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Inducing An Axillary Bud of *Dendrobium* Red Emperor 'Prince' With An Addition of Bap In Vitro

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ABSTRACT

The purpose of this study is to determine the effect of the addition of BAP and the position of nodus on the formation of axillary shoots and to know the optimum concentration of BAP for the induction of *Dendrobium* Red Emperor 'Prince' axial shoots. The research design used is a Complete Factorial Randomized Design consisting of one treatment, namely: concentration of growing regulatory substances (ZPT) BAP (0 ppm, 1 ppm, 2 ppm, and 3 pmm), each treatment repeated 5 times. The explant of the nodus were taken from plants produced by previous in vitro culture having 5 nodi. The basic medium used is New Phalaenopsis (NP) + Coconut Water + 1 ppm 2.4-D). The growth of axillary bud growth is measured based on the time of the bud emergence, growth of crown, and that of root. Data obtained were then analyzed using ANOVA. If there was a significant difference, the analysis was continued with a test of DMRT with a significant level of 5%. The results showed the addition of concentrations of 1 ppm and 2 ppm BAP influenced the time of the bud emergence, growth of crown, and that of the roots. The optimum concentration of BAP to induce the orchid axillary shoots is 2 ppm.

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1. INTRODUCTION

Orchids are ornamental plants with high economic value and good market demand. Orchids have beautiful colors and varied shapes, with some species still rare. Besides being potential ornamental plants, orchids are also utilized for medicinal purposes, making orchid cultivation in in the agriculture sector highly sought after. Heyne (1987) reported that in Sumatra, more than 10 species of orchids from the genus *Dendrobium* are used as medicines. In Vietnam, orchids from the genus *Nervilia* are utilized (Huyen, 2003). The utilization of orchids as medicinal ingredients is suspected due to the presence of bioactive compounds such as flavonoids, tannins, carbohydrates, terpenoids, and cyanogenic glycosides (Maridass et al., 2008). Orchids are used as medicine for various purposes including fever, stamina enhancement, cancer, stomachache, aphrodisiac, and boils (Silalahi, 2015).

One of the orchid genera favored by consumers is *Dendrobium*, especially the *Dendrobium* Red Emperor 'Prince'. According to the Agricultural Development Agency (2017), *Dendrobium* orchids dominate the orchid demand in Indonesia at 34%, followed by Oncinidium Golden Shower (26%), Cattleya (20%), Vanda Douglas (17%), and other orchids (3%). *Dendrobium* Red Emperor 'Prince' is a hybrid orchid resulting from the crossbreeding of D. New Comet x D. Benikujyaku, known for its unique colors and flower shapes. Orchids from the genus *Dendrobium*, including *Dendrobium* Red Emperor 'Prince', have potential medicinal properties. In China, orchids of this type are used as herbal medicines, known *as Dendrobium huoshanense* herbal medicine.

According to BPS (2013), orchid demand in 2010 was 14,050,445 stems, which increased to 15,490,256 stems in 2011. However, the high demand for orchids is not met with an adequate supply

of orchid seedlings. Various efforts are made to meet consumer demand for new and increasingly attractive and unique orchid varieties. These efforts include mutation techniques, hybridization (crossbreeding), and transgenics. Through these efforts, it is hoped that new positions with high-quality physiological and biological properties will be obtained.

After obtaining new orchid positions, propagation efforts are needed. Ex vitro orchid propagation takes a long time and produces plants with diverse flowers (Rianawati, et al., 2009). However, consumers desire plants with immediate availability and uniform flowers. Therefore, an alternative method is needed, namely vegetative propagation using in vitro techniques. In vitro culture can produce orchids in large quantities with the same characteristics as the parent plant and relatively uniform growth (Sandra, 2003).

