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The Effect of IAA and BAP on Root Induction of Cattleya Orchids

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Abstract

Orchid is an ornamental plant commodity that has an important meaning in international trade. Until now, orchids are still the center of attention of farmers and lovers of ornamental plants, because of their bright potential as cut flowers and potted plants. The popular and widespread type of orchid is Cattleya sp. This orchid is also called the queen of orchids because of its colorful flowers. Cattleya sp orchids take a long time to propagate by seed, about 4 to 7 years, so other methods are needed to deal with them. Tissue culture is a method for isolating plant parts such as cells, tissues or organs, and cultivating them in an aseptic environment. The purpose of this study was to decide the optimal interaction of growth regulators indole acetic acid (IAA), benzyl amino purine (BAP), and their interactions with the root induction of Cattleya sp. Cytokinins communicate with auxin in figuring out where the cells are going to differentiate. In this research method, differences in concentrations of indole acetic acid (IAA: 0, 2, 4, 6 ppm) and concentrations of benzyl amino purine (BAP: 0, 0, 5, 1 ppm) were carried out. Parameters observed were the number of roots and root length. Data were analyzed descriptively and inferentially using ANOVA. The most extensive root system was obtained in the treatment medium containing 0.5 ppm BAP and 6 ppm IAA, which was 6.33. The highest root length was found in the B0I4 treatment (BAP 0 + IAA 4 ppm). The after effects of the investigation of the interaction variance between the IAA treatment and the BAP treatment were 0.005, intending that there was a significant effect of the combination of IAA and BAP on the leaf length of Cattleya sp.

Keywords: *Cattleya sp., Growth regulator, In Vitro, Orchid*

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INTRODUCTION

Orchid is an ornamental plant commodity that has an important meaning in international trade. Until now, orchids are still the center of attention of farmers and lovers of ornamental plants, because of their bright potential as cut flowers and potted plants. The economic potential of orchids as a horticultural commodity has been exploited and developed by many countries, including Indonesia. This shows that the flower business at the international level is very prospective. The development of the orchid business is expected to have an impact on increasing income, providing labor, and growing the national economy. However, the development of orchids in Indonesia is constrained by the limited quality of seeds, because the technology used is still traditional. Clonal propagation technology through tissue culture techniques can overcome the availability of quality seeds on a mass scale (Thengane et al. 2006). The famous and broad kind of orchid is Cattleya sp. Because of its flower-like flowers, the Cattleya orchid is also known as the "queen of orchids." Cattleya sp. has a bigger blossom shape than different orchids, has a brilliant and lovely tone, has a fragrant smell, and is impervious to high temperatures. *Cattleya* sp. orchids take a long time to propagate by seed, about 4 to 7 years, so other methods are needed to deal with them. Orchid seeds are produced in large quantities, 2-3 million per capsule, but it is difficult for orchids to germinate spontaneously due to lack of endosperm.

Tissue culture is a method for isolating plant parts such as cells, tissues or organs, and cultivating them in an aseptic environment (Sulistiani & Samsul 2015). In general, the source of the explants and the kind of media used in tissue culture are very important factors in determining whether or not plant propagation is successful. The wellspring of explants in tissue culture is the piece of the plant that is still effectively separating (meristem tissue). Shoots, leaves, roots, seeds, shoots, cotyledons, hypocotyls, fruit, and ovaries are all examples of explants that can be used (Henuhili 2013).

In tissue culture, the growing medium has a significant impact on the development of the explants and the seeds they produce. The sythesis of the media utilized relies upon the sort of plant to be spread. Typically, agar, vitamins, mineral salts, and growth regulators make up the medium. Auxins and cytokinins are two types of growth regulators (PGR) that are frequently utilized in tissue culture media. Auxin is a PGR that assumes a part in prompting establishing in vitro spread, while cytokinin assumes a part in enlistment of explant shoots.

Cattleya orchid has always been a top priority for hobbyists and collectors alike. So that wherever these orchids are located they will always be hunted by collectors. Conventional Cattleya propagation through tillers takes a relatively long time to grow. Therefore, another alternative was carried out in Cattleya propagation by means of tissue culture techniques (in vitro) and added growth regulators. Cattleya propagation using tissue culture technique (in vitro) is carried out to obtain plant seeds in large quantities, the time needed to obtain plant seeds is not too long and the superior characteristics of the mother plant can be maintained.

