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ARTICLE

# Combination effects of NPK fertilizer and benzyl amino purine (BAP) in accelerating *Cattleya* Orchid vegetative growth

Efeitos combinados de fertilizante NPK e benzil amino purina (BAP) na aceleração do crescimento vegetativo da orquídea *Cattleya*

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**Abstract:** Vegetative growth of *Cattleya* orchids is influenced by environmental factors. In addition, growth and development are also determined by the availability of nutrients and hormones. One of the methods to accelerate the vegetative growth of orchids is by the application of NPK fertilizers and plant growth regulators such as benzyl amino purine (BAP). The objective of this study was to obtain the best concentration of NPK and BAP concentration on the vegetative growth of *Cattleya* orchids. The experiment was conducted at the screenhouse of the Experimental Garden of Bale Tatanen, Faculty of Agriculture, Padjadjaran University with a Factorial Randomized Block Design consisting of two factors, namely the concentration of NPK fertilizer (0, 1, 2, 3, and 4 g $L^{-1}$ of NPK-28:10:10 and 2 g $L^{-1}$ of NPK-32:10:10 as positive control), and the second factor was BAP concentration (0, 0.5 and 1 mg $L^{-1}$) with three replications. The results showed that there was an interaction effect between the application of 2 g $L^{-1}$ of NPK 28:10:10 and 1 mg $L^{-1}$ BAP on the number of pseudobulbs per clump. Independently, the treatment of 2 g $L^{-1}$ of NPK 28:10:10 showed the best results in the increase of plant height and the number of new shoots. While 0.5 mg $L^{-1}$ of BAP showed the best results in the increase of plant height, the number of new shoots and the SPAD value.
**Keywords:** cytokinin, foliar application, orchid, plant growth regulator.

**Resumo:** O crescimento vegetativo das orquídeas *Cattleya* é influenciado por fatores ambientais. Além disso, o crescimento e o desenvolvimento também são determinados pela disponibilidade de nutrientes e hormônios. Um dos métodos para acelerar o crescimento vegetativo das orquídeas é pela aplicação de fertilizantes NPK reguladores de crescimento vegetal, como benzil amino purina (BAP). O objetivo deste estudo foi obter a melhor concentração de NPK e concentração de BAP no crescimento vegetativo das orquídeas *Cattleya*. O experimento foi conduzido na estufa do Jardim Experimental de Bale Tatanen, Faculdade de Agricultura, Universidade Padjadjaran com um delineamento fatorial em blocos casualizados consistindo de dois fatores, a saber, a concentração de fertilizante NPK (0, 1, 2, 3 e 4 g $L^{-1}$ de NPK-28:10:10 e 2 g $L^{-1}$ de NPK-32:10:10 como controle positivo), e o segundo fator foi a concentração de BAP (0, 0,5 e 1 mg $L^{-1}$) com três replicações. Os resultados mostraram que houve efeito de interação entre a aplicação de 2 g $L^{-1}$ de NPK 28:10:10 e 1 mg $L^{-1}$ de BAP sobre o número de pseudobulbos por touceira. Independentemente, o tratamento de 2 g $L^{-1}$ de NPK 28:10:10 apresentou os melhores resultados no aumento da altura da planta e no número de novos brotos. Enquanto 0,5 mg $L^{-1}$ de BAP apresentou os melhores resultados no aumento da altura da planta, no número de novos brotos e no valor SPAD.
**Palavras-chave:** aplicação foliar, citocinina, orquídea, regulador de crescimento vegetal.

## Introduction

Orchids are one of the most of ornamental plants in the world due to the high appeal and aesthetic value. One type of orchid is *Cattleya* characterized by the fragrant smell, varied flower colors, and is larger than other orchids in size, so, the orchid has been designated as The Queen of Orchid (Harahap et al., 2023). Based on the growth type, *Cattleya* is categorized as sympodial orchids with more than one stem and one point growing upwards.

The need for orchids is increasing every year, therefore increasing flower quality and quantity is needed. To increase orchid plant growth, the fertilizer with NPK compound is mostly used (Sunawan et al., 2020). NPK fertilizer is a compound fertilizer containing the nutrients of N (Nitrogen), P (Phosphate), and K (Potassium) which are very useful for plants. The vegetative growth of *Cattleya* is generally grow very slow, so additional fertilizer especially a high nitrogen (N) content is needed. Hastuti et al. (2016) stated that foliar fertilizer by NPK with higher nitrogen (N) composition than other elements is needed during vegetative growth. Foliar fertilizer through leaves is an effective method through roots in accelerating the growth of orchid (Ayuningtyas et al., 2020). Sari et al. (2011) stated that fertilizing through leaves is able to absorb around 90% of nutrients, while fertilizing through roots is only able to absorb around 10% of nutrients.

