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Maturity Acceleration of Some Rubber Clones (Hevea brasiliensis L) with Plant Growth Regulator Treatment

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Abstract:

Background and Aims: Rubber is a principal raw material for industrial products such as tires, health facilities, and home appliances. This research paper focused on seeking the data of maturity acceleration of *Hevea brasiliensis*. Basically, the firm's growth and uniform immature tree (TBM) was studied to support productivity according to the genetic potential (clones). Mature rubber tree acceleration is an effort to reduce non-productive period.

Method: This experiment used five recommended rubber clones, a combination of IAA with Kinetin, and three paclobutrazol concentrations. It applied the growth parameters of several rubber clones by applying growth regulators during the immature tree period. It took a-7 month data collection in the Karang Inong Estate, PTPN-III, East Aceh Regency. The research was arranged in a Nested Design using three factors and two replications that nested in clones.

Findings: The obtained data showed that the growth of rubber clones was influenced by plant growth regulators (PGR). Paclobutrazol which applied through roots is more effective to inhibit plant high and girth increase than sprayed through leaves. The highest increase of girth was found in clones PB 330 and IRR 5 with PGR 500 ppm + Kinetin 60 ppm. The application of PGR IAA + Kinetin can accelerate the tapping maturity of each clone; at this design, the girth has a faster process to befall into mature tapping criteria (≥ 45 cm). Data found that the increase in stem girth did not have a significant difference. The combination of treatments tended to increase in clones PB 330 with IAA 600 ppm + PGR 60 ppm and soil application of paclobutrazol (K2H6P1) (± 49.05 cm) on rubber tree age of 46-month. Combination of clones, PGR and paclobutrazol (combination of three factors) was not significantly affecting the parameters of girth increased and accelerated maturity under 4 years.

Keywords: Growth of Rubber Clones; TBM; PGR IAA + Kinetin; Paclobutrazol.

1. Introduction

Rubber is one of the industrial raw materials that has been processed into many products, such as biodiesel with environmentally friendly catalysts [1]. Indonesia, Malaysia and Thailand are the largest rubber producers for industry. It is said that rubber is an export commodity that contributes to increasing the country's foreign exchange. The area of rubber plantations reaches 3.4 million hectares, and 80% is smallholder plantations with a national production of 3.16 million tons [2]. Indonesia, Malaysia and Thailand are the biggest producers of rubber for industry. Pre-treatment of high free fatty acid rubber seed oil through an esterification reaction using a pilot-scale hydrodynamic cavitation reactor (HC) provides three times shorter reaction times and HC

esterification efficiency than mechanical manual stirring [3]. It noted that rubber is an export commodity that contributes to increase Indonesia's foreign exchange.

The total area of rubber plantations reaches 3.4 million hectares, and 80% of the area is smallholder with a national production of 3.16 million tons [4]. Efforts to develop the potential and take advantage of the long-term opportunities of world natural rubber demand will continue to grow. [5] The catalyst loading has the greatest effect on FFA reduction, followed by the methanol to oil molar ratio. While the increase in temperature and reaction time has a nominal effect. The frequency and activation energy factors of rubber seed oil (RSO) Vietnam are about 1.8 and 1.2 times higher than RSO Malaysia.

The government has established a National Rubber Development Policy with a long-term national rubber production target of 3.80−4.00 million tons by 2025 [4]. National rubber development was constrained by the lack of interest of farmers to replant rubber trees. The period of immature rubber trees is longer than other plantation crops. Economical mature rubber trees are at 4.5 -5 years of age [5]. Such condition requires high investment costs and longer returns on capital compared to palm oil (3 years).

Research to accelerate stem girth was performed by combination of conventional and unconventional technologies. One of unconventional technologies that have not been implemented intensively is the use of growth regulator (PGR) on immature trees. Growth regulator activity is associated with plant growth and development, so it can be directed to secondary growth activities that lead to the development of girth [6, 7]. The use of PGR to replace the function of plant hormones is limited in number. [8] PGR is an alternative to accelerate tapping maturity and increase latex production.This secondary growth is the result of cambium activity [9], whose activity is regulated by growth regulator [10].

Auxins and cytokinins have important functions in plant growth and survival. Cambium cell division is also affected by auxin and cytokines [11, 12]. Matsumoto-Kitano *et al*. [13] stated that mutation of isopenteniltransferase gene that synthesizes cytokinin causes the branch and roots f *Arabidopsis* to be smaller than their natural types. Cytokinins and auxins has different role in cambium activity but interconnected. Cytokinins act to regulate the amplitude of auxin gradients in the cambium [14].

