

Micropropagation of Lacy Tree Philodendron (*Philodendron bipinnatifidum* Schott ex Endl.)

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Abstract. The present study aimed to optimize the micropropagation of lacy tree philodendron using shoot tip explants. Axillary shoot regeneration was investigated in Murashige and Skoog (MS) medium with different types and concentrations of plant growth regulators, varied levels of MS medium salt strength, sucrose concentration, and light intensity and culture type. Adding 6-benzylaminopurine (BAP; $1 \text{ mg}\cdot\text{L}^{-1}$) significantly increased shoot multiplication compared with other cytokinins, and the combination of cytokinins and auxins [indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA)], yielded more shoots than cytokinins alone, with the greatest number of axillary shoots (11.4 per explant) obtained using both BAP ($1 \text{ mg}\cdot\text{L}^{-1}$) and IBA ($0.5 \text{ mg}\cdot\text{L}^{-1}$). In addition, the use of half-strength salt concentrations significantly reduced shoot multiplication, and high sucrose concentrations ($>30 \text{ g}\cdot\text{L}^{-1}$) reduced explant growth. High light intensity also reduced shoot multiplication and growth, owing to photoinhibition, and shoot multiplication was more efficient in gelled culture, whereas shoot growth was greater in liquid/bioreactor culture. The best rooting success (100%) and greatest root number and fresh weight were obtained using MS medium supplemented with NAA ($1\text{--}2 \text{ mg}\cdot\text{L}^{-1}$). The resulting plantlets were successfully acclimatized, with a survival rate of 100%, and were morphologically similar to the mother plant.

The genus *Philodendron* (Araceae) includes 447 accepted species (The Plant List, 2019) that are native to tropical and subtropical regions of the Americas and West Indies (Mayo et al., 1997). Both vining and self-heading types of *Philodendron* spp. are highly prized for their attractive foliage and tolerance to indoor environments. The self-heading type has gained popularity

over the past 4 decades, owing to an increase in the introduction of new hybrids with red, yellow, or orange foliage (Chen et al., 2012). Lacy tree philodendron (*Philodendron bipinnatifidum* Schott ex Endl.; taxonomic serial no. TSN 506756, according to the *Integrated Taxonomic Information System*, 2019), formerly known as *Philodendron selloum* K. Koch (TSN 506759), is a

self-heading species that can reach heights of 4 to 5 m and possesses deeply cut, green to dark green leaves that can grow up to 1 m in length. Lacy tree philodendron is mainly propagated by seed, and growers maintain mature plants for seed production. However, seedling propagation does not encourage the multiplication of the species because the method is labor intensive and because the seeds are relatively short-lived unless properly processed and vacuum packed. In addition, conventional propagation, such as the use of cuttings, is not preferred for self-heading philodendron, owing to the slow growth, short internodes, and large stems and leaves of the species. Instead, in vitro propagation methods are widely used, as they facilitate the continuous generation of both high-quantity and high-quality plant materials over relatively short time periods. Previous studies have reported that most philodendron plants grown from seed or conventional cuttings produce few side shoots, whereas those produced by tissue culture possess basal shoots that help produce compact plants, even in small pots (Henley et al., 2005). Thus, tissue culture methods could be used to fulfill the demand for desired plant species in both domestic and export markets. Indirect shoot regeneration via callus formation at the base of intersected shoots has been reported in *P. selloum* (Kelie et al., 2004); however, few studies have investigated tissue culture propagation in other philodendron species, such as *Philodendron cannifolium* (Han and Park, 2008), *Philodendron micans* (Xiong, 2009), *Philodendron tuxilanum* (Jambor-Benczur and Marta-Riffer, 1990), *Philodendron xanadu* (Charan, 2009; Jirakiattikul and Limpradithanont, 2006), and various philodendron hybrids (Chen et al., 2012; Gangopadhyay et al., 2004; Sreeksumar et al., 2001; Ziv and Ariel, 1991).

Both the composition of tissue culture medium, including its carbon and plant growth regulator (PGR) contents, and light intensity play crucial roles in ensuring optimal growth and morphogenesis. Culture type also has a regulatory effect on in vitro cultures. For example, liquid/bioreactor systems are generally more cost-effective for micropropagation than gelled cultures (Paek et al., 2005). Moreover, the success of in vitro regeneration and propagation depends on a series of stages, each with specific requirements. In the present study, we aimed to establish an efficient micropropagation protocol for lacy tree philodendron using axillary shoot regeneration.

