

Effect of Plant Growth Regulator on Growth, Yield and Catechin Content of Tea (*Camellia sinensis* (L.) O.Kuntze)

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Abstract— Catechins are one of the secondary metabolites contained in tea leaves. Cultivation practices such as pruning affect the shoot production and quality of tea yield. The use of plant growth regulators is a new breakthrough in tea plant engineering. This research aims to determine the interaction effect of type, height of pruning, and concentration of Benzil Amino Purine (BAP) and Gibberellin (GA) concentrations on tea plants' growth and catechin content after pruning. The experiment was conducted at Tea and Cinchona Research Centre Gambung in June 2018 until October 2018, with a split-plot design consisted of three factors as followed: main factor (a) type of pruning (clean and lung pruning); subfactor (b) pruning height (40 cm, 50 cm, and 60 cm); sub-sub factor (h) plant growth regulator (0 ppm, 60 ppm BAP, 50 ppm GA, 60 ppm BAP + 50 ppm GA). The result showed that 60 cm pruning and 60 ppm BAP in the third month after pruning significantly affected the chlorophyll content index (91,581 cci). There was an interaction between the pruning height of 60 cm and 50 ppm GA on fresh shoots weight per bush on the fourth plucking. Based on the response curve, at clean pruning, the optimum value at pruning height of 51.5 cm and 66,63 ppm BAP contributes to the catechin content of 1.88% while at lung pruning, the minimum value pruning height of 50.73 cm and 7.238 ppm with catechin content of 0.776%.

Keywords— Benzil Amino Purine (BAP); catechins; gibberellin (GA); pruning.

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I. INTRODUCTION

Tea is a superior commodity that has a significant role in Indonesia, contributing to the country's foreign exchange and absorbs much labor. As a world tea producer, Indonesia produced 140 thousand tons made tea per year and exported 49 thousand tons in 2018. The average tea production is produced from a number of farmers, the private sector, and State-Owned Enterprises. About 70% of the raw materials for tea are produced by many West Java tea farmers, while the remaining 30% from farmers in Central Java, East Java, North Sumatra, and Bengkulu [1]. Global tea consumption is projected to increase by almost 3% annually over the coming decade [2]. Catechins are one derivative of polyphenols that have high antioxidant properties. Catechins play an important role in determining flavor and taste. In terms of health, the higher catechins are, the more beneficial for health.

The constituents of polyphenolic compounds, especially individual catechins, depend on altered with the change in cultivation altitude. According to Jiang *et al.* [3], the catechin

content in tea leaves varies depending on the season; fresh leaves plucked in spring season produced the strongest theaflavins (the catechin component). The percentage of catechins to total polyphenol was increased with increasing altitude. The temperature is being increased due to climate change. Rising temperatures at lower altitudes will deteriorate tea quality because of climate warming [4]. Furthermore, in tea leaves, high contents of certain individual catechins lead to a high yield of theaflavin. Although processing technologies impact the quality of tea, according to Zhang *et al.*, geographical location and other environmental factors significantly affect the quality of tea [5].

In addition to proper plucking, the yield of the tea plant can be increased through optimal maintenance, agronomic practices, including regular pruning, and application of plant growth regulators to initiate shoot growth after pruning. According to productivity and quality of tea in the field are affected with 35% cultivation techniques, 25% clones, 25 fields managerial, and 15% climate [6]. The results of Wakamatsu *et al.* showed that catechin content in green tea

leaves varies according to cultivation conditions such as intensity of solar radiation, temperature, and precipitation. Thus, there is ambiguity about the best harvest time for obtaining optimal functional effects [7]. Firouzi and Azarian showed that timely pruning of tea bush has desirable effects on the quantity and quality of the final product, and tea bushes are pruned in three forms of light prune, medium prune, and collar prune [8].