Research on the role of PGR in increasing stem growth was pioneered by Pakianathan and Wain [15]. The study found that 3-year-old of rubber clone RRIM 600 that was applied by Indole-3 Acetic Acid (IAA) show increased the size of stem girth by 55% than the control. Gibberellins acid (GA) actually suppresses the size of girth. Koryati [16] suggested the need of further research on the use of paclobutrazol in combination with PGR IAA and Kinetin until mature rubber tree and produce latex. High concentrations of Paclobutrazol has physiological activity that inhibits growth, accelerate flowering and increasing vigor of rice. The interaction between paclobutrazol and other PGRs has a variety of responses. Paclobutrazol at low concentrations increases the number of shoots in Rhizome of *Cucurma* sp [17] especially in rubber trees.

The long period of immature trees (TBM) is very closely related to the size of girth. Stem girth has been known related to production [18]. Production parameters such as the beginning of latex flow, total volume of latex, and clogging index (CI) is indication of the importance of the girth

criteria for tapping [19]. This experiment aimed to study the growth parameters of several rubber clones by application of growth regulators during the period of the immature tree.

2. Materials and Methods

The materials used are: clones, growth regulators (IAA, kinetin), and paclobutrazol. The clones used in this study were five rubber clones, namely PB 260, PB 340, PB 330, IRR 5, and IRR 107, these clones were used because they were clones found at the research site and were commercially recommended clones (latex-producing clones and latex-wood). The clone used was a 28-month-old rubber plant (planting year December 2014). Data was observed in April 2016-October 2017.

The use of paclobutrazol in this study was due to the results of previous research by Pakianathan and Wain (1975), that the administration of Gibberellin acid (GA) could reduce the size of the stem coils. paclobutrazol inhibits the biosynthesis of gibberellins (ICI, 1986; Sponsel, 1987 and Davis, et al. 1988). The results of Koryati's research (1998) showed that the administration of paclobutrazol to immature rubber plants could suppress the increase in plant height and increase the increase in stem girth. From the results of previous studies, it is necessary to support further research on the use of paclobutrazol with a combination of growth regulators IAA and Kinetin with the aim of accelerating the ripening of tapping.

3. Methods

This research has been conducted for 8 months (April 2016-Oktober 2017) at KSO Karang Inong plantation, PTPN-I and PTPN-III, East Aceh Regency using clones PB-260, PB 340, PB 330, IRR 5 and IRR 107. The age of rubber trees is. ± 28 months (planting year December 2014). This research is three factors of Nested Design, namely factor clone, factor PGR and factor paclobutrazol.

The clones used for testing were PB 260, PB 330 and PB 340, IRR 107 and IRR 5. The factor combination of PGR (IAA and Kinetin) consisted of 7 levels namely Control (without IAA and Kinetin), IAA (400 ppm) + Kinetin (50 ppm), IAA (400 ppm) + Kinetin (60 ppm), IAA (500 ppm) + Kinetin (50 ppm), IAA (500 ppm) + Kinetin (60 ppm), IAA (600 ppm) + Kinetin (Kinetin) (50 ppm) and IAA (600 ppm) + Kinetin (60 ppm). The third factor is paclobutrazol (P), consisting of 3 levels, namely; P0 = Control (without paclobutrazol), P1 = 2 ml/l of water (500 ppm) applied through soil and P2 = 2 ml/l water (500 ppm) applied through leaves.

Data from each treatment were tested by analysis of variance. If the test shows significant or very significant effect, the Duncan's Multiple Range Test is performed. Experimental plots in the planting area were chosen by criteria rubber stand relatively close together. Each clone selected for 168 trees that have relatively high homogenity. Criteria for selecting trees include plant height, stem size, crown condition and free from root and leaf disease. The number of plants was divided into two groups as replicates (84 trees). Each clone/treatment contained 2 replications, and for every replication, there were 84 plants. The number of experimental units was 7 x 5 x 3 x 2 = 210, each experimental unit consists of 4 rubber trees. The total number of plants used in total is 840 plants.

The PGRs treatment was carried out by mixing lanolin (a skin softening agent so that the PGRs compound is easily absorbed) with IAA and Kinetin, then smeared on a circle of bark that had been rubbed with sandpaper. Smearing area is 2.5 cm around the stem in two positions for each plant, the first is at an 70 cm above the ground level and the second is 30 cm above the first position.

Application interval is once a month for 8 applications. Paclobutrazol is applied according to the treatment. Application through soil is given only twice during the experiment and 6 months after treatment (BSP) while application through spraying the leaf surface is given 8 times from the beginning of experiment at once a month interval.

