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Influence of the cut length of plagiotropic branches in conilon coffee stem cuttings for the seedling production

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ABSTRACT

In the literature, no studies investigated which portion of the plagiotropic branches should be removed by pruning conilon coffee stem cuttings, as well as the effect of the remaining length of these branches on rhizogenesis, growth, and physiology of clonal seedlings. Thus, this work aimed to evaluate the remaining length of plagiotropic branches of conilon coffee stem cuttings on the growth and physiology of conilon coffee clonal seedlings. The experiment was conducted in the Fazenda Experimental de Marilândia, ES, Brazil, under a completely randomized design with five treatments and fifteen repetitions. The treatments referred to the length of plagiotropic branches (0.5, 1.0, 1.5, 2.0, and 2.5 cm from the point of insertion with the stem). After 120 days of cultivation, vegetative growth and physiological aspects of the seedlings were evaluated. The remaining plagiotropic branches between 1.5 and 2.0 cm long provide the best vegetative growth and physiological response of the conilon coffee seedlings. The lengths that provide the worst response, both in terms of growth and physiological aspects, were 0.5 and 2.5 cm.

Key words: Asexual propagation; Cloning; Seedling quality; Cuttings.

1 INTRODUCTION

The *Coffea canephora* species is of great social and economic relevance, as it is the second most widely cultivated species from the genus *Coffea* in the world (Ferrão et al., 2019). Knowledge related to factors affecting plant development is essential to improve nursery techniques (Trautenmulle et al., 2017). *Coffea canephora* Pierre ex A. Froehner presents the phenomenon of self-incompatibility, i.e. a physiological mechanism that prevents a fertile plant from forming viable seeds when fertilized by its pollen (Devreux et al., 1959; Schifino-Wittmann; Dall'agnol, 2002). Conilon coffee self-incompatibility is gametophytic, which paralyzes the pollen tubes development of the pollen grains, making fertilization of the female gametophyte impossible (Devreux et al., 1959; Nowak et al., 2011). For this reason, the natural reproduction of the species is allogamous and, to avoid variability from genetic segregation, the commercial propagation of conilon coffee is predominantly asexual (Fonseca et al., 2019).

The asexual propagation technique used in the conilon coffee plant, that uniform plants, is the stem cutting, which consists of the segmentation of young orthotropic shoots so that each segment contains a pair of leaves reduced to one-third of its size and a pair of plagiotropic branches (Fonseca et al., 2019).

Until now, the guidelines for the setup of the cuttings was, among other things, that the cuttings should have a whole node and that plagiotropic branches should be eliminated by pruning (Paulino; Matiello; Paulini, 1985). However, no study in the literature investigates which portion of the plagiotropic branches is more recommended to be removed by pruning, as well as the implications of the remaining length of these branches on rhizogenesis, growth, and physiology of seedlings.

In this sense, studies are needed to standardize the setup of stem cuttings for the production of clonal conilon coffee seedlings. Thus, this work aimed to test the lengths of the remaining plagiotropic branches in conilon coffee stem cuttings on the growth and physiological aspects of clonal seedlings.

2 MATERIAL AND METHODS

2.1 Experimental design

The experiment was conducted in the nursery for the production of Conilon coffee plantlets, covered by a polyethylene screen to promote the level of 50% shade, under controlled conditions at the Marilândia Experimental Farm (Fazenda Experimental de Marilância, FEM), an agricultural research base managed by INCAPER (Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural), located in the municipality of Marilândia, Northwest Region of Espírito Santo State, Southeast Region of Brazil, at the geographic coordinates 19º24'26.09"S and 40º32'26.83"W, and altitude of 89 m above sea level. The cultivar used in the trials was the clonal cultivar "Vitória Incaper 8142". The experiment followed a completely randomized design, with five treatments referring to the length of remaining plagiotropic branches from stem cuttings (0,5; 1,0; 1,5; 2,0 e 2,5 cm from the point of insertion with the stem) using 15 replicates and experimental plots composed of four plantlets.

2.2 Seedlings production

The stem cutting came from the central part of the well-developed shoots that were randomly collected from adult parent-plants from the cultivar "Vitória Incaper 8142" grown in a clonal garden, cultivated with the technique of bending orthotropic branches to stimulate the emission of shoots. The preparation of the cuttings altered, using pruning, the length of the pair of plagiotropic branches of the stem cutting (0.5; 1.0; 1.5; 2.0 and 2.5 cm from the insertion point with the stem) (Figure 1). The cut of the five treatments was straight cut and every stem cutting exhibited a pair of leaves per stem cutting, which were cut of about a third of its original area, apex 1 cm long using the bevel cut, and basal stem 4 cm long using straight cut at the base.

