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In vitro Mass Propagation of *Philodendron* cv. 'Birkin' through Direct and Indirect Organogenesis

Nafisa Tasnim, Kazi Tanbir Rahman, Snigdha Sarker, Md. Raihan Iqbal Raju* and Mohd. Talim Hossain

Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

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Abstract

This study established both direct and indirect *in vitro* regeneration protocol for *Philodendron* cv. 'Birkin' using shoot tips, leaf lamina and stem nodal segments. Direct shoot induction was highest in MS medium with 2.5 mg/l BAP, resulting in an 86.67% response with an average of 9.20 shoots per shoot tip and 75% response with 12.60 shoots per nodal segment. Callus induction from leaf lamina was most effective (72%) on MS medium containing 2.0 mg/l 2,4-D, leading to rapid and healthy callus formation. Indirect shoot organogenesis was optimized with 3.0 mg/l BAP, providing an 85% response with 25.80 shoots per callus. The combination of 0.5 mg/l NAA with 3.5 mg/l BAP produced longest shoots, measuring 5.12 cm. This combination of NAA and BAP also promoted the multiplication of direct and indirect shoots, resulting in 100% explant response with an average of 33.60 shoots per culture. In addition, supplementation with 1.5 mg/l 2-iP produced the longest shoots, measuring 7.58 cm. The shoot number reached a maximum of 37.40 during 4th subculture, and then slightly declined to 35.20 in the 5th subculture. This response was further improved by adding 10% coconut water, leading to an average of 38.20 shoots per culture, measuring 7.06 cm in length, with a maximum of 9-11 aerial roots. Sucrose levels below or above 3% led to poor shoot development. Rooting was observed in 93.33% of cultures using ½MS medium with 1.0 mg/l IBA, resulting an average of 17.80 roots per culture, and the combination of 1.0 mg/l IBA and 0.5 mg/l NAA produced the longest roots at 6.18 cm. Acclimatization in a garden soil-compost-coal pieces-moss mix (1: 1: 1: 1) led to 96.67% seedling survival, indicated by new root and leaf growth.

Introduction

Philodendron Schott ranks as the 2nd most diverse genus within the Araceae family, after *Anthurium* (Canal et al. 2018). There are 621 accepted species of the genus *Philodendron*,

*Author for correspondence: <raihan1792@gmail.com>.

which are indigenous to tropical and southern subtropical parts of the Americas and the West Indies (Plants of the World Online 2024). *Philodendron* cv. Birkin is quite a new plant that is currently trending as a houseplant owing to its highly attractive, oval-shaped, medium-sized, deep-green leaves that are richly striped with creamy white or pale yellow extending from the midrib to the margin, which gives the plant a distinctive and elegant appearance (Akramian et al. 2024, Mongkolsawat et al. 2023). This compact, upright plant thrives in bright, indirect light and humid environments, making it ideal for indoor decoration. Beyond aesthetics, *Philodendron* 'Birkin' contributes to improved indoor air quality and offers psychological benefits by promoting a calming indoor atmosphere (Oboni and Hossain 2025).

The ornamental industry is currently facing a scarcity of planting materials because it relies on traditional propagation methods, causing plant prices to rise. Commercial propagation of *Philodendron* 'Birkin' is primarily done through traditional methods such as stem cuttings, division, air layering, and seeds (Christensen 2023). These techniques are easy and effective but often hindered by slow growth, short internodes, and inconsistent leaf variegation in offspring (Klanrit et al. 2023). This difficulty has led to a shortage of high-quality planting materials and a subsequent rise in market prices. To meet the increasing demand and ensure uniformity in variegation, *in vitro* propagation is emerging as a promising alternative. It enables mass production of genetically consistent plants within a shorter timeframe, ensuring commercial viability.

Despite the success of *in vitro* techniques in other *Philodendron* species, limited research exists for *Philodendron* 'Birkin'. Only two published studies have investigated its tissue culture propagation, both emphasizing shoot tip-based direct regeneration without addressing indirect regeneration (Akramian et al. 2024, Mongkolsawat et al. 2023). Therefore, the present study aims to establish an efficient and reproducible *in vitro* regeneration protocol for *Philodendron* cv. 'Birkin' through both direct and indirect organogenesis, using shoot tips, leaf lamina, and stem nodal segments as explants to support large-scale production and meet market demand.

