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Micropropagation of banana cv. Williams through temporary immersion system: Response to explant density and plant growth regulators

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ABSTRACT

Banana presents issues with conventional propagation due to a low multiplication rate and diseases. Micropropagation allows the mass production of seedlings with better agronomic characteristics under controlled laboratory conditions, it uses explants and plant growth regulators (PGR) at different stages. Plant tissue culture (PTC) allows nutrients to be provided through the use of bioreactors with a temporary immersion system (TIS), the immersion of the plant material in the culture medium periodically prompts better individuals by facilitating the interaction of the plant material with the culture medium. The aim of this study was to evaluate the response to micropropagation of banana cv. Williams during the multiplication stage using RITA® (Recipient for Automated Temporary Immersion) bioreactors. For this purpose, two experiments were carried out: one using different explant density and the other one using different concentrations of plant growth regulators. The explants of banana correspond to the fourth subculture obtained from shoot meristems. The results obtained after 21 days of culture showed high multiplication rates and highlighted the usefulness of bioreactors with temporary immersion systems (TIS) to successfully propagate banana.

Keywords: banana; plant biotechnology; temporary immersion system; explant density; plant growth regulators.

1. Introduction

Banana has a rather slow plant propagation, and the use of conventional propagation methods leads to a low multiplication rate with an impact on genetic material, field uniformity and production (Hussein, 2012). Micropropagation through plant tissue culture allows an explant to be grown into a plant under *in vitro* conditions, overcoming environmental and physiological limitations (Nielsen et al., 2019; Bhowmik & Rahman, 2020). Individuals can be cloned and manipulated using *in vitro* techniques (Ramírez-Mosqueda et al., 2020).

The culture medium is composed of growth regulators, inorganic salts, carbon source and gelling agent; amino acids and antibiotics can be added. The formulation of the culture medium influence explant development (Alvarez et al., 2019). Liquid culture medium is ideal for the temporary immersion system (TIS) due to its characteristics (Carvalho et al., 2019). Plant growth regulators influence the process of obtaining new individuals (Katel et al., 2022), moderate the physiological functions of plants and are active in small quantities (Kumari et al., 2018). Likewise, these compounds affect the conditions of metabolism (Sharma et al., 2020).

The excess of one hormone generates hormonal imbalance, having an effect on another hormone and its action (Sosnowski et al., 2023; Zhang et al., 2022). Furthermore, the addition of hormones induces the plant to produce shoots sooner (Cardoso et al., 2020). Within plant hormones, auxins mainly control cell division and expansion (Grieneisen et al., 2007). The most commonly used auxin is 2,4-D due to its effectiveness; however, regulators such as IAA (Indole-3-butyric acid) and IBA (Indole-3-butyric acid) are also frequently used (Raghavan, 2004). On the other hand, cytokinins promote shoot formation and lateral root development; BAP (6-Benzylaminopurine) stands out for its wide use (Wybouw & De Rybel, 2018; Lakho et al., 2023).

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The temporary immersion system (TIS) requires a liquid culture medium with periodic immersion of the explant where nutrient absorption is directly related to the immersion time (Vidal & Sanchez, 2019); this system allows the production of excellent individuals without pests or diseases (Bello-Bello et al., 2021). The system increases efficiency through uniform contact between the plant material and the culture medium (Bozkurt et al., 2023). Specifically, bioreactors represent a sterile and closed system. These containers have an air inlet and outlet and have an automatic or semi-automatic control system (Mamun, 2015; Krol et al., 2021). The RITA® bioreactor has two compartments, the lower compartment contains the culture medium and the upper chamber encloses the plant material. Due to the pressure exerted by the injection of air, the culture medium rises to the upper compartment coming into contact with the explants and descending when the pressure is reached (Teisson et al., 1996).

Therefore, it is necessary to improve micropropagation in banana, thus optimizing the process. Consequently, this study aimed to evaluate the response of banana to micropropagation during the multiplication stage using RITA® bioreactors with a temporary immersion system (TIS) at different explant density and different concentrations of plant growth regulators.

