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In vitro propagation of an ornamental aquatic plant, *Anubias barterii* Var. Nana petite

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Abstract

An efficient protocol for rapid multiple shoots proliferation from the basal buds of a valuable ornamental aquatic plant, *Anubias barteri* var. nana petite was developed successfully. Treating the explants with 15% concentrated solution of commercial bleach (Robin liquid bleach, Reckitt Benckiser, India) for 10 mins followed by a quick dip in 70% ethanol was proved to be the best sterilization procedure to obtain clean cultures. After proper *in vitro* stabilization on plant growth regulator-free (PGR-free) Murashige and Skoog (MS) basal medium, earliest shoot bud regeneration was obtained on a medium containing full strength MS inorganic salts, 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 3% sucrose, 0.8% agar as gelling agent and supplemented with 0.25 mg/l 6-Benzylaminopurine (BAP). Maximum multiplication of the *in vitro* shoots was obtained on MS medium supplemented with 0.2 mg/l BAP. Lowering the concentration of BAP from 0.2 mg/l favoured shoot elongation and maximum elongation of the *in vitro* developed shoots was obtained on PGR-free MS basal medium. PGR-free MS basal medium as well as MS medium supplemented with a combination of 0.1 mg/l BAP and 0.1 mg/l NAA were equally good for producing maximum number of *in vitro* roots. The *in vitro* raised plantlets were successfully hardened and planted out in the aquarium.

Keywords: *Anubias barterii* var. nana petite; *in vitro* propagation; ornamental; aquatic plant

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Introduction

The family Aracea includes many familiar decorative plants, both terrestrial and aquatic. One genus that is particularly desirable for aquarium use is Anubias. The genus *Anubias* comprises eight species. Of these, *Anubias nana* Engler (Dwarf Anubias) is commercially cultivated for aquaria (Muhlberg, 1982). The plant was first discovered by Adolf Engler in 1899 from Cameroon as *Anubias nana* and the species was reduced to varietal status as *Anubias barteri* var. *nana* in 1979 (Karen, 1998). *Anubias barteri* var. *nana* ''petite'' is a man-made cultivated variety originally developed from *Anubias barteri* var. *nana* and has great aquascaping potential for every aquarium but can be especially useful in nana aquascapes. *Anubias barteri* var. *nana* petite is composed of several small leaves and a hard stalky stem called a rhizome. The entire plant grows to approximately 2 inch

wide by 2 inch tall (5 cm). The ½ inch wide (1 cm) leaves are beautifully round and dark green. These tiny leaves sprout from a rhizome which extends roots that anchor the plant to rocks and the substrate. Like most *Anubias* species, the plant does not require much attention and have high demands in the aquarium industry because of its minimal light requirements, hardy nature and attractive growth patterns.

Traditionally, *Anubias* is propagated vegetatively by rhizome division (Rataj and Horeman, 1977; Allgayer and Teton, 1986). Propagation of this particular variety without ensuring genetic uniformity will result in the production of undesired phenotypes, which will influence the quality and regeneration potential of the plants. Therefore, tissue culture technique is preferable for its propagation as the technique enables the production of large number of genetically uniform, vigorous, disease-free and virus-free plants (Alistock and Shafer, 2006). Moreover, tissue cultured water plant species show a more bushy growth with more adventitious shoots, a quality that many aquarists appreciate (Christensen, 1996). In spite of a considerable progress in developing *in vitro* micropropagation protocols in a variety of land plants (Ozcan et al., 1993, 1996; Khawar and Ozcan, 2002), aquatic plants in general have lagged behind to a considerable extent. The present study describes a rapid, simple and efficient micropropagation system from the basal shoot tip explants of *A. barteri* var. nana petite. The protocol developed by this study can be utilised for the commercial production of *A. barteri* meet the demands of aquarium industry.

