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Physiological development of red anthurium (*Anthurium andreanum* Linden) var. Tropical in three *in vitro* culture systems

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ABSTRACT

Objective: To evaluate the physiological development of red *Anthurium andreanum* L. var. Tropical in three *in vitro* culture systems: semi-solid, partial immersion and RITA® bioreactor.

Design/methodology/approach: A completely randomized design with three treatments, semi-solid medium, partial immersion and RITA® bioreactor, and four repetitions each was used. Vitroplants of anthurium were selected with a size of 0.5 cm from the stem to the highest leaf, with three leaves in each specimen. Morphometric, chlorophyll content and hormone content analyses were carried out after 60 days of sowing. Analysis of variance and means comparison tests were performed on the data obtained through Kruskal-Wallis and Tukey, respectively, using the statistical software R-STUDIO.

Results: The highest shoot rate and root length were obtained in partial immersion; however, the number of leaves, shoots and root multiplication did not show differences with the RITA® bioreactors. The highest concentration of chlorophylls and indole acetic acid was observed when using RITA® bioreactors.

Study limitations/implications: The results are favorable for the in vitro production of anthurium, although the use of RITA® bioreactors for commercial production is a high cost in the initial investment.

Findings/conclusions: With the results obtained, it is considered that the RITA® bioreactors obtained the best results for the production of anthurium, followed by the partial immersion system. This is due to the liquid medium and better gas exchange, which favors the development of plants.

Keywords: *Anthurium andreanum* L.; chlorophyll; phytohormones; immersion systems.

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INTRODUCTION

The bract of the anthurium plant is highly valued in the ornamental market, due to its beauty and vase life; in addition, the leaves are also marketed as foliage (Mireles-Ordaz *et al.*, 2015). The flower stems reach prices of $25 (SNIIM, 2019). The optimal temperature for the development of this plant is 20 to 35 °C and relative moisture between 70 and 80%. Its production requires for the climate requirements of light,

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height, temperature, etc., to be fulfilled (Gayosso-Rodríguez *et al.*, 2013). In Mexico, the central region of the state of Veracruz has the soil-climate conditions that are apt for its cultivation (García-Alonso *et al.*, 2014).

Traditionally, anthurium is propagated by seed and stem segmentation; however, these techniques are slow and present high genetic variability (Teixeira da Silva *et al.*, 2015). This translates into marketing problems, and therefore the tools for *in vitro* culture are advisable, which allow obtaining specimens in less time, which are genetically homogeneous, free of disease; these tools also allow controlling growth speed and tissue regeneration (Rangel-Estrada *et al.*, 2018). The semi-solid medium is effective, basic and provides support to the tissues; however, higher production has been reported in various species when using liquid medium, due to the contact that is maintained between the medium and the explants, which makes the absorption of nutrients more efficient (Ontaneda *et al.*, 2020).

Among the plant tissue cultivation systems, temporary immersion systems outperform those mentioned. Since they are semi-automatized, they decrease the maintenance costs and renew the gaseous atmosphere avoiding the accumulation of toxic gases within the system (Rosales *et al.*, 2018); in addition, a higher contact between the cultivation medium and the plant, good gas exchange, as well as the system's automatization increase the multiplication rates. Among these systems, the Automatized Temporary Immersion Recipients, RITA® (for its initials in Spanish, *Recipientes de Immersión Temporal Automatizado*), represent a technological advancement that allows an improvement in plant tissue culture (Alamilla-Magaña *et al.*, 2019); however, the results of the use of TIS are limited by factors such as the composition of the culture medium, which is reflected in the amount of nutrients that the plant can absorb affecting its quality (Vilchez & Albany, 2014).

The atmosphere created in the temporary immersion systems promotes physiological processes such as photosynthesis, respiration, chlorophyll synthesis, and stomata functioning (Ramírez -Mosqueda *et al.*, 2019); therefore, it is important to analyze the concentration of chlorophyll in plant tissues (Marín-Garza *et al.*, 2018). In addition, phytohormones have mechanisms of action capable of triggering effects and physiological changes; however, in in vitro culture the plants do not produce them in sufficient amount, which is why they must be aggregated to the culture medium (Elías & Padrón, 2020). Nevertheless, the concentration and absorption by the plant tissue can generate both an improvement in micropropagation and alterations in tissues such as de-differentiation between them, and this is why knowing the content of absorption by the plant tissue allows understanding its interaction and the response that it generates (Aguilar *et al.*, 2019).

