

## ***In vitro* Propagation of *Philodendron* spp.: Development and Future Perspectives**

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### **Abstract**

High consumer demand for variegated *Philodendron* cultivars outpaces the slow multiplication rates of conventional propagation. This review examines recent advances in *Philodendron* micropropagation as a strategy to overcome this production constraint. Optimized culture protocols incorporating specific cytokinin and auxin combinations have enabled efficient shoot multiplication and vigorous root development. Furthermore, innovations such as the usage of silver nanoparticles (AgNPs) for aseptic establishment and arbuscular mycorrhizal fungi (AMF) for acclimatization significantly improve survival rates of plants. While Temporary Immersion Systems (TIS) offer promising scalability, verifying genetic fidelity remains critical. Collectively, these advanced tissue culture techniques provide a reliable, scalable framework for producing true-to-type, disease-free *Philodendron* clones for the global ornamental market

**Keywords:** Micropropagation, Genetic fidelity, Silver nanoparticles (AgNPs), Acclimatization, Ornamental

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## Introduction

Ornamental plants represent an important segment of the horticultural industry, serving as a significant source of income through their commercial production and growing consumer demand. *Philodendron* spp. is one of the high demand genera in the ornamental horticulture market. *Philodendron* is a diverse ornamental genus with many foliage cultivars that attract high market value, particularly due to their attractive variegation patterns and adaptability to indoor environments (Kang & Sivanesan, 2025; Krishna et al., 2016). Increasing consumer demand for rare cultivars of *Philodendron* such as 'Pink Princess', 'White Knight', and 'Birkin' requires efficient propagation systems capable of producing high-quality plants at scale. Conventional propagation by seed and stem cuttings is labor-intensive, slow, and frequently fails to meet market demand due to limited healthy source material, short seed viability and the tendency of cuttings to produce very few lateral shoots (Kang & Sivanesan, 2025; Krishna et al., 2016).

Furthermore, seasonal constraints and variability in quality associated with conventional methods have increased reliance on biotechnology industry, contributing to its development into a multibillion-dollar industry, particularly within the ornamental sector (Majumder et al., 2025). To date, micropropagation by using tissue culture technology is widely used for many *Philodendron* species to mass-produce planting material, ensure a year-round supply, and maintain uniform genetic traits (Chen et al., 2012; Hwang et al., 2022). Consequently, many commercial *Philodendron* cultivars are produced mainly via *in vitro* culture.

Recent studies have well documented the optimization of every stage of the *in vitro* process to enhance propagation efficiency. Advances include highly specific cytokinin and auxin regimes for shoot multiplication and rooting, the use of silver nanoparticles (AgNPs) to dramatically improve aseptic culture establishment, and arbuscular mycorrhizal fungi (AMF) to enhance acclimatization success. Furthermore, while liquid bioreactors have definitively improved growth in species like *P. bipinnatifidum*, Temporary Immersion Systems (TIS) are increasingly highlighted as a promising, general technology for the mass micropropagation of this ornamental plants, though research documenting its specific application to *Philodendron* species is still emerging (Méndez-Hernández & Loyola-Vargas, 2024; Ferreira et al., 2023). In the study conducted by Vy et al. (2025), the optimal condition of shoot (1.0 mg/L 6-benzylaminopurine) and root (0.5 mg/L indole-3-acetic acid) multiplication using TIS. Finally, maintaining genetic fidelity and minimizing somaclonal variation remains a critical focus when scaling up using these technologies.

To meet the booming commercial demand for rare *Philodendron* cultivars, the industry is shifting from conventional propagation to advanced biotechniques. While traditional methods are slow and labor-intensive, modern micropropagation that supported by optimized plant growth regulators, nanoparticles, and mycorrhizal fungi ensured a scalable, year-round production. Although innovations like Temporary Immersion Systems (TIS) offer promising avenues for large-scale mass propagation, mitigating somaclonal variation and maintaining genetic fidelity remain critical priorities for future commercial success. Therefore, this review provides an overview of current developments in *Philodendron* tissue culture including shoot regeneration, rooting, acclimatization, and bioreactor approaches, summarizes key advances in propagation

technology and discusses future prospects and challenges such as somaclonal variation and large-scale commercialization.

