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Scientific Note

Physiological ages of uninodal segments in the production of cassava seedlings[1]

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ABSTRACT

Rapid propagation technologies have been used to increase the supply of basic cassava (*Manihot esculenta* Crantz) planting material, with emphasis on the production of etiolated seedlings, use of mini-cuttings, and reduction in the size of conventional stem cuttings. This study aimed to propose an accelerated multiplication protocol based on uninodal segments (single bud), as well as to evaluate whether the physiological age of the source material affects seedling establishment and development. The experiment was conducted using a completely randomized design, in a 3 × 3 factorial arrangement, with the cultivars BRS Prata, BRS Poti Branca, and Eucalipto and three physiological ages (top, middle, and base). The basal and middle segments provided a higher survival, whereas apical segments promoted a greater vegetative performance, with plant height reaching up to 57.41 cm, in addition to greater induction of tuberization in the Eucalipto cultivar. The protocol proved to be technically feasible and allowed the estimation of a production of 343 seedlings per year from a single mother plant, exceeding the multiplication rate attributed to mini-cuttings and the conventional method.

KEYWORDS: *Manihot esculenta* Crantz, vegetative propagation, accelerated multiplication protocol.

RESUMO

Idades fisiológicas de segmentos uninodais na produção de mudas de mandioca

Tecnologias de propagação rápida têm sido empregadas para ampliar a oferta de material básico de mandioca (*Manihot esculenta* Crantz), com ênfase na produção de mudas estioladas, no uso de miniestacas e na redução do tamanho das manivas convencionais. Objetivou-se propor um protocolo de multiplicação acelerada baseado em segmentos uninodais (gema única), bem como avaliar se a idade fisiológica do material de origem interfere no estabelecimento e desenvolvimento das mudas. O experimento foi conduzido em delineamento inteiramente casualizado, em esquema fatorial 3 × 3, utilizando-se as variedades BRS Prata, BRS Poti Branca e Eucalipto e três idades fisiológicas (topo, meio e base). Segmentos da base e do meio proporcionaram maior sobrevivência, enquanto os do topo promoveram maior desempenho vegetativo, com altura de até 57,41 cm, além de maior indução de tuberização na variedade Eucalipto. O protocolo mostrou-se tecnicamente viável e permitiu estimar a produção de 343 mudas por ano a partir de uma única planta-matriz, superando a taxa de multiplicação atribuída às miniestacas e ao método convencional.

PALAVRAS-CHAVE: *Manihot esculenta* Crantz, propagação vegetativa, protocolo de multiplicação acelerada.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) occupies a strategic position in food security and tropical agricultural systems because of its high production of starch-rich storage roots and its versatility for feeding and industrial processes (Nina et al. 2021). Despite this relevance, the availability of high-quality propagative material remains a bottleneck for the expansion and renewal of cultivated areas.

One of the most sensitive stages of the cassava production system is plant multiplication, which is traditionally performed by vegetative propagation using stem cuttings. Although widely adopted, this

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method has a low multiplication rate, phytosanitary limitations, and logistical constraints related to the transport and storage of stems (Oliveira et al. 2020, Rocha et al. 2021).

To overcome these limitations, rapid multiplication strategies associated with seedling production under greater phytosanitary control have been developed, such as the mini-cutting system disseminated by the Reniva Network (Rocha et al. 2026). This technology is based on the use of plants subjected to induced etiolation, which enables an expanded supply of propagative material with greater uniformity and sanitary quality (Rocha et al. 2021).

In this context, the use of uninodal segments represents a promising alternative to optimize the use of stems obtained from etiolated plants, increasing the number of propagules obtained per mother plant and potentially improving the efficiency of the multiplication process.

However, the quality of seedlings obtained by this method may vary according to bud position on the stem, because different portions of the stem differ in their degree of lignification, reserve accumulation, and physiological activity (Aquiles et al. 2021).

The hypothesis was that bud physiological age and genotype directly affect sprouting, rooting, and initial growth of seedlings produced from uninodal segments, thereby influencing vegetative multiplication.

Therefore, this study aimed to evaluate the effect of the physiological age of uninodal segments in three cassava cultivars on seedling survival, production, and initial development, with a view to developing a promising alternative for rapid cassava multiplication.

The experiment was carried out at the Biofábrica da Bahia, in Uruçuca, Bahia state, Brazil, from December 2024 to March 2025, in a greenhouse, under partially controlled environmental conditions.

