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RESEARCH ARTICLE

Axillary shoots: Initiation to acclimatization on micropropagation of *Philodendron* 'Super Atom'

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Abstract

Philodendron 'Super Atom' is among Indonesia's most favoured ornamental foliage plants, yet traditional propagation methods pose challenges. Establishing an *in vitro* propagation protocol for this plant was crucial to sustainably produce high-quality planting materials to meet consumer demand. All experiments were conducted using a completely randomized design (CRD) with four replicates. High axillary shoots with regeneration issues were used as explant sources, initiated on Murashige and Skoog (MS) medium containing 1 mg L^{-1} thidiazuron (TDZ) and 3 mg L^{-1} N6-benzylaminopurine (BAP). A total of 4.8 shoots per explant were regenerated on MS medium supplemented with 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA. A slight enhancement in axillary shoot proliferation was noted on MS medium with 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} TDZ, and 0.025 mg L^{-1} α-naphthalene acetic acid (NAA), yielding 6.7 shoots $explant^{-1}$, and 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} indole-3-butyric acid (IBA), yielding 6.9 shoots $explant^{-1}$. A significant improvement was achieved on MS medium fortified with 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} indole-3-acetic acid (IAA), resulting in 13.6 shoots $explant^{-1}$. Rooted shoots were readily observed in the proliferation medium. A 100% survival rate of plantlets was successfully acclimatized using a mixture of burned rice husks, hyacinth organic manure, and cocopeat (1:2:1, v/v/v), as well as burned rice husks and hyacinth organic manure (1:1, v/v). The findings of this study can serve as a model for developing and establishing new *in vitro* mass propagation protocols for other Philodendron species with appropriate modifications.

Keywords: axillary shoot; acclimatization; *in vitro* culture; plant growth regulator; *Philodendron selloum* K. Koch; Lacy tree philodendron.

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1. Introduction

Philodendron bipinnatifidum Schott ex Endl., previously known as *P. selloum* K. Koch or the 'Lacy tree philodendron,' is a self-heading ornamental plant species that can grow to heights of 4 – 5 m, featuring deeply cut green to dark green leaves (Alawaadh et al., 2020; Alwahibi et al., 2022). *P. bipinnatifidum* is a popular decorative plant cultivated both indoors and outdoors worldwide, exhibiting a variety of growth habits, including epiphytic, hemi-epiphytic, and terrestrial, and holds significant economic value (Surve, 2022; Dewir et al., 2023). Numerous hybrids exist with leaves in shades of green, red, yellow, and orange (Dewir et al., 2023). *The P. bipinnatifidum* 'Atom' is a compact philodendron that produces glossy green leaves with ruffled edges. Internationally, the potted 'Atom' is priced between US$16.00 and 374.35. In Indonesia, it is also known as 'Super Atom' and is sold for IDR 600,000 to 3,000,000 pot^{-1}. Although no quantitative data on annual plant demand has been published, the active agribusiness sector and relatively stable plant prices each year underscore the need to support the sustainable production of quality seedlings annually.

The Lacy tree philodendron is typically propagated through seeds and cuttings (Alawaadh et al., 2020). While growers often maintain mature plants for seed production, this approach is labour-intensive, and the seeds have a relatively short lifespan. Cut-

tings are not favoured for self-heading philodendrons due to the species' slow growth, short internodes, and large stems and leaves. Consequently, in vitro propagation methods are extensively employed to enable the rapid production of large quantities of high-quality plant materials. The superiority of *in vitro* propagation over traditional methods has been confirmed by Alawaadh et al. (2020), Seliem et al. (2021) Alwahibi et al. (2022), Dewir et al. (2023). This method also meets the demand for plants in both domestic and export markets.

Several *in vitro* propagation protocols for *P. bipinnatifidum* have been documented. Shoot tips were cultured on Murashige and Skoog (MS; Murashige & Skoog, 1962) medium containing 1 mg L^{-1} BAP and 0.5 mg L^{-1} IBA to produce high axillary shoots during initiation and multiplication. MS medium supplemented with 1 - 2 mg L^{-1} was used for rooting, followed by removing the medium from roots, dipping them in a fungicide solution, culturing them in a peat moss and perlite mixture (1:1), and regularly watering them with a half-strength MS basal salts solution (Alawaadh et al., 2020). Nodal explants cultured on MS medium fortified with 4 mg L^{-1} BAP for initiation, MS medium augmented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} IAA for multiplication, and MS medium with 1 mg L^{-1} IBA for rooting were established by Surve (2022). The highest number of shoots of *Philodendron erubescent* 'Pink Princes' on MS medium containing 0.5 mg L^{-1} N6-benzyladenine (BA) was consistently observed at 60.44 shoots nodal malondialdehyde^{-1} (MDA) content (1.91 nmol g^{-1} FW) and phenolic content (9.51 mg GAE g^{-1} FW) (Chiewchan et al., 2023). MS medium supplemented with 1.5 mg L^{-1} BA achieved the highest shoot induction, resulting in more shoots and leaves shoot^{-1} than any other treatment. The optimal medium for root induction was MS medium supplemented with 0.5 mg L^{-1} NAA (Khamrit & Jongrungklang, 2024). The best shoots, up to three explant^{-1} of *Philodendron billietiae*, were established on MS medium supplemented with 3 mg L^{-1} BA, while MS medium with 2 mg L^{-1} NAA produced 6.1 roots explant^{-1} (Maikaeo et al., 2024). Liquid MS medium containing 1.0 mg L^{-1} BAP resulted in 21 shoots explant^{-1} of *P. erubescens* 'Pink Princess'. MS medium supplemented with 0.5 mg L^{-1} IAA was suitable for root formation with three roots per explant, and a substrate mix of peat moss, perlite, and vermiculite (2:1:1, v/v/v) ensured a 100% survival rate (Vy et al., 2025). To date, no new in vitro propagation research has been published on similar Philodendron species.

The present research revealed new findings in the *in vitro* propagation of Philodendron 'Super Atom' in different steps of the *in vitro* culture process, starting from initiation, proliferation, and acclimatization, which was the main objective of this study. The findings are discussed in detail in this paper.

2. Methodology

2.1. Plant materials, preparation, sterilization, explant source preparation, and incubation

The donor plants utilized in this study were *Philodendron* "Super Atom" (Figure 1A), sourced from Babadan Nursery in Babadan, Beji village, Ungaran Timur Subdistrict, Semarang Regency. These plants were cultivated in plastic pots measuring 15 cm in both diameter and height, filled with a mixture of burned rice husks, raw husks, bamboo moss, and organic manure in equal proportions (1:1:1:1, v/v/v/v). The plants received regular watering tailored to their specific needs.