Materials and Methods

Plant material. Lacy tree philodendron shoots were maintained on MS (Murashige and Skoog, 1962) solid medium that included $1 \text{ mg}\cdot\text{L}^{-1}$ BAP, $30 \text{ g}\cdot\text{L}^{-1}$ sucrose, and $8.0 \text{ g}\cdot\text{L}^{-1}$ agar-agar for 8 weeks, and regenerated shoots were kept routinely by subculturing once every 4 weeks on MS medium without PGR

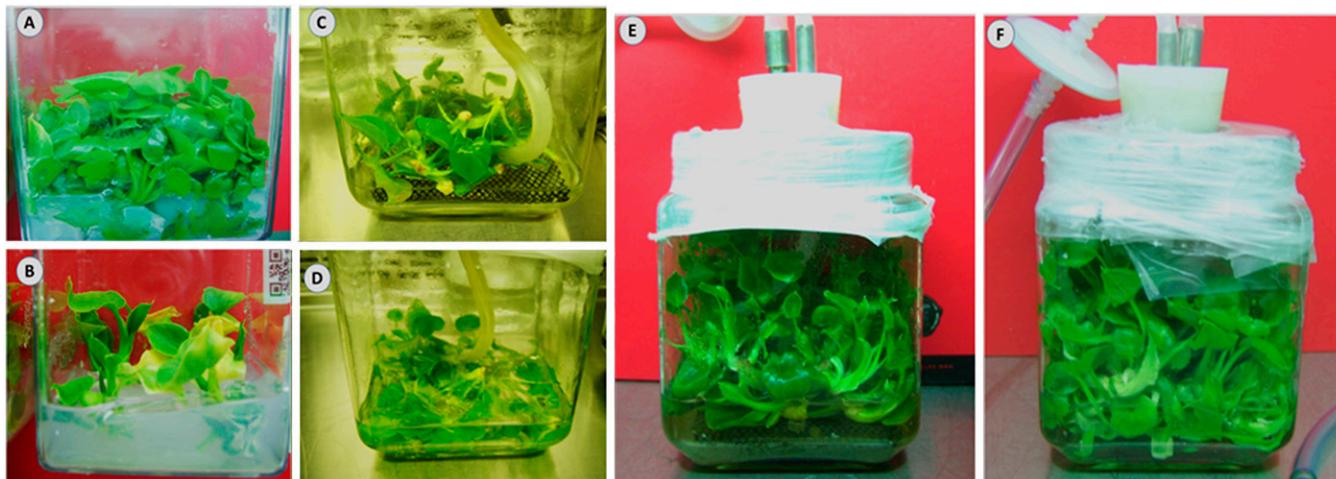


Fig. 1. Axillary shoot multiplication of lacy tree philodendron. (A) Shoot multiplication in gelled culture, full-strength Murashige and Skoog medium that contained 6-benzylaminopurine ($1 \text{ mg}\cdot\text{L}^{-1}$) and indole-3-butyric acid ($0.5 \text{ mg}\cdot\text{L}^{-1}$), after 6 weeks of culture. (B) Photoinhibition of shoot multiplication in gelled culture that was incubated under a photosynthetic photon flux density of $55 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, after 6 weeks of culture. (C, D) Shoot multiplication in liquid/bioreactor cultures with and without netting, after 0 d of culture. (E, F) Shoot multiplication in liquid/bioreactor cultures with and without netting, after 6 weeks of culture.

and used as plant materials to conduct the experiments.

Culture conditions. The cytokinins and auxins were added to MS basal medium, thereafter, the pH of all media was adjusted to 5.8 before autoclaving ($121 \text{ }^\circ\text{C}$ and $1.2 \text{ kg}\cdot\text{cm}^{-2}$ for 15 min), and the cultures were incubated at $25 \pm 2 \text{ }^\circ\text{C}$ under a 16-h photoperiod of $25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (PPFD) provided by cool white fluorescent tubes. PPFD was measured using a luminous intensity meter (Testo 545; Testo, Melrose, MA).