Pruning carried out cyclically to provide vegetative growth stimuli, rectify bush architecture, and maintain the perfect frame height of the bushes for efficient plucking. Pruning attempts to break the apical dominance and promote lateral growth during the growing season [9]. The difference lies in many leaves and branches that must be left behind and the high level of pruning from the ground surface. A study conducted by Mohale *et al.* [10] showed that the pruned (top) and unpruned tea plants exhibited higher levels of metabolites than the basal and middle pruned. Pruning bush tea showed a significant effect on the accumulation of secondary metabolites and thus could enhance bush tea quality. Top pruning (apically pruned) resulted in improved metabolite accumulation than other treatments and can be recommended in bush tea cultivation.

Shoot growth is a process that is controlled by interactions between hormones, nutrients, and environmental factors [11]. Until recently, shoot growth after pruning occurred naturally without the addition of growth regulators (ZPT). Cytokinin, which triggers cell division, controls every aspect of plant growth and development, including meristem function, vascular development, stress responses, senescence, and no less important, maintaining the stem cell population in the SAM (shoot apical meristem) [12].

Some research results show satisfactory results in terms of the use of cytokinin and gibberellin. Benzyl Amino Purine (BAP) is a cytokinin that is often used because it is most effective for stimulating the formation of shoots, is more stable and resistant to oxidation, and is the cheapest among other cytokinin's. The use of 200 or 400 mg.L⁻¹ 6-BA significantly increased spring tea yield by 28.9% or 13.3%, respectively as compared with the control. 6-BA at the four concentrations promoted dwarfing and the formation of productive lateral branches and increased the spring yield, and 200 mg.L⁻¹ 6-BA exerted the best comprehensive effect [13]. According to Anjarsari *et al.* [14], BAP application can improve plant growth and development. This indicated that the provision of growth regulators can be used to accelerate the growth of tea plants after being pruned. The results of Rosniawaty *et al.* [15] research that cytokinin derived from coconut water or BAP applied in tea plants after centering was only effective up to 3 months after application. At 1 and 3 months after application, 50% coconut water or BAP 60 ppm increased the length of stem diameter, a number of leaves, shoot length, and number of shoots. Another growth regulators used are Giberelin (GA). GA is one of the phytohormones that regulate numerous important biological processes in plant development [16]. GA has been shown to regulate plant shade response positively [17], and GA also controls certain biological processes in response to stress [18].

The background of this study is that the use of growth regulators in plantations in immature plants and mature plants applied to pruned tea plants is a new breakthrough in plant

cultivation engineering, especially tea as an annual plant, to increase growth and yield. This study aims to determine the interaction effect of type, height of pruning and BAP, and GA concentrations on tea plants' growth and catechin levels after pruning. An important point in this research is that pruning, and the application of growth regulators can affect the parameter studied. Application of BAP and GA to tea plants after pruning is expected to affect catechin content positively.

II. MATERIALS AND METHOD

A. Materials and Plot Preparation

The study was carried out at the Indonesian Research Institute for Tea and Chincona Gambung Ciwidey (1200 m above sea level) from July to October 2018 and the soil type of Andisol. The seven years old pruned tea plants of GMB7 clones are used as planting material. Benzil Amino Purine (BAP) and Gibberellin (GA) used as plant growth regulators. The materials used for catechin analysis are CHCl₃ p.a, Ethyl acetate p.a, Solven Polyphenol (acetonitrile mixture: ethyl acetate: H₃PO₄ 85% = 12: 2: 86), and standard substances. The materials used for the molecular experiment were ethanol, isopropanol, phenol, chloroform, liquid nitrogen, CTAB buffer, PCR kit, primer, aquades, TE buffer, and milliQ. The experiment designed into split-plot consisting of three factors as followed: main factor (a) (two type of pruning consist of clean and lung pruning); sub-factor (b) pruning height consist three levels (40 cm, 50 cm and 60 cm from ground level); sub-sub factor (plant growth regulator (PGR) consist four-level (0 ppm, 60 ppm BAP, 50 ppm GA, 60 ppm BAP + 50 ppm GA).

