Revista Ciência Agronômica, v. 54, e20228404, 2023
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Scientific Article
ISSN 1806-6690

Propagation potential of commercial pineapples and impact of the subculture interval on production planning[1]

Potencial propagativo de abacaxizeiros comerciais e impacto do intervalo de subcultivos no planejamento da produção

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ABSTRACT - Measuring the propagation potential of any variety, considering micropropagation to obtain the seedlings, has received little attention from researchers. The use of unusual statistical techniques, such as geometric growth rate and exponential regression, can produce important information for planning and applying subcultures based on their *in vitro* behaviour, which may indicate the need to improve the protocol and to better understand the effects of interval trials and subcultures. The aim of this study was to evaluate the effects of three different subculture intervals on the propagation potential of commercial pineapple cultivars, with the aim of optimising micropropagation protocols and planning for seedling production on a commercial scale. Axillary buds from the Perola, BRS Imperial and Smooth Cayenne cultivars were used for *in vitro* establishment and multiplication in trials with a subculture interval of 30, 45 and 60 days, in six subcultures. The BRS Imperial cultivar had the best results in the 30-day trial. Although the number of shoots increases as the subcultures progress, the propagation potential is lower. Longer subculture intervals show lower shoot production and propagation potential, as demonstrated by the geometric growth rate and the Poisson log-linear models. The trials and statistical tools employed showed that the protocol needs adjusting to improve production in the Smooth Cayenne cultivar, which had the lowest propagation potential.

Key words: *In vitro* behaviour. Geometric growth rate. Poisson log-linear models.

RESUMO - A medida do potencial propagativo de uma variedade, considerando a micropropagação para a obtenção das mudas, tem tido pouca atenção dos pesquisadores. O uso de técnicas estatísticas pouco usuais, a exemplo da taxa de crescimento geométrico e regressão exponencial, pode produzir informação relevante para o planejamento e aplicação nos subcultivos baseado no comportamento *in vitro*, o que poderá indicar a necessidade de melhorar o protocolo, assim como a influência dos ensaios de intervalos e repicagens precisa ser mais bem compreendido. O objetivo do trabalho foi avaliar os efeitos de três diferentes intervalos entre subcultivos no potencial propagativo de cultivares comerciais de abacaxizeiros visando otimização dos protocolos de micropropagação e no planejamento para a produção de mudas em escala comercial. Foram utilizadas gemas axilares das cultivares Perola, BRS Imperial e Smooth Cayenne para o estabelecimento e multiplicação *in vitro*, em ensaios com 30, 45 e 60 dias como intervalo de repicagem em seis subcultivos. A cultivar BRS Imperial apresentou os melhores resultados no ensaio de 30 dias. Embora o número de brotos cresça com o avanço dos subcultivos, há maior queda no potencial propagativo. Maiores intervalos de subcultivos apresentam menor produção de brotos e potencial propagativo, como ficou demostrado pela taxa de crescimento geométrico e uso de modelos log-linear de Poisson. Os ensaios e as ferramentas estatísticas utilizadas permitiram evidenciar que o protocolo necessita de ajustes para melhorar a produção da cultivar Smooth Cayenne que apresentou menor potencial propagativo.

Palavras-chave: Comportamento *in vitro*. Taxa de crescimento geométrico. Modelos log-linear de Poisson.

DOI: 10.5935/1806-6690.20230023
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Received for publication 18/03/2022; approved on 09/08/2022
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INTRODUCTION

Pineapple (*Ananas comosus* L., Merrill), belongs to genus *Ananas*, is the most important member of family Bromeliaceae. The fruit is widely grown in Brazil (COPPENS D'EECKENBRUGGE; GOVAERTS, 2015), which is the second largest producer in the world, with a production of 2.45 million tons, while global production reached 27.8 million tons in 2020 (FAO, 2022).

Propagation in the pineapple is vegetative, with seedlings obtained from suckers and/or shoots (REINHARDT *et al*., 2018). The use of these seedlings can spread the pathogen that causes fusariosis (*Fusarium subglutinans*) and pineapple wilt caused by the mealybug (*Dysmicoccus brevipes*) (DEY *et al*., 2018), which can result in significant production losses.

