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The Effect of Auxin and Cytokinin on Black Orchid Hybrid (*Coelogyne pandurata* Lindley) in Vitro

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***Abstract*—The Orchid is an ornamental plant with high economic value due to the emergence of unique varieties. These varieties are produced from conventional breeding methods of crossing and selection. Meanwhile, the propagation of orchids through in vivo hatcheries is constrained because the seeds are too small to contain the endosperm. Tissue culture is an alternative for the propagation of orchids Efforts to propagate orchids through tissue culture is carried out by adding Growth Regulatory Substances such as auxins groups, namely NAA, IBA, IAA, and cytokinin groups, namely BA, BAP, kinetin, and TDZ to MS media, to enable the plants to take up adequate nutrients for growth. Therefore, this study aims to determine the effect of various concentrations of NAA and BA on the best ½ MS media for the growth of orchid subcultures. A completely randomized design pattern (CRD) of 2 factors with 12 treatment combinations and four replications were used, totaling 48 experimental units. The results showed that the addition of 1ppm NAA had the highest number of roots and length, while the addition of BA at 3ppm had the highest height, and the combination of 1ppm NAA and BA 3ppm treatment produced the highest number of leaves (42 sheets) and shoots. Based on the results, the addition of NAA above 3ppm followed by BA at 6ppm can produce more leaves and shoots, while NAA above 3ppm can produce higher plant height.**

***Keywords*— Effect; NAA; BA; coelogyne pandurate; coelogyne rumphii.**

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I. INTRODUCTION

Orchid plants belong to the Orchidaceae family, and Indonesia has one of the largest tropical forests with a rich diversity of orchid species. The potential of orchids as ornamental plants with high economic value is due to the emergence of unique varieties produced from conventional breeding methods of crossing and selection. Besides, the diversity of orchids is used to make new types of plants through the crossing. One of such crossings has been carried out by [1], which form the species *Coelogyne pandurata* and *Coelogyne rumphii*. Furthermore, the *Coelogyne pandurata* is a black orchid native to Borneo.

Propagation through in vivo hatcheries is hindered due to the absence of endosperm in small orchids. The seeds require association with mycorrhizae for natural germination because of the long time necessary for singular germination. Orchids' seeds lacking endosperm culminate in hatcheries with low life expectancy, and only a few survive. This is in accordance with [2], which stated that the seed germination percentage in vivo is low because it contains little or no food reserve. Hence, tissue culture is an alternative to the propagation of orchids.

Tissue culture has the advantage of sprouting new plants within a relatively short time in large quantities, with the parent plants' exact physiological and morphological characteristics. In addition, it is a production technology used for plant propagation in large numbers [3]. The success of a plant tissue culture depends on the media used and the growth regulators added. According to Bamatov *et al.* [4], Murashige and Skoog (MS) media are the most widely used in-vitro culture due to the required mineral salt, vitamin, and phytohormones content for plant growth. The chemical concentration of this media is changed into half of its usual concentration, known as ½ MS.

Furthermore, growth regulators regulate plant growth and cultivation in vitro [5]. Common examples often added to the media include the auxin and cytokinin groups. NAA (Naftaleine Acetate Acid) is an auxin growth regulator utilized as energy for plant growth. Meanwhile, cytokinin growth regulators include BA, zeatin, and kinetin, each plays an important role in enhancing the cell division process,

especially shoot regeneration, lateral growth, and production of multiple shoots. The ratio between cytokinin and auxin needs to be considered to determine growth direction [6]. The orchid subculture in this study originated from the crossing of *Coelogyne pandurata* and *Coelogyne rumphii* with various combinations of NAA and BA concentrations. This study aims to determine the effect of NAA and BA addition on the growth of orchid subcultures resulting from the crossing of *Coelogyne pandurata* and *C. rumphii*. The addition of NAA and BA in the ½ MS media is expected to influence the growth of the orchid subculture.

II. Material and Method

This study was conducted between May and October 2019 at the Sublab Network Culture, Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, in Sebelas Maret University, Surakarta. It used an orchid plantlet produced by crossing *Coelogyne pandurata* and *Coelogyne rumphii* on ½ MS medium with various NAA and BA concentrations combinations. The research scheme can be seen in Fig. 1.

