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Vegetative propagation strategies in commercial pineapple cultivars

Estratégias de propagação vegetativa em cultivares comerciais de abacaxizeiro

Maria do R. A. de Almeida[1], Everton H. de Souza[1], Eva M. R. Costa[2], Fernanda V. D. Souza[2]*

[1]Universidade Federal do Recôncavo da Bahia, Cruz das Almas, BA, Brazil. [2]Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brazil.

ABSTRACT - The production of healthy pineapple seedlings for commercial plantations has been one of the main limiting factors of the species, and the search for improvements to meet this demand has been constant. Therefore, the present study aimed to compare different propagation strategies (natural tillering, stem sectioning, and micropropagation) in three commercial cultivars 'BRS Imperial', 'Pérola', and Gold 'MD-2' of pineapple. Three methods of vegetative propagation were evaluated: conventional, stem sectioning, and micropropagation. The evaluations consisted of the number of seedlings produced by each method and the period for their development until planting in the field. According to the conventional propagation technique results, the young seedling type in the cultivar 'Pérola' was more abundant. In the propagation by sectioning the stem, the 'BRS Imperial' cultivar presented the highest number of seedlings. Regarding the micropropagation technique, the highest production of shoots was observed in the third subculture for all cultivars, especially for 'BRS Imperial', which presented the highest total number of shoots in all subcultures. It was concluded that the micropropagation technique proved efficient in producing uniform seedlings on a large scale, in addition to having an advantage in the greater number of seedlings produced compared to the other methods studied.

Keywords: *Ananas comosus*. Conventional propagation. Stem sectioning. Micropropagation.

RESUMO - A produção de mudas sadias de abacaxizeiro para plantios comerciais tem sido um dos principais fatores limitantes para a cultura e a busca de melhorias para atender essa demanda tem sido constante. Diante disso, objetivou-se neste trabalho comparar diferentes estratégias de propagação em três cultivares comerciais de abacaxizeiro 'BRS Imperial', 'Pérola' e Gold 'MD-2'. Foram analisados três métodos de propagação vegetativa: convencional, seccionamento do talo e micropropagação em relação as características número de mudas produzidas por cada método e o período para o seu desenvolvimento das mudas até o plantio em campo. De acordo com os resultados da técnica de propagação convencional, a muda tipo filhote, na cultivar 'Pérola', foi a que produziu em maior quantidade. Na propagação por seccionamento de talo, a cultivar 'BRS Imperial' foi a que apresentou o maior número de mudas. Em relação à técnica de micropropagação a maior produção de brotos foi observada no terceiro subcultivo em todas as cultivares, principalmente a 'BRS Imperial' que apresentou o maior número total de brotos. Concluiu-se que, a técnica de micropropagação de abacaxizeiro foi eficiente para a produção de mudas uniformes em larga escala, além de apresentar maior número de mudas produzidas em comparação aos outros métodos estudados.

Palavras-chave: *Ananas comosus*. Propagação convencional. Seccionamento do talo. Micropropagação.

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***Corresponding author:**
<fernanda.souza@embrapa.br>

INTRODUCTION

The pineapple tree *Ananas comosus* (L.) Merr is the most economically important member of the Bromeliaceae family, within which numerous species have food, pharmaceutical, and ornamental value (ORLANDI-MATTOS et al., 2019; DEBNATH et al., 2019; SILVA et al., 2021). The world's fruit production has grown by more than 10% in the last decade, and Brazil occupies the third position in this ranking, preceded only by Costa Rica and the Philippines (TRIDGE, 2021).

The most relevant cultivars currently on the international and Brazilian market are Smooth Cayenne, Pérola, Gold 'MD-2', and Hawaii, which have characteristics appreciated for fresh consumption, however, they are susceptible to fusariosis, the main disease of the crop that affects both the fruits and the propagation material (DANTAS et al., 2015; REINHARDT et al., 2018). To overcome these phytosanitary difficulties, some cultivars were launched, such as 'BRS Imperial' (CABRAL; MATOS, 2009), 'BRS Vitória' (VENTURA; CABRAL; MATOS, 2009), 'BRS Ajubá' (REINHARDT et al., 2012), and 'IAC Fantástico' (IAC, 2010) resistant to fusariosis and with desirable characteristics for fresh consumption.

Vegetative propagation is the predominant method for establishing commercial pineapple cultivations (SANTOS et al., 2015). Sexual propagation is used in breeding programs to obtain hybrids between different genotypes since the species has self-incompatibility (SOUZA et al., 2017). The possibility of multiplication through seedlings allows large-scale production for expanding cultivation and launching new hybrids, considering the high number of plants per hectare. However, production success depends on the genetic and sanitary quality of the seedlings (TOMAZ et al., 2014).

Conventionally, pineapple plantations are carried out with seedlings of ground sucker, aerial sucker, and crown types, which develop on the parent plant, therefore, commercial plantations generally use propagation material purchased from the field. Considering that this process takes a long time, it produces a smaller amount of seedlings, and in addition, if they do not have a good origin, there is a risk of spreading pests and diseases (OLIVEIRA-CAUDURO et al., 2016).