B. Research Procedures

The first treatment for tea plants is pruning. Production pruning that is generally done is clean pruning and pruning or lung pruning. After pruning, the tea plant is then initiated by shoot growth by providing growth regulators (BAP and GA). Tea shoot plucking is done after 6 times of tipping with plucking cycle once every 14 days done as much as 6 times plucking. Application of Benzil Amino Purine (BAP) and Gibberellin (GA) is carried out six times in the plucking period by spraying BAP and GA solution in the tea bush canopy.

C. Growth and Yield Measurement

Chlorophyll measurement of tea leaves was carried out on the mature leaves in the production plucking phase using SPAD type chlorophyll meter three times to obtain the index data of the ovary leaves' chlorophyll content *cci* units (chlorophyll content index). Fresh shoot weights produced from production (g), are carried out after six plucking times with a 14-day plucking cycle. Wet shoot weights are weighed each time plucking. Dry shoot production weight (g), carried out at the same time as in the case of observation of fresh shoot weights. Fresh shoot from the plucking medium is taken as much as 100 g and then dried in the oven for approximately 48 hours ± 80°C until the weight is constant, then weighed.

D. Catechin Analysis

Catechin analysis was carried out at the Bogor Agricultural Post-Research Center Research and Development

Laboratory, and Catechin Analysis was accumulated every four times. The initial step in the catechin analysis is to dry the shoot samples of 100 g using an oven at 80°C for 10 hours. After that, puree the sample until it is completely smooth. Catechin analysis was performed using an HPLC tool. The method used according to the Official Method of Analysis of the Association of Official Analytical Chemists (AOAC) [19]. The stages are shown in Figure 1 below:

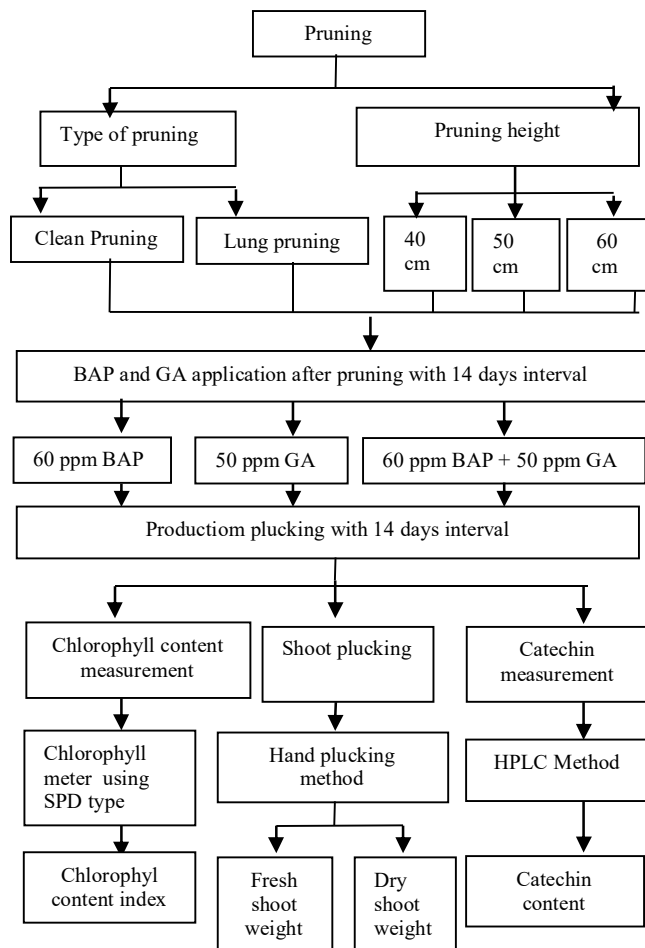


Fig. 1 Flowchart of research method

The initial stage in catechin analysis was to dry the shoot sample as much as 100 g using an oven at 80°C for 10 hours. After that, grind the sample until it is completely smooth. Catechin analysis was performed using HPLC tools. The first step is making a standard curve by making a standard series of c. Measure the standard solution according to the HPLC requirements and create a calibration curve and determine the value of b through zero. Dissolve the solvent and shake until homogeneous, then filter with 0.45 µl milex HA into a special (closed) test tube. The next step is to do sample preparation by weighing 1 g of fine tea and put it in a 500 ml Erlenmeyer flask. Put the distilled water to boil ± 200 ml and boil for 5 minutes then cool (assisted with cooling). Put it in a 250 ml volumetric flask, mark it with distilled water, and then shake it until it is homogeneous. The next step is to filter the solution into 100 ml Erlenmeyer to obtain ± 100 ml filtrate, then pipette 25 ml of the solution obtained and put it into a 250 ml separating funnel. Add 50 ml of CHCl₃ p.a to the solution in a 250 ml separating funnel and then shake it for 2 minutes (do

