

Vaccinium corymbosum shoot growth in culture media with different inorganic salts and pH

Crecimiento de brotes de *Vaccinium corymbosum* en medios de cultivos con diferentes sales inorgánicas y pH

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ABSTRACT

In vitro shoot proliferation of *Vaccinium corymbosum* var. Biloxi was evaluated in culture media that varied in inorganic salts and pH levels. Stem segments 2 cm long that had only axillary buds, and other segments had apex and axillary buds, were established on culture media containing inorganic salts WPM50 % (Woody Plant Medium), MS50 % (Murashige and Skoog) and the combination MS50 %-WPM50 % at different pH levels (4.5, 5.0, and 5.5), and contained 25 g L⁻¹ sucrose, 100 mg L⁻¹ myo-inositol, 0.4 mg L⁻¹ thiamine-HCL, 2 mg L⁻¹ 2iP, 0.5 mg L⁻¹ pyridoxine, 0.5 mg L⁻¹ nicotinic acid, 2 mg L⁻¹ glycine, 5.7 g L⁻¹ agar. The variables shoot length, number of leaves, and number of shoots were evaluated at 40, 80, and 120 days of incubation. The experiment was set up in a completely randomized design, with a 3×3×2 factorial arrangement. Stem segments established in culture media with MS50 %-WPM50 % inorganic salts and pH levels 4.5 or 5.0, developed axillary shoots that were larger (5.5 and 5.8 cm) and number of leaves (11.5 and 11.8).

KEY WORDS: Blueberry, micropropagation, MS inorganic salts, WPM inorganic salts.



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RESUMEN

Se evaluó la proliferación *in vitro* de brotes de *Vaccinium corymbosum* var. Biloxi, en medios de cultivo que variaron en sales inorgánicas y niveles de pH. Segmentos de tallo de 2 cm de longitud que solo tenían yemas axilares, y otros segmentos tenían ápice y yemas axilares, se establecieron en medios de cultivo con sales inorgánicas WPM50 % (Woody Plant Medium), MS50 % (Murashige y Skoog) y la combinación MS50 %-WPM50 % a diferentes niveles de pH (4.5, 5.0 y 5.5), y contenían 25 g L⁻¹ de sacarosa, 100 mg L⁻¹ de myo-inositol, 0.4 mg L⁻¹ de tiamina-HCl, 2 mg L⁻¹ 2iP, 0.5 mg L⁻¹ de piridoxina, 0.5 mg L⁻¹ de ácido nicotínico, 2 mg L⁻¹ de glicina, 5.7 g L⁻¹ de agar. Se evaluaron las variables longitud de brote, número de hojas y número de brotes a los 40, 80 y 120 días de incubación. El experimento se estableció de acuerdo con un diseño completamente aleatorio, con arreglo factorial 3×3×2. Los segmentos de tallo que se establecieron en medios de cultivo con sales inorgánicas MS50 %-WPM50 % y con niveles de pH 4.5 o 5.0, desarrollaron brotes axilares que fueron mayores en tamaño (5.5 y 5.8 cm) y número de hojas (11.5 y 11.8).

PALABRAS CLAVE: Arándano, micropropagación, sales inorgánicas MS, sales inorgánicas WPM.

Introduction

Blueberry (*Vaccinium* sp.) cultivation has gained importance worldwide due to its cultural, nutritional, and economic value (Hine-Gomez & Abdelnour-Esquivel, 2013; Chen *et al.*, 2018; Wang *et al.*, 2019; Georgieva & Kondakova, 2021). The main producing countries worldwide are the United States, Canada, Chile, Peru, and Spain (Zarate *et al.*, 2017; USDA, 2021). Mexico ranks sixth in blueberry production, in an area of 11,400 ha, and during 2021, 66,482 t were produced. The states with the highest production are Jalisco, Michoacán, and Sinaloa (SIAP, 2022). Although there are no production records in Oaxaca, there are regions with appropriate soil and climatic conditions for its cultivation. To ensure its productive success, it is necessary to have local producers of plant material to establish commercial orchards with plants that have genetic, morphological, physiological, and phytosanitary quality, such material is produced using the plant tissue culture technique that allows the production of a large number of genetically uniform and pathogen-free plants. Thus, it is essential to generate a massive plant propagation protocol from stock plants or ortets of successful varieties in Oaxaca.

The factors that influence the *in vitro* growth and development of an explant are: genotype, health, and physiological condition of the ortet plant, culture medium composition, and incubation environment (Greenway *et al.*, 2012; Bhojwani & Dantu, 2013). Culture media are composed of water, carbohydrates, growth regulators, vitamins, and essential mineral nutrients specific to each plant species and propagation stage (George & Klerk, 2008; Greenway *et al.*, 2012). The availability of essential elements is critical and is determined by the pH and osmotic potential of the growing medium resulting from the source, concentration, and total ionic strength of the growing medium (Bonga & Durzan, 1987; Morard & Henry, 1998; Molinos-da Silva *et al.*, 2004). Woody Plant Medium, WPM (Lloyd & McCown, 1980; Wolfe *et al.*, 1983) has a low ion concentration (42.39 mM L^{-1}) and is one of the most commonly used formulations for *in vitro* blueberry propagation. The MS culture medium (Murashige & Skoog, 1962) whose ion concentration is 94.25 mM L^{-1} , was designed for tobacco (*Nicotiana tabacum* L) tissue cultures, and is frequently used because it has proven to be efficient for cell multiplication, morphogenesis, and multiplication of propagules of different species (George *et al.*, 2008; Martínez-Villegas *et al.*, 2015), *Agave angustifolia* Haw, *A. potatorum* Zucc, *A. fourcroydes* Lem, *A. tequilana* Weber, *A. grijalvensis* B. Ullrich, *A. americana* var. *oaxacensis* Gentry (Enriquez-del Valle *et al.*, 2018), *Hylocereus monacanthus* (Lem) Britton & Rose (Montiel-Frausto *et al.*, 2016), *Laelia halbingeriana* Salazar & Soto Arenas (García-González *et al.*, 2020). Blueberry is considered a calcifuge plant, since the optimum level of *in vitro* growth of blueberry shoots ranges with pH from 4.5 to 5.5 (Retamales & Hancock, 2012), while for *in vitro* culture of other species such as: *Beaucarnea inermis* (S. Watson) Rose the pH of the culture medium is 5.7 (Guillén *et al.*, 2015), and for *Persea americana* Mill (Ibarra-López *et al.*, 2016), *Agave potatorum* Zucc (Enriquez-del Valle *et al.*, 2016), *Echinocactus platyacanthus* Link & Otto (López-Escamilla *et al.*, 2016), *Myrmecophila grandiflora* Walter Hood Fitch (Chavez-Cruz *et al.*, 2022), *Malus domestica* Borkh (Cabral-Miramontes *et al.*, 2022), culture media with pH 5.7 to 6.0 are used. Therefore, the present study aimed to evaluate the *in vitro* growth of axillary buds of *Vaccinium corymbosum* var. Biloxi, established in culture media that varied in the formulation of inorganic salts MS50 %, WPM50 %, and the combination MS50 %-WPM50 %, with three pH values (4.5, 5.0, and 5.5).

