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In vitro propagation of avocado (*Persea americana* Mill.) cv. Hass through morphogenesis

Propagación *in vitro* de aguacate (*Persea americana* Mill.) cv. Hass vía morfogénesis

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Abstract

Given the importance and potential development of avocado (*Persea americana* Mill.) cultivation in Colombia and its problems for an efficient field cultivation, which includes the genetic variability of propagated material and adverse phytosanitary conditions, the aim of this study was to develop a method for *in vitro* clonal propagation for avocado cv. Hass. *In vitro* introduction of apical explants and nodal segments was carried out from grafted parent plants. Etiolation pretreatment effect on the number of shoots formed and *in vitro* phenolization was evaluated in grafted parent plants and in explants under *in vitro* conditions, subject to photoperiod (12/12 hours) and darkness. Darkness conditions reduced significantly plant phenolization, and these in turn formed new shoots, which were higher and were also formed in a shorter time. During multiplication stage, using a WPM culture medium an effective control of phenolization was obtained. During subculturing there was a decreasing in apical explant elongation, but there was a significant induction of new shoots from nodal segments. However, only some segments reached the required height to keep the multiplication rate throughout subcultures. Finally, 82% survival rate of vitroplants was obtained during hardening.

Keywords: Acclimatization, etiolation, greenhouse, *in vitro,* photoperiodicity, plant propagation, rooting.

Resumen

Teniendo en cuenta la importancia y el potencial de desarrollo del cultivo de aguacate (*Persea americana* Mill.) en Colombia, y los problemas que se presentan para el cultivo eficiente en campo, principalmente por la variabilidad genética del material de propagación y por condiciones fitosanitarias adversas, el presente trabajo tuvo como finalidad desarrollar una metodología para la propagación clonal *in vitro* del cultivar "Hass". La introducción *in vitro* de explantes apicales y segmentos nodales se realizó a partir de plantas madres injertadas. El efecto del pretratamiento de etiolación sobre el número de brotes desarrollados y el porcentaje de fenolización *in vitro*, se evaluó sometiendo las plantas madre y los explantes introducidos *in vitro* a condiciones de fotoperiodo (12/12h) y oscuridad; esta última condición es la que redujo significativamente la fenolización de los explantes, y estos a su vez formaron un mayor número de brotes nuevos con mayor altura y en menor tiempo. En la etapa de multiplicación en el medio de cultivo WPM se obtuvo un efectivo control del proceso de fenolización. Durante los subcultivos hubo disminución de elongación de los explantes apicales, y a partir de los segmentos nodales, se logró una inducción importante de nuevos brotes; sin embargo, solo algunos alcanzaron la altura requerida para mantener la tasa de multiplicación a través de los subcultivos. Finalmente durante el endurecimiento, se obtuvo un 82 % de supervivencia de las vitroplantas.

Palabras clave: Endurecimiento, enraizamiento, etiolización, fotoperiodicidad, *in vitro*, invernadero, propagación vegetal.

Introduction

Avocado (*Persea americana* Mill.) is a plant species that belongs to the botanical family Lauraceae. According to its genetic composition the species comprises four races with different origins: one comes from Mexico, a second one comes from Guatemala, a third one from the Antilles and the fourth one comes from Costa Rica. Avocado cv. Hass contains between 85 to 90 % Guatemalan race and 10-15 % Mexican race. Its fruits have a high nutritional value due to its high content of different oils, proteins, carbohydrates, minerals and vitamins. According to FAOSTAT (FAO, 2016), Colombia is the fourth country in terms of annual avocado production with 279.274 t. However, productivity and competitiveness problems in Colombia are linked to low technological development that occurs in this sector, as well as to deficiencies in commercialization channels, lack of health guarantees, genetic variability of the propagation material found in greenhouses, and high losses in the field associated to plant material that comes from poor quality seed.

