Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Article

Culture media for seed germination and seedling growth of *Laelia autumnalis*

Marcela Cabañas-Rodríguez[1]
María Andrade-Rodríguez[1,§]
Oscar Gabriel Villegas-Torres[1]
José Antonio Chávez-García[1]
Iran Alia-Tejacal[1]
Porfirio Juárez-López[1]

1 Facultad de Ciencias Agropecuarias. Universidad Autónoma del Estado de Morelos. Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos, México. CP. 62209. (marcela.cabanasr@uaem.edu.mx; iran.alia@uaem.mx; antonio.chavez@uaem.mx; porfirio.juarez@uaem.mx; oscar.villegas@uaem.edu.mx).

Autora para correspondencia: maria.andrade@uaem.mx

Abstract

The orchid *Laelia autumnalis* is endemic to Mexico and is under special protection. *In vitro* culture has contributed to improving the propagation of orchids from seeds. In order to micropropagate this species, an experiment was established in 2023 to evaluate eight culture media: Murashige and Skoog (MS at 100, 75, 50 and 25%), Knudson C, Dalla Rosa and Laneri, Vacint and Went, and Lindemann; all media were supplemented with 0.5 mg L^{-1} thiamine HCl, 100 mg L^{-1} myo-inositol and 3% sucrose to determine the best medium for germination of this species. Seeds were disinfected with 0.3% sodium hypochlorite (NaClO) and sown in the media in a completely randomized design with 10 replications per treatment. Germination start and percentage were recorded; seedling height, leaf length and number, root number and length, and pseudobulb diameter were evaluated at 90 days. The 25 and 50% MS media generated a higher percentage of germination (84.57 and 72.54%, respectively), which began 29 days after seeding. Growth in height (5.6 mm), leaf length (4.54 mm) and root length (5.2 mm) were higher in the MS medium at 50%, where seedlings were green. It was concluded that the best culture medium for germination and growth of *L. autumnalis* seedlings was the MS at 50% concentration.

Keywords:

day of the dead orchid, MS medium, orchid seedlings.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Introduction

The Orchidaceae family is one of the most important in Mexico, surpassed only by the Asteraceae and Fabaceae families (Villaseñor, 2016). Mexico ranks eleventh in the world in terms of richness of wild orchids (Hágsater *et al*., 2005), with around 1 315 species (Soto *et al*., 2007; Solano *et al*., 2019).

At the state level, Oaxaca and Chiapas rank first in orchid diversity, both with more than 700 species (Solano *et al*., 2019), and Veracruz is in third place with 432 taxa (421 species, 4 varieties, 2 hybrids and 5 subspecies), representing 32% of the country's orchids.

Laelia is a genus of mostly epiphytic orchids, so they are found living on trees; in Mexico, 12 species and 2 subspecies have been classified; one of them is *Laelia autumnalis*, native to Mexico, whose wild populations have been subject to change in land use and massive and illegal extraction of plants in the reproductive stage to meet market demand.

If this activity continues, it will soon be part of the list of threatened species (Hernández *et al*., 2013). Due to the magnitude of the extraction of plants of this species, in the update of NOM-059-SEMARNAT-2010, it appears in the category of special protection (Pr) (DOF, 2019).

Orchids are species that are difficult to reproduce naturally because their tiny seeds have little or no nutrient reserves and require symbiosis with mycorrhizal fungi for germination, which are not always present due to the disturbance of their habitat. In addition, not all seeds in a capsule are fully formed or fertile (Verma *et al*., 2014).

These plants stand out for their beauty and attractive shapes; however, they are one of the families most affected by anthropocentric activities, plundering, and illegal trade (Castillo-Pérez *et al*., 2018). *In vitro* culture techniques have contributed to improving the propagation of orchids from seeds, both in native species and in hybrids.

In the genus *Laelia*, *in vitro* germination has been achieved in *L. speciosa* using the Murashige and Skoog (MS) culture medium at 50 and 100% of its components (Murashige and Skoog, 1962) and the Knudson C (KC) medium at 50 and 100% of its components (Knudson, 1946) (Aguilar and López Escamilla, 2013), in *L. anceps* ssp. *Dawsonii*, in which the formation of somatic embryos was achieved using the culture media of Knudson C (1946) (KC), Vacin and Went (1949) (VW), Murashige and Skoog (1962) (MS) (Lee *et al*., 2010), and in *L. eyermaniana*, where morphological development was observed from germination to *ex vitro* acclimatization in Murashige and Skoog (MS) culture medium at 50% (Francisco *et al*., 2011).

