

# AVOCADO SEEDLINGS MULTIPLE STEMS PRODUCTION<sup>1</sup>

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**ABSTRACT** - This study aimed to evaluate the potential of multi-stems in avocado seeds according to their mass as well as the adventitious rooting of multi-stem budding with or without the use of auxin. The research was carried out at the Vegetation House of Federal Institute of Espírito Santo, Campus Santa Teresa -ES, with seeds of different masses: <60 g, 61 to 80 g, 81 to 100 g and >100 g, in which each experimental unit was made of five seeds, distributed within five repetitions, under a completely randomized design. The seeds were put to germinate and the percentage number of emergence and multiple stems were evaluated. After 150 days, the following evaluations were carried out: survival of rooted cuttings; number of leaves; stem diameter; root length; root volume; root and shoot fresh mass; root and shoot dry mass; shoot height; absolute growth and shoot growth rate; shoot dry weight/root dry mass ratio; shoot height/stem diameter ratio; shoot height/root length and Dickson's quality index ratio. Avocado seeds with mass over 100 g and between 81-100 g presented higher percentage of multiple stems. Rods over 20 cm that were not treated with IBA (indole-3-butyric acid) resulted on avocado plants of better quality. The use of IBA (2000 mg L<sup>-1</sup>) does not affect the rooting and growth of avocado's multi-stem plants.

**Index terms:** Avocado, seeds, multi-stem, rooting, auxin, *Persea americana* Miller.

## PRODUÇÃO DE MUDAS POLICAULICAS DE ABACATEIRO

**RESUMO** – Objetivou-se avaliar o potencial de policaulia em sementes de abacateiro de acordo com a massa das sementes e o enraizamento adventício das brotações policaulicas com e sem o uso de auxina. O trabalho foi realizado na casa de vegetação do IFES, Campus Santa Teresa-ES, com sementes de diferentes massas: <60 g; 61 a 80 g; 81 a 100 g e >100 g, em que, cada unidade experimental foi composta por cinco sementes, distribuídas em cinco repetições, num delineamento inteiramente casualizado. As sementes foram colocadas para germinar, e foram avaliados o número de hastes emergidas, a porcentagem de emergência e de policaulia. Após 150 dias, foram avaliados: sobrevivência das estacas enraizadas; número de folhas; diâmetro do coleto; comprimento da raiz; volume de raízes; massa fresca das raízes e da parte aérea; massa seca das raízes e da parte aérea; altura da parte aérea; crescimento absoluto e taxa de crescimento da parte aérea; relação da massa seca da parte aérea com a massa seca de raízes; relação da altura da parte aérea com o diâmetro do coleto; relação da altura da parte aérea com o comprimento da raiz, e índice de qualidade de Dickson. Sementes de abacate com massas acima de 100 g e entre 81-100 g apresentam maiores percentuais de policaulia. As hastes acima de 20 cm não tratadas com o AIB resultaram em mudas de abacate de melhor qualidade. O uso de AIB (2.000 mg L<sup>-1</sup>) não afeta o enraizamento e o crescimento das mudas policaulicas de abacateiro.

**Termos para indexação:** Abacate, semente, policaulia, enraizamento, auxina, *Persea americana* Miller.

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## INTRODUCTION

The avocado belongs to the Lauraceae family and is cultivated in all tropical and subtropical regions of the world, particularly in Mexico, Indonesia, the United States, Brazil, Chile, Colombia, Dominican Republic, Peru and Ethiopia. The Brazilian production is distributed in the Southeast, Northeast and South regions, and the state of São Paulo is the biggest producer followed by the states of Paraná and Espírito Santo. The differences in harvest yields between the states are due to the cultivation forms, cultural tract, and beyond the diversity of cultivars according to consumer preferences in different regions (VALE, 2010; FAO, 2013).

The avocado is a berry, which depending on the vegetable race to which it belongs may have piriform, spherical, elliptical or oval shape (DAMIÃO FILHO; MORO, 2005), with eurypermic, exalbuminous, of brown-medium color seeds. The avocado consumption in Brazil is preferably in the natural form that is rich in fibers that can help to prevent diseases such as diverticulitis, colon cancer, obesity, cardiovascular disease and diabetes (SALGADO et al., 2008), and presents antioxidant activity and phenolic compounds (WANG et al., 2010).

At the beginning and during many years, the avocado commercial culture in Brazil occurred due to the acquisition of great numbers of hybrid by seminiferous propagation and today they are cultivated with monoembryonic seeds, generating different plants (VALE, 2010). Although, the avocado has only one embryo, there were doubts and disagreements on the number of avocado embryos, which was later found the occurrence of multi-stems, in other words, there is the occurrence of multiple stems from a single embryo axis (OLIVEIRA et al., 2010).

