BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 31 No. 2, 2024: 349 - 358
DOI: 10.11598/btb.2024.31.2.2214

Research Article

IN VITRO PLANT REGENERATION THROUGH PROTOCORM-LIKE BODIES DERIVED FROM STEM THIN LAYER OF *Anubias barteri* var. *nana* Petite

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ARTICLE HIGLIGHTS

- Effective induction of PLBs from stem thin layer and shoot regeneration using specific BAP and auxin combinations.
- Significant role of potato extract in enhancing shoot regeneration.
- Highest shoot regeneration rate achieved with 3 mg/L BAP concentration.

Article Information

Received 5 March 2024
Revised 1 April 2024
Accepted 3 April 2024

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ABSTRACT

Anubias barteri var. *nana* Petite, a highly valued ornamental aquatic plant within the *Araceae* family, encounters challenges in vegetative propagation due to its slow multiplication rate. This study aimed to identify the optimal concentrations of plant growth regulators and potato extract for the micropropagation of this species through shoot tips and protocorm-like bodies (PLBs). Initially, shoot tips were sterilized and cultured on the Murashige and Skoog medium (MS) with varying concentrations of 6-Benzylaminopurine (BAP) to induce growth and multiplication. Furthermore, the induction of PLBs and shoot regeneration was facilitated using BAP in combination with auxins, including Naphthaleneacetic Acid (NAA), Indole-3-acetic Acid (IAA), or Indole-3-Butyric Acid (IBA), in various combinations. The study also explored the impact of potato extract (PE) on shoot regeneration and the significant role of IBA in root development. Results indicated that the highest shoot regeneration rate from shoot tips was achieved at a BAP concentration of 3 mg/L. Effective induction of PLBs from stem explants, followed by shoot regeneration, was achieved with 1.5 mg/L BAP combined with 0.5 mg/L NAA or 0.5 mg/L IAA, and 3 mg/L BAP with 1.0 mg/L IBA. The inclusion of 50 g/L potato extract significantly enhanced shoot proliferation, and 0.5 mg/L IBA proved vital for root development. The plantlets acclimatized successfully in aquariums with a 100% survival rate. Future research will aim to further enhance PLB multiplication. The developed micropropagation protocol offers a promising approach for the mass production of *A. barteri* var. *nana* Petite, effectively overcoming the limitations of natural propagation methods.

Keywords: *Anubias barteri* var. *nana* Petite, plant growth regulators, protocorm-like bodies (PLBs), shoot induction, shoot regeneration

INTRODUCTION

Anubias barteri var. *nana* Petite holds significant commercial importance as a highly sought-after decorative aquatic plant within the *Araceae* family. This cultivated variant, renowned for its slow growth and cluster formation, typically ranging from 1 cm to 5 cm in height, has garnered popularity in both aquatic and ornamental settings (George *et al.* 2015).

Despite its aesthetic appeal, the natural propagation of *A. barteri* has proven inefficient, leading to numerous studies on micropropagation techniques, including *in vitro* multiplication (George *et al.* 2015; Huang *et al.* 1994; Kanchanapoom *et al.* 2012; Rittirat *et al.* 2021), *in vitro* organogenesis (Surendra *et al.* 2019; Rittirat *et al.* 2023), and hydroponic culture (Sholichah *et al.* 2020). However, these efforts have resulted in an average shoot production ranging between 2 shoots to 5 shoots from a single shoot.

To address this limitation, the utilization of protocorm-like bodies (PLBs) presents a promising approach for mass production because of their rapid formation, uniform characteristics, disease-free nature, and sustainability (Cardoso *et al.* 2020; Sheelavanthmath *et al.* 2005). Noteworthy success

has been achieved in the utilization of PLBs for the proliferation and multiplication of ornamental plants within the *Araceae* family. For example, *Anthurium andreanum* cv. CanCan exhibited 97.8% rate of PLB induction, averaging 120 PLBs for each explant over a period of 50 days in cultivation (Gantait *et al*. 2012; Yu *et al*. 2009). Similarly, TDZ was shown effective for inducing PLBs from leaf explants of *Cattleya tigrine* with 17.5% induction rate, and GA_3 significantly increases shoot regeneration from PLBs with approximately 220 developed plantlets per culture flask (Fritsche *et al*. 2022).

Despite these successes, there is currently no documented report on regeneration from PLBs on *Anubias barteri* var. *nana* Petite. Given the documented success of PLBs in various plant species, this study aimed to identify the optimal concentrations of plant growth regulators and potato extract for propagating this species through shoot tips and PLBs. The development of this micropropagation protocol provides a methodical approach to augment the propagation of *Anubias barteri* var. *nana* Petite, effectively overcoming the limitations of natural propagation methods.