For the germination of orchid seeds, some means of widespread use are reported, such as Knudson C (1946), Vacin and Went (1949), Murashige and Skoog (1962), Dalla Rosa and Laneri (1977) (Dutra *et al*., 2009; De Menezes *et al*., 2016). The main difference in these is the composition of minerals, the simplest is Knudson C and the richest in nutrients is Murashige and Skoog, which includes macro- and microelements; the latter is the most commonly used for orchid cultivation; Lallana *et al*. (2020) point out that 50% MS has given good results for the most common genera.

The composition of the culture medium is one of the factors that determine the success of generating and propagating seedlings, every medium is basically composed of macronutrients, micronutrients, vitamins, amino acids and a source of carbon (De Menezes *et al*., 2016). Therefore, the composition of culture media can vary in content of salts (macro and microelements), sugars, organic compounds, and gelling agents. The requirements for orchids can be diverse, even in species of the same genus (Mayo *et al*., 2010).

Based on the importance of this orchid species and the situation it has in its habitat, the present research aimed to evaluate the *in vitro* germination of *Laelia autumnalis* seeds in eight culture media, as well as the initial growth of seedlings, to determine the most suitable for this species.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Materials and methods

This research was conducted in the Plant Propagation Laboratory of the Faculty of Agricultural Sciences, Autonomous University of the State of Morelos, Cuernavaca, Morelos, Mexico, from September 2023 to January 2024.

Biological material

L. autumnalis capsules were collected from plants under cultivation belonging to a private producer in Tepoztlán, Morelos, Mexico. The seeds were dried at room temperature (29 °C), stored in airtight plastic tubes and kept in a refrigerator at 5 °C for 20 days.

Culture media

Mineral salts from eight culture media commonly used for the germination of orchid seeds were employed: Murashige and Skoog (1962), in four concentrations, 25 (MS25), 50 (MS50), 75 (MS75), and 100% (MS100) of macro and micronutrient concentration; Knudson C (1946) (KC); Vacin and Went (1949) (VW); Lindemann (1970) (L); Dalla Rosa and Laneri (1977) (DRL) (Table 1).

Table 1. Formulation of the culture media used in the *in vitro* culture of orchids, according to Mayo *et al*. (2007).

Compound (mg L^{-1})	Murashige and Skoog (1962)	Knudson C (1946)	Vacint and Went (1949)	Lindemann (1970)	Dalla Rosa and Laneri (1977)
NH_4NO_3	1 650				
KNO_3	1 900		525		
$CaCl_2$ $2H_2O$	332.2				1000
$MgSO_4$	370	250	122.1	58.62	250
KH_2PO_4	170	250	250	135	250
$(NH_4)_2$ SO_4		500	500	1 000	500
$Ca(NO_3)_2$ $4H_2O$		1 000		347.2	
H_3BO_3	6.2			1.014	
$MnSO_4$ $4H_2O$	22.3	5.682	5.68	0.05	7.5
$ZnSO_4$ $7H_2O$	8.6			0.565	
Na_2 MoO_4	0.25				
$CuSO_4$ $5H_2O$	0.025			0.019	
$CoCl_2$ $6H_2O$	0.025				
KI	0.83			0.099	
$FeSO_4$ $7H_2O$	27.85	25			27.85
Na_2EDTA $2H_2O$	37.27			1 050	37.25
Ferric tartrate			28		
Tribasic calcium phosphate			200		
Nickel chloride				0.03	
Ferric citrate				4.4	

All media were supplemented with 0.5 mg L^{-1} thiamine HCl, 100 mg L^{-1} myo-inositol, and 3% sucrose; the pH of the media was adjusted to 5.7. Likewise, 0.7% agar was added. The medium was dosed into 150 ml bottles, placing 15 ml of culture medium and sterilizing for 18 min at 121 °C and 15 lb pressure.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Seed disinfection

Before sowing, the seeds of *L. autumnalis* were disinfected in the laminar flow hood, using the syringe method, which consisted of the following: a piece of cotton was placed in the nozzle of a 20 ml syringe, the seeds were put inside the syringe and the disinfectant solution with 0.3% sodium hypochlorite (NaClO) and sterilized distilled water were added, then the plunger was placed and the syringe was shaken vertically for 5 min, three rinses were made with sterile distilled water and finally, 20 ml of sterile distilled water was added to the syringe to keep the seeds suspended.