Parameter observations were carried out at intervals of every 3 months, namely: observations at the age of 28 months (early), 31 months, 34 months, 37 months, 40 months, 43 months, and 46 months. in the field, namely the observation of plant growth including:

1. The nature of plant growth as measured by using bamboo or wood that has been given a size/meter. Measured above the elephant's foot to the top of the tallest plant on a straight plant and
2. Growth properties include stem girth (cm) measured at a height of 100 cm above the soil surface

The data was analyzed by MS Excel 2016 and SPSS ver. 24, a further test was correlation analysis and interaction among treatments.

4. Results and Discussion

3.1. Plant height

Application of IAA + Kinetin and paclobutrazol has significant effect on growth of several rubber clones by increasing plant height. Mean different tests of plant height (m) on clones, PGR and paclobutrazol treatments at the age of 31-46 months were presented in Figure 1 and Table 1.

Table 1. Average Plant Hight Inncrease at 31–46 months in Treatment of Clones, PGR and Paclobutrazol.

Treatment	Height increase of plant (m) age observation (months)					
Clones (K)	31	34	37	40	43	46
PB 260	0.66^{a}	1.18	1.62^{c}	2.02^{b}	2.49^{c}	3.00 ^{b}
PB 330	0.59^{b}	1.34	1.93^{a}	2.25^{a}	2.81^{a}	3.45 ^{a}
PB 340	0.38^{d}	1.31	1.76^{bc}	2.18^{a}	2.71^{ab}	3.26 ^{a}
IRR 107	0.41^{cd}	1.27	1.79^{ab}	2.17^{a}	2.78^{a}	3.39 ^{a}
IRR 5	0.44^{c}	1.21	1.66^{bc}	2.02^{b}	2.62^{bc}	3.28 ^{a}
PGR						
IAA+ Kinetin (H)						
Control	0.48	1.19	1.61	1.98	2.49^{c}	3.06^{c}
400 ppm+50 ppm	0.50	1.15	1.70	2.09	2.57^{bc}	3.09^{c}
400 ppm+60 ppm	0.48	1.29	1.83	2.16	2.77^{a}	3.39^{ab}
500 ppm+50 ppm	0.52	1.23	1.77	2.17	2.71^{ab}	3.27^{abc}
500 ppm+60 ppm	0.49	1.36	1.84	2.22	2.82^{a}	3.46^{a}
600 ppm+50 ppm	0.51	1.35	1.80	2.20	2.78^{a}	3.44^{ab}
600 ppm+60 ppm	0.47	1.26	1.72	2.07	2.64^{abc}	3.23^{c}
Paklobutrazol(P)						
P_0= control	0.56^{a}	1.39^{a}	1.93^{a}	2.42^{a}	3.12^{a}	3.85^{a}
P_1= soil	0.43^{c}	1.07^{b}	1.54^{c}	1.82^{c}	2.27^{c}	2.76^{c}
P_2= leaf	0.50^{b}	1.32^{a}	1.79^{b}	2.14^{b}	2.65^{b}	3.22^{b}

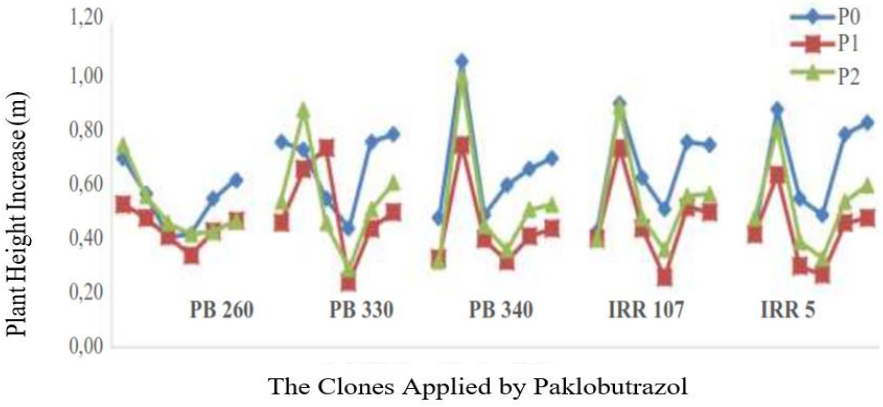
Noted: The number followed the same alphabet in the same coloum shows not significantly different at the level of 5 % based on DMRT test.

