ISSN: 1412-4718, eISSN: 2581-138x

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The effect of topping and various cytokinin-based plant growth regulators applications on immature Liberica coffee growth

Abstract. The growth of Liberica coffee in the 2-year immature plant phase can be improved by applying cytokinin-based plant growth regulators (PGRs) and topping. Toppings applied to plants can inhibit apical dominance, while cytokinin PGRs such as 6-Benzylaminopurine (BAP) and coconut water can stimulate plant shoot growth. This research aims to determine the effect of topping and PGR application on the vegetative growth of Liberica coffee plants. This experiment was carried out at the Ciparanje experimental field, Universitas Padjadjaran, West Java, with an elevation of ± 750 meters above sea level from March to June 2024. The experimental design used a randomized block design with six treatments: control (no topping and no added PGR), topping, topping + 60 ppm BAP, topping + 50% coconut water, 60 ppm BAP, and 50% coconut water. Each treatment was repeated four times. The results showed the best results were (a) topping treatments (topping, topping+coconut water, and topping+BAP) on canopy width and primary branch length; and (b) a combination of 50% coconut water and 60 ppm BAP on increasing the number of branches. This study provides information on efficient maintenance methods for Liberica coffee plant growth in the immature phase.

Keywords: Benzyl Amino Purine · Coconut water · Cytokinin · Vegetative growth

Submitted: 21 December 2024, Accepted: 28 April 2025, Published: 30 April 2025
DOI: https://doi.org/10.24198/kultivasi.v24i1.62677

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Jurnal Kultivasi Vol. 24 (1) April 2025
ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Coffee is one of the important plantation commodities in Indonesia. Indonesia was ranked the fourth largest coffee producer (Tampubolon et al., 2023). Several coffee species are well-known and produced in Indonesia: Arabica (*Coffea arabica* L.), Robusta (*Coffea canephora)*, and Liberica (*Coffea liberica* L.). Liberica coffee is cultivated on a small scale compared to Arabica and Robusta in the case of Indonesia (Davis et al., 2022; Maxiselly et al., 2023a). It is caused by the Liberica's taste being more bitter than other species, so the consumer has more specific preference (Maxiselly et.al., 2023b). However, Liberica coffee has the potential for resistance to leaf rust disease and to thrive under drought stress (Wibowo et al., 2021; Wahibah et al., 2023).

The Indonesian Bureau of Statistics data from 2021 to 2023 showed a declining trend in national coffee production. In 2023, there was a decrease in coffee production by 27.46 thousand tons compared to the total production in 2021 of 786.19 thousand tons (BPS, 2024). Therefore, maintenance practices need to pursue increasing coffee production.

One crucial on-farm maintenance in coffee production is pruning during the immature phase. Pruning is one of the cultivation methods that contributes to increasing plant production and is one way to harvest plants effectively (NRCS, 2009). Pruning stimulates lateral shoot growth, inhibits apical dominance, and forms an optimal canopy on the coffee plantation (Li et al., 2021; Kawabata et al., 2022). One type of pruning is a topping. Topping is cutting part of the top of primary branches or shoots at a certain height (Hamdani et al., 2021).

Vegetative growth after pruning typically occurs without the need for plant growth regulators (PGRs). However, PGRs may be required to enhance vegetative growth following pruning treatments. PGRs are organic compounds that regulate physiology in small amounts. PGRs include auxin, gibberellin, cytokinin, ethylene, and abscisic acid (ABA) (Fardha, 2024). In this study, cytokinin-based PGRs were used, as this compound promotes lateral shoot growth and stimulates flushing in plants (Purcell, 2017; Sosnowski et al., 2023).

Cytokinin plays a role in cell division that stimulates shoot growth. Cytokinin can be obtained from nature, such as coconut water. The chemical compounds in coconut water include purine, glucose, amino acid, nucleic acid, alcohol, vitamins, minerals, and PGR (Yong et al., 2009; Rosniawaty et al., 2018). Besides cytokinin, the coconut water contained various PGRs, including auxin, ABA, and gibberellin (Emilda, 2020). In addition, synthetic cytokinin can be found in 6-Benzylaminopurine (BAP). BAP is a regulator, stimulant, and inhibitor of plant growth and development (Ramayana et al., 2022).

