In vitro formation of adventitious shoots on caulinary tissue of physiologically contrasting *Agave angustifolia* plants

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ABSTRACT

To micropropagate agave plants, somatic tissue is obtained from selected plants that are conditioned for 2 to 6 months to improve their physiological condition and health before *in vitro* cultivation. The objective of this study was to evaluate the physiological condition of *Agave angustifolia* plants in terms of its effect on organogenic response in somatic tissue taken from these plants when they are established in similar culture media. In a nursery, growth of four groups of plants was evaluated when they were subjected to different types of irrigation for seven months: 1) water; 2) NS-50% (fertigation with nutrient solution at 50% strength); 3) NS-75%; and 4) NS-100%. At the end of the period, it was found that supplying nutrients is important for plants to achieve better physiological condition. The unfertilized plants and those that received NS-75% had increases of 3.8 and 7.8 leaves, 6.5 and 12.5 cm in length of the largest leaf, and 1610.3 and 4401.4 cm² in leaf area. Stem tissue was obtained from these stock plants and cultured for 90 days in *in vitro* culture, and formation of adventitious shoots was assessed. The results showed that the magnitude of organogenesis in stem tissue for formation of adventitious shoots was positively related to the physiological condition of the stock plant. Explants taken from unfertilized stock plants formed 14.6 total shoots and 3.8 shoots on each explant, while those fertigated at 100% concentration of nutrients formed 32.7 total shoots and 8 shoots on each explant.

Keywords: Adventitious shoots; Caulinary tissue; Nutrient solution (NS); Organogenesis

INTRODUCTION

In the state of Oaxaca, Mexico, several species of cultivated and wild agaves are used as raw material for produce a distillate beverage named mescal. During the period 2006 to 2013, *Agave* plantations covered 13,572 ha. Most were mature crops, and so around four thousand ha were harvested yearly (SAGARPA, 2013). *Agave angustifolia* Haw. is economically the most important species. It occupies 77.4% of the area under agave, and is established at a density of 2000 plants/ha (CONABIO, 2006; Aguirre and Eguiarte 2013). In 2014 Oaxaca produced 2.7 million liters of mescal and exported 96.1% of the total production (Nolasco, 2015), which had a total value of U.S. $ 8.9 million.

Agave angustifolia has a production cycle that varies from seven to nine years (Antonio and Ramírez, 2008). It is

necessary to propagate approximately 3.7 million plantlets yearly to replace harvested plants. Because of the intensity the areas were harvested during 2013 to 2015, the state's agave inventory decreased notably. The descriptions of Arizaga and Ezcurra (2002) and García-Mendoza (2004) reveal that the plants used in plantations are obtained from shoots produced by the rhizome and from bulbils produced by the inflorescence.

Today, it is essential to satisfy the demand for quality plants to establish plantations, increment productivity and reduce production costs. Thus, the use of biotechnological tools, such as *in vitro* culture of plant cells, tissues or organs, PTC, is a complementary alternative to conventional methods. PTC takes advantage of the totipotency of plant cells to express their capacity to regenerate plants through embryogenesis or organogenesis (Fortes and Pais, 2000;

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Zeng et al., 2007). This technology permits designing intensive plant production systems with duly controlled propagation environment in which somaclonal variation is minimized (Fortes et al., 2010). The technique consists of aseptic culture of different explants in culture medium and incubation to induce cell division, organogenesis and plant development (Morales et al., 2009; Kehie et al., 2012; Siddique et al., 2015). For this purpose, it is necessary to select plants in the field that are outstanding for their productivity and harvest quality to obtain clonal populations for establishment in plantations (Domínguez-Rosales et al., 2008a; Koné et al., 2013; Sagare and Mohanty, 2015).