In vitro propagation protocol for *Philodendron* 'Super Atom' was a process started from culturing shoot tips on MS medium containing 1 mg L^{-1} TDZ, 3 mg L^{-1} BAP, and 350 mg L^{-1} myoinositol to produce high number of initial axillary shoots, followed by subculturing initial axillary shoots on optimal regeneration medium, shoot rooting and acclimatization of plantlets to *ex vitro* environment. To establish the protocol, paying more attention, in fact, was focused regeneration of initial axillary shoot to produce normal shoots as a critical problem, involving 4 experiments. While rooting shoots to produced well performances of plantlets and acclimatization of them were easily carried out with maximal results.

Shoot tips approximately 2.5 cm long, each with three to four leaves, were cut using a tissue culture blade. The leaves were then carefully removed, leaving the young shoot exposed. These shoots were subsequently prepared for sterilization. Initially, the shoots were pretreated by immersing them in a solution containing 100 mg L^{-1} Ribavirin (200 mg) (Farmasi Kesehatan Prima, Ltd, Jakarta-Indonesia), 100 mg L^{-1} Rifampicin (500 mg) (Armoxindo Farma, Ltd, Cianjur, West Java-Indonesia), 100 mg L^{-1} Ceftriaxone (1 g) (Hexpharm Jaya, Ltd, Bekasi, West Java-Indonesia), and 500 mg L^{-1} systemic fungicide (Benomyl 50) (Dupont Indonesia, Ltd, South Jakarta, Jakarta-Indonesia) for 3 hours. The shoots were then rinsed with clean water to remove any residual fungicide, ribavirin, rifampicin, and ceftriaxone. Following this, the shoots were soaked in a 1% sunlight solution for 20 minutes with manual shaking and then rinsed with clean water until the

sunlight solution was completely washed away. The explants were sterilized by immersing them in 0.025% mercury chloride ($HgCl_2$; 271.52 g mol^{-1}) (LabMart Indonesia, Ltd, West Jakarta, Jakarta-Indonesia) for 10 minutes, followed by a rinse with sterile distilled water (SDW) for 3 minutes. They were then soaked in 0.005% $HgCl_2$ for 20 minutes and rinsed 5-6 times with SDW for 3 minutes each. The young leaves on the sterile shoot tips were carefully removed using a tissue culture blade. Finally, the sterile shoot tips were trimmed to reduce the explant size to approximately 0.5 mm in length and 1.0 cm in diameter and then cultured on the initiation medium (Figure 1B).

The prepared shoot tips were cultured on Murashige and Skoog (MS) medium (Phytotech Labs, Lenexa, KS, United States), which contained 1 mg L^{-1} TDZ (Phytotech Labs, Lenexa, KS, United States), 3 mg L^{-1} BAP (Phytotech Labs, Lenexa, KS, United States), 350 mg L^{-1} myoinositol (Phytotech Labs, Lenexa, KS, United States), and 1.8 g L^{-1} gelrite (MB Cellular, Inc; New York, United States) to regenerate early initial axillary shoots. After approximately 1.5 months of incubation, the shoot tips with early initial shoots (Figure 1C) were sliced longitudinally into four parts of nearly equal size (Figure 1D) and then periodically subcultured in a similar medium. This process was continued to multiply the number of explant sources until a sufficient quantity was available for the next experiment, given the limited number of donor plants.

All cultures were incubated in an incubation culture room for a 16-h photoperiod under a cool fluorescent lamp with 13 µmol m^{-2} s^{-1} light intensity at 24 ± 1 °C and 70% relative humidity.

2.2. Initial axillary shoot regeneration of *Philodendron* 'Super Atom' on different initiation culture media

The explants prepared earlier were utilized in the experiment, with MS medium serving as the base. Various combinations and concentrations of plant growth regulators (PGR) were applied: 0.3 mg L^{-1} BAP and 0.01 mg L^{-1} IAA (PCC-1), 0.25 mg L^{-1} BAP and 0.01 mg L^{-1} IAA (PCC-2), 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} IAA (PCC-3), 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA (PCC-4), 0.25 mg L^{-1} BAP and 0.005 mg L^{-1} IAA (PCC-5), and 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA (PCC-6). Each medium was supplemented with 30 g L^{-1} sucrose and 1.8 g L^{-1} gelrite, with the pH adjusted to 5.8. The experiment followed a completely randomized design (CRD), with four replicates per treatment. Each replicate was represented by three jam jars, each containing 5-6 cultured explants. All initial shoots subcultured in the experiment served as explant sources for the subsequent experiment.

2.3. Axillary shoot regeneration and proliferation of *Philodendron* 'Super Atom' on new combinations and concentrations of PGR

Explant samples of nearly identical size to those from the previous experiment were utilized. The base medium employed was MS medium. New combinations and concentrations of PGRs (BAP, TDZ, IAA, and NAA; NCC) were prepared as follows: NCC-1 consisted of 0.5 mg L^{-1} BAP and 0.25 mg L^{-1} TDZ; NCC-2 included 0.5 mg L^{-1} BAP, 0.25 mg L^{-1} TDZ, and 0.025 mg L^{-1} IAA; NCC-3 comprised 0.5 mg L^{-1} BAP, 0.25 mg L^{-1} TDZ, and 0.025 mg L^{-1} NAA; NCC-4 contained 0.5 mg L^{-1} BAP and 0.5 mg L^{-1} TDZ; NCC-5 was identical to NCC-3 with 0.5 mg L^{-1} BAP, 0.25 mg L^{-1} TDZ, and 0.025 mg L^{-1} NAA; NCC-6 included 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} TDZ, and 0.025 mg L^{-1} NAA. All media were supplemented with 30 g L^{-1} sucrose and 1.8 g L^{-1} gelrite, with the pH adjusted to 5.8. The experiment was organized in a completely randomized design (CRD) with four replicates. Each treatment involved three jars, with each jar containing 5-6 cultured explants.

