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CROP PRODUCTION

Adaptation of the rapid multiplication method: selecting stem cuttings based on their number of leaves for cassava seedling production

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ABSTRACT. A study was conducted during four growing seasons to investigate the rooting capacity and survival percentage of cassava seedlings from stem cuttings selected based on their number of leaves. The experimental design was a randomized block design with five replications in a factorial scheme (4 × 2 × 2), totalling 16 treatments. Treatments consisted of combinations of growing season (September 22, October 7 and 19, and November 25), stem cuttings smaller (15 to 19.99 mm) and larger (20 to 25 mm) than 20 mm in diameter, and number of leaves on the stem cuttings (3 to 5 and 6 to 8 leaves). The experiment was carried out during the four growing seasons in a Van der Hoeven greenhouse. The greenhouse, as well as the benches on which the treatments were placed, had a north-south orientation. The greenhouse has an automatic mist irrigation system, and the mean temperature was 25 °C. The plant height at planting, number of leaves at planting, and number of leaves at 7 days after planting were measured. The final number of leaves, final plant height, shoot dry matter, root dry matter, and total dry matter were measured after acclimatization. Stem cuttings between 20 and 25 mm in diameter should be used to produce cassava seedlings with the rapid multiplication method. Cuttings with 6 to 8 visible leaves should be collected, and the cuttings should be planted at the end of September.

Keywords: *Manihot esculenta* Crantz; vegetative propagation; physiological age; development.

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Introduction

Cassava (*Manihot esculenta* Crantz) belongs to the Euphorbiaceae family. This species is grown in many countries around the world and contributes to food security due to its production of tuberous roots that have high commercial interest (Rangel, Fey, Neubert, & Fidalski, 2018). Cassava is an important source of energy and nutrition that has good palatability; cassava root is well accepted by several herd animals, such as cattle, and cassava peel is an alternative for sheep feed (Faria et al., 2011). In addition, cassava has a wide potential for ethanol production (Salla & Cabello, 2010).

Cassava has high socioeconomic importance, especially in tropical regions, due to its high rusticity, ease of propagation, low soil fertility demands, and tolerance of water stress. The average cassava yield on Brazilian family farms is approximately 5,770 kg ha^{-1}, as it is mainly grown in areas with poor edaphic quality (Brasil, 2009). Low-quality propagation material and insufficient planting and management methods have contributed to low cassava yields (Silva et al., 2013). Therefore, the use of alternative methods is an effective and cost-effective way to increase yield.

The traditional and most commonly used planting method for cassava is the propagative method, which consists of planting stem cuttings with approximately six buds in unfertilized furrows and spacings that vary according to the cultivar and available technology. This method presents a low annual multiplication rate per plant, with a value of approximately 1:10 (Santos et al., 2009). This is characterized as an important limiting factor on the utilization of high-quality propagation materials and according to Ceballos, Ramirez, Bellotti, Jarvis, and Alvarez (2011), the use of rapid multiplication techniques is important for increasing the cassava multiplication rate.

Therefore, the rapid multiplication method developed by the International Center for Tropical Agriculture (CIAT) in Colombia is an alternative to the traditional planting method, allowing an increased in the utilization rate of sanitary and physiological quality stems. It consists of planting two-bud stem cuttings in beds covered with transparent plastic film to promote rooting. Sprouts are cut at the base (1 to 2 cm above the soil surface) when they reach approximately 15 cm in height and are placed in containers with water to stimulate adventitious rooting. After rooting begins, these stem cuttings are planted in containers with a substrate for seedling establishment. According to Santos et al. (2009), the use of this method allows an increase in the multiplication rate of up to 16 times compared to that in the traditional method. In addition, according to Piza and Pinho (2002), the rapid multiplication method is particularly important in cases of disease, pest attack, or frost damage, which drastically reduce the quantity and quality of propagation materials. In these cases, the use of the rapid multiplication method results in an improvement in the utilization rate of the available propagation material.