After preparing, the stem cuttings had 2/3 of their length buried, in a vertical position, in plastic tubets with a volume of 280 cm^3, using a mixture with 70% commercial substrate and 30% coffee husk obtained from the processing of coffee harvested during the previous year (Verdin Filho et al., 2018). The plantlets were cultivated in a nursery for 120 days, and their nutrition, irrigation, and phytosanitary management were carried out according to the recommendations to produce plantlets of Conilon coffee of Ferrão et al. (2012) and Fonseca et al. (2019).

2.3 Evaluations

After 120 days of cultivation, the seedlings were evaluated for growth parameters: height of plantlet (HGT) using a graduated ruler (precision 0.1 cm); diameter of the stem (DIA) using a digital pachymeter at a height of approximately 1.5 cm above the button insertion, leaf area (TLA) obtained by the non-destructive method of linear dimensions (Barros et al., 1973; Brinate et al., 2015). To physiological parameters, evaluations were performed on the third leaf pair of the plagiotropic branch of the middle third of the crown, and gas exchange was evaluated using a portable infrared gas analyzer (IRGA, Licor 6400XT) between 9:00 and 11:00 am on sunny days. The photosynthetically active radiation was standardized to 1000 µmol (photons) $m^{-2}s^{-1}$ and CO_2 concentration in the chamber at 400 ppm. We also evaluated the net CO_2 assimilation rate (A; µmol m^{-2} s^{-1}), stomatal conductance (g_s; mol H2O $m^{-2}s^{-1}$), transpiration rate (E; mmol H_2O $m^{-2}s^{-1}$), the substomatal concentration (Ci; µmol mol^{-1}) and instantaneous water use efficiency (A/E; µmol $mmol^{-1}$). In the same leaves and times, we evaluated the total chlorophyll index (CHT; ICF) obtained by reading the foliar chlorophyll content using the chlorophyll meter "ClorofiLOG" (Falker model FL1030).

After these analyses, the plants were collected, separated into the stem, leaf, and root, and dried in an oven with forced air circulation at 65 ºC ± 2 ºC, until a constant mass was obtained and then weighed on an electronic precision balance (0.0001 g) to obtain the total dry matter. The Dickson quality index (DQI) The DQI, which evaluates the quality of seedlings, was calculated using the method proposed by Dickson, Leaf and Hosner (1960), from the formula: DQI = [total dry mass (g)/(RAD+RPAR)], where, RAD represents the ratio between the height (cm) of the seedling and the diameter (mm) of the collar of the seedlings; and RPAR, the ratio of the dry mass (g) of the aboveground part to the dry mass (g) of the root.

2.4 Statistical analysis

The data were subjected to the assumptions of normality and homogeneity. Then, analysis of variance (ANOVA) was performed. In the significant effect of the treatments, the data were submitted to regression analysis (p≤0.05). The chosen regression model was based on the significance of the angular

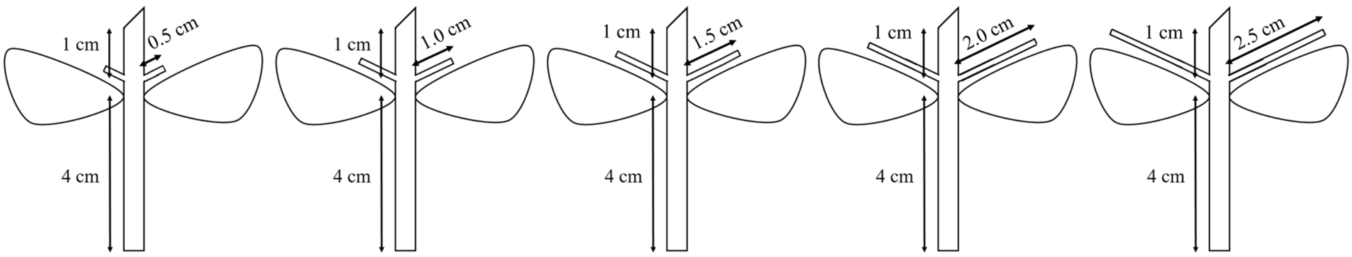

Figure 1: Illustrative scheme of the conformation of the conilon coffee stems used in the experiment. Demonstration of the measurement starting point of the length of the apex (1 cm) and basal stem (4 cm), as well as, the types of bevel cut and straight cut, respectively. Demonstration of the measurement start with the length of the plagiotropic branches pair (0.5; 1.0; 1.5; 2.0; 2.5 cm from the insertion point with the stake).

coefficients and the values of the coefficients of determination (R^2). The data were analyzed using the software Sisvar version 5.6 (Ferreira, 2011).

