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Micro propagation of *Bucephalandra sp*

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Exploration of the *Bucephalandra sp* plant in its natural habitat will threaten the sustainability of this species, therefore an effective method of propagation is needed to maintain its sustainability. Propagation of plants with organo genic pathways can produce plants that are the same as its parent and in large quantities. The purpose of this study was to obtain appropriate media formulations for shoot induction, shoot multiplication and root induction of *Bucephalandra sp*. This study consisted of 3 main activities, namely shoot induction, shoot multiplication and root induction. The results showed that The best media formulation for bud induction of *Bucephalandra sp* in vitro was MS added BA 2 mg/l. Optimal media formulation for shoot multiplication is MS added BA 2 mg/l and Thidiazuron 0.1 mg/l and for root induction is MS added IBA 2 mg/l.

Keywords: BA, IBA, *In vitro* ,Thidiazuron

INTRODUCTION

Bucephalandra sp belongs to an endemic plant, which is found on the island of Borneo known as Borneo (Yengand Boyce, 2014). The *Bucephalandrasp* is the most promising plant in the Araceae family to be kept in a decorative home aquarium (Araki, 2018). Small size, various shapes and colors, slow growth, ability to grow on stone and driftwood (Tustin, 2013)

This *Bucephalandra sp* plant looks similar to the Anubias and Cryptocoryne species. When compared with the Cryptocoryne species, *Bucephalandra sp* has similarities such as small leaves and color variants. However, this *Bucephalandra sp* has a rhizome and roots that can hold/cling according to the characteristics of Anubias. *Bucephalandra sp* is a beautiful genus and is still relatively difficult to find, this plant that can captivate many scalpers around the world thanks to a very attractive appearance. (Tustin, 2013)

This plant has a high economic value where the export value is around $ 300 / rhizome. (Ministry of Maritime Affairs and Fisheries, 2018). Given the high economic value of this plant, many exploit it directly from their habits. This will threaten the sustainability of this commodity. Besides that, plants that are exploited directly from nature are still contaminated with other organisms such as nematode, or any other organism, so it is not suitable for export. One of the requirements that must be fulfilled by ornamental plants to be suitable for export is to comply with phytosanitary regulations, to prevent the entry and spread of plant and insect diseases into new areas (Liu, 2007).

To preserve *Bucephalandra sp* in nature, water ornamental plant farmers must propagate *Bucephalandrasp*and not direct exploitation from nature. Propagation method used must be able to produce plants that are able to produce large quantities, fast, do not require a large place and are

free from pathogens and other organisms.

In vitro propagators, also known as micropropagations, are the most popular technology to overcome this obstacle. The advantage of propagation with in vitro technology includes the expansion technology. It does not require a large space or land because this technology is carried out on culture bottles in a sterile room. This technology will produce plants similar to their parents and free from plant-disturbing organisms. This is because the propagation process is carried out in aseptic conditions, so the resulting seedlings are free from disturbing organisms (Iliev et al., 2014). Production with this technology does not depend on the season or climate so that multiplying activities can be done at any time (Yildis, 2014).

Planting in vitro can be done through two pathways, namely somatic embryogenesis and organogenesis. Somatic embryogenesis is the induction of vegetative tissue to form an embryonic callus where growth leads to somatic embryo formation. Propagation by this method has been tried on several cultivation crops including magical plants (*Garciniamangostana* L.) (Rohani et al., 2012) *Citrus Limon L* (Goswami et al., 2013).Oganogenesis is the induction of vegetative tissue forming organs and forming plantlets with roots and shoots. The purpose of this study is to obtain the right media formulation for micropropagation of *Buchephlandra sp* plants through organogenesis

MATERIALS AND METHODS

The study was conducted from January to November 2018 in Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRAD).

Plant material used in this study was in vitro culture of *Bucephalandrasp* (L.). Plants from the field are sterilized by using 70% alcohol, Clorok 30 and 20% and rinsed with sterile aquadest. The sterilized plant material was cultured on MS base media (MurashigeandSkoog, 1962), enriched with vitamins and supplemented with 3% sucrose, and made solid by adding 0.2% phytagel. Cultures were incubated in the culture room at 25 ± 20C with a radiation intensity of 1.000–2.000 lux for 16 hours. After the culture is duo weeks old and sterile (no contamination) culture was sub-cultured on shoot induction media.

This study consisted of three activities, namely (1) shoot induction (2) shoot multiplication (3) rooting induction.

