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The Effect of Benzyl Amino Purine (BAP) Concentration on the Growth Amount of the Explant of *Dendrobium spectabile* Orchid by *In-Vitro*

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ABSTRACT
Dendrobium spectabile orchid is an ornamental plant of high economic value, having relatively durable properties, beauty, and attractiveness of orchids lie in the diverse shapes and colors of the flowers. Therefore, it requires rapid and efficient propagation techniques. One of them is in-vitro vegetative propagation. Plant propagation using the in-vitro technique has the opportunity to produce a large number of plant seeds in a relatively short time. This study aims to determine the correct concentration of Benzyl Amino Purine (BAP) on the growth amount of the explants of Dendrobium spectabile orchid by in-vitro. The research was conducted at the Biotechnology Laboratory of the Regional Technical Implementation Unit (UPTD) of the Aloe Vera Center (AVC) Pontianak. This research was conducted from January 1, 2020, to March 30, 2020. The method used was a Completely Randomized Design (CRD). The treatment consisted of 6 levels of treatment, namely b1 = 0.75 ppm, b2 = 1.00 ppm, b3 = 1.25 ppm, b4 = 1.50 ppm, b5 = 1.75 ppm, b6 = 2.00 ppm. All treatments were repeated 4 times for each treatment consisting of 3 plant samples. The research finding showed that the treatment of BAP concentration 1.50 was the best in increasing the average number of leaves 4.92 strands, the average number of shoots 4.25 buds, and the average leaf length was 2.44 cm.

INTRODUCTION

Orchid plants have a diverse amount of 700 genera and 35,000 species spread throughout the world (Puspitaningtyas and Mursidawati, 1999). Orchids are ornamental plants with high aesthetic value. The beauty and attractiveness of orchids lie in the various shapes and colors of flowers. Types of

orchid plants favored by many orchid enthusiasts include *Dendrobium* (34%), *Oncidium Golden Shower* (26%), *Vanda* (17%), and other types of orchids (3%) (Ditjen P2HP, 2010).

According to Widyastuti (2000), the *Dendrobium spectabile* orchid is an orchid that has varied properties and flower colors and is relatively more durable so that it has the potential to be an ornamental plant with high economic value, causing this orchid in nature to be overexploited and its existence begins to decrease. If allowed to continue, it can lead to extinction, if not balanced with conservation efforts. Conservation efforts to conserve *Dendrobium spectabile* orchids require a fast and efficient propagation technique. Propagation of orchids using conventional seed germination techniques takes a long time and produces plants with various flower colors. *In-vitro* vegetative propagation can be used as an alternative to overcome this problem.

Plant propagation using the *in-vitro* technique has the opportunity to produce a large number of plant seeds in a relatively short time. In addition, *in-vitro* propagation can be done all the time and is not affected by the season. The seeds are produced simultaneously and uniformly. *In-vitro* propagation is inseparable from growth influencing substances that contain essential nutrients (macro and micro), a source of energy, and vitamins which are useful for explant development. One of the most important plant-regulating substances in the *in-vitro* technique is cytokinins. Cytokinins play a role in the process of cell division, axillary shoot poly person, and stimulate shoot growth. The type of cytokinin that is most often used is Benzyl Amino Purine (BAP). BAP plays a role in stimulating cell division and formation because of its high effectiveness. This study aims to determine the correct BAP concentration on the growth amount of *Dendrobium spectabile* explants by *in-vitro*.

METHOD

The research was carried out in the Biotechnology Laboratory of the Regional Technical Implementation Unit (UPTD) of the Aloe Vera Center (AVC) Pontianak. The research time was for 3 months, namely from January 1, 2020, to March 30, 2020. The materials used in this study were the *Dendrobium spectabile* orchid, a stock solution. Murashige and Skoog (MS) media nutrients, Benzyl Amino Purine (BAP), KOH, and Hydrochloric Acid (HC1), sucrose, agar, sterile distilled water, NaOH, alcohol, 70% and 90% alcohol, tissue, and masks. The tools used in this study were Laminar Air Flow Cabinet (LAFC), autoclave, rotating shaker, analytical balance, stirrer, stroller, culture bottle, Erlenmeyer, pH indicator, measuring cup, cup, Petri dish, pipette, tweezers, scalpel, bunsen, hand sprayer, magnetic stirrer, knife, scissors, and other supports.

The method used was a one-factor Completely Randomized Design (CRD). The treatment consisted of 6 treatments, each treatment was repeated 4 times and each replication consisted of 3 samples, as for the treatment in question, namely b1 = 0.75 ppm, b2 = 1.00 ppm, b3 = 1.25 ppm, b4 = 1.50 ppm , b5 = 1.75 ppm, b6 = 2.00 ppm. The research implementation stage included sterilization of the workspace and tools, making Murashige and Skoog (MS) media stock solutions, making growth media, preparing for planting, and planting explants. The observed variables were number of leaves, number of shoots, length of leaf, number of roots, and length of the root.