To make more efficient the use of *in vitro* tissue culture techniques, especially the use of immersion systems, the objective of this study was to evaluate the physiological development of *A. andreanum* micropropagated in three culture systems: semi-solid medium (SS), partial immersion (PI), and in RITA® bioreactors.

MATERIALS AND METHODS

This research study was conducted in the LADISER (Laboratory of Teaching, Research and Services - *Laboratorio de Docencia, Investigación y Servicios*) of Plant Biotechnology and

Cryobiology at the School of Chemical Sciences, in Orizaba, Veracruz, which depends on Universidad Veracruzana.

Morphometric characteristics were measured (number of shoots, size of shoots, size of root in cm, number of leaves and number of roots), content of chlorophylls (mg/g^{-1} PF) and phytohormones (mg L^{-1}) in leaves from anthurium vitroplants. A completely random design with four repetitions was used; the treatments were PI systems, RITA® and semi-solid medium. With the data obtained, a Kruskal-Wallis one-way analysis of variance was conducted and Tukey's means comparison tests through the minimum significant difference ($P \leq 0.05$), using the R-STUDIO statistical software version 2019 for Windows.

Vitroplants of *A. andreanum* were micropropagated in MS culture medium (Murashige & Skoog, 1962) supplemented with 1mg L^{-1} of 6-Benzylaminopurine (BAP), 30 g L^{-1} of sucrose. For the semi-solid medium, 3 g L^{-1} of phytagel were used, while the pH of the medium was adjusted to 5.7 with a potentiometer Brand HANNA, and sterilized at 121 °C during 15 min in a vertical autoclave brand EVAR. The plants were incubated at 24±2 °C with a photoperiod of 16 h light and 8 h darkness. The light intensity was 36 μmol m^{-2} s^{-1}, supplied by white fluorescent lamps.

Vitroplants were micropropagated in MS medium, and with a size of 3.5 cm, three leaves and no roots were sown in the different systems. For the semi-solid and partial immersion mediums, four glass containers were used with capacity of 250 mL to which 25 mL of culture medium were added. Four explants were placed in each container. In four RITA® temporary immersion systems, 200 mL of culture medium were added and four plants per bioreactor. These were connected to an air system which came from a compressor brand Adir with a maximum entry flow of 5.89 with pressure of 29 psi, and the air entered to the bioreactors through a Midisart® 2000 filter of 0.2 μm (PTFE and polypropylene). The immersions in TIS were carried out in intervals of four hours, six immersions per day, which had a lapse of two minutes, according to what was described by del Rivero-Bautista *et al.* (2004) for the *in vitro* propagation of anthurium.

After 60 days the morphometric development of the anthurium vitroplants was analyzed through the number of shoots, leaves, roots, length of shoots and roots, using graph paper. The quantification of chlorophylls was done following the methodology described by Harborne (1973) in a spectrophotometer (Thermo Scientific®, Genesys 10S UV-VIS) at 645 and 665 nm of absorbance.

The hormonal analysis was carried out with the technique described by Pan (2010) using leaf samples, through HPLC (Agilent Techonologies 1200 serie).

RESULTS AND DISCUSSION

Morphometric analysis

For the analysis of the morphometric data (number of shoots, number of leaves, number of roots, size of shoots and size of root), a Kruskal Wallis non-parametric test and analysis of variance were conducted, as well as a means comparison through the t-student test ($P \leq 0.05$). Significant statistical differences were obtained for the morphometric development of anthurium cultivated in the different systems according to the Kruskal

Wallis test, regarding the systems which consist in a liquid medium compared to the treatment with semi-solid medium.

The development of plants is very plastic, which allows them to react to the environmental changes in size, morphology, etc. (Escaso Santos *et al.*, 2011). The treatment with the highest values for length of shoots was partial immersion. However, for number of shoots, number of leaves and number of roots, the RITA® and PI systems were statistically equal. No differences were observed between treatments for root length (Table 1).

The use of the liquid medium in the PI and RITA® systems favored the morphometric development of anthurium vitroplants. These results agree with what was reported by García *et al.* (2015), who mention that a higher rate of explant development was obtained in the systems where a liquid medium was used because they allow a higher contact of the explants with the culture medium, allowing a better absorption of nutrients and maximizing their development. In addition, the temporary immersion system allows supplying the culture medium to the explants by time lapses, and this allows the renovation of the gaseous atmosphere avoiding the hyper hydricity in the plant tissue with it, as well as the accumulation of toxic gases within the systems.

