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Original article

Ex-situ propagation of *Pogostemon helferi* (Hook. f.) Press using tissue culture and a hydroponics system

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ABSTRACT

Pogostemon helferi (Hook. f.) Press, locally known as "dao-noi" is a rare Thai indigenous aquatic plant that is popular for use in aquaria and water gardens. To address its scarcity and to make the plant more readily available, two experiments were conducted to find the optimum conditions for *ex-situ* propagation. The first experiment aimed to determine the concentration of growth regulators for its micropropagation. Sterile explants were cultured using a combination of 3-indoleacetic acid (IAA) and 6-benzylaminopurine (BA) supplements in Murashige and Skoog media (MS). MS media containing IAA 0.1 mg/L and BA 3 mg/L induced the highest percentage of callus formation (93.33%). In addition, MS media with IAA added at 0.3 mg/L significantly ($p < 0.05$) increased the number of new shoots appearing and their length after 8 wk. The second experiment aimed to determine the optimum electrical conductivity of the nutrient solution and the humidity level for *P. helferi* growth within a hydroponics system. The results showed that an electrical conductivity of 1.6 mS/cm and 80% humidity were optimal for *P. helferi* growth and production to a marketable size. These methods should enable the production of *P. helferi* appropriate to support market demand and thus can reduce the current practice of harvesting wild plants in their natural habitat.

Introduction

Pogostemon helferi (Hook. f.) Press is an ornamental plant, belonging to the family Lamiaceae, which is commonly known by its local name of "dao-noi", which means "little star" and is distributed across Myanmar and western Thailand (Tarepunda, 2004). A survey conducted by Bongcheewin (2005) in Thailand found it at sites in north, north-east, east and west Thailand. However, a more recent national survey undertaken by Christensen et al. (2007) only found *P. helferi* within Kanchanaburi province which lies close to the border with Myanmar. In Thailand, it is a popular indigenous aquatic plant for decorating aquaria, making an attractive mid-to foreground plant perhaps because of its bushy appearance, curly leaves and aesthetically pleasing green coloration and these striking features have created a high demand for *P. helferi* within the aquarium trade (Prasartkul, 2004). At the present time though, the increasing popularity of nano-aquaria is generating further demand for bushy aquarium plants. The current artisanal production of *P. helferi* is not sufficient to meet the demands of the aquarium market. The economic value of national *P. helferi* production annually can be estimated at approximately THB 252,000 (equivalent to USD 7010 at current exchange rates) whereas the annual market demand for *P. helferi* is more than THB 500,000 (USD 13,910) according to C. Tienrungsri (personal communication). In Europe, this aquatic plant retails at GBP 3.25–3.49 (USD 4.92–5.29) according to Aquarium Gardens (2015). However, most stocks of this plant sold on the market are harvested from nature for commercial purposes. In addition, deforestation and damage to natural ecosystems has increased. As a result, *P. helferi* has become rare in the wild and as there is no compensatory planting to counter the decline, local extinction is emerging as a possibility. Unfortunately, numerous specific conditions are required for successful *P. helferi* propagation in both the wild and under artificial culture conditions and yet research regarding appropriate propagation techniques is limited. Tissue culture techniques offer an alternative tool for the rapid multiplication of plants within a short period. This approach could provide an alternative methodological approach for the

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large-scale propagation of *P. helferi*. Tissue culture techniques have been reported for some aquatic plants, for example, *Juncus effuses* (Sarma and Rogers, 2000), *Scirpus robustus* (Wang et al., 2004), *Myriophyllum spicatum* and *Potamogeton crispus* (Zhou et al., 2006), *Porphyra yezoensis* (Liu et al., 2004), *Halophila decipiens* (Bird et al., 1998), *Cymodocea nodosa* (Garcia-Jimenez et al., 2006), *Posidonia oceanica* (Balestri and Cinelli, 2001), *Cryptocoryne lucens* (Kane et al., 1990) and *Cryptocoryne wendtii* (Kane et al., 1999). Thus, the culture of *P. helferi* within a hydroponics system using tissue culture techniques to increase efficiency offers a realistic technological approach to meet the current market demands for this aquatic plant and by switching to artificial culture practices would reduce harvest from the wild. This approach would also create opportunities for commercial exports.