Materials and Methods

In this study, young, disease-free leaf lamina (1.5-2 cm^2), stem nodal segments (2-3 cm) and healthy shoot tips (1.5-2 cm) from a potted *Philodendron* cv. 'Birkin' were used as explants and surface-sterilized using the method described by Raju et al. (2022). For direct shoot induction, shoot tips and stem nodal segments were individually inoculated into culture bottles containing MS medium with different concentrations (0-3.5 mg/l) of BAP. Meanwhile, leaf lamina were individually inoculated onto MS medium with various concentrations of 2,4-D (0-4.0 mg/l) for callus induction. Healthy calli were excised into small pieces (1.5-2 cm^2) and transferred to a gelled MS medium containing BAP (0-4.0 mg/l), either alone or in combination with NAA (0.5 mg/l), for indirect shoot morphogenesis.

Directly and indirectly induced shoots were aseptically removed from the culture vessels, segmented, and re-cultured on freshly prepared media containing varying concentrations and combinations of cytokinins (BAP, Kn, TDZ and 2-iP) and auxin (NAA) to promote shoot development and further multiplication. This study also investigated the effect of consecutive subculture cycles on shoot multiplication by culturing newly formed shoots on MS medium containing the optimal hormone concentration. For further multiplication of regenerated shoots, the effects of varying concentrations of sucrose (2-4%) and coconut water (5-10%) were also evaluated. Cultures were maintained at 25 ± 2°C under a 16/8 h light/dark photoperiod with 2000-3000 lux light intensity in a growth chamber. An aseptic environment was ensured throughout the entire process.

Following five subcultures, shoots reaching 6-8 cm were aseptically excised and transferred to half-strength MS rooting medium containing varying concentrations and combinations of IBA, NAA and IAA (mg/l). Cultures were maintained under dim light for 3 days to induce rooting and then shifted to standard light conditions. Plantlets with well-developed roots were removed from the culture vessels and acclimatized following the protocol described by Tazmin et al. (2024). After 30 days, acclimatized plantlets were transferred to larger pots containing an optimal potting mixture for further growth. Data were analyzed using one-way ANOVA in SPSS version 16.0, followed by Duncan's Multiple Range Test (DMRT) for post hoc comparison at a significance level of 0.05.

Results and Discussion

The inclusion of cytokinin in the basal medium is crucial for direct shoot induction, as the hormone-free MS medium showed poor response (Table 1). Both shoot tips and nodal segments exhibited the best results at a concentration of 2.5 mg/l BAP. Specifically, shoot tips showed an 86.67% response rate, producing an average of 9.20 ± 1.71 shoots per explant with an average length of 4.82 ± 0.36 cm (Fig. 1a-b). Whereas nodal segments had a 75% response rate, yielding an average of 12.60 ± 1.78 shoots per explant and an average length of 4.48 ± 0.35 cm (Fig. 1c-d). Higher BAP levels reduced shoot induction, indicating an inhibitory effect. Consistent with this study, lower BAP concentrations (0.5-2.0 mg/l) have been found more effective for direct shoot induction in various *Philodendron* species, including *P. billietiae* (Khamrit and Jongrungklang 2024), *P. erubescens* 'Pink Princess' (Klanrit et al. 2023), *P. bipinnatifidum* (Alawaadh et al. 2020), and *P. cannifolium* (Han and Park, 2008). However, Han et al. (2004) reported successful shoot formation in *Philodendron* 'wend-imbe' at 5.0 mg/l BAP.

Leaf lamina cultured on hormone-free gelled MS medium did not show any growth, highlighting the necessity of appropriate growth regulators for callus induction (Table 2). However, among the various concentrations of 2,4-D tested, the highest callus induction frequency of 72% was achieved at 2.0 mg/l. This concentration also resulted in the fastest initiation time (22.2 ± 2.20 days) and exhibited excellent callus formation, characterized

Table 1. Effects of varying concentrations of BAP in MS medium on direct shoot induction from shoot tip and stem nodal segments of *Philodendron* 'Birkin'.