Since 1986, research in Oaxaca has been conducted to develop a methodology of *in vitro* propagation of A. angustifolia. In 1987, the first 12 thousand micropropagated plants were established in a nursery, and in 1988 in the field. Now, we have a methodology that is technically viable but can be improved. Data have been published regarding the stages of propagule multiplication: shoot rooting in preparation for transplanting to soil (Enríquez-del Valle et al., 2005), acclimatization of plants in the greenhouse (Enríquez-del Valle et al., 2009), and development in the nursery (Enríquez-del Valle et al., 2012). However, during stage I of the methodology, establishment of aseptic cultures, at least 50% of the explants are contaminated by bacteria and fungi. Mroginski and Flaschland (2010) mention the difficulty of quantifying the impact of contamination, but they estimate that, on average, in laboratories dedicated to micropropagation, around 10% of the cultures are lost. It is of utmost importance to establish aseptically the highest percentage possible of explants that are viable for cell division and morphogenesis to occur.

The physiological condition of the stock plant influences the morphogenetic capacity of its somatic tissues when they are established *in vitro* (Styer and Chin, 1984). For this reason, it is recommended that in the nursery the stock plant, from which somatic tissues are to be extracted, be subjected to a treatment to improve its nutrient and physiological condition before tissue extraction for *in vitro* culture. Therefore, to implement Stage I, a high percentage of explants in *in vitro* culture must be aseptic but also viable to assume cell division and morphogenesis (Niubó et al., 2004). In this regard, there are no data on *A. angustifolia* Haw. and for this reason the objective of this study was to evaluate the effect of the physiological condition of *Agave angustifolia* Haw. stock plants on the organogenic response of their somatic tissues when they were established under similar conditions of culture medium. And the hypothesis was that the physiological condition of the stock plant from which the explants were obtained had effect on the stem tissue capacity to assume a process of organogenesis.

MATERIALS AND METHODS

The study consisted of two stages, each with its respective experiment. Experiment 1 evaluated growth and condition of the stock plants subjected to different nutrient supplies in the nursery. Experiment 2 assessed the organogenic response *in vitro* of explants obtained from stock plants that exhibited different growth vigor in the nursery.

Experiment 1. Evaluated growth and condition of the stock plants

Experiment 1 initiated with 40 plants obtained from bulbils from *A. angustifolia* Haw. inflorescences. The plants were 18 months old and had been established in the nursery in individual 4 dm³ pots with sandy loam textured soil. Four groups were prepared, each of which received a different irrigation treatment twice a week for seven months. Treatment 1 was irrigation with only water. The other treatments were nutritive solution (NS) at different dilutions of the Steiner formulation (1984): Treatment 2) NS-50%, Treatment 3) NS-75%, and Treatment 4) NS-100%. The NS-100% contains in mg/L: 166.42 N, 30.68 P, 276.44 K, 182.34 Ca, 49.09 Mg, 111.15 S, 1.25 Fe, 0.21 Mn, 0.025 Zn, 0.076 B, 0.005 Cu y 0.021 Mo. Also, all of the groups were sprayed with N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide fungicide (Bayer®) and a systemic antibiotic agricultural terramycin, 5% oxitetracyclin (Zoetis®), twice a week. After seven months under these conditions, the plants were harvested, and height and number of leaves were quantified and leaf area determined with a Laser Area Meter (CID Bio-Science, Copyright ©2016 CID Bio-Sciencie Inc., Camas, WA, USA). Root and leaf volume (cm³) were determined by submerging them in a known volume of water in a graduated test tube. Leaf and root fresh weight (g) were determined on a triple bar balance (IROSA® 700 PPW, Mexico) with 0.1 g precision. The leaves and roots were then placed separately in labeled paper bags and dried in a convection oven (Felisa® Fabricantes Feligneo, S. A. de C.V. Mexico) for two weeks at a temperature of 65 °C. After this time, leaf and root dry weights were determined on a balance. The experiment was established under a completely randomized design. The experimental unit was one plant, and there were 10 replications per treatment. The data were subjected to analysis of variance and comparison of means.