2.4. Axillary shoot regeneration and proliferation of *Philodendron* 'Super Atom' on new and different proliferation media

Explants of nearly the same size as those from the previous experiment were utilized. The base medium employed was MS medium. New combinations and concentrations of PGR for the new proliferation media (NPM) were prepared as follows: NPM-1, 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} NAA; NPM-2, 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IBA; NPM-3, 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA; NPM-4, 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} NAA; NPM-5, 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} IBA; and NPM-6, 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} IAA. All media were supplemented with 30 g L^{-1} sucrose and 1.8 g L^{-1} gelrite, with the pH adjusted to 5.8. Each treatment had four replicates, with each replicate represented by four replications. Each treatment consisted of three jars, and each jar contained 5 - 6 cultured explants. The experiment was conducted twice to verify the effect of the new proliferation media on shoot proliferation.

2.5. Plantlet preparation

During the proliferation stage, most regenerated axillary shoots developed roots on the proliferation medium (Figure 1H). A single regenerated shoot can produce 1 - 4 roots of varying lengths. To enhance plantlet performance, the rooted shoots

were harvested, trimmed to nearly uniform sizes and performances, and then subcultured on half-strength MS medium, free of PGR, with 1% activated charcoal (AC) (Figures 1I and 1J). The shoot cultures were incubated for 30 days. New plantlets, each with 1 - 3 new roots, were harvested, immersed in clean water (Figure 1K), treated with a 1% pesticide solution for approximately 3 minutes, and then planted in different acclimatization media.

2.6. Acclimatization of *Philodendron* 'Super Atom' plantlets on different acclimatization media

Plantlets, measuring 2 - 4 cm in height and featuring 2 - 4 leaves and 1 - 3 roots, were harvested from the previous experiment to serve as explant sources. The acclimatization media (AM) tested in this stage included: AM-1, a mix of burned rice husk (a local product from Semarang, Central Java, Indonesia), hyacinth organic manure (also from Semarang, Central Java, Indonesia), and cocopeat (sourced from Gunung Kidul, Yogyakarta, Indonesia) in a 1:1:1 ratio (v/v/v); AM-2, a combination of burned rice husk, hyacinth organic manure, and cocopeat in a 1:2:1 ratio (v/v/v); AM-3, a blend of burned rice husk and cocopeat in a 1:1 ratio (v/v); AM-4, a mixture of burned rice husk and hyacinth organic manure in a 1:1 ratio (v/v); AM-5, a combination of burned rice husk and hyacinth organic manure in a 2:1 ratio (v/v); AM-6, a mix of burned rice husk and hyacinth organic manure in a 1:2 ratio (v/v); and AM-7, a blend of burned rice husk and sheep organic manure in a 1:1 ratio (v/v). The experiment was organized in a completely randomized design (CRD) with four replicates. Each treatment involved a plastic tray containing approximately 25 plantlets.

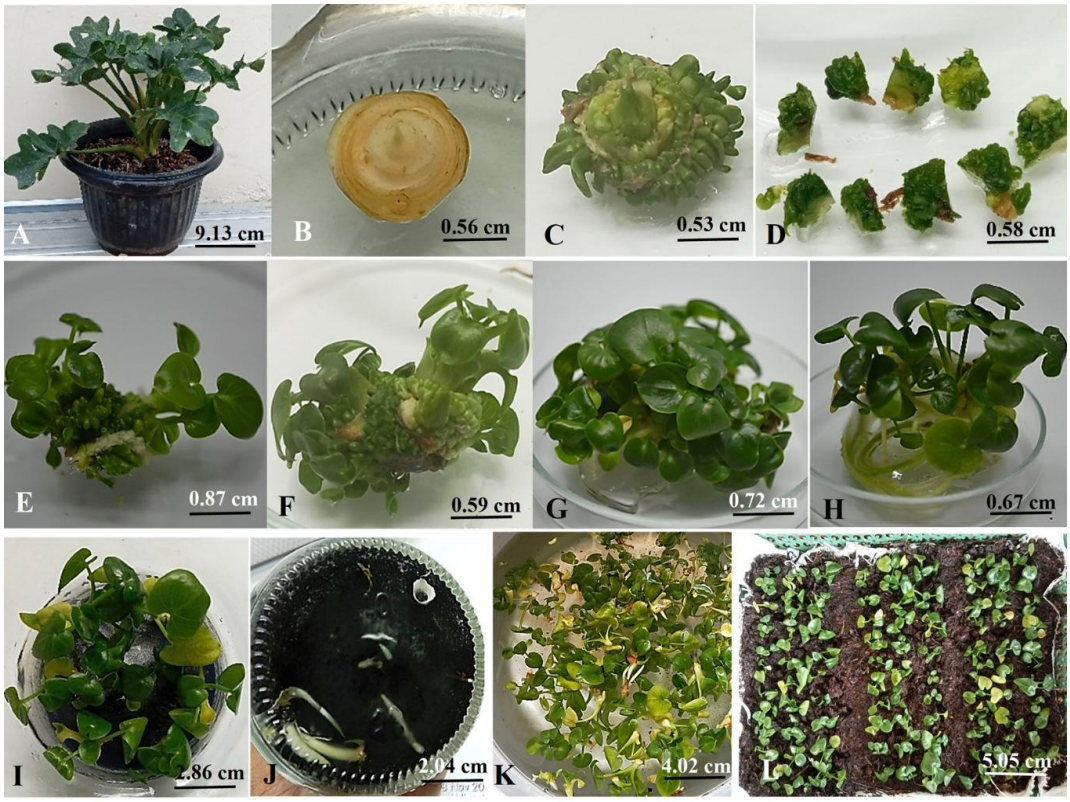

Figure 1. Axillary shoots: *In vitro* propagation of *Philodendron* 'Super Atom' from explant preparation to plantlet acclimatization. A. Donor plant of *Philodendron* 'Super Atom'. B. Shoot explant in initial culture. C. Initial axillary shoots regenerated from MS medium containing 1 mg L^{-1} TDZ and 3 mg L^{-1} BAP, approximately 1.7 months after culture initiation. D. Explant sources derived from regenerated initial axillary shoots. E. Low regeneration of axillary shoots on MS medium supplemented with 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA, about 1.5 months after subculture of explant on axillary shoot formation. F. Slight improvement in the number of regenerated axillary shoots on MS medium fortified with 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} TDZ, and 0.025 mg L^{-1} NAA, approximately 1.5 months after subculture of explant on initial proliferation. G. A high number of regenerated axillary shoots produced by the explant on MS medium augmented with 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA during the third proliferation phase. H. Regenerated axillary shoots easily rooted on proliferation medium, especially on MS medium containing low BAP and IAA, 2 months after subculture. I. Rooted shoots on half-strength MS medium, PGR-free, containing 1% AC, approximately 30 days after subculture. J. New roots regenerated from harvested shoots during the proliferation stage on half-strength MS medium, PGR-free, 30 days after subculture. K. Newly harvested plantlets with 1-3 roots per shoot were acclimatized. L. Surviving plant performances on a mixture of burned rice husk, hyacinth organic manure, and cocopeat (1:2:1, v/v/v) one month after acclimatization.

