



# Temporary Immersion System in Plant Tissue Culture: Applications, Technical Factors, and Bottlenecks for Efficient Micropropagation

Murgayanti<sup>1\*</sup>, Alifiyan Anur<sup>2</sup>, Salwa Septiani Rahayu<sup>3</sup>

<sup>1</sup>Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang  
45363, West Java, Indonesia

<sup>2,3</sup>Agrotechnology Study Program, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor,  
Sumedang 45363, West Java, Indonesia

\*Correspondence E-mail: murgayanti@unpad.ac.id

**Article History: Received: July 12, 2026; Accepted: September 04, 2026**

## ABSTRACT

Temporary Immersion System (TIS) has emerged as an efficient platform for micropropagation and conservation of medicinal and horticultural plants. Periodic immersion can facilitate nutrient uptake and gas exchange, with reported effects on shoot multiplication, rooting, and plantlet quality varying according to plant species and culture protocols. Key technical factors, including immersion frequency, immersion duration, bioreactor type, medium volume, explant density, and plant growth regulators, can substantially influence micropropagation efficiency. TIS offers potential for scalable propagation and labor reduction, although hyperhydricity may remain a challenge depending on system configuration and culture conditions. Applications range from high value horticultural crops to medicinal plants, supporting uniform propagation, germplasm conservation, and nursery scale production. Current bottlenecks include standardization of operational parameters, hyperhydricity management, acclimatization success, and integration of technological innovations such as automation, sensors, and data driven control. Future research should focus on optimizing these parameters for diverse species, developing smart TIS systems, and evaluating long term genetic stability and physiological performance to improve its applicability in commercial propagation and conservation. Overall, TIS represents a promising tool for advancing plant tissue culture efficiency and sustainable propagation strategies.

**Keywords:** Acclimatization; Bioreactor; Micropropagation; Plant Tissue Culture; Temporary Immersion System

## 1. INTRODUCTION

The global horticultural industry continues to face increasing demand for high-quality, uniform, and disease-free planting materials. This demand is driven by the need to improve productivity, maintain crop quality, and meet domestic and export standards, particularly for horticultural and medicinal crops. However, conventional propagation methods are often constrained by seasonal dependence, relatively low multiplication rates, variability in plant quality, and the potential transmission of pathogens across generations. Repeated vegetative propagation can facilitate pathogen accumulation and transmission, potentially compromising the health and



quality of planting materials over time. Therefore, advanced propagation technologies are needed to support the rapid, efficient, and large-scale production of healthy planting materials (Shah et al., 2020).

Plant tissue culture has become an important biotechnological approach for producing disease-free and genetically uniform plantlets under aseptic conditions. Micropropagation allows rapid multiplication of elite genotypes, supports conservation of valuable germplasm, and provides planting materials with better uniformity and quality. This technique is widely applied in horticultural and medicinal plants because it can overcome several limitations of conventional propagation and support mass production in limited space and time (Kunakhonnuruk et al., 2019; Swaraz et al., 2020; Zafarullah et al., 2021). However, conventional micropropagation systems still require large numbers of culture vessels, semi-solid media, and intensive manual handling. These requirements increase labor demand and production costs, making large-scale commercial application less efficient without further technological improvement (Rico et al., 2022).

Semi-solid culture systems using agar or other gelling agents also contribute to production costs during commercial micropropagation. In addition, conventional culture vessels can limit automation and reduce the flexibility of medium replacement and culture handling (Zhang et al., 2018; Kim et al., 2020). Liquid culture systems have been introduced to improve nutrient availability while eliminating the need for agar or other gelling agents. However, continuous exposure of plant tissues to liquid medium may induce physiological disorders such as hyperhydricity, depending on the plant species and culture conditions. Hyperhydric plantlets generally exhibit abnormal morphology, weak tissue structure, and reduced acclimatization capacity. These limitations highlight the need for culture systems that combine the advantages of liquid media with controlled exposure to the medium, adequate aeration, and reduced physiological stress.

The Temporary Immersion System (TIS) was developed as a bioreactor-based culture system to address some of the limitations associated with semi-solid and continuous liquid culture. In TIS, explants are periodically immersed in liquid medium and subsequently exposed to air during the drainage phase. This alternating immersion and drainage process provides intermittent access to liquid nutrients while allowing periods of aeration. Depending on the plant species, vessel configuration, and operating conditions, TIS can improve culture aeration and gas exchange and may help reduce the risk of hyperhydricity compared with continuous liquid culture (Kim et al., 2020; De Carlo et al., 2021). TIS may also facilitate contact between explants and the culture medium; however, the uniformity of medium distribution depends on factors such as vessel



geometry, explant loading, and immersion conditions. In some systems, improved control of the culture environment may also influence the accumulation of phenolic compounds and other metabolites. These characteristics have contributed to the potential of TIS as an alternative platform for large-scale plant micropropagation.