Since avocado cultivation in Colombia has excellent near future perspectives, it is necessary to implement efficient and effective techniques for its propagation and cultivation. An important biotechnological tool is the *in vitro* techniques that have emerged to have achieve, among other things, selection of clonal multiplication material and to scale. Various authors have already carried out trials to propagate avocado material using tissue culture (Wessels, 1996; Rodríguez, Capote & Zamora, 1999; Premkumar, Barceló-Muñoz, Pliego-Alfaro, Quesada & Mercado, 2002, Nhut, Thi, Khiet & Luan, 2008; Zulfiqar, Akhtar, Ahmad & Ahmed, 2009); notwithstanding, several limitations affect multiplication rate and rooting of in vitro material, and this in turn influences plant hardening. To solve these limitations several growth regulators and other additives have been evaluated in different studies. However, important factors as light conditions before and during *in vitro* cultivation have not been taken into account, and these play an important role in growth and development responses. Some studies have demonstrated that light conditions, especially in woody plants, have a positive effect in shoot induction and rooting *in vitro*; conversely, high phenolization that regularly occurs in these plant species is reduced (Rodríguez et al., 1999; Rache-Cardenal, Pinzón, & Maldonado, 2008). Therefore, considering the aforementioned, the aim of this study was to establish an effective methodology for *in vitro* clonal propagation of avocado "Hass" cultivar through morphogenesis, assessing the effect of etiolation, plant growth regulator combinations and two culture media in shoot induction and development under greenhouse and *in vitro* conditions.

Materials and methods

This research was carried out in the Plant Biotechnology laboratory of Corporación para Investigaciones Biológicas (CIB), Medellin, in the department of Antioquia (Colombia).

Plant material

Plants grafted in a conventional way, i.e. employing the terminal spike method, using "Hass" as the graft or scion on a "Criollo" rootstock of one (1) year of age and maintained under semicontrolled greenhouse conditions, were the source material for the explants used (apical sprouts and nodal segments) for *in vitro* essays.

Culture media composition and growth conditions

Two basal culture media were used for different *in vitro* cultivation phases: MS (Murashige & Skoog, 1962) and WPM (Lloyd & McCown, 1980). Both culture media were supplemented with 20 $g.L^{-1}$ of sucrose. Moreover, different growth regulator concentrations were added: IBA (indolbutyric acid), BAP (6-Benzylaminopurine) and GA_3 (gibberellic acid) according to the treatments described in Table 1 and 2; and as an antioxidant agent 2 $g.L^{-1}$ of activated charcoal were also added.

Table 1. Hormonal treatments evaluated for the induction of *in vitro* shoots

Treatment	BAP ($mg.L^{-1}$)	GA3 ($mg.L^{-1}$)	IBA ($mg.L^{-1}$)
T1	0	0	0
T2	0.5	0.5	-
T3	1.0	0.5	-
T4	1.5	0.2	-
T5	0.5	-	0.5
T6	-	0.2	-
T7	0.5	-	-

Table 2. Hormonal treatments evaluated in the MS and WPM basal culture means in the *in vitro* multiplication stage

Treatment	BAP ($mg.L^{-1}$)	(GA_3 $mg.L^{-1}$)	IBA ($mg.L^{-1}$)
T1	0	0	0
T2	0.5	0.5	-
T3	1.0	0.5	-
T4	1.5	0.2	-
T5	2.0	0.2	-
T6	0.5	-	0.5
T7	2.0	-	0.5

Adjustment of pH was to 5.7 ± 0.1 and sterilization was carried out in an autoclave at 120 °C with 20 pounds of pressure during 20 minutes. Cultures

were maintained at a temperature of 21°C ± 1 with a photoperiod of 12 hours light; fluorescent white light lamps (Phillips TL5 14W/840) were used that gave an average light intensity of 32.43 μmol m^{-2} s^{-1}. To establish total darkness conditions a black canvas was used to cover the shelves used in the trials.

Effect of light conditions on shoot formation under greenhouse conditions

One hundred (100) grafted plants were taken to a dark chamber (etiolation chamber) and the same numbers of plants were maintained in greenhouses under a photoperiod (12 hours of light/12 hours of darkness) for seven weeks. New shoot formation was stimulated by making cuts on the leaves and on the apical meristem, and bending the branches to a horizontal position. The variables evaluated were as follows: number of shoots per plant, number of internodes and shoot height.