Micropropagation techniques allow proven healthy and pathogen-free seedlings to be obtained from donor parent plants (GUERRA *et al*., 2021). The immediate consequence is more-uniform plantations, with the potential to respond to cropping treatments designed especially for the crop (SHERER *et al*., 2013; TURNER-HISSONG *et al*., 2020).

However, despite all the advances in this technique, a series of factors require further study and better adaptation, especially in the way experiments are conducted and the statistical tools used to analyse the data.

In the more specific case of tissue culture, the biological behaviour of plant cells, organs and tissue does not always appear to be properly evaluated, despite the so-called 'controlled' conditions to which they are subjected, and which, in theory, should result in a low coefficient of variation, which does not appear to happen (WERNER *et al*., 2012). In the case of micropropagation, this method is even more pronounced. Data that generally do not present a normal distribution, and even using transformations to achieve a normal distribution, do not reflect observed biological behaviour, need to be suitably manipulated.

In a study of more than 60 pineapple genotypes from the Active Germplasm Bank of Embrapa Mandioca e Fruticultura, Silva *et al*. (2016) showed that despite a normal distribution in the micropropagation data of accessions from four subcultures, the MSD (Minimum Significant Difference) generated by ANOVA was very high and did not allow visible significant differences to be expressed between the botanical varieties. Mendes *et al.* (1999) propose the use of Poisson regression to examine multiplication rates in the banana, which is a far more appropriate technique for use with quantitative data.

In a study by Silva *et al*. (2016) of geometric growth in the pineapple, they employed a tool that is generally used to measure population dynamics by translating the percentage increase in population that shows a trend towards stability in human populations. Adapting this type of measurement to quantify the propagation potential of plants *in vitro* has proved interesting, and reveals data which is more consistent and in line with reality. Another aspect that seems to significantly influence the results of micropropagation relates to subcultures: not only how many subcultures (transplants) should be carried out, but the interval between them, which can interfere with the end results (HAMAD; TAHA, 2008).

The aim of this study was to evaluate the effects of three different subculture intervals (days) on the propagation potential of commercial pineapple cultivars in order to optimising micropropagation protocols.

MATERIAL AND METHODS

Plant material, bud disinfestation and in vitro establishment

Adult plants of the BRS Imperial, Perola and Smooth Cayenne pineapple cultivars (four plants of each cultivar) were used as donor plants, obtained from the experimental area of Embrapa Mandioca e Fruticultura, in Cruz das Almas, Bahia. The leaves of each plant were removed to excise the axillary buds, which were washed in distilled water and commercial detergent for later asepsis in a laminar flow chamber by immersion in 70% alcohol for five minutes. They were then immersed for 20 minutes in a sodium hypochlorite aqueous solution at 1% active chlorine with three drops of Tween-20 detergent, followed by three rinses in sterile distilled water. The buds were inoculated in test tubes, 14 mm in diameter and 100 mm in height, containing MS culture medium (MURASHIGE; SKOOG, 1962) with no growth regulator and the addition of 30 g L^{-1} sucrose and 2.4 g L^{-1} Phytagel®, with the pH adjusted to 5.8 prior to autoclaving. The cultures were kept in a growth room for 60 days at 27 ± 1 ºC, a photon flux density of 22 µE m^{-2} s^{-1} and photoperiod of 16 hours.

Subcultures and Multiplication

After 60 days cultivation, buds were transferred to flasks containing 25 mL solid MS culture medium supplemented with 0.5 mg L^{-1} BAP, 0.02 mg L^{-1} ANA, 30 g L^{-1} sucrose and 2.4 g L^{-1} Phytagel®, with the pH adjusted to 5.8 prior to autoclaving.

Three different subculture intervals were established: 30, 45 and 60 days for the three cultivars, when the number of shoots was counted for each subculture.

During the establishment stage, the number of tumescent buds, oxidised buds, fungal contaminations (%) and bacterial contaminations (%) were counted. During

the multiplication stage, the number of initial buds varied depending on the cultivar, since buds were lost throughout the establishment stage. Due to the large loss of buds of the Perola cultivar during establishment and at the start of multiplication, the cultivar was used just in the interval of 30 days, leaving only two cultivars for the analysis.

For the two cultivars, the tumescent buds were divided proportionally over the three subculture intervals adopted; six subcultures were carried out, each time removing the shoots from the flasks and cutting them longitudinally into equal parts for subculturing in flasks containing fresh multiplication medium. At the end of each subculture, the number of shoots was counted and the number of contaminations per flask was recorded.