Despite adaptations of this method to cultivation conditions in the Northeast, North and South regions of Brazil, the trait used to select stem cuttings is the height, regardless of the cultivar and growing season. According to Streck, Weiss, Xue, and Stephen Baenziger (2003), the number of leaves reflects the physiological age of the plants and, thus, may be an appropriate trait for selecting cassava stem cuttings when using the rapid multiplication method, regardless of the cultivar. Replacing rooting in water with direct planting in the substrate may be an alternative for reducing the time required to obtain seedlings when the rooting potential of the cassava cuttings is known (Rodrigues et al., 2008).

Rapidly obtaining seedlings is important, especially in southern Brazil, where there is a dormancy period from winter to early spring due to low temperatures (Fagundes et al., 2009). In this context, studies on the use of adaptations of the rapid multiplication method in controlled environments during winter to produce cassava seedlings require attention because they address the possibility of obtaining seedlings for planting in the recommended seasons. In addition, the scarce information in the literature on cassava seedling production by the rapid multiplication method as well as the absence of a defined morphological character that can serve as an indicator for the choice and collection time for cuttings used for rooting are gaps that hinder the greater use of the rapid multiplication methodology.

This study aimed to verify the rooting capacity and survival percentage of cassava stem cuttings selected based on their number of leaves and planted in trays with a substrate in four growing seasons.

Material and methods

Stem cuttings of the cultivar Apronta Mesa for shoot collection were planted in July, August, September, and October in 15-cell black plastic trays with dimensions of 34 cm long × 21 cm wide × 7.8 cm high. The cells were 6.2 cm at the top × 5.0 cm square at the bottom × 7.8 cm in height, with five 6-mm holes to drain excess water applied via irrigation. The trays were filled with the commercial substrate Mec Plant®.

The experiment was carried out in 2017 during four growing seasons (September 22, October 7 and 19, and November 25) in a Van der Hoeven greenhouse. The greenhouse, as well as the benches on which the treatments were placed, had a north-south orientation. The structure has an automatic mist irrigation system. The mean temperature was 25°C. Irrigation was applied nine times each day, totalling approximately 6 mm day^{-1}.

The experimental design was a randomized block design with five replications in a factorial arrangement (4 × 2 × 2), totalling 16 treatments. Treatments consisted of the combination of growing season (September 22, October 7 and 19, and November 25), stem cuttings smaller (15 to 19.99 mm) and larger (20 to 25 mm) than 20 mm in diameter, and number of leaves (3 to 5 and 6 to 8 leaves).

Stem cuttings were collected using a stiletto knife disinfected with 70% alcohol. The cut was performed one centimetre above the substrate surface. Each stem cutting was immediately planted in a 15-cell black plastic tray with the same dimensions as those described previously. The trays were filled with previously moistened Mec Plant® commercial substrate to prevent the dehydration of tissues at planting. A one-centimetre deep furrow was opened, and one stem cutting was planted in each cell. The experimental unit consisted of a tray of 15 plants. Planting was carried out in the morning at a maximum temperature of 25°C to minimize the risk of tissue dehydration.

Plant height at planting (PHP) was measured from the base to the last visible leaf, and the number of leaves at planting (NLP) was counted. The leaf was considered visible when the edge of one of the leaf lobes did not touch the other leaf lobes (Schons, Streck, Kraulich, Pinheiro, & Zanon, 2007). The number of visible leaves was counted again at 7 days after planting (NL 7 DAP).

The seedlings were removed from the greenhouse at 30 DAP and taken to an agricultural greenhouse for a minimum of 5 days of acclimation, except in the November 25 growing season, when the seedlings were acclimated at 23 DAP. The final number of leaves (FNL), final plant height (FPH), shoot dry matter (SDM), and root dry matter (RDM) were determined in each growing season (September 22, October 7 and 19, and November 25, i.e., at 48, 47, 44, and 31 DAP, respectively). The total dry matter (TDM) was obtained by adding the shoot and root dry matter. All weights were determined on a precision scale with a 0.01 g precision. Two plants from each experimental unit were assessed for the traits measured in a destructive assessment (SDM and RDM), with values expressed in mg plant^{-1}. All plants in each experimental unit were considered for the final survival percentage (FS%), which was calculated by dividing the number of plants that survived until the end of acclimation by the total number of plants and multiplying the result by 100.