Disinfection and *in vitro* culture of apical buds and nodal segments

Disinfection of apical buds and nodal segments of ca. 6 cm were obtained from parent plants established in greenhouses; this was carried out according to the following protocol and also to the results obtained in previous essays. The protocol is as follows: initially, plant material was submerged in a iodine solution for an hour, then in a laminar flow chamber maintained in 2 $ml.L^{-1}$ of a timorex solution (terpinen-4-ol and γ-terpinene) for two hours; these were then submerged in 50 $mg.L^{-1}$ of vancomycin and 100 $mg.L^{-1}$ of cefotaxime for three hours, and then in ethanol 70 % for a minute, and followed by sodium hypochlorite at 2 % for ten minutes. Subsequently, dead tissue was cut on plant material edges leaving a ca. 4centimeter long explant for *in vitro* cultivation. In total, 420 explants per replicate were evaluated; essays were carried out per triplicate and contamination percentage 15 days after planting was established.

Nodal segments response to *in vitro* shoot induction

In this stage, nodal segments were cultivated in basal MS medium with different growth regulators (Table 1).

Explants used came from greenhouse plants subject to darkness as well as to light conditions (photoperiod of 12 hours light/12 hours darkness), and these in turn were subject to equal light conditions when cultivated *in vitro;* this was carried out to establish the effect on phenolization and development of lateral buds. Explants subject to *in vitro* darkness remained under these conditions for only 15 days and were then placed under photoperiod conditions. For each treatment with growth regulators and light conditions, 15 nodal explants were used, i.e. a total of 420 explants per replicate, as the essays were carried out by triplicate. In this phase, the following variables were measured: phenolization and callus formation percentage, number of shoots per explant, shoot height (cm), and the number of plants that developed leaves and roots for each treatment and per light condition (after 60 days of planting).

Multiplication stage

To establish the multiplication coefficient *in vitro*, initial explant used was an apical sprout of 4 cm whose roots and expanded leaves had been eliminated. Subcultures in fresh medium were carried out every six weeks. In each subculture new shoots were separated; these had to be larger or at least have the same size, i.e. 4 cm, to guarantee their further development. Interaction of plant growth regulators in MS and WPM basal media were assessed. The following variables were considered: number of shoots, and height and root formation in each subculture. For each treatment and basal medium 15 explants were evaluated for a total of 210 per replicate as the essay was carried out per triplicate.

***Ex vitro* acclimatization or hardening**

In this stage, one hundred (100) vitroplants of an approximate size of 6-7 cm and with roots were removed from their recipients, and their roots were washed with abundant water. In the mesh house the plants were sown in individual pots (9 x 10 cm); plants were sown in a chopped mixture of peat, sand and *Pinus patula* bark (3:1:1) previously sterilized in autoclave. Plants were covered with a plastic cup with holes that were then removed after 30 days. Every four days the substrate was moistened, and every three weeks these were fertilized with WPM basal salts. To evaluate growth and development during hardening the following variables were registered after three months: survival percentage, height, number of leaves, shoots and roots.

Statistical analysis

Data normality was tested with the Shapiro Wilk test. The differences between group pairs were evaluated using the student's *t*-test when the data behave according to a normal distribution, but the Mann-Whitney test was used when this is not the case. Differences among more than two groups were evaluated with the Kruskal-Wallis test or

with an ANAVA in case of normality, followed by a Pairwise Wilcoxon Rank Sum or a Tukey HSD test, respectively, when at least one group was different ($\alpha < 0.05$). Additionally, sampling techniques with bootstrapping replacement and with 10.000 permutations was carried out. All the statistical analyzes were carried out using the software R 3.1.2 R© Core Team 2014.

Results

Effect of light conditions in shoot formation under greenhouse conditions

Formation of new shoots in both conditions (photoperiod and darkness) was initiated after the second week that the essay was established; after the seventh week a higher number of shoots in average was obtained in the photoperiod (5.49) condition compared to the darkness (1.79) condition ($p < 0.05$). Plants under darkness conditions developed shoots whose tissues had a whitish color due to lack of chlorophyll, and also showed small and non-expanded leaves (Figure1A).

