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Growth and physiological quality in clonal seedlings of Robusta coffee[1]

Crescimento e qualidade fisiológica de mudas clonais de cafeeiros Robusta

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ABSTRACT - The intrinsic characteristics of the vegetative propagule may influence the rooting speed and shoot growth, as well as the final physiological quality of clonal seedlings of *Coffea canephora*. The aim of this study was to evaluate the growth and physiological quality of 'Robusta' coffee seedlings produced from propagules (stem cuttings) with different cutting ages. The study was conducted in a greenhouse, in the district of Ouro Preto do Oeste, in Rondônia, Brazil (10˚45'43" S and 62˚15'10" W). The ages of the cuttings were 30, 60, 90, 120 and 150 days, corresponding to five positions on the secondary orthotropic stem (sprout), from the apex to the base. Dry matter accumulation, and relative and absolute growth rates were evaluated for 188 days after cutting, as well as the vegetative characteristics of the seedlings at 125 days after cutting. It appears that growth can be divided into three phases: 1) Initial: slow growth, lasting approximately 83 days; 2) Intermediate: fast growth, lasting approximately 40 days; and 3) Final: slow growth, starting approximately 125 days after cutting. With physiological quality, although all the cuttings showed similar growth curves, those of 60, 90 and 120 days produced, 125 days after cutting, the best vegetative performance in the seedlings.

Key words: Rubiaceae. *Coffea canephora*. Cuttings. Maturation stage. Weight accumulation.

RESUMO - As características intrínsecas do propágulo vegetativo podem influenciar na velocidade de enraizamento e crescimento da parte aérea, bem como, na qualidade fisiológica final de mudas clonais de cafeeiros (*Coffea canephora*). Diante disso, objetivou-se avaliar o crescimento e a qualidade fisiológica de mudas de cafeeiros 'Robusta' produzidas a partir de propágulos (estacas caulinares) com diferentes idades. O estudo foi conduzido em casa de vegetação, no município de Ouro Preto do Oeste, Rondônia, Brasil (10˚45'43" S e 62˚15'10" W). As idades de formação das estacas foram: 30, 60, 90, 120 e 150 dias, o que correspondeu a cinco posições na haste ortotrópica secundária (broto), do ápice para a base. Foram avaliadas as taxas de acúmulo de massa seca e de crescimento relativo e absoluto durante 188 dias após o estaqueamento, bem como, características vegetativas das mudas aos 125 dias após o estaqueamento. Sobre o crescimento conclui-se que este pode ser dividido em três fases: 1) Inicial: crescimento lento, com duração de aproximadamente 83 dias; 2) Intermediária: de rápido crescimento, com duração de aproximadamente 40 dias; 3) Final: de crescimento lento, que tem início aproximadamente 125 dias após o estaqueamento. Sobre a qualidade fisiológica conclui-se que, apesar de todas as idades das estacas [cuttings] apresentarem curvas de crescimento semelhantes, as estacas [cuttings] de 60, 90 e 120 dias são as que proporcionam o melhor desempenho vegetativo das mudas aos 125 dias após o estaqueamento.

Palavras-chave: Rubiaceae. *Coffea canephora*. Estaquia. Estádios de maturação. Acúmulo de massa.

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INTRODUCTION

Coffea canephora, when propagated from seeds, results in uneven and heterogeneous crops. This is due to characteristics of genetic self-incompatibility and cross-fertilisation that are specific to the species, and which result in plants that are heterozygous and distinct. As such, to guarantee the permanence of specific characteristics from each genotype, cloning using cuttings is the most widely used method of propagation of the species in the main producing regions in Brazil (VERDIN FILHO *et al*., 2014).

This method (the rooting of stem cuttings) is important because it is physiologically viable (CHAGAS *et al*., 2012) and allows the genotypic value of the parent plant to be fully exploited (RAMALHO *et al*., 2016). As such, genetic characteristics are maintained, creating elite clones with desirable agronomic characteristics that include from precocity, homogeneity, productive stability and fruit quality to resistance to pests and diseases (ANDRADE JUNIOR *et al*., 2013; PARTELLI *et al*., 2006). In addition, the possibility of multiplying seedlings, regardless of the time of year, together with low production costs, has consolidated the use of this technology (cuttings) by coffee growers.