Experiment 2. Organogenesis in stem tissues

The 40 stock plants that in experiment 1 were separated into groups of 10 subjected to different dosages of fertigation were harvested to evaluate their leaves and root. The stem was taken to the laboratory and superficially disinfected using the following sequence: 1) rinsing with running water for 5 min; 2) immersion and

washing in soapy water for 15 min, 3) immersion in a 2.5 g/L solution of N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide fungicide (Bayer®) for three min; 4) three rinses with distilled sterilized water; 5) immersion for 2 min in 90% ethanol; 6) immersion for 15 min in a 0.6% solution of sodium hypochlorite; 7) five rinses with distilled sterilized water. Step four and thereafter of the disinfection process were conducted in aseptic conditions in a horizontal laminar flow bell. The disinfected stems were placed in sterilized glass Petri dishes of 10 x 100 mm and, with sterilized forceps and scalpel, cut into 1.5×1.5 cm and 0.3 cm thick fragments. Each fragment was placed in a 145 cm^3 culture recipient containing 20 mL sterilized geled culture medium prepared with MS (Murashige and Skoog, 1962) mineral salts, 1 mg/L thiamine-HCl, 100 mg/L de myo-inositol, 30 g/L sucrose, 1.5 mg/L N^6- bencylaminopurine, 1 mg/L indol-3-acetic acid, 40 mL/L coconut milk, 5.6 g/L agar, and 250 mg/L chloramphenicol. After establishing the stem tissues *in vitro*, the recipients were closed with a polypropylene top and sealed with adherent polyethylene. The cultures were placed in the area of incubation, exposed to white fluorescent lighting 35 μmol/m2/s in 16 h photoperiods/8h darkness with temperature ranging from 15 to 29 ºC. The number of tissue segments (explants) obtained from each stem was counted. During the incubation period, qualitative changes occurring in the tissue fragments in the culture media were observed: cell proliferation (callus), change in pigmentation, and shoot formation. After the eleven weeks, the number of viable uncontaminated stem segments achieved were quantified, as well as the total number of adventitious shoots and the average number of shoots per stem segments (explants) obtained from one stock plant. The experiment was established according to a completely randomized design. The experimental unit was one stem obtained from each stock plant, with 10 replications per treatment. The variables contaminated explants, viable uncontaminated explants, and percentage of contamination were transformed to *log10(x)* to comply with the assumptions of normality and homogeneity of variances. The data were subjected to analysis of variance and comparison of means (Tukey, 0.05). The statistical analysis routines of each experiment were performed using SAS® Statistical software, version 9.0.

RESULTS

The substrate was sandy loam in texture so this did not supply enough nutriments to the plants. The physiological condition of the stock plants was determined by the magnitude of their growth, observing that the nutrient supply did influence plant growth; fertigated plants grew larger than unfertilized plants. Later, it was observed that the condition and different growth vigor of the stock plant did indeed affect the magnitude of organogenic response of the *in vitro* cultured stem tissue obtained from these plants.

Experiment 1. Condition of stock plants in response to the level of nutrient supply in the nursery

Initially, the *Agave angustifolia* Haw. plants had 14 to 20 leaves and were 25 to 35 cm tall. After seven months, the plants supplied with different concentrations of nutrients exhibited different sizes in accord with the dosages of fertigation they received. In our study, the analysis of variance showed that the dosage of fertigation had significantly different effects (P ≤ 0.05) on leaf number and length, root and leaf volume, and monthly root and leaf volume increase, and a highly significant effect (P≤ 0.01) on leaf area (Table 1).

The plants that received 75% nutrient solution, compared with those that did not receive NS, had increments of 7.8 and 3.8 in total number of leaves and 10.5 and 6.5 cm in length of largest leaf, 1136.1 and 695.5 cm^3 leaf volume, and monthly increases in leaf volume of 162.3 and 99.3 cm^3 (Table 2). These values were statistically different (Tukey, 0.05). Most of these characteristics in plants fertigated with 100% NS did not show additional growth, relative to the plants supplied with 75% NS; the exception was leaf area (4401.4 cm^2), which was 2.73 time larger and significantly different (Tukey, 0.05) than the leaf area of unfertigated plants. It was also observed in all of the groups that the plants began rhizome shoot development with 1.6 to 2.0 shoots per plant, numbers that were not significantly different (Tukey, 0.05) among groups.

Experiment 2. Organogenesis in stem tissue

Data on time and percentage of contamination reveal that 75.6% of the explants from unfertigated *A. angustifolia* stock plants became contaminated during the first 9.6 days of incubation. In contrast, 69.5% of the explants from stock plants that received 75% NS became contaminated during the first 6.2 days of incubation.

