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Research Article
Effect of Pruning and Cytokinin Application on the Growth of Tea GMB 7 Clone

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Abstract

Background and Objectives: The use of growth regulators in pruned tea plantation in Indonesia is still very rare. One method that can be applied to increase growth is through the application of cytokinin such as Benzil amino purine (BAP). The objective of this study was to determine whether the combination of pruning and application of cytokinin BAP can increase the growth of tea. **Materials and Methods:** This experiment was conducted in Tea and Chinchona Research Centre, Gambung, west Java with an altitude of 1,250 masl. Randomized block design (RBD) was used with pruned height treatment, pruning type and cytokinin dosage combined into 8 treatments. Materials were used 7 year old GMB 7 clone and BAP. All parameters were tested by analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at the 5% significance level. **Results:** The results showed that type and height of pruning and application of cytokinin BAP affect the growth of pruned tea plants. The combination of clean pruning treatment, a pruning height of 60 cm and cytokinin usage in 60 mL produced optimal results for increased number of buds and active shoot (pecco) in pruned tea plants. **Conclusion:** The application of BAP can improve plant growth and development. This indicates that the provision of growth regulators has the potential to be used to accelerate the growth of tea plants after being pruned.

Key words: Active shoot (pecco), Benzil amino purine (BAP), clean pruning, cytokinin, GMB 7 clone

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesian tea production in 2017 experienced a decline, one of reason being unoptimal agronomic handling in the field which causes decreased production and shoot quality[1]. A number of agronomic techniques are needed to help improve tea growth and development. One of the tea clones in Indonesia with high yield potential and potential to be developed is GMB 7 clone. Slow shoot growth in tea after being pruned is one of the reasons to study the use of growth regulators. Pruning method and application of plant regulators also improve several agronomic traits in the plant one of them that tree species having light canopy is suitable since they allow easy penetration of sunlight, while tree of dense canopy required heavy pruning[2].

The mechanism of the pruned plant response is important for the recovery and sustainability of tea as the perennial crop. Several efforts have been made to maximize the production of shoots through pruning and plant growth regulators. Pruning can initiate shoot growth to a greater extent as well as widen the field of plucking, while application of cytokininis is intended to further stimulate shoot growth after pruning through cell division activity and cell extension, help overcome shoot dormancy, supporting photosynthesis activity the formation of chlorophyll and leaf expansion[3]. Kumar *et al*.[4] research showed that pruning and skiffing can increase the growth and productivity of Darjeeling tea. According to Sato and Mori[5], exogenous hormones like cytokinin have an important role in regulating axillary bud growth. Cytokinins applied in 0.5-1.5 mg L^{-1} doses *in vitro* resulted in multiplication of shoots[6] from 3.5-11. The results of the study showed that the use of 30 ppm kinetin can increase the number of buds that appear per sample, leaf area and chlorophyll content[7]. Cortleven and Valcke State in Autio and Day[8] stated that exogenously applied cytokinin stimulates the cell of elongation zone in plants. Cytokinins have implicated in diverse essential processes of plant growth and development as well as in regulation of key genes responsible for the metabolism and activities of plants[9]. Cytokinin are known to play essential roles in maintaining the size and activity of the shoot apical meristem (SAM) and root apical meristem (RAM)[10].

Consequently through the pruning, it is expected that the production of shoots will decrease. This condition will improve through the provision of growth regulators like cytokinin. Scientific research is expected to be useful in contributing to the development of plant crop science, especially on selecting the type of pruning and the use of cytokinin in tea plants to increase the growth so as to accelerate the growth of shoots in tropical plantation. The objective of this study is to determine whether the combination of different types and height of pruning and application of cytokinin BAP can increase the growth of tea. Through applications in the field, the results of this study can contribute to the improvement of the growth, yield and quality of tea.

MATERIALS AND METHODS

Place and time of experiment: The experiment was conducted at B5 experimental plantation of the Gambung Tea and Quinine Research Centre with an altitude of 1,250 m above sea level, Andisol soil ordo and B type precipitation. The experiment was carried out from September, 2017-February, 2018.