For the establishment of the experiment, stems were collected from healthy mother plants grown in a nursery and previously subjected to etiolation, with approximately 120 days of age and about 1.0 m in length. Each stem was divided into three physiological portions. Starting at 15 cm above the collar and proceeding toward the apical end, the basal third was defined. Subsequently, 25 cm were delimited to compose the middle third, and the remaining portion was considered the upper third (top). One uninodal segment was removed from each portion by making cuts at 1 cm above and 1 cm below the bud. The segments were then classified according to their position on the stem: base, middle, and top (Figure 1).

The uninodal segments were sown in individual polypropylene tubes with a volumetric capacity of 280 cm³, filled with a commercial substrate based on peat and coconut fiber, and enriched with slow-release fertilizers. The proportions used to fill 180 tubes were as follows: 7.41 kg of Carolina Soil® plant substrate; 3.33 kg of coconut fiber powder; 145.83 g of PG Mix R® fertilizer; 145.83 g of Osmocote R®; and Monceren 250 SC® at 0.1%, diluted in 20 L of water.

Sprinkler irrigation was performed to maintain 70 % of the moisture considered adequate for rooting and seedling growth. During the experimental period, the environment was monitored to ensure homogeneous conditions among the treatments.

The experiment was conducted in a completely randomized design, in a 3 × 3 factorial arrangement,

Photos: Bruno Conceição Reis Cazaes/Herminio Souza Rocha (a)

Figure 1. Protocol for obtaining uninodal segments from cassava plants stimulated to etiolation. A) Cassava plants subjected to etiolation and divided into three portions; B) cutting of uninodal segments; C) separation of segments according to their position on the stem; D) planting in trays for rooting.

combining three cassava cultivars (BRS Prata, BRS Poti Branca, and Eucalipto) and three positions of the uninodal segment on the stem (base, middle, and top). Twenty replicates were used per treatment, totaling 180 experimental units.

At 120 days after the beginning of the experiment, the following variables were evaluated: plant height (cm), measured with a measuring tape; stem diameter (mm), measured at 5 cm above the collar with a digital caliper; number of internodes; distance between buds; survival; shoot biomass and root biomass; and number of tuberous roots per plant.

Biomass was determined by measuring the fresh shoot and root mass, followed by dry mass determination after drying the material in a forced-air circulation oven at 60 ºC, for 72 h.

Data were subjected to the Shapiro-Wilk and Levene tests to verify the assumptions of normality and homogeneity of variance. For variables that met these assumptions, analysis of variance was performed, and means were compared by the Tukey test ($p \leq 0.05$). Biomass variables, which did not meet the assumptions of parametric statistics, were analyzed using the Kruskal-Wallis test followed by the Dunn's test with Bonferroni correction ($p \leq 0.05$).

The results for shoot morphological traits showed a significant effect of the physiological age of the propagative material. According to the analysis of variance (Table 1), a significant interaction between cultivar and physiological age of the segment was observed for plant height, stem diameter, and distance between buds. For number of internodes, however, no significant interaction between factors was detected, and only the isolated effect of segment physiological age was observed.

Higher survival rates were observed in segments from the base and middle of the stem, with a reduction in segments from the top (Figure 2c). It should be emphasized that the survival rate considered the seedlings effectively formed at the end of the experiment.

For seedlings originating from basal segments, mean survival ranged from 70 to 85 %, with the highest value recorded for the BRS Prata cultivar. In middle segments, BRS Poti Branca and BRS Prata showed 80 % of survival, whereas Eucalipto showed 60 %. The lowest values occurred in apical segments, ranging from 45 % in Eucalipto to 70 % in BRS Poti Branca (Figure 2c).

The marked reduction in survival in apical segments, especially for the Eucalipto cultivar, may be associated with the greater structural fragility of these tissues. The lower degree of lignification, greater susceptibility to dehydration, and greater vulnerability to pathogen attack tend to compromise the establishment of younger propagules (Leão et al. 2018, Neves et al. 2020).

The performance observed for the BRS Prata cultivar in the basal position supports the interpretation that more mature segments have greater availability of organic reserves, favoring sprouting and the initial establishment of seedlings (Munhoz et al. 2016). In cassava, the parenchyma of these more lignified portions may act as a source of essential carbohydrates before the consolidation of photosynthetic autonomy.