Coconut water and BAP function by stimulating shoot formation and vegetative growth at certain concentrations. Based on previous research, 50% coconut water has been proven to increase shoot length during the seedling phase in the Robusta coffee plant (Arpansori & Febrialdi, 2020). Furthermore, applying 50% coconut water in the immature phase of tea plants gave an increase in stem diameter, shoot length, number of leaves, and number of shoots (Rosniawaty et al., 2018). Meanwhile, applying BAP 60 ppm has improved shoot growth in tea plants (Ayuningsari et al., 2017; Rosniawaty et al., 2018). Maxiselly et al. (2021) reported that applying 30, 60, and 90 ppm BAP to the cinchona plant significantly responded to increased branch length, number of leaves, and stem diameter.

Several prior studies have reported the combination of pruning treatment and application of PGR. Anjarsari et al. (2021) used pruning techniques with cytokinin and gibberellin on tea plants by applying PGRs after the pruning treatment. De-topping pruning, accompanied by applying 500 ppm and 100 ppm GA, can increase the number of shoots on papaya plants on the 15th and 45th days after treatment (Das et al., 2018). The vegetative growth resulting from PGRs treatment should be compared with pruning treatments, including a combination of both, as no recent study as assessed these treatments on Liberica coffee plants during the immature phase. Based on the explanation above, topping and cytokinin-based PGRs can be combined to increase growth during the 2-year immature plant phase (IPP-2) of Liberica coffee. This study focuses on identifying the impact of topping and/or cytokinin growth regulators on the growth of IPP-2 Liberica coffee. The expected outcome is the discovery of an efficient and applicable method to improve the coffee growth performance in the immature phase.

Materials and Methods

This research was conducted from March to June 2024 at the Ciparanje experimental field, Universitas Padjadjaran, West Java, with an elevation of ± 750 meters above sea level. The materials were a 2-year-old Liberica coffee var Meranti 1 (LIM 1), BAP as synthetic PGR, coconut water, and water. The experiment was arranged in a randomized block design (RBD) with six treatment combinations. Each treatment was replicated four times, including control (no topping and no added PGR), topping (T), topping + 50% coconut water (T+CW), topping + 60 ppm BAP (T+BAP), no topping + 50% coconut water (CW), and no topping + 60 ppm BAP (BAP).

Liberica was planted 2 x 2.5 m. Topping was conducted on the plant that had reached a height of 130 cm or more. The technique was to cut the primary branches to a height of 130 cm using pruning shears. PGR formulation was carried out using the procedure: Coconut water was collected at a concentration of 50% (50% of coconut water: 50% of water). Meanwhile, the BAP concentration of 60 ppm is made by mixing 6 ml of BAP into 1000 ml of water. First, the dose to be sprayed is calibrated one day before spraying PGR. BAP and coconut water are applied by spraying them all over the plant canopy using a hand sprayer. The application is performed 0 weeks after treatment (WAT), 2 WAT, 4 WAT, 6 WAT, 8 WAT, and 10 WAT. The time for applied growth regulators is around 8.00-10.00 a.m.

The observed variables included the increase in canopy width (cm), number of branches, stem diameter (mm), branch length (cm), and chlorophyll content index (CCI) (units). The canopy-wide measurement measures the length between the outer branches in opposite directions. The number of branches counted are primary branches. The stem diameter was measured using a caliper at a height of 3 cm from the ground surface. The branch length measured was one primary branch on each plant. A selected primary branch is below the third branch from the top and then marked using a label. The chlorophyll content was measured using a digital chlorophyll meter. The way to use the tool is that the tool was clamped on the leaf of the sample plant until a number appeared on the monitor, and the result is expressed in units of chlorophyll content index (CCI). All observations are identified every 2 weeks from 2 to 12 WAT. The measured observations are then subtracted from the parameter appearance at 0 WAT to obtain the value of the parameter increase.

