In Vitro Cellular & Developmental Biology - Plant (2026) 62:657–667
https://doi.org/10.1007/s11627-026-10628-7

PLANT TISSUE CULTURE

Micropropagation of *Vanilla planifolia* using the Biotilt™ temporary immersion system

Mariana Cruz de Souza[1] · Autumn Reeves[2] · David Beleski[2] · Michele Valquíria dos Reis[1] · Wagner Vendrame[2]

Received: 15 September 2025 / Accepted: 3 January 2026 / Published online: 24 February 2026 / Editor: Jose Carlos Lorenzo
© The Author(s) 2026

Abstract

Vanilla planifolia Jacks. ex Andrews is an orchid species of great economic importance is responsible for the production of fruits rich in vanillin and has a high global demand. Conventional propagation has limitations for production and contributes to pressure on natural populations. In this context, tissue culture emerges as a viable strategy for clonal propagation of Vanilla, and temporary immersion bioreactor systems stand out by offering greater efficiency, sustainable management, and conservation potential for the species. The objective of this study was to evaluate the efficiency of the Biotilt™ bioreactor system compared to the conventional semi-solid system and to identify the best adjustments of immersion frequency and medium volume to optimize the *in vitro* multiplication of *V. planifolia* explants. Two experiments were conducted. The first experiment assessed the morphophysiological performance of explants cultivated in Biotilt™ (liquid medium) versus the semi-solid system, considering growth variables, physiological quality, efficiency indices, and correlation analysis. The second experiment investigated combinations of medium volume and immersion frequency in Biotilt™ in a factorial design, aiming to optimize culture conditions. Results indicated that Biotilt™ promoted higher explant length, number of shoots, leaves, and roots, as well as higher chlorophyll content, thus demonstrating greater multiplication and physiological efficiency. The analysis of composite indices reinforced the superiority of the temporary immersion system for explant development. Finally, although not statistically significant, the tested combinations of volume and immersion frequency maintained a positive performance trend, contributing to future protocols. These results highlighted the potential of Biotilt™ as an efficient alternative for the micropropagation of *V. planifolia*, combining increased productivity with conservation practices.

Keywords Tissue culture · Immersion frequency · Orchid · Plant biotechnology · Bioreactors

Introduction

The genus *Vanilla Plum. ex Mill.* (Orchidaceae) comprises approximately 120 species distributed across the tropics of the Americas, Africa, and Asia (Cameron 2018; Karremans *et al*. 2020; Powo 2023). Brazil has the highest diversity within the genus, with 30 recorded species, 13 of which are endemic (Reflora 2020; Nadal *et al*. 2025). Only this restricted group produces fruits rich in vanillin, making *Vanilla* the most economically relevant genus among orchids, with *V. planifolia* as the main cultivated species, followed by the hybrid *Vanilla* × *tahitensis* (Flanagan *et al*. 2018; Karremans *et al*. 2020; Perez-Silva *et al*. 2021).

The increasing demand for natural Vanilla puts pressure on large scale production, reinforcing the ecological importance of the genus and the need for conservation and sustainable management strategies (Simas *et al*. 2024). Conventional propagation faces limitations related to growth, rooting, and susceptibility to pathogens, in addition to encouraging the collection of wild plants (Tan and Chin 2015; Kadir *et al*. 2019). In this context, tissue culture emerges as an alternative to enable the production of healthy and uniform plantlets, ensuring a continuous supply without compromising native populations (Manokari *et al*. 2021; Ramirez-Mosqueda *et al*. 2022; Rodriguez-Demeneghi *et al*. 2023).

The micropropagation of *Vanilla planifolia* has been widely studied, and new technologies, such as temporary immersion bioreactors (TIBs), show potential for large scale

✉ Michele Valquíria dos Reis
michele.reis@ufla.br

[1] Department of Agriculture, Federal University of Lavras, Lavras, MG, Brazil

[2] Horticultural Sciences Department, University of Florida/IFAS, Gainesville, FL, USA

production, making *in vitro* biofactories feasible. These systems offer advantages, such as pest free plantlets, greater shoot multiplication and elongation, improved survival during acclimatization, and better explant oxygenation, thus reducing hyperhydricity (Ramirez-Mosqueda and Bello-Bello 2021; Rodriguez-Demeneghi *et al*. 2022; Baltazar-Bernal *et al*. 2024).

TIBs are semi-automated systems that alternate immersion in a nutrient solution for short periods of time with exposure to air. Among the various available models, the Biotilt™ stands out for its simplicity and efficiency, with two interconnected containers that reduce the complexity of aseptic cultivation in liquid medium (Plant Cell Technology 2025).

Although TIBs offer advantages, new technologies need to be compared to validate their efficiency and optimize protocols. Considering the importance of Vanilla and the challenges of traditional propagation methods, evaluating the potential of Biotilt™ as an efficient and simplified solution is essential not only to advance optimized production and reduce dependence on wild plant collection, but also to support the conservation and sustainable management of this species.

This study aimed to evaluate the efficiency of the Biotilt™ system using liquid medium compared to the conventional semi-solid system. In addition, it sought to identify the best combinations of immersion frequency and medium volume to optimize the *in vitro* multiplication of *V. planifolia* explants.

Materials and methods

Plant Material and Cultivation Conditions Nodal segments of *Vanilla planifolia* were collected from *in vitro* established plants. The explants were standardized to an average length of 1.5 ± 0.3 cm and randomly distributed across the evaluated culture systems. The culture medium used was Murashige and Skoog (MS; Murashige and Skoog 1962), supplemented with B5 vitamins (1.0 mL L^{-1}), myo-inositol (100.0 mg L^{-1}), 2iP (2iP (2.5 mg L^{-1}, obtained by adding 2.5 mL L^{-1} of a 1 mg mL^{-1} stock solution)), and sucrose (30.0 g L^{-1}), with the pH adjusted to 5.7. For cultivation in semi-solid medium, 2.5 g L^{-1} of Phytagel™ (Sigma-Aldrich, St. Louis, MO) was added prior to autoclaving. All treatments were maintained in a growth chamber (Percival Scientific, Perry, IA) at 25 ± 2 °C under a 16 h light/8 h dark photoperiod, with an irradiance of 50 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps (General Electric, Boston, MA).

Comparison between Different Culture Systems To assess the viability of the temporary immersion system BioTilt™ (Plant Cell Technology, Washington, DC), two types of culture systems were tested. These included a semi-solid (conventional method) medium with Phytagel (Sigma-Aldrich) and a liquid medium using the BioTilt™ system.

