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Effect of Auxin and Cytokinin on Orchid Subculture Resulting from Coelogyne Crosses

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Abstract
The black orchid or is one of the native orchids of Kalimantan, widely cultivated in Indonesia. New superior-quality Coelogyne can be obtained by crossing. Modifying the media, such as giving PGR, is necessary for good cross plant multiplication results. This experiment aimed to determine the effect of PGR *Benzyl Adenine* (BA) and *Naphtalene Acetic Acid* (NAA) on the growth of orchid subcultures from a cross between *Coelogyne pandhurata* and *Coelogyne rumphii*. The study used a completely randomized design (CRD) with 2 factors, namely PGR BA and NAA, each having a concentration of 0 ppm, 0.2 ppm, 0.4 ppm, and 0.6 ppm with 16 treatment combinations and 4 replications. They were implemented at the Laboratory Plant Physiology and Biotechnology, Faculty of Agriculture, UNS Surakarta, from November 2020 to March 2021. The variables observed included the number of shoots, plant length, plant weight, number of leaves, number of roots, root length, when roots appeared, and when shoots appeared. The results showed that BA 0.6 ppm gives the best output on the growth of the number of shoots, and NAA concentration of 0.2 ppm gives the best results on the variable number of roots. The combination of BA 0.6 ppm and NAA 0.2 ppm gave the best result on plant weight growth. BA and NAA were able to accelerate plant growth of Coelogyne cross multiplication.

Keywords: *Benzyl Adenine*; *Naphthalene Acetic Acid*; multiplication

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Introduction

Orchid cultivation can be done in two ways: conventional and tissue culture. Tissue culture is a plant cultivation technique that utilizes various plant parts such as leaves, stems, roots, and shoots. One of the stages of orchid seed growth is protocorm enlargement, which will later grow into plantlets. These plantlets can be grown in large numbers through sub-culture. The type of media that is widely used for orchid sub-cultures is MS (Murashige and Skoog). According to (1), MS media is optimal for shoot proliferation, but differences in salt contained in it will result in differences in shoot proliferation.

The success of shoot and root multiplication depends on the balance of auxin and cytokinin PGR. Cytokinin and auxin hormones are often used in plantlet development in in vitro culture (2). The Benzyl Adenine (BA) balance and Naphthalene Acetic Acid (NAA) are among them. BA is a hormone from the cytokinin group that can regenerate shoot growth. The speed of the growth process in orchid sub-cultures is influenced by either the media or growth regulators. Meanwhile, NAA is a hormone from the auxin group that can support cell division, stimulate callus growth, and stimulate root growth. Their research (3) resulted in the optimal number of shoots on the growth of Vernonia amygdalana using BA 0.5 mg-l-1. These growth regulators can affect the outgrowth in cell, tissue, or organ culture.

Coelogyne is one of the Indonesian native orchids that are widely cultivated. Orchids from crosses between *Coelogyne pandurata* and *Coelogyne rumphii* could become a new superior plant. There has yet to

be a study regarding adding BA and NAA in the multiplication of *Coelogyne pandurata* and *Coelogyne rumphii* crosses using MS media.

Therefore, it is necessary to research to determine the best concentration of PGR on the growth of sub-cultures of orchids from crosses between *Coelogyne pandurata* and *Coelogyne rumphii* to obtain maximum results. In this study, several media with different concentrations of PGR were studied. The PRG used were *Benzyl Adenine* (BA) and *Naphthalene Acetic Acid* (NAA).

Materials And Methods

The ingredients used in this experiment included orchid plantlets from crosses of *Coelogyne pandurata* and *Coelogyne rumphii*, MS media components, agarose, sucrose, BA and NAA, sterile distilled water, 70% alcohol, NaOH solution, and HCl solution.

The research was implemented from November 2020 to March 2021 at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, UNS Surakarta. The study utilizes a factorial, completely randomized design (CRD). The first treatment is the dosage of BA (*Benzyl Adenine*), which consists of 4 levels: obiter 0, 0.2, 0.4, and 0.6 ppm. The second treatment is the concentration of NAA (*Naphthalene Acetic Acid*), which consists of 4 levels: obiter 0, 0.2, 0.4, and 0.6 ppm. Each treatment was carried out with 4 replications.

Culture media was prepared by giving the treatment at various dosage levels. MS media was sterilized using an autoclave at 121^{o}C, 15 lbs, for 30 minutes. Then, subculture the planlet from crosses between *Coelogyne pandurata* and *Coelogyne rumphii* to media that has been given treatment. The planlets were incubated for 20 weeks under controlled room conditions. Planets were observed by measuring the number of shoots, roots, leaves, plant length, plant weight, and root length.

