Article

# Sample size to estimate the average of variables agronomic in cassava

André Schoffel[1]
Sidinei José Lopes[1]
Jana Koefender[2]
Alessandro Dal'Col Lúcio[1]
Juliane Nicolodi Camera[2]
Diego Pascoal Golle[2]

[1]Federal University of Santa Maria. Av. Roraima núm. 1000, Santa Maria, Rio Grande do Sul, Brazil. (andre-schoffel@hotmail.com; sjlopes2008@gmail.com; adlucio@ufsm.br). [2]University of Cruz Alta. Municipal road Jacob Della Méa km 5.6, Parada Benito, Cruz Alta, Rio Grande do Sul, Brazil. (jkoefender@unicruz.edu.br; ju_camera@yahoo.com.br; dgolle@unicruz.edu.br).

[§]Corresponding author: andre-schoffel@hotmail.com.

## Abstract

In agricultural experiments, measuring all plants that constitute an experimental unit is the most appropriate method for estimating averages. This study analyzed sample sizes (number of plants) for estimating averages of agronomic traits in cassava. The experiment for seedling production was conducted on four sowing dates: September 22, October 7, October 19, and November 25, 2017. The evaluated characters were plant height at planting, number of leaves at planting, number of leaves at 7 days after planting, number of leaves at transplanting, and plant height at transplanting. The seedlings were transplanted to the field on November 9, November 24, December 3, and December 26. Root characteristics and root yield were measured at harvest. The following statistics were measured: minimum, maximum, range, mean, median, variance, standard deviation, and coefficient of variation. The sample size was determined by resampling with replacement when the range of the 95% confidence interval (CI) was 10%, 20%, 30%, and 40% of the average estimate. Larger sample sizes were needed to estimate trait averages with a 95% CI range of 10% of the average estimate, i.e., when estimation accuracy was higher. In contrast, lower sample sizes were required to estimate averages of all characters in all planting times with a 95% CI range of 40%. For measuring seedling production and stem and root yield, 63 and 153 plants are sufficient to estimate averages when the range of the 95% confidence interval was 20%.

**Keywords:** *Manihot esculenta* Crantz, experimental design, rapid multiplication method, sample size and vegetative propagation.

Reception date: January 2021
Acceptance date: March 2021

# Introduction

Cassava belongs to the Euphorbiaceae family and is an important plant source for producing starchy roots, which contribute to food security in many countries. Cassava plants are highly adapted to different cultivation conditions and constitute the third most important source of food energy in the tropics (Hasibuan and Nazir, 2017). Moreover, the plants can grow in chemically and physically degraded soils and under water stress conditions (Egesi *et al*., 2007).

Approximately 87% of cassava production in Brazil comes from family farms, and the average yield in this type of property is 5 770 kg ha$^{-1}$ (Brasil, 2009). In addition to the limitations in the cultivation area and low technological investment, propagating materials of low physiological and sanitary quality are used in sequential harvests, which increases the risk of diseases that significantly impact plant development and yield (Silva *et al*., 2013). Therefore, implementing and improving management and cultivation techniques that allow the optimal utilization of high-quality propagating materials is crucial.

The rapid multiplication method developed by the International Center for Tropical Agriculture (CIAT) consists of planting cuttings with two to three buds and harvesting stems, which are rooted in pots with water and are later used for producing cassava seedlings in containers filled with substrate. This method allows the best use of stems as well as obtaining and selecting seedlings with good physiological and sanitary quality, especially seedlings without diseases disseminated by propagative material. Yuguda *et al*. (2013) have shown that limited knowledge about management techniques is responsible for the low productivity of cassava.

For transplanting of cassava seedlings to production fields, plant growth and development traits should be accurately assessed during seedling production period. Moreover, the measurement of root characters is crucial, especially rooting capacity, which dictates seedling quality. However, technical information on the growth, development, and yield of cassava stems and roots using the rapid multiplication method is scarce. Santos *et al*. (2009) have shown that planting in the first season is an alternative for producing stems because the roots have small size and uneven shape.

In agricultural experiments, measuring all plants that constitute an experimental unit is the most appropriate method for estimating averages. However, given the limited time, financial, and labor resources, a given population is represented by sampling. In these cases, estimating and using the appropriate sample size is necessary to increase sample representativeness (Storck *et al*., 2011). Confidence intervals determined by resampling are used to calculate the sample size. This technique is independent of the probability distribution of the data and has been used to estimate trait averages in white lupin (Burin *et al*., 2014).

Sample size is not always chosen according to data variability and the desired level of accuracy of the estimate, which may compromise the quality of the study. Schoffel *et al*. (2020) determined the sample size for estimating averages of agronomic traits in cassava seedlings originating from stem cuttings with a different number of leaves and different diameters.

Despite the existence of adaptations in the application of the rapid multiplication method, few studies have investigated relevant definitions, such as sample size, and experimental designs for cassava cultivation. The objective of this study is to determine the sample size (number of plants) for estimating trait averages in the production of cassava seedlings, stems, and roots of cassava seedlings transplanted on four different dates.