The BioTilt™ system (Fig. 1) operated with an immersion frequency of 5 min every 2 h, as reported by Baltazar-Bernal *et al*. (2024) for orchid micropropagation, using 150 mL of culture medium per vessel (37.5 mL per explant). In the conventional system, explants were individually cultivated in glass bottles ("baby food jars"), with 45 mL of culture medium per jar. Both systems were maintained under the environmental conditions previously described, and explants remained in culture for a period of 40 days (d).

Culture Medium Volume and Immersion Frequency in the BioTilt™ System To evaluate the combined effect of culture medium volume and immersion frequency on explant performance, a 2×2 factorial experiment was conducted using the BioTilt™ system. Treatments consisted of two culture medium volumes, 150 mL (37.5 mL per explant) and 300 mL (75 mL per explant), combined with two immersion frequencies: every 1 h and every 3 h, totaling four treatment combinations. The immersion duration was fixed at 5 min for all treatments. All other experimental conditions, including medium composition, cultivation environment, and total cultivation period (40 d), were kept constant.

Analyzed Variables, Experimental Design, and Statistical Analysis At the end of the 40-day experimental period, multiplication rate, propagation efficiency, and physiological quality of the explants were evaluated in all treatments to compare the efficacy of the culture systems. Morphometric parameters were measured through image analysis using ImageJ software (Schneider *et al*. 2012), including explant length and number of leaves; number, length, and diameter of shoots; and number and length of roots. In addition, stratified samples from each system were used to determine fresh and dry biomass, as well as chlorophyll content using a portable SPAD-502 chlorophyllmeter (SPAD-502, Minolta Co., Ltd., Osaka, Japan).

All experiments were conducted in a randomized complete block design, with four explants per block and five blocks per treatment, totaling 20 explants per treatment. The data were subjected to analysis of variance (ANOVA), and the assumptions of normality and homogeneity were verified using the Shapiro–Wilk and Bartlett tests, respectively. When significant differences were detected, means were compared using Tukey's test at a 5% probability level.

Additionally, a Pearson correlation analysis was performed to assess linear relationships among the evaluated traits across culture systems. Three composite indices were also calculated to summarize treatment performance: i) a multiplication index, based on normalized means of the

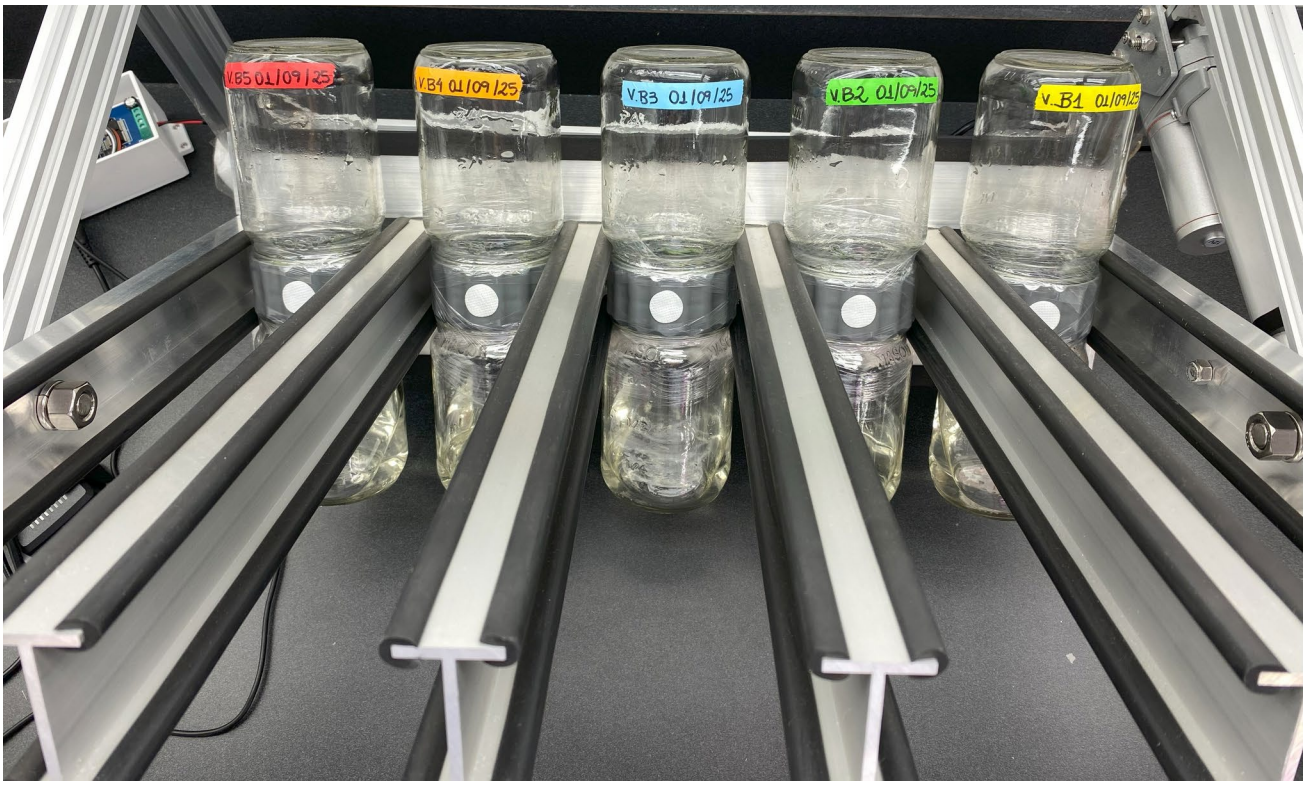


Figure 1. Biotilt™ temporary immersion bioreactor system containing *Vanilla planifolia* Jacks. ex Andrews explants.

number of shoots, leaves, and roots; ii) a physiological quality index, composed of shoot length and diameter, root and explant length, chlorophyll content, and fresh and dry weight; and iii) a global index, integrating all assessed morphophysiological variables. All values were standardized (z-score) to prevent distortion caused by different measurement scales.

To highlight the overall performance trend of each combination of immersion frequency and medium volume, a heatmap was generated based on the standardized means (z-scores) of the morphophysiological variables. All statistical analyses were performed using R software (R Core Team 2025).

Results

Comparison Between BioTilt™ and the Semi-solid System Analysis of variance (ANOVA), followed by Tukey's test, revealed significant differences ($p < 0.05$) among the culture systems. These differences were observed for explant length, number of shoots, number of leaves, number of roots, and chlorophyll content (Table 1).

