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Histology and Physiology of BPM1 Clones with Different Exploitation Systems

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Abstract

This research aimed to study the characters of histology and physiology of BPM1 rubber clones to obtain the right exploitation system in increasing production in accordance with the character of the clones. The research results are expected to serve as a useful technology package which can be applied not only in the community rubber plantations but also in private as well as government large rubber plantations. The study was conducted in the rubber plantation of PT Perkebunan Nusantara III (Persero), Deli Serdang, North Sumatra, from 5 April to 28 December 2014. This study used a randomized block design with exploitation systems as the treatments, and each treatment was repeated three times. The exploitation systems employed were: tapping system (P) with four levels (P_1 : S/2 d3 BI-1, P_2 : S/4 d3 BI-1, P_3 : S/2U d3 H0-1, P_4 : S/4U d3 H0-1, and types of stimulant (S) with four levels (S_0 : Etepon 2.5%, S_1 : Gas stimulant every 9 days, S_2 : Gas stimulant every 18 days, S_3 : Gas stimulant every 27 days). Before the application of the treatments, observations of histological characters were first performed on all experimental units to determine the number and diameter of the latex vessels of renewable bark (the tapping on the lower stem (BI-1) and virgin bark (the tapping on the upper stem (H0-1)).

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The results showed that the number and diameter of latex vessels were not significantly different between the renewable bark and the virgin bark, while the character of physiology showed a significant difference between the treatments given. The exploitation systems which were relatively safe for BPM1 clone were the treatments with the tapping systems of P_4S_0 (S/4 U + ET 2.5%, every 15 days) and P_2S_2 (S/4D + ETG, every 18 days).

Keywords: sucrose; Pi; thiol; histology; BPM1 clones; exploitation system.

1. Introduction

Each clone has a different metabolic level, resulting in a different tapping system (exploitation of production). The tapping which is not based on clonal typology will cause excessive tapping (over exploitation) or lack the intensity of exploitation (under exploitation). Tapping process inaccuracies will bring unfavorable consequences both to the tree itself and its production. This will cause a waste and bark damage that will have an impact on the shortening of the plant economic life and the decrease in the production, making the company suffer losses [1].

The clones used in this study are BPM1 clones, which belong to the low metabolic rubber. Based on their metabolic system, the clones are divided into two: fast metabolic clone (quick starter = QS) and slow metabolic clone (slow starter = SS). Clones with low to moderate metabolism describe the speed of poly-isoprene formation (latex) from basic materials of carbohydrates in form of sucrose as a result of photosynthesis. Clones with high metabolism describe the process of poly-isoprene (latex) formation which is faster [2].

In addition to using clonal typology, the characters of physiology and histology, as well as the exploitation system can be used as the parameters to find out the rubber productivity. The physiological characters observed related to rubber production are the levels of sucrose, Pi and thiol, whereas the histological characters are the number and diameter of latex vessels [3]. Each rubber clone is different in the number and length of latex vessels. [4], who conducted research on three rubber clones, found that production, vascular cell length, number of rings, and vessel diameter of RRIM clone were higher than GT1 and Fx2261. The diameter size of latex vessels will determine the number of vessel cells and the number of production rings.

Dry rubber content (DRC) is the rubber solidity content per unit of weight as calculated in a unit of percent (%). Dry rubber content in the latex depends on several factors such as the clone type, tree age, tapping time, season, temperature, and the height from the sea level. Latex quality is largely determined by the dry rubber content. [5] classified the quality of latex into two groups, namely quality I with at least 28% dry content and quality II with a dry content of at least 20% or below 28%. The DRC value becomes one indicator of latex quality because DRC describes the level of the water content in the latex. According to [6], DRC showed a latex regeneration balance between tapping activities. The low DRC shows that tapping frequency is so close that the plant does not have sufficient time to carry out a synthesis fully on the latex that has been harvested. DRC could be an indicator that reveals how heavy the exploitation activity, which in a serious condition can drain all the carbohydrate reserves in the bark tissues and wood.

Latex sucrose levels are closely related to the exploitation rate applied since the results of photosynthesis are

translocated to other plant organs in the form of sucrose. Source-sink mechanism is an assimilate partition which is generally derived from the canopy to latex-producing bark when tapped [7]. The content of sucrose in the latex vessels decreases with the increased exploitation intensity which is caused by the liquid discharged from the latex vessel containing much serum [8].

Inorganic phosphate (Pi) is an indicator of metabolic activity which describes the plant ability to change the raw material (sucrose) into the rubber particles. A high level of Pi shows a high metabolic activity and vice versa. In general, the higher the level of Pi in latex, the higher the production level of the rubber plant [9].