Data were analyzed using the analysis of variance (ANOVA) with the F test at the 5% level. A Duncan multiple range test (DMRT) was performed at 95% confidence when the variable data had significant differences between treatments.

Results and Discussion

Coefficient variation of observed variables. The data in Table 1-3 and Figure 1-2 showed the result of the observed variables. The observed variables were canopy width, number of primary branches, branch lengths, stem diameter, and chlorophyll content. All of that shows their increasing value. The observed data shows that accuracy starts from CV below 20%. However, various data have found that CV values are more than 20%. There are: increasing canopy wide at 2 weeks after treatments (WAT) and 4 WAT (Table 1); all observed data in Table 2 have CV more than 20%; data of 2, 4, and 6 WAT on increasing branch length (Table 3); and chlorophyll content at 2 WAT and 6 WAT (Figure 2). A CV value of more than 20% indicates high criteria. These data were influenced by external factors, including environmental factors (Utami et al., 2023). Maxiselly et al. (2021) assessed variables in immature coffee plants with a CV of more than 20%, indicating the accuracy of their data.

Increase in canopy-wide. Table 1 shows that the topping and topping with BAP treatments significantly differed from PGRs, with only a group of PGR treatments, such as coconut water and BAP treatments at 6 WAT and 8 WAT. The best treatment to increase the canopy width in those weeks was topping treatments. Although it was not significantly different from the control, it was topped with coconut water and BAP. In 12 WAT, topping and topping with BAP treatments had significant differences from the control, coconut water, and BAP treatments. The best method to increase the canopy width that week is topping with BAP, topping, and topping with coconut water.

The plant is generally composed of two metabolite groups, i.e., primary metabolites and

secondary metabolites. Primary metabolites are directly involved in developing plant growth, while secondary metabolites indirectly affect plant growth (Salam et al., 2023). Primary metabolites decode the genetic code that produces amino acids, proteins, and carbohydrates (Julianto, 2019). This can regulate the distribution of primary metabolites by pruning to focus on the desired targeted organ. Prior research by Kumar et al. (2017) stated that the growth of the canopy was affected by pruning due to a shift in plant metabolite allocation to support vegetative growth. Topping pruning can stop apical dominance, which inhibits vertical growth (Obasi & Msaakpa, 2005).

In addition, BAP after topping treatment has a greater increase in canopy width than topping treatment only at 10 and 12 WAT. Although statistically, there is no significant difference between them. Cytokinin treatment after topping can increase the number of lateral buds. The addition of exogenous cytokinins increased the amount of cytokinins in the canopy (Di Benedetto et al., 2015). Anjarsari et al. (2021) reported that the increasing number of lateral buds makes the canopy structure wider on the tea plants.

Increase in the number of primary branches. Table 2 shows that the BAP treatment has better results than other treatments. Meanwhile, topping with BAP had the lowest result in increasing the number of primary branches. Statistically, there was a significant difference between the coconut water and BAP treatments for topping and topping with BAP treatments at 6 WAT and 8 WAT. In 10 WAT, BAP was not significantly different from the control and coconut water treatments. However, the BAP treatment had no significant difference from the coconut water treatment at 12 WAT in the number of primary branches.

The cytokinin content in BAP and coconut water could cause an increase in the number of primary branches. Cytokinin is a plant growth regulator that plays an important role in stimulating the growth of lateral shoots, triggering chloroplast development, and encouraging leaf expansion and cell division in plant tissue (Ramayana et al., 2021).

Table 1. Increase in canopy width from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in canopy wide (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	11.63	16.75	20.00ab	26.00ab	32.25	41.50b
T	11.08	20.38	29.38a	33.88a	39.38	51.375a
T+CW	4.63	17.50	19.75abc	23.50ab	36.50	49.25ab
T+BAP	1.75	13.25	25.00a	33.25a	45.13	56.25a
CW	3.38	3.25	12.50bc	19.50b	32.00	41.00b
BAP	5.25	7.13	10.25c	17.75b	28.75	40.25b
CV (%)	44.06	26.57	15.85	13.89	11.53	17.80