## Material and methods

Cassava seedlings of the cultivar Apronta Mesa were planted on four sowing dates in 2017 (September 22, October 7, October 19, and November 25) in a greenhouse model Van der Hoeven with an automatic mist irrigation system, with a total irrigation depth of approximately 6 mm day$^{-1}$ at an average temperature of 25 ºC. On each sowing date, 300 cuttings were planted and collected when they contained three to nine visible leaves. The leaf was considered visible when the edges of one of the lobes did not touch each other (Schons *et al*., 2007).

The sprouted cuttings were planted in 15-cell black plastic trays with the following dimensions: length, 34 cm; width, 21 cm; height, 7.8 cm. The dimension of each cell was 6.2 cm (upper diameter), 5 cm (lower diameter), and 7.8 cm (height), and each cell had five 6-mm holes at the base for water draining. The trays were filled with previously irrigated Mec Plant® commercial substrate to prevent tissue dehydration. After that, the sprouted cuttings were planted (one per cell) in 1 cm deep furrows in the morning at a maximum temperature of 25 ºC to minimize tissue dehydration.

At the time of planting, plant height at planting (PHP) (from the base to the last visible leaf) and the number of leaves at planting (NLP) were measured. At 7 days after planting (DAP), the number of visible leaves (NL7DAP) was counted again. At 30 DAP, the seedlings were removed from the greenhouse and transferred to a hatchery for acclimation for a minimum of 5 days, except on November 25, when the seedlings were acclimated at 23 DAP. The number of leaves at transplanting (NLT) and plant height at transplanting (PHT) were measured on each planting date (September 22, October 7, October 19, and November 25) at 48, 47, 44 and 31 DAP, respectively.

Desiccation of the winter crop formed by the intercropping of cover crops (*Avena strigosa* Schreb. + *Raphanus sativus* L.) was carried out using the herbicide Glyphosate (3 L ha$^{-1}$). Conventional tillage was performed by plowing and harrowing. Seedling transplanting was performed on November 9, November 24, December 3, and December 26 (240 seedlings per date) in 15 cm deep unfertilized furrows.

Soil fertility in the experimental area was analyzed in a sample collected at a depth of 0-20 cm. The sample presented the following characteristics: pH in water, 5.5; clay, 50%; organic matter, 3.3%; phosphorus, 26.2 mg dm$^{-3}$; potassium, 96 mg dm$^{-3}$; calcium, 5.9 cmol dm$^{-3}$; magnesium, 2.4 cmol dm$^{-3}$; aluminum, 0 cmol dm$^{-3}$, and base saturation, 68.7%.

Fertilization was conducted according to the recommendation of the Soil Chemistry and Fertility Commission (2016) for cassava cultivation. Topdressing fertilization was performed during transplanting using 334 kg ha$^{-1}$ of mineral fertilizer (5-20-20). Urea nitrogen fertilization (46-00-

00) (70 kg ha$^{-1}$) was carried out either at 15 days after transplanting or at the beginning of starch accumulation. In cassava crop, the latter occurs when the plants present 21 visible leaves on the main stem (Schons *et al*., 2007).

Weeds were controlled manually on a weekly basis until the complete closure of the rows (up to 3 months after transplanting). In the absence of rainfall, sprinkler irrigation was performed daily until day 15 after transplanting. Water supply is essential for producing cassava seedlings using the rapid multiplication method.

The plants were harvested on May 30, and the duration of the growth cycle in each transplanting time (November 9, November 24, December 3, and December 26) was 203, 188, 179, and 156 days, respectively. Other measured traits were the branching height of the main stem (BHMS) (cm), number of branches (NB), number of buds per stem (NBS), stem length (SL) (cm), stem diameter (SD) (mm), root fresh weight (RFW) (g plant$^{-1}$), root length (RL) (cm), root diameter (RD) (mm), and number of roots (NR).

Diameter parameters were measured using a digital caliper. SD was calculated by the mean of the basal, intermediate, and upper diameter, and RD was measured in the upper third of the roots. The roots were weighed using a digital scale with a resolution of 0.01 grams. The following statistics were analyzed: minimum, maximum, range, mean, median, variance, standard deviation, and coefficient of variation (CV). The means were compared using a *t*-test for independent samples at a level of significance of 5% ($p< 0.05$).

A total of 264, 288, 264, and 244 plants were measured on the planting dates September 22, October 7, October 19, and November 25, respectively, for estimating NLP, NL7DAP, PHP, PHT, and NLT. A total of 213, 231, 232, and 179 plants were measured on the transplantation dates November 9, November 24, December 3, and December 26, respectively, for estimating BHMS, NB, NBS, SL, SD, RFW, RL, RD, and NR.

Based on these data, 999 sample sizes were planned, with an initial sample size of two plants, and the other sample sizes were obtained by sequentially adding one plant up to a maximum size of 1 000 plants. After that, iterative resampling was performed for each sample size using 2 000 resamples with replacement. Therefore, 2 000 trait averages were obtained for each sample size. The following statistics were analyzed based on average data: minimum value, 2.5% percentile, mean, 97.5% percentile, and maximum value. The range of the 95% confidence interval was calculated by the difference between 97.5% and 2.5% percentiles.