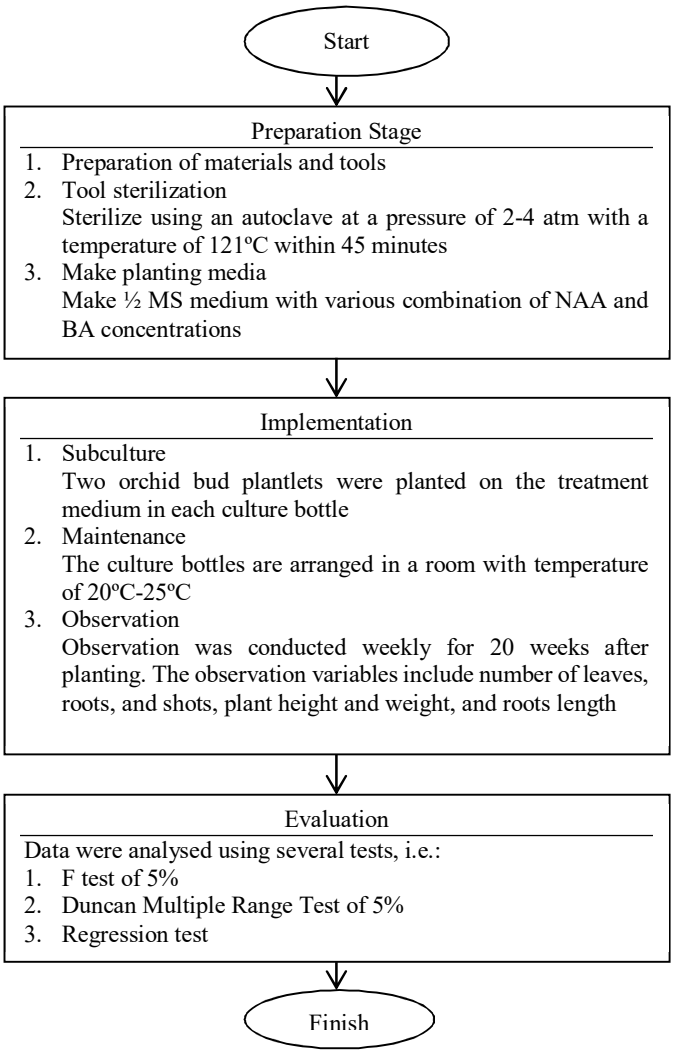

Fig. 1 Research Flow Diagram

The experimental method with a completely randomized design (CRD) was used with 2 factors, 12 treatment combinations, and 4 replications. Furthermore, the first factor was NAA 0ppm (N0), NAA 1ppm (N1), NAA 2ppm (N2), and NAA 3ppm (N3); meanwhile, the second factor was BA 0ppm (B0), BA 3ppm (B1), and BA 6ppm (B2).

The observed variables include the number of leaves, roots, shoots, height, weight, and root length. Data analysis was done using the F test of 5% significance level, and when there is a significant difference, it was followed by a DMRT (Duncan's Multiple Range Test) of 5% level to compare between treatments and then a regression test.

III. Results and Discussion

The orchid subculture produced from a cross between *Coelogyne pandurata* and *C. rumphii* is a complex and difficult propagation technique. Cultivation of orchids produced from a cross between the two species is carried out qualitatively and quantitatively using ½ Murashage and Scoog (MS) media containing plant growth regulators in the form of auxins and cytokinins. This study used PGR for plant growth in the in-vitro orchid culture and had good results. Meanwhile, NAA is more effective than 2,4-D when combined with other cytokinins in inducing PLB [7]. PLB formation increased by adding a combination of TDZ and NAA 5µM to MS media [8], while the addition of BA up to 1mg/L, to shoots of *Paphiopedilum villosum* had the highest yield [9]. Furthermore, the addition of BA 2,5 ppm showed optimal results in the leaf area of *Trillium govanianum* leaves [10]. The independent effect of NAA and BA and the interactions produced different results measured in the form of numbers of leaves, roots, and shoots and plant height and root length.