Plant preparation: The plant material to be used in this research is GMB 7 clone (7 years old), 320 plants (one plot of 10 tea bush) with a spacing of 75 cm×75 cm×120 cm, cytokinin (6-Benzylaminopurine), alcohol 96%, water, urea fertilizer (N) 30 kg, KCl (K_2O) 5 kg, TSP (P_2O_5) 5 kg, (Dose per plant 32 g) round up 1 mL L^{-1}.

Experimental design: Randomized block design (RBD) was used and it consisted of 8 combinations of pruned type treatments (cleaned pruning and lung pruning), pruning height (40 and 60 cm) and doses of cytokinin (0 and 60 mL) repeated 4 times which contained 32 treatment units. The treatments are: (A) Clean pruning+high pruning 40 cm+BAP 0 mL, (B) Clean pruning+high pruning 40 cm+BAP 60 mL, (C) Clean pruning+high pruning 60 cm+BAP 0 mL, (D) Clean pruning+high pruning 60 cm+BAP 60 mL, (E) Lung pruning+high pruning 40 cm+BAP 0 mL, (F) Lung pruning+high pruning 40 cm+BAP 60 mL, (G) Lung pruning+high pruning 60 cm+BAP 0 mL and (H) Lung pruning+high pruning 60 cm +BAP 60 mL.

Analysis of endogenous cytokinin levels in tea leaves: Cytokinin was analyzed using TLC (Thin Layer Chromatography) method scanner using Tie-Xin and Hong[11] method in Balittro Laboratory Bogor, west Java, Indonesia.

Analysis of starch content in roots before treatment: The root samples were analyzed in a Laboratory in the Faculty of Food Science Technology, Padjadjaran University. The tests conducted using SNI-01-2891-1992 method[12].

Soil analysis before experiment: The soil samples were analyzed in Soil Fertility Laboratory and Plant Nutrition Department of Soil Science and Agricultural Resources, Faculty of Agriculture Padjadjaran University.

Plant growth analysis

Number of bud: Observation was conducted for 2 months with an interval of 7 days.

Height of bud (cm): Observation was conducted for 2 months with an interval of 7 days by marking from the base until the point of growth.

Number of pecco (active shoots): Observation was conducted for 2 months with the interval of 7 days by observing the number of leaves that are perfectly open on the buds that grow well on branches of plants.

Statistical analysis: One ways ANOVA was conducted to observe data from growth variables including: Number of bud, number of pecco and height of shoot. The influence factor to the growth of tea plant were analyzed on 5% significance and subsequently followed by Duncan's multiple range test (DMRT) at 5% significance[13].

RESULTS

Number of buds: Statistical analysis results showed in Table 1 that the application of pruning and cytokinin significantly affected the average number of shoots at week 2-8.

This condition is possible because the plant is still in the stage of recovering after pruning. In the 2nd-8th week of observation, treatment D (clean pruning+height of pruning 60 cm+cytokinin 60 mL) statistically shows the highest number of shoots compared to other treatments.

Height of buds: The results of statistical analysis (Table 2) showed there is no significant effect of pruning and cytokinin application on the average height of buds per week. The growth of new shoots is visible and can be measured at 2 months after pruning. In this parameter, cytokinin was not able to trigger high growth of shoots for 8 weeks of observation. It seems that the high growth of shoots in this case is not only controlled by the application of cytokinin, but is also affected by interactions between hormones, nutrients and environmental conditions. Biomass lost due to pruning can also cause plants to become stressed so that in the early stages of growth it does not show significant differences in shoot growth after being pruned.

