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The Acclimatization Stage Growth Of Black Orchid (*Coelogyne Pandurata* Lindl.) Plantlet By Biostimune Extract Gotu Kola (*Centella Asiatica* L.) Treatment

Hani, Zulfa Zakiah*, Mukarlina
Biology Study Program, Faculty of Mathematics and Natural Sciences,
Tanjungpura University,
Jl. Prof. Dr. H. Hadari Nawawi, Kota Pontianak, Kalimantan Barat 78124
*email: zulfazakiah@gmail.com

ABSTRACT

Black orchid plantlets have a relatively slow growth at the acclimatization stage. The growth of black orchid plantlets can be increased by giving biostimulants. Biostimulants are bioactive compounds, and when they are applied, they can increase plant growth. This study aims to determine the effect of biostimulant Gotu kola extract (Centella Asiatica L.) on the growth of black orchid plantlets (Coelogyne Pandurata Lindl.) at the acclimatization stage. The research was conducted at the Biology Department's greenhouse from September until December 2020. The study used a completely randomized design with a factorial pattern. The first factor is the concentration of biostimulants consisting of 5 levels, e.g: 0 mg/L; 25 mg/L; 50 mg/L; 75 mg/L; 100 mg/L. The second factor is the application time which consists of 2 levels: once a week and once every two weeks. The results showed that the application of biostimulant Gotu kola extract had no significant effect on plantlet height, leaf length, leaf width, number of leaves, number of tillers, and number of plantlet roots of black orchid in the acclimatization stage.

Keywords: *acclimatization, biostimulant, black orchid, Gotu kola*

1. INTRODUCTION

The black orchid (*Coelogyne Pandurata* Lindl.) is one of the orchid species protected by the Indonesian government, based on Government Regulation No. 7/1999. This orchid type can be found in the Kalimantan forest. Black orchids have high economic value because of the beauty of their flowers which have a characteristic green color with a black labellum that hangs in the middle of the flower. The existence of black orchids is currently threatened and rarely found even in their natural habitat (Hartati et al., 2017). Forest fires, logging, and land conversion are severe threats to the black orchid population and impact the shrinking of Kalimantan's forests and the original habitat of black orchids (Kustini, 2011). In Indonesia, around 57% of the deforestation is caused mainly by converting land to oil palm plantations, and another 20% comes from pulp and paper (Wahyuni & Suranto, 2021). Another factor that causes the lack of orchid population is that orchid seeds are tiny and do not have food reserves (endosperm), so they are difficult to germinate and must be in symbiosis with mycorrhizae as a provider of nutrients for orchid growth (Handini, 2019).

One of the efforts that can be made to prevent the extinction of black orchids is by using in vitro propagation (tissue culture) techniques. Orchid propagation through in vitro culture has several stages. One final and critical stage of in vitro propagation is the acclimatization stage. Orchid plants from in vitro culture (plantlets) are very vulnerable to the external environment because in vitro culture conditions are controlled, so plantlets require an acclimatization stage before being planted in the field (Hardjo, 2018). Acclimatization is the plants' adaptation stage from tissue culture from in vitro to ex vitro conditions (Prasetyorini, 2019). The acclimatization stage is primarily influenced by several factors, including humidity, light intensity, temperature, media type, acclimatization method, and nutrition (Izudin, 2013). The acclimatization that has been carried out has given good results on the survival percentage of black orchid plantlets derived from explants with the addition of sweet potato extract, which is 100% (Untari et al., 2007). Adi et al. (2014) also obtained the highest percentage of live black orchid plantlets on a combination of wood charcoal and coconut fiber media, 57.14%. Given the previous research, Nugroho & Raden (2021), the percentage value of live black orchid plantlets obtained the highest yield of 72.22%, which was acclimatized to cocopeat+charcoal media.

The vegetative growth of orchid plants at the acclimatization stage is relatively slow, so special treatment is needed to stimulate its growth. One alternative that can be done to overcome this is the addition of biostimulants. Biostimulants are materials and microorganisms that are applied to increase the efficiency of nutrient absorption, tolerance of abiotic stress, and improve crop quality (Du Jardin, 2015). Biostimulants can be obtained from bacteria, yeast, seaweed, and higher plants. Compounds acting as biostimulants include amino acids, humic acids, fulvic acid, chitosan, protein hydrolysates, and secondary metabolic products (hormones, alkaloids, phenols, flavonoids, terpenoids, steroids, and saponins) (Bulgari et al., 2019; Rafiee et al., 2016).