Fig. 2 Treatment of 1 ppm NAA and 3 ppm BA at 20 Weeks After Planting

Fig. 3 Treatment of 0 ppm NAA and 3 ppm BA at 20 Weeks After Planting

A. Number of Leaves

The leaf is a plant organ that functions as a site for photosynthesis to produce energy in the form of carbohydrates. This compound is important as an energy source for plants. Plant growth is enhanced or inhibited depending on the number of leaves obtained photosynthates. The number of leaves is also a growth indicator and further explains the growth process. The addition of NAA and BA can promote a number of orchid leaves.

TABLE I
EFFECT OF INTERACTION OF NAA AND BA ON THE AVERAGE NUMBER OF LEAVES (STRANDS) AT 20 WEEKS AFTER PLANTING

Treatment	B0 (0ppm)	B1 (3ppm)	B3 (6ppm)	Average of NAA
N0 (0 ppm)	17.5 bcd	41.5 g	34.75 fg	31.25
N1 (1 ppm)	22.75 cde	42 g	14.25 bc	26.33
N2 (2 ppm)	3.5 a	28.75 ef	11,25 ab	14.50
N3 (3 ppm)	9.25 ab	8.75 ab	23,75 de	13.91
Average of BA	13.25	30.25	20.98	

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

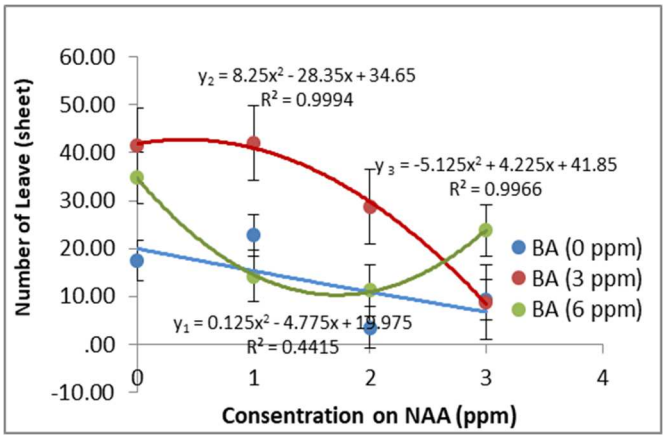

Fig. 4 Regression analysis of NAA and BA interactions on the average *Coelogyne pandurata* and *Coelogyne rumphii* at 20 weeks after planting

The analysis of variance using the F test with a significance level of 5% showed that the interaction of NAA and BA in the ½ MS medium significantly affected the number of leaves. Meanwhile, DMRT follow-up tests (Table 1) showed that the number of leaves with the combination of 1 ppm NAA and 3 ppm BA concentration was not significantly different from that of NAA 0 ppm, and BA 3 ppm was significantly different from other treatments. The highest number of leaves was obtained from a combination of 1ppm NAA and 3 ppm concentration of BA with 42 leaves. Auxin and cytokinin are ZPT used in inducing plant morphogenesis. A cytokinin concentration ratio higher than auxin increased the number of leaves, while low exogenous auxin treatment also produced a higher number of leaves because it provided a balance with the desired cytokinin in the plantlet.

The effect produced by the interaction of NAA, and BA is shown in the regression test (Figure 3). Equations obtained from the NAA administration without BA show the coefficient of determination R2 = 0.441 with the equation y = 0.125x2 - 4.775x + 19.975 and produce a regression graph. This graph indicates that increasing NAA concentrations without BA lower leaf numbers. Furthermore, a combination of NAA with BA 3ppm gives the equation y = 8.25x2 - 28.35x + 34.65 with a coefficient of determination R2 = 0.999, indicating that the interaction of NAA and BA has a significant influence on the number of leaves. The interaction of NAA with BA 3 ppm resulted in a decreasing graph which means that a higher concentration of NAA reduced the number of leaves. Higher administration of NAA with 6ppm BA produced the equation y = -5.125x2 + 4.225x + 41.85 with a coefficient of determination R2 = 0.996. Therefore, the regression results showed that higher BA administration produced a higher number of leaves. According to Asa and Kaviani [11], the type and concentration of PGR alone or in combination have an effect on plant growth. This depends on several factors, such as the plant species used.