3 RESULTS

3.1 Growth

No significant effect ($p \leq 0.05$) of the plagiotropic branches length of the stem cuttings was observed on the height of plantlet (HGT) and stem diameter (DIA), with mean values of 10.45 cm; 3.78 mm, respectively (Figure 2A and 2B).

The variables total leaf area (TLA), total dry matter (TDM), and Dickson quality index (DQI) were influenced by the lengths of plagiotropic branches. For the variables TLA and TDM, it was observed that the highest mean leaf area (226.85 cm²) in branches, which length was estimated at 1.42 cm, and greater total dry mass (2.19 g) at an estimated branch length of 1.78 cm. Lengths of plagiotropic branches greater than 1.42 and 1.78 cm resulted in decreased leaf area and total dry mass, respectively (Figure 2C and 2D).

For DQI, the length of the plagiotropic branches influenced the seedlings' response: the increase of this variable with the increase of the length of the plagiotropic branch. The maximum value of DQI (0.42) was observed in cuttings whose plagiotropic branch length was equal to 2.5 cm (Figure 2E).

3.2 Physiological properties

Regarding the physiological properties, the length of the plagiotropic branches of the stem cuttings influenced the responses of the seedlings (Figure 3), except in variables Total Chlorophyll Content (CHT) and Water use efficiency (A/E), which showed average values of 47.41 IFC and 3.09 μmol mmol^{-1} respectively (Figure 3A and 3B). In the net assimilation rate of CO_2 (A), there was a quadratic adjustment in the regression indicating the increase of A as a function of increasing branch length, with the maximum rate observed (7.43 μmol CO_2 m^{-2} s^{-1}) in cuttings with the length of plagiotropic branches estimated at 1.38 cm (Figure 3C). From this length on, A started to decrease.

Stomatal conductance (gs) and transpiration rate (E) indicated similar results with quadratic regression adjustment. We observed an increase as the length of the plagiotropic branches increased until the estimated length of 1.09 cm for stomatal conductance (0.18 mol H2O m^{-2}s^{-1}) and 1.25 cm for transpiration rate (2,35 mmol H_2O m^{-2}s^{-1}). Plagiotropic branch lengths greater than 1.09 and 1.25 cm provided reduced stomatal conductance and transpiration rate respectively (Figure 3D and 3E).

The substomatal concentration of CO_2 (Ci) showed a negative linear response in the regression analysis, where the highest concentration (316.54 μmol mol^{-1}) was observed in cuttings with plagiotropic branches of 0.5 cm in length and the lowest (277.30 μmol mol^{-1}) in cuttings with 2.5 cm long branches length (Figure 3F).

4 DISCUSSION

4.1 Growth

There was no effect of the treatments on plant height and on the stem diameter; all treatments showed mean values of the height of 10.45 cm (Figure 2A) and mean values of the stem diameter of 3.78 mm (Figure 2B). This height found in this work is higher than those of Silva et al. (2010) and Dardengo et al. (2013), who, when evaluating the initial growth of conilon coffee seedlings under the same conditions as in this research, obtained plants with heights of 8.15 and 7.34 cm, respectively. Regarding the stem diameter, this results found in this work, is higher than those of Dardengo et al. (2013), who, obtained the value of 3.2 mm as the largest diameter in their work.

Among the parameters for determining plant growth, leaf area is of fundamental importance (Schmildt et al., 2017). The leaf area can be a very relevant result in the initial phase of seedling development since the leaves make up the main organ responsible for CO_2 fixation. CO_2 is an essential compound for the formation of sugars, which in the reproductive phase of plants will be in great demand and, along with other factors, will determine the productive potential of the adult plant (Ribou et al., 2013). In the present study, the leaf area was higher in cuttings with a plagiotropic branch of 1.5 cm, from this length on, the values of this variable decreased (Figure 2C). This possibly occurred due to the reduction of the leaf area, which caused a decrease in transpiration and photosynthesis per unit area, and with the limited photosynthetic activity in cuttings with the greatest branch length, the production of dry matter was consequently reduced.