Material and Methods

Plant material

The present study was conducted during 2022-2023 in the Plant Tissue Culture Laboratory of the Instituto Tecnológico del Valle de Oaxaca, located in Santa Cruz Xoxocotlán, Oaxaca, Mexico. Micropropagated blueberry *Vaccinium corymbosum*, var. Biloxi plants were purchased from BIOTEC MARPA SPR DE RL DE CV®, at Ziracuaretiro municipality, Michoacán. The plants were established in black polyethylene pots of 30.8 dm^3 , in a substrate that was a mixture of peat 35 %, coconut fiber 35 %, and perlite 30 %; they were kept under shade with 35 % mesh, where they received irrigation with a manual watering can and fertigation once a week with Steiner's universal solution (1984).

Establishment of aseptic cultures

For the establishment of *in vitro* cultures, culture medium was prepared with a mixture of mineral salts MS (Murashige & Skoog, 1962) and WPM (Lloyd & McCown, 1980) each at 50 % (**Table 1**) (Bonga & Durzan, 1987), supplemented with 25 g L⁻¹ sucrose, 1.5 mg L⁻¹ of 2iP, 100 mg L⁻¹ of myo-inositol, pyridoxine 0.5 mg L⁻¹, nicotinic acid 0.5 mg L⁻¹, glycine 2 mg L⁻¹. The pH of the medium was adjusted to 5, with HCl or NaOH 1 N, before adding 5.7 g L⁻¹ of agar. The agar was dissolved and 20 mL was distributed into each 145 cm³ glass bottle, then each bottle was closed with a polypropylene lid. The flasks with culture medium were autoclaved at 1.2 kg cm⁻² pressure at 120 °C for 17 min.

For the establishment of aseptic *in vitro* cultures, from de plants in the nursery, vigorous and healthy branches were selected which were cut 10 cm long, with apex and axillary buds; these were placed in polyethylene bags for transport to the laboratory, where they were cut into segments of 5 cm in length, placed in a glass container of 9 cm in height and 7 cm in diameter, to be subjected to surface disinfection consisting of washing in water with 0.5 % w/v detergent, and rinsed with potable water; then they were immersed for 15 min in a 0.3 % solution of sodium hypochlorite, followed by three rinses with sterilized water. This last step was carried out in a horizontal laminar flow filtered air hood. Stem segments with axillary buds were placed in sterilized 10×100 mm glass Petri dishes and cut into 3 cm stem segments with one or two axillary buds, and two explants (stem segments) were established per 145 cm³ culture flask containing 20 mL of culture medium. The explants were incubated for 60 days in a temperature range of 15-28 °C, and LED illumination 35 μmol m⁻² s⁻¹, in 16/8 h light/dark photoperiods.

Table 1. Mineral composition of three nutrient solutions used in *in vitro* blueberry culture.

Ion	MS	WPM	MS-WPM	Ion	MS	WPM	MS-WPM
	50 %	50 %	50 %		50 %	50 %	50 %
Ions mE L ⁻¹				Ions mE L ⁻¹			
NH ₄ ⁺	10.3	2.4	12.7	MoO ₄ ⁼	0.51	0.51	1.03
NO ₃ ⁻	19.7	4.8	24.5	Fe EDTA ⁼	0.05	0.05	0.11
PO ₄ ⁼	0.6	0.6	1.2	Cl ⁻	3	0.65	3.65
SO ₄ ⁼	0.8	3.7	4.6	Na ⁺⁺	0.11	0.11	0.22
K ⁺	10.0	6.3	16.3	BO ₃ ⁼	0.05	0.05	0.1
Ca ⁺⁺	1.4	1.5	2.9	Mn ⁺⁺	0.06	0.06	0.13
Mg ⁺⁺	0.7	0.7	1.5	Cu ⁺⁺	0.05	0.05	0.1
				Zn ⁺⁺	0.01	0.01	0.02
				Co ⁺⁺	0.05	---	0.05

Continuation

Table 1. Mineral composition of three nutrient solutions used in *in vitro* blueberry culture.

Ion	MS	WPM	MS-WPM	Ion	MS	WPM	MS-WPM 50 %
	50 %	50 %	50 %		50 %	50 %	50 %
Ions mE L ⁻¹				Ions mE L ⁻¹			
				I ⁻	2.5	---	2.5
				Ni ⁺⁺	---	---	---
Total N					30.005	7.29	37.295
Total					47.125	21.195	68.32

Source: Bonga and Durzan, 1987.