B. Number of Roots

The root takes a role in the absorption of nutrients. The number of roots formed during the tissue culture process is very important for plantlets due to optimal absorption from nutrient media. The higher the number of roots formed, the wider the area covered for optimal nutrient absorption. This shows that the root is important because it enhances plantlet growth during the propagation process through tissue culture. The addition of the correct concentration of NAA gives many roots.

TABLE III
EFFECT OF NAA ON THE NUMBER OF ROOTS AT 20 WEEKS AFTER PLANTING

Concentration on NAA (ppm)	Number of roots
0	4.33 a
1	10.17 b
2	3.75 a
3	3.50 a

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

The analysis of variance through the F test at a significant level of 5% showed no significant effect in the number of roots with the interaction of NAA and BA. Meanwhile, significant effects occurred with the singular addition of NAA. Further DMRT test results showed that 1ppm NAA concentration had significantly higher results than other treatments (Table 2). This treatment resulted in the number of roots within the range of 10-17. However, high NAA concentrations result in inhibition of root growth.

An increase influenced an increase in the number of roots in NAA concentration. Higher NAA concentrations resulted in a lower number of roots. Plants can produce endogenous auxins; meanwhile, the addition of auxin to culture media tends to cause unbalanced interactions with endogenous auxins. This results in relatively no increase in the number of roots. The administration of auxin at higher concentrations inhibits rooting. Notwithstanding, roots are formed even though exogenous auxins are added in low concentrations because of plantlets' already high auxin levels. NAA is a type of growth regulator in the auxin group that stimulates root initiation [12]. According to Rodrigues *et al* [13], adding auxin to culture media is very important for induction and high production of roots.

C. Number of Shoots

The formation of shoots determines the success of tissue culture plant multiplication. The concentration of the growth regulator specifically determines shoot formation, namely auxin and cytokines added. The concentration determines the magnitude of plant growth and influences the number of shoots formed. This study shows that the interaction of NAA and BA affects shoots multiplication. The BA 3 ppm and NAA 1 ppm combination give the highest number of shoots.

TABLE IIIII
EFFECT OF NAA AND BA INTERACTION ON THE AVERAGE NUMBER OF SHOOTS AT 20 WEEKS AFTER PLANTING

Treatment	B0 (0ppm)	B1 (3ppm)	B2 (6ppm)	Average of NAA
N0 (0 ppm)	5.25 ab	13 de	13.25 e	10.5
N1 (1 ppm)	5.75abc	14.5 e	4 ab	8.08
N2 (2 ppm)	0 a	9.75 cde	3 ab	4,25
N3 (3 ppm)	3 ab	1.5 a	7.75 bcd	4,08
Average of BA	3.5	9.68	7	

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

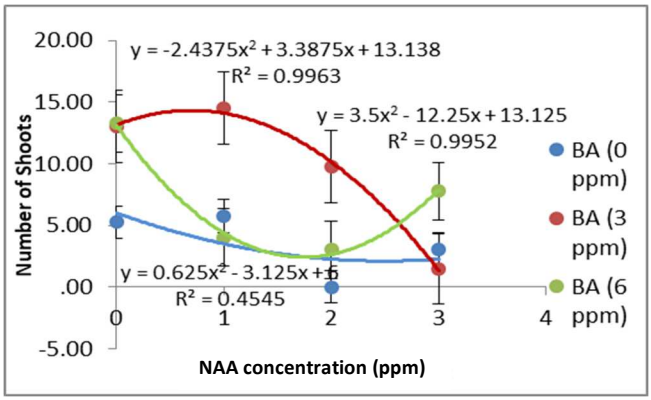

Fig. 5 Regression analysis of NAA and BA interactions on the number of shoots in the orchid subculture plants from the crossing of *Coelogyne pandurata* and *Coelogyne rumphii* at 20 Weeks After Planting