The application of TIS offers important operational and economic benefits for plant tissue culture. The system can reduce manual labor because explants do not need to be carefully positioned on gelled media, and liquid medium can be replenished more easily. TIS also supports automation, scale-up, longer subculture intervals, and improved production efficiency, making it attractive for commercial micropropagation (Ramírez-Mosqueda et al., 2016; De Carlo et al., 2021). Nevertheless, several challenges remain, including the optimization of immersion frequency and duration, medium volume, explant density, bioreactor type, plant growth regulators, and acclimatization protocols. Species-specific responses and limited standardization across different TIS platforms also make it difficult to establish universal protocols.

Therefore, this review aims to synthesize recent studies on Temporary Immersion System for plant micropropagation and conservation, with emphasis on bioreactor types, operational mechanisms, technical factors, growth performance, regeneration, plantlet quality, applications, bottlenecks, and future technological innovations. This review is expected to provide a comprehensive understanding of TIS as a strategy to improve the efficiency of mass propagation and production of high-quality planting materials in horticultural and medicinal plants.

## 2. RESEARCH METHOD

The literature search for this review was conducted using Google Scholar and four academic databases: Scopus, ScienceDirect, SpringerLink, and Web of Science. The search was conducted on 12 Juni 2026 using combinations of the following keywords: “temporary immersion system,” “temporary immersion bioreactor,” “TIS,” “TIB,” “plant tissue culture,” “micropropagation,” “medicinal plants,” “horticultural crops,” “hyperhydricity,” and “acclimatization.” The search strategy was designed to identify literature addressing the application and development of temporary immersion systems in plant tissue culture, particularly for micropropagation, conservation, biomass production, plantlet regeneration, and improvement of in vitro culture efficiency.

Publications from 2016 to 2026 were prioritized to capture recent developments and current applications of temporary immersion systems. However, older foundational studies were retained when they provided important information on the development, principles, mechanisms,



or early applications of TIS. Thus, the 2016–2026 period was used as the primary range for recent evidence rather than as an absolute exclusion criterion for earlier literature.

### **Eligibility and Study Selection**

The inclusion criteria comprised original research articles, review papers, book chapters, and conference proceedings that addressed temporary immersion systems in plant tissue culture, particularly those related to micropropagation, conservation, biomass production, plantlet regeneration, and in vitro culture efficiency. Studies were prioritized when they reported technical parameters or outcomes relevant to TIS performance, including immersion frequency, immersion duration, aeration, medium volume, explant density, plant growth regulators, bioreactor type, plantlet quality, hyperhydricity, and acclimatization.

Studies focusing on medicinal and horticultural plants were prioritized. Studies involving other plant species were included when they provided comparative or mechanistic information considered relevant to TIS-based micropropagation. Publications were excluded when they were unrelated to plant tissue culture or temporary immersion systems, did not provide information relevant to the objectives of this review, or were duplicate records.

The retrieved literature was screened based on title and abstract, followed by assessment of the full text for studies considered potentially relevant. The final selection was based on the relevance of each publication to the objectives and scope of the review.

### **Data Extraction and Synthesis**

Relevant information from the selected literature was extracted and organized according to plant species, culture objective, TIS or bioreactor configuration, immersion frequency and duration, aeration conditions, culture medium, plant growth regulators, explant type, and reported outcomes. Particular attention was given to shoot multiplication, rooting, biomass production, hyperhydricity, acclimatization, and plantlet quality. The findings were synthesized descriptively by comparing reported TIS configurations, operational parameters, plant responses, limitations, and technological developments across studies. Because TIS responses are influenced by plant species and culture conditions, reported effects and optimal parameters were interpreted as study-specific rather than as universally applicable settings.

## **3. RESULTS AND DISCUSSION**

### **Conceptual Framework of Temporary Immersion System in Plant Tissue Culture**

Temporary Immersion Systems (TIS) represent a bioreactor-based approach in plant tissue culture that combines the use of liquid culture medium with intermittent exposure of explants to



air. In TIS, explants are periodically immersed in liquid nutrient medium and subsequently exposed to air during the drainage phase. This alternating immersion and drainage process can facilitate nutrient delivery while providing periods of aeration. Depending on plant species, vessel configuration, and operating conditions, TIS has been reported to influence nutrient uptake, gas exchange, plantlet development, and the occurrence of physiological disorders such as hyperhydricity (Ahmadian et al., 2017; Mirzabe et al., 2022). The intermittent exposure of explants to the culture medium may also influence the distribution of nutrients within the vessel; however, the uniformity of medium contact depends on factors such as vessel geometry, explant loading, and immersion conditions.

