

Perfecting Explants Sterilization Procedure and Multiple Shoot Induction Medium for *In-Vitro* Propagation of *Lagenandra* Species

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Introduction

Lagenandra species are important aquatic plants in the aquaculture industry of Sri Lanka (Yapabandara and Ranasinghe, 2006). In the absence of a regular supply due to lack of effective propagation methods, *Lagenandra* species are indiscriminately harvested from the wild to supply to the export market. In order to overcome the problem of species loss and inadequate supply to the local and foreign markets, an in vitro micro- propagation method was developed for *Lagenandra lancifolia* and *Lagenandra ovata*.

The maintenance of aseptic or sterile conditions is essential for successful tissue culture procedures. Various sterilization agents are used for surface sterilization the tissues. These disinfectants are also toxic to the plant tissues, hence proper concentration of disinfectants, duration of exposing the explants to the various disinfectants, the sequences of using these disinfectants has to be standardized to minimize explants injury and achieve better survival (CPRI, 1992). Mercuric chloride and sodium hypochlorite were used for the present study to standardize the best sterilization protocol for in vitro culture of *Lagenandra ovata* and *Lagenandra lancifolia*.

Culture initiation and multiplication, to a great extent, are dependent on the type and genotype of explants as well as the type of hormone and their concentration. Many commercial ornamental plants are being propagated by in-vitro culture on the culture medium containing auxins and cytokinins (Peril, 2003). BAP (6- Benzylaminopurine) and IAA (Indole acetic acid) hormone with different concentration were used in the present study to perfect the best combination of IAA and BAP in shoot multiplication procedure.

Methodology

The research was conducted at the tissue culture laboratory, Floriculture research and development unit, Royal botanic gardens, Peradeniya. The rhizomes (1 cm - 2 cm pieces) were used as explants. In the first experiment, explants were soaked in different concentrations of sodium hypochlorite (15%, 20%, and 25%) and mercuric chloride (0.1%, 0.2%, and 0.4%) to perfecting explants sterilization procedure. Each explant was soaked for twenty minutes in Sodium hypochlorite solution and two minutes in Mercuric chloride solutions. Number of contaminated cultures was observed after two weeks from explants establishment in hormone free Murashige and Skoog medium.

In second experiment, effect of different hormone combinations (hormone free MS medium, BAP 1 mg l⁻¹, BAP 2 mg l⁻¹, BAP 3 mg l⁻¹, BAP 5 mg l⁻¹, BAP 7 mg l⁻¹, BAP 1 mg l⁻¹ + IAA 0.1 mg l⁻¹, BAP 2 mg l⁻¹ + IAA 0.1 mg l⁻¹, BAP 3 mg l⁻¹ + IAA 0.1 mg l⁻¹, BAP 5 mg l⁻¹ + IAA 0.1 mg l⁻¹, BAP 7 mg l⁻¹ + IAA 0.1 mg l⁻¹) in 0.5 MS semi solid medium, full MS semi solid medium and full MS liquid medium for shoot initiation and multiplication were evaluated. Number of shoot initiation, shoot length, number of shoots per culture and number of leaves per culture were recorded after six weeks from explants establishment in MS medium. All the data were analyzed using ANOVA in SAS and Minitab statistical package.

Results and Discussion

The ten experiments conducted to determine the best sterilization method for *Lagenandra ovata* were significantly different ($p < 0.05$). The highest mean number of uncontaminated cultures was found when it was treated with 0.2 % mercuric chloride and 25% sodium hypochlorite (0.6667). When used Sodium hypochlorite only could no non infection cultures were obtained. Mohanram and Agrawal also proved that (1999), the rhizome explants were successfully sterilized by using sodium hypochlorite with mercuric chloride.

Table 1. Effect of different hormone concentration for mean number of shoot initiation mean shoot height and mean number of leaves in both semi solid and liquid media.