A specific objective of the current study was to investigate the effects of the plant growth regulators 3-indoleacetic acid (IAA) and 6-benzylaminopurine (BA) on *P. helferi* callus induction and on shoot proliferation when reared in tissue culture. The study also investigated the effect of differing levels of electrical conductivity of the nutrient solution and humidity conditions within a hydroponics system on the growth of *P. helferi* with a view to developing a practical propagation method. The hypothesis under evaluation was whether the micropropagation of *P. helferi* integrated with hydroponics would increase production of quality specimens appropriate to meet the current demands of the aquarium market trade. In addition, tissue culture of *P. helferi* offers the means to mass produce specimens which could be exported with fewer biosecurity risks than from specimens harvested from the wild.

Materials and methods

Tissue culture

The axillary bud explants of *P. helferi* which measured approximately 3 mm in length were thoroughly washed under running tap water, then surface-sterilized by dipping each explant in a detergent solution for 10 min and then washed in two changes of sterile distilled water. Thereafter, the explants were subjected to a treatment of 10% sodium hypochlorite for 20 min followed by a 15 min treatment with 5% sodium hypochlorite and then they were rinsed with three changes of sterile distilled water. The surface-sterilized explants were then placed on sterilized filter paper, to remove the excess moisture, and then cultured in the Murashige and Skoog (MS) media (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.16% gelrite as a solidifying agent. The pH of the media was adjusted to 5.6 with 10% HCl and 2 M NaOH. The media were used in combination with two growth regulators: either 0 mg/L, 0.1 mg/L, 0.2 mg/L or 0.3 mg/L IAA or 0 mg/L, 1 mg/L, 2 mg/L or 3 mg/L BA. A volume of 30 mL MS was dispensed into 340 mL-sized bottles, with plastic caps, and then sterilized at 121 °C under 1 kg/cm^2 pressure for 15 min. The explants were inoculated on the surface of the media under aseptic conditions. The bottles were incubated at 25 °C under a 12 h photoperiod of light at an intensity of 2200 lux provided by fluorescent tubes in a growth room. The data (callus, the number of shoots, and the height of the plants) were recorded on a weekly basis for 8 wk. A factorial experimental design was used, arranged in a completely randomized design with 15 replications in each test group. Each explant was cultured in a separate bottle.

Hydroponics system

Samples of *P. helferi* from tissue culture with a height of approximately 5 cm (Fig. 1A) were cleaned and cut into root and leaf parts. Thereafter, the samples were cultured with rock wool as a planting material, and then transplanted into pots and transferred to a greenhouse for 2 wk of adaptation before the start of the experiment (Fig. 1B).

Nutrient concentration

A recirculation system incorporating a deep-flow technique (DFT) was set up in a greenhouse using KMITL 2 formula prepared as presented in Table 1. The experiment used a completely randomized design, which included three replications with 15 samples per replication. Five electric conductivity (EC) levels of nutrient solution, (0.4 mS/cm, 0.8 mS/cm, 1.2 mS/cm, 1.6 mS/cm and 2.0 mS/cm) were used to investigate the growth performance of *P. helferi*. The hydroponics system used fifteen 2 m-long polyvinylchloride pipes, each 5 cm in diameter, and 15 evenly spaced holes, each 5 cm in diameter, were made in each pipe into which the plant culture pots were inserted. Plants of a uniform size were selected and transferred into the hydroponics system at 60% humidity after running a test on the system for 1 d.

Humidity

Using the optimal EC levels of nutrient solution determined from the previous experiment, a subsequent trial explored four humidity levels, i.e. 90%, 80%, 70% and 60%, to determine the best level for the culture of *P. helferi* using a DFT-based hydroponics system. The experiment was conducted using a completely randomized design incorporating 3 replications with each replication consisting of 15 samples.

Statistical analysis

All tissue culture data, (callus development, number of shoots and the height of plants) and the data from the growth trial within the hydroponics system (the height, diameter and number of shoots on each plant) were analyzed using ANOVA in the SPSS version 11 software (SPSS Inc., Chicago, IL, USA). Duncan's new multiple range test was used for the comparison of means at a significance level of $p < 0.05$.