### **Micropropagation techniques and recent advances**

Micropropagation has been the predominant approach in most of plant tissue culture research, with shoot tips, nodal segments, petioles, and protocorm-like bodies commonly used as explants. To support the growth and regeneration of these explants, Murashige and Skoog (MS) medium and its modified formulations have been the most widely employed basal media for culture establishment and plant regeneration.

#### *Sterilization of explant*

Establishing aseptic cultures remains a critical step in successful micropropagation, and recent studies have introduced innovative approaches to improve sterilization efficiency. For example, the application of 40 mg/L silver nanoparticles for 60 minutes resulted in 100% aseptic petiole explants in *Philodendron* 'White Knight', demonstrating the potential of nanotechnology in contamination management (Kang & Sivanesan, 2025).

#### *Plant Growth Regulator*

Shoot multiplication was the primary focus of most studies, with propagation efficiency largely dependent on the optimization of plant growth regulator combinations. Species- and cultivar-specific responses were observed, highlighting the need for tailored culture conditions (Table 1). In *Philodendron bipinnatifidum*, optimal shoot proliferation was achieved using 1 mg/L 6-benzylaminopurine and 0.5 mg/L indole-3-butyric acid (Krishna et al., 2016). Similarly, *Philodendron* 'Birkin' produced up to 38 shoots per culture through direct and indirect organogenesis on MS medium supplemented with 3.5 mg/L 6-benzylaminopurine, 0.5 mg/L  $\alpha$ -naphthaleneacetic acid, and 10% coconut water (Hwang et al., 2022; Tasnim et al., 2025). In *Philodendron* 'White Knight', approximately 34 shoots per shoot tip were generated on MS medium containing 5  $\mu$ M 2-isopentenyladenine and 2.5  $\mu$ M  $\alpha$ -naphthaleneacetic acid (Kang & Sivanesan, 2025). These findings demonstrate that careful manipulation of plant growth regulator concentrations can substantially enhance shoot multiplication across different *Philodendron* cultivars.

#### *Rooting and Acclimatization*

Rooting is typically induced using reduced-strength MS media supplemented with auxins. For instance, *Philodendron* 'Birkin' achieves 93% rooting on  $\frac{1}{2}$  MS with 1 mg/L IBA, while 'White Knight' roots successfully on  $\frac{1}{2}$  MS with 10  $\mu$ M IBA. Following successful *in vitro* rooting, acclimatization procedures transfer plantlets into porous substrate mixes. *Philodendron* 'Birkin' showed approximately 97% survival in a soil, compost, coal, and moss mix, whereas *Philodendron* 'White Knight' achieved 100% survival in a peat, orchid stone, and coconut husk substrate (Hwang et al., 2022; Kang & Sivanesan, 2025). Well-documented research on *P. bipinnatifidum* demonstrates that inoculation with arbuscular mycorrhizal fungi (AMF) significantly enhances growth and improves root architecture during *ex vitro* acclimatization.

#### *Scale-up approaches in micropropagation*

Beyond solid media, liquid and bioreactor cultures have been extensively evaluated for scaling up production. In *P. bipinnatifidum*, liquid and airlift-type bioreactor cultures

significantly improved shoot growth compared to gelled media, though gelled media remained superior for initial multiplication (Krishna et al., 2016). In contrast, Temporary Immersion Systems (TIS) remain a general, promising technology. TIS are widely reported in broader micropropagation literature to increase multiplication rates, improve *ex vitro* performance, and reduce labor costs across many ornamental species (Majumder et al., 2025; Karunananda et al., 2021).

Although considerable progress has been made in developing micropropagation protocols for *Philodendron* species, recent studies indicate a shift from protocol establishment toward production optimization. Earlier investigations focused primarily on identifying suitable explants and plant growth regulator combinations for shoot regeneration and rooting, whereas more recent studies have explored advanced propagation technologies such as Temporary Immersion Systems. These systems improve nutrient uptake and gaseous exchange, resulting in higher multiplication rates and reduced production costs. Such developments demonstrate the increasing emphasis on scalability and commercial application within *Philodendron* tissue culture research.