Shoot Induction

In shoot induction activities using sterile explant in vitro. The media used were MS media (Murashigedan Skoog media**)** which added BA (Benziladenin) at a concentration of 0.0; 0.3; 0.5; 0.7 mg/l. Each treatment consisted of 10 replications. Variables observed were number of shoots, number of leaves formed

Shoot multiplication

The shoots produced in the shoot induction activity are transferred to multiplication media. Media for multiplication are basic media of MS which added BA at a concentration of 0; 1; 1.5 and 2 mg/l and combined with Thidiazuron at se veral concentrations namely 0.0; 0.1, and 0.3 mg/l. Each treatment consisted of 10 replications. Variables observed included number of shoots, number of leaves and visual appearance.

Rooting Induction.

The shoots which have a height of ± 5 cm, are transferred to rooting media. rooting media induction media MS which added IBA at several levels of concentration that is 0.0; 0.5; 1.0 mg/l. Each treatment consisted of 30 replications. The observed variables were the number of roots and root length after 4 weeks of age.

Data analysis

This study uses a randomized plan design. Each data obtained from each phase of the activity is analyzed statistically and if it is significantly different will be carried out further tests using the Duncan's multiple-range test

RESULTSAND DISCUSSION

Four weeks after planting, explants were able to produce shoots but the number of shoots produced varied. The addition of BA to 1 mg/l was able to induce shoots more than any other treatment, which was 1.5 shoots (figures 1 and 2). BA treatment 1.5 mg/l also resulted in an average number of leaves more than other treatments, namely 3.3 leaves. Some research results show that in general, water plants are propagated in vitro using explants in the form of stems with bud (Vijayakumar et al., 2010; Pandiyan and Selvara, 2012; Kapiland Sharma, 2014)

An increase in BA treatment to 1.5 mg/l resulted in a decrease in the number of shoots and leaves (number of shoots 1 and number of leaves 2.4). This shows that the addition of high amounts of BA can inhibit the formation of shoots of *Bucephalandra sp.*

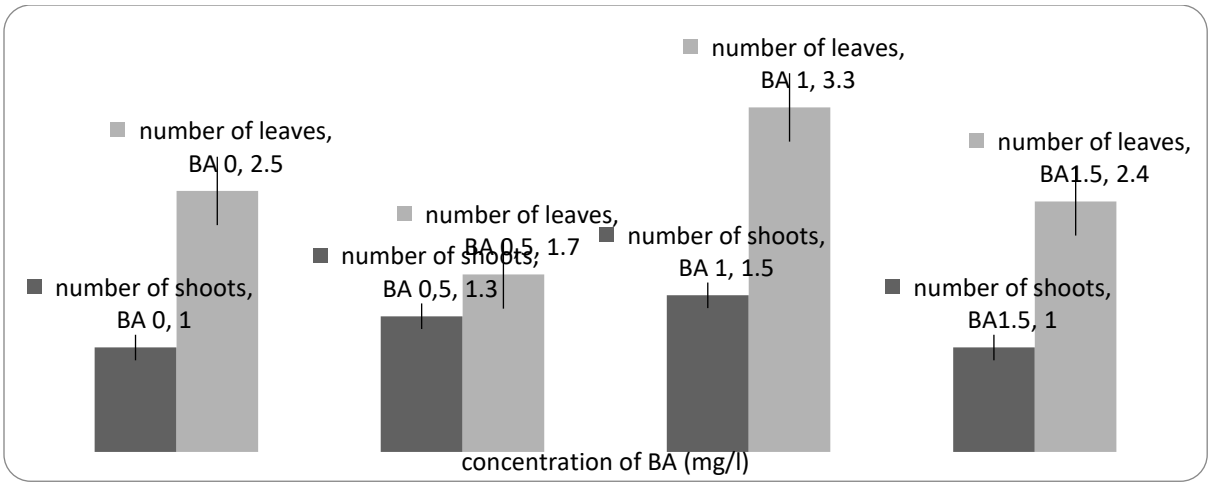

Figure1: Effect of BA treatment on number of shoots and number of leaves on in vitro shoots *Bucephalandrasp*

Figure 2: *Bucephalandra sp.* Shoot growth on MS media added BA 2 mg / l and Thidiazuron 0.1 mg / l