The BioTilt™ temporary immersion bioreactor demonstrated superior performance across all significantly affected variables compared to the traditional semi-solid system. Although some traits exhibited higher standard error in this system compared to the semi-solid medium, for example, number of leaves (SE = 0.39 vs. 0.33), the elevated means reflected a substantial improvement in explant development,

Table 1. Mean ± standard error (SE) of morphophysiological variables of *Vanilla planifolia* Jacks. ex Andrews explants grown in temporary immersion bioreactor system (BioTilt™) and semi-solid system (Phytagel™)

Variables	Treatments	
	Temporary immersion bioreactor	Semi-solid system
Explant length (cm)	8.85 ± 0.69 a	3.50 ± 0.31 b[a]
Number of shoots	5.25 ± 0.71 a	2.82 ± 0.30 b
Shoot length (cm)	1.41 ± 0.26 a	1.58 ± 0.25 a
Shoot diameter (cm)	0.28 ± 0.04 a	0.28 ± 0.04 a
Number of roots	4.80 ± 0.29 a	1.95 ± 0.29 b
Root length (cm)	1.76 ± 0.32 a	2.11 ± 0.21 a
Number of leaves	3.30 ± 0.39 a	1.00 ± 0.33 b
Chlorophyll (SPAD)	20.18 ± 1.18 a	13.52 ± 1.84 b
Fresh weight (g)	1.90 ± 0.56 a	1.12 ± 0.27 a
Dry weight (g)	0.19 ± 0.05 a	0.10 ± 0.02 a

[a]*Different letters* within the same *row* indicate significant differences between treatments according to Tukey's test at 5% probability

confirming the efficiency of the temporary immersion system for the micropropagation of *V. planifolia*.

The data revealed that the number of roots (4.80), number of shoots (5.25), and explant length (8.85 cm) per explant were markedly higher in the BioTilt™ system compared to the semi-solid system, which showed values of 1.95, 2.82, and 3.50, respectively. Figure 2 illustrates how growth differences between treatments were evident not only in quantitative terms but also visually perceptible.

To identify possible associations among the analyzed variables, a Pearson correlation analysis was performed. Figure 3 presents the correlation coefficients, allowing the observation of positive and negative relationship patterns among the evaluated parameters.

The analysis revealed values indicating strong and consistent correlations. Biomass variables (fresh and dry) showed highly significant correlations ($r = 0.99$), indicating proportional biomass accumulation. The number of shoots was strongly correlated with dry biomass ($r = 0.87$) and chlorophyll content ($r = 0.82$), suggesting a direct association between physiological activity, shoot proliferation, and biomass accumulation.

The number of leaves was also positively correlated with explant length ($r = 0.89$) and number of roots ($r = 0.82$), indicating that a more developed aerial part tends to support greater root formation. However, a potential trade-off between root and shoot development was observed, as root length was negatively correlated with explant length ($r = -0.47$) and number of leaves ($r = -0.53$).

Overall, the results indicate a strong interdependence among the morphophysiological parameters, reinforcing the consistency of the observed responses. The standardized multiplication, physiological quality, and global indices presented in Fig. 4 summarize all variable based results and support the overall assessment of each system's effectiveness in the micropropagation of *V. planifolia*.

The composite indices reinforced the superior performance of the BioTilt™ bioreactor compared to the semi-solid system. The multiplication index was the most prominent in distinguishing between the systems, with a score of 0.8 for BioTilt™ and −0.8 for the semi-solid system. Subsequently, the global and physiological quality indices further highlighted the overall effectiveness of the temporary immersion bioreactor, with positive scores of 0.54 and 0.29, respectively. These results demonstrated the consistency between the evaluated indices and the observed performance patterns across the systems.

Medium Volume and Immersion Frequency in the BioTilt™ System Based on the factorial analysis, significant differences were observed for explant length, shoot diameter, and chlorophyll content. These differences were influenced by both the medium volume and the immersion frequency factors (Fig. 5).

The explant length was influenced by frequency and medium volume individually, since the interaction between the factors was not significant. The frequency of 3 h was superior to 1 h, and the volume of 300 mL was superior to 150 mL. Similarly, the interaction between the factors was

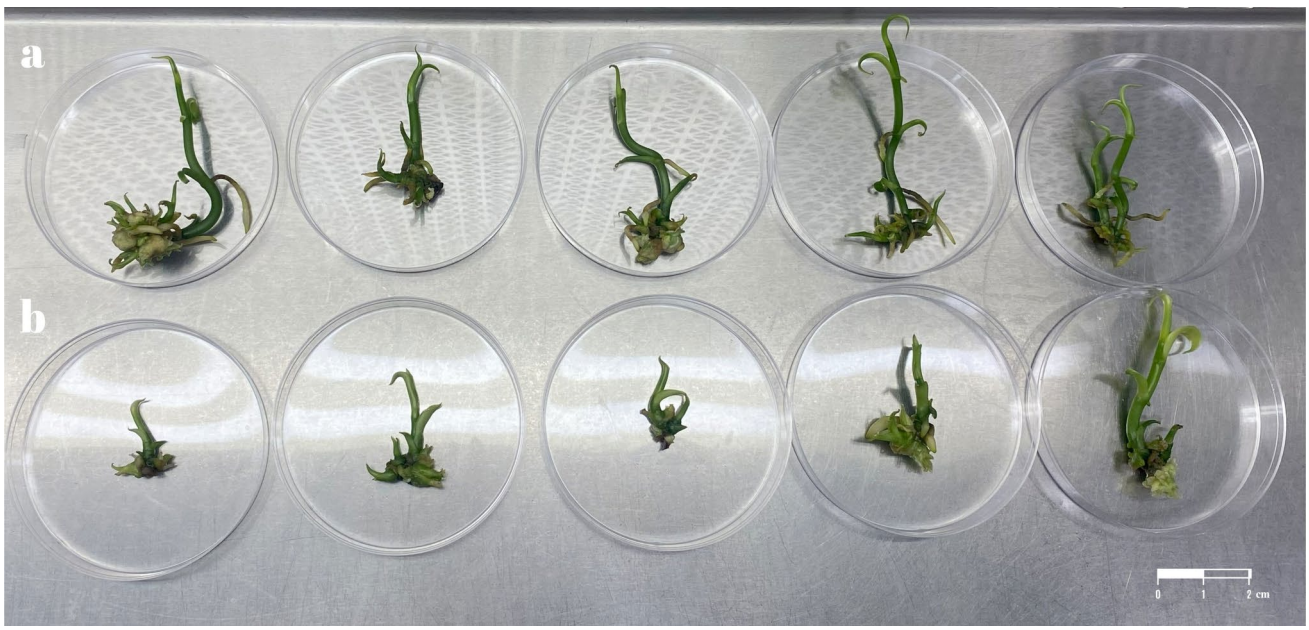


Figure 2. Growth of *Vanilla planifolia* Jacks. ex Andrews explants after 40 d of culture in the Temporary Immersion Bioreactor (*a*) and Semi-Solid (*b*). The image represents the five experimental blocks of each system. *Scale bar* = 2 cm.