Fertilizer dosage and concentration are important to be considered when applied by foliar method through leaves. Excessive foliar fertilizer will be toxic, on the other hand, if it is not given, it will inhibit plant growth and reduce the number of shoot (Hartati et al., 2019). The growth response of orchid plants to fertilizer application will be different for each orchid species. In dendrobium, fertilization of 1.5–4 g $L^{-1}$ increases the number of shoots, plant height, plant fresh weight, amount of chlorophyll and number of leaves (Prasetyo, 2019; Ayuningtyas et al., 2020; Fadhila and Nurul, 2020). Whereas in *Cattleya*, 2 g $L^{-1}$ of foliar fertilizer increase leaf length (Irsyadi and Mulanjari, 2023).

*Cattleya* is characterized as sympodial orchids, so the number of shoots will be the main parameter of plant growth. To stimulate shoot growth, nitrogen fertilization is mostly used, but some of the treatments can be applied by using plant growth regulator (PGR), such as cytokinin. Cytokinins consist of several types, including Benzylaminopurine (BAP), Kinetin, and Thiadiazuron (TDZ). BAP has a similar structure to kinetin,

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but BAP is more effective than kinetin because it has a benzyl group. Talukdar et al. (2022) stated that cytokinin groups such as BAP have a role in stimulating RNA and protein synthesis in various tissues which in turn encourages cell division and induces shoot formation.

As with fertilization, the effectiveness of PGR is greatly influenced by the dose and concentration. Nasution et al. (2022) stated that benzyl amino purine (BAP) and the interaction of both on the growth induction of *Cattleya* sp. Cytokinin interacts with auxins in determining the direction of cell differentiation. BAP of 1 mg $L^{-1}$ and NAA of 0.25 mg $L^{-1}$ affected in the number of shoots of dendrobium plantlets (Sakina et al., 2019). In other study reported that BAP 1-2 mg $L^{-1}$ has a positive effect on the time of shoot emergence, time of leaf emergence, number of leaves, and number of shoots of moon orchid (Syamsiah et al., 2020). Seeing the importance of using foliar fertilizer and BAP, this research aimed to identify the combination effect of foliar fertilizer and BAP on the growth of *cattleya* orchids.

## Materials and Methods

### Plant preparation

*Cattleya* 'Hybrid' at 5 months old after acclimatization was used in this experiment. Orchid plants were cultivated on sterilized fern chopping media. The plants are stored and cultivated in the Greenhouse Bale Tatanen Padjadjaran, Faculty of Agriculture, Universitas Padjadjaran at an altitude of 730 m above sea level, a temperature of 28.5 ± 2 ºC and a relative humidity of 75.0 ± 10%. The experiment consisted of six NPK concentrations (0, 1, 2, 3, and 4 g $L^{-1}$ of NPK-28:10:10 and 2 g $L^{-1}$ of NPK-32:10:10 as positive control (Mubarok et al., 2024)) with the combination of three concentration of BAP (0, 0.5 and 1.0 mg $L^{-1}$). Those treatment was repeated three times.

### NPK Fertilizer and BAP Application

The NPK fertilizer and BAP were applied as a foliar application based on predetermined concentrations. Each treatment was sprayed to orchid leaves with an equal amount, 15 mL per plant. The application of NPK fertilizer application is carried out in the morning, with an interval of application once a week according to the method described by Mubarok et al. (2024), whereas BAP treatment was applied once in two weeks.

### Plant Growth Analysis

The plant growth was analyzed according to the method described by Mubarok et al. (2024) with the modification as follows:

a. The plant height is measured the length of the plant using a meter from the base of plant near to the root to the tallest leaf in a clump of *cattleya* plant measured at 16 weeks after treatment (WAT)

b. Increase in the leaf width. Leaf width is measured using a ruler. Measurements were made on the widest part of the leaves. The Increase in the leaf width was measured at 16 WAT.

c. The number of pseudobulbs is determined by calculating the difference of the number of pseudobulbs at 16 WAT compared to 0 WAT.

d. The shoot number is determined by calculating the shoot that grows from 0 to 16 WAT.

### Chlorophyll Content Index (CCI) Analysis

CCI measurements were carried was measured at 16 WAP using a by using *Soil Plant Analysis Development* (SPAD) in three positions of the leaf, namely at the base, middle, and tip of the leaf. CCI was described as the SPAD value that described the chlorophyll levels of leaves in plants.

