Stimulation effect of synthetic plant growth regulator (GA₃ and BAP) on young cinchona plant (Cinchona ledgeriana) grown in lowland

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Abstract. Cinchona is a pharmaceutical plant that produces medical substance. This plant contains copious type of alkaloid such as quinine, quinidine, cinchonine, and cinchonidine. Several obstacles might restrict cinchona cultivation, mainly climatic factors such as temperature and rainfall. One of the efforts to improve cinchona growth in lowland through the application of synthetic plant growth regulator (PGR). Two stages of experimental design were used in this study: first stage was the application of GA₃ in six levels from October to December 2017 and the second stage was six levels of BAP concentrations treatment from July until October 2018. The experimental design used was randomized block design (RBD) with 4 replications at low altitude area for cinchona plant. The results showed GA₃ applications have a significant effect towards leaves width and plant height. The 225 ppm of GA₃ dosage produced maximum leaf width, 100ppm yielded the maximum number in plant height, and 150ppm produced the highest leaf count. While GA₃ as a PGR improved the vegetative characters, various concentration of BAP had positive impact for stem diameter, leaves number, leaves area, and chlorophyll content on young cinchona plant grown in lowland area.

1. Introduction

Cinchona is one of the plantation commodities that contain high alkaloid. Alkaloids such as quine, quinidine, cinchonine, cinchonidine can be used as medicinal ingredient for malaria and cardiovascular diseases. It is also an important commodity for beverage industry as a bitter sensation in the soft drink, and as fluorescence dye in textile industry. Cinchona is originated from the Andes highland of Southern America that is high in humidity, low in temperature, and receive low sunlight intensity from tropical rain forest [1]. In order to cultivate the plant, modification of environment and cultivation is needed more. Indonesia is one of cinchona producer and the biggest exporter during the Dutch colonization in the 19th century. Cinchona production were cut off during Japanese colonization in the World War II era, and has caused a significant decline in cinchona plantation development [2]. Recently, cinchona plantation has decreased due to land use alterations, despite the high demand for the medicinal substance by pharmacological sector. As the consequence, cinchona crystal demand has to be fulfilled through export from other countries such as Congo and Kenya. Although quinine content from Africa is lower (5%) than Indonesian species (7%) exported cinchona crystal supply is relatively stable [3]. Deploying the plant in other location will be a solution to re-start cinchona cultivation in Indonesia, but some environmental modification such as shading level, fertilizer
management and plant growth regulators stimulation is needed, mainly because the majority of areas in Indonesia have low and medium altitude, that is different to the original habitat [4].

Plant growth regulators (PGR) are chemical substances with hormonal effect towards plant. PGR encourages, decreases, and modifies plant metabolism process. PGRs can promote plant growth as good as cytokines, auxin, and gibberellin hormone, but also some kinds of PGRs can inhibit plant development because has similar function like abscisic acid and ethylene. For instance, gibberellin can be substituted by GA₃ or stem elongation and stimulate flowering stage [5]. BAP as synthetic cytokines can aid cell divisions and protein synthesis especially in meristem tissue [6]. According to Mayerni et al. [7] BAP has stable character and act as quickly and effective as PGRs in supporting cell and tissue growth.

The stimulation of young cinchona plant cultivated in lower altitude with some PGRs is crucial to observe because unfavourable habitat can inhibit of plant development. This study provides the information and technique for cinchona plantation expansion in unusual environment to gain best concentration for PGR treatment.

2. Material and Methods

The experiment was divided in two stages. The first stage was GA₃ treatment and BAP as the second trial. The first step was conducted from October to December 2017 and the second stage was done from February until September 2018 in Trial Field of Agricultural Faculty, Universitas Padjadjaran with the altitude of approximately 725 meters above the sea level (categorize of low land for cinchona plant is 400-800 meters above the sea level based on Central of Tea and Cinchona Research Gambung, 1995) and type C rainfall accordingly Smith and Fergusson classification (1951).

2.1 Tools

Field tools used in the experiment were: digital calipers, metre, analytical scales, measuring glass 1000 mL, chlorophyll meter SPAD, 2000mL pressure sprayer, and knapsack sprayer. Cinchona (Cinchona ledgeriana) used in this study was Cib 5 clone obtained from Central of Tea and Cinchona Research Gambung, Indonesia range aged between 1 - 2 years old, cow manure as organic fertilizer, NPK fertilizer, GA₃, BAP, 95% Ethyl Alcohol, and distilled water.

