 20 and 30% in the evaluation of papaya genotypes (Oliveira et al., 2014; Dantas et al., 2015; Luz et al., 2015). In both experiments, there was a significant difference between the means for the assayed genotypes with productivity of the first trial of 79 and of 66 t ha<sup>-1</sup> in the second experiment, always for the first year of cultivation. There was a significant difference between the means by the Scott-Knott grouping test in two experiments (Table 3).

Productivity ranged from 55 t ha<sup>-1</sup> (for UENF/Caliman 01 genotype to 97 t ha<sup>-1</sup> and CR2 x SS32 genotype in experiment 1) to 37 t ha<sup>-1</sup> (for Golden THB genotype to 104 t ha<sup>-1</sup> and CR3 x Maradol genotype in experiment 2). Most genotypes have a productivity average above Brazilian and world average of 50 and 31 t ha<sup>-1</sup>, respectively (FAO, 2014) are higher than the productivity of other genotypes verified in different studies (Oliveira et al., 2014; Dantas et al., 2014). Consequently, from the point of view of the experimental quality, the results of both experiments present credibility for study of the determination of optimum plot size.

The analyses of the data show that intra-block correlation ( $\hat{\rho}$ ) was 0.1809 and 0.1045 in experiments 1 and 2, respectively, which allowed to obtain soil heterogeneity index (b) 0.5693 and 0.6901, respectively (Table 2). As recommended by Lin and Binns (1986) when b value is between 0.2 and 0.7, the researcher should plan a suitable combination between the number of replicates and plot size.

In research involving yield production of papaya in the field, one of the researcher's wishes is also to reduce the experimental area. This can be obtained, according to equation 5 presented by Hatheway (1961): through, reducing the value of  $t_1$  which is achieved by increasing the number of treatments and/or repetitions; increasing the number of repetitions (J); by decreasing the accuracy or increasing the difference between the means (increasing the value of d). These options can be taken individually or all together as presented in Table 4, when b = 0.6307 and  $CV_{exp} = 23.14\%$ .

**Table 2.** Number of genotypes (ng), genotypes mean square (GMS), blocks mean square (BMS), residue mean square (RMS), productivity (Prod, in t ha<sup>-1</sup> in the first year), intra-block correlation ( $\hat{\rho}$ ), heterogeneity index (b) and coefficient of variation ( $CV_{exp}$ , in %) in two experiments of papaya genotypes following an experimental randomized blocks design.

| Experiment | ng | BMS    | GMS        | RMS    | Prod  | ô      | b      | CV <sub>exp</sub> |
|------------|----|--------|------------|--------|-------|--------|--------|-------------------|
| 1          | 11 | 856.83 | 807.66**   | 249.86 | 79.34 | 0.1809 | 0.5693 | 19.92             |
| 2          | 12 | 727.54 | 1,834.30** | 303.13 | 66.05 | 0.1045 | 0.6921 | 26.36             |
| Average    | -  | -      | -          | -      | 72.70 | 0.1427 | 0.6307 | 23.14             |

\*\* significant at 1% probability by F test.

Table 3. The first year average productivity of the genotypes evaluated in two papaya experiments.