This research aims to develop in vitro plant propagation techniques by inducing axillary buds from the nodes of *Dendrobium* Red Emperor 'Prince' orchids. Inducing axillary buds in *Dendrobium* Red Emperor 'Prince' orchids is the initial stage of multiplication. Axillary buds are lateral shoots that grow in the leaf axils (Rohayati, 2009). The addition of appropriate plant growth regulators (PGRs) can increase the emergence of axillary buds. The more axillary buds that emerge, the more orchid seedlings are produced. Therefore, axillary bud induction was chosen for this research due to its role in providing seedlings.

To improve the success of in vitro culture, medium modification is necessary by adding PGRs. One of the PGRs commonly used in in vitro culture is 6-benzylaminopurine (BAP) from the cytokinin group. Cytokinins stimulate lateral shoot growth, increase leaf chlorophyll, and slow down senescence in leaves, fruits, and other organs (Wattimena, 1988). Benzylaminopurine is a synthetic cytokinin PGR known for its stability, resistance to degradation during sterilization, and relatively low cost (Zulkarnain, 2009).

Benzylaminopurine applied to P. amabilis orchid explants at a concentration of 2.5 ppm influenced the growth and development of P. amabilis orchids, resulting in the best number of protocorm-like bodies (PLBs), emergence time of shoots, number of shoots, and number of leaves (Fitriyandini, et al., 2015). In the induction of shoots from *Dendrobium* sp. protocorms, using 3 ppm BAP resulted in the tallest plants and the highest number of leaves (Bakar, 2015). Based on the above description, research on the induction of axillary buds in *Dendrobium* Red Emperor 'Prince' orchids with the addition of BAP is needed. In this research, various concentrations of BAP were used to find the optimal concentration for inducing axillary bud growth in *Dendrobium* Red Emperor 'Prince' orchids. This research aims to determine the effect of adding BAP on the emergence time of orchid shoots, shoot growth, and root growth. It also aims to determine the optimum BAP concentration for inducing axillary shoot growth in *Dendrobium* Red Emperor 'Prince' orchids in vitro.

2. RESEARCH METHOD

This study is an experimental research using a Completely Randomized Design (CRD) factorial design consisting of 1 factor, namely: variations in BAP concentration.

2.1. Time and Location of Research

The research was conducted at the Plant Tissue Culture Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Yogyakarta State University from January 2021 to May 2021

2.2. Procedure

This research uses a Completely Randomized Design consisting of treatments as follows:

A= NP Media + AK + 1 ppm 2,4-D + 0 ppm BAP
B= NP Media + AK + 1 ppm 2,4-D + 1 ppm BAP
C= NP Media + AK + 1 ppm 2,4-D + 2 ppm BAP
D= NP Media + AK + 1 ppm 2,4-D + 3 ppm BAP

Each treatment was repeated 5 times, resulting in 20 culture petri dishes. In each culture petri dish, 3 explants of *Dendrobium* Red Emperor 'Prince' orchid nodes were grown. Thus, there were 60 *Dendrobium* Red Emperor 'Prince' orchid explants.

2.2.1 Medium Preparation

The treatment medium used was New Phalaenopsis (NP) + coconut water (CW) + 1 ppm 2,4-D supplemented with various concentrations of BAP. To make 100 ml of medium, 2.22 grams of NP medium (22.2 g.L-1) was needed. NP medium was placed into an Erlenmeyer flask, then sterile distilled water was added up to 70 ml. The medium was heated using a hotplate magnetic stirrer. 15 ml of coconut water was added and homogenized (150 ml.L-1). Plant growth regulators 2,4-D and BAP were added to the NP+CW medium for each treatment as follows:

Table 1. Variations in Treatment Combinations

Treatment Code	2,4-D (ppm)	BAP (ppm)
A	1	0
B	1	1
C	1	2
D	1	3

For all the prepared media, the acidity level was tested using a pH indicator. The pH of the medium was adjusted to 5.8 by adding KOH if it was too acidic or HCl if it was too basic. Sterile distilled water was then added up to 100 ml, followed by the addition of 0.7 grams of agarose (7 g.L-1). The medium was homogenized and heated to boiling. It was then sterilized using an autoclave at 121°C and 1 atm pressure for 15 minutes. The sterilized medium was poured into petri dishes with a volume of 20 ml per petri dish. The medium was stored in a storage room for 1 week before being used to detect contamination.