2.2 Experimental Design

Experimental design of two trials was randomized block design (RBD) and each trial consisted of six treatments with four replications. The treatment on first trial was control (without GA₃), 75 ppm, 100 ppm, 125 ppm, 150 ppm, and 225 ppm of GA₃. The second experiment consisted of control (without BAP), 30 ppm, 60 ppm, 90 ppm, 120 ppm, and 150 ppm of BAP. Data were analysed by variance test (F-test) and continued by Duncan Multiple Range Test (DMRT) with 5% significance level if differences were detected.

2.3 Field Transplanting

Manipulation was needed due to different environmental condition with cinchona habitat. The first modification added was shading (60% shelter density) and protective plant cultivation (Gliricidia sepium) to reduce sunlight intensity and maintain humidity. The next modification was tillage type, zero or minimum tillage to full tillage with 1 to 1 m distance between planting hole for root respiration and development. Planting holes were 30 x 30 x 40 cm as recommended for cinchona plant. Cow manure was added into the hole and incubated until one week prior to seedling transplantation. After incubation, cinchona plants were incubated and NPK fertilizer was added.

2.4 Treatment Application

PGRs solution was made by mixing PGRs (based on concentration) with 2 - 3 drops of 95% Ethyl Alcohol before the addition of distilled water until 1000 ml. Calibration technique was done to observe assure the accuracy of PGR prior to treatment. PGR was applied once every two weeks started from 6 weeks after transplantation around 08.00 AM every day. Observation was done 8 weeks after the first
application that consisted of height plant (cm), number of leaves, stem diameter (cm), leaf width (cm²), and chlorophyll content (for BAP measurement).

3. Results and discussion

3.1 Results

Result of experiment was illustrated in two tables. Table 1 was about GA₃ effect and table 2 describes BAP effect in young cinchona growth. All of tables have coefficient variance value (CV) that started from 2% to 30%. According to Gasperz [9] proper field trial was shown by CV value around 20% or less. Mostly experiment outcome indicated in decent performing because had CV value less than 20%, only on broad leaves and number of leaves had shown more than 20%. Table 1 revealed four variables that had been observed and significant effect on 2 parameters, plant height added and leaves broad. Plant height development on young cinchona plant treated with GA₃ showed the best impact on 100 ppm despite the no significant value compared to 225 ppm and 125 ppm, with highest leaf width from 225 ppm treatment. This result illustrated that some treatments of GA₃ have better effect than without GA₃.

**Table 1. The effect of GA₃ concentrations at young cinchona on several growth parameters**

| Treatments       | Addition of Plant Height (cm) | Addition of Stem diameter (cm) | Leaves number | Leaves Area (cm²) |
|------------------|-------------------------------|--------------------------------|---------------|------------------|
| Control (GA₃ 0 ppm) | 3.75 bc                       | 0.07                           | 1.50          | 186.55 b         |
| GA₃ 75 ppm       | 2.55 bc                       | 0.06                           | 3.29          | 52.94 c          |
| GA₃ 100 ppm      | 6.92 a                        | 0.03                           | 3.83          | 142.21 b         |
| GA₃ 125 ppm      | 4.23 abc                      | 0.04                           | 3.13          | 115.02 bc        |
| GA₃ 150 ppm      | 2.65 c                        | 0.05                           | 2.88          | 155.92 b         |
| GA₃ 225 ppm      | 5.34 ab                       | 0.03                           | 2.17          | 459.68 a         |
| CV               | 19.32%                        | 2%                             | 30%           | 24.12%           |

Note: Different significant parameter based on analysis of variance (0.05) and followed by Duncan Test (0.05).

**Table 2. The effect of BAP concentrations at young cinchona on several growth parameters**

| Treatments       | Addition of Plant Height (cm) | Leaves Number | Addition of Stem diameter (cm) | Content of Chlorophyll (unit) | Leaves Area (cm²) |
|------------------|-------------------------------|---------------|--------------------------------|-----------------------------|------------------|
| Control (BAP 0 ppm) | 1.15                          | 3.4 c         | 0.08 b                         | 48.55 ab                    | 347.077 ab       |
| BAP 30 ppm       | 1.42                          | 9.32 ab       | 0.13 a                         | 50.225 ab                   | 273.515 ab       |
| BAP 60 ppm       | 1.6                           | 5.55 bc       | 0.11 ab                        | 54.4 a                      | 592.066 a        |
| BAP 90 ppm       | 1.17                          | 9.9 ab        | 0.08 b                         | 48.525 ab                   | 330.596 ab       |
| BAP 120 ppm      | 1.6                           | 10.77 ab      | 0.09 b                         | 44.925 b                    | 268.469 b        |
| BAP 150 ppm      | 1.15                          | 13.62 a       | 0.11 ab                        | 52.15 ab                    | 569.31 a         |
| CV               | 15.32%                        | 22.53%        | 7.67%                          | 9.65%                       | 9.59%            |
Table 2 described the effect of BAP application for young cinchona growth. All of parameters except plant height were significantly influenced in accordance to various BAP concentrations. Number of leaf treated with BAP in all concentrations showed significant differences compared to control, stem diameter treated with BAP 30 ppm displayed the best result but not significantly to BAP 60 ppm and 150 ppm treatments. For other leaves characters such as chlorophyll content and leaf width, BAP 60 ppm showed the best result of chlorophyll content and leaf width and BAP 150 ppm yielded in highest leaf width, but they had slightly effect than the other concentrations especially for control that only different with BAP 120 ppm.