BAP (mg/l)	% of responded explant	No. of shoot per explant ($\bar{X}$ ± SE*)	No. of leaf per shoot ($\bar{X}$ ± SE*)	Shoot length (cm) ($\bar{X}$ ± SE*)
Shoot tip				
0.0	13.33	1.60^{c} ± 1.03	2.00^{d} ± 1.26	0.86^{d} ± 0.53
1.0	46.67	4.80^{bc} ± 0.66	3.80^{cd} ± 0.37	2.38^{c} ± 0.22
1.5	60.00	6.20^{ab} ± 0.97	4.40^{bc} ± 0.60	3.04^{c} ± 0.24
2.0	80.00	8.00^{ab} ± 1.38	6.20^{ab} ± 0.58	4.14^{ab} ± 0.43
2.5	86.67	9.20^{a} ± 1.71	6.80^{a} ± 0.37	4.82^{a} ± 0.36
3.0	66.67	6.40^{ab} ± 1.03	4.60^{bc} ± 0.60	3.34^{bc} ± 0.29
3.5	53.33	5.60^{ab} ± 0.75	4.20^{bc} ± 0.58	2.84^{c} ± 0.17
Stem nodal segment				
0.0	0.00	0.00	0.00	0.00
1.0	35.00	3.60^{c} ± 0.51	4.00^{c} ± 0.55	2.78^{c} ± 0.25
1.5	55.00	6.00^{bc} ± 0.84	4.80^{bc} ± 0.58	3.32^{bc} ± 0.33
2.0	70.00	8.60^{b} ± 1.60	6.20^{ab} ± 0.37	4.06^{ab} ± 0.46
2.5	75.00	12.60^{a} ± 1.78	7.60^{a} ± 0.60	4.48^{a} ± 0.35
3.0	60.00	9.40^{ab} ± 1.12	5.80^{b} ± 0.58	3.52^{abc} ± 0.30
3.5	45.00	7.00^{bc} ± 1.10	4.20^{c} ± 0.37	3.14^{bc} ± 0.33

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

Table 2. Effects of varying concentrations of 2,4-D in gelrite-gelled MS medium on callus formation from the leaf lamina of *Philodendron* 'Birkin'.

2,4-D mg/l	Days required for callus initiation ($\bar{X}$ ± SE*)	Callus induction frequency (%)	Intensity of callus formation *	Nature of callus
0.0	-	0.00	-	-
1.0	28.40^{ab} ± 3.26	32.00	+	Creamy in color and compact in texture
1.5	25.20^{ab} ± 1.98	56.00	++	
2.0	22.20^{a} ± 2.20	72.00	+++	
2.5	24.60^{ab} ± 3.01	68.00	+++	Greenish in color and friable in texture
3.0	27.00^{ab} ± 3.15	52.00	+	
3.5	27.60^{ab} ± 2.16	40.00	+	Brownish in color and compact in texture
4.0	32.40^{b} ± 2.54	40.00	+	

Callus formation intensity (*): No response (-), Moderate (+), Good (++), Excellent (+++); the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

by a creamy color and compact texture (Table 2, Fig. 1 e-f). Similarly, Haring et al. (2023) reported enhanced callus induction in *Amorphophallus muelleri* petiole segments using 2.0 mg/l 2,4-D. In contrast, Tazmin et al. (2024) and Raju et al. (2022) found that the optimal concentrations of 2,4-D for inducing callus from *Alocasia baginda* 'Silver Dragon' and *A. amazonica* leaf tissue were 3.0 mg/l and 4.0 mg/l, respectively.

This study demonstrated that cytokinin levels significantly influenced shoot induction from callus tissues, with no morphogenetic response occurred in the absence of hormones (Table 3). However, 3.0 mg/l BAP alone produced the best outcome for shoot morphogenesis, with 85% response rate and an average of 25.80 ± 1.85 shoots per callus (Table 3, Fig. 1g-h), while the combination of 3.5 mg/l BAP with 0.5 mg/l NAA significantly improved shoot development by increasing the number of leaves per shoot (9.60 ± 1.36) and shoot length (5.12 ± 0.32 cm) (Table 3, Fig. 1i). The findings of Tazmin et al. (2024) on *A. baginda* 'Silver Dragon' align with the present study, as they reported successful indirect shoot organogenesis on MS medium with 3.5 mg/l BAP. Conversely, studies by Heedchim et al. (2022) in *Caladium bicolor* and Bhavana et al. (2018) in *Anthurium andreanum* demonstrated that lower concentrations of BAP (0.1-0.5 mg/l) significantly enhanced the number of shoots produced from callus. On the other hand, Raju et al. (2022) in *A. amazonica*, Jazib et al. (2019) in *Dracaena fragrans* and Thokchom and Maitra (2017) in *Anthurium andreanum* found that BAP in conjugation with NAA positively influenced indirect shoot organogenesis, which was also consistent with the present findings.