2. Methodology

Experiment location and plant material

In this study, two experiments were conducted at the tissue culture laboratory facilities of the Institute of Biotechnology, located at the National Agrarian University La Molina, Peru. These experiments were carried out consecutively to determine the ideal values of explant density and concentrations of plant growth regulators necessary to obtain the largest number of shoots at a temperature of 22 °C and a 16 h photoperiod.

The explants corresponded to the fourth subculture of the insertion of plant material obtained from shoot meristems of banana cv. Williams. The banana explants were placed in the bioreactors maintaining asepsis in order to avoid contamination in the experiment.

Experimental design

The experiment was divided into two.

In the first experiment, a Completely Randomized Design (CRD) was used, which consisted of four treatments and five repetitions. Since each repetition is equivalent to one bioreactor, there were 20 bioreactors overall.

In the second experiment, a Completely Randomized Design (CRD) was used, considering four treatments with four repetitions each and, in this case, there were 16 bioreactors.

The plant material was set in a laminar flow chamber using sterile instruments. In the RITA® bioreactors (capacity of 0.9 L) 200 mL of culture medium was added (Figure 1). An immersion frequency of 3 hours (8 immersions per day) was established and the established immersion time was 3 minutes. At the end of the 21 days, the seedlings were evaluated (Figure 2).

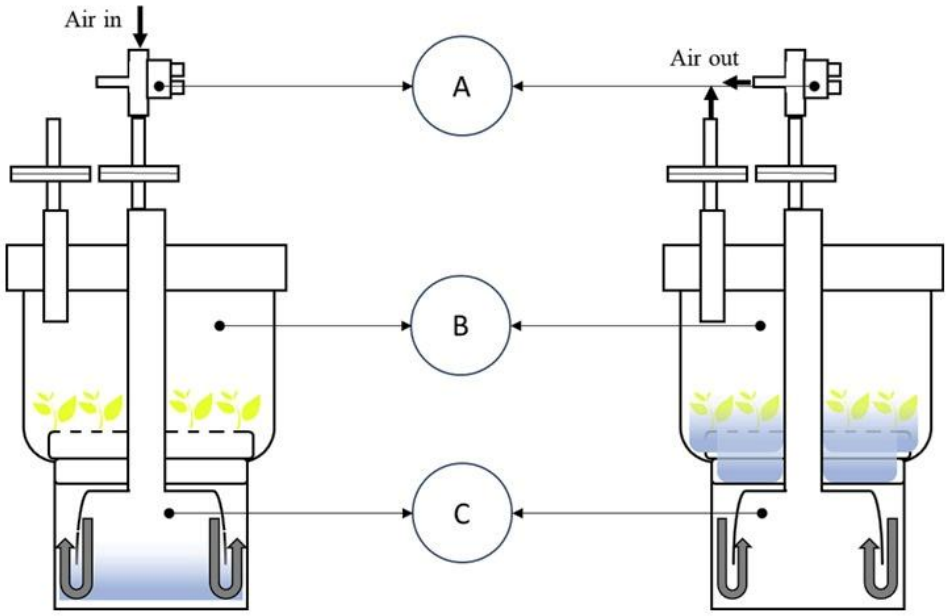

Figure 1. Diagram of the RITA® bioreactor during air inlet and outlet. (a) three-way solenoid valve, (b) culture chamber and (c) medium compartment.

Figure 2. (A) RITA® bioreactor with the culture medium and (b) RITA® bioreactor with banana seedlings.

In experiment 1, the aim was to determine the response to different explant density in banana cv. Williams. A liquid culture medium was used, based on MS culture medium (Murashige & Skoog, 1962) supplemented with 5.0 mgL^{-1} of IBA, 5.0 mgL^{-1} of BAP and 30 gL^{-1} of sucrose (Table 1).

Table 1
Treatments evaluated in explant density experiment

Treatment	Explant density	Culture medium
T0	1.00	MS + 5.00 mgL^{-1} BAP + 5.0 mgL^{-1} IBA + 30.00 gL^{-1} sucrose
T1	2.00	
T2	4.00	
T3	8.00	

In experiment 2, the aim was to determine the response to MS liquid culture medium with different concentrations of plant growth regulators in banana cv. Williams. Four different liquid culture media were used from an MS solution and four explants were used for each RITA® bioreactor (Table 2).