#### *Genetic Stability and True-to-Type Propagation*

Finally, ensuring genetic stability is important, particularly because variegated cultivars like 'Birkin' and 'White Knight' are propagated *in vitro* specifically to address the inconsistent leaf variegation seen in conventional propagation and to ensure true-to-type plant as well as uniform clones (Hwang et al., 2022). Broad reviews caution that *in vitro* culture especially callus mediated routes or high PGR exposure can induce genetic and epigenetic changes that threaten clonal fidelity (Alwahibi et al., 2022; Bello-Bello et al., 2025). However, well-designed protocols successfully mitigate this risk. RAPD marker analysis in micropropagated *P. bipinnatifidum* is well documented, demonstrating nearly 100% genetic similarity between the clones and the mother plant (Alawaadh et al., 2020). Collectively, these optimized protocols for shoot induction, rooting, and aseptic establishment demonstrate that advanced micropropagation can reliably scale *Philodendron* production while maintaining the genetic fidelity and ornamental value demanded by the global market.

#### *Challenges and Future Perspectives*

Despite significant progress in *Philodendron* micropropagation, several technical and biological challenges continue to limit its large-scale commercial application. Contamination during culture establishment continues to reduce propagation efficiency, particularly when explants are collected from field-grown plants. Genotype-dependent responses also affect the reproducibility of protocols among different *Philodendron* species and cultivars. Furthermore, the maintenance of desirable ornamental characteristics such as variegation is particularly important in commercially valuable cultivars. Research on leaf-colour differentiation has highlighted the complex genetic mechanisms underlying variegation, indicating that further studies are needed to ensure trait stability during mass propagation. Addressing these limitations will be essential for improving the consistency and economic viability of commercial *Philodendron* spp. plant production.

Table 1 summarized the previously published studies on the micropropagation of *Philodendron* and related species. The table provides a comparative summary of the *in vitro* propagation methods, media formulation, and reported outcomes, serving

as the basis for the subsequent analysis and discussion of current developments in *Philodendron* tissue culture.

**Table 1: Summary of *in vitro* propagation for various *Philodendron* species.**

Species / Cultivar	<i>In vitro</i> propagation	Media Formulation	Growth Rate / Response	Reference
<i>Philodendron cannifolium</i>	Shoot	MS + BA or TDZ; rooting on MS + IBA	Up to 30 shoots on BA medium	Han & Park (2008)
<i>Philodendron bipinnatifidum</i>	Shoot	MS + 1 mg/L BAP + 0.5 mg/L IBA for best multiplication; rooting best on MS + 1–2 mg/L NAA	11.4 shoots/explant at the best multiplication treatment	Alawaadh et al. (2020)
<i>Philodendron bipinnatifidum</i>	Genetic fidelity	Followed based on Alawaadh et al. (2020) micropropagation system	Not reported	Alwahibi et al. (2022)
<i>Philodendron erubescens</i> 'Pink Princess'	Organogenesis	Best shoot proliferation on MS + 1.0 mg/L BAP; liquid MS + 1.0 mg/L BAP; rooting on MS + 3 mg/L IBA	11.2 shoots/explant and 4.7 leaves/explant in liquid MS	Klanrit et al. (2023)
<i>Philodendron</i> 'White Knight'	Shoot regeneration	Highest multiplication on MS-B + 5.0 $\mu$ M 2-iP + 2.5 $\mu$ M NAA; induction optimized separately on 20 $\mu$ M 2-iP + 5.0 $\mu$ M NAA	Up to 34 shoots per shoot tip during multiplication; 13.9 shoots per petiole at induction	Kang & Sivanesan (2025)
<i>Philodendron</i> 'Birkin'	Organogenesis	Direct multiplication optimized with 3.5 mg/L BAP + 0.5 mg/L NAA; further improved with 10% coconut water; rooting on 1/2MS + 1.0 mg/L IBA	Up to 38.2 shoots/culture with coconut water; 93.33% rooting; 96.67% acclimatization survival	Tasnim et al. (2025)
<i>Philodendron erubescens</i> 'Pink Princess'	Temporary Immersion System	Liquid MS in TIS	Not stated	Vy et al. (2025)
Hybrid <i>Philodendron</i> cultivars	Molecular analysis	Not stated	Not stated	Chen et al. (2026)
<i>Philodendron erubescens</i> 'Gd'	Mutation breeding	Standard culture medium after $^{60}\text{Co}$ gamma irradiation	Not stated	Karunananda et al. (2021)