Sowing

Each of the bottles with culture medium was added with 250 µl of water with approximately 250 suspended seeds, which were counted with a hemocytometer. The bottles were covered and sealed with adhesive plastic, and finally, they were placed in the incubation area under controlled conditions with a photoperiod of 16 h light and 8 h dark at 24 ±1 °C and luminous intensity of 32 µE m^{-2} s^{-1}.

Experimental design

A completely randomized design with 10 replications per treatment was used to assess seed germination and seedling growth (10 seedlings per replication). Each replication was a bottle of culture.

Variables evaluated and statistical analysis

Seed germination

Two germination variables were evaluated; the start of germination was considered once the seed showed rupture of the testa; the percentage of germination was also determined, the germinated seeds were counted one month after sowing in four fields by replication with a stereoscopic microscope.

Seedling growth

Ten seedlings were taken per replication to assess growth; height (mm) was measured from the base to the part where the leaves open; leaf length (mm) was measured from the base to the leaf tip; root length (mm) was measured from the base to the root tip; these three variables were measured with a piece of millimeter graph paper; number of leaves and number of roots were counted; protocorm diameter (mm) was also measured. The data obtained were studied by analysis of variance (Anova); in the variables where there was an effect of the treatments, Tukey's comparison of means test ($p \leq 0.05$) was performed with the SAS v. 9.2 statistical package (SAS, 2023).

Results and discussion

Seed germination

The analysis of variance for the start of germination and total germination indicated that there was a highly significant effect of the culture media ($p \leq 0.01$). The R^2 values were 0.96 and 0.88, indicating that the statistical model used for the analysis of variance was adequate (Table 2).

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i6.3811

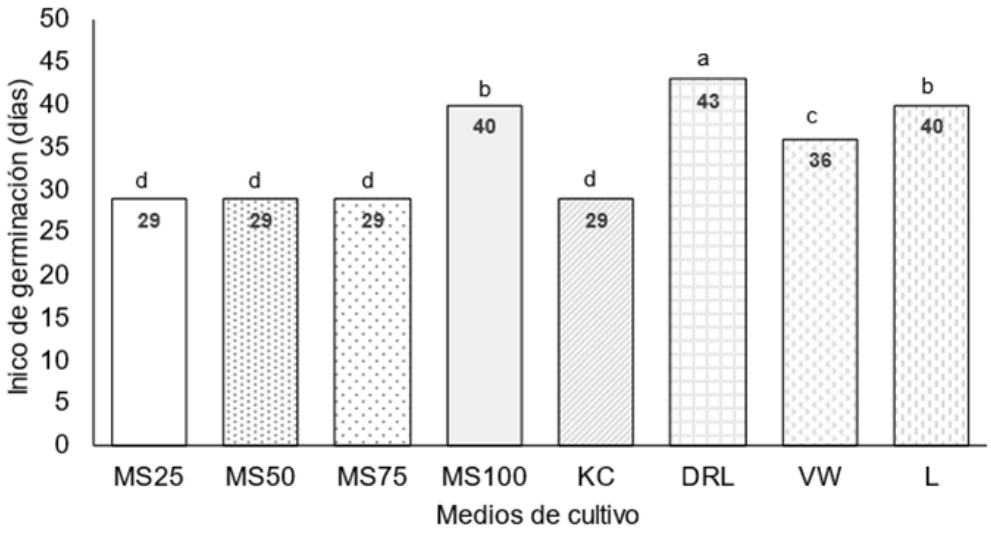
Table 2. Mean squares of the analysis of variance for start of germination and total germination in eight media of *in vitro* culture of *L. autumnalis*.

Source of variation	DF	Start of germination (days)	Total germination (%)
Culture media	7	365.53**	3 328.79**
Error	72	1.44	93.61
R^2		0.96	0.88
CV (%)		3.49	24
Average		34.37	40.31

DF= degrees of freedom; CV= coefficient of variation; R^2= coefficient of determination; **= highly significant effect ($p \leq 0.01$).

The germination of the seeds was observed with the naked eye when they changed from cream to green structures and the size of the embryo increased, later the embryo broke the testa, which could be observed under a microscope. This process varied depending on the culture media; seeds began germinating in less time in the MS media at 25, 50, 75% and the KC medium (at 29 days), whereas in the MS at 100%, DRL, VW and L media, it occurred after 35 days; in DRL, it took 14 days longer (Figure 1).