Directly and indirectly induced shoots showed the highest multiplication rate (100%) with a combination of 3.5 mg/l BAP and 0.5 mg/l NAA, which was significantly more effective than 3.5 mg/l BAP alone. The combination resulted in an average of 33.60 ± 1.17 shoots per culture, with 14.40 ± 0.87 leaves per shoot, an average shoot length of 6.44 ± 0.48 cm and 7-9 aerial roots (Table 4, Fig. 2a). Similarly, Akramian et al. (2024) reported improved shoot multiplication in *Philodendron* 'Birkin' using 3.0 mg/l BAP with auxin (0.5 mg/l IBA). Likewise, Alawaadh et al. (2020) in *P. bipinnatifidum* found that 1.0 mg/l BAP enhanced shoot multiplication, and combining BAP with auxins (IBA or NAA) resulted in more shoots than BAP alone.

The combined effects of BAP with Kn or TDZ failed to produce satisfactory results for shoot multiplication, with all BAP-TDZ combinations inducing callus formation at the level of the nodes (Table 4). Similarly, Alawaadh et al. (2020) reported lower shoot multiplication with Kn and TDZ compared to BAP in *P. bipinnatifidum*. TDZ also showed limited effectiveness in studies by Chen et al. (2012), who noted nodal callus and globule-like structures in TDZ-treated *Philodendron* cultivars. Additionally, 1.5 mg/l 2-iP in this study promoted the highest shoot elongation (7.58 ± 0.45 cm) and leaf formation (15.00 ± 1.34) but was less effective for inducing multiple shoots (Table 4, Fig. 2c). These findings align with those of Alawaadh et al. (2020) in *P. bipinnatifidum*, who reported that Kn and 2-iP resulted in lower shoot multiplication than BAP but produced taller and heavier shoots compared to BAP, Kn, and TDZ treatments.

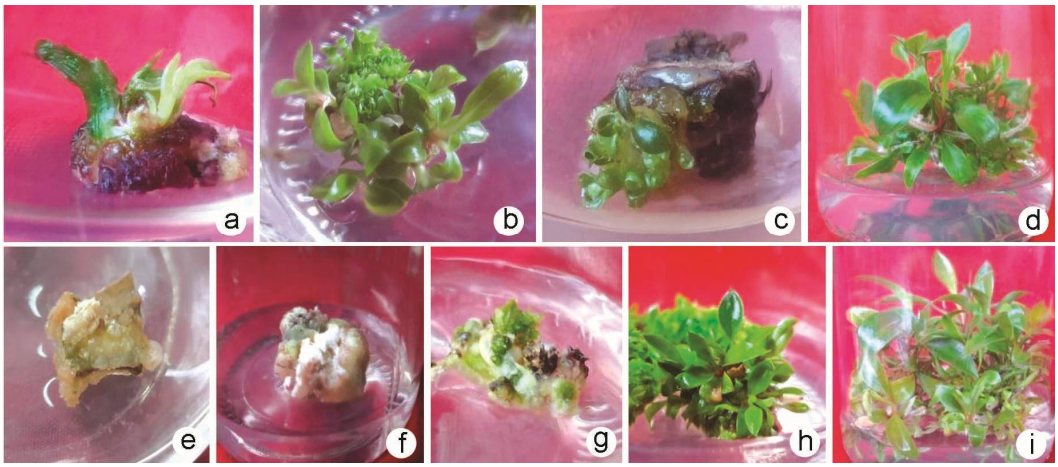

Fig. 1 (a-i). *In vitro* direct and indirect shoot induction from various explants of *Philodendron* cv. 'Birkin': (a-d) Multiple direct shoot inductions from (a-b) Shoot tip and (c-d) Stem nodal segment on MS + 2.5 mg/l BAP (e-f), (e) Callus induction from leaf tissue and, (f) Multiplication of leaf tissue-derived callus on MS + 2.0 mg/l 2,4-D (g-i) Multiple shoot induction from callus after, (g) 15 days and, (h) 30 days on MS + 3.0 mg/l BAP, (i) 30 days on MS + 3.5 mg/l BAP + 0.5 mg/l NAA.

Table 3. Effects of varying concentrations of BAPin MS medium alone or in conjunction with NAA on indirect shoot induction from leaf lamina-derived callus of *Philodendron* 'Birkin'.