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

Table 2. Increase in the number of primary branches from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in the number of primary branches					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	0.50	0.50	1.25a	1.50ab	1.75ab	1.75bc
T	0.00	0.00	0.00b	0.50bc	0.75bc	0.75bcd
T+CW	0.00	0.00	0.25ab	1.00abc	1.00bc	0.50cd
T+BAP	0.00	0.00	0.00b	0.00c	0.00c	0.00d
CW	0.50	0.50	1.00ab	1.50ab	2.00ab	2.50ab
BAP	0.50	0.50	1.50a	2.50a	2.75a	4.25a
CV	25.24%	25.24%	22.70%	21.12%	21.84%	23.35%

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

As a synthetic PGR, BAP was thought to trigger shoot growth that would become primary branches. Prior research confirmed that applying 60 ppm and 120 ppm BAP positively responded to increasing the number of shoots on tea plants (Saefas et al., 2017). Khalil et al. (2021) stated that BAP treatments amplify the expansions of vascular strands and xylem differentiation to increase the number of branches. As a natural PGR, coconut water contains two types of cytokinin, namely zeatin and kinetin, which accelerate shoot growth (Setyowati et al., 2023). Lazim et al. (2015) stated that the cytokinin content in coconut water could function in cell division, which helps shoot establishment.

Non-topping treatments have caused an increase in the number of branches. The number of branches obtained is greater than the topping treatments. Unpruned plants will produce non-productive branches with larger distances (Takur et al., 2018). The topping treatments did not increase the number of lateral shoots. The pruning, such as topping, will depress apical dominance, elongating the lateral shoot instead of elevating the number of branches (Kebrom, 2017).

Damayanti et al. (2022) stated that the increasing need for sinks influences the photosynthesis rate. A large number of branches and leaves can cause competition for nutrients and the rate of photosynthesis to be less than optimal (Yang et al., 2022). Therefore, a small number of branches after topping can reduce competition between branches and achieve optimal productivity.

Increase in branch length. Table 3 showed insignificant differences among all treatments, from 2 WAT to 8 WAT. Thus, the treatment given to Liberica coffee plants requires time to show its results. Korte et al. (2025) stated perennial plants require treatment time to show the effect that occurs.

The topping treatment differed significantly from that without topping, such as control, coconut water, and BAP treatments, at 10 WAT. The topping and topping+BAP also showed significant differences from control, coconut water, and BAP on 12 WAT. Pruning can influence the growth of branches by switching the allocation of photosynthate in the plant (Dong et al., 2019). The topping pruning treatments made branches grow stronger and longer. Topping can affect assimilation distribution from the apical bud to lateral growth (Hamdani et al., 2021).

PGR can play a role in stimulating the vegetative growth of plants. BAP and coconut water had cytokinin, which supported vegetative growth (Zanirah et al., 2023). Besides cytokinin, coconut water also contains the hormone auxin. These hormones play a role in the cell division process, which helps with bud formation. Cytokinin promotes cell division, while auxins stimulate cell elongation.

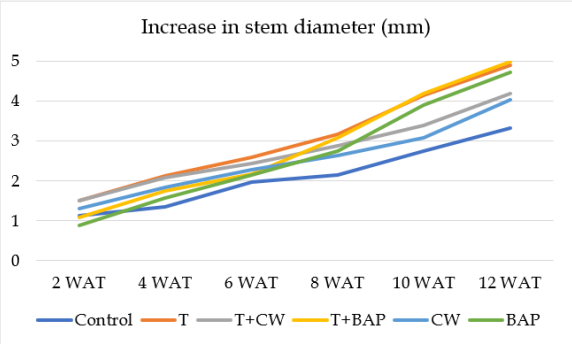
Table 3. Increase in branch length from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Treatments	Increase in branch length (cm)					
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
Control	2.50	4.75	6.00	7.50	8.75b	9.75b
T	2.38	5.25	8.38	11.25	14.13a	16.75a
T+CW	1.13	2.50	4.75	9.38	10.75ab	12.63ab
T+BAP	3.30	5.55	6.80	9.80	11.68ab	16.18a
CW	0.25	2.88	4.00	7.38	8.50b	10.50b
BAP	2.13	4.13	5.13	7.50	10.25b	11.63b
CV	31.68%	28.42%	22.62%	12.86%	8.88%	11.11%

Note: Means followed by the same notations in each column were not significantly different based on DMRT at a 95% confidence level. Code: WAT=Week after treatment, T=Topping, CW= 50% Coconut Water, BAP= 60 ppm 6-Benzylaminopurine, CV=coefficient of variation.