Experimental phase

Aseptic *in vitro* cultures of shoots developed from axillary buds were used to set up the experiment. Nine variants of culture media (CM) were prepared containing: 1) 25 g L⁻¹ sucrose, 100 mg L⁻¹ myo-inositol, 0.4 mg L⁻¹ thiamine-HCL, 2 mg L⁻¹ 2iP, 0.5 mg L⁻¹ pyridoxine, 0.5 mg L⁻¹ nicotinic acid, 2 mg L⁻¹ glycine. 2) Some mineral salts formulation, either MS50 % or the WPM50 % formulation, or the MS50 %-WPM50 % mineral salt combination. The total volume of each mineral salt variant was divided into three to adjust to different pH levels (4.5, 5.0, and 5.5), and 5.7 g L⁻¹ of agar was added, which was dissolved with heat and agitation, and 20 mL of culture medium was distributed to each 145 cm glass bottle, polypropylene lid was placed and autoclaved for 17 min at 120 °C and 1.2 kg cm⁻² pressure. Under aseptic conditions provided by the horizontal laminar flow filtered air hood, with the use of sterilized dissecting forceps and scalpel, the shoots were removed from the establishment of aseptic cultures vessel and placed in 10x100 mm, sterilized glass Petri dishes. Each shoot was cut into 2 cm segments. Some stem segments had only axillary buds, while other segments had the apex and axillary buds. Two stem segments were established in each flask with any of nine variants of culture medium to promote shoot development. One of the segments had only axillary buds and the other segment with the apex and axillary buds. The stem segments in an upright position and with the lower third inserted into the gelled culture medium. After establishing the stem segments in the culture medium, the lid was replaced and sealed with adherent polyethylene, and then incubated for 90 days, under conditions of 15-28 °C, and LED illumination 35 μmol m⁻² s⁻¹, in 16/8 h light/dark photoperiods.

Data management and analysis

The experiment was established according to a completely randomized design, with a 3×3×2 factorial arrangement, three levels of the factor mineral salts (MS50 %, WPM50 %, and the combination MS50 %-WPM50 %), three levels of the factor pH (4.5, 5.0, and 5.5) and two levels

of the factor type of stem segment (axillary or apical), consequently, there were 18 treatments. The experimental unit was a stem segment, and there were eight replicates per treatment.

Variables evaluated

Forty days after the establishment of the experiment, the first measurement was done, and later at 80 and 120 days, in the period November 2022 to January 2023, in which the following were quantified: height of the largest shoot (cm), obtained with a graduated ruler; number of leaves and number of shoots. The data were checked for assumptions of normality and homogeneity of variances of errors using Shapiro Wilks and Bartlett's tests ($\alpha \leq 0.05$), variables that did not meet these assumptions were transformed to $(x+1)^{0.5}$. Morphological and growth variable data were subjected to analysis of variance and comparison of means (Duncan, 0.05). The statistical analysis routines were performed with the SAS Statistical Analysis System software (SAS Institute, 2014).

Results and Discussion

In the aseptic culture establishment stage, 40 % of the stem segments that were established were aseptic and viable, which developed axillary shoots that were used for the propagule multiplication stage (**Figure 1**).

ANOVA showed that the inorganic salts types in the culture medium (CM), had highly significant effects ($p \leq 0.01$) on shoot length at 40, 80, and 120 days of incubation; significant effects ($p \leq 0.05$) on shoot number at 40 days; and showed high significance ($p \leq 0.01$) at 80 days. Stem segment type factor levels had highly significant ($p \leq 0.01$) effects on shoot number at 40, 80, and 120 days. The CM \times pH interaction showed highly significant effects on leaf number at 40 days, and significant effects ($p \leq 0.05$) on shoot length at all three dates, leaf number at 80 days, and shoot number at 40 and 80 days (Table 2).



Figure 1. *In vitro* propagation process of blueberry plants.

a) Orthotic plant, b) apical and axillary stem segments, c) *in vitro* stem segment with new axillary shoots, d) axillary shoots obtained *in vitro* placed in Petri dish to be cut into segments with axillary buds, and establish them in culture medium for propagule multiplication.

Stem segments with axillary buds and segments with apex and axillary buds were 2 cm in size when established on culture media with the mineral salts WPM50 %, MS50 %, or MS50 %-WPM50 %, and over 120 days developed shoots that had different sizes, leaves number and shoots number. At 120 days of incubation the stem segments that were established in culture medium with the mineral salts MS50 %-WP50 % with pH 4.5 or 5.0 developed on average 1.4 and 1.3 shoots, 5.5 and 5.8 cm in height, with 11.5 and 11.8 leaves, respectively; values significantly greater (Duncan, 0.05) than the 0.8 new shoots, 1.6 cm tall and with 7.8 leaves that developed from stem segments that were established on culture media with WPM50 % inorganic salts at pH 5.5. For blueberry shoot growth, the type of inorganic salts and pH level were important conditions for shoot development. The best condition for shoot development was the MS50 %-WPM50 % inorganic salt mixture and pH values in the range of 4.5 to 5.

Table 2. Summary of nine analyzes of variance of *in vitro* shoot development of *Vaccinium corymbosum* var. Biloxi from axillary or apical stem segments established in culture media that varied in type of mineral salts and pH.