The conceptual basis of TIS lies in balancing nutrient delivery with adequate aeration and controlled exposure of plant tissues to liquid medium. Continuous or excessive exposure to liquid medium can restrict gas exchange and increase the risk of physiological abnormalities under certain culture conditions, whereas programmed immersion and drainage cycles provide alternating periods of liquid contact and air exposure. These conditions may support shoot proliferation, rooting, biomass accumulation, and plantlet development, although responses vary according to plant species, explant type, culture medium, plant growth regulators, and TIS operating parameters (Baltazar et al., 2026; Nongdam et al., 2023). Therefore, TIS should be considered a flexible culture platform whose effectiveness depends on appropriate optimization of system configuration and operating conditions rather than as a universally superior alternative to conventional semi-solid culture.

Historically, the adoption of TIS emerged from the need to improve productivity and scalability of in vitro propagation. Early bioreactor designs progressed from simple liquid immersion to more sophisticated systems that control immersion frequency, duration, and aeration patterns. The development of variants such as twin flask, automated air pressure systems, and integrated nutrient reservoirs illustrates the evolution of TIS technology toward higher efficiency and reduced labor input, particularly for commercial applications (Baltazar-Bernal et al., 2024; Etienne et al., 2002; Mirzabe et al., 2022).

The general workflow of TIS-based micropropagation is captured in Figure 1, beginning with explant selection and placement into the bioreactor, followed by programmed cycles of immersion and aeration that encourage nutrient uptake and controlled growth. After sufficient shoot multiplication and rooting, plantlets are typically acclimatized to ex vitro conditions, a phase where TIS derived plantlets often display improved survival due to better physiological conditioning during in vitro culture (Baltazar et al., 2026; Nongdam et al., 2023).

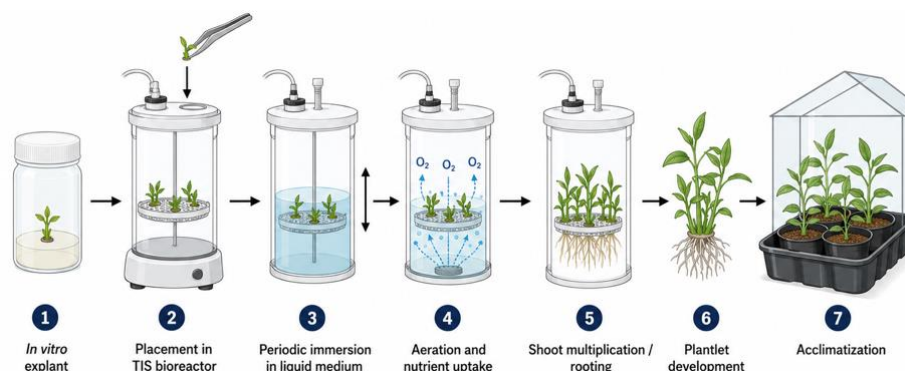


Figure 1. General workflow of a Temporary Immersion System (TIS) in plant tissue culture.

Adapted from Gomes et al., (2025); Hwang et al., (2022)

### Types of Temporary Immersion Bioreactors and Operational Mechanisms

Temporary immersion systems have been developed in different bioreactor designs to improve nutrient delivery, aeration, and handling efficiency during *in vitro* plant propagation. Although all TIS platforms work through intermittent contact between explants and liquid medium, each system differs in vessel structure, liquid movement, aeration control, culture capacity, and operational complexity. Several commonly used TIS bioreactors include RITA®, SETIS™, Plantform™, twin-flask or BIT systems, and modified low-cost TIBs, each with specific operational strengths and limitations.

RITA® is one of the most widely reported TIS bioreactors because it allows liquid medium to rise periodically and immerse the explants before draining back to the reservoir. This design supports controlled immersion and reduces continuous exposure of tissues to liquid medium, which may contribute to maintaining plantlet quality under appropriate culture conditions (Hwang et al., 2022; Ramírez-Mosqueda et al., 2016). However, the occurrence of hyperhydricity is influenced by immersion frequency, immersion duration, plant species, and other culture conditions. Inappropriate immersion regimes may increase the risk of hyperhydricity in susceptible species, particularly when exposure to liquid medium is excessive (Hwang et al., 2022; Ramírez-Mosqueda et al., 2016). SETIS™ works through an air-pressure mechanism that regulates immersion and aeration in connected culture vessels. Its larger culture capacity and improved aeration may facilitate scale-up, although the system requires appropriate equipment control and strict sterilization procedures (Bello-Bello et al., 2021; Ramírez-Mosqueda et al., 2016).

Plantform™ bioreactors use support grids and automated liquid movement to expose explants uniformly to the medium. This system is useful for commercial micropropagation because it improves nutrient contact and reduces labor compared with conventional semi-solid culture



(Hwang et al., 2022; Nongdam et al., 2023). However, its efficiency still depends on immersion frequency, medium volume, and explant density. Twin-flask or BIT systems use two connected vessels to separate the explants from the medium, with liquid transfer controlled by air pressure during immersion cycles. This system is simpler and more flexible for laboratory-scale optimization, but it is generally less standardized than commercial bioreactors (De Carlo et al., 2021; Uma et al., 2021).