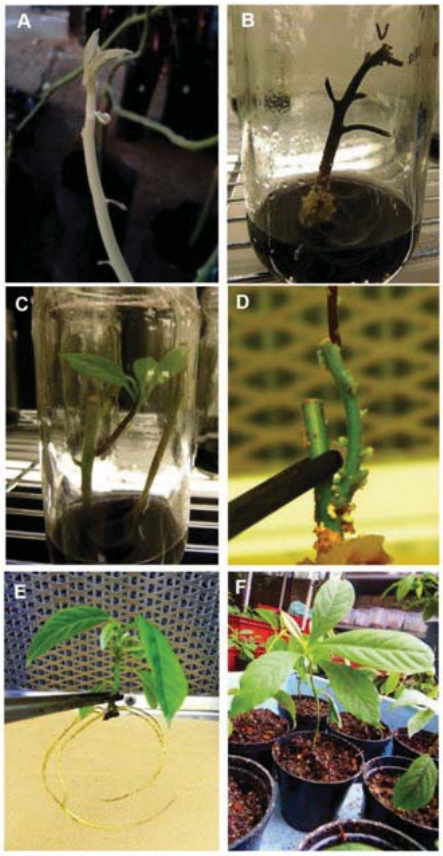

Figure 1. A. Etiolated shoots after week seven. **B.** Phenolized explant. **C.** *In vitro* shoot induction in darkness condition. **D.** Formation of shoots in nodal segments. **E**. Formation of roots in apical sprouts. **F.** Hardened vitroplants

Moreover, elongated buds reached an average height of 14.01 cm; on the contrary, plants maintained under photoperiod conditions only reached a height of 5.0 cm ($p < 0.05$). Furthermore, internodes of etiolated shoots elongated producing a lower number of internodes (1.85) compared to photoperiod condition (3.48) ($p < 0.05$). These results agree with what was reported for the "Duke 7" cultivar by Pullaz (2011), in relation to plants subject to total darkness.

Disinfection of apical sprouts and nodal segments

In this stage, a high disinfection percentage was reached both for apical sprouts as well as for nodal segments; solely a low contamination percentage (7.51 %) was registered. According to the type of explant, apical sprouts showed negative responses; however, after the disinfection process, all of these explants (100 %) became necrotic and died. Therefore, only nodal segments were used for the *in vitro* culture essays.

Effect of culture conditions and growth regulator combinations in shoot induction and development

In the explants stemming from photoperiod in greenhouse and maintained *in vitro* with the same light conditions, a higher phenolization percentage (59.29 %) was found (Figure1B). On the contrary, explants that were under darkness conditions in greenhouses had in average, a low phenolization percentage (1.50 %) for two light conditions under *in vitro* conditions ($p < 0.05$).

On the other hand, in this research we found significant differences in the induction and growth (height) stages of the new shoots under *in vitro* conditions, finding that under darkness conditions the highest height values were registered, i.e. 1.52 cm (Table 3).

Table 3. Average number of shoots and height achieved in different light conditions (photoperiod and darkness) evaluated *in vitro* and in greenhouse

Type of cultivation		Average number of shoots/ explants	Average height of shoots/explants
Greenhouse	*in vitro*		
Darkness	Photoperiod	0.62 ± 0.36a	1.19 ± 1.08a
	Darkness	0.59 ± 0.34a	1.52 ± 1.40a
Photoperiod	Photoperiod	0.42 ± 0.27b	0.61 ± 0.58b
	Darkness	0.27 ± 0.27c	0.36 ± 0.49c

Different letters indicate significant differences ($p < 0.05$). Values represent means ± standard error of mean.

The response time for the induction of new shoots under *in vitro* conditions was variable; for the explants that came from darkness conditions it initiated after eight days (Figure 1C), while for the explants that came from photoperiod

it began only after 20 days. Adding the above mentioned the darkness pretreatment under greenhouse conditions stimulated the formation of roots (until a 36 %), and the foliar lamina of the shoot developed under *in vitro* conditions. However, explants that came from photoperiod under greenhouse conditions did not show this response; on the contrary, abundant callus was formed affecting the development of new shoots.

On the other side, Gonzáles & Salazar (1984), obtained better rooting values (70 %) with partial light (6.76 μmol.m^{-2}.s^{-1}) in *Persea americana* cv. *americana* belonging to the Antillean race.

Statistical analysis showed that an induction of shoots was not influenced by combination of growth regulators ($p < 0.05$) (Table 4). For height variable, differences among hormonal treatments were found; highest values were registered for MS basal medium supplemented with 1 mg.L^{-1} of BAP and 0.5 mg.$L^{-1}$of GA_3 (1.74 cm in average).