Table 2. Average Increase of Plant Height (m) for observation at 46 months on the Interaction of Clones with PGR

IAA + Kinetin	Clones				
	PB 260	PB 330	PB 340	IRR 107	IRR 5
Control	1.42 ^{h}	1.90^{c-g}	1.93^{b-g}	2.10^{a-g}	1.88^{c-g}
400 ppm +50 ppm	1.78 $^{e-h}$	1.90^{c-g}	1.86^{d-g}	2.34^{abc}	1.70^{gh}
400 ppm +60 ppm	1.74^{fgh}	2.36^{ab}	1.88^{s-g}	2.23^{a-e}	2.31^{a-d}
500 ppm+ 50 ppm	1.98^{a-g}	2.16^{a-f}	2.03^{a-g}	1.90^{c-g}	1.96^{a-g}
500 ppm+60 ppm	2.03^{a-g}	2.39^{a}	1.91^{b-g}	1.81^{e-h}	1.98^{a-g}
600 ppm+ 50 ppm	1.87^{d-g}	2.11^{a-g}	2.24^{a-e}	2.01^{a-g}	2.19^{a-f}
600 ppm + 60 ppm	1.83^{e-h}	1.92^{b-g}	1.83^{e-h}	2.19^{a-f}	2.07^{a-g}

Note: Figures followed by the same letter, show no significant difference at the 5% level based on the DM Test

Paclobutrazol



a. PGR IAA + Kinetin

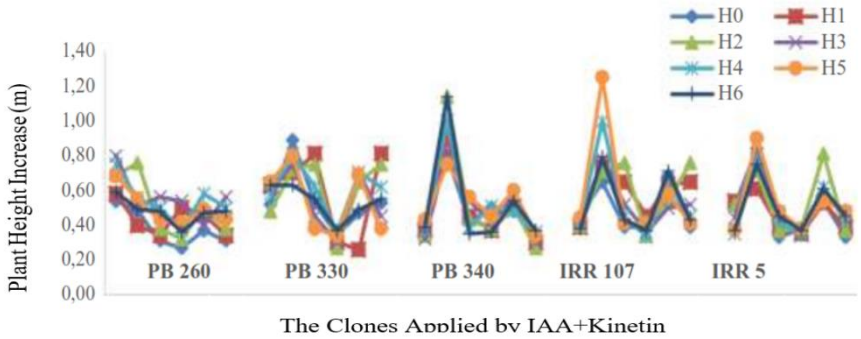


Figure 1. Pattern of Plant Height Increase fr Clones PB 260, PB 330, PB 340, IRR 107 and IRR 5 with Treatment: a) Paclobutrazol and b) PGR IAA + Kinetin per 3 months, period April 2016 - October 2017.

During the observation, increase in the plant height of the tested clones is varied and fluctuated. Dramatic increase in plant height occurs in the second observation or two months after the treatment and decreases sharply in the third observation. This might occur due to dry season in January to February (for 2 months there is no rain). At the end of observation or 46 months, clones

PB 260 had the lowest increase in height compared to other clones. Canopy shape of clone PB 260 is evergreen type with one main branch and little branch is self pruning. Because it has one main branch, the high inhibition of clone PB 260 is more pronounced than other clones that have a canopy with several main branches.

The PGR treatment began to have a significant effect on increasing plant height after five months (43 years old). Without PGR treatment, increasing plant height was lower than PGR application. The highest increase response was in the treatment of IAA 400 + kinetin 60 ppm and continued to increase with increasing IAA and kinetin concentrations. Peaks of increase were obtained in the combination treatment of IAA (500 ppm) + Kinetin (60 ppm), but the high increase tends to decrease with increasing IAA concentration. Application of high concentrations IAA is known inhibit shoot development due to cell extension, although the mechanism cannot be explained [20]. Current research related to the role of IAA in cell elongation known through apoplastic pH regulation [21].

Meanwhile, the increase in plant height in response to paclobutrazol application both through soil (P1) and leaves (P2) was significant since the first observation. Observations for paclobutrazol treatment showed the lowest average inhibition of plant height. Application through roots (P1) is more suppressed plant height compared to leaves (P2). Control (without paclobutrazol, P0) shows higher increase in plant height and significantly different than paclobutrazol application both through soil and leaves. The difference in height increases during observation. The height difference between control and paclobutrazol application is about 0.62 m by spraying and 1.02 m by soil application. Other studies also reported that paclobutrazol only affected plant heights, and did not affect the number of leaves and tillers in Sanseviera plants and the higher concentration of paclobutrazol the stronger the inhibition [22].

Interaction of clone and PGR had significant effect on increasing plant height during observation. The lowest increase in plant height is in clones PB 260 compared to other clones without PGR with an increase is only 1.42 m. Meanwhile, PGR application on clones at various levels showed varying height increases. The highest increase found in PB 330 with treatment of IAA 500 ppm + Kinetin 60 ppm, that is 2.39 m for 5 months of observation. In general, combination of IAA 400 ppm + kinetin 60 ppm has shown a high increase in most clones except PB 340.

Interaction of clone and paclobutrazol also had significant effect on plant height increase and the mean difference test was presented in Table 3.