Axillary shoot multiplication in gelled culture. Shoot tips (2.5–3.0 cm; four per culture vessel) were cultured in Magenta GA-7 culture vessels ($77 \times 77 \times 97 \text{ mm}$; Magenta LLC, Chicago, IL) that each contained 60 mL MS medium with $30 \text{ g}\cdot\text{L}^{-1}$ sucrose and $8 \text{ g}\cdot\text{L}^{-1}$ agar-agar. In the first experiment, the medium also contained different concentrations of cytokinins: BAP ($0.1, 0.5, 1, \text{ and } 2 \text{ mg}\cdot\text{L}^{-1}$), kinetin ($1, 3, 5, \text{ and } 7 \text{ mg}\cdot\text{L}^{-1}$), 2-isopentenyl adenine (2ip; $1, 3, 5, \text{ and } 7 \text{ mg}\cdot\text{L}^{-1}$), and thidiazuron (TDZ; $0.05, 0.1, 0.5, \text{ and } 1 \text{ mg}\cdot\text{L}^{-1}$). In the second experiment, BAP ($1 \text{ mg}\cdot\text{L}^{-1}$), kinetin ($5 \text{ mg}\cdot\text{L}^{-1}$), 2ip ($3 \text{ mg}\cdot\text{L}^{-1}$), and TDZ ($0.1 \text{ mg}\cdot\text{L}^{-1}$) were combined with auxins (i.e., NAA and IBA at $0.5 \text{ and } 1 \text{ mg}\cdot\text{L}^{-1}$). In the third and fourth experiments, shoots were cultured on MS medium that contained BAP ($1 \text{ mg}\cdot\text{L}^{-1}$) and IBA

($0.5 \text{ mg}\cdot\text{L}^{-1}$), to test the effects of MS medium salt concentration (full or half strength), sucrose concentration ($30, 40, 50, \text{ or } 60 \text{ g}\cdot\text{L}^{-1}$), and PPFD ($25, 40, \text{ and } 55 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on shoot multiplication. For all the experiments, shoot number, height, and fresh weight were recorded after 6 weeks of culture.

Axillary shoot multiplication in liquid/bioreactor culture. To compare the shoot multiplication and growth of a continuous immersion culture system (manually designed airlift type with or without net; Fig. 1C–F) to that of gelled culture, shoot tips (16 explants per bioreactor; 2.5 cm in length) were inoculated into 1.2-L glass vessels that contained 240 mL MS liquid medium with sucrose ($30 \text{ g}\cdot\text{L}^{-1}$), BAP ($1 \text{ mg}\cdot\text{L}^{-1}$), and IBA ($0.5 \text{ mg}\cdot\text{L}^{-1}$). In the immersion (without net) culture vessel, shoot tips were submerged in liquid during the whole period, whereas in the immersion (with net) culture vessel, a supporting net was used to prevent complete submersion of the plant material in the liquid medium. The air input was adjusted to 1.0 vvm (air volume/medium volume/min). The pH of the medium was adjusted to 5.8 before autoclaving ($121 \text{ }^\circ\text{C}$ and $1.2 \text{ kg}\cdot\text{cm}^{-2}$ for 30 min), and all cultures were maintained at $25 \text{ }^\circ\text{C}$ under a 16-h photoperiod of $25 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD. Shoot number, height, and fresh weight were recorded after 6 weeks of culture.

In vitro rooting and acclimatization. Axillary shoot clumps proliferated on MS medium containing BAP ($1 \text{ mg}\cdot\text{L}^{-1}$) and IBA ($0.5 \text{ mg}\cdot\text{L}^{-1}$) were divided and transferred to MS medium that contained indole acetic acid (IAA), IBA, or NAA at concentrations of $0.1, 0.5, 1, \text{ or } 2 \text{ mg}\cdot\text{L}^{-1}$, and auxin-free MS medium was used as a control treatment. All cultures were maintained at $25 \text{ }^\circ\text{C}$ under a 16-h photoperiod of $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD. After 6 weeks, the rooting success (rooting percentage, root length, and number of roots per explant) and fresh weight were recorded.

For acclimatization, the rooted plantlets were gently removed from the medium, rinsed using tap water, briefly (for few seconds) dipped in a fungicide solution ($0.5 \text{ g}\cdot\text{L}^{-1}$ Aromil-Plus 50 WP; Mobedco-Vet, Amman, Jordan), and, finally, transplanted into plastic pots (10 cm in diameter) that contained a sterile 1:1 (v:v) mixture of peatmoss and perlite. The potted plants were incubated for 30 d at $25 \pm 2 \text{ }^\circ\text{C}$ and 50% to 60% relative humidity under a 16-h photoperiod of $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD provided by white fluorescent lamps in a growth chamber (Model KBWF 720; Binder, Tuttlingen, Germany), with the pots being covered with transparent polyethylene for the first 10 d. The plantlets were regularly irrigated using a nutrient solution that contained half-strength MS basal salts, and plantlet survival was evaluated at 30 d.