Results and discussion

Tissue culture

At the end of the eighth week of *in vitro* culture in the *P. helferi* experiment, no interaction between IAA and BA in callus induction was observed (Table 2). Callus initiation was observed within 1 wk; the plant growth regulator BA induced callus formation, whereas the media lacking BA did not induce the formation of callus. Each treatment of MS media containing a different concentration of IAA but without BA did not significantly promote the induction of callus. In general, the ratio of auxin and cytokinin controls the differentiation of organogenesis and morphogenesis in plant cell cultures. If the ratio of auxin is more than cytokinin, then it can induce root formation. However, if the amount of auxin is less than that of cytokinin, then it can induce shoots or plantlets and if the amounts of auxin and cytokinin are equal, then it can induce the formation of callus (Skoog and Miller, 1957).

In this study, the formation of *P. helferi* calli increased with increasing concentrations of BA (Table 3), a finding which is consistent with those of Hembrom et al. (2006) who reported that increasing concentrations of BA produced a concomitant increase in callus formation in *Pogostemon heyneanus*. In addition, the current trial results did not align with the hypothesis given above, in that when the ratio of auxin was less than that of cytokinin, callus

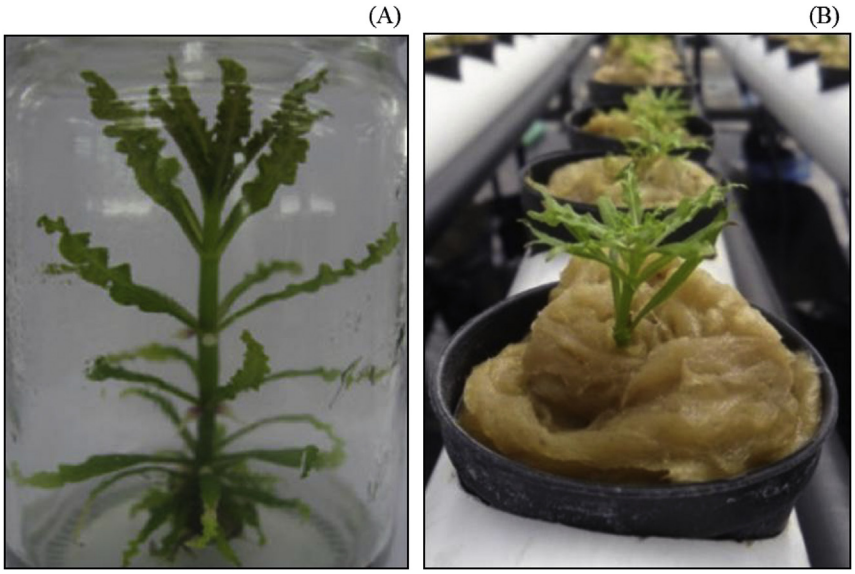

Fig. 1. (A) Specimen of *Pogostemon helferi* measuring approximately 5 cm in length derived from tissue culture and on-grown within a hydroponics system. (B) Specimens of *P. helferi* transferred to pots for experimental purposes within a greenhouse facility.

Table 1
Composition of the nutrient solution "KMITL 2 formula".

Chemical compound	Quantity/20 L
Soluble A	
1. Calcium nitrate	3.767 kg
2. Iron chelate	0.303 kg
Soluble B	
1. Potassium nitrate	1.769 kg
2. Potassium phosphate	0.653 kg
3. Magnesium sulfate	1.037 kg
4. Zinc sulfate	4.756 g
5. Copper sulfate	1.016 g
6. Manganese sulfate	14.194 g
7. Boric acid	8.894 g

Table 2
Tests investigating the effect of 3-indoleacetic acid (IAA) and indole 6-benzylaminopurine (IBA) on callus induction in *Pogostemon helferi* reared using a tissue-culture-based method. Each data point is derived from 15 replicates.

