Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Evaluation of bioreactors with and without air injection for the micropropagation of *Vanilla planifolia* G. Jackson

Clara Anabel Arredondo-Ramírez[1]
Gregorio Arellano-Ostoa[1,§]
Sergio Humberto Chávez-Franco[1]
Eduardo García-Villanueva[1]
María del Carmen López-Reyna[2]
José Humberto Caamal-Velázquez[3]

1 Postgrado de Recursos Genéticos y Productividad-Fruticultura-Campus Montecillo-Colegio de Postgraduados. Carretera Mexico-Texcoco km 36.5, Montecillo, Texcoco, Estado de México México. CP. 56230. Tel. 55 58045900. (anabel-is01@hotmail.com; sergiocf@colpos.mx; egarciav@colpos.mx).

2 Postgrado en Socioeconomía, Estadística e Informática-Economía. Campus Montecillo-Colegio de Postgraduados. Carretera Mexico-Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México. Tel. 55 58045900. (creyna@colpos.mx).

3 Bioprospección y Sustentabilidad Agrícola en Trópico-Campus Campeche-Colegio de Postgraduados. Carretera Haltunchén-Edzná, Champotón, Campeche. México. CP. 24450. (hcaamal@colpos.mx).

Autor para correspondencia: arellano@colpos.mx.

Abstract

The species *Vanilla planifolia* G. Jackson has a high commercial value in the food, pharmaceutical and cosmetic industries. Temporary immersion systems or bioreactors allow for faster and more controlled *in vitro* propagation under laboratory conditions. Nonetheless, due to the high costs of commercial bioreactors, such as the Rita® model, one of the most widely used for the micropropagation of several plant species, cheaper alternatives are sought. *In vitro* multiplication of vanilla was carried out in two types of temporary immersion bioreactors in order to evaluate the efficiency of a mechanical bioreactor without air injection compared to a Rita® bioreactor that uses air injection; the research was conducted between 2023 and 2024. The semi-solid culture system was used as a control. After 30 days of culture, the following physiological variables were analyzed: number of shoots, number of leaves, shoot length, and growth index; likewise, biochemical variables, such as total contents of chlorophylls α and β and phenol and carbohydrate contents, were quantified. The results obtained indicated that the BWA bioreactor was statistically equal ($p \leq 0.05$) to the Rita® bioreactor in the variables of number and length of shoots. Both were statistically different ($p \leq 0.05$) from the semi-solid system in most of the variables assessed. This suggests that the use of a mechanical bioreactor without air injection can be used as an alternative for the micropropagation of various species due to its low cost.

Keywords:

liquid media, mechanical, micropropagation, Rita®, vanilla.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Introduction

The reproduction of plant species is undoubtedly key to human development. Trying to cover the requested demands both in number of species and in cost reduction, large-scale micropropagation has been addressed using temporary immersion systems (TISs); this technique performs periodic and semi-automated immersions based on the alternation of cycles of temporary immersion of plant tissue grown in a liquid medium (Georgiev *et al*., 2014).

That is, temporary immersion bioreactors operate through immersion and emersion cycles; by means of a compressor, air is injected into the bioreactor and when it comes into contact with the liquid medium, it sprays the shoots. This cycle is repeated several times, creating an ideal environment for the efficient growth of the shoots. The immersion period is usually short, only a few minutes, whereas the exposure period is longer. The length of the immersions depends mostly on the species being worked with. In these systems, greater availability of all the components of the medium is allowed.

A temporary immersion system (TIS) has been highlighted as a resource to facilitate the micropropagation of various agricultural and forest species (Etienne *et al*., 1997), increase the multiplication rate and reduce the time required for this process. In addition, it offers significant advantages by reducing intensive manual handling, reducing production costs, and improving the quality of plant material (Etienne and Berthouly, 2002).

The bioreactor of the Récipient à Immersion Temporaire Automatique (Rita®, by its French acronym) type, developed at the end of the 90s, is the most widely used. Several models have been developed based on it, which can be divided into two categories: open bioreactors that have air injection, such as twin bottles (Escalona *et al*., 1999), Plantima® (Wu *et al*., 2018), Setis™ (Lotfi and Werbrouck, 2020), to mention a few. On the other hand, there are closed bioreactors that do not use air injection, such as the We Vitro by Magenta® and the national bioreactor BioMint™ (Robert *et al*., 2006).