Another method used in the propagation of the pineapple plant is the sectioning of the stem, where the axillary buds from pieces of the stem or crowns and shoots of the parent plant are used (REINHARDT et al., 2018). According to Freitas et al. (2012), this method is quite simple, however, to improve its efficiency, it is necessary to improve and incorporate new techniques in the production process.

Another method that can be used to optimize the production of healthy seedlings is micropropagation, which allows obtaining a large number of clonal seedlings with genetic stability at any time of the year (VILLA; PASQUAL; SILVA, 2014). However, factors such as genotype, source of explants, and cultivation conditions can affect *in vitro* plant regeneration. The high cost of seedling production and the somaclonal variations that may occur over subcultures also limit the use of this technique commercially (SAMARFARD et al., 2014).

Therefore, this study aimed to compare different propagation strategies (natural budding, stem sectioning, and micropropagation) in three commercial pineapple cultivars: 'BRS Imperial', 'Pérola', and Gold 'MD-2'.

MATERIAL AND METHODS

Different propagation methods were evaluated for the cultivars 'BRS Imperial' (Figure 1A), 'Pérola' (Figure 1B), and 'MD-2' (Figure 1C) to compare the propagation potential from each applied technique. The description of the cultivars studied in this study can be found in Table 1. The study was conducted at the Embrapa Cassava & Fruits in Cruz das Almas, Bahia. The average annual rainfall in the region is 1,224 mm, the average annual temperature is 23.8ºC, the relative humidity of 80%, latitude 12° 40′ 12″ S, longitude 39° 6′ 7″ W, and an altitude of 220 meters. The climate of the region is classified as tropical hot and humid.

Figure 1. Commercial pineapple cultivars. A) 'BRS Imperial'; B) 'Pérola'; C) 'MD-2'.

Table 1. Description of evaluated pineapple cultivars.

Cultivars	Genealogy	Description
'BRS Imperial'	Hybrid between 'Perolera' and 'Smooth Cayenne' was developed and launched by Embrapa Cassava & Fruits.	Resistant to fusariosis, yellow flesh, and cylindrical fruit.
'Pérola'	Brazilian traditional cultivar. Selected by indigenous people of Brazil.	Susceptible to fusariosis, white flesh, conical fruit.
Gold 'MD-2'	It is a double hybrid, descended from hybrids of the 'Smooth Cayenne' variety.	Susceptible to fusariosis, yellow flesh, and cylindrical fruit.

Conventional propagation

After fruiting and fruit harvesting of the pineapple cultivars, the plants remained in the field for three months, when natural seedling formation was recorded by type of seedling: ground sucker, aerial sucker, slip, crown, and slip-crown. The average number of seedlings/plants was calculated for each type of seedling per variety. The seedlings were established in seedbeds until they reached between 30 and 40 cm in height, then transferred to the field.

The experimental design was completely randomized and arranged in a 3 x 5 factorial scheme (three cultivars x five types of seedlings) with 20 replications, each replication consisting of one plant. Data were submitted for analysis of variance and means compared by the Tukey test ($p<0.01$) with the aid of the R statistical software (R CORE TEAM, 2021).

Propagation by stem sectioning

Adult plants, 120 days after fruiting and fruit harvesting, were used for stem sectioning. The roots, peduncle, and leaves were carefully cut to expose the pseudostem with the buds (Figure 2A). After removing the leaves and exposing the stems with the buds, the length and diameter (cm) of the stems were evaluated, which were then sectioned longitudinally (Figure 2B) according to the methodology described by Matos et al. (2009). The sectioned stems were immersed in a fungicide solution for five minutes (Figure 2C) and cultivated in seedbeds with commercial substrate Plantmax® (Figure 2D). After 40 days of implantation, the emergence of the seedlings was counted, which were removed when they reached a height of 10 cm. Then they were planted in trays containing the same substrate in a greenhouse and later transferred to the seedbed.

For sectioning the stems, the design was completely randomized with 10 repetitions, each consisting of 2 sections of the stems, as the stems were subdivided to expose the buds. Data were subjected to analysis of variance and means compared by the Tukey test ($p<0.01$). The following descriptive statistics were calculated: average, minimum, maximum, standard deviation, and coefficient of variation. The analyzes were performed using the R statistical software (R CORE TEAM, 2021).

Figure 2. Stem sectioning. A) Plants taken from the field with roots, peduncles, and leaves cut. B) Stems sectioned lengthwise. C) Sections immersed in fungicide mixture D) Sections planted in seedling production seedbeds.

Micropropagation

For the *in vitro* establishment, six adult plants of each variety from the experimental fields of Embrapa Cassava & Fruits were used. The plants had their leaves removed, the stem was washed with abundant water under laboratory conditions to expose the axillary buds used as an explant for multiplication. With tweezers and a scalpel, the buds were removed from deep cuts in the stem and washed with running water and neutral detergent. Subsequently, they were identified and transferred to a laminar flow chamber under aseptic conditions (Figure 3).