Experimental design and statistical analysis. The experiments were set up using a completely randomized design. For all the gelled culture experiments, there were three replicates in each treatment and each replicate was represented by a culture vessel (Magenta GA-7) containing four explants (shoot tips) rendering a group of 12 explants per treatment. For the liquid culture experiment, there were two bioreactors in each system treatment (with or without net). Twelve randomly selected explants were used for recording data after 6 weeks of culture. All data expressed as percentages were arcsine-transformed before analysis (Compton, 1994), and data were subjected to analysis of variance and Tukey's range tests using SAS statistical software (version 6.12; SAS Institute, Cary, NC).

Results and Discussion

Axillary shoot multiplication and growth in gelled culture. The multiplication and growth of lacy tree philodendron shoots were significantly influenced by the type and

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Table 1. Effect of cytokinins on shoot multiplication and growth of lacy tree philodendron after 6 weeks of culture.

Cytokinin (mg·L ⁻¹)	Shoots (no./explant)	Shoot length (cm)	Fresh wt (g/clump)
MS without PGR	1.0 f ^z	6.24 ab	0.93 g
BAP			
0.1	3.8 cde	5.67 bc	1.55 defg
0.5	4.8 bc	6.09 ab	1.93 cde
1.0	6.3 a	4.29 efg	2.45 abc
2.0	4.3 cde	3.65 g	2.11 bcd
Kinetin			
1.0	3.3 de	4.93 cde	1.15 fg
3.0	3.9 cde	4.49 def	1.61 defg
5.0	4.7 bc	5.11 cd	1.72 def
7.0	4.4 cde	4.27 efg	1.56 defg
2ip			
1.0	4.6 bcd	5.12 cd	1.69 def
3.0	5.8 ab	6.55 a	2.67 ab
5.0	4.7 bc	5.54 bc	2.76 ab
7.0	4.8 bc	4.96 cde	2.84 a
TDZ			
0.05	3.0 e	5.15 cd	1.15 fg
0.1	3.9 cde	4.34 efg	1.30 efg
0.5	4.0 cde	3.79 fg	1.22 fg
1.0	3.5 cde	3.65 g	0.89 g
Significance ^y			
Cytokinin type (CT)	***	***	***
Cytokinin concn (CC)	***	***	***
CT × CC	NS	***	NS

^zValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

^yNS, ***Nonsignificant or significant at $P \leq 0.001$, respectively.

PGR = plant growth regulator; BAP = 6-benzylaminopurine; 2ip = 2-isopentenyl adenine; TDZ = thidiazuron.

concentration of cytokinin added to the culture medium (Table 1). However, the number of axillary shoots per explant generally only increased up to certain concentrations, which depended on the type of cytokinin, and no proliferation was observed in the absence of cytokinin (control treatment). Supplementation of the medium with BAP (1 mg·L⁻¹) yielded the greatest mean shoot number (6.3), when compared with the other treatments. The application of kinetin and 2ip yielded lower multiplication rates than BAP. This might be partly due to the presence of a double bond in the chemical structure of 2ip, which makes it vulnerable to the action of cytokinin oxidases (Kaminek, 1992). However, 2ip treatment also yielded taller and heavier axillary shoots than the BAP, kinetin, and TDZ treatments. The TDZ-treated explants exhibited the lowest number of axillary shoots and produced stunted shoots with narrow leaves. Han and Park (2008) reported that TDZ is less effective than BAP for inducing shoot proliferation of *P. canifolium* and that TDZ-proliferated shoots were necrotic and associated with the formation of hard callus at their basal parts. Meanwhile, TDZ-treated *Philodendron* 'Imperial Red' and 'Imperial Rainbow' explants were reported to form numerous leaf-like structures (Chen et al., 2012). Dewir et al. (2018) suggested that TDZ-induced abnormalities and inhibition of shoot proliferation are due to overdose or continuous exposure for a long duration until organogenesis. BAP was more effective for inducing the shoot multiplication of lacy tree philodendron than the other cytokinins, as previously reported for other *Philodendron* species (Gangopadhyay et al., 2004; Han and Park, 2008; Jambor-Benczur and Marta-Riffer, 1990; Sreekumar et al.,

2001). The different activities of individual cytokinins could be due to differences in uptake rates (Blakesley, 1991), rates of translocation to meristematic regions, and metabolism (Kaminek, 1992; Sakakibara, 2010).