The plantlets were carefully removed from the culture vessel, followed by gentle root washing to remove all traces of the growth medium. The roots were then immersed in a 1% fungicide and bactericide solution for about 3 min, air-dried on paper briefly, and subsequently cultured in plastic trays containing the acclimatization media. The trays with the planted plantlets were covered with transparent plastic for 7 days and placed in a greenhouse area with reduced light.

2.7. Experiment Variables

Variables observed in the experiment were (1) number of initial shoots explant^{-1}, (2) percentage of shoot regeneration (%), (3) number of shoots explant^{-1}, (4) Number of leaves shoot^{-1}, (5) shoot height (mm), (6) leaf length (mm), (7) leaf width (mm), (8) percentage of plantlet survivability (%), (9) plant height (mm), (10) number of leaves plant^{-1}, (11) leaf length (mm), (12) leaf width (mm), (13) number of roots plant^{-1}, and (14) root length (mm).

$$\text{Percentage of shoot regeneration} = \frac{\text{Number of regenerated explant}}{\text{Total explant cultured}} \times 100\% \quad (Eq.\ 1)$$

$$\text{Percentage of plantlet survivability} = \frac{\text{Number of survived plantlets}}{\text{Total plantlets acclimatized}} \times 100\% \quad (Eq.\ 2)$$

2.8. Data analysis

All data collected from all experiments were analyzed using analysis of variance (ANOVA) with Professional SmartstatXL V.3.6.5.4. The statistical significance of differences between means was further evaluated with Tukey's test with a value of $p \leq 0.05$.

3. Results and discussion

3.1. Initial axillary shoot regeneration of *Philodendron* 'Super Atom' on different PGR combinations and concentrations

Periodic observations revealed that initial axillary shoots began producing regenerated shoots 20 days after being cultured. As growth continued, some of these initial shoots expanded in size and developed young leaves, while others remained in their original axillary shoot state. The number of initial shoots ranged from 16 to 27, with a regeneration rate of 10–24%. The regeneration of initial axillary shoots into normal shoots posed a significant challenge in the study (Figure 2A-D). This issue was likely influenced by the elevated application of TDZ and BAP during the early stages of the research, reaching concentrations of up to 1 and 3 mg L^{-1}, respectively. These high levels of TDZ and BAP, which enhanced cell division and shoot organogenesis, resulted in the prolific production of multiple axillary shoots, thereby inhibiting their regeneration.

Despite the high concentrations of TDZ and BAP in the initial stage, the removal of TDZ and reduction of BAP concentration, along with its combination with low concentrations of IAA across six initiation culture media, did not significantly affect most of the observed variables. However, based on the number of shoots explant^{-1}, PCC-4, an MS medium supplemented with 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA, proved to be a suitable medium for shoot formation, yielding 4.8 shoots explant^{-1} (Figure 1E), although it showed lower values in other variables (Table 1). The lowest number of shoots explant^{-1} was recorded for PCC-3, with 2.2 shoots explant^{-1}.

Although the experiment failed in regenerating optimal axillary shoots, subculturing the initial shoots could mitigate the high suppression effect of the initial shoots, aiding in their regeneration.

3.2. Axillary shoot regeneration and proliferation of *Philodendron* 'Super Atom' on new combinations and concentrations of PGR

New combinations and concentrations of various PGRs, including BAP, TDZ, IAA, and NAA, significantly enhanced axillary shoot growth and proliferation, leading to an increased percentage of shoot regeneration, a higher number of shoots explant^{-1}, and greater shoot height. The improved response of axillary shoots in regenerating and forming normal shoots was likely due to transitioning the growth conditions from high concentrations of TDZ and BAP to lower levels of BAP and IAA. The NCC-6 medium, which contains 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} TDZ, and 0.025 mg L^{-1} NAA, proved to be an effective combination and concentration of PGRs for promoting shoot growth and proliferation.

Figure 2. Shoot regeneration performance was derived from the initial shoots. A-B were easily found in the first and second experiments. C-D, performance of regenerated initial shoots in three to four experiments.

NCC-6 achieved 36.9% shoot regeneration, with 6.7 shoots explant^{-1}, 2.6 leaves shoot^{-1}, shoot heights of 9.1 mm, leaf lengths of 5.9 mm, and leaf widths of 4.8 mm (Table 2). Although the improvement was slight, there was a noticeable increase in the number of regenerated shoots in response to the modified growth conditions. The second-best results were observed with NCC-3, while the lowest results were recorded for NCC-2. The experiment also demonstrated that the combination of BAP, TDZ, and NAA, despite showing no significant difference from the previous experiment, could enhance the number of proliferated shoots.

3.3. Axillary shoot growth and proliferation of *Philodendron* 'Super Atom' on new and different proliferation media

In the third experiment, the new proliferation medium (NPM) for growth and axillary shoot proliferation did not significantly enhance the percentage of shoot regeneration, axillary shoot proliferation, or growth performance. However, applying similar BAP concentrations with varying auxin concentrations led to statistically significant differences in leaf width. The experimental findings confirmed that the diverse roles of auxin in promoting shoot elongation and further growth were not observed. NPM-5, an MS medium enriched with 0.2 mg L^{-1} BAP and 0.01 mg L^{-1} IBA, demonstrated optimal results for axillary growth and proliferation. This medium increased the percentage of shoot regeneration to 26%, with 6.9 shoots explant^{-1} (Figure 1F), a shoot height of 8.4 mm, 2.7 leaves shoot^{-1}, a leaf length of 6.4 mm, and a leaf width of 4.8 mm (Table 3).

Interestingly, the results varied when explants were resub-cultured in similar media. The proliferation media significantly affected only the number of shoots explant^{-1}, while other variables showed no significant differences. The NPM-3 medium, which is MS medium enhanced with 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA, yielded the highest number of shoots explant^{-1}, reaching 13.6 shoots (Figure 1G), and showed significantly different results compared to other media (Table 4). The second-best results were observed with NPM-6, whereas the lowest results were obtained with explants cultured on NPM-4. Two simultaneous experiments demonstrated that replacing IBA with IAA and reducing the IAA concentration from 0.01 to 0.005 mg L^{-1} significantly enhanced axillary shoot growth and proliferation. From the second subculture of explants, it was confirmed that although IBA, as a synthetic auxin, had a strong effect on shoot morphogenesis, it was not suitable for axillary shoot regeneration of Philodendron 'Super Atom.' The use of IAA as a natural auxin, along with a reduction in its concentration, proved more effective for significantly increasing axillary shoot regeneration.