Materials and Methods

Culture establishment: Mother plants (Fig. 1) were obtained from local dealers by ensuring that, they are visibly healthy and free from any signs of stress or surface blemishes. The selection was further carried out by growing the plants under environmentally controlled fresh water aquariums at 28 ± 2^0C with 12 hrs photoperiod. Lateral shoots containing the apical bud (Fig. 2) were excised from the mother plants and were washed under running tap water for 10 mins, soaked in 5% detergent solution (Cleansol, India) for 10 mins and again washed thoroughly in running tap water to remove the superficial dirt. Surface sterilants tried to obtain axenic cultures included 0.1% mercuric chloride ($HgCl_2$) and commercial bleach containing 5.25% sodium hypochlorite as active ingredient (Robin Liquid Bleach, Reckitt Benckiser, India). To standardize the most suitable surface sterilization procedure, explants were treated with 0.1% $HgCl_2$ for 5 different durations (1, 2, 3, 4 and 5 mins) and with six different dilutions of commercial bleach solution (5, 10, 15, 20, 25 and 30 %) for six different durations (1, 5, 10, 15, 20 and 25 mins). Each treatment was done with 10 replications and number of survival and formation of healthy cultures with each treatment was recorded after 4 weeks of explant inoculation. After discarding the surface sterilant, explants were washed thrice with sterile water, subjected to a quick dip in 70% ethanol, and again washed thrice with sterile water. Inside the laminar air chamber, the basal buds were isolated and inoculated on medium containing full strength Murashige and Skoog (MS) inorganic salts (Murashige and Skoog, 1962), 100 mg/l myo-inositol, 0.1 mg/l thiamine-HCl, 3% sucrose, 0.8% agar as gelling agent and devoid of any plant growth regulators (PGRs). The pH of the media was adjusted to 5.8 using 0.1 N HCl, dispensed in 150×25 mm culture tubes (15 ml medium per tube), and autoclaved at 121^0C for 15 mins. Cultures were maintained in a culture room at

25 ± 2^0C under a 16 hrs photoperiod with light intensity of 35 µmol photons $m^{-2}s^{-1}$ from Philips cool white fluorescent tubes. The cultures were subcultured thrice at an interval of 4 weeks on fresh media with the same media composition for attaining *in vitro* stabilization. The *in vitro* derived explants (basal buds) were then transferred to media containing various PGRs at varying concentrations to study the effect of various PGRs and their concentrations in the MS medium on shoot multiplication, shoot elongation and *in vitro* root formation for medium standardization.

Fig. 1. Mother plant of *Anubias barteri* var. nana petite

Fig. 2. Lateral shoots containing the basal bud

Media standardization

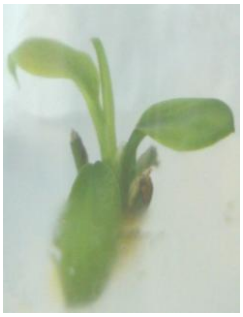
The media tried for earliest culture initiation and shoot regeneration included PGR-free MS basal medium and MS media supplemented with 0.1 to 0.3 mg/l (at an interval of 0.05) 6- Benzyl aminopurine (BAP), 0.1 to 0.3 mg/l (at an interval of 0.05) kinetin and 0.1 to 0.3 mg/l BAP in combination with 0.1mg/l α-naphthalene acetic acid (NAA). The basal buds from the *in vitro* rhizomes produced on each media were subjected to repeated subculture on the same media to test for consistency in shoot multiplication rate. Observations on culture initiation, shoot multiplication, shoot elongation and root formation on each media were recorded after 4 weeks of third subculture on each media.

To analyze the effect of 5 different concentrations of BAP, 5 different concentrations of kinetin and 5 different concentrations of BAP in combination with 0.1 mg/l NAA on culture initiation, three experiments were set up separately in a Completely Randomized Design (CRD), each with 6 treatments (including PGR-free MS basal as control) and 10 replications. Number of days taken for bud release on each media was taken as the observation and media on which shoot initiation occurred at earliest was standardized as most appropriate medium for culture initiation. In a similar way, three experiments each were set up to standardize the shoot multiplication medium and shoot elongation medium. To standardize medium for *in vitro* root formation, there was only a single experimental set up which was in CRD with 6 treatments (0 to 0.3 mg/l BAP in combination with 0.1 mg/l NAA) and 10 replications. To standardize best medium for shoot multiplication, number of shoots formed on various media after 4 weeks of third subculture was taken as observation. The length (cm) of the *in vitro* shoots (length of stolon) and number of *in vitro* roots per shoot on each medium after 4 weeks of third subculture were taken as observations to standardize the media for shoot elongation and *in vitro* root formation respectively. Data were subjected to square root transformation (except shoot elongation data) and analyzed by Univariate Analysis of Variance using SPSS ver. 20. Significant differences if any between means were compared by Tukey HSD (Zar, 1999).