Variables	DF	Mean squares							Error
		CM	pH	SS	CM×pH	CM×SS	pH×SS	CM×pH×SS	
TSL40	17	1.91**	1.01*	0.44 ^{ns}	0.66*	0.14 ^{ns}	0.19 ^{ns}	0.08 ^{ns}	0.26
TSL80	17	2.58**	1.22 ^{ns}	0.56 ^{ns}	1.05*	0.19 ^{ns}	0.13 ^{ns}	0.10 ^{ns}	0.43
TSL120	17	4.10**	1.10 ^{ns}	0.29 ^{ns}	1.65*	0.40 ^{ns}	1.11 ^{ns}	0.62 ^{ns}	0.58
TNL40	17	0.33 ^{ns}	1.14 ^{ns}	2.89*	2.13**	0.34 ^{ns}	0.41 ^{ns}	0.70 ^{ns}	0.64
TNL80	17	1.08 ^{ns}	2.28 ^{ns}	1.33 ^{ns}	3.09*	0.40 ^{ns}	0.62 ^{ns}	0.90 ^{ns}	1.15
TNL120	17	1.72 ^{ns}	2.03 ^{ns}	0.41 ^{ns}	1.89 ^{ns}	0.93 ^{ns}	2.84 ^{ns}	1.20 ^{ns}	1.53
TSN40	17	0.70*	0.49 ^{ns}	25.92**	0.51*	0.47 ^{ns}	0.04 ^{ns}	0.20 ^{ns}	0.17
TSN80	17	1.21**	0.25 ^{ns}	16.26**	0.62*	0.81*	0.02 ^{ns}	0.25 ^{ns}	0.21
TSN120	17	0.21 ^{ns}	0.52 ^{ns}	9.16**	0.24 ^{ns}	0.59 ^{ns}	0.18 ^{ns}	0.31 ^{ns}	0.31

DF= degrees of freedom; CM= growing medium; pH= hydrogen potential; SS= stem segment; CM×pH, CM×SS, pH×SS, CM×pH×SS= interactions; TSL= shoot length at 40, 80, and 120 days (transformed data); TNL= number of leaves at 40, 80, and 120 days (transformed data); TSN= shoots number at 40, 80, and 120 days (transformed data). ns= not significant F value ($p > 0.05$); *= significant F value ($p \leq 0.05$); **= highly significant F value ($p \leq 0.01$).

Stem segments with the apex maintained their apical dominance since few of these segments sprouted axillary buds, so the main shoot continued to grow and only developed an average of 0.5 axillary shoots, while in stem segments with only axillary buds, 1 to 2 buds sprouted, with an average of 1.2 new shoots. The type of stem segment, apical or axillary, did not determine the level of shoot growth under the various mineral salt and pH conditions.

When the data were sorted according to the type of explant that was established, those stem segments that initially had axillary buds and those stem segments that had the apex, when 40 days of incubation had elapsed, showed respectively 1.3 and 0.3 new shoots, with 7.5 and 6.1 leaves, which in each case were significantly (Duncan, 0.05) different. Shoots were 2.5 and 2.2 cm in height, not statistically (Duncan, 0.05) different. After 120 days, the shoots that developed from these explants were 3.2 and 3.4 cm tall with 9.1 and 9.3 leaves. When the data were sorted according to the inorganic salts used in the growing medium, at 120 days of incubation, the shoots obtained from stem segments established on the culture medium with the MS50 %-WPM 50 % mixture had on average 1.1 shoots, with the largest shoot being 4.7 cm long, and 10.2 total leaves (**Table 3**).

In apical and axillary stem segments that were established on culture media with the combination of inorganic salts MS50 %-WPM50 % at pH 5.0, 4.5 new shoots were formed and these were 3.6 and 3.8 cm at 40 days, as well as 8 and 6.4 cm at 120 days, values that are 4.3 and 3.5 times the heights of axillary shoots that developed in apical stem segments that were on culture medium WPM50 % with pH 5.5.

Table 3. Characteristics of *Vaccinium corymbosum* var. Biloxi shoots grown *in vitro* at 40, 80, and 120 days of incubation, as a function of factor levels.

Factor SS				
Variable	Axillary	Apical	DCR	
SL40 (cm)	2.5±0.2 ^a	2.2±0.2 ^a	0.41	
SL80 (cm)	3.2±0.2 ^a	2.8±0.2 ^a	0.59	
SL120 (cm)	3.2±0.3 ^a	3.4±0.4 ^a	0.81	
NL40	7.5±0.4 ^a	6.1±0.4 ^b	1.08	
NL80	9.2±0.5 ^a	8.2±0.6 ^a	1.57	
NL120	9.1±0.7 ^a	9.3±0.7 ^a	2.08	
SN40	1.3±0.1 ^a	0.3±0.1 ^b	0.24	
SN80	1.3±0.1 ^a	0.4±0.1 ^b	0.27	
SN120	1.3±0.1 ^a	0.6±0.1 ^b	0.34	
Factor CM				
Variable	WPM	MS	MS-WPM	DCR
SL40 (cm)	1.7±0.1 ^c	2.4±0.2 ^b	3.0±0.2 ^a	0.53
SL80 (cm)	2.1±0.2 ^b	3.2±0.3 ^a	3.7±0.3 ^a	0.76
SL120 (cm)	2.1±0.2 ^c	3.3±0.4 ^b	4.7±0.5 ^a	1.05
NL40	6.4±0.5 ^a	7.0±0.5 ^a	7.1±0.5 ^a	1.39
NL80	7.8±0.8 ^a	9.1±0.7 ^a	9.3±0.7 ^a	2.03
NL120	8.6±0.8 ^a	8.9±1.0 ^a	10.2±0.8 ^a	2.68
SN40	0.6±0.1 ^b	0.9±0.1 ^a	1.0±0.2 ^a	0.31
SN80	0.5±0.1 ^b	1.0±0.2 ^a	1.0±0.2 ^a	0.35
SN120	0.8±0.1 ^a	0.8±0.2 ^a	1.1±0.2 ^a	0.43

Continuation

Table 3. Characteristics of *Vaccinium corymbosum* var. Biloxi shoots grown *in vitro* at 40, 80, and 120 days of incubation, as a function of factor levels.