Jurnal Kultivasi Vol. 24 (1) April 2025
ISSN: 1412-4718, eISSN: 2581-138x

Increase in stem diameter. Figure 1 shows that all treatments gave insignificant differences in increasing stem diameter. Several previous studies confirmed that pruning treatment has no relation to increased stem diameter. Prior research confirmed that topping did not respond to tomato stem diameter (Nkansah et al., 2021). Gebisa (2023) reported that stem diameter had no response to various pruning treatments on the coffee plant. Apart from environmental factors, genotype factors also influenced the increase in stem diameter parameters. The statement is also supported by Lhamo et al. (2022) that growth is influenced more by cultivars than by pruning treatment. The various pruning methods did not influence the stem diameter in Arabica coffee species, as the diameter develops slowly in woody plants (Wisdawati, 2023). The application of cytokinin, such as BAP and coconut water, influences stem diameter by promoting cell division. Apical dominance leads to competition between the apical and lateral buds during vegetative growth, causing the plant's growth and development to focus more on new shoots and branches (Ayunda et al., 2021).



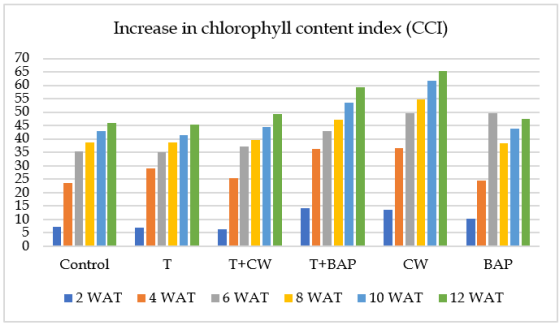
	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
CV	15.96%	13.92%	12.76%	14.1%	17.21%	14.89%

Note: WAT=Week after treatment, CV=Coefficient of Variation, T=, CW= 50%Coconut Water, BAP= 60 ppm 6-Benzylaminopurine.

Figure 1. Increase stem diameter from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Increase in chlorophyll contents. Figure 2 shows that the topping and/or application of PGRs had no significant effect. This is because the treatments given are not related to sunlight entering easily. Topping is a technique for manipulating the ideal environmental conditions and supporting photosynthesis. The leaves under the stem that have been pruned act as food reserves produced from the process of photosynthesis (Anjarsari et al., 2021). These food reserves play a role in the growth of new shoots and leaf development. Grati et al. (2016) stated that the pruning that changes the plant's shape to let sunlight in more easily could affect the chlorophyll content.

It has been found that there is an increase in the chlorophyll content index with leaf age. Wanda et al. (2022) stated that leaf age is a significant factor in increasing a leaf's chlorophyll index, apart from sunlight. Metabolites in leaves that are degraded due to senescence are then distributed to other tissues that are still actively growing (Kanojia et al., 2021).



	2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT
CV	32.99%	19.34%	20.69%	18.28%	16.28%	15.78%

Note: WAT=Week after treatment, CV= Coefficient of variation, T=Topping, CW= 50%Coconut Water, BAP= 60 ppm 6-Benzylaminopurine.

Figure 2. Increase in chlorophyll content from 2 to 12 WAT influenced by topping and cytokinin-based PGRs

Conclusion

The topping and/or cytokinin-based PGR treatments influenced three variables: canopy width, branch length increase, and number of branches. Plants treated with topping, topping + coconut water, and topping + BAP showed the best growth performance on canopy width and increased branch length. Treatments of coconut water and BAP responded best to increase the number of branches.

Acknowledgments

We would like to thank Universitas Padjadjaran, which supported this research through the HRU RPLK 2024 scheme.

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