3 times). The bottom layer of CHCl₃ is accommodated into a 100 ml beaker glass. The sample solution (top layer) was extracted with 3 x 50 mL ethyl acetate p.a, ethyl acetate solution (top layer containing polyphenol) was collected into a boiling flask. Steam the ethyl acetate solution with a Roravapor tool until it is almost dry, dissolve it with solvent into a 50 ml volumetric flask, mark it, and shake it until it is homogeneous. Filter the solution with Milex HA 0.45 µl, then inject it into the HPLC equipment.

E. Data Analysis

Parameter data is analyzed statistically with the F test (Fisher Exact Test) at 5% level; if the difference between treatments appears, Duncan at level 5% test will be conducted. The flowchart of the research method is illustrated in Fig. 1.

III. RESULTS AND DISCUSSION

A. Chlorophyll Content Index of Mature Leaves

Chlorophyll concentrations undergo significant increases as the leaf develops, while polyphenol decreases as the leaf mature [20]. Permanent leaves are used as maintenance leaves to ensure productivity and survival. The upper layers of maintenance leave mainly supply photosynthates for shoot growth, whereas the lower layer supplies the branches, stems, and roots.

Table I shows the leaf chlorophyll content index in the third month. There was an interaction effect between pruning height and PGR concentration on the chlorophyll index of mature leaves. Pruning height of 60 cm combined with a BAP of 60 ppm has a significant effect on the increase in chlorophyll content with a value of 91.58 cci. Application 50 ppm GA prevents its effect on 50 cm pruning height. The combination of 60 ppm BAP and 50 ppm GA did not have any effect at various clipping heights.

TABLE I
INTERACTION EFFECT OF PRUNING HEIGHT AND PGR CONCENTRATION ON THE AVERAGE CHLOROPHYLL CONTENT INDEX (CCI) OF 3-MONTH MATURE LEAVES

Pruning height	PGR concentration			
	0 ppm	60 ppm BAP	50 ppm GA	60 ppm BAP + 50 ppm GA
40 cm	88.06b B	86.97a AB	86.32a AB	84.14a A
50cm	84.00a A	86.88a A	89.13a B	85.72a A
60 cm	87.75ab A	91.58b B	88.25a A	87.25a A

Note: The average number marked with the same letter (uppercase horizontal and lowercase vertical) is not significantly different according to Duncan's test of 5%.

According to Davies [21], cytokinin can play a role in leaf expansion which is caused by cell enlargement. The leaf area is adjusted to account for root growth, reflected by the cytokinin amount that reaches the shoot [22]. Cytokinin contributes to chloroplast development, where its application accumulates chlorophyll and increases the conversion rate of etioplasic to chloroplasts.

Chloroplasts provide energy for plants by producing sugar during photosynthesis. To adapt to various environmental and

developmental conditions, plants have developed specific strategies to control chloroplast homeostasis in cells, including chloroplast degradation during leaf senescence and the transition of chloroplast into other types of plastids during the day-night cycle [23]. Chloroplast contains chlorophyll, is the place of photosynthesis, and is also involved in the biosynthesis of many important primary and secondary metabolites [24]. Cytokinin prolongs antioxidant-based protection in chloroplasts, extending their lifespan [25]. The chlorophyll content in tea increases gradually as the leaves mature and significantly improves photosynthesis [26].

