Sample size for estimation of averages of agronomic traits in cassava seedlings

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ABSTRACT

The rapid multiplication method improves the use of propagating material. However, improving management and experimental techniques is crucial for assessing plant production and quality. This study calculated the sample size for estimating averages of agronomic characters in cassava seedlings originating from stem cuttings with a different number of leaves and different diameters. A total of 600 cuttings (each with two buds) with a diameter of 15.00–19.99 mm (N = 300) or 20.00–25.00 mm (N = 300) of the cultivar Apronta Mesa were planted in different growing seasons (July, August, September, and October). On each planting season (September 22, October 7, October 19, and November 25), 300 cuttings, with three to five or even more than five visible leaves, were collected. The following statistics were analyzed: minimum, maximum, range, mean, median, variance, standard deviation, and coefficient of variation. The sample size was determined by resampling using 2,000 resamples, with replacement, and was defined by the number of plants from which the range of 95% confidence interval was 10%, 15%, and 20% of the average estimate. The use of stem cuttings larger than 20 mm in diameter and with more than five leaves was suitable for producing cassava seedlings. Irrespective of the range in cutting diameter and leaf number, 87 plants were enough to estimate trait averages when the range of the confidence interval was 20% of the average estimate.

Keywords: *Manihot esculenta* Crantz, rapid multiplication method, sampling, experimental design.

1. INTRODUCTION

Cassava plants are native to the Amazon and produce starch-rich roots that are used primarily as human and animal food and as an alternative source for producing bioethanol (Bennett 2015). Cassava plants have high rusticity and adaptability to different cultivation conditions (Delaquis et al. 2018) and constitute the third most important food source in the tropics (Hasibuan & Nazir 2017). In addition, cassava is a significant source of income for family farms (Egesi et al. 2007) because of its flexible harvest period and adequate yield under drought and in soils with poor nutritional and physical conditions (Adeniji et al. 2011). Cassava is grown using a traditional planting method with stem cuttings. This method is widely used but limits producing propagating materials with high sanitary quality (Ogero et al. 2012). The average yield is 5,770 kg ha^{-1} in family properties where this method is used (Brazil 2009) and the adoption of management techniques is limited (Khanthavong et al. 2016). Albuquerque et al. (2014) have shown that low crop yield can be attributed to the restricted use of management techniques at the right time, including the control of weeds, pests, and diseases.

Despite its social importance and contribution to national food security, few studies have evaluated strategies to increase cassava yield (Silveira et al. 2012). In this context, the rapid multiplication method developed by the International Center for Tropical Agriculture (Centro Internacional de Agricultura Tropical–CIAT) aims to increase the utilization of high-quality physiological and sanitary propagating materials. The method consists of planting cuttings with two to three buds in propagation beds and, after reaching the harvesting height, collect the cuttings and place them in containers with water to promote rooting. After that, the cuttings are cultivated in pots containing a soil substrate to produce seedlings. Although the method has the adaptation proposed by Koefender et al. (2015) for use in southern Brazil, there are few studies on experimental designing and sample size determination for research using adaptations of fast-multiplication methods for cassava seedlings.

The production of cassava seedlings requires selecting the propagating material to obtain vigorous cuttings with good rooting capacity. For this purpose, cuttings with different diameters should be evaluated because this parameter affects the physiological quality of stems (Bezerra 2012). The criterion used for selecting stems for implementing the rapid multiplication method is height, which usually ranges from 10 to 12 cm. However, the number of leaves is the character that best represents the physiological age of the plants (Streck et al. 2003) and may be a good criterion for collecting stems and obtaining high-quality seedlings. For this reason, it is critical to measure biological parameters to determine stem growth and development during plant production with an adequate degree of accuracy.

In agricultural experiments, measuring all plants that make up an experimental unit is the most appropriate method for estimating averages. However, given the limited time, financial, and labor resources, a given study 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 obtained by resampling have been used to calculate the sample size. This technique is independent of the probability distribution of data (Ferreira 2009) and is utilized to calculate the sample size to estimate trait averages in flax crop (Cargnelutti Filho et al. 2018a). The objective of this study is to calculate the sample size (number of plants) to estimate averages of agronomic traits in cassava seedlings obtained from cuttings with different diameters and a different number of leaves.

