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Propagation

Production of shoots from 'Smooth Cayenne' pineapple crowns with nitrogen fertilization

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Abstract - Pineapple fruit crowns are rarely used as planting material in Brazil. However, they can be used to produce plantlet in situations of scarcity of planting material of adequate quality. The aim of this work was to evaluate the production and nutritional status of early shoots of different sizes produced from 'Smooth Cayenne' pineapple crowns with nitrogen fertilization after the removal of its apical meristem. The experimental design adopted was that of randomized blocks in a 4x4 factorial scheme, with four nitrogen doses (5, 10, 15 and 20 g of urea per pot), four minimum shoot lengths at harvesting (10, 15, 20 and 25 cm), with four replicates. Under full sun cultivation conditions, crowns produced 18.11 and 6.83 shoots with 10 and 25 cm in length, respectively, up to 380 days after planting and removal of the apical meristem. Pineapple shoots harvested at 180 days presented better biometric quality and can be harvested up to 380 days after crown planting. Increasing urea doses reduce P, K and Ca levels in the dry mass of shoots. **Index terms:** *Ananas comosus*, plantlet, propagation, urea.

Produção de rebentos em coroas de abacaxi 'Smooth Cayenne' com adubação nitrogenada

Resumo – As coroas de frutos de abacaxi são muito pouco utilizadas como material de plantio no Brasil. No entanto, podem servir para a produção de mudas em situações de escassez de material de plantio de qualidade adequada. O objetivo deste trabalho foi avaliar a produção e o estado nutricional de rebentos precoces de diferentes tamanhos produzidos em coroas de abacaxi com adubação nitrogenada, bem como verificar as variáveis morfológicas e o estado nutricional das coroas de abacaxi da cv. Smooth Cayenne, após a extirpação de seu meristema apical. Ainda, avaliou-se o efeito de doses crescentes de adubo nitrogenado sobre o rendimento, o vigor e à qualidade nutricional das mudas. O delineamento experimental adotado foi o de blocos ao acaso, em esquema fatorial 4x4, sendo quatro doses de nitrogênio (5; 10; 15 e 20 g de ureia por vaso), quatro comprimentos mínimos para colheita de rebentos (10; 15; 20 e 25 cm), com quatro repetições. Em condições de cultivo em pleno sol, as coroas produziram 18,11 e 6,83 rebentos, com 10 e 25 cm de comprimento, respectivamente, até 380 dias após o plantio e a extirpação do meristema apical. Os tamanhos de colheita dos rebentos influenciam sobre a massa seca da raiz e do caule das coroas. A adubação nitrogenada aumenta os teores de N e influencia negativamente os teores foliares de P, K, Ca e S das coroas do abacaxizeiro 'Smooth Cayenne'.

Termos para indexação: Ananas comosus, mudas, propagação, ureia.

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Introduction

Brazil is the world's third largest pineapple producer, and in 2017, the largest producers in order of importance were: Costa Rica, Brazil, Philippines, Thailand and Indonesia, which together accounted for about 46% of global production. In that same year, Brazilian production was approximately 2.4 million tons (FAOSTAT, 2017), with planted area of 66,723 ha (IBGE, 2019). The number of pineapple plantlet required for planting this area was approximately 2.5 billion plantlet, since in Brazil, around 25 and 35 thousand plantlet are used per hectare (REINHARDT; CUNHA, 2000; GOMES et al., 2003).

The production of pineapple plantletplantlet of sufficient quality and quantity to supply this demand has long been an obstacle for crop growth and expansion (TEIXEIRA et al., 2001; CUNHA; REINHARDT, 2004), mainly due to low seedling production, notably for the 'Smooth Cayenne' cultivar, and the long period for obtaining plantlet by the conventional propagation method (REINHARDT; CUNHA, 1999).

Considering the above, the challenge is to increase the production of plantlet with superior characteristics to supply the scarcity of propagating material. To this end, several studies have been carried out to improve fast pineapple multiplication methods for the production of plantlet with superior vigor and health such as: micropropagation (OLIVEIRA et al., 2015), stem sectioning (COELHO et al., 2009; FREITAS et al., 2014) and breaking of the apical crown dominance (COELHO et al., 2007a; SANTOS et al., 2014; REINHARDT et al., 2018).