B. Fresh Shoot Weight

Shoot wet weight is a production parameter that is generally analyzed in studies related to tea to determine treatment effects on tea yield. Table II shows an interaction effect between pruning height and PGR concentration on the fresh weight of the tea shoot. The treatment of 60 cm pruning height with 50 ppm GA showed a significant effect on wet production weight and does not significantly differ from 40 cm pruning height and 50 ppm GA. This shows that the application of GA can increase tea shoot production per bush. Gibberellin intermediate is a precursor of GA that is not biologically active and is a product of biosynthesis in the active GA biosynthetic pathway. Efflux GA carriers, also known as transporters, can transport GA from the cytosol to apoplast (GA exporters). GA is transported through proteins across the plasma membrane from apoplast to cytosol (GA importer) [27].

TABLE II
INTERACTION EFFECT OF PRUNING HEIGHT AND PGR CONCENTRATION ON SHOOT FRESH WEIGHT PER BUSH (g)

Pruning height	PGR concentration			
	0 ppm	60 ppm BAP	50 ppm GA	60 ppm BAP + 50 ppm GA
40 cm	8.55a A	12.24b BC	15.22b C	9.62a AB
50 cm	13.15b B	13.47b B	13.79b B	12.43b B
60 cm	9.64ab AB	8.31a A	16.10b C	12.32a B

Note: The average number marked with the same letter (uppercase horizontal and lowercase vertical) is not significantly different according to Duncan's test of 5%

GA is applied to regulate the bud activity-dormancy transition in tea plants. Suppressing the growth of *banjhi* shoots indirectly stimulates *pecco* growth, increasing shoot wet weight [28]. According to Shu *et al.* [29], GA is one of the phytohormones that regulate biological processes crucial to plant development and has positively regulated plant shade and tea plant response [30]. Another research showed that the application of GA 100 mg. L⁻¹ increased the rate of net photosynthesis (Pn), chlorophyll content, and chlorophyll fluorescence parameters of the 1st and 6th leaves of *Camellia oleifera* [31].

Exogenous gibberellins begin the transport of photosynthesis from leaves to buds from the source sink relationship regulator. Photosynthates produced in maintained leaves have many uses inside the leaves and are also converted to sucrose in sinks. The process that allocates the amount of carbon is called photosynthate or carbon

partitioning. After arriving at the sink, photosynthates are partitioned again to be used as an energy source for growth or for food reserves in the form of carbohydrates, proteins, and oils [32].

The type of shoots produced determines tea yield; the younger the shoots are picked, the higher the quality. Shoot growth and contents that determine tea quality are influenced by plant conditions, soil fertility, season, age after pruning, and altitude. Rough plucking yields a high production with low shoot quality, while fine plucking yields a low production with high shoot quality [33]. In general, tea plantations utilize a medium plucking method. Therefore, the plucking method directly determines shoot quality (percentage of young shoots). According to Mitrowihardjo [34], quality correlates significantly with taste. Approximately 50-60% of tea leaf quality is influenced by post-process appearance, which is greatly influenced by the weight of existing *pecco*.

C. Dry Shoot Weight

The result of the analysis (Table III) showed that there was no significant difference in shoot dry weight during production plucking, presumably due to water absorption and limited nutrients in the dry season.

TABLE III
INDEPENDENT EFFECTS OF PRUNING HEIGHT AND PGR CONCENTRATION OF ON AVERAGE SHOOT DRY WEIGHT (g)

Pruning type	Pruning height	PGR concentration			
		0 ppm	60 ppm BAP	50 ppm GA	60 ppm BAP + 50 ppm GA
Clean	40 cm	26.84a	23.00a	25.33a	26.37a
Pruning	50 cm	26.65a	26.01a	26.49a	25.35a
	60 cm	21.93a	24.45a	27.17a	23.99a
lung pruning	40 cm	25.02a	25.61a	24.21a	24.21a
	50 cm	26.12a	25.18a	26.52a	24.20a
	60 cm	25.96a	26.76a	24.18a	26.98a

Note: The average number marked with the same letter is not significantly different according to Duncan's test of 5%

Limited water during low rainfall causes fertilizers to be insoluble and not absorbed by plant roots and decreases photosynthesis rates in maintenance leaves. Low soil moisture causes plants to lose more water than the amount absorbed by roots, which causes wilting leaves, closing stomata, and reduced carbohydrate production due to decreased photosynthesis. Carbohydrates are needed as energy to absorb nutrients so that a lack of water in plants results in reduced absorption of CO₂, water, and nutrients [35].