This type of technology has become an inaccessible option due to its high costs and use of specialized facilities. Therefore, new bioreactor prototypes that use more economical technology are required to meet demand.

This research aimed to evaluate a mechanical bioreactor without air injection, designed to be more accessible due to the use of low-cost materials compared to the Rita® bioreactor, which operates with air injection. For the assessment, shoots of vanilla (*Vanilla planifolia* G. Jackson) established *in vitro* were used, whose propagation importance has been described, and its efficiency was determined against the Rita® bioreactor compared to the semi-solid medium during the multiplication stage.

Materials and methods

Plant material

For the evaluation, the vanilla was multiplied *in vitro*, using vanilla nodal segments approximately 4 cm long, previously established *in vitro* in a semi-solid medium.

Culture medium

The liquid culture medium for MS (Murashige and Skoog, 1962) multiplication was supplemented with 0.2 mg L^{-1} BA (Benzyl adenine), 0.02 mg L^{-1} NAA (naphthalene acetic acid), and 30 g L^{-1} sucrose, whereas the semi-solid culture medium was added with 7 g L^{-1} Sigma® agar as a gelling agent plus 1 g L^{-1} of activated carbon. The pH of the media was adjusted to 5.7 before sterilization.

The medium was sterilized in an autoclave at 1.5 kg cm^{-2} pressure at 121 °C for 20 min. Cultures were incubated at 24 ±2 °C and kept under Led light at 55 µmol m^{-2} s^{-1}, with a photoperiod of 16:8 h light/dark. In an incubation room, this protocol was developed in the bioreactor laboratory of PREGEP-Fruticultura.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Treatments

The behavior of the mechanical bioreactor BWA was evaluated by comparing it with a Rita® commercial bioreactor and a control in a semi-solid medium (SS). The evaluation was conducted during two successive subcultures of thirty days, with three replications per treatment. The immersion frequency used was two minutes every four hours for 30 days for all immersion treatments. For the control treatment, the explants were in contact with the gelled medium throughout the experiment.

Mechanical bioreactor BWA (T1). It is a container with a cylindrical body made of Pyrex glass, which consists of an airtight polypropylene plastic lid. It includes a 0.2 µm air filter that allows the respiration of plant material. Its immersion mechanism operates through a mechanical system that is controlled by a stepper motor, which, when activated, immerses the explants that are on a plastic platform at rest in the culture medium.

Rita® bioreactor (T2): (Récipient à Immersion Temporaire Automatique). The features are described in Etienne *et al*. (1997).

Control in a semi-solid medium SS (T3): it consists of a glass bottle containing the explants that are in constant contact with the semi-solid culture medium. The specifications of each treatment are shown in Table 1.

Table 1. Characteristics of the treatments (T) used for the evaluation (total volume, volume of the medium, number of explants per bioreactor, and volume of the medium per explant).

Type of bioreactor	Total container volume (ml)	Culture medium volume (ml)	No. of explants per replication	Volume of the medium per explant (ml)
T1 BWA	1 700	500	14	35.71
T2 Rita®	940	350	10	35
T3 SS control	455	100	10	10

Variables evaluated

After 30 days of culture with the conditions described above, the variables evaluated were:

Physiological variables

Number of shoots per bioreactor, number of leaves per explant, shoot length, and plant material growth index GI=

$$\frac{\text{Final fresh weight- Initial fresh weight}}{\text{Initial fresh weight}}$$

Biochemical variables

Quantification of chlorophylls α and β: according to the methodology proposed by Lichtenthaler (1987). This process involves extracting the plant pigments using an organic solvent, such as acetone, and then measuring the absorbance of the extracted solutions at specific wavelengths with a spectrophotometer. This experiment used 0.1 g of fresh vanilla shoots in vitro, which was pulverized with acetone, and the absorbance was measured at 645 nm for chlorophyll α and at 661 nm for chlorophyll β.

Quantification of phenols: the methodology used was that by Singleton and Rossi (1965) with Folin-Ciocalteu. This method indicates that the Folin-Ciocalteu reagent is reduced in the presence of phenolic compounds, which generates a change in color that can be quantified by spectrophotometry.