Source of variance	df	SS	MS	F	*p*-value
Treatment	15	246,500.00	16,433.33	10.46	0.00
IAA	3	4833.33	1611.11	1.03	0.38
BA	3	237,833.33	79,277.78	50.45	0.00
IAA × BA	9	3833.33	425.93	0.27	0.98
Error	224	352,000.00	1571.43		
Total	240	1,140,000.00			

df = degrees of freedom; SS = sum of squares; MS = mean square; F = F test value.

formation in *P. helferi* could be induced. Elsewhere, the research findings of Tang et al. (2009) reported that MS media containing 0.5 mg/L 2, 4-D and 2.0 mg/L BA was appropriate for inducing callus formation in *Momordica charantia*. Erisen et al. (2010) reported 100% callus formation in *Astragalus nezaketae* within 4 wk when grown in media with 0.5 mg/L NAA and 4 mg/L BA. Satyavani et al. (2011), who studied the tissue culture of *Citrullus colocynthis* in MS media containing IAA and 0.5 mg/L 2, 4-D combined with 1 mg/L BA, found that 80% of the stem explants were able to produce callus. However, the occurrence of callus depends on many factors, as variability exists among genera, species, explants, the age or stage of the plants and even cultivars in the type and amount of auxin and cytokinin required for induction of morphogenesis (Skoog and Miller, 1957). In the present study, the treatment of *P. helferi* cultured on 3 mg/L BA with 0.1 mg/L IAA in MS media gave the highest percentage (±SE) of callus formation (93.33 ± 6.67%) in 3 wk (Table 3). The calli obtained were mostly green and compact in appearance (Fig. 2A). They were then sub-cultured for further proliferation in the same media for a further 3 wk, during which a large number of new shoots were seen to develop from the calli (Fig. 2B). The MS media without BA had a significant number of induced new shoots. *P. helferi* axillary buds given a treatment of 0.3 mg/L IAA without BA were able to produce the highest number (±SE) of new shoots per explant of 6.58 ± 0.85 (Table 4). However, an increasing concentration of BA resulted in a decreasing number of new shoots, possibly due to the inhibition of organogenesis and

Table 3
Occurrence of *Pogostemon helferi* callus induction (percent) after 3 wk when grown in Murashige and Skoog media containing various concentrations of 3-indoleacetic acid (IAA) and indole 6-benzylaminopurine (BA). Each data point is derived from 15 replicates.

BA (mg/L)	IAA (mg/L)				Mean ± SE
	0.0	0.1	0.2	0.3	
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[d]
1	33.33 ± 12.60	46.67 ± 13.33	40.00 ± 13.09	40.00 ± 13.09	40.00 ± 6.38[c]
2	53.33 ± 13.33	73.33 ± 11.82	73.33 ± 11.82	66.67 ± 12.60	66.67 ± 6.14[b]
3	80.00 ± 10.69	93.33 ± 6.67	86.67 ± 9.09	73.33 ± 11.82	83.33 ± 4.85[a]
Mean ± SE	41.67 ± 6.42[a]	53.33 ± 6.49[a]	50.00 ± 6.51[a]	45.00 ± 6.48[a]	

Mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

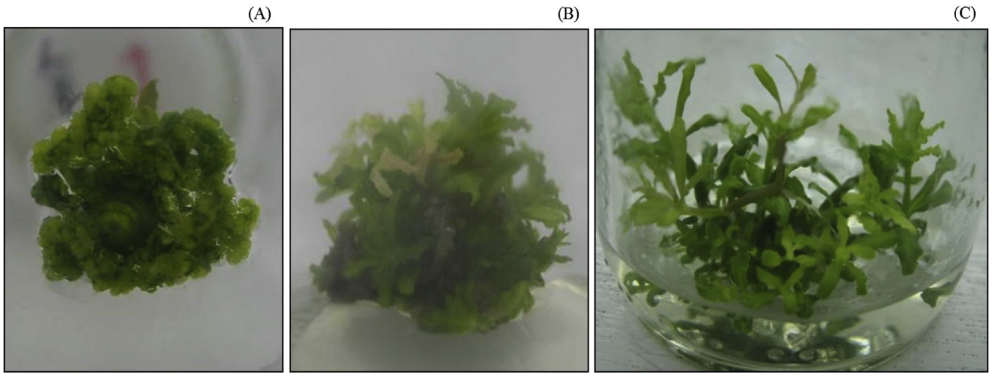

Fig. 2. (A) Callus induction of *Pogostemon helferi* occurred within three weeks when grown in Murashige and Skoog (MS) media containing 3-indoleacetic acid (IAA) 0.1 mg/L and 6-benzylaminopurine 3 mg/L; (B) Callus with new shoots after a further 3 wk; (C) Specimen of *P. helferi* cultured in MS media containing IAA 0.3 mg/L for 8 wk.