Table 2
Treatments evaluated in plant growth regulators experiment

Treatment	Explant density	Culture media
T0	4.00	MS
T1	4.00	MS + 5.00 mgL^{-1} BAP + 5.0 mgL^{-1} IBA + 30.00 gL^{-1} sucrose
T2	4.00	MS + 6.00 mgL^{-1} BAP + 2.0 mgL^{-1} IAA + 30.00 gL^{-1} sucrose
T3	4.00	MS + 6.00 mgL^{-1} BAP + 2.0 mgL^{-1} IBA + 30.00 gL^{-1} sucrose

Evaluation variables

These variables were evaluated: shoot multiplication coefficient, calculated from the number of final shoots with regard to the number of initial shoots; seedling height (cm), calculated taking the base of the seedling as a starting point; number of leaves per seedling (leaves/seedling), calculated counting all the true leaves present in the seedling; seedling fresh weight (g), calculated taking the plant material from the RITA® bioreactor, which was weighed with the use of an analytical balance; seedling dry weight (g), calculated placing the fresh plant material from the RITA® bioreactor in the oven at 70 °C for 3 days and recording the weight with the use of an analytical balance.

Statistical evaluation

For the analysis, the Duncan Multiple Range (DMR) test was used with a significance level of 0.05 for the variables evaluated. The analysis was performed using the RStudio software.

3. Results and discussion

The explant can be obtained from different parts of the plant. High-quality plant material allows for successful bioreactors culture (Coetser et al., 2022). Numerous types of temporary immersion systems (TIS) are often used for clonal propagation, with RITA® and TIB (Temporary Immersion Bioreactor) being the most common, guaranteeing plant material with better agronomic characteristics (Thanonkeo et al., 2024).

Response to explant density

Considering that the initial explants were quite similar, different shoot multiplication coefficients were obtained. It is important to standardize the number of explants per bioreactor to obtain a greater number of shoots.

Table 3 presents the data obtained for the variables at different explant density by applying the Duncan test with a significance level of 0.05. In this experiment, significant differences were observed between treatments. Treatment T2 (4 explants) obtained a significantly higher value in the shoot multiplication coefficient compared to the other treatments. However, it obtained a value with no significant differences in seedling height and number of leaves per seedling compared to the other treatments. In these variables, the higher values were obtained by treatments T1 (2 explants) and T3 (8 explants) respectively. In the case of the variable seedling fresh weight and seedling dry weight, treatment T3 (8 explants) was the one that revealed the highest value, being 3.63 g and 0.26 g respectively.

Culture time and explant density improve *in vitro* explant culture. Explant density influences the quantity and quality of the new shoots obtained.

Table 3
Determination of the response to different explant density

Treatment	Shoot multiplication coefficient	Seedling height (cm)	Number of leaves per seedling	Seedling fresh weight (g)	Seedling dry weight (g)
T0	5.60 ± 1.14 bc	4.19 ± 0.30 a	2.40 ± 0.54 a	1.90 ± 0.56 b	0.16 ± 0.03 b
T1	4.90 ± 0.65 c	4.57 ± 0.74 a	2.50 ± 0.50 a	3.12 ± 0.58 a	0.23 ± 0.04 a
T2	8.95 ± 0.48 a	4.29 ± 0.81 a	2.50 ± 0.40 a	2.86 ± 0.40 a	0.22 ± 0.03 a
T3	6.55 ± 0.35 b	4.53 ± 0.39 a	2.72 ± 0.35 a	3.63 ± 0.51 a	0.26 ± 0.04 a

T0: 1 explant, T1: 2 explants, T2: 4 explants, T3: 8 explants. Means with the same letter in each column are not statistically different ($p < 0.05$).

In addition, it controls physiological aspects during micropropagation (Monja-Mio et al., 2021). Low or high density is counterproductive for explant development due to poor use of resources (Bello-Bello et al., 2021). It is necessary 21 days of culture in temporary immersion bioreactors (TIS) for banana and there is an important relationship between explant density or number of explants per culture recipient in the formation of new shoots (Uma et al., 2021).

