IJANR
International Journal of Agriculture and Natural Resources

Int. J. Agric. Nat. Resour. 47(2):105-116. 2020
www.ijanr.cl

ORNAMENTAL CROPS, PLANT BREEDING

DOI 10.7764/ijanr.v47i2.2086

RESEARCH PAPER

Mineral salts and growth regulators for micropropagation of *Laelia halbingeriana* Salazar & Soto Arenas

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Abstract

R. García-González, J.R. Enríquez-del Valle, G. Rodríguez-Ortiz, G.V. Campos-Angeles, E.A. Pérez-García, and J. Ruiz-Luna. 2020. Mineral salts and growth regulators for micropropagation of *Laelia halbingeriana* Salazar & Soto Arenas. Int. J. Agric. Nat. Resour. 105-116. *Laelia halbingeriana* Salazar & Soto Arenas is a wild epiphytic orchid endemic to Oaxaca, Mexico, that is collected with no plans for its management or conservation. The objective of this study was to propagate and evaluate the *in vitro* development of this species in the culture media with a variety of mineral salts and growth regulators. Groups of three plants, 1.2 to 1.4 cm tall, were established in receptacles with culture medium containing 30 g L^{-1} sucrose, 1 mg L^{-1} thiamine, 100 mg L^{-1} inositol and 0.5 mg L^{-1} benzyl aminopurine (BAP). Proportions of Murashige and Skoog (1962) or Knudson (1946) mineral salts were varied (100, 66 and 33%). The growth regulators indoleacetic acid (IAA) and kinetin (Kin) were used at various concentrations (0.5 + 0.5 mg L^{-1}, 1 + 1 mg L^{-1} and 2 + 2 mg L^{-1}). pH was adjusted to 5.8, and 5.5 g L^{-1} agar was added. After 120 days of incubation, the plants that were cultivated in 66% and 100% Knudson media were 2.1 cm tall and had 4.8 roots, while the plants in 100% Murashige and Skoog media were 1.5 cm tall and had 2.5 roots. For 120 days, the plants had a logarithmic trend in vertical growth ($R^2 \geq 0.96$). Several plant characteristics were evaluated simultaneously using a cluster analysis, which found that the best culture medium for *Laelia halbingeriana* development contains 100% Knudson mineral salts, 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} IAA, and 0.5 mg L^{-1} KIN.

Keywords: Growth regulators, mineral salts, plant development, propagation.

Introduction

Laelia halbingeriana (Salazar & Soto Arenas) is a wild epiphytic species of the Orchidaceae family endemic to the Biosphere Tehuacán-Cuicatlán Reserve, a protected natural area in the state of Oaxaca. The plant grows adhered to the trunks and branches of *Quercus* spp. The plant is an attractive ornamental and thus, large quantities of this species are collected, and wild

Received Feb 26, 2019. Accepted May 11, 2020.
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populations are dwindling (Salazar *et al*., 2006). This species should be considered "subject to special protection" because its distribution area of less than 5,000 km^2 is severely fragmented and reduced to approximately 1,800 km^2 (Salazar *et al*., 2014). Development of *in vitro* propagation and cultivation in greenhouses and nurseries has been suggested as an alternative to collecting wild orchids. The objectives of conservation and commercial production can be attained within these projects. Procedures for propagation of several species have been described. Seed germination of *Oncidium stramineum* Lindl and plant development in the culture medium containing MS mineral salts (Murashige & Skoog, 1962; Flores-Escobar *et al*., 2008) have been evaluated. Development of *Laelia speciosa* (Kunth) Schltr protocorms and seedlings was evaluated in MS culture media with various concentrations of growth regulators (GR), naphthaleneacetic acid, (NAA) and benzyl aminopurine (BAP) (Avila-Díaz *et al*., 2010).

Orchids grow slowly, and in the wild, it takes approximately seven years to flower after seed germination. On the other hand, if the plants are cultured in a greenhouse nursery, they can flower in four to six years depending on the species. *Laelia halbingeriana* fruits contain thousands of viable seeds that germinate under optimal conditions; however, there is no information on the time of development from seed germination to reproductive age (Hágsater *et al*., 2006). It is possible to achieve asymbiotic germination of the seeds and *in vitro* development of *L. halbingeriana* seedlings (Heredia-Rincón *et al*., 2009). The goal of the present work was to evaluate the components of the *in vitro* culture medium, in particular the concentrations of mineral salts, MS salts and GR that contribute to optimization of the growth and development of this species.