2.2.2 Explant Preparation

The explants used were *Dendrobium* Red Emperor 'Prince' orchid explants in vitro culture, which had uniform characteristics (5 nodes).

2.2.3 Planting

The *Dendrobium* Red Emperor 'Prince' orchid explants were removed from the culture bottle and placed in petri dishes. The seedlings were removed by opening the bottle and passing the bottle lip through a Bunsen burner flame in a circular motion, then gripping the seedlings between the roots and leaves. The seedlings were then pulled out, ensuring that the roots came out first. Clumped explants were separated from other explants, as well as from the medium still attached to the explants. The explants were cut at the top (a), middle (m), and bottom (b) nodes, each explant segment consisting of 1 node with a length of 1 cm. Nodes from the same explant were planted in the same petri dish. The petri dishes were passed through a Bunsen burner flame, wrapped, and labeled.

2.2.4 Incubation

Incubation was carried out by placing the culture bottles on a cleaned incubation rack, labeled according to the previously made completely randomized design. The room temperature was set to 18°C.

2.2.5 Observation

Observations were conducted once a week on the same day for 90 days. Observations included the emergence time of shoots, and shoot growth (number of shoots, shoot height, number of leaves). On the 90th day, shoot growth (leaf length, leaf width, stem diameter) and root growth (number of roots, root length, root diameter) were measured.

2.3 Data, Instruments, and Data Collection Techniques

2.3.1 Data

The data collected included shoot emergence time, growth (number of shoots, shoot height, number of leaves, leaf length, leaf width, stem diameter), and root growth (number of roots, root length, root diameter).

2.3.2 Instruments

The tools used in this study included: petri dishes (Pyrex), measuring glasses (Pyrex), scalpel, forceps, spirit burner (Bunsen), Laminar Air Flow (LAF), autoclave, hotplate magnetic

stirrer, magnetic rod, micropipette, blue tip, Erlenmeyer flask (Pyrex), analytical balance, and calipers.

The materials used in this study included: NP medium, 2,4-D, BAP, coconut water, sterile distilled water, agarose, commercial activated charcoal (Norit), pH indicator, 70% alcohol, 96% alcohol, 1N HCL solution, and 1N KOH solution.

2.3.3 Data Collection Techniques

Shoot emergence time was determined from the time the first green conical protrusion appeared or when the tip became greener with a length of 1 mm. The number of shoots was counted as the total number of shoots that grew on each node. Shoot height was measured by measuring the height of the shoot from the base to the highest tip. Measurements were taken every week using a millimeter block attached to the culture dish. On the final observation day, calipers were used.

The number of leaves counted was the total number of leaves that emerged during the observation period. Leaf length, leaf width, and stem diameter were measured in the 12th week by removing the shoots from the culture dish using forceps. Leaf length, leaf width, and stem diameter were measured using calipers. Three leaves were taken from each shoot as a sample for leaf length and width measurement. Root diameter measurement was done on the largest part of the stem. The total number of roots was counted. Observations were made in the 12th week by removing the shoots from the culture dish using forceps, then cleaning them from the remaining medium and counting the roots. Root length and root diameter were measured using calipers. Three roots were taken from each shoot as a sample for root length measurement.

2.3.4 Data Analysis Technique

Data analysis was performed using two-way Analysis of Variance (ANOVA). Further analysis was conducted using Duncan's Multiple Range Test (DMRT) if there were differences between treatments at a significance level of 5% to determine differences between each treatment group.