Regarding biomass production, TDM was higher in cuttings with plagiotropic branches of 2.0 cm (Figura 2D), indicating that seedlings maintaining this branch length have better conditions for growth and development. This hypothesis is confirmed by the DQI, in which the best indexes were observed in cuttings with plagiotropic branches of 2.0 and 2.5 cm (Figure 2E). Biomass production is an important characteristic and of great consistency in the evaluation of the growth and development of plant species (Paiva et al., 2009).

The DQI was calculated based on the measured data and is an indicator of seedling quality that considers in its calculation the production of dry mass and the balance of the distribution of this biomass among the plant organs. In this study, the treatment that presented the highest value for DQI was the one that contained the cuttings with remaining plagiotropic branches of 2.5 cm in length. The value presented in this treatment was 0.42 and was lower than the value of

0.68, which was established by Dardengo et al. (2013) for conilon seedlings grown under 50% shade. But, on the other hand, Azevedo et al. (2014) observed DQI values no higher than 0.15 in *Coffea canephora* seedlings grown in different containers (bag or tube) in the presence or absence of hydro retainer.

4.2 Physiological properties

The cuttings with 1.0 cm plagiotropic branches showed higher stomatal conductance (Figure 3D), meaning that in these cuttings the stomata remained open. Due to this greater opening of the stomata, there was a greater gas exchange that resulted in increasing transpiration rate and photosynthesis until reaching the maximum rates in cuttings with plagiotropic branches of approximately 1.5 cm. From this length on, stomatal control was observed, resulting in the closure of the stomata and, consequently, in reductions in stomatal conductance, transpiration rates, and photosynthesis.

Thus, the reduction in stomatal conductance adopted by the cuttings from plagiotropic branches of greater length (2.0 and 2.5 cm) can be understood as a water-saving mechanism against tissue dehydration. Peloso et al. (2017) working with Arabica coffee plants under water deficit, found that the reduction in available water did not allow plants to maintain a continuous transpiratory flow, leading to the closure of stomata and temporarily limiting the assimilation of atmospheric CO_2.