For *in vitro* propagation of *Oncidium bifolium* Sims, MS mineral salts diluted to a half of the full concentration were more beneficial to the plant growth than the full concentration (Dalzotto, 2013). Good plant development has been achieved in culture media with other mineral salt formulations, such as Knudson (KN), Hutner, Dalla Rosa, Phytamax, and Morel, which have lower total ion concentrations than that in the full-strength MS (Hossain *et al*., 2009). Moreover, various concentrations of GR were evaluated for *in vitro* propagation of *Euchile mariae* (Ames) Withner and it was found that the plants grown in culture media with BAP developed more protocorm-like bodies, PLBs (Suárez-Quijada *et al*., 2007). Lozano-Rodríguez *et al*., (2015), cultured *Vanilla planifolia* Jacks ex Andrews *in vitro* and reported that 8.88 µM BAP promoted the formation and development of adventitious shoots. Thus, this study aimed to evaluate the *in vitro* development and growth of *Laelia halbingeriana* established in the culture media with variable composition and concentrations of mineral salts and GR. Our hypothesis was that the concentrations of mineral salts and GR have different effects on *Laelia halbingeriana* development and growth.

Materials and methods

Handling of experimental material

Plants of *Laelia halbingeriana* were collected in the La Cañada region, Oaxaca, Mexico, in the areas of wild vegetation affected by construction of power transmission lines. The plants were established in the botanical garden of the Technological Institute of the Valley of Oaxaca; since 2009, these plants have developed flowers and fruits. Since 2009, seed germination and *in vitro* propagation have been carried out. This study was conducted in the laboratory of plant tissue culture of the Instituto Tecnológico del Valle de Oaxaca, Mexico, located at 17° 04´ N and 96° 43´ W at an altitude of 1,519 m (INEGI, 2004). The plants of various sizes (0.5 to 4 cm tall) were grown in an aseptic gelatinous culture medium, which contained MS mineral salts, 1 mg L^{-1} thiamine-HCl, 25 g L^{-1} sucrose, 0.5 mg L^{-1} BAP, and 5.7 g L^{-1} agar.

For the experiment, the plants with similar characteristics were selected based on height (from 1.2 to 1.3 cm) and number of leaves. Aseptic conditions were achieved with a horizontal laminar flow chamber (Forma Scientific, model 1839, Marietta, OH-USA), sterilized dissection tools, forceps, scalpels and 10 × 100 mm glass Petri dishes; the selected plants were transferred to 145 cm^3 glass receptacles that contained 20 mL of culture medium containing 1 mg L^{-1} thiamine, 30 g L^{-1} sucrose, 100 mg L^{-1} inositol, and 0.5 mg L^{-1} BAP. The following conditions were varied: 1) factor formulations of mineral salts, MS or KN; each formulation was used at three different concentrations (MS=100, 66 and 33% and KN=100, 66 and 33%) and 2) factor growth regulator, GR, at three levels of the mixtures (GRM1 = 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} KIN + 0.5 mg L^{-1} IAA; GRM2= 0.5 mg L^{-1} BAP + 1 mg L^{-1} KIN + 1 mg L^{-1} IAA; and GRM3 = 0.5 mg L^{-1} BAP + 2 mg L^{-1} KIN + 2 mg L^{-1} IAA). pH of the culture media was determined with a Conductronic pH 120 potentiometer and adjusted to 5.8 with 1N HCl and NaOH before the addition of 5.7 g L^{-1} agar. The formulations of the GR mixtures were investigated during four years of *in vitro* cultivation of *Laelia halbingeriana* during investigation of the conditions of the minimal proliferation of propagules; formulations used in the present work enhanced growth of the propagules. All substances were weighed with an analytical balance (ADAM EQUIPMENT CO., LTD.) with total capacity of 300 g and accuracy of 0.1 mg. Agar was dissolved by heating and shaking on an electric hotplate (CIMAREC, Barnstead Thermolyne). Culture medium (20 mL) was placed into each 145 cm^3 receptacle, which was closed with a polypropylene stopper and sterilized for 17 min in an autoclave (AESA MOD. CV 300) at 121 ºC and pressure of 1.2 kg cm^{-2}. Under the aseptic conditions, the plants were extracted from their initial culture medium and nine plants were distributed per receptacle for treatment with the variants of the culture medium. The receptacles were covered, sealed with adhering polyethylene and transferred to the incubation area where they were incubated for 120 days exposed to diffused solar radiation and white fluorescent illumination at intensity of 2,000 lux (37 µmol s^{-1} m^{-2}) at photoperiods of 16 h and 8 h darkness and temperature from 16 to 28 ºC.

Management and data analysis

The experiment was conducted under a completely randomized design with a 6 × 3 factorial array of the treatments; the factor mineral salt type and dilution were used at six levels and the factor GR were used at three levels. The experimental unit included a single receptacle containing nine plants; however, the data were collected only from three plants; an average was calculated for each characteristic. There was a total of 11 replicates per treatment.