RESULTS AND DISCUSSION

Based on the results of the analysis of diversity, it showed that giving various concentrations of BAP had a significant effect on the variable number of leaves (JD), number of shoots (JT), number of roots (JA), root length (PA) and leaf length (PD). Furthermore, the variables that have a real effect are continued with the BNJ 5% test to see the differences in each treatment which can be seen in Table 1.

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Table 1
BNJ 5% Test on Effect of BAP Concentration on *Dendrobium spectabile* Orchids

The concentration of BAP (ppm)	Average				
	JD (strands)	**JT (bud)**	**PD (cm)**	**JA[*)] (root)**	**PA[*)] (cm)**
0.75	3.92 c	2.50 b	1.51 c	1.10 a	0.79 a
1.00	4.08 c	3.00 b	1.68 bc	0.81 b	0.73 b
1.25	4.17 bc	3.09 b	1.71 bc	0.78 b	0.73 b
1.50	4.92 a	4.25 a	2.44 a	0.76 b	0.71 b
1.75	4.75 ab	3.25 b	2.19 a	0.71 b	0.71 b
2.00	4.29 abc	3.17 b	2.08 ab	0.71 b	0.71 b
BNJ 5%	**0.63**	**0.88**	**0.44**	**0.17**	**0.03**

Information: Numbers followed by the same letter in the same column are different real on the BNJ 5% test
[*)]Data Transformation

The 5% BNJ test results in Table 1, show that the number of *Dendrobium spectabile* orchids leaves formed by giving BAP as much as 1.50 ppm were significantly different from the number of leaves given BAP as much as 1.25 ppm, 1.00 ppm, and 0.75 ppm, but not significantly different from the number of leaves given BAP as much as 1.75 ppm and 2.00 ppm. The administration of 1.25 ppm BAP is not significantly different from the provision of BAP as much as 1.75 ppm and 2.00 ppm. The least number of leaves formed in the BAP treatment was 0.75 ppm, namely an average of 3.92 strands, and the highest number of leaves in the BAP treatment was 1.50 ppm, namely an average of 4.92 strands.

The number of *Dendrobium spectabile* orchids shoots that appeared on BAP were 1.50 ppm significantly different from the number of shoots in the BAP administration of 1.75 ppm, 2.00 ppm, 1.25 ppm, 1.00 ppm, and 0.75 ppm. The least number of shoots formed in the BAP treatment was 0.75 ppm, namely an average of 2.50 buds, and the highest number of shoots in the BAP treatment was 1.50 ppm, namely an average of 4.25 buds.

Leaf length of *Dendrobium spectabile* orchids that were formed were given BAP as much as 1.50 ppm was significantly different from the length of the leaf in the BAP administration of 1.25 ppm, 1.00 ppm, and 0.75 ppm, but it was not significantly different from the length of the leaf in the BAP administration of 1.75 ppm and 2.00 ppm. The length of the leaf with the application of 2.00 ppm BAP was not significantly different with the provision of BAP as much as 1.25 ppm and 1.00 ppm and the administration of BAP as much as 1.25 ppm was not significantly different from the BAP administration of 1.00 ppm and 0.75 ppm. The shortest leaf in the BAP treatment was 0.75 ppm, namely an average of 1.51 cm and the longest leaf in the BAP treatment was 1.50 ppm, namely an average of 2.44 cm.

The number of *Dendrobium spectabile* orchids roots, namely the BAP administration of 0.75 ppm, significantly different from the number of roots in the BAP administration of 1.00 ppm, 1.25 ppm, 1.50 ppm, 1.75 ppm, and 2.00 ppm. The least formed was in the BAP treatment of 1.75 ppm and 2.00 ppm, namely an average of 0.71 roots and the highest number of roots in the BAP treatment of 0.75 ppm, namely an average of 1.10 roots. The results of the average number of roots obtained from the transformation data. Root length of *Dendrobium spectabile* orchids based on the results of the 5% BNJ test, namely the BAP administration of 0.75 ppm was significantly different from the root length of the BAP administration of 1.00 ppm, 1.25 ppm, 1.50 ppm, 1.75 ppm, and 2, 00 ppm. The shortest root of orchids in the BAP treatment was 1.50 ppm, 1.75 ppm, and 2.00 ppm, namely an average of 0.71 cm and the longest root in the treatment of 0.75 ppm BAP was an average of 0.79 cm. The results of the average root length were obtained from the transformation data.

