

## Matrix management interferes in yield and quality of cocoa seedlings

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**Abstract-** The influence of two managements on yield and quality of plagiotropic mini-cuttings of four cacao clones was evaluated. The design was completely randomized in a 4 x 2 factorial scheme with 4 clones x 2 mother plant management (apical pruning and bending), with 10 replicates and one plant as experimental unit. Seedlings used were propagated by rooting of semi-woody cuttings of plagiotropic branches and at seven months of age, two seedlings of each clone were transplanted to 12 L pots filled with organomineral substrate. After transplantation, the apex of one of the plants was cut based on the count of the first six leaves from the apex. For bending, stems were folded and horizontally fixed. The first collection of mini-cuttings was performed 30 days after transplantation and the others during the following seven months. The yield of mini-cuttings was estimated during collections and part was used for rooting and quality evaluation of seedlings. After 90 days, 10 mini-cuttings of each clone were evaluated for quality using the Dickson quality index (DQI). Another 10 rooted mini-cuttings were transplanted to 1.5 L polyethylene bags and kept in greenhouse for another 120 days until final quality evaluation at 210 days. Data were submitted to analysis of variance and Tukey test at 5% probability. Shapiro-Wilk normality test and Pearson correlation analysis were performed. Bending was more efficient in the average production of mini-cuttings especially for BN 34 clone. The Dickson quality index was correlated with root dry mass, at both 90 and 210 days, and the bending technique was responsible for the best DQI. Variable height was not adequate to estimate the quality of cocoa seedlings.

**Index Terms:** quality index, apical pruning, minicuttings

## Manejo de matrizes interfere no rendimento e na qualidade de mudas de cacauzeiros

**Resumo-** Foi avaliada a influência de dois manejos no rendimento e na qualidade de miniestacas plagiotrópicas de quatro clones de cacauzeiro. O delineamento utilizado foi inteiramente casualizado, em esquema fatorial 4 x 2, sendo 4 clones x 2 manejos da planta-matriz (poda apical e envergamento), com 10 repetições e uma planta como unidade experimental. As plantas-matrizes usadas foram propagadas por enraizamento de estacas semilenhosas de ramos plagiotrópicos, e, aos sete meses de idade, duas mudas de cada clone foram transplantadas para vasos de 12 L preenchidos com substrato organomineral. Após o transplante, foi realizado o corte do ápice de uma das plantas, baseado na contagem das seis primeiras folhas a partir do ápice. Para o envergamento, os caules foram dobrados e fixados em posição horizontal. A primeira coleta de miniestacas foi realizada 30 dias após o transplante, e as outras, durante os sete meses seguintes. O rendimento de miniestacas foi contabilizado nas coletas, e parte foi usada para enraizamento e avaliação da qualidade de mudas. Após 90 dias de enraizadas, 10 miniestacas enraizadas de cada clone foram avaliadas quanto à qualidade, usando-se como referência o índice de qualidade de Dickson (IQD). Outras 10 miniestacas enraizadas foram transplantadas para sacos de polietileno de 1,5 L e mantidas em casa de vegetação por mais 120 dias, até à avaliação final da qualidade, aos 210 dias. Os dados foram submetidos à análise de variância e ao teste de Tukey, a 5% de probabilidade. Foi realizado teste de normalidade de Shapiro-Wilk e análise de correlação de Pearson. O manejo com envergamento foi mais eficiente na produção média de miniestacas, especialmente para o clone BN 34. O índice de qualidade de Dickson foi correlacionado com a massa seca da raiz, tanto aos 90 quanto aos 210 dias, sendo o envergamento responsável pelos melhores IQDs. A variável altura não se mostrou adequada para estimar a qualidade de mudas de cacauzeiro.

**Termos para indexação:** índice de qualidade, poda apical, miniestacas.

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## Introduction

The production of cocoa seedlings in quantity and quality has been an important support factor for the cacao activity in the state of Bahia, which is distributed in more than 50 municipalities and accounts for more than 50% of the production of dried cacao almonds in Brazil (SODRÉ et al., 2017).

