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Plantlet quality of *Coffea canephora* propagated vegetatively from different stem cutting types

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Abstract

Coffea canephora trees are grown from cutting-derived plantlets. Thus, the objective was to evaluate the performance of *C. canephora* plantlets propagated by stem cuttings with different models. The experiment to assess the plantlet performance in the nursery stage was arranged in a completely randomized design and that of the early field development in randomized blocks. The four evaluated treatments consisted of different cutting types with different number of nodes, number of leaves and presence or absence of the apical bud. In the nursery phase, characteristics related to the shoot and root development of the plantlets and the shoot characteristics of the early field development were evaluated. The data were subjected to analysis of variance by the F test ($p \leq 0.05$) and the means compared by the Tukey test at 5% probability. The hierarchical clustering method UPGMA was applied to detect the similarity between treatments considering all studied variables. The plantlets grown in the treatments with cuttings with two leaf pairs, with and without the apical bud, and the treatment with three leaf pairs with the apical bud were superior to those grown by the conventional method in the nursery phase. The plantlets grown from cuttings with two leaf pairs and the presence of the apical bud stimulated the early field development of coffee trees. Thus, it is suggested not to cut the apical bud on the cutting, since it would bring better seedling quality and without additional costs for its production.

Keywords: Conilon coffee; Vegetative propagation; Clonal plantlets; Cloning; Cuttings.

Introduction

Worldwide, around 174 million bags of coffee are produced annually, with shares of 59.8% of the coffee species Arabica (*Coffea arabica* L.) and of 40.2% of Robusta/Conilon (*C. canephora*) (USD 2020). Both species are important for generating income in various sectors of the economy, with annual revenue of US$ 172 billion (ICO 2020). Brazil, where the two species are grown, is the world's largest producer and accounts for approximately 32% of the global production (CONAB, 2020).

Coffee is a perennial crop and all of its growth phases have to be planned, particularly those directly related with the formation and establishment of the plantation (Dardengo et al., 2013). In practice, the production begins with the development of plantlets that are later established in the field to initiate the coffee plantation. Therefore, this stage is fundamental. Producing plantlets with high genetic and phytosanitary quality is the basic prerequisite for the establishment of crops with high yield potential and maximized land use efficiency, making this agribusiness more competitive and sustainable (Verdin Filho et al., 2014).

For the species *C. canephora*, plantlets can be grown from seeds or by vegetative propagation. However, commercial crops consist predominantly of vegetatively propagated plantlets. Several factors have made this the main propagation method of the species in production systems. These factors are related to the reproductive characteristic of the species *C. canephora*, an allogamous plant reproduced by cross-fertilization (Conagin and Mendes, 1961; Nowak et al., 2011; Vázquez et al., 2019). Thus, the heterogeneity of the vegetative and reproductive development of crops grown from seed-derived plantlets is great, making the management difficult and above all, decreasing the mean crop yield. In contrast, the size of vegetatively propagated *C. canephora* plants is more homogenous and fruit maturation is more uniform, while yields are higher due to the use of fixed selected genotypes (Partelli et al., 2014; Partelli et al., 2019, Partelli et al., 2020).

Among the different vegetative propagation techniques, cuttings are the most commonly used in the main production regions. Several studies were carried out to improve this plantlet production system of *C. canephora*. They investigated the ideal time of cutting the orthotropic branches to produce the cuttings (Bazoni et al., 2020), the way the base of the cuttings is trimmed (Verdin Filho et al., 2014; Aquino et al., 2017), the most beneficial substrates for plantlet development (Alves et al., 2016), the most suitable tube types (Jaeggi et al., 2018; Espindula et al., 2018), optimal shading levels (Dardengo et al., 2013) and weed control in coffee nurseries (Silva et al., 2019). However, characteristics of the cuttings, e.g., their length and number of nodes and leaves have not yet been evaluated for the production of *C. canephora* plantlets.

According to Shi and Brewbaker (2006), leaving the leaves on the cuttings is important to stimulate rooting and root growth. Similar to the presence of leaves, different numbers of leaves on the cuttings can alter the plantlet development and affect rooting (Fochesato et al., 2006). Cutting length is also an important characteristic since it can influence the initial plantlet growth, by modifying the efficiency of dry weight accumulation in roots and shoots (Lima et al., 2010).