Biostimulants derived from plant extracts have been used to promote plant growth. One of the plant extracts that can be used as a biostimulant is the Gotu kola plant extract. Giving Gotu kola extract can increase plant height, leaf area, wet weight, dry weight, root length, chlorophyll content, and carotenoid levels in tomato plants (Lycopersicum Esculentum) (Sri, 2010). Based on research conducted by (Zakiah et al., 2017), biostimulant treatment from the crude extract of Gotu kola (Centella Asiatica L.) has been shown to increase the height and leaf area of

soybean plants (Glycine max Merr.).

Biostimulants are widely used to increase plant growth, but the application of biostimulants at the acclimatization stage is rarely carried out. The biostimulant Gotu kola extract treatment, especially on black orchid plantlets, has never been done, so this research needs to be done to prove the effectiveness of the application of Gotu kola extract (Centella Asiatica L.) on the growth of black orchid plantlets (Coelogyne Pandurata Lindl.) at the acclimatization stage.

2. MATERIAL AND METHOD

The study was carried out in September-December 2020. Acclimatization was carried out at the Kasa House, Department of Biology, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. The evaporation of Gotu kola extract was carried out at the Chemical-Biochemical Laboratory, Department of Agricultural Technology, Faculty of Agriculture, Pontianak State Polytechnic. The tools used in this study consisted of an autoclave, plastic cup, measuring cup, hand sprayer, lux meter, plastic pot, and thermohygrometer. The materials used were aquadest, Gotu kola extract, black orchid plantlets obtained from in vitro propagation at the Orchid Center UPTD Agribusiness Pontianak and aged 9-12 months, Gandasil-D fertilizer, and sawdust.

Figure 2. Black Orchid Plantlet

The study was conducted using a completely randomized design of 2 factors. The first factor was the concentration of Gotu kola extract with 5 levels of treatment: without being given a biostimulant (control); 25 mg/l; 50 mg/l; 75 mg/l; 100 mg/l. The second factor is the time of application of Gotu kola extract with two levels of treatment: once a week and once every two weeks (Aulya et al., 2018; Ummah et al., 2017; Zakiah et al., 2017). Environmental factors are measured weekly, including temperature, humidity, and light intensity. Temperature and humidity were measured using a thermohygrometer, while light intensity was measured using a lux meter. Observation parameters consisted of plantlet survival percentage (%), plantlet height (cm), leaf length (cm), leaf width (cm), number of leaves (strands), number of tillers (fruit), and number of roots (fruit).

Quantitative data from measurements of plant height, leaf number, leaf length and width, number of tillers, and number of roots at the end of the observation were statistically analyzed by analysis of variance (ANOVA). If there was a significant effect, then continued with the DMRT test at a significance level of 5% (Hidayat et al., 2018).