Cutting is the propagation method frequently used for cacao, however, mini-cutting is a recent technique that has been successfully used in order to maximize the process of clonal propagation of some forest species such as *Eucalyptus* (ALMEIDA et al., 2007), *Toona ciliata* (SILVA et al., 2012; SOUZA et al., 2014) and the propagation of some fruit trees, *Annona muricata* L. (FIGUEIREIDO et al., 2013), *Passiflora edulis* (CARVALHO et al., 2007), and *Theobroma cacao* (SODRÉ and CORÁ, 2007).

The use of mini-cutting as a tool for plant propagation was intensified in Brazil in the 1990s for the genus *Eucalyptus* and placed Brazil among the most efficient producing countries (ALFENAS et al., 2004, ASSIS, 2014). Since then, its application has allowed the propagation of genotypes of other species, among them cacao, with an increase in the percentage of rooted mini-cuttings and improvement of the root system, directly influencing the performance of field seedlings.

The growing concern with the yield and quality of cocoa seedlings has led to the adoption of managements for commercial production and changes in the production system, such as the use of clonal gardens in nurseries and potted mother plants (SODRÉ, 2013), always aiming greater uniformity of the material to be worked.

Among managements used, the bending of stems of juvenile mother plants in order to fix them in a horizontal position, and the apical pruning that consists of the decapitation of the plant apex stand out. In both situations, the break of apical dominance provides alterations in the hormonal balance, mainly between auxin and cytokinin, which in turn change the apical dominance of the plant, promoting the growth of lateral buds (TAIZ and ZEIGER, 2017).

In determining the quality of seedlings, both morphological characteristics, which are based on phenotypical aspects, as physiological characteristics, which are defined by biochemical mechanisms of plants, can be used. The morphological characteristics are still the most used to determine the quality of seedlings because they have greater acceptance by nurserymen; however, they still lack definitions that can respond to survival and initial growth, due to the adversities that are found in the field after planting (ELOY et al., 2013). In the context of the quality analysis aiming the selection of seedlings for planting, the Dickson quality index (DICKSON et al., 1960) is indicated as a good indicator.

The aim of this work was to evaluate the influence of

two managements on the yield and quality of plagiotropic mini-cuttings of four cacao clones.

## Material and Methods

The experiment was conducted in greenhouse at the Cacao Research Center (CEPEC / CEPLAC), located in the municipality of Ilhéus, Bahia (14° 45 'S and 39° 40' W), from December 2015 to July 2016. Four *Theobroma cacao* clones L (PS-13.19, CEPEC 2002, BN 34 and CCN 51) were evaluated, which were recommended by CEPLAC as of high production and resistance to witches' broom disease.

The design was completely randomized in a 4 x 2 factorial scheme, 4 clones and 2 management methods of the mother plant (apical pruning and bending), with 10 replicates and one plant as experimental unit.

Cacao mother plants used in the experiment were initially propagated by rooting of semi-woody cuttings of plagiotropic branches at the "Biofábrica do Cacao" Institute (IBC) in Uruçuca, Bahia, using methodology described by Sodr e (2013). Two 7-month-old seedlings were transplanted into 12 L pots, using as substrate the mixture of 25 liters of Carolina ® + 25 L of Biomix ® (v / v), enriched with Osmocote ® slow release fertilizer (25 g), PG mix (25 g) and single superphosphate (100 g).

After transplantation, the apex of one of the plants was cut based on the count of the first six leaves from the apex. For bending, the stems were folded and horizontally fixed. Plants were supplemented every 30 days with complete nutrient solution of Hoagland (1950) at the dose of 15 mL per pot. During the experiment, the substrate was monitored for pH and conductivity.

The first collection of mini-cuttings was performed 30 days after the transplantation of the mother plant and the others during the following seven months, as shoots reached 8 cm and had at least 3 mature leaves. The yield of mini-cuttings was counted during collections and part was used for rooting and quality evaluation. In order to avoid losses due to transpiration during preparation, mini-cuttings had the first leaf, from the base reduced by 50% of the leaf area and the others by 80%.