Modified or low-cost TIB systems have also been developed to reduce production costs and adapt TIS technology to species-specific protocols. These systems commonly use bottles, tubes, pumps, timers, or air pressure to control immersion cycles. They are useful for laboratories with limited facilities and can support large-scale propagation when the immersion cycle, medium volume, and explant density are properly adjusted (Bello-Bello et al., 2021; Thanonkeo et al., 2024; Uma et al., 2021). However, reproducibility and contamination control remain major limitations because each laboratory may use different vessel designs and sterilization procedures.

#### **Technical Factors Affecting Micropropagation Efficiency in TIS**

The efficiency of micropropagation in temporary immersion systems is strongly influenced by operational and culture-related factors. Unlike conventional semi-solid culture, TIS requires precise control of immersion frequency, immersion duration, medium volume, aeration, explant density, plant growth regulators, and vessel design. The main technical factors affecting plant growth and regeneration in temporary immersion systems are summarized in Table 1.

Table 1. Technical factors affecting plant growth and regeneration in temporary immersion systems

| <b>Technical Factor</b>        | <b>Common Setting</b>                                                                                                           | <b>Effect on Culture Performance</b>                                                                                                             | <b>Reference</b>                             |
|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|
| Immersion frequency & duration | 1–2 min per immersion; reported frequencies vary among studies, including 2–3 immersions per day or approximately every 6–8 h   | Supports shoot proliferation, rooting, and ex vitro acclimatization; effects on hyperhydricity depend on immersion regime and culture conditions | Thanonkeo et al., (2024); Uma et al., (2021) |
| Medium volume & composition    | 250 mL liquid medium; 0.5% sucrose, 2 µM meta-topolin                                                                           | Adequate nutrients improve shoot growth, leaf number, and rooting response                                                                       | Rico et al., (2022); Uma et al., (2023)      |
| Plant growth regulators (PGRs) | BAP 7.5 mg/L for shoot multiplication and IBA 0.5 mg/L for rooting, as reported for the specific plant species/cultivar studied | May enhance shoot multiplication, shoot development, and rooting efficiency depending on plant species, cultivar, PGR type, and concentration    | Thanonkeo et al., (2024)                     |



|                                       |                                                                                                                                                                                |                                                                                                                                                                                                                         |                                                       |
|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|
| Explant density loading               | 5–8 explants per vessel, as reported in specific studies; appropriate loading varies according to bioreactor type, vessel capacity, plant species, and explant characteristics | Appropriate explant density may support shoot multiplication and biomass production, whereas excessive loading may increase competition for nutrients and space and may contribute to reduced growth or tissue necrosis | Bello-Bello et al., (2021); Rico et al., (2022)       |
| Bioreactor design & immersion control | RITA®, Plantform™, TIB, or low-cost modified system; immersion adjusted by timer/air pressure                                                                                  | Ensures uniform nutrient contact, reduces labor, controls hyperhydricity, and supports plantlet quality                                                                                                                 | Hwang et al., (2022); Ramírez-Mosqueda et al., (2016) |

Immersion frequency and medium volume are among the important parameters in TIS-based micropropagation. Uma et al. (2021) reported that banana explants cultured in a temporary immersion bioreactor with 250 mL of liquid medium and 2 min immersion every 6 h showed efficient shoot proliferation and rooting. These findings demonstrate that the reported immersion regime supported shoot multiplication and rooting under the conditions tested. Although periodic exposure to liquid medium may facilitate access to nutrients and water, direct enhancement of nutrient uptake should not be inferred unless nutrient uptake was specifically measured. Therefore, immersion conditions should be carefully regulated because excessive exposure to liquid medium may increase the risk of hyperhydricity and negatively affect plantlet quality, depending on the plant species and culture protocol.

Plant growth regulators and explant density are important factors affecting plantlet multiplication in TIS. In banana cv. Kluai Numwa Pakchong 50, Thanonkeo et al. (2024) reported that 7.5 mg/L BAP supported shoot multiplication, whereas 0.5 mg/L IBA was used to promote rooting under the conditions tested. The study also evaluated explant density and reported differences in multiplication response among the tested loading levels. These findings suggest that PGR concentration and explant loading should be optimized together according to the plant species, cultivar, and culture conditions to support effective plantlet development.

Bioreactor type and explant loading further influence culture performance. Rico et al. (2022) demonstrated that RITA® and Plantform™ bioreactors could support axillary shoot proliferation of *Cannabis sativa*, with differences in explant capacity and immersion settings between the systems. Their study also reported that 1 min immersion three times per day, combined with 0.5% sucrose and 2 µM meta-topolin, supported shoot proliferation under the conditions tested. These findings suggest that TIS performance depends not only on the immersion cycle but also on vessel capacity, carbon source, cytokinin type, and other culture conditions.