The combination of cytokinins and auxins (IBA and NAA) yielded greater shoot number, length, and fresh weight than treatments with cytokinins alone, and the greatest number of shoots (10.9 per explant) was obtained using a combination of BAP (1 mg·L⁻¹) and IBA (0.5 mg·L⁻¹; Table 2). Similarly, the combination of 2ip (3 mg·L⁻¹) and IBA yielded high numbers of shoots, and the combination of 2ip (3 mg·L⁻¹) and NAA yielded shoots with relatively high length and fresh weight values. The addition of NAA and IBA to kinetin treatments also resulted in moderate increases in shoot number, whereas the least substantial increase was observed when NAA or IBA was added to TDZ treatments. The proportions of auxins and cytokinins is a determinant for meristem formation (George, 1993). Previous studies have reported that the combination of cytokinins and auxins exerts a synergistic and positive influence on the shoot proliferation of Araceae plant species, for example, *Aglonema* 'Valentine' (El-Mahrouk et al., 2016), *Alocasia amazonica* (Jo et al., 2008a), *Philodendron* 'Serratium' (Patel and Shah, 2004), and *Spathiphyllum canifolium* (Dewir et al., 2006).

Different MS medium salt strengths and sucrose concentrations were tested to identify the ideal conditions for stimulating shoot multiplication (Table 3). Reducing MS salts to half strength significantly reduced shoot number and fresh weight but failed to affect shoot length. Higher sucrose concentrations (4% to 6%) also reduced shoot fresh weight but had no effect on shoot number or length.

Meanwhile, full-strength MS was suitable for shoot multiplication and growth (Fig. 1A). Furthermore, increased light intensity (>25 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) drastically reduced shoot multiplication and growth (Table 4), and explants grown under 55 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF yielded the lowest shoot number, length, and fresh weight values and exhibited yellowing, which is a symptom of photo-inhibition (Fig. 1B). It is well-known that the concentrations of minerals in tissue culture medium have a strong effect on organogenesis, and in vitro-cultured plantlets generally exhibit low photosynthetic ability and are unable to establish a positive carbon balance. Therefore, the culture medium must be supplemented with saccharides (mainly sucrose), which function as a carbon and energy source for sustaining photomixotrophic metabolism. Indeed, several other studies also have reported that shoot multiplication and growth are influenced by medium salt strength (Kozak and Wnuk, 2012; Shaik et al., 2010), sucrose concentration (Dewir et al., 2006; Jo et al., 2009), and light intensity (Jo et al., 2008b; Lobna et al., 2008).

Axillary shoot multiplication and growth in liquid/bioreactor culture. The comparative study of gelled and liquid/bioreactor continuous immersion culture (with or without net) revealed that shoot multiplication was greater in the gelled culture (Table 5). The greatest number of shoots (10.4 shoots per explant) was obtained using gelled culture, whereas immersion culture with and without netting yielded only 7.6 and 8.5 shoots per explant, respectively. Interestingly, shoot growth (length and fresh weight) was greater in the liquid/bioreactor culture than in gelled culture and was greatest in the immersion system without netting. In liquid cultures,

Table 2. Effect of different auxins in combination with different cytokinins on shoot multiplication and growth of lacy tree philodendron after 6 weeks of culture.

Cytokinin (mg·L ⁻¹)	Auxins		Shoots (no./explant)	Shoot length (cm)	Fresh wt (g/clump)	
	IBA	NAA (mg·L ⁻¹)				
BAP	1.0	0.5	10.9 a ^z	5.4 c	3.40 cde	
		1.0	9.5 ab	6.8 abc	3.31 cdef	
		—	0.5	8.3 abc	6.9 abc	3.48 cde
		—	1.0	9.4 ab	6.3 abc	3.63 cd
Kinetin	5.0	0.5	6.1 cde	6.0 abc	1.68 g	
		1.0	7.5 bcd	6.0 abc	1.88 g	
		—	0.5	7.6 bcd	5.6 bc	2.57 defg
		—	1.0	7.8 bcd	5.8 abc	2.45 efg
2ip	3.0	0.5	9.5 ab	6.9 abc	2.29 fg	
		1.0	9.7 ab	6.3 abc	3.23 cdef	
		—	0.5	8.3 abc	7.0 ab	4.75 ab
		—	1.0	7.5 bcd	7.2 a	5.04 a
TDZ	0.1	0.5	4.4 e	5.6 bc	4.08 abc	
		1.0	5.7 cde	7.2 a	3.76 bc	
		—	0.5	5.4 de	5.9 abc	3.62 cd
		—	1.0	6.1 cde	5.9 abc	3.75 c
Significance ^y						
Cytokinin type (CT)			***	*	***	
Auxin type (AT)			NS	NS	***	
Auxin concn (AC)			NS	NS	NS	
CT × AT			NS	NS	***	
AT × AC			NS	NS	NS	
CT × AC			NS	NS	NS	
CT × AT × AC			NS	NS	NS	

^zValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

^yNS, *, ***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

BAP = 6-benzylaminopurine; 2ip = 2-isopentenyl adenine; TDZ = thidiazuron; IBA = indole-3-butyric acid; NAA = naphthalene acetic acid.

