In Vitro Multification of *Dendrobium* sp. with Plant Growth Regulator

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Abstract, Orchids are plants that has aesthetic value and high economic important. Included to ornamental plant whose existence is diminishing and endangered. To preserve this plant, it needs to in vitro technique. The aim of this study was to determine the best interaction of growth regulator Benzyl amino purine, coconut water and the interaction of both multiplication of *Dendrobium* sp. The research method used a completely random design with various concentrations of Benzyl amino purine (BAP; 0,1,2,3 mg/L) and coconut water concentrations (0%, 5%, 10%). The parameter observation are number of buds, leaves and roots. The data were analyzed descriptively and inferentially with ANOVA. The results shows the real effect of BAP interaction, between coconut water buds and leaves, but did not significantly affect the number of roots. Within 12 weeks of observation the highest number of buds and leaf obtained from a combination of 5% coconut water + BAP 0 mg/L were 12.00 and 5.00. The lowest number of buds and leaves was obtained in combination of 5% coconut water + BAP 2 mg/L, 6.00 and 1.00.

1. Introduction

Orchid is a plant that has aesthetic value and high economic important. One of the many popular types of orchid is *Dendrobium* [1]. Orchid is ornamental plant species whose existence in the wild has decreased and endangered. Central Statistics Agency and the Directorate General of Horticulture 2014 [2], explained that the production of orchids from 2010-2014 experienced volatile. Orchid production in Indonesia in 2014 decreased to 19,739,627. therefore the need for the preservation and cultivation of orchids, especially *Dendrobium sp.*

Tissue culture is a business that can be done to preserve genetic resources one way of plant propagation in vitro. Propagation through tissue culture is also an alternative technique to solve this problem [3] and can also help the production of seedlings more uniform and higher quality [4].

The success of plant propagation in vitro is influenced by several factors such as the selection of explants used, sterilization of explants, the composition of the basic medium, the use of plant growth regulators (PGR), especially auxin and cytokinin and environmental factors in which the culture is placed [5] [6].
Plant growth regulators have a role in the process of growth and development for the survival of plants [7]. Plant growth regulator cytokinin group that is often used in tissue culture is BAP and kinetin. Cytokinin BAP is often used as the most effective to stimulate the formation of buds, more stable and resistant to oxidation as well as the cheapest among cytokinin lainny [8]. While natural plant growth regulators or hormones may be obtained from coconut milk, tomato juice extract, bananas and so on [9]. Coconut water contains various substances, including the hormone cytokinin and auxin [10]. Benzyl amino purine (BAP) is one kind of plant growth regulator synthetic class of cytokines that are often used in plant growth [11]. The positive response to the application of plant growth regulator is influenced by several factors, including the type of crop, crop growth stages, types of plant growth regulators, concentration and how the application of growth regulators [12]. The influence of growth regulator concentrations of lead concentration needs to be determined when making an application to the plant.

[13] BAP 2 ppm able to accelerate time to germinate. [14] The addition of coconut water in tissue culture media provide a real influence on the growth of plantlets and number of buds orchid *dendrobium anosmum*. [15] The results empirically on tobacco culture Nicotinna tabacum var. shows a concentration of 4 ppm BAP produces buds with the highest average number of buds produced was 52.5%. This causes the need for research to determine the interaction of a combination of BAP and coconut water on the growth of *Dendrobium sp.*

2. Research Method
This research was conducted at YAHDI Tissue Culture Laboratory, started from May until October 2019.

The material used in this study are erlenmeyer, Laminar Air Flow Cabinet (LAFC), volume pipette, measuring cup, funnel glass, autoclave, pH meter, stir bar, spatula, tweezers, scissors, bunsen, culture bottles, analytical scales, petri dishes, millimeter paper, and cultures shelves. The materials are *Dendrobium sp.* orchid plantlet in vitro. The basic media used is Murashige-Skoog (MS) Media + coconut water (0; 5; 10%) + BAP (0; 1; 2; 3 ppm). media combination scan be seen in the table below:

| Coconut water | 0      | 5    | 10    |
|---------------|--------|------|-------|
| 0 ppm         | BAP 0 ppm; coconut water 0% | BAP 0 ppm; coconut water 5% | BAP 0 ppm; coconut water 10% |
| 1 ppm         | BAP 1 ppm; coconut water 0% | BAP 1 ppm; coconut water 5% | BAP 1 ppm; coconut water 10% |
| 2 ppm         | BAP 2 ppm; coconut water 0% | BAP 2 ppm; coconut water 5% | BAP 2 ppm; coconut water 10% |
| 3 ppm         | BAP 3 ppm; coconut water 0% | BAP 3 ppm; coconut water 5% | BAP 3 ppm; coconut water 10% |