Figure 1. Start of germination of *L. autumnalis* seeds by effect of eight culture media. HMSD= 1.67.



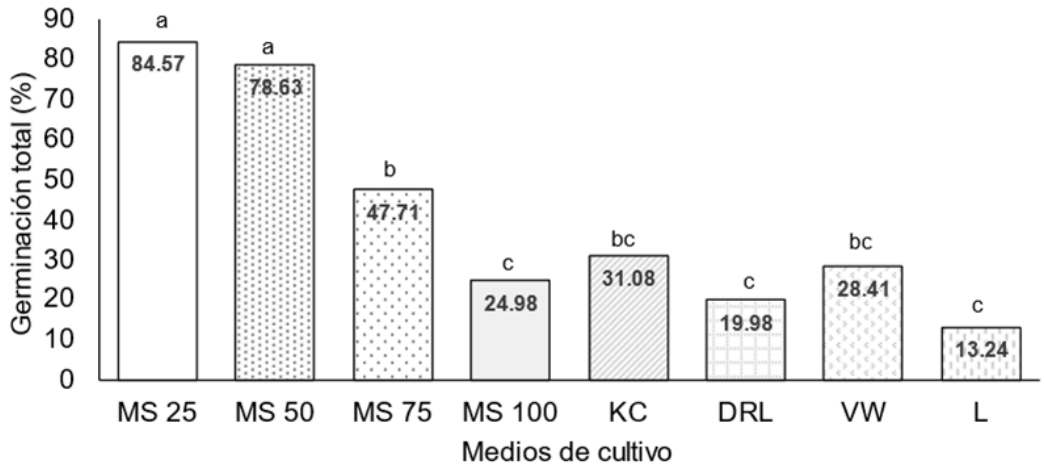
In this species, seed germination was a little slower compared to what was reported by Aguilar and López Escamilla (2013) for *Laelia speciosa*, which germinated in 10 and 16 days in 50 and 100% MS media; nevertheless, they indicate that in 50 and 100% KC media, germination occurred at 114 and 178 days, respectively. This indicates that the germination of orchid seeds varies depending on the culture medium and species.

The percentage of germinated seeds of *L. autumnalis* was higher when the macro- and micronutrients of the MS medium were used at 25 and 50% concentration, with no statistical differences between both culture media (84.57 and 78.63%, respectively); the tendency was to decrease germination as the concentration of salts in the MS medium increased, this happened because there was greater availability of water for the process to occur and as Martínez-Villegas *et al*. (2015) mention, as salts increase, the osmotic potential becomes more negative.

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v16i6.3811

In the VW, KC, and 75% MS media, germination ranged from 28.41 to 47.71%. The media with the lowest germination values were 100% MS, DRL and L, which presented 59.59, 64.59 and 71.33% less germination than that obtained with the MS medium at 25% concentration (Figure 2).

Figure 2. Effect of culture media on total germination of *L. autumnalis* seeds. HMSD= 19.82.



After the change in green, small protocorms formed in the seeds, from which the first small leaf was observed. Growth was also observed with the emission of new leaves, the increase in height and thickness of stems and the growth of the rhizoid, thus forming a seedling. Seeds germinated in the 25% MS medium generated yellowish-green seedlings, indicating nutrient deficiency due to low macro- and micronutrient concentration.

Seedling growth

Seeds germinated in the Vacin and Went (1949) and Lindemann (1970) media did not generate seedlings, so they were excluded from the growth results. The analysis of variance for the growth variables of seedling height, leaf length, number of leaves, number of roots, root length, and diameter of pseudobulbs showed that the composition of the culture media had a highly significant effect ($p \leq 0.01$).

The coefficients of variation were from 18.13 to 22.42, the values of R^2 were from 0.88 to 0.94 (Table 3), indicating that the statistical model used for the analysis of variance was adequate. The height of the seedlings was higher in the MS media at concentrations of 25 to 75%; in contrast, the height was lower when any of the other media was used. The length of leaves was longer in the 50 and 75% MS media, and the culture media that led to lower growth were MS at 100% and KC.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Table 3. Mean squares of the analysis of variance for the variables of *in vitro* growth of *L. autumnalis* seedlings, three months after planting.