Table 3. Effect of medium salt strength and sucrose concentration on shoot multiplication and growth of lacy tree philodendron after 6 weeks of culture on Murashige and Skoog medium containing 6-benzylaminopurine (1 mg·L⁻¹) and indole-3-butyric acid (0.5 mg·L⁻¹).

Medium salt strength	Sucrose (g·L ⁻¹)	Shoots (no./explant)	Shoot length (cm)	Fresh wt (g/clump)
Full strength	30	11.0 ab ^z	5.1 a	3.39 a
	40	11.9 a	5.5 a	3.53 a
	50	10.7 ab	5.7 a	3.47 a
	60	8.8 bc	4.9 a	2.37 b
Half strength	30	8.9 bc	5.0 a	2.54 b
	40	7.6 c	5.1 a	2.53 b
	50	7.4 c	5.2 a	2.16 b
	60	7.2 c	5.7 a	1.88 b
Significance ^y				
Salt strength (A)		***	NS	***
Sucrose concentration (B)		NS	NS	*
A × B		NS	NS	NS

^zValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

^yNS, *, ***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table 4. Effect of light intensity on shoot multiplication and growth of lacy tree philodendron after 6 weeks of culture on Murashige and Skoog medium containing 6-benzylaminopurine (1 mg·L⁻¹) and indole-3-butyric acid (0.5 mg·L⁻¹).

Light intensity (PPFD)	Shoots (no./explant)	Shoot length (cm)	Fresh wt (g/clump)
25	11.2 a ^z	5.0 a	3.42 a
40	6.4 b	4.7 ab	1.99 b
55	5.6 b	4.4 b	1.44 b

^zValues followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

PPFD = photosynthetic photon flux density.

Table 5. Effect of culture type on shoot multiplication and growth of lacy tree philodendron after 6 weeks of culture on Murashige and Skoog medium containing 6-benzylaminopurine (1 mg·L⁻¹) and indole-3-butyric acid (0.5 mg·L⁻¹).

Culture type	Shoots (no./explant)	Shoot length (cm)	Fresh wt (g/clump)
Gelled culture	11.4 a ^z	5.2 c	3.53 b
Liquid/bioreactor culture (immersion)	8.5 b	8.7 a	7.13 a
Liquid/bioreactor culture (immersion with net)	7.6 b	7.7 b	3.72 b

^zMeans followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

explants are continuously in contact with the medium, which enables a constant supply and high absorption of nutrients, as well as suitable aeration, which apparently enhance ex-

plant growth. Improved growth of explants in liquid culture also has been reported for other Araceae species [e.g., *A. amazonica* (Jo et al., 2008a) and *S. canniifolium* (Dewir et al.,

2006)]. Axillary shoot multiplication of philodendron is greatly influenced by cytokinin concentration (Table 1). The low multiplication rate observed in liquid culture could be

Table 6. Effect of auxins on rooting and growth of lacy tree philodendron after 6 weeks in culture.

Auxins (mg·L ⁻¹)	Rooting (%)	No. of roots	Root length (cm)	Fresh wt (g/plantlet)
MS without PGR	38 c ^e	2.5 d	11.0 a	0.96 fg
IBA				
0.1	75 b	2.3 d	10.3 ab	1.01 fg
0.5	94 ab	2.3 d	9.1 ab	1.45 efg
1.0	94 ab	6.1 c	10.6 ab	1.87 de
2.0	94 ab	7.1 c	12.0 a	2.35 cd
NAA				
0.1	100 a	7.7 c	11.0 a	2.69 c
0.5	100 a	9.8 b	7.8 bc	3.51 b
1.0	100 a	11.6 ab	5.5 cd	5.02 a
2.0	100 a	13.1 a	3.5 d	5.12 a
IAA				
0.1	100 a	2.3 d	10.9 a	1.68 def
0.5	100 a	2.1 d	11.5 a	1.40 efg
1.0	100 a	2.7 d	12.1 a	1.76 def
2.0	94 ab	2.2 d	10.6 ab	0.83 g
Significance ^y				
Auxin type (AT)	NS	***	***	***
Auxin concn (AC)	*	***	NS	***
AT × AC	NS	***	***	***

^zMeans followed by the same letter in the same column are not significantly different at $P \leq 0.05$ level, according to Tukey's range test.