2. MATERIAL AND METHODS

A total of 600 cuttings (each with two buds) with a diameter of 15.00–19.99 mm (N = 300) or 20.00–25.00 mm (N = 300) of the Apronta Mesa cultivar were planted in different growing seasons (July, August, September, and October). Planting was carried out 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 (top), 5.0 cm (bottom), and 7.8 cm (height), and each cell had five 6-mm holes at the base for water draining. The trays were filled with the commercial substrate Mec Plant®. After planting, the cuttings were kept in a hatchery (model Van der Hoeven) with sprinkler irrigation.

On each planting season (September 22, October 7, October 19, and November 25), 75 cuttings with a different number of visible leaves (3-5 or >5) and different diameters (<20 mm or >20 mm) were planted, totaling 300 cuttings. The leaf was considered visible when the edges of one of the lobes did not touch each other (Schons et al. 2007). After collection, the cuttings were planted in 15-cell plastic trays filled with wet Mec Plant® substrate to prevent tissue dehydration. Cuttings were planted (one per cell) in 1 cm-deep furrows in the morning at a maximum temperature of 25 ºC. After planting, the trays were kept in a greenhouse (model Van der Hoeven) with an automatic mist irrigation system, with a total irrigation depth of approximately 6 mm of water day^{-1}, at an average temperature of 25 ºC.

At the time of cultivation, 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 (NL7) 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. On each planting season, at 48, 47, 44, and 31 DAP, respectively, the number of leaves at transplanting (NLT) and plant height at transplanting (PHT) were measured in centimeters.

The following statistics were analyzed: minimum, maximum, range, mean, median, variance, standard deviation, and coefficient of variation (CV). The averages were compared using a *t*-test for independent samples at a level of significance of 5%. A total of 66, 72, 66, and 61 plants were evaluated on September 22, October 10, October 19, and November 25, respectively, for estimating trait averages in seedlings originating from cuttings smaller than 20 mm in diameter and with either three to five leaves or more than five leaves.

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 consecutively adding one plant, up to a maximum size of 1,000 plants. Therefore, the planned sample sizes varied from 2 to 999 plants. After that, for each sample size, iterative resampling was performed using 2,000 resamples with replacement. Therefore, 2,000 average estimates of each parameter were obtained for each sample size (Ferreira 2009). The following statistics were determined 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 the 97.5% and 2.5% percentiles.

The sample size was determined by the number of plants from which the range of the 95% confidence interval was equal to 10%, 15%, and 20% of the average estimate. Statistical analyses were performed using R software (R Development Core Team 2014).

3. RESULTS AND DISCUSSION

Standard deviations were higher for PHT in the cultivations carried out on September 22, October 10, and November 25. On October 19, the standard deviations were higher for both PHT and NLT (Tables 1 and 2). Moreover, ranges were higher for PHT and NLT, indicating higher variability in these parameters on the evaluated transplanting dates and the need to calculate adequate sample sizes to estimate average values.

Table 1: Minimum (min), maximum (max), range (ran), mean (m), median (med), standard deviation (SD), and coefficient of variation (CV) of the number of leaves at planting (NLP), number leaves at 7 days after planting (NL7), plant height at planting (PHP), plant height at transplanting (PHT), and number of leaves at transplanting (NLT) of cassava seedlings originating from cuttings with either three to five visible leaves or more than five leaves and a diameter of < 20 mm or > 20 mm planted on September 22 and October 7.