Variable	pH			DCR
	5.5	5.0	4.5	
SL40 (cm)	2±0.2 ^b	2.5±0.2 ^{ab}	2.7±0.2 ^a	0.53
SL80 (cm)	2.59±0.3 ^a	3.2±0.3 ^a	3.3±0.3 ^a	0.76
SL120 (cm)	2.90±0.4 ^a	3.7±0.5 ^a	3.4±0.4 ^a	1.05
NL40	6.37±0.6 ^a	6.7±0.4 ^a	7.4±0.5 ^a	1.39
NL80	7.85±0.8 ^a	9.0±0.6 ^a	9.3±0.7 ^a	2.03
NL120	8.5±1.0 ^a	10.0±0.8 ^a	9.1±0.8 ^a	2.68
SN40	0.7±0.1 ^b	0.8±0.1 ^b	1.1±0.2 ^a	0.31
SN80	0.7±0.1 ^b	0.9±0.1 ^{ab}	1.0±0.2 ^a	0.35
SN120	0.7±0.1 ^b	0.9±0.2 ^{ab}	1.1±0.2 ^a	0.43

SS= stem segment; CM= culture medium; pH= pH level; WPM= Woody Plant Medium (Lloyd and McCown, 1980); MS= Murashige and Skoog (Murashige and Skoog, 1962); MS-WPM= of inorganic salts MS50 % and WPM50 %; SL= shoot length at 40, 80 and 120 days; NL= number of leaves at 40, 80, and 120 days; SN= shoot number at 40, 80, and 120 days; DCR= Duncan's critical range. Means with the same letter in the rows and factor levels are not significantly different (Duncan, $P \leq 0.05$); mean \pm standard error.

At 120 days of incubation, the axillary shoots that developed in the culture media MS50%-WPM50% pH 5.0 and MS50%-WPM50% with pH 4.5 had an average of 6.3 and 9.3 leaves at 40 and 120 days of incubation, as well as as 12.6 and 10.5 leaves, respectively, amounts that were 2.2 and 2.0 times the number of leaves that had apical shoots that developed in culture media with inorganic salts WPM50% with pH 5.5.

After 120 days of incubation, from the stem segments with only axillary buds established in culture media with the combination of inorganic salts MS50%-WPM50% at pH 4.5, they developed an average of 2.2 shoots, an amount that was significantly greater than the 0.1 new shoots that developed from the apical segments established in culture media with WPM50% inorganic salts with pH 5.5 (Table 4).

Obtained data suggest that it is possible the *in vitro* clonal propagation of blueberry *Vaccinium corymbosum* var. Biloxi, from stem segments obtained from stock plants in nursery conditions. The growth of the shoots in height and their number of leaves is important, since at

the base of each leaf there is an axillary bud, and for propagation purposes, it is possible to obtain new shoots that determine an increment factor in each cycle of multiplication of propagules *in vitro*. Hence, it is estimated that, once the first stage of establishment of aseptic cultures has been overcome, in each replicating cycle, which is carried out in periods every two months, cutting the plant material in segments of 2 cm in length containing 3 leaves and axillary buds on average, in a year, five cycles of propagule multiplication could be obtained. After a year, there would be 283 plants derived from each segment of the initial stem.

Tetsumura *et al.* (2008) evaluated four blueberry genotypes in WPM, MS, and MS-WPM combination media, the results were similar to those obtained in the present study, as it was determined that the MS50 %-WPM50% combination was the best condition for shoot growth at the propagule multiplication stage. Likewise, Li *et al.*, (2021) compared WPM, DKW, and LP culture media, obtaining the best shoot proliferation of *V. arboreum*. Fan *et al.* (2017) compared MS, WPM and Anderson media in the *in vitro* multiplication and rooting stage, the Anderson culture medium showed greater efficiency in sprout induction, this result coincides with Ruzić *et al.* (2012), and is related to the mineral composition of the medium and its total ionic concentration (86.48 mEq L⁻¹) (Bonga & Durzan, 1987).

Table 4. Characteristics of blueberry shoots that developed in culture medium with different inorganic salts and pH.

TREATMENTS	VARIABLE						
	SS/iS/pH	SL40	SL120	NL40	NL120	SN40	SN120
AP/MS-WPM/5.0		3.6±1.4 ^{ab}	7.8±2.7 ^a	7.8±2.0 ^a	13.7±1.8 ^a	0.6±0.9 ^{bcd}	0.8±1.1 ^{ab}
AX/MS-WPM/4.5		3.8±1.3 ^a	6.4±1.2 ^{ab}	9.3±2.7 ^a	12.6±2.9 ^a	2.2±1.1 ^a	2.1±0.9 ^a
AP/MS-WPM/4.5		3.5±0.7 ^{ab}	4.6±1.6 ^{abc}	8.1±2.7 ^a	10.3±3.4 ^a	0.3±0.7 ^{bcd}	0.6±0.8 ^{ab}
AX/MS/5.5		2.6±1.4 ^{ab}	4.6±2.4 ^{abc}	7.6±2.9 ^a	11.3±6.2 ^a	1.2±0.4 ^{abcd}	1.0±0.6 ^{ab}
AX/MS-WPM/5.0		2.9±1.3 ^{ab}	4.4±2.9 ^{abc}	6.3±2.5 ^a	10.5±4.5 ^a	1.6±1.0 ^{ab}	1.6±1.2 ^{ab}
AP/MS/5.0		1.8±1.2 ^{ab}	3.9±2.5 ^{abc}	4.0±2.1 ^a	9.6±5.4 ^a	0.1±0.3 ^{cd}	0.6±0.8 ^{ab}
AP/MS/5.5		1.8±1.4 ^{ab}	3.7±3.3 ^{abc}	6.8±3.5 ^a	9.0±7.9 ^a	0.3±0.5 ^{bcd}	0.3±0.5 ^{ab}
AP/MS-WPM/5.5		1.7±1.5 ^{ab}	3.2±3.1 ^{abc}	4.0±3.1 ^a	7.1±4.9 ^a	0.1±0.3 ^{cd}	0.1±0.4 ^b
AP/MS/4.5		2.5±1.1 ^{ab}	3.0±1.3 ^{abc}	6.3±3.2 ^a	10.0±5.4 ^a	1.3±1.8 ^{abc}	1.0±1.5 ^{ab}
AP/WPM/5.0		1.7±0.8 ^{ab}	2.7±1.1 ^{bc}	7.2±3.1 ^a	11.1±4.6 ^a	0±0 ^d	0.1±0.4 ^b
AX/WPM/4.5		1.7±1.0 ^{ab}	2.5±1.4 ^{bc}	5.7±3.3 ^a	7.8±4.3 ^a	1.0±0.5 ^{abcd}	0.8±0.4 ^{ab}
AX/MS-WPM/5.5		2.4±1.6 ^{ab}	2.4±2.0 ^{bc}	6.7±4.3 ^a	7.8±6.5 ^a	1.1±0.6 ^{abcd}	1.0±0.8 ^{ab}
AX/MS/5.0		2.9±1.5 ^{ab}	2.3±2.3 ^{bc}	7.8±3.4 ^a	7.5±6.5 ^a	0.8±0.3 ^{bcd}	0.6±0.5 ^{ab}