Chlorophyll content

When it comes to chlorophyll content, significant statistical differences were found in the three systems. The highest concentrations of chlorophylls a, b and total were observed in the RITA® bioreactor (Table 2).

Table 1. Morphometric development of seedlings of *Anthurium andreanum* cultivated *in vitro* for 60 days, in three systems.

System	Sprouts (number)	Sprouts (cm)	Leaves (number)	Root (number)	Root (cm)
SS	1.81b	2.59b	6.19b	1.40b	3.05b
IP	2.93a	3.28a	9.25a	2.11a	4.06a
RITA®	3.19a	2.40b	10.25a	1.98a	3.90ab

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor. Different letters in the columns indicate significant statistical differences between treatments by Kruskal Wallis test for ($P \leq 0.05$).

Table 2. Effect of the different *in vitro* cultivation systems in the concentration (mg/g^{-1} PF) of chlorophylls a, b and total in anthurium plants cultivated for 60 days.

System	Chlorophylls (mg g^{-1} PF)		Total
	a	b	
SS	0.38±0.06c	0.14±0.02c	0.46±0.071c
IP	0.58±0.05b	0.21±0.017b	0.80±0.07b
RITA®	0.97±0.04a	0.35±0.01a	1.34±0.06a
DMSH/HSD	0.196	0.070	0.24

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor, DMSH=minimum significant honest difference, different letters in columns indicate significant statistical differences between treatments ($P \leq 0.05$).

According to the means comparison, the highest leaf content of chlorophylls a, b and total (0.97±0.04, 0.35±0.01, 1.34±0.06 mg g^{-1} PF respectively) was obtained when using the RITA® systems, compared to other treatments, and this is because the conditions of moisture and gas exchange inside the systems foster the synthesis of chlorophylls and, with that, photosynthesis. These results agree with what was reported by Ramírez-Mosqueda *et al.* (2019) who reported different contents of chlorophyll in different bioreactors of temporary immersion.

In addition, the chlorophyll content probably contributes to *in vitro* growth and the adaptation to an autotroph environment (Martins *et al.*, 2015).

Content of phytohormones

Phytohormones, in addition to their important role in the regulation of growth and development, are related to mechanisms that allow the plant to respond to the changes to which they are subjected. After 60 days of culture of *in vitro* anthurium seedlings, significant statistical differences were obtained in the content of kinetin (KIN), abscisic acid (ABA) and indole acetic acid (AIA) in the leaf tissue. The highest contents for KIN and ABA were observed in the semi-solid medium, of 24.98±0.92 mg L^{-1} and 13.55±0.42 mg L^{-1}, respectively. Meanwhile, for AIA the highest result was obtained in the RITA® bioreactor, of 24.33±1.64 mg L^{-1}.

The different treatments had significant statistical differences according to the means comparison obtained for KIN (24.98±0.92) and ABA (13.55±0.42) in the semi-solid culture medium, which contrast with the lowest morphological results obtained in this system. The development of the plant is influenced by the coordination of positive and negative regulators. Cytokinins, although they stimulate cell division, seem to do it at low levels in the root (Escaso Santos *et al.*, 2011). On the other hand, ABA was generally an antagonist and growth inhibitor, which explains the results obtained. Meanwhile, AIA is related to cell division, growth and differentiation, favoring the growth of roots, shoots and stems (Azcón-Bieto and Talón, 2003); this explains the higher statistical result obtained for the RITA® bioreactor (24.33±1.64). These results agree with what was reported by Aguilar Jiménez & Rodríguez De la O (2018), who described that the presence of AIA had a favorable effect in the *in vitro* growth and development of agave shoots and roots.

Table 3. Content of KIN, ABA, and AIA in leaf tissue of anthurium seedlings grown *in vitro* for 60 days.

	Phytohormones (mg L^{-1})		
System	**Kinetin**	**ABA**	**AIA**
SS	24.98±0.92 a	13.55±0.42 a	10.58±0.45 b
IP	2.82±0.06 c	1.55±0.016 c	3.86±0.01 c
RITA®	7.58±1.40 b	9.17±0.04 b	24.33±1.64 a
DMSH/HSD	4.2	1.05	4.26

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor, DMSH=honest least significant difference, different letters in the columns indicate statistically significant differences between treatments (P≤0.05).