3.4. Plantlet preparation

Root initiation, particularly the growth and development of adventitious roots, is typically stimulated by the presence of PGRs like IAA, IBA, and NAA, either individually or in combination with cytokinins. Following the application of PGRs, the strength of the culture medium is generally reduced, and the subculture period is shortened to enhance root formation.

Table 1
Different PGR combinations and concentrations on initial axillary shoot regeneration of *Philodendron* 'Super atom'

PGR combination-concentration	Number of initial shoots	Shoot regeneration (%)	Number of shoots explant^{-1}	Number of leaves shoot^{-1}	Shoot height (mm)
PCC-1	26.0 ± 9.8	10.48 ± 3.39	2.8 ± 0.6 ab	2.85 ± 0.39	9.13 ± 3.02
PCC-2	18.0 ± 6.8	23.58 ± 16.62	3.3 ± 1.5 ab	2.90 ± 0.84	6.48 ± 1.39
PCC-3	20.8 ± 6.7	17.33 ± 10.16	2.2 ± 0.6 b	3.48 ± 1.98	7.40 ± 1.71
PCC-4	23.3 ± 3.9	21.30 ± 8.07	4.8 ± 1.0 a	2.45 ± 0.33	5.65 ± 0.99
PCC-5	16.8 ± 3.3	15.40 ± 7.94	2.5 ± 0.9 b	3.93 ± 0.98	9.43 ± 4.85
PCC-6	21.3 ± 4.6	17.93 ± 4.37	3.8 ± 1.0 ab	2.48 ± 0.88	5.80 ± 1.04

Means followed by the same letter in the same column are not significantly different based on the Tukey test (p=00.5). Values reflect the means and standard errors of the cultured explants, with n = 72 for each treatment.

Table 2
Axillary shoot growth and proliferation of *Philodendron* 'Super Atom' on new combinations and concentrations of varied PGRs of BAP, TDZ, IAA and NAA

New combinations and concentrations of PGR	Shoot regeneration (%)	Number of regenerated shoots explant^{-1}	Shoot height (mm)	Number of leaves shoot^{-1}	Leaf length (mm)	Leaf width (mm)
NCC-1	25.1 ± 6.0 ab	4.0 ± 0.8ab	4.2 ± 0.6 b	2.5 ± 0.4	6.2 ± 1.4	4.8 ± 0.8
NCC-2	17.9 ± 10.5 b	3.1 ± 1.2 b	8.3 ± 2.6 b	2.5 ± 0.4	5.1 ± 1.7	4.1 ± 1.0
NCC-3	28.3 ± 8.8 ab	4.3 ± 1.9 ab	11.8 ± 4.1 b	2.0 ± 0.5	6.4 ± 1.3	4.9 ± 1.0
NCC-4	28.1 ± 9.3 ab	4.0 ± 1.7 ab	10.4 ± 1.1 b	2.3 ± 0.4	6.2 ± 1.1	4.6 ± 0.9
NCC-5	18.6 ± 9.1 b	3.5 ± 1.7 b	22.7 ± 9.5 a	2.7 ± 0.6	5.7 ± 0.8	4.1 ± 0.6
NCC-6	36.9 ± 6.6 a	6.7 ± 0.9 a	9.1 ± 2.9 b	2.6 ± 0.1	5.9 ± 1.9	4.8 ± 1.4

Means followed by the same letter in the same column are not significantly different based on the Tukey test, *p=00.5*. Values reflect the means and standard errors of the cultured explants, with n = 72 for each treatment.

Table 3
Axillary shoot growth and proliferation of *Philodendron* 'Super Atom' on new combinations and concentrations of PGR as new proliferation media (NPM)

New Proliferation media	Shoot regeneration (%)	Number of regenerated shoots explant^{-1}	Shoot height (mm)	Number of leaves shoot^{-1}	Leaf length (mm)	Leaf width (mm)
NPM-1	20.3 ± 6.9	4.0 ± 1.6	10.1 ± 3.7	2.8 ± 0.5	6.8 ± 1.9 ab	5.7 ± 1.61 ab
NPM-2	27.8 ± 12.2	5.7 ± 2.4	9.0 ± 1.8	2.9 ± 0.5	5.8 ± 1.1 b	4.7 ± 0.86 b
NPM-3	25.2 ± 9.8	5.8 ± 1.8	11.0 ± 1.5	3.2 ± 0.5	8.1 ± 1.1 a	7.1 ± 0.70 a
NPM-4	18.2 ± 8.0	5.3 ± 2.6	9.7 ± 2.6	2.8 ± 0.8	6.0 ± 1.3 b	5.2 ± 1.13 b
NPM-5	26.0 ± 7.7	6.9 ± 1.9	8.4 ± 1.2	2.7 ± 0.5	6.4 ± 1.0 ab	4.8 ± 0.51 b
NPM-6	19.8 ± 7.2	5.7 ± 2.23	9.6 ± 2.7	3.3 ± 0.3	7.3 ± 1.6 ab	6.3 ± 1.75 ab

Means followed by the same letter in the same column are not significantly different based on the Tukey test, *p=00.5.* Values reflect the means and standard errors of cultured explants, with n = 72 for each treatment.

Table 4
Axillary shoot growth and proliferation of *Philodendron* 'Super Atom' on new combinations and concentrations of PGR as new proliferation media (NPM)

New Proliferation media	Shoot regeneration (%)	Number of regenerated shoots explant^{-1}	Shoot height (mm)	Number of leaves shoot^{-1}	Leaf length (mm)	Leaf width (mm)
NPM-1	46.8 ± 8.33	8.4 ± 2.30 b	14.7 ± 2.52	3.3 ± 0.43	10.3 ± 2.35	8.9 ± 1.36
NPM-2	41.9 ± 10.74	9.2 ± 2.77 ab	14.7 ± 3.22	3.3 ± 0.29	8.1 ± 1.26	7.9 ± 1.35
NPM-3	50.1 ± 15.02	13.6 ± 2.70 a	12.8 ± 2.17	3.1 ± 0.30	8.1 ± 0.87	7.3 ± 1.13
NPM-4	42.5 ± 13.72	8.6 ± 3.58 b	12.9 ± 2.58	3.4 ± 0.25	8.3 ± 1.06	8.0 ± 1.07
NPM-5	45.0 ± 12.47	9.0 ± 0.71 ab	13.9 ± 2.89	3.2 ± 0.19	8.4 ± 1.08	7.9 ± 1.63
NPM-6	55.7 ± 14.43	11.0 ± 1.87 ab	12.8 ± 3.46	3.0 ± 0.39	8.7 ± 2.29	8.0 ± 1.63

Means followed by the same letter in the same column are not significantly different based on the Tukey test, *p=00.5.* Values reflect the means and standard errors of cultured explants, with n = 72 for each treatment.