Thiol functions to activate enzymes that play a role in environmental stress conditions. Stress conditions will activate the formation of this compound. The size of thiol concentration shows plant responses to stress exploitation. Thiol levels are inversely proportional to the intensity of exploitation. The higher the exploitation intensity, the lower the thiol level [10]. Therefore, this research was conducted to study the various exploitation systems to discover the right one for BPM1 clones through histological and physiological observations.

2. Materials and Methods

The experiment was conducted at two locations: (1) Experimental Plantation of Research Institute of Sungei Putih, Rubber Research Center of North Sumatra, and (2) the rubber plantation of PT. Perkebunan Nusantara III (Persero), Sungai Putih Plantation, Deli Serdang, North Sumatra, from April 5 to December 28, 2014.

The research materials used in the field were rubber plant clones of BPM1 (aged 15 years (planted in 1999), with a spacing of 2.5 x 5 m (with a population of 800 trees ha-1). The research observations included histological variables and analysis of latex physiology which were conducted at The Laboratory of Research Institute Sungai Putih.

2.1. Experimental Design

Field experiments used factorial randomized block design with three replications employing exploitation systems as the treatments. The exploitation systems applied consisted of two treatments, namely tapping system (P) and types of stimulant (S), each comprising four levels. The tapping system applied was as follows: P_1= S/2 d3 BI-1, P_2 = S/4 d3 BI-1, P_3 = S/2U d3 H0-1, P_4 = S/4U d3 H0-1, and types of stimulant (S) used were as follows: S_0 = Etepon 2.5%, S_1 = Gas stimulant every 9 days, S_2 = every 18 days, S_3 = Gas stimulant every 27 days. The variables measured in the field were latex physiology to determine the levels of the DRC, sucrose, P-inorganic (Pi) and thiol which were analyzed in the laboratory. For histological observation variables (number and diameter of latex vessels), prior to the analysis in the laboratory, bark tissue samples in the field were first taken.

2.2. Analysis of Plant Histology

The bark tissue samples are taken 1-2 punctures with a diameter of 8 mm, thickness 5-7 mm with a tapping area the edge using a cork-borer for each experimental sample. Each puncture will produce 0.6 g fresh weight of

tissue. The fresh bark tissue is then taken to the laboratory for histological analysis using a method of [11] by fixing the bark tissue with FAA for 1 night, then washed with running water for 5 minutes, dried with filter paper before being put into a solution of 15% KOH for 1 hours. After that, the tissue is washed again with running water for 5 minutes, dried with filter paper and put into a solution of HNO_3 for 2 hours. After 2 hours, the bark tissue is washed again with running water for 5 minutes, dried with filter paper and put into 70% alcohol for 15 minutes before being added to a solution of sudan III for 30 minutes. After that the tissue is thinly sliced crosswise or lengthwise using a razor blade and observed under a microscope.

2.3. Analysis Physiology

Levels of sucrose, inorganic phosphate, and thiol are measured by means of latex diagnosis using samples of latex serum TCA (trichloro-acetic acid). The latex serum TCA is made by mixing 1 ml latex and 9 ml TCA thoroughly in the film bottle until a rubber clump and TCA serum form. Then, the rubber clump in the solution is taken and TCA serum is filtered using filter paper, and then analyzed.

The analysis of sucrose concentration (mM) can be measured by Anthrone method: TCA serum is taken as much as 150 µL, (added by 2.5% TCA solution until the total volume is 150 mL) and added by 3 ml anthrone per reaction, then stirred until well blended using a vortex. After that, it is heated by soaking in boiling water for 15 minutes and cooled it back by soaking in the water
with a Beckman spectrophotometer DU 650 [12].

Absorbance. T

Sucrose Concentration * 33.3 (1)

The analysis of the levels of inorganic phosphate (Pi) was performed using the method of [13], based on the principle of binding by ammonium molybdate which was reduced by FeSO4 in acidic reaction so that a blue color forms, then the absorbent was measured at λ 627 nm with a Beckman spectrophotometer DU 650

Concentration Pi * 50 (2)

The analysis of the levels of thiol (R-SH) samples was conducted by taking 1.5 ml (added by 2.5% TCA solution until the total volume was 1.5 ml), plus DTNB of 10 mM and plus Tris buffer solution of 0.5 M 75 mL as much as 1.5 ml, then stirred until well blended using a vortex. Then, the solution was left at room temperature for 30 minutes
measured from TCA serum based on the principle of its reaction using dithiobis-nitrobenzoic acid (DTNB) to form a yellow TNB absorbed at λ 421 nm using Beckman spectrophotometer DU 650 [13].

absorbance. Ab

Concentration of thiol * 10/1000 (3)

The analysis of dry rubber content (DRC) (%) was carried out by adding 10 g of fresh latex to 0.5 mL of 2% formit acid, letting it in the oven for 5 minutes to separate the clumps of latex and serum. The clumps of latex were then milled for 10 repetitions using a roller with a thickness of ± 2 ml, then put in the oven with a temperature of about 70-80 °C until the weight remained. DRC was calculated using the equation [14].