During observation, factors that influenced the absence of interaction between pruning method and height are rainfall, temperature, and humidity. The temperature ranged between 19.26-19.29°C, which is slightly above the ideal temperature for the growth of tea plants (13-15°C), and relative humidity ranged from 87.54 to 89.04% during the day, which is higher than the optimal humidity of 70%.

According to Loveless [35], a lack of water will cause protoplasm dehydration in leaves, which causes the stomata to eventually close and consequently inhibit the absorption of CO₂. The dry weight of cultivated plants is the accumulation of net results of CO₂ assimilation during growth, resulting

from solar energy absorption and caused by sunlight. The main factors affecting plant dry weight are solar radiation absorption and the energy use efficiency of CO₂ fixation.

Decreased plant growth can be caused by growth period decreases, low temperatures, limited groundwater, oxygen, limited root system nutrient supply, and limited root system activity. Groundwater plays a central role in these limiting factors, as it affects soil compaction for plant growth and biological functions related to water [36].

Plant growth periods are directly affected by climatic conditions such as changes in maximum and minimum daily temperatures and rainfall rates [37]. The reduction of water absorption decreases tissue water content which causes turgor loss. Likewise, drought stress also decreases assimilation and metabolites needed for cell division. This disrupts mitosis, cell extension, and ultimately plant growth [38].

During the plucking period, the weather is quite dry, which indirectly reduces the availability of groundwater. According to Marcelis *et al.* [37], a decrease in groundwater reduces the

water absorption of plants and disrupts physiological and metabolic processes, altering plant morphology and physiology. In the dry season, the temperature in tea plantations is quite high. The temperature range strongly influences the growth of tea plants. Tea yield is susceptible to an increase in average monthly temperature, and a sustained increase further reduces tea yield [39].

D. Catechin content

The results of catechin analysis on picking production are displayed in Table IV (Fig.2 and Fig. 3), showing an interaction effect between pruning methods, pruning height, and PGR concentration on tea catechin levels. Catechin was initially known to accumulate during defense responses, fighting abiotic and biotic stress and also insects. Catechin found in tea plants act as anti-microbial (bacteria and viruses), antioxidants, anti-radiation, blood vessel strengtheners, urine secretion facilitators, and cancer cell inhibitors [40,41].

TABLE IV
INTERACTION EFFECT OF PRUNING METHOD, PRUNING HEIGHT AND PGR CONCENTRATION ON CATECHIN CONTENT

Pruning type	Plant Growth Regulator Concentration											
	0 ppm			60 ppm BAP			50 ppm GA			60 ppm BAP + 50 ppm GA		
	Pruning height			Pruning height			Pruning height			Pruning height		
	40 cm	50 cm	60 cm	40 cm	50 cm	60 cm	40 cm	50 cm	60 cm	40 cm	50 cm	60 cm
Clean pruning	1.21b	1.58b	1.07a	1.24a	2.14b	0.93a	1.48b	1.61b	1.51b	1.34a	1.59a	0.83a
	<u>B/P</u>	<u>C/P</u>	<u>A/Q</u>	<u>B/PQ</u>	<u>C/Q</u>	<u>A/P</u>	<u>A/R</u>	<u>A/P</u>	<u>A/R</u>	<u>B/Q</u>	<u>C/P</u>	<u>A/P</u>
Lung pruning	0.92a	0.80a	1.39b	1.51b	1.05a	1.40b	1.22a	0.81a	0.86a	1.44a	1.46a	2.23b
	<u>A/P</u>	<u>A/P</u>	<u>B/Q</u>	<u>B/R</u>	<u>A/Q</u>	<u>B/Q</u>	<u>B/Q</u>	<u>A/P</u>	<u>A/P</u>	<u>A/R</u>	<u>A/R</u>	<u>B/R</u>