Culture system design is another important factor, especially for laboratories aiming to reduce production costs. Modified or low-cost TIS/TIB systems can improve scalability and reduce labor compared with conventional semi-solid culture, but they require careful control of contamination and reproducibility (Bello-Bello et al., 2021; Pozoga et al., 2024). In addition, immersion control remains essential for limiting excessive liquid exposure and improving plantlet quality, particularly in species that are sensitive to hyperhydricity (Hwang et al., 2022; Ramírez-Mosqueda et al., 2016).

### **Growth Performance, Regeneration, and Plantlet Quality in TIS**

Growth performance is one of the main indicators used to evaluate the effectiveness of Temporary Immersion Systems in plant micropropagation. In TIS, periodic contact with liquid medium can improve nutrient uptake, water availability, and culture uniformity, which may increase shoot multiplication, shoot elongation, rooting, and biomass accumulation. However, improved growth is not only determined by the availability of liquid medium, but also by the ability of the system to maintain normal morphology and avoid excessive tissue hydration. Therefore, plantlet quality should be evaluated together with multiplication rate and regeneration response.

Several recent studies indicate that TIS can improve shoot proliferation and plantlet development in horticultural and ornamental species. In *Sorbus domestica*, the use of TIS was reported as an innovative approach for in vitro propagation, supporting its potential for the propagation of underutilized fruit tree species (Gianguzzi & Sottile, 2024). In *Crocus sativus*, TIS produced higher growth parameters than semi-solid medium, including microcorm, shoot, and root number, suggesting that temporary immersion can enhance regeneration and biomass formation in high-value horticultural crops (Tarraf et al., 2024). Similar benefits were reported in *Colocasia esculenta*, where different semi-automated bioreactors were compared for in vitro propagation, highlighting the importance of bioreactor choice in determining multiplication and plantlet performance (Mancilla-álvarez et al., 2021).

Plantlet quality is another important outcome in TIS-based micropropagation. High shoot number alone is not sufficient if regenerated plantlets show weak rooting, abnormal morphology, or low survival after transfer to ex vitro conditions. In *Anthurium andreaeanum*, temporary immersion improved in vitro multiplication and acclimatization, showing that TIS can support both propagation efficiency and post-culture establishment (Martínez-Estrada et al., 2019). For ornamental grasses such as *Pennisetum × advena* 'Rubrum', TIS also supported efficient plant production and was associated with lower production costs compared with agar-based culture,



indicating that improved plantlet production can be linked with practical scalability (Pozoga et al., 2024).

### Applications of TIS in Medicinal and Horticultural Plants

Temporary Immersion System has been applied to different plant groups, including medicinal plants, horticultural crops, and ornamental species. Its main applications include shoot multiplication, rooting, biomass production, plantlet quality improvement, and large-scale propagation. The applications of TIS in medicinal and horticultural plants are summarized in Table 2.

Table 2. Applications of temporary immersion systems in medicinal and horticultural plants

| Plant Group           | Example Species                      | TIS Application                                                | Main Outcome                                                                                                                                                                                                          | Reference                       |
|-----------------------|--------------------------------------|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Medicinal plants      | <i>Cannabis sativa</i>               | Shoot multiplication in RITA® and Plantform™ bioreactors       | Shoot multiplication using RITA® and Plantform™ bioreactors. Shoot proliferation and rooting responses under the tested TIS configurations, with differences observed between bioreactor types and culture conditions | Rico et al., (2022)             |
| Medicinal plants      | <i>Agave angustifolia</i>            | Shoot multiplication using low-cost TIS                        | Increased shoot proliferation and improved physiological quality                                                                                                                                                      | Monja-Mio et al., (2021)        |
| Horticulture crops    | <i>Musa</i> spp. (banana)            | Multiplication in TIB system with controlled immersion and PGR | Improved shoot multiplication, uniform rooting, successful ex vitro acclimatization                                                                                                                                   | Uma et al., (2021)              |
| Horticulture crops    | <i>Hylocereus undatus</i> (pitahaya) | Mass propagation using automated TIS                           | Reduced hyperhydricity, higher multiplication rates, uniform plantlets                                                                                                                                                | Bello-Bello et al., (2021)      |
| Ornamental plants     | <i>Vanilla planifolia</i>            | Multiplication using SETIS™ bioreactor                         | Higher shoot number, improved shoot length, scalable propagation                                                                                                                                                      | Ramírez-Mosqueda et al., (2016) |
| Woody/biomass species | <i>Salix viminalis</i>               | Micropropagation in RITA®                                      | Shoot proliferation and growth responses under temporary immersion culture, as reported under the tested culture conditions                                                                                           | Regueira et al., (2018)         |

As shown in Table 2, TIS has strong potential for medicinal plant micropropagation. In *Cannabis sativa*, the use of RITA® and Plantform™ bioreactors improved shoot multiplication, rooting, and plantlet quality compared with semi-solid culture (Rico et al., 2022). Similar benefits were observed in *Agave angustifolia*, where a low-cost TIS increased shoot proliferation and improved physiological quality (Monja-Mio et al., 2021). These findings indicate that TIS can



support the propagation of medicinal plants that require uniform, vigorous, and high-quality planting material.