The unfolding of the interaction for plant height showed that physiological age influenced growth in all cultivars (Figure 2a). In BRS Poti Branca and BRS Prata, top segments produced taller plants, with means of 55.40 and 57.48 cm, respectively, differing statistically from the base and middle positions. In Eucalipto, plants originating from the top (51.52 cm) and middle (42.52 cm) surpassed those from the base (37.66 cm). When the cultivars were compared within the different stem portions, differences occurred only in the middle position, in which BRS Prata had a lower plant height than the others.

Table 1. Summary of the analysis of variance (mean squares) for plant height (PH; cm), stem diameter (SD; mm), number of internodes (NI), and distance between buds (DB; cm) in cassava cultivars as a function of the physiological age of the uninodal segment.

Source of variation	DF	PH	SD	NI	DB
Cultivar (C)	2	7.64^{ns}	3.41***	118.70***	2.75***
Physiological age (A)	2	3,621.37***	2.25**	70.10***	6.35***
C × A	4	199.44*	1.74**	17.26^{ns}	1.16***
Residual	118	70.72	0.44	8.03	0.14
CV (%)		31.78	13.96	16.90	14.45

^{ns}, *, **, and ***: not significant and significant at 5, 1, and 0.1 % of probability by the F test, respectively. CV: coefficient of variation; DF: degrees of Freedom.

Regarding stem diameter, BRS Poti Branca and BRS Prata showed higher values in top segments, differing from middle segments (Figure 2b). For Eucalipto, the top also promoted the highest means, whereas base and middle segments did not differ from each other. When the cultivars were compared within each physiological age, a significant difference was observed only in the basal position; in the middle and top positions, cultivars were statistically similar.

Unlike the pattern observed for survival, shoot growth variables, such as plant height and stem diameter, were favored by the use of top segments. Although more sensitive, juvenile tissues have high cellular plasticity and intense meristematic activity, which favor initial growth (Taiz & Zeiger 2013).

The higher concentration of endogenous auxins in shoot apices favors cell elongation and helps to explain the higher plant height means observed for BRS Poti Branca and BRS Prata when derived from the top. This hormonal gradient along the stem may also contribute to the greater number of internodes observed in apical materials (Taiz & Zeiger 2013).

There was an isolated effect of physiological age on number of internodes (Figure 3). Top segments had the highest mean (18.03), differing from middle segments (15.48), whereas the base (17.00) showed intermediate behavior and did not differ from the other ages by the Tukey test ($p \leq 0.05$).

For distance between buds, the interaction between factors indicated different responses among the cultivars (Figure 4). In BRS Poti Branca, the highest values occurred in top segments (3.11 cm), differing from the middle and base. In BRS Prata, the top also resulted in a greater distance between