Treatment	Hormone Concentration (mg l ⁻¹)		Semi solid media		Liquid media	
	BAP	IAA	Mean number of shoot initiation	Mean shoot height (cm)	Mean shoot height (cm)	Mean number of leaves
	1	0	0	0.0000 ^e	0.0000 ^e	0.0000 ^f
2	1	0	0.0000 ^e	0.0000 ^e	0.3000 ^e	0.0000 ^c
3	2	0	0.1003 ^{cde}	1.1000 ^{bcd}	0.5000 ^{cde}	0.0000 ^c
4	3	0	0.2509 ^{bc}	1.2666 ^{abc}	0.6666 ^{bcd}	0.2594 ^{ab}
5	5	0	0.4601 ^a	1.8000 ^a	1.0000 ^a	0.4184 ^a
6	7	0	0.2300 ^{bcd}	1.0000 ^{bcd}	0.6000 ^{bcd}	0.2007 ^{abc}
7	1	0.1	0.0502 ^{de}	0.8000 ^{cd}	0.3666 ^{de}	0.0000 ^c
8	2	0.1	0.1799 ^{bcd}	0.6333 ^d	0.6000 ^{bcd}	0.2007 ^{abc}
9	3	0.1	0.1297 ^{bcd}	1.3333 ^{abc}	0.8000 ^{abc}	0.4184 ^a
10	5	0.1	0.3096 ^{ab}	1.6000 ^{ab}	0.8666 ^{ab}	0.3181 ^{ab}
11	7	0.1	0.2300 ^{bcd}	1.1333 ^{bcd}	0.6333 ^{bcd}	0.1003 ^{bc}

Means followed by the same letter in a column are not significantly different.

There were significant differences between treatment on number of shoot initiation, shoot length, number of shoots per culture and number of leaves per culture ($p < 0.05$). The result revealed that the all explants which cultured in liquid medium were initiated shoots except in hormone free media within six weeks. But explants which cultured in semi solid MS medium with 5 mg l⁻¹ BAP shown the highest number of shoot initiation (0.4601). There were not shoot initiation in hormone free semi solid medium and medium which contain 1 mg l⁻¹ BAP. The results of this study indicate that, the growth regulator supplementation is essential for the micropropagation of *Lagenandra* species. This was confirmed by the results of Hereath et al. (2008), who developed the protocol for *Cryptocoryne bongeri* and *Cryptocoryne becktii*.

Both semi solid and liquid media which contain 5 mg l⁻¹ BAP recorded the highest mean shoot height (Table 1.). The highest mean shoot height of liquid culture was 1 cm and semi solid culture was 1.8 cm. However, the shoot length of liquid agitated culture was less than semi solid culture. In liquid culture, disappearance or lesser expression of activity of apical dominances could be seen due to continuous shaking condition of the tissues in the medium which generally leads to the induction and proliferation of numerous auxiliary buds (Chu *et al.*, 1993). Therefore, height of the shoots could be reduced due to break down of the apical dominancy as shown by the results of this study.

Though there was no leaf formation in semi solid MS media, 3-4 leaves per plant was observed in liquid media. The shoots have initiated in all liquid media except in hormone free media (T1). The maximum mean number of leaves was obtained in media which contains 5 mg l⁻¹ BAP and

also media which contains 3 mg l⁻¹ BAP with 0.1 mg l⁻¹ IAA (0.4184)). There was no leaf formation in hormone free media, media which contains 1 or 2 mg l⁻¹ BAP and basal media which contained 1 mg l⁻¹ BAP with 0.1 mg l⁻¹ IAA (T1, T2, T3 and T7).

Sterilization procedure for mean number of uncontaminated explants in *Lagenandra lancifolia* was significantly different at 1% significant level (p=0.001). The highest number of uncontaminated explants for *Lagenandra lancifolia* was obtained when they were exposed to 0.2 % mercuric chloride and 20 % sodium hypochlorite (T7). However, there was no significant difference in sterilization procedure between the treatments with sodium hypochlorite at 20 % or 25 % with mercuric chloride 0.2% or 0.4 % (T7, T8, and T9). The Shoots have sprouted only on full MS liquid media contain 5 mg l⁻¹ BAP within six weeks period.

Conclusion

According to present study, the most suitable sterilization procedure for *Lagenandra ovata* was 25% sodium hypochlorite with 0.2% mercuric chloride. The better culture establishments of rhizome segments was observed on Murashige and Skoog (MS medium) medium with 5 mg l⁻¹ BAP with presence or absence of IAA. Length of shoot was maximized on full MS semi solid medium containing 5 mg l⁻¹ BAP. Number of leaves was maximized on full MS liquid media containing 5 mg l⁻¹ and 3 mg l⁻¹ BAP with 0.1 mg l⁻¹ IAA. Shoot multiplication could not be observed in any hormone combinations which were used in this study within a period of six weeks. The most suitable surface sterilization procedure for *Lagenandra lancifolia* was 20% sodium hypochlorite and 0.2% mercuric chloride.

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