According to Coelho et al. (2007a) and Santos et al. (2014), pineapple crown can be used as a source of propagating material in nurseries for seedling production by breaking its apical dominance, which stimulates the growth of lateral buds which provide for the production of shoot-type plantlet. The management of crown-grown shoots and the application of balanced fertilization can accelerate sprout growth.

Nitrogen fertilization is one of the factors responsible for the quality of plantlet and vegetative growth, since nitrogen is the nutrient that has an effect on the development of pineapple plants, both in the seedling production and growth, fruit development and productivity (COELHO et al., 2007b; BREGONCI et al., 2008; RIBEIRO et al., 2011; FREITAS et al., 2012).

The efficiency of fast multiplication techniques can be greater with the knowledge of nutrient absorption and nutritional requirements of this species, aiming to promote future seedling production programs in nurseries that can assist the producer in decision making to increase seedling availability. However, there are still few studies related to fertilization during the nursery phase, notably of shoots from the pineapple crown.

In view of the above, the aim of this work was to evaluate the production and nutritional status of early shoots of different sizes produced from 'Smooth Cayenne' pineapple crowns with nitrogen fertilization after the removal of the apical meristem.

Material and methods

The experiment was conducted in the municipality of Campos dos Goytacazes - RJ on stands under full sun located on the campus of the "Darcy Ribeiro" State University of the Northern State of Rio de Janeiro (UENF) at coordinates Latitude 21°19 ', Longitude 41°10' and Altitude of 14 m. The experimental design adopted was that of randomized blocks in a 4x4 factorial scheme, with four nitrogen doses (5, 10, 15 and 20 g of urea per pot), four minimum shoot length at harvesting (10, 15, 20 and 25 cm), with four replicates. The experimental unit consisted of two pots with capacity of 10 L containing two crowns each.

Crowns came from fruits harvested in commercial orchard in the municipality of São Francisco de Itabapoana, state of Rio de Janeiro. After removing crowns from fruits, the apical meristems were removed according to methodology adopted by Coelho et al. (2007a).

After breaking the apical dominance, crowns were planted in pots containing the following substrate: mixture of washed sand + Basaplant® vegetable substrate in the proportions of 1:1 (v/v), respectively, presenting the following parameters: pH: 4.5; K = 3.2 mmolc dm⁻³; Ca = 26.5 mmolc dm⁻³; Mg = 12.9 mg dm⁻³; M

Top-dressing nitrogen fertilization of pineapple crowns was carried out in the solid form every 60 days, from the 40th day to the 220th day after planting. At 180, 300 and 380 days after planting, four shoots harvested per pot were evaluated, determining their leaf area using bench measurer model LI-3100 LICOR, Lincoln, NE, USA; and the dry mass of shoots was measured in precision analytical scale after drying in a forced air circulation oven at 70°C for 72 h. This material was also submitted to macro and micronutrient nutritional analysis.

Up to 300 days after planting, the number of days necessary for shoots to reach the stipulated height was determined, considering the period from bud emission to its harvest, according to proposed treatments. Total number of shoots produced per crown was recorded at 180, 240, 300 and 380 days after planting. For nutritional analysis, samples were ground in a Wiley mill with 20 mesh sieve and stored in airtight containers. Leaf samples from each treatment were submitted to chemical analysis

to determine nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and (B) levels in the Mineral Plant Nutrition sector of the Laboratory of Plant Science (UENF). In the case of N, the material was submitted to sulfuric digestion and the nutrient was determined by the method of Nessler (Jackson, 1965). P, K, Ca, Mg, S and B levels were determined using the Shimadzu® plasma equipment (ICPE-9000) after digestion with HNO $_3$ and H $_2$ O $_2$ in an open digestion system according to Peters (2005).

Data were submitted to analysis of variance, using the SANEST software. For growth evaluations performed in four times (180, 240, 300 and 380 days) and three evaluation times (180, 300 and 380 days), data were analyzed in a split-time scheme. For quantitative factors, polynomial regression analysis was used, including the F test of regression analysis. For qualitative factors, the Tukey test at 5% probability was used.