The choice of an appropriate volume of culture medium for the bioreactor size was essential to avoid undesirable characteristics such as hyperhydricity and excessive growth (Kikowska et al., 2022). The temporary immersion system (TIS) solves problems of asphyxia and hyperhydricity compared to other systems, highlighting the improvement obtained by using liquid medium over semi-solid medium (Carvalho et al., 2019; San José et al., 2020), making it possible to obtain greater biomass for certain species (De Carlo et al., 2021). Using TIS, a higher biomass production can be obtained. The high presence of secondary metabolites such as flavonoids and phenolic acids would be a response to temporary immersion and oxygen availability (Makowski et al., 2023); and it allows to obtain seedlings with a lower incidence of chlorosis and necrosis (Bayraktar, 2019).

The characteristics of the explant culture process are related to the volume of the medium, immersion time and immersion frequency (Melviana et al., 2021). The use of TIS bioreactors improves shoot multiplication compared to the semisolid system (SS) (Kunakhonnuruk et al., 2019). This coincides with what has been reported using TIS compared to SS in *Epipactis flava* (Kunakhonnuruk et al., 2019) and *Stevia rebaudiana* (Melviana et al., 2021). In the shoot multiplication coefficient variable, the treatment T2 (4 explants) stood out. Therefore, the treatment T2 with a value of 8.95 shoots per explant turned out to be the best treatment.

Response to plant growth regulators

The different concentrations used were reflected in the characteristics of the explants. An optimal culture medium must be established for this multiplication stage. Table 4 presents the data obtained for the variables at different concentrations of plant growth regulators by applying the Duncan test with a significance level of 0.05. In this experiment, significant differences were observed between treatments. Treatment T2 (MS + 6.00 mgL^{-1} BAP + 2.00 mgL^{-1} IAA + 30 gL^{-1} sucrose) obtained a significantly higher value in the shoot multiplication coefficient compared to the other treatments. For the variables seedling height and number of leaves per seedling, no significant statistical differences were found between the treatments and the control group, however, the treatment T2 (MS + 6.00 mgL^{-1} BAP + 2.00 mgL^{-1} IAA + 30 gL^{-1} sucrose) presented the highest value again, 3.56 cm and 2.69 respectively. On the other hand, for the variables seedling fresh weight and seedling dry weight, the treatments were statistically different from the control group, the treatment T1 (MS + 5.00 mgL^{-1} BAP + 5.00 mgL^{-1} IBA + 30 gL^{-1} sucrose) being the one that exhibited the highest value in both variables, 2.64 g and 0.20 g respectively.

Table 4
Determination of the response to different concentrations of plant growth regulators

Treatment	Shoot multiplication coefficient	Seedling height (cm)	Number of leaves per seedling	Seedling fresh weight (g)	Seedling dry weight (g)
T0	4.19 ± 0.63 c	2.92 ± 0.38 a	2.12 ± 0.32 a	1.46 ± 0.22 b	0.12 ± 0.01 b
T1	7.38 ± 1.33 ab	3.06 ± 0.29 a	2.31± 0.24 a	2.64 ± 0.45 a	0.20 ± 0.02 a
T2	8.62 ± 0.49 a	3.56 ± 0.46 a	2.69 ± 0.43 a	2.51 ± 0.33 a	0.18 ± 0.01 a
T3	6.19 ± 0.66 b	3.36 ± 0.60 a	2.62 ± 0.14 a	2.55 ± 0.16 a	0.19 ± 0.004 a

T0: MS, T1: MS + 5.00 mgL^{-1} BAP + 5.00 mgL-1 IBA + 30 gL^{-1} sucrose, T2: MS + 6.00 mgL^{-1} BAP+ 2.00 mgL^{-1} IAA+ 30 gL^{-1} sucrose, T3: MS + 6.00 mgL^{-1} BAP+ 2.00 mgL^{-1} IBA+ 30 gL^{-1} sucrose. Means with the same letter in each column are not statistically different ($p < 0.05$).

