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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