The sample size for estimating averages was calculated by the number of plants, from which the range of the 95% CI was 10%, 20%, 30%, and 40% of the average estimate. Statistical analyses were performed using R software (R Development Core Team, 2014).

## Results and discussion

There were significant differences in trait averages between the four sowing dates (Table 1). NLP on October 7 was lower than that on the other planting dates, without significant differences. Many leaves fell from cuttings planted on October 7, which can be confirmed by the average NL7DAP.

NL7DAP was higher in cuttings planted on November 25, possibly due to environmental conditions, especially temperature, which favored the development of seedlings on this planting date. Moreover, a few leaves fell from cuttings planted on November 25.

**Table 1. Descriptive statistics on the number of leaves at planting (NLP), number of leaves at 7 days after planting (NL7DAP), plant height at planting (PHP) (cm), plant height at transplanting (PHT) (cm), and number of leaves at transplanting (NLT) of cassava seedlings cultivated on September 22, October 7, October 19, and November 25.**

| Statistic | NLP | NL7DAP | PHP | PHT | NLT |
|---|---|---|---|---|---|
| September 22 | | | | | |
| Minimum | 3 | 1 | 0.4 | 3.3 | 3 |
| Maximum | 9 | 9 | 6.4 | 19.6 | 13 |
| Range | 6 | 8 | 6 | 16.3 | 10 |
| Mean | 5.33* a | 4.5 c | 2.68 a | 10.14 a | 8.74 b |
| Median | 5.5 | 4 | 2.6 | 9.35 | 9 |
| Standard deviation | 1.34 | 1.45 | 1 | 3.76 | 1.58 |
| Coefficient of variation (%) | 25.1 | 32.28 | 37.41 | 37.06 | 18.11 |
| October 7 | | | | | |
| Statistic | NLP | NL7DAP | PHP | PHT | NLT |
| Minimum | 3 | 1 | 0.4 | 2.8 | 3 |
| Maximum | 8 | 7 | 5.5 | 16.5 | 12 |
| Range | 5 | 6 | 5.1 | 13.7 | 9 |
| Mean | 4.92 b | 3.91 d | 2.3 b | 8.15 b | 8.16 c |
| Median | 5.5 | 4 | 2.2 | 7.6 | 8 |
| Standard deviation | 1.42 | 1.31 | 0.86 | 3.05 | 1.56 |
| Coefficient of variation (%) | 28.87 | 33.61 | 37.34 | 37.5 | 19.14 |
| October 19 | | | | | |
| Statistic | NLP | NL7DAP | PHP | PHT | NLT |
| Minimum | 3 | 2 | 0.6 | 2.4 | 4 |
| Maximum | 8 | 7 | 4.3 | 11.5 | 11 |
| Range | 5. | 5 | 3.7 | 9.1 | 7 |
| Mean | 5.17 a | 4.89 b | 1.96 c | 5.78 d | 8.15 c |
| Median | 6 | 5 | 1.8 | 5.4 | 8 |
| Standard deviation | 1.31 | 1.19 | 0.77 | 1.77 | 1.43 |
| Coefficient of variation (%) | 25.24 | 24.25 | 39.31 | 30.64 | 17.6 |
| November 25 | | | | | |
| Statistic | NLP | NL7DAP | PHP | PHT | NLT |
| Minimum | 3 | 2 | 0.9 | 3.1 | 5 |
| Maximum | 8 | 8 | 5.5 | 12.6 | 13 |

| Statistic | NLP | NL7DAP | PHP | PHT | NLT |
|---|---|---|---|---|---|
| Range | 5 | 6 | 4.6 | 9.5 | 8 |
| Mean | 5.16 a | 5.33 a | 2.03 c | 6.37 c | 9.08 a |
| Median | 5.5 | 6 | 2 | 6.2 | 9 |
| Standard deviation | 1.26 | 1.03 | 0.73 | 1.66 | 1.24 |
| Coefficient of variation (%) | 24.39 | 19.33 | 36.27 | 26.12 | 13.63 |

*= the averages not followed by the same letter in each column were not significantly different from each other using the *t*-test for independent samples at a level of significance of 5% ($p < 0.05$).

PHP and PHT were higher on September 22 (2.68 cm and 10.14 cm, respectively). The averages of these characters decreased in later planting dates, indicating that early planting favored the growth of cuttings planted on September 9. Despite the higher PHP in September, the value of 2.68 cm was significantly lower than that recommended for cuttings planted using the rapid multiplication method (10-12 cm).

NLT was higher in cuttings planted on November 25, suggesting that the development of seedlings was higher in later planting times. Environmental conditions on later sowing dates favored the development of cassava plants. These results demonstrated that plant height as a criterion for collecting stems may present limitations because PHP varied between planting dates and may vary among cultivars. In addition, leaf number adequately represents the physiological age of the plant (Streck *et al*., 2003).

The averages of BHMS were higher in seedlings transplanted on November 9 and December 3. Nonetheless, SL was higher in seedlings transplanted on December 3 (Table 2). This result may have been affected by the cutting height of the stems at the time of harvest. The cut height was standardized to 15-20 cm above the soil surface.