Data was analyzed with ANOVA (Analysis of Variance) and then examined using the DMRT α 5% test to determine the treatment that gives the best growth in planlet.

Results And Discussion

Genetic factors, such as temperature, humidity, light, growth media, and growth regulators, determine orchid growth through vegetative or generative. Their research (4) said that the administration of growth regulators can provide different responses depending on the type and dose. The use of growth regulators in a medium can be calculated for its kind and concentration to function correctly on plantet growth.

Table 1. Effect of single BA and NAA on the average number of shoots, number of roots, and number of leaves at 20 weeks after planting

Concentration of BA	Number of shoots	Number of roots	
0 ppm	3,46 b	4,19 b	
0,2 ppm	4,07 b	3,14 a	
0,4 ppm	4,82 ab	2 a	
0,6 ppm	6,59 a	2,2 a	
Concentration of NAA	Number of shoots	Number of roots	Number of leaves
0 ppm	3,64 a	1,82 b	14,25 a
0,2 ppm	3,75 a	4,60 a	11,83 a
0,4 ppm	4,22 a	3,88 a	13,12 a
0,6 ppm	7,35 b	2,29 b	20,93 b

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test

Application of BA to the culture medium can increase the number of shoots. This is in line with the states of (5) and (6) that applying BA to the culture medium stimulates the formation of new shoots. BA 0,6 ppm gave the highest number of shoots, with 6,59 shoots, and the lowest result was found at 0 ppm concentration. The results showed that increasing the concentration of BA can increase the number of shoots of Coelogyne crosses. This is in line with the research of (7), which claims that a BA of 0,6 ppm also has the best effect on the number of shoots and leaves. BA application did not affect the number of roots of Coelogyne crosses. The results showed that increasing BA concentration suppressed root growth.

NAA application affects the number of shoots and the number of leaves but does not affect the number of roots of Coelogyne crosses. Applying NAA at a concentration of 0.6 ppm gave the most optimal results on the number of shoots and leaves, with 7.35 shoots and 20.93 leaves. Based on a statement from (8),

endogenous and exogenous growth regulators interact in regulating plant cell growth, including leaf formation.

The average shoot growth begins at week 4 after planting, while root formation starts at week 6 after planting. In contrast, (9) stated root growth started at week 4 after planting. This could be due to differences in age or type of plantlets. The appearance of shoots and roots indicates that plantlet growth has begun. (10) said cytokinins absorbed by the roots (exogenous) from the media could stimulate plant shoots' growth. (11), their research stated that adding combined growth regulators BA and NAA could inhibit shoot growth.

Roots are one of the main variables in observing growth. The appearance of the roots is marked by white protrusions that will gradually elongate to form roots. Applying single PGR BA and NAA to the orchids from the cross of *Coelogyne pandurata* and *Coelogyne rumphii* significantly impacted the number of roots. According to (12), the NAA growing substance with optimum concentration has a more characteristic function in forming plant roots. The best root formation was obtained at a concentration of 0.2 ppm NAA with an average number of roots of 4.60. According to (13), giving NAA to explants can form more roots according to the function of the NAA compound, namely initiating root apical meristem (RAM) or so-called root candidates, and concentrations of NAA that are too high can inhibit root growth. This is because NAA works at low concentrations.

The decrease in the number of roots at the addition of higher PGR concentrations could be due to the availability of hormone content in the plant. So, the content becomes unbalanced when cytokinins and auxins (BA and NAA) are added from the outside. A study conducted by (14) and (15) stated that the best root formation process was obtained on MS media enriched with PGR auxin (NAA, IBA, IAA). Furthermore, (16) noted that the concentration of IAA was better in maximizing the number and length of roots compared to IBA and NAA, while (17) stated that IAA and NAA were more optimal in growing Dendrobium sp. roots.

Table 2. Effect of the combination of BA and NAA on the average plant height (cm), plant weight (g), and root length at 20 weeks after planting