TIS is also widely used in horticultural crops that need rapid and large-scale multiplication. In *Musa* spp., controlled immersion combined with plant growth regulators improved shoot multiplication, rooting uniformity, and ex vitro acclimatization (Uma et al., 2023). In pitahaya (*Hylocereus undatus*), automated TIS supported mass propagation by increasing multiplication rates, reducing hyperhydricity, and producing uniform plantlets (Bello-Bello et al., 2021). These results show that TIS can improve propagation efficiency in crops where conventional micropropagation is often labor-intensive and costly.

For ornamental and multipurpose plants, TIS also provides advantages in culture uniformity and production scalability. In *Vanilla planifolia*, the SETIS™ bioreactor increased shoot number and shoot length, showing its potential for scalable propagation of high-value ornamental and horticultural species (Ramírez-Mosqueda et al., 2016). In *Salix viminalis*, RITA® supported uniform shoot multiplication and faster growth, indicating that TIS can also be applied to woody plants with medicinal, horticultural, or biomass-related value (Regueira et al., 2018).

### **Bottlenecks and Future Technological Innovations**

Despite its advantages, Temporary Immersion System still faces several bottlenecks that limit its broader application in commercial micropropagation (Sota et al., 2025). The key factors and bottlenecks influencing TIS-based micropropagation are summarized in Figure 2. The most common technical constraints include hyperhydricity, contamination risk, high initial equipment cost, limited protocol standardization, and species-specific responses. Hyperhydricity remains a major problem because excessive immersion or poor aeration can increase water uptake and produce abnormal plantlets. Therefore, immersion frequency, immersion duration, vessel design, aeration, and medium volume must be optimized carefully for each species (Carvalho et al., 2019; Mirzabe et al., 2022; Bello-Bello et al., 2025).

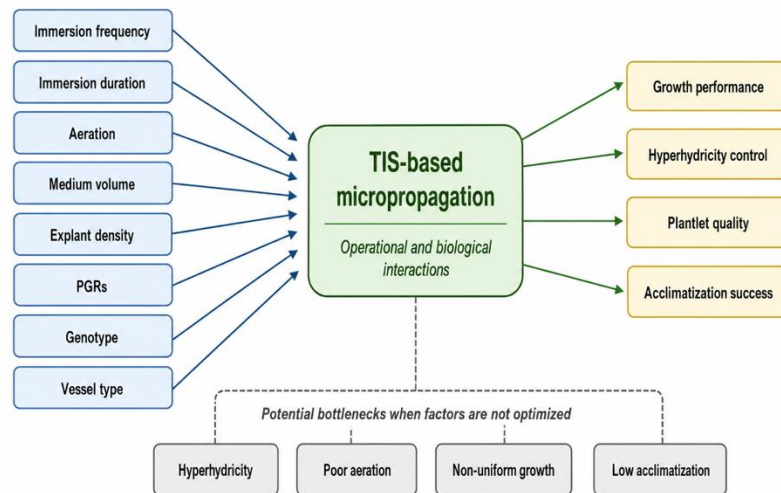


Figure 2. Key Factors and Bottlenecks Influencing TIS-Based Micropropagation

Although the Temporary Immersion System (TIS) offers advantages for large-scale plant propagation, including enhanced shoot multiplication and the potential to reduce labor/input costs, several challenges may limit its application under commercial conditions (De Carlo et al., 2021; Mirzabe et al., 2022). Hyperhydricity represents a major bottleneck and is a physiological disorder associated with excessive water uptake or an imbalance in tissue water relations, resulting in translucent, brittle, and morphologically abnormal tissues. Its occurrence is strongly influenced by immersion frequency and duration, as excessive exposure to liquid medium may disrupt water relations and gas exchange within the culture system (Mirzabe et al., 2022). Ramírez-Mosqueda et al. (2016) demonstrated that a 2-min immersion every 8 h in a RITA® system resulted in the highest shoot multiplication of *Stevia rebaudiana*, highlighting the importance of optimizing the immersion regime. In addition to hyperhydricity, liquid culture systems require appropriate control of immersion and aeration conditions to maintain adequate oxygen availability and nutrient supply for explant growth (De Carlo et al., 2021). Excessive or insufficient immersion may affect water relations, gas exchange, and nutrient availability, thereby compromising shoot development. The importance of optimizing TIS operating conditions has also been demonstrated in *Bletilla striata* and *Cannabis sativa*, where appropriate culture parameters improved propagation performance (Zhang et al., 2018; Rico et al., 2022). Therefore, precise control of immersion frequency, immersion duration, and aeration is essential for maximizing multiplication efficiency while minimizing hyperhydricity and other physiological abnormalities.

