Effect of auxin type and concentration on the induction of *Alternanthera Reineckii* roots in vitro

R Yunita* and M F I Nugraha

1Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development, Jl. Tentara Pelajar No. 3A Bogor Indonesia 16111
2Indonesian Research Center for Ornamental Fish - Research and Human Resources Agency-Ministry Marine and Fisheries, Jl. Perikanan No 13 Pancoran Mas Depok Indonesia

Abstract. *Alternanthera reineckii* is an aquatic plant that is used as an aquascape plant. This commodity is in great demand so that its needs continue to increase. To meet market demand, technology that can produce large quantities of seedlings in a short time is needed. In vitro propagation is a technology that can be used to produce uniform plants with a relatively high level of multiplication. The aim of this study was to obtain the appropriate type and concentration of auxin for the in vitro induction of *Alternanthera reineckii* roots. In this study, a factorial completely randomized design was used. The first factor is the type of auxin (IBA, NAA and IAA) and the second factor is the concentration of auxin (0, 0.5, 1.0 and 1.5 mg/l) with 10 replications. The results showed that the best auxin for *Alternanthera reineckii* root induction was IBA at a concentration of 1 mg/l.

1. Introduction
Aquatic plants are plants that live in water that have interesting morphology and character so that they can be used for ornamental aquatic plants and have a high selling value. *Alternanthera reineckii* is a freshwater ornamental plant native to South America. It is widely used as an aquascape plant and has high economic value and a large market demand. However, market needs have not been fully met.

Generally, aquaculture business is carried out conventionally. This is because the cultivation techniques only refer to the experiences that farmers get from time to time. If there are pests, diseases and bacteria or fungi in the plant for export, then the plants considered do not meet export standards. It requires cultivation technology for large-scale clonal propagation to meet a stable supply throughout the year. The method that can be applied is in vitro propagation (micropropagation). This technique can shorten the time to produce plants of sufficient quality and quantity so that they can meet market demand [1,2].

This technology can be used for uniform propagation of good quality seeds, plants can be reproduced at any time as needed, regardless of season [3,4]. The regeneration system used to produce plantlets through in vitro culture is recommended in the form of direct formation from plant organs or “direct organogenesis” [5]. The multiplication stages go through several stages, namely shoot induction, shoot multiplication, shoot elongation and root induction. Root induction is one of the most important steps to produce a complete plantlet.
One of the important factors that determine the success at each stage of in vitro propagation is the content of growth regulators in the media. The type and concentration of growth regulators used will depend on the purpose and stage of the culture. The growth regulators commonly used for measuring induction are auxin groups such as IBA, IAA and NAA [6]. The aim of this study was to obtain the appropriate type and concentration of auxin for in vitro induction of Alternanthera reineckii root.

2. Materials and methods
This research was conducted at the tissue culture laboratory, Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development. The plant material used in this study was the one week old Alternanthera reineckii plant culture on MS medium. The part used was in vitro shoots which have four leaves.

The experimental design used in this study was a factorial Completely Randomized Design (CRD). The first factor is the type of auxin (IBA, IAA and NAA) and the second factor is the concentration of auxins (0, 0.5, 1 and 1.5 mg / l). The combination obtained was 12 experimental units, with 10 replications. The basic medium used was MS medium with 3% sucrose added to 0.8% agar and the pH of the media was adjusted to + 5.7 with the addition of 0.1 N KOH or HCl. The observed parameter was the time the roots appeared, number of roots, root length, and root visualisation. Observation of the emergence of shoots was observed every day. For the parameters of the number of roots, root length and root visualisation were observed weeks after planting (MST). The culture was incubated in the culture room under bright conditions for 16 hours a day (with light intensity 1000-1400 lux) and the culture room temperature was +25 °C.

3. Results and discussion

3.1. The first day the roots appear
Shoots of Alternanthera reineckii cultured on media without auxin appeared more slowly than in media with auxin which was on the tenth day after planting. This is because the amount of endogenous auxins in plants is relatively small, so it is still able to stimulate the roots but it takes longer. The IBA treatment of 1 mg / l was faster than the other treatments for 5 days (Table 1). The increase in IBA at a higher rate tends to inhibit the root emergence speed. The efficiency and effectiveness of IBA used for root induction at low concentrations means that the endogenous auxin found in plants is able to stimulate roots, so it only requires a low concentration to increase the number of roots [7].

Root initiation can be stimulated with auxins. IBA is a type of auxin that is most often used to induce roots because it has a high ability to control root initiation [8]. IBA is more stable and has a lower level of toxicity than NAA and IAA [9]. Giving IBA to Ficus deltoidea has a significant effect on the number of roots, root length, and root initiation time. The 0.1-0.3 ppm IBA concentration is the most effective in pule pandak plants with an average percentage of successful root growth of 70%-100% [8].

| Auxin concentration (mg/l) | Auxin types | Auxin types | Auxin types |
|----------------------------|-------------|-------------|-------------|
| 0                          | 10          | 10          | 10          |
| 0.5                        | 5           | 6           | 6           |
| 1                          | 4           | 5           | 6           |
| 1.5                        | 5           | 6           | 6           |

3.2. Number and length of roots
In the variable number of roots, it was seen that all the treatments given were able to induce roots. Treatment without IBA can induce roots even though in a small amount compared to all treatments with an average of 1.5. At a concentration of 1 mg/l IBA produced a fairly good number of roots, namely 4.4 (Figure 1).
IBA can indeed trigger root growth, so that the number of roots produced is greater. However, at concentrations that are too high, it can inhibit root growth as seen at a concentration of IBA 1.5 it produces shoots of 3.7 while in concentration of 1 mg/l can produce 4.4 roots. This is consistent with the statement of Abidin [10] that stated IBA has activity as a root hormone, so that IBA activity can affect the number of roots. If IBA administration is too high, it can inhibit root growth, it is also suspected that the availability of endogenous auxins is available so that the addition of exogenous auxins can inhibit the root formation process [11].

In the root length variable, it was found that the IBA treatment of 1 mg/l produced the longest root that was 1.53 cm (Figure 2). The shortest root length of 0.94 cm was found in the treatment without IBA. This occurs because the optimum endogenous auxin is not available in the plant so it requires exogenous auxin to produce a greater number of roots. The higher the IBA level given, the lower the average length value that was generated. This result is the same in the gladiolus culture conducted by Badriah et al [12], that found high concentrations of IBA inhibited root elongation, while lower concentrations produced longer roots.

**Figure 1.** Effect of auxin type and concentration on the number of 4 MS roots

**Figure 2.** Effect of auxin type and concentration on root length
3.3. Root visualizations
Based on the results of visual observations, it appears that the explants induced by various auxin concentrations have varied roots. In Figure 3, it can be observed that the root growth on media containing IBA is better than the roots in other treatments. Where the number of roots is more and the growth is uniform. Root growth on IAA and NAA media showed that the number of roots produced was not a lot and the growth was not uniform where there was one root that grows dominant and longer. The colour of the roots looked different, on IBA media the roots appeared white and had many branches, while in the other treatments the roots were brown and had few branches.

![Figure 3. Visualization of roots in auxin treatment. A. 1 mg / l IBA, B. IAA 1 mg / l, C. 1 mg / l NAA](image)

4. Conclusion
This study found that the best auxin for *Alternanthera reineckii* root induction in vitro was IBA at a concentration of 1 mg/l. In IBA treatment 1mg/l was able to produce roots in a relatively fast time with larger number and longer roots

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