MATERIALS AND METHODS

Shoot Sterilization

Shoots of *Anubias barteri* var. *nana* Petite were subjected to surface sterilization using soap for 15 minutes, followed by exposure to an antifungal agent (Mancozeb, India) for 10 minutes, washed with running water, and further rinsed with 70% alcohol for 30 seconds. Subsequently, explants were soaked in a commercial bleach solution (5% sodium hypochlorite) at concentrations of 10%, 20%, 30%, and 40% (v/v) for 15 minutes, followed by a 30-minute soak with an antibiotic solution (1 mg/mL). After disinfection, 3-5 mm shoot tip explants were isolated and placed on MS (Murashige and Skoog, 1962) basal medium.

Shoot Multiplication from Shoot Tips

Shoot tips free of microbial infection were cultured in MS media supplemented with (0, 1.0, 2.0, 3.0, 4.0 mg/L) BAP for 6 weeks for shoot induction. In a subsequent experiment, a single shoot (approximately 0.8 cm in height) was transferred to MS medium supplemented with various BAP concentrations (2.0, 3.0, 4.0 mg/L) for multiplication over 4 weeks. Shoot multiplication parameters, including the number of shoots, shoot height, number of leaves per shoot, and leaf length were recorded.

Protocorm-like Bodies (PLBs) Induction, Proliferation and Shoot Regeneration

For PLB induction, *in vitro* stems were separated from the cluster, leaves were removed, and the stem was cut into 1-2 mm transverse slices. These slices were placed on MS medium containing 10% (v/v) coconut water (CW), 2% (w/v) sucrose, and complemented with (0.5-3.0 mg/L) BAP along with (0.5-2.0 mg/L) NAA. The percentage of PLB induction and the number of PLBs per explant were recorded after 6 weeks of dark culturing. PLB morphology and counting were performed at 2X to 4X magnification under a stereo microscope (Olympus SZ51, Japan).

Secondary PLB Induction and Shoot Regeneration

For PLB proliferation, PLB clusters were separated into individuals and placed on MS medium containing 10% (v/v) CW, 2% (w/v) sucrose, added (0.5-1.5 mg/L) BAP and (0.2-0.5 mg/L) IAA. After 3 weeks, the secondary PLB number and diameter, indicating PLB growth speed, were recorded.

To regenerate shoots from PLBs, tuber-shaped PLBs were separated and transferred to MS medium containing 10% (v/v) CW, 2% (w/v) sucrose, 1.0 g/L activated charcoal, 2 g/L peptone, and 1-3 mg/L BA and 0.5 mg/L IBA. After 3 weeks, shoot height was measured, and shoot quality was determined by leaf color, stem diameter, and leaf length.

Effect of Potato Extract on Shoot Proliferation

To assess the impact of potato extract on shoot proliferation, PLBs were cultured on MS medium complemented with 1.5 mg/L BAP and 0.5 mg/L NAA ranging from 10 g/L to 50 g/L of PE. Data on shoot number and individual shoot height were collected after the designated culture period.

Rooting

In vitro plantlets (1-1.5 cm) regenerated from PLBs were cultured on MS medium supplemented with 0.1% (w/v) activated carbon, 10% (v/v) CW, and Indole-3-Butyric Acid (IBA) at different concentrations (0.5, 1.0, 1.5, 2.0 mg/L). After 4 weeks, the number of roots per shoot, root length, shoot height, and number of leaves per shoot were recorded.

Experimental Design and Statistical Analysis

All experiments were conducted in a Completely Randomized Design (CRD) with three repetitions for each treatment, and each repetition included five jars, each containing at least one explant. The data underwent basic statistical analysis and were examined using ANOVA. Mean values were compared using Duncan's method at 5% probability level. The data were presented as the average followed by the standard deviation (M±SD).

RESULTS AND DISCUSSION

Explants Sterilization

To prepare explants, decontamination was performed using various concentrations of commercial bleach (5% sodium hypochlorite) for a 15-minute disinfection. Explants were crucially transferred to clean water for at least one week before sterilization to avoid a high infection rate. A 10% bleach solution yielded a 40% disinfection rate, but higher concentrations (up to 40%) were necessary to achieve a 100% sterilization rate, ensuring successful decontamination. The shoot tips, retaining their green color, initiated new shoots.