The assumptions of the mathematical model for the homogeneity of treatment variances and normality of errors were verified before the analysis of variance by applying the Bartlett and Shapiro-Wilk tests, respectively, at 0.05 error probability. A Box-Cox procedure (Box & Cox, 1964) was used when the assumptions were not met to verify the appropriate transformation of the obtained data using Action software (Equipe Estatcamp, 2014). The results were subjected to analysis of variance, and means were compared by the Scott-Knott test at 0.05 probability using the statistical package Sisvar 5.6 (Ferreira, 2011).

Results and discussion

None of the nine assessed traits had significant triple interactions. A significant double interaction was observed only between plant height at planting (PHP) and number of leaves at planting (NLP). An analysis of the interaction was carried out between growing seasons and diameter and between growing seasons and number of leaves. An analysis of the main effects of the significant factors was performed for the number of leaves at 7 days after planting (NL 7 DAP), the final plant height (FPH), the final number of leaves (FNL), and the final survival percentage (FS%).

The Box-Cox procedure was applied to verify the appropriate transformation of the shoot (SDM), root (RDM), and total dry matter (TDM) (destructive assessments) data because the data did not meet the assumptions of the mathematical model. A significant interaction effect on root dry matter was observed between the growing season and the number of leaves. The main effects of the significant factors were analysed for the shoot and total dry matter.

The number of leaves at planting trait affected the number of leaves of seedlings factor; seedlings from cuttings with 6 to 8 leaves had more leaves than seedlings from cuttings with 3 to 5 leaves in all growing seasons (Table 1). This result confirmed the difference between the two levels of the number of leaves factor. Seedlings from cuttings with 3 to 5 leaves and 6 to 8 leaves had the highest number of leaves when they were planted on September 22, with values of 4.21 and 6.44 leaves, respectively.

Table 1. Number of leaves at planting (NLP), plant height at planting (PHP), and root dry matter (RDM) of cassava seedlings from stem cuttings smaller (< 20 mm) and larger (> 20 mm) than 20 mm in diameter and with 3 to 5 and 6 to 8 leaves planted in four growing. seasons.

	NLP		PHP (cm)		RDM (mg plant^{-1})	
	Number of leaves		Diameter		Number of leaves	
Season	3 a 5 leaves	6 a 8 leaves	< 20 mm	> 20 mm	3 a 5 leaves	6 a 8 leaves
September 22	4.21*aB	6.44 aA	2.48 aB	2.88 aA	239.50 aA	337.00 aA
October 7	3.64 cB	6.19 bA	2.01 bB	2.58 aA	122.50 bB	194.00 bA
October 19	3.95 bB	6.25 bA	1.45 cB	2.40 bA	169.50 aB	274.00 aA
November 25	4.03 bB	6.24 bA	1.81 bB	2.21 bA	94.00 bB	237.50 aA
CV (%)	3.65		15.23		12.25	

*Means followed by different lowercase letters in the column and uppercase letters in the row for each trait differ from each other by the Scott-Knott test at 0.05 probability.

For the plant height at planting, stem cuttings larger than 20 mm in diameter provided larger cuttings in all growing seasons. The plant height was higher at planting on September 22 of all seasons for both diameter intervals but did not differ significantly from that carried out on October 7 for stem cuttings larger than 20 mm. In general, plant height decreased for both assessed diameter intervals over the growing seasons. A reduction in the stem cutting weight and the accumulation of reserve substances as storage time increased may have affected the growth of cuttings and, consequently, the plant height at planting. According to Alves

(2006), shoot and root system growth is mainly influenced by the nutritional reserves of stem cuttings until approximately 30 days after planting.