During the organogenesis process of *A. angustifolia*, the explants established *in vitro* were initially cream colored and smooth. In the first 8 to 15 days of incubation, the explants increased in size by 10%, relative to their initial size, and their surfaces acquired a granular texture. Also, as of day 15 up to day 40 of incubation, the explant showed beginnings of green coloring, initially pale green and changing gradually to lime green. On day 50 small shoots could be observed. The analysis of variance showed that the condition of vigor of *A. angustifolia* stock plants that received different dosages of nutrient solution had a

Table 1: Summary of the analysis of variance of the characteristics of *Agave angustifolia* Haw. plants fertigated with different concentrations of nutrients for seven months in the nursery

SV[a]	DF[b]	Mean Squares and significance					
		IL[c]	LL	DL	NS	RV	VL
CSn	3	29.2*	73.3*	0.4ns	0.3ns	56444.4*	470643.9*
Err	36	10.7	24.1	0.3	2.5	17049.0	132153.3
Total	39						
Sv	DF	Mean Squares and significance					
		MIVL	LFW	MILW	RFW	MIRW	LA
CSN	3	9605.0*	91309.9ns	1863.4ns	31205.8ns	636.8ns	5587267.9**
Error	36	2697.0	378846.4	7731.5	11711.7	239.0	566153.0
Total	39						

[a]SV: source of variation, CSn: solution concentration. [b]DF: degrees of freedom. [c]IL: increase in number of leaves, LL: length of largest leaf, DL: diameter of the largest leaf, NS: number of shoots, VR: root volume, MIVR: monthly increases in root volume, VL: leaf volume, MIVL: monthly increases in leaf volume, LFW: leaf fresh weight, MILW: monthly increases in leaf weight, RFW: root fresh weight, MIRFW: monthly increases in root fresh weight, LA: leaf area. [d]ns: non-significant ($P>0.05$), *: significant effects ($P \leq 0.05$), **: highly significant effects ($P \leq 0.01$) (Tukey test, $P<0.05$)

Table 2: Characteristics of *Agave angustifolia* Haw. plants fertigated with different concentrations of nutrients for seven months in the nursery

	Treatments, dilutions (%) of Steiner nutrient solution[b]			
Variable[a]	Water	50	75	100
IL	3.8±1.7 b	6.6±4.4 ab	7.8±4.3 a	5.4±1.0 b
LL (cm)	6.5±4.0 b	7.6±3.8 b	10.5±6.9 a	12.5±4.1 a
DL (cm)	2.2±1.3 a	2.0±0.6 a	2.5±0.3 a	2.3±0.6 a
NS	1.7±53.8 a	1.6±1.2 a	1.6±1.5 a	2.0±2.0 a
RV (cm³)	142.8±53.8 b	285.0±140.3a b	269.9±154.6 ab	312.0±147.2 a
MRV (cm³)	20.4±7.6 b	40.7±20.0 ab	38.5±22.0 ab	44.5±21.0 a
LV (cm3)	695.5±318.1 b	714.0±355.9 ab	1136.1±476.9 a	999.0±270.5 ab
MLV (cm³)	99.3±45.4 b	102.0±50.8 ab	162.3±68.1 a	142.7±8.6 ab
LFW (g)	1145.7±525.2 a	1220.0±754.6 a	1339.1±747.2 a	1129.4±334.0 a
MILFW (g)	163.6±75.0 a	174.2±107.8 a	191.3±106.7 a	161.3±47.7 a
RFW (g)	142.1±50.0 b	175.1±118.8 ab	208.8±113.1 ab	272.9±131.9 a
MIRFW (g)	20.3±7.1 b	25.0±16.9 ab	29.8±16.1 ab	38.9±18.8 a
LA (cm²)	1610.3±1008.5 b	2342.3±218.0 b	2735.3±540.7 b	4401.4±952.5 a

[a]IL: increase in number of leaves, LL: length of largest leaf, DL: diameter of largest leaf, NS: number of rhizome shoots, RV: root volume, MRV: monthly increase in root volume, LV: leaf volume, MLV: monthly incrase in leaf volume, LFW=leaf fresh weight, MILFW: monthly increase in leaf fresh weight, RFW: root fresh weight, MIRFW: monthly increase in root fresh weight, LA: leaf area. [b]In each column, values with the same letter are not statistically different (Tukey test, $P<0.05$), mean±standard deviatio.