Statistical design and analysis

The experimental design was completely randomised in a 2 x 3 x 6 factorial scheme (two cultivars, three subculture intervals and six subcultures), with four replications per treatment, each replication comprising one plant. The geometric growth rates and the mean number of shoots per cultivar were calculated from the data from each subculture. The geometric growth rate (r) measures the propagation potential of the cultivars between two subsequent subcultures, using the expression:

$$r = \frac{\left(\sqrt[t]{Vf\,/Vi} - 1\right)X100}{Vi} \quad (1)$$

where:

Vf – Number of shoots in the following subculture;

Vi – Number of shoots in the previous subculture;

t – Intervals of 30, 45 and 60 days between the six subcultures.

For both cultivars under evaluation (BRS Imperial and Smooth Cayenne), Poisson log-linear models were fitted to the resulting data (MENDES *et al*., 1999), considering each subculture from one to six as an independent variable (linear and quadratic effects) and the mean number of shoots as a dependent variable, for each of the subculture intervals used (30, 45 and 60 days), given by NB=exp(a+bx+cx^2), where *NB* is the mean number of shoots (dependent variable); *a*, *b* and *c* are the parameters to be estimated; and *x* are the subcultures (independent variable). The statistical analysis was carried out using the Statistica v7.1 (STATSOFT INC., 2005) and the R (R CORE TEAM., 2018) statistical software.

RESULTS AND DISCUSSION

The establishment stage is crucial for the success of the work and to evaluate the behavior of the buds at the beginning of the process, and can be an indicator of the behavior of each pineapple material. Table 1 shows the results at 60 days, expressed as a percentage and in absolute numbers, for different variables related to the buds from the first stage. The remaining buds comprised the initial explants of the experiment that aimed at evaluating the effect of the subculture interval on the large-scale production of seedlings for commercial varieties of pineapple.

The results indicate a difference in the number of buds during establishment, mainly due to a characteristic of the cultivars, which naturally have different multiplication rates when conventionally propagated (SANTOS *et al*., 2015; SENA *et al*., 2015; SOUZA *et al*., 2012). The Perola cultivar in particular shows high seedling production under the conventional system (BARTHOLOMEW, 2014; FRANCO *et al*., 2014; REINHARDT *et al*., 2012), but with a lower number of buds per stem, which was seen in the present study. For initial growing, 'Smooth Cayenne' showed the best response, with 80% of the buds responding well to the culture medium, followed by 'BRS Imperial' with 68% and 'Perola' with 42%.

The contamination, caused a loss of buds due to fungi or bacteria. The Perola cultivar had the highest percentage (33%) compared to 'Smooth Cayenne', where none of the buds were lost, despite showing an oxidation rate of 20%. Bacterial contamination was more frequent

Table 1 - Axillary buds of pineapple cultivars inoculated in MS culture medium with no growth regulator, divided into 30, 45 and 60 days

Cultivar	Inoculated buds	Tumescent buds (%)	Contaminated buds fungus/bacteria (%)	Total contaminated buds (%)	Oxidised buds (%)	Buds to be used as initial explant (30, 45 and 60 days)
BRS Imperial	60	68	12	25	6	15, 13, 13**
Total	(60)	(41)*	(7)	(15)	(4)	(41)
S.Cayenne	60	80%	0/0%	0	20%	16, 16, 16
Total	(60)	(48)	(0/0)	(0)	(12)	(48)
Perola	48	42%	0/33%	33%	25%	9, 6, 5
Total	(48)	(20)	(0/16)	(16)	(12)	(20)

* numbers in parentheses are absolute values; ** the division refers to the number of buds used for each subculture interval (30, 45 and 60)

than fungal contamination, which in most cases is not related to the disinfestation procedure, but to the fact that the bacteria may be an endophytic, coexisting with the plant in nature without causing damage, but may express themselves *in vitro*. This contamination is one of the difficulties in the establishment phase and control may depend on using antibiotics, which work not as bactericides, but as bacteriostatics. This means that the bacteria are not eliminated, only pressured temporarily to prevent multiplication, and in subsequent subcultures may reappear and cause damage to a large amount of already propagated material (PEREIRA; MATTOS; FORTES, 2003).