However, despite its consolidated position and the widely recognised advantages for seedling production, the use of cuttings is a method that can be affected by various factors, such as environmental conditions (substrate, luminosity, relative humidity and temperature) and the physiological quality of the vegetative propagule (ROSA *et al*., 2017). The importance of the physiological quality of the vegetative propagule is even more relevant, since its intrinsic characteristics (quantity of carbohydrates, presence of auxins, degree of lignification and predisposition to moisture loss) have an initial influence on adventitious-root emission and shoot formation (HUSEN; PAL, 2007).

Research carried out with the species *Coffea arabica* have shown that the part of the orthotropic branch from which the cuttings are taken has a distinct influence on the potential for root emission (REZENDE *et al*., 2010), and on the growth and development of the clonal seedlings (REZENDE *et al*., 2017). However, in *Coffea canephora*, information on the physiological quality of the propagules is still scarce. As such, information on the maturation stage of propagules can help to define propagules that would result in seedlings of higher physiological quality.

The aim of this study, therefore, was to evaluate the growth and vegetative characteristics of clonal seedlings of ‘Robusta’ coffee produced from propagules with different cutting ages.

MATERIAL AND METHODS

The experiment was conducted from November 2017 to April 2018 in a greenhouse located in the experimental area of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), in Ouro Preto do Oeste, Rondônia (10˚45’43” S, 62˚15 ‘10” W, at an altitude of 300 m). According to the Köppen classification, the climate is of type Am (ALVARES *et al*., 2014), with average annual temperature of 25 °C and average rainfall of 2,000 mm yr^{-1}. The rainy season extends from October-November to April-May.

The experimental design consisted of five treatments corresponding to the age of the stem cuttings on the parent plant (30, 60, 90, 120 and 150 days), originating with the first cutting from the apex containing two plagiotropic branches. The evaluation periods were zero (dry matter from the cutting), 41, 62, 83, 104, 125, 146, 168 and 188 days after cutting (DAC). The experiment was set up in a completely randomised design, arranged in a 5 × 9 factorial scheme with five replications. Each experimental plot comprised six cuttings/seedlings.

The cuttings were taken from parent plants of *C. canephora*, of the botanical variety Robusta, belonging to the breeding program of Embrapa Rondônia. The clonal garden of parent plants was five years old and had been cultivated following the technical recommendations for the crop (ESPINDULA *et al*., 2015a).

The cuttings were formed from a segment of orthotropic stem, detached from the original stem by a straight cut at the top, 1 cm above the insertion of the plagiotropic branches, with a straight cut 5 cm below the node (leaf insertion). The plagiotropic branches were removed, and the length of the leaves remaining on the cutting was reduced by 2/3 to minimise water loss and prevent dehydration (DIAS *et al*., 2012).

To obtain stem cuttings with different ages, 150-day-old orthotropic stems were used. These stems had five nodal segments, each with one pair of fully expanded leaves, where they were divided into five parts considering both their position on the branch and the period of growth. The first segment, formed from the basal region of the stem, made up the 150-day-old cutting; the segment formed just above that, in the semi-basal region, made up the 120-day-old cutting; the next segment, formed from the middle region of the stem, comprised the 90-day cutting; the segment formed from the semi-apical region comprised the 60-day cutting, and finally, the segment formed just below the apical meristem became the 30-day-old cutting.

The individual cuttings were placed in tubes with a capacity of 280 cm^3 filled with Vivatto Plus® commercial substrate (composed of a ground mixture of charcoal, pine bark and peat). Osmocote Plus® 5M controlled-release fertiliser was added to the substrate (15% N, 9% P_2O_5, 12% K_2O, 1.3% Mg, 6% S, 0.05% Cu, 0, 46% Fe, 0.06% Mn and 0.02% Mo) together with triple superphosphate in the proportion of 1 and 0.5 kg for each 100 kg of substrate respectively. The tubes containing the substrate were placed on suspended benches in a greenhouse and irrigated for 24 hours to saturate the micropores before receiving the cuttings.