Since vegetative propagation is used for large-scale production of *C. canephora* plantlets, it is relevant to determine a set of characteristics of cuttings that contribute to the best plantlet development. Therefore, the objective of this study was to evaluate the development of *C. canephora* plantlets in the nursery phase propagated by different cutting types, as well as to observe the early field development.

Results and discussion

Nursery phase

Analysis of variance detected a significant effect for all characteristics of plantlets evaluated 200 days after planting the cuttings, except for the number of leaves, number of roots and dry root weight (Table 1). This indicates that the different vegetative propagation methods by cuttings for plantlet production influence the development in terms of plantlet height, stem diameter, number of branches and weight, aside from the DQI.

For the plantlet height, treatments T2, T3 and T4 were statistically superior to treatment T1, and induced a mean increase of approximately 26% in plantlet height (Table 2). The plantlet height was measured considering the full shoot height, i.e., from plant base to tip. The results showed that treatments T2, T3 and T4 are more advantageous than the conventional treatment, since the cuttings already have a gain for this characteristic at planting. Plantlet height is a characteristic of agronomic interest, easy to measure and recommended to technically certify plantlet quality (Andrade Júnior et al., 2013). According to Bonomo et al. (2004), plant height is an important component because it usually indicates a higher number of plagiotropic branches, which are the productive branches of coffee trees. In an evaluation of coffee trees, Martinez et al. (2007) observed a high correlation between plant height and yield in the first harvest. These factors motivated the evaluation of larger cuttings for plantlet production as one of the key aspects of this study. The results confirmed the advantages of this practice, making it possible to take larger plantlets to the field than when using the conventional cutting pattern.

At 200 days after planting the cuttings, the stem diameter was larger in treatment T4 than treatments T1and T3and statistically equal to treatment T2(Table 2). Stem diameter is an important plant trait. According to Sun et al. (2019), plant vigor and growth can be evaluated by the parameter stem diameter, which is directly related to plant biomass accumulation. For coffee trees, Carvalho et al. (2010) observed that the trait stem diameter (*C. arabica* L.) is positively correlated with coffee yield. Therefore, treatments that induce an increase in this plant trait are important.

For shoot dry weight, the means were highest in treatments T2, T3 and T4 and lowest in treatment T1 (Table 2). The SDW of 13.36, 14.14 and 13.47 g. plant^{-1}, respectively, in treatments T2, T3 and T4 exceeded the values reported by Covre et al. (2016) in an evaluation of the plantlet development of *C. canephora* genotypes, confirming the superior plantlet development in response to the treatments. The greater number of leaves and longer cutting length in treatments T3 and T4 than T1 can improve plant biomass formation in the production of plantlets (Fochesato et al., 2006). These results could be explained by the greater amount of reserves stored in these cuttings (Costa et al., 2007).

With regard to shoot dry weight, the highest mean of the characteristic total dry weight was observed in treatments T2, T3 and T4 and the lowest in treatment T1 (Table 2). Dry weight accumulation has been used as a parameter of the vegetative development of *C. canephora* (Partelli et al., 2013; Dubberstein et al.,

Table 1. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), number of roots (NR), root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW), number of plagiotropic branches (NPB) and Dickson quality index (DQI) for *Coffea canephora* cuttings by different vegetative propagation methods.

Variables	Mean square		Mean	CV (%)
	Treatments	Error		
PH	84.12**	7.56	35.41	7.77
SD	1.09**	0.18	5.26	8.09
NL	30.41ns	13.33	20.32	17.97
NR	1.48ns	1.44	12.05	9.95
RDW	0.60ns	0.20	5.30	8.53
SDW	41.49**	0.56	12.23	6.14
TDW	42.77**	0.62	17.53	4.48
NPB	7.14*	1.54	3.67	33.81
DQI	0.25**	0.03	1.94	9.51

ns - not significant; ** and * - significant at 1 and 5% probability, respectively, by the F test.