The research using RAL (Complete Random Design). Combination treatment amounted to 12 with each treatment amounted to 3 repetitions. The steps of research started from sterilization, media preparation, sterilization of explants, planting, and observation of the bud *dendrobium sp.* The observation parameter consists of a number bud, leaves and root. Data obtained from the observation results were analyzed by ANOVA. If the results show significant differences, then the analysis is forwarded by using DMRT (Duncan's Multiple Range Test).
3. Results And Discussion

The observation of the parameters of the number of buds, number of leaves, and number of roots in the treatment with various concentration levels of BAP (0, 1, 2, 3 ppm) and coconut water (0, 5, 10%) with the basic MS medium can be seen in Table 2 below:

**Table 2.** Average Number of Buds, Leaves And Roots Total *Dendrobium* Sp. On 12 week After Planting

| Combination          | Number of Buds | Number of Leaves | Number of roots |
|----------------------|----------------|------------------|-----------------|
| MS + B₀ + AK₀       | 3.6667 a       | 11.333           | 1.0000          |
| MS + B₁ + AK₅       | 3.6667 a       | 11.000           | 1.0000          |
| MS + B₂ + AK₁₀      | 2.6667 ab      | 11.333           | 0.6667          |
| MS + B₃ + AK₀       | 2.3333 ab      | 9.333            | 0.6667          |
| MS + B₄ + AK₅       | 2.3333 ab      | 7.667            | 0.6667          |
| MS + B₅ + AK₁₀      | 2.3333 ab      | 7.333            | 0.3333          |
| MS + B₀ + AK₁₀      | 2.0000 ab      | 6.667            | 0.3333          |
| MS + B₁ + AK₅       | 2.0000 ab      | 6.667            | 0.3333          |
| MS + B₂ + AK₁₀      | 1.3333 b       | 5.667            | 0.0000          |
| MS + B₃ + AK₅       | 1.3333 b       | 5.667            | 0.0000          |
| MS + B₄ + AK₁₀      | 1.0000 b       | 5.333            | 0.0000          |

Information: The numbers, accompanied by the same letters in the same column, states are not significantly different by Duncan test (DMRT) 5%

3.1. Number of bud

The results of analysis variance showed that the combination of BAP and coconut water can provide a real influence on the number of buds. On average the highest number of buds 3.6667 obtained from media MS + BAP + 1 ppm AK 5%. However, the average number of buds are no different from media MS + BAP 2 ppm + AK₁₀; MS + BAP 3 + AK₀; MS + BAP 0 + AK₅; MS + BAP 0 + AK₁₀. MS + BAP 2 + AK₀; MS + BAP 3 + AK₅ and MS + BAP 0 + AK₁₀.[13] As the average number of buds highest in broccoli plants with a concentration of 2.5 ppm BAP is 17 buds. [14] BAP concentration of 2 mg / l is the effective concentration for the growth and development of pineapple buds. [12] In tobacco plants get a concentration of 4 ppm BAP + 0.1 ppm NAA had the highest average of 52.5 buds, this shows that the BAP as cytokinin very effective to initiate buds directly and indirectly.

From the research results mentioned above can be said that the BAP can increase the number of buds. It cause related to the physiological function of cytokinins can stimulate cell division and the role of cytokines in the morphogenesis is the formation of buds[15].

The average number of buds show that coconut water treatment significant effect on the number of buds average number of buds that 3.6667. [16] to get on the addition of coconut water and 45% were able to increase the number of buds chrysanthemum 10.2 buds / explant. This shows that the addition of coconut water can increase the number of buds. The average number of buds of BAP combination treatment with coconut water are presented in Table 2.