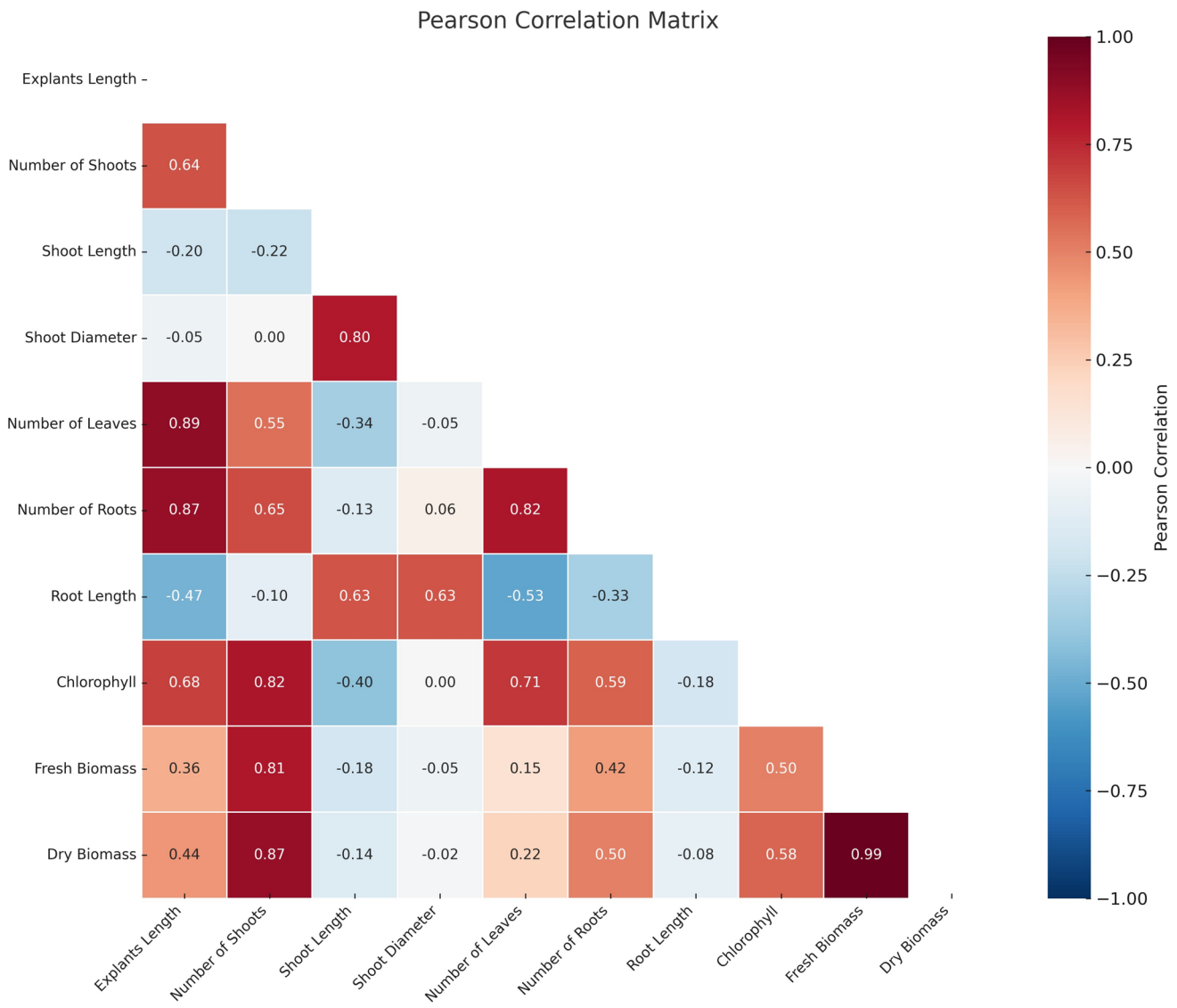


Figure 3. Pearson correlation matrix between morphophysiological variables of *Vanilla planifolia* Jacks. ex Andrews explants cultivated under both culture systems. The values indicate the Pearson correlation coefficient (r), ranging from –1 to 1. *Darker red tones* represent stronger positive correlations, and *blue tones* represent negative correlations.

not significant for shoot diameter; however, individually, both factors showed significant differences.

The variable root length was influenced only by the medium volume, with the longest lengths observed in the treatments with 150 mL. Meanwhile, the chlorophyll content showed a significant effect only for the immersion frequency. It was observed that the correlation was not significant, and for the frequency factor, there was no significant difference.

Although the other variables did not show significant differences (Table 2), the overall analysis of the means, summarized in the heatmap (Fig. 6), enabled a comparative evaluation of treatment performance that goes beyond isolated statistical significance. It is observed that although there are isolated points in the other treatments indicating good values, the treatments with immersions every 3 h stood out, especially the treatment with a volume of 300 mL, which showed a consistency of higher means, except for shoot length, which stood out at the 1 h frequency.

The results observed in the heatmap indicated that the combination of longer immersion frequency (every 3 h) and higher medium volume may favor the overall performance of the explants, which was a particularly and highly relevant result for practical purposes in defining more efficient protocols. However, most of the evaluated variables did not show statistically significant differences. Therefore, these observations should be interpreted as trends rather than conclusive results.