Table 4. Effect of growth regulators in the induction and height of shoots cultivated *in vitro*

Treatment	Average number of shoots/explants	Average height of shoots/explants
T1 (Control)	0.63 ± 0.35a	1.48 ± 1.29ab
T2 (BAP 0.5 + GA_3 0.5 mg.L^{-1})	0.65 ± 0.33a	1.54 ± 1.27bc
T3 (BAP 1 + GA_3 0.5 mg.L^{-1})	0.58 ± 0.27a	1.74 ± 1.44b
T4 (BAP 1.5 + GA_3 0.2 mg.L^{-1})	0.54 ± 0.36a	1.63 ± 1.49ab
T5 (BAP 0.5 + IBA 0.5 mg.L^{-1})	0.60 ± 0.37a	0.92 ± 0.9a
T6 (GA_3 0.2 mg.L^{-1})	0.51 ± 0.35a	1.19 ± 1.19ab
T7 (BAP 0.5 mg.L^{-1})	0.71 ± 0.38a	1.01 ± 0.92ac

Different letters indicate significant differences ($p < 0.05$). Values represent means ± standard error of mean.

Effect of different culture media and growth regulator combinations in shoot multiplication and development

When the shoots developed from nodal segments were subcultured in the MS medium, phenolization was observed in 40 % of the explants; on the contrary, in the subcultures with WPM media, phenolization was not observed; this agrees with the results obtained with MS basal media in other avocado varieties (Dalsaso & Guevara, 1998; Barceló-Muñoz, Encina, Simón-Pérez & Pliego-Alfaro, 1999; Nhut et al., 2008).

Although no significant differences for the height variables between treatments and control were found during the subculture stage, in this last one, explants reached a higher height in average, i.e. 5 cm and 4 cm in the MS and the WPM media, respectively. In the following subcultures the height reached in these apical sprouts was lower for all treatments (Figure 2A and 2B). These results agree with what has been reported by Rohim, Wanas, Abou-Aziz, El-Hamid & Abd (2013), for the "Hass" cultivar; however, for this last one a growth decrease was found only in the sixth subculture (from 3.8 cm to 1.47 cm).

Formation of new shoots under *in vitro* conditions was influenced by the type of explant, being the nodal explants the ones that showed the highest number of new shoots (Figure 1D). However, the elongation of these until they reached 4 cm lasted in average 60 days, and this in turn affected the multiplication period estimated in 45 days.

In this work, combination of growth regulators evaluated did not show significant differences for multiplication rates. However, the BAP and IBA combination was selected, both with 0.5 mg.L^{-1}, aiming at achieving in the lowest period of time the minimum height required, and at the same time, guarantee the survival during propagation stage (Figure 3).

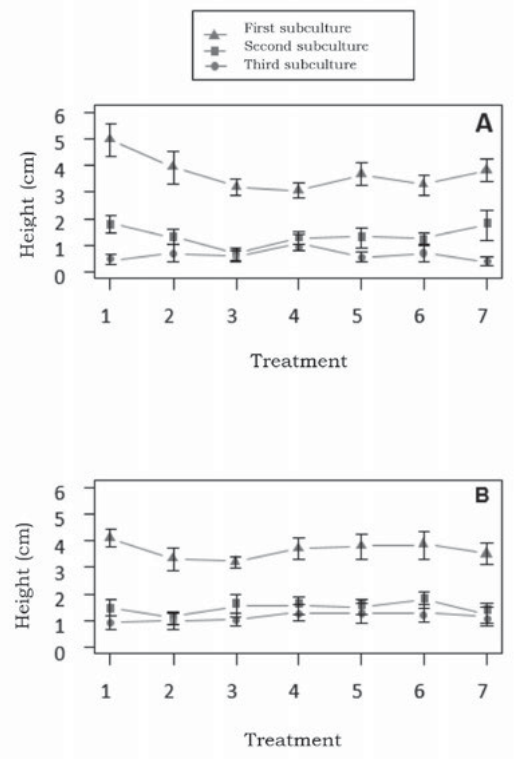

Figure 2. Height reached by the apical explants during three subcultures in different treatments with growth regulators and the control. **A.** In the MS medium **B.** In the WPM medium