At the beginning of the experiment and every two months thereafter, height (cm) was measured with an electronic Vernier caliper and recorded. The number of leaves, roots, shoots and new plants was quantified. The rates of monthly increments were calculated based on the data on the number of leaves, shoots, and roots on the initial date and after 120 days of incubation. The data on the initial and last dates and the increment rates of each variable were subjected to an analysis of variance and comparison of the means (Tukey; α=0.05) and to cluster analysis. The data on plant height in each treatment were analyzed as a function of time (four months) by regression. The logarithmic model $PH = b^0(Age)^{b1}$ provided the best fit.

The variables, including height, number of leaves, number of roots, and number of shoots, were analyzed simultaneously with cluster analysis. The Statistical Analysis System software (SAS Institute Inc. 2004) was used for routine types of analysis including PROC ANOVA, GLM, NLIN and CLUSTER procedures.

Results

After 120 days of incubation (February to June 2017), the analysis of variance (Table 1) showed that the evaluated GR mixtures had no significant effect (*P*>0.05) on height, shoot proliferation, leaves and roots. The types and concentrations of mineral salts (MS) had highly significant effects (*P≤0.01*) on plant height at four months of incubation (PHJ), monthly growth rate in height (MGRH), number of shoots in the fourth month (NBJ), monthly rate of increase in the number of leaves (MGRL), number of roots in the fourth month (NRJ) and monthly rate of increase in the number of roots (MGRR). The interaction of MGR×MS had significant effects (P<0.05) on plant height in the fourth month of incubation (NBJ) and number of roots in the fourth month (NRJ) and highly significant effects (P≤0.01) on the monthly rate of increase in the number of leaves (MGRL) and monthly rate of increase in the number of roots (MGRR).

The results were grouped as a function of the principal factors, including Msalts and mixtures of GR (Table 2); at the beginning of the experiment (February), the plants under various conditions had statistically similar values in all variables: PHF, NSF, NLF, and NRF. In June, after 120 days, significant differences were observed (Tukey, 0.05) in all variables (Figure 1).

The plants established in the culture media with mineral salts KN-100 and KN-66 had initial height of 1.27 cm and had an increase in height by 0.22 cm/month; thus, after 120 days, these plants were 2.15 cm tall. These values were significantly higher (*P<0.05*) than those detected in the plants established in MS-100, MS-66 and MS-33 (0.09 to 0.14 cm/month and 1.57 to 1.74 cm total height) (Tables 2 and 3). The number of shoots (NSJ) and the monthly rate of increase in the number of shoots, MGRS, were different due to the effect of the mineral salts since the plants established in the culture medium with KN-100 had 6.6 new shoots, NSJ, which was significantly higher (*P<0.05*) than 1.83 NSJ formed on the plants grown in the MS-100 culture medium. The plants established in KN-100 and KN-66 formed 1.10 and 1.21 roots/month, respectively.

Table 1. Summary of analysis of variance (mean squares) of the variables of *Laelia halbingeriana* cultivated *in vitro* in various culture media containing varied mixtures of growth regulators and mineral salts and the interactions of the factors.

SV	DF	PHF	PHJ	MGRH (cm/month)	NSF	NSJ	MGRS (shoots/ month)
GRM	2	0.03^{ns}	0.19^{ns}	0.005^{ns}	0.08^{ns}	0.22^{ns}	0.007^{ns}
Msalts	5	0.07^{*}	2.13^{**}	0.08^{**}	0.01^{ns}	112.38^{**}	6.91^{**}
GRM×Msalts	10	0.05^{*}	0.21^{*}	0.01^{ns}	0.03^{ns}	9.29^{ns}	0.61^{*}
Error	180	0.02	0.11	0.006	0.06	5.60	0.32
Total	197						
SV	DF	NLF	NLJ	MGRL (leaves/ month)	NRF	NRJ	MGRR (roots/month)
GRM	2	0.71^{ns}	0.89^{ns}	0.03^{ns}	0.39^{ns}	7.32^{ns}	0.27^{ns}
Msalts	5	0.26^{ns}	0.61^{ns}	0.09^{**}	0.30^{ns}	35.24^{**}	1.94^{**}
GRM×Msalts	10	0.62^{ns}	1.14^{*}	0.07^{**}	0.14^{ns}	8.72^{*}	0.49^{**}
Error	180	0.51	0.58	0.02	0.20	4.24	0.22
Total	197						

SV=source of variation, GRM=growth regulator mixtures, Msalts=mineral salts. DF=degrees of freedom. PHF=plant height in February, PHJ=plant height in June, MGR H, S, L, R= monthly rate of increase (in height, shoots, leaves and root), NSF=number of shoots in February, NSJ=number of shoots in June, NLF=number of leaves in February, NLJ=number of leaves in June, NRF=number of roots in February, and NRJ=number of roots in June.

ns= nonsignificant (P>0.05); *= significant effects (P≤0.05); **= highly significant effects (P≤0.01) (Tukey test, P<0.05).