Note : Figures followed by the same letter notation are not significantly different based on the Duncan test at 5% significance level; lowercase (a, b, c) are read vertically according to each column of pruning height which is a comparison between the stages of pruning type at each high level of pruning and concentration of PGR; Uppercase letters (A, B, C) are read horizontally according to each row type of pruning in each column of PGR concentration which is a comparison between high levels of pruning at each stage of pruning type and PGR concentration; underlined uppercase letters (P, Q, R) are read horizontally according to each row type of pruning across the PGR concentration column is a comparison between the levels of PGR concentration at each level (pruning type : pruning height)

The results showed that the treatment of clean pruning with 60 ppm BAP at a pruning height of 60 cm provided the highest catechin content with a value of 2.14%. In the pruned plot, the highest catechin content was obtained from the treatment of 60 cm pruning height and PGR concentrations of 60 ppm BAP + 50 ppm GA with a value of 2.23%. Several research results show that the biosynthesis of secondary metabolites depends on various environmental cues (light, temperature, CO₂, dryness, salinity, ozone, UV radiation) and endogenous signals (hormones and signaling molecules). Even if other factors remain constant, changes to only one factor can substantially change the concentration of endogenous secondary metabolites [42].

When depicted in the response surface graph, the catechin content of plants that underwent clean pruning shows a regression equation: $Z = (-6.7122) + (0.328)x + (0.00398)y + (-0.00329)x^2 + (-0.00009)y^2 + (0.00015)xy$ where Z = catechin levels (%); x = pruning height (cm); y = PGR concentration (ppm).

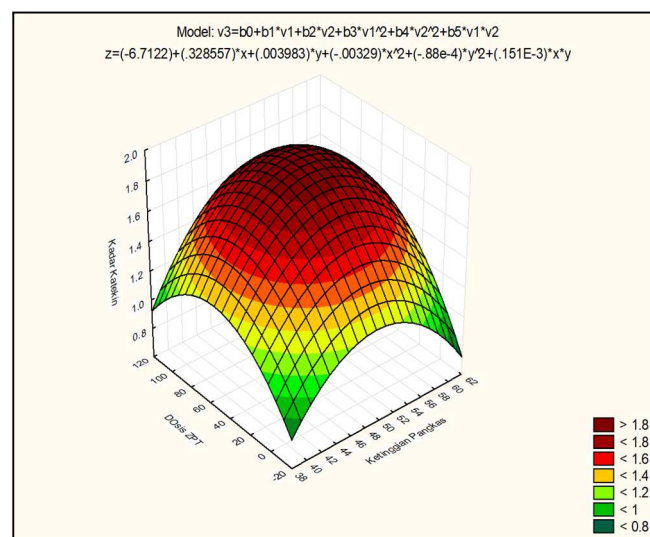


Fig. 2 The graphic surface response of pruning height and concentration of growth regulators on clean pruning to tea catechin content

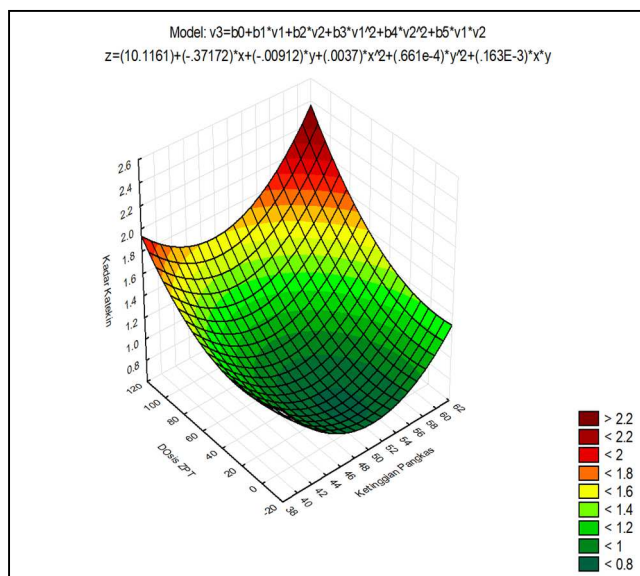


Fig. 3 The graphic of the surface response of pruning height and concentration of growth regulators on lung pruning to tea catechin content