**Table 2. Descriptive statistics of yield characteristics of stems and roots of cassava seedlings transplanted on four different dates (November 9, November 24, December 3, and December 26).**

| Statistic | BHMS | NB | NBS | SL | SD | RFW | RL | RD | NR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | November 9 | | | | | |
| Min | 0.8 | 2 | 26 | 0.65 | 11.16 | 74.7 | 10.4 | 19.84 | 1 |
| Max | 2.12 | 4 | 52 | 1.9 | 29.31 | 3807 | 38.03 | 42.93 | 17 |
| Range | 1.32 | 2 | 26 | 1.25 | 18.15 | 3732.3 | 27.63 | 23.09 | 16 |
| Mean | 1.42* a | 2.94 b | 39.34 a | 1.24 b | 21.26 a | 1181.55 a | 22.09 a | 30.23 a | 8.53 a |
| Median | 1.4 | 3 | 39 | 1.22 | 21.43 | 997.1 | 21.6 | 29.67 | 9 |
| SD | 0.29 | 0.35 | 4.76 | 0.29 | 3.1 | 730.25 | 4.96 | 3.64 | 3.05 |
| CV (%) | 20.49 | 11.96 | 12.09 | 23.2 | 14.59 | 61.8 | 22.44 | 12.05 | 35.8 |

| Statistic | BHMS | NB | NBS | SL | SD | RFW | RL | RD | NR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | November 24 | | | | |
| Statistic | BHMS | NB | NBS | SL | SD | RFW | RL | RD | NR |
| Min | 0.89 | 2 | 30 | 0.74 | 13.36 | 13.31 | 9.83 | 21.97 | 2 |
| Max | 1.97 | 4 | 48 | 1.8 | 27.38 | 3703.6 | 39.5 | 42.99 | 18 |
| Range | 1.08 | 2 | 18 | 1.06 | 14.02 | 3690.29 | 29.67 | 21.02 | 16 |
| Mean | 1.36 b | 3.03 a | 39.1 a | 1.19 c | 21.67 a | 1207.7 a | 22.67 a | 31 a | 8.39 a |
| Median | 1.3 | 3 | 39 | 1.14 | 21.7 | 1084.2 | 22.18 | 30.63 | 8 |
| SD¹ | 0.22 | 0.29 | 3.3 | 0.23 | 2.3 | 629.01 | 4.73 | 3.87 | 2.86 |
| CV (%)¹ | 16.43 | 9.71 | 8.43 | 18.92 | 10.61 | 52.08 | 20.86 | 12.48 | 34.09 |
| | | | | | December 3 | | | | |
| Statistic | BHMS | NB | NBS | SL | SD | RFW | RL | RD | NR |
| Min | 1.04 | 2 | 30 | 0.83 | 12.53 | 81.6 | 10.25 | 17.44 | 1 |
| Max | 2.17 | 4 | 49 | 1.97 | 26.73 | 2546.4 | 37 | 36.16 | 16 |
| Range | 1.13 | 2 | 19 | 1.14 | 14.2 | 2464.8 | 26.75 | 18.72 | 15 |
| Mean | 1.48 a | 3.02 a | 37.94 a | 1.30 a | 20.23 b | 773.27 b | 20.06 b | 26.39 b | 8.21 a |
| Median | 1.51 | 3 | 38 | 1.34 | 20.49 | 693.15 | 19.59 | 26.37 | 8 |
| SD | 0.22 | 0.29 | 3.48 | 0.22 | 2.26 | 455.05 | 4.27 | 3.29 | 3.01 |
| CV (%) | 14.98 | 9.46 | 9.18 | 17.04 | 11.17 | 58.85 | 21.31 | 12.48 | 36.7 |
| | | | | | December 26 | | | | |
| Statistic | BHMS | NB | NBS | SL | SD | RFW | RL | RD | NR |
| Min | 0.72 | 0 | 16 | 0.09 | 7.91 | 35.8 | 8.8 | 18.1 | 1 |
| Max | 1.96 | 4 | 42 | 1.73 | 22.48 | 1540.5 | 36.8 | 36.96 | 15 |
| Range | 1.24 | 4 | 26 | 1.64 | 14.56 | 1504.7 | 28 | 18.86 | 14 |
| Mean | 1.16 c | 2.61 c | 29.08 b | 0.92 d | 16.76 c | 496.39 c | 19.35 b | 26.51 b | 5.34 b |
| Median | 1.1 | 3 | 29 | 0.9 | 17.42 | 430.1 | 18.88 | 26.47 | 5 |
| SD | 0.25 | 0.78 | 5.91 | 0.29 | 3.4 | 311.86 | 4.87 | 3.14 | 2.65 |
| CV (%) | 21.2 | 29.94 | 20.33 | 31.63 | 20.28 | 62.83 | 25.15 | 11.85 | 49.54 |

*= the averages not followed by the same letter in each column were not significantly different from each other using the *t*-test for independent samples at a level of significance of 5% ($p< 0.05$). Min= minimum; Max= maximum; SD= standard deviation; CV= coefficient of variation. BHMS= branching height of the main stem (cm); NB= number of branches; NBS= number of buds per stem; SL= stem length (cm); SD= stem diameter (mm); RFW= root fresh weight (g $plant^{-1}$); RL= root length (cm); RD= root diameter (mm); NR= number of roots per plant.