Disinfestation consisted of treating the buds with a 70% (v/v) ethanol solution for 5 minutes, followed by immersion in a 2.0 and 2.5% sodium hypochlorite solution containing three drops of Tween® detergent per liter, for 20 minutes, after three washes with distilled water. After this procedure, the buds were reduced, removing excess tissue, and later placed in test tubes containing MS culture medium (MURASHIGE; SKOOG, 1962) supplemented with 3% sucrose, 0.5 mg L^{-1} of BAP, 0.01 mg L^{-1} of NAA and solidified with 2.5 g L^{-1} of Phytagel® previously autoclaved at 120 ºC for 20 minutes.

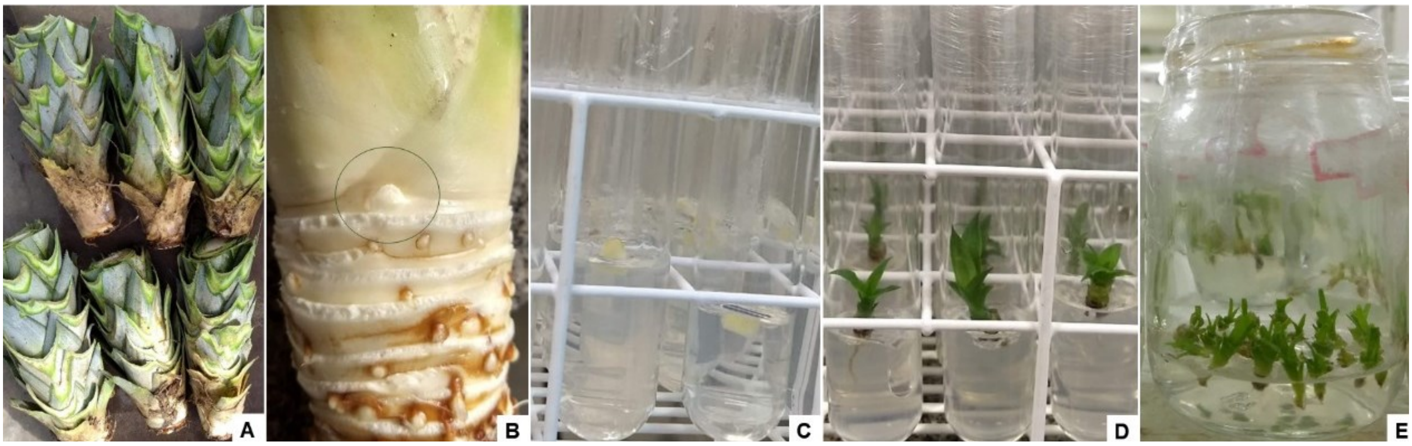

Figure 3. Steps of micropropagation. A) Removal of plants from the field with roots, peduncles, and leaves cut. B) Axillary bud for *in vitro* establishment. C) Buds established in the culture medium in test tubes. D) Sprouts at 45 days after *in vitro* cultivation. E) Sprout multiplication stage.

The test tubes were identified and randomly distributed in a growth room with incubation conditions of 27 ± 1 ºC, photoperiod of 16 hours, and photon flux density of 40 µmol m^{-2} s^{-1}. At 45 days, the following were evaluated: the total number of buds, the percentage of contaminated buds (%), the percentage of oxidized buds (%), and the percentage of surviving buds (%).

After the establishment stage, the micropropagation stage began with the transfer of twenty plants, originating from the development of buds, to a culture medium for multiplication, composed of MS salts and vitamins supplemented with 3% sucrose, 0.5 mg L^{-1} BAP, 0.2 mg L^{-1} NAA, and solidified with 2.4 g L^{-1} Phytagel®. Four subcultures were carried out at intervals of 45 days, evaluating the number of lateral shoots formed (n° of shoots) at each period.

The propagative potential of micropropagation was measured using the geometric growth rate (GGR) between two successive subcultures and between the first and last subcultures, given by the expression:

$$GGR = -\sqrt[t]{V_e/V_i} - 1 \times 100$$

Where:

GGR is the geometric growth rate;
V_e is the number of plants at the end of each subculture;
V_i is the number of plants at the beginning of each subculture;
t is the time between subcultures in days.

A Poisson log-linear model was adjusted for the number of shoots produced according to the four subcultures, considering the number of shoots as an independent variable (quadratic effects) and the shoot multiplication data as a dependent variable for each cultivar studied. All analyzes were performed using the R statistical program (R CORE TEAM, 2021).

RESULTS AND DISCUSSION

Conventional propagation

According to the analysis of variance performed, there was a significant interaction between the cultivars and the five types of structures (Figure 4). The cultivar that produced the most seedlings was 'Pérola', with slip-type structures, followed by 'BRS Imperial', with an average number of 8.5 and 6.4, respectively. Gold 'MD-2' showed the lowest seedling production regardless of the origin in the parent plant.