Shoot Multiplication

In this experiment, varying concentrations of BAP were added to the MS medium to promote shoot formation and multiplication (Table 1). During the shoot induction stage, shoot tips showed distinct responses to different BAP concentrations after 10 days of culture. PGR-free MS media resulted in minimal shoot formation (1.13 shoots/explant), significantly increasing with higher BAP concentrations. The maximum shoot yield (5.33 shoots/explant) was observed after 6 weeks on a medium with 3 mg/L BA, showcasing superior quality in terms of average shoot height (1.01 cm), leaves per shoot (4.92), and leaf length (1.03 cm) (Table 1; Figs. 1a-c). Subsequent stages confirmed the efficiency of 3 mg/L BAP in shoot multiplication, with clusters displaying large, green leaves, and long roots (Figs. 1d-f).

The response to BAP concentration observed in this study aligns with previous research on *Araceae* species, such as *A. barteri* var *nana* (Kanchanapoom *et al.* 2012; Rittirat *et al.* 2021), *Cryptocoryne wendtii* (Rittirat *et al.* 2020), and *Anubias heterophylla* (Rittirat *et al.* 2021). These findings highlight the significant impact of BAP concentration on shoot regeneration in *A. barteri* from shoot tips. However, adaptation to high PGR concentrations is not universal. For successful shoot regeneration from basal buds of *A. barteri* var. *nana*, it is necessary to use a minimal concentration of BAP (0.2 mg/L), and shoot elongation was obtained on PGR-free MS basal medium (Sheeja *et al.* 2015). Other studies within the *Araceae* family, utilizing lower BAP concentrations, resulted in fewer shoots (George *et al.* 2015).

In general, BA, with or without auxin supplementation, serves as the primary plant growth regulator in shoot induction and multiplication. MS medium supplemented with 3 mg/L BAP has proven suitable for maintaining long-term *in vitro* cultures. Shoot quality remained stable after four subculture cycles.

Protocorm-like Body (PLB) Induction

After 10 days of culture, transverse slices exhibited callus formation. After four weeks of cultivation, PLBs began to emerge, initially appearing opaque white and assuming a tuber-like shape (Table 2; Fig. 2). The ANOVA and subsequent post hoc analysis demonstrated a highly significant influence of BAP and NAA, and their interaction, on explant responses. For BAP, three distinct groups revealed concentrations that induced significant PLB formation. Similarly, specific NAA concentrations significantly impacted explant responses, particularly in PLB induction.

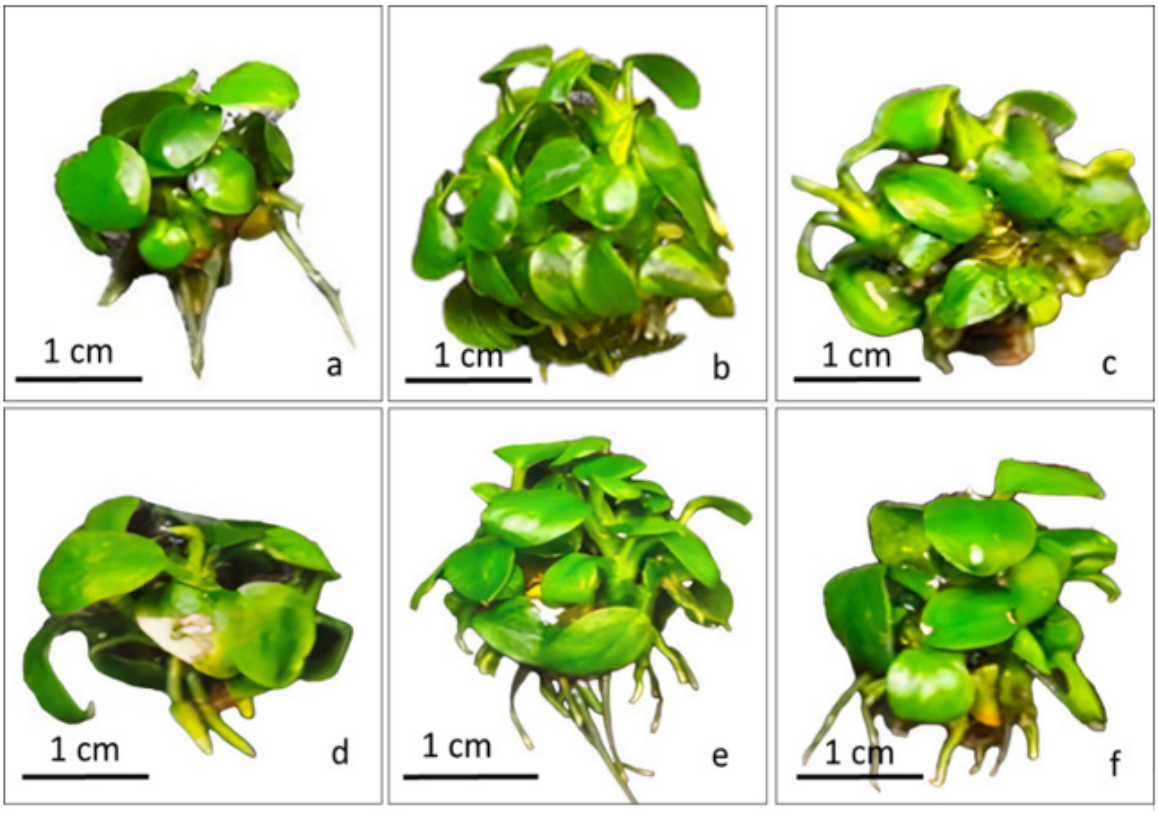
After 6 weeks of culture in media containing either 0.5 or 1.5 mg/L BAP combined with 0.5 mg/L NAA (P1, P3) resulted in underdeveloped buds on the browned callus, whereas 1.5 mg/L BAP with 1.0 or 1.5 mg/L NAA (P9, P15) induced callogenesis without PLBs (Figs. 2a-b). Conversely, treatments P2, P5, P8, P11, P12, P14, P19, P20, P21, and P23 led to significant PLB induction, showing variations in leafy plantlet, globular callus, and adventitious roots. Treatments P4, P6, P10, P13, P16, P17, P18, P22, P24 resulted in tiny PLB germination (Figs. 2c-e). Specific combinations of BAP and NAA concentrations (1.0 mg/L BAP + 0.5 mg/L NAA; 3.0 mg/L BAP + 1.5 mg/L NAA; 1.0 mg/L BAP + 0.5 mg/L NAA) significantly enhanced PLB formation and leafy plantlet development (Figs. 2 f-h). However, concentration-dependent effects were observed, leading to callusing or limited PLB growth as certain concentrations. Notably, a BAP concentration of 1.5 mg/L, combined with 0.5 mg/L NAA, showed a tendency to promote a robust PLB induction rate.