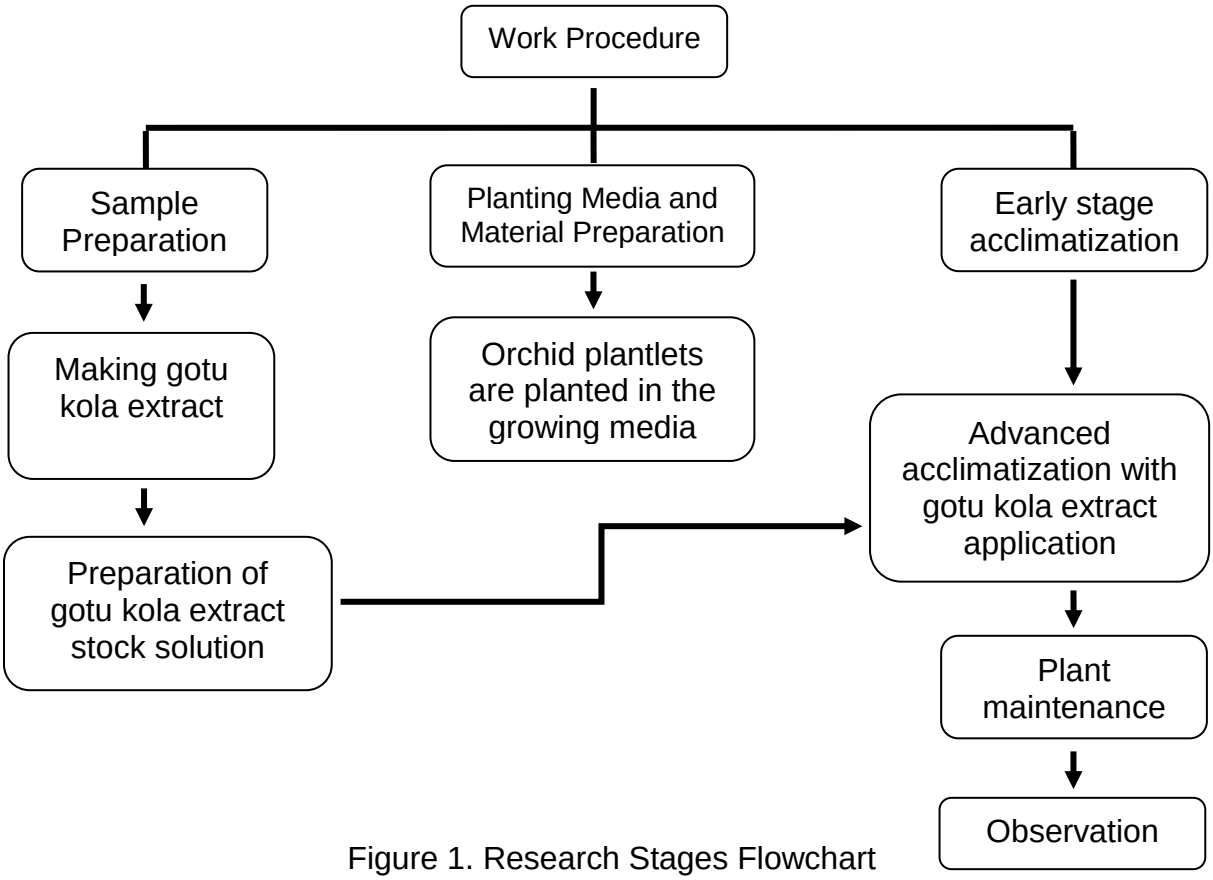
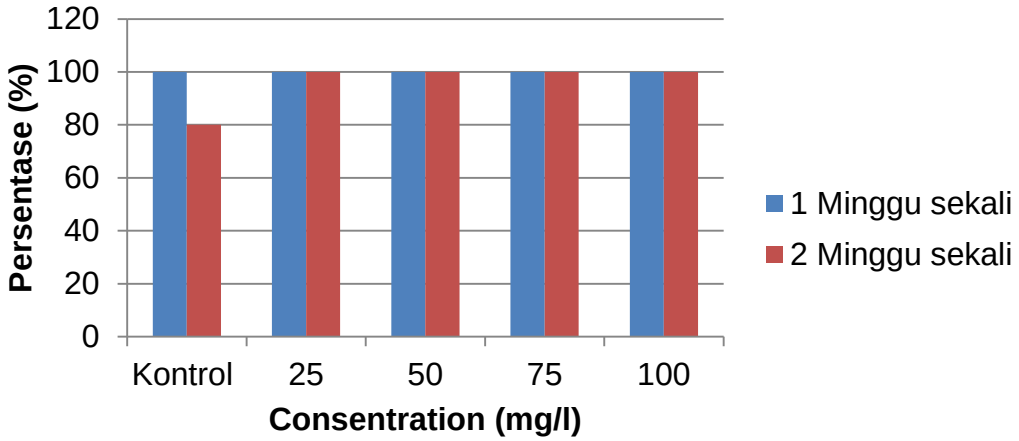


Figure 1. Research Stages Flowchart

3. RESULT AND DISCUSSION

The percentage of plantlet survival, when acclimatized until the end of the observation for each treatment, was 100%, but in one control treatment, the percentage of life was lower than in the other treatment, namely 80%, because the plantlets died (Table 1).