In the greenhouse, seedling management followed the technical recommendations for the crop (ESPINDULA *et al*., 2015b). Irrigation was intermittent, by means of sprinklers, with an automatic timer programmed to activate the system for 10 seconds every 7 minutes for the first 40 days, 20 seconds every 20 minutes from 40 to 80 days, and 30 seconds every 30 minutes from 80 to 120 days. After 120 days, the seedlings were watered by hand twice a day, at 8 am and 4 pm.

At 41, 62, 83, 104, 125, 146, 168 and 188 DAC the seedlings were removed from the tubes, washed to remove any substrate and taken to the laboratory to determine the dry matter accumulation (DMA) using an analytical balance (0.001 g). The material was then packed in paper bags and placed in a forced air circulation oven at 65 ºC to constant weight. From this dry matter data, the physiological indices of absolute growth rate (AGR) and relative growth rate (RGR) were determined using formulae by Peixoto, Cruz and Peixoto (2011).

At 125 days after cutting, the average time for the formation of clonal seedlings of *C. canephora* in the state of Rondônia (ESPINDULA *et al*., 2015b), the following vegetative characteristics were determined: 1) number of roots greater than 0.5 cm (NR), by counting; 2) root-system volume (RV), determined in a graduated cylinder from the difference in displaced volume; 3) stem length (SL), determined by measuring the cutting from the point of sprout insertion to the apical meristem; 4) leaf area (LA), determined using the DDA - Digital Area Determination software (FERREIRA; ROSSI; ANDRIGHETTO, 2008); 5) root dry matter (RDM), 6) shoot dry matter (SDM) and 7) total dry matter (TDM), determined by analytical balance after drying in a forced air circulation oven at 65 ºC to constant weight; and 8) the Dickson quality index (DQI) (DICKSON; LELA; HOSNER, 1960).

The data were submitted to analysis of variance using the Genes® software (CRUZ, 2013), and regression analysis using the SigmaPlot® Software (SYSTAT SOFTWARE, 2006). The mathematical models were chosen considering the highest values for the coefficients of determination (r^2/R^2), and the significance of the regression coefficients (βi) and F-test, both at 5% probability, as well as by the behaviour of the biological phenomenon.

RESULT AND DISCUSSION

The dry matter accumulation (DMA), and the absolute (AGR) and relative (RGR) growth rates showed common growth patterns regardless of the age of the cutting. For DMA, non-linear sigmoidal behaviour was seen, with reduced accumulation rates until 83 DAC, followed by a rapid increase between 83 and 125 DAC, and then by a period with little dry matter accumulation, from 125 to 188 DAC (Figure 1A).