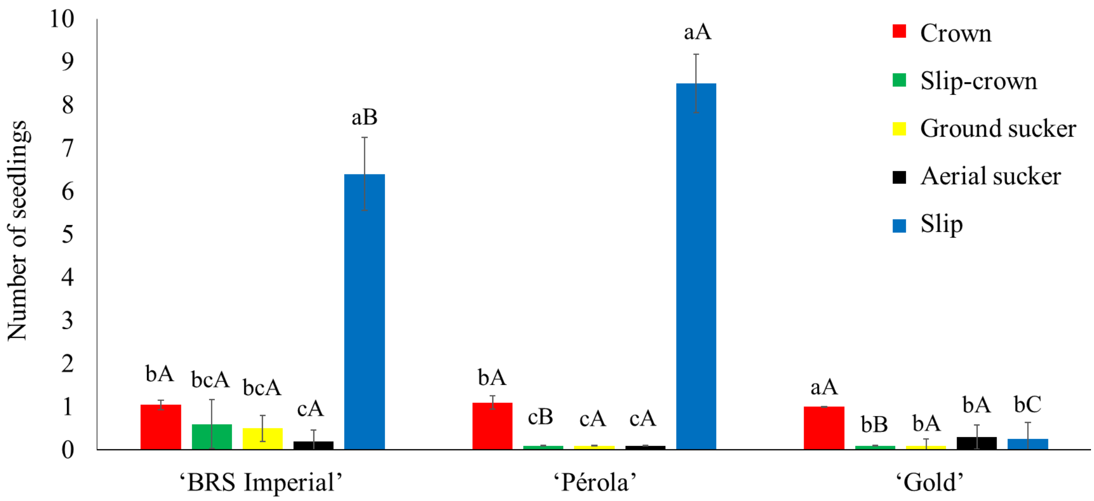

Figure 4. Average number of seedlings per plant from conventional propagation in three pineapple cultivars. Equal lowercase letters do not differ in the types of seedlings and uppercase letters in the cultivars by the Tukey test ($p<0.05$).

The crown-type structures result in one seedling per plant, highlighting the practical absence of fasciation an anomaly that begins during floral differentiation and can reach the crown, the fruit, and the peduncle. The simplest type is fruit formation with a double crown, which did not occur in this work. Other anomalies can appear in the seedling, depending on the variety, ranging from the appearance of thorns, their irregular distribution, fruits without a crown, or the occurrence of more than one crown, as mentioned above (REINHARDT et al., 2012).

Similar results were observed by Caetano, Ventura, and Balbino (2015), where 'Pérola' produced the highest average number of seedlings, with 10.6 seedlings per plant, followed by 'BRS Imperial', and Gold 'MD-2' cultivars. According to the authors, 'BRS Imperial', despite producing a good number of seedlings, shows high adherence of the seedling type to the base of the fruit, which leads to the total or partial loss of this type of seedling at harvest, being a negative characteristic in this cultivar of pineapple with this aspect.

The number and type of seedlings produced conventionally varied according to the cultivars and environmental conditions, mainly during floral differentiation and with the cultural practices carried out at harvest (WILLIAMS et al., 2017). The 'Pérola' produces many slip-type seedlings, but it produces few shoots, and when it does, they are late. The 'Smooth Cayenne', one of the parents of the 'BRS Imperial', as well as the Gold 'MD-2', produces a little more shoots when compared to the 'Pérola'. However, this characteristic was not inherited by the 'BRS Imperial', which has a good production of slips (REINHARDT et al., 2012).

On the other hand, Gold 'MD-2', also derived from hybridizations with 'Smooth Cayenne', produces few seedlings of the slip type, which under normal conditions, rarely exceeds three but presents a good formation of shoots, which results in the possibility to explore another cycle of the plant, called ratoon pineapple. In Brazil, the low production of seedlings of the Pérola cultivar and fusariosis do not allow the use of one more plant cycle (REINHARDT et al., 2012).

Propagation by stem sectioning

In propagation by stem sectioning, a significant difference between cultivars was observed, demonstrating a different behavior from that observed for conventional seedlings. The 'BRS Imperial' was the one that presented the highest average number of seedlings (about 13), statistically differentiating itself by the Tukey test at 5% probability of the cultivars 'Pérola' and Gold 'MD-2', which presented, respectively, the production of 2 and 3 seedlings by this method (Figure 5). Considering that each bud has the potential to generate a seedling, the theoretical yield would be approximately equal, however, this yield, or the number of shoots, is a trait of the cultivar and is directly related to the development and age of the parent plant, and the position of the buds on the plant, since younger buds sprout less than the more mature ones (MARULANDA; GUTIÉRREZ; MÁRQUEZ, 2005; MATOS et al., 2018).

Propagation by stem sectioning is one of the alternative methods for producing pineapple seedlings that can be easily reproduced by the producer. It is a simple method that allows the formation of seedlings through the development of axillary buds from pieces (sections) of the stem of the parent plant. The buds move from the dormant state to the physiologically active state by eliminating the dominant hormonal action of the apical meristem (MATOS et al., 2009). However, to improve the efficiency of the method, it is necessary to improve and incorporate new techniques in the production process (FREITAS et al., 2012).