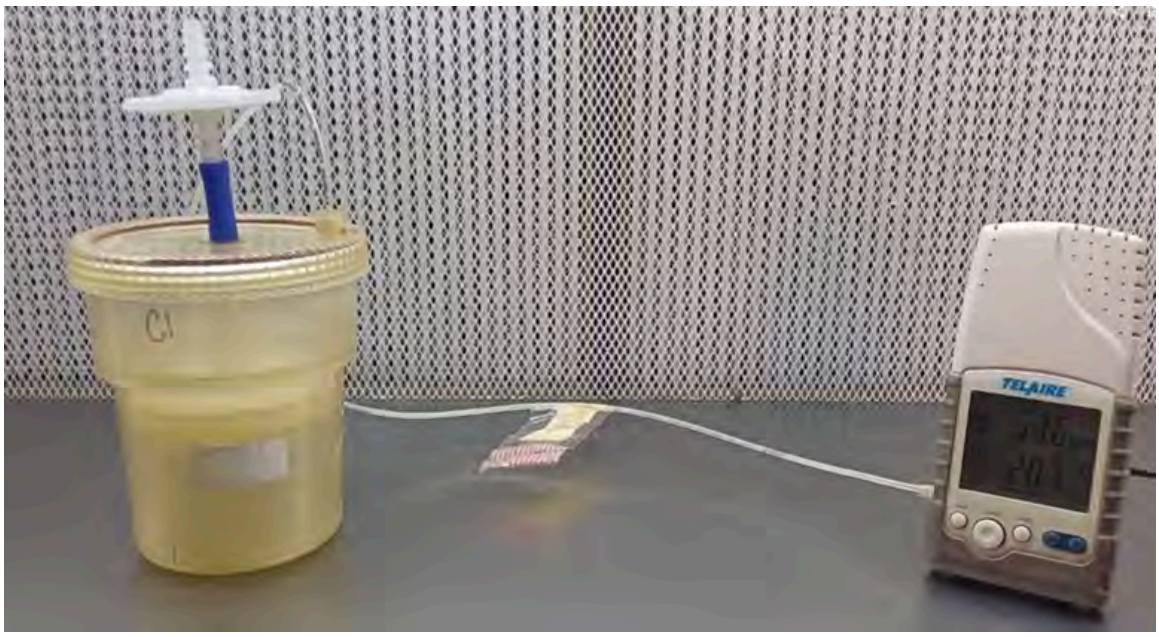
DOI: https://doi.org/10.29312/remexca.v16i6.3804

For the experiment, 0.1 g of fresh sample pulverized with alcohol was used, to which Folin-Ciocalteu and calcium carbonate ($CaCO_3$) were added until a blue coloration was obtained. Absorbance was measured at 760 nm to determine the total concentration of phenolic compounds and evaluate their antioxidant capacity.

Carbohydrate quantification: the methodology by Whitman *et al*. (1971) was used. One gram of fresh leaves was weighed and treated with anthrone reagent in sulfuric acid to form a green compound, the absorbance of which was measured at 660 nm.

Another part of the study consisted of quantifying the concentration of CO_2 at time zero, which is the beginning of the multiplication period and then evaluations were made every 15 days until the end of the period. A Telaire 2001 CO_2 monitor (Figure 1) was used, which measures temperature and CO_2 simultaneously within a maximum period of 30 s. Both types of bioreactors were under the same incubation conditions described above. It was ensured that the air conditioning was turned off and that there were no people in the incubation room during the readings.

Figure 1. Connection of the Telaire meter to the bioreactor by means of a silicone tube.



The meter was allowed to stabilize for 10 minutes before recording the readings. Then, measurements were taken every minute for 2 h to observe the behavior of the gas in the bioreactors.