Table 4
Number of new shoots appearing on *Pogostemon helferi* specimens when grown in Murashige and Skoog media containing various concentrations of 3-indoleacetic acid (IAA) and 6-benzylaminopurine (BA) after 8 wk. Each data point is derived from 15 replicates.

BA (mg/L)	IAA (mg/L)				Mean ± SE
	0.0	0.1	0.2	0.3	
0	4.08 ± 0.76	3.50 ± 0.66	4.17 ± 0.51	6.58 ± 0.85	4.58 ± 0.38[a]
1	4.08 ± 0.38	3.75 ± 0.57	2.00 ± 0.51	3.17 ± 0.61	3.25 ± 0.28[b]
2	4.17 ± 0.91	3.33 ± 0.96	3.33 ± 0.91	1.83 ± 1.05	3.17 ± 0.48[b]
3	3.42 ± 0.98	5.75 ± 0.57	4.92 ± 0.86	5.25 ± 0.73	4.83 ± 0.41[a]
Mean ± SE	3.94 ± 0.39[a]	4.08 ± 0.37[a]	3.60 ± 0.38[a]	4.21 ± 0.48[a]	

Mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

the induction of callus formation. Likewise, Swamy et al. (2010) reported a decrease in shoot production in *Pogostemon cablin* at higher concentrations of BA. Stunted shoots that developed in the medium corresponding to increased concentrations of BA have been reported for *Orthosiphon stamineus* (Lai-Keng and Leng, 2004) and in the same manner as *Eupatorium triplinerve* (Martin, 2004). It was also found that calli that were grown on MS media containing 3 mg/L BA and 0.3 mg/L IAA were able to develop 5.25 ± 0.73 shoots per explant (±SE).

In addition, BA was also observed to affect the height of *P. helferi*, with MS media containing IAA without BA generating the tallest plants as shown in Table 5. The results showed that the stems and leaves were noticeably larger in the MS medium with 0.3 mg/L IAA added (Fig. 2C). Effective regeneration and healthy shoot development were also observed in these treatments as the plant growth regulator IAA affected the expansion of plant cells (Skoog and Miller, 1957).

Hydroponics system

Effect of nutrient solution on the growth of P. helferi

The growth of *P. helferi* was studied using the DFT involving nutrient solutions at five different EC levels from 0.4 mS/cm to 2.0 mS/cm. It was found that the differing EC levels significantly affected the height and shoot number of *P. helferi* but did not significantly affect the diameter of the shrubs (Table 6). The specimens of *P. helferi* cultured at EC levels of 1.6 mS/cm had the greatest growth with respect to their height with a significant increase in the number of shoots (Table 6). After 6 wk, the specimens of *P. helferi* cultivated at the EC level of 1.6 mS/cm began to grow better than those under the other treatments. The height increased to be 7.47 ± 0.44 cm taller than the plants of other treatments experiment which was significant until the experiment was terminated at 10 wk.

For the specimens of *P. helferi* grown at different levels of EC for 10 wk, there was a noticeable difference in the diameter of the shrubs by the second week which resulted in different sizes among different EC treatments in the second week. However, by the end of the fourth week and throughout the remaining culture period, there was no significant difference in the diameter of the shrubs.

A comparison of the average number of new shoots on the *P. helferi* grown in nutrient solutions with differing EC levels found that there were significantly more shoots on the plants grown at an EC of 1.6 mS/cm at the start of week 8 than on any other plants.

Previous studies have reported that some species of aquatic plants can grow well in EC conditions of 0.5–1.0 mS/cm for example, *Bolbitis heteroclita*, one popular freshwater aquarium plant, can grow well in sand culture without soil at an EC level of

Table 5
Length of *Pogostemon helferi* shoots (in centimeters) after 8 wk when grown in Murashige and Skoog media containing various concentrations of 3-indoleacetic acid (IAA) and 6-benzylaminopurine (BA). Each data point is derived from 15 replicates.