Table 3. Average Increase of Plant Height (m) for observation at 46 months on the Interaction of Clones and Paclobutrazol

Clone	Paklobutrazol (P)		
	$P_{0=}$ control	$P_{1=}$ Through the root	$P_{2=}$ Through the leave
PB 260	3.26 ^{b}	2.62 ^{d}	3.12 ^{bc}
PB 330	4.02 ^{a}	3.04 ^{bc}	3.28 ^{b}
PB 340	3.98^{a}	2.63 ^{d}	3.17 ^{b}
IRR 107	4.02 ^{a}	2.86 ^{cd}	3.31 $_{b}$
IRR 5	3.99 ^{a}	2.63 ^{d}	3.23 ^{b}

Note: Figures followed by the same letter, show no significant difference at the 5% level based on the DMRT Test

Interaction of clone and paclobutrazol at the sixth month observation cause a significant increase in plant height. Clones PB 330 without paclobutrazol showed a normal increase, but application with paclobutrazol through leaves and roots will suppres increase in plant height. Application of paclobutrazol through roots is stronger in suppressing the increase in clone height compared to leaves. The lowest increase in plant height was found in clone PB 260 with application of paclobutrazol through soil. Paclobutrazol has physiological impact as opposed to combination of IAA and kinetin. Sakhdin & Suparto [23] reported that application of paclobutrazol cause decreasing concentration of giberelin and kinetin, but increasing the abscisic acid content. Decreasing gibberellins and kinetin at the shoots is likely to be a factor in decreasing plant height. Its inhibition occurs on the trajectory of kaurenoic formation from kaurenat oxidation, so this trajectory changes its role to carry out biosynthesis of abscisic acid [24, 25, 26]. The impact of this process is suppress the growth of vegetative cell elongation and in turn inhibit the growth of plant height during immature trees and has effect on stem enlargement and bark thickness due to switching of photosynthate use to other tissues.

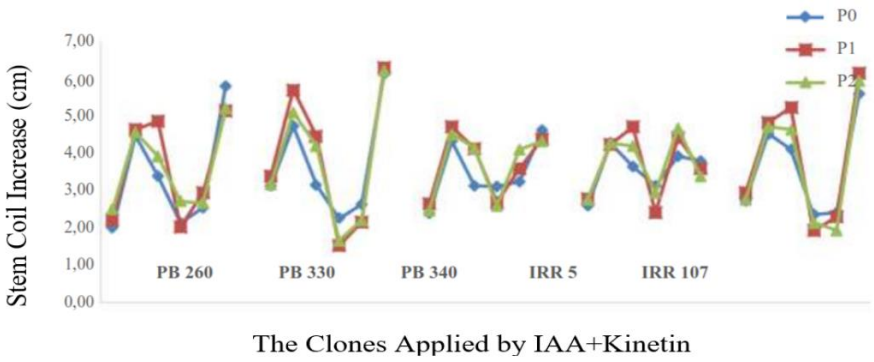
3.2. Stem Girth (cm)

The results of the analysis of variance showed that clones, PGR and paclobutrazol significantly affect the increase of stem girth in the first observation up to the sixth month, except for the fourth month. Application of PGR is not yet significantly affects the increase of the girth. The increase of girth among clones is varied. The largest increase is found in clones PB 330 and the lowest is IRR 230. Such girth increase is associated with increased carbohydrate deposits into xylem tissue. Accumulation of carbohydrates in rice seeds increases by application of IAA and kinetin [27].

All combinations of IAA with kinetin effect the increase of girth, especially at the fourth observation. Plants response to PGR application is relatively fast as indicated by increasing stem girth at the first observation. The first to the third observation was still show variation in stem girth, but after fourth observing, stem girth increase tend to uniform among PGR treatments. Cytokinins affect the activity and development of cambium [10], whereas combination of IAA and kinetin induces metaxilem maturation and accelerates the increase in stem diameter of *Cicer arietinum* L [28].

The changes pattern of girth increase average per three months of each clone with application of PGR and paclobutrazol for six months observations can be seen in Figure 3.