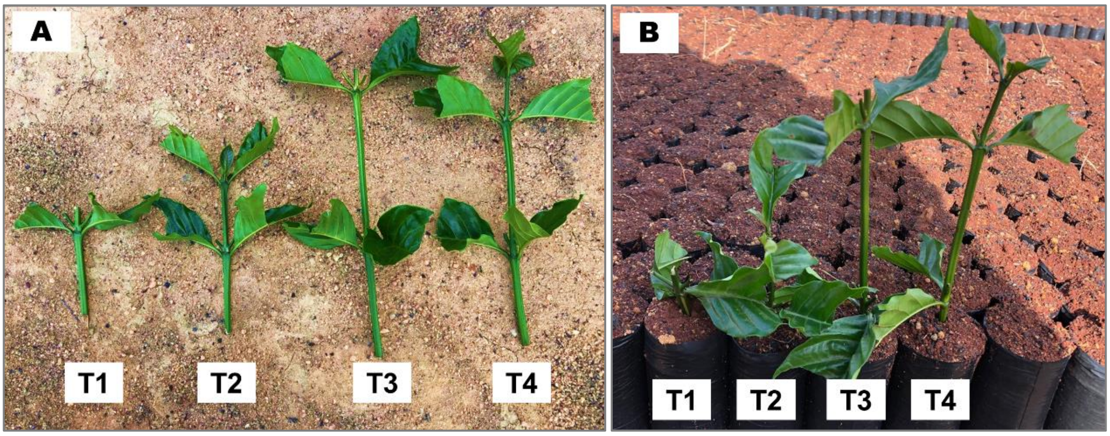

Fig 1. Cuttings in pre-planting treatments (A) and post-planting cuttings in the tubes (B). T1: cutting with one pair of leaves, without apical bud (conventional cutting); T2: cutting with two leaf pairs and the apical bud; T3: cutting with two leaf pairs, without apical bud; T4: cutting with three leaf pairs and the apical bud.

2017; Bote et al., 2018). It is an important variable, since plant biomass formation indicates a good functioning of the physiological system (DaMatta et al., 2007).

For the number of plagiotropic branches, treatments T3 and T4 were superior to treatment T1and statistically equal to treatment T2(Table 2). This characteristic is positively correlated with coffee yield. According to Rodrigues et al. (2012) and Assis et al. (2014), coffee yield is correlated with the number of plagiotropic branches. Although the characteristic grain yield is not evaluated in plantlets, it is known that high-quality plantlets have a direct positive effect on coffee development and yield.

For Dickson's quality index, the treatment means ranged from 1.74 to 2.14 (Table 2). The values in treatments T2and T4 were higher than in treatment T1and T3. For coffee plantlets propagated by grafting, DQI values above 1.00 (up to 1.42) were also reported by Andrade Júnior et al. (2013). The plantlet quality is positively correlated with a higher DQI index since in the Dickson equation, robustness, represented by total dry weight and the balance of biomass distribution, is taken into consideration (Dardengo et al., 2013). In other words, a higher DQI indicates a better plantlet performance (Dickson et al., 1960). The DQI values of 2.14 and 2.12 in treatments T2and T4, respectively, are higher than those reported in other studies focused on the production of *C. canephora* plantlets (Dardengo et al., 2013; Verdin Filho et al., 2014; Covre et al., 2016; Aquino et al., 2017; Espindula et al., 2018; Jaeggi et al., 2018).

Early field evaluation

The seedlings that make up the treatments were planted in the field, and 200 days after planting, the vegetative development was evaluated. Analysis of variance indicated no significant effect between treatments for the evaluated characteristics, except number of leaves and length of the longest plagiotropic branch (Table 3).

In treatment T2, the trait number of leaves of plants in the field was statistically superior to treatment T3 and equal to the other treatments (Table 4). The number of leaves on a plant is an important characteristic, since leaves are the specialized organs for light capture and gas exchange, constituting the photosynthetic apparatus that is responsible for the

Table 2. Plant height (PH), stem diameter (SD), number of leaves (NL), number of roots (NR), root dry weight (RDW), shoot dry weight (SDW), dry weight total (TDW), number of plagiotropic branches (NPB) and Dickson's quality index (DQI) for *Coffea canephora* cuttings by different vegetative propagation methods.