Explant density, immersion frequency, and plant growth regulators influence the success of TIS. Regarding PGR, using the cytokinin BAP guarantees better results in TIB compared to traditional methods (Thanonkeo et al., 2024). Plant hormones can promote or inhibit development and growth in plants. Cytokinins promote shoot growth and auxins shoot elongation. Their use has the adverse effect of presenting a greater number of phenolic compounds; yet they have a widespread use and ways of application (Sosnowski et al., 2023). The importance of the culture system, regulators, and genotype in the production of phenolic compounds is highlighted (Clapa et al., 2022).

Seedlings exhibit genetic similarity. The importance of using BAP for shoot production was demonstrated (Ramírez-Mosqueda et al., 2016). The non-use of IBA allows to obtain seedlings with better characteristics such as number of shoots, number of leaves and vigor (Khafri et al., 2023; Husen et al., 2024). Applying IBA in rooting had little relevance (Capaci et al., 2024). The presence of sucrose directly influences shoot multiplication and the importance of using bioreactors for later stages in micropropagation (Gago et al., 2022).

Temporary immersion bioreactors induce the formation of a high number of new banana shoots and achieve a high *ex vitro* survival (Uma et al., 2023). Totipotency capacity of plants due to somatic embryogenesis and suggests a relationship with growth regulators (Su et al., 2021). This is consistent with what has been reported using TIS compared to SS in *Cannabis sativa* (Rico et al., 2022) and *Chrysanthemum morifolium* (Hwang et al., 2022).

In the shoot multiplication coefficient variable, the treatment T2 (MS+ 6.00 mgL^{-1} BAP+ 2.00 mgL^{-1} IAA+ 30 gL^{-1} sucrose) stood out, with this treatment finally being the best treatment with an average of 8.62 shoots per explant.

4. Conclusions

The use of temporary immersion systems (TIS) compared to other systems guarantees better results in the micropropagation of numerous species, including banana. The initial density of explants influences the proliferation of shoots during the multiplication stage, as well as the availability of oxygen and consequently affects the biochemical reactions. On the other hand, the amount of plant growth regulators (PGR) used to prepare the liquid culture medium affects their impact on banana explants. Auxins and cytokinins have an effect on the development of explants and their concentration influences their action, therefore it is important to consider the ratio for optimal development. Future studies should address the influence of hyperhydricity and phenolization on the optimization of micropropagation.

References

Alvarez, S. P., Tapia, M. A. M., Vega, M. E. G., Ardisana, E. F. H., Medina, J. A. C., Zamora, G. L. F., & Bustamante, D. V. (2019). Nanotechnology and Plant Tissue Culture. In R. Prasad. (Ed.), *Plant Nanobionics* (333–370). Berlin, Alemania: Springer. https://doi.org/10.1007/978-3-030-12496-0_12

Bayraktar, M. (2019). Micropropagation of *Stevia rebaudiana* Bertoni Using RITA® Bioreactor. *HortScience horts, 54*(4), 725-731. https://doi.org/10.21273/HORTSCI13846-18

Bello-Bello, J. J., Schettino-Salomón, S., Ortega-Espinoza, J., & Spinoso-Castillo, J. L. (2021). A temporary immersion system for mass micropropagation of pitahaya (*Hylocereus undatus*). *Biotech, 11*(10), 437. https://doi.org/10.1007/s13205-021-02984-5

Bhowmik, T. K., & Rahman, M. M. (2020). Micropropagation of commercially important orchid *Dendrobium palpebrae* Lindl. through *in vitro* developed pseudobulb culture. *Journal of Advanced Biotechnology and Experimental Therapeutics, 3*(3), 225–232. https://doi.org/10.5455/jabet.2020.d128

Bozkurt, T., İnan, S., & Dündar, İ. (2023). Comparison of Temporary Immersion Bioreactor (SETISTM) and Classical Solid Culture in Micropropagation of 'Grand Naine'(*Musa* spp.) Banana Cultivar. *Journal of Agricultural Science, 15*(12). https://doi.org/10.5539/jas.v15n12p51