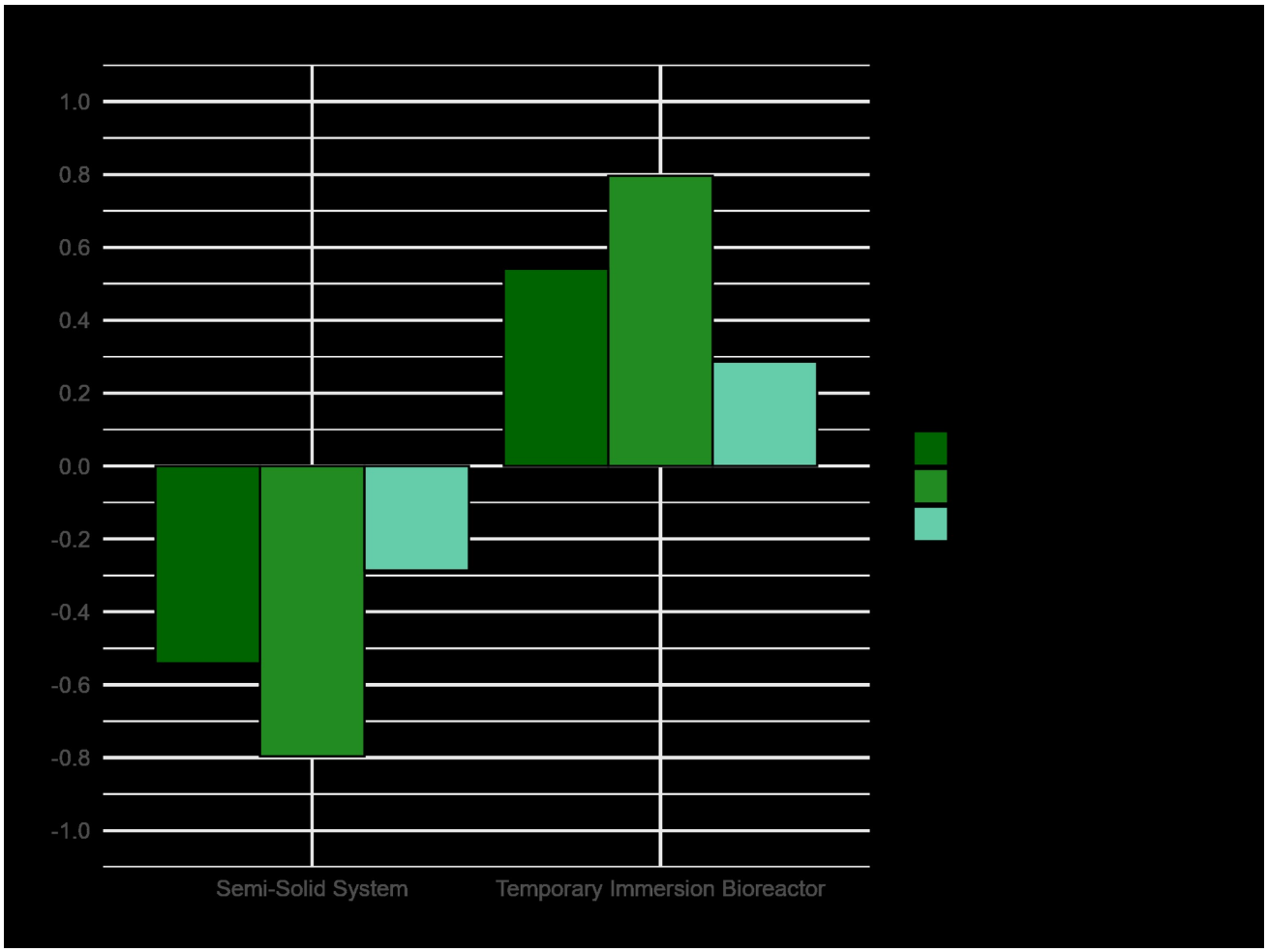


Figure 4. Mean values of three composite indices for *Vanilla planifolia* Jacks. ex Andrews cultured in two systems. The Multiplication Index reflects propagation capacity, the Physiological Quality Index represents morphophysiological vigor, and the Global Index integrates all measured variables.

Discussion

Performance of the Biotilt™ System Versus Semi-solid Medium The superior performance of the explants in the temporary immersion system (Table 1) indicated greater physiological and multiplicative efficiency of the protocol. This performance is possibly explained by the greater contact surface between the explant and the nutrients, allowing better absorption by all tissues that were in interaction with the medium. In the semi-solid system, on the other hand, the availability of nutrients was restricted to the base of the explant, since only this part remained in direct contact with the culture medium (Ramírez-Mosqueda and Bello-Bello 2021; Vendrame *et al*. 2023; Cabrera *et al*. 2024).

Shoot length and shoot production per explant were the variables that stood out the most in the Biotilt™ system, presenting mean values more than twice as high for length and almost double for the number of shoots compared to the semi-solid system. Similar patterns of superiority of temporary immersion systems were also observed in *Epipactis flava* Seidenf., which promoted significant increases in the multiplication rate and shoot elongation (Kunakhonnuruk *et al*. 2019).

In line with this, Ramírez-Mosqueda (2021) reported comparable gains in Vanilla using SETIS™ bioreactors, attributing such results to the greater availability of nutrients and to the design of each bioreactor in relation to light availability. Similarly, Leyva-Ovalle *et al*. (2020) concluded that the temporary immersion system in *Guarianthe skinneri* was efficient for generating a higher number of shoots, standing out as a potential protocol to meet the commercial demand for ornamental and conservation purposes.

This capacity to amplify plant material in a shorter time is highly desirable in micropropagation, since each shoot is a potential unit for the formation of new plants. In this same context, the positive correlation between the number of