Results and discussion

The number of shoots produced per crown increased linearly as a function of evaluation time for all shoot sizes (Figure 1). However, shoot production was higher the smaller its size at the time of crown removal, indicating that early removal stimulates the emission of new sprouts.

The total number of shoots produced per crown at 380 days after planting was high, reaching 18.1; 12.7; 9.0 and 6.8 for shoots harvested at 10, 15, 20 and 25 cm in height, respectively (Figure 1). In this context, it is known that auxins produced by meristematic cells at the stem apex promote apical dominance, inhibiting the development of axillary buds (TAIZ et al., 2017). In addition, the decapitation of the crown stem apex (AGOGBUA; OSUJI, 2011) and removal of shoots (COELHO et al., 2007a)

affect the action of auxins, favoring the breaking of the dormancy of lateral buds present in the crown stem.

Coelho et al. (2007a) found that 'Smooth Cayenne' pineapple crown can produce 5.2 shoots with length of 35.9 cm in 360 days. Santos et al. (2011) observed that in 420 days, pineapple crowns produced 25.75 shoots with length of 10 cm, suggesting that pineapple crowns have potential to be used as mother plants for a period longer than 420 days. However, the longevity of the pineapple crown is still unknown.

It was observed that as the shoot size increased, the production time was longer; adding about 32 days to harvest 25-cm shoots when compared to those of 10 cm. The first emissions were obtained 98 days after crown planting (data not shown).

It was found that the total time for seedling production by the method of removal of the crown apical meristem is reduced and that the technique shows efficiency in the production of shoot-type plantlet, when compared to the conventional method of pineapple propagation. According to Reinhardt and Cunha (1999), the amount of plantlet produced in the field by the conventional propagation method depends on the cultivar, climate and cultural treatments, which influence plant growth. 'Pérola' pineapple produces, on average, five to nine slips and one shoot per plant. For 'Smooth Cayenne' pineapple, production varies on average from zero to three slips and from one to two shoots per plant. The growth period for these plantlet can vary (REINHARDT; CUNHA, 1999; GOMES et al., 2003) for slip-type plantlet, from one to six months, and for shoot-type plantlet, from two to 12 months after the harvest of fruits (REINHARDT; CUNHA, 1999). In addition, it has been elucidated that conventional plantlet are more vigorous and they should have at least 30 cm to be considered suitable for planting (REINHARDT et al., 2018).

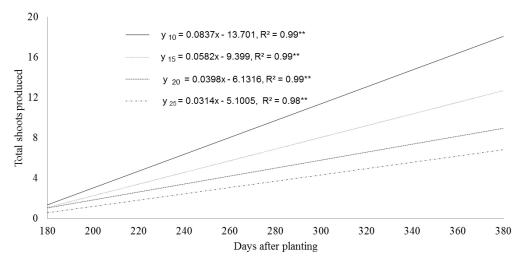


Figure 1. Total shoots produced in different sizes (10, 15, 20 and 25 cm) per 'Smooth Cayenne' pineapple crown according to four evaluation times (180, 240, 300 and 380 days after planting). ** significant at 1% probability level, respectively, by the F test.

Additionally, it was observed that the matrices whose shoots were 10 cm in size produced, simultaneously, from 3 to 6 shoots per crown, where most shoots could be harvested in the same weekly harvest. For 20 and 25 cm shoots, the amount of plantlet attached to crowns varied from 1 to 3 shoots per crown. The staggered production of pineapple plantlet of different sizes can be interesting for

the nursery producer, allowing meeting market seedling demands at different times.

Table 1 shows that the number of days for shoot growth to reach minimum sizes of 10, 15, 20 and 25 cm was different, being necessary 23,17; 32.35; 43.04 and 55.21 days, respectively (Table 1).

Table 1. Number of days required for the growth of 'Smooth Cayenne' pineapple shoots as a function of different shoot sizes harvested at 380 days after planting.

Shoot size (cm)	Number of days for shoot growth	
10	23.17 a	
15	32.35 b	
20	43.04 c	
25	55.21 d	
Mean	38.44	
CV (%)	9.37	

Averages followed by the same lower case letter in the column do not differ at 5% significance by the Tukey test.