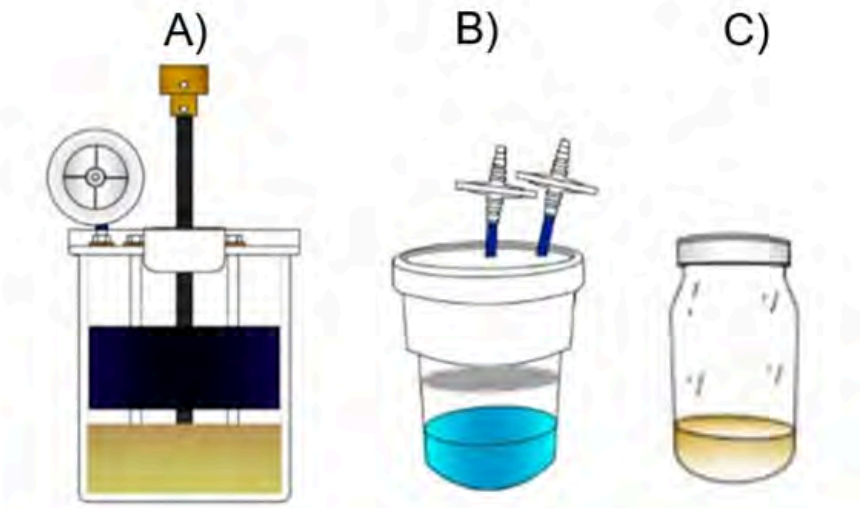
Experimental design

A completely randomized experimental design (CRED) was used, considering a bioreactor as a replication (R) and an explant as an experimental unit (10XR), three replications per treatment (T) were used, and the results were analyzed using analysis of variance ($p\#$ 0.05). The normality of the data was determined with a Shapiro-Wilk test.

In case of significant differences, a Tuckey mean comparison test was applied ($p\#$ 0.05). The data were analyzed using the RStudio® statistical package for Windows. The entire experiment was repeated twice over time by means of successive subcultures. Figure 2 shows the different treatments evaluated.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Figure 2. Diagram of the bioreactors evaluated. A) BWA bioreactor; B) Rita® bioreactor and C) control in SS.



Results and discussion

Number of shoots and leaves

It was observed that the two treatments were statistically different from the control, there were no significant differences between them. In temporary immersion systems, the explants have constant contact with nutrients and regulators in the liquid medium, whereas in the semi-solid medium, they only receive them at the base of the explant (Etienne and Berthouly, 2002). It can be seen that the frequency of immersion was the same in both treatments; nevertheless, there were differences in the total volume of the container vessel between them, the Rita® bioreactor has a volume of 940 ml and 350 ml of culture medium (35 ml/explant) was added, whereas the BWA bioreactor has a total volume of 1 700 ml and its added volume of medium was 500 ml (36 ml/explant).

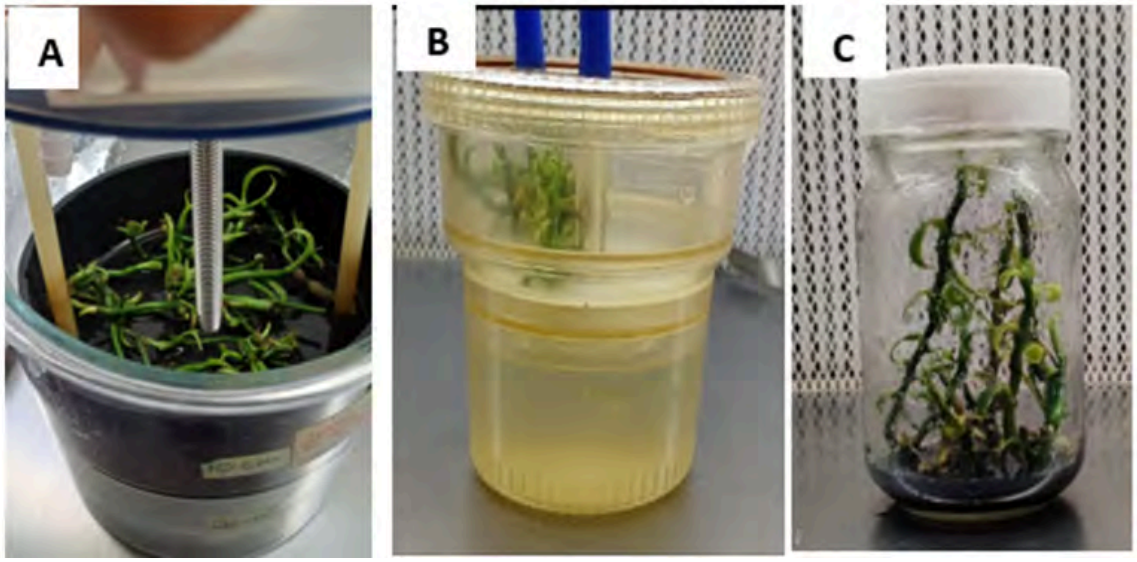
Studies show that the volume of the medium per explant is crucial in micropropagation in TIS. In bananas (*Musa* spp.), it was observed that 30 ml of medium per explant had the highest multiplication rate (Roels *et al*., 2005); in rootstocks of grapes (*Vitis vinifera*), using an air-lift balloon-type bioreactor, sprouting was doubled when using 65 ml of medium per explant compared to 25 ml with different air injection frequencies, such as 50, 100, 150, and 200 ml min^{-1} (Jin *et al*., 2013).