Hardening

Hardening was done by culturing the rooted plantlets on reduced concentration ($1/10^{th}$) of MS salts devoid of any plant growth regulators, agar or sucrose for the first 4 weeks and then on distilled water for the next 4 weeks under controlled conditions of 24 ± 2^0C under a 12 hrs photoperiod with light intensity of 35 µmol photons $m^{-2}s^{-1}$. The plants were then planted in small plastic cups containing Metro Mix-500 planting medium. A total of 20 plants were subjected to the hardening procedure at a time and the number of survival after 8 weeks of their final transfer to aquaria was considered as a measure of the procedure's reliability. The acclimatization experiments were repeated once.

Fig. 3. Culture initiation on FMS basal medium



Results and Discussion

It was observed that, when the basal bud explants were treated with 0.1% $HgCl_2$, shorter treatment durations (1 min) resulted in contaminated cultures and longer treatment durations (above 1 min) resulted in scorching of the tissue. Only 2 out of 10 explants treated with 0.1% $HgCl_2$ for 1 min produced contamination-free healthy cultures and none of the explants were survived when treated with 0.1% $HgCl_2$ for more than 1 min. Explant sterilization using 0.1 to 0.8% $HgCl_2$ have been reported in *Piper longum* (Sonia and Das, 2002), *Spilanthes acmella* (Haw and Keng, 2003) and *Bacopa monnieri* (Sharma et al., 2010). But in the present study, $HgCl_2$ was not found as suitable for producing axenic cultures from the explants. Among the various concentrations of commercial bleach and various durations of treatment tried, 90% of the apical bud explants treated with 15% concentrated solution of commercial bleach (0.79% Sodium hypochlorite as active ingredient) for 10 mins followed by a quick dip in 70% ethanol produced contamination-free healthy cultures (Table 1). When the treatment duration and bleach concentration decreased from the optimum level, rate of contamination was steadily increased; whereas, scorching of the tissue was observed with increase in bleach concentration and treatment duration (Table 1). Reports on explant sterilization using the appropriate concentration of commercial bleach are available on *Ludwigia repens* (Ozturk et al., 2004), *Phyla nodiflora* (Ahmed et al., 2005), *Rosa damascene* (Nikbakht et al., 2005), *Limnophila aromatica* (George et al., 2014a) and *Echinodorus argentinensis* (George et al., 2014b). Present result also support the use of alcohol in combination with commercial bleach for surface sterilization of explants as advocated by Bonga (1982), Maroti et al. (1982), Jenks et al. (2000) and Maridass et al. (2010).

Shoot initials without any callus formation were clearly visible on all the basal bud explants cultured on PGR-free MS basal medium within 4-6 weeks of explant inoculation, which subsequently developed into normal shoots after 6-8 weeks of culture initiation (Fig. 3). Time taken for bud release was decreased with subsequent sub culturing in the same medium as well as upon transfer to PGR-containing media. The results of the experiments conducted for the determination of appropriate concentration/combination of PGRs in the MS medium for earliest culture initiation, maximum multiple shoot proliferation and maximum shoot elongation are shown in

Table 1. Response shown by the basal bud explants of *A. barteri* to sterilization with various concentration of bleach for various durations

Concentration of bleach solution (%)	Duration of treatment (minutes)	Rate of contamination (%)	Uncontaminated cultures	
			Rate of Healthy cultures (%)	Rate of Scorching (%)
Control	-	100	0	0
10	10	60	40	0
10	15	50	40	10
10	20	50	30	20
10	25	50	30	20
15	10	10	90	0
15	15	0	70	30
15	20	0	50	50
15	25	0	40	60

Table 2. *In vitro* responses shown by the basal bud explants of *A. barteri* to various concentrations/combinations of PGRs in the MS medium