Figure 2. Percentage of plantlet survival (%) of black orchid after Gotu kola extract treatment



Internal and external factors influence the success of acclimatization. The low percentage of plantlet survival was thought to be due to internal factors such as plantlet genetic conditions. The plantlets used are plantlets obtained from

propagation through seeds. Bhattacharyya et al. (2017) & Konar et al. (2018) stated that plantlets obtained through axillary budding or somatic embryogenesis were considered the most genetically uniform. Referring to (Hazarika, 2006), the acclimatization process depends on several important factors, including genotype, which affects not only the response of explants to different culture media but also organogenesis and plant survival in ex vitro conditions.

Plantlets that died during acclimatization indicated that these plantlets were still not fully adapted to the ex vitro environment. Plantlets from tissue culture are still susceptible to changes from the in vitro environment to the ex vitro. According to Irsyadi (2021), the stomata on plantlets from in vitro culture do not function perfectly for the photosynthesis process and have not been able to absorb nutrients optimally. This condition resulted in plantlets' death, so the percentage of plantlet survival was low. This study's percentage of plantlet life has given better results than Adi et al. (2014) research results, which obtained the highest percentage of black orchid plantlet life of 57.14% with the addition of orchid fertilizer plus.

The success of acclimatization is influenced by several external factors, one of which is environmental conditions. The environmental conditions at the time of the research have supported the acclimatization process of black orchids, with temperatures between 28^{o}C-31^{o}C, humidity 72%-88%, and light intensity 2970-4720 lux (Table 2). Based on research by Managanta & Pangli (2014), *Coelogyne Pandurata (black orchid) grows in climatic conditions with a temperature of 19-32^{o}C*. Ramadanil (2010) also reported that the orchid coelogyne Celebensis grew in an environment with an average relative humidity of 85.17%. Coelogyne orchids grow epiphytes by attaching to tree branches. Referring to Nawawi (2014), Epiphytic orchids generally require sunlight intensity of around 2500-5000 lux.

Table 1. Results of Environmental Parameter Measurement

Weeks	Temperature (^{o}C)	Moisture (%)	Light Intensity (lux)
1	30	86	3140
2	31	77	3340
3	29	87	3290
4	28	79	2930
5	30	85	3220
6	31	78	3400
7	30	86	3350
8	31	72	3150
9	29	88	3950
10	31	80	4720
11	28	77	2970
12	28	81	3240

Another factor that also affects the success of acclimatization is the planting medium. The planting medium used must be able to support the growth of black orchid plantlets. This study uses a planting medium derived from sawdust wood. Based on the preliminary tests that have been carried out, sawdust media produced the highest percentage of survival, namely 100%, compared to other media used, including rice husks, cocopeat, coconut fiber, and bagasse. According to Lakitan (1995), Agustin et al. (2014), and Sandra (2005), sawdust has an excellent water-

binding capacity and contains lignin, oil, fat, and resin, so it is not easy to decompose. Waluya (2009) also added that a smooth and soft planting medium is needed for the acclimatization process so that the roots can grow optimally. The media used must be smooth enough, can bind water well, and be free from fungus and disease.

The acclimatization method plays an essential role in the success of an acclimatization process. This study uses the method of acclimatization with a hood system. Through this system, the hood is opened gradually so that the plants can slowly accept the external environmental conditions. Izudin (2013) explained that one technique to obtain the appropriate humidity is using a closed system with clear plastic (mask). This system gives better results in a more stable temperature and humidity.

Statistical analysis showed that the Gotu kola extract treatment had no significant effect on plantlet height, leaf length, leaf width, Leaf Quantity, tillers Quantity, and plantlet total roots of black orchid. Although the survival percentage has a value of 100%, morpho-physiologically, the plantlets are still slow in responding to the application of biostimulants. This is evidenced by all growth parameters not significantly affected by the application of Gotu kola extract biostimulants (Table 2).