Therefore, the number of initial explants in the proposed assay varied according to the losses of each cultivar, as shown in Table 1. A large amount of buds of the Pérola cultivar were contaminated, leaving only those from the 30-day interval. Surviving buds were transferred to the multiplication medium, and the number of shoots per subculture was recorded.

Table 2 shows the results for the number of shoots in each subculture, as well as the geometric growth rate between subcultures, which determines the propagation potential of the cultivar under the established conditions and for the three subculture intervals chosen.

For the absolute number of shoots, growth is exponential, and all the cultivars showed similar behaviour. The absolute number of shoots increases as the number of subcultures progresses, as well as the inverse behavior was observed as the subculture interval increases. These differences are more pronounced in 'BRS Imperial', with the production of 10,000 plants in the 5th subculture in the the 30-day interval, compared to half that production (5,360) in the the 60-day interval.

For the Perola cultivar, it was possible to carry out the trial just with 30 days interval, since contamination rates in the establishment were high, and not enough buds survived to set up the trial for the other subculture intervals. However, comparing the 30-day interval with the other cultivars, 'Perola' had the lowest number of shoots. This behaviour, registered in the *in vitro* conditions, is not the same as observed in the field, where 'Smooth Cayenne' barely proliferates, and 'BRS Imperial' and 'Perola' show similar rates of propagation.

During the multiplication stage, in addition to recording the number of shoots of each subculture, the percentage of fungal and bacterial contamination resulting from endogenous contamination or inadequate handling during the transfer of the material to the new culture medium were also computed (Figure 1).

Figure 1 shows a non-standard distribution of contamination between the different subculture intervals, although there is a prevalence of contaminations in the fifth subculture regardless of the interval.

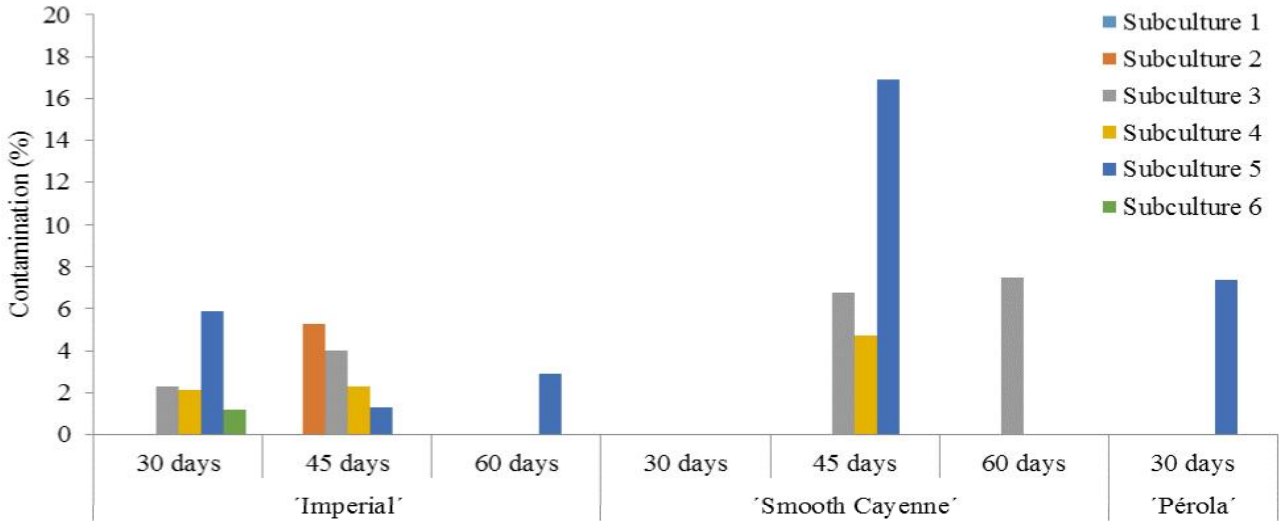
Contamination during the subcultures results in shoot losses that can compromise the entire process, as occurred with the Perola cultivar in the 45 and 60-day trials, with shoot loss in the first subculture, compromising the continuity of the study.