Capaci, P., Barozzi, F., Forciniti, S., Anglana, C., Iuele, H., Accogli, R. A., Carra, A., Lenucci, M. S., del Mercato, L. L., & Di Sansebastiano, G. P. (2024). RITA® Temporary Immersion System (TIS) for Biomass Growth Improvement and *Ex Situ* Conservation of *Viola ucriana* Erben & Raimondo. *Plants, 13*(24), 3530. https://doi.org/10.3390/plants13243530

Clapa, D., Nemeș, S.-A., Ranga, F., Hârța, M., Vodnar, D.-C., & Călinoiu, L.-F. (2022). Micropropagation of *Vaccinium corymbosum* L.: An Alternative Procedure for the Production of Secondary Metabolites. *Horticulturae, 8*(6), 480. https://doi.org/10.3390/horticulturae8060480

Cardoso, J. C., Zanello, C. A., & Chen, J. T. (2020). An overview of orchid protocorm-like bodies: Mass propagation, biotechnology, molecular aspects, and breeding. *International Journal of Molecular Sciences, 21*(3), 985. https://doi.org/10.3390/ijms21030985

Carvalho, L. S. O., Ozudogru, E. A., Lambardi, M., & Paiva, L. V. (2019). Temporary immersion system for micropropagation of tree species: a bibliographic and systematic review. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47*(2), 269-277. https://doi.org/10.15835/nbha47111305

Coetser, E., du Toit, E. S., & Prinsloo, G. (2022). An Investigation into Using Temporary Immersion Bioreactors to Micropropagate *Moringa oleifera* Lam. Callus, Roots, and Shoots. *Agronomy, 12*(11), 2672. https://doi.org/10.3390/agronomy12112672

De Carlo, A., Tarraf, W., Lambardi, M., & Benelli, C. (2021). Temporary immersion system for production of biomass and bioactive compounds from medicinal plants. *Agronomy 11*(12), 2414. https://doi.org/10.3390/agronomy11122414

Gago, D., Sánchez, C., Aldrey, A., Christie, C. B., Bernal, M. Á., & Vidal, N. (2022). Micropropagation of Plum (*Prunus domestica* L.) in bioreactors using photomixotrophic and photoautotrophic conditions. *Horticulturae, 8*(4), 286. https://doi.org/10.3390/horticulturae8040286

Grieneisen, V. A., Xu, J., Marée, A. F. M., Hogewep, P., & Scheres, B. (2007). Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature, 449*(7165), 1008–1013. https://doi.org/10.1038/nature06215

Hwang, H. D., Kwon, S. H., Murthy, H. N., Yun, S. W., Pyo, S. S., & Park, S. Y. (2022). Temporary immersion bioreactor system as an efficient method for mass production of *in vitro* plants in

horticulture and medicinal plants. *Agronomy, 12*(2), 346. https://doi.org/10.3390/agronomy12020346

Husen, S., Purnomo, A. E., Tina, S. A., Iriany, A., Wahyono, P., & Roeswitawati, D. (2024). Liquid culture for efficient *in vitro* propagation of potato (*Solanum tuberosum* L.) using bioreactor system. *Australian Journal of Crop Science, 18*(6), 365-373. https://doi.org/10.21475/ajcs.24.18.06.pne63

Hussein, N. (2012). Effects of nutrient media constituents on growth and development of banana (*Musa* spp.) shoot tips cultured *in vitro*. *African Journal of Biotechnology, 11*(37), 9001-9006. https://doi.org/10.5897/AJB11.4173

Katel, S., Yadav, S. P. S., & Sharma, B. (2022). Impacts of plant growth regulators in strawberry plant: A review. *Heliyon 8*(12), e11959. https://doi.org/10.1016/j.heliyon.2022.e11959

Khafri, A. Z., Zarghami, R., Ma'mani, L., & Ahmadi, B. (2023). Enhanced efficiency of *in vitro* rootstock micro-propagation using silica-based nanoparticles and plant growth regulators in myrobalan 29C (*Prunus cerasifera* L.). *Journal of Plant Growth Regulation, 42*(3), 1457-1471. https://doi.org/10.1007/s00344-022-10631-3