DRC (%) = (netto latex / bruto latex) * 100% (4)

2.4. Statistical Analysis

In the experimental design, the data were Analyzed using the Statistical Analysis System (SAS) Software 9.1, SAS Institute Ltd., USA. Mean comparisons were made using the Duncan's multiple range tests at the 0.05 level of probability based on the analysis of variance [15].

3. Results and Discussion

3.1. Histology of BPM1 Clones

The bark which has been tapped is then restored commonly referred to as renewable bark, while the tree bark which is tapped for the first time is referred to virgin bark [16]. The analysis results of virgin and renewable barks showed that the bark tissues of BPM1 clones had no difference from the number of latex vessels and vessel diameters of virgin and renewable barks (Table 1).

Table 1: Histology of BPM1 clones at the age of 15 years

Bark	∑ Latex Vessels	Diameter Latex Vessels (mμ)
Virgin bark	15.00	23.38
Renewable bark	11.42	24.15

The number of latex vessels in virgin bark was higher compared to renewable bark; however, based on the diameter, the number of latex vessels of renewable bark was higher although statistically they were significantly different. Physiologically, the thickness of virgin bark is related to the age of the plant. The older the plant is, the thicker the bark will be. The number and the diameter of latex vessels, in principle, is the characteristic of a clone, but its development depends on the level of plant growth which is influenced by environmental factors and the genetic of each [6].

3.2. Physiology of BPM1 Clones

The exploitation system can provide physiological stress on the rubber plant, so it is necessary to observe physiological parameters through the diagnosis of latex with the aim of determining the effects of the exploitation system on the plant health condition [17].

The role of dry rubber content (DRC) shows a balance in latex regeneration between tapping activities. Low DRC shows that the frequency of taping is too close, not allowing sufficient time for the plant to perform the latex synthesis [6].

The research results in Table 2 show that the exploitation system significantly affects the DRC, so that the highest DRC was obtained in the treatment of P_2S_1 (39%) and the lowest DRC was in the treatment of P_3S_2 (28%). The high DRC in the treatment of P_2S_1 (S/4 + ETG every 9 days) was probably caused by a short tapping cut and the application of gas stimulant every 9 days. The shorter the cut, the faster the flow pressure rate of the

latex and the smaller the disruption to assimilate transport (18). These results are consistent with the study conducted by [19] that showed that the tapping with short cuts combined with the use of the gas stimulant of ethylene (S/4U d3 ETG 20 / y (2w) could increase the productivity of approximately 66.1- 76.2%.

The low DRC in the treatment of P_3S_2 (S/2 U + ETG every18 days) was caused by long tapping cuts and the application of gas stimulant every 18 days. The long tapping cuts were thought to cause the rate of low latex flows, subsequently supported by the low application frequency of stimulant, leading to low DRC (28%). The application of stimulant every 18 days was probably unable to activate the pump of proton H+ to increase the flow rate of the latex. Pi content also had a low value (Table 2), which is in line with the results of the study carried out by [20] on RRIM clone that showed that with the tapping system S/8 U, DRC was lower than the control (S / 3D).

Sucrose is the basic ingredient of latex formation. Based on the results in Table 2, it could be seen that the exploitation system had a significant effect on the sucrose content of BPM1 clone latex.

The highest sucrose content was obtained in the treatment of P_3S_2 (S/2U + ETG every 18 days) and the lowest in the P4S1. The highest sucrose content in the treatment of P_3S_2 was thought to be caused by low Pi (18.80 mM). Pi is the driving force or energy source that converts sucrose into latex, and low levels of Pi would result in a shortage of energy in plant for the process of changing sucrose into latex. This is consistent with the statement of [10] that the changes of sucrose into dry materials were very dependent on the availability of Pi.

The application of gas stimulant ETG every 18 days in the tapping system S/2U was also less effective to be used for rubber clones with slow metabolism. The results of several studies conducted by [21] showed that the use of gas stimulant was effective in the tapping system with short cuts. For rubber clones with slow metabolism, the tapping system with double cut 2x S/4UD d3.ET 2.5% was recommended.