Growth regulators (mg/l)		% of responded explant	No. of shoot per callus ($\bar{X}$ ± SE*)	No. of leaf per shoot ($\bar{X}$ ± SE*)	Shoot length (cm) ($\bar{X}$ ± SE*)
0.0		0.00	0.00	0.00	0.00
BAP					
1.0		25.00	$6.00^{d} \pm 1.22$	$3.40^{b} \pm 0.51$	$1.96^{b} \pm 0.19$
1.5		40.00	$9.40^{cd} \pm 1.66$	$3.80^{b} \pm 0.37$	$2.36^{ab} \pm 0.25$
2.0		55.00	$12.80^{bc} \pm 1.62$	$4.60^{ab} \pm 0.81$	$2.70^{ab} \pm 0.38$
2.5		70.00	$18.40^{b} \pm 1.96$	$6.40^{ab} \pm 1.17$	$3.02^{ab} \pm 0.48$
3.0		85.00	$25.80^{a} \pm 1.85$	$7.80^{a} \pm 1.07$	$3.44^{a} \pm 0.46$
3.5		65.00	$14.00^{bc} \pm 2.98$	$7.20^{a} \pm 1.62$	$3.20^{ab} \pm 0.53$
4.0		50.00	$8.40^{cd} \pm 1.63$	$6.00^{ab} \pm 0.95$	$3.04^{ab} \pm 0.43$
BAP	NAA				
1.0	0.5	20.00	$3.60^{c} \pm 0.68$	$4.00^{c} \pm 0.84$	$2.30^{d} \pm 0.22$
2.0	0.5	30.00	$5.00^{c} \pm 0.89$	$4.80^{bc} \pm 1.02$	$2.84^{cd} \pm 0.27$
2.5	0.5	40.00	$5.80^{c} \pm 1.43$	$6.80^{abc} \pm 1.02$	$3.26^{bcd} \pm 0.43$
3.0	0.5	55.00	$10.00^{b} \pm 1.58$	$8.00^{ab} \pm 1.05$	$4.38^{ab} \pm 0.54$
3.5	0.5	75.00	$16.20^{a} \pm 1.69$	$9.60^{a} \pm 1.36$	$5.12^{a} \pm 0.32$
4.0	0.5	60.00	$11.40^{b} \pm 1.17$	$6.40^{abc} \pm 0.81$	$3.88^{bc} \pm 0.52$

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

Table 4. Effects of varying concentrations and combinations of growth regulators containing MS medium on the proliferation of directly and indirectly induced shoots of *Philodendron* 'Birkin.