Based on the surface graph response of clean pruning optimal value is obtained at 51.5 cm pruning height and 66.63 ppm BAP which means that both factors (pruning height and BAP concentration) contribute greatly in increasing catechin levels to 1.88%. Furthermore, based on the surface graph response of lung pruning (Figure 3) with the equation: $Z = (10,116) + (-0,371) * x + (-0,091) * y + (0,0037) * x^2 + (0,00007) * y^2 + (0,00016) * x * y$ where Z = catechin levels (%); x = trimming height (cm); y = PGR concentration (ppm), the minimum catechin level was obtained at 50.73 cm pruning height and 7.238 ppm PGR with a value of 0.776%. This means that both factors (pruning height and plant growth regulator concentration) contribute to the reduction of catechin levels to 0.776%.

In young (immature) leaves, a relatively large proportion of assimilated carbon is added to flavanols (catechins) and compounds to be used in situ (amino acids, organic acids). In mature leaves, most of the assimilated carbon is placed into a substance that is easily moved, such as sugar. Therefore, mature leaves in shrubs maintain immature plant parts by supplying young leaves with carbon compounds.

Based on a response surface chart, clean pruning at 51.5 cm greatly contributes to increasing tea catechins' levels. This is in line with the results of Johan's research which shows that a pruning height of 50 cm in tea seeds can increase the growth of shoots. Nutrient status and other environmental factors affect plant metabolism and growth, influence the synthesis and distribution of growth substance, and over time, hormones can direct the translocation and accumulation of plant nutrients [43].

The results show that growth environment and harvest time significantly impacts carbohydrate partitioning and bush tea quality [44]. Fresh leaves plucked in spring produced the strongest theaflavins (catechin component). In tea leaves, high contents of certain individual catechins lead to a high yield of theaflavin [45]. According to Samantha et al, a metabolic balance between chlorophyll and polyphenols in tea leaves is reached at different stages [46]. In intact shrubs, carbon assimilated by mature leaves support the flush and

roots. De Costa *et al.* states that photosynthates produced in immature leaves do not move. However, older leaves do not parasitize other leaves when they become unproductive [47].

The percentage of catechins in the GMB 7 clone shows that the results of Sriyadi's research is p + 2 dry, which is around 0.0001% [48]. GMB 7 is a superior variety of tea with high productivity, possess antioxidant potential, and can grow well in low, medium, and high altitudes [49]. Although processing technologies have an impact on the quality of tea, according to Zhang *et al.* [50], geographical location and other environmental factors also have significant effects. The constituents of polyphenolic compounds, especially individual catechins, depend on cultivation altitude; higher altitude equals a higher catechin count.

Furthermore, according to Han *et al.*, temperatures have seen an increase due to climate change which means that temperatures are also increasing at low altitudes and will decrease tea quality. Other negative impacts of global warming on the production and quality of tea, especially regarding rising temperatures include unpredictable rainfall trends and increased frequency of extreme weather events such as drought and frost [51]. In most plants, physiological and biochemical responses to combat the effects of environmental stress include lowering cellular growth and net photosynthesis rates, stomatal closure, and the accumulation of organic solutes [52].

IV. CONCLUSION

Interaction between 60 cm pruning height and 60 ppm BAP increase chlorophyll content index (91.581 cci) in 3rd month, meanwhile interaction of 50 ppm GA at the same level pruning height influence shoot fresh weight per bush at on the 4th plucking. Based on the response curve analysis, clean pruning type with optimum pruning height at 51.5 cm and 66.63 ppm BAP contribute to highest catechin content (1.88%). This research concludes that the application of 60 ppm BAP on 60 cm clean pruning height at the 3rd month and follow with 50 ppm GA at the 4th plucking, recommended to increase vegetative growth and catechin content of tea shoot after pruning.

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