Table 2. ANOVA analysis results of black orchid plantlet growth after Gotu kola extract treatment

No	Parameter	F-hit	KK (%)
1	Plantlet Height (cm)	0.681^{ns}	7.98
2	Leaf Length (cm)	0.822^{ns}	8.56
3	Leaf Width (cm)	0.579^{ns}	8.42
4	Leaf Quantity (ea)	1.319^{ns}	16.36
5	Tillers Quantity (ea)	0.806^{ns}	14.34
6	Total Roots (ea)	0.307^{ns}	20.53

Remark ns = not significant

KK = Coefficient of Diversity

Table 3. Growth of black orchid plantlets after Gotu kola extract treatment

Extract Concentration	Application Time		Average
	Once a Week	Every two weeks	
Plantlet Height (cm)			
0 mg/l	3.28^{ns}	3.52^{ns}	3.05^{ns}
25 mg/l	3.64^{ns}	3.48^{ns}	3.56^{ns}
50 mg/l	3.60^{ns}	3.66^{ns}	3.63^{ns}
75 mg/l	3.16^{ns}	3.06^{ns}	3.11^{ns}
100 mg/l	3.22^{ns}	3.96^{ns}	3.59^{ns}
Average	3.38^{ns}	3.39^{ns}	
Leaf Length (cm)			
0 mg/l	2.78^{ns}	3.00^{ns}	2.59^{ns}
25 mg/l	3.08^{ns}	2.80^{ns}	2.94^{ns}
50 mg/l	3.16^{ns}	3.04^{ns}	3.10^{ns}
75 mg/l	2.66^{ns}	2.64^{ns}	2.65^{ns}
100 mg/l	2.66^{ns}	3.38^{ns}	3.02^{ns}
Average	2.86^{ns}	2.85^{ns}	
Leaf Width (cm)			
0 mg/l	0.54^{ns}	0.56^{ns}	0.49^{ns}
25 mg/l	0.67^{ns}	0.60^{ns}	0.63^{ns}
50 mg/l	0.57^{ns}	0.65^{ns}	0.61^{ns}
75 mg/l	0.56^{ns}	0.52^{ns}	0.54^{ns}
100 mg/l	0.64^{ns}	0.60^{ns}	0.62^{ns}
Average	0.59^{ns}	0.56^{ns}	
Leaf Quantity (ea)			
0 mg/l	5.00^{ns}	4.25^{ns}	4.20^{ns}
25 mg/l	5.40^{ns}	6.00^{ns}	5.70^{ns}
50 mg/l	6.20^{ns}	5.60^{ns}	5.90^{ns}
75 mg/l	6.20^{ns}	5.40^{ns}	5.80^{ns}
100 mg/l	6.60^{ns}	3.80^{ns}	5.20^{ns}
Average	5.88^{a}	4.84^{b}	
Tiller Quantity (ea)			
0 mg/l	1.60^{ns}	1.40^{ns}	0.50^{ns}
25 mg/l	1.60^{ns}	1.20^{ns}	0.40^{ns}
50 mg/l	1.40^{ns}	1.80^{ns}	0.60^{ns}
75 mg/l	1.20^{ns}	1.20^{ns}	0.20^{ns}
100 mg/l	1.40^{ns}	1.60^{ns}	0.50^{ns}
Average	1.44^{ns}	1.44^{ns}	
Total Roots (ea)			
0 mg/l	4.40^{ns}	5.75^{ns}	4.50^{ns}

25 mg/l	6.00^{ns}	6.60^{ns}	6.30^{ns}
50 mg/l	8,40^{ns}	8,20^{ns}	8,30^{ns}
75 mg/l	6.20^{ns}	5.20^{ns}	5.70^{ns}
100 mg/l	7,40^{ns}	5.40^{ns}	6.40^{ns}
Average	6.48^{ns}	6.00^{ns}	