Variables	Treatments			
	T1	T2	T3	T4
PH	29.50 b	35.80 a	38.40 a	37.95 a
SD	4.92 bc	5.61 ab	4.79 c	5.71 a
NL	17.30 a	20.00 a	20.70 a	23.30 a
NR	11.70 a	12.80 a	11.60 a	12.10 a
RDW	5.21 a	5.48 a	4.86 a	5.65 a
SDW	7.94 b	13.36 a	14.14 a	13.47 a
TDW	13.15 b	18.85 a	19.00 a	19.12 a
NPB	2.10 b	3.40 ab	4.50 a	4.70 a
DQI	1.75 b	2.14 a	1.74 b	2.12 a

Means followed by the same letter in a row do not differ by the Tukey test at 5% probability. T1: cutting with one pair of leaves, without apical bud (conventional cutting); T2: cutting with two leaf pairs, with the apical bud; T3: cutting with two leaf pairs, without apical bud; T4: cutting with three leaf pair, with the apical bud.

Table 3. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), number of plagiotropic branches (NPB) and length of the longest plagiotropic branch (LLPB) for *Coffea canephora* cuttings by different vegetative propagation methods.

Variables	Mean square		Mean	CV (%)
	Treatments	Error		
PH	137.44^{ns}	62.21	64.33	12.26
SD	2.88^{ns}	4.31	19.27	10.77
NL	913.85*	184.67	251.87	5.40
NPB	52.78^{ns}	27.64	24.33	21.61
LLPB	118.37**	10.67	44.71	7.31

^{ns} - not significant; ** and * - significant at 1 and 5% probability, respectively, by the F test.

production of carbohydrates to be directed to the formation of vegetative and reproductive plant organs (Testone et al., 2019).

The treatments differed in the length of the longest plagiotropic branch, with highest values in treatments T2and T4, statistically superior to treatments T1and T3 (Table 4). This characteristic is directly related to the yield potential of coffee trees (Rodrigues et al., 2012; Assis et al., 2014). The greater length of the plagiotropic branch in treatment T2associated with the number of leaves in this treatment indicated a greater number of productive nodes, which together increased the potential for a higher coffee yield in this treatment in the first harvest.

Considering all variables analyzed in the nursery phase and early field phase, the similarity between treatments was analyzed by the hierarchical clustering method UPGMA. The results showed similarity of treatments T2, T3 and T4 in the nursery phase, which were considered to belong to the same group if any value above 50% dissimilarity was set as dendrogram cut-off level. Conversely, treatment T1is the most divergent, even at high dissimilarity levels in the dendrogram (Figure 2A). These results are due to the poorer performance of treatment T1 in the nursery stage than of the other treatments.

For plant evaluations in the initial field phase, the dendrogram indicated greater similarity between treatments T1, T3 and T4, and treatment T2 as the most divergent in this period, isolated from the others even at high dissimilarity levels between treatments (Figure 2B). This result demonstrates that the early field development of plantlets grown from the cutting types of treatments T1, T3 and T4 are similar, which shows the good development in treatment T1, which in the nursery phase performed worse than the other treatments. The high dissimilarity in treatment T2 was due to the initial field performance, with good results for variables such as number of leaves and length of the longest plagiotropic branch.

In the nursery phase of both evaluated periods, the conventional treatment performed worse than treatments T3 and T4. Despite the better performance of treatments T3 and T4 compared to the treatment with conventional cuttings, the use of these treatments as a method of producing *C. canephora* plantlets would not be justified, since the performance in the initial field stage was similar to that of the conventional treatment. Moreover, it is emphasized that these treatments would increase the production costs, as they require twice as many cuttings as would be spent by the conventional method.

Based on the set of data, of both the nursery period and the early field development, treatment T2 was identified as a vegetative propagation method of *C. canephora* that provides superior plantlets, mainly compared to the conventional method. In this treatment the apical bud is maintained, so that from

Table 4. Plant height (PH), stem diameter (SD), number of leaves (NL), number of plagiotropic branches (NPB) and length of the longest plagiotropic branch (LLPB) for *Coffea canephora* cuttings by different vegetative propagation methods.