a. Paclobutrazol



b. PGR IAA + Kinetin

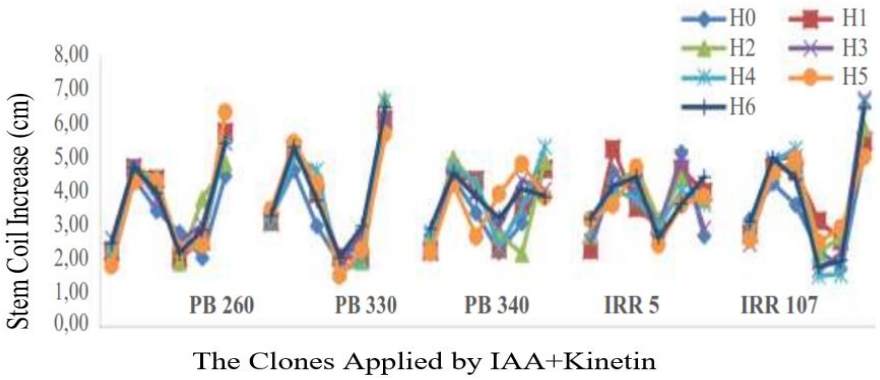


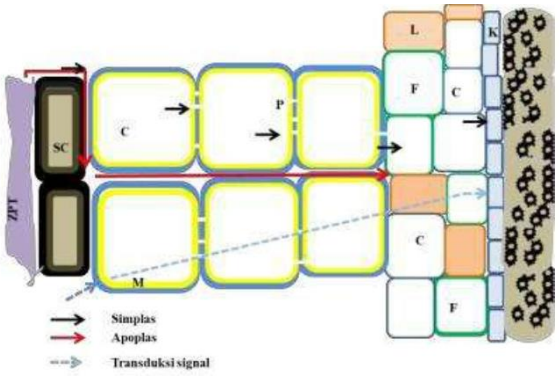
Figure 3. *Pattern of Girth Increase from Clone PB 260, PB 330, PB 340, IRR 5 and IRR 107 with Treatment : a) Paclobutrazol and b) PGR IAA + Kinetin per 3 months, period April 2016-October 2017*

Responses of each clone were almost the same for stem girth both paclobutrazol and PGR. Out of the six observations, at the fourth observation (37 months), each clone with paclobutrazol and PGR showed decreasing average of girth increase. Allegedly the decline occurred due to dry season in Aceh region in January to February.

Table 4. Average of girth increase at 31-46 months in the treatment of clones, PGR and Paclobutrazol

Treatment	The Increase of Stem Twist (cm) on Age (Months)					
Clone (K)	31	34	37	40	43	46
K_1 = PB 260	2.25 ^{d}	6.81 ^{c}	10.86 ^{b}	13.37 ^{d}	16.10 ^{c}	21.58 ^{c}
K_2 = PB 330	3.23 ^{a}	8.42 ^{a}	12.34 ^{a}	14.17 ^{ab}	16.49 ^{c}	22.74 ^{a}
K3 = PB 340	2.50 ^{c}	7.03 ^{c}	10.92 b	13.62 ^{cd}	17.28 ^{b}	21.73 ^{bc}
K_4 = IRR 230	2.82 ^{b}	7.47 ^{b}	12.09 a	13.80 ^{bc}	18.07 ^{a}	21.39 ^{c}
K_5 = IRR 5	2.71$^{b\,c}$	6.88 ^{c}	11.13 b	14.31 ^{a}	16.54 ^{c}	22.44 ^{ab}
PGR IAA+Kinetin(H)						
$H_{0=\text{ control}}$	2.66 ^{b}	7.01	10.20 ^{c}	12.97 ^{b}	15.83 ^{b}	20.31 ^{b}
$H_{1=400\text{ ppm}+50\text{ ppm}}$	2.51 ^{b}	7.37	11.56 ^{ab}	13.98 ^{a}	17.02 ^{a}	22.26 ^{a}
$H_{2=400\text{ ppm}+60\text{ ppm}}$	2.72 ^{ab}	7.42	11.90 ^{a}	14.03 ^{a}	16.98 ^{a}	22.07 ^{a}
$H_{3=500\text{ ppm}+50\text{ ppm}}$	2.60 ^{b}	7.31	11.67 ^{ab}	14.01 ^{a}	17.47 ^{a}	22.43 ^{a}
$H_{4=500\text{ ppm}+60\text{ ppm}}$	2.81 ^{ab}	7.46	11.89 ^{a}	14.07 ^{a}	16.76 ^{a}	22.29 ^{a}
$H_{5=600\text{ ppm}+50\text{ ppm}}$	2.64 ^{b}	7.05	11.37 ^{b}	13.80 ^{a}	17.01 ^{a}	21.96 ^{a}
$H_{6=600\text{ ppm}+60\text{ ppm}}$	2.99 ^{a}	7.66	11.69 ^{ab}	14.11 ^{a}	17.21 ^{a}	22.54 ^{a}
Paklobutrazol(P)						
P_0= control	2.58 ^{b}	7.04 ^{b}	10.43 ^{c}	13.15 ^{c}	16.09 ^{b}	21.30 ^{b}
P_1= Soil	2.80 ^{a}	7.62 ^{a}	12.48 ^{a}	14.40 ^{a}	17.45 ^{a}	22.50 ^{a}
P_2 = Leave	2.74 ^{ab}	7.31 ^{b}	11.5 ^{b}	14.02 ^{b}	17.15 ^{a}	22.13 ^{a}

Note: Figures followed by the same letter, show no significant difference at the 5% level based on the DMRT Test



C : cortex cells

F : fluem,

L : laticifer,

M : cell membrane,

K : cambium,

P : plasmodesmata,

Sc : sclerenkim,

X : xilem,

PGR : an exogenous growth agent

Figure 4. Hypothetic mechanism for PGR to enters into bark tissue and gives effect to cambium activity.

