Optimization of Sterilization of the Explants of *Cryptocorynewendtii* and Selection of a suitable Hormone combination for the Initiation of Shoots from the Explants.

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Abstract

Cryptocoryne species are important aquatic plants for aquaculture industry. These are indiscriminately harvested from the wild for export market. In order to overcome the problems of species loss and inadequate supply to the local and foreign markets, an *in vitro* method for the micro-propagation of *Cryptocoryne wendtii* has been developed. Contamination of the explants in culture is a major problem in micro-propagation of aquatic plants. However, optimum number of aseptic cultures of *C. wendtii* rhizome segments could be developed by the explants with 40 % Clorox, a commercial bleaching solution followed by 10 minutes and 20 % Clorox solution for 7 minutes. These explants were cultured on Murashige and Skoog basal (MS, 1962) medium, supplemented with 1, 2, 4 or 10 mg/l of BAP (6-Benzyladeninepurine) along with and 1mg/l IAA (Indole-3-acetic acid). The cultures were monitored daily for fourteen days for the shoot initiation. Shoot initiation was the best on the medium containing 4 mg/l BAP and 1 mg/l IAA.

Keywords: *Cryptocoryne wendtii*, endemic, micropropagation, rhizome, hormone

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Introduction

The genus *Cryptocoryne*, a member of the family Araceae is represented by more than 50 species that are distributed throughout South Asia (Wijesundara and Shantha Siri, 2004). More than twenty species of the genus are being used in aquariums. Because of slow propagation by rhizomes and infrequent seed production (due to polyploidy), these species have become endangered. Ten species are endemic to Sri Lanka, and all species are listed in International Union for Conservation of Nature (IUCN) Red Book as endangered species (IUCN Red list 2012). *C. wendtii*is one of the most common and widely used species in aquariums. It is also one of the most variable species with several colour variation. The species has become endangered because of its high demand and its incapability of producing seeds due to its triploid nature (Wijesundara and Shantha Siri, 2004). This study was conducted to develop a micro-propagation protocol for *Cryptocoryne wendtii,* with an immediate objective of optimizing sterilization method for the explants and determine the suitable hormone concentrations and combination for the initiation of shoots from the explants.

Material and Methods

Rhizome segments of the *C. wendtii*, was excised from the plant stock maintained at plant house in NARA. Four surface sterilization methods were tested in the first experiment (Table 1) and four hormonal combinations were tested using the best sterilization method obtained from

the experiment 1 (Table 2). Murashige and Skoog (MS,1962) medium supplemented with 3% sucrose and gelled with 8 % agar was used as the basal medium, which was supplement with different concentrations of BAP along with 1mg/l IAA. The cultures were incubated at 24 ± 1 °C under16 h photoperiod (1600 Lux). Cultures were observed at regular intervals for fourteen days. Each treatment consisted of ten replicates and the experiments repeated thrice.Different Clorox concentrations were used for treatments. To avoid the fungal contaminations explants were dipped in 0.1 % fungicide (Thiophanatemethyle) for overnight and washed under running tap water for 6 hours. Further to that explants were washed with 0.1 % Mercuric Chloride. TheKruskal-Wallis non parametric test used for statistical analysis.

Table 1. Treatments used for sterilization of the explants of *Cryptocoryne wendtii*.

Test	T1	T2	T3	T4
Clorox Concentration and time	6 % /15 min and 4 % / 10 minutes	20 % Clorox / 15 minutes	40 % Clorox / 15 minutes	40 % Clorox / 10 minutes+ 20 % Clorox / 7 minute

Table 2: The concentrations and combinations of growth regulators used for the induction of shoots from the rhizome explants of *Cryptocoryne wendtii.*

Test	Hormone Combination
T1	0 mg/l BAP + 0mg/l IAA
T2	1 mg/l BAP + 1mg/l IAA
T3	4 mg/l BAP +1 mg/l IAA
T4	10 mg/l BAP + 1mg/l IAA

Results and Discussion

The best Surface sterilization procedure was adopted to get around 96% of contaminant-free explants of *Cryptocoryne* species using T4 treatment (Table 1)and least (6%) contaminant-free explants were observed in the procedure of T1 treatment. Most submerged aquatic plants retain high level of microbes on their wet surfaces. Under warm tropical conditions a higher number of microorganisms are present in the aquatic environments than under temperate conditions. Rhizomes of the *Cryptocoryne* species used in this study have an uneven and a hairy surface preventing contact between the rhizome and the sterilizing agent. This results in retention of microorganisms and ultimately contamination of the culture medium. This is common problem with rhizomes of tropical aquatic plants. (Mohanram and Agrawal, 1999). Dissanayake *et al.* (2007) obtained successful surface sterilization in *C.wendtii*by using 5% Clorox for 15 minutes with vacuum infiltration followed by 95 % ethanol for minute and finally in 0.1 % mercuric chloride for 30 sec. In that study they were able to reduce the percentage of contamination up to 65%. According to Herath *et al.* (2008) surface sterilization of *Cryptocoryne* spp was successful using 75 % ethanol for one minute and 20 % Clorox solution for 15 minutes.

The best Surface sterilization procedure was adopted to get around 96 % of contaminant-free explants of *Cryptocoryne* species using T4 treatment (Table 1) and least (6 %) contaminant-free explants were observed in the procedure of T1 treatment. *C. wendtii* are generates new shoots MS supplemented with growth regulators. The best hormone combination was adopted to get around 78 % shoot initiation using T3 treatment (Table 2) and least (3.5 %) shoot initiation were observed in the T1 treatment (Table 2).

The effect of growth regulators for shoot initiation of *Cryptocoryne spp*has been reported by Herath *et al.*(2008). According to them, 5 mg/l BAP with 0.1 mg/l IAA induced maximum number of shoots in *C. beckettii* and *C. bogneri.* According to the Dissanayake *et al.* (2007), maximum number of shoots of *C.wendtii* was regenerated in MS medium supplemented with BAP 44 µM and 66 µM of Naphthalene acetic acid (NAA) The concentrations of the BAP hormone which above mentioned researchers have used much similar to the results in this study which was 4mg/l.

Conclusion

The most suitable surface sterilization for *C. wendtii* explants was obtained with treatment with 40% Clorox solution for 10 minutes and 20% Clorox solution for 7 minutes.*C. wendtii* yielded the highest shoot initiation in the presence of 4mg/l BAP and 1mg/l IAA in the medium.

References

Dissanayake, C. Hettiarachchi, M and Iqbal M.C.M 2007. Sustainable use of *Cryptocorynewendtii*and *Echinodoruscordifolius* in the aquaculture industry of Sri Lanka by micro-propagation. *Sri LankaJournal of Aquatic Science***12**:89-101

Herath, H.M.I. Krishnarajah, S.A and Wijesundara, D.S.A 2008. .Micro-propagation of two endemic threatened *Cryptocoryne* species of Sri Lanka.*Journal of Tropical Agricultural Research and Extention***11:** 19-24.

IUCN 2012. The National Red List of Sri Lanka. Conservation status of the Fauna and Flora.186.

Mohanramand,A, A. 1999. Tissue culture of aquatic plants and its applications. Agro Botanical Publishers, India: 111-112.

Murashige, T and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassay with Tobacco Tissue Culture. *Journal of Plant Physiology* **15**:473-497.

Wijesundara, D.S.A and Shantha Siri, I.G. 2004. Some selected Aquatic Ornamental Plants of Sri Lanka. P 34-94. National Science Foundation.