REVISTA BRASILEIRA DE FRUTICULTURA
Published by the Brazilian Society of Fruit Crops
ISSN 0100-2945

Economy - Original Article - Edited by: Alessandro Dal Col´Lucio

Optimal plot size estimation in field experiment with purple passion fruit

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Abstract: The species P. edulis Sims f. edulis, native to Brazil, known as purple passion fruit, has purple fruits and lower acidity. With the growing demand for passion fruits, there is greater need for research on their cultivation to reduce production costs and improve fruit quality. The adequate determination of the size and number of plots has been a fundamental limitation in studies with several crops, as it is difficult to obtain constant data on plants per plot in most experiments, making it impossible to use usual methodologies for data analysis. As a result, testing can be performed with less labor and implementation costs, making plot size optimization a step of interest. Thus, this work aims to determine the ideal size of experimental plots with purple passion fruit in the field using three methods. The variables analyzed were fruit length, fruit diameter, peel thickness, juice yield, soluble solids content, citric acid, number of fruits, and average fruit weight. The use of optimal plot size of six basic units for fruit-related variables, five for pulp-related variables, and seven basic units for production variables, is recommended.

Index terms: *Passiflora edulis* Sims f. *edulis*, experimental planning, blank test, maximum curvature, quadratic plateau.

Estimação do tamanho ótimo de parcela em experimento em campo com maracujá-roxo

Resumo: A espécie *P. edulis* Sims f. *edulis* é nativa do Brasil, conhecido como maracujá-roxo, possui frutos roxos e menor acidez. Com a crescente procura pelo maracujazeiro, há maior necessidade de pesquisas sobre seu cultivo para reduzir os custos de produção e melhorar a qualidade do serviço. A determinação adequada do tamanho e do número de parcelas tem sido uma limitação fundamental no estudo de várias culturas, pois é difícil obter dados de plantas por parcela, que sejam constantes, na maioria dos experimentos realizados, impossibilitando a utilização de metodologias habituais de análise de dados. Como resultado, os testes podem ser realizados com menos mão de obra e despesas de implementação, tornando a

Rev. Bras. Frutic., v.45, e-753 DOI: *https://dx.doi.org/10.1590/0100-29452023753*
Received 23 Jan, 2023 ● Accepted 16 May, 2023 ● Published Nov/Dec, 2023. Jaboticabal - SP - Brazil.

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otimização do tamanho da parcela uma etapa de interesse. Com isso, tem-se que o objetivo do trabalho foi determinar o tamanho ideal de parcelas experimentais com maracujá-roxo em campo, utilizando três métodos. As variáveis analisadas foram: comprimento do fruto, diâmetro do fruto, espessura da casca, rendimento do suco, teor de sólidos solúveis, ácido cítrico, número de frutos e peso médio dos frutos. Recomenda-se utilizar o tamanho ótimo de parcelas de seis unidades básicas para as variáveis relacionadas ao fruto, sendo cinco para as variáveis relacionadas à polpa e sete unidades básicas para as variáveis de produção.

Termos para indexação: *Passiflora edulis* Sims f. *edulis*, planejamento experimental, ensaio em branco, máxima curvatura, platô quadrático.

Introduction

Originating in Tropical America, the various passion fruit species are produced on a large scale in Brazil, Peru, and Colombia, and on a small scale in countries such as the United States, Argentina, Asia, and Australia (FALEIRO et al., 2017a), which also have some native species. Approximately 525 species of the genus *Passiflora* have been cataloged, of which 145 species are native to Brazil (FALEIRO et al., 2019).

Of Brazilian origin, *P. edulis* Sims f. *edulis*, known as purple passion fruit, has purple fruits and low acidity and is highly cultivated in Colombia, where it is commonly called gulupa. The fruit grows well in high-tech orchards in countries such as Holland, Germany, Belgium, the United Kingdom, Canada, and Switzerland. Among these, Colombia stands out for being the world's largest passion fruit producer, promoting its trade as exotic fruits. For this species, soils need light texture and pH between 5.5 and 7.0, altitude above 1000m and with ideal temperature between 15 °C and 20 °C. Fruits are round and vary in color from green to purple when ripe. Fruits are usually consumed fresh, as it is less acidic and stands out for its flavor and aroma (FALEIRO et al., 2017b).