The root dry matter was higher in seedlings from cuttings with 6 to 8 leaves in all growing seasons and did not differ significantly from that in cuttings with 3 to 5 leaves in the planting carried out on September 22. The root dry matter for seedlings from cuttings with 3 to 5 and 6 to 8 leaves was 94 and 237.50 mg plant^{-1}, respectively, on November 25. Stem cuttings smaller than 20 mm in diameter planted in October that generated the cuttings planted on November 25 had a lower stem cutting weight and, consequently, a lower carbohydrate contribution. This negatively influenced the rooting capacity of seedlings during this season since, during the rooting period, there is a great nutritional demand in order to maintain the physiological growth and development processes (Neves, Diniz, & Oliveira, 2018). Carbohydrate and auxin synthesis occurs in leaves, and their presence in plant cuttings favours survival and rooting (Pacheco & Franco, 2008). In addition, the vigour and nutritional characteristics of the parent plant influence the rooting of cuttings from different plant species (Fachinello, Hoffmann, Nachtigal, Kersten, & Fortes, 1995; Hartmann, Kester, Davies, & Geneve, 2002).

The seedlings produced from cuttings with 6 to 8 leaves showed the best results for the number of leaves at 7 days after planting, final number of leaves, final plant height, and shoot, root, and total dry mass. This result is an indication that cuttings with 6 to 8 leaves produced cassava seedlings with better growth and development in their shoot and root system (Table 2). The root differentiation capacity is influenced by endogenous factors of each species and exogenous factors related to the conditions of the rooting medium, such as temperature, light, and moisture. Endogenous factors include substances synthesized by leaves and carried by vascular vessels, such as carbohydrates, plant hormones, nitrogen compounds, and vitamins (Barbosa & Lopes, 2007).

Table 2. Number of leaves at 7 days after planting (NL 7 DAP), final number of leaves (FNL), final plant height (FPH), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM) and final survival percentage (FS%) of cassava seedlings from stem cuttings smaller (< 20 mm) and larger (> 20 mm) than 20 mm in diameter and with 3 to 5 and 6 to 8 leaves.

Number of leaves				Diameter			
Trait	3 a 5 leaves	6 a 8 leaves	CV (%)	Trait	< 20 mm	> 20 mm	CV (%)
NL 7 DAP	3.93* b	5.26 a	11.56	NL 7 DAP	4.47 b	4.72 a	11.56
FNL	7.98 b	8.98 a	7.68	FNL	8.19 b	8.78 a	7.68
FPH	6.71 b	8.56 a	28.67	FS (%)	88.00 b	92.83 a	10.97
SDM	355.87 b	606.50 a	10.88	SDM	430.62 a	531.75 a	10.88
RDM	156.37 b	260.62 a	12.25	RDM	191.37 a	225.62 a	12.25
TDM	512.25 b	867.12 a	15.96	TDM	622.00 b	757.37 a	15.96

*Means followed by different letters differ from each other by the Scott-Knott test at 0.05 probability.

Among plant hormones, cytokinins are responsible for cell multiplication in the shoot apical meristem but decrease the size of the root apical meristem when they are present at high levels (Taiz & Zeiger, 2013). Auxin is responsible for cell division in the root apical meristem. Although the shoot apex is the main source of auxin for the whole plant, basipetal polar transport contributes to its arrival in the roots. Adventitious root formation and root growth are favoured when the concentration of endogenous auxin is higher than that of cytokinins (Moubayidin et al., 2010; Taiz & Zeiger, 2013). On the other hand, the presence of more cytokinins than auxins favours adventitious sprouting (Hartmann et al., 2002). Because auxin synthesis occurs in meristems and young dividing tissues, especially in the stem apex, the higher number of leaves may have favoured auxin synthesis and, consequently, favoured adventitious rhizogenesis in seedlings from cuttings with 6 to 8 leaves.