^yNS, *, ***Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

MS = Murashige and Skoog; PGR = plant growth regulators; IBA = indole butyric acid; NAA = naphthalene acetic acid; IAA = indole acetic acid.

due to the absorption of high doses of BAP (Table 5); therefore, further experiments are needed to investigate the effects of inoculum size, BAP concentration, and aeration. Ziv and Ariel (1991) reported that leaves of *Philodendron hastatum* × *imble* × *wendlandii* 'Burgundy' became necrotic during the proliferation stage in agitated liquid culture. However, no symptoms of necrosis, hyperhydricity, or malformation were observed in the present study.

In vitro rooting and acclimatization. The addition of auxins to the rooting medium significantly improved the rooting responses of the philodendron shoots, when compared with the control treatment (Table 6). However, rooting percentage and root length were not significantly affected by auxin type or auxin concentration, respectively. In fact, the best rooting responses were observed on MS medium that was supplemented with NAA (1–2 mg·L⁻¹), which yielded 100% rooted shoots with greatest root number and fresh weight values in the various treatments (Fig. 2A and B). In addition, NAA was more effective than both IBA and IAA, regardless of concentration, and low-auxin (≤ 0.5 mg·L⁻¹) treatments yielded fewer, but longer, roots. Together, these results clearly demonstrate that exogenous auxin treatment is necessary to improve philodendron rooting. Previous studies have reported that the addition of NAA (0.5 mg·L⁻¹) to half-strength MS medium is effective for rooting *P. tuxtilanum* (Jambor-Benczur and Marta-Riffer, 1990) and *P. xanadu* (Jirakiattikul and Limpradithanont, 2006), whereas the addition of IBA (0.1–1 mg·L⁻¹) is more effective for rooting philodendron microshoots ['Imperial green', 'Imperial Red', and 'Imperial Rainbow' (Chen et al., 2012)]. In contrast, Sreekumar et al. (2001) reported that microshoots from six philodendron cultivars rooted easily in MS medium without PGR supplementation and that the resulting plantlets acclimatized with 90% survival. In the present study, in



Fig. 2. In vitro rooting and acclimatization of lacy tree philodendron. (A, B) In vitro plantlets rooted on Murashige and Skoog medium that contained naphthalene acetic acid (1 mg·L⁻¹), after 6 weeks of culture. (C) Acclimatized plantlets in a 1:1 (v:v) mixture of peatmoss and perlite, after 30 d.

vitro-rooted lacy tree philodendron plantlets were transplanted to a mixture of peatmoss and perlite and acclimatized over a 30-d period (Fig. 1 C).

The plants were successfully acclimatized, exhibited a survival rate of 100%, and were morphologically similar to the mother plant.