This phenomenon may be due to the removal of gaseous components, such as carbon dioxide and ethylene, in higher aeration volumes (Gao and Lee, 1992). In bioreactors, aeration influences oxygen transfer, turbulence and recirculation of the medium (Wu *et al*., 2018).

Figure 3 shows the vanilla shoots multiplied in the different treatments (bioreactors and semi-solid medium). It can be seen that there were variations in the growth and development of leaves and stems with different degrees of sprouting.

Revista Mexicana de Ciencias Agrícolas
DOI: https://doi.org/10.29312/remexca.v16i6.3804

Figure 3. View of treatments evaluated in the *in vitro* multiplication of vanilla. A) BWA bioreactor; B) Rita® bioreactor and C) SS control.



Shoot length

It was observed that there were no significant differences between the treatments evaluated, the length varies between 1.2 and 1.34 cm. According to Debabrata et al. (1997), inoculation density is a key factor influencing crop growth during micropropagation. Hahn and Paek (2005) found that 80 chrysanthemum nodes inoculated in 10 L column bioreactors with a volume of 4 L of medium was the best density for growth and multiplication. Jin et al. (2013) mention that the results depend on the volume of the bioreactor; for instance, in grapevine, it was shown that the density of the inoculum affected micropropagation, where the inoculation densities of 50 and 65 explants in a 5 L balloon-type bioreactor were optimal for seedling growth and higher biomass.

Quantification of chlorophylls α and β

The results obtained show that there were no significant differences ($p \leq 0.05$) between the types of bioreactors; in terms of quantification of chlorophyll α, the treatment with the BWA bioreactor is observed with 0.09 mg g-1 FW and the Rita® bioreactor with 0.07 mg g-1 FW; however, these were statistically different from the control (0.042 mg g-1).

In the case of the quantification of chlorophyll β, the observed results indicate that there were no significant differences between the treatments, including the control. This indicates that all treatments had the conditions for the development of vanilla shoots during in vitro multiplication despite the fact that the bioreactors used have different characteristics.

On the other hand, there are studies that have used temporary immersion bioreactors, and it has been shown that there are differences in the quantification of chlorophylls, such as in yams (Jova et al., 2011) or in apple tree (Dewir et al., 2006), indicating that the increase in photosynthetic pigments present in the 5 L air-lift balloon-type bioreactor with 2 L of medium could be due to the amount of air supplied externally and the high availability of nutrients (Roels et al., 2005).

Gas exchange probably caused increased photosynthetic activity; for example, Aragon et al. (2005) showed that TIS has a positive influence on the photosynthesis process during banana micropropagation; in the case of vanilla, it was reported that there was a higher chlorophyll content in the ebb-and-flow bioreactor compared to the temporary immersion bioreactor (BIT®) and the Rita® bioreactor. On the other hand, in apple tree, Dewir et al. (2006) found that shoots grown in temporary immersion bioreactors showed higher contents of chlorophylls α and β than shoots grown in a continuous system.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

temporary immersion bioreactors showed higher contents of chlorophylls # and # than shoots grown in a continuous system.

Carbohydrate quantification

In the two systems evaluated (TIS) and semi-solid medium (SS), the same amount of sucrose was provided to the culture medium (30 g L^{-1}); nonetheless, the carbohydrate contents in the vanilla shoots recorded at the end of the culture (30 days) were higher in the temporary immersion systems and significantly different ($p \leq 0.05$) from the control (SS).

Table 2 shows the results obtained in the different treatments, where it can be seen that there are no significant differences between the temporary immersion treatments (BWA and Rita®); nevertheless, there is a difference between bioreactors and the control, which means that the response in bioreactors with or without air injection is still more efficient than the semi-solid system (agar).

Table 2. Evaluation of the morphological and biochemical variables of the *in vitro* culture of vanilla (*Vanilla planifolia*) in two types of temporary immersion bioreactors and the control in a semi-solid system.