Stem cutting diameters larger than 20 mm provided seedlings with a higher number of leaves at 7 days after planting, final number of leaves, final survival percentage, and shoot, root, and total dry matter. However, shoot and root dry matter showed no significant differences in stem cuttings smaller than 20 mm in diameter. Despite the absence of a significant difference, the total dry matter was higher in seedlings from stem cuttings larger than 20 mm in diameter than in seedlings from stem cuttings smaller than 20 mm in diameter, and seedlings from the thicker cuttings had a higher final survival percentage (92.83%). This result indicates that stem cuttings larger than 20 mm in diameter resulted in larger and better quality seedlings for transplanting to production fields. Smaller-diameter stem cuttings from herbaceous stems have a high water content and reduced accumulation of reserve substances, resulting in plants that are less resistant to climate adversity. Vigorous shoot development is important for the production and accumulation of the reserves used at the beginning of plant growth, and the selection of quality stem cuttings is essential for increasing cassava yield (Yomeni, Akoroda, & Dixon, 2012).

The number of leaves at 7 days after planting was the highest in the planting carried out on November 25. Planting on October 7 led to the worst result for this trait. The result may have been influenced by the lower leaf fall in seedlings planted on October 7; leaf fall was observed to a lesser extent during other growing seasons (Table 3).

Table 3. Number of leaves at 7 days after planting (NL 7 DAP), final number of leaves (FNL), final plant height (FPH), final survival percentage (FS%), shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) of cassava seedlings grown in four seasons.

Season	NL 7 DAP	FNL	FPH (cm)	FS (%)	SDM	RDM	TDM
September 22	4.43* c	8.68 a	10.44 a	94.66 a	659.00 a	288.25 a	947.25 a
October 7	3.88 d	8.11 b	8.15 b	97.33 a	348.75 b	158.25 b	507.00 c
October 19	4.78 b	8.07 b	5.67 c	85.33 b	419.00 b	221.75 a	640.75 b
November 25	5.29 a	9.05 a	6.28 c	84.33 b	498.00 a	165.75 b	663.75 b
CV (%)	11.56	7.68	28.67	10.97	10.88	12.25	15.96

*Means followed by different letters differ from each other by the Scott-Knott test at 0.05 probability.

The best results for the final number of leaves were observed in the cultivation periods starting on September 22 and November 25. Despite the significant differences for this trait, the number of leaves between growing seasons ranged from 8.07 to 9.05 leaves plant^{-1}, i.e., the final number of leaves on seedlings ranged from 8 to 9 leaves plant^{-1} for the cultivar Apronta Mesa between the assessed growing seasons (September 22 to November 25). This result indicated that the development of cassava seedlings was favoured when they were planted during this period. This significant difference is unlikely to have a positive or negative effect on seedlings after transplanting to the field from a biological point of view; the observed amplitude was only one leaf, and transplanting conditions such as air and soil temperature, moisture and physicochemical characteristics can be important for the survival, growth, and development of seedlings.

The highest final plant height was observed in the cultivation starting on September 22. Despite having a longer duration from planting the cutting to the end of acclimation, at 48 days (Figure 1), this result may not be due to this fact alone. Planting cuttings during this season also favoured cassava plant growth because of the climate conditions. Thus, plantings at the beginning of the preferential growing season favoured cassava seedling growth under the rapid multiplication method. Temperature substantially impacts sprouting speed and, consequently, sprout growth (Keating & Evenson, 1979).

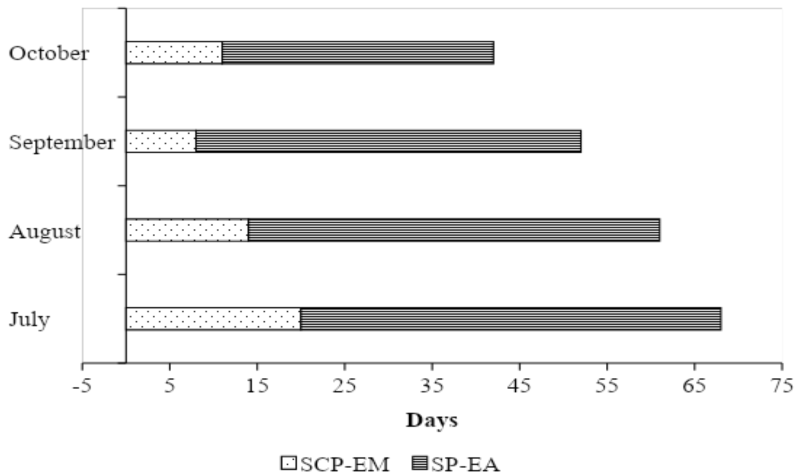

Figure 1. Time from stem cutting planting to sprout emergence (SCP–EM) and sprout planting to the end of acclimation (SP–EA) in four growing seasons.