METHOD

From October 2021 to January 2022, the plant tissue culture laboratory at Yayasan Hidayatul Islam was the setting for this study. Autoclave, measuring cup, culture bottles, heater (stove), beaker, Laminar Air Flow Cabinet (LAFC), pH meter, volume syringe, Bunsen, a culture rack, an analytical balance, and a stirring rod, scalpel, knife, tweezers, sprayer, refrigerator, and petri dish were all used in this study to collect Cattleya orchid buds made from in vitro-grown orchid seeds. Murashige-Skoog (MS) + Indole acetic acid (IAA) (0; 2; 4; 6 ppm) was the primary medium + benzyl amino purine (BAP) (0; 0.5; 1ppm). Table 1 depicts the combination of these media.

Table 1. *Cattleya* sp. in Combination Media for Orchid Root Induction.

IAA (ppm) / BAP (ppm)	0	2	4	6
0	B_0I_0	B_0I_2	B_0I_4	B_0I_6
0,5	$B_{0,5}I_0$	$B_{0,5}I_2$	$B_{0,5}I_4$	$B_{0,5}I_6$
1	B_1I_0	B_1I_2	B_1I_4	B_1I_6

The study's design (CRD) was completely random. Twelve treatments were combined, with three replications. The initial steps in this study were sterilization, preparation of the media, sterilization of the explants, observing and planting. The observation's specifics were the total quantity of roots, shoots, and leaves. ANOVA was used to analyze observational data. The Duncan's Multiple Range Test (DMRT) is used to continue the analysis if there is a significant difference in the results.

RESULTS AND DISCUSSION

The parameters observed were the number of roots and root length in the combination treatment of IAA (0; 2; 4; 6 ppm) and BAP (0; 0.5; 1 ppm) with MS basic media.

Number of Roots

The average number of roots can be seen in the figure 1. The highest number of roots was obtained in the treatment medium containing 0.5 ppm BAP and 6 ppm IAA, which was 6.33, while the lowest number of roots was obtained in explants with 0.5 ppm BAP and 0 ppm IAA, which was 3.33. This shows that the presence of auxin for induction of rooting is very important. One of the roles of giving auxin is that it can stimulate the formation and elongation of roots at low concentrations, while high concentrations can inhibit it. The main role of auxin in plant propagation is to stimulate roots in stem and leaf cuttings and to increase root branching. Early root formation begins with the metabolism of nutrient reserves in the form of carbohydrates which produce energy which then encourages cell division and forms new cells in the network. Visually, the roots begin to form with a bulge on the surface of the stem, then elongates and forms intact roots.

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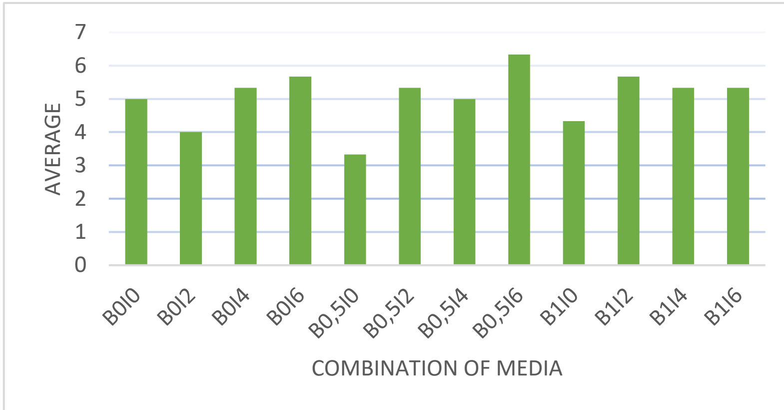

Figure 1. Average Number of Orchid Roots *Cattleya* sp.