When analyzing shoot growth, difference was observed in relation to the size of shoots harvested in the three evaluation times for variables leaf area and dry mass of shoots (Table 2). It was found that, regardless of length, plantlet had smaller leaf area and dry mass of the aerial part in the last two evaluation times (300 and 380).

days after planting) compared to the first evaluation time (180 days) (Table 2). This suggests that shoot emission and growth implies the consumption of crown reserves over time, affecting the development of late shoots (SANTOS et al., 2011).

Table 2. Leaf area and dry mass of the aerial part of shoots from 'Smooth Cayenne' pineapple crown according to size at harvest (10, 15, 20 and 25 cm in height) and times (180, 300, 380 days after planting) for seedling evaluation.

		Leaf area (cm ²)				
Shoot size (cm)		Time (days)		Mean		
	180	380				
10	116.06 Ad	85.92 ABd	71.75 Bd	91.24		
15	219.78 Ac	165.81 Bc	128.71 Bc	171.43		
20	344.11 Ab	247.68 Bb	199.66 Cb	263.82		
25	466.58 Aa	336.86 Ba	326.79 Ba	376.74		
Mean	286.63	209.07	181.73			
CV (%)		16.72				
	Dr					
Shoot size (cm)		Time (days)		Mean		
	180	300	380	_		
10	2.14 Ad	0.61 Bd	0.68 Bd	1.14		
15	3.90 Ac	1.37 Bc	1.32 Bc	2.20		
20	6.24 Ab	2.34 Bb	2.34 Bb	3.64		
25	8.43 Aa	3.33 Ca	4.38 Ba	5.38		
Mean	5.18	1.91	2.18	_		
CV%	19.82					

Averages followed by the same lowercase letter in the column and uppercase in the row do not differ at 5% significance by the Tukey test.

Table 3. Dry mass of aerial part, phosphorus, potassium and calcium content in shoots produced in 'Smooth Cayenne' pineapple crowns as a function of nitrogen fertilization (g of urea per pot).

Analyzed variable	Polynomial regression for nitrogen doses	\mathbb{R}^2
Dry mass content (g)	$Y = 2.552 + 0.103N - 0.004N^2$	0.66**
Phosphorus (g plant ⁻¹)	Y = 2.807 - 0.013N	0.79**
Potassium (g plant ⁻¹)	Y = 27.63 - 0.240N	0.90**
Calcium (g plant ⁻¹)	Y = 4.028 - 0.065N	0.99**
Sulfur (g plant ⁻¹)	Y = 1.73	-

^{**} significant at 1% probability level, respectively, by the F test.

The dry mass of shoots as a function of N doses showed a quadratic response, reaching higher value in crowns treated with 12.88 g urea pot¹ (Table 3). Nitrogen fertilization tends to accelerate growth, reducing the nursery period for pineapple plantlet, as verified by Silva et al. (2016) in micropropagated plantlet in the acclimatization phase and by Coelho et al. (2007b) in stem section plantlet.

Increasing N doses resulted in reduction in leaf P, K and Ca levels of shoots, but did not affect the S content, which reached average value of 1.73 g kg⁻¹ (Table 3). On the other hand, nitrogen fertilization promoted linear increase in the N content in the dry matter of shoots in all evaluation times, with greater effect observed at 300 days (Figure 2). Although plantlet showed less vigor at 300 days compared to that observed at 180 days (Table 2), their N levels were above the critical limit, 11 g kg⁻¹ of dry mass, suggested by Coelho et al. (2010) to restrict vegetative growth.

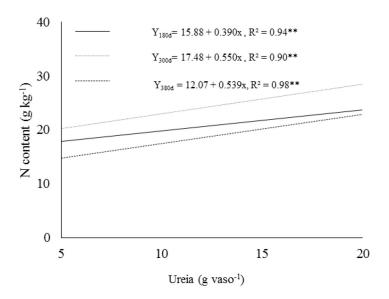


Figure 2. N content (g kg⁻¹) in the dry mass of the aerial part of shoots produced from 'Smooth Cayenne' pineapple crowns as a function of nitrogen fertilization (g of urea per pot containing 2 crowns), at different evaluation times (180, 300, 380 days after planting).