Variables	Treatments			
	T1	T2	T3	T4
PH	57.50 a	68.67 a	65.33 a	65.83 a
SD	20.00 a	19.33 a	18.33 a	19.43 a
NL	243.83 ab	277.00 a	237.33 b	249.33 ab
NPB	23.83 a	26.67 a	26.50 a	20.33 a
LLPB	41.83 b	48.50 a	40.00 b	48.50 a

Means followed by the same letter in a row do not differ by the Tukey test at 5% probability. T1: cutting with one pair of leaves, without apical bud (conventional cutting); T2: cutting with two leaf pairs, with apical bud; T3: cutting with two leaf pairs, without apical bud; T4: cutting with three leaf pairs, with apical bud.

Table 5. Results of soil chemistry analysis in the experimental area at a depth of 0-20 cm.

Chemical characteristics												
---------------------------mg/dm³ -------------------------									-----------cmolc/dm³--------			
P	K	Na	S	B	Zn	Mn	Cu	Fe	Ca	Mg	Al	H + Al
58,5	86	3	18	0.4	7.4	9.1	0.9	48	1.8	0.3	0.2	4.5
pH			V%			S.B (cmolc/dm³)			MO (dag dm-3)			
5			34			2,33			2,0			

pH: in H_2O 1:2.5; P, K, Zn, Mn, Cu and Fe (phosphorus; potassium; zinc; manganese; copper, iron) Extraction: Mehlich-1; S (sulfur): Acetic acid monocalcium phosphate; Ca and Mg (calcium; magnesium) Extraction: KCl 1mol/L; Al+H (aluminum, hydrogen) Titration; V% (base saturation); S.B (base sum); OM (organic matter): Embrapa Method.

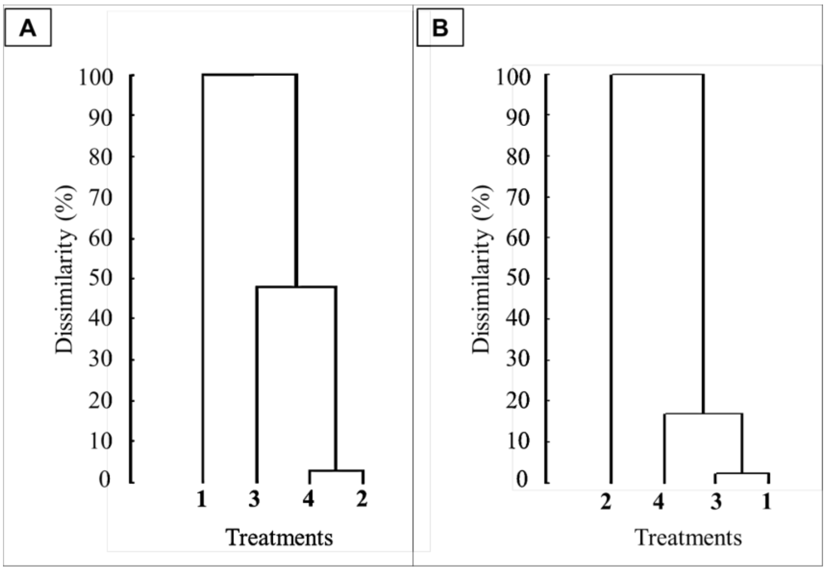

Fig 2. Representative dendrogram of the dissimilarity between the cuttings by different vegetative propagation methods based on UPGMA clustering and Mahalanobis' generalized distance, considering development traits of the shoot and root system of *Coffea canephora* plantlets evaluated in the nursery stage (A) and during the early field development (B). T1: cutting with one pair of leaves, without apical bud (conventional cutting); T2: cutting with two leaf pairs, with apical bud; T3: cutting with two leaf pairs, without apical bud; T4: cutting with three leaf pairs, with apical bud.

each orthotropic branch removed from the matrix plant, a cutting with this pattern can be formed, while the other vegetative parts of the branch can still be used for cuttings with another pattern. So, it is suggested that the apical bud should not be removed from the cutting, which can be interesting, since the plantlet quality could be improved without additional production costs.