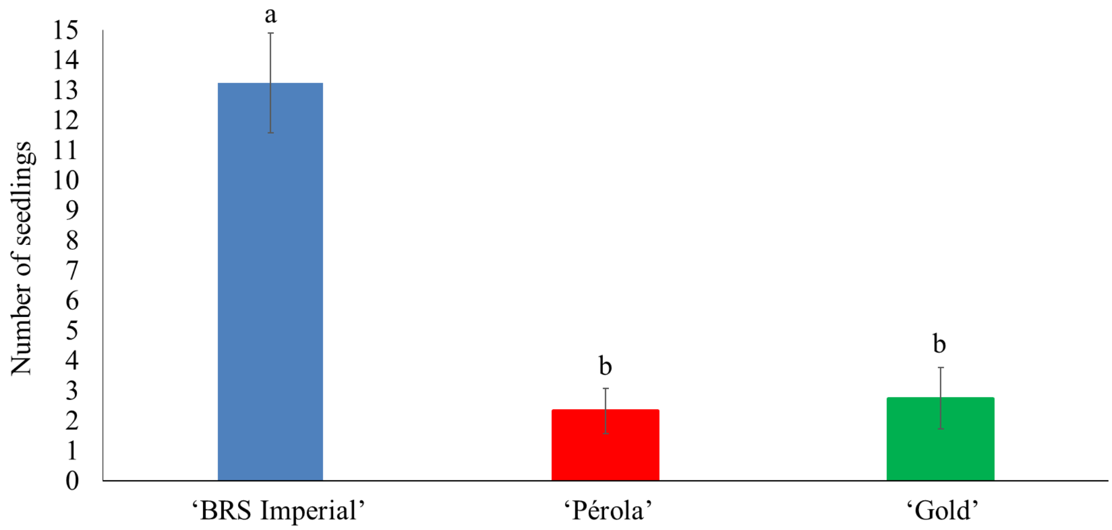

Figure 5. Average number of seedlings from a stem sectioning in three commercial pineapple cultivars. Equal letters do not differ by the Tukey test ($p<0.05$).

Studies that have been developed with promising results is the treatment of the stems with growth-promoting bacteria isolated from the pineapple itself (SOUZA et al., 2019). The buds of the stems seem to respond well to the inoculation of these microorganisms, maintaining the growth improvement in later stages (unpublished data).

Table 2 presents the descriptive statistics for the length, diameter, and average number of seedlings/stems of the three evaluated cultivars. These data are important because they allow a correlation between the size and diameter of the stem and the production of seedlings, making it possible to make predictions in production fields, in addition to being relevant information to obtain by variety. Regarding stem length, the highest amplitudes were observed for Gold 'MD-2' and 'BRS Imperial', with a coefficient of variation of 18% and 16%, respectively. The Pérola cultivar was the one that presented the smallest variation for this trait, presenting greater homogeneity in the size of the plants, allowing, therefore, greater predictability in the production of seedlings. The minimum value of 11.20 cm was observed for Gold 'MD-2' and the maximum value of 27.50 cm for 'BRS Imperial'.

Table 2. Stem length and diameter and the average number of seedlings obtained in propagation by stem sectioning in three commercial pineapple cultivars.

Cultivars	Average	Minimum	Maximum	SD*	CV (%)
			Stem length (cm)		
'BRS Imperial'	21.85	16.80	27.50	3.63	16.60
'Pérola'	18.28	15.30	20.40	1.67	9.15
Gold 'MD-2'	15.15	11.20	19.80	2.82	18.64
			Stem diameter (cm)		
'BRS Imperial'	5.15	5.00	5.30	0.14	2.68
'Pérola'	5.15	4.60	5.70	0.37	7.24
Gold 'MD-2'	6.81	6.40	7.60	0.43	6.25
			Seedlings/stem section		
'BRS Imperial'	13.00	9.00	20.00	3.33	25.16
'Pérola'	2.00	1.00	5.00	1.50	64.17
Gold 'MD-2'	3.00	1.00	6.00	2.05	74.56

*Standard deviation.

Regarding stem diameter, the mean values of 'BRS Imperial' and 'Pérola' were similar, but the coefficient of variation of the latter was higher, making clear the greater inequality between plants. Stem diameter is one of the most evaluated variables in studies with pineapple since it is related to vigor, as more vigorous plants have a larger diameter and, consequently, a greater number of buds. It is worth noting that these traits may change due to cultural practices and planting density, among other factors (CARDOSO et al., 2013).

For the number of seedlings, 'BRS Imperial' had the highest production, with an average of 13 seedlings per stem section. This number is much lower than that obtained by Oliveira, Pádua, and Matos (2013), with stems of this cultivar without performing the longitudinal cut, in which 46 seedlings were obtained per stem after 4 months. This difference is probably due to the shorter period in which the stems were obtained in the present study, which was 40 days.

In studies with the 'Pérola' cultivar, Oliveira (2017) evaluated different environments for stem sectioning of this variety and found that in the greenhouse environment, the plants had a longer shoot length (13 cm) when the largest stem section was used, which was 20 cm. There was an increase in the number of shoots with the increase in stem length, with a maximum value of 3.2 shoots found 57 days after planting in the shaded environment. These results are close to those observed in the present study, where the average value for stem length was 18 cm, and the average number of seedlings was 2 seedlings per stem section.

A positive and significant correlation was observed between the stem length and the number of seedlings (r = 0.84**). This correlation suggests that longer stems have a greater number of seedlings, that is, the larger the area, the greater the number of buds, similar to what was found by Oliveira (2017). In the present study, significant and negative correlations were also observed between stem diameter and length (r = -0.85**) and stem diameter and number of seedlings (r = -0.43**).