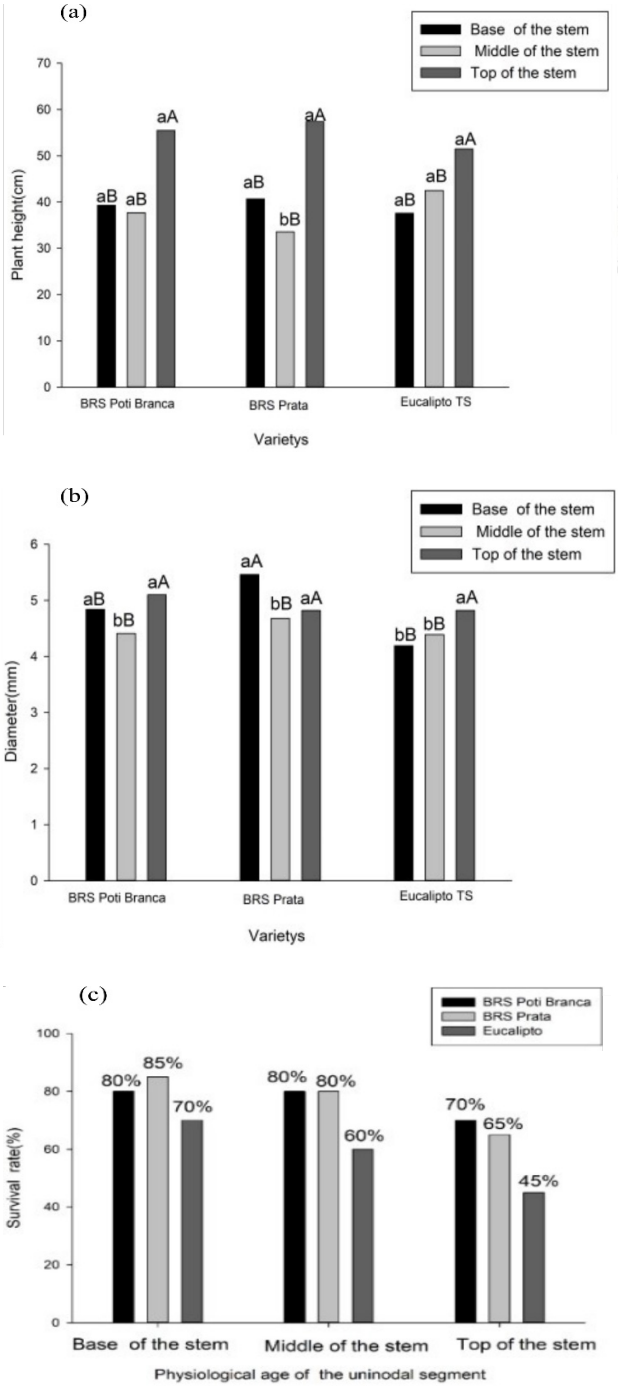

Figure 2. Plant height (a), stem diameter (b), and survival rate (c) of *Manihot esculenta* Crantz seedlings as a function of cultivar and physiological age of the uninodal segment. Uppercase letters compare physiological ages within each cultivar. Lowercase letters compare cultivars within the same physiological age by the Tukey test ($p \leq 0.05$).

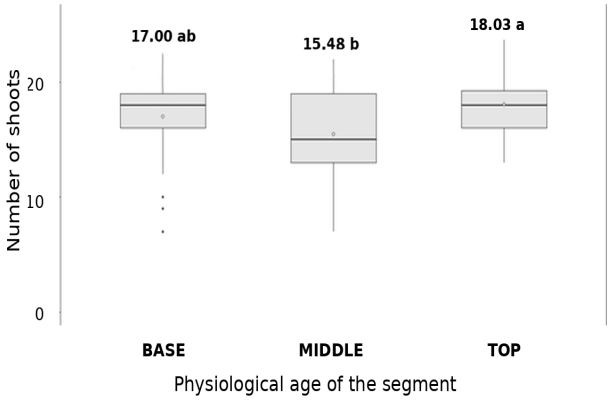

Figure 3. Mean number of internodes in *Manihot esculenta* Crantz seedlings as a function of the physiological age of the uninodal segment. Means followed by the same letter do not differ by the Tukey test ($p \leq 0.05$).

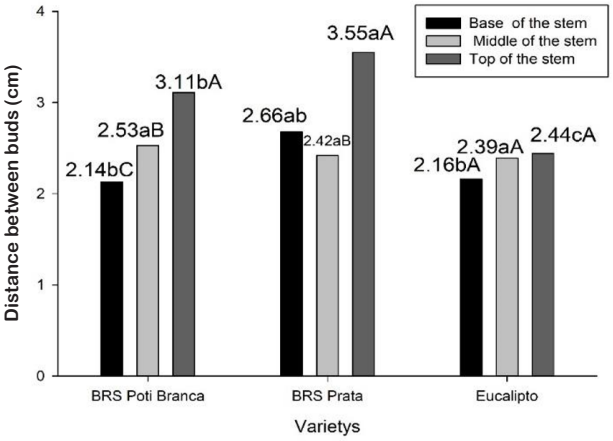

Figure 4. Mean distance between buds as a function of cultivar and physiological age of the cassava uninodal segment. Uppercase letters compare physiological ages within each cultivar. Lowercase letters compare cultivars within the same physiological age by the Tukey test ($p \leq 0.05$).

buds (3.55 cm), when compared with the other positions. For Eucalipto, no significant differences were detected among physiological ages. When the cultivars were compared, BRS Prata showed a greater distance between buds in the top and base positions than the other cultivars.

The significant interaction observed for distance between buds demonstrates that the response to physiological age is genotype-dependent (Figure 4). While the Eucalipto cultivar maintained a greater stability for this trait, BRS Prata stood out for its superiority in the top and base positions. Apical tissues have intense meristematic activity and rapid responses to hormones, especially auxins and gibberellins, which promote cell elongation and longitudinal stem growth (Shi & Vernoux 2022).