### Statistical Data Analysis

The obtained data were analyzed by using F-test and then continued by Duncan's Multiple Range Test at 5%. The statistical software used in the present experiment was Statistical Tool for Agricultural Research (STAR) (Shrestha, 2019), version 2.0.1.

## Results

### Plant height

Based on statistical data analysis showed that the combination of NPK fertilizer and BAP significantly affect the plant height. There showed the interaction effect between NPK and BAP in the increasing plant height (Fig. 1). However, an increase in plant height can be seen based on the independent influence of the application of NPK fertilizer. Among all fertilizer treatments, the application of NPK fertilizer with a concentration of 2 g $L^{-1}$ with or without BAP was able to provide a significant increase in plant height. The height of orchid plants with this fertilizer treatment reached 10.6 cm, while the application of a higher dose or around 4 g $L^{-1}$ gave a negative response to the height of orchid plants with a plant height of around 8.3 cm. Meanwhile, BAP application did not provide a significant response to orchid plant height at various BAP concentrations.

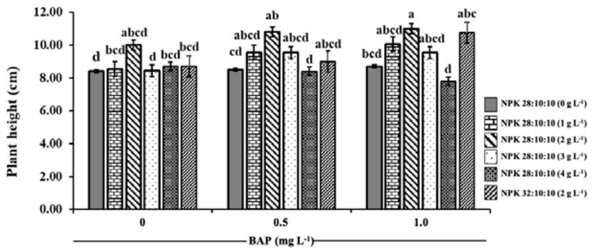


**Fig. 1.** The effect of different NPK and BAP concentrations on the plant height of *Cattleya* Orchid at 16 WAT. The mean value of SE (n = 3) followed by the same letter did not differ significantly based on Duncan's Multiple Range Test at the level of 5%.

### Leaf width

In contrast to plant height, the application of a combination of NPK fertilizer and BAP at certain doses showed an interaction that was able to significantly influence the growth of orchid plant leaf width. However, individually, the application of BAP at certain doses can increase the width of orchid plant leaves (Fig. 2). In this research, the role of BAP application provided a more impactful response compared to the role of NPK fertilizer. The application of BAP at a concentration of 0.5 mg $L^{-1}$ was able to increase the diameter of orchid leaves in all fertilizer concentration treatments compared to the control treatment except for the application of 2 g $L^{-1}$ fertilizer. Meanwhile, the addition of BAP concentration or 1 mg $L^{-1}$ did not show a significantly different response when compared to the application of BAP at the previous concentration.

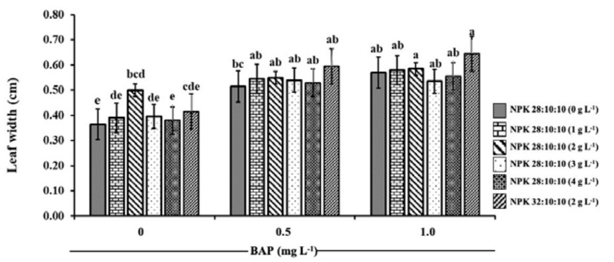


**Fig. 2.** The effect of different NPK and BAP concentrations on the leaf width of *Cattleya* Orchid at 16 WAT. The mean value of SE (n = 3) followed by the same letter did not differ significantly based on Duncan's Multiple Range Test at the level of 5%.

### Number of Pseudobulb

Based on statistical data analysis showed that the combination of NPK fertilizer with BAP was able to have a significant influence on the number of pseudobulbs of *Cattleya* plants. Untreated BAP plants that applied with NPK fertilizer at a concentration of 5 g $L^{-1}$ was able to produce the highest number of pseudobulbs compared to other NPK concentrations with a total pseudobulb of 1.75. However, this the number of pseudobulb decreased significantly when BAP was applied combination with NPK. Meanwhile, the application of fertilizer at a certain concentration increased with an increase in the concentration of BAP. The highest pseudobulb number was found in the combination of BAP 1 mg $L^{-1}$ and NPK 2 g $L^{-1}$ treated plants, but it was not significantly different compared with 3 and 4 g $L^{-1}$ (Fig. 3).

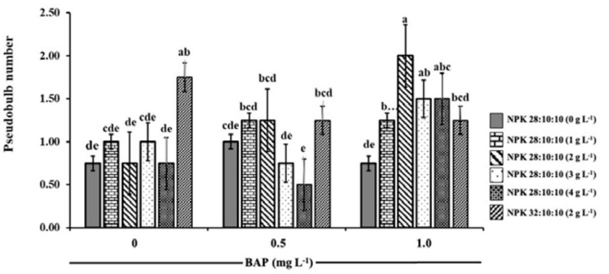


**Fig. 3.** The effect of different NPK and BAP concentrations on the pseudobulb number of *Cattleya* Orchid at 16 WAT. The mean value of SE (n = 3) followed by the same letter did not differ significantly based on Duncan's Multiple Range Test at the level of 5%.