SV	DF	SH	LL	NL	NR	RL	PD
Culture media 5		51.46**	33.25**	16.53**	2.53**	51.14**	3.64**
Error	54	0.64	0.27	0.13	0.02	0.26	0.04
R^2		0.88	0.92	0.92	0.89	0.94	0.89
CV (%)		22.42	18.31	18.13	22.22	18.51	21.41
Average		3.57	2.86	2.05	0.76	2.79	0.96

DF= degrees of freedom; CV= coefficient of variation; R^2= coefficient of determination; **= highly significant effect ($p \leq 0.01$); SH= seedling height; LL= leaf length, NL= number of leaves; NR= number of roots; RL= root length; PD= pseudobulb diameter. N= 100 seedlings per treatment.

The number of leaves per seedling was also affected by the culture medium; seedlings growing in the DRL medium had the highest number of leaves (3.03 mm per seedling), which were statistically different only from seedlings growing in the MS medium at 25%, which had the lowest number.

The diameter of pseudobulbs was statistically the same with most of the media, except for seedlings that grew in the MS medium at 25%, which had the lowest values (Table 4). The average number of roots per seedling ranged from 0.75 to 1.24. The KC, MS75 and MS50 media were statistically equal, with the highest root average (Figure 3). The lowest number of these organs occurred in the MS100 medium. Root length was greater in the 50% MS medium, with no statistical difference from the KC and 25% MS media, and the 100% MS medium had the lowest values (Table 4).

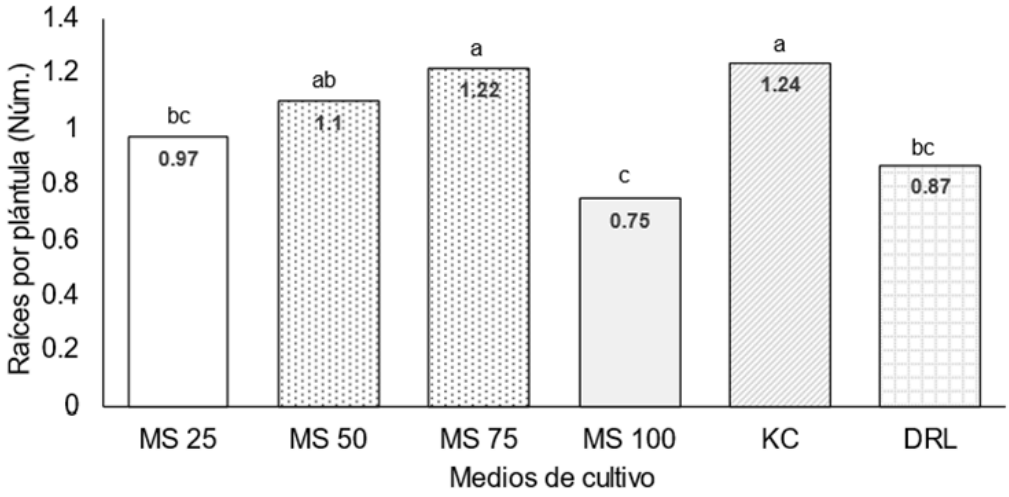
Table 4. Effect of culture media on growth variables of *L. autumnalis* seedlings, three months after planting.

Culture medium (%)	Seedling height (mm)	Leaf length (mm)	Leaves (num.)	Protocorm diameter (mm)	Root length (mm)
MS25	4.79 a	3.77 bc	2.31 b	1.09 b	4.64 a
MS50	5.6 a	4.54 a	2.87 a	1.3 ab	5.2 a
MS75	5.32 a	4.33 ab	2.92 a	1.3 ab	3.89 b
MS100	4.04 b	3.14 c	2.75 ab	1.49 a	0.66 d
KC	4.13 b	3.4 c	2.52 ab	1.21 ab	5.08 a
DRL	4.71 ab	3.7 bc	3.03 a	1.29 ab	2.89 c
HMSD ($p \leq 0.05$)	1.12	0.73	0.52	0.29	0.72

HMSD= means with the same letter in each column are statistically equal according to Tukey's test ($p \leq 0.05$). N= 100 seedlings per treatment.

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v16i6.3811

Figure 3. Effect of culture media on the number of roots of *L. autumnalis*, three months after planting. HMSD= 0.23.