Contamination prevailed in the Smooth Cayenne cultivar for the 45-day interval, with a propagation potential of 0.82 (Table 2). According to the prediction, this average number of shoots would have been approximately 1.28, if the percentage of contamination had not been so high. For the Perola cultivar, there were additional losses due to the occurrence of oxidative processes, probably due to the reduced size of the buds. The release of polyphenols, creating a large halo around the explant, which may difficult growth, may occur due to injuries possibly caused at the time of incision, or when the buds are too small. According to Cid and Teixeira (2010), phenolic oxidation is attributed to the polyphenol oxidase enzyme (PPO), which is released by cells after tissue injury and can be toxic, compromising swelling of the buds and their subsequent development.

Table 2 - Number of shoots in the six subcultures with the geometric growth rate, in three pineapple cultivars from the field (AGB), at different subculture intervals.

Cultivar	Interval (days)	Number of shoots						Geometric growth rate				
		Initial buds	Subcultures					S1-S2	S2-S3	S3-S4	S4-S5	S5-S6
			1	2	3	4	5					
BRS Imperial	30	15	102	517	1,860	3,580	10,255	6.60	5.56	4.36	2.21	3.57
BRS Imperial	45	13	71	342	1,465	3,465	7,005	3.84	3.56	3.29	1.93	1.58
BRS Imperial	60	13	60	380	1,055	2,962	5,360	2.58	3.12	1.72	1.74	0.99
S.Cayenne	30	16	46	121	255	624	1,078	3.58	3.28	2.52	3.03	1.84
S.Cayenne	45	16	42	136	305	440	759	2.17	2.65	1.81	0.82	1.22
S.Cayenne	60	16	27	124	206	575	852	0.88	2.57	0.85	1.73	0.66
Perola	30	9	18	54	130	312	635	2.34	3.73	2.97	2.96	2.40

Figure 1 - Percentage of contaminated shoots in the three cultivars during the multiplication stage of the pineapple



On the other hand, the same table shows the geometric growth rate, which expresses the propagation potential of the cultivars. The geometric growth rate is a measure used to estimate population growth that shows an exponential pattern but tends to remain stable in human populations, where it is translated into percentage values. An adaptation of this method to measure the propagation potential in micropropagation was first used by Silva *et al.* (2016), who revealed that despite the number of produced shoots showing exponential behaviour, propagation potential tends to reduce as the subcultures progress. This type of analysis can help in planning seedling production by providing practical information that can be applied to the subcultures. The value of the geometric growth rate corresponds to two subcultures, and reveals the same behaviour in studies by Mendes *et al.* (1999) and Hamad and Taha (2008) with the banana and pineapple, respectively, when a reduction in multiplication rates affected the propagation potential of the material along the subcultures.One hypothesis for this reduction in propagation potential is that it may be caused by damage to areas of tissue with potential buds for the development of new shoots. In the literature, this occurs more frequently at intervals of 30 and 45 days (HAMAD; TAHA, 2008; MENDES *et al*., 1999; SILVA *et al*., 2016).

The differences of each genotype when expressing its propagation potential *in vitro* are reported by Grattapaglia and Machado (1998) as a genotype-dependent relationship in *in vitro* multiplication resulting from the characteristics of the genotype. This behaviour can be seen in the pineapple and other crops, since the multiplication rate varies between genotypes (FARAHANI, 2014; MENDES *et al*., 1999; NELSON; ASARE; ARTHUR JUNIOR, 2015; SILVA *et al*., 2016; USMAN *et al*., 2013).

Another argument that can be made to explain the reduction in propagation potential seen in this study, is the increase in plant density inside each flask. The number of shoots per flask in the earlier subcultures was lower than in the later subcultures. A density of 15 shoots per flask (100 mm in height and 218 mm in diameter) was adopted during establishment and for the first subculture (S1), 45 shoots per large flask (130 mm in height and 272 mm in diameter) for the second and third subcultures (S3 and S4), and 60 shoots per large flask for S6 (Figure 2). Shoot development may therefore have been compromised by competition for space and nutrients, leading to a drop in the geometric growth rate.

After S3, a higher shoot density was adopted as a matter of logistics and not of study, especially for S4 and S5, since there was a high number of plants and limited space in the laboratory. The competition for nutrients may have had even more impact in trials with longer intervals between subcultures, especially 60 days, which may explain the exponential drop in propagation potential as the subculture interval increased (Table 2). This hypothesis of densification may have a negative correlation with the rate of multiplication, and should also take into account other factors involved, as mentioned above.