3. RESULTS AND ANALYSIS (11 Pt)

3.1 The Effect of Variation in BAP Concentration on the Emergence Time of Shoots in *Dendrobium* Red Emperor 'Prince' Orchids

The ANOVA results indicate that variations in BAP concentration have a significant effect on the emergence time of axillary shoots. The emergence time of shoots for all tested treatments occurred almost simultaneously, between weeks 2 and 3 after planting (Table 2).

Table 2. Average Emergence Time of Axillary Shoots in *Dendrobium* Red Emperor 'Prince' Orchids at Various BAP Concentrations

BAP Concentrated(ppm)	Shoot Emergence Time			
	Nodus Position			Average
	a	t	b	
0	2.6	2.2	2.8	2.53ab
1	2.2	2.2	2.0	2,13a
2	2.4	2.4	2.0	2.27a
3	4.2	2.4	2.2	2.93ab
Rata-rata	2.85b	2.30a	2.25a	

The balance between the hormones 2,4-D and BAP is needed to induce axillary shoots in vitro in the *Dendrobium* Red Emperor 'Prince' orchid. Benzyl amino purine plays a role in stimulating cell and morphogenesis, while 2,4-D regulates cell growth and elongation. Brault (1999) argues that cytokinin (including BAP) is an important component involved in controlling shoot development, especially cell growth. In cell growth, cytokinin controls gene expression, chloroplast development, secondary metabolite synthesis, and secondary metabolite growth in tissue culture (Kieber, 2002). According to Mahadi (2016: 3), shoot formation occurs due to cell elongation, cell division, morphogenesis, and growth regulation, which are important processes followed by shoot formation.

This indicates that in shoot formation, auxin (including 2,4-D) and cytokinin (including BAP) play important roles.

The addition of BAP at a concentration of 3 ppm tends to induce the shoots of *Dendrobium* Red Emperor 'Prince' orchids to emerge the slowest. The addition of PGRs exceeding the required concentration often inhibits growth (Santoso and Nursandi, 2004). According to Zulkarnain (2009), shoot regeneration tends to be inhibited if the addition of PGRs is not appropriate. The development of shoots is determined by the balance of endogenous PGRs present in the explants with exogenously added PGRs in the medium. If there is no balance between auxin and cytokinin PGRs, then the treatment is not able to induce shoot growth.

Axillary shoots are the result of the elongation of bud primordia in the leaf axils (Figure 2). Axillary shoots are formed due to the activity of meristematic cell division and elongation, which differentiate into axillary buds. Shoots are characterized as green cone-shaped protrusions, or with greener tips, measuring 1 mm in length, which then elongate and produce leaves (Prihatmanti, 2004).

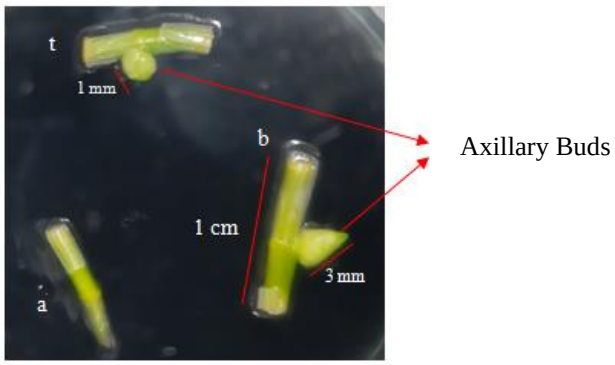

Figure 2. Axillary Buds *Dendrobium* Red Emperor 'Prince'

Observation results of shoot emergence time show that in the first week after planting, the explants appeared swollen and showed bud primordia (nod), indicating the role of apical meristem. In the second week, potential micro-shoots became visible on the swollen part, marked by the emergence of bumps in the leaf axils as seen in Figure 2. The node code "t" (middle) indicates the appearance of cone-shaped bumps with potential leaflets after the third week after planting, while on the node code "b" (bottom), cone-shaped bumps with potential leaflets emerge after the third week after planting. In the formation of shoot primordia, the hormone involved is cytokinin. Exogenous plant growth regulators can induce shoot formation by influencing reactions that occur within cells, ultimately leading to shoot emergence. Cytokinin given to plants can affect biochemical reactions and alter the composition of plant PGRs, causing the protoplasm inside cells to enlarge, leading to cell wall enlargement, a process that results in cell proliferation and reflects shoot formation in plants (Mahadi, 2016: 4).