3.2. Number of leaves

The results of analysis variance showed that the combination of BAP and coconut water does not provide a real influence on the number of leaves where Fhitung < Ftabel the trust level of 5%. but descriptively there are changes in the number of leaves during 12 weeks of observation. The average number of leaves of BAP combination treatment with coconut water are presented in Table 2, showed that the highest average number of buds on the observations contained in the 12-week combination treatment MS + BAP 0 + AK₀ and MS + BAP 2 + AK₁₀.
This is due to the addition of water head up to 40% can improve plant height and number of buds chrysanthemum [16]. Coconut milk contains the active substance for the development of the embryo such as cytokines, cytokines affect nucleic acid metabolism and the synthesis of proteins that affect cell division. Plant differentiation show that the average number of leaves on the highest combination treatment of MS + 2 ppm BAP + 10% coconut water is 11,333, although this treatment is not different from 0 ppm water treatment BAP + 0% coconut water, BAP 1 ppm + 5% coconut water, however coconut water tends to increase the number of leaves [17]. That the use of BAP at a concentration of 1.5 mg / l was reported capable of producing the highest number of leaves on the plant Telfairia occidentalis Hook [18]. The use of BAP 3 ppm / l resulted in the highest number of leaves of orchids (Dendrobium sp) with an average of 9.81 the number of leaves per bud [19].

Coconut water at a concentration of 10% can stimulate the formation of buds on the 4 var. green bean plants, thus indirectly used treatment can increase the number of leaves that are formed due Coconut Water has a high K element followed by Na, Mg and other elements. The formation process of the leaves in vitro with the use of Coconut Water with regard to the existence of elements of K and Mg are relatively high on the media used. Based on the mineral composition and hormones present in the alleged awarding Coconut Water Coconut Water can provide a good influence on the average number of leaves per explant [20].

3.3. number of roots
The results of analysis variance showed that the combination of BAP and coconut water no significant effect on the number of leaves where F_{count}<F_{table} with level of trust is 5%. The average number of roots of the treatment of MS + BAP + coconut water are presented in Table 2 where the average of the highest root number 1,000. this is in line with the opinion [21], BAP has properties that are very active role in cell differentiation, triggering the growth of buds, axillary bud proliferation and precisely minimize root formation. The use of BAP as cytokinin basically sheath serves to trigger the outbreak of plant buds and buds, than that BAP will prevent the apical dominance, so that the growth of side buds are not hampered [22].

4. Conclusion
Based on the research of addition of BAP and coconut water, shows that there is real effect on the number of buds, but no significant effect for the number of leaves and number of roots. The highest average number of buds is 3,6667 from combination media MS + BAP 1ppm + 5% coconut water. Description is changes for number of leaves during 12 weeks of observation. the highest average of number of shoots obtained on MS + BAP 0 ppm + 0% coconut water and MS + BAP 2 ppm + 10%. namely 11,333.

Reference
[1] Widiastoety, D, & Numralinda. 2010. Potensi Anggrek Dendrobium dalam Meningkatkan Variasi dan Kualitas Anggrek Bunga Potong, Jurnal Litbang Pertanian, vol.29, no.3, hal.101-106.
[2] Badan Pusat Statistik dan Direktorat Jendral Hortikultura. 2014. Produksi anggrek menurut provinsi. 2010-2014. Sub sector hortikultura. Diakses dari. http://www.pertanian.go.id/ap-pages/mod/datahorti.
[3] Harahap, F., D, S, Diky., Poerwanto, R., N,A, Nanda & H, M,R, Fadhilah. (2019). In Vitro Callus Induction Of Sipahutar Pineapple (Ananas Comosus L.) From North Sumatra Indonesia. Pakistan Journal Of Biological Sciences. 22 : 518-526
[4] Harahap, F., Poerwanto, R., Sobir, Hasruddin, Suriani, C., Siiallagan, J & Rohyan. (2015). Sterilization of Pineapple Explant from Sipahutar, North Sumatra, Indonesia (Ananas comosus L.) and In Vitro Growth Induction. Asian Jr. of Microbial. Biotech. Env.Sc. 17 (2) : 470-477.
[5] Harahap, F., Roedhy, P., Suharsono., Cicik, S., and Suci, R. (2014). In vitro Growth and Rooting of Mangosteen (Garcinia mangostana L) on Medium with Different Concentrations of Plant Growth Regulator. HAYATI Journal of Biosciences. 21(4): 151-157.

[6] ZulkARNAIN. 2013. Kultur Jaringan Tanaman. Solusi Perbanyakan Tanaman Budidaya, Jakarta: Bumi Aksara.

[7] Harahap, F. (2011). Kultur Jaringan. Medan: FMIPA Unimed.

[8] Nurjanah, E. (2009). Pengaruh Kombinasi NaCl dan ZPT IBA pada Media MS Terhadap Pertumbuhan Gatal Mutan Padi Secara In Vitro. Prodi Biologi, Fakultas Islamiyah Syarif Hidayatullah.