Table 2. Characteristics of *Laelia halbingeriana* plants established in culture media with various types and concentrations of mineral salts (Msalts) and growth regulators (GR).

Factor Msalts	PHF (cm)	PHJ (cm)	MGRH (cm/month)	NSF	NSJ	MGRS (shoots/month)
MS100	1.18±0.18[a]	1.57±0.21[c]	0.09±0.04[c]	0.13±0.20[a]	1.83±1.39[c]	0.42±0.43[c]
MS66	1.16±0.14[a]	1.64±0.21[c]	0.12±0.04[c]	0.18±0.30[a]	2.60±1.41[bc]	0.60±0.32[bc]
MS33	1.18±0.15[a]	1.74±0.31[bc]	0.14±0.06[bc]	0.18±0.26[a]	2.97±1.39[bc]	0.69±0.34[bc]
KN100	1.27±0.15[a]	2.15±0.37[a]	0.22±0.09[a]	0.19±0.23[a]	6.66±4.03[a]	1.61±0.98[a]
KN66	1.26±0.19[a]	2.15±0.49[a]	0.22±0.12[a]	0.18±0.27[a]	5.49±2.65[a]	1.32±0.63[a]
KN33	1.21±0.18[a]	1.95±0.41[ab]	0.18±0.09[ab]	0.16±0.25[a]	3.65±2.29[b]	0.87±0.55[b]
GRM						
1	1.24±0.17[a]	1.93±0.44[a]	0.17±0.10[a]	0.15±0.24[a]	3.80±2.46[a]	0.91±0.61[a]
2	1.20±0.14[a]	1.82±0.40[a]	0.15±0.09[a]	0.21±0.27[a]	3.91±2.73[a]	0.92±0.65[a]
3	1.19±0.19[a]	1.85±0.40[a]	0.16±0.08[a]	0.15±0.24[a]	3.89±3.47[a]	0.93±0.85[a]
Factor Msalts	NLF	NLJ	MGRL (leaves/month)	NRF	NRJ	MGRR (roots/month)
MS100	4.05±0.78[a]	5.61±1.01[a]	0.39±0.18[a]	0.19±0.18[a]	2.51±1.25[c]	0.58±0.29[b]
MS66	4.10±0.77[a]	5.74±0.83[a]	0.41±0.19[a]	0.33±0.39[a]	3.14±1.43[c]	0.70±0.35[b]
MS33	4.10±0.62a	5.74±0.65[a]	0.41±0.13[a]	0.43±0.51[a]	3.47±1.41[bc]	0.76±0.30[b]
KN100	4.17±0.66[a]	5.44±0.69[a]	0.33±0.13[ab]	0.43±0.44a	4.83±2.39ab	1.10±0.54a
KN66	4.29±0.72[a]	5.41±0.69[a]	0.28±0.12[b]	0.39±0.59a	5.24±3.57a	1.21±0.80a
KN33	4.21±0.74[a]	5.53±0.81[a]	0.33±0.14[ab]	0.28±0.39a	3.80±1.70abc	0.88±0.40ab
GRM						
1	4.04±0.64[a]	5.47±0.71[a]	0.35±0.13[a]	0.25±0.32[a]	3.45±1.48[a]	0.80±0.36[a]
2	4.16v0.74[a]	5.70±0.76[a]	0.38±0.17[a]	0.40±0.43[a]	4.09±2.51[a]	0.92±0.56[a]
3	4.25±0.75[a]	5.60±0.85[a]	0.33±0.16[a]	0.37±0.47[a]	3.95±2.69[a]	0.89±0.62[a]

Msalts=mineral salts; GR=growth regulators; PHF=plant height in February, PHJ=plant height in Jun, MGR H, S, L, R=monthly rate of increase (in height, shoots, leaves and root), NSF=number of shoots in February, NSJ=number of shoots in June, NLF=number of leaves in February, NLJ=number of leaves in June, NRF=number of roots in February, and NRJ=number of roots in June.

In each column, the values with the same letter are not significantly different (Tukey test, $P<0.05$); mean ± standard deviation.