Planting season										
	September 22					October 7				
	3 - 5 leaves / < 20 mm diameter					3 - 5 leaves / < 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	3.00	1.00	0.40	3.30	3.00	3.00	1.00	0.40	2.80	3.00
Max	5.00	6.00	4.00	17.50	11.00	5.00	6.00	5.40	11.90	10.00
Ran	2.00	5.00	3.60	14.20	8.00	2.00	5.00	5.00	9.10	7.00
M	4.14 b	3.92 c	2.01 d	9.52 b	8.06 c	3.50 b	3.03 b	1.61 d	6.30 d	7.11 c
Med	4.00	4.00	2.00	7.65	8.00	3.00	3.00	1.50	6.10	7.00
SD	0.70	1.04	0.79	4.57	1.42	0.63	0.95	0.72	1.62	1.53
CV	16.90	26.56	39.21	48.00	17.66	17.94	31.34	44.86	25.65	21.57
	> 5 leaves / < 20 mm diameter					> 5 leaves / < 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	6.00	2.00	1.10	3.40	5.00	6.00	2.00	1.00	4.20	5.00
Max	9.00	9.00	6.40	18.40	12.00	8.00	6.00	4.50	15.80	12.00
Ran	3.00	7.00	5.30	15.00	7.00	2.00	4.00	3.50	11.60	7.00
M	6.39 a	4.67 b	2.97 b	9.88 b	8.64 b	6.13 a	4.72 a	2.42 b	8.62 b	8.72 a
Med	6.00	5.00	2.90	9.40	9.00	6.00	5.00	2.50	7.50	9.00
SD	0.70	1.71	1.02	3.46	1.58	0.37	1.12	0.64	3.02	1.32
CV	10.93	36.70	34.35	35.03	18.24	6.09	23.64	26.43	35.06	15.18
	3 - 5 leaves / > 20 mm diameter					3 - 5 leaves / > 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	3.00	1.00	0.80	5.60	6.00	3.00	1.00	1.00	3.10	4.00
Max	5.00	6.00	3.60	13.80	11.00	5.00	5.00	3.80	16.50	11.00
Ran	2.00	5.00	2.80	8.20	5.00	2.00	4.00	2.80	13.40	7.00
M	4.24 b	4.05 c	2.28 c	8.76 c	8.42 b	3.78 b	3.25 b	2.17 c	7.44 c	7.72 b
Med	4.00	4.00	2.40	8.30	8.00	4.00	3.00	2.05	7.00	8.00
SD	0.63	1.06	0.64	2.22	1.23	0.79	0.96	0.65	3.41	1.52
CV	14.94	26.17	28.13	25.38	14.58	20.94	29.55	30.00	45.79	19.71
	> 5 leaves / > 20 mm diameter					> 5 leaves / > 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	6.00	3.00	1.80	6.40	7.00	6.00	2.00	1.60	4.50	7.00
Max	9.00	9.00	5.50	19.60	13.00	8.00	7.00	5.50	16.30	11.00
Ran	3.00	6.00	3.70	13.20	6.00	2.00	5.00	3.90	11.80	4.00

M	6.53 a	5.35 a	3.45 a	12.39 a	9.83 a	6.28 a	4.64 a	2.99 a	10.22 a	9.07 a
Med	6.00	5.00	3.45	12.35	10.00	6.00	5.00	3.00	10.35	9.00
SD	0.77	1.44	0.83	3.43	1.53	0.54	1.21	0.79	2.41	0.98
CV	11.78	26.94	23.98	27.71	15.51	8.55	26.17	26.45	23.56	10.84

*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%.

Table 2: Minimum (min), maximum (max), range (ran), mean (m), median (med), standard deviation (SD), and coefficient of variation (CV) of the number of leaves at planting (NLP), number leaves at 7 days after planting (NL7), plant height at planting (PHP), plant height at transplanting (PHT), and number of leaves at transplanting (NLT) of cassava seedlings originating from cuttings with either three to five visible leaves or more than five leaves and a diameter of < 20 mm or > 20 mm planted on October 19 and November 25.

Planting season										
October 19						November 25				
	3 - 5 leaves / < 20 mm diameter					3 - 5 leaves / < 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	3.00	2.00	0.60	2.40	4.00	3.00	2.00	0.90	3.10	5.00
Max	5.00	6.00	2.70	6.20	9.00	5.00	7.00	2.10	9.00	11.00
Ran	2.00	4.00	2.10	3.80	5.00	2.00	5.00	1.20	5.90	6.00
M	3.67 d	3.94 d	1.17 d	4.33 d	6.98 c	3.93 d	4.51 c	1.38 d	5.39 b	8.74 c
Med	4.00	4.00	1.10	4.30	7.00	4.00	4.00	1.40	5.00	9.00
SD	0.67	1.02	0.37	0.90	1.22	0.77	0.94	0.31	1.55	1.30
CV	18.35	25.79	31.33	20.81	17.49	19.62	20.90	22.52	28.78	14.91
	> 5 leaves / < 20 mm diameter					> 5 leaves / < 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	6.00	2.00	1.00	3.30	5.00	6.00	4.00	1.30	4.50	7.00
Max	7.00	7.00	2.90	8.80	10.00	7.00	7.00	4.30	12.60	13.00
Ran	1.00	5.00	1.90	5.50	5.00	1.00	3.00	3.00	8.10	6.00
M	6.17 b	5.36 b	1.72 c	5.62 b	8.26 b	6.11 b	5.92 b	2.25 b	7.19 a	9.21 b
Med	6.00	6.00	1.70	5.30	8.00	6.00	6.00	2.10	6.70	9.00
SD	0.38	0.95	0.40	1.38	1.18	0.32	0.49	0.61	1.60	1.25
CV	6.09	17.80	23.36	24.61	14.30	5.26	8.33	27.12	22.30	13.60
	3 - 5 leaves / > 20 mm diameter					3 - 5 leaves / > 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	3.00	2.00	1.20	3.00	5.00	3.00	3.00	0.90	3.50	5.00
Max	5.00	6.00	3.70	8.30	10.00	5.00	6.00	2.60	8.90	11.00
Ran	2.00	4.00	2.50	5.30	5.00	2.00	3.00	1.70	5.40	6.00
M	4.23 c	4.27 c	1.98 b	5.23 c	7.95 b	4.21 c	4.77 c	1.75 c	5.67 b	8.82 c
Med	4.00	4.00	1.90	5.10	8.00	4.00	5.00	1.70	5.60	9.00
SD	0.72	0.89	0.50	1.22	1.33	0.69	0.84	0.39	1.16	1.22