**Shoot number**

Based on statistical data analysis showed that there was no interaction effect between NPK fertilizer and BAP in shoot number. However, the independent application of BAP showed a significant effect in increasing the shoot number (Fig. 4). NPK fertilizer in all Applications of foliar fertilizer did not have a significantly different effect on the addition of new shoots. However, independently, the application of BAP showed significantly different results on the addition of new shoots. The application treatment with a concentration of 1 mg $L^{-1}$ BAP showed the greatest increase in new shoots compared to the control treatment and 0.5 mg $L^{-1}$ BAP. The highest shoot number of shoots was found in the combination of BAP 1 mg $L^{-1}$ and NPK 2 g $L^{-1}$ treated plants.

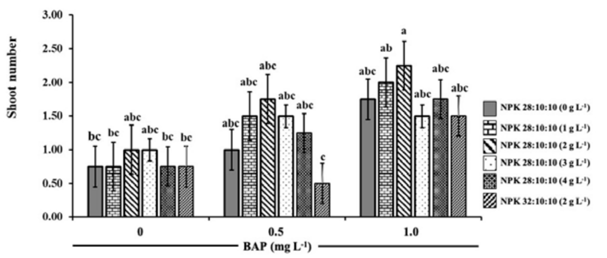


**Fig. 4.** The effect of different NPK and BAP concentrations on the shoot number of *Cattleya* Orchid at 16 WAT. The mean value of SE (n = 3) followed by the same letter did not differ significantly based on Duncan's Multiple Range Test at the level of 5%.

**Chlorophyll Content Index (CCI)**

CCI was represented by SPAD value. Based on statistical data analysis showed that the foliar application of NPK fertilizer showed a significant influence on the CCI (Fig. 5). However, the effect of NPK varied among the different concentrations of BAP treatment. In untreated BAP plants, the NPK at 3 g $L^{-1}$ treated plants resulted in the highest CCI index but it is not significantly different compared with all NPK concentrations. Increasing the concentration of BAP up to 2 mg $L^{-1}$ significantly affected in the increasing CCI, but it was different among the NPK concentrations. The highest CCI was observed from 2 and 4 g $L^{-1}$ NPK-treated plants with an additional of BAP 2 mg $L^{-1}$ (Fig. 5).

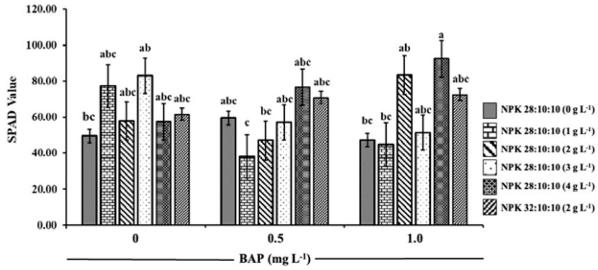


**Fig. 5.** The effect of different NPK and BAP concentration on the SPAD Value of *Cattleya* Orchid at 16 WAT. The mean value of SE (n = 3) followed by the same letter did not differ significantly based on Duncan's Multiple Range Test at the level of 5%.

## Discussion

BAP belongs to cytokinin which plays a role in various plant metabolisms such as plant growth, development, cell division, inhibition of senescence, and regulation of apical bud dormancy (Pérez-León et al., 2023). Laurensi (2018) states that BAP has a major role in cell division, encouraging morphogenesis, budding, and chloroplast formation. Based on our study, Individually, BAP can increase the size of the leaf width, and increase the number of shoots, and together with the NPK, it can increase the number of pseudobulbs.

The enlargement of leaf width is related to lighting conditions. The enlargement of leaf width in the BAP-treated plants due to the acceleration of cell division. It will affect the increase in the efficiency of light capture and the photosynthesis process. Dash et al (2022) reported that the reduction of light intensity due to the shading net stimulated the increasing leaf size. Another study reported by Annisa et al. (2023) that BAP application triggered effective shoot growth in propagating dendrobium plants. Andaryani et al. (2019) stated that BAP is able to stimulate plant cell division and differentiation. The role of BAP in increasing the number of growing shoots was demonstrated also in the propagation of turmeric (Vaze et al., 2024) and mulberry (Zhang et al., 2022).