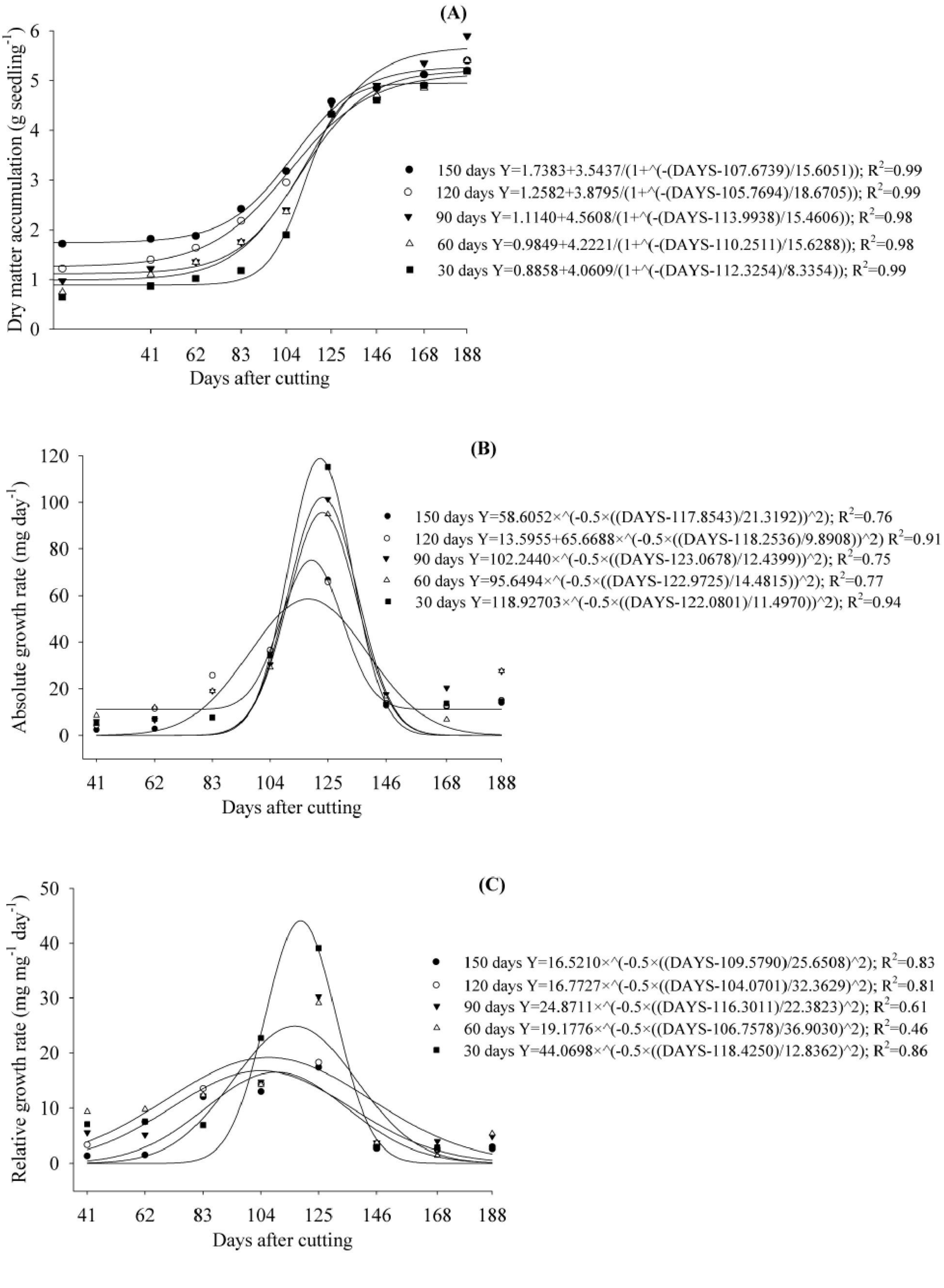
Despite the cuttings showing a common pattern for dry-matter accumulation, the curves showed inflection points for different phases of the seedling growth period. This difference was more pronounced in the youngest cuttings (30 days of age), where the rapid accumulation phase occurred between 83 and 104 DAC, whereas in the other cuttings, the rapid accumulation phase started earlier, between 62 and 83 DAC.

Non-linear Gaussian peak behaviour was seen for the absolute and relative growth rates, characterised by a period of slow growth (up to approximately 83 DAC), followed by a period of intense growth (between 83 and 125 DAC), and then a marked reduction up to 146 DAC, with no further growth after this period (Figures 1B and 1C).

For the absolute growth rate, despite cuttings with different ages all showing Gaussian behaviour with a peak in growth close to 118 DAC, these peaks were of varying intensity. This difference was more pronounced between cuttings with a greater age difference, 30 and 150 days, showing a peak in growth of 118.92 mg day^{-1} and 58.60 mg day^{-1} respectively (Figure 1B).

Similar to the AGR, different intensities of peak growth were also seen in the RGR, especially between

Figure 1 - Dry matter accumulation (A), absolute growth rate (B) and relative growth rate (C) in seedlings of *C. canephora* formed from cuttings of different ages



cuttings with the greatest age difference, 30 and 150 days, where the peaks in growth were 44 and 19 mg mg^{-1} day^{-1} respectively. However, in contrast to the AGR, the peaks in RGR occurred during different growth periods, at 118 DAC for the 30-day cuttings and 106 DAC for 150-day cuttings (Figure 1C).

The curves for dry matter accumulation and growth show that seedling development in the nursery is divided into three distinct phases. During the initial phase, until 83 DAC, growth rates are reduced and, consequently, dry matter accumulation is low. This reduced growth is the result of a lack of absorbing roots to remove water and nutrients from the substrate; this phase is therefore considered as the critical phase of vegetative propagation employing cuttings (LIMA *et al*., 2006). In addition, as carbohydrate availability influences rooting speed, the seedlings direct their reserves to the formation of adventitious roots, resulting in a reduction in shoot matter accumulation (LIMA *et al*., 2011).