[9] Sulistyorini, I., dewi, S., & syafaRuddin. 2012. Penggunaan Air Kelapa Dan Beberapa Auxins Untuk Induksi Multiplikasi Tunas Dan Perakaran Lada Secara In Vitro. Buletin RISTRI 3 (3), 231-238.

[10] Yong, J.W.H., L. Ge., Y.F. Ng., and S.N. Tan. 2009. The chemical composition and biological properties of coconut (Cocos nucifera L) water. [Online]. Available: http://www.mdpi.com. Diakses pada 30 September 2019.

[11] George, E.F., M.A. Hall., and G.J. De Klerk. 2008. Plant Propagation by Tissue Culture. Third edition. Springer. [Online]. Available: http://citeseerx.ist.psu.edu. Diakses pada 28 September 2019.

[12] Fahmi, Z. I. 2014. Direktorat Jenderal Pertanian. Kajian pengaruh auxin terhadap perkecambahan benih dan pertumbuhan tanaman. Tersedia: http://ditjenbun. pertanian.go.id. Diakses 30 September 2019

[13] Tilaar, W., & Rantung, J. L. (2013). Induksi Kalus dan Tunas dar Ekspalan Pucuk Brokoli dalam Media MS yang diberikan NAA dan BAP. Manado: Eugenia

[14] Tuhuteru, S., Dkk. 2012. Pertumbuhan Dan Perkembangan Angrek Dendrobium Anosmum Pada Media Kultur In Vitro Dengan Beberapa Konsentrasi Air Kelapa. Agrologia. Vol 1(1): 1-12.

[15] Nisak, K, Tutik N, & Kristanti I. 2012. Pengaruh Kombinasi Konsentrasi ZPT NAA dan BAP pada Kultur Jaringan Tembakau Nicotianana tabacum var. Prancak 95. Jurnal Sains dan Seni Pomits 3(2): 1-6.

[16] Tilaar, W., Sumeru, A., Bagyo, Y., & Polii -Mandang, J. (2012). Synthesis of Sulforaphan during the Formation of Plantlets from Broccoli (Brassica oleracea L var italic)a In Vitro. Manado: International Journal of Engineering & Technology IJET-IJENS Vol: 12 No; 03.

[17] Al-Saif, A.M.H. 2011. Effect Of Plant Growth Regulators On Fruit Growth And Quality Development Of Syzygium samarangense (Water Apple/Wax Apple). University Of Malaya. Kuala Lumpur

[18] Tilaar, W. 2016. Mikropropagasi Brokoli (Brassica oleracea L var. Italic Plenck) dan Peningkatan Sulforafan Selama Pembuatan Planlet.Disertasi. Pascasarjana Universitas Brawijaya. Malang

[19] Mandang, J.P.1993. Peranan Air Kelapa Dalam Kultur Jaringan Tanaman Krissan (Chrysanthemum morifolium Ramat). Disertasi Program Pasca sarjana, Institut Pertanian Bogor.113p

[20] Wattimena, G. A. 1992. Zat Pengatur Tumbuh Tanaman. Pusat antar Universitas Institut Pertanian Bogor. Bogor.

[21] Balogun, MO, SR Akande, And BA Ogunbodede. 2007. Effect Of Plant Regulators On Callus, Bud, And Root Formation In Fluted Pumpkin (Telfairia Occidentalis). Afric. J. Biotech 6(4): 355-358.

[22] Bakar, Meklin., Dkk. 2016. Penggunaan BAP Dan Kinetin Pada Induksi Tunas Dari Protocorm Angrek Dendrobium (Dendrobium sp.) Pada Kultur In Vitro. Fakultas Pertanian : Universitas Sam Ratulangi Manado
[23] Amutha, S, A Ganapathi, And M Muruganatham. 2003. *In Vitro* Organogenesis And Plant Formation In *Vigna Radiata (L.)* Wilczek. Plant Cell, Tissue And Organ Cultur 72: 203-207
[24] Wattimena, GA. 1988. Zat Pengatur Tumbuh Tanaman. Pusat Antar Universitas Institut Pertanian Bogor. Bogor
[25] Salisbury, FB & Ross, CW, 1992, Fisiologi Tumbuhan III edisi ke-4, Penerjemah Lukman, DR & Sumaryono, ITB, Bandung.