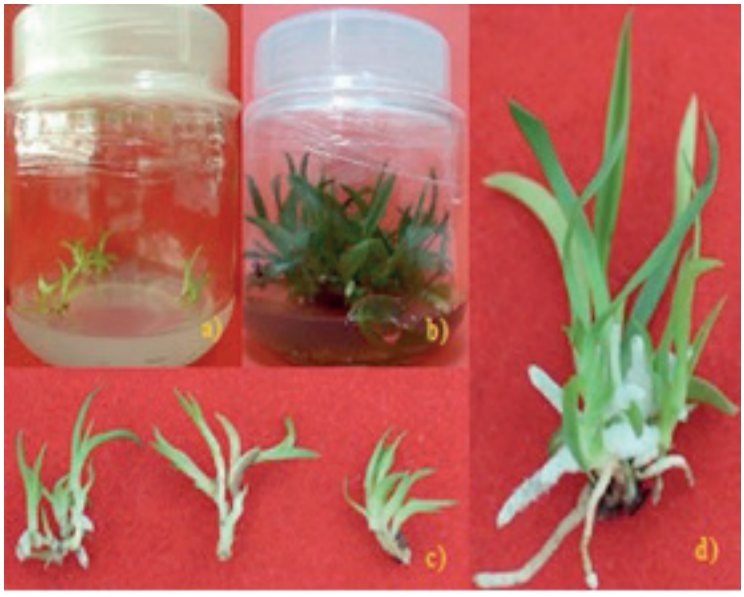

Figure 1. *In vitro* propagation of *Laelia halbingeriana*; a) plants at the beginning of the experiment; b) plants at the end of the experiment in T16; c) groups of three plants at the beginning and d) height and roots at the end of the experiment; treatment with KN66% + GRM1.

These values were significantly higher (Tukey, 0.05) than 0.58 to 0.78 roots/month formed by the plants grown in MS-100, MS-66 and MS-33 media (Tables 2 and 3).

The plants established in culture media with mineral salts MS-100 or MS-66 grew slowly; these media had 104.92 and 69.2 meq L^{-1} total ions, respectively (Table 4). These data indicate that when the number of ions is relatively high, the growth of *L. halbingeriana* is inhibited.

Regression analysis was used for additional description of the plant growth intensity during 120 days of incubation (Figure 2) as a function of the conditions of the growth culture medium (Table 5). The data indicate that the plants in T4 (KN-100 + 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} KIN + 0.5 mg L^{-1} IAA) had higher growth rate than the plants established in T1 (MS-100 + 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} KIN + 0.5 mg L^{-1} IAA); the amount of total ions was the only difference between these treatments. The growth in height of *L. halbingeriana* during 120 days was fit to a logarithmic model. The logarithmic equation for estimating the height showed a suitable goodness of fit ($R^2 \geq 0.96$) and mean square of the error ≤ 0.43, and the regression parameters β_0 and β_1 were highly significant ($P \leq 0.01$).

The plants subjected to the best treatments (4, 5, 6 and 11), which included mineral salts KN-100 or KN-66, grew faster than the plants subjected to the treatments with the culture medium with MS-100 mineral salts (Figure 2).

Table 3. Propagule size of Laelia halbingeriana grown for four months in the culture media with various types and concentrations of inorganic salts (Msalts) and mixtures of growth regulators (GRM).

Treatment Msalts-GRM	Height (cm)	Number of shoots	Number of leaves	Number of roots
1) MS100-GRM1	1.60 c	1.81 c	5.55 a	2.32 b
2) MS100-GRM2	1.55 c	1.86 c	5.67 a	2.64 ab
3) MS100-GRM3	1.56 c	1.85 c	5.62 a	2.57 ab
4) MS66-GRM1	1.67 bc	2.57 bc	5.68 a	2.95 ab
5) MS66-GRM2	1.62 c	2.62 bc	5.80 a	3.27 ab
6) MS66-GRM3	1.63 c	2.61 bc	5.75 a	3.20 ab
7) MS33-GRM1	1.77 abc	2.94 bc	5.68 a	3.28 ab
8) MS33-GRM2	1.72 abc	2.99 bc	5.80 a	3.60 ab
9) MS33-GRM3	1.73 abc	2.98 bc	5.75 a	3.53 ab
10) KN100-GRM1	2.18 a	6.63 a	5.38 a	4.64 ab
11) KN100-GRM2	2.13 ab	6.68 a	5.50 a	4.96 ab
12) KN100-GRM3	2.14 ab	6.67 a	5.45 a	4.89 ab
13) KN66-GRM1	2.18 a	5.46 ab	5.35 a	5.05 ab
14) KN66-GRM2	2.13 ab	5.51 ab	5.47 a	5.37 a
15) KN66-GRM3	2.14 ab	5.50 ab	5.42 a	5.30 ab
16) KN33-GRM1	1.98 abc	3.62 abc	5.47 a	3.61 ab
17) KN33-GRM2	1.92 abc	3.67 abc	5.59 a	3.93 ab
18) KN33-GRM3	1.94 abc	3.66 abc	5.54 a	3.86 ab

Table 4. Mineral composition (meq L^{-1}) of the MS and KN nutrient solutions used for *in vitro* culture of *Laelia halbingeriana.*

IC	MS-100/meq L^{-1}=OP (MPa)	MS-66/meq L^{-1}=OP (MPa)	MS-33/meq L^{-1}= OP (MPa)	KN-100/meq L^{-1}/= OP (MPa)	KN-66/meq L^{-1}= OP (MPa)	KN-33/meq L^{-1}= OP (MPa)
A	52.46	34.60	18.90	27.96	18.45	9.22
C	52.47	34.60	18.05	27.96	18.45	9.22
TI	104.92=-0.23	69.2=-0.15	36.1=-0.08	55.92=-0.12	36.90=-0.08	18.44=-0.04

IC: ion concentration; A: anions; C: cations: TI: total ions. MS: Murashige and Skoog; KN: Knudson; OP: osmotic potential.