Table 3: Summary of the analysis of variance of *in vitro* shoot formation on stem tissue obtained from *Agave angustifolia* Haw. stock plants that received different nutrient supply in the nursery

SV[a]	DF[b]	Mean Squares[c] and significance[d]					
		EC	UCVE	TSVE	ASVE	DC	DS
CSn	3	0.03 ns	0.20ns	812.5**	32.0*	21.0 ns	2524.8**
Error	36	0.06	0.19	160.8	11.0	7.8	406.5
Total	39						

[a]SV: source of variation, CSn: concentration of nutrient solution. [b]DF: degrees of freedom. [c]EC: contaminated explant, UCVE: uncontaminated viable explant, TSVE: total number of shoots in viable explant from each donor plant, ASVE: Average number of shoots on each viable explant, DC: days to appearance of contamination, DS: days to appearance of shoots. [d]ns: non-significant F value ($P>0.05$), *: significant F value ($P \leq 0.05$), **: highly significant F value ($P \leq 0.01$) (Tukey test, $P<0.05$)

significant effect ($P \leq 0.05$) on stem tissue capacity for organogenesis, formation of adventitious shoots, and a highly significant effect ($P \leq 0.01$) on total number of shoots formed on the viable explants obtained from each mother plant and on days to shoot formation (SF) (Table 3).

Stem tissue in the culture medium exhibited different levels of organogenic response, depending on the physiological condition of the stock plants from which they were obtained. Comparing explants obtained from unfertigated stock plants with those from plants fertigated with 100% nutrient concentration, of the total viable explants, 14.6 and

52

Emir. J. Food Agric ● Vol 30 ● Issue 1 ● 2018

Table 4: Percentages of contamination, viable explants, total number of shoots, and average number of shoots on *in vitro* cultured explants from stem tissue obtained from *Agave angustifolia* that received different nutrient supply in the nursery

NS (%) [a]	ExpO[b]	CExp	DOS	DC	TSE	SE
Water	37.6±10 a[c]	28.0±1.3 a	84.5±17.7 a	9.6±4.0 a	14.6±7.3 b	3.8±3.3b
NS-50%	35.5±11.2 a	25.1±15.2a	57.5±23.0 b	7.7±2.9 ab	20.2±10.3 ab	4.8±3.7ab
75%	29.7±13.7 a	11.5±8.2 a	47.7±18.5 b	6.2±1.7 b	32.3±14.0 a	4.2±2.4ab
100%	30.2±10.2 a	25.2±12.2a	56.8±20.9 b	7.0±1.6 ab	32.7±16.8 a	8.0±3.5 a

[a]NS: nutrient solution dilution. [b]ExpO: explants obtained from each plant, [c]Exp: contaminated explants, DOS: days to observation of shoots, DC: days to occurrence of contamination, TSE: total shoots on the explants from one plant, SE: shoots on each explant. cIn each column, means with the same letter are not significantly different (Tukey test, P<0.05), means are ± the standard deviation

32.7 shoots were formed, visible at 84.6 and 47.7 days of *in vitro* culture, respectively. In both cases, the differences were significant (Tukey, 0.05) (Table 4).

Nutrient supply to stock plants in the nursery had an effect on their physiological condition, evaluated by several characteristics of size and later, during *in vitro* culture, by the number of shoots formed on the explants. Table 4 shows that differences between the explants obtained from each of the unfertigated stock plants and those from plants fertigated with 100% NS were significant (Tukey, 0.05): a total of 14.6 *vs* 32.7 adventitious shoots and averages of 4.5 and 8 shoots per explant. There was a positive relationship between formation of a larger number of total shoots and average number of shoots per explant and the dosage of fertigation that the stock plant received in the nursery.

DISCUSSION

Experiment 1. The results show that agave plants supplied with an appropriate quantity of nutrients reached bigger size than the unfertilized plants, coinciding with (Enríquez-del Valle et al. 2000, 2009, 2013). According to Zúñiga (2013), the nutrients form part of the plant's metabolic compounds and increase their capacity for photosynthesis and synthesis of structural compounds, thus increasing rates of formation and growth of their organs.