Remark: ns = not signifikan

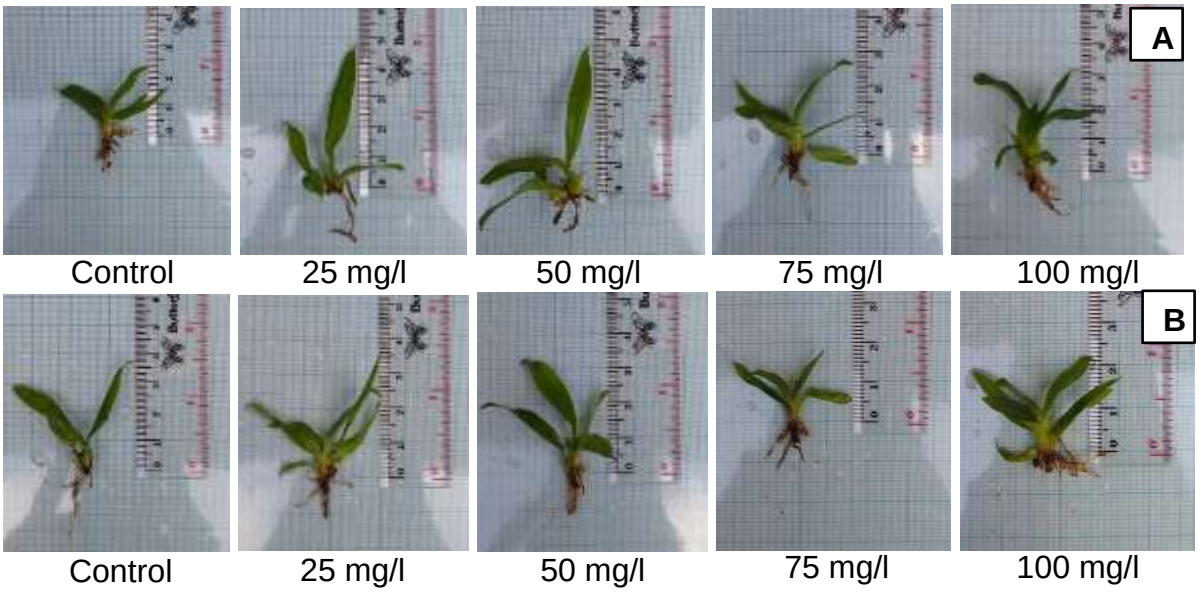


Figure 3. Comparison of observations on the growth of black orchid plantlets between treatments

Remarks:

A = Biostimulant application once a week

B = Biostimulant application every two weeks

Some of the morpho-physiological abnormalities often shown by in vitro cultured plantlets include a thin cuticle layer, vascular tissue that is not fully developed, stomata are not functioning optimally, and photosynthetic activity is still low (Hardjo, 2018). This will affect the plant's metabolic processes, one of which is inhibiting the photosynthesis process in the leaves. Photosynthate will be used for the plantlet growth process. Paradikovic et al. (2018) explained that biostimulants also help increase nutrient absorption, root system development, and photosynthetic activity and stimulate growth.

The plantlets will absorb the applied biostimulant through the roots and leaves. Van Oosten et al. (2017) explained that applying foliar biostimulants will lead to two targets, i.e., roots and shoots. Biostimulants absorbed through the leaves regulate stomata opening and closing and aid the fluid flow through the xylem. Biostimulants absorbed through the roots will increase the activity of the hormones ethylene and auxin in the roots to stimulate root growth and absorb more water and nutrients. Silva et al. (2013) explained that biostimulants applied to plants would facilitate the uptake of nutrients from the substrate by stimulating root growth, thereby increasing water absorption capacity.

The activity of opening and closing stomata is related to the entry of fluids into the leaves, such as water, nutrients, and biostimulants. The plantlets will absorb the nutrients and water to increase growth and development, while the biostimulant will regulate the opening and closing stomata. Drew et al. (1992) in Silva et al. (2017) stated that in vitro cultured plantlets showed characteristics such as the inability of stomata to close and open more, and the cuticle layer was not fully formed. This condition causes excessive evapotranspiration, so the plantlets will wither and even die.

Black orchid plantlets are thought to have not been optimal in transporting water and nutrients absorbed by roots and leaves. This is due to the vascular network between the roots and shoots of plantlets that have not developed properly. This condition causes plants to lack the nutrients needed for growth. Ziv & Chen (2008) *in* Silva et al. (2017) explained that in vitro cultured plantlet roots had limited epidermal and peridermal layers, immature bundles of vessels, limited cambium activity, and underdeveloped xylem, and phloem vessels, and few root hairs.

Lindsey et al.'s (1998) research showed that the application of Ecklonia maxima extract did not give a significant difference in the fresh weight and dry weight of Scilla Krauss and Kniphofia Pauciflora plantlets at the acclimatization stage.