The analysis of variance using the F test with a level of 5% showed that the interaction between NAA and BA in the ½ MS media had a significant effect on the number of shoots. Further DMRT test showed that the highest number of shoots was obtained with the combination of NAA and BA at 1 ppm and 3 ppm respectively, producing 14.5 shoots, which was not significantly different at 0ppm and 6ppm which yielded 13.25 buds (Table 3), but significantly different from the other treatments. The plant growth and development in culture were influenced by the balance of exogenous growth regulators produced by the plants themselves (endogenous). Higher cytokinin concentrations stimulates bud development, while higher auxin levels enhance root growth. Furthermore, Juras *et al* [14] reported that the growth of *C. xanthina* orchid buds using the MS media produced the best growth with cytokinin administration compared to auxin. It was also reported that increasing the concentration of NAA reduces shoot regeneration in each treatment with a combination of NAA in MS medium [15].

The equation obtained from the addition of NAA without BA is y = 0.625x2 - 3.125x + 6 (Figure 4), which shows a decreasing graph with a coefficient of determination R2 = 0.454. A higher concentration of NAA without the addition of BA reduced the number of shoots, but insignificantly. The interaction between NAA and BA with a concentration of 3ppm shows a decreasing graph with the equation y = -2.4375x2 + 3.3875x + 13.138, the coefficient of determination R2 = 0.996 indicating that the interaction of NAA and BA greatly influenced the number of shoots. This means that the concentration of 3ppm BA and the addition of NAA with a higher concentration result in fewer shoots. Furthermore, the equation obtained from the interaction of NAA with BA concentration of 6 ppm is y = 3.5x2 - 12.25x + 13.125 with a coefficient of determination R2 = 0.995. An increase in NAA concentration in combination with BA produces a higher number of shoots. A previous study stated that the use of a combination of NAA and BA is beneficial for the regeneration and multiplication of the *Paphiopedhilum insigne* orchid [16].

D. Plant Height

An increase in plant height is caused by cell division and elongation that occurs in tissue meristems located at the point of stem growth. Plant height is an easily observable indicator of growth for measuring applied treatment or ascertaining environmental influences. Growth in a plant is identified by constant observation of the height. This study shows that concentration on BA affects plant height better than control. While the addition of NAA causes plant height stunted.

TABLE IVV
EFFECT OF NAA AND BA ON AVERAGE PLANT HEIGHT (CM) AT 20 WEEKS AFTER PLANTING

Concentration on NAA (ppm)	Plant height (cm)
0	2.71 b
1	2.62 b
2	1.78 a
3	2.29 ab
Concentration on BA (ppm)	
0	2.08 a
3	2.82 b
6	2.15 a

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

The F test at a significance level of 5% showed that the interaction between NAA and BA in the ½ MS medium showed no significant effect, while the administration of NAA and BA independently showed a significant effect on the height of the orchid subculture. Further DMRT tests showed that the 2 ppm NAA treatment was significantly lower compared to others. The treatment without NAA, or NAA 0 ppm, yielded the highest plant height of 2.71 cm, which was not significantly different from 1 ppm NAA (Table 4). These results show that increased concentration of NAA causes lower plant height. The addition of exogenous auxins causes an imbalance between auxin and cytokinin in culture, which suppresses plant growth. Hence, NAA is more favorable for root growth but does not affect the height of orchid plants. The addition of plant growth regulators in the media is important for further growth and development.

Further DMRT tests showed that 3 ppm BA produced a plant height of 2.82 cm, which was significantly higher at 0 ppm and 6 ppm (Table 4). Increase in BA concentration has

a significant effect on plant height. BA administration in ½ MS media increased the height of the orchid subculture. while increasing concentrations of BA resulted in lower plant height. Therefore, it is suspected that the explants used produces endogenous cytokinins that stimulate cell division. According to Haque *et al* [17], BA is a growth regulator widely used in orchid culture, although the type and concentration differs according to type or variety.