The analysis of variance and comparison of the means describe the responses of each variable individually thus limiting the selection of the best condition for quality plant development. The results obtained with the cluster analysis were useful for identification of the most appropriate culture medium conditions for rapid development of the quality plants by simultaneously varying several plant characteristics. The cluster analysis used an array of five variables recorded over 120 days including the monthly growth rate. At a distance of 4.89, two main groups, A and B, and their subgroups were identified. Group A included the plants that had the best growth; all of which were cultured in KN mineral salt media, while group B included all plants cultured in MS media at any concentration and the plants in the culture media with KN-33 mineral salts. The plants with the slowest growth were maintained in the culture medium with the mineral

Table 5. Parameters of the logarithmic regression model of vertical growth of *Laelia halbingeriana* plants established in 18 different culture media.

Treat	R^2_{adj}	MSRE	β_0	β_1	Treat	R^2_{adj}	MSRE	β_0	β_1
1	0.97	0.22	1.28**	0.25**	10	0.97	0.27	1.21**	0.44**
2	0.99	0.11	1.12**	0.30**	11	0.96	0.35	1.16**	0.55**
3	0.97	0.24	1.17**	0.31**	12	0.97	0.23	1.17**	0.34**
4	0.97	0.30	1.22**	0.57**	13	0.99	0.43	1.07**	0.26**
5	0.96	0.33	1.27**	0.45**	14	0.98	0.20	1.12**	0.38**
6	0.97	0.28	1.20**	0.50**	15	0.97	0.27	1.21**	0.36**
7	0.99	0.13	1.16**	0.26**	16	0.98	0.21	1.23**	0.45**
8	0.98	0.18	1.25**	0.26**	17	0.96	0.34	1.20**	0.47**
9	0.97	0.23	1.12**	0.38**	18	0.97	0.28	1.18**	0.45**

Treat: treatment. R^2_{adj}: adjusted regression coefficient; MSRE: mean square root of error; $\beta_{0,1}$ = parameters of the regression model. **: highly significant (t-Student, P≤0.01).

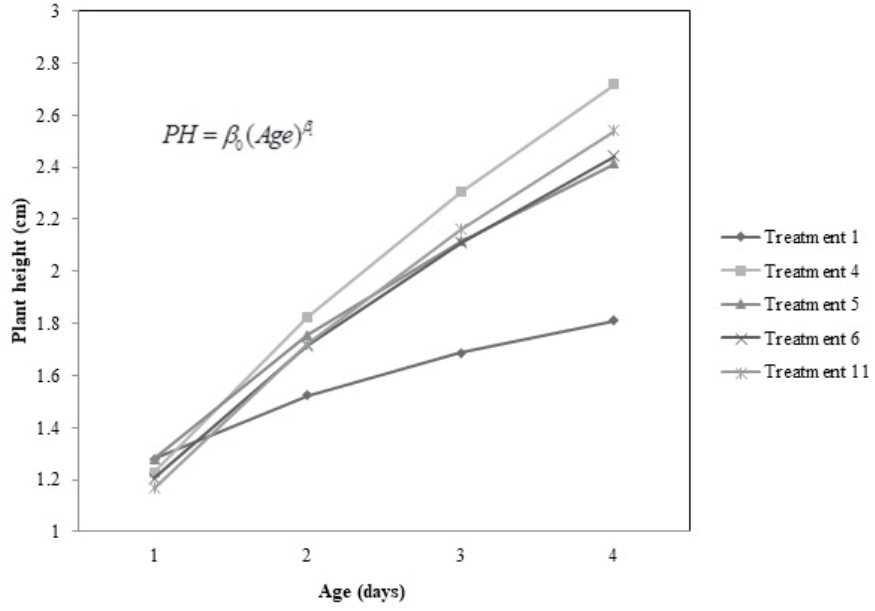

Figure 2. Vertical growth (PH) of *Laelia halbingeriana* plants cultured *in vitro* for four months under the conditions that produced significant effects.