Interaction between PGR and paclobutrazol had significant effect on stem girth increase, as well as interaction of clone and PGR at 37 months. Interaction clone and paclobutrazol as well as PGR and paclobutrazol did not significantly affect the increase in stem girth. Mean different test of combination of clones and PGR IAA + Kinetin was only significant at 37 months as presented in Table 5.

Table 5. Average of Girth Increase (cm) at 37 months on Interaction of Clones and IAA + Kinetin PGR

PGR IAA+Kinetin (H)	Clone (K)				
	PB 260 (K_1)	PB 330 (K_2)	PB 340 (K_3)	IRR 230 (K_4)	IRR 5 (K_5)
H_O= Control	9.87 ^{tu}	10.38 $^{p-u}$	10.17 ^{stu}	10.25 $^{r-u}$	10.32 $^{q-u}$
H_1=400ppm+50 ppm	11.28 $^{j-p}$	12.26 $^{a-i}$	10.98 $^{m-s}$	11.21 $^{k-q}$	12.05 $^{c-l}$
H_2=400ppm+ 60 ppm	11.33 $^{i-o}$	12.98 ^{abc}	11.68 $^{e-n}$	11.30 $^{j-p}$	12.21 $^{b-j}$
H_3=500ppm 50 ppm	11.09 $^{m-r}$	12.53 $^{a-e}$	10.77 $^{n-s}$	11.50 ^{s}	12.47 $^{a-f}$
H_4=500 ppm+60 ppm	11.12 $^{l-r}$	12.73 $^{a-d}$	11.81 $^{d-m}$	10.69 $^{o-t}$	13.10 ^{ab}
H_5=600ppm+ 50 ppm	10.32 $^{q-u}$	13.15 ^{a}	9.75 ^{u}	11.54 $^{f-o}$	12.07 $^{c-k}$
H_6=600ppm+ 60 ppm	11.00 $^{m-s}$	12.32 $^{a-h}$	11.27 $^{j-p}$	11.46 $^{h-o}$	12.41 $^{a-g}$

Note: Figures followed by the same letter, show no significant difference at the 5% level based on the DMRT Test

Combination of IAA and kinetin has a positive effect on the increase of stem girth. The increase girth is varies. Clones PB 330 and IRR 5 have higher response than other three clones. Concentration of kinetin has more role for rubber girth. This was shown by larger girth at kinetin concentration 60 ppm compared to 50 ppm at the same IAA concentration. Matsuto-Kitano et al. [20] reported that kinetin has important role in the development of cambium and its activities. Cambium cells division which followed by differentiation will results in enlargement of the girth. This IAA support cells elongation in caloptil and plant segments, cell elongation occurs in vertical direction followed by cell enlargement [21, 22]. Increase in biomass occurs due to nutrition mobilization in tissues with active IAA activity [23]. Auxin also has indirect role for girth increase that is through induction of better root development [24]. Auxin is transported naturally to roots and regulates root growth [25]. Mean different test of combinations PGR IAA + Kinetin and paclobutrazol (HxP) at observations of 46 months are presented in Table 6.

Table 6. Average of Girth Increase (cm) at 46 months on Interaction of PGR IAA + Paclobutrazol

PGR IAA+ Kinetin	**Paclobutrazol**		
(H)	P0 = Control	P1 = Soil	P2 = Leaf
HO = Control	**16.69** [b]	**22.37**[a]	**21.87**[a]
H1 = 400 ppm + 50 ppm	**22.11**[a]	**22.44**[a]	**22.22**[a]
H2 = 400 ppm + 60 ppm	**21.97**[a]	**21.97**[a]	**22.29a**
H3 = 500 ppm+ 50 ppm	**21.80**[a]	**22.92**[a]	**22.57**[a]
H4 = 500 ppm+ 60 ppm	**22.61**[a]	**21.96**[a]	**22.29**[a]
H5 = 600 ppm+ 50 ppm	**21.67**[a]	**22.54**[a]	**21.66**[a]
H6 = 600 ppm + 60 ppm	**22.28**[a]	**23.31**[a]	**22.03**[a]

Note: Numbers followed by the same letters is not significant difference at the 5% level based on the DMRT Test

Interaction between PGR and Paclobutrazol does not significantly affect the increase of the girth. This is evidenced by increasing girth at without paclobutrazol treatment is almost the same as paclobutrazol. Naturally, paklobutrasol is a retardant which causes growth inhibition. Nazaruddin et al. [45] reported that paclobutrazol effectively inhibits plant height but interferes xylem but this study does not show this.