Literature Cited

- Blakesley, D. 1991. Uptake and metabolism of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. *Plant Cell Tissue Organ Cult.* 25:69–74.
- Charan, A. 2009. Studies on cheaper alternatives in micropropagation of philodendron (*Philodendron xanadu*). Univ. of Agr. Sci., Bengaluru, India, MS Thesis.
- Chen, F.C., C.Y. Wang, and J.Y. Fang. 2012. Micropropagation of self-heading *Philodendron* via direct shoot regeneration. *Scientia Hort.* 141:23–29.
- Compton, M.E. 1994. Statistical methods suitable for the analysis of plant tissue culture data. *Plant Cell Tissue Organ Cult.* 37:217–242.
- Dewir, Y.H., D. Chakrabarty, E.J. Hahn, and K.Y. Paek. 2006. A simple method for mass propagation of *Spathiphyllum cannifolium* using an airlift bioreactor. *In Vitro Cell. Dev. Biol. Plant* 42:291–297.
- Dewir, Y.H., Nurmansyah, Y. Naidoo, and J.A. Teixeira da Silva. 2018. Thidiazuron-induced abnormalities in plant tissue cultures. *Plant Cell Rep.* 37:1451–1470.
- El-Mahrouk, M.E., Y.H. Dewir, and Y. Naidoo. 2016. Micropropagation and genetic fidelity of the regenerants of *Aglaonema* 'valentine' using randomly amplified polymorphic DNA. *Hort-Science* 51:398–402.
- Gangopadhyay, G., T. Bandyopadhyay, S.B. Gangopadhyay, and K.K. Mukherjee. 2004. Luffa sponge - A unique matrix for tissue culture of *Philodendron*. *Curr. Sci.* 86:315–319.
- George, E.F. 1993. *Plant propagation by tissue culture*. Exegetics Ltd., Westbury, UK.
- Han, B.H. and B.M. Park. 2008. *In vitro* micropropagation of *Philodendron cannifolium*. *J. Plant Biotechnol.* 35:203–208.
- Henley, R.W., A.R. Chase, and L.S. Osborne. 2005. *Philodendrons - Self-heading types*. CFREC-A Foliage Plant Research Note RH-91-27. 8 Sept. 2019. <<https://mrec.ifas.ufl.edu/Foliage/fofnotes/phil-sh.htm>>.
- Integrated Taxonomic Information System. 2019. 15 Sept. 2019. <<https://www.its.gov/>>.
- Jambor-Benczur, E. and A. Marta-Riffer. 1990. In vitro propagation of *Philodendron tuxtlanum* Bunting with benzylaminopurine. *Acta Agron. Hung.* 39:341–348.
- Jirakiattikul, Y. and P. Limpraditthanont. 2006. Shoot multiplication and rooting of *Philodendron xanadu* cultured *in vitro*. *Songklanakarin J. Sci. Technol.* 8:79–86.
- Jo, E.A., H.N. Murthy, E.J. Hahn, and K.Y. Paek. 2008a. Micropropagation of *Alocasia amazonica* using semisolid and liquid cultures. *In Vitro Cell. Dev. Biol. Plant* 44:26–32.
- Jo, E.A., R.K. Tewari, E.J. Hahn, and K.Y. Paek. 2008b. Effect of photoperiod and light intensity on in vitro propagation of *Alocasia amazonica*. *Plant Biotechnol. Rep.* 2:207–212.
- Jo, E.A., R.K. Tewari, E.J. Hahn, and K.Y. Paek. 2009. In vitro sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets. *Plant Cell Tissue Organ Cult.* 96:307–315.
- Kaminek, M. 1992. Progress in cytokinin research. *Trends Biotechnol.* 10:159–162.
- Kelie, L.L., L.I. Zhiying, X.U. Li, and M.A. Qianquan. 2004. Tissue culture and rapid propagation of *Philodendron selloum*. *Chinese J. Trop. Agr.* 24:21–23.
- Kozak, D. and K. Wnuk. 2012. The influence of the salt composition of basal medium and growth regulators on in vitro growth and development of *Tibouchina urvilleana* (DC.) Cogn. *Acta Sci. Pol. Hortorum Cultus* 11: 59–68.
- Lobna, S., M.M. Taha, S. Ibrahim, and M.M. Farahat. 2008. Micropropagation protocol of *Paulownia kowakamii* through in vitro culture technique. *Aust. J. Basic Appl. Sci.* 2:594–600.
- Mayo, S.J., J. Bogner, and P.C. Boyce. 1997. *The genera of Araceae*. Royal Botanical Gardens, Kew.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473–497.
- Paek, K.Y., D. Chakrabarty, and E.J. Hahn. 2005. Application of bioreactor system for large scale production of horticultural and medicinal plants, p. 287–300. In: A.K. Hvoslef-Eide and W. Preil (eds.). *Liquid culture systems for in vitro plant propagation*. Springer, New York.
- Patel, R.M. and R.R. Shah. 2004. Micropropagation of *Philodendron* var. 'Serratum'. *J. Ornament. Hort.* 7:338–340.
- Sakakibara, H. 2010. Cytokinin biosynthesis and metabolism, p. 95–14. In: P.J. Davies (ed.). *Plant hormones*. Springer, Dordrecht.
- Shaik, S., Y.H. Dewir, N. Singh, and A. Nicholas. 2010. Micropropagation and bioreactor studies of the medicinally important plant *Sutherlandia frutescens* L. *S. Afr. J. Bot.* 76:180–186.
- Sreekumar, S., S. Mukunthakumar, and S. Seeni. 2001. Morphogenetic responses of six *Philodendron* cultivars in vitro. *Indian J. Exp. Biol.* 39:1280–1287.
- The Plant List. 2019. *Philodendron*. 17 Sept. 2019. <<http://www.theplantlist.org/browse/A/Araceae/Philodendron/>>.
- Xiong, Z.J. 2009. Regeneration of *Philodendron micans* K. Koch through protocorm-like bodies and improvement of plant form using growth regulators. Univ. of FL, Gainesville, MS Thesis.
- Ziv, M. and T. Ariel. 1991. Bud proliferation and plant regeneration in liquid-cultured philodendron treated with ancymidol and paclobutrazol. *J. Plant Growth Regul.* 10:53–57.