There are reports in the literature concerning the effect of the subculture interval on the multiplication rate. In one of these studies, Kofi and Adachi (1993), evaluating the effect of two subculture trials, at 30 and 60 days, in the pineapple, found that the longer trial produced a greater number of shoots for the same number of subcultures, a different result to that found in this study.

Figure 2 - *In vitro* multiplication in the pineapple: 'BRS Imperial' at 30 days (a), 'Smooth Cayenne' at 45 days (b), and 'BRS Imperial' at 60 days, showing the second, fourth and sixth subculture of each cultivar in the growth room

Hamad and Taha (2008) confirmed this behaviour, obtaining a greater number of shoots in the Smooth Cayenne cultivar with an interval of 75 days, carrying out four subcultures at intervals of 30, 45, 60 and 75 days. These results were not corroborated in the present study, where the result was the opposite, with the greatest number of shoots for the shortest interval between subcultures. According to the authors, as the subcultures progress, transplanting slows down the maturation of shoot development, requiring a longer interval between subcultures to compensate for this effect and produce fully developed shoots.

As such, studies reported by the authors explain the reduction after the fourth transplant as due to the lack of shoot formation from 30 to 60 days. Among the few reports comparing the subculture interval in micropropagation of the pineapple, there is reference to greater shoot formation for the 60-day interval, compared to 30 days; this may be related to the cultivar, i.e. to genotype dependency. (KOFI; ADACHI, 1993).

On the other hand, in addition to the trials are the statistical procedures used as a tool for evaluating these trials. Most of the time, the analysis does not express the biological behaviour shown by the genotypes *in vitro*. During the subcultures, the number of shoot does not generally follow a normal distribution, which makes the use of ANOVA questionable in this type of evaluation, even when using data transformations, despite it having been used, as mentioned above.

However, some studies have provided more suitable statistical tools for analysing the data referring to multiplication rates or shoot counts, such as the work of Mendes *et al.* (1999), who propose the use of the Poisson regression model. In the present study, the data obtained in the six subcultures, considering the three subculture intervals, were analysed using the Poisson log-linear model.

Figures 3 and 4 show the graph resulting from regression analysis using the Poisson log-linear model, where the curves correspond to the subculture intervals and to the six transplants that were carried out, showing the difference between the trials under study and the mean number of shoots expressed over the course of these subcultures. Despite the difference in the number of shoots obtained at the end of the six subcultures, the biological behaviour was the same, showing exponential growth. Exponential growth for the mean number of shoots was expected due to shoot accumulation over the course of the subcultures, behaviour confirmed by the curves, which show an increasing variation in terms of production between the subcultures and between the intervals. The results also indicate an increase in rates after the sixth subculture for the intervals of 30 and 45 days, as observed in the ascending curves, and it is not possible to determine in which subculture the production of shoots begins to reduce.

The exponential model shows to a possible reduction in multiplication rate after the fifth subculture for the interval of 60 days, suggesting a quadratic function after reaching a peak in production, and tending to fall following the behaviour seen in biological models, and better reflecting that which occurs naturally. Some authors state that this reduction occurs from the fourth subculture onwards (HAMAD; TAHA, 2008; SILVA *et al*., 2016) due to internal and external factors that control or inhibit growth, such as the hormone concentration (KOFI; ADACHI, 1993).

The results of this study show the difference between cultivars in relation to the number of shoots produced, as well as differences within the same cultivar for the subculture intervals under study. Mendes *et al.* (1999), in a study on the dynamics of multiplication rates in six families of the Apple (Maçã) cultivar found variable shoot production over the course of six subcultures, showing a reduction in the rate after the fourth subculture, except for family B, which continued to increase following the sixth subculture. The authors state that such variability within a genotype may result from physiological differences in the shoots, which are located at different donating regions on the parent plant.

The buds that were excised to carry out this study were also distributed over two different regions of the matrix plant, the middle and upper regions of the stem, The buds that were excised to carry out this study were also distributed over two different regions of the matrix plant, the middle and upper regions of the stem, which explains the variability in plant development due to the different endogenous levels of growth regulators in the the shoots. The buds from the base of the stem are not used, as they use to show high rates of fungal and bacterial contamination.