In the process of plant metabolism, cytokinin works together with auxin in the process of plant height elongation. Kurepa and Smalle (2022) reported that cytokinin and auxin work synergistically, with cytokinin increasing cell division through mitosis and auxin increasing cell elongation through the process of cell osmosis. The administration of BAP increases endogenous auxin and accelerating the cell division at the base of the leaf (Larenkeng et al., 2020). The leaf development in monocots such as orchids is regulated spatially, namely cell division mainly occurs at the base of the leaf, cell expansion in the middle of the leaf, and cell maturation at the tip of the leaf Wu et al. (2021).

The application of BAP at optimum concentration can accelerate the cell division process, the formation of shoots and finally will increase the number of pseudobulbs. In addition, the combination of NPK and BAP can increase the efficiency of the photosynthesis process. In plant metabolism, phosphorus plays a role in ATP synthesis in the process of photosynthesis or in the process of catabolism. The availability of ATP in cells increases plant growth and development. Lestari et al. (2017) reported that the availability of ATP in cells can increase plant growth and development, one of which is the pseudobulb in orchids which functions as a carbohydrate storage organ.

The impact of BAP application is greatly influenced by the plant stage. In the reproductive phase, BAP will initiate flowering and increasing the number of flower bud, but will affect the decrease in the increase in the number of shoots and plant height (Li et al., 2016), while, during the vegetative phase, BAP accelerates the growth of lateral shoots, branches and enlargement of leaf width (Werner et al., 2021). Ramy et al. (2019) reported that the application of BAP in the generative phase causes energy and photosynthate allocation to focus more on flowering initiation and flower bud formation.

Apart from BAP, this study reported that the application of NPK plays a role in increasing plant height, number of pseudobulbs, and chlorophyll in *Cattleya*. A similar study reported by Sunawan et al. (2020) that individual application of NPK fertilizer was able to increase plant height and leaf width in *Phalaenopsis amabilis* L. The increase in chlorophyll content could be caused by the composition of the NPK fertilizer applied being dominated by nitrogen compounds which are closely correlated with the formation of chlorophyll compounds (Saleem et al., 2021). The chlorophyll content in plant leaves is greatly influenced by the availability of nitrogen and phosphor. The nitrogen plays an active role in enzyme activation and chlorophyll formation so increased nitrogen absorption will increase the efficiency of plant metabolism processes (Liu et al., 2022), while phosphor plays an active role in the formation of chlorophyll a and b owned by plants so that their photosynthetic ability increases. Plant photosynthesis is directly related to the ability of plants to grow and grow taller (Kalaitzoglou et al., 2019). In addition, potassium plays an important role in the growth of meristem tissue and functions to maintain cell turgidity which is needed for cell elongation (Kumar et al., 2022).

Meanwhile, the increase in plant height could be influenced by the phosphorus content in NPK which can trigger plant height (Fathi and Afra, 2023). On the other hand, applying NPK fertilizer containing high levels of nitrogen can increase the effectiveness and efficiency of the photosynthesis process (Satari et al., 2022), so that the process of storing food reserves and dividing them in pseudobulbs can increase. The division process is supported by the provision of BAP (Pérez-León et al., 2023) so that the results of pseudobulb division can be maximized. Andriyani and Yanti (2006) stated that the efficiency of fertilizer application through leaves is greatly influenced by the concentration and dosage of the fertilizer. Fertilizer concentrations that are too high cause an imbalance in the plant's metabolic processes, both in photosynthesis and respiration, which will then disrupt plant growth (Agussimar, 2016).

## Conclusions

BAP is able to increase the width of the leaves and the number of shoots, while NPK fertilizer is able to increase plant height and chlorophyll content. Independently, the best NPK treatment was at a concentration of 2 g $L^{-1}$, while the best BAP treatment was at a concentration of 0.5 mg $L^{-1}$. The use of these two combinations can increase the vegetative growth of *Cattleya* Orchid plants and increase the production of pseudobulbs for propagation purposes.

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**Author Contribution**

**SM:** conceptualization, data curation, funding acquisition, methodology, supervision, validation, visualization, writing – original draft, writing – review & editing. **AA:** formal analysis, investigation, methodology, software, validation, visualization, writing – original draft. **DD:** formal analysis, investigation, methodology, software, validation, visualization, writing – original draft. **FF:** conceptualization, data curation, formal analysis, supervision, validation, writing – review & editing. **AN:** conceptualization, data curation, formal analysis, investigation, writing – review & editing. **HIJ:** data curation, formal analysis, software, validation, visualization; SA: validation, writing – review & editing.

**Conflict of Interest**

No potential conflict of interest was reported by the authors.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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