Continuation

Table 4. Characteristics of blueberry shoots that developed in culture medium with different inorganic salts and pH.

TREATMENTS	VARIABLE						
	SS/iS/pH	SL40	SL120	NL40	NL120	SN40	SN120
AX/WPM/5.0		1.6±1.1 ^{ab}	2.0±1.5 ^{bc}	6.8±3.2 ^a	8.6±4.3 ^a	1.2±0.4 ^{abcd}	1.5±0.5 ^{ab}
AX/MS/4.5		2.7±0.9 ^{ab}	1.9±2.3 ^{bc}	9.3±3.0 ^a	5.6±6.8 ^a	1.5±0.7 ^{ab}	1.1±1.1 ^{ab}
AX/WPM/5.5		1.8±0.8 ^{ab}	1.9±0.9 ^c	7.6±2.8 ^a	9.5±3.9 ^a	1.2±0.4 ^{abcd}	1.3±0.5 ^{ab}
AP/WPM/4.5		1.5±0.9 ^b	1.8±1.4 ^c	5.2±3.0 ^a	8.0±4.6 ^a	0.1±0.3 ^{cd}	1.0±1.5 ^{ab}
AP/WPM/5.5		1.5±1.2 ^b	1.3±1.5 ^c	5.3±5.6 ^a	6.1±7.2 ^a	0±0 ^d	0.1±0.4 ^b
DCR		1.23-1.52	2.45-3.01	3.24-3.99	6.27-7.70	0.73-0.90	1.02-1.25

SL= shoot length (cm) at 40 and 120 days; NL= number of leaves at 40 and 120 days; SN= shoot number at 40 and 120 days. Trat= treatment; SS= stem segment; AP= apical, AX= axillary; iS= inorganic salts; MS= Murashige and Skoog; WPM= woody plant medium; pH (4.5, 5.0, and 5.5). DCR= Duncan's critical range. In each column, means with the same letter are not significantly different (Duncan, 0.05).

Meanwhile, Wang *et al.* (2019) reported that olive culture medium (OM) supplemented with 2.0 mg L⁻¹ of Zeatin (ZT); 2.0 mg L⁻¹ of naphthaleneacetic acid (NAA), and 0.05 mg L⁻¹ of kinetin, (KT) increased the *in vitro* shoot proliferation coefficient of highbush blueberry in 60 days.

In the *in vitro* blueberry culture, there are references for the use of various formulations of mineral salts (WPM; MS; W-M; B5; MO; White; Anderson; Driver; DKW, and LP) for propagation purposes (Debnath, 2007; Tetsumura *et al.*, 2008; Ruzić *et al.*, 2012; Chen *et al.*, 2018; Wang *et al.*, 2019; Li *et al.*, 2021), however in this study, shoot growth was obtained, this happened in the culture media with the MS50 %-WPM50 % mixture, due to the higher amount of macro and micronutrient minerals, the total ions is 68.32 mEq L⁻¹, amount that is 1.45 times than MS50 % (47.125 mEq L⁻¹) and 3.22 times than WPM50 % (21.195 mEq L⁻¹). Another difference between the media used was that the WPM formulation does not contain I⁻ and Co⁺⁺ ions and both the MS and WPM formulation do not present Ni⁺⁺ ions; Ramage & Williams (2002), suggesting that mineral nutrients constitute an important component of culture media, but are often overlooked as morphogenic elicitors.

In vitro sprouts of *V. corymbosum* proliferated at pH levels of 4.5-5.0, a value similar to that obtained by Li *et al.* (2021) for *Vaccinium arboreum*. While for the *in vitro* culture of blueberry sprouts (*V. corymbosum* and *V. virgatum*) varieties 'Berkeley', 'Bluecrop', 'Earliblue, and O'Neal', it is mentioned that pH has a notable effect on proliferation of shoots, since the greatest propagule

multiplication response was obtained in culture medium with pH 5.0 (Ostrolucká *et al.*, 2004a), Li *et al.* (2021) point out that when the pH of the medium was increased, the proliferation of shoots decreased, and the leaves changed color, from green to reddish yellowish, due to lower availability of nutrients. The pH value is specific to each genotype (Ostrolucká *et al.*, 2010b).

Conclusions

From stem segments with axillary buds obtained from blueberry plants in the nursery, it was possible to establish 40 % success *in vitro* aseptic cultures, in which axillary shoots developed. The culture medium with inorganic salts MS50 %-WPM50 % and pH level 4.5-5.0 provided the best conditions for the growth of blueberry shoots at the propagule multiplication stage.