Table 1 Effect of BAP added to culture media on shoot formation after 6 weeks of culture

BAP (mg/L)	Number of shoots	Shoot height (cm)	Number of leaves	Leaf length (cm)
0.0	1.13^{d}± 0.12	0.89^{b}± 0.09	4.1^{c}± 0.55	0.93^{d}± 0.04
1.0	2.07^{c}± 0.12	0.89^{b}± 0.05	4.33^{bc}±0.31	0.94^{cd}± 0.02
2.0	3.87^{b} ±0.12	0.92^{b}± 0.02	4.63^{ab} ±0.30	0.96^{bc}± 0.03
3.0	5.33^{a} ±0.31	1.01^{a}±0.06	4.92^{a} ±0.30	1.03^{a}± 0.01
4.0	4.20^{b}± 0.20	0.93^{b}± 0.03	4.71^{ab} ±0.21	0.98^{b}± 0.03

Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.05).

Figure 1 Shoot induction from shoot tips (a-c) and multiplication (d-f) in response to varying BAP concentrations after 6 weeks



Notes: (a, d) = 2 mg/L BA; (b, e) = 3 mg/L BA; (c, f) = 4 mg/L BA.

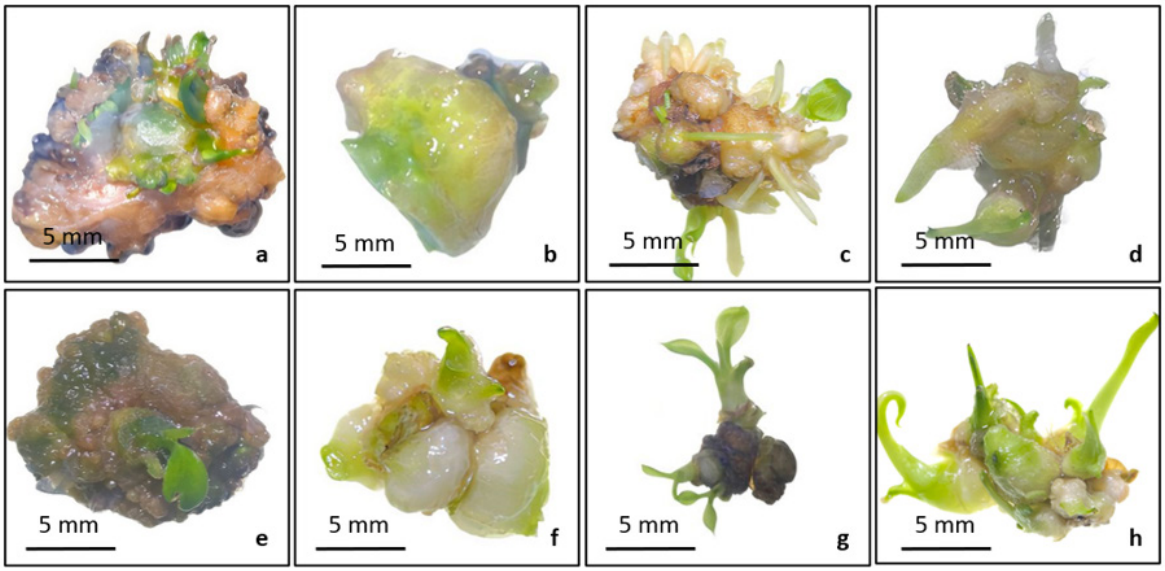
Table 2 Effect of BAP combined with NAA on callus/PLBs/shoot formation after 6 weeks of culture