The passion fruit planting area is approximately 47 thousand hectares. Among the major regions, the Northeastern region of Brazil stands out as the largest passion fruit producer, with production of about 491 thousand tons, followed by the Southeastern region with about 84 thousand tons, the Southern region, with 65 thousand tons, the Northern region, with 36 thousand tons and finally, the Midwestern region, with around 14 thousand tons. The states with the largest planting areas are Bahia (17,414 ha), Ceará (8,278 ha), and Pernambuco (2,637 ha) (SIDRA – IBGE, 2021).

However, according to Storck et al. (2014), passion fruit production requires a considerable amount of labor and is considered an agricultural activity requiring large growing areas. With the increasing demand for passion fruits, there is greater need for research on their cultivation to reduce production costs and improve fruit quality. Field test procedures are crucial tools to ensure the reliability of results; therefore, adequately planned, implemented and evaluated experiments generate reliable results.

The appropriate determination of the size and number of plots has been a fundamental limitation in the study of several crops, since it is challenging to obtain sustained data on the number of plants per plot in most experiments, impairing the use of usual methodologies for data analysis (RAMALHO et al., 2012). As a result, testing can be performed with less labor and implementation costs, making plot size optimization a step of interest.

In fruticulture, optimal plot size has been calculated for Passiflora in the field (STORCK et al., 2014), Passiflora in vitro (PEIXOTO et al., 2011; FARIA et al., 2020ab), banana (DONATO et al., 2008; SILVA et al., 2019a), greenhouse papaya (BRITO et al., 2012; CELANTI et al., 2016ab; FARIA et al., 2020c), papaya in the field (SILVA et al., 2019b) and castor bean (PALUDO et. al., 2015).

Thus, this work aimed to determine the ideal plot size for experiments with purple passion fruit in the field using the maximum curvature, modified maximum curvature, and segmented quadratic model with plateau methods.

Material and methods

Data used in this work come from an experiment carried out with *Passiflora edulis* Sims f. *edulis* (purple passion fruit); whose original data were collected in 2008 at Embrapa Mandioca e Fruticultura, located in the municipality of Cruz das Almas, Bahia. The experiment was carried out in a completely randomized design, conducted as a blank (or uniformity) test. Since this species presents allogamy, a mechanism that leads to self-incompatibility, 77 plants from 12 distinct families have been chosen to carry out uniformity tests. Seedlings were planted in a vertical spreader at spacing of 2.0 x 5.0 m.

The variables analyzed were the main agronomic characteristics: fruit length (FL, mm), fruit diameter (FD, mm), peel thickness (PT, mm), juice yield (JY, mL), soluble solids content (Brix, °Bx), citric acid (acidity, % citric acid), number of fruits (NF) and average fruit weight (FW, g).

In the experiment, 77 replicates were obtained, in which each plant was considered a basic unit (bu) distributed in 11 rows by seven columns. Different combinations between row (X_1 basic units) and column (X_2 basic units) were used; thus, plot sizes were simulated by grouping X_1 and X_2 so that $X_1 X_2 = x$ represents x plot sizes in basic units. Thus, 16 plot sizes (X) were simulated with 28 different shapes, where the number of plots varied from 77 to 2 and the plot size from 1 to 30 basic units per plot (Table 1).

Table 1. Number of simulations (NS), number of plots (NP), plot size (PS) and plot shape (PSH) for the basic units of a field experiment with passion fruit in the field.

NS	NP	PS	PSH
1	77	1	1x1
2	30	2	2x1
3	30	2	1x2
4	20	3	3x1
5	21	3	1x3
6	15	3	2+1
7	15	3	1+2
8	15	4	2x2
9	9	5	2x2+1
10	9	6	2x3
11	10	6	3x2
12	6	7	2x3+1
13	6	7	3x2+1
14	6	8	2x4
15	5	8	4x2
16	6	10	2x5
17	5	10	5x2
18	4	12	3x4
19	3	12	4x3
20	4	15	3x5
21	3	15	5x3
22	2	16	4x4
23	2	18	3x6
24	3	18	6x3
25	2	20	4x5
26	2	20	5x4
27	2	25	5x5
28	2	30	6x5

1x1, reads: one row value by one column value, 2x1, reads: two row values added in each column; 2+1, reads: add two row values adding one more column value; 2x2+1, reads: sum of two row values, two column values adding one more unit. Source: Authors.

Based on the different plot sizes and shapes, of same size, the averages of the coefficients of variation for each variable under study were calculated.

Methods to estimate the optimum plot size

Maximum curvature method (MC)

The maximum curvature method, proposed by Federer (1963), uses data from blank tests, which is one of the first methods applied to field experiments.