There was interaction of shoot size and evaluation time factors on N, P, K, Ca, Mg and S levels in the dry mass of the aerial part of shoots (Table 4). N and P content verified in the dry mass of shoots harvested at 180 and 380 days after planting were lower than those obtained at 300 days after planting, regardless of shoot size (Table 4). However, at 380 days after planting, it was observed

that the N content found in the dry mass of shoots was higher for 10 and 15 cm size shoots (Table 4). Regarding P content and shoot size, it was found that, at 180 days, there was no difference in the P content in relation to size. However, at 300 and 380 days, 10 cm shoots showed the highest P levels (Table 4).

^{**} significant at 1% probability by the F test.

Table 4. Content of macronutrients and micronutrients in the dry mass of shoots produced from 'Smooth Cayenne' pineapple crowns according to shoot size (10.15, 20 and 25 cm in height) and evaluation times (180, 300 and 380 days after planting).

		Nitrogen (g kg ⁻¹)			Phosphorus (g kg ⁻¹)		
Shoot Size (cm)	Time (days)			Time (days)			
	180	300	380	180	300	380	
10	20.92 Ba	24.08 Aa	20.68 Ba	2.04 Ca	3.43 Aa	3.07 Ba	
15	20.14 Ba	23.87 Aa	19.12 Bab	1,96 Ca	3.15 Abc	2.78 Bł	
20	21.24 Ba	24.39 Aa	17.43 Cb	1.96 Ca	3.21 Aab	2.59 Bł	
25	20.79 Ba	25.12 Aa	18.04 Cb	2.01 Ca	2.90 Ac	2.56 Bl	
CV (%)		11.03			11.22		
	F	Potassium (g kg ⁻¹)			Calcium (g kg ⁻¹)		
Shoot Size (cm)		Time (days)			Time (days)	
	180	300	380	180	300	380	
10	28.84 Aa	27.33 Aa	28.40 Aa	2.99 Aa	3.08 Aa	2.63 Al	
15	28.86 Aa	25.35Bab	24.58 Bb	2.94 Aa	3.39 Aa	3.05 Al	
20	27.32Aab	23.90 Bb	21.09 Cc	3.19 ABa	3.67 Aa	2.98 Bl	
25	25.58 Ab	19.00 Bc	15.31 Cd	3.29 Ba	3.41 ABa	3.87 Aa	
CV (%)		11.02			20.89		
	N	Iagnesium (g	kg-1)		Sulfur (g kg	-1)	
Shoot Size (cm)		Time (days)		Time (days)			
	180	300	380	180	300	380	
10	1.88 Bb	2.70 Ac	2.50 Ac	1.46 Bab	1.99 Aa	2.05 Aa	
15	2.04 Bab	3.26 Ab	3.04 Ab	1.38 Bb	1.95 Aa	1.95 Aa	
20	2.27 Bab	3.68 Aab	3.30 Ab	1.60 Bab	1.91 Aa	1.70 Bl	
25	2.40 Ca	3.81 Ba	4.39 Aa	1.64 Aa	1.62 Ab	1.47 A	
CV (%)		17.06			13.83		
a. a.	Boron (mg kg ⁻¹)						
Shoot Size		Time (·		
(cm)		180		300		380	
10	19.37 Ba		24.57 Aa 17.45 B		17.45 Bb		
15	 19.00 Ba		25.50 Aa		19.5 Bb		
20	20.18 Ba		26.93 Aa 20.69 I		20.69 Bb		
25	21.25 Ba		27.62 Aa		27.44 Aa		
CV (%)	20.35						

Averages followed by the same lowercase letter in the column and uppercase in the row do not differ at 5% significance by the Tukey test.

According to Malavolta et al. (1997), the appropriate N content range for pineapple is from 20 to 22 g kg⁻¹. Under the conditions of this experiment in all evaluation times, with the exception of the second evaluation time, the N content in the dry mass of shoots was within or lower than levels considered appropriate by Malavolta et al. (1997). Only at 380 days in shoots of sizes 20 and 25 cm, reductions in N levels were found. However, considering N levels from 15 to 17 g kg⁻¹ as ideal by Malézieux and

Bartholomew (2003) for 'Smooth Cayenne' pineapple, leaf N levels in the present study, as a function of shoot size were above levels considered adequate.