4. CONCLUSION

The Gotu kola extract biostimulant treatment did not affect all growth parameters of black orchid plantlets at the acclimatization stage. Plantlet morpho-physiological conditions that are not perfect, such as low photosynthetic efficiency, lack of stomata function, and a thin cuticle layer, cause the applied biostimulants not to be used optimally by black orchid plantlets.

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REFERENCE

Adi, N., Astarini, I., & Astiti, N. (2014). Aklimatisasi Anggrek Hitam (Coelogyne pandurata Lindl.) Hasil Perbanyakan In Vitro pada Media Berbeda. *Jurnal Simbiosis*, *2*(2), 203–214.

Agustin, A., Riniarti, M., & Duryat. (2014). Pemanfaatan Limbah Serbuk Gergaji dan Arang Sekam Padi sebagai Media Sapih untuk Cempaka Kuning (Michelia champacea). *Jurnal Sylva Lestari*, *2*(3), 49–58.

Aulya, N., Noli, Z., Bakhtiar, A., & Mansyurdin. (2018). Effect of Plant

Extracts on Growth and Yield of Maize (Zea mays L.). *Pertanika J. Trop. Agric. Sc*, *41*(3), 1193–1205.

Bhattacharyya, P., Ghosh, S., Mandi, S., Kumaria, S., & Tandon, P. (2017). Genetic Variability and Association of AFLP Markers with Some Important Biochemical Traits in Dendrobium thyrsiflorum threatened Medicinal Orchid. *S. Afr. J. Bot*, *109*, 214–222.

Bulgari, R., Franzoni, G., & Ferrante, A. (2019). Biostimulants Application in Horticultural Crops under Abiotic Stress Condition. *Journal of Agronomy*, *9*(306), 1–30.

Drew, A., Kavanagh, K., & Maynard, C. (1992). Acclimatizing Micropropagated Black Cherry by Comparison with Half-Sib Seedlings. *Physiol Plant*, *86.* 459–464.

Du Jardin, P. (2015). Plant Biostimulants: Definition, concept, main categories, and regulation. *Scientia Horticulturae*, *196.* 314.

Handini, E. (2019). Penyimpanan biji dan mikropropagasi anggrek pensil (Papilionanthe hookeriana) di Kebun Raya Bogor. *Pros Sem Nas Masy Biodiv Indon*, *5*(1), 7–12.

Hardjo, P. H. (2018). *Kultur Jaringan Anggrek: Embriogenesis somatik Vanda tricolor (Lindl.) var. Pallida*. Graha Ilmu.

Hartati, S., Nandariyah, Yunus, A., & Djoar, W. (2017). Short Communication: Cytological studies on black orchid hybrid (Coelogyne pandurata Lindley). *BIODIVERSITAS*, *18*(2), 555–559.

Hazarika, B. (2006). Morpho-physiological Disorders in In Vitro Culture of Plants. *Sci Hort*, *108*, 105–120.

Hidayat, S., Saputri, W., & Astriani, M. (2018). *Metodologi Penelitian Biologi*. Universitas Muhammadiyah Palembang Press.

Irsyadi, M. B. (2021). Factors That Effect of the Optimal Plantlet Growth from Tissue Culture on the Acclimatization Stage. *Proc. Internat. Conf. Sci. Engin*, *4.* 100–104.

Izudin, E. (2013). Teknik Aklimatisasi Tanaman Hasil Kultur Jaringan. *Jurnal Informasi Teknis*, *11*(2), 49–56.

Konar, S., Karmakar, J., Ray, A., Adhikari, S., & Bandyopadhhyay, T. (2018). Regeneration of Plantlets Through Somatic Embryogenesis from Root Derived Calli of Hibiscus sabdariffa L. (Roselle) and Assessment of Genetic Stability by Flow Cytometry and ISSR Analysis. *PLoS One*, *13*.

Kustini, S. J. (2011). *Factsheet Anggrek Hitam*. Yayasan WWF Indonesia.

Lakitan, B. (1995). *Fisiologi Pertumbuhan dan Perkembangan Tanaman*. Raja Grafindo Persada.

Lindsey, K., Jager, A., & van Steden, J. (1998). Effect of A Seaweed Concentrate on Acclimatization of In Vitro Grown Planlets of Kniphofia pauciflora and Scilla krausii. *S Afr J Bot*, *64*(4), 262–264.