Treatment	NS[1]	NL[2]	SL[3] (cm)	GI[4]	C α[5] (mg g^{-1} FW)	C β[6](mg g^{-1} FW)	TP[7] (mg g^{-1} FW)	CH[8] (mg g^{-1} FW)
T1 BWA	23.66[a]	2.9[a]	1.3[a]	5.816[b]	0.09[a]b	0.0338[a]	0.0202[a]	9.76[a]
T2 Rita®	20.33[ab]	2.6[a]	1.34[a]	8.566[a]	0.0739[ab]	0.0403[a]	0.0119[a]	10.384[a]
T3 control SS	12[b]	1.9b	1.2[a]	5.34[b]	0.042[b]	0.0289[a]	0.0119[a]	4.2031[b]

Letters with the same letter within columns indicate that there are no statistical differences $p < 0.05$ according to Tukey test. 1) average number of shoots/bioreactor; 2) average number of leaves/explant; 3) average shoot length/explant; 4) growth index/bioreactor; 5) contents of chlorophyll α/bioreactor, evaluated using the methodology by Lichtenthaler (1987); 6) contents of chlorophyll β/bioreactor, evaluated using the methodology by Lichtenthaler (1987); 7) total phenol contents/bioreactor, evaluated using the methodology by Singleton and Rossi (1965) and 8) carbohydrate contents/bioreactor, evaluated using the methodology by Whitman *et al*. (1971).

Although there are few studies on carbohydrate quantification, there are records where it was observed that pineapple seedlings assimilated nutrients better, where 275 mg g^{-1} of carbohydrates was obtained compared to the conventional culture in a semi-solid system, which obtained 16.8 mg g^{-1} (Escalona *et al*., 2003).

In this study, there was no significant difference between the different bioreactors tested, it is even observed that the carbohydrate contents were very similar between the Rita® bioreactor (10.384 mg g^{-1} FW) and the BWA bioreactor (9.76 mg g^{-1} FW). These results suggest that temporary immersion systems promote greater photosynthesis in tissues together with the fact that, in these systems, there is also a higher content of chlorophylls, and Arencibia *et al*. (2013) mention that, since photosynthesis is a complex process in which a series of environmental factors intervene that determine the assimilation of carbon, a CO_2 concentration of 550 ppm per volume of container could be optimal for the best development of tissues cultured *in vitro*.

The method of injecting air into bioreactors has a significant impact on plant growth. In their studies carried out with cocoa and yams Trauger *et al*. (2022) showed that the injection of air with 40% oxygen increased the response of the shoots compared to those grown without aeration. This method promotes better oxygenation and the development of the shoots.

The BWA system showed that despite not requiring air injection, this can be an effective option. As a result of the CO_2 readings (Table 3) taken, it can be observed that, in the BWA bioreactor, there is an average concentration of 404 ppm compared to the final evaluation, where there is a concentration of 378 ppm.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Table 3. CO_2 readings from the RITA bioreactor and the BWA bioreactor.

RITA®	D0 (ppm)[1]	D15 (ppm)[2]	D30 (ppm)[3]	BWA	D0 (ppm)[1]	D15 (ppm)[2]	D30 (ppm)[3]
Initial reading#	590	530	560	Initial reading#	402	468	360
Adjustment reading#	533	500	520	Adjustment reading#	359	346	364
Beginning of immersion#	892	800	900	Beginning of immersion#	340	351	282
End of immersion#	980	1001	987	End of immersion#	355	360	285
Time of high concentration (10 min)#	930	900	945	Stable reading#	426	408	378
Stable reading#	330	320	350				

1) CO_2 concentration in ppm at day 0 of multiplication, average of six immersions in 24 h; 2) CO_2 concentration in ppm on day 15 of multiplication, average of six immersions in 24 h; 3) CO_2 concentration in ppm on day 30 of multiplication, average of six immersions in 24 h; 4) initial reading at the time of connecting the meter; 5) stable reading after 10 min of the start of the process; 6) reading at the beginning of the immersion; 7) reading at the end of the immersion after 2 min of immersion; 8) CO_2 concentration that remains unchanged after 10 min of air injection and 9) stable end reading, average of six immersions in 24 h.

This indicates that, as time progresses, the concentration of CO_2 decreases; this may be due to an increase in the amount of plant material inside the bioreactor, indicating that the concentration of CO_2 decreases as the explants grow, remembering that there is no direct air injection, but there is air intake through the 0.2 µm filter that helps its continuous absorption, although to a lesser extent than in the Rita® bioreactor, where there is air injection through the compressor.