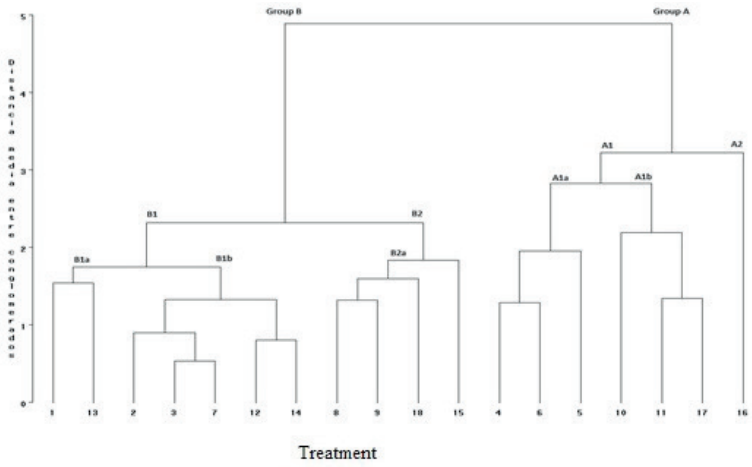

Figure 3. Cluster analysis of the variables and growth rates. Letters A and B represent the main groups; letters with the numbers represent the subgroups formed within each of the main groups (treatments numbering is described in to Table 3).

salts MS at 100% (T1, T2 and T3) as shown on the far left of the dendrogram (Figure 3); the conditions can be improved to yield the plants with better growth culminating in KN 100 (T16, T17 and T18) (treatment numbering refers to description of Table 3).

Discussion

This study demonstrates that the growth of the *L. halbingeriana* plants is influenced by the type and concentration of mineral salts to a greater extent than it is by the effects of GR. Thus, it is necessary to evaluate not only GR but also mineral salts and other components of the culture medium. Ferreira *et al* (2011) reported differences in propagule growth in *Dendrobium* due to the effect of various quantities of sucrose in the culture medium.

Plants established in the Knudson (1946) media

Orchid growth depends on the concentrations of solutes present in the culture medium. In this study, the plants that had faster growth were established in KN media similar to the results of Rodrigues *et al.* (2009) who found that *Cattleya loddigesii* (Lindl.) plants established in KN-200% had faster growth and the plants in KN-100% achieved better shoot proliferation. Seeds of *Cyrtopodium saintlegerianum* (Carlos-de Sousa *et al*., 2017) cultured in the media with KN mineral salts had a higher germination rate (80%) at seven days, and the plants grew taller and had longer shoots than the plants grown in the culture media with MS salts.

Culture media with KN-100% influence seed germination and later plant growth. Ferreira and Suzuki (2008) found that the KN culture medium improved *Hadrolaelia tenebrosa* (Rolfe) Chiron & V.P. Castro seed germination. In the case of *Cyrtopodium saintlegerianum* Rchb.f seeds established in KN media (Afraire *et al*., 2015), *Cattleya* (Lindl.) seeds in MS culture media (Schneiders *et al*., 2012), *Coelogyne flaccida* (Lindl.) and *Dendrobium lasianthera* J.J.Sm, shoot development occurred in the culture media with Vacin and Went mineral salts complemented with 2 g L^{-1} peptone (Utami *et al*., 2017).

Plants established in the Murashige and Skoog (1962) media

Plants grown in the culture medium with MS-66% mineral salts achieved better values in most of

the variables, except the number of leaves, than plants grown in the culture media with MS-100%. The formulation MS-100 has 1.51 and 1.87 times more total ions than the formulations MS-66% and KN-100%. These results agree with the study of Sabarimuthan *et al*. (2013), who reported that *Eulophia cullenii* (Wight) Bl. grew vigorously and produced roots in the media with MS diluted by half. In the case of propagation of *Cyrtopodium brandonianum* Barb.Rodr (Flachsland *et al.,* 2011) and *Cymbidium bicolor* Lindl. (Mahendran & Bai, 2012), the plants established in the culture media with diluted MS mineral salts grew faster and had more roots than the plants grown in the culture medium with MS at full concentration. In propagation of *D. densiflorum* Lindl. ex Wall. (Jian-Ping *et al*., 2008) development of protocorm-like bodies (PLB) of *Cymbidium aloifolium* (L.) Sw. and *Dendrobium nobile* (Nayak *et al*., 2001), the growth of the propagules was better in the case of a combined effect of the MS mineral salts and BAP.