In rubber trees, the standard provisions for initial tapping (exploitation) is 70% of trees in the location have mature or able to produce [46]. The criteria is if the girth as measured at 100 cm above grund level has reached 45 cm with a minimum bark thickness of 7 mm [44, 47, 48, 49]. Thus, acceleration of girth increase for each clone is very important to accelerate the criteria. In addition, combination of PGR applications can increase girth above 70% so that the rubber yield in the first year is higher. Rubber stem girth have heretability of 0.32 - 0.66 and interaction between girth and rubber yields has close relationship with the genetic namely 0.64 - 0.92 and phenotic 0.63 - 0.89 [50].

Correlation analysis shows that stem girth is negatively correlated to the tested clones except PB The highest negative correlation is PB 340 (-0.44), IRR 5 (-0.40), IRR 230 (-0,26), and PB 330 (-0.24). Clones PB 260 has negative correlation between girth and plant height, namely -0.01. Efforts to suppress height of rubber trees can encourage the increase of girth. This condition is

allegedly due to division of carbon from carbon addition biomass pathways for height which transferred for the addition of biomass in the stem.

PGR application is an alternative to trigger the increase and acceleration of stem girth. However, clones response to PGR is influenced by planting environment. Gonçalves., *et al*. [45] reported that the development of several clones varied depending on agro ecological conditions. Rouf., *et al*. [51] suggested that extra treatment should be carried out on plants aged 12 - 36 months, that is plants still in the logarithmic phase.

The application is relatively easy to do by large plantations and farmers, that is by circle smearing PGR around 2.5 cm wide on stem at 70 cm above the elephant's foot. Further testing can be focused on younger plants (12 months). There are three hypothetic mechanisms for PGR to enter into bark tissue and gives effect to cambium activity. The first is apoplast mechanism, namely diffusion from high concentrations to the lower. PGR diffuses through cell walls or cell space to cambium.

Secondly, PGR diffuses through sclerenchymalcells, then enters to the cell and passes through plasmodesmata to cambium. Third, combinations between the two and after passing through the cell wall, PGR is received by receptors within the cell membrane and passed through signal transduction to reach the cambium cell [52]. Auxin transport between cells is aided by a transporter protein in the cell (known as PIN1) with a feedback regulation system by auxin-binding Protein (ABP1) [53, 54]. Cambium activity increases and then cell division occurs to forms skin and woods cells. Such activity causes the plants experience faster girth increase than untreated plants.

Generally, rubber clones give a positive response in terms of increasing plant height and girth if treated with combination of auxin and cytokinin. For 18 months, clones that show faster girth increase is PB 330 (K2 = 46.28 cm), followed by IRR 5 (K5 = 46.15 cm), PB 340 (K3 = 45.48cm), IRR 107 (K4 = 45.21 cm) and PB 260 (K1 = 45.20 cm) at 46 months in the field. Thus the circle of girth for the tested clones has meets the criteria for mature rubber tree. Sundiandi, et al. [49] stated that shortening TBM period will accelerate the investment return period and can save costs by 18-19%. It is assumed that the clones tested were commercial recommended clones grouped into two. The first is clones PB 330, PB 260 and PB 340 which included as latex producing clones and secondly clones IRR 5 and IRR 107 as latex and wood producing clones [54, 47].

Although the girth increase was not significant, combination of treatments tended to increase in clones PB 330 with PGR IAA 600 ppm + Kinetin 60 ppm and paclobutrazol (K2H6P1) through soil application (± 49.05 cm) at 46 month age. Thus, the use of PGR (PGR IAA + Kinetin and paclobutrazol) in immature rubber trees has very positive effect because accelerating rubber maturity under 4 years and save maintenance costs for 1 year.

5. Conclusions

Paclobutrazol which applied through roots is more effective to inhibit plant high and girth increase than sprayed through leaves. Application of IAA with kinetin showed optimal activity at a concentration of 60/400 (ppm/l), but increased of IAA concentration did not significant increase of girth of stem. Combination of clones, PGR and paclobutrazol (combination of three factors) was not significantly affecting the parameters of girth increase.

Growth pattern of several immature rubber clones with application of growth regulators has very positive effect because it can accelerate maturity under 4 years.

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