Treatment	BAP (mg/L)	NAA (mg/L)	PLB induction rate (%)	PLBs/explant	Morphogenesis of explant
P0	0.0	0.0	0	0±0	Callusing
P1	0.5	0.5	100	5.89^{bc}±0.81	Tiny buds undeveloped, callus brown
P2	1.0	0.5	100	6.56^{ab}±0.85	PLBs, leafy plantlets
P3	1.5	0.5	100	7.78^{a}±0.93	Tiny buds undeveloped, callus brown
P4	2.0	0.5	100	3.44^{de}±0.62	Tiny PLBs sprout
P5	2.5	0.5	100	2.56^{def}±0.53	PLBs, leafy plantlets
P6	3.0	0.5	100	3.67^{def}±0.64	Tiny PLBs sprout
P7	0.5	1.0	100	4.56^{cd}±0.71	Globular callus, adventitious buds

Treatment	BAP (mg/L)	NAA (mg/L)	PLB induction rate (%)	PLBs/explant	Morphogenesis of explant
P8	1.0	1.0	100	4.44^{cd}±0.70	PLBs sprout
P9	1.5	1.0	0	0^{g}±0	Callusing
P10	2.0	1.0	100	3.00^{def}±0.58	Tiny PLBs sprout.
P11	2.5	1.0	100	1.22^{fg}±0.37	PLBs, leafy plantlets
P12	3.0	1.0	100	3.00^{cdef}±0.58	PLBs, leafy plantlets
P13	0.5	1.5	100	3.00^{def}±0.58	Small globular PLBs, adventitious roots
P14	1.0	1.5	100	3.22^{de}±0.60	PLBs sprout
P15	1.5	1.5	0	0^{g}±0	Callusing
P16	2.0	1.5	100	1.78^{efg}±0.44	Small PLB
P17	2.5	1.5	100	1.00^{efg}±0.33	Tiny PLBs sprout.
P18	3.0	1.5	100	1.11^{efg}±0.35	PLBs
P19	0.5	2.0	100	2.33^{def}±0.51	PLBs, adventitious roots
P20	1.0	2.0	100	2.00^{efg}±0.47	PLBs sprout
P21	1.5	2.0	100	3.78^{de}±0.65	PLBs, adventitious roots
P22	2.0	2.0	100	1.56^{def}±0.42	Tiny PLBs sprouted.
P23	2.5	2.0	100	2.44^{defg}±0.52	PLBs, leafy plantlets
P24	3.0	2.0	100	2.67^{def}±0.54	Tiny PLBs sprouted, adventitious roots.

Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.01).

Figure 2 Morphogenesis of transverse slices after 6 weeks of culture in the presence of different concentrations of BAP and NAA



Notes: a = underdeveloped buds on the browned callus; b = callogenesis without PLB; c-e = PLB induction with various patterns, i.e., leafy plantlet, globular callus, adventitious roots, and tiny PLB germination; f-h = PLB formation and leafy plantlet development.

Research has showcased the efficacy of utilizing both NAA and BAP in stimulating PLB induction across different species within the *Araceae* family. Rittirat *et al*. (2021) demonstrated the number of shoots per explants (3.60±0.24) was formed in the culture medium containing 1.0 mg/L NAA in combination with 1.0 mg/L BAP, followed by culture medium with 3.0 mg/L BAP (2.40±0.24). Mondal *et al.* (2014) found that a combination of 2 mg/L NAA and 2 mg/L BAP was most effective for PLB induction in *Doritis pulcherrima* Lindl. Sarma and Tanti (2017) further supported these findings, showing that a combination of 3.0 mg/L BAP and 1.0 mg/L NAA was most successful in inducing shoot formation in *Aristolochia saccate*. These studies collectively demonstrated that a combination of NAA and BAP is a popular approach for promoting PLB induction and plantlet regeneration in *Araceae* species.