In the intermediate phase, from 83 to 125 DAC, intense growth and matter accumulation are seen. During this period, the root system has already begun to grow, allowing the seedlings to absorb water and nutrients (CARDOSO *et al*., 2011), and contributing to shoot growth. As the shoots develop, in addition to the reserves of carbohydrates and nitrogen compounds absorbed by the root system, the seedlings begin the production of carbohydrates (PEIXOTO; CRUZ; PEIXOTO, 2011), with the nutrients absorbed by the root system and the sugars produced via photosynthesis both being directed to seedling growth.

The final phase, following 125 DAC, is marked by stable seedling growth. This behaviour is probably related to the physical limitation imposed by the container and the substrate, where, despite the supply of nutrients from the fertilisers continuing to promote growth, the physical limitation imposed by the 280 cm^{-3} of the cylinder results in excessive confinement of the root system over long periods in the nursery (ESPINDULA *et al*., 2018; REZENDE *et al*., 2010).

Although the growth curves are similar, the age of the cuttings resulted in different intensities and peaks in growth. This result may be related to the levels of carbohydrates available in each type of cutting, due to a concentration gradient that forms along the orthotropic stem, lower in the region close to the apex and higher near the base (CUNHA *et al*., 2015; HUSEN; PAL, 2007).

It is also important to note that other factors, such as hormonal balance and the stability/reversibility of the tissues (HARTAMANN; DAVIES JUNIOR; GENEVE, 2011) may be associated with the growth rate of the seedlings. This can result in different growth rates and, consequently, peaks in growth throughout the period of seedling formation.

Each of the vegetative characteristics of the seedlings (SL, LA, NR, RV, SDM, TDM and TDM) was influenced by the age of the cutting. This behaviour fitted the quadratic polynomial model, with maximum points obtained with the 90-day-old cuttings (Figure 2A, B, C, D and E). The Dickson quality index (DQI) did not adjust to any mathematical model, with a mean value of 0.62 attributed to each seedling, regardless of the age of the cutting (Figure 2F).