In this study, a specific experiment to investigate the effect of culture media on the rooting of regenerated shoots was not conducted. This was because, during shoot proliferation under subculture activities, the regenerated shoots readily rooted on various proliferation media (NPM-1 to NPM-6). The number of roots per shoot ranged from 1 to 4 (Figure 1H). However, to prepare better-rooted shoots with new roots, the regenerated shoots were subcultured on half-strength MS medium without PGRs and with 1% activated charcoal. The number of new roots from subcultured shoots varied from 1 to 3 per shoot, with lengths ranging from 0.2 to 2.5 cm. The results confirmed that for in vitro Philodendron 'Super Atom,' a specific experiment on the roots of shoots was unnecessary.

3.5. Acclimatization of *Philodendron* 'Super Atom' plantlets on different acclimatization media

Transferring plantlets to *ex vitro* conditions often poses a significant challenge in *in vitro* plant culture due to issues such as imperfect stomatal function, reduced leaf epicuticular wax, increased temperature and light intensity, and decreased relative humidity. To mitigate the high mortality rate of plantlets, various treatments and acclimatization processes, detailed in the Materials and Methods section, were implemented in this study. The acclimatization media had varying impacts on plantlet growth and their ability to adapt to ex vitro conditions. Overall, all acclimatization media proved suitable for plantlet acclimatization, with a high survival rate of 95%–100%. The highest plantlet survivability, reaching up to 100%, was observed in AM-2, which consisted of burned rice husk, hyacinth organic manure, and cocopeat (1:2:1, v/v/v) (Figure 1L), and AM-4, composed of burned rice husk and hyacinth organic manure (1:1, v/v) (Table 5). Conversely, the lowest survival rate of 58.9% was recorded for AM-1, which included burned rice husks, hyacinth organic manure, and cocopeat (2:1:2, v/v/v).

Table 5
Acclimatization of *Philodendron* 'Super Atom' plantlets under different acclimatization media

Acclimatization media	Plant survivability (%)	Plant height (mm)	Number of leaves plant^{-1}	Number of roots shoot^{-1}	Root length (mm)
AM-1	58.9 ± 6.2 b	13.8 ± 0.91 c	5.4 ± 0.49	3.9 ± 0.81 a	23.1 ± 3.60
AM-2	100.0 ± 0.0 a	14.5 ± 0.51 c	6.3 ± 2.55	1.9 ± 0.35 bc	19.8 ± 3.61
AM-3	97.8 ± 2.6 a	17.0 ± 1.61 ab	5.3 ± 0.68	3.2 ± 0.85 ab	20.2 ± 4.94
AM-4	100.0 ± 0.0 a	15.1 ± 0.73 bc	5.5 ± 0.38	2.6 ± 0.60 abc	28.2 ± 8.06
AM-5	98.5 ± 2.1 a	19.1 ± 0.40 a	5.1 ± 0.47	2.9 ± 0.19 abc	20.5 ± 3.18
AM-6	95.8 ± 8.4 a	12.6 ± 1.75 c	6.9 ± 1.31	2.8 ± 0.82 abc	23.7 ± 6.78

Means followed by the same letter in the same column are not significantly different based on the Tukey test, *p=00.5.* Values reflect the means and standard errors of the cultured explants, with n = 100 for each treatment.

Based on vegetative growth performance, AM-5, comprising burned rice husk and hyacinth organic manure (2:1, v/v), promoted optimal plantlet growth, with plant height reaching 19.1 mm, leaf length 18.5 mm, and leaf width 17 mm, showing significant differences compared to other acclimatization media (Table 5).

The experiment confirmed that there were no critical issues with the acclimatization of *Philodendron* 'Super Atom' plantlets. Different combinations and proportions of burned rice husks, hyacinth organic manure, cocopeat, and sheep organic manure did not significantly affect plantlet survivability.

Axillary shoots from initiation to acclimatization were successfully established for *Philodendron* 'Super Atom' (Figure 1), despite encountering significant challenges with axillary shoot regeneration. TDZ and BAP are the most effective PGRs for in vitro plant culture (Agustina et al., 2020; Nautiyal et al., 2022; 2023; Lamboro et al., 2022). TDZ (N-phenyl-N'−1,2,3-thiadiazol-5-yl urea) is the most widely used PGR, known for its ability to promote callus formation, somatic embryogenesis, shoot organogenesis, and regeneration across a diverse range of species, including medicinal, horticultural, woody, ornamental, and crop plants (Wu et al., 2021; Nautiyal et al., 2022; 2023). BAP is an effective PGR for promoting cell division and protein synthesis in meristematic tissues, stimulating meristematic activity in explants as a crucial step for the formation of multiple shoot buds (del Rosario Cárdenas-Aquino et al., 2022; Talukdar et al., 2022), and inducing cell proliferation due to its stability, resistance to oxidation, and difficulty for plants to degrade (Agustina et al., 2020). However, in the present study, although the application of TDZ and BAP on *Philodendron* 'Super Atom' at concentrations up to 1 and 3 mg L^{-1}, respectively, resulted in high axillary shoot formation, the combination inhibited the regeneration capacity of axillary shoots to produce normal shoots, as also reported for Chrysanthemum (Sjahril et al., 2016; Sushmarani et al., 2021).