Authors' contribution

Work conceptualization, author 2, author 3; methodology development, author 1; software management, author 5; experimental validation, author 2, author 4, author 5; analysis of results, author 1, author 2, author 5; data management, author 1, author 2, author 4, author 5; manuscript writing and preparation, author 1, author 2, author 4, author 5; drafting, revising and editing, author 1, author 2, author 4; project manager, author 2; fund acquisition, author 2.

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Conflict of interest

The authors declare that they have no conflicts of interest.

References

- Bonga, J. M., & Durzan D. J. (1987). Cell and Tissue Culture in Forestry. Volumen 1: General Principles and Biotechnology. Springer-Science +Business Media. <https://doi.org/10.1007/978-94-017-0994-1>
- Bhojwani, S. S., & Dantu P. K. (2013). Plant Tissue Culture: An Introductory Text. Springer. <https://doi.org/10.1007/978-81-322-1026-9>
- Cabral-Miramontes, J. P., Chávez-Simental J. A., Pulido-Díaz C., González-Portillo M., Goche-Télles J. R., & Barragán-Hernández V. M. (2022). Propagación in vitro de manzano a partir de embriones cigóticos maduros. *Revista Mexicana de Ciencias Agrícolas*, 13(4), 603–616. <https://doi.org/10.29312/remexca.v13i4.2164>
- Chávez-Cruz, I. L., Enríquez-del Valle J. R., Hernández-Santiago E., & Rodríguez-Ortiz G. (2022). Sales minerales y reguladores de crecimiento en medios de cultivo para desarrollo de *Myrmecophila grandiflora*. *Revista Mexicana de Agroecosistemas*, 9(S1), 108–116. <https://rmae.voaxaca.tecnm.mx/volumen-9-s-2/>
- Chen, H. Y., Liu J., Pan, C., Yu, J. W., & Wang, Q. C. (2018). In vitro regeneration of adventitious buds from leaf explants and their subsequent cryopreservation in highbush blueberry. *Plant Cell Tissue and Organ Culture*, 134(2), 193–204. <https://doi.org/10.1007/s11240-018-1412-y>
- Debnath, S.C. (2007). Propagation of *Vaccinium* in vitro: a review. *International Journal of Fruit Science*, 6 (2), 47–71. https://doi.org/10.1300/J492v06n02_04
- Enríquez-del Valle, J. R., Alcará Vázquez, S. E., Rodríguez-Ortiz G., Miguel-Luna, M. E., & Vázquez, Calep M. (2016). Fertirriego en vivero a plantas de *Agave potatorum* Zucc micropropagadas-aclimatizadas. *Revista Mexicana de Ciencias Agrícolas*, 7(5), 1167-1177. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S200709342016000501167&lng=es&tlng=es
- Enríquez-del Valle, J. R., Rodríguez-Ortiz, G., Ruiz Luna, J., Pacheco-Ramírez, A. J., & Vásquez-Vásquez, L. (2018). Crecimiento y condición nutrimental de plantas micropropagadas de *Agave angustifolia* abonadas y fertirrigadas en vivero. *Revista Mexicana de Agroecosistemas*, 5(2), 106–115. https://rmae.voaxaca.tecnm.mx/wp-content/uploads/2020/11/4-2018_RMAE-28-Agave-to-edit.pdf
- Fan, S., Jian, D., Wei, X., Chen, J., Beeson, RC, Zhou, Z., & Wang, X. (2017). Micropropagation of blueberry ‘Bluejay’ and ‘Pink Lemonade’ through *in vitro* shoot culture. *Scientia Horticulturae*, 226, 277–284. <https://doi.org/10.1016/j.scienta.2017.08.052>
- García-González, R., Enríquez-del Valle J. R., Rodríguez-Ortiz G., Campos-Ángeles G. V., Pérez-García E. A., & Ruiz-Luna J. (2020). Mineral salts and growth regulators for micropropagation of *Laelia halbingeriana* Salazar & Soto Arenas. *International Journal of Agriculture and Natural Resources*, 47(2), 105–116. <https://doi.org/10.7764/ijanr.v47i2.2086>
- George, E. F.; Hall, M. A., & De-Klerk, G. J. (2008). Chapter 3: The Components of Plant Tissue Culture Media I: Macro-and Micro-Nutrients. *Plant propagation by tissue culture*. 3rd. Ed. Springer, Dordrecht. pp. 65–113. https://doi.org/10.1007/978-1-4020-5005-3_3
- Georgieva, M., & Kondakova, V. (2021). In vitro propagation of *Vaccinium corymbosum* L. Bulgarian *Journal of Agricultural Science*, 27 (2), 323–327. <https://www.agrojournal.org/27/02-11.pdf>
- Greenway, M. B., Phillips, I. C., Lloyd, M. N., Hubstenberger, J. F., & Phillips, G. C. (2012). A nutrient medium for diverse applications and tissue growth of plant species *in vitro*. *In Vitro*