In our study, after seven months of fertigation, the plants were of different sizes, positively related to the quantity of nutrients they received. This is in agreement with data reported by Enríquez-del Valle et al. (2016), who demonstrated the positive effect of fertigation on growth of *A. potatorum* plants to which Steiner (1984) NS was administrated at different dilutions (1, 20, 40, 60, 80 and 100%). Plants fertigated at 1% NS increased the number of leaves by 9.8 and leaf area, LA, by 125.53 cm², while those fertigated at 100% increased the number of leaves by 17 and leaf area by 392.35 cm². The data obtained on leaf area, in agreement with Warnock et al. (2006), suggest that the magnitude of LA has a positive relationship to nutrient supply and, in turn, LA is related to the capacity to intercept photosynthetically active radiation (PAR) and fixing CO_2 to synthesize carbohydrates and accumulate biomass.

Experiment 2. The evidences show the difficulty in obtaining high percentages of aseptic cultures during the first stage of *in vitro* culture of *Agave* stem tissue since the tissues are close to the substrate and to populations of microorganisms, originating a high degree of endogenous and exogenous contamination. For this reason, for this type of plant some of the management conditions should be modified in the nursery stage, as described by Deberg and Maene (1985). However, when the stage of establishing aseptic cultures *in vitro* is over and explants that responded to organogenesis stimulus are obtained, in the next stage of propagule multiplication it is expected that the levels of contamination decrease drastically and the number of shoots on each explant increase notably. During the *in vitro* establishment of aseptic cultures of node segments of the woody species *Legrandia concinna* (Phil.), Uribe-Moraga and Cifuentes (2004) reported that of all explants, 81% died and 19% were viable. In species such as *Musa* spp, during *in vitro* establishment of aseptic cultures, Ortega et al. (2012) obtained 6.6% contaminated and 93.4% aseptic cultures when they tested six methods of disinfecting explants. Niubó et al. (2004) described a methodology for decontaminating sugarcane of endophyte and exophyte microorganisms in *in vitro* culture, combining a surface-action biocide with a broad-spectrum systemic biocide. They obtained 90% of the explants free of exophyte contamination by using mercury chloride (0.005 % for 10 min) and 92% explants free of endophyte contamination three months after removing gentamicin sulfate (30 mg/L for 14 d). Rache and Pacheco (2010) applied fungicides to explant donor *Vaccinium meridionale* plants to reduce levels of contamination in the stage of aseptic culture establishment *in vitro*. When the explant donors were not sprayed with fungicides, 80-100% of the *in vitro* explants became contaminated, while only 20% of the explants from sprayed donors became contaminated.

In our study explants obtained from nursery stock plants with the highest nutrient supply formed more shoots in a shorter time than the explants obtained from unfertigated plants or plants supplied with nutriments under the optimum level. No previous studies had shown differences in *in vitro* organogenic response of explants from *Agave* plants that varied in physiological condition. It is much

Emir. J. Food Agric ● Vol 30 ● Issue 1 ● 2018

53

more frequent to find assessments of variations in the composition of the culture media, mostly of growth regulators. Thus, in *A. tequilana* different number of shoots were formed from explants that were subjected to pulses that consisted of different concentrations (2.3, 4.5, 6.8 and 9.0 µM) of 2,4-D during different periods (one, three, and six days) as a step prior to establishing them in culture media with 0.113 µM 2,4-D plus 4.44 µM BA (Ramírez-Malagón et al., 2008). Aureoles-Rodríguez et al. (2008) assessed shoot formation of two types of explants, tissue from stem and axillary buds of *Agave inaquidens*, which were established in culture media that varied in type and concentration of cytokinins. They proved that formation of the highest number of shoots occurred from stem tissue established in culture media with 3 mg/L cytokinin benzyladenine. Domínguez-Rosales et al. (2008b) also varied the type and concentration of cytokinins for *in vitro* propagation of *Agave cupreata*, *A. difformis*, *A. karwinskii*, *A. obscura* and *A. potatorum*. As explants they used meristematic tissue extracted from plantlets obtained from seeds germinated *in vitro*. The explants from *A. cupreata* and *A. karwinskii* established in culture medium with 1.5 and 1 mg/L BA formed 10.5 and 6.1 shoots per explant, respectively. *A. difformis* and *A. obscura* formed 8.5 and 11 shoots per explant in medium with 0.2 mg/L TDZ, while *A. potatorum* formed 6.9 shoots in medium with 3 mg/L kinetin. Valenzuela-Sánchez et al. (2006) described the proliferation of *A. tequilana* shoots via indirect organogenesis, evaluating explants from leaf segments and stem meristem tissue. The greatest proliferation of shoots occurred in three months from meristem segments and calluses established in culture medium with 1.1 µM 2,4-D and 44 µM 6-benzylaminopurine (BAP).