Future development of TIS technology is increasingly focused on improving bioreactor design, process control, sterilization, and scalability. Computational Fluid Dynamics (CFD) has



been proposed to optimize bioreactor configuration and fluid distribution, while improved sterilization approaches and hybrid systems combining TIS with other bioreactor configurations, such as mist bioreactors, have been identified as potential directions for future development (Mirzabe et al., 2022). In parallel, sensor-based monitoring and automated control may provide greater control over culture conditions and improve process reproducibility. The use of easily sterilizable or disposable components and advanced LED lighting may also offer opportunities to simplify operation and improve environmental control; however, these approaches require further validation of their effects on propagation efficiency and production costs. Thus, although some engineering approaches have been explored, several of these innovations remain prospective rather than established features of TIS technology, highlighting the need for further research to integrate engineering advances with biological optimization for efficient and scalable plant propagation (De Carlo et al., 2021; Mirzabe et al., 2022). Overall, the future of TIS in plant micropropagation lies in integrating automation, digital sensors, CFD modeling, and next-generation bioreactor designs to reduce hyperhydricity, improve aeration, and facilitate industrial-scale culture management, positioning TIS as a major platform for mass, economical production of high-quality, disease-free planting materials.

#### 4. CONCLUSIONS

Temporary Immersion System (TIS) is a promising platform for the efficient and scalable micropropagation of medicinal and horticultural plants. Its performance is species- and protocol-dependent and is influenced by bioreactor configuration, immersion frequency and duration, culture medium composition, explant density, and plant growth regulator concentration. Appropriate optimization of these factors is essential to achieve effective shoot multiplication, rooting, plantlet development, and acclimatization while managing physiological disorders such as hyperhydricity. Although TIS has demonstrated potential for large-scale propagation and biomass production, its advantages cannot be generalized across species or culture conditions. Further research should prioritize species-specific optimization, improved bioreactor design, process automation and monitoring, and evaluation of long-term genetic stability and physiological performance. These efforts are important for establishing reliable and reproducible TIS protocols for commercial propagation and plant conservation.

**REFERENCES**

- Ahmadian, M., Babaei, A., Shokri, S., & Hessami, S. (2017). Micropropagation of carnation (*Dianthus caryophyllus* L.) in liquid medium by temporary immersion bioreactor in comparison with solid culture. *Journal of Genetic Engineering and Biotechnology*, 15(2), 309–315. <https://doi.org/10.1016/j.jgeb.2017.07.005>.
- Baltazar, E., Correia, M., Lopes, T., Martins, J., Canhoto, J., & Correia, S. (2026). Sweet cherry rootstock micropropagation using SETIS<sup>TM</sup> bioreactor: evaluation, challenges, and biochemical characterization of regenerated shoots. *Plant Cell, Tissue and Organ Culture*, 164(3), Article 68. <https://doi.org/10.1007/s11240-026-03399-x>.
- 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*). *3 Biotech*, 11(10), 437. <https://doi.org/10.1007/s13205-021-02984-5>.
- De Carlo, A., Tarraf, W., Lambardi, M., & Benelli, C. (2021). Temporary immersion system for production of biomass and bioactive compounds from medicinal plants. In *Agronomy*, 11(12), 2414. <https://doi.org/10.3390/agronomy11122414>.
- Etienne, H., Berthouly, M. Temporary immersion systems in plant micropropagation. *Plant Cell, Tissue and Organ Culture* 69, 215–231 (2002). <https://doi.org/10.1023/A:1015668610465>
- Gianguzzi, V., & Sottile, F. (2024). Temporary Immersion System as an innovative approach for in vitro propagation of *Sorbus Domestica* L. *Horticulturae*, 10(2), 164. <https://doi.org/10.3390/horticulturae10020164>.
- Gomes, H. T., Monteiro, T. R., Camillo, J., Nogueira, G. F., da Silva Costa, F. H., de Araújo Silva-Cardoso, I. M., & Scherwinski-Pereira, J. E. (2025). Integrated seed cryopreservation, bioreactor and conventional micropropagation, and genetic stability assessment by flow cytometry of *Uncaria guianensis* (Aubl.) J.F.Gmel, a high-value Amazonian medicinal plant. *Plant Cell, Tissue and Organ Culture*, 161(2), article 45. <https://doi.org/10.1007/s11240-025-03077-4>.
- 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>.
- Kim, N. Y., Hwang, H. D., Kim, J. H., Kwon, B. M., Kim, D., Park, S. Y. (2020). Efficient production of virus-free apple plantlets using the temporary immersion bioreactor system. *Horticulture, Environment, and Biotechnology*, 61, 779–785. <https://doi.org/10.1007/s13580-020-00257-3>.
- Kunakhonnuruk, B., Kongbangkerd, A., & Inthima, P. (2019). Improving large-scale biomass and plumbagin production of *Drosera communis* A.St.-Hil. by temporary immersion system. *Industrial Crops and Products*, 137, 197–202. <https://doi.org/10.1016/j.indcrop.2019.06.054>.