Managanta, A., & Pangli, M. (2014). Studi Habitat dan Inventarisasi Anggrek Hitam (Black Orchid) di Kawasan Hutan Cagar Alam Bancea Kecamatan Pamona Selatan, Kabupaten Poso. *Jurnal Agro Pet*, *11*(1), 19–45.

Nawawi. (2014). Jenis-Jenis Anggrek Epifit pada Kawasan Hutan Bremi Distrik Manokwari Utara. *Jurnal Biodiversitas*, *1*(2), 7–10.

Nugroho, C., & Raden, I. (2021). Aklimatisasi Tiga Jenis Anggrek pada Media Tanam yang Berbeda. *Jurnal Pertanian*, *12*(2), 109–107.

Paradikovic, N., Teklic, T., Zeljkovic, S., Lisjak, M., & Spoljarevic, M. (2018). Biostimulants Research in Some Horticultural Plant Species. *Food and Energy Security*, 1–17.

Prasetyorini. (2019). *Kultur Jaringan* (1st ed.). Lembaga Penelitian dan Pengabdian Masyarakat

Universitas Pakuan.

Rafiee, H., Naghdi, B., Mehrafarin, A., Qaderi, A., Zarinpanjeh, N., Sekara, A., & Zand, E. (2016). Application of Plant Biostimulants as New Approach to Improve the Biological Responses of Medicinal Plants. *Journal of Medicinal Plants*, *15*(59), 6–39.

Ramadanil. (2010). Beberapa Aspek Botani Anggrek Endemik Coelogyne celebensis J.J. Sm dari Taman Nasional Lore Lindu Sulawesi Tengah. *Jurnal Biocelebes*, *4*(1), 1–14.

Sandra, E. (2005). *Membuat Anggrek Rajin Berbunga*. Agromedia Pustaka.

Silva, J., Hossain, M., Sharma, M., Dobranszki, J., Cardoso, J., & Songjun, Z. (2017). Acclimatization of in Vitro-derived Dendrobium. *Horticultural Plant Journal*, *3*(3), 110–124.

Silva, S., Brown, P., & Ponchet, M. (2013). *First World Congress on the Use of Biostimulants in Agriculture*. International Society of Horticultural Science.

Sri, G. (2010). *Pengaruh Ekstrak Pegagan (Centella asiatica (L.) Urban) sebagai Alelokemi terhadap Perkecambahan dan Pertumbuhan Bayam Duri (Amaranthus spinosus) serta Tomat (Lycopersicum esculentum)* [Skripsi]. Univrsitas Sebelas Maret.

Ummah, K., Noli, Z., Bakhtiar, A., & Mansyurdin. (2017). Effect of Certain Plant Crude Extracts on the Growth of Upland Rice (Oryza sativa L.). *Int. J. Curr. Res. Biosci. Plant Biol*, *4*(9), 1–6.

Untari, R., Sandra, E., & Puspitaningtyas, D. (2007). Aklimatisasi Bibit Anggrek Hitam (Coelogyne pandurata Lindl.). *Buletin Kebun Raya Indonesia*, *10*(1), 13–19.

Van Oosten, M., Pepe, O., Pascale, S., Silletti, S., & Maggio, A. (2017). The Role of Biostimulants and Bioeffectors as Alleviators of Abiotic Stress in Crop Plants. *Chem Biol Technol Agric*, *4*(5), 1–12.

Wahyuni, H., & Suranto. (2021). Dampak Deforestasi Hutan Skala Besar terhadap Pemanasan Global di Indonesia. *Jurnal Ilmiah Ilmu Pemerintahan*, *6*(1), 148–162.

Waluya, A. (2009). *Aklimatisasi Planlet Hasil Perbanyakan secara Kultur Jaringan*. Departemen Agronomi dan Hortikultura Institut Pertanian Bogor.

Zakiah, Z., Suliansyah, I., Bakhtiar, A., & Mansurdin. (2017). Effect of Crude Extracts of Six Plants on Vegetative Growth of Soybean (Glycine max Merr.). *Journal of Advances in Agricultural Science an Technology*, *4*(7), 1–12.

Ziv, M., & Chen, J. (2008). The Anatomy and Morphology of Tissue Cultured Plants. *Springer*, *1*(3), 465–477.