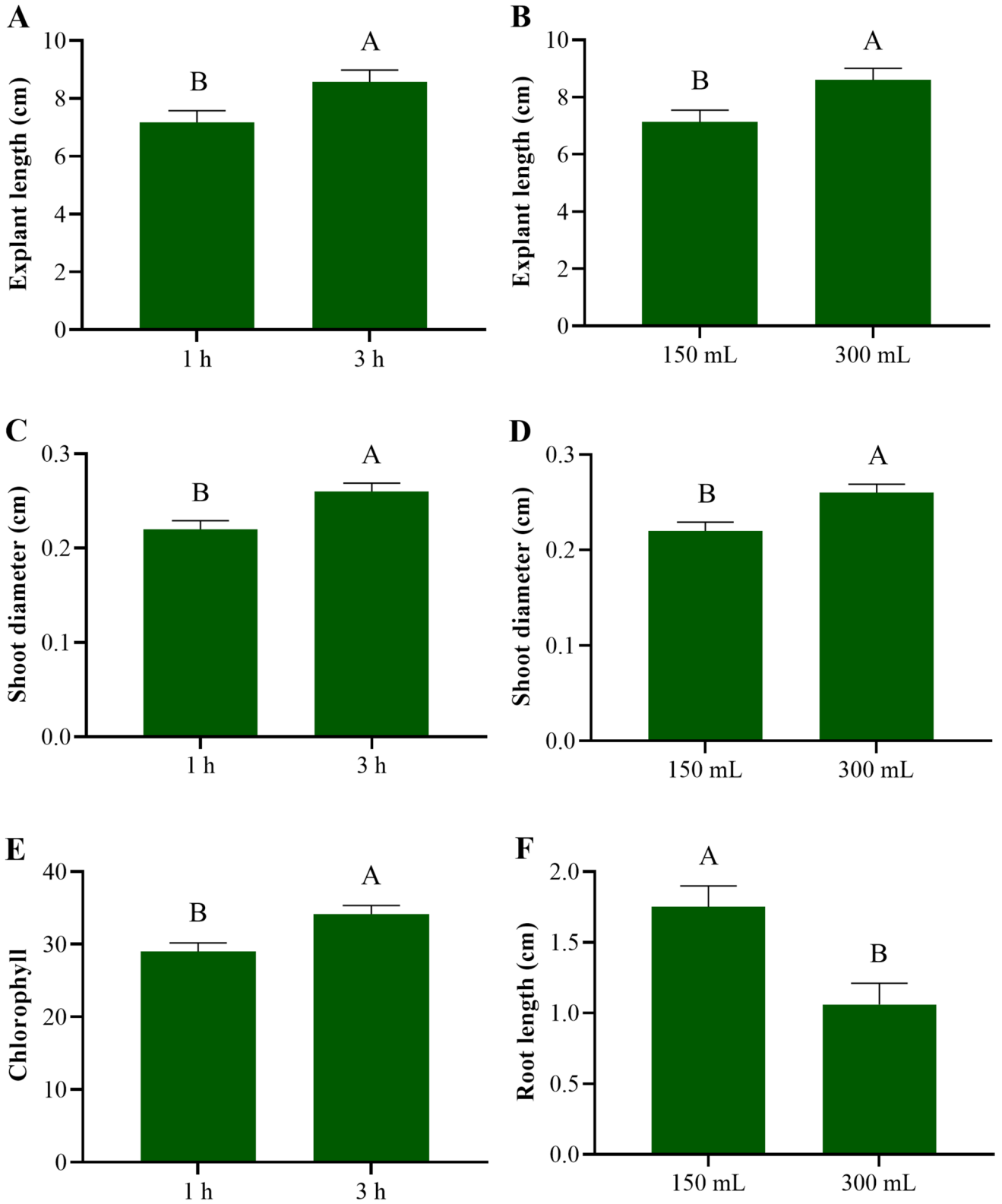


Figure 5. Explants of *Vanilla planifolia* Jacks. ex Andrews cultured under different immersion frequencies (1 h and 3 h) and medium volumes (150 mL and 300 mL). (*A–B*) Explant length, (*C–D*) shoot diameter, (*E*) chlorophyll content, and (*F*) root length. Treatments followed by different letters differ significantly according to Tukey's test at 5% probability. *Error bars* represent the standard error.

Table 2. Mean ± standard error (SE) of morphophysiological variables of *Vanilla planifolia* Jacks. ex Andrews cultivated in the BioTilt™ system under different medium volumes (150 and 300 mL) and immersion frequencies (1 h and 3 h)

Variables	Treatments				
	Frequency 1 h		Frequency 3 h		Significance[a] (A/B/A×B)
	150 mL	300 mL	150 mL	300 mL	
Number of shoots	3.50 ± 0.74	4.65 ± 0.95	5.15 ± 0.74	4.85 ± 0.29	ns/ns/ns
Shoot length (cm)	1.58 ± 0.20	1.87 ± 0.35	1.69 ± 0.12	1.62 ± 0.04	ns/ns/ns
Number of roots	3.95 ± 0.33	2.80 ± 0.15	3.00 ± 0.61	3.60 ± 0.43	ns/ns/ns
Number of leaves	3.00 ± 0.41	4.00 ± 0.27	3.95 ± 0.31	3.85 ± 0.17	ns/ns/ns
Fresh weight (g)	4.63 ± 0.88	4.70 ± 0.63	4.28 ± 0.55	5.34 ± 0.36	ns/ns/ns
Dry weight (g)	0.42 ± 0.07	0.45 ± 0.06	0.39 ± 0.04	0.52 ± 0.03	ns/ns/ns

[a]Refers to the main effects of immersion frequency (A), medium volume (B), and the interaction between them (A × B). (*): significant at 5% ($p < 0.05$); (**): significant at 1% ($p < 0.01$); (ns): not significant