BA (mg/L)	IAA (mg/L)				Mean ± SE
	0.0	0.1	0.2	0.3	
0	10.68 ± 2.10	6.56 ± 2.47	8.53 ± 2.77	12.76 ± 2.65	9.63 ± 1.26[a]
1	1.43 ± 0.75	4.57 ± 1.02	3.60 ± 1.16	3.05 ± 1.42	3.16 ± 0.56[b]
2	2.59 ± 1.22	1.53 ± 0.45	1.73 ± 0.73	0.82 ± 0.75	1.67 ± 0.41[b]
3	1.38 ± 0.75	3.13 ± 0.89	3.19 ± 0.89	2.31 ± 1.15	2.50 ± 0.46[b]
Mean ± SE	4.02 ± 0.86[a]	3.95 ± 0.74[a]	4.27 ± 0.86[a]	4.73 ± 1.05[a]	

Mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

Table 6
Comparison of growth of *Pogostemon helferi* cultivated in nutrient solutions of differing electrical conductivity after 10 wk. Each data point is derived from 3 replicates.

Nutrient solution (mS/cm)	Height (cm)	Shrub diameter (cm)	Shoots (*n*)
0.4	4.03 ± 0.43^{b}	0.76 ± 0.13^{a}	2.18 ± 0.23^{c}
0.8	4.74 ± 0.46^{ab}	0.85 ± 0.08^{a}	4.36 ± 0.20^{b}
1.2	5.45 ± 0.60^{ab}	1.05 ± 0.10^{a}	4.82 ± 0.30^{ab}
1.6	5.89 ± 0.44^{a}	1.06 ± 0.16^{a}	5.45 ± 0.47^{a}
2.0	5.45 ± 0.60^{ab}	0.93 ± 0.12^{a}	4.27 ± 0.27^{b}

Mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

Table 7
Comparison of growth of *Pogostemon helferi* grown under different humidity conditions for 10 wk. Each data point is derived from 3 replicates.

Humidity (%)	Height (cm)	Shrub diameter (cm)	Shoots (*n*)
60	4.65 ± 0.34^{a}	5.22 ± 0.19^{b}	5.73 ± 0.40^{a}
70	2.79 ± 0.15^{b}	5.70 ± 0.23^{b}	5.82 ± 0.43^{a}
80	2.44 ± 0.38^{bc}	8.61 ± 0.34^{a}	6.00 ± 0.53^{a}
90	1.78 ± 0.14^{c}	8.04 ± 0.34^{a}	5.73 ± 0.48^{a}

Mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

0.5 mS/cm (Wangwibulkit and Laohavisuti, 2009). Likewise, the Java fern *Microsorium pteropus* can grow well when bound onto wood and then cultured underwater in ponds at an EC level of 1.0 mS/cm (Wangwibulkit and Laohavisuti, 2006). Wangwibulkit (2003) reported that culturing *Cryptocoryne balansae* at an EC level of 0.5 mS/cm using the DFT provided the highest overall production and *Barclaya longifolia* can grow well at an EC level of 0.75 mS/cm (Kasam, 2009). Some other aquatic plants, however, have been reported to require more nutrients, such as *Echinodorus africanus*, which requires an EC level of 2.0 mS/cm (Mitrnoi, 2007). The nutrient requirement of plants depends on many factors and variability exists among genera, species and even ages. In the current trial, the most suitable nutrient solution that was tested for *P. helferi* growth in a hydroponics system was found to be at an EC level of 1.6 mS/cm, which provided the best growth for a 10 wk culture period. The plants were observed to grow well, with height (±SE) increasing to be 7.47 ± 0.44 cm taller than the plants of the other treatments and the average number of shoots (±SE) increased to 5.45 ± 0.47. However, the differing ECs did not significantly affect the diameter of the shrubs.

Effect of humidity on the growth of P. helferi

The effect of relative humidity was studied at four levels (60%, 70%, 80% and 90%) in the DFT system over a period of 10 wk. The results showed that the humidity did not affect shoot multiplication significantly (Table 7). During the first 2 wk, the height of the *P. helferi* plants cultured at the four levels of relative humidity were not significantly different. However, by the trial end, the *P. helferi* cultured at 90% humidity had grown to a height of 3.08 ± 0.22 cm whereas those grown at 60% humidity produced the tallest plants at 6.15 ± 0.33 cm. This result was consistent with the findings of Nelson et al. (2003) in which calcium uptake and its accumulation in tulips (*Tulipa gesneriana*) was lower at high relative humidity (82%) than in those reared at low humidity (42%). However, in *Hydrangea macrophylla* culture, a decrease in the relative humidity from 80% to 50% was reported to lead to a 38% decrease in height (Codarin et al., 2006). Mortensen and Gislerod (1990) reported that the height of some foliage species from warm and humid conditions increased when the humidity was elevated.