PGR	Conc. (mg/l)	Average no. of days taken for bud release (Mean±SD)	Average no. of Shoots/explant (Mean±SD)	Average length of the shoot (cm) (Mean±SD)
None	-	5.4659 ± 0.16475^{e}	1.3535 ± 0.27486^{a}	5.1390 ± 0.20712^{e}
BAP	0.10	4.6648 ± 0.21024^{d}	1.9196 ± 0.12943^{b}	4.6500 ± 0.35978^{d}
BAP	0.15	4.3109 ± 0.13457^{c}	2.5019 ± 0.21263^{de}	3.8400 ± 0.25033^{c}
BAP	0.20	3.8581 ± 0.12848^{b}	2.7687 ± 0.19543^{e}	2.2750 ± 0.31380^{b}
BAP	0.25	3.2365 ± 0.16708^{a}	2.3315 ± 0.26651^{cd}	1.2100 ± 0.32128^{a}
BAP	0.30	3.2373 ± 0.14904^{a}	2.0644 ± 0.20567^{bc}	1.1300 ± 0.21628^{a}
kinetin	0.10	4.6760 ± 0.19774^{c}	1.9904 ± 0.20593^{b}	4.5600 ± 0.29136^{c}
kinetin	0.15	4.1889 ± 0.24265^{b}	1.9904 ± 0.20593^{b}	4.1500 ± 0.46488^{c}
kinetin	0.20	3.6045 ± 0.09253^{a}	2.2956 ± 0.18373^{b}	2.6300 ± 0.62191^{b}
kinetin	0.25	3.6595 ± 0.09636^{a}	2.0358 ± 0.24793^{b}	2.1900 ± 0.42019^{b}
kinetin	0.30	3.6181 ± 0.10221^{a}	1.6074 ± 0.35919^{a}	1.6000 ± 0.24495^{a}
BAP+NAA	0.10+0.10	5.4579 ± 0.11264^{a}	1.6367 ± 0.15353^{abc}	1.9000 ± 0.36515^{c}
BAP+NAA	0.15+0.10	5.6372 ± 0.15497^{ab}	1.4999 ± 0.23622^{a}	1.7600 ± 0.62397^{ab}
BAP+NAA	0.20+0.10	5.5851 ± 0.08250^{a}	1.9914 ± 0.38608^{bc}	1.7500 ± 0.92045^{ab}
BAP+NAA	0.25+0.10	5.9463 ± 0.51792^{bc}	1.6164 ± 0.45603^{ab}	1.2900 ± 0.47947^{ab}
BAP+NAA	0.30+0.10	6.2015 ± 0.21542^{c}	2.0172 ± 0.18494^{c}	1.0800 ± 0.10328^{a}

Means of 10 observations. Means with different superscripts in the same column of the same experiment are significantly different ($p< 0.05$). Effect of various concentrations of BAP, kinetin and BAP+NAA are taken as separate experiments

Table 3. Effect of various concentrations of BAP in combination with 0.1 mgl^{-1} NAA on root formation in *A. barteri*

Treatments	Concentration of PGRs(mgl^{-1})	Average number of roots/shoot Mean ± SD (cm)
MS basal (PGR free)	-	2.3855 ± 0.10309^{c}
MS + BAP + NAA	0.10, 0.1	2.3887 ± 0.19729^{c}
MS + BAP + NAA	0.15, 0.1	1.8497 ± 0.29574^{b}
MS + BAP + NAA	0.20, 0.1	1.6049 ± 0.16413^{b}
MS + BAP + NAA	0.25, 0.1	1.8025 ± 0.23839^{b}
MS + BAP + NAA	0.30, 0.1	1.1975 ± 0.27094^{a}

Table 2 Effect of various concentrations of BAP in combination with 0.1 mg/l NAA on *in vitro* root formation is provided in Table 3. Statistical analysis of the data showed that, the average number of days taken for axillary bud release, average number of shoots formed per explant, average length (cm) of the shoot and average number of roots per shoot vary significantly ($p< 0.05$) with various concentrations/combinations of cytokinins in the MS media (Table 4). It took an average of 5.4659 days for sprouting on MS basal medium and when 0.1 to 3.0 mg/l BAP or kinetin was added to the medium, average time taken for sprouting become lowered. Even though, the best result among the different concentrations of kinetin tried was observed with 0.2 mg/l (an average of 3.6045 days for sprouting), Post Hoc test revealed that, culture initiation on media containing 0.25 and 3 mg/l kinetin are also at par (Table 2). Among the media containing various concentrations of BAP or kinetin, culture initiation occurred at the earliest (an average of 3.2365 days) on MS medium supplemented with 0.25 mg/l BAP. When the concentration of BAP in the medium was increased from this optimum level, time taken for bud release was also increased (Table 2). Callus formation from the explant base was also observed with higher concentrations (≥ 0.3 mg/l) of BAP or kinetin in the media. With all the combinations of BAP and NAA tried, average numbers of days taken for bud release were greater than that on PGR-free MS basal medium. Present observations support the opinion that, incorporation of PGRs into the medium favours early bud break from the explants (Patnaik and Chand, 1996; Tiwari et al., 1998). Huang and Chang (1994) have reported that, micropropagation of *Anubias barteri* var. undulata was achieved from the lateral shoot tip explants cultured on MS medium supplemented 0.3 mg/l BAP and 0.1 mg/l NAA. But in the present study, media containing 0.1 mg/l NAA in combination with 0 to 0.3 mg/l BAP was not favourable for inducing bud break at the earliest. The present result is also contradicted by the reports on the efficiency of auxin-cytokinin combinations on culture establishment in *Acorus calamus* (Anu et al., 2001), *L. repens* (Ozturk et al., 2004), *P. nodiflora* (Ahmed et al., 2005), *R. damascene* (Nikbakht et al., 2005), *Cryptocoryne wendtii* and *Echinodorus cordifolius* (Dissanayake, 2007). Present result is supported by the reports on early induction of shoot buds on MS medium supplemented with BAP alone in the micropropagation of *Myriophyllum* sp. (Kane and Gilman,