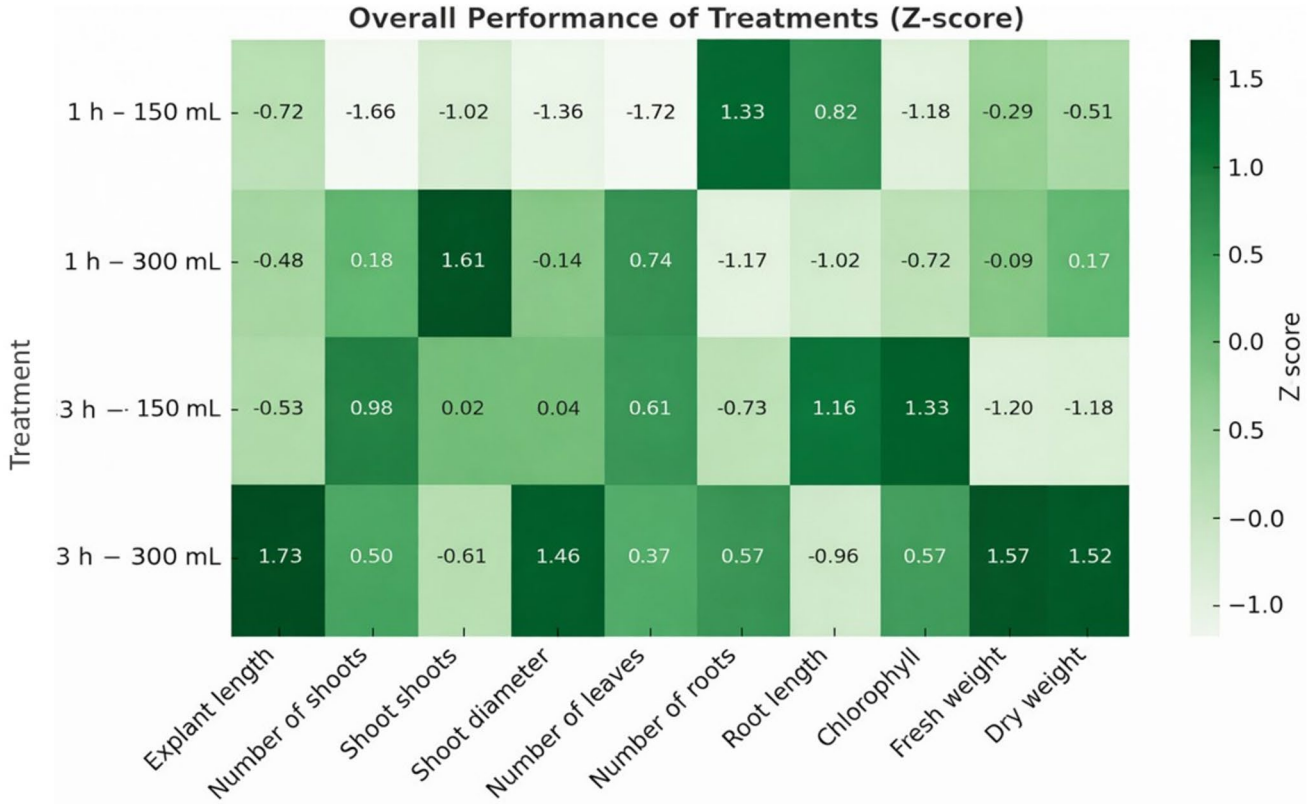


Figure 6. Overall performance of temporary immersion bioreactor treatments based on standardized Z-scores of variables measured in *Vanilla planifolia* Jacks. ex Andrews. Positive values *(dark green)* indicate performance above the global mean, while negative values *(light green)* represent below-average performance.

shoots, leaves, and chlorophyll content shows that these characteristics together reinforce multiplication efficiency, as they indicate metabolically active explants capable of photosynthetic activity (Fig. 4). Studies with orchids, such as *Spathoglottis plicata,* highlight that temporary immersion systems can enhance this performance by optimizing the efficiency of photosynthetic pigments. This set of characteristics can directly contribute to the survival and quality of the plantlets during the acclimatization process (Shekhawat *et al*. 2021).

Additionally, there was also a positive correlation of these variables with the number of roots and biomass, which demonstrated vigorous initial growth. Such a pattern reinforced the physiological efficiency of the temporary immersion system, which has proven effective in promoting greater vigor in ornamental plants. For example, Zhang *et al*. (2022) demonstrated that the use of temporary immersion resulted in greater plant biomass accumulation in *Dendrobium nobile*, while Martínez-Estrada *et al*. (2019)

reported significant improvements in the micropropagation of *Anthurium andreanum*, in terms of rooting and physiological aspects, using the same principle.

The calculated composite indices reinforced the superior performance of the temporary immersion system compared to the semi-solid system (Fig. 5). The multiplication index was the main discriminating factor between the systems, which consolidated the greater multiplicative potential of the Biotilt™ system for the propagation of *Vanilla planifolia*. Additionally, the global and physiological indices complemented these results, demonstrating that this system also promoted better physiological quality of the explants.

Several studies have already demonstrated the performance of the temporary immersion system for Vanilla propagation (Ramírez-Mosqueda *et al*. 2019; Ramírez-Mosqueda and Bello-Bello 2021; Spinoso-Castillo and Bello-Bello 2023). However, it is worth noting that this is the first report on the use of the Biotilt™ bioreactor model for Vanilla. Considering practical implications, conventional micropropagation tends to be more laborious and costly compared to this system, as it requires the maintenance of many culture vessels and the use of gelling agents in semi-solid media. These agents increase the costs of *in vitro* production and still limit the potential for large scale automation.

The coherence between the composite indices and the data obtained from individual variables reinforced the reliability of the results, providing an integrated view of the performance of the tested systems. These findings not only highlighted the superiority of the temporary immersion based protocol but also strengthened its applicability as a more efficient, practical, and economically viable alternative.

In addition, the Biotilt™ system also shows potential for broader applicability, as it can be used for other orchid species and even for different plant groups that benefit from temporary immersion, such as *Phalaenopsis*, *Dendrobium*, or *Anthurium*. Due to its simple design, with fewer structural components and low maintenance requirements, it offers practical advantages compared to more complex temporary immersion systems, which generally require higher operational costs and technical adjustments. Therefore, it represents an alternative particularly suitable for laboratories or commercial facilities seeking scalable, user friendly bioreactors that optimize production and research processes.

Medium Volume and Immersion Frequency in the BioTilt™ System The medium volume and immersion time were essential parameters in this type of protocol, as they directly influenced the exposure of the explants to nutrients, growth regulators, and adequate oxygenation. Inadequate immersion frequencies may result in hyperhydricity and asphyxia, while excessive or insufficient medium volumes compromise explant formation, reducing their growth and vigor (Murthy *et al*. 2023; Nongdam *et al*. 2023). However, in this study, hyperhydricity and asphyxia were not observed as problems, since these events did not occur, even though they are commonly reported in the literature.

In general, when analyzing the results through the heatmap, a trend of better performance was observed with the three hours frequency. In other reports with *Vanilla*, better performances were obtained with four hours frequencies compared to longer frequencies, but under different experimental conditions and using other types of media and systems, such as SETIS™ and RITA® (Ramos-Castellá *et al*. 2014; Ramírez-Mosqueda and Iglesias-Andreu 2016; Ramírez-Mosqueda *et al*. 2022). Thus, the three hours frequency tested in this study represents a promising alternative, especially since it concerns a relatively new bioreactor model, whose results revealed superior trends, particularly when compared to those observed in the conventional semi-solid system.

Furthermore, the heatmap analysis with standardized values (z-score) showed that, within the three hours frequency, the volumes of 150 and 300 mL presented similar performance (Fig. 6). However, a greater intensity of values was observed for 300 mL (75 mL per explant), indicating a trend toward a better response with this medium volume. It is important to emphasize that the ideal volume may vary according to the bioreactor model and the species cultivated. For example, for *Vanilla* in the SETIS™, Ramírez-Mosqueda *et al*. (2021) reported better performance with 50 mL per explant; for *Phalaenopsis* protocorms, success was achieved with 10.5 mL per explant in the RITA® (Mohammadpour Barough *et al*. 2024), while for *Agave*, also using the RITA®, the ideal volume was 10 mL per explant (Monja-Mio *et al*. 2021).

It is important to note that, despite the observed tendencies, several variables did not reach statistical significance in the factorial analysis. The heatmap interpretation therefore serves mainly to visualize the overall behavior of treatments rather than to confirm statistically supported differences. This limitation highlights the need for further experiments with larger sample sizes or additional replicates to reinforce these preliminary findings. Considering that the treatments showed similar performance for most variables, the choice of the most suitable protocol may consider factors such as cost, medium availability, and operational practicality.

Conclusion

The Biotilt™ temporary immersion system, with a frequency of 3 h and 300 mL of medium, proved to be a practical, efficient, and economically viable alternative when compared to conventional micropropagation for the multiplication of *Vanilla planifolia*. The results reinforced the

system's potential to produce new plants, offering greater operational flexibility and the possibility of automation, in addition to reducing costs associated with gelling agents and individual culture vessels. This study provided new evidence for the use of temporary immersion systems, strengthening their practical applicability and opening perspectives for future advances in the production of high-quality planting material.