In contrast, in the Rita® bioreactor, the average concentration of CO_2 is higher than that of the BWA bioreactor. It was observed that in Rita®, the injection of air causes a considerable increase in CO_2 concentration.

Daily readings start at an average of 333 ppm. Although at the end of each cycle the stabilizations of the readings are lower than in the BWA bioreactor, it is evident that the air injection contributes to a greater multiplication of explants, reaching an average of 870 ppm at the beginning of the injection and 989 ppm at the end of each immersion, until the CO_2 concentration stabilizes again within the bioreactor. On the other hand, in the BWA bioreactor, the CO_2 concentration remains relatively stable for most of the time, even during immersion periods.

Conclusions

It is shown that a similar efficiency existed between the Rita® bioreactor and the BWA bioreactor during the *in vitro* multiplication of vanilla and both were superior to the control cultured in agar. The BWA bioreactor showed promising potential in shoot multiplication. It is essential to continue with the optimization of this system through additional research based on the results obtained with the aim of getting closer and closer to the standards set by commercial bioreactors. This will benefit both the scientific community and growers by obtaining plants at a lower cost.

CO_2 measurements show that gas exchange within the bioreactor atmosphere plays a crucial role in yield. An increase in CO_2 concentration in the Rita® bioreactor is observed after each injection, which could represent an advantage by acting as a form of CO_2 fertilization. It is also recommended to perform more replications of the experiments performed in this study to obtain more robust and accurate results.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Bibliography

1 Aragón, C. E.; Escalona, M.; Capote, I.; Pina, D.; Cejas, I.; Rodríguez, R.; Jesús-Cañal, M.; Sandoval, J.; Roels, S.; Debergh, P. and González-Olmedo, J. 2005. Photosynthesis and carbon metabolism in plantain (*Musa*AAB) plantlets growing in temporary immersion bioreactors and during*ex vitro*acclimatization. *In Vitro* Cellular & Developmental Biology-Plant. 41(4):550-554. https://doi.org/10.1079/IVP2005640.

2 Arencibia, A. D.; Vergara, C.; Quiroz, K.; Carrasco, B.; Bravo, C. and Garcia-Gonzales, R. 2013. an approach for micropropagation of blueberry (*Vaccinium corymbosum* L.) plants mediated by temporary immersion bioreactors (TIBs). American Journal of Plant Sciences. 4(5):1022-1028. https://doi.org/10.4236/ajps.2013.45126.

3 Debabrata, S.; Ramesh, C. and Prakash, S. N. 1997. Effect of inoculation density on potato micropropagation. Plant Cell, Tissue and Organ Culture. 48(1):63-66.

4 Dewir, D. H.; Chakrabarty, D.; Hahn, D. and Paek, E. J. 2006. A simple method for masspropagation of *Spathiphylium cannifolium* using an airlift bioreactor. *In vitro* Cellular & Developmental Biology-Plant. 42(3):291-297.

5 Escalona, M.; Lorenzo, J. C.; González, B.; Daquinta, M.; Gonzalez, J. L.; Desjardins, Y. and Borroto, C. G. 1999. Pineapple (*Ananas comosus* L. Merr.) micropropagation in temporary immersion systems. Plant Cell Reports. 18(9):743-748. https://doi.org/10.1007/s002990050653.

6 Escalona, M.; Samson, G.; Borroto, C. and Desjardins, Y. 2003. Physiology of effects of temporary immersion bioreactors on micro propagated pineapple plantlets. *In vitro* Cellular & Developmental Biology Plant. 39(6):651-656. https://doi.org/10.1079/IVP2003473.

7 Etienne, H. and Berthouly, M. 2002. Temporary immersion systems in plant micropropagation. Plant Cell, Tissue and Organ Culture. 69:215-231.

8 Etienne, H.; Lartaud, M.; Michaux-Ferriere, N.; Carron, M. E.; Berthouly, M. and Teisson, A. 1997. Improvement of somatic embryogenesis in *Hevea brasiliensis* (Mt) ll. Arg.) using the temporary immersion technique. *In vitro* Cellular & Developmental Biology- Plant. 33(2):81-87.

9 Gao, J. and Lee, J. M. 1992. Effect of oxygen supply on the suspension culture of genetically modified tobacco cells. Biotechnology Progress. 8(4):285-290. https://doi.org/10.1021/bp00016a004.