- Mancilla-álvarez, E., Pérez-Sato, J. A., Núñez-Pastrana, R., Spinoso-Castillo, J. L., & Bello-Bello, J. J. (2021). Comparison of different semi-automated bioreactors for in vitro propagation of taro (*Colocasia esculenta* L. Schott). *Plants*, 10(5), 1010. <https://doi.org/10.3390/plants10051010>.
- Martínez-Estrada, E., Islas-Luna, B., Pérez-Sato, J. A., & Bello-Bello, J. J. (2019). Temporary immersion improves in vitro multiplication and acclimatization of *Anthurium andreaeanum* Lind. *Scientia Horticulturae*, 249, 185–191. <https://doi.org/10.1016/j.scienta.2019.01.053>.
- Mirzabe, A. H., Hajiahmad, A., Fadavi, A., & Rafiee, S. (2022). Temporary immersion systems (TISs): A comprehensive review. In *Journal of Biotechnology*, 357, 56–83. <https://doi.org/10.1016/j.jbiotec.2022.08.003>.
- Monja-Mio, K. M., Olvera-Casanova, D., Herrera-Alamillo, M., 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(2), 77. <https://doi.org/10.1007/s13205-020-02604-8>.
- Nongdam, P., Beleski, D. G., Tikendra, L., Dey, A., Varte, V., EL Merzougui, S., Pereira, V. M., Barros, P. R., & Vendrame, W. A. (2023). Orchid micropropagation using conventional semi-solid and temporary immersion systems: a review. In *Plants*, 12(5), 1136. <https://doi.org/10.3390/plants12051136>.
- Požoga, M., Olewnicki, D., & Latocha, P. (2024). A temporary immersion system as a tool for lowering planting material production costs using the example of *Pennisetum × advena* 'Rubrum.' *Agriculture (Switzerland)*, 14(7), 1177. <https://doi.org/10.3390/agriculture14071177>.
- 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.2016.07.015>.
- Regueira, M., Rial, E., Blanco, B., Bogo, B., Aldrey, A., Correa, B., Varas, E., Sánchez, C., & Vidal, N. (2018). Micropropagation of axillary shoots of *Salix viminalis* using a temporary immersion system. *Trees – Structure and Function*, 32(1), 61–71. <https://doi.org/10.1007/s00468-017-1611-x>.
- 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/10.36899/JAPS.2020.1.0012>.
- Shah, S. H., Khan, N., Memon, S. Q., Latif, M., Zia, M. A., Muhammad, A., Nasir, K., & Zafarullah. (2020). Effects of auxins and cytokinins on in vitro multiplication of banana (*Musa* spp.) variety 'W-11' in Pakistan. *Journal of Animal and Plant Sciences*, 30(1), 98–106.
- Sota, V., Wilms, H., Yücesan, B., Mendi, Y. Y., Christie, B., Nisler, J., Aslan, Ş. E., Purmale-  
Trasune, L., Silvestri, C., Werbrouck, S., Fischerová, L., Aronen, T., Cvjetković, B., &



- Lambardi, M. (2025). Challenges in the micropropagation of economically important fruit species in Europe. *Plant Cell, Tissue and Organ Culture* (PCTOC), 162(3), 53. <https://doi.org/10.1007/s11240-025-03165-5>.
- Swaraz, A. M., Sumi, S. K., Sultana, F., Hasan, M., Islam, M. M., Bari, M. W., Islam, M. A., Satter, M. A., Ahmed, K. S., & Hossain, M. H. (2020). Bioactive compound and bioactivity fidelitous micropropagation method of *Blumea lacera* (Burm. f.) DC.: a large scale production potential. *Industrial Crops and Products*, 151, 112370. <https://doi.org/10.1016/j.indcrop.2020.112370>.
- Tarraf, W., İzgü, T., Şimşek, Ö., Cicco, N., & Benelli, C. (2024). Saffron in vitro propagation: an innovative method by temporary immersion system (tis), integrated with machine learning analysis. *Horticulturae*, 10(5) 454. <https://doi.org/10.3390/horticulturae10050454>.
- 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/10.1038/s41598-021-99923-4>.
- 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). <https://doi.org/10.1016/j.sajb.2023.04.006>.
- Zafarullah, Hussain, Z., & Mehmood, K. (2021). Micropropagation of disease-free banana genotype 8818-William for field cultivation. *Advances in Agriculture and Biology*, 4(1), 41–47.
- Zhang, B., Song, L., Bekele, L. D., Shi, J., Jia, Q., Zhang, B., & Chen, J. (2018). Optimizing factors affecting development and propagation of *Bletilla striata* in a temporary immersion bioreactor system. *Scientia Horticulturae*, 232, 121–126. <https://doi.org/10.1016/j.scienta.2018.01.007>.