Secondary PLB Induction and Shoot Regeneration

PLBs were subcultured on MS media with varying BAP and IAA concentrations, leading to secondary PLB (sPLB) formation (Table 3). At 0.5 mg/L BAP and 0.2 mg/L IAA, a 25.0% PLB induction rate was observed, with a mean diameter of 0.61 cm. However, the quantity of sPLB generated by each explant was 0.33, indicating moderate development. Increasing BAP to 1.0 mg/L whereas maintaining 0.2 mg/L IAA resulted in a notable decline in the PLB induction rate (0.00%), along with a decrease in both the number of secondary PLBs and their diameter, suggesting a dose-dependent negative effect of BAP on PLB formation at this concentration. Interestingly, at 1.0 mg/L BAP with an elevated IAA concentration of 0.5 mg/L, the PLB induction rate rebounded to 33.3%, with an increase in both the number of sPLB and their diameter, suggesting a potential synergistic effect between BAP and IAA. At the highest concentrations of both BAP (1.5 mg/L) and IAA (0.5 mg/L), the PLB induction rate reached 68.1%, demonstrating substantial improvement. The number of sPLBs increased to 1.55, and the diameter of the PLBs reached 0.67 cm, indicating a robust response to the combined higher concentrations of BAP and IAA. These results highlight the significant influence of the interplay between BAP and IAA concentrations on PLB induction and subsequent development. The optimal combination for enhanced PLB formation appears to be 1.5 mg/L BAP and 0.5 mg/L IAA, offering valuable insights for optimizing micropropagation protocols for this specific plant.

The impact of different concentrations of BAP and IBA on shoot regeneration from PLBs is presented in Table 4. At 1.0 mg/L BAP and 0.5 mg/L IBA, shoots reached a height of 0.65 cm. Increasing BAP to 1.5 mg/L whereas maintaining IBA at 0.5 mg/L resulted in a slightly improved shoot length of 0.68 cm and enhanced shoot quality. Similarly, at BAP concentrations of 2.0 mg/L with 0.5 mg/L IBA, shoot length remained consistent at 0.68 cm. At higher BAP concentrations (2.5 mg/L) and 0.5 mg/L IBA, the shoot length increased to 0.83 cm. Notably, increasing IBA to 1.0 mg/L with BAP at 1.0 mg/L significantly improved shoot length to 1.23 cm, with good quality, indicating vigorous shoot regeneration. For BAP ranging from 1.5 mg/L to 3.0 mg/L with 1.0 mg/L IBA, shoot lengths varied from 0.94 cm to 1.57 cm, and the shoot quality was good.

These results emphasized the crucial role of the interaction between BAP and IBA concentrations in shoot regeneration potential from PLBs. The combination of BAP and IAA is effective in promoting shoot proliferation and elongation in *Quercus suber* L. (Romano *et al*. 1992). This combination also enhances shoot growth and proliferation in *Aegle marmelos* (Ajithkumar & Seeni 1998). In the production of PLBs in orchids, BAP is the most effective growth regulator, followed by kinetin, NAA, IAA, 2,4-D, and gibberellic acid (GA3) (Saiprasad *et al*. 2002). In the case of pointed gourd, BAP has been found to induce callus formation, whereas the addition of IAA has been shown to enhance rooting (Komal 2011). These findings suggested that the combination of BAP and IAA may have a positive effect on PLBs multiplication and shoot regeneration in *Araceae*, but further research is needed to confirm this.

Impact of Potato Extract on Shoot Regeneration

The impact of varying potato extract (PE) concentrations on shoot regeneration from PLBs is shown in Table 5. Without PE, an average of 2.33 shoots was noted for each explant, accompanied by an average shoot length of 1.21 cm. However, at 10 g/L, 20 g/L, and 30 g/L PE, the number of shoots per explant nearly doubled to 4.19, 4.37, and 4.62, and the shoot height increased to 1.72, 2.08, and 2.36 cm. The most significant enhancement in shoot regeneration occurred at 40 g/L and 50 g/L PE concentrations. At 40 g/L, shoots per explant increased to 5.52, and the shoot height reached

2.74 cm. The highest concentration, 50 g/L, yielded the most favourable outcome, with 6.55 shoots per explant and a maximum shoot height of 2.80 cm (Fig. 3). This highlighted a positive correlation between PE concentration and both the quantity and quality of shoots regenerated from PLBs, suggesting a potential role of specific compounds within the PE in promoting shoot development.