10 Georgiev, V.; Schumann, A.; Pavlov, A. and Bley, T. 2014. Temporary immersion systems in plant biotechnology. Engineering in Life Sciences. 14(6):607-621. https://doi.org/10.1002/elsc.201300166.

11 Hahn, E. J. and Paek, K. Y. 2005. Multiplication of *Chrysanthemum* shoots in bioreactors as affected by culture method and inoculation density of single node stems. Plant Cell, Tissue and Organ Culture. 81(3):301-306. https://doi.org/10.1007/s11240-004-6655-0.

12 Jin, M. Y.; Piao, X. C.; Xiu, J. R.; Park, S. Y. and Lian, M. L. 2013. Micropropagation using a bioreactor system and subsequent acclimatization of grape rootstock '5BB'. Scientia Horticulturae. 164:35-40. https://doi.org/10.1016/j.scienta.2013.09.004.

13 Jova, M. C.; Kosky, R. G. and Cuellar, E. E. 2011. Effect of liquid media culture systems on yam plant growth (*Dioscorea alata* L. Pacala Duclos). Biotechnology, Agronomy, Society and Environment. 15(4):515-521.

14 Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *In*: Lester Packer and Roland Douce. Methods in Enzymology, Plant Cell Membranes. 148:350-382. Academic Press. https://doi.org/10.1016/0076-6879(87)48036-1.

15 Lotfi, M. and Werbrouck, S. P. O. 2020. SETIS™, a novel variant within the temporary immersion bioreactors. Acta Horticulturae. 30(1285):253-258. https://doi.org/10.17660/ActaHortic.2020.1285.37.

DOI: https://doi.org/10.29312/remexca.v16i6.3804

16 Murashige, T. and Skoog, F. 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15(3):473-497. https://doi:10.1111/j.1399-3054.1962.tb08052.x.

17 Robert, M. L.; Herrera-Herrera, J. L.; Herrera-Herrera, G.; Herrera-Alamillo, M. Á. and Fuentes-Carrillo, P. 2006. A new temporary immersion bioreactor system for micropropagation. *In*: Loyola-Vargas, V. M. and Vázquez-Flota, F. Methods in Molecular Biology. 318(2):121-130. Humana Press. https://doi.org/10.1385/1-59259-959-1:121.

18 Roels, S.; Escalona, M.; Cejas, I.; Noceda, C.; Rodriguez, R.; Canal, M. J.; Sandoval, J. and Debergh, P. 2005. Optimization of plantain (*Musa* AAB) micropropagation by temporary immersion system. Plant Cell, Tissue and Organ Culture. 82(1):57-66. https://doi.org/10.1007/s11240-004-6746-y.

19 Singleton, V. L. and Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 82(1):57-66. https://doi.org/10.1007/s11240-004-6746-y.

20 Trauger, M.; Hile, A.; Sreenivas, K.; Shouse, E. M.; Bhatt, J.; Lai, T.; Mohandass, R.; Tripathi, L.; Ogden, A. J. and Curtis, W. R. 2022. CO_2 supplementation eliminates sugar-rich media requirement for plant propagation using a simple inexpensive temporary immersion photobioreactor. Plant Cell, Tissue and Organ Culture (PCTOC). 150(1):57-71. https://doi.org/10.1007/s11240-021-02210-3.

21 Whitman, F. H.; Blaydes, D. F. and Devlin, R. M. 1971. Experiments in plant physiology. Monograph Wageningen University, Ed. Van Nostrand Rteinhold. New York. 245 p.

22 Wu, H. C.; Kuo, M. L. and Chen, C. M. 2018. Promotion of vegetative growth in force-ventilated *Protea Cynaroides* L. explants cultured in modified temporary immersion culture vessels. HortScience. 53(2):231-235. https://doi.org/10.21273/hortsci12513-17.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i6.3804

Evaluation of bioreactors with and without air injection for the micropropagation of *Vanilla planifolia* G. Jackson

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 June 2025
Date accepted: 01 August 2025
Publication date: 07 October 2025
Publication date: Aug-Sep 2025
Volume: 16
Issue: 6
Electronic Location Identifier: e3804
DOI: 10.29312/remexca.v16i6.3804

Categories

Subject: Articles

Keywords:

Keywords:

liquid media
mechanical
micropropagation
Rita®
vanilla

Counts

Figures: 3
Tables: 3
Equations: 2
References: 22
Pages: 0