During the initiation stage, it was hypothesized that suboptimal axillary shoot regeneration was significantly influenced by the high application of 1 mg L^{-1} TDZ and 3 mg L^{-1} BAP in the axillary shoot formation step. At these high concentrations, TDZ and BAP significantly increased cell division and meristematic activity in explants, leading to a greater proliferation of shoots rather than their elongation, regeneration, and further development (Agustina et al., 2020; del Rosario Cárdenas-Aquino et al., 2022; Talukdar et al., 2022). This condition resulted in a low regeneration capacity. The use of MS medium supplemented with 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA, known as PCC-4, proved to be an appropriate medium, yielding 4.8 regenerated shoots $explant^{-1}$ and showing a statistically significant difference compared to other media. However, there was no significant difference in the number of initial shoots, percentage of shoot regeneration, number of leaves per shoot, and shoot height. PCC-4 was the optimal PGR combination and concentration for inducing maximal axillary shoot regeneration. In a similar Philodendron, the highest number of axillary shoots, reaching 11.4 shoots $explant^{-1}$, was achieved on MS medium containing 1 mg L^{-1} BAP and 0.5 mg L^{-1} IBA (Alawaadh et al., 2020). After approximately 6 weeks of incubation, 10 shoots $explant^{-1}$ with a length of 3.2 cm were obtained on MS medium supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} IAA (Surve, 2022).

The optimal combination and concentration of PGRs, which ensures a balanced role of PGRs in cell division, proliferation, and shoot formation and regeneration—primarily influenced by cytokinin and auxin (Nautiyal et al., 2022; 2023; Sosnowski et al., 2023; Lamboro et al., 2022; Bhat & Shahzad, 2026) will result in maximal shoot initiation and regeneration. In other Philodendron species, the highest number of shoots per explant, reaching up to 4.8 shoots for *Philodendron* 'Birkin', was achieved on MS medium supplemented with 2 mg L^{-1} BA (Mongkolsawat et al., 2023). MS medium enhanced with 1.0 mg L^{-1} BAP produced the highest number of shoots and leaves, yielding 7.7 shoots $explant^{-1}$ and 4.1 leaves $explant^{-1}$ for *Philodendron erubescens* 'Pink Princess', compared to other BAP concentrations (Klanrit et al., 2023). A total of 3.1 shoots $explant^{-1}$ for *Philodendron erubescens* 'White Princess' and *P. verrucosum*, along with a shoot height of 5.5 mm, were observed in liquid MS medium without growth regulators in the Temporary Immersion Bioreactor System (Sangchanjiradet et al., 2024). In other Araceae plants, MS media supplemented with 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA achieved the highest culture establishment with 2.2 shoots $explant^{-1}$ on Aglaonema in 7.2 days (Karthik et al., 2023). The highest number of shoots $explant^{-1}$, up to 17.9 shoots, for *Theriophonum sivaganganum* was observed on MS medium fortified with BA and 0.5 mg L^{-1} of 1-naphthaleneacetic acid (NAA) (Anbazhakan et al., 2025). MS medium supplemented with 0.5 mg L^{-1} NAA and 3.0 mg L^{-1} of TDZ produced 7.14 shoots $explant^{-1}$ of *Cryptocoryne crispatula* var. yunnanensis (Sakulsathaporn & Mapanao, 2025).

Regeneration and multiplication of both axillary and adventitious shoots are typically influenced by

applying various combinations and concentrations of PGR (Alawaadh et al., 2020; Chen et al., 2020). The use of exogenous PGR, which often modifies the endogenous hormonal balance, must be fine-tuned to establish optimal conditions for all in vitro processes involving regeneration and multiplication (Li et al., 2026). At this stage, employing a similar basal medium with an appropriate and/or balanced combination and concentration of PGR generally promotes high shoot regeneration and multiplication (Martini et al., 2022; Pasternak & Steinmacher, 2024; Capotescu et al., 2025; Bhat & Shahzad, 2026). In a balanced combination and concentration, cytokinin and auxin play crucial roles in cell proliferation, elongation, and subsequent shoot growth. The optimal and balanced PGR concentrations for achieving high shoot regeneration and multiplication vary among different plants. Seliem et al. (2021) reported that an MS medium fortified with 5 mg L^{-1} and 35 mg L^{-1} yielded the highest number of proliferated shoots, reaching up to 25 shoots. In *Philodendron erubescens* 'Pink Princess', an initiation medium of MS medium supplemented with 1.0 mg L^{-1} BAP successfully proliferated shoots, achieving 11.2 shoots and 4.7 leaves $explant^{-1}$ (Klanrit et al., 2023). The highest shoot multiplication in *Philodendron* 'Birkin' was attained by culturing explants in MS medium containing 3 mg L^{-1} BA and 0.5 mg L^{-1} IBA, resulting in an average of 16.7 shoots $explant^{-1}$ over a four-week period (Akramian et al., 2024). In other Araceae plants, the highest shoot proliferation rate, up to four new shoots of *Eminium rauwolffii* (Blume) Schott var. R. rauwolffii, was observed on MS medium supplemented with 4 mg L^{-1} BAP and 1 mg L^{-1} NAA (Güney, 2023). Three experiments on shoot proliferation were conducted in this study, yielding different results (Tables 2, 3, and 4). However, the highest-proliferated shoots, reaching 13.6 shoots per explant, showed significantly different results from other media, where MS medium was augmented with 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA (NPM-3).

De novo adventitious roots represent root organogenesis following tissue injury and are a form of plant regeneration. Plants possess a remarkable ability to perceive external stimuli (Zhi & Hu, 2023). The formation of adventitious roots on *in vitro* cutting explants typically involves several key processes and factors, including the wounding response, auxin metabolism, phenolic compounds, ethylene signalling, gene expression dynamics, and transcriptomic analysis (Mhimdi & Pérez-Pérez, 2020; Ayala et al., 2022; Zhang et al., 2025). However, in shoot subcultures, the formation of adventitious roots is influenced by a decrease in IAA and abscisic acid due to periodic subcultures of shoots (Lakho et al., 2023; Shintiavira et al., 2024; Zhang et al., 2025). Regenerated axillary shoots from the proliferation stage readily produced roots (Figure 1H) on NPM-1 to NPM-6 media and in half-strength MS medium PGR-free with 1% AC (Figure 1I and 1J). The number of roots per shoot varied from 1 to 3, with differing lengths. In various studies with similar Philodendron species, a comparable rooting medium stimulated four $roots\ shoot^{-1}$, each 3 cm in length (Surve, 2022). MS medium containing 2 mg L^{-1} IAA resulted in 13 $roots\ shoot^{-1}$ (Alawaadh et al., 2020). In *Philodendron erubescens* 'Pink Princess', 3.2 $roots\ shoot^{-1}$ were regenerated on MS medium fortified with 3 mg L^{-1} IBA (Klanrit et al., 2023), while 2.7 $roots\ shoot^{-1}$ were observed in similar Philodendron on MS medium with 1 - 2 mg L^{-1} BA (Kuathan & Judsri, 2023). For *Philodendron* 'Birkin', 6.8 $roots\ shoot^{-1}$ were produced on MS medium augmented with 1 mg L^{-1} IBA (Mongkolsawat et al., 2023), and 6.1 $roots\ shoot^{-1}$ were induced on MS medium with 1 mg L^{-1} IBA in similar *Philodendron* (Akramian et al., 2024). In *Alocasia amazonica*, the best response for root induction (85%) was achieved on ½ MS medium fortified with 3.0 mg L^{-1} IBA, 2.0 mg L^{-1} IAA, and 2.0 mg L^{-1} NAA, with an average of 22.2 $roots\ shoot^{-1}$ (Raju et al., 2022). In *Alocasia amazonica*, the best response for root induction (85%) was achieved on ½ MS medium fortified with 3.0 mg L^{-1} IBA, 2.0 mg L^{-1} IAA, and 2.0 mg L^{-1} NAA, with an average of 22.2 $roots\ shoot^{-1}$ (Kharrazi et al., 2023), and 3.3 $roots\ shoot^{-1}$ established on MS medium containing 1 mg L^{-1} NAA (Pourhassan et al., 2023). These findings confirm that shoot rooting is not a significant challenge in the *in vitro* culture of Philodendron.