Growth regulators (mg/l)		% of responded explant	No. of shoot per culture ($\bar{X}$ ± SE*)	No. of leaf per shoot ($\bar{X}$ ± SE*)	Shoot length (cm) ($\bar{X}$ ± SE*)	Presence of aerial root (*)
Control		0.00	0.00	0.00	0.00	-
BAP						
1.5		50.00	7.80^{c} ± 1.62	6.60^{bc} ± 1.36	2.80^{b} ± 0.28	++
2.0		50.00	8.00^{c} ± 1.10	6.20^{bc} ± 0.66	2.62^{b} ± 0.33	+
2.5		65.00	14.80^{b} ± 0.97	6.60^{bc} ± 1.12	3.04^{ab} ± 0.07	+
3.0		75.00	21.40^{a} ± 1.29	7.80^{b} ± 0.80	3.46^{ab} ± 0.25	++
3.5		90.00	24.20^{a} ± 1.93	11.60^{a} ± 0.75	3.94^{a} ± 0.67	+++
4.0		60.00	11.40^{bc} ± 1.99	5.40^{bc} ± 0.81	2.52^{b} ± 0.29	-
5.0		45.00	8.80^{c} ± 2.03	4.2^{c} ± 0.49	2.34^{b} ± 0.27	-
BAP	NAA					
2.0	0.5	55.00	8.60^{de} ± 1.96	6.60^{bcd} ± 0.75	3.10^{cd} ± 0.26	++
2.5	0.5	70.00	15.20^{c} ± 0.97	7.00^{bc} ± 0.89	3.86^{bc} ± 0.27	+
3.0	0.5	85.00	23.40^{b} ± 1.17	8.60^{b} ± 0.98	4.62^{b} ± 0.45	+
3.5	0.5	100.00	33.60^{a} ± 1.17	14.40^{a} ± 0.87	6.44^{a} ± 0.48	++++
4.0	0.5	50.00	11.80^{cd} ± 1.62	5.80^{cde} ± 0.66	2.92^{cd} ± 0.39	+
4.5	0.5	40.00	6.80^{e} ± 0.86	4.40^{de} ± 0.68	2.38^{d} ± 0.20	-
5.0	0.5	35.00	6.60^{e} ± 0.93	3.60^{e} ± 0.24	2.26^{d} ± 0.29	-
BAP	Kn					
2.0	0.5	65.00	10.40^{ab} ± 1.81	5.80^{b} ± 1.20	2.96^{a} ± 0.36	+
2.5	0.5	75.00	16.20^{a} ± 0.97	9.20^{a} ± 1.32	3.52^{a} ± 0.37	+++
3.0	0.5	60.00	14.80^{a} ± 1.02	8.40^{ab} ± 1.81	3.30^{a} ± 0.43	+
3.5	0.5	55.00	11.60^{ab} ± 3.25	6.60^{b} ± 0.93	3.10^{a} ± 0.47	+
4.0	0.5	40.00	8.40^{b} ± 1.75	6.40^{b} ± 0.87	2.30^{a} ± 0.40	-
BAP	TDZ					
-	1.0	0.00	0.00	0.00	0.00	-
-	2.0	0.00	0.00	0.00	0.00	-
2.5	0.5	45.00	6.40^{bc} ± 1.50	5.80^{ab} ± 0.73	2.80^{a} ± 0.36	++
2.5	1.0	65.00	13.60^{a} ± 0.87	8.60^{a} ± 0.93	3.46^{a} ± 0.31	+
2.5	1.5	35.00	4.80^{c} ± 0.86	6.00^{ab} ± 1.30	2.66^{a} ± 0.40	++
3.0	0.5	45.00	4.00^{c} ± 0.84	3.20^{bc} ± 0.80	2.56^{a} ± 0.32	+
3.0	1.0	55.00	8.20^{b} ± 1.02	4.80^{bc} ± 0.97	3.04^{a} ± 0.32	-
3.0	1.5	35.00	3.60^{c} ± 0.68	2.40^{c} ± 0.75	2.42^{a} ± 0.28	+
2-iP						
0.5		60.00	4.20^{b} ± 0.73	7.40^{b} ± 1.40	4.90^{b} ± 0.45	+
1.0		75.00	6.40^{b} ± 0.68	12.60^{a} ± 1.33	6.50^{ab} ± 0.54	++
1.5		85.00	9.60^{a} ± 1.03	15.00^{a} ± 1.34	7.58^{a} ± 0.45	++
2.0		65.00	6.80^{ab} ± 1.20	12.60^{a} ± 1.72	6.56^{ab} ± 0.75	-

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; presence of aerial root (*): No root (-); 1-3 root (+); 3-5 root (++); 5-7root (+++); 7-9 root (++++); the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

New shoots from the multiplication stage were cultured on an optimal combination of growth regulators, 3.5 mg/l BAP and 0.5 mg/l NAA, for periodic sub-culturing. An increase in shoot number was noted from the 2nd to 4th subcultures, followed by a subsequent decline in the next subculture (Table 5). The 4th subculture cycle recorded the highest shoot number and length per culture, with values of 37.40 ± 1.63 and 7.26a ± 0.46 cm, respectively (Fig. 2b). These findings align with Tazmin et al. (2024) for *A. baginda* 'Silver Dragon' and Kakuei and Salehi (2015) for *Dracaena sanderiana*, who also observed a significant effect of sub-culturing on multiplication potential after the 3rd cycle. In contrast, Mariani et al. (2011) reported maximum shoot proliferation in *Aglaonema* at the 5th subculture.

Table 5. Effects of consecutive sub-cultures on shoot multiplication of *Philodendron* 'Birkin' in gelrite-gelled MS media containing 3.5 mg/l BAP and 0.5 mg/l NAA.