The best emergence time of axillary shoots in *Dendrobium* Red Emperor 'Prince' orchids is at a BAP concentration of 1 ppm, consistent with the study by Alitalia (2008) in Sagai, et al. (2016: 4), where the use of 1 ppm BAP in Nepenthes gracilis explants resulted in the fastest initiation of shoots, 17 days after planting. Increasing the use of BAP to 2 ppm actually delays shoot emergence. According to Anisa, et al. (2016: 593), the best result for shoot emergence time in Black Orchid (Coelogyne pandurata Lindl) explants is at a concentration of 1.5 ppm BAP. Khoiriyah (2013) also stated that a BA concentration of 1 ppm resulted in shoot emergence 4 days after planting from rose stem explants. In contrast, the study by Yanti and Mayta (2021: 55) stated that the fastest shoot emergence in kasturi lime induction was at the 2 ppm BAP treatment, with shoot emergence occurring 12 days after planting. For pineapple shoot induction, a concentration of 0 ppm BAP showed the highest average, with shoots emerging 10.03 days after initiation (Purita, et al., 2017: 1209).

3.2 The Effect of Variation in BAP Hormone Concentration on the Growth of Shoot Apex in *Dendrobium* Red Emperor 'Prince' Orchids

ANOVA results show that the addition of BAP has a significant (significant) effect on shoot apex growth except for stem diameter. The growth of axillary shoots in *Dendrobium* Red Emperor 'Prince' orchids in various treatment media (12 weeks after planting) (Figure 3).

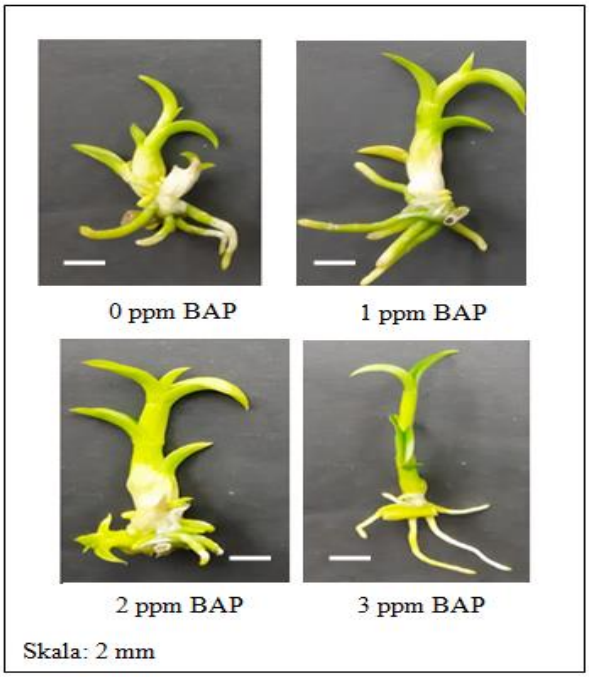

Figure 3. Axillary Shoots Growth Anggrek *Dendrobium* Red Emperor 'Prince'

3.3 Number of shoots in *Dendrobium* Red Emperor 'Prince' Orchids

There is a difference in the number of axillary shoots in each treatment. Differences in shoot number growth are due to the growth and organogenesis of explants requiring a balance between cytokinin and auxin hormones. The addition of BAP at concentrations of 0 ppm and 2 ppm shows almost the same results in inducing axillary shoots in *Dendrobium* Red Emperor 'Prince' orchids. At a concentration of 0 ppm BAP, 1.73 shoots were produced, and at a concentration of 2 ppm, 1.87 shoots were produced (Table 3).