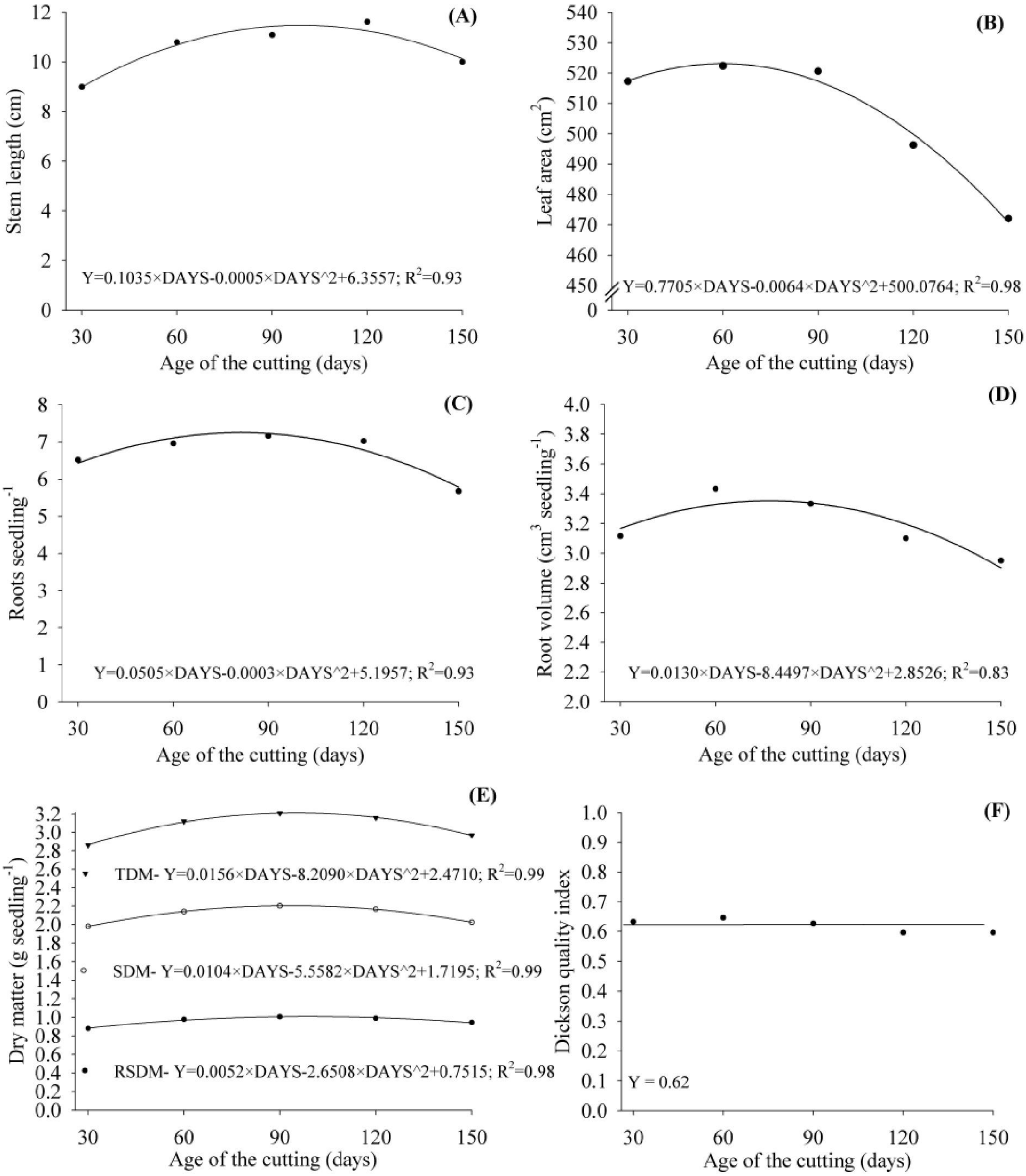
The greater development shown by seedlings from the 90-day cuttings is related to the physiological condition of the vegetative propagule, due to the cuttings being removed from the middle region of the orthotropic stem, the region that presents the greatest lignification and nutritional and hormonal balance (REZENDE *et al*., 2010). In general, vegetative characteristics show higher values in seedlings propagated from cuttings of intermediate age (COELHO; AZEVEDO, 2016; PAULINO *et al*., 2011; REZENDE *et al*., 2017).

Even with similar growth behaviour (Figure 1), the physiological condition of the 30-day and 150-day-old cuttings had a negative influence on the vegetative attributes of the seedlings. In the case of the 150-day-old cuttings, this may have been due to their being removed from the basal region of the stem, which contains a large amount of carbohydrates; however, the tissues are generally very lignified, which can hamper or prevent rooting (PAULINO *et al*., 2011; REZENDE *et al*., 2010).

In the case of cuttings produced from the apical region, such as those of 30 days of age, despite a high concentration of auxin, an essential hormone for rooting, they have a low reserve of carbohydrates, which can lead to low survival rates due to depletion of these reserves. In addition, the cuttings are more prone to dehydration due to moisture lost to the environment, a result of their smaller diameter (COELHO; AZEVEDO, 2016; SANTOS *et al*., 2016).

It can be inferred from the results that the Dickson quality index (DQI) alone is not enough to define the quality of *C. canephora* seedlings; despite showing a mean value of 0.62, the physiological condition of the 30-day and 150-day-old cuttings had a negative effect on the vegetative characteristics of both the shoots and roots. The DQI is an indicator of seedling quality and robustness, and considers the distribution of dry matter between the root system and the shoots. It is therefore necessary to evaluate other vegetative characteristics to determine the end quality of the seedlings.

Figure 2 - Vegetative characteristics in seedlings of *C. canephora* formed from cuttings of different ages, at 125 days after cutting: Stem length (A), leaf area (B), number of roots (C), root-system volume (D), shoot dry matter, root system dry matter and total dry matter (E), and Dickson quality index (F)



CONCLUSIONS

1. Regardless of the age of the vegetative propagule (stem cutting), growth in seedlings of *C. Canephora* can be divided into three distinct phases: slow growth (lasting up to 83 days); rapid growth (lasting 40 days) and slow growth (starting 125 days after cutting);
2. Seedlings formed from 30-day and 150-day-old cuttings result in lower values for the vegetative attributes of the roots and shoots at 125 days after cutting.

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