1991), *B. monnieri* (Tiwari et al., 1998; Sharma et al., 2010), *S. acmella* (Haw and Keng, 2003) and *Myriophyllum aquaticum* (Smitha et al., 2005). Culture initiation from the lateral shoot tip explants of *Anubias barterii* var. nana on MS medium containing 3.0 mg/l BAP alone have been reported by Kanchanapoom and Chunui (2012). Even though, such a higher concentration of BAP was fatal to the explants in the present study, the results support the use of BAP alone in the MS medium for culture initiation.

Among the various media tried for multiple shoot proliferation, the best result was obtained with MS medium containing 0.2 mg/l BAP (Table 2, Fig. 3). A gradual reduction in average shoot number was observed with increase in the concentration of BAP from the optimum level and when the concentration of BAP or kinetin in the medium was increased beyond 0.3 mg/l, 90% of the explants showed basal callusing. Similar tendency was also visible with kinetin-media too. Post Hoc test showed similarity in shoot multiplication with all the concentrations of kinetin tried except 0.3 mg/l (Table 2). In the present study, addition of 0.1 mg/l NAA into the BAP-containing media did not show any superior effect on the number of shoots formed per explant. Present observations on shoot multiplication are in line with the reports on enhanced release of multiple shoots on MS media containing optimum concentration of BAP in the micropropagation of *B. monnieri* (Tiwari et al., 1998; Shrivasthava and Rajani, 1999; Sharma et al., 2010), *Avicenia marina* (Al-Bahrany and Al-Khayri, 2003), *P. nodiflora* (Ahmed et al., 2005), *M. aquaticum* (Smitha et al., 2005), *L. aromatica* (George et al., 2014a) and *E. argentinensis* (George et al., 2014b). Kanchanapoom and Chunui (2012) has reported culture initiation and shoot multiplication from the lateral shoot tip explants of *A. barterii* var. *nana* on MS medium containing 3mg/l BAP. But in the present study, when the BAP concentration exceeded 1 mg/l, the cultures were wilted, showing the toxic effect of high concentrations of BAP in *Anubias barterii* cultures. The difference in hormone requirement may be due to difference in the endogenous hormone levels in these two varieties. Another reason for difference in the hormonal requirement of *A. barterii* var. *nana* and *Anubias barterii* var. *nana* petite may be that, species that have conditioned in different environments have altered sensitivity to hormones for shoot induction and proliferation as opined by Dissanayake et al. (2007).

Fig. 4. Multiple shoots on MS media containing 0.2 mgl^{-1} kinetin

Fig. 5. Shoot elongation and *in vitro* root formation on FMS basal medium

The *in vitro* shoots elongated to an average of 5.139 cm on PGR-free MS basal medium. Average lengths of the *in vitro* shoots on media containing 0.1 mg/l BAP and 0.1 mg/l kinetin were 4.65cm and 4.56 cm respectively.