Sub-culture cycle	No. of shoot per culture ($\bar{X}$ ± SE*)	No. of leaf per shoot ($\bar{X}$ ± SE*)	Shoot length (cm) ($\bar{X}$ ± SE*)
1st	33.60^{b} ± 1.17	14.40^{a} ± 0.87	6.44^{a} ± 0.48
2nd	34.40^{b} ± 0.93	14.20^{a} ± 1.53	6.58^{a} ± 0.37
3rd	35.80^{ab} ± 1.11	14.80^{a} ± 1.77	6.82^{a} ± 0.41
4th	37.40^{a} ± 1.63	15.20^{a} ± 1.66	7.26^{a} ± 0.46
5th	35.20^{b} ± 1.02	13.40^{a} ± 1.03	6.32^{a} ± 0.53

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; in each sub-cultural cycle, 7-9 aerial roots have been found per shoot; the different letters within a column are significantly different at $p \leq 0.05$ based on DMRT analysis.

This study also examined the impact of coconut water (CW) and sucrose, in combination with 3.5 mg/l BAP and 0.5 mg/l NAA on shoot multiplication. Supplementation with 10% CW and 3% sucrose resulted in the highest shoot production (38.20 ± 3.47), enhanced shoot elongation (7.06 ± 0.59 cm), and the highest number of aerial roots (9-11) (Table 6; Fig. 2d). Similar findings were reported by Raju et al. (2022) for *A. amazonica*, where 10% CW and 3% sucrose promoted satisfactory shoot growth and development. Whereas Jazib et al. (2019) reported that 10% CW with 4% sucrose promoted shoot growth in *D. fragrans* but Tazmin et al. (2024) found higher levels of CW (>5%) and sucrose (>3%) inhibited shoot development in *A. baginda* 'Silver Dragon'.

Newly grown shoots developed roots best in a gelled ½MS medium with 1.0 mg/l IBA, resulting in the highest rooting rate of 93.33% and an average of 17.80 ± 1.20 roots per culture (Table 7, Fig. 2e), while IAA and NAA were less effective individually. However, combining 1.0 mg/l IBA with 0.5 mg/l NAA significantly increased root length to 6.18 ± 0.55 cm (Table 7, Fig. 2f). Consistent with these findings, Akramian et al. (2024) and Chen et al. (2012) reported maximum rooting in *Philodendron* 'Birkin' and *Philodendron* 'Imperial Red,' respectively, on ½MS medium with 1.0 mg/l IBA. Similarly, several studies, including Tazmin et al. (2024) for *A. baginda* 'Silver Dragon', Klanrit et al.

(2023) for *P. erubescens*, Ali et al. (2022) for *C. bicolor* and Jazib et al. (2019) for *D. fragrans*, found optimal rooting using IBA alone (0.2-3.0 mg/l). Additionally, Swaranjali and Abhishek (2023) and Barakat and Gaber (2018) reported that 0.5 mg/l IBA with 0.25 mg/l NAA was most effective for rooting in *Aglaonema commutatum*.

Table 6. Effects of coconut water (CW) and sucrose on shoot multiplication of *Philodendron* 'Birkin' in gelrite-gelled MS media containing 3.5 mg/l BAP and 0.5 mg/l NAA.

Media composition	No. of shoot per culture ($\bar{X}$ ± SE*)	No. of leaf per shoot ($\bar{X}$ ± SE*)	Shoot length (cm) ($\bar{X}$ ± SE*)	Presence of aerial root (*)
CW (%)				
0.0	33.60^{b} ± 1.17	14.40^{a} ± 0.87	6.44^{a} ± 0.48	++++
5.0	34.80^{b} ± 2.37	14.00^{a} ± 0.84	6.46^{a} ± 0.50	++++
10.0	38.20^{a} ± 3.47	14.80^{a} ± 1.24	7.06^{a} ± 0.59	+++++
Sucrose (%)				
2.0	18.00^{c} ± 1.05	7.20^{b} ± 1.28	3.26^{b} ± 0.44	++
3.0	33.60^{a} ± 1.17	14.40^{a} ± 0.87	6.44^{a} ± 0.48	++++
4.0	25.60^{b} ± 2.93	8.80^{b} ± 0.37	4.36^{b} ± 0.60	+++

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; presence of aerial root (*): 3-5 root (++), 5-7 root (+++), 7-9 root (++++), 9-11 root (+++++); the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

Table 7. Effects of different auxins in gelrite-gelled ½MS medium on rooting of in vitro grown shoots of *Philodendron* 'Birkin'.