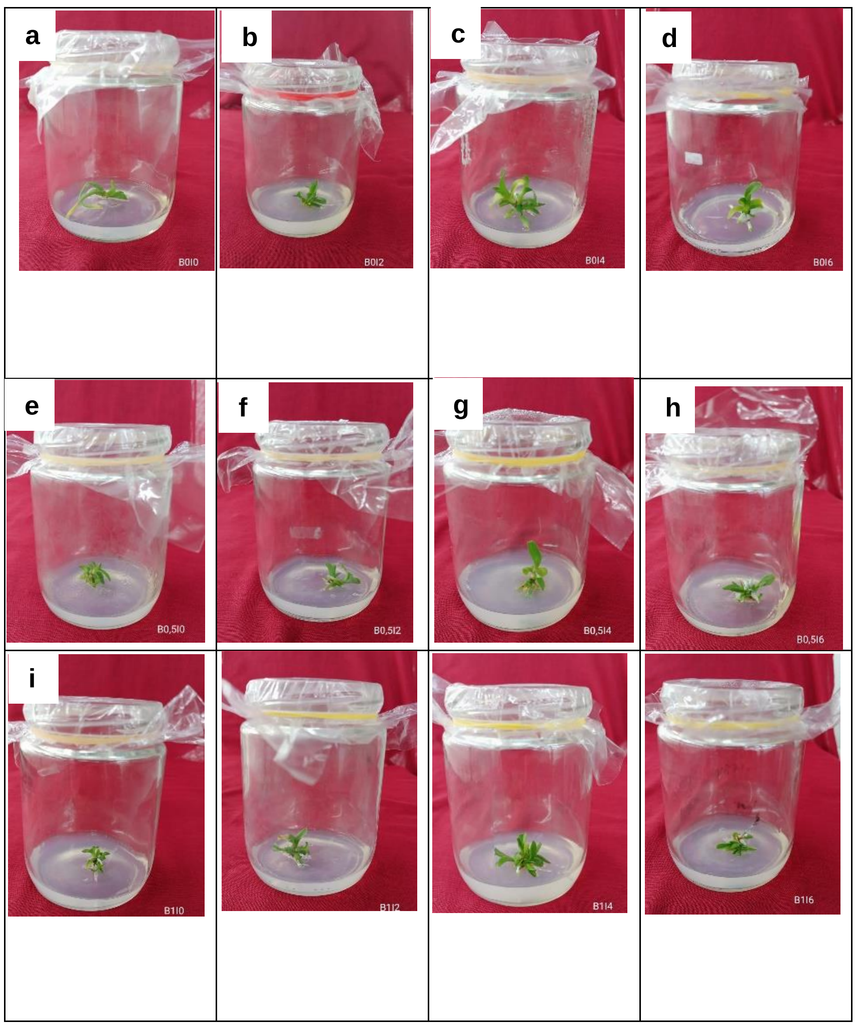

Figure 2. Results of *Cattleya* sp. Root Induction Research. combination of BAP and IAA: a. B_0I_0, b. B_0I_2, c. B_0I_4, d. B_0I_6, e. $B_{0,5}I_0$, f. $B_{0,5}I_2$, g. $B_{0,5}I_4$, h. $B_{0,5}I_6$, i. B_1I_0, j. B_1I_2, k. B_1I_4, l. B_1I_6

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Root Length

Root development in explants can be constrained by managing auxin bunch development controllers. The auxin group used in tissue culture is IAA (Indole Acetic Acid). Figure 3. shows that the highest root length was found in the B0I4 treatment (BAP 0 + IAA 4 ppm). This shows that IAA is an auxin group that can induce rooting. The lowest root length was found in the B0I0 treatment (BAP 0 + IAA 0 ppm) and in the B0.5I0 treatment (BAP 0.5 ppm + IAA 0 ppm).

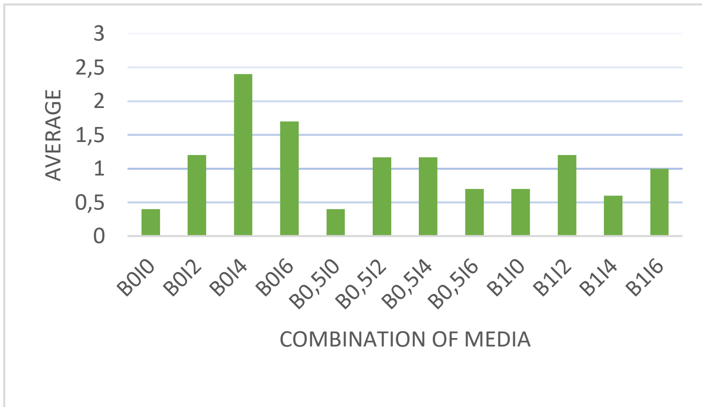

Figure 3. Average Root Length of *Cattleya* sp.

