# Effect of growth regulators on in-vitro multiplication of *Lagenandra ovata* and *Lagenandra lancifolia*

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## Introduction

Sri Lanka is a country which consists with number of endemic aquatic plants. These endemic aquatic plants are having high demand in aquatic plant industry (Galapitagedra, n.d.). Due to the lack of effective propagation methods plant collectors collect plant from wild to fulfill the market demand. It leads to the depletion of natural plant stock and bio diversity. *Lagenandra* species only can observe in Sri Lanka, Southern India and Bangladesh (Dassanayake *et. al*., 2001). In Sri Lanka there are seven species and six species are considered as endemic. Red List of International Union for the Conservation of Nature in 2013, categorized five of those endemic species under the highly threatened category. Main purpose of this study is to develop a proper method for micro propagation of *Lagenandra ovata* and *Lagenandra lancifolia* to overcome inadequate supply and depletion of natural plant stock. Present study was carried out to evaluate effect of different hormone concentrations in basal media for shoot initiation and multiplication of rhizome explants,to identify the best explant of *L. lancifolia* for micro propagation and to identify the best medium for *L. ovata* seed culture.

## Methodology

Present study was carried out at Royal Botanic Gardens, Peradeniya. Seven experiments were conducted in order to achieve the objectives of the study. Explants of *L. ovata* (rhizome and seeds) and *L. lancifolia* (rhizome and smaller plantlets) were sterilized using standard procedures. *L. ovata* rhizomes were placed in Ms Semi-solid media with different cytokinins such as BAP, Kinetin and TDZ with the presence of IAA. Different concentrations of BAP and Kinetin such as 0.4, 0.8, 2, 5 and 8 $mgl^{-1}$ and 0.4, 0.8, 1.6 and 2 $mgl^{-1}$ of TDZ were used. Grown plants of *L. ovata* were transferred in to multiplication media with 1, 2 and 3 $mgl^{-1}$ BAP hormone concentrations. Smaller plantlets of *L. lancifolia* were placed in MS semi solid media with and without growth regulators. Different concentrations of cytokinins such as 0.4 and 0.8 $mgl^{-1}$ of BAP and Kinetin concentrations and IAA were added to the medium. Grown Plantlets of *L. lancifolia* were transferred in to multiplication medium with 1, 2 and 3 $mgl^{-1}$ BAP hormone levels. Survival rate of *L. lancifolia* plantlets and rhizomes were measured weekly in order to identify the best explant. The best medium for *L. ovata* seed germination was identified by placing seeds in different media such as; solid medium, semi-solid medium, liquid medium and sterilized distilled water medium. After the germination seeds were transferred in to solid or semi-solid medium for further growth.

## Results and Discussion

This study shows the importance of growth regulators for the shoot initiation of *L. ovata* rhizome culture. According to the one way ANOVA, there was a significant effect ($p<0.05$) of growth

regulators such as BAP, Kinetin and TDZ on shoot initiation. The highest mean number of shoot initiation of *L. ovata* rhizomes was observed at 0.4 mgl $^{-1}$ Kinetin with the presence of 0.1 mgl $^{-1}$ IAA. The second highest mean number of shoot initiation was observed at 0.8 mgl $^{-1}$ TDZ with the presence of 0.1 mgl $^{-1}$ IAA. According to the data lower concentrations of growth regulators stimulate shoot initiation highly. Highest shoot multiplication was observed at 2 mgl $^{-1}$ BAP level. According to the one way ANOVA there was a significant effect of hormone treatments for the shoot multiplication ($p < 0.05$).

Table 1: Effect of different BAP hormone concentrations for the mean number of new shoot regeneration after three weeks of culture establishment

| **Treatment** | BAP hormone concentration (mgl $^{-1}$) | Mean number of new shoot regeneration |
|---|---|---|
| **Control** | 0 | 0.000 $^{b}$ |
| **$T_1$** | 1 | 1.000 $^{b}$ |
| **$T_2$** | 2 | 2.667 $^{a}$ |
| **$T_3$** | 3 | 0.667 $^{b}$ |

Means that do not share a letter are significantly different

As indicate by the table there was a significant difference between treatment 2 and all other treatments. Hormone free MS media did not show any shoot initiation or multiplication. The highest seed germination of *L. ovata* was observed in sterilized distilled water medium at the second week of culturing. Low contamination possibility and low cost are main advantages of sterilized distilled water medium. There was a significant difference of seed germination between sterilized distilled water and all other treatments.

Table 2: Mean number of seed germination in different media after two weeks of culture establishment

| **Treatment** | Media type | Mean number of seed germination |
|---|---|---|
| **T1** | Solid media | 2.400 $^{c}$ |
| **T2** | Semi- Solid media | 2.600 $^{bc}$ |
| **T3** | Liquid media | 4.800 $^{b}$ |
| **T4** | Sterilized distilled water | 8.200 $^{a}$ |

Means that do not share a letter are significantly different

The best shoot initiation of *L. lancifolia* smaller plantlet culture was observed at 0.4 mgl $^{-1}$ Kinetin with the presence of 0.1 mgl $^{-1}$ IAA hormones.

Table 3: Effect of different BAP hormone concentrations for mean number of new shoot regeneration of *L. lancifolia* after three weeks of culture establishment

| **Treatment** | BAP hormone concentration (mgl-1) | Mean number of new shoot regeneration |
|---|---|---|
| **Control** | 0 | 0.000 [c] |
| **T1** | 1 | 1.333 [b] |
| **T2** | 2 | 2.667 [a] |
| **T3** | 3 | 1.000b [c] |

Means that do not share a letter are significantly different

As indicated by the above table there was a significant difference in new shoot regeneration of both treatment 1 and 2 when compare to the other treatments. The highest shoot multiplication of *L. lancifolia* was observed at 2 mgl $^{-1}$ BAP level. Therefore treatment 2 was considered as the best treatment for new shoot regeneration of *L. lancifolia*. The survival rate of *L. lancifolia* rhizome was below 30% and survival rate of plantlets was above 80% at the fifth week of culture establishment. Therefore, smaller plantlets of *L. lancifolia* were recommended as the best explants source.

## Conclusion

Hormone treatments should be used for the shoot initiation of *L. Ovata* rhizome cultures. According to which obtained 0.4 mgl $^{-1}$ Kinetin with presence of 0.1 mgl $^{-1}$ IAA suitable or both *L. ovata* rhizomes and *L. lancifolia* smaller plantlets culture. The maximum shoot multiplication was observed at 2 mgl $^{-1}$ BAP level. Smaller plantlet of *L. lancifolia* is recommended as the best explants source for micropropagation based on the survival rate. Best medium for *L. ovata* seed germination is sterilized distilled water.

## References

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