On the other hand, the greater uniformity observed in the Eucalipto cultivar indicates morphological stability along the stem, a desirable characteristic for rapid multiplication programs because it provides a greater standardization of the segments used in seedling production. Thus, the results suggest that the choice of cultivar and the physiological position of the segment should consider not only sprouting capacity and seedling growth, but also the multiplication efficiency provided by stem architecture, a determining factor for large-scale production systems (Neves et al. 2020).

Overall, the results show a physiological trade-off between survival and vigor: top segments produce seedlings with greater shoot development but a higher risk of losses during initial establishment, whereas basal and middle segments show greater survival stability.

Thus, the evaluated morphological variables indicate that the physiological age of the uninodal segment directly interferes with the formation and initial vigor of cassava seedlings (Figure 5). The position of the segment on the source stem influences the future behavior of the seedling because different plant regions present distinct levels of physiological maturity, reserves, and hormonal balance (Taiz & Zeiger 2013).

The results for seedling biomass demonstrate that initial vigor, both in the shoot and root system (Figure 6), is influenced by bud position on the stem and by cultivar (Table 2). For shoot fresh mass, the greatest accumulations were recorded in treatments with top buds of the cultivars BRS Poti Branca and BRS Prata, with values equal to or greater than 11.40 g. In BRS Poti Branca, the superiority of the top was statistically greater than that of the middle portion, which showed 6.55 g.

The same pattern of superiority of apical segments was also observed for shoot dry mass, reinforcing that the greater initial growth of materials originating from the top was not restricted to elongation, but involved a greater biomass accumulation.

When root biomass is analyzed together with the frequency of tuberous root emission, it is

Photos: Bruno Conceição Reis Cazaes

Figure 5. Cassava seedlings of the BRS Poti Branca cultivar produced from uninodal segments derived from the base and top portions of the stem.

observed that tuberization initiation and root mass accumulation are related processes, although not necessarily simultaneous (Tables 2 and 3; Figure 7).

It was found that plants can present considerable values of root fresh and dry mass without effective tuberization initiation, suggesting a predominance of fibrous absorbing roots at this stage. This behavior was clear in the BRS Prata cultivar, in which the top of the stem generated an expressive root biomass (7.10 g of fresh mass), but showed the highest percentage of plants without initial tuberization (53.8 %).

For the Eucalipto cultivar, the influence of physiological age on the root system was even more dependent on genetic material. The use of basal and middle segments resulted in the absence of tuberization in more than 70 % of the seedlings, and in lower root dry mass values. This scenario differed in seedlings propagated from top segments, in which the absence of tuberization was reduced

Table 2. Medians of shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM), and root dry mass (RDM) in cassava seedlings subjected to physiological ages of the uninodal segment.

Treatment	SFM (g)	SDM (g)	RFM (g)	RDM (g)
BRS Poti Branca - base	7.20 ab	2.57 ab	5.50 ab	1.00 a
BRS Poti Branca - middle	6.55 b	2.29 b	8.30 ab	1.71 a
BRS Poti Branca - top	11.75 a	4.09 a	11.20 a	2.54 a
BRS Prata - base	11.60 ab	3.51 ab	9.40 ab	1.46 a
BRS Prata - middle	8.40 ab	2.35 b	5.10 ab	0.83 a
BRS Prata - top	11.40 a	3.20 ab	7.10 ab	0.72 a
Eucalipto - base	7.45 ab	2.39 ab	3.15 b	0.51 a
Eucalipto - middle	9.50 ab	2.95 ab	4.10 ab	0.67 a
Eucalipto - top	11.40 ab	4.08 a	11.15 ab	1.77 a

Different letters in the same column indicate a statistically significant difference by the Dunn's test with Bonferroni correction ($p < 0.05$).

Table 3. Relative frequency of the number of tuberous roots per plant in cassava seedlings of different cultivars and stem portions.

Cultivar	Physiological age	0 root (%)	1 root (%)	2 roots (%)
BRS Poti Branca	Base	37.5	62.5	0.0
BRS Poti Branca	Middle	37.5	37.5	25.0
BRS Poti Branca	Top	38.5	46.2	15.4
BRS Prata	Base	40.0	40.0	20.0
BRS Prata	Middle	36.4	54.5	9.1
BRS Prata	Top	53.8	30.8	15.4
Eucalipto	Base	71.4	28.6	0.0
Eucalipto	Middle	72.7	27.3	0.0
Eucalipto	Top	22.2	66.7	11.1

Photos: Bruno Conceição Reis Cazaes

Figure 6. Callus formation and root primordia in cassava mini-cuttings.