Hamad and Taha (2008) justifyed a long multiplication interval, explaining that, in general, 35% of shoot formation occurs during the first 30 days, with 40% occurring during the last 15 days of a 75-day incubation period. These peaks

Figure 3 - Poisson log-linear model for mean number of shoots as a function of the subculture (transplant) for each of the intervals used between subcultures (30, 45 and 60 days), in the BRS Imperial pineapple cultivar

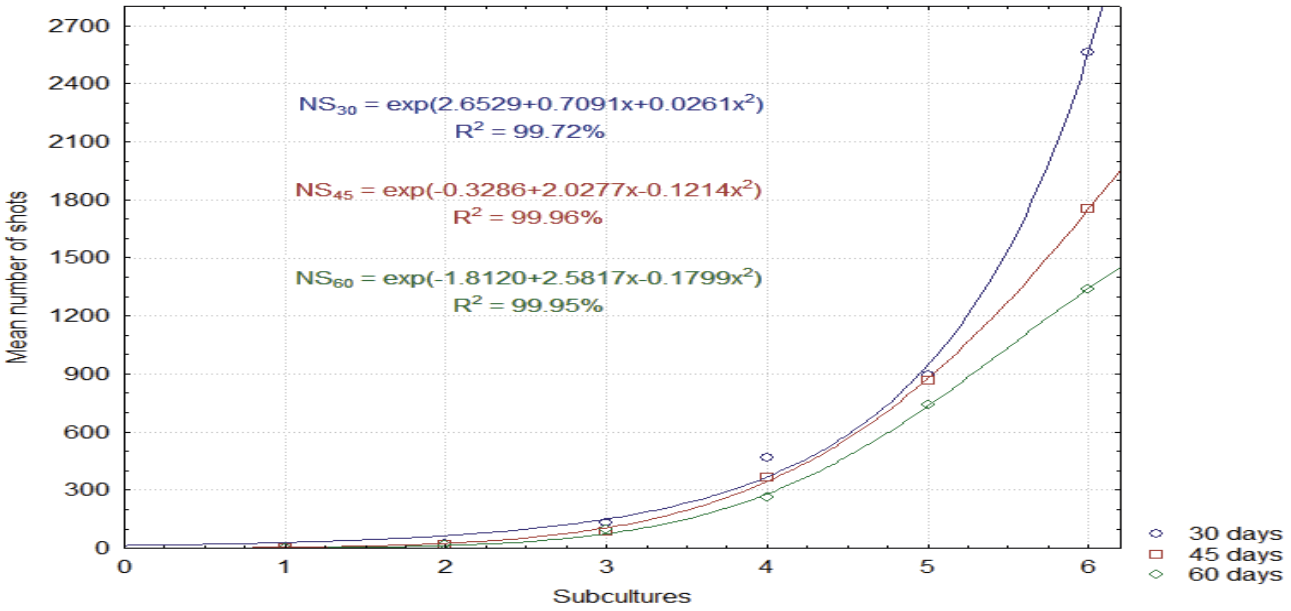
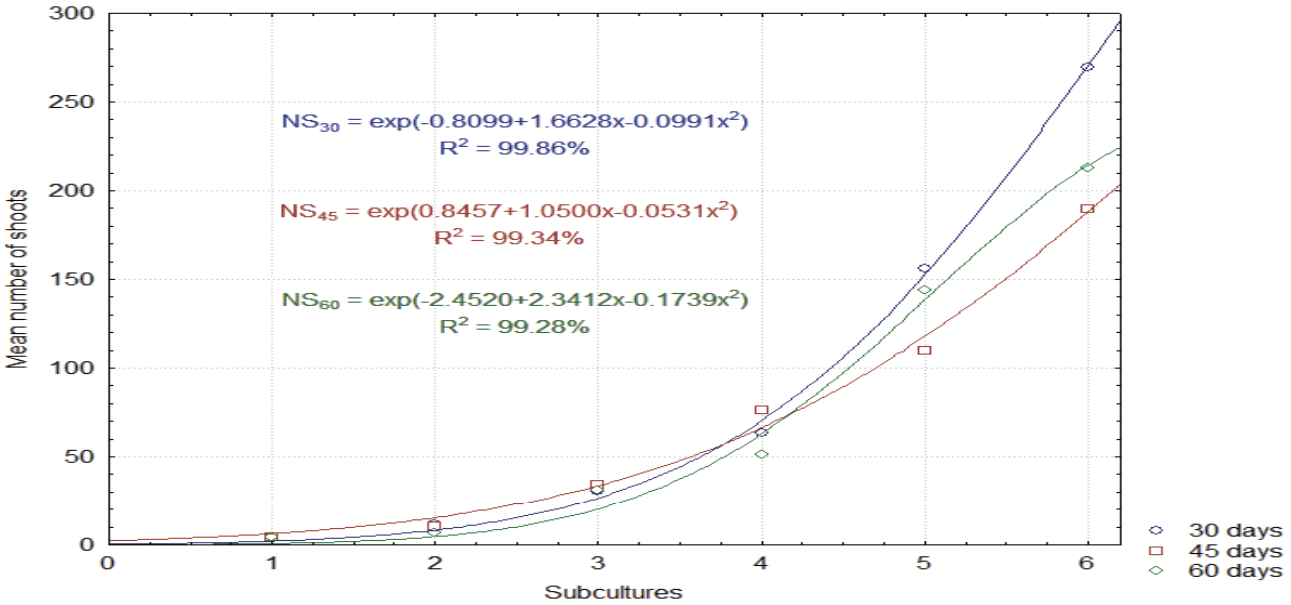