Studies indicate that plantlet regeneration from protocorm-like bodies in orchids is significantly enhanced by the application of PE. Rahman (2004) found that PE enhanced plantlet regeneration and growth, with the optimum concentration being 100 mL/L. This finding was further supported by Islam *et al.* (2012), who reported that PE enhances the germination of seeds and the growth of seedlings of Vanda *roxburgii* orchids. The beneficial impact of PE on the growth and development of PLBs was also demonstrated by Lee (2003) in *Cypripedium formosanum* (Lee & Lee 2003). Collectively, these studies suggest that PE can be a valuable supplement in the medium for regenerating of plantlets from PLBs in orchids.

Effects of IBA on Rooting and Plantlet Growth

The impact of IBA on the root and plantlet development of *A. barteri* after a 4-week culture period is shown in Table 6.

With IBA of 0.5 mg/L, all parameters showed significant improvement. The number of roots increased to 6.19, with a longer average root length of 2.50 cm. Shoot height and leaf number also increased to 4.72 cm and 6.81, respectively. At 1.0 mg/L IBA, whereas the number of roots slightly decreased to 4.81, both root length (2.14 cm) and shoot height (4.80 cm) remained relatively higher compared to the control, with a leaf number of 6.11. Further increases in IBA concentration to 1.5 mg/L and 2.0 mg/L led to a gradual decline in the number and length of roots, shoot height, and leaf number. The results indicated that IBA positively influences root and plantlet development in *A. barteri* var. *nana* Petite with an optimal response observed at 0.5 mg/L IBA. Higher concentrations, beyond 0.5 mg/L, showed diminishing returns, suggesting a dose-dependent effect.

Table 3 Effect of BA and IAA added to secondary PLB induction after 4 weeks of culture

Plant growth regulators (mg/L)		sPLB induction rate (%)	sPLBs/explant	Diameter of PLB (cm)
BA	IAA			
0.5	0.2	25.0^{bc}±14.4	0.33^{bc}±0.33	0.61^{abc}±0.10
1.0	0.2	0.00^{d}±0.00	0.00^{c}±0.00	0.50^{c}±0.06
1.5	0.2	8.33^{cd}±8.33	0.33^{bc}±0.33	0.51^{bc}±0.01
0.5	0.5	25.0^{bc}±14.4	1.00^{ab}±0.00	0.56^{abc}±0.03
1.0	0.5	33.33^{b}±8.33	1.00^{ab}±0.00	0.65^{ab}±0.03
1.5	0.5	68.06^{a}±3.67	1.55^{a}±0.29	0.67^{a}±0.16

Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.05).

Table 4 Effect of BAP combined IBA on shoot formation from protocorm-like bodies

BAP (mg/L)	IBA (mg/L)	Shoot height (cm)	Shoot quality
1.0	0.5	0.65^{b}±0.09	Poor
1.5	0.5	0.68^{b}±0.09	Fair
2.0	0.5	0.68^{b}±0.09	Fair
2.5	0.5	0.83^{b}±0.08	Fair
3.0	0.5	0.74^{b}±0.14	Fair
1.0	1.0	1.23^{ab}±0.20	Excellent
1.5	1.0	0.94^{ab}±0.27	Fair
2.0	1.0	1.00^{ab}±0.24	Fair
2.5	1.0	0.91^{ab}±0.09	Good
3.0	1.0	1.57^{a}±0.55	Fair