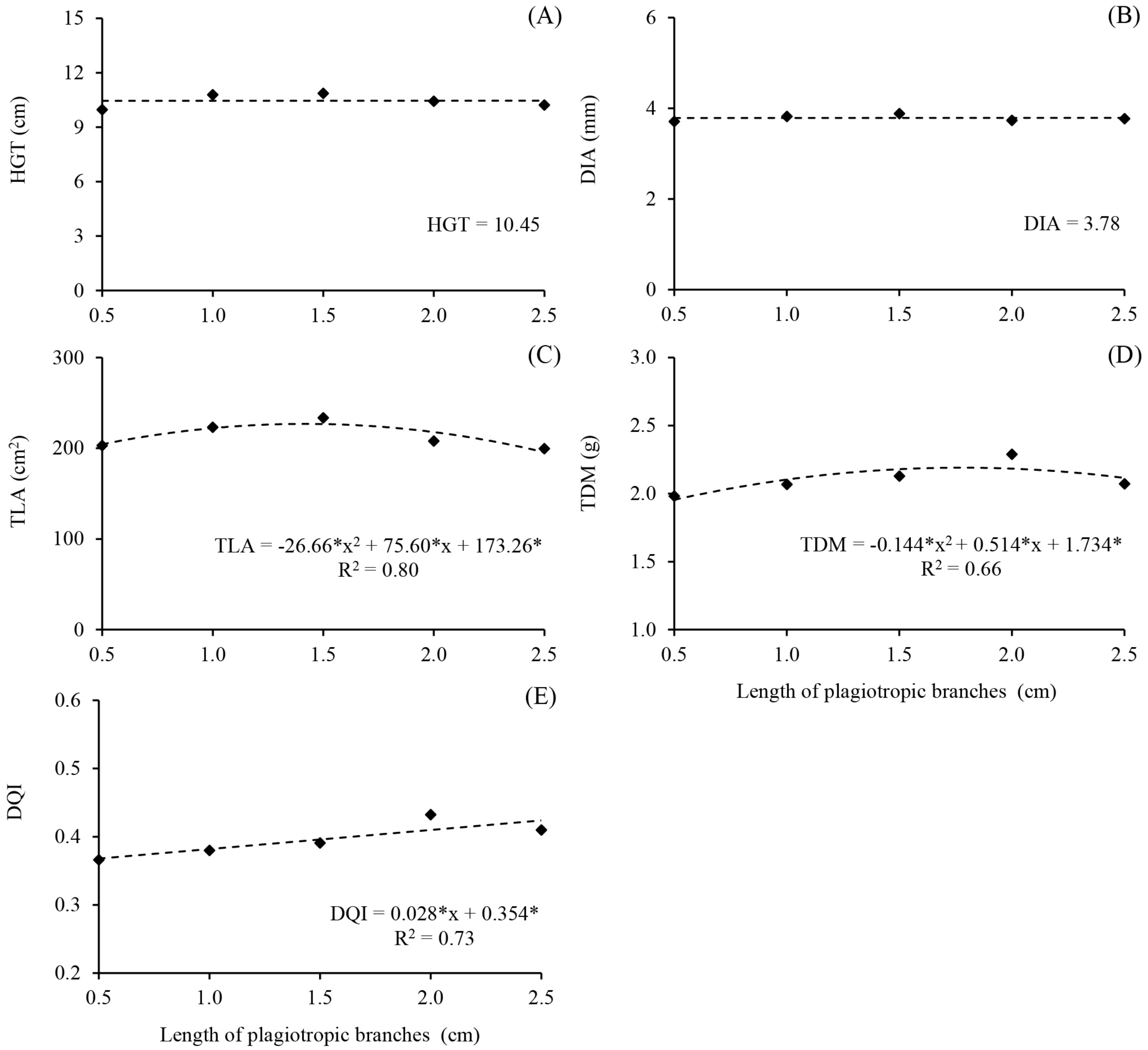

Figure 2: Height of plantlet (A), stem diameter (B), total leaf area (C), total dry matter (D), Dickson quality index (E) of plantlets of Conilon coffee produced using stem cuttings, at 120 days of cultivation, as a function of the length of the remaining plagiotropic branches produced in Marilândia-ES (regression coefficient is significant, at 5% of probability, by the t-test).

