Development of micropropagation protocols for two aponogeton species of Sri Lanka (*Aponogeton crispus* and *Aponogeton natans*)

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Introduction

Sri Lanka being a tropical country contributes for a highly diversified flora and fauna. These climatic conditions have become a critical factor for the occurrence of highly diversified aquatic flora as well. *Aponogeton* is genus of aquatic plants which belongs to the family Aponogetonaceae. According to Bruggen there are four *Aponogeton* species occur in Sri Lanka (Dassanayake and Fosberg, 1987). Among these species *Aponogeton jacobsenii and A. rigidifolius* are endemic to Sri Lanka while *A. natans* and *A. crispus* are native plants (Wijesundara and Shantha Siri, 2004).They are having a high demand as an aquarium decorative plant in the export market. This study was carried out to develop a micropropagation protocol for mass production of *A. crispus* and *A. natans* and *A. jacobsenii* species.

Methodology

The study was carried out at tissue culture laboratory of Royal Botanic Gardens, Peradeniya. Mother plants were collected from different areas of Sri Lanka. Experiments were done to determine hormonal effect on shoot initiation and multiplication using rhizomes of *Aponogeton crispus* and *A. jacobsenii*; leaf and leaf stalks of *A. natans* and *A. jacobsenii* and seeds of *A. crispus* and *A. natans.* For the Sterilization of *A. crispus* and *A. jacobsenii* rhizomes were kept under running tap water for 3 hours and dipped in a fungicide for 30 minutes. Then disinfected using 20 % NaOCl (Clorox) with 2 drops of Tween twenty for 10 minutes and washed with 0.1 % $HgCl_2$ for 7 minutes. Finally rhizomes were washed 5 times thoroughly with distilled water per 5 minutes. For seeds sterilization seeds were kept under running tap water for 10 minutes, disinfected using 5 % Clorox with 2 drops of Tween twenty for 15 minutes and washed 3 times with distilled water. Three sterilization procedures were carried out for leaves and leaf stalks. Firstly leaves kept under running tap water for 1 ½ hours and dipped in fungicide (topsin) for half an hour. Then washed with 5 % of NaOCl (Clorox) for 10 minutes and washed with 0.1 % $HgCl_2$ for 3 minutes. Finally leaves washed 3 times with distilled water. Again sterilization procedure was tested by using 5 % NaOCl for 5 minutes and 0.1 % $HgCl_2$ for 2 minutes. As the final method leaves were sterilized with 2 % of NaOCl for 2 minutes 0.1 % $HgCl_2$ for 1 minute. Basal full strength MS medium supplemented with 20 mgL^{-1} of sucrose and 7 gL^{-1} of agar was used. pH was adjusted to 5.6. Different levels of BAP and IAA hormones were tested for the experiments (Figure 1, 2, 3). Cultures were maintained under controlled condition of 26 ^0C +/- 2 ^0C temperatures and white fluorescent light with 16 hour photoperiod for shoot regeneration. Ten replicates per each treatment were maintained and media without adding hormones used as the control. Experiment was arranged in factorial Complete Randomized Design (CRD). Data analyzed using ANOVA and Tukey's Test using MINITAB 17 software.

Results and Discussion

In the study only rhizome culture of *Aponogeton crispus* and seed culture of *A. crispus* and *A. natans* were succeeded. Shoot initiation was not observed in rhizome culture of *Aponogeton jacobsenii* within six week time period. Leaf and leaf stalk culture was unsuccessful due to over sterilization. The effects of the hormone concentration on shoot regeneration of *Aponogeton crispus* had shown a significant difference ($p < 0.05$). Maximum mean number of shoot initiation was observed in culture medium T6 (Figure 1). Minimum number of shoot initiation was observed in T7 (Figure 1). Shoot regeneration was not observed in hormone free MS medium. There was a significant different between T1, T4 and T6 (Figure 1).