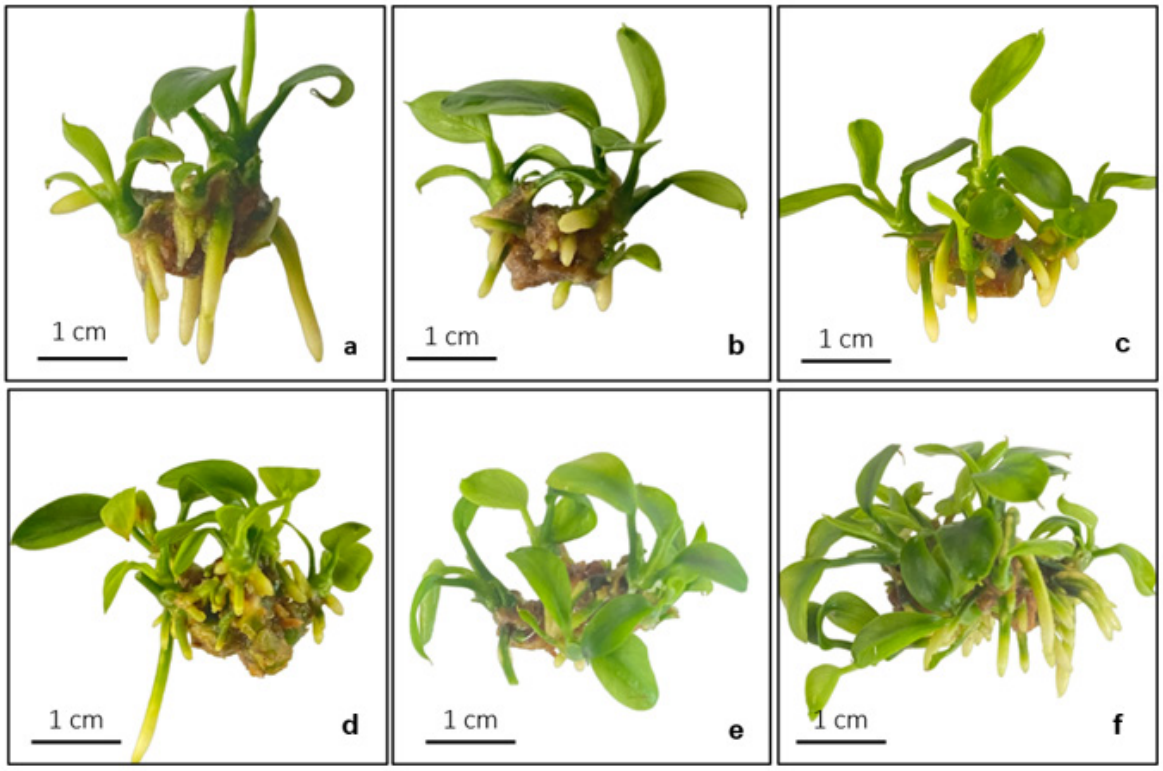
Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.05).

Table 5 Impact of potato extract on the quantity and quality of shoots

Potato extract (g/L)	Shoots/explant	Shoot height (cm)
0	2.33^{e}±0.11	1.21^{e}±0.06
10	4.19^{d}±0.13	1.72^{d}±0.13
20	4.37^{cd}±0.06	2.08^{c}±0.07
30	4.62^{c}±0.17	2.36^{b}±0.11
40	5.52^{b}±0.23	2.74^{a}±0.07
50	6.55^{a}±0.19	2.80^{a}±0.04

Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.05).

Figure 3 The effect of potato extract on the growth of PLBs derived plantlets



Notes: a = 0 g/L; b = 10 g/L; c = 20 g/L; d = 30 g/L; e = 40 g/L; f = 50 g/L.

Table 6 Effects of IBA on root and plantlet after 4 weeks of culture

IBA (mg/L)	No. of roots	Root length (cm)	Shoot height (cm)	No. of leaves
0.0	4.22^{bc}±0.29	1.64^{c}±0.33	4.12^{b}±0.31	5.22^{bc}±0.40
0.5	6.19^{a}±0.56	2.50^{a}±0.11	4.72^{a}±0.14	6.81^{a}±0.45
1.0	4.81^{b}±0.39	2.14^{ab}±0.19	4.80^{a}±0.19	6.11^{ab}±0.51
1.5	3.33^{cd}±0.22	1.74^{bc}±0.07	4.19^{b}±0.07	4.74^{cd}±0.17
2.0	2.70^{d}±0.17	1.05^{d}±0.02	3.94^{b}±0.09	4.00^{d}±0.22

Notes: Means followed by different letters indicate significant differences (Duncan's test, P = 0.05).

CONCLUSION

This study successfully developed a micropropagation protocol for *Anubias barteri* var. *nana* Petite, enabling rapid and efficient multiplication. Through precise sterilization and optimized growth conditions, including the use of 3 mg/L benzyl adenine (BAP), the protocol demonstrated high rates of shoot regeneration. Additionally, the induction of protocorm-like bodies (PLBs) from stem explants and subsequent shoot regeneration highlighted the effectiveness of 1.5 mg/L BAP combined with 0.5 mg/L NAA or 0.5 mg/L IAA; 3 mg/L BAP plus 1.0 mg/L IBA. The study also demonstrates the impact of 50 g/L of potato extract on shoot proliferation and 0.5 mg/L of indole-3-butyric acid (IBA) in root development. This improved method presents a promising approach for the mass production of *A. barteri* var. *nana* Petite, addressing the challenges associated with natural propagation and meeting market demands for this valued ornamental aquatic plant.

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