Conc. of Auxins (mg/l)			Days required for root initiation ($\bar{X}$ ± SE*)	Rooting (%)	Number of roots/culture ($\bar{X}$ ± SE*)	Root length ($\bar{X}$ ± SE*)
IBA	IAA	NAA				
1.0	-	-	14.40^{a} ± 1.47	93.33	17.80^{a} ± 1.20	3.40^{b} ± 0.26
2.0	-	-	17.80^{ab} ± 1.20	76.67	10.20^{b} ± 1.39	2.64^{b} ± 0.33
-	1.0	-	24.40^{cd} ± 1.29	70.00	4.80^{c} ± 0.37	3.02^{b} ± 0.36
-	2.0	-	26.00^{cd} ± 2.07	56.67	3.60^{c} ± 0.51	2.56^{b} ± 0.37
-	-	1.0	27.60^{de} ± 1.86	50.00	4.00^{c} ± 1.05	2.82^{b} ± 0.44
-	-	2.0	32.20^{e} ± 1.80	36.67	2.80^{c} ± 0.58	2.16^{b} ± 0.33
1.0	-	0.5	20.60^{bc} ± 2.16	80.00	9.40^{b} ± 0.81	6.18^{a} ± 0.55
2.0	-	0.5	25.40^{cd} ± 1.96	53.33	5.00^{c} ± 0.71	3.16^{b} ± 0.50

Values are means (± SE* = Standard Error) obtained from 5-independent treatments; the different letters within each column represent statistically significant differences at $p \leq 0.05$ according to DMRT analysis.

In vitro regenerated plantlets (Fig. 2g) acclimatized best in a ratio of 1 : 1 : 1 : 1 mixture of garden soil, compost, coal pieces and moss, showing the highest survival rate (96.67%) (Table 8, Fig. 2h). Similarly, Tazmin et al. (2024) reported 100% survival of *A. baginda* 'Silver Dragon' using a mix of compost, coco husk chips, coal, and moss (2 : 1 : 1 : 1). Similarly, Klanrit et al. (2023) and Alawaadh et al. (2020) observed 90-100% survival

in *P. erubescens* 'Pink Princess' and *P. bipinnatifidum* grown in peat moss. Other studies (Ali et al. 2022 in C. bicolor and Abdulhafiz et al. 2020 in A. longiloba) also highlighted peat moss as an ideal substrate for acclimatizing aroids due to its water retention. After 30 days, the plants were transplanted into larger pots and successfully produced new leaves (Fig. 2i). Well-established plants exhibit full growth and variegation under moderate light and water stress (Fig. 2j).

Fig. 2 (a-j). *In vitro* multiplication, regeneration, and acclimatization of directly and indirectly induced shoots of *Philodendron* cv. 'Birkin' (a-b) Multiplication on MS + 3.5 mg/l BAP + 0.5 mg/l NAA, after 30 days of (a) 1st and, (b) 4th subcultures, (c) Multiplication on MS + 1.5 mg/l 2-iP, after 30 days of culture, **(**d) Rapid multiplication on MS + 3.5 mg/l BAP + 0.5 mg/l NAA + 3% sucrose + 10% CW (e-f) Rooting on ½MS with, (e) 1.0 mg/l IBA and, (f) 1.0 mg/l IBA + 0.5 mg/l NAA, (g) Complete plantlets, (h) Acclimatization in a potting mixture combining garden soil, compost, coal pieces and moss (1 : 1 : 1 : 1), (i) One-month-old acclimatized plant with leaves starting to show variegation (arrows), (j) Two-month-old plant in larger pot, showing deep green leaves with creamy white stripes.

Table 8. The effects of different potting mixtures on the acclimatization of *Philodendron* 'Birkin' plantlets grown *in vitro*.

Potting mixtures (PM)	Mixture ratio	Survival (%)
Garden soil + Compost + Sand	2 : 1 : 1	66.67
Garden soil + Compost + Coco husk chips	2 : 1 : 1	80.00
Garden soil + Compost + Coal pieces + Moss	1 : 1 : 1 : 1	96.67

For acclimatization, 30 rooted shoots were transplanted into each potting mixture, and data were recorded after 6-weeks of culture in the potting mixtures.

This study highlights the efficiency of both direct and indirect *in vitro* propagation protocols as a viable and efficient method for rapidly regenerating *Philodendron* cv. 'Birkin' at a significantly faster rate compared to any traditional propagation method. The success of this protocol not only provides a reliable solution for the large-scale production of this popular ornamental species but also serves as a foundation for applying similar techniques to other economically and aesthetically important leafy-ornamental plants.

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