The beginning of emergence occurs after 33 days of the sowing and its stabilization after the eighth week, with approximately 80%, reaching 86% after the fifteenth week and it shows the multi-stems phenomenon (OLIVEIRA, 2006; OLIVEIRA, et al., 2010).

The use of seeds with higher number of emerged sprouts, from the commercial point of view, from the rooting of cuttings obtained from multi-stems will result in a higher number of seedlings to be grafted, and may be characterized as an advantage process, since several of them are clones that are likely to give rise to clonal gardens, which later may provide uniform material for obtaining rootstocks.

This study aimed to evaluate the multi-stems potential in avocado seeds according to the mass

seeds and adventitious rooting of multiple-stem budding with or without the use of auxin.

## MATERIAL AND METHODS

The experiments were conducted in the production nursery of seedlings covered with polyolefin fabric with 50% shadowing, in the fruit-growing sector of the Federal Institute of Santa Teresa (IFIST), Campus Santa Teresa-ES, Brazil, located in the Espírito-Santo region, Santa Teresa, geographical coordinates 19°56'12"S and 40°35'28"W, with an altitude of 665m. The region's climate is Cwa, mesothermal, with a dry season in the winter and heavy rainfall in the summer (Köppen classification), average annual rainfall of 1,404.2 mm and average annual temperature of 19.9 °C, with a maximum average of 32.8 °C and minimum of 10.6 °C (INCAPER, 2011).

Seeds taken from freshly picked fruits of donors-plant from the variety Collinson (*Persea americana* Miller) (Antillean race, group A). The seeds were weighed and defined in treatments in mass function: < 60 g, from 61 to 80 g, from 81 to 100 g and > 100 g. Five seeds distributed in five repetitions (25 seeds) composed each experimental unit in a completely randomized design (CRD). The sowing was done in plastic bags of 12x25x0.07 cm, prepared with a substrate produced from a mixture of three parts of sifted subsoil with a part of cattle manure hardened and sifted (mash four).

In the second experiment, ninety days after the emergence, the multi-stem rods were selected in < 10 cm, from 11 to 20 cm and > 20 cm, counted, detached and the leaves removed, measured and treated with 4-indole-3-butyric acid (IBA) at concentration of 2000 mg L<sup>-1</sup>, and untreated cuttings as control. Later, the cuttings were planted in bag and similar substrates to the ones from the first experiment. The experimental designed used was randomized block in factorial 3x2x10 (rods size x presence or absence of IBA x rods number), with four repetitions and sixty rods per block.

The mixture was classified in the physical analysis as loamy textural class (400.94 g kg<sup>-1</sup> sand; 54.63 g kg<sup>-1</sup> silt; 544.43 g kg<sup>-1</sup> loam) and in the chemical analysis showed the following nutritional values: P = 56.79 mg dm<sup>-3</sup>; K = 836 mg dm<sup>-3</sup>; Ca = 3.18 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 2.77 cmol<sub>c</sub> dm<sup>-3</sup>; H+Al = 1.90 cmol<sub>c</sub> dm<sup>-3</sup>; TB = 8.59 cmol<sub>c</sub> dm<sup>-3</sup>; CEC = 8.59 cmol<sub>c</sub> dm<sup>-3</sup>; CEC at pH 7 = 10.49 cmol<sub>c</sub> dm<sup>-3</sup>; OM = 27.16 g kg<sup>-1</sup>; pH = 6.47.

The variables evaluated: emergence

percentage (E), rods per seed number and multi-stem percentage (MS) and after 150 days according to the rod size: rooted rods survival (SR); leaves number (LN); stem diameter (SD); root length (RL), root volume (RV); root fresh mass (RFM) and shoot fresh mass (SFM); root dry mass (RDM) and from the shoot (SDM); shoot height (SH); absolute growth (AG) and shoot growth rate (GR) in 90; 120; 135 and 150 days; relation of the shoot dry mass with the root dry mass (RSDMRDM); the relation of shoot height with stem diameter (RSHSD); the relation of shoot height with root length (RSHRL) and DQI (Dickson's quality index) obtained by the following equation:

$$DQI=[(RDM+SDM).(RSHSD+RSDMRDM)^{-1}]$$

(DICKSON et al., 1960).

The data were submitted to analysis of variance and the averages compared by the Tukey test at 5% probability, through the GENES program (CRUZ, 2013).