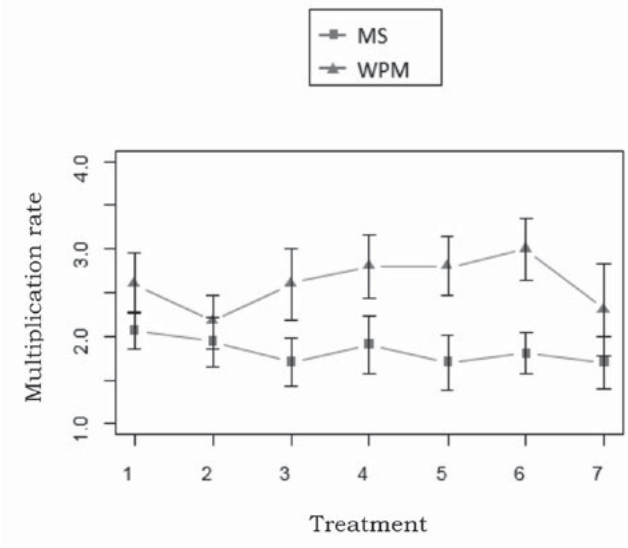

Figure 3. Multiplication rate in the MS and in the WPM media with different growth regulator combinations and control

Ex vitro acclimatization or hardening

In this phase a survival percentage of 82 % was achieved in *ex vitro* conditions, and showing an active growth (Figure1F). Average height reached after three months was 7.51 cm; likewise, an increase in number of leaves, roots and shoots per plant were also found (Table 5).

Table 5. Survival percentage, height averages, number of leaves, roots and shoots of rooted vitroplants after 90 days of hardening

	Survival %	Average height	Average leaves	Average roots	Average shoots
Vitroplants	82.0 (74-89)	7.5 ± 1.0	3.1 ± 1.1	1.2 ± 0.6	1.2 ± 0.6

Values in parenthesis indicate bootstrapping confidence intervals at 95 % confidence. Values represent means ± standard error of mean.

Discussion

In this study explants that were cultivated under photoperiod in greenhouse and maintained *in vitro* with the same light conditions, a higher phenolization percentage was found. Conversely, explants that were under darkness in greenhouses had a lower phenolization percentage for the two light conditions under *in vitro* conditions. In the *in vitro* avocado cultivation, the release of phenolic substances is common when the tissue is hurt; these oxidized compounds can inhibit the enzymatic activity and in occasions, these can be lethal for plant development. This growth inhibition is more severe in species that contain high levels of tannins or other hydroxyphenols. Light conditions play a critical role in the production of these substances, whether increasing or offsetting production (Azofeifa, 2009). In this study the production was significantly reduced when donor plants were subject to darkness. Similar results were described for parent plants of avocado "West Indian" and "Colin V-33" patterns, where pretreatment with darkness have allowed the elimination of oxidation from explants (Solorzano, 1989). Nevertheless, Dalsaso & Guevara (1998), who worked with the cultivar "Fuerte" did not apply etiolation pretreatment; however, they described an oxidation of only 20% in explants. These results show that the phenolization responses of the explants compared to light conditions also depends on the genotype; therefore, the need to standardize the methodology in each cultivar.

On the other hand, significant differences in induction and plant growth stages of the new shoots under *in vitro* conditions were found, observing that under darkness conditions the highest height values were found. Highest number of shoots and height in the explants subject to etiolation, both *ex vitro* as well as *in vitro,* are due to de-etiolation; this process from growing under continuous darkness conditions to then growing under light conditions triggers some morphological and molecular changes. Some of these are: chlorophyll synthesis is activated; the photosynthetic apparatus is organized and pigments as anthocyanin are synthesized; and a large number of genes increase expression due to a higher transcription rate. Levels of mRNA of the rubisco enzyme, of the proteins that links chlorophyll a and b to the chloroplast of thylakoid membrane, and those of the nitrate reductase enzyme, among others, are strongly augmented (Azcón-Bieto & Talón, 2008).

Taking into account the above mentioned, the darkness pretreatment under greenhouse conditions stimulated roots formation, and foliar lamina of the shoots also developed under *in vitro* conditions. Rooting found only in darkness pretreated explants is due to fact that an etiolated tissue is less lignified, i.e. there is a higher auxine content due to the absence of IAA oxidases that probably helps ease rhizogenesis (Basuk & Maynard, 1987). According to what Wessels (1996) reported for the "Duke 7" cultivar, etiolation was necessary for rooting to occur in 20 % of the plants.