Table 4. ANOVA of the experiments to analyze the effect of various concentrations/combinations of PGRs on culture initiation, shoot multiplication, shoot elongation and root formation in *A. barteri*

Experiment	Sum of Squares	df	Mean Square	F	Sig
Effect of various concentrations of BAP on culture initiation[1]:					
Treatment	37.724	5	7.545	290.02*	.000
Error	1.405	54	.026		
Total	39.129	59			
Effect of various concentrations of kinetin on culture initiation[1]:					
Treatment	28.145	5	5.629	220.138*	.000
Error	1.381	54	.026		
Total	29.526	59			
Effect of various concentrations of BAP and 0.1 mg/l NAA on culture initiation[1]:					
Treatment	4.412	5	.882	13.742*	.000
Error	3.468	54	.064		
Total	7.880	59			
Effect of various concentrations of BAP on shoot multiplication[1]:					
Treatment	12.341	5	2.468	51.235*	.000
Error	2.601	54	.048		
Total	14.942	59			
Effect of various concentrations of kinetin on shoot multiplication[1]:					
Treatment	5.728	5	1.146	17.873*	.000
Error	3.461	54	.064		
Total	9.190	59			
Effect of various concentrations of BAP and 0.1 mg/l NAA on shoot multiplication[1]:					
Treatment	3.554	5	.711	7.810*	.000
Error	4.915	54	.091		
Total	8.470	59			
Effect of various concentrations of BAP on shoot elongation:					
Treatment	152.201	5	30.440	377.763*	.000
Error	4.351	54	.081		
Total	156.553	59			
Effect of various concentrations of kinetin on shoot elongation :					
Treatment	102.264	5	20.453	126.874*	.000
Error	8.705	54	.161		
Total	110.969	59			
Effect of various concentrations of BAP and 0.1 mg/l NAA on shoot elongation:					
Treatment	111.932	5	22.386	81.240*	.000
Error	14.880	54	.276		
Total	126.812	59			
Effect of various concentrations of BAP and 0.1 mg/l NAA on root formation[1]:					
Treatment	9.009	5	1.802	36.746*	.000
Error	2.648	54	.049		
Total	11.656	59			

[1]Data subjected to square root transformation * Treatments are significantly different ($p< 0.05$)

The shoot length further decreased with increase in the concentration of BAP or kinetin in the medium. Post Hoc test showed similarities between the average shoot lengths observed on MS medium supplemented with 0.25 mg/l BAP and MS medium supplemented with 0.30 mg/l BAP. The results also revealed that, addition of 0.1 mg/l

NAA to the BAP-containing media suppressed shoot elongation and caused wilting of the *in vitro* shoots. Russel and Mc Cown (1986), Singha and Bhatia (1988), Fasolo et al. (1989), Preece and Imel (1991) and Ozturk et al. (2004) have opined that, presence of growth regulators is not essential for the growth of the *in vitro* developed shoots. The present study is in support of this opinion as the regenerated shoots elongated to the maximum on PGR-free MS basal media (Table 2, Fig. 4). Gradual decrease in shoot length was observed when 0.1 to 0.3 mg/l BAP or kinetin was incorporated in to MS media and shoot elongation was least at 0.3 mg/l BAP and kinetin (Table 2). Similar observations on suppressive effect of higher concentrations of cytokinins on shoot elongation were also reported in *L. repens* (Ozturk et al., 2004), *L. aromatica* (George et al., 2014a) and *E. argentinensis* (George et al., 2014b). Shoots developed in the presence of cytokinin

Fig. 6. Plantlet after acclimatization

generally lack roots (Fasolo et al. 1989; Yunsita et al., 1990; Preece et al., 1991; Huetteman and Preece, 1993). But in the case of *A. barteri*, root formation was observed on all the shoot multiplication media. As the media supplemented with various concentrations of BAP in combination with 0.1 mg/l NAA seemed to produce more number of healthy roots than the media supplemented with BAP or kinetin alone, number of roots produced on media containing various concentrations of BAP in combination with 0.1 mg/l NAA was statistically analyzed. Average number of roots formed per shoot on each medium is given in Table 3. Analysis of variance showed that, average number of roots/shoots differ significantly ($p< 0.05$) with various concentrations of BAP + 0.1 mg/l NAA (Table 4). Among the various media tried, average number of roots/shoot was highest on medium supplemented with 0.1 mg/l BAP + 0.1 mg/l NAA. As the Post-hoc revealed PGR-free MS basal medium as equally good for inducing *in vitro* root formation (Table 3), it is preferred as the rooting medium considering the cost-effective aspect. 100% of the acclimatized plantlets (Fig. 6) were survived and established well in the aquarium.

Conclusion

Anubias barterii var. *nana* petite has high demands in the aquarium industry. While its characteristics are like that of *Anubias barteri* var. *nana* in almost every aspect, its smaller stature is displaced by its disproportionately larger price tag and lack of availability. The *in vitro* propagation protocol described here can be utilized for the large scale production of this plant so as to increase its availability in the market at cheaper cost.

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