The average NB ranged from 2.61 in seedlings transplanted on December 26 to 3.03 in seedlings transplanted on November 24. The cultivar Apronta Mesa has three branches in the main stem. This result indicated that NB was affected by the time of transplanting and that late transplanting decreased the averages of this trait. Similarly, the average NBS on the last transplanting date was 29.08.

On the first three dates, NBS ranged from 37.94 to 39.34 and did not differ significantly between these dates, indicating that late transplanting of seedlings limited stem development. Given that NBS is a relevant parameter in the rapid multiplication method because it represents the amount of propagative material harvested for use in the next harvest, seedling transplanting on the third week of December limited bud production per unit area.

SD was decreased in seedlings transplanted in December. The higher SD in plants transplanted in November may be related to the increased leaf area development for the accumulation of reserve substances in the stems. This trait improves cassava stem quality for storage and propagative material selection for use in the next harvest. Higher SD is a result of the higher amount of storage material and water, which are essential for sprouting and rooting.

The medulla has a high concentration of water and is surrounded by the cortex, which has a high concentration of reserve material. SD and the relationship between the cortex and medulla affect the sprouting and rooting capacity of cuttings after planting. RL was higher in seedlings transplanted in November and was significantly different from that in seedlings transplanted in December, with averages ranging from 22.09 to 22.67 cm, a minimum of 9.83, and a maximum of 39.5 cm.

Nevertheless, the range on the four transplanting dates ranged from 26.75 to 29.67 cm, indicating that there was variability in RL and that delayed transplanting reduced the average RL. After morphological definition of the amount of storage roots, there is a significant increase in RL (Figueiredo *et al*., 2014). On the first three transplanting dates, the average NR ranged from 8.21 to 8.53. On the last transplanting date, NR was 5.34, indicating the lower development of the root system, especially storage roots, in seedlings transplanted late.

RFW was higher in seedlings transplanted in November and decreased with delayed transplanting. Figueiredo *et al*. (2014) have shown that root productivity parameters are defined in the following order: number, length, diameter, and fresh weight of roots. Therefore, the reduction in NR may directly affect RFW, as demonstrated in this study and the decrease is attenuated as transplanting time is delayed. Therefore, NR is the most significant limiting factor for cassava yield in late seedling transplanting seasons.

Larger sample sizes (247 plants) were needed to estimate trait averages with a 95% CI range of 10% of the average estimate, i.e., when estimation accuracy was higher (Table 3). In contrast, 16 plants were required to estimate averages of all characters in all planting times with a 95% CI range of 40% (lower accuracy). Therefore, determining sample size at different degrees of accuracy allows researchers to choose the most appropriate size for measuring agronomic traits.

Larger sample sizes were required to estimate trait averages when planting was performed on October 7, except for PHP, whose CV was higher (39.31%) on October 19, and 247, 63, 27, and 16 plants were measured to estimate averages with 95% CI ranges of 10%, 20%, 30%, and 40%, respectively. This result demonstrated the relationship between sample size and CV, in which CVs were higher for NLP, NL7DAP, PHP, PHT, and NLT, on October 7, corresponding to 28.87%, 33.61%, 37.34%, 37.5%, and 19.14%, respectively. Cargnelutti Filho *et al*. (2018a) observed that larger sample sizes were needed to estimate traits with higher CVs and that sample size was variable in pigeon pea (*Cajanus cajan* (L.) Millsp) seeds.

**Table 3. Sample size (number of plants) with 95% confidence interval ranges of 10%, 20%, 30%, and 40% of average estimates for number of leaves at planting (NLP), number of leaves at 7 days after planting (NL7DAP), plant height at planting (PHP), plant height at transplanting (PHT), and number of leaves at transplanting (NLT) of cassava seedlings on four sowing dates (September 22, October 7, October 19, and November 25).**

| | 10% | 20% | 30% | 40% |
|---|---|---|---|---|
| | | September 22 | | |
| NLP | 106 | 25 | 11 | 6 |
| NL7DAP | 173 | 41 | 18 | 11 |
| PHP | 234 | 53 | 24 | 13 |
| PHT | 220 | 54 | 23 | 14 |
| NLT | 53 | 13 | 6 | 4 |
| | | October 7 | | |
| NLP | 138 | 33 | 13 | 9 |
| NL7DAP | 182 | 45 | 19 | 11 |
| PHP | 227 | 56 | 24 | 14 |
| PHT | 220 | 54 | 24 | 15 |
| NLT | 61 | 14 | 7 | 4 |
| | | October 19 | | |
| NLP | 100 | 27 | 11 | 6 |
| NL7DAP | 94 | 22 | 11 | 6 |
| PHP | 247 | 63 | 27 | 16 |
| PHT | 145 | 35 | 17 | 9 |
| NLT | 47 | 12 | 6 | 4 |
| | | November 25 | | |
| NLP | 92 | 25 | 10 | 6 |
| NL7DAP | 62 | 15 | 7 | 4 |
| PHP | 208 | 52 | 22 | 13 |
| PHT | 112 | 27 | 13 | 7 |
| NLT | 29 | 8 | 3 | 2 |

Smaller sample sizes were necessary to estimate trait averages on November 25, and 10, 7, 22, 13, and 3 plants were necessary to estimate NLP, NL7DAP, PHP, PHT, and NLT, respectively, with a 95% CI range of 30%. This result showed that on this planting date, there was less variability between the measured characters, indicating the higher homogeneity between plants and consequently the need to use smaller sample sizes to estimate averages. In contrast, Bandeira *et al*. (2018a) found that data variability in rye culture (*Secale cereale* L. *cv* BRS Progresso) was higher on the first and last growing dates and, consequently, larger sample sizes were required for estimating parameter averages on these dates.