E. Root Length

A longer plant root guarantees wider absorption of nutrients. The optimal absorption of nutrients from the growing media maintains nutrient availability for planlet. Root growth occurs because the process of tissue cleavage is supported by the presence of organic and inorganic compounds in the media. Moreover, root formation is also associated with endogenous auxin and cytokinin content in plant tissue, followed by cell lengthening and enlargement processes. In this study, root length is affected by NAA and BA.

TABLE V
EFFECT OF NAA AND BA ON AVERAGE ROOT LENGTH (CM) AT 20 WEEKS AFTER PLANTING

Concentration on NAA (ppm)	Root Length (cm)
0	0.96 a
1	2.07 b
2	0.88 a
3	0.95 a
Concentration on BA (ppm)	
0	1,04 a
3	1,87 b
6	0,73 a

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

The ANOVA test showed no significant effect of NAA and BA interaction in ½ MS media on the root length of the orchid subculture. Meanwhile, a significant effect was observed with the independent administration of NAA and BA. The DMRT follow-up test showed that 1 ppm NAA yielded significantly different results compared to other treatments (Table 5). A concentration of 1 ppm NAA produced a root length of 2.07 cm, the increase in NAA concentration significantly influenced root growth. According to Gaurav and Bijaya [18], media lacking the NAA hormone result in improper roots development due to plants' genetic makeup and endogenous growth regulators.

The regression results show that an increasing concentration of NAA produces longer roots but inhibits root growth. Increasing concentration does not continuously affect the root length after attaining optimal concentration because the need for NAA has been fulfilled endogenously or exogenously. According to Juras *et al* [14], auxin is very important for root induction as observed in *Cyrtopodium saintlegerianum.* Nevertheless, increasing its concentration causes inhibition of root length. A study Devi *et al* [19] on *T. spathulata* cultured on ½ MS medium with the addition of low concentrations of NAA produced a better root system.

The independent addition of BA in the ½ MS medium significantly influenced root length. Further DMRT tests showed that a concentration of 3 ppm BA yielded significantly different results than other treatments (Table 5). The 3 ppm BA treatment resulted in a root length of 1.87 cm. A great influence on the length of the root. The addition of BA produces longer roots, but an increase in concentration further lowers the root length. Root lengthening does not require further cytokinins' additions because it inhibits root length in high concentrations. Hence, high cytokinin concentrations are more favorable for shoot growth. A study on *Cyrtopodium saintlegerianum* showed that the best response to the variable number of roots occurs at lower concentrations of NAA and BA [11].

F. Plant Weight

Plants with significant growth undergo an increase in weight. This occurs due to the increasing number of leaves, roots, and plant height. The ANOVA results showed that the interaction of NAA and BA had no significant effect on plant weight. Meanwhile, independent administration of NAA significantly increased plant weight. Further DMRT tests showed that the absence of NAA produced the highest plant weight of 0.2758 g which was significantly different compared to other treatments (Table 6).

TABLE VI
EFFECT OF NAA ON AVERAGE PLANT WEIGHT (GRAMS) AT 20 WEEKS AFTER PLANTING

Concentration on NAA (ppm)	Plant Weight (gram)
0	0.2758 b
1	0.0550 a
2	0.0508 a
3	0.0475 a

Note: Numbers with the same letters indicate no significant difference based on DMRT level of 5%.

The addition of auxin at high concentrations inhibited tissue growth, consequently producing plants with lower weight. Endogenous auxins present in the plantlet accumulated with the given exogenous auxin, thereby resulting in an imbalance between auxin and cytokinin levels. Furthermore, there is a competition between exogenous and endogenous auxins in penetrating cell membranes, therefore, external addition of auxin does not affect cell growth and development. Increase in NAA concentration significantly influenced weight loss. According to Parthibhan *et al* [20], auxin and cytokinin influence the fresh weight of a plantlet. Furthermore, explant multiplication can increase fresh weight [21].

IV. CONCLUSIONS

Based on the results, the addition of 1 ppm NAA yielded the highest results in terms of number of roots (10 roots) and length (2.07 cm), while the addition of 3ppm BA had the highest result in form of plant height (2.82 cm). The combination of 1ppm NAA and 3ppm BA treatment produced the highest number of leaves (42 sheets) and shoots (14.5 shoots). Therefore, the addition of NAA above 3ppm followed BA at 6ppm have the potential to produce more leaves and shoots, while the addition of NAA above 3ppm has the potential to produce greater plant height.