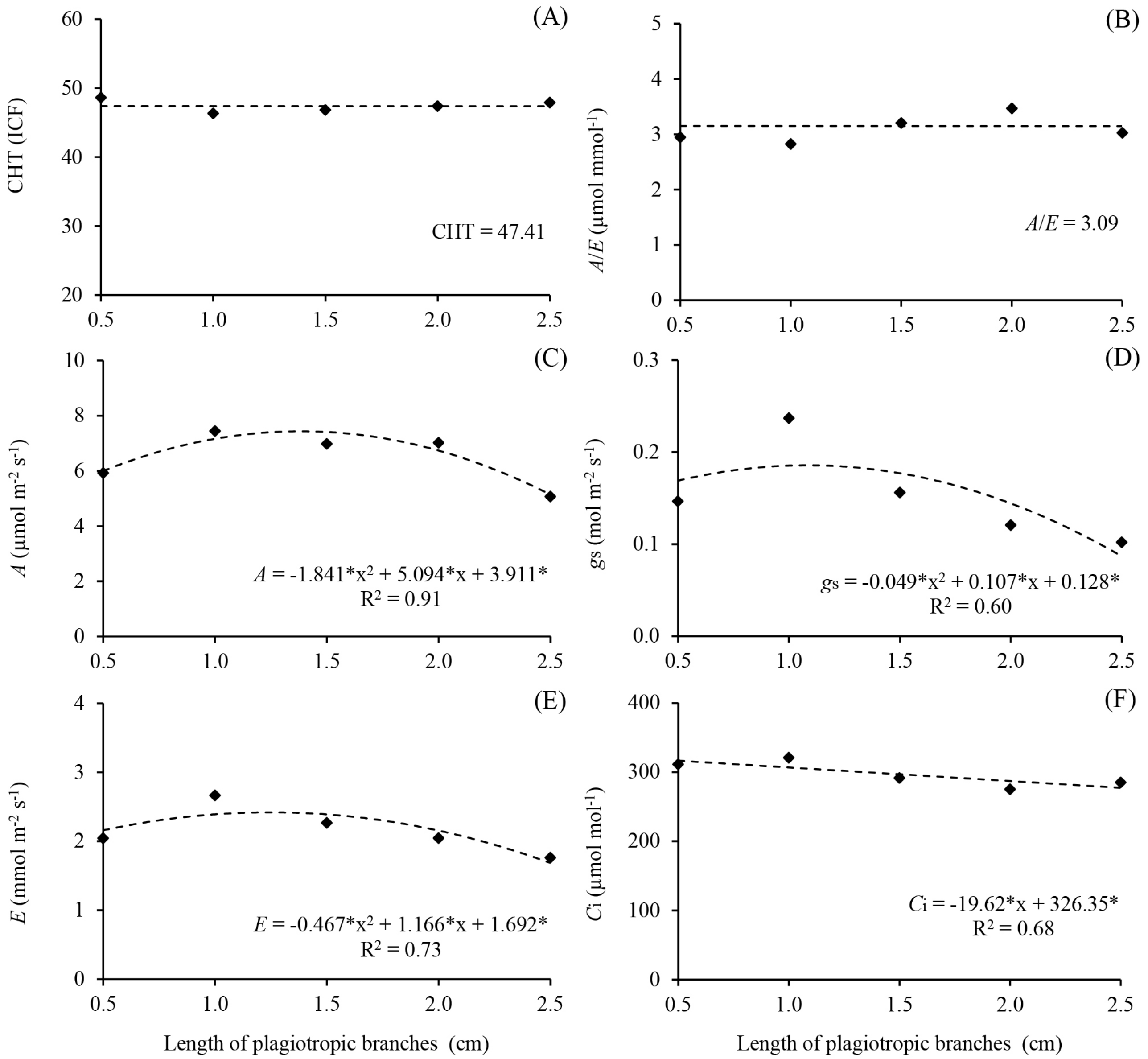

Figure 3: Total chlorophyll content (A), water use efficiency (B), net assimilation rate of CO2 (C), stomatal conductance (D), transpiration rate (E), and substomatal concentration of CO_2 (F) of plantlets of Conilon coffee produced using stem cuttings, at 120 days of cultivation, as a function of the length of the remaining plagiotropic branches produced in Marilândia-ES (regression coefficient is significant, at 5% of probability, by the t-test).

The seedlings with plagiotropic branches of 2.0 and 2.5 cm besides presenting the lowest values of stomatal conductance, also presented the lowest values of transpiration rate, (Figure 3D and 3E), both reductions possibly due to stomatal closure. The stomatal structure has a dynamic behavior and can lead to an increase or decrease in resistance to the passage of gases by controlling the water potential of the guard cells (Barbosa; Porto; Bertolde, 2019).

The substomatal concentration of CO_2 (Ci) also tended to reduce as a function of the increase in length of the plagiotropic branch (Figure 3F). This fact can be associated with the results found for stomatal conductance since the entrance of CO_2 into the plant occurs through the opening of the stomatal cavity. Thus, the limiting factor of photosynthesis of the cuttings is based on the limitation of CO_2 diffusion as a function of stomatal control. Considering that the coffee stem cuttings are young shoot segments that have undergone incisions in the internode, leaf lamina, apex, and plagiotropic branch, as well as having a growing root system, stomatal closure for short periods can be an important tool against dehydration. Verdin Filho et al. (2020) observed an average Ci value of 280.91 µmol mol^{-1} similar in Conilon coffee seedlings in an experiment with similar conditions to this work.

Regarding the instantaneous water use efficiency (A/E) and the total chlorophyll content, there was no significant difference between the treatments, presenting average values of 3.09 µmol $mmol^{-1}$ and 47.42 CHT respectively (Figure 3B and 3A). The instantaneous water use efficiency is calculated by dividing the photosynthetic rate by the transpiration and denotes the water savings made by the plant (Flexas, 2016). The chlorophyll index is related to the photosynthetic potential of the plant as well as its development through the production of photoassimilates (Taiz; Zeiger, 2017).

Analyzing the variables studied together, we found that the length of the remaining plagiotropic branches between 1.5 and 2.0 cm favors leaf expansion, biomass accumulation, higher photosynthetic rates, better stomatal control, and results in seedlings with higher quality indexes.

5 CONCLUSIONS

The remaining plagiotropic branches between 1.5 and 2.0 cm long provide the best vegetative growth and physiological response of the conilon coffee seedlings. The lengths that provide the worst response, both in terms of growth and physiological aspects, were 0.5 and 2.5 cm.

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