In relation to K content, the larger the minimum seedling size at harvest, the lower the average levels of this nutrient, which intensified during the experimental period, except for 10 cm plantlet (Table 4). Malézieux and Bartholomew (2003) established that the ideal K contents are within the range from 22 to 30 g kg⁻¹. Coelho

et al. (2010) considered K content of 16 g kg⁻¹ as most suitable for 'Smooth Cayenne' plantlet harvested in the ninth month after the planting of stem sections, showing the need for potassium fertilization between 180 and 300 days after planting for pineapple crowns, when minimum seedling size at harvest of 25 cm is established.

Ca levels in the dry matter of shoots varied according to size only at 380 days, with higher content of this nutrient being found in 25 cm shoots when compared to those of 10, 15 and 20 cm (Table 4). This effect may have occurred due to a possible interference of the number of days for shoot collection in relation to the different sizes. At 380 days, the redistribution of greater amounts of this nutrient from crowns to shoots may have been limited in shoots of sizes 10, 15 and 20 cm until bud collection. Thus, as 25 cm shoots remained in the crown for more days, greater Ca amount may have been allocated to these shoots. This effect was similar to that observed in the B content in the dry mass of shoots (Table 4).

In this context, these results corroborate Ramos et al. (2011), who evaluated Ca and B levels in pineapple 'D' leaves at 12 months after transplanting and found that they were higher than those obtained at nine months. The authors attributed this effect to the low mobility of these nutrients in the phloem. The drain strength in different plant parts causes Ca to be concentrated in leaves and branches, where there is greater transpiration than in fruits (KLUGE et al., 2003).

Regarding the Mg content, it was found that as the shoot size increased (10, 15, 20 and 25 cm) and also according to evaluation times (180, 240, 300 and 380 days after planting), the Mg content increased in the dry mass of shoots (Table 4). In contrast, the S content was higher at 300 and 380 days after planting in 10 and 15 cm size shoots. In 25 cm shoots, no statistical difference in relation to the S levels and evaluation times was found. However, in 20 cm shoots, the highest S content was obtained at 300 days after planting (Table 4). Unlike other nutrients, S levels were not within the range considered adequate by Malavolta et al. (1997), which vary from 2 to 3 g kg⁻¹. However, the values found in the present work were close to those found in the dry mass of shoots obtained by the sectioning of stems fertilized with urea and boric acid (COELHO et al., 2010).

Regarding the nutrient ranges obtained for pineapple and considered as adequate by different authors, Ramos et al. (2011) pointed out that, if concentrations found are within adequate ranges, plant shows no deficiencies. However, to be considered deficient, the critical level or critical range must be taken into account, which according to these authors, is rarely available in literature.

In view of the above, this work showed that shoots obtained from pineapple crowns can be an interesting alternative to solve bottlenecks in the production chain of

quality pineapple plantlet in sufficient quantity to supply the demand. However, the K content both in crown and shoots may have caused a limiting effect for seedling production. It should also be taken into account that information related to nutrient content ranges considered suitable for pineapple plantlet obtained from pineapple crowns is not available in literature. For this reason, further studies on seedling nutrition and early production of pineapple shoots should be carried out.

Conclusions

'Smooth Cayenne' pineapple crowns with average weight of 200 g after breaking the apical dominance by removing their apical meristem produce 18.11; 12.72; 8.99 and 6.83 shoots with 10, 15, 20 and 25 cm in height, respectively, up to 380 days after planting.

'Smooth Cayenne' pineapple shoots have better biometric quality, regardless of minimum harvest size at 180 days after planting.

'Smooth Cayenne' pineapple shoots can be harvested up to 380 days after crown planting.

Shoots harvested with sizes of 20 and 25 cm in height have reduced K content.

Increasing urea doses reduce P, K and Ca levels in the dry matter of the aerial part of 'Smooth Cayenne' pineapple shoots.

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