Transitioning plantlets from a controlled environment to *ex vitro* conditions poses a significant challenge in *in vitro* plant culture. This issue arises from impaired stomatal regulation, reduced or absent leaf epicuticular wax, increased temperature and light intensity, and decreased relative humidity, all contributing to a high mortality rate during acclimatization (Mohammed et al., 2023; Nishesh et al., 2023). However, acclimatizing plantlets derived from the *in vitro* culture of Philodendron is generally not problematic (Alawaadh et al., 2020; Klanrit et al., 2023; Akramian et al., 2024; Kang & Sivanesan, 2025). The high survival rate of plantlets transferred to *ex vitro* conditions is supported by effective acclimatization processes. Alawaadh et al. (2020) began by gently removing plantlets from the culture vessel, washing the roots to remove all growth medium remnants, immersing the roots in a 1% fungicide and bactericide solution for

approximately 3 minutes, air drying them on paper, then culturing them in plastic trays with acclimatization media, covering them with transparent plastic for seven days, and placing them in a reduced-light greenhouse area. Klanrit et al. (2023) applied a method of gently collecting plantlets from the culture vessel, rinsing the roots under tap water, and placing the acclimatized plantlets in a growth chamber. Akramian et al. (2024) carefully rinsed the plantlets with tap water to remove adhering gel, transferred them to acclimatization media, and kept them in a greenhouse at 18 - 25 ˚C with a relative humidity of about 70%. Kang & Sivanesan (2025) separated plantlets from the rooting medium, thoroughly washed them to remove any traces of the medium, and transplanted them into trays containing a mixture media. These were placed in a greenhouse at a temperature of 20 – 25°C and a relative humidity of 95% – 100% for two weeks, which was gradually reduced to 60%, and fertigated with ¼ MS nutrients.

Rooted plantlets underwent successive acclimatization, achieving a 100% survival rate on a peat moss and perlite mixture (1:1, v/v), and they were morphologically similar to the mother plant (Alawaadh et al., 2020). The use of peat moss and perlite (1:1, v/v) led to a 100% survival rate for *Philodendron selloum* (Seliem et al., 2021). Similarly, a 100% survival rate was observed for Philodendron 'Pink Princess' plantlets on peat moss and vermiculite (Klanrit et al., 2023). *Philodendron* 'Birkin' plantlets were successfully acclimatized in a greenhouse, achieving a 100% ex vitro survival rate on a cocopeat and perlite mixture (2:1, v/v) (Akramian et al., 2024). A 100% survival rate was also established for plantlets of *Philodendron erubescens* 'White Princess' and *P. verrucosum* on a peat moss-perlite mixture (4:1, v/v) (Sangchanjiradet et al., 2024). In this study, plantlets exhibited 100% survivability in AM-2, burned-rice husk, hyacinth organic manure, and cocopeat (1:2:1, v/v/v) as well as in AM-4, burned-rice husk, and hyacinth organic manure (1:1, v/v).

4. Conclusions

An *in vitro* mass propagation protocol through axillary formation and proliferation up to plantlet acclimatization was successfully developed. The initial establishment of high axillary shoots in MS medium containing 1 mg L^{-1} TDZ and 3 mg L^{-1} BAP faced critical challenges due to difficulties in regenerating normal shoots. Axillary shoot regeneration was initially achieved on MS medium supplemented with 0.3 mg L^{-1} BAP and 0.005 mg L^{-1} IAA. Highly regenerated and proliferated shoots were established on MS medium containing 0.2 mg L^{-1} BAP and 0.005 mg L^{-1} IAA. There was no need for a plantlet preparation stage, as the shoots readily rooted in the proliferation media. A 100% survival rate of plantlets was successfully attained when transferred to *ex vitro* conditions using a mixture of burned rice husks, hyacinth organic manure, and cocopeat (1:2:1, v/v/v; AM-2) and burned rice husks and hyacinth organic manure (1:1, v/v; AM-4).

Developing an *in vitro* mass propagation protocol for the Araceae plant group, including Philodendron, presents significant challenges, particularly in creating effective sterilization methods and selecting suitable initiation culture media, which are crucial for achieving high initiation of axillary shoots. Utilizing MS medium, either at full or ¾ strength, with 1 mg L^{-1} TDZ and 3 mg L^{-1} BAP and/or 350 mg L^{-1} myoinositol, is effective for promoting high axillary shoot formation in several Araceae plants. However, it is important to note that using high concentrations of TDZ and BAP in the initiation culture may inhibit axillary shoot regeneration in subsequent culture processes. Future research should focus on determining the optimal concentrations of TDZ and BAP for initial axillary shoot formation, the duration for maintaining initial axillary shoots in high concentrations of TDZ-BAP, and identifying a more effective medium for axillary shoot regeneration.

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Author contributions

B. Winarto: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – original draft, Writing – review & editing. **F. Rachmawati:** Conceptualization, Methodology, Investigation, Writing – review & editing. **S. Rianawati:** Investigation, Formal analysis, Validation, Writing – original draft. **Fitrahtunnisa:** Methodology, Investigation, Writing – review & editing. **J. Pramono:** Investigation, Resources, Writing – review & editing. **S. Handoko:** Investigation and Writing-review and editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

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