The low sucrose content was obtained in the exploitation system of P_4S_1 (S/ U + ETG every 9 days) (3.56 mM) (Table 2). The low sucrose content in the treatment of P_4S_1 was probably caused by the clones used in this study, that is, BPM1 clones with the low metabolic rate where the formation of their dry materials was low. Clones with slow metabolism indicate that the formation rate of poly-isoprene (latex) from carbohydrates as the basic materials in form of sucrose as result of photosynthesis is slow to moderate [2]. The application of gas stimulant (ETG every 9 days) was so intensive that it caused the plant did not have enough time to transform sucrose material into latex. The low level of sucrose can also indicate that exploitation is so intensive that it is necessary to reduce it by lowering the intensity of tapping and stimulation [10].

Based on the statistical analysis, the exploitation system had a significant effect on the levels of Pi of BPM1 clones. The highest level of Pi was obtained in the treatment of P_3S_1 (36.30 mM), while the lowest was in the exploitation system of P_1S_1 (12.00 mM) (Table 2). Pi levels indicate the ability of plants to change raw materials into solid particles (latex). Plants with a high content of Pi have enough energy to alter sucrose. The results of this study also indicated the same pattern. The high levels of Pi gave a positive contribution to the improvement of DRC and the decrease of sucrose content. The low Pi level in the exploitation system of P_1S_1 was thought to

be caused by the rubber clones used to be slow in metabolism. The high intensity due to the application of stimulant was also unable to activate the proton pump.

Table 2: Diagnosis of BPM1 Clone Latex with various exploitation systems

Exploitation Systems	Analisys Physiology			
	DRC (%)	Sucrose	Pi	R-SH
		……mM…….		
S/2 + ET 2.5% every 15 days	36.58 cde	6.89 defg	24.70 f	0.43 def
S/2 + ETG every 9 days	31.94 gf	8.26 cd	12.00 j	0.45 d
S/2 + ETG every 18 days	35.71 e	5.26 ghi	21.00 g	0.45 d
S/2 + ETG every 27 days	33.07 f	7.13 def	35.70 a	0.48 c
S/4 + ET 2.5% every 15 days	38.18 abc	9.26 bc	20.35 gh	0.44 de
S/4 + ETG every 9 days	39.92 a	6.59 efgh	26.85 e	0.39 gh
S/4 + ETG every 18days	39.11 ab	8.09 cde	24.50 f	0.36 i
S/4 + ETG every 27 days	36.12 de	10.22 ab	31.85 b	0.41 fg
S/2 U + ET 2.5% every 15 days	34.91 e	10.89 a	28.35 d	0.44 de
S/2 U + ETG every 9 days	32.09 fg	5.93 fghi	36.30 a	0.42 ef
S/2 U + ETG every 18 days	28.53 i	11.66 a	18.80 i	0.42 ef
S/2 U + ETG every 27 days	35.17 e	5.00 hij	30.05 c	0.51 b
S/4U + ET 2.5% every 15 days	38.48 abc	5.46 ghi	25.40 f	0.55 a
S/4 U + ETG every 9 days	37.84 bcd	3.56 j	19.30 hi	0.32 j
S/4U + ETG every 18 days	29.78 hi	8.33 cd	24.80 f	0.38 hi
S/4 U + ETG every 27 days	30.86 gh	4.50 ij	35.45 a	0.44 de

Note : Data in the same column followed by the common letters are not significantly different at the P = 0.05 level according to the Duncan's Multiple Range Test.

The research results in Table 2 shows that the highest stress level was obtained in the treatment of exploitation system P_4 S_1 (S/4U + ETG every 9 days). Although the stress levels were relatively high in this study, they were still relatively safe for latex production plantations. This is consistent with the results of the study conducted by [8] who stated that the thiol levels ranging from 0.4 - 0.9 mM were still considered relatively safe for slow rubber metabolism. The low stress levels were obtained in the treatment of P4S0 with a thiol level of 0.55 mM. The exploitation system of P_4S_0 (S/4U + ET 2.5% every15 days), etepon stimulant in the form of paste that was used in this exploitation system could not be directly available to the plant, because it takes time for the hydrolysis process. Applying etepon stimulant every 15 days provides sufficient time for the formation of sucrose to become latex so that the stress level could be controlled.

Viewed from the thiol levels, the exploitation systems of P_2 (S/4 d3) and P_4 (S/4U d3) and the stimulant applications of treatments S_1 (ETG 9 every days), S_2 (ETG every 18 days) showed relatively low stress because the thiol value ranged from 0.36 to 0.39 mM, but the DRC was quite high (39%). The results indicated that short cuts produce relatively high DRC. This is in line with the statement made by [22], who stated that one alternative to optimize the production was by reducing the length of cuts and the use of gas stimulant technology. Short cuts on each tree tapping could be done more quickly and the effect of gas stimulant was quite effective on the plant productivity.