The general analysis of the results obtained for the various variables allows us to point out that, for the *in vitro* germination of *L. autumnalis* seeds, the 25, 50 and 75% MS culture media and the KC medium were where germination began in the shortest time; nevertheless, the total percentage was higher in the MS media at 25 and 50%. Regarding seedling growth, most variables indicate that it was better and similar in MS media at 25, 50 and 75%.

Nonetheless, seedlings were green in the 50% MS medium; in contrast, seedlings that grew in the 25 and 75% MS media began to show violet or reddish color after 107 days. Therefore, it is considered that the best culture medium for germination and growth of *L. autumnalis* seedlings was MS at 50% nutrient concentration. Dalzotto (2013) reported that the plant height, number of roots, and dry matter of *Oncidium bifolium* were higher in the 50% MS medium, which coincides with what was obtained in the present research.

Murashige and Skoog (1962) medium has been used in the germination of several species of orchids in different proportions and supplemented with growth regulators and other substances. The results obtained regarding the beginning of germination and total germination of this research could be due to the fact that the Murashige and Skoog medium contain the necessary macronutrients and micronutrients compared to the other media.

The study by Aguilar and López Escamilla (2013) used *L. speciosa* seeds and reported that germination occurred 10 days after sowing in 50% MS medium and 16 days after in 100% MS medium, and germination was 100% in both cases, which coincides with the results obtained in this work since the 50% MS medium was the best for the germination of *L. autumnalis* seeds.

For their part, Hernández *et al*. (2017) used capsules of *Laelia autumnalis* which were irradiated with gamma rays and were later grown in Murashige and Skoog (1962) culture medium and observed that the imbibition process occurred between 5 and 40 days and the formation of photosynthetic protocorms began at 10 days and concluded until 60 days.

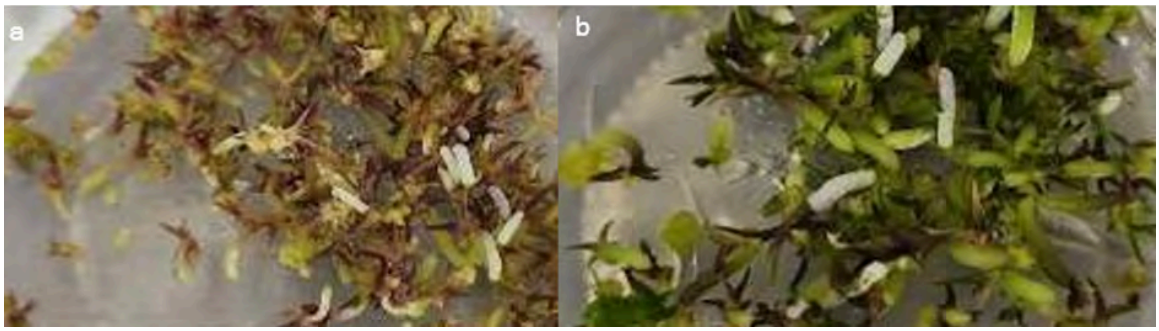
Findings of Francisco *et al*. (2011) did not report the days to germination of *Laelia eyermaniana*; however, the culture medium used to germinate the seeds was Murashige and Skoog at 50%. Jara *et al*. (2007) used 50% and 100% MS, KC and Morel media for the germination of *Chloraea virescens*, *Chloraea lamellate*, and *Gavilea araucana* and report that the 50% MS medium gave the best results.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

In their research, Ávila *et al*. (2009) germinated seeds of *Laelia speciosa* using 100 and 50% Murashige and Skoog (1962) and Knudson C (KC) media added with sucrose, and report that the 100 and 50% MS media generated the best response in seed establishment for this species since the first stages of development were better in the MS medium compared to the KC medium.

Regarding seedling height, leaf length, number of leaves, root length, number of roots, root length and pseudobulb diameter, it was observed that the 25% MS medium was not suitable for seedling growth. It is important that the culture media have the right amount of nutrients so that the seedlings do not present nutritional deficiency and have better development, as occurred in the 25% MS medium (Figure 4).

Figure 4. Growth of *L. autumnalis* seedlings. a) medium MS (25%) and b) medium MS (50%).



The Vacin and Went (1949) and Lindemann (1970) media were the least suitable for the germination of *L. autumnalis* since they took longer to germinate; likewise, in the percentage of germination, they presented the lowest values compared to the other media; on the other hand, the seeds died as time went by so there was no growth of seedlings.