Table 1: Effect of pruning and cytokinin on average number of bud per week

	Average number of bud per week							
Treatments	1	2	3	4	5	6	7	8
A	32.17^{a}	35.17^{b}	37.42^{abc}	42.25^{abc}	42.36^{bc}	47.67^{ab}	47.88^{b}	47.95^{bc}
B	26.83^{a}	38.00^{b}	38.42^{abc}	40.50^{abc}	42.25^{bc}	42.58^{b}	45.67^{b}	49.50^{bc}
C	31.42^{a}	44.17^{ab}	45.67^{abc}	46.42^{abc}	47.75^{bc}	50.83^{ab}	50.17^{b}	50.67^{ab}
D	40.33^{a}	63.67^{a}	65.58^{a}	67.08^{a}	68.42^{a}	69.33^{a}	72.58^{a}	73.83^{a}
E	28.58^{a}	32.25^{b}	33.50^{bc}	33.58^{c}	36.11^{bc}	37.38^{b}	41.92^{b}	47.29^{bc}
F	24.83^{a}	34.75^{b}	34.80^{bc}	37.08^{bc}	38.97^{bc}	39.67^{b}	41.75^{b}	43.83^{c}
G	27.42^{a}	46.92^{ab}	48.58^{abc}	52.83^{ab}	52.94^{b}	53.75^{ab}	55.58^{b}	59.00^{ab}
H	37.25^{a}	46.83^{ab}	48.08^{abc}	48.17^{abc}	52.28^{b}	55.00^{ab}	55.50^{b}	58.42^{ab}

Values in the same column followed by different letters show significant differences at 5% DMRT

Table 2: Effect of pruning and cytokinin on height of bud per week

	Height of bud per week							
Treatments	1	2	3	4	5	6	7	8
A	2.41^{a}	2.41^{a}	10.35^{a}	15.71^{a}	21.15^{a}	27.13^{a}	32.88^{a}	38.63^{a}
B	3.54^{a}	2.73^{a}	12.29^{a}	18.04^{a}	23.65^{a}	28.29^{a}	32.42^{a}	38.71^{a}
C	2.92^{a}	2.22^{a}	7.67^{a}	11.71^{a}	16.73^{a}	22.29^{a}	28.17^{a}	34.42^{a}
D	2.88^{a}	2.25^{a}	9.77^{a}	15.58^{a}	22.14^{a}	27.58^{a}	32.96^{a}	38.38^{a}
E	1.73^{a}	1.89^{a}	7.54^{a}	10.04^{a}	12.23^{a}	14.04^{a}	15.54^{a}	19.96^{a}
F	2.54^{a}	2.25^{a}	8.11^{a}	12.54^{a}	17.58^{a}	21.75^{a}	26.96^{a}	32.75^{a}
G	2.96^{a}	2.45^{a}	11.04^{a}	16.13^{a}	20.75^{a}	26.73^{a}	31.75^{a}	36.17^{a}
H	2.2^{a}	1.94^{a}	6.21^{a}	9.00^{a}	12.55^{a}	15.96^{a}	17.72^{a}	24.04^{a}

Values in the same column followed by different letters show significant differences at 5% DMRT

Table 3: Effect of pruning and cytokinin on average number of pecco shoots

	Average number of shoots per week							
Treatments	1	2	3	4	5	6	7	8
A	1.17^{a}	0.73^{a}	15.71^{a}	17.25^{a}	26.75^{ab}	23.92^{ab}	27.92^{ab}	28.75^{ab}
B	0.67^{a}	0.57^{a}	20.25^{a}	19.67^{a}	24.50^{ab}	23.33^{ab}	23.33^{abc}	23.38^{abc}
C	0.75^{a}	0.43^{a}	19.71^{a}	22.54^{a}	28.50^{ab}	29.92^{ab}	28.17^{ab}	28.50^{ab}
D	2.00^{a}	0.71^{a}	22.75^{a}	29.00^{a}	31.08^{a}	36.92^{a}	37.33^{a}	37.83^{a}
E	0.50^{a}	0.50^{a}	10.29^{a}	8.38^{c}	11.83^{c}	10.88^{c}	13.33^{c}	14.04^{c}
F	0.25^{a}	0.25^{a}	13.58^{a}	15.67^{a}	18.17^{ab}	18.25^{bc}	19.75^{bc}	21.17^{abc}
G	2.08^{a}	1.35^{a}	22.92^{a}	23.17^{a}	24.42^{ab}	26.58^{ab}	27.67^{ab}	27.88^{ab}
H	1.50^{a}	1.54^{a}	18.17^{a}	22.54^{a}	29.58^{ab}	29.54^{ab}	30.04^{bc}	31.21^{ab}

Values in the same column followed by different letters show significant differences at 5% DMRT

Table 4: Root starch content of GMB 7 tea plant before pruning (%)

	Root starch content (%)		
Type of sample	August	September	Increase (%)
1	9.16	10.21	11.46
2	5.82	9.64	65.63
3	8.98	9.25	3.20
4	6.99	9.61	3.48
Average	7.7375	9.6775	

Table 5: Endogenous cytokinins level in tea leaves GMB 7 clone before pruning

Type of sample	Cytokinin endogen content (%)
1	0.0016
2	0.0017
3	0.0018
4	0.0019
Average	0.0018

Number of pecco shoots: The results of statistical analysis showed that the number of pecco shoots was significantly affected by pruning and cytokinin application in the 5th until the 8th week of observation (Table 3).