3.2 Discussion

Gibberellin and cytokines are plant hormone which is important for vegetative development. Gibberellin function is controlling many factor for plant development such as stem elongation, germination, and transition stage between vegetative to flowering because one particularly effect of gibberellin can stimulate elongation cells and cell division [10,11]. Environment also gives impact for gibberellin works for instance light and temperature. When physiological condition of plant change cause of dormancy during unfavourable weather such as too cold or drought, gibberellin treatment has been proved to break dormancy on bud and seed plant effectively for long day, temperature and light effect [6].

Based on the result of GA$_3$ treatment in young cinchona plant on low altitude has different of sunlight intensity and temperature which has significant effect for plant height and leaves broad. Meanwhile, the concentration of GA$_3$ that has significantly impact was caused by the highest concentration (225 ppm) but by 100 ppm of GA$_3$. It will be supported by Khuanakaew et al. [12] that reported about GA$_3$ concentration influence to curcuma plant height, the research described some of GA$_3$ application have effect for plant height development not only caused by the highest concentration but also another concentration. The impact of GA$_3$ for stem elongation also depend on the plant condition, it will incline significantly when gibberellin endogenous is limited but it does not have dramatically effect when gibberellin in the plant still available enough [12]. For other trait which did not have significant effect cause of GA$_3$ application, it will be caused by growth characteristic. Cinchona as perennial plant has slowly movement for stem diameter growth even is stimulated by PGRs [4].

Cytokines is hormone that concern on physiological and development of plant including leaf senescence, apical dominance, apical meristem activity, breaking of bud dormancy. It also appears for chloroplast differentiation, leaf and cotyledon expansion and also autotrophic metabolism developing [11]. This could be caused the research parameter which has significant effect due to BAP application. Leaves number in young cinchona has shown the influence of BAP concentration that increase strikingly than the control. Based on reported by Purwanto [13] in BAP application on mangosteen multiplication showed the number of leaves upward rapidly and another information from Saefas et al. [14] on young tea plant that was given various BAP concentrations reveals significantly difference some of BAP concentrations to number of leaves. It also supported by Naqvi [6] statement that said one of cytokines main sink is young leaves as one part of meristem tissue in plant. Cytokinin also can be natural regulator when is sprayed on the leaf and can delay senescence period and keep content of chlorophyll [11] it could be caused significantly effect on chlorophyll content variable of young cinchona by BAP application. Based on the research of Rulcova and Pospisilova [15] the application of BAP can produce higher chlorophyll content in the leaves of beans than plants that are not applied by BAP. Chlorophyll pigments (chlorophylls) are a group of green pigments that can be found in photosynthesizing tissues.

They constitute a key element of photosynthesis, one that is needed for light absorption [16] Originally, the term chlorophyll was only used to refer to the green pigments which enter into photosynthesis in higher plants; later it was extended to include all photosynthetic porphyrin pigments [17]. On the other hand, BAP treatments did not have influence for plant height of young cinchona that might be caused by endogenous cytokines contents. According to Mayerni [7] stem elongation did
not involve high concentration cytokines or just need low exogenous cytokines when the endogenous cytokines has adequate.

Application of PGRs on young cinchona at low altitude could be solution for increasing development of young plant to productive plant. Even though, the applications have to concern about concentration precision to make effective and efficient procedures. It is caused by the different concentration of PGRs would be have various effect for plant growth including on young cinchona. According to Werner and Schmülling [18] the hormone cytokines is part of an intrinsic genetic network controlling organ development and growth in these two distinct environments that plants have to cope with. Cytokines also mediates the responses to variable extrinsic factors, such as light conditions in the shoot and availability of nutrients and water in the root, and has a role in the response to biotic and abiotic stress. Together, these activities contribute to the fine-tuning of quantitative growth regulation in plants.

4. Conclusion
The effect of synthetic plant growths (GA$_3$ and BAP) increases some vegetative character on young cinchona plant in low altitude area, such as leaves broad and plant height addition for GA3 application and the best concentration is 225 ppm. BAP application strongly influenced for stem diameter, leaves number, leaves area, and chlorophyll content with various concentration of BAP.

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