For rooting, the mini-cutting base was immersed for 3 seconds in 2% Carbendazim solution and then in a hydroalcoholic solution (1: 1) of indolebutyric acid (IBA) at concentration of 6000 mg L<sup>-1</sup>. Then, they were cut in 150 cm<sup>3</sup> tubes using as substrate the same mixture used in mother plant pots. After cutting, they were kept in nebulization chamber with sprinkling regime of 5 seconds every 10 minutes between 08:00 am and 05:00 pm, for 90 days. The internal chamber temperature in the experimental period varied between 27 and 32°C.

After 90 days, 10 rooted mini-cuttings of each clone were evaluated for quality, using the Dickson quality index (DQI) as reference. Another 10 rooted mini-cuttings

were transplanted to 1.5 L polyethylene bags and kept in greenhouse for another 120 days until final quality evaluation.

To evaluate DQI, the stem diameter (SD) and height (H) of plants were obtained. To obtain shoot dry mass (SDM) and root dry mass (RDM), plants were cut and roots were washed in running water for substrate removal and placed in an oven at 65°C for 72 hours and then weighed in analytical scale with precision of 0.01 g. The total dry mass (TDM) was obtained by summing the SDM and RDM values. To calculate the quality index of seedlings, the formula of Dickson et al. was used (1960).

$$DQI = \frac{TDM}{\left(\frac{H}{SD}\right) + \left(\frac{SDM}{RDM}\right)}$$

Data were submitted to analysis of variance and when the test presented significance ( $p < 0.05$ ), Tukey's test ( $p < 0.05$ ) was applied to compare the means of the levels of interactions or levels of each factor studied. The Shapiro-Wilks normality test and Pearson's correlation analysis between the Dickson Quality Index (DQI), diameter (SD), plant height (H), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), shoot height / diameter ratio (SHDR) and shoot / root ratio (SRR). The correlation coefficients were verified by Student's t-test ( $p < 0.05$ ). Statistical analyses were performed using the R software (FERREIRA et al., 2011).

## Results and Discussion

There was interaction ( $p < 0.05$ ) between levels of clone and management factors of mother plants for variables average yield, DQI-90 and DQI-210 days after cutting (DAC). When assessed separately, clone and management factors significantly affected ( $p < 0.05$ ) SD, H, SDM, SDR and TDM variables (Tables 1 and 2).

The average production of mini-cuttings varied between managements and clones. Forwarded plants produced significantly more mini-cuttings in relation to pruned plants (Table 3). This result suggests that bending is more efficient in promoting stimulation for the production of new shoots. According to Morais et al., (2012), the use of plant bending induces the highest number of shoots during the formation of clonal coffee plantations. Schmidt et al., (2015) also observed that the bending of clonal coffee trees promoted greater stimulus in the vegetative growth of new shoots in relation to apical pruning.

The bending of 'Valencia' orange seedlings grafted by budding on 'Swingle' citrumelo, Pereira and Carvalho, (2006) verified greater vigor in shoots compared to apical pruning of the plant, both of which were applied to the rootstock.

In BN 34 clone, higher production of mini-cuttings was observed in plants submitted to bending, followed by CEPEC 2002, PS 13.19 and CCN 51 clones (Table 3). Also for pruned mother plants, BN 34 produced greater number of mini-cuttings, there were no statistical differences between CEPEC 2002 and PS 13.19 clones and in CCN 51, the lowest production was verified.

Although being currently recommended only for small-scale planting, BN 34 clone has been shown to be precocious in high field conditions, with plants already showing fruits in the first year of planting, which is not usually the case with most clones grown today in the state of Bahia, including clones used in this experiment. The results indicate that this precocity also appears in vegetative propagation and that this clone is more efficient in modifying the apical dominance of the plant, promoting the growth of lateral buds and consequently improving the yield of mini-cuttings.