The performance of the cultivars, considering the stem sectioning method, can be influenced by several factors, ranging from the cultivation environment to the physiological conditions of the plants used as parent plants for the production of seedlings. The process begins with selecting these parent plants, still in the production phase, marking the most vigorous ones and fruits characteristic of the variety (MATOS et al., 2009).

Micropropagation

The establishment results can be seen in Table 3. The percentages of bacterial contamination ranged from 13% in Gold 'MD-2' to 19% in the other two cultivars. Despite not compromising the establishment, these contamination rates are considered relatively high compared to other results obtained using this establishment methodology. The protocol used in this study for micropropagation is based on Souza et al. (2013), which generally results in high establishment rates above 90%.

Table 3. Total number of buds established *in vitro* (Total number of buds), surviving buds (Number of buds), fungal contamination (Fungal Count %), and bacterial contamination (Bacterial Count %) in three pineapple cultivars.

Cultivars	Total number of buds	Number of buds	Fungal Cont. (%)*	Bacterial Cont. (%)*
'BRS Imperial'	93	20	10 (11)	18 (19)
'Pérola'	90	20	0	17 (19)
Gold 'MD-2'	92	20	0	12 (13)

(*) percentage of contamination over the total number of established buds.

However, success in the establishment stage may depend on several factors ranging from the age of the plant to its phytosanitary condition and the location of the stem from which the bud is excised. Buds from the lower third of the stem should only be used in case of scarcity of propagation material, as this part of the stem is in direct contact with the soil. On the other hand, older plants may have more contamination problems at this stage after fruiting, which may explain the results obtained.

Table 4 shows the values referring to the total number of shoots obtained for each cultivar during the four subcultures and the geometric growth rate between the intervals of subcultures.

Table 4. Total number of shoots and geometric growth rate among the four subcultures for the three pineapple cultivars.

Commercial Cultivars	……...Total number of shoots………					…..……..Geometric growth rate…………			
	S0	S1	S2	S3	S4	S0-S1	S1-S2	S2-S3	S3-S4
'BRS Imperial'	20	96	270	481	2743	1.16	0.64	1.95	0.94
'Pérola'	20	49	257	447	1650	1.86	0.62	1.46	0.98
Gold 'MD-2'	20	45	122	195	688	1.11	0.52	1.41	0.76

In the first subculture, the cultivars Pérola and Gold ‘MD-2’ showed similar results, and half of the shoots were registered for the ‘BRS Imperial’. Over the four subcultures, this response changed, and Gold 'MD-2' recorded the lowest number of shoots (688) at the end of the fourth subculture. The highest number of shoots was obtained with 'BRS Imperial' (2,743), followed by 'Pérola' with 1,650 shoots after the fourth subcultivation. This behavior of the cultivars, about the production of shoots in response to the protocol used, is well represented in the Poisson log-linear regression model (Figure 6), which provided the best fit to represent the multiplication rate in each subculture for the evaluated cultivars. The coefficients of determination (R^2) were above 95%, showing the reliability of data adjustment. Despite the similar behavior of linear growth as one progresses in the subcultures, the differences in the production of sprouts are quite evident for the three cultivars.

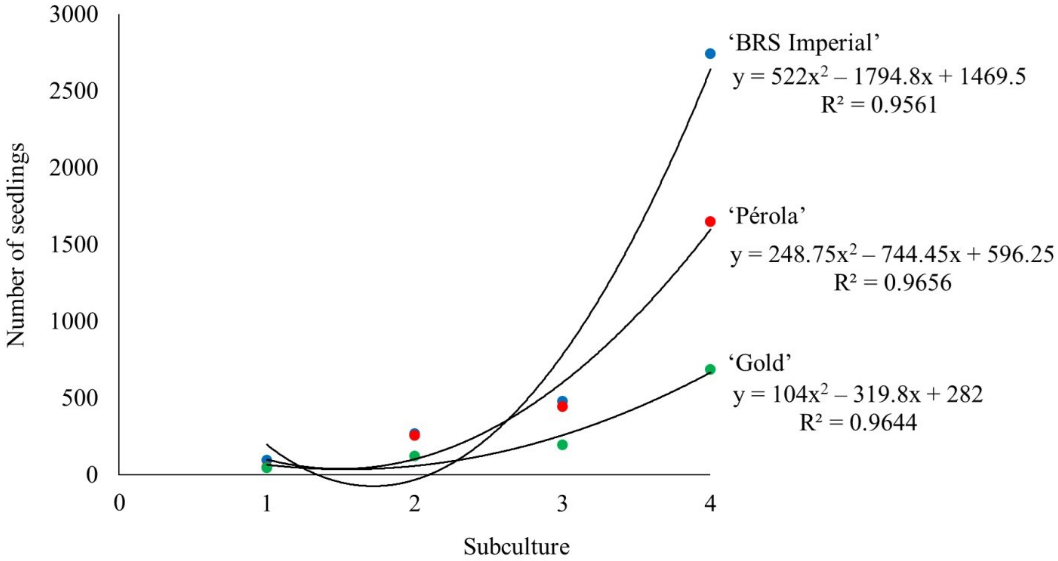

Figure 6. The Poisson log-linear regression model produced and estimated the number of shoots according to the four subcultures in three pineapple cultivars.