Photos: Bruno Conceição Reis Cazaes

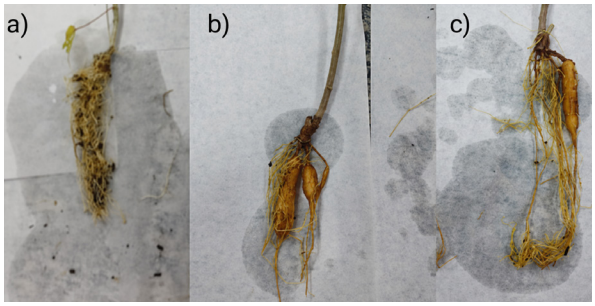

Figure 7. Frequency of tuberous root appearance. a) Plants without tuberous roots; b) with one tuberous root; c) with two tuberous roots.

to 22.2 %, and root biomass accumulation was approximately tripled.

For the BRS Poti Branca cultivar, the behavior was distinct. Although the top accumulated the highest total root mass (11.20 g of fresh mass), this result did not translate into a greater number of tuberous roots when compared with the middle third of the stem. This result reinforces that root vigor and tuberization onset should be interpreted as complementary, but not equivalent, variables.

The high multiplication capacity of the propagative material is one of the main advantages of the method. For this comparative analysis (Table 4), a single cassava mother plant was considered, and its capacity to generate new plants over one year (365 days) was estimated, considering a 120-day cycle for each plant to reach 1 m in length and become able to provide propagation material.

The Reniva Network approach, using mini-cuttings, represents an advance, if compared with the conventional method (Rocha et al. 2021). In this model, a 1-m stem obtained in 120 days is divided into mini-cuttings of approximately 12 cm, containing two to three buds. Thus, each stem can generate four propagation units per cycle.

In the conventional system, considering only the use of the viable middle third for producing seed stem cuttings, multiplication capacity remains limited. In a practical estimate, from a single mother plant, the conventional method would allow the production of approximately six new plants per year.

Maintaining the same 120-day cycle for each new plant to become a mother plant, multiplication occurs in a network: generation 1 (120 days) produces 4 plants; generation 2 (240 days) produces 16 new plants; and generation 3 (360 days) produces 64 new plants. At the end of one year, the mini-cutting-based method would result, cumulatively, in approximately 84 plants from a single initial mother plant.

The uninodal segment method is based on maximizing the stem use, especially stems from plants subjected to etiolation. As observed in the field, an etiolated stem of 1 m in length produced in 120 days can contain 12 to 15 viable internodes for the production of uninodal segments. For this comparative analysis, a conservative scenario was adopted, considering a survival rate of 60 % for the segments, regardless of physiological age. Under this premise, each stem with 12 buds would generate approximately 7 viable seedlings.

Applying the same network multiplication logic, starting from a single initial plant, generation 1 (120 days) would produce 7 viable seedlings; generation 2 (240 days) 49 new plants; and generation 3 (360 days) 343 plants. These values demonstrate the multiplicative potential of the method.

The possibility of generating hundreds of seedlings from a single mother plant in less than one year highlights the potential of the method for large-scale production of propagative material. Therefore, the implementation of the seedling production system based on uninodal segments represents a relevant technological advance to overcome limitations of the traditional method and may contribute to increasing the availability of propagative material with greater operational efficiency.

Basal and middle segments provide higher survival rates in the formation of cassava seedlings.

Apical segments, although associated with higher mortality, produce plants with greater initial vegetative performance and a greater number of internodes.

The propagation method using uninodal segments has a high multiplication potential, with an estimated production of 343 seedlings annually from a single mother plant.

Table 4. Comparison among the conventional propagation, mini-cutting, and uninodal segment methods.

Propagation method	Seedlings produced in 1 year (from 1 initial plant)	Observations
Conventional	6 seedlings	Limited multiplication, with sanitary and logistical challenges
Reniva/biofábrica da Bahia (mini-cutting)	84 seedlings	Significant improvement, but with a lower multiplication ceiling than that observed for the uninodal segment
Uninodal segment	343 seedlings	Exponential multiplication, even under a conservative scenario (60 % of survival), with high efficiency in the use of genetic material and potential for seedling selection based on vigor or survival

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