Kikowska, M., Danek, K., Gornowicz-Porowska, J., & Thiem, B. (2022). Application of temporary immersion system RITA® for efficient biomass multiplication and production of artificial seeds for *ex situ* conservation of *Linnaea borealis* L. *Plant Cell, Tissue and Organ Culture (PCTOC), 151*(3), 673-680. https://doi.org/10.1007/s11240-022-02381-7

Krol, A., Kokotkiewicz, A., Szopa, A., Ekiert, H.M., & Luczkiewicz, M. (2021). Bioreactor-Grown Shoot Cultures for the Secondary Metabolite Production. In K. G. Ramawat, H. M. Ekiert, S. Goyal (Eds.), *Plant Cell and Tissue Differentiation and Secondary Metabolites* (187–247). Cham, Suiza: Springer. https://doi.org/10.1007/978-3-030-30185-9_34

Kumari, S., Bakshi, P., Sharma, A., Wali, V. K., Jasrotia, A., & Kour, S. (2018). Use of plant growth regulators for improving fruit production in sub tropical crops. *International Journal of Current Microbiology and Applied Sciences, 7*(3), 659-668. https://doi.org/10.20546/ijcmas.2018.703.077

Kunakhonnuruk, B., Inthima, P., & Kongbangkerd, A. (2019). *In vitro* propagation of Rheophytic Orchid, *Epipactis flava* Seidenf. - A Comparison of Semi-solid, Continuous Immersion and Temporary Immersion Systems. *Biology, 8*(4), 72. https://doi.org/10.3390/biology8040072

Lakho, M. A., Jatoi, M. A., Solangi, N., Abul-Soad, A. A., Qazi, M. A., & Abdi, G. (2023). Optimizing *in vitro* nutrient and *ex vitro* soil mediums-driven responses for multiplication, rooting, and acclimatization of pineapple. *Scientific Reports, 13*(1), 1275. https://doi.org/10.1038/s41598-023-28359-9

Makowski, W., Królicka, A., Tokarz, B., Szopa, A., Ekiert, H., & Tokarz, K. M. (2023). Temporary immersion bioreactors as a useful tool for obtaining high productivity of phenolic compounds with strong antioxidant properties from *Pontechium maculatum*. *Plant Cell, Tissue and Organ Culture (PCTOC), 153*(3), 525-537. https://doi.org/10.1007/s11240-023-02487-6

Mamun, N.H.A., Egertsdotter, U., & Aidun, C.K. (2015). Bioreactor technology for clonal propagation of plants and metabolite production. *Frontiers in Biology, 10*(2), 177–193. https://doi.org/10.1007/s11515-015-1355-1

Melviana, A. C., Esyanti, R. R., Mel, M., & Setyobudi, R. H. (2021). Biomass enhancement of *Stevia rebaudiana* Bertoni Shoot culture in temporary immersion system (TIS) RITA® bioreactor optimized in two different immersion periods. *EDP Sciences 226*(7), 1-9. https://doi.org/10.1051/e3sconf/202122600007

Monja-Mio, K. M., Olvera-Casanova, D., Herrera-Alamillo, M. Á., Sánchez-Teyer, F. L., & Robert, M. L. (2021). Comparison of conventional and temporary immersion systems on micropropagation (multiplication phase) of *Agave angustifolia* Haw. 'Bacanora'. *3 Biotech, 11*, 77. https://doi.org/10.1007/s13205-020-02604-8

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

Nielsen, E., Temporiti, M. E. E. & Cella, R. (2019). Improvement of phytochemical production by plant cells and organ culture and by genetic engineering. *Plant Cell Reports, 38*(10), 1199–1215. https://doi.org/10.1007/s00299-019-02415-z

Raghavan, V. (2004). Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of *Arabidopsis*: cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. *American Journal of Botany, 91*(11), 1743–1756. https://doi.org/10.3732/AJB.91.11.1743

Ramírez-Mosqueda, M. A., Sánchez-Segura, L., Hernández-Valladolid, S. L., Bello-Bello, E., & Bello-Bello, J. J. (2020). Influence of silver nanoparticles on a common contaminant isolated during the establishment of *Stevia rebaudiana* Bertoni culture. *Plant Cell Tissue and Organ Culture, 143*(3): 609-618. https://doi.org/10.1007/s11240-020-01945-9