To simulate experimental plots of different sizes, the method establishes basic units of blank tests so that at least one measure of variability can be found, which could be the coefficient of variation, standard error of the mean, or plot size variance. After simulation, a two-dimensional graph is constructed, where the plot size (X) is on the abscissa axis (x) with its corresponding coefficients of variation (CV_x) on the ordinate axis (y). Therefore, the plot size is determined by visual inspection, where the point of maximum inflection of the curve (point of maximum curvature) corresponds to the stability of the curve; therefore, the optimal plot size is given by the value found in the abscissa (MOREIRA et al., 2016).

Graphs were constructed considering the CV values calculated with their respective plot sizes, thus obtaining the curve that illustrates the relationship between them for each variable under study.

For this purpose, the original method for constructing graphs (freehand) was not used. Graphs were generated with the aid of the R software (R DEVELOPMENT CORE TEAM, 2020), and an analysis of the optimal plot size was performed according to Federer (1963) and LeClerg (1966), who consider that the optimal size is determined where there is greater influence on CV caused by the increase in plot size.

Modified maximum curvature method (MCM)

The modified maximum curvature method proposed by Meier and Lessman (1971) and the exponential model to be used to estimate the optimal plot size considering the relationship between coefficient of variation (CV) and plot size with X basic units is explained by

$$CV = aX^{-b} \qquad (1)$$

where a and b are the parameters to be estimated. From the curvature function given by this model, the value of the abscissa at which the point of maximum curvature occurs was determined, given by:

$$X_C = \left[\frac{a^2b^2(2b+1)}{b+2}\right]^{\frac{1}{2b+2}} \qquad (2)$$

where X_c is the value of the abscissa at the point of maximum curvature, which corresponds to the optimal size estimation of the experimental plot (MEIER; LESSMAN, 1971). Coefficients of determination are also estimated to verify the goodness of the model's fit.

Segmented quadratic model method with plateau (QMP)

The quadratic model segmented with plateau (PARANAÍBA et al., 2009) has two segments, in which the first consists of $X \leq X_C$ presenting CV values explained by a quadratic model, and in the second segment, when $X > X_C$, it could be said that the explanatory equation is constant and parallel to the abscissa (MOREIRA et al., 2016).

Therefore, the following regression model was used:

$$CV_X = \begin{cases} \beta_0 + \beta_1 X + \beta_2 X^2 + \varepsilon_i, se\ X \leq X_C \\ P + \varepsilon_i, se\ X > X_C \end{cases} \qquad (3)$$

where β_0, β_1, and β_2 are the parameters to be estimated. X_c is the optimal plot size to be estimated, in which the quadratic model becomes a plateau, with respect to the abscissa.

The optimal plot size can be calculated as follows: $X_C = -\frac{\beta_1}{2\beta_2}$. Replace X_c in expression (3) to obtain the value corresponding to the plateau, given by $P = \beta_0 - \frac{\beta_1^2}{4\beta_2}$.

Therefore, it could be considered that such parameters are effective, since X_c and P are determined by β_0, β_1, and β_2.

Results and discussion

Maximum curvature method (MC)

For the maximum curvature method, 28 simulations were performed in different ways, and the coefficients of variation were calculated for each one, resulting in 16 plot sizes. Figure 1 was generated considering the relationship between CV and its respective plot size. It was observed that as the plot size values increase, the CV values decrease; however, at some point, the curve reaches particular stability, suggesting that from this point on, there is no gain in experimental precision, and larger plot sizes are not required.