Conclusions

Based on the results of this work, *in vitro* germination of *L. autumnalis* seeds was better in the MS culture media at 25 and 50% since the start of germination occurred in less time and in a higher percentage. Seedling growth was best in the 50% MS medium, in which seedlings were green. Therefore, the best growing medium for germination and growth of *L. autumnalis* seedlings was MS at 50% nutrient concentration.

Acknowledgements

To the National Council of Humanities, Sciences and Technologies (CONAHCYT), for its acronym in Spanish for the scholarship to the master's student Marcela Cabañas-Rodríguez.

Bibliography

1 Aguilar-Morales, M. A. y López-Escamilla, A. L. 2013. Germinación *in vitro* de *Laelia speciosa* (Kunth) Schltr., una herramienta para su conservación *ex situ*. *In*: estudios científicos en el estado de Hidalgo y zonas aledañas. Pulido F. G. y Monks S. (eds.) USA. Zea Books, University of Nebraska. Volumen II. 18-24 pp. https://digitalcommons.unl.edu/hidalgo.

2 Ávila-Diaz, I.; Oyama, K.; Gómez-Alonzo, C. and Salgado-Garciglia, R. 2009. *In vitro* propagation of the endangered orchid *Laelia speciosa*. Plant Cell Organ Culture. 99(3):332-343. Doi:10.1007/s11240-009-9609-8.

3 Castillo-Pérez, L. J.; Martínez-Soto, D.; Maldonado-Miranda, J. J.; Alonso-Castro, A. J. and Carranza-Álvarez, C. 2018. The endemic orchids of Mexico: a review. Eslovaquia. Biología. 74:1-13. 10.2478/s11756-018-0147-x.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

4 Dalla-Rosa, M. and Laneri, U. 1977. Modification of nutrient solutions for germination and growth "*in vitro*" of some cultivated orchids and for the vegetative propagation of *Cymbidium* cultivars. American Orchid Society Bulletin. 46(9):813-820.

5 Dalzotto, C. A. 2013. Efecto de medios de cultivo en el crecimiento *in vitro* de *Oncidium bifolium* sims. "federal". Revista Científica Agropecuaria. 17(1-2):7-15.

6 De Menezes, G. L.; Machado, M. F.; Ballesta, P.; Mora, F.; Milaneze, G. M. A. y Mangolin, C. A. 2016. Suplementos orgánicos para el cultivo *in vitro* del híbrido *Laeliocattleya* (Orchidaceae). Chile. Idesia (Arica). 34(1):47-54. https://dx.doi.org/10.4067/S0718- 34292016000100006.

7 DOF. 2019. Diario Oficial de la Federación. Modificación del anexo normativo III, lista de especies en riesgo de la Norma Oficial Mexicana NOM-059-SEMARNAT-2010, protección ambiental-especies nativas de México de la flora y fauna silvestre- categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo, publicada.

8 Dutra, D.; Kane, M. and Richardson, L. 2009. Asymbiotic seed germination and *in vitro* seedling development of *Cyrtopodium punctatum*: a propagation protocol for an endangered Florida native orchid. USA. Plant Cell Tissue and Organ Culture. 96(3):235-243. 10.1007/s11240-008-9480-z.

9 Francisco-Nava, J. J.; Jiménez-Aparicio, A. R.; De Jesús-Sánchez, A.; Arenas-Ocampo, M. L.; Ventura-Zapata, E. y Evangelista-Lozano, S. 2011. Estudio de la morfología y aclimatación de plantas de *Laelia eyermaniana* Rchb. f. generadas *in vitro*. Polibotánica. 32(16):107-117.

10 Hágsater, E.; Soto-Arenas, M. Á.; Salazar-Chávez, G. A.; Jiménez-Machorro, R.; López-Rosas, M. A. y Dressler, R. L. 2005. Orquídeas de México. México. Instituto Chinoín México, . 304 p.

11 Hernández-Muñoz, S.; Pedraza-Santos, M. E.; Morales-García, J. L.; Guillén-Andrade, H.; López, P. A. and Téllez-Velasco, M. A. A. 2013. Phenotypic characterization of Mexican orchid *Laelia autumnalis*. Bélgica. Acta Horticulturae. 977:245-252. 10.17660/ActaHortic.2013.977.28.

12 Hernández-Muñoz, S.; Pedraza-Santos, M. E.; López P. A.; Cruz-Torres, E.; Martínez-Palacios, A.; Fernández-Pavía, S. P. y Chávez-Bárcenas A. T. 2017. Estimulación de la germinación y desarrollo *in vitro* de *Laelia autumnalis* con rayos gamma. México. Revista Fitotecnia Mexicana. 40(3):271-283.