Combination		Plant height	Plant weight	Root length
BA	NAA			
0 ppm	0 ppm	2,00 ± 0,082 a	2,34 ± 0,582 bcd	0,92 ± 0,283 abc
	0.2 ppm	1,78 ± 2,060 a	0,73 ± 1,198 abc	0,78 ± 0,903 abc
	0.4 ppm	4,25 ± 0,265 e	1,15 ± 0,819 abc	0,85 ± 0,412 c
	0.6 ppm	3,25 ± 0,656 c	1,96 ± 1,823 abcd	0,80 ± 0,408 ab
0,2 ppm	0 ppm	2,13 ± 0,456 ab	0,93 ± 1,044 abc	2,03 ± 0,763 bc
	0.2 ppm	2,23 ± 1,758 ab	0,29 ± 0,084 a	0,60 ± 0,408 a
	0.4 ppm	2,48 ± 0,946 b	1,03 ± 1,001 abc	0,48 ± 0,457 a
	0.6 ppm	3,83 ± 0,936 e	1,73 ± 0,275 abcd	1,08 ± 0,818 abc
0,4 ppm	0 ppm	2,35 ± 0,823 b	0,56 ± 0,764 ab	0,93 ± 1,209 a
	0,2 ppm	3,53 ± 1,328 cd	1,98 ± 1,289 abcd	1,23 ± 0,608 abc
	0.4 ppm	1,90 ± 1,428 a	1,30 ± 1,538 abcd	0,45 ± 0,332 a
	0.6 ppm	3,70± 0,906 cde	2,65 ± 3,725 cd	0,93 ± 0,585 abc
0,6 ppm	0 ppm	3,13 ± 0,189 c	2,10 ± 1,374 abcd	0,58 ± 0,419 ab c
	0,2 ppm	3,78 ± 0,954 de	3,43 ± 0,479 d	1,23 ± 0,465 abc
	0.4 ppm	2,75 ± 0,635 b	0,53 ± 0,403 ab	1,08 ± 0,350 abc
	0.6 ppm	3,00 ± 0,938 c	1,13 ± 0,377 abc	2,20 ± 0,909 c

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significance in the DMRT α5% test

The balance of application auxin and cytokine types of hormones can affect plant growth. Plants will respond to growth if the hormone applied is to their needs. According to (18), cytokinins and auxins affect root and shoot growth. The addition of a combination of PGR BA and NAA was able to have a significant effect on plant height, plant weight, and root length. This indicates different results from the research (19) that adding NAA or BA did not significantly affect the planlet weight. The average plant height increased every week. The highest planets were found in the combination of BA 0.2 ppm and NAA 0.6 ppm, with 3.83 cm. According to (20), stem elongation occurs due to the division and the elongation of new cells found in the meristems and stem segments, which can cause a plant to grow taller. (21) discovered that MS medium with NAA 0.6 mg/l gives a higher stem length in the tested plants.

The results of DMRT 5% showed that the combination of BA 0.6 ppm and NAA 0.2 ppm gives the best results on the weight of orchid planlets from the cross of *Coelogyne pandurata* and *Coelogyne rumphii*, with 3.43 g. Their research (22) revealed that the sucrose content in the media absorbed by plants could increase the weight of a plant itself. The sucrose content in tissue culture medium serves as a provider of energy and carbon for plantlets. Other variables also influence the weight of a plant in the plant.

In addition to plant height and weight, the combination of BA and NAA also significantly affects root length. The longest roots in the cross of Coelogyne pandurata and Coelogyne rumphii were obtained by combining BA 0.6 ppm with NAA 0.6 ppm with an average root length of 2.20 cm. Based on this value, it can be concluded that the best root elongation was obtained in BA and NAA, which were given at the same concentration. This study's results align with research (23), which found the longest roots in the combination of NAA 0.5 mg/l and BA 0.5 mg/l. This is because NAA can increase plant rooting in vitro in various types of plants (24).

Conclusion

The addition of BA and NAA in the culture medium of crosses of *Coelogyne pandurata* and *Coelogyne rumphii* subculture can promote the growth of plantlets. A single addition of BA dose 0.6 ppm gave the optimum output on the development of the shoots (6.59 shoots), while the NAA 0.2 ppm gave the optimum result on the variable number of roots (4.6 roots). The combination of BA 0.6 ppm and NAA 0.2 ppm gave the best results on plant weight growth (3.43 g).

Suggestion

Based on the research that has been done, suggestions that can be given for further research are that additional research is needed on orchid subculture from *Coelogyne pandurata* and *Coelogyne rumphii* on MS media with PGR BA concentration 0,6 ppm and NAA 0,2 ppm to obtain optimal results.

References

1. Rezaei A, Hosseipour B. In vitro propagation and rooting of garnem rootstock. Plant Cell Biotech Mol Bio. 2015; 16(1-2): 41 – 47.
2. Feng J, Shi Y, Yang S, Zuo J. Hormone metabolism and plant signaling. Academic Press. 2017; 77-106.
3. Mutasim M, Eisa K, Elgaali I. In vitro multiple shoot regeneration from nodal explants of *Vernonia amygdalana,* an important medicinal plant. Af Crop Science. 2017; 8(2): 747–752.
4. Nurana AR, Wijana G, Dwiyani R. Effect of 2-ip and NAA on plantlet growth of hybrid dendrobium orchids at the subculture stage. Agrotrop. 2017; 7(2): 139–146.
5. Ni Made, Hartini M, Rauf G. In Vitro Proliferation of Plb Orchid Dendrobium lasianthera Result of Genetic Mutation Induction with Colchicine Through Addition of Benzyl Adenine. Bul Agrohorti. 2017; 5(2): 146-156.
6. Kumari N, Pragiti M. Mass in vitro micropropagation of banana Musa sp. International J of Plant Protection. 2016; 9(1): 204–210.
7. Indriani BS, Enni S, Krispinus. Efektivitas substitusi sitokinin dengan air kelapa pada multiplikasi tunas krisan secara in vitro. J Life Science. 2017; 3(2): 148–155.
8. Zahara M, Avishek D, Patchareeya B. Effect of plant growth regulators on the growth and direct shoot formation from leaf explants of the hybrid *phalaenopsis* ‘pink.’ Acta Agriculturae Slovenica. 2018; 111(1): 5–16.
9. Kartiman R, Dewi S, Syarifah IA. Multiplikasi in vitro anggrek hitam (*Coelogyne pandurata* lindl.) pada perlakuan kombinasi NAA dan BAP. J