Materials and methods

Experimental location

The experiment was evaluated in two stages: the plantlet phase in the nursery and initial field development. The first was carried out in a plantlet nursery with 50% shading, in the district of Vila Valério, in the northwest of the state of Espírito Santo, Brazil, at approximately 130 m asl (latitude 18º54'35.83" S, longitude °14'31.89" W). On the same property where the plantlets were produced, less than 100 m away from the nursery, the early field development was observed. The soil at the planting site was classified as Latossolo Vermelho-Amarelo, distrófico, with a clayey texture and wavy relief (Santos et al., 2018). The chemical characteristics of the soil are in table 5. According to the Köppen climate classification, the regional climate is Aw, characterized by hot, humid summers and dry winters. The mean annual temperature is 23ºC and the maximum and minimum temperatures are 29ºC and 18ºC, respectively (Alvares et al., 2013).

Material and experimental design

The cuttings for plantlet production were taken from orthotropic branches of Conilon coffee trees with phytosanitary stability and balanced nutrition status. To grow the clonal plantlets, polyethylene tubes with drainage holes (width 11 cm, height 20 cm) were used as recipients. As substrate, a proportion of 800 L sieved soil from a slope, 200 L bovine manure, 0.5 kg potassium chloride and 1.5 kg dolomitic limestone were used. Irrigation was applied with a micro sprinkler system controlled by timers. The activation time of the irrigation system was determined to ensure moisture maintenance, with small droplets on the leaves of the coffee plantlets.

A completely randomized design with 10 replications and 4 treatments was used in the nursery phase, with one plantlet per experimental unit. The early field development was laid out in a randomized block design with six replications, evaluating one plant per plot. The treatments consisted of different cutting types, which were taken from a single genotype to ensure standardization, namely from genotype A1, of cultivar Tributun (Partelli et al., 2020) or Andina (Partelli et al., 2019). In treatment T1, we used cuttings with one pair of leaves, without the apical bud (conventional cutting); in treatment T2, cuttings with two leaf pairs and presence of the apical bud; treatment T3, cuttings with two leaf pairs, without apical bud; treatment T4, cuttings with three leaf pairs and with the apical bud (Figure 1). The treatments were evaluated in two stages, in the nursery phase and in the field planting.

Evaluated characteristics

The development of the shoots and root system of the plantlets was evaluated 200 days after planting the cuttings, based on the following characteristics: plantlet height (cm), stem diameter (mm), number of leaves, number of roots, root dry weight ($g.plant^{-1}$), shoot dry weight ($g.\ plant^{-1}$), total dry weight ($g.\ plant^{-1}$), number of plagiotropic branches and the Dickson quality index (1960). The following characteristics of the early field development were evaluated 200 days after planting: plantlet height (cm), stem diameter (mm), number of leaves, number of plagiotropic branches and length of the longest plagiotropic branch (cm).

The plant height (PH) was measured from the plant base to the apical bud with a ruler. The stem diameter (SD) was measured at the plant/base with a digital caliper. The fully expanded leaves were counted to determine the number of leaves (NL) and the roots longer than 01 cm for the number of roots (NR). The shoot dry weight (SDW) and root dry weight (RDW) were weighed on a precision scale after oven-drying to constant weight at 65ºC with forced air ventilation. The total dry weight (TDW) was computed as the sum of SDW plus RDW. The Dickson quality index was calculated by the formula: $DQI = TDW/[(PH/SD) + (SDW/RDW)]$ (Dickson et al., 1960). The length of the longest plagiotropic branch (LLPB) was measured with a ruler and the number of plagiotropic branches (NPB) was counted directly.

Statistical analysis

The data of the nursery phase and early field development were subjected to analysis of variance by the F test ($p \leq 0.05$). Since the results were significant, the means were compared by the Tukey test at 5% probability. To check the similarity between treatments considering all variables analyzed in each stage simultaneously, the hierarchical method UPGMA (unweighted pair-group method using arithmetic averages) was applied based on the generalized distance of Mahalanobis. Software Genes (Cruz et al., 2016) was used to run the analyses.

Conclusion

In the nursery stage, cuttings by the different studied vegetative propagation methods produce better *C. canephora* plantlets than cuttings by the conventional method. Plantlets grown from cuttings with two leaf pairs and the presence of the apical bud improved the initial development of coffee trees in the field.

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