The geometric growth rate (Table 4) has been used to measure the propagation potential of a variety over several subcultures from an interval between one subculture and another. The geometric growth rate is the average increment in the number of shoots between two subcultures and indicates the pace of this multiplication, allowing future estimates and projections to be made. The rate is influenced by the loss of shoots or their non-development, which does not allow their subdivision and, therefore, the formation of new shoots.

When the first shoots or groups of buds appear in micropropagation, they must be subdivided and transferred to a new culture medium in 30 to 45 days, characterizing the interval between subcultures. According to Silva et al. (2016), cutting the shoots during transfer can affect the tissues or meristematic regions, influencing the multiplication results.

The geometric growth rates found in this study show an atypical behavior of what has been shown in other studies, such as Silva et al. (2016) and Souza et al., (2018), which observed a decrease in propagation potential in pineapple after the third subculture.

According to the data presented in Table 4, it is observed that there was a reduction between S1 and S2, followed by a significant increase in S2-S3 and a new reduction in S3-S4. The results show the same behavior for the three cultivars, which reinforces the possibility that some change in the culture medium contributed similarly to the behavior of the three cultivars. The propagation potential is directly related to the genotype response, the type of explant used, and the *in vitro* culture conditions. With the advancement of subcultures, the emergence of somaclonal variations should also be considered, which are undesirable because they do not maintain the genetic stability of the materials (SAMARFARD et al., 2014).

Differences between the methods, considering the total number of seedlings and the time to obtain them, make the marked difference between the three ways of obtaining propagation material very clear (Table 5). Regarding the propagation method, the number of seedlings generally increases in conventional propagation, followed by sectioning the stem with the highest number of seedlings for micropropagation (Figure 7). An exception is registered for the Pérola cultivar, which registered more seedlings by the conventional method than by stem sectioning.

Table 5. Comparison of the number of seedlings produced and time required by the different propagation techniques of commercial pineapple cultivars until planting in the field.

Propagation Method	'BRS Imperial'	'Pérola'	Gold 'MD-2'	Time (days)[4]
Crown[1]	21	22	20	120
Slip-crown[1]	12	0	0	120
Ground sucker[1]	10	0	2	120
Aerial sucker[1]	4	0	6	120
Slip[1]	128	170	5	120
Total seedlings produced				
Conventional propagation [1]	175	192	33	120
Stem sectioning[2]	530	130	116	360
Micropropagation[3]	2743	1650	688	480

[1] = 20 plants; [2] = 10 plants/stems subdivided in half (n = 20); [3] = 20 Buds from six plants; [4] = Days after harvesting the fruit until planting in the field: Conventional propagation = removal of seedlings and direct planting in the field. Stem sectioning = harvesting the stem until the sprouts are removed = 180 days + from the removal of sprouts to the seedbed = 180 days. Micropropagation = establishment phase = 60 days + Subcultures (4 x 45 days = 180 days) + Acclimatization = 120 days + Seedlings in the seedbed = 120 days.