Table 2. Analysis of Variance (ANOVA) Effect of Giving IAA and BAP on Root Length of Cattleya sp Orchid Plants

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.959	123.309[b]	4.000	21.000	.000
	Wilks' Lambda	.041	123.309[b]	4.000	21.000	.000
	Hotelling's Trace	23.487	123.309[b]	4.000	21.000	.000
	Roy's Largest Root	23.487	123.309[b]	4.000	21.000	.000
Treatment_BAP	Pillai's Trace	.917	4.659	8.000	44.000	.000
	Wilks' Lambda	.231	5.676[b]	8.000	42.000	.000
	Hotelling's Trace	2.689	6.723	8.000	40.000	.000
	Roy's Largest Root	2.425	13.336[c]	4.000	22.000	.000
Treatment _IAA	Pillai's Trace	.475	1.083	12.000	69.000	.388
	Wilks' Lambda	.578	1.073	12.000	55.852	.400
	Hotelling's Trace	.642	1.052	12.000	59.000	.416
	Roy's Largest Root	.467	2.683[c]	4.000	23.000	.057
Treatment _BAP * Treatment _IAA	Pillai's Trace	1.122	1.559	24.000	96.000	.068
	Wilks' Lambda	.229	1.630	24.000	74.470	.057
	Hotelling's Trace	2.019	1.640	24.000	78.000	.054
	Roy's Largest Root	1.155	4.619[c]	6.000	24.000	.003

a. Design: Intercept + treatment_BAP + treatment _IAA + treatment _BAP * treatment_IAA
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table 2 shows that the significant value of the results of the analysis of the various effects of the IAA treatment on root length was 0.625 (above 0.05), meaning that the administration of IAA had no significant effect on the root length of *Cattleya* sp. The results of the analysis of the variety of BAP treatments on root length were 0.000, meaning that giving BAP had a significant effect on the root length of *Cattleya* sp.,

Table 3. Root length of *Cattleya* sp. 12 Weeks after Planting

IAA (ppm) / BAP (ppm)	0	2	4	6	Average
0	$3{,}20^{d}$	$1{,}20^{ab}$	$2{,}40^{cd}$	$1{,}70^{bc}$	2,13
0,5	$0{,}40^{a}$	$1{,}17^{ab}$	$1{,}17^{ab}$	$0{,}70^{ab}$	0,86
1	$0{,}70^{ab}$	$1{,}20^{ab}$	$0{,}60^{ab}$	$1{,}00^{ab}$	0,88
Average	1,43	1,19	1,39	1,13	

Note: Numbers followed by different letters in the same column or row show significantly different (P>0.05) in the DMRT test at the 5% level

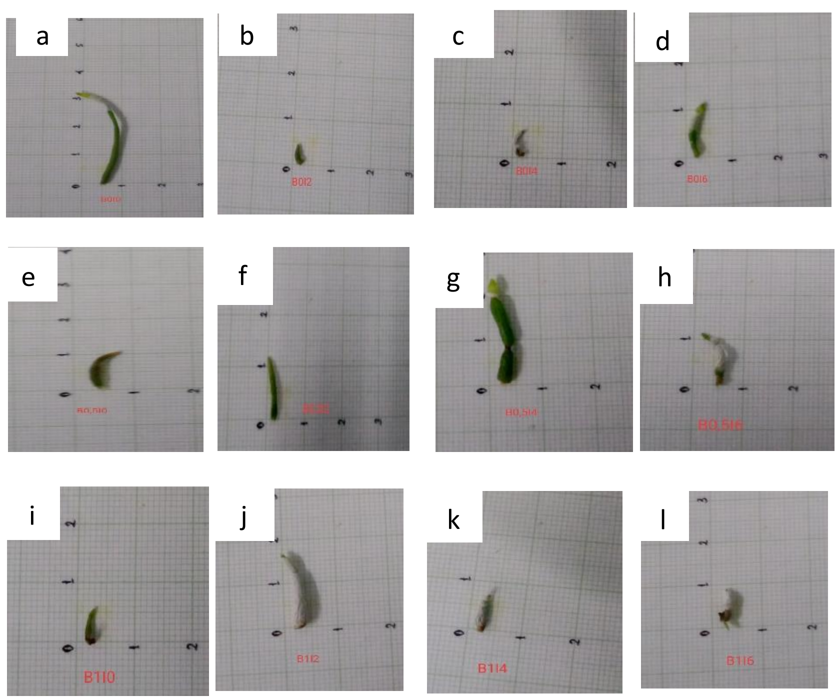

Figure 4. Root length results of *Cattleya* sp. combination of BAP and IAA: a. B_0I_0, b. B_0I_2, c. B_0I_4, d. B_0I_6, e. $B_{0,5}I_0$, f. $B_{0,5}I_2$, g. $B_{0,5}I_4$, h. $B_{0,5}I_6$, i. B_1I_0, j. B_1I_2, k. B_1I_4, l. B_1I_6