Ramírez-Mosqueda, M. A., Iglesias-Andreu, L. G., Ramírez-Madero, G., & Hernández-Rincón, E. U. (2016). Micropropagation of *Stevia rebaudiana* Bert. in temporary immersion systems and evaluation of genetic fidelity. *South African Journal of Botany, 106*, 238-243. https://doi.org/10.1016/j.sajb.2016.07.01

Rico, S., Garrido, J., Sánchez, C., Ferreiro-Vera, C., Codesido, V., & Vidal, N. (2022). A temporary immersion system to improve *Cannabis sativa* micropropagation. *Frontiers in Plant Science, 13*, 895971. https://doi.org/10.3389/fpls.2022.895971

San José, M. C., Blázquez, N., Cernadas, M. J., Janeiro, L. V., Cuenca, B., Sánchez, C., & Vidal, N. (2020). Temporary immersion systems to improve alder micropropagation. *Plant Cell Tissue and Organ Culture, 143*, 265-275. https://doi.org/10.1007/s11240-020-01937-9

Sharma, L., Priya, M., Kaushal, N., Bhandhari, K., Chaudhary, S., Dhankher, O. P., Prasad, P. V. V., Siddique, K. H. M., & Nayyar, H. (2020). Plant growth-regulating molecules as thermoprotectants: functional relevance and prospects for improving heat tolerance in food crops. *Journal of Experimental Botany, 71*(2), 569–594. https://doi.org/10.1093/jxb/erz333

Sosnowski, J., Truba, M., & Vasileva, V. (2023). The impact of auxin and cytokinin on the growth and development of selected crops. *Agriculture, 13*(3), 724. https://doi.org/10.3390/agriculture13030724

Su, Y. H., Tang, L. P., Zhao, X. Y., & Zhang, X. S. (2021). Plant cell totipotency: Insights into cellular reprogramming. *Journal of Integrative Plant Biology, 63*, 228-240. https://doi.org/10.1111/jipb.12972

Teisson, C., Alvard, D., Berthouly, B., Cote, F., Escalant, J., Etienne, H., & Lartaud, M. (1996). Simple apparatus to perform plant tissue culture by temporary immersion. *Acta Horticulturae, 440*, 521-526. https://doi.org/10.17660/ActaHortic.1996.440.91

Thanonkeo, S., Kitwetcharoen, H., Thanonkeo, P., & Klanrit, P. (2024). Temporary Immersion Bioreactor (TIB) System for Large-Scale Micropropagation of *Musa* sp. cv Kluai Numwa Pakchong 50. *Horticulturae, 10*(10), 1030. https://doi.org/10.3390/horticulturae10101030

Uma, S., Karthic, R., Kalpana, S., & Backiyarani, S. (2023). Evaluation of temporary immersion bioreactors for *in vitro* micropropagation of banana (*Musa* spp.) and genetic fidelity assessment using flow cytometry and simple-sequence repeat markers. *South African Journal of Botany, 157*, 553-565. https://doi.org/10.1016/j.sajb.2023.04.006

Uma, S., Karthic, R., Kalpana, S., Backiyarani, S., & Saraswathi, M. S. (2021). A novel temporary immersion bioreactor system for large scale multiplication of banana (Rasthali AAB—Silk). *Scientific Reports, 11*(1), 20371. https://doi.org/10.1038/s41598-021-99923-4

Vidal, N., & Sánchez, C. (2019). Use of bioreactor systems in the propagation of forest trees. *Engineering in Life Sciences, 19*(12), 896-915. https://doi.org/10.1002/elsc.201900041

Wybouw, B., & De Rybel, B. (2018). Cytokinin – A Developing Story. *Trends in Plant Science, 24*(2), 177-185. https://doi.org/10.1016/j.tplants.2018.10.012

Zhang, Q., Gong, M., Xu, X., Li, H., & Deng, W. (2022). Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. *Cells, 11*(17), 2761. https://doi.org/10.3390/cells11172761