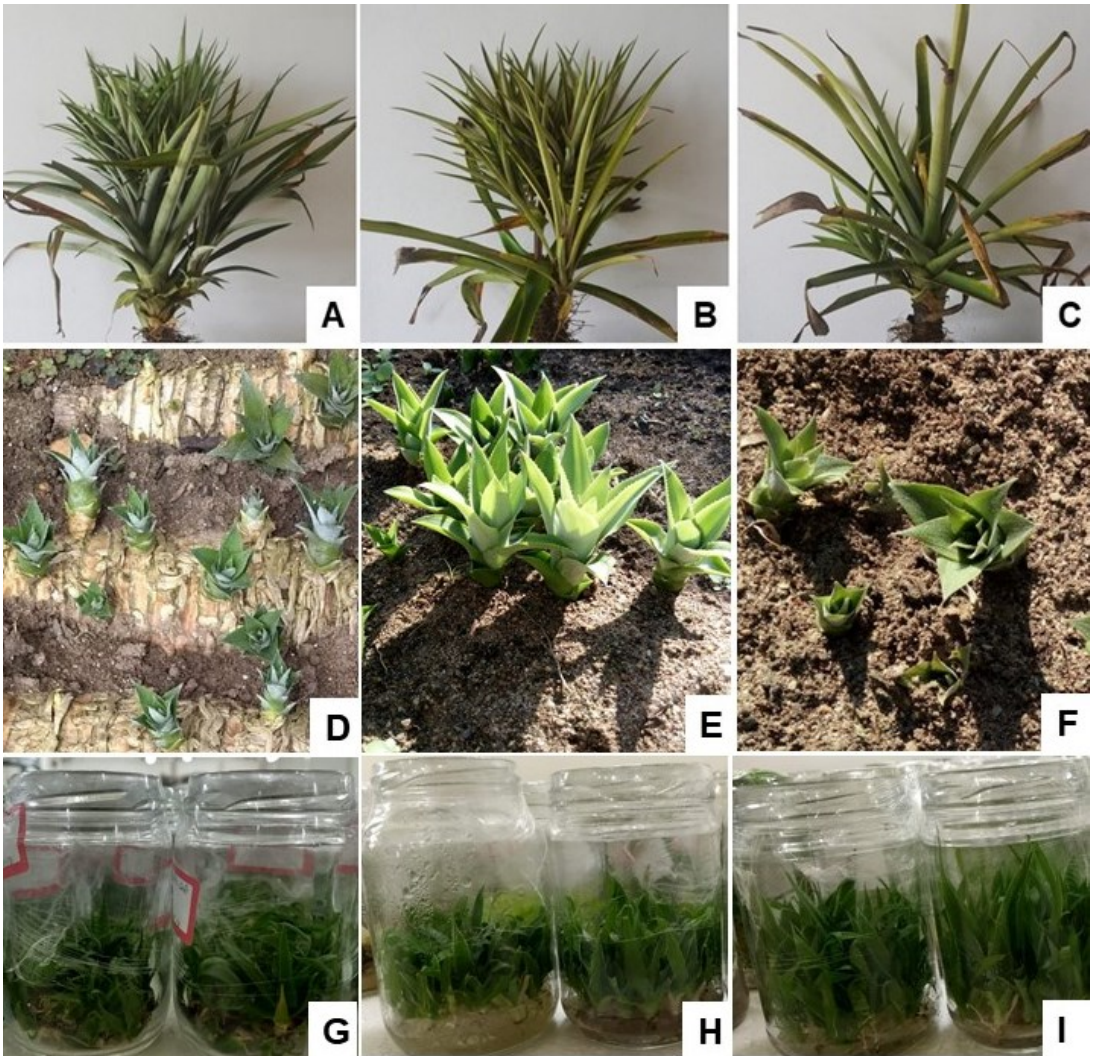

Figure 7. Comparison of different propagation techniques of commercial pineapple cultivars 'BRS Imperial' (A, D, G), 'Pérola' (B, E, H), and Gold 'MD-2' (C, F, I). A-C) Conventional propagation at 120 days after fruit removal; D-F) Stem sectioning after 45 days of establishment in seedbeds. G-I) Micropropagation in the fourth subculture at 45 days.

The production yield of seedlings from sectioning the stem depends on factors such as cultivar, section size, stem vigor, and adopted cultural practices. Considering the cultivars, the 'BRS Imperial' was the one that obtained the best results, both in stem sectioning, with 530 seedlings in 360 days, a result far superior to that obtained in cultivars 'Pérola' (130) and Gold 'MD-2' (116). With micropropagation, 2,743 seedlings were obtained, followed by 'Pérola' with 1,650 seedlings and Gold 'MD-2' with 688 seedlings at 480 days (Table 5).

This study observed the advantages of *in vitro* multiplication in producing pineapple seedlings, such as large-scale production, high multiplication rate, and propagules free of pests and diseases, as reported by Santos et al. (2015). Another advantage of this method is its use for commercial seedlings and the reproduction and multiplication of parent plants in breeding programs, where a single plant with desirable traits is obtained (SOUZA et al., 2013). Another aspect being considered is the health of the vegetative material. Seedlings from the conventional method can be contaminated with fusariosis and infested by pests (REINHARDT et al., 2018). For stem sectioning, it is necessary to ensure that the matrices used are disease-free. Cutting the stem makes it possible to identify the presence of fusariosis and proceed with the disposal of infected plants. However, it is impossible to identify pineapple wilt caused by the PMWaV viral complex (*Pineapple mealybug wilt associated virus*). As fusariosis is a limiting factor for pineapple production, 'Pérola' has a disadvantage to 'BRS Imperial', as 'Pérola' is susceptible to fusariosis, which makes its propagation by the conventional method a risky activity (MATOS et al., 2009).

The better performance of 'BRS Imperial' in the stem sectioning and micropropagation techniques is not only conditioned to obtaining the highest number of seedlings respect to the other evaluated cultivars but also by the phytosanitary condition of these seedlings obtained by these techniques. According to Reinhardt et al. (2018), the stem sectioning technique and *in vitro* propagation provide planting material free of diseases disseminated by using contaminated seedlings, crowns, or shoots. Once again, the need for viral detection methods before using the plant as a multiplication matrix is highlighted. However, according to Reinhardt et al. (2018), to ensure that micropropagated plants are disease-free, it is recommended that the parent plant be virus-indexed, especially for the cochineal-associated pineapple wilt virus, PMWaV.

At the Embrapa Cassava & Fruits, indexing for the wilt virus is routinely performed via RT-PCR (GUERRA et al., 2021; SILVA et al., 2021). As for the time to obtain the seedlings, micropropagation takes the most time but compensates for the number of plants produced.

CONCLUSION

The 'BRS Imperial' cultivar showed satisfactory performance in most propagation techniques studied, except for the conventional method, in which the 'Pérola' cultivar was superior and obtained better performance combined with the use of the slip-type structure. Among the propagation methods studied, micropropagation is the one that provides the highest number of seedlings. As for the time to obtain seedlings, all three techniques obtained satisfactory results, with micropropagation demanding more time, making up for the number of plants produced.

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