Among the analyzed traits, a larger sample size was needed to estimate PHP and PHT on all planting dates. In contrast, smaller sample sizes were necessary to estimate discrete traits, especially NLT, corresponding to 13, 14, 12, and 8 plants on September 22, October 7, October 19, and November 25, respectively, with a 95% CI range of 20%. Storck *et al*. (2007) and Toebe *et al*. (2014) found that variability was higher in characters obtained by weighing compared to those obtained by counting or measuring in maize crop. The variability in sample size for estimating trait averages in cassava seedlings was low, allowing the researcher to choose the sample size considering only the set of traits and the desired level of accuracy.

The transplanting performed on November 9 and December 26 required larger sample sizes compared to transplanting carried out on November 24 and December 3 for measuring characters at different levels of accuracy (Table 4). Two to 50 plants were required to measure shoot and root system parameters on November 24, whereas 3 to 68 plants were required to measure these parameters on December 26 with a 95% CI range of 30%. Bandeira *et al*. (2018b) found that there was variability in sample size according to the analyzed agronomic parameters and sowing dates in rye (*Secale cereale* L.) crops.

**Table 4. Sample size (number of plants) with 95% confidence interval ranges of 10%, 20%, 30%, and 40% of average estimates for stem and root yield of cassava seedlings on four transplanting dates (November 11, November 24, December 3, and December 26).**

| | 10% | 20% | 30% | 40% |
|---|---|---|---|---|
| | | November 11 | | |
| BHMS | 65 | 16 | 8 | 5 |
| NB | 24 | 6 | 3 | 2 |
| NBS | 22 | 6 | 3 | 2 |
| SL | 86 | 21 | 9 | 6 |
| SD | 35 | 8 | 4 | 3 |
| RFW | 617 | 153 | 70 | 40 |
| RL | 84 | 20 | 8 | 5 |
| RD | 23 | 6 | 3 | 2 |
| NR | 206 | 50 | 24 | 13 |
| | | November 24 | | |
| BHMS | 44 | 11 | 5 | 3 |
| NB | 17 | 4 | 3 | 2 |
| NBS | 13 | 3 | 2 | 2 |
| SL | 57 | 14 | 6 | 4 |
| SD | 17 | 5 | 2 | 2 |
| RFW | 444 | 107 | 50 | 25 |
| RL | 70 | 18 | 7 | 5 |
| RD | 25 | 6 | 3 | 2 |
| NR | 186 | 46 | 20 | 12 |

| | 10% | 20% | 30% | 40% |
|---|---|---|---|---|
| | | December 3 | | |
| BHMS | 37 | 9 | 4 | 2 |
| NB | 17 | 4 | 3 | 2 |
| NBS | 14 | 4 | 2 | 2 |
| SL | 44 | 11 | 6 | 3 |
| SD | 20 | 5 | 2 | 2 |
| RFW | 545 | 132 | 62 | 33 |
| RL | 73 | 18 | 8 | 5 |
| RD | 25 | 7 | 3 | 2 |
| NR | 219 | 51 | 24 | 14 |
| | | December 26 | | |
| BHMS | 70 | 18 | 8 | 4 |
| NB | 149 | 31 | 16 | 7 |
| NBS | 69 | 16 | 8 | 4 |
| SL | 163 | 38 | 20 | 10 |
| SD | 66 | 15 | 7 | 4 |
| RFW | 643 | 153 | 68 | 37 |
| RL | 100 | 26 | 11 | 7 |
| RD | 21 | 6 | 3 | 2 |
| NR | 402 | 103 | 42 | 23 |

BHMS= branching height of the main stem (cm); NB= number of branches; NBS= number of buds per stem; SL= stem length (cm); SD= stem diameter (mm); RFW= root fresh weight (g plant$^{-1}$); RL= root length (cm); RD= root diameter (mm); NR= number of roots per plant.

In addition, the authors observed that intermediate sowing dates required smaller sample sizes, indicating higher homogeneity of the plants on these dates, similar to the present study. These results demonstrated that there was variability between the sowing times and stress the importance of studies using different sample sizes and sowing dates for estimating trait averages (Bandeira *et al*., 2018a).

Among the stem parameters, larger sample sizes were needed to estimate BHMS and SL on the four transplanting dates. In contrast, smaller sample sizes were required for estimating NB and NBS, except for NB on the last transplanting date, which presented a sample size of 149 plants, whereas the size was 24, 17, and 17 plants on November 9, November 24, and December 3, respectively, with a 95% CI range of 10%.