With *Vaccinium meridionale*, Rache and Pacheco (2010) obtained up to 4.5 shoots per viable explants from caulinary apexes of adult plants established in culture medium with different concentrations of mineral salts. To propagate four varieties of *Vigna subterranea* (L.) Verdc., Koné et al. (2013) used explants from cotyledons established in culture media with different types and concentrations of growth regulators, achieving 20 to 30% viable explants on which an average of six to ten shoots were formed in culture media with 3 mg/L benzylaminopurine alone or combined with 0.5 mg/L α-naftalenoacetic acid.

Sánchez-Hernández (2009), working with *in vitro* propagation of squash found differences in the level of organogenic response due to genotype; explants of *Cucurbita pepo*, variety round zucchini, produced 2 shoots per explant, and *Cucurbita maxima*, variety Brujita, produced 2.7 shoots after 22 days in similar culture medium. Rosa-Carrillo et al. (2012) evaluated the capacity of regeneration and *in vitro* propagation of 14 species of *Turbinicarpus* (Cactaceae).

The efficiencies per species in a single proliferation cycle of 50 to 60 days were from 4.0 shoots per explant in *T. hoferi* and up to 26.3 per explant in *T. pseudomacrochele* subsp. lausseri. Martínez-Palacios et al. (2003) assessed indirect organogenesis in *A. victoriae reginae*, in which regeneration of multiple shoots was induced from axillary buds from stem segments cultured in MS medium with 2,2-4,4 µM BA. Arzate-Fernández and Mejía-Franco (2011) used zygotic embryonic axes from mescal agave (*Agave angustifolia* Haw.) as explants and achieved formation of 40 to 216 somatic embryos *in vitro*. Summarizing, the results obtained in our study and in those we mentioned conducted with diverse species show that levels of organogenic response depend on several factors: species, genotype within the species, type of explant, physiological condition of the stock plant, composition of the culture medium and physical conditions of incubation, coinciding with Pierik (1987), Domínguez-Rosales et al. (2008a), Ramírez-Malagón et al. (2008).

The process of organ formation on somatic tissues was described in *Nicotiana* (Thorpe and Murashige, 1968; Bertrand-Garcia et al., 1992) and Pinus sp. (Villalobos et al., 1984; Thompson and Thorpe, 1997). The process occurs through a sequence of events: parenchima cells are induced to division to form groups of dediferenciated cells, meristemoids formation, meristems and shoots. This process consume a great quantity of energy and occurs active synthesis of nucleic acids, and proteins. At present work the differences in magnitude of organogenesis in *Agave angustifolia* explants cultured *in vitro* probably could be affected by the physiological condition of the stock plants from which these were taken.

CONCLUSIONS

Agave angustifolia plants that were fertigated in the nursery for seven months had better growth than unfertigated plants. Plant vigor was positively related to the quantity of nutrients received in fertigation. When stem tissue explants taken from the more vigorous plants were established *in vitro*, they produced a larger number of adventitious shoots in a shorter time than explants taken from the less vigorous plants.

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Authors' Contributions
S.C.R.R and J.R.E.V: Growth of nursery plants, application of fertilization doses and tissue culture

technique. G.R.O: Experimental design planning and statistical analysis of data. J.R.L and V.A.V.V: Revision and writing design.

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Emir. J. Food Agric ● Vol 30 ● Issue 1 ● 2018

55

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56

Emir. J. Food Agric ● Vol 30 ● Issue 1 ● 2018