CV	17.01	20.75	25.34	23.39	16.71	16.28	17.70	22.16	20.40	13.81
	> 5 leaves / > 20 mm diameter					> 5 leaves / > 20 mm diameter				
Estat	NLP	NL7	PHP	PHT	NLT	NLP	NL7	PHP	PHT	NLT
Min	6.00	3.00	1.40	3.80	6.00	6.00	4.00	1.40	5.20	6.00
Max	8.00	7.00	4.30	11.50	11.00	8.00	8.00	5.50	11.30	11.00
Ran	2.00	4.00	2.90	7.70	5.00	2.00	4.00	4.10	6.10	5.00
M	6.35 a	5.80 a	2.85 a	7.69 a	9.20 a	6.38 a	6.13 a	2.72 a	7.25 a	9.56 a
Med	6.00	6.00	2.85	7.40	9.00	6.00	6.00	2.60	7.10	10.00
SD	0.59	0.83	0.64	1.51	1.13	0.61	0.67	0.72	1.40	1.01
CV	9.37	14.25	22.32	19.62	12.25	9.57	10.93	26.49	19.29	10.55

*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%.

The CV was lower for NLP, ranging from 5.26% to 20.94% in seedlings originating from cuttings with three to five leaves or more than five leaves and a diameter of <20 mm or >20 mm on the four planting season. In contrast, the CV was higher for PHP (22.16–44.86%) and PHT (19.29–48.00%). Furthermore, the CV was higher for parameters obtained by measurements compared to those obtained by counting, suggesting that at a given degree of accuracy, a larger sample size is required for characters obtained by measurement compared to those obtained by counting. In contrast, in jack beans (*Canavalia ensiformis*), the average CV in morphological characters was 21.70%, except for the number of leaves (95.65%) (Cargnelutti Filho et al. 2018b).

For the five measured traits, there were significant differences among the averages for combinations between observed trait for cutting collection (number of leaves) and stem cutting diameters in the four planting seasons. Seedlings produced from cuttings with three to five leaves and with a diameter of <20 mm had lower averages for all parameters, regardless of the planting season. In turn, seedlings from cuttings with more than five leaves and with a diameter of >20 mm had higher averages for all parameters on the four planting seasons. This result indicated that seedlings from cuttings with a diameter of >20 mm and with more than five leaves showed better growth and development. The success of vegetative propagation is conditioned by the level of nutrient storage in plant tissues for plant growth and development (Neves et al. 2018).

The sample size necessary to estimate trait averages on different cultivation dates was highly variable among the four types of seedlings produced (Table 3). When the range of the confidence interval was 10% of the average estimate, in seedlings originating from cuttings with either three to five leaves or more than five leaves and with a diameter of <20 mm on the four planting seasons, the sample size ranged from 35 to 361 plants and from 2 to 215 plants, respectively. In seedlings from cuttings with either three to five leaves or more than five leaves and with a diameter of >20 mm, the sample size ranged from 29 to 354 plants and 12 to 124 plants, respectively. Sample size variability was also observed in parameters from pecan (Cargnelutti Filho et al. 2014), black oats (Cargnelutti Filho et al. 2015), and crotalaria (Toebe et al. 2018).

Table 3: Sample size (number of plants) for the confidence interval ranges of 10%, 15%, and 20% of the average estimate of plant height at planting (PHP), number of leaves at planting (NLP), number of leaves at 7 days after planting (NL7), plant height at transplanting (PHT), and number of leaves at transplanting (NLT) of cassava seedlings originating from cuttings with either three to five leaves or more than five leaves and with a diameter of either <20 mm or >20 mm on four planting seasons.