The diametric growth of shrubs specimens of *P. helferi* cultivated at different humidity levels was measured every 2 wk during the experiment and development was observed in the second week. The shrubs of *P. helferi* grown at 80% and 90% humidity levels were significantly wider compared to those grown in humidities of 60 and 70%. In general, various plants grown under high humidity conditions can lose stomatal control of transpiration and suffer detached leaves as was reported in an earlier study conducted by Mortensen and Fjeld (1998) on cut roses. In the present study, *P. helferi* was able to grow well under high humidity without its leaves detaching as it is an aquatic plant so that high humidity should be normal. Moreover, under these conditions, the increased diameter of the of *P. helferi* can produce denser shrubs (Fig. 3). These meet the market demand as the bushy form of *P. helferi* provides attractive foreground in aquaria.

This study identified an efficient method to produce the aquarium plant *P. helferi* using tissue culture and callus induction through the use of MS medium containing 0.1 mg/L IAA and 3 mg/L BA for a period of 3 wk. The calli on-grown for a further 3 wk could develop new shoots. In addition, the optimal conditions for the culture of *P. helferi* in a hydroponics system involves an EC level of 1.6 mS/cm at 80% humidity. These conditions can positively improve the quality and aesthetic

Fig. 3. Diameter of shrubs increased at high humidity.

appeal of *P. helferi* plants when they are marketed. The height and diameter of the shrubs can be manipulated to produce a bushy form that is preferred by the aquarium trade. These data could also be used to support commercial *P. helferi* production for export and to decrease the current practice of harvesting *P. helferi* plants from their natural habitat and so thereby contribute to effective resource management and environmentally friendly land use.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Aquarium Gardens 2015. Pogostemon helferi. http://www.aquariumgardens.co.uk/pogostemon-helferi-465-p.asp, 25 August 2015.

Balestri, E., Cinelli, F. 2001. Isolation and cell wall regeneration of protoplast from *Posidonia oceanica* and *Cymodocea nodosa*. Aquat. Bot. 70: 237–242.

Bird, K.T., Johnson, J.R., Jewett-Smith, J. 1998. *In vitro* culture of the seagrass *Halophila decipiens*. Aquat. Bot. 60: 377–387.

Bongcheewin, B. 2005. The Tribe Satureineae (Lamiaceae) in Thailand (M.Sc. thesis). Khon Kaen University, Khon Kaen, Thailand.

Christensen, C., Anderson, T., Pederson, O. 2007. Hunting for *Pogostemon helferi*. http://www.bio-web.dk/ole_pedersen/pdf/tag_2008_2.pdf, 23 November 2014.

Codarin, S., Galopin, G., Chasseriaux, G. 2006. Effect of air humidity on the growth and morphology of *Hydrangea macrophylla* L. Sci. Hortic. 108: 303–309.

Erisen, S., Yorgancilar, M., Atalay, E., Babaoglu, M., Duran, A. 2010. Callus induction and plant regeneration of the endemic *Astragalus nezaketae* in Turkey. Electron. J. Biotech. 13: 1–7.

Garcia-Jimenez, P., Navarro, E.P., Santana, C.H., Lugue, A., Robaina, R.R. 2006. Anatomical and nutritional requirements for induction and sustained growth *in vitro* of *Cymodocea nodosa* (Ucria) Ascherson. Aquat. Bot. 84: 79–84.

Hembrom, M.E., Martin, K.P., Patchathundikandi, S.K., Madasser, J. 2006. Rapid *in vitro* production of true-to-type plants of *Pogostemon heyneaus* through dedifferentiated axillary buds. In Vitro Cell. Dev. Biol. Plant 42: 283–286.

Kane, M.E., Davis, G.L., McConnell, D.B., Gargiulo, J.A. 1999. *In vitro* propagation of *Cryptocoryne wendtii*. Aquat. Bot. 63: 197–202.

Kane, M.E., Gilman, E.F., Jenks, M.A., Sheehan, T.J. 1990. Micropropagation of the aquatic plant *Cryptocoryne lucens*. HortScience 25: 687–689.