Bioteknologi dan Biosains Indonesia. 2018; 5(1): 75–87.
10. Garvita ES. Effect of 6-benzyl amino purine on the structure of photosynthetic apparatus of faba bean (Vicia faba L.). Appl Biochem Microbiol. 2019; 39: 411–417.
11. Jitsopakula N, Kachit T, Keiko L. Efficient adventitious shoot regeneration from shoot tip culture of *Vanda coerulea*, a Thai orchid. Science Asia. 2013; 39(3): 449–455.
12. Bohidar S, Thirunavoukkarasu M, Roa TV. Effect of plant growth regulators on in vitro micropropagation of 'garden rue' (R. graveolens L.). International Journal of Integrated Biology. 2018; 3(1): 36-43.
13. Kriswanto B, Sigit S, Didik PR. The propagation of orchid through formation of protocorm like bodies (plb) for supporting the resque of phalaenopsis orchid in Indonesia. Proceedings of the international conference of Fossa. J Biosfera. 2017; 34(1): 9–14.
14. Hajare ST, Nitin MC, Girum C. Effect of Growth Regulators on *In Vitro* Micropropagation of Potato (*Solanum tuberosum* L.) Gudiene and Belete Varieties from Ethiopia. The Scientific Word Journal. 2021; 1(1): 1–8.
15. Ngomuo M, Emerald M, Patrick N. The effects of auxins and cytokinin on growth and development of (Musa sp.) Var. "Yangambi" Explants in Tissue Culture. American Journal of Plant Sciences. 2018; 4(2): 2174-2180.
16. Maharjan S, Pradhan S, Thapa BB, Pant B. In Vitro Propagation of Endangered Orchid, Vanda pumila Hook.f. through Protocorms Culture. American Journal of Plant Sciences. 2019; 10(7): 1220-1232.
17. Pradhan S, Tiruwa BL, Subedee BR. Efficient plant regenration of Cympidium aloifolium, a threaened orchid of nepal through artificial seed technology. American J of Plant Science. 2017; 7(2): 1964–1974.
18. Ansari ZNE, Amina EM, Ihtissam B. Effect of macronutrients, cytokinins, and auxins on in vitro organogenesis of *thymus vulgaris* L. American Journal of Plant Sciences. 2019; 10(9): 1482-1502.
19. Siron U, Noertjahyani, Yana T, Romiyadi. Pengaruh konsentrasi zat pengatur tumbuh naphthalene acetic acid dan benzil amino purin terhadap pertumbuhan protokorm anggrek *Dendrobium spectabile* pada kultur in vitro. Paspalum: Jurnal Ilmiah Pertanian. 2019; 7(1): 16–23.
20. Shoemaker RC, Amberger LA, Palmer RG. Effect 2.4-dichlorophenoxy acetic acid concentration on somatic embryogenesis and heretable variation in soybean (*Glycine max* (L.) Merr)', In vitro Cell Dev. Biology. 2015; 27(2): 84-89.
21. Shah ZM, Quraishi A, Hassan G. Meristem culture of potato (*Solanum tuberosum* L.) for producing virus-free plantlets. Journal of Biological Sciences. 2018; 1(2): 898-899.
22. Pather C. Effect of naphtaleine acetic acid (NAA) on somatic embryogenesis of the orchid *Phalaenopsis amabilis* (L.). Biodiversitas. 2017; 5(1&2): 30-35.
23. Goswami K, Yasmin S, Nasruddin KM. In Vitro Regeneration of Dendrobium sp. of Orchid Using Leaf Tip as Explant. J Environ Sci Natural Resources. 2015; 8(2): 75–78.
24. Hussein N. Effects of nutrient media constituent on growth and development of banana (Musa sp.) shoot tips cultures in vitro. Africal J of Biotechnology, 2021; 11(37): 233–239.