| Experiment 1                           |                    | Experiment 2                              |                     |  |  |
|--|--------------------|---|---------------------|--|--|
| Genotype                               | Average            | Genotype                                  | Average             |  |  |
| CR3 x SSAM                             | 73.62 <sup>b</sup> | CR1 x São Mateus                          | 58.54 <sup>b</sup>  |  |  |
| CR3 x UENF/Caliman 01                  | 93.52 <sup>a</sup> | CR1 x Improved Sunrise Solo Line 72/12 66 |                     |  |  |
| CR3 x JS 12                            | 84.29 <sup>a</sup> | CR2 x São Mateus                          | 64.56 <sup>b</sup>  |  |  |
| CR3 x Improved Sunrise Solo Line 72/12 | 94.21 <sup>a</sup> | CR3 x São Mateus                          | 64.03 <sup>b</sup>  |  |  |
| CR3 x Progeny Tainung                  | 87.02 <sup>a</sup> | CR1 x Maradol                             | 86.84 <sup>a</sup>  |  |  |
| CR1 x Sekati                           | 81.33 <sup>a</sup> | CR2 x Sekati                              | 96.01 <sup>a</sup>  |  |  |
| CR1 x Progeny Tainung                  | 56.35 <sup>b</sup> | CR3 x Maradol                             | 104.18 <sup>a</sup> |  |  |
| CR1 x JS 12                            | 76.89 <sup>b</sup> | CR1 x UENF/Caliman 01                     | 45.56 <sup>b</sup>  |  |  |
| CR2 x SS32                             | 97.12 <sup>a</sup> | CR3 x Sekati                              | 80.14 <sup>a</sup>  |  |  |
| JS12 x SSAM                            | 73.32 <sup>b</sup> | CR1 x SSAM                                | 49.98 <sup>b</sup>  |  |  |
| UENF/Caliman 01                        | 55.06 <sup>b</sup> | BSA x Golden PC                           | 45.41 <sup>b</sup>  |  |  |
|  |                    | Golden THB                                | 36.59 <sup>b</sup>  |  |  |

Averages of genotypes followed by the same letter but do not differ by Scott-Knott's cluster test at 5% probability.

When working with d, the optimum plot size ( $X_0$ ) is larger and the value of d is lower (higher precision) considering the same number of treatments and replications (Table 4). In the experiment, considering 10 genotypes and 4 replications,  $X_0$  is 2, 4 and 16 plants by plot for d = 40, 30 and 20%, respectively. It seems reasonable for the researcher to assume a value of d = 30% because any further increase in the accuracy will result in a large increase in plot size. Similar results were observed by other researchers using Hatheway's method (Muniz et al., 2009; Celanti et al., 2016b).

Hence, assuming d = 30%, and if the researcher intends to use 10 genotypes, the optimum size of two plants per plot in seven replications implies the use of 14 plants per genotype in the experiment. In order to keep d = 30%, the researcher could use 3 plants per plot and five replications (15 plants per genotype in the experiment), or 7 plants per plot and three replications (21 plants per genotype) (Table 4). Therefore, for the same precision, smaller plots and larger number of replications are more efficient for the use of the same experimental area, as observed by Muniz et al. (2009) in eucalyptus and Souza et al. (2015) in sunflower.

Concerning the number of genotypes involved, with d = 30% and four replicates, X<sub>0</sub> is four plants per plot when evaluating 8 to 35 genotypes in the experiment in a randomized blocks design (Table 4). Schmildt et al. (2016) suggested 6 plants per plot using 3 replications and a difference of 30% between means (d), similar to the results obtained in this work using 25 to 35 genotypes per block (Table 4). However, for better use of the experimental area, it is recommended to design experiments with four replications and four plants per plot because this will require 16 plants per plot it require 18 plants per genotype.

From the results presented in Table 4 we deduce, larger changes in the optimum plot size with changes of d and J than with changes of I, as observed by other researchers with different crops (Storck et al., 2007; Cargnelutti Filho et al., 2014; Souza et al., 2015). The results showed that Lin and Binns (1984) and Hatheway (1984) methods should be used together as observed by Storck et al. (2007, 2009). The results of the present