REFERENCES

[1] S. Hartati, Nandariyah, A. Yunus, and D. W. Djoar, "Hybridization Technique of Black Orchid (Coelogyne pandurata Lindley) to Enrich the Genetic Diversity and to Rescue the Genetic Extinction," *Bulg. J. Agric. Sci.*, vol. 25, no. 4, pp. 751–755, 2019, Accessed: May 12, 2021. [Online]. Available: https://www.agrojournal.org/25/04-20.html.S.

[2] Hartati, R. B. Arniputri, L. Soliah, and O. Cahyono, "Effects of Organic Additives and Naphthalene Acetid Acid (NAA) Application on the in vitro Growth of Black Orchid Hybrid (Coelogyne pandurata Lindley).," *Bulg. J. Agric. Sci.*, vol. 23, no. 6, pp. 951–957, 2017, Accessed: May 12, 2021. [Online]. Available: https://www.cabdirect.org/cabdirect/abstract/20183048901.

[3] M. Faisal, N. Ahmad, M. Anis, A. A. Alatar, and A. A. Qahtan, "Auxin-cytokinin synergism in vitro for producing genetically stable plants of Ruta graveolens using shoot tip meristems," *Saudi J. Biol. Sci.*, vol. 25, no. 2, pp. 273–277, Feb. 2018, doi: 10.1016/j.sjbs.2017.09.009.

[4] I. Bamatov, E. Butsaeva, M. Arsanov, and D. Bamatov, "The modification of murashige and skoog media for efficient cultivation of gizella-5 and vsl-2 rootstocks in vitro," in *IOP Conference Series: Earth and Environmental Science*, Aug. 2019, vol. 315, no. 4, p. 42015, doi: 10.1088/1755-1315/315/4/042015.

[5] A. Kumari *et al.*, "Plant growth regulator interactions in physiological processes for controlling plant regeneration and in vitro development of Tulbaghia simmleri," *J. Plant Physiol.*, vol. 223, pp. 65–71, Apr. 2018, doi: 10.1016/j.jplph.2018.01.005.

[6] R. Mastuti, A. Munawarti, and E. R. Firdiana, "The combination effect of auxin and cytokinin on in vitro callus formation of Physalis angulata L. - A medicinal plant," in *AIP Conference Proceedings*, Nov. 2017, vol. 1908, no. 1, p. 40006, doi: 10.1063/1.5012721.

[7] S. M. Haque and B. Ghosh, "Regeneration of Cytologically Stable Plants Through Dedifferentiation, Redifferentiation, and Artificial Seeds in Spathoglottis plicata Blume. (Orchidaceae)," *Hortic. Plant J.*, vol. 3, no. 5, pp. 199–208, Sep. 2017, doi: 10.1016/j.hpj.2017.10.002.

[8] N. P. Huy *et al.*, "In vitro polyploid induction of Paphiopedilum villosum using colchicine," *Sci. Hortic. (Amsterdam).*, vol. 252, pp. 283–290, Jun. 2019, doi: 10.1016/j.scienta.2019.03.063.

[9] P. Bhattacharyya, V. Kumar, J. Grúz, K. Doležal, and J. Van Staden, "Deciphering the phenolic acid reserves and antioxidant activity within the protocorm like bodies of Ansellia africana: A vulnerable medicinal orchid," *Ind. Crops Prod.*, vol. 135, pp. 21–29, Sep. 2019, doi: 10.1016/j.indcrop.2019.03.024.

[10] H. K. Chauhan, A. K. Bisht, I. D. Bhatt, and A. Bhatt, "Protocol for vegetative propagation of Trillium govanianum Wall ex D. Don," *J. Appl. Res. Med. Aromat. Plants*, vol. 16, p. 100233, Mar. 2020, doi: 10.1016/j.jarmap.2019.100233.