The higher variability in branching emission in plants grown on the last transplanting date required measuring a larger number of plants for analyzing this trait and is demonstrated by a CV of 29.94% on the last date compared to 9.46-11.96% on the other dates. Haesbaert *et al*. (2017) highlighted that different sample sizes should be used at the same level of accuracy for different characters. However, given the limitations of this method, the largest sample size should be used to estimate averages to account for trait variability.

With a 95% CI range of 20%, the sample size for ST was 8, 5, 5, and 15 plants on November 9, November 24, December 3, and December 26, respectively. For this character and others, the sample size was reduced or unchanged from the first to the third transplantation date. This trend was not maintained on December 26, and the number of plants analyzed was increased, except for RD, which remained unchanged on the four dates at the analyzed levels of accuracy.

For RD, the minimum sample size was three plants on each transplantation date with a 95% CI range of 30%. Nevertheless, the sample size varied between traits on the same transplantation date at a given degree of accuracy, as also observed by Toebe *et al*. (2014) and Bandeira *et al*. (2018a). The CV for RFW ranged from 52.08% to 62.83%, suggesting that a larger sample size is necessary to estimate RFW at a given level of accuracy.

These results are similar to those of Cargnelutti Filho *et al*. (2018b), in which the CV of yield parameters in flax crop (*Linum usitatissimum* L.) ranged from 58.81% to 68.87%. Cargnelutti Filho *et al*. (2018c) also found that variability in yield traits was higher than that in morphological traits in jack beans (*Canavalia ensiformis*). The higher the CV presented by a character, the larger is the sample size needed to estimate averages (Toebe *et al*., 2014).

Regardless of the time of transplanting and the selected level of accuracy, estimating RFW required larger sample sizes. Most root characters required larger sample sizes, except for RD. This result is related to the CV in RD, which ranged from 11.85% to 12.48% across the four transplanting dates. Cargnelutti Filho *et al*. (2018b) found that the CVs of morphological traits ranged from 14.50% to 48.30%, similar to the present results.

The high number of plants measured and the existing variability suggests that the analyzed data set allowed calculating sample size by resampling, as also observed by Cargnelutti Filho *et al*. (2018c) and Bandeira *et al*. (2018a). Except for RFW, which required larger sample sizes on each transplanting date with a 95% CI range of 20%, measuring 21 and 51 plants is sufficient for estimating stem and root characters, respectively.

Therefore, if traits of these two groups have to be measured, the researcher may choose a minimum sample size of 51 plants for transplanting between November 9 and December 3, i.e., in an experiment with four repetitions, 13 plants need to be sampled per repetition to estimate the average of one treatment. In transplanting performed on December 26, the required sample size was 38 and 103 plants for shoot and root characters, respectively, except for RFW, which required measuring 153, 107, 132, and 153 plants on November 9, November 24, December 3, and December 26 at a level of accuracy of 20%. It is evident that the choice of the degree of accuracy depends on the researcher's knowledge about data variability and the desired accuracy for estimating averages of morphological and productive characters in cassava plants propagated using the rapid multiplication method.

## Conclusions

Sixty-three plants are sufficient to estimate averages of seedling production traits with a 95% CI range of 20%, and 153 plants are sufficient to estimate averages of stem and root yield traits with a 95% CI range of 20%.

## Acknowledgements

To the National Council for Scientific and Technological Development (CNPq) for the scholarship granted to first author.

## Cited literature

Bandeira, C. T.; Cargnelutti, F. A.; Carini, F.; Schabarum, D. E.; Kleinpaul, J. A. and Pezzini, R. V. 2018a. Sample sufficiency for estimation of the mean of rye traits at flowering stage. J. Agric. Sci. 10(3):178-186. https://doi.org/10.5539/jas.v10n3p178.

Bandeira, C. T.; Cargnelutti, F. A.; Follmann, D. N.; Bem, C. M.; Wartha, C. A. and Thomasi, R. M. 2018b. Sample size to estimate the mean of morphological traits of rye cultivars in sowing dates and evaluation times. Semina: Ciênc. Agrár. 39(2):521-532. http://dx.doi.org/10.5433/1679-0359.2018v39n2p521.

Brasil. 2009. O censo agropecuário 2006 e a agricultura familiar no Brasil. Brasília, MDA. 96 p.

Burin, C.; Cargnelutti, F. A.; Toebe, M.; Alves, B. M. and Fick, A. L. 2014. Dimensionamento amostral para a estimação da média e da mediana de caracteres de tremoço branco (*Lupinus albus* L.). Comum. Sci. 5(2):205-212. https://doi.org/10.14295/cs.v5i2.317.

Cargnelutti-Filho, A.; Alves, B. M.; Follmann, D. N.; Bem, C. M.; Kleinpaul, J. A.; Pezzini, R. V. and Simões, F. M. 2018a. Tamanho de amostra para a estimação da média de caracteres de sementes de feijão guandu anão. Agrarian. 11(41):294-298. https://doi.org/10.30612/agrarian.v11i41.4577.