When shoots developed from nodal segments were subcultured in MS medium, phenolization was observed in 40 % of the explants. Favorable results obtained with the WPM basal medium are probably due to the lower nitrogen concentration found in both sources (NH_4NO_3 and $Ca(NO_3)2.4H_2O$), that is toxic for some tree species when incorporated in high concentrations, as

is the case of the MS culture medium. In this study, high phenolization followed by necrosis in explants cultivated in MS medium show the influence of concentrations of mineral salts on explant response.

Difference found in the number of new shoots induced can be explained by differences in the morphogenic potential, *i.e.* difference in the meristematic activity of nodes belonging to the same branch and plant, compared to its position. This also suggests a differential response of nodal segments in the same vitroplant. Likewise, and according to Zulfiqar *et al.* (2009), accumulation of inhibitors in the axillary buds can occur, and these reduce elongation.

Using plant growth regulators in this stage has demonstrated to be important to improve the multiplication rate. In this sense, Dalsaso and Guevara (1998) and Rodríguez *et al.* (1999), found in other avocado cultivars, the number and height of the new shoots was superior when benzoic acid (BA) alone or in combination with GA_3 was added to the basal culture medium.

Formation of new shoots under *in vitro* conditions was influenced by the type of explant, being the nodal explants the ones that showed the highest number of new shoots. This difference found in the number of new shoots induced can be explained by differences in morphogenic potential, i.e. difference in the meristematic activity of nodes belonging to the same branch and plant, compared to its position. This also suggests a differential response in nodal segments in the same vitroplant. Likewise, and according to Zulfiqar *et al.* (2009), accumulation of inhibitors in the axillary buds can occur, and these reduce elongation.

Given that a high rooting percentage was obtained in the multiplication phase and through subcultures (82.2 % in average), we decided to eliminate the rooting phase under *in vitro* conditions. This high rooting percentage was obtained in the three subcultures with the treatment that had 1 $mg.L^{-1}$ of BAP and 0.5 $mg.L^{-1}$ of GA_3 (Figure 1E). Although, in some studies gibberellins have been reported as being capable of inhibiting root production, in other species as *Prunus avium* (L.), there was an increasing in 85 % in root production when cuttings were treated with GA_3. The relation between this growth regulator and rooting can involve factors as IAA biosynthesis stimulation or the polar transport of this hormone. Furthermore, in some species it might be able to induce rejuvenation that is associated to plant capacity to produce roots (Ford, Taylor, Blake & Marks, 2002).

During the acclimatization phase, survival is of utmost importance due to light excess and low relative humidity in the *ex vitro* environment. In the essays carried out, the mechanism to control water loss in plants was to cover them with a plastic cup; moreover, the substrate used have allowed to maintain an adequate humidity in roots during the acclimatization period. In this regard, there are very few reports that describe micropropagation of avocado plants until the hardening phase with high survival rates. For the "Hass" cultivar, Rohim *et al.* (2013), described the use of a similar substrate to the one use in this work (sand:peat:vermiculite in a 1:2:1 proportion) but achieving a survival rate of only 48.4 %.

Conclusions

In this study *in vitro* propagation protocols through complete, effective and reproducible morphogenesis for avocado cv. "Hass" is described, obtaining high percentage of plants adapted to *ex vitro* conditions. This success is however achieved when mother plants under greenhouse conditions and explants under *in vitro* conditions receive a previous etiolation treatment that eliminates phenolization in explants during *in vitro* establishment, decreases callus formation and increases the number of shoots and their height.

The apical explants are not an explant to recommend in the multiplication phase since their height is restricted through the subcultures, on the contrary with the nodal segments a greater number of new shoots are obtained and a high percentage of rooting in the medium of culture added with BAP (1 mg.L-1) and GA3 (0.5 mg.L-1), making it possible to eliminate the phase of rooting in vitro. Effective control of phenolization in this last stage is achieved using the WPM basal medium instead of MS independent of the growth regulators used.

Massive plant production of avocado "Hass" cultivar that can be used as rootstock or scion and become the baseline for further work can be achieved by using the *in vitro* protocol assessed in this research.

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