The DQI values of seedlings varied with the management of mother plants and clones, in the two evaluations (Table 4). At 90 DAC, BN 34 clone presented significantly higher DQI values ( $p < 0.05$ ), both for management with bending and for apical pruning. At 210 DAC, BN 34 and PS 13.19 clones showed significantly higher DQI values than CEPEC 2002 clone for plants from mother plants that underwent bending, whereas for apical pruning, PS 13.19 clone was superior to BN 34 and CEPEC 2002 clones, and did not differ significantly ( $p > 0.05$ ) from CCN 51.

It was found that the DQI increased between evaluations of 90 and 210 DAC, for example, the CCN 51 clone ranging from 0.03 to 0.81 (Table 4). This result indicates that at 90 days, the DQI of seedlings has not yet shown a tendency to stabilize, and therefore, is not an adequate age to assess quality. The DQI values at 210 DAC were higher than those reported by Sodr e et al., (2016), who evaluated DQI in grafted cacao seedlings. However, considering that the values tend to increase, it may be suggested that the maintenance time of nursery seedlings should be extended in order to improve some morphological characteristics, such as height and diameter, before being taken to the field.

SDM, RDM and TDM variables were significantly correlated ( $p < 0.05$ ) with DQI at 90 days. On the other hand, the height of plants did not present significant correlation with the study variables (Table 5). Although height is easy to measure and is considered a good reference for estimating the quality standard of nursery seedlings, this variable did not explain the DQI under the conditions of this study, probably because it is a characteristic influenced by environmental variables.

Root dry mass (RDM) showed significant correlations with SDM, TDM, SRR and DQI, suggesting to be the most appropriate variable to estimate DQI at 90 DAC (Table 5). It is noteworthy that for SRR, the

correlation was negative. This study corroborates Binnoto et al., (2010), who observed that the root dry mass was the variable most related to DQI in *Eucalyptus grandis* W. Hill ex maid and *Pinus elliottii* var. *elliottii* seedlings.

Except for height, all variables evaluated at 210 days were significantly correlated with DQI (Table 6).

SDM was the characteristic that most significantly correlated among the study variables. According to Caldeira et al. (2008) and Gomes and Paiva (2006), the shoot dry mass must be considered because it indicates the rusticity of seedlings. According to these authors, there is a relationship between the factors that influence the growth in height and the dry matter gain of shoots, which could be related to the availability of nutrients of substrates used.

RDM is an index that similarly to DQI, can define the quality of cocoa seedlings. Caldera et al. (2008) consider RDM as an adequate variable to estimate survival and initial growth of seedlings in the field. Thus, the root system is important because it provides better development of plants when transferred to the field at the seedling stage, increasing the survival probability (ALMEIDA et al., 2005).

No significant differences were observed at 210 DAC in relation to clones for the HD variable (Table 7). It should be noted that in this period, seedlings had already been transplanted in polyethylene bags, which possibly allowed better utilization of the space, substrate and nutrients by the root system.

For SDM at 210 DAC, significant differences were verified for the BN 34 clone in relation to CEPEC 2002 (Table 7). BN 34 clone also stood out for management using bending and higher RDM values at 90 DAC. However, at 210 DAC, there were no significant differences between clones and management. This result suggests that as seedlings grew, the required amounts of nutrients increased and larger containers along with greater availability of nutrients reduced the differences between clones and management.

Regarding TDM, BN 34 clone at 90 DAC differed statistically from the other clones when mini-cuttings were collected in mother plants submitted to bending. At 210 DAC, this clone did not differ statistically from PS 13.19, but stood out from CCN 51 and CEPEC 2002 with higher values for the bending management.

**Table 1.** Analysis of variance for mean production of mini-cuttings and quality index (DQI) of seedlings originated from mother plants of four cacao clones submitted to bending and pruning.