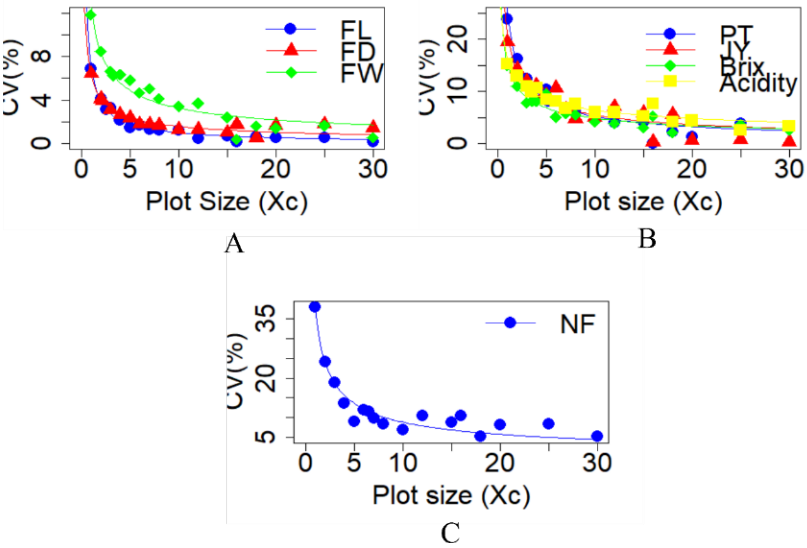

Figure 1. Relationship between coefficient of variation ($CV\%$) and plot size (X_c), by the maximum curvature method, in basic units for the variables under study in the uniformity test with purple passion fruit. A) FL: fruit length (mm), FD: fruit diameter (mm), and FW: average fruit weight (g); B) PT: peel thickness (mm), JY: juice yield (mL), Brix: soluble solids content (°Bx), and Acidity: citric acid (%); C) NF: number of fruits (NF). Source: Authors.

Therefore, this method consists of visual inspection (FEDERER, 1963) to identify the optimal plot size, which does not guarantee the accuracy of results, since it depends on the criterion used for decision-making.

Thus, the optimal plot size, following the criterion of Federer (1963) and LeClerg (1966) (which is the point of greatest change in the coefficient of variation curve regarding the plot size), is six basic units (bu) per plot for variables FL, PT and Brix and seven bu for variables FD, JY, acidity, NF and FW (Figure 1). Therefore, optimal plot size that cover all the variables under study is recommended, that is, seven bu. Therefore, it could be said that from seven bu onwards, there is no accuracy gain.

Separating variables into groups of agronomic characteristics of interest, the optimal plot size for fruit-related variables (FL, FD, PT) is seven bu, equal to the optimal plot size for pulp-related variables (JY, Brix, Acidity), and production variables (NF, FW).

Modified maximum curvature method (MCM)

The lowest coefficient of determination (R^2) value was found for variable JY (85%); therefore, the model explains at least 85% of the variability and the highest R^2 value for variable CF (98%). However, except for the JY variable, all variables have R^2 greater than or equal to 90%, so only 10% of the variability was not explained by the model, which guarantees good fit for the method used (Table 2).

Table 2. Estimates of the parameters of the model, by the modified maximum curvature method, coefficient of variation corresponding to the maximum curvature point (CV%), the value of the abscissa at which the maximum curvature point (X_c) occurs, coefficient of determination (R^2) for the variables under study in the uniformity test with purple passion fruit.

Variables	$\hat{a}$	$\hat{b}$	CV (%)	X_C	R^2
FL	7.04	0.63	3.15	2.59	0.98
FD	6.21	0.59	3.95	2.15	0.92
PT	24.79	0.68	8.16	5.17	0.95
JY	20.89	0.58	8.63	4.59	0.85
Brix	15.15	0.49	8.10	3.56	0.90
Acidity	16.31	0.41	9.85	3.47	0.92
NF	36.53	0.61	16.36	6.54	0.93
FW	12.45	0.58	6.22	3.30	0.93

FL: fruit length (mm); FD: fruit diameter (mm); PT: peel thickness (mm); JY: juice yield (mL); NF: number of fruits (NF); FW: average fruit weight (g). Source: Authors.