Likewise, Flores-Mora *et al.* (2015) describe that there are advantages in temporary immersion systems compared to the other tissue culture systems that have an impact on the response in the absorption of plant hormones making it faster due to the atmosphere created inside the systems and the type of immersion.

CONCLUSIONS

The morphometric development of anthurium vitroplants var. Tropical was favored using partial immersion systems and the RITA® bioreactor where the highest number of leaves, number and size of shoots were seen. The higher content of chlorophylls a, b and total, as well as indole acetic acid in the anthurium leaf tissue was favored when using those bioreactors in comparison to other treatments; meanwhile, the absorption of kinetin and abscisic acid was favored by the semi-solid medium. According to the results obtained, the RITA® system was considered to be the most viable option for *in vitro* culture of anthurium, since as a whole the characteristics studied will foster the best development of plants and, with that, the most desirable characteristics for their production.

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REFERENCES

Aguilar, M. E., Garita, K., Kim, Y. W., Kim, J. A., & Moon, H. K. (2019). Simple Protocol for the Micropropagation of Teak (*Tectona grandis* Linn.) in Semi-Solid and Liquid Media in RITA® Bioreactors and ex Vitro Rooting. https://doi.org/10.4236/ajps.2019.107081

American Journal of Plant Sciences, 10(07), 1121.

Aguilar Jiménez, D., & Rodríguez De la O, J. L. (2018). Micropropagación y aclimatación de Maguey Pitzometl (*Agave marmorata* Roezl) en la Mixteca Poblana. Revista Colombiana de Biotecnología, *20*(2), 124–131. https://doi.org/10.15446/rev.colomb.biote.v20n2.77084

Alamilla-Magaña, J. C., Caamal-Velázquez, J. H., Criollo-Chan, M. A., Vera-López, J. E., & Reyes-Montero, J. A. (2019). Biofactory and temporary immersion bioreactor: *In vitro* propagation of *Anthurium andreanum* L., and economic viability. *Agro Roductividad, 12*(10), 23–29. https://doi.org/https://doi.org/10.32854/agrop.vi0.1457

Azcón-Bieto, J. (2000). Fundamentos de fisiología vegetal (No. 581.1 F981f). Madrid, ES: McGraw-Hill Interamericana.

del Rivero Bautista, N., Quiala, E., Agramante, D., Barbón, R., Camacho, W., Morejón, L., & Pérez, M. (2004). Empleo de sistemas de inmersión temporal para la multiplicación *in vitro* de brotes de *Anthurium andraeanum* Lind. var. Lambada. *Biotecnología Vegetal, 4*(2). Recuperado de https://revista.ibp.co.cu/index.php/BV/article/view/393/362

Elías, I., & Padrón, S. (2020). TEJIDOS VEGETALES. Retrieved from https://repositorio.unicordoba.edu.co/handle/ucordoba/2553

Escaso Santos, F., Martínez Guitarte, J. L., & Planello Carro, M. del R. (2011). Fundamentos básicos de fisiología vegetal y animal. Retrieved from https://www.biblionline.pearson.com/Pages/BookDetail.aspx?b=661

Flores-Mora, D. M., Chacón-Cerdas, R., Alvarado-Marchena, L., Schmidt-Durán, A., & Alvarado-Ulloa, C. (2015). Enraizamiento de vitroplantas de membrillo (*Cydonia oblonga*) por medio de inmersión temporal automatizada y su aclimatación. DOI https://dx.doi.org/10.1590/0100-2945-184/14

García-Alonso, Goméz-Gomez, L. E. Chalita-Tovar, J. J. Brambila-Paz y R. (2014). Factibilidad financiera por opciones reales para la producción de Anturio (*Anthurium andreanum* Lindem Ex Andre) en Cuitláhuac, Veracruz. Scielo.Org.Mx. Retrieved from http://www.scielo.org.mx/scielo.php?pid=S20070934201400 1001467&script=sci_arttext

García, A. V., Sandrea, Y., Gonzalez, O., Diaz, A., Albarran, J. G., Schmidt, A., Marin R, C. (2015). Micropropagación de plantas de lechosa en recipientes de inmersión temporal a partir de brotes axilares Micropropagation of papaya plants in temporary immersion recipients from axilary shoots. *Revista Colombiana de Biotecnologia, 17*(1), 70–78. https://doi.org/10.15446/rev.colomb.biote.v17n1.50718