Kasam, W. 2009. Micropropagation and the Optimum Conditions for Orchid Lily *Barclaya longifolia* (Wallich, 1827) Growth (MSc. thesis). King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Lai-Keng, C., Leng, W. 2004. Plant regeneration from stem nodal segments of *Orthosiphon stamineus* Benth a medicinal plant with diuretic activity. In Vitro Cell. Dev. Biol. Plant 40: 115–118.

Liu, H., Yu, W., Dai, J., Gong, Q., Yang, K., Lu, X. 2004. Cryopreservation of protoplast of the alga *Porphyra yezoensis* by vitrification. Plant Sci. 166: 97–102.

Martin, K.P. 2004. Rapid axillary bud proliferation and *ex vitro* rooting of *Eupatorium triplinerve*. Biol. Plant. 47: 589–591.

Mitrnoi, M. 2007. Tissue Culture and Factors Affecting Growth of African Sword Plant (*Echinodorus africanus* K. Ratag) in Deep Flow Technique System (M.Sc. thesis). King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Mortensen, L.M., Fjeld, T. 1998. Effects of air humidity, lighting period and lamp type on growth and vase life of roses. Sci. Hortic. 73: 229–237.

Mortensen, L.M., Gislerod, H.R. 1990. Effects of air humidity and supplementary lighting on foliage plants. Sci. Hortic. 44: 301–308.

Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473–497.

Nelson, P.V., Kowalczyk, W., Niedziela Jr., C.E., Mingis, N.C., Swallow, W.H. 2003. Effect of relative, humidity, calcium supply, and forcing season on tulip calcium status during hydroponics forcing. Sci. Hortic. 98: 409–422.

Prasartkul, A. 2004. *Pogostemon helferi* (Hook. f.) Press (Lamiaceae), eine neue Aquarienpflanze aus Thailand. Aqua Planta 29: 143–148 (in German).

Sarma, K.S., Rogers, M.D. 2000. Plant regeneration from seedling explants of *Juncus effuses*. Aquat. Bot. 68: 239–249.

Satyavani, K., Ramanathan, T., Gurudeeban, S. 2011. Effect of plant growth regulators on callus induction and plantlet regeneration of bitter apple (*Citrullus colocynthis*) from stem explants. Asian J. Biotechnol. 3: 246–253.

Skoog, F., Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant culture *in vitro*. Symp. Soc. Exp. Biol. 11: 118–131.

Swamy, M.K., Balasubramanya, S., Anuradha, M. 2010. *In vitro* multiplication of *Pogostemon cablin* Benth. through direct regeneration. Afr. J. Biotechnol. 9: 2069–2075.

Tang, Y., Li, H., Liu, J., Liu, B., Luo, H.P. 2009. Callus formation from anther culture in balsam pear (*Momordica charantia* L.). Am. Eurasian J. Agric. Environ. Sci. 6: 308–312.

Tarepunda, N. 2004. Little Star, Aqua Star-the Magnificent One, *Pogostemon helferi*. http://www.aquarticles.com/articles/plants/Nid_Little_Star.html, 23 January 2014.

Wang, J., Seliskar, D.M., Gallagher, J.L. 2004. Plant regeneration via somatic embryogenesis in the brackish wetland monocot *Scirpus robustus*. Aquat. Bot. 79: 163–174.

Wangwibulkit, M. 2003. Micropropagation of Crypts *Cryptocoryne balansae* Gagnepain, 1941. Technical Paper No. 16/2003. Inland Fisheries Research and Development Bureau, Department of Fisheries, Bangkok, Thailand, pp. 23.

Wangwibulkit, M., Laohavisuti, N. 2006. Propagation of Java fern *Microsorium pteropus* (Blume) Ching, 1961. Technical Paper No. 20/2006. Inland Fisheries Research and Development Bureau, Department of Fisheries, Bangkok, Thailand, pp. 36.

Wangwibulkit, M., Laohavisuti, N. 2009. Propagation of *Bolbitis heteroclita* (Presl) Ching, 1934. In: Proceedings of the Annual Conference on Fisheries. Bangkok, Thailand, pp. 267–277.

Zhou, C., An, S., Jiang, J., Yin, D., Wang, Z., Fang, C., Sun, Z., Qian, C. 2006. An *in vitro* propagation protocol of two submerged macrophytes for lake revegetation in East China. Aquat. Bot. 85: 44–52.