Acknowledgements The authors would like to thank the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Research Support Foundation of the State of Minas Gerais (FAPEMIG) for financial support. The authors also acknowledge Plant Cell Technology for the development of the Biotilt™ bioreactor system, which was employed in this study. This work is supported by the USDA National Institute of Food and Agriculture, Hatch project 1012202.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614).

Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Baltazar-Bernal O, Mora-González EG, Ramírez-Mosqueda MA (2024) Orchid micropropagation using temporary immersion systems: A review. In: Ramírez-Mosqueda MA, Cruz-Cruz CA (eds) Micropropagation methods in temporary immersion systems, Methods in molecular biology, vol 2759. Humana, New York, pp 227–244

Cabrera IC, Hernández KV, Burgos LS, Torre MH (2024) Use of temporary immersion systems for the micropropagation of *Puya alpestris* (Poepp.) Gay as a source to produce proteolytic enzymes. In Vitro Cell Dev Biol - Plant 60:880–890

Kadir NA, Naher L, Sidek N (2019) Economical important phytopathogenic diseases in *Vanilla planifolia*: a review paper. J Trop Resour Sustain Sci 7:77–82

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 8:72

Leyva-Ovalle OR, Bello-Bello JJ, Murguía-González J, Núñez-Pastrana R, Ramírez-Mosqueda MA (2020) Micropropagation of *Guarianthe skinneri* (Bateman) Dressler et WE Higging in temporary immersion systems. Biotech 10:26

Manokari M, Priyadharshini S, Phanikanth J, Abhijit D, Mahipal S (2021) Shekhawat meta-topolin and liquid medium mediated enhanced micropropagation via *ex vitro* rooting in *Vanilla planifolia* Jacks. ex Andrews. Plant Cell Tissue Organ Cult 146:69–82

Martínez-Estrada E, Islas-Luna B, Pérez-Sato JA, Bello-Bello JJ (2019) Temporary immersion improves *in vitro* multiplication and acclimatization of *Anthurium andreanum* Lind. Sci Hortic 249:185–191

Mohammadpour AB, Daylami SD, Fadavi A, Vahdati AK (2024) Enhancing *Phalaenopsis* orchid production: a comparative study of permanent and temporary immersion bioreactors. In Vitro Cell Dev Biol - Plant 60:523–537

Monja-Mio KM, Olvera-Casanova D, Herrera-Alamillo MA, Sánchez-Teyer FL, Robert ML (2021) Comparison of conventional and temporary immersion systems on micropropagation (multiplication phase) of *Agave angustifolia* Haw. 'Bacanora.' Biotech 11:1–8

Nadal MC, Teixeira GN, Coronado JFO, Ochiutto AA, Cunha Neto AR, Reis MV (2025) Potential of Brazil's native Vanilla species a study on distribution and research gaps. Ornam Hortic 31:1–9

Plant Cell Technology (2025) Available in: < Available in: https://plantcelltechnology.com/pages/biotilt > Cited 15 July 2025

Ramírez-Mosqueda MA, Bello-Bello JJ (2021) SETIS™ bioreactor increases *in vitro* multiplication and shoot length in *Vanilla* (*Vanilla planifolia* Jacks. Ex Andrews). Acta Physiol Plant 43:52

Ramírez-Mosqueda MA, Bello-Bello JJ, Armas-Silva AA, Rodríguez-Deméneghi MV, Martínez-Santos E (2022) Advances in somatic embryogenesis in Vanilla (*Vanilla planifolia* Jacks.). In: Ramírez-Mosqueda MA (ed) Somatic embryogenesis, methods in molecular biology, vol 2527. Humana, New York, pp 29–40

Ramírez-Mosqueda MA, Iglesias-Andreu LG (2016) Evaluation of different temporary immersion systems (BIT®, BIG, and RITA®) in the micropropagation of *Vanilla planifolia* Jacks. In Vitro Cell Dev Biol - Plant 52:154–160

Ramírez-Mosqueda MA, Iglesias-Andreu LG, Favián-Veja E, Silva JAT, Leyva-Ovalle OR, Murguía-González J (2019) Morphogenetic stability of variegated *Vanilla planifolia* Jacks. plants micropropagated in a temporary immersion system (TIB®). Rend Lincei Sci Fis Nat 30:603–609

Ramos-Castellá A, Iglesias-Andreu LG, Bello-Bello JJ, Lee-Espinosa H (2014) Improved propagation of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. In Vitro Cell Dev Biol - Plant 50:576–581

Reflora (2020). Instituto de Pesquisas Jardim Botânico do Rio de Janeiro. Programa Reflora. Available in: < Available in: http://reflora.jbrj.gov.br > Cited 15 July 2025

Rodríguez-Deméneghi MV, Ramírez-Mosqueda MA, Armas-Silva AA, Aguilar-Rivera N, Gheno-Heredia YA (2022) Biofábricas de Vanilla (*Vanilla planifolia* Jacks.) en México como oportunidad de desarrollo agrário. Cuad Biodivers 63:49–54

Rodríguez-Deméneghi MV, Rivera NA, Gheno-Heredia YA, Armas-Silva AA (2023) Vanilla cultivation in Mexico: typology, characteristics, production, agroindustrial prospective and biotechnological innovations as a sustainability strategy. Sci Agropecu 14:93–109

Schneider C, Rasband W, Eliceiri K (2012) NIH image to ImageJ: 25 years of image analysis. Nat Method 9:671–675

Simas DLR, Silva TR, Vicintim AB, Silva AJR, Camargo LEV (2024) Espécies de baunilha (Vanilla) do Brasil com potencial valor econômico. Rev Virt Quim 16:436–444

Spinoso-Castillo JL, Bello-Bello JJ (2023) CO2-enriched air in a temporary immersion system induces photomixotrophism

during *in vitro* multiplication in Vanilla. Plant Cell Tissue Organ Cult 155:29–39

Tan BC, Chin CF (2015) *Vanilla planifolia*: an economically important orchid and its propagation. Minerva Biotecnol 27:107–116

Vendrame WA, Xu JJ, Beleski DG (2023) Micropropagation of *Brassavola nodosa* (L.) Lindl. using SETIS™ bioreactor. Plant Cell Tiss Org Cult 153:67–76

Zhang B, Niu Z, Li C, Hou Z, Xue Q, Liu W, Ding X (2022) Improving large-scale biomass and total alkaloid production of *Dendrobium nobile* Lindl. using a temporary immersion bioreactor system and MeJA elicitation. Plant Methods 18:10

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.