SV	DF	Mean square		
		Production	DQI – 90 DAC	DQI -210 DAC
Clones	3	25488.6*	0.2184*	0.215333 <sup>ns</sup>
Manegement	1	31462.3*	0.1357*	0.159819 <sup>ns</sup>
Clones*Manegement	3	1940.2*	0.0616*	0.06731*
Residue	72	31.8	0.0019	0.00212
VC (%)		9.2	33.4	29.4

\* F Test ( $p < 0.05$ ); DAC: Days after cutting; ns: not significant; SV: Source of variation; DF: Degree of freedom; VC: Variation coefficient

**Table 2.** Analysis of variance for diameter (SD), plant height (H), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) of mini-cuttings originating from four cacao clones submitted to bending and pruning.

90 DAC						
FV	GL	Mean square				
		SD	H	SDM	RDM	TDM
Clones	3	0.017 <sup>ns</sup>	0.433*	0.046*	0.141*	0.339*
Manegement	1	0.029 <sup>ns</sup>	0.053 <sup>ns</sup>	0.011 <sup>ns</sup>	0.075*	0.074*
Clones*Manegement	3	0.017 <sup>ns</sup>	0.352 <sup>ns</sup>	0.001 <sup>ns</sup>	0.003 <sup>ns</sup>	0.005 <sup>ns</sup>
Residue	72	0.089	0.042	0.005	0.002	0.015
VC (%)		20.9	7.3	30.6	30.2	27.1
210 DAC						
Clones	3	1.38 <sup>ns</sup>	367.89*	11.88*	0.39 <sup>ns</sup>	13.14*
Manegement	1	5.44*	132.00*	12.10*	0.67 <sup>ns</sup>	14.42*
Clones*Manegement	3	0.87 <sup>ns</sup>	25.14 <sup>ns</sup>	0.29 <sup>ns</sup>	0.09 <sup>ns</sup>	0.98 <sup>ns</sup>
Residue	72	1.01	26.85	2.7	0.39	4.19
VC (%)		16.4	24.1	29.5	42.3	23.4

\* F Test ( $p < 0.05$ ); DAC: Days after cutting; ns: not significant; SV: Source of variation; DF: Degree of freedom; VC: Variation coefficient

**Table 3.** Average yield per plant of mini-cuttings originated from mother plants of four cacao clones submitted to bending and pruning.

Clones	Management	
	Bending	Pruning
BN 34	146.0 Aa	78.4 Ba
CEPEC 2002	74.0 Ab	36.8 Bb
PS1319	65.0 Ac	32.9 Bb
CCN 51	40.0 Ad	18.2 Bc
VC (%)	9.2	

Means followed by the same uppercase letters in the rows and lowercase in the columns do not differ by the Tukey's test ( $p < 0.05$ )

**Table 4.** Dickson quality index (DQI) of seedlings produced at 90 and 210 days after mini-cutting rooting of four cacao clones originating from mother plants submitted to bending and apical pruning.

Clones	90 DAC	
	Management	
	Bending	Pruning
BN 34	0.41 Aa	0.16 Ba
CEPEC 2002	0.11 Ab	0.06 Bbc
PS13.19	0.13 Ab	0.11 Aab
CCN 51	0.05 Ac	0.03 Ac
VC (%)	33.48	
210 DAC		
BN 34	0.94 Aa	0.74 Bb
CEPEC 2002	0.77 Ab	0.75 Ab
PS13.19	0.89 Aa	0.88 Aa
CCN 51	0.85 Aab	0.81 Aab
VC (%)	29.4	

\*Means followed by the same uppercase letters in the rows and lowercase in the columns do not differ by the Tukey's test ( $p < 0.05$ )

**Table 5.** Pearson correlation coefficients between the Dickson Quality Index (DQI); diameter (SD); plant height (H), shoot dry mass (SDM); root dry mass (RDM); total dry mass (TDM); shoot height / diameter ratio (SHDR) and shoot / root ratio (SRR) in seedlings at 90 DAC of mini-cuttings of four cacao clones originated from mother plants submitted to bending and apical pruning.