The D treatment (Clean pruning+high pruning 60 cm +cytokinin 60 mL) statistically at week 4, 5, 6, 7 and 8 after pruning tended to significantly increase the amount of pecco. In general, treatment E (Lung pruning+high pruning 40 cm +cytokinin 0 mL) showed a lower number of pecco than other treatments at week (4-8).

Root starch content, endogenous cytokinin and soil analysis before experiment: Observation of root starch contents before pruning is needed to determine the contents of the tea before being pruned. Starch level is useful as a food reserve as long as the tea plant is recovered after being pruned for tea plants that experience clean pruning.

The results of the initial analysis of the average root starch content can be seen in Table 4 which showed that the average condition of root starch in each replication is below 12%. This condition can be caused by several things, namely plant genetics and root starch levels which are dismantled for shoot growth. It can be seen in Table 4 that the results of the analysis of root starch content in August and September increased after the plants were rested for one month. It can be seen that the percentage of root starch in the four samples above experienced varied increase in September, ranging from 3.2-65.63%.

The observation of endogenous cytokinin was carried out to determine the initial data on the status of endogenous cytokinin in tea leaves. This may be needed to be further researched to determine the exact concentration of cytokines that can be given to the tea plant that will be pruned to trigger new shoots faster. The results of the initial analysis can be seen in Table 5 which showed that the levels of cytokinin were in the range of 0.0016-0.0019%.

Soil analysis pre-treatment was carried out to determine the nutrient status at the experimental site. This is needed to determine nutrient requirements to ensure the plant does not experience a deficiency during the recovery process.

The results showed that before the experiment, Andisol Gambung soil has a pH of 6.22 (rather sour), 1.73% organic C (low), C /N ratio 7 (low), P_2O_5 HCl 25% 285.58 mg/100 g (very high), soil fertility in various tea plantations varies according to soil type (potential fertility) and nutrient content which are available in the soil (Table 6). Andisol soil is most suitable for the growth of tea plants, but because it is continuously cultivated, for example continuous fertilization and continuous harvesting, the ideal conditions can change.

The results of soil analysis of Andisol Gambung can be seen in Table 6.

The soil pH of 6.22 is still classified as a suitable pH for tea plant growth. Cation exchange capacity was observed at 38.83 cmol kg^{-1}. Cation exchange capacity is very close to soil fertility, where land with high CEC is better in providing nutrients compared to land with low CEC.

Table 6: Chemical and physical soil properties of andisols soil of gambung

Parameters	Results	Criteria
pH:H_2O	6.22	Sour
pH:KCl 1 N	4.86	-
C-organic (%)	1.73	Low
N total (%)	0.25	Medium
C/N	7.00	Low
P_2O_5 HCl 25% (mg/100 g)	285.58	Very high
P_2O_5 bray (ppm P)	29.43	Very high
K_2O HCl 25% (mg/100 g)	20.01	Low
Cation		
K-dd (cmol kg^{-1})	0.39	Medium
Na-dd (cmol kg^{-1})	0.27	Low
Ca-dd (cmol kg^{-1})	4.56	Low
Mg-dd (cmol kg^{-1})	0.69	Low
CEC (cmol kg^{-1})	38.83	High
Base saturation (%)	15.22	Very low
Al-dd (cmol kg^{-1})	0.05	-
H-dd (cmol kg^{-1})	0.00	-
Al saturation (%)	0.84	Very low
Texture (%)		
Sand	26.00	Dusty clay
Dust	62.00	
Clay	12.00	

CEC: Cation exchange capacity

DISCUSSION

The combination of clean pruning, 60 cm pruning height with 60 mL cytokinin significantly affected the number of buds, but not its height. A clean pruning with a light pruning height (60 cm) allowed the tea plants to still exhibit rapid shoot growth because it is possible that the starch content in the roots is still sufficient for new shoot growth. The existence of the lung pruning that leaves 2 twigs of leaves as a means of photosynthesis does not guarantee the growth of tea after being pruned. This is also due to the low pruning height (40 cm) which caused the growth of the plant to be slower than the tea plant that is pruned at a height of 60 cm.