13 Jara, G.; Seemann, P.; Durán, C. y Soto, S. 2007. Multiplicación *in vitro* y caracterización citológica de dos especies de orquídeas nativas (*Chloraea* y *Gavilea*) de la provincia de Valdivia, Chile. Agro Sur. 35(2):43-44. http://revistas.uach.cl/pdf/agrosur/v35n2/art21.pdf.

14 Knudson, C. 1946. A new nutrient solution for germination of orchid seed. American Orchid Society Bulletin. 15(10):214-217.

15 Lallana, V. H.; Billard, C. E.; Reinoso, P. D.; Martínez, V. A. y García, L. F. 2020. Banco de germoplasma de orquídeas nativas de la región litoral. Suplemento Ciencia. Docencia y Tecnología. 10(10):84-110.

16 Lee-Espinosa, H. E.; Laguna-Cerda, A.; Murguía-Gonzáles, J.; Iglesias-Andreu, L.; García-Rosas, B.; Escobedo-López, D.; Martínez-Ocampo, Y. M.; Barredo-Pool, F. A. y Santana-Buzzy, N. 2010. Un protocolo de embriogénesis somática para la regeneración y caracterización *in vitro* de *Laelia anceps* ssp. *Dawsonii*. México. Revista Fitotecnia Mexicana. 33(4):323-332. https://www.scielo.org.mx/pdf/rfm/v33n4/v33n4a10.pdf.

17 Lindemann, E. G. P.; Guncke, J. E. and Davidson, W. O. 1970. Meristem culture of *Cattleya*. American Orchid Bulletin. 39(11):1002-1004.

DOI: https://doi.org/10.29312/remexca.v16i6.3811

18 Martínez-Villegas, Y. M.; Andrade-Rodríguez, M.; Colinas-León, M. T.; Villegas-Torres, O. G.; Castillo-Gutiérrez, A. and Alia-Tejacal, I. 2015. Efecto de las sales inorgánicas del medio de cultivo en el crecimiento de pascuita (*Euphorbia leucocephala* Lotsy). Revista Fitotecnia Mexicana. 38(4):369-374.

19 Mayo M. A.; Cazares, J. G. C.; Cruz, E. L. y Flores, A. H. 2010. Germinación *in vitro* de semillas y desarrollo de plántulas de orquídeas silvestres de Tabasco. Universidad Juárez Autónoma de Tabasco. 32 p.

20 Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. USA. Physiologia Plantarum. 15(3):473-497. 10.1111/j.1399-3054.1962.tb08052.x.

21 Solano, G. R.; Salazar, C. G. A.; Huerta, E. H.; Hágsater, E. and Jiménez, M. R. 2019. Diversity of Mexican orchids: synopsis of richness and distribution patterns. Guayaquil. Proceedings of the 22th World Orchid Conference. 255-270 pp.

22 SAS Institute Inc. 2023. SAS/STAT User #s guide, Version 9,2. SAS Institute. Cary, NC.

23 Soto, A. M. A.; Solano, G. R. and Hágsater, E. 2007. Risk of extinction and patterns of diversity loss in Mexican orchids. Costa Rica. Lankesteriana. 7(1-2):114-121.

24 Verma, J.; Sharma, K.; Thakur, K.; Sembi, J. K. and Vij, S. P. 2014. Study on seed morphometry of some threatened western himalayan orchids. Turquía. Turkish Journal of Botany. 38(2):234-251. https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article=1560&context=botany.

25 Villaseñor, J. L. 2016. Checklist of the native vascular plants of Mexico. México. Revista Mexicana de Biodiversidad. 87(3):559-902. https://doi.org/10.1016/j.rmb.2016.06.017.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i6.3811

Culture media for seed germination and seedling growth of *Laelia autumnalis*

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 May 2025
Date accepted: 01 August 2025
Publication date: 01 October 2025
Publication date: Aug-Sep 2025
Volume: 16
Issue: 6
Electronic Location Identifier: e3811
DOI: 10.29312/remexca.v16i6.3811

Categories

Subject: Articles

Keywords:

Keywords:

day of the dead orchid
MS medium
orchid seedlings

Counts

Figures: 4
Tables: 4
Equations: 0
References: 25
Pages: 0