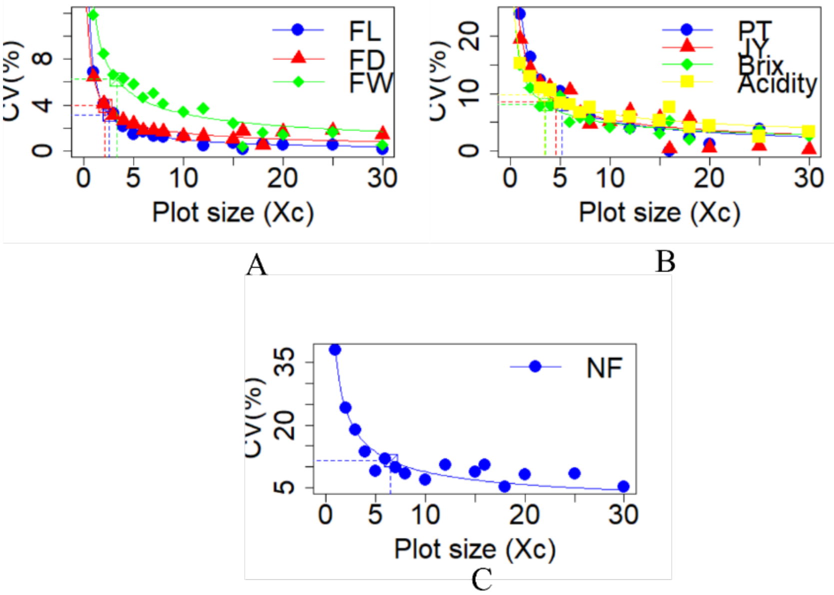

Figure 2. Relationship between the coefficient of variation (CV%) and plot size (Xc), by the modified maximum curvature method, in basic units for the variables under study in the uniformity test with purple passion fruit. A) FL: fruit length (mm), FD: fruit diameter (mm), and FW: average fruit weight (g); B) PT: peel thickness (mm), JY: juice yield (mL), Brix: soluble solids content (°Bx), and Acidity: citric acid (%); C) NF: number of fruits (NF). Source: Authors.

Evaluating the estimation of $\hat{a}$ there is a wide variation in values, since the lowest value is 6.21 (FL) and the highest value is 36.53 (FW), suggesting great divergence between variables. As for the estimation of $\hat{b}$ it was observed that only variables Brix and acidity showed low variability ($\hat{b}$ < 0.5), suggesting that the other variables are possibly heterogeneous data. It appears that the optimal plot sizes ranged from 2.15 (FD) to 6.54 (NF) (Table 2, Figure 2).

From figure 2, it is possible to visually identify the optimal plot sizes (X_c) with their corresponding points of maximum curvature (CV (%)) where the lowest X_c value of 2.15 corresponds to a point of maximum curvature CV (%) of 3.95 and the highest X_c value of 6.54 bu corresponds to CV (%) of 16.36. Therefore, optimal plot size of seven bu is recommended, which is the minimum required plot size that includes all variables using the modified maximum curvature method (Table 2, Figure 2).

However, separating variables into groups of agronomic characteristics of interest, the optimal plot size for fruit-related variables such as FL, FD, and PT is six bu for pulp-related variables such as JY, Brix, and acidity the optimal plot size is five bu, and for production variables such as NF and FW the optimal plot size is seven bu.

Segmented quadratic model method with plateau (QMP)

Coefficient of determination (R^2) values ranged from 85% (JY) to 95% (NF), thus ensuring good fit. The optimal plot sizes ranged from 5.79 bu (NF) to 17.16 bu (FW), which compared to the previous model, generated higher X_c values. There is also a wide variation in the coefficient of variation, ranging from 0.72 to 8.56 (Table 3).

The segmented quadratic model with plateau response determines the optimal plot size at the meeting between curves generated by the quadratic model and the plateau, so after this point, there is no experimental accuracy gain. Figure 3 shows the point at which the optimal plot size for each variable under study occurs. The lowest X_c value is 5.79 bu (NF) with P value corresponding to 8.56, and the highest X_c value is 17.16 (FW) with P value corresponding to 1.42.

Table 3. Estimates of the parameters of the segmented quadratic model method with plateau. coefficient of variation corresponding to the point of maximum curvature (P), the value of the abscissa at which the point of maximum curvature (X_c) occurs, coefficient of determination (R^2) for the variables under study in the uniformity test with purple passion fruit.

Variables	$\hat{\beta}_0$	$\hat{\beta}_1$	$\hat{\beta}_2$	P	X_c	R^2
FL	8.25	-2.02	0.07	0.72	7.43	0.94
FD	7.69	-1.81	0.13	1.46	6.89	0.93
PT	24.78	-3.90	0.17	3.04	11.16	0.93
JY	18.58	-1.94	0.06	2.33	16.77	0.85
Brix	15.33	-2.02	0.09	3.63	11.57	0.88
Acidity	16.08	-1.66	0.06	4.74	13.63	0.89
NF	49.93	-14.28	1.23	8.56	5.79	0.95
FW	10.90	-1.11	0.03	1.42	17.16	0.92

FL: fruit length (mm); FD: fruit diameter (mm); PT: peel thickness (mm); JY: juice yield (mL); NF: number of fruits (NF); FW: average fruit weight (kg). Source: Authors