Cargnelutti-Filho, A.; Alves, B. M.; Santos, G. O.; Wartha, C. A.; Kleinpaul, J. A. and Silveira, D. L. 2018b. Sample size to estimate the mean and median of traits in flax. Revista Brasileira de Ciências Agrárias. 13(1):5492. https://doi.org/10.5039/agraria.v13i1a5492.

Cargnelutti-Filho, A.; Alves, B. M.; Toebe, M.; Burin, C. and Wartha, C. A. 2018c. Sample size to estimate the mean of traits in jack bean. Revista Brasileira de Ciências Agrárias. 13(1):1-7. https://doi.org/10.5039/agraria.v13i1a5505.

Comissão de química e fertilidade do solo. 2016. Manual de calagem e adubação para os estados do Rio Grande do Sul e de Santa Catarina. Sociedade Brasileira de Ciência do Solo, Núcleo Regional Sul. Comissão de Química e Fertilidade do Solo RS/SC. 376 p.

Egesi, C. N.; Ilona, P.; Ogbe, F. O.; Akoroda, M. and Dixon, A. 2007. Genetic variation and genotype × environment interaction for yield and other agronomic traits in cassava in Nigeria. Agron. J. 99(4):1137-1142. https://doi.org/10.2134/agronj2006.0291.

Figueiredo, P. G.; Bicudo, S. J.; Moraes-Dallaqua, M. A.; Tanamati, F. Y. and Aguiar, E. B. 2014. Componentes de produção e morfologia de raízes de mandioca sob diferentes preparos do solo. Bragantia. 73(4):357-364. http://dx.doi.org/10.1590/1678-4499.0150.

Haesbaert, F. M.; Lopes, S. J.; Mertz, L. M.; Lucio, A. D. and Huth, C. 2017. Tamanho de amostra para determinação da condutividade elétrica individual de sementes de girassol. Bragantia. 76(1):54-61. http://dx.doi.org/10.1590/1678-4499.389.

Hasibuan, S. and Nazir N. 2017. The development strategy of sustainable bioethanol industry on iconic Sumba island, Eastern Indonesia. Int. J. Adv. Sci. Eng. Inf. Technol. 7(1):276-283. http://dx.doi.org/10.18517/ijaseit.7.1.1796.

R-Development Core Team. 2014. R: a language and environment for statistical computing. Vienna, Áustria.

Santos, V. S.; Souza, A. S.; Viana, A. E. S.; Ferreira-Filho, J. R.; Souza, K. A. S. and Menezes, M. C. 2009. Multiplicação rápida, método simples e de baixo custo na produção de material propagativo de mandioca. Boletim de Pesquisa e Desenvolvimento 44. Embrapa Mandioca e Fruticultura Tropical. 24 p.

Schoffel, A.; Lopes, S. J.; Koefender, J.; Lúcio, A. D.; Camera, J. N. and Golle, D. P. 2020. Sample size for estimation of averages of agronomic traits in cassava seedlings. Inter. J. Innovation Education Res. 8(5):73-82. https://doi.org/10.31686/ijier.vol8.iss5.2312.

Schons, A.; Streck, N. A.; Kraulich, B.; Pinheiro, D. G. and Zanon, A. J. 2007. Emissão de folhas e início de acumulação de amido em raízes de uma variedade de mandioca em função da época de plantio. Cienc. Rural. 37(6):1586-1592. http://dx.doi.org/10.1590/S0103-84782007000600013.

Silva, C. A. D.; Medeiros, E. V.; Bezerra, C. B.; Silva, W. M.; Barros, J. A. and Santos, U. J. 2013. Interferência da incorporação de matéria orgânica no solo no controle da podridão negra da mandioca, causada por *Scytalidium lignicola*. Biosci. J. 29(6):1823-1831.

Storck, L.; Garcia, D. C.; Lopes, S. J. and Estefanel, V. 2011. Experimentação vegetal. 3 (Ed.). UFSM. Santa Maria, UFSM. 198 p.

Storck, L.; Lopes, S. J.; Cargnelutti Filho, A.; Martini, L. F. D. and Carvalho, M. P. 2007. Sample size for single, double and three-way hybrid corn ear traits. Sci. Agr. 64(1):30-35. http://dx.doi.org/10.1590/S0103-90162007000100005.

Streck, N. A.; Weiss, A.; Xue, Q. and Stephen-Baenziger, P. 2003. Incorporating a chronology response into the prediction of leaf appearance rate in winter wheat. Ann. Bot. 92(2):181-190. http://dx.doi.org/10.1093/aob/mcg121.

Toebe, M.; Cargnelutti-Filho, A.; Burin, C.; Casarotto, G. and Haesbaert, F. M. 2014. Tamanho de amostra para estimação da média e do coeficiente de variação em milho. Pesq. Agropecu. Bras. 49(11):860-871. http://dx.doi.org/10.1590/S0100-204X2014001100005.

Yuguda, R. M.; Girei, A. A.; Dire, B. and Salihu, M. 2013. Socio-economic factors and constraints influencing productivity among cassava farmers in Taraba State, Nigeria. Int. J. Adv. Agric. Sci. Technol. 1(1):1-15.