Table 3. Average Number of shoots in *Dendrobium* Red Emperor 'Prince' Orchids in Different Concentration of BAP

BAP Concentration (ppm)	Number of Shoot			Average
	Nodus Position			
	a	t	b	
0	2.60	1.60	1.00	1.73b
1	1.00	0.80	0.80	0.87a
2	1.60	1.60	2.40	1.87b
3	1.00	1.00	0.40	0.80a
Average	1.55	1.25	1.15	

Cytokinin (BAP) will increase its performance in stimulating shoot growth if there is auxin in the media and absorbed by the plant. The research results of Elismani, et al. (2006) as cited in Fathurrahman, et al. (2012) reported that the addition of 2 ppm 2,4-D and 1 ppm BAP resulted in the highest number of shoots in Dutch young seed culture, namely 1.90 shoots. According to

Wattimena (2002), low auxin and higher cytokinin tend to suppress adventitious shoot formation and axillary shoot proliferation.

At a concentration of 3 ppm BAP, the results showed the lowest number of shoots with an average of 0.80 shoots. The administration of excessively high exogenous BAP concentrations inhibits shoot growth, thus not influencing the number of shoots. Exogenous cytokinin causes lateral cell expansion, resulting in thicker stem tissues (Miryam, 2008).

In contrast, the study by Purita (2017: 1211) stated that in pineapple shoot induction (*Ananas comosus* L. Merr), the highest number of shoots appeared in the treatment with 1 ppm BAP, with a total of 4 shoots. According to Sagai (2016: 4), the administration of 1 ppm BAP resulted in the highest number of shoots, namely 1.92 shoots in broccoli shoot explants (*Brassica oleraceae* L. var. Italica Plenck), while at 2 ppm BAP, there was a decrease. The differences in the number of shoots results from the differences in the types of explants used, thus also varying the endogenous hormone content they possess.

3.4 Axillary bud height of *Dendrobium* Red Emperor 'Prince'

The addition of BAP at a concentration of 2 ppm had the best effect in stimulating axillary bud height growth with a height of 2.14 cm (Table 4). Plant height growth occurs due to cell division and elongation, resulting in the stem becoming longer. According to Gardner et al. (1985) cited in Purnamasari (2020:168), stem elongation occurs due to the process of division, elongation, and enlargement of new cells that occur in the shoot apical meristem, resulting in increased plant height. The addition of a combination of BAP had an effect on the growth of the axillary bud height of *Dendrobium* Red Emperor 'Prince'. The higher the concentration of BAP given, the higher the buds that grew. Optimal axillary bud height growth occurred at a concentration of 2 ppm BAP. Giving a higher concentration of BAP than the optimal concentration indicates a decrease in bud height.

Table 4. Average Growth of Axillary Bud Height of *Dendrobium* Red Emperor 'Prince'

BAP Concentration (ppm)	Bud Height (cm)			
	Nodal Position			Average
	a	t	b	
0	1.54	1.96	1.54	1.68ab
1	1.58	1.97	1.67	1.74ab
2	2.41	2.14	1.86	2.14b
3	1.24	1.67	0.61	1.17a
Average	1.69	1.94	1.42	

Axillary bud height increase occurs due to cell division and elongation in the shoot apical meristem. Elongation or increase in axillary bud height is due to the presence of auxin and cytokinin hormones. Increasing the exogenous cytokinin level (BAP) can increase cell division activity in meristem tissue. The increase in axillary bud height is also caused by the role of auxin hormone (2,4-D) in cell elongation. The role of auxin in cell elongation results in the production of many primary cell wall materials which are then transferred to both ends of the cell. Subsequently, the cell structure undergoes stretching, forming more cell walls. Thus, cell elongation occurs at the bud tip (Mulyono, 2010 cited in Setiawati, 2016: 150).