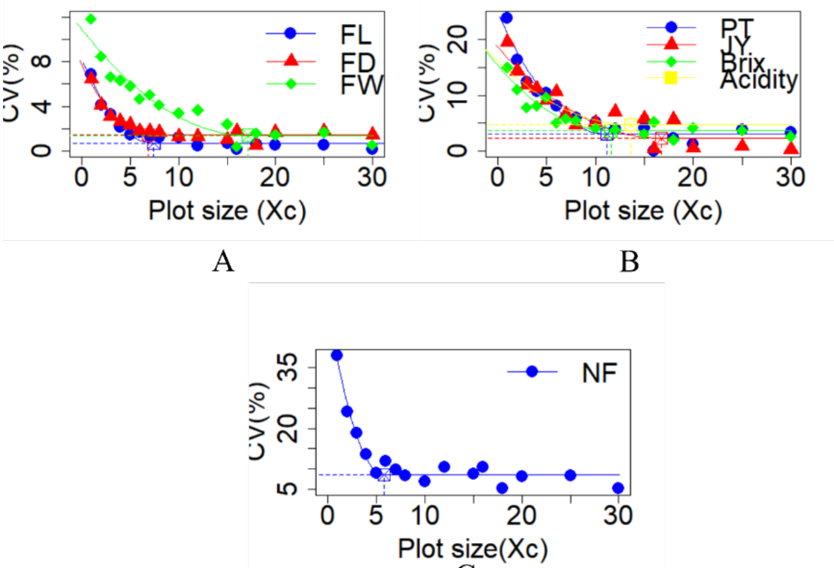

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Figure 3. Relationship between the coefficient of variation (CV%) and plot size (X_c) by the Segmented quadratic model with plateau, in basic units for the variables under study in the uniformity test with purple passion fruit. A) FL: fruit length (mm), FD: fruit diameter (mm), and FW: average fruit weight (g); B) PT: peel thickness (mm), JY: juice yield (mL), Brix: soluble solids content (°Bx) and Acidity: citric acid (%); C) NF: number of fruits (NF). Source: Authors.

Comparing models it is possible to detect that the coefficient of determination (R^2) values are on average higher (better adjusted) than in previous models. In this case, it is recommended to use the average between optimal plot sizes in order to recommend the one that would bring the lower financial impact; therefore, the ideal size would be 12 basic units.

Separating the variables into groups of agronomic characteristics of interest, the optimal plot size for the fruit variables (FL, FD, PT) is 12 bu with average of nine bu, for pulp variables (JY, Brix, Acidity) it is 17 bu with average of 14 bu. As for production variables (NF, FW) the optimal plot size is 18 bu, with average of 12 bu.

A comparison analysis was performed between methods (Table 4), where the model that presented the best fit was the segmented quadratic model with plateau; however, not very different from the modified maximum curvature model. Nonetheless, when using segmented models a "false plateau" can occur, since the simulated plot sizes do not guarantee amplitude capable of reaching a response plateau (PEIXOTO et al., 2011).

Table 4. Range of variation of the coefficient of determination (R^2) for the modified maximum curvature method (MCM), and segmented quadratic model with plateau (QMP) method.

Methods	R^2
MCM	85 a 98%
QMP	85 a 95%

Source: Authors.

The modified maximum curvature method works better for determining the ideal plot size, since it avoids under or overestimating the optimum plot size which can occur with the linear plateau and quadratic plateau methods (SOUSA et al., 2015). The modified maximum curvature approach is one of the most popular methods for assessing plot size, and it can to be used to compare one or more methods for estimating the size of experimental units (BAKKE, 1988).

According to Henriques Neto et al. (2004), the modified maximum curvature method estimates small plot size values. with the possibility of decreasing the coefficient of variation with increasing plot size in the region above the maximum curvature point, a behavior also observed by Faria et al. (2020a). However, as the plot size is algebraically estimated, the values obtained are not necessarily integers, which leads to the possibility of rounding up the numbers.

Optimal plot size studies with purple passion fruit were not found; however, Storck et al. (2014) with yellow passion fruit. observed that the optimal plot size is less than five plants per plot. In other fruit trees, such as papaya, eight plants per plot was found by Silva et al. (2019), and Schmildt et al. (2015) observed six plants per plot. For castor beans, Paludo et al. (2015) observed eight plants per plot.

Conclusion

In experiments with purple passion fruit. the optimal plot size varied according to each method. However, the use of optimal plot size based on the modified maximum curvature method is recommended, which showed the best results: six plants per plot for fruit-related variables (length, diameter. and peel thickness), five plants per plot for pulp-related variables (juice yield, soluble solids contente, and citric acid), and seven plants per plot for production variables (number of fruits, and average fruit weight).

Acknowledgements

The authors would like to thank the financial support provided by the Coordination for the Improvement of Higher Education Personnel (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* - CAPES) - Financing Code 001 and the authors would also like to thank "Embrapa Mandioca e Fruticultura" (Cruz das Almas. BA. Brazil) for technical support.

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