Pruning height also affected the number of shoots produced. Lower heights (40 cm) caused bud breaking to take longer, which produces an inversely proportional number of shoots at 60 cm pruning height. According to Hossain[14] bud breaking takes longer in brown wood present in the lower part of the thickened sticks. Research conducted by Saifuddin *et al.*[15] showed that different pruning methods can modify the roots and initiate the appearance of buds, as well as affect the physiological and biochemical properties of plants. According to Ashraf and Ashraf[16], pruning can lead to minimal or depressed vegetative growth and reduced supply of photosynthate for growth during the recovery period.

The physiological balance between nutrients, starches and hormones appeared to greatly affect the rate of growth of buds. Some environmental factors will also greatly affect the rate of growth of shoots, including rainfall, light intensity and humidity. No significant differences in pruning and cytokinin addition to height of buds parameters can be attributed to several factors such as genetic factors and plant metabolism undergoing changes during recovery after pruning.

The plant growth hormone, cytokinin, is known to play essential roles in maintaining the size and activity of the SAM (shoot apical meristem) and RAM (root apical meristem)[17]. Clean pruning treatments do not always adversely affect plant growth. The results of this study showed that clean pruning treatment with a height of 60 cm pruning and 60 mL cytokinin concentration can increase the growth of the number of shoots and pecco in tea plants. It relied heavily on plant health and the starch content in the roots. According to Zeing[18] plants have a functional equilibrium between their above ground parts (leaves) and below ground parts (roots). If plants are pruned, the starch reserves in the roots are utilized for shoot growth to maintain the equilibrium. The recovery speed from pruning a tea bush depends on the plant's starch reserves in the roots[19]. Endogenous levels of cytokinin are strongly influenced by metabolic processes that take place in plants. Nutrient status and other environmental factors affect metabolism and plant growth, affecting the synthesis and distribution of growth substances.

Climate factors during growth greatly affect the physiological response of plants, in addition to genetic factors, cultivation techniques and soil nutrient content[20]. According to Effendi *et al.*[21], the ideal rainfall for tea plant growth of 2000 mm/year or 60 mm/month is not more than 2 months, a daily air temperature ranging from 13-15°C and relative humidity during the day[21] above 70%. It can be seen that in August the precipitation is quite low and the average temperature is above the recommended temperature for tea, yet the humidity is relatively optimal.

Referring to soil as a medium for growing tea, soil texture is categorized as dusty clay with a composition of 26% sand, 62% dust and 12% clay, which is close to ideal conditions for plant growth[22]. A low C to N ratio indicates that the soil has enough nutrients and is ready to be used by plants because the process of short composition of materials in the soil has occurred[23].

Type and height of pruning combined with BAP affect the growth of pruned tea plants. Consequently, the number of bud and active shoot (pecco) in pruned tea plants can be increased. The implications of the increasing number of bud increased of active shoot (pecco) as target organ in tea plants which in turn ultimately increases the productivity of tea plants. Here it appears that the role of cytokinin in bud outgrowth or initiating shoots is quite large. However, the role of nutrition and environment contributed to trigger the bud outgrowth[24].

CONCLUSION

The application of cytokinin can improve plant growth and development of tea plant. This indicates that the provision of cytokinin plant growth regulators has the potential to be used to accelerate the growth of tea plants after being pruned.

SIGNIFICANT STATEMENT

This study aims to discover the potential use of cytokinin BAP which, when combined with pruning types and pruning height can be beneficial to accelerate the growth of tea plants after being pruned. This study will help uncover the critical areas of tea growth after being pruned that many researchers were unable to explore. A new theory arises , that after pruned, the tea plant growth can be increased through the use of cytokinin BAP with the appropriate type and height of pruning.

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