## RESULTS AND DISCUSSION

The seedlings emergence of the avocado variety Callinson started at 30 days after the sowing and stabilized between the eighth and the ninth week, with an average of 88% (Table 1). According to Oliveira et al. (2010), in the avocado variety Hass, the seedlings emergence occurred 33 days after the sowing, with the beginning of emergence stabilization in the eight week, with 88%. According to Petry et al. (2012), the seedlings emergence of the avocado variety Baronessa started 30 days after the sowing, extending for more 30 days. In this study, the seeds with mass lower than 60g; 61-80 g; 100 g and over 100 g did not show statistic difference in the seedlings emergence, with values of 88; 84; 92 and 88%, respectively, observing that, for the avocado, the seed weight is not the fact that characterizes the increase in germination.

The avocado seeds showed hypogeal germination, agreeing with Oliveira et al. (2010) that verified in the variety Hass monoembryonic and exalbuminous seeds and hypogeal germination. Oliveira et al. (2010) conclude by the anatomical studies of the embryonic axis that the vascular bundles come from a single embryo, occurring the branching of the Epicotyl in the collar, originating multiple stems in the avocado seed, characterizing as multi-stem and not polyembryony. The results found by Oliveira et al. (2010) supported the results obtained in this study, in which the higher multi-

stem percentage occurred in the masses over 100 g (64%), but without statistically differ from seeds with masses between 81 and 100 g (52%) (Table 1). The avocado seed sizes between 81-100 g and over 100 g gave the higher number of rods (50) (Table 1), which is very important to increase the propagules number and at the same time to enable the production of a higher number of seedling per seed unit. In the Jaboticaba plant cvs. Sabará and Cabinho, the bigger seeds showed higher number of seedlings per seed (1.53 seedlings per seed) (WAGNER JÚNIOR et al., 2011). The success in germination and establishment of seedlings is associated to size and quantity of seed reservations (CÓRDULA et al., 2014). In the case of avocado, the obtained result should be associated with a greater amount of nutritive reserves in bigger seeds, which stimulates the production of multi-stem rods. According to Oliveira et al. (2010), the greater avocado rods production occurred in the emergence period, around the fourth week and this fact can be assigned to the greater quantity of reserves in seeds, with early emergence.

In this study, the cuttings smaller than 10 cm treated with IBA showed lower survival percentage values, stem diameter, root volume, shoot height, absolute growth, fresh and dry mass of the shoot and root, relation of shoot height with stem diameter and DQI (Table 2). The resulting index of the relation between shoot height and stem diameter expresses the balance in growth, also known as sturdiness quotient and it is considered one of the most accurate indexes (ANDRADE JÚNIOR et al., 2013).

The highest survival percentages of rooted stems, leaves number, root fresh mass and shoot fresh mass (Table 2) occurred in rods bigger than 20 cm treated with IBA. However, the stem diameter characteristics, root volume, shoot height and Dickson Quality Index were higher in rods bigger than 20 cm, independent of the IBA application. And the higher root dry mass and shoot dry mass values were obtained in rods bigger than 20 cm, without the use of auxin (Table 2). The auxin, depending on the concentration, can stimulate and prevent the cell distension; even in roots that have a higher sensibility, but there was no significant effect to the increase of its mass or shoot mass. These results suggest that it is unnecessary the use of auxin, 2000 mg L<sup>-1</sup>, in the option for rods bigger than 20 cm of length, mainly because the highest Dickson quality index was obtained regardless the use or not of the auxin.

According to Gomes (2001), the equation that determines the DQI is balanced because it includes morphological parameters of height, diameter and biomass, and the higher the index value is, the

higher the quality standard of the seedlings will be. The avocado seedlings also need to grow in diameter when considering that they are produced by grafting. Another very important characteristic is the relation of the shoot height with the stem diameter because as closer to one (1) it means that the growth is allometric. This growth is directly related to the ability of capture resources, on other words, bigger nutrition and shoot expansion. As an example, we can quote the plants that invest in the increase of the photosynthetic area to allocate important assimilates in the root growth.

Young rods above 20 cm have higher content of nutrient reserves, increasing the resistance to external factors period, with easier emission of root, regardless of the use of auxins (Table 2). The best indexes observed in the bigger rods can be related to leaves production (Table 2), where there is the increase in photosynthesis performed by the seedlings, with higher carbohydrates production and nutritional reserves, favoring the production of roots.

In azalea, the cutting survival percentage and leaf retention reached values of 100%, regardless of the use of IBA. And in relation to the rooted cuttings percentage, there was no significant interaction between the IBA concentrations and the substrate, with averages between 96 and 99%, respectively (LONE et al., 2010). According to Ramos et al. (2003), the supply of auxin, in certain amounts, can promote hormonal change, favoring

or not the cuttings. This was observed in Oliveira et al. (2008) study, in which the use of IBA (0; 1000, 3000 and 5000 mg kg<sup>-1</sup>) did not promote the rooting of air laying in avocado variety 'Duke 7'. However, etiolated sprout of avocado variety 'Duke' treated with 5000 and 10000 mg L<sup>-1</sup> of IBA showed 43.3 and 56.6% of rooting, respectively, with significant differences (ESCOBEDO; ESCOBEDO, 2011). In this case, it may be during the peroxidase activity, the root formation was strongly associated with the growth process and differentiation, for example, the cambial and xilogenesis cell division (GARCIA-GOMEZ et al., 1995).