Generally, Cytokinins are used to stimulate the formation of shoots, influence cell metabolism, and stimulate dormant cells and their main activity is to promote cell division. In line with the opinion of George and Sherington (1993) on tissue culture, cytokinins play a role in promoting cell or tissue division used as explants and stimulating the development of shoots. In in-vitro plant propagation, cytokinins are used to overcome apical dormancy and enhance lateral bud branching from the axillary. According to Zulkarnain (2009), BAP is a type of ZPT group that is often used in tissue culture, BAP is a class of cytokinin hormones that functions to increase cell division, shoot proliferation, and shoot morphogenesis.

The number of *Dendrobium spectabile* shoots formed based on the 5% BNJ test results showed that the BAP concentration of 1.75 ppm was significantly different from other treatments. It is assumed that the cytokinin content in BAP applied through *in-vitro* growing media can divide cells, especially shoot formation. This is in line with the results of Harahap's research (2010) which showed that the higher the BAP concentration is given will increase the increase in the number of shoots in the *Nepenthes gracilis* plant. Hartman et al. (1997) also stated that the addition of cytokinins to the in-vitro culture medium could induce more shoots in high concentrations.

Growth regulators through BAP are widely used for the induction and shoot multiplication of various types of plants. This is because the main role of BAP is to stimulate the growth and development of shoots which in subsequent development produce leaves. According to Sukendah (2009), BAP affects shoot growth, namely the length of shoots in *kopyor* coconut sprouts explants. According to the opinion of Mok, Martin, and Mok (2000) which stated that BAP is an adenine-type cytokinin that increases cell division and cell enlargement in plant culture.

The number of leaves of the orchid plant *Dendrobium spectabile* which was formed based on the results of the 5% BNJ test showed that the treatment of various BAP concentrations showed significant differences. It is suspected that the BAP applied at a concentration of 1.50 ppm was able to stimulate the formation of orchid leaves by *in-vitro*. Increasing the concentration of BAP also plays an important role in the growth and development of explants, the higher the availability of cytokinins will stimulate the explants to grow faster and develop into plantlets. However, the use of growth regulators in *in-vitro* culture at certain limits can stimulate growth and can act as an inhibitor if used beyond the optimum concentration (George and Sherrington, 1984).

The effect of exogenous growth regulators in-vitro media is determined by the content of the same or different endogenous growth regulators (in plant tissue). This means that the effect of exogenous BAP in the growing media on the growth of the number of leaves is determined by the BAP content or other endogenous cytokinin groups. Leaves are an important factor in plant growth, namely as a place for photosynthesis to take place and the formation of carbohydrates. Sitompul and Bambang (1995) argued that the number of leaves is needed as a growth indicator so that it explains the growth processes that occur such as in the formation of plant biomass. The more leaves that appear on the explants, the better the explant growth.

Based on the results of the 5% BNJ test on the leaf length of the orchid plant, it was shown that the BAP treatment with a concentration of 1.50 ppm was not significantly different from the treatment concentration of 1.75 ppm and 2.00 ppm and was significantly different from other treatments. This indicates that BAP is effective in influencing the longest leaf length in moderate concentrations. Yelnititis, Bermawie, and Syafaruddin (1999) stated that the addition of cytokinins could increase the number and size of leaves. Hariyanti, Nirmala, & Rudarmono (2004) stated that cytokinins play a role in cell division not in cell elongation, especially in high concentrations.

The results of the research on the number of roots and length of orchids by *in-vitro* showed that 0.75 ppm BAP treatment was the best concentration and was significantly different from other treatments. This indicates that the higher the concentration of BAP applied to the *in-vitro* media can cause more

stunted plant root growth. According to Karjadi and Buchory (2007), plant tissue culture generally requires auxin in root formation whereas at high cytokinin concentrations it will usually inhibit the formation or growth of explant roots.

The number of roots and the length of the roots of the plant indicate how wide the reach of the plant is to absorb nutrients, so that the longer the roots, the wider the reach of the plant and the more nutrients that can be absorbed. In addition, the number of roots in the growth utilizing tissue culture showed that the explants were healthy and able to optimally absorb nutrients from the media. According to Zulkarnain (2009), roots function as a tool to absorb nutrients and nutrients and as a support for the plant body.

The potential yield of *Dendrobium spectabile* orchid explants with various concentrations of BAP treatment can be seen from the production of the number of shoots formed. The results showed that giving BAP with a concentration of 1.50 ppm was the best concentration in increasing the growth of *Dendrobium spectabile* explants by *in-vitro* with an average of 4.25 buds. Following the statement of Hoesen (2001) that high concentrations of cytokinins will increase the number of shoots formed on explants.

CONCLUSION AND SUGGESTION

Based on the results of the research, the effect of various BAP concentrations on the growth of *Dendrobium spectabile* explants by *in-vitro* could be concluded that: (1) Giving various concentrations of BAP on orchid explants by *in-vitro* could increase the number of leaves, number of shoots, number of roots, length of root, and length of the leaf. (2) The best BAP concentration in increasing the number of orchid explant growth by *in-vitro* was 1.50 ppm based on the variable number of shoots formed.

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