| <u> </u> | J | d = 20% | d = 30% | d = 40% | I  | J | d = 20% | d = 30% | d = 40% |
|----------|---|---------|---------|---------|----|---|---------|---------|---------|
| 3        | 7 | 7       | 2       | 1       | 15 | 2 | 52      | 14      | 6       |
| 4        | 5 | 13      | 4       | 1       | 15 | 3 | 25      | 7       | 3       |
| 4        | 6 | 9       | 2       | 1       | 15 | 4 | 15      | 4       | 2       |
| 4        | 7 | 7       | 2       | 1       | 15 | 5 | 10      | 3       | 1       |
| 5        | 4 | 18      | 5       | 2       | 15 | 6 | 8       | 2       | 1       |
| 5        | 5 | 12      | 3       | 1       | 15 | 7 | 6       | 2       | 1       |
| 5        | 6 | 9       | 2       | 1       | 20 | 2 | 49      | 14      | 5       |
| 5        | 7 | 7       | 2       | 1       | 20 | 3 | 24      | 7       | 3       |
| 6        | 4 | 17      | 5       | 2       | 20 | 4 | 15      | 4       | 2       |
| 6        | 5 | 11      | 3       | 1       | 20 | 5 | 10      | 3       | 1       |
| 6        | 6 | 8       | 2       | 1       | 20 | 6 | 8       | 2       | 1       |
| 6        | 7 | 6       | 2       | 1       | 20 | 7 | 6       | 2       | 1       |
| 7        | 3 | 29      | 8       | 3       | 25 | 2 | 48      | 13      | 5       |
| 7        | 4 | 17      | 5       | 2       | 25 | 3 | 23      | 6       | 3       |
| 7        | 5 | 11      | 3       | 1       | 25 | 4 | 14      | 4       | 2       |
| 7        | 6 | 8       | 2       | 1       | 25 | 5 | 10      | 3       | 1       |
| 7        | 7 | 6       | 2       | 1       | 25 | 6 | 7       | 2       | 1       |
| 8        | 3 | 28      | 8       | 3       | 25 | 7 | 6       | 2       | 1       |
| 8        | 4 | 16      | 4       | 2       | 30 | 2 | 46      | 13      | 5       |
| 8        | 5 | 11      | 3       | 1       | 30 | 3 | 23      | 6       | 3       |
| 8        | 6 | 8       | 2       | 1       | 30 | 4 | 14      | 4       | 2       |
| 8        | 7 | 6       | 2       | 1       | 30 | 5 | 10      | 3       | 1       |
| 9        | 3 | 27      | 7       | 3       | 30 | 6 | 7       | 2       | 1       |
| 9        | 4 | 16      | 4       | 2       | 30 | 7 | 6       | 2       | 1       |
| 9        | 5 | 11      | 3       | 1       | 35 | 2 | 46      | 13      | 5       |
| 9        | 6 | 8       | 2       | 1       | 35 | 3 | 23      | 6       | 3       |
| 9        | 7 | 6       | 2       | 1       | 35 | 4 | 14      | 4       | 2       |
| 10       | 2 | 60      | 17      | 7       | 35 | 5 | 10      | 3       | 1       |
| 10       | 3 | 26      | 7       | 3       | 35 | 6 | 7       | 2       | 1       |
| 10       | 4 | 16      | 4       | 2       | 35 | 7 | 6       | 2       | 1       |
| 10       | 5 | 11      | 3       | 1       |    |   |         |         |         |
| 10       | 6 | 8       | 2       | 1       |    |   |         |         |         |
| 10       | 7 | 6       | 2       | 1       |    |   |         |         |         |

**Table 4.** Optimal size of plots ( $X_0$ ), in number of plants per plot estimated by the method of Hatheway in an experimental randomized blocks design, in different scenarios formed by the combinations of I genotypes, J replications, and d differences between the means of the genotypes, to be detected as significant at the 5% probability, expressed as a percentage of the overall mean of the experiment (precision) for yield produced by different papaya genotypes.

study are useful to guide researchers in papaya field experiments with several genotypes, since there is no standard number of plants by plot (Oliveira et al., 2014; Luz et al., 2015; Silva et al., 2017).

### Conclusion

The optimum plot size for field genotypes papaya experiments is four plants by plot, using four replications assuming a precision of 30% in the difference among means. Lin and Binns (1984) and Hartheway (1984) methods are complementary and should be used together in the determination of the plot size.

#### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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