One of the roles of giving auxin is that it can stimulate the formation and elongation of roots at low concentrations, while high concentrations can inhibit it. The benefits of the rooting hormone are overall expanding the level of establishing, speeding up root commencement, expanding the number and nature of roots, and empowering uniform establishing. The primary job of auxin in plant spread is to animate roots in stem and leaf cuttings and to increment root fanning. Early root formation begins with the metabolism of

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nutrient reserves in the form of carbohydrates which produce energy which then encourages cell division and forms new cells in the network.

Number of Shoots

The number of shoots was observed from 1 week after planting (MST) to 12 weeks after planting (MST). The number of shoots is obtained by counting the shoots that grow from explants. The emergence of shoots is marked by the presence of green protrusions and forms leaves.

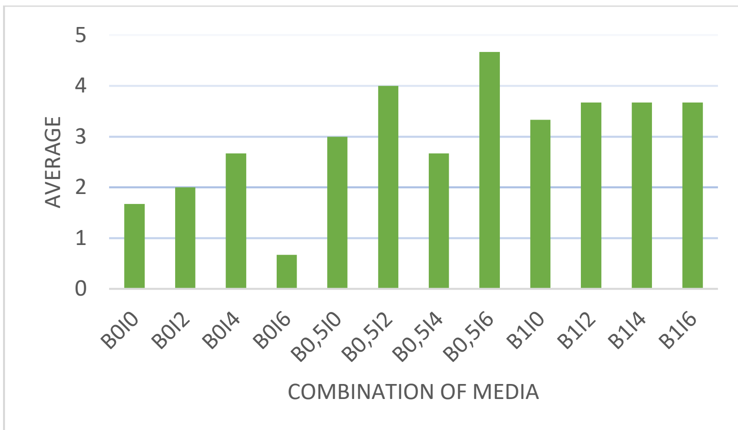

Figure 5. The Average Number of *Cattleya* sp. Orchid Shoots.

The highest number of shoots, namely 4.67, was found in the MS+BAP 0.5 ppm +IAA 6 ppm (B0.5I6) treatment medium, while the lowest number of shoots was found in the MS+BAP 0.5 ppm +IAA 6 ppm (B0I6) media, namely 0 ,67. Giving IAA didn't altogether influence the quantity of shoots of *Cattleya* sp. and BAP did not significantly affect the number of shoots of *Cattleya* sp. Auxin can generally encourage cell enlargement, whereas when combined with cytokinins it can trigger shoot growth by spurring plant morphogenesis. The formation of shoots and leaves relies heavily on the combination of high cytokinins and low auxin. If utilized in the appropriate concentration, both of these growth regulators play an important role in promoting tissue growth.

Number of Leaves

Leaves are one of the most important organs in plants. Leaves act as energy-providing organs through the process of photosynthesis. The biggest number of leaves was gotten in the media treated with B1I4 with an average of 21, while the most minimal number of leaves was in the media treated with B0I6 with an average of 8.3. The emergence of leaves begins with the emergence of shoots. The shoots elongate and then develop into leaves. Together with auxins, cytokinins stimulate cell division and influence differentiation pathways. The addition of growth regulators can affect RNA metabolism which plays a role in protein synthesis through the process of transcription of RNA molecules. Increase in protein synthesis as a source of energy used for growth. Balance and interaction between growth regulators absorbed from the media and those contained in the explant itself (endogenous) determine the number of leaves produced on each planted explant (exogenous). Auxin can trigger cell enlargement, but at low concentrations NAA combined with BAP can trigger the formation of shoots and leaves.