Figure 4 - Poisson log-linear model for mean number of shoots as a function of the subculture (transplant) for each of the intervals used between subcultures (30, 45 and 60 days), in the Smooth Cayenne pineapple cultivar



in formation may be related to the maturation state of the shoots, since, according to the authors, the shoots formed during the first 30 days come from axillary buds located in regions where the maturation state is more advanced, and complete their development during incubation, these shoots being counted every week of the study.

This formation pattern continued in the works of Hamad and Taha (2008) over four subcultures, followed by a reduction in the multiplication rate attributed to a lack of promoter or of shoot formation during the 30- and 60- day intervals, the effects of which are still not well understood despite influencing the development process, as seen in the subcultures.

This phenomenon of declining in the multiplication rates after the fourth subculture is reported by Mendes *et al*. (1999) in the banana. As the multiplication speed decreases, a deceleration occurs after a production peak, and this transition is demonstrated after the derivation of each regression-fitted equation.

Both authors report that despite a reduction in the speed of multiplication as a function of time, the number of shoots continues to increase. The speed of multiplication, defined as such by the authors, reflects what was reported by Silva *et al*. (2016) and adopted in the present study, the potencial of propagation. Recognising this potential and how it is expressed over the time depends on several factors, but may be important in planning and scheduling the *in vitro* production of seedlings. Based on the results of this study, it can be seen that the growing conditions were quite satisfactory for the BRS Imperial cultivar, but need to be optimised for the other varieties.

CONCLUSIONS

1. The biological behaviour of the BRS Imperial and Smooth Cayenne pineapple cultivars are similar, considering exponential growth in the number of shoots up to the fifth subculture, with a reduction in propagation potential as the subcultures progress, despite the number of shoots and propagation potential of the BRS Imperial variety being significantly higher than those of the Smooth Cayenne cultivar;
2. The Geometric Growth Rate and Poisson log-linear model prove to be suitable tools for evaluating the present research, aiding prediction models and indicating improvements in the protocol;
3. The trials and statistical tools employed allowed that the *in vitro* behaviour to be expressed, showing that the protocol needs adjustment to improve production in the Smooth Cayenne cultivar, which showed less propagation potential as the subculture interval increased and the cultivars progressed. The results confirm the genotype dependency mentioned above in the BRS Imperial cultivar, with better results in the 30-day trial.

ACKNOWLEDGEMENTS

The authors would like to thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB – no. BOL0065/2017), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Finance Code 001, PNPD/ UFRB - 88882.315208/2019-01) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for their financial support. We would also like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity grant awarded to the authors (F.V.D.S - CNPq-304269/2018-2) e CASL (Process 306003/2018-0). Finally, our thanks go to the Universidade Federal do Recôncavo da Bahia (EHS – Visiting Professor) and Embrapa Mandioca e Fruticultura, which provided the plant material and experimental, technical and financial support.

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