Shoot and rhizome development of *Geodorum densiflorum* was obtained in culture media with MS mineral salts and BAP (Roy & Banerjee, 2001). Formation of the pseudobulbs of *Cymbidium finlaysonianum* Lindl. was stimulated in the culture media with MS mineral salts supplemented with 1.0 to 1.5 mg L^{-1} BAP; the culture medium with 0.75 mg L^{-1} NAA stimulated multiplication and size of the shoots (Islam *et al*., 2015). In *Dendrobium*, PLB were formed and later development of the shoots and rooting occurred in the culture medium with MS mineral salts and 44.4 µM BAP and 6.97 µM KIN (Martin & Madassery, 2006), while *Eulophia nuda* Lindl. shoots multiplied when they were established in the culture media with MS and BAP (Dawande & Gurav, 2015). The orchid *Laelia halbingeriana* grows slowly, and the results of our study suggest that it is possible to accelerate plant growth by modifying not only GR but also the mineral salt composition.

The culture media with KN 100% and KN 66% mineral salts have a total ions of 55.92 and 36.90 meq L^{-1}, which correspond to the osmotic potentials, OP, of -0.122 and -0.080 MPa; the plants grew better in these media than the plants cultured in media with MS 100% mineral salts. The data reported by Cárdenas and Villegas (2002) show that an increase in the concentration of solutes results in more negative osmotic potential of the culture medium and therefore plant growth is inhibited. The MS inorganic salts at 100% concentration generate an OP of -0.23 MPa, and 30 g l^{-1} (87.7 mM) of sucrose generates an OP of -0.45 MPa; the combination of these factors in the culture medium creates an OP of -0.68 MPa (Miguel-Luna *et al*., 2014).

The models of logarithmic regression that describe vertical plant growth as a function of time show that the plants in the T16 culture medium had an average growth rate of 0.25 cm/month during 120 days of incubation. The orchids grew slowly, and the nonlinear regression models were useful for describing their growth and predicting the behavior of the species (Rodríguez-Ortiz *et al*., 2012). The logarithmic fit model describing the vertical growth during 120 days has a suitable goodness of fit ($R^2 \geq 0.96$). The regression models are useful for describing and predicting growth over a given time; thus, these models are frequently used for forest species in the field (Santiago-García *et al*., 2015). However, these models are also useful for description of the behavior of the *in vitro* cultures of *Laelia halbingeriana*.

The type and concentration of mineral salts is an important condition to improve *in vitro* growth of *Laelia halbingeriana* plants. Culture media with Knudson (1946) mineral salts provided better conditions for plant growth compared to those obtained in the culture media with Murashige and Skoog mineral salts. The plants established in the culture media with inorganic Knudson salts at 100 and 66% supplemented with 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} KIN and 0.5 mg L^{-1} IAA showed faster growth and development. The mixtures of the GR did not have differential effects on the growth and development of *Laelia halbingeriana*.

Resumen

R. García-González, J.R. Enríquez-del Valle, G. Rodríguez Ortiz, G.V. Campos-Angeles, E.A. Pérez-García, y J. Ruiz-Luna. 2020. Sales minerales y reguladores de crecimiento para la micropropagación de *Laelia halbingeriana* Salazar & Soto Arenas. Int. J. Agric. Nat. Resour. 105-116. *Laelia halbingeriana* es una orquídea epífita silvestre, endémica de Oaxaca, México, colectada sin planes de manejo, y este trabajo tuvo el objetivo de propagar y evaluar el desarrollo *in vitro* de esta especie en medios de cultivo que variaron en sales minerales y reguladores de crecimiento. Grupo de 3 plantas de 1.2 a 1.4 cm de altura se establecieron en cada frasco con medios de cultivo que tenian 30 g L^{-1} de sacarosa, 1 mg L^{-1} de tiamina, 100 mg L^{-1} de inositol y 0.5 mg L^{-1} de bencilaminopurina, BAP, variando las sales minerales de Murashige y Skoog (1962) o Knudson (1946) (100, 66 y 33%) los reguladores de crecimiento: ácido indolacético, AIA, y kinetina, Kin, y su concentración (0.5+0.5 mg L^{-1}, 1+1 mg L^{-1} y 2+2 mg L^{-1}). El pH se ajustó a 5.8, y agregó 5.5 g L^{-1} de agar. Transcurridos 120 días de incubación, las plantas que estuvieron en medios Knudson 100 y 66 %, tuvieron 2.1 de altura y 4.8 raíces, mientras las plantas en medios Murashige y Skoog 100 % tuvieron 1.5 de altura y 2.5 raíces. Durante 120 días, las plantas mostraron crecimiento en altura con una tendencia logarítmica ($R^2 \geq 0.96$). Al evaluar simultáneamente varias características de plantas mediante análisis clúster, se determinó que el mejor medio de cultivo para el desarrollo de *Laelia halbingeriana* tenía las sales minerales Knudson al 100%, 0.5 mg L^{-1} de BAP, 0.5 mg L^{-1} de AIA, 0.5 mg L^{-1} de KIN.

Palabras clave: Desarrollo, propagación, reguladores de crecimiento, sales minerales.

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