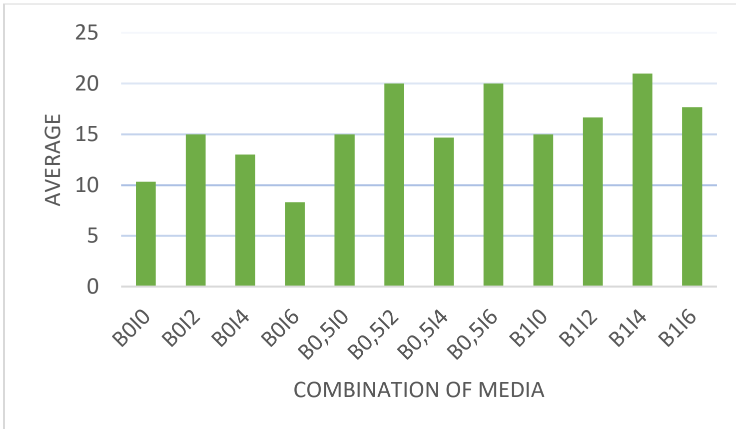

Figure 6. The Average Number of *Cattleya* sp. Orchid Leaves

One of the roles of giving auxin is that it can stimulate the formation and elongation of roots at low concentrations, while high concentrations can inhibit it. Due to competition with endogenous auxin for the position of the cell membrane signal receiver, the addition of high concentrations of auxin inhibits tissue growth, preventing the addition of external auxin from affecting cell growth and development (Paramartha, 2012). The primary job of auxin in plant spread is to animate roots in stem and leaf cuttings and to increment root fanning. Early root formation begins with the metabolism of nutrient reserves in the form of carbohydrates which produce energy which then encourages cell division and forms new cells in the network. In addition to giving auxin, according to Aiken & Smucker (1996) states that root growth is also influenced by stimulation from the surrounding environment such as the availability of water, nutrients or nutrients, light, as well as biochemical signals from the hormones cytokinin and abscisic acid which are transmitted from the roots to function as regulators. active various physiological processes that affect the growth and multiplication of roots. Then in the journal Novak (2014) also stated that the success of plant explants in the root induction process is determined by the ability of explants to produce their own auxin. After the plant roots are able to produce auxin independently, these conditions allow it to be selected as a root induction explant. In orchid roots, there are usually symbiont fungi and bacteria that will synthesize auxin as a factor supporting the growth and multiplication of orchid roots.

Epigenetic conditions of plants also play an important role in tissue culture. Arabidopsis plants in the aging process experience a 16% decrease in gene expression so that it is no longer effective when used as tissue culture explants. In orchids, the expression of the root growth gene is most active during the early stages of the development of the root functional system. When the orchid seed germinates, it will produce one cotyledon leaf which serves as the initial energy source for the plant. After the cotyledons of the orchid are used up, it will build its root system to start absorbing nutrients, water, and in this phase gene expression starts to be active. There is no specific age for this plant phase, but this phase certainly occurs in young plants. When the expression of the root growth gene is active, this plant is suitable for use as an in vitro culture explant (Miryeganeh, 2022).

Meanwhile, according to Ibrahim (2010) the meristem size for in vitro plant culture is most effective in the range of 0.25-0.5 cm, which is obtained from medium-sized shoots, the larger the shoot size. While shoots that are too small, of course the culture process is easier but the chances of regeneration are small. Small sized explants are relatively easy to go through the sterilization stage and do not need a large space or a lot of media. Meanwhile, large explants tend to be difficult to sterilize, have a lot of contaminants and require a lot of media in the culture process. However, when viewed from the regeneration ability, large-sized explants will be far more optimal than small-sized explants (Basri, 2016).

CONCLUSION

The highest number of roots was obtained in the treatment medium containing 0.5 ppm BAP and 6 ppm IAA, which was 6.33, while the lowest number of roots was obtained in explants with 0.5 ppm BAP and 0 ppm IAA, which was 3.33. The highest root length was found in the B0I4 treatment (BAP 0 + IAA 4 ppm). The lowest root length was found in the B0I0 treatment (BAP 0 + IAA 0 ppm) and in the B0.5I0 treatment (BAP 0.5 ppm + IAA 0 ppm). The results of the analysis of the variety of BAP treatments on root length were 0.000, meaning that giving BAP had a significant effect on the root length of *Cattleya* sp.. The highest number of shoots, namely 4.67, was found in the MS+BAP 0.5 ppm +IAA 6 ppm (B0.5I6) treatment medium, while the lowest number of shoots was found in the MS+BAP 0.5 ppm +IAA 6 ppm (B0I6) media, namely 0 ,67. The highest number of leaves was obtained in the media treated with B1I4 with an average of 21, while the lowest number of leaves was in the media treated with B0I6 with an average of 8.3.