	SD	H	SDM	RDM	TDM	SHDR	SRR	DQI
SD	---	---	---	---	---	0.97*	---	---
H	---	---	---	---	---	---	---	---
SDM	---	---	---	0.68*	0.90*	---	---	0.61*
RDM	---	---	0.68*	---	0.92*	---	-0.71*	0.84*
TDM	---	---	0.90*	0.92*	---	---	---	0.79*
SHDR	0.97*	---	---	---	---	---	---	---
SRR	---	---	---	-0.71*	---	---	---	---
DQI	---	---	0.61*	0.84*	0.79*	---	---	---

\* Student's t-test ( $p < 0.05$ )

**Table 6.** Pearson correlation coefficients between the Dickson Quality Index (DQI); diameter (SD); plant height (H), shoot dry mass (SDM); root dry mass (RDM); total dry mass (TDM); shoot height / diameter ratio (SHDR) and shoot / root ratio (SRR) in seedlings at 210 DAC of mini-cuttings of four cacao clones originated from mother plants submitted to bending and pruning.

	SD	H	SDM	RDM	TDM	SHDR	SRR	DQI
SD	---	0.35*	0.63*	0.37*	0.65*	---	---	0.55*
H	0.35*	---	0.71*	0.24*	0.67*	0.84*	---	---
SDM	0.63*	0.71*	---	0.38*	0.96*	0.36*	0.28*	0.42*
RDM	0.37*	0.24*	0.38*	---	0.62*	---	-0.40*	0.91*
TDM	0.65*	0.67*	0.96*	0.62*	---	0.32*	---	0.63*
SHDR	---	0.84*	0.36*	---	0.32*	---	---	-0.27*
SRR	---	---	0.28*	-0.40*	---	---	---	-0.34*
DQI	0.55*	---	0.42*	0.91*	0.63*	-0.27*	-0.34*	---

\* Student's t-test (p<0.05)

**Table 7.** Mean diameter (SD), plant height (H), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) values of seedlings produced at 90 and 210 days after rooting of mini-cuttings of four cacao clones originated from mother plants submitted to bending and pruning.

90 DAC					
	SD	H	SDM	RDM	TDM
FACTOR	CLONE				
BN 34	1.38 <sup>ns</sup>	5.37 b	0.29 a	0.24 a	0.53 a
CEPEC 2002	1.43 <sup>ns</sup>	5.41 b	0.22 b	0.12 c	0.34 b
PS1319	1.43 <sup>ns</sup>	5.60 ab	0.21 b	0.18 b	0.39 b
CCN 51	1.45 <sup>ns</sup>	5.80 a	0.19 b	0.05 d	0.24 c
FACTOR	MANAGEMENT				
Bending	1.45 <sup>ns</sup>	5.62a	0.24a	0.17 a	0.41 a
Pruning	1.41 <sup>ns</sup>	5.41a	0.21a	0.12 b	0.33 b
VC(%)	20.92	7.30	30.62	30.22	27.15
210 DAC					
	SD	H	SDM	RDM	TDM
FACTOR	CLONE				
BN 34	5.94 <sup>ns</sup>	25.3 a	4.91 a	1.52 a	6.43 a
CEPEC 2002	5.81 <sup>ns</sup>	14.8 b	3.08 b	1.27 a	4.35 b
PS1319	6.36 <sup>ns</sup>	21.8 a	4.28 ab	1.45 a	5.73 ab
CCN 51	6.48 <sup>ns</sup>	16.4 b	3.23 ab	1.76 a	4.99 b
FACTOR	MANAGEMENT				
Bending	6.39 a	23.2 a	4.71 a	1.58 a	6.29 a
Pruning	5.83 b	19.6 b	3.72 b	1.39 a	5.11 b
VC (%)	16.4	24.11	29.54	42.33	23.45

\*Means followed by the same uppercase letters in the rows and lowercase in the columns do not differ by the Tukey's test (p <0.05)

## Conclusions

The management of mother plants was more efficient than the apical pruning in the average production of cacao mini-cuttings, especially for the BN 34 clone.

The Dickson quality index was correlated with root dry mass both at 90 and 210 days, and the forwarding technique resulted in higher DQI values.

The height variable was not adequate to estimate the quality of cocoa seedlings originated from mini-cuttings of mother plants submitted to both forwarding and apical pruning.

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