## Discussion

*Philodendron* species are valued for their vigorous vegetative growth. The relatively high nitrogen content of Murashige and Skoog (MS) medium, supplied as both ammonium and nitrate ions, supports the synthesis of chlorophyll, amino acids, proteins, and nucleic acids, thereby promoting cell division, shoot proliferation, and leaf development (Sriskandarajah & Skirvin, 1991). However, the overall growth response also depends on factors such as plant growth regulators, explant type, and genotype.

Silver nanoparticles (AgNPs) offer a promising method for achieving fully sterile petiole explants in *Philodendron* 'White Knight'. Nevertheless, their use requires precise concentration management. Low levels safely promote growth and eliminate bacteria in tissue culture, while high levels can cause phytotoxic side effects like DNA damage, oxidative stress, and mitochondrial dysfunction.

Future developments in *Philodendron* tissue culture are expected to follow broader advances in commercial plant micropropagation. One of the most promising areas is the adoption of bioreactor technologies and Temporary Immersion Systems (TIS), which can reduce labor requirements, improve space utilization and lower production costs while enhancing shoot multiplication and plant quality. Recent studies have demonstrated the potential of TIS to significantly increase propagation efficiency in *Philodendron*. However, further optimization of immersion frequency, medium composition, and explant density will be necessary to establish reliable large-scale production systems. The development of automated bioreactor platforms tailored to *Philodendron* cultivars could further improve commercial propagation efficiency.

Another important direction is the maintenance of genetic fidelity during *in vitro* culture. Commercial cultivars such as 'Pink Princess', 'White Knight', and 'Birkin' derive much of their value from unique variegation patterns and ornamental characteristics. Consequently, minimizing somaclonal variation remains a major priority. Future propagation systems may incorporate routine molecular screening techniques, including DNA-based markers, to verify clonal uniformity throughout the production process. At the same time, controlled somaclonal variation may be explored as a potential breeding strategy for the development of novel ornamental traits and improved cultivars.

Technological advances are also expected to transform the tissue culture management. Artificial intelligence, machine learning, sensor technologies, and digital imaging systems could be used to optimize culture conditions, monitor plant growth, detect contamination, and automate subculture scheduling. In addition, tissue culture may become increasingly integrated with modern biotechnological tools such as gene editing, cryopreservation, and synthetic seed technology. These approaches could facilitate the development of improved cultivars with enhanced ornamental value and stress tolerance while supporting long-term germplasm conservation. Collectively, these innovations have the potential to make *Philodendron* micropropagation faster, more cost-effective and more reliable for large-scale commercial production.

## Conclusion

In conclusion, *Philodendron* tissue culture has emerged as an effective propagation method for the large-scale production of high-quality, disease-free, and genetically uniform plants. From this review, it demonstrates significant progress in the development of micropropagation protocols, including improved shoot multiplication, rooting, and acclimatization techniques for commercially important cultivars such as 'Pink Princess', 'White Knight', and 'Birkin'. Recent innovations, particularly the use of Temporary Immersion Systems and enhanced culture media formulations, have increased propagation efficiency while maintaining desirable ornamental traits.

Despite these advances, challenges remain in ensuring genetic stability, reducing contamination, and optimizing protocols for diverse *Philodendron* species and cultivars. Future developments are expected to focus on automated bioreactor systems, molecular tools for genetic fidelity assessment, artificial intelligence assisted culture optimization, and the integration of advanced biotechnological approaches such as gene editing and germplasm conservation. Overall, continued research and technological innovation will further strengthen the commercial production, conservation, and sustainable development of *Philodendron*, supporting the growing global demand for these valuable ornamental plants.

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