REFERENCES

Basry, Arie. (2016). Study of the Utilization of Tissue Culture in Virus-Free Plant Propagation. bppsdmp.pertanian.go.id. p 64-73.

Central Bureau of Statistics and Directorate General of Horticulture. (2014). Orchid Production by Province. 2010-2014. Horticulture Subsector. Accessed from. http://www.pertanian.go.id/ap-pages/mod/datahorti .

Gunawan, LW. (1987). Tissue culture techniques. Bogor. Plant Tissue Culture Laboratory. 89 page

Harahap, F., Roedhy P., Suharsono, Cicik S. and Suci R. (2014). In Vitro Growth and Rooting of Mangosteen (Garcinia mangostana L.) on Medium With Various Concentrations of Growth Regulators. *Journal of Biological Biosciences. Vol 21*(4), 151-158

Harahap, F. (2010) Practical Techniques to Make Orchids Always Flower. Community service. 16(61):41-50.

Harapan, F. (2011). Plant tissue isolation method. Medan : United Press.

Harahap. F., Poerwanto. R., Spbir., Hasruddin., Suriani. C., Siallagan, J & Rohyan. (2015). Sterilization of Pineapple Explan from Sipahutar, North Sumatra, Indonesia (*Ananas comosus L*) and *In vitro* Growth Induction. *Asian Jr. of Microbal. Biotect. Env.Sc.* 17 (2) : 469-478

Henuhili V. (2013). Plant Tissue Culture. Yogyakarta: UNY Press. 88 p

Isda, MN, and Fatonah, S. (2014). Root induction in Orchid shoot explants Grammatophylum scriptum var. In Vitro Oranges on MS Media with the Addition of NAA and BAP. *Journal of Biology Al Kauniyah. 7*(2): 53-57

Iswanto . (2007). Orchid care instructions. Jakarta: Agromedia Pustaka. 124 p

Karjadi, AK & Buchory, C. (2007). Effect of NAA and BAP on Garlic Meristem Tissue Growth in B5 Media. *J. Hort , 17*(3): 217-223

Lestari, EG (2011). The Role of Growth Regulatory Substances in Plant Propagation Through Tissue Culture. *Journal of AgroBiogen, 7*(1): 63-68

Miryeganeh, Matin, (2022). Ephigenetic Mechanism of Senescence in Plants. *Cells*. 11(2) : 251. doi.10.3390/cells11020251.

Nika, SL, Siregar, LAM, Kardhinata, EH, (2018). Success in the form of Orchid Microshoots (Cattleya trianae Lindl & Rchb.fil.) in several composition media. *Journal of Agroecotechnology* FP USU, 6(1): 113-117.

Novak, Stacey. Lila J Luna. Roshan. (2014). Role of Auxin in Orchid Development. *Plant Signaling & Behavior*. 9(10).

Panjaitan, E. (2005). Growth Response of Orchid (Dendrobium sp.) to Administration of BAP and NAA In Vitro. *Journal of Agricultural Science Research, 3*(3): 45-51.

Paramartha, Aisya., Dini Ermavitalini, Siti Nurfadilah. (2012). The Effect of the Addition of NAA and BAP ZPT Concentrations on the Growth and Development of Dendrobium taurulinum J.J Smith Seeds in Vitro. *Journal of Science and Arts ITS. 1*(1) : E40-E43.

Sulistiani E, Samsul AY. (2015). Production of Plant Seeds Using Tissue Culture Techniques. Bogor: SEAMEO BIOTROP.

Thengane, SR, Deodhar, SR, Bhosle, SV & Rawal, SK. (2006). Direct somatic embryogenesis and plant regeneration in Garcinia indica Choiss, *Current Science, vol. 91*(8), pp 1074-8.

Widiastoety, D. (2014). Effect of Auxin and Cytokinin on the Growth of Mokara Orchid Plantlets (Effect of Auxin and Cytokinin on the Growth of Mokara Orchid Plantlets), *J. Hort. 24*(3):230-238.

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