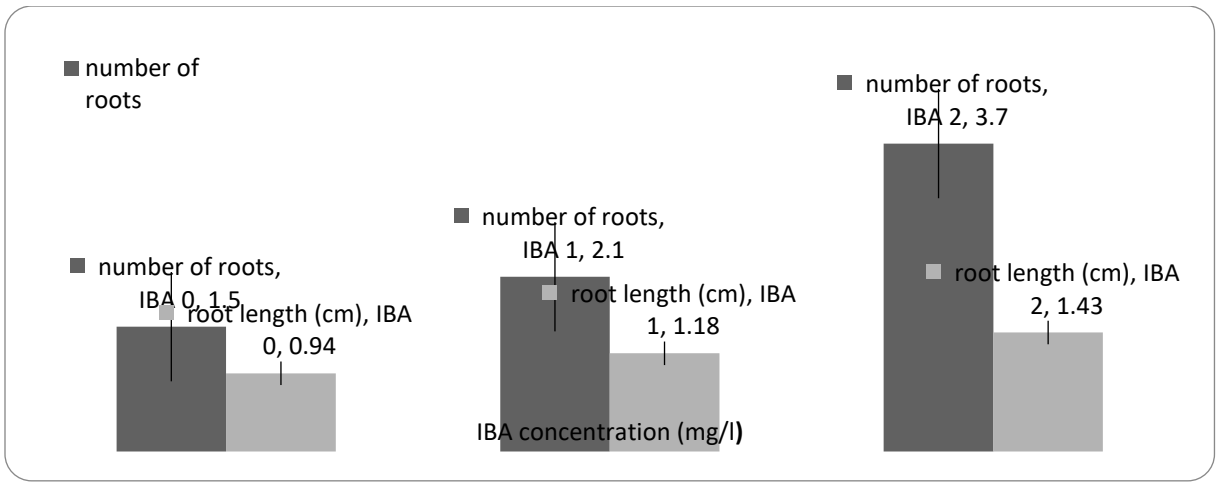

Figure 3: Effect of IBA treatment on root formation in in vitro shoots of *Bucephalandra sp*

Tabel 1: The effect of BA and thidiazuron treatment on the multiplication of *Bucephalandra sp* shoots

Thiadizuron Concentration (mg/l)	BA Concentration mg/l			
	0	1	1,5	2
0.0	1.1a	1.5a	1.1a	1.4a
0.1	1.1a	2.4b	4.3c	5.3d
0.3	1.2a	4.4c	4.1c	2.2b

Remarks: Values followed by the same letter in the same row and column are not significantly different at the 5% level, according to Duncan's multiple-range test

Tabel 2: The effect of BA and thidiazuron treatment on leaf formation in *Bucephalandra sp* shoots

Thiadizuron Concentration (mg/l)	BA Concentration mg/l			
	0	1	1,5	2
0.0	2.0a	3.4a	2.3a	3.0a
0.1	2.7a	4.9ab	13.0b	13.8b
0.3	2.5a	12.3b	11.2b	5.4ab

Remarks: Values followed by the same letter in the same row and column are not significantly different at the 5% level, according to Duncan's multiple-range test

Shoot Multiplication

ANOVA analysis showed that the interaction of BA and Thidiazuron treatment had a significant effect on the number of shoots and number of leaves. The best treatment for multiplication of shoots is a combination of BA 2 mg/l and Thidiazuron 0.1 mg/l. From this treatment it can produce 5.3 (Table. 1) and 13.8 leaves (Table 2). This treatment has a significantly different effect on other treatments (Figure 1). The number of shoots that is more important in plant micro propagation activities because it will determine the number of seeds produced.

Increasing the concentration of Thidiazuron to 0.3 mg / l will decrease the explant's ability to form shoots and leaves, although not significantly different from the Thidiazuron treatment of 0.1 mg / l. The same thing also happens to several types of plants including *Plumbagozeylanica*, *Rauwolfiaserpentina* L, Bacopa where the administration of suitable thidiazuron will increase the multlipication of shoots. This is because Thidiazuron has the ability to induce cell division, but at high concentrations it will inhibit cell growth (Syahidand Kristina, 2008; Yunitaand Lestari, 2011;Yunita et al., 2018).

Root induction

Buchephlandrasp was not difficult to form roots, as evidenced when 70% of shoots cultured on MS media without IBA were able to produce roots while those treated with IBA were able to form 100% forming roots. This is thought to be because this plant contains enough endogenous auxin to induce root formation.

Figure 3 shows that the use of IBA 2 mg / l has a significant effect on the average number and length of roots where the average number of roots is 3.7 and the average root length is 1.43. The IBA treatment of 2 mg / l is the best treatment for induction of root in vitro *Bucephlandra sp*. IBA is an auxin commonly used to induce roots in vitro. The results of research on *Rutagraveolens* with the treatment of IAA (Indole-3-acetic acid), IBA and NAA (Naphthalene acetic acid) at a concentration of 0.25-1 mg / l show IBA gives the fastest response in inducing roots (Bohidar et al., 2008)

CONCLUSION

The best media formulation for bud induction of *Bucephalandra* sp in vitro was MS added BA 2 mg/l. Optimal media formulation for shoot multiplication is MS added BA 2 mg/l and Thidiazuron 0.1 mg/l and for root induction is MS added IBA 2 mg/l.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

RY is main author. RY designed and performleed the experiments and also wrote the manuscript. MFIN and EGL designed experiments and reviewed the manuscript. All authors read and approved the final version.

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