Cuttings from cultivations carried out in July and August provided the best results for the final seedling survival percentage. Survival may be related to the nutritional supply from stem cuttings, which was lower in the stem cutting cultivations carried out in September and October and influenced the vigour and survival of cuttings after planting. Ribeiro et al. (2016) observed a mortality percentage of

3.09% in cassava seedlings of the cultivar Platinão propagated by the rapid multiplication method without indole-3-butyric acid (IBA). This result is similar to that found in the present study for cultivations carried out in July and August.

The planting performed on September 22 provided the best results for the shoot, root, and total dry matter, though these results did not differ significantly from those of November 25 and October 19 for the shoot and root dry matter, respectively. Ribeiro et al. (2016) measured 0.41 g of shoot dry matter in cassava seedlings from cuttings 10 to 12 cm high planted in 32-cell plastic trays and assessed at 90 days after planting in the state of Bahia. This is within the range observed for shoot dry matter in seedlings by destructive assessment at 48 days after planting for all the different growing seasons, diameter limits, and cutting collection traits (number of leaves). In fact, the shoot dry matter in this study was 60.73% higher and 15.12% lower than the highest and lowest values found in the previously mentioned study, respectively. This indicates that selecting stem cuttings based on the number of leaves rather than on the height was adequate and contributed to the growth and development of cassava seedlings, resulting in high-precocity and vigorous seedlings.

Leaf fall and the slow initial establishment of seedlings in the planting on October 7 carried out with cuttings from Augulst may have impaired the results observed for the traits measured in the destructive assessment, mainly root dry mass, which is related to the quality of vegetatively propagated seedlings. Leaf presence is important for the rooting process in plant cuttings (Dias, Takahashi, Duarte Filho, & Ono, 2012; Vignolo, Picolotto, Gonçalves, Pereira, & Antunes, 2014). Despite the higher result for shoot dry matter, the root dry matter on November 25 was 165.75 mg plant^{-1}, indicating that the rooting capacity of the cassava seedlings was impaired as the growing season delayed.

In addition to the 300 seedlings planted as described in the methodology, additional cuttings were planted to assess the multiplication capacity of the adapted rapid multiplication method (Table 4). Planting additional seedlings was possible in all growing seasons, except for the November 25 season. The October 19 growing season, with cuttings from the planting carried out in September, exhibited the highest number of planted (390) and surviving (344) seedlings. On the other hand, the planting performed on November 25 provided the lowest values for planted (300) and surviving (253) seedlings.

Table 4. Number of cassava seedlings generated from the adapted rapid multiplication method planted and surviving in four growing seasons.

Growing season	Planted seedlings	Surviving seedlings	Survival (%)
September 22	315	299	94.92
October 7	315	307	97.46
October 19	390	344	88.20
November 25	300	253	84.33

The actual survival percentages were 94.92, 97.46, 88.20, and 84.33% for seedlings planted on September 22, October 7 and 19, and November 25, respectively. The high seedling survival during the September 22 and October 7 growing seasons may be related to the higher nutritional reserves of stem cuttings planted in July and August. Neves et al. (2018) obtained cassava seedlings through an in vitro micropropagation technique with leaf buds and obtained an increase of five times in the multiplication rate when compared to that under the traditional method. However, the micropropagation technique has limitations, such as the need for skilled labour, laboratory facilities, and higher financial expenditures for seedling production (Ogero, Gitonga, Ombori, & Ngugi, 2010).

Conclusion

Stem cuttings between 20 and 25 mm in diameter should be used for producing cassava seedlings through the rapid multiplication method. Cuttings (sprouts) with 6 to 8 visible leaves should be collected, and the cuttings should be planted at the end of September.

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