	PHP			NLP			NL7			PHT			NLT		
3 - 5 leaves / < 20 mm diameter															
Season	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%
Sep 22	250	105	60	44	21	11	114	46	29	361	163	87	49	21	12
Oct 7	329	135	80	58	23	12	155	69	40	103	44	26	76	30	19
Oct 19	160	67	40	55	24	14	110	47	25	68	31	16	49	22	13
Nov 25	77	33	18	60	27	16	69	35	17	127	55	31	35	14	9
> 5 leaves / < 20 mm diameter															
	PHP			NLP			NL7			PHT			NLT		
Season	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%
Sep 22	187	85	45	18	8	4	215	93	52	196	88	47	50	22	13
Oct 7	107	46	26	4	3	2	85	39	23	190	86	46	36	17	9
Oct 19	84	38	20	2	2	2	51	22	13	93	41	23	31	15	7
Nov 25	113	52	29	2	2	2	11	6	3	75	35	18	28	13	8
3 - 5 leaves / > 20 mm diameter															
	PHP			NLP			NL7			PHT			NLT		
Season	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%
Sep 22	123	55	30	38	15	10	111	46	28	102	45	25	32	16	9
Oct 7	144	64	35	78	31	19	139	60	32	354	141	84	60	27	15
Oct 19	105	44	24	45	21	10	71	30	17	88	39	21	41	19	11
Nov 25	76	32	19	41	18	10	49	21	12	67	28	16	29	13	8
> 5 leaves / > 20 mm diameter															
	PHP			NLP			NL7			PHT			NLT		
Season	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%	10%	15%	20%
Sep 22	93	38	24	23	10	4	115	53	28	124	53	31	37	16	10
Oct 7	108	48	27	12	5	2	108	45	25	91	38	21	18	9	5
Oct 19	78	35	19	15	6	3	32	14	8	60	28	14	20	11	5
Nov 25	107	47	27	15	7	3	22	9	5	56	24	15	17	8	5

Larger sample sizes were required to estimate parameter averages at the highest accuracy, that is, when the range of the confidence interval was 10% of the average estimate. Smaller sample sizes were necessary for estimating characters measured in later cultivation times, whereas larger sample sizes were required in cultivations performed on September 22 and October 7. Accordingly, a sample size of 28, 25, 8, and 5 plants was used to estimate NF7DAP on September 22, October 7, October 19, and November 25, respectively, in seedlings originating from cuttings with more than five leaves and with a diameter of >20 mm when the range of the confidence interval was 20% of the average estimate. This difference in sample size can be explained by the lower variability in this trait in seedlings cultivated on October 19 and November 25 (Table 2).

Smaller sample sizes were required to evaluate parameters obtained by counting compared to those obtained by measurements. A total of 37, 18, 20, and 17 plants were measured to estimate NLT on September 22, October 7, October 19, and November 25, respectively, in seedlings derived from cuttings with more than five leaves and with a diameter of >20 mm when the range of the confidence interval was 20% of the average estimate. In contrast, 124, 91, 60, and 56 plants were measured to estimate PHT under the same conditions as NLT. Given that there was variability in sample size between characters, seedling origin, and time of planting, sample sizes should be chosen considering these factors and the degree of accuracy set by the researcher. Variability in sample size for estimating morphological characters was also reported by Kleinpaul et al. (2017) in millet crops (*Pennisetum glaucum* (L.) R. Brown) and by Schabarum et al. (2018) in crotalaria (*Crotalaria juncea*).

There was a significant and positive correlation between the CV and sample size. The CV for PHT ranged from 19.29% to 48.00%, and the sample sizes for this trait at these two limits of variation for the confidence interval ranges of 10%, 15%, and 20% were 56, 24, and 15 plants and 361, 163, and 87 plants, respectively. Toebe et al. (2014) found that morphological traits with higher CVs required larger sample sizes to estimate averages. Bandeira et al. (2016) observed that the increase in variability increased the sample size for measuring traits in passion fruit culture (*Passiflora caerulea*).

These results allow researchers to choose the sample size and degree of accuracy according to the financial and technical resources for data collection and the available experimental area. Measurements with higher accuracy produce more reliable estimates but require the analysis of a larger number of plants. Moreover, estimating sample size at different levels of accuracy allows researchers to choose the level that best fits the research conditions. It is known that, in models with low accuracy, the number of sampled plants is decreased, but the reliability of average estimates is also decreased.

4. CONCLUSIONS

Regardless of the range in stem cutting diameter and leaf number when selecting cuttings, 87 plants are enough to estimate average values of morphological characters in cassava when the range of the confidence interval was 20% of the average estimate.

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