3.3. Relationship between KKK (%) and Thiol (R-SH) (mM) and Pi (mM)

The increased DRC due to exploitation treatment is related to the character of the physiological balance which is very complex and specific in producing latex [17]. The decline in the physiological conditions of the plant could be determined by observing the DRC and the latex diagnosis. In the event of severe stress exploitation, the latex DRC will decrease drastically. The results of a regression-correlation analysis between DRC (%) and thiol level (mM) presented in Figure 1 A shows that the relationship between DRC (%) and latex thiol was relatively low, as can be seen from the relatively small value of R^2 (R^2 = 0.1). The R^2 = 0.1 means that the contribution of thiol level influenced DRC by 1%.

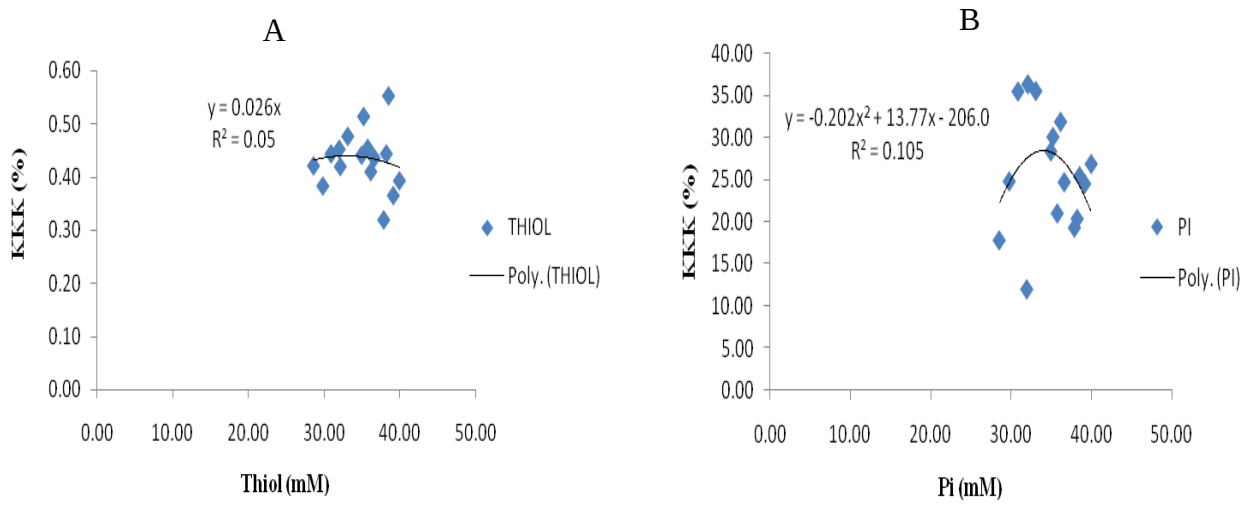

Figure 1: Relationship between KKK (%) and Thiol (mM) (A) and Pi (mM) (B)

Inorganic phosphate (Pi) is an indicator of metabolic activity. It is a description of the plant ability to change the raw material (sucrose) into the rubber particles. A high Pi level shows a high metabolic activity and vice versa [21]. In general, the higher the Pi level in latex, the higher the crop production will be. The results of a regression-correlation analysis between DRC (%) and Pi level (mM) presented in Figure 1 B shows that the relationship between DRC(%) with Pi (mM) was relatively small (R^2 = 0.1). The relationship between the levels of DRC (%) and Pi (mM) is quadratic in form, which means that by increasing the level of Pi (mM) until the

optimum limit, the DRC (%) will also increase. According to [6] the level of DRC (%) maximum for a healthy plant is 25 mM. In general, the study results show that the Pi levels of BPM1 clones ranged from low to high (12.00 mM – 36.30 mM). According to Table 2, based on the treatment of P_3S_1 (S/2U d3 3 tapping activities per ETG application), Pi levels was high (36.30 mM). The increase in the provision of stimulant S_1 (3x tapping activities per ETG application) could improve the metabolism of latex cells, resulting in the increase in KKK [9].

4. Conclusion

The research results showed that there was no difference in the number and diameter of latex vessels between virgin bark and renewable bark of BPM1 clones.

Short cuts (S / 4) with up or down directions produce the highest DRC.

The exploitation systems for BPM1 clones which were relatively safe were P_4S_0 (S/4U + ET 2.5% every15 days), and P_2S_2 (S/4 + ETG every 18days) .

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