[11] M. Asa and B. Kaviani, "In vitro propagation of orchid Phalaenopsis amabilis (L.) Blume var. Jawa," *Iran. J. Plant Physiol.*, vol. 10, no. 2, pp. 3113–3123, Apr. 2020, doi: 10.22034/IJPP.2020.672571.

[12] R. V. Garvita and Sahromi, "Plant Regeneration Through Direct Somatic Embryogenesis from Leaf Explants of Paraphalaenopsis Labukensis P. S. Shim," in *IOP Conference Series: Earth and Environmental Science*, Dec. 2019, vol. 394, no. 1, p. 012053, doi: 10.1088/1755-1315/394/1/012053.

[13] L. A. Rodrigues *et al.*, "Propagação in vitro de Cyrtopodium saintlegerianum rchb. F. (orchidaceae), uma orquídea nativa do cerrado Brasileiro," *Crop Breed. Appl. Biotechnol.*, vol. 15, no. 1, pp. 10–17, 2015, doi: 10.1590/1984-70332015v15n1a2.

[14] M. C. R. Juras, J. Jorge, R. Pescador, W. De Melo Ferreira, V. Tamaki, and R. M. Suzuki, "In vitro culture and acclimatization of Cattleya xanthina (Orchidaceae), an endangered orchid of the Brazilian Atlantic Rainforest," *Rodriguesia*, vol. 70, 2019, doi: 10.1590/2175-7860201970014.

[15] F. A. Ozdemir, "Effects of 6-benzylaminopurine and α-naphthalene acetic acid on micropropagation from ten days old cotyledon nodes of Mentha spicata subsp. spicata received for.," *Rom. Biotechnol. Lett.*, vol. 22, no. 3, pp. 12554–12559, 2017, Accessed: May 12, 2021. [Online]. Available: https://www.cabdirect.org/cabdirect/abstract/20183041025.

[16] C. R. Deb and H. Y. Jakha, "Factors affecting asymbiotic immature seed culture and in vitro propagation of Paphiopedilum insigne (wall. Ex. Lindl.) pfitzer –a horticultural important vulnerable orchid," *Plant Cell Biotechnol. Mol. Biol.*, vol. 21, no. 15–16, pp. 129–141, Jul. 2020, Accessed: May 12, 2021. [Online]. Available: https://ikprress.org/index.php/PCBMB/article/view/5178.

[17] S. M. Haque, S. Kazuhiko, and S. J. Nahar, "Combination treatment of 6-benzylaminopurine (BA) and hyaluronic acid (HA) on plb culture of Cymbidium spp. In vitro," *Am. J. Agric. Environ. Sci.*, vol. 16, no. 7, pp. 1335–1340, 2016, doi: 10.5829/idosi.aejaes.2016.16.7.12948.

[18] P. Gaurav and P. Bijaya, "In vitro seed germination and seedling development of the orchid Coelogyne stricta (D. Don) Schltr.," *African J. Biotechnol.*, vol. 15, no. 5, pp. 105–109, Feb. 2016, doi: 10.5897/ajb2015.14870.

[19] N. P. Devi, B. Lisipriya, and N. Bai, "Asymbiotic seed germination and mass multiplication of Taprobanea spathulata (L.) Christenson (Asparagales: Orchidaceae): a medicinally important epiphytic orchid," *Brazilian J. Biol. Sci.*, vol. 2, no. 4, pp. 271–286, Dec. 2015, Accessed: May 12, 2021. [Online]. Available: http://revista.rebibio.net.

[20] S. Parthibhan, M. V. Rao, and T. Senthil Kumar, "In vitro regeneration from protocorms in Dendrobium aqueum Lindley - An imperiled orchid," *J. Genet. Eng. Biotechnol.*, vol. 13, no. 2, pp. 227–233, Dec. 2015, doi: 10.1016/j.jgeb.2015.07.001.

[21] S. Konar, Adhikari S, Karmakar J, Ray A, and Bandyopadhyay TK, "Evaluation of subculture ages on organogenic response from root callus and spar based genetic fidelity assesment in the regenerant of Hibiscus sabdariffa L. " *J. Industrial Crops & Products*, vol. 135, no. 1, pp. 321-329, 2019.