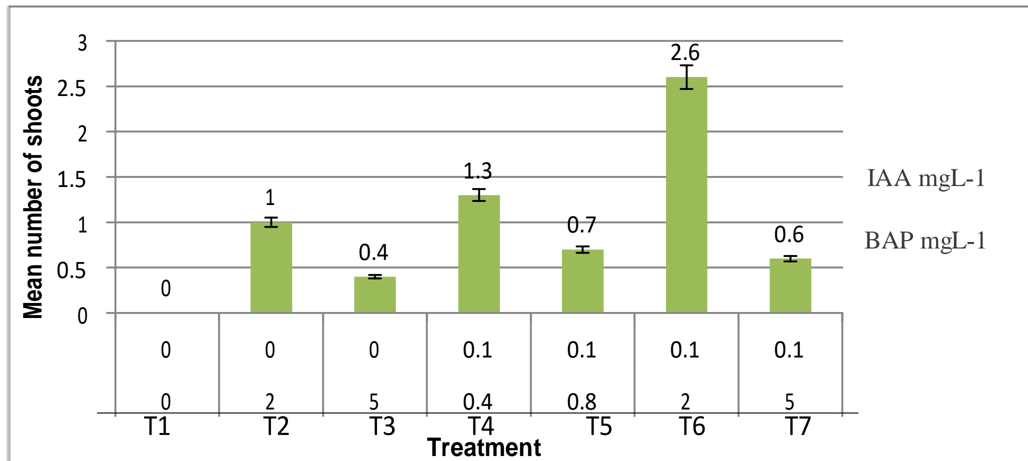

Figure 3 : Effect of hormone concentration on shoot initiation of *Aponogeton*

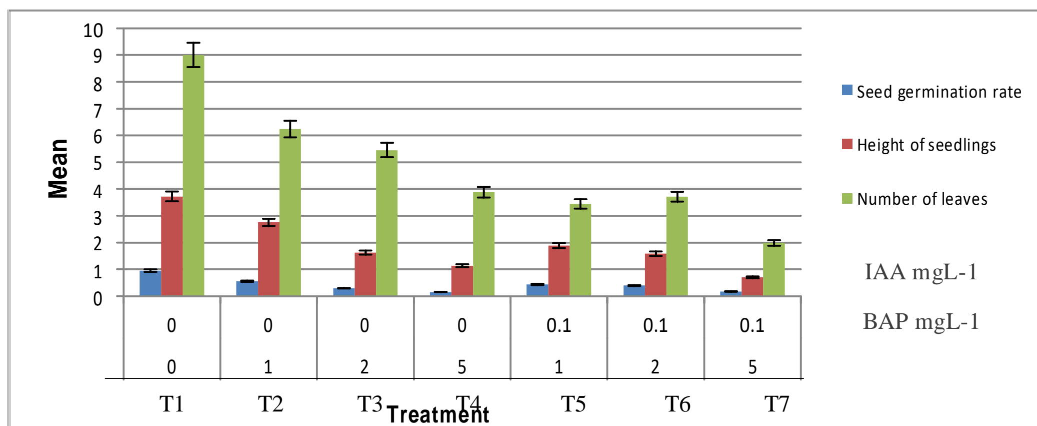

Figure 4: Effect of different hormone concentration for Seed culture of *Aponogeton natans*

Seed culture of *Aponogeton natans* and *A. crispus* were succeeded. The data analysis of *Aponogeton natans* seed culture had shown a significant difference ($p < 0.05$) in mean of seed germination rate, mean height of seedlings and mean number of leaves. Highest mean of seed germination rate was observed in hormone free MS media (T1). Minimum mean number of seed germination was observed in medium supplemented with 5 mgL^{-1} of BAP. There was a significant difference among T1, T2 and T4. The maximum mean height of seedlings and the maximum mean number of leaves were observed in hormone free MS medium followed by T2, T3, T4 and T6 (Figure 2). The minimum height of

seedlings and the minimum mean number of leaves were observed in T7 medium which was supplemented with high concentration of BAP (5 mgL^{-1}) and IAA (0.1 mgL^{-1}). (Figure 2)

There was a significant difference in treatments for seed germination ($p < 0.05$) of *A. crispus*. The maximum seed germination was recorded in hormone free MS medium (T1). The minimum seed germination was observed in T4 medium which was supplemented with 1 mgL^{-1} of BAP and 0.1 mgL^{-1} of IAA. The maximum mean height of seedlings and the maximum mean number of leaves were observed in hormone free MS medium (T1). The minimum mean height of seedlings and the minimum mean number of leaves were observed in medium which contained 1 mgL^{-1} of BAP and 0.1 mgL^{-1} of IAA. (Figure 3)

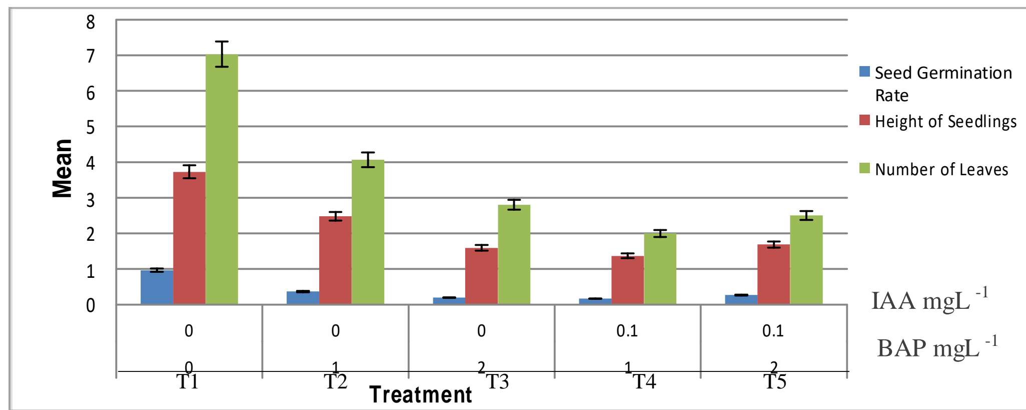

Figure 3: Effect of different hormone concentration for Seed culture of *Aponogeton crispus*

Medium supplemented with 2 and 5 mgL^{-1} of BAP were used for shoot multiplication. Data analysis of *A. crispus* and *A. natans* had shown a significant difference ($p < 0.05$) among hormone treatments. Maximum was obtained in the medium supplemented with 2 mgL^{-1} of BAP. The toxicity caused by an excess of growth regulators in the culture medium, or the extended period of time in which the culture was exposed to them, might lead to genetic, physiological and morphological changes, resulted in a reduction of the proliferation rate in vitro (Narayanaswamy, 1977). It is therefore important to evaluate their effects on plant regeneration.

Conclusion

Among selected hormone concentrations 2 mgL^{-1} of BAP and 0.1 mgL^{-1} of IAA is the most suitable combination for shoot regeneration of *A. crispus*. MS medium without hormones is more effective for seed culture of *A. crispus* and *A. natans*. Culture medium supplemented with 2 mgL^{-1} of BAP is more suitable for multiplication of seedlings of *A. natans*.

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