At a concentration of 3 ppm BAP, the lowest axillary bud height growth occurred, which was 1.17 cm. This indicates that giving a higher concentration of cytokinin than the optimum dose will result in lower axillary bud height growth because essentially cytokinin is already present in the explant, so with the addition of 3 ppm BAP, the concentration of endogenous cytokinin increases. The high cytokinin content and low auxin content required for bud cell elongation may inhibit bud elongation. Buds will grow taller if the cytokinin concentration is lower (Indriyani, 2013 cited in

Setiawati, 2016:150-151). Another study showing similar results is Yuniastuti's research (2010:3) that the addition of 2 ppm BAP resulted in the best bud height of 0.9 cm in Anthurium buds (Anturium andraeanum Linden). In Kasturi lime (Citrus microcarpa Bunge.) nodal explants, the longest buds were produced with the addition of 1 ppm BAP, which is 1.75 cm (Yanti, 2021:56).

3.5 Number of leaves in *Dendrobium* Red Emperor 'Prince' buds

Not all explants grow leaves. Only explants that grow buds also grow leaves. Leaf growth is relatively fast, in the second week after planting, explants with leaves have emerged. The counted leaves are those that have opened and are still attached to the bud stem. Based on the ANOVA results, it can be seen that the concentration factor has a significant effect on the number of axillary bud leaves of *Dendrobium* Red Emperor 'Prince'. The addition of BAP concentration has a different effect at each concentration. The 2 ppm BAP concentration produced the highest number of leaves, namely 4.73 leaves, while the 3 ppm BAP concentration produced the lowest number of leaves, namely 2 leaves (Table 5).

Table 5. Average Number of Leaves on Axillary Bud of *Dendrobium* Red Emperor 'Prince'

BAP Concentration (ppm)	Number of Leaves			
	Nodal position			Average
	a	t	b	
0	3.20	5.00	2.80	3.67ab
1	3.40	4.20	2.40	3.33ab
2	4.80	4.40	5.00	4.73b
3	2.00	3.20	0.80	2.00a
Average	3.35	4.20	2.75	

Adding cytokinin (BAP) in high concentrations cannot serve as a reference for increasing the number of leaves even though cytokinin functions to stimulate cell division and the formation of organs, including leaves. This is because of differences in the concentration of endogenous hormones in each explant. Hardjo (1994) states that there is a difference in explant responses given cytokinin; explants with low endogenous cytokinin will give a positive response, and if the explant has enough endogenous cytokinin, it will not respond or even give a negative response.

Lestari (2016:595) reported that in the multiplication of black orchids (Coelogyne pandurata Lindl), the addition of 1.5 ppm BAP was the best treatment with an average of 3.50 buds/explant, which is in line with the results of Purita's research (2017:1211), the most leaves appeared in the treatment of 1.5 ppm BAP in pineapple plants (Ananas comosus L. Merr).

Talukder et al (2003) in Purwanto's research (2018:13) found that the addition of 2.0 ppm BAP in mini bananas (Musa acuminata colla AA Group) produced the highest number of shoots, which was 6.36 shoots/explant. This is because at a low cytokinin concentration, auxin and cytokinin interact optimally, so that leaf growth is maximal, while at a high concentration of BAP, the function of auxin in cell division will be inhibited, resulting in the inhibition of leaf growth.

4. CONCLUSION (11 Pt)

The addition of 1 ppm BAP to the NP+AK medium + 1 ppm 2,4-D is capable of inducing the fastest bud emergence time and results in the highest stem diameter, leaf length, root length, and root diameter growth. A concentration of 2 ppm BAP can produce the highest number of buds, leaf

count, leaf width, and root quantity. A concentration of 2 ppm BAP is optimal for inducing axillary buds in vitro for the *Dendrobium* Red Emperor 'Prince' orchid.

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