The natural rejuvenation, in which the more mature plant parts, the reproductive organs produce the younger ones, the embryos through the sexual reproduction, it is an efficient form to maintain the youthfulness (WENDLING et al., 2014b). Considering that the progression of juvenile phase to more advanced phases of maturation in woody plants is combined with several changes in individual characteristics, such as the adventitious rooting capacity (WENDLING et al., 2014a) and depending in how this progression occurs, the rooting capacity can be reduced. In the avocado case, a woody tree, the ability to produce seeds that show high potential to grow sprouts from emerged seedling is a strategy to increase the number of young propagules with high rhizogenic capacity, maximizing the seedlings production.

**TABLE 1** - Total number of rods, percentage of emerged seedlings and multiple stems, from different masses of avocado seeds of Collinson variety.

Evaluated characteristics	Seeds mass (g)			
	< 60	61-80	81-100	> 100
Total number of rods	28 c*	32 b	50 a	50 a
Percentage of emerged seedlings	88 a	84 a	92 a	88 a
Percentage of multiple stems	20 b	28 b	52 ab	64 a

\*Averages followed by the same letter in line do not differ from each other by Tukey test at 5% probability.

**TABLE 2** - Rooted rods survival (RS); leaves number (LN); stem diameter (SD); root length (RL), root volume (RV); shoot height (SH); absolute growth (AG); root fresh mass (RFM); root dry mass (RDM) shoot fresh mass (SFM); shoot dry mass (SDM); relation of the shoot dry mass with the root dry mass (RSDMRDM); the relation of shoot height with stem diameter (RSHSD); the relation of shoot height with root length (RSHRL) and DQI (Dickson's quality index) of the avocado Collinson variety, depending on the size of multiple stems rods and on the presence or not of IBA.

Multi-stem rods	IBA (2000 mg L <sup>-1</sup> )	RS (%)	LN	SD <sub>&lt;</sub> <sub>&gt;</sub> (cm) <sub>&lt;</sub>	RL (cm)	RV (cm <sup>3</sup> )
< 10	Absence	87.5e*	10.4 b	0.4655 b	16.68 a	1.8 c
10 to 20		90.6 d	14.6 ab	0.5502 b	22.16 a	4.8 b
> 20		94.8 b	15.6 ab	0.7361 a	24.24 a	8.2 a
< 10	Presence	86.9 f	11.2 ab	0.4456 b	13.68 a	1.2 c
10 to 20		92.1 c	14.6 ab	0.5526 b	17.62 a	4.8 b
> 20		100.0 a	16.6 a	0.7898 a	24.40 a	8.8 a
Multi-stem rods	IBA (2000 mg L <sup>-1</sup> )	SH (cm)	AG (cm)	RFM (mg)	RDM (mg)	SDM (mg)
< 10	Absence	8.8 d	1.7 e	1806 e	221 e	1998 e
10 to 20		17.1 c	1.8 d	4311 c	462 c	5374 d
> 20		27.1 a	1.9 c	7312 b	1096 a	12127 b
< 10	Presence	7.6 d	1.5 f	1147 f	123 f	1902 f
10 to 20		18.8 b	3.9 a	3893 d	340 d	5377 c
> 20		28.0 a	3.1 b	7651 a	944 b	12693 a
Multi-stem rods (cm)	IBA (2000 mg L <sup>-1</sup> )	SDM (mg)	RSDMRDM	RSHSD	RSHRL	DQI
< 10	Absence	441 e	1.99 f	0.1890 b	0.53 a	0.4655 b
10 to 20		1240 c	2.68 e	0.3108 a	0.77 a	0.5502 b
> 20		3191 a	2.91 c	0.3681 a	1.18 a	0.7361 a
< 10	Presence	434 f	3.53 a	0.1706 b	0.55 a	0.4456 b
10 to 20		943 d	2.77 d	0.3402 a	0.83 a	0.5526 b
> 20		2931 b	3.10 b	0.3545 a	1.15 a	0.7898 a

\*Averages followed by the same letter in the column do not differ from each other by Tukey test at 5% probability of error.

## CONCLUSIONS

Avocado seeds with mass above 100 g and between 81-100 g show the highest percentages of multi-stem.

The rods above 20 cm result in avocado seedling of better quality.

The use of IBA (2000 mg L<sup>-1</sup>) does not affect the rooting and growing of the avocado multi-stem seedlings.

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