Gayosso-Rodriguez, & García, T. H. . Hernández-Hernández y M. A. Estrada-Botello (2013) El cultivo del anturio (*Anthurium andreanum*). División de ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco. Vilahermosa, Tabasco, México. 36 p. Sidalc.Net. Retrieved from http://www.sidalc.net/cgibin/wxis.exe/?IsisScript=sibe01.xis&method=post&formato=2&cantidad=1&expresion=mfn=034934

Harborne, J. B. (1973). Phenolic Compounds. In *Phytochemical Methods* (pp. 33–88). https://doi.org/10.1007/978-94-009-5921-7_2

Marín-Garza, T., Gómez-Merino, F. C., Aguilar-Rivera, N., Murguía-González, J., Trejo-Téllez, L. I., Pastelín-Solano, M. C., & Castañeda-Castro, O. (2018). Bioactive composition of coffee leaves during an annual cycle. *Revista Fitotecnia Mexicana, 41*(4), 365–372. https://doi.org/10.35196/rfm.2018.4.365-372.

Martins J.P.R., Schimildt E.R., Alexandre R.S., Falqueto A.R., Otoni W.C. (2015). Chlorophyll a fluorescence and growth of *Neoregelia concentrica* (Bromeliaceae) during acclimatization in response to light levels. *Vitro Cell Dev Biol Plant* 51:471–481. https://doi.org/10.1007/s11627-015-9711-z

Mireles-Ordaz, Julieta, Arellano-Perusquia, Abraham, Espinal-Centeno, Annie, Sánchez-Segura, Lino, Estrada-Luna, Andrés Adolfo, & Cruz-Ramírez, Alfredo. (2015). Reprogramación celular de embriones de *Anthurium andraeanum* por fitohormonas para micropropagación masiva. *Nova scientia, 7*(15), 49-67. Recuperado en 25 de octubre de 2021, de http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S2007-07052015000300049&lng=es&tlng=es..

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum, 15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Ontaneda, A. L. C., Herrera, A. M., & Batista, R. M. G. (2020). Eficiencia del sistema de inmersión temporal frente al método de propagación convencional *in vitro*. *Revista Metropolitana de Ciencias Aplicadas, 3*(2), 173-182. Remca.Umet.Edu.Ec. Retrieved from http://remca.umet.edu.ec/index.php/REMCA/article/view/284

Pan, X., Welti, R., & Wang, X. (2010). Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography–mass spectrometry. *Nature protocols, 5*(6), 986. https://doi.org/10.1038/nprot.2010.37

Ramírez-Mosqueda, M. A., Cruz-Cruz, C. A., Cano-Ricárdez, A., & Bello-Bello, J. J. (2019). Assessment of different temporary immersion systems in the micropropagation of anthurium (*Anthurium andreanum*). *3 Biotech, 9*(8), 3–9. https://doi.org/10.1007/s13205-019-1833-2

Rangel-Estrada S.E., Hernández-Meneses, J. Canul-Kú, E. J. Barrios-Gómez, M. C. G. López-Peralta y L. Tapia-Jaramillo (2018). Germinación, viabilidad y organogénesis *in vitro* de *Anthurium schlechtendalii* Kunth subsp. schlechtendalii. *Revista Bio Ciencias* 5:e475, https://doi.org/10.15741/revbio.05.nesp.e475

Rosales, C., Brenes, J., Salas, K., Arce-Solano, S., & Abdelnour-Esquivel, A. (2018). Micropropagación de *Stevia rebaudiana* en sistemas de inmersión temporal para incursionar en la producción hortícola. *Revista Chapingo, Serie Horticultura, 24*(1), 69–84. https://doi.org/10.5154/r.rchsh.2017.08.028

SE-SNIIM (Secretaría de Economía-Sistema Nacional de Información e Integración de Mercados). 2019. Precio del anturio flor de corte en las centrales de abastos nacionales www.economia-sniim.gob.mx, (consultado junio 15, 2020).

Teixeira da Silva, J. A., Dobránszki, J., Winarto, B., & Zeng, S. (2015). Anthurium *in vitro*: A review. *Scientia Horticulturae*, 186, 266–298. https://doi.org/10.1016/j.scienta.2014.11.024

Vilchez, J., & Albany, N. (2014). Multiplicación *in vitro* de *Psidium guajava* L. en sistemas de inmersión temporal *In vitro* multiplication of *Psidium guajava* L. in temporary immersion systems. *Rev. Colomb. Biotecnol.* Diciembre, XVI(2), 96–103. https://doi.org/10.15446/rev.colomb.biote.v16n2.42180