- Cellular and Developmental Biology - Plant*, 48(4), 403–410. <https://doi.org/10.1007/s11627-012-9452-1>
- Guillén, S., Martínez-Palacios, A., Martínez, H., & Martínez-Ávalos, J. G. (2015). Organogénesis y embriogénesis somática de *Beaucarnea inermis* (Asparagaceae), una especie amenazada del noreste de México. *Botanical Sciences*, 93(2), 221–230. <https://doi.org/org/10.17129/botsci.129>
- Hine-Gómez, A., & Abdelnour-Esquivel, A. (2013). *In vitro* establishment of blueberry (*Vaccinium corymbosum* L.). *Tecnología En Marcha*, 26(4), 64–71. <https://doi.org/org/10.18845/tm.v26i4.1584>
- Ibarra-López, A., Ojeda-Zacarías M. C., García-Zambrano E. A., & Gutiérrez-Diez A. (2016). Inducción *in vitro* de brotes de dos cultivares de aguacate raza Mexicana *Persea americana* var. *drymifolia* Schlttdl. & Cham. *Revista Mexicana de Ciencias Agrícolas*, 7(2), 337–347. <https://doi.org/org/10.29312/remexca.v7i2.348>
- Li, Q., Yu, P., Lai, J., & Gu, M. (2021). Micropropagation of the potential blueberry rootstock—*Vaccinium arboreum* through axillary shoot proliferation. *Scientia Horticulturae*, 280, 109908. <https://doi.org/10.1016/j.scienta.2021.109908>
- López-Escamilla, A. L., López-Herrera M., & Loaiza-Alanís C. (2016). Efecto de diferentes agentes gelificantes en la germinación y desarrollo *in vitro* de plántulas de *Echinocactus platyacanthus* link Et Otto (Cactaceae). *Polibotánica*, 42 (21), 153–166. <https://doi.org/10.18387/polibotanica.42.8>
- Lloyd, G., & McCown, B. H. (1980). Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Combined proceedings, International Plant Propagators Society*, 30, 421–427.
- Martínez-Villegas, Y. M., Andrade-Rodríguez M., Colinas-León M. T., Villegas-Torres O. G., Castillo-Gutiérrez A., & Alía-Tejacal I. (2015). Efecto de las sales inorgánicas del medio de cultivo en el crecimiento de pascuíta (*Euphorbia leucocephala* Losty). *Revista Fitotecnia Mexicana*, 38(4) 369– 374. <https://www.scielo.org.mx/pdf/rfm/v38n4/v38n4a4.pdf>
- Molinos-da Silva, C., Villegas-Monter A., Sánchez-García P., Alcántar-González G., Rodríguez-Mendoza M. N., & Ruiz-Posadas L. M. (2004). Efecto del potencial osmótico y contenido de CA en el medio de cultivo sobre la distribución de Ca²⁺ y K⁺, producción de biomasa y necrosis apical de VID R110. *Interciencia*, 29(7), 384-388. <https://www.redalyc.org/articulo.oa?id=33909407>
- Montiel-Frausto, L. B., Enríquez-del Valle J. R., & Cisneros A. (2016). Propagación *in vitro* de *Hylocereus monacanthus* (Lem.) Brittony Rose. *Biotecnología Vegetal*, 16(2), 113–123. <https://revista.ibp.co.cu/index.php/BV/article/view/516/pdf>
- Morard P., & Henry M. (1998). Optimization of the mineral composition of *in vitro* culture media. *Journal of Plant Nutrition*, 21(8), 1565-1576. <https://doi.org/10.1080/01904169809365504>
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 15(3), 473–479. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Ostrolucká MG, Libiaková G, Ondrušková E., & Gajdošová A. (2004^a). *In vitro* propagation of *Vaccinium* species. *Acta Universitatis Latviensis Biology*, 676, 207-212. <https://eeb.lu.lv/EEB/2004/Ostrolucka.pdf>
- Ostrolucká, M., Gajdošová, A., Ondrušková, E., Latečková, M., & Libiaková, G. (2010^b). Effect

- of Medium pH on Axillary Shoot Proliferation of Selected *Vaccinium vitis-idaea* L. Cultivars. *Acta Biologica Cracoviensia Series Botanica*, 52(2), 92-96. <https://doi.org/10.2478/v10182-010-0029-1>
- Ramage, C. M., & Williams R. R. (2002). Mineral nutrition and plant morphogenesis. *In Vitro Cellular & Developmental Biology -Plant*, 38 (2), 116-124. <https://doi.org/10.1079/IVP2001269>
- Retamales, J. B., & Hancock J. F. (2012). Blueberries. <https://books.google.com.mx>
- Ruzić, D., Vujović, T., Cerović, R., Ostrolucka, MG., & Gajdosova, A. (2012). Micropropagation in vitro of highbush blueberry (*Vaccinium corymbosum* L.). *Acta Horticulturae*, 926(36), 265–272. <https://doi.org/10.17660/ActaHortic.2012.926.36>
- SAS Institute. (2014). *Statistical Analysis System (SAS) User's Guide*. SAS/ETS® 9.4. SAS Institute Inc. Cary, North Carolina, USA.
- Servicio de Información Agroalimentaria y Pesquera (SIAP). (2022). Panorama agroalimentario. <https://www.gob.mx/siap/acciones-y-programas/panorama-agroalimentario-258035>
- Steiner A. A. (1984). The universal nutrient solution. Sixth International Congress on Soilless Culture. Wageningen, The Netherlands. pp: 633-650.
- Tetsumura T, Matsumoto Y, Sato M, Honsho C, Yamashita K, Komatsu H, Sugimoto Y. & Kunitake H. (2008). Evaluation of basal media for micropropagation of four highbush blueberry cultivars. *Scientia Horticulturae*, 119(1), 72-74. <https://doi.org/10.1016/j.scienta.2008.06.028>
- United States Department of Agriculture (USDA). (2021). Blueberries Around the Globe-Past, Present, and Future. United States Department of Agriculture. <https://www.fas.usda.gov/data/blueberries-around-globe-past-present-and-future>
- Wang, Y., Dong, X., Huang, H.-Y., & Wang, Y.-Z. (2019). Establishment of efficient adventitious shoots induction system and ex vitro rooting in *Vaccinium corymbosum* (Ericaceae). *Botanical Sciences*, 97(2), 180-191. <https://doi.org/10.17129/botsci.2135>
- Wolfe, D.E., Eck, P., Chin, C.-K. (1983). Evaluation of seven media for micropropagation of highbush blueberry. *HortScience*, 18(5), 703–705. <https://doi.org/10.21273/HORTSCI.18.5.703>
- Zárate, N. B., Yescas, A. A. & Morales, D. V. J. (2017). Manejó agronómico del cultivo de arándano (*Vaccinium corymbosum* L.) en la Sierra Norte de Oaxaca. *Universidad & Ciencia*, 6, 138–155. <http://revistas.unica.cu/index.php/uciencia/article/view/707>